

# Integrated patch amplifier ELECTROPHYSIOLOGY

# **PATCH-CLAMP SYSTEM**

WITH

SUTTERPATCH<sup>™</sup> SOFTWARE

# Operation Manual Rev. 1.0.6 (20160729)





# **One Digital Drive** Novato, CA 94949 USA

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



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# DISCLAIMER

The **IPA** consists of one electronic amplifier with integrated digitizer and one headstage. 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 using 110 – 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.

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

Line Voltage: 110 – 240 VAC				
Fuse	Manufacturer Examples			
Rating	RoHS Compliant (Lead Free)			
T2.0, 250V	Bussmann: GMC-2-R, S506-2A Littelfuse: 239.002.P			

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, S506-2ALittelfuse:239.002.P

- Avoiding Electrical Shock and Fire-related Injury
- Always use the grounded power cord provided to connect the system's power adapter to a grounded/earthed mains outlet (3-prong). 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.
- <sup>1</sup> 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 reserves the right to change specifications without prior notice.
- Use of this instrument is for research purposes only.

# **Handling Micropipettes**

Failure to comply with any of the following precautions may result in injury to the users of this instrument as well as those working in the general area near the instrument.

- The micropipettes used with this instrument are very sharp and relatively fragile. Avoid contact with micropipette tips to prevent accidentally impaling yourself.
- Always dispose of micropipettes by placing them into a well-marked, spill-proof "sharps" container.

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# 1. INTRODUCTION

Welcome to a new era in Patch-Clamp Technology! Our passion is creating the next generation of electrophysiology research instrumentation. With over two centuries of combined experience from across the patch-clamp industry, our experienced team has designed a new microelectrode amplifierrecording system that is powerful enough to satisfy experienced patch-clampers, yet easy-to-use for new entrants, such as Molecular Biologists.

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 now bring this same commitment to the next generation of patch-clamp instrumentation.

# Overview

# Features

- Single program for data acquisition, data analysis and amplifier control
- Sophisticated data file structure with context retention and metadata capabilities
- Both online and offline data analysis
- Complex experimental control

# **Advanced Design**

The IPA<sup>™</sup> Integrated Patch Amplifier is the world's first fully integrated microelectrode patchclamp system, which minimizes and streamlines your experimental setup. All of the electronics and cabling used in stimulating, monitoring, compensating and recording from cells are integrated by design into a single-board instrument, the IPA.

The accompanying SutterPatch<sup>™</sup> 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 7 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, the IPA was designed as a fully integrated computer-controlled patch-clamp amplifier-recording system. Your immediate benefits are an affordable, easier-to-use, easier-to-setup, more robust, and more flexible patch-clamp system for your electrophysiology experiments.

# Software Feature Comparison

The SutterPatch program is a full-featured electrophysiology software package. In spite of a number of conceptual similarities that help shortening the training, there are several major differences in how the SutterPatch software compares to the Axon Instruments conventional electrophysiology software, and some smaller differences compared to the HEKA electrophysiology software:

Topic	Sutter	Axon	HEKA	Sutter
	Instrument	Instruments	Elektronik	Advantages
Software Programs	SutterPatch	pCLAMP: Clampex, Clampfit; MultiClamp 700B Commander	PATCHMASTER, FITMASTER	A single program for amplifier con- trol, data acquisi- tion, data man- agement and data analysis
Data Files	One "experiment" data file (*.pxp) for all data record- ings per cell or per day	One data file (*.abf) per protocol execution. Multi- ple data files be- longing to the same recording have no intrinsic connection.	One "bundle" data file (*.dat) for all data recordings per day or a longer pe- riod	All acquisition controls and con- texts saved with data in a compre- hensive experiment file
Flow Control	Paradigms: com- plex conditional links	Sequencing Keys: simple "go to" links	Protocols: complex conditional links	Create sophisticat- ed assays and ex- perimental pathways
Sampling Rates	Differing input and output rates	Same input and output rates	Same input and output rates	Flexible acquisi- tion parameters for data through- put and data stor- age concerns
Command Waveform	50 "segments" per sweep	10 "epochs" per sweep	Unlimited "segments" per sweep	Extended resolu- tion of the com- mand waveform

Table 1-1. Software Comparison

# **1.1 Experiment Structure**

#### Experiment:

An Experiment is the highest-level structure in the SutterPatch world. An Experiment file (\*.pxp) can encompass all SutterPatch activity for the entire day, such as instructions (Paradigms), settings, data acquisition parameters (Routines), recorded data (Series), program history and comments. During reanalysis, data can be included from multiple experiments.

Some users may prefer to start a new Experiment for each new preparation, or even each new cell they record. To avoid clutter, it is recommended not to collect data from more than one day in a single Experiment file.

#### Paradigm:

A Paradigm is a sequence of control instructions in an Experiment. Every Experiment contains at least one Paradigm.

Multiple Paradigms can exist in a Paradigm "pool". To acquire data, a Paradigm runs one or multiple Routines.

Paradigms can contain simple or sophisticated control structures using a rich set of operators. Some of the operations include conditional processing via if-then statements, nested loops, userdefined variables and hardware commands.

#### Paradigm Data:

The collection of all data points and metadata event tags acquired during the execution of a paradigm is referred to as Paradigm Data. A copy of the paradigm is stored as part of the Paradigm Data and enables repetition of the Paradigm the same way it was originally executed.

#### Routine:

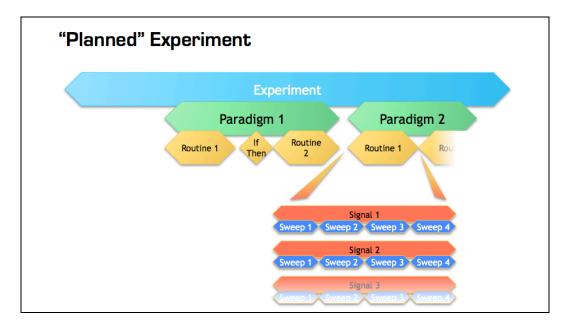
A Routine is the set of data acquisition and online data analysis parameters that control input and output channel timing, triggering, command waveforms, leak subtraction and display.

Multiple Routines can be held in a Routine "pool" for rapid access and execution.

#### Series (= Routine Data):

Acquiring data produces a Series composed of sweeps of data from all input signals. Multiple runs of a Routine produce multiple Series of data. All Series are automatically stored in the Experiment file.

In a planned Experiment, running a Paradigm will automatically execute any included Routines. However, if a Routine is manually executed while no Paradigm is running, an "auto-triggered" Paradigm is started; this default Paradigm ensures that each Series is associated with a Paradigm in the context of an Experiment. See Figure 1-1



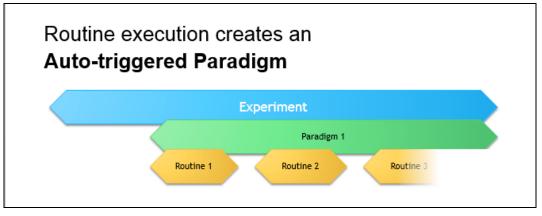


Figure 1-1. SutterPatch execution paths

Top: A planned Experiment with two Paradigms that automatically control Routines. Bottom: Manual execution of a Routine outside a Paradigm creates a default "auto-triggered" Paradigm for that Routine.

#### Channel:

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

Input channels are used to record data, and are displayed in their own panes in the Scope window. There are two dedicated analog input channels for Current and Voltage measurements from the IPA headstage, and four Auxiliary analog input channels. These are connected to BNC cables using either the standard breakout cable or the available Sutter Patch Panel.

Output channels are used to send electrical stimuli, such as analog command waveforms. There is one dedicated, internally configured, analog output channel for Stimulus commands to the IPA headstage, and two Auxiliary (BNC) analog output channels. These are connected to BNC cables using either the standard breakout cable or the available Sutter Patch Panel.

Digital Output bits are also referred to as TTL output "channels". There are 8 digital output channels available, which are connected to BNC cables using either the standard breakout cable or the available Sutter Patch Panel.

#### Signal:

Named analog input and output channels are referred to as Signals in the program. A Signal is either the logical representation of a physical channel, or the result of a computation that generates an equivalent number of samples.

#### Sweep:

A Sweep is the data from all Signals, triggered from a time zero, that occur before the next trigger.

#### Segment:

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

#### Metadata:

Metadata are additional information associated with stored raw data. These can include information about preparation (cell/tissue/animal), instrumentation (hardware and software), environmental parameters (temperature, atmospheric composition, etc.), stimuli (chemical compounds, light, acoustic, etc.) and other parameters. Metadata information is associated with each paradigm and dynamically recorded with a timestamp during an experiment. Information that can automatically be determined, such as the SutterPatch version, user Login Name, or the change of a digital output level, are automatically recorded without user intervention. See the Metadata section for more details.

# **Terminology Comparison:**

A table of equivalent terms to other electrophysiology software packages:

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

Table	1-2	Software	Termino	logv
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# 2. INSTALLATION

# 2.1 System Requirements

Operating System:	Windows 7, 8, 10: 64-bit versions
Check your OS version via:	Control Panel > System settings.
Mac OS X:	10.11 (El Capitan)
Check your OS version via:	Apple > About this Mac menu
CPU (Central Processing Unit):	Dual-core
RAM (Random Access Memory):	3 GB
Hard Disk Free Space:	$500  ext{ GB}$
Display Resolution:	1024 x 768 (XGA)
Computer Ports:	(1) USB 2 high-speed or USB 3 port*

\* USB 2 computer ports are usually implemented with a 'high-speed' transfer rate, but a slower 'fullspeed' specification can sometimes be found on older PC (Windows) computers or USB add-in cards. To check for high-speed USB 2 or USB 3 on a PC computer running Windows, look in the Control Panel / Device Manager / Universal Serial Bus controllers section for "*Enhanced*" host controllers. As this does not provide any mapping information to the computer's physical ports, and there can be a mix of USB port versions, you should check individual USB ports for USB 2/3 high-speed operational performance.

# 2.2 SutterPatch System Environment

Igor Pro 7 is the system environment that runs the SutterPatch software on your Windows or Mac OS X computer. Igor Pro is widely used to acquire and analyze scientific data, and create publicationquality presentation graphics. Igor Pro 7 is automatically installed with the SutterPatch software.

Igor Pro features include:

- High-quality presentation graphics
- High-speed data display
- Large data set handling
- · An extensive set of built-in data analyses-
- Waveform arithmetic
- · Image display and processing
- · Graphical and command-line user interfaces
- Automation

• Extensibility via C and C++ modules

Igor functionality can be further explored via its 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 space. (4) Rack screws are needed (not 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, 120 V 50 Hz, 240V

The IPA amplifier will run with either 120 or 240 VAC power, no switches need be set.

However, the AC power should be as clean as possible. If you experience brownouts or voltage sags, a switching power supply (SPS) can be used to supply clean power to your instruments. In case of brief power interruptions, a universal power supply (UPS) can provide stable power levels. At a minimum, a surge protector should be used. The building's electrical grid should have an excellent earth ground; if not, consider creating your own physical earth ground for your laboratory rigs.

- 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.

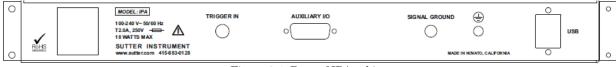


Figure 2-1. Rear of IPA cabinet

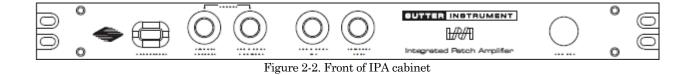
Power Switch

A power button for the IPA amplifier is located on its front panel. The silver power button lights up blue when the power is ON.

#### 2.5 Install Hardware

- 1. Start with the IPA power button OFF (unlit position).
- 2. Plug the IPA headstage into the HEADSTAGE port on the front of the IPA amplifier.
- 3. Plug the IPA breakout cable into the AUXILIARY I/O port on the back of the IPA amplifier's rear panel.

4. Connect the supplied USB cable to the computer's USB and the IPA amplifier's rear panel USB port.



# Initial Set-Up

# 2.6 Install Software

- 1. Power on the computer.
- 2. It is strongly recommended to use the latest SutterPatch Software version available online. Download the latest version of SutterPatch installer software and USB driver from <u>www.sutter.com/AMPLIFIERS/SutterPatch.html</u> and choose the "Download" tab. Navigate your file browser to the downloaded files.
- 3. If internet access is not available, attach the included USB flash drive to your computer's USB port, and navigate to the flash drive.

#### Windows

- 1. Double click on 'SutterPatch\_installer\_with\_Igor' to install the software for 'All Users'.
- 2. Follow the installer prompts.
- i. If an old version of Igor is found, choose to delete or keep it.
- ii. If an existing version of Igor 7 is found, choose to replace or keep it.
- 3. The installer should report a successful installation.
- 4. Launch Igor Pro and activate the Igor license as instructed. You will need your Igor Pro 7 Serial Number and Activation Key from the sticker included in the Quick Start Guide.
- 5. Safely eject the Flash Drive and remove it from the computer.

#### Macintosh

- 1. Double click on 'setupIgor7.exe' to install the software for 'All Users'.
- 2. Follow the installer prompts
  - i. If an old version of Igor is found, choose to delete or keep it.
  - ii. If an existing version of Igor 7 is found, choose to replace or keep it.
- 3. The installer should report a successful installation.

- 4. Launch Igor Pro and activate the Igor license as instructed. You will need your Igor Pro 7 Serial Number and Activation Key from the sticker included in the Quick Start Guide.
- 5. Safely eject the Flash Drive and remove it from the computer.

# 2.7 INSTALL DRIVER

# Windows

- 1. Power on the IPA amplifier.
- 2. Wait  $\sim$ 3 seconds for the USB module to load.
- 3. If asked for driver files:
  - a. Browse to the "C:/Program Files/SutterPatch" folder on your computer.
  - b. Select 'Next'.
  - c. You should see 'Windows has successfully updated your driver software'.
- 4. If computer does not ask for files:
  - a. Open the Windows Device Manager.
  - b. Right-click on 'Sutter Patch Clamp Amplifier-IPA'.
  - c. Click 'Update Driver'.
  - d. Select 'Browse my computer for driver software', and browse to the "C:/Program Files/SutterPatch" folder on your computer.
  - e. Select 'Next'.
  - f. You should see the message "Windows has successfully updated your driver software".
- 5. Safely eject the flash drive and remove from the computer.

#### Macintosh

No driver installation is necessary on a Macintosh computer.

#### 2.8 Test System

#### Install Model Cell

- 1. Attach the model cell to the headstage and tighten its collar.
- 2. Attach the 1 mm ends of the supplied grounding wire into the gold sockets on the headstage and model cell.
- 3. Completely surround the model cell/headstage assembly with electromagnetic shielding, such as aluminum foil.

