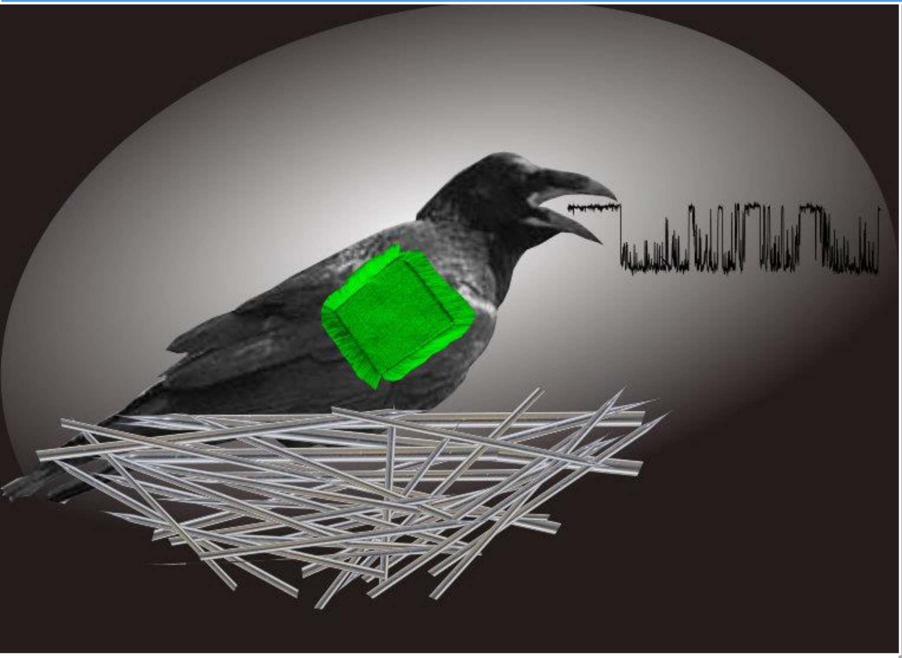
Nest-o-Patch 1.2

User's guide



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CONTENTS

I. PURPOSE AND SCOPE OF THE PROGRAM.

Nest-o-Patch was designed mainly for "preconditioning" and basic analysis of electrophysiological data. Although developed mainly with patch-clamp data and analysis of single-channel events in mind, it may be (and actually was) successfully used for analysis of whole-cell data, obtained either in patch-clamp or two-electrode voltage clamp experiments. Currently, the program is able to open files, created by PatchMaster or Pulse software from HEKA Electronic company. Unlike software from HEKA itself, Nest-o-Patch can open several series or even groups at once, which makes it indispensable for analysis of lengthy experiments, including several solution exchanges and several series recorded from one cell. Single channel conductance, kinetics, NP_o and open probability may be estimated. The program focused mainly on the analysis of level dwellings distribution in a recording with several channels and estimate of the number of channels (N) and open probability (P_o). Advanced event detection algorithms and analysis of recordings with different conductances are planned in the future – look for new versions.

Tip: you can measure whole-cell current amplitude with the same tool as is used for measuring unitary conductance, or even mark levels of current during a pulse protocol to get an I/V plot. Other possibility is a use of Average Current tool; see "How to…" chapter.

In fact, when text file is used as an input, *Nest-o-Patch* itself does not make any assumptions about nature and units of the recordings, it deals only with X-values and Y-values which may represent absolutely everything. Correct conversions of units is therefore the responsibility of a user.

II. GLOSSARY, USE AND CONVENTIONS OF THE MANUAL.

TYPING CONVENTIONS

Mentioned elements of interface (like menu items) are printed in century, special terms in definitions are in **bold,** *italic* is used for general emphasis and for special terms, which are defined in this guide. Tips, notes and warnings are typed in blue.

GLOSSARY

Comma separated file (csv): table data file in a text format where fields are separated by a special delimiter (comma or semicolon). Originally comma, but in the majority of European countries semicolon is used instead, since comma may be used as a separator of integer and decimal parts of a number. Nest-o-patch can use both semicolon- (default option) and comma-separated CSV files.

Conductance level (or just Level) Value of a registered current which corresponds to a certain number of opened ion channels. Level 0 is "all closed"; level 1 means that 1 channel is opened and so on.

Event: opening or closing of an ion channel (that is, transition from one level to another).

HEKA File: Pulse or PatchMaster file (see below).

Linear sections view: X values (usually, time values) monotonically increase from the beginning to the end of a **Trace**. All sweeps are shown consecutively.

N: (in most cases) number of channels in a patch.

NP_o: product of **N** and channel open probability (**P_o**).

Overlayed sections view: X values (usually, it is time) begins from "0" for each sweep (or series, or group), such that several sweeps are shown in parallel, like in **HEKA files**.

PatchMaster: PatchMaster software from HEKA Elektronik Dr. Schulze GmbH.

PatchMaster file: Binary data file, created by PatchMaster. Actually includes 3 files: *.dat (rough data), *.pul (tree structure with the various information about the experimental conditions) and *pgf (information about stimulation protocol). However, they can be saved in a one, so-called bundle, file.

PatchMaster trace: PatchMaster software is able to aquire data simultaneously from up to 16 channels. Recording of each such channel is called *Trace* in terms of PatchMaster. It should not be confused with **Trace** as a data set in Nest-o-Patch (see below).

P_o: open probability of an ion channel.

Pulse: Pulse software from HEKA Elektronik Dr. Schulze GmbH.

Pulse file: Binary data file, created by Pulse. Actually includes 3 files: *.dat (rough data), *.pul (tree structure with the various information about the experimental conditions) and *pgf (information about stimulation protocol).

Section: any section of HEKA file: group, series or sweep.

The program: *Nest-o-Patch*, which we describe in the present guide.

Trace: Two-dimentional (e.g. Time-Current) data, loaded and analyzed by the Program. Can be taken from an ASCII (text) file, complete HEKA file, or several sections of it. Sections in the latter case may be contiguous or not.

TIPS ON THE USE OF THE MANUAL

Chapters I-IV of the Manual are short, traditional for every software, and do not require any tips. Chapter V describes what you can get from Nest-o-Patch and explains all formulas which are used for the calculations. In some cases it gives also derivation of the formulas. This chapter is not necessary for the operation of the program, but helps to understand what are you really doing. Chapter VI explains in detail how every control in every tab acts and what you can do with it. Chapter VII guides you through the process of data preconditioning and analysis, showing how Nest-o-Patch is typically used. Maybe, it is a best place to start and get familiar with the program. Chapter VI focuses more on the operation of interface controls; chapter VII – on the sense of the actions. Important tips, notes and warnings are given in blue. I strongly recommend reading all stuff in blue across the whole manual before you start to use Nest-o-Patch for real analysis of your data.

III. SYSTEM REQUIREMENTS AND INSTALLATION

Operating system.

Windows XP or higher. Tested at XP, Windows 7, Windows 8.1 and under Wine Windows emulator under Linux.

Hardware.

Nothing special. *Nest-o-Patch* will run at any computer with the abovementioned operating systems.

Package content.

Nstpatch.exe – main executable and the only required file.

Readpulse.dll – library, needed to open directly Pulse files.

ReadPMaster.dll – library, needed to open directly PatchMaster files.

NstPatchGuide.pdf – this manual.

Installation.

Simply copy nstpatch.exe and (optionally) readpulse.dll and readpmaster.dll into a desired directory. No special installation is needed. However, the program will create configuration file nstpatch.ini in a user's software configuration directory (normally, c:\users\<username>\AppData\Local\Nest-o-patch\ ).

IV. GETTING SUPPORT

First, read this manual.

Second, visit the official site of Nest-o-patch:

https://sourceforge.net/projects/nestopatch/

Use bug tracker tickets system if you want to report a bug;

Use forums if you want to ask the author a question or want to request a feature.

In the About box you will find buttons, which open these web pages.

V. OVERVIEW OF THE FEATURES.

1. SUPPORTED DATA FILE FORMATS. READING AND SAVING DATA.

Reading data. Nest-o-Patch is able to open text files with one or two columns of numbers. The program tries to determine number of columns automatically. Space, tab or CSV delimiter (comma or semicolon) may be used as delimiters. If two columns are found, the first one contains X-value, the second one – Y-value. When only one column is found in a file, *Nest-o-Patch* uses line numbers for X-values. Lines, which the program cannot recognize as numbers or pairs of numbers (for example, a title or subtitles) are ignored.

One important special case: program understands and uses information, contained in the title and sections of the files, exported from *Pulse* program by HEKA Elektronik Dr. Schultze company.

Moreover, *Nest-o-Patch* opens binary data files, created by *Pulse* or *PatchMaster* for Windows. **ReadPulse** library (normally included in the package) is needed to open Pulse files, **ReadPMaster** – to open PatchMaster files. Normally both libraries are included in the package. Initially *Nest-o-Patch* loads and displays a pulse tree. Further, any file section (series or sweep), several selected sections (contiguous or not) or a complete group may be loaded at once as a **Trace**. In case of PatchMaster file, a user selects which PatchMaster trace to load. Only one PatchMaster trace may be loaded at a time. During testing, The program was able to load and process successfully a recording with 6 million points. Larger recordings were not tested. The program reads Sampling rate information for each *series* and Time field for each *sweep.* Based on these data, time coordinate is calculated for each data point. Therefore, recordings with varying sampling rate are handled correctly. Time scale can be set relative to a beginning of a file, a group, a series, or a sweep, providing possibility to overlay different sections.

Pulse for Mac is not supported in the current version.

Saving modified traces. By default, *Nest-o-Patch* saves *.csv files with X value in the first column and Y-value in the second. The program does NOT save back HEKA files, so, original data are always preserved unaltered. Complete Trace or selection can be saved. In addition, multi-column file can be saved if a Trace is viewed in *overlaid sections* mode.

2. DATA PROCESSING.

Compression. Sometimes, you need to make a figure from a very long recording with millions of points, or want to analyze slow processes in a recording with high time resolution, which is excessive for your purpose. In such cases, you may open a file (or a section of a HEKA file) with compression. You must specify a compression factor, which may be any positive integer number. See also `Open with compression` dialog and `Pulse tree` tab in this guide.

Data filtering. In patch-clamp analysis, low-pass filters are used almost universally. This program implements Moving Average and Gaussian filters. Select one in `Options` dialog.

Units conversion. One may convert between any units (Volts to mV, cm to inches, seconds to years), just entering factor by which X or Y data are multiplied. Use of correct factors is a responsibility of a user. In addition, you may "shift" numbering, entering second parameter, free member, which is added to initial values. This may be useful when, for example, you want to show fragment of a recording in a middle of your file, but want its numbering to start from zero. See `Conversion` dialog.

Correction of a "floating" baseline. Unfortunately, not all recordings are ideal. Sometimes you have a drift of a baseline, not always linear. However, a stable baseline is vitally important for an analysis of single channel events. This program allows to eliminate this drift.

"Wipe" artifacts. If a recording contains false spikes, solution exchange artifacts, or whatever you want to get rid of, select and "wipe" unwanted part of the recording. Correct timing is preserved.

3. DATA ANALYSIS.

Simple measuring of X- and Y-distances. To measure X-distance, select part of the recording between points of interest. X-coordinates of the first and ending points of the selection and length of the selection will be sent to the Notebook. Alternatively, simply point with a mouse cursor to a point of interest and look at the lower left corner of the program window. Coordinates of the mouse are shown there. To measure Y-distance, select Set levels in the Analysis menu and set them; y-coordinates of all levels are recorded in the Notebook.

Find mean current over interval. Select part of a recording, press  button. Mean current over selected interval is sent to Notebook. If *Levels* were set prior to the calculation, mean current is found relative to Level "0", otherwise absolute value of mean current is calculated.

Calculate current amplitude histogram. *Select* part of a recording, optionally set *borders* with 2 borders. They will serve as upper and lower limit of the histogram. Select  Amplitude histogram in the Analysis menu or press corresponding button on a toolbar. See Amplitude Tab. Change number of bins to change the resolution. Histogram is calculated as probability density, pA^{-1} ((or other current units of your choice)$^{-1}$).

