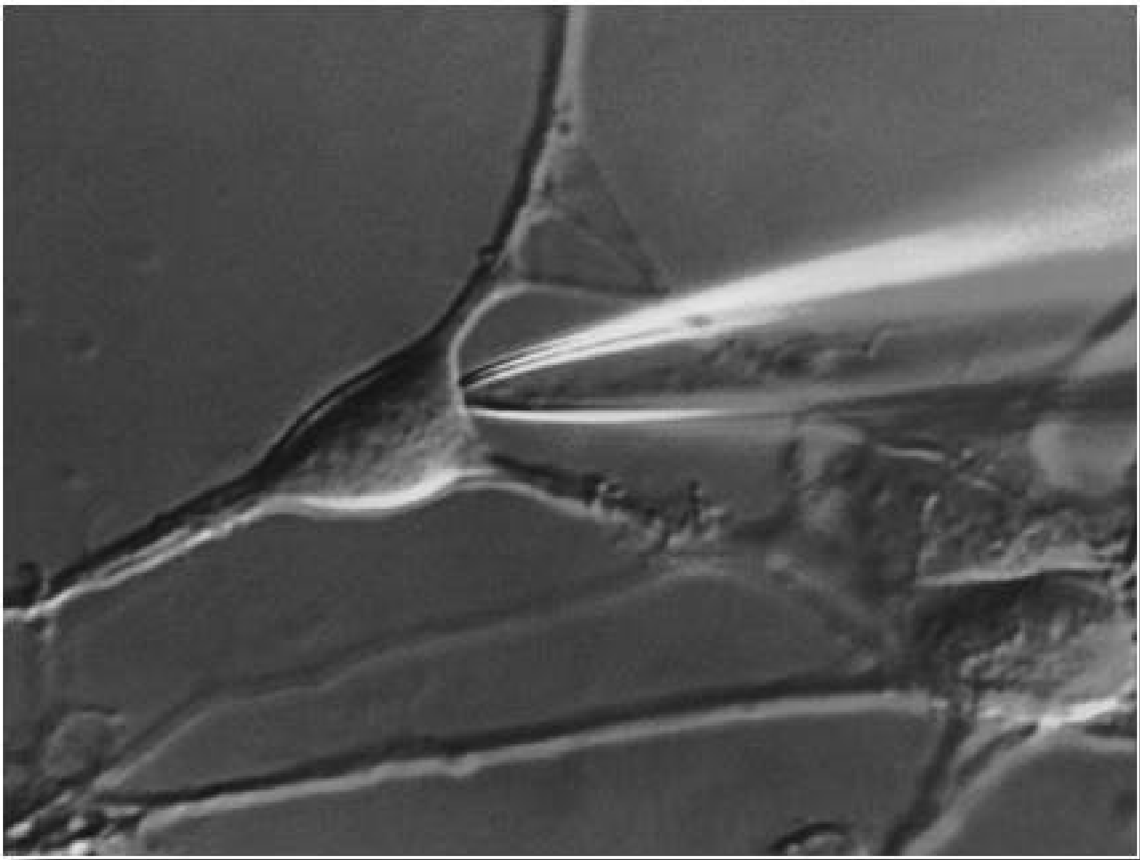
8.0
PulseFit Manual



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1. Introduction

Data files acquired by *Pulse* can be reviewed and edited using *Pulse* as described in the *Pulse Manual*. Most of the features already found in *Pulse* are also present in *PulseFit*. In principle, *PulseFit* extends these capabilities by adding more specific analysis features and the generation of an analysis tree.

The way *PulseFit* works is tightly linked to the tree structure of data generated by *Pulse*. Hence, *PulseFit* has equivalent hierarchical levels of analysis corresponding to the tree levels featured in the *Replay* data tree. The analysis is organized in a structured way, relating to *Sweeps*, *Series* or *Groups*. These analyses are summarized below and discussed in more detail in the following chapters.

Sweep Fit: The lowest level is the analysis of a raw data trace, a *Sweep*. The *Sweep Fit* dialog provides tools for selecting a section of the trace for analysis and specifications of the type of analysis to be performed (e.g., measuring the peak current within a certain time window).

Series Fit: The values determined by this sweep analysis can be further processed based on a parameter specified for one family of sweeps, i.e., within a *Series*. For this purpose the *Series Fit* dialog allows to display one of the *Sweep Fit* results (in our example the peak current) as a function of one out of several possible parameters (e.g., the potential of the *Relevant X-Segment*). Several fit functions can now be used to describe this ensemble of data points, again yielding data fit results (e.g., the reversal potential of a current-voltage relationship).

Group Fit: The results of a *Series Fit* can now be further analyzed on the level of a *Group*. The *Group Fit* dialog allows therefore to select one of the *Series Fit* results and to display it as a function of a list of various parameters specific to the series (e.g., temperature at time of series acquisition). Again, several fit functions can now be used to describe these data.

Because of this order, it is of key importance to select first the right target from the data tree (*Sweep*, *Series*, or *Group*) and then switch to the analysis dialogs corresponding to this kind of target (*Sweep Fit*, *Series Fit*, or *Group Fit*, respectively).

2. The Analysis File

For each data file to be analyzed, an *Analysis File* (extension ".ana") is created whenever a data file is loaded for analysis. This file has the same general structure as the *Pulsed File* (extension ".pul"). Each *Series* carries two arrays of descriptors (ASCII strings); one describes the analysis data on the level of a series (e.g., the result of an I-V fit), the other one describes the analysis of the raw data sweeps (e.g., a complete Hodgkin & Huxley parameter set). The *Group* contains the group descriptors and the corresponding analysis. The analysis parameters are stored as entries in the corresponding tree levels. Each entry consists of a *LongReal* number (the parameter) and an *enumerated* variable, indicating the status of the parameter (*Fit*, *Hold*, or *Off*). The data structure is defined in the module "Analysis.de" (see Pulse Manual *Chapter 18 - Data Structure*).

Upon start of *PulseFit* a data file has to be selected for analysis. If a matching analysis file exists, it is loaded; if not, a new analysis tree is created. Be sure not to edit data files with *Pulse* (e.g., deleting sweeps) after an involved analysis was performed with *PulseFit*, because this will cause a mismatch between the pulsed data tree ("*.pul") and the analysis tree ("*.ana"): Use *PulseFit* for data editing purposes whenever there is an analysis file created.

All fit results are entered to copies of the analysis file structure. Before program termination or before a new file is opened the user has to decide if the original analysis file should be updated. The message is: "Analysis file exists - overwrite?". This is an important question, don't forget to enter "Yes", if you want to save your new analysis results.

***Note**:* *PulseFit running in "Demo" mode is restricted to analyze the file "Demo.dat", which is supplied with the software package.*

3. The First Analysis

Before the functions of *PulseFit* are described in more detail, we go through several steps of a simple analysis. It is briefly illustrated how one can obtain a fit to a current-voltage relationship of peak sodium currents as stored in the “Demo.dat” file. This section is not thought to explain specific fit functions; it should rather help to get a feeling of what the sequential order of steps are that are necessary to use *PulseFit* efficiently.

Start PulseFit

There are two ways to start *PulseFit*:

- **Application**: *PulseFit* is started by double-clicking on the *Pulse+PulseFit* icon and then choosing *PulseFit* in the alert box.
- **Configuration**: One can directly start up *PulseFit* by double-clicking on the “DefaultFit.set” icon.

At first, the user is prompted to open a data file. Initially, it may be useful to open the demonstration file supplied (“Demo.dat”). If you open it with the option *Modify*, the data file itself can be modified in *PulseFit* in the same way as in *Pulse* (see Pulse Manual *Chapter 12 - Replay*).

When the program comes up, several windows are open. Most of them are identical to those of *Pulse*. There is no Amplifier window but, instead, there is a so-called *Sweep Fit* window.

Select a Sweep

You will find that the functions provided by the *Replay* window and the dropdown menu *Tree* are just as in *Pulse*. Click on a *Sweep* (e.g., Sweep 1) of the first series and it will be displayed. Make sure that the oscilloscope was set to *Zero Subtract*, otherwise you will analyze non-subtracted traces.

Select Fit Type

We want to determine the peak of the current trace within a specified time window. Therefore we could use the result of the online analysis (if ordinate is set to *Extremum*), for example. It is more precise, however, if we try to fit a function to the trace around the peak and then determine the maximum / minimum from this fit. For that, we have to select a function in the *Sweep Fit* window (e.g., *Polynomial*). The order of the polynomial should not be lower than 3.

Set Cursors

We have selected and displayed a sweep which consists of three segments. The relevant segment is the second one. If you click on *Cursor* in the *Oscilloscope* window, you can now select a time range for analysis. Move the left cursor a bit to the right, and the right one a bit to the left in order to get rid of some small artifacts in the beginning and at the end of the test segment.

