New Product News

New Integrated Systems for Concurrent Imaging and Electrophysiology

Axon Instruments now offers two new Integrated Systems ready for concurrent Ion Imaging and Electrophysiology. The Integrated Imaging and Electrophysiology (IIE) package includes a complete hardware and software package for simultaneously running the Axon Imaging Workbench and Clampex 7: our integrated ion imaging system, the Digidata 1200 interface, pCLAMP 7, and an Axopatch 200B. If you already have an amplifier, save by ordering the Integrated Imaging and Data Acquisition System (IIDA), which does not include the amplifier. These packages include on-site installation and check-out. Let Axon Instruments put all the pieces together for you!

For more information and a detailed quote tailored to your needs, contact Axon Instruments and ask for the Integrated Imaging Systems program.

Visit the Axon Instruments Booth at ...

**German Neurological Society**
Hanover, Germany • June 2-16, 1998

**The 1998 Forum of European Neuroscience**
Berlin, Germany • June 27 to July 1, 1998

**Congress of Neurological Surgeons**
Seattle, WA • October 3-8, 1998

**The Society for Neuroscience '98**
Los Angeles, CA • November 7-12, 1998

**American Society for Cell Biology**
San Francisco, CA • December 12-16, 1998

Finally Married!
Simulation in AXOVACS 3: a Teaching and Research Tool

AXOVACS 3 now provides an easy to use Windows application that simulates a wide variety of channel models. Originally developed to teach students the ionic and channel basis of the action potential, it models the Hodgkin and Huxley equations.

The product comes with both DOS and Windows versions which can simulate and display on-line channel behavior that would be recorded in a variety of experiments. Both ligand gated and voltage gated channels can be simulated, and this version of the program adds the modeling of synaptic potentials and channel kinetics. The kinetic simulation employs multi-state (Markov) models for channel gating, and allows the user to establish transition probabilities between states in a spreadsheet-like grid.

The parameter file for a given model can be saved as a file, and for research purposes the simulated data can be saved either as raw data or as an events list, for subsequent analysis in pCLAMP. A second application, PRI2Dat, inputs an events list, adds simulated noise and instrumentation filtering, and produces an Axon Binary Format File.

Marketing and Scientific Application Staff Grows

In our continuing commitment to the technical support and development of Axon Instruments’ products, we have again expanded our Marketing and Scientific Applications (MSA) staff.

Please welcome Chang Wang, Ph.D., the newest Application Scientist in the MSA Department, who will assist you in setting up and running experiments with the Axon Imaging Workbench and pCLAMP.

Prior to joining Axon, Chang was a postdoctoral fellow at the Howard Hughes Medical Institute at Northwestern University, and then at the University of California at Berkeley. She has extensive experience with imaging and electrophysiology, and has published many articles on axonal transport, ion channel activity and synaptic transmission. Her latest study of synaptic transmission using FM1-43 and a confocal imaging system will be published in the June issue of Neuron.

Her recent publications include:


Axobits

Copyright 1998, Axon Instruments, Inc.

Editor .......................... Heather Evans
Axon Contributors .......... Al Walter, Ph.D.
                  Cliff Christian, Ph.D.
                  Enrique Chang
                  James Fox, Ph.D.
                  Marc Paron

Additional Contributors .... E. Yoder, Ph.D.
                  R. Nyffenegger
**Q: What should I do when I try to configure my Digidata 1200A in Clampex 7 and am told that no IRQs are available?**

**A:** First look at the IRQ usage in the System Device Manager: in the Control Panel choose System, select computer and view its properties. There must be at least one free IRQ among the group 10, 11, 12 or 15. As the Digidata 1200 A or B board is not plug and play, other devices on your computer may pre-empt these IRQs before you can configure the digitizer. It may be necessary to shut down your computer, remove one device card which is using one of the required IRQs, and reboot. Consult the Device Manager again to see if an IRQ has been freed up. It may be necessary to reserve an IRQ in the computer’s BIOS setup program which can be usually accessed during boot up with an <F1> or <Del> keystroke. In addition you can reserve IRQs at the Windows level, again in the system Device Manager. If you are ordering a new computer, insist that it be configured such that at least one required IRQ is free. For further details, click Help/Latest Web Info in Clampex 7.

