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A Message from the Director of Microarray Technology

GenePix Pro 4.0…GenePix Personal 4100A …Acuity 1.1—this has been a busy and exciting quarter for the Axon Genomics team!

GenePix Pro software is becoming the preferred standard for microarray analysis. So much so that other suppliers of microarray tools are modifying their products to accommodate GenePix Results (GPR) and GenePix Array List (GAL) file formats. We released version 4.0 in March, and we're already putting finishing touches on version 4.1.

Version 4.1 will control the newest addition to our GenePix microarray scanner product line—the GenePix Personal 4100A. Axon's top-quality engineers have built this scanner to the same exacting performance standards as the popular GenePix 4000B, keeping its cost low by providing a basic essential feature set. Especially considering last year's economic slump, we hope this very affordable scanner will make microarray technology available to more investigators for a wider range of applications. See the announcement in this issue for more details.

Acuity 1.1 is the second release of our brand-new bioinformatics software for data storage, analysis and visualization. Axon engineers developed Acuity during several years of close consultation with recognized microarray scientific leaders and inventors of gene expression profiling methods. It is extremely flexible and powerful, and we expect Acuity to follow in the footsteps of GenePix Pro to become another microarray analysis standard.

So it's been a busy quarter, but the year has only just begun. We have a long list of new features for Acuity. You'll see some of these soon in version 1.2, and more of them with each of several Acuity releases planned throughout the year. GenePix hardware engineers are also busy with new ideas for future scanners. And Axon is always seeking new opportunities for collaboration in genomics, cellular neurosciences, and high-throughput screening. With Axon's strong history in neurosciences, and our more recent expansion into microarray technology, applications that span both genomics and cell-based technologies are especially exciting to us. We'd be happy to discuss any of your ideas, and hear about how we can tailor our products to better meet your needs.

Siobhan Pickett
Director, Microarray Technology
Axon Instruments announces the release of the GenePix Personal 4100A microarray scanner.

The modest price and benchtop footprint of the GenePix Personal microarray scanner belies the power under its hood. This scanner has the sensitivity, throughput, reliability and flexibility that you absolutely require for your microarray-based research, be it in genomics, proteomics or drug discovery. The GenePix Personal microarray scanner includes an eight-position filter wheel. Built on Axon’s industry-leading optical, electronic and software design, the GenePix Personal microarray scanner has all the quality and ease-of-use features found in more expensive scanners but at a more affordable price. This even includes eight user-selected emission interference filters, allowing one to explore alternative dyes.

How is all of this possible? The sequential scanning mode incorporated in the GenePix Personal scanner allowed Axon to reduce the development and manufacturing cost without sacrificing the superb quality that you’ve come to expect from Axon.

The GenePix Personal microarray scanner has comparable repeatability, sensitivity, and linear range to the GenePix 4000B. You need only ask your colleagues about the outstanding industry-leading quality of the GenePix 4000B to appreciate the significance of this feat.

Finally, there is a scanner perfect for individual lab use. Achieve your personal best with the GenePix Personal microarray scanner!

**Acuity 1.1**

**High Performance Microarray Informatics Software for Windows**

Axon Instruments is pleased to announce the release of Acuity 1.1, Axon’s flexible and powerful genomic and proteomic expression analysis program that has been optimized for Windows with performance, speed and scalability in mind. Acuity securely stores your data, mines this data for the answers to your advanced questions, and provides you with sophisticated 2-D and 3-D visualizations that help you track down elusive correlations in your data.

Acuity continues to evolve by providing the most significant new bioinformatics tools for mining genomic and proteomic data.

**New features in Acuity 1.1 include:**

**PDF Export of all visualizations** - Easily create publication-quality graphics of all your Acuity visualizations.

**Dendrograms can be sorted by Self-Organizing Map order** - The order of branches in a dendrogram is arbitrary. Use the ordering produced by a Self-Organizing Map analysis to order the branches and reveal hidden structure in the data.

**Batch Normalization** - With one click of a button, Acuity can normalize thousands of imported microarrays at once.

**More flexible dataset creation with column logic** - New enhancements in Acuity provides more extensive control over data set creation, so that only spots passing strict quality control criteria can be analyzed.

**Web links** - Like GenePix Pro, Acuity can be used to submit substance names or IDs directly to web-based databases, or browse online databases of pathway maps such as the Kyoto Encyclopedia of Genes and Genomes (KEGG).

**Data type management tools** - New data management tools strengthen the availability and integrity of your data in microarray databases.

