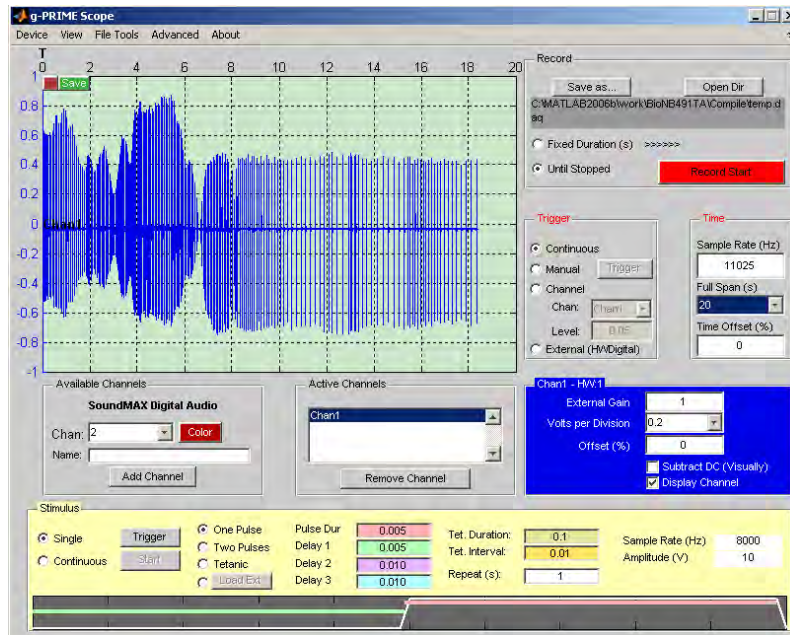


g-PRIME

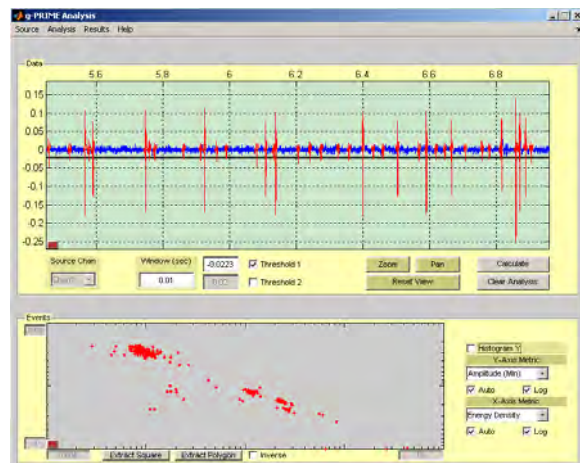
Physiology Recording & Identification of Multiple Events

Written by Gus K. Lott
User Manual



g-PRIME is a data acquisition and analysis package designed for use in physiology labs where continuous multi-channel amplitude data is acquired and discrete events are analyzed.

This software was written in spring of 2007 by Gus Lott as a component of his PhD process at Cornell University Program code construction details are outlined in his dissertation. This program was written to include many of the functions of previously implemented programs, StimScope and FreqHisto, which were written by Dr. Bruce Land for use in the neurophysiology lab course BioNB 4910 taught by Dr. Bruce Johnson.



This program is copyright 2007 Gus Lott. Free distribution is allowed for any and all university research and educational purposes as long as authorship is referenced. Industrial use is prohibited.

Contents

g-PRIME Features

1. Visualizing and Acquiring Data

- 1.1.Connecting to Hardware
- 1.2.Visualizing Data
- 1.3.Sweep Trigger
- 1.4.Recording Data
- 1.5.Example Recording Sessions
- 1.6.Advanced Interface Control
- 1.7.Spectrogram and FFT Visualization
- 1.8.File Tools, Report Generation, & Trace Measurement

2. Stimulus Generation

- 2.1.Single, Double, or Tetanic Pulse Generation & Arbitrary File Loading
- 2.2.Advanced Stimulus Control

3. Real-Time Analysis

- 3.1.Connecting to Active Signal and Visualizing
- 3.2.Signal Conditioning
- 3.3.Amplitude Threshold Event Detection (Single & Double)
- 3.4.Analysis Metrics (the **g-PRIME** transform)
- 3.5.Visualizing Analysis Parameters
- 3.6.Analysis Parameter Thresholding
- 3.7.Real-Time Event Correlation (Autocorrelation or Cross Correlation)
- 3.8.Saving Analysis
- 3.9.Histogram Generation

4. Offline Analysis

- 4.1.Loading a File
- 4.2.Differences from Real-Time Analysis Features
- 4.3.Extracting Result Subsets
- 4.4.Visualizing & Grooming Extracted Analysis Subsets
- 4.5.Saving a Subset (Analysis & Raw Traces)
- 4.6.Offline Event Correlation

g-PRIME History & Acknowledgments

g-PRIME Features

Scope Features

- Virtual oscilloscope with multi-channel support, independent channel zoom, and sweep width control from .05 to 30 seconds.
- Spectral visualization with real-time FFT or spectrogram
- Visually reject the DC level of a given sweep for display purposes
- Multiple trigger modes including Continuous, Manual, and Channel voltage level
- Advanced Gain Control to utilize internal pre-amps in data acquisition hardware
- Sample rate control and acquisition to a user selected file in a native data format to the hardware interface.

Stimulation Features

- Single, double, or tetanic pulse train generation with fixed parameters for stimulus delivery in a variety of environments.
- Arbitrary signal loading and stimulation
- Single channel signal generation and an associated channel for triggering stimulus start.
- Operates in parallel with acquisition and analysis features

Analysis Features

- Real-Time Event Thresholding and Analysis
- Real-Time Event Triggered Correlation with an Arbitrary Source
- Real-Time Band Pass Filters
- Event Rejection based on Multi-Threshold Levels in Many Parameters
- Analysis Results:
 - Threshold Cross Time
 - Maximum Window Amplitude, Minimum Window Amplitude
 - Time of Maximum and Minimum Values
 - Inter-Event Interval/Rate
 - Window Energy Density
 - Frequency of Peak FFT Value
- Arbitrary Parameter Pair Clustering
- Parameter Histograms
- All Real-Time Features Available for Offline Analysis
- Analysis Subset Selection and Grooming Tools
- Analysis Results Storage Functions for Further External Analysis
- Raw Event Export for External Analysis
- Report Generation Functions Create Publication Quality Graphics

1. Visualizing & Acquiring Data

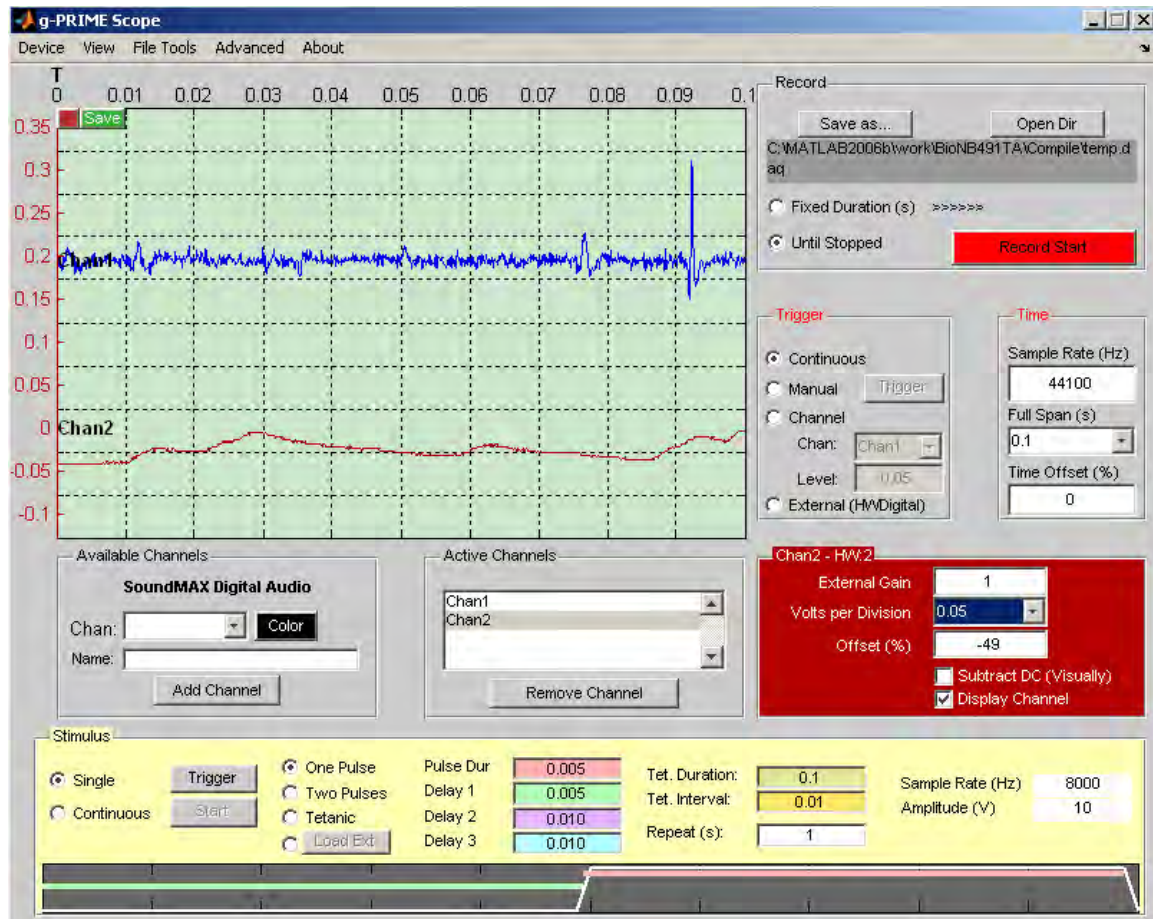


Figure 1. The g-PRIME software oscilloscope interface.

