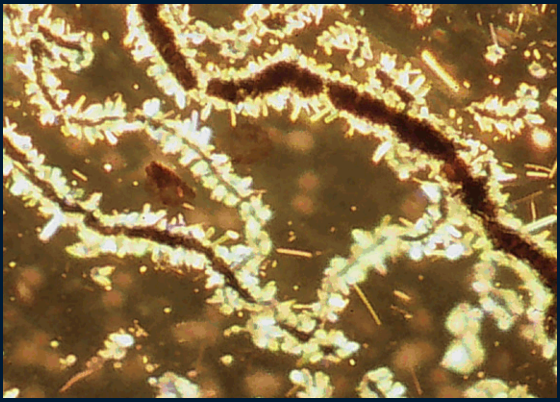
Tutorial 2.20

FITMASTER

Tutorial



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Title Page: Blood Vessels of the Retina; courtesy of Max-Planck-Institute for Brain Research, Frankfurt

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1. Introduction

1.1 Prerequisites

For demonstrating FitMaster, please

1. **Install** FitMaster version 2.05 from the HEKA CD-ROM or download a web installer from our web site.

   The installation contains:

   - a default Online Analysis file `DefAnalFit.onl`
   - a default Key file `FitMaster.key`
   - a default setting file `FitMaster.set`

   all located in the Fitmaster folder.

2. **Demo Data** In addition, we provide a demo data set for the Fitmaster program. In case you use downloaded Fitmaster from out web site, please also download the zip file containing demo data. Please copy the file `DemoFitData` in the C:/HEKA/Data folder.

   This `DemoFitData` data set includes the following series:

   - IV
   - Hinf (3 Series)
   - Recovery
   - Onrate
   - Trains (3 Series)

3. **Dongle** A dongle is requires if you would like to save your analysis results. The demo data set can be loaded without a dongle.

1.2 Window Placement

For demonstrating FitMaster you just need to display the following windows:

- `TraceFit`
- `Oscilloscope`
- `Replay`
- `OnlineAnalysis`
- `Notebook`

We recommend the following window placement.

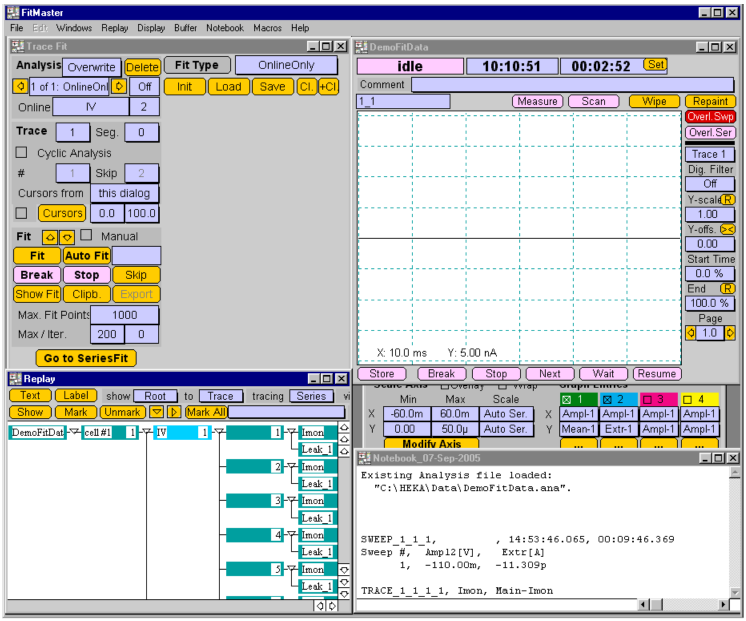

Figure 1.1: Recommended window placement for demonstrating FitMaster

1.3 Start-up

Double click the FITMASTER program executable and start the demonstration with using FITMASTER'S default settings.

Then, load the file `DemoFitData.dat` in `Modify Analysis` mode. Please double check if the default Online Analysis is loaded `DefAnalFit.onl`.

The `OnlineAnalysis` should be set to `Automatic Stimulus Control`. This way the default analysis is always selected correctly and if you want you do not have to worry about the online analysis at all.

