New Product Announcements

Optiscan’s F900e Confocal Microscope

The F900e is a full-featured Laser Scanning system that provides high-resolution confocal fluorescence and reflected-light images. This advanced system has the unrivaled performance offered by a totally new approach to confocal design. Incorporating Optiscan’s patented fiber optic technology, the F900e is able to provide flexibility and reliability for the most challenging applications. Axon Instruments has the exclusive U.S. marketing rights for this product and will provide technical support to our customers.

Excellence in Design

A prestigious winner in the world renowned International R & D 100 Award, earlier versions of this microscope achieved recognition as “One of the 100 Most Technologically Significant New Products of the Year.” With a revolutionary approach, Optiscan’s winning technology does away with the necessary vibration-free benches and the rigidly mounted bulky hardware found on most conventional Laser Scanning Confocal Microscopes. Optiscan’s F900e incorporates a modular design philosophy. This not only allows direct access to all instrument components, but also allows for relocating the assemblies to provide an ergonomically efficient work environment.

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Optiscan’s MS-Windows™ based software follows a tradition of inherent simplicity, power and flexibility. The operating system provides fully integrated software for system control, 3D image acquisition, data analysis and reporting. Information is presented to you in logically organized MS-Windows™ dialog boxes, in most cases prompting the user with recommended values.

Designed for Performance

Optiscan’s MS-Windows™ based software follows a tradition of inherent simplicity, power and flexibility. The operating system provides fully integrated software for system control, 3D image acquisition, data analysis and reporting. Information is presented to you in logically organized MS-Windows™ dialog boxes, in most cases prompting the user with recommended values.

Powerful Workstation

A Pentium™ 133 MHz PCI local bus computer is the powerful workhorse for this confocal microscope, with two high-resolution monitors (Working screen and Live Image display) and an advanced framegrabber as standard equipment.

Microscope Control Unit

The Microscope Control Unit was designed with “Control” as the important issue. The advanced plug-in card design facilitates a once only instrument calibration that provides a drift-free instrument for continuous trouble-free operation.
New Product Announcements . . .

**Laser Launch/Detection Unit**

Multilabel imaging gives you an effective technique to distinguish and characterize structural and functional elements within tissue preparations. Two fluorescence detection channels with simultaneous acquisition are standard. An advanced photomultiplier detector (with a high quantum efficiency photocathode) provides each channel with excellent sensitivity and a high signal-to-noise ratio. An optional third transmission channel is available to cope with diverse requirements.

**Scanning Assembly**

Extremely compact, the advanced design scanning head, using high-speed crystal-locked galvanometers, scans your sample with superior precision and reproducibility. The multi-layer dielectric-coated silver mirrors with super reflective surfaces (reflectance in excess of 99.8%) provide the highest possible pixel resolution.

Come to the November Society for Neuroscience meeting and see the F900e Confocal Microscope at Optiscan’s booth #726 located adjacent to Axon’s booths 718-724. Call Axon for further details.

**Axon DigiPack 1200 for Windows**

**LOOK FOR THE DIGIPACK 1200 MAILING!**

Axon Instruments and Microcal Software have teamed up to bring you the DigiPack 1200 product bundle. Included in this powerful product bundle are AxoScope 1.0, the Digidata 1200A, and Origin 4.0.

**AxoScope 1.0**

AxoScope 1.0 is the culmination of a significant development effort at Axon Instruments and represents the first of Axon’s upcoming MS Windows™ and Windows ’95 data acquisition and control products. In AxoScope 1.0, we have taken the best features of AxoTape 2.0 and Fetchex 6.0 (from the pCLAMP suite), and your valuable suggestions, and combined them with a powerful user interface, resulting in a modern, professional research laboratory tool. The enhanced interface and features have been added without sacrificing the excellent performance of AxoTape 2.0 and Fetchex 6.0. Indeed, AxoScope is even faster than its DOS predecessors. To facilitate data file exchange, AxoScope is designed to output data in a variety of formats (including all formats used by the pCLAMP 6 DOS programs) and to read all previous and current pCLAMP and AxoTape file formats. If you have not received an AxoScope 1.0 brochure detailing the many powerful new features in AxoScope, call the Sales Department.

**Digidata 1200A**

The Digidata 1200A is the direct successor to the Digidata 1200 and features the same low noise and synchronous performance necessary for electrophysiology. It is the best 12-bit data acquisition board on the market for MS Windows™ and Windows ’95. Through the addition of large memory buffers and enhanced dual-DMA data handling capabilities, we have specifically engineered the Digidata 1200A to perform robust real-time data acquisition and control under the MS Windows environment. In addition, the Digidata 1200A now comes with complete MS Windows drivers for C/C++.
Visual Basic, and LabVIEW. Please note that the Digidata 1200A is required in order to run AxoScope. We are providing an upgrade from the Digidata 1200 to the Digidata 1200A at a substantially lower price than a new Digidata 1200A.

**Origin 4.0**

To round out this powerful data acquisition combination we are pleased to offer Microcal Software’s new Origin 4.0 technical analysis and graphing program, along with their pClamp support module. Origin 4.0 with the pClamp support module allows you to directly read Axon data files, perform statistical analyses on the data, and produce publication-quality plots.

**DigiPack 1200 Purchase and Upgrade Pathways**

To celebrate the introduction of AxoScope 1.0 and the Digidata 1200A, we are pleased to announce that the DigiPack 1200 bundle will be available at a significant savings. To show our appreciation to Axon’s loyal customers, owners of TL-1, TL-2 and Digidata 1200 systems will enjoy even further savings. Those of you who have already purchased the Digidata 1200A will automatically receive AxoScope 1.0 and the MS Windows drivers for the board at no extra charge. The following bundle and bundle upgrade prices apply:

**DigiPack 1200-1**  $3300
Includes AxoScope 1.0 acquisition software, Digidata 1200A data acquisition system, MS Windows drivers, Origin 4.0 technical analysis and presentation graphics software.