4. Connect the shielding material to the headstage ground connector. This can be done using a short wire with alligator clips on both ends.

### Startup

- 1. Power ON the IPA amplifier by pressing the silver POWER button on its front it lights up.
- 2. Launch the SutterPatch application.
- 3. Click on the 'Start' button in the SutterPatch Welcome window.
- 4. SutterPatch loads up in DEMO mode if the IPA amplifier is OFF or disconnected from the computer.
- 5. Create an Experiment file name when prompted.
- 6. Check the IPA Control Panel.
  - a. If the title bar in the IPA Control Panel reads DEMO (see **Error! Reference source not found.**), uncheck SutterPatch > Demo Mode and wait for about 3 seconds.
  - b. Make sure that the IPA Control panel is in the voltage-clamp operational mode - click on the VC button at the top of the IPA Control panel.

IPA Control DEMO
V 0.0 mV I 0.0 pA VC CC ■ Seal (1) Offset lock
VC CC I/O hide
Holding (mV) 0
Cell Compensation Rs (MΩ) 0.0 ♀ Cm (pF) 0.0 ♀
Rs Correction Corr (%) 0
Gain/Filter Gain 5 mV/pA ▼
Filter 5 kHz 💌

Figure 2-3. IPA Control Panel

- 7. Run a Membrane Test
  - 1. Go to the Dashboard window:

#### Click on the Acquire Data icon.

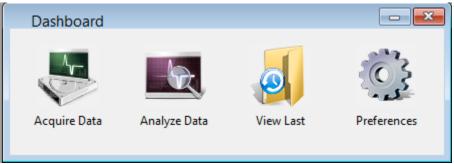


Figure 2-4. Dashboard – Acquire Data

Click on the Membrane Test icon.

Dashboard	uire Data		
Membrane Test	Routines	Paradigms	Set Metadata

Figure 2-5. Dashboard – Membrane Test

The Membrane Test is used to do a quick check of the basic IPA functionality. It tests the three basic steps necessary for recording in a whole-cell configuration. These test values assume a 5 kHz filter.

2. Select the BATH mode.

This mode tests simulating an electrode in the bath solution and sending a pulse through the solution.

Set the Model Cell switch to 'Bath'. Click on the Analysis window 'Bath' button.

Verify this reading:

- Pipette Resistance:  $\sim 10 \text{ M}\Omega$
- 2. Select the SEAL mode.

This mode tests simulating an electrode after making contact with a cell and forming a high-resistance gigaohm seal to the membrane.

Set the Model Cell switch to 'Seal'. Click on the Analysis window 'Seal' button.

Verify this reading:

- Seal Resistance: > 10,000 MΩ ٠
- 3. Select the CELL mode.

This mode tests simulating an electrode after breaking into a cell and achieving a successful whole-cell patch.

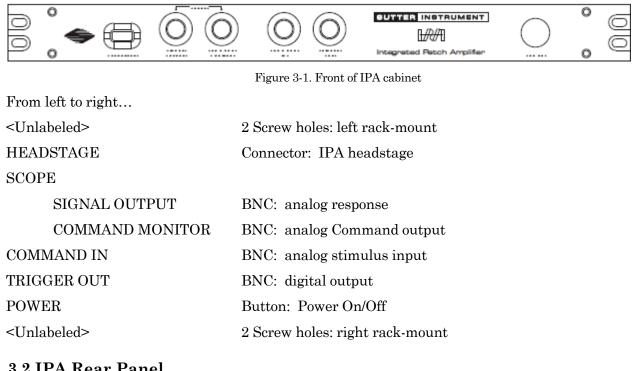
Set the Model Cell switch to 'Cell'. Click on the Analysis window 'Cell' button.

Verify these readings:

- Series Resistance: • ~10 MΩ
- Membrane Resistance:  $\sim 500 \text{ M}\Omega$
- Membrane Capacitance: ~28 pF •

# **3. HARDWARE OPERATION**

#### **3.1 IPA Front Panel**



# **3.2 IPA Rear Panel**

0	$\checkmark$	MODEL: IPA 100-240 V- 50/60 Hz T2.0A, 250V			USB	0
0		SUTTER INSTRUMENT www.sutter.com 415683-0128		MADE IN NOVATO, CALIFORNIA	<u> </u>	0

#### Figure 3-2. Rear of IPA cabinet

<unlabeled></unlabeled>	Power and fuse receptacle
TRIGGER IN	BNC: digital input
AUXILIARY I/O	D-Sub 15 connector: analog and digital channels
SIGNAL GROUND	Banana socket
EARTH GROUND	Banana socket: chassis ground to earth
USB	Type B receptacle: computer communications

# **3.3 IPA Control Panel**

This is the software interface to manually control the IPA amplifier operations. It replaces all of the physical knobs, dials and meters found on non-computer-controlled amplifiers.

Tip: Most of these settings can be programmatically controlled via a Paradigm.

V I	The 'V' meter displays the reading of the Voltage input channel. The 'T' meter displays the reading of the Current input channel.		
VC CC	<ul> <li>The 'CC' button swith</li> <li>The 'Reset USB' but channel between the</li> <li>A green button in System has been</li> </ul>	icches the IPA amplifier into Voltage Clamp mode. Icches the IPA amplifier into Current Clamp mode. Icches the IPA amplifier into Current Clamp mode. Icches the state of the USB communication e computer and the IPA system. Indicates that a stable USB connection to the IPA in established. Iccates that there is no USB connection to the IPA	
Seal (n)	panel. Use to monit	cton to conveniently run a seal test from the IPA Control for seal formation, from pipette resistance in the bath, to in contacting the cell.	
	This button displays the seal test pulse value. The slider beneath it indicates if it is the 'low' or 'high' level of the test pulse. Switch between the low/high states by clicking the slider's 'low' or 'high' region, or by clicking and dragging the slider bar.		
	VC Mode: CC Mode:	`The low/high values will be in mV. The low/high values will be multiplied by 2 pA.	

View the Seal signals by connecting the IPA amplifier 'SCOPE' BNC's to an oscilloscope. Numeric responses are also displayed in the Control panel V and I meters.

Note: The hardware seal test in the IPA Control panel uses different settings than the Membrane Test described in Chapter 4.

#### Right-click menu:

•	Reset USB	Re-establish t	he USB	connection
---	-----------	----------------	--------	------------

• Set Seal Test Configure the IPA amplifier seal test pulse amplitudes. Both high and low values can be specified. The actual amplitude of the seal test pulse depends on the selected gain.

> VC mode: The value will be multiplied by 1 mV. CC mode: The value will be multiplied by 2 pA.

- Reset Control Panel Set the IPA Control panel back to its factory default settings.
- Offset A pipette offset should initially be applied in voltage-clamp mode when the pipette is placed into the bath solution. This adjusts the pipette current signal to zero, to provide a proper reference baseline for accurate measurements. This offset counteracts any inherent hardware circuitry offsets, as well as pipette-in-thesolution offsets.

The amount of offset applied is displayed in the numeric field below the Offset button.

- Click on the Offset button to auto-zero the signal. (In Demo mode, the value is set to -0.2 mV.) This is an approximate value and might need further adjustment.
- For fine adjustments, use the numeric field up and down spinners to increase or decrease the setting by  $\sim 0.015$  mV.
- For moderate adjustments, increase the spinner increment to ~0.15 mV by holding down the Shift key and simultaneously clicking on the spinners.
- For fastest operation, hover over the numeric field or spinners, and hold down the Shift key while simultaneously scrolling up or down with the mouse wheel.
- Numeric values can also be directly typed into the numeric field.
- Range: ±500 mV
- Lock Once a pipette offset has been applied, use the 'Lock' check box to prevent any accidental changes to the Offset.

VC The 'VC' tab displays the Voltage Clamp controls of the IPA:

Holding (mV):  $\pm 1000$ 

After achieving a seal, set the cell's equilibrium or membrane "resting" potential (typically -60 to -80 mV for neurons).

#### Electrode Compensation

Mag (pF):	0 - 25.0
Phase (%):	0 - 100
Auto	Automatically sets approximate values

After seal formation (i.e., in a cell-attached patch configuration), compensate for the microelectrode pipette capacitive transients by iteratively adjusting the controls. For a square pulse (Seal Test) command, the goal is to eliminate the sharp (fast) transients and reproduce a square waveform without distortion.

Note: Rs Correction must be disabled during Electrode Compensation

#### Cell Compensation

Rs (M $\Omega$ ):	0 - 100.0
Cm (pF):	0 - 100.0
Auto	Automatically sets approximate values

After breaking into a cell (going "whole cell"), the membrane of the entire cell generates capacitive spikes that include a significant slow decay phase on the signal. Iteratively adjust the controls until adequate compensation results in a flat response.

Note: Rs Correction must be disabled during Cell Compensation

#### **Rs** Correction

Corr (%):	0 - 100
Pred (%):	0 - 99
Lag (us):	20 - 200

Series Resistance Correction is used to speed up a slowed rise time of the current response. As the Rs Correction/Prediction settings are increased to sharpen the rise time, transients will start to develop; avoid oscillation of the signal by adjusting the lag setting.

#### Gain/Filter

Gain (mV/pA):	(Range)
0.5	±20 nA
1	±10 nA
2.5	±4 nA
5	±2 nA
10	±1 nA
25	±400 pA
Filter (kHz):	0.5, 1, 2, 5, 10, 20

The IPA digitizer gain and filter settings apply to all input and output INTEGRATED PATCH AMPLIFIER OPERATION - MANUAL - REV. 1.0.6 (20160729)

channels.

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

H. Current (pA):  $\pm 1000$ 

A non-zero Holding Current can be pre-set here. Use the Enable button to activate it when desired.

Electrode Compensation Mag (pF): 0-25.0Phase: 0-100

> After seal formation (i.e., in a cell-attached patch configuration), compensate for the microelectrode pipette capacitive transients by iteratively adjusting the controls. For a square pulse (Seal Test) command, the goal is to eliminate the sharp (fast) transients and reproduce a square waveform without distortion.

#### Bridge Balance

Resistance (M $\Omega$ ): 0 - 200.0

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

#### Tracking

Potential (mV):  $\pm 100$ 

Tracking is used to keep the membrane voltage at a preset level without drifting over time. Set the desired voltage potential.

#### Gain/Filter

Gain (mV/mV)	(Range)
10	$\pm 1 \text{ V}$
20	$\pm 500 \text{ mV}$
50	$\pm 200 \text{ mV}$
100	$\pm 100 \text{ mV}$
200	$\pm 50 \text{ mV}$
500	$\pm 20 \text{ mV}$

I/O

The 'I/O' tab displays the Digital Outputs controls, and the Auxiliary controls:

#### **Digital Output**

This section controls the Digital Output holding bit pattern generated by the amplifier. Eight 'high' and 'low' values are displayed, based on the slider beneath it. Switch between the two states by clicking the slider's 'low' or

'high' region, or by clicking and dragging the slider bar.

1 - 8	digital channels		
High:	red dot =	$+5 \mathrm{V}$	
Low:	black dot =	0 V	

Auxiliary I/O

The IPA system can be used as a digitizer for external instruments. To support this usage, analog holding levels can be set on the two auxiliary analog output channels.

Aux Out 1 & 2:	±10.000 Select an auxiliary analog output channel from the drop-down list. Directly edit the holding value, or use the spinners to change the holding value by 0.100 increments.
Set	Click the 'Set' button to apply the value to the selected output channel.
External input char	nnels can also be monitored here. This is useful to take a single reading of a channel, such as a temperature.
Aux In 1 - 4:	[±10.000] (Read Only)
	Select an auxiliary analog input channel from the drop- down list.
Read	Click the 'Read' button to display the level of the selected input channel.

.

The Show/Hide button collapses or expands the VC / CC / I/O panes.

# 3.4 Peripheral I/O

A variety of external instruments can be controlled by the IPA system. These include items such as:

- solution changers
- wavelength switchers
- pulse generators

Both analog and digital I/O signals can be used to control these items. Digital output patterns are configured in the Routine Editor's 'Acquisition & Routine Parameters' section, and Output Channels & Waveform / Waveform Editor.

Analog and digital holding levels are set in the IPA Control panel I/O tab.

# **3.5 External Amplifiers**

The IPA system can support external (non-IPA) amplifiers by operating as a stand-alone digitizer. Use the BNC's located on the Auxiliary cable (attached to the IPA back panel Auxiliary I/O port).

Analog and digital holding levels are set in the IPA Control panel I/O tab.

Analog stimulus waveforms can be sent from a SutterPatch routine via two auxiliary analog output channels:

AuxOUT 1 & 2

Digital output patterns can be sent from a SutterPatch routine via eight digital output channels:

DigOUT 1 - 8

Input signals can be read and recorded by four auxiliary analog input channels:

AuxIN 1-4

# **3.6 External Digitizers**

The IPA system can also be operated as a stand-alone amplifier interfacing to non-SutterPatch data acquisition systems.

Connect the external digitizer's analog channels to the IPA amplifier front panel:

BNCChannelCOMMAND IN:to the preparationSCOPE - SIGNAL OUTPUT:from the preparationSCOPE - COMMAND MONITOR:from the Command signal

You continue to control the IPA amplifier via the IPA Control panel.

# 4. SOFTWARE OPERATIONS

# WINDOWS DIALOGS AND PANELS

# 4.1 Dashboard Panel

The Dashboard panel provides a convenient gateway to key areas of the SutterPatch application:

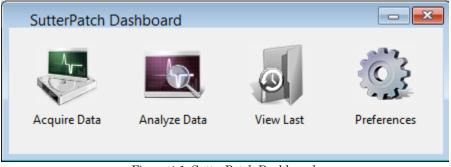


Figure 4-1. SutterPatch Dashboard. INTEGRATED PATCH AMPLIFIER OPERATION - MANUAL – Rev. 1.0.6 (20160729)

- Acquire Data: Panel to configure the recording of data
  - Analyze Data Data tree structure of the experiment's data
  - View Last Opens the last recorded data Series in the Experiment
  - Preferences Configuration of program preferences

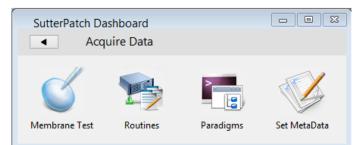


Figure 4-X. SutterPatch Dashboard.

 Acquire Data: Membrane Test Routines
 Paradigms Set Metadata
 Seal formation and cell health Recording & analysis settings
 Flow control steps
 Data-associated information

# 4.2 Scope Window - Data Acquisition

The Scope window provides the central view of your data, for data acquisition, data analysis, and Membrane Test signals. Note that while many of the window controls are common to all Scope windows, each type of Scope window has its own set of active buttons.