Fit Sweeps

This first trace basically shows no inward current, therefore the setting of the cursor is not that critical. Click on the *Fit* control in the *Sweep Fit* window. The fit will be superimposed on the data. Now click on *Next* to display the next sweep of the series. Readjust the cursors so as to get a smooth fit around the peak current. Proceed through all sweeps.

Because the time to peak changes from sweep to sweep, the cursors have to be readjusted quite often. In other experiments where this peak is not changing (steady-state inactivation curves as shown in Series 2 (*Hinf*) in the “Demo” file) one would do the fit for the first sweep and then click on *Auto*, i.e., the remaining sweeps of a series are fitted automatically.

Select a Series

The result of the previous analysis has yielded amplitudes of peak currents for all sweeps of the first series. Now, select the series as new target by clicking on it in the *Replay* window.

Display Sweep Fit Results

A series is selected as target, so you can go ahead and switch to the series dialog, which allows to handle the *Sweep Fit* results on the level of a series: click on *Series Fit*. In the *Series Fit* dialog there is a list of parameters on the left-hand side, which are the results of the *Sweep Fit*. In our case of *Polynomial* fit, we are interested in the parameter *IPeak*. Click on *IPeak* in order to select it for display (the control will get highlighted). Now you have to decide which abscissa to use for display of *IPeak*. Since we want to plot a current-voltage relationship, select *Voltage* from the list of abscissas. Now *Series Fit* knows that it is supposed to display *IPeak* from all sweeps as a function of the *Voltage* of their relevant segment: create this display by clicking on *Show Data*. The data points are now displayed as squares using an automatic scaling of the graph. In the left lower corner of the graph the comment to the series is shown.

Fit Series

From the list of available fit functions (*Type*) we select *Current-Voltage*. Several controls are set up describing parameters used for a current-voltage relationship for voltage-activated ion channels considering voltage-dependent block. Since in this example we are dealing with voltage-activated sodium channels, we assume that there are three activation gates, i.e., we select "3" in the control called "*m*" (*m* is the classical descriptor for the number of activation gates in the Hodgkin-Huxley theory for ion channel gating). We further assume that the single-channel current-voltage relationship obeys the Goldman-Hodgkin-Katz equation, thus we select *GHK IV* from the list left of "*m*". We do not consider any voltage-dependent block, so the controls *Pos. Block* and *Neg. Block* are not checked.

Now click on *Fit* and the program will iterate the free parameters. Upon convergence the fit function will be superimposed on the data points. The convergence criterion is set in the *Sweep Fit* window. You can manually stop the fit by pressing [Space]. The resulting new fit parameters are updated in the fit dialog controls.

Export Fit

Depending on the setting of the *Export Format* in the drop-down menu *Tree*, the *Export* control will output the data points and the fit function.

Next Series

If we had analyzed the sweeps of more than the first series, we could proceed in this dialog to the analysis of the *Sweep Fit* results of the next series by clicking on the *Next* control below the parameter list.

Group Fit

The display and fit of results obtained by fitting the *Sweep Fit* results on the level of a series, i.e., the *Series Fit* results, may be further analyzed on a higher tree level (*Group*) in analogy to what has been described above. Select a group in the *Replay* tree, click on *Group Fit*, select a parameter from the list of *Series Fit* results, select an abscissa for this parameter, display it (*Show Data*), select a fit function, adjust the fit parameters, and finally do the fit.

Exit

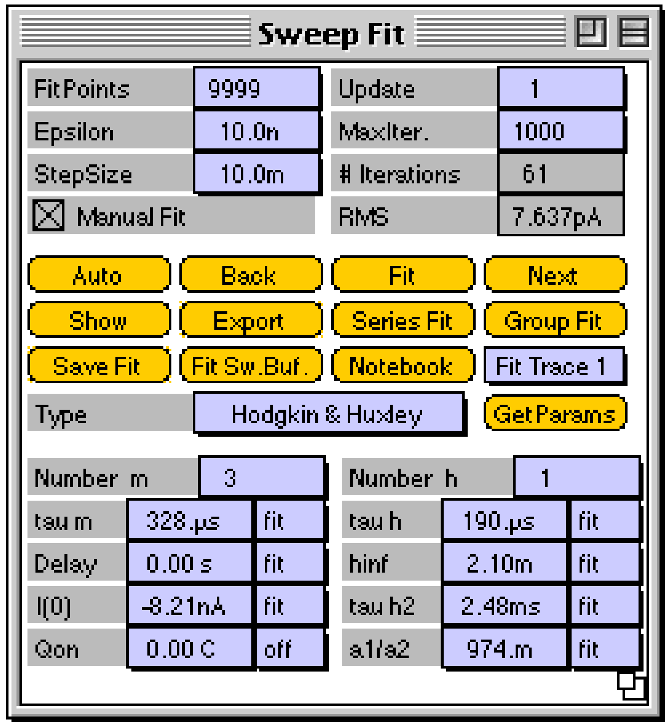
All fit results are stored in a copy of the analysis file. Note that old fit results are overwritten, whenever a new fit is done. When *PulseFit* is exit, or whenever a new input file is loaded, *PulseFit* saves an analysis file to disk. If there is an existing analysis file, you have to choose whether to overwrite the old one or to discard the results obtained in the new analysis session.

4. Sweep Fit

The *Sweep Fit* window contains three sections:

- Simplex data fit control
- Sweep Fit control
- Sweep Fit types

The *Simplex* data fit control functions are used to set the parameters for the optimization procedure. They are also used by the dialogs, which operate on a higher level: *Series Fit* and *Group Fit*. *Sweep Fit* control and *Fit Type* functions are specific to the fit of data traces.



Simplex Data Fit Control

The data fit in *PulseFit* is done according to a *Simplex* optimization algorithm (M. S. Caceci & W. P. Cacheris, 1984, Byte, 340 ff). This algorithm is quite fast and uses no analytical derivative of the fit function. The draw-back is that there is no direct error estimation of the optimized parameters. To get an idea of the error, one would need to repeat the fit very often starting from random initial conditions and then evaluate the statistics of fit results. This is not straightforward. Thus, if you wish to have precise error estimates of the fit results, export the data and use other algorithms, e.g., the method of steepest descent offered by *Igor*. This method provides error estimates; but since it is much slower than the method used here, it may not be an ideal solution for fit of raw data traces with many data points.

Fit Start and Termination

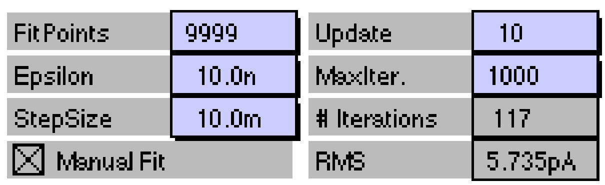
In all three fit dialogs (*Sweep Fit*, *Series Fit*, and *Group Fit*) there are controls that start the fit (*Fit*). The number of iterations and the actual residuals (see below) are then continuously updated in these windows. The fit is terminated if:

- the maximal number of iterations is exceeded,
- the convergence criterion is met,
- the user exit event has been encountered (Space, Control S or Control B, click on buttons *Break* or *Stop*).

After termination of the fit, parameters are shown in the fit menus. These and some more derived parameters are then stored in the analysis tree to be available for further analysis on the next higher tree level.

In the following, the functions of the *Simplex* fit parameters are described. The initial step size, the absolute convergence criterion, the maximal number of iterations as well as the number of iterations per display update can be set.

Simplex Fit Parameters



Fit Points: This variable is used for speed enhancement. If *Fit Points* is smaller than the total number of points in the selected interval, the fit points are exponentially distributed. This feature is quite useful for fits of exponentially decaying functions where a higher density of data points in the beginning is desired. The default setting of this parameter is 9999. If the number is greater than the actual number of data points, all points are considered for data fit.

Epsilon: Fit termination criterion. If the residual (see *RMS*) does not change more than *Epsilon* in relative terms between two successive iterations, the fit is considered to have converged and is terminated.

Note*: The default setting of 10.0n (10-8) is a quite tight criterion; for many fits you may want to increase this number.*

Step Size: Relative step size for changing the fit parameters for the first *Simplex* iteration. The next step sizes for the following iterations are calculated automatically by the *Simplex* algorithm. If one thinks that the fit ended up in a local rather than in a global minimum, one can try to increase *Step Size* and compare the results. If the fit is already quite good and one just wants to run some more refinement iterations, *Step*

Size should be rather small in order to avoid the risk of finding a completely different local minimum. The default value of 1% appears to be a reasonable approach for most problems.

Update: Number of fit iterations between display and window update. This parameter is only active for *Sweep Fit* operations.

Max. Iter.: Maximal number of fit iterations. When this number of iterations is reached, the fit will be terminated.

 # Iterations: Iterations counter. This field is continuously updated during data fit in steps of the *Update* to indicate progress of the fit.