Interface Sections

- Available Channels
- Active Channels
- Channel Controls (Titled Chan2 – HW2 above)
- Trigger Controls
- Time Controls
- Recording Controls
- Stimulus Generation
- Scope Window

1.1 Connecting to Hardware

Supported Devices are automatically detected at startup and listed in the device menu. Select an interface from the **Device** menu to connect.

Available channels on the selected device are listed in the “**Available Channels**” panel below the scope window. Give the channel a name or just click the “**Add Channel**” button to select a channel. If no channel name is specified, one is automatically generated. A color for the channel trace and control window may also be selected from the “**Available Channels**” interface panel.

1.2 Visualizing Data

In order to modify a channel’s display properties, select it in the “**Active Channels**” interface panel. Once selected, the channel’s controls appear to the right of the “**Active Channels**” panel and the vertical scale axis associated with the channel is visible in the scope window. The channel may be moved vertically by **clicking on the channel name** text in the scope window and dragging up and down.

Display “**volts per division**” may be modified in fixed increments from the pop-up menu in the channel controls panel. An **external gain value** may also be entered to the channel control window. If an external gain greater than 1 is set, the trace will be scaled down in amplitude to match values associated with the unamplified signal. Note that this is for visualization only and will not be saved to streamed data files when recorded.

The DC Value (average value) of a sweep may be visually rejected by selecting the “**Subtract DC (Visually)**” check box in the channel control panel. The trace may also be removed from display by unselecting the “**Display Channel**” checkbox in the channel control panel. Neither the “Subtract DC” functionality nor the “Display Channel” functionality have any effect on recorded data or on voltage level based triggering. These features are for visualization only.

The **Time** panel contains controls that modify all channels of the interface. Here, one may set the system **sample rate** per channel, sweep width in the scope window, and time offset for channel based and manual trigger mode. Sweep widths faster than 200ms will refresh at 200ms divisions of their full period creating a quasi-continuous display of real time data in the scope window. Center point for channel based level triggering (i.e. how much data is buffered and displayed before a trigger event) may be set by entering a value in the time offset box as a % of the screen width or the **T** above the scope display may be dragged left or right to set the time center.

1.3 Sweep Trigger

Continuous triggering will provide a continually updating display of the data on the selected input channels.

Manual trigger allows one sweep to be acquired when the user clicks on the trigger button in the trigger window. When the trigger button is pressed, the program will acquire one screen width of data and then wait for another trigger event.

Channel based triggering updates the display only as a set threshold level is crossed by the input signal. The voltage level and source channel may be selected in the trigger panel. The trigger level will appear as a horizontal line in the scope display that may be manually dragged up and down. The time center for the signal in this display mode may be set by dragging the “**T**” above the scope left and right. The trigger event will be centered on this time=0 point and will have data preceding it in the visual display.

External (HWDigital) mode is only supported by data acquisition boards with hardware based trigger lines. A rising edge transition of a TTL voltage will activate a sweep.

1.4 Recording Data

Select a recording file by clicking the “**Save as...**” button in the **Recording** panel. The current recording file is listed in the **Record** interface panel in the upper right of the interface. The default file name is “**temp.daq**” and will be overwritten whenever the “**Record Start**” button is activated. If the file name is set to anything other than temp.daq, a new file will be created with an indexed suffix each time “Record Start” (red button) is pressed.

Data may be acquired until the “Record Stop” button (same as the “Record Start” button) is pressed or for a fixed interval. Select the desired recording method from the interface. For a fixed interval recording, enter the recording time in the interface box after selecting the “**Fixed Duration**” recording option.

Initiate recording by clicking the “**Record Start**” button in the record panel. The “Record Start” button will turn green and the total recording duration in seconds will be displayed on the button. Recording will not begin until the selected trigger condition is met. Data is streamed to disk and to the scope window. If fixed trigger duration is selected, a manual/channel trigger will record for that fixed duration and then wait for another trigger and record again. You must manually stop recording in a channel or manual trigger mode in either fixed or unlimited duration recording.

1.5 Example Recording Sessions

Note: In all cases, setting the time span of the scope window has no bearing on the data acquired. It will only change the way in which the data is displayed during the active recording interval. It may not be changed once recording begins..

Example: Continuous Recording for 10 seconds

- 1) Select Continuous Trigger Mode
- 2) Select “Fixed Duration” in the Recording Panel
- 3) Enter 10 into the duration box above the “Record Start” red button
- 4) Click the Record Button.
- 5) Data will stream for 10 seconds and then the system will exit record mode

Example: 5 Manual Triggers for 1 second (total of 5 seconds of data)

- 1) Select Manual Trigger Mode
- 2) Select Fixed Duration in the Recording Panel
- 3) Enter 1 into the duration box above the “Record Start” red button
- 4) Click the Record Button
- 5) Click the Manual Trigger Button
- 6) Data will stream to target file for 1 second
- 7) Repeat from step 5 five more times

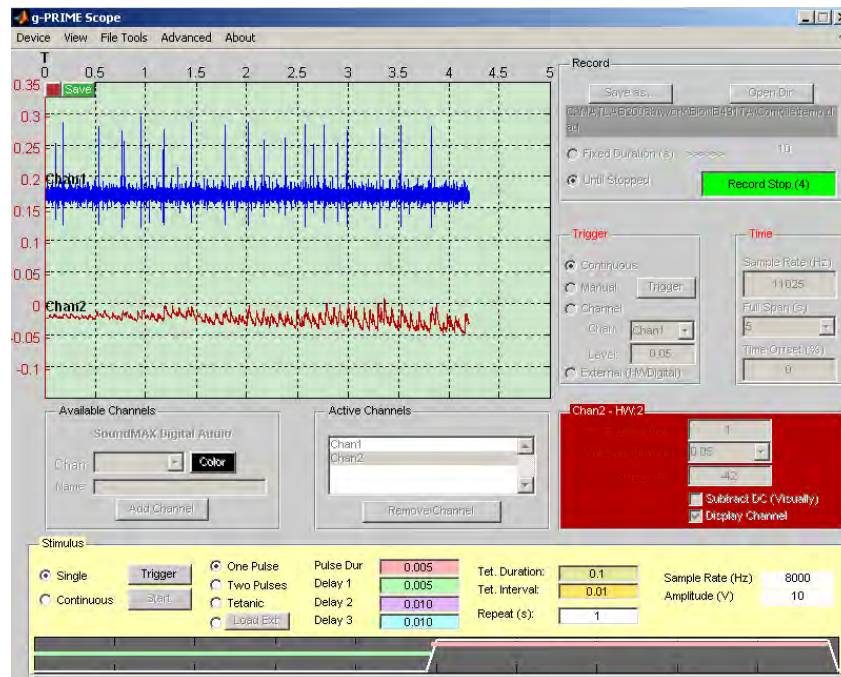


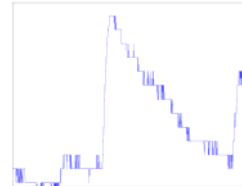
Figure 2. An active 2 channel recording session set to record until stopped with a display sweep width of 5 seconds.

1.6 Advanced Interface Control

Channel Input Gain Controls

Many data acquisition systems have internal amplifiers that allow for pre-digitization amplification of signals. If the amplifier has available gain input ranges, they will be listed in an associated menu in the “**Advanced**” drop down menu. The indicated range is the voltage difference over which the bits of the analog input channel will be quantized. Modifying this value will change the voltage detection range of the system and may allow for the resolution of smaller signals.

$$\text{Quantization level} = \frac{\text{Voltage Range}}{2^{(\text{bit depth of interface})}}$$

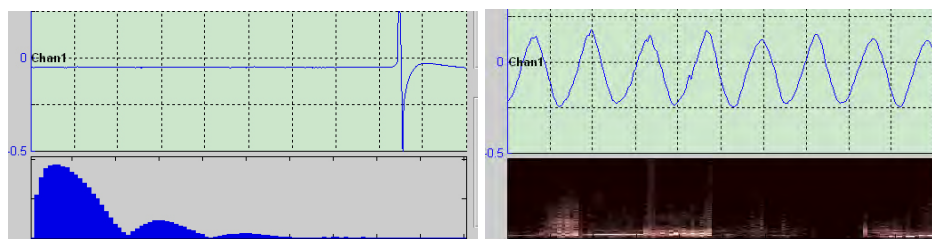


1.7 Spectrogram and FFT Visualization

The Fast Fourier Transform (FFT) of a signal is an expression of the amplitude of the components of a sinusoidal series representation of a signal. It is a useful means to express signals found in nature because most physical processes have some form of oscillating process as their basis. Frequency content of signals can be used as an effective means of differentiating overall signal shape.

There are 2 visualization options for frequency elements in the scope display. An FFT is calculated and then interpolated into 100 bins. The resulting frequency bins may be visualized in a **per-sweep FFT** or in a running 100 sweep width **frequency spectrogram**. In both cases, the amplitude of the sweep FFT slowly scales to match the max value of the frequency components over time. Visualization may be activated from the “**View**” menu in the main scope window.

