PatchXpress® 7000A Automated Parallel Patch-Clamp System

User Guide

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Molecular Devices

For research use only. Not for use in diagnostic procedures.

Product manufactured by Molecular Devices, LLC.
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Contents

1 Introduction

The PatchXpress® 7000A Automated Parallel Patch-Clamp Systemfrom Axon Instruments (now part of Molecular Devices) is a fully automated 16-channel parallel patch clamp system. The glass pipette of a conventional patch-clamp rig is replaced by an AVIVA Biosciences' *Seal*chip$_{16}$™ 16-channel planar electrode array. During an experiment, the PatchXpress 7000A system automatically positions a *Seal*chip$_{16}$ onto the recording headstages, transfers solutions, and cells to the *Seal*Chip$_{16}$ wells, forms a gigaohm seal, and perforates the cell membrane for high quality whole cell recordings. Dedicated wash probes extend into each well of the *Seal*Chip$_{16}$ for extracellular solution perfusion, and aspiration.

Typical cells used with the PatchXpress 7000A system are HEK, RBL, CHO, and other standard tissue culture cells expressing ion channels. The flexibility of the system allows users to run multiple stimulation protocols on the same cell, record during compound additions, or tailor procedures using the powerful system-control software, PatchXpress Commander Software. If a cell remains healthy after a procedure is complete, the procedure can be run again using a different test compound. Once an assay is developed, you can program the PatchXpress 7000A system to automatically process a batch of compounds, including replicate tests.

The 16-channel *Seal*chip$_{16}$™ planar electrode used with the PatchXpress 7000A system is supplied exclusively to Molecular Devices by AVIVA Biosciences Corp. Each well of the *Seal*chip$_{16}$ consists of a chamber with a tapered hole (~2 µm) traversing the base of the chamber to the underside. Cells in solution are added to each chamber, and sucked down towards the hole. One cell fits over the hole, and interacts with the specially treated surface to form a gigaohm seal. Increased suction then ruptures the patch of membrane covering the hole to gain electrical access to the cell. A tube protruding into the intracellular cavity from below serves as both the clamp electrode, and the conduit for suction. The wash station probe in solution above serves as the reference electrode to complete the electrical circuit across the cell membrane.

Figure 1-1 The PatchXpress 7000A system

Once cells are patched a robotic fluid handler transfers compounds from the compound plates to the *Seal*Chip$_{16}$ wells. Head stages for all 16 channels connect back (via a modified Digidata 1322A digitizer) to eight MultiClamp dual-channel patch-clamp amplifiers, delivering command signals, and recording the response in each chamber.

The PatchXpress 7000A system combines the best of automation, and heightened throughput without any loss of quality in the data it produces. Its architecture, and software allow you to configure complex experimental routines that can automatically test large numbers of compounds before any intervention is necessary. The high quality data produced by the PatchXpress 7000A system is automatically imported into the DataXpress database where you can readily analyze, view, and sort it.

With the PatchXpress 7000A system an exciting new era in ion channel drug discovery has begun—we wish you every success as you explore the possibilities that the PatchXpress 7000A system opens.

About this Guide

This guide documents technical features, and specifications of the PatchXpress 7000A system hardware, and software, with assistance also for equipment installation, and maintenance. It provides full instructions for the configuration, and running of experiments. If you are a new user of the PatchXpress 7000A system, we recommend that you take time to familiarize yourself with the instrument, and software using this document as a reference.

The User Guide is provided both as a printed manual, and in PDF format on the PatchXpress computer. The PDF file is accessed from within PatchXpress Commander Software, via the question mark tool button, and from the PatchXpress programs folder under the Start menu. As well as the table of contents, you can use the Find command in the Adobe Acrobat Reader to search the PDF version for words or phrases.

The PDF manual can be updated in PatchXpress Commander Software updates, and so may have revisions not present in the printed manual that you receive.

Here is a brief summary of the contents of this guide:

- Chapter 1 is a general introduction to the PatchXpress 7000A system, and describes the documentation that comes with it.
- Chapter 2: Getting Started on page 11, contains a quick checklist of the steps taken in a PatchXpress 7000A system experiment. Detailed instructions are provided in Chapter 5. A second section describes PatchXpress 7000A system setup if you ever need to relocate the machine.
- Chapter 3: System Components on page 13, describes the hardware components of the PatchXpress 7000A instrument.
- Chapter 4: PatchXpress Commander Software on page 31, describes the software loaded on the PatchXpress 7000A system computer, with emphasis on PatchXpress Commander Software, which controls the instrument itself. The chapter includes a section on the file types used, and produced by the program.
- Chapter 5: Setup to Acquisition on page 61, is a detailed progression through the steps involved in running an experiment. It begins with instructions for hardware setup, and consumables preparation, and then moves to general software setup, and experiment configuration, finishing with a description of the running of an experiment.
- Chapter 6: System Maintenance on page 125, has instructions for the care, and maintenance of the PatchXpress 7000A instrument, and describes how to access, and replace user-serviceable, and user-changeable components.
- Chapter 7: Reference on page 141, has a description of the lowpass filtering, and instructions for cell preparation.

- Chapter 8: Specifications on page 151, provides detailed specifications of the PatchXpress 7000A system.
- The Glossary on page 157 defines technical terms used in the manual.

Software Updates

Molecular Devices provides new bug-fix versions of PatchXpress Commander Software as required, so check regularly to see if a new version is available. Access the web download site from the Software Upgrades link in the Help menu (the book with a question mark on the cover) in the main toolbar in PatchXpress Commander Software.

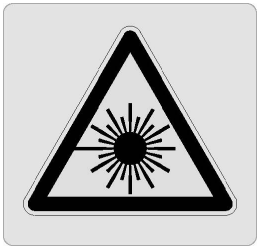
Activating Your Software License

PatchXpress Commander Software version 2.0 runs license-free for a thirty day trial period. When the trial period expires the software license needs activation to continue functioning.

To activate the software license:

1. Start the PatchXpress Commander Software application.
2. Click the **Settings** > **License Configuration**.
3. If you have Internet connectivity, type the provided **Product Key** in the field, and click **Activate Online**, and then follow the on-screen instructions.
4. If you do not have Internet connectivity, click **Activate Offline**, and follow the on-screen instructions. Activate Off line, requires the following:
   - Your product key
   - A separate computer with Internet connectivity
   - A USB drive for transferring files between the computers

Safety Warnings



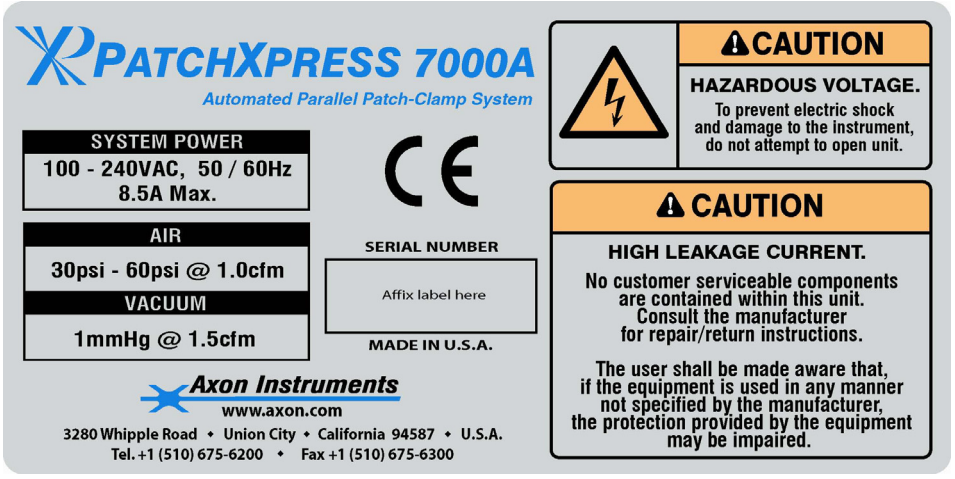
Caution: Laser Light

Label for Red Emergency Stop Button on front panel of cabinet. Push Red Emergency Stop Button to stop movement of robotic arm on liquid handler. Rotate Red Emergency Stop Button clockwise to release, and allow reactivation of robotic arm on liquid handler.

Caution: Risk of Electric Shock.

Label on the back instrument panel.

Label around the outside of the patch compartment.



Label on side next to power input to system.

Label on front of intracellular fluid injector (Filler Robot).

Getting Started

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The first section in this chapter is a quick checklist of steps taken to run an experiment. The second section covers PatchXpress installation, should you ever need to relocate the instrument.

Running an Experiment

Below is a quick checklist of steps taken to run an experiment. For detailed explanation of these steps, see Chapter 5: Setup to Acquisition on page 61.

1. Prepare cells, test compounds, and buffer solutions.
2. Start PatchXpress 7000A hardware, computer, vacuum pump, and air pressure unit.
3. Load intracellular solution, and extracellular buffer solution.
4. Load tips.
5. Open PatchXpress Commander Software.
6. Run start-of-day initialization.
7. Select screening or assay development mode.
8. Configure the Experiment dialog, including:
   a. Identify, and load compound plates
   b. Select a procedure
   c. Select patch settings
9. Press **Start Experiment** button.
10. Load Sealchip16 electrode when prompted.
11. Load cells when prompted.

Basic Installation

A Molecular Devices field service engineer installs each PatchXpress® 7000A Automated Parallel Patch-Clamp System on site, however the following information is provided in case you need to relocate the instrument.

Position

The PatchXpress 7000A instrument should be located in a laboratory in a position with adequate free floor space. The dimensions are 1.473 m

(58”) wide by 0.762 m (30”) deep by 1.676 m (66”) high, plus the 61 cm (24”) monitor, and keyboard connected to the left side.

The PatchXpress 7000A instrument generates a fair amount of heat so should be placed in a reasonably large room, ideally with temperature control.

Before positioning the PatchXpress 7000A instrument, ask the following questions:

- Is the tissue culture facility nearby?
- Are there unusual sources of vibration or noise in the room?
- Is there sufficient room around the machine to operate?

Connect the PatchXpress 7000A instrument directly to a 110 V or 240 V power source (see Chapter 8: Specifications on page 151, for details), optionally via a surge protector. The power cord is connected on the left side of the instrument, secured with a bracket.

Connecting Vacuum and Air Pressure Lines

- Connect the thick-walled rubber tubing to the vacuum pump provided by Molecular Devices. The vacuum pump should reside on the floor on the left side of the PatchXpress 7000A instrument, connected directly to a power source. Turn the pump on, and off independently from the instrument. A manual for the vacuum pump is included; please consult this for operation details.
- Connect the red tubing to the air compressor provided by Molecular Devices. The compressor should reside on the floor on the left or right of the PatchXpress 7000A instrument, connected directly to a power source. Turn the compressor on, and off independently from the PatchXpress 7000A instrument. A manual for the air compressor is included; please consult this for operation details.
- If house air is available, and preferred, connect it to the yellow tubing that comes from the power panel on the left side of the PatchXpress 7000A instrument. A ¼” adapter might be necessary to connect the air line to the house air source. Be sure that no leaks occur, as this will affect the performance of the PatchXpress 7000A instrument.

System Components

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This chapter describes the hardware components of the PatchXpress® 7000A Automated Parallel Patch-Clamp System. Description of the PatchXpress Commander Software is given in Chapter 4: PatchXpress Commander Software on page 31, while detailed specifications are provided in Chapter 8: Specifications on page 151.

Overview

Most components of the PatchXpress 7000A instrument are contained in a metal cabinet with four front-opening doors and removable side and rear panels. The top two Plexiglas doors open the main workbench of the unit. Attached on the outside left of the cabinet an adjustable arm supports the computer monitor and keyboard. A barcode reader is attached to the arm. All components within the cabinet, including the system computer, use one power source.

In addition to the cabinet and arm, a separate vacuum pump and air compressor are provided with all PatchXpress 7000A instruments. The vacuum pump and air compressor use independent power supplies. They should be located close to the left or right side of the cabinet.

Cabinet

The PatchXpress 7000A instruments metal cabinet stands 1.473 m (58”) wide by 0.762 m (30”) deep by 1.676 m (66”) high. A 61 cm (24”) computer monitor and keyboard are attached to the side of the cabinet on an adjustable arm.

The top half of the cabinet contains the main workbench, enclosed behind Plexiglas doors. Sensors on the doors stop all motion inside the cabinet when the doors are open.

The entire left-hand end of the workbench consists of a platform with positions for compound well plates, tip trays and cell vials. At the back right-hand corner of this rack is a holder for loading *Seal*chip$_{16}$ electrodes. The rear wall of the bench area supports two robotic arms—on the left the fluid handler that brings cell-bearing solution and well plate compounds to *Seal*chip$_{16}$ electrodes during an experiment, and on the right the gripper that picks up and loads electrodes. Two syringe pumps on the rear wall control fluid delivery in the fluid handler and in the intracellular solution filling station respectively.

To the right of the syringe pumps a rack holds the intracellular solution vial.

A number of subunits used in electrode preparation and fluid delivery are positioned about the recording station located in the center at the right-hand end of the bench. Behind the recording station is an electrode rack for automatic replacement of electrodes (this feature is not implemented in the first version of the PatchXpress instrument). Along the right-hand end of the bench, from back to front, are located the blank *Seal*chip$_{16}$ electrode holder, the *Seal*chip$_{16}$ electrode drying station, intracellular solution filling station, loading clamp, and *Seal*chip$_{16}$ electrode waste chute. A flipper arm moves electrodes between the loading clamp and recording station, and within this the wash station is located. Near the center of the bench at the front is a waste chute for used tips.

Two lights are located at the top right and left sides of the upper cabinet. The light switches are in the middle at the top of the cabinet, behind the point where the upper panel doors come together. The lights can contribute heat to the cabinet and can be turned off at the user's discretion.

Underneath the bench are two front-opening compartments. The left-hand compartment houses eight MultiClamp amplifiers, a 16-channel digitizer, and the system computer. The right-hand compartment has fluid containers for water, extracellular solution and waste, as well as the master controller for pressure and fluidics control to the electrode chambers. Fixed at the top of the compartment directly beneath the recording station a sealed unit holds the amplifier headstages.

On the rear of the cabinet two removable panels give access to the back panels of the MultiClamps, digitizer and computer, as well as to fluidics and pressure control components and circuits.

Emergency Stop Button

Located below the right-hand glass door on the front of the main cabinet is an emergency stop button. This cuts 24 V power to the system, stopping most of the motors immediately. Some of the pumps and valves are not stopped by the button, but these components are not accessible to the user and so do not present any danger. There is no easy recovery from pressing the stop button as moving components lose their positions, and so the experiment is aborted. Turn the button clockwise to reset after it has been depressed.

Sealchip16 Planar Electrode

The disposable *Seal*chip$_{16}$ planar electrode is the interface between the cell and the PatchXpress 7000A instrument electronics and fluidics. Each electrode has 16 chambers, for 16 cells to be patch clamped.

*Seal*chip$_{16}$ planar electrodes are manufactured for Molecular Devices under sole license by AVIVA Biosciences Corp., San Diego, CA, USA. AVIVA's expertise in microfabrication techniques is used in the creation of the minute electrode holes and special surface treatment needed to ensure high success rates for strong, reliable cell sealing. *Seal*chip$_{16}$ electrodes are available to PatchXpress 7000A system customers from Molecular Devices.

Each *Seal*chip$_{16}$ chamber has a volume of ~80 µL, however with displacement by the wash station nozzle and disposable tip (Figure 3-2 on page 16), 50 µL is the effective maximum volume, and compound additions without suction are limited to 45 µL. With the wash station height in its default position approximately 5 µL of fluid remains at the bottom of the chamber after aspiration. The chambers have a crescent shaped ledge close to the bottom to optimize fluid circulation and replacement during overhead compound addition and washouts (Figure 3-3 on page 17).

A 1 µm to 2 µm diameter hole extends from the bottom of each chamber through to the base of the electrode. When an electrode is loaded for an experiment each hole is aligned with a cavity that is subsequently filled with intracellular solution, in the flipper arm gasket. When the flipper arm moves over onto the recording clamp the solution-filled cavities fit over the Ag/AgCl electrode tubes on the electrode base plate. These tubes are also used for pressure control. Once the assembly is in place over the electrode tube plate, the wash station heads are lowered into the chambers of the *Seal*Chip$_{16}$ and used to introduce extracellular buffer into each chamber. After this priming step, cell-containing solution is added to the chambers (Figure 3-2 on page 16) and a cell is sucked down onto the hole, sealed there tightly then ruptured. This gives the electrode suction tubes below, via the intracellular solution in the flipper gasket cavities, access to the interior of the cell. The wash station nozzles, immersed in extracellular solution on the upper side of the *Seal*chip$_{16}$ electrode, complete the circuit across the sealed cell's membrane, allowing delivery of stimulus commands and electrical recording.

The chambers in a *Seal*chip$_{16}$ electrode number from 1 at the end nearest the back of the machine to 16 at the front.

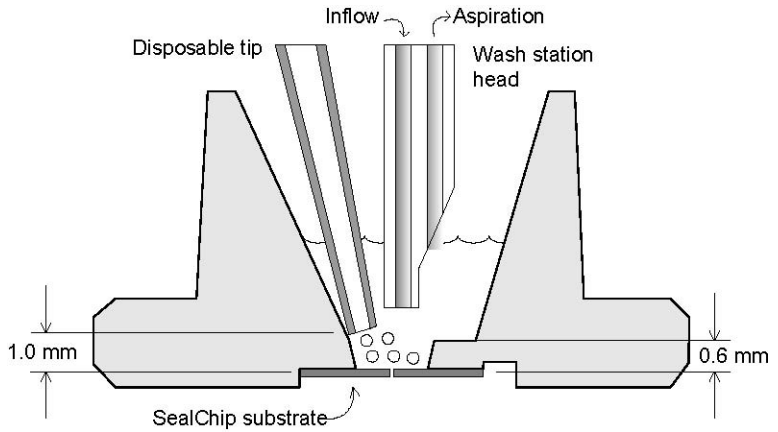


Figure 3-1 Cross-section of Sealchip$_{16}$ electrode chamber with wash station and disposable tip in position for cell addition.

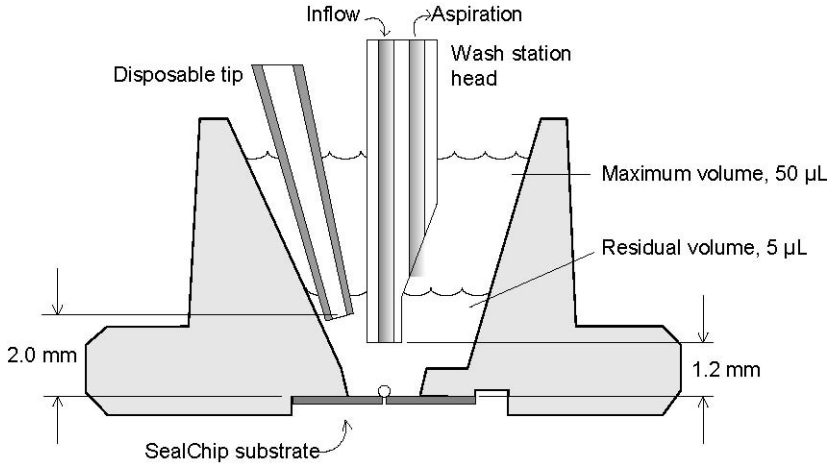


Figure 3-2 Cross-section of Sealchip$_{16}$ electrode chamber with wash station and disposable tip in position for compound addition.

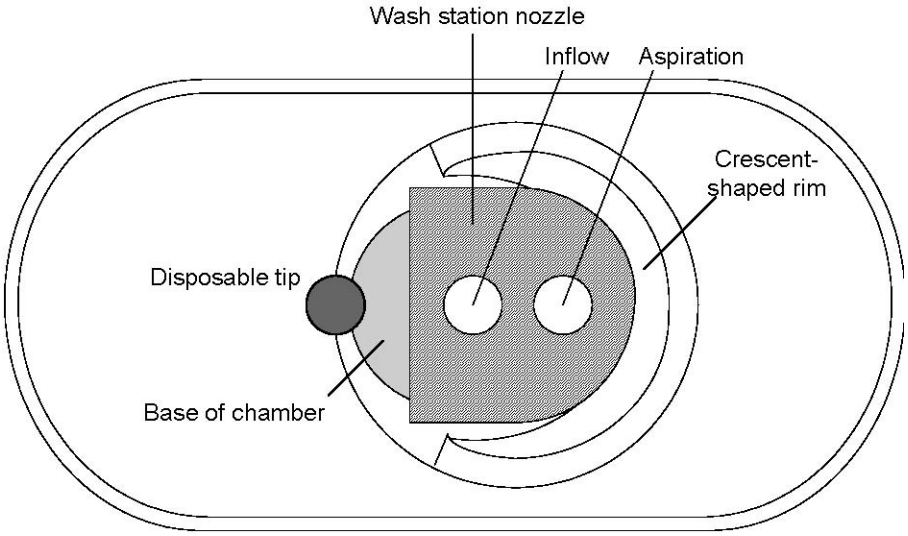


Figure 3-3 Top view of Sealchip$_{16}$ electrode chamber showing wash station and cell or compound-dispensing tip.

Packaging and Handling

*Seal*chip$_{16}$ electrodes are supplied one each in solution-filled glass containers. The electrodes can be safely removed from the containers for brief periods, but should never be out of the packaging solution long enough to dry out. Once dry they must be used immediately or rehydrated in ultra-pure deionized water. If dried for long they should be discarded.

It is safe to handle the *Seal*chip$_{16}$ electrodes by gripping them at either end or along their sides. Use the tweezers supplied for this purpose in the accessories pack. Electrodes should not be touched or set down on their bases as any impurities on them could impair the seal when they are loaded in the recording station, contaminate the intracellular solution, or lead to cross-talk between channels. To prevent contamination and damage to the surface treatment on the chamber bottom, never put anything into the *Seal*chip$_{16}$ electrode chamber.

When an electrode is removed from its container it should be shaken to remove large water droplets before placing on the loading mount. This is important to reduce the chance that the electrode may not be entirely dried, which could then lead to cross-talk when the experiment is run.

Each glass container has a barcode with the electrode lot number. A barcode reader is provided to facilitate the entry of this data at the start of each experiment, for each *Seal*chip$_{16}$ electrode used. The barcode number becomes a queryable attribute in DataXpress.

Barcode numbers are of the form '051028001-59008', for example, date (YYMMDD), batch number for that day (001), and the part number (59008).

*Seal*chip$_{16}$ Electrode Holders

Located near the back center of the main PatchXpress 7000A instrument bench above the cell vial rack is a raised holder to place the single *Seal*chip$_{16}$ electrode to be used in an experiment. Always wait until the prompt tells you to place an electrode on this holder. This ensures that the electrode stays out of its storage solution and exposed to the air for the minimum time possible.

Using the tweezers provided, place the electrode in the holder upside down (chambers facing downwards), with the beveled end pointing towards you. Remember to shake excess solution from the electrode before placement.

The electrode rack at the back of the bench will be used for automatic electrode loading in a later version of the PatchXpress instrument.

Blank Cartridge

A second single *Seal*chip$_{16}$ electrode holder is located on the far back-right of the bench, beside the drying station. Use this for the *Seal*chip$_{16}$ 'blank cartridge', used in automatic start-of-day initialization and end-of-day shutdown routines. These cartridges are black, for easy recognition.

Ensure you have a blank cartridge in position in its holder whenever you run an experiment. In normal operation the same cartridge can be kept in position in the holder all the time. Like the front electrode holder, place the blank cartridge upside down, with the beveled end towards you.

Sponge

The blank electrode holder has a lid with a sponge on its bottom surface. The gripper shifts this over the electrodes in the recording station during 'blow out electrode tubes' actions, to absorb any fluid expelled.

When the blank cartridge is to be used, the gripper moves the lid/sponge to the loading position for normal *Seal*chip$_{16}$ electrodes, at the middle back of the main bench.

Recording Station

The *Seal*chip$_{16}$ electrode is held in the recording station during an experiment. As such, the recording station is the point towards which all the pressure, fluidics, and electrical systems in the PatchXpress 7000A instrument are directed.

The recording station consists of the electrode base plate, a clamp, and the flipper arm, which serves as an extension of the *Seal*chip$_{16}$ electrode when an experiment takes place, as well as a device for loading and removing *Seal*chip$_{16}$ electrodes.

Electrode Base Plate

The base plate is a rectangular aluminum block with 16 Ag/AgCl tubes protruding from it—in line, to match the placement of chambers in the *Seal*chip$_{16}$ electrode. The tubes serve both as electrodes, playing the role of the pipette electrode in a conventional patch clamp, and as tubes to convey the pressure commands used to seal, rupture and hold cells.

A temperature sensor is attached to the base plate. Temperatures measured here are generally within 1º C to 2º C of the temperature of the cells in the electrode chambers. The temperature is recorded at the start of each cell procedure and passed to DataXpress, where it is a queryable attribute. Apart from this, you can view the temperature in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog, in PatchXpress Commander Software.

The electrode base plate must be kept clean at all times. If solution spills on to it, wipe it clean with a lint-free cloth wetted with deionized water to prevent accumulation of salts, then completely dry the tubes before proceeding with the next experiment.

With normal use it is inevitable that the sealing surface of the base plate will wear and be affected by contaminants that cannot be fully removed. Deposits may also occur on the electrode tubes. For these reasons, the base plate should be replaced every three months. See Chapter 6: System Maintenance on page 125, for directions.

Flipper Arm

The flipper arm picks up new, dried *Seal*chip$_{16}$ electrodes from the loading clamp and flips them over into position on the recording station. After an electrode has been used the flipper arm returns it to the loading clamp, from where it is pushed to the disposal chute.

Besides transporting *Seal*chip$_{16}$ electrodes, the flipper arm contains the intracellular compartment gasket (‘gasket’). This component serves as an integral part of the planar electrode when the flipper is locked into position in the recording station.

The gasket contains 16 small cavities, open to both sides of the gasket. When a fresh *Seal*chip$_{16}$ electrode slides into position in the flipper arm the chamber holes on the base of the electrode line up with the openings on one side of the gasket. The electrode is clamped securely against the gasket in the flipper arm. The cavities in the gasket thus form an extension to the base holes in the *Seal*chip$_{16}$ electrode, albeit broader than the holes in the electrode, to provide a reservoir for intracellular solution. The cavities hold 15 µL of solution each.

When the flipper arm flips over to the recording station, the openings on the other side of the gasket fit tightly over the electrode suction tubes in the electrode base plate. In this position each intracellular cavity is securely enclosed, with access only via the electrode suction tube—transmitting pressure and electrical commands—and the hole leading through to the patched cell in the *Seal*chip$_{16}$ electrode chamber.

Both facing surfaces of the gasket have silicon coatings to ensure a tight seal with the electrode and the base plate. It is essential to keep these surfaces clean. Use a lint-free cloth dipped in deionized water to wipe if necessary and then dry thoroughly. The gasket should be replaced monthly to ensure tight seals, contamination-free cavities, and to avoid cross-talk between electrode channels. See Chapter 6: System Maintenance on page 125 for instructions.

Wash Station

Placed between the arms of the flipper arm, the wash station has 16 twin-tube fluid-delivery and aspiration heads that lower into the electrode chambers when the *Seal*chip$_{16}$ electrode is in the recording station. The unit is fixed so that all tube heads are always in the same position in each chamber. The longer of the two tubes in each head delivers extracellular buffer solution to the wells, while the shorter removes fluid during washout steps and, if configured, during compound addition steps.

Attached to each wash station head an Ag/AgCl pellet serves as the ground electrode so that when lowered into fluid in the electrode chambers the heads complete the electrical circuit across the cell membrane.

The wash station has three positions—fully retracted, and two lowered positions. When an electrode is first loaded the wash station lowers enough to add buffer solution to the wells. In this position the heads are low enough to be in contact with the solution—allowing the membrane test to be run—but not so low as to inhibit cell access to the electrode holes. Following a user-defined period after cell addition (set in the **Patch Settings** dialog) the wash station lowers further into the wells bringing the tube tips quite close to the well bottom and the patched cell (1.2 mm from the chamber bottom by default, but this position can be varied between 0.1 mm and 2 mm in the **Settings** dialog).

Gripper

Mounted on the rear wall of the cabinet, the electrode gripper is a robotic arm that takes a *Seal*chip$_{16}$ electrode from the back-center electrode holder and places it in position on the bolt at the rear right of the bench. The bolt takes the electrode along the right-hand end of the

bench through the drying station to the loading clamp, and after an experiment to the electrode waste chute.

During start-of-day initialization and end-of-day shutdown, the gripper takes the blank cartridge from its holder near the drying station and places it on the same path leading to the drying station. At the end of each initialization or shutdown, the gripper returns the blank cartridge back to its holder location.

Drying Station

The drying station blow-dries each *Seal*chip$_{16}$ electrode in preparation for use in an experiment. The electrode, positioned on the bolt, moves slowly through the drying station where it is exposed to a series of air diffusers. After drying the bolt moves the electrode to the loading clamp.

Filling Station

The filling station consists of a small robotic arm with a thin vertical delivery tube. The tube is connected, via a three-way valve, to intracellular solution, water, and vacuum sources. After a *Seal*chip$_{16}$ electrode has been dried it moves to the loading clamp where it is gripped in the flipper arm and pressed tightly against the flipper gasket. The delivery tube injects intracellular solution into the flipper gasket cavities now directly over the underside of each electrode chamber. When the electrode has been used and the flipper returned to the loading clamp with the spent electrode, the arm removes intracellular solution from the gasket cavities. It then injects water into each of the cavities to rinse them, and then again removes the water. If another electrode is to be loaded, the process begins again with fresh intracellular solution.

Intracellular solution for the filling station is loaded in the vial on the back wall of the cabinet, usually a 50 mL plastic tube. Solution from this source passes through a degasser before it is injected into the gasket cavities, to avoid formation of bubbles in the small cavities of the gasket.

The right-hand syringe pump on the rear wall of the cabinet controls fluid delivery in the filling station.

Fluid Handler

A single-head robotic fluid handler operates over the left-hand side of the main bench, picking up disposable plastic tips to bring cells and compounds to the *Seal*chip$_{16}$ chambers. Used tips are dropped into the tip waste bin after each delivery.

The left-hand syringe pump on the rear wall of the PatchXpress 7000A instrument controls uptake and delivery of the fluids transported in the arm, allowing precise control of the volumes and flow rates of the solutions dispensed. The default delivery rate for compound addition is 100 µL/s, while volumes are configured independently for each add compound step of the procedure. The delivery rate can be adjusted in the **Settings** dialog **Add Compound** tab. Command steps in the procedure can be configured to change this value during an experiment, in which case the delivery rate changes at the appropriate point in the procedure independently in each chamber.

The teflon tube in the fluid handler is filled with deionized water, with a 115 µL air gap at the tip end. Sometimes during fluid delivery a small air bubble can form at the tip of the nozzle, inside the disposable tip. The bubble may burst as fluid is forced from the tip. This generally only happens after most of the compound has been dispensed from the tip. With most of the water spraying onto the walls of the tip (and this of insufficient quantity to run down into the compound) only a very small quantity is ever likely to enter the compound, diluting it negligibly.

As well as conveying cells and compounds to the *Seal*chip$_{16}$, the fluid handler uses cycles of 'suck and spit' to mix solutions—routinely in the case of cells to resuspend them before they are taken up into the tip, and, if enabled, to mix compound during pickup from the compound well plates and/or after deposition in the *Seal*chip$_{16}$ chamber. Mixing of cell-bearing solution is configured in the **Patch Settings** dialog (open from the **Define Experiment** dialog), and compound mixing in the **Settings** dialog (main toolbar).

Once the arm has delivered cell-containing solution to the all chambers of a new *Seal*chip$_{16}$ electrode and cell procedures begin, the fluid handler (under default settings) adds compound to the chambers on a 'first come, first served' basis. There is always at least a 10 to 11 second wait between an add compound procedure step calling the arm—while the arm picks up a tip and the compound—but with many cell procedures running at once cells often have to wait longer than this.

The arm can be locked to each chamber for a sequence of compound addition steps, minimizing the wait between these. This means, however, that cells (after the first one to patch) typically have to wait longer until the arm comes to them. Configure this behavior with procedure command step 'Lock the compound robot' (Command on page 88).

The fluid handler arm is also locked to each chamber for 'multi dose' compound addition steps. These dispense compound two or more times for each add compound step that they are configured for. After the first delivery the fluid handler picks up a new tip (or not, as configured), picks up the same volume of compound again from the same well, and returns to the chamber for the second and following deliveries. A specific delay between multidose additions can be configured, with a

minimum of 11 seconds if there is no compound mixing. This minimum setting does not change if the tip is reused.

The name and number of arms of the pipetting system are reported in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog in PatchXpress Commander Software.

Tip, Well Plate and Cell Vial Rack

The entire left-hand end of the workbench is occupied by racks for disposable plastic tips, compound well plates, and cell vials.

Disposable Tips

The back section of the rack holds eight 96-tip containers, for 50 µL plastic disposable tips. Tip container locations are numbered as shown in Figure 3-4. At the start of an experiment tips are always taken first from the container in position one, before moving on to other containers in their numbered order. Within a container tips are taken starting from the rear-left corner, moving one row at a time left to right until the last, front-right tip is used.

Standard pipetting tips are required for use with the PatchXpress 7000A instrument. Molecular Devices recommends:

- BioRobotix pipetting tips from Molecular BioProducts (Pure 50 µL, presterilized, CAT # 902-261). Nonsterilized tips from the same source (CAT # 902-262) can also be used.