RMS: Root mean square deviation between fit and data. The *RMS* value is used as residual for the *Simplex* fit algorithm. If it changes less than *Epsilon* in relative terms between two successive iterations, the fit will be terminated, i.e., convergence is assumed. Be aware, however, that the resulting set of parameters can correspond to a local minimum.

Manual Fit: If this checkbox is set, the fit function is continuously updated and shown as the parameters in the fit menus are changed (dragged or typed). Since this operation is time consuming, simultaneous function update while dragging a parameter value will only work well on fast CPUs. Manual fit is only possible for non-linear parameters; e.g., the time constants of an exponential function can be dragged, while the amplitudes (the linear parameters) are calculated automatically (see below).

Since the *Simplex* fit - like any other fit algorithm - relies on reasonable starting values, it is recommended to enter good guesses for the parameters before the first fit operation. Check if the guess is reasonable by using the *Show* function (if *Manual Fit* is on, the result is shown automatically). Make fine adjustments with the *Manual Fit* option by dragging the parameters with the mouse until you reach a fit that reasonably approximates the data. Then you can run the actual fit.

***Note**: Always make sure that the initial parameters are not set to zero when fitting a non-linear function. For exponential fits, do not set the time constants to 1 (time constants are converted to log during the fit).*

Sweep Fit Control

Raw data traces (sweeps) of the first and second acquired channel are accessible to fittting. A sweep has to be selected using the *Replay* window functions. Cursors can be set in order to define a time window for data analysis. The cursor settings determine the window for data analysis, based on the relevant segment specified in the *Pulse Generator*. If you want to analyze a different segment, make use of the segment offset functions provided by the *Online Analysis* window.

You can use the display scaling functions of the *Oscilloscope* the same way as in *Pulse*. Be aware, however, that your cursor settings may be outside the window range if you change the scaling of the time axis. Try to first set the cursors roughly to what they are supposed to be, then expand the time scaling if desired. Now you can do the fine adjustments of the cursors with higher display resolution.

The fit is done to both, leak-corrected as well as non-corrected data as selected by the *Leak Sub* option in the *Oscilloscope* window. *Zero Sub* can be either on or off. The fit will then be done to the data with or without zero subtraction, respectively.

For display of the fit result, the raw data are shown in the P/n color in the background. The button *Show P/n* does not need to be on for that; in fact, it will be disabled whenever a fit result is displayed. The fit function is inserted into another data trace inside the selected time window of the fit and it is shown in the trace color in the foreground. That is, one only sees the raw data outside the selected time window; inside this window the smooth fit function is superimposed on the (usually noisy) raw data.

***Note**:* *Whatever the display scaling is, the actual fit is not affected by it; only the cursor settings are important.*

The following controls are used to control the *Sweep Fit* and to switch to the higher-level analysis windows.

Sweep Fit Control Buttons

Auto: Fit sweeps of a series starting from the currently selected sweep. This function is quite useful for cases where the range of a trace to be fitted remains constant from sweep to sweep. Examples are the determination of plateau currents at the end of the relevant stimulation segment or the compilation of steady-state inactivation curves, where only the amplitude of the measured signal changes. The currently fitted sweep is automatically highlighted in the *Replay* window, i.e., after the *Auto* function has come to an end, the tree pointer is directed to the last fitted sweep. A mouse-click on the *Stop* button in the *Oscilloscope* window (or Control S) interrupts the Auto procedure after the currently fitted sweep. Clicking on the *Break* button (or Control B) interrupts the procedure instantly, i.e., it also stops the fit of the currently active sweep. Thus, the functions are analogous to the data acquisition controls *Stop* and *Break* in *Pulse*.

Back: Select and display previous tree entry.

Fit: Fit first or second trace of currently selected sweep (or first possible sweep if a series or a group is selected) within the selected bounds. The trace to be fitted is selected by the *Fit Trace 1 / Fit Trace 2* option. For selection of the fit range use the *Cursor* function in the *Oscilloscope* window. If the cursors do not appear in the data section of interest, i.e., if the *Relevant Y-Segment* as specified in the *Pulse Generator* is not equal to the segment to be analyzed here, one can use the variable *Y-Seg.* in the *Online Analysis* window to define a segment offset relative to the *Relevant Y-Segment*.

Next: Select and display next tree entry.

Show: Display data and fit function according to the selected fit type and parameters.

Export: Output selected sweep and fit according to output settings (*Export Format* in drop-down menu *Tree*). The fit is exported as P/n trace.

Series Fit: Switch to *Series Fit* window in order to display and fit the results as determined on the level of sweeps (see *Chapter 5 - Series Fit*). Select a series in the data tree before you enter the *Series Fit* window.

Group Fit: Switch to *Group Fit* window in order to display and fit the results as determined on the level of series (see *Chapter 6 - Group Fit*). Select a group in the data tree before you enter the *Group Fit* window.

Save Fit: Overwrite raw data by fit result. This operation is dangerous, because it modifies the raw data; it is recommended to use this feature on copies of data files only (for creating leak templates, for example).

Fit Sw. Buf.: When selected, the data in the *Sweep Buffer* will be fitted. Please note, that *PulseFit* will use whatever target sweep is presently selected.

Notebook: Writes the result of a fit to the *Notebook*.

Get Params: Retrieve fit parameters of the selected sweep from analysis file and put them as new initial values into the fit menu. *Get Params* only appears for the fit types *Exponentials* and *Hodgkin & Huxley*, which contain non-linear parameters. The function is useful if one wants to resume analysis of an old file, continuing with already stored parameters.

Fit Trace 1 / Trace 2: Selects the trace to be fitted.

Sweep Fit Functions

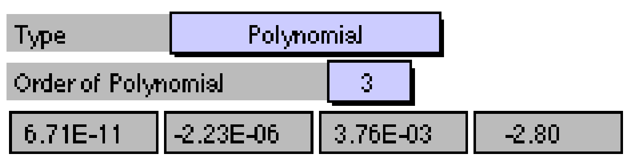
The *Fit Type* determines the fit function to be used. Note that *PulseFit* only contains very dedicated functions tailored to analyze voltage-dependent channel kinetics.) Whenever a *Fit Type* is selected, a corresponding fit menu will appear at the bottom. It contains the free parameters of the fit. They can be set to off, hold, or fit. Note that an initial parameter that is supposed to be fit should not be zero (for exponential functions the time constant should not be 1). The available fit types are:

- **Polynomial**
- **Exponentials**
- **Hodgkin & Huxley**
- **Gaussian**
- **Online**
- **Measure**

Most functions have linear and nonlinear parameters. The nonlinear parameters are fit by a *Simplex* algorithm; therefore reasonable start values have to be provided. The linear parameters are calculated and displayed.

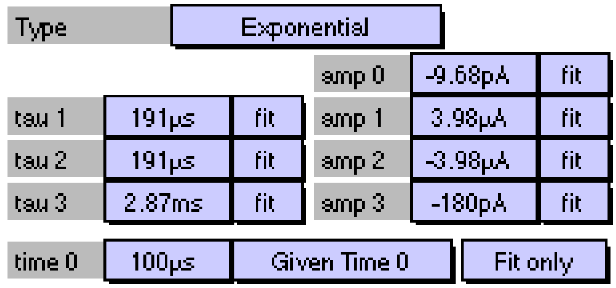
Polynomial: A direct evaluation of the best parameters of a polynomial function up to the order of 9 is provided.

$$I(t) = \sum_{i=0}^{n} a_i t^i$$



The extreme value within the fit interval is determined from the coefficients (similar to the *Online Analysis* fit procedure). This fit option is suited for determining mean values (order = 0), linear regressions (order = 1), or the higher polynomial functions. It is also useful for determining peak currents (e.g., for h_∞) by fitting a smooth curve to the data trace; the peak is then determined from the smooth curve rather than from the raw data. The determination of the parameters of a polynomial is much faster than the iterative procedures as described below.

Exponentials: The parameters should be self-explanatory. For convenience, there are two types of exponential fits. There are no fundamental differences in these two types of fits. Anyone of them could be used to fit any type of exponential data (by tweaking the parameters):



- **Exponential**: This is useful for fitting inactivation times of currents.

$$I(t) = a_0 + a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)$$

- **1 - Exponential**: This is useful for fitting activation times of currents.

$$I(t) = a_0 + a_1(1 - \exp(-t/\tau_1)) + a_2(1 - \exp(-t/\tau_2)) + a_3(1 - \exp(-t/\tau_3))$$

The main difference between these fits is that for exponential, *amp 0* determines the start of the exponential, whereas for 1 - exponential, it determines the value to which the exponential decays or rises. The amplitudes of the exponentials are extrapolations to **time 0**. This can be the beginning of the relevant segment, the left cursor position, or a given time (see below).

tau 1-3 / amp 0-3: There are up to three exponential components with amplitudes and time constants and one starting or ending amplitude (*amp 0*).

