IPA® / DOUBLE IPA®

Integrated Patch Amplifier

ELECTROPHYSIOLOGY
PATCH-CLAMP SYSTEM
WITH
SutterPatch® SOFTWARE

Operation Manual

SUTTER INSTRUMENT
One Digital Drive
Novato, CA 94949 USA

Voice: +1 415-883-0128 Web: www.sutter.com
Fax: +1 415-883-0572 Email: info@sutter.com

CE EU Declaration of Conformity

Application of Council Directives:
2014/30/EU (EMC), 2014/35/EU (LVD), and 2015/863/EU (RoHS 3)

Manufacturer's Name:	Sutter Instrument Company
Manufacturer's Address:	One Digital Drive Novato, CA. 94949 USA Tel: +1 415 883 0128
Equipment Tested:	**IPA** and **Double IPA** Integrated Patch Amplifiers
Model(s):	Controller, headstage, and expansion panel
Conforms to Standards:	EMC IEC 61326-1: 2020, including: EMC Emissions: EN 55011: 2016+A2:2021, Class A RE & CE, EN 61000-3-2:2019+A1:2021, & EN 61000-3-3:2013+A1:2019 EMC Immunity: EN 61000-4-2:2009, EN 61000-4-3:2021, EN 61000-4-4:2012, EN 61000-4-5:2014+A1:2017, EN 61000-4-6:2013, EN 61000-4-8:2010, & EN 61000-4-11:2020 LVD (Safety): EN 61010-1:2010+A1:2016 Cor. 1/2019
Tested/Verified (based on dPatch) By:	ITC Engineering Services, Inc. 9959 Calaveras Road, PO Box 543 Sunol, CA 94586-0543 USA Tel. +1 925 862 2944 Fax: +1 925 862 9013 Email: itcemc@itcemc.com Web: www.itcemc.com Sutter Instrument
Test Report(s):	EIC 61326-1:2020 EMC requirements 20210922-01; EIC 610101-1:2010 LVD requirements 20210922-01; and RoHS Compliance Statement

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Sutter Instrument Company hereby declares that the equipment specified above was tested and conforms to the EU Directives and Standards listed above, and further certifies conformation to the requirements of the European Union's Restriction on Hazardous Substances in Electronic Equipment Directive 2015/863 (2011/65/EU Annex II) for RoHS 3.

Project Engineer:

Mark Flaming
President

SUTTER INSTRUMENT®
One Digital Drive, Novato, CA 94949 USA Phone: +1 415 883 0128 Fax: +1 415 883 0572
Email: info@sutter.com Web: http://www.sutter.com

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 Sutter 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 the newest breakthroughs 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 provide 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: "IPA" 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 per day. These saved Experiments can then be imported into a larger combined Experiment for data analysis. For large files, this helps to keep the saved data manageable.

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.

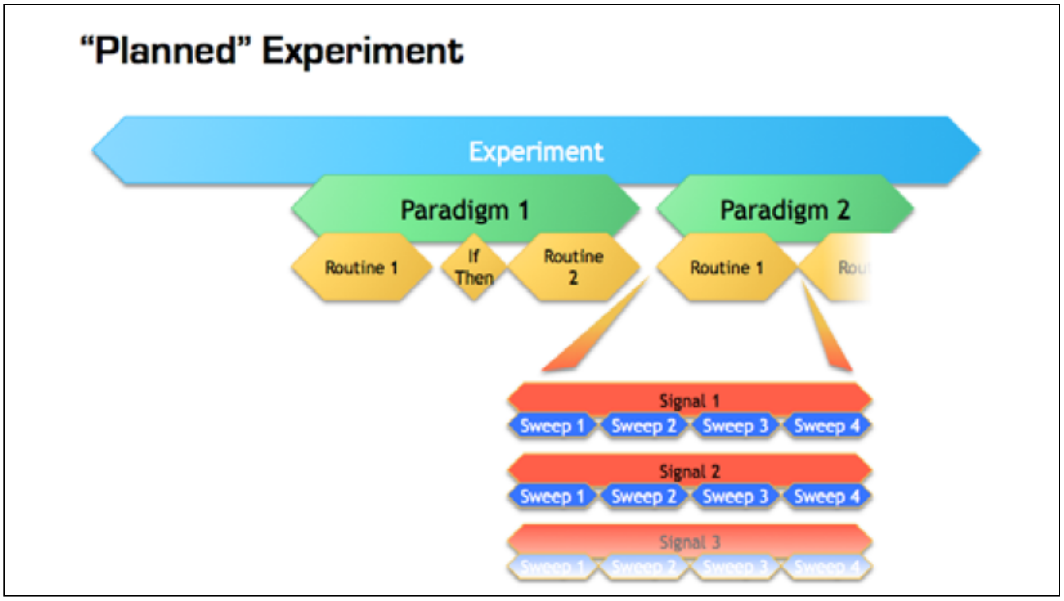


Figure 1-1. Data Structure - Planned Paradigms

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.

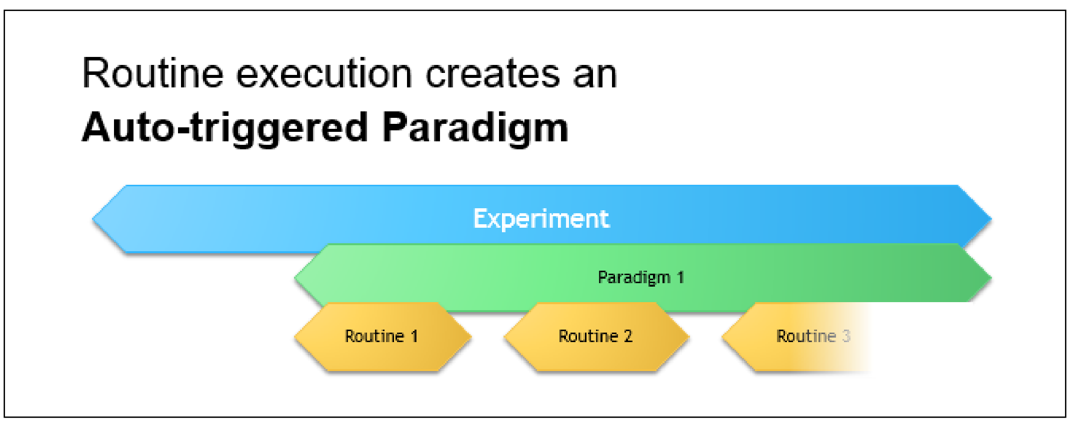


Figure 1-2. Data Structure - Auto-triggered Paradigms

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 metadata.

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 (digital-to-analog), or a physical (analog-to-digital) or virtual input of the IPA system.

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

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

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

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

Signal:

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

Sweep:

A Sweep is the sum off all data points from all Signals, acquired 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, amplitude and duration.

SutterPatch Metadata

Metadata are additional information associated with stored data. These can include such information as the preparation (cell, tissue, animal), instrumentation (hardware, software), environmental parameters (temperature, atmospheric composition), stimuli (chemical compounds, light, acoustic) 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

OS (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) to 10.15 (Catalina) (listed in OS: Apple > About this Mac)
	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 The drive should be configured as the primary system drive.	
Display Resolution:	XGA (1024 x 768)	
Computer Ports:	(1) USB 2.0 High Speed port To check for High Speed USB 2.0 ports on a PC computer, look in the Windows Control Panel / Device Manager / Universal Serial Bus controller section for “Enhanced” host controllers. The newer USB 3.x ports are backwards compatible to USB 2.0 ports, with an appropriate cable. Cables extenders, cables longer than 10 feet, external USB hubs and docking stations are not supported, as they can cause timing issues. Computer USB add-in cards or adapters are not recommended, as compatibility can be problematic. Note: USB 2.0 computer ports are usually implemented with a 'High Speed’ transfer rate, but a slower	

‘Full Speed’ specification can sometimes be found on old computers or USB 2.0 add-in cards.

Also, sometimes BIOS settings, virus scanners and/or Windows updates can put a USB port to sleep. (See Troubleshooting: Startup Q&A ‘USB Communication Fails’.)

Recommended Configuration	(for Bandwidths > 50 kHz)
CPU (Central Processing Unit):	Note: Mac computers based on the Apple Silicon M1 architecture are not yet fully supported by Igor Pro. See https://www.wavemetrics.com/news/igor-and-apple-arm-processors for technical details. Preliminary experiments indicate that SutterPatch Software runs on these computers, both under Igor Pro 8 and 9, and with each of the Sutter Amplifier Systems connected. However, as with each new technology, we cannot fully exclude incompatibilities at this early stage.
RAM:	8 GB
Hard Disk (Free Space):	SSD (Solid State Drive) 500 GB or greater. The drive should be configured as the primary system drive.
Display Resolution:	Full HD (1920 x 1080) 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 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.

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

AC Power:	60 Hz
	50 Hz

The IPA amplifier runs on AC power from 100 to 250 VAC - no switches need to be set.

The AC power should be as clean as possible:

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

2.5 Install Hardware

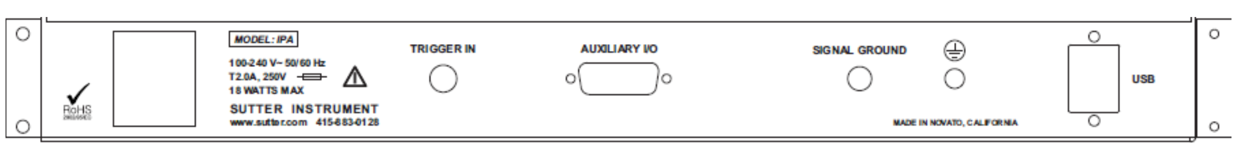


Figure 2-1. Rear of IPA Cabinet

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

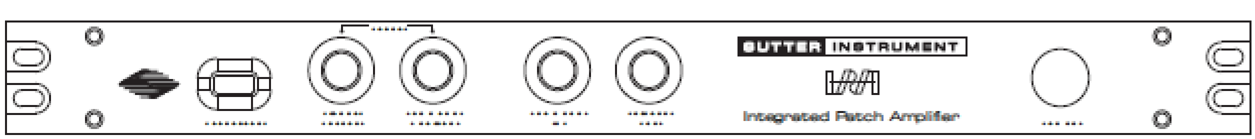


Figure 2-2. Front of IPA Cabinet

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

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

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

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

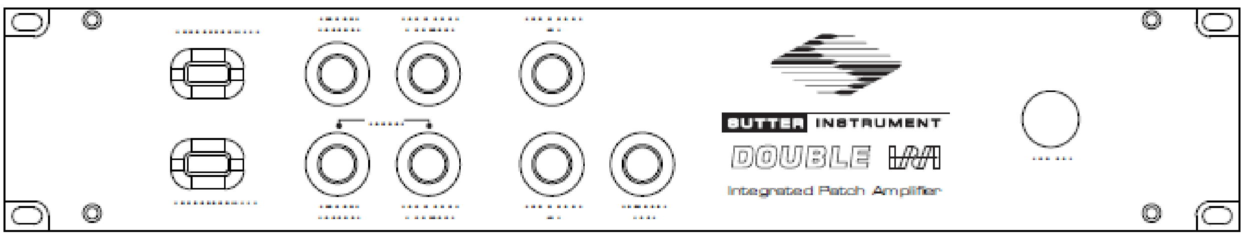


Figure 2-3. Front of Double IPA Cabinet

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

2.6 Install Software

A. Locate the Files

Use your web browser to locate the latest version of the SutterPatch v2.3 installer software at:

https://www.sutter.com/AMPLIFIERS/SutterPatch.html

and choose the ‘Download’ tab.

If internet access is not available, attach the included USB flash drive to your computer USB port, and navigate to the flash drive installer files.

The SutterPatch 'Release Notes' PDF file for recent fixes and changes to the software is also found here.

B. Choose Installer File

Windows	macOS
Full: SP + Igor 9	Full: SP + Igor 9
Full: SP + Igor 8	Full: SP + Igor 8
Updater: SP (Igor 9)	
Updater: SP (Igor 8)	

Separate Full installers (4) are provided for Windows OS and macOS for Igor Pro 9 and Igor Pro 8: both the Igor Pro and SutterPatch software are updated.

It is strongly recommended to run the **Igor Pro 9 Full installer** for optimum data processing performance.

However, if you do not want to upgrade to Igor Pro 9, the last version of Igor Pro 8 (v8.0.4) still supports SutterPatch v2.3.

Additionally, multiple versions of Igor Pro and SutterPatch can be installed on your computer, each with independent settings and parameters.

Full

The Windows "Full" installers install both 64-bit and 32-bit English-language versions of Igor Pro. Only the 64-bit version supports SutterPatch. The 32-bit version is for any Windows 3rd party applications that require it.

The macOS Full installers install a 64-bit English-language version of Igor Pro. For macOS 32-bit support, you need to run Igor Pro 7.

Update

Separate Updater installers (2) are provided for Windows OS running SutterPatch on Igor Pro 9 or Igor Pro 8: only the existing SutterPatch software is updated.

We do not recommend using the SutterPatch Updater on an existing "standalone" installation of Igor Pro. If you already have Igor Pro (without SutterPatch) on your computer, please use the full installer (for the same Igor Pro version) to update the existing version of Igor Pro and install SutterPatch v2.3.

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

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

C. Install the Software

Warning! Before launching the installer, make sure that SutterPatch is not running, or file version errors will occur, and require a re-install.

Use your file browser to navigate to the downloaded installer file and run it.

1. Install the full software for 'All Users' by double-clicking on:
   - Windows: sutterpatch_win_full
   - macOS: sutterpatch_mac_full

2. Follow the installer prompts:
   - We recommended replacing any prior versions of Igor Pro with the latest version of Igor Pro 9, after making a backup copy of all user files and parameter files in the program folder and its sub-folders.
   - If an existing version of Igor Pro is found, the Igor Pro Preferences are overwritten.
   - If an existing version of SutterPatch is found, SutterPatch sample files are overwritten.

3. Upon completion, the installer will report a successful installation. The following files and folders are also installed:

• IPA QuickStart Guide	PDF file with installation instructions and the Igor Pro 9 Serial Number and Key.
• SutterPatch manual	PDF file of the IPA Operation manual.

Windows folders

SutterPatch data:	C:\Users\<User Account Name>\Documents\SutterPatch\Data\
SutterPatch code:	C:\Program Files\Wavemetrics\Igor Pro 9 Folder\

macOS folders

SutterPatch (sample) data & Pools	Applications/SutterPatch2/SutterPatch /Data/
SutterPatch code:	Applications/SutterPatch2 /SP_Code/

Applications/SutterPatch2/SP_Drivers/
(XOP)

4. Launch Igor Pro by clicking on its icon:

5. Activate its license as instructed.

   You will need to enter the Igor Pro Serial Number and Activation Key found in your printed IPA Quick Start Guide.

6. “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 alternative electromagnetic shielding, such as aluminum foil, and connect the shielding material to the headstage ground - a short wire with a metal alligator clip on each end makes a convenient shield-ground connector.

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. Click on the ‘Igor Pro 9’ icon:

An Igor Pro “splash” screen displays while compiling Igor Pro files:

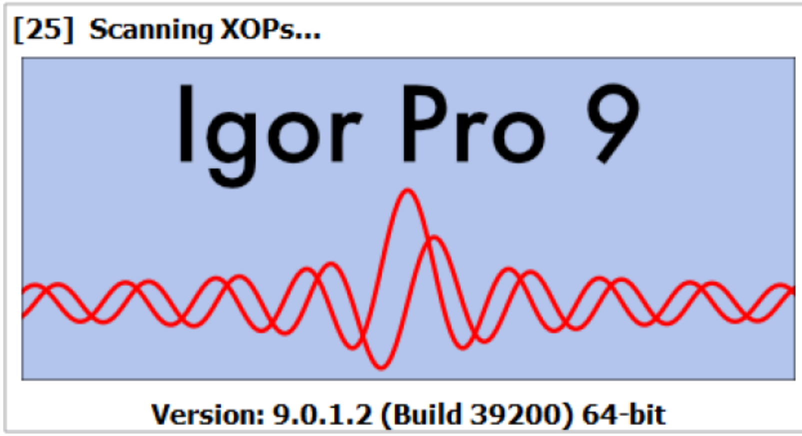


Figure 2-4. Igor Pro Splash Screen.

Then the 'Welcome to SutterPatch' screen displays with launch options:

Figure 2-5. Welcome Screen.

- Igor Only Run Igor Pro (without launching SutterPatch).
- Open Launch SutterPatch from a saved Experiment file.
- Start Launch SutterPatch for a new Experiment.

Don't wait at Startup

Skip this Welcome screen and automatically start a new SutterPatch experiment during startup.

3. Click the 'Start' button and a progress bar displays while compiling SutterPatch files, the Igor Pro Command window displays, and then the Welcome screen closes.

4. Next, the Igor Pro Command window opens.

   Then, if the IPA amplifier is OFF or disconnected from the computer, the 'No USB Connection' pane allows you to re-establish the USB connection, or to select a hardware-emulation mode:

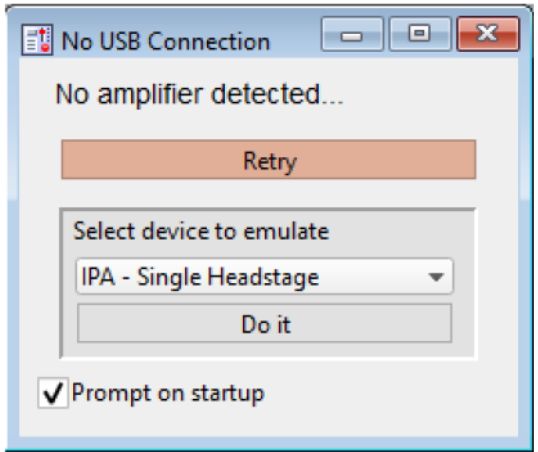


Figure 2-6. Emulation Modes.

a. Reconnect the amplifier, and then click 'Retry', or

b. Click in 'Select device to emulate' and select 'IPA' or 'DIPA', then click 'Do it'.

Note: If amplifier hardware is not attached, and 'Prompt on startup' is disabled, the program will automatically start up in the last known emulation state.

5. A SutterPatch splash screen temporarily displays:

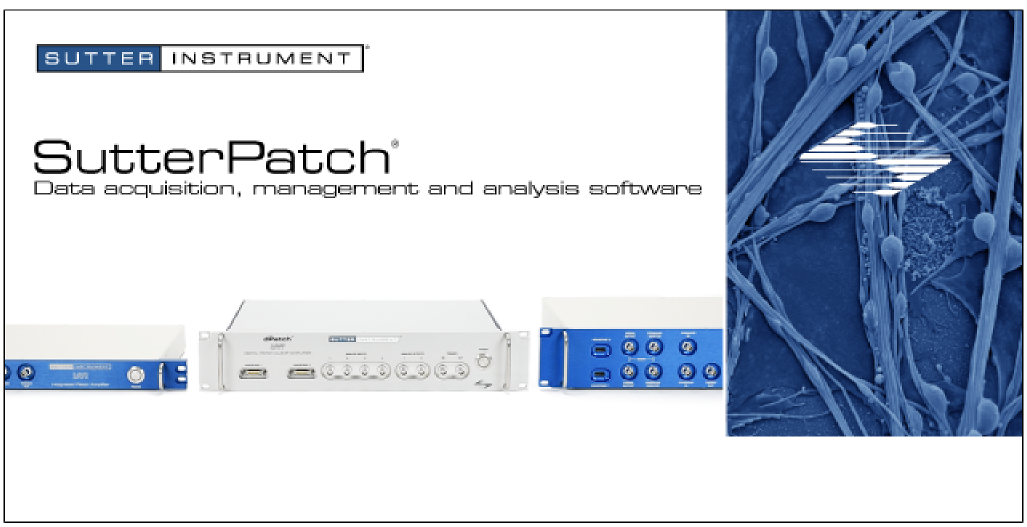


Figure 2-7. SutterPatch Splash Screen.

and then a 'Summary of Major Preferences' window displays:

Major Preferences as set in the "SutterPatch" menu, entry "Set Preferences":
Configuration pool: not used
Selected amplifier: IPA
Automatic experiment naming is ON: <pathname>
Save data to separate HDF5 file is OFF
Save entire experiment after each routine

Warn when file size exceeds limit of 500 MB
Maximal sweeps displayed in persistence display during acquisition: 30
Maximal sweeps displayed in persistence display during reanalysis: 30
Show event tags in reanalysis scope: ON
Number format: Use exponential notation (e.g., 10e-3 V)

Show on startup	Enable display of the Preferences "Summary" window at startup.

6. Other windows that also display are:

   Dashboard
   Acquisition Control Panel
   dPatch Control Panel

   and any other windows that were open in the prior Experiment session.

7. Click on the 'Acquire Data' icon:

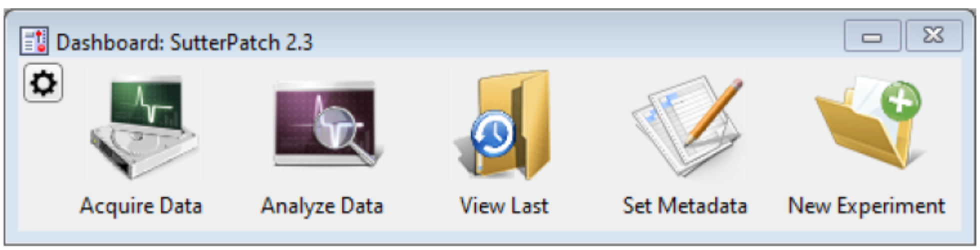


Figure 2-8. Dashboard.

and a second level of the Dashboard is displayed:

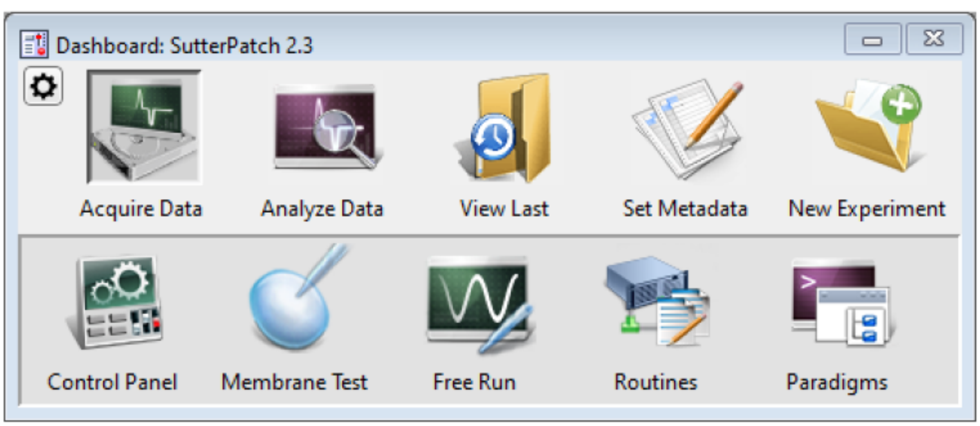


Figure 2-9. Acquisition Dashboard.

8. Click on the 'Control Panel' icon, and the IPA Control Panel is displayed :

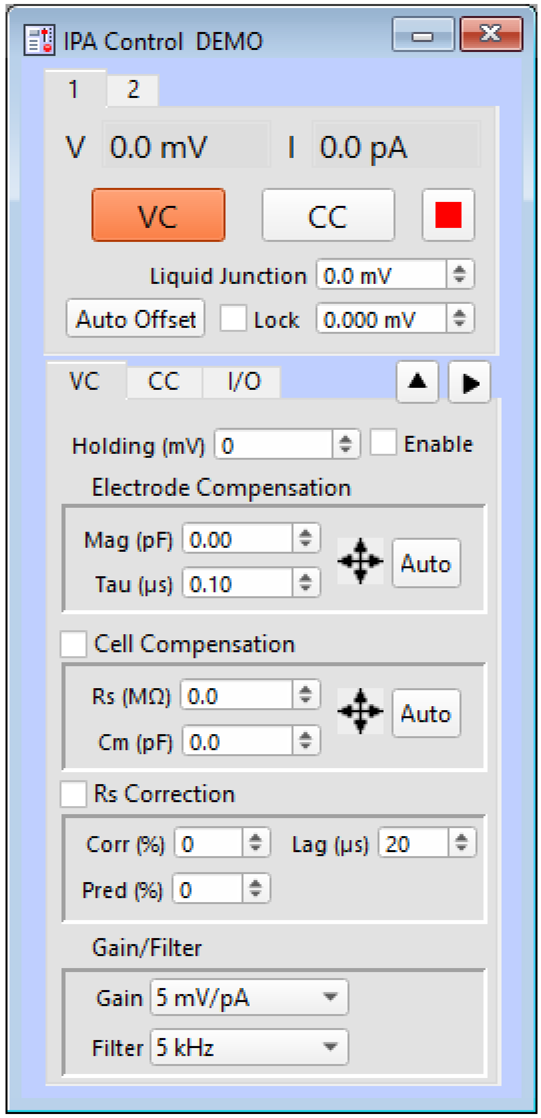


Figure 2-10. IPA Control Panel

a. If "DEMO" displays in the IPA Control Panel title bar, you are running in a hardware emulation mode. To run the physical instrument, ensure that the amplifier is powered on and its USB cable is connected, then choose "New Experiment" from the Dashboard and select "IPA".

b. For the next step, make sure that the IPA Control Panel is in voltage-clamp mode – the "VC" button at the top of the IPA 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 2-11. Dashboard

2. Click on the 'Membrane Test' icon.

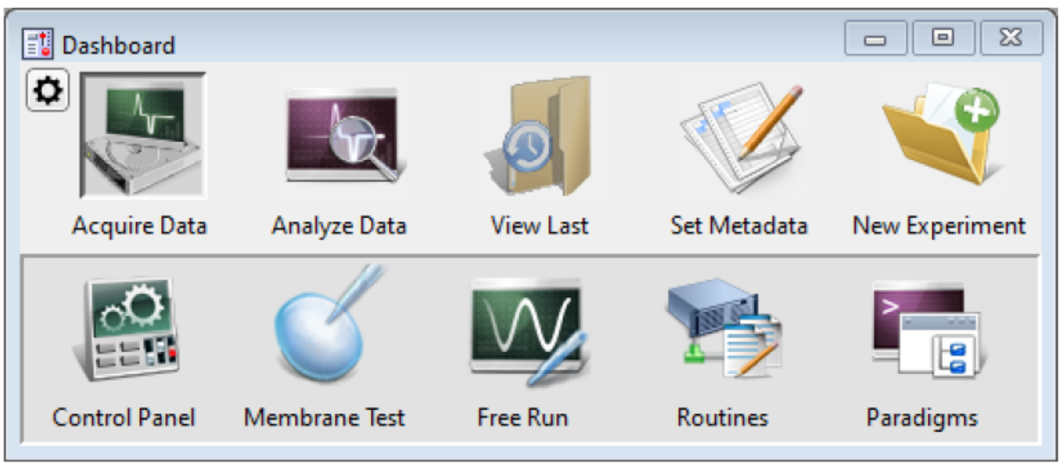


Figure 2-12. Dashboard – Acquisition

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: ~10 MΩ

4. Test the SEAL mode:

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

   a. Set the Model Cell switch: Seal

   b. Click on the Membrane Test ‘Seal’ button.

   c. Verify reading: Seal Resistance: ~1 GΩ to 1 TΩ

5. Test the CELL mode:

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

   a. Set the Model Cell switch: Cell

   b. Click on the Membrane Test ‘Cell’ button.

   c. Verify readings:

	Series Resistance:	~10 MΩ
	Membrane Resistance:	~500 MΩ
	Membrane Capacitance:	~28 pF

6. For a dual-headstage system:

   a. Move the model cell, ground wires and shielding to Headstage 2.

   b. Set the Acquisition scope window to ‘Headstage 2’.

   c. Repeat steps 3 – 5.

.

3. HARDWARE OPERATION

3.1 IPA Front Panel

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

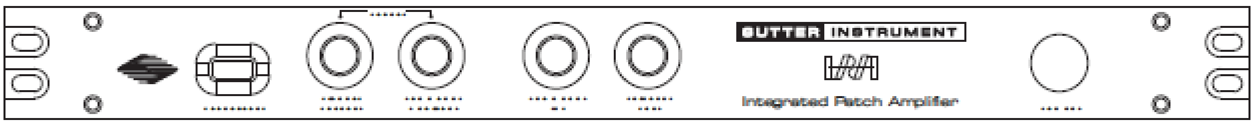


Figure 3-1. Front of IPA Cabinet

The front panel from left to right:

HEADSTAGE:	HDMI-style Type A		For IPA headstage.
SCOPE-SIGNAL OUTPUT:	BNC		A scaled analog output signal of the headstage response signal.
• VC mode:	mV/pA		Variable gain. (Amplifier Control Panel)
	Example:		(for 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
• CC mode:	mV/mV		Variable gain. (Amplifier Control Panel)
	Example:		
	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:		
		Command voltage:		10 mV
		Scaled output voltage:		V * Gain
			=	10 mV * 10 mV/mV
			=	100 mV
•	CC mode:	0.5 mV/pA		Constant gain.
		Example:		
		Command current	=	200 pA
		Scaled output voltage	=	I * Gain
			=	200 pA * 0.5mV/pA
			=	100 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:		
		External Command	=	5 mV
		Stimulus signal	=	20 mV
		Total stimulation	=	(V * Gain) + StimOUT
			=	(5 mV* 10 mV/mV) + 20 mV
			=	70 mV
•	CC mode:	2 pA/mV		Constant gain.
		Example:		
		External Command	=	1 mV
		Stimulus signal	=	5 pA
		Total stimulation :		(V * Gain) + StimOUT
			=	(1 mV * 2 pA/mV) + 5 pA
			=	7 pA

TRIGGER OUT:	BNC	Digital Trigger pulse output. A 100 µs square pulse is 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

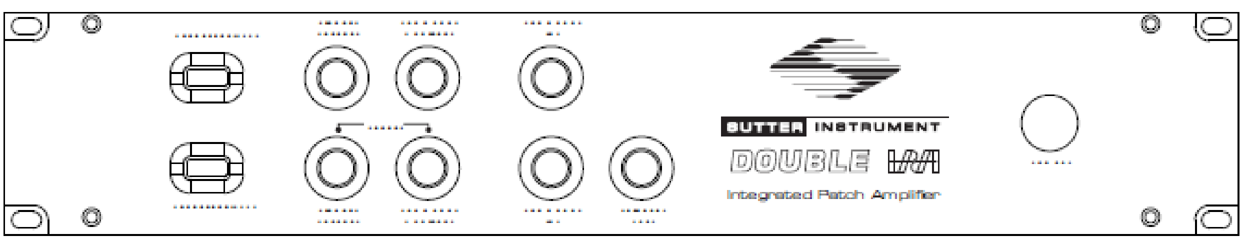


Figure 3-2. Front of Double IPA Cabinet

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.

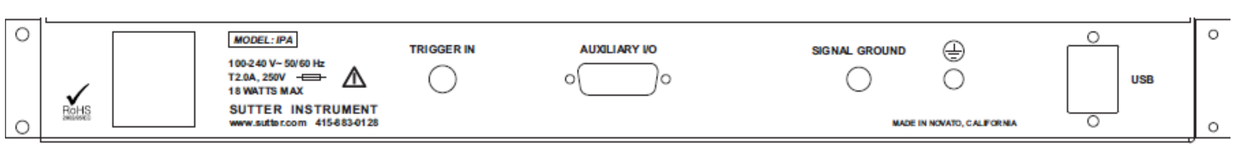


Figure 3-3. Rear of IPA Cabinet

[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

Proper grounding is essential for the integrity of an electrophysiology laboratory setup. It greatly affects the "noise" within your system, and hence the quality of your data recordings. Low noise levels are especially needed for miniature post-synaptic recordings. While AC (mains) line-noise can be software-filtered out of a data signal, it is much more desirable to have a well-grounded electromagnetically clean hardware environment to start with.

For a properly grounded laboratory, an electrical connection is needed from your laboratory's electrical system to an "earth" ground. If your building's electrical grid does not provide an good earth ground, you can create your own earth ground by making use of the building's plumbing system, or by inserting a heavy metal bar 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 the earth ground also helps to prevent ground loops. Consider standardizing your setups by using a GP-17 Ground Point on each rig.

"Signal" ground is a sensitive ground for very low voltages:

- BNC shields: Hard-wired to signal ground (single-ended).
- Bath ground electrode: Connect to the headstage signal ground jack.
- Shielding (Faraday cage): Connect to the rear panel SIGNAL GROUND socket.

However, due to the complexity of grounding factors, you may need to test various strategies for the best grounding configuration for your system. For example, when multiple headstages are used, one or both headstages might need to be grounded.

A grounded power cable is provided with this instrument.

3.5 Headstage

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

Feedback resistor:	500 MΩ	
Whole-cell capacitance compensation:	0 – 100 pF	
Current-clamp rise time:	17.5 µs	(100 MΩ load, 20 kHz filter)

The headstage noise, as measured with an 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	< 1.4 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.

Note: 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

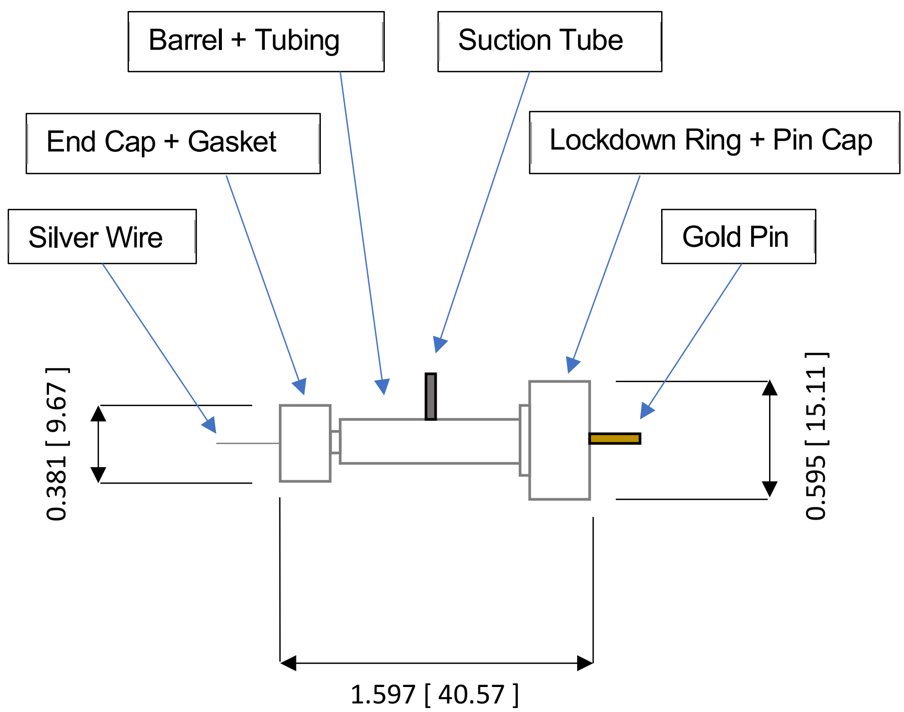


Figure 3-4. Electrode Holder

Figure dimensions are in "inches [mm]".

Assembly Tips

- Silver wire should be kept straight – do not bend or twist it.
- Fire-polish glass electrodes on both ends to prevent scratching the silver wire or the holder barrel.

1. Cut the silver wire to size. Check for proper tubing height and wire-crimping length - the depth it extends into the pipette plus half the length of the barrel. Avoid excess or insufficient amounts of wire.
2. Chloride the silver wire. (see below)
3. Thread the silver wire through the barrel.
4. 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.
5. Thread the small piece of tubing over the silver wire into the end-cup.
6. Crimp the end of the silver wire just slightly over the end of the tubing.
7. Slide the lockdown ring over the tubing-side of the barrel, with the ring’s threads facing outwards.
8. Insert the gold pin into the recessed end of the pin cap - push it through the pin hole until it stops.
9. Screw the pin cap onto the barrel so that pressure from the compressed snippet of tubing ensures good electrical contact between the silver wire and the gold pin.

   For the most stable configuration, before screwing the pin cap onto the barrel, solder the crimped silver wire to the end of the gold pin. Apply only a small bead of solder in the very middle of the top of the pin to avoid any excess solder interfering with the parts properly mating, as excess solder can result in air or solution leaks.

10. Find a silicone gasket with an ID (inner diameter) just greater than your pipette OD (outer diameter):

Gasket ID	Color
1.1 mm	Clear
1.2 mm	Green
1.5 mm	Orange-Red
1.75 mm	Blue

Note: The rubber gasket will wear out over time and need to be periodically replaced.

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

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 desiccant.

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 desiccant overnight.

After daily use:

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

   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.

2. Blot dry.

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 desiccant overnight.

3.7 IPA Amplifier Control Panel

SutterPatch: Hardware Control: 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

<u>General Controls</u>

[Headstage #]   tabs [1 – 4]

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.

 Show additional headstages in their own control panel.

V	‘V’ meter:	Displays the Voltage input channel level.
I	‘I’ meter:	Displays the Current input channel level.
VC	VC button:	Switches the IPA amplifier from Current Clamp to Voltage Clamp mode.
CC	CC button:	Switches the IPA amplifier from Voltage Clamp to Current Clamp mode.
	Warning!	If the headstage is left in open-circuit current-clamp mode for an extended period of time, there is a possibility that internal components can be damaged.
		As a precaution, the resistance meters will display “OVLD”, and the Scope VU meters show a red bar. After 5 minutes in this condition, an alert displays; after 10 minutes, the headstage is automatically switched into voltage-clamp mode.

The button for the active mode (VC or CC) is highlighted in red.

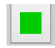
A “gentle” switch is used to protect cells against transition spikes that could degrade the electrode seal and integrity of the recording. The amount of current to hold the voltage steady is automatically injected into the cell during the transition (< 100 ms), before stepping to the new current command level.

If the mode is switched during acquisition, tags are inserted into the data record for the new Control Panel settings.

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

- All USB channels are reset.
- A green button indicates that a stable USB connection to the

amplifier has been established.



- It can take several seconds for the USB connection to be re-established.
- A red button indicates that there is no USB connection to the amplifier.
- When multiple amplifiers are connected, if any one instrument loses its connection, the Reset button turns red.

If an amplifier is attached while in Demo mode, to exit Demo mode and run the hardware, you need to start a new Experiment.

Right-click menu   Click on any blank space in the Amplifier Control Panel to access:

- Reset USB   (same as above).
- Reset Amplifier Controls

  Reset all hardware settings of attached Sutter amplifiers and their headstages to factory defaults.

Liquid junction   [±250.0 mV]

Enter a pre-calculated Liquid Junction Potential (LJP) value for the bath and pipette solutions in use. This value is used by ‘Auto Offset’ to apply “online” LJP correction for whole-cell experiments.

A liquid junction offset occurs when an “open” micropipette is placed into the bath, and an ionic potential forms between the two dissimilar solutions in the bath and micropipette. This potential contributes to the system offset of the amplifier, and needs to be specially handled.
Commercial calculators are available to determine a correct LJP value. An LJP value of “+5” to “+15” mV is not uncommon.

Note: ’Liquid Junction Potential (LJP)’ values are calculated, by convention (Barry), with an opposite polarity to membrane voltage measurements and system offsets. This LJP polarity difference is accounted for by ‘Auto Offset’ for the whole-cell patch configuration.

Auto Offset   [first set the ‘Liquid junction’ value]

This feature requires an “open” pipette in the bath, in voltage-clamp mode.

Click the ‘Auto Offset’ button to apply “online” Liquid JunctionPotential (LJP) correction, so that voltage and current levels are accurate when recording from a whole-cell patch.

The amplifier is automatically tuned, and the hardware portion of the system offset populates the Offset field.

Note: Once a seal forms on a cell, a liquid junction and its offset no longer exist. This is corrected for, in the whole-cell patch configuration, where:

$V_{memb} = V_{cmd}$ - LJP

(See the ‘Liquid Junction’ section for additional information.)

(See the SutterPatch Algorithms appendix for part of the Auto Offset algorithm.)

Lock — Once a system offset has been applied, use the ‘Lock’ check box to prevent accidental changes to the “Offset” value.

The ‘Lock’ is automatically enabled whenever a Routine starts to acquire data.

[<Offset>] — [±250.000 mV]

The “Offset” field is to the right of the ‘Lock’ button, and is independent of the holding potential.

“Auto” Use

An ‘Auto Offset’ automatically populates this field with the hardware offset portion of the system offset.

“Manual” Use

Use for manual tuning of the amplifier.

Place an “open” micropipette into the bath, run ‘Free Run’, and adjust the “Offset” field until the ‘Current’ signal is zero - this compensates for the hardware and liquid junction portions of the system offset.

- Values can be directly typed into the numeric field.
- For fine adjustments, use the up / down spinners to increase / decrease the setting by ~0.015 mV.

  The Offset spinner step size is based on the 16-bit resolution of a 1 V DAC. The actual spinner step size resolution is 0.01525878 mV.
- For moderate adjustments, increase the spinner increment by 10x (~0.15 mV) by holding down the Shift key and 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.

Voltage Clamp Controls

VC The 'VC' tab displays amplifier Voltage Clamp controls:

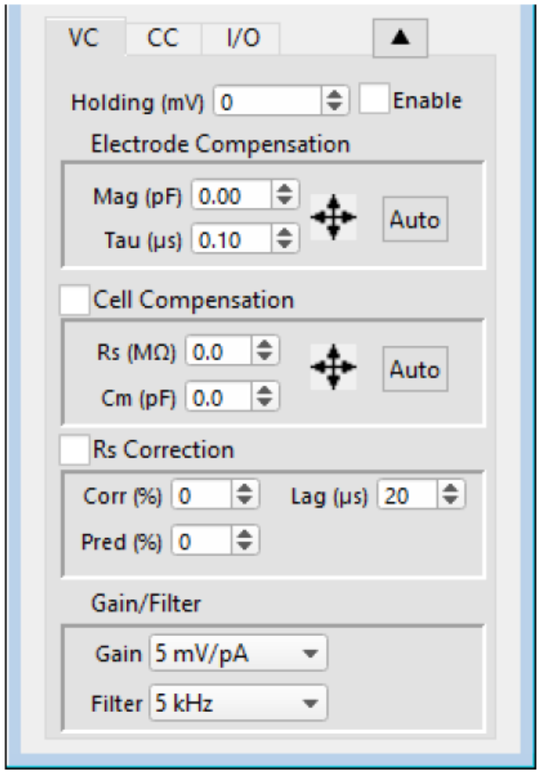


Figure 3-6. VC Control

Holding (mV): [±1000]

After achieving a seal, the holding voltage is typically set to the cell's equilibrium or "resting" membrane potential (typically -60 to -80 mV for neurons). This control is active during acquisition - changes are applied to the next sweep.

- 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.

Enable — Use the Enable checkbox to activate Holding.

If Holding is not enabled, the holding level is zero volts.

The holding value is written at the start of Routine acquisition to the metadata as 'Command Holding Value'.

Whenever this button is activated or de-activated during acquisition, a 'Command Holding Value' tag is inserted into the data recording and written to the metadata.

<u>Electrode Compensation</u>

Electrode capacitance compensation section.

Mag (pF): [0.00 – 25.00] Magnitude

[shared with CC mode]

Tau (μs): [0.10 – 4.50]

Opens a 2-D slider panel for simultaneous tuning of both parameters.

Alert! When dragging with a mouse, slow down when approaching panel boundaries, else undershoot or overshoot of the values can occur.

Auto — Automatically sets approximate values.

After making an on-cell gigaohm seal, large microelectrode capacitance spikes are visible. To remove the transients, click the 'Auto' button. Then, zoom in on the signal,, and if needed, adjust the 'Mag' and 'Tau' controls (separately, or with the slider panel for a combo control), until the signal is adequately compensated. For a square pulse command (such as a Membrane Test 'Seal command), the goal is to eliminate the edge-effect spike transients.

Note: IPA Compensation controls do not affect demo data.

<u>Cell Compensation</u>

Enable whole-cell capacitance compensation.

Rs (MΩ): [0 – 100.0] Series Resistance

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

Opens a 2-D slider panel for simultaneous tuning of both parameters.

Alert! When dragging with a mouse, slow down when approaching panel boundaries, else undershoot or overshoot of the values can occur.

Auto

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

After breaking into a cell, i.e., going “whole cell”, additional large capacitive transients are now generated by the entire membrane of the cell.

To remove the transients, click the ‘Auto’ button. Then, zoom in on the signal, and if needed, adjust the ‘Rs’ and ‘Cm’ controls (separately, or with the slider panel for a combo control), until the signal is adequately compensated. For a square pulse command, the goal is to eliminate the edge-effect transients with minimal distortion of the response signal.

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

Alert! ‘Cell Compensation’ should be disabled when running a Membrane Test in Cell mode, or results will not be valid.

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

<u>Rs Correction</u>

Enable whole-cell Series Resistance compensation.

“Rs Correction” is used to correct command potential voltage drops, to minimize rise-time delays and slow decay phases in the current response, and to reduce unwanted filtering effects, caused by Series resistance.

Corr (%):	[0 – 100]	Correction
Pred (%):	[0 – 99]	Prediction
Lag (μs):	[20 – 200]	RC filter component Control the speed of the correction vs. possible oscillations. (see the Algorithms Appendix)

Rs Correction requires that the Electrode and Cell Compensations are first applied.

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

Next, increase the Correction (Corr) current injected into the membrane to sharpen the rise time. As the Corr setting is increased, the current response transients also increase in size. 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. Then adjust the Cell Compensation ‘Rs’ setting again until the best result is achieved, or try over again with lower Prediction/Correction settings.

Alert! ‘Rs Correction’ should be disabled when running a Membrane Test in Cell mode, or results will not be valid.

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

Gain/Filter

Gain

Analog Input Gain

mV/pA	Signal Range
0.5	± 20 nA
1	± 10 nA
2.5	± 4 nA
5	± 2 nA
10	± 1 nA
25	± 400 pA

New voltage-clamp gain settings are applied to headstage Current input signals when new Acquisition: Routine scope windows are created.

Filter

Input Filter (low-pass 4-pole Bessel)

kHz	Data Point Interval
0.5	2 ms
1	1 ms
2	500 μs
5	200 μs
10	100 μs
20	50 μs

This low-pass filter is applied to the active headstage input signals, and serves to reduce the headstage input sampling rate (as set in Routine Editor / Acquisition & Routine Parameters.)

Filter settings are shared between the voltage- and current-clamp modes. New filter settings are applied when new Acquisition: Routine scope windows are created.

Tip: For experiments where the shape of the response is of interest, an input filter rate of 10 kHz is commonly used.

However, for very long stimuli, you might want to use a lower input filter rate.

Note: The Demo mode does not apply filtering - demo data uses the sampling rate timing (set in Routine Editor: Acquisition & Routine Parameters, or Membrane Test Editor: Settings: Signal Parameters).

Current Clamp Controls

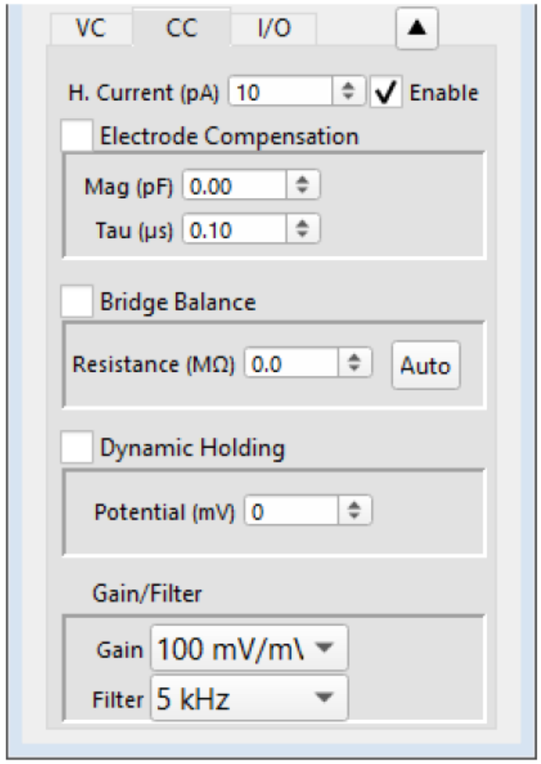


Figure 3-7. CC Control

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

H. Current (pA): [±1000]

A Holding Current can be preset here.

Enable — Use the 'Enable' checkbox to activate H. Current.

If I-holding is not enabled, the holding level is zero amperes. The holding level value is written at the start of Routine acquisition to the metadata as 'Command Holding Value'.

Whenever this button is activated or de-activated during acquisition, , a 'Command Holding Value' tag is inserted into the data recording and written to the metadata.

Electrode Compensation

Enable electrode capacitance compensation.

Mag (pF): [0.00 – 25.00] Magnitude

[shared with VC mode]

Tau (µs)	To remove microelectrode capacitance-charging transients and reduce their filtering effects (increased signal amplitudes and rise-times), click the 'Auto' button. Then autoscale the signal, and if needed, adjust the 'Magnitude' control.

CC mode Electrode Compensation 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.

Note: IPA Compensation controls do not affect demo data.

Bridge Balance	Enable "bridge balance" correction.
Resistance (MΩ):	[0 – 200.0] [shared with VC mode 'Cell Compensation']

[Bridge Balance correction requires Electrode Compensation to be enabled and set.]

Bridge Balance is an adjustment to remove voltage-drop effects due to the electrode Series resistance, when command current is flowing into the preparation. Voltage readings from the cell during current flow (injection) are corrected.

If you are simply recording voltage (I=0) without any current injection, then Bridge Balance can be ignored or left disabled.

To manually determine the Bridge Balance 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 be a small glitch at the beginning of the rise, due to electrode and headstage capacitance that doesn't entirely go away. (Further adjustments to the Electrode Compensation may need to be made.) In some cases, it can be difficult to determine the exact Bridge Balance value, but as long as the Series resistance is significantly smaller than the cell resistance, the errors are very small.

Auto	The Bridge Balance 'Auto' button can be used to approximate the correction value. For larger steps, it is recommended to run the 'Auto' function twice in a row.

Dynamic Holding

Enable dynamic holding.

Potential (mV): [±1000]

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

The tracking time constant (tau) is ”how fast” it takes to reduce the difference between the actual voltage and the target voltage by 64%. The IPA ‘tau’ is ~18 ms.

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

If the amplifier is accidentally disconnected, then when the USB connection is restored, Dynamic Holding will be disabled.

Gain/Filter

Gain

Analog Input Gain

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

New current-clamp gain settings are applied to headstage Voltage input signals when new Acquisition: Routine scope windows are created.

Filter

Input Filter (low-pass 4-pole Bessel)

kHz	Data Point Interval
0.5	(2 ms)
1	(1 ms)
2	(500 µs)
5	(200 µs)
10	(100 µs)
20	(50 µs)

Apply a low-pass filter to the active headstage input signals.

Filter settings are shared between the voltage- and current-clamp modes. New filter settings are applied when new Acquisition: Routine scope windows are created.

In general, it is recommended that the Input Filter Rate be greater than or equal to the Output Sampling Rate.

Tip: For experiments where the shape of the response is of interest, an input filter rate of 10 kHz is commonly used.

However, for very long stimuli, you might want to use a lower input filter rate.

Note: Filtering is not applied in Demo mode. Demo data uses the sampling rate, as set in Routine Editor / Acquisition & Routine Parameters, or in Membrane Test Editor: Settings: Signal Parameters.

Input/Output Controls

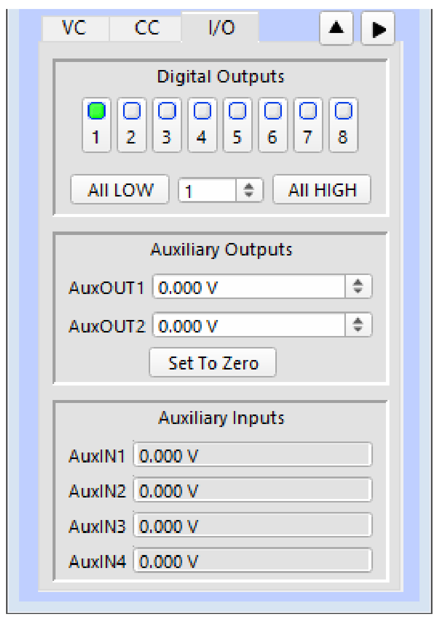


Figure 3-8. I/O Control

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 Outputs

This section controls the holding bit pattern generated by the Digital Outputs of the IPA amplifier. Eight TTL-compatible digital channels are displayed.

[1 – 8] Switch between digital states by clicking on a channel button. When a dot changes color, its channel state changes to:

- **Green** dot On High (+3.3 V)
- White dot Off Low (0 V)

All LOW Set all digital channels 'Off'.

[digital word] Displays the decimal value of the bit pattern.

Or, enter a decimal value to set the bit pattern.

All HIGH Set all digital channels 'On'.

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

<u>Auxiliary Outputs</u>

General purpose "auxiliary" analog 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, then click the 'Set' button to apply the value.

Note: a) Demo-mode output channels are reset to zero when selected.

b) If an auxiliary output (holding) level is changed during continuous acquisition, the only system notification of this is a tag in the metadata of the recording.

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

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

<u>Auxiliary Inputs</u>

AuxIN 1 – 4: [±10 V]

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 tabbed controls below this button.

The horizontal "Show/Hide" button displays/hides the Double IPA input monitor, which displays the real-time Voltage and Current input channel values for both headstages.

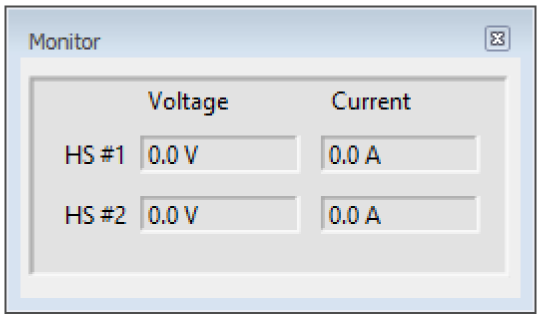


Figure 3-9. Headstage Input Monitor

3.8 Liquid Junction Potentials

A "liquid junction" creates an electrochemical boundary between two solutions (actually a liquid-liquid junction), which produces an unwanted voltage offset between the two liquids. When this occurs between the bath and electrode solutions, the liquid junction potential (LJP) is included in the System hardware offset. As this distorts headstage output levels, the LJP should be corrected for.

<u>Online LJP Correction</u>

SutterPatch LJP correction is applied "on line", i.e., during acquisition, for automatic correction of whole-cell voltage levels.

1. Place an "open" micropipette into the bath in voltage-clamp mode.

2. Disable the Amplifier Control Panel offset 'Lock' control.

3. In the Amplifier Control Panel 'Liquid junction' field, enter your calculated bath-pipette solutions LJP value.

4. Click the Amplifier Control Panel 'Auto Offset' button.

   The amplifier is automatically tuned and LJP correction applied, and the hardware portion of the system offset populates the Offset field.

The amplifier is now ready for recording in a whole-cell patch-clamp configuration.

3.9 Lock-In Adjustments

SutterPatch: Hardware Control: Lock-In Adjustments

The "lock-in" system is used to detect very small changes in membrane capacitance measurements, such as for single-vesicle fusion and retrieval 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. Measurements, such as membrane conductance, are 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 (amperage) 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.
Phase Delay Adjustment	[±1.00 µs] Apply a phase delay to the calculations.
Reset	Reset to ‘0.00 s’.
Attenuation Adjustment	[0.001 – 9.999] Apply a gain to the calculations.
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 - run the electrode compensation on a pulse, and then disable it, 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.

3.10 System Integration

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

3.10.1 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; the command output patterns are configured in the accessible Waveform Editor.

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

3.10.2 Using Multiple Sutter Amplifiers

Any combination of two IPA or Double IPA amplifiers can be connected to the same computer and run simultaneously by the SutterPatch program.

When a computer is powered on, its USB ports (and attached hardware) are detected by the operating system and enumerated in a particular order. The resulting amplifier sequence numbers are listed with their associated model and serial number in the Help / About SutterPatch dialog. This sequence should not change after installation, unless the attached equipment is changed or the USB ports are re-configured.

The amplifier with sequence number 1 is designated the "Main" amplifier, and provides a trigger signal to start a sweep acquisition by the secondary "Triggered" 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 multiple Sutter amplifiers are attached, the Routine Editor also displays their sequence in the 'Acquisition & Routine Parameters' section, along with their serial and headstage numbers in the 'Routine Settings' overview of the 'Input Channels' and 'Output Channels Waveform' sections.

Digital Outputs are only available from the "main" amplifier (#1).

When more than one IPA headstage is attached, the Amplifier Control Panel displays each headstage in a numbered tab [1 – 4].

Up to 16 analog input channels can be configured, using a mix of headstage, auxiliary and virtual input channels from either amplifier.

Note: Demo mode does not support multiple-amplifier configurations.

3.10.3 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 0.5%.

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

3.10.4 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

BNC	Channel
COMMAND IN	Stimulus to the preparation. Combines the analog input signal from an external source with the stimulus (command

	waveform) sent to the preparation. The Command In external signal is summed with the IPA internal StimOUT output signal, and is then sent to the IPA headstage.
SCOPE - SIGNAL OUTPUT	Data from the preparation. This BNC supplies the response from the IPA preparation. The current or voltage response from the headstage is directly available from this analog BNC output, and can be connected to an external data acquisition system for digitization and recording.
SCOPE - COMMAND MONITOR	Data from the Stimulus signal. This BNC allows you to monitor the stimulus channel. The analog stimulus delivered to the IPA headstage (voltage or current) is directly available on this BNC, and can be connected to an external data acquisition system for digitization and recording.

3.11 IPA Maintenance

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

3.11.1 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.

3.11.2 Cleaning

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

3.11.3 Calibration

The IPA amplifiers do not support user calibration.

4. SOFTWARE OPERATION

4.1 Acquisition

SutterPatch acquisition operations.

4.1.1 Acquisition Control

SutterPatch: Acquisition Control

The interactive acquisition controls for both Routines and Paradigms are grouped into this control panel.

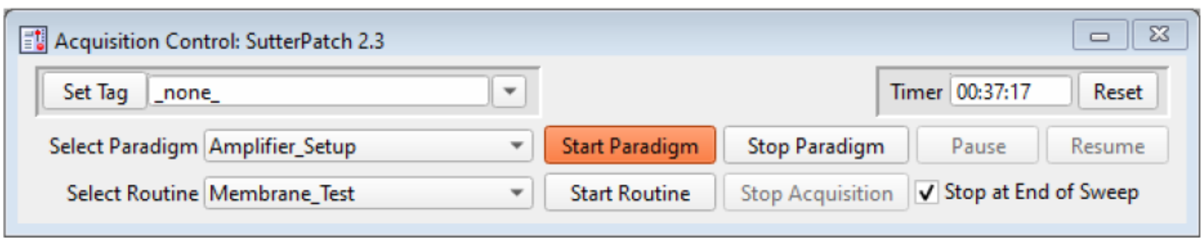


Figure 4-1. Acquisition Control

Set Tag

Click the Set Tag button to create a time-stamped text comment in the Paradigm metadata at any time.

Enter the comment text into the field, or select a comment from a drop-down list of recently used entries The drop-down list is saved with the SutterPatch preferences.

When run during acquisition, the comment tag is also written to the Routine. When the data is opened in a Reanalysis Scope window, the tags are only visible in the Time Course and Concatenated display modes. Tags are also visible in the Data Navigator's Paradigm Review and Routine Review windows.

none	A special case text entry, as a tag is not generated.
Clear Menu	Erase the text comments from the drop-down list.

Cycle to Next	Cycle through the drop-down list of text comments each time the Set Tag button is clicked, starting from the displayed comment. When the last comment in the list is reached, it cycles back to the first comment in the list.

Note: Tag timing is not accurate in hardware emulation mode.

Time	[hh:mm:ss] The system time. [only displays if enabled in Set Preferences / General / 'Show time in Acquisition Control window']
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.
Select Paradigm	The active Paradigm is selected from the loaded 'Paradigm Pool' list. See the Paradigm Editor to load a different Paradigm Pool.
Start Paradigm	Manually run the selected Paradigm. Any existing acquisition in a Scope window is stopped, and a "planned" (user named) Paradigm is created and executed.
Stop Paradigm	Terminate the current Paradigm. The next Paradigm to run starts a new "planned" named Paradigm. Otherwise, the next Routine to run starts a new date/time-stamped "auto-triggered" Paradigm.
Pause	Temporarily halt a running Paradigm or Routine.
Resume	Continue running the paused Paradigm or Routine.
Select Routine	[Membrane_Test, Free_Run, < Routine list >] Select an Acquisition type or an active Routine from the loaded 'Routine Pool' list. See the Routine Editor to load a different Routine Pool.
Start Routine	Manually run the selected "Routine" item. Any acquisition in a Scope window is stopped.

When you click the 'Start' button, the Scope window is cleared, and data recording starts after ~300 ms. When acquisition is running, the Scope window updates every 200 ms.

If the Sweep Start-to-Start time is ≥ 5 s, the "Time to next sweep: # s" is reported below the Start / Stop buttons.

If Metadata prompts are configured for Routines or Paradigms, the Confirm Metadata Settings dialog displays just before recording begins.

If measurement graphs are enabled, a docked "child" Analysis window opens and plots sweep-by-sweep measurements.

If no prior auto-triggered Paradigm is running, a new date/time-stamped Paradigm is created.

Stop Acquisition	Terminate any running data acquisition.
Stop at End of Sweep	When 'Stop at End of Sweep' is enabled, and you click the 'Stop Acquisition' button in the middle of a sweep, the sweep completes before data acquisition is stopped; otherwise, when disabled, acquisition stops immediately and the partial sweep is discarded.

Note: Data files are stored in the file path specified in the menu item SutterPatch / Set Preferences / Files and Naming.

Default file path

Windows: C:\Users\<User Account Name>\Documents\SutterPatch\Data\

macOS: Applications/ SutterPatch2/SutterPatch/Data/

4.1.2 Acquisition Measurements & Graphs

Make real-time changes to the online measurements and graphs, even during data acquisition, with this dialog.

