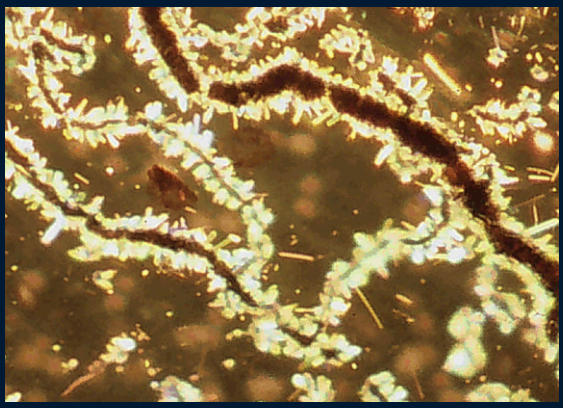
Tutorial 2.73

Fitmaster

Tutorial



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

COFITU/3

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1. Introduction

1.1 Prerequisites

For demonstrating FITMASTER, please

1. Install the latest FITMASTER version. It can be download from our web site (http://www.heka.com/download/download.html#fitmaster). For this tutorial we used FITMASTER v2x73.

   The installation contains:

   - the FITMASTER executable (`FitMaster.exe`)
   - a default Online Analysis file (`DefAnalFit.onl`)
   - a default Key file (`FitMaster.key`)
   - a default Help file (`FitMaster.hlp`)
   - a default Equations file (`Equations.txt`)

   all files are located in the `Fitmaster` folder.

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

   This `DemoFitData` data set includes 5 *Groups*:

   (a) cell#1
   - IV
   - Hinf_LQT (3 Series)
   - recovery
   - Onrate
   - Train (3 Series)

(b) slow

- Test (5 Series)

(c) faster

- Test (5 Series)

(d) 2 channels

- Test (5 Series)

(e) E-1

- CClamp (3 Series)

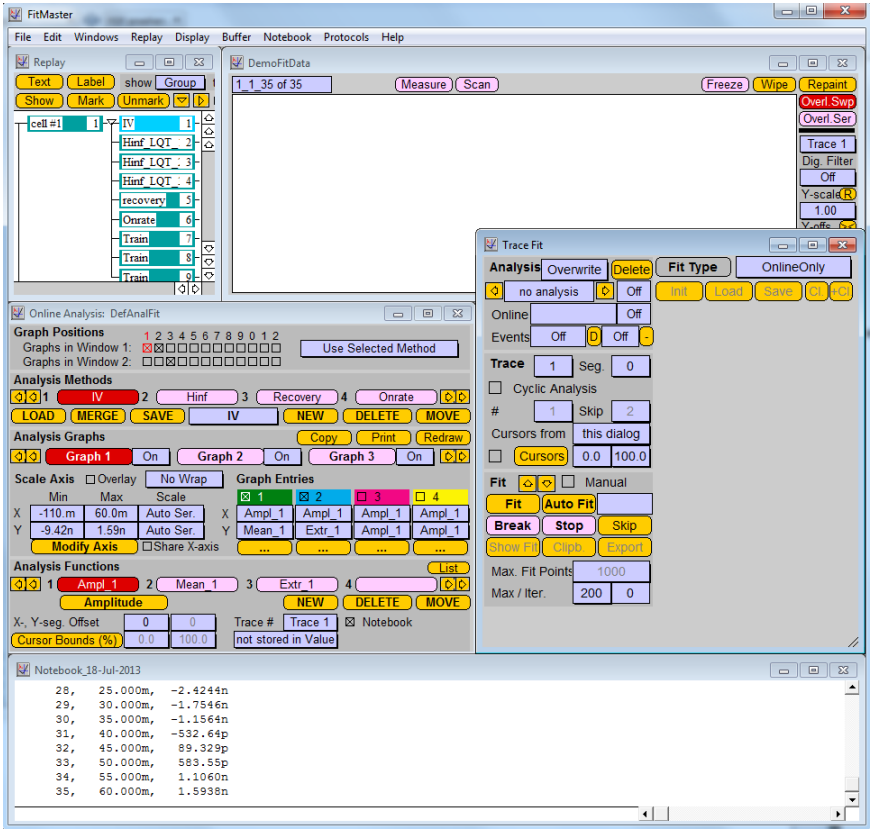
3. A dongle is required if you would like to save your analysis results. The demo data set can be loaded without a dongle.

1.2 Window Placement

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

- TraceFit
- Oscilloscope
- Replay
- Online Analysis
- Notebook

We recommend the following window placement.



1.3 Start-up

Double click the FITMASTER program executable and start the demonstration with using FITMASTER 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 Online Analysis should be set to *Automatic Stimulus Control*. This way the default analysis is always selected correctly and 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 the Series Fit window. Parameters might have the same name for different *Series*. Therefore, please always have a look at the *Series* selection in the *Series Fit* window (top left).