**DigiPack 1200-2**  $2900
Includes AxoScope 1.0 acquisition software, Digidata 1200A data acquisition system, MS Windows drivers.

**Digidata 1200 to DigiPack 1200-1 Upgrade**  $1300
Includes AxoScope 1.0 acquisition software, Digidata 1200A board only, MS Windows drivers, Origin 4.0 technical analysis and presentation graphics software.

**Digidata 1200 to DigiPack 1200-2 Upgrade**  $900
Includes AxoScope 1.0 acquisition software, Digidata 1200A board only, MS Windows drivers.

**TL-1/TL-2 to DigiPack 1200-1 Upgrade**  $2575*
(25% discount!)
**TL-1/TL-2 to DigiPack 1200-2 Upgrade**  $2175*
(25% discount!)

*Requires registered TL-1 or TL-2 interface serial number.

**AxonGraph 3.0**

Axon Instruments is pleased to announce the latest Macintosh and Power Macintosh data analysis software. While AxonGraph 3.0 may not look very different from version 2.0, it is full of many new features, program modules for analysis and performance enhancements.

**Performance**

You wanted Power Macintosh compatibility, and now it’s here. AxonGraph 3.0 runs in the 680X0 emulation mode on Power Macintosh computers, and is faster than the previous version 2.0 running on Quadras.

Run your custom analyses 10 to 100 times faster! Compiled analysis modules are now supported for programs written in C, Pascal or Fortran. And for faster programming, the built-in programming environment has been upgraded to run up to 30% faster.

Additional performance enhancements have been added in the form of keyboard shortcuts and display optimizations.

**New Features & Feature Enhancements**

**Artifacts?**  Now you can zero, blank or interpolate them.
**Publishing?**  Display error bars and interval bars w/data.
**Notes?**  Add multiple lines of notes to a graph.
**Fitting?**  Exponential fitting allows up to 6 exponentials.
**Slopes?**  Calculate the maximum slope with the Max Slope analysis.
**Binning?**  The Amplitude Histogram has the choice of zero-centered or zero-aligned bins.
**Large files?**  The decimate option allows you to reduce your graph file sizes.

If this AxoBits was not addressed to you . . .

and you enjoy reading AxoBits, make sure you don’t miss an issue! To receive AxoBits and other mailings from Axon Instruments, fill out the form on the back of this AxoBits and return it to Axon.
Many X-axes? Combine files with different X-axes for data with different sampling rates, such as I-V curves with different voltage protocols or dose response curves with different drug concentrations.

Programming? Many new internal procedures and functions as well as bit and byte functions.

Program Modules
- Automated mPSC detection and measurement
- Classical quantal analysis with statistical confidence measures
- Operational model analysis of dose-response data
- A vesicle release model incorporating several vesicle pools
- Current-voltage (I-V) and population spike analyses are improved

Compatibility
Make your own! If there is a particular binary data file format not currently supported by AxoGraph, you can create drop-in modules that automatically import them using several new file import commands. However, built-in supported file formats now include Igor, Chart 3.4 with tags, and space-delimited text. Also, importing text data with line feeds now runs up to 5 times faster.

AxoGraph Programming Service
How many of you have tried the programming options available with AxoGraph? If programming is not your cup of tea, help is here! Dr. John Clements, the developer of AxoGraph, is offering to share his considerable expertise in programming and AxoGraph, to help researchers customize or develop their own analyses for AxoGraph.

This service will be contracted directly with Dr. Clements. There will be a minimum base fee, with a charge of $50/hr thereafter. The preferable method of communication with Dr. Clements will be via email and ftp. Please contact Axon Instruments for further details.

**Digidata 2000 Image Lightning**

**High Performance Image Acquisition and Processing Board**

Axon Instruments proudly announces the Image Lightning, the first member of the Digidata 2000 family of ground-breaking boards for cellular imaging. The Image Lightning represents a new concept in interfaces for high-quality imaging and device control. This board’s main functions are to:

- acquire and process high-resolution analog and digital images in real time;
- store data to host computer memory at high speed over the PCI bus; and
- simultaneously perform A/D and D/A conversion and external device control with excellent precision and time resolution.

The Image Lightning replaces traditional single-function frame grabber boards with a state-of-the art, multi-purpose feature set, at a very modest price. Stop by the Axon booths (718-724) at the Society for Neurosciences meeting for a demo, or contact Axon Instruments.

**A Feature Set Unlike Any Other Image Processor's**

- Works with standard and custom analog and digital camera images at high rates, up to 40 Mixels/s.
- High resolution digitization (10 bits) and ultra-low pixel jitter (±4 ns).
- Digital camera input via an inexpensive interface module.
- Image size up to 2048 × 2048.
- Accumulates or averages images on the board for low-light imaging.
- Region of Interest (ROI) Bitmask allows transfer of image data to the host computer just for pixels that lie inside arbitrarily shaped ROIs. This saves host computer processing power, time and storage space.
- Transfers image data to host computer memory over the PCI bus at high rates, up to 80 MB/s sustained; transfer a full RS-170 image in much less than 33 ms.
- Includes a separate set of A/D and D/A Converters and Digital Input/Output lines, controlled by a 80386 microprocessor using DMA for gap-free acquisition, stimulation or device control.
- Includes a serial communications port for camera control.

**Applied Scientific Instrumentation, Inc. (ASI)**

Axon Instruments has formed relationships with companies that now offer Axon Instruments’ Cellular Imaging products. These Approved Resellers (ARs) of Axon Instruments offer significant expertise in cellular imaging and/or offer their own line of imaging-related products. Here we introduce our first AR: Applied Scientific Instrumentation, Inc., of Eugene, OR. Stop by their booth #320 at the Society for Neurosciences Meeting.

ASI offers products for low-light video microscopy and fluorescence imaging. ASI integrates imaging systems, manufactures precision x-y stages and focus/shutter controls, and offers low-light cameras from Videoscope and Dage-MTI. ASI manufactures low-cost pressure (MPPI-2) and iontophoresis (IPI-1) injectors for use with fluorescence probes.

