dPatch[®] Digital Patch Amplifier

ELECTROPHYSIOLOGY PATCH-CLAMP SYSTEM WITH

SutterPatch® SOFTWARE

Operation Manual





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The dPatch system has two international safety certifications.

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



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



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DISCLAIMER

The dPatch system consists of one electronic amplifier with integrated digitizer, and one or two headstages. 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.

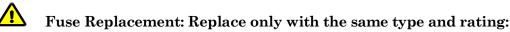
Do not allow an unauthorized and/or untrained operative to use this instrument.

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



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

Table 0-1. dPatch Fuses

 Type: 5 x 20 mm glass tube, Medium Time Delay (Slow Blow), RoHS compliant.
 Rating: T2.0A 250V (Time Delay, 2 Amps, 250 Volts)
 Examples: Bussmann: GMC-2-R, S506-2A Littelfuse: 239.002.P

Avoiding Electrical Shock and Fire-related Injury

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

Operational

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

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

Other

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

Handling Micropipettes

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

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

TABLE OF CONTENTS

DISCLAIMER	3
SAFETY WARNINGS AND PRECAUTIONS	3
Electrical	
Operational	
Other	4
Handling Micropipettes	4
1. INTRODUCTION	<u>14</u>
1.1 Overview	<u>14</u>
1.2 Software Highlights	
1.3 Experiment Structure	
2. INSTALLATION	
2.1 Computer Requirements	<u>19</u>
2.2 SutterPatch System Environment	
2.3 Mounting Instructions	
2.4 Electrical Connections	
2.5 Install Hardware	
2.6 Install Software	
2.7 Test System	
2.7.1 Install Model Cell	
2.7.2 Startup 2.7.3 Run a Membrane Test	
3. HARDWARE OPERATION	
3.1 dPatch Front Panel 3.2 dPatch Rear Panel	
3.3 Grounding	
3.4 Headstage	
3.5 Holder	
3.5.1 Assembly	
3.5.2 Chloriding Silver Wire	
3.5.3 Holder Maintenance	<u>36</u>
3.6 dPatch Amplifier Control Panel	
3.7 Lock-In Adjustments	
3.8 Dynamic Clamp Editor	
3.9 System Integration	
3.9.1 Using Peripheral Equipment	
3.9.2 Using Multiple Sutter Amplifiers	
3.9.3 Using Non-Sutter Amplifiers 3.9.4 Using Non-Sutter Data Acquisition Systems	
3.10 dPatch Maintenance	
3.10.1 Inspection	
3.10.2 Cleaning	
3.10.3 Calibration	
4. SOFTWARE OPERATION	
4.1 Acquisition	· · · · · · · · · · · · · · · · · · ·
• • • •	

4.1.1 Camera Module	
4.1.2 Free Run	
4.1.3 Membrane Test Windows	
4.1.4 Paradigm Editor	
Amplifier	
Each Sweep	
Routine	
Analysis	
Camera	
Clear Key	
Execute	
Export	<u>96</u>
Front Window	<u>97</u>
Hide Window	<u>98</u>
Reset Timer	
Scope Operation	
Set Axis	
Set Checkbox	
Set Metadata	
Set Solution	
Set Tag	
Set Variable	
Sound	
View Last	
Write to Log Alert	
Aler t Beep	
Comment	
Wait	
Break	
Chain	
For Loop	
Jump	
Label	
If	
Elself	
Else	
4.1.5 Routine Editor	<u>128</u>
Routine Editor: Acquisition & Routine Parameters	<u>135</u>
Routine Editor: Input Channels	<u>138</u>
Routine Editor: Output Channels & Waveform	
Routine Editor: Waveform Editor	
Routine Editor: Real Time Measurements & Graphs	
Routine Editor: Routine Variables	
4.1.6 Real Time Measurements & Graphs (Scope)	
4.1.7 Scope (Acquisition)	<u>175</u>
4.1.8 Solution Editor	<u>186</u>
4.1.9 Template Editor	
4.2 Data Analysis	
4.2.1 Action Potential Analysis	
4.2.2 Analysis Editor	
4.2.3 Analysis Window	
4.2.4 Data Browser	
4.2.5 Data Navigator	<u>205</u>

4.2.6 Data Table	
4.2.7 Equation Editor	<u></u>
4.2.8 Event Detection (synaptic)	
4.2.9 Igor Analyses	<u></u>
4.2.10 Layout Window	
4.2.11 Metadata Review	<u></u>
4.2.12 Paradigm Review	
4.2.13 Reanalysis Measurements & Graphs	
4.2.14 Routine Review	<u>241</u>
4.2.15 Scope (Analysis)	
4.2.16 3D View Window	<u>247</u>
4.2.17 Set Metadata	<u>249</u>
4.2.18 Single Channel Analysis	<u>259</u>
4.3 General	
4.3.1 Command Window	
4.3.2 Dashboard Panel	
4.3.3 File Import/Export	
4.3.4 Log Window	<u>272</u>
4.3.5 Menus	
4.3.6 Preferences	
i. General	<u>278</u>
ii. Files and Naming	
iv. Hardware	
v. Control Panel	
vi. Scope vii. Metadata	
viii. Graphs and Layouts	
ix. Factory Reset	
4.3.7 Shortcut Editor	
4.3.8 Sample Files	
4.3.9 Startup	
5. Programming	
5.1 Data Format	
5.2 Data Structure	
5.3 Data Paths	
5.4 User Functions	<u>298</u>
6. TROUBLESHOOTING.	<u>300</u>
6.1 Technical Support	<u>300</u>
6.2 Manual	<u>300</u>
6.3 Online Help	<u>301</u>
6.3.1 Error Messages and Notifications	<u>301</u>
6.4 Startup Issues	<u>301</u>
6.5 Acquisition Issues	<u>303</u>
6.6 Analysis Issues	
6.7 General Issues	
APPENDIX A: LIMITED WARRANTY	<u>309</u>
APPENDIX B: SOFTWARE LICENSE	<u>310</u>
APPENDIX C: ACCESSORIES	<u>317</u>

APPENDIX D: FUSE REPLACEMENT	<u>318</u>
APPENDIX E. TECHNICAL SPECIFICATIONS	<u>319</u>
APPENDIX F. SutterPatch Algorithms	
Auto Electrode Compensation Algorithm	
Auto Cell Compensation Algorithm	
LockIn Computation	
Event Detection Reference	
Single Channel Fitting	

TABLE OF FIGURES

Figure 1-1. Data Structure - Planned Paradigms15
Figure 1-2. Data Structure – Auto-triggered Paradigms16
Figure 2-1. Rear of dPatch Cabinet
Figure 2-2. Front of dPatch Cabinet
Figure 2-3. Splash Screen
Figure 2-4. Welcome Screen
Figure 2-5. Emulation Modes
Figure 2-6. Dashboard
Figure 2-7. Acquisition Dashboard
Figure 2-8. Amplifier Control Panel
Figure 2-9. Dashboard
Figure 2-10. Acquisition Dashboard
Figure 3-1. Front of dPatch Cabinet
Figure 3-2. Rear of dPatch Cabinet
Figure 3-3. Amplifier Control Panels
Figure 3-4. Headstage Monitor
Figure 3-5. VC Control Panel
Figure 3-6. CC Control Panel
Figure 3-7. Dynamic Clamp Control Panel
Figure 3-8. I/O Control Panel
Figure 3-9. Calibration
Figure 4-1. Membrane Test Monitor
Figure 4-2. Paradigm Editor
Figure 4-3. Step: Amplifier
Figure 4-4. Step: Each Sweep

Figure 4-5. Step: Routine	
Figure 4-6. Step: Analysis	
Figure 4-7. Step: Camera	
Figure 4-8. Step: Execute	
Figure 4-9. Step: Export	
Figure 4-10. Step: Front Window	
Figure 4-11. Step: Hide Window	
Figure 4-12. Step: Scope Operation	
Figure 4-13. Step: Set Axis	
Figure 4-14. Step: Checkbox	
Figure 4-15. Step: Select Metadata Group	
Figure 4-16. Step: Set Solution	
Figure 4-17. Step: Set Tag	
Figure 4-18. Step: Set Variable	
Figure 4-19. Step: Sound	
Figure 4-20. Step: Write to Log	
Figure 4-21. Step: Write to log window	
Figure 4-22. Step: Alert	
Figure 4-23. Step: Comment	
Figure 4-24. Step: Wait	
Figure 4-25. Step: Break	
Figure 4-26. Step: Chain	
Figure 4-27. Step: For Loop	
Figure 4-28. Step: Jump	
Figure 4-29. Step: Label	
Figure 4-30. Step: If	
Figure 4-31. Step: Else If	
Figure 4-32. Checkboxes	
Figure 4-33. Paradigm Variables	
Figure 4-34. Routine Editor	
Figure 4-35. New Routine Pool	
Figure 4-36. Waveform Preview Pane	
Figure 4-37. Routine Settings	
Figure 4-38. Acquisition & Routine Parameters	
Figure 4-39. Input Channels	

Figure 4-40. Output Channels & Waveform	
Figure 4-41. Output Channels & Waveform with Leak	153
Figure 4-42. Waveform Editor	156
Figure 4-43. Template Waves	
Figure 4-44. Real Time Meaqsurement Settings	
Figure 4-45. Real Time Graph Settings	
Figure 4-46. Routine Variables	
Figure 4-47. Real Time Measurements & Graphs (Scope)	
Figure 4-48. Scope (Acquisition) Window	
Figure 4-49. Signal Layout	
Figure 4-50. Axis Magnification	
Figure 4-51. Axis Scroll Bars	
Figure 4-52. Center Button	
Figure 4-53. Autoscale Axes	
Figure 4-54. Amplitude Meters	
Figure 4-55. Solution Editor	
Figure 4-56. Template Editor	
Figure 4-57. Action Potential Analysis	
Figure 4-58. Action Potential Measurements	
Figure 4-59. Analysis Editor	
Figure 4-60. Analysis Window	
Figure 4-61. Data Browser	
Figure 4-62. Data Navigator	
Figure 4-63. Data Table	
Figure 4-64. Equation Editor	
Figure 4-65. Event Detection	
Figure 4-66. Reanalysis Measurements & Graphs	
Figure 4-67. Edit Virtual Signals	232
Figure 4-68. Routine Review	
Figure 4-69. Scope (Reanalysis) Window	
Figure 4-70. Navigator Pane	
Figure 4-71. 3D Axes Definition	
Figure 4-72. 3D View	
Figure 4-73. Single-Channel Scope	
Figure 4-74. Current Amplitude Histogram Controls	

Figure 4-75. Transition Controls	. 262
Figure 4-76. Plots and Tables Controls	. 264
Figure 4-77. Command Window	. 267
Figure 4-78. Dashboard	. 268
Figure 4-79. Dashboard - Acquire Data	. 269
Figure 4-80. Preferences Settings	. 278
Figure 4-81. Shortcuts Editor	. 289
Figure E-1. Electrode Holder	. 326
Figure E-2. dPatch Expansion Panel	. 327
Figure E-3. Ground Point	. 328

TABLE OF TABLES

Table 1-1. Software Terminology	
Table 3-1. Headstage Specifications	
Table 3-2. Dynamic Clamp Pool	
Table 3-3. Main Unit Calibration	
Table 3-4. Headstage Calibration	61
Table 4-1. Routine Files and Pools	
Table 4-2. Routine Editor Buttons	
Table 4-3. Other Scope Buttons	
Table 4-4. Equation Parser	
Table 4-5. Engineering Notation	
Table 4-6. Scope Window Buttons	
Table 4-7. Metadata Parameters	
Table D-1. dPatch Fuses	
Table E-1. dPatch Amplifier - Physical	
Table E-2. dPatch Headstage - Physical	
Table E-3. dPatch Headstage - Operational	
Table E-4. dPatch Headstage – Open Circuit Noise	
Table E-5. dPatch Headstage – RMS Noise	
Table E-6. dPatch Headstage - Risetime	
Table E-7. dPatch Data Acquisition	
Table E-8. dPatch Electrical	
Table E-9. dPatch Expansion Panel	

1. INTRODUCTION

Welcome to the new standard in low-noise patch-clamp technology! Our expert team has created an ultra-fast electrophysiology recording system. Its breakthrough digital design extends the limits of low-noise single-channel and whole-cell recording.

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

1.1 Overview

Advanced Design

The dPatch[®] Digital Patch Clamp Amplifier is a complete digital microelectrode patch-clamp system for ultra-fast high-fidelity voltage- and current-clamp recordings. Most of the electronics (amplifier and digitizer) used in stimulating, compensating and recording from cells are integrated into a single printed circuit board. An extremely high base digitization rate (5 MHz) can extend data precision up to 22-bits of resolution.

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

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

From concept to production, from hardware to software, this fully integrated patch-clamp system is the ultimate in easy-to-use, ultra-fast, very low-noise cellular recording systems.

1.2 Software Highlights

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

Convenient:

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

Comprehensive:	All data recordings, analyses, graphs, layouts, configurations and con- trols are saved in a single experiment file. This ensures that data are always kept together with their complete contexts.
Automation:	Automate your experiment using a rich set of data acquisition, data analysis, and amplifier controls. Create complex "Paradigms" that can respond to changing conditions via conditional steps and loops.

1.3 Experiment Structure

Experiment:

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

Paradigm:

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

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

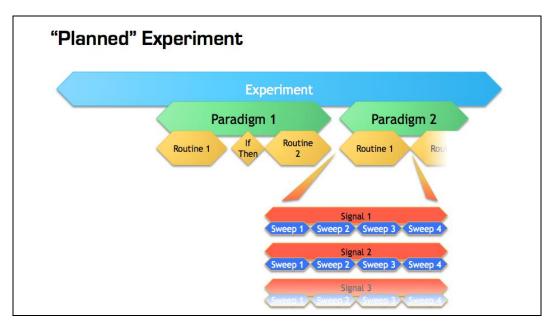


Figure 1-1. Data Structure - Planned Paradigms

An Experiment with two "Planned" Paradigms running Routines.

However, if a Routine is manually run, an "auto-triggered" Paradigm is created to maintain the Experiment structure. This default Paradigm ensures that each Series is associated with a Paradigm in the context of an Experiment. If an auto-triggered Paradigm is already the active Paradigm, it is used for subsequent manually-run Routines.

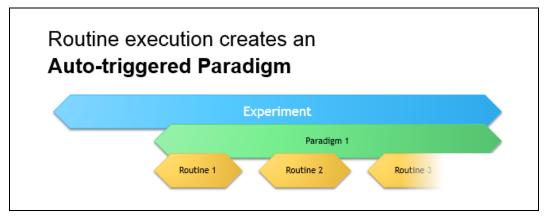


Figure 1-2. Data Structure – Auto-triggered Paradigms

An Experiment with manually run Routines uses an "auto-triggered" Paradigm.

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

Routine Parameters:

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

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

Series (Routine Data):

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

Channel:

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

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

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

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

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

Signal:

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

Sweep:

A Sweep is the sum of all data from all Signals, acquired from time zero, for a single fixed duration. In SutterPatch software, the Sweep duration is determined by the length of the longest command waveform.

Trace:

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

Segment:

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

SutterPatch Metadata:

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

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

Terminology Comparison:

A table of equivalent terms to other electrophysiology software packages:

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

Table 1-1. Software Terminology

2. INSTALLATION

2.1 Computer Requirements

Minimum Configuration

Operating System:	Windows:	Version 10 (64-bit versions)			
		Most language packs are compatible.			
		(listed in OS: Control Panel > System)			
	macOS:	Version 10.11 (El Capitan) or later			
		(listed in OS: Apple > About this Mac)			
	Note:	Virtual machines and OS emulators, such as Parallels and VMWare Fusion, are not supported.			
CPU (Central Processing Unit):	Dual-core i5				
RAM (Random Access Memory)	: 6 GB	6 GB			
Hard Disk Free Space:	SSD (Solid State Drive) 500 GB				
Note:	The drive she drive.	ould be configured as the primary system			
Display Resolution:	1024 x 768 (X	KGA)			
Computer Ports:	(1) USB 3.0 S	SuperSpeed port			
Note:	-	SB 3.0 add-in card adapters are not recom- ompatibility can be problematic.			
	dows Control	SuperSpeed USB 3.0 ports, look in the Win- l Panel / Device Manager / Universal Serial er section for "USB 3.0" host controllers.			
	for the compu- physical USF	es not provide any USB version information uter's USB ports, you might need to test the 3 ports for operational performance. Also, dates can sometimes put a USB 3.0 port to			
	External US	B hubs are not supported.			

Recommended Configuration (for Bandwidths > 50 kHz)

CPU: As fast and powerful as possible. RAM (Random Access Memory): 16 GB (or more for very large data sets) **Display Resolution:**

1920 x 1080 (Full HD)

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

2.2 SutterPatch System Environment

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

Igor Pro Features

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

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

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

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

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

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

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

2.3 Mounting Instructions

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

2.4 Electrical Connections

Typical AC Power: 60 Hz, 120 V

50 Hz, 240V

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

The AC power should be as clean as possible:

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

2.5 Install Hardware



Figure 2-1. Rear of dPatch Cabinet

- 1. Plug the female end of the power cord into the dPatch rear panel power receptacle.
- 2. Plug the male end of the included power cord into a grounded electrical mains outlet.
- 3. Push the dPatch power button to OFF (unlit position).



• WARNING! Hot-swapping of headstages should be avoided, or a software crash with data loss may occur. Turn off the dPatch system power before altering headstage connections.



Figure 2-2. Front of dPatch Cabinet

- 4. Plug the dPatch headstage(s) into the HEADSTAGE port(s) on the front of the dPatch amplifier each headstage is independently tuned, so any port can be used.
- 5. Plug the included Screw Terminal Panel or optional dPatch Expansion Panel into the DIG-ITAL OUTPUTS port on the back of the dPatch amplifier.
- 6. Connect the supplied USB 3.0 cable to your computer's USB 3.0 port and the dPatch amplifier's rear panel USB port.
- 7. Connect the included electrode holder to the headstage. See the Holder section for holder assembly instructions.

2.6 Install Software

Power on the computer.

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

Download the latest version of SutterPatch installer software from:

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

and choose the "Download" tab. Navigate your file browser to the download file and run it.

- B: If internet access is not available, attach the included USB flash drive to your computer USB port, and navigate to the flash drive.
 - 1. Install the software for 'All Users' by double-clicking on:
 - Windows: SutterPatch_installer_with_Igor
 - Macintosh: sutterpatch_mac_full
 - 2. Follow the installer prompts:
 - If an existing version of Igor Pro 7 is found, we recommend to replace it with Igor Pro 8. However, first make a backup copy of all user files and parameter files in the program folder and its sub-folders.

- However, older versions of Igor Pro can be retained if desired, as different versions of Igor Pro can coexist on the same computer.
- If an existing version of Igor Pro 8 is found, SutterPatch sample files are overwritten.
- The new install uses the Preferences from prior SutterPatch installations.
- 3. Upon completion, the installer will report a successful installation. The following files and folders are installed:
 - dPatch QuickStart Guide (PDF) (Includes Igor Pro 8 Serial Number and Key.)
 - Release Notes (PDF)
 - SutterPatch manual (PDF)
 - SutterPatch folder (Includes sample Routine Pool, Paradigm Pool, and Experiment data files).
 - SP_Code folder
 - SP_Drivers folder (XOPs)
- 4. Launch Igor Pro 8 and activate its license as instructed.

You will need to enter the Igor Pro 8 Serial Number and Activation Key found in your dPatch Quick Start Guide.

5. "Eject" the flash drive - wait for the "Safe to Remove Hardware" prompt, and then unplug it from the computer.

2.7 Test System

2.7.1 Install Model Cell

- 1. Attach the model cell to the Headstage 1 holder and tighten the screw collar.
- 2. Plug the supplied 1 mm grounding wire into the gold sockets on the headstage and model cell.
- 3. If the headstage is not inside a Faraday cage, completely surround the model cell/headstage assembly with electromagnetic shielding (such as aluminum foil), and connect the shielding material to the headstage ground wire or connector short wire with an alligator clip on each end makes a convenient shield-ground wire.

2.7.2 Startup

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



A SutterPatch splash screen displays while opening files.

Figure 2-3. Splash Screen

Then the Welcome to SutterPatch "start" window displays.



Figure 2-4. Welcome Screen

- 3. Click on the 'Start' button in the 'Welcome to SutterPatch' window, and the application begins compiling. This process may take several seconds.
- 4. Specify the Experiment file name and storage location when prompted.

5. If the dPatch amplifier is OFF, or disconnected from the computer:

No USB Connecti				
Retry				
Select device to emulate				
IPA - Single Headstage Do it				
✓ Prompt on startup				

Figure 2-5. Emulation Modes

- a. Power cycle or reconnect the amplifier and click 'Retry', or
- b. Click in 'Select device to emulate' and select 'dPatch'.
- c. If amplifier hardware is not attached, and 'Prompt on startup' is disabled, the program automatically starts up in the last known emulation state.
- 6. The Dashboard panel is displayed.

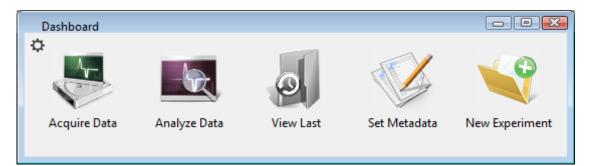


Figure 2-6. Dashboard.

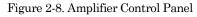


7. Click on the 'Acquire Data' icon, and a second level of the Dashboard is displayed.

Figure 2-7. Acquisition Dashboard.

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

📑 dPatch Control D 💷 🔳 🖾
Headstage 1 Headstage 2
V 0.0 V I -1.1 pA
VC CC
Range +/- 20 nA 💌 Reset
Hide Controls Show Monitor
Liquid junction 0.0 mV Auto Offset Lock 0.0 mV
VC CC DynC I/O
V-holding 0.0 mV 🖨 Enable
I-Filter 10 kHz 👻
Electrode Compensation
Mag 0.00 pF
Tau 4.00 μs 🗘
Cell Compensation
Rs 0.10 MΩ 🗘
Cm 2.00 pF
Rs Correction
Corr 0 %
Lag 6 µs



- a. If "CONTROL D..." is displayed in the Amplifier Control Panel title bar, you are running in a hardware emulation mode ensure that the amplifier is "on" and its USB 3.0 cable is connected, and then run a "New Experiment".
- b. For the next step, make sure that the Amplifier Control Panel is in voltageclamp mode – the "VC" button at the top of the Amplifier Control Panel should be highlighted in red.

2.7.3 Run a Membrane Test

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

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

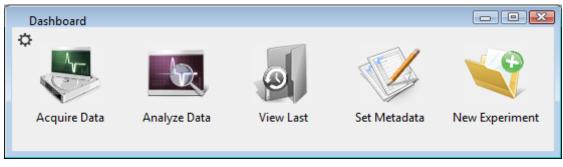


Figure 2-9. Dashboard

2. Click on the 'Membrane Test' icon.

Dashboard		6		
Acquire Data	Analyze Data	View Last	Set Metadata	New Experiment
0	ď	VV	23	
Control Panel	Membrane Test	Free Run	Routines	Paradigms

Figure 2-10. Acquisition Dashboard.

The following test values assume a 5 kHz filter.

3. Test the BATH mode:

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

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

28

4. Test the SEAL mode:

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

- a. Set the Model Cell switch: Seal
- b. Click on the Membrane Test 'Seal' button.
- c. Verify value: Seal Resistance: $> 10,000 \text{ M}\Omega$
- 5. Test the CELL mode:

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

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

c.	Verify values:	Series Resistance:	$\sim 10 \text{ M}\Omega$
		Membrane Resistance:	$\sim 500 \ M\Omega$
		Membrane Capacitance:	$\sim \!\! 28 \ \mathrm{pF}$

- 6. For a dual-headstage system:
 - a. Move the model cell, ground wires and shielding to Headstage 2.
 - b. Set the Scope window to 'Headstage 2'.
 - c. Repeat steps 3-5.

3. HARDWARE OPERATION

3.1 dPatch Front Panel

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

° p		itch' Æ	SU	TTER	INST	RUMEN	т. ⁻					90
		U U LAMP AMPLIFIER	1	ANALO 2	g INPUTG 3	4	ANALOG 1	e outputs a	TRI IN	oger Out	POWER	
	HEADSTAGE 1		6	6	0	6	0	6	6	6	U	
06			-					-		•	4	-
				5		a d	<i>₩₩</i> ₽ •		AR	Æ	4	

Figure 3-1. Front of dPatch Cabinet

The front panel, from left to right:

HEADSTAGE 1 & 2:	HDMI ports	For dPatch headstages.
AUXILIARY INPUTS 1 – 4:	BNCs	Analog inputs from external sources.
AUXILIARY OUTPUTS 1 & 2:	BNCs	Analog outputs to external targets.
TRIGGER IN:	BNC	Digital input trigger.
TRIGGER OUT:	BNC	Digital trigger output pulse
		Automatically sent at the start of continuous acquisition or each triggered sweep (including Membrane Test).
POWER:	Button	Turn power to unit On/Off. Lights up blue when 'On'.

3.2 dPatch Rear Panel

The rear panel of the dPatch system is used for grounding, USB connections, and signal I/O.



Figure 3-2. Rear of dPatch Cabinet

[Unlabeled]:	Power-entry receptacle	AC power cord.
SIGNAL GROUND:	4 mm Banana socket	Low-voltage grounding.
EARTH GROUND:	4 mm Banana socket	Instrument grounding.
USB-3:	USB Type B receptacle	USB 3.0 computer communication.
AUXILIARY I/O:	HDMI port	Expansion Panel analog signals.
FIRMWARE UPDATES:	Chip	Firmware code – keep chip installed.
AUXILIARY INPUTS 5 – 8:	BNCs	Analog inputs from external sources.
AUXILIARY OUTPUTS 3 - 4:	BNCs	Analog outputs to external targets.
DIGITAL OUTPUTS 1 – 16:	DC-37 D-Sub connector:	Digital output channels.
		(See Appendix E for pin

3.3 Grounding

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

definitions.)

The equipment in a rig should all be grounded to a single point to avoid ground loops. Installing a bus bar to an earth ground can make this easier to accomplish. "Signal" ground is a sensitive ground for very low voltages:

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

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

3.4 Headstage

The dPatch headstage supports both voltage- and (true) current-clamp in the same headstage. Multiple capacitor and resistor feedback elements optimize both single-channel and whole-cell recordings.

Feedback Element	Recording Amplitude Range	Analog Bandwidth	RMS Noise*	Pipette Compensation Range	Series Resistance Range	Cell Capacitance Range
1 pF Capacitor	±20 nA	> 500 kHz	< 0.22 pA	20 pF	N/A	N/A
500 MΩ Resistor	±20 nA	> 250 kHz	< 0.7 pA	20 pF	100 MΩ	100 pF
50 MΩ Resistor	±200 nA	> 250 kHz	< 2.3 pA	20 pF	10 MΩ	1000 pF

Table 3-1. Headstage Specifications

* The headstage RMS (Root-Mean-Square) noise is measured with an "open circuit" configuration at 10 kHz with an 8-pole Bessel filter. Measuring "open-circuit", i.e., with no attachments so the headstage input is exposed to the air, provides a fairly consistent baseline for such headstage noise measurements. Conversely, measuring noise with an electrode in the bath generates the worst noise conditions.

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

One or two dPatch headstages can be attached in any order to either HEADSTAGE port.

The dPatch headstage cable length cannot be increased.



WARNING! Hot-swapping of headstages should be avoided – data loss can occur. Turn off the dPatch system power before handling headstages.

The dPatch Amplifier uses two modes of headstage operation:

1)	"Capacitive" mode:	Single-channel patch-clamp recording.
	Feedback element:	1 pF Capacitor.
	Advantages:	Less noise (thermal, correction circuitry).
		Faster response (increased bandwidth).
		Better linearity.
		A capacitive ("integrating") headstage has the benefit of lower noise over resistive headstages. However, it also requires pe- riodic "resets" of its feedback capacitor. As current flows into the feedback capacitor, the capacitor charges up to its limit, and then discharges (resets) to zero, before starting the next round of measurements.
		During the reset period, the data is not valid, however, the re- set transients can be optionally left unaltered, "blanked", or "masked", during or after data acquisition, This SutterPatch software implementation is much more flexible than hard- ware implementations, which can only mask the data (sam- ple-and-hold).
		The frequency of automatic resets is proportional to the amount of current (amplitude) in the recorded signal. As the capacitor rating is 1 pF, a current of 1 pA will cause the reset to occur after 700 ms (when the capacitor reaches 70% of its maximal charge.)
2)	"Resistive" mode:	Whole-cell patch-clamp recording.
	Feedback element:	$500 \ \mathrm{M\Omega}$ and $50 \ \mathrm{M\Omega}$ resistors.
	Advantages:	No capacitive "resets".
		Extended input range of ±200 nA.

3.5 Holder

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

The standard pipette holder included with the dPatch amplifier is composed of low-noise polycarbonate and Teflon. A suction tube projects at a right-angle from the middle of the barrel. An ultra-low-noise quartz pipette holder is optionally available. While polycarbonate is a proven material for patch pipette holders, it undergoes significant thermal expansion. Uneven warming can lead to motion of the pipette tip, and is often incorrectly perceived as drift in the micromanipulator. Quartz has a significantly lower thermal expansion coefficient, and virtually eliminates thermal drift.

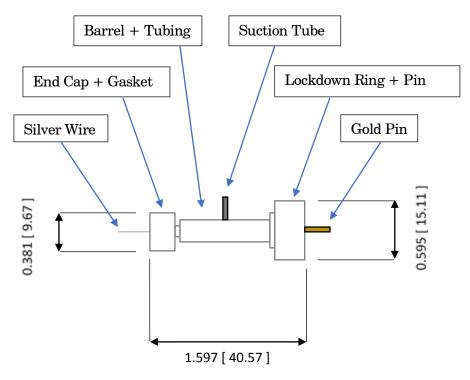
WARNING! Quartz is fragile, and can crack or shatter on impact. Treat your quartz pipette holder with the same care as with an optical component.

3.5.1 Assembly

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

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

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



Dimensions are in inches [mm].

- 1. Cut the silver wire to size: the depth it extends into the pipette plus half the length of the barrel.
- 2. Thread the silver wire through the barrel.
- 3. Cut a small piece of clear tubing sized to fill the tiny "end-cup" in the pin-side of the barrel the end with the narrower shaft.
- 4. Thread the small piece of tubing over the silver wire into the end-cup.
- 5. Crimp the end of the silver wire just slightly over the end of the tubing.

- 6. Slide the lockdown ring over the tubing-side of the barrel, with the ring's threads facing outwards.
- 7. Insert the gold pin into the recessed end of the pin cap push it through the pin hole until it stops.
- 8. Screw the pin cap onto the barrel. Pressure from the compressed snippet of tubing ensures electrical contact between the silver wire and the gold pin.
- 9. Find a silicone gasket with an ID (inner diameter) just greater than your pipette OD (outer diameter):

<u>Gasket ID</u>	Color
1.1 mm	Clear
1.2 mm	Green
$1.5 \mathrm{mm}$	Orange-Red
$1.75 \mathrm{~mm}$	Blue

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

Assembly tips

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

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

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

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

3.5.2 Chloriding Silver Wire

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

Chemical Method

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

Electrochemical Method

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

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

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

Re-Chloriding Silver Wire

- 1. Pass the used silver wire through a flame the wire should become bright silver in color. Alternatively, use a razor blade or fine sandpaper to scrape off any existing chloride.
- 2. Chloride the wire as described above.

3.5.3 Holder Maintenance

Holders must be properly maintained for good noise performance. Storage:

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

Before 1st time use:

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

After daily use:

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

Caution! Washing with soapy water can leave a film.

Continual cleaning with ethanol can degrade the polycarbonate parts.

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

Weekly Cleaning:

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

3.6 dPatch Amplifier Control Panel

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

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

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

Image: Headstage 1 Headstage 2 Image: Headstage 2 V 0.0 V I -1.1 pA VC CC Image: CC <th>Image: Headstage 1 Headstage 2 Headstage 1 Headstage 2 V 1.8 mV I 0.0 A VC CC Image Range +/- 20 nA Reset Hide Controls Show Monitor</th>	Image: Headstage 1 Headstage 2 Headstage 1 Headstage 2 V 1.8 mV I 0.0 A VC CC Image Range +/- 20 nA Reset Hide Controls Show Monitor
Liquid junction 0.0 mV Auto Offset Lock 0.0 mV VC CC DynC I/O V-holding 0.0 mV Enable I-Filter 10 kHz Electrode Compensation Mag 0.00 pF Tau 4.00 μs Auto Cell Compensation Rs 0.10 MΩ Cm 2.00 pF Auto Corr 0 % Liquid junction Corr 0 % Liquid junction Auto Corr 0 % Liquid junction Auto Corr 0 % Auto Lag 6 μs Auto	 Liquid junction 0.0 mV Auto Offset Lock 0.0 mV VC CC DynC I/O I-holding 0.0 pA Enable Subtract pipette offset V-Filter 5 kHz Electrode Compensation Magnitude 0.00 pF Bridge Balance Correction Percentage 100 % Dynamic Holding Target 0.0 mV V-hold Speed Slow V-mon Enable during acquisition

Figure 3-3. Amplifier Control Panels

General Controls

Headstage 1 - 2 Headstage # tabs

Up to 2 headstage tabs will display. Each headstage maintains its own settings.

	Clicking on a headstage tab will open its last-used active mode (VC, CC) settings - the tab and its background are set to matching colors, and the power light of the attached amplifier blinks.	
	Note: If a headstage is unattached while the amplifier is in use, its channel will be at "ground".	
	Show the second head	dstage in its own control panel.
V I	V' meter: I meter:	Displays the Voltage input channel level. Displays the Current input channel level.
VC	VC button:	Switch the dPatch amplifier from Current Clamp to Voltage Clamp mode. The button for the active mode is highlighted in red.
		A "gentle" switch is used to protect cells against transition spikes that could degrade the electrode seal and integrity of the recording. The amount of voltage to hold the current steady is automatically injected into the cell during the transition (< 100 ms), before stepping to the new voltage command level.
CC	CC button:	Switch the dPatch amplifier from Voltage Clamp to Current Clamp mode. The button for the active mode is highlighted in red.
		A "gentle" switch is used to protect cells against transition spikes that could degrade the electrode seal and integrity of the recording. The amount of current to hold the voltage steady is automatically injected into the cell during the transition (< 100 ms), before stepping to the new current command level.
	Reset USB button:	Click to re-establish the USB connection to the amplifier.
	 All USB channels are reset. 	
	 A green button indicates that a stable USB connection to the amplifier (or hardware emulation mode) has been established. 	
	 A red button indicates that there is no USB connection to the amplifier. 	
	 It can take see established. 	everal seconds for the USB connection to be re-
	-	er is attached in demo mode, a USB reset will disable and run the hardware "live.

Feedback Mo	Mode The headstage feedback mode applies to voltage-clamp input current-clamp output channels.	
	Capacitive Mode	± 20 nA range, for low-noise single-channel recording.
		Feedback element: 1 pF Capacitor.
		The headstage feedback capacitor, while providing very low-noise circuitry, also generates periodic "reset" artifacts in the recording. When at least one reset occurs in a sweep, a small gold box flashes in the Amplifier Control Panel to the left of the Range field. When more than one reset occurs in a sweep, the gold box will flash twice with the sweep. The number of resets for the sweep will display at the bottom of the Amplifier Control Panel as "Cap. Discharges: #".
		While capacitor resets affect the recorded signal, they do not affect the preparation. For single-channel re- cordings, currents are typically so small, that capacitor resets are infrequent enough to be essentially ignored.
		(See the Headstages section for more information.)
		The raw data always includes the actual capacitive re- set transients, however the transients can also be op- tionally hidden via Virtual Input Channels to reduce their impact during acquisition or analysis. Use a Vir- tual Input Channel (Math Type: Bessel Filter or Smooth) to manually set an arbitrary 'Integrator Reset' duration to blank or mask the transient.
		For Routines that contain inter-sweep intervals, if the headstage capacitor is reset during this interval, there is no interference with the acquired data. Such capacitor resets can also be forced to occur by running the Paradigm step 'Amplifier / Reset Cap'.
	Note:	The Virtual Input Channel 'Integrator Reset' controls are only visible when the Routine data are acquired in Capacitive mode.
	Range ± 20 nA	Amplitude range for whole-cell recording.
		Feedback element: $500 \text{ M}\Omega$ Resistor.
	Range ± 200 nA	Extended amplitude range for whole-cell recording.

Reset Reset the active headstage's controls to default settings.

• Safe mode: Initiate with a mouse left-click.

Disable these controls:

Offset 'Lock'

VC mode

Cell Compensation Rs Correction

CC mode

Electrode Compensation Bridge Balance Correction Dynamic Holding

Reset these controls to defaults:

VC mode

I-filter

CC mode

V-filter

• Default mode: Initiate with a mouse right-click or shift-click.

Reset all fields to default settings, except:

Liquid junction Offset value

VC mode

V-holding value

CC mode

Subtract Pipette Offset

Show/Hide Controls	Display or hide the general and tabbed controls below this
	button.

Show/Hide Monitor Display or hide the dPatch monitor, to view real-time Voltage

and Current input values for both headstages and all auxiliary input channels.

dPatch Monitor		E
	Voltage	Current
Headstage 1	0.0 V	241.4 fA
Headstage 2	0.0 V	-668.1 fA
AuxIN-1/2	0.001 V	-0.001 V
AuxIN-3/4	-0.001 V	0.001 V
AuxIN-5/6	0.002 V	-0.004 V
AuxIN-7/8	-0.000 V	-0.006 V

Figure 3-4. Headstage Monitor

dPatch Settings

Enable Headstage 1 Configure the control panel to include the headstage.		
Enable Headstage 2	Configure the control panel to include the second headstage.	
Note:	At least one headstage always remains enabled.	
Live Demo Mode	Allows an interactive demo mode, but consumes extra system resources.	
Read Signal Inputs	Enable the dPatch Monitor panel to read input signals.	
Show Bridge Balance as %	Rs compensation	
Show Bridge Balance magnitude in $M\Omega$		
Combine RsCorrection and RsPrediction		
Separate RsCorrection and RsPrediction		
Display the 'Prediction' component separately.		

When disabled, the input bandwidth is limited to 50 kHz or less. This restriction against very high throughput levels helps prevent inadvertently overloading the system resources during data acquisition and analysis.

Liquid junction [±250.0 mV]

Enter the liquid junction potential values to correct for - these are the ionic voltage differences between the pipette solution and the bath and cellular solutions. To correct for this, an offset is automatically applied to the stimulus voltage.

- Note: A liquid junction potential calculator should be used to estimate the liquid junction potential values.
- Auto Offset Click on the Auto Offset button to apply a potential to auto-zero the current signal. The auto-offset value is an approximation that might need further adjustment.

The Offset value is to the right of the Auto Offset button. This value is independent of the holding potential.

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

Auto Algorithm

- Input is acquired with a 10 kHz filter bandwidth and a 100 kHz sampling rate.
- A 40 ms interval is acquired, of which the second half, i.e. 20 ms, is averaged and used as the offset to be nulled.
- The iteration is repeated until the offset is less than full-scale/10000,

i.e. 0.2 mV in VC-mode, or after 20 iterations.

Lock Once an electrode offset has been applied, use the 'Lock' checkbox to prevent accidental changes to the Offset.

 $[\pm 250.0 \text{ mV}]$ Offset values can be directly typed into the numeric field, or

 $\circ~$ For fine adjustments, use the up and down spinners to increase or decrease the setting by 0.1 mV.

• For fastest operation, select the offset field, hover the cursor over the numeric field or spinners, and hold down the Shift key while simultaneously scrolling up or down with the mouse wheel.

Voltage Clamp Controls

VC CC DynC I/O
V-holding 0.0 mV 🖨 Enable
I-Filter 10 kHz 💌
Electrode Compensation
Mag 0.00 pF
Tau 4.00 μs 🗢
Cell Compensation
Rs 0.10 MΩ 🗘 🕂 Auto
Cm 2.00 pF 🖨
Rs Correction
Corr 0 %
Lag 6 µs
I

Figure 3-5. VC Control Panel

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

V-holding [±750 mV]

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

Note: The holding level is restricted to ± 750 mV, because when used in conjunction with a hardware offset of ± 250 mV, it can reach the circuitry's analog output limit of ± 1 V.

Enable Use the Enable checkbox to activate V-holding.

If V-holding is not enabled, the holding level is zero volts.

- I-Filter Low-pass 8-pole Bessel filter bandwidth.
 - [100 Hz, 200 Hz, 500 Hz, 1 kHz, 2 kHz, 5 kHz, 10 kHz, 20 kHz, 50 kHz, 100

kHz, 250 kHz, 500 kHz, 1000 kHz]

This VC-mode filter setting is applied to the active headstage "Current" input channel when a Scope acquisition window is created.

The dPatch system has a fixed headstage sampling rate of 5 MHz. Analog input channels from the headstage are then low-pass filtered by the Control Panel I-Filter (or the Routine Editor Input Channels 'Filter Bandwidth'.)

During acquisition, the Filter control is locked, as it affects the sampling rate.

Note: Demo mode bypasses the filter.

Electrode Compensation: Electrode capacitance compensation.

Magnitude:	[0.00 - 20.00 pF] [shared with CC mode]
Tau:	[1.00 – 10.00 µs]
÷	Opens a 2-D slider panel for simultaneous tuning of both parameters.
Auto	Automatically sets approximate values.

Compensate for the microelectrode capacitive transients by clicking the 'Auto' button, and then using the 2-D slider panel or iteratively adjusting the 'Mag' and 'Tau' controls. For a square pulse command (like a Seal Test), the goal is to eliminate the sharp (fast) transients and reproduce a square waveform with minimal distortion.

(See the SutterPatch Algorithms appendix for the algorithm.)

Cell Compensation Whole-cell compensation.

Rs:	Series Resistance
	$[0.10 - 100.00 \text{ M}\Omega]$
Cm:	Membrane Capacitance
	$[\ 1.00 - 500.00 \ \mathrm{pF} \]$
- + +-	Opens a 2-D slider panel for simultaneous tuning of both parameters.
Auto	Automatically sets approximate values using small "gentle" steps to avoid hyperpolarization.

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

	To remove the transients, click 'Auto', then use the 2-D slider panel or iteratively adjust the 'Rs' and 'Cm' controls until the signal is adequately compensated, i.e., smooth transitions without transients.		
	The dPatch system is optimized for real-world measurements from electrodes. So, when used with the model cell, the compensation might need several more 'Auto' adjustments to compensate the model-cell capacitance.		
Note:	Disable 'Cell Compensation' while the amplifier is in 'Capacitive' mode. 'Cell Compensation' should also be disabled when measuring Series Resistance or Membrane Capacitance in the Membrane Test (Cell mode), so that the calculations are based on uncompensated cell activity.		
	However, you can leave 'Cell Compensation' enabled when using Membrane Test Pulse Type 'Lock-in' frequencies up to 2 kHz. At higher Lock-in frequencies, we recommend disabling Cell Compensation, else the Lock-in phase delay is affected and requires frequent manual adjustment.		
	(See the SutterPatch Algorithms appendix for the algorithm.)		
Rs Correction	ion Whole-cell series resistance compensation.		
	"Rs Correction" speeds up the rise time of the current response, reduces unwanted filtering effects, and corrects voltage drops.		
	Corr:	[0-99%] Correction	
		[disabled in Capacitive mode when "Corr" and "Pred" are displayed separately]	
	Pred	[0-99%] Prediction	
		[displays if enabled in dPatch Settings menu]	
	Lag:	$[~1-100\mu{ m s}~]$ (RC filter component)	
		Lag = 1 / (2 * <i>π</i> * Bandwidth)	
		Control the speed of the correction vs. possible oscillations.	
	+	Opens a 2-D slider panel for simultaneous tuning of both parameters.	
	Auto	Automatically sets an approximate value for "Correction". "Lag" is a critical factor that should be manually set by the user.	
		[disabled in Capacitive mode when "Corr" and "Pred"	

are displayed separately]

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

Small transients might be visible at the beginning and end of the current response. Increase the Correction (Corr) current injected into the membrane. As the Corr setting is increased to sharpen the rise time, the current response transients increase in size. Try to avoid overshooting; if the correction is set too high, internal feedback can lead to oscillation of the circuit, i.e. "ringing", and loss of the patch. Reduce oscillation of the circuit by adjusting the 'Lag' setting - larger values increase the stability of the circuit, but also increase the rise time.