2. Analysis and Fit of a Current-Voltage Relationship

In this chapter we want to demonstrate how to simply create a current-voltage relationship in FITMASTER. Therefore we show you how to:

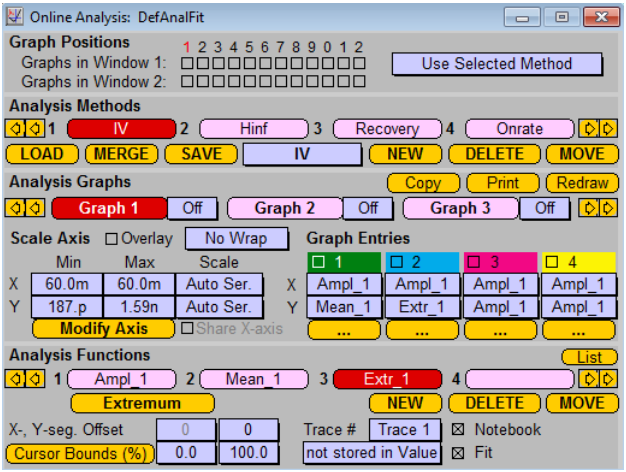
- set the *Analysis Function* in the Online Analysis.
- generate the analysis results with the Trace Fit window.
- plot the data in the Series Fit window.
- fit the current-voltage relationship in the Series Fit window.

2.1 Online Analysis

- Open the `DemoFitData` data.
- Open the Online Analysis window and open the `DefAnalFit.onl` file (default file).
- Select the first *Analysis Method*, named "IV".
- As you can see there are already three *Analysis Functions* inside this *Analysis Method*: "Amplitude", "Mean" and "Extremum". All set for *Trace 1* which is ok. The name of the corresponding Online Analysis was already set in the PGF. Therefore, when *Automatic Stimulus Control* is enabled, the user does not have to care about the proper *Analysis Method* selection.
- Please check if the option *Notebook* for "Ampl_1" and for "Extr_1" is activated.

> ***Note:*** *It is important for later steps that the analysis values are written into the* Notebook.

- If desired you can also plot the results in an Online Window but this is not necessary here.



That is so far all we have to do in the Online Analysis.

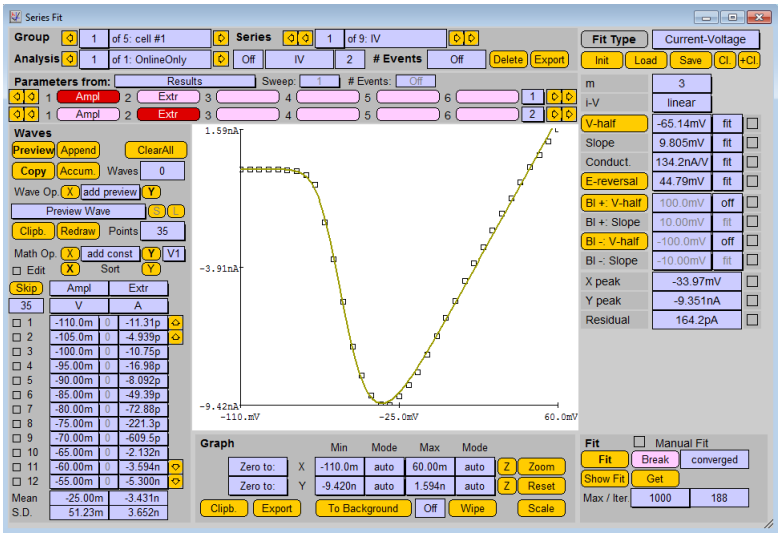
2.2 Trace Fit Window

- Open the Trace Fit window (or activate it).
- Set the *Fit Type* to *OnlineOnly*.
- In the Replay window select the first *Sweep* of the IV *Series*.
- Click *Auto Fit* in the Trace Fit window.

Next, we open the Series Fit window.

2.3 Series Fit Window

- Go to the Series Fit window.
- Select from the parameters "Ampl" in the first row and "Extr" in the second row.
- Make sure that for *Parameters from* "Results" is selected.
- Press *Preview* in the "Waves" section to display the data in the *Series Graph*.
- Select the *Current-Voltage* function from the *Fit Type* selection and press *Fit*.



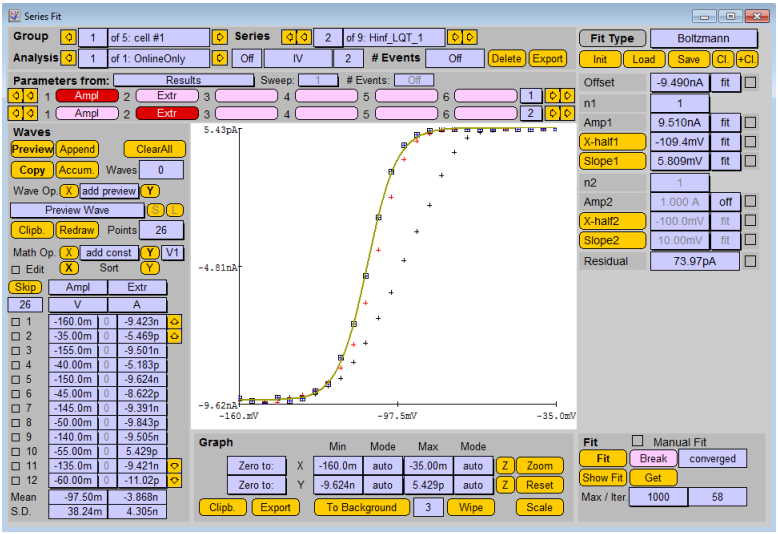
The result is a very nice fit of the current-voltage relationship. If necessary the fit parameters can be modulated in the "Fit" section.