The Polychrome I is a high speed illumination system for exciting fluorescent probes. Developed by Dr. Rainer UhI, it uses a fast galvanometer to select wavelength. It switches quickly (3 ms over the range 260-680 nm), has high power output (2-3 mW over 12 nm bandwidth) and uniform spatial output.

Applied Scientific Instrumentation, Inc.
3770 West 1st Ave., Eugene, OR 97402
(503) 485-2284, (800) 706-2284 e-mail: info_asi@rain.com
Controlled devices (e.g. wavelength changers) can be precisely synchronized to video signals or external events, overcoming large variable latencies inherent in multi-tasking operating systems such as Microsoft® Windows or Windows ’95.

A Feature Connector passes image data to a separate Image Processing Accelerator module (available in the future).

The number of frames that can be acquired quickly is limited only by the size of host computer memory.

1 MB of First-In, First-Out (FIFO) Memory holds image data to prevent its loss if the computer is momentarily too busy to receive the data.

Ideal for Applications in Neurosciences and Biology

The Digidata 2000 feature set was designed by biological scientists who wish to acquire and process high-quality images in real time while controlling external devices and acquiring waveforms, all precisely synchronized to external events such as video capture. Typical applications include ion/analyte concentration imaging with control over drug delivery systems and wavelength switchers, or concurrent electrophysiology and imaging. The board can control fast (~1-3 ms) wavelength switchers, ensuring that motor movements initiate precisely at the start of the video vertical blanking interval.

Software support will shortly be available in Axon Imaging Workbench version 2, a Windows program for ion/analyte concentration imaging. A high- and low-level driver is available in DLL form for support of other applications. The Digidata 2000 Image Lightning will be available in January 1996 for a price of $3500.

New Version of Axon Imaging Workbench
Ion Imaging for Windows

A new version of Axon Imaging Workbench (AIW) has recently implemented a number of most-wanted features requested by users interested in AIW’s common-sense, low-cost approach to ion and analyte concentration imaging for Microsoft® Windows. A few of these improvements are profiled here. Stop by the Axon booths (718-724) at the Society for Neurosciences meeting for a demo, or get in touch with Axon Instruments.

New Low-Cost PCI Bus Frame Grabber

One exciting addition is support for the DT3155, an inexpensive PCI bus frame grabber card from Data Translation. This new card digitizes standard monochrome video images with scientific-grade accuracy and stores them to host computer memory at real-time rates: 30 images/s on RS 170 or 25 images/s on CCIR. The surprisingly low board cost of $995 is possible because very little expensive image memory or processing circuitry is present; the host computer does all image processing such as averaging and ratio calculations. Optimized program code in AIW running on e.g. a 90 MHz Pentium computer allows in-host image averaging to take place in real time, and performs 4 full screen ratio images per second with display. The DT3155 dramatically reduces the costs of getting into ion imaging since board costs have previously been about $5000 or up. It can be purchased from Axon Instruments.

New Zone and Region of Interest Paradigms

AIW has always allowed use of both Regions of Interest (ROIs) and Zones. If only portions of the field of view need to be acquired, the user may define multiple ROIs. Only image data inside a ROI are processed and stored to disk, thus improving performance and decreasing disk storage. The average image intensity, ratio or ion concentration is calculated over each Zone and can be exported to a graph and a text file.

AIW 1.1 allows new shapes for ROIs and Zones. Arbitrary polygons are made up of joined straight lines that enclose an area, and are useful to enclose a cell or other irregular shape. Profiles of intensity, ratio or ion concentration can be drawn along a set of joined straight lines, and are useful to visualize e.g. [Ca^{2+}] diffusing from the periphery to the center of a cell, or along a nerve.

More Cameras and New Fast Wavelength Changers

AIW now supports selected current digital cameras of Photometrics Ltd., as well as new fast (1-3 ms) wavelength switchers such as the Olympus Corp. OSP-EXA and models from T.I.L.L. Photonics and Stanford Photonics. Support is also available for the Sutter Instrument Lambda 10-2, including the version with two coupled filter wheels.

Buffer Images to Memory

When performing episodic acquisition of images, the data can be stored at high speed to host computer memory instead of to the slower hard disk. The image data is saved to the hard disk during inter-episode times. This is useful for bursts of high-speed image acquisition.

AIW version 1.1 is a no-charge upgrade to purchasers of version 1.0. For more information on AIW’s Ion Imaging for Windows, contact Axon Instruments. Full-featured demonstration versions of AIW are available.
Q: Can your new ProCFE carbon-fiber electrodes be reused to make electrochemical recordings on a single cell or on many cells?

A: Yes, you may make multiple recordings with ProCFE electrodes, but you must carefully note any consequent degradation in their performance.

The results obtained by electrochemistry are dependent on the state of the electrode surface. However, we have found that touching the cell surface does affect an electrode’s properties, but in a predictable fashion. Below are ProCFE recordings of single vesicle contents released from adrenal chromaffin cells provided by Zhuan Zhou. Reported are peak height, the half width (t½) or speed of each event, and peak area, or total charge (Q). Let’s examine the effect on t½ first. Figure (1) shows the response of a ProCFE after touching the tip of the electrode to the cell surface two times; it resembles data obtained from new ProCFEs.

Figure 1

<table>
<thead>
<tr>
<th>peak=154 pA</th>
<th>t½=8 ms</th>
<th>charge=1.96 pC</th>
</tr>
</thead>
</table>

Figures 2 and 3 show a typical response of the ProCFE after 8 and 10 touches to the cell surface, respectively. As can be seen, the response has slowed considerably. What happens to Q? The affect on Q is relatively small because Q is determined by the integration of the event. The change with use in peak height and speed may affect your ability to resolve individual events, but the charge, and hence a measurement of vesicle content, is conserved.

If you are careful in your electrode placement, we find that each ProCFE can be used with as many as five cell experiments.

Q: I wrote a protocol using the Split-Clock feature of Clampex and I am a little confused about the Epoch duration values reported in the Parameters waveform table.