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

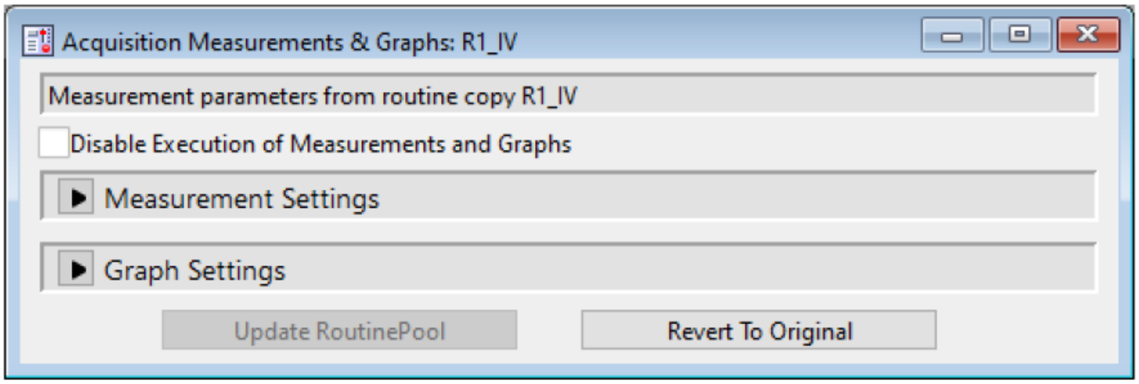


Figure 4-2. Acquisition Measurements & Graphs

This dialog is the same as in the Routine Editor / Real Time Measurements & Graphs dialog, with two extra buttons:

- Update Routine Pool Save your edits to the Routine.
- Revert to Original Discard any edits.

4.1.3 Acquisition: Routine Scope

The Acquisition: Routine Scope window is used for viewing and recording digitized time-series data, displayed as a smooth interpolated line.

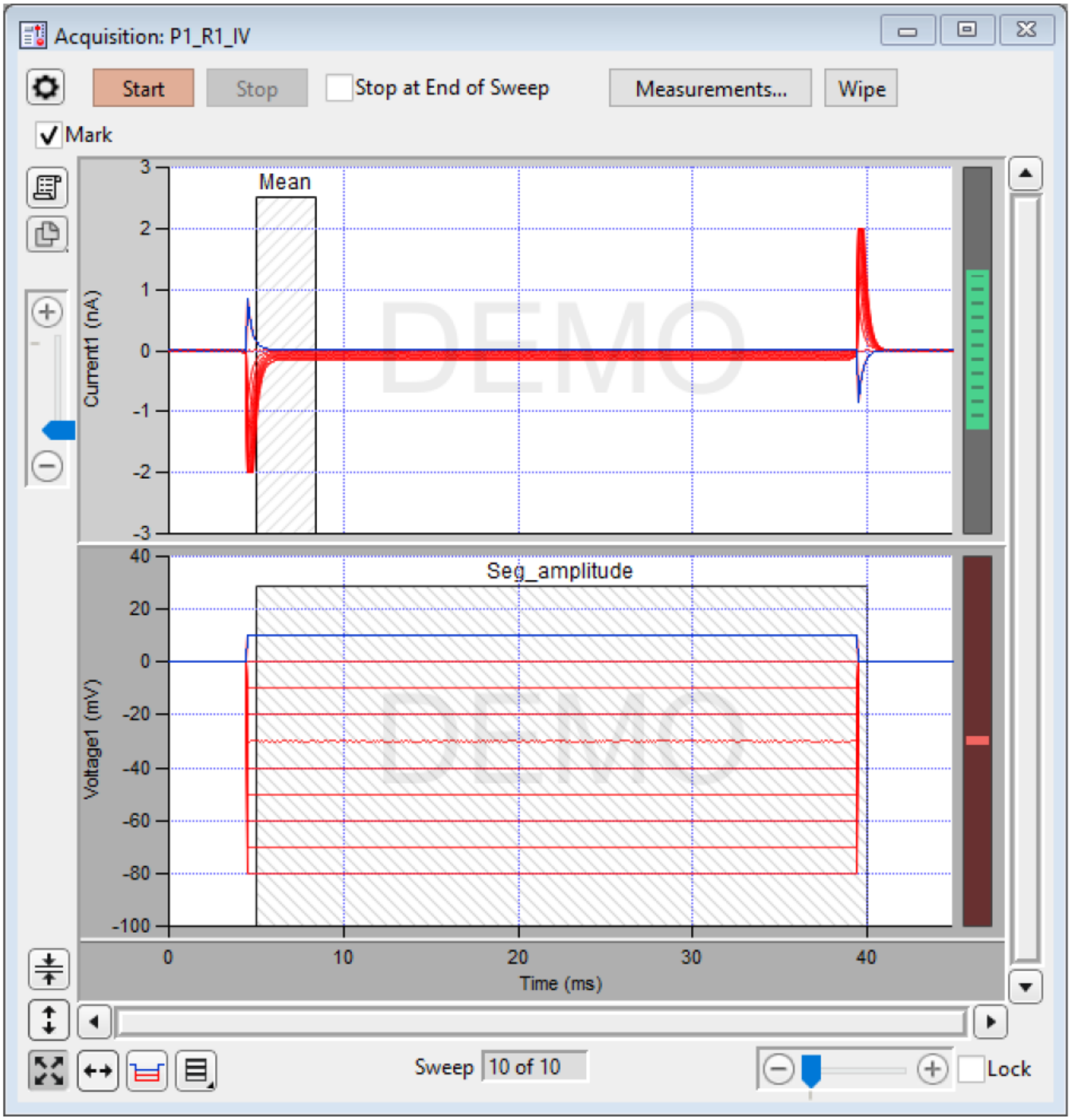


Figure 4-3 Acquisition: Routine Scope Window

The Scope window is titled with the active Paradigm sequence number + Routine name.

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

Signals

The central area of the Scope window graphically displays input data in up to 16 separate signal panes. Click on a signal pane to make it “active” - the Y-axis border area displays in a lighter color, and the Y-axis controls (magnify, scroll) apply to it. Non-active panes display with a darker Y-axis border area.

If multiple signals are displayed stacked on top of each other, you can vertically resize the panes by clicking and dragging them with a resizing cursor. Position the mouse cursor over a pane separator (the horizontal area between panes) to change it to the resizing cursor (a horizontal line with a vertical double-headed arrow.)

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

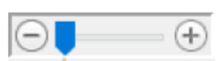
Cursors

Measurement cursors are visible as light gray vertical bars in the signal panes. Each measurement region is bounded by a start-time cursor (the left edge) and an end-time cursor (the right edge).

To move a measurement region, click and drag it with the mouse - the region briefly turns dark when selected.

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

Signal Controls

- 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 Y-axis magnification only controls the active pane; the X-axis magnification controls all signals.

- Lock

  Enable to retain the X-axis scaling and position for the next activated or created scope window (Acquisition, Analysis, Free Run, Membrane Test).

  However, any changes to the X-axis duration (rescaling or autoscaling) or position (scrolling) disables the ‘Lock’ option.

- Axis Zoom

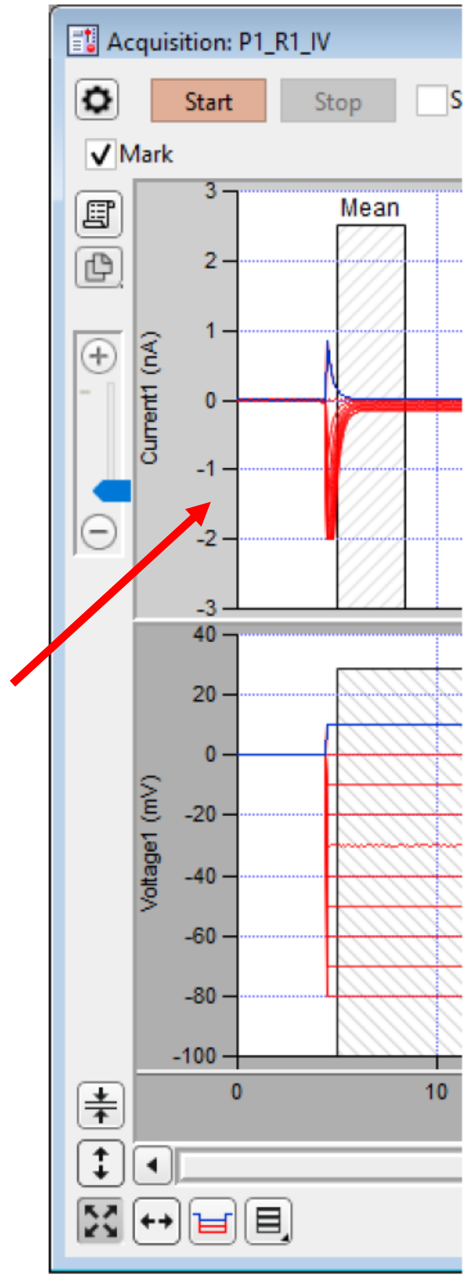


Figure 4-4. Axis Magnification

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-axes or the active Y-axis.

- Area Zoom

  Any area of interest in a signal pane can be graphically selected and expanded:

1. Move the mouse cursor into a signal pane - it changes into a large "+".

2. Click and drag a bounding box around the desired data. (This box is also referred to as a "marquee".)

3. Right-click in the marquee and select the desired action.

Expand	Applies to all signals.
Horiz Expand	Applies to all signals.
Vert Expand	Applies to active signal.
Shrink	Applies to all signals.
Horiz Shrink	Applies to all signals.
Vert Shrink	Applies to active signal.

- Axis Scroll Bars

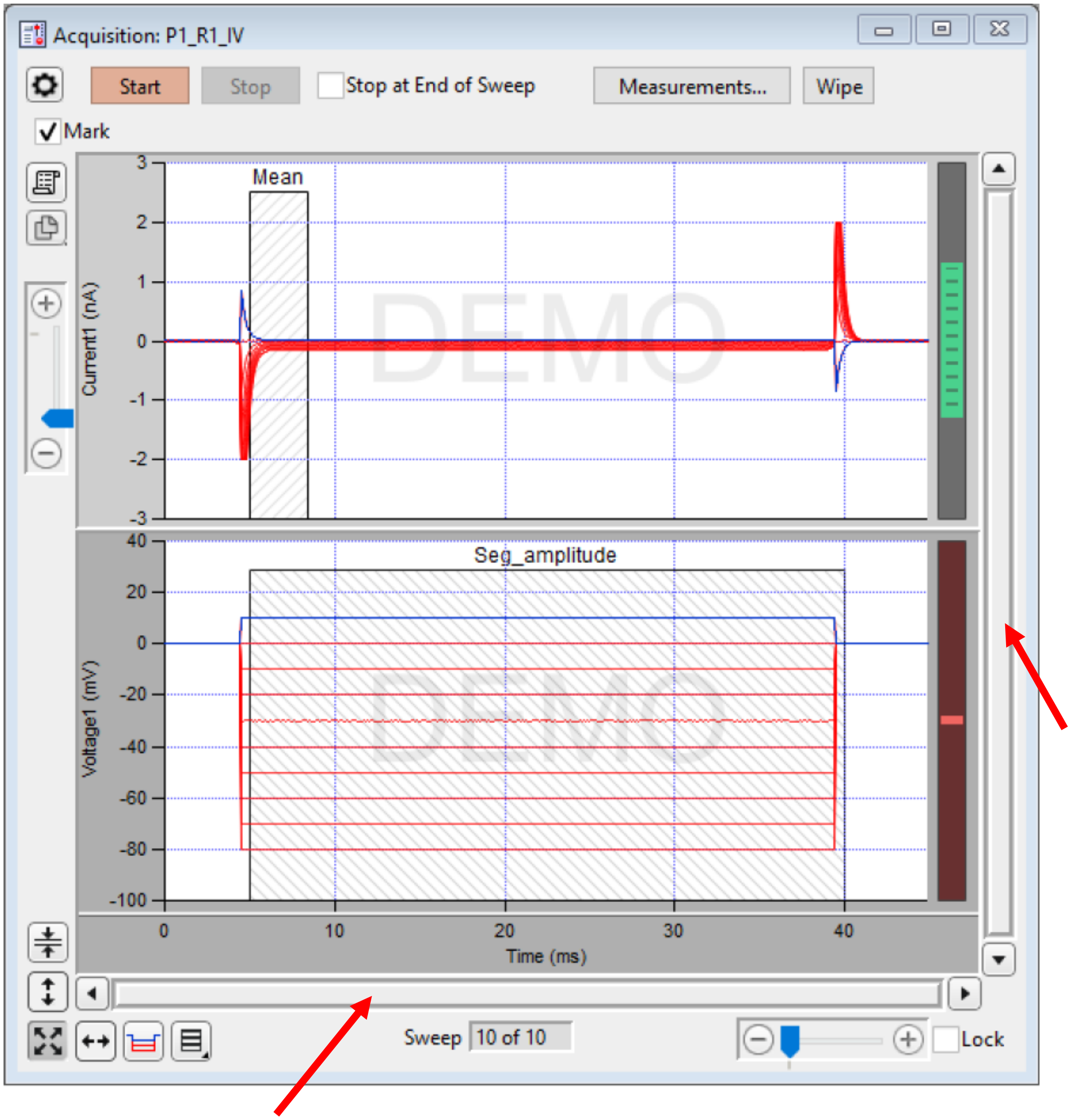


Figure 4-5. Axis Scroll Bars

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. Click and drag the scroll bar slider buttons, or use their directional buttons to move the displayed signals in the desired direction. (The size of the X- and Y-axis scroll bar slider buttons reflects the amount of signal magnification.) The Y-axis scroll bar controls the active signal pane; the X-axis scroll bar controls all panes.

- Center:

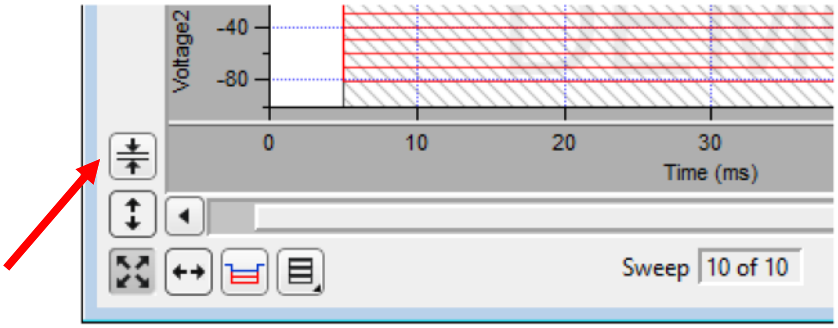


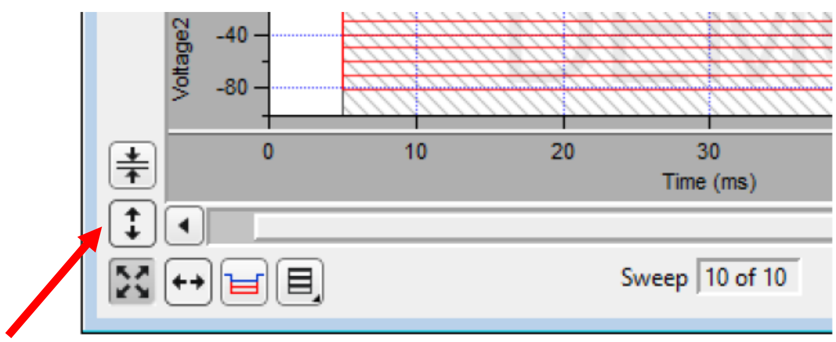
Figure 4-6. Center Button

Center signal.

Center the Y-range of the X-axis data in the active signal pane. The Y-axis offset is automatically adjusted, while the X-axis scaling is unchanged.

To center all signals, shift-click the button.

- Y-Autoscale



Autoscale Y-axis.

Click to autoscale the Y-axis of the selected signal to its visible sweeps data limits.

To autoscale the Y-axes of all visible signals, in “Windows” Shift-click the button, or in “macOS” Control-click the button.

To include the zero amplitude in the Y-ranges, enable “Include zero when autoscaling” in Set Preferences / Scope Window / General.

Tip: To invert the Y-axis of the active signal, such as for data with reversed polarity from an outside-out patch, right-click in the Y-axis of the signal and select Axis Properties / Axis Range. Either reverse the Manual Range Settings / Minimum and Maximum values, or disable the Manual Range and enable the Autoscale Settings / Reverse axis.

- Autoscale Axes

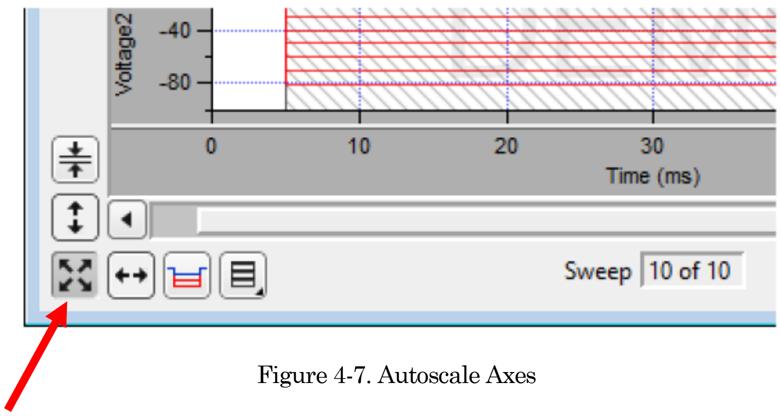
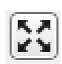


Figure 4-7. Autoscale Axes



Autoscale all axes.

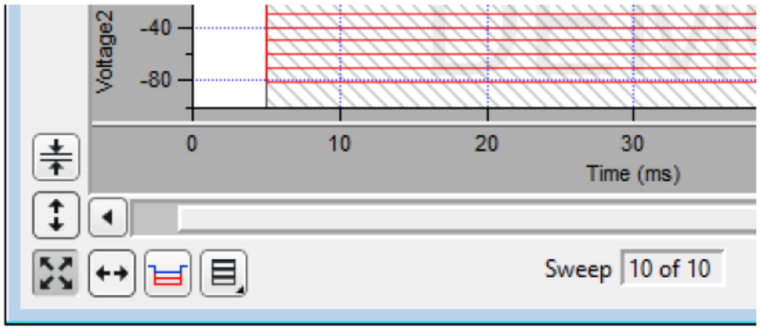
This autoscale setting is used by the Acquisition, Membrane Test, and Free Run Scope windows.

Click to autoscale the Y-axes of all signals to their visible sweeps data limits, and to set the X-axis range to the maximum defined sweep duration for all signals.

To continuously autoscale the Y-axes of all visible signals during acquisition, in “Windows” right-click (or Shift-click or Ctrl-click) the button, or in “macOS” Control-click the button; this also sets the X-axis range to the maximum defined sweep duration for all signals. The Autoscale button remains depressed (grayed) in this state. However, continuous autoscaling is disabled by any changes to the Scope window Y-axis scaling or offset.

To include the zero amplitude in the Y-ranges, enable “Include zero when autoscaling” in Set Preferences / Scope Window / General.

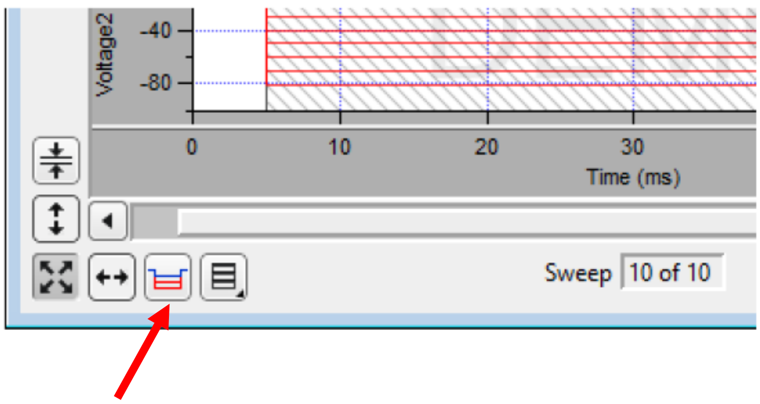
- X-Scale

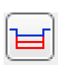


Autoscale all X-axes.

Set the X-axis range to the maximum defined sweep duration for all signals.

- Persistence





Persistence data display.

Enable: Overlay each new sweep of data onto the display of any prior sweeps. (per Scope Preferences limits).

Disable: For each new sweep, all prior sweeps are cleared, and only the newest sweep is displayed.

Applies to the Scope window, and its (right-click) graphs:

Parametric Plot

Amplitude Histogram Plot.

- Signal Layout

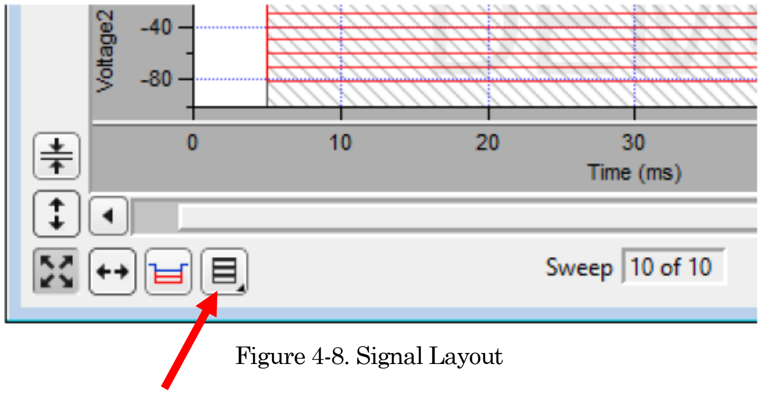


Figure 4-8. Signal Layout

Signal pane layout.

Set how the input signals are graphically arranged:

- Stack Vertical column of signals
- Single Only the active signal
- [m x n] Tiled array of signals in ‘m’ rows and ‘n’ columns

- Amplitude Meters

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

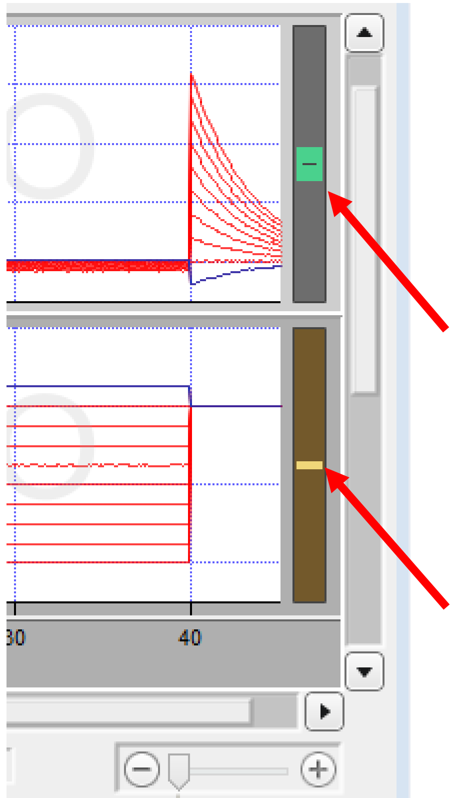


Figure 4-9. Amplitude Meters

For the Triggered Sweeps acquisition mode, each displayed signal has its own Y-axis amplitude meter on the inner-right side of its 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) If too little hardware gain is applied, and 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. If the data saturates, those data points are substituted with the maximum amplitude of their input channel. 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

	Scope Settings Marks selectively flag sweeps for later reanalysis. • Set all marks in sweeps of active series • Clear all marks in sweeps of active series • Set all marks in sweeps of active series by equation (see below)

	------------------ • Begin with all marks set (in sweeps of active series) • Begin with all marks cleared (in sweeps of active series)
[ ] Mark	Enable/disable to "mark/unmark" the current (or upcoming) sweep. This is useful for quality control during slow acquisition of signals. See the Data Navigator 'Available Actions' or use the Reanalysis Scope window to analyze or process marked sweeps.
Start	Start recording and displaying digitized analog data in the Scope window input channels. When you click the 'Start' button, the Scope window is cleared, the Control Panel Offset is locked, and data recording starts. When acquisition is running, the Scope window updates every 200 ms. If the Sweep Start-to-Start time is ≥ 5 s, the "Time to next sweep: # s" is reported below the Start / Stop buttons. If Metadata prompts are set for Routines or Paradigms, the Confirm Metadata Settings dialog displays just before recording occurs. If measurement graphs are enabled, a docked "child" Analysis window opens and plots sweep-by-sweep measurements. If no paradigm is running, an "Auto-triggered Paradigm" is generated and assigned a Paradigm name with the current Date/Time.
Stop	Stop recording data immediately. If in the middle of a sweep, the partial sweep in progress is also saved. If external triggering is configured, after clicking 'Stop', click the 'Do Trigger' button, and then 'Stop' again.
☐ Stop at End of Sweep	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. The message 'Waiting to stop' displays below the Stop / Start buttons, until the last sweep completes and acquisition stops. If no sweep is in progress, acquisition stops at the end of the next sweep to be recorded.
Measurements	Show Cursors: Display measurement cursors in the Scope window. Hide Cursors:

	Do not display cursors in the Scope window. Button displays as "Measurements(H)". Lock Cursors: Prevent cursors from being moved or altered. Button displays as "Measurements(L)". No Measurements or Graphs Analyze with Active Measurements Analyze with Original Routine Measurements Analyze with Routine Last Executed Measurements Analyze with Saved Default Measurements Save as Default Measurements Edit Measurements: Open a special Reanalysis Measurements & Graphs dialog, where all edits apply instantly to the measurements and graphs, even during acquisition. These changes override the loaded routine for quick interactive control. Parametric Plot Plot the relationship between two signals. (see below) Amplitude Histogram Plot Plot an amplitude histogram. (see below) Color Plot Map the data to a color table. (see below)
Wipe	The 'Wipe Scope' button clears the Scope window of all sweeps before the active sweep, and also clears any corresponding measurements from the Analysis window.
Do Trigger	This green button appears when acquisition is started for a Routine configured with an external trigger, and provides a manual trigger option. The message "Waiting for trigger…" also displays.
Copy to Layout 	Copy all visible Scope signals and analyses into a new Layout window, or append to an existing Layout page.

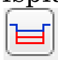
Copy to Clipboard 	Copy the active signal to the system clipboard or, if the 'Shift' key is pressed, the complete Scope window.
Persistence Display 	Enable: Overlay each new sweep onto the display of any prior sweeps (per Scope Preferences limits). Disable: For each new sweep, all prior sweeps are cleared, and only the newest sweep is displayed. Applies to the Scope window, and its (right-click) graphs: Parametric Plot and Amplitude Histogram Plot.
Sweep # of #	The active sweep number vs. the total number of configured sweeps is reported. If multiple cycles are set, the active sweep cycle number is inserted between them.
Lock	

Table 4-1. Other Scope Buttons

Scope window "Gear" Settings

Set all marks in sweeps of active series by equation

Opens the 'Sweep Mark: Equation Editor' to enter an equation for the sweeps to mark.

Equation [ ]

Undo Remove all edits to the equation.

Check Equation Check the equation syntax. The equation is evaluated, and if valid, it reports "Syntax is ok."

Insert special identifier

sweep
Odd(sweep)
Even(sweep)

Do Mark Evaluate the equation and update the sweep marking.

[Status message]

A value >= 0.1 = 1 (marked)

A value < 0.1 = 0 (unmarked)

Scope window Measurements button menu:

Parametric Plot — Display a graph of X vs. Y input signals in a separate window.

If this window is left open when the Scope window is closed, it will also close - then re-opening the Scope window will also re-open the Parametric Plot window.

- **Y-signal** — Select an input signal for the Y-axis.
- **X-signal** — Select an input signal for the X-axis.
- **Plot** — Update the plot using the new parameters.
- **Copy to Layout** — Copy the Parametric Plot graph into a new Layout window, or append to an existing Layout page.
- **Copy to Clipboard** — Copy the Parametric Plot graph to the system clipboard.
- **Time Range** — The time range of the data to be plotted.
  - **Full Trace** — Use the entire trace for the time range.
  - **Sweep Time** — Set relative to the start time of a sweep (time zero).
    - **Start Time** — Set the starting time.

      Once the Start Time is within 2 ms of the End Time, further Start Time increments will increase the End Time by the same amount.
    - **End Time** — Set the ending time.

      Once the End Time is within 2 ms of the Start Time, the End Time cannot be decremented.
  - **Segment Time** — Set the time range as a ratio of the Segment duration.
    - **Segment** — Select the Segment number.
    - **Start Ratio** — [0 = beginning of Segment]

      Set the starting time ratio.

End Ratio	[1 = end of Segment]
	Set the ending time ratio.

Measurements can be made on the parametric graph using Igor cursors (Ctrl-I). The cursor measurement is written to the SutterPatch Notebook window.

Amplitude Histogram Plot

Open a real-time histogram plot window. The amplitude data are plotted in "real time" as samples are acquired and binned. The window is cleared at the start of a new Series.

If this window is left open when the Scope window is closed, it will also close - then re-opening the Scope window will also re-open the Amplitude Histogram Plot window.

Y-signal	Select the input signal to be analyzed.
Time Range	The time range of the data to be plotted.

- Full Trace — Use the entire trace for the time range.
- Sweep Time — Set relative to the start time of a sweep (time zero).

Start Time	Set the starting time.
End Time	Set the ending time.

- Segment Time — Set the time range as a ratio relative to the Segment duration.

Segment	Select the Segment number.
Start Ratio	[0 = beginning of Segment]
	Set the starting time ratio.
End Ratio	[1 = end of Segment]
	Set the ending time ratio.

Histogram Bins	[50, 100, 200, 500, 1000, 2000, 4000]
	Select the number of bins for the amplitude range (X-axis). Changes instantly update the plot.
Plot	Refresh the plot for any Time Range settings changes.

 Copy to Layout

Copy the Amplitude Histogram Plot graph into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard

Copy the Amplitude Histogram Plot graph to the system clipboard.

[graph pane]

Measurements can be made on the amplitude histogram graph using Igor Pro cursors (Ctrl-I). The cursor measurement is written to the SutterPatch Notebook window.

Color Plot

Plot amplitude data in a false-color graph of Sweep vs. Time. This "heat map" display mode is commonly used in fast-scan cyclic voltammetry.

If this window is left open when the Scope window is closed, it will also close - then re-opening the Scope window will also re-open the Color Plot window.

Note: The data display for a sweep is centered on its Y-axis whole number tick mark (±0.5).

Signal — List of available signals.

The color graph is based on the selected input signal name.

If no such signal name exists in the current Series, the color graph is blank.

[range] — Select the Y-range to be used for a Plot.

- Auto Y Range — Use an autoscaled Y-axis range for the data.
- Scope Y Axis min and max

  Use the existing Y-range for the data.
- Given Y min and max

  Set the upper and lower Y-axis boundaries.

Color Table — List of color lookup tables.

Reverse — Reverse the color lookup table.

Plot — Plot the selected signal as a false-color graph using the selected Y-range and color lookup table.

 Copy to Layout

Copy the Color Plot graph into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard

Copy the Color Plot graph to the system clipboard.

Measurements can be made on the color graph using Igor Pro cursors (Ctrl-I). The cursor measurement is written to the SutterPatch Notebook window. Once set, clicking and dragging a cursor will dynamically update its measurement value in the notebook.

<u>Right-click Menus</u>

X Axis

Autoscale All Axes — Scale all signals Y-axes to their data, and set the X-axis range for all signals to the maximum defined sweep duration.

Autoscale X Axis — Set the X-axis range for all signals to the maximum defined sweep duration.

Set X Scale… — Manually set the X-axis range.

- X-min — The minimum X-axis value.
- X-max — The maximum X-axis value.

Axis Properties… — Modify the axes style and components.

Y Axis

Autoscale All Axes — Scale all signals Y-axes to their existing data, and set the X-axis range for all signals to the maximum defined sweep duration.

Continuous Autoscale Y Axis — Continuously scale the signal's Y-axis to its data until the end of the recording.

Autoscale Y Axis — Autoscale the signal's Y-axis to its existing data.

Full scale Y Axis — Set the signal's Y-axis to its full-scale range.

Use Last Y Scale	Maintain the Y-axis scaling at its existing range, overriding any prior Y-axis scaling settings.
Set Y Scale…	Manually set the Y-axis range.

Y-min — The Y-axis minimum value.

Y-max — The Y-axis maximum value.

Copy Y scale of signal

[input signals list]

Apply the Y scaling from another signal.

Axis Properties… — Modify the axis style and components.

To reverse the Y-axis polarity (such as for inside-out or cell-attached patches)…

Axis Range tab

Manual Range Settings

Minimum: Enable and enter a positive number.

Maximum: Enable and enter a negative number.

Or, if Y-axis autoscaling will be used:

Click the 'Uncheck Both' button, and…

Autoscale Settings

Autoscale Only Visible Data: Disable.

Reverse axis: Enable.

Hide Signal <name>	Hide the selected signal in the scope window.
Show Signal <name> Only	Show the selected signal in the scope window, hide all other signals.
Stack All Signals	Display all signals in a single column.

Main Window

Limited data modification menu

Right-click in the blank area in a signal pane.

Tip: If you click too close to the signal data, the full data modification menu displays instead; if this occurs, click near a horizontal or vertical edge of the signal pane.

Autoscale All Axes	Scale all signals Y-axes to their data, and set all signals X-axes to their full-scale range.
Add Annotation	Add a floating text-box label to the signal pane. To edit or delete an annotation, double-click on it.
Export Graphics	Copy the signal and open in a separate window. Saves to Windows / Graph Macros.
Toggle Cursor Info	Show the Cursor Info pane to measure X-Y data points or set a fitting range. (See the ‘Signal data’ section below.) Select ‘Toggle Cursor Info’ again to hide the Cursor Info pane, and any cursor symbols in the active pane.
Colors	Adjust the colors used by the active signal pane:
graph background	The background of the pane.
all axes	The X- and Y-axis areas.
all grids	The grid lines in the pane.
all tick labels	The tick labels in the X- and Y-axis areas.
all axis labels	The axis labels in the X- and Y-axis areas.
Hide Signal ‘<name>’	Hide the selected signal in the scope window.
Show Signal ‘<name>’ Only	Show the selected signal in the Scope window, and hide all other signals.

Show Last Sweep of ‘<name>’ Only

[displays when “All Sweeps” are shown]

Display only the last [marked] sweep of the selected signal.

or

Show All Sweeps of ‘<name>’

[displays when “Last Sweep” is shown]

	Restore the display of all [marked] sweeps in the selected signal.
Stack All Signals	Display all signals in a stacked signal layout.

Marquee

Click and drag the mouse to surround a region of interest, and right-click for a context menu:

Expand	Set the signal's Y-axis range from the marquee vertical data limits, and set all signals X-axes ranges from the marquee horizontal data limits.
Horiz Expand	Set all signals X-axes ranges from the marquee horizontal data limits.
Vert Expand	Set the signal's Y-axis range from the marquee vertical data limits.
Shrink	Move the signal's Y-axis current limits to the position of the marquee vertical data limits, and move all signals X-axes current limits to the position of the marquee horizontal data limits.
Horiz Shrink	Move all signals X-axes current limits to the position of the marquee horizontal data limits.
Vert Shrink	Move the signal's Y-axis current limits to the position of the marquee vertical data limits.
Extract Template	Copy the last sweep to the Template Editor.
Extract To Graph	Display the first trace in a floating window, using all data within the X-range.

Signal Data

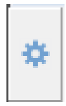
Full data modification menu

Right-click on or near the data to display this context menu, which includes options to modify sweeps and data points, such as marker symbols and lines.

To manually measure X-Y data values, or to set a fitting range, open a Cursor Info pane.

Toggle Cursor Info	Select 'Toggle Cursor Info' to Show/Hide the Cursor Info pane, and any cursor symbols in the active pane.

Cursor Info pane

 Options menu

-  One Mover Moves All — Draggable cursor mover tool moves all cursors together with a single control.
- All Styles — Change the cursor symbol style.
- Show Cursor Pairs — Display up to 5 sets of cursor symbol pairs.

Cursor A

⊕	Cursor symbol for data point 'A'.
A:	Symbol letter (beginning cursor of the pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.
pnt:	Data point number (starting from zero).
X:	X-axis value of data point 'A'.
Y:	Y-axis value of data point 'A'.
ΔY	Difference of the cursor pair Y values.

Cursor B

⊠	Cursor symbol for data point 'B'.
B:	Symbol letter (ending cursor of the pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.

pnt: Data point number (starting from zero).

X: X-axis value of data point ‘B’.

Y: Y-axis value of data point ‘B’.

ΔX Difference of the cursor pair X values.

Cursor Instructions

1. Click on symbol ‘A’ to enable it.
2. Manually drag the highlighted symbol onto a data point in the signal pane, or enter the data point number in the ‘pnt’ field.
3. Click on symbol ‘B’ to enable it.
4. Manually drag the highlighted symbol onto a data point in the signal pane, or enter the data point number in the ‘pnt’ field.

   X- and Y-measurements are displayed for the cursor pair data points.
5. Fitting can also be applied to the cursor pair data. Right-click on the data, and select ‘Quick Fit’ for a list of built-in Igor Pro fitting functions.

   The fit is displayed in the graph, and the fitting information is written to the Command window.

4.1.4 Camera Control

SutterPatch: Camera Control

The Camera Control window displays still pictures or live video.

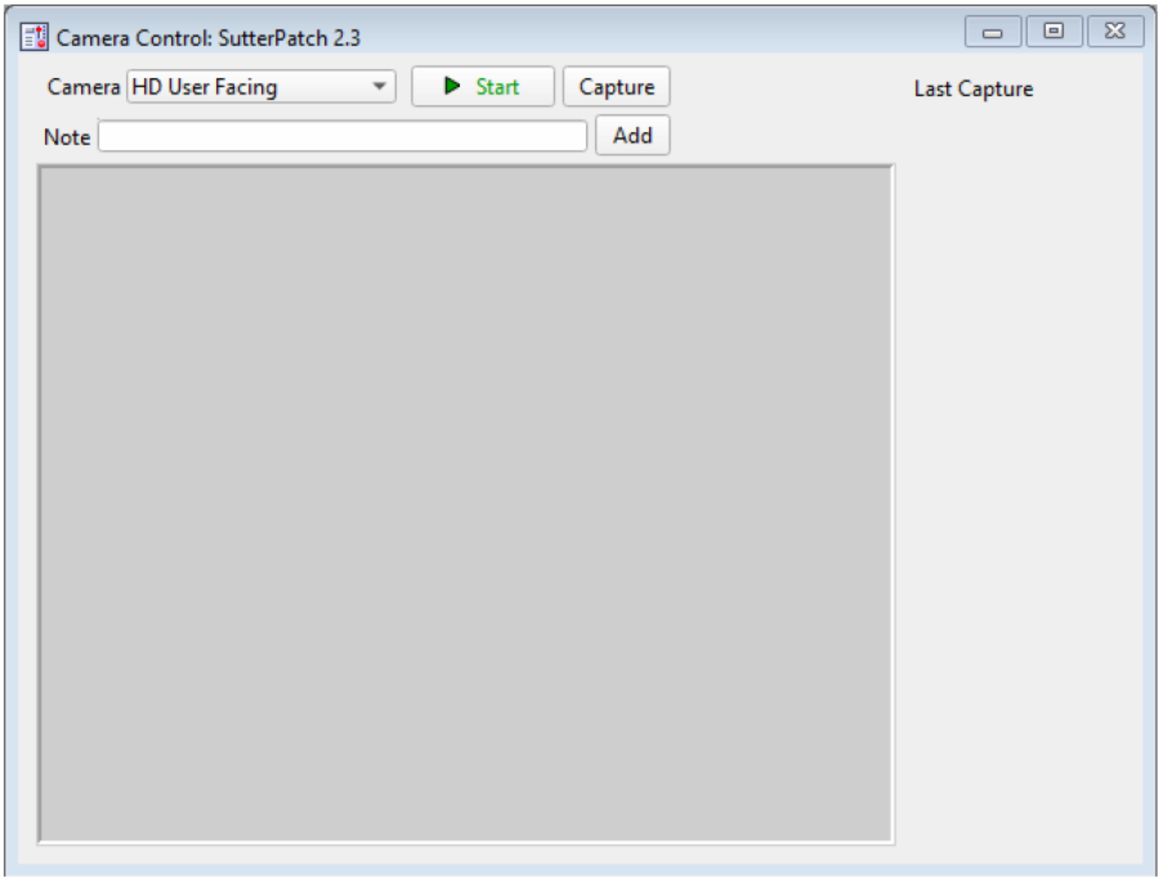


Figure 4-10. Camera Control

Camera	Select a camera name from those attached to the computer.
Start	View live video. This data is not stored.
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.
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 the Data Navigator and select a Paradigm or Routine. Any associated images are listed in the Preview pane. Click on an image name to display the image.
Note	Enter a text message for the ‘Last Capture’ image.

Add	Update the ‘Last Capture’ image with the Note text. Notes are visible in the Data Navigator ‘Images’ preview windows.

[video screen]

Tip: For dark-room experiments, the window background color can be adjusted by the operating system.

Windows:	In the Windows 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

4.1.5 Free Run (Scope)

SutterPatch: Free Run (Scope)

The Acquisition: Free Run (Scope) window simulates a one-channel oscilloscope, and is a quick method of viewing repetitive data.

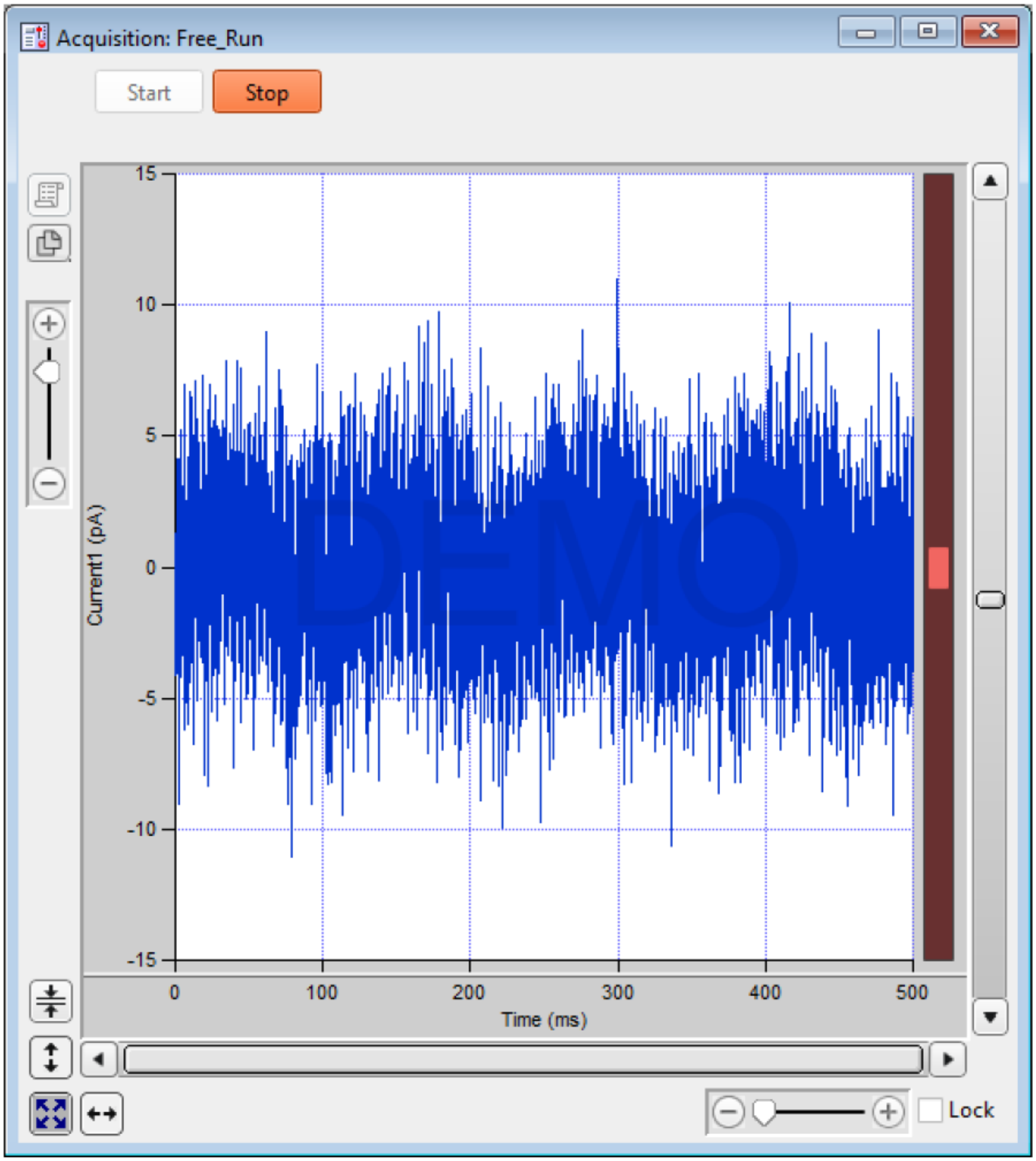


Figure 4-11. Free Run Scope

This window operates similarly to the Acquisition: Routine Scope window, with unsupported controls removed or disabled.

However, when this Scope window is initially created, the Autoscale button is set to the last used state, instead of using a Preferences setting.

Note: Copying to the clipboard can temporarily pause the Free Run display for several seconds, until the display catches up to the actual acquisition.

<u>Free Run Settings Window</u>

Signal Parameters

Channel: Select an input channel to monitor:

Current1 - 2

Voltage1 - 2

AuxIN1 - 4

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

Duration: The duration of the data sweep.

[100, 200 ms, 0.5 , 1 , 2 s]

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

4.1.6 Membrane Test

SutterPatch: Membrane Test

The Membrane Test is primarily used to monitor seal formation and cell health in a voltage-clamp whole-cell patch-clamp configuration.

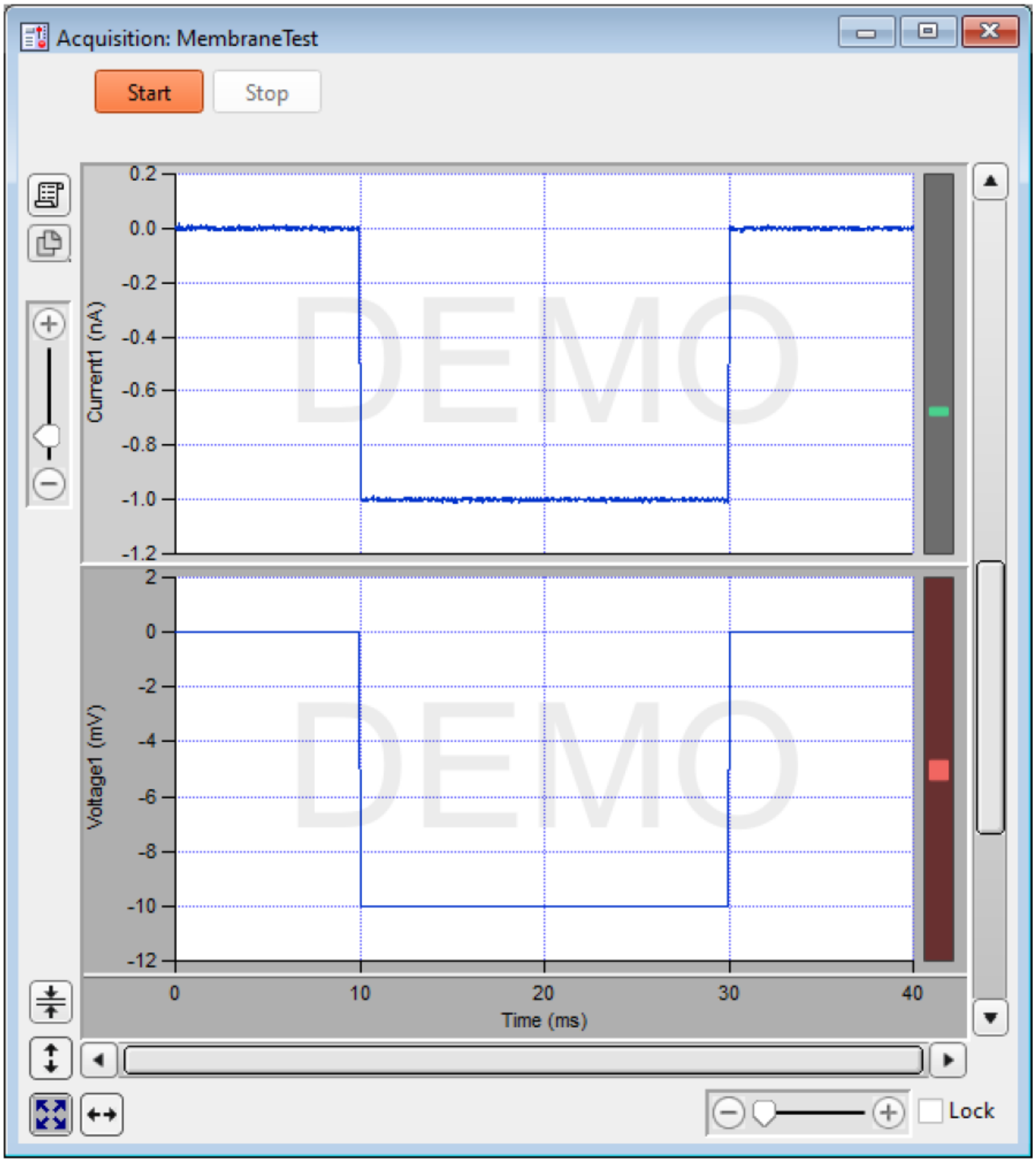


Figure 4-12. Membrane Test Scope

Acquisition: Membrane Test

This Scope window operates similarly to the Acquisition: Routine Scope window. By default, this Scope window's top pane displays the Current signal from the active Sutter headstage, and the pane beneath it displays the corresponding Voltage command signal.

Differences to the Scope window for the Membrane Test:

- Unsupported buttons and controls were removed (Persistence Display, Signal Layout, Sweeps Counter).

- The Sweeps Counter only displays when the Membrane Test is run from a Paradigm with numbered “Repeats” configured.
- The Membrane Test opens in a “running” state.
- During an Experiment, the Autoscale button is set to the last used state, instead of using a Preferences setting.
- Closing the Membrane Test Settings panel halts acquisition.
- If a signal is hidden via the right-click menu, retrieve it with the ‘Stack All Signals’ right-click menu item.

Membrane Test Settings

Configure all Membrane Test parameters in the Membrane Test Settings panel. This panel automatically opens when the Membrane Test is opened or started.

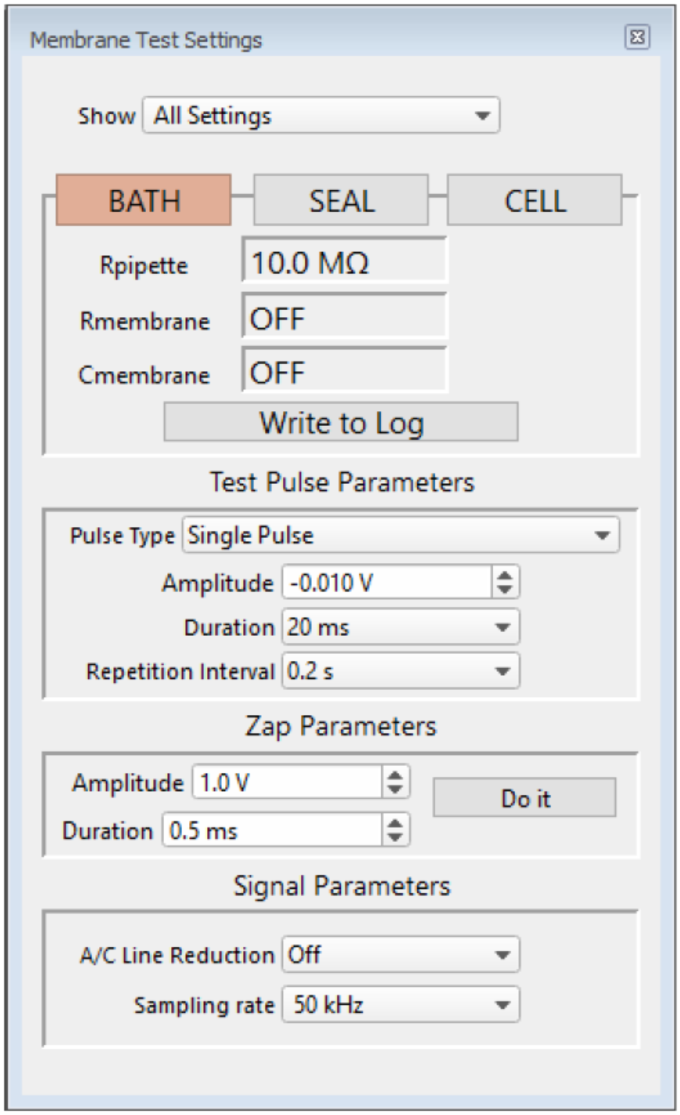


Figure 4-13. Membrane Test Settings

Show

- Monitor Only
- Monitor + Test Pulse
- All Settings

The Membrane Test Analysis panel contains “mode” buttons for 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.

For whole-cell patch clamping of dissociated/cultured cells, typical pipette resistances are 1 – 5 MΩ. For brain slice recordings, pipette resistances up to 20 MΩ or higher are used.

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 GΩ.

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.

The Membrane Test calculations are displayed in real-time numeric fields. (These values are automatically written to the metadata when the test mode is switched.)

Bath	Rpipette (MΩ)	Pipette Resistance meter. [Model cell = ~10 MΩ]
Seal	Rseal (MΩ)	Seal Resistance meter. [Model cell = ~1 GΩ to 1 TΩ] (open circuit)
Cell	Rseries (MΩ)	Series Resistance meter. [Model cell = ~10 MΩ]
	Rmembrane (MΩ)	Membrane Resistance meter. [Model cell = ~500 MΩ]
	Cmembrane (pF)	Membrane Capacitance meter. [Model cell = ~28 pF]

Capacitance values should be periodically checked to monitor the health of the cell due to osmosis and swelling.

Monitoring the Series Resistance is also helpful, as if it increases by more than 5%, the electrode tip might be clogged.

Tip: 'Series Resistance' and 'Access Resistance' are equivalent terms.

Alert! For the Membrane Test 'Cell' mode, disable 'Cell Compensation' and 'Rs Correction' (in the Amplifier Control Panel), so that uncompensated whole-cell capacitance spikes are generated for the calculation of the Series Resistance (Rseries) and Membrane Capacitance (Cmembrane) values, else these fields are reported as "OFF".

Note: Demo values for Cell mode can vary from the model cell values. Reported values are dependent upon experimental variables and settings, such as cell and pipette size, solution conductivity, test pulse duration, etc.

Parameters used in "Cell" mode measurements:

The demo input signal is computed dynamically for the selected bath/seal/cell configuration and the following parameters:

Sampling rate: 10 kHz	Set in Amplifier Control Panel.
Averaging: 10	Set in MT Measure Parameters 'Num to Average'.

Also, the amplifier adjustments, as set in the control panel, are applied to the given simulation:

CSlow = 30 pF	
RSeries = 10 MOhm	Cell Compensation Rs
CFast = 5 pF	Electrode Compensation Mag
CFastTau = 4 µs	Electrode Compensation Tau
PipResistance = 10 MOhm	Rpipette
SealResistance = 1 GOhm	Rseal
CellResistance = 500 MOhm	Rmembrane

Write to Log Click the 'Write to Log' button to write the last acquired measurements for that mode to the Log window. Valid measurements are logged for the active head-stage.

Test Pulse Parameters

Pulse Type

- Single Pulse — A single monopolar square pulse.
- Double Pulse — A symmetrical bipolar (biphasic) square pulse.
- Triangle — [dPatch only] A train of 5 symmetrical bipolar triangular pulses.
- Sine — [dPatch only]

A train of 5 sine wave pulses.

- RMS Noise — No pulse – the holding level is output. The RMS noise of the signal is measured.

  The noise is measured 5 – 10x per second, and averaged from 10 repeats. The bandwidth is 5 kHz.

Amplitude

VC mode: [±1.00 V]

A pulse amplitude is required.

Any value less than |0.1| mV (absolute) is reset to ±0.10 mV.

CC mode: [±2000.00 pA]

A pulse amplitude is required.

Any value less than |0.1| pA (absolute) is reset to ±0.10 pA.

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 to disrupt the cell membrane, 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.

Signal Parameters

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

4.1.7 Paradigm Editor

SutterPatch: 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.

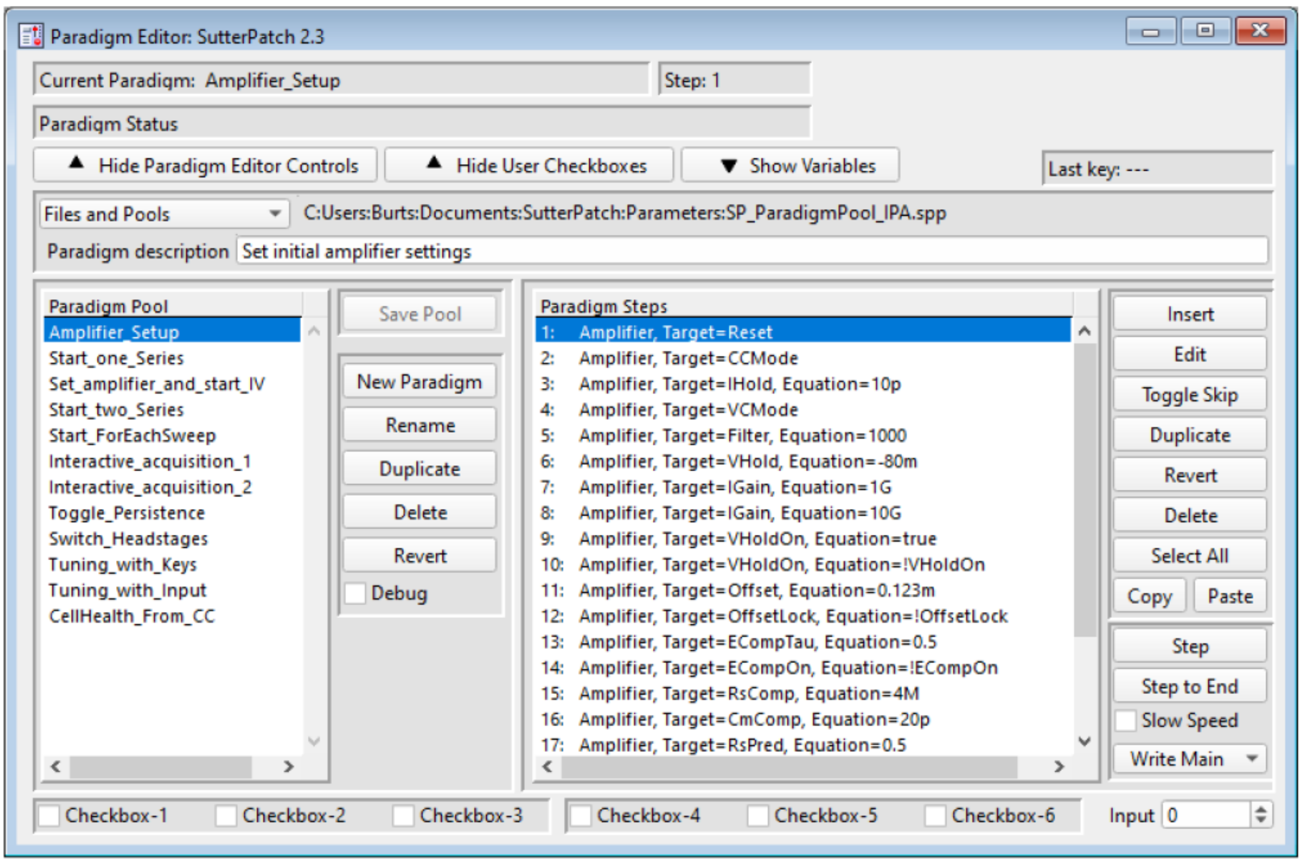


Figure 4-14. Paradigm Editor

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.

<u>Controls</u>

Start/Stop Paradigm, Set Tag and Reset Timer controls are located in the Acquisition Control panel.

Current Paradigm:	The name of the currently loaded Paradigm.
Step:	The highlighted Paradigm Step.

Paradigm Status: Status information about Paradigm execution.

Show/Hide Paradigm 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.

Files and Pools These file operations affect "Paradigm Pools", which can contain multiple Paradigms.

[ ]	List of the 5 most recently used Paradigm Pool files.
Load Paradigm Pool	Load the Paradigms of a previously saved Paradigm Pool file into the 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.
Get Sample Paradigm Pool	Load the IPA factory defaults into the sample Paradigm Pool file (SP_ParadigmPool_IPA.spr).
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.
Send Last Used List to Command	Copy the pathname of the Files and Pools' last used Paradigm Pools list to the Command window history.
Clear Last Used List	Clear the "Last Used" Pool list of all entries.

[ ] Pathname of the loaded Paradigm Pool file.

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.

- Valid characters are alphabetic and numeric (A-Z, a-z, 0-9), and the underscore "_".
- Names starting with a number are prepended with an 'x' to the name.

- Invalid characters at the start of a name are replaced by an 'x'; invalid characters and spaces within a name are replaced by an underscore.

- The maximum name length is 26 characters; extra characters are truncated.

- The minimum name length is 2 characters; a single character is appended with an 'x'.

- Duplicate names are not allowed in a Paradigm Pool; an underscore and autoincrement number are appended 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.

- 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".

<u>Step Buttons</u>

Note: Most of these buttons can handle multiple steps. To select multiple steps, click each step with a Ctrl-click.

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

Set Write Steps

Sound

Start New Paradigm Data

Update Inputs

View Last

Write to Log

Write to Notebook

Alert

Beep

Comment

Pause

Wait

Wait for Trigger

Flow Control

Break

Chain

For Loop

Jump

Label

Condition

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.

The following buttons (Toggle Skip, Duplicate, Delete, Copy, Paste) can handle multiple steps. To select multiple steps, click each step with a Shift-click.

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 after the selected step. Multiple selected steps are inserted after the last 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.
Copy	Select a step to copy to the clipboard.
Paste	Select a step and paste the copied step below it. Multiple steps are pasted as a group.
Delete	Delete the selected step. For multi-line steps, optionally delete the step without deleting the contents of the step.
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.

<u>Write to Log</u>

• Write None	Write No Steps
• Write Main	Write 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

- Write All — Write 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.

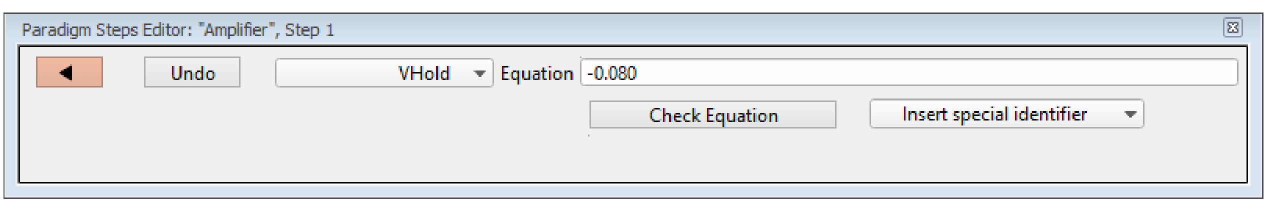


Figure 4-15. Paradigm Step: Amplifier

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

 Close the 'Paradigm Steps Editor'.

Undo — Remove any unsaved edits to this step.

Target — Amplifier settings:

Headstage

SelectProbe (select active headstage)

[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".

