IGOR Macros for the Automatical Analysis of Amperometric Spikes

by

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1- Aim and purposes of these Macros

These macros have been created for the automatical analysis of secretory spikes resulting from amperometric detection. They calculate the kinetic parameters of spikes and produce histograms for their characterization.

Macros permit noise analysis, digital filtering, spike identification and its kinetics characterization. In addition, they allow modification of the type and the levels of digital filtering, the spike identification criteria. They also provide easy manual correction of data.

2- Computer and software requirements

Macros can be used on either Power Macintoshes or PC-based platforms (Pentium and above). The speed of processing will logically be increased with the use of faster computers. These macros only run on the IGOR program. You can purchase this directly from the company (http://www.wavemetrics.com).

IGOR may present problems with the use of comas/dots for number formats within Spanish countries. Please ensure that your files are captured, stored or exported in the same format (i.e. 1,000.48).

Macros have been created using IGOR pro 3.13 for Macintosh and were tested successfully on Windows 95/98 (3.11 IGOR) version. It is most probably that they will continue to work with the newer versions of IGOR.

Optimal RAM requirements are over 16 Mb although it probably can work with less.

If you want to modify the RAM memory assigned to IGOR in a Mac, please look for and open the IGOR Pro folder, click once on IGOR program icon and then push ⌘ + I. There you can modify the amount of memory assigned. We recommend assigning a value of above 10 Mb.

Data acquisition can be assessed by many programs provided that they can produce files in recognized data format (i.e. IGOR binary format, delimited text, general text). We currently use Labview® (National Instruments) which directly produces IGOR binary files. In the case that IGOR binary format is not used, (i.e. general text). The files must be converted into IGOR waves (.bwav (Mac) or .Ibw (PC)) prior to proceeding with the analysis.

Macros should be stored in an empty folder and the user must be ensure that no others macros were loaded. This can produce conflicts related to variable names.

3- How to acquire and store files

Data files have to be saved unpacked, in other words, each wave must be stored as a separated wave.

We strongly recommend numbering the waves consecutively, i.e. Exp01, Exp02... it helps the macros to analyze different files. Macros are able to operate with files with different names (Control, Drug#1, etc...) but this will result in extra work for typing in all the names in the tables.

The easier way is storing all the files (for instance obtained on a particular day or under certain experimental conditions) in a folder called "Original waves". Create an empty folder called "Processed waves" or a name that can be distinguished from the raw data. This will be the destination folder for the files treated by the macros (filtered waves, spike data, etc...).

Once waves have been processed, they will be stored in the “Processed waves” folder. Macros will save a file where all the parameters used for the analysis are stored (“Parameters_Exp03”, etc…) therefore you can, of anytime, locate where your waves were processed.

Once you have done the manual corrections you can store all the files in single experiments (“Results” folder) where all the spikes obtained under the same conditions are grouped.

4- How macros work

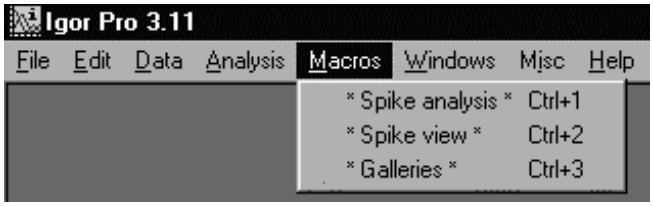
Macros convert raw data into files containing spike kinetic characteristics. For a description of the filter, spike identification algorithms and spike classification used please refer to:

Segura, F., Brioso, M.A., Gómez, J.F., Machado J.D. and Borges, R. (2000) Automatic Analysis for Amperometrical Recordings of Exocytosis. (In press).

5- Getting started

1- Double clicking on the IGOR icon, the computer will open the program.

2- Close procedure window. Choose “hide” in the dialog box, not kill. Macro options should be now present under the Macros menu: spike analysis, spike view and galleries.