Clicking on the FFT Visualization window region will convert the scope display from a *linear* to a *logarithmic* distribution of frequencies between a low frequency and the Nyquist frequency (half the sample rate).



**Figure 3. 100 point FFT displayed as single sweep value (left)
or as spectrogram with 100 sweep width (right)**

1.8 File Tools, Report Generation, & Trace Measurement

File Conversion

Files may be converted from the native Matlab data acquisition toolbox format (*.daq) to a variety of other file types using options in the “**File Tools**” drop down menu.

- The files may be converted into **Matlab (*.mat)** files if you have Matlab but do not have the data acquisition toolbox functions to read the .daq files.
- The files may be converted into **raw text** files with columns consisting of time, followed by data channels for loading into an arbitrary program as a comma delimited file.
- The files may also be converted into a **wave audio** file (up to 2 channels) for audio playback or filtering/analysis in an audio manipulation program or for multimedia presentation elements.

Basic Report Generation Tools

Acquired *.daq files may be reloaded through the “**File Tools**” menu into a figure window for review or figure generation. The figure may be modified (i.e. zoom/scaled & colored) and saved in a variety of graphics formats including **JPEG**.

Scope capture

The current scope window contents may also be captured to a figure for report generation. As with all windows in **g-PRIME**, a **small maroon button** appears in the corner of a window containing data. Pushing this button will launch the data window’s contents into a separate figure for report generation. In all cases, a data cursor may be used in the external window to label the values of elements of a graph and the trace may be zoomed/framed for graphic generation.

Trace Measurement Functions

The trace measurement option in the “**View**” drop down menu activates a rudimentary point to point draw utility with amplitude reported at the bottom of the main scope trace. Any offloaded graph may be labeled with the data cursor (option at the top of the offloaded figure).

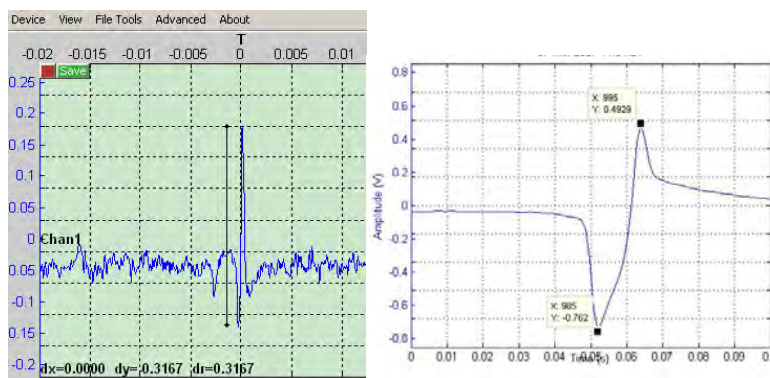


Figure 4. Measurement tool reporting the value of the displayed trace

2. Stimulus Generation

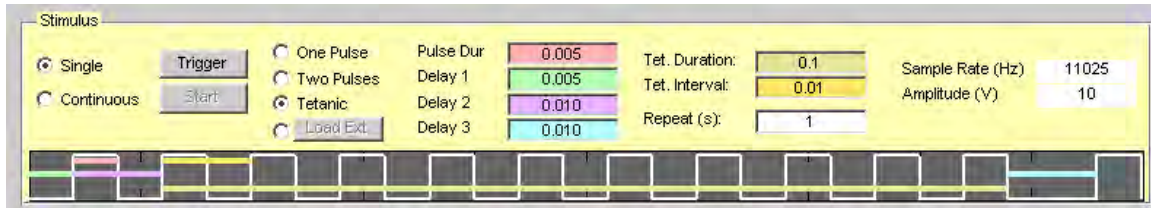


Figure 5. Stimulus generation interface

2.1 Generating a Stimulus

The stimulus generation portion of the interface is found at the bottom of the main scope window. Two channels of stimulation information are generated. The first two output channels available on the selected interface are activated for stimulus generation. The first channel (Left for sound card and 0 for a NIDAQ board) contains the generated signal and the **second channel contains a trigger signal** that is a 5ms square pulse with 5V amplitude (depending on interface output range). The trigger signal's rising edge is in sync with the first sample of the generated stimulus. A signal may be output by the manual "**Trigger**" button in single stimulus mode. The output may be repeated at a fixed interval (set in the repeat interface box in the stimulus panel) and activated using the "**Start**" button when the **Continuous** option is selected.

There are 4 modes for stimulus generation. Timing parameters are illustrated in pulse generation mode by colored bars overlaying the signal with colors corresponding to those seen in the interface option boxes.

- 1) **One Pulse** – In this mode, a single pulse is generated with pulse width set by "**pulse dur**" interface option and pulse delay (relative to the second channel trigger signal) set by **delay 1** interface option.
- 2) **Two Pulse** – Two pulses may be generated the output is identical to the one pulse mode. The **delay 2** interface option sets the time between the rising edges of two pulses.
- 3) **Tetanic** – A series of multiple pulsed stimuli may be generated. The tetanus is spanned by the two pulses. **Delay 3** controls the time after the last tetanus pulse of the rising edge of the final pulse. **Tetanus duration** indicates the length of time to present the repeated pulse stimulus and the time between tetanic pulses is indicated by the **tetanic interval**. An example of an application of tetanic stimulus on a study of synaptic potentiation is illustrated in figure 6.
- 4) **Arbitrary Stimulus Presentation** – An arbitrary signal may be loaded into the interface from a variety of file types including .wav, .daq, .mat, and raw text. Only the first channel of any file type is loaded as an output signal.

2.2 Advanced Stimulus Controls

The **sample rate** and **pulse amplitude** of the stimulus interface may be modified. You must activate them as interface inputs from the advanced drop down menu in the main scope program window.

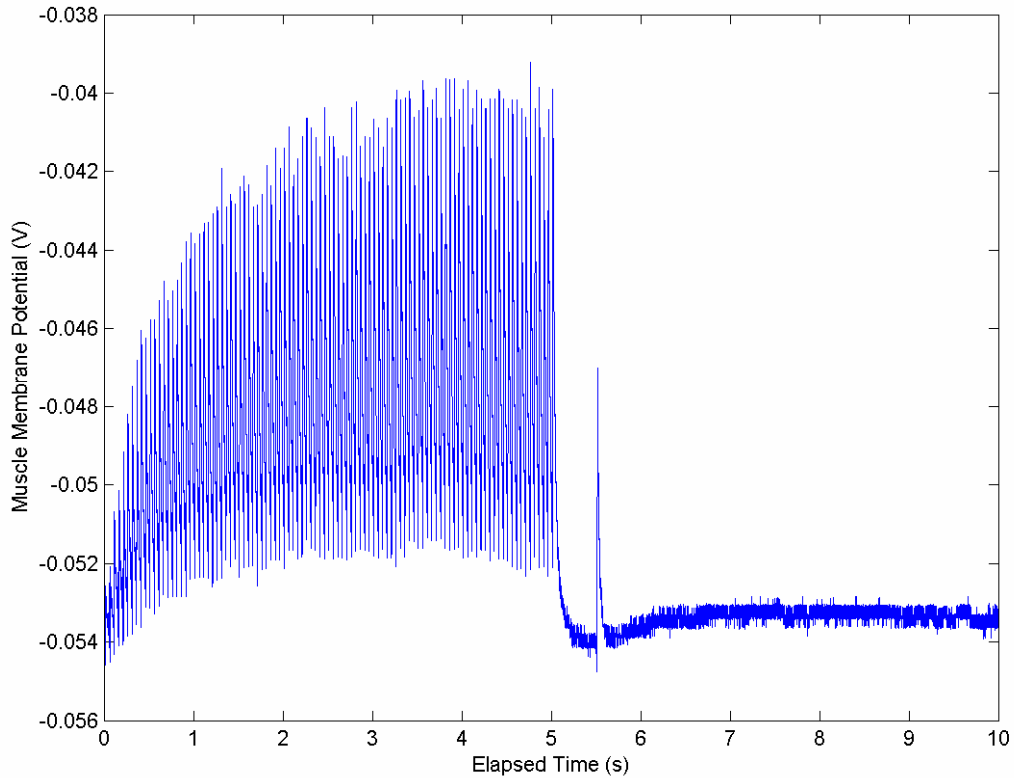


Figure 6. Posttetanic potentiation data. Note the large post synaptic potential (PSP) after the tetanic train compared to the initial PSP size at the beginning of the tetanus. This response decays over time. The increased level of response is due to increased neurotransmitter release from the presynaptic cell. This increased release is due to residual calcium in the presynaptic cell that enhances the activation of exocytotic events. A tetanic stimulus was delivered to the presynaptic motor neuron to produce these potentials (recorded in the post-synaptic muscle tissue).