IPA Settings

CCMode (amplifier current clamp)

Place the amplifier into Current-Clamp mode.

VCMode (amplifier voltage clamp)

Place the amplifier into Voltage-Clamp mode.

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

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

Set the active headstage holding level.

IHold (amplifer holding current, A)

[± 0.000,000,020 A (±20 nA)]

Set and enable 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)]

Set and enable the active headstage

holding level in Voltage-Clamp mode.

VHoldOn (amplifier holding voltage On)

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

IGain (amplifier current gain, V/A)

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

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

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

VGain (amplifier voltage gain, V/V)

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

- 10 mV/mV
- 20 mV/mV
- 50 mV/mV
- 100 mV/mV
- 200 mV/mV
- 500 mV/mV

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

Gain setting.

Filter (amplifier input filter, Hz)

Apply a preset filter level to the input channels:

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

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

Offset (amplifier pipette offset, V)

[±0.5 (±500 mV)]

OffsetLock (amplifier pipette offset lock On)

[1 = On, 0 = Off]

IPA Compensation

ECompMag (amplifier electrode compensation magnitude, F)

ECompTau (amplifier electrode compensation tau, s)

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]

Bridge (amplifier bridge balance, Ohm)

BridgeOn (amplifier bridge balance On)

IPA Correction

RsCorr (amplifier Rs correction, fraction)

[0.00 – 1.00] Converted to a percentile

RsPred (amplifier Rs prediction, fraction)

RsLag (amplifier Rs correction lag, s)

RsCorrOn (amplifier Rs correction On)

[1 = On, 0 = Off]

IPA Auto and Reset Functions

AutoEComp (amplifier auto electrode compensation)

AutoCellComp
(amplifier auto cell compensation)

Reset (reset amplifier controls)

Dynamic Holding

DynHoldOn (amplifier dynamic holding On)

DynHold (amplifier dynamic potential, V)

Auxiliary Output

AuxOUT1 (Auxiliary Output-1, V)

AuxOUT2 (Auxiliary Output-2, V)

Digital Output

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)

<u>Lock-In</u>

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.]

Values are configured in standard units (Amperes, Volts).

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

Insert special identifier
SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)

<u>**Each Sweep**</u>

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.

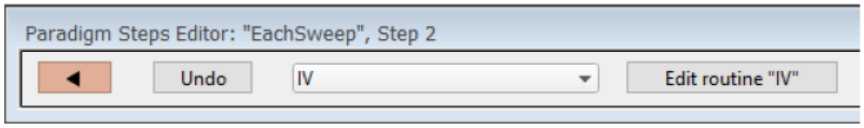


Figure 4-16. Paradigm Step: Each Sweep

Default Setting: *ForEachSweep*
EachSweep, Target= Amplitude_Equations
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.

Edit routine ‘<name>’
Open for editing in the Routine Editor.

Note: When using ‘Each Sweep’ to record data, the minimum sweep start-to-start time is +200 ms.

Routine

Start a Routine, Membrane Test or Free Run.

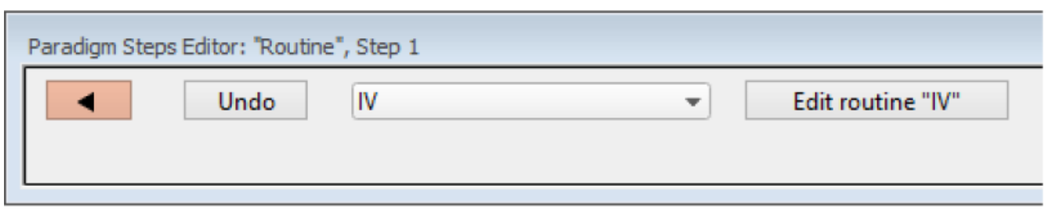


Figure 4-17. Paradigm Step: Routine

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.

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

- Membrane Test

Repeats	[1 – 999]
Indefinitely	Repeat an "infinite" number of times.
Write to Metadata	Write any measurements to the Log window.
Test Amplitude	[No change, ±100 mV, ±2000 pA]
Test Duration	[No change, 0.5 – 500 ms]
Pulse Type	[No change, Single Pulse, Double Pulse, Triangle, Sine, RMS Noise]
Configuration	[No change, Bath, Seal, Cell]
Add Channel	[Clear] Clear the selected channels list. [Headstage1 – 2, AuxIN1 – 8] Available input channels.
[selected channels]	

- Free Run

Total Duration	[100 ms – 999.9 s]
Indefinitely	Acquire for an "infinite" duration.
Store	Save the recorded data.
Add Channel	[Clear] Clear the selected channels list. [Headstage1 – 2, AuxIN1 – 8] Available input channels.
[selected channels]	

- List of Routine names from the loaded Routine Pool.

  [selected Routines]

  Edit routine “<name>” Open for editing in the Routine Editor.

Edit Routine “Routine name”
Open the Routine Editor with the selected Routine loaded.

Stop at end of sweep
[No change, off, on]

If enabled, only full sweeps of data are acquired.

Note: “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.

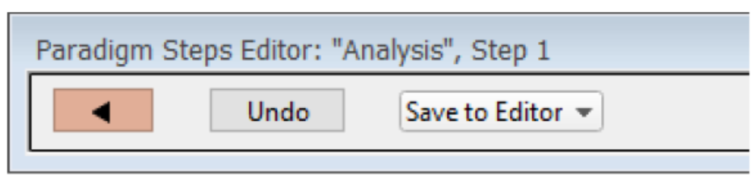


Figure 4-18. Paradigm Step: Analysis

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

 Close the ‘Paradigm Steps Editor’.

Undo Remove any unsaved edits to this step.

[Operations]

- Save to Editor Save the latest analysis.
- Append to Last Append to the prior analysis.
- Average with Last Average with the prior analysis.

- Concatenate — Concatenate with the prior analysis.

- Show Table — Display the analyses as a numeric table.
- Show Graph-[1 – 8] — Display the analysis as a visual graph.

Camera

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

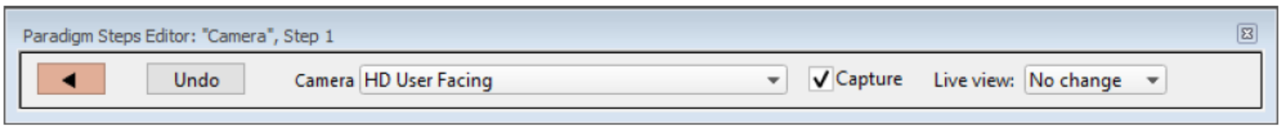


Figure 4-19. Paradigm Step: Camera

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 Pro command.

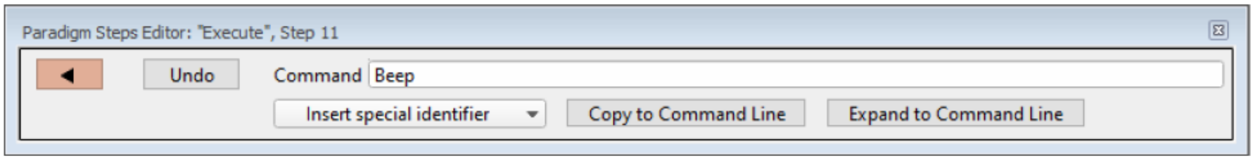


Figure 4-20. Paradigm Step: Execute

Default Setting: *Execute, Command=Beep*

Close the 'Paradigm Steps Editor'.

Undo

Remove any unsaved edits to this step.

Command

[ ]

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

Note: Igor Pro 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.

Insert Special Identifier

Special references can also be used within commands:

- s[series-count, sweep-count, trace-count, routine name]

  (trace of specified series)

  Reference an input trace in an open Scope window via counts of Series #, Sweep #, Trace # (scope channel position), and the Routine name.

Acquisition: Routine Scope window:
The active trace has count values of zero. If a count number is non-zero, it is used as a relative offset (positive or negative) from the active trace.

Reanalysis Scope window:
The first Series/sweep/trace has count values of one. All counts are positive relative to the first trace.

Any fractions in count numbers are truncated to integers.

If the Routine name is left blank, the active Routine is used.

Ex: s[0,0,0,]

The Acquisition: Routine Scope window active Series, active sweep, and active trace of the active Routine.

- t[#] (trace of current sweep of current series)

  Reference the input trace in the open Scope window channel position "#", for the active sweep of the active Series of the active Routine.

- p[1..16] (n'th Paradigm variable)

- eq[equation] (result of the given equation)

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, i.e., 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 Pro 'FFT' command is run, and "t[2]" retrieves the Acquisition: Routine Scope's second input trace.

Export

Export data graphs into a new or open Layout window.

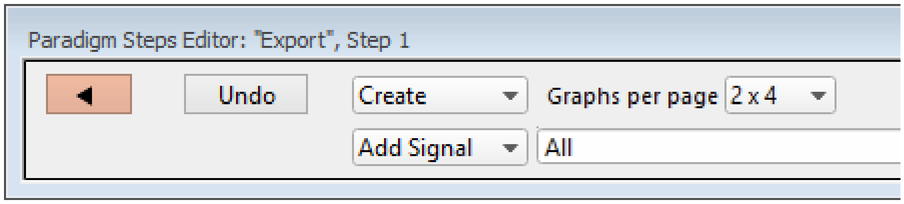


Figure 4-21. Paradigm Step: Export

Default Setting: *Export, Signal=Layout*

 Close the 'Paradigm Steps Editor'.

Undo — Remove any unsaved edits to this step.

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

[Add Signal 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.

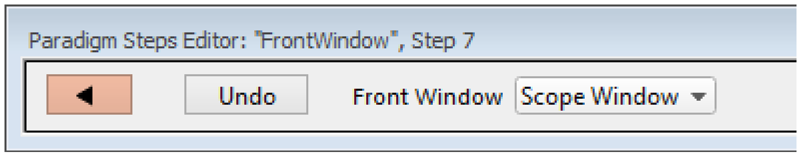


Figure 4-22. Paradigm Step: Front Window

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 4-23. Paradigm 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.

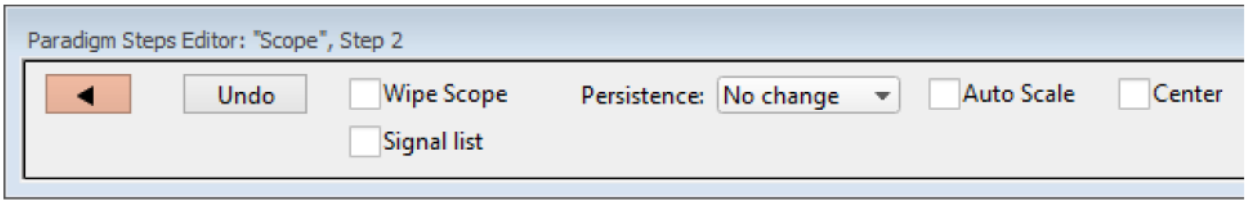


Figure 4-24. Paradigm Step: Scope Operation

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 all sweeps, except the last one.

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 possible 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 — Center 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 selected signals in the open Scope window.

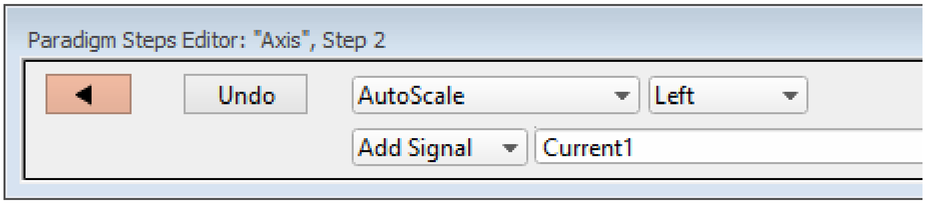


Figure 4-25. Paradigm Step: Set Axis

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] [Left]

Select the Y-axis scaling to apply.

- Autocenter Center the Y-range of the X-axis data.
- Autoscale Match the axis range to the data range.
- Autoscale from Zero

  Display from zero to the largest absolute value.
- Full scale Display the full range of the axis.
- Set scale Enter custom range settings:

  Min.

  Max.

[drop-down list] Select the axes orientation.

- Left Set the Y-axes on the signal list.
- Bottom Set the X-axes of all signals.

[drop-down list] [Bottom]

Select the X-axis scaling to apply.

- Full scale Display the full range of the axis.
- Set scale Enter custom range settings:

  Min.

  Max.

[drop-down list] [only displays for 'Left' axis]

Add the selected signals to the list.

- Clear Clear the signal list.
- All Signals Select all signals.
- [list of all available input signals]
  Select individual signals.

[a list of the selected signals]
[only displays for 'Left' axis]

Signal names can be directly edited; user-defined signal labels can be used.

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".

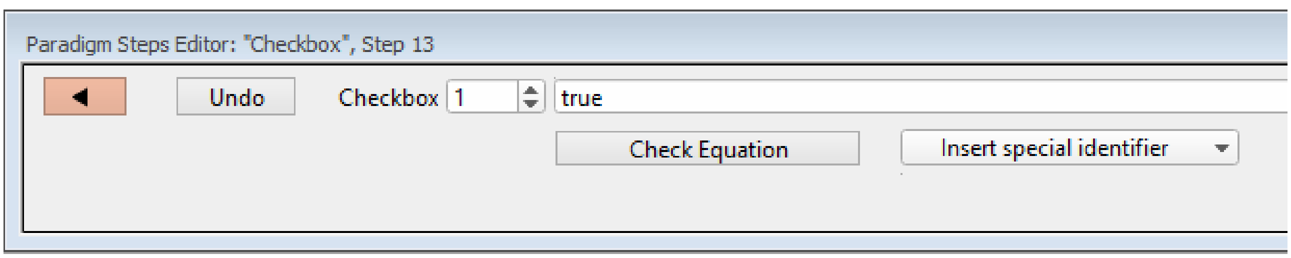


Figure 4-26. Paradigm Step: Set Checkbox

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

 Close the 'Paradigm Steps Editor'.

Undo Remove 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: 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

SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)

Set Mark

The 'Set Mark' step marks (or unmarks) the active sweep for later processing by the Data Navigator.

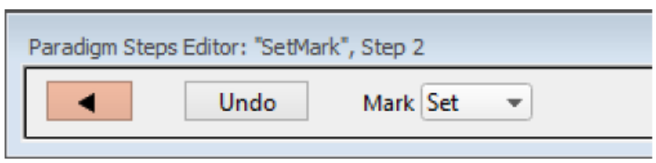


Figure 4-27. Paradigm Step: Set Mark

Default Setting: *SetMark, Value=Set*

 Close the 'Paradigm Steps Editor'.

Undo — Remove any unsaved edits to this step.

Mark

- Set
- Clear
- Toggle

"Marking/unmarking" a sweep marks/unmarks that sweep in all signals in the same Series. Marked sweeps are loaded into the Data Navigator as "marked".

Use within a Paradigm step to mark or unmark a sweep, based upon experimental conditions.

For example, when used within a paradigm 'If' step, if the leak current is too high, unmark the sweep, else mark the sweep. This is an easy way to process just the sweeps that have a reasonable leak current.

Marks are used by the Data Navigator 'Available actions':

Action Potential Analysis

Synaptic Event Analysis

Average Selected Sweeps

Display Signal/Sweep

Export Data

Set Metadata

Define Metadata parameter values to apply to the data during acquisition.

The "Set Metadata Paradigm Step Value" dialog opens for configuration:

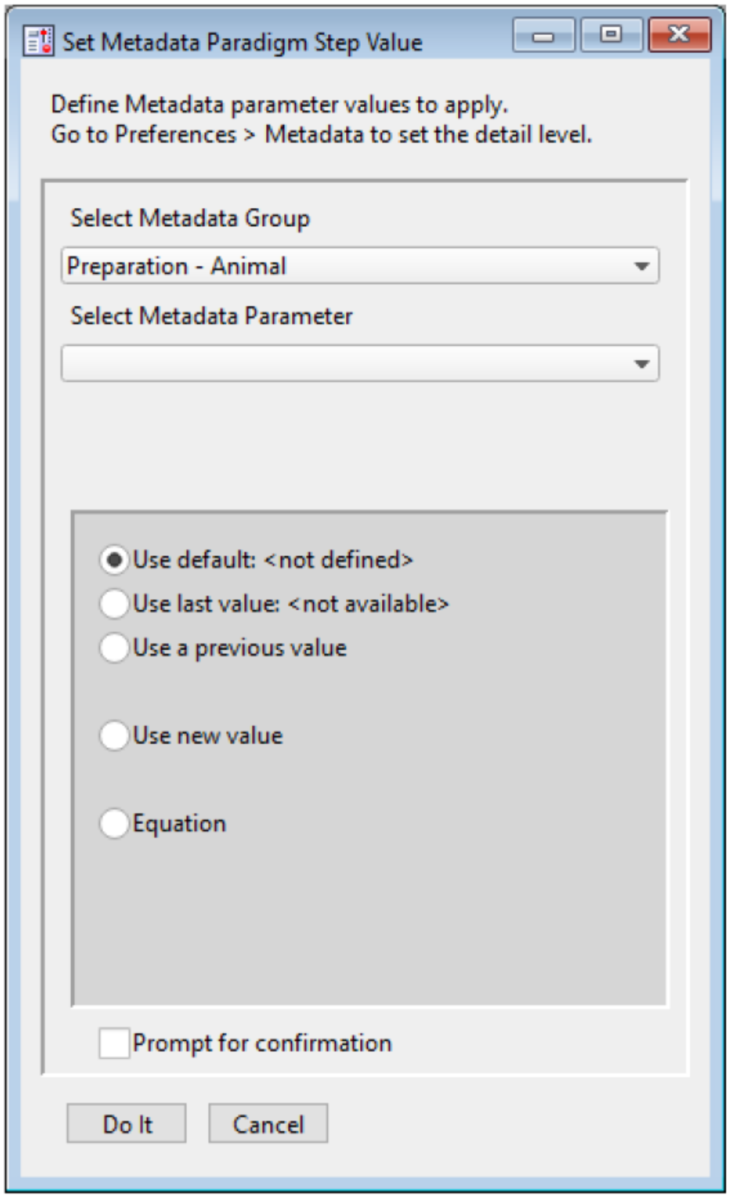


Figure 4-28. Paradigm Step: Set Metadata

Select Metadata Group

Tto change the metadata detail level, go to Preferences > Metadata.

• 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	Entries depend on the selected Group.

[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.

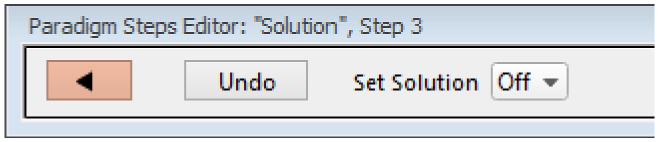


Figure 4-29. Paradigm Step: Set Solution

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 is automatically written to the Paradigm metadata with this step. Enter the comment into the ‘Tag text’ field.

When run during acquisition, the comment tag is also written to the Routine metadata, and when the data is opened in a Reanalysis Scope window, a black vertical cursor displays at that time point.

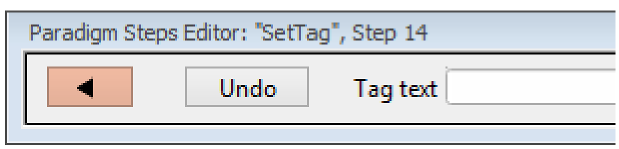


Figure 4-30. Paradigm Step: Set Tag

Default Setting: *SetTag, Text=*

 Close the ‘Paradigm Steps Editor’.

Undo — Remove any unsaved edits to this step.

Tag text — Enter the comment text.

Note: The comment text for this Paradigm step is maintained separately from the manually triggered Acquisition Control ‘Set Tag’ button text.

Set Variable

Variables allow flexible control of any operation using equations.

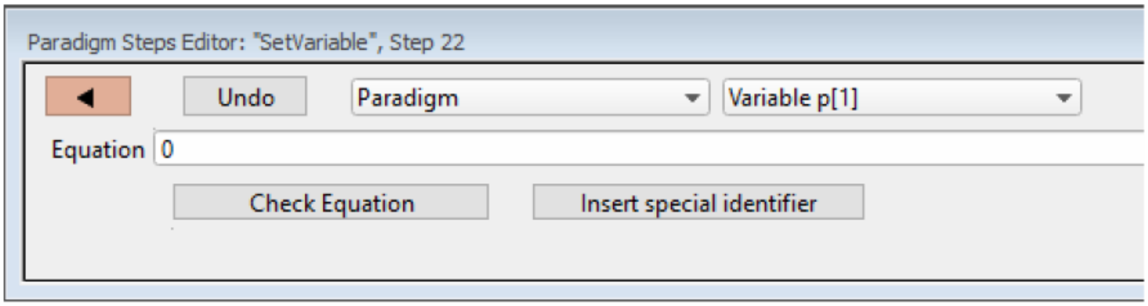


Figure 4-31. Paradigm Step: Set Variable

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

SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)

Set Write Steps

Configure the level of logging Paradigm metadata.

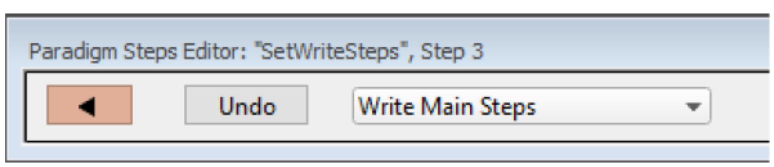


Figure 4-32. Paradigm Step: Set Write Steps

Default Setting: *SetWriteSteps, Value=Main*

 — Close the 'Paradigm Steps Editor'.

Undo — Remove any unsaved edits to this step.

[Drop-down list]

- Write No Steps
- Write 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

- Write All Steps

  Log the main steps and additional steps into the Paradigm metadata (visible in the Metadata 'By Event' view.)

Sound

Output a note from the computer speaker.

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

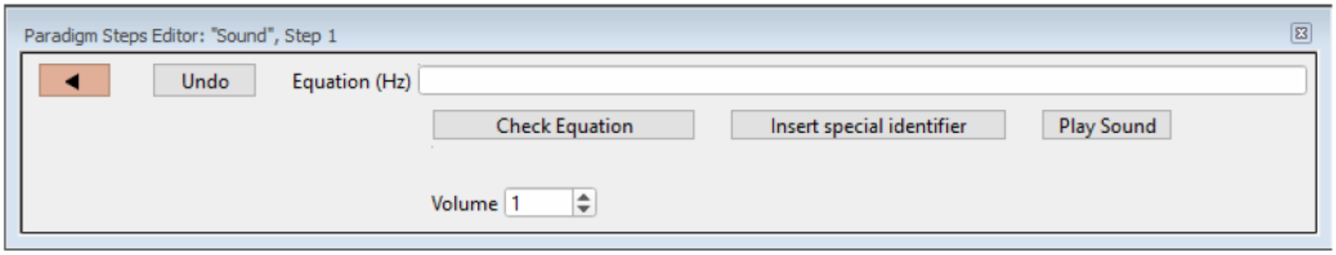


Figure 4-33. Paradigm Step: Sound

Default Setting:	*Sound, Equation=, Volume=1*
	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
Equation (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 250 Hz – 8 kHz frequency tone > 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	SutterPatch 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 Pro 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 Pro 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)

Start New Paradigm Data

Stop the current Paradigm and start a new Paradigm.

This forces a new Paradigm node to be created in the Data Navigator.

Update Inputs

Read a “live” data point from all auxiliary and headstage monitor input channels. This is useful for monitoring slowly changing parameters, such as temperature, without acquiring an entire sweep of data.

View Last

Display the last recording in a Reanalysis Scope window.

Write to Log

Enter text to be written to the Log window.

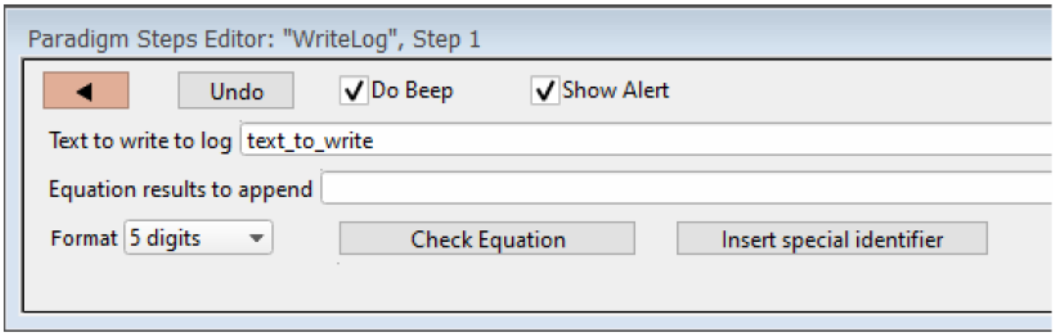


Figure 4-34. Paradigm Step: Write to Log

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

SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)

Paradigm: Write to log

This run-time dialog displays when the Paradigm step is executed:

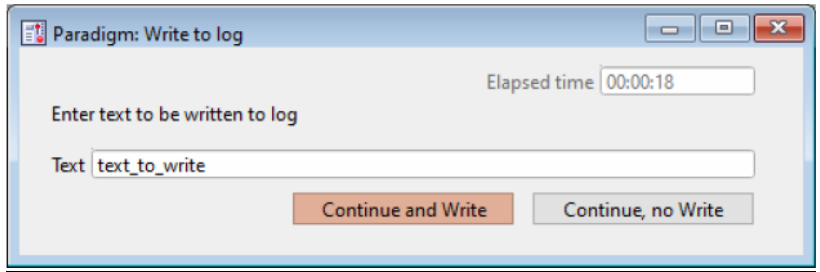


Figure 4-35. Paradigm Write to Log Run-Time Window

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.

Write to Notebook

Enter text to be written to the Notebook.

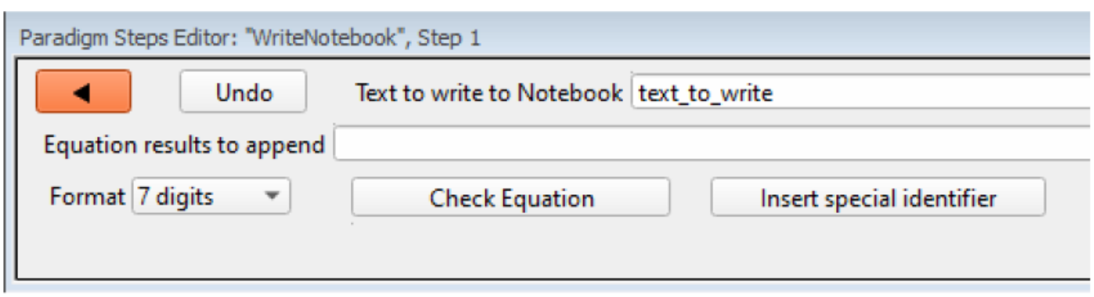


Figure 4-36. Paradigm Step: Write to Notebook

Default Setting: *WriteNotebook, Text=text_to_write, Equation=*

 Close the ‘Paradigm Steps Editor’.

Undo Remove any unsaved edits to this step.

Text to write to Notebook

[ ]

Equation results to append

[ ]

Format [Time, Date, 3 – 12 digits]

For the 'Time' format, seconds are converted into Hours:Minutes:Seconds.Milliseconds.

For the 'Date' format, seconds are converted into Year-Month-Day as XXXX-XX-XX. The starting date is "1904-01-01". No rounding is done.

For numbers, set the number of significant digits to display in scientific exponential notation.

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

Insert special identifier

SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)

Alert

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

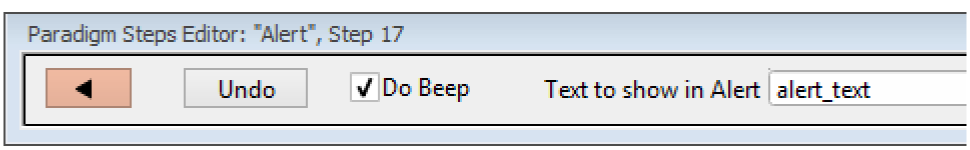


Figure 4-37. Paradigm Step: Alert

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.

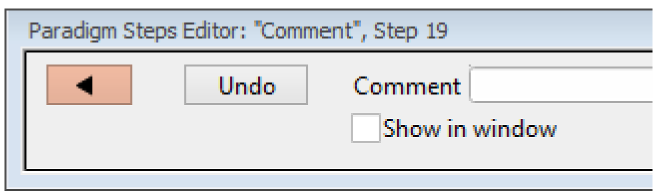


Figure 4-38. Paradigm Step: Comment

Default Setting: *Comment, Text=*

 Close the 'Paradigm Steps Editor'.

Undo — Remove any unsaved edits to this step.

Comment — Enter the comment text.

To display multiple lines of text (up to 3), use "\r" as a line separator. Enter up to 40 characters per line, with a maximum of 100 characters per Comment.

Note: Text characters are from the ANSI character set.

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

Pause

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

Wait

Temporarily pause execution of the Paradigm for a defined duration.

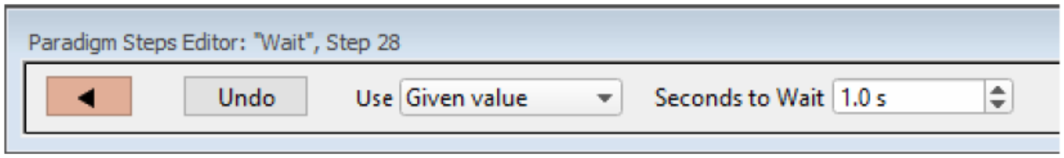


Figure 4-39. Paradigm Step: Wait

Default Setting:	*Wait, Time=1*
	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
Use	[Given value, Variable p[1] – p[16]]
Seconds to Wait	[displays for "Given value"] Click the spinners for 0.1 s increments, or type in a value. The precision of the wait time is 5 ms.

Wait for Trigger

Temporarily pause execution of the Paradigm until an external input trigger is received.

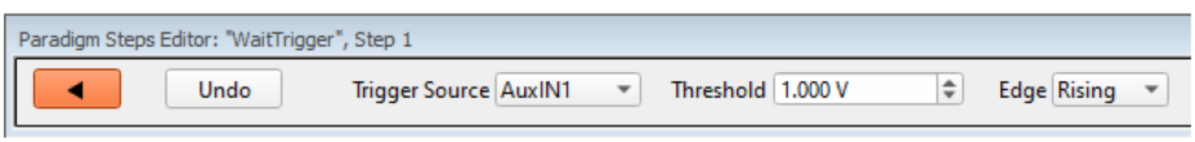


Figure 4-40. Paradigm Step: Wait for Trigger

Default Setting:	*WaitTrigger, Source=AuxIN1, Threshold=1, Edge=Rising*
	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.

Trigger Source [AuxIN1 - 4]

Threshold [±5.000 V]

Click the spinners for 0.001 V increments, or type in a value.

Edge [Rising, Falling]

Flow Control: Break

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

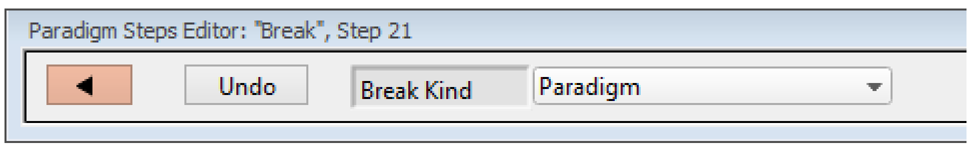


Figure 4-41. Paradigm Step: Break

Default Setting: *Break, Kind=Paradigm*

 Close the ‘Paradigm Steps Editor’.

Undo Remove any unsaved edits to this step.

Break Kind: Paradigm
ForLoop

Flow Control: Chain

Use to link step execution to another Paradigm.

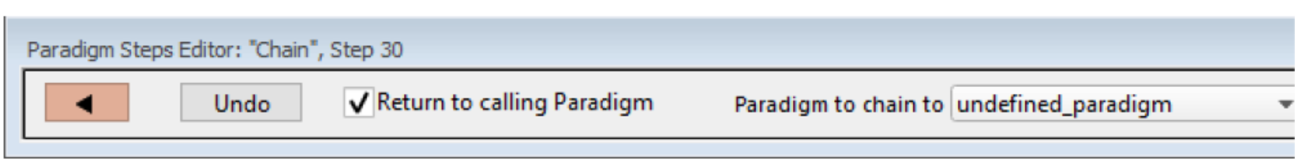


Figure 4-42. Paradigm Step: Chain

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.

Edit paradigm “<selected Paradigm>“

Load the selected Paradigm for editing.

Flow Control: For Loop

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

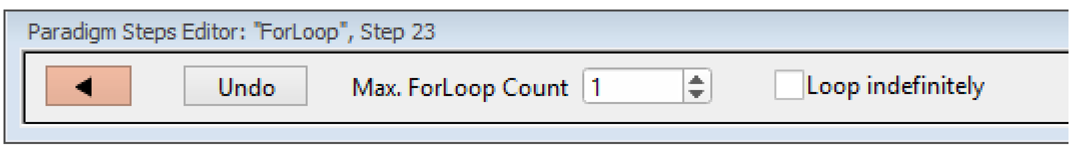


Figure 4-43. Paradigm Step: For Loop

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.

Flow Control: Jump

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

Figure 4-44. Paradigm 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.

Flow Control: Label

Create a Label for a Jump step.

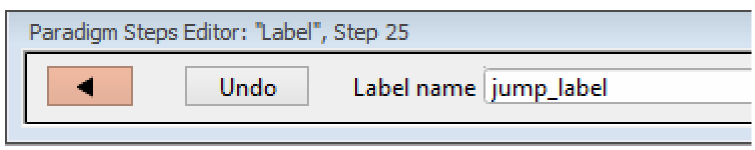


Figure 4-45. Paradigm Step: Label

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.

Condition: If

This step allows conditional Paradigm flow control between multiple choices.

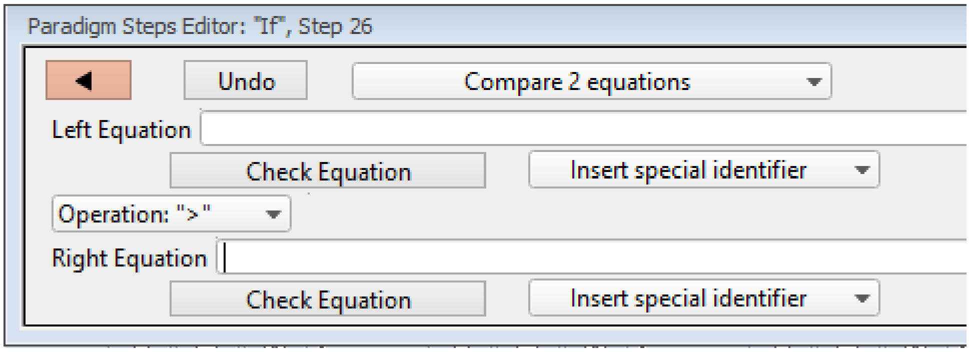


Figure 4-46. Paradigm Step: If

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	
	SutterPatch 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	SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)
• Check for key pressed	The "Last key" typed on keyboard. The "Last key" field is cleared at the start of a Paradigm.
Key to check for	Enter a text key, or insert a "special" key.
Insert special key	Use a "non-text" key.
• 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.

<u>Run-time dialog</u>

Elapsed Time — A time counter for the alert.

Yes — 'Yes' button (value = 1)

No — 'No' button (value = 0)

Stop Paradigm — Manually abort the Paradigm.

<u>Condition: ElseIf</u>

Allow conditional Paradigm flow control between multiple choices.

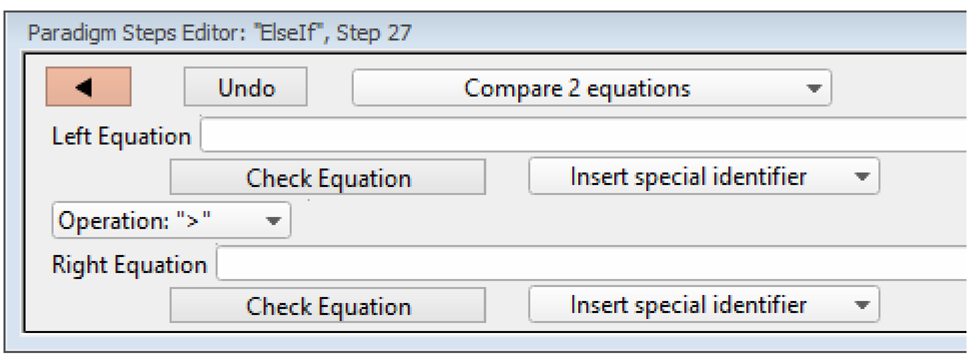


Figure 4-47. Paradigm Step: Else If

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	SutterPatch 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	SutterPatch acquisition, amplifier and reference settings are available for use in equations. (See list below.)

- Check for key pressed — The “Last key” typed on the keyboard.

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

Key to check for	Enter a text key, or insert a “special” key.

Insert special key	Use a "non-text" key.
• 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, and are cleared at the start of a Paradigm.
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.

<u>Condition: Else</u>

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.

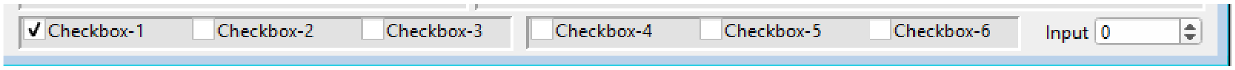


Figure 4-48. Paradigm Checkboxes

[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 / Insert special identifier / Paradigm Parameters’.

[-1.00 – 1.00]

This value is restricted to ±1.00 to enable scrolling through a defined range of values. It can be rescaled when used in an equation.

Paradigm Variables

The Paradigm Variables table displays at the bottom of the Paradigm Editor.

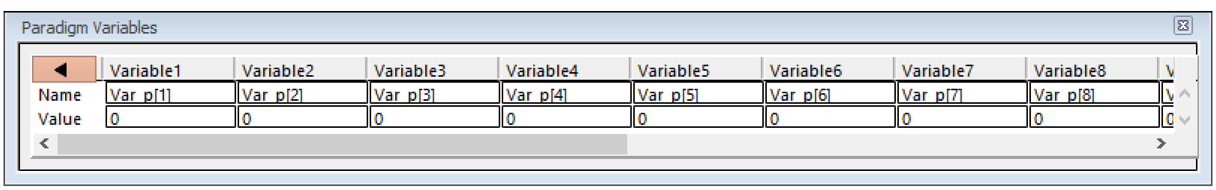


Figure 4-49. Paradigm Variables

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

 ‘Close’ button Closes the Variables table.

Variable[1 – 16] 16 columns of Paradigm Variables.

Name: Var_p[1 – 16] Paradigm Variable names can be edited to any text.

Note: These names are for display only, and are notr supported in equations.

Value: [ ] Numeric values can be manually entered, or programmatically set via the paradigm step ‘Set Variable’.

Special identifiers List

SutterPatch acquisition, amplifier and reference selections are appended to an equation with a “plus” sign.

Timing

Time	(present date-time, s)
Timer	(timer time, s)
ParadigmTime	(time at start of paradigm, s)
RoutineTime	(time at start of routine, s)

Paradigm Parameters

Loop (active paradigm ForLoop count)

Sweep (active paradigm EachSweep count)

Sweep count of the active sweep in the scope window.

LastSweep (active paradigm sweep count of last sweep)

During acquisition, this is set according to the routine parameters. Once acquisition terminates, this is replaced by the count of the last acquired sweep, i.e., the last sweep in the stored series.

Processing can occur before or after the last sweep of a series.