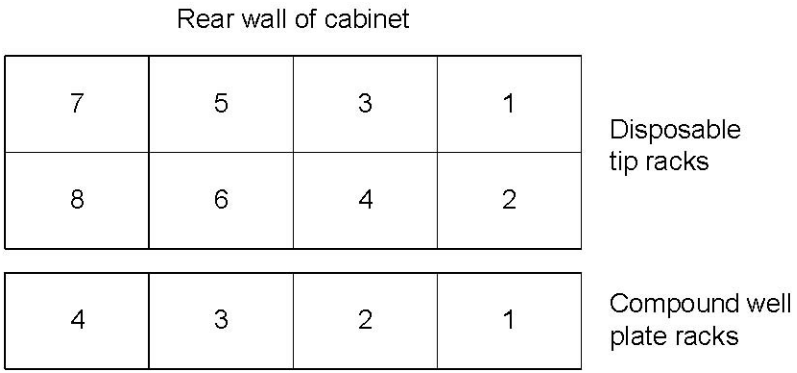


Figure 3-4 Positions of disposable tip and compound well plate racks, showing order of use.

If the arm goes to pick up a tip at an empty location it will try the next two locations in the tray. If a tip is not found in these it goes to the next tray and attempts the first three positions there. If no tips have been located after three trays have been tested this way, the experiment pauses.

When a tip container has been emptied the tip icon in PatchXpress Commander Software starts to flash yellow. It continues to do this until you indicate to the software—in the **tip** dialog opened from the icon—that you have replaced the empty trays. Always replace either all the completely empty tip trays only, or all of these and the trays with some tips used. You must then indicate which option you have taken in the dialog. If partially used trays are kept the software keeps track of where it was up to in these and goes immediately to the next tip when it has worked back to the half-used tray.

It is possible to replace empty tip trays during an experiment, by pausing the experiment. There can be a small time advantage in doing this, by keeping tip pick-up locations close to the recording station.

Well Plates

The rack has positions for four compound well plates, numbered from right to left. Compounds are taken from these as they are called during cell procedures, so wells in the different plate locations might be accessed at any time during an experiment.

Compound plates should be placed with well A1 in the rear left position. See Add Compound on page 84, for a description of how well locations are specified during experiments, and consequently how compounds should be arranged in well plates.

Standard 6-well, 24-well, 96-well, and 384-well compound plates can be used.

Cell Vials

Located in the right side of the tip and compound plate positions are multiple cell vial positions. Cells are always picked up from position 1, nearest to the front of the rack.

Cells should be placed into the vial rack in a 1.5 mL plastic Eppendorff-style tube (the same type used during installation to calibrate the system).

Pneumatics and Fluidics Control System

The systems for controlling pneumatics and fluidics in the recording station are located in the bottom right compartment of the cabinet. These systems provide suction for cell patching, and fluid delivery and removal to and from electrode chambers via the wash station.

Two main pressure vessels, one each for positive and negative pressure, are attached to the right hand wall of the compartment. These supply vacuum and pressurized air to another set of regulators located inside the master controller at the rear of the compartment. A HEPA filter inside the positive pressure vessel removes small particles that can harm the pneumatics control or lodge within the *Seal*chip$_{16}$

holes. On the front, a double-filter for oil vapor and odor conditions air coming into the system. Oil vapor can affect sealing on the *Seal*chip$_{16}$ electrodes.

Pressure controllers in the master controller precisely control pressure to the intracellular chambers, in the flipper gasket, under each *Seal*chip$_{16}$ chamber, via the electrode suction tubes in the electrode base plate. At the front of the master controller 16 peristaltic pumps control delivery of extracellular solution through the wash station, to each extracellular chamber. Similarly, 16 valves in the unit control aspiration from each chamber. When the valves are open, aspirated fluid is deposited to a small 'local' waste container. A peristaltic pump at the front of the master controller removes fluid from here into the main waste container, held at room pressure.

Pressures in various parts of the system are reported in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog, in the PatchXpress Commander Software. The same dialog reports model and serial numbers, and firmware version, of the master controller.

Fluid Containers

The bottom right compartment of the PatchXpress cabinet has four fluid containers:

- 1 L container for extracellular solution.
- 2 L container for deionized nanopore-filtered water, for the fluid handler hydraulics, and flushing intracellular gasket cavities and the wash station.
- 250 mL Pyrex local container (pressurized) for waste fluid—waste fluid passes into this before being pumped into the main waste container.
- 5 L container for waste fluid.

All four containers have sensors located at the top or bottom to indicate when levels are low, or in the case of the waste containers, high. PatchXpress Commander Software issues a warning message when one of these levels is reached. Levels can also be checked in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog.

If levels become too high or low during an experiment you can pause the experiment to replace or empty the container. If, at the start of an experiment, the waste bottle is sensed as full or the water or extracellular solution bottles sensed at near empty, the experiment will not start.

Waste Bins

The PatchXpress 7000A instrument has two waste bins, both located just under the main bench at the front center and front right-hand end. The center bin is for used plastic tips and the right-hand bin takes used *Seal*chip$_{16}$ electrodes. Both bins should be lined with biohazard bags for easy and safe removal of these waste products, which could have hazardous compounds adhering to them. Waste bags should be checked at the end of each day's operation as part of normal system maintenance.

MultiClamps

The PatchXpress 7000A instrument uses eight dual-channel Axon Instruments MultiClamp amplifiers to deliver command waveforms and record current from the 16 *Seal*chip$_{16}$ chambers. The MultiClamps are located in the bottom left-hand compartment of the cabinet.

Command waveforms defined in the protocols that you incorporate within procedures in PatchXpress Commander Software pass into each MultiClamp channel, via the digitizer, at the COMMAND BNC for the channel. The amplifier then passes this to the channel's CV-7A headstage, under the recording station, feeding the command to the *Seal*chip$_{16}$ electrode chamber that it serves. The response returns via the headstage to the amplifier. The SCALED OUTPUT gain is left at unity and the headstages are typically operated in the 500 MΩ feedback resistor position for a full-scale range of –20 nA to +20 nA nA in Voltage Clamp mode and -10000 mV to 10000 mV in Current Clamp mode. The scaled output filter is set at or near 6 kHz Bessel. It is used to provide signal filtering at minimum bandwidth and to provide antialiasing filtering. When lower bandwidths are required these are implemented in a cascaded, lowpass, 8-pole software Bessel filter (see Lowpass Filtering on page 141.)

Electrode and whole cell capacitance compensation, as well as electrical access resistance (Ra) correction, are also carried out in the amplifier if these have been enabled in the **Procedure** dialog in the software. See Whole-Cell Compensation on page 94, and Electrical Ra Correction on page 95.

In general, amplifier settings made in PatchXpress Commander Software apply to all the MultiClamps together, for example, the holding potential (set in the **Patch Settings** dialog) and whole-cell compensation and electrical Ra correction (enabled on the **Initial Settings** tab of the **Procedure** dialog) set values that apply to all 16 channels together. However, command steps that change these settings for individual channels can be included in the procedure. The same set of procedure steps must be applied to all channels, but because the procedure is applied to each successfully patched cell independently, these command steps can change amplifier settings asynchronously.

The PatchXpress Commander Software sets the MultiClamp RAW OUTPUT, SCOPE OUTPUT and SCALED OUTPUT BNCs to different signals, depending on the amplifier mode. You can connect an independent oscilloscope or digitizer to one of these if you want to monitor a particular channel. In Voltage Clamp mode sets the RAW OUTPUT BNCs to Vp, the pipette voltage measured at the top of the headstage. You can connect an independent oscilloscope to one of these if you want to monitor this parameter for a particular channel. The SCOPE OUTPUT BNCs carry Im, the same signal as the SCALED OUTPUT.

In Current Clamp mode, RAW OUTPUT BNCs carry Ip, the pipette current measured at the top of the headstage. The SCOPE OUTPUT BNCs carry Vm, the same signal as the SCALED OUTPUT.

MultiClamp and DSP (digital signal processor) firmware version numbers are reported in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog in PatchXpress Commander Software.

Digitizer

One modified Axon Instruments Digidata 1322A digitizer performs all digital-to-analog and analog-to-digital conversion for the 16 channels of the PatchXpress 7000A instrument. The digitizer is located in the bottom left-hand cabinet, in the middle of the MultiClamp amplifiers. The digitizer converts both input and output signals, on all 16 channels, at 31.25 kHz (32 µs per sample), for each channel. When lower sampling rates are specified in the acquisition protocol, these are achieved by software decimation, after applying the software filter.

The digitizer range is –10 V to +10 V. With the scaling factors used in the PatchXpress 7000A instrument, this gives an output command range of –200 mV to +200 mV, and an input range of –20 nA to +20 nA.

The PatchXpress 7000A instrument Digidata differs from standard units of this model by having 16 output channels rather than the usual two.

Serial number and product and firmware version numbers for the Digidata are reported in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog in PatchXpress Commander Software.

Computer

The main case of the PatchXpress 7000A instrument computer is mounted inside the bottom left compartment of the main cabinet, to the left of the MultiClamp and digitizer stack. The keyboard and 61 cm (24”) flat panel LCD monitor are mounted on the adjustable Ergotron arm attached to the outside of the main cabinet.

Computer specifications may vary to reflect the latest standards. Dual-processor computers are used, with a minimum 1 GB RAM. The computer comes with an on-site warranty from the supplier. Basic specifications for the computer are reported in the **Hardware Diagnostics** dialog, opened from the **Hardware and Fluidics** dialog in PatchXpress Commander Software.

See Chapter 4: PatchXpress Commander Software on page 31, for information about PatchXpress Commander Software and other programs loaded on the computer.

Barcode Reader

A hand-held barcode reader for reading *Seal*chip16 electrode lot numbers and compound well plate barcodes is attached to the adjustable arm. The reader copies the barcode number that is read to the cursor position in any editable text field in the software. During experiment setup, use the barcode reader to enter well plate barcodes in the **Compound Plates** dialog, opened from the **Define Experiment** dialog. Having enabled a well plate location by checking the appropriate tab in the dialog, click in the **Plate ID** field. Aim the reader at the barcode and press the trigger to insert the barcode number in the field. Provided that the compound file for that plate has been saved to the appropriate folder, the well plate contents are displayed in the dialog. Similarly enter *Seal*chip16 electrode lot numbers from the *Seal*chip16 vials when prompted during experiments. For full instructions on the use of compound plate files, see the Compound Plates on page 63.

Accessories

The PatchXpress 7000A instrument comes with an accessories kit comprised of a model cell, some spare parts, and a number of specialized tools.

MC-PX Model Cell

See Model Cell on page 127 for circuit diagrams and instructions for use.

- Channels 1–15 mimic the whole-cell configuration (Ra = 5 MΩ; Rm = 500 MΩ and Cm = 15 pF).
- Channel 16 has two configurations: bath setting (Re, Rseal = 2 MΩ, 20 MΩ, 100 MΩ, 500 MΩ, 1200 MΩ, or 1700 MΩ) and membrane setting
(fixed Cm = 15 pF and Rm = 500 MΩ) and variable resistance values (Ra = 3.3 MΩ, 5 MΩ, 10 MΩ, 15 MΩ or 50 MΩ).

Spare Parts

- intracellular gasket × 5
- electrode base plate × 1
- ¼" tubing for air line × 5'
- tubing for waste container
- sponge x 1
- blank electrode cartridges × 2
- biohazard waste bags

Tool Kit

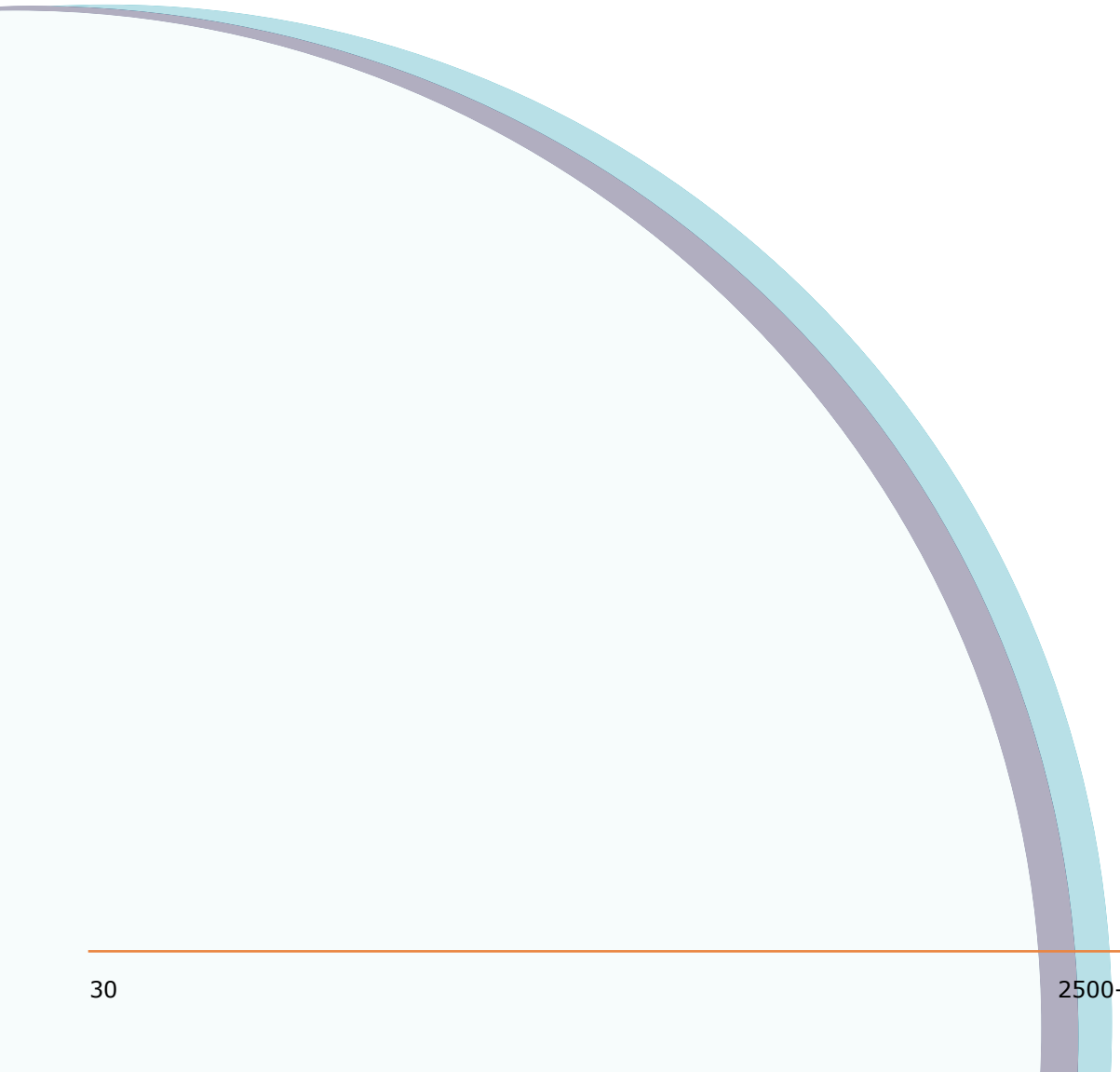
- intracellular gasket removal
  - small flat-head screwdriver
  - gripper tool
- electrode base plate removal
  - extracting tool
  - Allen screwdrivers
- tweezers (for removing *Seal*chip$_{16}$ electrodes from containers)
- toolbox

Vacuum Pump

A separate vacuum pump is provided with the PatchXpress 7000A instrument. By having a vacuum pump dedicated to the PatchXpress 7000A instrument alone you are better able to ensure a stable vacuum source, as opposed to house vacuum systems that are more prone to fluctuate. The PatchXpress 7000A instrument should be used with the pump provided, and not with house vacuum.

Air Compressor

An air compressing unit is provided with the PatchXpress system. Like the vacuum pump, the air compressor stands outside the main PatchXpress 7000A instrument cabinet.



PatchXpress Commander Software

4

This chapter provides an overview, warnings, and general advice with respect to the programs loaded on the PatchXpress 7000A system computer. The greater part of the chapter, in the third section, is dedicated to a description of the PatchXpress Commander Software program, which controls the instrument. This focuses on display, and other software options not directly related to hardware control or the running of an experiment. Details of how PatchXpress Commander Software is used to set up, and control an experiment are included in Chapter 5: Setup to Acquisition on page 61.

The fourth section of the chapter describes the different file types used, and produced by PatchXpress Commander Software.

General Information and Warnings

The PatchXpress 7000A system computer comes preloaded with all the software you should need on the computer. The computer is an integral part of the PatchXpress 7000A instrument, and as such, should be used only for controlling the instrument, and other matters immediately related to the PatchXpress 7000A system.

We strongly advise against changing the software configuration of the computer by loading any programs other than those included when the system is first installed, or by changing any configuration settings likely to affect computer performance. If additional software is loaded, and used, and leads to a performance problem requiring on-site service for its resolution, the service call will not be covered by the service contract, and will be charged for at standard rates.

It is important that when an experiment is in progress only the PatchXpress Commander Software, and DataXpress Importer Software are running—the computer runs near to capacity acquiring data from 16 channels, and any other processes occurring at the same time could lead to data loss.

Antivirus software is not installed on the computer because of potential negative impact on performance during data acquisition. For this reason you should minimize exposure to potential sources of virus attack, for example, we advise against using the computer for email or general internet browsing. If antivirus software is installed this is the responsibility of the customer.

Keep the computer as independent as possible from the network, for example, do not map network drives to it.

It is necessary to include the computer in a network in order to move output data to the DataXpress database, however the PatchXpress

7000A system can be run if the network is down—files will simply accumulate in the designated folder on the PatchXpress 7000A system computer until the network is restored, at which time the DataXpress importer clears the backlog. Connection of the computer to the network is the user's responsibility.

By default, the computer is configured with two logical hard drives, C, and D. Most of the software, including all the executable files, is installed on the C drive. The D drive is used to store the various files used in experiment configuration, as well as the directories for output files. In normal use, you save, and open files just from the D drive.

Multiple Users and Computer Security

On delivery, the PatchXpress 7000A system computer is configured in Windows 7 for a single user with administrator privileges. This user can create new, standard users, with rights to configure, and run PatchXpress 7000A system experiments. Having created a standard user, the administrator must ensure that the new user has modify privileges for the application directory (**C: \Axon**), and the data, and configuration files directories (by default in **D:\PatchXpress**). Any changes that are made in the access, and security setup of the computer beyond the addition, and removal of standard users is the customer's responsibility. We recommend that the delivered configuration not be changed beyond addition, and removal of standard users.

PatchXpress Commander Software, and DataXpress Software do not require or use individual Windows 7 user names to track user ID's or privileges. Instead, user names are set, and tracked from within the PatchXpress Commander Software, and DataXpress Software programs.

Preloaded Programs

The PatchXpress 7000A system computer is provided with the following programs installed:

- Microsoft Windows 7 operating system
- PatchXpress Commander Software
- DataXpress Software client, and Importer
- pCLAMP (includes Clampfit)
- AxoTrace
- Microsoft Office

Windows 7 is installed with automatic upgrade options disabled. Do not enable these as an upgrade installation occurring during an experiment could lead to data loss.

AxoTrace is a small utility that records events occurring in Axon applications on the computer. View the record by double-clicking the icon in the System Tray.

PatchXpress Commander Software

PatchXpress Commander Software is the single control interface for the PatchXpress instrument. Use it to configure, and run experiments, run start, and end-of-day routines, and control individual hardware components when maintaining your instrument.

Demo & Model Cell Modes

As a useful training, and familiarization tool, PatchXpress Commander Software can be run in demo mode, disconnected from any PatchXpress 7000A instrument. In demo mode the program operates as if connected to an instrument, generating simulated data in dummy experiments.

To run in demo mode, select the **PatchXpress Demo** icon on your desktop, or press **PatchXpress Demo** in the PatchXpress Commander Software submenu in **Start / Programs / Axon Laboratory**. PatchXpress Commander Software reports that it is in demo mode in the title bar.

Trial data simulated by PatchXpress Commander Software in demo mode is a copy of the command waveform, in current units, with artificial noise added.

You can also run PatchXpress Commander Software connected to the instrument, but without using real cells, with the model cell (see Model Cell on page 127).

PatchXpress Commander Software Interface

The PatchXpress Commander Software interface has five main sections, as illustrated in Figure 4-1:

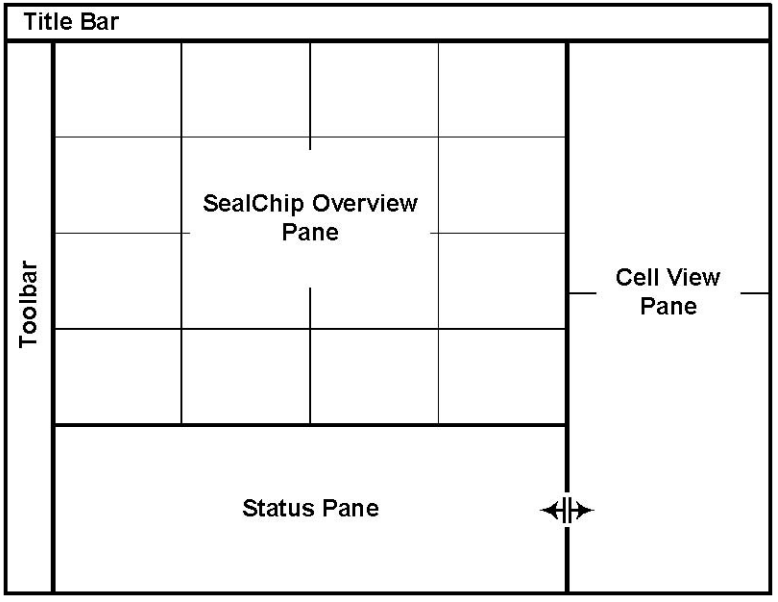


Figure 4-1 Overview of the PatchXpress Commander Software interface.

The **SealChip Overview** pane has three tabs, while the **Status**, and **Cell View** panes have four tabs each. The **Cell View** pane is separated from the **Overview**, and **Status** panes by a dragable splitter bar, but otherwise the basic interface configuration is fixed.

Descriptions of the interface sections are given in the following sections.

Title Bar

The program has a standard Windows title bar that displays the PatchXpress Commander Software version number, the current user's name, and also indicates when the program is in demo or model cell modes.

Toolbar

The main PatchXpress Commander Software toolbar extends down the left-hand side of the interface. All machine command, and experiment configuration dialogs are opened from buttons on the bar.

The toolbar buttons are placed in the order that they are likely to be used to set up an experiment.

Logon

The button opens an **authentication** dialog to log a user on to a selected DataXpress Software server. The PatchXpress Commander Software operator must already exist as a user on that server.

The PatchXpress Commander Software user is reported as the 'Operator' attribute in DataXpress, always using the user's full name rather than their login name.

Hardware and Fluidics

This button opens a dialog from which you can start machine maintenance, and error recovery routines, and control specific machine actions. The toolbutton flashes yellow if you are overdue to run an automatically scheduled maintenance routine. The **Hardware Log**, and a **Hardware Diagnostics** dialogs also open from this dialog.

Details of the commands in this dialog are provided in Hardware and Fluidics Dialog on page 129.

The **Hardware and Fluidics** dialog cannot be opened while an experiment is running.

Settings

A range of PPatchXpress Commander Software configuration settings are made in the **Settings** dialog:

- System mode—run experiments in a selected number of chambers or in the higher throughput screening mode
- Raw data viewing options
- Feedback resistor selection—alters amplifier current range
- File locations
- Membrane test configuration
- Well plate definition
- Add compound configuration—mixing, and tip positioning
- Washout configuration—adjust wash station height to vary volume in well after suction

See Basic Settings on page 66, for detailed instructions for this dialog.

Define Experiment

The great majority of configuration options for an experiment are made from the **Define Experiment** dialog. Use this dialog (or dialogs opening from it) to configure:

- The experimental procedure of compound additions, electrical stimulation, and recording (**Procedure** dialog)
- The electrical stimulus, and acquisition protocols used in the procedure (**Protocol** dialog, opened from the **Procedure** dialog)
- Whole-cell capacitance compensation, and access resistance compensation (**Initial Settings** tab of **Procedure** dialog)
- Scripts incorporated into the procedure, to change machine settings or for conditional wait steps (**Scripts** tab of **Procedure** dialog)
- Suction, voltage, and other parameters used in the patching process (**Patch Settings** dialog)
- Cell, and intracellular, and extracellular solution information (**Cell and Solutions** dialogs)
- Cell reuse, and replicate options
- Compound plate selection, and layout configuration (**Compound Plate Configuration** dialog)
- **Project**, and **Screen** selections
- A comment for the experiment

New users can view a sample configuration by pressing the **Sample** button at the bottom of the dialog. The **Clear** button clears all these fields.

Patch settings, and procedure definitions—including, within the latter, protocol definitions, and scripts—are saved in files. These files can be saved, edited, and reselected.

A summary of the experiment is provided at the bottom of the dialog, along with a report of the experiment's validity (for example, whether it is configured in a way that will allow it to run), with its current settings.

For details of experiment configuration, see Define Experiment Dialog on page 71.

Start Experiment

The triangular **Start** button starts the experiment configured in the **Define Experiment** dialog, in the operation mode (screening or assay development) defined in the **Settings** dialog.

The **Define Experiment** dialog—in read-only format—is displayed for last-minute checking before you commit to running the experiment.

As well as offering the opportunity for a manual check of your experiment settings the program runs a number of automatic checks to

ensure all aspects of the system are ready for an experiment. For example, if the system is due for wash station priming you are warned, and given the opportunity to carry this out before the experiment begins. Similarly, disk space on the PatchXpress 7000A system computer is checked.

Pause

The **Pause** button safely pauses an experiment so that it can later be restarted at the point it stops. Depending on when the button is pressed, the system may complete the operation it is performing before it comes to rest.

The **Pause** button flashes red when an experiment is paused.

If pause is pressed during data acquisition the experiment continues until the trials that are running are finished.

Stop Experiment

The **Stop** button opens a dialog with stop options.

In assay development mode there is one option only—to stop the experiment immediately. The *Seal*chip$_{16}$ electrode remains in place in the recording clamp when you stop an experiment this way, so if you no longer want to use this electrode you should remove it using the **Hardware and Fluidics** dialog command.

In screening mode you can stop the experiment immediately, and unload the current *Seal*chip$_{16}$ electrode, or opt to have the experiment stop at the point the currently loaded electrode is completed.

System Monitor

The **system monitor** is a view-only dialog that reports on the progress of hardware operations such as those started from the **Hardware and Fluidics** dialog, or during an experiment.

The cogs in the tool button rotate when a hardware process is running.

Refill Tip Racks

The **tip** button flashes once the first disposable tip tray is emptied during an experiment. If you have full tip trays in other, following locations in the tip tray racks you can leave the experiment to run, as tip pick-up automatically proceeds to these trays.

When you get the chance to replace the used tips (ideally, between experiments, but otherwise while the experiment is paused) click on the **tip** button to open the **information** dialog. You have two tip-replacement options:

- Replace only tip trays that have been fully used
- Replace all tip trays that have had any tips used

Once you have done this, dismiss the dialog with the appropriate button. PatchXpress now goes back to collecting tips from the first location. If you left a partially used tray the position where pick-up had previously advanced to is remembered, and the fluid handler goes straight to the next tip when the tray is reached, ignoring the empty spaces in the tray.

If tips run out during an experiment the experiment automatically pauses for you to replace the tips.

If you do not replace empty tip trays after opening the tip dialog be sure to cancel out of the dialog to dismiss it—pressing **OK** sends the fluid handler to location 1 in the tip tray rack.

Manuals and Web Support

Open a PDF version of this User Guide, or the scripting interface document. There are also links to PatchXpress 7000A system web pages at the Molecular Devices web site, including the download page for PatchXpress Commander Software updates.

About PatchXpress

The **i** button opens a dialog reporting the PatchXpress Commander Software version, and machine serial number.

Emergency Stop Button

The red **emergency stop** button immediately stops all movement of the PatchXpress 7000A instrument, and, if running an experiment, aborts the experiment.

SealChip Overview Pane

The **SealChip Overview Pane** displays raw current, user-defined measurements, and cell health measurements (one tab each) for each of the16 *Seal*chip$_{16}$ chambers. Measurements are updated in real time.

Graphs in the Overview pane cannot be resized. For a closer view of a particular channel, single- or double-click in its graph region to open it in the Cell View pane on the right:

- Single-click: displays the data type already upmost in the Cell View pane.
- Double-click: displays the data type on view in the Overview pane

During an experiment, a moving tip icon in the left-hand border of a graph indicates the next chamber to receive compound.

Sections below describe each of the three tabs in the Overview pane, popup menu options (with special sections for cell marking, and cell suspension), color-coding in the tabs, and graph scaling.

Raw Tab

The **Raw** tab shows raw data current traces from each of the 16 electrode chambers.

When P/N leak subtraction on page 106 or Resistive Leak Subtraction on page 94 is enabled you can choose to view your data leak-corrected or not, from the **View Settings** dialog opened from the **Cell View** pane toolbar (both corrected, and uncorrected data are always recorded, regardless of this selection).

The **Raw** tab graphs display:

- during the patching process: the response to the membrane test,
- between trials, once a procedure has started: the response to the membrane test,
- when a trial is running: the response to the command waveform

Note: if the membrane test is enabled to run between sweeps these responses are not displayed.

By default, only the most recent sweep of the membrane test is displayed, while all sweeps recorded during trials persist. The most recent sweep is blue, while past traces are gray. Sweep persistence can be changed in the **Settings** dialog **Options** tab.

Note: When a trial is running, only the response to the main stimulus waveform is displayed—response to any pre-sweep train or P/N leak subtraction subsweeps is not displayed.

Measurements Tab

Measurements taken from a selected sweep region during trials, are plotted against time in the **Measurements** tab. Compound additions are also displayed in these graphs.

When P/N or resistive leak subtraction is enabled, measurements are taken from the raw or corrected signal as set in the **Edit Protocol** dialog **Measurements** tab.

Each selected measurement type is displayed in a separate pane within the graph. To save space, the panes are not labeled. To see which measurements are displayed, double-click in a graph to view it, enlarged, in the Cell View pane.

Measurements viewed in this tab are not recorded, however you can re-create the measurements in DataXpress with the Measurements script (DataXpress 1.0) or the 'Use acquisition measurements settings' option in the **Measurements** dialog (DataXpress 2).

Cell Health Tab

This tab shows membrane test measurements for each channel, plotted against experiment time. You can choose which of these to display in the **View Settings** dialog opened from the **Cell View** pane toolbar.

Available measurements are:

- membrane resistance (Rm in MΩ)
- access resistance (Ra in MΩ)
- holding current (IHold in nA)
- membrane voltage (Vm)
- pressure applied to the cell (in mmHg)

The membrane test runs continuously on each channel throughout the experiment, except when trials are running. Even then, it can be configured to run between sweeps (see Membrane Test Between Sweeps on page 93).

The stimulus pulse for the membrane test is configured in the **Toolbar Settings** dialog.

Popup Menu

A range of scaling, and other options are available from popup menus for each chamber display:

- The **Copy** command copies the graph to the Windows clipboard.
- Graph scaling options are described in Graph Scaling on page 42, below.
- **Examine cell** displays the graph in the **Cell View** pane.
- The **Log entry** command allows you to write a comment to the log for that cell. The comment appears in the **Log** tab in the **Cell View** pane, and then passes to the **Cell Procedure Log** in DataXpress Software.
- **Cell suspension**, and **marking** options are described in the sections below.

Marking Cells

The **Overview** pane popup menu allows you to 'mark' cells good, bad or outstanding. These marks are queryable attributes in DataXpress.

You can change the mark on a cell while the procedure is running—the last mark before the cell procedure ends is the one that goes through to DataXpress. Each change is recorded in the cell log.

If you are reusing cells, you can mark different cell procedures on the same cell differently, for example the first cell procedure on a cell might be good, but the following one bad.

Note: If you mark a cell in one cell procedure that mark persists onto the following cell procedure unless you change the mark.

Suspending Cells

If a cell is generating poor data you can suspend the procedure on that cell with the **Suspend cell** option in the popup menu for the channel. Should the cell recover, you can undo cell suspension by clicking **Suspend cell** again.

When a cell is suspended, active procedure steps continue to run, but no new steps are started. If a trial is in progress it will continue acquisition, and similarly other steps in progress will run to completion. A multidose add compound step will complete all the deliveries within the step.

If the cell remains suspended until all the other channels on the *Seal*chip$_{16}$ electrode have completed, then the compounds that were to be used for the cell are requeued for another cell on the next *Seal*chip$_{16}$, providing sufficient volume of compound remains.

If the cell is unsuspended, the procedure starts again at the next step.

Tab Color Codes

The graphs on all three tabs of the **Overview** pane are color-coded to indicate the state of the corresponding cell, and its progress through the experiment, as shown in Table 4-1:

Table 4-1 Data, and background color codes for SealChip Overview Pane.

Trace and Background Colors	Status
Black/gray* on light gray	Membrane test during patching
Black/gray* on white	Membrane test after procedure has started
Blue/gray* on white	Recording trial
Light gray on dark gray	Channel terminated
White on light gray	Unused channel (in assay development mode)
Red on white	Error condition: check the Cell View Log tab

* when 'show history' is selected in the Settings/Options tab, previous sweeps persist in gray.

Graph Scaling

Graphs in the **Overview** pane can be scaled with:

- X-axis, and Y-axis arrowhead buttons in the graph interface
- dragging along an axis to zoom into the region demarcated
- right-click popup menu options
- the **continuous autoscale** button, in the top-right in the **Cell View** pane toolbar (see Cell View Pane Toolbar on page 49)

Arrowhead Buttons

Y-axis: zoom in or out on the Y-axis about the midpoint of the data currently on display.

On the **Raw** tab, all channels are locked together to keep the same scale. Similarly, on the **Cell Health**, and **Measurements** tabs, channels are locked together but zoom applies just to the selected measurement pane.

X-axis: zoom in, and out on the X-axis keeping the leftmost point of the current range fixed.

Scaling applies only to the channel where adjustment is made.

Axis Dragging

Move the cursor over any axis in the graphs in the **Overview** pane, and when it turns to a magnifying glass with a '+' in it, drag along the axis to define the range of that axis that you want to view.

Y-axis dragging sets the range for all channels, while X-axis dragging sets the range for only the selected channel.

Popup Menu Options

Autoscale Y: autoscale the Y-axes of all the panes in the selected channel, and then apply the same Y-axis range for each pane to all the other channels (for example, each channel is rescaled to be consistent with the selected, autoscaled, channel; only the selected channel is itself autoscaled).

