

1- Author

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2- Scope

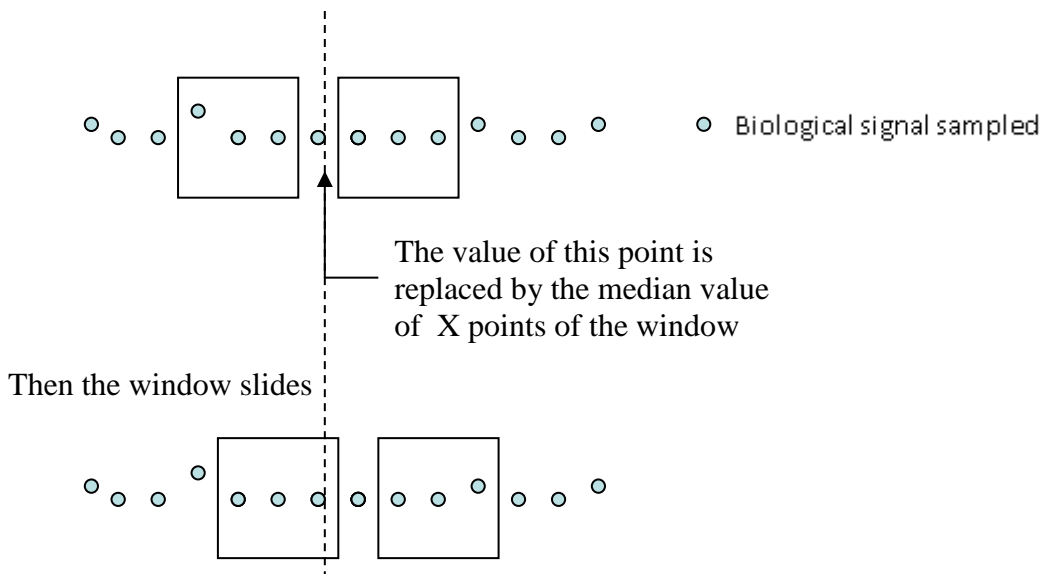
The Community of electro-physiologist who used pClamp (©Molecular Devices) for acquisition. Version 9 or 10 is supported.

3- Method used

This program was developed using Labview (National Instruments Corporation © 2012)

Detection

The principle used is the median filter. The program takes a window which the experimenter sets the width and for each point of the biological signal, it calculates the median of values in the window before and after the point.



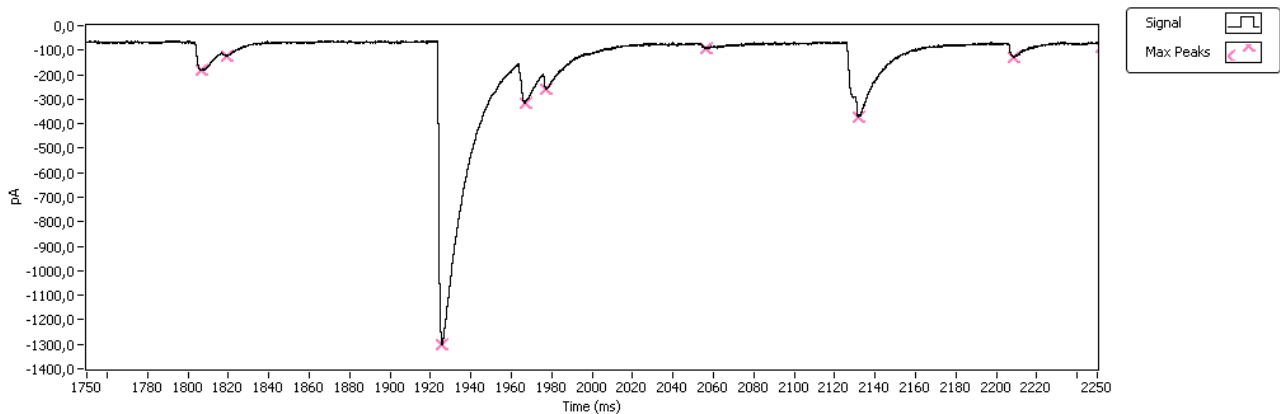
The detection is not made on the biological signal but on another signal (*Detection Signal*), calculated from the difference between the filtered signal and the baseline signal.