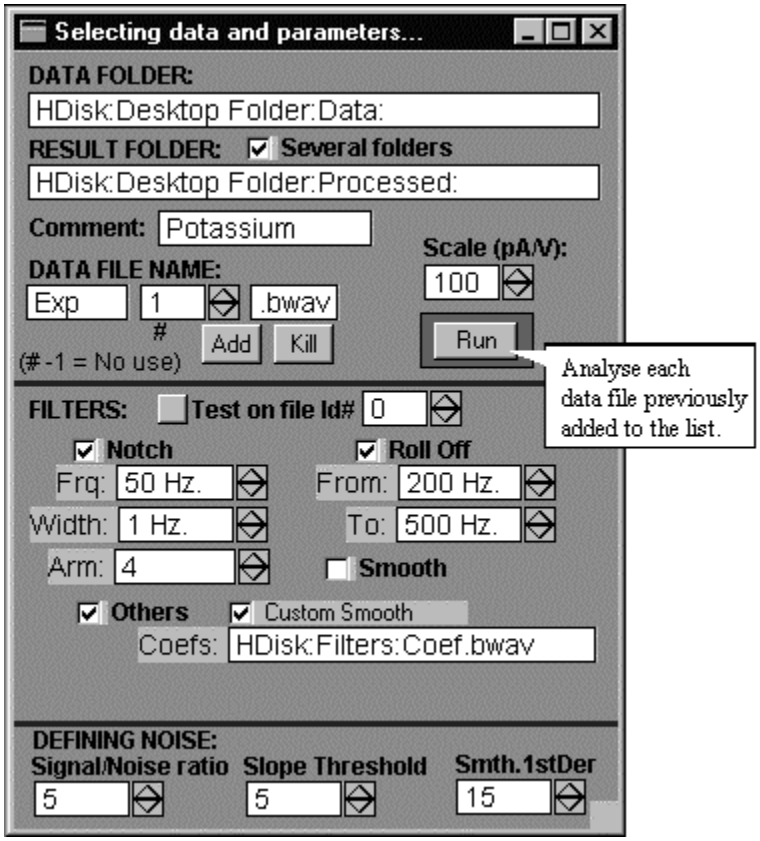


3- Prepare a folder to store the analyzed files.

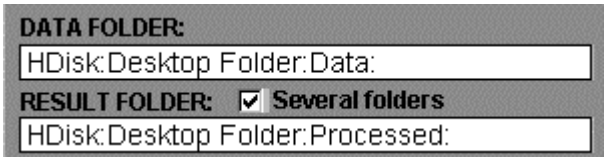
4- Select “Spike analysis” from the Macros window.

6- Spike analysis

You will now see the following window. You can get on-line help about macro functions by pressing HELP in the IGOR menu and getting balloon help (Mac) or by pressing **F1** after placing the mouse on the desired field (PC).



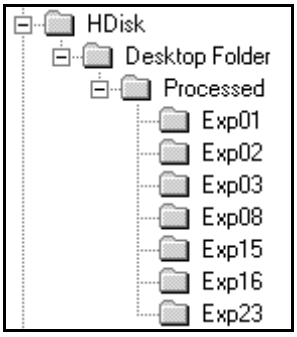
Now you may localize the folder where the raw data is stored and the path of the folder that the macro must use to save the processed data.



DATA FOLDER: please type, in this box, the full path of the folder where data files are stored.

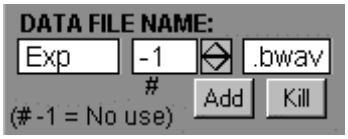
RESULT FOLDER: type in this box the full path of the folder where the results obtained will be saved.

Several folders check: When clicked, the results of each data file will be saved in different folders. In the result folder, typed previously, subfolders will be created for saving result waves of every experiment. Each experiment will be saved in its own folder when this check is clicked. The following image is an example for seven experiments (i.e.in the folder "Exp01" the program will save the result waves of data file "Exp01.bwav"):



How to introduce the name of the different data files.

DATA FILE NAME:



It is assumed that data files are called with an initial name plus a final number, i.e. Exp01, Exp05, Control53, Exp103, etc. Although files without numbers are accepted as well. In addition, file names may have extensions, i.e.: Exp01.bwav, Control08.ibw, etc.