3. Real-Time Analysis

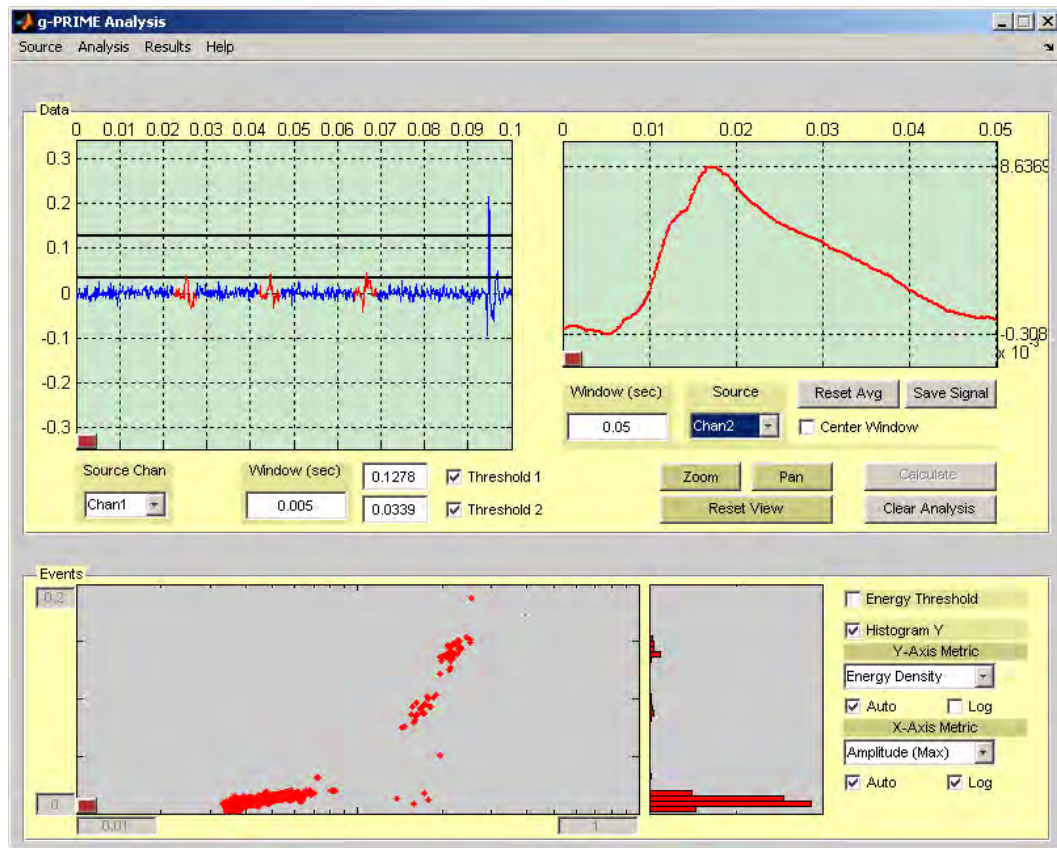


Figure 7. The Fully Activated Real-Time Analysis Interface. Raw data display with events colored red (upper left). Active correlation trace (event triggered average, upper right). The bottom graph region contains analysis values and a histogram functionality.

3.1 Connecting to Active Signal and Visualizing

The analysis window is accessed through the “**Advanced**” drop-down menu in the main **g-PRIME** scope window. The user may link the analysis window to a real time source by selecting the “**Scope Channel**” option from the **Source** menu in the **g-PRIME** Analysis window. Once the “Scope Channel” mode is selected, the user may select which of the active traces to connect to from the “**Source Chan**” pop-up menu under the data display trace.

The user may frame the real time data through the **Zoom/Pan** function in the main **Data** panel. Zoom to specify a vertical range on an active data trace. When the next sweep occurs, the data trace horizontal limits will be reset to the sweep width.

Depending on the time base of the main scope display, the real time analysis window will have 50ms, 100ms, or 200ms of data per sweep. Any scope sweep width higher than 200ms will report 200ms sweep widths to the analysis interface for calculations.

3.2 Signal Conditioning

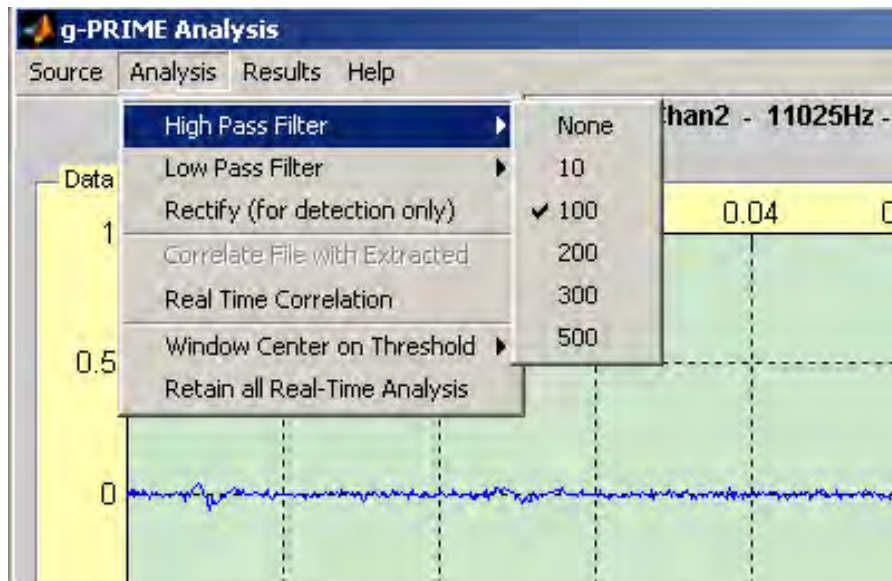


Figure 8. Analysis Features for Signal Conditioning. Band Pass Filtering and Rectification

The user may apply **high and low pass filters** by selecting from the drop down “**Analysis**” menu (Figure 8). The critical frequencies for the filters are indicated. These filters are third order “Butterworth” digital filters with critical frequency indicated by the drop down menu value. During real-time signal analysis, the filter is immediately applied when selected.

The user may also “**Rectify**” the signal for easy threshold based detection (i.e. negative sweeping values will be captured by a positive threshold level). When “**Rectify (for detection only)**” is selected from the “**Analysis**” menu, the absolute value of signal amplitudes is displayed and will be used to detect events. *Analysis metrics are calculated from un-rectified data.*

3.3 Amplitude Threshold Event Detection (Single & Double)

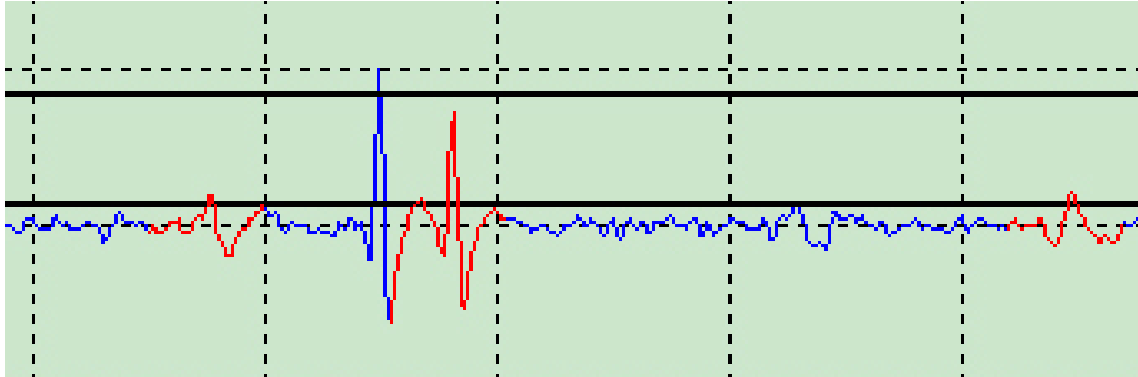


Figure 9. Two trigger levels. Acquired events are indicated by a red trace overlay in the acquisition window. Values with peaks outside of the two threshold levels are rejected. Threshold levels are indicated by two solid black lines across the image.

Setting Thresholds

Thresholds are set by either manually entering a value in the edit box next to the threshold activation check box or by dragging the horizontal threshold line up or down on the data visualization display.

Two thresholds are available for event detection. **Threshold crosses are detected in a direction relative to the mean value of the signal.** If the threshold is placed below the mean of the signal, falling edge events are detected which cross the negative threshold relative to the mean. If the threshold is placed above the mean of the signal, rising edge events are detected.

The active threshold closest to the mean value of the signal will trigger all event detection above that level. Analysis is carried out on all of these events. If a second threshold level is set, detected events with peak amplitudes between the two thresholds are kept while values that peak outside of the threshold range are discarded.

g-PRIME will detect events that cross the threshold and center the analysis window based on the selection from the “**Window Center on Threshold**” menu under the “**Analysis**” menu. The default is 50% of the window before the threshold cross.

3.4 Analysis Metrics (the g-PRIME transform)

When an event is detected, several analysis values are calculated and may be displayed in the analysis graph at the bottom of the screen.

1. Event Time (sec)

- Relative to start of the last input reset or beginning of the file

2. Rate Between Events (Hz)

- Inverse of difference between event times

3. Interval Between Events (sec)

- Difference between event times

4. Minimum Amplitude in Window (V)

- This value is used to reject events outside of the two-threshold range if thresholds are set below the mean value of the signal

5. Maximum Amplitude in Window (V)

- This value is used to reject events outside of the two-threshold range if thresholds are set above the mean value of the signal

6. Peak Frequency Component (Hz)

- The mean value of the window is subtracted from the signal and the Fast Fourier Transform (FFT) is taken. The peak value of the FFT is calculated. This value tends to be highly quantized at low frequencies.

7. Energy Density

- Energy density is the sum of the FFT divided by the number of points in the FFT. This number yields a general “Size” description of your event. It is a fairly clean relative measure of signal energy as noise sources will tend to have constant energy density. The energy density of signals relative to one another then depends only on the contribution from the signal source. This is the best “amplitude” measurement in most cases.

3.5 Visualizing Analysis Parameters

The analysis display is manipulated by controls in the bottom right of the analysis window.

Histogram Y

Selecting histogram display casts the data values displayed on the vertical axis of the analysis plot into a 50 bin histogram spread over the range of all events in the data set for that given parameter.