[TIP: Only one Scope window can be open at a time. If the Scope window is open for data acquisition, running a Membrane Test or opening data for analysis will close the acquisition Scope window and open a new Scope window for the Membrane Test or data analysis.]

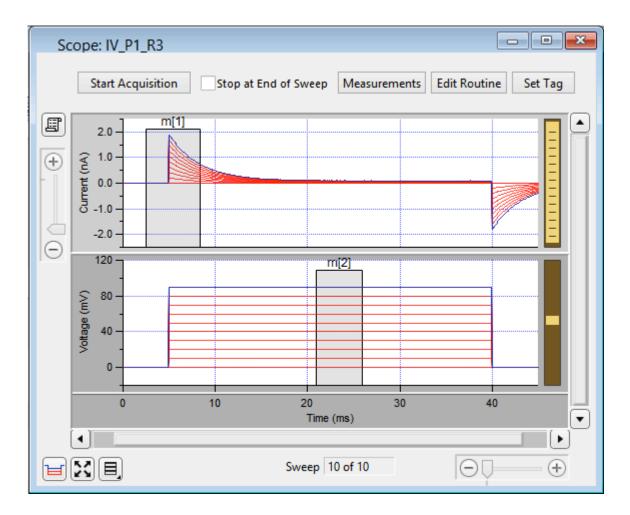
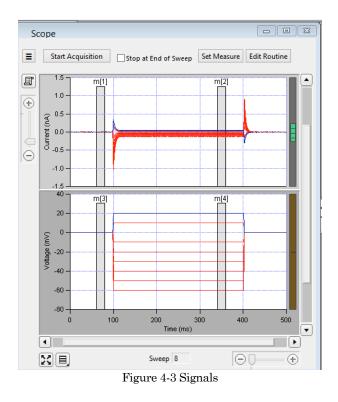


Figure 4-2 Scope View of SutterPatch

#### Signals

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

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



Select signal display mode

Signal pane arrangements are available via the 'Select signal display mode' button in the lower left corner of the Scope window:

- Single pane (applies to the active pane)
- Vertically stacked panes
- Tiled configurations: [m x n]

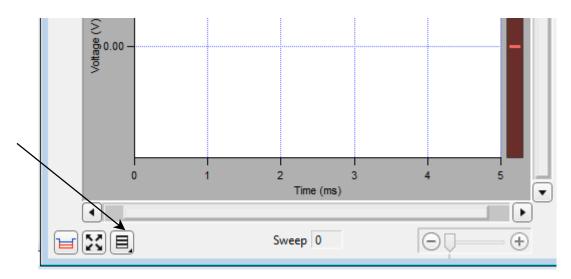


Figure 4-4. Signal Display Mode

#### Magnification & Scrolling

Signals can be magnified or unmagnified using several X- and Y-axis display controls in the Scope window:

Magnification combo:



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

• Axes zoom:

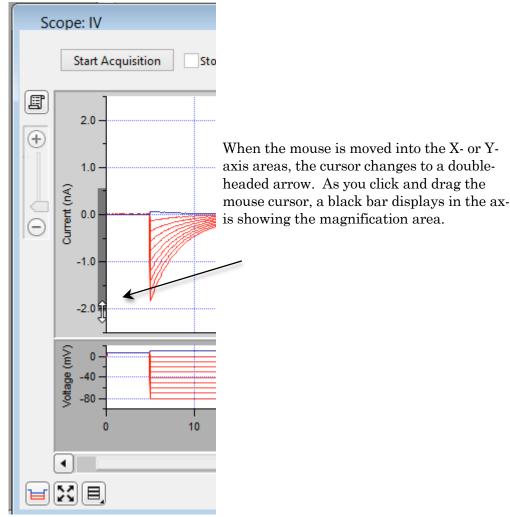


Figure 4-5. Axis magnification

• Area zoom:

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

- 1. Move the mouse cursor into a signal pane, and it changes into a large "+".
- 2. Click and drag a bounding box around the area of interest.
- 3. Right-click in the bounding box and select 'Expand'.
- 4. The selected area fills the entire pane.

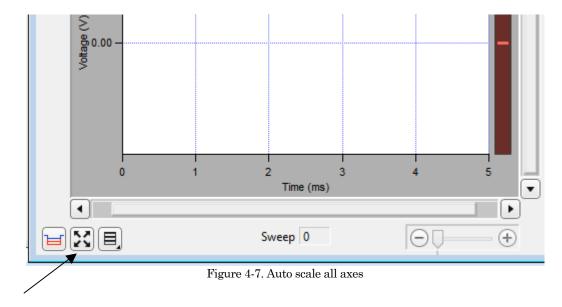
Note: For signal panes without an X-axis bar, the zoomed data is displayed independently of any X-axis.

 Image: second second

• Axis scroll bars:

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

Autoscale all axes:



The 'Autoscale all axes' button is a convenient way to return magnified signals to an auto-scaled view. This button applies to all visible signals.

#### Amplitude Meters

Amplitude meters occupy the right border of signal panes. They provide visual feedback on the integrity of 'Triggered Sweeps' data recording, similarly to how audiometers monitor audio signals.

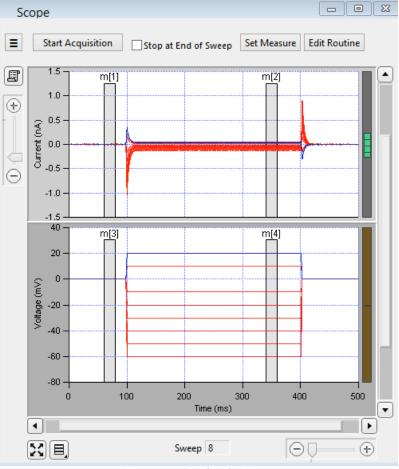


Figure 4-8. Amplitude Meters

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's "health".

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

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



When the recorded data is within proper amplitude limits, the amplitude meter will be displayed in green.



If too much hardware gain is applied, the recorded signal will "saturate", i.e. your data will exceed the amplitude limits of the digitizer. In this case, those data points are substituted with the maximum amplitudes of their input channel, thus creating a straight horizontal line, and the amplitude meter will be displayed in red, showing that the data went out of range and are not valid.

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When there is a danger that saturation will occur, and the data is near saturation levels, the amplitude meter will be displayed in yellow, as a warning sign to decrease your hardware gain.

If too little hardware gain is applied, the recorded signal may have insufficient amplitude resolution. This means that the digitizer is not utilizing enough bits to accurately represent the data, and your signal of interest may be contaminated with digitizer noise. When this occurs, the amplitude meter will display a thin yellow line, as a warning to increase your hardware gain.

# Cursors

Vertical measurement cursors can be set within the input signal panes. Each measurement cursor has a measurement width, with a start time and an end time, over which analyses can be made.

In the Scope window, measurement cursors can be graphically repositioned - left-click on a measurement cursor to select it (the shaded cursor area turns black) and hold and drag the mouse cursor to the new location, and when you release the mouse button, the measurement cursor moves to the new position.

#### Buttons

Start Acquisition	Clicking the button displays digitized analog data in the Scope win- dow. In the data acquisition and membrane test modes, live digitiza- tion occurs for your input signals.
	When you click the 'Start Acquisition' button, data recording starts, and the button then changes into the 'Stop Acquisition' button. For both the online data acquisition and offline re-analysis modes, an asso- ciated Analysis window is displayed that plots measurements made upon the digitized data.
	If the ' <b>Stop Acquisition</b> ' button is clicked, data acquisition will stop, and the button will change back to the ' <b>Start Acquisition</b> ' button
Stop at End of Sweep	Determines how data acquisition is terminated. When the 'Stop Ac- quisition' button is clicked, data acquisition is immediately halted, even if this occurs in the middle of a sweep.
	In this case, any partial sweep that was in progress is not saved with the data.
	However, if the <b>'Stop at End of Sweep'</b> checkbox is enabled, then the current sweep will complete before data acquisition is stopped, and the last recorded sweep will be a complete sweep of data. If no sweep is in progress, acquisition will stop at the end of the next sweep to be recorded.
Measurements	Opens the Real Time Analysis dialog, in which you configure the Scope window cursors and their analysis measurements, or more-complex user-defined equations, for graphing in the Analysis window.
	See the Routine Editor: Real Time Measurements dialog for details.
	When accessed via the Set Measure button, changes to the dialog apply to the active routine in the Scope window, but are not written to the Update button in the dialog allows

Edit Routine	Opens the Routine Editor dialog, for configuration, management and activation of routines. Routines can be configured for:	
	Data acquisition parameters	
	Input channels	
	Output channels	
	Stimulus waveforms	
	Analysis measurements	
Set Tag	A time tag is stored in the data at the time you click this button. An optional text comment can be included. A black vertical cursor marks a tag in the data; see the metadata for any tag text.	
	Note: in "demo" mode, tag timing is not accurate, and no tags are set in the first sweep.	

# **Right-Click Menus**

Different areas of the Scope windows support additional Igor functionality though right-click menus. Note that these are not supported by Sutter Instrument, so use at your own discretion.

# 4.3 Scope Window - Membrane Test

The Membrane Test Scope window is used to monitor seal formation and cell health for a whole-cell voltage-clamp configuration.

The top signal pane displays a Current signal, corresponding to one of the three basic Membrane Test steps:

Bath	With the pipette in the bath solution, a square pulse is visible.
Seal	When an on-cell patch is formed between the pipette and the cell, sharp spikes occur at the square-pulse edges.
Cell	After a whole-cell patch is created, the capacitance spikes are ana- lyzed for membrane resistance and capacitance measures.

The bottom signal pane displays the Voltage command signal.

The Membrane Test Scope window operates similarly to the Data Acquisition Scope window, with some unused controls removed. The Persistence Display button is disabled, as the Membrane Test only supports non-persistent mode.

Membrane Test acquisition parameters are set in the Preferences dialog.

# 4.4 Scope Window - Free Run (Scope)

The Free Run (Scope) window simulates a one-channel oscilloscope. This provides you with a quick method of viewing repetitive data.

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

Select channel:Select an input channel from a list of all input channels.Select sampling frequency:1, 2, 5, 10, 20, 50 kHzInput sweep length (s):

The Free Run (Scope) window operates similarly to the Data Acquisition Scope window, with some unused controls removed. The Persistence Display button is disabled, as Free Run only supports non-persistent mode.

# 4.5 Scope Window - Data Analysis

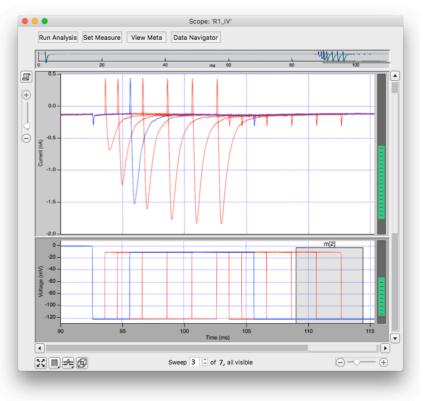


Figure 4-9. Reanalysis View of Scope Window

The analysis version of the Scope window is used to display and re-analyze stored data. Both physical and virtual channels can be displayed here. Measurement cursors are used as previously described in the data acquisition version of the Scope window.

The window controls that are different from the online data acquisition version of this window are described below.

# Navigation pane:

The Navigation pane is unique to the analysis Scope window. It provides an overview of the active signal's data, overlaid with a gray magnification box corresponding to the magnification applied to the signal.

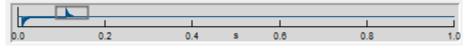


Figure 4-10. Navigation Pane.

The magnification box can be used as an alternate method of scrolling through the signal's data. When the mouse cursor is placed over the magnification box, it changes into a 'hand' icon, which can then be used to click and drag the magnification box throughout the navigation pane, in both the X and Y directions. As the magnification box is moved in the navigation pane, the active signal's data is scrolled to match its view.

	701 1 44	· · · · · · · · · · · · · · · · · · ·	
Run Analysis	The button re-runs previously defined analyses for the data series, and graphs the results in the Analysis window.		
Set Measure	Operates as previously described for the Scope window.		
View Meta	Displays any extra information (metadata) associated with the cur- rent data Series, such as the operator, preparation details, solution information, etc.		
Data Navigator	Opens a Data Navigator window. All of your experiment's data and metadata are available in a tree structure, such as Routine Series data and Paradigm data.		
Persistence Display	In persistence display mode, recorded sweeps are kept on display un- til another acquisition is started. Otherwise prior sweeps are cleared from the Scope window whenever a new sweep is recorded.		
Autoscale	Rescale the X and Y axes so that all data is visible within 90% of the Y-axis and 100% of the X axis.		
Signal Display	Select how input signals are graphically arranged.		
	Stack: a vertical column of signals		
_	Single: just show the active signal		
	m x n: tiled configurations of signals		
Sweeps Display	The 'Select sweeps display mode' button has 3 states		
	Sweeps	Each trace is displayed as starting at time zero, and overlaid for the duration of the ac-	

<b>A</b>	tive waveform, as defined in the routine's Waveform parameters.
Continuous	Sweeps are displayed from left to right against a continuous linear time axis. Any time between sweeps without data is left blank.
Concatenated	Sweeps are displayed similar to the Continu- ous mode, however any blank spaces between sweeps are replaced by a vertical line

Ø	The 'Show 3D view of current signal' button brings up a separate 3D display window attached to the right of the Analysis window. The Sweeps data is color coded for amplitude, and its 3D graph can be ro- tated in any direction.
Sweep #:	The 'Sweep #' display at the bottom of the Scope window indicates the active sweep, the total number of sweeps in the Series, and the number of visible sweeps.

# **Right-click Menus**

Different areas of the Scope windows support additional Igor functionality though right-click menus. Note that these are not supported by Sutter Instrument, so use at your own discretion.

# 4.6 Analysis Window

Analysis windows are docked to the right side of an associated Scope window. Although an Analysis window can be resized or closed, it is cannot be undocked from the Scope window, even when dragged. However, if the Analysis window is closed, it will not be included as a graph in a Layout.

1. Data Acquisition - Online Analysis

Online measurements are plotted in real-time in the Analysis window.

Analysis measurements are defined in the Set Measure dialog, accessed from the Scope window, or the routine editor Real Time Analysis section, or the paradigm editor. A separate pane is created in the Analysis window for each measurement enabled for graphing.