Autoscaling adjusts the Y-axis range to show the full Y-range of the data using all the vertical space available. If zero does not fall within the data range the axis range is extended to include it.

Autoscale X: autoscale the X-axis of the selected channel (for example, set the X-axis range to show all the data using all the horizontal space available—zero is not necessarily included in this range). On the **Raw** tab only the selected channel is affected. On the **Measurements**, and **Cell Health** tabs the same X-axis range is applied to all the other channels.

Full scale Y-axis: sets the Y-axis for all panes in all channels to its 'full range', however this is defined.

On the **Raw** tab, full range is –20 nA to 20 nA or –200 nA to 200 nA, depending on the selection in the **Settings** dialog **Options** tab. For the **Measurements** tab full range is arbitrarily defined as –200 to 200 for each measurement type, while each of the **Cell Health** tab measurements has its own defined range.

Full scale X-axis: for the **Raw** tab the X-axis is set at the full duration of the membrane test pulse, or of the sweep when a protocol is running. Only the selected channel is rescaled.

On the **Measurements**, and **Cell Health** tabs, the data are X-axis autoscaled, and that range up to the next one-minute (60 seconds) boundary is displayed. All channels are set to the same range as the selected channel.

Status Pane

The **Status** pane takes its name from the **Status** tab, which presents real-time data on the progress of an experiment, for each *Seal*chip$_{16}$ electrode used, in a tabular format. The pane also includes the **Plate** tab, showing compound usage in a diagram of the compound well plate, the **Procedure** tab, that gives a static display of the procedure, and each channel's progress through it, and the PatchXpress Lab Book.

Plate Tab

A diagram of the compound well plate currently in use is color-coded to indicate progress through the experiment. Tooltips show the contents of each well.

Compound groups, as defined in compound plate layouts, are shown in gray around the wells that they include. When one of these compound sets is being applied its color on the **Plate** tab matches coloration of chamber views in the other panes, so you can see which compounds are going to which chamber.

Color codes for the wells themselves are explained in Table 4-2. Where replicate applications have been selected, each well divides pie-wise into as many sections as there are replicates, with color coding applied to each section.

Table 4-2 Well location color codes on Progress tab.

Well Color	Explanation
White	No compound or, no compound that will be used in this experiment.
Blue	Well locations that will be used in the current experiment.
Yellow	Well locations queued for use on the current *Seal*chip$_{16}$ electrode. If cells fail to patch or the procedure fails to complete on a cell, the well is requeued for the next electrode.
Green	Well locations from which compound has been successfully dispensed.
Red	Error conditions: see the Cell View Log tab.

Status Tab

The **Status** tab displays key parameters of each channel of a *Seal*chip$_{16}$ electrode, updated in real time, to provide a valuable indicator of the progress of an experiment, especially during patching. When an experiment runs to a new electrode the tab clears, and then populates with values for the new electrode.

Most columns in the table are relevant only for a part of the patching process. Columns remain clear until they have relevant data to show. Columns showing data that becomes irrelevant as the experiment proceeds are grayed to indicate they will not be further updated.

One special case of values being frozen is when a cell fails cell health limits set in the patch settings, but where you have opted to continue the procedure on such cells, in the **Procedure** dialog.

A summary of the **Status** tab column information is provided in Table 4-3.

Table 4-3 Columns in the Status tab .

Column	Explanation
Ch.	*Seal*chip$_{16}$ electrode chamber (or channel) number. Chamber 1 is nearest the door.
mmHg	Pressure applied to cell. Negative pressure draws the cell into the electrode hole.
Re (MΩ)	Electrode resistance. This column displays the total resistance, measured from the start of the experiment (before an electrode is loaded) until the electrode is loaded, and extracellular solution added—to complete the circuit, and provide a measure of electrode resistance. The value is frozen at the electrode resistance measurement. Values may be displayed with a 'greater than' or 'less than' sign if resistance goes over 100,000 MΩ or under -100,000 MΩ.
Ce (pF)	Electrode capacitance. This column displays the total capacitance measured until the cell registers as having ruptured. Total capacitance is then reported in the Cm column, but reverts to the Ce column if whole cell is not verified.
Rseal (MΩ)	Seal resistance. The total resistance, displayed from the time cells are added until verified as whole cell. Prefixes show: • Premature = went whole cell before verified gigaseal attained • Sealed = went whole cell after verified gigaseal attained • (2nd) Sealed = went whole cell after the 'second chance' seal resistance attained. • Lost = after reaching either seal threshold, dropped below second-chance threshold At whole cell verification the Rseal value reverts to: • Premature = the last Rseal value before whole cell • Sealed = the verified gigaseal value The values are frozen at this point.

Table 4-3 Columns in the Status tab (cont'd).

Column	Explanation
Cm (pF)	Membrane capacitance. The total capacitance measurement once cell is registered as whole cell. If whole cell is not verified, total capacitance measurement reverts to the Ce column. Values may be displayed with a 'greater than' sign if the membrane test response tau is too long to accurately derive Cm. Once whole cell is verified capacitance measurements are shown only in this column for the remainder of the cell procedure.
Rm (MΩ)	Membrane resistance. Displayed when the cell first registers as whole cell. Rm ceases to be reported if whole cell is not verified. Once whole cell is verified, resistance measurements are shown only in this, and the Ra column for the remainder of the cell procedure.
Ra (MΩ)	Access resistance. Displayed when the cell first registers as whole cell. Ra ceases to be reported if whole cell is not verified. Values may be displayed with a 'greater than' or 'less than' sign if the membrane test response tau is too long or short to accurately derive Ra. For Cm values of about 7 pF to 10 pF, Ra is overestimated about 10%. Over 10 pF, the error drops to 5% less. Once whole cell is verified, resistance measurements are shown only in this, and the Rm column for the remainder of the cell procedure.
Ra range (MΩ)	The minimum, and maximum Ra values from the time the procedure begins (after patching) until the end of the cell procedure.

Table 4-3 Columns in the Status tab (cont'd).

Column	Explanation
Vm (mV)	A direct measurement of the average value of the membrane voltage. When the Amplifier Mode is Current Clamp, values display in the Vm column, and the Cm, Rm & Ra measurements are balnk. Similarly, when the Amplifier Mode is Voltage Clamp the Vm column is blank, and there measurements display in the Cm, Rm & Ra columns.
Sweep	When a trial is running, the current sweep number is reported, with the total number of sweeps in the trial protocol.
Trial	The number of the trial currently running, or that was last run, in the procedure.
Step	The procedure step currently running, with the total number of steps in the protocol.
State	Description of the current state of the channel, or operation being carried out on it. Experiment time (time since the Start button was pushed) is also displayed while the experiment is running.

Procedure Tab

This tab lists the steps in the procedure. Blue bars to the left indicate where in the procedure each channel is.

This tab does not carry information about the progress of the patching process, so only becomes useful once the procedure begins. Progress through the procedure is also indicated on the **Status** tab, but with a rapidly updating interface it can be difficult to gauge exactly your position within the procedure on that tab.

Lab Book

The Lab Book keeps an automatic record of significant events in the PatchXpress program, as well as providing a location to store user-entered comments.

The **Lab Book** tab itself displays recent entries (one to a row), but offers no configuration options. These are accessed from the Lab Book window, opened with the button in the top left of the tab.

The **Lab Book** window shows the same information as the tab, but includes more entries, a panel for displaying the body of user comments (rather than just the descriptive summary seen in the listed

entries), and a range of configuration options. There are four verbosity settings, and two sets of filter options. The first set of filter options allows you to show any combination of:

- system information—hardware functions such as wash station priming, start up procedure, etc.,
- experiment information—when experiments are started, and stop, when new electrodes are loaded, and
- user-entered comments.

The second filter sets the time range you want to view, with choices to show entries from the entire Lab Book, the current session (for example, since PatchXpress Commander Software was last started) or the current experiment.

Lab Book verbosity, and filter settings do not alter the quantity of data recorded—they are display options only.

To add a comment to the Lab Book press the second (lower) button on the left hand side of the **Lab Book** tab, or the **Add Entry** button in the **Lab Book** window, to open the **Lab Book Entry** dialog. The dialog has two fields—one for a summary of your comment, and the second for your comments at length. The summary appears in entry lines in the Lab Book, while the second field can only be viewed in the lower pane of the Lab Book window.

Once a user comment has been added, it cannot be deleted or edited.

Each Lab Book entry has a priority rating. Most entries have rank 'i', for information. Entries marked with a red 'x' report a malfunction.

While the Lab Book keeps a program-wide record of events, there are also separate logs for each cell, viewed on the **Log** tab in the **Cell View** pane.

There is also a separate **Hardware Log** in the **Hardware and Fluidics** dialog.

Cell View Pane

The **Cell View** pane is in the right-hand side of the main display interface, separated from the **Overview**, and **Status** panes by a vertical splitter bar. The pane displays enlarged versions of graphs selected in the **Overview** pane, and channel logs.

A toolbar on the right of the pane has display options that apply to both the **Cell View**, and (for some commands) Overview panes.

The **Cell View** pane can have one to four subpanes which can each display information from different channels, or different tabs for the same channel (set with the '**+**', '**–**', and **padlock** buttons in the toolbar).

Popup menus on the graph tabs offer the same options as the Overview popup menus, allowing rescaling, copying, and cell suspension, and marking (see Overview Pane section Popup Menu on page 40 above).

Cell View Pane Toolbar

This toolbar has commands for viewing options in the **Cell View**, and (for some commands) **Overview** panes.

- The uppermost button sets **continuous autoscale** for the Y-axis, and continuous full scale for the X-axes, for all the graphs in the **Measurements**, and **Cell Health** tabs in both the **Cell View**, and **Overview** panes (see Graph Scaling on page 42, Popup Menu Options on page 43). Autoscaling is updated with each new point that is added, when the button is first pressed, and when a new channel is selected.
- The next two buttons are an **autoscale** vs. **full scale** toggle pair that apply just to the **Raw** tab of the selected **Cell View** subpane.
- The **padlock** button locks all subpanes to one channel—useful when you want to see all the relevant information, on the different tabs, for a selected channel.
- The +, and **–** buttons control the number of subpanes in the **Cell View** pane.
- The bottom button opens the **View Settings** dialog where you select;
  - membrane test values to view in the **Cell Health** tabs in both the **Cell View**, and **Overview** panes,
  - leak-corrected or raw data traces to view in the Raw tabs in both the **Cell View**, and **Overview** panes.

Clocks

Three clocks in the top left-hand corner of the **Cell View** pane report different durations:

- **Experiment**: in both hours, minutes, and seconds, and seconds only, this clock shows time from the start of an experiment, for example, from when the experiment **Start** button was pressed.
- **SealChip**: the time since the current *Seal*chip$_{16}$ electrode was loaded. In screening mode this is always less than the experiment time, and restarts within an experiment as new electrodes are loaded. In assay development mode several experiments might be run on an electrode, with waiting periods in between. In this case, the SealChip clock continues reporting while the experiment clock stops, and starts.
- **Cells added**: the time since cells were added to the *Seal*chip$_{16}$ electrode.

Selecting Channels to View

To select a channel to see in a **Cell View** subpane first click in the subpane, to give it focus, and then on the channel you want to view, in

the **Overview** pane or **Status** or **Procedure** tabs. You can also click on a compound set in the **Plate** tab, and the channel receiving compound from that set is selected. For example, with two **Cell View** subpanes open, it takes four clicks to view channels 5, and 9 in the subpanes—in order, click:

- the top **Cell View** subpane
- channel 5 in the **Overview** pane, row 5 in the **Status** tab, column 5 in the **Procedure** tab, or the appropriate compound set in the **Plate** tab
- the lower **Cell View** subpane
- channel 9 in the **Overview** pane, row 9 in the **Status** pane, column 9 in the **Procedure** tab, or the appropriate compound set in the Plate tab

Since the last **Cell View** subpane you gave focus to was the lower one, if you subsequently click on a further channel in the Overview pane or Status, Procedure, or **Plate** tabs, this channel will replace channel 9 in the lower subpane.

The **padlock** button in the **Cell View** pane locks all the subpanes to show just one (the selected) channel. This is useful if you want to see different tabs for the same channel at the same time, all controlled by a single click.

If you select a channel to view from the Overview pane, a single click changes the **Cell View** subpane to the selected channel, retaining the tab that was uppermost in the **Cell View** subpane when the selection was made. In contrast, a double-click ensures that the **Cell View** subpane changes to the same tab type displayed in the **Overview** pane.

Channels selected for viewing in the **Cell View** pane are given the same border or line color in each of the **Cell View**, **Overview**, and **Status** panes.

Log

The **log** tab contains a read-only record, updated in real time, of all events pertaining to the cell in the selected chamber. This includes details of the patching process, compound applications, when trials were started, and stopped, and when scripts were run. You can copy the contents displayed in the tab to the Windows clipboard with the button in the top right-hand corner of the tab.

The **Log** tab has four verbosity options—terse, normal, verbose, and very verbose. Terse shows when an electrode is loaded, the configured procedure steps, and procedure end, as well as any error messages. The other verbosity settings include increasing detail about the patching process, and intermediate steps within the procedure, up to ‘very verbose’, which includes all this as well as membrane test readings for the whole cell procedure. These parameters are itemized below.

The log entries use experiment time, for example, time since the experiment **start** button was pressed.

Log data are saved in the cell procedure **Params** file exported to DataXpress. In DataXpress they can be viewed in a similar format to the PatchXpress **Log** tab, with the exception of the membrane test parameters seen in very verbose mode. You can open the Params file in DataXpress if you want to see these values in a graphical format.

Membrane Test Parameters Reported in Very Verbose Option

In **very verbose** mode all membrane test parameters for each channel can be viewed on the **Log** tab. Some of these parameters are plotted on the **Cell Health** tabs (in both the **Overview**, and **Cell View** panes), and some are reported in the **Status** tab, and **Cell View Raw** tab. The **Log** tab is the only place in PatchXpress Commander Software where all the parameters can be viewed together. The reported parameters are:

- Re: electrode resistance
- Rseal: seal resistance
- Ce: electrode capacitance
- Rm: membrane resistance
- Ra: access resistance
- Cm: membrane capacitance
- Vm: membrane voltage
- Vhold: holding voltage
- Ihold: holding current
- mmHg: pressure on the cell
- Irms: noise in the membrane test response signal
- Rt: total resistance
- Ct: total capacitance

Raw Tab

The **Raw** tab displays the raw current trace for the selected cell (corrected for leak if this is selected in the **View Settings** dialog, opened from the **Cell View** toolbar).

Electrode resistance, and capacitance, and seal resistance, from the cell's patching process, are reported in the border above the display. The most recent membrane capacitance, membrane resistance, and access resistance are also shown.

Scaling in the tab is independent of other subpanes in the **Cell View** pane. As well as arrowhead, axis, and popup menu scaling options similar to those provided in the **Overview** pane (see Graph Scaling on page 42) the tab includes horizontal, and vertical scroll bars, and you

can use the **autoscale**, and **full scale** buttons in the toolbar to the right of the pane.

Measurements and Cell Health Tabs

In the **Measurements** tab, measurement types selected in the **Protocol** dialog—from a defined region of each trial sweep—are plotted in real time, with names, and units. Measurements of amplitude used in different trials in different **Amplifier Mode** display in a new measurement pane. **Voltage Clamp** measurements display in nanoamps (nA), and **Current Clamp** measurements display in millivolts (mV).

On the **Cell Health** tab, membrane resistance, access resistance, holding current, membrane voltage, and pressure for the selected channel can be viewed (select which from the **View Settings** dialog, opened with the bottom button in the **Cell View** toolbar).

On both tabs the X-axis shows experiment time. Each measurement or cell health parameter is displayed in its own horizontal pane within the tab, on the same X-axis.

Both tabs have similar scaling options to the **Raw** tab, except that setting the X-axis to full scale first autoscales the axis, then rounds up to the next 300 s interval.

File Management

PatchXpress Commander Software uses five file types to run an experiment:

- Procedure, PXP: contain procedural steps. These are text files that can be read in Notepad as well as opening into the PatchXpress Commander Software **Procedure** dialog.
- Protocol, PRO: contain electrical stimulation, and acquisition configuration. Readable in the **Protocol Editor** in PatchXpress Commander Software, Clampex, and ClampXpress.
- Compound plate contents, TXT: contain compound names, concentrations, and other values. Tab delimited text files that can be written in Notepad or Excel, and are read into the PatchXpress Commander Software C**ompound Plates** dialog.
- Patch settings, PXS: contain configuration parameters for the patching process. Text files readable in Notepad as well as opening into the PatchXpress Commander Software **Patch Settings** dialog.
- Script, TXT: contain scripted instructions for application during experimental procedures. These text files can be read in Notepad or any code-editing application such as PatchXpress Commander Software **Script Editor** (Experiment / Procedure / Scripts).

Three principle file types are output:

- Experiment, PXX: contain experiment-wide attributes, for example, Operator. These are text files that can be read in Notepad. They can also be accessed through DataXpress Software.
- Cell logs, or 'Params' files, ABF: contain cell health values throughout the life of one cell, with compound addition information, and when trials were run. Can be viewed in Clampfit, and DataXpress Software.
- Trials,ABF: contain raw data traces. Can be viewed in DataXpress Software, and Clampfit.

For each one of these files that is generated, an encrypted DXI file is also produced. This ensures secure passage of its associated data file into the DataXpress Software database.

In addition to the data files the program has two Lab Book files—one each for demo, and normal modes. These files, called **PatchLabBook 001.rec** reside in **D:\PatchXpress\DemoData**, and **D:\PatchXpress\Data** respectively. The file contents can only be viewed from the PatchXpress Commander Software interface. Be sure not to accidentally delete these files.

File Types Used in an Experiment

Procedure Files

The sequence of steps defined in the **Procedure** dialog is saved in a 'procedure' (PXP) file. Procedure configurations must be saved before they can be used in an experiment.

To save the procedure currently defined (in the **Procedure** dialog) use either the **Save** or **Save As** option in the menu opened from the **Procedure** button in the **Define Experiment** dialog. A directory with the same name that you give the procedure is automatically created. This contains the procedure (PXP) file itself, and as many protocol (PRO), and script (TXT) files as are defined within the procedure. By default, procedure directories are saved under file path:

D:\PatchXpress\Procedures

You can change the directory path in the **Settings** dialog if you wish, although the **Procedures** directory name cannot be changed.

Protocol Files

Protocol (PRO) files define stimulus, and acquisition parameters for the current recordings you make within a procedure. They also define online measurements you want to take from these recordings, pre-sweep trains (to stimulate the cell prior to recording), and P/N leak subtraction sweeps (also not recorded). In PatchXpress Commander Software, protocols cannot be run independently of a procedure. Like

procedure files they must be saved before an experiment using them can be run, however this is done automatically when the procedure is saved.

Protocol files can be imported into a procedure or created anew, copied, edited, and renamed, all within the **Procedure** dialog, for **Start trial** steps. Protocol files that are used in a procedure are saved in the same directories as the PXP (procedure) files they belong to. If you reuse a particular protocol in several procedures, the protocol is saved separately in the folder for each procedure.

By default, the directory:

D:\PatchXpress\Protocols

is created during installation as a general storage location for protocols. Store protocols that you wish to reuse here so that they are easy to find.

Plate Contents Files

Information about the compounds in well plates used in experiments must be written into ASCII tab-delimited text files. The information for each compound addition is transferred into the appropriate cell procedure log, which is then passed to DataXpress.

The default location for plate contents files is:

D:\PatchXpress\PlateFiles

Plate content files should be named with the well plate barcode number.

To point PatchXpress to a particular plate contents file, open the **Compound Plates** dialog from the **Define Experiment** dialog, select the plate location you are using, and then place the cursor in the **Plate ID** field. Scan the well plate barcode. The barcode number is written into the field, the associated file found, and the plate contents information displayed in the dialog.

Alternatively, select the file name in the Plate ID field list box.

It is not necessary to use plate contents files to run an experiment, though you must provide a well plate name in the Plate ID field. In this case just plate IDs, and well locations are carried through to DataXpress. These can be used for later manual identification of the compounds used.

Plate Contents File Configuration

Plate contents files must be ASCII tab-delimited text files with a ‘txt’ extension. They can be created in Notepad, or in word-processing or spreadsheet programs like Word or Excel, provided they are saved correctly as tab-delimited text files—that is, do not save the files as formatted DOC or XLS files. File names must be 64 characters or less.

A simple plate contents file, containing only well location, compound name, and compound concentration can be set up without column headers. In this case columns must be in the following order, left to right:

- well location (in the form ‘A1’ or ‘A01’)
- compound name
- concentration (in µM)

Within the columns compounds can be listed in any order. Empty wells do not need to be included.

Compound names may not include commas, periods, equals signs, slashes, quotes, question marks or arrows.

Note: DataXpress truncates compound names over 50 characters long.

All compound concentrations must be given in micromolars. Values with decimal places can be entered.

An example of a simple plate contents file is given in Figure 4-2:

```
A1 Risperidone 0.1
B1 Risperidone 0.3
C1 Risperidone 1.0
D1 Risperidone 3.0
```

Figure 4-2 Example of simple plate contents file.

If you want to include additional compound parameters then you must use column headers. PatchXpress Commander Software recognizes seven of these:

- Well
- CompoundName
- Concentration
- Batch
- Lot
- SaltCode
- Custom

These can be included in any order.

In addition, plate contents files can be linked to specific compound layouts, as defined in the **Compound Plates** dialog Layout Manager, with specified volume per well:

- In the top line of the file, type **Layout ID**, tab, then give the name of the layout in double quotes.
- In the second line of the file type VolumePerWell (uL), tab, then enter the volume of compound per well in microliters.

This way, when the plate contents file is selected inPatchXpress Commander Software, it automatically gets the chosen layout. See Compound Layouts on page 74.

An example of a plate contents file with layout configuration identification, volume per well, and column headers is given in Table 4-4:

Table 4-4 Example of plate contents file with compound layout identification, volume per well, and column headers.

Layout ID "Axon 16 x5 Test, 1 Control" VolumePerWell (uL) 300						
Well	CompoundName	Concentration	Batch	Lot	SaltCode	Custom
A1	DMSO		22/5a	p77836m		
A2	ketamine	0.01	24/5a	TR994821	TYL53	2
A3	ketamine	0.1	24/5a	TR994821	TYL53	2
A4	ketamine	0.3	24/5a	TR994821	TYL53	2
A5	ketamine	1	24/5a	TR994821	TYL53	2
A6	ketamine	10	24/5a	TR994821	TYL53	2
A7	DMSO		22/5a	p77836m		
A8	atropine	0.01	24/5m	739001c	MBY82	5
A9	atropine	0.1	24/5m	739001c	MBY82	5
A10	atropine	0.3	24/5m	739001c	MBY82	5
A11	atropine	1	24/5m	739001c	MBY82	5
A12	atropine	10	24/5m	739001c	MBY82	5

Patch Settings Files

Patch settings (PXS) files are text files that store the complete set of amplifier, and other hardware settings configured from the **Patch Settings** dialog, opened from the **Define Experiment** dialog. These include times, pressures, and voltages used during cell patching, as well as the final voltage holding level, and fluidics pressure maintained once cells are patched, and the procedure run.

Your current patch settings must be saved in a PXS file before you can run an experiment with them. Use the **Define Experiment** dialog Patch settings menu options **Save** or **Save As** to do this. The default location for patch settings files is:

D:\PatchXpress\PatchSettings

You can change the file path on the PatchXpress 7000A system computer in the **Settings** dialog, but not the directory name, **PatchSettings**.

Script Files

Script (TXT) files can be configured as steps within the procedure. They are used to send commands to PatchXpress, for example to change settings such as the voltage holding level or to turn Ra correction on or off, or change its configuration. Scripts can also be used to monitor the cell, for example pausing the procedure until a steady state is achieved in response to a regular stimulus.

Script files can be imported into a procedure or created anew, copied, edited, and renamed, all within the **Procedure** dialog, for **Command** steps. Script files that are used in a procedure are saved in the same directories as the PXP (procedure) files they belong to. If you reuse a particular script in several procedures, the script is saved separately in the folder for each procedure.

By default, the directory:

D:\PatchXpress\Scripts

is created during installation as a general storage location for scripts. Store scripts that you wish to reuse here so that they are easy to find.

Output Files

Each PatchXpress Commander Software experiment creates three types of output file: experiment files, cell logs (‘params’ files), and trials. These file types fall into a natural hierarchy, with trials always belonging to cell procedures (recorded in the params files), and cell procedures belonging to experiments.

When an experiment is run the three file types are all deposited into the same ‘Screen’ directory, within a ‘Project’ directory, as selected in the **Project and Screen** dialog opened from the **Define Experiment** dialog. By default, project and screen directories are located in the file path:

D:\PatchXpress\Data

or, for data generated in demo mode:

D:\PatchXpress\DemoData

For each output file a corresponding ‘instructions’ file (file extension DXI) is also produced. This file contains, encoded, the user name, and the database they logged into when they began the session.

Under normal conditions files remain in this directory for only a short time before they are copied into the DataXpress database by the DataXpress Importer. It uses the DXI instructions file to direct the files to the correct database. Once files are verified as having been successfully transferred to the database, they are removed from the original directory, and either placed into a second directory on the PatchXpress 7000A system computer:

D:\DataXpress_Imported\PatchXpress\Data

or deleted. This option is set in the DataXpress Importer. The instructions files are deleted.

When files are copied to a second location the original project, and screen directory hierarchy is maintained.

If for some reason the DataXpress Importer is unable to transfer a file into the database, depending on the configuration of the Importer, importing stops or the file is removed from its original location, and placed in a directory in the path:

D:\DataXpress_Errors\ PatchXpress\Data

again maintaining the original project, and screen hierarchy.

Experiment Files

Each experiment produces one experiment (PXX) file. This is a simple text file reporting properties applying to the whole experiment, such as the user, PatchXpress 7000A instrument ID, and procedure details. The experiment file is generated first, before any other files.

In DataXpress, experiment files corresponding to a query you have run are listed on the **Experiment** tab, from where they can be opened for viewing. The files are named in PatchXpress using the following format:

COMPUTERNAME_YYYY-MM-DD_HH-MM-SS.pxx

for example, the name of the PatchXpress 7000A system computer plus the time the experiment started given by date (year, month, and day), and 24-hour clock time (hours, minutes, and seconds).

Cell Logs

Cell log, or ‘params’ files are ABF (Axon Binary Format) files that record the history of a single cell during an experiment. They do this both by plotting a range of measurements taken throughout the patching process, and during the cell procedures run on a cell, and with timed tags that report all the significant events that occur to the cell—for example, stages of the patching process, and steps in the defined procedure. The tags are used by DataXpress Software to reconstruct the history of each cell for display, and analysis.

In normal operation it is not necessary to view cell log files themselves. However, in the case that you want to see cell health details the files can be opened in DataXpress or Clampfit.

Each cell log starts at the experiment start time, and records up to the end of the last cell procedure that is run on the cell, plus about a second of final cell health measurements. Because all the params files start at the experiment start time, those that log cells from the second, and later *Seal*chip$_{16}$ electrodes used in an experiment have a section of empty data at their beginning, this being the time while preceding electrodes were being processed.

The measurements plotted in the cell logs are:

- Rt: total resistance, in MΩ
- Ct: total capacitance, in pF
- Re: electrode resistance, in MΩ
- Ce: electrode capacitance, in pF
- Rseal: seal resistance, in MΩ
- Ra: access resistance, in MΩ
- Rm: membrane resistance, in MΩ
- Cm: membrane capacitance, in pF
- Vm: membrane voltage, in mV
- VHold: holding potential, in mV
- IHold: holding current, in nA
- Noise: noise in current response, in nA rms
- Pressure: intracellular-extracellular pressure, in mmHg

Most of these measurements come from the membrane test, which is not run while trials are being recorded, hence the log file has gaps in the data where trials were run.

As well as the plotted measurements, and tag information, cell logs carry a great deal of information in their headers, and as annotations. Many of these entries are taken up as queryable attributes in DataXpress, but the original headers, and annotations can be viewed in the **file properties** dialog in Clampfit or DataXpress Software.

Cell log files are named using the format:

YYYY-MM-DD_HH-MM-SS_SCnn_Cmm_Params.abf

This has the start date, and time of the experiment that the log comes from, given by year, month, and day; hour, minute, and second. This time is shared with the experiment file name, and all other params, and trial file names within the experiment.

Following this is the number of the *Seal*chip$_{16}$ electrode, within the experiment. The ‘C’ value is the chamber number.

Trials

Trials are ABF (Axon Binary Format) files recording raw current traces, as viewed on the **Raw** tabs of the **Overview**, and **Cell View** panes

during an experiment. Each trial corresponds to a protocol run, with separate trials produced for each cell.

Trials record only the response to the stimulus command configured in the Waveform section of the **Trial Setup** tab, in the **Protocol** dialog. The response to P/N leak subtraction subsweeps, and Pre-sweep trains, configured on the **Stimulus** tab, is not recorded.

PatchXpress Commander Software trials always record current in nanoamperes, and have one signal, except when P/N or resistive leak subtraction have been enabled. In this case the trials have two signals—the first leak corrected.

Trials all start, internally, at time zero. Tags within the trials, for compound addition, and wash steps, also use trial time, however they display time from the start of the sweep when the trial is viewed in sweeps mode (as opposed to continuous or concatenated modes).

The start time of each trial since the start of the experiment is passed to DataXpress where it appears as the 'Stopwatch' attribute.

A trial is created if one complete sweep of data is recorded—for example, if for any reason recording stops before the first sweep is completed, no trial is generated. Similarly, if recording cuts off midsweep at any time after the first sweep, data from the final, uncompleted sweep are lost.

All PatchXpress Commander Software trials are recorded using sweeps, however with minimum sweep start-to-start times the sweeps adjoin, effectively giving gap-free recording. Alternatively, a recording of one long sweep can be used.

Like params files, trials carry information in their headers, and as file annotations. This information, from which a number of DataXpress Software attributes are drawn, can be viewed with the **file properties** dialog in DataXpress Software, and Clampfit.

Trials are named using the format:

YYYY-MM-DD_HH-MM-SS_SCnn_Cmm_Rpppp_Sqq.abf

The date, time, *Seal*chip$_{16}$, and chamber number components of this are the same as for cell log file naming (above). The four-digit 'R' number is the cell procedure 'run'—the number of the cell procedure within the experiment. Trials from the same cell procedure share this number. The final 'S' number is the procedure step number that the trial comes from.

Note: 'Run' here does not refer to the run in protocol definition.

Setup to Acquisition

5

This chapter describes in detail the stages involved in running an experiment. Many of the steps involve system configuration that need be done rarely, so for a quick summary of the steps associated with running an experiment, see Chapter 2: Getting Started on page 11.

Running PatchXpress Commander Software in Demo mode is a good way to gain practice with software settings independently of the PatchXpress hardware.

Experiment Plan

As with all experimental science, it is important to establish a plan for the type of experiment you will run, as well as a basic set of expectations for the data that will be generated. The PatchXpress 7000A system generates data comparable to that obtained from a conventional electrophysiology workstation, however there are some key differences that make the planning stage somewhat more important. Specifically, once you start the experimental run it is not possible to alter the procedures, and protocols that will be executed during the experiment. Moreover, the instrument is limited in its capacity to verify that the elements required to execute the experiment are available.

Prior to starting work on the PatchXpress 7000A system, make sure that sufficient quantities of the consumables required are available, and situated ready for use. This includes intracellular, and extracellular solutions, deionized water, AVIVA *Seal*chip$_{16}$ electrodes, cells, and test compounds. Other disposables are listed in Table 5-1:

Table 5-1 Consumables quicklist.

1. T ips: 50 mL polypropylene, Molecular BioProducts # 902-261 (nonsterile, MBP# 902-262)
2. 1 liter disposable bottles, 45 mm neck. (Nalgene part # 455-1000 preferred, Filter Unit Receiver (1L), 45 mm neck)
3. 50 mL conical tubes (Corning, Falcon or others)
4. Microcentrifuge tubes, 1.5 mL (Eppendorff or similar)
5. 96-well compound plates, for example:

   a) STANDARD 96 well: Corning Cat #3595, FLAT, polystyrene

   b) GLASS COATED: SUN SRI Cat #400 062, FLAT, polystyrene/coated with glass

   c) 96 well with glass vials: BioTech Solutions Cat # 9905-812, FLAT,0.5mL glass vials
6. *Seal*chip$_{16}$ electrodes (AVIVA Biosciences, ordered through Molecular Devices)

Solutions

The solutions used in the PatchXpress 7000A instrument are the same as those you would use for a conventional electrophysiology. During a normal working day, you can expect to use 30` mL to 50` mL of intracellular solution, and approximately 700 mL to 1000 mL of extracellular solution. If the solutions are stored refrigerated or frozen, the solutions need to be warmed to room temperature or above before use. As solutions warm it is common for air bubbles to form. While the PatchXpress 7000A instrument has been designed to minimize the impact of gases within the solution, in some cases it may be necessary to take extra measures to degas the solutions. Often this can be accomplished by simply heating the solution to a slightly higher temperature than the operating temperature. Alternatively one can use a mild vacuum to degas the solution. See the Molecular Devices Knowledge Base article for more details (http://mdc.custhelp.com/app/answers/detail/a_id/17259/kw/PatchXpress%20solutions). Filtering the solutions before use is recommended.

The whole-cell patches formed on the AVIVA Sealchip$_{16}$ electrodes are slightly more sensitive to the osmotic balance between the intracellular, and extracellular solutions than patches formed with a conventional glass capillary microelectrode. Measuring the osmolarity of the solutions is recommended. The solutions used in the Molecular Devices laboratory typically have osmolarities of 285 mOsm for the intracellular solution, and 290 mOsm for the extracellular solution.