In the first box, where "Exp" appears, you must enter the data file name, i.e.: Exp, Control, File, etc.

In the adjacent box, key the number of the experiment. The program adds a zero when the number in this box consists of only one digit. In the case that your files are not numbered or are numbered with only one digit (i.e. Control, Exp7, etc.), please type **–1** in this box, this indicating that the whole name, including number, is typed in the first box.

In the third box, where .bwav appears, type the extension of the data file: .bwav (Mac), .ibw (Windows). If file has no extension, this box must be blank.

Add: Add a data file for being processed in the list of files shown on the adjacent table (shown below).

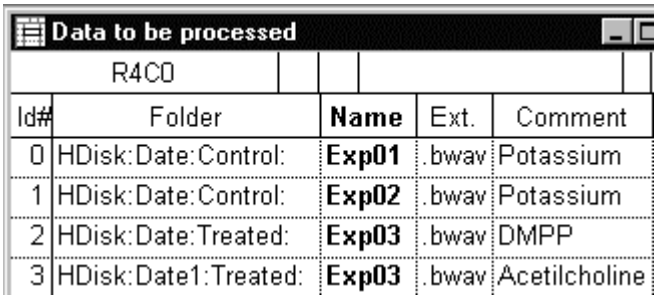
Kill: Remove data from table.

Examples of data file name:

Data file name	First box (initial name)	Second box (number)	Third box (extension)
Exp05.bwav	Exp	5	.bwav
Control32	Control	32	
Exp8.ibw	Exp8	-1	.ibw
Exp10Dic	Exp10Dic	-1	
Ctrol234.bwav	Control	234	.bwav

You can too introduce waves with the same name located in different folders.

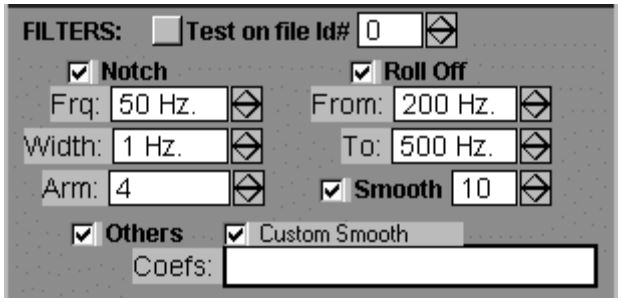
This image shows the adjacent table that program displays with an example of data files to be processed:



Choosing the filter.

Digital filtering is usually required for spike analysis. You will be able to choose the type of filter or a combination of filters. This operation is carried out before the

program starts to find spikes. Macro allows the visualization of the effect produced by a given filter on your data



Test on file Id#: Program filters the data and returns both the original and the filtered wave in a graph. You only have to choose the wave that you want to filter. This wave must be on the list of files already added to the list. **File Id#** corresponds to the row number of the data file present in the "Data to be processed" table. This row number is labeled under Id# column. This option allows the choice of a proper filter for a specific purpose.

NOTCH

Frq Select the main notch frequency to be removed (i.e. 50 Hz).

Width This is the band-width that will be filtered in the frequency domain around the Notch frequency (i.e. 1 Hz means that will be filtered from 49 Hz until 51 Hz).

Arm To select the number of odd harmonic of the main frequency that will be filtered. Program will remove all the harmonic frequencies having an exclusion width equal to that used for the main. In the example given, it will remove four odd harmonics, so bands of 50±1, 150±1, 250±1, 350±1 and 450±1 Hz.

ROLL OFF

From Select the initial frequency from which roll off filter will be applied

To Select the final frequency to which roll off filter will be applied.

This is a very gradual low pass filter. In the image shown above program will start to remove progressively frequencies from 200 to 500 Hz. Subsequently program will suppress all the frequencies above 500 Hz.