Each episode consists of 2560 samples (only one channel is recorded)
- The first sampling interval is 200 ms
- The second sampling interval is 50 ms
- The sampling rate changes after sample 1000

The protocol is as follows:

<table>
<thead>
<tr>
<th>Type</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>FirstLevel (mV)</td>
<td>0</td>
<td>-60</td>
<td>10</td>
</tr>
<tr>
<td>#Level (mV)</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>First duration (samples)</td>
<td>500</td>
<td>850</td>
<td>1000</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>100</td>
<td>111.5</td>
<td>50</td>
</tr>
</tbody>
</table>

I understand the 100 ms corresponding to the 500 samples of EpochA and the 50 ms associated with the 1000 samples of EpochC. However, I am confused by the 111.5 ms for EpochB.

The way I see things is that 500 samples of EpochB should be sampled at 200 ms/sample while the remaining 350 samples (850-500) should be sampled at 50 ms/sample. Then the duration of EpochB should be 100 + 17.5 ms = 117.5 ms and not 111.5 ms. There appears to be a 6 ms problem.

A: Your confusion arises because there are 40 initial samples that Clampex assigns to your protocol that were not chosen by you. A little noticed feature of Clampex is that ALL protocols start and end at the holding potential; regardless of what the user chooses, each episode starts and ends with a few samples at the holding potential in order that there will be some baseline data in every record. The number of samples at the beginning of the episode at the holding potential is calculated from the TOTAL SAMPLES/64 (please see pages 190-191 in the pCLAMP User's Guide) . Thus, in your protocol, with 2560 samples per episode, the first 40 samples will be at the holding potential, and then Epoch A will start (at sample 41). The sampling rate for these initial samples is the first sampling rate.

Thus, the actual table is as follows:

<table>
<thead>
<tr>
<th>Epoch</th>
<th>Initial</th>
<th>Epoch A</th>
<th>Epoch B</th>
<th>Epoch C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>40</td>
<td>500</td>
<td>850</td>
<td>1000</td>
</tr>
<tr>
<td>Rate</td>
<td>0.2 ms</td>
<td>0.2 ms</td>
<td>0.2 ms and 0.05 ms</td>
<td>0.05 ms</td>
</tr>
<tr>
<td>1st holding level</td>
<td>8 ms</td>
<td>40 samples @ 0.2 ms</td>
<td>500 samples @ 0.2 ms</td>
<td></td>
</tr>
<tr>
<td>Epoch A</td>
<td>100 ms</td>
<td>390 samples @ 0.05 ms</td>
<td>19.5 ms</td>
<td></td>
</tr>
<tr>
<td>Epoch B</td>
<td>111.5 ms</td>
<td>(460 samples @ 0.2 92 ms)</td>
<td>390 samples @ 0.05 19.5 ms)</td>
<td></td>
</tr>
<tr>
<td>Epoch C</td>
<td>50 ms</td>
<td>1000 samples @ 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Axon’s Educational Programs

We were pleased to note the considerable interest in pCLAMP shown by the number of people attending the free lectures on “Using pCLAMP 6” at past scientific meetings. These lectures will again be offered at the

Society for Neuroscience meeting
Room 11A, San Diego Convention Center
Saturday, November 11, 1995, 9 AM to 4:30 PM

Biophysical Society meeting
Baltimore, Maryland
Saturday, February 17, 9 AM to 4:30 PM

Topics to be covered will include Clampex and Clampfit in the morning, and Fetchex, Fetchan and pSTAT in the afternoon. We will also demonstrate our new Windows acquisition program, AxoScope 1.0, running under Windows ‘95. Although no prior experience with pCLAMP is assumed, even users with familiarity and experience with these programs will benefit from the lecture.

In addition, Axon Instruments will sponsor the Satellite Symposium, “Chemical Microsensor Techniques, Problems and Solutions”, at the Society for Neuroscience meeting. The symposium will be chaired by Dr. Kirk Kawagoe, an electrochemistry researcher at Axon Instruments.

The invited speakers will describe and discuss techniques for chemical microsensing of brain analytes. Dr. Zhuan Zhou (Washington U.) will discuss “Detection of Quantal Neurotransmitter Release by Amperometry”; Dr. Werner Kuhr (U.C. Riverside) will discuss “Sinusoidal Voltammetry at Enzyme-modified Microelectrodes for the Detection of Glutamate Dynamics”; Dr. James Eberwine (University of Pennsylvania) will discuss “Molecular Biology of Single Neurons”; and Dr. Joseph Justice (Emory University) will discuss “Quantitative Microdialysis Measurements in Brain”.

The symposium will be held in Room 4 of the San Diego Convention Center, on Tuesday, November 14 between 7 and 10 PM. No preregistration is required.

We welcome your comments on our lectures and symposia, and invite your suggestions for these and other activities we might offer to the public.

Sincerely,

James Fox, Ph.D.
Head, Educational Programs

Employment Opportunities at Axon

Axon Instruments is the world leader in the design and manufacture of instrumentation and software for electrophysiology and biophysics. At present, our primary product lines are voltage-clamp and patch-clamp amplifiers, signal conditioners and digitizers, and data acquisition and analysis programs. We are also developing two new and exciting product lines employing electrochemical and video imaging technologies. If you are looking for a career in industry in such areas as product development, software programming, or marketing please send us your resume c/o Personnel Department/ab17.

Distribution of Our Products

A recent incident prompts us to clarify our methods of distribution. As most of you know, Axon Instruments sells direct to its customers worldwide. We do not have any formal distribution agreements in any country except Japan. We do, however, understand that some institutions outside the United States may find it convenient to use the services of a local scientific instruments distributor. As such, Axon Instruments provides a discount to “non-official” distributors who act as facilitators for these institutions. It is, however, important to note that these relationships are really between the institution and the non-official distributor. There is no formal relationship between these non-official distributors and Axon Instruments. That is, these non-official distributors do not act as agents of Axon Instruments. Technical support continues to be provided directly from Axon Instruments to the customer in these cases, therefore it is important that even if you purchase through a non-official distributor you should return your warranty registration card directly to Axon Instruments. This information is simply a statement of our distribution philosophy and is not meant to judge the capabilities of the non-official distributors favorably or unfavorably.

