

## ARTICLE

## g-PRIME: A Free, Windows Based Data Acquisition and Event Analysis Software Package for Physiology in Classrooms and Research Labs

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We present g-PRIME, a software based tool for physiology data acquisition, analysis, and stimulus generation in education and research. This software was developed in an undergraduate neurophysiology course and strongly influenced by instructor and student feedback. g-PRIME is a free, stand-alone, windows application coded and "compiled" in Matlab (does not require a Matlab license). g-PRIME supports many data acquisition interfaces from the PC sound card to expensive high throughput calibrated equipment. The program is designed as a software oscilloscope with standard trigger modes, multi-channel visualization controls, and data logging features. Extensive analysis options allow real time and offline filtering of signals, multi-parameter threshold-and-window based

event detection, and two-dimensional display of a variety of parameters including event time, energy density, maximum FFT frequency component, max/min amplitudes, and inter-event rate and intervals. The software also correlates detected events with another simultaneously acquired source (event triggered average) in real time or offline. g-PRIME supports parameter histogram production and a variety of elegant publication quality graphics outputs. A major goal of this software is to merge powerful engineering acquisition and analysis tools with a biological approach to studies of nervous system function.

*Key words: physiology software, data analysis, data acquisition, crawdad, student laboratory software*

As the modern life sciences mature as technical fields, experimental biologists require tools developed by electrical engineers and physicists for their communications and information processing systems. These tools can be just as powerful when applied in reverse to deconstruct the behavior of physiological systems. In particular, we are applying these tools in educational and research settings towards understanding the information processing mechanisms of the nervous system. Today, biological curricula are adapting to advances in computer and sensor technology. Bridges are needed between classical biology training and new engineering resources for faculty and students as future biological scientists. In order to remain competitive in their research fields, students and investigators must be connected with powerful signal processing tools.

Paralleling (and possibly driving) this paradigm shift in the life sciences is a miniaturization and refinement of technology, and powerful, more user-friendly software tools to control this technology. Sophisticated electronic equipment can be bought off the shelf by biologists, and scientific development environments have grown from raw computer language roots into user-friendly and empowering experiment design and data analysis tools. These new scientific programming environments, such as Matlab (Mathworks Inc., Natick, MA) and LabVIEW (National Instruments, Austin, TX), free the engineer from overhead associated with a deep computer architecture understanding. These relatively new scientific computing languages allow a new breed of hybrid engineer/biologists to construct the bridge between classical biology and modern engineering. Instead of preoccupation with the underlying software/hardware architecture, biomedical engineers and physiologists may focus on an efficient user

interface and specific analysis algorithms for their acquired data.

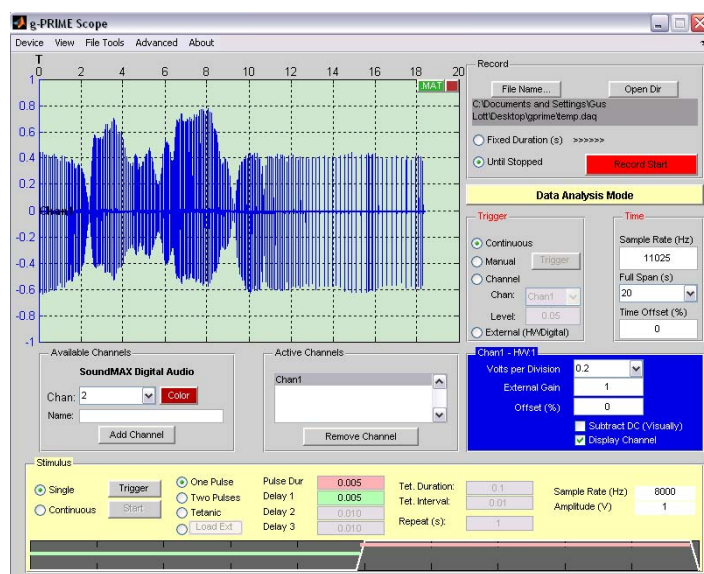
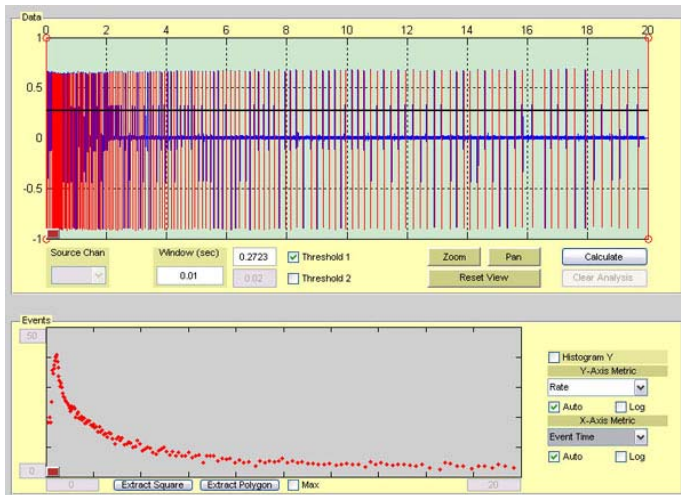