Remove the Correction transients in the signal by reducing the Cell Compensation 'Rs' setting until a minimum value is found.

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

Current Clamp Controls

VC CC DynC I/O		
I-holding 0.0 pA 🜲 Enable		
Subtract pipette offset		
V-Filter 5 kHz 💌		
Electrode Compensation		
Magnitude 0.00 pF 🜲		
Bridge Balance Correction		
Percentage 100 %		
Dynamic Holding		
Target 0.0 mV 🗘 V-hold		
Speed Slow V-mon		
Enable during acquisition		

Figure 3-6. CC Control Panel

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

I-holding: [±20,000 pA]

A holding current can be preset here.

Enable Use the Enable checkbox to activate I-holding.

If I-holding is not enabled, the holding level is zero amperes.

Subtract pipette offset

Subtracting the pipette offset adjusts the electrode current signal to zero, providing an accurate reference baseline for measurements. This offset counteracts any inherent hardware circuitry offsets, as well as electrode-in-the-solution offsets.

The amount of offset applied is displayed in the numeric field to the right of the 'Lock' checkbox.

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

V-Filter Low-pass 8-pole Bessel filter bandwidth.

[[100 Hz, 200 Hz, 500 Hz, 1 kHz, 2 kHz, 5 kHz, 10 kHz, 20 kHz, 50 kHz, 100 kHz, 250 kHz, 500 kHz, 1000 kHz]

This CC-mode filter setting is applied to the active headstage 'Voltage' input channel when a Scope acquisition window is created.

The dPatch system has a fixed headstage sampling rate of 5 MHz. Analog input channels from the headstage are then low-pass filtered by the Control Panel Filter (or the Routine Editor Input Channels 'Filter Bandwidth'.)

During acquisition, the Filter control is locked, as it affects the sampling rate.

Note: Demo mode bypasses the filter.

Electrode Compensation

Magnitude: [0.00 - 20.00 pF] [shared with VC mode]

After seal formation (i.e., in a cell-attached patch configuration), compensate for the microelectrode capacitive transients by adjusting this control. For a square pulse command (like a seal test), the goal is to eliminate the sharp (fast) transients and produce a square waveform with minimal distortion. This function is also known as capacitance neutralization.

Tip: If you consistently lose cells when switching into CC mode, consider adjusting the CC mode electrode compensation value in Set Preferences / Hardware / Stability Control. Bridge Balance Correction is an adjustment to remove the voltage effects of series resistance on current clamp recordings when current is injected. It corrects distortion of the signal due to a voltage drop across the electrode during current flow (injection).

[format set in dPatch Settings menu]

- Percentage [0-200%]
- Magnitude $[0.00 100.00 \text{ M}\Omega]$
- Note: If you are simply recording voltage (I=0), then Bridge Balance can be left disabled, i.e., completely ignored.

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

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

Dynamic Holding

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

Target	[±750 mV]	Target voltage.
	Enter the voltage level to l	be maintained.
Speed • Slow • Medium • Fast	Track the holding potential	at multiple speeds.
V-hold	Set the target value from t setting.	the amplifier V-holding
V-mon	Set the target value to the seen in the headstage volt	cell's existing potential, as age monitor.

Enable during acquisition

Dynamic Clamp Controls

The dPatch system can be setup to perform full dynamic-clamp experiments. Configuration is done in the Dynamic Clamp Editor via the menu item SutterPatch / Hardware Control / dPatch Dynamic Clamp.

VC CC DynC I/O
Dynamic Clamp Active
Model
Mode During Sweeps 💌
Update Rate 100 kHz 💌
Voltage Source Headstage 1 👻
Error

Figure 3-7. Dynamic Clamp Control Panel

DynC The 'DynC' tab contains the "dynamic clamp" controls.

Dynamic Clamp Active Enable the Dynamic Clamp controls.

Model [1-99]

Mode

- During Sweeps
- Run Continuously

Update Rate

[1, 2, 5, 10, 20, 50, 100, 200, 250, 500 kHz]

Voltage Source

- Headstage1
- Headstage2

Error

Error mode indicator.

I/O Controls

VC CC DynC I/O
Digital Output
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 All low
11 12 13 14 15 16 All low All high
Auxiliary Output
AuxOUT1 0 mV
AuxOUT2 0 mV 🜲
AuxOUT3 0 mV 🜲
AuxOUT4 0 mV 🜲
All to zero
,

Figure 3-8. I/O Control Panel

I/O The 'I/O' tab contains the digital output and auxiliary analog output controls.

Digital Output [1-16]

This section controls the holding bit pattern generated by the Digital Outputs of the amplifier. Sixteen TTL-compatible digital channels are displayed. Switch between digital states by clicking on a channel.

• On	high	(+3.3 V)
• Off	low	(0 V)
All low	Set all	l digital channels 'Off'.
All high	Set all	l digital channels 'On'.

Auxiliary Output General purpose "auxiliary" analog output channels are available.

AuxOUT1 - 4 [±10000 mV]

Select an auxiliary analog output channel and directly edit its voltage level, or use the spinners to change the value in 1 mV increments.

- Tip: When the dPatch system is used as a data acquisition system for external instruments, the auxiliary outputs can be used as holding levels.
- All to zero Sets all auxiliary outputs to zero.

3.7 Lock-In Adjustments

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

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

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

Math Type	LockIn
Current Channel	Select a (source) input channel with a "current: signal.
Trace Kind	Select the LockIn measurement to display.
	The selected 'Trace Kind' is automatically set as the Virtual Channel label.
$\mathbf{C}\mathbf{M}$	Computed membrane capacitance.
GM	Computed membrane conductance.
\mathbf{GS}	Computed series conductance.
DC	DC component of measured signal.
RealY	Real number part of the lock-in response signal.
ImagY	Imaginary number part of the lock-in response signal.
Cycles to Average	[1 – 1000]
Cycles to Skip	[1 – 1000]
V-reversal	[±1000 mV]

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

3) The lock-in phase detection can be manually tuned via the menu item SutterPatch / Hardware Control / Lock-In Adjustments. Phase adjustments are made to optimize the headstage signals, and should be done in voltage-clamp mode.

Enable Manual Adjustments	Adjustments can be made using direct field edit- ing, spinners, or a field right-click slider panel.
Absolute Values	
Phase Delay Adjustment	Apply a phase delay to the calculations.
	[±1.00 µs]
Reset	Reset to '0.00 s'
Attenuation Adjustment	Apply a gain to the calculations.
	[0.001 - 9.999]
Reset	Reset to '1.000'
List Results	Display results in the Command window.
Auto Adjust	Automatically adjusts the phase delay so the lock- in absolute capacitance is equal to the cell capaci- tance value in the Amplifier Control Panel. Re- quires the Membrane Test to be running a Lock-In test pulse pattern.

The lock-in computation is quite stable - its calibration values do not change day-to-day. However, lock-in measurements can be affected by experimental conditions, including the amplifier itself. In particular, the electrode compensation has a large influence on the results, and needs to be properly set. Due to its sensitivity, run the electrode compensation on a pulse before running the LockIn adjustments.

When tuning, start with the Phase Delay Adjustment, and then follow with the Attenuation Adjustment.

The sensitivity of the SutterPatch software lock-in results is as good as for a hardware lock-in amplifier. However, for higher frequencies (5 kHz and higher), the lock-in sine frequency is increasingly sensitive to the lock-in phase adjustment. In such cases, you should tune-up the lock-in adjustments more frequently.

When making absolute capacitance measurements, you can improve the consistency of the measurements by adjusting the lock-in phase adjustment to a known reference capacitance, such as with the model cell. The SutterPatch lock-in calculations are based on the Lindau/Neher method of time-resolved capacitance measurements in single cells. (See the Algorithms appendix for the reference.)

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

3.8 Dynamic Clamp Editor

The dPatch system can now perform dynamic-clamp experiments, an extension of the currentclamp technique, with your existing dPatch amplifier and SutterPatch software. This all-inone dynamic-clamp implementation uses a voltage feedback from the cell to replicate the effects of simulated voltage-gated channels, ligand-gated channels, gap-junctions and synaptic inputs on living cells. A fast feedback loop monitors the voltage of the cell under study, and calculates the corresponding simulated conductance to apply to the cell on a point-by-point basis. This ultra-fast implementation easily simulates fast sodium conductances and complex Ornstein-Uhlenbeck Markov calculations, as it can process data in real-time up to a 500 kHz rate.

The simpler leak and linear conductance clamps are also fully supported.

Configuration is done in the Dynamic Clamp Editor via the menu item SutterPatch / Hardware Control / dPatch Dynamic Clamp.

Each headstage supports a pool of 32 coefficients for usage. Multiple equations can be defined per headstage. One equation can have multiple conductances; set a conductance to zero to remove it.

Dynamic clamp equations can be modified in the field via firmware updates.

Status Field	Notifications on edits a	nd Dynamic Clamp names are displayed here.
Files and Pools	[drop-down list]	
	Most recently used list	of the last 5 Dynamic Clamp Pool file names.
	Load Dynamic Clamp Pool	Load a previously saved Dynamic Clamp Pool file into the Dynamic Clamp Pool.
	New Default Dynam- ic Clamp Pool	Create a new Dynamic Clamp Pool either with a default Dynamic Clamp, or populated from the currently loaded Dynamic Clamp Pool.
	New Dynamic Clamp Pool	Create a new Dynamic Clamp Pool either with a default Dynamic Clamp, or populated from the currently loaded Dynamic Clamp Pool.
	Get Default Sample Dynamic Clamp Pool	
	Revert to Last Saved	Undo any unsaved changes to the Dynamic Clamp Pool
	Save Dynamic Clamp Pool	Save the Dynamic Clamp Pool using its exist- ing file name and path.

Save Dynamic Clam Pool As	p Save the Dynamic Clamp Pool to a new file, and switch to the new file. The default file name has an increment number appended to the original file name.
Save Dynamic Clam Pool Copy	p Save the Dynamic Clamp Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to the original file name.
Merge Dynamic Clamp Pools	Insert the Dynamic Clamp Pool from a saved Dynamic Clamp Pool file into the loaded Dy- namic Clamp Pool.
Send Last Used List to Command	Copy the pathname of the last used Dynamic Clamp and paste it into the Command window history.

[Pathname of the loaded Dynamic Clamp Pool file.]

Dynamic Clamp Model Description

Dynamic Clamp Pool [A list of names in the loaded Pool.]

Activate	Run the Dynamic Clamp.
Deactivate	Stop running the Dynamic Clamp.
Save Pool	Save the Dynamic Clamp Pool using its existing file name.
New	Add a default Dynamic Clamp to the Dynamic Clamp Pool and open it for editing. The default name is "untitled" with an increment number appended.
Duplicate	Add a copy of the selected Dynamic Clamp to the Dynamic Clamp Pool. The Dynamic Clamp name-number is appended or increment- ed.
Delete	Remove the selected Dynamic Clamp from the Dynamic Clamp Pool.
Revert	Discard any unsaved changes to the selected Dynamic Clamp.

Table 3-2. Dynamic Clamp Pool

Headstage 1 / Headstage 2 tabs

Activate Headstage

Model		s' equations are functions of voltage. The equations upon activation in the Amplifier Control Panel	
•	Predefined Hodgkin-Hu	xley This model is used for testing.	
•	Hodgkin-Huxley	This model simulates sodium conductance.	
•	Goldman-Hodgkin-Katz		
•	Variable Conductance		
•	Markov Model	This model of state transitions is compu- ting intensive.	
Active Mo	de		
•	During Sweeps		
•	Run Continuously		
Update Ra	te $[1, 2, 5, 10, 20]$	$0, 50, 100, 200, 250, 500 \mathrm{kHz}$]	
Voltage Si	gnal Source		
•	• Headstage 1		
•	Headstage 2		
Edit Mode	l Parameters	[not enabled for predefined models]	
Ch	annel Settings		
	Channel	Ion channel ID.	
	Active	Enable execution of channel in the equation.	
	Label	Enter a custom label for the state.	
	Copy Channel	Copy channel parameters from an activated state.	
	G_maximum (S)		
	V_reversal		
Ga	te Settings		
	Gate	Ion channel gate ID.	
	Active	Enable execution of gate in the equation.	

DPATCH-OPERATION MANUAL-REV. 2.0.4 (2020-2-5)

L	abel	Enter a custom label for the gate.
C	Copy Gate	Copy gate parameters from an activated state.
E	Exponent	
I	nitial State	
α	L	Alpha rate constant.
в		Beta rate constant
τ		Tau rate constant.
Copy Headstage	1 Copy settings	from headstage 1.
Headstage # Equation(s	s) Equations for	Headstage #.
Allow Editing	Allow table ed	liting.
Copy/app	end to layout wind	DW.
Copy to c	elipboard.	
Channel		
Gate #		

3.9 System Integration

The dPatch system can be integrated with other suitable laboratory equipment.

3.9.1 Using Peripheral Equipment

The dPatch system can control peripheral equipment, such as:

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

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

The digital command output can be formatted as either a single "bit" or a 16-bit "word", as selected in the Routine Editor / Output Channels & Waveform section. Actual command output patterns are configured in the Routine Editor / Output Channels & Waveform / Waveform Editor.

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

3.9.2 Using Multiple Sutter Amplifiers

The dPatch amplifier is not supported for multiple-amplifier configurations.

3.9.3 Using Non-Sutter Amplifiers

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

The dPatch digitizer interfaces to external amplifiers via front panel BNC connections:

ANALOG OUTPUTS 1 & 2	These two auxiliary analog output channels can be used to send stimulus waveforms to external instruments, such as non-Sutter microelectrode amplifiers.
ANALOG INPUTS 1 – 4	These four auxiliary analog input channels can be used to digitize signals from external instruments, such as non-Sutter microelectrode amplifiers.

The dPatch digitizer can also interface to external instrumentation via additional rear-panel analog BNC connectors, and the Digital Outputs port optional 'dPatch Expansion Panel' BNC connectors or the included Screw Terminal Board.

ANALOG OUTPUTS 3 & 4	These two auxiliary analog output channels can be used to send stimulus waveforms to external instruments, such as non-Sutter microelectrode amplifiers.
ANALOG INPUTS 5 – 8	These four auxiliary analog input channels can be used to digitize signals from external instruments, such as non- Sutter microelectrode amplifiers.
DIGITAL OUTPUTS	Digital output patterns can be sent via sixteen TTL- compatible digital output channels to a variety of

peripheral equipment.

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

Note:Sutter amplifier output levels into Sutter systems attenuate by < 0.2%.</th>HEKA amplifier output levels into Sutter systems attenuate by 0.%.Axon amplifier output levels into Sutter systems attenuate by 5%.

3.9.4 Using Non-Sutter Data Acquisition Systems

Due to the ultra-high bandwidth of the system, the dPatch system does not operate as a stand-alone amplifier or interface to non-Sutter data acquisition systems.

3.10 dPatch Maintenance

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

3.10.1 Inspection

Periodically inspect all cables and connections to make sure that all cables are sound and that all connections are firm and evenly seated.



Warning! Turn off the dPatch power before plugging/unplugging headstages.

3.10.2 Cleaning

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

3.10.3 Calibration

The dPatch amplifier and headstages can be calibrated, or reset to defaults, via the SutterPatch / Hardware Control / dPatch Maintenance menu. Calibrate with the model cell attached.

📑 dPatch M	🗓 dPatch Maintenance								
Calibration									
Main Unit	Main Unit Headstage 1 Headstage 2						<u>e</u>		
	Gain		Offset (m	V)		Gain		Offset (r	nV)
AuxOUT1	1.000	\$	0.00	\$	AuxOUT2	1.000	\$	0.00	\$
AuxOUT3	1.000	\$	0.00	\$	AuxOUT4	1.000	\$	0.00	\$
AuxIN1	1.000	\$	0.00	\$	AuxIN2	1.000	\$	0.00	\$
AuxIN3	1.000	-	0.00	\$	AuxIN4	1.000	\$	0.00	\$
AuxIN5	1.000	-	0.00	\$	AuxIN6	1.000	\$	0.00	\$
AuxIN7	1.000	\$	0.00	\$	AuxIN8	1.000	\$	0.00	\$
Serial No DPA/E-10010? Part No SA-B000730-REV-G Firmware Version 0 dPatch XOP Version 1.39									
Set Defaults Read from EEPROM Write to EEPROM									
Tools									
Calibrate	Aux I/C)	Updat	e F	irmware				
Status									
Use User EEPROM Copy Factory to User EEPROMs To User Print Main Unit EEPROM									

Figure 3-9. Calibration

Copy to clipboard Copy present calibration window to clipboard.

Main Unit tab

	Gain	Offset (mV)
AuxOUT 1 – 4	0.500 - 1.500	±5000
AuxIN 1 - 8	0.500 - 1.500	±5000

Table 3-3. Main Unit Calibration

Serial No	Serial Number of the dPatch amplifier system.
Firmware Version	Firmware code version of the dPatch amplifier system.
Part No	Part Number of the dPatch amplifier system.
dPatch XOP Version	External Operations code version of the dPatch system.

Set Defaults	The dPatch system default firmware is available for retrieval.
Read from EEPROM	Retrieve the system firmware settings from non-volatile user memory (Electrically Erasable Programmable Read Only Memory).
Write to EEPROM	Store the system firmware settings in non-volatile memory (Elec- trically Erasable Programmable Read Only Memory).

Headstage 1 & 2 tabs

Active

Select a numbered Headstage tab to make it the active headstage here, as well as in the Control Panel.

Voltage Clamp	Gain	Offset (pA)
Capacitive Mode	0.500 - 1.500	±500.00
20 nA Mode	0.500 - 1.500	±5000.0
200 nA Mode	0.500 - 1.500	±5000
Current Clamp	Gain	Offset (mV)
20 nA Mode	0.500 - 1.500	±500.000
200 nA Mode	0.500 - 1.500	±5000.000
Voltage Clamp	Gain	Offset (mV)
Stim DAC	0.500 - 1.500	±500.000

Table 3-4. Headstage Calibration

Serial No	Serial Number of the dPatch headstage.
Headstage Part No	Part Number of the dPatch headstage.
PreAmp Part No	Part Number of the dPatch pre-amplifier.

Set Defaults	Set the dPatch headstage firmware to defaults.
Read from EEPROM	Retrieve the headstage firmware settings from non-volatile memory.
Write to EEPROM	Store the headstage firmware settings in non-volatile memory.

Tools				
Calibrate Aux I/O	[Main Unit tab]			
	Calibrate the Auxiliary Input/Output channels when a dPatch Expansion Panel is connected.			
	Calibrate Headstages	[Headstage 1 & 2 tabs]		
Update Firmware	The dPatch firmware is field files.	l-updatable via electronic		
<u>Status</u> Status	s field of maintenance operatio	ons.		
[]				
Use User EEPROMUse Factory EEPROM				
Print Main Unit EEPROM [Main Unit tab]				

Print Headstage EEPROM [Headstage 1 & 2 tabs] Copy Factory to User EEPROMs To User

4. SOFTWARE OPERATION

4.1 Acquisition

SutterPatch acquisition operations.

4.1.1 Camera Module

The SP_Camera window displays:						
Camera	Select a came	Select a camera name from those attached to the computer.				
Start	View live vid	eo.				
Capture	Take a single picture. If live video is running, this will take a picture while live video continues to run. The image time-stamp is reported in the Log window.					
[video screen]						
Last Capture	A thumbnail	of the last picture taken in the Experiment is displayed.				
All pictures are	stored in the c	urrent Experiment. To review pictures, go to:				
Data Navigator		Select a Paradigm or Routine. Any associated images are listed in the Preview pane. Click on an image name to display the image.				
Data Bro		Navigate to the Data / Images folder. Right-click on an image name, and select 'New Image'.				
-	For dark-room experiments, the window background color can be adjusted by the oper- ating system.					
Windows:	In the Control Panel / Appearance / Personalization window, scroll down and select the High Contrast Black theme, or use the Magnifier tool with option 'Turn on color inversion' enabled.					
macOS:	Press 'Control-Option-Command-8' to set the System Preferences / Accessibility / Display / Invert Display colors option, or open its menu with 'Command-Option-5'.					

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

Sentech drivers:

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

Photometrics PVCAM drivers :

Photometrics:

Prime 95B Prime 95B 25mm

Qimaging:

Electro

4.1.2 Free Run

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

Record streaming data as sweeps into the current Experiment. The button turns green, and the Scope window displays a sweep counter with the maximum number of sweeps.

Note: The button also displays "H5" when run in an HDF5 Experiment.

Move (dock) the Free Run Scope (and Monitor) windows next to the Amplifier Control Panel.

Show / Hide the Free Run Monitor panel.

Monitor Panel

Stored / Recorded:	The cumulative stored recording time. / The cur- rent or last recording time duration.				
Maximum Duration		The maximum amount of time that can be recorded with the current set of recording parameters.			
Signal Parameters					
First Channel	Select	Select an input channel to monitor:			
	Active Headstage				
	Headstage1				
	Headstage2				
	AuxII	N1 - 8			
*][+	-	ay compression is applied to the signal in the window to help reduce the load on computer rces.			
		the mouse over the Compression button to be computed compression factor.			
	When compression is enabled, the button color is darkened.				
	The minima and maxima from two groups of 50 samples are displayed per screen pixel.				
Bandwidth	For headstage channels, this is a read-only field automatically set by 'I-Filter'.				
	For auxiliary channels, select from list:				
	[1, 2, 5, 10, 20, 50, 100, 200 kHz]				
Nyquist Factor	[for Headstage (Current, Voltage) and Virtual input channels]				
	To manage the quantity of data being processed, the hardware sampling rate can adjusted in accordance with the Nyquist Ra				
	2	The Nyquist-limited minimum sampling rate is twice the filter rate, which downsamples the data by 80% from the maximum sampling rate.			
	5	The sampling rate is five times the filter rate; the amount of data to process is cut in half from the maximum allowable.			

10 Use the maximum hardware sampling rate of ten times the filter rate; no downsampling of data occurs.

<u>1 MHz Filter Bandwidth</u>

	2.5	2.5 The Nyquist Factor of '2.5' downsa the data by 50%.		
	5	The highest hardware sampling rat MHz; the maximum Nyquist Facto limited to '5', where no downsampli data occurs.		aximum Nyquist Factor is
		Warni	ng!	Processing very large data sets can overload system resources.
Integrator Reset	[capa	citive m	ode onl	y]
	When using the amplifier's Capacitive mode, choose how to handle undesirable transients in the data generated by capacitor discharges (re- sets).			
	Ignore		Capacitive mode reset transients are displayed in the data.	
	Blank		The data during capacitive transients are made invisible by replacing those data points with NANs (Not A Number).	
	Mask		transi data v discha	ata during capacitive ents are replaced by the last alue before the transient rge, simulating a sample- old operation.
Blank/Mask Duration	n [cap	acitive r	node or	lly]
	[50.00) μs – 1.0	00 s]	
		the rese		500 μs should completely sient. Otherwise, adjust as
Second Channel	Select an input channel to monitor:		nel to monitor:	
	Active	Headst	age	
	Heads	stage1		
	Heads	stage2		

+][+	-	ay compression is applied to the signal in the window to help reduce the load on computer rces.				
	Hover the mouse over the button to see the com- puted compression factor.					
		When compression is enabled, the button color is darkened.				
		ninima and maxima from two groups of 50 les are displayed per screen pixel.				
Bandwidth	For headstage channels, this is a read-only field automatically set by 'I-Filter'.					
	For au	For auxiliary channels, select from list:				
	[1, 2,	5, 10, 20, 50, 100, 200 kHz]				
Nyquist Factor	[for Headstage (Current, Voltage) and Virtual input channels]					
	To manage the quantity of data being processed, the hardware sampling rate can be adjusted in accordance with the Nyquist Rate.					
	2	The Nyquist-limited minimum sampling rate is twice the filter rate, which downsamples the data by 80% from the maximum sampling rate.				
	5	The sampling rate is five times the filter rate; the amount of data to process is cut in half from the maximum allowable.				
	10	Use the maximum hardware sampling rate of ten times the filter rate; no downsampling of data occurs.				
<u>1 MH</u>	z Filter	Bandwidth				
	2.5	The Nyquist Factor of '2.5' downsamples the data by 50%.				
	5	The highest hardware sampling rate is 5 MHz; the maximum Nyquist Factor is limited to '5', where no downsampling of data occurs.				

	Warni	ing!	Processing very large data sets can overload system resources.	
Integrator Reset	[capacitive mode only]		y]	
	choose how to	nplifier in Capacitive mode, undesirable transients in y capacitor discharges (re-		
	Ignore	-	itive mode reset transients splayed in the data.	
	Blank	transi replac	ata during capacitive ents are made invisible by ing those data points with (Not A Number).	
	Mask	transi data v	ata during capacitive ents are replaced by the last alue before the transient, ating a sample-and-hold tion.	
Blank/Mask Duration [Capacitive mode only]				
	[50.00 µs – 1.00 s]			
	The default value of 500 μ s should completely cover the capacitive reset transient. Otherwise, adjust as needed.			
Scope Duration	[0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0 s; Other: 100.00 ms – 100.00 s]			

4.1.3 Membrane Test Windows

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

Scope (Membrane Test)

This Scope (Membrane Test) window operates similarly to the Scope: Acquisition window. The default Scope window top pane displays the Current signal from the active Sutter headstage, and the pane beneath it displays the corresponding Voltage command signal.

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

Additional controls were also added:



Move (dock) the Membrane Test windows next to the Amplifier Control Panel.



Show the Membrane Test Monitor panel.

The first time the Membrane Test is opened, it opens in a "stopped" state, to allow setup and configuration. Thereafter, re-opening the Membrane Test automatically uses the last acquisition status of the Scope window.

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

Membrane Test Monitor

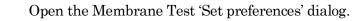
Configure all Membrane Test settings in the Membrane Test Monitor window.

Membrane Test					
Show All Settin	igs	- 0			
BATH	SEAL	CELL			
	Headstage1	Headstage2			
Rpipette	0.00 MΩ	OFF			
Rmembrane	OFF	OFF			
Cmembrane	OFF	OFF			
Write To Log					
	Test Pulse Parame	eters			
Pulse Type Sir	ngle Pulse	•			
Amplitude -5	mV 💌 -	-5.00 mV 🗘			
Duration 5 m	; • • E	5.00 ms 🌲			
Repetition Inte	erval 1.0 s	• 0			
	Zap Parameters				
Amplitude 1.00 V 🗢 Zap 1st Channel 👻					
Duration 500.00 µs 🗢 Do it					
Signal Parameters					
First Channel	Active Headstage	- 📌 🕀			
LFR Off	 Displ 	ay Stimulus			
Second Channel Inactive Headstage 🕞 📌 🕂					
Bandwidth Set by I-Filter (10 kHz)					
LFR Off Display Stimulus					
Measure Parameters					
Measurements From Both Channels					
Num to Average 1 Display OFF					
Resistance Monitor Audio OFF -					
Hz/MΩ Factor 10.00					

Figure 4-1. Membrane Test Monitor

Show

Monitor Only Monitor + Test Pulse All Settings



	Second Membrane Test channel					
	MT analysis on second channel					
	ZAP controls					
	Sound controls					
	Line Frequency Reduction options					
	A/C Line Frequency	$50 \mathrm{Hz}$				
		$60\mathrm{Hz}$				
	Running Average Trace	The average of the last "N" traces, as set in 'Measure Pa- rameters'.				
	Line Thickness	[0.25, 0.50, 0.75, 1 - 10]				
	Color	Open a color selection panel.				
Average Traces		The last "N" traces used in the running average.				
	Line Thickness	$[\ 0.25,\ 0.50,\ 0.75,\ 1-10\]$				
	Color	Open a color selection panel.				
	Capture Trace	Set which trace is used by the "push pin" snapshot control in the Signal Parameters section.				
	Source	SignalAverage	Use the raw Membrane Test trace. Use the running average trace.			
	Line Thickness	[0.25, 0.50, 0.75, 1 – 10]				
	Color	Open a color selection panel.				

The Membrane Test monitor panel contains the three basic steps to patch-clamp a cell:

With a new pipette in the bath solution, a low-resistance square pulse is visible. The pipette resistance should be very low if the tip is not clogged.
With dissociated cells, typical pipette resistances are $1-5~M\Omega$. In slice patch experiments, pipette resistances well above 10 $M\Omega$ are not uncommon.
When an on-cell patch is formed between the pipette and the cell, voltage transition spikes are visible. The seal resistance increases as the seal forms. The goal is to achieve a "gigaseal" with a resistance above $1 \text{ G}\Omega$.
After breaking through the cell membrane and creating a whole-cell patch, membrane resistance and capacitance measures are calculated

0

Membrane Test Preferences

from the resulting capacitance spikes. These values can be periodically checked to monitor the health of the cell.

The Membrane Test calculations are displayed in real-time numeric fields:

Bath	Rpipette (M Ω) [Model cell = ~10 M Ω]		Pipette Resistance meter			
Seal	Rseal (M Ω) [Model cell = 2.05 G Ω]		Seal Resistance meter			
Cell	Rseries (MΩ) [Model cell =	~10 MΩ]	Series Resistance meter			
	Rmembrane ([Model cell =		Membrane Resistance meter			
	Cmembrane (pF) [Model cell = ~28 pF]		Membrane Capacitance meter			
	Warning: In Cell mode, disable the Amplifier Control Panel 'Cell Compensation' and 'Rs Correction' to allow capacitance spikes to be generated for the calculation of the Series Re- sistance and Membrane Capacitance values.					
Note: 'Series Resistance' and 'Access Resistance' are equivalent terms. It is helpful to monitor the Series Resistance, as if it increases by more than 5%, the electrode tip might be clogged.						
[Write To Log] Click the 'Write To Log' button to copy the measurements to the Log window. The monitor results are logged for Headstage 1 and/or Headstage 2.						
<u>Test Pulse Parameters</u>						
Pulse Type						
	• Sin	ngle Pulse	A single monopolar square pulse .			
	• Do	ouble Pulse	A symmetrical bipolar (biphasic) square			

pulse.

	73
• Triangle	A train of 5 symmetrical bipolar triangular pulses.
• Sine	A train of 5 sine wave pulses.
RMS Noise	No pulse – the holding level is output. The RMS noise of the signal is measured.
	In capacitive mode recording, 500 µs of masking is automatically applied to the capacitor reset transients in the data.
• Lock-in	Measurements are based on a phase anal- ysis of a software lock-in amplifier.
	When running the Membrane Test to measure Series Resistance and Membrane Capacitance, the whole-cell capacitance compensation is usually disabled. However, when using Membrane Test Pulse Type 'Lock-in' frequencies up to 2 kHz, the amplifier's 'Cell Compensation' can be enabled. At higher lock-in frequencies, we recommend disabling Cell Compensation, else the lock-in phase delay is affected and requires manual adjustment.
• Lock-in 2.0 m	s (500 Hz)
• Lock-in 1.0 m	s (1 kHz)
• Lock-in 0.5 m	s (2 kHz)

- Lock-in 0.2.ms (5 kHz)
- Lock-in 0.1 ms (10 kHz)
- Lock-in 0.048 ms (20.8 kHz, OnCell only)
- Lock-in: OnCell mode

Amplitude	VC mode:	[Other, -50, -20, -10, -5, -2, -1, 0.1, 1, 2, 5, 10, 20, 50 mV]
		Other: [±75.00 mV] Manually entered value.
		A pulse amplitude is required. Any value less than ± 0.1 mV (absolute) is reset to ± 0.10 mV.
	CC mode:	[Other, -2000, -1000, -500, -200, -100, -50, -20, -10, 0.1, 10, 20, 50, 100, 200, 500, 1000, 2000 pA]
		Other: [±2000.00 pA] Manually entered value.
		A pulse amplitude is required. Any value less than ±0.1 pA (absolute) is reset to ±0.10 pA.

	Amplitude is relative to the 'Holding' level in the Amplifier Con- trol Panel.		
Duration	[Other, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500 ms]		
	Other: Manually entered value.		
Repetition Interval	[Shortest Possible, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10 s]		
0	Oval marker blinks green at the start of each sweep.		
<u>Zap Parameters</u> (This feature is only	active in voltage-clamp mode.)		

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

Amplitude	Set the amplitude of the square wave zap pulse. [$\pm 750.00 \text{ mV}$]		
Duration	[0.10 – 10000.00 ms]		
Zap	1 st Channel 2 nd Channel Both		
Do It	Click the 'Do It' button to send a single square wave voltage pulse from the dPatch headstage to the preparation to disrupt the cell membrane.		
Signal Parameters			
First Channel	Active Headstage	The headstage that is selected or highlighted.	
	Headstage1		
	Headstage2		
* %	Take a snapshot of the next incoming trace and "pin" it as a sta "captured" trace in the Scope window background. Use the cap tured trace to compare signals before and after compensations are applied.		

	"Cut" and remove the	captured trace from the display.		
*[+	Display compression is applied to the signal in the Scope window, to help reduce the load on computer resources.			
	Hover the mouse over sion factor.	the button to see the computed compres-		
	When compression is	enabled, the button color is darkened.		
	The minima and maxima from two groups of 50 samples are dis- played per screen pixel.			
m LFR	Off	Line Frequency Reduction Off.		
	Replace Signal	Display the signal with LFR On.		
	Add Signal	Add a channel for LFR signal.		
Display Stimu	ulus Add a channel	for the stimulus signal.		
Second Channel	No Channel			
	Bandwidth	OFF		
	Inactive Headstage	The headstage that is not selected or high- lighted.		
	Headstage1 - 2			
	Bandwidth	VC mode: Set by I-Filter (x.x kHz) CC mode: Set by V-Filter (x.x kHz)		
	AuxIN1 - 8			
	Bandwidth	[1, 2, 5, 10, 20, 50, 100, 200 kHz]		
* 2	Take a snapshot of the next incoming trace and "pin" it as a s "captured" trace in the Scope window background. Use the ca tured trace to compare signals before and after compensations are applied.			
	"Cut" and remove the captured trace from the display.			
*][+	Display compression is applied to the signal in the Scope window, to help reduce the load on computer resources.			
	Hover the mouse over the button to see the computed compression factor.			
	When compression is enabled, the button color is darkened.			
	The minima and max played per screen pixe	ima from two groups of 50 samples are dis- el.		
Bandwidth	[Set by I-Filter (# kH	Iz)]		

75

LFR Display Stimu <u>Measure Parameters</u>	Off Replace Signal Add Signal Ilus Add a channel for th	Line Frequency Reduction Off. Display the signal with LFR On. Add a channel for LFR signal. ne stimulus signal.
		splays when the Membrane Test Preferences ane Test channel' / 'MT analysis on second
Num to Average Display	for Lock-in and Nois	derived from the last averaged trace, except se measurements. The averaged trace dis- verage is reset when the main mode is al - Cell).
	 Signal Average Signal + Avg Avg + Signal All 	 The raw data trace is displayed. The averaged data trace is displayed. The signal trace is displayed on top of the average trace. The average trace is displayed on top of the raw data trace. The raw data trace is displayed on top of the averaged trace. Prior data traces have persistence, and are displayed as grayed out in the background.
Rm Sound		Membrane Test sound monitor, which beeps er at the beginning of each Membrane Test

	• OFF
	No Sound
	• First Channel
	Second Channel
$Hz/M\Omega$ Factor	[0.01 – 10000.00]
	Hertz / resistance factor to control the pitch of the sound monitor beep in relation to the membrane resistance. Suitable values are typically less than 10.
Volume	$[\ 1.00 - 100.00\ \%\]$
	Sound monitor speaker volume .

4.1.4 Paradigm Editor

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

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

Start Paradigm Stop Paradig	m Pause Resu	me Stop Acquisition Stop at End of Sweep Timer 01:	12:57 Reset
Current Paradigm: Amplifier_Set	up	Step: 1 Set Tag _none_	
Paradigm Status			
Hide Editor Controls	Hide User Ch	eckboxes Variables	y:
Paradigm Pool Files 🚽 👻 C:U	Jsers:EmeraldCity:Do	cuments:SutterPatch:Parameters:SP_ParadigmPool_dPatch.spp	
Paradigm description Set initial	amplifier settings		
aladigin description Set Initial	ampimer seconds		
Paradigm Pool	Save Pool	Paradigm Steps	Insert 💌
Amplifier_Setup		1: Amplifier, Target=Reset	E 10
Start_one_Series		2: Amplifier, Target=CCMode	Edit
Set_amplifier_and_start_IV	New Paradigm	3: Amplifier, Target=IHold, Equation=10p	Toggle Skip
Interactive_acquisition_1	Rename	4: Amplifier, Target=VCMode	
Start_two_Series	Kename	5: Amplifier, Target=Filter, Equation=1000	Duplicate
Start_ForEachSweep	Duplicate	6: Amplifier, Target=VHold, Equation=-80m	Revert
Interactive_acquisition_2		7: Amplifier, Target=IGain, Equation=1G	Kevert
Tuning_with_Input	Delete	8: Amplifier, Target=IGain, Equation=10G	Delete
Toggle_Persistence	Revert	9: Amplifier, Target=VHoldOn, Equation=true	
Switch_Headstages		10: Amplifier, Target=VHoldOn, Equation=!VHoldOn	Step
Tuning_with_Keys		11: Amplifier, Target=Offset, Equation=0.123m	P
		12: Amplifier, Target=OffsetLock, Equation=!OffsetLock	Step to End
		13: Amplifier, Target=ECompMag, Equation=5p	Slow Speed
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		14: Amplifier, Target=ECompTau, Equation=1µ	·
< >		15: Amplifiar Taraat-ECompOn Equation=ECompOn	Log Main 📼

Figure 4-2. Paradigm Editor

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

### **Controls**

Start Paradigm		Run the selected Paradigm highlighted in the Paradigm Pool list. A "Planned Paradigm" is created, which terminates after the end of the last paradigm step.	
		Acquisition in the Scope window (for Membrane Test or Free Run) is stopped.	
Stop Paradigm		Stop a running Paradigm.	
Pause		Manually pause a running Paradigm.	
Resume		Manually resume running the Paradigm.	
Stop Acquisition		End a Routine's acquisition.	
Stop at End of Sweep		Wait until the sweep-in-progress has completed before stopping an acquisition.	
Timer		A running clock displays the time in "hh:mm:ss" since the last timer reset, or since a new experiment established a USB connection or emulation mode.	
Reset		Reset the Timer to 00:00:00.	
Current Paradigm:		The name of the currently loaded Paradigm.	
Step:		The highlighted Paradigm Step.	
Paradigm Status		Status information about Paradigm execution.	
Set Tag		A comment tag can be manually written to the Paradigm metadata with this button. Enter the comment text in the field, or select text from a drop-down list of the most recently used entries. Click this button to write the tag text. The last se- lection is used for all subsequent entries within the same Par- adigm.	
No	ote:	In emulation mode, tag timing is not accurate, and no tags are set in the first sweep.	
${ m Ti}_{ m j}$	p:	When using the Set Tag button, you can minimize the rest of the Paradigm window by hiding its Editor Controls and Variables.	

Show/Hide Editor Controls The Paradigm Editor controls (and checkboxes) for the

	Paradigm Pool and Paradigm Steps can be displayed or hidden.
Show/Hide User Checkboxe	s Checkbox controls are displayed at the bottom of the Paradigm Editor controls, for use in conditional Paradigm step execution. This display is dependent upon Show Editor Controls.
Show/Hide Variables:	A Variables table can be displayed at the bottom of the Paradigm Editor. These paradigm variables can be utilized in any equation.
	Variable names can be edited to any label, but they are only informational, and are not supported in equations.
Last key:	The last key (or key combination) pressed on the keyboard is displayed here, such as used in Shortcuts or the 'If' and 'ElseIf" Paradigm steps. (See sample Paradigm 'Tuning with Keys'.)
Note:	Function and Control (Ctrl/Cmd) Shortcut key combinations are not displayed.
Paradigm Pool Files	These operations affect the entire "Paradigm Pool".
New Paradigm Pool	Create a new blank Paradigm Pool and optionally copy Paradigms into it from the existing Paradigm Pool.
	The suggested name is auto-incremented from the previously loaded Paradigm Pool name.
Load Paradigm Pool	Load the Paradigms of a previously saved Paradigm Pool file into the Paradigm Pool.
Revert to Last Saved	Undo any unsaved changes to the Paradigm Pool.
Save Paradigm Pool	Save the Paradigm Pool using its existing file name and path.
Save Paradigm Pool	As Save the Paradigm Pool to a new file, and switch to the new file. The default file name is the same as the original file name.
Save Paradigm Pool Copy	
	Save the Paradigm Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to it.
Merge Paradigm Poo	
	Insert the Paradigms from a previously saved Paradigm Pool file into the loaded Paradigm Pool.

[The file path and file name of the loaded Paradigm Pool file are displayed.]			
A user description of the active paradigm.			
A column of paradigm names from the loaded Paradigm Pool.			
• Click on a paradigm name to highlight it as the active paradigm and display its steps.			
• Double-click on a paradigm name to start execution of the paradigm and display its steps.			
• Click-and-drag a paradigm name to change its position in the column.			

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

Save Pool Save the Paradigm Pool using its existing file name.

Create a new blank Paradigm in the Paradigm Pool. New Paradigm

Rename the selected Paradigm.

- Valid characters are A-Z, a-z, 0-9, and "".
- Special characters are not allowed.
- Spaces are replaced by an underscore.
- The name cannot start with a number such entries will have an 'X' prepended to the name.

Duplicate Add a copy of the selected Paradigm to the Paradigm Pool. The Paradigm name number is appended or incremented. Delete Remove the selected paradigm from the Paradigm Pool. Revert Select a paradigm and click the 'Revert' button. All editable steps are reset to their originally loaded values, as long as the Paradigm Pool has not been saved.

- Paradigm Steps A column of instructions from the active paradigm is displayed. These instructions are sequentially run by the paradigm.
  - Click on a paradigm step to highlight it as the active • step.

Rename

	• Double-click on a paradigm step to view or edit its settings.	
	• Click-and-drag a paradigm step to change its position in the column.	n
	Note: Step values are usually in standard units, i.e. "Volts" and "Amperes".	
<u>Step Buttons</u>		
Insert	Inserts a new command Step into the Paradigm Steps column:	
	Amplifier	
	Each Sweep	
	Routine	
	Analysis	
	Camera	
	Clear Key	
	Execute	
	Export	
	Front Window	
	Hide Window	
	Reset Timer	
	Scope Operation	
	Set Axis	
	Set Checkbox	
	Set Metadata	
	Set Solution	
	Set Tag	
	Set Variable	
	Sound	
	View Last	

82

	Write to Log
	Alert
	Beep
	Comment
	Wait
	Pause
	Break
	Chain
	For Loop
	Jump
	Label
	If
	ElseIf
	Else
	(See details in Insertable Steps list below.)
Edit	If a highlighted Step is configurable, clicking the Edit button (or double-clicking the step) will open it in the Paradigm Steps Editor for configuration.
	Also, if a highlighted Step's text is partially hidden, use the Edit button to view the entire entry.
Toggle Skip	Mark a step so it is not executed.
	A semicolon is prepended to the Step number to "comment out" the instructions, and a Skip status is appended to the Step text.