When Axon Instruments establishes formal distribution relationships, as we have with Inter Medical in Japan and as we will for our imaging products, we will make sure that we publicize the names of the distributors.

Visit the Axon Instruments Booth

<table>
<thead>
<tr>
<th>The Society for Neurosciences</th>
<th>American Society for Cell Biology</th>
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<tr>
<td>San Diego, California</td>
<td>Washington, D.C.</td>
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<td>November 11-16, 1995</td>
<td>December 9-13, 1995</td>
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<td>Booths 718-724</td>
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<th>Biophysical Society</th>
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<tr>
<td>Baltimore, Maryland</td>
</tr>
<tr>
<td>February 18-22, 1996</td>
</tr>
</tbody>
</table>
Focus on Methods

Identification of Chemical Substances by Electrochemistry

R. Mark Wightman and Karin Pihel
Department of Chemistry and Neurobiology Curriculum
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-3290

Previous articles in this series have described recent advances in the measurement of secretion at the level of single vesicular events. Such measurements are made possible with a microelectrode fabricated from a single carbon fiber and placed adjacent to the cellular plasma membrane. Detection of secreted molecules is based on the chemical processes of oxidation and reduction. A potential is applied to the carbon-fiber electrode versus a reference electrode using instrumentation identical to that used in a voltage clamp experiment. If this potential is sufficient to extract (oxidize) or donate (reduce) electrons from the secreted chemical species, then a current will flow. If all the secreted species are electrolyzed then the integral of the current is directly proportional to the number of molecules detected via Faraday’s law. A number of molecules can be detected in this way. Examples that have been demonstrated include catecholamines from chromaffin cells (1), PC12 cells (2), invertebrate neurons (3), and sympathetic neurons in culture (4). The electrochemical reaction that occurs in this case is illustrated in Figure 1. Other species detected are the secretion of 5-hydroxytryptamine (serotonin) (5) and histamine from mast cells (6); and insulin from β cells isolated from human pancreas (7).

![Figure 1. The electrochemical oxidation reaction for catecholamines. For norepinephrine, R=H and for epinephrine, R=CH₃.](image1)

The technique described above (and in more detail in AxoBits 16) is referred to as an amperometric experiment. In addition to reporting the existence of individual secretion events, such experiments provide the opportunity to probe the kinetics of secretion after vesicle and cell membrane fusion, the quantity of material secreted, and the frequency and modulation of these events. However, amperometry is very unspecific with respect to the chemical nature of the species detected. Quite simply, the current could arise from any molecule that is electroactive at the potential applied to the carbon-fiber electrode. This failure to obtain chemical identity may be acceptable in a well characterized, highly purified cell preparation, but it leads to considerable uncertainty when measurements are made in heterogeneous cell cultures, tissue slices or in intact animals. However, the technique of cyclic voltammetry provides a route to chemical identification.

Cyclic voltammetry has been used by electrochemists for more than thirty years to characterize the redox behavior of compounds; detailed descriptions exist in textbooks on this subject (8). It is actually a simple technique that provides information concerning the identity and concentration of species in the region of the electrode surface. A potential waveform in the form of a triangular wave is applied to the carbon-fiber electrode and the measured current is plotted as a function of potential. Usually, the potential scan rate is several hundred volts per second in biochemical applications so that the entire scan has a duration of only a few milliseconds. For measurements of secretion, the waveform is repeated at intervals that are regularly spaced in time, as illustrated in Figure 2 with a protocol that we have used for the detection of catecholamine secretion from chromaffin cells. As reported in AxoBits 16, the experiment is quite easily done with a CyberAmp or patch clamp instrumentation.

![Figure 2. Voltage waveform applied to the carbon fiber microelectrode.](image2)

The current has two origins: the first is current that is required to charge the capacitance arising from the double layer of the electrode, and the second is the current caused by chemical redox processes occurring at the electrode surface. These are illustrated in the form of current-voltage curves, or cyclic voltammograms, in Figure 3. Panel A shows a voltammogram recorded in a physiological buffer solution which we refer to as a background voltammogram. Although the primary contributor to the current in this case is capacitance, waves can be seen superimposed on the capacitive current that arise from oxidation and reduction of functional groups on the carbon surface (9). A cyclic voltammogram in the presence of a catecholamine is shown in Panel B of Figure 3. (The voltammograms are plotted according to the sign conventions used by electroanalytical chemists: positive potentials to the left and oxidation current down.) Careful inspection shows that additional features are present in the current. These are more clearly revealed when background cyclic voltammograms are subtracted to reveal the contribution of the catecholamine (Panel C). A remarkable feature of carbon electrodes first exploited by Julian Millar is that the background is stable on the minute time scale so that the background-subtraction procedure is very reproducible (10).
The background-subtracted cyclic voltammogram contains considerable information (11). Note that no current occurs until a potential of approximately 200 mV has been reached. At this potential, the electrode has sufficient oxidizing power that conversion of the catecholamine to an o-quinone can commence, i.e., the forward reaction in Figure 1. The current increases to a peak which then decays towards the baseline. When the direction of the voltage scan is reversed, a peak-shaped current is observed which has the opposite polarity. This indicates that a reduction is occurring at the electrode surface. For catecholamines, this occurs as a result of the o-quinone, which was electrogenerated on the positive going scan, undergoing an electrochemical reduction to regenerate the original catecholamine, the reverse reaction of Figure 1. The peaks on the forward and reverse scans occur at different potentials in part because of the diffusion of the reactants and products in the solution adjacent to the electrode tip.

Several other factors affect the position of the voltammetric waves and these provide information that can aid in chemical identification. Primary among these is the standard reduction potential or \( E^0 \). Values of standard reduction potentials are compiled for a number of compounds of biological interest in reference texts. Since many compounds have similar \( E^0 \)s, this information does not provide absolute identification, but it does allow a large number of molecules to be eliminated from consideration. The peak position is also affected by compound-specific interactions of the molecule and the rate of electron transfer with the electrode surface. The ratio of the cathodic peak current to the anodic peak current provides further identifying information. For example, the product of the oxidation of ascorbate is chemically unstable, and its cyclic voltammogram does not exhibit any current on the reverse scan.