Measure single channel current amplitude. Set *levels,* and average distance between them is recorded in the Notebook as *Single channel current*.

Measure single channel kinetics, if only one channel is present in a patch. (Menu Analysis -> Kinetics). Before pressing it, select interval for analysis (Select…) and define 3 borders (Set borders). Upper and lower borders will show limits of "true data"; everything below and above will be treated as artifacts and excluded from analysis; crossing of the middle border will be used for the detection of opening/closing events. Table of all events is generated; distribution of closed state life-times, distribution of opened state life-times, frequency of opening/closing events, average lifetime of closed and opened states, total time spent in open and closed states are calculated; NP_o calculated as

$$NP_o = \frac{t_{open}}{t_{open} + t_{closed}} \tag{1}$$

And if you believe that there is indeed only one channel and not just only one visible level, then this value is equal to its P_o. In addition, table of all opening/closing events is generated and may be saved and analyzed by any software. Likelihood that there is more than one channel may be tested with a technique described below.

If there is more than one level, other techniques of analysis are available. One can measure *single channel current* as a distance between *conductance levels*; calculate the *average current* as a current at each point minus current at "all closed" level.

NPo is calculated by two formulas. First, as

$$NP_{o(mean)} = \frac{\bar{I}}{i} \tag{2}$$

where $\bar{I}$ – average current, *i* – single channel current (for this calculation Levels must be set, see Main Window section). Second, NP_o from the distribution of levels:

$$NPo = \frac{\sum_{i=1}^{L} iT_i}{T} \tag{3}$$

where *L* – number of levels (1 corresponds to one opened level); *i* – number of each given level (0 for "all closed") ; T_i – total time when *i* channels were opened (that is, level *i* was registered); *T* – total time of the recording. Of course, in a good recording, both techniques of NP_o estimation should give very similar results. Generally, $NP_o(mean)$ is sensitive to the correct determination of single channel current amplitude and works only for the recording with one type of conductance, while NP_o from the distribution of levels is more sensitive to clear distinction between the levels.

Number of channels in a patch (N) and average open probability (Po) are estimated by the analysis of total dwelling time at each *conductance level*. First, empirical distribution of total dwelling time at each level is calculated (eq.4); best fitted binomial distribution can be found by one of three methods: weighted least squares, unweighted least squares or by classical Maximal Likelihood Estimation (MLE).

Calculations are following. Empirical probability for each level *i* (P_i) is found as:

$$P_i = \frac{t_i}{T} \tag{4}$$

where t_i is total time dwelling at a level *i*; $\sum_{i=0}^{L} t_i = T$; T – duration of the analyzed recording, *i*=0 corresponds to "all closed" level. Binomial distribution which fits best this empirical distribution is found in iterative tests for *N* varying from *L* (number of observed levels) to 100 (by default; a user may specify another value). Parameters of each distribution under test are found as follows:

$$P_o = \frac{NP_o}{N} \tag{5}$$

$$Pt_i(N,P_o) = \binom{N}{i} {P_o}^i (1-P_o)^{N-i} \tag{6}$$

where $Pt_i(N,P_o)$ is a theoretical probability of i-th level according to a binomial distribution for N channels and open probability P_o.

Weighted sum of squared residues is calculated for each of the tested distributions as

$$SSr = \sum_{i=0}^{L} \left[(Pt_i - P_i)^2 \cdot \frac{1}{Pt_i} \right] + \sum_{k=L+1}^{N} Pt_i \tag{7}$$

Parameters of a binomial distribution which gives a minimal SSr are regarded as the best estimates for N and P_o. Weighted squares technique equally takes into account discrepancy between observed and theoretical distribution for the levels which have high and very low probability. Under ideal conditions, this approach gives more accurate results then unweighted squares. However, estimates of probability of the the levels which are seldom observed, may be unreliable due to very limited number of observations. This would, in turn, make unreliable weighted squares statistics. Therefore, Nest-o-Patch offers also estimate of binomial distribution by unweighted squares:

$$SSr = \sum_{i=0}^{N} [(Pt_i - P_i)^2] \tag{8}$$

Alternatively, Maximal Likelihood Estimate (MLE) may be used to find a best fitting binomial distribution. Likelihood function for each tested distribution is calculated as:

$$LE(N,P_0) = \prod_{i=0}^{L} Pt_i(N,P_0)^{P_i} \tag{9}$$

Distribution, for which this function has a maximal value, is the most likely distribution according to MLE.

Underlying theory. Likelihood function for each tested hypothesis (given binomial distribution in our case) is equal to the probability of the observed data set under tested hypothesis. This probability in turn is a product of probabilities of each individual point in the dataset. In case of binomial distribution, probability of each point is equal to the value of probability density function for the level to which this point belongs. Therefore, if there are levels from $level_0$ to $level_n$ with theoretical probabilities Pt_0 to Pt_n, and if k_0 to k_n points are observed for each level, probability of such dataset is:

$$P(dataset) = \prod_{i=0}^{n} (Pt_i(N,P_0))^{k_i} \tag{10}$$

We use P_i instead of k_i. However, $P_i = k_i/M$ where M is the number of observations in the dataset. Substituting P_i in Eq. 9 we have:

$$LE(N,P_0) = \prod_{i=0}^{L} (Pt_i(N,P_0))^{\frac{k_i}{M}} \tag{11}$$

And, finally,

$$LE(N,Po) = \sqrt[M]{P(dataset)} \tag{12}$$

Since $f(x) = \sqrt[n]{x}$ is monotonically increasing for all positive *x*, *LE(N,Po)* reaches maximum at the same point as *P(dataset)*, therefore it may be used as MLE function.

One important limitation of these techniques is that if *N* are large and P_o – small enough, then binomial distribution approximates Poisson distribution, which has only one parameter (*P*), so, estimates of *N* become unreliable. Often, “Best N” in this situation is infinite. Thus, in this situation it is impossible to estimate N, but one may conclude that P_o is small (<0.1), because this is a necessary condition for converging of a binomial distribution to Poisson distribution.

VI. USER INTERFACE AND PROGRAM OPERATION.

1. MAIN WINDOW

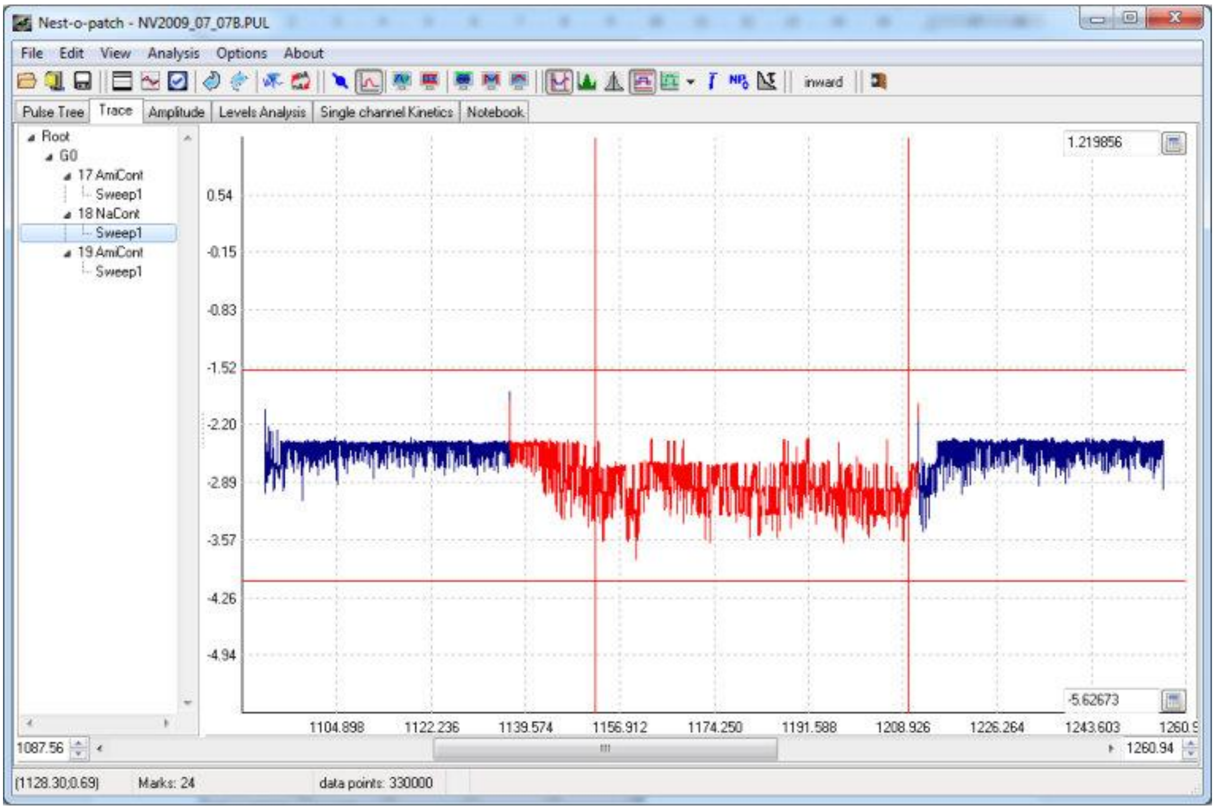


Figure 1. Main window, Trace tab is opened, general view.

Main window has 6 tabs.

Pulse Tree tab (Figure 2) shows information about the structure and experimental conditions of a loaded HEKA file and allows to select which sections of the file will be loaded.

Trace tab (shown in Figure 1 and Figure 4 - 5) shows the Trace itself (right panel, which typically occupies most of the screen) and a tree of loaded sections. By selecting a section in the tree, a user can highlight it (in the picture above, Sweep1 from Series 18 NaCont is highlighted). One can *highlight* some loaded *section* of a HEKA file; this is possible only when a *Trace* is taken from an opened *HEKA file* or ASCII file exported from *Pulse*. **Selection** is any part of a trace, which is selected for analysis. It can include several sections, or part of a section or whatever.

Amplitude tab (Figure 6) shows a *current amplitude histogram* of a selected portion of a trace. Note, that **Highlighted** and **Selected** is not the same.

Levels analysis. (Figure 7) Shows tools and results of the analysis of levels probability distribution to estimate *N* and P_o.

Kinetics. (Figure 8) Shows tools and results of kinetic analysis in a recording with one level (and supposedly one channel) only.

Notebook. Contains log of the analysis, including actions of a user and results of the analysis. Can be edited manually. Pretty much the same as Notebook in the *Pulse or PatchMaster software*.

Main menu and Toolbar give access to all the commands and options of the program (will be described below). Statusbar shows current position of a mouse (in the coordinates of a trace; in the shown example – seconds and pA); number of existing (or existed) marks for the correction of the baseline; number of loaded data points and, in the last section, displays a tip for a selected tool button, menu item, or any other controls or warning messages. In this version of the program, this is the only Help available.

2. MAIN MENU AND COMMANDS OF NEST-O-PPATCH.

Here commands accessible from main menu are described; these are almost all commands existing in the program. So, explore main menu and experiment with its commands to get familiar with main features of Nest-o-Patch. Below, each command is described; hot key, if exists, shown in brackets.

`File submenu.` Operations with the file of Trace.

Open (F3). Displays open dialog. Default file type for this dialog (Pulse (*.pul), PatchMaster (*.dat) or text files (*.asc;*.txt;*.csv) can be set in Options dialog. In case of *.csv, semicolon or comma, as set in Options dialog, are expected as a delimiter. Decimal separator (“.” Or “,”) depends on Windows settings.

Warning: if you save a file on a computer where “.” is a decimal separator and then try to open the file on another computer where separator is “,” or vice versa, it would not work. Workaround: change (temporarily?) Windows settings (Control panel, Language and Regional standards) on one of the computers, or use a text editor to replace separators.

Other choices of file types in the file open dialog are “PatchMaster files” (.dat files are shown), “pulse files” (.pul files) and “all files”. If the extension of a file selected for loading is .pul, the program will try to open it as Pulse file; if .dat, then as PatchMaster file, in other cases – as text file.