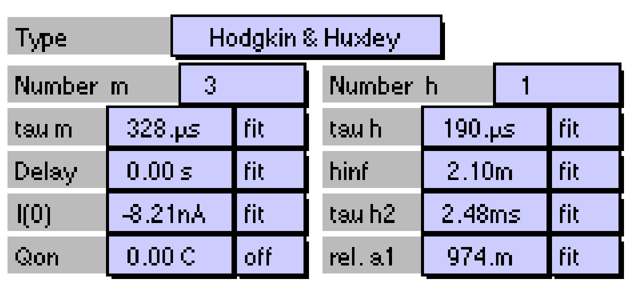
time 0: Time zero of the fit can be specified as:

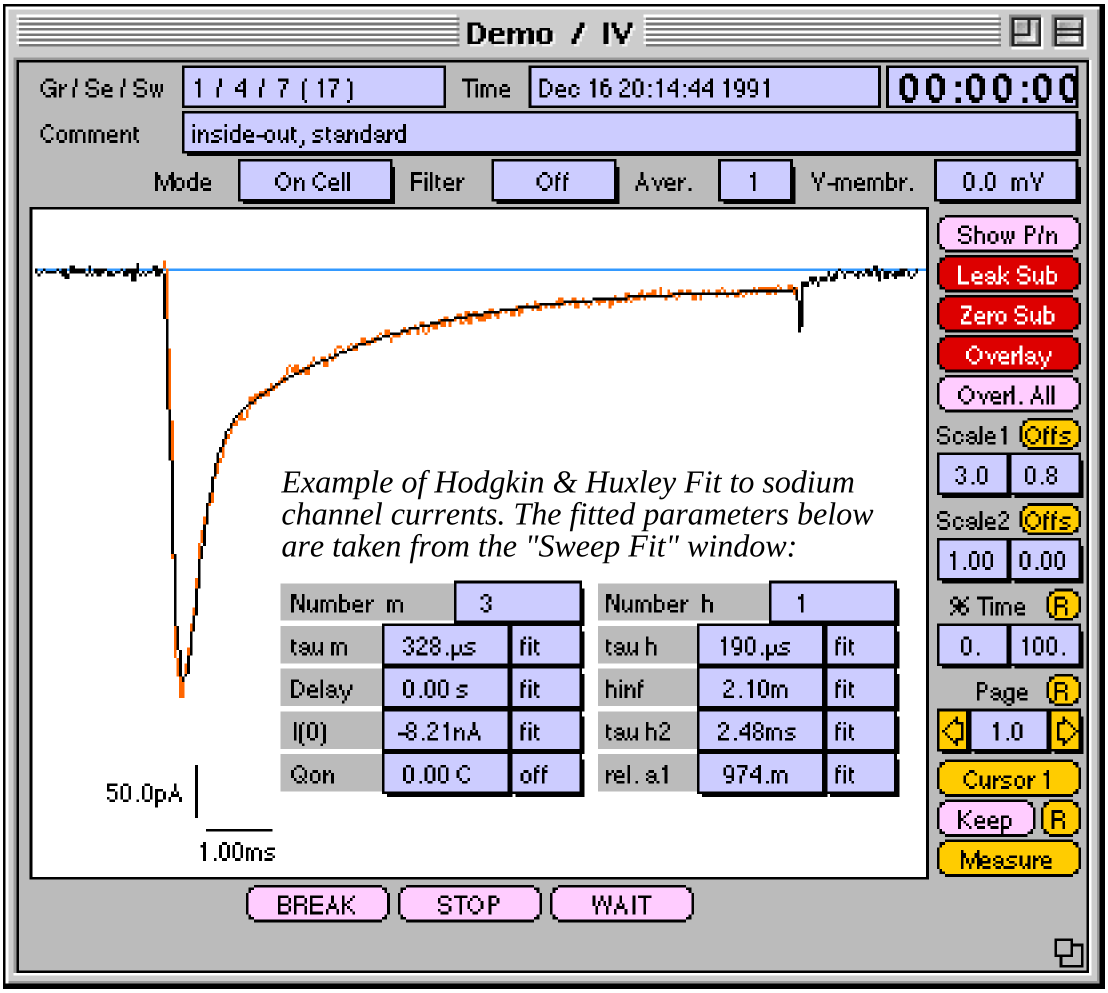
- **Segment Start**: Beginning of the relevant segment.
- **Cursor Position**: Location of the left cursor.
- **Given Time 0**: The time specified (within the relevant segment).

Fit display: This entry specifies how the fit should be plotted:

- **Fit Only**: Draws the fit according to the boundaries set.
- **Full Fit**: Draws the fit across the entire relevant segment.

Hodgkin & Huxley: This fit is done according to a general Hodgkin & Huxley formalism with an arbitrary number of activation (*m*) and inactivation (*h*) gates. *m* and *h* have to be specified as constants; they are not adjusted by the fit procedure.





The numerical expression of the function is:

$$I(t) = 0 \qquad \text{for } t < \text{Delay}$$

$$I(t) = I_{gat} + I_{ion} \qquad \text{for } t > \text{Delay}$$

$$I_{gat}(t + \text{Delay}) = Q_{on}/\tau_m \exp(-t/\tau_m)$$

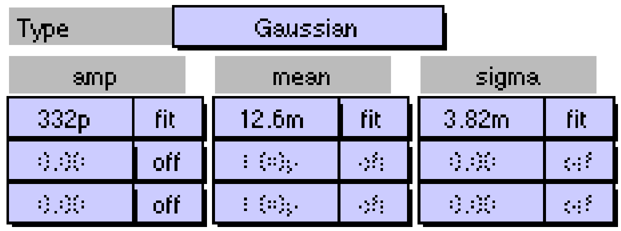
$$I_{ion}(t + \text{Delay}) = I_0 m(t)^m h(t)^h$$

$$m(t) = 1 - \exp(-t/\tau_m)$$

$$h(t) = h_\infty + rel a_1 \exp(-t/\tau_h) + (1 - rel\ a_1)\exp(-t/\tau_{h2})$$

The time constants τ_m and τ_h determine the activation and inactivation kinetics, h_∞ determines the steady-state inactivation. The steady-state activation, m_∞, is inherently given by the expected steady-state current, *I*. τ_{h2} is a second inactivation time constant which is weighted relative to τ_h by the variable a_1/a_2. *Delay* is a constant time delay and Q_{on} is a gating charge assumed to decay with a single exponential time course with $\tau_g = \tau_m$.

Gaussian: This option allows fitting of up to three Gaussian distributions:



$$f(x) = \sum_{i=1}^{3} amp_i \ \exp^{-\left(\frac{x - mean_i}{2\sigma_i^2}\right)^2}$$

The parameters *amp*, *mean*, and *sigma* correspond to the amplitude, the midpoint, and the width of the bell-shaped Gaussian distribution, respectively.

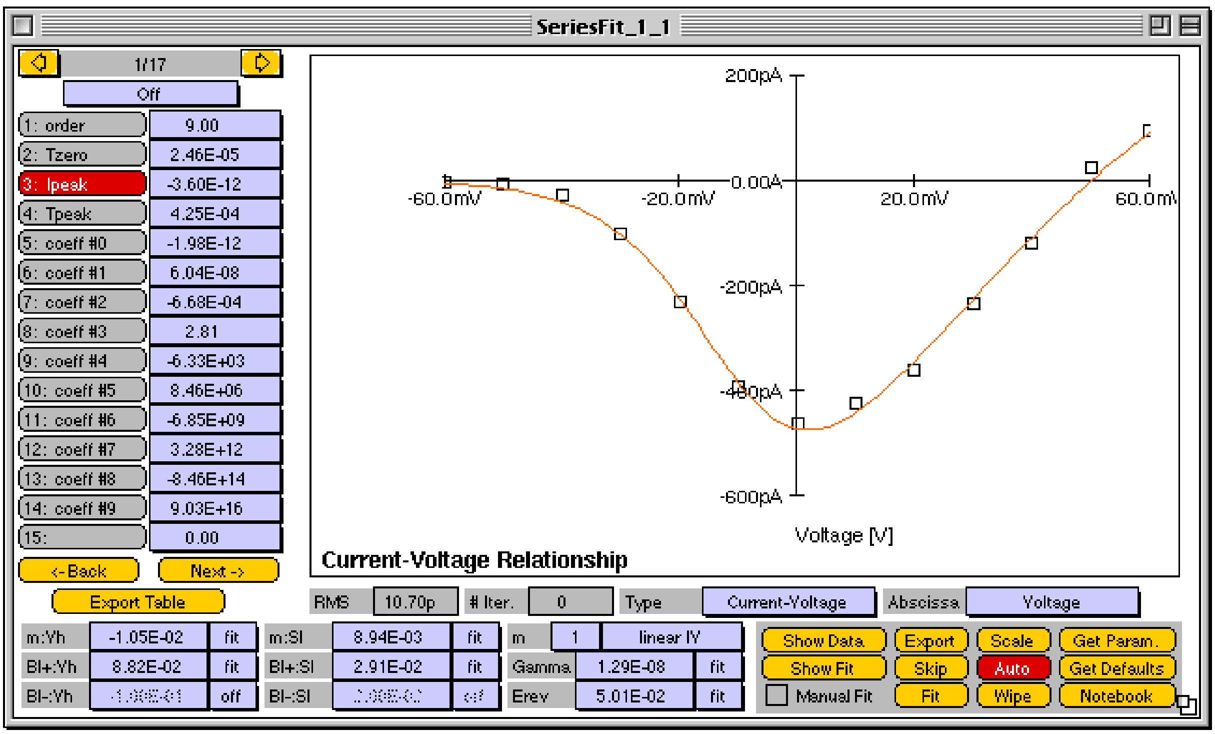
Online: The values determined by the *Online Analysis* are written as the two last entries of the analysis tree. Usually these are the peak current and the charge. Select this type and click on *Fit* or *Auto* to execute this procedure. This function is used to transfer the *Online Analysis* result, which quite often is precise enough, into the analysis tree for further analysis on higher tree levels (*Series Fit*, *Group Fit*).