Figure 1. g-PRIME scope graphical interface. In this example: electric organ discharges from a weakly electric fish (Carl Hopkins, Cornell University). Stimulus generation tools and data logging controls are visible.

g-PRIME is such a tool, created by our working group, which has both strong engineering (GKL and BRL) and biology backgrounds (BRJ, RHB and RRH). The program creates an intuitive connection to hardware data acquisition interfaces as a software oscilloscope with data logging from an arbitrary number of channels and stimulus generation. In addition to input/output functionality, g-

PRIME supports real-time and offline analysis of data. Filters of arbitrary shape may be applied to acquired data, events may be detected by multi-threshold crossing discrimination in multiple analysis parameter spaces, and detected events may be clustered and cross correlated with simultaneously acquired channels. All of these features are wrapped into a user-friendly graphical interface and all results may be exported as publication quality graphics files as well as Matlab or Excel (Microsoft, Redmond, WA) supported data formats.



**Figure 2.** Offline analysis of the firing rate of a crayfish tail proprioception system as it adapts to a stretch stimulus. Spikes were detected by a single threshold (top panel) and the inter-event rate was displayed versus event time to illustrate adaption of a sensory system (bottom panel).

## PSEUDOCODE

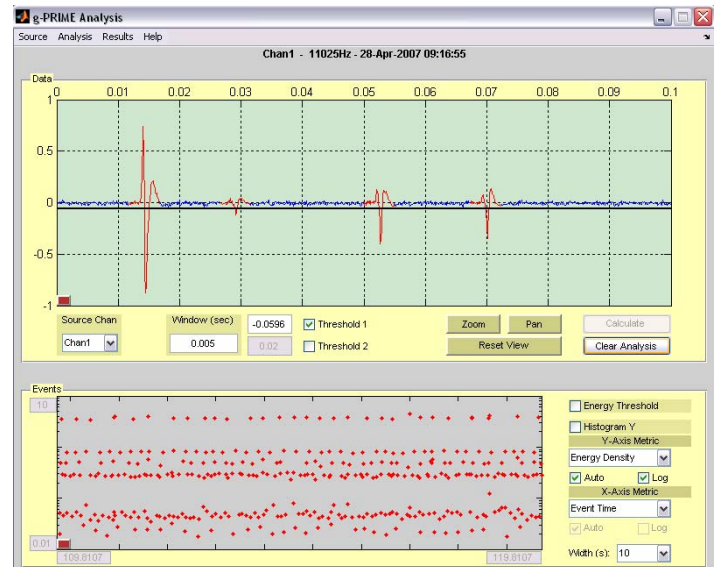
### Interface development

Installed and supported devices include, in our teaching setting, a National Instruments (NI) data acquisition board and the sound port of a PC laptop computer. The user may select a single interface from the pull down Device menu, and connect to an arbitrary number of channels. Each channel may be independently controlled with a visualization offset, color, name, display scale, input gain range, and a display option (see Fig. 1).