Signal Rise, Width mini, Filter signal, Filter baseline Threshold

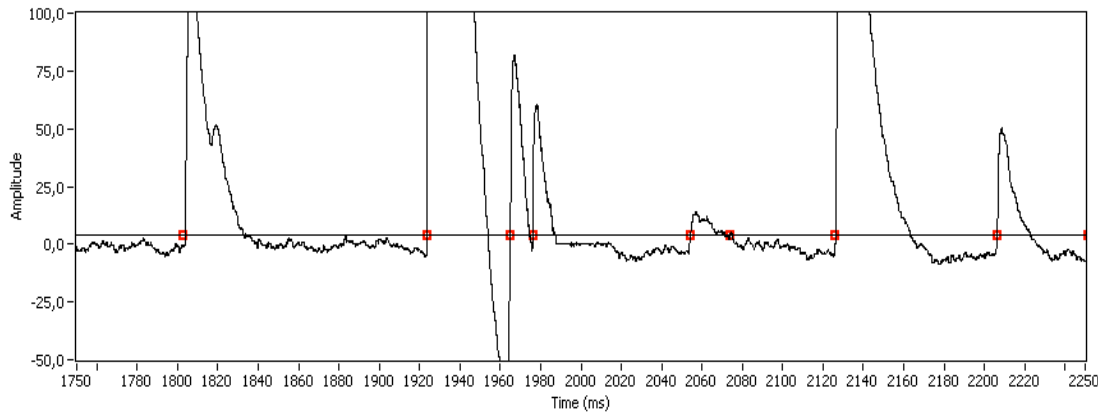
Five parameters are used for the detection:

- Signal Rise (EPSC) or Fall (IPSC)
- Width mini*: Removes artifacts very fast without filtering.
- Filter signal*: Filter the high frequency signal (for the smooth and thus reduce noise). Applies only to the *detection signal*.

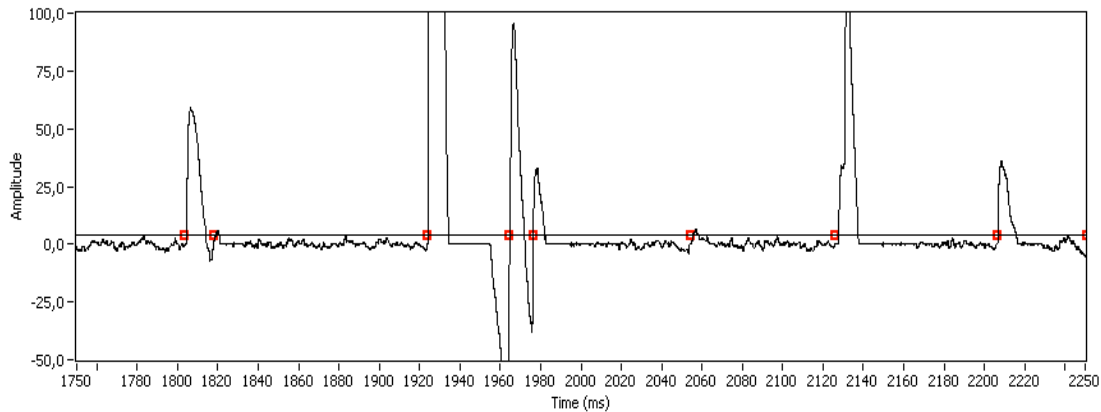
- d) *Filter baseline*: This is the heart of this program. It creates the signal baseline to be subtracted from the filtered signal to create the *signal detection*.
Applies only to the *detection signal*.
- e) *Threshold* : Defines the detection threshold which applies to the signal detection.
The value that is given is not that of the minimum peak that can be detected.
95% percentile to estimate this threshold:
Choose a portion of the signal without biological event (only noise).
Click on *show detection*, *Percentile 95%* shows the value of noise on the signal detection (95% peak to peak noise). The threshold can then be set between 1.5 and 3 times this value.