Example: Use in a ‘ForEachSweep’ loop Routine, to compare an ‘If’ step equation to the sweep number.

```
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
Stimulant	(last applied stimulant concentration)
	From the Solution Editor 'Concentration' setting, for a solution configured as a 'Chemical Stimulant'.
Input	(Input variable on paradigm window)
Hold[1..4]	(holding of n'th output channel)
p[1..16]	(n'th paradigm variable)
r[1..16]	(n'th routine stimulus variable)

<u>Analysis Results</u>

m[1..16]	(n'th analysis measurement value)
gx[1..16]	(n'th analysis graph x value)
	The X-value of the last data point in the latest version of gaph[#].
gy[1..16]	(n'th analysis graph y value)
	The Y-value of the last data point in the latest version of graph[#].

<u>Signal Readings</u>

AuxIN[1..4]	(auxiliary input, V)
	A single-point reading from an Auxiliary Input channel, 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)

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)

Headstage

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.

IPA Settings

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 (amplifer 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)
	Read the gain of the active voltage-clamp ‘Current’ input channel.
VGain	(amplifier voltage gain, V/V)
	V/V evaluates to mV/mV.
	Read the gain of the active current-clamp ‘Voltage’ input channel.
Filter	(amplifier input filter in VC- and CC-mode, Hz)
	Read the low-pass filter of the input channels.
IFilter	(amplifier input filter in VC-mode, Hz)
	Read the low-pass filter of the ‘Current’ input channels.
VFilter	(amplifier input filter in CC-mode, Hz)
	Read the low-pass filter of the ‘Voltage’ input channels.
Offset	(amplifier pipette offset in VC-mode, V)
OffsetLock	(amplifier pipette offset lock On in VC-mode)

<u>IPA Compensation</u>

ECompMag	(amplifier electrode compensation magnitude, F)
ECompTau	(amplifier electrode compensation tau, s)
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)

Bridge	(amplifier bridge balance, Ohm)
BridgeOn	(amplifier bridge balance On)

IPA Correction

RsCorr	(amplifier Rs correction, fraction)
RsPred	(amplifier Rs prediction, fraction)
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On)

Dynamic Holding

DynHoldOn	(amplifier dynamic holding On)
DynHold	(amplifier dynamic holding potential, V)

Membrane Test

Relectr[1..2]	(electrode/seal/access resistance, Ohm) Value from last Membrane Test.
Rmemb[1..2]	(membrane resistance (cell mode), Ohm) Value from last Membrane Test.
Cmemb[1..2]	(membrane capacitance (cell mode), F) Value from last Membrane Test.
RMSNoise[1..2]	(membrane test RMS noise, A) Value from last Membrane Test.

Lock-In

LockInPhaseAdj	(Lock-In phase delay adjustment, s)
LockInAttenAdj	(Lock-In attenuation adjustment)

4.1.8 Routine Editor

SutterPatch: 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.

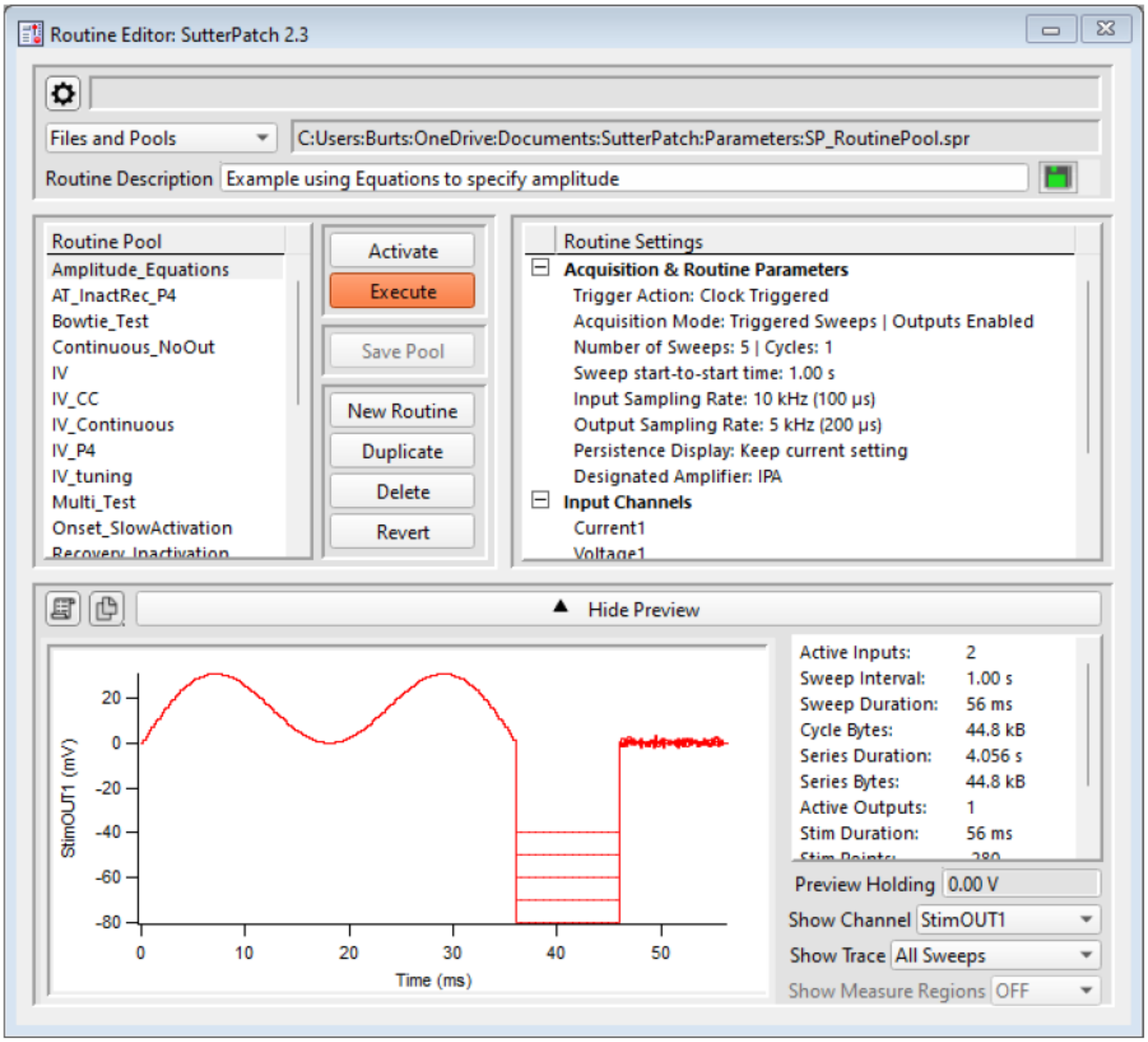


Figure 4-50. Routine Editor

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.

Routine Settings	Set Membrane Test Segment Amplitude.	
[ ]	Status field: Notifications on edits and Routine names are displayed.	
Files and Pools	[drop-down list …]	
	List of the 5 most recently used Routine Pool files. To remove a file from the list, Shift-click it.	
	Load Routine Pool	Load the Routine Pool of a previously saved Routine Pool file.
	New Routine Pool	Create a new Routine Pool with a default Routine, or populated with Routines from the currently loaded Routine Pool. (see below)
	Get Sample Routine Pool	Load the IPA factory-default sample Routine Pool file: SP_RoutinePool.spr SP_RoutinePool_DIPA.spr
	Revert to Last Saved	Undo any unsaved changes to the Routine Pool.
	Save Routine Pool	Save the Routine Pool using its existing filename and path.
	Save Routine Pool As	Save the Routine Pool to a new filename, and switch to the new file. The default filename has an increment number appended to the original filename.
	Save Routine Pool Copy	Save the Routine Pool to a new file, but do not switch to the new file. The default filename has ‘Copy of’ prepended to the original filename.
	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. Re-assign the analog input and output channels in a ‘Mappings’ dialog that opens.

		[The amplifier hardware must be attached to enable this option.]
	Convert Routine Pool	Convert the loaded Routine Pool (designed for other instruments) to be compatible with the attached amplifier or emulation mode. The original file is overwritten. All conversion changes are written to the Command window.
	Send Last Used List to Command	Copy the pathname of the 'Files and Pools' last used Routine Pool into the Command window history.
	Clear Last Used List	Clear the "Last Used" Pool list of all entries.
	Sort Routine Pool – Ascending Order	Sort the 'Files and Pools' list in increasing order.
	Sort Routine Pool – Descending Order	Sort the 'Files and Pools' list in decreasing order.

Table 4-2. Routine Files and Pools

New Routine Pool dialog:

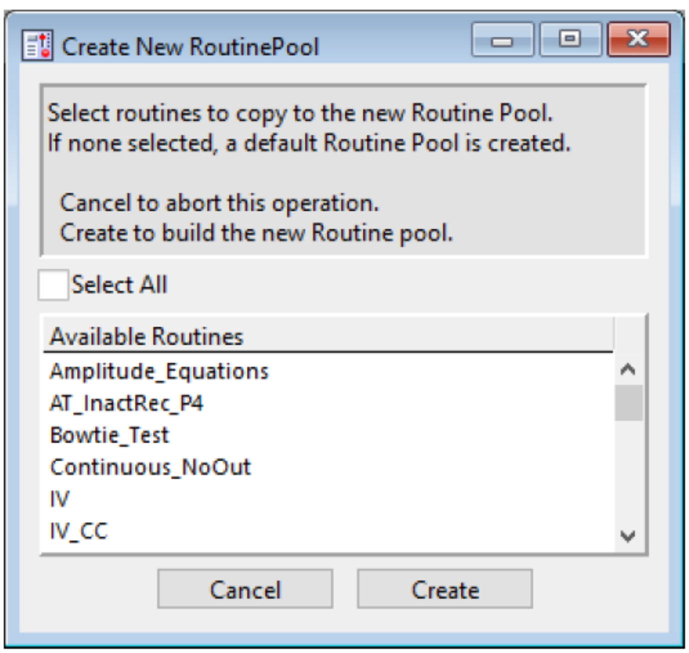


Figure 4-51. New Routine Pool

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

[ ]

File path of the loaded Routine Pool file.

Routine Description

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

 Store data during Routine execution.

This button is green when enabled, and red when disabled.

<u>Routine Pool</u>

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.

To rename a Routine, double-click to select it, then rename or click in it to edit:

- Valid characters are alphabetic and numeric (A-Z, a-z, 0-9), and the underscore "_".
- Names starting with a number are prepended with an 'x' to the name.
- Invalid characters at the start of a name are replaced by an 'x'; invalid characters and spaces within a name are replaced by an underscore.
- The maximum name length is 22 characters; extra characters are truncated.
- The minimum name length is 2 characters; a single character is appended with an 'x'.
- Duplicate names are not allowed in a Routine Pool; an underscore and auto-increment number are appended to the name.

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.

Activate	Open or refresh the Acquisition: Routine Scope window with the latest Routine settings, but do not start acquisition. This button is renamed to "In Progress" during a recording.
Execute Convert	Open or refresh the Acquisition Scope window and immediately start recording. The latest Routine settings are applied to the Scope window. This button is renamed to "Convert" if the selected routine was designed for a different amplifier type than the current Experiment uses. Routine conversion changes are written to the Command window.
Save Pool	Save the Routine Pool using its existing file name.
New Routine	Add a default 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 4-3. Routine Editor Buttons

Waveform Preview

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

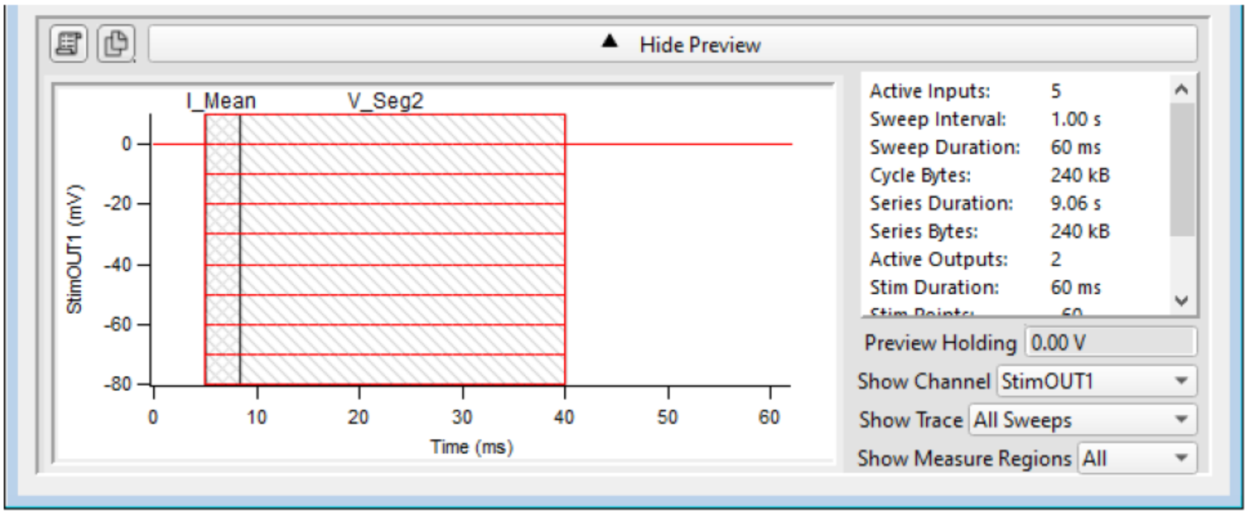


Figure 4-52. Waveform Preview Pane

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

Measurement regions can be manually repositioned in the Preview pane. Click and hold a measurement region to highlight it in black, then drag it to a new position, and release. This also updates its Measurement Settings / Region Timing 'Start/End Time' settings.

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 to Layout — Copy the stimulus graph into a new Layout window, or append to an existing Layout page.

Copy to Clipboard — Copy the stimulus graph 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'.

Active Inputs:	The number of enabled input channels.
Sweep Interval:	The interval of time between the starts of consecutive triggered sweeps (Sweep Start-to-Start Time) in the active Routine. When set to 'Shortest', this equals the longest Sweep Duration + 200 ms.
Sweep Duration:	The amount of time in a sweep during which signal recording occurs with the active Routine. • Outputs enabled The sweep duration is based upon the longest stimulus waveform duration set in Output Channels & Waveform / Waveform Editor. • Outputs disabled The sweep duration is based upon the longest duration set in Input Channels / Edit Signals / Waveform Editor.
Cycle Bytes:	The number of bytes of data in a cycle.
Series Duration:	The amount of time for the Series.
Series Bytes:	The number of bytes of data in the Series.
Active Outputs:	The number of enabled output channels.
Stim Duration:	The maximum amount of time during which output stimulation occurs in a sweep. Set in Output Channels & Waveform / Waveform Editor / Duration.
Stim Points:	The number of points in the output stimulation.
Cycle Duration:	The amount of time for a cycle. Set in Acquisition & Routine Parameters.

Cycle Points:	The number of points in a cycle.
Preview Holding:	The holding level in the Amplifier Control Panel.

User selectable settings

Show Channel: A list of output channels to preview.

- All Channels — Preview all analog output channels..
- All Dig Bits — Preview all digital output bits.
- [list] — Select from the enabled output channels.

Show Trace: Select how to display autoscaled sweep traces in the preview pane.

- 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 five main sections. Click on a section header or item to open its sub-window.

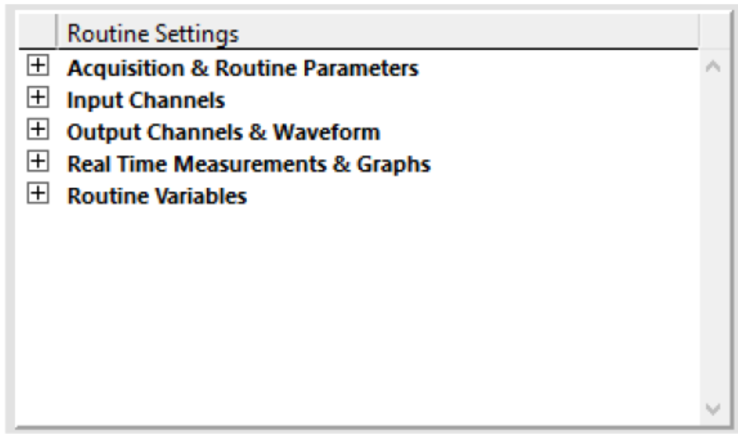


Figure 4-53. Routine Settings

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.

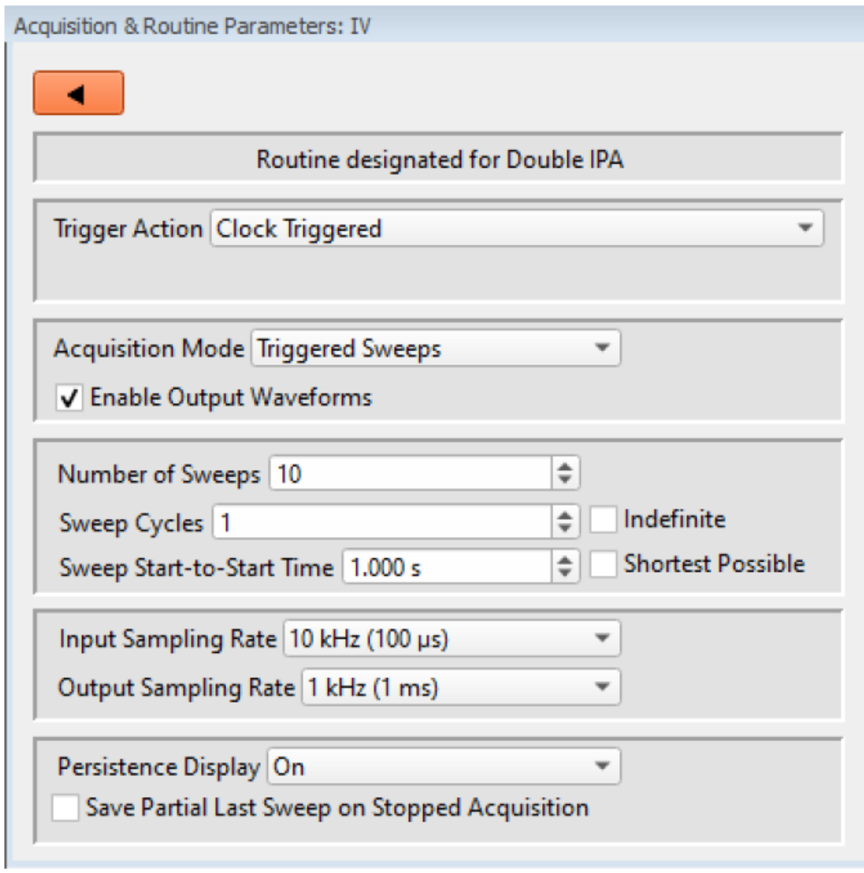


Figure 4-54. Acquisition & Routine Parameters

Trigger Action

Control how and when recordings occur.

- Clock Triggered: Start a recording timed by the SutterPatch program. Hardware trigger inputs are ignored.
- Externally Triggered Sweep:

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

  Connect the external trigger to the 'Trigger In' BNC on the rear panel of the IPA amplifier.

  However, if a Routine is run from within a Paradigm via an ‘Each Sweep’ step, then the hardware trigger is substituted by a software trigger generated by the Paradigm.

Once a sweep has been triggered, additional triggers are locked out, until the sweep has been completed. The refractory period, i.e., the time during which another event trigger cannot occur, is the same as the ‘Sweep Start-to-Start Time’.

Note: If this option is grayed out, first set the Trigger Action to ‘Clock Triggered’ or ‘Externally Triggered Series’, then change the Acquisition Mode from ‘Continuous Sweeps’ to ‘Triggered Sweeps’, to ungray the option.

- Externally Triggered Series:

  Use an external signal from other laboratory equipment to trigger the start of a Series, then operate like Clock Triggered. A command waveform is only generated for the first cycle.

  Connect the trigger signal to the ‘Trigger In’ BNC on the rear panel of the IPA amplifier.

  However, if a Routine is run within a Paradigm ‘Each Sweep’ step, then the hardware trigger is substituted by a software trigger generated by the Paradigm.

- Event Triggered: Use an amplitude event in an input signal to trigger data acquisition of a sweep.

  Event triggering is useful to reduce extraneous data when infrequent events occur during long recordings.

  The refractory period, i.e., the time during which another event trigger cannot occur, is the same as the ‘Sweep Start-to-Start Time’.

  Note: The Acquisition Mode is reset to ‘Continuous Sweeps’, to continuously monitor the signal for an event trigger.

<u>Event Triggered Settings</u>

Input Channel To Scan — Trigger on this channel. The unit of the input channel is used for the trigger threshold.

Pre-Trigger Duration	[0 – 56.00 ms] The portion of the sweep duration that is recorded before the event trigger.	
Trigger Threshold	[±20.000 nA]	current input
	[±0.75 V]	voltage input
Trigger Polarity	Rising	▲
	Falling	▼
Minimum Trigger Duration	[100 µs – 56.00 ms]	

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 (~200 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 to the 'Sweep Duration'.

  Data are displayed as successive sweeps, not as a continuous "rolling" display.

  This option does not support:

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

  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.

If outputs are disabled, sweep and segment durations for analysis measurements can be configured in the Input Channels / Edit Signal / Waveform Editor.

If disabled in Continuous Sweeps mode, holding levels can be controlled via the Amplifier Control Panel. And, while metadata settings are only written at the beginning of a Routine, tags are inserted for such additional changes. Also, the amplifier VC/CC mode is set here, as the Output Channels section is unavailable when outputs are disabled.

Restrict To — Ensures that the matching headstage is in the proper VC/CC mode, else the Routine cannot be activated or executed.

- VC Mode — The Amplifier Control Panel matching headstage must be in VC mode to run the routine.

  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.

- CC Mode — The Amplfier Control Panel matching headstage must be in CC mode to run the routine.

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

Number of Sweeps [1 – 65000]

The number of sweeps to record.

Note: a) When allocating large memory blocks, if more than 1 mega-sample of memory is allocated for the Routine, it can take several minutes to load, and a message displays "Allocating acquisition buffers, please wait…"

b) The largest signal size that SutterPatch can record is 2.5 Gsamples, with up to 16 signals (data waves) recorded at a time. This signal limit is independent of the OS file size limit.

Sweep Cycles [0, 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.

A value of zero is replaced by "indefinite", and the Indefinite checkbox is enabled.

Indef Post Stim [for 'Continuous Sweeps' with Output Waveforms]

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

Sweep Start-to-Start Time ['s' or 'ms']

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

Shortest Possible [for Triggered Sweeps]

Set to 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 (as configured in the Waveform Editor.)

Note: Demo mode sweep start-to-start times can vary during acquisition, especially on slower computers.

Input Sampling Rate: Drop-down list applies to all input channels.

According to the Nyquist sampling theorem, the input *sampling* rate should oversample the input *filter* rate (set in the Amplifier Control Panel) at a minimum of 2x. However, the Nyquist Factor is typically implemented at 5x - 10x for cellular responses, due to their complex shapes.

Also, if the input filter rate is greater than the input sampling rate, the filter rate is ignored.

Sampling Rate	(Sample Interval)
100 Hz	(10 ms)
200 Hz	(5 ms)
400 Hz	(2.5 ms)
500 Hz	(2 ms)
1 kHz	(1 ms)
2 kHz	(500 µs)
5 kHz	(200 µs)

10 kHz	(100 µs)
25 kHz	(40 µs)
50 kHz	(20 µs)

Output Sampling Rate: Drop-down list applies to all output channels.

Sampling Rate	(Sample Interval)
100 Hz	(10 ms)
200 Hz	(5 ms)
400 Hz	(2.5 ms)
500 Hz	(2 ms)
1 kHz	(1 ms)
2 kHz	(500 µs)
5 kHz	(200 µs)
10 kHz	(100 µs)

Note: New Routines use a 1 kHz default output channel sampling rate, as command waveforms usually do not require high-resolution time changes. Increase the sampling rate as needed for more complex waveforms.

In general, it is recommended that the Output Sampling Rate be equal to or faster than the Input Filter Rate (set in the Amplifier Control Panel).

Channel Timing

IPA amplifiers record both stimulus and response signals via physical analog channels, so the start times of all recorded signals are in sync, with no timing delays between them.

Persistence Display

Control which sweeps are displayed in the Scope window

- Off — For each new sweep, all prior sweeps are cleared, and only the newest sweep is displayed.
- On — Overlay each new sweep onto the display of any prior sweeps (per Scope Preferences limits).
- Keep current setting

  Do not change the Scope window's prior settings.

Input Channels

Configure the input channels.

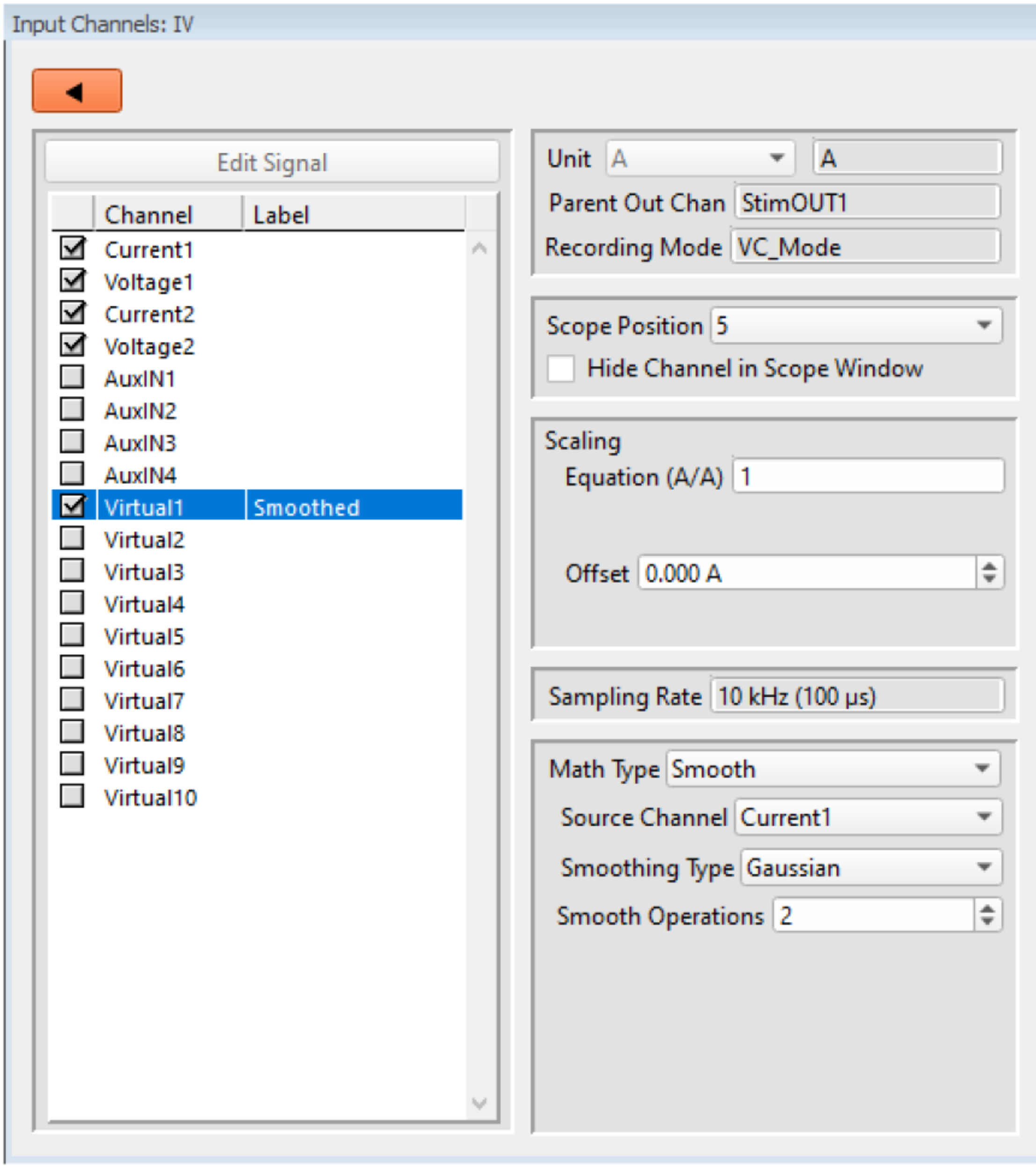


Figure 4-55. Input Channels

Edit Signal

[for disabled Output Waveforms]

When Output Waveforms are disabled in the Acquisition Parameters section, segment timing can still be controlled via a modified Waveform Editor. This signal editor is a “timing only” version of the Waveform Editor, and only controls the duration of segments, to facilitate segment-based measurements.

When this button is enabled, double-clicking a channel will open the signal Waveform Editor.

Channel

Enable up to sixteen 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 its 'Label' field, 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, a default 'Math Type' label is automatically generated for it.

Unit The base unit of measurement.

• Headstage Channels	[read-only] Fixed at 'A' for Current channels, and 'V' for Voltage channels. The unit resolution is automatically adjusted in the signal.
• 'AuxIN' Channels	Enter the base unit of measurement from a drop-down list. (Default is 'V'.) The unit resolution is automatically adjusted in the signal. [A, V, S, Ohm, °C, °K, °F] Or edit the text field to add new nomenclature to the list.
• 'Virtual' Channels	[only editable for Math Type 'Equation'] Enter the base unit of measurement from a drop-down list. (Default is 'V'.) The signal's unit resolution is automatically adjusted. [A, V, S, Ohm, °C, °K, °F] Or edit the text field to add new nomenclature to the list. [read-only for all other Math Types, where the unit is the same as its 'Source' channel]

Parent Out Chan

This is the output channel associated with the selected input channel. The ouput channel timing is also used for measurements with 'Cursors Relative to Segments'.

[for headstage input channels]	Displays its associated headstage output channel.
[for Auxiliary input channels]	Select any output channel from the list.
[Virtual input channels]	Displays its 'Source' channel's Parent Output channel.

Recording Mode

[only editable for Auxiliary input channels]

Displays the patch-clamp recording mode assigned at the start of acquisition.

• VC_Mode	Voltage-Clamp mode
• CC_Mode	Current-Clamp mode

Scope Position

The input channel panes can be repositioned in the Scope window.

Position "1" is the top pane.

Hide Channel in Scope Window

The selected input channel is hidden in the Scope window.

Scaling [only displays for Auxiliary and Virtual input channels]

Equation ('Unit'/V)

Enter a Scaling factor as an equation (or a fixed value).

(See the Equation Editor for more details.)

Factor ('Unit'/V)

[only displays for "non-unity" evaluated equations]

The input channel Scaling factor is evaluated from the equation. Raw values are converted to input units.

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.

Offset (V) Apply an amplitude offset to the input signal, before any scaling.

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

To use 'pA' units, enter: '#p' or '#e-12'

Scaled Offset

[only displays for "non-unity" evaluated equations]

The amplitude offset of the input signal, after any scaling. Raw offset values are converted to input units.

Sampling Rate

[read-only field]

[kHz (μs)] The sampling rate (and sampling interval), before low-pass filtering.

Virtual Input Channels

Virtual input channels allow you to perform a variety of mathematical transformations on input signals in real time. When a Virtual input channel is enabled and selected, its configuration fields are ungrayed.

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

BaselineSubtract

BesselFilter

CycleAverage

Differentiate

Downsample

Equation

Integrate

Leak

LineFreq

LockIn

Smooth

Stimulus

SweepAverage

SweepSubtract

- BaselineSubtract

  Subtract a fixed value from all data points in an input trace.

  This is useful for adjusting an offset or resetting a baseline.

  Post-analysis can be limited to marked sweeps via the Reanalysis Scope Measurements button / Edit Virtual Signals.

  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.

  - 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. A higher number provides a sharper (more accurate) response, but consumes more processing time and system resources.

Apply Filter Delay Correction

[only for ‘LowPass’ filter setting]

Correct the signal for estimated digital filtering delays by shifting the signal forwards in time.

Cutoff Frequency [0.01 Hz to < ½ the sampling rate]

Restrict frequencies from this boundary point onwards.

- CycleAverage Apply averaging across cycles for each numbered sweep.

  Post-analysis can be limited to marked sweeps via the Reanalysis Scope Measurements button / Edit Virtual Signals.

  Source Channel Select an input channel to average.

- Downsample Apply downsampling to an input signal, i.e., reduce the sampling rate of the signal data.

  Source Channel Select an input channel to downsample.

  Source Sampling Interval [read-only field (μs)]

  Reduction Factor [2 – 100]

  Only whole numbers are used; non-whole numbers are rounded up or down.

  Sampling Rate [read-only field (Hz)]

  New sampling rate of the reduced signal.

- 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.

  Post-analysis can be limited to marked sweeps via the Reanalysis Scope Measurements button / Edit Virtual Signals.

  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 — A limited set of identifiers are available for virtual equation traces. However, special references can also be used within commands.

  - s[series-count, sweep-count, trace-count, routine name]

    Trace of specified series.

    Access an arbitrary input trace (data wave) via counts of Series #, Sweep #, Trace # (scope channel 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 channel 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.

- p[#] n'th paradigm variable.
- if[selector ? true-branch : false-branch]

  Conditional processing.

- eq[equation] Result of the given equation.

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	60 Hz	Canada, (Caribbean), Central America, (Japan), Mexico, (South America), South Korea, Taiwan, USA. Some (regions) include both 50 Hz and 60 Hz frequencies.
	50 Hz	Most of rest of world.

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.

Note: When using short sweeps or slow sampling, performance might improve with a larger number of sample points, such as from an increased sweep duration or filter frequency.

Warning! Do not apply to sweeps of 3 minutes or more, or problems will occur. Either apply offline, or reduce the sweep duration.

- 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' 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 reference and 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:

- Gausian — 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.

  Post-analysis can be limited to marked sweeps via the Reanalysis Scope Measurements button / Edit Virtual Signals.

  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 — [for Run Average]

  Start Sweep — Sweep number to start sweep averaging.

  Set Sweep < Start Sweep To NAN

  Sweeps prior to the Start Sweep are set by default to the initial source sweep. Enable to set these pre-sweeps to NaNs .

  End Sweep — Sweep number to end sweep averaging.

  Set Sweep > End Sweep To NAN

Sweeps after the End Sweep are set by default to the initial source sweep. Enable to set these post-sweeps to NaNs.

- SweepSubtract Subtract a sweep from the input trace.

  Post-analysis can be limited to marked sweeps via the Reanalysis Scope Measurements button / Edit Virtual Signals.

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.

Output Channels & Waveform

Configure the output channels and command waveforms.

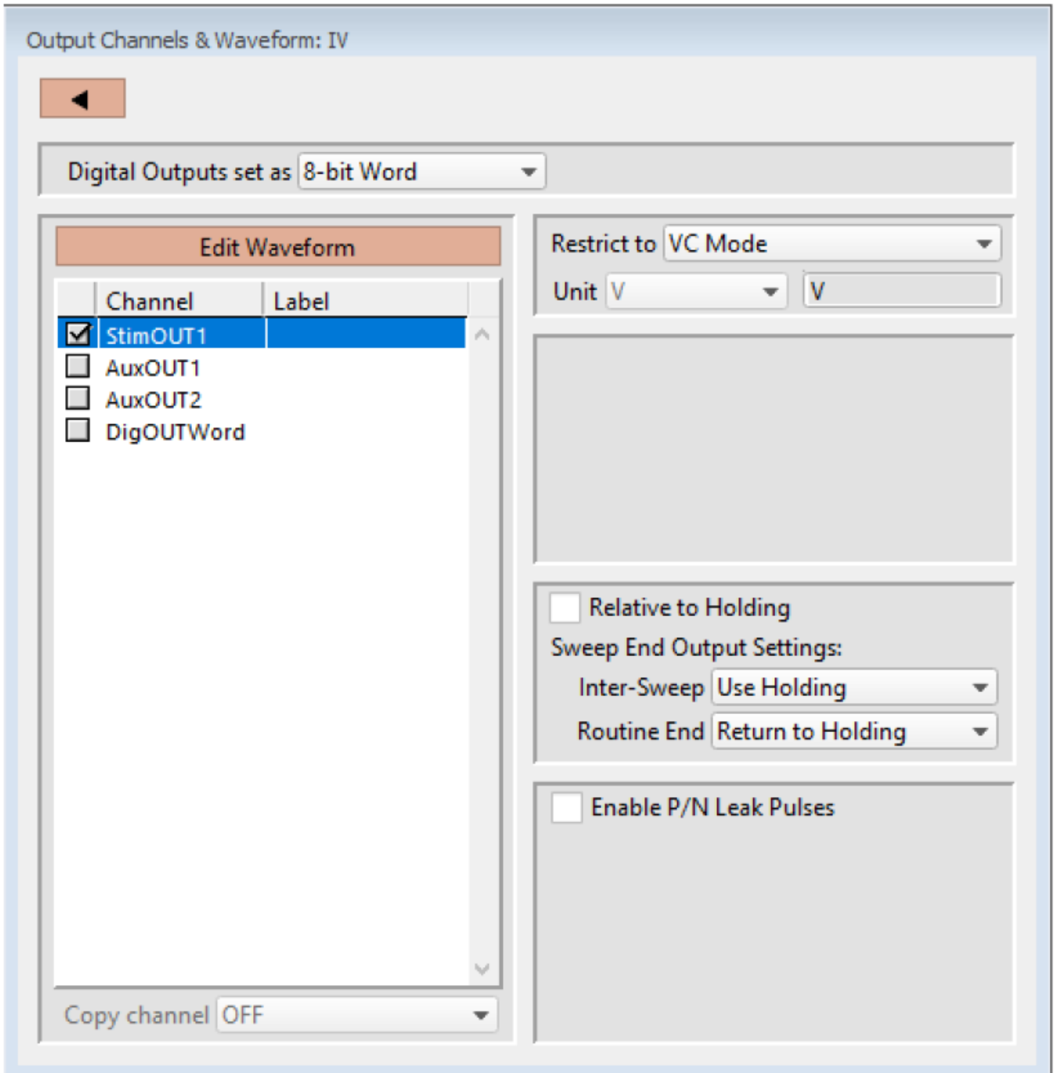


Figure 4-56. Output Channels & Waveform

[Status field] Hardware information displays for the highlighted channel.

Digital Outputs set as

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

- Individual bits DigOUT[1 – 8]

  Each bit is individually set in its own Waveform Editor table.

The waveform preview uses the bit's binary word decimal value for its Y-axis value, i.e. if bit 3 is 'HIGH', it has a "word" value of 4.

- 8-bit Word [0 – 255]

The 8-bit digital output pattern is controlled by a single decimal number, 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', it has a value of 5.

Edit Waveform

Click the Edit Waveform button to access the Waveform Editor table and create a stimulus waveform. (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 an enabled channel name to highlight and select it – the channel output parameters are displayed for configuration. Double-click an enabled channel name to open its stimulus waveform in the Waveform Editor.

StimOUT — The default StimOUT channnels are hardwired to the IPA headstage.

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

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 experiments that only need a holding level (vs. a stimulus waveform), instead of using StimOUT (which generates a waveform that increases loading time), disable the Acquisition & Routine Parameters 'Enable Output Waveforms' control, and use an Amplifier Control Panel 'V-holding' or 'I-holding' level.

AuxOUT — The AuxOUT auxiliary analog output channels can be used to send stimulus or timing waveforms to external instruments.

DigOUT — 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.

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 its Label field 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., “StimOUT”, “AuxOUT” or DigOUT). 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

[for headstage channels only]

Ensures that the matching headstage is in the proper VC/CC mode, else the Routine cannot be activated or executed (or started).

- VC Mode — The Amplifier Control Panel matching headstage must be in VC mode to run the Routine.

  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.

  “Tag” Recording Mode: 1

- CC Mode — The Amplfier Control Panel matching headstage must be in CC mode to run the Routine.

  “Tag” Recording Mode: 2

However, the IPA amplifier can be switched into any mode (VC or CC) while a recording is in progress. Recording Mode tags are inserted into the signal to assist you, but it is your own responsibility to correctly interpret data with mixed recording modes.

Unit

[read-only for StimOUT channels]

Fixed at 'V' for voltage-clamp experiments, and 'A' for current-clamp.

[only editable for AuxOUT channels]

[A, V, S, Ohm, °C, °K, °F]

Enter the base unit of measurement from a drop-down list. (Default is 'V'.) The unit resolution is automatically adjusted in the signal.

Or edit the text field to add new nomenclature to the list.

Scaling

[only displays for AuxOUT channels]

Equation (Unit/V)

Enter a scaling factor as an equation (or a fixed value).

(See the Equation Editor for more details.)

Factor [only displays for "non-unity Equation" channels]

Read-only field of the evaluated Scaling equation.

Offset [±10.000 V]

Applies an amplitude offset to the output channe, before any scaling.

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

To use 'pA' units, enter: '#p' or '#e-12'

Scaled Offset [only displays for "non-zero Offset" headstage channels]

Read-only field of the scaled Offset value. Raw offset values are converted to output units.

Relative to Holding

StimOUT 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.

Relative to I/O Setting

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

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

Note: When enabled, the holding level output is immediately updated by any changes in the Amplifier Control Panel.

Sweep End Output Settings

[for headstage StimOUT channels]

Control how the amplifier output levels (including I/O Auxiliary and Digital Output levels) are handled when the system is not acquiring data.

Inter-Sweep

This is the time between sweeps - after a sweep ends, but before the next sweep starts.

- Use Waveform Value — Set the output signals to their last values in the command waveform, at the end of a sweep.

  Use to avoid generating a short (potentially disruptive) glitch in your preparation, caused by returning to holding levels at the end of a sweep.

- Use Holding — Set the output signals to the Amplifier Control Panel “holding” levels, at the end of a sweep.

  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.

Routine End

This is the time after the Routine ends, until the next Routine starts.

- Use Waveform Value — Set the output signals to their last values in the command waveform, at the end of a Routine.

  Use to avoid generating a short (potentially disruptive) glitch in your preparation, caused by returning to the holding levels at the end of a Routine.

- Return to Holding — Set the output signals to the Amplifier Control Panel “holding” levels, at the end of a Routine.

This ensures that your cells are kept in a resting state as much as possible.

Note: In demo mode, holding levels are only updated when a Routine is activated, unless 'Relative to Holding' is enabled - then the 'Return to Holding' levels are updated immediately.

Enable P/N Leak Pulses

Displays the P/N Leak Pulses section.

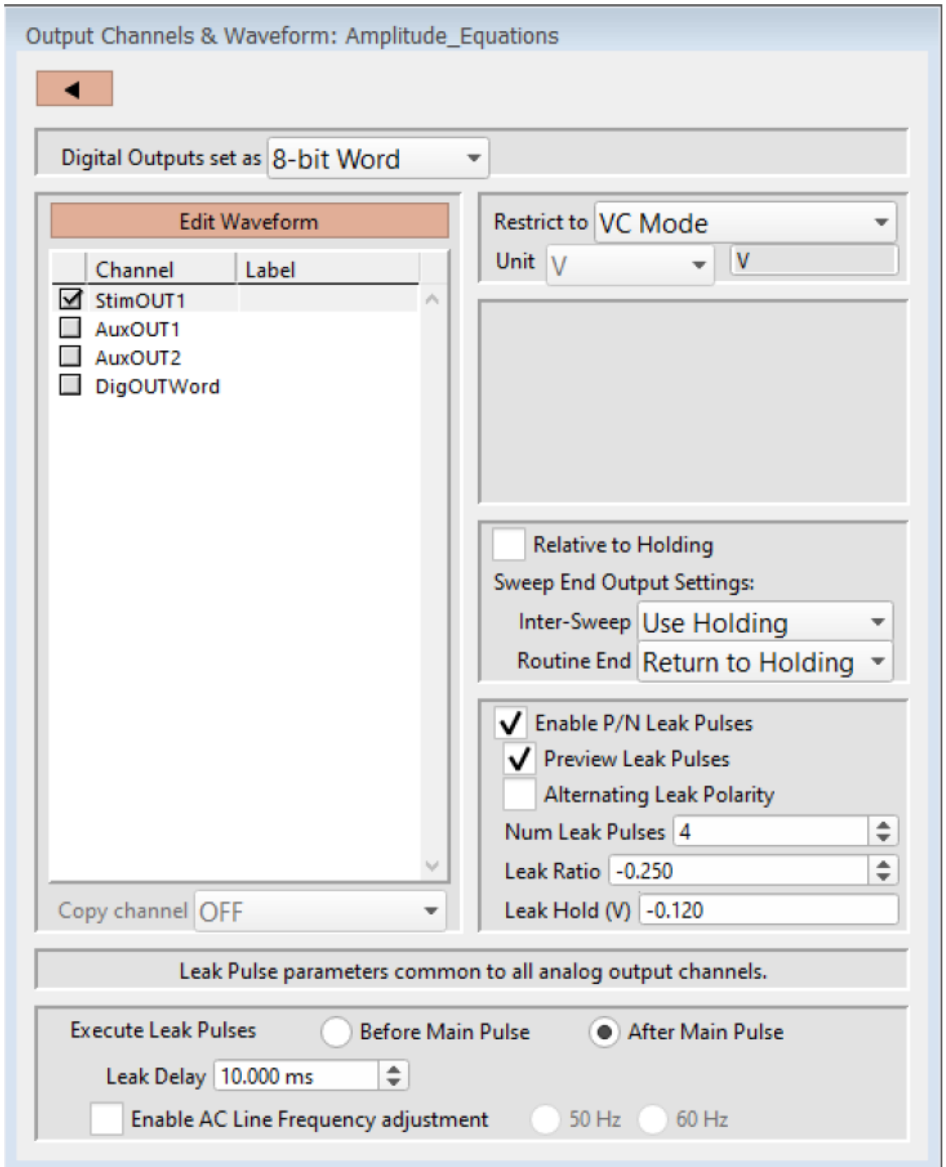


Figure 4-57. Output Channels & Waveform with Leak

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

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. Due to the high precision of modern 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 ¼ the amplitude of the main stimulus waveform, while a Leak Ratio of 0.2 will generate leak pulses at 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 "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

Execute Leak Pulses

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.

Leak Delay [0 – 1,000.000 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 amplifier's 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 recording:

- 60 Hz — Enable the reduction of 60 cycle AC line noise.
- 50 Hz — Enable the reduction of 50 cycle AC line noise.

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

Waveform Editor

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

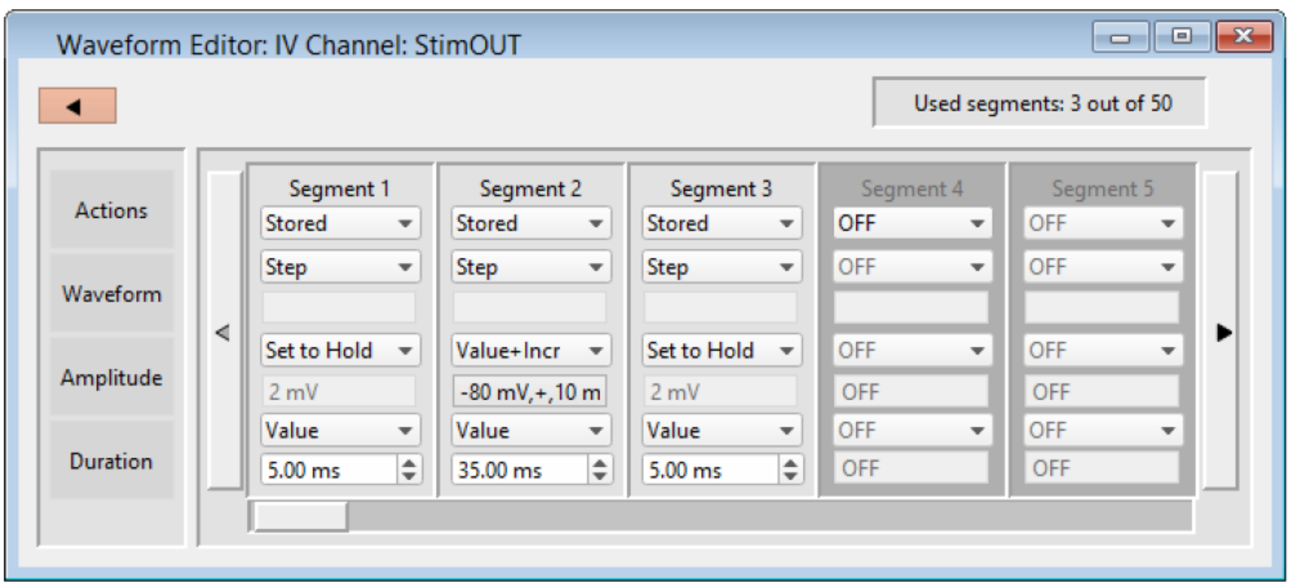


Figure 4-58. Waveform Editor

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

Bit Pattern Segment # [for digital Words only]

A visual chart of the selected Segment's digital bit pattern. Only displays settings for Bit Word "Values"

(not for lists, increments, etc.)

Used segments: [# out of 50]

Displays how many of up to 50 contiguous Segments are configured in the 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 — If P/N LeakPulses are enabled, this will optionally exclude the Segment from being generated in the P/N Leak Subtraction output wave.

This is useful for inactivation or recovery studies, when commands do not change for long periods of time.

Insert — Insert a default Segment into the current position, and increment the position of the following Segments, i.e., move them to the right.

Copy — To copy a Segment, click the segment's Actions list and select 'Copy'. A copy is inserted as the next Segment.

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.

Delete — To remove a Segment, select its 'Delete' Action.

If there is only one Segment, it cannot be deleted - there is always at least one Segment enabled.

To remove multiple Segments, select the desired Segments. Then, click any Segment's Actions list and select 'Delete'. All selected Segments are deleted.

To select multiple Segments, in Windows use Ctrl-click, or in macOS use Command ⌘-click, to highlight each segment, or use Shift-click to highlight a range of Segments.

	Any following Segments shift their Segment #'s down by the number of deleted Segments.		
Waveform	Select the waveform shape. For Waveform types Sine / Chirp / Squarewave / Template / Triangle, a 'Parameters' field displays below this field, to allow quick access to their parameters.		
Step	The waveform amplitude rapidly jumps from a pre-existing level to the new level within one sample point, and stays at the new level for the duration of the Segment. The resulting waveform shape looks like a step. The first segment typically consists of a Step waveform set to the holding level amplitude (Set to Hold).		
Ramp	The waveform amplitude goes from the previous amplitude to the new amplitude as a smooth straight line - the sloping line looks like a ramp. Tip: To create a sawtooth pattern, follow a ramp segment with a zero duration Step segment. This sharply resets the ramp amplitude to the baseline level – the next ramp will start from the baseline level again.		
Constant	[for dPatch only] For each sweep, download and repeat a set value for all stimulus points in the last Segment of the output signal. This can help to reduce stimulus loading time.		
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 sensitive capacitance measurements. A corresponding virtual input LockIn channel also

needs to be enabled.

LockIn sine waves cannot be mixed with non-LockIn sine waves.

Multiple sine LockIn Segments in a waveform share the same settings (except duration) for each segment.

Amplitude	Amplitude of the first peak from the sine wave baseline. Tip: To offset a sine wave from the default baseline (0 units), set the Segment Amplitude value, or enable Routine Editor / Output Channel 'Relative to Holding'. For LockIn measurements, the larger the sine wave amplitude, the better the signal-to-noise ration for the measurements, just be sure to avoid the activation range of voltage-gated ion channels.
Cycle Duration	[for LockIn only] One cycle length (ms). A preset list of cycle durations: 2.0 ms (500 Hz) 1.0 ms (1 kHz)
Ramp Increment	[±1.00 V, ±20.0 nA] Apply the sine wave onto a ramp "baseline". [for multiple cycles]
Segment Duration	Sine wave duration (ms).

Square Pulses

The waveform generates a train of rectangular pulses.

Base Amplitude Increment

[±1.00 V, ±20.0 nA]

Increment the baseline amplitude for each succesive pulse.

Step1 Amplitude [±1.00 V, ±20.0 nA]

Amplitude of first pulse.

- Value
- Var_r[1] – [16]

Step1 Width Duration of first pulse (ms).

- Value
- Var_r[1] – [16]

Step2 Amplitude [±1.00 V, ±20.0 nA]

Amplitude of second pulse.

- Value
- Var_r[1] – [16]

Step2 Width Duration of second pulse (ms).

- Value
- Var_r[1] – [16]

Pulse Frequency [read only]

Segment Duration Square wave duration (ms).

Chirp

This waveform generates a sinusoidal wave that changes its frequency over time.

Chirp Type

Linear A linear change in frequency.

Geometric A geometric change in frequency.

Note: For a geometric chirp, a minimal

frequency spread is enforced: the End Frequency has to be at least double the Start Frequency, or half or less than the Start Frequency.

Amplitude	[±1.00 V, ±20.0 nA]
Start Frequency	[1 – 50000 Hz]
End Frequency	[1 – 50000 Hz]
Segment Duration	Chirp wave duration (ms).

Template — Assign an arbitrary waveform to a Segment.

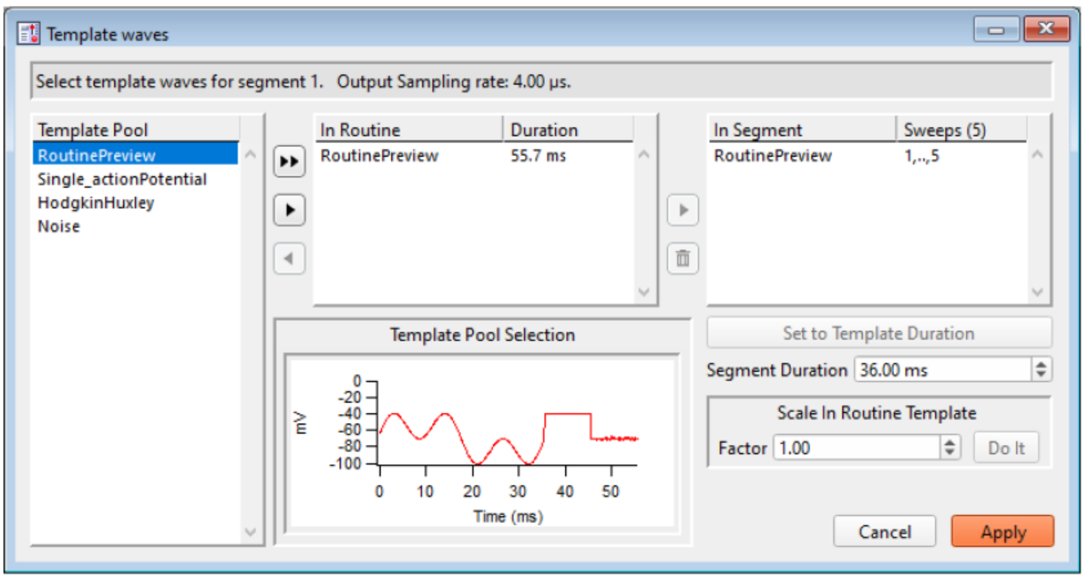


Figure 4-59. Template Waves

[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.

Note: Changes made in the Template Editor are only applied to Routines when the modified template in the Template Editor Pool list is copied into the Routine.

Template Editor Pool

Lists the templates loaded in the Template Editor, plus any extracted templates.

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

This button is enabled if the ‘Number of Sweeps’ allows more Segment templates.

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

Up to 16 template waves can be loaded.

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

Copied In Routine

Lists the templates copied from the Template Editor Pool and loaded into the Routine.

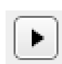
Each output channel can have a maximum of 16 template waves loaded in its routine. Each template can be used in multiple Segments, each Segment can use multiple templates.

While the most used case will probably be a single template paired with a single Segment, the possibilities are endless.

Note: To avoid unnecessary increase in the size of the Routine Pool, only include templates that are actually going to be used in a Segment.

Duration (ms)

The duration of the template trace.



Copy the selected template in the Routine into the Segment.

Remove the template from the Segment, or remove an unused Routine template.

Used In Segment

Lists the loaded templates that are actually used in the Routine. Each segment can use multiple templates.

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

If multiple templates are copied into the Routine Segment, they will be executed in sequential order, one template per sweep.

Sweeps — Number of sweeps in the Routine.

Sweeps are assigned to templates in sequential order. If the number of sweeps is greater than the number of templates, the sweep number cycles back to the first template and continues incrementing the templates, etc.

Template Pool Selection

A preview of the selected template signal.

Set to Template Duration

Set the Segment duration to match the template (sweep) duration.

Segment Duration — The Segment duration can be manually adjusted here.

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).

Scale In Routine Template

Factor — Set a scaling factor for the amplitude of the template signal.

Note: When using templates in Igor Pro 8, close the Scope and Data Navigator windows before saving the Experiment, else the Experiment might not re-open properly.

Triangle Pulses — The waveform generates a train of triangular pulses.

Base Amplitude Increment

[±1.00 V, ±20.0 nA]

Increment the baseline amplitude for each succesive pulse.

Peak Amplitude — [±1.00 V, ±20.0 nA]

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.

Membrane Test — The Membrane Test runs in ‘Cell’ mode.

The waveform applies a predefined negative pulse step (-5 mV) with a 50% duty cycle for Routine-based measurements.

Use with the Real Time Measurements ‘Analysis Functions’:

- MT Series Resistance
- MT Membrane Capacitance
- MT Membrane Resistance

Warning! For valid results, ‘Cell Compensation’ and Rs Correction should be disabled in the Amplifier Control Panel.

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

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

For Auxiliary output channels, when the Output Channel / Scaling Factor is not “1”, i.e., when scaling is applied to the signal, then a non-editable scaled output field is also displayed below the amplitude value field.

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

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 might be recorded, such as from tail currents.

Tip: To help intepret your data, record an initial baseline in Segment 1, and/or a final baseline in the last segment.

Value — Use a single number for the Segment amplitude.

Value List	Set an arbitrary Segment amplitude for each numbered sweep.	
	[Sweep | Value]	[# | ±1.00 V, ±20.0 nA] 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	• Holding • Value [±1.00 V, ± 20.0 nA]
	Increment Value	[±1.00 V, ± 20.0 A]
	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.	
	[ ]	A free-form text field for writing equations.
	[ ]	Syntax messages are reported here.
	Check Equations	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 in Equation Editor.)

Undo — All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

Warning! Computing an equation for an output wave consumes significant computing power, as every data point needs to be computed by the CPU. For larger acquisitions, this can generate significant delays to the start of acquisition.

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

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

Bit — Set the digital level for an individual bit.

- LOW = 0
- HIGH = 1

Bit Word — [0 – 255]

Value is the decimal number of an 8-bit digital word.

Duration — Set the Segment duration.

Value — [0 – 12 ks]

Use a single number for the Segment duration.

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] — [# | 0 – 12 ks]

For each numbered sweep, enter a value.

(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.

[ ]A free-form text field for writing equations.

[ ] Syntax messages are reported here.

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 in Equation Editor.)

Undo All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

Warning! Computing an equation for an output wave consumes significant computing power, as every data point needs to be computed by the CPU. For larger acquisitions, this can generate significant delays to the start of acquisition.

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

Segment Controls

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

Standard mouse behaviour is used to select multiple Segments:

•	Individual Segments:	Windows:	Ctrl-click
		macOS:	command (⌘)-click
•	Range of Segments	Both:	Shift-click

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

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

Real Time Measurements & Graphs

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

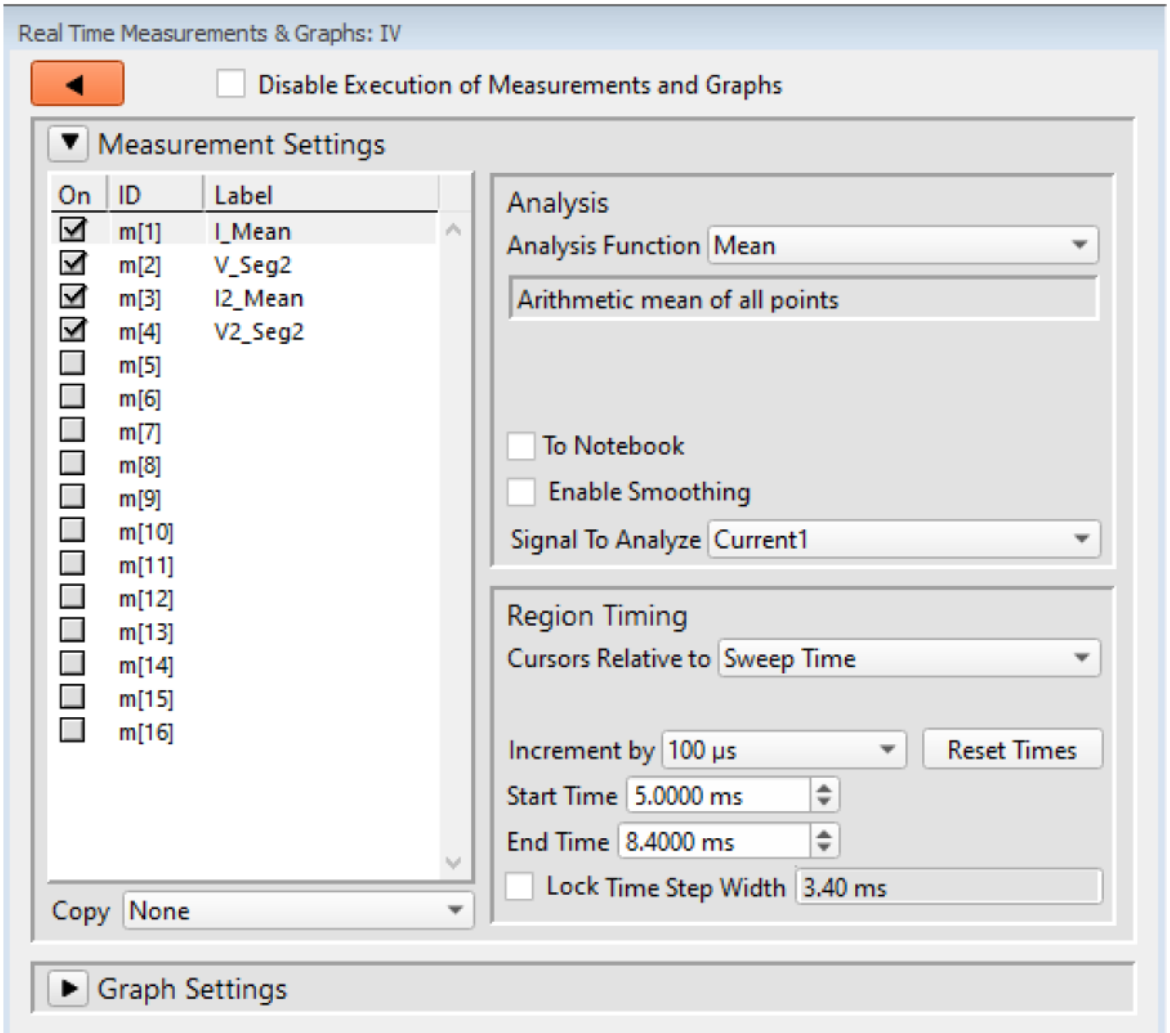


Figure 4-60. Real-Time Measurement Settings

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.

<u>Analysis</u>

Be sure to set appropriate Region Timing (below) for the following analyses.

Analysis Function

Select a predefined Analysis statistics for each measurement:

Absolute area	Negative area values are converted to positive and summed with the positive area values.
Absolute peak	Largest absolute value.
AP Duration	Action potential duration (by percentiles).
Area	Signed area - negative values negate positive values.
Decay time	10 - 90% decay (fall) time of "peak to end".
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 positive sample.
Mean	Arithmetic mean of the samples.
Min value	Value of smallest sample.

[only available when an output channel Segment uses a 'Membrane Test' wave-form]

MT Series Resistance

Membrane test 'Rseries' value of a patch.

MT Membrane Capacitance

Membrane test Cmembrane' value of a whole-cell patch.

MT Membrane Resistance

Membrane test 'Rmembrane' value of a whole-cell patch.

Rise Tau	Time constant of 'Rise time'.
Rise time	10 - 90% rise time of "start to peak".

RMS noise	Root-Mean-Square noise.
Segment amplitude	Amplitude of the specified 'Out Channel' Segment.
Segment duration	Duration of the specified 'Out Channel' Segment.
Slope	Slope of simple linear regression fit.
Std deviation	Standard deviation of the samples: √(variance)
Stimulus at absolute peak	Stimulus amplitude at time of the largest absolute sample.
Stimulus at min	Stimulus amplitude at time of the smallest sample.
Stimulus at threshold	Stimulus amplitude at time of the first threshold crossing.
Time of absolute peak	Time from sweep start to largest absolute value.
Time at 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. Area / Peak - y0 (based on end of measurement).

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

Many other SutterPatch settings and readings can be plotted, without defining an Analysis measurement, through the Graph Settings axes source equations.

Note: The first sample point is used for any needed baselines.

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

Threshold This amplitude level needs to be crossed by the signal to trigger measurements for:

- AP Duration
- 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.

AP Duration [for AP Duration only]

[20, 30, 40, 50, 60, 70, 80, 90, 100 %]

The action potential amplitude-percentile setting, to calculate the associated AP Duration width.

To Notebook Copy the measurement result to the Notebook window during acquisition:

Experiment File:			<Experiment name>	
Paradigm:			<Paradigm name>	
Series:			<Series name>	
Sweep	#1	m[#]	<analysis function>	<analysis value>
Sweep	#2	m[#]	<analysis function>	<analysis value>

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 which signal is to be measured from the list of Input Channels. "Membrane Test" signals are restricted to the headstage “Current” inputs.

A measurement made on one input channel can be used in multiple graphs.

Region Timing

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

Cursor Start times cannot be greater than their End times.

Warning! Beware of boundary issues, where sharp transitions can be unexpectedly included or excluded in measurements. Due to the various input and output sampling rates and time durations of the actual signal, data points might not exactly match up with defined measurement regions.

You might need to adjust the measurement region to be one sample (or more) greater or less, than the target region, depending on whether you want to exclude or include the initial response. Otherwise, for example, a spike at the beginning of a Segment could skew measurement amplitudes to be larger, or a transition at the beginning of a Segment could be missed in a threshold crossing, thus lowering a Frequency count.

- Sweep Time — Set relative to the start time of a sweep (time zero).

Increment by: [ ]

The 'Start Time' or 'End Time' spinners increment by this amount.

The listed time values depend upon the input filter bandwidth.

Reset Times — Set the cursor Start / End Time to the beginning and ending of the sweep.

Start time — Set the left cursor start time (s).

End time — Read-only field of the right cursor 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 Time' is updated.

[ ] The width of the cursors in seconds.

The minimum width size is 2 sample points.

- Segment Time — Set the time range as a ratio of the Segment duration.

  Uses the Segment timing from the input signal's "Parent Output Channel".

  Out Channel — [Output Channel list] [Segment #]

  Increment by: — [0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5]

  Increment the Start / End Ratios by a relative amount.

  Reset Ratios — [0.0000] / [1.0000]

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

  Tip: If unwanted Segment boundary issues occur, where measurements are affected by data in a neighboring Segment, increase the Start Ratio or decrease the End Ratio until the issue is resolved.

  Start Ratio — [0 = beginning of Segment]

  Set the left cursor as a ratio of the Segment duration.

  Time [0.0000 s] Cursor start-time read-only 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.

  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.

  Cursor Time Width — The width of the measurement region is reported.

Graph Settings

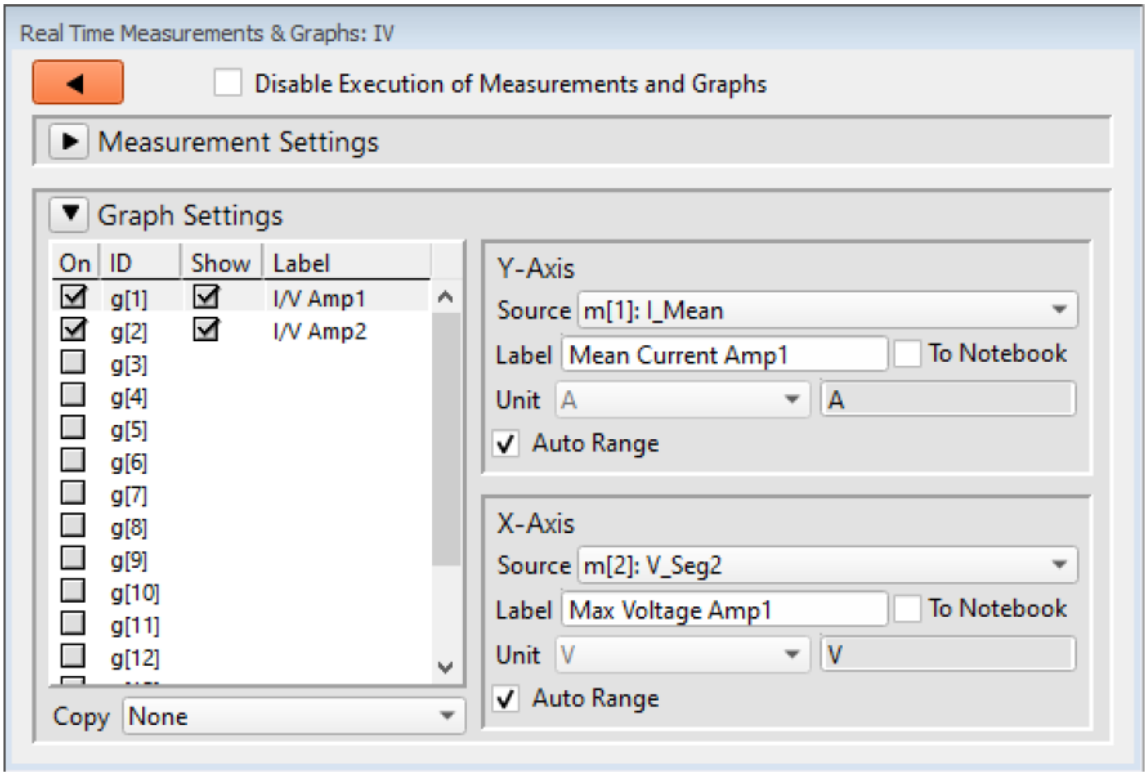


Figure 4-61. Real Time Graph Settings

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 sub-window.

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

Source: Set up the source of the Y-axis numbers

- Equation: Use an equation for a customized Y-axis plot. (See the Equation Editor for details.)

  Many SutterPatch settings and readings can be plotted, without defining an Analysis measurement, by using Special IDs in the equation.

- <m[#]: *Name*> Select a Measurement ID for the Y-axis.

Label — A Y-axis label is automatically generated from the Measurement label. Directly edit to customize the Y-axis graph label.

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.

Auto Range — Restrict the graph Y-axis range.

Y-min — Lower limit of the Y-axis.

Y-max — Upper limit of the Y-axis.

<u>X-Axis</u>

Source — Set up the source of the X-axis numbers:

- Equation — Use an equation for a customized X-axis plot. (See the Equation Editor for details.)

  Many SutterPatch settings and readings can be plotted, without defining an Analysis measurement, by using Special IDs in the equation.

- Time — Use a standard time-base.
- <m[#]: *Name*> Select a Measurement ID for the X-axis.

Label — Enter a customized name for the X-Axis.

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.

Auto Range — Restrict the graph X-axis range.

X-min — Lower limit of the X-axis.

X-max — Upper limit of the X-axis.

Routine Variables

Up to 16 Routine Variables can be configured for use in Routines. These variables allow manual or automatic control of certain Routine settings.

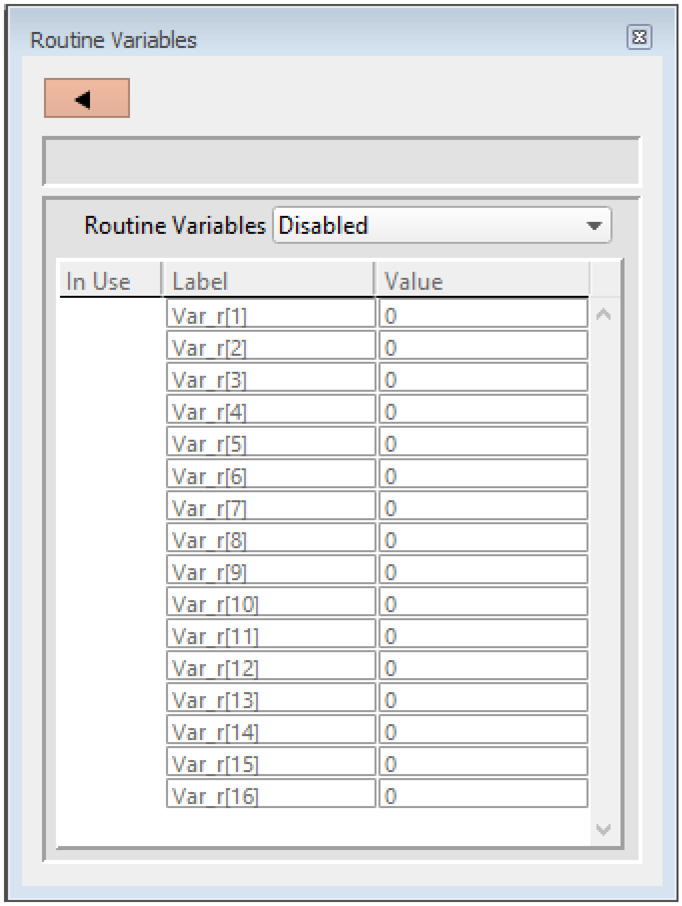


Figure 4-62. Routine Variables

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 the Routine Variable is “active”, i.e., set to a non-zero value, or is being used in a Routine setting or equation field.

Label — Var_r[1 – 16] — Edit the default Routine Variable name if desired.

Value Numeric values can be manually entered here, or automatically set by the Paradigm step 'Set Variable'.

Routine Variables can be used in:

Input Channels / AuxIN / Scaling

Input Channels / Virtual Channel / Equation

Output Channels / Enable P/N Leak Pulses / Leak Hold

Output Channels / Waveform Editor / Amplitude

Output Channels / Waveform Editor / Duration

Measurements / AP Duration / Threshold

Measurements / Frequency / Threshold

Measurements / Time to Threshold / Threshold

If a Waveform Editor / 'Amplitude' or 'Duration' is set to a Routine Variable, and then changed to a value, the Waveform Editor converts its 'Var_r[#]' settings to 'Value' settings, using the last enabled value.