Data Logging, time series configurations (sample rate and visualization width), and trigger modes are all implemented in the user interface. Parameters are error checked for appropriate values before setting, and descriptive errors are returned to the user if values are selected outside of allowed ranges (i.e. typos in sample rates, etc). Configuration parameters are also limited, in many cases, to a set of discrete values in pop-up menus (such as the “volts-per-division” field). This allows intuitive interaction with the scope feature, and keeps the user from being overwhelmed by displayed options. By far, the largest portion of the program code base is dedicated to user interface functionality.

The data display is updated at 100ms intervals (10Hz update rate) utilizing the Analog Input “Samples Acquired Function” feature of the Matlab Analog Input Objects. Data

is streamed to memory (or memory & disk when logging), and is extracted from the buffer and displayed in the associated channel trace in the software oscilloscope window. This function is also the launching point for the real-time analysis features of the program. After data is displayed, the raw data values are passed to the analysis function for filtering and event detection. Analysis features in g-PRIME primarily support filtering and threshold based event detection.



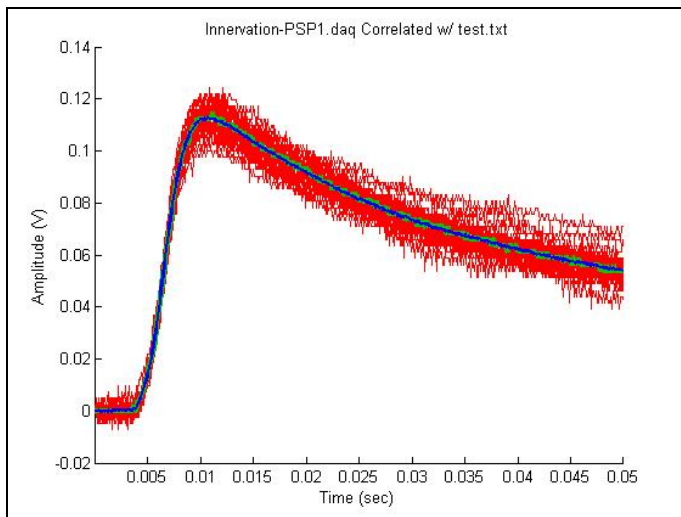
**Figure 3.** Real-time spike detection from a 6-unit tonic nerve in the crayfish tail (superficial flexor system). All six units can be clearly discerned in this energy density vs time representation of the entire recording (bottom panel). Four spike classes are visible in the trace segment (top panel).

### Real-Time & Offline Analysis Features

A number of pre-calculated low and high-pass filters can be applied to data streams for data visualization and event extraction. Pre-calculated filters are constructed as 3rd order Butterworth IIR filters at discrete frequencies between 10 Hz and 10 kHz. A 60 Hz IIR -3dB notch filter with a quality factor of 15 filters power line noise.

The user may also activate and place several amplitude threshold levels within which events will be logged and analysis results will be displayed (Fig. 2). The most fundamental of these thresholds is the minimum absolute amplitude threshold. When an input signal level crosses this threshold level, an “event” is detected. A window of data points is collected around this event and the threshold cross point is placed at a certain location within the window of points such that 0, 25, 50, or 75% of the window will precede the threshold cross in order to capture pre-trigger components of an event. This could be the negative sweep of a multiphasic pulse, for example (Fig. 3). Once a threshold crossing is detected, this window region is considered a single event, and other events must be located outside of the window region for detection.

Window maximum and minimum amplitudes are calculated and compared to a second amplitude threshold (if activated). If the event amplitude exceeds the



**Figure 4.** Student report generated figure of a spike-triggered average between two channels. A single spike class from a multi-unit motor nerve in the crayfish (see Fig. 3.) was selected via multiple threshold discriminations (including the user's own subjective analysis), and the corresponding intracellular muscle synaptic potentials from a second data channel were averaged. One can clearly see the averaged propagation delay, PSP membrane depolarization rate, PSP amplitude, and PSP rise time associated with the firing of a single presynaptic motor neuron. A student may then include this figure in their report as a TIFF or JPEG image.

secondary threshold, the event is discarded, saving only events between the two amplitude threshold levels. Once an amplitude-discriminated set of events is detected, the samples within the events are further analyzed. The Fast Fourier Transform (FFT) of the event samples (windowed with Blackman window) is calculated, and the energy density is calculated by summing FFT amplitude components and normalizing to the window width. Peak frequency component is also calculated to describe the primary frequency component of the signal, and an inter-event interval is calculated between the current and previous event threshold crossing time.