Measure: The value determined with the measure function (see *Oscilloscope* window) is written as the last two entries of the analysis tree. Select this type and click on *Fit* to get into the measuring mode. The measured results are stored automatically when measuring is terminated.

Note: *The "Measure" option in the "Oscilloscope" window does not store the results in the analysis tree.*

5. Series Fit

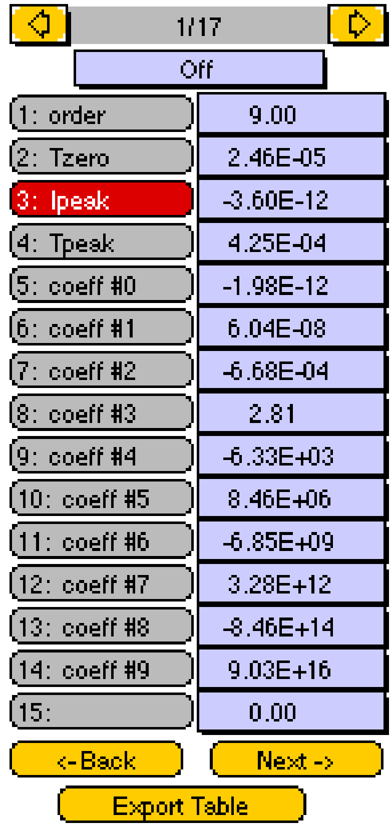
The results of the *Sweep Fit* are stored in the analysis tree, i.e., there are two arrays: one with a string identifying what kind of parameter was stored, the other one with the value of that parameter. These parameter identifiers and the corresponding values are shown as a list in the *Series Fit* window.



In this section it is described how data from these lists are extracted, displayed and fit to a selection of functions.

Data Selection

One parameter of the parameter list generated by the *Sweep Fit* can be displayed as a function of parameters as specified by the *Abscissa* control (including other parameters of the list; see *Abscissa… List Entry*) by clicking on the button *Show Data*. The parameters of the list are selected by clicking on them. If more than one parameter is selected, the last one will be used. A graph with automatic scaling is set up and the data points are shown as squares. Except for a general polynomial, functions are offered that may be fit to the data using the *Simplex* algorithm with the criteria set in the *Sweep Fit* fields.



Parameter List: The arrows at the top of the parameter list are used to browse through the sweep analysis records of a series. In the example to the right, the result of a *Polynomial* fit to sweep 1 of a series containing 17 sweeps is shown with the selected parameter *Ipeak*.

The value fields can be edited. So if necessary, you can modify data points within the analysis tree.

In addition to the parameters from the *Sweep Fit* results, there are other parameters that may be used as the *Ordinate*. These are essentially identical to the *Abscissa* entries and may be selected from the pop-up menu:

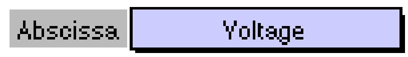
- **Off**: Data selected in parameter list
- **Voltage**: Potential of relevant *X-segment*
- **Duration**: Duration of relevant *X-segment*
- **Sweep Index**: Index of sweep in series
- **Time**: Time relative to start of series
- **Real Time**: Realtime of sweep execution (s)
- **C-slow**: Capacitance of *C-slow* compensation (F)
- **G-series**: Series conductance of *C-slow* compensation (S)
- **Rs-fraction**: % series resistance compensation (%)

Back / Next: These are used to select the previous or the next series within a group, respectively. The title of the *Series Fit* dialog identifies the currently selected series; e.g., if the third series of the fourth group is selected, the title window title will be “Series Fit_4_3”.

Note: *Series Fit does not make any assumptions on what kind of parameters are lumped together for display. Therefore, make sure that you do not fit sweeps of one series with different functions so that the parameters provided for Series Fit are incompatible. You can check this by browsing through the table with the arrows at the table head and checking if the parameter identifiers are all the same. They are blank and the values are zero if no analysis was performed for the corresponding sweep.*

Export Table: This button is used to output an ASCII table of the selected parameters for the entire series. In the example below the beginning of such a table with the parameter *I_peak* being selected is shown. The first three columns of the table are always: Sweep index, potential, and duration of the relevant *X-segment* (the segment number is given in parentheses). Another column holds the value of the selected abscissa (see below) if this is neither potential nor duration. To select more than one parameter, hold down Shift while you select additional parameters.

```
Series No.   1 :
Sweep   V( 2)[mV] T( 2)[ms]   I_Peak
   1      -60.0      10.0    -11.231p
   2      -50.0      10.0    -13.642p
   3      -40.0      10.0    -36.195p
   4      -30.0      10.0    -110.02p
   5      -20.0      10.0    -240.97p
   6      -10.0      10.0    -393.54p
```

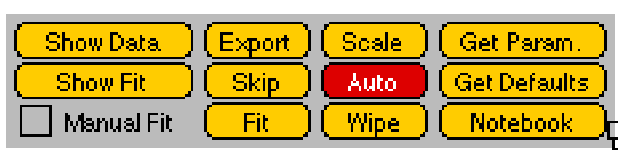


Abscissa: For the abscissa the following parameters are available:

- **Voltage**: Potential of relevant *X-segment* (mV)
- **Duration**: Duration of relevant *X-segment* (ms)
- **Sweep Index**: Index of sweep in series
- **Time**: Time relative to start of series execution
- **Real Time**: Realtime of sweep execution (s)
- **C-slow**: Capacitance of *C-slow* compensation (F)
- **G-series**: Series conductance of *C-slow* compensation (S)
- **Rs-fraction**: Percent series resistance compensation (%)
- **List entry…**: One entry of the parameter table (1-15)

Measuring with the mouse: One can read out X- and Y-values in the graph by clicking the mouse pointer on the desired position in the graph.

Series Fit Control



The following functions provide control of display and fit operations.

Show Data: Display selected parameter as a function of the selected *Abscissa*.

Show Fit: Display fit function according to the selected fit function type (see below) and the fit parameters. The fit function is calculated inside the currently selected bounds of the graph as an array of 100 entries.

Manual Fit: Enable manual fit option similarly to the function in *Sweep Fit*. Any change of the fit parameters (by typing or mouse drag) will cause an update of the fit function display.

Export: Generate output file of displayed data and fit. In case of *Igor* output, *Igor* tags are output that describe the fit result (e.g., Vrev = 45.5 mV) together with the data points and the fit function. Fitted data points and skipped data points are output as separate waves. By default they are displayed as filled and open circles, respectively. Note that the wave names of skipped data have the identifier "nf" (not fit) attached to it. Also note that the minimum number of entries per wave in *Igor* is 2; i.e., if only one point is skipped, it will be exported twice in the same wave. Fit curves are waves of twice 100 points, for the X- and Y-values.

Skip: Data points to be excluded from data fit can be skipped by specifying their number. A text box will ask for the points to skip/resume. Either the indices of individual points can be entered, e.g., 2, 4, 7, a range of points, e.g., 2-13, or both. These disabled data points are then shown as asterisks. Reselection of the same number activates the data points again. If "none" is entered, all data points are activated. Export functions consider active and disabled data points as separate waves (see *Export*).

Fit: Fit selected function to active data points using the *Simplex* fit settings as specified in the *Sweep Fit* window (i.e., *Max. Iterations*, *Epsilon*, *Step Size*).

Note: *Data have to be displayed before they can be fit.* [Space] *terminates the fit. At the end, the fit function is superimposed on the data.*

Auto: When selected, it will auto-scale the graph according to the extreme values in the data set.