**View multiple stages of data analysis alongside a complete history of analyses** - Acuity allows you to discover hidden relationships in your data by seamlessly moving between different stages of data analysis: from microarray images, to raw data, and higher level analysis such as hierarchical and non-hierarchical clustering or principal components analysis.
Introduction

Modern drug discovery and development programs require rapid analysis of newly synthesized compounds. Hundreds, often thousands of compounds need to be screened in an effort to identify ideal pharmacological candidates. This can only be accomplished efficiently with the use of high throughput techniques.

Ion channels represent therapeutic targets for which a variety of non-mechanistic, high-throughput assays currently exist. These include the use of flux assays, radiolabeled probes, binding assays, and fluorescent-based methods of detecting changes in membrane potential or ion concentration. Since these methods measure ion channel activity indirectly, secondary assays must be invoked to confirm “hits.” Ultimately, to obtain the highest quality information about drug-ion channel interaction, the traditional patch-clamp technique is utilized. This technique is the gold standard for determining ion channel function at the single-cell or single-channel level. It allows for the direct measurement of current carried by ions through channels in the membrane while simultaneously controlling the membrane potential. Traditional patch clamping is anything but high throughput. At best, a seasoned patch clamper can make measurements from 10 to 15 cells per day.

A promising solution to this bottleneck would be the development of an automated chip-based patch-clamping device, whereby many cells are recorded in parallel until such a product becomes commercially available, an immediate way to achieve an increase in throughput, without sacrificing data quality, is to record from two cells simultaneously. The dual channel MultiClamp 700A patch-clamp amplifier is ideally suited for such experiments. It combines all the features of two microelectrode patch-clamp amplifiers into one unit. Data can be acquired in parallel from two individual cells, effectively doubling throughput.

Methods

In our setup, each of the MultiClamp 700A headstages (MultiClamp 700A amplifier, Axon Instruments, Inc., Union City, CA) was mounted to a piezoelectric micromanipulator that was, in turn, mounted to the microscope stage. The manipulators were placed on opposite sides of the microscope. This configuration provided the greatest amount of functional working space around the microscope stage. The bath was grounded by connecting a reference (ground) electrode to one headstage (either Channel 1 or 2, Figure 1).

There was no need to connect a reference electrode to both headstages, since grounds are shared in the MultiClamp 700A. This setup produced noticeably less channel-to-channel artifacts than similar experiments performed with two separate amplifiers.

Two separate cells on the same culture dish were patch clamped simultaneously. The two cells were within close proximity (<200 µM) but did not physically border each other. Any electrophysiologist will recall the time when they first learned to patch clamp a cell. Some of those awkward moments are likely to be encountered again when learning to do double patch-clamp experiments. Our routine was to first locate two cells in the culture dish. The electrodes were then placed within close proximity of each cell, GΩ seals were formed on the cell membranes and light suction was applied to rupture the membrane under the seals.

All electrophysiological recordings were performed in the whole-cell patch-clamp configuration at room temperature (23 °C). Pipette solution contained (in mM) 115 K-gluconate, 15 KCl, 2 MgCl₂, 11 EGTA, 5 K₂ATP, 10 HEPES with pH adjusted to 7.25 and an osmolarity of 292. Bath solution contained (in mM) 149 NaCl, 3.25 KCl, 2 CaCl₂, 1 MgCl₂, 11 D-glucose, 10 HEPES.
with pH adjusted to 7.32 and an osmolarity of 331. Patch pipettes were fabricated from N51A glass (Garner Glass, Clairmont, CA), coated with silicone elastomer (Sylgard 184, Dow Corning, Midland, MI) and fire polished. Pipette resistance varied from 1 MΩ to 3 MΩ. Capacitive transients were neutralized electronically, and series resistance compensation was used at 80 to 90%. Membrane currents were filtered at 2 kHz. Data were acquired using Clampex 8 software through a Digidata 1322A and analyzed using Clampfit 8 (Axon Instruments, Inc.). An inhibition curve was created and fit to the Hill equation with a base line: \[ I = \frac{(100-b)}{(1+(x/IC_{50})^n)} + b \], where \( x \) is the compound concentration, \( b \) is the maximal inhibition level and \( n \) is the slope factor.

A cell line stably transfected with a cationic channel of interest was used in these experiments. The day prior to use, cells were dissociated using enzymatic digestion and mechanical trituration and plated at a density of 40,000 cells/ml onto 12 mm coverslips coated with poly-D-lysine. Cells were visualized by phase-contrast optics. A programmable, computer-controlled perfusion system was used to exchange extracellular solutions.

Results and Discussion

Using the MultiClamp 700A and the MultiClamp Commander to perform dual channel recordings offers several advantages over the use of two separate patch-clamp amplifiers. The MultiClamp Commander provides access to all the amplifier features in one convenient location, making it easier to control and keep track of the activities in both channels. We found that the automatic series resistance and capacitance compensation features were handy and freed up valuable time for experiments.