Filter baseline = 40 ms



Filter baseline = 10 ms

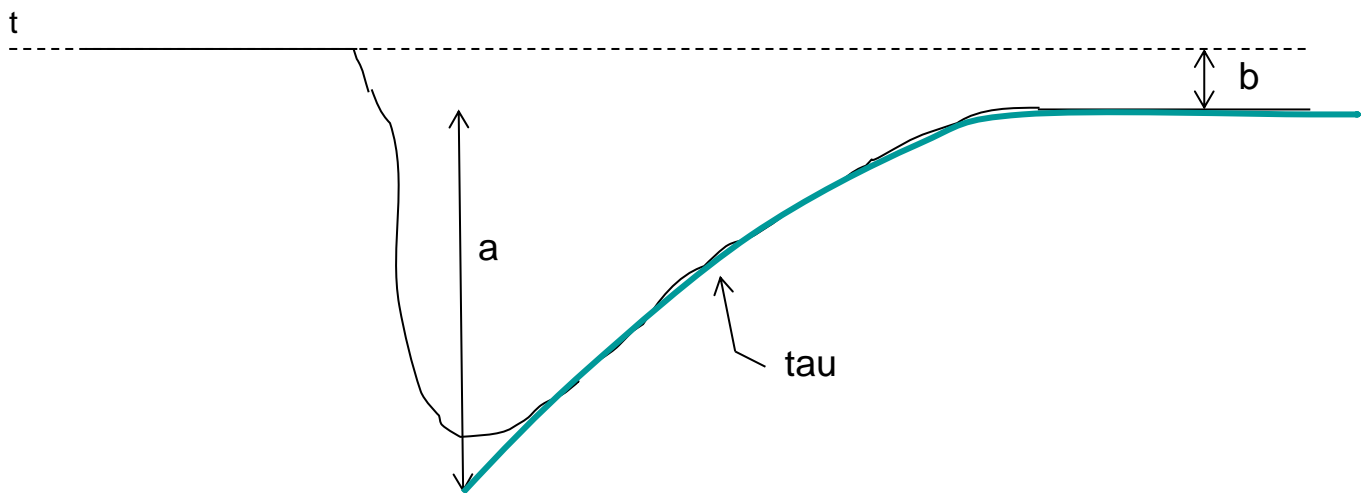


Fit

The biological signal is fitted with an exponential:

$$U(t) = a e^{-\frac{t}{\tau}} + b \text{ for the EPSC}$$

$$U(t) = -a e^{-t/\tau} - b \text{ for the IPSC}$$



$$U(\tau) = 0.37 + b$$

$$U(3 * \tau) = 0.05 + b$$

$$U(5 * \tau) = 0.006 + b$$

If the previous event is over $5 * \tau$ ($n-1$), the amplitude and rise time is not disturbed by this event.

In the result file, this event tagged is 2 or 3.

If the next event is over $5 * \tau$ (n), the fit is not disturbed by this event.

In the result file, this event tagged is 1 or 3.

If the event preceding is over $5 * \tau$ ($n-1$) and the next was over than $5 * \tau$, in the result file, this event tagged is 3.

4- Users manual

a) Reading the data file pClamp

Click to File:Open on menu bar and select your file in the dialog box.

After reading, *Nb channel*, *Sampling period* and *Nb point by channel* is shown.

You can see in the main window your biological signal.

Clicking on *first 10s*, this window shows the first 10 seconds of the record.

Graph palette:

With the graph palette, you can move cursor, zoom, and pan the display.



- **Cursor Movement Tool** (graph only)—Moves the cursor on the display.
- **Zoom**—Zooms in and out of the display.
- **Panning Tool**—Picks up the plot and moves it around on the display.

Y Auto Scale:

The chart can automatically adjust their vertical scale to fit the data.

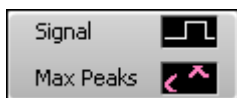
Customizing Graph and Chart Appearance:

Customize the appearance of a graph or chart by showing or hiding options. Right-click the graph or chart and select **Visible Items** from the shortcut menu to display or hide the following options:

- **Plot Legend**—Defines the color and style of plots. Resize the legend to display multiple plots.
- **Scale Legend**—Defines labels for scales and configures scale properties.
- **Graph Palette**—Allows you to move the cursor and zoom and pan the graph or chart while a VI runs.
- **X Scale and Y Scale**—Formats the x- and y-scales.
- **Cursor Legend** (graph only)—Displays a marker at a defined point coordinate. You can display multiple cursors on a graph.
- **X Scrollbar**—Scrolls through the data in the graph or chart. Use the scroll bar to view data that the graph or chart does not currently display.
- **Digital Display** (waveform chart only)—Displays the numeric value of the chart.

Plot Legends:

Using the Plot Legend to Customize the Plot Appearance



Customizing the plots that appear in the plot area of a graph or chart helps distinguish each plot from another. Use the plot legend to customize how each plot appears in the plot area of a graph.

Click a glyph in the plot legend and select from the following options in the shortcut menu to customize how the plot appears in the plot area of a graph or chart:

Common Plots: Provides options for the plot type. You can select a line, scatter, line and symbol, fill, comb, or bar plot type.

Color: Displays the color picker so you can select the color of the plot.

Line Style: Provides solid and dashed line styles.

Line Width: Provides line widths.

Anti-Aliased: Place a checkmark next to this item to make line plots appear smoother. Anti-aliased line drawing can be computation intensive. Using anti-aliased line plots can slow performance.

Bar Plots: Provides bar plot options.