Cells

The quality of the cells is one of the most important variables governing the success of experiments. For examples of the cells used in the Molecular Devices laboratory, and how the cells are prepared, see Cell Preparation on page 142. Approximately 1 million cells are needed per run, so 5 to 10 million cells per day is sufficient for normal use. A T-75 flask that is 80% confluent often has 2 to 4 million cells. Each chamber of the *Seal*chip$_{16}$ will receive 20 to 30 thousand cells. One may find that lower cell densities can be used, however this cell density should be viewed as a good starting point. The cells need to be in suspension prior to starting an experiment, however this does not mean that the cells need to be cultured in suspension. After adherent cells are lifted from their culturing substrate, they should be resuspended in extracellular buffer, but take care to minimize the time that the cells are suspended in extracellular buffer prior to being applied to the *Seal*chip$_{16}$.

AVIVA *Seal*chip$_{16}$

The AVIVA *Seal*chip$_{16}$ electrodes have a shelf life of at least 3 months from the time of delivery. If the *Seal*chip$_{16}$s are to be stored for an extended period, they should be stored at 4° C. Prior to use, they should be allowed to equilibrate at room temperature. It is common practice to leave all of the *Seal*chip$_{16}$ electrodes that will likely be used in the course of a week at room temperature.

Disposable Tips

Tips are taken first from the rear right-hand tray location, then the front right-hand location, working in this fashion from right to left. The PatchXpress Commander Software maintains a log of the tips that have been used. When empty tip racks are replaced, it is important to update the status within the software. A button for this purpose is located in the panel on the left of the PatchXpress Commander Software main window (see also Disposable Tips on page 23).

Compound Plates

Have your compound plate or plates ready to put in position in the PatchXpress 7000A instrument. You can place these in any of the four locations for this, as later you will need to input the plate locations you are using into PatchXpress Commander Software. If you want compound information to be available in DataXpress ensure you have contents files for the plates as well (see Plate Contents Files on page 54).

If the procedure has more than one compound addition step, with each cell receiving compound from more than one well when the procedure is executed, it pays to arrange compounds in the well plate in a way that makes stipulation of the compound additions (later, in procedure

configuration) convenient. See Plate Contents File Configuration on page 54, for a description of this.

Start PatchXpress

Startup procedure summary

1. Turn on the vacuum pump.
2. Turn on the air compressor (if configured).
3. Turn on the PatchXpress 7000A instrument power first and then the computer.
4. Bring the extracellular and intracellular solutions and the *Seal*chip$_{16}$ electrodes to room temperature.

The main power switch for the PatchXpress 7000A system is located on the left-hand side panel of the instrument. When PatchXpress Commander Software is opened, the PatchXpress 7000A instrument hardware is initialized. During the initialization process, each of the robots, and amplifiers is tested, and any failures reported. The MultiClamps, Digidata, and master controller have indicator lights to show when they are powered on.

Start Computer

When the computer has booted up, open PatchXpress Commander Software from the icon on the desktop.

Before the program opens you must log on to a DataXpress server. To operate PatchXpress Commander Software you must already be a user on the selected server.

PatchXpress Commander Software keeps a local record of users for each DataXpress server that it has been connected to, so if the network is down when you log in, provided you were a user on the server the last time PatchXpress Commander Software was connected to it, logon is still allowed. If the PatchXpress Commander Software remains off the network for the duration of your session, and is not reconnected until a second person has logged on, your data are still sent to the server of your choice under your user name, even if the second user is sending their data to a different server.

If you have never connected to a DataXpress server, and hence have no locally-cached username, you can cancel the **logon** dialog. You will later have to use the **Sample** button in the Define Experiment Dialog on page 71) to be able to run an experiment, using a default name.

On opening the program you are prompted to begin start-of-day initialization. If you have not already done it, this is your last chance to

load solutions, and a blank *Seal*chip$_{16}$ electrode cartridge for this process, before pressing **OK** in the **initialization prompt** dialog.

Prepare Fluidics

Place intracellular solution in a 50 mL tube on the mount for this on the rear wall of PatchXpress platform. Ensure the tubing to the filling station is in solution in the tube, and that the lid of the tube is off. Place the 1 L Nalgene bottle containing the extracellular solution on the mount located under the main deck on the right-hand side of the instrument. There are capacitively coupled sensors to monitor the levels of the extracellular solution, the 5 L deionized water reservoir, and the waste fluid container. There is no level sensor for the intracellular solution.

Inspect the level of waste in the large waste fluid container to ensure that there is sufficient headroom for the day's waste. There should be room for at least 2 L for an average day's experiments.

Blank Cartridge

Check that there is a black 'blank cartridge' Sealchip16 electrode in the mount for this near the drying station. This cartridge is used during the initialization process to contain fluid pumped through the wash station heads. On a periodic basis rinse the blank Sealchip16 with 70% isopropanol, and allow drying just prior to loading on the PatchXpress 7000A instrument. This is to ensure that the blank Sealchip16 is not carrying contaminants to the flipper arm gasket. Two additional blank cartridges are included in the PatchXpress 7000A system toolkit should the original blank cartridge become damaged.

Start-of-Day Initialization

Start-of day initialization automatically checks the major hardware components of the PatchXpress 7000A instrument, and primes the fluidics systems ready to run an experiment. The process takes about 13 minutes to complete. If you do not run start-of-day initialization when prompted at program start-up you can start it from the **Hardware and Fluidics** dialog later.

PatchXpress Commander Software will not allow you to run an experiment if initialization has not been carried out—if you attempt to start an experiment in this case you are prompted to run the initialization first, after which the experiment immediately starts, or you can cancel out of the experiment.

It is essential that water, and extracellular, and intracellular solutions are loaded prior to initialization, and that the blank electrode cartridge is in place on the mount near the drying station.

In the initialization process the PatchXpress 7000A instrument primes the compound dispensing robot arm by flushing ~ 6 mL of deionized water through the syringe pump, and the probe.

The intracellular solution injector is primed with water, and then intracellular solution, removing any air bubbles that may have built up in the line since the last end-of-day procedure.

The blank cartridge is loaded into the recording clamp, and extracellular solution is flushed through the wash station inflow, and aspiration nozzles for about 6 minutes, removing water left in the lines from the last end-of-day procedure, and ensuring that the nozzles are clear.

At the end of initialization the blank cartridge is returned to the blank cartridge holding chamber, and the lid is returned. Once this process is complete the PatchXpress 7000A instrument hardware is ready for an experiment.

Basic Settings

With hardware, and consumables ready, and before moving to the details of experiment configuration, you may need to check some basic settings in the **Settings** dialog. You should always check that you are in the correct system mode, but may also occasionally want to make adjustments to file locations, the membrane test, and the compound addition settings.

Sections below cover all the **Settings** dialog tabs.

System Mode

The **System Mode** tab is used to select between screening, and assay development modes.

Screening Mode

In screening mode the system uses all 16 channels of each *Seal*chip$_{16}$ electrode, loading new electrodes until all the compounds configured for an experiment have been tested or have run out. Near the end of an experiment, if there are more successfully ruptured cells than sets of compounds remaining to be tested, the remaining compound sets are applied to more than one cell (provided there are sufficient quantities of compound). This maximizes the use of the electrode, as well as the probability of getting successful cell procedures for the remaining compounds.

Sets of compounds, defined in the sequence of add compound steps in the experiment procedure, are applied in order as cells rupture, and become ready for the procedure to be applied to them. If a cell procedure runs to completion, that compound set is marked as successful. If the cell procedure is not completed the compound set is requeued for application to another patched cell, provided a sufficient quantity of compound remains.

If the procedure contains a loop then unsuccessful procedures use ‘autocompletion’ on the second, and following attempts. Rather than

run the full procedure on the new cell, successfully completed loops are excluded.

Assay Development Mode

Assay development mode allows you to run experiments in individual chambers in a *Seal*chip$_{16}$ electrode. You can elect to use any number of the 16 chambers on one electrode for each experiment. This allows other chambers in the same electrode to be used in further assay development experiments, or, if all 16 chambers are used, ensures that the system does not load a second *Seal*chip$_{16}$ electrode.

Note: The electrode remains loaded between experiments if fewer than 16 chambers are selected. To avoid aging of the electrode it is not recommended to use fewer than 4 chambers at a time. If too few chambers are used at a time, and the electrode remains exposed for too long, success rates are likely to drop significantly for the final chambers used.

Chambers are used in order, from number 1 to 16.

If only one set of compounds is defined for an experiment, these are applied to all the chambers selected. If the procedure defines more than one set of compounds the PatchXpress 7000A system attempts to apply all of them, provided enough chambers have been selected, and enough cells patch successfully. If fewer compound sets are defined than chambers, the sequence of compounds is repeated to the extent that the number of successfully patched cells, and the volume of compound, allows.

Options

In the **Raw data windows** section, set trace persistence options for the **Raw** tabs in both the **Overview**, and **Cell View** panes. You can set different options for the membrane test, and trials. Unchecked, each sweep replaces the previous sweep so that only the most recent sweep is visible. Checked to show history, earlier sweeps remain in view, but are grayed.

Select the headstage feedback resistor to use in the **Input range** section: the default 500 MΩ resistor gives a current recording range of –20 nA to 20 nA, while the 50 MΩ resistor, for large cells, has a range from –200 nA to 200 nA.

File Locations

Use the **File Locations** tab to stipulate directory locations for files used, and produced by the PatchXpress Commander Software.

For data files you can choose the drive on the PatchXpress 7000A instrument computer to which to send files. The data are sent to the file path:

X:\PatchXpress\Data

X:\ PatchXpress\DemoData

depending on whether data are created in demo mode or not, with drive D the default. Data files themselves are located in project, and screen subdirectories under these paths.

Note: If you change the drive here you also need to reconfigure the DataXpress Importer to pick up data from the new directory.

The various types of configuration files used by PatchXpress Commander Software each have their own directories, which must all be located together on the same drive or in the same directory. You can select any directory on any drive on any computer within the network for these.

Default settings place the directories in the path:

D:\PatchXpress

Membrane Test

The membrane test generates a regular square pulse centered on the voltage holding level. This pulse is output from the time each *Seal*chip$_{16}$ electrode is loaded until—independently for each chamber—a second or so after the final cell procedure is completed, barring times when trials are run. There is an option to run the test within trials, between sweeps (see Membrane Test Between Sweeps on page 93).

The current response to the test is used to calculate the resistance, and capacitance values shown on the **Cell Health** tabs in the **Overview**, and **Cell View** panes, and also reported in the **Status** tab, and **Cell View**, **Raw**, and **Log** tabs.

During cell patching, changes in these values trigger the steps of the patching process, for example, cells are considered ruptured when capacitance rises above a level set in the patch settings. The membrane capacitance value measured in the test is used to set the amount of whole cell (capacitance) compensation applied, and the membrane, and access resistance values (with the membrane capacitance) are used to monitor cell health.

Note: For Cm values of about 7 pF to 10 pF, Ra is overestimated by about 10%. Over 10 pF this drops to about 5%, and becomes less as the Cm increases.

You can set both the peak-to-peak amplitude of the membrane test pulse, and the duration of one pulse cycle. The default values are 20 mV (for example, 10 mV above, and below the holding level), and 12 ms for one cycle. The duration of the pulse cycle should be at least 10 times the expected time constant of the cells you are working with.

In addition, you can average the measurements of a stipulated number of cycles, mitigating the effects of noise on the test measurements. By selecting a higher number of pulses to average, and by increasing the pulse duration, you reduce the rate at which measurements are reported. Since the PatchXpress 7000A system hardware, and software have a limited rate at which pressure changes, and other settings can be applied you should average enough cycles to yield an update rate of 200 ms or longer. At default settings the membrane test measurements are updated four or five times a second.

When the amplifier is in Voltage Clamp mode, membrane test is active whenever a trial is not running.

Membrane test is not active when a trial is running, unless the trial is configured to run the test between sweeps.

When the amplifier is in Current Clamp mode a static holding level is output whenever a trial is not running and also between sweeps when a trial is running.

Plates

Use the **Plate** tab to enter measurements for the compound well plates you are using. This ensures there are no collisions between tips, and well plates when picking up compound.

Note: The well volume entered here is the total capacity of the wells, and not the volume of compound added to the wells for the experiment.

Plate dimensions, manufacturer, material etc., entered on this tab, are passed to DataXpress (from version 2) where they are queryable attributes.

Add Compound

The **Add Compound** tab sets physical parameters for compound additions. These include rate of flow into the chamber, tip position during delivery, compound mixing options, and an option to apply pressure (for example, suction) to the cell during addition.

Set the rate of flow of compound out of the tip during compound addition in the first field. The default rate is 100 µL/s. The value selected here applies for the entire experiment, unless changed with a procedure command step, compound **Delivery rate** (see Command on page 88).

You can enable compound mixing to occur both in the well plate during compound pickup, and/or in the chamber following compound release. Mixing consists of one or more cycles of compound being drawn into the disposable tip, and ejected again.

Mixing during pickup can be turned on or off, but allows no further configuration. Pickup mixing runs for two cycles with a dead volume of 5 µL, and flow rate (in, and out of the tip) of 50 µL/s.

Post-delivery mixing in the *Seal*chip$_{16}$ chamber requires you to set the number of mixing cycles, and determine the volume of compound to be drawn up in each mix cycle. Less volume than was added to the chamber should be drawn up during mixing, in order to avoid air being drawn into the tip. Thus, enter the volume to be deducted from the addition volume (the 'dead volume') to determine how much will be taken up in the mixing cycles, for example, if you add 45 µL, and set the dead volume to 5 µL, 40 µL is drawn up during mixing.

Flow rate in, and out of the tip during post-delivery mixing is 10 µL/s.

Both mixing options can be turned on, and off during the procedure, so that some compounds are mixed, and others not. Use a procedure command step for this.

You can also configure to apply suction to the cell membrane for the duration of the compound delivery, to help ensure it is not displaced.

On the right-hand side of the tab you can make fine adjustments to the positioning of the disposable tips in the Sealchip$_{16}$ chambers during compound addition. Position can be varied on two axes—to raise or lower the tips with the Z-offset, and to shift the tips left or right across the chamber—to be closer to or further from the wash station nozzles—with the X-offset setting. On the vertical Z-axis, positive values raise tips, and negative values lower them from their default position. On the X-axis, positive values move the tips to the right, towards the wash station nozzles, and negative values shift them left.

X-axis, and Z-axis positions can be changed from those set on this tab during the running of an experiment using a command procedure step. Changes are made individually for each chamber at the time the procedure reaches the command step.

See Sealchip16 Planar Electrode on page 14, for diagrams illustrating disposable tip positioning.

All the values in this tab are carried over to DataXpress where they are experiment-level attributes. In cases where the values are changed by command steps while the procedure is running, the initial values (set on the tab) become the DataXpress attributes, however the change of values is reported in the cell procedure logs.

Washout

Adjust the **wash station working height** when it is in position lowered into the chambers. This alters the volume of fluid left in the chambers after suction. The wash station height may also influence compound potency or the likelihood of cell displacement during compound addition by shielding the cell when the position is low.

To accelerate the onset of washout steps, the wash station should be lowered to about 0.6 mm for experiments with ligand-gated currents. The X-axis, and Z-axis positions must then be altered accordingly.

Define Experiment Dialog

All the settings that most directly define an experiment are brought together in the **Define Experiment** dialog. The current settings values, or—where settings are saved in files—the selected files' names, are reported in blue. In most cases you can access editing options for these by pressing buttons beside the fields.

The top left-hand corner of the dialog reports the current user, the DataXpress server that PatchXpress Commander Software is logged into, and to which output data will be sent, and file paths for output data, and configuration files.

Some experiment settings are relatively straightforward, while others offer a great range of configuration options. This section covers the simpler experiment configuration categories:

- Project, and Screen
- Compound plates
- Comment
- Cells
- Intracellular, and extracellular solutions
- Cell reuse
- Replicates

Sections following this one deal with procedure, and protocol configuration (Procedure Configuration on page 80, Edit Protocols on page 98), and the patching process (The Patching Process on page 110).

The **Clear** button clears all the fields in the dialog except the data file paths. Use this to clear settings for a fresh start.

The **Sample** button fills all the fields with sample settings. For those fields that refer to files, default sample files, provided with the program, are selected. The user, database, project, and screen are not changed if there is a connection to a DataXpress server. However, if there is no connection to a DataXpress server, and you did not log on with cached DataXpress credentials (see Start Computer on page 64), a sample logon is used. Data acquired under these conditions is not

automatically imported into a DataXpress database by the DataXpress Importer, however it can be imported from within a DataXpress client.

Experiment Validity & Saving Experiment Configurations

PatchXpress Commander Software automatically checks the experiment configuration in the dialog for consistency, and reports it as valid or invalid in the bottom right of the dialog.

If the current configuration is invalid, the problem is reported in the same place.

Also, when the configuration is invalid, the dialog's **OK** button changes to **Close**. This is to indicate that you can close the dialog, and save the current invalid configuration, but that the configuration cannot be used to run an experiment.

All the fields in the dialog, apart from the comment, must have values entered before an experiment can be run.

Project and Screen

The **hammer** button opens a list of projects, and screens—in the DataXpress database currently logged in to—that the current user has permission to access. Select a project, and screen to which data output in the experiment will belong.

Users with permission levels Administrator, and Lab Head can create new projects, and screens, while Read/Write, and Read Only users must select from the existing list. New projects, and screens are checked against the DataXpress database to ensure unique names are chosen.

Projects, and screens are two high-order category levels enforced for all data output from PatchXpress Commander Software in order to help manage these data. A project might be used to contain results relating to a particular target, while screens might subdivide this according to the compound libraries used. It is preferable not to create a new screen for every experiment, especially if these are named by date, and time, as this simply replicates functionality already in DataXpress Software, and you lose the higher level of categorization that project, and screen are intended to give. However you are free to use these categories in whatever way best suits your purposes.

Projects, and screens are independent queryable attributes in DataXpress.

Note: Data security is set in DataXpress using project, and screen categories. Thus, users are given rights to view all the data in a particular screen or project, or are excluded from entire screens or projects—they cannot be given rights to just some of the data in a screen.

Projects and screens are two category levels enforced within PatchXpress in order to help manage output data.

Create new projects and screens sparingly, and do not duplicate existing attributes when naming them.

Data are shared in DataXpress as entire projects or screens.

Compound Plates

The **Compound Plates** dialog has one tab for each of the four plate locations on the PatchXpress 7000A instrument. Check the tabs for the locations you will use, then configure the tabs.

Well plate configuration consists of two parts:

- Select a **plate contents file**: this file reports the compounds, concentrations, and other parameters, for the contents of a specific well plate (see Plate Contents Files on page 54 for details).
- Select or configure a **compound layout**: this identifies wells in the plate as containing test or control compound. Compounds must have been added to the plate in a regular pattern that allows definition of a compound layout within this dialog (see Compound Layouts on page 74).

Note: It is possible to specify a particular compound layout, and volume per well within a plate contents file, in which case the fields for entry of these in the **Compound Plates** dialog are automatically filled when the contents file is selected.

Having checked a plate location to enable it, enter or select the name of the well plate you are using in the **Plate ID** field. The list box in this field shows all the txt-extension files in (by default) the **D:\PatchXpress\PlateFiles** folder, where compound plate files should be stored. If you have barcoded plates, place the cursor in the **Plate ID** field, and read the barcode to automatically write the barcode into the field.

You must provide a plate ID for all enabled well plate locations in order to run an experiment, but it is not necessary to have a plate contents file corresponding to these. If you run an experiment without plate contents files the well location is recorded for each compound addition. This information is passed to DataXpress, so, provided you have an independent record of the contents of the plates, there is enough information to be able to later identify (manually) all the compounds added.

When a compound plate file is found it is read into the dialog. The field in the lower left of the dialog lists the contents of the plate, while the well plate diagram to the right grays the wells that contain compound. Hold the cursor over a gray well to see its contents in a tooltip.

If necessary you can open the plate contents file in Excel, directly from the dialog, where you can edit it.

With a plate contents file selected, choose a compound layout (see below) from the top field in the right of the dialog. If the layout has been written into the plate contents file, this will be automatically selected. If no layout is specified in the plate contents file, and there are no satisfactory compound layouts in the list, you will need to open the **Layout Manager** (button at the bottom of the **Compound Plates** dialog) to configure one.

Next enter the volume of compound per well. As for the compound layout, the field is automatically filled if this value is specified in the plate contents file.

Note: In screening mode this value, along with the volume of compound you apply in compound additions, determines the number of times a compound will be attempted if cells fail during the cell procedures.

The specified volume is not corrected for residual volume that remains in the compound well. The residual volume depends on the geometry of the compound plate. You should specify the usable volume, for example, the well volume less the residual volume, in the plate setup. The residual volume is typically 10 µL to 20 µL in conical or round-bottom well plates, and about 50 µL in flat-bottom plates.

Compound Layouts

Each compound plate requires a ‘compound layout’. Compound layouts divide the wells on the plate into ‘sets’, such that compound from a set is applied to one cell in the application of the procedure to that cell. Within sets the constituent wells are identified as belonging to specific ‘compound groups’—for example*.,* as test or control compounds. Compound groups are generic classifications that can be applied to

different individual compounds, so long as they play the appropriate role within the experiment design.

Compound group classifications defined in the layout are used to configure the sequence of compound additions later in the experiment procedure, and after acquisition are important attributes for analysis in DataXpress.

Compound layouts are defined, and stored in the **Compound Layout Manager**, opened from the **Compound Plates** dialog. Existing layouts can be edited, deleted, renamed, and copied to provide the basis of a new layout. New layouts can be created afresh.

Most layouts define a set of wells to be used in the application of the procedure to a single cell, and then repeat this set across the plate. An alternative layout type has just one well for each of the different compound groups used, for example only one well containing test compound, one well containing Control A, etc. Instructions for both of these types of layout are presented below.

Repeated-Set Layouts

For the more common, repeated-set type of layout, configuration consists of:

- definition of a first, single set of wells, for use on one cell during a procedure
- iteration of that set across, and down the plate

The steps below set this out in closer detail.

1. Select plate size (for example, 96-well, 384-well, etc.).
2. In the far right section of the dialog, select the **Cell procedures use compounds according to a common pattern** option (this is the default).
3. Now create the first set in the plate diagram using the **Compound Groups** section of the dialog. The first set should be created in the upper left of the diagram.
4. In **Compound Groups**, check the compound group that you want for the first well or wells in the set.
5. Click on the **Select Range** button for that type.
6. Move the cursor up to the diagram, and drag it over the well or wells you want to define as being of that group—the wells are given a colored border to indicate the group type.
7. Go back to the **Compound Groups** section, and repeat for other compound groups included in the set.

   The wells defined in this process should be adjoining, in a pattern likely to maximize the number of such sets that can be fitted onto the plate.
8. When you have finished the first set, enter the number of times the set should be repeated, horizontally across the well plate,

and vertically down it, or use the **Fill Entire Plate** button, in the bottom right-hand section of the dialog.

Single-Well Layouts

To configure single-well layouts, where only one well of each compound group is used in the experiment:

1. Select the **Cell procedures use compounds from the same, shared well** option in the right of the dialog.
2. Check the first compound group you will identify in the diagram.
3. Press the **Select Range** button for that group.
4. Move the cursor up to the well plate diagram, and click on the single well that will contain compound of the selected group.
5. Repeat as required to select other wells for other compound groups.

Compound Groups

There are four compound groups available: Controls A, B, and C, and Test. Control A might be negative control, and Control B positive control. There is only one group category for test compound, Test.

Having only one Test category is intended to help reinforce that you should configure procedures with only one test compound.

DataXpress is unable to deal effectively with multiple test compounds per cell procedure, making all but the first invisible to queries. If your experimental conditions are such that they allow the testing of multiple compounds on single cells, configure a procedure that tests just one compound at a time, and turn on cell reuse.

Within a well set there can be more than one well for each compound group. In this case the wells within the group are indexed for full identification. A test compound well range, for example, might have test compound at different concentrations, for example, for IC50 analysis.

Compound Groups for Experiments on Ligand-Gated Channels

For ligand-gated experiments, where the test compound is sometimes added pure (at some concentration), and sometimes added mixed with a second compound—for example, an agonist:

- Make wells that contain only test compound 'Control'
- Make wells that contain test compound mixed with second compound 'Test'

These attributions will make output data more easily analyzable in DataXpress.

For example: testing inhibitor bicuculline with agonist GABA:

- Wells containing only bicuculline should be 'Control' (A, B, or C)
- Wells containing bicuculline and GABA should be 'Test'

It is advisable to name the mixture with the test compound first, for example 'bicuculline + GABA 10 uM'.

As an example of a compound group layout, consider a five-point IC50 experiment, with negative, and positive controls. The well set will consist of seven wells:

1. Control A: for negative control,
2. Test x 5, for test compound at increasing concentrations,
3. Control B for positive control.

These could be placed horizontally across the well plate but that would allow only eight compounds to be tested per plate, so the better arrangement is vertical, as illustrated in Figure 5-1:

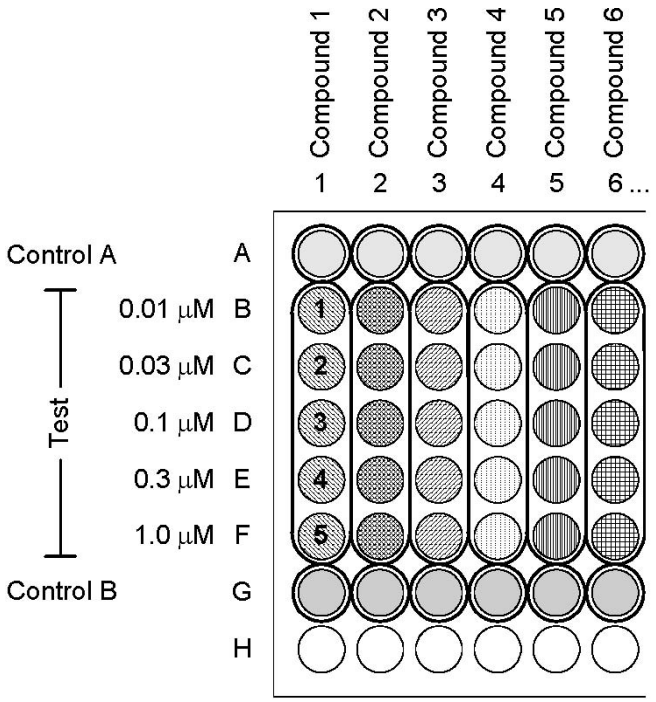


Figure 5-1 Illustration of a compound layout for a five-point IC50.

Once the well plates are set up like this, with the compound layout illustrated, it is easy to define the sequence of compound additions in the **Procedure** dialog, simply using the labels 'Control A', 'Test 1' to 'Test 5', and finally 'Control B'.

Comment

You can enter a comment of up to 200 characters for each experiment. This is passed to DataXpress where it can be viewed, and queried on.

The comment field does not take carriage returns—if you use the **<Enter>** key this is registered as equivalent to hitting the **OK** button, and closes the **Experiment** dialog.

Procedure

See Procedure Configuration on page 80.

Cells

Enter the name of the cell type, ion channel type, and up to five channel subtypes in the **Cells** dialog. You can also enter a comment of

up to 200 characters. All of these are queryable attributes in DataXpress.

You can create a list of cell, and channel types that you commonly use to store in the dialog, and then choose from these each time you set up an experiment. You can add, remove, or edit names in the lists at any time.

Intracellular and Extracellular Solutions

Use the **Solutions** dialog to enter all the details of your intracellular, and extracellular solutions. Each complete set of solutions parameters, incorporating both intracellular and extracellular solutions, is identified with a user-entered 'Solutions' name, selected in the 'Solution Pairs' section at the top of the dialog. Names for the intracellular, and extracellular solutions used are reported alongside the Solutions name in the same list. These are selected, and configured in their respective sections in the bottom of the dialog.

You can add, edit, and remove **Solutions** names from the top list. You can also enter a comment for the solution pair, and the liquid junction potential of the intracellular solution relative to the extracellular solution (see note in the box below).

While the top section of the dialog names a complete set of intracellular, and extracellular parameters, details of the two constituent solutions are named in their respective sections below. Again, the names are saved to an editable list. For each extracellular or intracellular solution you can list the constituent chemicals used in the solution, along with their concentrations, and a comment for each.

Note: Concentration values here are given in millimolars, as opposed to the standard micromolar units for compounds. Besides this there are fields for the solution pH, osmolarity, and the chemical used to adjust pH.

All of the parameters entered in the dialog are queryable entities in DataXpress, though it should be noted that the intracellular, and extracellular chemical lists are recorded as one long text string, for

example, you are unable to query on an individual chemical component of a solution, though the full set of chemicals can be viewed.

The liquid junction potential is the potential across the liquid-liquid interface between two solutions with different ionic compositions. This is not the same as the solid-liquid junction potential between the Ag/AgCl electrode, and solution. The solid-liquid interfacial potential is compensated for the with Pipette Offset (or Junction Null). The calculated liquid junction potential is applied after whole-cell conditions are established. If you have not calculated a liquid junction potential or if you do not wish to apply this correction during data acquisition, make sure that the value is set to 0 mV.

Patch Settings

See The Patching Process on page 110.

Cell Reuse

If a cell remains healthy following completion of a procedure you can have a further procedure run on it—continuously, until the cell dies or the experiment finishes. **Health**, here, is defined by the parameters set on **In Procedure** Tab 7 in the **Patch Settings** dialog, where membrane resistance, access resistance, and membrane capacitance thresholds are set.

If cell reuse is not enabled, only one procedure is run on any cell.

Replicates

You can test each compound set defined in the procedure on two or more cells by enabling this option, and setting the number of replicates you want. Compound is drawn from each set of wells defined in the compound layout as many times as you have replicates set, and applied to different cells. Thus you must ensure that the wells contain sufficient volume of compound for the number of replicates you select.

When both replication, and cell reuse are enabled PatchXpress Commander Software ensures that replicate cell procedures are run on different cells—it will not allow the same compound set to be applied twice to the same cell.

Procedure Configuration

The procedure is the sequence of actions applied to each cell once it is successfully patched. As well as standard steps such as add compound, and start trial, command steps allow you to change settings during the cell procedure, and perform customized steps.

Each run of the procedure on an individual cell is termed a 'cell procedure'. With cell reuse enabled, multiple cell procedures are run on cells that pass cell health thresholds.

IC_{50}-type procedures that require additions of test compound from different wells can be simplified by the insertion of a loop. The set of test-compound wells to be drawn from can then be stipulated in a single step, significantly shortening the procedure length, and the time required for configuration. Inserting a loop into a procedure also turns on cell procedure autocompletion, described below.

The currently loaded procedure is reported beside the **Procedure** button in the **Define Experiment** dialog. You can open the procedure to edit it with either **Edit** in the **Procedure** button menu, or by double-clicking on the button. You can select to open a previously saved procedure from this menu, or build up a new one from a blank slate with **New**. To base a new procedure on another one, open the existing procedure, and immediately save it under another name. You can then make the changes you want in the new procedure.

Rules for how many cells the procedure is applied to depend on the system mode selected; screening or assay. For details, see System Mode on page 66.

Autocompletion

When running a procedure that includes a loop (in screening mode), PatchXpress Commander Software applies 'autocompletion' for cell procedures that fail to complete.

With autocompletion PatchXpress Commander Software automatically attempts to complete only the parts of the procedure that were not carried out on the previous cell. Rather than run the procedure in its entirety on a new cell (which occurs in screening mode when autocompletion is not in operation), the procedure runs as usual up until the start of the loop, and then skips as many of the loop cycles as were previously successfully performed, starting at the one during which the procedure previously failed.

The result, then, is two (or more) cell procedures, in which, for example, the first might include the first three concentrations of an IC_{50}, and the second the final three. With proper procedure configuration, both cell procedures will include response to control compound (because the addition of control should be applied in a step before the loop), allowing results to be normalized for combination in an IC_{50} calculation. See Loop Boundary on page 91.

The Procedure Dialog

Configure procedures in the **Procedure** dialog.

The dialog has a table at the top-left where procedure steps are displayed. Buttons to add, delete, and move steps are located above the table, while step configuration is performed in the space beneath

the table. Tabs on the right are for configuration of scripts, and protocols that can be called on in the procedure, and the **Initial Settings** tab allows configuration of some commonly used settings.

Subsections below cover each of the right-hand tabs, and the step table (with a special section for step timing). Details of procedure configuration, with explanation of each of the step types, is provided in the Step Configuration on page 83.

The Step Table

The step table shows, for each step, its number in the sequence, step type, configuration details, and duration.

Use the **Append** button to add new steps to the bottom of the procedure list, selecting the step type from the button menu.

The **Insert** button adds a new step above the currently selected step.

The **Duplicate** command in both menus adds a new step the same as the currently selected one, appending or inserting the new step as appropriate.

Unwanted steps can be removed, and you can move steps up or down the sequence with the up, and down arrows.

If you include a loop in the procedure, the last button toggles between an expanded, and collapsed view of these.

Step Timing

When the procedure is run, each step must be complete before the next step begins. There are two important points to note with respect to this:

1. **Start trial steps start a trial only, with a duration that is effectively nil, so that following steps are likely to occur concurrently with the running of the trial.**
2. **Where step durations are set, these are a minimum and actual durations may be longer.**

You can set up procedures with the intention of using the behavior noted in the first point, for example, by starting a trial, waiting a short time, adding compound, and then washing until the end of the trial. This will give you a trial that contains data from before, during, and after compound addition. However, due to the second point, there is a danger that unless the trial is long it could finish before the other steps occur. This is especially a problem for compound additions, for which there can be relatively long waits before compound is brought to a chamber. This is reflected in the duration range reported for compound additions in the step table—the fluid handler services all 16 channels, so when a compound addition is called it could have to wait for

previously queued compound additions before it receives the compound.

One way to overcome this problem is to configure very long protocols, so that you are sure to record the effects of all the following steps, and then put in a stop trial step.

Note: The corresponding trials from each cell procedure will be of different lengths under this system.

Another means of being sure you capture the effects of compound addition, especially useful for experiments on ligand-gated channels, is to use the compound-triggered protocol acquisition mode. In this mode each compound addition triggers a sweep of the selected protocol, see Compound Triggered Mode on page 99.

Step Configuration

Having added a step with the **Append** or **Insert** buttons, configure it while it is selected, and the area beneath the procedure table displays options appropriate for a step of its type.

