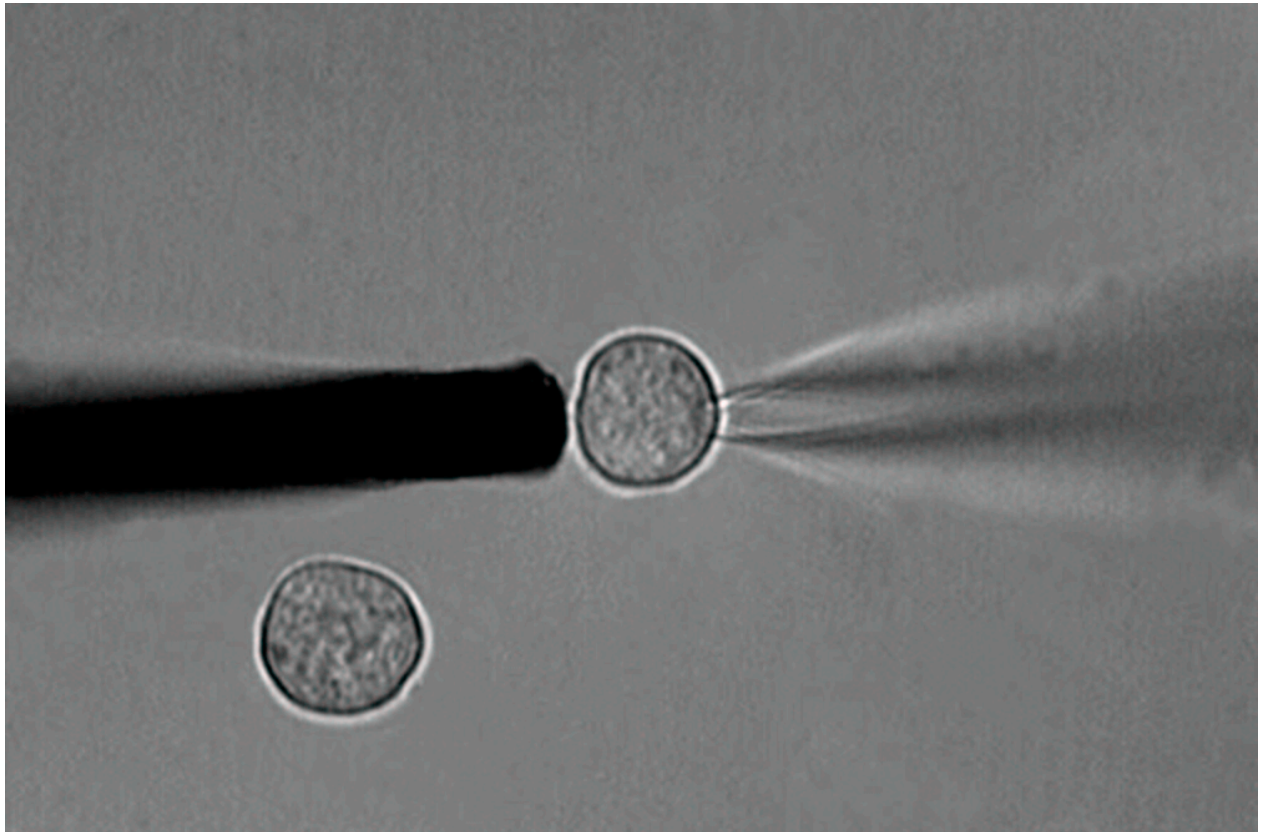


Patcher's Power Tools

An Igor Pro™ Tool Collection (not only) for Electrophysiologists



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Introduction

Patcher's Power Tools (PPT) is a software package that adds further functionality to *IGOR Pro*, a scientific analysis program from WaveMetrics, Inc. running on Macintosh OS 9 and OS X and Windows (visit <http://www.WaveMetrics.com> for more details and to download a demo). Although *IGOR Pro* is very powerful and comes already with several built in features useful for the scientists' everyday life, its abilities still can be further expanded with so called "*macros*" and "*external operations*".

Macros contain instructions or "commands" written in a programming language that *IGOR Pro* understands, very similar to batch files or scripts known from operating systems like *Unix* or *MS-DOS*. The commands are interpreted by *IGOR Pro* and translated into executable code whenever you start a new experiment (in case of "functions") or when the instructions are to be executed ("procedures"). Since macros are simple text files, they only need *IGOR Pro* to run and can be executed within any environment (Windows or Macintosh) almost without further modification. Macros don't need to be installed in any way, you can open the files containing them and run them at any time within *IGOR Pro*. However, if you want them to be loaded "automatically" during startup so they are accessible every time you run *IGOR Pro* you have to put them into the "Igor Procedures Folder" which you can find within the "Igor Pro Folder" (see below).

XOPs (=eXternal OPeration) in contrast to macros are extensions to *IGOR Pro*. They contain executable code compiled specifically for the target machine. They are designed to run upon and have been written in a higher programming language like 'C'. XOPs communicate with *IGOR Pro* through a defined protocol. Once installed, they behave as if they were a part of the program themselves, so you can't distinguish what comes from *IGOR Pro* and what from the XOP. XOPs have many advantages over macros: they run faster since they don't have to be compiled during runtime any more, also modern development environments usually create much better code than *IGOR Pro* could. There are almost no limitations to what an XOP can do, communication with the operating system, file I/O and several other things that are hard or impossible to deal with macros can be done. However, XOPs are harder to develop, you have to be familiar with *IGOR Pro*, 'C', the development system (incl. the protocol defined by the XOP toolkit), and the target operating system in order to create them.

Overview

The XOP(s) add the following functionality to Igor:

- Statistics of multiple waves
- Plotting of multiple waves against one x-axis
- Wave slimming
- Calculation of buffered calcium concentration, liquid-junction potential, and patcher's calculator
- Direct loading of HEKA's Pulse™-, PatchMaster™-, and X-Chart™ files, incl. preview and calculation of lockin data

The macros add the following functionality to Igor:

- Renaming/killing of multiple waves

- Drawing scale bars, zero lines and time markers
- Setting the cursor range to NaN
- Control bar for easier modification of graph axes
- Control panel for automated peak analysis
- Control panel for automated single- and double dose-response fit
- Control panel for automated Boltzman fits
- Control panel for loading Pulse-/X-Chart files

Installation

There are two packages available, one for Macintosh and one for Windows. The macros and experiments will run on both Igor platforms, Macintosh and Windows. Some of the macros added by the PPT require *IGOR Pro 3.13* or later to run, whereas the Igor extensions (XOPs) will be compatible with *IGOR Pro 3.16* or higher.

The whole package consists of the files:

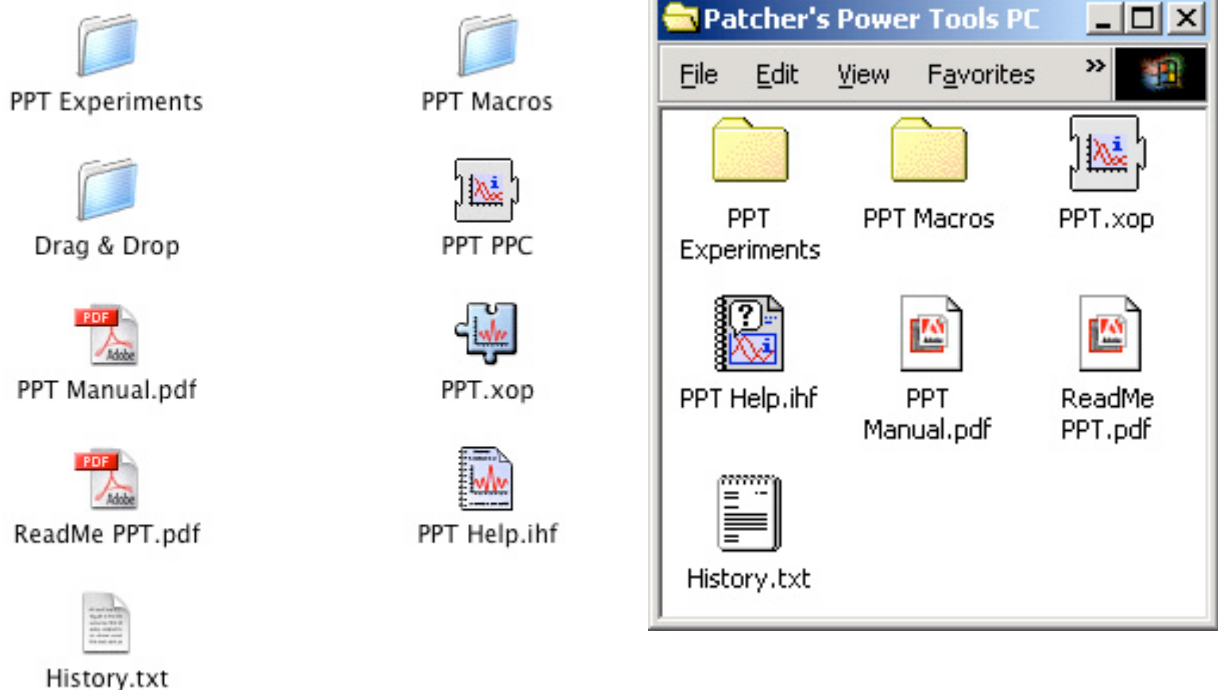


Fig. Contents of the PPT distribution package (Mac and Windows)

- **PPT.xop** – the Igor extensions for Mac or Windows
- **PPT PPC** – the Igor extensions for Mac Classic
- **PPT Manual.pdf** – this Manual
- **PPT Help.ihf** - a help file explaining the added external operations
- **ReadMe PPT.pdf** - a short description of PPT and the installation
- **History.txt** - a file explaining the changes from version to version
- **Drag & Drop** - D&D utilities to convert text files from Pulse *.pgf and *.pul files (Macintosh only)
- **PPT Experiments** - a folder containing Igor experiments demonstrating some features

- **PPT Macros** - a collection of useful Igor macros including:
 - **PPT Control Panel** - a control panel for easily modifying (chart) graphs
 - **PPT Peak Analysis** - a control panel for analyzing peaks (incl. a demonstration experiment)
 - **PPT Dose Response** - a control panel for dose-response fits (incl. a demonstration experiment)
 - **PPT Boltzmann Fit** - a control panel for Boltzmann fits (incl. a demonstration experiment)
 - **PPT Pulse Utilities** - macros extending the Pulse-/X-Chart Loader
 - **PPT Utilities** - general macros and routines needed by the other macros

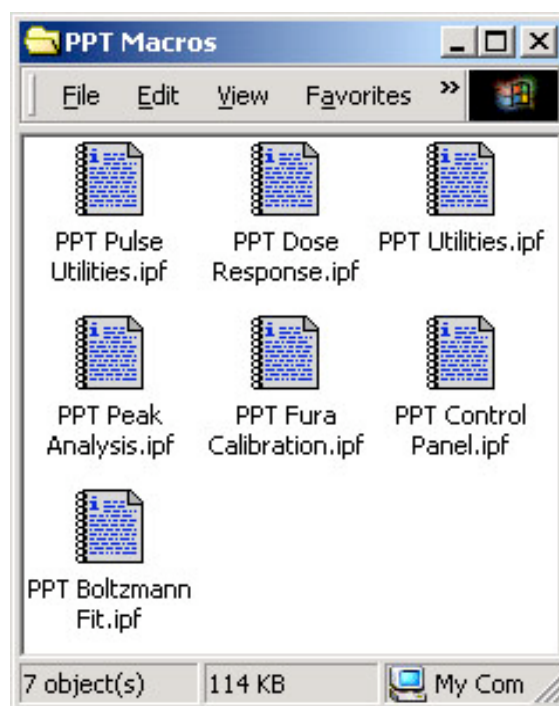
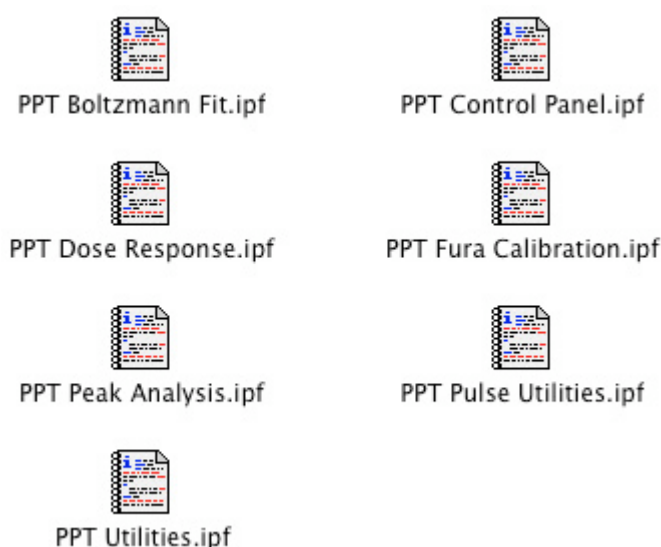


Fig. Contents of the PPT Macros folder

To install the package move **PPT PPC** (Macintosh package classic version) or **PPT.xop** (Macintosh package carbon version) or **PPT.xop** (Windows package) to the folder **Igor Extensions** inside your **Igor Pro** folder. Alternately you can create aliases/shortcuts instead. If you create aliases/shortcuts, you must not move the helpfile **PPT Help.ihf** to the **Igor Extensions** folder.