The amplitude of the current at the peak of the forward scan of the cyclic voltammogram is directly proportional to the concentration of electroactive species at the tip of the electrode. The peak current from successive scans provides information concerning the local fluctuations in concentration. This is illustrated in Figure 4 in which voltammograms were recorded while catecholamine was transiently applied to a carbon fiber electrode via iontophoresis.

Figure 4. Response to epinephrine applied onto the electrode by iontophoresis for 167 ms. The individual data points (solid circles) were obtained from the current in successive voltammograms at the potential (500 mV) where catecholamines are oxidized.

Figure 5 shows results obtained by cyclic voltammetry at the surface of a bovine chromaffin cell. Individual vesicular secretion events are clearly resolved. In addition, the cyclic voltammograms reveal that each secretion event is the release of a packet of catecholamines.

Unlike amperometry, not all of the secreted, electroactive molecules are oxidized with cyclic voltammetry. This is because the waveform is only operant for a fraction of the time, and the majority of the electrochemically generated o-quinone is restored to its original reduced form during the negative going portion of the scan and the interval between scans. The electrolysis that occurs during the positive-going portion of the triangular wave generates a local concentration gradient of catecholamine in the vicinity of the electrode tip (Figure 6). Since the concentration gradient exists only for 3.5 ms with the conditions given in Figure 2, then it only extends approximately three microns from the electrode surface at a scan rate of 800 V/s. For this reason, the electrode should be placed approximately this distance from the cell to prevent interaction of the concentration gradient with the cell. Note that cyclic voltammetry reports the local concentration of electrolyzed species while amperometry measures the flux of released substances.
In summary, cyclic voltammetry with carbon-fiber electrodes is a powerful technique for the identification of electroactive species secreted from individual cells. We have used it to distinguish norepinephrine from epinephrine secretion from chromaffin cells (13) and to simultaneously observe histamine and serotonin (6) released from mast cell vesicles. The technique sacrifices temporal resolution, however, to obtain the chemically specific information. Therefore amperometry and cyclic voltammetry should be thought of as complementary techniques to probe vesicular secretion events.

Acknowledgement. Our research in this area has been supported by the Office of Naval Research.


Combining Patch Clamp and Optical Methods in Brain Slices

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Introduction

Individually, patch clamp or optical imaging methods have much to offer when studying the functional properties of neurons. The combination of these two approaches can enhance the usefulness of each. This article explains some of the practical considerations of which one should be aware when beginning these experiments.

Design of a Patch Clamp/Imaging System

1. The Importance of Section Thickness

One must decide whether one’s experiments will be performed on thin or thick slices. Patch clamping is easier when performed on thick slices (those 300-500 µm thick) (Blanton et al., 1989). Thick slices limit optical resolution because light scattering through tissue retards and blurs optical signals. While it is possible to perform optical imaging experiments using thick slices (e.g., Muller & Connor, 1991), we have adopted the thin slice approach developed by Edwards et al. (1989). Under a compound microscope, we visualize sections whose thicknesses are on the order of 100 µm and whose neurons, therefore, are easily identifiable.
2. Microscope Considerations

The microscope is the critical interface that physically links optical and electrophysiological components. Ideally, this microscope should be equipped with both objective and condenser lenses of high numerical aperture (NA). The NA of a lens is a measure of its ability to deliver and collect light (Inoue, 1986). In order to take full advantage of the optical benefits of thin slices, a high-quality compound microscope is needed. Although a lens with high NA is optically preferable, high NA lenses must be positioned very close to the specimen; often much less than a millimeter distance for an objective with a NA of 1 or higher. This means that combining patch clamping with optical imaging forces a compromise in the choice of optical components in order to allow pipette access to the preparation.

For many applications, such as epifluorescence imaging, the optical properties of the condenser are less critical than those of the objective. In such cases, one option is to use an inverted microscope with a low NA condenser, thereby sacrificing the light-delivering ability of the condenser in return for a very large (many cm) working distance. This arrangement permits the use of very powerful objectives with high NAs, such as those in the lower half of Table 1. However, light scattering from tissue will tend to degrade the optical signals before they reach the objective.

If one uses an upright microscope to view the slice from its upper surface, the optical compromise is to trade objective NA for working distance. For imaging single neurons, the 40x water-immersion objectives listed in Table 1 are most popular; these have NAs ranging from .55-.75 but offer 1.6-3.2 mm of working distance. With this amount of working distance, the patch pipette can be placed between the objective and the slice so that it is straightforward to view a cell during patch clamping. However, the relatively low NA of these objectives often limits the resolution and light delivering ability of the optical system. Although it requires some practice to maneuver patch pipettes within the available space, this approach is used in all labs currently performing patch clamp experiments on thin slices.

Upright microscopes present several micromanipulator options:

(A) Moveable stage: Manipulator must be mounted directly on the stage. Movable stages tend to have a limited load-carrying capacity, requiring lightweight manipulators in order to prevent stage drift.

(B) Moveable upper half of microscope: Manipulator may be attached directly to the platform on which the microscope rests (usually an isolation table), posing little hazard associated with changing the focus (or even the objective) during an electrophysiological recording. However, heavy optical elements, such as video cameras resting on the moving part of the microscope, may produce drift.

(C) Nosepiece moves: An upright microscope in which only the nosepiece (objective-holding part) moves during focusing prevents the opportunities for drift present in options (A) or (B).

Imaging Applications

Using the approaches described, it is possible to image brain cells from which patch clamp recordings are being made. There are two classes of such imaging experiments: with indicator dye; and without. Both types of experiments can provide information about the morphology of the cell from which the patch clamp recording has been made. Using a dye that reports on some dynamic physiological parameter — such as membrane potential, cytoplasmic pH, or intracellular Ca levels — allows one to answer many questions about the relationship between this parameter and the electrical activity of the cell.