The step types include the following:

Start Trial

Start trial configuration consists in specifying a protocol to be run. The protocols available for selection in the Protocol list box beneath the table are those listed on the **Protocols** tab on the right. Thus, to locate, edit, or create a new protocol, use this tab.

See Edit Protocols on page 98, for full discussion of protocol configuration.

If you want no fluidics changes while a trial is running, make the step following the trial start a wait step, configured to wait until the end of the trial.

If a start trial step occurs while another trial, started earlier in the procedure, is still running, the first trial is stopped, and the second started.

Stop Trial

Stop trial steps stop any trial that is running. If you want a protocol to run to completion, there is no need to use a stop trial step.

This step type is most useful in procedures with drug additions occurring during recording. Because the timing of drug addition steps may undergo a delay (see Add Compound below), you cannot know how long you must configure a trial to run in order to capture all the drug addition steps that you want to record. The answer is to configure the protocol to run a long time—longer than you envisage will be

required—include as many add compound steps as you want, and then finish with a stop trial step.

Add Compound

Add compound configuration consists of selection of the type of compound to be added—using the compound group defined in the Compound Layouts on page 74—and of how addition is to be carried out.

To select compound to add in the step you can click directly in a well of the appropriate group in the compound layout diagram. This sets the first two fields: compound group, and compound index.

Within a loop, you can set one compound addition to deliver from a range of Test wells. This needs to be specified directly in the second compound index field that is enabled in this situation.

Next set the volume of compound to add. This can be varied for each compound addition within the procedure.

Note: It is acceptable to return to wells already used within a procedure—you just need to ensure that there is sufficient compound for all the steps that the wells are accessed.

Suction

Compound additions can be made with concurrent aspiration (suction) through the wash station aspiration nozzle. In this case compound flows out of the dispensing tip, across the cell, and then out of the wash station nozzle on the other side of the chamber. The total amount of fluid within the chamber remains at about 7 µL to 10 µL (at default wash station height).

With suction off, the entire volume of compound is added to the chamber, mixing with the 7 µL to 10 µL of fluid already present.

Note: This setting is different from the suction option in the **Settings** dialog **Add Compound** tab, which applies negative pressure to the cell during compound additions.

Having suction on during compound addition has two advantages:

- Compound dilution is close to nil
- Compound contact with the sides of the chamber is kept to a minimum, increasing the likelihood of the compound's complete removal in a following washout step. There is also some evidence that suction during compound addition may increase compound potency.

Suction cannot be applied if postdelivery compound mixing has been enabled in the **Settings** dialog.

Note: Fluid is always removed from the chamber before any new compound addition. So, if suction was not enabled for a previous add compound step, most of this is automatically removed before the next compound is added.

Multi-Dose

For some compounds drug potency has been found to increase if two or more applications are made for each compound, one following shortly after the other. If multi-dose application is configured the single add compound step in the procedure becomes, in its application, two or more dispense actions.

Once the fluid handler comes to a chamber that is queued for a multi-dose application it does not leave to service other chambers, allowing specification of a fixed time between the consecutive dispenses within the step. The shortest possible time between dispenses—for example, when no compound mixing, and tip reuse is enabled—is 11 seconds. Compound mixing can increase this minimal interval, though tip reuse will not decrease it.

If tip reuse is not enabled, the fluid handler picks up a clean tip for each of the multi-dose applications. With tip reuse the same tip is used, but the handler picks up only as much compound as is dispensed per delivery, returning to the compound plate to pick up compound for each subsequent delivery.

Ligand-Gated Precise Dose Duration

Under this option a washout is automatically triggered at a precise time following the start of the compound dispense, thus exposing the cell to compound for a precise, known duration. This is often useful when testing ligand-gated channels.

When this option is selected configure a washout step to follow the add compound step—not necessarily immediately. The washout started in the add compound step will continue until the end of the washout step.

Compound Additions Within Loops

Multiple compound additions can be created within a loop, however only one of them—delivering test compound—can have more than one

well location defined for it. This step determines the number of cycles in the loop, for example, the loop cycles as many times as there are wells defined for the step. For further discussion, see Loop Boundary on page 91.

Timing of Compound Addition Steps

Note: The timing of add compound steps cannot be stipulated exactly in procedure configuration, because the fluid handler that delivers the compounds serves all 16 electrode chambers, and so must queue the cells as calls are made for it.

Actual compound addition always occurs at least four to five seconds after the end of the step prior to the add compound step—effectively inserting an additional wait step in the interim—as the fluid handler picks up a tip, and compound, and brings them to the chamber. Depending on the length of the queue, waits can be many seconds longer.

The duration of an add compound step is determined by the volume of compound being delivered, set in the **Procedure** dialog, the compound delivery rate, set on the **Add Compound** tab in the **Settings** dialog, and whether compound mixing is enabled.

The step following an add compound step begins after compound addition, including in-chamber mixing, is completed. For example, if you set a start trial step after a compound addition, recording will only begin once all the compound has been added, and mixed.

Wait

Wait steps are periods when there is no fluid exchange—the cell sits in the fluid in the well, with no circulation, as either a trial or the membrane test is run.

Wait steps can be used to give time for cells to settle after stimulation, and to space out compound additions. If a voltage trial is not active the membrane test is run, and measurements of whole-cell parameters are updated.

During configuration be aware of the amount of fluid in the chamber during wait steps. If a wait step follows an add compound step that had suction off, the cell waits in the full contents of the compound addition, plus the approximately 7 µL to 10 µL of fluid that was present before the compound was added. Otherwise the cell waits in just the 7–10 µL of fluid in the chamber at the time—extracellular solution at the start of a procedure (before compound additions) or following a washout, or the last compound that was added.

Besides setting an absolute wait time, you can opt to **wait until a trial finishes**, or until a script-defined condition is met.

For simple conditions, that require a true or false response to a query that can be posed directly to PatchXpress Commander Software, the script property can be entered directly, for example 'RaAtTarget' entered in this field returns FALSE if Ra is over the limit set for acceptable Ra in patch settings, and TRUE if under. The wait step will continue until it receives a TRUE (or until the step times out, see below).

More complex wait conditions might require a script function to be called, in which case the name of that function should be entered in the script condition field, and the function must be included in a script file associated with the procedure. See Scripts Tab on page 97, and the PatchXpress Scripting Interface PDF.

Two fields below the script field allow you to stipulate how long to wait for a script condition to be met, and what action to take if it is not.

All the wait options can be scripted—the script equivalent of each is shown in the bottom field.

Always bear in mind that when following a wait step with an add compound step, the wait may be extended beyond the time you specify because add compound steps need to be queued while the compound fluid handler serves other chambers.

Washout

Washout steps rinse extracellular solution through the chamber in a sequence of fill, and aspiration steps, to wash out compound from previous add compound steps. To ensure complete exchange a period of no less than 45 seconds should be used, but 17 seconds is the shortest duration PatchXpress can perform a washout.

Because the inflowing buffer washes directly over the cell the onset of complete washout in the vicinity of the cell is very fast, however it takes many seconds for the compound outside the vicinity of the cell to be replaced by buffer. It should be noted, moreover, that 45 seconds might still be insufficient for some 'sticky' compounds, that might attach to the chamber sides, and wash station nozzles.

Washout steps are similar to wait steps in that they have a defined duration, and they offer the same configuration options as well, for example, you can stipulate a washout duration, wash until the end of a trial, or use a script call that defines a condition to be met to stop washout, and start the next procedure step.

A full washout cycle consists of repetitions of 'wash', and 'fill' phases, always finishing with a 'dry' phase. Because of the cyclical nature of the washouts only specific durations can be configured for these steps—the shortest time is 17 seconds, and thereafter increments in multiples of 7 seconds.

- Wash phases: Both inflow, and aspiration are active, flushing extracellular solution through the chamber. The rate of aspiration is greater than the buffer inflow so that if the level of fluid in the chamber is higher than the aspiration nozzle opening at the start of a wash step, it is brought down to the level of the nozzle by the end.
- Fill phases: Aspiration stops but extracellular solution continues to feed into the chamber, raising the fluid level above the aspiration outlet. Most fill steps in a wash deliver a bit under 20 µL of extracellular solution to the chamber, but the second fill step always has double this amount.
- Dry phases: Aspiration only occurs, removing all fluid above the level of the aspiration nozzle.

Command

Commands are used to change basic instrument settings or call script functions to perform custom procedures. As with all procedure steps, the **Command** is applied to each chamber individually as it reaches the step. For example, at any one time some cells might be at one holding potential, and others at another, because the first cells have reached a command step in the procedure that changes the potential, and the other cells have not.

Most of the predefined commands change settings that have input interfaces elsewhere in PatchXpress Commander Software. Two exceptions to this are:

1. **Lock the compound robot**: this command locks the fluid handling arm to a particular chamber so that successive compound additions occur without the delays that can occur when the arm is servicing all the chambers. Other chambers have to wait for the handler to be unlocked before they can lock it for their sequence of add compound steps.

Note: you must always include a second command to unlock the fluid handler, following the sequence of compound additions during which it is to be locked. If you do not do this, the handler will not progress from the first cell.

2. **Wash**: this command starts a wash that continues until there is another Wash command to disable it, or a standard washout or add compound procedure step. To compare this command with a normal washout step: if a start trial step is configured to follow a normal washout, the trial does not start until the washout is complete. If a start trial step is placed after a command Wash step, the trial starts immediately, recording as the wash proceeds.

To configure a command step select the basic command type from the **Command** field, then configure it in the field that is displayed after your selection. Typically the configuration options simply enable or disable a function, or you can enter a new setting. For scripts, enter the name of a script function that you want to call. The function must be included in one of the script files included in the procedure.

Script equivalents for all the commands in the list are displayed when the commands are selected. Users creating their own script functions can copy these to build the commands into the functions.

Predefined commands are:

- **Call script**: call any script function (enter the function name, not the name of the script file). The function must be in one of the script files included in the procedure, listed in the **Scripts** tab (see Scripts Tab on page 97).
- **Pneumatic Ra control**: turn on or off, changing the original setting made on the **Procedure** dialog **Initial Settings** tab.
- **Whole-cell compensation**: turn on or off, changing the original setting made on the **Procedure** dialog **Initial Settings** tab. Disabling also turns electrical Ra correction off, if it is enabled.
- **Electrical Ra correction**: turn on or off, changing the original setting made on the **Procedure** dialog **Initial Settings** tab. Enabling turns on whole-cell compensation if it is disabled.
- When electrical Ra correction is enabled it uses the correction percentage value in the **Initial Settings** tab. If you intend turning correction on in a command step, set the percentage in the **Initial Settings** tab before the experiment, but then disable correction in the tab. You can also set the percentage in a command step if you wish (next command).
- **Electrical Ra correction percentage**: set a new electrical Ra correction, and prediction percentage, changing the original setting made on the **Procedure** dialog **Initial Settings** tab.
- **Set holding pressure**: set a new holding pressure, changing the original setting made on the Patch Settings Dialog on page 110 **Whole-Cell** tab (5.2.2).
- **Set MeasurementsHistory sweeps**: change the number of measurements in the moving window used to calculate MeasurementHistory script properties, originally set on the **Procedure** dialog **Scripts** tab.
- **Resistive leak subtraction**: turn on or off, changing the original setting made on the **Procedure** dialog **Initial Settings** tab.
- **Membrane test between sweeps**: turn on or off, changing the original setting made on the **Procedure** dialog **Initial Settings** tab.

- **Log comment**: enter a comment to be written to the cell log when the procedure reaches this step.
- **Mix compounds during pickup**: turn on or off, changing the original setting made on the **Settings** dialog Add Compound on page 69.
- **Mix compounds during delivery**: turn on or off, changing the original setting made on the **Settings** dialog **Add Compound** tab.
- **Compound delivery rate**: set a new compound delivery rate changing that originally set on the **Settings** dialog **Add Compound** tab.
- **Compound delivery X offset**: set a new X offset for tip position during compound delivery, changing the original setting on the **Settings** dialog **Add Compound** tab.
- **Compound delivery Z offset**: set a new Z offset for tip position during compound delivery, changing the original setting on the **Settings** dialog **Add Compound** tab.
- **Lock the compound robot**: lock the fluid handler so that it serves only the current chamber until it is unlocked again in another application of this command.

Note: Be sure to set the unlock step or else the experiment will not progress past the first cell to receive compound.

- **Terminate unhealthy cells**: turn on or off, changing the original setting made on the **Procedure** dialog **Initial Settings** tab.
- **Set cell health maximum Ra**: set a new maximum Ra (used for cell health monitoring) changing the original setting made on the **Patch Settings** dialog **In Procedure** tab.
- **Set cell health minimum Rm**: set a new minimum Rm (used for cell health monitoring) changing the original setting made on the **Patch Settings** dialog **In Procedure** tab.
- **Set cell health minimum Cm**: set a new minimum Cm (used for cell health monitoring) changing the original setting made on the **Patch Settings** dialog **In Procedure** tab.
- **Set user list for next trial**: enter user list parameter values to apply in the next trial to run. The protocol for the trial must have the user list enabled, with the parameter to change already selected. If the protocol already has parameter values entered, these are overridden by the values you enter here.
- **Wash**: start a wash that continues until it is stopped by either a later command Wash (disable) step, or by the start of a normal washout or add compound procedure step.

When the setting that is changed in a command step is one that is passed to DataXpress to become a queryable attribute, the original setting, that applied at the start of the experiment, is the one that becomes the attribute. The change of setting is recorded in the individual cell procedure log, which can be viewed in DataXpress.

Loop Boundary

Procedure step loops are designed specifically to make IC50-type procedures shorter, and easier to configure.

Rather than having to include separate add compound steps for each of the test compound concentrations—as well as steps of other types (for example wait) to be performed with each addition—looping allows all these steps to be configured once only. Steps to be repeated are bounded by loop boundary steps, and then iteration of the loop cycle is determined by stipulation of a range of test compound well locations in the add compound step within the loop.

When loop boundary steps are added to the procedure a dotted line on the left of the procedure table clearly marks the loop's range. An add compound step added within the loop will allow selection of a range of test compound wells. This step, which is marked with a key icon, determines the number of loop cycles—as many cycles as there are wells in the range defined for the step.

Usually, only one add compound step is placed within a loop. Additional add compound steps can be added, but these can have only a single well location each. This means that within each cell procedure the same one well will be returned to in each cycle of the loop. If you configure the procedure this way you must be sure to add enough compound to this well for all the cycles.

Excluding all but the essential steps, a typical loop procedure might have the following structure:

1. Start trial (a long-running trial)
2. Add compound: Control A (negative control)
3. Loop boundary
4. Add compound: Test indices 1 to 5 (the 'key' step)
5. Loop boundary
6. Add compound: Control B (positive control)

With five test compounds configured at step 4, the loop will cycle step 4 five times.

Only one loop can be included in a procedure.

When a loop is configured the **Expand loops** button above the table can be used to display all of the steps of the procedure as they will be applied.

Note: When a loop is included in a procedure PatchXpress uses Autocompletion on page 81.

Amplifier Mode

Adding an **Amplifier Mode** step to your procedure lets you control whether the amplifier is in Voltage Clamp or Current Clamp mode. Voltage Clamp is the default mode selection. Use **Voltage Clamp** to measure current. Select **Current Clamp** to measure voltage. If you use Current Clamp as a procedure step you must follow it later in the procedure with a resetting to Voltage Clamp before ending the procedure in order to ensure cell health measurements are performed.

Holding Level

The **Holding Level** step changes the default setting. The new setting applies immediately, even if a trial is in progress. If the amplifier is in **Voltage Clamp** mode, the Holding level changes the Holding Potential. If the amplifier is in **Current Clamp** mode, the Holding Current is changed.

Find Threshold

Use a **Find Threshold** step to identify the action potential threshold of a cell. In the **Find Threshold** step, designate the **Action Potential Duration** (milliseconds), and the number of sweeps. The sweep number determines the number of required consecutive attained threshold matches.

The **Find Threshold** step must be preceded by a **Start trial** step, and the **Amplifier mode** must be set to **Current Clamp**. There must also be a protocol associated with the trial that includes the following conditions (set using the **Edit Protocol** dialog):

- **Sweeps/run** must be set to a number greater than the designated number in the **Find Threshold** step.
- **Epoch** waveform **Type** must be set to **Step**.
- **Epoch** waveform **Delta level** must be a positive value.
- **Event duration** measurement must be selected from the **Measurements** tab section.

The process for **Find Threshold** can be followed in the **log** tab window. The process involves finding the designated number of consecutive matching threshold sweeps. When the requested number of matching sweeps are found, the trial stops, and the command output level from the selected Epoch in the last sweep is used for the matching Epoch in

all trials until the Amplifier mode is changed. If fewer than the designated number of sweeps match when the trial finishes, the procedure terminates by default unless **Continue procedure** is selected in the **Step** definition section of the **Procedure** dialog in the **Find threshold** settings.

Initial Settings Tab

Enable a range of cell health, and recording options on the **Initial Settings** tab. The settings made here apply to all channels from the start of the procedure, but can be changed with command steps built into the procedure.

The various Initial Setting functions only apply if the amplifier is in Voltage Clamp mode, and have no effect while the amplifier is in Current Clamp mode.

Pneumatic R_a Control

When **Pneumatic Ra** control is enabled each chamber's Ra value is monitored, and a sequence of suction steps automatically applied to the cell if Ra rises above a stipulated level, in an attempt to clear the electrode hole, and reduce the resistance. The force, duration, and frequency of the suction pulses varies automatically with Rm, and Ra. Configure the settings for this on **In Procedure** Tab 7 in the **Patch Settings** dialog; see Pneumatic Ra Control on page 93 for details.

If enabled, pneumatic Ra control is applied whenever Ra rises above the threshold you set, including when a trial is running. If you want to avoid its application during a trial, include a wait step before the start of the trial waiting for script condition 'RaAtTarget'. The wait step will continue while the Ra remains above range, and cease once the script condition returns TRUE.

Membrane Test Between Sweeps

Enable generation of the membrane test pulse between the sweeps of a trial while it is running. This allows cell health measurements to be updated continuously throughout the trial, in turn allowing the possibility of triggering pneumatic Ra control or cell termination.

For this setting to be effective, protocols must be configured with non-minimum sweep start-to-start times, leaving time between the

sweeps for the membrane test to run; the test is not automatically inserted between sweeps that are configured to run continuously.

Note: If you work with very slowly deactivating currents, such as hERG, Membrane Test Between Sweeps may deliver falsely low values of the membrane resistance. The first few measurement cycles would reflect the still active current of interest, which deactivates or inactivates with a time constant of up to many seconds (depending on the channel, and protocol used). If Membrane Test Between Sweeps is used in combination with Terminate Unhealthy Cells on page 96, this scenario may lead to unintended termination of cells that are actually viable.

Resistive Leak Subtraction

Resistive leak subtraction subtracts an estimate of the leak current from the membrane current, based on Rm.

Like P/N leak subtraction on page 106, configured in the **Edit Protocol** dialog, trials recorded using this option have two signals: one each for the raw, and corrected response. Select which to view from the **View Settings** dialog in the **Cell View** toolbar.

If resistive leak subtraction is enabled, and the procedure runs a protocol in which P/N leak subtraction is configured, resistive leak subtraction is automatically disabled for that trial.

Leak subtraction works by scaling the command potential waveform by the membrane resistance (Rm) to obtain a time-varying estimate of the leak current, which is then subtracted from the membrane current. Thus the leak current that is subtracted varies as the command waveform varies. The Rm value used is updated continually from the membrane test, so with membrane test enabled to run between sweeps, it will update within a trial.

Whole-Cell Compensation

With whole-cell compensation enabled, combined membrane, and electrode capacitance is measured by the membrane test prior to each trial in the procedure. The trial is then compensated accordingly, removing transients due to membrane capacitance, and the fast component of electrode capacitance from recorded traces. This is done by charging, and discharging the capacitance through headstage circuits separate from those relayed to the amplifier outputs.

Measurements for whole-cell compensation are made once only for each trial: they are not updated during the trial even in if you have configured the membrane test between sweeps.

Electrical Ra Correction

If whole-cell compensation is enabled you can also enable automatic correction for access resistance. Access resistance—the resistance between the headstage, and the membrane—has the effect of slowing the speed at which command voltage changes are felt at the membrane, and of making the steady-state voltage at the membrane different from the command. As well as this, Ra, in parallel with cell membrane capacitance, forms a filter that restricts recording bandwidth.

Electrical Ra correction has two components:

- Prediction: in order to speed the rate of response to voltage changes the prediction component adds a transient signal to the command potential. The distorting effects that access resistance has on the time course of voltage changes as they are felt at the membrane are removed up to the percentage setting specified. For example, with a prediction setting of 90% the time-course effects of Ra are equivalent to those that would be due to an uncorrected Ra that was 10% of the actual value.
- Correction: in order to mitigate the effects of access resistance on steady-state membrane potential, and filtering effects, the correction component feeds back a portion of the measured membrane current, adding it to the command potential. The correction percentage setting determines how much of the measured Ra is compensated for, for example, if Ra is measured at 10 MΩ, a 90% correction effectively reduces this to 1 MΩ.

In the PatchXpress Commander Software, the correction, and prediction percentage settings are locked together at the same value, hence there is only one field to set both parameters. While the prediction setting is limited only by the headstage circuitry, positive feedback in the correction circuitry can mean that with too high a correction oscillations occur. Take care not to set too high a percentage in the correction, and prediction field, for this reason.

The correction signal passes through a one-pole RC filter before being summed with the command potential. As well as the correction percentage setting, lower cutoff settings in this filter help ensure stability when large amounts of correction are used. The –3 dB cutoff frequency for this filter is set automatically at half the analog bandwidth for each trial. This setting should help minimize the chances of setting up oscillations in the circuit.

If the time constant for the Membrane test is too fast to measure (due to low Ra), and no Ra value is returned, electronic Ra compensation is not applied.

The analog bandwidth is set up in the **Protocol** dialog, see Filtering on page 102, for details.

Terminate Unhealthy Cells

This option automatically terminates cell procedures on cells that become **unhealthy** as defined by the **cell health configuration** fields in **In Procedure** Tab 7 of the **Patch Settings** dialog. These set minimum acceptable membrane resistance, and capacitance values, and maximum acceptable access resistance.

Failure to meet cell health thresholds is reported in the cell log whether cell termination is enabled or not.

When a cell is terminated it cannot be restored in order to complete the procedure (in contrast to Suspending Cells on page 41). The procedure running on the cell immediately ceases. This means that, if the membrane test is run between sweeps, a cell could fail part way through a trial.

Note: Care should be taken if this feature is combined with Membrane Test Between Sweeps on page 93. With slowly deactivating or inactivating channels, this combination may lead to unintended termination of healthy cells.

Protocols Tab

The **Protocols** tab lists all the protocols associated with the current procedure. To include a protocol in a procedure the protocol must first be placed in the list. It will then appear in the **Protocol** list box for start trial step configuration, allowing its selection in the procedure.

All the protocols in the **Protocols** tab list are saved with the procedure, including any that may not actually be used in the procedure.

A range of editing, import, and export tools are available for protocols on the tab. You can open a protocol to edit it by double-clicking on it, or select it, and press the **Edit** button. See next section, Edit Protocols for full details on protocol configuration. The **New** button creates a new protocol with default values. Having created a new protocol you must name it, then open it to edit it.

If you want to base a new protocol on one already included in the procedure, select the existing protocol then press the **Copy** button. Name the new, copied, protocol, then open it for editing. Any protocol can be renamed, or deleted from the procedure.

If you want to copy an existing protocol into the procedure, use Import to find the protocol on the PatchXpress 7000A system or a networked computer. The Import dialog defaults to:

D:\PatchXpress\Protocols

This directory is intended as a common location in which to save protocols that you might want use in multiple procedures. It is also the default directory for the **Export** command, which copies a protocol that

is in a procedure to another directory, from which it can be imported into a second procedure.

When you select a protocol a number of key features of the protocol are reported in the bottom of the tab.

Scripts Tab

The **Scripts** tab lists the script (TXT) files available in the current procedure. These files are saved in the procedure folder.

When a script is included in a procedure, the functions in the script are available for wait, washout, and command steps, which can all call these functions. When you create the step, select the script option, and then type, or copy, and paste, the function name into the relevant field. You can copy function names by opening scripts from the **Scripts** tab.

When a script function is called, all the script files in the procedure are searched to locate that function.

Note: The fields where you enter the function names take VB Script, so you can use this to call more than one script function in a particular logical relation. For example, you can insert 'AND' or 'OR' between two named functions.

Script file management mimics that of protocol files on the **Protocols** tab. Double-click files to open them into a simple editing window, or select, and press the **Edit** button. The **New** button creates a new, empty, script file, that you need first to name, then can open to write the script. Copy, and rename an existing script in your procedure if you want a new script based on another. Scripts can be copied into the procedure with the **Import** button, and copied out with **Export**. The default directory for both import, and export is:

D:\PatchXpress\Scripts

This folder is intended as a common location to store scripts likely to be used in a range of different procedures.

MeasurementsHistory Setup

The MeasurementsHistory scripting object operates a moving window on peak or mean measurements generated during trials. The bottom section of the **Scripts** tab has two setup options for this object:

- Select to use peak or mean measurements in the moving window
- Set the number of measurements within the moving window

Values calculated from the moving window can be accessed in scripts. For example, with peak measurements selected, and a window size of five measurements, you can obtain the mean, slope, or standard deviation of the last five peak measurements, updated for each sweep

in the trial. See the *PatchXpress Scripting Interface PDF* (opened from the main toolbar **Manuals**, and **Web Support** button) for a full list of **MeasurementsHistory** properties you can call.

Note: The measurement you want to use—peak or mean—has to be enabled in the protocols that you run in the procedure, and that these measurements are available to the **MeasurementsHistory** object only while the trials are running.

Two scripts with functions that you may find useful are supplied with PatchXpress Commander Software. **Stability and Baseline Utilities.txt**, for example, contains a number of functions that can be used to pause your procedure until response to a command stimulus reaches steady-state or returns to a baseline. Many of these functions use the **MeasurementsHistory** object.

Typically, when using script steps of this type you will need to use a protocol with many sweeps so that its full length is longer than you anticipate the cell procedures to last. This ensures that the protocol runs continuously while you monitor values.

Edit Protocols

Whenever you include a start trial step you must select a protocol to define the stimulation, and acquisition properties of the trial. Protocol configuration also includes real-time measurements (for display in the **Measurements** tabs), P/N leak subtraction, and presweep trains.

Select the protocol you want from the **Protocol** list box below the table, when the start trial step is selected. The list box includes all the protocols on the **Protocols** tab. Use the **Protocol** tab to import, edit, and create new protocols—see the section Protocols Tab on page 96 above.

To configure a protocol, double-click on it in the **Protocols** tab, or select it, and press **Edit**. This opens the **Edit Protocol** dialog. The dialog has three tabs:

- **Trial Setup**—to set basic trial properties like acquisition mode, trial structure, sampling rate, and filtering.
- **Waveform**—the stimulus waveform is configured on this tab
- **Stimulus**—to configure P/N leak subtraction, and presweep stimulation pulses. The user list on the tab gives control of one protocol parameter, allowing entry of arbitrary user-entered values for that parameter in each sweep of the trial.
- **Measurements**—configure measurements within each sweep of the recorded traces, such as peak, and slope, for real-time display on the **Measurements** tabs in the **Overview**, and **Cell View** panes.

In addition, a waveform preview window shows a graphical display of the command stimulus. Preview **Auto-update** can be turned on or off by selecting the check box. By default, **Auto-update** is on. When **Auto-update** is off, click the **Update** button to refresh the preview.

Acquisition Mode

Set on the **trial setup** tab, PatchXpress Commander Software has two basic acquisition modes—**Internally triggered**, and **Compound triggered**. A command waveform can be generated in the first mode but not the second, however in compound-triggered mode each sweep of the trial is triggered by an add compound step, making this mode a convenient format for testing ligand-gated channels. Each sweep of resulting trials shows the effects of one compound addition.

The mode distinctions do not mean that compound cannot be added while a command waveform is running—it can—but you cannot trigger a recording sweep by adding compound, and deliver a stimulus waveform at the same time. If you select internally triggered mode to deliver a command waveform drug additions cannot be timed to occur accurately at a particular time within the trial.

Internally Triggered Mode

In this mode a trial, once started, runs to completion—or until it is stopped—according to the timing you stipulate when the protocol is configured. Each recording sweep can be configured to deliver a stimulus waveform. Sweeps can be run with minimum start-to-start time, effectively providing gap-free recording, or be spaced apart.

If **P/N Leak Subtraction** is enabled the subsweeps used to calculate the leak are delivered between the recorded sweeps. Similarly, **Presweep trains** are delivered between each recorded sweep.

Internally triggered mode is the **episodic stimulation** mode familiar to pCLAMP users. In DataXpress acquisition mode is a queryable attribute, and trials recorded in **Internally triggered** mode are labeled **episodic stimulation**.

Compound Triggered Mode

In this mode each sweep of a trial is triggered by an add compound step. No command stimulus can be delivered, and **P/N Leak Subtraction**, **Presweep trains**, and the user list are not available.

Sweeps are triggered precisely by the compound delivery, however recording in each sweep can be set to begin prior to this—the **Pretrigger duration**.

Note: The **Pretrigger duration** is included in the duration configured for the sweep—for example, a trial with a sweep duration of 30 seconds, and a pretrigger duration of five seconds has 25 seconds of recording following compound addition. For this reason the pretrigger duration cannot exceed the sweep duration.

To ensure that pretrigger data are available to record, and that these are as stable as possible, it is advisable to configure procedures to have wait periods prior to compound additions when operating in this mode. This is most important for the first sweep, because if you immediately follow the start trial step with an add compound step—triggering the first sweep—you limit the time of actual pretrigger recording to the time it takes for the fluid handler to pick up compound, and bring it to the chamber. This normally takes a minimum of four or five seconds, but this may be less than what you had configured as your pretrigger duration.

Compound triggered mode is the **high-speed oscilloscope** mode familiar to pCLAMP users. In DataXpress acquisition mode is a queryable attribute, and trials recorded in **Compound triggered** mode are labeled **high-speed oscilloscope**.

Trial Structure

Define the structure of trials on the **Trial Setup** tab. Set the sampling rate here, the number of samples per sweep, sweeps per run, and runs per trial, which together constitute the basic architecture of the trials that the protocol will create. Also set sweep, and run start-to-start times for internally triggered acquisitions.

Sampling Rate

Working from the bottom up, the first element of trial configuration is the sampling rate. The **Sampling rate** selected is used for both analog-to-digital acquisition sampling, and digital-to-analog command sampling. Enter the rate, in kilohertz, that you want to sample at, up to the fastest rate of 31.25 kHz. The nearest rate to the value you enter that the digitizer can work at is immediately displayed to the right of the field, as well as the time between each sample at that rate, in microseconds.

Lowpass filtering is set in the field immediately below the sampling rate field—see the Filtering on page 102 for details.

Sweeps

Next comes sweep definition. First set **Sweep** duration, in seconds. Again, the nearest duration possible, at the selected sampling rate, is displayed, to the nearest 0.1 millisecond. The total number of samples that this equates to is also displayed, beneath the **Waveform** bar at the bottom of the trial setup section.

When a trial is running, acquisition only occurs during sweeps, for the entire sweep, and this is also when the command stimulus waveform is delivered. However, about three percent of the waveform sweep—one and a half percent each at the start, and end—is reserved to be held at the **Intersweep holding level**, with the remaining 97 percent available for waveform configuration. This division is illustrated with the three bars in the trial setup section—first holding, waveform, and last holding—with the duration, and number of samples in each displayed.

As you build up a command waveform in the **Waveform** tab table, the proportion of that part of the sweep dedicated to the waveform is displayed in the waveform bar. Any unused remainder in this part of the sweep effectively becomes part of the last holding.

Sweeps are spaced apart by entering a sweep **Start-to-start interval** on the right of the tab. If you want sweeps to run continuously, check the **Use minimum** box, otherwise you need to enter duration, in seconds. Plainly the **Start-to-start** time has to be at least as long as the sweep itself, but if you have **P/N leak subtraction** or a **Presweep train** enabled there must be time to run these between sweeps as well. An error message is generated if you set an insufficiently long **Start-to-start** interval.

The **Use minimum** option gives clean, effectively gap-free recording, as the time between the end of one sweep, and the start of the next is a single sample interval.

Intersweep Holding Level

The first, and last holding periods in each sweep default to the holding level set at step 4.6 in the **Patching Process** dialog, displayed on the **Trial Setup** tab on the right-hand side. Alternatively, set the intersweep holding level, in the same section, to **Use last epoch**.

On **Use last epoch** the last stipulated level of the command waveform is used as the last holding level for each sweep, maintaining this through any intersweep period (except where **P/N leak subtraction** or **Presweep trains** are run), and on to be the first holding level of the following sweep. Thus, if a waveform is configured with steps of incrementing level, the result is a step-wise command output, stepping up a level in each sweep without returning to the general holding level in between.

Use last epoch is only available for **Internally triggered** acquisition.

Runs and Trials

One trial (in one ABF data file) is recorded for each start trial step.

Note: Trials only ever contain complete sweeps: if a trial is stopped midway through a sweep then all the data from that sweep are lost. If that sweep is the first sweep in the trial then no ABF file is created. Thus, though you can get what is effectively gap-free recording by configuring a trial with one, long sweep, be aware that this sweep must run to completion or no data will be recorded.

Where you set more than one run in a trial the recordings from the corresponding sweeps of each run are averaged. Only the averaged sweeps are saved, so a completed trial always has the number of sweeps you set in the sweeps/run field.

Filtering

PatchXpress does not allow independent control of the lowpass filtering cutoff frequency, instead using set ratios of the sampling frequency. Three ratios are available—one tenth, one fifth, and one to 2.5 of the sampling frequency. Select one of these values in the analog bandwidth ratio field below the sampling rate field in the top left-hand corner of the **Trial Setup** tab. The effective cutoff frequency, given the sampling rate, and the bandwidth ratio, is displayed to the right of the field.