If you want to permanently access the macros move the folder **PPT Macros** (or of the macros you wish to use) to the folder **Igor Procedures** (again you can create aliases/shortcuts instead). When Igor is started the next time, a new menu will be added, called "PPT" and the macros will be installed in Igor's "Macros"-menu (both systems, Macintosh and Windows).

Getting Help

If you need help: all control panels added by the macros support balloon help. If you want to include PPT's help into Igor's help, place an alias of **PPT Help.ihf** in the **Igor Help Files** folder. You can get help from the Command Help of the Help Menu. In Igor version 5, the help file stays in the Igor Extensions folder.

Loading Pulse- and X-Chart Data directly into Igor Pro

Although *Pulse*, *PatchMaster* and especially *PulseFit* and *PulseTools* do a great job on analyzing electrophysiological data - even online during a running experiment - these programs of course, cannot offer every imaginable kind of analysis for modern applications of the patch-clamp technique. Therefore sometimes it might become desirable to use *Igor's* enhanced flexibility in order to handle more complex experiments or to do some unusual analysis. *Pulse* offers for this purpose the possibility to export its data into a format that *Igor* can easily read. Unfortunately, this export consumes extra time to write the data and additional hard drive space since it produces an extra copy of your data in the old "unpacked" *Igor* format (i.e. one file per sweep). It would be handier and it could save you a lot of time if you could import the requested data directly into *Igor*.

The PPT XOP allows you to import all kind of raw data generated by *HEKA's* *Pulse-* and *PatchMaster-package* for electrophysiologists directly into *Igor Pro* without having to export them within *Pulse*, *PatchMaster*, or *X-Chart*. The XOP supply both, an easy-to-use *Igor* styled graphical user interface allowing you to preview your experiment and load data with one mouse click as well as a command line interface for the more experienced user. The supported data formats include raw current traces from original electrophysiological recordings together with their leak current and second trace, *X-Chart*-data generated either by *X-Chart Standalone* or the *Pulse* extension, as well as Lockin- and Fura-data. However, at the moment the fast *X-Chart* trace is not supported yet, but you can easily load it using *Igor's* *GBLoadWave* command. The data can be read on and from either computer platform supported by *Pulse* and *PatchMaster*, Apple Macintosh (PowerPC-format) or Windows (Intel-format with reversed byte order) in 16- or 32-Bit format. You can load single traces, sweeps, series, groups or even a whole experiment with one single call to the XOP. If you load *X-Chart* data *Igor* will recreate a graph window just as you specified it within *X-Chart* - if you wish so.

Importing Data generated by Pulse or PatchMaster

Calling **Load PULSE File** from the PPT menu brings up an *Igor* styled dialog allowing you to select the data file to import from and to specify the required options. After selecting all options, the dialog will generate a command line to be executed by *Igor Pro*. Alternatively you can write this command and call the XOP directly from the command line or from within an *Igor* procedure/function in case you want to automate analysis of electrophysiological experiments. You can also call back an earlier created command and edit it to load different sweeps, series or data from different experiments. Together with the macros supplied by the procedure file *PPT Pulse Utilities* you can easily reconstruct a complex electrophysiological experiment consisting of hundreds of sweeps containing *Pulse-*, *PatchMaster-*, *X-Chart-*, *lockin-* and *fura-data*, within *Igor Pro*

The *Load PULSE File* dialog window consists of several parts. The button **File...** will call the file selector allowing you to specify the desired *Pulse/Patchmaster* experiment (it uses the file extension ***.dat**). The latter will also happen if you call the XOP and no file had been specified so far or in an earlier *Igor* session (the XOP remembers together with its settings the last *Pulse/PatchMaster* file you were using). Below that button you can see four popup menus, each one of them representing the group-, series-, sweep-, and trace-number to be loaded. The popups will reflect the *names* of the actual group or series, which are read out of the experiment. Since this may consume some time to build the popups you can speed this up by disabling the naming feature by selecting the **Fast Popups** checkbox. In this case the popups will only show the *numbers*.

Load Pulse™ File

Select previous/next series/sweep/trace
Specify the file to be loaded from

File... 140Frank_HD1:Data:050610003.dat

If checked, popups will show numbers instead of names

Group 1: E-1 Fast Popups
Series 3: CaCu10ms
Sweep 1
Trace all_

Specify the object (group, series, sweep or trace) to be loaded

Stimulus
passiv
1-33msCaCu
CaCu10ms
IV10ms

Exclude or include sweeps with the selected stimuli

Include

Buttons to control the oscilloscope

Options

Base name of waves to be created

Base name \$

Range

☐ Load Leak Trace
☐ Get Stimulus Template
☐ Load 2nd Trace
☒ Overwrite Waves
☐ Plot Traces
☐ Absolute Time
☐ Load Fura
☒ Calculate Lockin
☒ Prevent History Printing
☐ Subtract Zero-Data

Specifies the loading options

Phase Shift and V-reversal can be specified for Lockin calculation

Phase Shift
V-reversal

Command line to be executed

LoadPulse /b /q /s /h=1 /B=3 /b=1 /H= '050610003' "140Frank_HD1:Data:050610003.dat"

These buttons have the same functions as in Igor

Do It To Cmd Line To Clip Cancel

Root, Group- and Series-Info

Sweep- and Trace-Info

Oscilloscope window

Series Info

%Start Time: Fri Jun 10 2005 17:15:47
Mode: WholeCell
Comment: 0
Internal: 0
Hold. Pot.: -80 mV
Sequence: CaCu10ms
User Par 1: User Param 1 0.0000000 V
User Par 2: User Param 2 0.0000000 V
Temp.: 20 C
Bandwidth: 4500 Hz
Cell Pot.: 0 mV
R-Pip: 1.0 MOhm
R-Seal: 0.9 GOhm
Phase:

Sweep Info 1(1)/3(15)/1(12), Pulse Time 00:42:40
Timer: 02:11:28.796
Stim Count: 3
Sweep Count: 1
Average Count: 1
Has leak: yes
Has second trace: no
Label: 19000
Data points: 16.13 pF
CSlow: 11.484 MΩ
RSeries: 5.731 MΩ
Stimulus: CaCu10ms

You can select the whole experiment or number of consecutive groups, series, sweeps or traces

as defined by the text item **Range**. If no trace is specified, the whole sweep will be loaded, if no sweep is specified, the whole series will be loaded, if no series is selected, the whole group will be loaded and if no group is selected, the XOP will load the whole experiment. On a Macintosh you can interrupt the loading process in *Igor* at any time by pressing the Macintosh interrupt hotkey **Cmd+dot**, which is especially useful if you accidentally selected the whole experiment.

The upper two boxes in the right half of the dialog show some information about the current selection as specified by the four popups. If this is the whole experiment (*group = all*) you will get general information about the experiment in the left box like time and date of the experiment as well as the total number of groups, series, sweeps, and traces. If the current selection reflects a group, you will be informed about the group settings time, label, text, number of internal and external solution and the name of the pulse protocols of each series. If you select a whole series (*sweep = all*), the left box will display some EPC9 parameters. If you select a single sweep to load, the left box will show the series info and the right box the sweep info or the trace info for PatchMaster files. The lower box in the right half will represent a graphical preview of the traces or sweeps and also the Capacitance, if *Calculate Lockin* is selected and if there is lockin data available, as can be seen in the *Pulse/PatchMaster* oscilloscope window. In this case you can scale this preview by using the buttons below the box.

The bottom box shows the composed command line, which gets updated as you select one of the options. If you click the **Do It** button, the dialog will be closed and Igor will execute this command immediately. "**To Cmd Line**" will copy this command into the command line, allowing to edit (or finetune) it before execution. If you want to create an Igor macro you can also copy this line to the clipboard (**To Clip**) and paste it into a procedure window. Clicking one of these three buttons will also save the current settings in the XOPs resource fork for a later call of the dialog. Clicking **Cancel** will close the dialog without doing anything and without saving the settings. Here is the complete syntax of the command if you want to call the Pulse/PatchMaster loader from the command line.

LoadPulse

```
[/A=groupNum /B=seriesNum /C=sweepNum /R=rangeNum
/N=baseName /L/S/I/O/P/T/G /H=phaseShift /V=V-reversal /F
/J=stimulusName /J=$stringValue /K=stimulusName
/K=$stringValue /Q] fileNameStr
```

LoadPM

```
[/A=groupNum /B=seriesNum /C=sweepNum /D=traceNum
/R=rangeNum /N=baseName /I/O/P/T/G /H=phaseShift /V=V-
reversal /J=stimulusName /J=$stringValue /K=stimulusName
/K=$stringValue /Q /Z] fileNameStr
```

Parameters

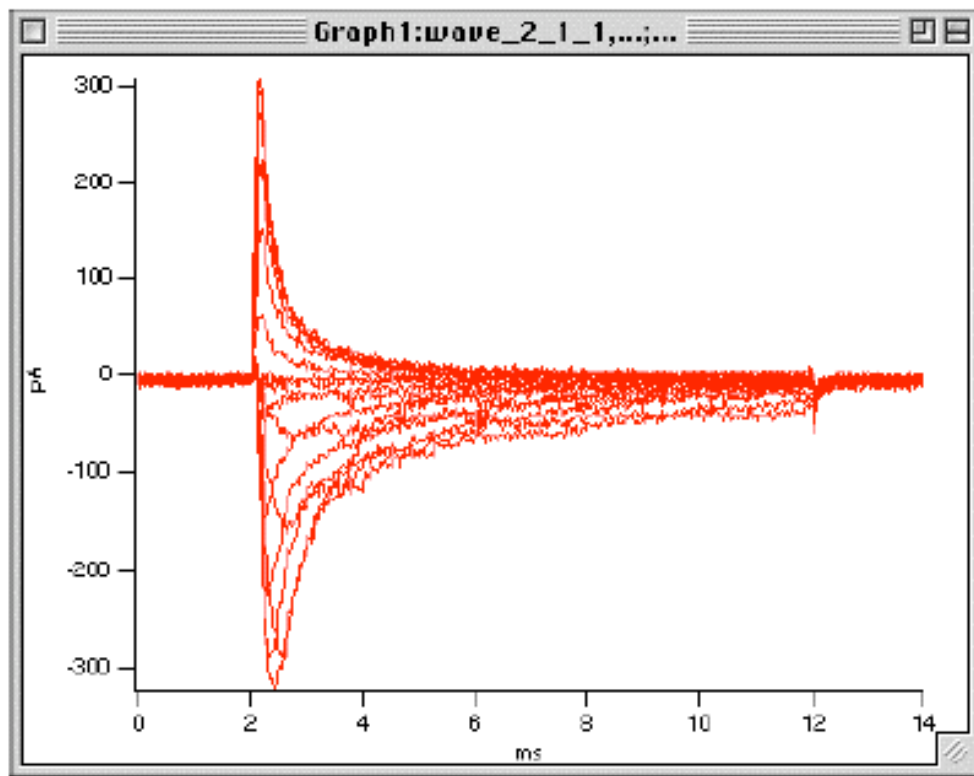
fileNameStr is the full file name specification of an existing Pulse™/PatchMaster™ file. When loading, the filename will be truncated after the right most dot and "dat" will be appended, so please make sure, your naming matches this convention.