3. Demonstration of Background Traces

- Close the Series Fit window.
- In the Replay window select the first *Sweep* of the second *Series* ("Hinf_LTQ2") and go back to the Trace Fit 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 Series Fit 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 *Auto Fit* from the Trace Fit window. Go to Series Fit, automatically the results from the last analysis is presented. Also the selection might be correct already (Ampl vs. Extr). 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*.

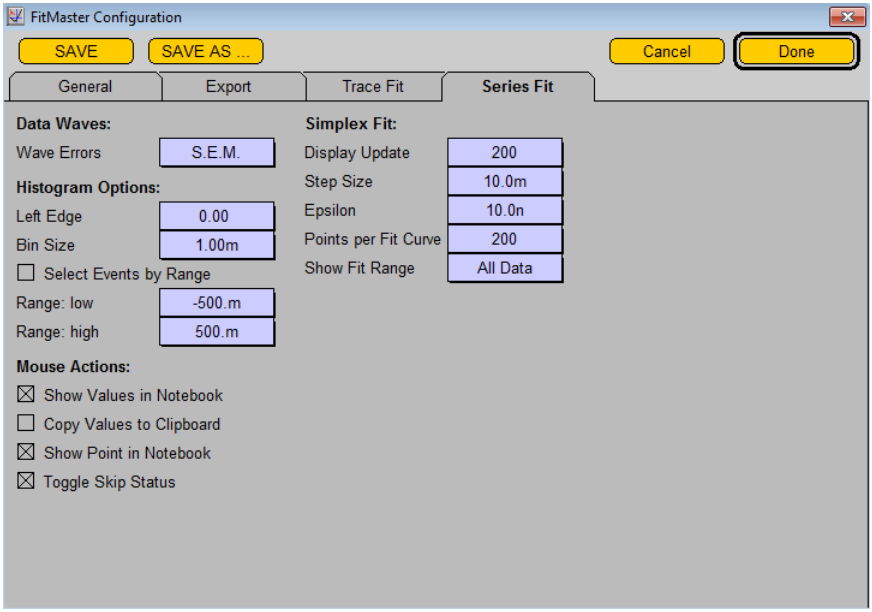


Once the results from the third *Series* are displayed together with the two background waves, you can play around with retrieving fit results form the data file. E.g. go to the *Series* selection on the top of the Series Fit window and select another one of the three "Hinf" *Series* (e.g. click on the 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 Fit Configuration from the Windows menu and select the tab *Series Fit*. 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 "Hinf" curve.

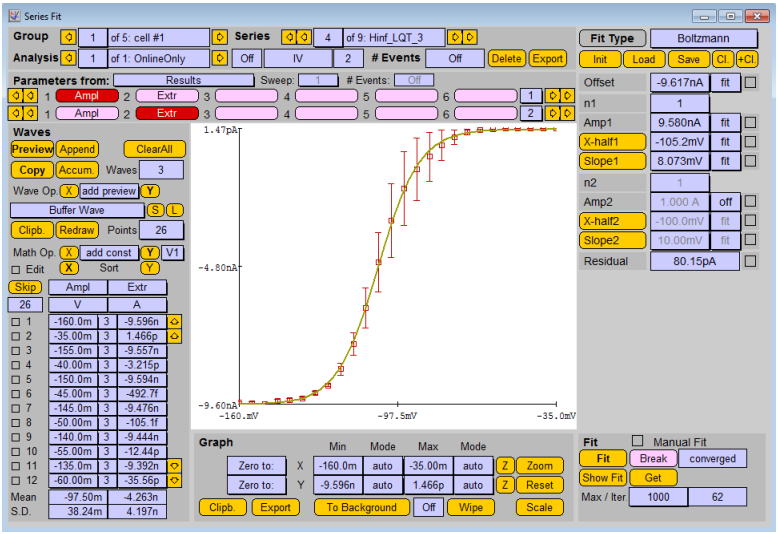


- We assume that the three *Series* "Hinf" have been analyzed with Trace Fit already.

- Go to the Series Fit 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 *Series Graph*.
- 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*.
- Select "Boltzmann" fit type to fit the average of the three results.
- Press the *Export* button to save the averaged results as well as the fit in the ASCII format.