Analog bandwidth is a queryable attribute in DataXpress.

Lowpass filtering is shared between the MultiClamp hardware filters, and software filtering. See Lowpass Filtering on page 141, for details.

Waveform Configuration

For internally triggered acquisition mode, configure the stimulus command in the **Waveform** table in the **Waveform** tab. The sweep can be divided into up to ten sections—'epochs' A to J—each independently configurable as a step, ramp, or one of four train types.

Note: A little less than the entire sweep is available for waveform configuration, see Sweeps on page 101 section above.

You can view a graphical display of the waveform configuration in the **Waveform Preview** window. This has manual, and automatic update options.

There is no need to use all epochs in the waveform table, nor to configure the combined duration of these to be equal to the sweep length available—if your waveform is shorter than the time available to

it in the sweep, output is held at the **Intersweep holding** level for the remaining time.

the **Waveform summary** shows duration, and voltage output ranges for the entire waveform over the full number of sweeps. A separate amplitude level range is given for **P/N leak subtraction**. If you opt to deliver leak subtraction subsweeps in opposite polarity to the waveform, output during these can go outside the level range for the waveform itself. All voltage output levels throughout the protocol must fall within the range –200 mV to 200 mV.

Epoch Configuration

For each **Epoch** you must select a waveform **Type**, an amplitude **Level** (in millivolts), and **Event duration** (in milliseconds). The meaning of the epoch level differs according to the waveform type that is selected—this is explained below in sections for each waveform type. Epoch durations must be a whole number of samples, so when you enter a time the software automatically adjusts this to the nearest product of the sampling interval. This value is displayed in the **Epoch summary** below the table.

Both the amplitude level, and duration for each epoch can be varied in regularly incrementing or decrementing steps, sweep by sweep, with an associated 'delta' setting. For example, a level of –100 mV with a delta level of 10 mV will have the level for the first sweep go to –100 mV, for the second sweep –90 mV, then –80, –70, etc., for as many sweeps as are configured for the trial.

If you want to change the levels or durations of an epoch in an irregular way, this is possible with the user list, on the **Stimulus** tab, which can control one parameter at a time.

The level, and duration ranges are reported in the epoch summary.

There are six waveform types available for each epoch. When you select one of the train options you need to define the train frequency, in hertz, and also the duration of the **Pulse** as opposed to the 'baseline' component of the cycle, in milliseconds. The meaning of **Pulse**, and 'baseline' differs depending on the type of train you select. Again, this is explained below in sections on each train type.

As with other timing-related fields on the tab, the nearest possible train frequency, and pulse width values to the ones that you enter—given the granularity imposed by the sampling interval—are automatically used. When you enter a **Train frequency** the **Epoch summary** below reports the number of pulses this will produce in the **Epoch**. It also reports the train period, which is the duration of one train cycle, and the actual frequency that will be applied. The last row of the summary shows the actual **Pulse width**, and the proportion of the cycle that the **Pulse** constitutes, as a percentage.

The six waveform types available for each **Epoch** are explained in the following sections.

Step

A step setting holds the output voltage constant at the nominated level for the duration of the **Epoch**.

Ramp

In a **Ramp** epoch the voltage increases or decreases linearly from the level setting of the previous epoch (or first holding period), to the level set for the current ramp **Epoch**.

Pulse Train

This option generates a series of square waves, each consisting of first a period at the 'level' amplitude for the epoch (the pulse), then a period at the **Level** of the previous epoch (which becomes the baseline for the current epoch).

The **Train period** is the duration of one train cycle (pulse, and baseline). If, given the frequency you set, train cycles do not fit evenly into the epoch, the remaining time, if sufficient, is used to generate the **Pulse** component of the cycle, with a shortened period at baseline. If there is not time for another **Pulse** the baseline segment of the final cycle is extended to the end of the **Epoch** (see Figure 5-2).

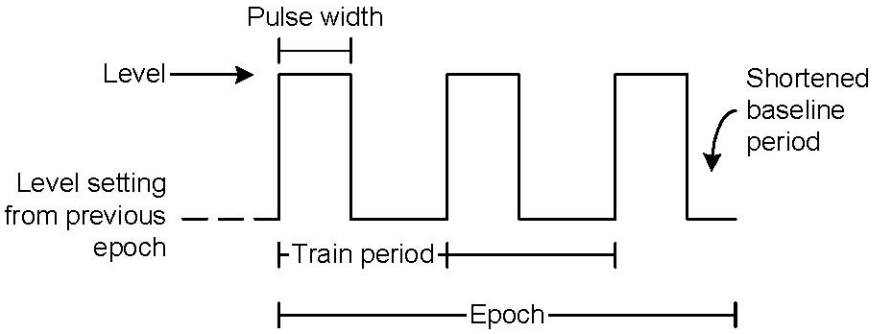


Figure 5-2 Pulse Train.

Triangle Train

A sequence of regular triangular pulses is generated with this option. For any sweep, the level setting of the previous epoch serves as the baseline, and the pulse rises (or falls) to the amplitude determined by the level setting in the current triangle epoch.

The duration of one cycle of the train is the train period, determined by the frequency setting, while the pulse width is the duration of the rise (or fall) from baseline to the nominated amplitude level. For triangle trains, any time remainder insufficient for another cycle is held at the baseline amplitude.

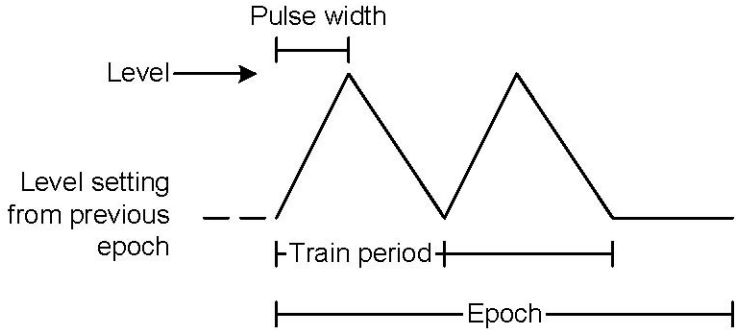


Figure 5-3 Triangle Train.

Cosine Train

PatchXpress generates a regular sinusoidal pulse for this type of train. The level setting of the previous epoch serves as the epoch baseline, output rising or falling to the level setting of the current **Cosine** train epoch, then back to the baseline level, within each train period (similar to the triangle train, but with a curved trace).

No pulse width is entered for cosine trains as the waves are regular, with equal rise, and fall times. Like triangle trains, any time remainder in an epoch insufficient for a full cycle is held at the epoch baseline level.

Biphasic train

Biphasic trains produce rectangular waveform trains in which the pulse width component of the train period is equally divided between the level amplitude you set, and an amplitude equidistant from but of reverse polarity to the baseline amplitude. For example, if the level setting from the previous epoch is 20 mV, a level setting of 30 mV steps the pulse first to 30 mV, then to 10 mV, then returns it to 20 mV for any remainder of the train period, for example, the pulse goes 10 mV above, and then 10 mV below the baseline value of 20 mV, in the first pulse component of the train cycle (see Figure 5-4).

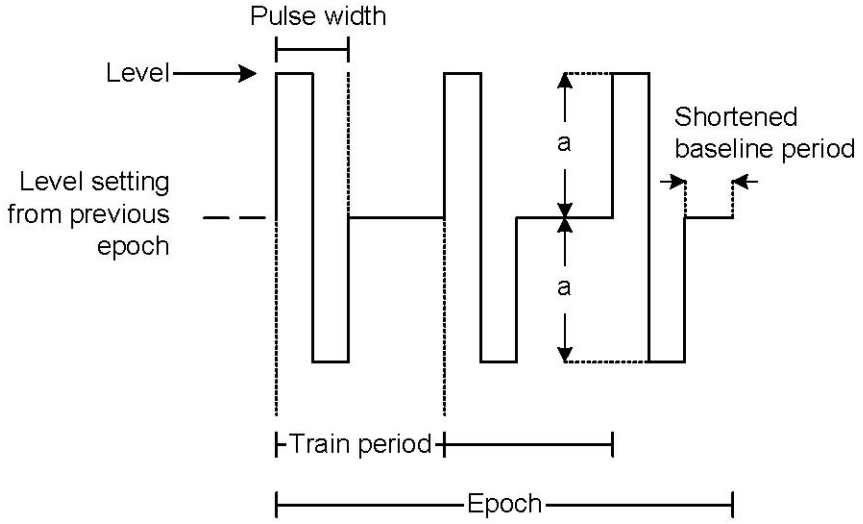


Figure 5-4 Biphasic train.

Any time remainder shorter than a train period is used to generate a final biphasic pulse, if sufficiently long, or held at the epoch baseline level.

P/N leak subtraction

PatchXpress supports **P/N leak subtraction** when a waveform is being generated. This is set on the **Stimulus** tab in the **Protocol** dialog. Scaled down copies of the command waveform, termed 'subsweeps', are generated either before or after the waveform, with the same or opposite polarity as the waveform, in order to ascertain the amount of current in the response that is due to leakage rather than ion channel activity. This is then subtracted from the current response.

By generating waveforms of lesser amplitude than the command stimulus, it is intended that no ion channel activity is elicited. Thus, when the leak current that is calculated from the subsweep responses is subtracted from the raw data response of the main sweep, only currents due to biological activity should remain.

PatchXpress also has Resistive Leak Subtraction (**Procedure** dialog **Initial Settings** tab). If enabled, this is automatically turned off for trials where **P/N leak subtraction** is set.

In PatchXpress you can opt to show your data leak-corrected or not (from the **Cell View** toolbar **View Settings** dialog), but in either case both uncorrected, and corrected data are saved as separate signals in resulting trials.

Leak subtraction subsweeps are not recorded or displayed in PatchXpress as they are run.

The **P/N leak subtraction** subsweeps can be generated relative to a subsweep holding level, different from the normal holding level for the protocol. This can help to ensure that ion channels are not activated. The subsweep holding level is used for any pre-subsweep 'settling time' you may configure, and for the periods between subsweeps determined by your subsweep start-to-start time. If you set a settling time for P/N subtraction, the period you define is applied both before generation of the subsweeps, at the subsweep holding level, and then again after the subsweeps, at the normal holding level.

The amplitudes used in leak subtraction subsweeps are relative to the number of subsweeps selected. For example, if the number of subsweeps is two, the relative amplitude of the subsweeps is half that of the command waveform, if five, then one fifth. Each subsweep runs for the same duration as the command sweep.

Presweep Train

In internally triggered mode you can stimulate the cell with a 'presweep train', then let it settle for a stipulated period at a particular voltage, before each sweep of the trial. Presweep trains are configured on the **Stimulus** tab of the **Protocol** dialog.

The trains consist of square waveform pulses alternating between (first) a baseline, and then a step level. Both of the amplitude levels, and the duration at each, are user-defined. The pulse frequency is reported, given the duration values you enter, in milliseconds.

If **P/N leak subtraction** is enabled to run prior to the main sweep, and you have a presweep train enabled as well, the leak subtraction subsweeps are run before the presweep train.

Presweep trains are not recorded, nor are they displayed in PatchXpress Commander Software as they run.

All presweep train settings can be overridden with the **User list**, on the same tab.

User List

The **User list** on the **Protocol** dialog **Stimulus** tab allows you to select one protocol setting, and vary this, for each sweep of the trial, with arbitrary values you enter in the **List of parameter values** field. It is available in internally triggered mode, but not compound-triggered mode.

Having selected the parameter you want to control from those available in the list box, enter the values (in the units used in the main field for this parameter), separated by commas, for each sweep of the run. If you want to repeat a sequence of values you need to enter the sequence only once, then check the **Repeat parameter values** option.

All of the presweep train parameters can be controlled with the user list, as can the number of subsweeps for **P/N leak subtraction**, and the main sweep start-to-start time. In addition, level, and duration settings for each of the command waveform epochs can be controlled this way.

If you enable the user list but enter fewer values in the list than sweeps in the protocol, the last value in the list is reused until the end of the protocol. If there are more entries than sweeps, the final entries are ignored.

Note: You can use different sets of user list values in second, and following runs of the protocol, providing the new values in a Command procedure.

Measurements

A range of nineteen different types of measurement, including peaks, mean amplitudes, and slope measurements, can be made within a specified region of the raw or leak-corrected data trace in each sweep. Configure these measurements on the **Measurements** tab in the **Edit Protocol** dialog.

The measurements are for real-time display purposes only, on the **Measurements** tabs in both the **Overview**, and **Cell View** panes. They are not saved. However, the measurement configuration settings that you use pass to DataXpress, where you can reproduce the on-line measurements. You can also configure new measurements in DataXpress. Since you can always get any additional measurements that you want in DataXpress, only select a small number of measurements in the PatchXpress Commander Software protocol, that you can effectively monitor while the experiment is running.

You must stipulate the region of the sweep you want to search for the measurement types that you select. You can search the whole sweep, any particular epoch, or an arbitrary range, defined by sample number. The search region is shown in the waveform preview window used to display the command waveform.

Peak (nA/mV), **Mean (nA/mV)**, and **Area (nA/mV - ms)** measurements can be made relative to the mean amplitude in a baseline region within the sweep. If you select to have no baseline region, absolute measurements, using Y-axis values, are used. Like the search region, the baseline region is shown in the waveform preview window.

Amplitude measurement values will depend on which **Amplifier Mode** is in use. **Voltage Clamp** measurements display in nanoamps (nA), and **Current Clamp** measurements display in millivolts (mV).

Several measurement types use the peak polarity setting required on the **Measurement** tab. With a positive peak polarity selection the

sample point in the search region with the highest value is the peak, and the lowest the antipeak. With negative polarity these attributions are reversed. With absolute polarity, the sample furthest from the baseline value is the peak, and the sample furthest from the peak the antipeak. Rising slope is always the slope prior to the peak, be this a positive or negative peak, and decay slope the slope following the peak.

Event duration (ms), measures the time difference between the beginning, and the end of the stimulus response determined by the specified percentage of the Peak amplitude.

A simple 'boxcar' filter—the 'smoothing window'—can be used to reduce the effect of noise on your measurements. This filter does not affect the raw data at all, either as it is displayed in PatchXpress, or as it is recorded in the ABF file. With setting '1', no filtering takes place. With setting '3', each sample is averaged with the sample on either side of it, for example, three points are averaged. For '5' it is two points on either side, up to '21', where 10 points on either side are included.

The Patching Process

Amplifier, fluidics, and pressure settings used to patch cells (and beyond), are set in the **Patch Settings** dialog, opened from the **Define Experiment** dialog. More specifically, settings are made here for:

- preparation, and checking of electrodes
- the pick-up, and deposition of cells into the electrode chambers
- the sequencing of pressure steps, and ramps, as well as voltage settings, used to seal, and rupture cells
- thresholds for the failure or success of cell patching
- the voltage holding level, and fluidics pressure maintained for the experiment once cells are ruptured
- configuration of pneumatic Ra control pulses, and setting of cell health thresholds

The next section, Patch Settings Files on page 110, describes management of the files in which these settings are contained. The section following that, Patch Settings Dialog on page 110, provides some detail about the dialog itself.

Patch Settings Files

The complete set of configuration options you select within the Patch Settings dialog must be saved, as a PXS file, before the settings can be used in an experiment. The currently loaded PXS file is reported beside the **Patch settings** button, marked with an asterisk if unsaved changes have been made. The button opens a submenu with management options for these files. When you opt to create a new patch settings file the dialog opens populated with default values.

The default location for **PXS** files, changeable in the **Settings** dialog, is: **D:\PatchXpress\PatchSettings**

Double-clicking on the **Patch settings** button opens the **Patch settings** dialog directly, populated with settings from the currently selected **PXS** file.

Patch Settings Dialog

The **Patch Settings** dialog is organized to take you through the cell patching process, each tab corresponding to a separate stage. Some steps are descriptive only, provided to give a complete picture of the processes that are applied. Where appropriate, spinner boxes allow you to set parameters appropriate to the step. The dialog thus provides a complete explanation of the patching process, as well as serving as a configuration interface.

On most tabs the right-hand half of the tab has a **Test** section for you to enter threshold values for key parameters within the patching

process. These values have to be met for the process to advance to the next stage. Some tabs also have sets of subsidiary settings on the right-hand side—for example, configuration settings for the pressure ramps used to seal, and rupture cells are on the right-hand side of their tabs.

The following provides a brief discussion of the parameters you can adjust within the Patch Settings dialog, assuming you do not want to accept the default settings. See the next section, Run the Experiment on page 117, for a full description of the patching process that follows the sequence of steps in the dialog closely.

1. Initialize SealChip

The first tab in the dialog deals with the tests carried out on the newly loaded Sealchip16 electrode to ensure each chamber can adequately generate data. Chambers that fail any of these tests are not used.

There is just one parameter to set on the tab—the electrode resistance (Re) test threshold.

The Re Test value is the largest electrode resistance you will accept, measured once extracellular solution has been added to chambers to complete the patching circuit through the intracellular, and extracellular solutions. Too high an electrode resistance suggests that the electrode hole is blocked, and cannot be used for a successful patch or recording, so any chambers in the electrode with resistances higher than your threshold are not used.

There is also a non-configurable lower limit for electrode resistance, set at one megohm.

2. Add Cells

After a chamber's electrode resistance is verified as good, cells can be added. There is no test for the cell addition stage—cells are added using parameters set on the tab, and then the process proceeds to the next, cell detection, stage.

Cell dispersion pressure is applied immediately following a high pressure purge through the electrode holes (to ensure that these are clear), and before chambers are filled with extracellular solution in preparation for cell addition. It continues up until cell addition, and for a user-specified time after that. Before cells are added electrode offsets are nulled, and electrode resistance measured.

Only positive pressure can be set, forcing intracellular fluid out into the main chamber. A low pressure—10 mmHg is the default—ensures that intracellular solution is not contaminated by extracellular solution through the electrode hole.

The second option on the tab is to pause the experiment before cells are added, to give you time to put a cell vial in place. This option allows you to bring cells to the PatchXpress 7000A system just before they are needed. It is especially useful for large screening mode experiments,

allowing you to bring out fresh cells for each new electrode used in the experiment. If the checkbox is not checked PatchXpress Commander Software assumes that the cell vial is in place, and proceeds to pick up cells without pause.

Step 2.6 has options for agitation of the cell-bearing solution before it is drawn into the tip for deposition into the electrode chambers. This step is purely to mix cells, likely to have settled on the bottom of the Eppendorff tube, into the solution before this is drawn up into the tip for delivery to the electrode chambers. Sufficient solution for 16 chambers (at the volume stipulated for each chamber next on the tab) is sucked into the tip, and then 80% of this ejected again, for as many cycles as you stipulate, at the flow rate that you stipulate. The default is three cycles at 25 µL/s.

At **step 2.8** set the volume of cell-bearing solution to be delivered to each chamber, and the flow rate for this delivery. By default 3 µL are delivered per chamber, at 5 µL/s.

Step 2.9 allows you to set a waiting period to follow the end of cell addition, during which the cell dispersion pressure you set on the previous tab continues.

While cells are being added the wash station nozzles are in a raised position 1.6 mm above the chamber bottom, to allow room for cells to disperse. On the right-hand side of the tab you can enter a time period, to follow cell addition in the last chamber, after which the wash station lowers into its final position 1.2 mm above the chamber base. This gives time for cells to disperse, and then be attracted to the electrode hole. The timing for this wait is independent of the stage that each chamber is at, and is the same for all chambers, so when the wash station lowers after the stipulated period different chambers might be at different stages of the patching process, or could possibly even have finished patching. The procedure cannot start until the wash station is lowered, however.

3. Detect Cell

In the cell detection stage cells are left to disperse at a stipulated cell settling pressure before suction is applied to draw a cell to the hole. Enter a settling pressure (3.1), and waiting period at this pressure (3.2). This can be either positive or negative, and its purpose is to give you a fine-tuning capacity to optimally disperse cells prior to the suction that will shortly be applied. With good, even, cell dispersion, one (and hopefully, only one) nearby cell will be quickly drawn to the hole for sealing, and rupturing. The default cell settling pressure is the same as the default cell dispersion pressure, 10 mmHg.

After the period you designate to wait, suction is applied at the pressure you enter at step 3.3, and resistance through the electrode hole measured. Until a cell comes close to the hole this resistance is effectively just the electrode resistance measured earlier, but once a cell starts to block the hole a seal resistance component is added to

this. When the resistance continuously exceeds the test value for this stage, entered on the right-hand side of the tab, for the duration you stipulate, the system takes it that a cell is in position over the hole ready to be sealed—the next stage.

You can set a new pressure, and holding potential at this point, and hold these for a defined period in order to stabilize the cell before pressure ramps to fully seal the cell are applied.

4. Sealing

At the sealing stage repeated pressure ramps of uniformly increasing negative pressure draw the cell firmly into the electrode hole. At the top right of the tab enter the Test value for the step—the seal resistance you want to achieve, for example, one gigohm, before attempting to rupture the cell. Once this resistance is achieved the pressure ramps are stopped, and the seal must be maintained for a stipulated verification time, that you enter at step 4.4 on the left of the tab. You can allow some hysteresis during this period by entering a second, lower, test resistance threshold. If seal resistance falls lower than this during the verification period the system assumes the seal is not good, and restarts the pressure ramps, but if resistance remains between the two test values or higher, the procedure goes on to the next step.

Beneath the Test resistance values on the right of the tab are configuration options for the pressure ramps. Starting from the baseline **stabilization pressure** you entered on the previous tab, pressure progresses uniformly down to a stipulated level, over a stipulated time. The rate of pressure increase is reported. You can set a time to hold at the lowest pressure before returning to the baseline pressure, and a time to wait at the baseline pressure before starting the next ramp.

In **step 4.1.1**, set a threshold to automatically stop an individual pressure ramp if seal resistance falls while the ramp is being applied. This gives a short space of time for a failing seal to re-establish before suction begins again. The threshold you enter is a percentage of the maximum seal resistance attained since the current sequence of pressure ramps started, for example, since the start of the sealing stage, or since this threshold was last breached. The pressure drops back to the baseline pressure for the ramps (from **step 3.5**), a new maximum resistance value set, and then pressure ramps restart.

At **step 4.1.2** you can set configuration options to modify the holding potential as the seal resistance increases. When the sealing pressure ramps begin the holding voltage is at the stabilization level set at **step 3.5**. You can have this automatically change a specified number of millivolts for each increase of a specified number of megohms in seal resistance. These changes, usually to a more negative holding potential, will continue until the final holding potential (set lower on the tab) is reached.

For cells that have met the seal resistance test for the verification period, enter a new holding potential, and pressure, and a duration to wait at these. Following this wait the next set of pressure ramps, to rupture the cell, begins. This is the last point that holding potential is set in the **Patch Settings**. The potential set here is the one that the cell is ruptured at, and it is maintained for the duration of the procedure unless it is changed by a command step. The pressure value entered here, on the other hand, serves as the baseline pressure for the cell rupturing pressure ramps, but can be reset following whole-cell.

In the bottom right-hand corner of the tab there is an option to activate a **second-chance seal**. With this option enabled, if the seal resistance test condition has not been met after the number of seconds that you enter, then the test value is reduced, to a lower value that you stipulate. Cells that seal under these conditions are marked as such in the **Status** tab.

5. Whole-Cell

Once a cell is sealed, a further series of pressure ramps, optionally accompanied by voltage 'zaps', is used to rupture the membrane. The test criterion for this is a rise in capacitance, for which you must enter a threshold. An acceptable access resistance is also incorporated into the test conditions.

As for the sealing stage, pressure ramps are configured on the right-hand side of the tab. Configuration is similar to that for sealing ramps, except for the inclusion of zaps, which can be incorporated at the start, and end of each ramp (except for the first—a zap before the first ramp is enabled on the left-hand side of the tab). In a further difference, cell-rupture pressure ramps can begin at a pressure other than the baseline pressure (set at the end of the sealing stage). When a ramp begins, then, pressure jumps immediately to the 'starting' pressure, from there increasing smoothly to the peak pressure. A zap can be configured to occur when the peak pressure is reached, and the peak pressure can be held a stipulated time. Pressure then falls back to the baseline for a further defined period before, potentially with another zap, pressure jumps again to the starting pressure for the next ramp.

When you have enabled any zaps the magnitude, and duration of these is configured below the ramp configuration section.

At **step 5.2.1** enter a duration to maintain the pressure at which rupture occurred, starting immediately that the capacitance, and access resistance test values are met. At 5.2.2 enter the final holding pressure, applied following the period at 5.2.1. This is the final pressure setting for the patching process, maintained for the rest of the procedure unless it is changed in command steps.

Finally, at **step 5.2.3**, set a verification time for the whole-cell capacitance, and access resistance test values. If either of these values go outside their acceptable ranges the suction pulses are restarted. No

hysteresis is allowed for this test. If the test values are not breached during the verification period the patching process moves to the next stage.

6. Improve Whole-Cell

At the start of the Improve Whole-Cell stage correction for the liquid junction potential entered in the Solutions dialog occurs. Beyond that, the tab offers two means of improving the quality of the whole-cell by reducing access resistance.

At **step 6.4** set a period to hold the cell at rest, beginning once all stimulation within the patching process has been completed, up until the time the procedure is started. This is a final stabilization period you can use to ensure the cell is quite settled before stimulation for data recording begins. It is recommended that a final stabilization period is always set, with 60 seconds being a reasonable duration.

On the right-hand side of the tab, set a cell timeout period. If a cell has not completed all the configured stages of the patching process within this time, starting from cell addition up to the end of the final stabilization period set at 6.4, the cell is not used.

After setting the stabilization, and timeout values, you can choose to enable one or two access resistance-reducing strategies. **Pneumatic Ra** optimization applies strong suction pulses for a specified time. Pneumatic Ra control applies sequences of somewhat gentler, automatically varied suction pulses until specified test values are met or a timeout duration exhausted.

If enabled, pneumatic Ra optimization is applied first. This runs for the period you stipulate on the left of the tab, with pressure pulses configured on the right. From the baseline pressure (the final, procedure holding pressure of 5.2.2), ‘rectangular’ pressure pulses are applied. Set the duration of the pulses, and the pulse start-to-start times. You also set a pulse pressure, however the pressure used is the larger (for example, more negative) of this, and the pressure applied when the cell was ruptured.

To avoid applying these suction pulses to a poorly sealed cell you must also set a membrane resistance value below which the pulses are not delivered. Similarly, you must enter a percentage value such that if the membrane resistance falls below this percentage of its value when the cell first ruptured, the pulses are again skipped.

When you enable **pneumatic Ra** control at **step 6.3** the test conditions on the top right-hand corner of the tab are enabled, as is the timeout field for this setting, on the left. Pneumatic Ra control pulses are configured on **tab 7**. Within the period you designate, the Ra control pressure pulses are delivered until access resistance falls below the test value you stipulate, provided membrane resistance is above its test value. If the test conditions are not met within the period you have set then pulses are stopped, and the process moves to the final stabilization period prior to the procedure being run.

7. In Procedure

Tab 7, In Procedure, has configuration options for pneumatic Ra control, and cell health.

Pneumatic Ra Control

Pneumatic Ra control delivers automatically adjusted suction pulses in order to reduce access resistance. It can be enabled to run as the last active stage in the patching process just before the final stabilization period, or while the procedure is running. In the latter case the suction pulses are triggered by a stipulated, high, access resistance measured by the membrane test. It is enabled in the **Procedure** dialog **Initial Settings** tab.

Pneumatic Ra control suction pulses are 'rectangular' pulses from the baseline holding pressure set at **step 5.2.2**. The magnitude, duration, and frequency of the pulses are all automatically adjusted, within limits you define. Whether pulses are delivered at all is determined by the access resistance, which also helps determine the strength of the pulses. The frequency, and duration of the pulses, as well as their strength, is determined by the membrane resistance when pulses are triggered. Basically, the higher the membrane resistance, the shorter, and more frequent the suction pulses, and the higher the membrane resistance, and access resistance, the stronger the pulses.

The first set of configuration options on the bottom-left of the tab define limits for the pulse that is delivered. First nominate upper, and lower membrane resistance values, between which the pulse configuration will automatically, linearly, vary. If the membrane resistance is outside this range when pulses are triggered the pulses take the high or low Rm configuration, appropriately.

For each of the high, and low membrane resistance limits enter pulse duration, and start-to-start values—typically shorter, and quicker for the higher Rm. These values always only ever depend on Rm. For pulse pressure, enter two values for each of the Rm limits. The pressure actually delivered will fall between these limits according to the access resistance. Pulse pressure, then depends on both Rm, and Ra.

Next, in the top-right of the tab, enter two Ra values, 'top', and 'target'. The target value is the threshold for pneumatic Ra control—this must be crossed to generate any pulses. The top Ra value, which must be set higher than the target, defines a range within which the pulse strength is modified relative to Ra. At the top Ra the maximum pressure range defined in the previous fields is used. At the target Ra the minimum pressure range is used. Ra values between the target, and top use pressures between these limits, linearly scaled.

Cell Health

The **cell health** parameters set cell viability thresholds: minimum acceptable membrane resistance, and capacitance, and maximum acceptable access resistance.

If any of these measurements remains outside its threshold for more than two seconds it is reported in the cell log. If Terminate unhealthy cells is enabled in the Procedure dialog **Initial Settings** tab, the cell is 'terminated'—meaning the procedure is stopped for that cell (see Terminate Unhealthy Cells on page 96).

Run the Experiment

With the PatchXpress 7000A instrument primed, and compounds in position, cells prepared, the plate contents files selected, and the procedure, **Patch Settings**, and other experimental parameters defined, you are ready to run your experiment.

You can follow the experiment progress on the **Status** tab, and **Cell View Log** tabs, and once the procedure starts, on the **Procedure** tab as well.

Start Experiment

Click the **start experiment** button.

When you press the **Start** button the experiment clocks in the Cell View pane start, and an experiment file reporting experiment parameters is sent to DataXpress. On the **Status** tab the state for each channel is reported as 'Prepare *Seal*chip$_{16}$, and you are prompted to load a Sealchip16 electrode onto the loading mount.

At this point scan the barcode on the *Seal*chip$_{16}$ electrode container. It is automatically written into the Load SealChip dialog. Alternatively, type in the barcode. This is the SealChip lot number, a queryable attribute in DataXpress.

Having removed the electrode shake excess fluid out of the electrode chambers before placing it in its loading position. This is an important as large drops of fluid may not be entirely removed in the drying process, which can lead to channel cross-talk during the experiment. Make sure the beveled end of the electrode is towards you when you put it in place.

Click the dialog **OK** button.

Loading the SealChip

The gripper collects the electrode, and puts it into position to move into the drying station. The intracellular filler station is primed while the electrode is dried. Once dry, the electrode, upside down, is moved to

the loading clamp where the flipper grips it. Intracellular solution is injected into the flipper gasket cavity beneath each electrode hole.

The electrode is now flipped over into the recording clamp, the gasket cavity under each electrode hole fitting over a Ag/AgCl electrode/suction pipe on the electrode base plate.

Basic electrode preparation, and checking is now carried out. The membrane test starts, and for each chamber open circuit resistance is measured. Chambers with less than 5000 MΩ resistance are rejected.

Following this the channels are tested for cross-talk. Pulses are generated in every fourth channel, starting with the second, and the response recorded in the two channels on either side. The process is then repeated with a shift of one channel, for example, every fourth channel starting with channel three. With these two passes all neighboring chambers are tested for cross-talk. Any two chambers with less than 5000 MΩ resistance between them are rejected.

The SealChip clock in the Cell View pane is started now.

In the amplifiers, compensation for fast, and slow electrode capacitance is applied. Most electrode capacitance comes from the electrode headstages, and cabling from these to the electrode, with a small additional component from the electrode holes themselves. This capacitance is measured when the PatchXpress is set up, and the values recorded then compensated for now. The preset compensation value is applied now, until cells are successfully sealed, when the capacitance is measured, and compensation applied for the new, measured value. In the interim, electrode capacitance is reported on the **Status** tab, this being any remaining, uncompensated capacitance. With the compensated electrode capacitance typically around 20 pF, low capacitance values, less than 1 pF, should be reported. If cells rupture before attaining their nominated seal threshold, no new electrode capacitance measurement is made, and the original, set compensation is maintained for the cell procedure.

A holding potential of 0 mV is set.

At this point the wash station is lowered into the chambers, stopping with the nozzles in their lower, compound-addition position 0.6 mm above the chamber base.

Now an electrode hole purge starts. Strong positive pressure is applied forcing intracellular solution up through the electrode holes into the chamber, to clear these of any debris. The purge uses maximum pressure, 700 mmHg, for 60 seconds. The size of the electrode holes is such that only very little solution is used, with no danger of depleting the solution so that it will adversely affect your experiment.

One second after the purge starts the wash station begins a 15 second high speed wash, flushing extracellular solution through the chambers. Again, the purpose of this is to remove debris that may be sitting in the chamber.

As the wash proceeds the wash station nozzles are in solution, establishing the full circuit, and five seconds after wash starts electrode offsets are nulled. This is to prevent nanoampere-scale currents from flowing through the electrodes, which might affect the sealing characteristics of the electrode. This is a temporary null, and its value is not logged.

Once the purge finishes the cell dispersion pressure, set in tab 1 of the **Patch Settings** dialog, is applied. A second, normal-speed wash occurs. This removes intracellular solution from the chamber following the purge.

Next, resistance is measured across the electrode. Too low a resistance—less than one megohm—indicates a crack in the electrode or some other abnormal condition, and channels with this are rejected. Similarly too high a resistance suggests a blocked electrode hole. The upper resistance threshold is user entered, on the first tab of the Patch Settings. The average electrode resistance value is reported in the **Status** tab, where the value is grayed to show that it will not change again.

At this point any faulty chambers have been rejected, and remaining chambers are filled with extracellular solution in readiness for the addition of cells.

Cell Addition

If you have enabled it in the patching process, there is a pause at this point for you to load an Eppendorff tube with cells. You are prompted in the software, and asked to enter a cell split time. When you have placed the cells in position clear the dialog with the **OK** button, and the patching process continues. If you did not enable the pause the PatchXpress Commander Software assumes cells are already in position.