		Enomalo	A 'Poon' command in Danadian stan #2	
		Example:	A 'Beep' command in Paradigm step #2:	
			; 2 Beep, Skip=true	
			The leading semicolon ";" prevents this step from being executed by the instruction queue, and the 'Skip' status is displayed.	
Duplicate		Insert a copy	of the selected step.	
Revert		Editable field	to be reverted, and click the Revert button. ds are reset to their originally loaded values, as her Paradigm has not been loaded.	
Delete		Delete the selected step. For multi-line steps, optionally delete the entire group.		
Step		Execute the s	selected step, then move to the next step.	
		-	single step does not terminate a running ven if it is the last step in the Paradigm.	
	Note:	A 'For' loop is	s processed as a single step.	
Step to End		Execute the s system allow	selected step and all following steps as fast as the rs.	
Slow Speed		Execute 'Step	p to End' at ~1 second per step.	
Log Main Steps			ted steps are recorded in the Paradigm metadata e Metadata 'By Event' view):	
		Amplifier		
		Break		
		Camera		
		Chain		
		Execute		
		For Each Sw	eep	
		Reset Timer		
		Routine		
		Set Checkbox	x	
		Set Solution		

83

	Set Variable
	Wait
Log All Steps	Log the main steps and additional steps into the Paradigm metadata (visible in the Metadata 'By Event' view.)
Insertable Steps:	

<u>Amplifier</u>

Control the dPatch amplifier hardware.

Paradigm Steps Editor: "Amplifier", Step 1	B
Undo VHold Vequation -0.0	080
	Check Equation Insert special identifier 👻
Figure 4-3.	Step: Amplifier
Default Setting:	Amplifier, Target=VHold, Equation=-0.080
	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
[ drop-down list ]	Amplifier options
Selec	tProbe (select active probe)
	[1-2]
	Most Paradigm Step commands apply to the "active" probe, the Sutter headstage presently controlled by the Amplifier Control Panel. Select the target headstage.
	For a single headstage system, the active probe is always headstage number "1".
CCM	ode (amplifier current clamp)
	Place the amplifier into Current-Clamp mode.

VCMode	(amplifier voltage clamp)
	Place the amplifier into Voltage-Clamp mode.
Hold	(IHold in CC-mode, VHold in VC-mode)
	$[\pm 0.000,000,020 \text{ A} (\pm 20 \text{ nA}), \text{ or } \pm 1.000 \text{ V}]$
	Set the active headstage holding level.
IHold	(amplifer holding current, A)
	$[\pm 0.000,000,020 \text{ A} (\pm 20 \text{ nA})]$
	Set the active headstage holding level in Current-Clamp mode.
IHoldOn	(amplifier holding current On)
	[0 = Off, 1 = On
	Enable the active headstage holding level in Current-Clamp mode.
VHold	(amplifier holding voltage, V)
	[±1.000 V]
	Set the active headstage holding level in Voltage-Clamp mode.
VHoldOn	(amplifier holding voltage On)
	[0 = Off, 1 = On
	Enable the active headstage holding level in Voltage-Clamp mode.
IGain	(amplifier current gain, V/A)
	Set the gain for the active 'Current' input channel using standard unit numbers (V/A) or scientific notation (1 mV/pA = "1e9".). The value is converted to a preset Gain level:
	• 0.5 mV/pA
	• 1 mV/pA

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

To help prevent saturation, exceeding a 90% threshold between levels promotes the equation value to the next higher gain level.

VGain (amplifier voltage gain, V/V)

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

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

To help prevent saturation, exceeding a 90% threshold between levels promotes the equation value to the next higher gain level.

Feedback	(amplifier feedback mode 0, 1 or 2)
----------	-------------------------------------

Set the amplifier feedback mode.

- 0 Capacitive Mode
- 1 ±20 nA range
- 2 ±200 nA range

(amplifier input filter in VC- and CCmode, Hz)

Set the filter level of the active input channel:

Filter

	•	500	(500 Hz)
	•	1000	(1 kHz)
	•	2000	(2 kHz)
	•	5000	(5 kHz)
	•	10000	(10 kHz)
	•	20000	(20 kHz)
	a 109	% threshold p	er-filtering, exceeding comotes the equation ligher filter level.
IFilter	(amp	olifier input fil	ter in VC-mode, Hz)
		he filter level t channel:	of the active 'Current'
	•	500	(500 Hz)
	•	1000	(1 kHz)
	•	2000	(2 kHz)
	•	5000	(5 kHz)
	•	10000	(10 kHz)
	•	20000	(20 kHz)
	a 109 the e	% threshold be	er-filtering, exceeding etween levels promotes e to the next higher
VFilter	(amp	olifier input fil	ter in CC-mode, Hz)
	Set the filter level of the active 'Voltage' input channel:		
	•	500	(500 Hz)
	•	1000	(1 kHz)
	•	2000	(2 kHz)
	•	5000	(5 kHz)
	•	10000	(10 kHz)
	•	20000	(20 kHz)
			er-filtering, exceeding

a 10% threshold between levels promotes

the equation value to the next higher filter level.

Offset	(amplifier pipette offset in VC-mode, V)		
	[±0.5]		
	Adjust the active output channel to remove any hardware- and/or solution-related offsets.		
OffsetLock	(amplifier pipette offset lock On in VC- mode)		
	[0 = Off, 1 = On]		
	Prevent the offset value from being accidentally changed during a recording.		
SubtractPip(	Offset (subtract pipette offset On in CC- mode)		
LiquidJunc	(liquid junction potential, V)		
GentleSwitch			
GentleSwitch	mode) nV2C (gentle mode switch VC- to CC- mode)		
DynHold	(amplifier dynamic holding potential, V)		
DynHoldOn	(amplifier dynamic holding On)		
ECompMag	(amplifier electrode compensation mag, F)		
ECompTau	(amplifier electrode compensation tau, s)		
ECompOn	(amplifier electrode compensation On in CC-mode)		
	[ 0 = Off, 1 = On ]		
CmComp	(amplifier cell compensation Cm, F)		
	Set a cell capacitance value and enable cell capacitance compensation.		
RsComp	(amplifier cell compensation Rs, Ohm)		

	Set a series resistance value and enable cell capacitance compensation.
RsCompOn	(amplifier cell compensation On)
	[0 = Off, 1 = On]
RsCorr	(amplifier Rs correction, fraction)
	[0.00 - 1.00] Converted to a percentile
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On)
	[0 = Off, 1 = On]
Bridge	(amplifier bridge balance, Ohm)
BridgeOn	(amplifier bridge balance On)
	[0 = Off, 1 = On]
Reset	(reset amplifier controls)
ResetCap	(reset capacitor in capacitive feedback mode)
	Avoid capacitor reset transients in data recordings by generating a reset before each sweep; use the Paradigm 'Each Sweep' step with the Amplifier step option 'ResetCap' preceding the acquisition.
	A short waiting step between the capacitor reset and the acquisition should be set, as there is a small delay between sending the reset command to the dPatch headstage and the actual reset operation; a waiting period of 1 ms should be sufficient.
AutoEComp	(amplifier auto electrode compensation)
AutoCellCom	p (amplifier auto cell compensation)
AutoRsCorr	(amplifier auto Rs correction)
AutoOffset	(amplifier auto pipette offset)

[ 0 = Off, 1 = On ]

AutoSet and enable the offset.

AuxO	UT1 (Auxiliary Output-1, V)
AuxO	UT2 (Auxiliary Output-2, V)
AuxO	UT3 (Auxiliary Output-3, V)
AuxO	UT4 (Auxiliary Output-4, V)
DigOU	UTWord (Digital Output Word)
DigOU	UT1 - 16 (Digital Output-1 - 16)
LockI	nAdjustOn (set LockIn adjustments On)
LockI	nPhaseAdj (set LockIn phase delay adjustment)
LockI	nAttenAdj (set LockIn attenuation adjustment)
[Equation field]	A free-form text field. This field is evaluated and its value passed to the "target" function.
	[ Errors are reported under this field. ]
Note:	Values in the Equation field are rounded to whole numbers.
	While Amplifier steps are configured in standard units (Amperes, Volts), the Amplifier Control Panel displays values in scaled units.
Check Equation	Check the equation syntax. The equation is eval- uated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identi:	fier
-	Acquisition, amplifier and reference settings are available for use in equations. (See <u>list</u> below.)

### Each Sweep

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



Figure 4-4.	Step:	Each	Sweep
-------------	-------	------	-------

Default Setting:	ForEachSweep EachSweep, Target=untitled ForEachEnd
•	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
[ drop-down list ]	Select a Routine name from the loaded Routine Pool.

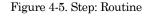
For example, when tracking whole-cell Rs values in real-time measurement graphs, update the Cell Compensation sweep-by-sweep, by inserting an Amplifier step (with its AutoCellComp command selected) within a For EachSweep loop,

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

#### Routine

Record data from a Routine.

Paradigm Steps Editor: "Rout	ine", Step 1		
Undo	IV	•	Edit routine "IV"



Default Setting:	Routine, Target=untitled
•	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
[ drop-down list ]	Select an acquisition type, or a Routine to record a data Series.

Membrane Test

Once

Clear

Indefinitely

Free Run

Duration

[ 100 ms – 999.9 s ] Indefinitely

Add Channel

List of input channels.

[ <selected channels>]

List of Routine names from Routine Pool

[ <selected Routines>]

Edit routine "xxx" Open the Routine into the Routine Editor for "last minute" changes.

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

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

### <u>Analysis</u>

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

Paradigm Steps Editor: "Analysis", Step 4

Figure 4-6. Step: Analysis

Default Setting:	Analysis, Operation=Save to Editor
•	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.

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

#### <u>Camera</u>

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

Paradigm Steps Editor: "Camera", Step 1				
Undo Camera TOSHIBA Web Camera - HD	Capture Live view: No change 💌			
Figure 4-7. Step: Camera				
Default Setting:	Camera, Camera=_Camera_Name, Capture =true			
	Close the 'Paradigm Steps Editor'.			
Undo	Remove any unsaved edits to this step.			
Camera	Select a camera on the computer system.			
Capture	Take a picture when executed.			
Live view:	Configure the state of the live view:			
No Change	Keep last settings.			
• Stop	Stop live view.			
• Start	Start live view.			

# <u>Clear Key</u>

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

# <u>Execute</u>

Extend the functionality of SutterPatch by running an Igor command.

Paradigm Steps Editor: "Execute", Step 10			
	Undo	Command Beep	
		To Command Li	ne

#### Figure 4-8. Step: Execute

Default Setting:	Execute, Command	=Beep
•	Close the 'Paradign	n Steps Editor'.
Undo	Remove any unsave	ed edits to this step.
Command	Run any Igor command accepted by the Command window, including user-created Functions.	
Note:	parentheses "()" be command, however	requires that open/close e appended to the end of a e exceptions include the commands, for which no ed.
	Special references of commands:	can also be used within
	• p[#]	n'th Paradigm variable.
	• s[series-count	t, sweep-count, trace-count, routine-name]
		Reference an input trace (data wave) via counts of Series #, Sweep #, Trace # (Scope position), and the Routine name.
		The "current" item is the "active" trace in the Scope

				ow, and has a count of zero.
			zero, t from t value in cou	count" number is non- it is used as an offset the current count of zero. Any fractions ant numbers are ated to integers.
			blank	routine name is left , the current routine is used.
			Ex:	s[0,0,0,]
				The current series, current sweep, current trace, of the current routine.
	• 1	:[#]	(data positi	ence the input trace wave) in Scope signal on "n", for the last o of the current Series.
Copy to Comm		Append the Co Command win		nd text to the command line.
Expand to Cor	mmand Line	Command w processing it Command w	indow [*] to be o indow	and entry to the 's command line after compatible with execution - any ced by their values.
Example 1:	Reset the Time	er.		
	Set the	Execute 'Con	nmand	' to:
		Paradigm_Re	setTin	ner()
	Note th	ne open and clo	ose pai	rentheses at the end.
Example 2:	Create an FF	Γ graph of yoι	ır data	a.
	The Pa	aradigm Steps	8:	
	1. For	EachSweep		
	2. E	EachSweep, Ta	arget=	YourRoutineName

- 3. Execute, Command=FFT/OUT=3/DEST=Voltage1_ FFT t[2]
- 4. If, Left=sweep, Operation="=", Right=1
- 5. Execute, Command=Display Voltage1_FFT
- 6. EndIf
- 7. Execute, Command=SetAxis Bottom 0,60
- 8. ResetTimer
- 9. ForEachEnd
- In Step 2: Replace "YourRoutineName" with your own Routine name, or use the sample "IV" Routine.
- In Step 3: The Igor 'FFT' command is run, and "t[2]" retrieves the Scope's second input trace.

#### **Export**

Export data graphs into a <u>Layout</u> window.

Paradigm Steps Editor: "Export"	, Step 1	
<b>■</b> Undo	Create 💌	Graphs per page 2 x 4 💌
	Add Signal 📼	All

Figure 4-9. Step: Export

Default Setting:	Export, Signal=Layout
•	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
[ drop-down list ]	Select the operation to perform.
Append	Append graphs to an open Layout window, or

	into a new Layout window.
Create	Create a new Layout window.
Graphs per page	Set the graph layout configuration for new Layout windows:
1	Graph fills entire page.
2	Graphs stacked.
3	Graphs stacked.
2 x 2	Matrix display.
2 x 4	Matrix display.
[ drop-down list ]	Select signals to be exported from a list of default names.
Clear	Clear the signal field, set it to 'off'.
All	Select all entries.
All Signals	Select all input signals.
[ list of input	signals ]
All Analyses	Select all Analysis graphs.
	vsis graphs ][ List of selected signals] User- can be directly entered into the signal field.
	of signals is not used for positioning in the ow – signal positioning is based on their Scope ence.

# Front Window

Set the specified window as the front window.

Paradigm Steps Editor: "FrontWindow", Step 7			
Undo Front Window Scope Window 💌			

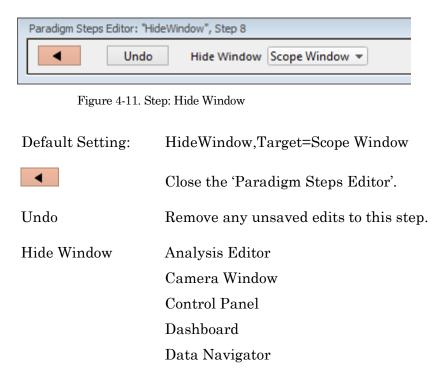
Figure 4-10. Step: Front Window

Default Setting: Front Window, Target=Scope Window

•	Close the 'Paradigm Steps Editor'.
Undo	Remove any unsaved edits to this step.
Front Window	Analysis Editor
	Camera Window
	Control Panel
	Dashboard
	Data Navigator
	Equation Editor
	Log Window
	Paradigm Editor
	Routine Editor
	Scope Window
	Shortcut Editor
	Solution Editor
	Template Editor

#### Hide Window

Hide the specified window.



Equation Editor Log Window Paradigm Editor Routine Editor Scope Window Shortcut Editor Solution Editor Template Editor

### Reset Timer

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

Default Setting: ResetTimer

### Scope Operation

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

Paradigm Steps Editor: "Scope", Step 2		
Undo Wipe	e Scope Persistence: No change al list	✓ Auto Scale Center

riguic 4-12, Step, Scope Operation	Figure 4-12.	Step: Sc	ope Operation
------------------------------------	--------------	----------	---------------

Default Setting:	Scope, Wipe=false		
•	Close the 'Paradigm Steps Editor'.		
Undo	Remove any unsaved edits to this step.		
Wipe Scope	Clear the Scope window of sweeps.		
Signal list:	Enable to display a	list of input signals.	
Add Signal	Clear	Clear the signal list.	
	All Signals	Select all signals.	
	[ list of all av	ailable input signals ]	

[list of selected input signals]

	You can directly edit the list. User- defined signal labels can also be used.
Persistence:	No change On Off
Autoscale	A one-time Autoscale of the Y-axes of all selected signals to their incoming data, i.e., to their visible sweeps data limits, and resets the X-axes to the full sweep duration.
Center	Center the active signal so the mean of the Y-axis data is vertically centered in the signal pane. Only the Y-axis offset is automatically adjusted, not the scaling; the X-axis is unaffected.

# Set Axis

Modify the Axis scaling of a signal.

Paradigm Steps Editor: "Axis", Step 2				
Undo	AutoScale Add Signal 💌	▼ Left ▼ Current1		

Figure 4-13.	Step:	Set Axis
--------------	-------	----------

Default Setting:	Axis, Axis=Autoscale, Kind=Left, Target=Current1			
•	Close the 'Paradigm Steps Editor'.			
Undo	Remove any unsaved edits to this step.			
[ drop-down list ]	Select the operation to perform			
Autoscale	Match the axis range to the data range.			
Autoscale fro	m Zero Display from zero to the largest value.			
Full scale	Display the full range of the axis.			
Use last	Keep using the last-used settings.			
[ drop-down list ]	Select the axis orientation.			

Left	Apply to the Y-axes on the signal list.
Bottom	Apply to the X-axes for all signals.
Add Signal	
Clear	Clear the signal list.
All Signals	Select all signals.
[ list of all available	e input signals ]
[ list of selected signals ]	Select an input signal to modify from the list, or,enter user-defined signal labels.

#### Set Checkbox

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

Paradigm St	eps Editor: "Check	oox", Step 13			
•	Undo	Checkbox 1	true	Check Equation	Insert special identifier 💌

#### Figure 4-14. Step: Checkbox

Default Setting:	Checkbox, Count=1, Equation=true
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Checkbox	Checkboxes $[1 - 3]$ are local: they are cleared whenever a Paradigm is started.
	Checkboxes $[4-6]$ are global: they are not automatically cleared, so their status persists across all Paradigms in the Experiment.
[Equation field]	A free-form text field, evaluated to a value, and applied to the Checkbox.
	[Errors are reported under this field.]

Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it repor "Syntax is ok."	
	•	The constant "True" evaluates to '1.000'.
	•	The constant "False" evaluates to '0.0000'.
Insert special identi	fier	Acquisition, amplifier and reference settings are available for use in equations. (See list below.)

<u>Set Metadata</u>

Define Metadata parameter values to apply.

Go to Preferences > Metadata to change the detail level.

Default Setting: Metadata, Value=

The 'Set Metadata Paradigm Step Value' dialog opens:

Set Metadata Paradigm Step Value
Define Metadata parameter values to apply. Go to Preferences > Metadata to set the detail level.
Select Metadata Group
Stimulus
Select Metadata Parameter
▼
[Metadata Parameter Info]
• Use default:
OUse last value:
OUse a previous value
OUse new value
OEquation
Prompt for confirmation
Do It Cancel

Figure 4-15. Step: Select Metadata Group

# Select Metadata Group

- Operator
- Preparation Animal
- Preparation Tissue
- Preparation Cell
- Experiment
- Electrode
- Recording Solutions
- Paradigm

- (Full detail level)
- (Basic detail level)
- (Basic detail level)
- (Basic detail level)
- (Buble detail level)

(Full detail level)

- (Basic detail level)
- (Extended detail level)
- (Extended detail level)

Cell Health / Quality Cont	rol (Full detail level)
• Series (= Routine Data)	(Full detail level)
• Stimulus	(Basic detail level)
Select Metadata Parameter	
[Metadata Parameter Info]	
• Use default:	
• Use last value:	
• Use a previous value	
• Use new value	
Equation	
[ <define equation=""> ]</define>	
Check	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Special identifier	Special functions for use in equations. (See list below.)
Prompt for confirmation	Display a metadata prompt before acquisition.

### Set Solution

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

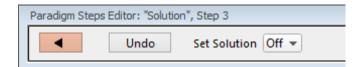


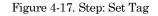
Figure 4-16. Step: Set Solution

Default Setting:	Solution, Target=Off
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Set Solution	[1-24]
	Select a solution number to activate its valve. The number of available solutions depends on the Solution Editor configuration.

# <u>Set Tag</u>

A comment tag can be automatically written to the Paradigm metadata with this step. Enter the comment text in the 'Tag text' field.

	Paradigm Steps	Editor: "SetTa	g", Step 14	
		Undo	Tag text	
1.1				



Default Setting:	SetTag, Text=
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Tag text	Enter the comment text.
	Note: "Step" comment text is maintained separately from the manually triggered 'Set Tag' control comments.

# <u>Set Variable</u>

# Variables allow flexible control of any operation that uses equations.

Paradigm Steps Editor: "SetVariable", Step 15		
◀ Undo Paradigm	•	Variable p[1]
Equation p[1]		
Check Equation	Insert special i	dentifier 💌
Check Equation		
Figure 4-18. St	tep: Set Variable	
Default Setting:	SetVariable, Equation=p[	Target=Paradigm, Count=1, 1]
•	Close the 'St	ep Parameters Editor'.
Undo	Removes any	v unsaved edits to this step.
[List of targets]		
• Paradi	gm	Set the value of a Paradigm Variable_p[#].
		[ 1 – 16. All Variables ]
		When 'All Variables' is selected, if varying values are desired, enter their values into the Equation field as a comma-separated list; simple equations (those without internal commas) can also be used in place of a value.) If there are more variables than list values, the "extra" variables are unchanged. If a list value is blank, the corresponding variable is unchanged.
• Paradi	gm_Input	Set the value of the Paradigm Editor 'Input' control.
• < Rout	ine Names >	Select a Routine and set the value of its Variable_r[#]
		[ 1 – 16, All Variables ]

		When 'All Variables' is selected, if varying values are desired, enter their values into the Equation field as a comma-separated list; simple equations (those without internal commas) can also be used in place of a value.) If there are more variables than list values, the "extra" variables are unchanged. If a list value is blank, the corresponding variable is unchanged.
Equation	Evaluates to a value, used to set variables (or the Paradigm Editor 'Input' control.)	
	inserti reads	an likewise set the value of a variable by ing special identifiers; for example, 'Input' the 'Input' control. (See sample Paradigm ng_with_Input'.)
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."	
Insert special identifier		Acquisition, amplifier and reference settings are available for use in equations.
		(See_list_below.)

### Sound

Output a note from the computer speaker.

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

Paradigm Steps Editor: "Sound", Step 2	×
Undo Frequency (Hz)	
Check Equation Insert special identifier  Play Sound	
Volume 1 🗢	

Figure 4-19. Step: Sound

Default Setting: Sound, Equation=, Volume=1

•	Close the 'Step Parameters Editor'.		
Undo	Removes any unsaved edits to this step.		
Frequency (Hz)	[ 250 - 8000 ]		
	Specify as an equation or fixed value.		
	The sound output has a linear frequency response range within its limits, else:		
	< 250 Hz two clicks > 8 kHz 8 kHz tone		
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."		
Insert special identi	fier Acquisition, amplifier and reference settings are available for use in equations. (See_list_below_)		
Play Sound	Test the sound output.		
Volume	[ 0.1 – 1.0 ]		
	Use the spinners for 10% increments, or directly edit the field.		
Output is via the sta	undard sound output that Igor uses:		
• Windows:	Built-in speakers, or a computer sound card with external speakers		
Note:	Lower frequency tones are attenuated in volume on lower-quality speakers		
• macOS:	Built-in speakers		
command. For insta	This paradigm step can also be utilized as an Igor programming command. For instance, using an equation, one could listen to the membrane resistance of the cell under investigation		

Example: Output a note.

Enter this equation in the Command window command line:

SutterPatch#Paradigm_PlaySound(400, 1)

<u>View Last</u>

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

## Write to Log

Enter text to be written to the Log window.

Paradigm Steps Editor: "WriteLog",	Step 1		
■ Undo	Do Beep	Show Alert	
Text to send to Log text_to_w	vrite		
Equation results to append			
Format 5 digits 💌	Check	Equation	Insert special identifier 🔹

Figure 4-20. Step: Write to Log

Default Setting:	WriteLog, Alert=true, Text=text_to_write, Equation=, DoBeep
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Do Beep	Generate a beep before writing.
Show Alert	Display and/or edit the Alert text, then write it to the Log window.
Text to send to log	[ ]
Equation result to ap	opend [ ]
	Multiple equations in a comma-separated list can be evaluated.
Format	Time Date 1 – 12 digits
Check Equation	Check the equation syntax for sweep #1. The

equation is evaluated, and if valid, it reports "Syntax is ok."

Insert special identifier

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

### Run-time dialog: Write to log

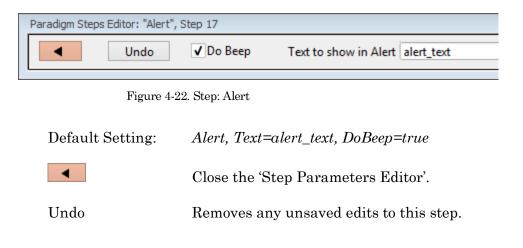
📑 Paradigm: Write to log	
	Elapsed time 00:00:14
Enter text to be written to log	
Text text_to_write	
	Continue and Write Continue, no Write

Figure 4-21. Step: Write to log window

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

## Alert

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



Do Beep Sound a "beep" from the computer.

Text to show in Alert Enter a message to the user.

## Beep

Generate a "beep" sound from the computer speaker.

Default Setting: Beep

## Comment

A text message can be displayed in a floating window.

Paradigm Steps	Editor: "Comm	ent", Step 19	
	Undo	Comment Show in window	
		Show in whicew	

Figure 4-23. Step: Comment

Default Setting:	Comment, Text=
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Comment	Enter the comment text.
Show in window	A 'Paradigm Comment' window is displayed with the comment text, and closes when the paradigm ends.

#### <u>Wait</u>

Temporarily pause execution of the Paradigm for a defined duration.

	Paradigm Steps Editor: "Wait", Step 20
Undo Seconds to Wait 1.0 s	Undo Seconds to Wait 1.0 s

Figure 4-24. Step: Wait

Default Setting:	Wait, Time=1
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Seconds to Wait	Click the spinners for 0.1 s increments, or type in a value. The precision of the wait time is 5 ms.

## <u>Pause</u>

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

#### <u>Break</u>

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

Paradigm Steps Editor: "Break", 3	Step 21	
■ Undo	Break Kind	Paradigm 💌
Figure 4-25.	. Step: Break	
Default Setting:	Break, Kind	=Paradigm
•	Close the 'Step Parameters Editor'.	
Undo	Removes any	v unsaved edits to this step.
Break Kind:	Paradigm	

## ForLoop

## <u>Chain</u>

Use to link step execution to another Paradigm.

Paradigm Ste	eps Editor: "Chain", S	tep 22		
	Undo	<b>√</b> Retur	n to calling Paradi	igm
		Paradig	m to chain to	undefined_paradigm 🔹
L				
	Fig	ure 4-26.	Step: Chain	
	Default Settin	ıg:	Chain, Targe Return=true	et=undefined_Paradigm,
	•		Close the 'Ste	ep Parameters Editor'.
	Undo		Removes any	v unsaved edits to this step.
	Return to calli	ing Pa	radigm:	
		C	Once executi	on of the target Paradigm has eturn execution to this Paradigm
	Paradigm to c	hain to	o: Paradigm e Paradigm.	execution will shift to the selected
	For multiple ( eight Paradig		(or recursive	calls), you can link a maximum o

## <u>For Loop</u>

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

Paradigm Steps Editor: "ForLoop", Step 23	
Undo Max. ForLoo	p Count 1 Coop indefinitely
Figure 4-27.	Step: For Loop
Default Setting:	ForLoop, Max=1 ForEnd
	Close the 'Step Parameters Editor'.

UndoRemoves any unsaved edits to this step.Max. ForLoop CountNumber of loop cycles to run.Loop IndefinitelySets 'Max. ForLoop Count' to 'inf".Note:A "For loop" is processed as one step.

### <u>Jump</u>

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

Para	adigm Steps	s Editor: "Jump",	, Step 24
	•	Undo	Jump Target jump_label

Figure 4-28. Step: Jump

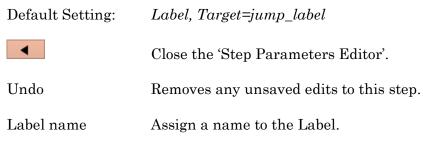
Default Setting:	Jump, Target=jump_label
•	Close the 'Step Parameters Editor'.
Undo	Removes any unsaved edits to this step.
Jump Target	Enter the Label of the step to jump to.

#### Label

Create a Label for a Jump step.



#### Figure 4-29. Step: Label



This step allows conditional Paradigm flow control between multiple choices.

Paradigm Steps Editor: "If", Step 26			
	✓ Undo Compare 2 equations ▼		
	Left Equation		
	Check Equation Insert special identifier 💌		
	Operation: ">"		
	Right Equation		
	Check Equation Insert special identifier 💌		

Figure 4-30. Step: If

Default Setting:	If, Left=, Operation=`>', Right= EndIf		
•	Close the 'Step Parameters Editor'.		
Undo	Removes any unsaved edits to this step.		
[ drop-down list ]	Operation se	lection.	
• Compare 2	equations		
Left Equation		Evaluated to a value.	
Check Equation		Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."	
Insert special identifier		Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)	
Operation:		Comparison operators.	
>		Greater than	
>=		Greater than or equal to	
=		Equal to	
!=		Not equal to	

<=	Less than or equal to
<	Less than
Note:	Be careful when comparing two floating-point numbers for equality, as minor variations in resolution can affect the outcome.
Right Equation	Evaluated to a value.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)
Check for key pressed	"Last key" typed on keyboard during the current Paradigm.
	The "Last key" field is cleared at the start of a Paradigm
Key to check for	Enter a text key, or insert a "special" key.
Insert special key	Use a "non-text" key.
o Space	
o Return	
$\circ$ Esc	
Check checkbox status	Select a checkbox to monitor for "on/off" status.
	Checkboxes are displayed at the bottom of the Paradigm Editor window.
Checkbox	
[1-3]	Paradigm-level "local" checkboxes, cleared at start of Paradigm.
[4-6]	Experiment-level "global" checkboxes, persists across Paradigms.

•

•

• Answer of yes-no-alert

Do Beep	Your computer beep the alert displays.	ps once when
Alert Text [ ]	Enter your alert question text.	
Run-time dialog		
Elapsed Time	A time counter for t	the alert.
Yes	'Yes' button	(value = 1)
No	'No' button	(value = 0)
Stop Paradigm	Manually abort the	Paradigm.

## <u>ElseIf</u>

Allow conditional Paradigm flow control between multiple choices.

Paradigm Steps Editor: "ElseIf", Step 27		
Undo Compare 2 equations	•	
Left Equation		
Check Equation Insert special identifie	er 💌	
Operation: ">"		
Right Equation		
Check Equation Insert special identifie	er 💌	

Figure 4-31. Step: Else If

Default Setting:	ElseIf, Left=,	Operation='>', Right=
Close the 'St		ep Parameters Editor'.
Undo Removes any unsaved edits to this step.		
[ drop-down list ] Operation se		lection.
• Compare 2 equations		
Left Equation		Evaluated to a value.
Check Eq	luation	Check the equation syntax. The equation is evaluated for sweep

	#1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations.

(See_list_below.)

	(Dee_list_below.)
Operation	Comparison operators.
>	Greater than
>=	Greater than or equal to
=	Equal to
!=	Not equal to
<=	Less than or equal to
<	Less than
Note:	Be careful when comparing two floating-point numbers for equality, as minor variations in resolution can affect the outcome.
Right Equation	Evaluated to a value.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)
Check for key pressed	"Last key" typed on the keyboard during the current Paradigm.
	The "Last key" field is cleared at the start of a Paradigm
Key to check for	Enter a text key, or insert a "special" key.
Insert special key	Use a "non-text" key.
$\circ$ Space	
o Return	
$\circ$ Esc	

• Esc

•

Check checkbox status	Select a checkbox t "on/off" status.	to monitor for
	Checkboxes are dia bottom of the Para window.	
Checkbox		
[1-3]	Paradigm-level "loo	cal" checkboxes.
[4-6]	Experiment-level ' checkboxes.	ʻglobal"
Answer of "Yes/No" alert		
Do Beep	Your computer been the alert displays.	eps once when
Alert Text [ ]	Enter your alert q	uestion text.
Run-time dialog		
Elapsed Time	A timer of how lon been displayed.	g the Alert has
Yes	'Yes' button	(value = 1)
No	'No' button	(value = 0)
Stop Paradigm	Manually abort the	e Paradigm.

## Else

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

Default Setting: Else

•

•

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

Checkbox-1	Checkbox-2	Checkbox-3 Ch	eckbox-4 Checkbox-5 Checkbox-6 Input 0
		Figure 4	-32. Checkboxes
		[1-3]	These "local" checkboxes are cleared when a Paradigm starts. They provide Paradigm- specific controls that are only valid for the current Paradigm session.

	[4-6]	These "global" checkboxes are cleared when an Experiment starts. They can be used across all Paradigm Pools for the entire Experiment.
Input	Routine and Paradigm variables can be set to this value. Manually enter a value, or set via the Paradigm step 'Set Variable'.	
	[-1.00-1.00]	This value is restricted to $\pm 1.00$ to enable scrolling through a defined range of values. It can be rescaled when used in an equation.

Paradigm Variables Pane The

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

Paradigm \	Variables								×
	Variable1	Variable2	Variable3	Variable4	Variable5	Variable6	Variable7	Variable8	V
Name	Var p[1]	Var p[2]	Var p[3]	Var p[4]	Var p[5]	Var p[6]	Var p[7]	Var p[8]	
Value	0	0	0	0	0	0	0	0	0 🗸
<									>

Figure 4-33. Paradigm Variables

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

	•	'Close' button	Closes the Variables table.
		Variable[1 – 16]	16 columns of Paradigm Variables.
	Name:	Var_p[1 – 16]	Paradigm Variable names can be edited to any text.
		Note:	These names are for display only, and cannot be used in equations.
	Value:	[ ]	Numeric values can be manually entered, or programmatically set via the Paradigm step 'Set Variable'.
lentifiers:	The fo	llowing acquisition, a	amplifier and reference

List of special identifiers:

The following acquisition, amplifier and reference settings are available for use in equations.

Loop	(active paradigm ForLoop count)		
Sweep	(active Routine sweep count)		
	Sweep count of the active sweep in the Scope window.		
LastSweep	(total number of sweeps in active Routine)		
	During acquisition, this is set according to the Routine parameters. Once acquisition terminates, this is replaced by the count of the last acquired sweep, i.e., the last sweep in the stored Series.		
	Processing can occur before or after the last sweep of a Series.		
	Example: Use in a 'ForEachSweep' loop Routine, to compare an 'If' step equation to the sweep number.		
	ForEachSweep EachSweep, Target=IV If, Left=sweep, Operation= '=', Right=LastSweep- 1 Alert, Text=LAST SWEEP, DoBeep=true EndIf ForEachEnd		
AqStopped	(last acquisition was stopped)		
	[ 0 = the last acquisition completed ] [ 1 = the last acquisition was stopped ]		
ParadigmTime	(time at start of paradigm, s)		
RoutineTime	(time at start of routine, s)		
Stimulant	(last applied stimulant concentration)		
	From the Solution Editor 'Concentration' setting, for a solution configured as a 'Chemical Stimulant'.		
Time	(present date-time, s)		
Timer	(timer time, s)		
m[116]	(n'th analysis measurement value)		
gx[18]	[18] (n'th analysis graph x value)		

		The X-value of the last data point in the latest version of graph[#]
Ę	gy[18]	(n'th analysis graph y value)
		The Y-value of the last data point in the latest version of graph[#]
1	c[116]	(n'th Routine stimulus variable)
ł	p[116]	(n'th Paradigm variable)
]	Hold[12]	(holding of n'th output channel)
		Headstage holding level.
]	Input	(Input variable on Paradigm window)
-		
1	AuxIN[18]	(reading of auxiliary input, V)
		A single-point reading.
]	lmon	(amplifier current reading, A)
V	Vmon	(amplifier voltage reading, V)
I	Mean[name or coun	t,start,width] (mean of given input signal)
		'name' = signal name
		'count' = window-signal position
		'start' = time of start, s (of measurement range)
		'width' = duration, s (of measurement range)
-		
1	ActiveProbe	(active probe)
		[1-2]
		The highlighted headstage number in the Amplifier Control Panel.
		For a single headstage system, the active probe is always headstage number "1".
]	NumProbes	(number of probes)

The number of dPatch headstages attached to the system.

-----

CCMode	(amplifier current clamp)		
	[0 = Off, 1 = On]		
VCMode	(amplifier voltage clamp)		
	[0 = Off, 1 = On]		
Hold	(IHold in CC-mode, VHold in VC-mode)		
	$[\pm 0.000,020 \text{ A} \ (\pm 20 \text{ nA}), \text{ or } \pm 1.000 \text{ V}]$		
	Headstage holding level.		
IHold	(amplifer holding current, A)		
	[±0.000,020 A (±20 nA)]		
IHoldOn	(amplifier holding current On)		
	[0 = Off, 1 = On]		
VHold	(amplifier holding voltage, V)		
	[±1.000 V]		
VHoldOn	(amplifier holding voltage On)		
	[0 = Off, 1 = On]		
IGain	(amplifier current gain, V/A)		
	The gain levels of the active voltage-clamp 'Current' input channel:		
	0.5 mV/pA		
	1 mV/pA		
	2.5 mV/pA		
	5 mV/pA		
	10 mV/pA		
	25 mV/pA		

VGain	(amplifier volta	ge gain, V/V)		
	V/V evaluates to mV/mV.			
	The gain levels input channel:	of the active current-clamp 'Voltage'		
	10 mV/mV	V		
	20 mV/mV	V		
	50 mV/mV	V		
	100 mV/mV	V		
	200 mV/mV	V		
	500 mV/mV	V		
Feedback	(amplifer feedba	ack mode: 0, 1, 2)		
	• 0	Capacitive Mode		
	• 1	±20 nA range		
	• 2	±200 nA range		
Filter	(amplifier input	t filter in VC- and CC-mode, Hz)		
	The filter level of the active input channel.			
	To help prevent over-filtering, exceeding a 10% threshold between levels promotes the equation value to the next higher filter level.			
	• 500	(500 Hz)		
	• 1000	(1 kHz)		
	• 2000	(2 kHz)		
	• 5000	(5 kHz)		
	• 10000	(10 kHz)		
	• 20000	(20 kHz)		
IFilter	(amplifier input	t filter in VC-mode, Hz)		
	Apply a filter to the current input channels.			
	over-filtering, exceeding a 10% een levels promotes the equation value er filter level.			

	500	(500 Hz)
	1000	(1 kHz)
	2000	(2 kHz)
	5000	(5 kHz)
	10000	(10 kHz)
	20000	(20 kHz)
VFilter	(amplifier in	put filter in CC-mode, Hz)
	Apply a filte	r to the voltage input channels.
	threshold be	ent over-filtering, exceeding a 10% tween levels promotes the equation value ligher filter level.
	500	(500 Hz)
	1000	(1 kHz)
	2000	(2 kHz)
	5000	(5 kHz)
	10000	(10 kHz)
	20000	(20 kHz)
Offset	(amplifier pi	pette offset, V)
OffsetLock	(amplifier pi	pette offset lock On)
	[ 0 = Off, 1 =	On]
SubtractPipOffset	(subtract pip	ette offset On in CC-mode)
	[ 0 = Off, 1 =	On]
LiquidJunc	(liquid juncti	ion potential, V)
GentleSwitchC2V	(gentle mode	e switch CC- to VC- mode)
GentleSwitchV2C	(gentle mode	e switch VC- to CC- mode)
DynHold	(amplifier dy	vnamic holding potential, V)

[ 0 = Off, 1 = On ]

-----

ECompMag	(amplifier electrode compensation magnitude, F)
ECompTau	(amplifier electrode compensation tau, s)
ECompOn	(amplifier electrode compensation On in CC-mode)
	[0 = Off, 1 = On]
CmComp	(amplifier cell compensation Cm, F)
RsComp	(amplifier cell compensation Rs, Ohm)
RsCompOn	(amplifier cell compensation Rs On)
	[0 = Off, 1 = On]
RsCorr	(amplifier Rs correction, fraction)
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On)
	[0 = Off, 1 = On]
Bridge	(amplifier bridge balance, Ohm)
BridgeOn	(amplifier bridge balance On)
	[0 = Off, 1 = On]
CapResets	(number of capacitor resets since last sweep start)
Relectr[12]	(electrode/seal/access resistance, Ohm)
	Value from last Membrane Test
Rmemb[12]	(membrane resistance (Cell mode), Ohm)
	Value from last Membrane Test
Cmemb[12]	(membrane capacitance (Cell mode), F)
RMSNoise[12]	(membrane test RMS noise, A)

	Value from last Membrane Test
LockinPhaseAdj	(LockIn phase delay adjustment, s)
LockinAttenAdj	(LockIn attenuation adjustment)

## 4.1.5 Routine Editor

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

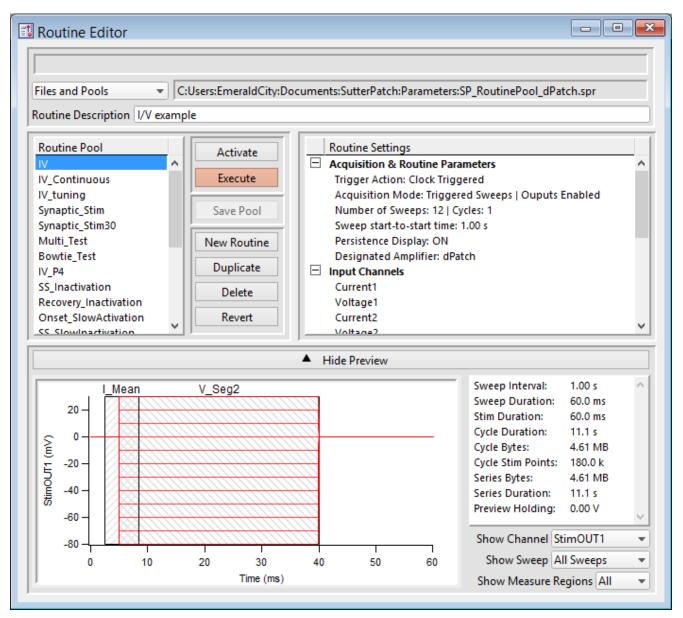


Figure 4-34. Routine Editor

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

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

<u>Status Field</u>	Notifications on edits and Routine names are displayed here.		
Files and Pools		[ drop-down list]	
	Most recently used list	of the last 5 Routine Pool files	
	Load Routine Pool	Load the Routines of a previously saved Rou- tine Pool file into the Routine Pool	
	New Default Routine Pool	Create a new blank Routine Pool.	
	New Routine Pool	Create a new Routine Pool optionally populated with Routines from the currently loaded Rou- tine Pool.	
	Get Default Sample Routine Pool	Load the Routines from the default dPatch sample Routine Pool file SP_RoutinePool_dPatch.spr.	
	Revert to Last Saved	Undo any unsaved changes to the Routine Pool	
	Save Routine Pool	Save the Routine Pool using its existing file name and path.	
	Save Routine Pool As	Save the Routine Pool to a new file, and switch to the new file. The default file name has an increment number appended to the original file name.	
	Save Routine Pool Copy	Save the Routine Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to the original file name.	
	Merge Routine Pools	Insert the Routines from a previously saved Routine Pool file into the loaded Routine Pool.	
	Merge PatchMaster PGF File	Insert the "Routines" (PGF Sequences) from a PatchMaster PGF file into the loaded Routine Pool. Re-assign the analog input and output channels in a 'Mappings' dialog that opens.	
	Convert Routine Pool	Convert the loaded Routines to be compatible with the attached amplifier or emulation mode.	
	Send Last Used List to History	Copy the pathname of the last used Routine and paste it into the Command window history.	