2. Re-Analysis - Offline Analysis

An Analysis window is also used for the display of previously recorded data. The last measurements applied to the data are automatically used to re-analyze the data, which are graphed in the Analysis window. Data can be selected for offline re-analysis via the Dashboard's Analyze Data / Data Navigator window.

3. Membrane Test

Select the Membrane Test mode via the Bath / Seal / Cell buttons in the Analysis window. The Membrane Test measurements are displayed in real-time numeric fields.

Bath	Pipette Resistance (M $\Omega$ )
	The pipette resistance should be very low if the tip is not clogged.
Seal	Seal Resistance (M <sub>Ω</sub> )
	The seal resistance increases as the seal forms. Hopefully, a good seal is achieved.
Cell	Series Resistance (M $\Omega$ )
	Membrane Resistance (M $\Omega$ )
	Membrane Capacitance (pF)
	The Series (Access) Resistance and Membrane Capacitance fields are not calcu- f 'Cell Compensation' is enabled in the IPA Control panel, and will display 'NaN' d.
Write Va	lues Click the 'Write Values' button to copy the measurements to the Log window.

The Membrane Test acquisition parameters are set in the Preferences dialog.

Note: In demo mode, the Cell Membrane Resistance value is 50 pF vs. the model cell value of 28 pF.

# 4.7 3D View Window

The 3D View window creates a 3D representation of your data, color-coded to show amplitude variations.

## 4.7.1 Axis Definitions

The axis definition in 3D View is based on the fact that it represents 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, therefore, 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 4-11).

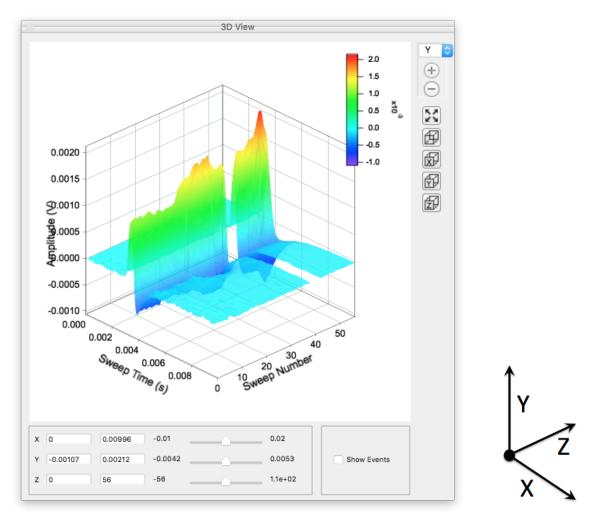


Figure 4-11. 3D View and axis definition.

- 1. A "heat map" bar illustrates the color measurement units.
- 2. Magnification buttons are located in the upper right corner of the window. A selection button allows X, Y, Z or "All" views to be selected for magnification. The Zoom In (magnify) and Zoom Out (unmagnified) buttons are located below, as well as the Autoscale button which resets all zoomed axes to the initial (default) magnification.

3. Axes limits can also be manually set to numeric values in the bottom section of the 3D View window. The 3D graph viewing angle can be changed with a set of 3D buttons:

Ø	= Default View	(X / Y / Z axes display)
Ø	X = Right View	(Y / Z axes display)
Ð	Y = Top View	(Z / X axes display)
Ð	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 during dragging, the 3D display will continue to rotate in the direction of the mouse drag.

The bottom section of the 3D View window has boxes containing the numeric axes limits, as well as scroll bars, for the X, Y and Z axes. The numeric axes limits update the visual graph limits, but are also updated by the axes zoom buttons. Clicking and dragging the scroll bars updates the visual graph, while maintaining the delta set in the numeric axes boxes.

'Show Events' checkbox

# 4.8 Layout Window

The Layout window is accessed from the Scope window 'Layout' button

The 'Layout window is used to prepare your data for publication. The Scope window input signals and associated Analysis window graphs are transferred to the Layout window for graphical arranging and editing.

A toolbar display in the upper-right corner of the window. There are two configuration buttons at the top: 'Operate Mode' and "Draw Mode'.

When you close the Layout window, you have the option of saving a name "window recreation macro". To later restore the window, go to the Igor menu 'Windows / Layout Macros" and select the macro name.

# 4.9 Command Window

The Command window is labeled with the currently loaded Experiment filename. This window is an Igor code interpreter, for programmatic interaction with SutterPatch.

Commands can be entered into the command buffer in the lower portion of the window. A history of commands and responses is displayed in the upper portion of the window.

A maximum of 400 characters can be entered into the command buffer, however they can be spread over multiple lines. Lines in the history section can be easily transferred into the command section for processing, by highlighting them and pressing the Enter key.

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 to change the relative sizes of these sections.

For more information, see the Programming chapter of the Igor Pro manual.

# 4.10 Log Window

The Log window lists a mix of time-stamped commands, responses, administrative information, and error messages. These entries provide a history of the experiment that is automatically saved with the experiment.

Default Log window columns

Date / Time:	The Date / Time-stamp format:	
	Year-Month-Day Hours:Minutes:Seconds	
	Example: 2016-04-25 14:21:34.478	
Event Type:	Log entries are assigned an Event Type:	
	Data Acquisition	
	History	
	Routine	
	Startup	
	WriteMeta	

Event Parameter:	A text description of the log entry line.
Paradigm Data / Series:	Paradigm, Routine and data Series names are displayed
Experiment Category:	The Experiment Category # value(s) set in the Metadata.

### Additional Log window columns

User Name:	The name of the user logged onto the computer.
Host Name:	The name of the computer.
OS & Version:	The operating system and its version number.
Software & Version:	The SutterPatch application name and version number.
Log ID:	A unique identifier to help sort Date / Time duplicates.

### Right-click menu (on a column header)

	Search for Text:	A Search Log dialog opens with multiple text search options.
	Select columns to show:	Enable or disable the display of any columns in the window.
	Sort on this column:	Alphabetically sorts the entire window based on the selected col- umn.
	Default view:	Restores the Log window's default view.
ht-	click menu (on a Series data :	row)

Right-click menu (on a Series data row)

Analyze Series: Opens the named Series data into a Scope window for analysis. Right-click menu (on a Paradigm data row)

Review Paradigm Data:

Opens the named Paradigm data into a Paradigm Review window for review.

Each column can be sorted by clicking on its column header. Clicking on the column header a second time reverses the sort order.

Note: The timestamp has a resolution of 1 ms, and multiple entries can occur within this timespan. This may create an unreliable sort order when sorting by Date/Time. To sort log entries in the order they were created, right-click on the column header and select Default View. Alternatively, right-click on the column headers, then click Select Columns to Show and check the Log ID column. This allows you to sort either ascending or descending.

Default View restores the default selection of columns and the default sort order (descending by time of creation). Any filtering applied by text search is cleared.

# DIALOGS

# 4.11 Routine Editor

The Routine Editor is the central place to create and manage your Routine Pools and key data acquisition settings, such as sampling rate, stimulus waveform and I/O channels.

SutterPatch Routine Editor	
Hide Routine Editor Activated Routine = NONE	0
Routine Pool Files 👻	
Comment	
	Routine Settings Acquisition & Routine Parameters
Save Pool	Input Channels Output Channels & Waveform
New Routine	Real Time Measurements & Graphs
Rename	
Duplicate	
Delete	
Revert <	>
▼ Hide Waveform Preview	
	Sweep Interval: 1 s
	Sweep Duration: 0.005 s
e	Min Stim Duration: 0.005 s
S 0.00	Max Stim Duration: 0.005 s
0.00	Max Stim Points: 25
	Holding (display): 0 V
	Show Sweep All
0 1 2 3	4 5 Show Channel Stimulus
Time (ms)	Show Measure Region All

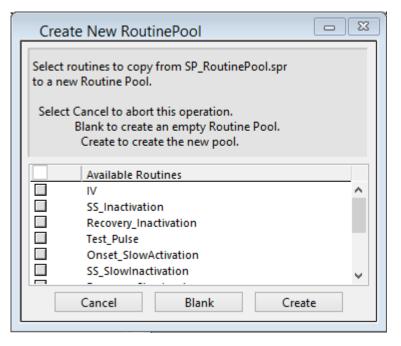
Figure 4-12. Routine Editor

### 46

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.

As the Routine Pool contents are held in memory, the execution times between routines are very fast. Routine names can include the following characters: A-Z, a-z, 0-9 and \_.

<u>Status Field</u>	A status field at the top of the dialog displays the name of the last load- ed, saved, or "activated" routine.	
<u>Routine Pool Files</u>	Drop-down list operations affect the entire "Routine Pool"	
	New Routine Pool	Create a blank Routine Pool, and optionally copy Routines over from the existing Routine Pool
	Load Routine Pool	Load the Routines of a previously saved Rou- tine Pool file into the Routine Pool
	Revert to Last Saved	Undo any unsaved changes to the Routine Pool
	Save Routine Pool	Save the Routine Pool using its existing file name and path.
	Save Routine Pool as	Save the Routine Pool to a new file, and switch to the new file. The default file name is the same as the original file name.
	Save Routine Pool Copy	Save the Routine Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to it.
	Merge Routine Pools	Insert the Routines from a previously saved Routine Pool file into the loaded Routine Pool



## File Path Field

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

### Comment

The Comment field allows you to save a text comment with a Routine Pool file.

### Routine Pool

The Routine Pool section lists the names of all currently loaded routines. Selecting a routine name loads it into the Routine Settings section for editing.

### Buttons

	Table 4-1. Toutine Editor Duttons
Activate:	Open the Scope window for recording using the selected Routine. This but- ton displays as "In Progress" during a recording. An activated routine has been loaded ('Activated') into the Scope window for data acquisition.
Save Pool:	Save the Routine Pool using its existing file name.
New Routine:	Add a blank Routine to the Routine Pool and optionally copy Routines over from the existing Routine Pool
Rename:	Edit the name of the selected Routine. Allowable characters are A-Z, a-z, 0-9, and "_".
Duplicate:	Add a copy of the selected Routine to the Routine Pool.
Delete:	Remove the selected Routine from the Routine Pool.
Revert	Discard any unsaved changes to the selected Routine.

Table 4-1. Routine Editor Buttons

# **Routine Settings**

The Routine Settings are split into 4 main sections. Click on a section header to display its sub-items and to open that section for editing.

Routine Settings	
Acquisition Parameters	$\sim$
Input Channels	
Current	
Voltage	
Output Channels & Waveform	
Stimulus	
🛨 Real Time Analysis	
	~

Figure 4-13. Routine Settings and sub-items

# **Waveform Preview**

A graphical preview of the stimulus waveform displays at the bottom of the Routine Editor. Some key settings and display controls are listed to the right of the waveform preview pane:

Sweep Interval:	The interval of time between the starts of consecutive sweeps, across all channels in the active routine.			
	section If the 'Sweep sta	he Acquisition & Routine Parameters art-to-start time' is set to 'Shortest Pos- is equal to the Min Stim Duration +		
Sweep Duration:	The amount of time in a sweep in which recording occurs with the active routine. This parameter can be set to either the Waveform Duration or the Acquisition Duration.			
	_	outine Settings in Output Channels & litor / Segment / Durations. It is always val.		
Min. Stim. Duration:	The minimum amount of time in which output stimulation occurs in a sweep.			
	This setting is based on Routine Settings in Output Channels & Waveform / Waveform Editor / Segment / Durations.			
Max. Stim. Duration:	The maximum amount of time in which output stimulation occurs in a sweep.			
	This setting is based on Routine Settings in Output Channels & Waveform / Waveform Editor / Segment / Durations.			
Max. Stim. Points:	The maximum number of sample points that can be output as a stimulus command.			
Show Sweep:	Control how sweeps are displayed in the preview pane. All displays are autoscaled. Sweep# a single sweep			
	All Sweeps Time Course:	<b>overlaid sweeps</b> all sweeps over a linear time axis		
Show Channel:	Select the output channel	to preview.		
Show Measure Region:	Select which measurement cursor to preview.			

# 4.12 Routine Editor: Acquisition & Routine Parameters

Many of the key acquisition parameters are controlled in this section. These include sweep duration and timing, digital output channel control, sampling rates, and leak subtraction.

Note that the controls in this section are common to all output channels; for control of individual output channels, see the Output Channels & Waveform section.

NewRoutine_2 Acquisition & Routine Parameters	×
External Trigger Action Nano	
External Trigger Action None 👻	
Acquisition Mode Stimulation	
✓ Digital Outputs set by 8-bit Word	
Use Waveform Durations	
Set Acquisition Duration (s)	
Number of Sweeps 1	
Sweep Cycles 1 🔷 Indefinite	
Sweep start-to-start time (s) 1	
Input Sampling Rate 5 kHz (200 µs) 💌	
Output Sampling Rate 5 kHz (200 µs)	
Enable Acquisition of Leak Pulses	
Leak Delay (ms)	
Enable AC Line Frequency adjustment	
50 Hz 60 Hz	
Execute Leak Pulses Before Main Pulse After Main Pulse	
Persistence Display	

Figure 4-14. Acquisition Parameters Menu

# **External Trigger Action**

Control how and when recordings occur using the external hardware digital trigger input (Trigger In' BNC) on the back panel of the IPA.

None:	Hardware trigger inputs are disabled.
Start Series:	A trigger pulse starts a recording, the same as clicking on the
	'Start' button
Start Sweep:	A trigger pulse starts a Series recording that halts after one sweep, and then repeatedly waits for the next trigger pulse to record the next sweep, until the routine is completed.

### Acquisition Mode

Triggered Sweeps:	Standard sweeps-oriented recording. Each sweep is started either by an internal trigger from a Routine or Paradigm, or an external trigger into the 'TRIGGER IN' connector on the back of the IPA amplifier. Each sweep corresponds to a single run of the Waveform Editor table. To account for triggering delays, there is a short gap (~100 ms) between sweeps.
Continuous Sweeps:	This option provides for continuous recording without any gaps between the data.

However, note that in this mode, the data recording is also specified in sweeps, as the Scope window uses a sweepsoriented display (vs. a continuous rolling display).

Note: in Demo mode, the Continuous data timing might not be accurate.

#### **Enable outputs**

Output channels are enabled by default, but can be optionally disabled during data acquisition.

#### Digital Outputs set by 8-bit Word

The 8-bit digital output channel pattern is controlled by a single decimal number from '0' to '255' in the Output Channels & Waveform / Waveform Editor. In the corresponding binary 8-bit number, each bit that is used corresponds to a high digital output signal (+5 V)

Otherwise, the Output Channel section lists each DigOut # (1 - 8) channel for setting to 'High' or 'Low' in its own Waveform Editor.

### **Acquisition Duration**

Waveform (s)

Acquire data for the duration of the waveform (set in the Output Channels & Waveform / Waveform Editor.)