4.1.9 Solution Editor

SutterPatch: Solution Editor

Create a named list of solutions to control physical valves in solution changers and perfusion systems.

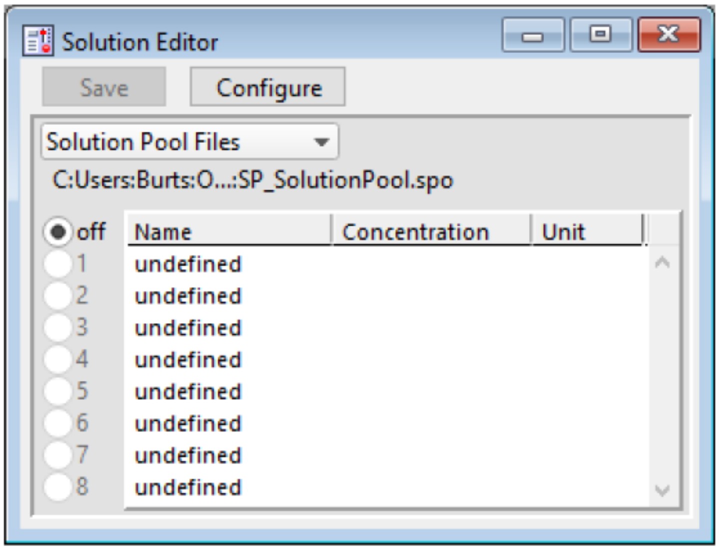


Figure 4-63. Solution Editor

Save	Save changes to the current Solution Pool file. This button becomes active once any changes are made in the Solution Editor.
Configure	Open the Configure Solutions dialog to categorize solution types and configure output channels.
Solution Pool Files	A Solution Pool file (*.spo) can contain multiple defined solutions.

New Solution Pool	Create a new Solution Pool, either blank or populated with selected Solutions.
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 filepath.
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 pathname of the loaded Solution Pool file is displayed.
[off, 1 – #]	Select a "valve" radio button to open a valve. This activates its corresponding solution configuration. A radio button is available (ungrayed) when its name is changed from 'undefined'.

Only one "valve" can be active at a time. The number of radio buttons is set in the Configure Solutions sub-window.

When set to 'off', all configured solution outputs are set to a zero amplitude.

Name — Double-click on a field to edit it; click-and-drag to move it up or down in the table.

Concentration — The concentration value for the solution.

Tip: You can access the concentration value from the last-used 'Test Compound' or 'Control' solution valve in any fields that accept the Special Identifier 'Paradigm Parameters: Stimulant'.

Unit — The unit type of the concentration.

<u>Configure Solutions</u>

 Close Dialog button.

[# Solutions] — Set the maximum number of solutions to configure. When this number is changed, a new Solution Pool is created.

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

Loading other Solution Pool files allows an unlimited number of solutions to be accessed in an Experiment.

Description — A text note for the solution.

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

- Initial Condition
- Washout
- Control

  Index [1 – 4] Distinguish between different Control solutions.
- Test Compound
- Not a Solution

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

- No Output
- AuxOUT[1 – 4] [±10.000 V] Analog output voltage.
- DigOUT Word [0 – 255] Decimal value of an 8-bit digital word.

- DigOUT[1 – 8]   A single digital bit is set "high".

4.1.10 Template Editor

SutterPatch: Template Editor

Templates allow any data waveform or portion of an existing data wave to be incorporated into a command waveform. The Template Editor can manage and manipulate such templates.

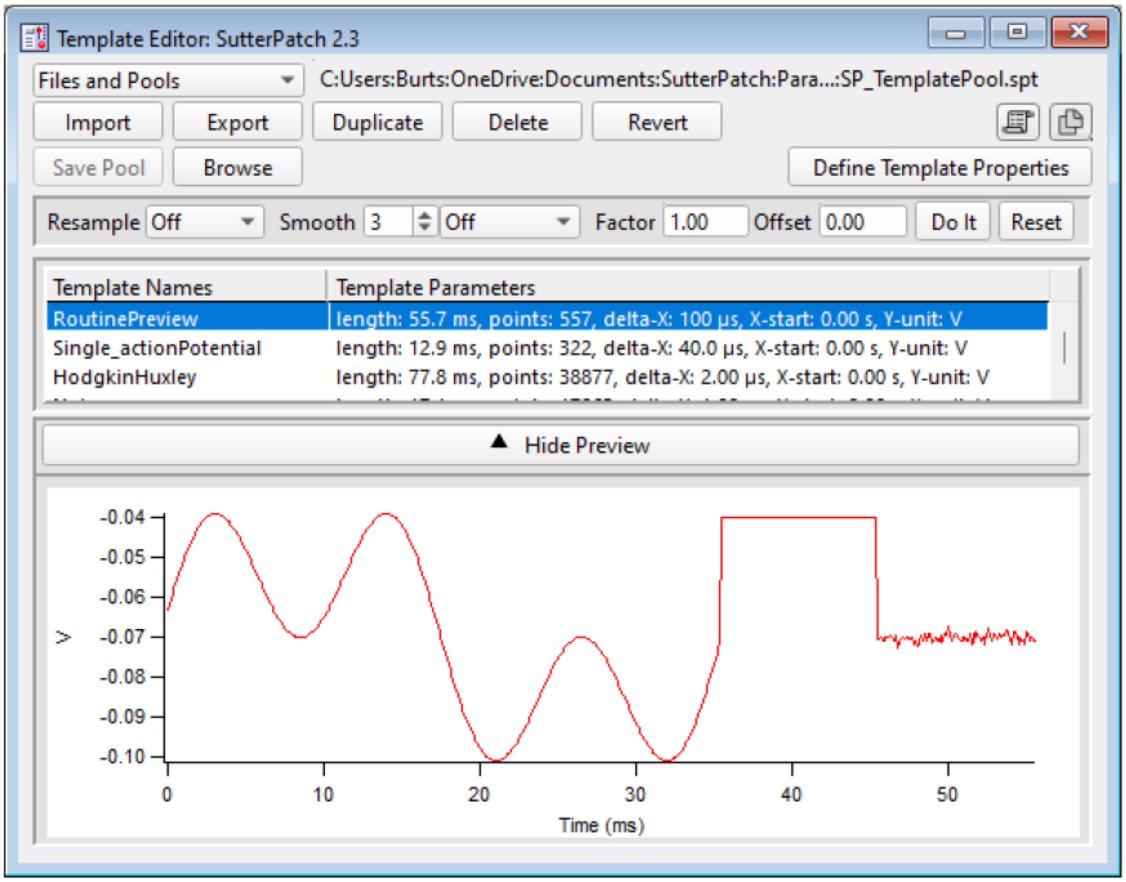


Figure 4-64. Template Editor

Note: Changes made in the Template Editor are only applied to Routines when the Routine Editor / Output Channels / Waveform Editor / 'Template wave' is used to copy the modified template in its Template Editor Pool list into the Routine.

Template Pool Files   A Template Pool file (*.spt) can contain multiple defined Templates.

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:

- Acquisition Scope window: Last sweep.
- Reanalysis Scope window: Selected sweep.
- Preview pane: Last or selected sweep.
- Analysis Editor Selected wave.

The Y-axis values are copied to the template; the X-axis values are reset in the template to start at zero.

Note: 'Extract Template' is not implemented for the Data Navigator preview pane. Also, it is only valid with monotonically increasing or decreasing X-axes.

Export	Export the selected template to an Igor Pro 1-D wave file (*.ibw).

To export a portion of a sweep, select the region of interest with the mouse, and use the marquee 'Extract Template' right-click command. The new wave can now be exported.

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.

Revert — Discard any unsaved changes to the selected template.

Delete — Remove the selected template from the list.

Copy to Layout — Copy the selected template graph into a new Layout window, or append to an existing Layout page.

Copy to Clipboard — Copy the selected template graph 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.)

Resample — [Off, (ms: 10, 5, 2.5, 2.0, 1.25, 1), (µs: 500, 250, 200, 125, 100, 50, 25, 20, 10, 5, 4, 2, 1), Other]

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.

- 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. To measure X-Y data points or set a fitting range, select ‘Toggle Cursor Info’ from the right-click menu. (See ‘Right-Click Menus’ for Scope windows.)

4.2 Data Analysis

Both online and offline data analyses are configured in these main SutterPatch Editors:

- Routine Editor (Real Time Measurements section)
- Paradigm Editor Note: For extra flexibility in performing data analysis, ‘Execute’ SutterPatch commands, Igor Pro analyses, or user-defined functions.

Additional online/offline plots can be configured via Scope window ‘Measurements’:

- Amplitude Histogram
- Color Plot
- Parametric Plot

Fitting can be applied to most displayed data.

Offline data processing is performed in the Analysis Editor:

- Average
- Concatenate
- Normalize

Offline analysis modules are provided via the Data Navigator ‘Available actions’:

- Action Potential Analysis
- Single-Channel Analysis
- Synaptic Analysis

Igor Pro also offers its own additional analyses in the Analysis main menu.

4.2.1 Action Potential Analysis

SutterPatch: Available Analysis Modules: Action Potential Analysis

Action potentials (APs) are analyzed offline with this window. Access via the Reanalysis Scope window ‘Measurements’ button, or the Data Navigator (signal) ‘Available actions’ menu.

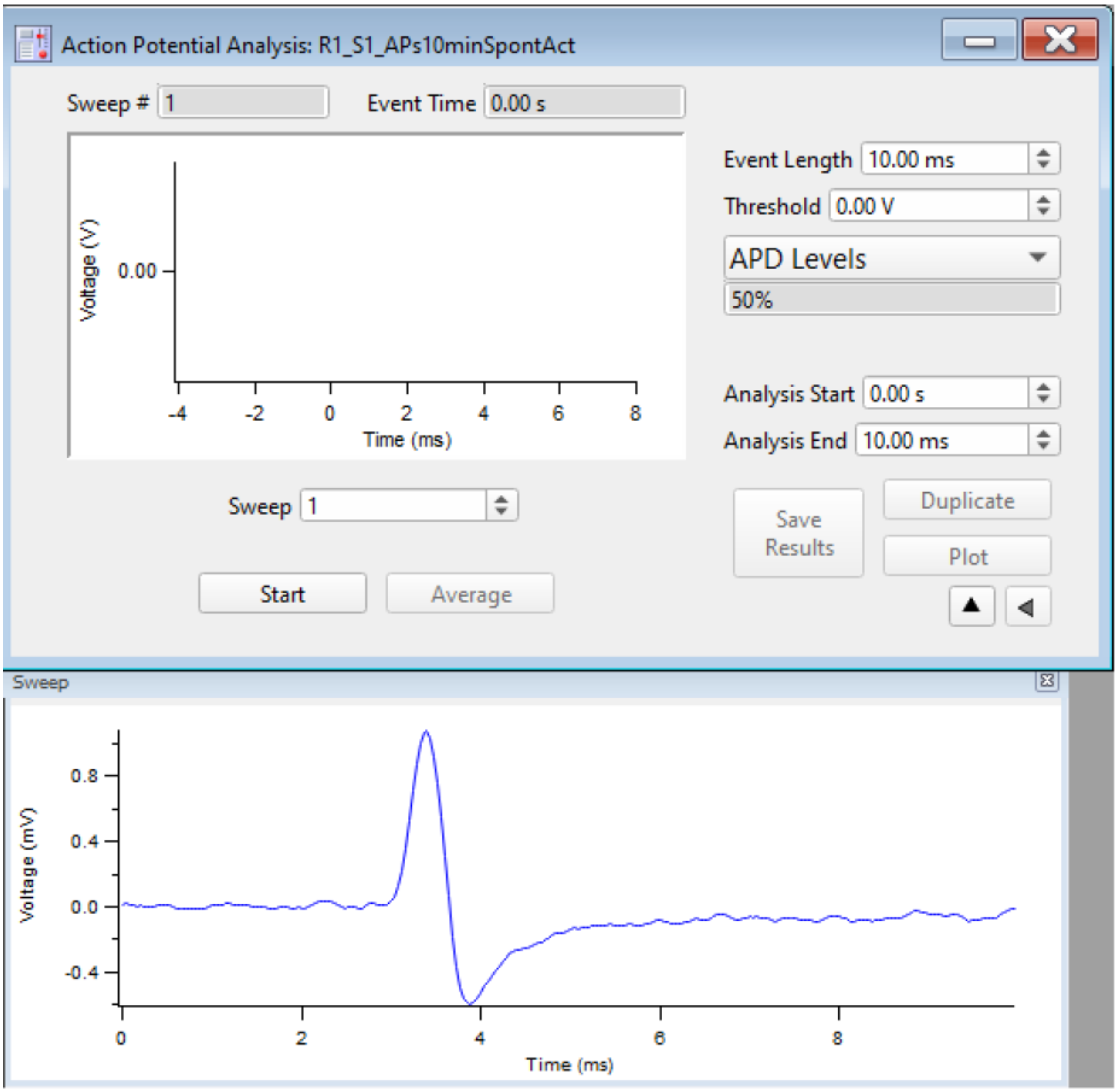


Figure 4-65. Action Potential Analysis

Sweep # — The sweep number of the selected action potential event.

When set to '0', this indicates that averaged Results measurements are being displayed.

Pre-select sweeps for processing by "marking" them in a Scope window or the Data Navigator.

Event Time — Time point when the potential of the selected event crosses the threshold.

[Event pane] — A graph of the selected event, with the X-axis zero point reset to the Threshold point.

To measure X-Y data points or set a fitting range, select 'Toggle Cursor

	Info' from the right-click menu. (See 'Right-Click Menus' for Scope windows.)
Event [# of #]	Event number vs. total events.
	Cycle through the analyzed events in the Event pane, with the event highlighted in red in the 'Sweep' pane,
or	
Sweep [#]	Cycle through the unanalyzed sweeps in the 'Sweep' pane.
[Sweep pane]	Displays a sweep of data colored in blue, with the selected event highlighted in red.
	To measure X-Y data points or set a fitting range, select 'Toggle Cursor Info' from the right-click menu. (See 'Right-Click Menus' for scope windows.)
Start	Click to find and analyze action potentials, and to display the Results pane.
Average	Click to display the averaged event (in Sweep # 0) in the Event pane.
	The Average Event Amplitude and plot are displayed in the Save Results layout window.
	Note: While the 'Sweep #' is set to '0' for averaged events, the last displayed Event # is unchanged.
Event Length (s)	The event duration in the event pane; the selected event is highlighted in red in the Sweep pane.
Threshold (V)	[±0.1000]
	This voltage level needs to be reached or exceeded for analysis of an event to be triggered.
APD Levels:	Set the Action Potential Duration percentile(s).
	Measures the duration of an event at percentiles of the event's repolarization amplitude.
	[20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 %] [Set to Default | Select All Odd | Select All Even]
Analysis Start (s)	[0+]
	Set the start time of the Sweep data to be analyzed.
Analysis End (s)	Set the end time of the Sweep data to be analyzed.

Save Results

The latest results are displayed in a Results table and a Layout window . Separate 'Average AP' and 'Phase plot' hidden graphs are also created, and can be accessed via menu item Windows / Graphs.

Results table

[ ]	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 at the AP repolarization percentile.
AHP (V)	Peak amplitude of the 'After Hyper-Polarization' phase.
AHP Time (s)	Time point of 'After Hyper-Polarization, when the event re-crosses the threshold amplitude at this time.
Absolute Event Time	The absolute time of the event from the start of the recording.
Interevent Interval	The time between adjacent events.
Max Slope (V/s)	The maximum slope of the event.
Min Slope (V.s)	The minimum slope of the falling phase.
AP Duration @ n% (s)	The action potential duration at the selected APD level.

'Action Potential Analysis Results' Layout window

Signal Pathname:	The Igor Pro experiment pathname for the analyzed signal.

Analysis Prefix:	The prefix for the signal's analysis objects in the Igor Pro 'Data:Analysis' folder.
Total time analyzed =	[s] Includes the Start / End times for all analyzed sweeps.
Number of events detected =	Total number of events found.
Event Frequency =	[Hz] The average frequency of the found events. Note: In a sweep, the time before the first event, and after the last event, are not included in this calculation.
All Sweeps analyzed	Every sweep was analyzed, or
Sweeps analyzed:	[list of analyzed sweep #s]
[Event graph]	[V vs. s] A graph of the averaged Event.
[Phase plot]	[dV/dt (V/s) vs. V] A graph of the phase plot for visual inspection of the derivatives.

Duplicate — Results are copied to a new Results table and a new Layout window.

Plot — The 'Plot sweeps' dialog displays to allow event sweeps to be plotted overlapping in a floating graph window.

Plot Sweeps — Enter a list of events separated by a comma "," and/or a range of events separated by a dash "-".

Show/Hide the Sweep pane (below).

Displays a graph of the sweep containing the selected event.

Show/Hide the Results pane (on the right).

Displays the Action Potential Measurements results.

Results pane — Measurement results are displayed for the event selected in the main window.

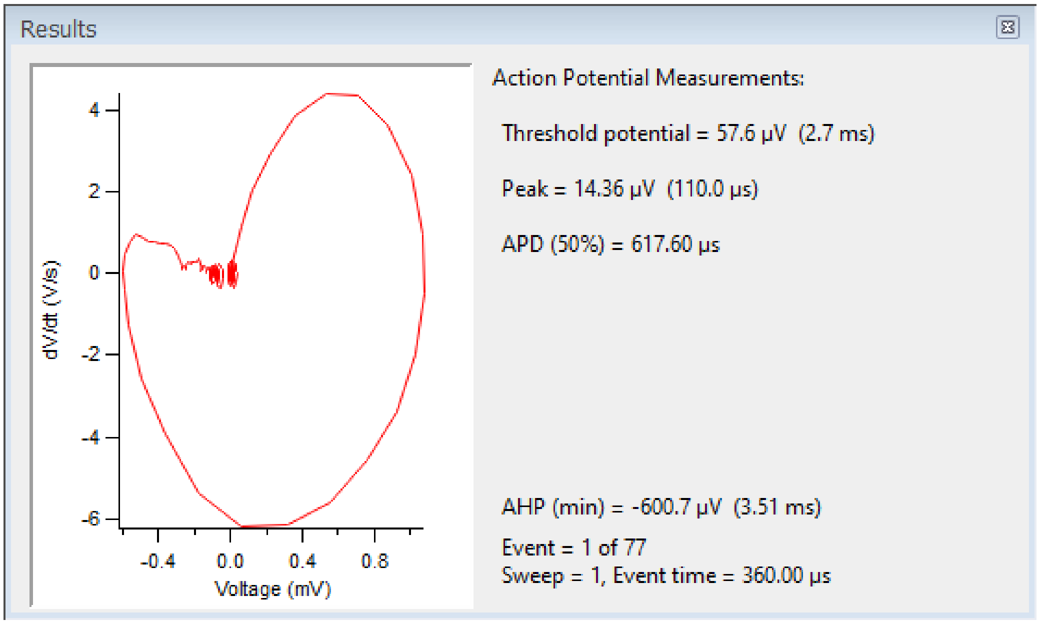


Figure 4-66. Action Potential Measurements

Phase plot — A graph of the phase plot for visual inspection of the derivatives.

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

To measure X-Y data points or set a fitting range, select 'Toggle Cursor Info' from the right-click menu. (See 'Right-Click Menus' for Scope windows.)

Threshold potential = [V (s)]

Event starting amplitude (time from Threshold setting).

The actual start of the selected event (the biological starting amplitude), and its timepoint relative to the 'Threshold' setting timepoint.

See the Algorithms appendix for more information.

Peak = [V (s)]

The largest amplitude excursion of the event (and the timepoint of the 'Threshold' setting).

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

APD (%) = [%, s]

	Action Potential Duration of the event at (n %) of amplitude re-polarization.
AHP (min) =	[V (s)]
	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.

[displays after ‘Start’, and when reviewing individual Events]

Event =	The analyzed event (of the total number of events) found in the data.
Sweep =	The sweep number of the analyzed event.
Event time =	The sweep time of the start of the analyzed event.

[displays for ‘Average’ and ‘Save Results’]

Events found =	The number of averaged events.
Event frequency =	The average frequency of the found events.

4.2.2 Analysis Editor

SutterPatch: Analysis Editor

View and manipulate the data in your Experiment's various analyses and graphs.

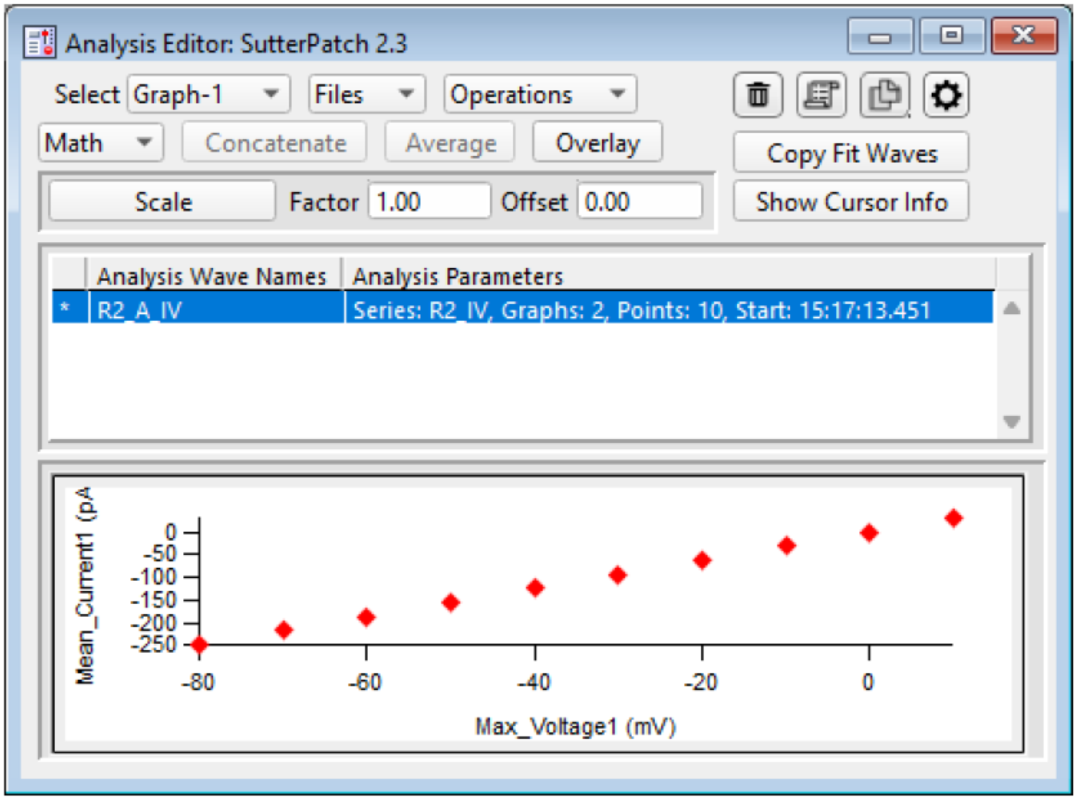


Figure 4-67. Analysis Editor

Select Choose how to view the data:

Table View a spreadsheet of the Analysis data

Warning! Editing the table will permanently alter its data.

Tip: As there is no "Undo", before making any changes, use Operations / Duplicate to make a working copy of the data that can be deleted later.

Top Row: Row Column numbers and value of the target cell.

Source row: Dimension labels: source routine name and source step number.

Header Row Analysis function measurement name.

Data Rows: Rows [0 – n]

[columns for time-based graphs]

First Column: Row numbers.

Second Column: "X \ Y" column label.
[has blank rows]

Column 0: X-data (Time) from the first graph: start times of the measurement sweeps.

Column 1: Y-data from the first graph: amplitudes of the measurements.

Columns m – n: Another pair of X- and Y-data columns displays for each additional time-based graph. However, the X-data columns are blank, as the Column 0 (Time) values apply instead.

[columns for X-Y graphs]

First Column: Row numbers.

Second Column: "Time" column.
Time data from the first graph: start times of the measurement sweeps.

Column 0: Y-data from the first graph: amplitudes of the Y-axis measurements.

Column 1: X-data from the first graph: amplitudes of the X-axis measurements.

Columns m – n: For each additional X-Y graph, a pair of Y- and X-data columns repeat.

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 row ungrays and the next row below is automatically grayed.

Graph-[1 – 8] Select an Analysis graph for the selected data wave. The graph number refers to its original Analysis window position.

Files — Import or export an analysis graph file.

Export Table to text file	The table data are written to a tab-delimited plain text file. Any column header information is lost. To preserve such metadata, export to the binary format.
Export Graphs as binary wave	Save the entire graph as a multi-dimensional Igor Binary Wave file (*.ibw).
Export Graph X-column	Save the X-column data, including labels, as a one-dimensional Igor Binary Wave file (*.ibw).
Export Graph Y-column	Save the Y-column data, including labels, as a one-dimensional Igor Binary Wave file (*.ibw).
Import Table from text file	Import numeric text data from comma- or tab-delimited columns.
Import Graphs	Open and display a saved graph. Note: Import of one-dimensional Igor Binary Wave files (*.ibw) is not supported.

Operations

Duplicate	Insert a copy below the highlighted item.
Delete Analysis or Table	Delete the entire analysis wave.
Delete Single Graph	Delete the selected graph. Note: If an analysis cannot be deleted, it likely exists in another Graph window or Layout page - first close the other analysis instance via menu items Windows / Graphs, or Windows / Layouts, or Windows / Layout Macros.

 Delete Analysis or Table — Click to delete the selected analyses or tables. Hold <Shift> to delete without verification.

 Copy to Layout — Copy the selected graph or table into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard — Copy the selected graph or table to the system clipboard.

Options

Show Fits	Display fit lines on the graph data.
Show Error Bars	Display SEM error bars for averaged data.
Show Axes Color	Display a background color for the axes.
Show Grid	Display X & Y grid lines in a graph.
Show Markers	Display data points with marker symbols.
Show Lines	Display a line between data points. (Toggle between 'markers' and 'lines', or both.)
Include Column Labels	Column labels appear on the first line of an exported table.

Math [for Graphs]

Normalize: zero to maximum

Rescale the absolute value data, so the zero point is maintained in, or relative to the data, and the largest absolute point is set to 1.0.

Normalize: minimum to maximum

Subtract the minimum value from all data points, so the smallest point is at the zero point, and rescale the data so the largest point is set to 1.0.

Tip: Use normalization to compare % of solution block.

Invert — Reverse the Y-axis sign of the data points.

Compute average of respective sweeps in a cycle

More than one full cycle of sweeps is required.

Compute average of all sweeps in a cycle

[for Tables]

Table selection average

Table selection sum

Concatenate To enable this button, select additional analysis waves using Shift-click. The newly concatenated wave is inserted below the last selected analysis wave; if the last selected wave is also a concatenated wave, the additional data is instead concatenated with the last selected wave. Time-course data are plotted relative to the loaded analysis wave's "time zero".

Average — Select an analysis wave (Shift-click in Windows) 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.

(See Appendix F: SutterPatch Algorithms for the SEM algorithm.)

Overlay — Select multiple analysis waves (Shift-click in Windows) and plot them in the same graph. When only a single analysis wave is selected, all graphs in the Editor are overlaid.

The initial wave is plotted with standard "diamond" symbols, while the added selections are plotted with smaller "plus" symbols.

Copy Fit Waves

Copy all Quick Fits (from all open SutterPatch windows) into their own analysis waves in the Analysis Editor, so that they persist during the entire experiment, and also are saved with the experiment. Otherwise, uncopied Quick Fits disappear when another Analysis Editor analysis wave is selected, or the Cursor pane is hidden, or the window is closed.

Scale and add

Use to combine available analysis 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 a data wave that will be added to the displayed data.

Values are displayed with SI unit prefixes.

To subtract a data wave, change the Factor to a negative number.

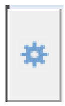
Offset — Set an offset for a data wave that will be added to the displayed data.

Values are displayed with SI unit prefixes.

Show/Hide Cursor Info

Open the Cursor Info pane to manually measure X-Y data values or to set a fitting range with Igor Pro cursor measurements. Hiding the Cursor Info pane also hides any cursor symbols in the active pane.

Cursor Info pane



Options menu

-  One Mover Moves All — Draggable cursor mover tool – move all cursors together with a single control.
- All Styles — Change the cursor symbol style.
- Show Cursor Pairs — Display up to 5 sets of cursor symbol pairs.

Cursor A

	Cursor symbol for data point 'A'.
A:	Symbol letter (beginning cursor of the pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.
pnt:	Data point number (starting from zero).
X:	X-axis value of data point 'A'.
Y:	Y-axis value of data point 'A'.
ΔY	Difference of the cursor pair Y values.

Cursor B

	Cursor symbol for data point 'B'.
B:	Symbol letter (ending cursor of the pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.

pnt:	Data point number (starting from zero).
X:	X-axis value of data point 'B'.
Y:	Y-axis value of data point 'B'.
ΔX	Difference of the cursor pair X values.

Cursor Instructions

1. Click on symbol A to enable it.
2. Manually drag the highlighted symbol onto a data point in the graph, or enter the data point number in the 'pnt' field.
3. Click on symbol B to enable it.
4. Manually drag the highlighted symbol onto a data point in the graph, or enter the data point number in the 'pnt' field.

   X- and Y-measurements are displayed for the cursor pair data.
5. Fitting can also be applied to the cursor pair data. Right-click in the graph, and select 'Quick Fit' for a list of built-in Igor Pro fitting functions.

   The fit is displayed in the graph, and the fitting information is written to the Command window.

Analysis Wave Names

Loaded analyses available for manipulation.

Analysis Parameters

- Series: R#_ — Series name of the wave.
- Graphs: # — Number of graphs in the wave.
- Points: # — Number of data points in the graph.
- Start: # — Start time of the analysis wave,

or

- SEM — Standard Error of the Mean 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.

The marquee tool is also supported in the Graph pane. Click and drag a bounding box around the region of interest, then right-click in it for magnification options.

To measure X-Y data points or set a fitting range, select 'Toggle Cursor Info' from the right-click context menu. (See 'Show/Hide Cursor Info' above.)

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.

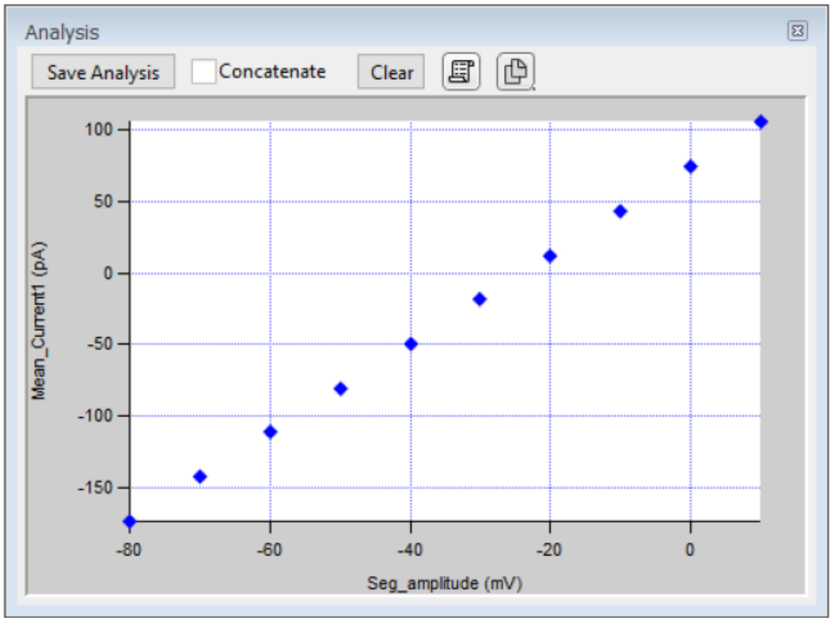


Figure 4-68. Analysis Window

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

Data can be opened for offline review or analysis via the Dashboard 'View Last' button or the Data Navigator. When stored data are rerun for analysis, the data displays in a Reanalysis Scope window, and the measurements are graphed in the 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 also the Data / Data Browser 'Data: Analysis' folder. Unmarked sweeps measurements are not visible in Analysis Editor tables.

Concatenate — Append new measurements to the existing measurements in the graph. [when docked to a Scope window]

Clear — Erase all measurements from the graph display.

 Copy to Layout — Copy the analysis graphs into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard Copy the analysis graphs to the system clipboard.

[Graph panes]

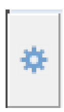
The Graph pane X and Y-axes can be magnified to be larger or smaller. Place the mouse cursor in the axis ticks region (do not include the tick labels or numbers), then scroll the mouse wheel up or down.

The marquee tool is also supported in the Graph pane. Click and drag a bounding box around the region of interest, then right-click in it for magnification options.

To manually measure X-Y data values or to set a fitting range with Igor Pro cursor measurements, right-click in the graph to display a data modification menu and select 'Toggle Cursor Info'. The 'Cursor Info' pane displays:

Select 'Toggle Cursor Info' again to hide the Cursor Info pane, and any cursor symbols in the active pane.

Cursor Info pane

 'Options' menu

-  One Mover Moves All — Draggable cursor mover tool - move all cursors together with a single control.
- All Styles — Change the cursor symbol style.
- Show Cursor Pairs — Display up to 5 sets of cursor symbol pairs.

Cursor A

 Cursor symbol for data point 'A'.

A: Symbol letter (beginning cursor of the A/B pair).

R1_A_IV (Default) wave name.

 Draggable cursor mover tool for the cursor pair.

pnt: Data point number (starting from zero).

X: X-axis value of data point 'A'.

Y:	Y-axis value of data point ‘A’.
ΔY	Difference of the cursor pair ‘Y’ values.

Cursor B

⊠	Cursor symbol for data point ‘B’.
B:	Symbol letter (ending cursor of the A/B pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.
pnt:	Data point number (starting from zero).
X:	X-axis value of data point ‘B’.
Y:	Y-axis value of data point ‘B’.
ΔX	Difference of the cursor pair ‘X’ values.

Cursor Instructions

1. Click on symbol A to enable it.
2. Manually drag the highlighted symbol onto a data point in the graph, or enter the data point number in the ‘pnt’ field.
3. Click on symbol B to enable it.
4. Manually drag the highlighted symbol onto a data point in the graph, or enter the data point number in the ‘pnt’ field.

   X- and Y-measurements are displayed for the cursor pair data.
5. Fitting can also be applied to the cursor pair data. Right-click in the graph and select ‘Quick Fit’ for a list of built-in Igor Pro fitting functions.

   The fit is displayed in the graph, and the fitting information is written to the Command windows.

Note: When applied to Analysis windows, the same Quick Fit is applied to all panes selected for fitting.

4.2.4 Data Browser

Data: Data Browser

The 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. Access it from the Data menu.

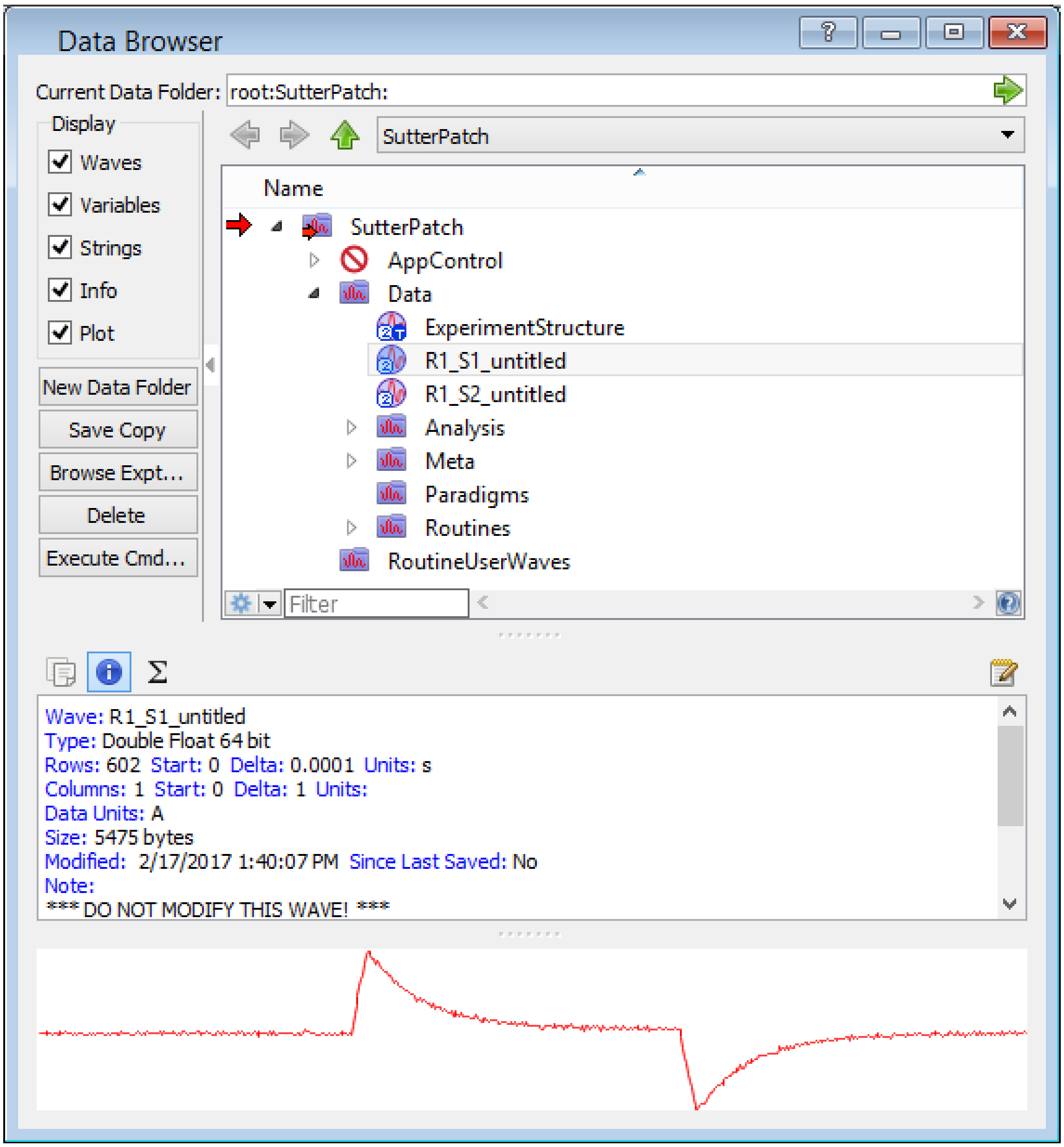


Figure 4-69. Data Browser

Objects are displayed in a tree structure, using a path "root" of 'SutterPatch'.

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

For the macOS: opening and closing windows can take a long time.

The ‘Data’ subfolder contains additional subfolders, followed by recorded data waves, arranged per Signal.

ExperimentStructure The sequence of Paradigms and Routines.

<Names of data Series…>

Analysis This folder contains data measurements, including results from fits, and Event tables.

- Wave names that include “_M_” contain the status of sweep marks. A marked sweep has a value of ‘1’, while an unmarked sweep has a value of ‘0’.
- Wave names that include “_A_” contain analysis measurements.
- Wave names that include “_df_” contain the differentiated average action potential (phase plot) waveform.

Images This folder contains stored images that display in the preview pane.

Meta This folder contains a table of general system metadata parameters (unformatted).

Routines This folder contains limited information on the used Routines.

<u>Right-click Menu</u>

Display Display the first sweep of the 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 permanently alters the raw data. Modify at your own risk!

Copy Full Path Copy the object’s pathname to the clipboard. This is in relation to an internal (hidden) Igor Pro data folder, not the computer’s file system. This pathname can be used by advanced Igor Pro users in user functions and executable commands.

4.2.5 Data Navigator

SutterPatch: Data Navigator

The Data Navigator window organizes and displays all levels of data for the current Experiment.

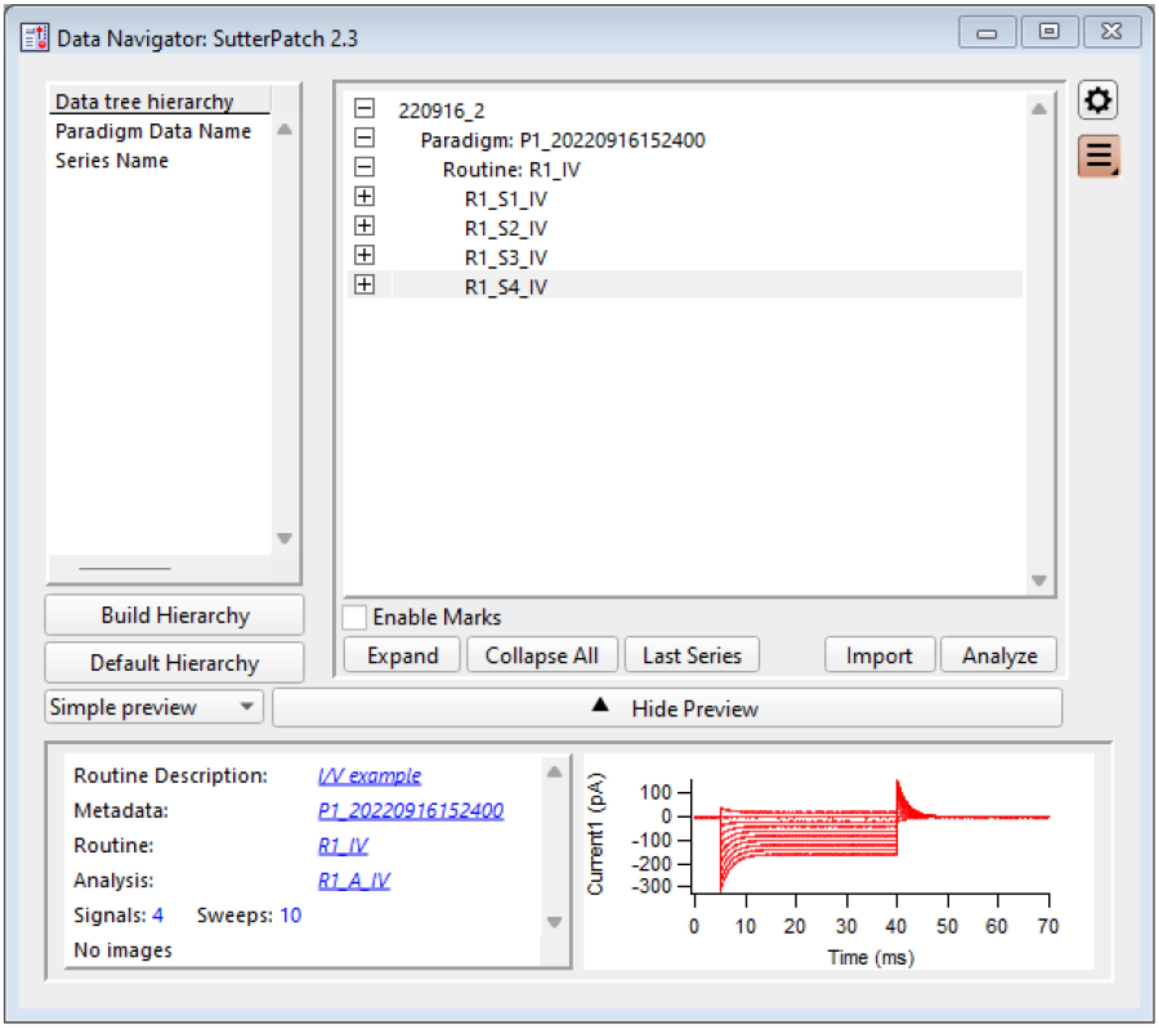


Figure 4-70. Data Navigator

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:

[Parameter availability depends on the selected 'Set Preferences / Metadata' detail level.]

All Categories

Frequently Used

Experiment Hierarchy

Tag

Operator

Preparation - Animal

Preparation - Tissue

Preparation - Cell

Experiment

Amplifier

Instrumentation and Software

Electrode

Recording Solutions

Paradigm

Cell Health / Quality Control

Series (= Routine Data)

Data Acquisition Settings

Imaging

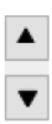
Stimulus

Available parameter — Click on a specific parameter from the selected group.

Click on the “copy” button to insert the selected parameter above the highlighted level in the Hierarchy pane.

Hierarchy — The Hierarchy pane displays the new sorting hierarchy for the current Experiment.

Remove the selected parameter from the list.



The sorting levels can be re-organized by selecting an entry and clicking on the Up/Down keys to reposition it, or using the ‘Del’ key to remove it.

Do It — Click on the ‘Do It’ button to apply these changes to the data tree.

Default Hierarchy — Restore the default experimental hierarchy:
Paradigm > Routine (> Signal > Sweeps)

Data Tree Window

The current Experiment's data are arranged in the data tree in five default levels:

Experiment
Paradigm
Routine
Signal
Sweep

Selecting a node level in the data tree highlights it in blue.

Enable Marks — Allow data to be marked in the data tree or Available Actions menu for analysis processing.

Marks enabled

- Node selected and marked.

  All levels and nodes below it included for processing.

- Node selected and unmarked.

  Only marked levels and nodes below it included for processing.

Marks disabled

- Node selected.

All levels and nodes below it included for processing.

Sweeps "marked" during acquisition are loaded into the Data Navigator as "marked".

"Marking/unmarking" sweeps in a signal "marks/unmarks" those sweeps in all signals in the same Series.

Mark a range: Highlight a marked starting selection, shift-click on the ending selection.

Clear a range: Highlight an unmarked starting selection, shift-click on the ending selection.

Expand — All nodes of the data tree are expanded down to the Signal level.

To expand a node to the next lower level, with a mouse click on a "+" node, or double-click the node name. With a keyboard, use the up/down arrow keys to select a "+" node and press the space key.

Collapse All | All nodes of the data tree are collapsed up to the Paradigm level.

To collapse the lower levels of a node, with a mouse click on a "-" node, or double-click the node name. With a keyboard, use the up/down arrow keys to select a "-" node and press the space key.

Last Series | The last Routine's first signal is highlighted in the data tree and displayed in the Preview pane.

Import | Select a previously saved Experiment, data or image file to add into the current SutterPatch Experiment.

Importing is not allowed during acquisition. The same data in an Experiment cannot be re-imported. For an Experiment file, only data are imported, i.e., no graphs or layouts.

Note: Imported Routine names are truncated to a maximum length of 28 characters, and special characters are replaced by an underscore.

- Import Igor Experiment File

  The same data set (Experiment) cannot be imported twice.

  When a Series name already exists in the Data Navigator, imported Series are renamed to avoid conflicts.

- Import SutterPatch HDF5 Data File

- Import PatchMaster Data File

  [This option is only available if Sutter Amplifier Systems hardware has been attached and detected by the SutterPatch software at any previous point in time for the current OS user.]

  Mappings

  Select the leak-subtraction mode when importing PatchMaster traces in SutterPatch.

  Main Pulse Traces:

  - Keep traces P/N-leak subtracted
  - Make P/N-leak unsubtracted traces

- Don't subtract zero-current from traces
- Subtract zero-current from traces

Set Defaults

- Import pClamp Data File

[This option is only available if Sutter Amplifier Systems hardware has been attached and detected by the SutterPatch software at any previous point in time for the current OS user.]

Import pCLAMP ABF version 1 and 2 data files.

Supported pCLAMP acquisition modes are 'Episodic Stimulation' and 'Gap-free' (equivalent to SutterPatch Clock Triggered modes 'Triggered Sweeps' and 'Continuous Sweeps').

- Import Image File

Review [for Paradigms]

Its signals are displayed in a Scope window as continuous data.

Note: This button changes to the Analyze button when a Routine, signal or sweep is selected.

Analyze [for Routines, Signals, Sweeps]

The Routine's signals are displayed in the Reanalysis Scope window and Measurements analysis is run.

Note: This button changes to the Review button when a Paradigm is selected or marked.

Simple preview / Full preview

[for Paradigms and Routines]

A "simple" preview displays a thumbnail sketch of the first signal's first Series (or sweep) of data, to the right of the preview information section.

A "full" preview displays all signals and Series in an attached pane.

Show Preview / Hide Preview

The displayed Preview information items are based upon the selected data tree level.

Note: The Data Navigator Preview panes do not support mouse operations.

Experiment

Experiment Name:	The experiment file name.
HDF5 File Name:	For optional HDF5 files.
Paradigms:	Total number of Paradigms in the Experiment.
Routines	Total number of Routines in the Experiment.
Total bytes in data waves:	Combined size of all data waves in the Experiment.

Paradigm

Paradigm Description:	Displays the Paradigm description text. Click to edit.
Metadata:	Click to display the Paradigm's metadata in a docked sub-window.
Images:	Open any saved images.
Simple preview	
Paradigm Preview window	Displays a thumbnail image of the first signal of the first Routine.
Full preview	
Paradigm Preview window	Displays all signals and all Routines in continuous mode in a docked Paradigm Preview window.

Routine

Routine Description:	Displays the Routine description text. Click to edit.

	Metadata:	Click to display the Routine's metadata in a docked sub-window.
	Images:	Open any saved images.
	Metadata:	Click to display the Routine metadata.
	Routine:	Click to display the named Routine parameters.
	Analysis:	Click an analysis name to open it into the Analysis Editor.
	Signals:	Number of signals in the Routine.
	Sweeps:	Number of sweeps in the Routine.
	Images:	Open any saved images.
		The image preview window text Note is editable.
	<u>Simple preview</u>	
	[Preview sub-pane]	Displays a thumbnail image of the first signal of the selected Routine.
	<u>Full preview</u>	
	Routine Preview window	Displays all signals in the Routine in continuous mode in a docked window.
<u>Signal</u>		
	[Preview pane]	Displays a thumbnail image of the selected Signal.
<u>Sweep</u>		
	[Preview pane]	Displays a thumbnail image of the selected Sweep.

Settings menu

Single Review Window	Re-use the same window for all Reviews.
Multiple Review Windows	Create a new window for each Review.

Review is Sweeps Display Mode

Review in Concatenated Display Mode

Review in Time Course Display Mode

Available Actions button

A menu lists various actions for the marked and/or highlighted data levels. These actions are also available via a right-click on the selected data level.

Experiment

Copy Signal Data Paths — Copy the Series internal Igor Pro path to the system clipboard:

root:SutterPatch:Data:Series_name

Export Data (See Preferences)

Export all marked data in the Experiment to file(s).

Uses the 'Set Preferences / Data Export' options.

When saving files, and the new filename is the same as an existing filename in the target folder:

- Choose a different folder, or
- First delete the older file via the OS file browser.

Analyze All [Marked] Routines

Run the reanalysis on all [marked] Routines in the Experiment.

[select options to be applied by this Action]

Store Analysis Waves	Append results to the Analysis Editor pool.

Copy Analysis Results to Clipboard

Copy Analysis Graphs to Layout Page

[the following options display when 'Enable Marks' is checked]

Mark All Paradigms	All Paradigms in the Experiment are marked.
Unmark All Paradigms	All Paradigms in the Experiment are unmarked.
Mark All Routines	All Routines in the Experiment are marked.
Unmark All Routines	All Routines in the Experiment are unmarked.
Mark All Signals	All signals in the Experiment are marked.
Unmark All Signals	All signals in the Experiment are unmarked.
Mark All Sweeps	All sweeps in the Experiment are marked.
Unmark All Sweeps	All sweeps in the Experiment are unmarked.

Set Routine Marks by Name Match

Opens the 'Routine Mark: Name Match Editor' to enter the Routine name to mark.

Set Sweep Marks by Equation

Opens the 'Sweep Mark: Equation

Editor' to enter an equation for the sweeps to mark.

Equation

[ ]

Undo

Remove all edits to the equation.

Check Equation

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

Insert special identifier

sweep

Enumerate the sweeps in the equation when applying marks.

Odd(sweep)

Set all odd sweeps to "1", and all even sweeps to "0".

Even(sweep)

Set all even sweeps to "1", and all odd sweeps to "0".

Do Mark

Evaluate the equation and update the sweep marking.

[Status message]

value >= 0.1 = 1 (marked)

value < 0.1 = 0 (unmarked)

Paradigm

Review Paradigm	Display all Series (with all signals and sweeps) in the

	Paradigm. Each signal displays in a pane in a Paradigm Review window. Note: This action is not supported with the HDF5 file preference “Keep only one Sweep in Memory”.
Average Marked Sweeps	The average sweep of all marked sweeps is copied to the Analysis Editor.
View Metadata	Display the Paradigm metadata in the Metadata Review sub-window, docked to the right of the Data Navigator window.
View Paradigm Steps	[only displays if ‘Enable Marks’ is disabled; only executes for “planned” Paradigms] Display the steps in the selected Paradigm in a ‘Paradigm Steps Review’ window.
	The Paradigm steps can be copied to the system clipboard. Hold the <Shift> key when clicking, to remove the line counts and step formatting. The Paradigm can also be copied to the Paradigm Pool,
Edit Paradigm Description	[only displays if ‘Enable Marks’ is disabled; only executes for “planned” Paradigms] Add or alter Paradigm Description text in the Preview window. Changes are appended to the metadata as tags.
Copy Signal Data Paths	Copy the Series internal Igor Pro path to the system clipboard: root:SutterPatch:Data:Series_name

Export Data (See Preferences)

Export the marked Paradigm data to file(s).

Uses the 'Set Preferences / Data Export' options.

When saving files, and the new filename is the same as an existing filename in the target folder:

- Choose a different folder, or
- First delete the older file via the OS file browser.

Discard Paradigm

Remove the highlighted Paradigm and its data from the Experiment. If marks are enabled, the selected Paradigm also needs to be marked.

If the last Paradigm is discarded, when acquiring another Paradigm in the same Experiment, the new Paradigm name will be incremented past the discarded Paradigm name.

Note: If any associated graphs are still open when trying to discard the Paradigm, an error message will display. To fix, close any associated Graph windows found in the main menu Windows / Graphs.

Analyze All [Marked] Routines

Run the reanalysis on all [marked] Routines in the Paradigm.

[Select options to be applied by this Action]

Store Analysis Waves — Append results to the

Analysis Editor pool.

Copy Analysis Results to Clipboard

Copy Analysis Graphs to Layout Page

[the following options display when ‘Enable Marks’ is checked]

Mark All Routines	All Routines in the Paradigm are marked.
Unmark All Routines	All Routines in the Paradigm are unmarked.
Mark All Signals	All signals in the Paradigm are marked.
Unmark All Signals	All signals in the Paradigm are unmarked.
Mark All Sweeps	All sweeps in the Paradigm are marked.
Unmark All Sweeps	All sweeps in the Paradigm are unmarked.

Set Routine Marks by Name Match

Opens the ‘Routine Mark: Name Match Editor’ to enter the Routine name to mark.

Set Sweep Marks by Equation

Opens the ‘Sweep Mark: Equation Editor’ to enter an equation for the sweeps to mark.

Equation

[ ]

Undo

Remove all edits to the equation.

Check Equation

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

Insert special identifier

sweep

Enumerate the sweeps in the equation when applying marks.

Odd(sweep)

Set all odd sweeps to “1”, and all even sweeps to “0”.

Even(sweep)

Set all even sweeps to “1”, and all odd sweeps to “0”.

Do Mark

Evaluate the equation and update the sweep marking.

[Status message]

value >= 0.1 = 1 marked)

value < 0.1 = 0 (unmarked)

Routine

Analyze Routine	Display the marked sweeps of all signals in the highlighted Series in a Reanalysis scope window.
Review Routine	Display all signals (with all sweeps) from the highlighted Series. Each signal displays in a pane in a Routine Review window. Note: This action is not supported with the HDF5 file preference “Keep only one Sweep in Memory”.
View Metadata	Display the highlighted Series’ metadata in the Metadata Review sub-window docked to the right of the Data Navigator window.

View Routine Settings	Display the highlighted Series' settings and preview in the Routine Settings window.
Edit Routine Description	[only displays if 'Enable Marks' is disabled; only executes for "planned" Paradigms] Add or alter Routine Description text in the Preview window. Changes are appended to the metadata as tags.
Copy Signal Data Paths	Copy the Series internal Igor Pro path to the system clipboard: root:SutterPatch:Data:Series_name
Show in Data Browser	Open Igor Pro's Data Browser window to examine the highlighted Series' data waves.
Export Data (See Preferences)	Export the marked Series to file(s). Uses the 'Set Preferences / Data Export' options. When saving files, and the new filename is the same as an existing filename in the target folder: - Choose a different folder, or - First delete the older file via the OS file browser.

Discard Routine	Remove the highlighted Series data from the Experiment. If the last Series is discarded, when acquiring another Series in the same Experiment, the new Series name will be incremented

past the discarded Series name.

Note: If any associated graphs are still open when trying to discard the Routine, an error message will display. To fix, close any associated Graph windows found in the main menu Windows / Graphs.

Concatenate Sweeps — Combine all sweeps into one sweep.

Restore concatenated Sweeps

Convert the concatenated sweep back to the original sweeps.

[the following options display when 'Enable Marks' is checked]

Mark All Signals	All signals in the Series are marked.
Unmark All Signals	All signals in the Series are unmarked.
Mark All Sweeps	All sweeps in the Series are marked.
Unmark All Sweeps	All sweeps in the Series are unmarked.

Set Sweep Marks by Equation

Opens the 'Sweep Mark: Equation Editor' to enter an equation for the sweeps to mark.

Equation

[ ]

Undo

Remove all edits to the equation.

Check Equation

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

Insert special identifier

sweep

Enumerate the sweeps in the equation when applying marks.

Odd(sweep)

Set all odd sweeps to “1”, and all even sweeps to “0”.

Even(sweep)

Set all even sweeps to “1”, and all odd sweeps to “0”.

Do Mark

Evaluate the equation and update the sweep marking.

[Status message]

value >= 0.1 = 1 (marked)

value < 0.1 = 0 (unmarked)

Signal

Action Potential Analysis	Analyze action potentials from the marked sweeps of the highlighted signal.
Single Channel Analysis	Analyze single-channel Events in the highlighted signal.
Synaptic Event Analysis	Analyze synaptic events (EPSPs, minis, etc.) from the marked sweeps of the highlighted signal
------------	
Analyze Routine	Display all signals in the Series, and their marked sweeps, in a

	Reanalysis Scope window.
Edit Signal	Display all sweeps of the highlighted signal as numeric columns in an editable table.
Display Signal	Highlighted signal: display the marked sweeps in the signal in a graph window. Marked signal: display all sweeps in the signal in a graph window.
Average All Sweeps	Average all sweeps in the highlighted signal and display in the Analysis Editor.
or	
Average Marked Sweeps	Average the marked sweeps in the highlighted signal and display in the Analysis Editor.
View Metadata	Display the highlighted signal's metadata in a Metadata Review sub-window docked to the right of the Data Navigator window.
View Routine Settings	Display the Series parameters in the Routine Settings window.
Copy Signal Data Path	Copy the Series internal Igor path to the system clipboard: root:SutterPatch:Data:Series_name
Show in Data Browser	Open Igor's Data Browser window to examine the highlighted signal's data waves.
Export Data (See Preferences)	
	Highlighted signal: Export the marked sweeps in the signal. Marked signal: Export all sweeps in the signal.

Uses the ‘Set Preferences / Data Export’ options.

When saving files, and the new filename is the same as an existing filename in the target folder:

- Choose a different folder, or
- First delete the older file via the OS file browser.

[the following options display when ‘Enable Marks’ is checked]

Mark All Sweeps	All sweeps in the Series are marked.
Unmark All Sweeps	All sweeps in the Series are marked.

Sweep

Extract Sweep	Create a graph of the highlighted sweep in the Analysis Editor.
Display Sweep	Display the highlighted sweep in a graph window.

Export Data (See Preferences)

Export the highlighted sweep.

To export a portion of a sweep, extract the data with the marquee tool, and then export from the Template Editor.

Uses the ‘Set Preferences / Data Export’ options.

When saving files, and the new filename is the same as an existing filename in the target folder:

- Choose a different folder, or
- First delete the older file via the OS file browser.

[the following options display when ‘Enable Marks’ is checked]

Mark All Sweeps	All sweeps in the Series are marked.
Unmark All Sweeps	All sweeps in the Series are unmarked.

4.2.6 Data Table

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

Table0:R1_S1_IV

R0 C0 | 5.2332054e-12

Row	R1_S1_IV[][0]	R1_S1_IV[][1]	R1_S1_IV[][2]	R1_S1_IV[][3]	R1_S1_IV[][4]
	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 4-71. Data Table

Warning! Editing data permanently alters the raw data. Modify at your own risk!

Data Tables are accessed from the Data / Data Browser. Select a Series from the Data folder, then right-click the 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 Edit Virtual Signals

The Reanalysis Scope window Measurements button provides access to the ‘Edit Virtual Signals’ dialog. Use it when applying different analysis scenarios to recorded data with “pseudo” input signals, in conjunction with the 'Reanalysis Measurements & Graphs' dialogs.

Virtual input channels allow you to perform a variety of mathematical transformations on input signals. To enable a virtual signal, highlight a signal name. When a virtual input channel is enabled, its configuration fields are ungrayed. Changes to the highlighted signal are saved when you click the ‘Do It’ button, and changes in unhighlighted signals are discarded.

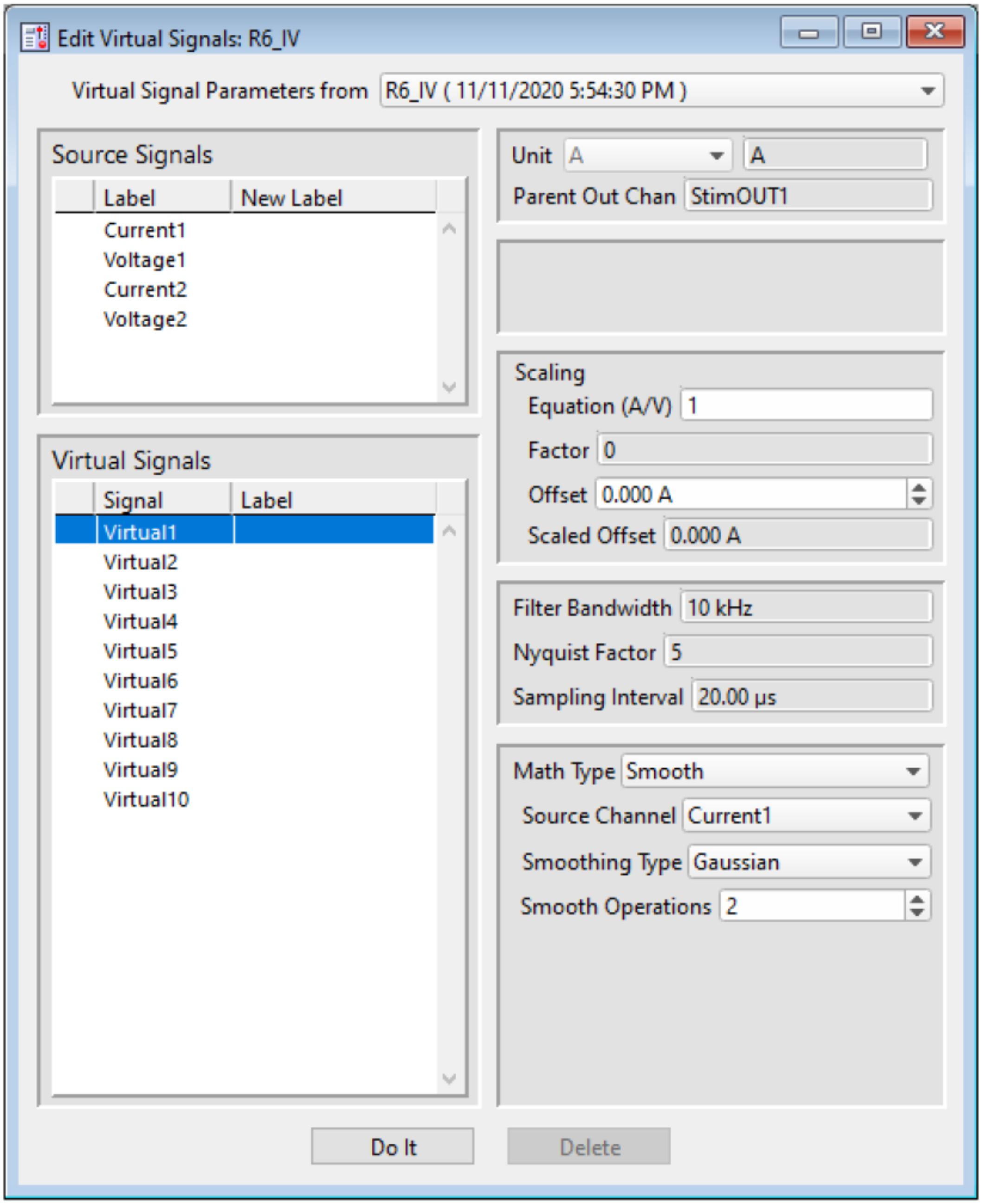


Figure 4-72. Edit Virtual Signals

Virtual Signal Parameters from

- [<Series name> (date/time stamp)]
- Overwrite with Original Routine

Source Signals

Label	The input signal name.
New Label	Double-click this field to edit the signal name, then click the 'Do It' button. Legal characters are A-Z, a-z, 0-9, and underscore "_". The label must start with a letter, be at least two characters long, and not be a duplicate of another label. Otherwise, the label will be automatically updated to a legal format. To see if automatic updates will be applied to the label, after making edits, first click the 'Enter' key, before clicking the ungrayed 'Do It' button.

Virtual Signals

Virtual signals can be added, edited, or removed from the Scope window.

To enable a virtual signal, highlight it and click the 'Do It' button. A check mark is displayed in the first column, and the signal is added to the Scope window.

To disable a virtual signal, highlight it and click the 'Delete' button. The check mark is removed from the first column, and the signal is removed from the Scope window.

Signal	The virtual signal name.
Label	Double-click this field to edit the signal name, then click the 'Do It' button. Legal characters are A-Z, a-z, 0-9, and underscore "_". The label must start with a letter and be at least two characters long, and not be a duplicate of another label. Otherwise, the label will be automatically updated to a legal format. To see if automatic updates will be applied to the label, after making edits, first click the 'Enter' key, before clicking the ungrayed 'Do It' button.

Unit — The base unit of measurement from its Source signal. The resolution of the unit is automatically adjusted in the signal.

Parent Out Chan

The “Parent Output Channel” shows which output channel is associated with which input channel.

Scope Position

Signal Action

- None
- Show
- Hide
- Move to >> [signal name]

<u>Scaling</u>

Equation — Apply scaling to interpret the input signal data. Specify as a numeric value or an equation for the scaling ratio.

Factor — [read only field of Equation result]

Note: The dPatch system acquires data with a high-resolution 16-bit ADC into 64-bit data words, so data resolution is not an issue when scaling input signals.

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

Scaled Offset — Raw offset values converted to input units.

[for AuxIN channels]

Sampling Interval

The duration of a single data sample.

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]

Limit to Marked Sweeps

Enable to limit this analysis to marked sweeps.

- 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 < ½ the sampling rate]

  Restrict frequencies from this boundary point onwards.

  Integrator Reset [for Capacitive Mode]

  - Ignore Capacitive-mode transients are displayed in the data.

  - Blank The data during capacitive transients are made invisible by replacing those data points with NaNs (Not a Number).

    Blank Duration [10 µs – 1 s]

  - Mask The data during capacitive transients are replaced by the last data value before the transient discharge, simulating a sample-and-hold operation.

    Mask Duration [10 µs – 1 s]

The default value of 500 µs should be sufficient to encompass the reset transient duration.

- CycleAverage Apply averaging across cycles for each numbered sweep.

  Source Channel Select an input channel to average.

  Limit to Marked Sweeps

  Enable to limit this analysis to marked sweeps.

- 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[#] nth 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

Undo All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

Limit to Marked Sweeps

Enable to limit this analysis to marked sweeps.

- 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 signal 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. 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	60 Hz	Canada, (Caribbean), Central America, (Japan), Mexico, (South America), South Korea, Taiwan, USA. Some (regions) include both 50 Hz and 60 Hz frequencies.
	50 Hz	Most of rest of world.

  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) insead 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.

(see the SutterPatch Algorithms appendix)

- Smooth — Smooth the data with a "moving average" noise-reduction filter.

  Source Channel — Select an input channel to smooth.

  Smoothing Type

  - Gausian — 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.

  Integrator Reset [for Capacitive Mode]

  - Ignore — Capacitive-mode transients are displayed in the data.

  - Blank — The data during capacitive transients are made invisible by replacing those data points with NaNs (Not a Number).

    Blank Duration

    [10 µs – 1 s]