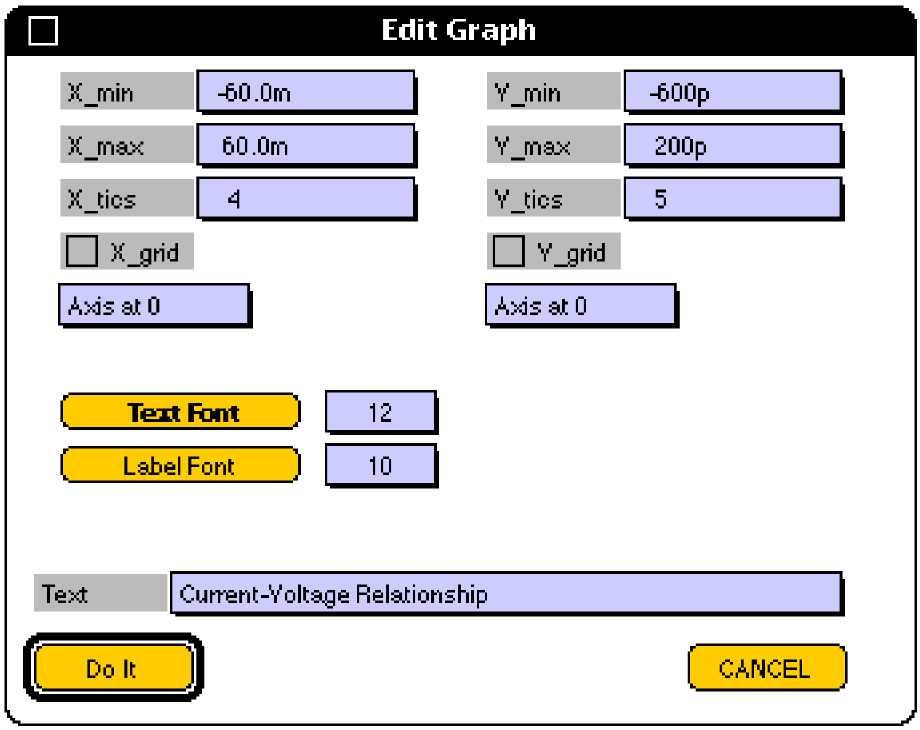
Wipe: Wipe graph display.

Get Parameters: This option will retrieve the parameters of the last fit applied to the selected data set.

Get Defaults: This option will retrieve the default values for a given fit type as stored in the *PulseFit* program. These defaults may or may not be useful for the given data.

Notebook: Writes the result of a fit to the *Notebook*.

Scale: Usually the scaling of the graph is set up according to the extreme values found in the selected data set. A new display scaling can be specified by clicking on *Scale*. The following dialog will appear:



The scaling menu contains the minimal and maximal values of the axes, the number of tics, a flag which specifies the use of a grid, a specification of the axis position (left, right, at zero) and a header text, which is by default the *Series Comment*. You may specify the font and size of the comment and the axis labels. The new header text does not overwrite the *Series Comment*; it is just active as long as you work with one series in the *Series Fit* dialog. If you select the next series and then come back to the last one, the old *Series Comment* will be restored.

After return from this graph scale menu the *Auto* button will be turned off. Thus, the next display is done with the same scaling. If *Auto* is activated again, an automatic scaling is performed for the next *Show* operation. The graph display is wiped before every drawing action when *Auto* is on.

Be aware of the danger of not seeing data points when a manual graph scaling is used. If you find that the fit function does not at all match the displayed data points, it may be that you selected the wrong function, it could also be that there are some strange, non-disabled data points outside the graph that distort the fit. Check by displaying everything with *Auto Scaling* turned on.

Series Fit Functions

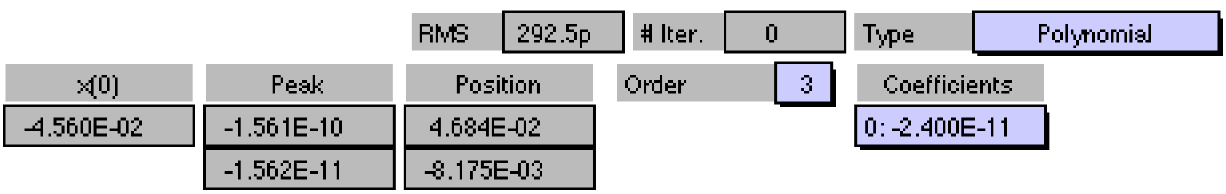
The available functions for data fit are:

- **Polynomial**
- **Exponentials**
- **Boltzmann**
- **Current-Voltage**
- **Copy Sweep**

Most functions have linear and nonlinear parameters. The nonlinear parameters are fit by a *Simplex* algorithm; therefore reasonable start values have to be provided. The linear parameters are calculated and displayed.

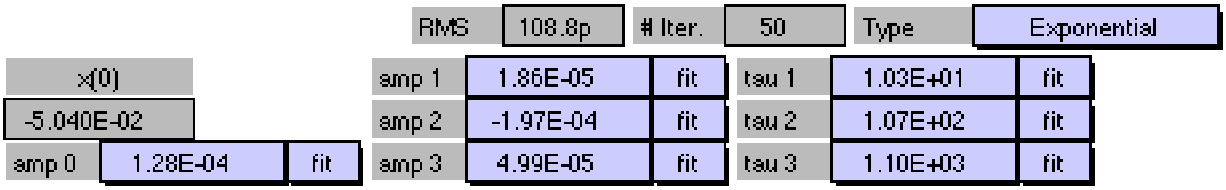
Polynomial: Polynomial of n-th order according to:

$$I(V) = \sum_{i=0}^{n} a_i V^i$$



The list of determined coefficients is output to the *Notebook*. For the order 0, the DC current value is displayed in a separate control. If the order is 1, the zero-crossing of the linear function (x(0)) is calculated and displayed. If the order is 2 or 3, extrema are determined analytically and shown in the dialog controls. For order = 2 there is one peak, for order = 3, there are two peaks. The order of the polynomial, the peak values and their positions, as well as the coefficients of the polynomial are written to the analysis file to be available for further analysis on the level of a group (*Group Fit*). The determined parameters of the polynomial are also written to the list control *Coefficients*.

Exponentials: The parameters should be self-explanatory.



For convenience, there are two types of exponential fits. There are no fundamental differences in these two types of fits. Anyone of them could be used to fit any type of exponential data (by tweaking the parameters):

- **Exponential**: This is useful for fitting inactivation times.

$$I(t) = a_0 + a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)$$

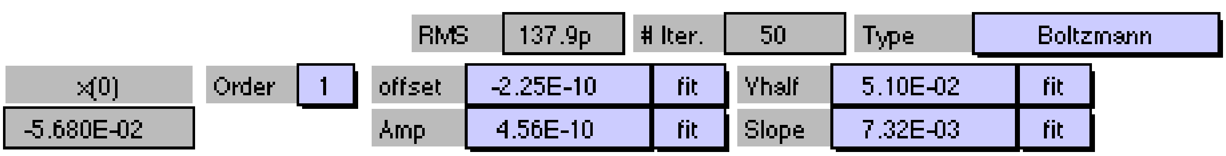
- **1 - Exponential**: This is useful for fitting activation times.

$$I(t) = a_0 + a_1(1 - \exp(-t/\tau_1)) + a_2(1 - \exp(-t/\tau_2)) + a_3(1 - \exp(-t/\tau_3))$$

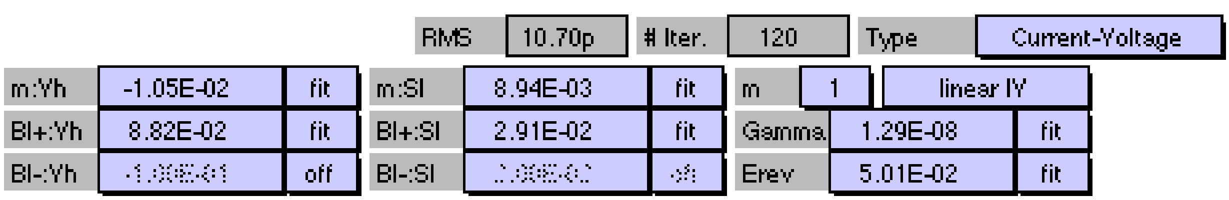
The main difference between these fits is that for exponential, *amp 0* determines the start of the exponential, whereas for 1 - exponential, it determines the value to which the exponential decays. The amplitudes of the exponentials can be set accordingly. There are up to three exponential components with amplitudes and time constants and one starting or ending amplitude (*amp 0*).

Boltzmann: General Boltzmann function according to:

$$I(V) = \text{Offset} + \frac{\text{Amplitude}}{1 + \exp(-(V - V_{half}) / \text{Slope})}$$



Current-Voltage: A current-voltage relationship can be composed of a variety of multiplicative contributions.



