New Product News

The New Digidata 1322A
Axon Instruments is proud to announce the latest improvement in the Digidata 1320 Series digitizers. These are 16-bit digitizers that interface with desktop or laptop computers via the SCSI bus. The Digidata 1320 Series all have 16 analog inputs, two analog outputs, 16 digital inputs, 16 digital outputs, and additional trigger inputs and outputs. The Digidata 1320A featured a 250 kHz maximum sampling rate and 2 mV peak-to-peak (p-p) input noise. The Digidata 1321A featured a 500 kHz maximum sampling rate and 1 mV p-p noise. The new Digidata 1322A features a 500 kHz maximum sampling rate and 2 mV p-p noise. Please note that the Digidata 1322A replaces both the 1320A and 1321A.

The Digidata 1322A is supplied with a SCSI adapter and cable. Windows users also receive the AxoScope for Windows acquisition program. The Digidata 1322A is also supported by pCLAMP 8 for Windows and AxoGraph Data Acquisition Package for the Mac. See the Axon Instruments web site for details and pricing.

Introducing the GenePix 4000B Microarray Scanner
Axon Instruments proudly announces the latest enhancement to its Genomics product line, the GenePix 4000B Microarray Scanner. In keeping with Axon Instruments commitment to innovation, the 4000B incorporates several new features that markedly enhance the flexibility of the GenePix 4000 product line.

User selectable scanning resolutions of 5 or 10 μm/pixel are available. A 100 μm feature scanned at 10 μm/pixel resolution contains approximately 78 pixels, which is a sufficient sampling size for reliable intensity measure-
ments. Analysis of features (DNA spots) smaller than 100 nm in diameter may be improved by scanning at 5 mm/pixel resolution. The increased resolution will also facilitate analysis of more densely packed microarrays, allowing closer microarray printing and, therefore, increased throughput. The scan time at the 5 mm resolution setting is about twice as long as that of 10 mm/pixel setting and leads to a four-fold increase in file size. The user must determine whether the sample and experiment justify this increase.*

* For features larger than 100 µm, the increased sampling size has a negligible effect on improving statistical analysis and is unlikely to justify the increase in file size.

**Marketing and Scientific Applications Staff Grows**

Dr. Shawn Handran recently joined the Marketing and Scientific Applications department of Axon Instruments. He has a diverse background in cellular imaging techniques including vital dye probes for intracellular calcium and mitochondrial membrane potential, confocal three-dimensional reconstruction of mitochondrial structure in living cells and Fluorescence Resonance Energy Transfer (FRET). His experiments focused on the pathophysiology of various neurological diseases. During his doctoral work at Washington University he developed a method for studying an in vitro stroke model. As a postdoctoral fellow in the laboratory of Dr. Anne B. Young at the Massachusetts General Hospital, Harvard Medical School, Shawn developed fluorescence-based assays using two different GFP variants to screen for neuroprotective agents in the treatment of Huntington’s disease. Two manuscripts are now in preparation that will describe his findings. Two of his most recent publications are:


The second new feature in the GenePix 4000B is the ability to adjust the focal plane of the excitation laser beam. This dramatically increases the flexibility of the 4000B in analyzing a variety of specimens such as slides that have a cover slip, or atypically thick specimens. In addition, this operation can be performed during the scan so that the optimal plane of focus can be identified.

Finally, the GenePix 4000B has the ability to attenuate excitation laser power of either laser, with two attenuation choices (thirty and ten percent) available for each. This allows for less light exposure to the specimen while focus adjustments are made, or for reducing photobleaching of slides that are dimly fluorescent or to be scanned multiple times.