If Pulse file type is specified in a dialog, while in fact it is PatchMaster file or vice versa, the program automatically detects the right format and opens it (provided, that necessary library is present).

Open compressed**.** Valid only for text files. If a file is too long, a user can opt for compression. Then, number of points equal to `Compression` factor is replaced with one point where Y value is equal to the average of Y-values of each point in a replaced group, and X-value is equal to the X-coordinate of the first point in the group. HEKA traces also can be compressed, but at the stage of sections loading (see **VI.3**).

Save as… (F2). Saves an opened trace as a text file, .csv by default. HEKA files are not saved.

Save selection… Same as previous item, but saves only Selected portion of the trace.

Export table… If a Trace is presented in overlayed view (see **VI.4**), this command allows to export it as a table, where first column is time, other columns represent Y values for each of the overlaid Trace sections.

Save notebook as… Saves content of the Notebook as a text file.

Open notebook… (Ctrl-O). Opens a text file and loads its content into the notebook.

Recent List of recently opened files.

Close file. Closes opened HEKA file without exiting the program.

Exit Quit the program.

Edit submenu. Commands of the processing of data file.

Select Allows to set X-borders of a selection for analysis. X-coordinates of Selection and its length are sent to the Notebook. Tip: Select command is a convenient way to measure time intervals (or, in a general case, distance in X coordinate).

Select all (Ctrl-A). Selects the whole Trace for Analysis. Enabled only when tab with a Trace is active. This allows to use Ctrl-A in a notebook in a usual way.

Select view. Selects part of a Trace contained in the screen for analysis.

Convert. Opens convert dialog for the conversion of units (min to sec; pA to nA or whatever) and shifts of the data. See description of the **Convert dialog** for details.

Set ideal baseline, Mark baseline, correct baseline (F9). These commands serve for the correction of a drifting baseline. Refer to the section about **Baseline correction** for details.

Undo baseline correction, Redo. These commands allow to undo and repeat last baseline correction. Only one correction may be undone or redone.

Tip: “Redo” may be used not only after “undo” command, but also after new loading of a Trace. This may be useful, for example, if you have corrected baseline, then filtered the Trace and found results of filtering to be unsatisfactory. Filtering cannot be undone; however, you can reload the Trace, redo database correction using “Redo” command and filter the Trace with better settings.

Filter data. Opens Filter dialog. Moving average or Gauss low pass filters are used (select one in Options dialog).

Important notice: in a definition of Hz, the Filter assumes that X-values are seconds. This is always correct if the Trace is part of a HEKA file and no conversion of X-values was performed. In all other situations, it is responsibility of a user to think about correct frequencies.

Wipe selection. If the *Trace* contains artifacts which would interfere with an analysis, or just are too ugly, a user can wipe them. Points inside the Selection are removed from the trace, but X-coordinates of all other points are preserved, so, empty space appears in the place of an artifact.

View submenu. What to show.

Complete trace, Selection, Highlighted. Set X-scale: corresponding parts of the Trace are expanded to the whole window.

Complete vertical scale, Y scale to borders. Set Y-scale: show the whole range of data or expand the outer Borders to the whole height of the window.

Show data markers. Show individual points of the Trace. However, this setting has an effect only if a number of points on the screen is less then width of the window in pixels.

(Sometimes complete Trace includes millions of points, attempt to draw markers for each of them would take too much time!) This setting may be useful for removal of short artifacts or for very detailed view of some very fast process.

Highlight sections. Switches Highlight on or off. This is possible only if a sectioned trace is loaded and a section is selected in the Loaded Pulses tree, left of the Trace on Trace Tab.

Connect sweeps Sometimes you can have considerable time gaps between neighboring sweeps. This setting defines if a line must be drawn, spanning these gaps or not. Default setting is NOT; this option MUST be set OFF in overlaid view. In all other cases it is just matter of taste.

Analysis submenu.

Amplitude histogram Calculates the binned current amplitude histogram of a selected portion of a trace and displays it in the **Amplitude** tab. If nothing is selected, emply histogram is shown.

Set Borders. Allows to set Y-*borders* for the analysis. Each border is set by left mouse button click; use right button to stop. *Borders* may be set either in the Trace tab or in the Amplitude tab. One can detect borders in any order; they are automatically sorted afterwards, considering the *Current Direction*. After initial setting, they can be dragged by a mouse. The *Borders* play a dual role: first and last borders instruct the program that all “real data” are inside them, everything which is outside, is considered to be an artifact and ignored. Second, and most important, they serve as the “borders” between levels, for the detection of opening and closure events (Analysis of kinetics) or calculation of level dwelling distribution (Distribution analysis).

Set levels. Allows to set *conductance levels,* corresponding to the different number of opened channels. Used to calculate single channel conductance and *NPo by average current* (see above). First level is considered to be “all closed” (and is therefore not “first”, but “zero” level). In terms of the Program, you have one level, if only one open channel is opened at any given time point, that is, when you have “zero” and “first” levels. Which level is “zero”, is defined by Current direction. As well as borders, levels may be set in the Amplitude or Trace tabs. In the Amplitude tab levels may be moved by mouse dragging afterwards. After levels are defined or moved, Y-values corresponding to each level and single channel current amplitude are automatically sent to the Notebook, as well as number of levels.

Add levels. Add new levels to an existing set.

Tip: sometimes, “all closed” level is never reached in a selected range of analysis, but its position is known from a prior or posterior inhibitor application and for correct calculation of NP_o, N and P_o you need all levels. In such a case, you may start with a selection of an interval where inhibitor is present, find “all closed” level and its neighbors, then move Selection to your interval of interest and use “Add levels” command to complete the set of levels.

Mean Current. Calculates mean current over Selection. If no Levels are defined, just absolute value is calculated, if Levels exist, than difference between absolute mean current and current, corresponding to “all closed” level, is shown.

NP_o. Interval for analysis and levels or borders (or both) should be set before one can use this item. If **Levels** are set, mean current over the interval and NP_o according to Eq. 2 is calculated. If Borders are set, number and frequency of events are found, distribution of level dwellings, according to Eq. 4 and NPo from levels probability distribution (Eq. 3) are calculated and results are sent into the Notebook. In addition, Level analysis is enabled.

Calculate kinetics. *Can be used if one and only one level is defined.* Meaningful, if you believe that there is indeed one channel active. All opening/closing events are found and sent into a table on Kinetics Tab, histogram of open and closed time dwelling times is calculated. In addition, mean open time, total open time, mean closed time, total closed time and NP_o according to Eq. (1) are calculated and results are sent into the Notebook.

Test binomial fits. This command calculates empirical distribution of total dwelling time at each current level (ie, of n channels simultaneously opened), finds NP_o and iteratively tries to fit these data with binomial distributions with N varying from 1 to 100 and Po=NPo/N for each N. Best fit may be determined by maximal likelihood estimate, weighted squares, unweighted squares. Details of mathematics are described in section V.3 of this manual, further details of operation – in VI.6.

Save Results submenu.

Save amplitude histogram Saves binned amplitude histogram as .csv file.

Save fit results as… Saves values of likelihood function for fitting experimental distribution of levels with different tested binomial distributions, as csv file.

Save openings… Saves histogram of opened state durations as csv files. Histogram is calculated by Calculate kinetics command.

Save closures. Same as previous item, but for closed state.

Save events table. Saves table of channel openings and closures, detected by border crossing method. Also calculated by Calculate kinetics command.

Options submenu

Toggle current direction. Toggles inward/outward current (may be set also by clicking corresponding button on the toolbar). This is important, since it determines where "all closed" is and what is the sign of current.

Options. Opens Options dialog, see below.

About. Opens About dialog.

3. STATUSBAR

Mouse coordinates, various information about the state of the program, length of the Trace in data points are shown in the Statusbar. Importantly, tips about almost each control, menu item or speedbutton are also shown there when mouse cursor hovers over that control. All fields of the Statusbar are shown in Figure 2.

4. PULSE TREE TAB.

On the left, tree of *HEKA file* is displayed, essentially the same as in "replay" window from HEKA programs. is a symbol of a Group, is a Series and is a sweep. In red are shown those sections which are currently loaded in the Trace. Blue is the selection to be loaded. A single section (group, series or sweep) may be selected by left mouse click on an item. To select a contiguous interval, select a first section of it by left-click, than press and hold Shift key and left-click last section of the desired interval. Both sections must be of the same level (ie, both groups, or series, or sweeps). To select multiple non-contiguus sections, left- click **node area** of each desired section. When the sections which one wants to load are selected,

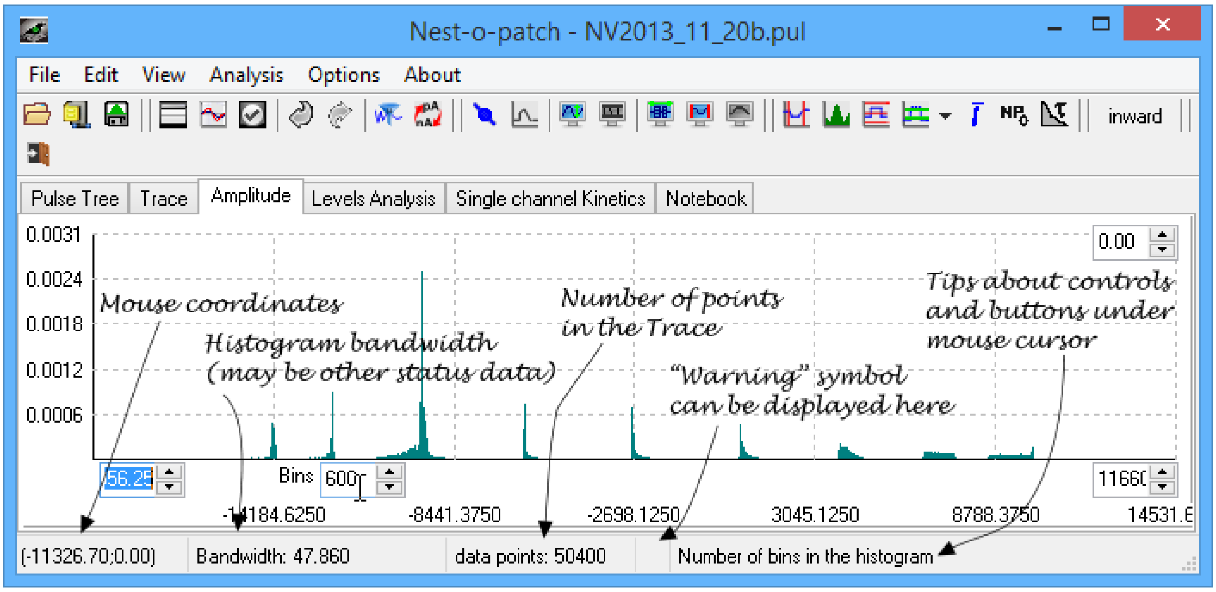


Figure 2. Main window, Amplitude tab opened, fields of StatusBar are described.

button allows to actually load them. Single section may be loaded simply by double-click on it in the Tree.