1.4 Hints and Tips

Please note the following:

- The analysis file `DemoFitData.ana` if present from a previous demonstration should be deleted or renamed. Starting the demonstration with an empty analysis file helps keeping an overview on all the results generated.
- A major difficulty is to navigate through your analysis results in `SeriesFit`. Parameters might have the same name for different Series. Therefore, please always have a look at the "Series" selection in the `SeriesFit` window (top left).

2. Analysis and Fit of a Current-Voltage Relationship

- Open the `TraceFit` window (or activate it).
- Set the `FitType` to `OnlineOnly`.
- In the `Replay` window select the first sweep of the IV series.
- Click `Auto Fit` in the `TraceFit` window. (IV parameters are analyzed.)
- Go to the `SeriesFit` window.
- Select from the parameters “Ampl” in the first row and “Extr” in the second row.
- Make sure that `Parameters from` *Results* is selected.
- Press `Preview` in the waves section to display the data in the `SeriesGraph`.
- Select `Current-Voltage` function from the `Fit Type` selection and press `Fit`.

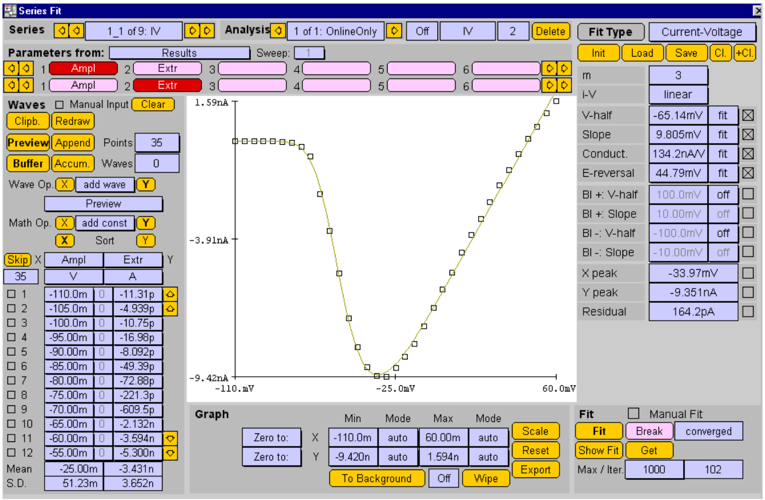

Figure 2.1: SeriesGraph: Results and Fit of an IV curve.

3. Demonstration of Background Traces

- Close the `SeriesFit` window.
- In the `Replay` window select the first sweep of the second series ("Hinf_LTQ2") and go back to the `TraceFit` window.

> ***Note:*** *Use the arrow keys to maneuver through the replay tree. To easily go from the last to the first sweep in a series, press the left and then the right arrow.*

- Press `Auto Fit` to analyze the whole series.
- Switch to the `SeriesFit` window.
- Select "Ampl" vs "Extr" and press `Preview`.
- Select `Boltzmann` function from the `Fit Type` selection and press `Fit`.
- Now, click `To Background` to add this wave to the background trace buffer.
- Then, close the window and select the first sweep of the second Hinf series and perform an AutoFit from the `TraceFit` window. Go to `SeriesFit`, automatically the results from the last analysis is presented. Also the selection might be correct already (Ampl vs Exts). Just press `Preview` to display the new results and the background wave. Press `To Background` again to copy the new results also to the background wave buffer.
- Then repeat the above procedure with the third series.

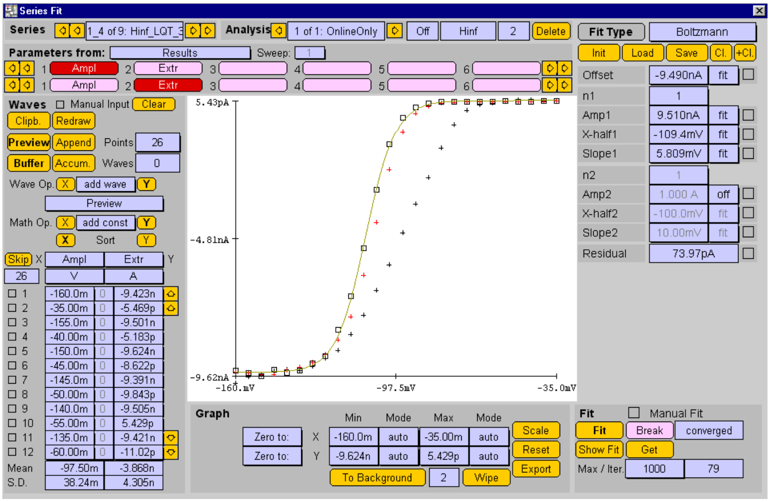

Figure 3.1: `SeriesFit` Window: Result, Fit and Background Traces displayed.