We’re excited to offer the GenePix 4000B Microarray Scanner, which includes three licensed copies of GenePix Pro (our powerful acquisition and analysis software) and expert technical support. In addition, we offer seven different lasers for custom configuration in order to meet the burgeoning demands of microarray and high-throughput screening experimentation. We think you’ll agree that the GenePix 4000 series is setting the standard in the Genomics industry and hope that you will consider Axon Instruments for your complete Genomics solution. Please write or call us if you would like more information about the GenePix 4000B Microarray Scanner or GenePix Pro software.
Q: At the beginning of a patch-clamp experiment, before forming a G\(\text{W}\) seal, electrophysiologists typically remove voltage offsets due to the electrodes by zeroing the current in V-clamp mode. One of the offsets arises from liquid-liquid junction potentials that form between bath and pipette solutions due to differing ion mobilities in the two solutions. After a G\(\text{W}\) seal is formed, the voltage offset from the liquid-liquid junction potential disappears but the correction remains. How do I adjust my results, recorded in V-clamp and I-clamp mode, to compensate for this correction?

A: In Axopatch amplifiers, current through the pipette is measured in the headstage with a current-to-voltage converter operational amplifier (“op amp”) configuration. The signal is connected to the inverting input of the op amp and command voltages are introduced at the non-inverting input. With the pipette in the bath and a command voltage of 0 mV, the liquid-liquid junction potential causes current to flow from the output of the op amp through the op amp feedback resistor and pipette to ground. Introducing a command voltage equal to the liquid-liquid junction potential decreases the current to zero. In V-clamp mode, the junction potential correction is not included in the \(V_m\) signal (displayed on the front panel meter or read from the BNC 10V_m output) even though it is summed with other voltage commands applied to the membrane. After G\(\text{W}\) seal formation, the liquid-liquid junction potential is believed to dissipate fairly rapidly, even for whole cell recordings of medium-sized and smaller cells. If a liquid-liquid junction potential of \(-10\) mV is compensated before seal formation and a command voltage of \(-60\) mV is applied to the inside of the membrane, as in whole cell and outside-out patch configurations, the actual membrane potential will be \(-70\) mV. The formula for calculating the voltage applied to the membrane is: \(V_{\text{mem}}\) (actual membrane potential) = \(V_m\) (applied voltage) + \(V_{\text{jp}}\) (junction potential correction). For cell-attached and inside-out configurations, voltage commands are applied to the opposite side of the membrane. To make the membrane potential equal to \(-60\) mV, it is necessary to apply \(+60\) mV. If a liquid-liquid junction potential of \(-10\) mV is initially compensated, the actual voltage applied to the membrane is \(+50\) mV and the actual membrane potential \((V_{\text{mem}})\) is \(-50\) mV. Thus, to achieve a \(V_{\text{mem}}\) of \(-60\) mV, the applied voltage \((V_m)\) must be \(+70\) mV.

Axon Supports Scientific Training

The commitment of Axon Instruments to academic research and training is an understandable goal, considering that Axon was born out of the very same community in need of quality instrumentation and software to foster scientific discovery. Our continued support is recognized annually in courses offered by renowned institutes such as Cold Spring Harbor Laboratory (CSHL) and Marine Biological Laboratory (MBL), where students perfect their research skills under the guidance of distinguished instructors.

This summer, CSHL and MBL each held several scientific training courses over a broad range of subjects, including developmental biology, genomics, and neurobiology. An international assembly of participants learned or perfected techniques in electrophysiology, imaging and microarray technology. Courses such as the “Physiological Approaches To Ion Channel Biology” at CSHL, as well as the “Neural Systems and Behavior” at MBL relied on Axon amplifiers, analog-to-digital converters and pCLAMP software for data acquisition.

A tremendous effort is required by all those involved to organize and equip such excellent training laboratories. Axon would like to salute course directors, lecturers, assistants and all participants for their efforts to advance scientific research through these training programs. We are proud to provide the best instrumentation and software available for these endeavors.
When recording in I-clamp mode with no current command or in I=0 mode, the actual resting membrane potential of the cell can be calculated from \( V_m \) as follows: 

\[
V_{\text{mem}} = V_m + V_{j0}
\]

(\( V_m \) is the sum of the measured voltage response, a component from the Series Resistance compensation circuit, and the pipette offset (junction null) correction (which is summed with a negative polarity or, in other words, subtracted). Therefore, in I-clamp whole cell recordings, if the correction for the liquid-liquid junction potential is -10 mV and the measured \( V_m \) is -50 mV, the actual resting membrane potential (\( V_{\text{mem}} \)) is -60 mV.