Fill Base Line: Provides options for filling in space below the plot with the same color as the plot. If the graph or chart has multiple plots, you can select another plot at the bottom of the shortcut menu to fill the space below the plot up to the other plot.

Interpolation: Provides interpolation options, including plotting only data points.

Point Style: Provides point styles for the data points.

X Scale: Sets the variable to associate with the x-axis.

Y Scale: Sets the variable to associate with the y-axis.

Export: Export your table in excel file or clipboard.

You can export the biological signal and signal detection into *bmp* formats (C:\Temp). Click to *Save bmp* on Signal Detection Tab.

Quality of Signal filters the biological signal by a median filter. The value given must be very low because the filter distorts the signal and influences on the results (peak, tau, ...)

b) Detected the Mini:

Click to *Detection* tab and enter the parameters *Signal Rise*, *Width mini*, *Filter signal*, *Filter baseline*, *Threshold* then click to Run:Detection on menu bar.

After detection, *Number of mini* is shown.

You can see in the main window the events detected (pink cross).

c) Removed the bad events:

To exclude an event, move the cursor to the cross and type on the spacebar.

You can exclude events, but not to include events that have not been detected.

d) Analysis :

Click to Run: Analysis on menu bar and the tab *Analyze* is show.

The left window *Mini* show the event.

The right window *Fit Mini* show the fit.

The parameter *Mini Max Length* must be set between 3 and 5 times the largest tau.

The result of the fit is Height, Offset, Position, Tau and R^2 .

R^2 is a measure of deviations between the points of the biological signal and calculate the curve. This is the square of Pearson correlation coefficient. Higher its value approaches 1, the more fit is good. A R^2 less than 0.8 should lead to reject him.

The causes may be a bad signal / noise has a very little Mini, *Mini Max Length* too high or the event after arriving too early.

If the next event is over $5 \cdot \tau$ (n), the fit is not disturbed by this event.

e) Export the result

Click to *Write result file* tab and enter the parameters *Path*.

To open the dialog box click to 

The windows are selected with *Write X max* and *Write X min*

Then click to Run:Writing on menu bar.

The data are exported in text file (.csv) for spreadsheet application (eg. Excel).

The processing can be long time.

File format:

The parameters used for this calculation are written on the both first line.

Version, Threshold, Filter Signal(ms), Filter Base Line(ms), Width mini(en ms), Length, Qual Signal, Number of Mini, Write X min, Write X max, Data File

Column

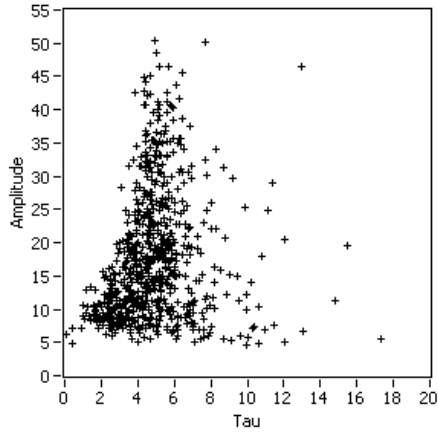
- ▶ Num: Event number in the list of events detected.

You may have jumps corresponding to remove event.

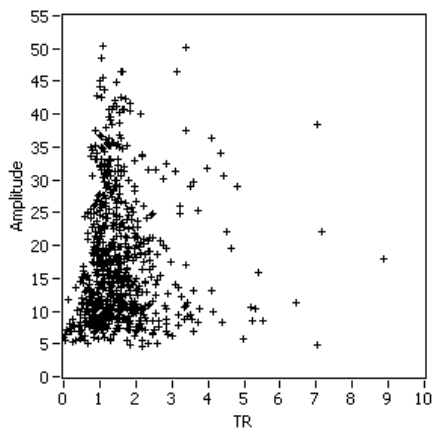
- ▶ Positions: the time of the peak.
- ▶ Max-BL: The peak amplitude (measured on the biological signal but not on fit signal)
- ▶ Baseline: Value of the baseline before the event.
- ▶ Rise time: Duration between the 10 and 90% of the peak amplitude
- ▶ Offset Exp: Value of the baseline of fit signal
- ▶ Amplitude Exp: Value of the amplitude of fit signal
- ▶ Tau exp: tau of exponential
- ▶ R^2 exp: R^2 , the square of Pearson correlation coefficient
- ▶ Higner $5 \cdot \tau$: 1 if the next event is over $5 \cdot \tau$ (n), the fit is not disturbed by this event.
- ▶ Higher $5 \cdot \tau$ _Exp(n-1): 1 if the previous event is over $5 \cdot \tau$ (n-1), the amplitude and rise time is not disturbed by this event.
- ▶ And: 1 or 3 if the next event is over $5 \cdot \tau$ (n)
2 or 3 if the previous event is over $5 \cdot \tau$ (n-1)
3 if the event preceding is over $5 \cdot \tau$ (n-1) and the next was over than $5 \cdot \tau$

After the writing, the XY graph tab is show with three plots.