**Q: We are presently using Axopatch 200B and 1D amplifiers to patch isolated cells, and we would like now to begin patching cells in intact tissue or slices. Can you give some general suggestions for making seals in tissue?**

**A:** In general, ways that keep the pipette tip clean help to improve seal formation. Use positive pressure inside the pipette while in the bath and in the tissue, until you are ready to try to obtain a seal. Then, with the pipette tip pressed against a cell membrane, try just releasing the positive pressure, rather than actively applying suction.

Sometimes including fluoride in the internal solution improves seals; however, it can create a precipitate with some bath cations which can clog the tip. Positive pressure may help here as well.


**Q: The Inkjet 1000 printer does not work with the Clampex 7 dongle. What can I do?**

**A:** Launch HPW3CFG.EXE (part of HP Inkjet 1000c installation CD) and uncheck the box for ‘birection printing’ you will then be able to use your printer with the Clampex 7 dongle.

**Do You Need a Manual for Our Software Applications?**

Axon Instruments has put a lot of effort into making the on-line help in our new Windows applications such as Clampex 7 as complete and helpful as possible. We now include the standard Windows searchable help facility, context sensitive help for all forms, a special Quick Start section in the help, a Tip of the Day, and a Clampex assistant. As we were putting the finishing touches on the manual for Clampex 7, we began to question if a manual for a professionally-done Windows application is really required. Given this on-line help facility, do you really derive any help from a printed manual? Could Axon perhaps reinvest the time it takes to prepare a printed manual in some other way that would benefit our customers? If you have an opinion, please reply by Fax [650-571-9500] or e-mail “MSA@axonet.com” to the following survey:

1. Do you need a manual in addition to the on-line help?
2. If you would like a manual, what content would you like to see in it?
   - Same content as the on-line help.
   - Introduction, configuration and getting-started alone.
   - Description of algorithms used in the software.
   - Reference section detailing operation of each command.
   - General chapters on experimental procedures, such as found in the Axon Guide.
Using the Axopatch 200B for Low Current Scanning Tunneling Microscopy

Ralph Nyffenegger
Park Scientific Instruments
1171 Borregas Avenue, Sunnyvale, CA 94089
e-mail: ralphn@park.com

Many samples of interest in biology and material science are poorly or non-conducting. Prior to the advent of non-contact atomic force microscopy (NC-AFM)\(^1\) low-current scanning tunneling microscopy (STM) was the method of choice to investigate the morphology of such samples. Even though contact AFM was available at that time, its application was limited for several reasons: the resolution on soft materials is rather low, and poorly adhering particles were pushed aside rather than being imaged. As NC-AFM has become more standard the interest in low-current STM has decreased. However, low current STM has again become the focus of some research groups because it can provide information unavailable with NC-AFM or regular STM. Some of these applications are listed below:

- **DNA sequencing**
  The resolution of both contact and non-contact AFM is not sufficient to recognize the different bases of a DNA string. Regular tunneling currents are too high to image the molecules correctly: the tip is so close to the surface that it often touches or even moves them around\(^2\).

- **Oxides**
  Oxides are often poorly conducting and regular STM cannot image their surfaces very well.
  An interesting application in this context is a combination of low current STM with contact AFM. While STM should reveal the underlying metal or semiconductor topography, AFM will show the topography of the oxide\(^3\).

- **Semiconductors**
  With biases below the band gap, the tunneling current is very small. In order to obtain information of trap states within the band gap, STM spectroscopy capable of measuring very small currents can be used.

- **“Noise measurements”**
  Theoretical considerations predict that the “noise” of the tunneling current contains physical information (e.g., in liquids the diffusion of single molecules in the tunneling gap\(^4\)). Clearly, this “noise” should be well resolved if such experiments are attempted.

- **Electrochemistry**
  Whereas on ideal surfaces like gold, STM imaging is easily performed with nA currents, on less noble surfaces that are susceptible to oxide formation, the conductivity is low and therefore low current STM is advantageous.
  Because of the proximity of the tip, diffusion of dissolved species in the vicinity of the tunneling gap is hindered. If STM can be performed with the tip further away (by controlling the STM with a small current) the diffusion rate should increase.