There are several good discussions of this topic in the literature, some of which are listed below. The polarity of the liquid junction potential compensation is determined not only by the intracellular and extracellular solutions used, but also by the patch configuration. A particularly good reference by Neher (1992) in Methods in Enzymology 207:123-131 gives equations for the different patch clamp configurations and outlines how best to measure liquid-liquid junction potentials for this purpose. Junction potentials can also be calculated using the Junction Potential Calculator in Clampex versions 7 and 8.

Other good references include:

For more details please see the following web site:
http://134.197.54.225department/kenyon/Junction_Potentials/primer.htm

**Q:** I get an Internet Explorer Script Error message stating “Library not registered” when I attempt to run a script in the Reports tab of GenePix Pro 3.0. I am using the Windows 2000 operating system and version 5 of Internet Explorer. Is there something wrong with GenePix Pro?

**A:** The Internet Explorer (IE) script error is due to a security characteristic of the Windows 2000 operating system. A user must have *Administrator* level privileges to install or upgrade GenePix Pro software, and in order to run IE scripts from the Report Tab of GenePix Pro, the user must be assigned *Administrator* or *PowerUser* level access. This is easily accomplished in the Control Panel settings for *Users and Passwords*. There is a Microsoft Wizard that guides you through a two-step process to add new users; the second page of the Wizard will prompt you for the level of access (note that *Standard Access* is the PowerUser level). PowerUser profiles must be set up in the *Users and Passwords* control panel for all users who wish to run scripts in GenePix Pro 3.0 (a simple solution is to make one generic user profile and password that anyone can use), but has to be performed by an Administrator level user. If your computer is part of a domain, Windows 2000 does allow any user with access to the domain to log into the local computer, however, unless the user has been specifically added to the PowerUser group, they will be assigned lower level access privileges and the IE scripting function will not work. Such a user will then have to contact the Administrator of the computer in question in order to be assigned to the PowerUser group, and once that has been accomplished, the script functionality in GenePix Pro 3.0 will operate normally.

**Q:** My experiments employ a non-ratiometric indicator, and I want to calculate \( \Delta F/F_0 \). Imaging Workbench 4 appears to support only \( \Delta F \). Is there a way to calculate \( \Delta F/F_0 \)?

**A:** Although \( \Delta F/F_0 \) cannot be calculated on-line with Imaging Workbench 4.0, it can be calculated off-line. During image acquisition, turn on the *Delta F Subtraction* feature in Acquire/Edit Protocol to compute the difference of the subsequent images relative to the first, \( \Delta F \). The workaround of calculating \( \Delta F/F_0 \) employs the Shading Correction, which is used to compensate for differences in brightness across the field of view due to factors such as uneven illumination. If the correction is used, each acquired image is divided by a shading reference image captured from a uniformly fluorescent sample. If \( F_0 \) is used as the *shading reference image*, then \( DF/F_0 \) images, corrected for shading, are actually \( DF/F_0 \) images. Note that when \( DF/F_0 \) is calculated, an actual shading correction is unnecessary since \( DF \) and \( F_0 \) have the same shading reference image, which is canceled in their ratio equation.

Here are the step-by-step instructions for calculating \( DF/F_0 \). During acquisition, turn on the *Delta F Subtraction* feature in Acquire/Edit Protocol. After acquisition, open the image file. In Playback/Settings, select *Enable Shading Correction* and *Use acquisition image*. In Acquire/Shading Correction, check the *Enable Shading Correction* box. Select *From File in Image to Use*. Click on *Browse* to view the all files, highlight the file to be analyzed and select the F(0) image in the list of images. The *Delta F Shading Corrected* image in the Playback window represents \( DF/F_0 \).
**Detection and Analysis of Individual RNA or DNA Molecules Using an Ion Channel**

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**Introduction**

Earlier this year, government and industry scientists announced completion of a rough draft of the human genome. Virtually all of the nucleotides sequenced in this endeavor were acquired using the dideoxy sequencing method first described by Sanger and colleagues. An essential component of this sequencing strategy is the electrophoretic separation of single stranded DNA molecules of different length as they migrate through pores in polyacrylamide slab gels or capillaries. A single fluorescent or radioactive band in a typical 6% gel represents a very large number of single-stranded DNA molecules each of which migrated through roughly $1 \cdot 10^7$ pores during electrophoresis.