Y-Axis Metric

Any of the parameters may be cast as the Y-axis value of the data display. Select a parameter from the pop-up menu and select an axis range mode (auto on/off) and an axis scale (log/linear) for display.

X-Axis Metric

Similarly, any parameter may be cast on the X-axis to form a 2 parameter space in an attempt to see differentiation amongst the data values. If **Event time** is selected during real-time analysis mode, this axis will be automatically controlled and actively changed as time passes. The axis will have a **fixed window width** defined by a selection in the displayed width pop-up menu and the data will scroll from right to left as events are detected and time passes.

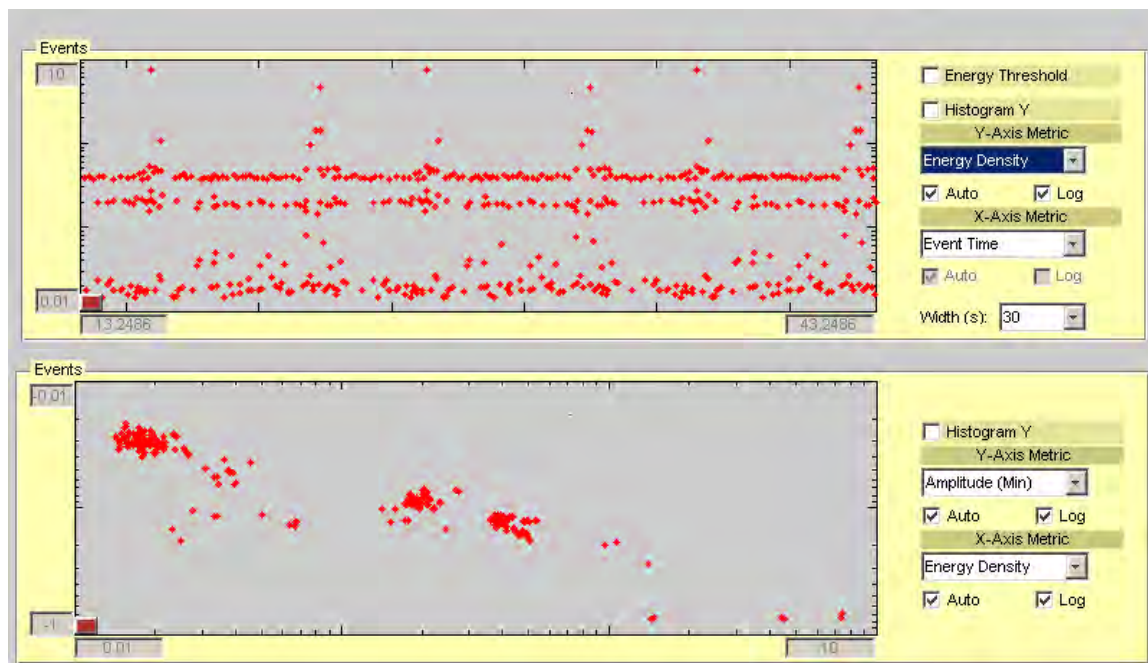
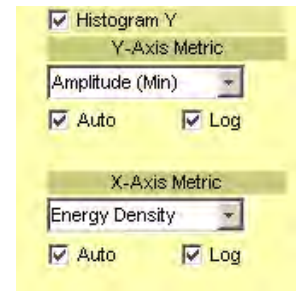


Figure 10. An example of the same events from a real-time data stream. Clear amplitude levels for multiple events may be seen in the Energy Density vs. Time display (Top) while clear clusters appear in the Minimum Amplitude vs. Energy Density display at right. A second threshold is used in the left image to allow for more resolution in the histogram. Rejecting the larger amplitude signals allows the histogram to be cast over a smaller range thus yielding higher resolution.

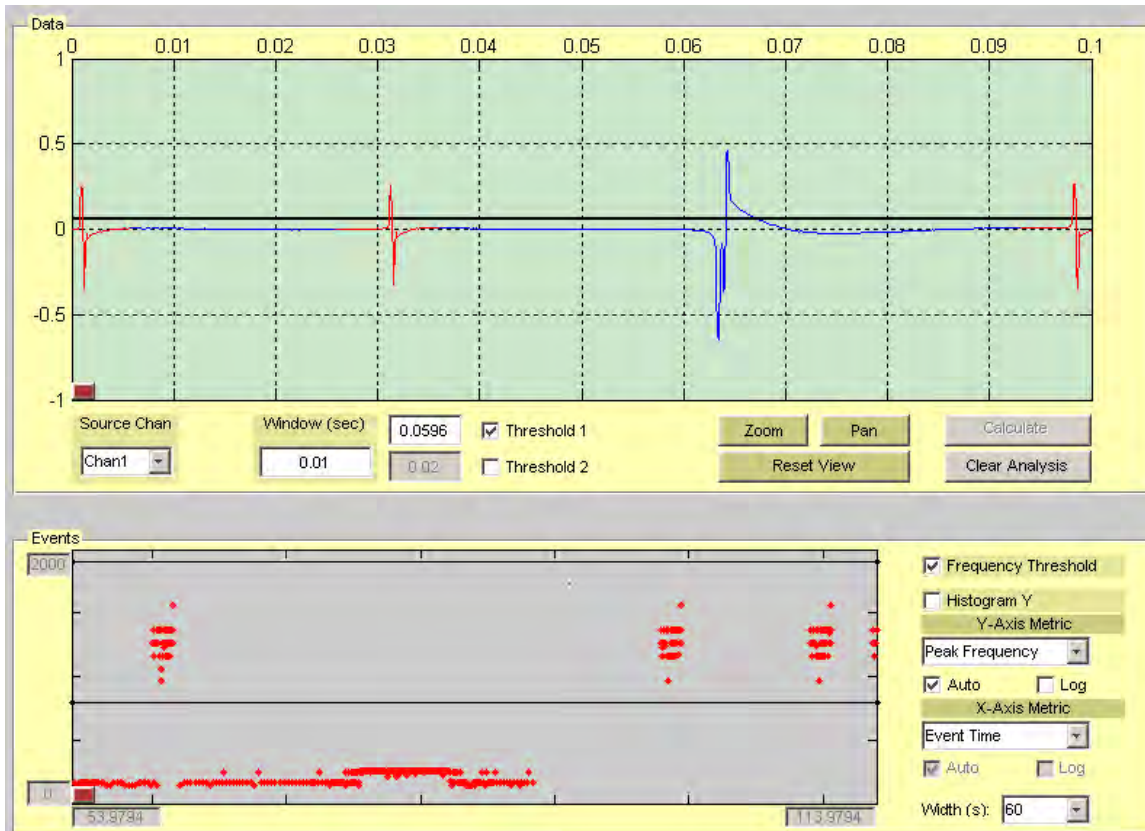


Figure 11. Activated frequency discrimination of real time data. Note the larger (lower frequency) pulse crosses the threshold but is not captured due to frequency threshold rejection (bottom pane). This is data from a high frequency (female) and low frequency (male) mildly electric fish in a tank. Frequency thresholds allow for discrimination and rate analysis of individual fish in the tank in real-time.

3.6 Real-Time Analysis Parameter Thresholding

While amplitude thresholds in the raw data trace offer some limited event discrimination, further event discrimination may be activated in terms of threshold ranges in the “**Energy Density**” and “**Peak Frequency**” space.

Select either parameter as the **Y-Axis Metric** value and independent checkboxes will appear which allow for threshold activation. When an analysis based threshold is activated, you may drag the two lines in the analysis plane to specify the acceptance region for events.

When an analysis parameter region is selected, you may then switch to another display mode such as Rate, Interval, or amplitude and monitor only the behavior of the subset of events.

3.7 Real-Time Event Correlation (Autocorrelation or Cross Correlation)

While the user will not have access to the raw trace information as displayed with offline analysis, the user may actively accomplish a **cross-correlation** or an **autocorrelation** of signals. Signals are actively acquired into a running **event triggered average**. The pair of thresholds may be used to limit the amplitude range of event for the real time correlation. Peak Frequency and Energy Density thresholds (as described in the last section) may also be activated to discriminate correlated events.

To activate the correlation interface, select “**Real Time Correlation**” from the “**Analysis**” menu. A trace window will appear and controls will activate that will allow you to select a window width for signal averaging, a source for the time triggered average, and whether or not to center the average window on the threshold cross or to use the threshold cross as the beginning of the average window. The user may also use the “**Reset Avg**” button to clear the contents of the correlation window. The threshold cross time amplitude value of the target region is set to zero in all extracted traces.