Once the results from the third series are displayed together with the two background waves, you can play around with retrieving fit results form the data file. E.g. go to the series selection on the top left of the `SeriesFit` window and select another one of the three Hinf series (e.g. click on the left arrow). Now go to the fit section and press `Get` to retrieve the fit parameters from the analysis file. You will see the parameters changing. Then press `Show Fit` and you will see the fit superimposed to one of the background waves. This way you can easily compare different fit results.

4. Averaging results from different Series

We will average the results of the Hinf series and fit a Boltzmann curve to the average and finally export the average with error bars, the fit and fit parameters.

First, we will open the `FitConfiguration` from the `Windows` menu and select the pane `SeriesFit`. Then, we set the `Waves Errors` to `S.E.M.´ to calculate and show the standard error of the mean of each point in the H_inf curve.

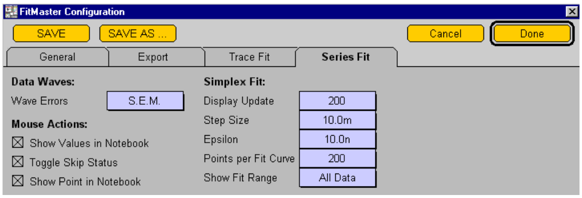

Figure 4.1: `FitConfiguration` Window: Result, set Waves Errors to S.E.M.

- We assume that the three series `Hinf´ have been analyzed with `TraceFit` already.
- Go to the `SeriesFit` window.

> ***Note:*** *To clean up the program form previous demonstrations you might* `Wipe` *the background buffer and* `Clear` *the waves buffer.*

- Then, select the first Hinf series.
- Press `Preview` to display the selection in the `SeriesGraph`.

- In case this is the correct graph, press `Accum.` to transfer the results of the first series to the waves buffer.
- Select the second "Hinf" series, press `Preview` and then `Accum.` to accumulate the second series to the buffer. Do the same with the third Hinf series.

Now, you can fit a Boltzmann function to the average of the three results by pressing `Fit`.

> ***Note:*** *The fit results will be stored with the series selected when pressing the* `Fit` *button.*

4.1 Export SeriesFit results with error bars in IgorPro format

Prerequisite: have at least a demo version of IGOR PRO installed on the demo computer.

- First, go the the `Replay` menu and set the export format to "Igor Pro".
- Go back to the `SeriesFit` window and display the averaged results with the fit. (All required data should be still in the waves buffer if you continue sequentially from the section before.)
- Press `Export` and save the Igor ∗.itx file.

Double click the ∗.itx file. Igor will automatically load and display the data.

Fit parameters are stored in the waves "∗_label" and "∗_res". Please make a new table (Windows/New Table ...) to show the two parameter waves.

5. Analysis of a Recovery Process and Fit of Exponential

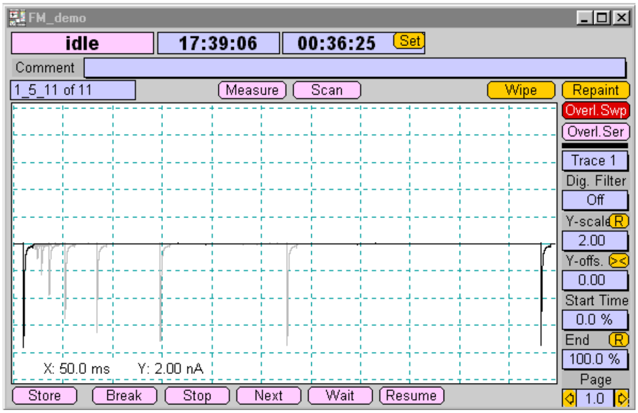

Figure 5.1: Display of the series Recovery in the oscilloscope.

- Analyze the series "Recovery". (Follow the procedure described above.)

- Go to `SeriesFit` and display "dt" versus "Extr". The recovery of current amplitude is plotted versus the duration of the time segment between the first (reference) stimulus and the test stimulus.