  - Mask — The data during capacitive transients are replaced by the last data value before the transient discharge, simulating a sample-and-hold operation.

Mask Duration

[10 µs – 1 s]

The default value of 500 µs should be sufficient to encompass the reset transient duration.

- 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

  Limit to Marked Sweeps

  Enable to limit this analysis to marked 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.

  Limit to Marked Sweeps

  Enable to limit this analysis to marked sweeps.

4.2.8 Equation Editor

SutterPatch: 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, or for data analysis.

Access the Equation Editor from the SutterPatch menu.

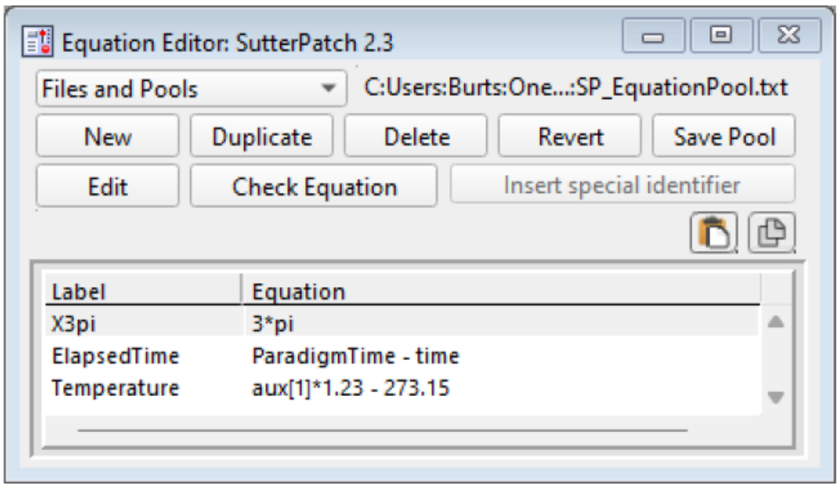


Figure 4-73. Equation Editor

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 (*.txt) 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 "Syntax is ok".

Insert special identifier

SutterPatch acquisition, analysis and reference settings are available for use in equations. (see list below)

Table

Label — A column of editable equation names, for easy usage in equations in place of the actual equation. (see 'Syntax' below)

Equation — A column of associated equations in free-form text fields, that evaluate as math expressions.

Equations are limited to a maximum of 80 characters, including white space.

Special Identifiers

SutterPatch acquisition, analysis and reference selections are appended to the equation with a "plus" sign.

Timing

Time — (present date-time, s)

Timer — (timer time, s)

ParadigmTime — (time at start of paradigm, s)

RoutineTime — (time at start of routine, s)

Paradigm Parameters

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.

Stimulant — (last applied stimulant concentration)

From the Solution Editor 'Concentration' setting, for solutions configured as a 'Chemical Stimulant'.

Input	(Input variable on paradigm window)
Hold[1..4]	(holding of n'th output channel)
p[1..16]	(n'th paradigm variable)
r[1..16]	(n'th routine stimulus variable)

Analysis Results

m[1..16]	(n'th analysis measurement value)
gx[1..16]	(n'th analysis graph x value)
gy[1..16]	(n'th analysis graph y value)

Signal Readings

AuxIN[1..4] — (reading of auxiliary input, V)

A single-point voltage reading from an Auxiliary Input channel, 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)

‘name’ = signal name

‘count’ = window-signal position

‘start’ = time of start, s (of measurement range)

‘width’ = duration, s (of measurement range)

<u>Headstage</u>

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.

<u>IPA Settings</u>

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	(amplifer 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.
VGain	(amplifier voltage gain, V/V)
	The gain of the active current-clamp ‘Voltage’ input channel.
Filter	(amplifier input filter in VC- and CC-mode, Hz)
	Read the low-pass filter of the input channels.
IFilter	(amplifier input filter in VC-mode, Hz)
	Read the low-pass filter of the ‘Current’ input channel in VC-mode.
VFilter	(amplifier input filter in CC-mode, Hz)
	Read the low-pass filter of the ‘Voltage’ input channel in CC-mode.
Offset	(amplifier pipette offset in VC-mode, V)
OffsetLock	(amplifier pipette offset lock On in VC-mode)

IPA Compensation

ECompMag	(amplifier electrode compensation magnitude, F)
ECompTau	(amplifier electrode compensation tau, s)
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)
Bridge	(amplifier bridge balance, Ohm)
BridgeOn	(amplifier bridge balance On)

<u>IPA Correction</u>

RsCorr	(amplifier Rs correction, fraction)
RsPred	(amplifier Rs prediction, fraction)
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On)

<u>Dynamic Holding</u>

DynHoldOn	(amplifier dynamic holding On)
DynHold	(amplifier dynamic holding potential, V)

<u>Membrane Test</u>

Relectr[1..2]	(electrode/seal/access resistance, Ohm) Value from last Membrane Test.
Rmemb[1..2]	(membrane resistance (cell mode), Ohm) Value from last Membrane Test.
Cmemb[1..2]	(membrane capacitance (cell mode), F) Value from last Membrane Test.
RMSNoise[1..2]	(membrane test RMS noise, A) Value from last Membrane Test.

<u>Lock-In</u>

LockInPhaseAdj	(Lock-In phase delay adjustment, s)
LockInAttenAdj	(Lock-In attenuation adjustment)

Other identifiers are forwarded to Igor Pro's ‘Execute’ command.

Paste from clipboard — Paste equations from the system clipboard.

Paste Equations — Select the paste action.

Append

Replace

Copy to clipboard — Copy the selected equations to the system clipboard.

Equation Usage

Arguments

X — The "X" (or “x”) specifier allows an argument to be passed to an equation. Insert “X” as the placeholder(s) in numeric expressions.

To call such an equation in other parts of the program, prepend a “#” to the equation label, and append the argument in parentheses.

Example 1 — Pass the value "1.7" to the named equation label “My_Equation”:

#My_Equation(1.7)

Example 2 — Send an AuxOUT voltage command to a piezo-drive controller in distance units, using the sample conversion formula:

volts = ((micrometers + 0.08) / 4.04) ^ 1.3

Instead of retyping this equation every time it is used, use an argument ‘X’ in the equation:

volts = ((X + 0.08) / 4.04) ^ 1.3

Label the equation as:

um2volt

Pass a distance of 10 micrometers to the labeled equation in a Routine (Routine Editor / Output Channels / Waveform Editor /

Amplitude Segment), or in a Paradigm (Paradigm Editor / Amplifier step / Auxiliary Output target) as:

#um2volt(10)

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)

Lists

Anywhere equations can be used, a list of comma-separated equations can also be used, to generate a sequence of values. If the sequence extends beyond the end of the list, the sequence wraps around and continues from the beginning of the list again, and so on.

Places used:

<u>Paradigm Steps</u>

Amplifier

Checkbox

Set Variable

Sound

Write Log

If

Else If

<u>Routine Editor</u>

Virtual Input Channel: Equation

Waveform Editor: Amplitude, Duration

Measurements: Time to Threshold

Graphs: X-Axis, Y-Axis

Example: Create a sequence of increasing values with a 1 / 2 / 5 progression, such as might be used to increase a Routine's waveform amplitude or duration, on a per sweep basis:

1m*10^ceil(sweep/3),2m*10^ceil(sweep/3),5m*10^ceil(sweep/3)

This will generate a sequence of values of: 10m, 20m, 50m, 100m, 200m, 500m, 1000m…

The 'ceil' function rounds up any fraction to the next higher whole number, and "sweep" is a special identifier that reports the active sweep number. So, for the first 3 sweeps (1, 2, 3), "ceil(sweep/3)" generates a '1'. As '10' raised to '1' is '10', the initial number (1, 2, 5) is multiplied by '10', resulting in values of "10m, 20m, 50m".

For the next 3 sweeps (4, 5, 6), the sequence wraps around the list, and now "ceil(sweep/3)" generates a '2'. As '10' raised to '2' is '100', the initial number (1, 2, 5) is now multiplied by '100', resulting in values of "100m, 200m, 500m".

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	^, <<, >>
6	Negation (logical) operations: Unary Negation, Logical Negation	-, !
5	Multiplication, Division, Remainder	*, /, %
4	Addition, Subtraction	+, -
3	Bitwise operations: And, Or, Nor, Xor	&, |, nor, %^
2	Comparison operations:	>, >=, <, <=, !=

<table>
<tr><td></td><td>Greater Than, Greater Than or Equal, Less Than, Less Than or Equal, Equal To, Not Equal To</td><td></td></tr>
<tr><td>1</td><td>Logical operations:
And, Or, Conditional</td><td>&&, ||, ? :</td></tr>
<tr><td>0</td><td>Other functions</td><td>abs, acos, asin, atan, ceil, cos, deg, exp, floor, ln, log, mlast, noise, odd, rad, random, round, sin, sqrt, tan, trunc</td></tr>
</table>

Table 4-4. Equation Parser

Comments are also processed differently between the SutterPatch equation parser and the Igor Pro command parser:

SutterPatch — All characters to the right of a semicolon are ignored

Igor Pro: — All characters to the right of a double slash "//" are ignored.

A semicolon separates multiple commands on the same command line.

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

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.

The function “mlast[count]” returns the measurement result of the previous sweep.

The function “Odd” returns a “1” when its argument is odd, and a “0” when it is even.

For expressions using Comparison and Logical operators, it is recommended to use parentheses to explicitly define the order of execution.

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”. For variable inputs, “#MyLabel(5)”passes the argument “5” to the equation labeled “MyLabel” for evaluation.

Example: Using an LED light source

To stimulate in increments of light intensity, use an equation to transform light intensity values in Routine variables into actual stimulus values with amplitudes in volts.

Build an equation in the equation pool as follows:

equation	=	ln(r[1]) * 2.55 + 3
		The natural log of the Routine Variable r[1] is multiplied by 2.55 and added to 3.
label	=	power_to_volts
		In the Waveform Editor, set a Segment Amplitude field to 'Equation', and enter the equation as "#power_to_volts".

Two SutterPatch Editors use a simplified version of the Equation Editor which allows equations and equation Labels to be used:

Paradigm Editor

Steps:	Amplifier, Checkbox, Set Variable, Sound, Write Log, If, Else If

Routine Editor

Input Channels:	Virtual Channels: Math Type: Equation
Output Channels:	P/N Leak Pulses: Leak Hold, Waveform Editor: Amplitude, Duration
Measurements:	Time to Threshold, X-Axis, Y-Axis

Note: For acquisition, computing an equation within a command waveform 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.

4.2.9 Igor Pro Analyses

Analysis

Numerous mathematical operations are found in the Analysis main menu. and are documented in the Igor Pro Help.

These built-in Igor Pro fitting analyses are also accessible via the "Scope" right-click menus:

- Curve Fitting — Create your own fitting equation
- Quick Fit — Use a pre-defined equation:

line	
poly [3 – 10]	
poly_XOffset [3 – 10]	
gauss	
Ior	
Voight	
exp_XOffset	
dblexp_XOffset	
exp	
dblexp	
dblexp_peak	
sin	
HillEquation	
Sigmoid	Use for Boltzmann function.
Power	
LogNormal	
poly2D [1 – 10]	
Gauss2D	

FitBetweenCursors	
Weight from Error Bar Wave	
Textbox Preferences	

Example: Perform a fit on a section of a sweep:

1. Open the data into a Reanalysis Scope window.
2. In the Scope window, right-click 'Toggle Cursor Info' to display the cursor pane.
3. Drag cursors 'A' and 'B' from the cursor pane onto the data to set the fitting range.

4. Right-click on the data, and select Quick Fit and the fit of your choice.
5. Fitting results are written to the Command window.

Other built-in Igor analyses include:

- Transforms
  - Fourier Transforms
  - Periodogram
  - Lomb Periodogram
  - MultiTaperPSD
  - Discrete Wavelet Transform
  - Continuous Wavelet Transform
  - Wagner Transform
  - Short-Time Fourier Transform
- Convolve
- Correlate
- Differentiate
- Integrate
- Smooth
- Interpolate
- Filter
- Resample
- Sort
- Histogram
- Compose Expression
- Packages
  - Average Waves
  - Batch Curve Fitting
  - Function Grapher
  - Global Fit
  - Igor Filter Design Laboratory
  - Median XY Smoothing
  - MultiPeak Fitting
  - Percentiles and Box Plot
  - Wave Arithmetic

4.2.10 Metadata Review

"Metadata" parameters describe the system environment, attached Sutter instrumentation, Paradigm and Routine acquisition settings, and tag information.

Metadata parameters can be retrieved several different ways:

Data Navigator

Open a Metadata Review docked sub-window.

When this docked window is open, it is linked to the Data Navigator window, where changing the Paradigm or Series node selection also updates the docked Metadata Review parameters.

- Select a Paradigm or Series and click on 'Metadata' in the Preview pane.
- Select a Paradigm or Series, then click the 'Available actions' button and select 'View Metadata'.
- Right-click a Paradigm or Series and select 'View Metadata'.

Reanalysis Scope window:

Open a Metadata Review floating sub-window.

Open a Series into a Reanalysis Scope window (from the Data Navigator 'Analyze' button or Action 'Analyze Routine'), and use the 'View Metadata' button to open a Metadata Review floating sub-window.

To simultaneously display metadata from two different Paradigms, use floating and docked windows:

Select the first Paradigm and the Action 'Review Paradigm'. Then, in the Paradigm Review window, click the 'View Metadata' button to open a Metadata Review floating sub-window. Next, select the second Paradigm, and select the Action 'View Metadata' to open a Metadata Review docked sub-window.

To simultaneously display metadata from two different Series, use floating and docked windows:

Select the first Series and the Action 'Analyze Routine' (or 'Review Routine'). Then, click the 'View Metadata' button to open a Metadata Review floating sub-window. Next, select the second Series, and the Action 'View Metadata' to open a Metadata Review docked sub-window.

Paradigm [ ] Displays the name of the Paradigm.

Routine [ ] Displays the name of the Series.

Signal [ ] Displays the name of the Signal selected in the Data Navigator.
[only if selected in the Data Navigator]

Copy to Clipboard
Copy the metadata settings to the system clipboard.

Metadata parameters from all detail levels are displayed (see Set Preferences / Metadata).

- By Event — Events are grouped by [time-stamp] [Event #] [Event type]. Highlighted values are editable.
  - Absolute Time — Display the event times in the computer system time.
  - Relative Time — Display the event times relative to the start of the Paradigm.
- By Parameter — Parameters are grouped into major categories. (see below)

  Parameters might also list “Prior” values. These include system and Paradigm parameters written before a Routine starts.

Expand All — All settings entries are displayed.

Two columns of information are presented (parameter name and value). If the first column’s text does not fully display, either increase the width of the window, or adjust the indentation of the second column – drag it when the mouse cursor turns into a double-headed arrow

Collapse All — All settings entries are hidden and collapsed up to the Event or Parameter level.

’By Parameter‘ Metadata Categories / Parameters

<u>Tag</u>
Tag Number
Tag Creation Timestamp
Timer Time at Tag Creation
Tag Signals
Tag Source Event

<u>Operator</u>
Login Name

<u>Experiment</u>

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 Firmware Version
Interface Serial Number
Interface Input Channel (physical)
Interface Signal Type
Interface Number of Digital Outputs
Computer Name
Physical Computer Memory
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
Paradigm Data Start Timezone

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	

<u>Sweep</u>

Sweep Number

<u>Data Acquisition Settings</u>

Active Headstage	
Recording Mode	
Current Gain	
Voltage Gain	
Headstage Gain	
Headstage Feedback Mode	[dPatch only]
Filter Cutoff Frequency	
Filter Type	
Input Offset Voltage	[VC mode, '0' in Demo mode]
Input Offset Lock On/Off	[VC mode]
Input Liquid Junction Potential	
Subtract Pip. Offset in Current Clamp	[dPatch]
Input Signal Name	
Input Signal Units	
Input Scaling Factor	[for AuxIN channels]
Input Full-scale Minimum	
Input Full-scale Maximum	
Input Sampling Rate	
Auxiliary Input Signal Offset	[for AuxIN only]
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 Subtract Baseline Type	[for Virtual BaselineSubtract]
Virtual Signal Subtract Baseline Start	[for Virtual BaselineSubtract]
Virtual Signal Subtract Baseline End	[for Virtual BaselineSubtract]
Virtual Signal Filter Type	[for Virtual BesselFilter]
Virtual Signal Filter Order	[for Virtual BesselFilter]
Virtual Signal Filter Cutoff Frequency	[for Virtual BesselFilter]
Virtual Signal Integrator Reset Strategy	[for Virtual BesselFilter & Smoothing, Capacitive mode]

Virtual Signal Integrator Reset Duration	[for Virtual BesselFilter & Smoothing, Capacitive mode]
Virtual Signal Leak Display On/Off	[for Virtual Leak]
Virtual Signal Leak Zero Segment	[for Virtual Leak]
Virtual Signal Line Frequency Base	[for Virtual LineFreq]
Virtual Signal LockIn Trace Kind	[for Virtual LockIn]
Virtual Signal LockIn Cycles to Average	[for Virtual LockIn]
Virtual Signal LockIn Cycles to Skip	[for Virtual LockIn]
Virtual Signal LockIn Reversal Potential	[for Virtual LockIn]
Virtual Signal Smoothing Algorithm	[for Virtual Smoothing]
Virtual Signal Smoothing Factor	[for Virtual Smoothing]
Virtual Signal Sweeps Processed	[for Virtual SweepAverage]
Virtual Signal Reference Sweep	[for Virtual SweepSubtract]
Electrode Fast Magnitude	[VC mode]
Electrode Fast Time Constant	[VC mode]
Whole-cell Compensation On/Off	[VC mode]
WC Comp – Series Resistance	[VC mode, if WC Comp On]
WC Comp – Membrane Capacitance	[VC mode, if WC Comp On]
Series Resistance Correction On/Off	[VC mode]
Series Resistance Prediction Value	[VC mode, if Rs Correction On]
Series Resistance Correction Value	[VC mode, if Rs Correction On]
Series Resistance Corr. Lag Time	[VC mode, if Rs Correction On]
Capacitance Neutralization On/Off	[CC mode]
Capacitance Neutralization Mag.	[CC mode, if Cap Neut On]
Capacitance Neutralization Tau	[CC mode, if Cap Neut On]
Bridge Balance On/Off	[CC mode]
Bridge Balance Resistance	[CC mode, if Bridge Balance On]
Current Clamp Dynamic Hold On/Off	[CC mode]
Current Clamp Dyn. Hold Potential	[CC mode, if Dynamic Hold On]
Current Clamp Dynamic Hold Speed	[CC mode, if Dynamic Hold On]
CC Dynamic Hold On for Acquisition	[CC mode, if Dynamic Hold On]
Command Signal Name 1	
Command Signal Unit 1	
Command Full-scale Minimum 1	
Command Full-scale Maximum 1	
Command Sampling Rate 1	
Command Holding Enabled 1	
Command Holding Value 1	['0' if Holding disabled]
Auxiliary Output Signal Name 1	[if AuxOUT1 enabled]
Auxiliary Output Scaling Factor 1	[if AuxOUT1 enabled]
Auxiliary Output Offset 1	[if AuxOUT1 enabled]
Auxiliary Output Holding Value 1	['0' in demo mode]
Command Signal Name 2	[if StimOUT 1 & 2 enabled]
Command Signal Unit 2	[if StimOUT 1 & 2 enabled]

Command Full-scale Minimum 2	[if StimOUT 1 & 2 enabled]
Command Full-scale Maximum 2	[if StimOUT 1 & 2 enabled]
Command Sampling Rate 2	[if StimOUT 1 & 2 enabled]
Command Holding Enabled 2	[if StimOUT 1 & 2 enabled]
Command Holding Value 2	['0' if Holding disabled]
Auxiliary Output Signal Name 2	[if AuxOUT2 enabled]
Auxiliary Output Scaling Factor 2	[if AuxOUT2 enabled]
Auxiliary Output Offset 2	[if AuxOUT2 enabled]
Auxiliary Output Holding Value 2	['0' in demo mode]
Digital Holding Pattern (1 → N)	[1 – 8]

Stimulus

Key Stimulus	[if Solution activated]
Compound Group	[if Solution activated]
	[if Solution activated: for Control and Test Compounds]
Compound Group Index	
Compound Identifier	
Compound Name	
Compound Description	
Compound Concentration	
Compound Concentration Unit	
Compound Batch	
Compound Lot	
Compound Preparation Date	
Compound Preparation Time	
Compound Counterion	
Compound Source	
Compound Solution	
Compd. Vehicle / Solubility Enhancer	
Compound Vehicle Concentration	
Compound Vehicle Conc. Unit	
Compound Reservoir Identifier	

4.2.11 Paradigm Review

'Paradigm Review' displays data from all Series within the Paradigm in a modified reanalysis Scope window, in a Time Course or Concatenated view. This view also displays tags that occur between Series.

Access this window from the Data Navigator 'Available actions' menu 'Review Paradigm'.

The state of the Autoscale button (one-time vs. continuous) applies to all Paradigm Review and Routine Review windows.

To display the Series_Signal_Routine name of the selected data at the bottom of the window, click on the data.

To open a Series into a Reanalysis Scope window, right-click on the Series data and select Analyze <Series Name>' from the menu list.

For more information on the window controls, see the Reanalysis Scope section below.

4.2.12 Reanalysis Measurements & Graphs

The Reanalysis Scope window Measurements button 'Edit Measurements' provides access to the 'Reanalysis Measurements & Graphs' dialog. Use it to apply different analysis scenarios to recorded data. Settings changes for input channel measurements and analysis graphs override the loaded Routine for quick interactive control.

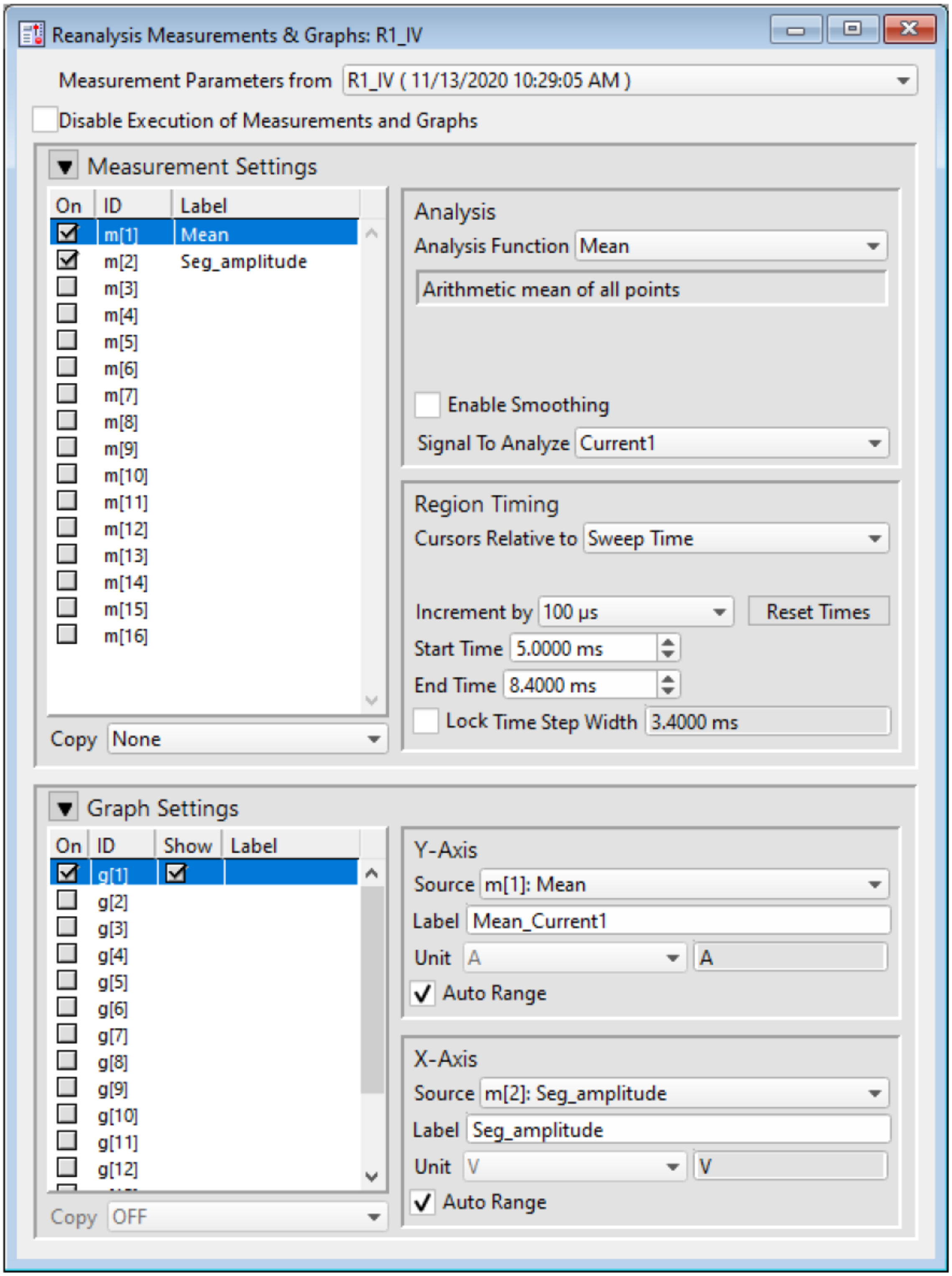


Figure 4-74. Reanalysis Measurements & Graphs

This dialog operates similarly to the Routine Editor: Real Time Measurements & Graphs dialog, with the addition of optional configurations:

Measurement Parameters from

The name of the Series data, and the date/time of the last update to the Measurement parameters.

The current parameters can be optionally overwritten (updated) from these sources:

- Overwrite Measurements from Original Routine
- Overwrite Measurements with Active Values

  The last run analysis values are remembered.
- Overwrite measurements from Saved Default

  [Available when previously saved in the 'Measurements' button drop-down list.]

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.

4.2.13 Reanalysis Scope

This analysis version of the Scope window is used to display and reanalyze stored data.

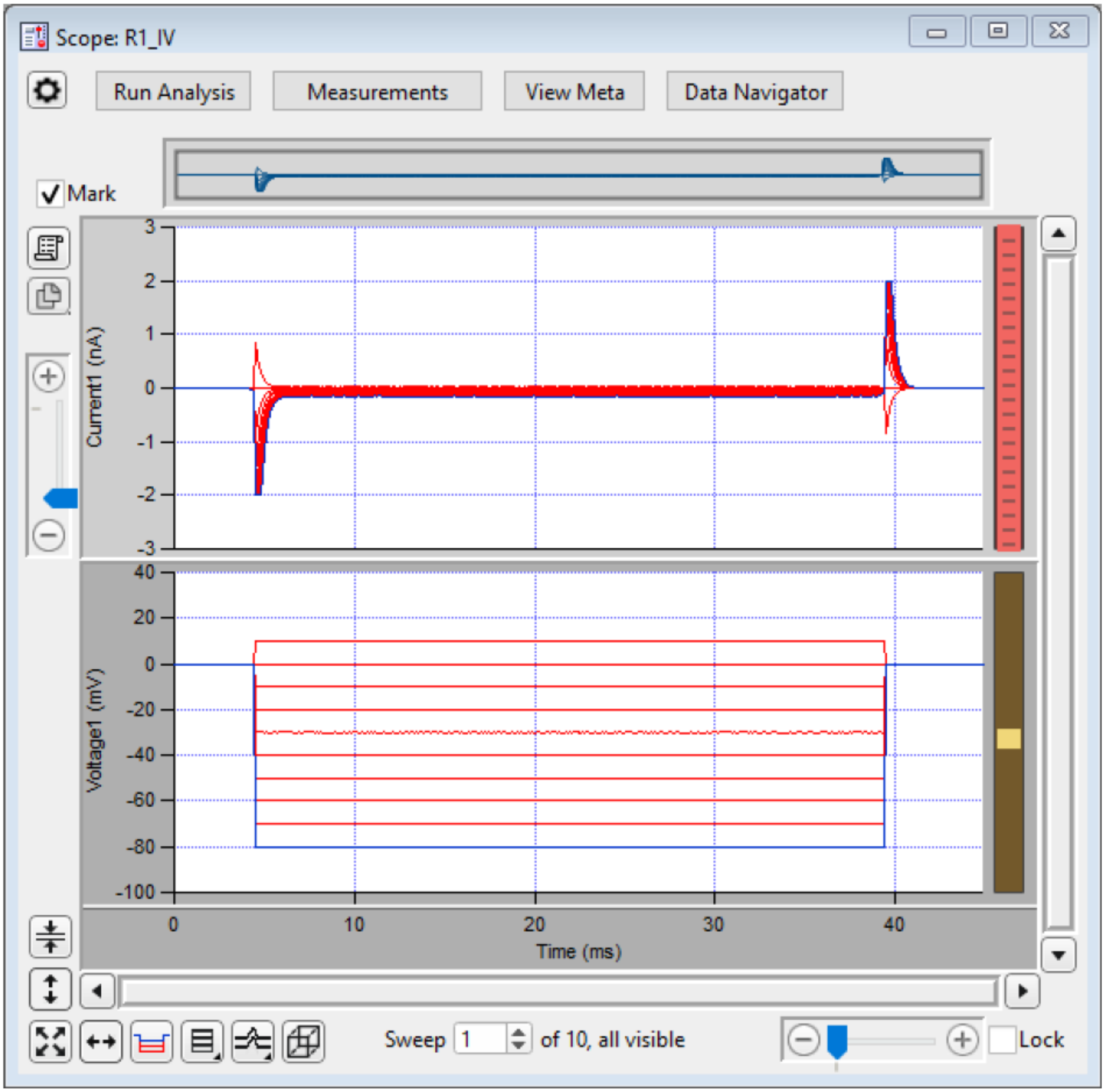


Figure 4-75. Reanalysis Scope Window

Both physical and virtual channels can be displayed and analyzed here.

Many window controls are the same as in the ‘Routine’ Scope window, however others have slight changes, and new controls were also added:

Navigation pane

The Navigation pane appears at the top of the Reanalysis Scope window. It displays an overview of the active signal's full-scale data, with a gray box surrounding the magnification area.

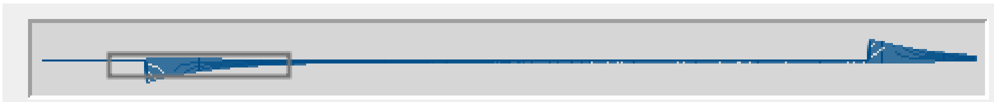
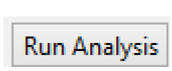
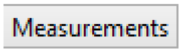


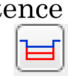
Figure 4-76. Navigation Pane

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.

Buttons

	Scope Settings: Show all sweeps — All sweeps are visible. Show marked sweeps Only marked sweeps are visible. The text "Showing Marked" displays above the Scope window 'Mark' checkbox. -- Set all marks in sweeps of active series Clear all marks in sweeps of active series Set all marks in sweeps of active series by equation (see below) Note: Analysis is only run on visible sweeps.
[ ] Mark	Enable/disable to "mark/unmark" the active sweep for display and/or analysis. The Data Navigator 'Available Actions' can process marked sweeps as a group. When the Scope Settings 'Show marked sweeps' is enabled, the text "Showing Marked" displays above the Scope window 'Mark' checkbox.
	Run the defined analysis for the displayed sweeps of the active 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.

	Show Cursors: Display measurement cursors in the Scope window. Hide Cursors: Do not display cursors in the Scope window. Button displays as “Measurements(H)”. Lock Cursors: Prevent cursors from being adjusted or moved. Button displays as “Measurements(L)”. No Measurements or Graphs Analyze with Active Measurements Analyze with Original Routine Measurements Analyze with Routine Last Executed Measurements Analyze with Saved Default Measurements Save as Default Measurements Edit Measurements: Open a special Reanalysis Measurements & Graphs dialog where all changes apply instantly to the measurements and the graphs, even during acquisition. These edits override the loaded routine for quick interactive control. Edit Virtual Signals: Open the virtual input signals panel for editing. Action Potential Analysis Analyze action potentials. Synaptic Event Analysis Analyze synaptic events (EPSPs, mEPSPs, etc.) Single Channel Analysis Analyze a single ion channel. Parametric Plot Plot the relationship between two signals. (see below) Amplitude Histogram Plot

	Plot an amplitude histogram. (see below) Color Plot Map the data to a color table. (see below)
View Meta	Display any extra information (metadata) associated with the displayed data Series, such as the operator, preparation details, solution information, etc., in a floating window.
Data Navigator	Open a Data Navigator window with all of your Experiment data and metadata available in a tree structure.
	Show and analyze [Previous / Next] Routine.
Y-Autoscale 	Click to autoscale the Y-axis of the selected signal to its visible sweeps data limits. To autoscale the Y-axes of all visible signals, in Windows Shift-click the button, or in macOS Control-click the button. To include the zero amplitude in the Y-ranges, enable “Include zero when autoscaling” in Set Preferences / Scope Window / General. Tip: To invert the Y-axis of the selected signal, such as for data with reversed polarity from an outside-out patch, right-click in the Y-axis of the signal and select Axis Properties / Axis Range. Either reverse the Manual Range Settings / Minimum and Maximum values, or disable the Manual Range and enable the Autoscale Settings / Reverse axis.
Autoscale 	Click to autoscale the Y-axes of all signals to their visible sweeps data limits, and to set the X-axis range to the maximum defined sweep duration for all signals. For persistent autoscaling, in “Windows” right-click (or Shift-click or Ctrl-click) the button, or in “macOS” Control-click the button (it turns dark gray). To include the zero amplitude in the Y-ranges, enable “Include zero when autoscaling” in Set Preferences / Scope Window / General.
Persistence Display 	Enable: Display all sweeps (per Marks and/or Scope Preferences). Disable: When disabled, only the active sweep is displayed. Applies to the Scope window, and its (right-click) graphs: Parametric Plot and Amplitude Histogram Plot.

Signal Layout 	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 data trace starts the first data point from time zero to the duration of the waveform. To view tags, switch to a ‘Time Course’ or ‘Concatenated’ display.
	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: a) The first data point is delayed by 10’s of ms after “time zero” due to Routine startup overhead time. b) 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 in time sequence on a single time axis. Portions without data, are replaced by a vertical line. Display sweeps in time sequence on a single time axis. Portions without data, are replaced by a vertical line. Note: a) The first data point is delayed by 10’s of ms after “time zero” due to Routine startup overhead time. b) Emulation mode has a minimum 0.5 s interval between sweeps, both triggered and continuous.
	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. (see below)	

Sweep #:	The ‘Sweep #’ display at the bottom of the Scope window indicates the ‘active sweep’ number, the total number of sweeps in the Series, and either “all” or the total number of visible sweeps (per Preferences).

Table 4-6. Reanalysis Scope Window Buttons

Tags are only shown in the Time Course and Concatenated display modes. They display as vertical blue lines at the tag time points in the data. Their associated text boxes are positioned in the top-most signal pane:

Event <#> Tag Comment

<Time stamp>

Comment: <text>

Set all marks in sweeps of active series by equation

Opens the ‘Sweep Mark: Equation Editor’ to enter an equation for the sweeps to mark.

Equation [ ]

Undo Remove all edits to the equation.

Check Equation Check the equation syntax. The equation is evaluated, and if valid, it reports "Syntax is ok.”

Insert special identifier

sweep
Odd(sweep)
Even(sweep)

Do Mark Evaluate the equation and update the sweep marking.

[Status message]

A value >= 0.1 = 1 (marked)

A value < 0.1 = 0 (unmarked)

Parametric Plot

Display a graph of X vs. Y input signals in a separate window.

If this window is left open when the Scope window is closed, it will also close; and re-opening the Scope window will also re-open the Parametric Plot window.

Y-signal — Select an input signal for the Y-axis.

X-signal — Select an input signal for the X-axis.

Plot — Update the plot using the new parameters.

 Copy to Layout — Copy the Parametric Plot graph into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard

Copy the Parametric Plot graph to the system clipboard, or if the 'Shift' key is pressed, the complete scope window.

Time Range — The time range of the data to be plotted.

- Full Trace — Use the entire trace for the time range.
- Sweep Time — Set relative to the start time of a sweep (as time zero).

  (Can also be set from the Scope window Marquee menu.)

  Start Time — Set the starting time.

  Once the Start Time is within 2 sample points of the End Time, further Start Time increments will increase the End Time by the same amount.

  End Time — Set the ending time.

  Once the End Time is within 2 ms of the Start Time, the End Time cannot be decremented.

- Segment Time — Set the time range as a ratio of the Segment duration.

Segment — Select the Segment number.

Start Ratio — [0 = beginning of Segment]
Set the starting time ratio.

End Ratio — [1 = end of Segment]
Set the ending time ratio.

Measurements can be made on the parametric graph using Igor Pro cursors (Ctrl-I). The cursor measurement is written to the SutterPatch Notebook window.

Amplitude Histogram Plot

Open a histogram plot window. The amplitude data are binned and plotted. The window is cleared at the start of a new Series.

If this window is left open when the Scope window is closed, it will also close; and re-opening the Scope window will also re-open the Amplitude Histogram Plot window.

Y-signal — Select the input signal to be analyzed.

Time Range — The time range of the data to be plotted.

- Full Trace — Use the entire trace for the time range.
- Sweep Time — Set relative to the start time of a sweep (as time zero).

  (Can also be set from the Scope window Marquee menu.)

  Start Time — Set the starting time.

  End Time — Set the ending time.

- Segment Time — Set the time range as a ratio relative to the Segment duration.

  Segment — Select the Segment number.

  Start Ratio — [0 = beginning of Segment]
  Set the starting time ratio.

  End Ratio — [1 = end of Segment]
  Set the ending time ratio.

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

Select the number of bins for the amplitude range (X-axis). Changes instantly update the plot.

 Copy to Layout Copy the Amplitude Histogram Plot graph into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard

Copy the Amplitude Histogram Plot graph to the system clipboard.

Plot Refresh the plot for any Time Range settings changes.

Cityscape Display the plot line using steps, no interpolation.

[graph pane]

Measurements can be made on the amplitude histogram graph using Igor Pro cursors (Ctrl-I). The cursor measurement is written to the SutterPatch Notebook window.

Color Plot Plot amplitude data in a false-color graph of Sweep vs. Time. This “heat map” display mode is commonly used in fast-scan cyclic voltammetry.

If this window is left open when the Scope window is closed, it will also close; and re-opening the Scope window will also re-open the Color Plot window.

Note: The data display for a sweep is centered on its Y-axis whole number tick mark (±0.5).

Signal List of available signals.

The color graph is based on the selected input signal name.

If no such signal name exists in the current Series, the color graph is blank.

<u>[range]</u> Select the Y-range to be used for a Plot.

- Auto Y Range Use an autoscaled Y-axis range for the data.
- Scope Y Axis min and max

  Use the existing Y-range for the data.

- Given Y min and max

Set the upper and lower Y-axis boundaries.

Color Table	List of color tables.
Reverse	Reverse the color lookup table.
Plot	Plot the selected signal as a false-color graph using the selected Y-range and color lookup table.

 Copy to Layout

Copy the Color Plot graph into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard

Copy the Color Plot graph to the system clipboard.

Measurements can be made on the color graph using Igor Pro cursors (Ctrl-I). The cursor measurement is written to the SutterPatch Notebook window. Once set, clicking and dragging a cursor will dynamically update its measurement value in the notebook.

Right-click Menus

X Axis

Autoscale All Axes	Scale all signals Y-axes to their data, and set the X-axis range for all signals to the maximum defined sweep duration.
Autoscale X Axis	Set the X-axis range for all signals to the maximum defined sweep duration.
Set X Scale…	Manually set the X-axis range.
X-min	The minimum X-axis value.
X-max	The maximum X-axis value.
Axis Properties…	Modify the axes style and components.

Y Axis

Autoscale All Axes	Autoscale all signals Y-axes to their data, and set the X-axis range for all signals to the maximum defined sweep duration.
Initial Autoscale Y Axis	Autoscale the signal's Y-axis based on the first 1% of the data.
Autoscale Y Axis	Autoscale the signal's Y-axis to its data.
Full scale Y Axis	Set the signal's Y-axis to its full-scale range.
Use Last Y Scale	Maintain the Y-axis scaling at its existing range, overriding any prior Y-axis scaling settings.
Set Y Scale…	Manually set the Y-axis range.
Y-min	The minimum Y-axis value.
Y-max	The maximum Y-axis value.
Copy Y scale of signal	[input signals list] Apply the Y scaling from another signal.
Axis Properties…	Modify the axis style and components.

To reverse the Y-axis polarity (such as for inside-out or cell-attached patches)…

Axis Range tab

Manual Range Settings

Minimum: Enable and enter a positive number.

Maximum: Enable and enter a negative number.

Or, if Y-axis autoscaling will be used:

Click the 'Uncheck Both' button, and…

Autoscale Settings

Reverse axis: Enable.

Hide Signal <name>	Hide the selected signal in the Scope window.

Show Signal <name> Only	Show the selected signal in the Scope window, hide all other signals.
Stack All Signals	Display all signals in a single column.

Main Window

Limited data modification menu

Right-click in the blank area in a signal pane.

Tip: If you click too close to the signal data, the full data modification menu displays instead; if this occurs, click near to a horizontal or vertical edge of the signal pane.

This context menu is the same as in the Acquisition: Routine Scope window (plus a couple additional items):

- Show All Sweeps (with triggered sweeps)
- Show Marked Sweeps (with triggered sweeps)

Autoscale All Axes	Scale all signals Y-axes to their data, and set the X-axis range for all signals to the maximum defined sweep duration.
Add Annotation	Add a floating text-box label to the signal pane. To edit or delete an annotation, double-click on it.
Export Graphics	Copy the signal and open in a separate window. Saves to Windows / Graph Macros.
Toggle Cursor Info	Show/Hide the Cursor Info pane to measure X-Y data points or set a fitting range. (See the 'Signal data' section below.)
Colors	Adjust the colors used by the active signal pane:

graph background	The background of the pane.
all axes	The X- and Y-axis areas.
all grids	The grid lines in the pane.
all tick labels	The tick labels in the X- and Y-axis areas.
all axis labels	The axis labels in the X- and Y-axis areas.

Hide Signal '<name>'	Hide the selected signal in the Scope window.
Show Signal '<name>' Only	Show the selected signal in the Scope window, and hide all other signals.
Show Last Sweep of '<name>' only	Display only the last [marked] sweep of the selected signal.
or	
Show All Sweeps of '<name>'	Restore the display of all [marked] sweeps in the selected signal.
Stack All Signals	Display all signals in a stacked signal layout.
Show All Sweeps	(with triggered sweeps)
Show Marked Sweeps	(with triggered sweeps)

Marquee

Click and drag the mouse to surround a region of interest, and right-click for a context menu:

Expand	Set the signal's Y-axis range from the marquee vertical data limits, and set all signals X-axes ranges from the marquee horizontal data limits.
Horiz Expand	Set all signals X-axes ranges from the marquee horizontal data limits.
Vert Expand	Set the signal's Y-axis range from the marquee vertical data limits.
Shrink	Move the signal's Y-axis current limits to the position of the marquee vertical data limits, and move all signals X-axes current limits to the position of the marquee horizontal data limits.
Horiz Shrink	Move all signals X-axes current limits to the position of the marquee horizontal data limits.
Vert Shrink	Move the signal's Y-axis current limits to the position of the marquee vertical data limits.
Extract Template	Copy the last sweep to the Template Editor.

Extract To Graph	Display the active trace in a floating window, using all data within the X- range.
Set Time Range of Amplitude Histogram	[only in Scope if an Amplitude Histogram is open]
Set Time Range of Analysis	[only in Scope if Single Channel Analysis is open]
Set Time Range of Parametric Plot	[only in Scope if a Parametric Plot is open]

Signal Data

Full data modification menu

Right-click on or near the data to display this context menu, which includes options to modify sweeps and data points, such as marker symbols and lines.

This menu is the same as in the Acquisition: Routine Scope window (plus a couple of other items):

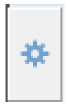
- Hide Sweep_# (with triggered sweeps)
- Show Sweep_# Only (with triggered sweeps)

Tip: To manually measure X-Y data values, or to set a fitting range, open a Cursor Info pane to use Igor Pro measurements.

- Toggle Cursor Info — Select 'Toggle Cursor Info' to show/hide the Cursor Info pane, and any cursor symbols in the active pane.

Note: For the display modes 'Time Course' and 'Concatenated', Igor Pro measurements are only correct within a single sweep. For measurements across multiple sweeps, use the 'Sweeps' display mode.

Cursor Info pane



Options menu

-  One Mover Moves All — Draggable cursor mover tool moves all cursors together with a single control.
- All Styles — Change the cursor symbol style.
- Show Cursor Pairs — Display up to 5 sets of cursor symbol pairs.

Cursor A

⊕	Cursor symbol for data point ‘A’.
A:	Symbol letter (beginning cursor of the pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.
pnt:	Data point number (starting from zero).
X:	X-axis value of data point ‘A’.
Y:	Y-axis value of data point ‘A’.
ΔY	Difference of the cursor pair Y values.

Cursor B

⊠	Cursor symbol for data point ‘B’.
B:	Symbol letter (ending cursor of the pair).
R1_A_IV	(Default) wave name.
	Draggable cursor mover tool for the cursor pair.
pnt:	Data point number (starting from zero).

X: X-axis value of data point 'B'.

Y: Y-axis value of data point 'B'.

ΔX Difference of the cursor pair X values.

Cursor Instructions

1. Click on symbol 'A' to enable it.
2. Manually drag the highlighted symbol onto a data point in the scope window, or enter the data point number in the 'pnt' field.
3. Click on symbol 'B' to enable it.
4. Manually drag the highlighted symbol onto a data point in the scope window, or enter the data point number in the 'pnt' field.

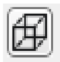
   X- and Y-measurements are displayed for the cursor pair data points.
5. Fitting can also be applied to the cursor pair data. Right-click on the data, and select 'Quick Fit' for a list of built-in Igor Pro fitting functions.

   The fit is displayed in the graph, and the fitting information is written to the Command window.

Channel Timing Delays

IPA amplifiers record both headstage stimulus and response signals via physical analog channels, so all recorded signals are precisely in sync, with no timing delays between them.

4.2.14 3D View Window



Show 3D View

The Reanalysis Scope 3D View window creates a 3D representation of your data, color-coded to show amplitude variations.

Note: If the Igor Pro/SutterPatch main window frame is not wider than the Reanalysis Scope window plus its Analysis sub-window, then clicking the 3D button will generate an error message, but the operation will still execute.

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.

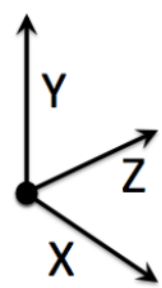


Figure 4-77. 3D Axes Definition

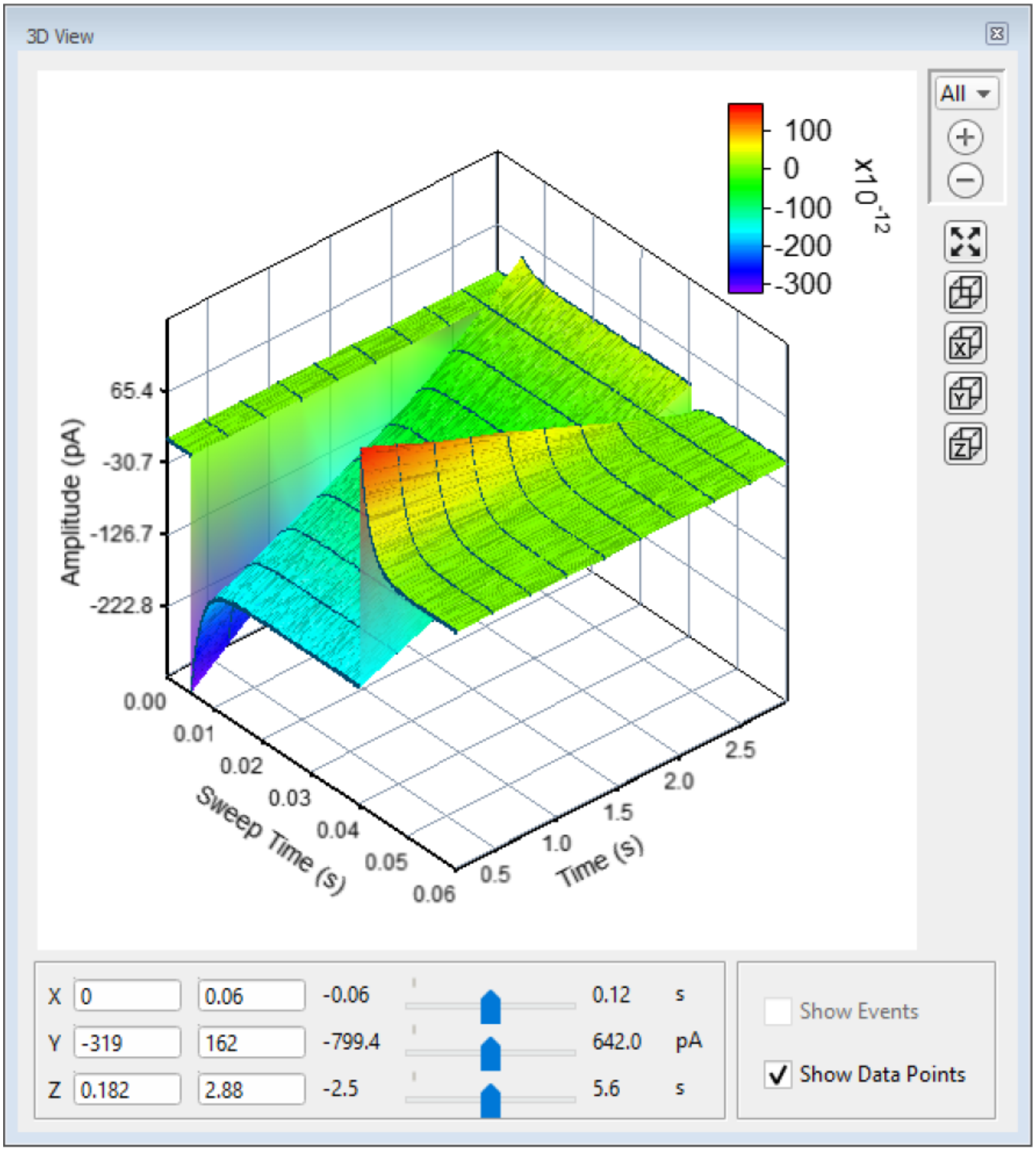


Figure 4-78. 3D View

A “heat map” bar illustrates the color measurement units.

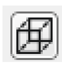
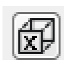
Magnification buttons are located in the upper right corner of the window for the selected axis:

- 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.

4.2.15 Routine Review

'Routine Review' displays the selected Series data in a modified Reanalysis Scope window, defaulted to the 'Time Course' display mode.

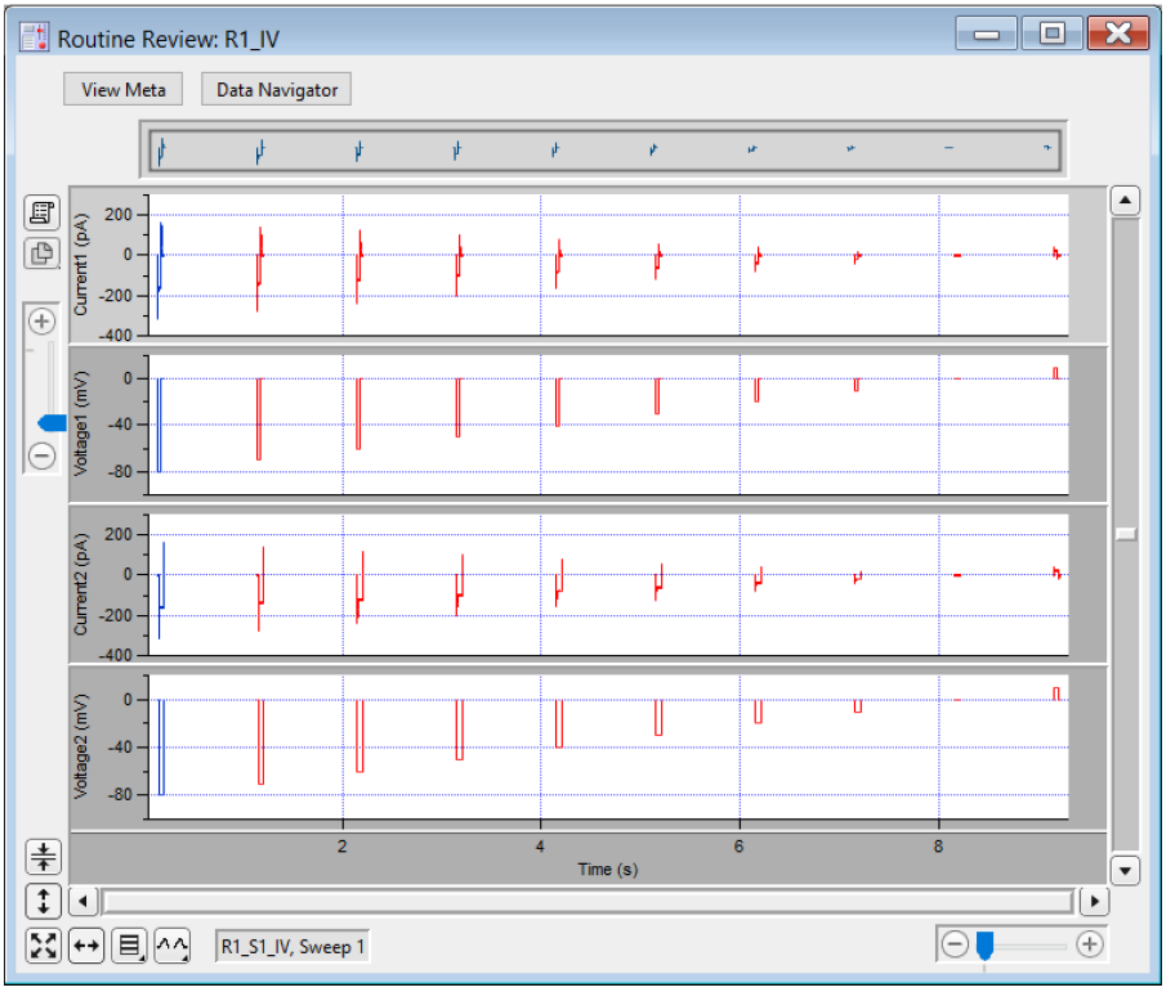


Figure 4-79. Routine Review

Open this window from the Data Navigator by highlighting a Series, and selecting the 'Review Routine' command from a right-click menu or the 'Available actions' button.

The first data point does not display at time = 0 in time course display mode, due to system overhead.

The state of the Autoscale button (one-time vs. continuous) applies to all Routine Review and Paradigm Review windows.

For information on the window controls, see the Reanalysis Scope section.

New Controls

- Click in a signal pane to display the name of the “Series_Signal_Routine”, or on the data to also include the “Sweep #” in a field at the bottom of the window.

- To reopen a Series in the Routine Review into a Reanalysis Scope window, right-click in a signal, and select ‘Analyze <Series Name>’ from the menu list.

4.2.16 Routine Settings

The Routine Settings window reports the same settings as would be seen in the Routine Editor / Routine Settings, however the preview pane does not support interactive dragging of measurement regions.

Open this window from the Data Navigator by highlighting a Series, and selecting the 'View Routine Settings' command from a right-click menu or the 'Available actions' button, or by selecting the 'Routine' name in the Data Navigator preview pane.

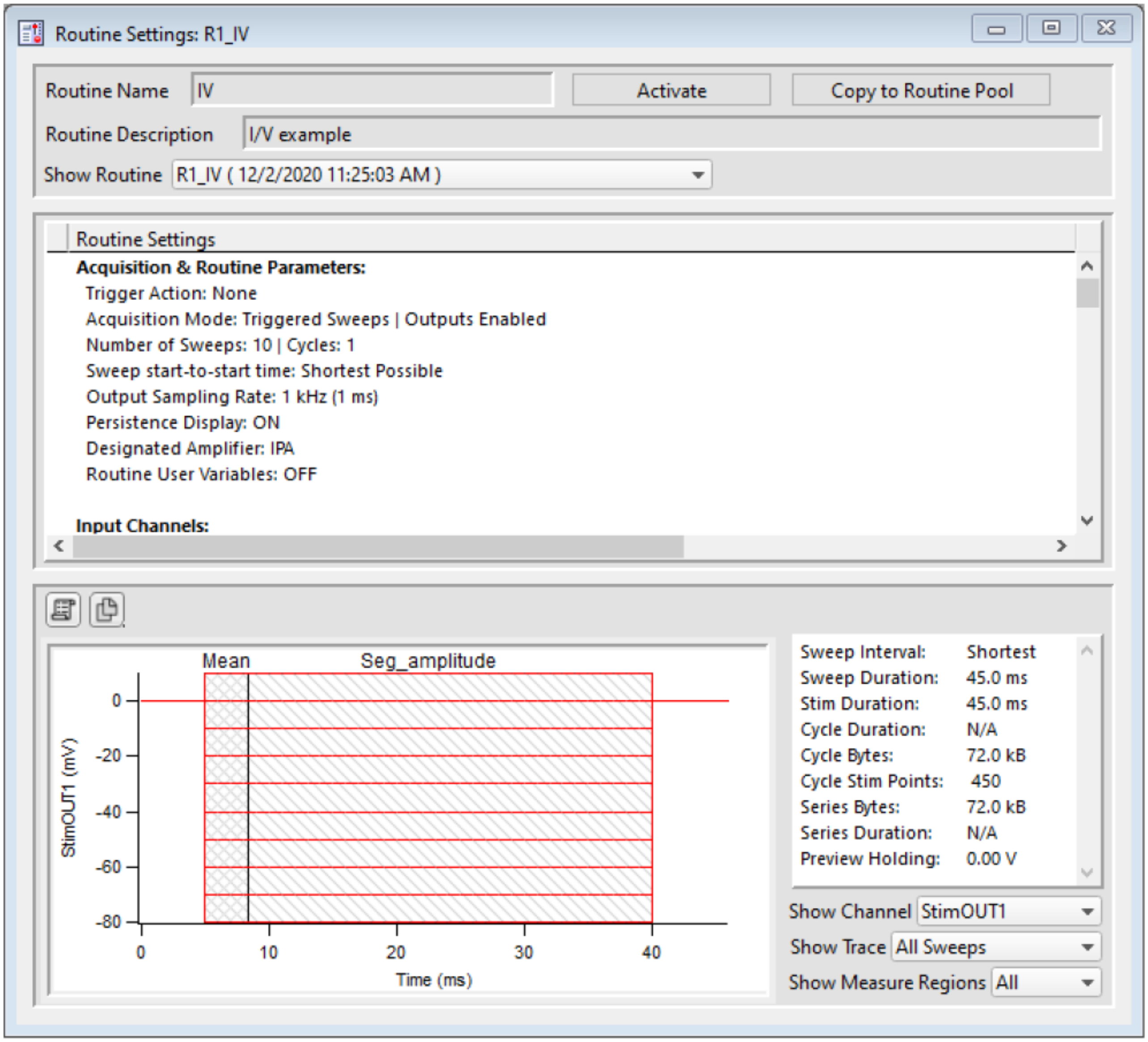


Figure 4-80. Routine Settings

Routine Name	Displays the Routine name.

Activate	Opens the Acquisition: Routine Scope window loaded with these settings.
Copy to Routine Pool	Adds this Routine to the loaded Routine Pool.
Routine Description	Displays the Routine description.
Show Routine Copy	
Routine Settings	Listing of all settings from all sections of the Routine.

Acquisition & Routine Parameters

Input Channels

Output Channels & Waveform

Real Time Measurements & Graphs

<u>Preview panel</u> Display of the stimulus waveforms.

Copy to Layout	Copy the visible stimulus waveforms into a new Layout window, or append to an existing Layout page.
Copy to Clipboard	Copy the visible stimulus waveforms to the system clipboard.

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

Show Channel	Select the output signals to display.
Show Trace	Select the output traces to display.
Show Measure Regions	Select the measurement regions to display.

4.2.17 Single Channel Analysis

SutterPatch: Available Analysis Modules: Single Channel Analysis

Perform analysis of low-noise currents from single ion channels.

Access single-channel analysis via:

- the Reanalysis Scope window 'Measurements' button, or
- the Data Navigator (signal) 'Available actions' menu, or
- the main menu SutterPatch > Available Analysis Modules > Single Channel Analysis.

Single Channel Analysis uses a special Scope window, where amplitude levels and transitions and are overlaid onto the raw data. When you click-and-drag in the scope window, the closest amplitude level is repositioned to the new amplitude. Because of this, to access the marquee tool in the Scope window, hold down the shift key when you click-and-drag the mouse.

Marquee Right-click Menu	Special addition to the menu.
Set Analysis Time Range	Sets the Single Channel Analysis 'Time Range' to 'Sweep Time', and the 'Start Time' and 'End Time' are set from the marquee range.

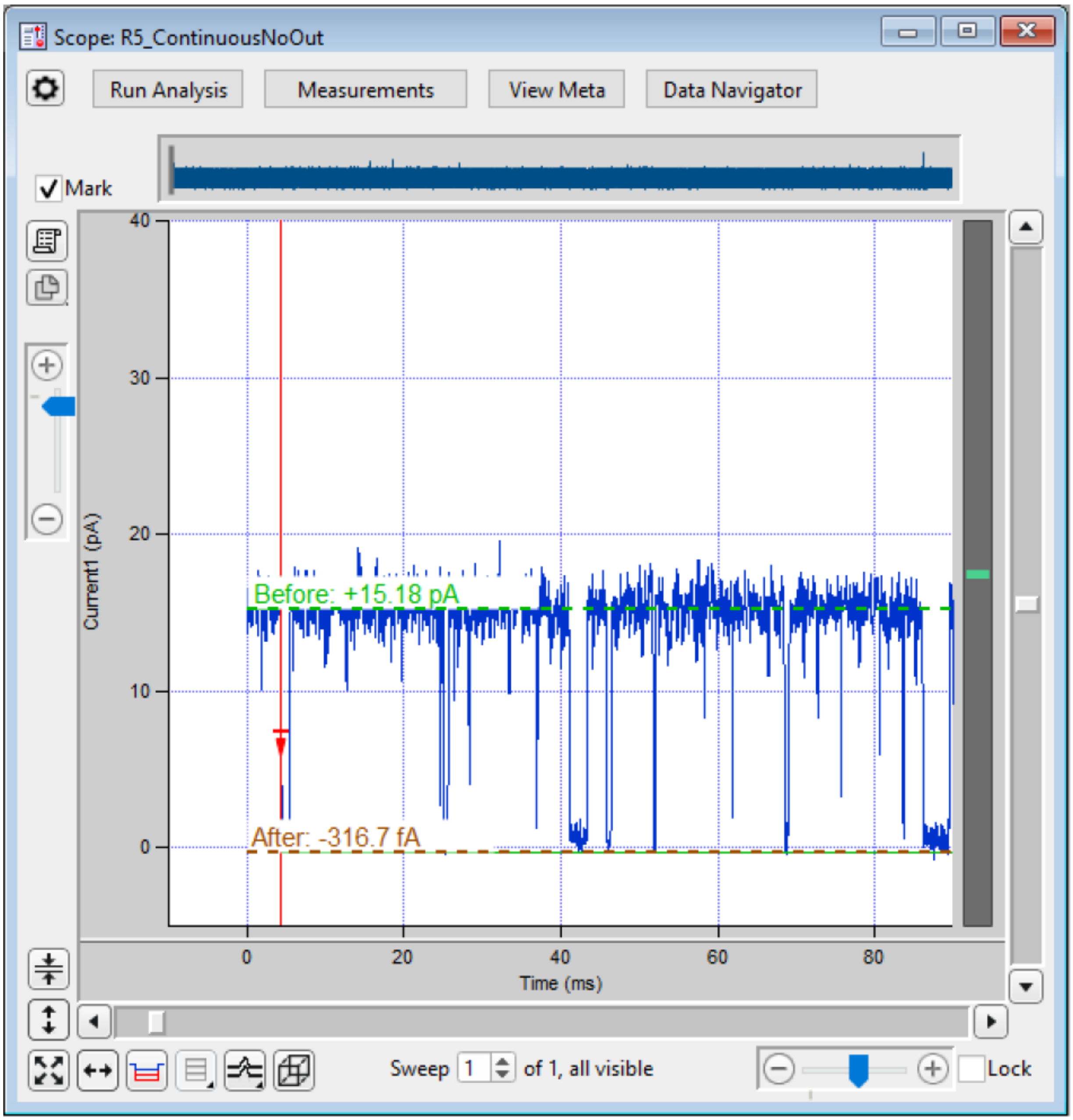


Figure 4-81. Single-Channel Scope

When single channel analysis is activated, a Single Channel Analysis control panel is opened, and the Reanalysis Scope window active signal is overlaid with the transition levels of the first single-channel opening or closing.

Note: Single-channel analysis only operates in the Scope 'Sweeps' display mode; the Concatenated and Time Course display modes are not supported.

Scope window levels

- A dashed **green** "Before" line displays the amplitude of the previous transition/event, i.e., the level before the transition point. Manually adjust by dragging with the mouse.
- A dashed **brown** "After" line displays the amplitude of the selected transition/event, i.e., the level after the transition point. Manually adjust by dragging with the mouse.
- A solid **green** line displays the idealized trace of the found transitions/events.

  At times, the dashed amplitude lines might superimpose onto the idealized trace.
- A vertical **red** line displays at the transition point between the two levels, with a red arrow indicating the direction of the transition.

If the initial levels are incorrect, a couple of basic settings need to be adjusted:

1. Determine the starting amplitude of the data before the first transition.

   Zoom in on the Scope data, so that the open and closed state amplitudes are well visualized. Or run the 'Plots and Tables' 'Current Amplitude Histogram' to find the amplitude peaks in the binned data.
2. Set the 'Current Transition Controls' estimated 'Amplitude' signed value for the first level in the data. (Use negative numbers for negative-going openings.)
3. Set the Start Level number for the initial data (0 = baseline state, 1+ = open states).
4. Click on the 'Find target transition' section 'Clear All' button.
5. The Scope window resets the "Before" and "After" transition levels to proper values.

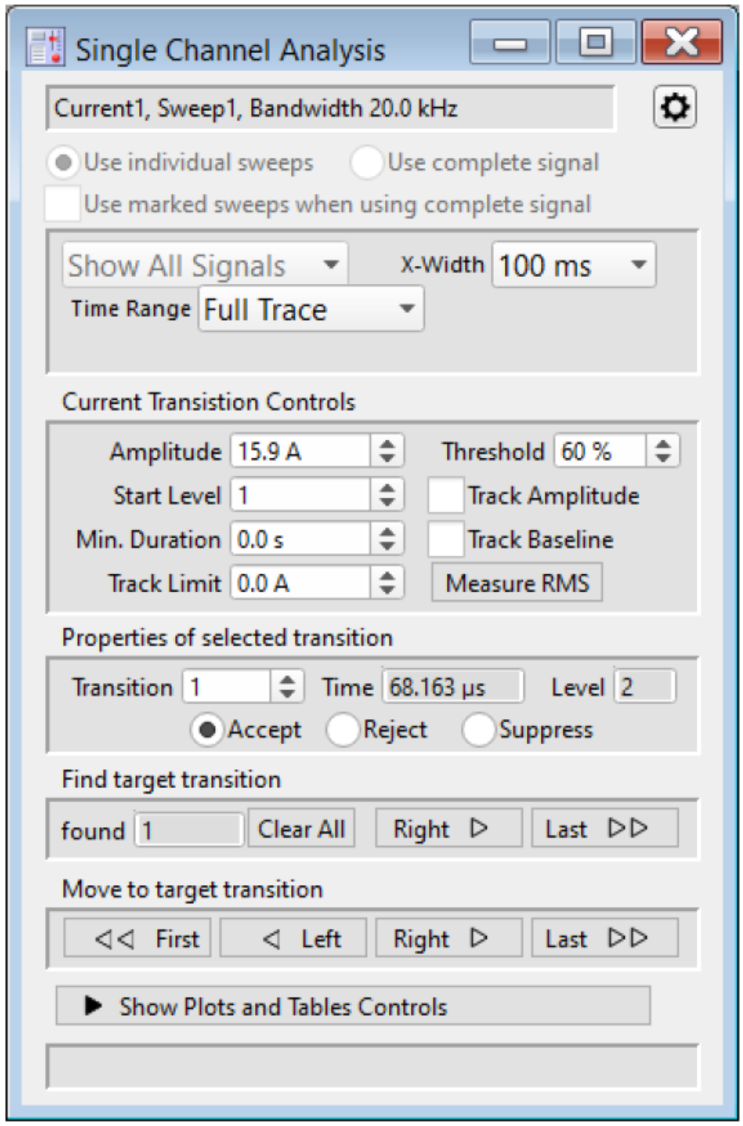


Figure 4-82. Single Channel Analysis

[Descriptive information]

 Single Channel Analysis Preferences

Dock to scope

Baseline Average Duration = x.x ms

Calculate the averaged baseline amplitude with the duration of the last baseline data (closed state) before the transition to an open state.

Mean Amplitude Duration = x.x ms

Calculate the mean amplitude using up to the duration of the open state data at that level, after a transition to that level.

Allow display compression

Signal Controls

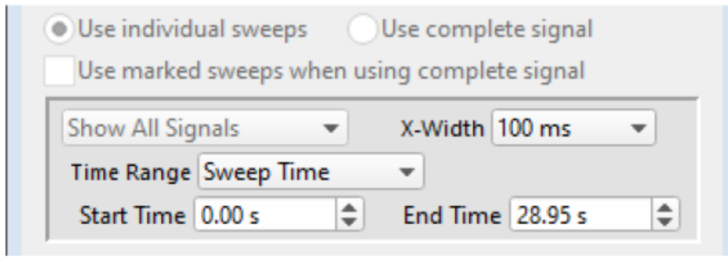


Figure 4-83. Signal Controls

Control the display of single channel data in the Scope window:

- Use individual sweeps — Perform analysis on a single sweep at a time.
- Use complete signal — For plotting - use found transitions from all sweeps in the active signal.

[ ] Use marked sweeps when using complete signal

Include transitions only from the marked sweeps in the active signal.

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 reset to this value whenever a 'Find' or 'Move' transition operation is performed.

Time Range Full Trace

Sweep Time	Start Time (s)	
	End Time (s)	
Segment Time	Start Ratio	Relative to the start of a Segment.
	End Ratio	Relative to the end of a Segment.

Current Transition Controls

Set up the basic level-detection parameters.

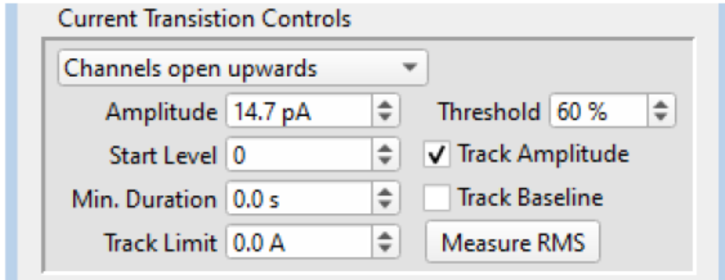


Figure 4-84. Current Transition Controls

Channels open upwards / Channels open downwards

Upwards opening channels only accept positive amplitudes.

Downwards opening channels only accept negative amplitudes.

Amplitude

Set to the expected transition size of the initial ion-channel level in the data.

For downwards-opening channels, use a negatively-signed value.

Start Level

The starting state of the ion-channel data:

Level 0	=	Closed state
Level 1	=	First open state
Level 2	=	Second open state
etc.		

If there are multiple levels in the data, the program will try to automatically detect them. However, overlapping channel openings are treated as a single combined level.

Min. Duration

[0.0 – ∞ s]

The minimum duration for a "found" transition.

The increment/decrement spinners use a step size of 100 µs.

Note: Displayed values are rounded up or down to one decimal point for the scaled unit of display. For example, for values greater than 1.0 s, the increment spinner does not update the displayed value until a rounding threshold is reached for the last digit, i.e., '1.5499' converts to '1.5', while '1.5500' converts to '1.6'.

Track Limit

[0 – 1.0 nA]

The maximum (absolute) amount that the Baseline level can change while being automatically tracked.

For baseline tracking, the value must be > 0. Set manually, or set to 3 * RMS via the 'Measure RMS' button.

Threshold

[50 – 90%]

The percentile of the Amplitude value (open state) that needs to be reached by the raw data to "find" a transition.

Track Amplitude

Store the amplitude of the measured event, instead of the theoretical (short) event, in the event transition table.

Track Baseline

Automatically adjust the baseline amplitude (Level 0) based on the prior data.

To use, the 'Track Limit' value must be > 0.

Measure RMS

To measure the RMS (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 channel 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.

<u>Properties of selected transition</u>

View or alter how a transition is processed by the analysis.

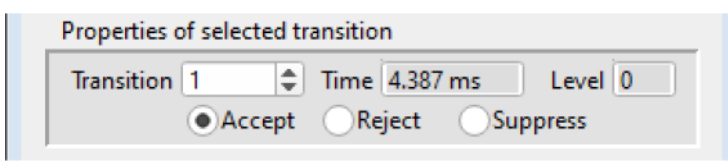


Figure 4-85. Properties of Selected Transition