The following functions are supported:

- **Open Channel (Linear IV)**: Γ is the conductance in Siemens:

$$I(V) = \Gamma(V - V_{rev})$$

- **Open Channel (GHK IV)**: Goldman-Hodgkin-Katz formalism:

$$I(V) = \Gamma V \frac{1 - \exp(-(V - V_{rev})/\ 25\ \text{mV})}{1 - \exp(-(V / 25\ \text{mV})}$$

- **Activation**: Boltzmann-type activation with power of m:

$$P_{activated}(V) = \left(\frac{1}{1 + \exp(-(V - V_{half}) / \text{Slope})} \right)^m$$

- **High-Voltage Block**: Voltage-dependent block increasing with voltage:

$$P_{unblocked}(V) = \left(\frac{1}{1 + \exp((V - V_{half}) / \text{Slope})} \right)$$

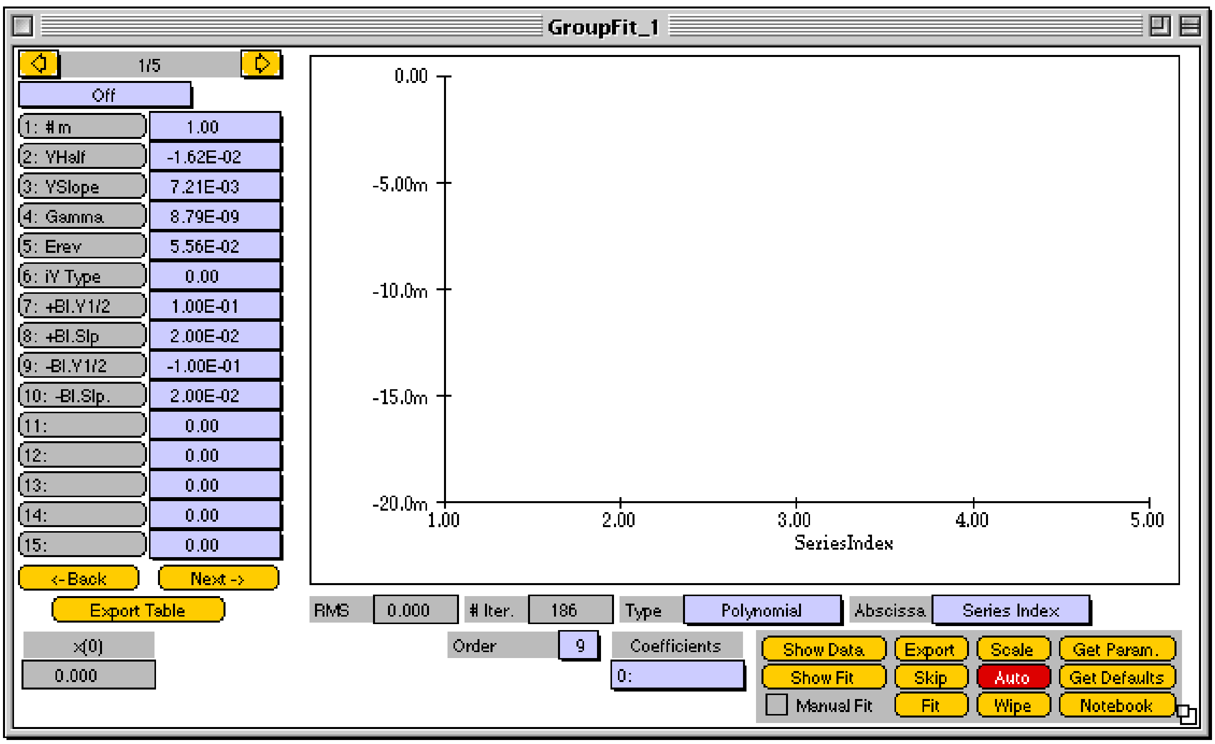
- **Low-Voltage Block**: Voltage-dependent block decreasing with voltage:

$$P_{unblocked}(V) = \left(\frac{1}{1 + \exp(-(V - V_{half}) / \text{Slope})} \right)$$

Copy Sweep: Occasionally, in particular when many series with one sweep were recorded, one may want to display or output results of the *Sweep Analysis* on the level of a group (see *Group Fit*). For this purpose, the results including descriptions of the given sweep in a series can be copied to the series analysis record to appear in the *Group Fit* dialog. Select *Copy Sweep* and the *Fit* button will change to *Copy*. Now click on it to copy the results. The sweep index to copy from is entered with the *Enter Sweep No* option. The *Copy All Sweeps* will copy all n-th sweep results of every series in the active group.

6. Group Fit

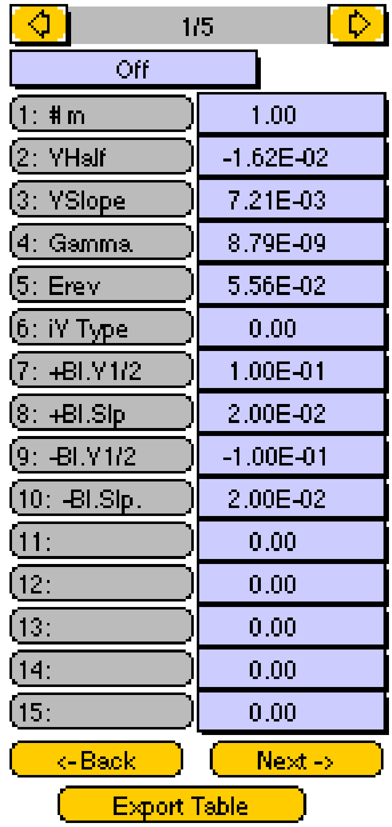
One parameter of the parameter list generated by the *Series Fit* can be plotted as function of parameters as specified by the *Abscissa* control by clicking on the button *Show Data*. The functions of the controls in this window are analogous to the controls of the *Series Fit* window.



Data Selection

Parameter List: The arrows at the top of the parameter list are used to browse through the series analysis records of a group. The value fields can be edited. So if necessary, you can modify data points within the analysis tree.

In addition to the parameters from the *Series Fit* results, there are other parameters that may be used as the *Ordinate*. These are essentially identical to the *Abscissa* entries and may be selected from the pop-up menu:



- **Off**: Parameter from the list as Ordinate
- **Series Index**: Index of series in group
- **Time**: Time relative to start of series execution
- **Real Time**: Realtime of series execution (s)
- **V-membrane**: Membrane potential (mV)
- **Temperature**: Temperature at start of series (°C)
- **Bandwidth**: Filter bandwidth (Hz)
- **V-pipette**: Pipette potential (V)
- **V-cell**: Cell potential (for 2-Electrode Clamp) (V)
- **Seal Resistance**: Seal resistance (Ω)
- **rms Background**: rms background noise (A)
- **Pip. Pressure**: Pipette pressure (mm H_2O)
- **User Parameter 1**: User parameter 1
- **User Parameter 2**: User parameter 2
- **Ext. Solution**: One parameter of the external solution
- **Int. Solution**: One parameter of the internal solution

Back / Next: These are used to select other groups within a root. The window title identifies the currently selected group; e.g., if the third group is selected the window title will indicate "GroupFit_3".

Export Table: This button is used to output an ASCII table of the selected parameters for the entire group. A dialog box will appear: "Output results of all Sweeps?". If *Yes* is selected, the *Series Fit* tables of the entire group are output (according to the settings in the *Series Fit* dialog). "No" means that only the parameters on the level of a *Series* are output. To select more than one parameter, hold down Shift, while you select additional parameters.

Abscissa: For the abscissa the following parameters are available:

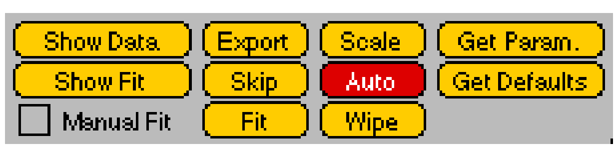
- **Series Index**: Index of series in group

- **Time**: Time relative to start of series execution
- **Real Time**: Realtime of series execution (s)
- **V-membrane**: Membrane potential (mV)
- **Temperature**: Temperature at start of series (°C)
- **Bandwidth**: Filter bandwidth (Hz)
- **V-pipette**: Pipette potential (V)
- **V-cell**: Cell potential (for 2-Electrode Clamp) (V)
- **Seal Resistance**: Seal resistance (Ω)
- **rms Background**: rms background noise (A)
- **Pip. Pressure**: Pipette pressure (mm H_2O)
- **User Parameter 1**: User parameter 1
- **User Parameter 2**: User parameter 2
- **Ext. Solution**: One parameter of the external solution
- **Int. Solution**: One parameter of the internal solution
- **List Entry**: One entry from the parameter table (1-15)

If the abscissa type is either *Ext. Solution* or *Int. Solution* an additional list containing the selectable solution parameters will be shown. The list entries are: *Numeric*, *pH*, *Osmol*, and the *Concentrations* of the individual ingredients. The abscissa type *List Entry* is selected from a drag field identifying the list entry number.

Measuring with the mouse: One can read out X- and Y-values in the graph by clicking the mouse pointer on the desired position in the graph.

Group Fit Control



The following functions provide control of display and fit operations.

Show Data: Display selected parameter as a function of the selected *Abscissa*.