- **Non-contact AFM**
  In non-contact AFM, the tip sample distance is larger than the usual tunneling distance of 1nm. Tunneling currents between the tip and the sample in this configuration are therefore very small. However, to better differentiate between topographical and electrical information, combined AFM/STM is a very desirable technique.

With the I/V converter in Park Scientific Instruments STM, a typical lower limit of the set current is about 50 pA - 100 pA. However, the above mentioned low current applications demand a set current of 1 pA or lower and obviously the background current has to be even lower.

Patch clamp amplifiers from Axon Instruments have performance levels that easily fulfill the requirements regarding amplification gain (≥ 10\(^9\)) and bandwidth (≥ 1 kHz) for low current STM. Hence it is logical to try to connect a patch clamp amplifier to an STM.

An Axon Instruments’ Axopatch 200B was interfaced with Park Scientific Instruments AutoProbe CP and VP2 for low-current STM. The CP is a general purpose research instrument that operates in air, the VP2 is a specialized instrument for ultra high vacuum applications (UHV).
Figure 1 shows the typical components of the CP. In STM, the length of the connection between the tip and the pre-amp should be kept as short as possible to minimize induced capacitive effects, hence the pre-amp is usually built directly into the STM head. The output of the pre-amp is connected through the base and a multipurpose signal cable to the AutoProbe electronics where it is used in the feedback circuitry.

The open design of the microscope allows a user to easily incorporate an external current pre-amplifier, such as the Axopatch 200B. The connection from the tip to the built-in pre-amp is disconnected. The Axopatch pre-amp is mounted on the STM head and connected to the STM tip and the Axopatch 200B as shown in Figure 2. Because of the size of the pre-amplifier, the regular cover was too small and a larger cover had to be constructed. We used stainless steel metal foil and µ-metal adhesive tape.

A signal access module (SAM) is installed between the AutoProbe electronics and the CP base. The SAM allows the user to disconnect the output from the internal pre-amplifier and to connect the output from the Axopatch 200B instead.

The implementation for the VP2 was similar. Ideally, the pre-amp would be placed as close to the tip as possible. However, the Axopatch pre-amp is not UHV compatible and it had to be mounted outside of the chamber and connected to the tip with a roughly 6” long coaxial cable as shown in Figure 3. This length of cable may cause some undesired capacitive effects that can be compensated with the Axopatch 200B. In this study we did not use this feature nor did we try to use twisted cable instead of the coaxial one.

Two additional measures were undertaken to lower the noise of the entire system: the tunneling bias provided by the computer was disconnected and the standard tip holder for the AutoProbe CP was replaced by a smaller one.

- For the CP, the tunneling bias was disconnected by switching a button (called EC/Norm) in the base of the CP. An external BNC connected the sample to the analog ground of the Axopatch 200B. For the VP, access to signals is given in the pre-amp, where the sample connection was physically disconnected from the bias and wired to the analog ground of the Axopatch. The Axopatch provides a built-in ±1V command voltage that can be used as tunneling bias and is applied to the tip. The range of ±1V is usually sufficient for applications in air, where higher bias voltages normally cannot be used because of electrochemical reactions that would occur at either the tip and/or at the sample (depending upon the polarity of the bias). For applications in water-free environment, e.g., dry argon atmosphere or vacuum, higher bias voltages can be used and are often also needed e.g., to image large band gap semiconductors. In this case, an external bias supply is needed. A battery is easiest to implement because it will not introduce additional ground loops.

- The regular tip holder for the CP has a large shaft and a set screw to tighten the metal wire (i.e., the tip). For low current STM, this tip holder was replaced with a gold-plated pin, of the type commonly used in Op Amp housings. The use of this smaller tip holder reduced the amount of unshielded metal, resulting in a much smaller background signal.

We had to try several alternatives to find the best wiring and routing for the ground connections which gave the lowest 60 Hz background signal. For example, when

**Laboratory Tip**

A clean pipette holder gives the cleanest recordings. For lowest noise, keep the pipette holder clean. Frequently rinse the holder with distilled water. If more thorough cleaning is required, briefly wash in ethanol or mild soapy water. Never use methanol or strong solvents.
Focus on Methods . . .