4.1 Export Series Fit results in IgorPro format

Note: *You need at least a demo version of* IGOR PRO *installed on the demo computer*

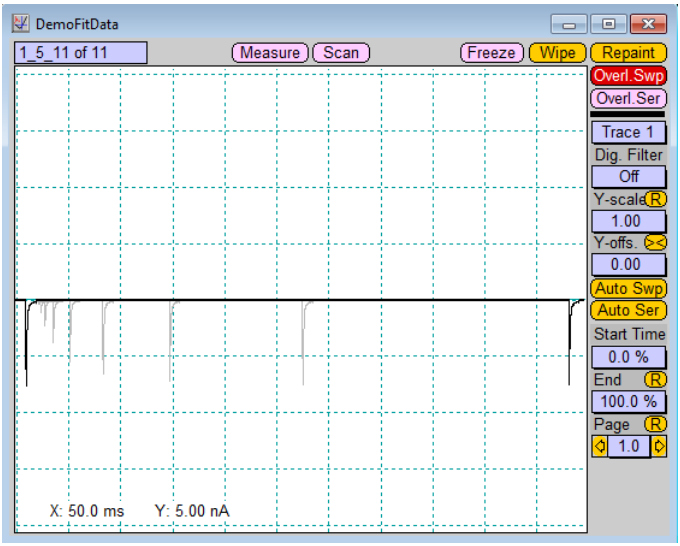
.

- First, go the the `Replay` menu and set the export format to "Igor Pro".
- Go back to the Series Fit 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 the *Export* button in the Series Fit window and save the Igor `*.itx` file.

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

5. Analysis of a Recovery Process and Fit of Exponential

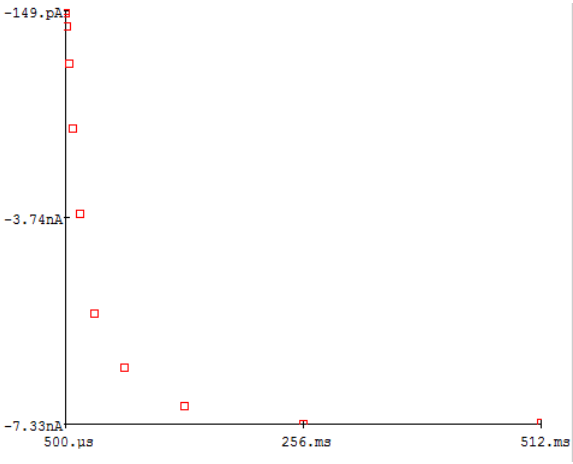
Display the *Series* "Recovery" in the Oscilloscope:



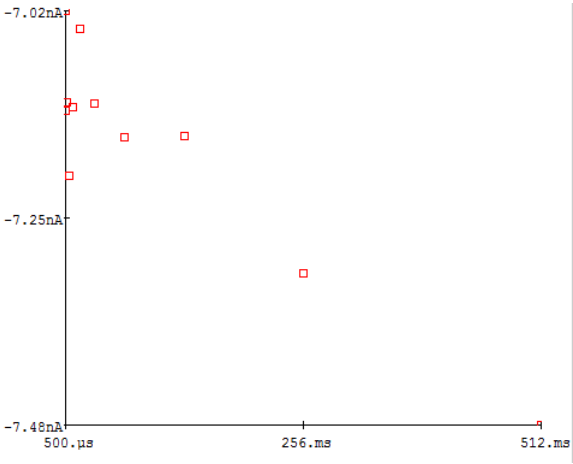
- Go to the Online Analysis window and add the new *Analysis Function* "Duration" and enable the checkbox *Notebook*.

- Analyze the *Series* "Recovery" in the Trace Fit window as described before.

- Go to Series Fit window 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.



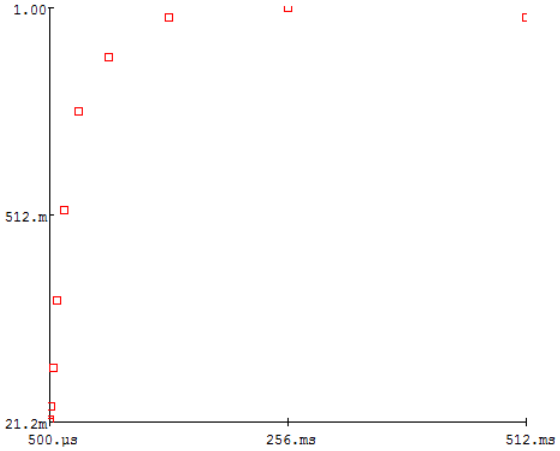
- The *Analysis Method* also analyzed the amplitude of the reference stimulus. Displaying "dt" versus "Ref" shows the amplitude of the first current.



- 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.

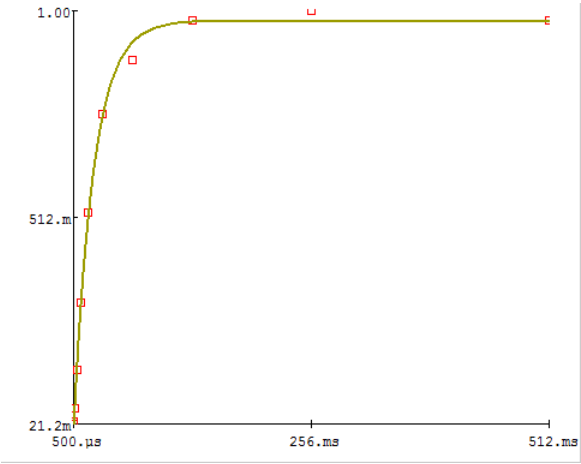
- The Online Analysis has also calculated the ratio of the "Extr" and "Ref" to get a normalized result for each *Sweep*. The function is named "Norm".