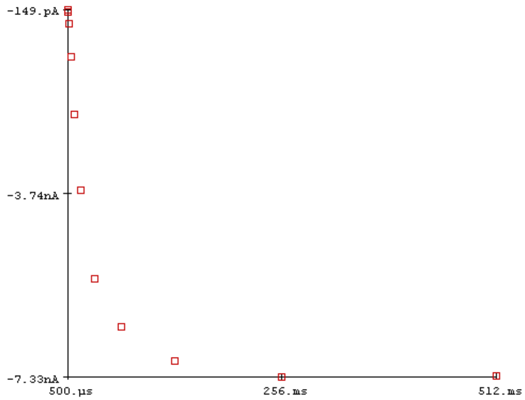

Figure 5.2: Display of “Extr” versus “dt” in the SeriesGraph.

- The online method also analyzed the amplitude of the reference stimulus. Displaying “dt” versus “Ref” shows the amplitude of the first current.

- At the bottom of the wave buffer table the mean and standard deviation of the current is displayed. The amplitude is -7.17 nA with a small S.D of 127 pA.

- The `OnlineAnalysis` has also calculated the ratio of the ”Extr” and “Ref” to get a normalized result (”Norm”) for each sweep.

- Please display “Norm” versus “dt”. You will see the recovery of the current with longer durations. (1 corresponds to 100%)

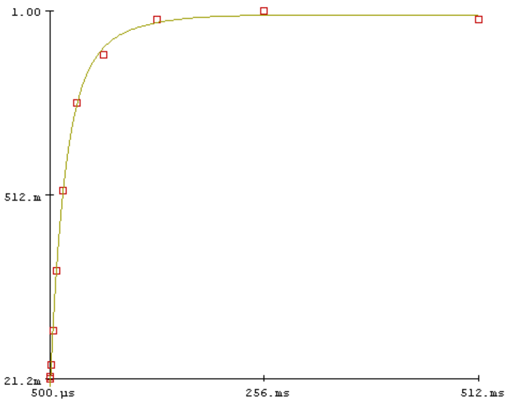

Figure 5.3: Display of the normalized current amplitude versus “dt” in the SeriesGraph. A double-exponential fit is superimposed.

5.1 Fitting of an exponential curve to the data

Select the function `1-exponential` from the `Fit Type` selection and fit the data. You might play around with one or two time constants.

5.2 Inspection of data by mouse click

Now click e.g. on the third data point from the right in the display. In the notebook you will see info on this point:

```
dt: 130.80ms   Norm:  979.71m   # 9=off
```

In addition you can toggle the skip status by clicking on the data point (you can configure this behavior in the `FitConfigurations`). A skipped data point is represented by a star. Just click on the data point again to enable this point.

6. Analysis of a Time Series

- Analyze the series "Onrate" with `TraceFit` using `OnlineOnly` as fit function.

- Go to the `SeriesFit` window and display "Extr" versus "SerTime". You will see the current amplitude decreasing rapidly at a time of about 90 seconds.

How do we analyze this set of data? We would like to

1. normalize the currents to the baseline current and measure the percentage of current reduction or percentage of remaining current.

2. measure the time constant of current reduction.

To measure the baseline current we can proceed as follows:

6.1 Normalization to baseline results

- In the next step we would like to normalize the current amplitude to the mean current amplitude before the current reduction. We would like to calculate the mean of the first 10 current responses.

- Click on the `Skip` button and enter "11-end" in the upcoming dialog box. This way we skip all entries except the first ten.

Skip X	SerTime	Extr	Y
10	s	A	
☐ 1	24.06m	-5.043n	
☐ 2	5.024	-4.991n	
☐ 3	10.02	-5.072n	
☐ 4	15.02	-4.918n	
☐ 5	20.02	-4.942n	
☐ 6	25.02	-4.879n	
☐ 7	30.02	-4.927n	
☐ 8	35.02	-4.850n	
☐ 9	40.02	-4.876n	
☐ 10	45.02	-4.891n	
☒ 11	50.02	-4.847n	
☒ 12	55.02	-4.926n	
☒ 13	60.02	-4.860n	
☒ 14	65.02	-4.719n	
☒ 15	70.02	-4.620n	
Mean	22.52	-4.939n	
S.D.	15.14	74.23p	