Flags

/A=groupNum number of group to be loaded. If not specified, the whole experiment will be loaded into RAM. **Cmd-dot** will cancel the

	operation on the Macintosh.
/B=seriesNum	number of <u>series</u> to be loaded. If not specified, the whole group will be loaded.
/C=sweepNum	number of <u>sweep</u> to be loaded. If not specified, the whole series will be loaded.
/D=traceNum	number of <u>trace</u> to be loaded. If not specified, the whole sweep will be loaded. (This flag works only for LoadPM.)
/R=rangeNum	<u>range</u> to load. Defines the number of objects (groups, serieses, sweeps, or traces) to be loaded beginning with the specified group, series, sweep or traces. (Traces work only with the LoadPM command.)
/N=baseName	is the <u>basename</u> for the waves to be created. If no basename is given, "wave" will be used instead. Wavenames will be generated according to the group/series/sweep number (e.g. "wave_1_5_34" for group 1, series 5, sweep 34). The basename must match the old Igor naming convention (no literal names with spaces or tabs). If a "\$" is specified as basename, the filename is taken as basename. This works only, if the filename match the old Igor naming convention.
/L	load <u>leak</u> trace. If you had leak-subtraction on during data acquisition, Pulse will store the leak subtracted currents together with the difference currents (leak traces) in order to recalculate the original currents which is the sum of the leak trace plus the raw current. With this setting the XOP will also load the leak currents so you can calculate the original current by just adding these traces to the "raw data". "_leak" will be added to the loaded waves. (This flag works only for LoadPulse.)
/S	load <u>second</u> trace. If this option is on, the XOP will load the second trace (e.g. second amplifier with amperometry recordings) in addition to the first trace (e.g. EPC9). "_2nd" will be added to the loaded waves. (This flag works only for LoadPulse.)
/I	calculate stimulus wave. With this option on, the XOP will generate a wave with equal dimensions from the pulse protocol according to the PGF entry. "_stim" will be added to the loaded waves.
/O	<u>overwrite</u> existing waves.
/P	<u>plot</u> traces. With this option the XOP will generate a new graph window displaying the loaded traces.
/T	absolute <u>time</u> . The wave scaling of each loaded wave will be set corresponding to the Pulse timer instead of starting at 0. This way consecutive waves can be concatenated and will be drawn consecutive instead of overlaid. The Pulse timer displays the number of seconds since 1.1.1900 when acquisition of the sweep started. In addition the XOP will generate a new global Igor variable pptPulseTime which contains the absolute time of the beginning of the experiment, when the internal Pulse was reseted the last time. Subtraction this value from the left x value of the new

	<p>wave, will scale the waves relative to the Pulse experiment. This option must be turned on, if you wish to concatenate Pulse data with data acquired within the X-Chart extension using the macros supplied by the procedure file PPTPulseUtilities (see below).</p>
/G	<p>calculate <u>lockin</u> data. If your raw data contains sweeps with sinewave segments (type: ConstSine or SquareWave), the XOP can calculate high time resolved traces containing membrane capacitance C_m, membrane conductance G_m and series conductance G_s (see below). In this case, three further waves per sweep will be generated. You can identify them by their extension “_Cm”, “_Gm” and “_Gs”. The code for the calculation was kindly provided by Dr. Tao Xu.</p>
/H=phaseShift	<p>value of the EPC9 <u>phase shift</u> during lockin measurements. If this flag is not set, 0 will be set as the default value (which is the default with the EPC9).</p>
/V=V-reversal	<p>V-reversal in Volts overrides the internal V-reversal in lockin measurements.</p>
/F	<p>calculate <u>fura</u> data. If your raw data contains fluorescence signals acquired by one of the supported Fura-extensions, the XOP will load these data and generate three further sweeps with the extension “_F1”, “_F2” and “_F3”. (This flag works only for LoadPulse.)</p>
/J=stimulusName	<p>include stimulus. Only sweeps with the specified stimulus name are loaded.</p>
/J=\$stringVariable	<p>include stimuli. Only sweeps with the stimuli names specified in the stringVariable separated by commas are loaded.</p>
/K=stimulusName	<p>exclude stimulus. Sweeps with the specified stimulus name are not loaded.</p>
/K=\$stringVariable	<p>exclude stimuli. Sweeps with the stimuli names specified in the stringVariable separated by commas are not loaded.</p>
/Q	<p>quiet mode, no messages of loaded files are printed to the history</p>
/Z	<p>subtracts a baseline offset. If this parameter is checked, PPT acts like PatchMaster. (This flag works only for LoadPM)</p>

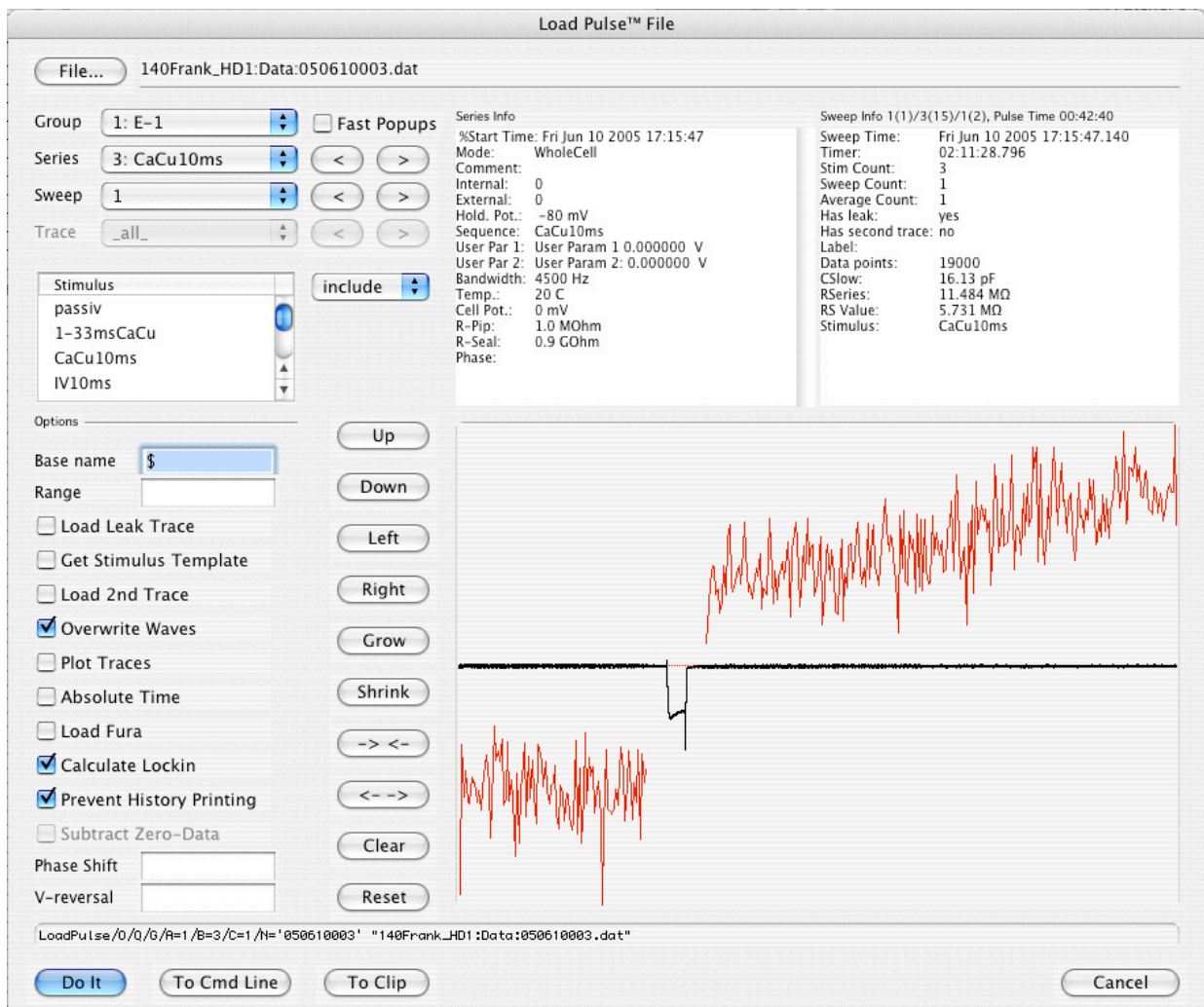


If you had option /P selected, Igor will plot the loaded current traces in a new graph. All new waves (raw, second, leak, stim and lockin results) except for the fura data will be shown in an X-Chart like graph.

Lockin-/Fura Extension

A special case occurs, if the *Pulse™* or *PatchMaster™*-file contains additional information generated by either the Lockin- or the Fura-extension. If the option /G (**Calculate Lockin**) was selected, the file loader will generate for each wave three further waves by calculating the corresponding membrane capacitance, series- and membrane conductance. Calculation - of course - will only be done for all segments of type *ConstSine* or *SquareWave*, the rest of the segments will be filled with NaNs so you can easily plot and analyze current traces, stimulus patterns and lockin waves together. The LockIn results will be printed to the history area during calculation of the waves.

If you acquired fluorescence data together with your *Pulse* data, you can load these by using the flag /F (**Load Fura**). In this case three further waves will be loaded which contain fluorescence values at wavelength 1, 2 and 3. You can identify those waves on their extension (“_F1”, “_F2” and “_F3”).