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



5.1 Fitting of an exponential curve to the data

Select "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: 129.87ms   Norm:  979.71m   # 9=off
```

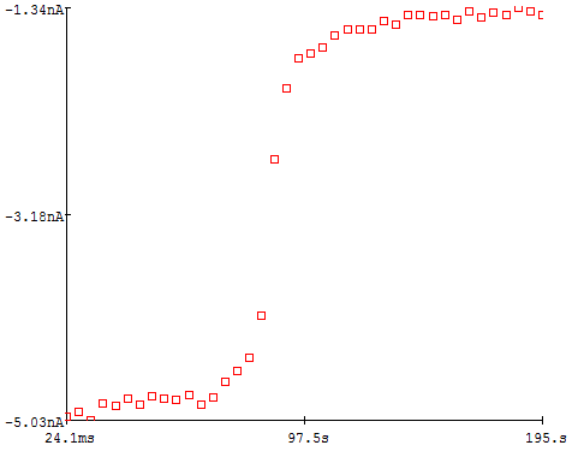
In addition you can toggle the skip status by clicking on the data point (you can configure this behavior in the Fit Configurations). A skipped data point is represented by a star. Just click on the data point again to enable this point.

6. Analysis of a Time Series

- Analyze the *Series* "Onrate" with Trace Fit using *OnlineOnly* as fit function.

- Go to the Series Fit window and display "SerTime" versus "Extr".

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

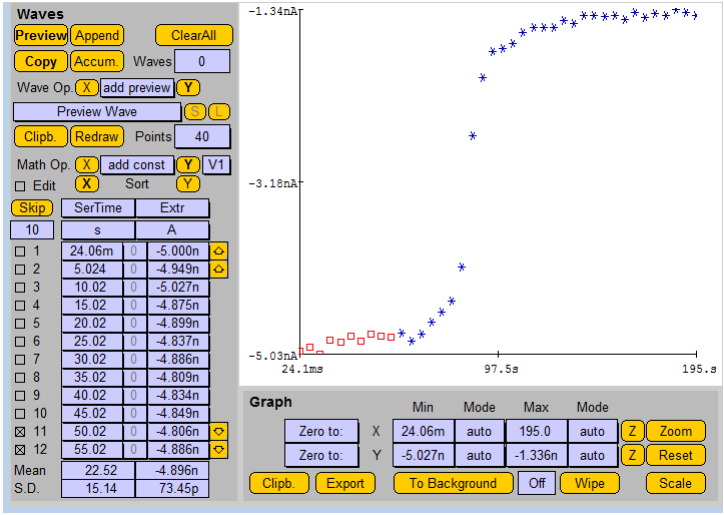
1. normalize the currents to the baseline current and measure the percentage of current reduction or percentage of remaining current.

2. measure the time constant of current reduction.

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.

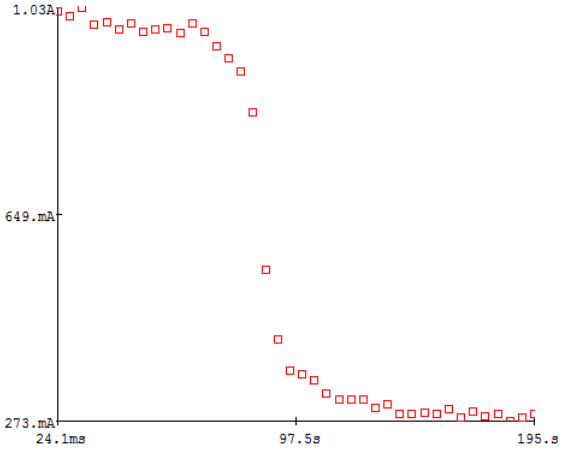
- Click on the *Skip* button and enter "11-end" in the upcoming dialog box. This way we skip all entries except the first ten.



- 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 Op.* selection and press the *Y* button. Enter "-4.9n" in the upcoming dialog box and click "OK".

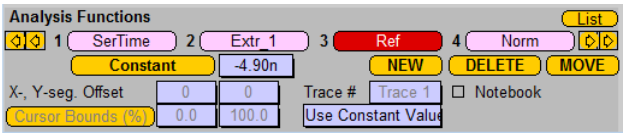


6.2 Normalization to baseline results, using Online Analysis

Alternatively, you can use the Online Analysis to perform the normalization. This procedure involves some more steps, but you will have the raw and normalized data available in Series Fit.

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

- Now we close the Series Fit window and bring the Online Analysis window to front. The function "Ref" is a *Constant*. We now manually assign the mean value to this constant (-4.9n).