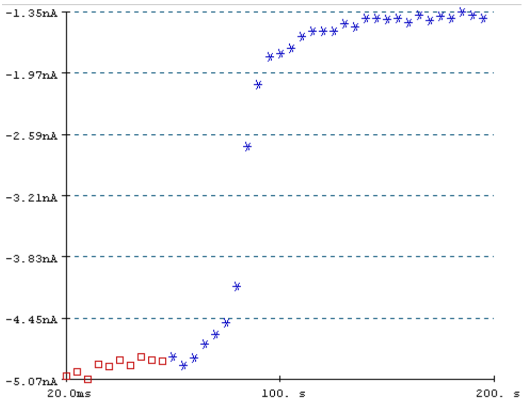

Figure 6.1: Left) Wave Buffer: Skipped entries are marked at the check-boxes, buffer statistics are shown on the bottom. Right) Display of "Extr" versus "SerTime". Skipped data points are marked with stars.

- At the bottom of the wave buffer the mean and SD of all non-skipped buffer entries is displayed. The mean baseline current is -4.9 nA.
- Now we set all data points active again. Click on the `Skip` button and enter "on" in the upcoming dialog box and click OK.
- We will divide all Y-values by -4.9 nA to get a normalized current. Therefore, select "div const" from the math selection and press the `Y` button. Enter "-4.9n" in the upcoming dialog box and click OK.

6.2 Normalization to baseline results, using Online Analysis

Alternatively, you can use the `OnlineAnalysis` to perform the normalization. This procedure involves some more steps, but you will have the raw and normalized data available in `SeriesFit`.

The description starts after reading the mean baseline current from the statistics field of the waves buffer.

- Now we close the `SeriesFit` Window and bring the `OnlineAnalysis` window to front. The function “Ref” is a constant. We now manually assign the mean value to this constant (-4.9n).

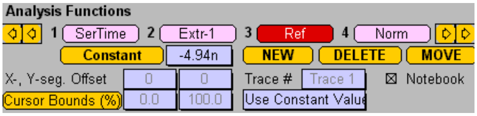

Figure 6.2: Online Analysis function. The mean baseline current is assigned to the function “Ref”.

- Now select again the first sweep of the “Onrate” series (click in the Replay window and press the left arrow key followed by the right arrow key) and analyze the series again with `TraceFit`.

- Go back to the `SeriesFit` window and display “Norm” versus ”SerTime”.

6.3 Adapting the Graph display

Now it is a good time to customize the graph display. Click on the `Scale` button to open the `Scale Property` window. Choose `Round to 0/1/2/5` for both axis, check the grid display for the Y-axis and set the number of Tics to 7 for the Y-axis.

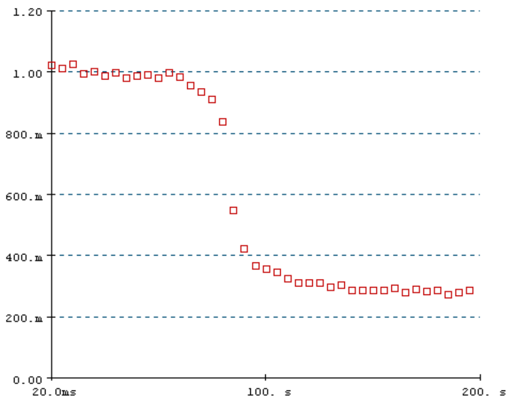

Figure 6.3: Reduction of the normalized current amplitude.

6.4 Analyzing the current reduction

At a time of about 90 seconds the current rapidly reduces from 100% (1) to about 30% (0.3). We would like to fit a curve to this time course.

In order to measure the time constant of the current reduction we will fit the time course with a $(exp(x - x0)^n)$ function. This function describes an exponential time course dropping from a baseline to another amplitude.

> ***Important note:*** *The initial fit parameters might not be suitable for a successful fit. Please first think, how you can optimize the starting parameters.*

- X0 (time of start of the decay): we guess 80s
- Amp0 (baseline): we guess 1.0
- n1: we use 1 and set the parameter to “hold”
- Amp1 (amplitude of decay): we guess 0.7
- Tau1 (time constant of decay): we guess 2.0s

Now let's try a fit. Press **Fit**. Successful, please see the resulting fit parameters below.