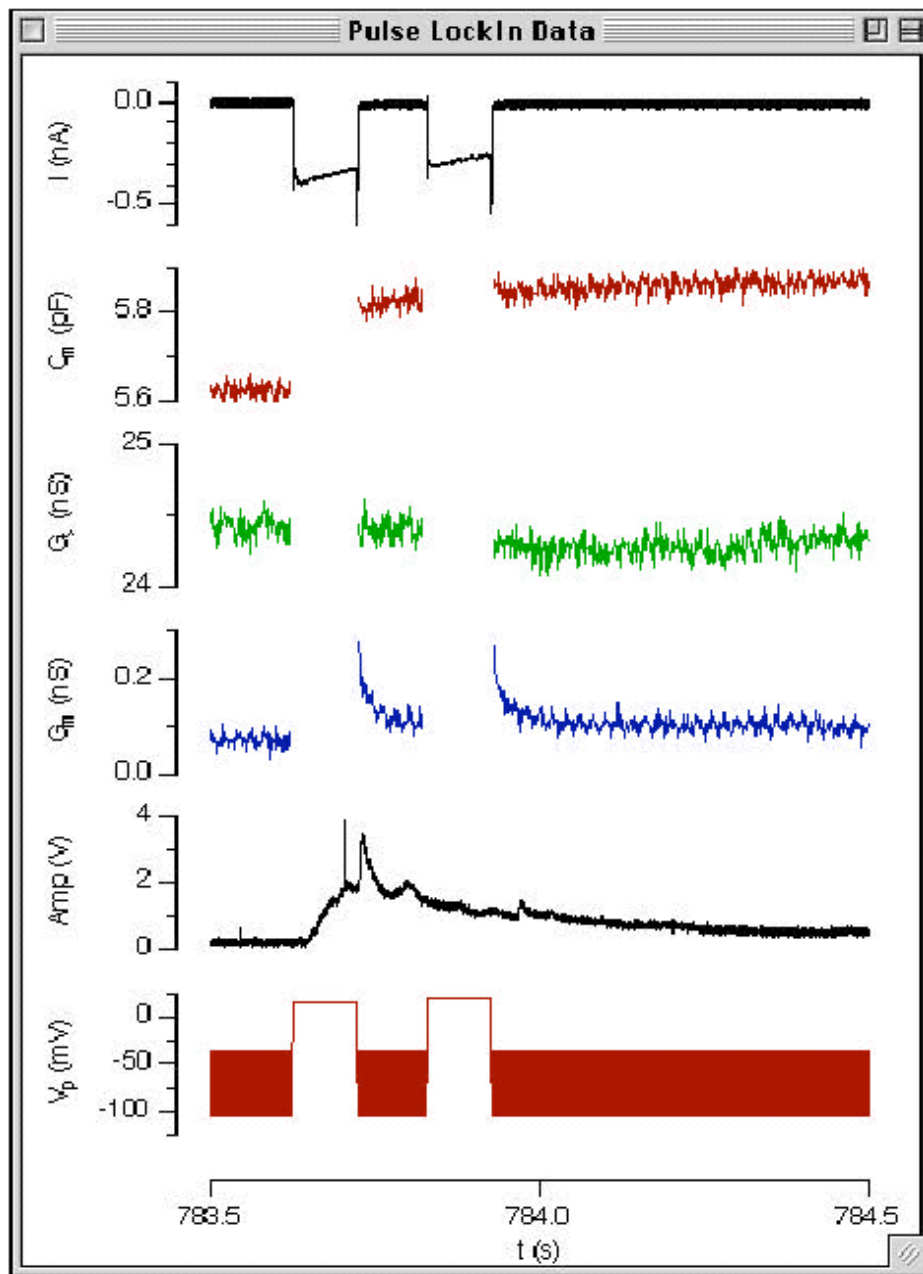
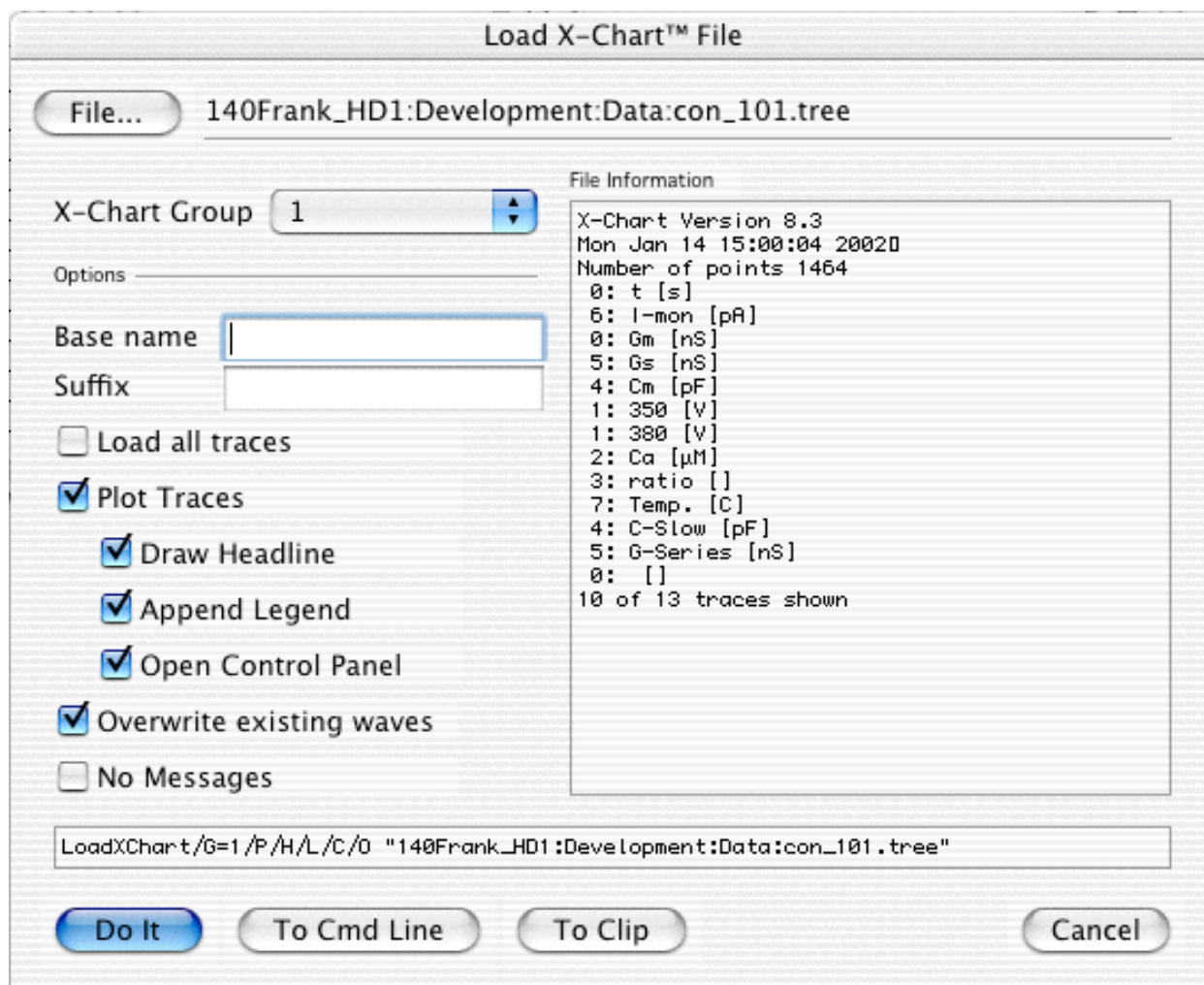


Figure: Example showing a Pulse sweep containing lockin data and a second trace. The upper trace gives the raw current (voltage dependent Calcium and Sodium currents) acquired by *Pulse* as the “first trace”. The next three traces are the lockin waves calculated by the XOP from trace one and the lockin parameters within the *Pulse* file (note the gaps due to segments containing no sinewave stimulation). The fifth trace is the “second trace” acquired by *Pulse* and shows amperometric events from a secreting chromaffin cell detected with a carbon fibre near by the patch-clamped cell. The bottom trace shows the underlying stimulus protocol with sinewaves and constant segments calculated from the corresponding PGF editor entry. The experiment was kindly provided by Dr. Corey Smith.

X-Chart Files

The **LoadXChart** operation loads data from the named *X-Chart* tree file. It can also create a graph according to the file settings specified by *X-Chart*. The fast trace is unsupported, but you can easily import those data using *Igor's* command *Load General Binary Data*.



LoadXChart

[/A/B=baseName/G=groupNum/O/P/Q] fileNameStr

Parameters

fileNameStr

is the full file name specification of an existing X-Chart treefile (*.tree, tested up to version 8.54). When loading, the filename will be truncated after the right most dot and "tree" will be appended, so please make sure, your naming matches this convention.

Flags

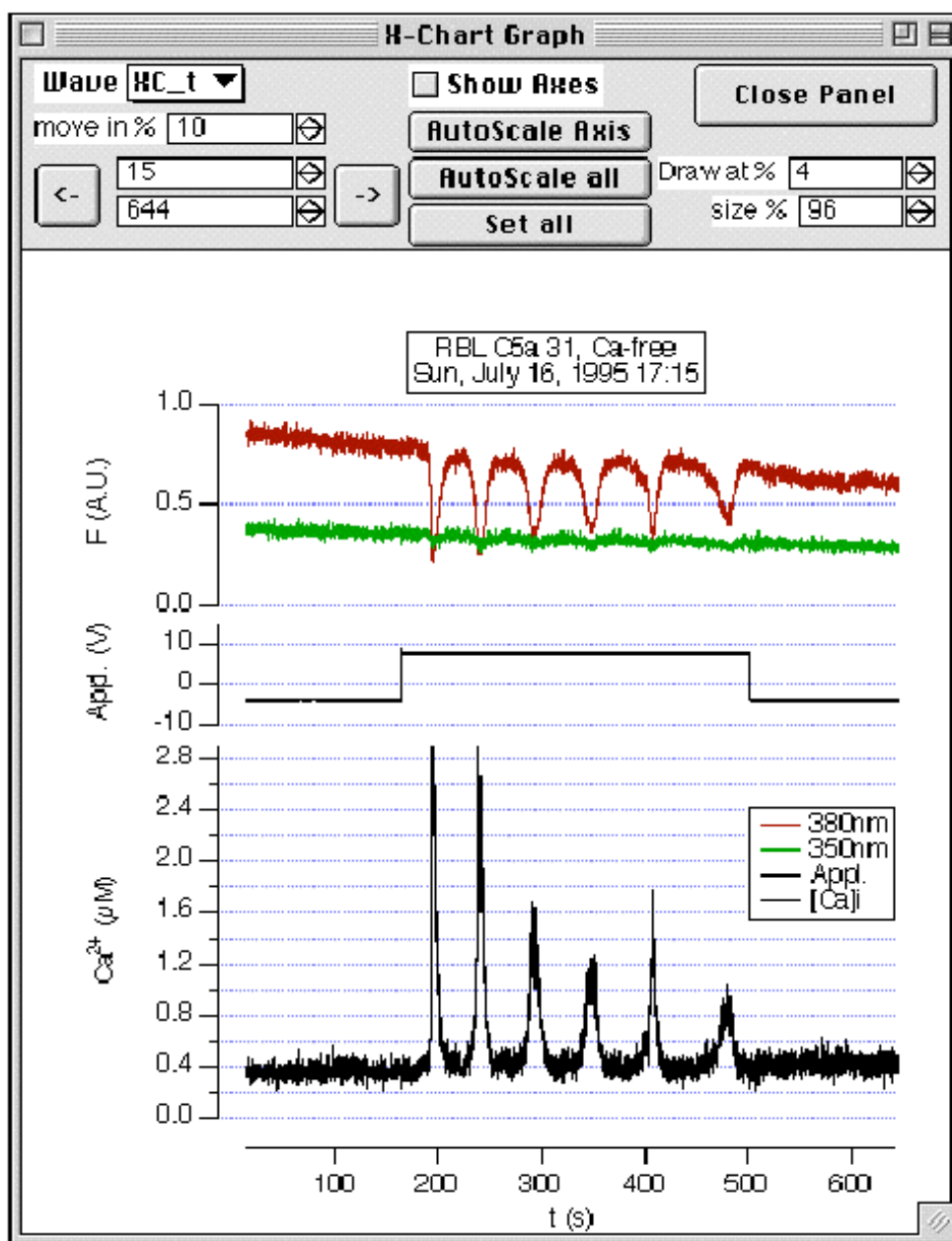
/A

load all waves. Without this flag only those traces will be loaded which are actually displayed.

/B=baseName

is the basename for the waves to be created. If no basename is given, "XC" will be used instead. Wavenames will be generated according to the trace descriptions by filtering out all illegal characters (e.g. "C

	Fast" will give "XC_CFast"). If a "\$" is specified as basename, the filename is taken as basename. This works only, if the filename match the old Igor naming convention.
/G=groupNum	number of the tree-group to be loaded (only of interest, if the data were acquired by the Pulse™-extension, the group number then corresponds to the desired Pulse group).
/O	new waves will overwrite existing once.
/P	the loaded file will be plotted in a new graph.
/H	a headline will be appended to the new graph, showing name, date/time and comments of the loaded X-Chart file.
/L	a legend will be added to the new graph.
/C	the X-Chart control panel will be added to the new graph (if the PPTControlPanel macro was loaded before). This allows you to quickly modify the appearance of the traces.
/Q	quiet mode. If this flag is set, no result will be printed in the history area.



Merging XChart and Pulse Data

Let's consider you have high-time resolved data acquired by *Pulse* and low-time resolved *X-Chart* data. In order to analyze them you want to combine these data into a single *Igor* wave. The first thing would be to load both, the *Pulse*- and the *X-Chart* data. Make sure to load the *Pulse* data using absolute timing of the waves (/T), otherwise, all sweeps would start at 0 and overlay in time, instead of being consecutively timed.

```
LoadPulse /T/G/N=Pulse "HD:Path:PulseFileName.dat"
```

```
LoadXChart /G=1/O/B=XChart "HD:Path:XChartFileName.tree"
```