Show Fit: Display fit function according to the selected fit function type (see below) and the fit parameters. The fit function is calculated inside the currently selected bounds of the graph as an array of 100 entries.

Manual Fit: Enable manual fit option similarly to the function in *Sweep Fit*. Any change of the fit parameters (by typing or mouse drag) will cause an update of the fit function display.

Export: Generate output file of displayed data and fit. In case of *Igor* output, *Igor* tags are output that describe the fit result (e.g., V_{half} = -62.5 mV) together with the data points and the fit function. Fitted data points and skipped data points are output as separate waves. By default they are displayed as filled and open circles, respectively. Note that the wave names of skipped data have the identifier “nf” (not fitted) attached to it. Also note that the minimum number of entries per wave in *Igor* is 2; i.e., if only one point is skipped, it will be exported twice in the same wave. Fit curves are waves of twice 100 points, for the X- and Y-values.

Skip: Data points to be excluded from data fit can be skipped by specifying their number. A text box will ask for the points to skip/resume. Either the indices of individual points can be entered, e.g., 2, 4, 7, a range of points, e.g., 2-13, or both. These disabled data points are then shown as asterisks. Reselection of the same number activates the data points again. If “none” is entered, all data points are activated. Export functions consider active and disabled data points as separate waves (see *Export*).

Fit: Fit selected function to active data points using the *Simplex* fit settings as specified in the *Sweep Fit* window (i.e., *Max. Iterations*, *Epsilon*, *Step Size*).

Note: *Data have to be displayed before they can be fit.* Space *terminates the fit. At the end, the fit function is superimposed on the data.*

Wipe: Wipe graph display.

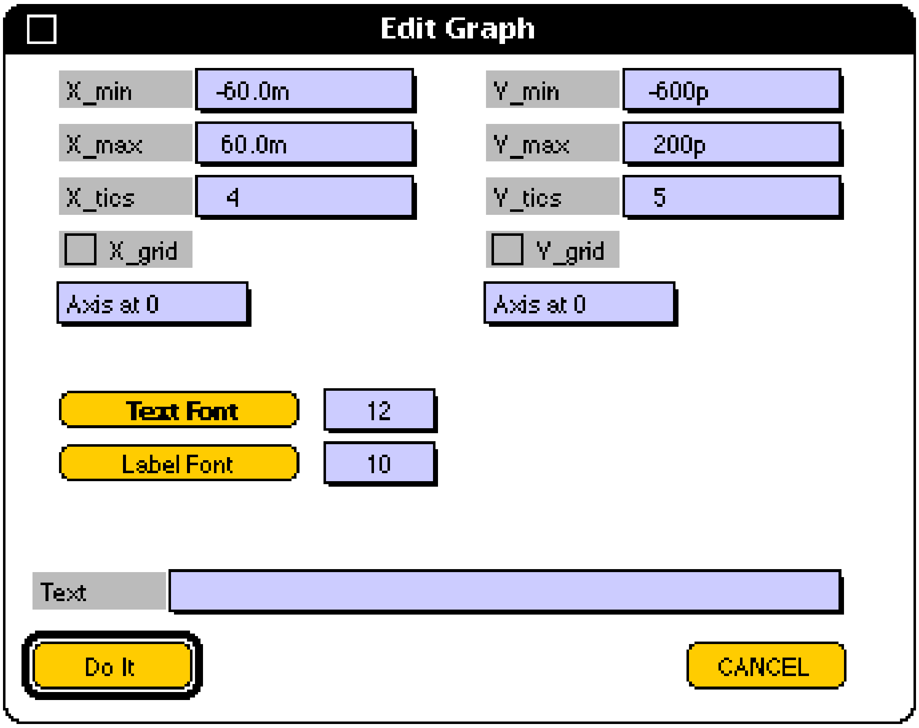
Auto: When selected, it will auto-scale the graph according to the extreme values in the data set.

Get Parameters: This option will retrieve the parameters of the last fit applied to the selected data set.

Get Defaults: This option will retrieve the default values for a given fit type as stored in the *PulseFit* program. These defaults may or may not be useful for the given data.

Notebook: Writes the result of a fit to the *Notebook*.

Scale: Usually the scaling of the graph is set up according to the extreme values found in the selected data set. A new display scaling can be specified by clicking on *Scale*. The following dialog will appear:



The scaling menu contains the minimal and maximal values of the axes, the number of tics, a flag which specifies the use of a grid, a specification of the axis position (left, right, at zero) and a header text, which is by default the *Group Comment* . You may specify the font and size of the comment and the axis labels. The new header text does not overwrite the *Group Text*; it is just active as long as you work with one series in the *Group Comment* dialog. If you select the next series and then come back to the last one, the old *Group Comment* will be restored.

After return from this graph scale menu the *Auto* button will be turned off. Thus, the next display is done with the same scaling. If *Auto* is activated again, an automatic scaling is performed for the next *Show* operation. The graph display is wiped before every drawing action when *Auto* is on.

Be aware of the danger of not seeing data points when a manual graph scaling is used. If you find that the fit function does not at all match the displayed data points, it may be that you selected the wrong function, it could also be that there are some strange, non-disabled data points outside the graph that distort the fit. Check by displaying everything with *Auto Scaling* turned on.

Group Fit Functions

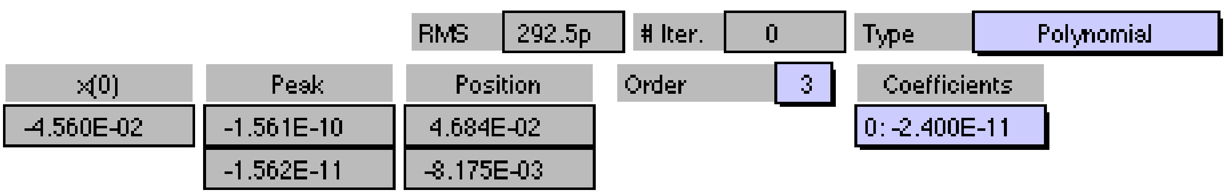
The available fit types are:

- **Polynomial**
- **Exponentials**
- **Boltzmann**
- **Dose-Response**

Most functions have linear and nonlinear parameters. The nonlinear parameters are fit by a *Simplex* algorithm; therefore reasonable start values have to be provided. The linear parameters are calculated and displayed.

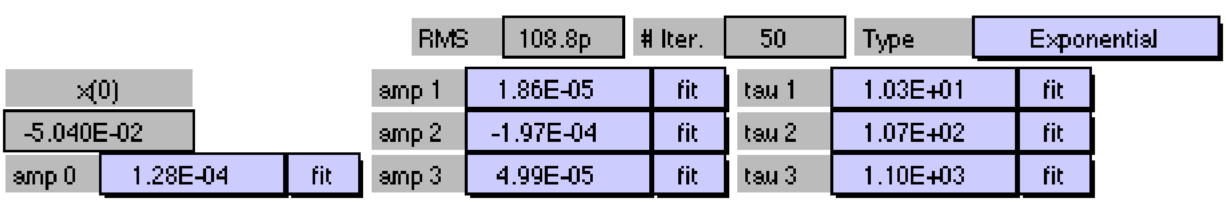
Polynomial: Polynomial of n-th order according to:

$$I(V) = \sum_{i=0}^{n} a_i V^i$$



The list of determined coefficients is output to the *Notebook*. For the order 0, the DC current value is displayed in a separate control. If the order is 1, the zero-crossing of the linear function (x(0)) is calculated and displayed. If the order is 2 or 3, extrema are determined analytically and shown in the dialog controls. For order = 2 there is one peak, for order = 3, there are two peaks. The order of the polynomial, the peak values and their positions, as well as the coefficients of the polynomial are written to the analysis file. The determined parameters of the polynomial are also written to the list control *Coefficients*.

Exponentials: The parameters should be self-explanatory.



For convenience, there are two types of exponential fits. There are no fundamental differences in these two types of fits. Anyone of them could be used to fit any type of exponential data (by tweaking the parameters):

- **Exponential**: This is useful for fitting inactivation times.

$$I(t) = a_0 + a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)$$

- **1 - Exponential**: This is useful for fitting activation times.

$$I(t) = a_0 + a_1(1 - \exp(-t/\tau_1)) + a_2(1 - \exp(-t/\tau_2)) + a_3(1 - \exp(-t/\tau_3))$$

The main difference between these fits is that for exponential, *amp 0* determines the start of the exponential, whereas for 1 - exponential, it determines the value to which the exponential decays. The amplitudes of the exponentials can be set accordingly. There are up to three exponential components with amplitudes and time constants and one starting or ending amplitude (*amp 0*).

Boltzmann: General Boltzmann function according to:

$$I(V) = \text{Offset} + \frac{\text{Amplitude}}{1 + \exp(-(V - V_{half}) / \text{Slope})}$$

Dose-Response: Dose-response relationship according to:

$$y(x) = \text{Offset} + \frac{\text{Amplitude}}{1 + (x/K_D)^{Hill}}$$