Figure 2 illustrates the experimental protocol used to measure the dose-dependence relationship. After a whole-cell recording was achieved, the cells were clamped at a holding potential of -80 mV and were continuously perfused with bath solution. Outward cationic currents were evoked by membrane potential steps to +40 mV for 100 ms. This stimulus was repeated at 0.1 Hz throughout the experiment on both headstage channels. A stable 5-minute baseline was established initially. This was followed by the addition of increasing concentrations of a compound. Each concentration was applied for 5 minutes. Finally, there was a 5 to 10 minute recovery period during which the compound was washed out of the bathing solution. The percent inhibition of the peak control current was determined at each concentration. In Figure 3, the percent inhibition of control current was plotted as a function of antagonist concentration and the IC_{50} of the compound was calculated from the curve fit.

It should be noted that not every dual-patch experiment could be carried to completion with both cells intact, due to differences in cell integrity. However, the loss of one cell does not hinder the collection of data from the other cell, which is extremely important for the continuity of experiments.

The experiments described here typically took ~1 hour to complete. Since two separate dual patch-clamp experiments (n=3 to 4) provide sufficient data for generating an accurate IC_{50} curve for a compound, 2 to 3 compounds could be analyzed on a daily basis. This is a significant difference especially when measured over the long run. Consider that in one month a person working 4 days a week using the dual patch-clamp technique can screen ~40 compounds vs. ~20 compounds patch-clamping a single cell at a time. While such productivity is still too low to serve as a primary screening method for drug discovery, it is an effective way to increase throughput using a single amplifier.

References


Exporting Graphics in GenePix Pro
GenePix Pro features several powerful graphical functions for generating images or graphs of publication quality. This technical note describes the various options available in GenePix Pro for optimal export of images, histograms, scatter plots and reports.

Image File Export
GenePix Pro exports ratio images in 24-bit color JPEG format. The color look-up table and contrast settings are preserved as viewed on the computer monitor. Color JPEG files are considerably smaller in size than TIFF files yet retain nearly identical color content and image resolution, making them perfect for use in third-party presentation programs. The smaller size of JPEG files also makes them more amenable to send to collaborators as an email attachment. Preview scan images are also exportable in color JPEG format. Note that GenePix Pro does not import color JPEG files, nor can you “re-import” JPEG images that were exported by GenePix Pro. Individual 635 nm and 532 nm grayscale images can and should be saved as TIFF, but can also be exported in JPEG format. However, since pixel intensity values can be lost or changed with JPEG compression, JPEG export should not be used for archiving image data.

Clipboard Export Options
Exchange of graphics via the Windows clipboard is available from all tabs (Image, Histogram, Lab Book, Results, Scatter Plot) except the Reports tab, with a click of the Copy button (see left) or by pressing Ctrl-C.

The content can be copied into any other Windows programs that support the Paste Special operation. The following clipboard formats are supported:

Picture and Picture (Enhanced Metafile)
“Picture” refers to the original metafile format, whereas “Picture (Enhanced Metafile)” refers to modifications and enhancements made to the original metafile standard. Picture is generally superior for images and Picture (Enhanced Metafile) for graphs. Either format can be used to create a vector-based object of a GenePix Histogram or Scatter Plot. Note that Scatter Plots containing large numbers of data points (e.g., 10,000) become extremely memory-consumptive when converting a graph into a vector object.

Bitmap and Device Independent Bitmap
Converts an image from the Image tab of GenePix Pro into a Windows-standard bitmap file. This format is larger (in byte size) than the Picture format.

Context-sensitive Data Export (Text format)
A powerful but less well-known function of GenePix Pro is the ability to directly export the actual x- and y-graph data in text format. This is useful for users who wish to use a third-party graphing program. This function is available for the Histogram or Scatter Plot in GenePix Pro; simply copy the graph to the clipboard and choose “Text” in the Paste Special options in the spreadsheet or graphing program of your choice.

Generating Portable Document Format (PDF) of Graphics
Included free with GenePix Pro version 4.0 is the PDF-generating utility program FinePrint pdfFactory. It emulates a printer and generates a PDF file from any Windows Print function. Printing is available from all GenePix Pro tabs in version 4.1 or later. In earlier versions of GenePix Pro, Reports can be printed by exporting the report in HTML format and opening it in your web browser. From there, the report can be converted to PDF via the print function.

Graphs or images can be seamlessly imported into other Adobe products such as Illustrator via PDF as well.

Using the GP-7 and GP-8 Calibration Slides:
With the release of GenePix Pro 4.0, Axon Instruments introduces a new procedure and a new set of calibration slides, the GP-7 (red) and GP-8 (green). Having two separate slides allows for a more precise calibration of the red and green channels. These slides are required for the new calibration procedure.