In a landmark paper, Kasianowicz et al. showed that conventional patch clamp instrumentation could be used to rapidly detect electrophoresis of one DNA or RNA molecule through one nanometer-scale pore. The pore they used is formed by α-hemolysin, a 33 kD, 293 amino acid protein isolated from *Staphylococcus aureus*. Seven α-hemolysin monomers self-assemble in a lipid bilayer to create a channel whose pore has a limiting aperture of 1.5 nm. In 1 M KCl at pH 7.5-8.0, this channel adopts a steady open conformation with a monovalent ion current of about 120 pA (120 mV at 22 °C). Capture of single-stranded polynucleotides substantially reduces this channel current with translocation times that are proportional to strand length. Recently we built upon this early work and showed that current blockades caused by homopolymers of polycytidylic acid (poly C), polyadenylic acid (poly A), and polyuridylic acid (poly U) are readily distinguished from one-another based on blockade amplitude or blockade kinetics. This note is in part a synopsis of that article.

**Experimental**

Our experiments are performed using an apparatus (Figure 1) that combines the advantages of a conical aperture with those of a horizontal bilayer. Bilayers formed across the 20 nm aperture of this device exhibit high resistance (>200 GΩ), low noise (< 0.6 pA rms at 5 kHz bandwidth in whole cell mode, 0.2 pA rms in patch mode using an Axopatch 200B amplifier), and are stable for many hours at applied voltages up to 240 mV. In addition, the bath can be tens of microliters in volume permitting analysis of small amounts of RNA or DNA. To form a bilayer, the Teflon aperture is coated by applying 1.5 ml of a 200 u/ml solution of diphytanoyl phosphatidylcholine in spectroscopy grade hexane which is then evaporated using a light stream of air. The chamber on both sides of the aperture is filled with 70 ml of buffer composed of 1.0M KCl, 5-10 mM HEPES/KOH at pH 7.5-8.0. A single one-centimeter-long bristle of a 000 brush is dipped into a 3 mg/ml diphytanoyl phosphatidylcholine/hexadecane bilayer is formed across this aperture, and one alpha hemolysin channel is inserted into the bilayer. At 120 mV, monovalent ion current through the pore is about 120 pA (center). When a single-stranded polynucleotide is captured and then traverses the pore, the ionic current decreases abruptly (right). Reprinted with permission from the Biophysical Society.

![Figure 1. Horizontal bilayer apparatus. A U-shaped Teflon tube connects two 70 microliter baths milled into a Teflon support (left). The baths and the Teflon tube are filled with 1 M KCl buffer and are connected to an Axopatch 200B amplifier by Ag/AgCl electrodes. One end of the Teflon patch tube has a conical tip that narrows abruptly to a 20 micrometer aperture. A Diphytanoyl phosphatidylcholine/hexadecane bilayer is formed across this aperture, and one alpha hemolysin channel is inserted into the bilayer. At 120 mV, monovalent ion current through the pore is about 120 pA (center). When a single-stranded polynucleotide is captured and then traverses the pore, the ionic current decreases abruptly (right). Reprinted with permission from the Biophysical Society.](image-url)
bilayer is formed, 20 ng of α-hemolysin (Calbiochem, La Jolla, CA) is added to the cis side of the bilayer and 120 mV potential is applied, cis side negative. After a few minutes, insertion of the assembled toxin heptamer is aided by passing an air bubble repeatedly across the bilayer. Although anomalous channels are common, a single stable α-hemolysin channel with the requisite conductance can be inserted within 30 minutes, after which the excess α-hemolysin is flushed from the system by perfusion with 3.0 ml of fresh buffer.