The **Status** tab state is now reported as ‘Preparing Cells’.

A further, regular-speed, five second wash occurs at this point, and electrodes are nulled again. This nulling is recorded in the **Cell View Log** tab. The **Status** tab state reports **Junction potential null** briefly (in **Voltage Clamp** mode, and **Current Clamp** mode), while offsets are measured, and removed.

The fluid handler now picks up a clean tip, and moves to the Eppendorff tube containing the prepared cells. Cell-bearing solution is drawn in, and expelled out of the tip to resuspend the cells, for the number of cycles, and at the flow rate set in tab 2 of the Patch Settings. Solution is then drawn up into the tip for deposition into the chambers. Meanwhile, the quantity of extracellular solution in each well is reduced in preparation for addition of the cell-bearing solution. The wash station probe shifts up to its cell-addition position, 1.6 mm from the chamber base.

When cells have been added to all chambers the Cells added clock starts. The **Status** tab state is now 'Cell dispersion', and seal resistance is reported on the tab.

Patching

After waiting a user-specified time at the 'cell dispersal' pressure entered earlier, a second 'cell settling' pressure is now applied for a further specified duration.

Following this a stronger 'cell attraction' pressure starts, to suck a cell to the electrode hole. This is reported as 'Waiting for cell detection' in the **Status** tab. When seal resistance remains above the level stipulated for this, for the stipulated period—suggesting that a cell has been sucked into position over the hole—the next, stabilizing period begins, applying the 'stabilization' pressure, and holding potential set on **Detect Cell Tab 3** of the patching process, for the stipulated time. The **Status** tab reports 'Stabilizing cell'.

After the stabilization period the suction ramps of the sealing stage begin. This is reported as 'Forming seal' on the **Status** tab, and if a cell has been cleanly attracted to a hole you should be able to see the seal resistance steadily increasing up to the threshold you have set. If configured in **Patch Settings**, the holding potential changes in response to the increasing seal resistance during this time.

Immediately the seal resistance threshold is met the suction ramps stop, and the system waits in 'Verifying seal' state to see if the seal is maintained, within the set hysteresis, for the verification period. If the seal threshold is not met PatchXpress 7000A system will continue attempting to form the seal until the overall patching process timeout is reached, at which time the procedure is terminated for that cell, or—if the 'second-chance' seal option is enabled—until its timeout is reached, whereupon a new seal threshold applies.

If the seal is verified, the final Rseal value in the verification period is stored. Rseal continues to be displayed in the **Status** tab as the next set of suction ramps is applied, to rupture the cell, but once the cell ruptures the displayed Rseal reverts to the value stored after seal verification. It is appended with 'Sealed', to show the nominated seal resistance was achieved, and is grayed, to indicate that the value can no longer be updated. Once a cell is ruptured membrane, and access resistance measurements replace the seal resistance measurement.

A cell may fail to gain the seal resistance threshold nominated, but might reach a second-chance seal threshold if this has been enabled. Reporting in this case is as for a normally sealed cell, but the Rseal value is prefixed '(2nd) Sealed'.

Sometimes a cell ruptures in response to the sealing ramps, before the seal threshold has been reached. In this case the process jumps to the appropriate stage for a newly ruptured cell. In the **Status** tab the final

seal resistance value before the cell ruptured is frozen (shown in gray to indicate this), and prefixed 'Premature'.

One further seal condition is reported in the **Status** tab. If a seal goes over the second-chance seal threshold but then, at any time prior to going whole cell, falls below this again, the Rseal value is reported with a 'Lost' prefix. Should the cell rupture after this, however, the prefix reverts to 'Sealed'.

A further stabilization pause follows verification of the seal—'Stabilizing seal' on the **Status** tab. The final holding potential is applied at this time. It can only be changed now by command steps within the procedure.

The next round of suction ramps, optionally with voltage zaps, now begins—'Attempting whole cell'. The primary indicator of a cell having ruptured is rise in capacitance, as the capacitance of the whole cell membrane takes effect. Once the stipulated capacitance threshold is met, provided the access resistance is below its stipulated level, the cell is taken to have, likely, ruptured, and the verification period for it begins—'Verifying whole cell'. There is no hysteresis for this threshold—the capacitance, and Ra values must each stay above, and below, respectively, their thresholds, for the duration of the verification period, otherwise cell-rupturing suction ramps are restarted.

Starting at the same time that the whole-cell verification period begins, another configured period may apply, for maintenance of the pressure when the cell first ruptured. Once this period ends the pressure drops to the final holding pressure that has been set. This pressure remains unchanged throughout the rest of the cell procedure unless it is changed by a command step in the procedure.

Now that the cell is ruptured, any liquid junction potential between the intracellular, and extracellular solutions disappears, the solutions being no longer in direct contact. Since this potential was present when the electrodes were nulled earlier in the patching process, it must now be added back. This occurs automatically, using the liquid junction potential value entered in the **Solutions** dialog.

Following this, further suction ramps may be applied in order to bring access resistance down. These may take two forms, 'pneumatic Ra optimization', which delivers comparatively strong pulses for a stipulated time, or 'pneumatic Ra control' which automatically adjusts pulse strength, duration, and frequency according to the membrane resistance, and access resistance. The **Status** tab reports 'Optimizing Ra' for these steps.

The final stabilization period now applies, holding the ruptured cell steady, with no stimulation, at the final holding potential, and holding pressure. Once this period ends, the procedure begins.

Running the Procedure

Once a cell passes the tests for having gone whole cell, the sequence of steps defined in the procedure is run. A procedure applied to a single cell is termed a 'cell procedure'. Major cell procedure events, and cell health parameters are recorded in the cell log (params) file, one for each cell.

Remember that add compound steps occur a variable amount of time after the time defined for them, because the compound-dispensing arm must serve all 16 wells, and so must queue add compound steps as they are required in the various cell procedures that are running. In the **SealChip Overview** display, the next chamber to receive compound has a moving tip displayed beside it.

As trials run, the resulting data traces are displayed in the **Raw** tabs in the **Overview**, and **Cell View** panes. Remember that only the main sweeps are displayed, and not the P/N leak subtraction subsweeps or pre-sweep trains. If leak subtraction is enabled (P/N or resistive) the corrected sweeps can be optionally viewed, as set in the **Cell View** toolbar **View Settings** dialog.

The **Raw** tabs also show the membrane test response when trials are not running. The **Measurements** tabs display measurements configured for the trials, while the access resistance, and other parameters measured by the membrane test are plotted on the **Cell Health** tabs.

Static Spikes at Compound Addition

When compound is added during recording, there is typically a fast spike of static discharge as the compound tip comes into contact with solution in the chambers. If this occurs during the main sweep, then when you review the file you will be able to see that the spike coincides with a compound addition. Sometimes, however, compound addition can occur during a leak subtraction subsweep. In this case, because the subsweeps are subtracted from the recorded trace, an artifact of the original spike becomes incorporated into the main sweep, even though the compound addition was earlier than this. These artifacts can be removed from the data traces in DataXpress.

Marking and Suspending Cells

As you watch trials being recorded you can mark cells as good, bad or outstanding. Right-click in the window for the particular cell, in the Cell View or Overview pane, and select the appropriate option. This adds the attribute to the cell procedure, (and following cell procedures run on the same cell), that can later be queried on, and displayed in DataXpress. You can label a following cell procedure on the same cell with a different mark.

Similarly, if a cell is producing poor results, you can stop the procedure on it with the Suspend command, thereby saving compounds that will

then be available for a new cell. Should the cell revive, Suspend can be undone, and the procedure will continue on the cell from where it left off. See Suspending Cells on page 41.

Watching Experiment Progress

The **Status** tab displays cell procedure progress, reporting the procedure step number, and for trials, the trial number within the cell procedure, and the sweep number within the trial. As this view is constantly changing, it can be easier to view procedure progress on the **Procedure** tab, where the procedure steps are displayed statically, and progress through them shown. The **Cell View Logs** also display the procedure steps as they occur.

To get an indication of your progress through the experiment on a higher scale, use the **Plate** tab, which shows how many compounds have been tested, and how many remain.

Removal of *Seal*chip$_{16}$ Electrode

When all the cell procedures on an electrode are completed or have timed out, a final wash is run to clean the wash station nozzles. The pressure is set to zero.

In assay development mode, if chambers remain unused, the electrode remains in place on the electrode plate so that another experiment can be run. When the final chamber on the electrode has been used, the electrode is automatically unloaded. If you want to unload the electrode before all the chambers have been used, use the Unload SealChip command in the **Hardware and Fluidics** dialog The electrode is also unloaded if you prime the wash station.

In screening mode, the electrode is automatically unloaded. You are prompted to load another if compounds remain to be tested. The wash station is withdrawn, the recording clamp releases the flipper, and gasket, and the flipper takes the electrode back to the filling station.

In the filling station intracellular solution is removed from the gasket, and the cavities washed with water. The electrode is then released, and pushed into the waste bin.

Experiment Duration

The overall duration of an experiment is determined by the length of the procedure, but also, in screening mode, by the number of compounds to be tested. If all the cell procedures started in an experiment are satisfactorily completed, there are (in screening mode) as many cell procedures as there are sets of well locations defined for the experiment. If some cell procedures fail to run to completion, for example because the cell dies part way through, then there will be additional cell procedures as the compound is tested on a new cell, provided there is sufficient compound for this.

New electrodes are loaded while compound remains to be tested. At the end of a screening mode experiment there may be more patched cells in the last electrode than compounds to test. In this case each of the remaining compounds is applied to more than one cell, provided there are sufficient quantities of the compound for this.

You can stop an experiment at any time with the software **Stop** button. This gives you options to stop immediately, or to stop once all the cells on the currently loaded *Seal*chip$_{16}$ electrode have been finished with.

The easiest way to get a broad overview of the progress of an experiment is to use the **Progress** tab. This shows the compound well locations in the compound well plates, color-coded to represent either that they have compound to be used during the experiment, or that it is being used, or that the compound in that location has been successfully tested or has run out.

Shutdown

Table 5-2 Shutdown summary.

1. At the end of each day of use, rinse the PatchXpress 7000A instrument fluidics system by running the End-of-day hardware shutdown procedure (in the **Hardware and Fluidics** dialog) or when prompted on exiting the program.
2. Discard unused extracellular, and intracellular solutions.
3. Return unused Sealchip16 electrode arrays to their original containers. Secure the lid tightly.
4. Inspect the pipette tip, and Sealchip16 waste bins. If they are more than ¾ full, remove the red biohazard waste bags, and discard appropriately. Replace the waste bags.
5. Turn off the vacuum pump. This will extend the lifetime of the pump.
6. Turn off the air compressor if configured.
7. Close the PatchXpress Commander Software, and turn off the computer. Then turn off the PatchXpress 7000A instrument.

System Maintenance

6

Daily, weekly, monthly, and three-monthly maintenance procedures are listed below in Regular Maintenance Procedures. A short section describes the Model Cell on page 127, and the Hardware and Fluidics Dialog on page 129 describes maintenance functionality run from PatchXpress Commander Software. Final sections give instructions for Replacing the Electrode Base Plate on page 135, and Check for Electrode Drift on page 137.

Regular Maintenance Procedures

Daily

Startup Procedure

1. Turn on the vacuum pump.
2. Turn on the air compressor.
3. Turn on the PatchXpress first, and then the computer.
4. Bring extracellular, and intracellular solutions, and the Sealchip16 electrodes to room temperature. Load solutions on the PatchXpress 7000A instrument. All solutions should be filtered (0.2 µm).
5. Run the Start-of-day initialization procedure (in the **Hardware and Fluidics** dialog). Periodically, rinse the blank *Seal*chip$_{16}$ electrode with 70% isopropyl alcohol, and allow to dry.

Shutdown Procedure

1. At the end of each day of use, rinse the PatchXpress 7000A instrument fluidics system with deionized water by running the End-of-day hardware shutdown procedure (in the **Hardware and Fluidics** dialog) or when prompted on exiting the program.
2. Discard unused extracellular, and intracellular solutions.
3. Return unused *Seal*chip$_{16}$ electrodes to their original containers. Secure the lid tightly.
4. Inspect the PatchXpress 7000A instrument working area, and clean up any spills. Rinse any saline solutions with water first before drying with a lint-free wipe or vacuum line.
5. Inspect the pipette tip, and *Seal*chip$_{16}$ waste bins. If they are more than ¾ full, remove the biohazard waste bags, and discard appropriately. Replace the bags.

6. Turn off the vacuum pump. This will extend the life of the pump.
7. Turn off the compressor.
8. Close the PatchXpress Commander Software, and turn off the computer. Then turn off the PatchXpress 7000A instrument.

Weekly

1. Inspect the liquid waste container. Empty if more than half full.
2. Inspect the pipette tip, and *Seal*chip$_{16}$ waste bins. Empty if necessary.
3. Inspect the water container. Empty, and refill with fresh deionized water if necessary.
4. Rinse all fluid lines, including the intracellular solution injector, with 70% isopropyl alcohol to inhibit mold growth, followed by 2-3 rinses with deionized water to remove the alcohol (use the **Hardware and Fluidics** dialog 'Alcohol flush', and 'End-of-week flush' for these operations).

Monthly

1. Replace the intracellular solution gasket. Two tools are provided for removing the gasket. The flat-head screwdriver is used to unclip the front end. The gripper-tool is used to pick up the gasket simultaneously. The old gasket should be discarded. A new gasket is inserted with the arrow pointing forward.
2. Inspect the waste container tubing. If this short stretch of tubing is coated with a visible accumulation of waste material, and/or mold growth the tubing needs to be replaced. Spare sections of tubing are included in the Spare Parts kit located in the Tool Box.
3. Inspect the waste transition bottle. If necessary, remove this bottle, and clean it out with bleach solution or replace with a new bottle.
4. Download the latest version of PatchXpress Commander Software. Open the **Manuals**, and **Web Support** dialog (the book icon), and go to the Software Upgrades link for this.

Every Three Months

1. Replace the electrode plate. A T-handle allen-wrench, and extracting tool are provided in the Tool Box. An electrode plate is located in the Spare Parts kit. The PatchXpress 7000A instrument should be turned off before removing the electrode plate. First remove the screws with the T-handle allen-wrench. Then use the extracting tool to carefully lift off the electrode plate. The old electrode plate should be returned to Molecular Devices for recycling. Take the new electrode plate, and carefully place it in position. Replace the screws. Initially just

put all eight screws in, then tighten them slightly, opposite screws first, then a little tighter with the same pattern, then completely tight. If this procedure is not followed the silver tubes could be bent, and/or the plate may be skewed.

2. Test the PatchXpress 7000A system with the model cell. Verify that the PatchXpress Commander Software is reporting values within 10% of the component values in the model cell. If not, contact Technical Support (see Technical Assistance on page 155.)

Model Cell

The PatchXpress 7000A system is supplied with a purpose-built model cell useful for isolating problems in the system. It allows you to test the instrument without the problems of live cells, and with known values for access resistance, membrane resistance, and membrane capacitance.

The model cell—more correctly, model cells—is a unit that sits over the electrode base plate, completing the circuit for each channel by making contact with the suction tube electrode, and the base of the wash station. Intervening circuitry loosely imitates the electrical properties of real cells. Channels 1 to 15 have fixed circuits, while channel 16 can be set to a range of resistance values in two configurations (see figures below).

To use the model cell:

- Close PatchXpress Commander Software
- Place the model cell into position
- Restart PatchXpress Commander Software

PatchXpress Commander Software senses the presence of the model cell when it starts, and reports this in the title bar. Similarly, PatchXpress Commander Software must be started again after removal of the model cell, to return it to its normal operational mode. If robots start to move, remove the model cell immediately, and contact Technical Support.

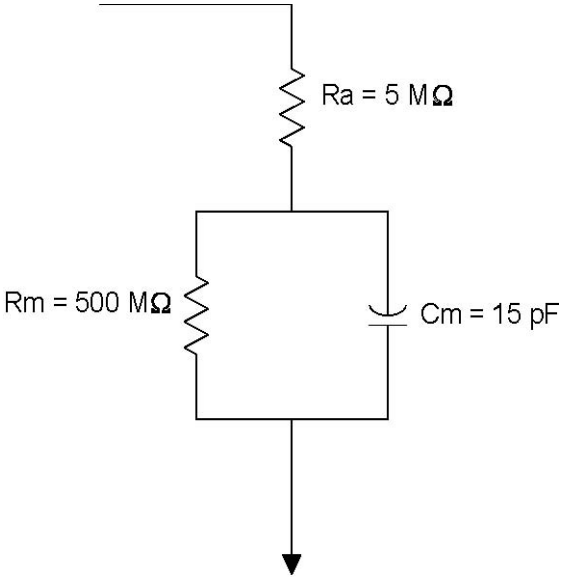


Figure 6-1 Circuit for model cell channels 1 to 15.

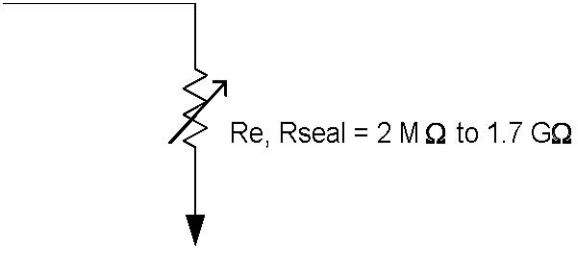


Figure 6-2 Model cell channel 16 circuit, configuration 1.

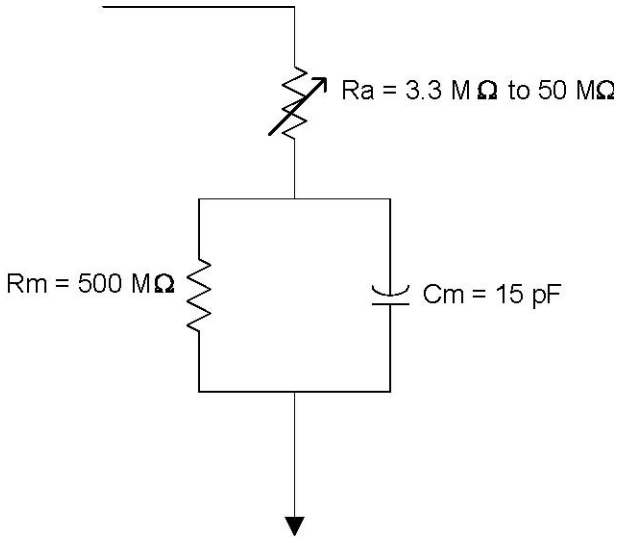


Figure 6-3 Model cell channel 16 circuit, configuration 2.

Note: You need to set electrode capacitance compensation values for the model cell, see Electrode Capacitance Compensation on page 133.

Hardware and Fluidics Dialog

The **Hardware and Fluidics** dialog has a range of commands to start fluidics priming routines, and to move specific hardware components for maintenance. It also contains a log of hardware events, and the **Hardware Diagnostics** dialog reports a large range of hardware parameters.

The dialog is opened from the **circular tool** button, second from the top, in the main toolbar.

Commands in the dialog fall into five categories, listed below.

Prime Fluidics

The commands in this section activate specific processes to prime, and clean the PatchXpress 7000A instrument fluidics systems. If a particular process is due (causing the **Hardware and Fluidics** button to flash yellow), the button for that process is highlighted when the dialog is opened. Durations for each process are reported beside the button.

Start-of-Day Initialization

This process checks hardware components, and prepares intracellular, extracellular, and compound-dispensing fluidics systems for operation. Intracellular, and extracellular solutions, and the blank electrode cartridge, must be in place.

You are prompted to run start-of-day initialization when you open PatchXpress Commander Software. Otherwise, it can be run at any time between experiments from this command. As the name implies, the procedure need normally be performed only once a day, however wash station priming, which occurs as a part of the start-of-day process, should sometimes be run additionally between experiments. Start-of-day initialization takes about 13 minutes.

End-of-Day Shutdown

End-of-day shutdown flushes water through the intracellular, and extracellular supply tubing, leaving the lines filled with water until the system is to be used again. It is necessary to replace the intracellular, and extracellular solutions with deionized distilled water for the procedure, which also requires the blank electrode cartridge.

You are prompted to run end-of-day hardware shutdown when you close PatchXpress Commander Software. If you want to run the shutdown procedure but not close the program use this command. The process takes about 14 minutes.

Prime Wash Station

Prime wash station flushes extracellular solution (or water, if it has been connected) through the wash station. Prime wash station occurs as part of the start-of-day routine, but is sometimes carried out during the day between experiments—especially if there is some time between experiments—to help ensure air bubbles do not build up in the system.

Prime wash station should also be carried out if changing the extracellular solution. It is usual to run the process first with water, to cleanse the tubing, and then with the new solution.

The blank electrode must be in position for prime wash station. The process takes about seven minutes.

Prime Dispensing Robot

This command forces water through the compound-dispensing fluid handler, to help keep the tubing clean, and to remove air bubbles. After the flush is complete, water is drawn back up from the tip providing an air space so that no water comes into contact with compounds drawn up into the disposable tips.

Prime dispensing robot is carried out as part of start-of-day initialization, and does not normally need to be carried out apart from this. The process takes about two minutes.

Prime Intracellular Fluidics

This command forces fluid through the intracellular filling station system to clean it, and remove air bubbles. It is carried out as part of start-of-day initialization, and is not normally required apart from this, unless you change intracellular solution. In this case you are prompted to provide water, and then the new intracellular solution, to first wash the system, and then prime it ready for the next experiment.

Prime intracellular fluidics takes about three minutes.

Alcohol Flush

Alcohol flush should be carried out weekly, to wash the intracellular, and extracellular systems with alcohol (70% isopropyl alcohol). It is, in effect, the combination of prime wash station, and prime intracellular fluidics, but carried out for longer. End-of-week flush (see below) should be run after alcohol flush to remove the alcohol from the system.

You are prompted to replace intracellular, and extracellular solutions with alcohol after the **alcohol flush** button is pressed. Ensure that the blank electrode is in place for the operation, which takes about 14 minutes.

End-of-Week Flush

After an alcohol flush has been executed, run the end-of-week flush to clear the alcohol from the intracellular, and extracellular lines with deionized water.

You are able to set the number of flush cycles performed, up to six. One cycle takes about 14 minutes, while six cycles takes about an hour, and a quarter. Three cycles are recommended.

General

Open/Close SealChip Tray Lid

These commands open, and close the lids of the trays for fresh *Seal*chip$_{16}$ electrodes positioned at the back of the main bench. These trays are not currently in use.

Information

Hardware Log

The **Hardware Log** dialog keeps a record of basic hardware events on the PatchXpress. It stands in contrast to the Lab Book in the **Status** pane, which records events carried out as part of the running of an experiment (though there is some overlap in the events reported in the two logs), and individual cell logs in the **Cell View** pane.

You can choose to show all events since the PatchXpress 7000A system was first installed, or just those in the current session. Each recorded event has a severity classification:

- 'i' for 'information', where an event is simply reported
- 'x', in red, for error conditions

Selected lines of the log can be copied to the Windows clipboard for pasting into other applications. Use the **Shift** or **Control** keys to select blocks of data or noncontiguous rows, or use the **Select All** button to select the entire contents of the log.

Hardware Diagnostics

The **Hardware Diagnostics** dialog reports a large range of hardware information, including:

- basic computer specifications
- model, serial, and firmware version numbers for the master controller, MultiClamps, and Digidata
- information on the pipetting system

The right column of the dialog reports information from a number of system sensors, including:

- pressures
- recording electrode base plate temperature
- system supply voltage
- liquid levels: these indicate when containers require filling or emptying. If the local waste bottle reports other than OK you should check that the waste pump is operating properly.
- upper door open or closed
- *Seal*chip$_{16}$ electrode loaded
- *Seal*chip$_{16}$ electrode overflow: detected if any chamber overflows. This could be caused by a blocked aspiration nozzle or adding too much solution in a compound addition.

If a value is out of range, or if a sensor indicates that a particular item needs attention, then the value or status appears in red, and a message appears in the **Devices** field at the bottom of the dialog.

Maintenance

Hardware Maintenance

Clean Vacuum Tubes: Move the wash station to a 45 degree angle above the recording station, to allow inspection, and cleaning of the nozzles.

Test Vacuum Tubes: This command tests all 16 lines for pressure leaks. Any blockages or leaks are reported.

Blow Out Electrode Tubes: If solution is spilled on the deck of the PatchXpress 7000A instrument it is important to make sure that it does not get into the electrode tubes. When the button is depressed, high-pressure flow is initiated on all 16 channels. For the first few seconds the sponge lid is moved into place above the tubes. High-pressure flow continues after the sponge lid moves back to its home position. Depress the button a second time to stop the high-pressure flow.

Dismantle Loading Clamp: Depressing this button moves the flipper arm to the recording position without a chip in place. This is useful if you need to dismantle the loading clamp or inspect the flipper arm.

Electrode Capacitance Compensation

Electrode capacitance compensation needs to be updated, using the **Electrode Capacitance Compensation** dialog opened from this button, whenever any part of the circuit from the headstage, through the *Seal*chip$_{16}$ electrode, and back, is changed. Most commonly this is after replacement of the electrode plate or wash station.

When the dialog is opened the system loads the blank *Seal*chip$_{16}$ electrode in order to complete the circuit through the electrode for measuring the capacitance. Ensure that the blank electrode is in its loading position for this. In addition, the PatchXpress 7000A system should be set up with the intracellular, and extracellular solutions that you most commonly use.

Electrode capacitance compensation works by supplying additional current to the cell at the start, and end of each voltage step, to charge, and discharge the electrode capacitance. The current is supplied through circuits that bypass the normal voltage clamp circuitry, so with proper compensation the charge, and discharge of the electrode capacitance is invisible to the user. The **electrode capacitance compensation** dialog is used to set the amount of capacitance that will be compensated for on each channel.

The dialog has fields for the magnitude (in picofarads), and time constant (microseconds) for both fast, and slow electrode capacitance components, for each channel. Auto All buttons for each of Ce Fast, and Slow measure these values for all the chambers, or separate Auto buttons measure them for single channels.

It can be a good idea to press the **Auto** buttons several times to see if there is much fluctuation in the values reported, always ending on Ce Fast. After doing this you may want to manually enter a value you estimate as an average of the values that have been measured.

When you press **OK** to close the dialog you set the electrode capacitance values that will be compensated for in every experiment until you next perform this operation. The blank SealChip is automatically unloaded, and returned to its home position.

Model Cell

Separate electrode capacitance values should be set for the model cell. Values set with the model cell in position—while PatchXpress Commander Software is in model cell mode—are remembered, and automatically used whenever PatchXpress Commander Software is in that mode. When you restart PatchXpress Commander Software after removing the model cell, the settings revert to their state prior to going into model cell mode.

In model cell mode the **electrode capacitance compensation** dialog sets just the fast component of electrode capacitance, and whole cell compensation, since the model cell imitates the presence of real cells. Only automatically measured values can be set in the whole cell compensation fields.

Note: The model cell settings are distinct from those for real solutions. The model cell has some extra stray capacitance (which is why the values for channel 16, which has the switch, are somewhat higher than for channels 1 to 15).

Error Recovery

Hardware Reset

Initialize the MultiClamp amplifiers, master controller, and fluid handler. This returns these components to default settings, where applicable. Use this option for error recovery.

Unload SealChip

This command is a direct hardware control to remove a *Seal*Chip$_{16}$ electrode from the recording station, and flip it over to the loading clamp. Use it if power loss or some other event causes the system to shut down without unloading the chip in the usual way.

Release Gripper

If the gripper arm encounters an error or if the system loses power when a chip is in the gripper, it may be necessary to manually remove the chip.

Correct SealChip State

PatchXpress Commander Software does not sense the presence of a *Seal*Chip$_{16}$ electrode, but keeps track of its own actions, and uses this to determine where the electrode is at any time, based on an initial setting. Some error situations can cause PatchXpress Commander Software to lose track of the electrode’s position, in which case you must use this dialog to reset it.

Use this setting with caution—it is best to contact Molecular Devices technical support before using it.

Replacing the Electrode Base Plate

This section describes how to replace the electrode base plate, and the tests, and configuration that must be performed following this.

Replace the Base Plate

1. Loosen all 8 screws of the base plate using the 3/32” hex driver (part of driver set 7700-0152 in the tool box), and carefully remove with the tweezers (7700-0150).

   CAUTION! Do not touch the tops of the electrode tubes with your fingers or the tools. Put the screws aside where they will not be scattered or lost inside the PatchXpress 7000A instrument.

2. Use the electrode base plate extraction tool (7700-0145) to remove the plate. Place the edge of the extraction tool under the base plate.

   Lift the plate straight up by rotating left on the tool arm. The electrode pipes will pull straight up, and out of the mating sockets in the interconnect block. Put the old plate aside.

Figure 6-4 Removing the electrode base plate with the extraction tool.

3. Get a replacement base plate from the tool box, and remove it from its plastic packaging tube.

Note: Some oxidation on the exterior of the silver tubes from chloriding is normal.

4. Place the old plate in the packaging tube. Old plates can be returned to Molecular Devices for refurbishment, which includes inspection of the part for wear, re-chloriding, and replacement of the Teflon gasket.
5. The base plate is symmetrical. In the event it needs to be rotated 180° for troubleshooting pressure leaks, mark the rear edge of the replacement electrode with a permanent marker.
6. Load the replacement plate vertically on the mating sockets of the interconnect block.
7. Get all 8 screws started in their threads, and tighten just until each screw head loosely touches the electrode plate.
8. Tighten screws: Begin with the screw in the upper right corner, and tighten sequentially in a clockwise pattern. Tighten each screw 1/4 turn only, and then move to the next, and repeat. It will take several passes of this clockwise pattern for all screws to be tight. By tightening in this method, the electrode is drawn down slowly, straight on the interconnect block. On the final pass, make sure each screw is 'finger tight'.

   It is not necessary to use great force in the final torque of each screw; 'finger tight' is sufficient.

Verify Alignment by Checking for Pressure Leaks

1. To test that the electrode has been installed correctly, and that the pipes are not tipped front-to-back or left-to-right (which could cause pressure leaks), load a blank electrode, and prime the fluidics by running the **Start of day initialization**. Leaks are reported at the bottom of the Status pane:

   Pressure leak detected

   In AxoTrace:

   AXPS ERROR –1052 on Ch15 : Pressure leak detected
2. Leaks caused by the electrode pipes not being straight typically affect several channels in a row, either at the center (channels 7–10), or at either end (channels 1–4 or 13–16). Single channel leaks are usually caused by a faulty pressure control module.
3. If several channels in a row have a pressure leak, remove the electrode using the procedure described above, and rotate it 180° (using the mark made in step 5 above for orientation).

Replace in the new alignment. Realignment solves the problem in almost all cases. If pressure leaks persist, contact Molecular Devices Technical Support.

Set Electrode Capacitance Compensation

After successfully replacing the electrode, set compensation for system capacitance. This is stray capacitance in the hardware components (electrode base plate, coaxial cables, etc.) that must be nulled.

See Electrode Capacitance Compensation on page 133, for directions.

Check for Electrode Drift

Electrode drift is caused by damage or wear of the chloride coating in the electrode tubes. You can measure electrode drift, and monitor its time course by running an experiment with cell-free external solution, and monitoring the holding current.

To test for electrode drift you need a test *Seal*Chip$_{16}$ electrode with an electrode resistance between 1, and 4 MΩ. A normal *Seal*Chip$_{16}$ electrode can be used as well. If you did not receive test chips, please contact Technical Support.

1. Start the PatchXpress 7000A system, and prime with the pair of solutions you use during experiments. A cell vial with 200 µL of external solution must be in position where cells are normally placed.
2. Set up a new experiment. You must assign a compound plate to make the experiment valid. No compound addition will actually take place, so you can use any sample plate definition. Remember, however, that the key compounds are imported into DataXpress, so it makes sense to use a plate file with something common like ‘DMSO’, ‘External Saline’ or ‘Dummy Compound’.

   It is also a good idea to assign the experiment to distinct Projects, and Screens, such as Project ‘Instrument Tests’, and Screen ‘Electrode Drift’. Open any of the sample procedures or your favorite procedure. It does not matter which, since no cell will go into its cell procedure. A valid procedure must be defined, however, to start the experiment.

   The Cell Type, and Channel should again refer to something that does not interfere with your actual experiments in DataXpress, so define something like ‘Cell-Free Saline’, and ‘No Channel’, respectively. The Solution Pair should reflect the solutions you actually use.

   The Patch Settings must contain a sufficiently long timeout to allow monitoring the holding current. You can download a suitable Patch Settings file from the Molecular Devices Knowledge Base at

http://mdc.custhelp.com/app/answers/detail/a_id/17232/kw/PatchXpress%20patch%20setting%20file), or create one yourself. Start from the default Patch Settings file by selecting Patch Settings / New. On tab 2 of the dialog (Add Cells), change the value for item 2.9 (Wait at Cell Dispersion Pressure for:) to 1200 s. This will insert a wait period of 20 minutes before PatchXpress Commander Software proceeds to the Cell Detection stage. No other changes are necessary, click **OK**, and save the **Patch Settings** using an suitable name.