Table 4-1. Routine Files and Pools

New Routine Pool dialog:

1	Create New RoutinePool	×
	Select routines to copy to the new Routine Pool. If none selected, a default Routine Pool is created.	
	Cancel to abort this operation. Create to build the new Routine pool.	
[	Select All	
	Available Routines	
	IV	^
	IV_Continuous	
	IV_tuning	
	Synaptic_Stim	
	Synaptic_Stim30	
	Multi_Test	¥
	Cancel Create	

Figure 4-35. New Routine Pool

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

[File Path field]

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

#### **Routine Description**

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

#### Routine Pool

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

Double click a Routine name to edit it.

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

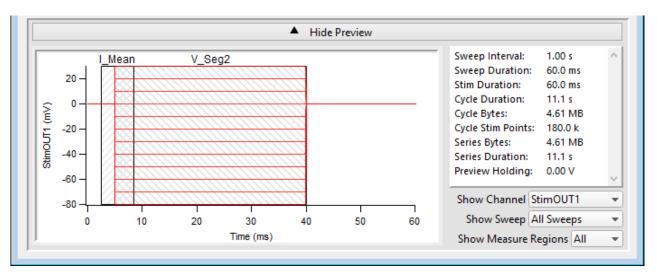
To select multiple Routines, use a Shift-Click mouse drag, or individually Shift-Click the Routine names. Multiple Routines can thus be deleted, or saved to a new Routine Pool.

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

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

Table 4-2. Routine Editor Buttons

### Waveform Preview



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

Figure 4-36. Waveform Preview Pane

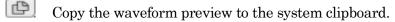
The waveform preview and its settings are updated live to reflect changes in the Waveform Editor and Amplifier Control Panel.

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

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

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

- Note: A "Cityscape" display mode is used, i.e. plotting with straight horizontal and vertical lines connecting the preview sample points (vs. smooth interpolated transitions).
  - Copy and paste the waveform preview into a Layout window. If no Layout window is open, a new one is created.



Show / Hide Preview Expand or collapse the Preview pane.

X- and Y-axis Control

囯

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

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

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

Sweep Interval:	The interval of time between the starts of consecutive triggered sweeps (Sweep Start-to-Start Time) in the active Routine.		
Sweep Duration:	The amount of time in a sweep during which signal recording occurs with the active Routine.		
Stim. Duration:	The maximum amount of time during which output stimulation occurs in a sweep.		
	Set in Output Chann tion.	nels & Waveform / Waveform Editor / Dura-	
Cycle Duration:	The amount of time	for a cycle.	
	Set in Acquisition & Routine Parameters.		
Cycle Bytes:	The number of bytes of data in a cycle.		
Cycle Stim Points:	The number of points in which output stimulation occurs in a cycle.		
Series Bytes:	The number of bytes of data in the Series.		
Series Duration:	The amount of time for the Series.		
Preview Holding :	The holding level in the Amplifier Control Panel.		
Show Channel:	A list of output channels to preview.		
Show Sweep:	Select how to display autoscaled sweep traces in the preview pane.		
	• Time Course	Display all traces in continuous linear time.	
	• All Sweeps	Display all traces overlaid from time zero.	
		(not available for "Show Channel: All Channels")	

• Sweep # Display a trace from a single sweep.

Show Measure Regions:

A list of measurement regions to preview.

•

•

- None No regions displayed.
- All All regions displayed.
- m[#] Select a single region to display.

## **Routine Settings**

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

Routine Editor					٥ [٢
Files and Pools	- · ·		ocum	ents:SutterPatch:Parameters:SP_RoutinePool_dPatch.spr	
Routine Pool IV IV_Continuous IV_tuning Synaptic_Stim Synaptic_Stim30 Multi_Test Bowtie_Test IV_P4 SS_Inactivation Recovery_Inactivation Onset_SlowActivation SS_SlowInactivation	~	Activate Execute Save Pool New Routine Duplicate Delete Revert		Input Channels Output Channels & Waveform Real Time Measurements & Graphs	~

Figure 4-37. Routine Settings

## Routine Editor: Acquisition & Routine Parameters

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

cquisition & Routine Parameters: IV	[
•	
Routine designated fo	or dPatch
Trigger Action Clock Triggered	<b>•</b>
Acquisition Mode Continuous Sweeps	✓ Auto Configure
<ul> <li>Enable Output Waveforms</li> </ul>	
Number of Sweeps 10	\$
Number of Sweeps 10 Sweep Cycles 1	•

Figure 4-38. Acquisition & Routine Parameters

Trigger Action

- Clock Triggered: Start a recording timed by the SutterPatch program.
- Externally Triggered Sweep:

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

Connect the trigger signal to the 'Trigger In' BNC on the front panel of the dPatch amplifier.

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

• Externally Triggered Series:

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

Connect the trigger signal to the 'Trigger In' BNC on the

front panel of the dPatch amplifier.

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

### Acquisition Mode

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

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

• Continuous Sweeps: Uninterrupted data without time gaps between sweeps are supported in this mode, when the Sweep Start-to-Start Time' is set equal to the 'Sweep Duration'.

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

#### This option does not support:

- Pausing of sweeps during recording.
- Paradigm step 'Each Sweep'.
- Very short sweeps.
- Warning! High data throughput has the potential to overload system resources and interfere with data processing.
- Auto ConfigureAutomatically re-configures the Acquisition Mode from<br/>Triggered Sweeps to Continuous Sweeps when the Sweep<br/>Start-to-Start Time' is 'Shortest Possible' or 'Sweep<br/>Duration' is enabled.

## Enable Output Waveforms

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

Number of Sweeps [1 - 65000]

The number of sweeps to record.

Sweep Cycles [1 - 65000]

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

		-
Indefinite	[ for 'Triggered Swe	eps' ]
		ep Cycles is undefined - sweeps continue to tion is manually stopped.
Indefinite Post Stir	nulation [ for 'C	ontinuous Sweeps' ]
		d number of Sweep Cycles is reached, the s disabled while Sweeps continue to record manually stopped.
The largest file size	e that SutterPatch can	record is 2.5 G samples.
Note: If more than 1 mega-sample of memory is allocated, a message panel will display "Allocating acquisition buffers, please wait" When allocating large memory blocks, the computer can appear to be "frozen" for several minutes.		
Post Stimulation Cycles	[ for 'Continuous Sw	eeps' with Outputs ]
After the specified with outputs disable		es is reached, continue acquiring cycles
Sweep Start-to-Start Time	e [ 's', or if < 1 s, then i	n 'ms' ]
The time from the s	start of recording a sw	eep to the start of the next sweep.
Shortest Possible	[ for 'Triggered Swe	eps']
	The longest wavefor processing time (200	rm duration in the Series + overhead 0 ms).
Sweep Duration	[ for 'Continuous Sw	veeps']
	The sweep duration Series.	is the longest waveform duration in the
	[Outputs enabled]	As set in Output Channels & Waveform / Waveform Editor.
	[Outputs disabled]	As set in Input Channels / Edit Signals / Waveform Editor.
	de, the sweep start-to- n slower computers.	start time can vary during acquisition,
Persistence Display		

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

• Off Set the Scope window into non-persistence display mode.

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

# Routine Editor: Input Channels

Configure the input channels.

Input Channels: IV	8
Edit Signal	Input Unit A
Channel Label	Scaling Offset 0.00 A Factor A/V 1 Parent Out Chan StimOUT1 Scope Position 5 Hide Channel in Scope Window Filter Bandwidth Use Control Panel Nyquist Factor 10
AuxIN8 Virtual1 Smoothed	Math Type Smooth 💌
Virtual2 Virtual3 Virtual4 Virtual5 Virtual6 Virtual8 Virtual9 Virtual9	Source Channel Current1  Smoothing Type Gaussian Smooth Operations 2 Integrator Reset Ignore

Figure 4-39. Input Channels

[Status field] Notifications on edits are displayed.

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

#### Channel

Enable up to 16 Input Channels for recording data:

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

Voltage# Analog input voltage channels hardwired from the dPatch

headstage.

AuxIN[1-4]	Four auxiliary analog input channels allow you to directly digitize and record input signals from connected non-Suttter external equipment.
Note:	In Emulation mode, the AuxIN channels display a ±20 mV sine wave.
Virtual[ 1 – 10 ]	Ten virtual channels are available.
	Virtual channel data are mathematically transformed data from another input channel, or are entirely computed from an equation.

#### Label

A user-editable signal name for a channel.

These labels are used in:

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

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

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

## Edit Signal

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

## Input Unit

The base unit of measurement.

• 'Voltage' Channels Fixed at 'V' for voltage.

• 'AuxII	N' Channels	Default is 'V', but is editable.
• 'Virtu	al' Channels	Same as its Source channel; is only editable for virtual Math Type 'Equation'.
Note: Th	ne resolution of the sig	gnal units are automatically set.
Scaling [ for A	uxIN and Virtual Inp	ut channels ]
Offset	Apply an amplitude	offset to an input signal (after any scaling).
Tip:	To use 'mV' units, er	nter: '#m' or '#e-3'
	To use 'pA' units, en	ter: '#p' or '#e-12'
Factor	Change the scaling f equation.	factor of input channels using a fixed value or
Note:		uses a high-resolution 16-bit ADC with 64-bit tion is not an issue when scaling input signals.
Parent Out Chan		
	associated with which	Channel" shows which output channel is ch input channel. The ouput channel timing is cements with 'Cursors Relative to Segments'.
	Auxiliary Input char	nnels allow you to change the 'Parent Out Chan'.
		annels cannot directly select Parent Output hey utilize their Source channel's Parent
Scope Position	The input channel p	anes can be repositioned in the Scope window.
Hide Channel in Sco	-	hannel is hidden in the Scope window.
Filter Bandwidth	[ for headstage analo	og input channels ]
	Set the input channe	el low-pass filter bandwidth:
	Use Control I	Panel Set the 'Current' and 'Voltage' channel bandwidths via the Amplifier Control Panel.
	[ 100, 200, 50	0 Hz; 1, 2, 5, 10, 20, 50 100, 250, 500, 1000 kHz ]
Note:	The headstage samp of the filter bandwid	oling rate is automatically adjusted to a mulitple th:

		100 Hz – 500 kHz filter 1 MHz filter	10x 5x	1 8
		vailability of higher filter fi uter system and display spe	-	cies can be limited by your
Sampling Rate	[1,2,	5, 10, 20, 50, 100, 200 kHz	]	
	[ for A	Auxiliary input channels ]		
	Comp rate:	press the Auxiliary input ch	annel s	signal from a 200 kHz sample
		sampling is done by simple width, each data point is the s.		
Nyquist Factor	[ for H	leadstage (Current, Voltag	e) and	Virtual input channels ]
		anage the quantity of data l ling rate can be adjusted in		rocessed, the hardware lance with the Nyquist Rate.
	2	The Nyquist-limited mini filter rate, which downsar maximum x10 sampling r	mples t	
	5			he filter rate; the amount of n the maximum allowable.
	10	Use the maximum hardw filter rate; no downsamp		npling rate of ten times the data occurs.
	<u>1 MH</u>	z Filter Bandwidth		
	2.5	The Nyquist Factor of '2.5	5' down	samples the data by 50%.
	5			rate is 5 MHz; the maximum vhere no downsampling of
Warr	ing!	Processing very large data	a sets c	an overload system resources.

## Virtual Input Channels

Virtual input channels allow you to perform a variety of mathematical transformations on input signals in real time. To enable a virtual signal, highlight a signal name. When a virtual input channel is enabled, its configuration fields are ungrayed.

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

- BaselineSubtract Subtract a fixed value from all data points in an input trace. This is useful for adjusting for an offset, or resetting a baseline. Source Channel Select an input channel to process. **Baseline** From Select how to calculate the subtraction value. Value Subtract a fixed value. . Value Spinner adjusts in 1 pA or 1 mV increments. Trace Subtract the average of the entire input trace. Sweep Time Subtract the average of the data between the Start Time and End Time. Start Time Set the starting time of the data to be averaged. End Time Set the ending time of the data to be averaged. Segment #s Subtract the average of a Segment. Start Ratio Set the starting time of the data to be averaged, as a ratio relative to the starting time of the Segment duration. Start Time [derived value] End Ratio Set the ending time of the data to be averaged, as a ratio relative to the ending time of the Segment duration. End Time [derived value] BesselFilter A frequency-domain filter with excellent response characteristics for preserving the shape of a biological signal. Source Channel Select an input channel to filter.
  - Filter Bandwidth Select a frequency range.
    - LowPass Allow signal frequencies less than the cutoff frequency, and block all higher frequencies, such as high frequency

	1.10
	noise.
• HighPass	Allow signal frequencies greater than the cutoff frequency, and block all lower frequencies.
Filter Order	[1,2,4,8]
	Number of "poles" in the filter. A higher number provides a sharper (more accurate) response, but consumes more processing time and system resources.
Cutoff Frequency (Hz)	[ 100 to < $\frac{1}{2}$ the sampling rate ]
	Restrict frequencies from this boundary point onwards.
Integrator Reset	For amplifier Capacitive mode.
• Ignore	Capacitive-mode transients are displayed in the data.
• Blank	The data during capacitive transients are made invisible by replacing those data points with NANs (Not A Number).
• Mask	The data during capacitive transients are replaced by the last data value before the transient discharge, simulating a sample-and-hold operation.
Blank/Mask Duration	[ 50 µs – 1 s ]
	The default value of 500 µs should be sufficient to encompass the reset transient duration.
• Differentiate Apply differentiation t of change in the signal	o an input signal. The instantaneous rate is displayed.
Source Channel	Select an input channel to differentiate.
• Equation Specify an equation to proc	ess an input signal.
Source Channel	Select an input channel to process.
Equation [ ]	Click field to access the 'Specify math equation' editor.

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

#### Specify math equation for virtual signal

[ <equation> ]</equation>	A free-form text field.
	Errors are reported under this field.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is "ok".

#### Insert special identifier

Special references can also be used within commands:

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

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

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

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

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

Ex: s[0,0,0,]

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

t[#] n'th input trace

Access the input trace (data wave) in Scope position "n" for the last sweep of the current Series. This numbering can

•

Undo

All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

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

Source Channel Select an input channel to integrate.

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

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

Source Channel	Select an input channel to process.
Show Leak	Displays the accumulated leak currents after the subtracted data in a sweep.
Leak Zero Segment	Identify a segment with no active cellular response to the command signal.
	When set to zero, the field is set to 'OFF'. To re-display the numeric spinners, enter a non-zero number into the field.
Note:	The mean of the second half of the specified segment is used to compute an averaged leak current, which is then used to correct the P/N leak average. This option reduces the influence of a constant leak-current, which is otherwise added to the leak current of

the main pulse.

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

Source Channel	Select an input channel to process.		
Line Frequency	$50~{ m Hz}$	Most of rest of world.	
	60 Hz	Canada, (Carribean),	

Central America, (Japan), Mexico, (South America), South Korea, Taiwan, USA.

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

- LockIn Measure cell characteristics (such as membrane capacitance) with high signal-to-noise sensitivity, using a dual-phase software lock-in amplifier.
  - Note: This feature is only enabled when the Routine includes an output channel with a waveform Segment set to 'Sine / Sine Wave Cycles / For LockIn.'

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

Current Channel	Select a (source) input channel with a "current" signal.	
Trace Kind	Select the LockIn measurement to display.	
	The selected 'Trace Kind' is automatically set as the Virtual Channel label.	
$\mathbf{C}\mathbf{M}$	Computed membrane capacitance.	
$\mathbf{G}\mathbf{M}$	Computed membrane conductance.	
$\mathbf{GS}$	Computed series conductance.	
DC	DC component of measured signal.	
RealY	Real number part of the lock-in response signal.	
ImagY	Imaginary number part of the lock-in response signal.	
Cycles to Average	[ 1 – 1000 ]	
Cycles to Skip	[ 1 – 1000 ]	
V-reversal	[±1000 mV]	
	When using a calculated stimulus trace, enter	

When using a calculated stimulus trace, enter the reversal potential for the ion under study,

such as for (Na+) sodium spikes or (K+) potassium tail currents.

- Note: See the SutterPatch Algorithms appendix for the math used in the LockIn computation.
- Smooth Smooth the data with a "moving average" noise-reduction filter.

Source Channel	Select an input channel to smooth.	
Smoothing Type:		
• Gausian	A standard filter with excellent $10 - 90\%$ rise-time response.	
Smooth Operations	[ 1-32767 ]	
	# of smoothing operations to perform.	
• Boxcar	A fast time-domain filter with excellent 0 $-100\%$ rise-time response.	
Smooth Repetitions	[ 1-32767 ]	
	# of smoothing repetitions to perform.	
Boxcar Window Point	s $[1-99]$	
	# of points in boxcar sliding window.	
	Note: For best performance, only odd values are used.	
Integrator Reset	For amplifier Capacitive mode 'Current' channels.	
• Ignore	Capacitive-mode transients are displayed in the data.	
• Blank	The data during capacitive transients are made invisible by replacing those data points with NANs (Not A Number).	
• Mask	The data during capacitive transients are replaced by the last data value before the transient discharge, simulating a sample-and-hold operation.	
Blank/Mask Duration	[ 50 us – 1 s ]	
	The default value of 500 µs should be sufficient to cover the reset transient	

### duration.

• Stimulus Replicate the command waveform.

Source Channel Se

Select an input channel – the waveform from its 'Parent Out Chan' is used.

• SweepAverage Average the input traces.

Source Channel	Select an input channel to average.		
Average Type	Cumulative	Average all processed sweeps together.	
	Run Average	Average the last "N" sweeps.	
		Number of Sweeps	

• SweepSubtract Subtract a sweep from the input trace.

Source Channel	Select an input channel to process.
Reference Sweep	Select a sweep to be subtracted from all other sweeps. If the sweep does not yet exist, no subtraction occurs.

## Routine Editor: Output Channels & Waveform

Configure the output channels and command waveform.

Output Channels & Waveform: IV
Digital Outputs set as 16-bit Word
Digital Outputs set as 16-bit Word     Edit Waveform     Channel     Label     StimOUT1   StimOUT2   AuxOUT1   AuxOUT3   AuxOUT4   DigOUTWord     V     Relative to Holding   V Return to Holding at Sweep End   Enable P/N Leak Pulses     Copy channel None

Figure 4-40. Output Channels & Waveform

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

Digital Outputs set as

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

• Individual bits Each DigOUT(1 - 16) bit is individually set in its own Waveform Editor table.

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

• 16-bit Word The 16-bit digital output pattern is controlled by a single decimal number (0 - 65,535), which is also the waveform preview amplitude value.

The waveform preview uses the binary bit pattern word

value for its Y-axis value, i.e., if bits '1' and '3' are 'HIGH', the word has a value of 5.

#### Edit Waveform

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

### Channel

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

The default StimOUT channnels are hardwired to the dPatch headstage.

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

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

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

- Tip: If a signal is connected to the front panel 'COMMAND IN' BNC, that signal is summed with the StimOUT waveform that is sent to the headstage.
- Note: For StimOUT channels, the actual DAC output signal is passed through a 20 kHz low-pass filter before entering the headstage.

#### Label

A user-defined signal name for the channel.

These are used in:

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

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

## Copy channel

Copies one channel's waveform to another channel, for output channels of the same type (i.e., "Stim", "Aux", or Digital). If a channel is enabled, then highlighting

another or blank channel of the same type ungrays the 'Copy channel' field, and changes it from 'OFF' to 'None', with a drop-down list of available channels to copy from.

#### Restrict to

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

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

- VC Mode The Amplifier Control Panel must be in VC mode to run this routine.
- CC Mode The Amplfier Control Panel must be in CC mode to run this routine.
- Note: The dPatch amplifier can be switched into any mode (VC or CC) while a recording is in progress, however it is your own responsibility to interpret data from mixed recording modes.

#### **Output Unit**

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

• StimOUT Channels

Fixed at 'V' for voltage clamp and "other" experiment types; fixed at 'A' for current clamp experiments.

• AuxOUT Channels

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

Scaling [for AuxOUT channels]

- Offset Apply an offset to the output channel (after any scaling.)
- Factor Apply a scaling factor as a fixed value or an equation.

(See the <u>Equation Editor</u> for more details.)

#### Relative to Holding

If 'Relative to Holding' is enabled, the headstage output signal is the command waveform summed with the "-holding" level in the Amplifier Control Panel. If the holding level is set to '0', this setting has no effect. For the Auxiliary channels, the command waveform is summed with the Amplifier Control Panel I/O Auxiliary Output settings.

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

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

Return to Holding at Sweep End

During a Series, set the output signal to the Amplifier Control Panel "-holding" level or I/O Auxiliary and Digital Output levels while the system is not acquiring data, i.e., the time between sweeps after a sweep acquisition ends, but before the the next sweep starts.

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

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

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

This setting is updated live by holding level changes in the Amplifier Control Panel if 'Relative to Holding' is enabled, otherwise it is only updated at the time of routine activation. [ The P/N Leak Pulses section is only displayed if this field is enabled. ]

Output Channels & Waveform: IV	X
Digital Outputs set as 16-bit Word	_
Edit Waveform Restrict to VC Mode	•
Channel     Label     Output Unit     V       StimOUT1     ^       StimOUT2	
□ AuxOUT2         □ AuxOUT3         □ AuxOUT4         □ DigOUTWord	
✓ Preview Leak Pulses         Alternating Leak Polarity         Num Leak Pulses	¢
Leak Ratio -0.250	\$
Copy channel None	
Leak Pulse parameters common to all analog output channels	_
Execute Leak Pulses     Before Main Pulse     After Main Pulse       Leak Delay     10.000 ms     \$       Enable AC Line Frequency adjustment     50 Hz     60 Hz	

Figure 4-41. Output Channels & Waveform with Leak

Endogenous leak currents can flow, even while a cell is in its resting state, from conditions such as an imperfect or leaky seal, or via existing ion channels, and affect response amplitudes. Online P/N leak subtraction automates the removal of such currents from the data.

If endogenous leak conductance is an issue with your cell type, and/or high temporal resolution is required along with a need to reduce capacitive transients (e.g., with voltage-gated sodium currents), click 'Enable P/N Leak Pulses' and configure its settings below.

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

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

**Preview Leak Pulses** 

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

Alternating Leak Polarity

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

Num Leak Pulses

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

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

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

#### Leak Ratio

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

- Note: The program scales the leak subtraction pulses based upon the Leak Ratio setting, not the number of Leak Pulses. This means that the Leak Ratio can be set independently from Num Leak Pulses, instead of those settings being interdependent.
- Tip: As an alternate way to avoid electrical activation of ion channels, use a negative ratio to reverse the polarity of the leak pulses relative to the main pulse.

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

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

(See the Equation Editor section for more details.)

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

## Leak Pulse parameters common to all D/A channels

Leak Delay ['s', or 'ms' if < 1 s]

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

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

Enable A/C Line Frequency adjustment

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

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

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

## **Execute Leak Pulses**

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

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

### Routine Editor: Waveform Editor

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

🚦 Waveform	Edito	or: IV Channe	el: St	imOUT1			
						Used	segments: 3 out of 50
		Segment	1	Segment 2	Segment 3	Segment 4	Segment 5
Actions		Stored	•	Stored 💌	Stored 💌	OFF 👻	OFF 👻
Waveform		Step	•	Step 💌	Step 💌	OFF 👻	OFF 👻
waverorm						OFF	OFF
Amplitude		Set to Hold	•	Value+Incr 💌	Set to Hold 💌	OFF 👻	OFF 🔹
Amplitude		0.00 V		-80.0 mV,10.0 r	0.00 V	OFF	OFF
Duration		Value	•	Value 💌	Value 🔻	OFF 🔻	OFF 🔻
Duration		5.00 ms	\$	35.00 ms 🌲	5.00 ms 🌲	OFF	OFF
		`@		,	,		
	$ \cup$						
,	,						

Figure 4-42. Waveform Editor

Close button **U**se this button to close the Waveform Editor window.

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

Actions

OFF

Unused segments are labeled as 'OFF'.

	Tip:	A segment with a Duration of '0' ms is equivalent to 'OFF'. This is a convenient way to skip a segment instead of deleting it.
Stored		Enable a segment for stimulation and recording.
Not In Leak		If P/N LeakPulses are enabled, this will optionally exclude the segment from being generated in the P/N Leak Subtraction output wave.
		This is useful for inactivation or recovery studies, when commands do not change for long periods of time.
Insert		Insert a default Segment into the current position, and increment the position of the following Segments, i.e. move them to the right.
Сору		To copy a segment, click the segment's Actions list and select 'Copy'. A copy is inserted as the next segment.
		To copy multiple segments, select the segments to be copied. Then, for the segment to be inserted before, click its Actions list, select 'Copy', and enter the number of times to copy the segments - the selected segments are inserted before the "Copy" segment.
Delete		To remove a segment, select its 'Delete' Action.
	Note:	If there is only one segment, it cannot be deleted - there is always at least one segment enabled.
		To remove multiple segments, select the desired segments. Then, click any segment's Actions list and select 'Delete'. All selected segments are deleted.
	Tip:	To select multiple segments, use the Windows CTRL- click / macOS \mathcal{H}-click to highlight segments, or SHIFT- click to highlight a range of segments.
		Any following segments shift their Segment #'s down by the number of deleted segments.
Waveform		Select the waveform shape.
Step		The waveform amplitude rapidly jumps from a pre- existing level to the new level within one sample point, and stays at the new level for the duration of the segment. The resulting waveform shape looks like a step.

The first segment consists of a Step waveform set to

the holding level amplitude (Set to Hold).

Ramp		The waveform amplitude goes from the previous amplitude to the new amplitude as a smooth straight line - the sloping line looks like a ramp.				
	Tip:	To create a sawtooth pattern, follow a ramp segment with a zero duration Step segment. This sharply resets the ramp amplitude to the baseline level – the next ramp will start from the baseline level again.				
Sine		The waveform is a sinusoidal wave.				
		Sine Wave Cycles	Multiple	One or more cycles.		
			Single	One cycle, where the Cycle Duration is equal to the Segment Duration.		
			For Lock-in	For capacitance measurements in a virtual input channel.		
				For multiple Sine Lock-In segments in a Routine waveform, use the same Cycle Amplitudes and Cycle Durations for each Segment.		
		Cycle Amplitude	First peak an baseline. [±750.00 mV	mplitude from 7, ±20 nA ]		
		Tip:	To offset a sine wave from the default baseline (0 units), set t segment Amplitude, or enable Routine Editor / Output Chann 'Relative to Holding'.			
		Cycle Duration	One cycle ler	ngth (ms).		
			[ For Lock-in	only]		
			A preset list of cycle durations /			

(frequencies):

Segment Duration Sine wave duration (ms).

2.0 ms	(500 Hz)
1.0 ms	(1 kHz)
$0.5 \mathrm{ms}$	(2 kHz)
0.2 ms	(5 kHz)
0.1 ms	(10 kHz)
$0.048 \mathrm{~ms}$	(20.83 kHz)

Squarewave	The waveform gene	rates a train	of rectangular pulses.
	Base Amplitude Increment 1		rement the baseline plitude for each cesive pulse.
		[±0	.75 V, ±20.000 nA ]
	Step1 Amplitude	Amplitude	of first pulse.
		Value [ ±0.	75 V, ±20.000 nA ]
		Var_r[1 - 1	16]
	Step1 Width	Duration o	f first pulse (ms).
		Value [ ]	
		Var_r[1-1	16]
	Step2 Amplitude	Amplitude	of second pulse.
		Value [ ±0.	75 V, ±20.000 nA ]
		Var_r[1-1	16]
	Step2 Width	Duration o	f second pulse (ms).
		Value [ ]	
		$Var_r[1-1]$	16]
	Segment Duration	Square wa	ve duration (ms).
Chirp	This waveform gene changes its frequen		
	Chirp Type	Linear	A linear change in frequency.
		Geometric	A geometric change in frequency.

Note: For a geometric chirp, a minimal frequency spread is enforced: the End Frequency has to be at least double the Start Frequency, or half or less of the Start Frequency.

Amplitude	[ ±750.00 mv, ±20 nA ]
Start Frequency	[1 - 50000  Hz]
End Frequency	[ 1 - 50000  Hz ]
Segment Duration	Chirp wave duration (ms).

## Template

Select template waves for segment 1. Output Sampling rate: 4e-06.         Template Pool         minis         R1_51_10minSpontAct         Template1_Sweep5         Image: Select template1_Sweep5         Image: Select template1_Sweep5         Image: Select template1_Sweep5	Template waves		
minis         R1_S1_10minSpontAct         Template1_Sweep5         Image: Section 100 ms         Image: Section 100 ms	Select template waves for seg	ment 1. Output Sampling rate: 4e-06.	
-40 - 41 - 41 - 4 - 4 0 10 20 30 40 50 60 Time (s) Cancel Apply	minis A R1_S1_10minSpontAct		Set to Template Duration Segment Duration 5.00 ms

Figure 4-43. Template Waves

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

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

Template Pool	Templates loaded in the Template Editor Pool plus extracted templates.
	Copy the selected template wave from the Tem- plate Pool into a Routine and Segment.
	Copy the selected template wave from the Tem- plate Pool into the Routine.
•	Copy the selected template wave from the Routine into the Template Pool.
In Routine	Templates stored in the Routine.
	Each output channel can have a maximum of 16 template waves stored in its routine. The same template can be used in multiple segments. Each segment can have one or multiple templates as- signed to it.

	Probably the most used case will simply be a sin- gle template paired with a single segment, but the possibilities are endless.
Note:	To avoid unnecessary increase in the sizes of rou- tines and Routine Pool files, only include tem- plates that are actually used by segments.
Duration (ms)	The duration of the template trace.
	Copy the selected template from a Routine to a Segment.
	Delete the template wave from a Segment or an unused Routine
In Segment	Each segment can have one or multiple templates assigned to it.
	If only one template is listed, then for any number of sweeps, the segment output wave will be the same for all sweeps.
	If more than one template is assigned to a seg- ment, and the number of sweeps is greater than 1, then an output wave is created as:
	1st sweep with the 1st template on the list, 2nd sweep with the 2nd template, etc.
	If there are more sweeps than templates, then the templates are reused (round robin) for each subsequent sweep.
Sweeps	Number of sweeps in the Routine.
Set to Template Dura	ation
	Set the segment duration to match the template (sweep) duration.
Segment Duration	The Segment Duration can be manually adjusted here.
	When typing in a value, if no unit type is entered, the unit type defaults to seconds (s). If you enter a number followed by an 'm' or 'ms', the unit type is milliseconds (ms).

Triangle	The waveform gene	The waveform generates a train of triangular pulses.	
	Base Amplitude Inc	Base Amplitude Increment	
		Increment the baseline amplitude for each succesive pulse.	
	Peak Amplitude	Amplitude of the triangle pulse.	
	Ramp1 Width	Duration of the initial phase.	
	Ramp2 Width	Duration of the secondary phase.	
	Segment Duration	Duration of the triangle train.	
Amplitude ( <i>analog</i> ) Se	t the waveform amplitud	le for a Segment.	
		he Chirp, Sine, Squarewave and Triangle waveforms, s used as a baseline offset.	
Set to Hold	-	Use the Amplifier Control Panel holding level for the Segment amplitude. Tip: Record an initial baseline Segment to help intepret your data. Use in Segment 1.	
		For voltage-clamp experiments, records the leak current along with the actual holding voltage.	
	-	For current-clamp experiments, records the actual cell potential along with the actual holding current.	
	0	Avoid using the last Segment for this, as post- stimulation data can be recorded, such as from tail currents.	
Value	Use a single numbe	Use a single number for the segment amplitude.	
	[±750.00 mV, ±20 m	[±750.00 mV, ±20 nA ]	
Value List	Set an arbitrary seg	Set an arbitrary segment amplitude for each sweep.	
	[Sweep   Value]	For each sweep, enter a number. [ ±750.00 mV, ±20 nA ]	
	Fill Remaining List	Copy the active value to all remaining sweeps in the list.	

	Segment Duration	Adjust the duration of the Segment.
	Number of Sweeps	Adjust the number of sweeps in the Routine.
Value+Increment	Increment the segm	ent amplitude for each sweep.
	Start Value	<ul> <li>Holding</li> <li>Value</li> <li>[+750.00 mW +20 nA ]</li> </ul>
		[±750.00 mV, ±20 nA ]
	Increment Value	[±750.00 mV, ±20 nA]
	Segment Duration	Adjust the duration of the Segment.
	Number of Sweeps	Adjust the number of sweeps in the Routine.
Equation	Specify the segment amplitude as an equation.	
	[ Equation field ]	A free-form text field.
		[±750.00 mV, ±20 nA]
		Errors are reported under this field.
	Check Equations	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is "ok".
	Insert special identifier	
	-	Acquisition, amplifier and reference settings are available for use in equations. (See list in Equation Editor.)
	Undo	All changes in the equation editing session are discarded.
	(See the Equation Editor for more details.) Note: Computing an equation for an output wave consumes sig- nificant computing power, as every data point needs to be computed by the CPU. While a slight update delay in such operations is expected, for computers with marginal	
Note:		

		e "beach ball" icon displays while the sive and busy processing.
Var_r[ 1-16 ]	Variable labels are d table is enabled.	lisplayed if the Routine Variables
Amplitude (digital) Digita	l settings are display	ed if digital outputs are enabled.
Bit	Set a digital level for	r an individual bit.
	<ul> <li>LOW = 0</li> <li>HIGH = 1</li> </ul>	
Bit Word	Values are the decin 65,535), also display	nal number of a 16-bit word (0 – ed as a bit pattern.
Duration Set th	e segment duration.	
Set to Hold	Use the Amplifier Co holding levels.	ontrol Panel to set the output
Value	Use a single number [ 0 – 12 ks ]	for the segment duration.
	unit type defaults to	lue, if no unit type is entered, the seconds (s). If you enter a an 'm' or 'ms', the unit type is
Value List	Set an arbitrary seg a list of numbers.	ment duration for each sweep from
	[Sweep   Value]	For each sweep, enter a number. [ 0 – 12 ks ]
		(Blank lines are removed.)
	Fill Remaining List	Copy the active value to all remaining sweeps in the list.
	Number of Sweeps	Adjust the number of sweeps in the Routine.
Value+Increment	Increment the segme	ent duration for each sweep.
	Start value	[ ms ]
	Increment value	[ ms ]

Number of Sweeps Adjust the number of sweeps in the Routine.

Equation

Specify segment duration as an equation.

[Equation field]	A free-form text field.
[ Errors are reporte	ed under this field. ]
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is "ok".
Insert special ident	tifier
	Acquisition, amplifier and reference
	settings are available for use in equations.
	(See list in Equation Editor.)
Undo	All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

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

#### Segment Controls

To copy or delete a Segment, click on its background area, and its color turns gold. This enables the additional Actions items: Copy and Delete.

Standard mouse behaviour is used to select multiple Segments:

•	Individual Segments:	Windows:	CTRL-click
		macOS:	command (光)-click
•	Range of Segments	Both:	SHIFT-click

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

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

## Routine Editor: Real Time Measurements & Graphs

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

Figure 4-44. Real Time Meaqsurement Settings

Figure 4-45. Real Time Graph Settings

## Disable Execution of Measurements and Graphs

Block execution of all measurements and analyses with one click.

## Measurement Settings

On	Enable an analysis to run.	
ID	Measurement regions are identified with an ID number: m[#]	
	[1-16]	
Label	These measurement labels display in the Waveform Preview and Scope windows, and can be used in equations. A Label name is automatically generated from the Analysis Function; double-click to manually edit.	
Сору	Copy to the selected Measurement another Measurement's settings.	
Clear All Measurements		
	All measurements and their settings are cleared.	
Analysis		
Analysis Fur	nction	
	Choose from 16 predefined Analysis statistics for each measurement:	
	Absolute area Absolute area (negative values are converted to positive.)	

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

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

- [Status field] A short description of the selected Analysis.
- Threshold This amplitude level needs to be crossed by the data to trigger measurements for:

Frequency, Time to threshold

Polarity The direction of a Threshold crossing.

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

These settings apply to measurements:

Rise/Decay tau & time, Frequency, Time to threshold

#### **Enable Smoothing**

[2-200]

Set the number of Gaussian smoothing operations per measurement.

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

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

#### Signal to Analyze

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

#### **Region Timing**

Cursors Relative to	Set the measurement boundaries with left/right cursors.		
Note:	te: Cursor Start times cannot be greater than their End times.		
•	Full Trace	Encompass the entire trace.	
	Start Time	Set the left cursor relative to the Trace start time (s).	
	End Time	Set the right cursor relative to the Trace start time (s).	
•	Sweep Time	Set relative to the start time of a sweep (time zero).	
	Increment by:	[ ]	
		When 'Lock Time step width' is enabled, the Start/End Time spinners increment by this amount.	
		The list of available values depends upon the in- put filter bandwidth.	

Start Time	Set the left cursor relative to the Sweep start time (s).	
End Time	Set the right cursor relative to the Sweep start time (s).	
Lock Time step wid	Ith Fix the width of the measurement region.	
	The measurement width is maintained at a con- stant value when the cursor 'Start/End Time' is updated.	
	[ ] The width of the cursors in seconds.	
	The minimum width size is 2 sample points.	
Segment Time	Set relative to the start time of a Segment #. Uses the segment timing from the input signal's "Par- ent Output Channel".	
Out Channel	[Output Channel list] [Segment#]	
Reset Ratios	[ 0.0000 / 1.0000 ]	
	Reset the Start/End Ratios to span the entire segment.	
Increment by	[].	
	When 'Lock step width' is enabled, the Start/End 'Time' spinners increment by this amount.	
	The list of available values depends upon the in- put filter bandwidth.	
Start Ratio	[ 0 = beginning of Segment ]	
	Set the left cursor as a ratio of the Segment dura- tion.	
Tim	e [0.0000 s] Cursor start-time field.	
End Ratio	[1 = end of Segment]	
	Set the right cursor as a ratio of the Segment du- ration.	
Tim	e [0.0000 s] Cursor end-time read-only field.	
Note	e: If the Start/End Ratios extend past the boundary of a Segment, and the measurement is switched to	

•

•

		a beginning or ending Seg tios are reset to '0' and '1'		
	Lock	me step width Fix the relative width of gion.	the measurement re-	
		The measurement width i stant ratio value when St ed.		
		E 3	ne cursors in seconds. width size is 2 sample	
<u>Graphs</u>				
On	Enable a grap	to configure its settings.		
ID	Graphs have	default ID (identification): g[1] - g[8]		
Show	Display this g	aph in an Analysis window during acquis	sition and analysis.	
	Note: If the Y	Axis 'Equation' field is blank, the graph v	vill also be blank.	
Label	Double-click	Double-click to manually enter a graph label for the Analysis.		
Сору	Use to transfer graph settings to a new graph. Highlight a Graph ID, then select from the drop-down list of enabled graphs.			
<u>Y-Axis</u>	Select a Measurement ID for the graph Y-Axis, or use an equation for a custom- ized Y-axis plot.			
	Source	Select a Measurement, or enter an equat	ion	
		(see the Equation Editor for more details	.)	
		For example, to monitor cell comp cial ID 'RsComp'. Then track cell sweep by running a planned Para step inside a 'For Each' step.	changes sweep-by-	
	Label	A Y-axis label is automatically generated label; directly edit to customize the label		
	Unit	Select a standard unit from the drop-dow unit type.	m list, or enter a custom	
	Note:	Standard unit resolutions, such as 'pA' or calculated and displayed in the graph.	r 'mV', are automatically	
<u>X-Axis</u>		uph X-axis with a standard time-base, or		

Source		Select a Measurement, or enter an equation.	
		(See the Equation Editor for details.)	
Label		Enter a customized name for the X-Axis.	
Unit		Select a standard unit from the drop-down list, or enter a custom unit type.	
	Note:	Standard unit resolutions, such as 'pA' or 'mV', are automatically calculated and displayed in the graph.	

## Routine Editor: Routine Variables

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

Routine Vari	ables		X
In Use	Label	Value	
	Var r[1]	0.00	∧
	Var r[2]	0.00	
	Var r[3]	0.00	
	Var r[4]	0.00	
	Var r[5]	0.00	
	Var r[6]	0.00	
	Var r[7]	0.00	
	Var r[8]	0.00	
	Var r[9]	0.00	
	Var r[10]	0.00	
	Var r[11]	0.00	
	Var r[12]	0.00	
	Var r[13]	0.00	
	Var r[14]	0.00	
	Var r[15]	0.00	
	Var r[16]	0.00	
			× .

Figure 4-46. Routine Variables

**Routine Variables** 

In Use	A checkmark means this Routine Variable is in use by the Waveform Editor.		
Label	Var_r[1 - 16]	Edit the default variable name if desired.	

Value	Numeric values can be manually entered or set by a Paradigm.
Note:	If Routine Variables are enabled and then disabled, the Waveform Editor converts its 'Var_r' settings to 'Value' settings using the last enabled values.

# 4.1.6 Real Time Measurements & Graphs (Scope)

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

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

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

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

🗓 Real Time Measurements & Graphs				
Parameters read from Routine copy: R3_ Measurement Settings	v.			
On       ID       Label <ul> <li>m[1]</li> <li>Mean</li> <li>m[2]</li> <li>Max</li> <li>m[3]</li> <li>m[4]</li> <li>m[5]</li> <li>m[6]</li> <li>m[7]</li> <li>m[8]</li> <li>m[9]</li> <li>m[10]</li> <li>m[11]</li> <li>m[12]</li> <li>m[13]</li> <li>m[14]</li> <li>m[15]</li> <li>m[16]</li> </ul> Copy         None <ul> <li>Clear All Measurements</li> </ul>	Analysis Analysis Function Mean Arithmetic mean of all points Enable Smoothing Signal To Analyze Current1 Region Timing Cursors Relative to Sweep Time Increment by 100 µs Start Time 2.5000 ms End Time 8.4000 ms Lock Time step width 5.9000 ms			
Graph Settings         On ID       Show       Label         ✓       g[1]       ✓         ✓       g[2]       ✓         ✓       g[2]       ✓         ✓       g[3]          ✓       g[4]          ✓       g[5]          ✓       g[7]          ✓       g[8]          ✓       g[9]	Y-Axis Source m[1]: Mean Label Mean_Current1 Unit A X-Axis Source m[2]: Max Label Max_Voltage1			

Figure 4-47. Real Time Measurements & Graphs (Scope)

## 4.1.7 Scope (Acquisition)

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

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

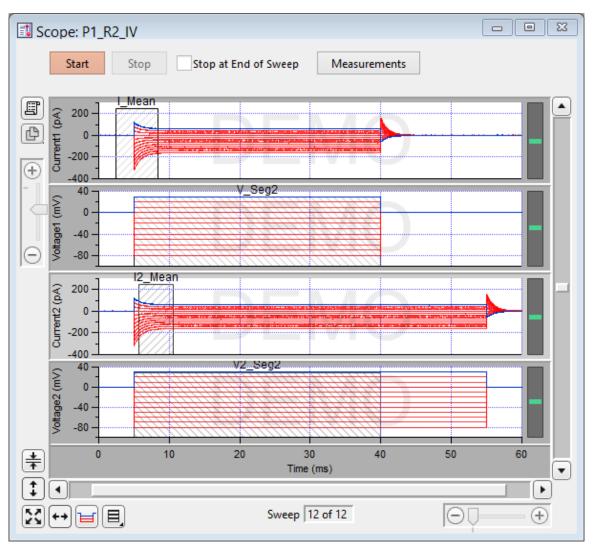


Figure 4-48. Scope (Acquisition) Window

The Scope window is titled with the active Routine name.