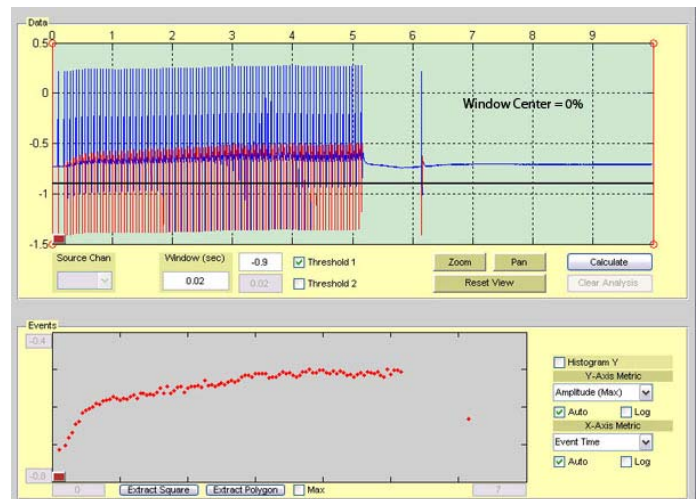
The user may choose to display any pair of these seven parameters (time, max, min, energy density, peak frequency, rate, interval) in a two-dimensional space where further parameter thresholds may be applied. In real-time analysis, threshold pairs may reject values in the energy density and peak frequency parameter space. In offline analysis, arbitrary boxes and polygons can be drawn to create a complex threshold in any two-dimensional data display space. Extracted event sets using this offline analysis method may be further groomed to remove outliers.

Once all analysis thresholds are applied, the user can correlate these events with the associated time points of another raw data channel currently connected in the scope, or to another logged data channel from an offline file (Fig. 4). This correlation consists of extraction of windows of samples at identical time points to detected events and a running average of these extracted points from event to event. Time correlated signals may rise out from noise sources, and both leading and lagging temporally

synchronized events may be discerned.

### Report Generation & Input/Output Formats

Users may also produce publication quality graphics or export/import a variety of file types for analysis. Associated with each data display graph is a button that will export the trace contents into a figure where a JPEG, PNG, or TIFF format image may be produced. Histogram bar graphs may also be generated for visualizing distribution of events in a single parameter space. When "compiled" to a stand-alone application, Matlab code does not support the graph customization functionality available in the licensed product (i.e. axis labeling, axis limiting, trace color, etc). We added a limited axis editing tool to g-PRIME that provides customization features to data plots for clean, annotated graphics.



**Figure 5.** A tetanic stimulus train was produced by g-PRIME to stimulate action potentials in an upstream neuron and short term potentiation of the post-synaptic response was characterized. In this example, PSP amplitude increases over stimulation time (bottom panel). The post tetanic PSP is larger than the initial pre-tetanic PSP and the difference varies as the student moves the post-tetanic stimulus in time. Large spikes in the top panel are stimulus artifacts.

There are several support input formats for offline data analysis. g-PRIME will load and parse Data Acquisition Toolbox default logging format (\*.daq) files, Wave Audio files (up to 2-channels \*.wav), Matlab data files (\*.mat) containing "data" and "time" vectors, and text files with two columns of numbers (first column representing a time vector). Limited support for Axon Instruments binary file format (ABF) is also included. Logged DAQ files may be exported as .wav files (single channel) or as text files with columns of time and data for import into programs such as Excel.