Set Duration (s)

Acquire data for a fixed length of time that is greater than the waveform duration. This option must be used when no output channels are enabled, as no waveforms or their durations exist.

Sweep start-to-start time (s)

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

Shortest Possible

Sets the Sweep start-tostart time to the longest Acquisition Duration time, plus 100 ms for overhead processing.

## Automatically set based on Acquisition duration

Number of Sweeps	The number of sweeps to record.		
Sweep Cycles	The number of times to automatically repeat the entire set of sweeps to be recorded within a single Series.		
	Indefinite	Record an unlimited number of sweep cycles within the same Series.	
Input Sampling Rate:			
input bainping flate.	100 Hz 200 Hz 400 Hz 800 Hz 1 kHz 2 kHz 4 kHz 5 kHz 8 kHz 10 kHz 20 kHz 25 kHz 40 kHz 50 kHz	(10 ms) (5 ms) (2.5 ms) (2 ms) (1 ms) (500 $\mu$ s) (250 $\mu$ s) (200 $\mu$ s) (125 $\mu$ s) (100 $\mu$ s) (50 $\mu$ s) (40 $\mu$ s) (25 $\mu$ s) (20 $\mu$ s)	
Output Sampling Rate:			
	100 Hz 200 Hz 400 Hz 800 Hz 1 kHz 2 kHz 4 kHz 5 kHz 8 kHz 10 kHz	<ul> <li>(10 ms)</li> <li>(5 ms)</li> <li>(2.5 ms)</li> <li>(2 ms)</li> <li>(1 ms)</li> <li>(500 μs)</li> <li>(250 μs)</li> <li>(200 μs)</li> <li>(125 μs)</li> <li>(100 μs)</li> </ul>	

Note: the Input and Output channels can use different sampling rates.

[Tip: In electrophysiology, 10 kHz sampling rates are most commonly used, with 20 kHz occasionally used for faster events, and 5 kHz for slower events.]

### **Enable Acquisition of Leak Pulses**

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Currents can occur while a cell is in its resting state, such as from an imperfect seal or from existing ion channels, and affect the amplitudes of responses. These endogenous leak currents can be automatically removed from the data by using online leak subtraction.

A leak "pulse" is a copy of the main pulse (the stimulus waveform), and is used to record a portion 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.

Leak Delay (ms)

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 the contamination of the leak pulses by any active currents or inactivation.

The number of leak pulse, their amplitudes, and their waveform order are set in the Output Channels & Waveform section.

Enable A/C Line Frequency adjustment

AC line frequency noise (hum) can be automatically removed from P/N leak subtracted recordings.

- 50 Hz Enable the online removal of 50 cycle AC line noise.
- 60 Hz Enable the online removal of 60 cycle AC line noise.

The line frequency is monitored, and the leak sub-pulses are aligned to the same AC phase as the main pulse, prior to subtraction. In-phase repetitive AC line frequency noise is removed from the recording for up to 11 harmonics.

### **Execute Leak Pulses**

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

- Before Main Pulse
- After Main Pulse

### Persistence Display

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

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

# 4.13 Routine Editor: Input Channels

NewRoutine Input Channel Param	eters		×
Input Channel Current Voltage AuxIN1 AuxIN2 AuxIN3 AuxIN4	<	Channel Units A Channel Scaling Factor 1 Parent Output Channel StimOUT Scope Position 1	

### Input Channel

Select the analog input channels to be digitized and recorded from.

The default Current and Voltage input channels are hardwired to the IPA headstage. The remaining four auxiliary input channels allow you to directly digitize and record analog input signals from non-IPA external data sources.

### **Channel Units**

The channel units ('A' for Amps and 'V' for Voltage) are automatically assigned to the default Current and Voltage input channels. For the Auxiliary input channels, enable an Input Channel and select 'A' or 'V' as its channel unit from the drop-down list.

### **Channel Scaling Factor**

The built-in Current and Voltage input channels have a fixed scaling factor of '1'. The Auxiliary input channels allow a scaling factor to be set between '-1' and '1'.

Parent Output Channel

When Outputs are enabled (in the Acquisition Parameters section), every analog Input channel is associated with a "parent" analog Output channel.

So, when using an external amplifier with the IPA digitizer, use this setting to assign an Auxiliary Input channel its matching Output (stimulus) channel.

<u>Scope Position</u> The Input Channel's signal pane can be re-positioned in the Scope window.

# 4.14 Routine Editor: Output Channels & Waveform

Output Channels & Waveform

NewRoutine Output Channel Paramete	ers 🖾
Output Channel         StimOUT         AuxOut1         AuxOut2         DigOut1         DigOut3         DigOut5         DigOut7         DigOut8	Edit Waveform         Restrict to VC Mode         Output Units         V         Relative to Holding         ✓ Return to Holding at Sweep End         Enable P/N Leak Subtraction         Preview Leak Pulses         Alternating Leak Polarity         Num Leak Pulses         Leak Ratio         Leak Holding (mV)

Figure 4-15. New Routine

### Output Channel

Enable the analog and digital output channels to be used in the routine.

The default StimOUT channnel is hardwired to the IPA headstage. If a signal is connected to the front panel 'COMMAND IN' BNC, that signal is added to the StimOUT waveform sent to the headstage.

The two rear panel auxiliary analog output channels (AuxOUT#) can be used to send stimulus waveforms to other external instruments.

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

### Edit Waveform

Click the Edit Waveform button to access the waveform editor table and create a stimulus waveform. [see the Waveform Editor section below]

Restrict to VC or CC Mode

This setting prevents VC/CC routines from being *activated* in the Routine Editor, unless the IPA amplifier is in a matching (VC/CC) hardware state.

- For Voltage Clamp experiments, set to 'Restrict to VC Mode', and the Output Units are fixed at 'V' for voltages.
- For Current Clamp experiments, set to 'Restrict to CC Mode', and the Output Units are fixed at 'A' for amperes.
- For other types of experiments, set to 'No restriction', INTEGRATED PATCH AMPLIFIER OPERATION - MANUAL – REV. 1.0.6 (20160729)

[Tip: While a recording is in progress, the IPA amplifier can be switched into any state (VC/CC). However, it is the researcher's own responsibility for maintaining the health of the cell and interpreting the results from recording in mixed states.]

#### **Output Units**

Select the base unit of measurement for the output channel. This is set to 'V' for voltage clamp experiments, and to 'A' (Amperes) for current clamp experiments. Additional base unit types can be added to this list via the Preferences menu.

Note that the resolution of the unit is automatically adjusted in the signal.

### **Relative to Holding**

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

This provides an alternate way to construct and edit stimulus waveforms, as relative to the holding level, when this is more convenient for your experiments.

#### Return to Holding at Sweep End

Use the holding level as the output signal when the system is not acquiring data, i.e. the "down time" after the Waveform/Acquisition Duration ends, but before the the next sweep starts (a minimum of ~100 ms per 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 amplitude (holding) level.

#### Enable P/N Leak Subtraction

If endogenous leak conductance is an issue with your seal, configure the "P/N" leak subtraction waveform settings per output channel

Note that this section is dependent upon the 'Enable Acquisition of Leak Pulses' setting in the Acquisiton & Routine Parameters section.

#### 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.

#### <u>Alternating Leak Polarity</u>

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

#### Num Leak Pulses

Set the number of leak pulses used to average out noise and leak conductance. Adjust this number in accordance with the amount of noise in the signal. With the precision of modern 16-bit digitizers, this number can sometimes be reduced to less than 4 leak sub-pulses. The default setting is '4', which when paired with the default Leak Ratio, is equivalent to commonly used P/4 settings.

Note that as each leak pulse replicates the stimulus waveform, larger numbers of leak pulses can greatly increase the total duration of a sweep during acquisition.

#### 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 eletrically-gated ion channels are activated. For instance, a Leak Ratio setting of 0.25 will generate leak pulses at <sup>1</sup>/<sub>4</sub> the amplitude of the main stimulus waveform, a Leak Ratio of 0.2 will generate leak pulses at 1/5 the main pulse.

Note that 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 if desired, instead of being coupled together.

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

#### Leak Holding

Set the leak pulses holding level. This can be set differently from the routine's main holding level, for flexibility in finding a suitable voltage range for the leak pulses.

# 4.15 Routine Editor: Waveform Editor

•						Used	segments: 0 out of 50
Actions		Segment Stored	1	Segment 2 OFF 👻	Segment 3 OFF 💌	Segment 4 OFF 🗸	Segment 5 OFF 🗸
Waveform		Step	-	Step 💌	Step 💌	Step 💌	Step 💌
Amplitude (mV)	∣₄	Value	-	Value 🔹	Value 🔹	Value 🔹	Value 👻
Amplitude (mv)	4	10	\$				
Duration (ms)		Value	-	Value 👻	Value 🔻	Value 🔹	Value 🔻
Duration (ms)		5	¢				
					•		
	able1	Variable	2			riable5	
Name <u>R V</u> Value 0	ar1	R Var2			R Var4 R V	Var5	
						<u> </u>	

Figure 4-16. Waveform Editor



Use this button to close the Waveform Editor window

# Used segments: "#" out of 50

Up to 50 segments can be enabled in a waveform. Enabled segments must be contiguous, however actual data recording can skip some segments.

### Actions

OFF	Unused segments are labeled as 'OFF'.
Not Stored	Stimulation and digitization will occur for this segment, but the data for this segment is not stored.
Stored	Enable a segment by changing its Actions setting from 'OFF' to 'Stored'.
Not In Leak	?
Сору	To copy and paste a segment, click in a Segment's Actions list and select 'Copy'. Next, enter the number of times to copy the segment.*
Delete	To remove a Segment, set it to 'OFF' by clicking on a Segment to highlight it, and then from the Segment's Actions list, select 'Delete'.*
	* To select multiple Segments, use the Windows controls CTRL-Click to highlight individual Segments, and SHIFT-Click to highlight a range of Segments.

Waveform	Select the waveform	n shape.		
Step	The waveform amplitude rapidly jumps from a pre- existing level to the new level within one sample point, and stays at the new level for the duration of the segment. The resulting waveform shape looks like a step.			
Ramp	The waveform amplitude increases from the pre- existing level to the new level over the duration of the segment as a smooth straight-line. The sloping line looks like a ramp.			
	segment to sharply baseline level – the	To create a sawtooth pattern, use a zero duration segment to sharply reset the ramp amplitude to the baseline level – the next ramp will start from the baseline level again.		
Sine	The waveform is a s	sinusoidal wa	ave	
	Sine wave cycle	Multiple Single	One or more cycles One cycle	
	Amplitude (mV)	Amplitude (mV) First peak amplitude from bas		
	<ul> <li>Wavelength (ms) One cycle length</li> <li>Segment Duration Sine wave duration For a single cycle, this is the wavelength [Same as the Waveform Editor / Duration field]</li> <li>Live Preview Update the Routine Editor previe in real time.</li> <li>Tip: To offset the sine wave from the (0 mV) default baseline, enable the Routine Editor / Output Channel 'Relative to Holding' setting</li> </ul>			
Squarewave	The waveform gene	erates a train	of rectangular pulses	
	Base Amplitude Increment (mV) Increment th baseline amplitude succesive pulse			
	Pulse1 Amplitude (	mV) Amp	litude of first pulse	
	Pulse1 Width (ms)	Dura	ation of first pulse	
INTEGRATED PATCH AM	Pulse2 Amplitude (mV) Amplitude of second pulse			

	Pulse Width (ms)	Dura	tion of second pulse	
	Segment Duration [Same as the Wave	-		
	Live Preview	Update the preview in 1	Routine Editor real time.	
Chirp This waveform generates a sinusoid increases in frequency over time.				
	Chirp Type	Linear frequency Geometric	A linear increase in A geometric increase in frequency	
	Amplitude (mV)	±1000		
	Start Frequency (Hz) $1 - 50,000$			
	End Frequency (H	z) $1 - 50,000$	)	
	Segment Duration Chirp wave duration [Same as the Waveform Editor / Duration field]			
	Live Preview	Update the preview in 1	Routine Editor real time.	
Template				
Constant				
Amplitude	Select the wavefor	m amplitude fo	or a Segment	
Set to Hold	Use the IPA Contr Segment's amplitu	-	ng level for the	
Tip:	When used in Segment 1 for voltage-clamp experiments, this allows you to record the leak current along with the actual holding voltage, For current-clamp experiments, this allows you to record the actual cell potential and holding current.			
	When used in the l stimulation is reco	-		

Value	Use a single number for th	ne segment's amplitude
Value List	Use a different segment an a list of arbitrary numbers	mplitude for each sweep, from
	<u>Create Value List dialog</u> Enter a list of comr for each sweep	na separated numbers, one
Value+Increment	Increment the segment's a (Max amplitude: ±1000 m <sup>3</sup>	
	<u>Value+Increment</u> Start value Operator Increment value	+, -
Equation*	Specify the segment ampli	itude as an equation
	[free-form Equation field]	
	Results and errors	are reported under this field.
	Check Equations	Test the equation
	Insert special identifier	Key words in equations
	Undo All changes in the e	editing session are discarded
* see the Equation	Editor section for more deta	ils
Duration	Set the segment's duration 65 s per segment.	n. The maximum duration is
Value	Use a single number for th	ne segment's duration.
Value List	Use a different segment de list of arbitrary numbers.	uration for each sweep, from a
	<u>Create Value List</u> Enter a list of comr for each sweep	na separated numbers, one
Value+Increment	Increment the segment's c (Max duration: 65,000 ms)	_
	<u>Value+Increment</u> Start value Operator	+, -
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Increment value

Equation	Specify segment duration as an equation		
	Equation field		
	Results and errors	are reported under this field.	
	Check Equations	Test the equation	
	Insert special identifier (see list)	Key words in equations	
	Undo All changes in the e	editing session are discarded	
Variable table			
Name			
Value			
Segment Selection Left-click Right-click Copy	Selects the segment Displays the Copy Segmer Displays the Copy Segmer		
Delete	Displays the Delete Segme	ents dialog	

# 4.16 Routine Editor: Real Time Measurements & Graphs

Online/offline graphs are created from the Measurements dialog, where you set up the inputs' measurement cursors and the analyses to be graphed. 'Measurement cursors' display in the Scope window, and their associated measurements are plotted in the connected Analysis window.

Measurements are also configured via the Scope window's Set Measure button.