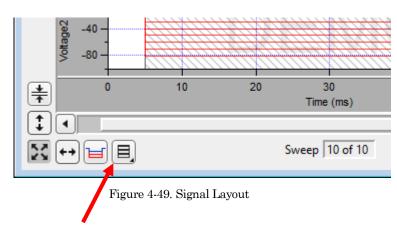
## Controls

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

> If multiple signals are displayed stacked on top of each other, their panes can be vertically resized by clicking and dragging the horizontal area between panes,

using the resizing mouse cursor (a horizontal line with a vertical double-headed arrow.) The standard mouse cursor will change to the resizing cursor when positioned over a pane separator.

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



Signal Display Mode

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

- Stack Vertical column of signals.
- Single Only the active signal.
- [m x n] Tiled configuration of signals with 'm' rows and 'n' columns.
- Cursors Vertical measurement regions can be set up for input signals. Measurement regions display as light gray vertical bars in the signal panes. Each measurement region has a measurement width, bounded by a start-time cursor and an endtime cursor - cursors are the left and right edges of these regions.

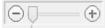
To graphically move a measurement region, click and drag them with the mouse (when selected, the region turns dark.)

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

#### Magnification and Scrolling

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

Magnification Combo



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

Axis Zoom

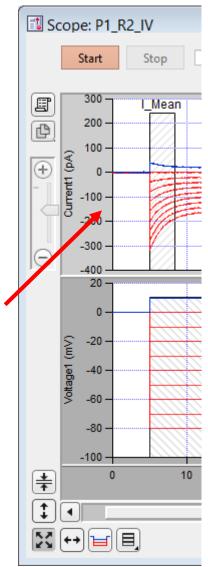


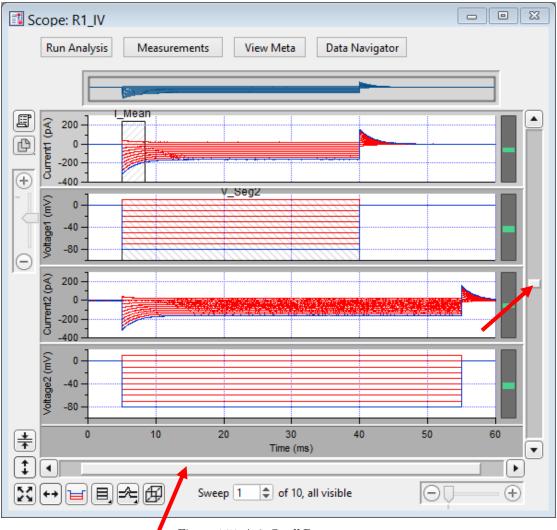
Figure 4-50. Axis Magnification

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

• Area Zoom:

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

- 1. Move the mouse cursor into a signal pane, and it changes into a large "+".
- 2. Click and drag a bounding box around a region of interest. The box is also referred to as a "marquee".
- 3. Right-click in the marquee and select 'Expand' or 'Shrink'.
  - Note: For signal panes without an X-axis bar (i.e., all except the bottom-most signal), the zoomed data are displayed independently of any X-axis, and the time synchronization between signals gets lost. Click the 'Auto-scale All' button to restore synchrony.



#### Axis Scroll Bars

Figure 4-51. Axis Scroll Bars

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

• Center:

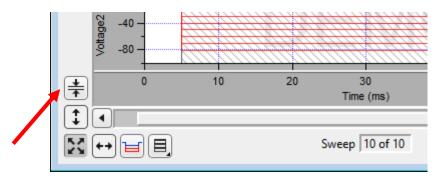


Figure 4-52. Center Button

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

-40 Voltage -80 20 0 10 30 * Time (ms) ‡ Sweep 10 of 10 23 Figure 4-53. Autoscale Axes X Click to instantly autoscale the Y-axes of all visible signals to their incoming data, i.e., to their visible sweeps data limits, and to reset all X-axes to the full sweep duration. To continuously autoscale the Y-axes, right-click the button in Windows, or Control-click in macOS - the Autoscale button remains depressed in this state. ‡` Click to autoscale the Y-axis of the selected signal. <del>++</del> Click to full-scale all X-axes.

Autoscale Axes

Amplitude Meters

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

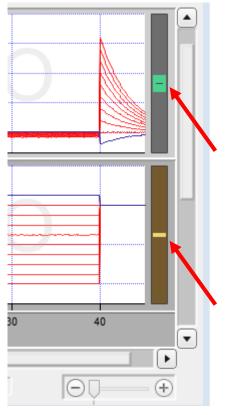


Figure 4-54. Amplitude Meters

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

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

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

•	Green:	Good	Signal within appropriate range.
			When the recorded data are within acceptable amplitude limits, the amplitude meter is green.
•	Yellow:	Caution	Signal approaching upper limit.

(within 10% of range limit) When there is a danger that saturation will occur, as the data are near the upper limit, the amplitude meter is yellow, as a warning sign to decrease your hardware gain. Danger Signal too large. Red: (within 1% of, or at range limit) When an amplitude meter is displayed in red, it indicates that the data might have gone out of range and be invalid. If too much hardware gain is applied, the recorded signal will be in danger of saturating, i.e. your data will exceed the amplitude limit of the digitizer. In this case, those data points are substituted with the maximum amplitude of their input channel.

# **Other Buttons**

Start	Start recording and displaying digitized analog data in the Scope win- dow input signals.
	When you click the 'Start' button, the Scope is cleared, and data recording starts after ${\sim}300~{\rm ms.}$
	When acquisition is running, the Scope window updates every 200 ms.
	If the Sweep Start-to-Start time is $\geq 5$ s, the "Time to next sweep: # s" is reported below the Start / Stop buttons.
	If Metadata prompts are configured for Routines or Paradigms, the Confirm Metadata Settings dialog displays just before recording be- gins.
	If measurement graphs are enabled, a docked "child" Analysis window plots sweep-by-sweep measurements.
	If no prior paradigm is running, an "Auto-triggered Paradigm" is generated and assigned a Paradigm name with the current Date/Time.

	Stop recording data.	
Stop	When you click the 'Stop' button, if 'Stop at End of Sweep' is enabled, the message 'Waiting to stop' displays below the Stop / Start buttons, until the last sweep completes and acquisition stops.	
Stop at End of Sweep	Determines how data acquisition is terminated. When the 'Stop Acqui- sition' 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 'Stop at End of Sweep' checkbox is enabled, then the current sweep will complete before data acquisition is stopped, and the last recorded sweep will be a complete sweep of data. If no sweep is currently in progress, acquisition will stop at the end of the next sweep to be recorded.	
	Show Cursors: Display measurement cursors in the Scope window.	
Measurements	Hide Cursors: Do not display cursors in the Scope window.	
	Button state is displayed as "Measurements(H)".	
	Lock Cursors: Prevent cursors from being adjusted or moved.	
	Button state is displayed as "Measurements(L)".	
	Edit Measurements:	
	Open a special Real Time Measurements & Graphs dialog where all changes apply instantly to the measurements and graphs, even during acquisition. These edits (temporarily) override the loaded routine for quick interactive analysis.	
	Edit Virtual Signals:	
	Open the virtual input signals panel for editing.	
Edit Routine	Open the Routine Editor dialog, for configuration, management and activation of Routines. Routine configuration includes:	
	Data acquisition parameters	
	Input channels	
	Output channels	
	Stimulus waveforms	
	Analysis measurements	
Layout	Copy and paste all visible Scope signals and analyses into a Layout window. If no Layout window is open, a new one is created.	
Copy to	Copy the Scope window to the system clipboard.	
	1	

Clipboard	
Persistence Display	When enabled, sweeps are not cleared from the display until a new acquisition is started. When disabled, all sweeps are cleared and only the next acquired sweep is displayed.
Sweep #:	The active sweep number vs. the total 'Number of Sweeps'; if multiple cycles are set, the active sweep cycle number is inserted between these.

Table 4-3. Other Scope Buttons

#### **Right-click Menus**

#### Scope (Acquisition) main window

- Note: If you don't click far enough away from the data, the data menu will display instead.
  - Autoscale All Axes Add Annotation Export Graphics Colors Hide Signal 'Signal Name' Show Signal 'Signal Name' Only Stack All Signals

## Y-Axis

Autoscale All Axes	Scale all signals X- and Y-axes.
Continuous Autoscale Axis	Continuously scale a signal's Y-axis.
Autoscale Axis	Scale a signal's Y-axis.
Full-scale Y Axis	Set a signal's Y-axis to the full-scale range.
Axis Properties	Modify the axis style and components.
Hide Signal <name></name>	Remove the signal from the Scope.
Show Signal <name> only</name>	Remove all other signals from the Scope.
Stack All Signals	Display all signals in a single column.

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

#### Data area

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

#### Marquee

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

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

## **Channel Timing Delays**

The dPatch amplifier uses analog output and input channels that pass signals through two filters, fixed DAC output stimulus filters and variable-bandwidth ADC input filters. These filters impose a time delay (also called a phase delay) on the physical input and output channels, which is reflected in the recorded response signals.

However, when stimulus signals are recorded (such as 'Voltage1' in voltage-clamp mode), their output and input channels are directly processed as digital streams. Thus, there are no analog filter delays in recorded stimulus signals.

This means that recorded response signals are time-delayed compared to recorded stimulus signals.

Therefore, when analyzing data that requires precise timing between stimulus and response signals, these timing delays should be taken into account:

Typical dPatch Filter Delays

<u>Input Bandwidth</u>	<u>Total Filter Delay</u>
1000 kHz	$16.2 \ \mu s$
$500 \mathrm{kHz}$	$17 \ \mu s$
$250~\mathrm{kHz}$	$18.5~\mu s$
$10\mathrm{kHz}$	$85 \ \mu s$

# 4.1.8 Solution Editor

Control perfusion systems with "named" solution control settings.

Solu 🖥	tion Editor		
Sav	e Configur	e	
Solution Pool Files			
C:User	s:EmeraldCity:Doc	umen:SP_Solutio	nPool.spo
<b>√</b> off	Name	Concentration	Unit
1	undefined		~
2	undefined		
3	undefined		
4	undefined		
5	undefined		
6	undefined		
7	undefined		
	undefined		

Figure 4-55. Solution Editor

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

Save Save the solution list as a "*.spo' file.

Configure Opens the Configure Solutions dialog to set solution types, channels, and outputs.

Solution Pool Files Drop-down list operations affect the entire "Solution Pool".

New Solution Pool	Create a blank Solution Pool.
Load Solution Pool	Load the Solutions of a previously saved Solution Pool file into the Solution Pool.
Revert to Last Saved	Undo any unsaved changes to the Solution Pool.

Save Solution Pool	Save the Solution Pool using its existing file name and path.
Save Solution Pool As	Save the Solution Pool to a new file, and switch to the new file.
Save Solution Pool Copy	Save the Solution Pool to a new file, but do not switch to the new file.

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

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

[ off, 1 – # ]	Manually select a valve radio button to activate its corresponding solu- tion configuration.
	Only one valve can be active at a time.
	The number of valve buttons is set by the number of "Configured" solution outputs.
	A valve button becomes available for selection (ungrayed) when its 'Name' is changed from 'undefined'.
Name	Double-click on a field to edit it, or click-and-drag to move it up or down in the table.
Concentration	Enter a concentration value for the solution.
	Access the concentration value from the last-used Solution valve with the Special ID 'Solutions' (for 'Test Compound' solution types only.)
Unit	The unit type of the concentration.

# **Configure Solutions**

Close Dialog button

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

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

The loading of other Solution Pool files allows a virtually unlimited number of solutions to be accessed in an Experiment.

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

[Solution Type list] A list of predefined solution types:

- Initial Condition
- Washout
- Control
- Test Compound
- Not a Solution

[Index #] Additional organizational levels.

• Index 1 - 5

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

- No Output
- AuxOUT1 4 [±10.000 V] Analog output voltage.
- DigOUTWord [0-255] Decimal value of an 8-bit digital word.
- DigOUT1 16 [Low / High ] A single digital bit is set "high".

# 4.1.9 Template Editor

Manipulate and manage templates for use in command waveforms.

Template Editor			
Template Pool Files 🔹 💌	ol Files  C:Users:EmeraldCity:SP_TemplatePool.spt		
Import Export	Rename Duplicate Delete Revert 🗐 🕒		
Save Pool Browse Define Template Properties			
Convert Off   Smooth 3  Off  Factor 1.00 Offset 0.00 Do It			
Template Names	Template Parameters		
minis	length: 60.0 s, points: 600000, delta-X: 100 µs, X-start: 0.00 s, Y-unit: A		
R1_S1_10minSpontAct	length: 570 ms, points: 14250, delta-X: 40.0 µs, X-start: 0.00 s, Y-unit: V		
Template1_Sweep5 length: 55.7 ms, points: 557, delta-X: 100 µs, X-start: 0.00 s, Y-unit: V			
▼ Show Preview			

Figure 4-56. Template Editor

Template Pool Files

New Template Pool

Create a blank Template Pool.

Load Template Pool Load the Templates of a previously saved Template Pool.

Revert to Last Saved	Undo any unsaved changes to the Template Pool.
Save Template Pool	Save the Template Pool using its existing file name and path.
Save Template Pool As	Save the Template Pool to a new file, and switch to the new file. The default file name is the same as the original file name.
Save Template Pool Copy	Save the Template Pool to a new file, but do not switch to the new file. The default file name increments.
Merge Template Pools	Insert the Template from a previously saved Template Pool file into the loaded Template Pool.

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

Import	Select a template file (*.ibw).		
	Alternatively, in a Scope window or preview pane, click and drag the mouse to surround a region of interest with a bounding box (the "marquee"). Right-click in the box and select 'Extract Template'. A template with the signal name is added to the template list.		
	An extracted template is compose	d of a single sweep:	
	• Scope window (Acquisition)	: Last sweep.	
	• Scope window (Analysis):	Selected sweep.	
	Preview pane:	Last or selected sweep.	
	Note: 'Extract Template' is not in or the Data Navigator pre- only valid with monotonica decreasing X-axes.	view pane. Also, it is	
Export	Export the selected template to a 1-D Igor wave file (*.ibw).		
	To export a portion of a sweep, first extract (import) a portion of a sweep.		
Rename	Edit the name of the selected template. Allowable characters are A-Z, a-z, 0-9, and "_". Special characters are not allowed; spaces are replaced by an underscore.		
Duplicate	Add a copy of the selected template to the list. The new template name's number is appended or incremented.		

Delete	Remove the selected template from the list.		
Revert	Discard any unsaved changes to the selected template.		
To Layout	Copy/append the template preview to the Layout window.		
To Clipboard	Copy the template to	o the system clipboard.	
Save Pool	Save the template po	ool using its existing file name.	
Browse	Create a template fr Browser.	om the Experiment data in the Data	
Define Template Properties	Update a data wave's X- and Y-axis parameters to be compatible with SutterPatch templates.		
Enter X-increment	The data point time interval is changed, which also adjusts the length of the trace.		
Enter X-start	The X-axis st	carting time for the data.	
Enter Y-unit	The Y-axis base unit (enclose between double quotes.)		
Convert	The data is interpolated to match the new sampling rate. While the number of samples is updated, the length of the trace is unchanged.		
Smooth	Apply smoothing to the template.		
	• Off		
	• Boxcar	A fast time-domain filter with excellent 0 – 100% rise-time response.	
	• Gaussian	A standard filter with excellent $10-90\%$ rise-time response.	
Factor	Adjust the template scaling factor.		
	Values are displayed with SI unit prefixes.		
Offset	Adjust the template offset.		
	Values are displayed with SI unit prefixes.		
Do It	Apply the adjustments to the template parameters.		

Template Names	A list of the loaded templates.	
	Click on a Template entry to make it the active one.	
	Double-click on a Template Name to rename it.	
	Click-and-drag a Template entry to reposition it in the list.	
Template Parameters	Parameter settings description.	
Show/Hide Preview	Display/Hide a preview pane with the selected template.	
	The preview pane X- and Y-axes can be controlled in two ways:	
	• Hover the mouse over an axis line until the cursor turns into a double-headed arrow, then scroll up or down to contract/expand the axis.	
	• In the preview, click and drag the mouse to surround the region of interest with a bounding box (the "marquee"). Right-click in the box and select one of the expand/shrink options.	

## 4.2 Data Analysis

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

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

# 4.2.1 Action Potential Analysis

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

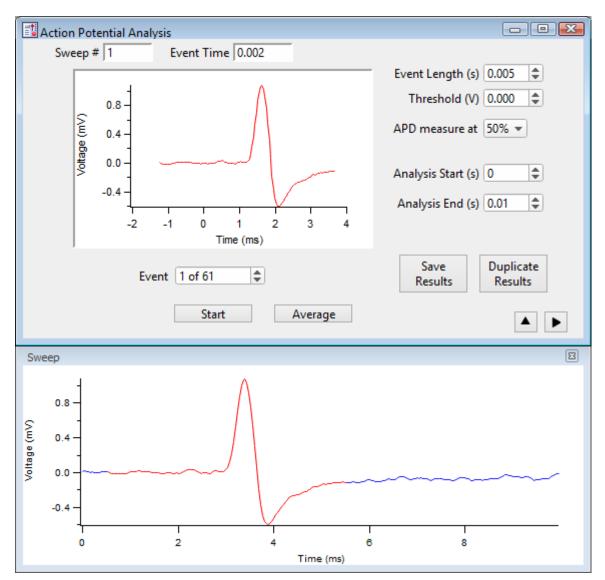


Figure 4-57. Action Potential Analysis

Sweep#	The sweep number of the selected event.	
	When set to '0', this indicates that averaged Results measurements are being displayed.	
Event Time	Timepoint when the potential of the selected event crossed the threshold.	
[Event pane]	A graph of the selected event.	
Event [#of#]	Selected event number vs. total events.	
Start	Run the Action Potential Analysis.	

DPATCH-OPERATION MANUAL - REV. 2.0.4 (2020-2-5)

		100	
Average	Display the averaged event in the Event pane, and its measurements in the Results panel, and set the 'Sweep #' to '0'.		
Event Length (s)	Event duration in the Event pane (also highlighted in red in the Sweep sub-window.)		
Threshold (V)	[±0.100]		
	This voltage level needs to be reach event to be triggered.	hed or exceeded for analysis of an	
APD measure at:	Set the Action Potential Duration p	percentile	
	This measures the duration of an e repolarization duration.	vent based on a percentile of the event	
	APD at % repolarization • 50% • 80% • 90%		
	• 100% (same as the	AP Duration)	
Analysis Start (s)	Start time for data analysis in a sw	7000	
Analysis End (s)		-	
Save Results	End time for data analysis in a sweep.		
Dave Results	Results are displayed in a Layout window and a table - prior Results from the loaded data signal are overwritten.		
Duplicate Results	Results are copied to a new Layout window and table, so prior Results are not overwritten.		
Results	A table of all events results.		
	[ ]	Row number, one row per event.	
	Sweep Number	Sweep number the event is in.	
	Event Time (s)	Time point of the event start.	
	Threshold (V)	Amplitude of the event threshold.	
	Threshold Time (s)	Time point of the "trigger" thresh- old time.	
	Peak (V)	Amplitude of the event peak.	
	Peak Time (s)	Time point of the event peak.	
	AP Duration (s)	Duration of the action potential.	
	AHP (V)	Peak amplitude of the After Hyper- Polarization phase.	
	AHP Time (s)	Time point of After Hyper- Polarization; the event re-crosses the threshold amplitude at this time.	

193

Action Potential Analysis Results	A Layout window rep	ort is created.
Tota	l time analyzed	Includes the Start/End times for all sweeps (s).
Num	nber of events detected	Total number of events found.
Ever	nt Frequency	(Hz)
[ Eve	ent graph ]	
[ Pha	ase plot ]	dV/dt (V/s) vs. mV

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

Displays a graph of the sweep containing the selected event.

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

Displays the sub-window Action Potential Measurements.

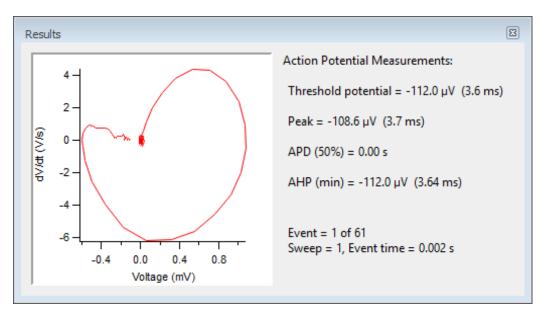


Figure 4-58. Action Potential Measurements

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

[Y-axis vs. X-axis]

Threshold potential =  $[mV (\mu s)]$ 

Event starting amplitude (time from Threshold setting).

•

	The actual biological start of an event, the reported 'Threshold potential', is calculated to be when the signal slope is > 1 mV/100 $\mu$ s (10 V/s), or when 25% of the maximum slope is reached, whichever is smaller. As this timepoint is reported relative to the 'Threshold' detection timepoint, it is a negative number.
Note:	The exact 'Threshold potential' timepoint is based upon differen- tials using a central differences algorithm:
Peak =	[ mV (ms)
	The largest amplitude excursion of the event (time from 'Threshold' setting).
	Two measures of an event's peak amplitude are reported, voltage and time. Time is reported relative to the 'Threshold' timepoint.
APD (%) =	[ %, ms ]
	Action Potential Duration of the event at (%) of amplitude repolarization.
AHP (min) =	[ mV (ms) ]
	The largest amplitude excursion of the "After HyperPolarization" phase of the event (time from 'Threshold' setting).
	Two measures of the AHP amplitude are reported, voltage and time. AHP is when the action potential repolarization phase drops to its lowest point below the resting membrane potential, i.e. during the hyperpolarized refractory period of the cell.
Event =	The analyzed event of the total number of events found in the analyzed data.
Sweep =	The sweep number of the analyzed event.
Event time =	The sweep time of the start of the event.
Event frequency =	The average event frequency.

# 4.2.2 Analysis Editor

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

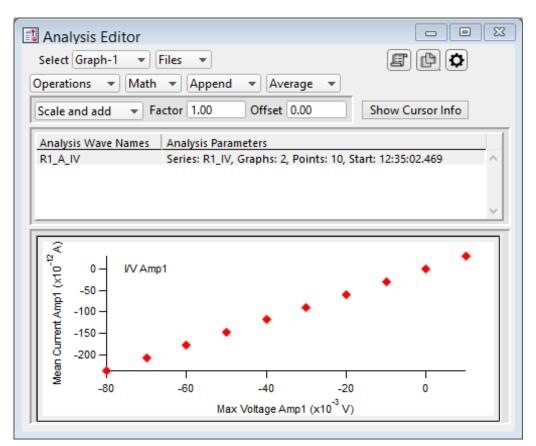


Figure 4-59. Analysis Editor

Select Choose how to view the data.

Table

View a spreadsheet of the Analysis data.

- Column 1: Row number.
  Column 2: X-data for the first graph.
  Column 3: Y-data for the first graph.
  Column m n: Pairs of X- and Y-data columns repeat for each additional graph.
  The first graph (X-Y-pair) that uses X-axis time units populates its X-data column with time values; subsequent graphs that use X-axis time units have blank X-data columns, as they use the first time-
- Note: The first data point is in row 0, so the last data point is in row [N-1].

column created.

		Row N is a blank row that contains grayed-out cells. It is used to manually add extra rows of data to the table. Once a number is entered into one of these cells, the blank (gray) row is automatically moved to the row beneath it.	
	Warning!	Editing the table will permanently alter the data.	
	Graph-[1 – 8 ]	Select an Analysis graph for the selected data wave. The graph number refers to its original Analysis window position.	
Files	Save or open an analysis grap	ph file.	
	Export Graphs	Save the entire graph as a multidimensional Igor Binary Wave file (*.ibw).	
	Export Graph X-column	Save the X-column data, including labels, as a 1-D wave file (*.ibw).	
	Export Graph Y-column	Save the Y-column data, including labels, as a 1-D wave file (*.ibw).	
	Import Graphs	Open and display a saved graph.	
	Note:	There is no import of 1-D wave data.	
To Layout Copy/append the selected table or graph to a Layout window.			
To Clipt	board Copy the selected table	e or graph to the operating system (OS) clipboard.	
© Options			
	Show Axes Color	Display a background color for the axes.	
	Show Grid	Display X & Y grid lines in the graph.	
	Show Error Bars	Display SEM error bars for averaged data.	
	Show Markers	Display data points with marker symbols.	
	Show Lines	Display a line between data points.	
		(Toggle between 'markers' and 'lines', or both).	
	Cell Separator: Tab	Use tab separators when exporting a table (e.g. to Excel).	
	Cell Separator: Comma	Use comma separators when exporting a table (e.g. to MS-Works).	

	Include Column Labels		nn labels appear on the first line of an ted table.
	Digits in table entries:	Digits	s = (3, 5, 7, 9, 11, 13, 15)
Operations	Duplicate	Appei	nd a copy of the graph.
	Delete Graph or Table	Delete	e the entire analysis wave.
	Delete Single Graph	Delete	e the selected graph.
	Note:		alysis cannot be deleted if it exists in a graph lysis window - it must first be closed.
Math	Normalize: zero to maximur	n	Rescale the largest point to 1.0.
	Normalize: minimum to may	kimum	Rescale the smallest point to zero, and the largest point to 1.0.
	Invert		Reverse the Y-axis sign of the data.
Append	Displays available analysis w	ave na	mes for appending.
	Select an analysis wave for a are plotted relative to the loa		ng with the loaded wave. Time-course data alysis wave's "time zero".
Average	Displays available analysis v	vave na	mes for averaging.
	•	-	ged with the loaded wave. A weighted er of data sets is accounted for when
	Two new entries are inserted	into th	e wave list after the loaded wave:
	1. The averaged wave.		
	<ul><li>2. The SEM (Standard Error of Means) data points wave.</li><li>If Options / Show Error Bars is enabled, the SEM data are used to display error bars in the corresponding averaged-data graph.</li></ul>		
	Standard Error of the Mean (SEM) Algorithm		
	$SEM = \sqrt{(SumSq - )}$	Mean ²	* N ) / (N-1)
	SumSq = sum of all s	squared	samples
	Mean = sum of all sa	mples /	Ν
			is similar to the Standard Deviation using 'Mean' vs. 'sum of all samples'.
	DPATCH_OPERATION M		EV 2 0 4 (2020-2-5)

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

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

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

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

Values are displayed with SI unit prefixes.

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

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

Values are displayed with SI unit prefixes.

Show/Hide Cursor Info

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

Cursor Bar

# *

Options menu

- One Mover Moves All
- All Styles Change the symbol graphics.
- Show Cursor Pairs Display up to 5 sets of cursor symbol pairs.
- A: The fit's beginning data-point symbol, and its (pnt:) data-point number.
- B: The fit's ending data-point symbol, and its (pnt:) data-point number.
  - 1. Select symbol A and drag it onto the beginning data point (or enter the data-point number in the 'pnt' field.)
  - 2. Select symbol B and drag it onto the ending data point (or enter the data-point number in the 'pnt' field.)
  - 3. The data points X- and Y-coordinates are displayed.
  - 4. Right-click in the graph and select 'Quick Fit' for a list of built-in Igor fitting functions.
  - 5. The fit is displayed in the graph, and the fitting information is written to the Log and Command windows.

Analysis Wave Names	Loaded analyses available for manipulation.	
Analysis Parameters		
	• Series: R#_	Name of the Series.
	• Graphs: #	Number of graphs in the analysis wave.
	• Points: #	Number of data points in the graph.
	• Start: #	Start time of analysis wave.
	• Average: #	Number of graphs averaged or appended.
Tip:	•	cameters text is not fully visible, increase the visible distribution window.
[ Graph & Table pane ]	Data point markers are plotted, or a numeric table is displayed.	
	X- and Y-axes can be magnified to be larger or smaller. Place the mouse cursor in the axis ticks region, then scroll the mouse wheel up or down. The axis ticks region does not include the tick label (numbers) area.	

## 4.2.3 Analysis Window

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

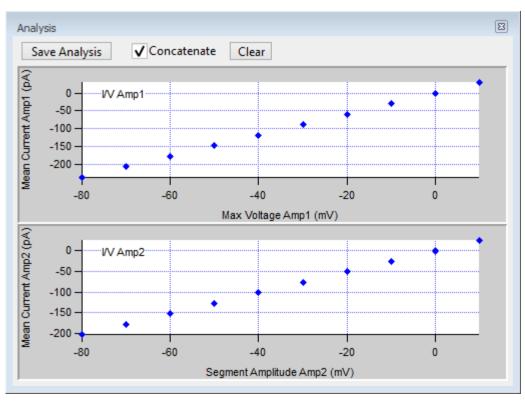


Figure 4-60. Analysis Window

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

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

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

Save Analysis	This button saves the displayed analyses with the Experiment.
	Saved analyses are viewable in the Analysis Editor or from the Data / Data Browser: Data / Analysis folder.
Concatenate	Display new measurements along with the previous measurements. [ only in the Acquisition Scope ]

- <u>Graph pane</u> X- and Y-axes can be magnified to be larger or smaller. Place the mouse cursor in the axis ticks region, then scroll the mouse wheel up or down.
  - Note: The axis ticks region does not include the tick labels (numbers).

Symbols can be manually overlaid onto Analysis window data points.

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

'Options' menu

- One Mover Moves All Move all symbols together with a control.
- All Styles Change the symbol graphics.
- Show Cursor Pairs Display up to 5 sets of cursor (symbol) pairs.
- Note: If the Analysis window is closed when a Layout graph is created, Analysis graphs will not be included in the Layout graph.

Symbols

# 4.2.4 Data Browser

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

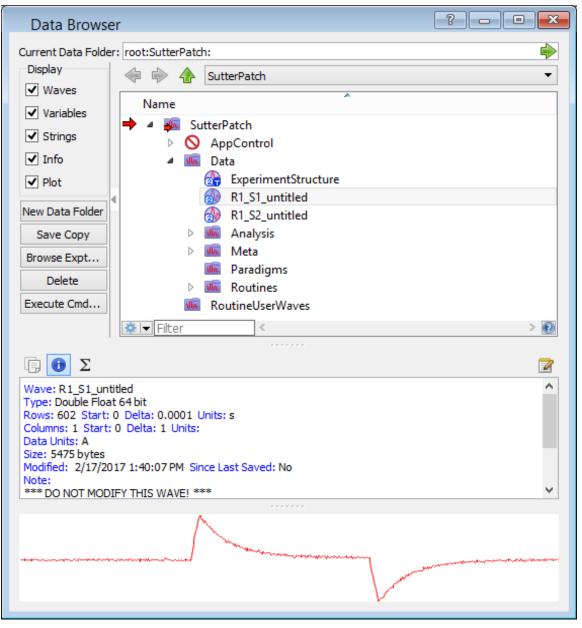


Figure 4-61. Data Browser

Objects are displayed in a tree structure, using a path "root" of 'SutterPatch'.

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

'Analysis' subfolder waves marked with "_En" contain tables of events; waves marked with "_Zn" contain analysis results, such as from fits.

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

# <u>Right-click Menu</u>

Display	Display the data in a visual graph.
Edit	Display the data in a numerical table.
	SutterPatch signal data are stored in two-dimensional data waves, with one column per trace, and one row per sample point.
Warning!	Editing data here will permanently alter the raw data. Modify at your own risk!
Copy Full Path	Copy the object's path to the clipboard. This refers to Igor's internal data folder in the Data Browser, not the computer OS file system. This path can be used by advanced Igor users in user functions and executable commands.

# 4.2.5 Data Navigator

The Data Navigator window organizes and displays all levels of data for the current Experiment.

Data Navigator          Data tree hierarchy         Paradigm Data Name         Series Name	Experiment         Paradigm: P1_20170419211648         Routine: R1_IV_1 <ul> <li>R1_S1_IV_1</li> <li>R1_S2_IV_1</li> </ul>	
Build Hierarchy		
Clear Hierarchy		
Default Hierarchy	Expand Collapse All Last Series	Import Review
	<ul> <li>Hide Preview</li> </ul>	
Metadata Wave Name: <u>P1_201704192</u> Images:	211648	~ ~

Figure 4-62. Data Navigator

Data tree hierarchy The hierarchy of sorting levels is displayed in this pane. **Build Hierarchy** Re-organize the data tree using custom settings. Select parameter group Organize by metadata parameters: (availability depends on Preferences) All Categories Frequently Used **Experiment Hierarchy** Tag Operator **Preparation - Animal Preparation** - Tissue Preparation - Cell Experiment Amplifier

	Data Acquisition Settings Imaging Stimulus
Available para	meter Click on a specific parameter to select it.
	Click on the "copy" button to insert the selected parameter into the data hierarchy.
Hierarchy	The Hierarchy pane displays the new sorting hierarchy. The entries can be re-organized by selecting an entry and clicking on the Up/Down keys to reposition them, or using the 'Del' key to remove them.
Del	
Clear Hierarchy	Remove the existing hierarchy. The current Experiment's raw data are organized in a simple time sequence.
Default Hierarchy	Restore the default experimental hierarchy: Experiment > Paradigm > Routine > Series > Sweeps
Do It	Click on the 'Do It' button to apply these changes to the data tree.
	The current Experiment's data are arranged in the data tree (down to the Sweep level.)
Expand	All closed nodes of the data tree are expanded down to the Signal level.
Collapse All	All nodes of the data tree are collapsed up to the Paradigm level.
Last Series	The last Routine's first signal is highlighted in the data tree and its data are displayed in the Preview pane below it.
Import	Select a previously saved SutterPatch experiment to incorporate into the current experiment.
Analyze	The selected Routine or Series signals are opened in the Scope window for analysis.

	201	
The selected Paradigm's signals and data are opened in the Scope window for review.		
e: Depending upon the data displays the Analyze or Re		
view The display is based up	pon the data level.	
e: The Preview pane does not	support mouse operations.	
Metadata Wave Name:	Click to display the Paradigm's metadata.	
Images:	Open any saved images.	
[Preview sub-pane]	Displays the first signal of the first Routine.	
Metadata Wave Name:	Click to display the Routine metadata.	
Signals:	Number of signals.	
Sweeps:	Number of sweeps.	
Routine Data Name:	Click to display the Routine parameters.	
Analysis:	Open any saved analyses into the Analysis Editor.	
Images:	Open any saved images.	
[Preview sub-pane]	Displays the first signal of the selected Routine.	
[Preview]	Displays the selected Signal.	
[Preview]	Displays the selected Sweep.	
	<ul> <li>the Scope window for revie</li> <li>Depending upon the data displays the Analyze or Review The display is based up</li> <li>the Preview pane does not Metadata Wave Name:</li> <li>Images: <ul> <li>[Preview sub-pane]</li> <li>Metadata Wave Name:</li> <li>Signals:</li> <li>Sweeps:</li> <li>Routine Data Name:</li> <li>Analysis:</li> <li>Images:</li> <li>[Preview sub-pane]</li> </ul> </li> </ul>	

# Available Actions button

Various actions can be performed on the selected data level. These actions are also available via a right-click on the selected data level.

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

Paradigm:	Review	Display the Series data in the Paradigm Review window.
	Display	Display each signal in its own graph window.
	View Metadata	Display the metadata in the Metadata Review window.
	Discard Paradigm Data	Remove the selected Paradigms and their Series from the Experiment.
		If the last Paradigm is discarded, then acquiring another Paradigm in the same Experiment session will increment the new Paradigm name.
		Otherwise, if the Experiment is closed and re-opened after the discard, then acquiring another Paradigm will use the last discarded Paradigm number.
	Export Data (See Preference	ces)
		Export the selected Paradigm data to file(s).
Routine:	Analyze	Display the Series data in a Scope (reanalysis) window.
	Display	Display each signal in its own graph window.
	View Metadata	Display the metadata in the Metadata Review sub-window.
	View Routine	Display the Routine parameters in the Routine Review window.
	Show Data in Data Browse	er Open Igor's Data Browser window.
	Discard Series	Delete the selected Series and remove from the Experiment.
		If the last Series is discarded, then acquiring another Series in

the same Experiment session will increment the new Series name.

Otherwise, if the Experiment is closed and re-opened after the discard, then acquiring another Series will use the last discarded Series number.

## Export Data (See Preferences)

Export the selected Series to file(s).

Signal:	Analyze	Display the Series data in a Scope (reanalysis) window. All signals and sweeps display.		
	Event Detection	Open to analyze synaptic events (minis, etc.).		
	Action Potential Analysis	Open to analyze action potentials.		
	Edit	Display the Series signals as numeric columns in an editable table.		
	Average Signal	Average all sweeps in the signal and display in the Analysis Editor <u>.</u>		
	View Metadata	Display the Series metadata in the Metadata Review sub-window.		
	Copy Full Path	Copy the Series internal Igor path to the clipboard. ( root:SutterPatch:Data:Series_na me )		
	Show Data in Data Browser			
		Open Igor's Data Browser window.		
	Export Data (See Preferences)			
		Export the selected Signals to file(s).		
Sweep:	Analyze	Display the Series data in a Scope (reanalysis) window.		
	Edit	Display the Series data as		

	numerie columns in a table.	
Extract Sweep	When a single sweep is selected, creates a graph of the sweep in the Analysis Editor.	
Average Selected Sweeps	When multiple sweeps are selected, create a graph of the averaged sweep in the Analysis Editor.	
Show Data in Data Browser		
	Open Igor's Data Browser window.	
Export Data (See Preferences)		
	Export the selected sweeps to file(s).	

numeric columns in a table.

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

#### 4.2.6 Data Table

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

□ □ ⊠ R0 C0 5.2332054e-12 ⊕ 🛱						
Row	R1_S1_IV[][0]	R1_S1_IV[][1]	R1_S1_IV[][2]	R1_S1_IV[][3]	R1_S1_IV[][4]	F
	0	1	2	3	4	
0	5.23321e-12	-5.63541e-14	1.63961e-12	3.32488e-12	8.47496e-13	^
1	-9.90098e-13	1.71653e-12	1.4612e-12	-2.72349e-12	-4.44117e-12	
2	-3.81879e-12	3.93903e-13	-2.90443e-12	4.29801e-13	6.7005e-13	
3	-7.61568e-12	-4.25361e-12	5.03512e-13	-2.13725e-12	-2.38818e-13	
4	1.77125e-12	-2.23609e-12	-9.99009e-13	5.83818e-13	-1.80796e-12	
5	-8.78851e-13	-1.72464e-12	5.22561e-12	-4.20016e-12	-2.72047e-12	
6	1.95622e-12	-4.37322e-12	-2.92145e-12	2.1944e-12	-1.13872e-13	
7	-2.17838e-12	-3.97302e-12	-1.08042e-12	-4.92174e-13	1.49426e-12	
8	3.4504e-12	-6.02635e-12	9.20459e-13	-2.37966e-12	-8.44283e-13	$\sim$
<						>

Figure 4-63. Data Table

Data Tables are accessed from the Data Navigator by highlighting a data Series, rightclicking on it, and selecting the 'Edit' command. Alternatively, use the menu item Data / Data Browser and select a Series from the Data folder; then use the right-click menu item 'Display'. To allow adding data to the table, the last row of data in the table is followed by a final row of blank (gray) cells. Manually entering data into the final blank row causes a new last row of data to be created in the table, followed by a new final blank row.

## 4.2.7 Equation Editor

The Equation Editor manages simple or complex expressions that evaluate to a value. Such math equations can be used to create stimulus waveforms, and for data analysis.

Access the Equation Editor from the SutterPatch menu.

Equation Edito	r 🗖 🗖 💌
Equation Pool Files	<ul> <li>C:Users:EmeraldCity:Documents::SP_EquationPool</li> </ul>
New Dup	licate Delete Revert Save Pool
Edit Ch	neck Equation Insert special identifier 💌
Label	Equation
label1	1*pi ^
label2	2*pi
label3	3*pi
	×

Figure 4-64. Equation Editor

Equation Pool Files	Equations are	created and saved in an Equation Pool file
New Equation	n Pool	Create a blank Equation Pool file.
Load Equatio	n Pool	Load the Equations of a previously saved Equa- tion Pool file into the Equation Pool.
Revert to Las	t Saved	Undo any unsaved changes to the Equation Pool.
Save Equation	n Pool	Save the Equation Pool using its existing file name and path.
Save Equation	n Pool As	Save the Equation Pool to a new file, and switch to the new file. The default file name is the original file name.

	Save Equation Pool Copy	Save the Equation Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to it.	
	Merge Equation Pools	Insert the Equation from a previously saved Equation Pool file into the loaded Equation Pool.	
Note:	Equation Pool files are simple	e text files that can be directly edited.	
New	Create a blank Equat	ion.	
Duplicate	Add a copy of the set	lected Equation to the Equation Pool.	
Delete	Remove the selected	Remove the selected Equation from the Equation Pool.	
Revert	-	Select an Equation and click the Revert button. All editable steps are re- set to their last saved settings.	
Save Pool	Save the Equation Po	Save the Equation Pool using its existing file name.	
Edit	Make edits to the 'Eq	Make edits to the 'Equation' field.	
Check Equat	ion The equation is evalu syntax is "ok".	The equation is evaluated (for sweep #1), and if valid, it reports that the syntax is "ok".	
Label	Column of editable equip	quation names, for quick usage in place of the equa-	
Equation	Column of equations	Column of equations in free-form text fields.	
т, ·	1 • 1		

Insert special identifier

The following acquisition, amplifier and reference settings are available for use in equations:

Loop	(active paradigm ForLoop count)	
Sweep	(active paradigm EachSweep count)	
LastSweep	(active paradigm sweep count of last sweep)	
	Processing c	an occur before or after the last sweep of a series.
	Example:	Compare 'sweep' numbers in a ForEachSweep loop in a Paradigm 'If' step:
		ForEachSweep EachSweep, Target=IV If, Left=sweep, Operation= '=', Right=LastSweep-1 Alert, Text=LastSweep, DoBeep=true EndIf

## ForEachEnd

AqStopped	(last acquisition was stopped)	
	The last Routine-Series did not complete by itself.	
ParadigmTime	(time at start of paradigm, s)	
RoutineTime	(time at start of routine, s)	
Stimulant	(last applied stimulant concentration)	
	From the Solution Editor 'Concentration' setting for solutions configured as a 'Chemical Stimulant'.	
Tme	(present date-time, s)	
Timer	(timer time, s)	
m[116]	(n'th analysis measurement value)	
gx[116]	(n'th analysis graph x value)	
gy[116]	(n'th analysis graph y value)	
r[116]	(n'th routine stimulus variable)	
p[116]	(n'th paradigm variable)	
Hold[12]	(holding of n'th output channel)	
	Headstage holding level.	
Input	(Input variable on paradigm window )	
AuxIN[18]	(reading of auxiliary input, V)	
	A single-point voltage reading from an Auxiliary Input channel of the dPatch system, such as from a slowly changing temperature probe.	
Note:	This usage does not require setting up a Routine Input Channel.	
Imon	(amplifier current reading, A)	
	In the Amplifier Control Panel (pA).	
DPATCH-OPERATION MANUAL-Rev. 2.0.4 (2020-2-5)		

Vmon	(amplifier voltage reading, V)			
	In the Amplifier Control Panel (mV).			
Mean[name or count, start,width] (mean of given input signal)				
ActiveProbe	(active probe)			
	[1-2]			
	The "active" probe number is the Sutter headstage presently controlled by the Amplifier Control Panel.			
	For a single headstage system, the active probe is always headstage number "1".			
NumProbes	(number of probes)			
	[1-2]			
	The number of dPatch headstages attached to the system.			
CCMode	(amplifier current clamp)			
VCMode	(amplifier voltage clamp)			
Hold	(IHold in CC-mode, VHold in VC-mode)			
	$[\pm 0.000,000,020~{\rm A}~(\pm 20,000~{\rm pA}),~{\rm or}\pm 1.000~{\rm V}~(\pm 1000~{\rm mV})~]$			
	Headstage holding level.			
IHold	(amplifer holding current, A)			
	$[\pm 0.000,000,020 \ (\pm 20,000 \text{ pA})]$			
IHoldOn	(amplifier holding current On)			
VHold	(amplifier holding voltage, V)			
	[±1.000 V (±1000 mV)]			
VHoldOn	(amplifier holding voltage On)			
IGain	(amplifier current gain, V/A)			
	The gain of the active voltage-clamp 'Current' input channel.			