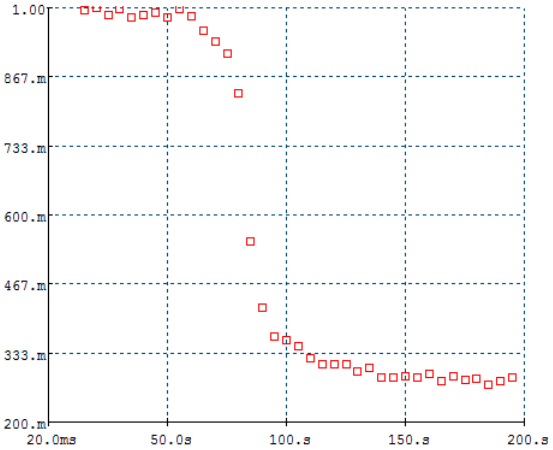


- Now select again the first *Sweep* of the "Onrate" *Series* and analyze the *Series* again with Trace Fit.

- Go back to the Series Fit window and display "SerTime" versus "Norm".

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 Series Fit Scale Properties window. Choose *Round to 0/1/2/5* for both axis in the *Axes and Scaling* tab, check the *Grid, Factor* for the Y-axis and set the number of tics to "7" for the Y-axis and to "5" for the X-axis.



6.4 Analyzing the current reduction

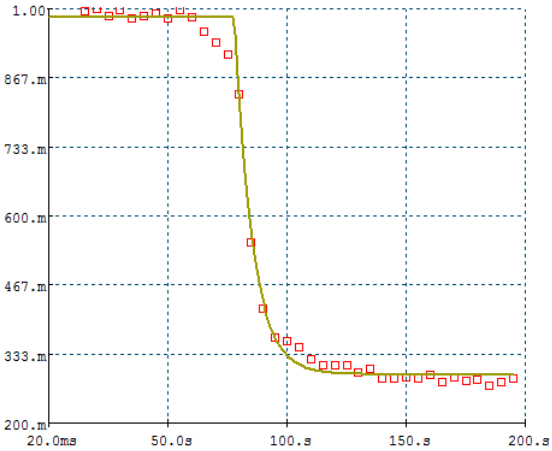
At a time of about 90 seconds the current rapidly reduces from 100 % (1) to about 30 % (0.3). We would like to fit a curve to this time course.

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.

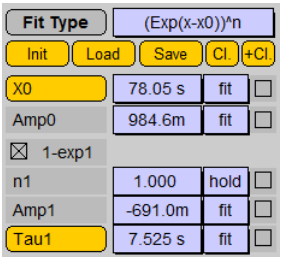
Important note: *The initial fit parameters might not be suitable for a successful fit. Please first think, how you can optimize the starting parameters.*

- X0 (time of start of the decay): we guess "80" s
- 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 "2.0" s

Now let's try a fit. Press *Fit*.



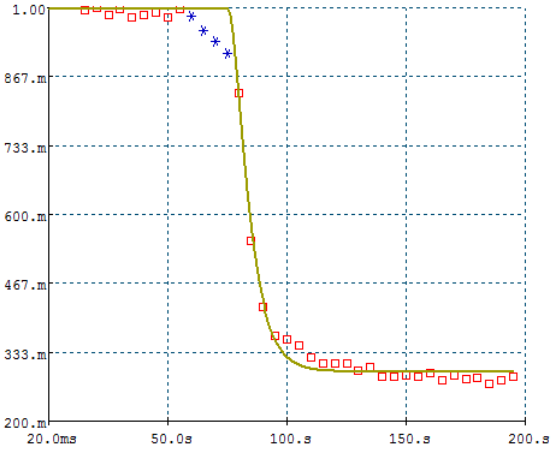
Successful, please see the resulting fit parameters below:



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

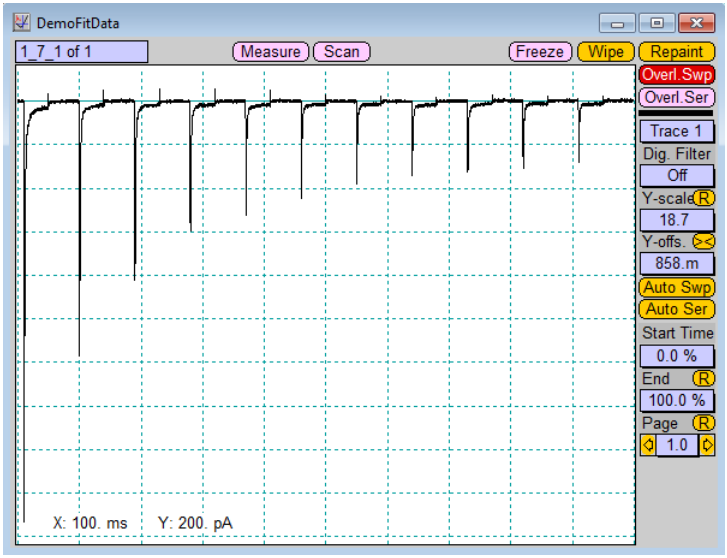
You might improve the fit by:

- 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. "2") to make the onset of the decay more sigmoidal.



7. Cyclic Analysis

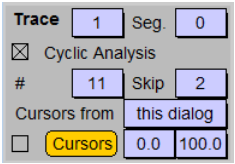
We have recorded a use-dependent inactivation of sodium currents. In our PGF sequence we have used 11 stimuli (depolarizations) separated by a waiting segment. The current response is displayed below.



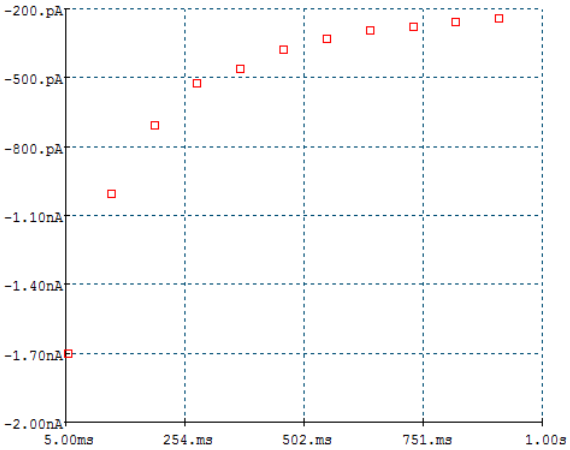