During calibration, the PMT voltages are adjusted such that the intensities read from the slides approach the benchmark values established at the factory for each channel.

Axon Instruments recommends performing calibrations the first time you use your scanner and on a monthly basis thereafter. To perform a calibration with the GP-7 (red) and GP-8 (green) slides, open GenePix Pro 4 and click on the Hardware Diagnostics button. In the dialog box that appears, click on Calibrate System and follow the prompts. Calibration can be used to restore a single scanner to benchmark performance, or to match the signal output from multiple scanners. Calibration can be removed at any time if the need arises. Please consult the GenePix Pro 4 Manual and on-line Help for more information about the calibration procedure.

Because these slides will be used in sensitive calibration procedures, treat them with the care that you would treat any calibration standard. The slides should be handled only by their edges. They should be stored in the protective box in a dark place at room temperature. If the surface becomes contaminated with fingerprints or dirt, use a cloth dipped in mild detergent to wipe off the contaminants. Do not use abrasive wipes such as paper towels or KimWipes™. Make sure that the cloth does not leave residues, such as lint. Rinse with distilled water and blow off excess water spots with clean compressed air. With careful handling your calibration slides will last indefinitely.
Q: Can dyes other than Cy3 and Cy5 be used in the GenePix scanners?

A: Yes, other dyes can be used with the GenePix scanners. Which dye depends upon how well the dye’s excitation and emission spectra correlates with the green and red channels of the GenePix 4000 scanners. The green channel excites at 532 nm and uses a 575DF35 (~557 nm to 592 nm) emission filter. The red channel excites at 635 nm and uses a 670DF40 (~650 nm to 690 nm) emission filter. Although these laser and emission filter combinations have been optimized for use with Cy3 and Cy5 dyes, any dye with spectra similar to Cy3 and Cy5 can be used. Besides the dye’s excitation and emission spectra, the performance of the dye will also depend upon such properties as its extinction coefficient and quantum yield. Biological considerations such as hybridization efficiency and sensitivity to environmental conditions such as quenching, fading, and stability are also important parameters for determining the dye’s effectiveness for use in the GenePix 4000B scanner.

The Alexa series of dyes, available from Molecular Probes (www.probes.com), have been used in flow cytometry and confocal microscopy for several years and are now receiving an increasing amount of attention for use in microarray research. Alexa 546 (ex: 553/ em: 573) and Alexa 647 (ex: 650/ em: 668) are two dyes that may be of special interest. The spectra of these dyes are similar to Cy3 and Cy5, plus the quantum yield and absorption coefficient of these dyes may actually make them brighter than Cy3 or Cy5. Please visit the Axon website to learn more about dye compatibility with the GenePix 4000B and 4100 scanners.

Q: What is the difference between the normalization factors reported in GenePix Pro version 3 and 4?

A: GenePix Pro 3.0 reports the ratio-based normalization factor, while GenePix Pro 4.0 reports the wavelength-based normalization factors.

In GenePix Pro 3.0, the normalization factor is calculated using the equation:

\[
\frac{1}{10^{\sum_{i=1}^{n} \log_{10} R_i}}
\]

where \(R_i\) is the ratio for the \(i\)th feature. This is the ratio-based normalization factor.

GenePix Pro 4.0 takes this ratio-based normalization factor and distributes it equally between the two wavelengths so that the normalization factor for one wavelength scales up by the square root of the ratio-based normalization factor and the other wavelength factor scales down by this amount. These are the wavelength-based normalization factors.

GenePix Pro 4.0 provides the wavelength-based normalization factors so that four-color data can be normalized.

For example, for ratios of wavelength 1: wavelength 2, if the ratio-based normalization factor calculated by the above equation is 1.44, then the normalization factor for wavelength 1 is \((1.44)^{1/2} = 1.2\) and the normalization factor for wavelength 2 is \(1/(1.44)^{1/2} = 0.833\).

The normalization factors for wavelength 1 and 2 given in the example above would be reported in the GPR file header as:

\[
\text{NormalizationFactors} = 1.2 \quad 0.833 \quad \text{n/a} \quad \text{n/a}
\]

The "n/a" in the above example indicates that 4-channel mode is turned on, but no data is available for channel 3 and 4.

In contrast to GenePix Pro 3.0 where the normalization factor is reported but not applied to the data, clicking the "Apply" button in GenePix Pro 4.0 automatically normalizes each wavelength's Feature and background intensities. As a result, the ratios derived from the normalized wavelength intensities are also normalized and are mathematically equivalent to those of GenePix Pro 3.0. In addition, this normalization in GenePix Pro 4.0 is reflected in the images for each individual wavelength and the ratio. Normalization can be reversed by clicking on the "Remove" button. Images and results can be saved in both the original and normalized state by using the "Save As" function.
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