SMOOTH

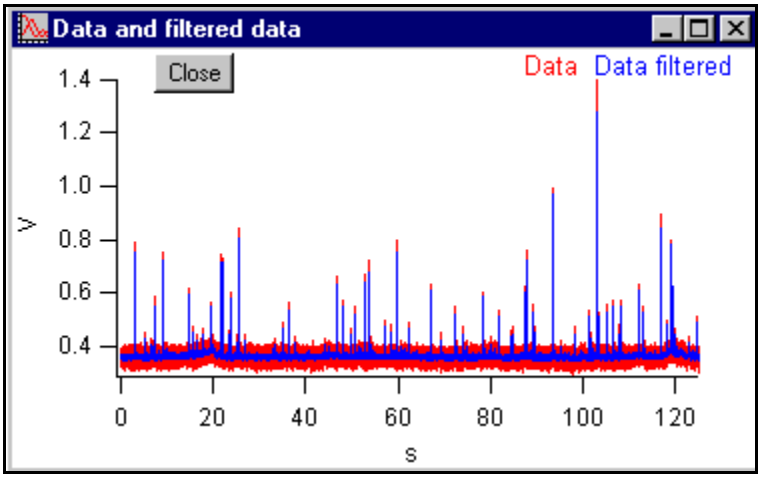
To indicate the smoothing factor. Please refer to the IGOR manual for description of the smoothing filtering properties.

OTHERS/CUSTOM SMOOTH

You can check this option to introduce the coefficient wave of a particular FIR filter. This filter is an igor custom smooth.

Coefs: Type in this field the file name (full path) in which coefficient wave for filtering is located.

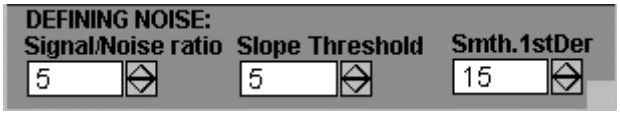
Example:



Effects of digital filtering on an amperometric wave. Raw data are displayed in red whereas filtered data are displayed in blue. Note noise reduction and that some spikes were altered. You can use cursors to zoom into a particular area of interest.

Defining noise.

This part of the main panel is used to define what is and what is not an amperometric spike. Therefore, the values selected are very important when program is finding spikes.



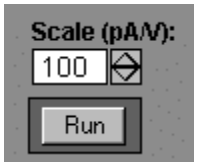
Signal/noise ratio: This value will be multiplied by the standard deviation of noise obtaining a threshold used to ignore small spikes. A bigger value will result in fewer spikes found.

Slope Threshold: This value will be multiplied by the standard deviation of noise slope giving a threshold, this then being used to find spikes in the first derivative of the data. A bigger value will result in fewer spikes found.

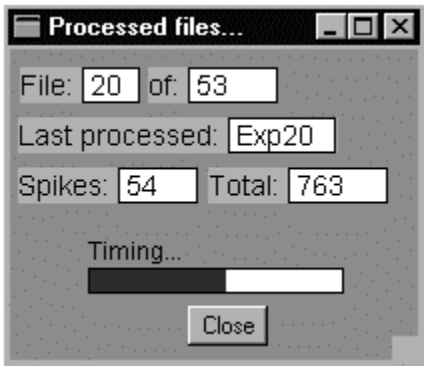
Smth. 1 stDer: This smoothing factor will be applied to the first derivative of data. This factor does not modify spikes and is only used to ignore noise peaks in the first derivative.

Choose the gain and start the analysis.

Scale pA/V: Amplifiers produce an output voltage signal. In order to calibrate the signal in pA, you have to set the gain in this box:



Now by clicking **Run** program starts the analysis. The status of the analysis can be viewed in the "Processed files…" window.



File / of: Number of files processed and total number of data files.

Last processed: Name of the last file processed.

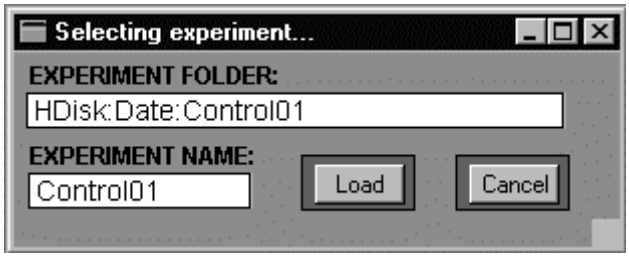
Spikes: Number of spikes found in the last processed file.