Transition

The count number of the selected (active) transition.

Time

The start time of the selected transition.

Level

The level number of the selected transition.

Note: If other unexpected levels are detected "below the baseline", they are assigned a negative number. Negative levels might be detected from noise, biological artifacts, or an incorrect initial ‘Start Level’ setting.

Status — The operational status of the selected transition.

- Accept — Terminates the preceding event and starts a new open/close time. The selected transition is included in the idealized trace and all Plots.
- Reject — Terminates the preceding event and starts a new open/close time. However, the selected transition is considered inappropriate for analysis, and is excluded from the idealized trace and all Plots.

  Events that border a rejected transition are also excluded from histograms.
- Suppress — Does not terminate the preceding event or start a new open/close time. A suppressed event is considered as “not having happened”. The selected transition is excluded from the idealized trace and all Plots.

Find target transition

Find a transition based on the ‘Current Transition Controls’, and process the transition based on the ‘Properties of selected transition’.

An “event” is a valid transition that is followed by another valid transition.

Figure 4-86. Find Target Transition

found	The total number of found transitions (including rejected and suppressed transitions).
Clear All	Reanalyze the data – reset the number of found transitions to zero, and move to the first found transition.
Right >	Find, move to, and process the next transition.
Last >>	Find and process all subsequent transitions, and move to the last transition.

Note: Multiple open levels are handled in a simplistic fashion. It is assumed that there is only one channel open, and that it is open for the same state throughout the duration of the Event.

Example:

Level 1 openings: The Event duration is from the transition to the Level 1 amplitude, to the next transition to a different Level amplitude.

Level 2 openings: The Event duration is from the transition to the Level 2 amplitude, to the next transition to a different Level amplitude.

Etc.

Move to target transition

Among the 'found' (processed) transitions, move to an adjacent transition, or jump to the beginning or ending transition.

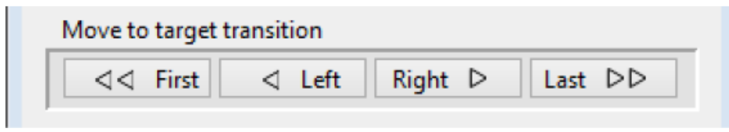


Figure 4-87. Move to Target Transition

<< 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.

Show/Hide Plots and Tables Controls

This button opens/closes the 'Tables and Plots' dialog, docked on the right of this dialog.

[ ] Total events in… Status bar for the number of events in a particular Plot.

Single Channels: Plots and Tables

Note: All plots and histograms support measuring X-Y data points or setting a fitting range via the 'Toggle Cursor Info' right-click menu entry (or Ctrl-I). (See 'Right-Click Menus' for Scope windows.)

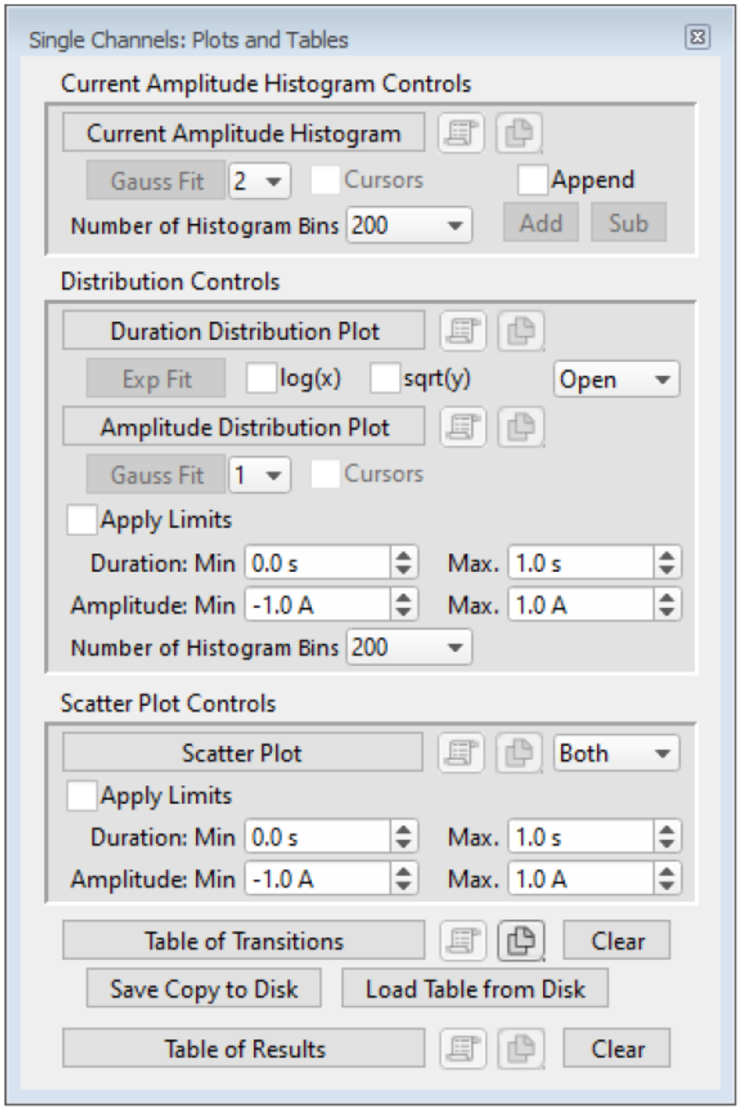


Figure 4-88. Plots and Tables Controls

<u>Current Amplitude Histogram Controls</u>

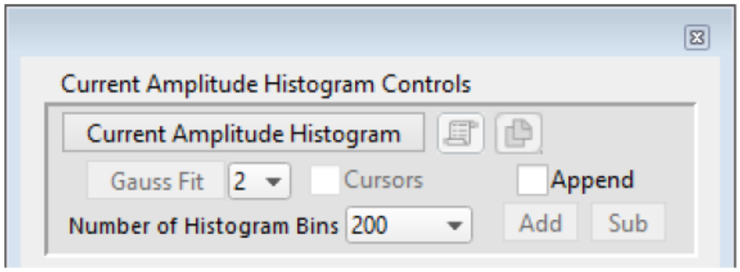


Figure 4-89. Current Amplitude Histogram Controls

A current amplitude histogram is often the first analysis performed on an uncharacterized channel, whereby all data points are binned by amplitude. 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 to create a histogram plot of the raw data. No prior settings are needed to run this.
Copy to Layout Page	Copy the ‘Current Amplitude Histogram’ into a new Layout window, or append to an existing Layout page.
Copy to Clipboard	Copy the ‘Current Amplitude Histogram’ to the system clipboard. With the ‘Shift’ key pressed: as a graph, otherwise as a table.
Gauss Fit	Click button to perform a Gaussian fit on the histogram. SutterPatch will automatically find and fit up to the three largest peaks. You can the fitting ranges as needed with manual cursors. To fit additional smaller distributions, reposition the cursors and click the Append button. See the ‘Table of Results’ for the fitting components.
[1, 2, 3]	Select the number of peaks (levels) to fit.

When more than one peak is selected, the graph reports amplitudes (in relation to the closed state peak) and P(open) and P(closed) values.

Cursors — Display fitting cursors in the plot window.

To reposition cursors, first create the histogram, and then enable 'Cursors' – this will open a Cursor Bar at the bottom of the plot window. Next, drag the cursor's symbol (labeled "A", "B", etc.) from the cursor bar to the new position (near to the X-axis) on the plot.

Append — Modify the 'Current Amplitude Histogram'.

Add — Add the current data to the existing 'Current Amplitude Histogram'.

Sub — Subtract the current data from the existing 'Current Amplitude Histogram'.

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

<u>Distribution Controls</u>

Create histogram plots of the found events.

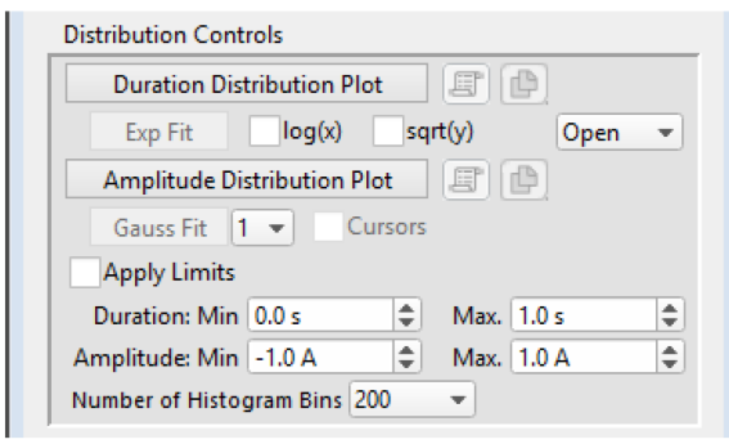


Figure 4-90. Distribution Controls

Duration Distribution Plot — Click to create a duration (dwell-time) histogram plot of the found Events. The histogram bin count is reported as 'Relative Frequency' (to 1.0) on the plot's Y-axis.

 Copy to Layout — Copy the 'Duration Distribution Plot' into a new Layout window, or append to an existing Layout page.

Copy to Clipboard — Copy the 'Duration Distribution Plot' to the system clipboard.

Exp Fit — Apply an exponential fit to the data.

log(x) — Set the X-axis to a log scale.

sqrt(y) — Use the square-root of the Y-axis data.

Open / Closed — Select open or closed state data for the Distribution plots.

Amplitude Distribution Plot — Click to create an amplitude histogram plot of the selected state's found Events. The histogram bin count is reported as 'Frequency' on the plot's Y-axis.

The Amplitude Distribution Plot bins "transition deltas", which measures the *directional change* in amplitude for each transition (not the raw amplitude).

For example, an opening transition to 15 pA bins on the X-axis at 15 pA, while a following closing transition back to 0 pA bins on the X-axis at -15 pA, i.e., the delta of the transition's Before and After amplitudes.

The histogram bins plot as colored lines:

Open = **red**

Closed= **blue**

Copy to Layout — Copy the 'Amplitude Distribution Plot' into a new Layout window, or append to an existing Layout page.

Copy to Clipboard — Copy the 'Amplitude Distribution Plot' to the system clipboard.

Gauss Fit — [1, 2, 3] — Select the number of components in the fit.

Cursors — When an Amplitude Distribution Plot exists, you can enable draggable fitting cursors in the graph.

To position a cursor, drag its cursor symbol (labeled "A", "B", etc.) from the cursor bar onto the data at the desired position.

Apply Limits — Apply data limits to the events used in distribution plots.

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]

<u>Scatter Plot Controls</u>

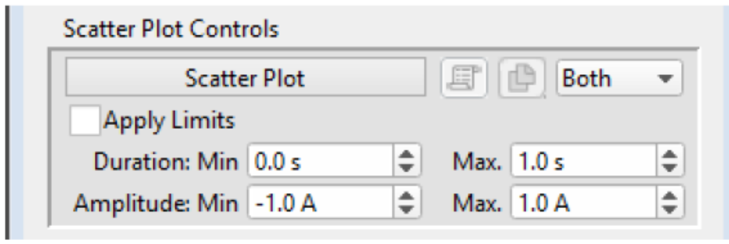


Figure 4-91. Scatter Plot Controls

The scatter plot uses "transition deltas" for event amplitudes, which plot on the Y-axis as the *directional change* in amplitude for each transition; the X-axis plots the duration of the event.

For example, an opening transition to 15 pA will plot on the Y-axis at 15 pA, while a following closing transition back to 0 pA plots on the Y-axis at -15 pA, i.e., the delta of the transition's Before and After amplitudes.

Events are plotted as colored symbols:

Open = **red**

Closed= **blue**

Selected event (transition) = **green**

[Scatter Plot] Click to create a scatter plot of the selected state's found events.

 Copy to Layout — Copy the 'Scatter Plot' into a new Layout window, or append to an existing Layout page.

 Copy to Clipboard — Copy the 'Scatter Plot' to the system clipboard.

Open / Closed / Both — Select which states are plotted.

Apply Limits — Apply data limits to the events used in scatter plots.

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]

Table Controls

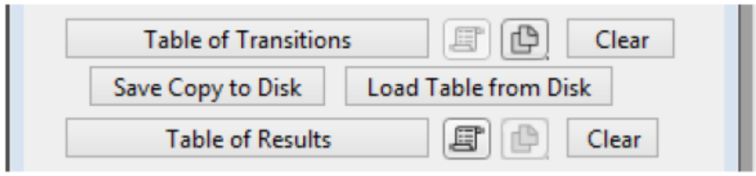


Figure 4-92. Table Controls

Table of Transitions — Click for a listing of all transitions.

Layout of the table

Top Row:	[Cell address | Cell value]
Column Number Row:	Column numbers. [0, 1, 2, …]
Row 0: Status	The operational status of the selected transition.
	1 = Accepted — Terminates the preceding event and starts a new open/close time. The selected transition is included in the idealized trace and all Plots.
	2 = Rejected — Terminates the preceding event and starts a new open/close time. However, the selected transition is considered inappropriate for analysis, and is excluded from the idealized trace and all Plots.
	3 = Suppressed — Does not terminate the preceding event or start a new open/close time. A suppressed event is considered as “not having happened”. The selected transition is excluded from the idealized trace and all Plots.
Row 1: Time	Time of the start of the transition, i.e., the transition point.
Row 2: Level	The open or closed state level number.
Row 3: Amplitude_Before	Amplitude of the level preceding the transition, i.e., the level before the transition point.
Row 4: Amplitude_After	Amplitude of the transition, i.e., the level after the transition point.

Row 5:	Duration	Duration of the transition. Note: The last column of transition data is preset to a zero duration.
Row 6:	Amplitude	Amplitude of the transition.
Row 7:	Amplitude_Valid	Include / Exclude the transition for processing 0 = Invalid A valid transition. 1 = Valid Not a valid transition. Note: The very first column of transition data is always defined to be 'Invalid', and is excluded from processing. Also, the last two columns of transition data are excluded from Plots.

Copy to Layout — Copy the 'Table of Transitions' into a new Layout window, or append to an existing Layout page.

Copy to Clipboard — Copy the 'Table of Transitions' to the system clipboard.

Clear — Clear all transitions from the table, and reset to the first found transition.

Save Copy to Disk — Save the 'Table of Transitions' to an Igor Pro wave (*.ibw) file.

Load Table from Disk — Load the 'Table of Transitions' from an Igor Pro wave (*.ibw) file.

Table of Results — Click for a listing of all fitting results.

Copy to Layout — Copy the 'Table of Results' into a new Layout window, or append to an existing Layout page.

Copy to Clipboard — Copy the 'Table of Results' to the system clipboard.

Clear — Clear all entries from the table, and reset to the first found transition.

Table Column Labels

Gaussian Fit

Header Row	Row 0
Source	Sweep_#
Analysis	Current Amplitude Histogram Fit, or Amplitude Distribution Fit
Label1	Amplitude
Value1	#
Label2	p(closed)
Value2	#
Label3	p(open)
Value3	#
Label4	Gauss_y0_1
Value4	#
Label5	Gauss_A_1
Value5	#
Label6	Gauss_x0_1
Value6	#
Label7	Gauss_width_1
Value7	#
Label8	Success_1
Value8	#
Label9	Gauss_y0_2
Value9	#
Label10	Gauss_A_2
Value10	#
Label11	Gauss_x0_2
Value11	#
Label12	Gauss_width_2
Value12	#
Label13	Success_2
Value13	#
Label14	Gauss_y0_3
Value14	#
Label15	Gauss_A_3

Value15	#
Label16	Gauss_x0_3
Value16	#
Label17	Gauss_width_3
Value17	#
Label18	Success_3
Value18	#

Linear Exponential Fit

Header Row	Row 0
Source	Sweep_#
Analysis	Duration Distribution Fit
Label1	Exp_y0
Value1	#
Label2	Exp_A
Value2	#
Label3	Exp_Tau
Value3	#
Label4	Exp_0
Value4	#
Label5	Success
Value5	#

Logarithmic Exponential Fit

Header Row	Row 0
Source	Sweep_#
Analysis	Duration Distribution Fit
Source	Sweep_#
Analysis	Duration Distribution Fit
Label1	LogNormal_k0
Value1	#
Label2	LogNormal_k1
Value2	#
Label3	LogNormal_k2
Value3	#

Label4	LogNormal_k3
Value4	#
Label5	Success
Value5	#

4.2.18 Synaptic Event Analysis

SutterPatch: Available Analysis Modules: Synaptic Event Analysis

Post-synaptic potentials and currents from excitatory and inhibitory events (EPSPs, EPSCs, IPSPs, IPSCs) are analyzed with this application module. Access via the Reanalysis Scope window 'Measurements' button or the Data Navigator (signal) 'Available actions' menu.

Spontaneous miniature events (mEPSPs, etc.), which generate small and often overlapping events, are detected with an innovative deconvolution algorithm. This technique finds Events with high temporal fidelity, while also improving the signal-to-noise ratio (SNR).

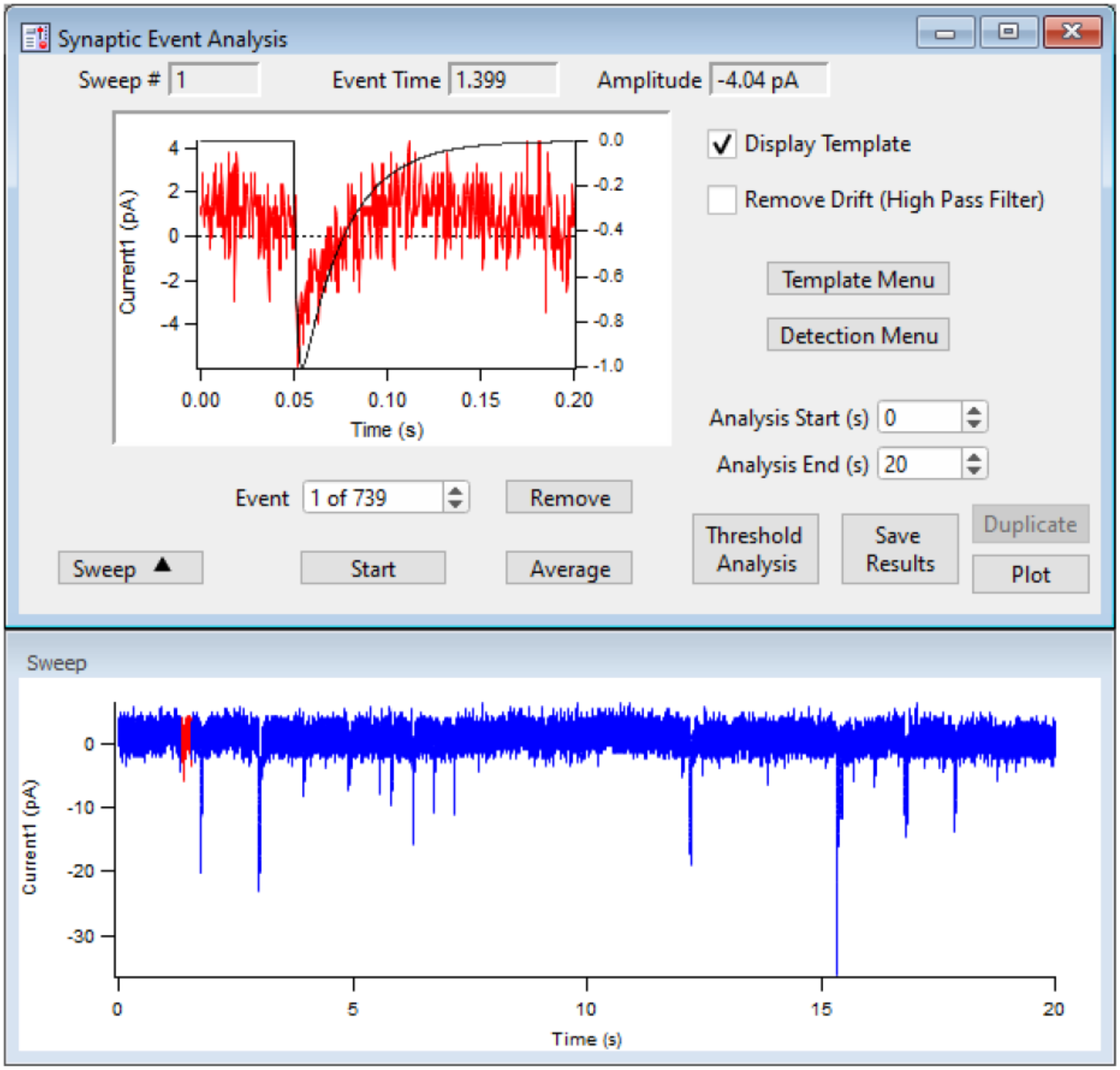


Figure 4-93. Synaptic Event Analysis

Sweep #	The sweep number of the displayed data.
	The Sweep # is set to '0' when the Average event is displayed in the Event pane (or when the template is initially created prior to analysis.)
	Select arbitrary sweeps for processing by "marking" them in the Data Navigator tree, or in a Scope window during acquisition or reanalysis.
Event Time	The time(s) of the event threshold crossing relative to the start of the sweep.
Amplitude	The amplitude averaged around the peak by ±1 ms.
[Event pane]	A graph of the selected event overlaid by the template, with the X-axis zero point reset to the template starting point.

To measure X-Y data points or set a fitting range, select 'Toggle Cursor Info' from the right-click menu. (See 'Right-Click Menus' for Scope windows.)

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.

Remove Drift (High Pass Filter)

A 1 Hz high-pass filter is applied to the signal to remove baseline drift.

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, -1]	1 = positive -1 = negative
Rise Time (µs)	[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 to create a custom event template.	
Use Average	Click to use the event Average as the event template.	
Realign average	Allow realignment of the 'Use average' template to keep the analysis from drifting.	

Detection Menu — Open the Levels sub-panel to configure detection levels.

Threshold (xSD) — A detection threshold represents the "Event Strength". A lower ("weaker") number finds more events, while a higher ("stronger") number finds less events. Adjust this threshold based on empirical testing of your data.

[0.1 – 10]	Lower # =	more events	(false-positives)
	Higher # =	less 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	[5e-13 – 1e-6] Set an amplitude threshold for the minimum size of events.
	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 pane. A right-click menu is available, as well as the click-and-drag marquee for magnification and extraction
[Sweep pane]	Displays a sweep of data colored in blue, with the selected event colored in red. To measure X-Y data points or set a fitting range, select 'Toggle Cursor Info' from the right-click menu. (See 'Right-Click Menus' for Scope windows.) To resize or extract data, click and drag a box around the data with the mouse marquee tool, and right-click for the marquee menu. The special menu option 'Add Mini' allows you to manually classify a raw data selection as an event during manual detection, or to include an event missed by the template detection. The new event is highlighted in red, and included in new Results tables. Note: Manually detected events do not have an 'Event Strength' entry in the Results table, as an algorithm was not used to detect them.
Start	Click to find and analyze synaptic events.
Average	Click to display the averaged event in the Event pane. The Sweep # is set to '0'.
Threshold Analysis	A scatter plot of the Event Strength vs. Current is displayed in a sub-panel. To measure X-Y data points or set a fitting range, select 'Toggle Cursor Info' from the right-click menu. (See 'Right-Click Menus' for Scope windows.)

Save Results — Results are displayed in a Layout page and a table.

Synaptic Event Analysis Results

Results are displayed in their own Layout window, accessible via Windows / Layouts.

Signal Pathname: The path from the Igor Pro internal root directory is displayed (see Data Browser.)

Total time analyzed = (s)

Includes the Start/End times for all sweeps.

Number of events detected =

Total number of Events found.

Event Frequency = (Hz)

Average Event Amplitude = (pA)

±1 ms peak average.

Standard Deviation of Event Amplitude = (pA)

Graphs: Cumulative probability vs. Amplitude.

Amplitude (Average) vs. Time.

Frequency vs. Sweep Number.

Amplitude vs. Sweep Number.

Note: The individual graphs are also accessible via Windows / Graphs.

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 the 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)	A continuous time scale from the start of acquisition, i.e., from the clicking of

the 'Start' button, prior to the initial Sweep/Series external trigger.

Event Inter Event Interval (s)

Duplicate	Display duplicate copies of the Results table and layout.
Plot	The 'Plot sweeps' dialog displays to allow event sweeps to be plotted overlapping in a floating graph window.
Plot Sweeps	Enter a list of events separated by a comma "," and/or a range of events separated by a dash "-".

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

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

Window: Command Window

This window is an Igor Pro code interpreter, providing programmatic interaction with SutterPatch. You can manually execute Igor Pro and user-defined assignments, functions and operations in this window.

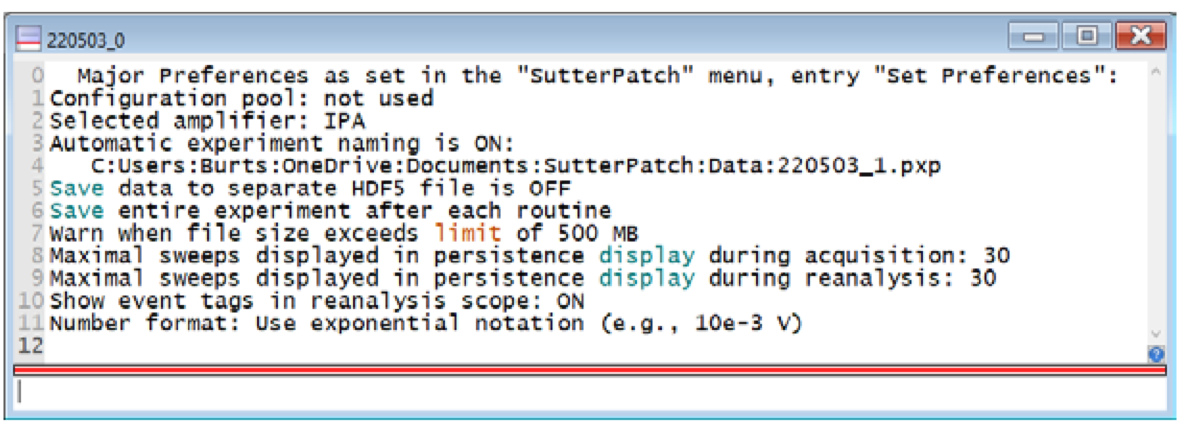


Figure 4-94. Command Window

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.

At program startup, some of the SutterPatch major preferences are written to the history:

Configuration pool: [filename]

Selected amplifier: dPatch

Automatic experiment naming is ON/OFF

[ON file path]

Save data to separate HDF5 file is ON/OFF

Save entire experiment after each routine

Warn when file size exceeds limit of # MB

Maximal sweeps displayed in persistence display during acquisition: #

Maximal sweeps displayed in persistence display during reanalysis: #

Show event tags in reanalysis scope: ON/OFF

Number format: (for table export)

The lower section is a command buffer with a "command line", where commands to be executed are entered. Commands can be entered into the command buffer in multiple ways:

- Manually type (or copy and paste) a line of text into the "command line" in the lower section of the window.
- Highlight lines in the history section and press the Enter key to copy them into the command buffer in the lower section of the window. To select the entire history, use 'CTRL-A'.
- Use the Paradigm Editor Execute step buttons 'Copy to Command Line' or 'Expand to Command Line' (for vars) to transfer the step command to the command line.

Commands in the command buffer are processed 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 Pro 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

SutterPatch: Dashboard

The Dashboard panel provides a convenient gateway to key areas of the SutterPatch program, or to the entire SutterPatch menu.

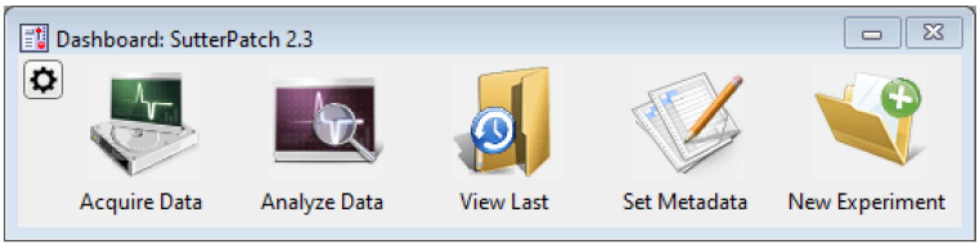


Figure 4-95. Dashboard

Note: To reposition the Dashboard to its default location (on upper-left of screen), Shift-click when selecting the 'Dashboard' menu item.

 Settings — Dashboard Settings menu.

Large — Display key areas as large icons in a Dashboard pane.

Vertical — When the Dashboard 'Acquire Data' sub-pane is open, dock it below the main Dashboard pane.

Horizontal — When the Dashboard 'Acquire Data' sub-pane is open, dock it on the right-side of the main Dashboard pane.

Small — Display the SutterPatch menu as small icons in a floating toolbar.

Vertical — Align the toolbar vertically, and dock it to the upper right corner of the computer screen.

Horizontal — Align the toolbar horizontally, and move it to the left-side of the computer screen.

Show Defaults — Amplifier Control Panel

Membrane Test

Free Run (Scope)

Paradigm Editor

Routine Editor

Data Navigator

Set Metadata

View Last

New Experiment / New HDF5 File

Show All Entries

Hide All Entries

Large Icons

 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. All sweeps (marked and unmarked) are visible in the initial display.

 Set Metadata

Configure metadata settings and values.

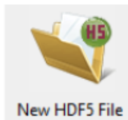
 New Experiment

Start a new Experiment, and/or switch the amplifier model or emulation mode.

Note: During the shutdown of the existing

Experiment, it is highly recommended to say “Yes” to save changes, even if no changes were made. This is used by an internal Igor Pro cleanup process to ensure proper file reopening.

or (set option in SutterPatch / Set Preferences / Files and Naming)



New HDF5 File

Start a new HDF5 file for the Experiment.

Clicking the Acquire Data icon opens an adjoining secondary pane:

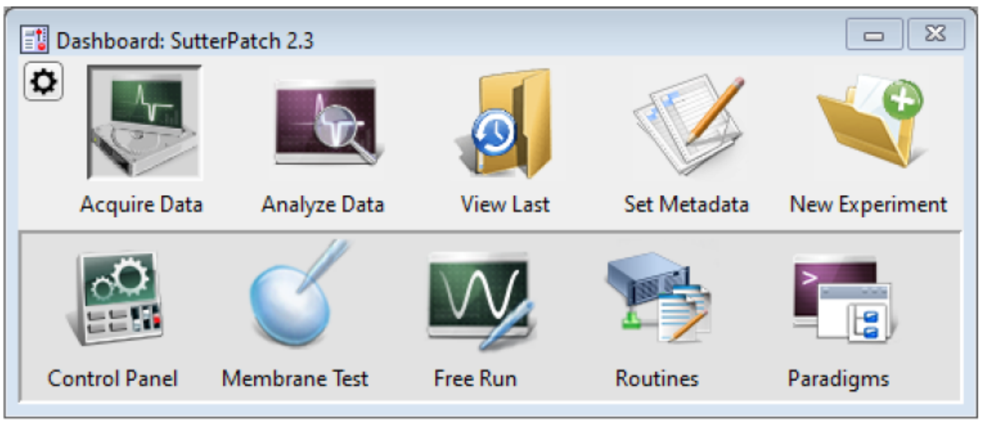
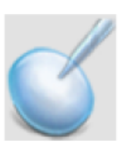


Figure 4-96. Dashboard – Acquisition

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.

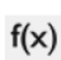
 Paradigms

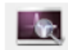
Control the execution of commands.

Small Icons

Display the SutterPatch dashboard as small icons in a floating toolbar.

Select the icons to display in the toolbar:

Icon	Icon Name	Shortcut Key
	Acquisition Control	Ctrl-0
	Scope Window	Ctrl-2
	Amplifier Control Panel	Ctrl-3
	Membrane Test	Ctrl-4
	Free Run (Scope)	Ctrl-5
	Paradigm Editor	Ctrl-6
	Routine Editor	Ctrl-7
	Template Editor	
	Equation Editor	
	Solution Editor	

	Camera Control	
	Data Navigator	Ctrl-8
	Analysis Editor	
	Layout Page	
	Set Metadata	Ctrl-9
	Set Preferences	
	Notebook	
	Shortcut Editor	
	View Last	FN-F2
	New Experiment / New HDF5 File	

4.3.3 Documentation

Help

Help: Help Topics

Full online Help for the SutterPatch software and all models of Sutter Amplifier Systems is available via Igor Pro's 'Help Browser'.

In the browser's 'Help Topics' tab:

1. Select a Help file starting with "SP_".
2. Select a Topic to display its Subtopics.
3. Select and click 'Show Selected Topic'.

Manual

The SutterPatch manual is customized to your IPA amplifier, and installed as a PDF file in the following folders:

Windows:	C:\Program Files\SutterPatch2\SutterPatch\Documentation\
macOS:	Applications/SutterPatch2/SutterPatch/

To display a PDF Table of Contents with links:

Windows:	In the PDF document, click the 'Contents' button on the left side of the Navigation Toolbar, and select the 'List' button.
macOS:	In the PDF document, click the 'Contents' button on the left side of the Navigation Toolbar, or the 'Page Thumbnails' button on the left of the sidebar, and select 'Table of Contents'.

QuickStart Guide

A printed "quick" installation guide for your Sutter hardware and software.

Important! Contains your Igor Pro 9 Serial Number and Registration Key.

Release Notes

A list of new feature and bug fix highlights for the SutterPatch software is posted on the SutterPatch web product page in the 'Download' tab:

www/sutter.com/AMPLIFIERS/SutterPatch.html.

4.3.4 File Types

Experiments & Data

The "packed" Igor Pro file format is recommended for saving an Experiment 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.

  A Preferences option now allows a packed Experiment to save data to an HDF5 file, which has the advantage of an unpacked Experiment (like fast saving), without the disadvantage of much larger file sizes.

- 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 during the SutterPatch start up. Or you can add the data only into the current Experiment via the Data Navigator ‘Import’ button.

Data file path defaults

Windows: C:\Users\<User Account Name>
\Documents\SutterPatch\Data\

macOS: Applications/ SutterPatch2/SutterPatch/Data/

Note: If a SutterPatch Experiment file is opened into Igor Pro without SutterPatch running, its graphs and layouts can be displayed with the menu items Windows / Graphs, or Windows / Layouts, or Windows / Layout Macros.

Axon Files

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

The Data Navigator ‘Import’ option for pCLAMP data is only available if Sutter Amplifier Systems hardware has been attached and detected by the SutterPatch software at any previous point in time for the current OS user. Only pCLAMP ‘episodic’ and ‘gap-free’ data import are supported.

HDF5 Files

HDF5 is a modern efficient file format for saving and managing high volumes of data.

Enable SutterPatch HDF5 files (*.h5) for Experiments with SutterPatch / Set Preferences / Files and Naming / ‘Save data to separate HDF5 file’.

Multiple SutterPatch HDF5 files can be created during an Experiment to segregate or manage data.
(See the File menu for additional options.)

HEKA Files

HEKA Elektronik 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.

The Data Navigator 'Import' option for PatchMaster data is only available if Sutter Amplifier Systems hardware has been attached and detected by the SutterPatch software at any previous point in time for the current OS user.

Igor Pro Files

Data can be selected and exported to the Igor Pro file format (*.ibw) via the Data Navigator 'Available Actions' menu. Select the Igor Binary format in SutterPatch / Set Preferences / Data Export.

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

Note: If data is imported from other (non-Sutter) Igor Pro programs, adjust the scaling of the data as needed.

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.

Graph, Table and Layout Files

Graphs in the Analysis Editor can be imported or exported as Igor Binary Wave (*.ibw) files, via the dialog's 'File's options.

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.

Individual graphs can also be saved with the experiment as Graph Macros - recall them via the Windows / Graph Macros menu.

Tables in the Analysis Editor can be imported or exported as text files.

The Layout window of the current experiment can be saved to several file formats via the main menu File / Save Graphics command. Various formatting options are available here.

4.3.5 Layout Window

SutterPatch: Layout Page: Show Layout

The Layout window is used to prepare your data for publication. Scope window input signals analysis graphs and other objects can be exported to a Layout window for graphical arrangement and editing.

A default Layout window is automatically created when SutterPatch is launched - display it with the menu command SutterPatch / Layout Page / Show Layout. Only one Layout window exists at a time. If no Layout window exists, it can be manually created via a 'Layout' button located in various windows.

The Layout window can also be created by running a Paradigm 'Export' step.

Note: Layout windows are sometimes created hidden behind other windows.

Clicking a Layout button appends its associated items into an existing Layout page (or a new Layout window.) Each signal and analysis graph is appended as an individual object.

The default configuration of "2 x 4" ('column' x 'row') objects per page can be changed in Preferences / Export Graphics or the Paradigm 'Export' step, and is applied when a new Layout window is created:

- 1 single pane
- 2 2 stacked panes
- 3 3 stacked panes
- 2 x 2 matrix
- 2 x 3 matrix
- 2 x 4 matrix

Once a Layout window page is filled, additional objects are automatically appended into additional Layout pages.

A toolbar displays in the upper-left edge of the Layout – the top two buttons reconfigure the toolbar buttons:

- Operate Selection tools and object insertion mode.
- Draw Mode Drawing tools mode.

The main menu 'Layout' command also displays to 'Show' or 'Delete' the Layout window.

The Layout window of the current experiment can be saved to several file formats via the main menu File / Save Graphics command. Various formatting options are available there.

Layout windows can also be saved with the Experiment as Layout Macros - recall them via the Windows / Layout Macros menu.

4.3.6 Log Window

SutterPatch: Log Window

The Log window displays time-stamped commands, responses, administrative information and error messages that provide a history of the steps having a possible influence on the execution of the experiment and its data. The Log window can also serve as a user laboratory notebook for free-form entries.

Figure 4-97. Log Window

At startup, the Log window displays the SutterPatch Version and Build numbers.

The following unnamed columns are used by the Log window:

Date & Time — Log entries are assigned a date/time stamp.

Day name, month name, day date, year date, time: hours:minutes:seconds, AM/PM

Event Type — Log entries are assigned an Event Type.

Data Acquisition	Acquisition operations, Membrane Test measurements.
Metadata	Tags.
Paradigm	Paradigm operations.
Startup	SutterPatch version information.
Unknown	Other operations.

Event Description — A text description of the log entry.

Each row with a Data Acquisition, Metadata or Paradigm entry is appended with the name of the appropriate Routine or Paradigm; if there is no value to list, just the name of the Routine or Paradigm is displayed.

4.3.7 Menus

The SutterPatch main menu item contains all of the SutterPatch-specific menu items. The rest of the main menu items provide the standard Igor Pro functionality. For documentation of the non-SutterPatch features, refer to the Igor Pro online help or manual.

Window/Dialog Controls

Keyboard "Return" key	=	'OK / Yes' buttons
Keyboard ESC key	=	'Cancel' button

File

Data file path defaults:

Windows:	C:\Users\<User Account Name> \Documents\SutterPatch\Data\
macOS:	Applications/ SutterPatch2/SutterPatch/Data/

New Experiment	Unload the current Experiment and start a new Experiment. It is recommended that you create one Experiment per cell, to keep file sizes manageable. Note: During the shutdown of the existing Experiment, it is highly recommended to say "Yes" to save changes, even if no changes were made. This is used by an internal Igor Pro cleanup process to ensure proper file reopening.
Open Experiment	Open a previously saved SutterPatch Experiment (*.pxp, *.uxp) file. If a SutterPatch Experiment is opened into an Igor Pro-only session, SutterPatch is automatically loaded. Note: During the shutdown of the existing Experiment, it is highly recommended to say "Yes" to save changes, even if no changes were made. This is used by an internal Igor cleanup process to ensure proper file reopening. If no active hardware is attached, the original amplifier configuration of the Experiment will be automatically used for the SutterPatch demo mode.

If the SutterPatch preference for HDF5 files was enabled, a SutterPatch Question will ask how to load the matching HDF5 file:

Load matching HDF5 File: [pathname]

- Load in modify mode, i.e., add new data, store changes in analysis files

  Open the HDF5 file in read-write mode, i.e., the original metadata and experiment structure, analysis results, images, etc. are overwritten when closing the present experiment. However, raw data are NEVER modified.

- Load in read-only mode, i.e., don't store any change back to the file

  Open the HDF5 file in read-only mode. Routine acquisition is disabled. Anything done in this session is lost when closing the Experiment.

- Cancel loading HDF5 file

  Do not open the HDF5 Experiment.

  A normal Igor session is launched. The SutterPatch menu is populated with blank submenus, and the command 'Reactivate SutterPatch' to re-open the HDF5 Experiment.

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.

Stores the Experiment data and temporary (input / output) waves, but does no other cleanup; HDF5 files are not updated to disk.

Save Experiment As

If Preferences are disabled for automatic file naming, the last used Experiment file name or the default name is displayed for renaming and saving. This then stores the Experiment data and temporary (input / output) waves, but does no other cleanup; HDF5 files are not updated to disk.

If Preferences are enabled for automatic file naming, an incrementing Experiment name is displayed for renaming and saving. However, nothing else in the Experiment gets saved.

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.

[The following HDF5 file options only display for Experiments started with the Preferences for Files and Naming / 'Save data to separate HDF5 file' enabled.]

Open SutterPatch HDF5 File	
New SutterPatch HDF5 File	Store all existing data into the present SutterPatch HDF5 file, clean up the Experiment, and create a new SutterPatch HDF5 file, so that acquisition can continue as if you had started a "New Experiment", but without starting a new SutterPatch session (or *.pxp file).
Update SutterPatch HDF5 File	[Only ungrays when data has been acquired or loaded.] Update the SutterPatch HDF5 data file without starting a new Experiment.
Compact SutterPatch HDF5 File	[Only displays when data has been discarded.] Remove discarded data from an the SutterPatch HDF5 data file without resaving the entire Experiment.

Data

Data Browser	Access all SutterPatch objects contained in the Experiment.

Analysis

The Analysis menu provides a wide assortment of mathematical transforms.

Curve Fitting	Create custom fitting equations.
Quick Fit	A variety of Igor Pro fitting equations.

Windows

The Windows menu provides access all windows controls.

Command Window	A quick code interpreter to manually process SutterPatch and Igor Pro 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 display and objects.

SutterPatch

Dashboard		Display icons for core program functions.
Acquisition Control		Open a control panel with Start/Stop and other interactive acquisition controls for Routines and Paradigms.
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	Manually tune the “lock-in amplifier” system.
	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 a window to load, edit and run Paradigms.
Routine Editor		Open a window to load and edit Routines.
Template Editor		Open a window to manage templates.
Equation Editor		Open a window to load and edit Equations.
Solution Control		Open a window to control solutions.

Camera Control		Open a window to capture images.

Data Navigator	Open a window to organize and display the experiment Paradigm, Routine and acquisition data in a tree structure.
Analysis Editor	Open a window to manage analysis graphs.
Layout Page	Show Layout
	Delete Layout

Set Metadata	Open a window to configure user-specified experimental information.
Set Preferences	Open a window to modify the default program settings.
Log Window	Open a window to display a history of program actions.
Notebook	Open a window for laboratory text entries and notations.

Shortcuts

Shortcut Editor	Open a window to manage keyboard shortcuts.

Action 1 [VHold+10mV|10pA:Right]	
Action 2 [VHold-10mV|10pA:Left]	
Action 3 [VHold+1mV|1pA:Right,shift]	
Action 4 [VHold-1mV|1pA:Left,shift]	
Action 5 [View last]	F2
Action 6 [Stop Acquisition]	F3
Action 7 [Start Routine]	F4
Action 8 [Stop Routine]	F5
Action 9 [Pause Paradigm]	F6
Action 10 [Resume Paradigm]	F7

Available Analysis Modules

- Action Potential Analysis
- Synaptic Event Analysis
- Single Channel Analysis

Help

Igor Help Browser	Igor Pro 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 X-Axis (right-click the X-axis)

- Autoscale All Axes
- Autoscale X Axis
- Set X Scale…
- Axis Properties…

Scope Y-Axis (right-click the Y-axis)

- Autoscale All Axes
- Continuous Autoscale Y Axis
- Autoscale Y Axis
- Full Scale Y Axis
- Set Y Scale…
- Axis Properties…
- Hide Signal ‘<signal name>’
- Show Signal ‘<signal name>’ Only
- Stack All Signals

Acquisition Scope main window — To display a limited data modification menu, right-click the blank area in a signal.

Note: If you click too close to the data, the full data modification menu displays instead. If you are having this issue, click near a horizontal or vertical edge of the signal pane.

- Autoscale All Axes
- Add Annotation
- Export Graphics — Copy the selected signal to a Graph window.
- Toggle Cursor Info — Manually measure X-Y data values or set a fitting range.
- Colors
- Hide Signal ‘<signal name>’
- Show Signal ‘<signal name>’ Only

- Stack All Signals

Reanalysis Scope main window	To display a limited data modification menu, right-click in the blank area in a signal pane. Note: If you click too close to the data, the full data modification menu displays instead. If you are having this issue, click near a horizontal or vertical edge of the signal pane.

- Autoscale All Axes
- Add Annotation
- Export Graphics — Copy the selected signal to a Graph window.
- Toggle Cursor Info — Manually measure X-Y data values or set a fitting range.
- Colors
- Hide Signal '<signal name>'
- Show Signal '<signal name>' Only
- Stack All Signals
- Show All Sweeps
- Show Marked Sweeps

Signal data	To display the full data modification menu, right-click on or near the data.

- Browse <signal name>
- Edit <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
- Quick Fit
- Parametric Plot
- Amplitude Histogram Plot
- Export Graphics
- Toggle Cursor Info — Manually measure X-Y data values or set a fitting range.
- Hide Signal '<signal name>'
- Show Signal '<signal name>' Only
- Stack All Signals
- Show All Sweeps
- Show Marked Sweeps

Scope Marquee window (click-and-drag in a signal)

- Expand
- Horiz Expand
- Vert Expand
- Shrink
- Horiz Shrink
- Vert Shrink

- Extract Template

4.3.8 Notebook

SutterPatch: Notebook

The SutterPatch Notebook is a free-form text-entry lab Notebook, that also receives Igor Pro cursor measurements, and optional system logging information.

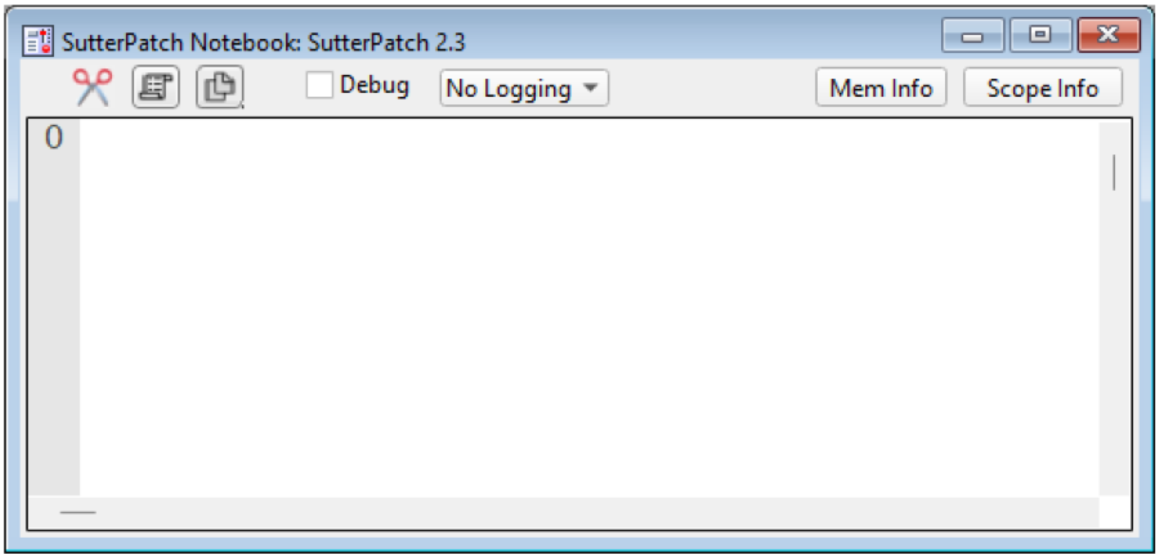


Figure 4-98. Notebook

	Clear	Clear the complete Notebook content.
	Copy to Layout	Click to copy the selected text or the (unselected) compete Notebook into a new Layout window, or to an existing Layout page, as a text annotation on top of the graph 1 location. Use a Shift-click to copy the text into successive graph locations.
	Copy to Clipboard	Copy the selected text or the (unselected) compete Notebook to the system clipboard.
[Text window]		Each line is numbered, and usually starts with the time in hh:mm:ss.xxx. All entries can be edited or erased.

4.3.9 Sample Files

Sample settings files (subject to change) are included in the ... / Documents / SutterPatch / Parameters folder:

<u>Compound Pool</u>

SP_CompoundPool.spl

1. undefined	No compounds are configured.

<u>Dynamic Clamp Pool</u>

SP_DynamicClampPool.spd [for dPatch systems]

<u>Equation Pool</u>

SP_EquationPool.txt

1. X3pi	3*pi
2. ElapsedTime	ParadigmTime - time
3. Temperature	aux[1]*1.23 – 273.15

<u>Paradigm Pools</u>

LockIn / LockIn_dPatch.spp [for dPatch systems]

LockIn / LockIn_IPA.spp [for D/IPA systems]

1. LockIn_Adjust_500Hz
2. LockIn _ Adjust_1kHz
3. LockIn _DoAdjust

SP_ParadigmPool_Dendrite.spp [for Dendrite systems]

SP_ParadigmPool_dPatch.spp [for dPatch systems]

SP_ParadigmPool_IPA.spp [for D/IPA systems]

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 stops 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 window 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.
12.	CellHealth_From_CC	Monitor the cell's resistance and capacitance in current clamp mode.

Routine Pools

LockIn / LockIn_DIPA.spr [for Double IPA systems]

1. phase_delay
2. LockIn_500Hz
3. LockIn_1kHz

LockIn / LockIn_dPatch.spr [for dPatch systems]

LockIn / LockIn_IPA.spr [for IPA systems]

1. phase_delay
2. LockIn_500Hz
3. LockIn_1kHz

SP_RoutinePool.spr [for IPA systems]

1.	Amplitude Equations	Equations for a variety of stimulus waveforms.
2.	AT_InactRec_P4	Inactivation with leak subtraction.
3.	Bowtie_Test	Multi-channel input with incrementing ramp waveforms.

4.	ContinuousNoOut	Acquisition without any output waveform.
5.	IV	I-V for voltage-clamp mode.
6.	IV_CC	I-V for current-clamp mode.
7.	IV_Continuous	I-V with continuous acquisition.
8.	IV_P4	I-V with four leak-subtraction pulses.
9.	IV_tuning	I-V for sample “tuning” paradigms.
10.	Multi_Test	Multi-channel input with an incrementing square-step waveform.
11.	Onset_SlowActivation	Onset Slow activation.
12.	Recovery_Inactivation	Recovery from inactivation.
13.	Recovery_SlowInact	Recovery from slow inactivation.
14.	SS_Inactivation	Steady-state inactivation.
15.	SS_SlowInactivation	Steady-state slow activation.
16.	Synaptic_Stim	Synaptic stimulation.
17.	Synaptic_Stim30	Synaptic stimulation for 30 s.
18.	Synaptic_StimPlusDig	Synaptic stimulation with digital output.
19.	Template_PlusVirtual	Template wave and recording virtual signals.
20.	Template_SpontAct	Template wave from a recorded signal.
21.	Template_Test	Template wave for waveform output.
22.	Test_Pulse	Test pulse.

SP_RoutinePool_Dendrite.spr [for Dendrite systems]

SP_RoutinePool_DIPA.spr [for Double IPA systems]

1.	Amplitude Equations	Equations for a variety of stimulus waveforms.
2.	AT_InactRec_P4	Inactivation with leak subtraction.
3.	Bowtie_Test	Multi-channel input with incrementing ramp waveforms.
4.	ContinuousNoOut	Acquisition without any output waveform.
5.	IV	I-V for voltage-clamp mode.
6.	IV_CC	I-V for current-clamp mode.
7.	IV_Continuous	I-V with continuous acquisition.
8.	IV_P4	I-V with four leak-subtraction pulses.
9.	IV_tuning	I-V for sample “tuning” paradigms.
10.	IV_VC_CC	IV for voltage- and current-clamp modes.

11. Multi_Test	Multi-channel input with an incrementing square-step waveform.
12. Onset_SlowActivation	Onset Slow activation.
13. Recovery_Inactivation	Recovery from inactivation.
14. Recovery_SlowInact	Recovery from slow inactivation.
15. SS_Inactivation	Steady-state inactivation.
16. SS_SlowInactivation	Steady-state slow activation.
17. Synaptic_Stim	Synaptic stimulation.
18. Synaptic_Stim30	Synaptic stimulation for 30 s.
19. Synaptic_StimPlusDig	Synaptic stimulation with digital output.
20. Template_PlusVirtual	Template wave and recording virtual signals.
21. Template_SpontAct	Template wave from a recorded signal.
22. Template_Test	Template wave for waveform output.
23. Test_Pulse	Test pulse.

SP_RoutinePool_dPatch.spr [for two-headstage dPatch systems]

SP_RoutinePool_dPatch_1HS.spr [for one-headstage dPatch systems]

Shortcut Pool

SP_ShortcutPool.sps

1. VHold + 10mV	Increase the Control Panel V-holding level by 10 mV.
2. VHold – 10mV	Decrease the Control Panel V-holding level by 10 mV.
3. VHold + 1mV	Increase the Control Panel V-holding level by 1 mV.
4. VHold – 1mV	Decrease the Control Panel V-holding level by 1 mV.
5. View last	Open the last acquired Series into a Reanalysis Scope window.
6. Stop Acquisition	Stop the acquisition of a Series.
7. Start Acquisition	Start the acquisition of a Series.
8. Stop Paradigm	Stop the execution of a Paradigm.
9. Pause Paradigm	Pause the execution of a Paradigm.
10. Resume Paradigm	Resume execution of a Paradigm.
11. Cursor Info	Toggle On/Off

12. Next Sweep
13. Previous Sweep

Solution Pools

SP_SolutionPool.spl	[for all Sutter amplifier systems from SutterPatch v2.3 onwards]
1. undefined	No solutions are configured.
SP_SolutionPool.spo	[for all Sutter amplifier systems from SutterPatch version 2.2.1 or earlier]
1. undefined	No solutions are configured.

Template Pool Sample Files

SP_TemplatePool.spt

1. RoutinePreview
2. Single_actionPotential
3. HodgkinHuxley
4. Noise

Experiment Sample Files

Sample data (subject to change) are included in the ... / Documents / SutterPatch / Example folder:

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

4.3.10 Set Metadata

SutterPatch: Set Metadata

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

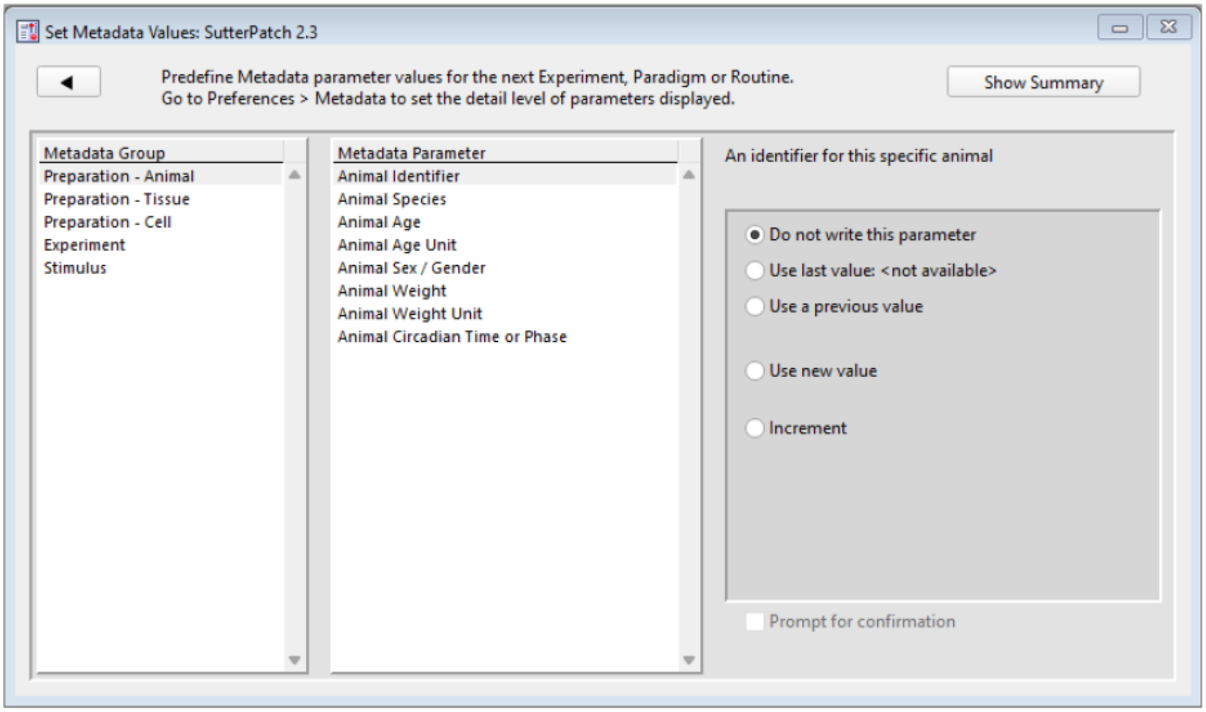


Figure 4-99. Set Metadata Values

Predefine the Metadata parameter values here.

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

Metadata Summary dialog

This list summarizes all Metadata parameters (from all Metadata detail levels) for which values have been defined. Double-click a line to show and edit the details for a parameter.

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	Availability is per the SutterPatch > Set Preferences > Metadata > ‘Metadata detail level’.

Basic Level 1	(default groups)

Preparation – Animal
Preparation – Tissue
Preparation – Cell
Experiment
Stimulus

Extended Level 2	(plus two more groups)

Electrode
Recording Solution

Full Level 3	(plus four more groups)

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

Note: Values in this dialog are updated by acquisition changes after the dialog is closed and re-opened.

- 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 — At the start of a named Paradigm (i.e., pre-planned, not auto-triggered by a Routine.)
- Routine — At the start of a Routine.

<u>Confirm Metadata Settings for</u> — This dialog displays whenever an Experiment, Paradigm or Routine is started with metadata prompts enabled.

Write	Enable to write the selected metadata parameter with the Experiment, Paradigm or Routine.
Metadata Parameter	The name of the selected metadata parameter.
Next Value	The metadata value to write.
Update	Enable so that edits made to 'Value' will update the 'last value' for the next prompt. This field is automatically disabled after each execution.
Prompt	Disable to remove the metadata parameter from those listed in the Confirm Metadata Settings dialog. The 'Confirm Metadata Settings' dialog only displays if a metadata parameter has been enabled for 'Prompt'.

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 Unit	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 Unit	
B	E	F	Animal Circadian Time or Phase	
	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: YYY-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 Unit	
	E	F	Tissue Incubation Temperature	
	E	F	Tissue Incubation Temperature Unit	
	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: YYY-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 Unit	
B	E	F	Cell Preparation Incubation Temperature	
B	E	F	Cell Prep. Incubation Temperature Unit	
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	
	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 Preheat Enabled	
		F	Pull Heat-on Time	
		F	Electrode Tip Diameter	
		F	Electrode Taper Length	
		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	
		F	Bath Solution Preparer	
	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	Pipette Solution Preparer	
		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 Unit	
		F	Ambient Temperature	
		F	Ambient Temperature Unit	
		F	Atmospheric Composition	
		F	Atmospheric Pressure	
		F	Atmospheric Pressure Unit	
		F	Atmospheric Humidity	% relative humidity ("-1" = uncontrolled)
		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	
		F	**SERIES (= ROUTINE DATA)**	
		F	Routine User Data	
		F	**IMAGING**	
		F	Image Comment	
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 Unit	
	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 Unit	
	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 Unit	
	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 Unit	
	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 Unit	
	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 Unit	°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 Unit	The intensity unit of an external electrical stimu-lus
	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	

Table 4-7. Metadata Parameters

4.3.11 Set Preferences

SutterPatch: Set Preferences

Preferences settings customize the default settings for several areas of the SutterPatch program. To access, go to the SutterPatch / Set Preferences menu.

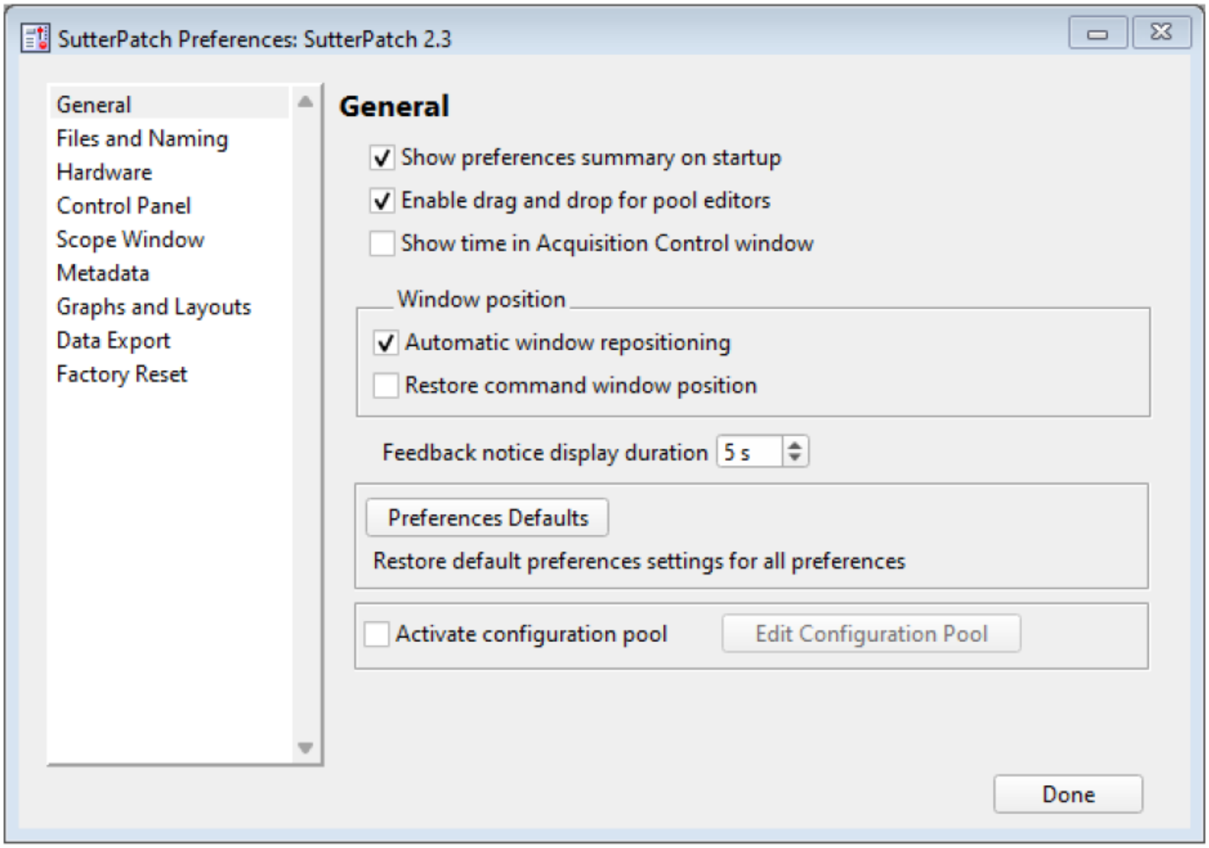


Figure 4-100. Preferences Settings

i. General

Don’t wait at Startup Panel

Skip the “Welcome” screen during startup and directly launch SutterPatch with a new Experiment. The options to startup just ‘Igor Pro’ or to re-open a saved Experiment are bypassed.

Show preferences summary on startup

Display the ‘Summary of Major Preferences’ pane when SutterPatch opens a new Experiment:

Configuration pool: <name> | not used

Selected amplifier: IPA

Automatic experiment naming is ON|OFF

<experiment file pathname>

Save data to separate HDF5 file is ON|OFF

Save entire experiment | (Don’t) Save to temp file after each routine

Warn when file size exceeds limit of 500 MB|OFF

Maximal sweeps displayed in persistence display during acquisition:

| all

Maximal sweeps displayed in persistence display during reanalysis:

| all

Show event tags in reanalysis scope:

ON|OFF

Number format:

Use exponential | engineering

and also display this summary in the startup Command window.

Enable drag and drop for pool editors

Most SutterPatch “Editors” load a set of named configurations from a main “Pool” file. These named items can be re-arranged in their Editor’s Pool list by clicking and dragging with the mouse.

This also applies to the Paradigm Editor list of Paradigm Steps.

Show time in Acquisition Control window

Display a system “Time" clock [hh:mm:ss] in the Acquisition Control panel.

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.

Restore command window position

Enable so the command window position is remembered for the next Experiment that opens. Otherwise, a new Experiment always returns the Command window to its default size and location.

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.

Feedback notice display duration

[1 – 30 s]

Control how long SutterPatch messages display for reading, before automatically closing.

Preferences Defaults — Restore default settings for all preferences.

Activate configuration pool — Display the Configuration loading window when starting a new Experiment.

Edit Configuration Pool — Create different user Preferences configurations, selectable for loading at the start of a new experiment.