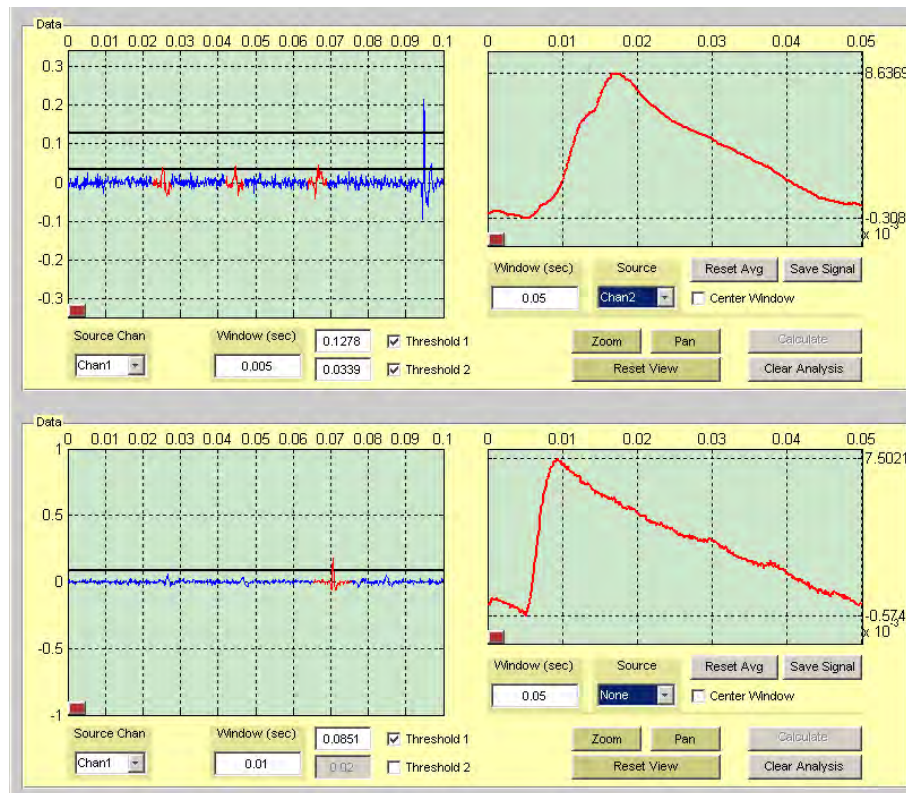


Figure 12. Real-time cross correlation between events detected in a channel of action potentials recorded (extracellularly) from a motor nerve and the intracellular potentials elicited by that neuron in the post-synaptic muscle. Multiple amplitude thresholds are used to select out different size classes of action potential amplitudes. Note the varying character of the post-synaptic potential in each of the example cases (small action potential, top) (large action potential, bottom).

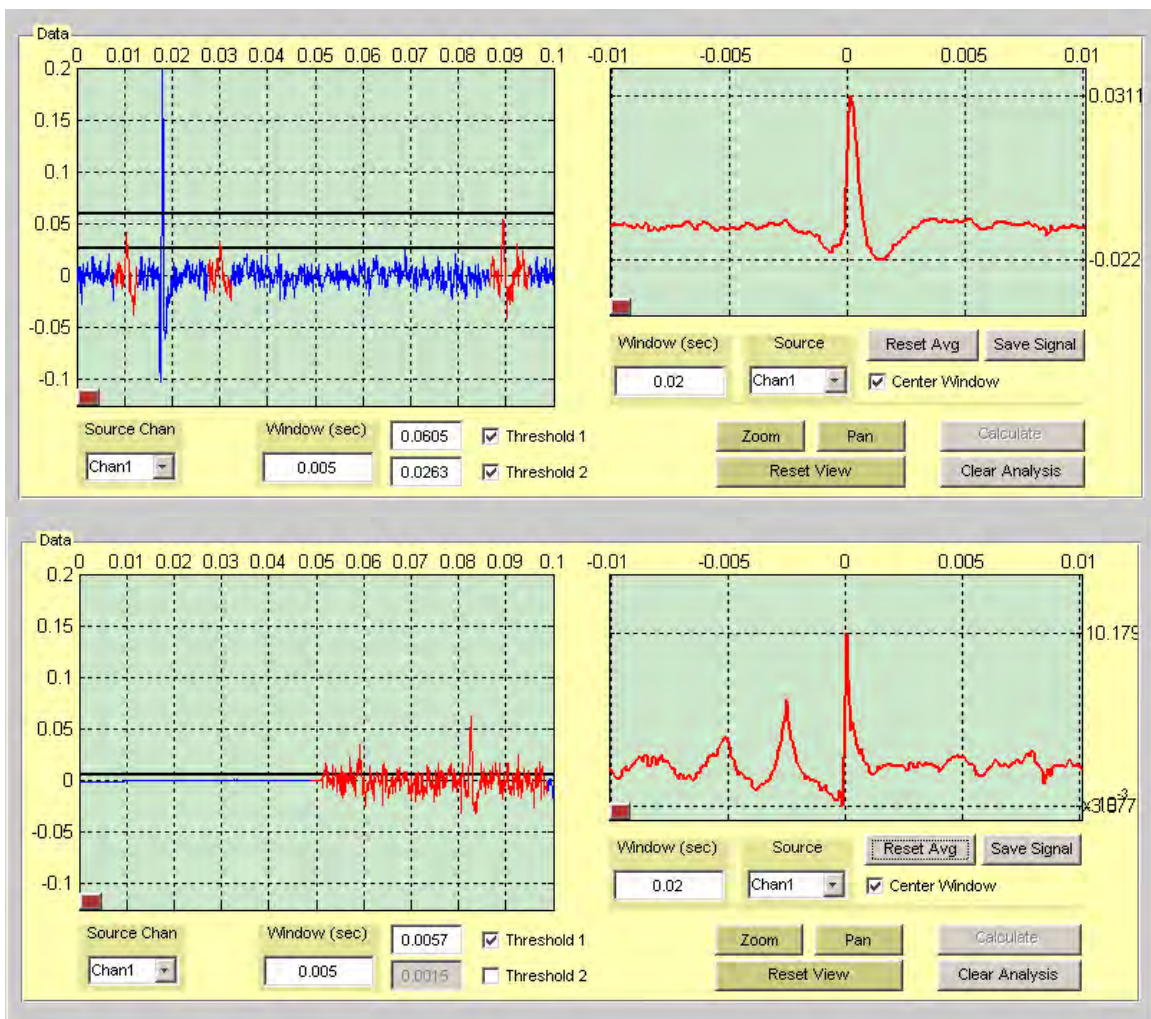


Figure 13. Real-time autocorrelation of detected (low signal/noise) events (top) illustrating a general pulse shape for a low signal level action potential. Also shown is the result of autocorrelation of uncorrelated noise which generates a signal with no distinct shape beyond the central triggered peak.

3.8 Saving Analysis

Saved Data Files

During Real-Time Analysis, only the 1000 latest events are stored unless the “**Retain all Real-Time Analysis**” option is checked in the “**Analysis**” menu (to prevent processor loading from graphics updates of many thousands of points). At any time, all stored analysis values may be saved to a text file (one event per row of columns) via the “**Save All Analysis**” option in the “**Results**” menu.

Saved .txt files with analysis parameters consist of 7 columns of data points and a number of rows of data equal to the number of detected events. Rate and Interval are not stored as these are easily obtained from the event time value. In addition to the above mentioned values, the time codes for the maximum and minimum amplitudes are stored. The columns in saved analysis text files are as follows:

Column 1: Time of Threshold Crossing
Column 2: Time of Maximum Window Amplitude
Column 3: Value of Maximum Window Amplitude
Column 4: Time of Minimum Window Amplitude
Column 5: Value of Minimum Window Amplitude
Column 6: Peak Frequency in FFT of the Event Window
Column 7: Energy Density of Event Window

This table is available in the help menu in the analysis window for reference.

3.9 Histogram Generation

Event histograms may be generated for any of the parameters in the interface. Selecting a specific variable from the “**Generate Histograms**” option in the “**Results**” menu launches a figure containing the histogram for saving as a graphic.

4. Offline Analysis

4.1 Loading a File

The user may also load a previously stored data file for offline analysis. In all cases, only a single file may be loaded at a time. If multiple channels of data are present, the interface will prompt for a channel selection. Supported formats are:

1. **(*.daq)** Raw Matlab data acquisition file format with data stored in interface native format. In addition to channel selection, you may also select individual triggered recording intervals if the session included multiple software voltage level or manual trigger events.
2. **(*.mat)** a Matlab MAT file containing the variables 'data' and 'time'. The variable 'time' should be a monotonically increasing sequence with a fixed sample rate and 'data' should be real numbers.
3. **(*.txt)** Raw text files may be loaded consisting of delimited values detected by the matlab "load" function automatically. Values should be arranged in columns. The first column will be treated as the time vector and subsequent columns will be treated as individual channels of data.
4. **(*.wav)** A Wave audio file may be loaded into the interface for analysis
5. **(*.nbb)** A file from "StimScope" written by Dr. Bruce Land (Cornell University) is also supported for offline analysis.

4.2 Differences from Real-Time Analysis Features

Offline analysis features are nearly identical to Real-Time analysis features with a few notable exceptions. When modifications are made to the band-pass filters and threshold levels, the user must click the "**Calculate**" button in the bottom right of the "**Data**" panel.

The user may actively extract subsets of values from clustered threshold results in the analysis window (as described in the next section), and event correlation (event triggered averaging) is applied by loading a subset of analysis events (text file) into an actively loaded target channel for correlation.

Report Generation features have more available graphics options in Offline Analysis Mode.

4.3 Extracting Result Subsets

When a file is loaded into the analysis window and a set of analysis values are generated, the user may select a sub-region of values in the space for display. Buttons appear under the analysis axis which will activate group selection and instructions appear above the axis.

Either a **square region** may be defined (2 points) or a **polygon** may be drawn to enclose oddly shaped distributions. When the region is defined, events corresponding to the points in the space are extracted from the dataset and overlaid in a separate report window. Activating the “**Inverse Selection**” checkbox will select all points outside of the selection polygon.