Total: Number of total spikes found.

Close: Button to close this window when process has finished.

7- Spike view

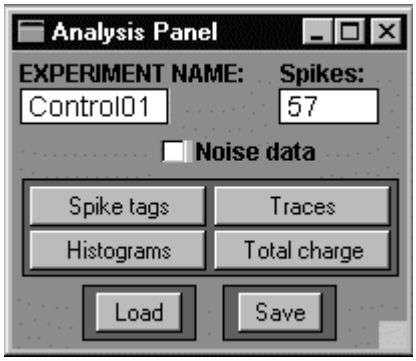
Once the automatic analysis has been performed, the researcher can check the spikes found. Manual corrections can be introduced along this part of the macro.



EXPERIMENT FOLDER: Type in this field the full path of the folder where experiment waves are saved. This folder should now contain a series of new files saved in it, which have completed spike analysis: Data_Control01, X_Beginning_Control01, X_Peak_Control01, X_Final_Control01, etc. Each wave name ends with the experiment name for easier identification.

EXPERIMENT NAME: Type in this field the name of the experiment of the original wave previous to the analysis, without extension, where raw data was located.

LOAD: With this button, all result waves from the folder and experiment typed are loaded in memory and four new windows will appear. One of them is:



EXPERIMENT NAME: In this box the current experiment name is displayed.

Spikes: Number of spikes found within the file.

Noise data: Click in this check in order to show/hide the information about the segment of noise located and analyzed in the spike analysis.

Spike tags: Click in this button for show/hide tags to the spikes in the graph on the right of this previous panel. This graph is shown below.

Traces: Click in this button to display graphs, in the case of they were closed.

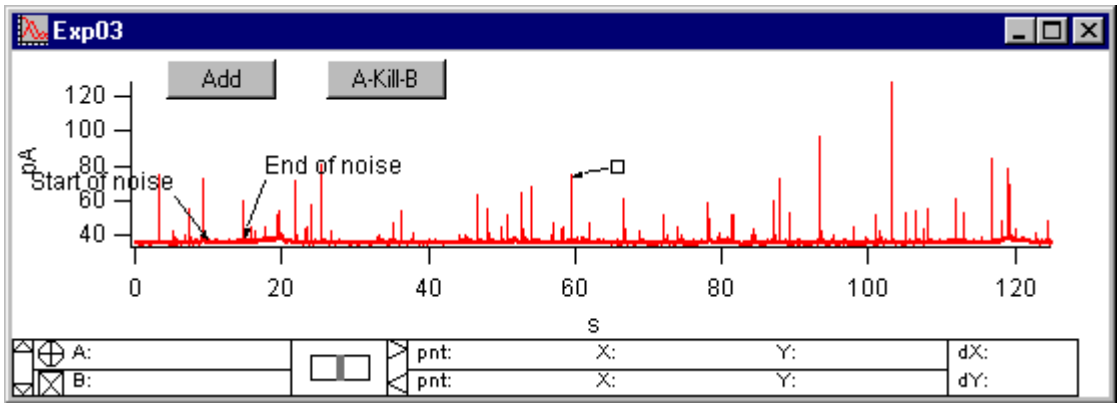
Histograms: Click in this button to see histograms obtained from spike parameters.

Total charge: Click in this button to see the cumulative charge of the spikes.