We will analyze the current amplitudes within this *Sweep* using the *Cyclic Analysis* of Trace Fit:

- Turn on the *Cyclic Analysis* by activation of the checkbox next to it.
- Enter the number of responses (#), here "11".
- Enter the number of *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 (here: "2").



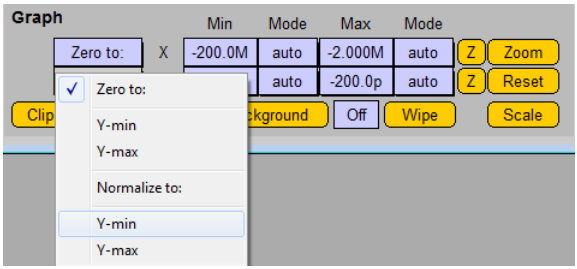
Make sure that the *Sweep* of the *Series* "Train" is activated in the Replay window. Then press *Auto Fit* to analyze the *Sweep*. Go to the Series Fit window, select *Parameters from* "All Events" and display "t_seg" versus "Extr".



7.1 Normalizing each inactivation curve

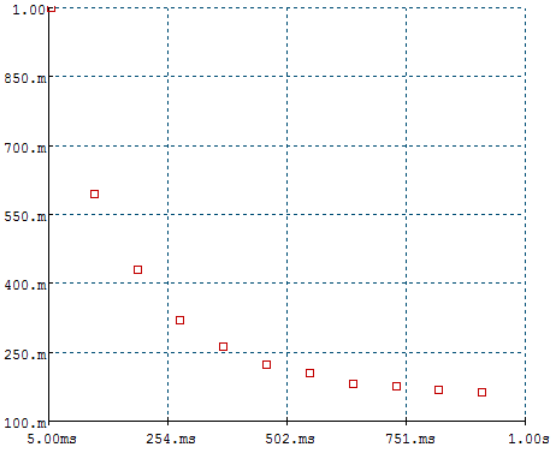
Now, we would like to analyze all three trains. In addition, we would like to normalize the currents to the first response in the train.

- First, we analyze all three trains with the *Cyclic Analysis* method in the Trace Fit window as described above.
- Then, we go to the Series Fit window, clear the waves buffer and preview the "t_seg" versus "Extr" 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.

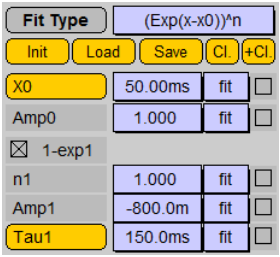
You will then see the averaged normalized inactivation curve.



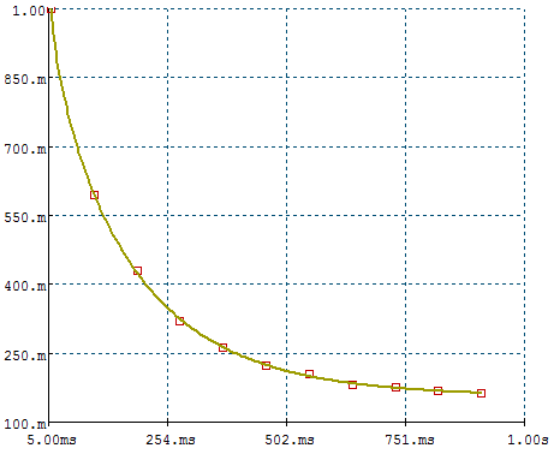
7.2 Averaging normalized inactivation curves and fitting an exponential

Now, you can fit a decaying exponential to the averaged data. We choose:

- "$(Exp(x - x0))^n$" from the *Fit Type* selection.
- *X0* = "50 ms".
- *Amp0* = "1".
- *n1* = "1".
- *Amp1* = "-0.8".
- *Tau1* = "150 ms".
- Set these values to "fit".



The fit matches very nicely for our inactivation curve data:



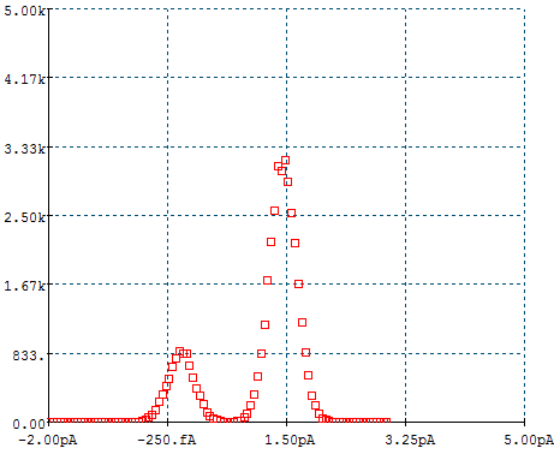
8. Amplitude Histograms

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.

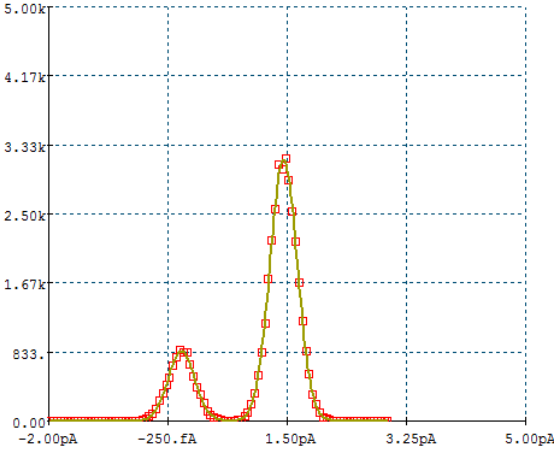