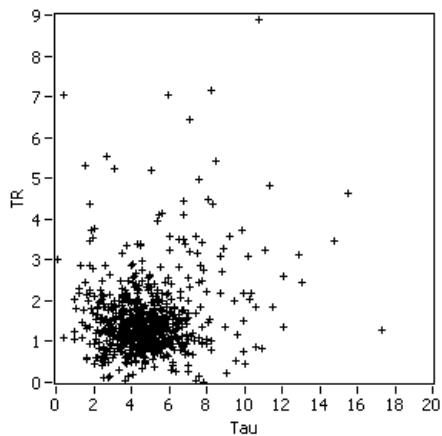
Amplitude vs Tau



Amplitude vs Rise Time



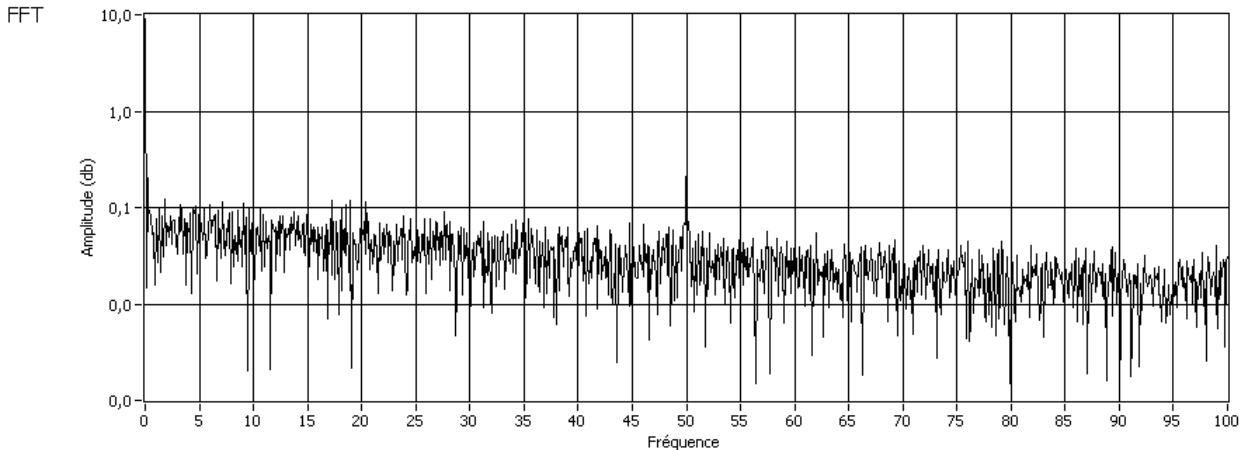
Rise Time vs Tau



You can export in spreadsheet file and image files.

f) Fast Fourier Transformer

It's the frequency domain representation of your signal.
Then click to Run:FFT on menu bar.
The plot of discrete Fourier transform is show in FFT tab



By analyzing the spectra of signals, you can see the dominant frequency (e.g. 50 or 60Hz).

g) Average Plot

Click to *Average trace* tab and enter the parameters of time windows used (X_{max} , X_{min}).
Enter the time window to plot (X_+ , X_-).

Select the filter

- *Mini good*: only the event not removed is used.
- *Mini good + sup 5 tau*: only the event not removed and tagged 3 is used.
You much have made a result file for this choice.

Select type of average

- Mean: In the window of plot, you average point to point all event filtered.
- Median: Same but with the median

You can export in spreadsheet file and image files.

h) Setting

You can save or load a setting file for each analysis (Setting:Save, Setting:Save as, Setting:Load).
You record also the removed event in this file (bad mini).

You can replay a setting with or without the removed event (Setting: Open+Replay with bad mini
or Setting: Open+Replay clear bad mini)

i) License key

To send the update, i want to know the people and computers that use my program.

If you have a file with invalid key license, the program is limited.

The file result is restricted to 50 events.

Click to Help:License Key and send an email to the author with your ID and Computer number.

5- Conclusion

I hope that is small program will allow you to write a good paper and it's save you a lot of time.

If you write a paper using this program, I would be very happy that i am in Acknowledgment

For a large diffusion, you should be specifying the name of this program name and the author.

Thanks'

M Goillandeau Michel