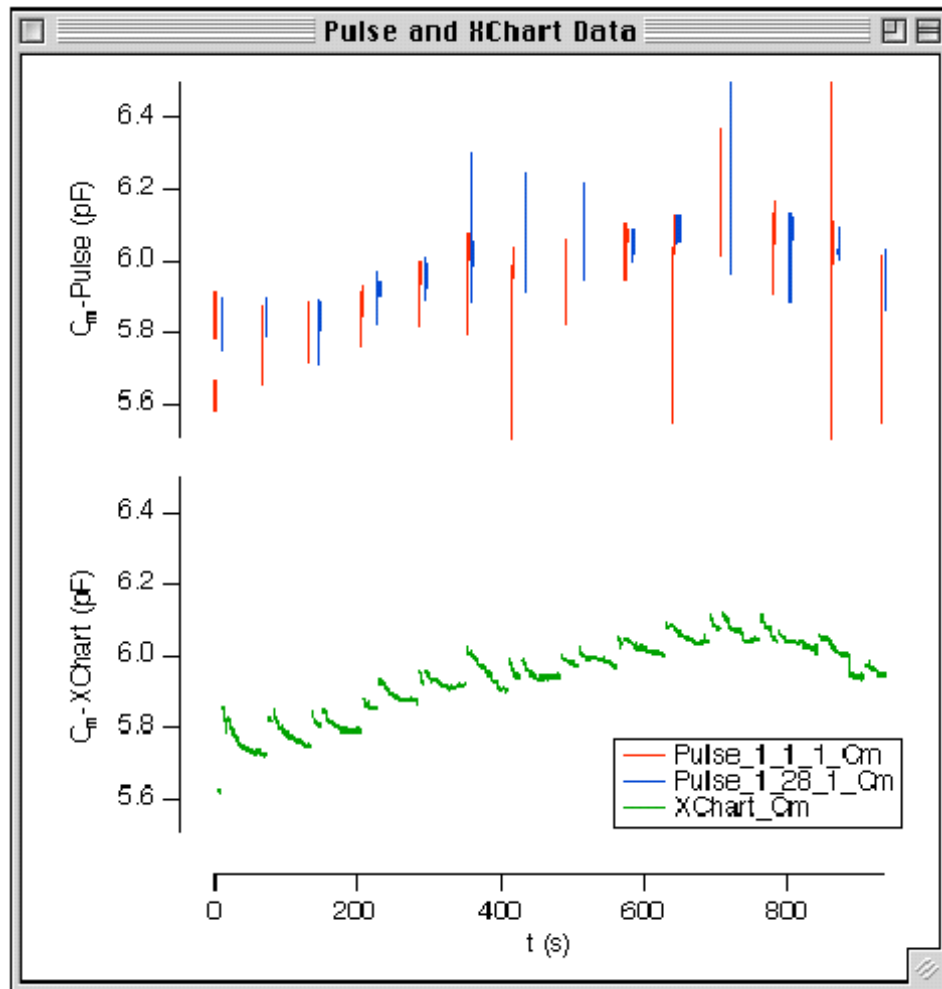


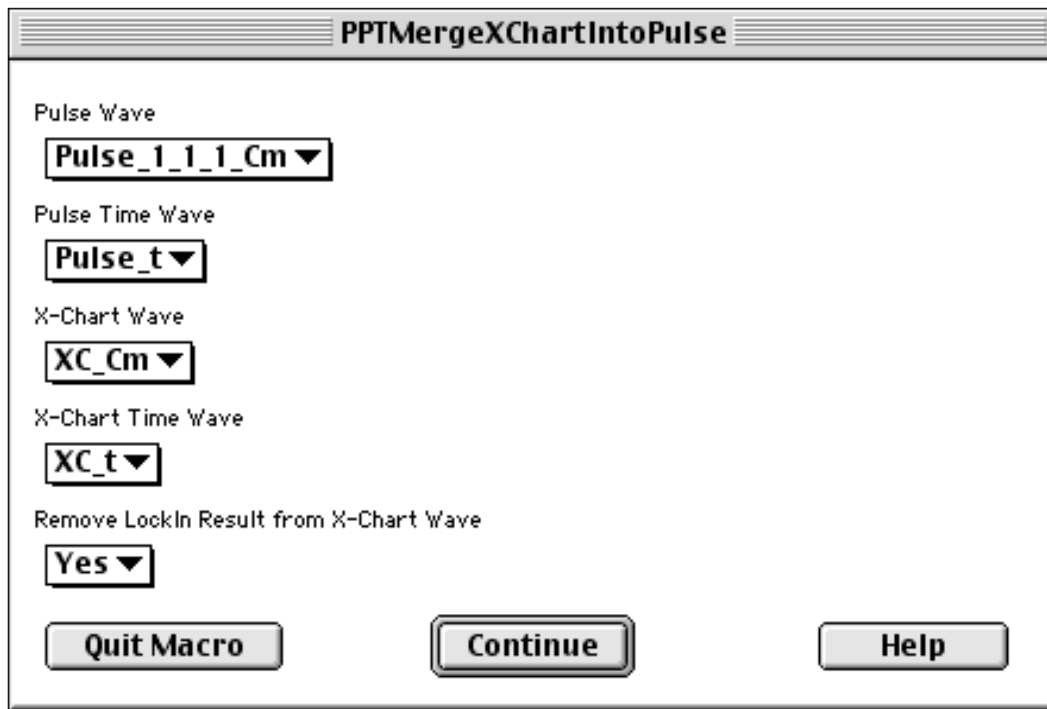
Figure: The upper trace shows 28 fast capacitance sweeps acquired with *Pulse*'s lockin extension and calculated by the *LoadPulse XOP* using the option */G* (frequency 800 Hz). For better clarity every second sweep is given in red or blue color. The bottom trace shows one wave representing the lockin result sampled by the *X-Chart* extension. The values were calculated by *Pulse* online during the experiment in between fast acquisition by applying short sinewave pulses at a frequency of 12 Hz. The example was kindly supported by Dr. Corey Smith.

The next step is to concatenate all desired *Pulse* sweeps (e.g. all sweeps containing the membrane capacitance) into one wave. You can do this by calling the macro *PPTConcatenatePulseWaves*.

PPTConcatenatePulseWaves ("Cm", "XChart", "Pulse_Cm", "Pulse_t")

The macro expects four string parameters: the first string identifies all waves to be concatenated. In this example, every wave containing "Cm" in its name would be considered as input wave (*Pulse_1_1_1_Cm* ... *Pulse_1_28_1_Cm*). Since you want to exclude the *XChart_Cm* wave from being concatenated, although its name contains also "Cm", you use "XChart" as the second parameter. This instructs the macro to ignore every wave containing this substring in its name from input. The result of the concatenation will be two waves, the concatenated *Pulse* data and a timing x-wave. The last two parameters are the names of these two waves.

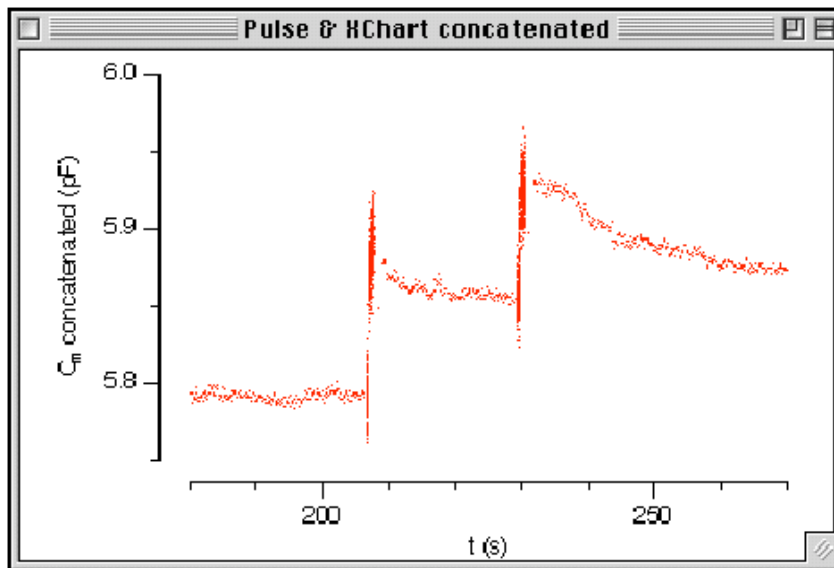
Now you can merge the *X-Chart* data- and time-wave into the two new waves by using the macro *PPTMergeXChartIntoPulse*. If you are merging lockin data you should set the "Remove LockIn Result from X-Chart Wave" popup to "Yes". The background for this is that *Pulse* will calculate the corresponding lockin values during high time resolved sinewave stimulation and will send them as **one single value** to *X-Chart*. These values represent the mean over the whole sinewave segment and interfere with the lockin values calculated from the raw current traces, since they would appear in the middle of the high time-resolved lockin wave.



```
PPTMergeXChartIntoPulse("Pulse_Cm","Pulse_t","XChart_Cm","XChart_t",2)
```

Finally let's see the concatenated result containing both, *Pulse* data in high and *X-Chart* data in low-time resolution:

Display `Pulse_Cm` vs `Pulse_t`



Load Pulse/X-Chart Panel

Users can use the *LoadPulse/X-Chart* control panel to determine interactively the files to be loaded and the loading parameters. The panel supplies access to all parameters for both, the *LoadPulse* and the *LoadXChart* external operation. When you click the buttons, it will generate the command lines according to the selected settings and then call the corresponding XOP. File calls the system's file selector so you can select the *Pulse* file to load (extension ".dat"). The corresponding *X-Chart* file will be found by replacing the extension by ".tree".

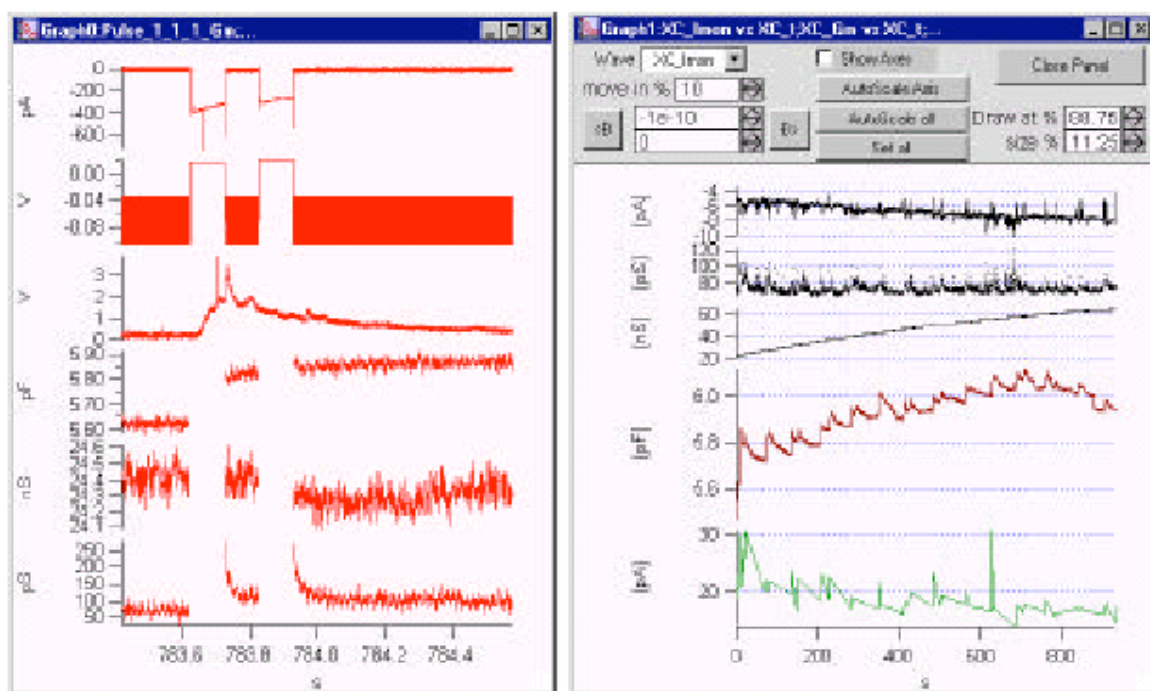
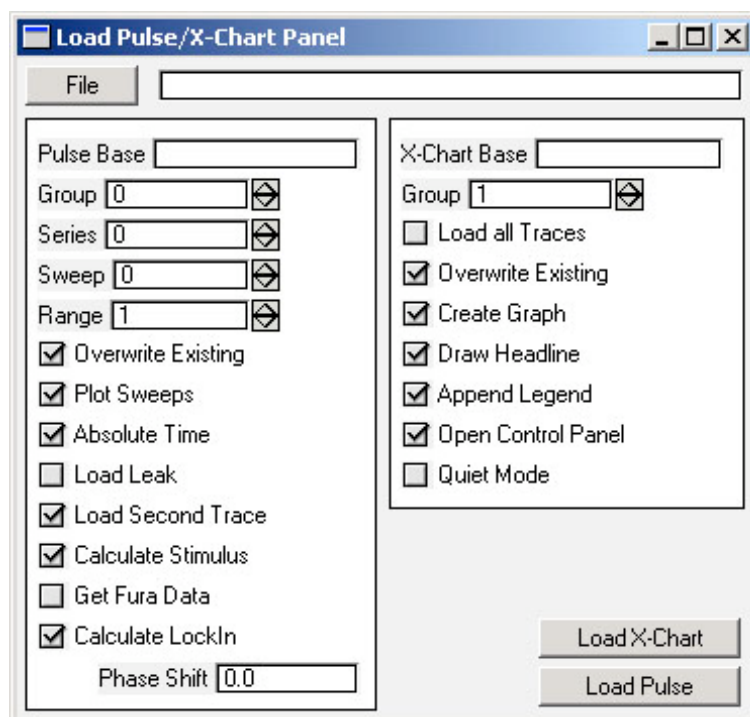


Fig.: Graphs showing loaded *Pulse* and *X-Chart* data from the example shown above. The pulse waves represent (from top to bottom) raw current, stimulus template, second trace and the lockin results.