---

Table 1 — Specifications of the Microscope Objectives Used in Our Lab

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<th>Objective</th>
<th>Mag.</th>
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<th>WD</th>
<th>UV transmission</th>
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Abbreviations:  
Mag. = magnification  
NA = numerical aperture  
WD = working distance  
UV = ultraviolet light (364 nm)  
ICS = infinity-corrected optics

* = only used objectives are sold now; infinity corrected replacements are available
1. Fluorescence Imaging

Fluorescent dyes offer a high signal/noise ratio. An epifluorescence microscope is needed for such applications. The primary choice here is between conventional and confocal microscopes. Conventional microscopes are less expensive (typically 1/3 to 1/10 the price of a confocal) and more readily available, while confocal microscopes offer improved spatial resolution.

The choice of objective is crucial for successful fluorescence microscopy. In addition to working distance, discussed above, three other factors weigh heavily in this choice:

(A) Numerical aperture. The intensity of a fluorescence signal increases roughly in proportion to the fourth power of the NA. Furthermore, spatial resolution is higher with a higher NA. Thus, the objective with the highest practical NA is preferred for epifluorescence microscopy.

(B) Magnification. The brightness of a fluorescent object decreases in proportion to the square of how much the object is magnified. Thus, for a given NA, signals are brightest with a lower magnification objective.

(C) UV transmission. Many interesting dyes, including some of the best Ca indicators, are excited only by ultraviolet (UV) light. However, some types of glass do not transmit UV light efficiently. The UV transmission properties of several objectives are compared in Table 1. In general, lower magnification objectives transmit UV light relatively well because they have fewer UV-absorbing lenses in their optical pathway. For high magnification lenses the composition of the glass in the lenses is critical; quartz objectives are the best but are very expensive.

2. Imaging Transmitted Light

Light transmitted through an unstained specimen can be used to visualize cell shape or structures within the cell. Nomarski differential-interference or Hoffman modulation-contrast optics are often used to enhance contrast. However, light scattering limits the resolution in living brain slices. One means of reducing the deleterious effects of this scattering is to illuminate the slice with infrared light (Dodd & Ziegglansberger, 1990; Stuart, Dodd, & Sakmann, 1993).

Flash Photolysis of Caged Compounds

One powerful method of combining patch clamp and optical methods comes from experiments employing "caged" molecules. These molecules are chemically inert until they are exposed to UV light. Caged neurotransmitter analogs (acetylcholine, glutamate), intracellular messengers (Ca, cyclic AMP, cyclic GMP) and nucleotides (ATP, GTPgS) are some of these molecules now available. Caged compounds may be used in kinetic and spatial distribution studies in brain slices.

One can not regulate the delivery of compounds to a cell via patch pipette since compounds begin to diffuse into the cell when the patch is ruptured. Flash photolytic activation of caged compounds allows one to control the timing of the physiological effects of these caged compounds. With this technique, one can compare electrophysiological parameters before, during and after compounds are activated.

UV transmission properties of the optical system are of paramount importance in these experiments because caged compounds are photolyzed only by UV light. Some confocal microscopes offer UV lasers as light sources. This opens up the possibility of combining high-resolution imaging with photolysis in time scales relevant to the electrophysiology of neurons and with spatial dimensions much smaller than a single dendrite.

An Application Example

This example considers the importance of Ca ions in triggering various forms of synaptic plasticity, such as long-term synaptic potentiation or depression. When trying to elucidate the roles of Ca in regulating synaptic function, it is essential to define the temporal relationship between synaptic activity and cytoplasmic Ca levels. Currently, only by combining electrophysiological and imaging methodologies can one hope to determine what synaptic activity does to Ca levels within the relevant spatial compartments of a neuron. A specific example of this is shown in Figure 1, which illustrates measurements of Ca levels within a single postsynaptic Purkinje cell in a cerebellar slice. Part A of this figure shows the morphology of this cell as revealed by dialysis of the fluorescent Ca indicator dye, fura-2, from a patch pipette to the interior of the Purkinje cell. Part B of the figure illustrates the spatial distribution of changes in Ca levels produced by activation of a strong excitatory synaptic input, called a climbing fiber, that innervates this Purkinje cell. The rise in Ca produced by climbing fiber is transient, large-ly recovering within a few seconds (Figure 1C). Patch clamp recording from the Purkinje cell allows simultaneous measurement of the electrical responses produced by the activity of another, weaker excitatory synapse, called the parallel fiber synapse. These recordings reveal that climbing fiber activity causes a long-term depression (LTD) of transmission at the parallel fiber synapse (Figure 2A). Thus, combining imaging with patch clamp recording establishes that induction of LTD is associated with a rise in postsynaptic Ca levels, particularly within the dendritic field of the Purkinje cell.

A correlation between changes in Ca levels and a physiological response motivates the hypothesis that Ca mediates the physiological response, but this correlation is not adequate to prove that there is a causal relationship between the two events. To establish whether or not a rise in postsynaptic Ca actually mediates LTD, it is necessary to manipulate postsynaptic Ca levels and determine the consequences of these manipulations for LTD. Fortunately, the combination of optical and patch clamp methods is also well-suited for this type of experiment. Dialysis of the Ca buffer, BAPTA, into the Purkinje cell, via the patch pipette, prevents the Ca signals normally associated with climbing fiber activity and also prevents induction of LTD at the parallel fiber synapse (Figure 2B). Further, depolarizing pulses applied through the pipette elevate Ca by opening voltage-gated Ca chan-
These rises in Ca also are capable of inducing LTD (Figure 2B). Thus, these two means of manipulating postsynaptic Ca offer a reciprocal to the Ca imaging experiments described above. Taken together, these experiments demonstrate that: (1) climbing fibers elevate postsynaptic Ca; (2) preventing a climbing-fiber-mediated rise in postsynaptic Ca prevents LTD induction; and (3) elevating postsynaptic Ca induces LTD. These results formally are sufficient to establish that elevated postsynaptic Ca causes LTD. It is important to note that photolysis of "caged Ca" or "caged Ca buffers" offers still other means of combining optical and patch clamp methods in experimental efforts to probe intracellular Ca signaling pathways (Adams & Tsien, 1993).