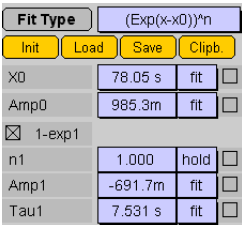

Figure 6.4: Fit parameters for the "Onrate" series.

The fit results show that the current is dropping by about 69% (see Amp1) with a time constant of 7.5 seconds (see Tau1).

You might improve the fit by

- Skip some points around the onset of the decay. During this time the drug concentration might not be constant.
- Set n1 to a higher value (e.g. n1 = 2) to make the onset of the decay more sigmoidal.

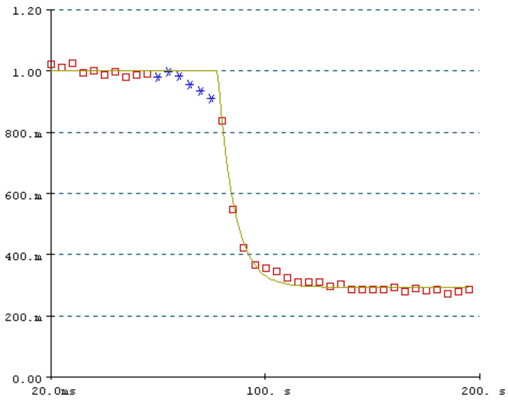

Figure 6.5: Fit of an exponential decay. Points at the onset are skipped.

7. Cyclic Analysis

We have recorded a use-dependent inactivation of sodium currents. In our pgf sequence we have used 11 stimuli (depolarizations) separated by a waiting segment. The current response is displayed below.

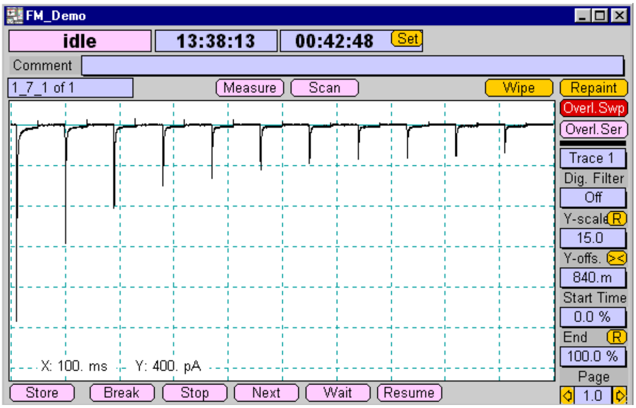

Figure 7.1: Use-dependent inactivation of sodium channels. 11 depolarizations within one sweep.

We will analyze the current amplitudes within this sweep using the `Cyclic Analysis` of `TraceFit`. Just turn `Cyclic Analysis` on by activation the checkbox next to it. Then enter the number of responses (results) and the number of skip segments. (If you are in the segment showing the first response. Then you have to increase the segment number by 2 to get to the segment of the next response. Skip = 2.)

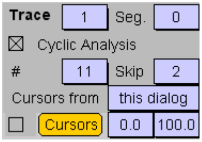

Figure 7.2: Turn on Cyclic Analysis, 11 results with skip segments 2.

Make sure that the sweep of the series "Train" is activated in the `Replay` window. Then press `Auto Fit` to analyze the sweep. Go to the `SeriesFit` window, select Parameters from - All Events" 

and display Extr" versus "t_seg".

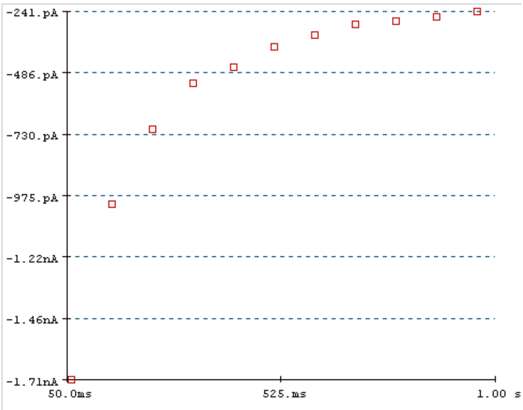

Figure 7.3: Use-dependent inactivation of sodium channels. Current amplitude versus Time.

7.1 Normalizing each inactivation curve

Now, we would like to analyze all three trains. In addition, we would like to normalize the currents to the first response in the train.