Dose Response Control Panel

Use this control panel to quickly fit dose-response curves interactively according to the equation

$$y = \frac{y_{\max}^1}{1 + \left(\frac{x}{K_D^1}\right)^{\text{hill}^1}} + \frac{y_{\max}^2}{1 + \left(\frac{x}{K_D^2}\right)^{\text{hill}^2}}$$

To start, you first have to create a graph containing your dose-response relationship, since all operations are done directly in the graph. You can use the example in the file *DoseResponse Demo* from the *PPT Experiments Folder*.

If checked, these parameters will be fixed, i.e. they won't be fitted

Restricts fit to the cursor range

Popup showing the waves currently displayed in the graph

Matching x-waves with same number of points as y-wave

PPT Dose-Response

☐ Max 77.7402 ☐ Max 2 0

☐ KD 10 ☐ KD 2 0

☐ Hill 1 ☐ Hill 2 0

☐ Use Cursors ☐ Double Dose-R.

y-Data Gd_mean x-min 0

x-Data Conc x-max 0

n 0

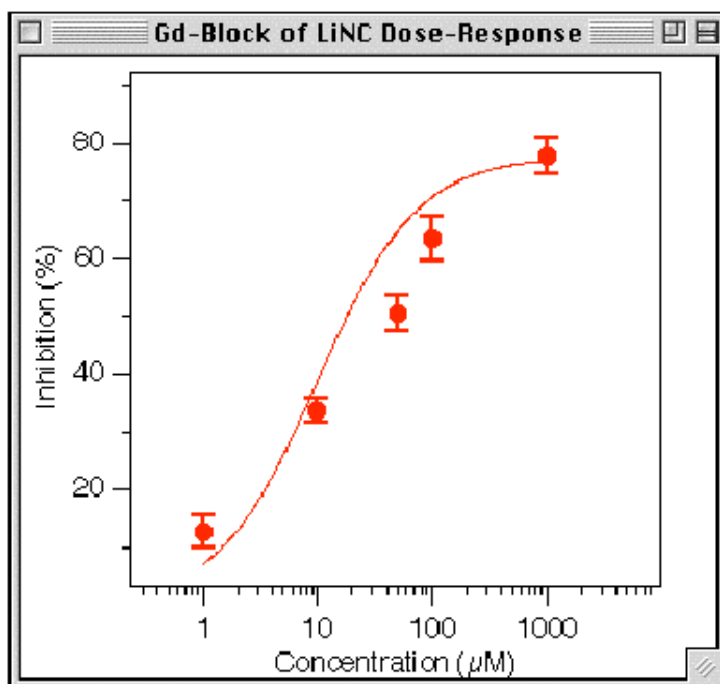
Append Legend Remove Legend

Fit Dose-Res. Auto Estimate Draw Fit

If checked, a double dose response will be fitted

Determines x-range and number of points of the fitted curve

Now you can open the control panel by choosing "Macros/Dose Response Panel". This control panel allows you to easily modify the fitting parameters, preview the fit and do the actual fit. First select the data you want to fit from the y-Data popup, which shows all the waves from the top (or target) graph. The x-Data popup shows all matching waves containing the same number of values as your selected y-wave. Select the corresponding x-wave. If you want to do a doubledose-response, click the Double Dose-Res. button.



PPT Dose-Response

<input type="checkbox"/> Max	87.313	<input type="checkbox"/> Max 2	0
<input type="checkbox"/> KD	23.4183	<input type="checkbox"/> KD 2	0
<input type="checkbox"/> Hill	0.572018	<input type="checkbox"/> Hill 2	0
<input type="checkbox"/> Use Cursors		<input type="checkbox"/> Double Dose-R.	

y-Data: **Gd_mean** ▼

x-Data: **Conc** ▼

x-min: 0.1

x-max: 10000

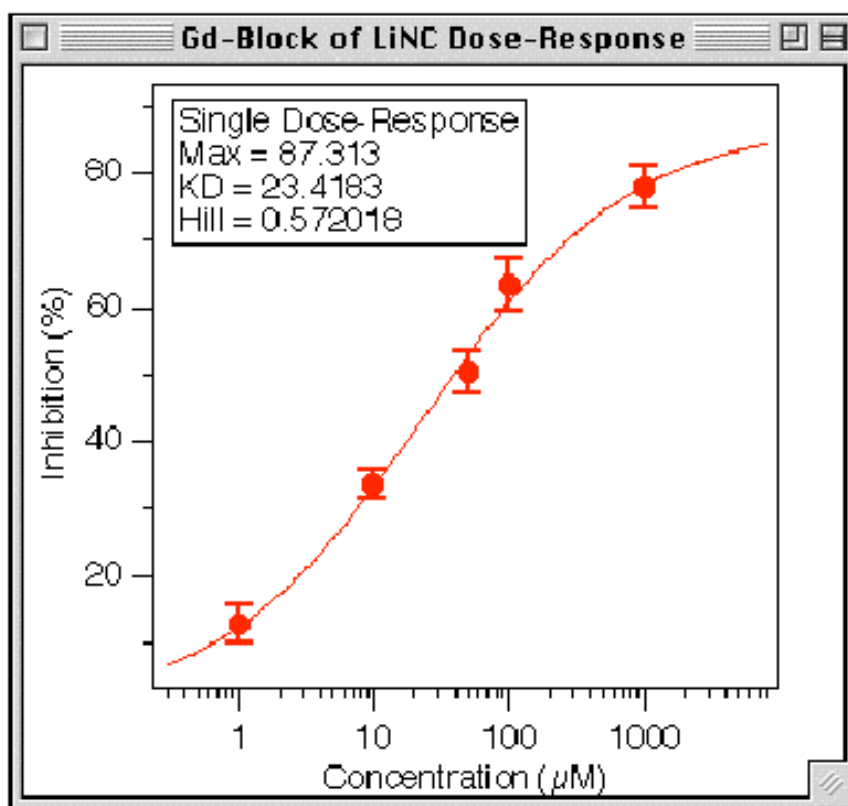
n: 400

Buttons: **Append Legend** **Remove Legend** **Fit Dose-Res.** **Auto Estimate** **Draw Fit**

Click the *Auto Estimate* button to get a primary guess for the fit parameters. Of course you can also type your initial guesses directly into the corresponding fields. If you do an auto estimation *KD* is approximated as the most middle value and *Max* is the greatest y-value in the wave. If you have an ascending wave, *Hill* will be set to 1, otherwise to -1 (for a single dose-response). Click the

Draw Fit button, to get a view of the quality of the current fit parameters. The fit will be drawn starting from *x-min* towards *x-max*. The wave containing the fit will automatically be created with *n* points - the bigger *n*, the smoother the curve will appear, 400 is always a good value. Actually, two curves are created: a wave containing the fitted y-values and a corresponding x-wave which will contain equally spaced values on a logarithmic scale over the drawn range. If the parameters *x-min*, *x-max* and *n* don't make any sense, they will be replaced by the left and right x-values (1 and 1000 in the example).

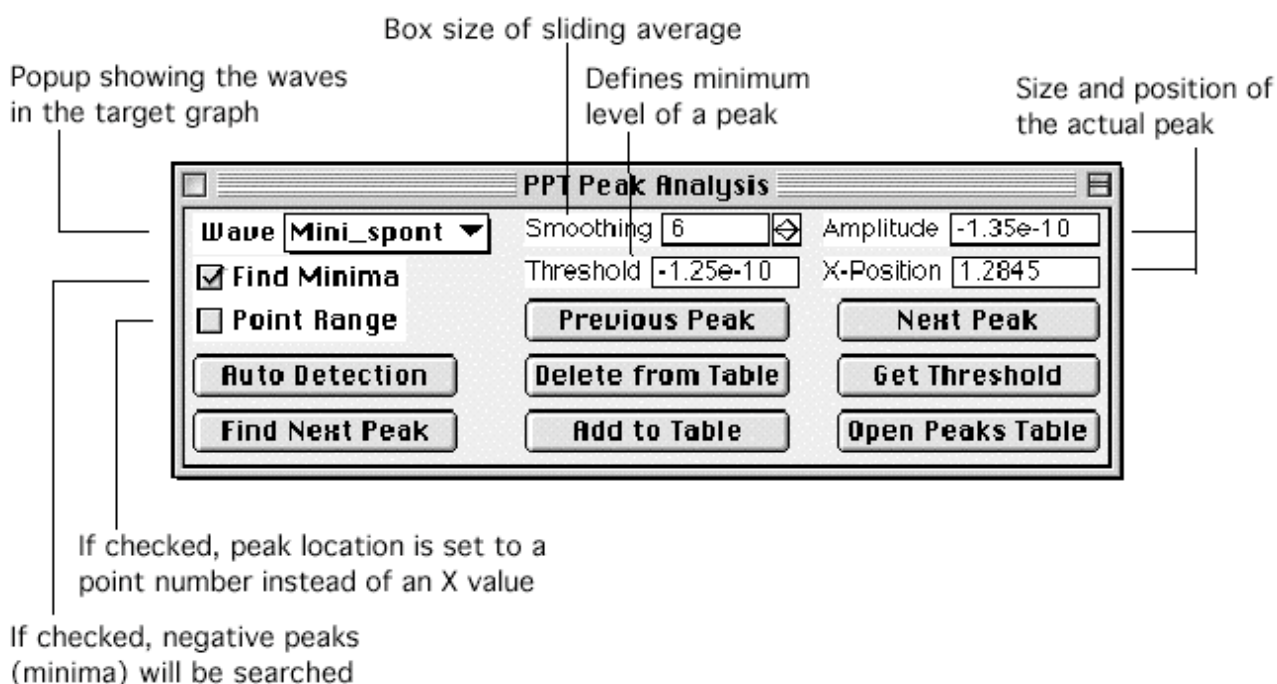
Start Igor's fit by clicking the *Fit Dose-Res.* button. You will need at least 3 data pairs to perform a single dose-response fit and 6 pairs for a double dose-response. If you want some parameters not to be fitted (e.g. you wish to set *Max* to 100% or *Hill* to 1) click the checkboxes left to the parameter so they are checked. After the fit, the values will be updated in the panel and the new fit will be drawn according to *x-min* and *x-max*. If you want a bigger range to be drawn, just increase these values accordingly. The button *Append Legend* draws a legend containing the fit parameters into the top graph. You can remove the legend any time by clicking *Remove Legend*. If you perform a new fit, the legend is not updated automatically. Just append a new legend, overwriting the old one.



Peak Analysis Control Panel

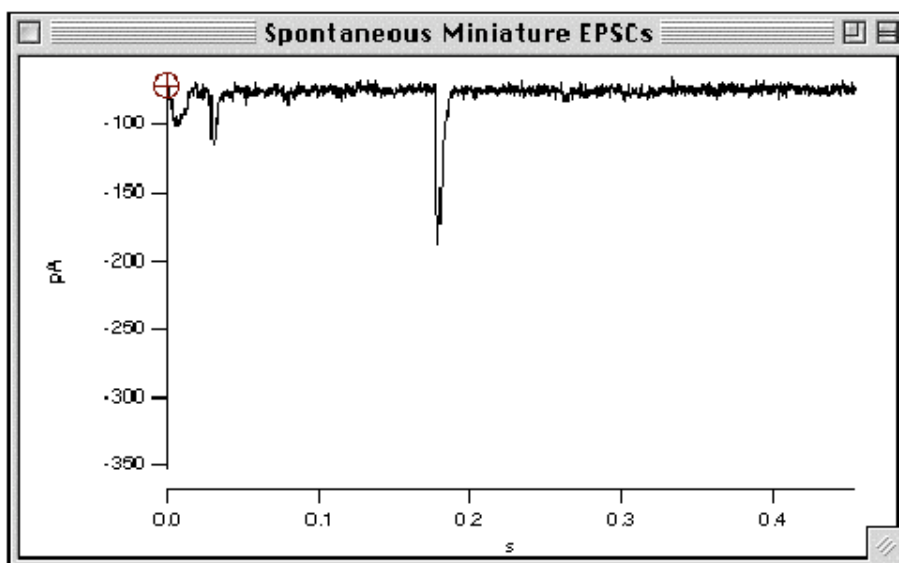
This control panel allows the user to automatically detect peaks in a wave (e.g. Calcium transients or postsynaptic currents/potentials). It uses Igor's built in *FindPeaks* operation, which is - due to the amount of options and required input parameters - a little bit unhandy and gives a quick interface to Igor's operation by allowing the user to specify all necessary parameters in an interactive way. The panel furthermore has some options for automatic analysis like generation of a table containing the size and location of found peaks.