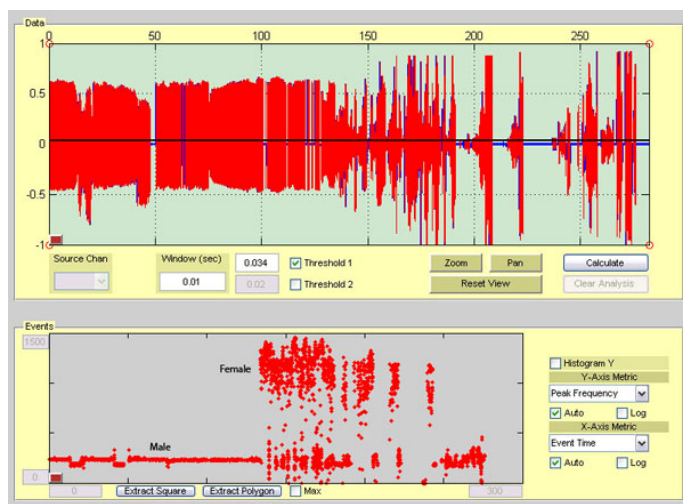
### Code Refinement, Compilation, & Distribution

As source code developed, efficient computation became an important factor for display of large numbers of events in real-time. The Matlab Profiler tool provides a line by line analysis of code execution times, and was instrumental in the development of streamlined analysis code. Pre-



allocation of large matrices of known sizes and elimination of unnecessary input format validations (such as in the “getdata” function) greatly increase the efficiency of the underlying analysis and acquisition code.

Once g-PRIME was in a stable configuration, a stand-alone executable could be exported using the Matlab Compiler Toolbox. Contrary to the toolbox name, the “compiler” does not actually compile the code. The Matlab “.m” file itself is bundled into an executable, and the user must install the “Matlab Runtime Components” associated with the correct release to enable execution of the code. This does, however, free GKL to release a version of the software free of the Mathworks product licensing rules for applications on many of our own computers or for distribution to colleagues or customers. In the case of g-PRIME, the software is released as freeware for general use.



**Figure 6.** Analysis of large data sets. This 5-minute recording of electric fish electric organ discharge (EOD) spikes contained spikes of varying amplitude due to fish distance from recording electrodes (top panel, see also Fig. 1). Each EOD spike, however, had a characteristic pulse width which varied between individual and gender. This inverse measurement of pulse width could be used to effectively track individuals when amplitude thresholds were not effective.

## RESULTS

g-PRIME was developed in a feedback loop with an upper level undergraduate neurophysiology lab course at Cornell University. The students were mostly undergraduates from majors with neuroscience interest (such as Biology, Psychology, Computer Science, and Math), different Engineering majors, and some first year graduate students from disciplines with neuroscience interest. During the semester, students studied various topics based on the *Crawdad* project (Wytttenbach et al., 1999). These ranged from the basics of membrane passive properties, extracellular recording of sensory and motor activity, and intracellular recording of action and synaptic potentials to the effects of neuromodulatory chemicals on synaptic dynamics and membrane ion currents (voltage clamping). The student preparations were model systems ranging from single celled plants to crayfish and snail brains. g-

PRIME was designed as a “just-right” solution for neuroscience education and research. While there are many commercially and freely available tools for signal analysis (mostly for audio applications), and many technical programming environments offer substrates for the development of customized packages, g-PRIME offered a “turnkey” solution designed with the classical physiologist and student in mind. Our goal with this project was to enable discovery while stimulating the budding neurobiologist’s interest to explore the nervous system with more powerful engineering tools.

Throughout the semester of development, students implemented existing laboratory protocols using new tools and techniques of g-PRIME. As bugs or feature limitations would appear (in actual applications to neurobiological preparations), on the fly corrections would be made and distributed to the students. As the students progressed through their course work, features were added that enabled new and more complex exploration of the teaching preparations. At the end of the semester, the students were fluent in the use of the program and produced some individual final projects on par with modern peer-reviewed work.