- First, we analyze all three trains with the `Cyclic Analysis` method in the `TraceFit` window as described above.

- Then, we go to the `SeriesFit` window, clear the waves buffer and preview the "Extr." versus "t_seg" of the first train.
- Use the Normalize to Y-min function to normalize the previewed graph.
- Use `Accum.` to accumulate the normalized series to the waves buffer.
- Repeat the procedure with train 2 and train 3.

You will then see the averaged normalized inactivation curve.

7.2 Averaging normalized inactivation curves and fitting an exponential

Now, you can fit a decaying exponential to the averaged data. We choose the function of type $exp(x-x0)$. Our data allow constraining the function. E.g. X0 = 50 ms, Amp0 = 1, n1 = 1. We set these values to "hold".

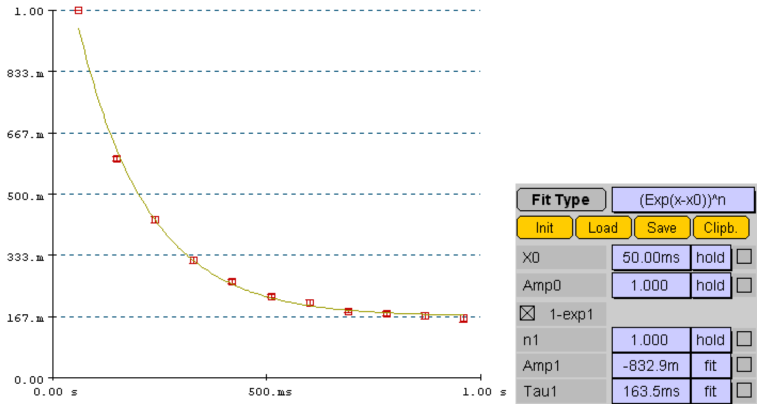

Figure 7.4: Exponential decay of the normalized current response. Data superimposed by decaying exponential. Fit parameters are shown on the right.

8. Amplitude Histograms

Amplitude histograms can e.g. be used to analyze the ratio of `Open` to `Close` time (as ration of `Amp0` over `Amp1` of a double-Gaussian fit), the mean current level of the `Open` and `Close` level, and the baseline noise in the recording.

> ***Note:*** *When analyzing single-channel data, please make sure that the option `Subtract Zero Offset` in the `Display` menu is turned off.*

Then, proceed as follows:

- Select the first sweep of a series containing single-channel data.
- Then, select `Amplitude Histogram` as `TraceFit` function and set the `Number of bins`, the `Lower Edge` of the first bin, and the `Bin Width` to appropriate values.
- Use `AutoFit` to analyze the complete series.
- Goto `SeriesFit`, select as parameters `Amplitude` and `Count`, and set the `Parameters from:` option to `Add All Events Sweepwise`. Then, press the `Preview` button.
- In the `SeriesFit` graph the cumulative histogram is shown.
- Choose `Gaussian` as `SeriesFit` function and adjust the initial parameters for the fit appropriately.
- Press `Fit`. The fit function is also shown in the `SeriesFi` graph.

9. Power Spectra Analysis

Power spectra can e.g. be used to easily measure the mean current through a single channel. The single channel current i can be calculated from the power spectrum as follows:

$$i = \sqrt{2\pi \int S(f)}$$

> ***Note:*** *For display and scaling of the power spectra it is required to set the* ***`Number Presentation`*** *(see* ***`FitConfiguration/General`****) to* ***`Scientific`****. To make this change to become effective restart of* FITMASTER *is required.*

- Select the first sweep of a series containing single-channel data.
- Then select `Power Spectra` as `TraceFit` function and set the `Number of Points` as high as the data allow (512 for `Singles_Demo.dat`).
- Use `AutoFit` to analyze the complete series.
- Goto `SeriesFit` and select as parameters `Frequency` and `SpecDens`, and set the `Parameters from:` option to `Avg All Events Sweepwise`. Then, press the `Preview` button.
- In the `SeriesFit` graph the averaged power spectrum is shown.
- Choose `Spectra` as `SeriesFit` function. Use the `Shot Noise` and the `Lorentzian` component of the function to fit the spectrum.
- Press `Fit`. The fit function is also shown in the `SeriesFit` graph.