To start, you first have to create a graph containing the desired wave(s), since found peaks will be marked directly in the graph. Also you can use the cursor to specify the threshold of the peak analysis. You can use the example in the file *Peak Analysis Demo* from the *PPT Experiments Folder*.

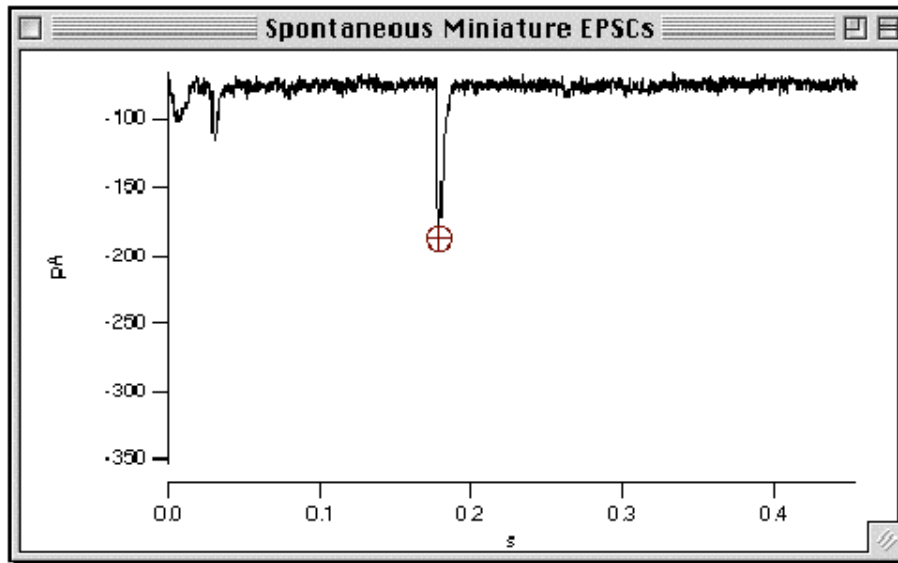


After selecting a wave using the popup, cursor *A* will be set to the beginning of that wave. If this point is out of the axis scaling the wave will be "scrolled" appropriately to show the cursor, so you can zoom the wave (especially if you have very long recordings). Type in a reasonable peak-threshold.

Alternatively, you can also drag the cursor within the wave to a



value determining your

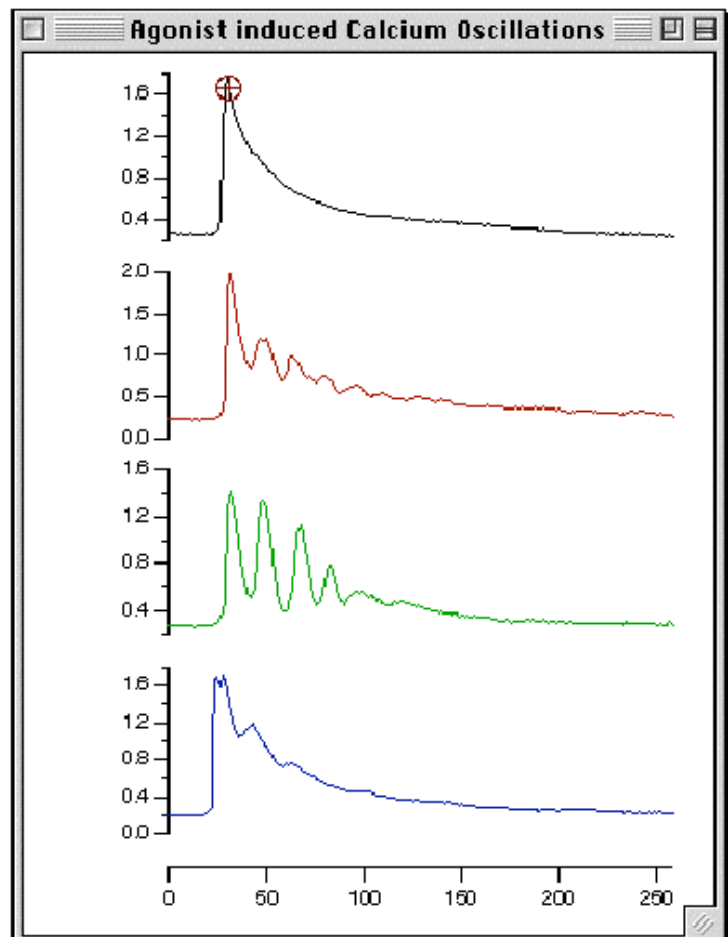


minimal amplitude defining a peak and subsequent click the *Get Threshold* button. The actual Y value of the cursor will be copied into the threshold field. Click the *Find Next Peak* button. This will start looking for the next peak matching the conditions from the **current** cursor location on. If no peak could be found, you will hear the system beep - you may

uncheck the *Point Range* option and/or increase *Smoothing* in order to get a better peak detection. Otherwise the cursor will be set to the found peak (if it is out of the visible range the wave will be scrolled) and its amplitude and location will be copied into the corresponding panel variables. If you want to keep those values you can copy them into a table by using the *Add to Table* button. This will create two waves - *WaveName_peak_x* and *WaveName_peak_y* were *WaveName* is the name of the actual wave containing the peaks, if they didn't exist before. You can see the table by clicking the *Open Peaks Table* button. If the current peak is already in the peak-table(s) a system beep will sound.

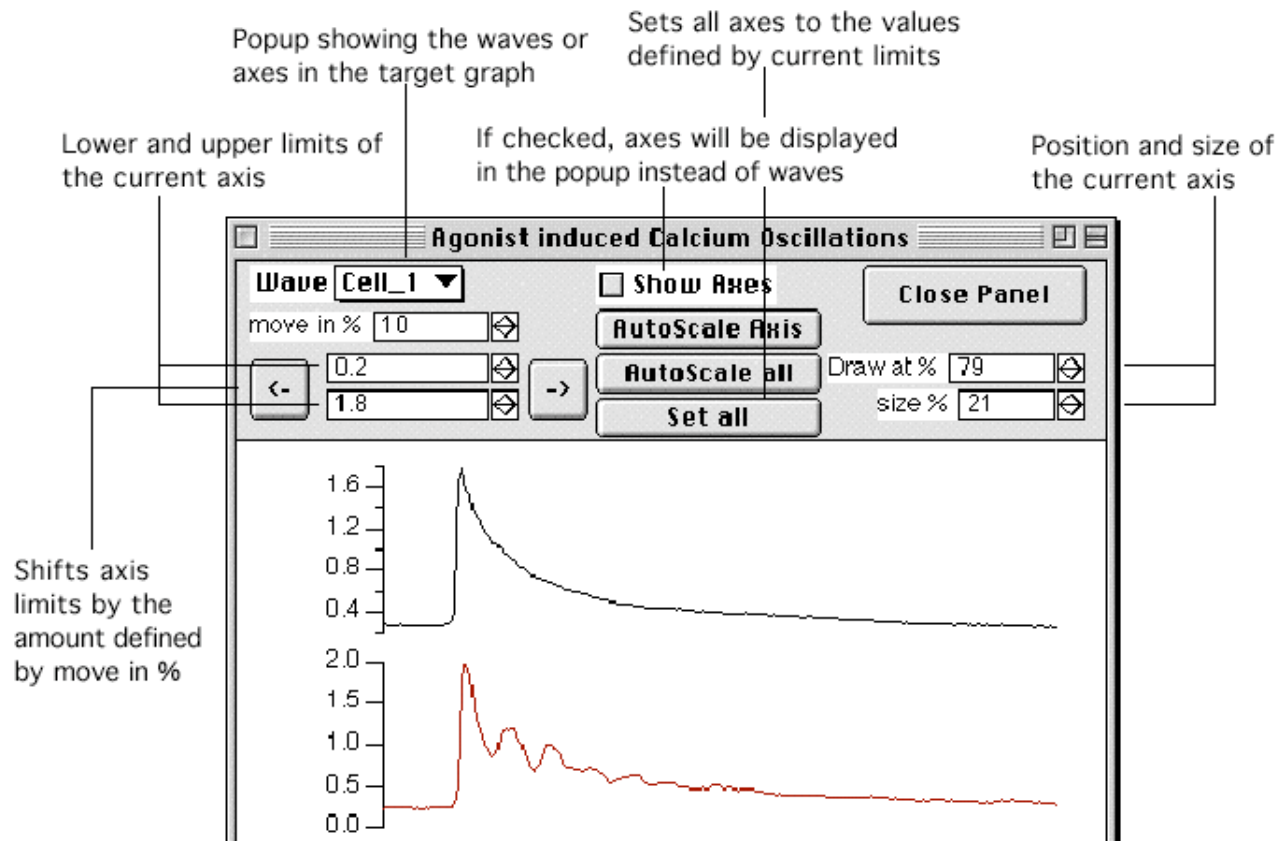
Once you determined the correct settings for a given peak-detection, you can automate the analysis by using the *Auto Detection* feature. This looks for all peaks in all waves within a target graph. The peak-tables will be generated automatically containing all found peaks. You can interrupt this process by hitting **cmd-dot** on the keyboard.

Use the *Previous Peek* and *Next Peek* buttons to browse through the found peaks in the current wave. If you want to get rid of a peak, click the *Delete from Table* button. This kills the peak marked by the current position of cursor *A* from the locations- and the amplitudes-table.



Graph Control Panel

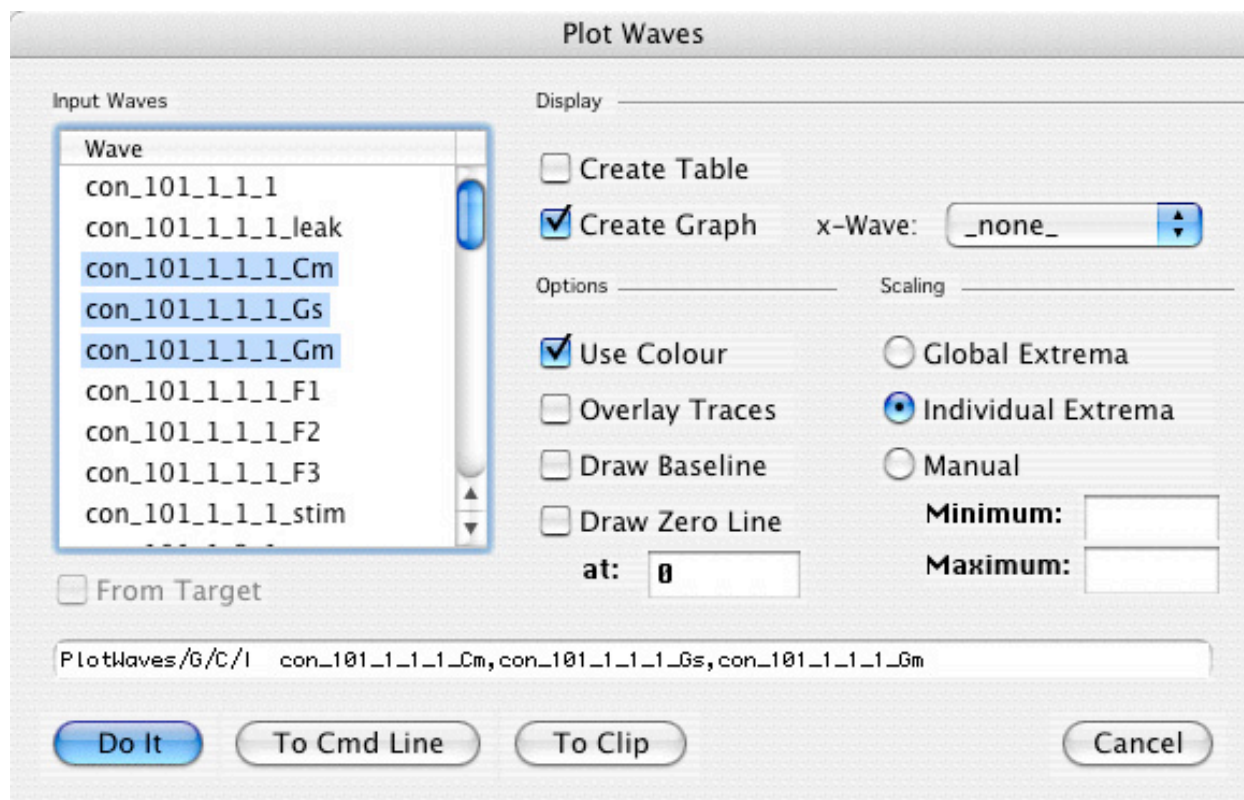
The graph control panel allows you to easily change most properties of the drawing axes within a given graph without having to use Igor's *Modify Axis* dialog. This control panel is especially useful, if you want to modify a chart-recorder type of graph, i.e. several traces plotted against one (time-) wave using separate axes (as i.e. created by the PPT's XOP-command *Plot Waves*).