Edits for global loading options can be found near the Load button. Trace # defines which HEKA trace to load (PatchMaster file can contain up to 16 traces, Pulse – up to 2 traces). Respectively, values from 1 to 16 may be specified. Global compression defines a factor, by which the Trace will be compressed during loading. Default is 1 (no compression), any positive integer N may be specified. Compression is achieved by averaging N neighboring points. Next to it, Units dropbox is located. Slightly simplifying, data are stored in .dat files from Pulse and PatchMaster software in Volts and Amperes. Units dropbox allows to convert them to anything from femtoamp (factor 10^{-15}) to amperes (no conversion, factor 1). Default is picoAmp. If one opens a trace recorded in current clamp mode, choice of picoAmp would give picoVolts and choice of mAmp – mV and so on. I.e., this combo defines just the coefficient for conversion. If you open a Trace and find that your choice of units was not optimal, it is always possible to use Units conversion dialog (see **VI.9**) for a loaded Trace. Time relative to: dropbox allows to select the origin of the time scale for a loaded Trace. If you select time relative to Sweep and load a whole series, all sweeps are shown overlaid, pretty much like in HEKA programs. However, Nest-o-Patch allows to show overlaid several series or even groups. Default setting is time relative to file, i.e., to the beginning of Group1.Series1.Sweep1, which means linear consecutive view of all sections. Below these controls, various information about an experiment is depicted. Date is a date of the recording (first sweep of the first series in the first group). Aplifier and Series/Sweep panels show information about a series and sweep currently highlighted in the Tree. *Mode* is a mode of recording (inside-out, whole-cell etc), *Vhold* – holding potential (mV) (or, in case of current clamp, holding current (pA)), *Rs* – series resistance, *C slow* – cell capacitance, *Gain* – gain setting of Multiplier, *Filter bandwidth* – frequency of the amplifier built-in analogue *filter*, *V-offset* – offset voltage, as was set by Multiplier. *Traces count* – number of PatchMaster traces in a selected sweep. *Time*: time between the beginning of a file and selected sweep, *Temp.* – temperature, *"0" current* – current value at the beginning of this

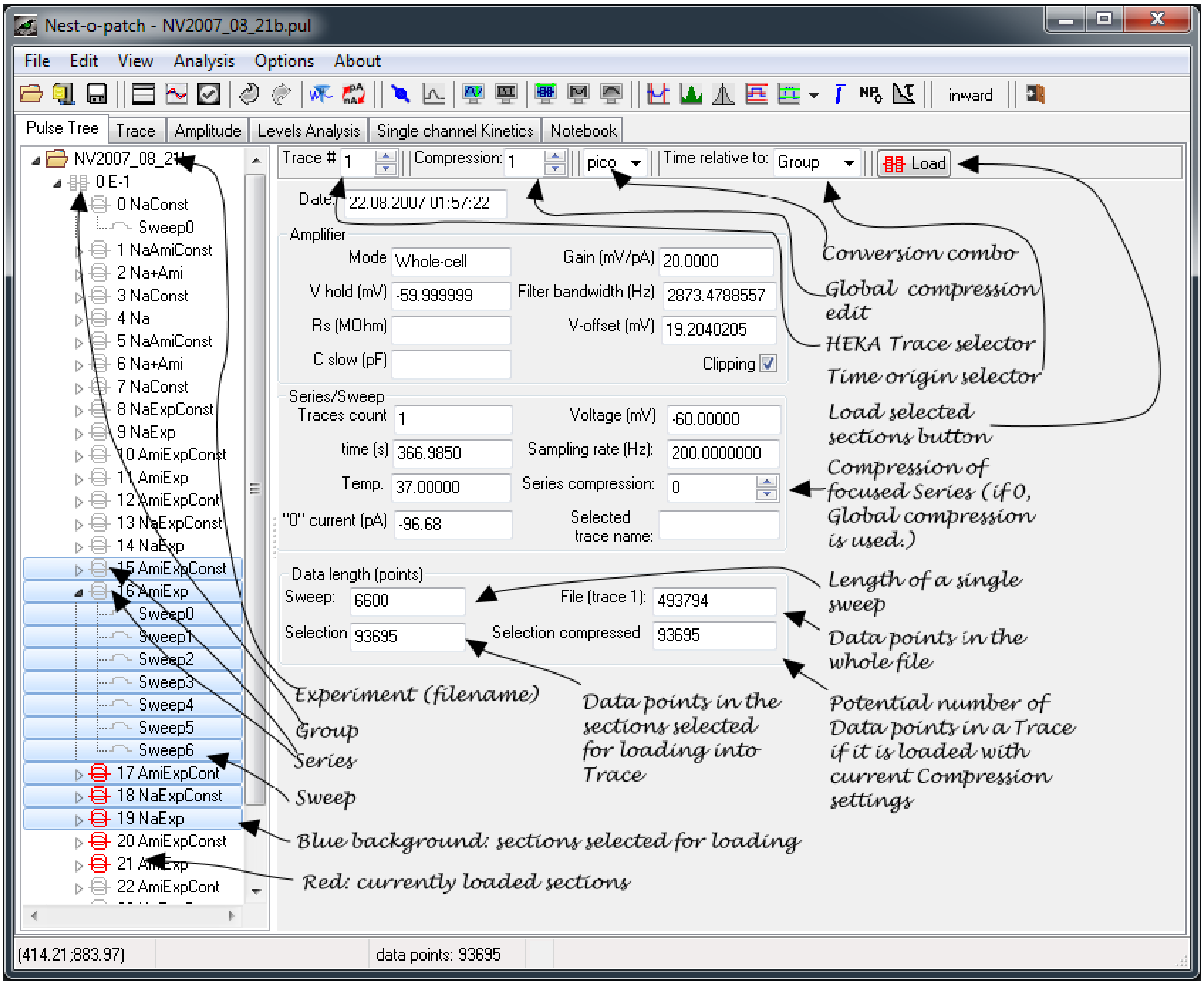


Figure 3. Main window, Pulse tree tab is opened. Experiment from a file named NV2007_08_21b is loaded. Partially expanded Tree is shown. Series 17 to 21 are loaded as a Trace; series 17 to 19 are selected and will be loaded when a user presses "Load" button.

sweep, *Voltage* – holding potential at this sweep (in pulse protocols different from global *V hold*), *Sampling rate* – digitalization frequency.

Series compression edit on the `Series/Sweep` panel allows to set a compression factor for highlighted series other than global compression. Variable compression be useful if different sampling rate was used for the recording of different series, and now you want to bring them all to the same format (for example, during the preparation of a figure). Default value for this edit is 0, which means the use of *Global compression* setting. *Selected trace name*: name of the PatchMaster trace, selected in *Trace #*.

Data length panel shows how many data points are there in the whole file, in a selected portion, in a selected portion after the compressions will be applied and what is the length of a sweep in a currently highlighted series. This information may be useful if you think how should a trace be compressed at a loading.

5. TRACE TAB.

Trace itself can be viewed and edited in this Tab. View and scaling may be changed with the following buttons and corresponding menu items: Show data markers, Highlight sections, Show full vertical scale, Y-Scale to borders, Show complete trace, show Selection, Show highlight. You can edit and prepare a trace for analysis with commands from Edit menu or corresponding speedbuttons.

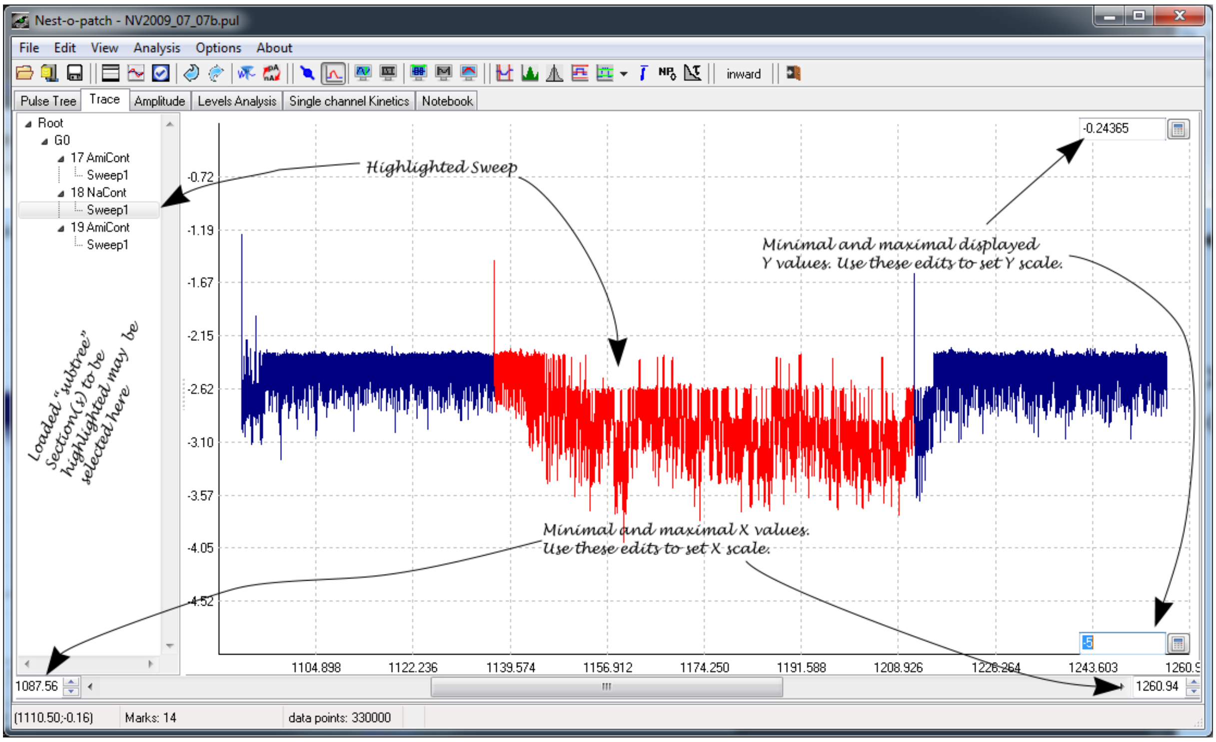


Figure 4. Main window, Trace tab. 3 consecutive Series (with 1 sweep each) are loaded as a Trace. Second sweep is highlighted.

buttons serve to correct a floating baseline (see **VI.2** for description of each command and chapter VII "How to…" for advice about the procedure).

applies a low-pass filter: moving average or gaussian. Type of the filter may be defined in Options dialog; other parameters – in corresponding filter dialogs after pressing this button.

Wipe selection command (available only from menu) allows to remove selected part of a Trace, for example, to get rid of an artifact. Correct timing is preserved. buttons correspond to the Analysis menu. Note arrow near the Levels button: Pressing this arrow you can select between defining completely new set of Levels (or undefining an existing one) and adding new Levels to an existing set.

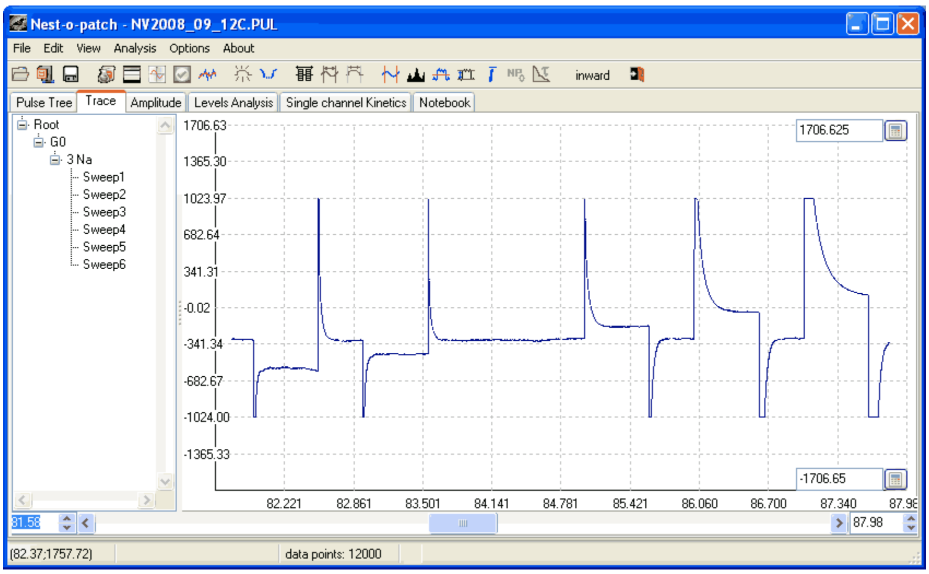


Figure 5. Trace in "linear sections view". Time scale is relative to the beginning of file; sweeps are shown one after another (v 1.1).