On-line Processing of Zone Data Files: Using KaleidaGraph with Axon Imaging Workbench

Elizabeth J. Yoder, Ph.D.
UCLA, Department of Neurology, Los Angeles, CA

Introduction

This article describes the concurrent use of KaleidaGraph with Axon Imaging Workbench for expedient on-line generation, saving, and printing of graphs of zone data acquired with Imaging Workbench. In some cases it may be difficult to distinguish the individual zone data within the Imaging Workbench graph window because the data from multiple zones overlap (see Figure 1). While the number of zones allowed in Imaging Workbench is unlimited, the number of colors used to distinguish zone data is limited to 16. KaleidaGraph, a commercially available software package, contains a “Plot Script” function which creates and archives new plots automatically. KaleidaGraph allows for easy viewing of such data, since zone data may be viewed individually or in small groups selectable by the user. KaleidaGraph can run simultaneously with Imaging Workbench, thereby allowing users to go back and forth between programs during experiments and plot zone data on-line. The “KaleidaGraph for Windows” system requirements are similar to those of Imaging Workbench. An additional 2.5 MB of hard disk space will be required for KaleidaGraph’s use.

Highly oriented pyrolithic graphite (HOPG) is a standard sample for STM imaging. Large atomically flat terraces prevail and a clean surface is easily obtained by simple cleaving. Good quality images could be obtained with a 0.4 pA set current. This is about 100 times lower than usually achievable with the built-in amplifier. Atomic resolution could only be obtained with currents ≥ 1 pA. This result does not necessarily mean that with 0.4 pA the background noise is too high. It could also mean that the tip-sample distance is simply too big to allow a higher resolution.

On the VP2, the tip is mounted on the piezo scanner tube. With the Axopatch we were able to measure currents that are induced through the scanning itself. Even though the scanner rests in a grounded housing we found that the rapid changes of the applied high voltage signals (that are used to drive the scanner) are sufficiently large to induce a capacitive current at the tip. The magnitude of this current obviously depends upon the scan rate and the scan size. For example, we found that scanning a 1 µm window at 10 Hz scan rate induced a background current of 0.15 pA, with an alternating sign between the forward and the backward scan. However, a 1 µm window is usually not scanned at a rate faster than at 1 Hz. With these more realistic settings no induced current could be detected.

In summary, we demonstrated that the Axopatch 200B can easily be connected to PSI’s scanning tunneling microscopes. The lower tunneling current limit was reduced by more than 100 times, i.e., from roughly 0.1 nA to 0.5 pA, while still using the same scan rates.

Acknowledgment

We would like to thank Dr. James Fox from Axon Instruments for encouragement and support.

References


Figure 1: Axon Imaging Workbench data may be saved as a text file, which can be directly imported into KaleidaGraph.
From Imaging Workbench to KaleidaGraph

Once the preferences have been set within each program, the transfer of data from Imaging Workbench to KaleidaGraph is straightforward. In Imaging Workbench, “Save zone averages to text file” is selected from the “Zones and Graphs” option under the Settings Menu. Data acquisition and zone selections and calculations are performed in Imaging Workbench as always. To save the data into a text file, the “save icon” in the Imaging Workbench Graph Window is clicked, as shown in Figure 1. Within KaleidaGraph, the “Import text” option under the File Menu is chosen. The “Text File Import Format” box which appears on the screen should be set as shown in Figure 2. KaleidaGraph will retain these settings once they have been entered.

Using KaleidaGraph

The expediency of KaleidaGraph stems from the use of templates and scripting. Templates for data, plots, layouts, and scripts may be customized and saved for repetitive use. Data file templates might include column titles such as “Time”, “Zone 1”, “Zone 2”, etc. Plot file templates have a number of preferences which will need to be selected for each plot type. Some of these preferences are line thickness, graph symbols and colors, labels and indicators of where drugs are added and removed, and axes characteristics. Layout file templates designate how many plots are printed per page, and the arrangement of plots on the page. Script file templates contain the Plotscript settings, which specify how the software should automatically generate, name, save, and print the plots from a data file.