In our setup, ionic current flux through the α-hemolysin channel is measured and recorded using an Axopatch 200B integrating patch clamp amplifier (Axon Instruments, Foster City, CA) in voltage clamp mode. Data are acquired at 10 ms intervals in the whole cell configuration and filtered at 10 kHz using a low-pass bessel filter, or at 3 ms intervals and filtered at 100 kHz. To date, our published data have been analyzed using Fetchan (Axon Instruments, Foster City, CA), however customized software coupling a finite state automaton with Support Vector Machine analysis will be reported shortly (Winters et al., manuscript in preparation).

Typical results comparing blockades caused by RNA homopolymers are shown in Figure 2. Poly C reduced the channel current significantly more than did poly A or poly U (ca. 95% and 91% blockades vs ca. 85% blockades, respectively). Although the current amplitudes of poly U blockades (18±3 pA residual current) and poly A blockades (17±1 pA residual current) were practically indistinguishable, the shorter durations of the poly U blockades were clearly distinct from those of poly A. For example, when polymers of essentially equal length were compared (175±50nt poly A and 150±50nt poly U), poly A blockades averaged 16 ms/nt at 22 °C while poly U blockade durations were centered at 1.4 ms/nt and 6 ms/nt11 (Figure 2).

The differences between these homopolymers can be explained by secondary structure. We argued that poly C causes a deeper blockade than do poly U or poly A because it exists primarily as a single-stranded, 1.3 nm in diameter helix at room temperature and neutral pH8,16, 3 that is narrow enough to traverse the α-hemolysin pore. Entry of this helix into the pore would cause greater blockage than an extended chain of nucleotides. This explanation is supported by the data in Figure 3 where we compared blockades caused by poly C with those caused by poly dC. Under the conditions of this study, the helical conformation of single-stranded poly dC is less stable than that of poly C1 resulting in 89% and 86% current blockades (similar to poly U). For the same α-hemolysin channel, poly C (130±15 nt) caused 95% and 91% as per usual.

Unlike poly C, poly U lacks ordered local structure6,7,10. Thus, the 85% blockade amplitudes characteristic of poly U most likely represent capture and translocation of a disordered, RNA random coil (Figure 4).

The slow traversal characteristic of poly A (average 16 msec/nucleotide at 22 °C) is not consistent with an adenosine-specific effect because poly-deoxyadenylic acid 100mers traverse the pore at an average rate of 3 ms/nt13. We postulate that the slow traversal of poly A can be accounted for by its helical structure at room temperature and neutral pH11,12,4, but unlike the narrow,
1.3 nm poly C helix, the poly A helix is believed to have a diameter of about 2.1 nm, with 7-to-50 nt segments of helix interspersed among unstacked segments. The 2.1 nm diameter is small enough to enter the α-hemolysin vestibule (limiting aperture ca. 2.6 nm, but too wide to traverse the 1.5 nm limiting aperture in the neck of the α-hemolysin channel (Figure 4). Thus, helical segments along a poly A strand must be partially unwound and extended to permit translocation through the channel pore. This accounts for both the slow traversal rate and 85% blockade amplitude characteristic of poly A.

![Figure 3](image-url)

**Figure 3.** Comparison between poly C and poly dC current blockades. The poly C was a 130±20 nt nominal-length strand purified by PAGE; the poly dC was a synthetic 100 mer purified by PAGE. a) Scatter plot in which each point corresponds to the amplitude and duration of a current blockade caused by a single polynucleotide passing through the α-hemolysin pore. b-c) Typical blockades caused by the poly dC and poly C molecules used in (a) above. The data in these experiments were digitally filtered at 50 kHz. Quiescent periods between events were spliced out so that numerous blockades could be presented in ‘b’ and ‘c’. Reprinted with permission from the Biophysical Society.

**Conclusion**

We conclude that nanometer scale pores coupled with conventional patch clamp instrumentation can be used to capture and analyze individual polynucleotide molecules. Our laboratory and others are now developing solid state pores, detectors, and software with the aim of analyzing individual DNA strands with greater precision.

![Figure 4](image-url)

**Figure 4.** Model of RNA homopolymer conformers that are captured and translocated across the α-hemolysin pore. Reprinted with permission from the Biophysical Society.

**References**


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