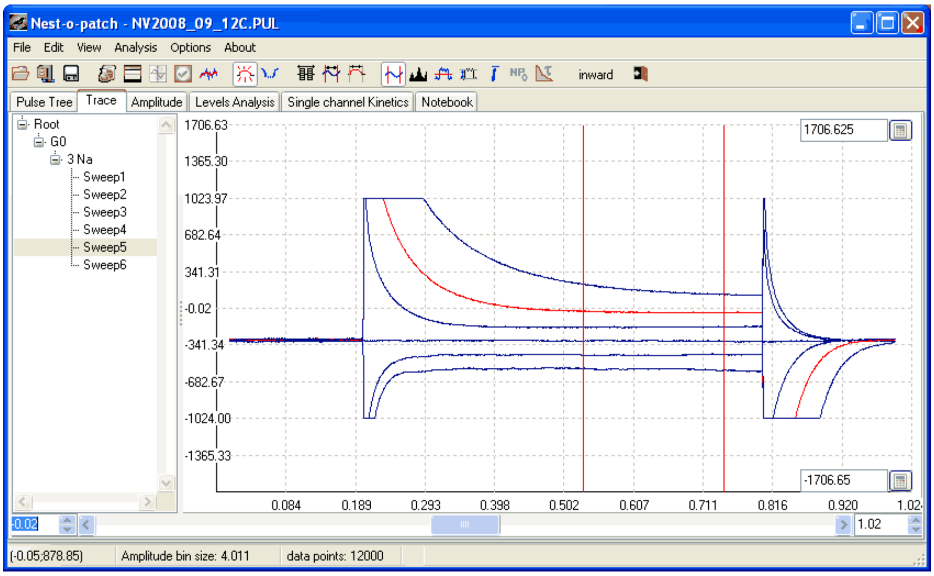


Figure 6. Trace in "overlaid sections view". Origin of time scale is set to the beginning of a sweep; respectively, all sweeps are shown in parallel. Sweep 5 is highlighted (v.1.1).

Different origins of Time scale and "overlaid view". By default, time "0" in Nest- o-Patch is a beginning of a file. (Technically, Group1.Series1.Sweep1.Time). In this setting, all sweeps of every series are shown one after another, as in fig. 5. However, one can want to look at a series with all sweeps overlaid, like in HEKA programs. To do so, define Time relative to: sweep in the Pulse Tree before loading a Trace.

Then, your Trace would look like shown in Fig. 6, with sweeps overlaid. All types of analysis can be used in this mode, but with some differences.

Average current will be calculated for a selected portion of each sweep; table with holding potentials and results is sent to the Notebook.

Current amplitude histogram is calculated either as a summary of selected fragments from every overlaid sweep or, **if one sweep is highlighted,** then for a selected fragment of a highlighted sweep only. NP_o and *single channel kinetic* can be calculated only for selected part of **highlighted** sweep. If no highlight exists, commands for these types of analysis are disabled. Most frequent use of overlaid sections view is showing of overlaid sweeps of one series; however, few series or even groups also may be overlaid, even if they have different length and/or sampling rates.

Warning: only one of overlaid segments (usually sweeps) must be highlighted for correct work of these types of analysis. Otherwise, you get incorrect results!

Saving overlaid sections to a file. Saving a Trace or its selected portion with Save as or Save Selection buttons would produce csv files with 2 columns; if you want to save a table where time is in a first column and each of overlaid section in a separate column, you must use Export Table command. Important: such Table can be opened with an external program, but cannot be read back to Nest-o-Patch.

Markers of individual points. For very detailed inspection of some event, a user can opt for displaying markers of individual points in a Trace with command. However, this command has an effect only if number of points per window width is lesser then this width in pixels (otherwise it would take too long to display let say 4 millions of points!).

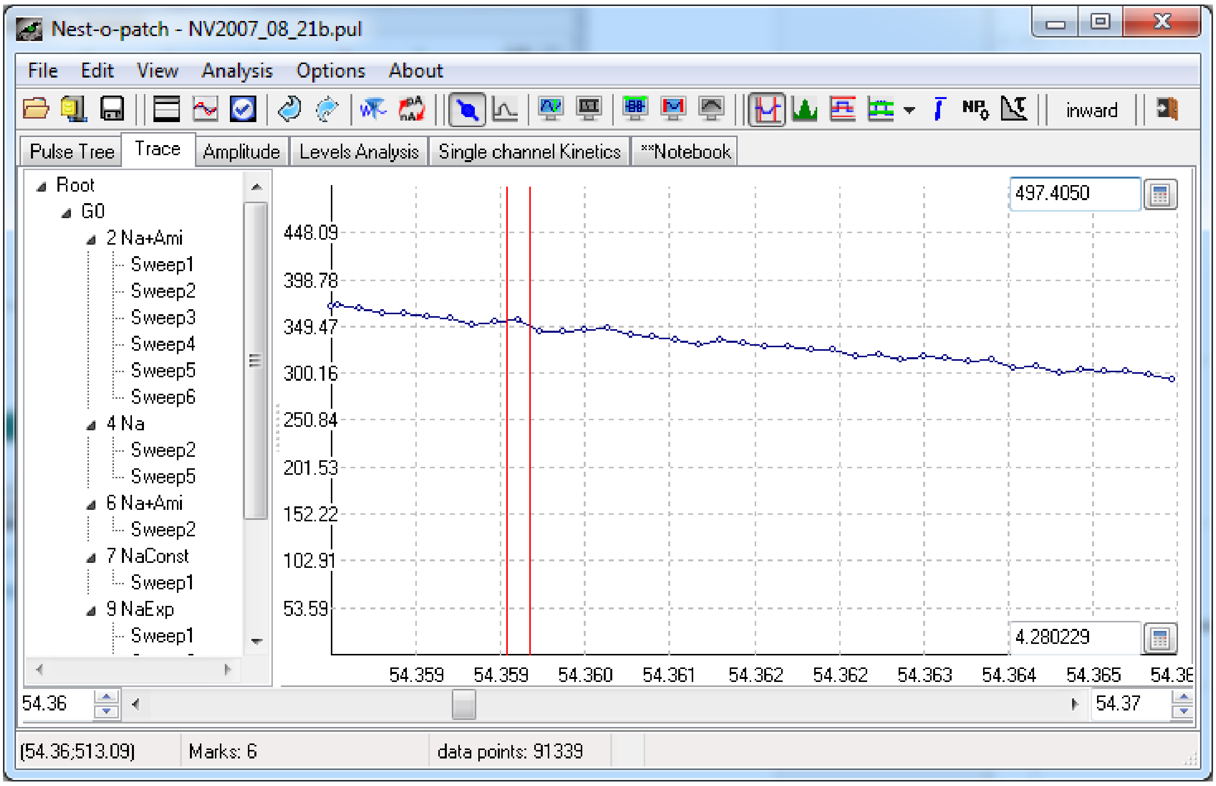


Figure 7. Individual points shown. A single point is selected.

6. AMPLITUDE HISTOGRAM TAB

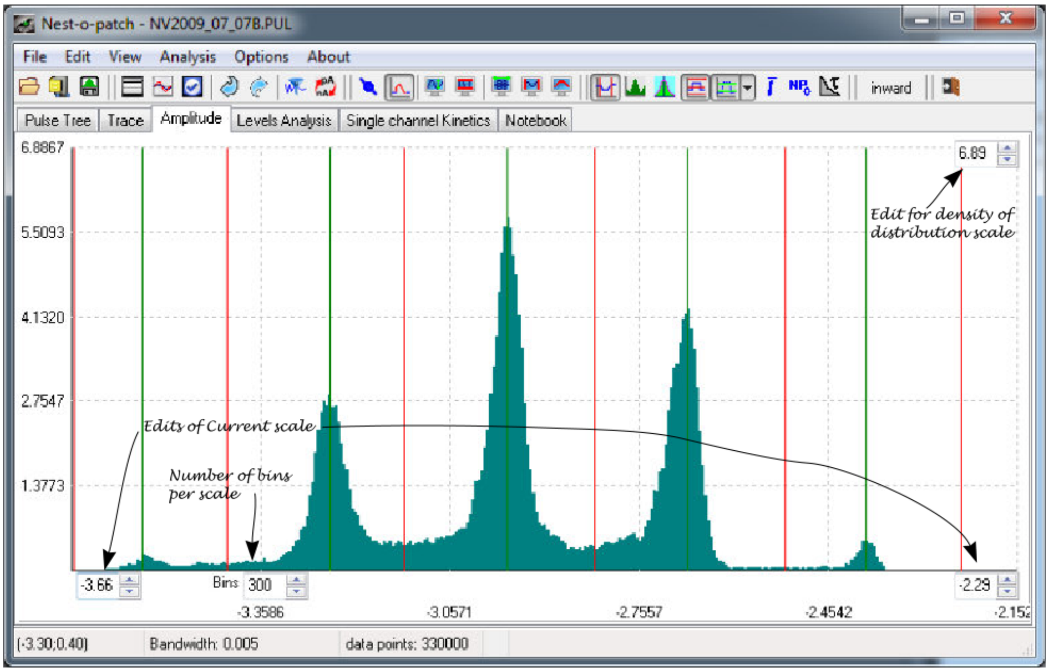


Figure 8. Main window, Amplitude histogram tab is opened and amplitude histogram of a recording with presumabely "all closed" plus 4 open levels is displayed. Levels (in green) and borders (red) are set.

Binned current amplitude histogram, generated after command Analysis|Amplitude histogram, is shown in this Tab. X axis is for current value, pA (or any other current unit), Y axis – density of probability, pA^{-1}. A user can change scale and number of intervals (bins) per X scale, using the edits, shown at the Fig.8. This Tab is probably the best place to set current *Levels* and *Borders*. Do it with the same buttons or menu commands as in the **Trace** Tab. After setting them, you may go to the Trace tab and inspect their position. Both Levels and Borders can be moved with a mouse in the Amplitude tab.

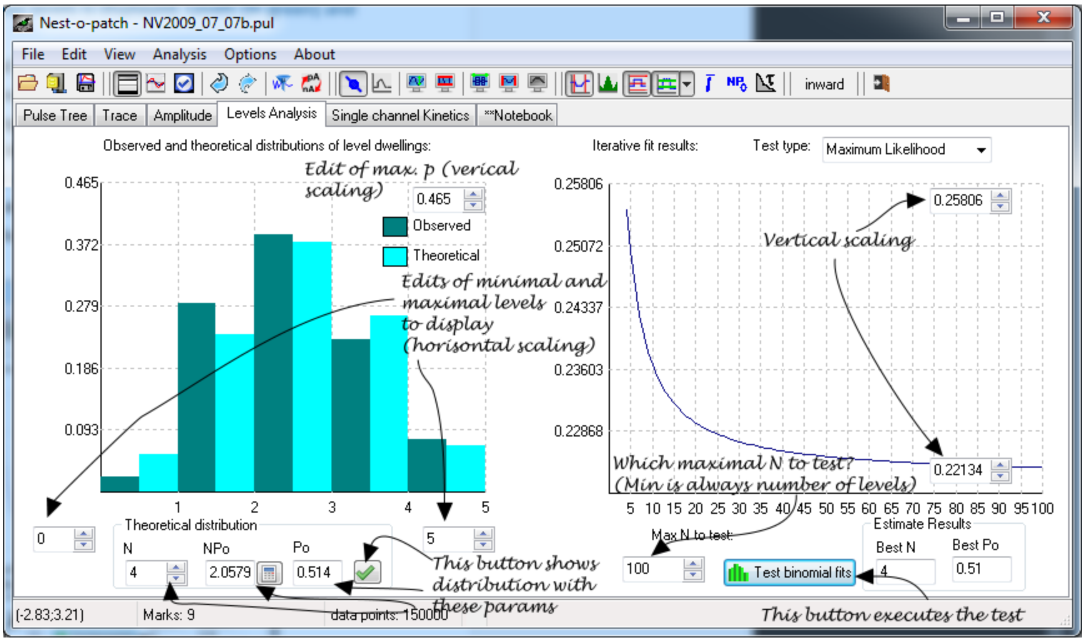


Figure 9. Levels analysis Tab. Left graphical panel shows observed and best fitting theoretical distributions of total time on each level. Right panel: likelihood function values plotted agains tested N.