Real Time Measurements & Graphs: Amplitude_Equations
Arithmetic mean of all points.
ID Input Channels Analysis
m[1] ^ Current ^ Off ^
m[2] Voltage Mean
m[3] Max value
m[4] Time of max m[5] Min value
m[6] Time of min
m[7] Absolute peak
m[8] Time of absolute peak
nin time
Cursors Relative to: Sweep Time
Step cursors by roumpic
Start time (s): 0.0000
End time (s): 0.0010
Threshold OFF Polarity OFF
Smoothing Factor OFF Enable
ON Graphs Y-Axis m[1]
Graph 2 Equation m[1]
Graph 3 Units A V
Graph 4
Graph 5 X-Axis time 💌
Graph 6
Cruch 8
Units s v s

Figure 4-17. Time Analysis Graph

# ID

Up to 16 measurement cursors can be enabled in the ID column (m[1] .. m[16]).

# **Input Channels**

For each measurement cursor, select a name from the Input Channels column. The corresponding Scope window signal pane will contain that measurement cursor.

This channel list corresponds to the input channels enabled in the routine's Input Channels dialog.

# Analysis

Choose from 16 pre-defined Analysis statistics for each measurement cursor:

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• Mean	Arithmetic mean of all points		
• Max value	Value of largest sample		
• Time of max	Time at which largest sample occu	rs	
Min value	Value of smallest sample		
• Time of min	Time at which minimum value occ	urs	
Rise time	10-90% rise time:	(polarity [1, 0, -1])	
• Decay time	10-90% decay (fall) time:	(polarity [1, 0, -1])	
• Slope	Slope of linear fit		
Std deviation	Standard deviation of all points		
Variance	Variance of all points		
• Area	Signed area		
Absolute area	Absolute area around zero		
• Frequency	Number of threshold crossings (Hz	): (threshold [n])	
• Time to threshold	Time of first threshold crossing:	(threshold [n])	
• Tau	Time constant		
• Weighted tau	Weighted time constant		

These analyses can be directly plotted, or used in more complex equations, as defined below in the equation table.

# Cursors Relative to

Select where a measurement cursor is positioned in the data.

• Trace	Selects the entire trace
Sweep Time	Set from the start time of a sweep
• Segment #	Selects an entire Segment
Step Cursors by	The Start/End time cursor spinners can be adjusted to increment from 1 to 20 samples. The increment time is displayed to the right.
Start time (s)	The start of the measurement cursor
End time (s)	The end of the measurement cursor
Width (s)	The width of the measurement cursor
lock	Keep the cursor width at a constant value. If the Width field is "locked", changing the Start time updates the cursor position while preserving the cursor width
Threshold	
Polarity	

Smoothing Factor"Smooth" the data by averaging from 2 to 100 samplesEnableActivate the smoothing factor for cursor statistical analyses

## Graphs

You enable the display of measurement graphs in the Analysis window by checking their 'ON' boxes - up to eight graphs can be displayed. Once enabled, select (highlight) a graph to edit its settings.

## X- & Y-Axis

The X- and Y-axes define what will be graphed. The X- and Y-Axis drop-down lists paste into the Equation fields.

Set a time-base from the X-Axis list:

time X-Axis time-base

and select a basic measurement from the Y-Axis list:

m[#] measurement ID

or create more complex X- and Y-equations:

Equation Equation Editor

### Equations

This lists the actual X- and Y-axis definitions. Click on this field to access the Equation Editor.

For compound equations, parsing is executed from left to right, processing the highest precedence operators first.

Note: See the Equation Editor section for syntax.

# Units

For a standard time-dependent X-axis, set "s" as the unit type.

In some cases you need to manually set the X- and Y-axis base units of measurement, such as 'A' for amps or 'V' for volts, or 's' for seconds. Choose the units type from a drop-down list, or just manually enter the units type. The proper units resolution, such as 'pA' or 'mV', is automatically calculated by the program and displayed in the Analysis window graph.

### **Basic Analysis**

For standard plots using our basic statistical analyses, simply open the Y-Axis drop-down list and select the corresponding cursor ID (m[#]). If needed, manually set the Units type.

When the Routine is activated and data is acquired, the Analysis window will display the realtime graph of this analysis.

Example 1

To plot the mean of data, with Measurement cursor m[1] configured as 'Mean', select 'm[1]' from the Y-Axis list. The Equation field is populated as:

m[1]

The X-Axis (and its Equation) should be set as 'time' and its Units as 's'.

### Example 2

To plot the amplitude of measurement cursor #1 minus measurement cursor #2, enter the Y-Axis equation as:

m[1] - m[2]

Adjust the Y-Axis Units if needed.

The X-Axis and Equation defaults are 'time', and the Units default is 's'.

### Free-form Equations

Various variables, special identifiers, and constants, are available for use in equations. See the Equation Editor section for details and examples, along with parsing and syntax information.

# 4.17 Paradigm Editor

Start Paradigm	Pause	Stop Acquisit	on 🗸 Stop at End	of Sweep	Timer 03:27:2	5 Reset
urrent Paradigm: Untitled aradigm Status		Show User Checkboxes	Step: 0	Set Tagnone_ Show Equations		
Paradigm Pool Files   Comment none Paradigm Pool Untitled			digm Steps e "Insert" to define a Ste	p>		Insert •
		New Rename Duplicate				Duplicate Revert Delete
		Delete Revert Debug				Step Step to End
						Log Main Slow Speed V Drag & Drop

Figure 4-18. Paradigm Editor

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

Start Paradigm	Run the selected Paradigm highlighted in the Paradigm Pool list. This button turns into a 'Stop Paradigm' button while the Paradigm is running.	
Pause	Manually pause a running Paradigm.	
Stop Acquisition	Manually stop a running Routine's acquisition.	
Stop at End of Sweep	Wait until the sweep-in-progress has completed before stopping an acquisition.	
Timer	A running clock displays in hh:mm:ss, from when the Paradigm Editor is first opened.	
Reset:	Press this button to reset the Timer to 00:00:00.	
Current Paradigm:	The name of the currently loaded Paradigm.	
Step:	The Paradigm Step number that is being activated.	
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Paradigm Status:	Status	s information about Paradigm execution.
Set Tag	??	
Show/Hide Editor Control		Paradigm Editor controls for the Paradigm Pool and digm Steps can be displayed or hidden.
Show/Hide User Checkbox	displa section	ckbox controls for conditional Paradigm execution can be yed or hidden below the main Paradigm Editor Controls n. If the Editor Controls are hidden, the User Checkboxes n is disabled, and the checkboxes are also hidden.
Show Variables:	_	s a table of global Paradigm variables for use in digm steps. (see below).
Paradigm Pool Files	Drop-	down list operations affect the entire "Paradigm Pool"
New Paradigm Poo	1	Create a blank Paradigm Pool and optionally copy Paradigms over from the existing Paradigm Pool
Load Paradigm Poo	ol	Load the Paradigms of a previously saved Paradigm Pool file into the Paradigm Pool
Revert to Last Save	ed	Undo any unsaved changes to the Paradigm Pool
Save Paradigm Poo	ol	Save the Paradigm Pool using its existing file name and path
Save Paradigm Poo	ol 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 Poo	ol 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 Po	ools	Insert the Paradigms from a previously saved Paradigm Pool file into the loaded Paradigm Pool
The path and file name of	the load	ded Paradigm Pool file is displayed.
Paradigm Description:		a description for the loaded Paradigm. The text is saved he Paradigm.
Paradigm Pool		
Save Pool	Save t	the Paradigm Pool using its existing file name
New Paradigm	over fi	e a blank Paradigm Pool and optionally copy Paradigms com the existing Paradigm Pool
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Rename	Rename the selected Paradigm. Allowable characters are A-Z, a-z, 0-9, and "_".
Duplicate	Add a copy of the selected Paradigm to the Paradigm Pool
Delete	Remove the selected Paradigm from the Paradigm Pool
Revert	Select a Paradigm to be reverted, 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.
Debug	?
Paradigm Steps	
Insert	Insert a Paradigm step from the drop-down list as the next step of the Paradigm instruction queue.
Amplifier	Amplifier, Target=VHold, Equation=0.080
digm Steps Editor: "Amplifier", Step 2	8
✓ VHold ▼ Equation	-0.080
	Check Equation Insert special identifier 💌

Select an amplifier target from the drop-down list of amplifier functions.

CCMode (current clamp)

Para

VCMode (voltage clamp)

IHold (amplifer holding current, A)

VHold (amplifier holding voltage, V)

IGain (amplifier current gain, V/A)

VGain (amplifier voltage gain, V/V)

Filter (amplifier filter, Hz)

--

Offset, (amplifier pipette offset, V)

VTrack (amplifier tracking potential, V)

AutoEComp (amplifier auto electrode compensation, F)

AutoCellComp (amplifier auto cell compensation, F)

ECompMag (amplifier electrode compensation magnitude, F)

ECompPhase (amplifier electrode compensation phase, fraction)

CompOn (amplifier cell compensation on)

RsComp (amplifier cell compensation Rs, Ohm)

CmComp (amplifier cell compensation Cm, F)

RsPred (amplifier Rs prediction, fraction)

RsCorr (amplifier Rs correction, fraction)

RsLag (amplifier Rs correction lag, s)

Bridge (amplifier bridge balance, Ohm) --AuxOut1 (Aux-Output-1, V)

AuxOut2 (Aux-Output-2, V)

DigOut1 (Dig-Outpput-1) DigOut2 (Dig-Outpput-2) DigOut3 (Dig-Outpput-3) DigOut4 (Dig-Outpput-4) DigOut5 (Dig-Outpput-5) DigOut6 (Dig-Outpput-6) DigOut7 (Dig-Outpput-7) DigOut8 (Dig-Outpput-8) DigOutWord (Dig-Output-Word)

Equation field

Check Equation

Insert special identifier (see list in Equation Editor section)

Each Sweep ForEachSweep EachSweep, Target=<Routine name> ForEachEnd

Paradigm Steps Editor: "EachSweep", Step 5	X

Select a Routine name to operate upon, from the loaded Routine Pool drop-down list. After this Paradigm Editor step executes, it is appended with the number of completed sweeps of the executed Routine.

	Routine	Routine, Target= <routine name=""></routine>
Parad	igm Steps Editor: "Routine", Step 4	X
		•
		Select a Routine name in the drop-down list, from the loaded Routine Pool.
	Clear Key	ClearKey
	Execute	Execute, Command=Beep
Parad	igm Steps Editor: "Execute", Step 1	8
	Command Beep	
		Command
	Export	Export, Signal=Layout
Paradigm Steps Ed	litor: "Export", Step 32	8
	dd Signal 💌 Layout	
	Add Signal:	
		Reset (off) Layout Current Voltage Aux-1 Aux-2 Aux-3 Aux-4
	Make Picture	Picture, Camera=_none_
Parad	ligm Steps Editor: "Picture", Step 2	X
	Camera	▼
		Take a single photo. Select a camera name from the drop-down list of installed cameras.
	Reset Timer	ResetTimer Reset the Paradigm Editor Timer to 00:00:00
	Scope Operation	Scope, Wipe-false
	Set Axis	Axis, Axis=AutoScale, Kind=Left,Target=Current

Paradigm Steps Editor: "Axis", Step 12			
AutoScale	▼] Left ▼] Current		
		AutoScale AutoZero FullScale Given	
		Left Bottom	
		Current Voltage Aux-1 Aux-2 Aux-3 Aux-4	
	Set Checkbox	Checkbox, Count=1, Equation=true.	
Paradigm Steps Editor: "Check	box", Step 3		
Checkbox 1	true  Check Equation		
		Set Checkbox allows programmatic control of a User Checkbox status, based upon simple or complex equations. Checkboxes are used in conditional (If, ElseIf) Paradigm steps for control of Paradigm steps execution. Checkbox Checkboxes [1 – 3] are cleared whenever a Paradigm is started. Checkboxes [4 – 6] are not cleared at the start of a Paradigm, and their status persists across all Paradigms and Paradigm Pools of the Experiment. [equation field]	
		Check Equation	
	Set Metadata Tag	MetadataTag, Text=	
Paradigm Steps Editor: "Metada	ataTag", Step 4	8	

	Set Variable	SetVariable, Target=Paradigm, Count=1,	Equation=p[1]
Paradigm Ste	ps Editor: "SetVariable", Step	5	8
	Paradigm	▼ Variable p[1] ▼	
Equation	p[1]		
	Check Equation	Insert special identifier 💌	

From the drop-down list, select 'Paradigm', or a Routine name from the loaded Routine Pool. Up to 16 Paradigm or Routine variables can be defined.

Variables allow flexible control of Paradigm steps and Routine settings, and can also be used in equations. (see Variables table below)

Equation

**Check Equation** 

Insert special identifier (\* see list below)

Write Log WriteLog, Alert=true, Text=text\_to\_write, Equation=, DoBeep

Paradigm Steps Editor: "WriteLog", Step 6			X
✓ Do Beep	✓ Show Alert		
Text to send to Log text_to_write			
Equation result to append			
	Check Equation	Insert special identifier	•

### Do Beep

Show Alert

Text to send to Log

Equation result to append

**Check Equation** 

Insert special identifier (\* see list below)

	Alert	Alert, Text=alert_text, DoBeep=true
Paradigm Ste	eps Editor: "Alert", Step 8	
	✓ Do Beep Text to	show in Alert alert_text
		Do Beep Text to show in Alert
	Beep	Beep
	Comment	Comment, Text=
Paradigm Ste	ps Editor: "Comment", Step 9	B
•	Comment Show in window	
		Comment Show in window
	Wait	Wait, Time=1
Paradigm Ste	eps Editor: "Wait", Step 10	8
	Time to Wait [s] 1	
		Temporarily pause execution of the Paradigm.
		Time to Wait [s] The spinner increments in 0.1 s steps.
	Break	Break, Kind=Paradigm
Paradigm Ste	ps Editor: "Break", Step 11	8
	Break Kind Paradig	<u>m</u>
		Break Kind: Paradigm ForLoop
		Use a Break step to stop the execution of a Paradigm, or to interrupt a Paradigm's For Loop or For Each Sweep constructs.
	Chain	Chain, Target=undefined_paradigm, Return=true

Paradigm Ste	ps Editor: "Chain", Step 12		
	✓ Return to calling Parad	ligm	
	Paradigm to chain to	undefined_paradigm 💌	
		Return to calling Paradigm: Once execution of the target Paradigm has compl return execution to this Paradigm.	leted,
		Paradigm to chain to: Select a target Paradigm name in the drop-down from the loaded Paradigm Pool; Paradigm step execution will shift to the selected Paradigm.	list
	For Loop	ForLoop, Max=1 ForEnd	
Paradigm Ste	ps Editor: "ForLoop", Step 13	B	
	Max. ForLoop Count 1	Loop indefinitely	
		Max. ForLoop Count Loop Indefinitely	
	Jump	Jump, Target=jump_label	
Paradigm Ste	ps Editor: "Jump", Step 14	B	
	Jump Target jump_label		
		The Jump Target is assigned a Label, which can inserted as another step. When executed, a jump to the step after the jump Label.	
	Label	Label, Target=jump_label	
Paradigm Ste	ps Editor: "Label", Step 15		
	Label name jump_label		
		Label name	
	If	If, Left=, Operation=>, Right= EndIf	

Paradigm Steps Editor: "If", Step 16	X
Compare 2 equations	
Left Equation	
Check Equation Insert special identifier 💌	
Operation: ">"	
Right Equation	
Check Equation Insert special identifier 👻	

Compare 2 equations Check for key pressed Check checkbox status Answer of yes-no-alert Left Equation

Check Equation

Insert special identifier

Operation: >, >=, =, !=, <=, <

**Right Equation** 

**Check Equation** 

Insert special identifier (\* see list below)

ElseIf

ElseIf, Left=, Operation=>, Right=

Paradigm Steps Editor: "ElseIf", Step 17	×
Compare 2 equations	
Left Equation	
Check Equation Insert special identifier 💌	
Operation: ">"	
Right Equation	
Check Equation Insert special identifier 👻	

(Same as 'If')

Else

Else

\*Special Identifiers

Edit	If the highlighted step has editable fields, the Paradigm Steps Editor is opened.	
Duplicate	Insert a copy of the selected step.	
Revert	Select a Step to be reverted, and click the Revert button. Editable fields are reset to their originally loaded values, as long as another Paradigm has not been loaded.	
Delete	Delete the selected step. For multi-line steps, optionally delete the entire group	
Step	Execute the selected step, then move to the next step.	
	Note: a 'For' loop is processed as one step	
Step to End	Execute the selected step and all following steps as fast as the system allows	
Slow Speed	Execute 'Step to End' at ~1 second per step.	
Log Main/All	Log Main Steps Log All Steps	

Checkbox-1 .. Checkbox-6

Checkboxes are useful for quick *ad hoc* conditional control of Paradigm steps. They are optionally displayed at the bottom of the Paradigm Editor Controls pane.