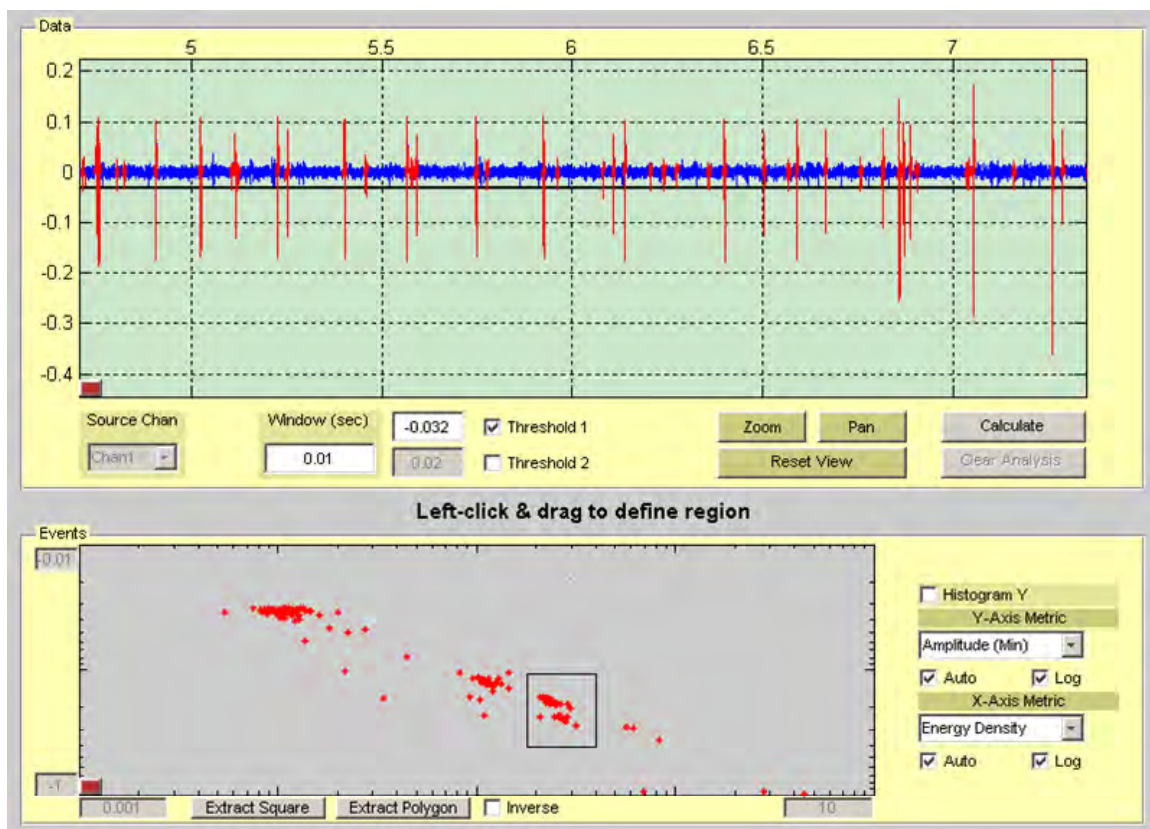


Figure 14. Selecting a square region subset of data points in a space of events that form clear clusters. Results are illustrated in figure 15.

The resulting window has several customization parameters in the “Options” menus.

Display Data (For Report Generation)

- Display source w/ extracted signals. The raw data trace is displayed with only the subset of events highlighted (red).

- Display cluster with subset illustrated. This is a copy of the analysis subset graph in the main analysis window with selected values colored red and unselected values colored black.
- Raw Traces are overlaid
- Both the event rate and interval may be visualized for the subset of values to see behavior of an individual unit.

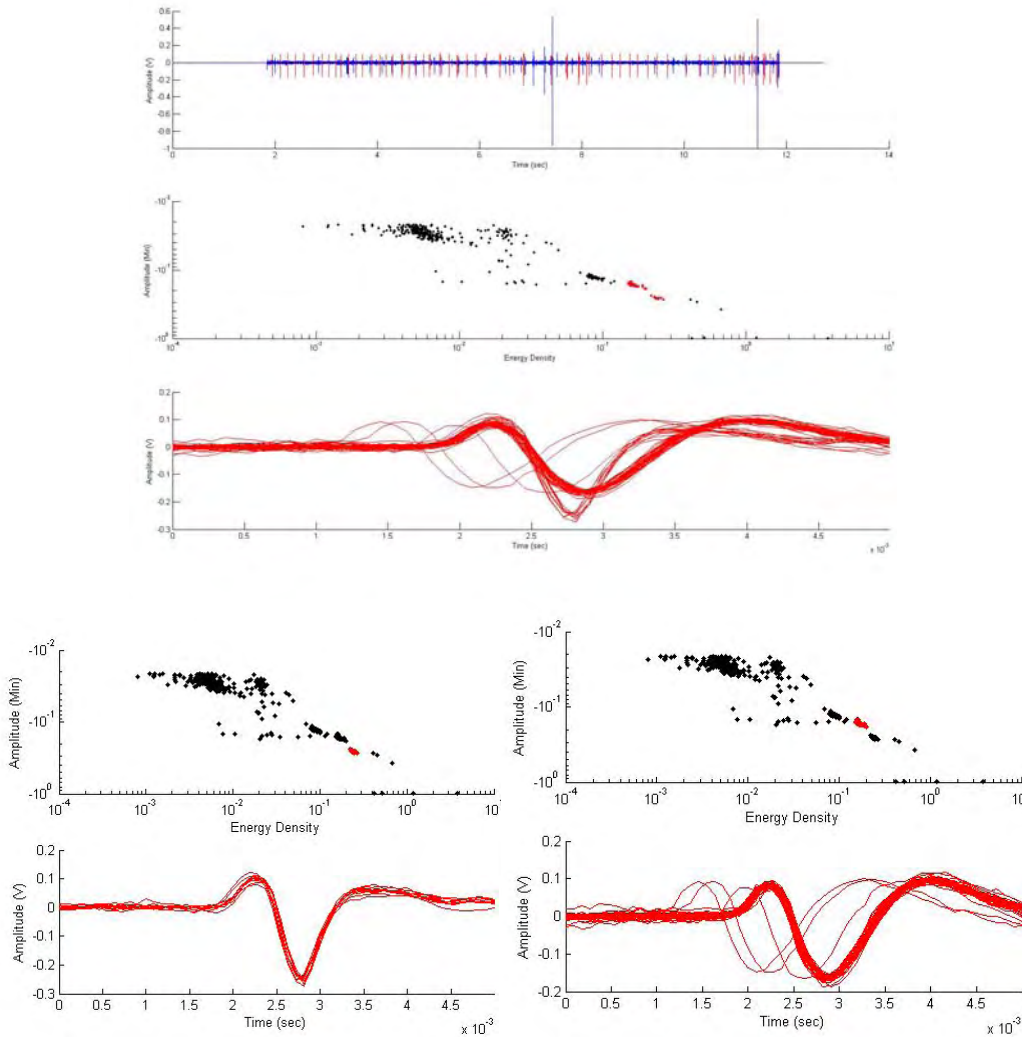


Figure 15. The results of a subset extraction from a multi-unit nerve recording (extracellular). Two clear action potential classes can be seen in the resulting data subset. Further refinement of subset (bottom images) illustrates the characteristics of the sub-clusters from the original selection. The raw data trace (top) may be displayed with only the subset of events highlighted.

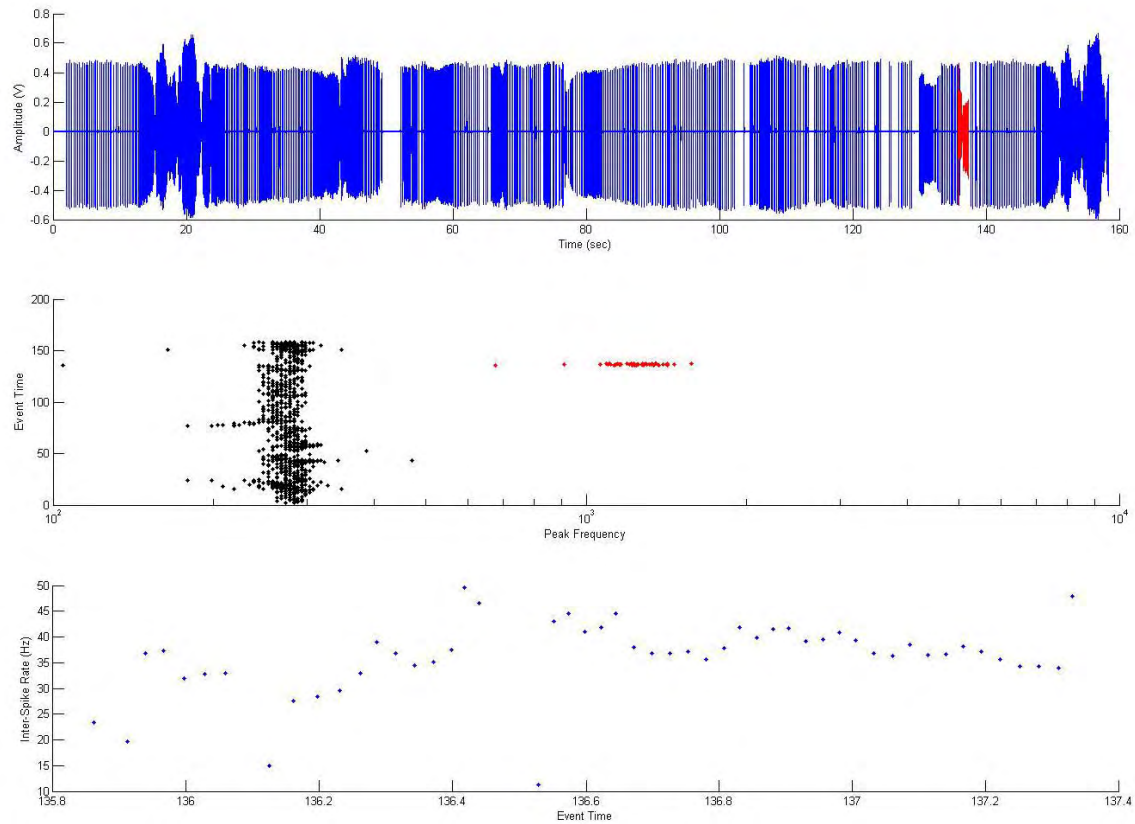


Figure 16. Electric fish data. One male electric fish was placed in a tank for a 2 minute period at which point a female was inserted. The female has a distinctly higher main frequency component signal compared to the male and the distinction readily appears in the analysis window while other parameters (such as amplitude) may make a relatively unclear clustering. The rate behavior of the only the female discharges may be readily illustrated.