	• 0.5 mV/pA		
	• 1 mV/pA		
	• 2.5 mV/pA		
	• 5 mV/pA		
	• 10 mV/pA		
	• 25 mV/pA		
VGain	(amplifier voltage gain, V/V)		
	The gain of the active current-clamp 'Voltage' input channel.		
	• 10 mV/mV		
	• 20 mV/mV		
	• 50 mV/mV		
	• 100 mV/mV		
	• 200 mV/mV		
	• 500 mV/mV		
Feedback	(amplifier feedback mode: 0, 1, 2)		
Filter	(amplifier input filter in VC- and CC- mode, Hz)		
	Apply a filter to the appropriate input channel in either VC- or CC-mode. See IFilter or VFilter below.		
IFilter	(amplifier input filter in VC-mode, Hz)		
	Apply a filter to the Current input channels.		
	Use a preset value, or a 10% threshold between the preset values is applied to the equation (to avoid over filtering).		
	• 500 (500 Hz)		
	• 1000 (1 kHz)		
	• 2000 (2 kHz)		
	• 5000 (5 kHz)		
	• 10000 (10 kHz)		
	• 20000 (20 kHz)		
VFilter	(amplifier input filter in CC-mode, Hz)		
	Apply a filter to the Voltage input channels.		
	Use a preset value, or a 10% threshold between the preset		

# values is applied to the equation (to avoid over filtering).

•	500	(500 Hz)
•	1000	(1 kHz)

- 2000 (2 kHz)
- 5000 (5 kHz)
- 10000 (10 kHz)
- 20000 (20 kHz)

Offset	(amplifier pipette offset in VC-mode, V)
OffsetLock	(amplifier pipette offset lock ON in VC-mode)
SubtractPipOffset	(subtract pipette offset ON in VC-mode)
LiquidJunc	(liquid junction potential offset, V)
ECompMag	(amplifier electrode compensation magnitude, F)
ECompTau	(amplifier electrode compensation tau. s)
ECompOn	(amplifier electrode compensation On in CC-mode)
CmComp	(amplifier cell compensation Cm, F)
RsComp	(amplifier cell compensation Rs, Ohm)
RsCompOn	(amplifier cell compensation Rs On)
RsCorr	(amplifier Rs correction, fraction)
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On)
Bridge	(amplifier bridge balance, Ohm)
BridgeOn	(amplifier bridge balance On)
Relectr[12]	(electrode/seal/access resistance, Ohm)
Rmemb[12]	(membrane resistance (Cell mode), Ohm)
Cmemb[12]	(membrane capacitance (Cell mode), F)
RMSNoise[12]	(membrane test RMS noise, A)

Other identifiers are forwarded to Igor Pro's 'Execute' command.

# **Equation Extras**

Constants			
	true	1	
	false	0	
	ON	1	
		T	
	OFF	0	
	The following	constants hav	e 27-digit precision:
	e	2.71	(Euler's number)
	pi	3.14	(п)

Parsing and Operators

Equation parsing is executed from left to right, processing the highest precedence level operators first, except for comparison and bitwise operators, which associate from right to left.

Precedence	Operation Type	Operator
8	Comment	;
7	Exponentiation, Arithme- tic operations: Left Shift, Right Shift	^, <<, >>
6	Negation operations: Unary Negation, Logical Negation	-, !
5	Multiplication, Division, Remainder	*, /, %
4	Addition, Subtraction	+, -
3	Bitwise operations: And, Or, Nor, Xor	&,  , nor, %^
2	Comparison operations: Greater Than, Greater Than or Equal, Less Than, Less Than or Equal, Equal To, Not Equal To	>, >=, <, <=, ==, !=

1	Logical operations: And, Or, Conditional	&&,   ,?:
0	All other operations	round, trunc, ceil, floor, exp, sqrt, ln, log, sin, cos, tan, asin, acos, atan, abs, rad, deq, noise, random

Table 4-4. Equation Parser

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

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

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

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

<expression>? <True>: <False>

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

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

For complex expressions, only the real portion is evaluated.

Note: The ": " is a colon with two blank spaces around it.

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

#/2^n

Syntax

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

Three kinds of brackets [ ], {}, ( ), can be used equivalently to improve the clarity of nested expressions.

Numeric values can be written in scientific E-notation using exponents:

5e-12 (5 picoamps)

or in equivalent engineering notation using unit prefixes:

5p

(5 picoamps)

Prefix	Exponent	Prefix Name	Prefix	Exponent	Prefix Name
k	10 ³	Kilo	m	10-3	milli
м	10 ⁶	Mega	μ (or u)	10 ⁻⁶	micro
G	10 ⁹	Giga	n	10 ⁻⁹	nano
т	10 ¹²	Tera	р	10 ⁻¹²	pico
Р	10 ¹⁵	Peta	f	10 ⁻¹⁵	femto
E	10 ¹⁸	Exa	а	10 ⁻¹⁸	atto
z	10 ²¹	Zetta	z	10 ⁻²¹	zepto
Y	10 ²⁴	Yotta	У	10 ⁻²⁴	yokto

Table 4-5. Engineering Notation

Insert an equation from the Equation Editor Pool into an Equation field by entering "#" followed by the label of the equation, e.g. "#MyLabel(5)". This passes the argument "5" to the equation labeled "MyLabel" for evaluation.

Example: Using an LED light source

To stimulate in increments of light intensity, use an equation to transform light intensity values in Routine variables into actual stimulus values with amplitudes in volts.

Build an equation in the equation pool as follows:

equation =

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

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

label = power_to_volts

In the Waveform Editor, set a Segment Amplitude field to 'Equation', and enter the equation as "#power_to_volts".

The following areas have a simplified version of the Equation Editor that allows Equations (and equation labels) to be used:

Paradigm Editor

Steps:	Amplifier, Checkbox, Set Variable, Sound, Write Log, If, Else If
Routine Editor	
Input Channels:	Virtual Channels: Math Type
Output Channels:	Leak Hold, Waveform Editor: Amplitude, Duration
Measurements:	Time to Threshold, X-Axis, Y-Axis

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

# 4.2.8 Event Detection (synaptic)

Post-synaptic potentials and currents from excitatory and inhibitory events (EPSPs, EPSCs, IP-SPs, IPSCs) are analyzed with this application module. Spontaneous miniature events ("minis"), which generate small and/or overlapping events, are also detectable with an innovative deconvolution algorithm. This technique finds events with high temporal fidelity, while also improving the signal-to-noise ratio (SNR).

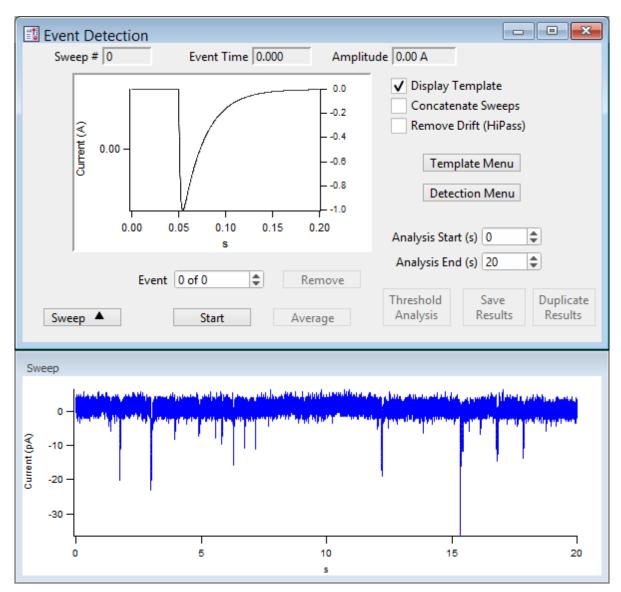


Figure 4-65. Event Detection

Sweep#	The sweep number of the displayed data.
Event Time	The time of the event's threshold crossing (s).
Amplitude	The amplitude averaged around the peak by $\pm 1$ ms.
Display Template	Display the ideal event's template on top of the selected event in the graph - its Y-axis displays on the right edge of the graph.

Tip:	To match the template to the data, hover the mouse cursor over the right Y-axis, and use the mouse wheel to rescale the template.	
Concatenate Sweeps		ine all sweeps into a single pseudo-sweep before processing. This be useful if data was collected using the continuous acquisition
Remove Drift (HiPass)		
Template Menu	Open	the Template sub-panel to configure a template.
		e a template of a typical event as a double-exponential curve. The vill be deconvolved to this template for further analysis.
Event Polarit	У	1 = positive
		-1 = negative
Rise Time (µs	)	[10-5,000]
		Time constant $(\tau)$ for the rising phase of the template event.
Decay Time (	us)	[ 100 – 100,000 ]
		Time constant $(\tau)$ for the falling phase of the template event.
Create Templ	late	Click this button to create the template.
Detection Menu	Open	the Levels sub-panel to configure detection levels.
Threshold (xS	SD)	A detection threshold representing the "Event Strength", where a lower ("weaker") number finds more events, while a higher ("stronger") number finds fewer events. Adjust this threshold based on empirical testing of your data.
		[0.1-10]
		Lower # = more noise-based events (false-positives) Higher # = more missed events (false negatives)
	Note:	The default threshold is set to 4 times the standard deviation of a Gaussian fit to an all-points histogram of the (Fourier) deconvolved data signal.
Ampl Thresh	old	Set an amplitude threshold for the minimum size of events.
		[5e-13-1e-6]
Decay tau (us	5)	[<,>] [500-1,000,000]
		Set the decay tau as "less than" or "greater than" the tau value.

Analysis Start (s)	Sweep time to start looking for an event threshold.
Analysis End (s)	Sweep time to stop looking for an event threshold.
Event Remove	The current event number vs. total number of events. Delete the current event from the analysis.
Sweep	Show/Hide the sweep preview panel.
Start	Run the Event Detection analysis.
Average	The averaged event displays in the graph.
Threshold Analysis	A scatter plot of the Event Strength vs. Current is displayed in a sub- panel.
Save Results	Event Detection Results A Layout window report is created.
	Total time analyzed = Includes the Start/End times for all sweeps (s).
	Number of events detected = Total number of events found.
	Event Frequency = $(Hz)$
	Average Event Amplitude = $\pm 1$ ms peak average (pA)
	Standard Deviation of Event Amplitude = (pA)
	Graphs: Cumulative probability vs. Amplitude
	Amplitude (Average) vs. Time
	Frequency vs. Sweep Number
	Amplitude vs. Sweep Number
	Results Table A table of columns is created.
	[ blank ] Row number with one row per Event.
	Sweep Number The sweep number the Event is in.
	Event Time (s) 'Time to event' from the start of sweep.
	Event Strength (xSD) A measure of how well the signal matches the template. (Lower is weaker, higher is stronger.)
	Event Amplitude (A) The Event peak amplitude $\pm 1$ ms average.
	Event Integral (A*s)
	10-90% Rise Time (s)
	Event Decay Tau (s)
	Absolute Event Time (s)

Note: For this measurement, events are measured relative to execution of the Routine's Start button, not to the sweep start.

### Inter Event Interval (s)

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

### 4.2.9 Igor Analyses

Curve fitting options are located in the Analysis main menu:

- Curve Fitting Create your own fitting equation.
- Quick Fit Use a pre-defined equation:

line poly poly_XOffset gauss Ior exp_XOffset dblexp_XOffset exp dblexp  $\sin$ HillEquation Sigmoid Power LogNormal poly2D Gauss2D FitBetweenCursors Weight from Error Bar Wave **Textbox Preferences** Example: Perform a fit on a section of a sweep: In the Data Navigator, select the sweep, and use the 'Ex-1. tract Sweep' command on it.

- 2. In the Analysis Editor, click the 'To Layout' button.
- 3. In the Layout window, right-click for the "Show Window" command.
- 4. With the Graph window active, select the main menu item Graph > Show Info.
- 5. In the Graph window cursor pane, drag cursors 'A' and 'B' onto the data.
- 6. Select main menu Analysis / Quick Fit and the fit of your choice.

Built-in Igor analyses are documented in the Igor Pro Help:

Fourier Transforms Convolve Correlate Differentiate Integrate Smooth Interpolate Filter Resample Sort Histogram **Compose Expression** Packages Average Waves Batch Curve Fitting **Function Grapher** Global Fit Median XY Smoothing

MultiPeak Fitting Percentiles and Box Plot

Wave Arithmetic

# 4.2.10 Layout Window

The Layout window is used to prepare your data for publication. Scope window input signals and associated Analysis window graphs are displayed in a Layout window for graphical arrangement and editing.

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

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

When a Layout window is open, the main menu "Layout" item displays. The 'Append to Layout' command allows you to append any graph object in the Experiment into the active Layout window, as does the Layout button in SutterPatch dialogs.

Once a Layout window is created and filled, additional graphs are automatically appended to additional pages. The default arrangement is "2 x 4" graphs per page.

New Layout windows allow page layout configuration for the number of graphs per page ('column' x 'row'). Control this setting in Preferences / Export_Graphics or the Paradigm 'Export' step.

- 1 single pane
- 2 stacked panes
- 3 stacked panes
- 2 x 2 matrix
- 2 x 3 matrix
- 2 x 4 matrix

A toolbar displays in the upper-right corner of the Layout window. There are two configuration buttons at the top, which display different sets of buttons:

- Operate Mode Selection tools and object insertion.
- 🔁 Draw Mode Drawing tools.

When you close a Layout window, you can choose to save it as a "macro". To restore a Layout window, go to the Igor Pro menu Windows / Layout Macros and select the macro name. Refer to the Igor Pro documentation for further details about macros.

### 4.2.11 Metadata Review

Metadata describes the system environment (computer and amplifier) and user settings of Paradigms and Routines.

Metadata parameters can be retrieved several different ways:

Data Navigator

- Select a Paradigm or Routine, and then its Metadata Wave Name in the Preview pane.
- Select a Paradigm or Routine, then click the 'Available actions' button and select 'View Metadata'.
- Right-click a Paradigm or Routine and select 'View Metadata'.

#### Scope (Reanalysis) Use the 'View Metadata' button in this window.

Note: User-defined parameters from all levels of the Set Preferences / 'Metadata detail level' are displayed along with the automatic metadata.

Paradigm Data Displays the name of the Paradigm.

Routine Data Displays the name of the Routine.

- By Event System settings, routine settings, and defined metadata events are displayed per Signal. Highlighted fields are editable.
- By Parameter Includes an expanded set of software parameters displayed per category.
- Expand All All parameter settings are displayed.

Collapse All All parameter settings are hidden.

Automatic Metadata Parameters "By Parameter"

<u>Tag</u> Tag Number Tag Creation Timestamp Timer Time at Tag Creation Tag Signals Tag Source Event <u>Operator</u> Login Name

<u>Experiment</u> Experiment Timestamp

Amplifier Amplifier Sequence Number Amplifier Manufacturer Amplifier Model Amplifier Revision Amplifier Firmware Version Amplifier Serial Number Amplifier Channel Number of Available Headstages Headstage Sequence Number Headstage Model Headstage Revision Headstage Preamplifier Model Headstage Preamplifier Revision

[ only with hardware attached ] [ only with hardware attached ]

[only with hardware attached]

Instrumentation and Software **Interface Sequence Number** Interface Manufacturer **Interface Model Interface Revision** Interface Serial Number Interface Input Channel (physical) Interface Out. Ch. (physical or logical) Interface Signal Type **Interface Number of Digital Outputs** Computer Name **Operating System Platform Operating System** Software Environment Software Environment Version Software Environment Build Software Environment Kind Software Environment Serial Number Data Acquisition Software Data Acquisition Software Version Data Acquisition Software Build Data Acquisition XOP Version

<u>Paradigm</u> Paradigm Data Sequence Number

Paradigm Data Base Name Paradigm Name Paradigm Description Paradigm Data Start Timestamp

<u>Series (= Routine Data)</u> Series Sequence Number Series Base Name **Routine** Name **Routine Acquisition Mode Routine Description** Routine Data Start Timestamp Routn. Completed / Terminated Early Number of Input Signals Sweep Sweep Number **Data Acquisition Settings** Active Headstage **Recording Mode** Current Gain Voltage Gain Headstage Gain Headstage Feedback Mode **Filter Cutoff Frequency** Filter Type Input Offset Voltage [VC mode] Input Offset Lock On/Off [VC mode] Subtract Pip. Offset in Current Clamp [ CC mode only ] Input Signal Name **Input Signal Units Input Scaling Factor** Input Full-scale Minimum Input Full-scale Maximum **Input Sampling Rate** Auxiliary Input Signal Offset Virtual Signal Scaling Offset [for Virtual Input channels] Virtual Signal Math Type [for Virtual Input channels] Virtual Signal Equation [for Virtual Input channels] Virtual Signal Source Channel [for Virtual Input channels] Virtual Signal Source Signal Name [for Virtual Input channels] Virtual Signal Leak Display On/Off [for Virtual Leak] Virtual Signal Leak Zero Segment [for Virtual Leak] Virtual Signal Sweeps Processed [for Virtual SweepAverage] Virtual Signal Reference Sweep [for Virtual SweepSubtract] Virtual Signal Line Frequency Base [for Virtual LineFreg] Virtual Signal Filter Type [for Virtual Filter/Smoothing] Virtual Signal Filter Order [for Virtual Filter/Smoothing] Virtual Signal Filter Cutoff Frequency [for Virtual Filter/Smoothing] Virtual Signal LockIn Delay [for Virtual LockIn] Virtual Signal LockIn Attenuation [for Virtual LockIn] **Electrode Fast Magnitude** [VC mode only] Electrode Fast Time Constant [VC mode only] Whole-cell Compensation On/Off [VC mode only]

WC Comp – Series Resistance	[ only if WC Comp On ]
WC Comp – Membrane Capacitance	[ only if WC Comp On ]
Series Resistance Correction On/Off	[VC mode only]
Series Resistance Prediction Value	[ only if Series RCorr On ]
Series Resistance Correction Value	[ only if Series RCorr On ]
Series Resistance Corr. Lag Time	[ only if Series RCorr On ]
Capacitance Neutralization On/Off	[ CC mode only ]
Capacitance Neutralization Mag.	[ only if Cap Neut On ]
Capacitance Neutralization Tau	[ only if Cap Neut On ]
Bridge Balance On/Off	[ CC mode only ]
Bridge Balance Resistance	[ only if Bridge Bal On ]
Current Clamp Dynamic Hold On/Off	[ CC mode only ]
Current Clamp Dyn. Hold Potential	[ only if CC Dyn Hold On ]
Current Clamp Dynamic Hold Speed	[ only if CC Dyn Hold On ]
CC Dynamic Hold On for Acquisition	[ only if CC Dyn Hold On ]
Command Signal Name 1	
Command Signal Units 1	
Command Full-scale Minimum 1	
Command Full-scale Maximum 1	
Command Sampling Rate 1	
Command Holding Enabled 1	
Command Holding Value 1	[ only if Cmd Holding 1 enabled ]
Auxiliary Output Holding Value 1	
Command Signal Name 2	
Command Signal Units 2	
Command Full-scale Minimum 2	
Command Full-scale Maximum 2	
Command Sampling Rate 2	
Command Holding Enabled 2	
Command Holding Value 2	[ only if Cmd Holding 2 enabled ]
Auxiliary Output Holding Value 2	
Auxiliary Output Holding Value 3	
Auxiliary Output Holding Value 4	
Digital Holding Pattern $(1 \rightarrow N)$	[1-16]
0 0 (	L - J

# 4.2.12 Paradigm Review

The 'Paradigm Review' Scope window displays a time-course of all data recorded with the Paradigm, including all executed Routines and their Series data.

This view also displays the tags that occur between Routines.

See the Scope Window for usage of the window controls.

# 4.2.13 Reanalysis Measurements & Graphs

The Scope (Analysis) window Measurements button allows editing of the 'Reanalysis Measurements & Graphs' panel. Use it to apply different analysis scenarios to recorded data by configuring measurements on input channels and analysis graphs.

🖪 Reanalysis Measurements & Gr	aphs 🗖 🗖 💌
Measurement Parameters from Last E	xecuted: R2_IV 🔹
Measurement Settings	
On ID Label	Analysis
✓         m[1]         I_Mean         ∧           ✓         m[2]         V_Seg2         ∧	Analysis Function Mean 💌
✓ m[3] l2_Mean ✓ m[4] V2_Seg2	Arithmetic mean of all points
m[5]	
□ m[7]	Enable Smoothing
m[9]	Signal To Analyze Current1
m[10] m[11]	Region Timing
m[12] m[13]	Cursors Relative to Sweep Time 💌
m[14] m[15]	Increment by 40.0 µs
□ m[16]	Start Time 2.5000 ms
Copy None 👻	End Time 8.4000 ms
Clear All Measurements	Lock Time step width 5,9000 ms
Graph Settings	
On ID Show Label	Y-Axis
g[1]      I/V Amp1     ↑     g[2]      I/V Amp2	Source m[1]: I_Mean
□ g[3] □ g[4]	Label Mean Current Amp1
g[7]	X-Axis Source m[2]: V_Seg2
	Label Max Voltage Amp1
Copy None	Unit V 👻 V

Figure 4-66. Reanalysis Measurements & Graphs

This dialog operates similarly to the Routine Editor: Real Time Measurements & Graphs dialog, with a few additions:

Measurement parameters from

Select which Routine settings to record with:

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

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

Edit Virtual Signals

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

Edit Virtual Signals	B
Edit Virtual Signals	Input Unit   A   Scaling   Offset   0.000 A   Factor A/V   1   Math Type   Smooth   ✓   Math Equation:   Smooth (Current1; Gaussian; 2)   Source Signal Current1   ✓   Smoothing type   Gaussian   ✓   Delete

Figure 4-67. Edit Virtual Signals

Virtual input channels allow you to perform a variety of mathematical transformations on input signals. To enable a virtual signal, highlight a signal name. When a virtual input channel is enabled, its configuration fields are ungrayed. Changes to the highlighted signal are saved when you click the 'Do It' button, and changes in unhighlighted signals are discarded.

Input Unit The base unit of measurement from its Source signal. The resolution of the unit is automatically adjusted in the signal.

Scaling

Apply an amplitude offset to the input signal (after any scaling). For "mV" units, append with 'm' or 'e-3'. For "pA" units, append with 'p' or 'e-12'. Example: 5 picoamps using engineering notation: 5p or in equivalent scientific E-notation: 5e-12 Apply scaling to interpret the input signal data. Specify as a numeric value or an equation. Note: The dPatch data acquisition system uses a high-resolution 16-bit ADC with 64-bit data, so data resolution is not an issue when scaling input signals. Math Type Apply a data transformation to a virtual input signal: BaselineSubtract Subtract a fixed value from all data points in an input trace. This is useful for adjusting for an offset, or resetting a baseline. Source Channel Select an input channel to process. **Baseline** From Select how to calculate the subtraction value. Subtract a fixed value. Value Value Spinner adjusts in 1 pA or 1 mV increments. Trace Subtract the average of the entire input trace.

Offset

Factor

- Sweep Time Subtract the average of the data between the Start Time and End Time.
- Start Time Set the starting time of the data to be averaged.
- End Time Set the ending time of the data to be averaged.
- Segment #s Subtract the average of a Segment from the input trace.
  - Set the starting time of the data to be Start Ratio

		averaged, as a ratio relative to the starting time of the Segment duration.
	Start Time	[ derived value ]
	End Ratio	Set the ending time of the data to be averaged, as a ratio relative to the ending time of the Segment duration.
	End Time	[derived value]
BesselFilter		filter with excellent response reserving the shape of a biological signal.
	Source Channel	Select an input channel to filter.
	Filter Bandwidth	Select a frequency range.
	• LowPass	Allow signal frequencies less than the cutoff frequency, and block all higher frequencies, such as high frequency noise.
	• HighPass	Allow signal frequencies greater than the cutoff frequency, and block all lower frequencies.
	Filter Order	[1,2,4,8]
		Number of "poles" in the filter
	Cutoff Frequency (H	Iz) [ 100 to < $\frac{1}{2}$ the sampling rate ]
		Restrict frequencies from this boundary point onwards.
	Integrator Reset	[ in capacitive mode ]
	• Ignore	Capacitive-mode transients are displayed in the data.
	• Blank	The data during capacitive transients are made invisible by replacing those data points with NaNs (Not a Number).
		Blank Duration [10 us – 1 s]
	• Mask	The data during capacitive transients are replaced by the last data value before the transient discharge, simulating a sample-and-hold operation.

Mask Duration [10 us - 1 s]

The default value of  $500 \ \mu s$  should be sufficient to encompass the reset transient duration.

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

•

	8	0	1 5
	Source Channel	Select	an input channel to differentiate
Equation	Specify an equation	to proce	ess an input signal.
	Source Channel	Select	an input channel to process.
	Equation [ ]		ield to access the 'Specify math on' editor.
	Note:	tool tip	ll equation is always visible as a o, by hovering the mouse cursor ne 'Math Equation' field.
	Specify math	equatio	on for virtual signal
	[ <equation>]</equation>	]	A free-form text field
			Errors are reported under this field.
	Check Equati	on	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is "ok".
	Insert special	identif	ier
			Special references can also be used within commands:
	• p[#]		n'th Paradigm variable <u>.</u>
	-	-count,	sweep-count, trace-count, routine
	name]		Access an arbitrary input trace

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

The "current" item is the "active" trace in the Scope window, and

has a count value of zero.

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

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

Ex: s[0,0,0,]

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

t[#] Access the input trace (data wave) in Scope position "n" for the last sweep of the current Series.

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

All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

Undo

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

Source Channel Select an input channel to integrate.

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

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

Source Channel	Select an input signal to process.
Show Leak	Display the accumulated leak currents after the subtracted data in a sweep. Display the average of the leak sub- pulses.
Leak Zero Segment	Identify a segment with no active

Leak Zero Segment Identify a segment with no active cellular response to the command signal.

When set to zero, the field is set to 'OFF'. To re-display the numeric spinners, enter a non-zero number into the field.

- Note: The mean of the second half of the specified segment is used to compute an averaged leak current, which is then used to correct the P/N leak average. This option reduces the influence of a constant leak-current, which is otherwise included in the current of the main signal.
- LineFreq Remove AC line frequency noise (hum) from the data signal.

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

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

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

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

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

Current Channel	Select a (source) input channel with a "current" signal.
Trace Kind	Select the LockIn measurement to display.
	The selected 'Trace Kind' is automatically set as the Virtual Channel label.

			СМ	Computed membrane capacitance.
			GM	Computed membrane
				conductance.
			GS	Computed series conductance.
			DC	DC component of measured signal.
			RealY	Real number part of the lock-in response signal.
			ImagY	Imaginary number part of the lock-in response signal.
		Cycles to Average	[1-1	000 ]
		Cycles to Skip	[1-1	000 ]
		V-reversal	[±100	0 mV ]
			enter f under	using a calculated stimulus trace, the reversal potential for the ion study, such as for (Na+) sodium or (K+) potassium tail currents.
	Note:	See the SutterPatch the LockIn computati	-	hms appendix for the math used in
•	Smooth	Smooth the data wit filter.	h a "mo	oving average" noise-reduction
		Source Channel	Select	an input channel to smooth.
		Smoothing Type		
		• Gausian		dard filter with excellent 10 – 90% ne response
		Smooth Oper	ations	[ 1-32767 ]
				# of smoothing operations to perform.
		• Boxcar		time-domain filter with ent 0 – 100% rise-time response
		Smooth Repe	titions	[ 1-32767 ]

i i

# of smoothing repetitions to perform.

Boxcar Window Points [1-99]

# of points in boxcar sliding "window".

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

#### **Integrator Reset**

- Ignore Capacitive-mode transients are displayed in the data.
- Blank The data during capacitive transients are made invisible by replacing those data points with NaNs (Not a Number).

Blank Duration [10 us - 1 s]

• Mask The data during capacitive transients are replaced by the last data value before the transient discharge, simulating a sample-and-hold operation.

Mask Duration [10 us - 1 s]

The default value of 500  $\mu s$  should be sufficient to encompass the reset transient duration.

• Stimulus Replicate the command waveform.

Source Channel	Select an input channel – the waveform
	from its Parent Out Chan is used.

• SweepAverage Average the input traces.

Source Channel	Select an input channel to average.
Average Type	Cumulative
	Average all processed sweeps together.

#### RunAverage

Average the last "N" sweeps.

Number of Sweeps

• SweepSubtract Subtract a sweep from the input trace.

Source Channel	Select an input channel to process.
Reference Sweep	Select a sweep to be subtracted from all other sweeps. If the sweep does not yet exist, no subtraction occurs.

# Analysis Examples

Example 1: Plot the mean of the data (using sample routine IV)

- 1. Set measurement m[5] to the 'Mean' analysis and select signal Current1.
- 2. Enable graph [g5].
- 3. From the graph's Y-Axis list, select m[5]. The Equation field displays: m[5]
- 4. Set 'X-Axis' to 'time'.
- 5. Run the analysis.
- 6. An Analysis window displays a graph of the mean vs. time.

Example 2: Plot the difference between two measurements

- 1. Set measurement m[5] to the 'Mean' analysis and select signal Current1.
- 2. Set measurement m[6] to the 'Mean' analysis, using the same signal.
- 3. Adjust the m[6] cursors Start/End times so they do not overlap with the m[5] cursors.
- 4. Enable graph [g6].
- 5. For the graph's Y-Axis, select 'Y-Equation' and enter the equation as: m[5]-m[6]
- 6. Set the X-Axis to 'time'.
- 7. Run the analysis.
- 8. An Analysis window displays a graph of the difference vs. time.

# 4.2.14 Routine Review

The Routine Review window is similar to the Routine Editor Routine Settings and Preview pane sections, except that this Preview pane does not include sweep- and region-selection controls.

Routine Review: R1_IV_1	
Routine Name IV_1	Activate Copy to Routine Pool
Routine Description I/V example	
Routine Parameters           Acquisition & Routine Parameters:           External Trigger Action: None           Acquisition Mode: Triggered Sweeps   Ouput           Digital Outputs set as: 8-bit Word           Number of Sweeps: 10   Cycles: 1           Sweep start-to-start time: Shortest Possible           Input Sampling Rate: 10 kHz (100 µs)           Output Sampling Rate: 1 kHz (1 ms)	ts Enabled
Persistence Display: ON Designated Amplifier: Double IPA Input Channels:	×
0 - (Âm) -20 - (Âm) -40 - (Am)	Sweep Interval: auto set       Sweep Duration: 0.0450 s       Min Stim Duration: 0.0450 s       Max Stim Duration: 0.0450 s       Max Stim Points: 45
-8080808080	Holding (display): 0 mV Display Signal StimOUT1 -

Figure 4-68. Routine Review

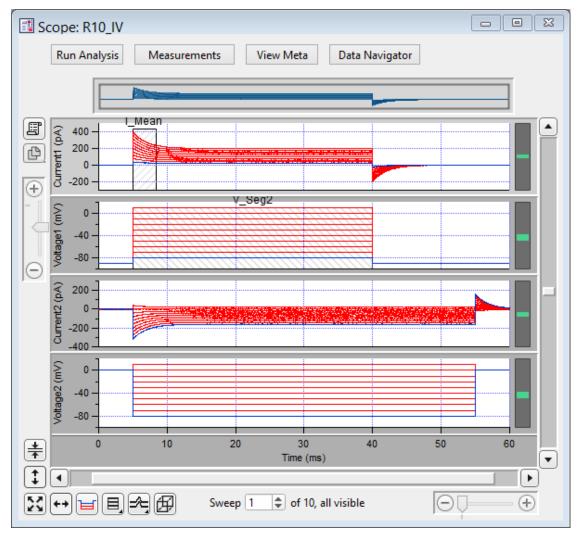
Open this window from the Data Navigator by highlighting a Routine, and selecting the 'View Routine' command from a right-click menu or the 'Available actions' button, or by selecting the Routine Data Name in the Data Navigator Preview pane.

**Routine Name** 

Displays the Routine name.

Activate	Opens the Scope window loaded with these settings.
Copy to Routine Pool	Adds this routine to the loaded Routine Pool.
Routine Description	Displays the Routine description.
Display Signal	Select the output signal to display.

# 4.2.15 Scope (Analysis)



This analysis version of the Scope window is used to display and reanalyze stored data.

Figure 4-69. Scope (Reanalysis) Window

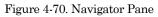
Both physical and virtual channels can be displayed here. Measurement regions are used as described for the Scope (acquisition) window.

Additional window controls are described below:

Navigation pane

The Navigator pane appears at the top of the Scope window. It displays an overview of the active signal's full-scale data, with a gray box surrounding the magnification area.





The Navigator pane "magnification" box can be used to scroll through the active signal's data. Place the mouse cursor over the magnification box and it changes into a 'hand' icon; click and drag the magnification box to scroll through the data.

Run Analysis	Run the defined analysis for the data series, and graph the results in the Analysis window. If this button is grayed out, edit the Meas- urements to activate analysis. Note: To stop a long-running analysis, click on the 'Abort' button in the bottom right corner of the main screen.
	Show Cursors: Display measurement cursors in the Scope window.
Measurements	Hide Cursors: Do not display cursors in the Scope window.
	Button state displays as "Measurements(H)".
	Lock Cursors: Prevent cursors from being moved or altered.
	Button state displays as "Measurements(L)".
	<ul> <li>Edit Measurements:</li> <li>Open a special Reanalysis Measurements &amp; Graphs dialog, where all changes apply instantly and interactively to the measurements and graphs, even during analysis. These edits (temporarily) override the loaded routine for fast response.</li> <li>Edit Virtual Signals:</li> <li>Open the virtual input signals panel for editing.</li> </ul>
	Single Channel Threshold Analysis: Configure single-channel analysis of ion channels.
View Meta	Display any extra information (metadata) associated with the dis- played data Series, such as the operator, preparation details, solu- tion information, etc.
Data Navigator	Open a Data Navigator window with all of your Experiment data and metadata available for analysis in a tree structure.
Layout	Copy and paste into a new or append to an existing Layout window all Scope signals and Analysis graphs.

Copy to Clipboard	Copy the Scope window to the system clipboard.		
Center	Center the mean of the data in the selected signal pane. The Y-axis offset is automatically adjusted, while the Y-axis scaling is unchanged.		
Autoscale	Autoscale all visible signals. Rescale the Y-axes to their displayed sweeps data limits, and the X-axes to their full sweep duration.		
1	Click to autoscale the Y-axis of the selected signal.		
<b>+</b> +	Click to full-scale all X-axes.		
Persistence Display	Display all sweeps (per Preferences settings). Otherwise when disabled, only one sweep is displayed at a time.		
Signal Display	Graphically arrange the input signals. Stack: A vertical column of signals. Single: Only the active signal. m x n: A tiled array of signals with 'm' rows and 'n' columns.		
Sweeps Display	This button has 3 modes:		
	Sweeps	Each trace starts from time zero to the dura- tion of the waveform.	
	Time Course	<ul> <li>Display sweeps in time sequence on a single time axis. Portions without data are left blank (such as the time between triggered sweeps.)</li> <li>Note: Emulation mode has a minimum 0.5 s interval between sweeps, both triggered and continuous. If the sweep duration is less than 0.5 s, the time between sweeps will be padded with "blank" time,</li> </ul>	
	Concatenated	Display sweeps similarly to the Time Course mode, but any blank portions 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 Sweep data are color-coded for amplitude, and their 3D graph can be rotated in any direction.
Sweep#:	The 'Sweep #' display at the bottom of the Scope window indicates the 'active sweep' number, the total number of sweeps in the Series, and either "all" or the total number of visible sweeps (per Prefer- ences).

Table 4-6. Scope Window Buttons

X- and Y-Axes	Same as the acquisition Scope <u>.</u>	
Main window	<ul><li>Additional items:</li><li>3D View</li><li>Zero Baselines</li></ul>	
	<ul><li>Show All Sweeps</li><li>Select Sweeps</li></ul>	(with triggered sweeps) (with triggered sweeps)
Data	<ul><li>Additional items:</li><li>Hide Sweep_#</li><li>Show Sweep_# Only</li></ul>	(with triggered sweeps) (with triggered sweeps)

### **Channel Timing Delays**

The dPatch amplifier uses analog output and input channels that pass signals through two filters, fixed DAC output stimulus filters and variable-bandwidth ADC input filters. These filters impose a time delay (also called a phase delay) on the physical input and output channels, which is reflected in the recorded response signals.

However, when stimulus signals are recorded (such as 'Voltage1' in voltage-clamp mode), their output and input channels are directly processed as digital streams. Thus, there are no analog filter delays in recorded stimulus signals.

This means that recorded response signals are time-delayed compared to recorded stimulus signals.

Therefore, when analyzing data that requires precise timing between stimulus and response signals, these timing delays should be taken into account:

Typical dPatch Filter Delays

<u>Input Bandwidth</u>	<u>Total Filter Delay</u>
$1000 \mathrm{kHz}$	$16.2 \ \mu s$

$500 \mathrm{kHz}$	$17 \ \mu s$
$250\mathrm{kHz}$	$18.5~\mu s$
$10\mathrm{kHz}$	$85~\mu s$

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

### 4.2.16 3D View Window

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

The axis definition in 3D View is based on the change of a waveform over the course of successive sweeps. In a two-dimensional display, the X-axis represents the Sweep Time, while the Amplitude is plotted on the vertical Y-axis. For consistency, the vertical axis in the SutterPatch 3D view is also defined as the Y-axis. In the default orientation of the 3D View, the Z axis, on which the Sweep Number is plotted, points backward and to the right.

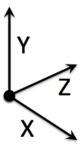


Figure 4-71. 3D Axes Definition

248

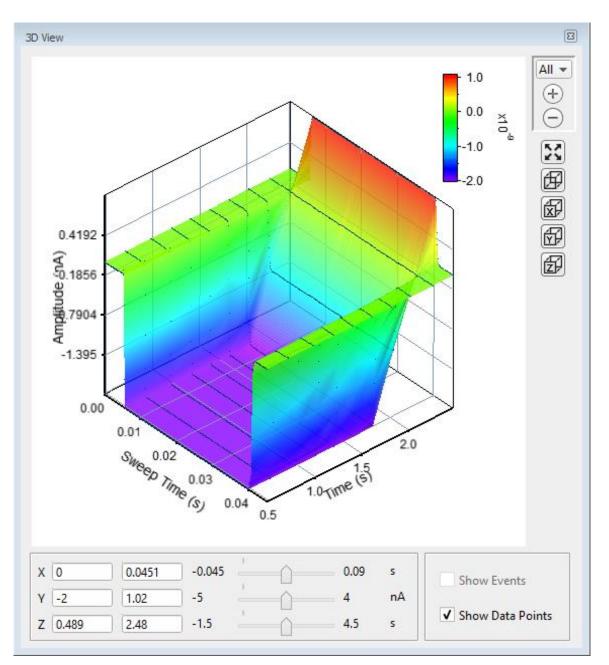


Figure 4-72. 3D View

A "heat map" bar illustrates the color measurement units.

Magnification buttons are located in the upper right corner of the window for the selected axis:

- All (All 3 axes)
- X (Sweep Time)
- Y (Amplitude)
- Z (Time)
  - Zoom in

(+)

(Magnify)

Autoscale

X, Y and Z axis limits can be set in the bottom section of the 3D View window. Their delta value is preserved when using the scroll bars to update the visual graph (and the numeric axes limits.)

(Set to the data limits)

The 3D graph viewing angle can be changed with a set of 3D buttons:

Ø	= Default View	(X, Y & Z axes display)
	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 while dragging, the 3D display will rotate in the direction of the mouse drag.

Show Events	Display tagged events in the 3D graph.
Show Data Points	Display data points as surface dots in the 3D graph.

# 4.2.17 Set Metadata

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

Predefine the Metadata parameter values here.

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

### Metadata Summary dialog

All parameters with defined values are displayed in the Summary, even from unselected 'Metadata detail levels'. Double-clicking on any line opens the Set Metadata dialog with the respective parameter selected.

Parameter value.

Metadata Parameter Parameter name.

Current Value

249

Increment Enabled	If enabled, double-click to review details.
Prompt before	Display metadata prompts before running:
<ul><li>Expt</li><li>Pdgm</li><li>Routn</li></ul>	Experiment Paradigm Routine
Metadata Group	(Per the Set Preferences > Metadata detail level)
1. BASIC Level	Default metadata groups:
	Preparation – Animal Preparation – Tissue Preparation – Cell Experiment Stimulus
2. EXTENDED Level	Additional metadata groups provided:
	Electrode Recording Solution
3. FULL Level	Even more metadata groups included:
	Operator Paradigm Cell Health / Quality Control Series (= Routine Data)
Metadata Parameter	The available metadata parameters are displayed by detail level, as set in SutterPatch > Set Preferences > Metadata.
[Parameter description]	
Configuration choices for th	e selected parameter:
• Do not write this pa	rameter This parameter is not stored.
	If this parameter was previously written in this Experiment, then its Previous Value is displayed.
• Use last value	The parameter used in the previous acquisition is written.
• Use a previous value	Select from a drop-down list of the previous 20 metadata values used for acquisition in the experiment.

- Use new value Enter a new value for the metadata parameter.
- Increment

Numerically increment the value.

By

•

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- Experiment At the start of each Experiment.
- Paradigm At the start of each Paradigm.
- Routine At the start of each Routine.

Prefix	Enter text to be prepended to the value.
Start value	The initial value (including decimals and negative numbers).
Increment:	Select an arithmetic operator [ +, -, *, / ].
[]	Enter the incremental amount.
Suffix	Enter text to be appended to the value.

Prompt for confirmation before:

• Experiment	At the start of an Experiment.	
• Paradigm	For "planned" or named Paradigms (i.e., not auto-triggered by Routines).	
Routine	At the start of a Routine.	
	This dialog displays when an Experiment, Paradigm or Routine is started while enabled for prompts.	
Write	Write the selected metadata parameter with the Experiment, Paradigm or Routine.	
Metadata Parameter	The selected metadata parameter.	
Next Value	The metadata value to write.	
Update	Store edited metadata values for future Experiments, Paradigms or Routines. This field is automatically disabled after each execution.	
Prompt	Include this metadata parameter in the Confirm Metadata Settings dialog. If no metadata parameters are enabled for Prompt, the Confirm Metadata Settings dialog will not display.	