Delete Configuration — Remove the named Configuration from the list.

Add New Configuration — Create a new Preferences Configuration for the existing Preferences settings.

Description — Enter the name of the new Preferences Configuration.

Update Active Configuration (#)

The "active" Configuration is updated with the existing Preferences settings.

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

Note: The 'Files and Naming' preference for HDF5 file saving cannot be disabled via running a Configuration; it must be manually disabled by the user in Set Preferences.

Also, while SutterPatch Preferences Configuration files use the *.spc file extension, this is reported by the OS as file type "PKCS #7 Certificates".

ii. Files and Naming

Data file path: — Browse to select a folder.

The data folder should reside on a local disk drive. We do not recommend using a mapped network drive, as speed/throughput bottlenecks can occur, including delays when saving experiments.

Default file paths

Windows: C:\Users\<User Account Name>\Documents\SutterPatch\Data\

macOS: Applications/SutterPatch2/SutterPatch/Data/

Enable automatic naming for experiment

Experiment file name example: (Maximum 34 characters)

[ ]

At least one of the following file name components must be enabled:

Text:	[ ]
	Include user text at the start of the file name. At least one character is required.
	Valid characters are A-Z, a-z, 0-9 and "_".
Separator	Separate the user text from the rest of the file name with an underscore.
Date: YYMMDD	Include the date in the file name.
Separator	Separate the date from the rest of the file name with an underscore.
Time: hhmmss	Include the time in the file name.

Save data to separate HDF5 file

Store the Experiment data waves using the HDF5 file format, a modern efficient format for managing and saving high volumes of data.

Note: This setting is disabled by a Factory Reset.

By default, the Experiment file is stored in a "packed" (*.pxp) experiment, where 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.

Enable the HDF5 file option to avoid such delays. A new setting becomes active after starting a New Experiment or a new SutterPatch session.

Then, whenever a new HDF5 file is created, SutterPatch 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 starting a new SutterPatch session.

The raw signal data are stored to the HDF5 disk file during acquisition after each sweep, instead of storing all data at the end of an Experiment, which can be a time-consuming experience.

Other waves from the SutterPatch Data folder (including metadata, Experiment structure, analysis results, Routines, Log, images) are stored to the HDF5 file at the end of a Routine or Experiment; items outside of the SutterPatch Data folder (such as graphs and layouts) are stored to the ".pxp" Experiment file.

Note: It is strongly advised to enable the "automatic naming" option above, so that '*.h5' HDF5 files and their parent '*.pxp' Experiment file are kept "in sync".

Keep only one Sweep in Memory

[only displays when "Save data to HDF5" is enabled]

For the leanest operation, only hold the wave of one sweep in memory, so memory buffers do not need to be re-allocated for the Experiment.

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

Update HDF5 file after each routine

[only displays when "Save data to HDF5" is enabled]

For efficient processing, while the raw signal data are written after each sweep during acquisition, the other SutterPatch Data folder information (metadata, Experiment structure, analysis results, Routines, Log, images, Dynamic Clamp conductances) is separately written to the HDF5 file.

The non-data information are automatically written to the HDF5 file at the end of an Experiment. However, if this option is enabled, the information is also automatically written to the HDF5 file at the end of each Series.

[save options] [displays when “Save data to HDF5” is enabled]

- Save data as double precision, 8-byte reals

The raw data are saved as reals instead of floats. This can help to reduce file sizes for large Experiments.

- Save data as single precision, 4-byte reals

The raw data are saved as reals instead of floats. This can help to file sizes for large Experiments.

[save options] [displays when “Save data to HDF5” is disabled.]

- 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.

Warn when file size exceeds limit

When a recording causes the Experiment to exceed the desired limit, a notification message displays after the Routine stops.

Limit [+10.0 MB]

Enter the limit.

Note: It is advised to disable the Igor Pro 9 'Autosave' feature to prevent delays during data acquisition. See Misc / Miscellaneous Settings / Autosave.

iii. Hardware

- Prompt for hardware on startup (if no Sutter hardware is found).

  When a new Experiment is started, if Sutter patch-clamp hardware is not connected to the computer and turned on, you are prompted to retry the USB connection or select an emulation mode.

- If no Sutter hardware is found, emulate:

  - IPA — Integrated Patch Amplifier system.
  - Double IPA — Dual-headstage IPA system.
  - dPatch — Digital Patch-clamp system.
  - Dendrite — Data acquisition 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 – 5.0] 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.

iv. Control Panel

Customize the active headstage tab's color in the Control Panel.

Headstage background color

Headstage 1	Color palette displays.
Headstage2	Color palette displays.

v. Scope

Scope setting changes are applied to an acquisition Scope window at the time of window creation, and to a reanalysis Scope window when data is loaded.

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.

Include zero when autoscaling

When using Scope window autoscaling, the Y-axes limits are rescaled with each sweep so that all data are visible. If the signal does not cross the zero line, the Y-axes ranges are extended to the zero amplitude,

Acquisition

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.

Allow Display Compression — Display compression is applied to the data in all “live” Scope windows (Acquisition / Free Run / Membrane

Test). This reduces the display processing load, and can improve system performance when resources are low.

When there are four times as many data points as the Scope width in pixels, the number of data points plotted are reduced, as the minima and maxima from two groups of up to 50 samples are displayed per screen pixel.

Reanalysis

Persistence

On

Off

Keep current setting

Maximal sweeps displayed in persistence display

[2 – 100] (30 = default value)

The last 'N' sweeps are displayed

[All] All sweeps are displayed.

Improve data readability by restricting the number of sweeps displayed. This can also improve system performance by reducing the display processing load.

Allow Display Compression

Display compression is applied to the data in the Reanalysis Scope window. This reduces the display processing load, and can improve system performance when resources are low.

When there are four times as many data points as the Scope width in pixels, the number of data points plotted are reduced, as the minima and maxima from two groups of up to 50 samples are displayed per screen pixel.

Note: Display compression is not applied to Single Channel Analysis data.

Show event tags

Display tag lines in the Reanalysis Scope window; also display tag text boxes in the

Continuous and Concatenated display modes.

Tag Position

- Frozen
- Movable

Tag types to show

- User
- Input-triggered
- System

Tag text box

- Relative Time — Time from beginning of Series. [hours to milliseconds]
- Absolute Time — Clock time [hours to milliseconds]
- Description — User Comment, from ‘Set Tag’.

Tag appearance

- Color by type
- Transparent

Appearance — Use a color palette to choose colors.

Active signal panel color	[light gray]
Inactive signal panel color	[dark gray]
Active sweep color	[dark blue]
Inactive sweep color	[red]
Event tag line color	[blue]
Drop-down color palette	Click on a color square in the palette to set it as the active color.
Other	Use a color dialog with more options.
Opaque	< unused >
[Preview Pane]	The selected signal and sweep colors are displayed in a preview pane.

Tip: For dark-room experiments, the window background color can be adjusted by the operating system:

- Windows: In the Control Panel / Appearance / Personalization window, scroll down and select the High Contrast Black theme, or use the Windows Magnifier tool with option 'Turn on color inversion' enabled.
- macOS: Press 'Control-Option-Command-8' to set the System Preferences / Accessibility / Display / Invert Display colors option, or open its menu with 'Command-Option-5'.

vi. Metadata

Metadata detail level:

Select levels for metadata setup categorization complexity.

Select which metadata groups and parameters are visible for configuration in 'Set Metadata' and in Data Navigator / 'Build Hierarchy'.

Basic	Show only the most essential parameters.
Extended	Show additional detail.
Full	Expose all available metadata parameters.

Note: This setting does not affect the Metadata Review dialog - all defined metadata are displayed.

This setting does not affect data acquisition metadata prompts – all configured prompts are always executed.

vii. 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 are supported by other programs.

Layouts

Changes are applied when a new Layout window is created.

Default graphs per new Layout page:

- 1
- 2
- 3
- 2 x 2 (Column x Row)
- 2 x 3 (Column x Row)
- 2 x 4 (Column x Row)

Graphs

left (the Y-axis)

Tick location:

- Outside
- Crossing
- Inside
- None

Labels:

- On
- Axis only Text labels only.
- Off

Grid:

- Off
- On
- Major only

bottom (the X-axis)

Tick location:

- Outside
- Crossing
- Inside
- None

Labels:

- On
- Axis only Text labels only.
- Off

Grid:

- Off
- On
- Major only

viii. Data Export

File Export

- Igor Binary Format Save the signal formatted as an "Igor Binary Wave" file (*.ibw).
    - Export all selected sweeps to one file per signal (one 2D wave)
    - Create individual files for each sweep (multiple 1D waves)
    - Export all selected sweeps to one concatenated file per signal
- 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).

  Uses the table formatting preferences below.

Table Formatting: Copy to Clipboard, Text Table Format

Cell separator

- Tab
- Comma
- Semicolon

New line

- <CR> (Igor Pro, macos)
- <CR> <LF> (Windows)

Invalid value

- Use NaN
- Use empty string
- Use zero

Digits in table entries [3 – 15]

Numeric format

- Use exponential notation (e.g., 10e-3 V)
- Use engineering prefix (e.g., mV)

  This uses “powers of 3” to determine the decimal prefix symbol (e.g., μ, m, n, p) for a non-zero number to the left of the decimal point with 1 – 3 digits.

ix. Factory Reset

[Factory Reset] Click this button to reset all SutterPatch preferences and settings to their defaults. 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.)

4.3.12 Shortcut Editor

SutterPatch: Shortcuts: Shortcut Editor

Keyboard control of SutterPatch is available by configuring keyboard shortcuts.

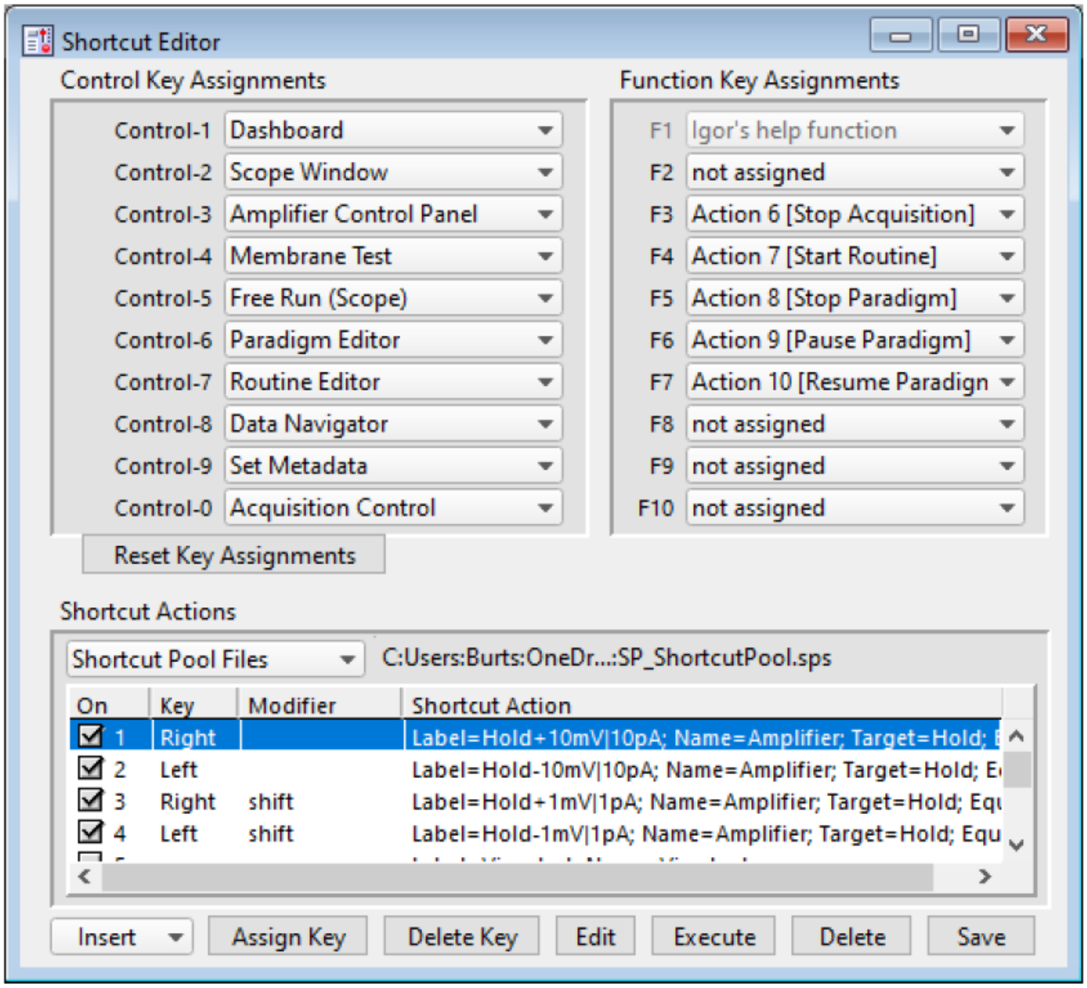


Figure 4-101. Shortcuts Editor

All assigned Control (Command), Function and Shortcut Action key combinations are automatically added to the main menu SutterPatch / Shortcuts submenu. All Control and Function Key Assignments are automatically saved when the program is closed.

The Shortcuts main menu list is shared by the Control and Function keys, so each menu item can only be assigned to a single key. Prior duplicate entries are replaced by “not assigned”.

Control Key Assignments / Command Key Assignments

A Control key assignment can be easily made by clicking on its drop-down list and selecting a new item from the list of SutterPatch menu items.

Use Control keys by holding down:

- Windows: Control key: Ctrl
- macOS: Command key: ⌘

and clicking its assigned number.

Control keys and their default SutterPatch menu assignments:

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	Acquisition Control	

Other SutterPatch menu items available for assignment:

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

Function Key Assignments

Computer keyboards usually include a set of Function keys [0 – 9] for special functionality. Configure a 'Function Key' assignment by clicking on its drop-down list and selecting a new menu item or Action item.

Function keys and Control keys share the same menu items list, and each menu item can only be only be assigned to a single key. So, after any new assignment, any duplicate key is changed to "not assigned".

Function keys can also be assigned from Shortcut Actions – see below.

Note: On some keyboards, you also need to press the ‘Fn’ key to use Function keys.

Also, macOS reserves nearly all Function keys for itself. In order to use Function keys for a macOS application, you must first check a checkbox in the macOS Keyboard control panel. Even then, macOS will intercept some Function keys.

Function Key F1 is not available for assignment, as it is reserved for Igor Pro’s Help function.

Default Functions

F1		Igor Pro’s Help function	
F2	Action 5	View last	
F3	Action 6	Stop Acquisition	
F4	Action 7	Start Routine	
F5	Action 8	Stop Routine	
F6	Action 9	Pause Paradigm	
F7	Action 10	Resume Paradigm	
F10	Action 11	Cursor Info	[target window must be open]

Reset Key Assignments

Reset all Control keys to their default settings. Function key “menu” items are reset to ‘not assigned’, while ‘Action’ items are unaffected.

Shortcut Actions

Up to 10 additional custom keyboard Actions can be created, managed and stored in a file.

[ ] Displays the last executed shortcut Action.

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	Append the Shortcut Actions from a previously saved Shortcut Pool file into the loaded Shortcut Actions table.

[   ]   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 for use with keys '0 – 9'.
- Alt — Keys '0 – 2' reserved by Igor Pro for File / Recent Experiments.
- Shift — Shift key.
- Fn — Function key.
- Caps Lock — Ignored.

macOS

- Command — Only for use with keys '0 – 9'.
- Option — Option key.
- Shift — Shift key.
- ctrl — Keys '0 – 2' reserved by Igor Pro for File / Recent Experiments.
- Caps Lock — Ignored.

Shortcut Action	An Action's instructions and settings.

Click a field in the table to highlight an Action and make it the active entry. Click-and-drag a row to reposition it in the table.

Predefined Shortcut Actions

1	[Hold+10mV|10pA: Right]	(keyboard arrow key)
2	[Hold-10mV|10pA: Left]	(keyboard arrow key)
3	[Hold+1mV}1pA: Right,shift]	(keyboard arrow key)
4	[Hold-1mV|1pA: Left,shift]	(keyboard arrow key)
5	[View last]	F2
6	[Stop Acquisition]	F3
7	[Start Routine]	F4
8	[Stop Routine]	F5
9	[Pause Paradigm]	F6
10	[Resume Paradigm]	F7
11	[Cursor Info]	F10
12	[Next Sweep]	In Reanalysis Scope
13	[Previous Sweep]	In Reanalysis Scope

The following buttons modify the Shortcut Actions:

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.
Cursor Info	Use cursors to set a fitting range for graphical data
Data Navigator	Open the Experiment's data management center.
Execute	Run an Igor Pro or SutterPatch command.
Export	Send graphs to the 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.

Select Series	Select the next/previous series in the Reanalysis Scope window.
Select Sweep	Set a sweep to be the “active” sweep.
Set Axis	Modify the axis scaling of a signal.
Set Checkbox	Set local and global checkboxes for conditional processing in Paradigm ‘If’ steps.
Set Mark	The selected sweep in the Scope window is “marked” or “unmarked” for processing by the Data Navigator.
Set Tag	Write a comment tag to the Paradigm metadata.
Set Variable	‘Label’ entry only displays in the Shortcut Action column.
Start Acquisition	In an open Acquisition Scope window.
Stop Acquisition	In an open Acquisition Scope window.
View Last	Display the last recording in a Reanalysis Scope 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.)

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.

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

Delete Key — Remove the Key entry for the selected Action.

Edit — Open the Shortcut Actions 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.

4.3.13 SutterPatch Startup

The SutterPatch application startup sequence:

1. Power-on the Dendrite system 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. Click on the 'Igor Pro 9' icon to launch SutterPatch: 

   Igor Pro opens with an Igor Pro "splash" screen displaying while Igor Pro files are compiled.

   Then the 'Welcome to SutterPatch' screen displays with launch options:

   - Igor Only — Run Igor Pro (without launching SutterPatch).
   - Open — Launch SutterPatch from a saved Experiment.
   - Start — Launch SutterPatch for a new Experiment.

   Don't wait at Startup

   Optionally skip this screen and automatically launch a new session of SutterPatch at startup.

3. Click its 'Start' button, and a progress bar displays while compiling SutterPatch files, then the Welcome screen closes.

4. Next, if no Sutter amplifier is detected, the 'No USB Connection' pane allows you to retry establishing the USB connection, or to select a hardware-emulation (demonstration) mode:

   - IPA - Single Headstage
   - DIPA – Double Headstage
   - dPatch – Single Headstage
   - dPatch – Double Headstage
   - Dendrite – Data Acquisition System

   In emulation mode, the Amplifier Control Panel and Acquisition Scope window signal panes are labeled with "Demo", the input and output channels use simulated data, and most SutterPatch functions are available.

5. The SutterPatch files are initialized and the Dashboard panel, Acquisition Control panel , and SP_Notebook and Command windows display, and additional SutterPatch windows display if they were open in the prior experiment.

   A 'Summary of Major Preferences' window opens:

   Major Preferences as set in the "SutterPatch" menu, entry "Set Preferences":

Configuration pool: not used

Selected amplifier: IPA

Automatic experiment naming is ON: <pathname>

Save data to separate HDF5 file is OFF

Save entire experiment after each routine

Warn when file size exceeds limit of 500 MB

Maximal sweeps displayed in persistence display during acquisition: 30

Maximal sweeps displayed in persistence display during reanalysis: 30

Show event tags in reanalysis scope: ON

Number format: Use exponential notation (e.g., 10e-3 V)

Show on startup	Enable display of the "Preferences Summary" window at startup. This window is only created at startup. If this window is closed, a copy of the "Preferences Summary" can be found in the Command window history.

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 Pro 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 Pro 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 references an internal Igor Pro "root" folder, and not the computer's file system. The Data Browser right-click 'Copy Full Path' command copies a Signal's data wave path to the system clipboard.

For advanced users, 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 Pro, 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 Startup Compiler Errors

Problem: The SutterPatch loading on Windows fails due to compiler errors.

Solution: Instead of using the SutterPatch updater, run the full SutterPatch installer.

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'.

6.4.3 Saved Experiment Not Loading

Problem: In Igor Pro 8, a saved Experiment does not open due to a 'Macro Recreation Error'.

Solution: When using templates in a Routine, close the Scope and Data Navigator windows before saving the Experiment.

6.4.4 Startup EEPROM Errors

Problem: Starting up SutterPatch and simultaneously powering on the amplifier generates an EEPROM error. Attached hardware such as headstages might use incorrect settings.

Solution: Close SutterPatch, power cycle the amplifier, and relaunch SutterPatch.

6.4.5 Startup Odd Errors

Problem: When starting up or running SutterPatch, odd program errors display.

Solution: If after a SutterPatch update, closing and re-opening SutterPatch sometimes clears up these errors. If after an OS update, roll back the OS software update.

6.4.6 USB Communication Fails

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

Solution: Power off the amplifier, then reseat the USB cable on both ends, and then power on the amplifier.

Or, 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".

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

6.4.7 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.

6.4.8 Igor Pro Crashes on Wake Up

Problem: When a computer with external monitors wakes up from sleep, Igor Pro crashes.

Solution: Close Igor Pro before letting the computer go to sleep, or disconnect the external monitors. This is an OS issue we have no control over.

6.5 Acquisition Issues

6.5.1 Routine Loading Delays I

Problem: Selecting a Routine in the Routine Editor temporarily hangs the SutterPatch program.

Solution: Hide the Routine Editor Preview pane.

If there is a very large number of data or sweeps to display, the Preview pane can take a long time to redraw, and the program becomes temporarily unresponsive.

6.5.1 Routine Loading Delays II

Problem: Starting a pre-loaded Routine takes the same amount of time to load into the SutterPatch program.

Solution: Any changes made to the Amplifier Control Panel 'Offset', 'Holding' or 'Compensation controls will invalidate a pre-loaded Routine, and reload the Routine.

6.5.2 Acquisition Does Not Restart

Problem: Unable to start an acquisition because SutterPatch thinks a prior acquisition is still in progress.

Solution: Use the menu command SutterPatch / Reset Acquisition to clear the acquisition status.

6.5.3 Sweep Loading Delays

Problem: Sweep-by-sweep loading takes longer than expected.

Solution: If the intersweep time is less than 1/5 of the sweep duration, sweep loading delays can occur.

6.5.4 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.

6.5.5 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.

6.5.6 Signal Flat

Problem: A Scope window input signal is completely flat during acquisition, i.e., zero amplitude.

Solution: The corresponding Sutter headstage might not be firmly attached to its port, as the headstage HDMI connectors do not "lock on".

Power off the IPA system and firmly reconnect the headstage.

6.5.7 Signal Saturated

Problem: A Scope input signal is completely saturated during acquisition.

Solution: The corresponding Sutter headstage is not attached to its port. Power off the IPA system and firmly reconnect the headstage cable.

Or, if the headstage is attached, the Gain setting might be set too high. Reduce the output gain on the Amplifier Control Panel.

6.5.8 Headstage Noise

Problem: The noise levels of the instrument suddenly and erratically increase.

Solution: If any attached headstages are touched, the noise level will greatly increase. Make sure all headstages are grounded and in a Faraday cage.

6.5.9 Headstage Noise IPA

Problem: The noise levels of the instrument suddenly and permanently increase from ~0.8 pA to 2 pA (with a 5 kHz input filter).

Solution: If early model headstages are "hot swapped", i.e., plugged in or unplugged while the instrument power is on, their headstage operational amplifiers might be damaged. Contact Sutter Technical Support for service.

Best practices is to turn off the instrument power before (un)plugging its head-stages.

6.5.10 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.

6.5.11 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.

6.5.12 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, such as saving to temporary files or separate HDF5 data files.

6.5.13 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.
Close:	Scope Analysis windows.
Disable:	Routine Editor / Input Channels / Virtual 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.

6.5.14 USB Errors

Problem: A USB communications error occurs.

Solution: In the amplifier Control Panel, click the Reset USB button . If the button does not turn from red to green, then try to isolate the problem.

a) Unplug and re-plug both ends of the USB cable from the amplifier to the computer.

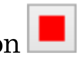
b) Try another USB cable.

c) Try another USB port

d) Remove any USB hubs.

6.5.15 System Freezes

Problem: The system hangs up after changing the filter setting or VC/CC mode selection.

Solution: Reset the USB port via the amplifier Control Panel USB Reset button  or the SutterPatch / Hardware Control menu.

6.5.16 IPA Amplifier Control Panel Issues

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

Solution: Reset all hardware controls to default settings by right-clicking on blank space in the Control Panel and selecting 'Reset Amplifier Controls', or via the menu item SutterPatch / Hardware Control./ Reset Control Panel.

:

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-axis and units are overlaid in the Scope window.

Solution: There is not enough room for the X-axis 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 Pro menu command Windows / Graph, or Windows / Layout or Layout Macros, to see the object. Right-click it to modify with Igor Pro 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, or reduce the Preference / Scope Window / "Maximal sweeps displayed in persistence display" setting.

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, Synaptic Event Analysis, Log, Paradigm Editor, Set Metadata, Set Preferences.) This is a reported Igor Pro issue.

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

6.7.4 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.

6.7.5 Windows Slowly Move Up or Down the Screen

Problem: Various SutterPatch windows in the Windows OS slowly creep up or down the screen, until they get to the top or bottom frame of the SutterPatch main window.

Solution: Click and hold the title bar of the window, or…

a) Disable the SutterPatch option Set Preferences / General / 'Automatic window positioning', or

b) Disable the Windows 10 option Start / Settings / Devices / Mouse / 'Scroll inactive windows when I hover over them', or

c) Reduce the Windows 10 option Start / Settings / System / 'Scale and layout' to 100%.

6.7.7 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 Pro issue.

6.7.8 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.

6.7.9 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.

6.7.10 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 Magnification Corrupts Window

Problem: After applying right-click Expansion to a window, returning to normal magnification corrupts the window.

Solution: Disable the Set Preferences / General / 'Automatic window repositioning'. Or, use an expansion factor which does not increase the window size beyond the screen size.

6.7.11 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.

6.7.12 Igor Pro Features

Problem: There are a large number of standard features in Igor Pro that can be used in conjunction with the SutterPatch application.

Solution: Refer to the Igor Help browser, or to Wavemetrics, Inc., regarding issues with Igor Pro features.

APPENDIX A: LIMITED WARRANTY

- Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair and replacement of defective components for two years from date of shipment, provided the instrument has been operated in accordance with the instructions outlined in this manual.
- Abuse, misuse, or unauthorized repairs will void this warranty.
- Warranty work will be performed only at the factory.
- The cost of shipment both ways is paid for by Sutter Instrument Company during the first three months this warranty is in effect, after which the cost is the responsibility of the customer.
- The limited warranty is as stated above and no implied or inferred liability for direct or consequential damages is intended.
- An extended warranty for up to three additional years can be purchased at the time of ordering, or until the original warranty expires. For pricing and other information, please contact Sutter Instrument Company.

APPENDIX B: SOFTWARE LICENSE

SutterPatch Software Licensing Agreement

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PLEASE READ THIS CONTRACT CAREFULLY. BY USING ALL OR ANY PORTION OF THIS PROPRIETARY SOFTWARE YOU ACCEPT ALL THE TERMS AND CONDITIONS OF THIS AGREEMENT. YOU AGREE THAT THIS AGREEMENT IS ENFORCEABLE LIKE ANY WRITTEN NEGOTIATED AGREEMENT SIGNED BY YOU. IF YOU DO NOT AGREE, DO NOT USE THIS SOFTWARE.

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Section 1.0. Defined Terms. Terms defined in this Article 1 shall have the meanings given below. Defined terms may be used in the singular or plural.

Section 1.1. “Agreement” means this Software License Agreement, which includes this Agreement’s terms and conditions, Schedules, Exhibits, Addenda, and Amendments, if any, which are incorporated in, and form an integral part of, this Agreement.

Section 1.2. “Confidential information” means any data or information, oral or written, of Sutter Instrument Corp., including without limitation, past, present, or future research, development or business activities, including any unannounced product(s) and service(s), and including any information relating to services, developments, inventions, processes, plans, financial information, customer lists, forecasts, and projections, including the terms of this Agreement.

Section 1.3. “End User” means the person or entity who is a client of Licensee and is authorized by User ID and Password to use the Product under Licensee’s authorization in the ordinary course of Licensee’s business.

Section 1.4. “Licensee” means a party or individual whose client has paid for and is using the Product or an End User, including any evaluation licensee.

Section 1.5. “Licensing Fees” mean the fees paid by a Licensee for the Product.

Section 1.6. “Product” means the software programs of Sutter Instrument Corp., including without limitation, SutterPatch software and corresponding documentation, printed materials and all updates or upgrades of the above that are provided to you, including without limitation, reports, graphs, test scores, interpretations and other information.

Section 1.7. “Term” means the length of time the License paid to use the Product (see current pricing schedule).

Section 1.8. "Website" means the Sutter Instrument Corp. website located at https://www.sutter.com and the Wavemetrics websites located at http://www.wavemetrics.com and https://www.igorpro.net.

2. GRANT OF LICENSE.

Section 2.1. Evaluation License. If the Product is used for evaluation, trial or demonstration purposes, Sutter Instrument Corp. grants such user a license solely for the purpose of evaluating, sampling, testing, or demonstrating the Product for the timeframe specified in the evaluation period. The evaluation license is subject to the following conditions: (i) Licensee hereby agrees to indemnify, defend and hold harmless Sutter Instrument Corp. and its officers, directors, employees and suppliers from and against any and all claims, damages, liabilities, costs and expenses, including without limitation, attorneys' fees and costs, arising from or in any way related to the use of the Product, and (ii) otherwise comply with the terms of this agreement.

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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 — Grounding point hardware.
- Patch Panel — Rack-mountable analog and digital I/O BNC panel.
- Quartz pipette holder — Ultra-low-noise low-drift pipette holder.

Ground Point GP-17

For system grounding, 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 ¼-20 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.

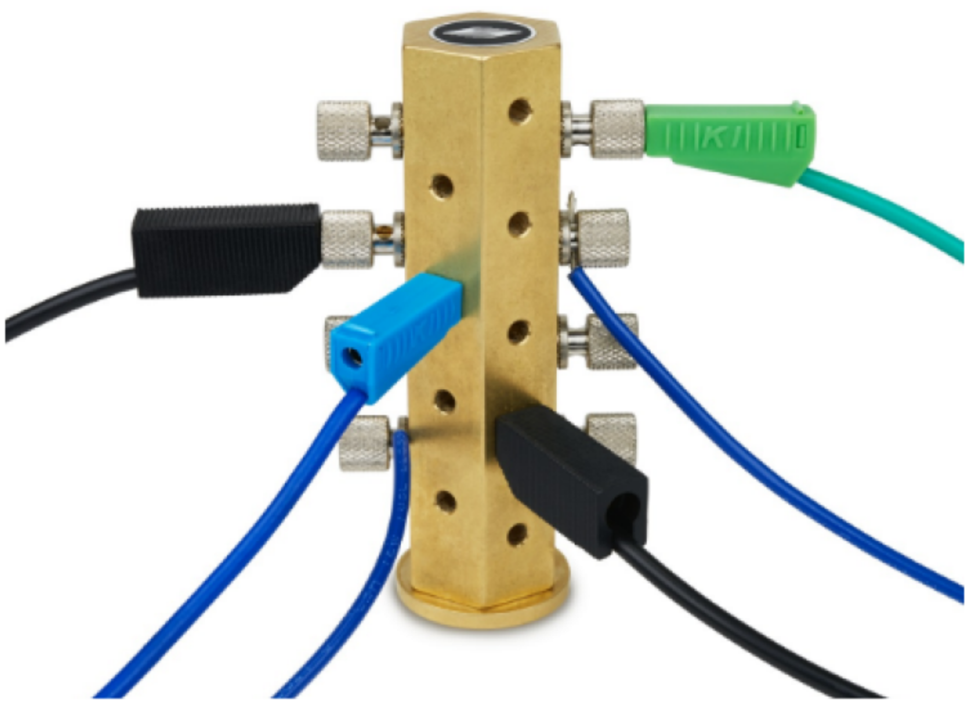


Figure C-0-1. Ground Point

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.

Patch Panel IPA-PCH

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

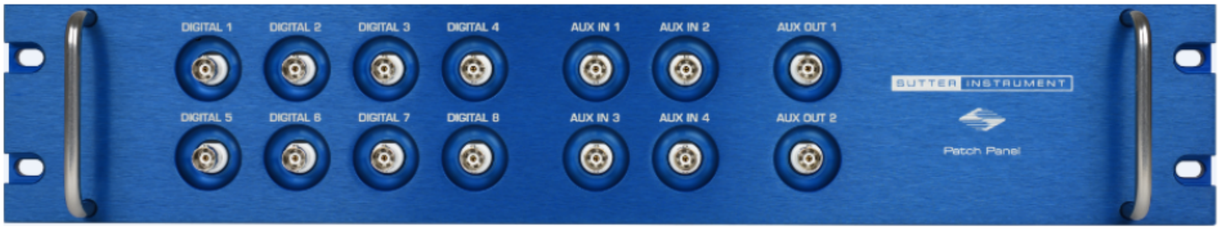


Figure C-0-2. Patch Panel

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 C-1. IPA Patch Panel

Quartz Pipette Holder EH-Q170

An ultra-low-noise quartz pipette holder is optionally available for very low-noise recording.

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.

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.

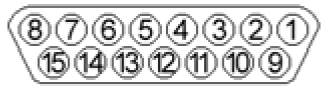
Mains Power Source	Fuses (Type: Time Delay/Time Lag, 5mm x 20mm, glass tube)	
	Fuse Rating	Manufacturer Examples
100 – 240 VAC	T2.0A, 250V	Bussmann: GMC-2-R, S506-2A, Littelfuse: 239.002.P

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	IPA: 2 SCOPE analog outputs (current sourcing: ±30 mA) IPA: 1 COMMAND analog input (impedance: 1 MΩ) DIPA: 4 SCOPE analog outputs (current sourcing: ±30 mA DIPA: 2 COMMAND analog inputs (impedance: 1 MΩ) 1 Digital output trigger (current sourcing: 20 mA) 1 Digital input trigger (impedance: 1 MΩ)
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 Port	DB-15 female connector 

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
Analog Output Gain	0 – 25x

Table E-1. IPA & DIPA Amplifier Specifications

IPA Headstage - Physical	
Construction	Anodized aluminum case
Dimensions (in.)	4.0 (L) 4.25 (L) w/threads x 1.375 (W) back end x 0.75 (H) x 0.825 (H) w/dovetail

Dimensions (cm)	10.16 (L) 10.795 (L) w/threads x 3.493 (W) x 1.9 (H) x 2.096 (H) w/dovetail
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 MΩ

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 Rates	0.1 - 50 kHz
Headstage Input Filter Bandwidth	0.5 - 20 kHz
Headstage Output Sample Rates	0.1 -10 kHz
Auxiliary In Sample Rates	0.1 - 200 kHz
Auxiliary Out Sample Rates	0.1 - 100 kHz
Digital In States	0 - 0.8 V = Low 2.0 – 5.5 V = High
Digital Out States	0 – 0.4 V = Low 2.4 – 3.3 V = High
Digital In Trigger Width	Edge triggered (ns)
Digital Out Trigger Width	100 µs
Digital In Sample Rates	0.1 – 50 kHz
Digital Out Sample Rates	0.1 – 10 kHz
Digital Out Current (max)	20 mA

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
• (1) Model Cell	MCELL
• (1) Auxiliary I/O Adapter Cable	
• (1) Rack-Mount Kit	RACK-PK
• (1) Power Cord	
• (1) USB 2.0 Cable	
• (1) Quick Start Guide	(with Igor Pro Serial #)
• (1) USB Flash Drive	SutterPatch and Igor Pro 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
• (2) Polycarbonate Pipette Holders	EH-P170
• (1) Model Cell	MCELL
• (1) Auxiliary I/O Adapter Cable	
• (1) Rack Mount Kit	RACK-PK
• (1) Power Cord	
• (1) USB 2.0 Cable	
• (1) Quick Start Guide	(with Igor Pro Serial #)
• (1) USB Flash Drive	SutterPatch and Igor Pro software.

Pipette Holder Parts

- End Cap
- Silicone Gaskets (O-rings, 6 ea.)

Gasket ID	Color
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

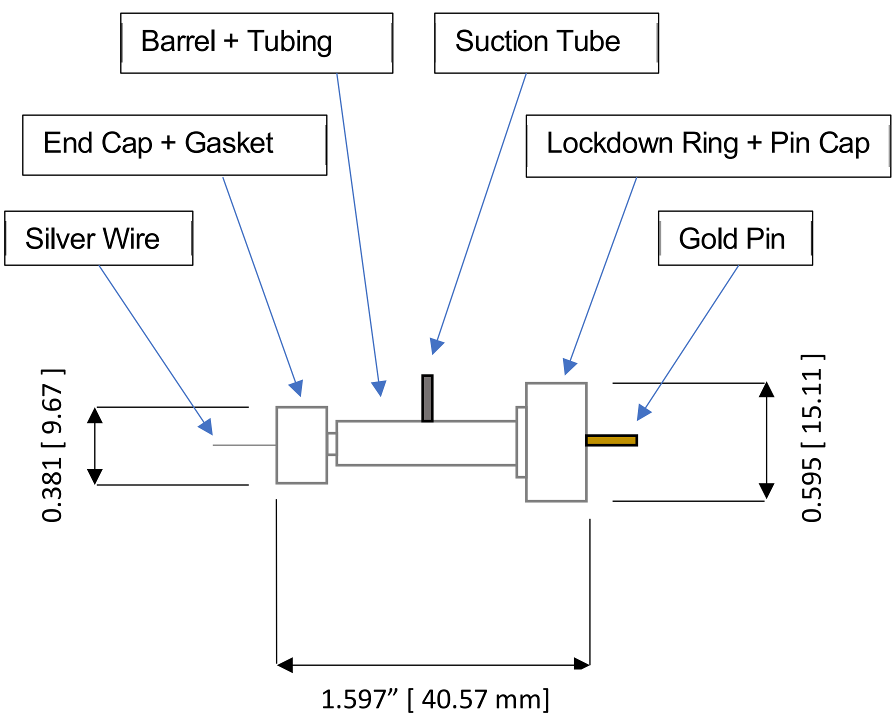


Figure E-0-1. Electrode Holder

Figure dimensions are in “inches [mm]”.

Model Cell Parts

- Model Cell
- Connector pins with crimp
- Ground wire

APPENDIX F. SUTTERPATCH ALGORITHMS

Action Potential Threshold Algorithm

[for Action Potential Analysis]

Results pane 'Threshold potential' computation:

The Event starts when the signal slope is > 1 mV/100 µs (10 V/s), or when 25% of the maximum slope is reached, whichever is smaller.

The exact 'Threshold potential' timepoint is based upon differentials using a central differences algorithm.

Liquid Junction Potentials

[for the Amplifier Control Panel 'Liquid junction' correction]

I Bath Offsets

Command Offsets

When a micropipette is placed into the bath, a voltage-clamp "zero-volt" stimulus command can still generate a small amount of unwanted current flow.

Correspondingly, a current-clamp "zero-current" stimulus command can generate a small amount of unwanted voltage charge.

In these cases, the stimulus command output is inaccurate, i.e., non-zero, due to hidden voltage offsets inherent in the physical system.

Offset Factors

Contributory factors to system offsets include the amplifier circuitry, the micropipette, the metal electrode, the liquid-metal junction of the electrode and the micropipette solution, and the liquid-liquid junction of the micropipette and bath solutions.

Liquid Junction Potential

Liquid-liquid solution offsets occur when two dissimilar solutions are in contact. Due to differences in their ion species charges, mobilities and concentrations, the two solutions create an unwanted voltage offset. The Liquid Junction Potential (LJP) is the reverse polarity calculation of the offset.

System Offset

The system's hardware-based offsets are fairly stable, and can be corrected for by a simple one-time tuning of the amplifier.

However, the solution-based liquid-junction offset is more complex, and requires further processing.

II Potential Polarities

Membrane Polarity

The electrical polarity of a cell membrane is defined as being measured in relation of the ***outside*** of the cell to the ***inside*** of the cell, i.e., the outside membrane is more positive compared to the inside membrane.

Inside of Cell → **Outside** of Cell
(Positive Polarity) →

Also by convention, the potentials on the outside of the cell membrane and the bath solution are equivalently at '0 V':

Inside of Cell → **Outside** of Cell | **Bath** (0 V)
(Positive Polarity) →

Membrane Potential

Membrane potential (V_m) is defined as:

$$V_m = V_{in} - V_{out}$$

for

V_{in}	Voltage on the cell inner membrane.
V_{out}	Voltage on the cell outer membrane.

As in the bath,

$$V_{out} = 0$$

then

$$V_m = V_{in} - 0$$

$$V_m = V_{in}$$

Liquid Junction

However, the polarity of LJP values is reversed from that of membrane potentials, as by historical convention (Barry), the LJP is defined as the potential of the ***bath*** solution with respect to the ***micropipette*** solution.

Bath ← Micropipette
← *(Positive Polarity)*

Therefore, as the liquid junction measurement direction (polarity) is reversed from that of V_m, the sign of LJPs needs to be properly addressed in membrane equations.

III Offset Changes

Seal

After a seal is formed on a cell, the micropipette's "open circuit" configuration changes to a 'cell-attached' patch configuration.

The micropipette solution now contacts the cell membrane, not the bath solution, so a liquid-liquid junction no longer exists. This means a liquid junction offset no longer contributes to the system offset. This situation occurs with all patch configurations, and is addressed by LJP correction.

IV Patch Configurations

Voltage Levels

In a patch-clamp experiment, baseline voltage levels change (pre- vs. post-seal) by the magnitude of the liquid junction offset. However, the polarity of the LJP correction is handled differently for the various patch configurations.

"Outside-Out" Configurations

Outside of cell membrane: in **bath**

Inside of cell membrane: attached to **micropipette** (or via ICF)

Micropipette → **Inside** of Cell → **Outside** of Cell | Bath
Outward Current → *(Positive Polarity)*

The **outside** of the cell membrane is measured in respect to the **inside** of the cell membrane for conventional polarity:

- "**Outward**" currents flow from the *inner* to *outer* membrane, so these currents flow *out* of the micropipette with a **positive** polarity.
- "**Inward**" currents flow from the *outer* to *inner* membrane, so these currents flow *into* the micropipette with a **negative** polarity.

These "outside-out" patch configurations use conventional cell membrane polarity:

Whole-Cell

[This is the only patch configuration supported by SutterPatch automatic LJP correction.]

A cell-attached patch is ruptured, and the cell membrane reseals onto the outside of the micropipette.

Outside of cell membrane: in **bath**

Inside of cell membrane: in contact with **micropipette** via intracellular fluid (ICF)

Note: The ICF-micropipette liquid junction is usually ignored, as it's offset is small compared to the bath liquid junction, and the micropipette and intracellular solutions equilibrate fairly quickly.

The polarity of calculated LJP values is reversed (by convention) from that of conventional cell membrane measurements. So, to "add" an LJP value into a membrane equation, you actually subtract it.

The Membrane voltage equals the Command voltage "minus" the LJP.

$$V_m = V_{cmd} - V_{LJP}$$

For whole-cell Current Clamp mode, where "V_{rec}" is the measured voltage from the cell:

$$V_m = V_{rec} – V_{LJP}$$

Outside-Out

A cellular patch is specially excised and reformed, so that:

Outside of excised membrane patch: in **bath**

Inside of membrane patch: in contact with **micropipette**

This configuration is useful for studying the effect of extracellular compounds, such as adding neurotransmitters to the bath.

However, the polarity of calculated Liquid-Junction Potential (LJP) values is reversed (by convention) from that of conventional cell membrane measurements. So, to "add" an LJP value into a membrane equation, you actually subtract it.

The Membrane voltage equals the Command voltage "minus" the LJP.

$V_m = V_{cmd} - V_{LJP}$

"Inside-Out" Configurations

The membrane orientation to the bath and micropipette is reversed from the "outside-out" configurations:

Inside of cell membrane: in **bath** (or **ICF**)

Outside of cell membrane: attached to **micropipette**

Micropipette → **Outside** of Cell → **Inside** of Cell
Inward Current → (Positive Polarity)

The **inside** of the cell membrane is measured in respect to the **outside** of the cell membrane, for a reversed polarity:

- "**Inward**" currents flow from the *outer* to *inner* membrane, so these currents flow *out* of the micropipette with a **positive** polarity.
- "**Outward**" currents flow from the *inner* to *outer* membrane, so these currents flow *into* the micropipette with a **negative** polarity.

These "inside-out" patch configurations utilize reversed polarity from "conventional" cell membrane polarity:

Inside-Out

This configuration is useful for studying single channel activity, while modifying the internal milieu.

A cellular patch is excised:

Outside of membrane patch: in contact with **micropipette**

Inside of membrane patch: in **bath**

As the polarity of an estimated LJP value is already reversed (by convention) from that of cell membrane values, the LJP value now matches V_m in reverse polarity. So, to "add" an LJP value into a membrane equation, you simply add it in.

The Membrane voltage equals the negative Command voltage plus the LJP.

$V_m = -V_{cmd} + V_{LJP}$

Cell-Attached

This is the initial patch configuration after making a seal on an intact cell membrane:

Outside of cell patch: in contact with **micropipette**

Inside of cell patch: in contact with **ICF**

As the polarity of a calculated LJP value is already reversed (by convention) from that of cell membrane values, the LJP value now matches V_m in reverse polarity. So, to “add” an LJP value into a membrane equation, you simply add it in.

The Membrane voltage equals the negative of the Command voltage, plus the LJP, plus the cell Resting Membrane Potential (RMP).

$$V_m = - V_{cmd} + V_{LJP} + V_{RMP}$$

Note: The RMP for most neuronal cells is between -50 mV to -90 mV.

LockIn Computation

[for Routine Editor Virtual Input Channels]

LockIn paper:

Lindau, M., Neher, E. Patch-clamp techniques for time-resolved capacitance measurements in single cells. Pflugers Arch. 411, 137–146 (1988). https://doi.org/10.1007/BF00582306

Math used in the LockIn computation:

Factor = (2.0 / SinePointsPerCycle) / sine_amplitude^2

A = Factor * ∑(current * stim_real)
∑ over one SinePointsPerCycle

B = Factor * ∑(current * stim_imag)
∑ over one SinePointsPerCycle

DC = 1/SinePointsPerCycle * ∑(current)
∑ over one SinePointsPerCycle

	VC-mode	CC-mode
Phase =	atan(B/A)	atan(B/A)
RealY =	A	A / (A^2 + B^2)

ImagY = B B / (A^2 + B^2)

Omega = (2 * pi) / SineCycleDuration

Gt = Idc / (Vdc – Et)

Gs = (A^2 + B^2 - A * Gt) / (A - Gt)

Gm = Gt * Gs / (Gs - Gt)

Cm = (A^2 + B^2 - A * Gt)^2 / ((A - Gt)^2 + B^2) / (Omega * B)

Membrane Test Calculations

[for Membrane Test in voltage-clamp "Cell" mode]

Parameters

The following parameters are calculated:

Exponential curve fitting

Transient peak amplitude (It)

Fitted curve time constant (τ or tau)

Electrode access resistance (Ra)

Membrane resistance (Rm)

Steady-state response (I1, I2)

Steady-state current (Iss)

Charge (Q)

Membrane capacitance (Cm)

Stimulus

The stimulus is a square-pulse wave, i.e., each pulse width is 50% of the pulse period (cycle), with the peak-to-peak amplitude centered around the cell's resting potential.

Fitting

Capacitive decay transients (current responses) from each pulse edge (positive and negative) are averaged (10x) for noise reduction and fit by a single (log) exponential.

Fitting range: 10 – 80%	The curve fit is applied to the data between the % amplitudes of the transient peak and its steady state response.

Note: The fit and decay time constant (tau) can be extremely sensitive to the electrode capacitance compensation.

Tau

The fitted curve decay time constant (tau) is calculated.

Peak

The fitted curve is also used to calculate the peak of the transient (It).

This theoretical calculation is used to indirectly measure the peak, as it is less sensitive to low-pass filtering effects.

Steady-State Response

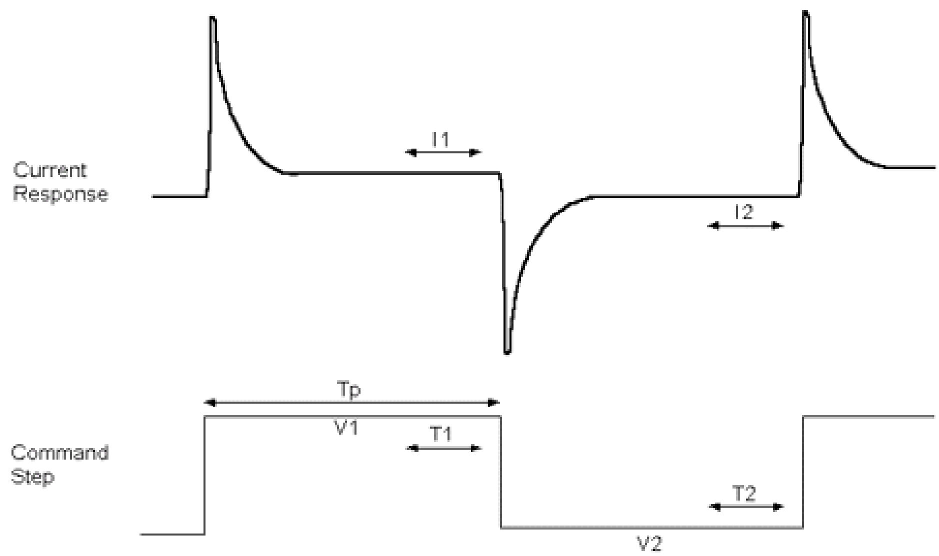


Figure 0-1. MT Command Pulse & Response

A square-pulse stimulation generates a pair of equal duration amplitude levels.

The pulse level amplitudes (V1, V2) should be centered around the cell's resting potential.

Note: The SutterPatch Membrane Test sets the pulse ***first*** level amplitude (V1) ***relative*** to 'V-holding', and the pulse ***second*** level amplitude (V2) ***at*** 'V-holding'.

The "steady-state response" current **(I1)** is averaged during the last 20% (T1) of the duration of the ***first*** level (V1) in the pulse, and is the baseline for the transient of the ***second*** level (V2) in the pulse.

Correspondingly, the "steady-state response" current **(I2)** is measured during the last 20% (T2) of the duration of the ***second*** level (V2) in the pulse, and is the baseline for the transient of the ***first*** level (V1) in the pulse.

Iss

The steady-state current (Iss) for the cell is calculated as the average of the steady-state responses I1 and I2:

$$Iss = (I1 + I2) / 2$$

Rt

The total resistance (Rt) is calculated from the steady-state response:

$$Rt = \Delta V / \Delta I, \qquad \Delta I = I1 - I2$$

Ra

The access resistance Ra is derived,

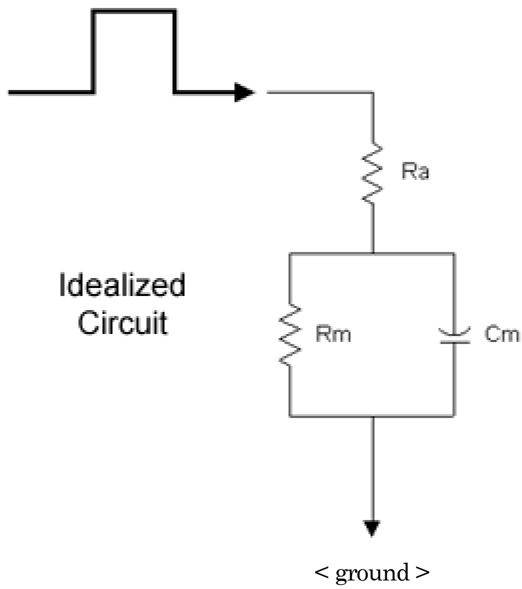


Figure 0-2. Idealized Membrane Circuit

as:

$$Tau / Cm = (Ra * Rm) / (Ra + Rm)$$

or:

$$(Ra + Rm) * (Tau/Cm) = Ra * Rm$$

Substituting in above for resistance terms:

$$Rt = Ra + Rm$$

$$Rm = Rt - Ra$$

$$\begin{aligned} Rt * (Tau/Cm) &= Ra * (Rt - Ra) \\ &= (Ra * Rt) - Ra^2 \end{aligned}$$

and:

$$\begin{aligned} (Rt * (Tau / Cm)) - ((Ra * Rt) - Ra^2) &= 0 \\ (Rt * (Tau / Cm)) - (Ra * Rt) + Ra^2 &= 0 \\ Ra^2 - (Ra * Rt) + (Rt * (Tau/Cm)) &= 0 \end{aligned}$$

Solve iteratively using the Newton-Raphson method.

Rm

The membrane resistance is derived:

Rt = Ra + Rm

Rm = Rt - Ra

<u>Cm</u>

Cell capacitance measurements are derived from the “area under the curve” charge calculations.

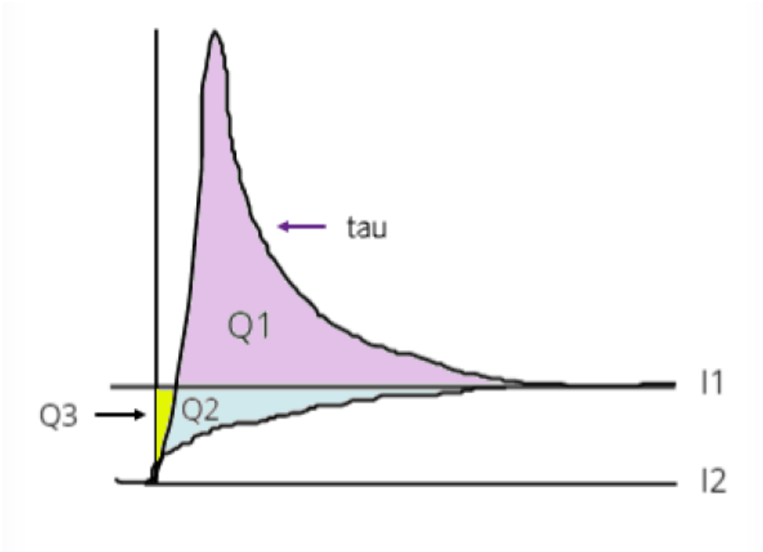


Figure 0-3. Membrane Charge

The total charge (Qt) is composed of three parts:

Q1	Main charge:	The area between a response transient and its steady-state response (I1).
Q2	Correction charge:	The area between the start of a pulse and the tau decay from its baseline (I2), relative to its steady-state response (I1). This area compensates for the settling time of the voltage step.
Q3	Error charge:	The area between the start of a pulse and its response transient, relative to its steady-state response (I1). This area represents the settling time of the voltage step before Q1, and is included in Q2.

For simplification, the small error charge (Q3) is ignored in our calculations.

So:

$Qt = Q1 + Q2$

The main charge (Q1) under the response transient is integrated. However, first the baseline steady-state response current (I1) is subtracted from the maximum peak response (Ip), so only the current difference (Id) is integrated:

$Id = Ip – I1$

The correction charge (Q2):

$Q2 = \Delta I * \tau,$ $\Delta I = I1 - I2$

Cm is derived from:

$Qt = Cm * \Delta V,$ $\Delta V = V1 – V2$

$Cm = Qt / \Delta V$

Rs Correction

[for Whole-cell Series Resistance compensation]

Math used in Lag:

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

Single Channel Fitting

[for Single Channel Analysis]

Math used in single channel fitting:

Gaussian Fit

$y = y0 + A * exp(-((x – x0) / width)^2)$

y0 = offset

A = height of curve's peak

x0 = position of center of peak

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

σ = standard deviation of the peak

Linear Exponential Fit

y = y0 + A * exp(-(x – x0) / tau

Logarithmic Exponential Fit

y = k0 + k1 * exp(-(ln (x / k2) / k3)^2)

Standard Error of the Mean (SEM) Algorithm

[for Analysis Editor Error Bars]

'Standard Error of the Mean' computation:

SEM = √ (SumSq – Mean2 * 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 "√ (variance)", but using 'Mean' vs. 'sum of all samples'.

Synaptic Event Detection Reference

[for Synaptic Event 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.