4.4 Visualizing & Grooming Extracted Analysis Subsets

When event subsets are extracted for analysis using polygon zones, further clarification of the group components may be carried out. For example, outliers and overlapped signals may be manually selected by **clicking on the trace** or the event in the cluster. When the trace is selected, the **event is highlighted blue**. If the user wishes to remove a selected event from a particular subset of events, the trace may be removed from the analysis values by pressing the delete key on the keyboard.

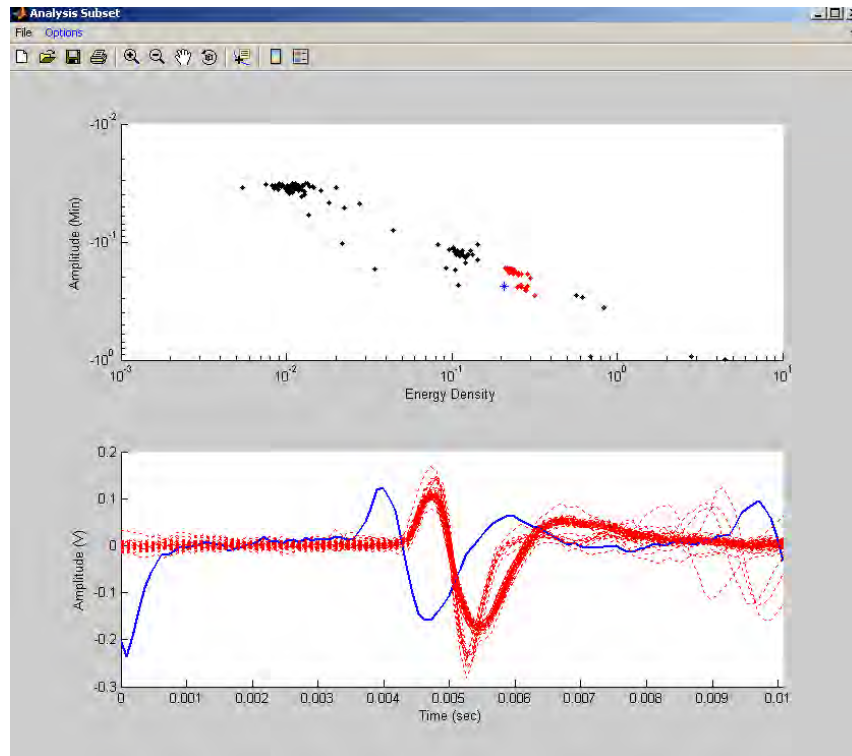


Figure 17. An outlying point is selected and may be extracted from the data set before saving.

4.5 Saving a Subset (Analysis & Raw Traces)

A selected and groomed subset of events may be saved to a file. Full 7-column analysis values corresponding with the selected subset may be saved to a text file as before and the raw traces may be saved to a text file formatted with a time vector as first column and subsequent columns representing amplitude values for each trace.

This subset of events may be reloaded later for offline correlation analogous to the real-time correlation function described earlier. Both data saving options are available from the “**Options**” menu in the “**Analysis Subset**” window.

4.6 Offline Event Correlation

Once a subset of events have been extracted and groomed from a data set based on a polygon threshold in an arbitrary event metric space, these events may be loaded back and correlated with corresponding time values in another data set.

In order to correlate an offline data set with extracted time points, load the file/channel containing the correlation target to the main analysis window. Select “**Correlate with Extracted**” from the “**Analysis**” menu in the analysis window. The user will be prompted to select a text file, a correlation window width, whether to center the window on the event or to only correlate after the time points in the file, and whether to include the raw traces in the background of the display for visualization of a correlation.

The result is displayed with all raw traces extracted and overlaid in the background (if selected) of a new figure. The mean value of all traces is displayed in blue and the median value is displayed in green. Alignment of the mean and median traces indicates high confidence in correlated values.

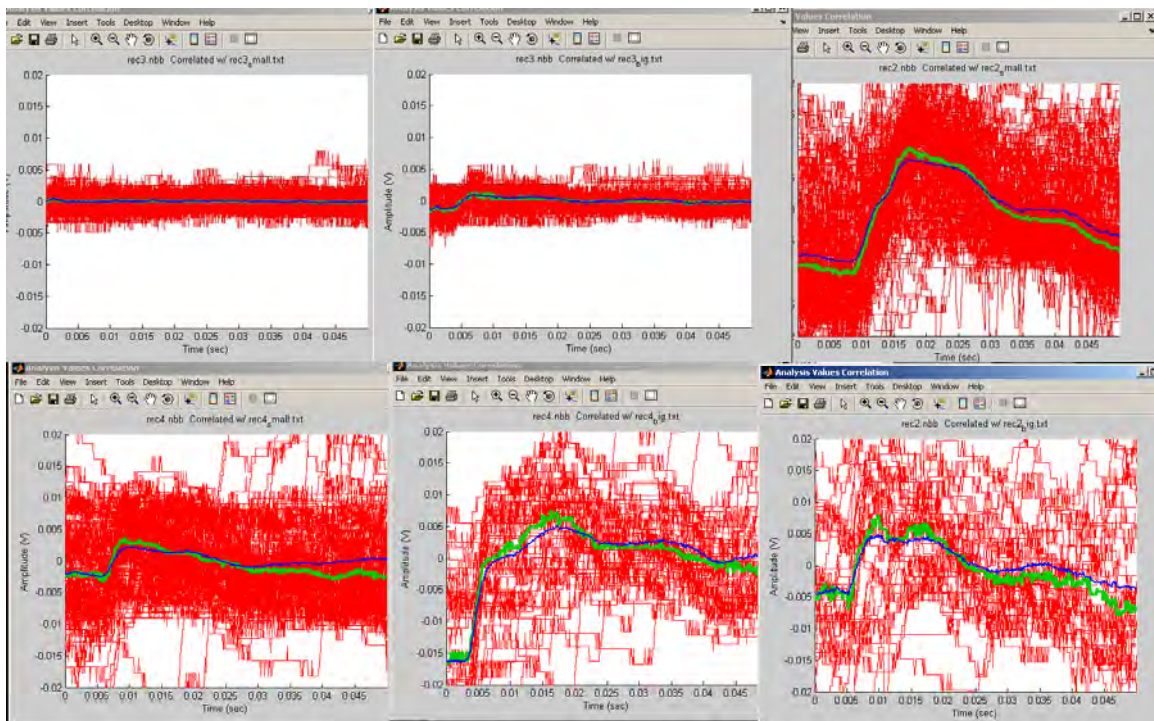


Figure 18. Examples of cross-correlations between subset classes of action potentials recorded from a motor-nerve and the resulting endplate potentials from the muscle innervated by the nerve. This time triggered average clearly illustrates a variety of amplitudes and delays corresponding to the different nerves innervating the muscle. In some cases, there are not enough points to form a clear display of the correlation and thus, the median diverges significantly from the mean. In one case, it seems that there is no correlation (top left).

g-PRIME History & Acknowledgments

History

g-PRIME was conceived in part when I took the Neurophysiology Lab course (BioNB491) at Cornell University in Spring 2003 and realized that the software driven component of the lab could use a “tech refresh.” Over the intervening years between 2003 and 2007, I developed instrumentation and MATLAB programming techniques that accomplished virtually all of the components of the program individually as I progressed through my PhD process in the related field of hybridizing a variety of engineering tools and techniques to neurophysiology problems.

My final term as a Doctoral Candidate at Cornell, I was given the chance to be a teaching assistant in that same course that I took four years earlier. At this point I was able to implement the changes with funding and feedback, support, and a target group of students to which I could apply the program.

As the program developed, I adapted a threshold detection functionality to apply to some electric fish recordings for Carl Hopkins. The program then advanced to a full fledged analysis and scope opus incorporating almost all of my MATLAB “work directory” from my work at Cornell. A large portion of the Neurobiology & Behavior Department jumped onboard the project and started offering frequent feedback and ideas directing g-PRIME into the event analysis tool that it is in this incarnation.

Acknowledgments

The Author, Gus Lott, wishes to thank Bruce Johnson, Bruce Land, Carl Hopkins, Rob Bonow, Ron Hoy, Sarah Matt and the students of the Spring 2007 BioNB491 course at Cornell. All of their feedback and support has made this project possible.

Contact

Author: Gus K. Lott III

Email: lottg@janelia.org