With this panel you can define and shift the axis-range, autoscale single or all axes and shift/resize the drawing position of any axis.

Wave Plotting

PlotWaves is similar to Igor's menu "New Graph...", except that many input waves can be plotted against one x-wave, and the waves are plotted in several traces (which can be overlaid optionally). At the same time, a table with the input waves can be opened.



PlotWaves

`[/G/X=xWave/T /C/O/U/D/R=(min,max)/Z=zeroValue]waveName1
waveName2 [...]`

Parameters

waveName1... are the single- or double-precision input waves.

Flags

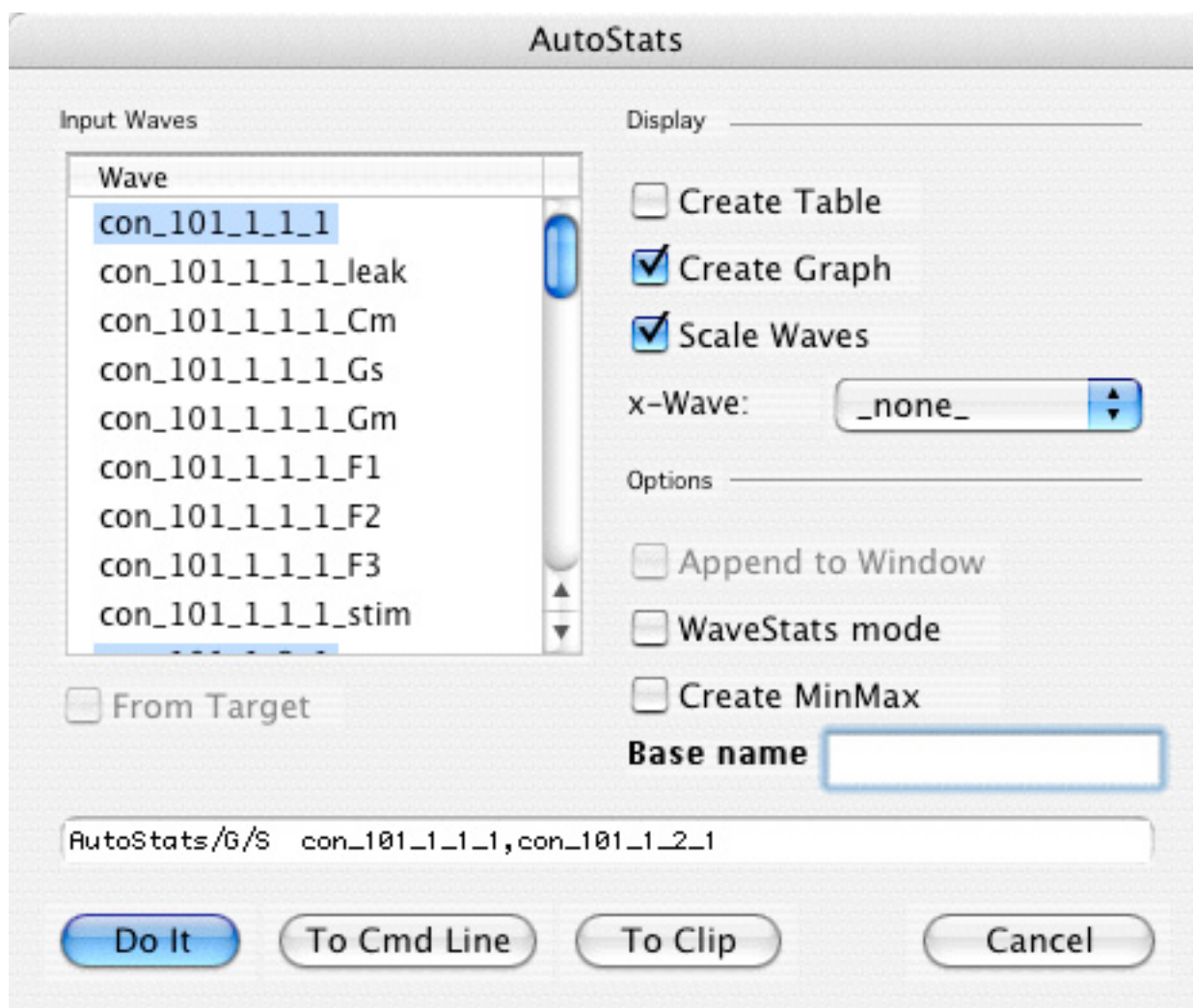
- /G** a graph window will be created plotting the input waves in separate traces (1st input wave on top)
- /X=*xWave*** if a graph is created, the input waves can be plotted versus an existing x-wave
- /T** a table window will be created showing the input waves. Since all waves from a window are already preselected when opening the dialog, you can easily edit all waves from a graph window with this option.
- /C** Draw all traces coloured (black, blue, red and green) with different line styles. Otherwise all waves are drawn black.
- /O** Overlay mode. All waves are drawn together instead of separate

traces.

- /U AutoScale waves. Without this option, y-dimensions of the largest wave are used.
- /R=(min,max) with no AutoScale option, *min* and *max* will be used as y-range instead of the largest y-dimensions
- /D Draw a zero line.
- /Z=zeroVal Draw the zeroline at *y=zeroVal*. If *zeroVal* is out of range, the wave's minimum will be used instead.

Wave Statistics

AutoStats calculates statistical parameters from several input waves. It creates 4 (or 6) new waves for **mean-value**, **standard deviation**, **standard error of the mean** and **number of values** (optional: **minima** and **maxima**). Furthermore, the function can handle very large waves. If option /W is used, the command does the same as the built in Igor function "WaveStats", except that multiple input waves are allowed and the result is also put into output waves instead of the terminal.



AutoStats [/G/S/X=xWave/T /A/M/W/N=baseName] waveName1 waveName2[...]

Parameters

waveName1... are the single- or double-precision input waves. At least 3 waves are required for statistics without option /W. The waves can be of different length and/or precision (32 and 64 bits), the XOP internally calculates with double precision and tries to get as much input data as possible - watch for the "_num" wave to see the number of processed input values (NaNs and undefined data are ignored).

Flags

/G a graph window will be created plotting mean-values together with their standard deviation

/S the waves are scaled as defined

/X=xWave if a graph is created, the mean values can be plotted versus an existing x-wave

/T a table window will be created showing the statistical output waves

/A if a table or graph is opened, the statistical output waves are appended automatically

/M minima and maxima of the input waves will also be calculated and put into two further output waves

/W Data is assumed to be oriented vertically instead of horizontally ("WaveStats" mode)

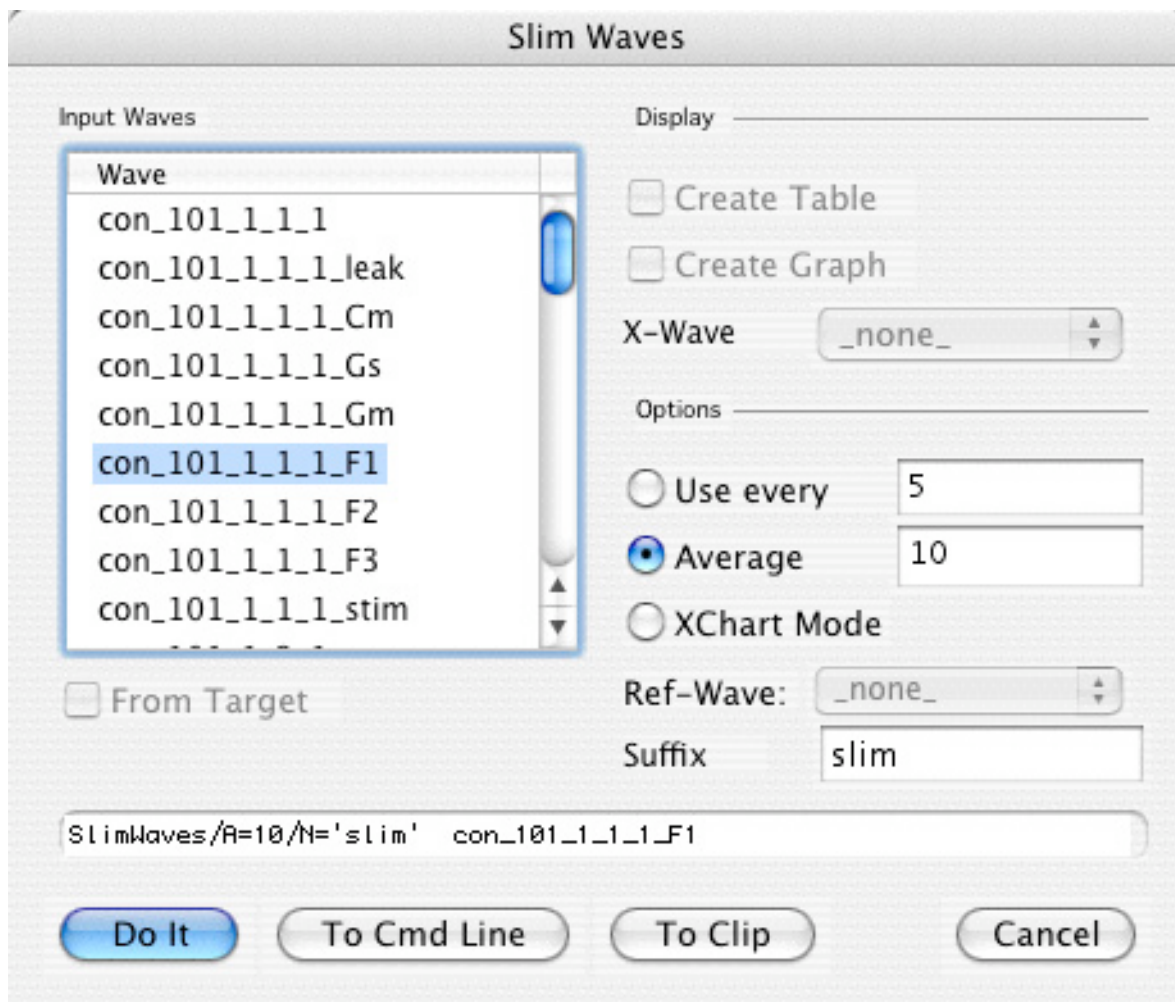
/N=baseName is the basename for the double-precision output waves. If no basename is defined, "swave" will be used. "_mean", "_sdev", "_sem", "_num", "_min" and "_max" are appended automatically to the output waves. If the output waves already exist, they are overwritten. The number of wave points is the same as the largest input wave.

Dialog

Invoking the "Wave Statistics" menu calls an Igor-styled dialog, where you can select the input waves from a wavelist of all existent waves (if there are no waves, an error is returned). If a graph or table is opened at that time, the corresponding waves are already selected and the "Append to Window" Button is checked (otherwise the button is disabled).

Wave Slimming

SlimWaves can be used to reduce data. Slimming can be performed either by using only every n^{th} point of an input wave, by averaging or by deleting all points not matching a reference wave.
 [/G/T /X=x-wave] wave1 wave2 ...



SlimWaves [/A=n|/U=n|R=RefWave /N=basename] waveName1 [...]

Parameters

waveName1... are the single- or double-precision input waves.

Flags

- /A=n Slimming will be performed by averaging every n points.
- /U=n Slimming will be performed by using every n^{th} point. Only those values will copied into the output wave.
- /R=RefWave this option slims down X-Chart™ waves. For each non-NaN value in the reference wave (e.g. the online analysis result of Pulse™), the corresponding values of the input waves will be copied into the output waves.
- /N=baseName is the basename for the double-precision output waves (by default "slim").