Analysis of levels distribution serves for the estimate of the number of channels *(N)* and *open probability* (P_o) in a recording when several channels are present. The program calculates experimental distribution of current over the levels (in Fig. 8 shown in dark green), finds NP_o and iteratively fits this observed distribution with different binomial distributions to find a best one. In theoretical distributions, N varies from number of observed levels (in this example, 5), because, obviously, number of channels in a patch cannot be lesser then number of observed levels, to N, defined by "Max N to test" edit. P of each tested distribution is NPo/N. Best fit can be estimated by maximal likelihood test, sum of weighted squares of residues or sum of simple squares of residues. Mathematics is described in more detail in chapter **V.3**. Type of analysis can be selected by Test type combo box. This analysis becomes available only after level dwellings themselves have been calculated. At least 2 of them must be non-zero. Therefore, before using this Tab one should:

1. Define at least 3 *Borders* (lower border of valid data; border between level "0" and level "1"; upper border of valid data). Of course, there may be more levels and respectively more Borders. For example, in the figure above, there are 5 levels and 6 borders. Typically one would define also *Levels* themselves, but for the analysis of distributions it is not required.
2. Press **NP_o** button or use Analysis | NP_o command. If *Borders* are defined, this command calculates empirical levels distribution and finds NP_o according to Eq. 3. Information about levels distribution is sent to the Notebook, but more important is that it is stored internally and may be used for Levels distribution analysis. (If *levels* were defined, it would find NP_o also according to Equation 2, but for analysis of levels distribution it is not important).
3. Now you may open the Levels analysis tab. On the left, bar diagram of the observed distribution of levels dwellings is displayed along with a theoretical binomial distribution.

After iterative test, parameters of the theoretical distribution correspond to a best fit found by the program; this distribution is sent also to the Notebook. However, a user can try different parameters, using the Theoretical distribution panel below the diagram. In fact, playing with theoreticial disribution is possible even if no Trace is loaded. Each time when a user presses the button in the Theoretical distribution pane, the generated distribution is displayed here and sent into the **notebook**. On the right, instruments for the estimate of best binomial distribution are placed.

Max N to test editbox allows to set any desired maximal *N*. Minimal tested *N* is always the number of observed levels. Button [Execute test] actually fulfills the analysis. Best *N* and best P_o according to the selected and completed test are shown on the right. As was already mentioned, corresponding theoretical distribution is shown in the left graphical pane.

8. SINGLE CHANNEL KINETICS TAB.

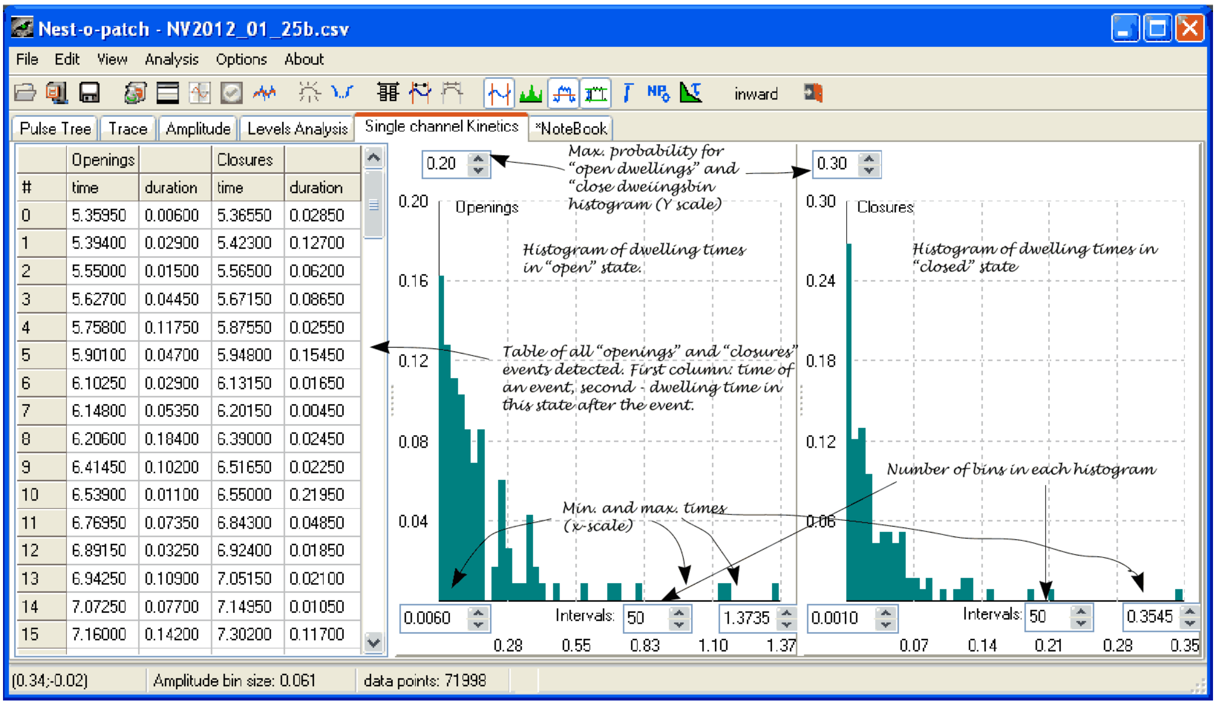


Figure 10. Main window, Single channel kinetics Tab (v. 1.1).

This Tab may be used when only one level (and when you believe that only one channel) is present in a recording. Analysis is invoked and the controls of the Tab are filled with data after one presses button.

Before it may be done, one should set 3 and only 3 *Borders*: upper border of true data, border between "open" and "closed" levels and lower border of true data. If *Selection* for the analysis is not set, the whole *Trace* is used. Crossing the middle *Border* is used for *event* detection. However, if values go outside the range between upper and lower borders, an event is disregarded as possible artifact and time when values are out of the range is not counted.

The **table of events** and the data of both histograms can be saved as .csv files. Besides that, following information is sent into the **Notebook** when the **Kinetics analysis** is invoked (as an example):

Mean open time: 0.24305 ;time always in seconds, if time scale was not modified

Total open time: 33.54050
Mean closed time: 0.09406
Total closed time: 13.07450
NPo: 0.71952
Events frequency: 5.94229 per second

9. NOTEBOOK TAB.

Not much can be said about the **Notebook**. Majority of the user's actions and results of analysis are sent here. Whenever a program sends something to the Notebook, it adds asterisk (*) to its title, so, it looks like '*Notebook' which means that there is something a user has not seen yet. Contents of the notebook may be freely edited by a user and saved as a text. A text file can be loaded to the Notebook once more (File | Save notebook… and File | Open notebook… commands).

10. UNITS CONVERSION

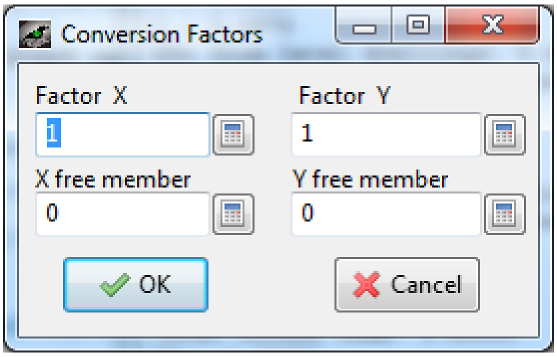


Figure 11. Conversion dialog

As was mentioned in Chapter I, this program does not make any assumptions about units of X or Y values of the data. Respectively, in the Conversion dialog you do not explicitly operate with units, but directly set coefficients for recalculation. The dialog for the whole Trace is invoked with a button: or Menu command Edit | Convert | Convert Trace… . Selected part of the *Trace* or a highlighted section may be converted by menu subitems Edit | Convert | Highlighted and Edit | Convert | Convert Selection.

Conversion dialog allows to recalculate data values of your trace:

$$NewVal = Factor \cdot OldVal + FreeMember$$

For example, to convert time from seconds to minutes, insert 0.0166 (1/60) into Factor X edit. button on the right of an Edit allows to invoke calculator and insert result of calculations into the Edit.

Tip 1 about conversions. Free member may be useful for example if you want to change Time offset. For example, you are preparing a figure and use a fragment of a recording from a middle of experiment, but want to have zero time point at the beginning of a fragment, which you show.

Tip 2 about conversions. Theoretically, it is possible to change both Factor and Free member at once. But even I do not remember which calculation is done first. Therefore, if you want to change both offset and units, it is advisable to invoke this dialog twice: once for the units (Factor), once for the offset (Free member).

11. DATA FILTERING

Nest-o-Patch uses moving average or Gaussian lowpass filters. Select one in the Options dialog. Data filter dialog may be invoked either from menu (Edit | Filter data command), or by pressing button.

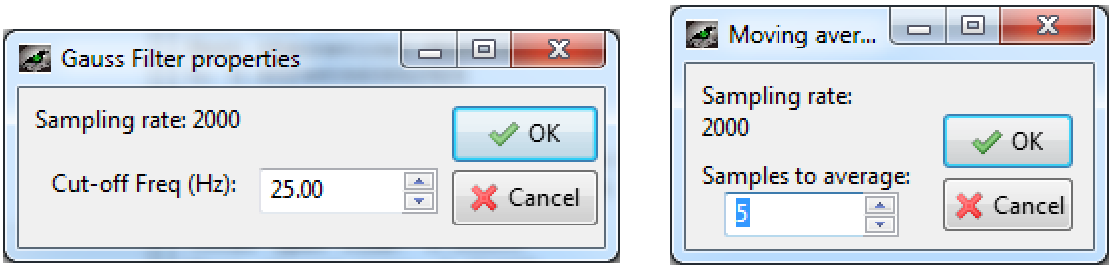


Figure 12. Gauss filter and Moving average filter dialogs.

Sampling rate is shown for the information. If time is in seconds, then it is in Hz. Samples to average in Moving average filter defines length of the averaging window.

Important note: both sampling rate and cut-off frequency are in Hz only if X-values are in seconds. HEKA files are loaded with this setting. However (1) if a text file was used as an input, the Program has no way to find out what units were used and (2) the program does not trace units conversions. Once again, it is a responsibility of a user to have correct dimensions.

12. OPTIONS DIALOG

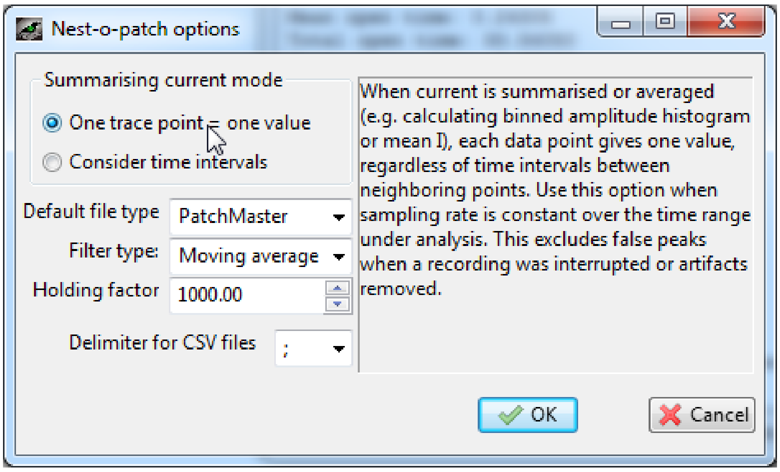


Figure 13. Options dialog. Help for "Consider time intervals" radiobutton is displayed.

"Options" dialog allows to define several important options.