BASIC	EXTENDED	FULL	GROUP / Parameters	NOTES
		F	OPERATOR /	
		F	Full Operator Name	
В	E	F	PREPARATION - ANIMAL /	
В	E	F	Animal Identifier	
В	E	F	Animal Species	Binomial species name
	E	F	Animal Strain	Strain, breed or variety characterizing the animal
	E	F	Animal Genotype	
В	E	F	Animal Age	
В	E	F	Animal Age Units	Ex.: h, d, m
В	E	F	Animal Sex / Gender	Ex.: 1: F, 2: M, 3: Unde- termined
В	E	F	Animal Weight	
В	E	F	Animal Weight Units	
	E	F	Animal Preparation Date	ISO Date, Format: YYYY- MM-DD
	E	F	Animal Preparation Time	Time of Day, Format: hh:mm[:ss.000]
	E	F	Animal User Parameter 1 Name	
	E	F	Animal User Parameter 1	
	E	F	Animal User Parameter 2 Name	
	E	F	Animal User Parameter 2	
	E	F	Animal User Parameter 3 Name	
	E	F	Animal User Parameter 3	
	E	F	Animal User Parameter 4 Name	
	E	F	Animal User Parameter 4	
	E	F	Animal User Parameter 5 Name	
	E	F	Animal User Parameter 5	
В	E	F	PREPARATION - TISSUE /	
В	E	F	Tissue Preparation Identifier	
В	Е	F	Organ	
	E	F	Organ Region	
	E	F	Preparation Method	
	E	F	Tissue Preparation Date	ISO Date, Format: YYY- MM-DD
	E	F	Tissue Preparation Time	Time of Day, Format: hh:mm[:ss.000]
	E	F	Tissue Incubation Duration	
	E	F	Tissue Incubation Duration Units	
	E	F	Tissue Incubation Temperature	
	E	F	Tissue Incubation Temperature Units	
	E	F	Tissue Incubation Solution	
	E	F	Tissue User Parameter 1 Name	
	E	F	Tissue User Parameter 1	
	E	F	Tissue User Parameter 2 Name	
	E	F	Tissue User Parameter 2	
	E	F	Tissue User Parameter 3 Name	

	F	-	Tions Hon Donomotor 2	
	E	F	Tissue User Parameter 3	
	E	F	Tissue User Parameter 4 Name Tissue User Parameter 4	
	E	F		
	E	F	Fissue User Parameter 5 Name Fissue User Parameter 5	
	E	F		
В	E	F	PREPARATION - CELL /	
В	E	F	Cell Preparation Identifier	
	E	F	Acutely Dissociated Cells	
	E	F	Cell Line	
	E	F	Slice Preparation	
	E	F	Whole-organ Preparation	
	E	F	In-situ Recording	
	E	F	Stem Cell Preparation	
	E	F	User-defined Preparation	
В	E	F	Cell Type	
В	E	F	Cell Identifier	
В	E	F	Cell Preparation Date	ISO Date, Format: YYY- MM-DD
В	E	F	Cell Preparation Time	Time of Day, Format: hh:mm[:ss.000]
	Е	F	Cell Dissociation Solution	
	E	F	Cell Preparation Dissociation Temperature	
	E	F	Cell Prep. Dissociation Temperature Units	
В	Е	F	Cell Preparation Incubation Duration	
В	Е	F	Cell Prep. Incubation Duration Units	
В	E	F	Cell Preparation Incubation Temperature	
В	Е	F	Cell Prep. Incubation Temperature Units	
В	E	F	Cell Preparation Incubation Solution	
В	Е	F	Ion Channel	
	Е	F	Cell Fluorescent Marker	
	E	F	Cell Diameter	
	E	F	Cell User Parameter 1 Name	
	E	F	Cell User Parameter 1	
	E	F	Cell User Parameter 2 Name	
	E	F	Cell User Parameter 2	
	E	F	Cell User Parameter 3 Name	
	E	F	Cell User Parameter 3	
	E	F	Cell User Parameter 4 Name	
	E	F	Cell User Parameter 4	
	E	F	Cell User Parameter 5 Name	
	E	F	Cell User Parameter 5	
В	E	F	EXPERIMENT /	
		F	Experiment Category 1 Name	
		F	Experiment Category 1	

		F	Experiment Category 2 Name		
		F	Experiment Category 2		
		F	Experiment Category 2 Experiment Category 3 Name		
		F	Experiment Category 3		
		F	Experiment Category 5 Experiment Category 4 Name		
		•	Experiment Category 4 Name		
		F	Experiment Category 4 Experiment Category 5 Name		
		F	Experiment Category 5 Name		
В	F		Experiment User Parameter 1 Name		
	E	F			
B	E	F	Experiment User Parameter 1		
B	E	F	Experiment User Parameter 2 Name		
B	E	F	Experiment User Parameter 2		
B	E	F	Experiment User Parameter 3 Name		
B	E	F	Experiment User Parameter 3		
В	E	F	Experiment User Parameter 4 Name		
В	E	F	Experiment User Parameter 4		
В	E	F	Experiment User Parameter 5 Name		
В	E	F	Experiment User Parameter 5		
		F	experiment Category Parameter 1 Name		
		F	Experiment Category Parameter 1		
		F	Experiment Category Parameter 2 Name		
		F	Experiment Category Parameter 2		
		F	Experiment Category Parameter 3 Name		
		F	Experiment Category Parameter 3		
		F	Experiment Category Parameter 4 Name		
		F	Experiment Category Parameter 4		
		F	xperiment Category Parameter 5 Name		
		F	Experiment Category Parameter 5		
	E	F	ELECTRODE /	LECTRODE /	
	E	F	Electrode Identifier		
	E	F	Electrode Glass Manufacturer		
	E	F	Electrode Glass Item Number		
		F	Electrode Glass Lot Number		
		F	Electrode Glass Material		
		F	Electrode Glass Item Outer Diameter		
		F	Electrode Glass Item Inner Diameter		
		F	Filamented Glass		
	E	F	Electrode Glass Ramp Test Value		
	E	F	Pipette Puller Manufacturer		
	E	F	Pipette Puller Model		
		F	Pipette Puller Serial Number		
		F	Puller Filament Type		
		F	Puller Filament Item Number		

	-	Dull Dromon Number	
	F	Pull Program Number	
	F	Pull Program Parameters	
	F	Pull Program Air Mode	
	F	Pull Program Air Pressure	
	F	Puller Heat-on Enabled	
	F	Puller Heat-on Time	
	F	Electrode Fire-polished	
	F	Electrode Coated	
	F	Electrode Coating Material	
	F	Electrode Beveled	
	F	Clectrode Bevel Angle	
E	F	Electrode User Parameter 1 Name	
E	F	Electrode User Parameter 1	
E	F	Electrode User Parameter 2 Name	
E	F	Electrode User Parameter 2	
E	F	Electrode User Parameter 3 Name	
E	F	Electrode User Parameter 3	
E	F	lectrode User Parameter 4 Name	
E	F	lectrode User Parameter 4	
E	F	ectrode User Parameter 5 Name	
E	F	ectrode User Parameter 5	
E	F	RECORDING SOLUTIONS	
E	F	Solution Pair Identifier	
E	F	Solution Pair Name	
E	F	ath Solution Identifier	
E	F	ath Solution Name	
	F	ath Solution Batch	
	F	ath Solution Composition	
	F	Bath Solution Preparation Date	
	F	Bath Solution Preparation Time	
	F	Bath Solution pH	
	F	Bath Solution pH Adjustment Agent	
	F	Bath Solution Osmolarity	
	F	Bath Solution Osmolarity Adj. Agent	
E	F	Pipette Solution Identifier	
E	F	Pipette Solution Name	
	F	Pipette Solution Batch	
	F	Pipette Solution Composition	
	F	Pipette Solution Preparation Date	
	F	Pipette Solution Preparation Time	
E	F	Pipette Solution pH	
	F	Pipette Solution pH Adjustment Agent	
E	F	Pipette Solution Osmolarity	

	-	D' a state Called's a Os seally state All' A seast	
	F	Pipette Solution Osmolarity Adj. Agent	
	F	Liquid Junction Potential, computed	
	F	Liquid Junction Potential, measured	
	F	Solution User Parameter 1 Name	
	F	Solution User Parameter 1	
	F	Solution User Parameter 2 Name	
	F	Solution User Parameter 2	
	F	Solution User Parameter 3 Name	
	F	Solution User Parameter 3	
	F	Solution User Parameter 4 Name	
	F	Solution User Parameter 4	
	F	Solution User Parameter 5 Name	
	F	Solution User Parameter 5	
	F	PARADIGM /	
	F	Bath Temperature	
	F	Bath Temperature Units	
	F	Ambient Temperature	
	F	mbient Temperature Units	
	F	Atmospheric Composition	
	F	Atmospheric Pressure	
	F	Atmospheric Pressure Units	
	F	Atmospheric Humidity	
	F	Paradigm User Comment	
	F	Paradigm User Parameter 1 Name	
	F	Paradigm User Parameter 1	
	F	Paradigm User Parameter 2 Name	
	F	Paradigm User Parameter 2	
	F	Paradigm User Parameter 3 Name	
	F	Paradigm User Parameter 3	
	F	Paradigm User Parameter 4 Name	
	F	Paradigm User Parameter 4	
	F	Paradigm User Parameter 5 Name	
	F	Paradigm User Parameter 5	
	F	CELL HEALTH / QUALITY CONTROL /	
	F	Cell Health User Parameter 1 Name	
	F	Cell Health User Parameter 1	
	F	Cell Health User Parameter 2 Name	
	F	Cell Health User Parameter 2	
	F	Cell Health User Parameter 3 Name	
	F	Cell Health User Parameter 3	
	F	Cell Health User Parameter 4 Name	
	F	Cell Health User Parameter 4	
	F	Cell Health User Parameter 5 Name	
	F	Cell Health User Parameter 5 Name	

		F	Cell Health User Parameter 5	
В	E	F	STIMULUS /	
	E	F	Key Stimulus	
	E	F	Stimulus Duration	
	E	F	Compound Group	
	E	F	Compound Group Index	
В	E	F	ompound Identifier	
В	E	F	Compound Name	
В	Е	F	Compound Concentration	
В	Е	F	Compound Concentration Units	
	E	F	Compound Batch	
	E	F	Compound Lot	
	E	F	Compound Salt Code	
	E	F	Compound Solution	
	E	F	Compound Vehicle / Solubility Enhancer	
	E	F	Compound Vehicle Concentration	
	E	F	Compound Vehicle Concentration Units	
	E	F	Compound Reservoir Identifier	
	Е	F	Application Tip Identifier	
	Е	F	Compound Plate Identifier	
	Е	F	Compound Plate Row	
	Е	F	Compound Plate Column	
	E	F	Chem. Stimulus User Parameter 1 Name	
	E	F	Chem. Stimulus User Parameter 1	
	E	F	Chem. Stimulus User Parameter 2 Name	
	E	F	Chem. Stimulus User Parameter 2	
	E	F	Chem. Stimulus User Parameter 3 Name	
	E	F	Chem. Stimulus User Parameter 3	
	E	F	Chem. Stimulus User Parameter 4 Name	
	E	F	Chem. Stimulus User Parameter 4	
	E	F	Chem. Stimulus User Parameter 5 Name	
	E	F	Chem. Stimulus User Parameter 5	
В	E	F	Light Stimulus Wavelength	
В	E	F	Light Stimulus Intensity	
В	E	F	Light Stimulus Intensity Units	
	E	F	Light Stimulus User Parameter 1 Name	
	E	F	Light Stimulus User Parameter 1	
	E	F	Light Stimulus User Parameter 2 Name	
	E	F	Light Stimulus User Parameter 2	
	E	F	Light Stimulus User Parameter 3 Name	
	E	F	Light Stimulus User Parameter 3	
	E	F	Light Stimulus User Parameter 4 Name	
	E	F	Light Stimulus User Parameter 4	

	E	F	Light Stimulus User Parameter 5 Name		
	E	F	Light Stimulus User Parameter 5 Name Light Stimulus User Parameter 5		
В	E	F	Mechanical Stimulus Intensity		
B	E	F	Mechanical Stimulus Intensity Mechanical Stimulus Intensity Units		
D		Г	Mechanical Stimulus User Parameter 1		
	E	F	Name		
	E	F	Mechanical Stimulus User Parameter 1		
	_	_	Mechanical Stimulus User Parameter 2		
	E	F	Name		
	E	F	Mechanical Stimulus User Parameter 2         Mechanical Stimulus User Parameter 3		
	E	F	Jachanical Stimulus User Parameter 3 Jame		
	E	F	Mechanical Stimulus User Parameter 3		
	_	_	Mechanical Stimulus User Parameter 4		
	E	F	Name		
	E	F	Mechanical Stimulus User Parameter 4 Mechanical Stimulus User Parameter 5		
	E	F	Name		
	E	F	Mechanical Stimulus User Parameter 5		
В	Е	F	Acoustic Stimulus Frequency		
В	Е	F	Acoustic Stimulus Intensity		
В	Е	F	Acoustic Stimulus Intensity Units		
	Е	F	Acoust. Stimulus User Parameter 1 Name		
-	E	F	Acoust. Stimulus User Parameter 1		
	E	F	Acoust. Stimulus User Parameter 2 Name		
	E	F	Acoust. Stimulus User Parameter 2		
	E	F	Acoust. Stimulus User Parameter 3 Name		
	E	F	Acoust. Stimulus User Parameter 3		
	E	F	Acoust. Stimulus User Parameter 4 Name		
	E	F	Acoust. Stimulus User Parameter 4		
	E	F	Acoust. Stimulus User Parameter 5 Name		
	E	F	Acoust. Stimulus User Parameter 5		
В	E	F	Thermal Stimulus Temperature		
В	E	F	Thermal Stimulus Temperature Units	°C, °F or K	
	E	F	Thermal Stimulus User Parameter 1 Name		
	E	F	Thermal Stimulus User Parameter 1		
	E	F	Thermal Stimulus User Parameter 2 Name		
	E	F	Thermal Stimulus User Parameter 2		
	E	F	Thermal Stimulus User Parameter 3 Name		
	E	F	Thermal Stimulus User Parameter 3		
	E	F	Thermal Stimulus User Parameter 4 Name		
	E	F	Thermal Stimulus User Parameter 4		
	E	F	Thermal Stimulus User Parameter 5 Name		
	E	F	Thermal Stimulus User Parameter 5		
В	E	F	Electrical Stimulus Frequency	The frequency of an exter- nal electrical stimulus	

В	E	F	Electrical Stimulus Intensity	The intensity of an exter- nal electrical stimulus
В	E	F	Electrical Stimulus Intensity Units	The intensity units of an external electrical stimu- lus
	E	F	Electrical Stimulus User Parameter 1 Name	
	E	F	Electrical Stimulus User Parameter 1	
	E	F	Electrical Stimulus User Parameter 2 Name	
	E	F	Electrical Stimulus User Parameter 2	
	E	F	Electrical Stimulus User Parameter 3 Name	
	E	F	Electrical Stimulus User Parameter 3	
	E	F	Electrical Stimulus User Parameter 4 Name	
	E	F	Clectrical Stimulus User Parameter 4	
	E	F	Electrical Stimulus User Parameter 5 Name	
	E	F	Electrical Stimulus User Parameter 5	
	E	F	Other Stimulus User Parameter 1 Name	
	E	F	ther Stimulus User Parameter 1	
	E	F	Other Stimulus User Parameter 2 Name	
	E	F	Other Stimulus User Parameter 2	
	E	F	Other Stimulus User Parameter 3 Name	
	E	F	Other Stimulus User Parameter 3	
	E	F	Other Stimulus User Parameter 4 Name	
	E	F	Other Stimulus User Parameter 4	
	E	F	Other Stimulus User Parameter 5 Name	
	E	F	Other Stimulus User Parameter 5	
		F	SERIES (= ROUTINE DATA) /	
		F	Routine User Comment	

Table 4-7. Metadata Parameters

# 4.2.18 Single Channel Analysis

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

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

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

260

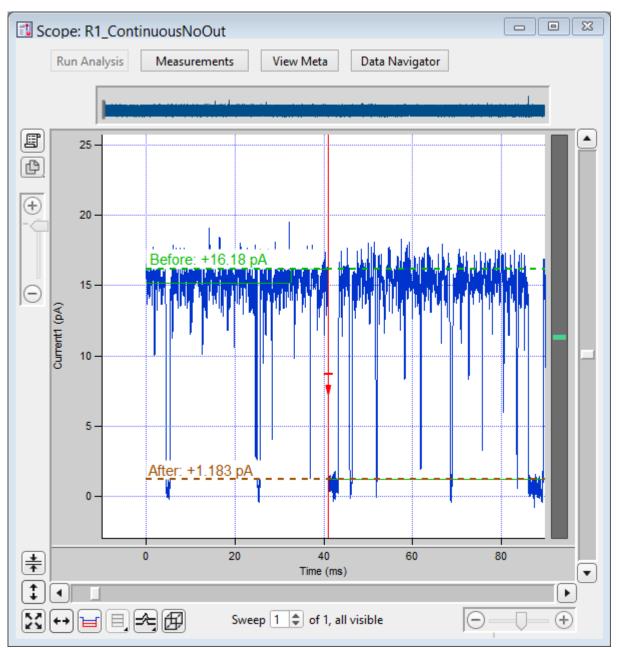


Figure 4-73. Single-Channel Scope

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

- Use individual sweeps
- Use complete signal

Perform analysis on a single sweep at a time. Perform analysis on the entire active signal.

[Descriptive information]

# Single Channel Analysis Preferences

Dock to Scope Baseline Average Duration = x.x ms Mean Amplitude Duration = x.x ms Cell Separator: Tab Cell Separator: Comma Invalid values: Use NaN Invalid values: Use empty string Digits in table entries: Digits = 3, 4, 5, 7, 9, 11, 13, 15

Show All Signals

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

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

Show/Hide Current Amplitude Histogram Controls

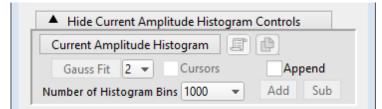


Figure 4-74. Current Amplitude Histogram Controls

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

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

Current Amplitude Histogram Click button to create a histogram plot.

Copy to Layout Page	Copy/append the current amplitude histogram to a Lay- out page.
Copy to Clipboard	Copy the current amplitude histogram to the clipboard.
Gauss Fit	Click button to perform a Gaussian fit on the histogram. SutterPatch will automatically find and fit up to the three

	largest peaks; adjust the fitting ranges as needed with manual cursors.
	To fit additional smaller distributions, reposition the cur- sors and click the Append button.
	Multi-peak amplitude (open level) differences and P(open) and P(closed) values are displayed in the graph. See the Table of Results for the fitting components.
[1,2,3]	Select the number of peaks (levels) to fit.
Cursors	Enable to display fitting cursors in the histogram plot. To reposition a cursor, drag its cursor symbol (labeled "A", "B", etc.) from the cursor bar onto the graph to the desired position.
Append	Modify the Current Amplitude Histogram.
Add	Add bins to the Current Amplitude Histogram.
Sub	Subtract bins from the Current Amplitude Histo- gram.
Number of Histogram Bins	[ 4000, 2000, 1000, 500, 200, 100, 50 ]

## Show/Hide Transition Controls

<ul> <li>Hide Transitions Controls</li> </ul>				
Amplitude -31.5 pA 🗢 Threshold 60 % 🗢				
Start Level 0				
Min. Duration 200.0 µs 🖨 🛛 Track Baseline				
Track Limit 1.1 pA 🗣 Measure Rms				
Properties of selected transition				
Transition 0 🗢 Time 0.000 s Level 0				
Accept Reject Suppress				
Move to target transition				
Move to target transition				
Move to target transition       Image: Constraint of the state of the				

Figure 4-75. Transition Controls

Amplitude

The expected transition size for the ion-channel opening. For upwards-opening (positive) channels, use an unsigned value. For downwards-

		263
		opening (negative) channels, use a negatively- signed value.
Threshold		[ 50-90% ]
		The percentile of the Amplitude value that needs to be reached by the data to trigger finding a tran- sition.
Start Level		The starting Closed or Open state of the ion- channel data under study. Closed = 0, Open = 1+.
	Note:	All open states should have a positive level num- ber. If transition levels are incorrectly reported as negatively signed, double-check the defined Am- plitude value and its sign.
Track Amplitude		Automatically adjust the expected transition Am- plitude based on the prior data.
Min. Duration		The minimum duration that the data needs to achieve at or above the Threshold level.
Track Baseline		Automatically adjust the baseline amplitude (Lev- el 0) based on the prior data.
Track Limit		The maximum amount that the Amplitude or Baseline measurements can change while being automatically tracked.
Measure RMS		To measure the Root-Mean-Square noise in the signal, adjust the signal trace in the Scope window such that it shows a stretch of current without any event activity (i.e., all channels are closed), then click on the 'Measure RMS' button.
		The RMS value is displayed at the bottom of the dialog, and a 3 x RMS value populates the Track Limit field.
Properties of selected transition		
Transition		Transition (event) number.
Time		Read-only field of time of transition start.
Level		Read-only field of open-channel level.
• Accept		
• Reject		
• Suppress		

Move to target transition	
<< First	Move to the first found event.
< Left	Move to the prior found event.
Right >	Move to the next found event.
Last >>	Move to the last found event.
Find target transition	
found	The number of found events.
Clear All	Reset the number of found events to zero and move to the first found event.
Right >	Find and move to the next event.
Last >>	Find all subsequent events and move to the last event.

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

## Show/Hide Plots and Tables Controls

<ul> <li>Hide Plots and Tables Controls</li> </ul>
Duration Distribution Plot
Exp Fit log(x) sqrt(y) Open 💌
Amplitude Distribution Plot
Gauss Fit 1 💌 Cursors
Apply Limits
Duration: Min 0.0 s 🗢 Max. 1.0 s 🗢
Amplitude: Min -1.0 A 🔷 Max. 1.0 A 🌲
Number of Histogram Bins 200 👻
Scatter Plot 📳 🕒 Both 💌
Apply Limits
Duration: Min 0.0 s 🗢 Max. 1.0 s 🗢
Amplitude: Min -1.0 A 🗘 Max. 1.0 A 🗘
Amplitude: Min     -1.0 A     Max.     1.0 A       Table of Transitions     Image: Clear
Table of Transitions     Image: Clear

Figure 4-76. Plots and Tables Controls

Duration Distribution Plot	Create a dwell-time histogram plot.
	Create a dweil-time instogram plot.
Copy to Layout	Copy/append the Duration Distribution Plot to a Layout page.
Copy to Clipboard	Copy the Duration Distribution Plot to the Clipboard.
Exp Fit	
$\log(\mathbf{x})$	
sqrt(y)	
Open / Closed	
Amplitude Distribution Plot:	
Gauss Fit [1, 2,	3]
Cursors	
Apply Limits	
Duration: Min [ 0.0 –	- 1.0 s ]
Max. [ 0.0 –	- 1.0 s ]
Amplitude: Min [-1.0-	– 1.0 A ]
Max. [-1.0-	– 1.0 A ]
Number of Histogram Bins [1000	), 500, 200, 100, 50, 20 ]
Scatter Plot	
Copy to Layout	Copy/append the Scatter Plot to a Layout page.
Copy to Clipboard	Copy the Scatter Plot to the Clipboard.
Open / Closed / Both	copy the Souther 1 ist to the Christian
Table of Transitions	
Copy to Layout	Copy/append the Table of Transitions to a Layout page.
Copy to Clipboard	Copy the Table of Transitions to the Clipboard.
Clear	

Table of Results

(I) Copy to Layout

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

Copy to Clipboard Clear

Table Column Labels

# **Gaussian Fit**

<u>Header</u>	Label
Source	Sweep_#
Analysis	Current Amplitude Histogram Fit /
	Amplitude Distribution Fit
Label1 / Value1	Amplitude
Label2 / Value2	p(closed)
Label3 / Value3	p(open)
Label4 / Value4	Gauss_y0_1
Label5 / Value5	Gauss_A_1
Label6 / Value6	Gauss_x0_1
Label7 / Value7	Gauss_width_1
Label8 / Value8	Success_1
Label9 / Value9	Gauss_y0_2
Label10 / Value10	Gauss_A_2
Label11 / Value11	Gauss_x0_2
Label12 / Value12	Gauss_width_2
Label13 / Value13	Success_2
Label14 / Value14	Gauss_y0_3
Label15 / Value15	Gauss_A_3
Label16 / Value16	Gauss_x0_3
Label17 / Value17	Gauss_width_3
Label18 / Value18	Success_3

## Linear Exponential Fit

<u>Header</u>	Label
Source	Sweep_#
Analysis	Duration Distribution Fit
Label1 / Value1	Exp_y0 / #
Label2 / Value2	Exp_A/#

Label3 / Value3	Exp_Tau / #
Label4 / Value4	Exp_0/#
Label5 / Value5	Success/#

### Logarithmic Exponential Fit

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

## 4.3 General

SutterPatch general operations.

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

## 4.3.1 Command Window

The Command window is labeled with the currently loaded Experiment filename. A history of commands and responses displays in the upper portion of the window. Some warning messages also display here.

Experiment	
0	^
	~
	0

Figure 4-77. Command Window

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

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

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

The Command window has a resizing line between the upper history section and the lower command section – the mouse cursor will change to a double-headed arrow.

For more information, see Section II-2 of the Igor Pro manual.

## 4.3.2 Dashboard Panel

The Dashboard panel provides a convenient gateway to key areas of the SutterPatch program.

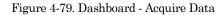
Dashboar	d					
Acquire	Data	Analyze Data	Viet	w Last	Set Metadata	New Experiment
		]	Figure 4-7	8. Dashboa	ard	
0	٥	Preferences				
		Icon Size		Large Small		
		Icon Orienta	ation	Vertica Horizo		
0	Acq	uire Data				on configuration.
0	Ana	lyze Data		-	_	its window is oper he Data Navigator

#### 268

- View Last Data Open the Experiment's last recorded data Series.
  - Set Metadata Configure metadata settings and values.
- New Experiment Start a new Experiment, and/or switch the amplifier model or emulation mode.

Clicking the Acquire Data icon opens an adjoining secondary pane:





0	Control Panel	Hardware control via the Amplifier Control Panel.
0	Membrane Test	Monitor seal formation and cell health.
		Button stays depressed while its window is open.
0	Free Run	Run an oscilloscope-style signal monitor.
		Button stays depressed while its window is open.
0	Routines	Configure Routine acquisition settings.
0	Paradigms	Control the execution of commands.

#### 4.3.3 File Import/Export

#### Experiments & Data

0

There are two formats for saving an experiment. The packed format is recommended for most purposes.

• Packed experiment: (*.pxp file)

A SutterPatch Experiment is saved by default as a "packed" (Igor Pro) experiment, which includes all data, analyses, graphs, routines, paradigms, etc., in one file.

• Unpacked experiment: (*.uxp file, experiment folder)

A SutterPatch Experiment can also be saved as an "unpacked" (Igor Pro) experiment, which saves all waves, procedure windows, and notebooks as individual files in an experiment or "home" Folder, along with an instruction (*.uxp) file.to recreate the experiment.

The advantage of an unpacked experiment is:

• Much faster saving of experiments that include very large numbers of waves (thousands or more), as existing data waves are not resaved to the experiment with each new recording.

The disadvantages of an unpacked experiment are:

- Much more disk space is used, especially for experiments that have a lot of small waves.
- The UXP format is more "fragile", as you need to keep the experiment file and its corresponding folder together when you copy or move the experiment.

Saved experiments can be re-opened into the current experiment via the Data Navigator 'Import' button.

Note: If a SutterPatch Experiment file is opened into Igor Pro without SutterPatch running, its graphs will not be visible – display them with the Igor Pro 'Windows / Graphs' or 'Windows / Layouts' menu items.

#### Axon Files

Data can be exported to the pCLAMP ABF or ATF file formats via the Data Navigator 'Available Actions' button (or right-click menu).

#### HDF5 Files

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

#### **HEKA Files**

PatchMaster Pulse Generator Files (*.pgf) can be opened in the Routine Editor 'Pools and Files' section and their Sequences merged with the current routine pool.

#### Igor Files

Igor Pro Binary Wave files (*.ibw) can be imported or exported.

#### Template Files

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

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

#### Graphs and Layouts

Import or export graphs for the current experiment via the Analysis Editor / Files options. Graph files are stored as Igor Binary Wave (*.ibw) files.

Note: Graph data for each axis can also be saved as Igor Pro 6 one-dimensional wave files, however files using this older format cannot be re-imported back into SutterPatch.

Graphs can be exported to a Layout window via:

Analysis Editor:	[ To Layout ] button
Scope window:	"Export a layout" button 💷
Paradigms:	Export step

Graphs exported to the Layout window use the Preferences / Export Graphics settings.

Individual graphs can be saved as Graph Macros - recall them via the Windows / Graph Macros menu.

Layout windows can be saved as Layout Macros - recall them via the menu Windows / Layout Macros menu.

# 4.3.4 Log Window

The Log window collects time-stamped commands, responses, administrative information and error messages in a structured format. These entries provide a history of the steps having a possible influence on the execution of the experiment and its data.

When SutterPatch starts up, the Log window displays the SutterPatch Version and Build numbers.

Columns

Date & Time	Day name, month name, day date, year date, time: hours:minutes:seconds, AM/PM		
Event Type	Log entries are assigned an Event Type:		
	Command	Command window execution.	
	Comment		
	Data Acquisition	Scope acquisition operations.	
	Data Management		
	Exit		
	History		
	Metadata		
	Paradigm		
	Routine	Routine Pool operations.	
	Startup	Startup / loading information.	
	Unknown		

Event Description A text description of the log entry.

## 4.3.5 Menus

The SutterPatch main menu item contains all of the SutterPatch-specific menu items. The rest of the main menu items provide the standard Igor Pro functionality. For documentation of the non-SutterPatch features, refer to the Igor Pro online help or manual.

Window/Dialog Controls

Keyboard "Return" key	=	'OK/Yes' buttons
Keyboard ESC key	=	'Cancel' button

### Menu Items

File

New Experiment	Unload the current Experiment and start a new Experi- ment.	
	It is recommended that you create one Experiment per cell, to keep file sizes manageable.	
Note:	Even if immediately selected after saving an Experiment, you will still be re-asked to save the prior Experiment (due to internal processes.)	
Open Experiment	Open a previously saved SutterPatch Experiment (*.pxp, *.uxp) file. If a SutterPatch experiment is opened into an Igor-only session, SutterPatch is automatically loaded.	
	If the Experient preference for HDF5 files is enabled, a SutterPatch Question will display regarding loading the matching HDF5 file:	
	• Load and store changes in analysis files	
	Open the HDF5 file in read-write mode, i.e. the original metadata and experiment structure, analysis results, images, etc. are overwritten when closing the present experiment. However, raw data are NEVER modified.	
	• Load in read-only mode, i.e. don't store any change back to the file	
	Open the HDF5 file in read-only mode. Nothing is stored, and the data file remains unaffected. Any- thing done in this session is lost when closing the Experiment.	
	Cancel loading HDF5 file	
	Do not open the HDF5 data file. Nothing is stored, and anything done in this session is lost when closing the Experiment.	
Save Experiment	If the current experiment is already named, it is immedi- ately saved. Otherwise, a 'Save experiment as' file dialog is displayed. If Preferences are enabled for automatic file naming, a default Experiment name is provided.	
Save Experiment As	Saves and renames the loaded Experiment to a new file name and/or format.	
Save Experiment Copy	Save the Experiment to another file name and/or format without closing the current session.	
Revert Experiment	Undo any unsaved changes to the Experiment.	
Recent Experiments	A list of recently used Experiments.	

Exit	An Experiment file 'Save' dialog is displayed before clos- ing the program. If an Experiment is not saved, global variables and window sizes/positions are lost.
New HDF5 File	( only displays when the HDF5 file in Set Preferences is enabled )
	Stores all existing data into the present HDF5 file, cleans-up the Experiment, and creates a new HDF5 file, so that acquisition can continue as if you had started a "New Experiment", but without restarting SutterPatch.

## Data

Data Browser	Access all SutterPatch objects contained in the experi-	
	ment.	

Load Waves / Packages / Install HDF5 Package

Load Waves / New HDF5 Browser

### Windows

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

Command Window	Process SutterPatch and Igor commands.
Control / Retrieve All Window	s Hidden unminimized windows can be brought into

## Layout

The Layout menu only displays when a Layout is the active window. Use it to modify the Layout window objects and display.

view with the menu command.

### SutterPatch

Dashboard	Display	y icons for core program functions.	
Scope Window	Bring an open Scope window to the front.		
Hardware Control			
Amplifier Control Panel Reset All Amplifier Settings		Open the hardware control panel.	
		Return the Amplifier Control Panel to its default settings for both headstages.	
dPatch Maintenance		Calibrate the headstages and auxiliary analog channels.	

dPatch Dynamic Clamp

Lock-In Adjustments	Manually tune the dPatch "lock-in amplifier" sys- tem.	
Reset USB	Re-initialize USB communication with the com- puter. If in Demo mode, you need to start a 'New Experiment' to access 'Reset USB'.	
Membrane Test	Open and run the Scope window to monitor seal for- mation and cell health.	
Free Run (Scope)	Open and run the Scope window in oscilloscope style.	
Reset Acquisition	Stop the Paradigm and/or data acquisition and clear cor- rupted acquisition settings.	
Paradigm Editor	Open the dialog to load, edit and run Paradigms.	
Routine Editor	Open the dialog to load and edit Routines.	
Template Editor	Open the dialog to manage templates.	
Equation Editor	Open the dialog to load and edit Equations.	
Solution Editor	Open the dialog to control solutions.	
Camera Module	Open the window to capture images.	
Data Navigator	Open the window to organize and display the experiment Paradigm, Routine and acquisition data in a tree struc- ture.	
Analysis Editor	Open the dialog to manage analysis graphs.	
Layout Page	Show	
	Delete	
Set Metadata	Open the dialog to configure user-specified experimental information.	
Set Preferences	Open the dialog to modify the default program settings.	
Log Window	Open the window to display a history of program actions.	
Shortcuts	Open the Shortcuts Editor dialog to manage keyboard shortcuts.	

Available Analysis Modules

Action Potential Analysis Synaptic Event Analysis Single Channel Analysis

## Help

Igor Help Browser	Igor and SutterPatch Help Topics.
About SutterPatch	SutterPatch version and contact information.

### Scope Right-click Menus

Different areas of the Scope windows support additional functionality through "right-click" menus in Windows, or "Command-click" menus in macOS.

Scope (Acquisition) main window (right-click a blank area in a signal)

- Note: If you don't click far enough away from the data, the Data menu will display instead.
  - Autoscale All Axes
  - Add Annotation
  - Export Graphics
  - Colors
  - Hide Signal '<signal name>'
  - Show Signal '<signal name>' Only
  - Stack All Signals

Scope data (right-click on the data)

- Browse <series signal name>
- Edit <series signal name>
- Remove Sweep_#
- Hide Sweep_#
- Duplicate Sweep_#
- Replace Wave
- Copy
- Modify Sweep_#
- Customize at Point
- Mode
- Line Style
- Line Size
- Markers
- Marker Size
- Color
- Bring to Front
- Send to Back
- Forward

- Backward
- Move to Opposite Axis
- Modify Contour
- Modify Image
- Modify Box Plot
- Modify Violin Plot
- Quick Fit
- Export Graphics
- Hide Signal '<signal name>'
- Show Signal '<signal name>' Only
- Stack All Signals

### Scope Y-Axis

- Autoscale All Axes
- Continuous Autoscale Axis
- Autoscale Axis
- Full Scale Y Axis
- Axis Properties...
- Hide Signal 'Signal Name'
- Show Signal 'Signal Name' Only
- Stack All Signals

#### Scope X-Axis

- Autoscale All Axes
- Full Scale X Axis
- Axis Properties...

Scope (Reanalysis) main window (additional items vs. Acquisition)

- 3D View
  - Zero Baselines The average of the first four data points is subtracted from the sweeps display. Does

not affect data values in measurements.

- Show All Sweeps
- Select Sweeps

## 4.3.6 Preferences

Preferences settings customize the default settings for several areas of the SutterPatch program. To access, go to the SutterPatch / Set Preferences menu.

Preferences Setting	S		3
General Files and Naming Hardware Control Panel Scope Metadata Graphics and Layouts Factory Reset	General         ✓ Enable drag and drop for pool editors         Window position         ✓ Automatic window repositioning.         Preferences Defaults         Restore default preferences settings for all preferences.         Edit Configuration Pool		
		Done	

Figure 4-80. Preferences Settings

### i. <u>General</u>

Enable drag and drop for pool editors

The Routine Editor loads a "pool" of Routines from a Routine Pool file. These Routines can be re-arranged in the Routine Pool list by clicking and dragging with the mouse.

The Paradigm Editor operates in the same manner, and also displays a list of Paradigm Steps, which can also be re-arranged by dragging-and-dropping.

Window Position

Automatic window repositioning

When SutterPatch windows or dialogs are opened or moved, when the action

is done, they are automatically repositioned to be fully visible. If a "child" window is opened, the parent window is moved to the left until the child sub-window is fully visible or the parent window reaches the left edge of the main window/screen.

Dual-monitor option	(macOS only)		
One screen	Prevents windows spanning across multiple monitors.		
	If a "parent" window is moved to another monitor, it fully displays in the new monitor, while any child sub-window remains behind fully displayed in the original monitor.		
Note: You can also bring all unminimized windows into view with the Windows > Control > Retrieve All Windows menu command.			
Preferences Defaults Restor	e default Preferences settings for all Preferences.		
Edit Configuration Pool Manag	ge user Preferences configurations.		
Delete Configuration	Remove the named Configuration from the list. The last remaining name is not deletable.		
Add New Configuration	Create a new Configuration name for the current Preferences settings.		
	Configurations are selected for loading at the start of a new experiment.		
Description	Enter the name for the Configuration (enclose in double quotes).		
Update Active Configuration (#)			
	The "active" Configuration is updated with the current Preferences settings.		
	This dialog opens with the active Configuration name listed. The number (#) indicates its position in the Configuration list.		
-	ration files use the *.spc file extension. However, he OS as file type "PKCS #7 Certificates".		

## ii. Files and Naming

Data files path:

Browse to select a folder.

Enable automatic naming for experiment

Experiment file name example: (Maximum 34 characters) ſ 1 Text: [ Legal characters are A-Z, a-z, 0-9 and "_". Include user text in the file name. Date: YYMMDD Include the date in the file name. Time: hhmmss Include the time in the file name. Save to separate HDF5 file Optional HDF5 file format for high volumes of data. The new setting will be active after restarting SutterPatch, or after starting a New Experiment. It is strongly advised to enable the "automatic naming" option above, so that the matching H5 and PXP files are kept "in sync". Data waves from the SutterPatch "data folder" (including raw data, metadata, Experiment structure, analysis results, images, etc.) are stored to a separate HDF5 file. Anything outside of the "data folder" remains part of the ".pxp" Experiment file. The File / New HDF5 File menu item only displays when the HDF5 file preference is enabled. This stores all existing data into the active HDF5 file, cleans-up the Experiment, and creates a new HDF5 file so that acquisition can continue as if you had started a "New Experiment", but without restarting SutterPatch. Keep only one Sweep in Memory [only displays when HDF5 is enabled] The new setting will be active after restarting SutterPatch, or after starting a New Experiment. Keeps only the wave of one sweep in memory. This minimizes memory allocation issues, as allocation of big buffers occurs only once, and these buffers can then be requested without re-allocating them,) Data are stored to disk while acquisition runs, eliminating the time required to store all the data at the end of an Experiment.

However, this also means that SutterPatch cannot handle the situation when data of multiple sweeps are needed at the same time, e.g., subtracting the data of a previous "reference" sweep from sweeps acquired later.

#### <u>Save Options</u> (non-HDF5)

• Save to temp file after each routine

The raw data are saved into a temporary file after each recording. This can help to speed up file-saving time for large Experiments composed of several smaller recordings.

The temporary file starting size is based on the starting size of the Experiment. The temporary data are re-saved to the main Experiment when the Experiment is closed and/or saved.

• Save entire experiment after each routine

This default option re-saves the entire Experiment after each recording (all data and Experiment information). This is the safest method of operation for data integrity, but can produce significant postrecording file-saving delays in larger Experiments.

• Don't save to temp file after each routine

Data and information are held in memory until the Experiment is explicitly saved; there are no file-saving delays after a recording is stopped. This provides the fastest method of operation when making multiple recordings, but is also the least secure, as data loss can occur if the computer encounters problems.

### iii. <u>Data Export</u>

- Igor Binary Save the signal formatted as an "Igor Binary Wave" file (*.ibw).
- ABF Format Save each signal of the routine formatted as an "Axon Binary File" v.1.8 (*.abf).
  - Export all selected sweeps to one file per signal
    - Ignore unselected sweeps
    - Replace unselected sweeps with NaN

- Create individual files for each sweep
- ATF Format Save the signal formatted as an "Axon Text File" (*.atf).

### iv. <u>Hardware</u>

• Prompt for hardware on startup (if no Sutter hardware is found).

When a new Experiment is started, if Sutter patch-clamp hardware is not connected to the computer and turned on, you are prompted to retry the USB connection or select an emulation mode.

- If no Sutter hardware is found, emulate:
  - IPA Integrated Patch Amplifier system
  - Double IPA Dual-headstage IPA system
  - dPatch Ultra-fast Digital Patch-clamp system

When a new Experiment is started, if Sutter patch-clamp hardware is not connected to the computer and turned on, automatically start up in the selected hardware emulation mode.

#### Stability Control

In CC mode reduce electrode compensation by [0.0 - 3.0] pF.

During whole-cell patching, if the Electrode Compensation control is set too high, oscillations can occur, and the patch-clamp seal can become unstable and be lost. As the Voltage Clamp mode typically operates with higher electrode compensation values than the Current Clamp mode, this preference promotes "safe" switching between the Voltage Clamp and Current Clamp modes.

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

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

Preserve clamp state when switching modes:

• Voltage Clamp to Current Clamp

The VC actual current level is used as the CC holding level.

• Current Clamp to Voltage Clamp

The CC actual voltage level is used as the VC holding level.

The current is held at 0 A for 50 ms between modes.

### v. <u>Control Panel</u>

Customize the active headstage tab's color in the Control Panel.

Headstage background color

Headstage 1	Color palette displays.
Headstage2	Color palette displays.

#### vi. Scope

To apply these settings, open or re-open the Scope window.

General

Time axis unit:

•	Auto-set	Sweep duration < 120 s, use "s"	
		Sweep duration >= 120 s, use "min"	
		Sweep duration $\geq$ 7200 s, use "h"	

• SI unit Always use standard SI base units, such as "s" for time.

Acquisition

Y axis autoscale settings:

٠	Continuous autoscale	The Y-axis limits are rescaled with
		each sweep so that all data are
		visible.

• Continuous autoscale from zero

One Y-axis limit is positioned at zero, while the other Y-axis limit is rescaled with each sweep. The Y-axis direction matches the sign of the largest absolute value in the sweep, can change during a sweep, and can be different for each sweep.

This is just a display setting - all data, both positive and negative, are recorded.

If Persistence is enabled, the direction of the first sweep is used for all subsequent sweeps.

- Full scale The full-scale range is used.
- Use last y-scale The last setting of the Scope window Yaxis scaling is used.

Maximal sweeps displayed in persistence display

	[2 - 100]
	All
	Improve data readability and system performance by restricting the number of sweeps displayed, which reduces the display processing load.
Display compression	Improve system performance by reducing the number of data points plotted, which reduces the display processing load.
Reanalysis	

Show tags by default

Y axis autoscale settings:

• Autoscale The Y-axis limits are rescaled with each sweep so that all data are visible.

Autoscale from zero One Y-axis limit is positioned at zero, while the other Y-axis limit is rescaled with each sweep. The Y-axis direction matches the sign of the largest absolute value in the sweep, and can change during a sweep, and can be different for each sweep.

> If Persistence is enabled, the direction of the first sweep is used for all subsequent sweeps.

- Full scale The full-scale range is used.
- Use last y-scale The last setting of the Scope window Y-

Signals Display

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

Example: 1,2,4-5

## Sweeps Display

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

separated by commas.

Example: 1,3,5-20

Appearance Use a color pa

Use a color palette to choose colors.

Active signal panel color	[ light gray ]
Inactive signal panel color	[ dark gray ]
Active sweep color	[ blue ]
Inactive sweep color	[ red ]
Drop-down color palette	Click on a color square to set it as the active color.
Other	Use a color dialog with more options.
Opaque	< unused >
[ Preview Pane ]	The selected signal and sweep colors are displayed in a preview pane.

- Tip: For dark-room experiments, the window background color can be adjusted by the operating system:
  - Windows: In the Control Panel / Appearance / Personalization window, scroll down and select the High Contrast Black theme, or use the Windows Magnifier tool with option 'Turn on color inversion' enabled.