> ***Note:*** *When analyzing single-channel data, please make sure that the option Subtract Zero Offset in the* `Display` *menu is turned off.*

Then, proceed as follows:

- Select the first *Sweep* of a *Series* containing single-channel data (here: "Test 3" *Series* of the *Group* "slow").
- Switch off the Online Analysis but keep the window open.
- Then, select *Amplitude Histogram* as Trace Fit function and set the *Number of bins*, the *Lower Edge* of the first bin, and the *Bin Width* to appropriate values.
- Use *Auto Fit* to analyze the complete *Series*.
- Go to Series Fit, select as parameters *Amplitude* and *Count*, and set the *Parameters from:* option to "Add All Events Sweepwise". Then, press the *Preview* button.
- In the *Series Fit* graph the cumulative histogram is shown.



- Choose *Gaussian* as Series Fit function and adjust the initial parameters for the fit appropriately.
- Press *Fit*. The fit function is also shown in the *Series Fit* graph.



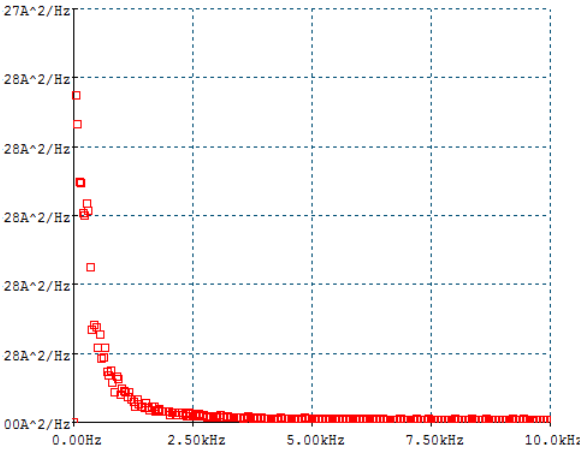
9. Power Spectra Analysis

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:

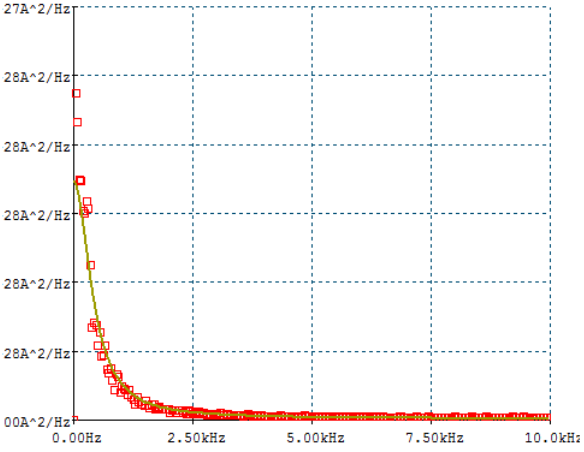
$$i = \sqrt{2\pi \int S(f)}$$

> ***Note:*** *For display and scaling of the power spectra it is required to set the number presentation in the* ***`Fit Configuration`*** *(General tab) 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 Trace Fit function and set the *Number of Points* as high as the data allow (512 for `Singles_Demo.dat`).
- Use *Auto Fit* to analyze the complete *Series*.
- Go to Series Fit 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 *Series Fit* graph the averaged power spectrum is shown.



- Choose *Spectra* as *Series Fit* 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 *Series Fit* graph.



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