This example of cerebellar Ca signaling, along with many other such studies underway in other labs (e.g., Alford et al., 1993), makes it clear that the combination of optical and patch clamp methods opens new avenues of inquiry into neuronal function.

Conclusions
The wedding of patch clamp and optical methods has the hallmarks of a long and successful marriage: many exciting fantasies and few practical obstacles. Some of the potential of this combination already is being realized, but the next few years promise to be especially good ones for this new approach to the study of brain physiology.

References


**pCLAMP Corner**

*How Does the Clampfit Peak Detector Work?*

It would appear that the task of locating the peak and measuring, for example, the rise and decay rate of the action potential displayed in Figure 1 would be easy. However, when this data segment is loaded into Clampfit, cursors 1 and 2 are set to bracket what is surely the peak, as shown, and Statistics Spikes is chosen, the following report is generated:

- **PEAK VALUE**: -70.662
- **TIME OF PEAK**: 80.997 ms
- **10-90% RISE TIME**: 12.768 ms
- **10-90% DECAY TIME**: 8.578 ms
- **HALF-WIDTH**: 13.566 ms
- **AREA**: -3284.074
- **MAX. L. SLOPE**: 6.902
- **TIME OF L. SLOPE**: 33.017 ms
- **MAX. R. SLOPE**: -8.415
- **TIME OF R. SLOPE**: 41.795 ms

The program cannot seem to even find the peak, no less measure anything about it. What happened? To understand what happened, we must understand a little about how the peak detection routine in Clampfit works. The peak detection algorithm searches for the sample point with the largest ABSOLUTE value between the cursors. Unfortunately, the largest absolute value between the cursors in this case occurs at the location of the #2 cursor at about -70 mV, and so the program happily reports this value for the peak amplitude, and reports the peak location at about 81 ms (the location of cursor #2). Since no real peak was found, the values for the other measured parameters are either impossible, as designated by the ********, or are wrong.

What to do? The answer is to bring the peak closer to the zero voltage level using either Analyze Operators or Analyze Expressions in Clampfit. It is not important that you perform this very accurately, just so that the absolute value of the actual peak is greater than the absolute value of the baseline. You must, of course, subtract (or add) the same value to each data segment that you wish to compare, and you must remember that value so that you can adjust the peak amplitude value. In this example we could simply add 50 mV to the data trace in Figure 1 giving the result shown in Figure 2.

If we now run the peak finding routine (Analysis, Statistics, Spikes), we are presented with the much more satisfying values:

- **PEAK VALUE**: 75.404
- **TIME OF PEAK**: 36.908 ms
- **10-90% RISE TIME**: 18.952 ms
- **10-90% DECAY TIME**: 8.578 ms
- **HALF-WIDTH**: 13.566 ms
- **AREA**: 739.473
- **MAX. L. SLOPE**: 6.902
- **TIME OF L. SLOPE**: 33.017 ms
- **MAX. R. SLOPE**: -8.415
- **TIME OF R. SLOPE**: 41.795 ms

Remember to subtract the appropriate factor from the peak value measurement, in this case 50 mV, to obtain the true peak value.
User-Submitted Software

Axon Instruments is always eager to provide researchers with the latest advances in electrophysiological analysis software. Although our commercial products such as pClamp are extremely flexible, there is no way that we can fulfill the analytical desires of everyone. Certain analytical techniques are simply too new or specialized for us to include them in our packages. We would, however, like to serve as a resource to the scientific community as a storehouse of analysis programs submitted by enterprising programmers. To this end, we have initiated a "User-Submitted Program" service. If you have written a program or utility that might be useful to electrophysiologists, we encourage you to send it to us for posting on our Web / FTP site. The procedure is simple: Send a copy of the executable program or utility to Andy Blatz at Axon via e-mail at: andyb@axonet.com or upload it to our Web / FTP site (http://www.axonet.com or FTP.axonet.com). You should copy your program into the write-only subdirectory "USERIN" found in the PUB directory. We will perform a precursory evaluation of the program and virus check and then post it onto the read-only "USERWARE" subdirectory. We will also send to the author of the program a brief agreement to sign which explains that Axon Instruments does not warrant nor will supply technical support for the submitted software, nor will we offer any payment for software.

We continue to serve our AxoBASIC customers who wish to share their programs. Look in the “AxoBASIC” subdirectory for available AxoBASIC programs. We offer:

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- synapse.bas — an acquisition and analysis of synaptic events program;
- axospike.bas — a program for the recognition and analysis of extracellular spikes.

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Orbital Stage Systems by Meridian Manufacturing, Inc.

Headstage vibration is a serious problem faced by researchers making electrical measurements from cells. Although air flotation tables isolate the microscope and micromanipulators from high frequency vibrations, these components can still undergo movement relative to one another. It is often desirable to rigidly attach micromanipulators to the microscope. The ability to move the microscope stage in the X-Y plane independent of the manipulator is also desirable.

The Orbital Stage was designed with these specific aims in mind. It provides rigid coupling between the microscope and manipulator, free movement of the microscope stage relative to the manipulator, and flexible positioning of the manipulator holder at almost any position around the optical axis. In addition, the manipulator holders of the Orbital Stage provide sufficient range of coarse motion so that high-quality manipulators with limited X-Y-Z range can often be used without additional coarse manipulators.

The Orbital Stage will accommodate headstages made by Axon Instruments and many other manufacturers. Models of the Orbital Stage are available for Nikon and Zeiss upright and inverted microscopes.

If you would like further information please contact: Lori Seabright, Meridian Manufacturing, Inc., (206) 854-9914 or e-mail address: merid@eskimo.com.
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- **Computer brand and type (286, 386, 486)**: 
- **A/D interface (Digidata, TL-1)**: 
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