### Student Applications

We present a few examples here of student data gathered and analyzed using g-PRIME with a NI A/D board running on a Dell desktop. Students initially learn extracellular recording using suction electrodes (Johnson et al., 2007) attached to either a small, purely motor nerve or a nerve containing large diameter stretch receptor neurons in the crayfish tail (Wytttenbach et al., 1999). The students examine real time responses of stretch receptor neurons to constant tail bending in the crayfish. The receptor response to stretch adapts over a span of 30 seconds, giving the students plenty of time to describe and acquire an intuition for the system’s adaptation rate (Fig. 2). Detected spike times and amplitudes are extractable to a comma delimited text file and loaded into Microsoft Excel for curve fitting and further analysis. In the motor nerve recordings, students acquire spikes from the six-unit tonic motor neuron bundle, visualize them in real time, and characterize network reflex activity in response to sensory stimulation. Frequently, the students are able to separate all six units utilizing g-PRIME’s energy density calculation instead of the classical “max amplitude” calculation for a spike event (Fig. 3). The energy density calculation (sum of the amplitude components of the Fourier transform of a window) provides a relatively noise invariant measure of spike amplitude (noise spectrum amplitude is constant and additive to spike amplitudes).

Later in the semester, students learn to achieve intracellular recordings from the crayfish tail muscle innervated by the six-unit tonic motoneuron bundle described above. They use the spike triggered averaging tools of g-PRIME to match individual synaptic potentials (Fig. 4) with extracellular AP amplitude classes, and map out muscle innervation patterns. In addition, students design g-PRIME controlled stimulation of the motor nerve to describe and explore synaptic plasticity at this

neuromuscular junction. Figure 5 shows an example student recording of synaptic facilitation caused by tetanic stimulation of the motor nerve, followed by a post-tetanic potentiation of the synaptic potential.

g-PRIME can record, manipulate and analyze any biological activity representable as a voltage change, even with large, complex data sets. For example, electric organ discharges from two different Morymrid fish can be discriminated by its analysis features (Fig. 6). Students have also recorded and analyzed sounds with g-PRIME, for example courtship songs of fruit flies. Students download g-PRIME (see below) onto their personal PC laptops for data analysis and report graphics presentation outside the teaching lab. The program does not work under Macintosh operating systems, but runs on any 32-bit Microsoft Windows based system.

## DISCUSSION

The g-PRIME project is a powerful tool for undergraduate education and professional research. It represents a hybridization of disciplines normally reserved for high level collaborative projects at research universities. By bridging commonly used engineering tools into biological applications, we hope to contribute to a new standard for neuroscience education. New researchers and teachers in the modern era are realizing that they require the information processing and systems analysis tools previously only wielded by electrical engineers. g-PRIME makes these powerful tools available to students and established professors/researchers in a user-friendly manner.

A compiled and license free version of g-PRIME and a g-PRIME users' manual are available as a free download to the general public at the "Crawdad" website: <http://crawdad.cornell.edu/gprime/>. The source code for this software can also be found here. g-PRIME is part of our continuing project to increase the availability of inexpensive and powerful technical resources for neuroscience teaching faculty (see also Land et al., 2001, 2004, Krans et al., 2006, and Johnson et al., 2007).

## REFERENCES

- Johnson BR, Hauptman SA, Bonow RH (2007) Construction of a simple suction electrode for extracellular recording and stimulation. *J Undergrad Neurosci Ed* 6:A21-A26.
- Krans J, Gilbert C, Hoy R (2006) Teaching insect retinal physiology with newly designed, inexpensive micromanipulators. *Adv Physiol Educ* 30:254-261.
- Land BR, Wyttenbach RA, Johnson BR (2001) Tools for physiology labs: an inexpensive high-performance amplifier and electrode for extracellular recording. *J Neurosci Methods* 106:47-55.
- Land BR, Johnson BR, Wyttenbach RA, Hoy RR (2004) Tools for physiology labs: Inexpensive equipment for physiological stimulation. *J Undergrad Neurosci Ed* 3:A30-A35.
- Wyttenbach RA, Johnson BR, Hoy RR. (1999) *Crawdad: a CD-ROM lab manual for neurophysiology*. Sunderland, MA: Sinauer Press.

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