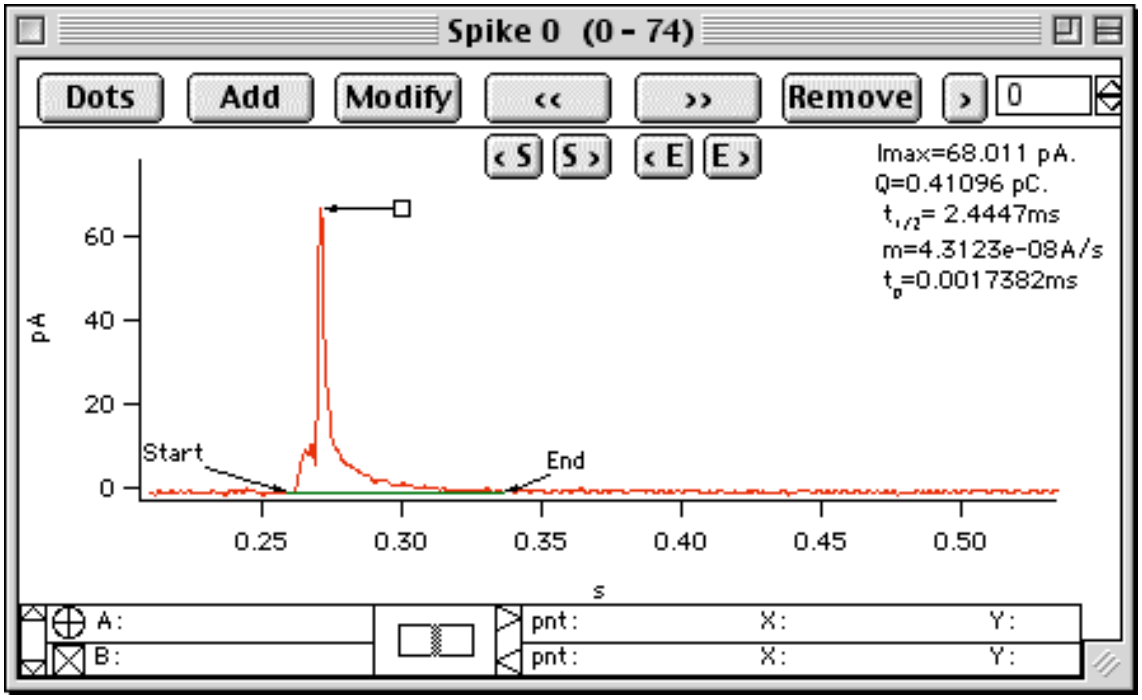
Load: Load new experiment resulting from "Spike analysis".

Save: Save corrected data.

Example: The following graph shows a trace where the baseline segment used for the computer to analyze the noise is shown. A secretory spike not detected by the spike analysis can now be added by placing cursors at initial and final points and pressing **Add**. Conversely, a group of undesired spikes can be removed placing cursors and pressing **A-Kill-B** button.



The lower windows will zoom, at two different scales, the spike marked with square in the upper graph.



This window shows the starting and the end points of the spike, as well as all of the kinetic parameters quantified.

Dots/Line: Change the graph to dots or line between dots mode.

Add: Work in a similar way as in the upper window by adding a new spike to the table. To add a new spike place cursors at both sides of the trace before pressing Add.

Modify: In the case that the beginning or the end of the spike were located in a wrong place, they can be modified by dragging the cursor(s) to the desired point(s) before pressing Modify.

<< Show previous spike.

>> Show next spike.

Remove: Remove shown spike from the record. It deletes the spike from the table of results.

> Choose the number of spike you wish to show before pressing >.

Besides these buttons, in the 1.1 version four new buttons have been added in this graph for modifying the beginning and final points of the spike in a quicker and more precise way. They are:

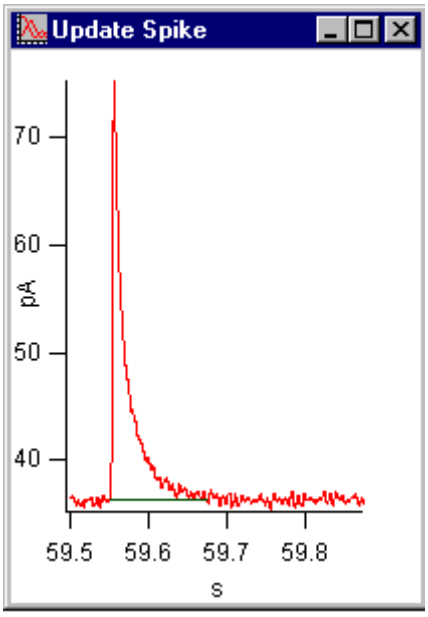
<S The start of the spike is moved backwards five points.

S> The start of the spike is moved forwards five points.