"Summarizing current mode" radiogroup influences calculation of mean current (command), calculating *NPo* (command) and calculating current amplitude histogram ( ). When "Consider time intervals" is selected, mean current over time is calculated as:

$$\bar{I} = \frac{\sum_{i=0}^{N} I_i (t_{i+1} - t_i)}{t_{N+1} - t_0}$$

Here nominator gives the charge, transferred over time of the fragment under analysis, while denominator is duration of the fragment. Advantage of this setting is that it allows calculating mean current over intervals with various sampling rate, since time interval for each point is found individually. Various sampling rate is possible if a fragment under analysis includes several *series.* However, if there was a break in data acquisition during the recording of a fragment under analysis, or if part of the data were removed by "wipe selection" command, then time interval for a point preceding this gap in data would include the whole gap and may be therefore largely overestimated, which would give a false peak in a histogram or incorrect calculation of mean current, as shown in fig. 13. However, situation with variable sampling rate appears relatively seldom, and much more simple, but robust approach to current averaging is possible, if sampling rate is constant.

$$\bar{I} = \frac{\sum_{i=0}^{N} I_i}{N}$$

That is, one trace point gives one value (first option in the Options dialog, “Summarizing current mode” radiogroup). With this approach, problem of false peaks is removed, but no analysis of intervals with variable sampling rate is possible. By default, this option is activated.

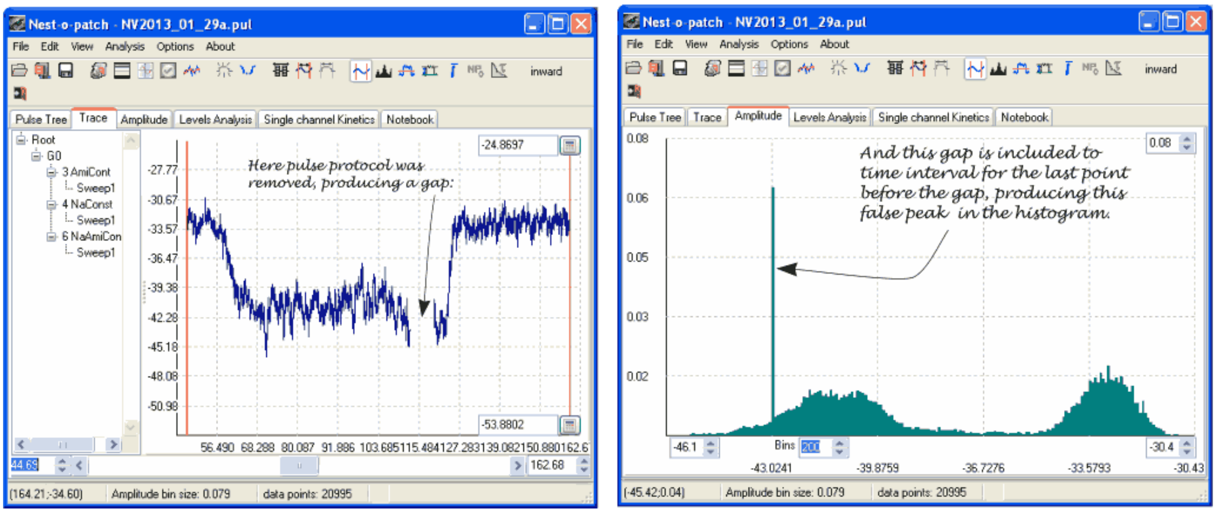


Figure 14. “Consider time intervals” option selected. Gap in the recording produces false peak in the histogram (ver 1.1).

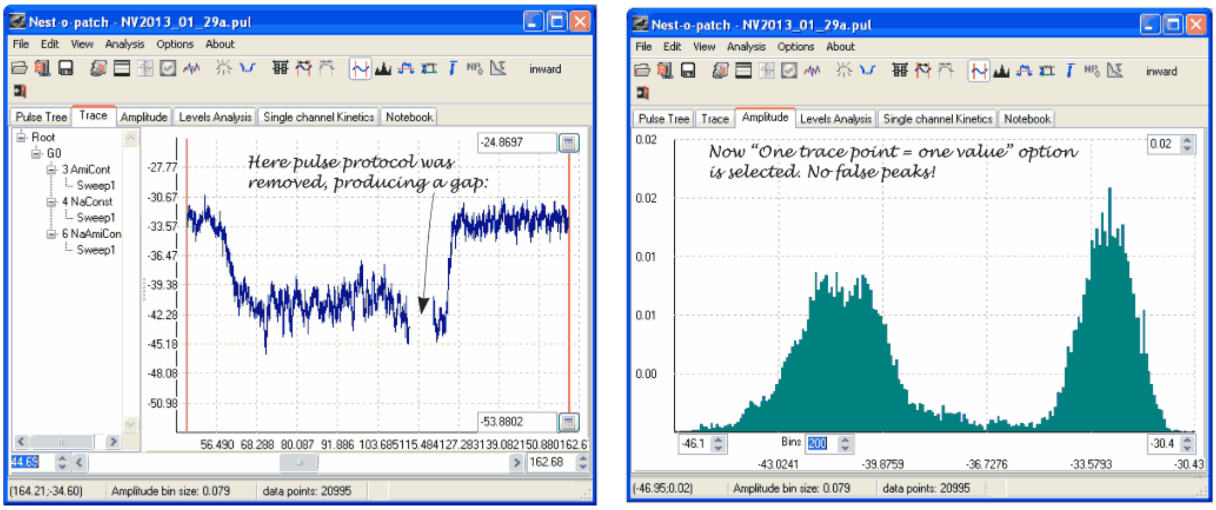


Figure 15. “One trace point = one value” option selected. False peak is removed (ver 1.1).

Other options:

Default file type: default file type in the Open file dialog (Pulse; PatchMaster; Text).

Filter type: Moving average or Gaussian.

Holding factor. All values are saved by Pulse or PatchMaster as Volts and Amperes. However, for patch-clamp in most cases it is more convenient to express them as mV and pA, respectively. Therefore, we multiply values for Holding potential, stored by Pulse or PatchMaster by a factor, which is defined here. Default value is 1000, which would give mV for experiment in voltage clamp mode, or mA, for experiment in current clamp mode. You may change this value for your own convenience, for example, set 1E12 to convert to pA, if you work in current clamp mode.

Delimiter for CSV files. CSV stands for Comma Separated Values. Original American standard uses indeed commas as delimiters between values. However, in most European contries, commas is used as a decimal separator and semicolon is used as a CSV delimiter. This option allows to toggle between comma and semicolon for this role. Semicolon is default value. Area in the right part of Options dialog is used to show help for an element under cursor.

VII. HOW TO… PROCESSING AND ANALYSIS OF SINGLE CHANNEL AND ENSEMBLE CURRENTS WITH NEST-O-PATCH.

In this chapter we will discuss how one can use Nest-o-Patch to prepare data and consequently perform an analysis of single channel events.

1. TRACE CONDITIONING

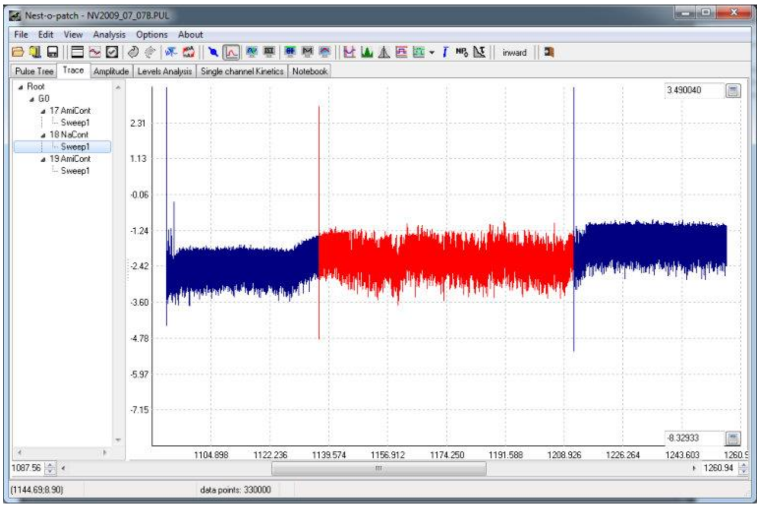


Figure 16. Unprocessed outside-out recording. Highlighted is a sweep when channel inhibitor was removed from the bath.

Let's consider an imperfect recording, shown in fig. 16., which despite its flaws contains valuable information about single channels behavior. Our task is to retrieve and use this information. Obviously, channel events are almost completely hidden in the high frequency noise. So, first of all, we apply filter. Click button and get a dialog similar to one of dialogs shown in Fig. 12. However, in this case Sampling rate would be 200. After application of a filter with cut-off frequency 80, we have a picture, shown in Figure 17.

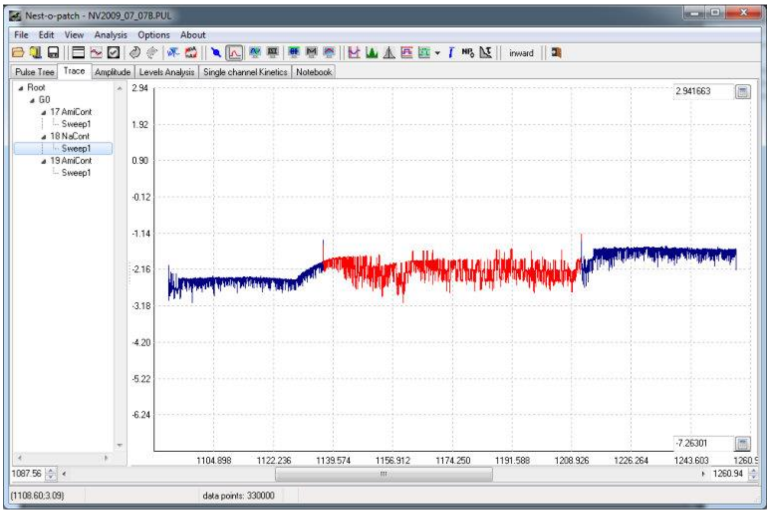


Figure 17. Digital filtering was applied, but huge drift of baseline prevents an analysis.

Now we clearly see single channel events, but very unstable baseline prevents us from their successful analysis.

This drift of baseline may be corrected in 2 steps. First, let's show the program where "true" baseline lies. Press button. When you hover with a mouse over the Trace, black horizontal line follows your cursor. Find the best location, click the left button – and true baseline found (Figure 18). Next, let's show where the actual drifting baseline runs. Press , then go to the Trace and, repeatedly pressing left button, draw the outline of this drifting baseline. Press Esc or click right button to leave this mode. If you made a mistake, last entered point may be deleted by pressing Backspace. As a result, you get something like Figure 19.

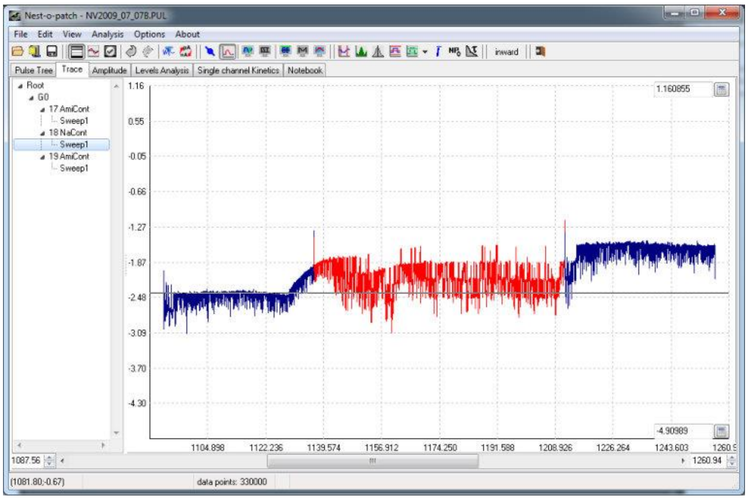


Figure 18. **"True baseline" defined.**

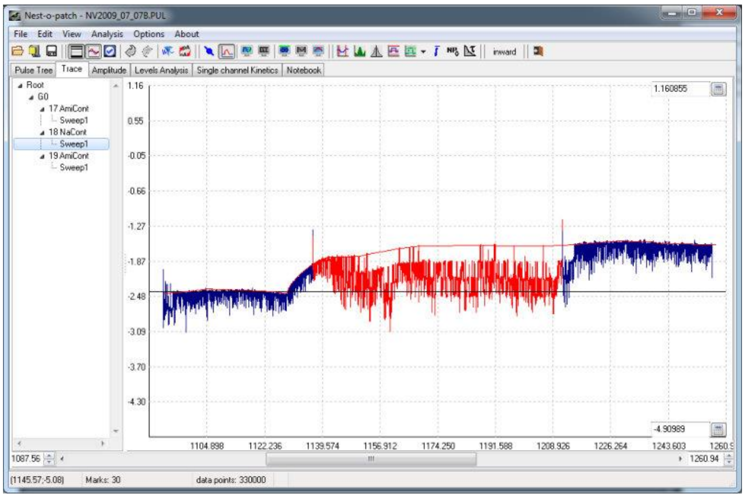


Figure 19. **Actual Baseline followed.**

Now, press – and baseline is magically corrected (Figure 20). Preprocessing of the data is over and we are ready for the analysis. But maybe it is a good idea to save the trace as .csv file (Menu File | Save as) or Save button.