Once the Imaging Workbench zone data has been imported, insure that all template files to be used are open. To use the plot template, select “template” from the Gallery Menu. To use the automated plotting function, select “Plotscript” from the Windows Menu. A sample plotscript menu is shown in Figure 3. An example of Imaging Workbench data processed in KaleidaGraph is shown in Figure 4. Both data and plot files may be exported in various formats.

Figure 2: When importing data from Imaging Workbench into KaleidaGraph, advance the “lines skipped” to the beginning of data for zone 1. Use tab-delimited import, separated by a comma, number=1. Do not select “read titles.” KaleidaGraph will retain these settings once they have been entered.

Figure 3: The Plot Script menu has a number of parameters which may be configured and saved into a script file for repeated use. “Graphic Template” designates which plot template to use. When selected, “Auto Print” will automatically print the plots as they are generated - either individually or in a layout of several plots (up to 16 plots per page). When selected, “Auto Save” will automatically save the plots as they are generated - with or without the data - using the Plot File Prefix in the file name.

Figure 4: An example of some Imaging Workbench zone data processed using KaleidaGraph.

Conclusion

While this article has focused on the use of KaleidaGraph to facilitate on-line graphing and viewing of Imaging Workbench zone data, KaleidaGraph is also well-suited for further data analysis. Standard statistical values are available from menu options. Additionally, “Formula Entry” and “Macro Calculator” features allow users to quickly perform customized analyses.

To obtain information on “KaleidaGraph for Windows”, including pricing and ordering information, please contact Synergy Software at (610) 779-0522 or visit their web site at http://www.synergy.com. To obtain further information about using KaleidaGraph with Imaging Workbench, or to see samples of KaleidaGraph templates used with Imaging Workbench data, please contact Axon Instruments Technical Support.
INTERNATIONAL TOLL-FREE NUMBERS

Belgium 11-8201
Denmark 8001-0306
Finland 9800-10039
France 05-90-1137
Germany 0130-81-0458
Israel 177-100-1504
Italy 1678-74-022
Netherlands 06-022-6850
Norway 050-12-042
Switzerland 046-05-7323
Sweden 020-795-661
United Kingdom 0800-89-1504

Worldwide sales are direct from the factory.

JAPAN

Our customers in Japan may also order from our local distributor, Inter Medical.

Phone: 052-937-7060
Fax: 052-937-5423

Toll-free fax number from Japan for direct sales and technical support from Axon Instruments is 00-66-33-800-102.

EUROPE

FOR CLINICAL AND IMAGING PRODUCTS IN EUROPE CONTACT:
European Office (except France)
Manager Director
Axon Instruments Europe GmbH
Holzdamm 40
D-20099 Hamburg
Germany
Phone: 40 2805 4979
Fax: 40 2805 4999
e-mail: info@axonet-europe.com

ALL FRENCH CUSTOMERS PLEASE CONTACT:
French Distributor
DIPS Industrie
Vecteur-Sud
70-86, Avenue de la Republique
F-92325 CHATILLON CEDEX
France
Telephone: 01 49 65 67 20
Fax: 01 49 65 67 29
e-mail: info@dipsi.com

If you would like your name added to the Axon Instruments mailing list or would like to join the pCLAMP Users Group, please photocopy this form, fill it out in print letters and fax or mail it to Axon Instruments. Or you can use our Literature Request page on our Web site at http://www.axonet.com

Check as appropriate:

☐ Add my name to your mailing list.
☐ I want to join the pCLAMP Users Group.

Axon Instruments, Inc.
1101 Chess Drive
Foster City, CA 94404
USA
Fax: +1(650)571-9500

General Information

Name _____________________________________
Department __________________________________
Institute ____________________________________
Street _______________________________________
City, State _________________ Zip _____________
Country ____________________________________
Phone ( ___ ) ______________  Fax ____________
e-mail: _____________________________________

pCLAMP Users Group only

Area of research interest ______________________
Computer brand and type (486, Pentium) __________
A/D interface (Digidata, TL-1) ______________________
pCLAMP version ______________________________

Axon Instruments, Inc.
1101 Chess Drive, Foster City, CA 94404 USA
Phone +1(650)571-9400    Fax +1(650)571-9500
e-mail: sales@axonet.com
http://www.axonet.com