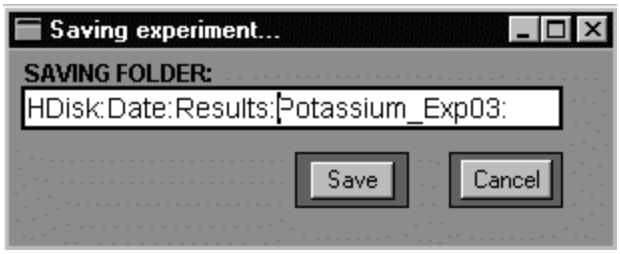
<E The end of the spike is moved backwards twenty points.

E> The end of the spike is moved forwards twenty points.

The right-lower graph offers a view of the spike in a different scale. It helps to check whether the initial and end points of the spike were correctly found**.**



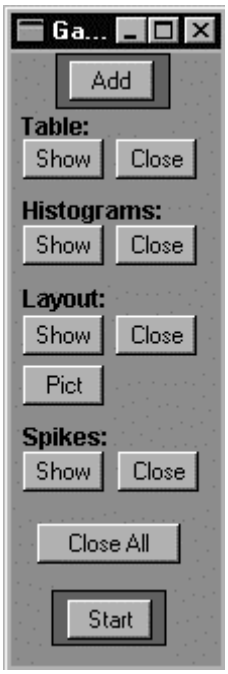
When the spike view has been finished you can save your work through the following dialog window which appears pressing Save button.



8- Galleries

This third part of the macro has been created to pool spikes from different files obtained under the similar conditions. You can make a new gallery by adding spikes to the table from different files. Firstly, you must locate the file where the analyzed spikes, obtained from the “Spike view” program, are present and then add them to the following

table in the next window. Once spikes have been pooled, this constitutes a new IGOR experiment file which can compile control files to be compared with another named, for instance, Drug#1. You can visualize the table of data and the histograms resulting from the pooled data. You can export histograms to a graphic program. This window also produces a preview of data which can be printed.



Add: Add spikes from one file to gallery.

TABLE

Show: Show the table with all spikes added and their kinetic parameters.

Close: Close the gallery table.

HISTOGRAMS

Show: Make histograms from pooled data.

Close: Close histogram windows.

LAYOUT

Show: Preview of data histograms and table. To get them on the printable sheet you must have all of the graphs and/or tables already displayed on screen. You can modify the presentation and introduce decorations and legends.

Close: Close layout sheet.

PICT: Save layout as PICT image.

SPIKES

Show: It shows the spikes added to the gallery.

Close: Close the window shown.

Close All: Close all graphic and table windows from this gallery

Start: Start a new gallery.

9- Displaying data

Macros allow to present data as histograms, tables, etc…This is very convenient as a working page resulting from a working day or for the internal use within your lab. These options are offered in the Gallery window. However, IGOR is not intended as a graphical program. Using the copy an paste option you can export graphs to these programs (Canvas, Adobe Photoshop, Adobe illustrator, Power Point, etc…).

10- Statistical analysis of data

IGOR allows the calculation of some descriptive statistic parameters. They are located in the "Analysis" menu in the "Wave stat…" option. However, most of statistic programs accept IGOR tables. We use the copy and paste option. Be sure that comas and periods are appropriately used.

11- Troubleshooting.

As it was mentioned, you can use the on-line help (Show balloons help in Mac or **mouse+F1** option in PC). For IGOR specific problems please referred to the IGOR manual.

Some old versions of IGOR could have problems to calculate the FFT provided they required a power of 2 number of points, please referred to the IGOR manual.

Macros can enter in conflict with others opened macros. Before start the analysis please close and kill all loaded macros. You can use the “New experiment” option to start from the beginning.

Mistakes typing the path of the file are very common. Please use “**:**” character between folders. In case that the problem persists it could occur when files are stored in a different disk (Zip, magneto-optical units, etc…) try to save them directly in your hard disk.

Macros work only with waves not with already made IGOR experiments. You must unpacking experiments prior to start the analysis.

Please try to fix the problem by yourself, you can, however, contact us (rborges@ull.es).