Checkboxes [1-3]These are cleared whenever a Paradigm is started, and they are only valid while a Paradigm is running.

Checkboxes [4-6]These are semi-permanent, and can exist across all Paradigms and Paradigm Pools for the entirety of an experiment,

✓ Checkbox-1	Checkbox-2	Checkbox-3	Checkbox-4	Checkbox-5	Checkbox-6	
--------------	------------	------------	------------	------------	------------	--

### 4.18 Variables Window

A table of global Paradigm Variables is displayed. These variable are available to all Paradigms loaded into the Paradigm Editor.

2	Edit Text Cell	Control of font type and size.
Var p	[1 - 16]	A column of 16 Paradigm variables.

## 4.19 Equation Editor

The Equation Editor maintains a list of equations that can be re-used in any equation. A convenient label is assigned to an equation, and used in an equation field in place of the stored equation. Your collection of equations is available wherever equations are used:

- Paradigm Editor / Steps: Amplifier, Set Variable, Write Log, If, Else If
- Routine Editor / Measurements: X-Axis, Y-Axis
- Routine Editor / Output Channels / Waveform Editor: Amplitude, Duration

The Equation Editor is accessed from the SutterPatch menu. Its equations are created and saved in an equation pool file - a simple text file which can also be directly edited with a text editor.

	New Equation Pool	Create a blank Equation Pool
	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 same as 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 Equa- tion Pool file into the loaded Equation Pool
New	Create a blan	k Equation Pool
Duplic	cate Add a copy of	f the selected Equation to the Equation Pool
Delete Remove the selected Equation from the Equation Pool INTEGRATED PATCH AMPLIFIER OPERATION - MANUAL – REV. 1.0.6 (20160729)		

**Equation** Pool Files

Revert	Select an Equation to be reverted, and click the Revert button. All editable steps are reset to their originally loaded values, as long as the Equation Pool has not been saved.
Save Pool	Save the Equation Pool using its existing file name
Edit	
Check Equation	
Insert special ident	tifier
The following	g special identifiers can be used as key words in equations:
loop (active )	paradigm ForLoop count)
sweep (activ	e paradigm EachSweep count)
lastsweep (a	ctive Paradigm sweep count of last sweep)
time (presen	at date-time, s)
timer (timer	time, s)
 m[116] (n't	h analysis measurement value)
r[116] (n'th	routine stimulus value)
p[116] (n'th	n paradigm value)
 aux[14] (re	ading of aux input, V) a single-point reading
Imon (ampli	fier current reading, A)
Vmon (ampl	ifier voltage reading, V)
 IHold (ampl	ifer holding current, A)
VHold (amp	lifier holding voltage, V)
IGain (ampl	ifier current gain, V/A)
VGain (amp	lifier voltage gain, V/V)
Filter (ampl	ifier filter, Hz)
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Offset, (amplifier pipette offset, V)

VTrack (amplifier tracking potential, V)

ECompMag (amplifier electrode compensation magnitude, F)

ECompPhase (amplifier electrode compensation phase, fraction)

CompOn (amplifier cell compensation on)

RsComp (amplifier cell compensation Rs, Ohm) CmComp (amplifier cell compensation Cm, F)

RsPred (amplifier Rs prediction, fraction)

RsCorr (amplifier Rs correction, fraction)

RsLag (amplifier Rs correction lag, s)

Bridge (amplifier bridge balance, Ohm)

[Additional Identifiers]

hold The amplifier holding level, in a Routine, using VHold or IHold, whichever is applicable

Other identifiers are forwarded to Igor's execute command.

Label	The name of the equation
Equation	Enter the equation here

#### **Equation Extras**

#### **Variables**

m[1]m[16]	The n'th measurement cursor's value	
p[1]p[16]	The n'th Paradigm Variable	(only in Paradigms?)
r[1]r[16]	The n'th Routine Waveform Variable	(only in Routines)
Input	Manual control of a Routine Variable	

#### Constants

е	2.71 (Euler's number)
pi	3.14 (п)

These constants have 27 digit precision.

#### Parsing

Equation parsing is executed from left to right, processing the highest precedence operators first:

Precedence	Туре	Operator
5	Unitary operations	-, !
4	Power	۸
3	Remainder	*, /, %
2	Addition/subtraction	+, -
1	Bit operations	& (and),   (or), nor, xor
0	Comparison operations	>, >=, <, <=, =, ==, !=
0	All other operations	round, trunc, ceil, floor, exp, sqrt, ln, log, sin, cos, tan, asin, acos, atan, abs, rad, deq, noise, random

#### Syntax

All equations use the same syntax as Igor, with a few additions:

- Three kinds of brackets '[], {}, ()' can be used equivalently to improve the clarity of nested expressions.
- Numeric values can use engineering units, e.g. '5p' is equivalent to '5e-12'.
- Equations follow a parsing sequence, where parsing is stopped at the end of a string, or at a semi-colon (";"), whichever comes first. Thus, comments can be added after a semi-colon.

#### Equation Pool Syntax

Insert an equation from the equation pool into an Equation field by entering "#" followed by the label of the equation, e.g. "#label(5)". This will use the result of the equation from the equation pool with label "Label", passing the argument "5" to that equation.

Example: using a LED light source.

To stimulate in increments of light intensity, one has to use an equation to transform the light intensity values in the routine to the actual stimulus values, with amplitudes in volts. One might build an equation in the equation pool as follows:

label = "power\_to\_volts"

equation = " $\ln(r[1]) * 2.55 + 3$ "

This equation takes the natural log of the Routine Variable r[1], multiplies it by 2.55, and adds 3.

Then, in the Waveform Editor, set a Segment's Amplitude field to 'Equation', and enter the equation as "#power\_to\_volts".

## 4.20 Data Navigator

The Data Navigator window organizes and retrieves your experimental data, as well as their associated control paths and data. Information is displayed from the current experiment, or the currently loaded experiment.

Main Window	The main window displays a "tree" structure arrangement of your experimental data, based upon the Data Tree Hierarchy.	
Expand	All nodes of the data tree are expanded, and their data are displayed down to the Routine-Signal level.	
Collapse	All nodes of the data tree are collapsed up to the top-level node(s) in the Experiment.	
View Last	The first signal of the last routine in the experiment is highlighted in the data tree, and that signal's data are displayed in the preview pane below it.	
Analyze	The signals from the highlighted routine are opened into the Scope window for analysis.	
Data Tree Hierarchy	The "sort" hierarchy is displayed in this window, and can be modified using the buttons below.	
Experiment Hierarchy	This button restores the default experimental hierarchy, which is Paradigm Name > Series Name.	
Clear Hierarchy	The sort hierarchy is removed, and the current experiment's raw data is displayed in the main window in a simple time sequence.	
Build Hierarchy	A custom sorting hierarchy can be built up from any associated metadata.	
Data Tree Hierarchy (Window) A convenient 'Data Tree Hierarchy' window opens to select and order parameters.		
Select parameter g list.	roup Select a Metadata parameter group from the drop-down	
Available Paramete	er Select a specific parameter to add to the data tree.	

Click on the "copy" button to move the selected parameter to the data tree.

Hierarchy The Hierarchy pane displays the new sorting hierarchy. The entries can be re-organized by selecting an entry and clicking on the Up/Down keys to reposition them, or using the 'Del' key to remove them.



Do It Click on the 'Do It' button to apply these changes to the data tree.

**Right-click Menu** 

Paradigm:	Review View Metadata
Routine:	Analyze View Metadata View Routine

Series:Analyze Edit View Metadata View Routine Copy Full Path

R0 (	_S1_IV C0	5.2332054e-12			¢ ۋ	3
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	

### 4.21 Data Table

Figure 4-19. Data Table Example

The Data Table provides direct access to the sample points in a data series, in a spreadsheet format. This is accessed from the Data Navigator, by highlighting a data series, right-clicking on it, and selecting the 'Edit' command.

## 4.22 Routine Review

This window is very similar to the main Routine Editor window, but without the ability to manipulate Routine Pools and files.

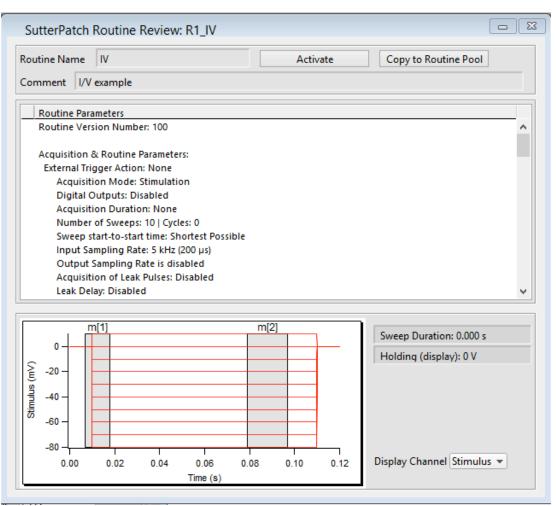


Figure 4-20. SutterPatch Routine Review

#### **4.23 Preferences**

Preferences settings customize the default settings for several areas of the SutterPatch program:

Acquisition Display File path Graphics export

To access, click on the Dashboard's Preferences icon, or go to the menu item SutterPatch/ Set Preferences.

General

Enable drag and drop for routine and paradigm editor

The Routine Editor loads a "pool" of Routines from a Routine Pool file. These Routines can be re-arranged in the Routine Pool list by clicking and dragging with the mouse. The Paradigm Editor operates in the same manner. However, the Paradigm Editor also displays a list of Paradigm Steps for the selected Paradigm, which can also be re-arranged by drag-and-drop. Files and Naming

Data files path: [pick a folder]

Data wave base name:

- Use routine name
- Use custom name: <Routine Name>

The data wave names are composed of:

**R#**\_ Routine Sequence Number

S#\_ Signal Number

**Base Name** 

Example: R3\_S2\_IV Curve

#### Scope

New Scope

Show tags by default

Y axis Autoscale settings: Autoscale

Autoscale from zero Fullscale

Signals Display

- Show all signals
- Use last selection
- Signals

Enter signal numbers and/or signal ranges separated by commas. For example, 1,2,4-5

Sweeps Display

- Show all sweeps
- Use last selection
- Sweeps:

Enter sweep numbers and/or sweep ranges separated by commas. For example 1,3,5-20

#### Configure

Time axis unit:	Auto-set
	SI unit

Colors

Active signal panel color Inactive signal panel color Active sweep color Inactive sweep color

#### Membrane Test

Sampling Rate:	20 us (50 kHz)
	50 us (20 kHz)

Duration:	LONG (200 ms)
	SHORT (20 ms)

Test Pulse Amplitude (V): 0.001 - 0.01 (CC: 20 pA/mV)

Line Frequency Reduction: Off

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	50 Hz 60 Hz	
Y axis Autoscale se	ettings: Autoscale Autoscale from zero Fullscale Use last setting	
Signals Display	Show all signals Use last selection Signals ranges separated by	Enter signal numbers and/or signal commas. For example, 1,2,4-5
Export Graphics Axis: left bottom		
Tick location:	Outside Crossing Inside None	
Labels:	On Axis only Off	
Grid:	Major only Off On	

## Hardware

Prompt on startup if no hardware is found.

When the SutterPatch program is started, if an IPA amplifier is not turned on and connected to the computer, you are prompted to retry the computer (USB) connection, or to startup in Demo mode.

No USB Connection

IPA not connected...