• macOS: Press 'Control-Option-Command-8' to set the System Preferences / Accessibility / Display / Invert Display colors option, or open its menu with 'Command-Option-5'.

#### vii.Metadata

Metadata detail level: Increasing levels of data categorization complexity.

Select which metadata groups and parameters are visible for configuration in Set Metadata and in Data Navigator / Build Hierarchy.

1	Basic	Show only the most essential parameters.
2	Extended	Show additional detail.

3 Full Expose all available metadata parameters.

Note: This setting does not affect the Metadata Review dialog - all user-defined metadata are displayed irrespective of the metadata detail level.

#### viii. Graphs and Layouts

These settings apply to Layout windows and stand-alone graph windows (not graph files).

Copy "To Clipboard" graph format

Several popular file formats are supported:

- PNG Portable Network Graphics
- PDF Portable Document Format
- TIFF Tagged Image File Format
- JPEG Joint Photographic Experts Group

Note: When pasting, not all formats may be supported by other programs.

Layouts

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

Default graphs per page

- 1
- 2

286

- 3
- 2 x 2 (Column x Row)
- 2 x 3 (Column x Row)
- 2 x 4 (Column x Row)

## Graphs

left

(typically the Y-axis)

## Tick location:

- Outside
- Crossing
- Inside
- None

## Labels:

- On
- Axis only
- Off

### Grid:

- Off
- On
- Major only

bottom

# m (typically the X-axis)

## Tick location:

- Outside
- Crossing
- Inside
- None

### Labels:

- On
- Axis only

• Off

Grid:

- Off
- On
- Major only

## ix. Factory Reset

[ Factory Reset ]	Click this button to reset SutterPatch to it default settings. SutterPatch will need to be exited and restarted to complete the factory reset.
Warning!	When you do a factory reset, you will lose ALL your configuration data (including Metadata, Scope, Preferences, etc.)

# 4.3.7 Shortcut Editor

Shortcut Edi	itor			
Control Key As	signments		Funct	ion Key Assignments
Control-1	Dashboard	•	F1	Igor's help function 🔹
Control-2	Scope Window	•	F2	not assigned 🔹
Control-3	Amplifier Control P	anel 💌	F3	not assigned 🔹
Control-4	Membrane Test	•	F4	not assigned 👻
Control-5	Free Run (Scope)	•	F5	not assigned 🔹
Control-6	Paradigm Editor	•	F6	not assigned 🔹
Control-7	Routine Editor	•	F7	not assigned 🔹
Control-8	Data Navigator	•	F8	not assigned 🔹
Control-9	Set Metadata	•	F9	not assigned 🔹
Control-0	Log Window	•	F10	not assigned 🔹
,		Reset Key As	signmer	nts
Shortcut Actions				
Shortcut Pool	·	erstEmeraldCity	Docum	ents:S:SP_ShortcutPool.sps
			Docum	
On Key	,	nortcut Action ame=ResetTimer		
		and resettine	,	
				×
<				>
Insert 💌	Assign Key De	elete Key Ec	lit E	xecute Delete Save

Keyboard control of SutterPatch is available by configuring keyboard shortcuts.

Figure 4-81. Shortcuts Editor

Note: All Control/Command, Function and Shortcut Action key combinations are automatically added to the SutterPatch / Shortcuts submenu.

Control Key Assignments / Command Key Assignments

A Control key assignment can be changed by clicking on its drop-down list and selecting a new menu item.

- Windows 'Control' key: CTRL
- macOS 'Command' key: #

Use a Control key by holding down the 'CTRL' (or 'Command') key while simultaneously clicking its assigned number.

A list of the Control keys and their default SutterPatch menu items:

Control-1	Dashboard
Control-2	Scope Window (Scope window must be open.)
Control-3	Amplifier Control Panel
Control-4	Membrane Test
Control-5	Free Run (Scope)
Control-6	Paradigm Editor
Control-7	Routine Editor
Control-8	Data Navigator
Control-9	Set Metadata
Control-0	Log Window

Other SutterPatch menu items available for Control key assignment:

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

#### Function Key Assignments

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

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

Function Keys:

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

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

macOS reserves nearly all Function keys for itself. In order to use Function keys for an application, you must first check a checkbox in the macOS Keyboard control panel. Even then, macOS will intercept some Function keys.

#### Reset Key Assignments

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

#### Shortcut Actions

Additional custom keyboard Actions can be created and managed.

Shortcut Pool Files

	New Shortcut	Pool	Create	a default Shortcut Pool.
	Load Shortcut	Pool		e Shortcuts of a previously saved at Pool file into the Shortcut Pool.
	Revert to Last Saved		Undo a: Pool.	ny unsaved changes to the Shortcut
Save Shortcut Pool		Pool		e Shortcut Pool using its existing file nd path.
	Save Shortcut	Pool As	switch	e Shortcut Pool to a new file, and to the new file. The default file name ame as the original file name.
	Save Shortcut Pool Copy		not swi	e Shortcut Pool to a new file, but do tch to the new file. The default file as 'Copy of" prepended to it.
	Merge Shortcu	ut Pools		he Shortcuts from a previously Shortcut Pool file into the loaded at Pool.
[Shortcut Pool file path]				
Shortcut Acti	ons Table			
Columns				
On		Enable/disab	le the Sł	nortcut Action.
Key		The assigned	l keyboar	rd key.
Modifier		The keyboard "modifier key" used in a key combination - all keys are simultaneously pressed.		•
		<u>Windows</u>		
		• CTRL		Only use with keys $0-9$ .

	• ALT	Keys '0 – 2' reserved by Igor for File / Recent Experiments.
	• SHIFT	Shift key.
	• FUNC	Function key.
	• CAPS LOCK	Ignored.
	<u>macOS</u>	
	• Command	Only use with keys $0-9$ .
	Option	Option key.
	• Shift	Shift key.
	• Control	Keys '0 – 2' reserved by Igor for File / Recent Experiments.
	• Caps Lock	Ignored.
Shortcut Action	An Action's instructio	ns and settings.

Click a field in the pool to highlight an Action and make it the active entry. Click-anddrag a field to reposition an Action in the pool.

Insert Adds an Action to the 'Shortcut Action' list and opens its Shortcut Actions Editor for setup.

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

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

		Set Checkbox	Set local and global checkboxes for condi- tional processing in Paradigm 'If' steps.
		Set Variable	Set Paradigm or Routine variables.
		Set Tag	Write a comment tag to the Paradigm metadata.
		Start Acquisition	In open Scope acquisition window.
		Stop Acquisition	In open Scope acquisition window.
		View Last	Display the last recording in a Scope rea- nalysis window.
		Write Log	Write a note to the Log window.
Assign Key		This button opens the Shortcut Key Input dialog (or double-click in a "Key" or "Modifier" field) to input the desired keyboard com- bination for a letter, number, or symbol.	
	Note:	computer to compute	etters, numbers, and symbols can vary from r, depending on the computer OS and Igor served keys typically open another window onsive.)
	Tips:	If the CAPS LOCK by case insensitive.	utton is on when assigning a key, the key is
		Keyboards often have cess to the Function k	e a Function (FN) button to allow special ac- xeys.
		Although the F1 func signed if used with a	tion key is reserved in Igor Pro, it can be as- modifier key.
Delete Key		Remove the Key entry	v for the selected Action.
Edit		Open the Shortcut Editor dialog (or double-click in a "Shortcut Ac- tion" field) to change the Action's parameters.	
Execute		Run the selected Action.	
Delete		Remove the selected A	Action from the 'Shortcut Action' list.
Save		Saves any changes to	the current Shortcut Pool file.

# 4.3.8 Sample Files

Sample settings files (subject to change) are included in the ... / Documents / SutterPatch / Parameters folder:

Equation Pool Sample File

 $SP_EquationPool.txt$ 

Equations for all amplifiers.

1. X3pi

3*pi

2.	ElapsedTime	ParadigmTime - time
3.	Temperature	aux[1]*1.23 – 273.15

# Paradigm Pool Sample Files

$SP_ParadigmPool_dPatch.spp$		Paradigms for dPatch amplifiers.
1.	Amplifier_Setup	Set initial amplifier settings.
2.	Start_one_Series	Start acquisition of one routine.
3.	Set_amplifier_and_start_l	V Set amplifier to a known state, then start a routine.
4.	Interactive_acquisition_1	Run an interactive acquisition stopping at a given analysis condition.
5.	Start_two_Series	Start acquisition of two subsequent routines.
6.	Start_ForEachSweep	Start acquisition of a routine, individually trig- gering each sweep.
7.	Interactive_acquisition_2	Run an interactive acquisition loop that selects between 2 routines, and manually stop via a Checkbox.
8.	Tuning_with_Input	Use the paradigm "Input" control to increment or decrement a Routine's stimulus output.
9.	Toggle_Persistence	Use a Checkbox to toggle Scope trace persistence while acquiring a routine.
10.	. Switch_Headstages	Switch between multiple headstages.
11.	. Tuning_with_Keys	Use the keyboard to increment or decrement a Routine's stimulus output by 10 mV.

#### LockIn_dPatch.spp

Paradigms for dPatch lock-in tuning.

- 1. LockIn_Adjust_500Hz
- 2. LockIn_Adjust_1kHz
- 3. LockIn_Adjust_2kHz
- 4. LockIn _ Adjust_5kHz
- 5. LockIn _ Adjust_10kHz
- 6. LockIn_Adjust_20kHz
- 7. LockIn_DoAdjust

SP_RoutinePool_dPatch.spr	Routines for two-headstage dPatch amplifiers.
SP_RoutinePool_dPatch_1HS.spr	Routines for one-headstage dPatch amplifiers.
1. IV	I/V for voltage-clamp mode.
2. IV_Continuous	I/V with continuous acquisition.
3. IV_tuning	I/V for sample "tuning" paradigms.
4. Synaptic_Stim	Synaptic stimulation.
5. Synaptic_Stim30	Synaptic stimulation.
6. Multi_Test	Multi-channel waveform.
7. Bowtie_Test	Multi-channel waveform.
8. IV_P4	I/V with 4 leak pulses.
9. SS_Inactivation	Steady-state inactivation.
10. Recovery_Inactivation	Recovery from inactivation.
11. Onset_SlowActivation	Onset Slow activation.
12. SS_SlowInactivation	Steady-state slow activation.
13. Recovery_SlowInact	Recovery from slow inactivation.
14. Test_Pulse	Test pulse.
15. ContinuousNoOut	Acquisition without any output waveform.
16. Template_SpontAct	Template wave from a recorded signal.
17. TemplateTest	Template wave for waveform output.
18. TemplateAndVirtual	Template wave and recording virtual signals.
19. IV_VC_CC	IV for voltage- and current-clamp modes.
	[ for two-headstage dPatch amplifiers ]
20. A_T_InactRec_P4	Inactivation with leak subtraction.

## $LockIn_dPatch.spr$

Routines for dPatch lock-in tuning.

- 1. phase delay
- 2. LockIn  $_500$ Hz
- 3. LockIn_1kHz
- 4. LockIn_2kHz
- 5. LockIn $_5kHz$
- 6. LockIn_10kHz
- 7. LockIn $_20kHz$

## Solution Pool Sample File

SP_SolutionPool.spo

Solutions for all amplifiers.

1. undefined

# Template Pool Sample File

SP_TemplatePool.spt

Templates for all amplifiers.

- 1. minis
- 2. R1_S1_10minSpontAct
- 3. Template1_Sweep5

Sample data (subject to change) are included in the  $\dots$  / Documents / SutterPatch / Example folder:

**Experiment Sample Files** 

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

# 4.3.9 Startup

The SutterPatch application startup sequence:

- 1. Click on the Igor Pro 8 icon to launch Igor Pro.
- 2. The application opens an Igor 'Command' window along with a "splash" screen displaying file opening information; both windows are then closed.
- 3. Next a 'Welcome to SutterPatch' screen displays a few launch options:
  - Igor Only Run Igor Pro (without launching SutterPatch).
  - Open Launch SutterPatch from a saved Experiment.
  - Start Launch SutterPatch for a new Experiment.
- 4. Click 'Start', an Igor 'Command' window opens, and the Welcome screen displays a progress bar while compiling the SutterPatch code; the Welcome screen is then closed.
- 5. A 'Save experiment as" dialog displays with standard file saving controls.
- 6. Next, if no Sutter amplifier is detected, the 'No USB Connection' pane allows you to try re-establishing the USB connections, or to select a hardware-emulation mode:
  - IPA Single Headstage

- DIPA Double Headstage
- dPatch Double Headstage
- Dendrite Data Acquisition System

In amplifier demonstration mode, the Amplifier Control Panel and acquisition Scope window are labeled with "Demo", the input and output channels use simulated data, and most SutterPatch functions are available (except dPatch Calibration and Dynamic Clamp).

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

# 5. PROGRAMMING

#### 5.1 Data Format

SutterPatch data are written in a 64-bit double-precision binary floating-point format. This supports a decimal precision of 17 significant digits.

The data are stored within an Igor Experiment (*.pxp) file.

For large data sets, an optional HDF5 file format will be available for streaming data acquisition without resaving the experiment at the end of a recording.

#### 5.2 Data Structure

SutterPatch recorded data are stored as multidimensional data waves, and are listed per signal in the Data Browser. Select a data wave in the Data Browser and right-click to 'Edit' the Signal data in a spreadsheet-style table. The two-dimensional data wave is displayed with one row per sample point and one column per trace, with the number of data table columns increasing with the number of sweeps.

- Warning! The raw data can be directly edited in the Data Browser this is not recommended, as it permanently alters the data.
- Note: While SutterPatch does not read the older Igor one-dimensional wave data-format, graph data for each axis can be separately exported to it. See the Analysis Editor / Files menu.

# 5.3 Data Paths

The Data Browser path uses a "root" of 'SutterPatch'. The Data Browser right-click 'Copy Full Path' command copies a Signal's data wave path to the clipboard.

Note: This path references Igor's internal data folders, and not the computer OS file system.

The object's path name can be used in user functions and executable commands. However, when referencing an active Scope window, the path name to the data wave can be substituted by "t[#]", where "#" refers to the signal position number in the Scope window.

# **5.4 User Functions**

SutterPatch functionality can be extended through the use of user-defined Functions.

To create a user Function:

- 1. Open the menu for Windows / Procedure Windows / Procedure Window.
- 2. Enter your user code into the Procedure window, following its #pragma' and #include' lines.

Example:

```
#pragma TextEncoding = "Windows-1252"
#pragma rtGlobals=3 // Use modern global access method...
#include "SP Globals", optional
```

```
Function SayHello()
```

DoAlert 0, "Hello World!"

End

Note: The Function name must include trailing open/close parentheses "()"

- 3. Click on the Compile button at the bottom of the window.
- 4. Enter the Function name (including parentheses) into the Command window and press 'Enter', or use it in a Paradigm 'Execute' step.

For more information on creating your own functions, see the Igor Help topics on Programming / User-Defined Functions, and Procedure Windows.

Warning! User-defined functions only exist during the Experiment. They are not stored when the Experiment is closed. If you plan to re-use them in other Experiments, save them to a separate file, such as with a word processor.

Also, while user-defined functions are stored internally by Igor, there is no visible list, so you will need to maintain such a list manually.

# 6. TROUBLESHOOTING

# **6.1 Technical Support**

Technical support is provided to customers at no charge.

Support hours:	8:00 AM - 5:00 PM PST (Pacific Standard Time).
Telephone:	(+1) 415.883.0128
Fax:	(+1) 415.883.0572
E-mail:	info@sutter.com
Address:	Sutter Instrument Company One Digital Drive Novato, CA 94949

When contacting us for technical support, please provide your SutterPatch version and "build" numbers to help us troubleshoot your situation. These numbers are found in the Start splash screen during program loading, or in the Log Window Startup events.

For issues regarding Igor Pro features (all non-SutterPatch menu items), please contact Wavemetrics, Inc. for technical support.

# 6.2 Manual

The dPatch manual is installed as a PDF file along with the SutterPatch software. The latest version of the manual can be downloaded from our web site: <u>https://www.sutter.com/AMPLIFIERS/SutterPatch.html</u>.

You can navigate through the PDF document using Table of Contents links, accessed via the Bookmarks tab on the left side of the PDF screen.

# 6.3 Online Help

Online help is available via the main Help menu, under 'Igor Help Browser' or 'Help Topics'. The SutterPatch Help Topics / Help File names start with "SP_". The online Help includes the same information as found in the PDF manual.

Most items in SutterPatch also include a short description as a tool tip. Hover the mouse over an item to see the tool tip.

# **6.3.1 Error Messages and Notifications**

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

## 6.4 Startup Issues

#### 6.4.1 Installation Fails

- Problem: The SutterPatch installation on Windows fails due to language pack incompatibilities.
- Solution: Support for foreign language packs (except Japanese) has been added. If foreign language versions still cause problems, please contact Sutter Technical Support.

#### 6.4.2 Sample Parameter Files Not Installed

- Problem: The SutterPatch installer fails to install the sample parameter files, as access is blocked to the Program Files or Users\...\Documents folders.
- Solution: Disable any virus scanners or firewalls. If that does not help, then manually copy the sample parameter files into the Users\..\Documents\SutterPatch \Parameters folder.

#### 6.4.3 Application Not Loading

- Problem: The SutterPatch application does not load the startup sequence only loads Igor Pro.
- Solution: If available, execute the Igor Pro menu command 'Macros / Autocompile'.

#### 6.4.4 EEPROM Startup Errors

- Problem: Starting up SutterPatch and simultaneously powering on the amplifier generates an EEPROM error. Attached hardware such as headstages might use incorrect settings.
- Solution: Close SutterPatch, power cycle the amplifier, and relaunch SutterPatch.

#### 6.4.5 Update <u>Startup Errors</u>

Problem: When starting up SutterPatch after installing a software update, program errors display.
Solution: Closing and re-opening SutterPatch sometimes clears up these errors. Otherwise, if possible, roll back the software update.

#### 6.4.6 USB Communication Fails

Problem:	When starting up SutterPatch in Windows, there is no USB communication with the computer.
Solution:	Power off the amplifier, then reseat the USB cable on both ends, and then power on the amplifier.
	Otherwise, the Windows "power plan" might have disabled the USB ports:
	1) Go to the Windows Start screen, and enter "edit power plan" in the Windows Search box.
	2) Click on "Change advanced power settings".
	3) Scroll down to "USB settings" and click on its [+] box.
	4) Click on the "USB selective suspend setting" [+] box.
	5) Change the "On battery" and "Plugged in" settings from 'enabled' to 'disabled', and click "OK".
	Also, sometimes a Windows 10 update can disable the computer's USB ports. Either browse the web to find Device Manager and driver solutions to the prob- lem, or restore the OS to an earlier version of Windows 10 until a Windows 10 update fix is released.

## **6.5 Acquisition Issues**

## 6.5.1 Acquisition In Progress

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

#### 6.5.2 Acquisition Windows Lock Up

Problem:	The Scope window, Routine Editor or Paradigm Editor lock up during acquisition.
Solution:	Use the menu command SutterPatch / Reset Acquisition to halt acquisition.
	A combination of SutterPatch-related and computer-related issues can contrib- ute to your system's performance. For suggestions to improve it, see the Trou- bleshooting item Sluggish Acquisition below.

#### 6.5.3 Acquisition Terminates

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

#### 6.5.4 Signal Flat

Problem:	A Scope input signal is completely flat during acquisition, i.e., zero amplitude.
Solution:	The corresponding headstage might not be attached to its port. The headstage HDMI connectors do not lock on - make sure they do not disconnect from their port.
	Power off the dPatch system and reconnect the headstage.

#### 6.5.5 <u>Headstage Noise</u>

Problem:	The noise levels of the instrument suddenly and erratically increase.
Solution:	If the headstages are touched, the noise level will greatly increase. Make sure you are grounded or working in a Faraday cage.

#### 6.5.6 Paradigm Sound Reduced

- Problem: The paradigm 'Sound' step volume is attenuated at lower frequencies.
- Solution: Upgrade the computer speaker, such as with add-on speakers.

#### 6.5.7 Offset Zero Delay

- Problem: The Offset button in the IPA Control Panel has a short delay before it responds.
- Solution: This can occur after running the Membrane Test due to internal processing.

#### 6.5.8 Post-Acquisition Delay

Problem:Every time acquisition completes, there is a delay with the program operations,<br/>as the entire *.pxp Experiment file is resaved when a recording stops.Solution:Create new Experiments more often, so that file sizes are smaller and more<br/>manageable.<br/>Or, change the file saving settings in Set Preferences / Files and Naming.

#### 6.5.9 Sluggish Acquisition

Data acquisiti	ion is sluggish.
The computer load.	's available resources need to be increased to handle the system
	n of SutterPatch-related and computer-related issues can contrib- stem's performance. Here are some suggestions to improve it:
Close:	Data Browser window – in Igor Pro 8, it consumes a lot of CPU time.
Disable:	Computer screen saver, and Power Save or Sleep modes.
Disable:	Scope window persistence display.
Disable:	Routine Editor / Input Channels / Virtual channels.
Reduce:	Routine / Acquisition & Routine Parameters / Output sampling rate.
Close:	Background software.
Remove:	Software for certain license protection USB keys (dongles).
Optimize:	Hard disk (defragment).
Upgrade:	Computer graphics card.
	The computer load. A combination ute to your sy Close: Disable: Disable: Disable: Reduce: Close: Remove: Optimize:

#### 6.5.10 USB Errors

6.5.11

Problem:	A USB communications error occurs.	
Solution:	Right-click in the Control Panel and select Reset USB. If the red USB button does not turn green, then try to isolate the problem.	
	a) Unplug and re-plug both ends of the USB cable from the amplifier to the computer.	
	b) Try another USB cable.	
	c) Try another USB port	
	d) Remove any USB hubs.	
<u>dPatch Ampli</u>	fier Control Panel Issues	

Problem: The dPatch Amplifier Control Panel is having odd problems.

Solution: You can reset the active headstage controls in two ways:

• Safe mode: Initiate with a mouse left-click to disable controls:

Offset 'Lock'

#### VC mode

Cell Compensation Rs Correction

#### CC mode

Electrode Compensation Bridge Balance Correction Dynamic Holding

and to reset controls to defaults:

<u>VC mode</u>

I-filter

#### CC mode

V-filter

• Default mode: Initiate with a mouse right-click or shift-click.

Reset all fields to default settings, except:

Liquid junction Offset value

VC mode

V-holding

CC mode

Subtract Pipette Offset

Bridge Balance: Show.M $\Omega$  vs. % Rs

# 6.6 Analysis Issues

6.6.2

6.6.3

6.6.1 <u>Analysis Not Deleted</u>

Problem:	An analysis cannot be deleted in the Analysis Editor.	
Solution:	The analysis is still in use, i.e. displayed in another window, such as a graph window - close the window to allow the analysis to be deleted.	
Signal Axes O	verlay	
Problem:	The X-axes and units are overlaid in the Scope window.	
Solution:	There is not enough room for the X-axes and units due to the number of signals displayed. Switch to a tiled signal layout, or reduce the number of visible signals by right-clicking a signal and selecting 'Hide Signal'.	
Graphs & Layouts Not Visible		
Problem:	Cannot see SutterPatch Experiment graphs or layouts on non-SutterPatch computers.	
Solution:	Use the Igor menu command Windows / Graph or Windows / Layout to see the object. Right-click it to modify with Igor options.	

## **6.7** General Issues

#### 6.7.1 <u>Buttons Unresponsive</u>

Problem:	When using a slower computer in emulation (demo) acquisition mode, acquisi-
	tion-related actions might be difficult, such as clicking the Stop button.

Solution: You may need to click the button more than once or hold it down longer than usual.

A combination of SutterPatch-related and computer-related issues can contribute to your system's performance. For suggestions to improve it, see the Troubleshooting item Sluggish Acquisition above.

#### 6.7.2 Slow Display of Sweeps

Problem:	When displaying a large number of sweeps, the display slows down.
Solution:	Disable Persistence display in the Scope window.

#### 6.7.3 <u>Window Maximizing</u>

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

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

#### 6.7.4 Command Window Frozen

Problem: The Command Window is blank and/or unresponsive.

Solution: Use Ctrl-J, or click on the Amplifier Control Panel, and the Command window is redrawn as an active window. This is a reported Igor issue.

#### 6.7.5 Slow Window Opening/Closing

- Problem: Window opening and closing is slow on the macOS.
- Solution: Close the SutterPatch Data Browser. This function consumes a lot of system resources.

#### 6.7.6 File Operations Crash

Problem: In Windows 10, file opening or saving crashes SutterPatch.

Solution: Remove the Dell Backup and Recovery utility v1.8, or upgrade it to a newer version.

# 6.7.7 <u>Wrong Preference Settings</u>

Problem:	Program preferences are non-standard or corrupted.
Solution:	Reset the SutterPatch preferences to their defaults via the SutterPatch / Set Preferences / General / Preferences Defaults button.

# 6.7.8 Font Size Too Large

Problem:	The font size is too large when using the display scaling in 'Scale and Layout'.
Solution:	This can occur on high-resolution monitors running on older versions of Win- dows 10. Upgrade to the current version of Windows 10.

# 6.7.9 <u>Weird Behavior</u>

Problem:	There is weird or buggy behavior with the SutterPatch program.
Solution:	Reset the SutterPatch program settings to their factory defaults via the
	SutterPatch / Set Preferences menu command, by performing a Factory Reset.

# 6.7.10 Igor Pro Features

Problem:	There are a tremendous number of standard features in Igor Pro that can be used in conjunction with the SutterPatch application.
Solution:	Please refer to the Igor Help browser, or to Wavemetrics, Inc., regarding issues with Igor Pro features.

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Section 10.8. The headings provided in this Agreement are for convenience and reference purposes only. In the event of a conflict between the terms and conditions listed in Articles 1 through 10, and the attached Schedules, the terms and conditions shall govern.

**Section 10.9.** A waiver of a breach, violation, or default under this Agreement shall not be a waiver of any subsequent breach, violation or default. Failure of either party to enforce compliance with any term or condition of this Agreement shall not constitute a waiver of such term or condition.

**Section 10.10.** All notices and communications shall be in writing and shall be deemed to have been duly given when delivered or three (3) Business Days after mailing by certified mail, return receipt requested, postage prepaid, addressed to the parties at their respective addresses or at such other addresses as the parties may designate by written notice in accordance with this section.

#### 316

**Section 10.11.** Any amendments or addenda to this Agreement, may be executed in counterparts, each of which will be considered an original, but all counterparts together will constitute one agreement. A facsimile of a signed copy of this Agreement, or an electronic or other digital signature imprinted on this Agreement, may be relied upon as an original.

# APPENDIX C: ACCESSORIES

- dPatch Expansion Panel
- Ground Point GP-17
- Quartz Pipette Holder
- Rack Pack

- Rack-mountable analog and digital I/O BNC panel  $% \mathcal{A}$
- Grounding point hardware
- Quartz pipette holder
- Rack mounting hardware

# APPENDIX D: FUSE REPLACEMENT

In the event that the instrument fails to power up when it is switched on, the power-line fuses should be checked to determine whether they have blown. Two fuses are located in the fuse holder in the power cord module on the rear of the amplifier.

To replace a fuse:

- 1. Unplug the power cord from the power entry module, revealing the fuse holder below.
- 2. Remove the fuse holder.
- 3. If a fuse is blown, it is recommended to replace both fuses.
- 4. Insert appropriately-rated replacement fuses (see below).
- 5. Replace the fuse holder in the power entry module and reconnect the power cord.

Mains		Fuses			
Power	(5 mm x 20 mm, glass tube)				
Source	Fuse Rating	Manufacturer Examples			
100 - 240	T2.0 A,	Bussmann: GMC-2-R, S506-2A,			
VAC	250 V (Time Delay)	Littelfuse: 239.002.P			

Table D-1. dPatch Fuses

# APPENDIX E. TECHNICAL SPECIFICATIONS



# **General Specifications**

	dPatch Amplifier – Physical			
Dimensions (in.) (includes handles & connectors)	19 (W) x 11 (D) x 3.5 (H)			
Dimensions (cm) (includes handles & connectors)	48.2 (W) x 28 (D) x 9 (H)			
Weight (lb) (with headstages)	15			
Weight (kg) (with headstages)	6.8			
Case	Aluminum			
Computer Com- munications	USB 3.0 port			
BNC Channels	<ul> <li>4 Auxiliary analog outputs (impedance: 16 Ω)</li> <li>8 Auxiliary analog inputs (impedance: 10 kΩ)</li> <li>1 Digital output trigger</li> <li>1 Digital input trigger</li> </ul>			
Rack use	19" rack-mount (2U)			
Benchtop use	4 Rubber feet			
Signal Ground	4 mm Banana socket			
Earth Ground	4 mm Banana socket			
Safety Signage	CE marking (Conformité Européenne)			
Digital Outputs	DC-37 female connector			

		0	
	PIN	DEFINITION	
	1	Digital Output 1	
	2	Digital Output 2	
	3	Digital Output 3	
	4	Digital Output 4	
	5	Digital Output 5	
	6	Digital Output 6	
	7	Digital Output 7	
	8	Digital Output 8	
	9	Digital Output 9	
	10	Digital Output 10	
	11	Digital Output 11	
	12	Digital Output 12	
	13	Digital Output 13	
	14	Digital Output 14	
	15	Digital Output 15	
	16	Digital Output 16	
	17	+5 V	
Digital Output	18	+5 V	
Pinout	19	+5 V	
	20	Ground	
	21	Ground	
	22	Ground	
	23	Ground	
	24	Ground	
	25	Ground	
	26	Ground	
	27	Ground	
	28	Ground	
	19	Ground	
	30	Ground	
	31	Ground	
	32	Ground	
	33	Ground	
	34	Ground	
	35	Ground	
	36	+5 V	
	37	+5 V	
Pin numbering		t, top to bottom.	

Table E-1. dPatch Amplifier - Physical

dPatch Headsta	ge - Physical
Construction	Anodized aluminum case
Dimensions (in) [ L x W x H ]	4.000 x 1.375 x 0.825
Dimensions (cm) [ L x W x H ]	10.160 x 3.493 x 2.096
Cable Length (ft) Headstage to Preamp Preamp to Port	6 6.56
Cable Length (m) Headstage to Preamp Preamp to Port	$\begin{array}{c} 1.83\\ 2\end{array}$
Weight (lbs) w/o cable w/cable	0.21 0.294
Weight (kg) w/o cable w/cable	0.095 0.133
Ground Socket (mm)	1

Table E-2. dPatch Headstage - Physical

	dPatch Headstage – Operational (Measured with 8-pole Bessel filter.)					
Feedback Element	Analog Bandwidth	Amplitude Range	Electrode Compensation Range	Series Resistance Compensation Range	Whole-cell Capacitance Compensation Range	
1 pF Ca- pacitor	> 500 kHz	± 20 nA	0.0 - 20 pF	N/A	N/A	

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500 MΩ Resistor	> 250 kHz	± 20 nA	0.0 - 20 pF	$0.1 - 100 \ M\Omega$	$1.0-500~\mathrm{pF}$
50 MΩ Resistor	> 250 kHz	± 200 nA	0.0 - 20 pF	$0.1 - 10 \ M\Omega$	10.0 – 1000 pF

Table E-3. dPatch Headstage - Operational

dPatch Headstage – RMS Noise (VC) (Measured with 8-pole Bessel filter, voltage clamp, open circuit.)						
Feedback Element10 kHz5 kHz1 kHz						
1 pF Capacitor	220 fA	95 fA	$25~{ m fA}$			
500 MΩ Resistor	750 fA	475 fA	200 fA			
50 MΩ Resistor	2.3 pA	1.5 pA	650 fA			

Table E-4. dPatch Headstage – Open Circuit Noise

dPatch Headstage – RMS Noise (CC) (Measured with 8-pole Bessel filter, current clamp, grounded input.)					
Feedback Element10 kHz5 kHz1 kHz					
$500 \ \mathrm{M\Omega}$ Resistor	1 μV	700 nV	400 nV		
50 MΩ Resistor	1 μV	700 nV	400 nV		

Table E-5. dPatch Headstage – RMS Noise

# dPatch Headstage – Risetime (CC)

(Measured	with	$\pm 20$	nA	range.)
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Series Resistance Rs	Pipette Capacitance	10-90% Risetime	Bandwidth
0 Ω	10 pF	250 ns	1.4 MHz
100 k $\Omega$	10 pF	$1\mu{ m s}$	350 kHz
1 MΩ	10 pF	< 5 µs	> 70 kHz
10 MΩ	10 pF	<10 µs	> 35 kHz
100 MΩ	10 pF	< 15 µs	>20 kHz

Table E-6. dPatch Headstage - Risetime

dPatch Data Acquisition		
Analog I/O Channel Type	Full Differential	
Analog I/O Channel Amplitude (voltage)	±10 V	
Analog I/O Channel Amplitude (current)	±20 nA, ±200 nA	
Headstage Input Channel Resolu- tion	18 bits	
Headstage Input Sample Rates	1 kHz - 5 MHz	
Headstage Input Filter Bandwidth	$100 \mathrm{Hz} - 1 \mathrm{MHz}$	
Headstage Output Channel Reso- lution	16 bits	
Headstage Output Sample Rate	$250 \mathrm{~kHz}$	
Auxiliary I/O Channel Resolution	16 bits	
Auxiliary Input Sample Rate	200 kHz	
Auxiliary Input Bandwidth	1 - 200 kHz	
Auxiliary Output Sample Rate	$250 \mathrm{~kHz}$	
Auxiliary Output Current (max)	20 mA	
Digital Input Trigger Width	100 ns	
Digital Input Level	+2.3 V	
Digital Output Sample Rate	$250~\mathrm{kHz}$	

Digital Output Trigger Width	1 ms
Digital Output Level	+3.3 V
Digital Output Current (max)	20 mA

Table E-7. dPatch Data Acquisition

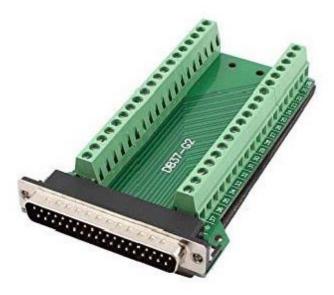


Figure E-1. Screw Terminal Board

The included Screw Terminal Board attaches to the dPatch rear panel DIGITAL OUTPUTS connector. Digital output signal wires need to be manually screwed into the appropriate pin number. An intervening 37-pin cable (not included) can also be used to extend the length of the Screw Terminal Board.

dPatch Electrical		
Power consumption	18 Watts maximum	
Mains fuse	250V 1A Slow Blow (5 mm x 20 mm) T2.0	
Cables	Shielded grounded power line cord	
Line Voltage	100 VAC – 240 VAC	

Table E-8. dPatch Electrical

# dPatch System Components

Carefully remove all components from the shipping container. The following are included for a two-headstage system:

٠	(1) dPatch Amplifier	DP/E-2	
•	(2) dPatch Headstages	DP-HS	
•	(2) Polycarbonate Pipette Holders	EH-P170	(standard)
	or Quartz Pipette Holders	EH-Q170	(optional)
•	(1) Model Cell	MCELL	
•	(1) Power Cord		
•	(1) USB 3 Cable		
•	(1) Rack Mount Kit	RACK-PK	
•	(1) Screw Terminal Board		
٠	(1) Quick Start Guide	(with Igor P	ro 8 Serial #)
•	(1) USB Flash Drive	(with Sutter	Patch and Igor Pro 8 software)

# **Pipette Holder Parts**

- End Cap
- Silicone Gaskets

(O-rings, 6 ea.)

- Gasket IDColor1.1 mmClear1.2 mmGreen1.5 mmOrange-Red1.75 mmBlue
- Silver Wire
- Body/Barrel
- Wire Seal
- Gold Pin
- Pin Cap
- Lockdown Ring

(standard: polycarbonate; optional: quartz) (tubing)

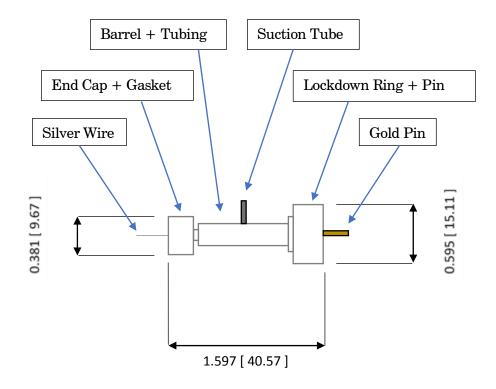


Figure E-1. Electrode Holder

# **Model Cell Parts**

- Model Cell
- Connector pins with crimp
- Ground wire

# dPatch ACCESSORIES

#### dPatch Expansion Panel

DPATCH-PCH

This optional rack-mountable panel provides BNC ports for easy access to all dPatch digital I/O channels and rear-panel auxiliary analog channels.



Figure E-2. dPatch Expansion Panel

dPatch Expansion Panel		
Dimensions (in)	18.8 x 2 x 3.5	
Dimensions (cm)	48 x 5 x 9	
Weight (lbs)	3.5	
Weight (kg)	1.6	
Digital Output BNCs	16	
Auxiliary Analog Output BNCs	2	
Auxiliary Analog Input BNCs	4	

Table E-9. dPatch Expansion Panel

This panel attaches to the dPatch rear panel via the included 50-pin D-sub cable for digital outputs and HDMI cable for analog I/O.

Digital channels with "high" outputs are indicated by a blue LED light.

#### **Ground Point**

This optional machined brass tower provides reliable low-resistance connections for electrophysiology setups The base plate mounts directly to air table tops (imperial and metric) with the included ¼-20 and M6 screws. The plated connectors accept up to 9 banana plugs and 8 bare wires (up to 10 gauge). A "star" ground configuration is used to avoid ground loops.

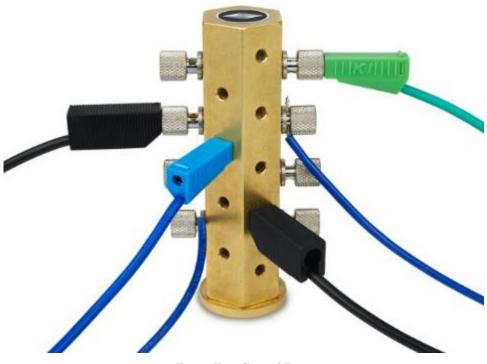


Figure E-3. Ground Point

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

# APPENDIX F. SUTTERPATCH ALGORITHMS

#### Auto Electrode Compensation Algorithm

[ for the dPatch Amplifier Control Panel ]

A train of 11 square pulses is applied with a cycle length of 2 ms each. Typically, the pulse amplitude is ±5 mV, i.e. 10 mV peak-to-peak. If saturation occurs, the routine reduces the pulse amplitude.

The resulting step responses are averaged, skipping the first transient and averaging the central 10 square cycles. Then the negative transients response is reversed in polarity and averaged with the positive transients response, resulting in an average composed of twenty transients.

The input is acquired with a 50 kHz filter bandwidth and a 500 kHz sampling rate.

The measured transient of the electrode capacitance starts to rise at about 22  $\mu$ s, measured with an apparent pipeline delay of 7 samples.

The transient terminates at about 40  $\mu$ s.

The transient is integrated from the 13th sample to the 21st sample, i.e. over 8 samples (sampling interval is 2  $\mu$ s per sample). The integration baseline is the mean of the samples after the transient.

The raw Integral is converted to capacitance by:

```
capacitance = (Integral * SampleInterval ) / ( PulseAmplitude * Cur-
rentGain )
```

where,

"PulseAmplitude" is the test pulse amplitude (typically 5 mV),

and

"CurrentGain" is the gain of the dPatch current input, 50 M $\Omega$  or 5 M $\Omega$ , as defined by feedback mode (or 50e6 V/A and 5e6 V/A, since an Ohm is defined as 1 V/A.)

The computed capacitance value is used as a correction value that is added to the electrode capacitance magnitude.

The measurement is repeated until the correction value gets too small (< 2% of electrode capacitance magnitude), or after 10 iterations.

The electrode capacitance tau is optimized by acquiring the same averaged transient while changing tau and measuring the RMS value, and using the tau giving the smallest RMS value. The first iteration starts at a tau value of 1  $\mu$ s, and increments by 2  $\mu$ s. Then it is refined, as the iteration cycle is repeated starting at the minimum tau minus 2  $\mu$ s and incrementing by 0.5  $\mu$ s, followed by an iteration incrementing by 0.1  $\mu$ s.

#### Auto Cell Compensation Algorithm

[ for the dPatch Amplifier Control Panel ]

A train of 11 square pulses is applied with a cycle length being 5 times the expected tau of the transient. The routine adjusts the pulse length, if the transient would be too short or too long. Typically, the pulse amplitude is  $\pm 5$  mV, i.e. 10 mV peak-to-peak. The routine reduces the pulse amplitude, if saturation would occur. See figure 3 for an overview.

The resulting step responses are averaged, skipping the first transient and averaging the central 10 square cycles. Then the negative transients response is reversed in polarity and averaged with the positive transients response, resulting in an average composed of twenty transients.

The input is acquired with a 10 kHz filter bandwidth and a 100 kHz sampling rate.

The measured transient of the whole cell capacitance starts to rise at about 60  $\mu$ s, measured with an apparent pipeline delay of 5 samples.

The time of half-decay is searched, starting at the first peak. The transients half-time must end in the first half of the stimulus pulse length. Otherwise, acquisition is repeated with an adjusted pulse length.

First estimates for Rs and Cm are computed:

Rs = exp(Xintercept)

Semi-logarithmic regression from the peak, over a time range of 4 * halfdecay time. The mean of the last 10% is used as the baseline.

Cm = Integral / dV

Integral from latency (i.e., pipeline samples plus stimulus-filter delay) over a time range of 4 * half-decay time.

These first estimates are iteratively improved by the method as described by Sigworth et al (1995), J Neurosci. Methods, 56:195-202.

Iterations are terminated when the improvements get less than 2%, or after 10 iterations.

Notes:

 $\circ~$  Estimating Rs by Rs = tau * dV / Qt (as used in the membrane test), underestimates Rs by about 20 %.

#### LockIn Computation

[ for Routine Editor Virtual Input Channels ]

Math used in the LockIn computation:

Factor = (2.0 / SinePointsPerCycle) / sine_amplitude^2

- $A = Factor * \sum (current * stim_real)$ \$\sum 0 over one SinePointsPerCycle
- $B = Factor * \sum (current * stim_imag)$  $\sum over one SinePointsPerCycle$
- $DC = 1/SinePointsPerCycle * \sum(current)$ \$\sum over one SinePointsPerCycle\$

Phase = $\frac{VC\text{-mode}}{\operatorname{atan}(B/A)}$	$= \frac{\text{CC-mode}}{\text{atan}(B/A)}$
Real $Y = A$	$= A / (A^{2} + B^{2})$
ImagY = B	$= B / (A^{2} + B^{2})$

Omega = (2 * pi) / SineCycleDuration

Gt = Idc / (Vdc - Et)

 $Gs = (A^2 + B^2 - A * Gt) / (A - Gt)$ 

Gm = Gt * Gs / (Gs - Gt)

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

## **Event Detection Reference**

[ for Event Detection Analysis ]

Deconvolution paper:

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

#### Single Channel Fitting

[ for Single Channel Analysis ]

Math used in single channel fitting:

Gaussian Fit  $y = y0 + A * exp(-((x - x0) / width)^2)$  y0 = offset A = height of curve's peak x0 = position of center of peak  $width = \sqrt{2} * \sigma$  $\sigma = standard deviation of the peak$ 

Linear Exponential Fit

y = y0 + A * exp(-(x - x0)) / tau

Logarithmic Exponential Fit

 $y = k0 + k1 * exp(-(ln(x / k2) / k3)^2)$