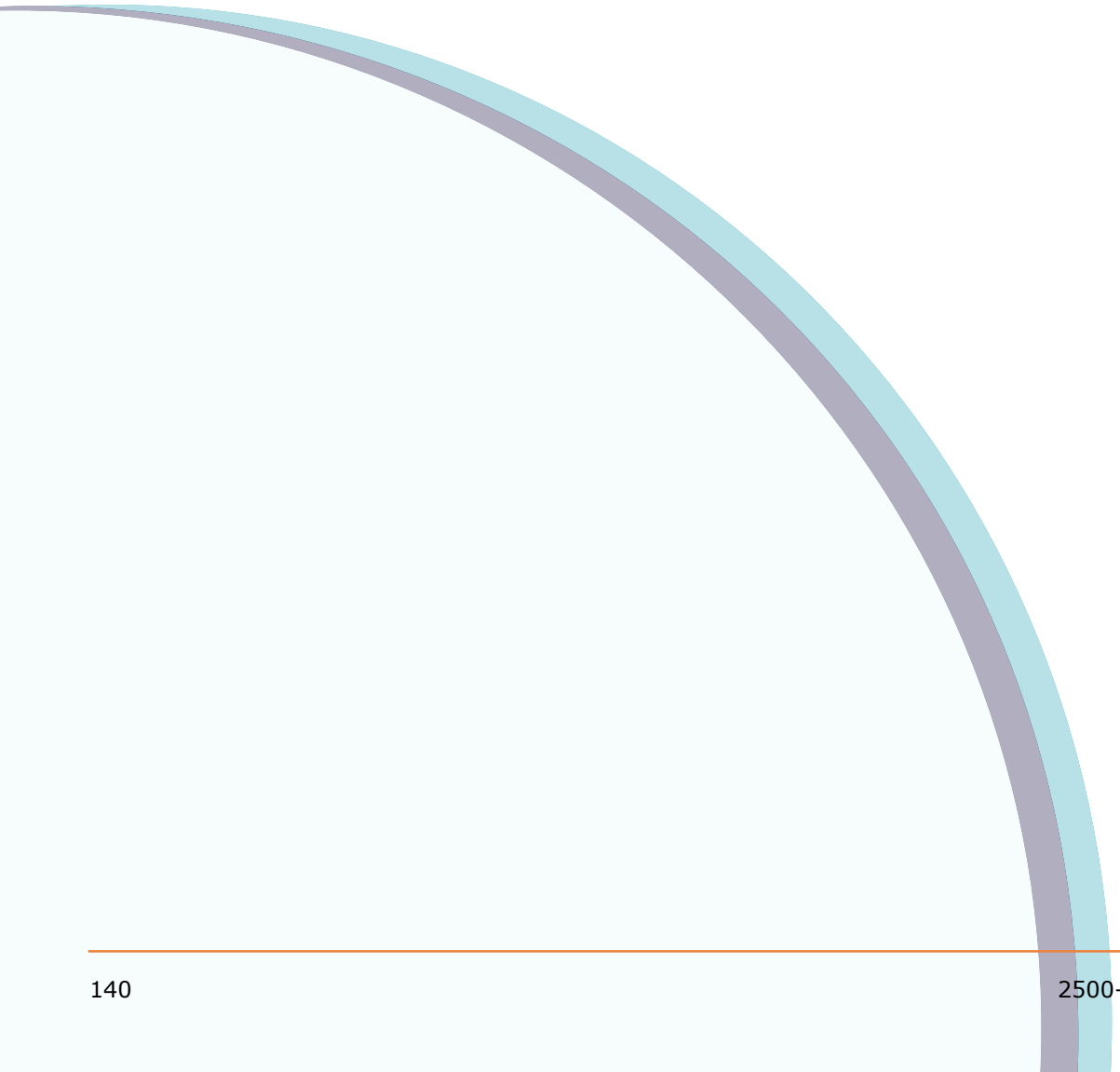
3. Now start the experiment you defined, just like any other experiment. PatchXpress Commander Software nulls the junction potential twice: once after the *Seal*Chip$_{16}$ electrode has been loaded, and a second time, just before the cells are added. You may delay the second junction null by waiting before you OK the **Add Cells** dialog. You can monitor the time course of the holding current on the **Cell Health** tabs in the **Overview or Cell View** panes.

Note: The Seal/Membrane Test pulse is applied to both polarities around the holding potential. The square current response is therefore symmetrical to 0 nA right after the offset is nulled.

4. Normally, there is a small amount of drift that settles in the course of several minutes, following a logarithmic time course. A drift of no more than 5 mV during the first 10 min. should be considered acceptable. Compute the electrode potential from the holding current using Ohm's Law. For example, at an electrode resistance of 1.8 MΩ, if the holding current stabilizes at 1.5 nA, the offset potential is 2.7 mV.
5. If the holding current (and along with it the electrode potential) exhibits an irregular time course, does not stabilize, or the value is too high, then the electrode plate needs to be replaced.
6. Keep a screenshot showing the time course of the holding currents for all channels on the **Overview** pane. When finished, stop the experiment using the **Stop** button. After 20 minutes (or

the time you specified in **Patch Settings**) the experiment will stop automatically, since no cell formed a seal.

Note: The *Seal*chip$_{16}$ electrode used in the test can be reused at a later date, however it is important to catch the chip as it is being ejected from the bolt towards the waste. The chip should be rinsed thoroughly with de-ionized water, and then placed back in a SealChip vial. Tap the vial to ensure that there are no air bubbles in the chambers. An electrode should be rehydrated for at least 24 hours before reuse. If the chip is not allowed to fully rehydrate a significant fraction of the holes are likely to have a higher than acceptable resistance. Eventually this may happen even if the chip is handled carefully each time after use, at which point the chip should be discarded.



Lowpass Filtering

The PatchXpress 7000A system uses a MultiClamp amplifier for each digitizer channel. The acquisition engine performs software Bessel filtering, and decimation from data acquired at a sample interval of 32 µs/sample to create data sampled at the requested sample interval, and filter frequency. A combination of hardware, and software filters are used for different combinations of sample interval, and requested analog bandwidth.

Software Bessel filters can cause overshoot if the cut-off frequency is greater than about one seventh of the sampling frequency. Therefore for higher filter frequencies it is preferable to use hardware filtering that does not suffer from this problem. Hardware filters can be noisy, and have baseline shifts at low frequencies, less than 1000 Hz. Therefore the ideal filter is a combination of hardware, and software filters.

The **Analog bandwidth ratio** field in the **Protocol** dialog allows users to specify the ratio between the sampling frequency, and the analog bandwidth. This ratio, and the sampling interval are used to calculate the appropriate values of the hardware, and software filters. The user chooses a ratio of 2.5, 5, or 10; the actual ratio is modified as needed to give round numbers for the analog bandwidth.

The formula for calculating the effective filter frequency is given below:

$$R = \frac{1}{\sqrt{\frac{1}{H^2} + \frac{1}{S^2}}}$$

where H is the hardware filter frequency, S is the software filter frequency, and R is the requested filter frequency.

For ease of computation, this calculation can also be done in terms of the time constant, tau:

$$\tau_R = \sqrt{\tau_H^2 + \tau_S^2}$$

where τH is the hardware filter time constant, τS is the software filter time constant, and τR is the requested filter time constant.

It would be preferable to use a single anti-aliasing filter of 12 kHz, and then use the software filter for all requested filter values below this.

However, this would cause unacceptable overshoot at frequencies above about 5 kHz.

The compromise here is to use the hardware filter alone if possible, or a combination hardware, and software filters for other frequencies. In practice, this means that frequencies of 4 kHz, and lower are realized by using a hardware filter of 6 kHz in conjunction with software filters, and frequencies of 6 kHz, and higher by hardware filtering only.

Cell Preparation

Preparation instructions for the three principal cell lines used with the PatchXpress 7000A system are provided in the following sections. Two alternative preparation techniques for CHO cell isolation are provided.

Note: The seeding protocol may vary depending on the company where the cell line originated.

For cell preparation we recommend using 4 mL cell suspension with a density of one million total cells (~2.5 x 10^5 cells/mL) per *Seal*chip$_{16}$ electrode. To achieve optimum cell isolation, split one flask at a time. A 70% confluent flask gives ~4 x 4 mL cell suspension in all cell lines. The seeding protocol in the instructions below should be scaled up depending on how many experiments are to be run per day—refer to the seeding density for each cell line.

CHO (Chinese Hamster Ovary) Cells—Method One

Medium and Reagents

- F12 media by Gibco, Cat# 11765
- 10% FBS – heat inactivated by Hyclone, Cat# SH30070H.01
- 1.0% Pen/Strep by Gibco, Cat# 15140-122
- 1.0% Geneticin by Gibco, Cat# 10131-035
- Trypsin (0.25% Trypsin with 1.0 mM EDTA) by Gibco, Cat# 25200-056
- DPBS without Ca++, and Mg++ by Sigma, Cat# D-8537

Materials

- T75 flasks
- 10 mL sterile pipettes
- 25 mL sterile pipettes
- 50 mL sterile pipettes

- 1 mL sterile aspirating pipettes
- 15 mL sterile centrifuge tube
- 50 mL sterile centrifuge tube

Procedure for Cell Passage

Note: Warm all media, and reagents prior to splitting the cells.

1. Split one T75 flask with 70% to 80% confluence.
2. Aspirate spent media.
3. Add 10 mL DPBS.
4. Tilt 1 to 2 times to cover the cell monolayer.
5. Leave flask undisturbed for 1.5 minutes.
6. Aspirate the DPBS.
7. Add 6 mL Trypsin.
8. Tilt 1 or 2 times to cover the cell monolayer.
9. Leave flask undisturbed for 1.5 minutes to trypsinize.
10. Add 10 mL F12 growth media.
11. Pipette up, and down to mix, and dissociate cell clumps.
12. Place cell suspension in a 15 mL sterile centrifuge tube.
13. Centrifuge for 1.5 minutes at 1000 RPM or 168g.
14. Aspirate supernatant without disturbing the cell pellet.
15. Add 4 mL to 6 mL F12 growth media to resuspend cells.
16. Add 30 mL of growth media/T75 flask to be seeded.

Note: Seeding density depends on the day split.

17. Place all T75 flasks in 37 °C, 5.0% CO2 incubator with caps slightly closed.

Seeding Density

For 1 day split = 1:2
For 2 day split = 1:3
For 3 day split = 1:10

Procedure for Cell Isolation

Note: Warm all media, and reagents prior to splitting the cells.

1. Split one T75 flask with 70% to 80% confluence.
2. Aspirate spent media from the flask.
3. Add 10 mL of DPBS.
4. Tilt 1 to 2 times to cover the cell monolayer for a quick wash, and aspirate.
5. Add 10 mL of DPBS.
6. Tilt 1 to 2 times to wash the cell monolayer.
7. Leave flask undisturbed for 1–2 minutes.
8. Aspirate the DPBS.
9. Add 10 mL of 1:10 dilution Trypsin with EDTA in DPBS.
10. Leave flask undisturbed for 1.5 minutes.
11. Aspirate the Trypsin.
12. Leave flask undisturbed for another 1.5 minutes.
13. Hit the side of the flask once to dislodge cells.
14. Add 10 mL of F12 growth media.
15. Place cell suspension to 15 mL sterile centrifuge tube.
16. Centrifuge to 1000 RPM or 168g for 1.5 minutes.
17. Aspirate supernatant.
18. Resuspend cells with 5 mL F12 growth media.
19. Perform cell count.
20. Place 4 mL cell suspension in a 15 mL centrifuge tube with a density = 2.5 x 105 cells/mL.

Note: The number of 15 mL centrifuge tubes with cell suspensions needed varies depending on cell confluence, and the number of experiments.

21. Place tubes in 37 °C, 5.0% CO2 incubator with caps slightly closed. Use cells after 15 minutes post-split for cells to recover. Cells can still be used 1 to 1.5 hours after incubation.

CHO (Chinese Hamster Ovary) Cells—Method Two

Medium and Reagents

- DMEM without Ca++ (GIBCO cat# 21068-028) with 10% FBS (GIBCO cat#10082-147)
- DPBS without Ca++, and Mg++ (VWR cat# 16777-149)
- Accumax (Innovative cell Tech, cat# AM105)

Procedure

1. Seed CHO-hERG into T-75 flask in culture medium, cells should be about 70% to 85% confluence the day of the isolation.
2. Warm all media, and reagents to room temperature prior to isolation.

Note: Please DO NOT thaw Accumax at 37o C.

3. For isolation cell from each T-75 flask, prepare digestion buffer as following:

   a. Add 5 mL Accumax into 15 mL of DPBS without Ca++, and Mg++, mix gently, and leave at room temperature for later use.
4. Remove medium, and wash twice with DPBS without calcium, and magnesium.
5. Add 10 mL digestion buffer (prepared at step 1), wait about 1 to 2 minutes at room temperature (cells should be turning round), tilt to remove the loose attached cells.
6. Aspirate supernatant, add 10 mL of digestion buffer (prepared at step 1), incubate cells at 37o C until 95% of cells dissociate from the flask. If it is difficult to form seals with the isolated cells, incubate the cells two to three minutes longer in the digestion buffer after detaching the cells from the flask.

Note: short digestion time generates cells with strong membranes that can make it hard to form seals, but the seals tend to last longer. Longer digestion time makes cells that are easier to seal but the seals tend to break more easily.

7. Add 9 mL of DMEM without Ca++ (GIBCO cat# 21068-028) with 10% FBS (GIBCO cat#10082-147) into the flask to stop the digestion.

Option: pass the cell suspension through the 20 µm filter (Spectrum Laboratories, Inc cat# 146510) into a 50 mL centrifuge tube.

8. Centrifuge 1000 rpm for 1.5 minutes, then carefully aspirate supernatant without disturbing the cell pellet.
9. Resuspend cells into 5 mL DMEM without Ca++ (GIBCO cat# 21068-028) with 10% FBS (GIBCO cat#10082-147), count the cell concentration**.**
10. Aliquot 1 x 10^6 cells into each 15 mL tube, incubate cells at 37° C with the desired volume of DMEM + 10% PBS (usually 2 to 4 mL). The cells can be used for up to 4 hours.

Cell Preparation Prior to Patching

1. Take one tube of cells out of incubator, centrifuge at 1000 rpm for 1 minute, discard supernatant.
2. Resuspend cells in the desired volume of extracellular solution for patch-clamp recording.

HEK (Human Embryonic Kidney) Cells

Medium and Reagents

- DMEM with high glucose, and L-glutamine by GIBCO, Cat# 11965-126
- 10% FBS– defined by Hyclone, Cat# SH30070.01
- 1.0% Pen/Strep by GIBCO, Cat# 15140-122
- DPBS without Ca++, and Mg++ by Sigma, Cat# D-8537
- Trypsin 0.25%, 1 mM EDTA.4Na by GIBCO, Cat# 25200-056

Materials

- T75 flasks
- 10 mL sterile pipettes
- 25 mL sterile pipettes
- 50 mL sterile pipettes
- 1 mL sterile aspirating pipettes
- 15 mL sterile centrifuge tube
- 50 mL sterile centrifuge tube

Procedure for Cell Passage

Note: Warm all media, and reagents prior to splitting the cells.

1. Split one T75 flask with 70% to 80% confluence.
2. Aspirate spent media.
3. Add 10 mL DPBS.
4. Tilt 1 to 2 times gently to wash the cell monolayer.

Note: Cells dissociate easily from the flask.

5. Leave flask undisturbed for 2 minutes.
6. Aspirate the DPBS.
7. Add 6 mL Trypsin.
8. Tilt once or twice to cover the cell monolayer.
9. Leave flask undisturbed for 2 minutes to trypsinize.
10. Dislodge cells from the flask.
11. Add 6 mL of DMEM growth media.
12. Pipette up, and down to mix, and dissociate cell clumps.
13. Place cell suspension in a 15 mL sterile centrifuge tube.
14. Centrifuge for 1.5 minutes at 1000 RPM or *168g.*
15. Aspirate supernatant without disturbing the cell pellet.
16. Add 4 mL to 6 mL DMEM growth media to resuspend the cells.
17. Add enough cell suspension (depends on the next splitting day) to each T75 flask with 20 mL to 30 mL DMEM growth media/flask.
18. Place flasks in 37 °C, 5.0% CO_2 incubator with caps slightly closed.

Seeding Density

For 1 day split = 1:2
For 2 day split = 1:4
For 3 day split = 1:10

Procedure for Cell Isolation

Note: Warm all media, and reagents prior to splitting the cells.

1. Split one T75 flask with 70% to 80% confluence.
2. Aspirate spent media from each flask.
3. Add 10 mL of DPBS.
4. Tilt 1 to 2 times gently to wash the cell monolayer for a quick wash.

Note: Cells dissociate easily from the flask.

5. Add 10 mL of DPBS.
6. Tilt 1 to 2 times gently to wash the cell monolayer.
7. Leave flask undisturbed for 1 minute.
8. Aspirate the DBPS.
9. Add 10 mL of 1:10 dilution Trypsin with EDTA in DPBS.
10. Leave flask undisturbed for 2 minutes.
11. Tilt flask to dislodge cells.
12. Add 10 mL of growth media.
13. Place cell suspension to 50 mL centrifuge tube.
14. Centrifuge to 1000 RPM or *168g* for 1.5 minutes.
15. Aspirate supernatant.
16. Resuspend cells with 5 mL growth media.
17. Perform cell count.
18. Seed 4 mL cell suspension with density = 2.5 x 10^5 cells/mL in a 15 mL centrifuge tube.

Note: The number of 15 mL centrifuge tubes with cell suspension depends on cell confluence, and the number of experiments.

19. Place tube/s in 37 °C, 5.0% CO_2 incubator with caps slightly closed. Use cells after 15 minutes post split for cells to recover. Cells can still be used 1–1.5 hours after incubation.

RBL-1 Cells

Medium and Reagents

- DMEM/F12 by GIBCO, Cat# 11330-032
- 15% FBS defined by Hyclone, Cat# SH30070.02
- 1% Pen/Strep by GIBCO, Cat# 15140-122
- 1% NEAA by GIBCO, Cat# 11140-050
- 1% NaPyruvate by GIBCO, Cat# 11360-070

Materials

- T75 flasks
- 10 mL sterile pipettes
- 25 mL sterile pipettes
- 50 mL sterile pipettes
- 1 mL sterile aspirating pipettes
- 15 mL sterile centrifuge tubes
- 50 mL sterile centrifuge tube

Procedure

Note: Warm the media prior to splitting the cells.

1. Split 1 x T75 flask.
2. Collect all spent media, and transfer to a 50 mL centrifuge tube.
3. Centrifuge for 1.5 minutes @ 1000 RPM.
4. Aspirate supernatant.
5. Resuspend cells with 4 mL to 6 mL DMEM/F12 growth media.
6. Perform cell count.

For Seeding

1. Seed enough T75 flasks for the next splitting day (refer to the seeding density below) with 40 mL growth media/flask.
2. Place all flasks in 37 °C, 5% CO_2 incubator with caps slightly closed.

Seeding Density

For 1 day split = 1:4

For 2 day split = 1:10

For 3 day split = 1: 40 (seed in the afternoon)

For PatchXpress Experiment

1. Make 4 mL cell suspension with density = 2.5 x 10^5 cells/mL, and place in the 15 mL sterile centrifuge tube.

Note: The number of 15 mL centrifuge tubes with cell suspension depends on cell confluence of the flask, and the number of experiments.

2. Place the 15 mL centrifuge tubes with cell suspension in 37 °C, 5% CO_2 incubator with caps slightly closed. Use cells after 15 minutes post split for cells to recover. Cells can still be used 2–3 hours after split.

Specifications

8

Major Components

- Computer-Controlled Dual Headstage Patch-Clamp Amplifiers (8)
- Digitizer
- Fluid Handler
- Wash Station
- Intracellular Injector Station
- Vacuum Pump
- Air Compressor
- Cabinet dimensions: 1.473 m (58”) x 0.762 m (30”) x 1.676 m (66”) (W x D x H)
  - Plus 24” monitor attached to side.
- Computer
- PatchXpress Commander Software
- pCLAMP 9.2 Software
- DataXpress 1.0 Software (Client, and Importer)

System Power Requirements

- 100 VAC to 240 VAC
- 50/60 Hz
- 8.5 A Maximum

Patch-Clamp Amplifiers

- MultiClamp 700A Computer-Controlled Patch-Clamp Dual Headstage Amplifiers (8)
- CV-7A Headstages (16)
- Headstage Feedback Resistors: Rf = 50 GΩ, 5 GΩ, 500 MΩ, 50 MΩ
- Scaled Output Filters: Four-pole Bessel or Butterworth lowpass filter with fifty-eight 3dB cutoff frequencies ranging from 2 Hz to 30 kHz plus bypass
- Postfilter Output Gain: 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000

Digitizer

- Customized Digidata 1322A with 16 Analog Output Channels
- Analog Input Channels: 16
- Analog Output Channels: 16
- Analog Input/Output resolution: 16 bits
- Digitizer Input range: ±10 V
- Maximum acquisition rate: 500 kHz or 31.25 kHz per channel
- Analog Input noise: 300 µVrms
- Analog Output noise: 100 µVrms

Barcode Reader

- External handheld for *Seal*chip$_{16}$ ID
- Minimum bar width: 0.127 mm
- 44 scans/second bidirectional
- Decoded scanners: UPC/EAN/JAN, UPC/EAN with supplementals, Code 128, Code 39, Code 39 Full ASCII, Code 93, MSI, Interleaved 2 of 5, Discrete 2 of 5, UCC/EAN 128, Codabar, Code 11, Coupon Code, Bookland EAN, RSS variants

Vacuum Pump

- Edwards model RV5
- 1 mmHg @ 1.5 cfm
- Pinch valves on lines - on/off
- Draws 28 A initially with 8 A stable maximum current (110 VAC/60 Hz)

Air Compressor

Use house air or pump supplied by Molecular Devices.

- SilentAire model # SIL-Tech 50-9-D
- 30-60 psi @ 1.0 cfm
- 10 A maximum current (110 VAC/60 Hz)

Computer

Supplied with the PatchXpress 7000A system:

- Windows 7
- Flat LCD 24” screen monitor
- DVD-RW drive

SealChip16 by AVIVA Biosciences

- 16 channel planar electrode array with polycarbonate cartridge upper chamber.
- Proprietary surface modification promotes seal formation, and stability with the quality of the conventional patch pipette.

Fluid Handler

- Uses disposable tips
- Probe positioning performance:
  - Accuracy: +/-0.4 mm in Z
  - Accuracy: +/- 0.2 mm X/Y

Syringe Pumps (2)

Intracellular Fluid

- Syringe volume 1.0 mL
- 0.05% CV
- flow rate 12 µL/s

Drug and Cell Delivery

- Syringe volume 0.5 mL
- 0.05% CV
- Flow rate selectable in software: 10 µL/s to 200 µL/s

Peristaltic Pumps (16)

- Independently-controlled for each of the 16 chambers
- Wash flow rate - 4 µL/s
- Tubing size 0.8 mm ID

Consumables

- AVIVA *Seal*chip$_{16}$ - 16 channels/chip
- Disposable tips, BioRobotix by Molecular Bioproducts catalog #902-261 or #902-262

Model Cell

- MC-PX - 16 channels
- Channels 1-15 with fixed values: Ra = 5 MΩ, Rm = 500 MΩ, and Cm = 15 pF in parallel to ground
- Channel 16 with switchable values:
  - configuration 1: Re, Rseal = 2 MΩ to 1.7 GΩ to ground
  - configuration 2: Ra = 3.3 MΩ to 50 MΩ, Rm = 500 MΩ, and Cm = 15 pF in parallel to ground

Toolkit

- Model cell
- System tools
- Electrode plate
- Gaskets

Technical Assistance

If you need help to resolve a problem, there are several ways to contact Molecular Devices:

Molecular Devices Customer Support

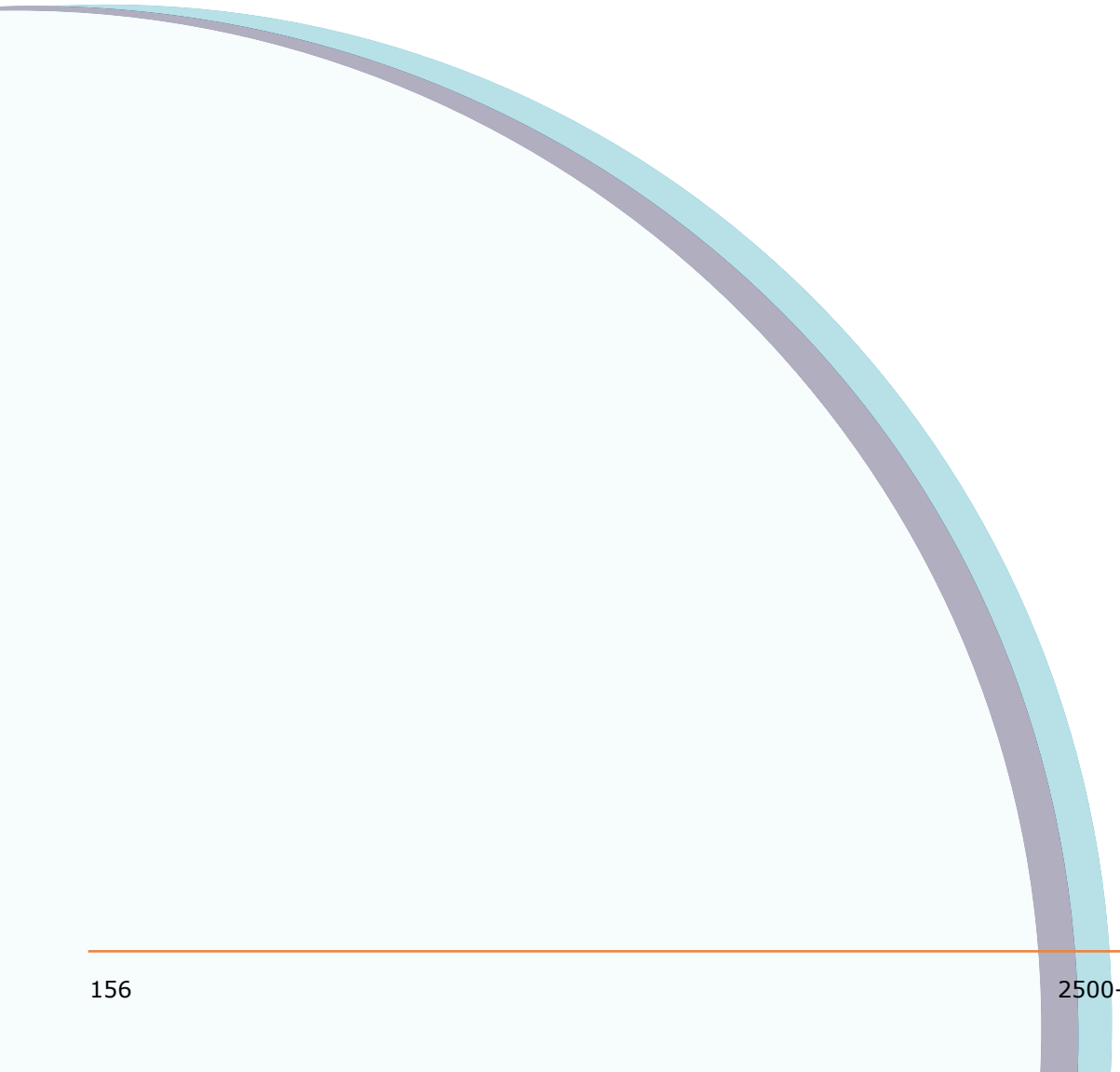
http://www.moleculardevices.com/support.html

Knowledge Base

http://mdc.custhelp.com/app/home

Telephone

(408) 747-1700
(800) 635-5577 (US, toll-free)
+44 (0) 118 944 8000 (Europe)



Glossary

ABF: Axon Binary File, the standard file format for Axon data files. PatchXpress's trial and cell procedure log (params) files are in ABF format.

Assay Development Mode: PatchXpress operating mode where experiment runs in just the number of channels (less than or equal to 16) that are specified. cf. screening mode.

Ce fast: The fast component of the electrode capacitance. The electrode capacitance is the lumped total capacitance of the headstage case, the connecting coaxial cable, the connector block and the electrode itself.

Ce slow: The slow component of the electrode capacitance.

Cell Procedure: The application of the procedure to an individual cell. Each cell procedure is recorded in a cell log (params) file, and one or more trials.

Chamber: The 16 cavities on the Sealchip$_{16}$ electrodes where cells are held as compounds are applied. Each chamber corresponds to a channel, with one patched cell in each.

Channel: The circuit on which one cell is tested, for example, each MultiClamp amplifier has two channels. 'Channel' can often be used interchangeably with 'chamber', as each chamber of a Sealchip$_{16}$ electrode corresponds to one circuit.

Cm: Membrane capacitance (only measurable during whole-cell mode).

Compound triggered acquisition mode: Term used in PatchXpress for high-speed oscilloscope mode (see glossary entry).

Current Clamp: Measures voltage.

Data point: Measure of analysis throughput. One data point is the single result value for a test compound at a given concentration. Averaged result values from replicates give one data point. Values for the same compound at different concentrations are different data points. Thus, a 5-point EC50 analysis is five data points, regardless of the number of replicates run to create it.

Depolarize: A positive current into the cell. It makes the membrane potential more positive. cf. hyperpolarize.

Electrical Ra Correction: Use of a circuit inside the MultiClamp to reduce the effective value of Ra. This does not reduce the actual value of Ra. cf. pneumatic Ra control and optimization.

Electrode: This term is used differently depending on context within this manual. Usually it refers to the 16-channel Sealchip$_{16}$ planar electrode, but might also be used to refer to individual channels of this. It can also refer to the Ag/AgCl suction tubes in the recording station base plate, or the wash station nozzles. 'Electrode hole' refers to the small

hole in the base of each Sealchip$_{16}$ chamber, where a cell is sealed.

Episodic Stimulation Mode : In PatchXpress, called 'internally triggered' mode. Noncontinuous acquisition where each sweep occurs at a predefined interval (set in Edit Protocol dialog). A stimulus can be delivered during the sweep cf. high-speed oscilloscope mode.

Epoch : A part of a sweep, used for configuration of the stimulus waveform. PatchXpress has 10 epochs per sweep.

Experiment : In PatchXpress an experiment is all that happens from when the Start button is pushed until all the compounds defined for the experiment have been tested, or the experiment is stopped manually. An experiment may consist of numbers of cell procedures, using numbers of Sealchip$_{16}$ electrodes.

An experiment (PXX) file is produced for each experiment.

High-Speed Oscilloscope Mode : In PatchXpress, called 'compound triggered' mode. Noncontinuous acquisition where each sweep is triggered by some event—in the case of PatchXpress, the addition of compound. No stimulus can be delivered in this mode. cf. episodic stimulation mode.

Hyperpolarize : A negative current into the cell. It makes the membrane potential more negative. cf. depolarize.

Im : Membrane current. It has both transient and steady-state components.

Internally triggered acquisition mode : Term used in PatchXpress for episodic stimulation mode (see glossary entry).

Mode : PatchXpress has two 'acquisition' modes and two 'system modes'.

Acquisition modes:

• Internally triggered (see entry for episodic stimulation)

• Compound triggered (see entry for high-speed oscilloscope)

System modes (see separate entries):

• Screening mode

• Assay development mode

Amplifier modes (see separate entries):

- Voltage Clamp

- Current Clamp

P/N leak subtraction : Software subtraction of the transient current that charges Cm and the steady-state current through Rm. Both of these are passive currents. By subtracting these passive currents the researcher can clearly observe ionic currents.

Pneumatic Ra Control : Pressure control algorithm to lower the Ra value. This reduces the actual value of Ra by producing suction pulses designed to clear the electrode hole. The pulses are sensitive in strength and duration to Rm and Ra.

Pneumatic Ra Optimization : Sequence of suction pulses to lower Ra. Similar to pneumatic Ra control, but the pulses do not automatically adjust.

Pre-sweep Train : A set of regular, rectangular pulses that can be delivered prior to each sweep in a trial, to stimulate the cell prior to further stimulation and recording in the main sweep.

Procedure : The sequence of steps applied to a cell once it is patched;

for example, start trial, add compound, wait, wash, etc. When a procedure is applied to an individual cell it is called a 'cell procedure'.

Project : The highest level of classification under which all experiments must be placed. Projects are attributes in DataXpress, where they (with Screens) are the units bywhich data is exposed or hidden to individual users or groups. cf. Screen

Protocol : Set of definition parameters for trials. Protocols determine sampling rate and filtering, trial timing, command waveform, P/N leak subtraction and pre-sweep trains, and on-line measurements.

Ra : Access resistance (only measurable during whole-cell mode). It is the series combination of the electrode resistance (Re) and the resistance of the ruptured patch.

Re : Electrode resistance. It is only measurable up until a cell lands.

Rf : Feedback resistor in the headstage.

Rm : Membrane resistance (only measurable during whole cell mode).

Rs : Ambiguous because it describes either the access resistance in series with the cell or the seal resistance. 'Rs' is used for the MultiClamp (for seal resistance) but its use is avoided with PatchXpress.

Rseal : Seal resistance. It is only measurable after cell landing is detected and before whole-cell mode is detected.

Rt : Total resistance. In whole-cell mode Rt = Ra + Rm.

Run : Subcomponent of a trial, between sweep and trial. The corresponding sweeps of each run are averaged, so the ABF file of the trial has only as many sweeps as were configured for a run, even though actual acquisition might have consisted of several runs.

Sample : The datum produced by one A/D (Analog-to Digital) or D/A (Digital-to-Analog) conversion. In trial definition you must set the rate at which samples are output and acquired.

Screen : The second highest level of classification (under Project) for an experiment. Screens are attributes in DataXpress, where they (with Projects) are the units by which data is exposed or hidden to individual users or groups. cf. Project.

Screening Mode : PatchXpress operating mode where experiment continues, replacing Sealchip_{16} electrodes as needed, until all compounds have been tested. cf. assay development mode.

Signal : Data streams from different sources, sharing the same time base, within a trial. In PatchXpress there is only ever one signal recorded per channel unless leak subtraction is enabled, in which case the raw and the corrected data are both recorded, one in each signal.

Sweep : A continuous sequence of data acquisition within a trial. Sweeps may be accompanied with delivery of a command waveform. In trials with multiple runs, corresponding sweeps in the different runs are averaged.

T_m : Tau, membrane time constant, measurable during whole-cell mode.

Trial : A data acquisition, often consisting of multiple sweeps, and possibly accompanied with delivery of a stimulus

waveform. Trials are defined in a protocol, and recorded in ABF files.

Voltage Clamp: Measures current.

Vcmd : Command voltage applied to the electrode.

Vm = Vcmd – Va.

Vm : Membrane potential. It has both transient and steady-state components. Vm can only be approximated during current flow because of the voltage drop across Ra.

Waveform : The voltage command stimulus delivered during the acquisition sweep.

Well : A fluid-containing cavity in a compound plate. cf. chamber.

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