- Retry
- Demo

Prompt on startup

If no IPA amplifier is detected at startup, display this dialog

### Metadata

Metadata detail level: Allows levels for data categorization complexity: 1 to 3 INTEGRATED PATCH AMPLIFIER OPERATION - MANUAL – REV. 1.0.6 (20160729)

- 2: Extended (recommended for advanced users)
- 3: Full (recommended for experts users)

## Factory Reset

When you do a factory reset, you will lose ALL you configuration data (including Metadata, Scope, Routine, Paradigm, Preferences ...) [Factory Reset]

## Help

Defaults

## 4.24 Set Metadata

#### Metadata Group: Metadata Parameter: Description

Tag

8	Predefined Comment	A user comment for the recording		
Preparation – Animal				
1	Animal Identifier	An identifier for this specific animal		
	Animal Species	Binomial species name		
	Animal Strain	Strain, breed or variety characterizing the		
		animal		
	Animal Age	The numerical age		
	Animal Age Units	The units, in which the age is given (e.g., h, d, m)		
	Animal Gender	E.g., 1: f, 2: m, 3: undetermined		
	Animal Preparation Date	ISO Date, Format: YYYY-MM-DD		
	Animal Preparation Time	Time of Day, Format: hh:mm[:ss.000]		
Prepa	aration – Tissue			
	Tissue Prep. Identifier	An identifier for this specific tissue sample		
	Organ	The organ which the sample came from		
	Organ Region	A region or sub-structure within the organ, if applicable		
	Preparation Method	Preparation method for this sample		
	Tissue Prep. Date	ISO Date, Format: YYY-MM-DD		
	Tissue Prep. Time	Time of Day, Format: hh:hh[:ss.000]		
Prepa	aration – Cell			
	Cell Preparation Identifier	An identifier for this specific cell preparation		
	Acutely Dissociated Cells	0: No, 1: Yes		
	Cell Line	0: No, 1: Yes		
	Slice Preparation	0: No, 1: Yes		
	Whole-organ Preparation	0: No, 1: Yes		
	In-situ Recording	0: No, 1: Yes		
	Stem Cell Preparation	0: No, 1: Yes		
	User-defined Preparation	User defined cell preparation technique		
	Cell Type	The cell type		
	Cell Identifier	An identifier for this specific cell		
	Cell Prep. Date	ISO Date, Format: YYY-MM-DD		

Cell Prep. Time

#### Experi

Experiment	
Experiment User	Comment
Experiment Cate	gory 1 Name
Experiment Cate	gory 1
	2 N
Experiment Cate	gory 2 Name
Experiment Cate	
Experiment Cate	
Experiment Cate	gory 5 Name
Experiment Cate	gory 3
Experiment Cate	
Experiment Oate	gory 4 Manne
<b>Experiment</b> Cate	gorv 4
Experiment Cate	0.
	gory o runne
<b>Experiment</b> Cate	gorv 5
1	0-0-
Stimulus	
Compound ID	
Compound Name	1
Compound Conce	ntration
Compound Conce	ntration Units
-	
Light Stimulus W	lavelength
Light Stimulus Ir	ntensity
Light Stimulus In	
	1 T / ·/

Mechanical Stimulus Intensity

Mechanical Stimulus Intensity Units

Acoustic Stimulus Frequency Acoustic Stimulus Intensity

Acoustic Stimulus Intensity Units

**Thermal Stimulus Temperature** 

**Thermal Stimulus Temperature Units Electrical Stimulus Frequency** 

**Electrical Stimulus Intensity** 

**Electrical Stimulus Intensity Units** 

A comment assigned to the Experiment A categorization level for the Experiment (e.g., Site) E.g., the name of a site in large institutions A categorization level for the Experiment (e.g., Department) E.g., the name of a department A categorization level for the Experiment (e.g., Lab) E.g., the name of a laboratory A categorization level for the Experiment (e.g., Project) E.g., the name of a project A categorization level for the Experiment (e.g., Screen) E.g., the identifier for a particular screen

> A code or identifier for the compound The name of the compound The concentration of the compound The units or the compound concentration The wavelength of a light stimulus The intensity of a light stimulus The intensity units of a light stimulus The intensity of a mechanical stimulus The intensity units of a mechanical stimulus The frequency of an acoustic stim The intensity of an acoustic stimulus The intensity units of an acoustic stimulus The temperature of a thermal stimulus °C, °F or K The frequency of an external electrical stimulus The intensity of an external electrical stimulus The intensity units of an external electrical stimulus

#### Metadata Settings

- Do not write this parameter
- Use last value
- Use a previous value
- Use new value
- Increment

By

- Experiment
- Paradigm
- Routine

Prefix	A free-form text field
Start value	A numeric field
Increment: +, -, *, /	A drop-down list A numeric field
Suffix	A free-form text field

Prompt for confirmation before:

•	Experiment	At the start of an Experiment
•	Paradigm	For "planned" or named Paradigms (not individual
		Routines)
٠	Routine	At the start of every Routine

Confirm Metadata Settings (Acquisition Dialog)

Uncheck to prevent new setting.
Apply to all future Routines
Enable/disable prompting for new setting

## 4.25 Review Metadata

Metadata settings are displayed by right-clicking on an item in the Data Navigator tree.

Metadata event tags document the running of a Paradigm.

Paradigm Name

Sorting drop-down list

- By Event
- By Parameter

## 4.26 Menus

The main menu at the top of the main window includes the full complement of standard Igor functionality. There is a tremendous number of features available in Igor, however these are not directly supported by Sutter Instrument, so use at your own discretion.

The major SutterPatch functions are listed in the SutterPatch menu item.

#### SutterPatch

----

Show Dashboard	Display icons to access core program functionality
Connect to Amplifier	Initialize the IPA amplifier if available.
Demo Mode	If no IPA hardware is available, run in demonstra- tion mode, i.e., acquire simulated input/output data
Reset USB	Re-initialize communications to the computer
IPA Control Panel	
Control Panel	Display the IPA amplifier software control panel.
Set Seal Test	Set the amplitudes of the Seal Test pulses
Reset Control Panel	Return the IPA Control panel to its default settings
Membrane Test	Display the Scope window to monitor seal for- mation and cell health (Bath/Seal/Cell)
Free Run (Scope)	Display the Scope window for oscilloscope-style op- eration
Input Channel	Select channel: [channel name from list]
	Select sampling frequency: [5 kHz from list]
	Input sweep length (s): <0.5 default >
Reset Acquisition	Stop and clear corrupted data acquisition settings
Paradigm Editor	Display the dialog to load, edit and run Paradigms
Routine Editor	Display the dialog to load and edit Routines
Equation Editor	Display the dialog to load and edit Equations
Data Navigator	Organize and display your experiment, Paradigm, Routine and acquisition data in a tree structure
Application Modules	Ad hoc specialty modules
Camera Module	Simple control of attached cameras
Event Detection	Analyze action potential and minis

Set Metadata	Configure user-specified experimental information to be associated with the acquired data
Set Preferences	Modify the default program settings
Log Window	Display a history of program actions and messages

## 4.27 Import/Export Data

Under development.

## 5. APPLICATIONS

#### 5.1 Data Acquisition

#### 1. External I/O (Front Panel)

Using the IPA system as a stand-alone amplifier with an external data acquisition system.

#### **Command In**

This allows you to stimulate the IPA preparation from other equipment.

An external analog command signal is passed to the internal StimOUT channel and sent to the headstage. The external signal is added to the StimOUT signal before they are sent to the headstage. The Command In signal bypasses the IPA digitizer D/A conversion.

#### Scope – Signal Output

This allows you to monitor the IPA preparation with other equipment.

The analog response from the headstage (current in voltage-clamp mode, and voltage in current-clamp mode) is directly available on this BNC, and can be sent to an external data acquisition system for digitization.

#### **Scope** –Command Monitor

This allows you to monitor the IPA stimulus with other equipment.

The analog stimulus delivered to the IPA headstage (voltage or current) is directly available on this BNC, and can be sent to an external data acquisition system for digitization.

2. External I/O (Rear Panel)

Using the IPA system as a standalone data acquisition system with external instruments.

#### Auxiliary I/O

The two rear panel auxiliary analog output channels (AuxOUT#) can be used to send stimulus waveforms to external instruments, such as microelectrode amplifiers.

The four auxiliary analog input channels (AuxIN#) can be used to digitize signals from other external instruments, such as microelectrode amplifiers.

- 3. Configuration
  - 1) Paradigms
  - 2) Routine Editor

The routine editor allows you to define acquisition parameters, set input and output channels, and to create stimulus waveforms.

4. Automation

## 5.2 Data Analysis

- 1. Online Analysis
- 2. Offline Analysis
- 4. Igor General Analyses
  - 1) Curve Fitting
  - 2) Quick Fit
  - 3) Fourier Transforms
  - 4) Convolve
  - 5) Correlate
  - 6) Differentiate
  - 7) Integrate
  - 8) Smooth
  - 9) Interpolate
  - 10) Filter
  - 11) Resample
  - 12) Sort
  - 13) Histogram
  - 14) Compose Expression
  - 15) Packages
  - 16) Statistics

## **5.3 Voltage-Gated Currents**

- 1. Methods
  - 1) I-V Plot

## **5.4 Ligand-Gated Currents**

## **5.5 Event Detection**

- 1. Action Potentials
- 2. Synaptic Events
- 3. References

Deconvolution paper:

Pernía-Andrade AJ, Goswami SP, Stickler Y, Fröbe U, Schlögl A, Jonas P. A Deconvolution-Based Method with High Sensitivity and Temporal Resolution for Detection of Spontaneous Synaptic Currents In Vitro and In Vivo. Biophys J. 2012 Oct;103(7):1429–39.

See also:

Guzman SJ, Schlögl A, Schmidt-Hieber C. Stimfit: quantifying electrophysiological data with Python. Front Neuroinform. 2014;8:16.)

## 5.6 Use Dependence

- 1. Use-dependent Block
- 2. Use-dependent Activation

## 6. MAINTENANCE

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

Periodically inspect all cables and connections to make sure that all connections are made well and that all connectors are well and evenly seated.

## 7. TECHNICAL SUPPORT

UNLIMITED TECHNICAL SUPPORT IS PROVIDED BY THE SUTTER INSTRUMENT COMPANY AT NO CHARGE TO OUR CUSTOMERS. OUR TECHNICAL SUPPORT STAFF IS AVAILABLE BETWEEN THE HOURS OF 8:00 AM AND 5:00 PM (PACIFIC TIME) AT +1 (415) 883-0128 OR E-MAIL QUERIES TO info@sutter.com.

1. Troubleshooting Tips

## APPENDIX A. SOFTWARE LICENSE

## APPENDIX B. 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 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.

## APPENDIX C. ACCESSORIES

Headstage

I/O Adapter Cable

USB Cable

Power Cord

Model Cell

Ground Wire

Electrode Holder

Stick-on Feet

## APPENDIX D. FUSE REPLACEMENT

In the event that the instrument fails to power up when it is switched on, the line power 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	Fuses	
Power	(Type: Time Delay/Time Lag, 5mm x 20mm, glass tube)	
Source	Fuse Rating	Manufacturer Examples
100 - 240	T2.0A,	Bussmann: GMC-2-R, S506-2A,
VAC	250V	Littelfuse: 239.002.P

## APPENDIX E. TECHNICAL SPECIFICATIONS



## E.1. IPA Amplifier

		General Specifications	
Dimensions (in.)	1.75 (H) x 15 (W) x 10.5 (D)		
Dimensions (cm)	4.5 (H) x 48.25 (W) x 29.2 (D)		
Weight (lb)		9.05	
Weight (kg)		4.1	
Communica- tions		USB 2 (High Speed)	
		1 analog input	
BNC		2 analog output	
Channels	1 digital input		
	1 digital output		
Configura-		Voltage-to-current	
tions	Current-to-voltage		
Rack use	19" rack-mount (1U)		
Benchtop use	Rubber feet		
Signal Ground	Banana connector		
Earth Ground	Banana connector		
Safety	CE marking (Conformité Européenne)		
Auxiliary I/O Port	87654321 1514131211109		
Auxiliary I/O	Pin	Definition	
Pinout	1	Digital Output 1	

INTEGRATED PATCH AMPLIFIER OPERATION - MANUAL - REV. 1.0.6 (20160729)

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	Aux Analog Input 1
10	Aux Analog Input 2
11	Aux Analog Input 3
12	Aux Analog Input 4
13	Aux Analog Output 1
14	Aux Analog Output 2
15	Signal Ground
	$ \begin{array}{r} & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & $

IPA Headstage		
Construction:	Anodized aluminum case	
Dimensions (in.)	0.8 (H) x 1 (W) x 1.375 (L)	
Dimensions (cm)	2 (H) x 2.54 (W) x 3.5 (L)	
Weight (lb)	0.21 / 0.294 (with cable)	
Weight (kg)	0.095 / 0.133 (with cable)	
Output Gain:	0-25x	
Feedback Resistor	500 MΩ	
Whole Cell Capacitan Compensation Range	ce 0 – 100 pF	
Current Clamp Rise Ti	ime 17.5 μs	

- Case: Connected to ground. Case jack for 1 mm plugs.
- Bandwidth (voltage-clamp mode):
  - $\circ$  Internal:
    - 35 kHz
  - $\circ$  External:
    - 20 kHz (limited by Bessel filter)
- Maximum instrument noise: Measured with 8-pole Bessel filter.

Voltage Clamp	
Bandwidth	Open-Circuit Noise (RMS)
0.1 - 10 kHz	1.40 pA

### Digitization:

Range (voltage)	±10 V
Range (current)	±20 nA
Resolution	16-bit
Frequency	20 kHz

### Electrical:

Power consumption	18 Watts maximum
Mains fuse	250V 1A Slow Blow (5 mm x 20 mm) T2.0
Cables	Shielded power line cord

Table 7-1. IPA cables and receptacles/connectors.

## E.2. Electrode Holder

Body

End Cap Pin Cap Lockdown Ring Gold Pin Silver Wire Wire Seal (tubing) O Rings (6 ea.) 1.1 mm ID Clear 1.2 mm ID Green 1.5 mm ID Orange-Red 1.75 mm ID Blue

#### E.3. Glossary of Terms

## E.4. Bioelectric Terminology

The IPA amplifier records currents and voltages from various preparations.

The interpretation and measurement of such currents and voltages depends upon the directional flow of either positively or negatively charged ions:

Bioelectric Term	<u>Ion Polarity</u>	Cell Membrane Direction
Positive (Outward) Current	: positive ions	inside to outside
	negative ions	outside to inside
Negative (Inward) Current:	positive ions	outside to inside
	negative ions	inside to outside
Positive Potential:	positive voltage at he	eadstage vs. ground
Negative Potential:	negative voltage at h	eadstage vs. ground

## E.5. Software Limits

## E.6. Hardware Limits

Electrode Compensation	$25\mathrm{pF}$
Cell Compensation (Cm)	$1-100 \mathrm{ pF}$

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Cell Compensation (Rs) $1 - 100 \text{ M}\Omega$ Series Resistance Prediction0 - 100%Series Resistance Correction0 - 100%Current Injection $\pm 20 \text{ nA}$ 

## APPENDIX F. PROGRAMMING

- a. SutterPatch Application
- b. Igor Pro 7 Environment
- c. File Formats
  - i. SutterPatch Format

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# **NOTES**

#### Components of the IPA System

Carefully remove all components from the shipping container. In addition to this manual, the following should be included:

- IPA Amplifier
   IPA-E
- IPA Headstage IPA-HS
- Electrode Holder EH-P170
- Model Cell
   MCELL
- Ground Wire
- Power Cord
- USB 2 Cable
- Auxiliary I/O Adapter Cable
- USB thumb drive for software