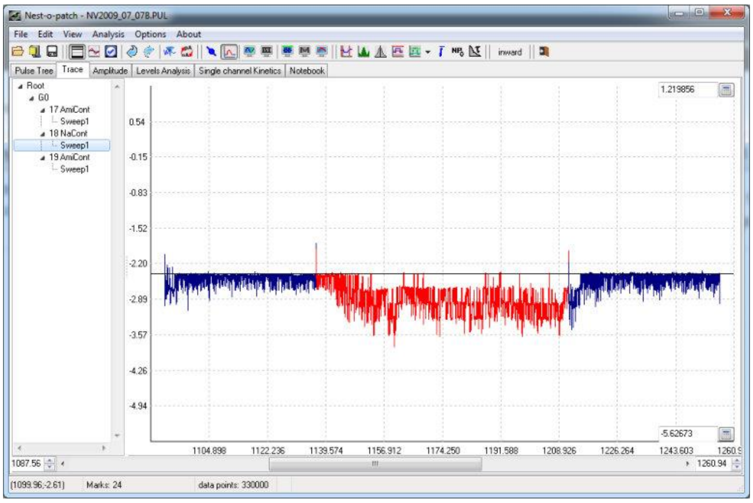


Figure 20. Baseline corrected.

Now, let's define horizontal and vertical ranges of the analysis. Horisontal: , find position of a first border, left click, then position of a second border, left click. No matter, which one is found first: left or right. Vertical: , first border, left click, second border, left click, then ight click to tell the program that you do not want further borders. Now your window looks like this:

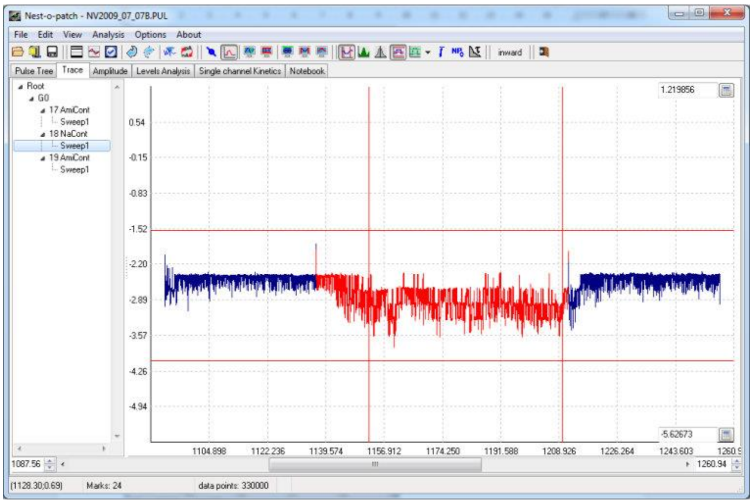


Figure 21. Upper and lower borders as well as left and right limits are set.

Next logical step is to build a binned current amplitude histogram. Press . The program generates the histogram and opens Amplitude Tab. Histogram is plotted as probability density function of current values, $pAmp^{-1}$.

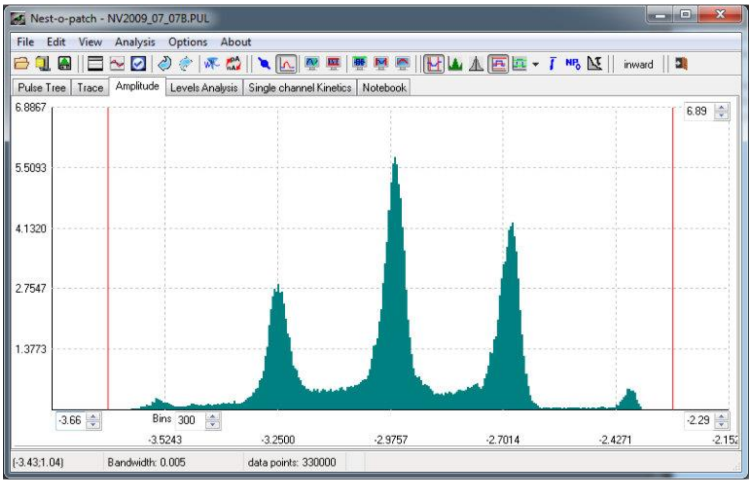


Figure 22. Amplitude histogram generated.

Note red lines (borders) at the borders of the histogram panel. They were used to define vertical range of the data, used to generate the histogram. Now you can define levels, corresponding to different number of open channels (at the histogram maxima) and borders between the levels (use either local minima or just middles between the Levels). To do it, press and mark Levels, using mouse. Left click sets a level, right click terminates the Levels definition process. Then press to define Borders and mark them, using similar technique.

Important: for the correct work of the analysis, make sure that Current direction selector is in the right position. Inward if you study inward currents, and outward otherwise. To change the position, simply left-click it.

Levels and Borders are now shown in the Trace Tab as well.

After the definition of Levels, information about value of current at each level was sent to the Notebook, along with the single channel current amplitude, which was calculated as an average distance between the levels. See Figure 24.

Hint: if you want to measure accurately a single channel current, don't use all possible levels: ones, visited too seldom may be poorly defined. Rather, use only prominent and sharp ones.

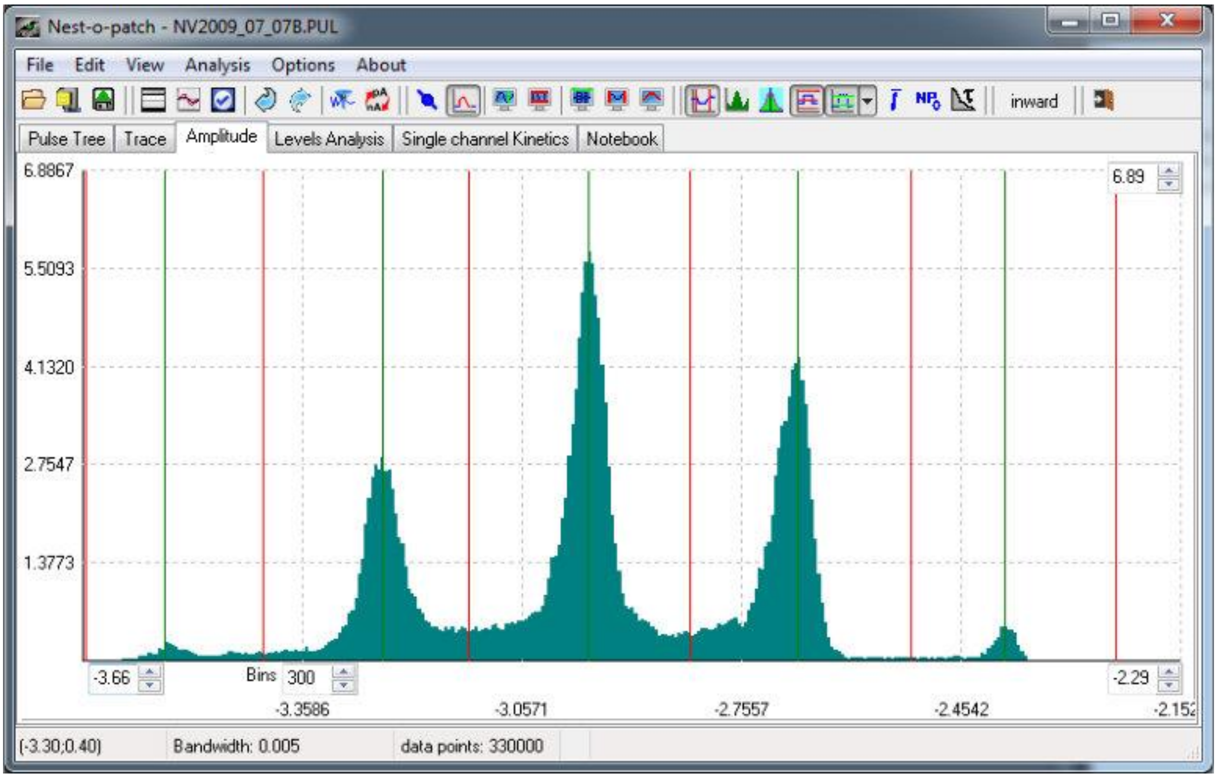


Figure 23. Amplitude histogram with Levels (green) and borders (blue). Note `Current direction selector`.

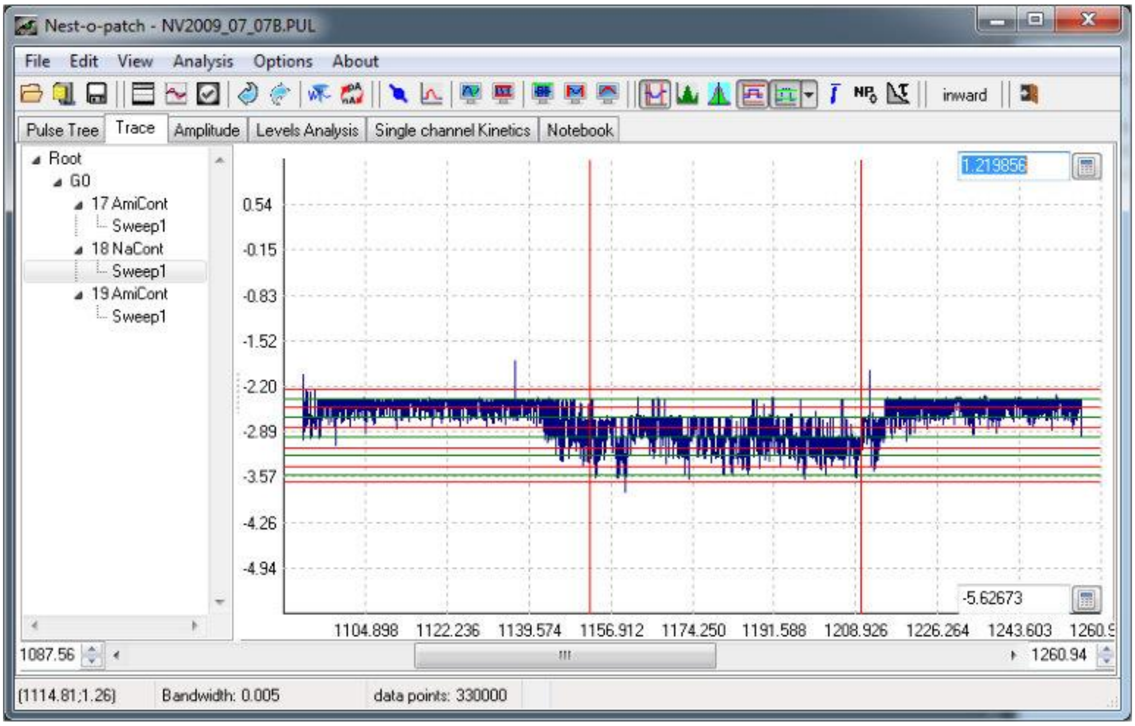


Figure 24. Trace tab shows Levels and Borders after they were defined in the Amplitude histogram.

To continue analysis, press **NPₒ** button. Values of average current, NPₒ calculated as $\bar{I}/i$, distribution of accumulated dwelling time at each level and NPₒ calculated by Eq. 3 are sent to the Notebook. Finally, you can go to Levels Analysis tab and press 

there. You will see following:

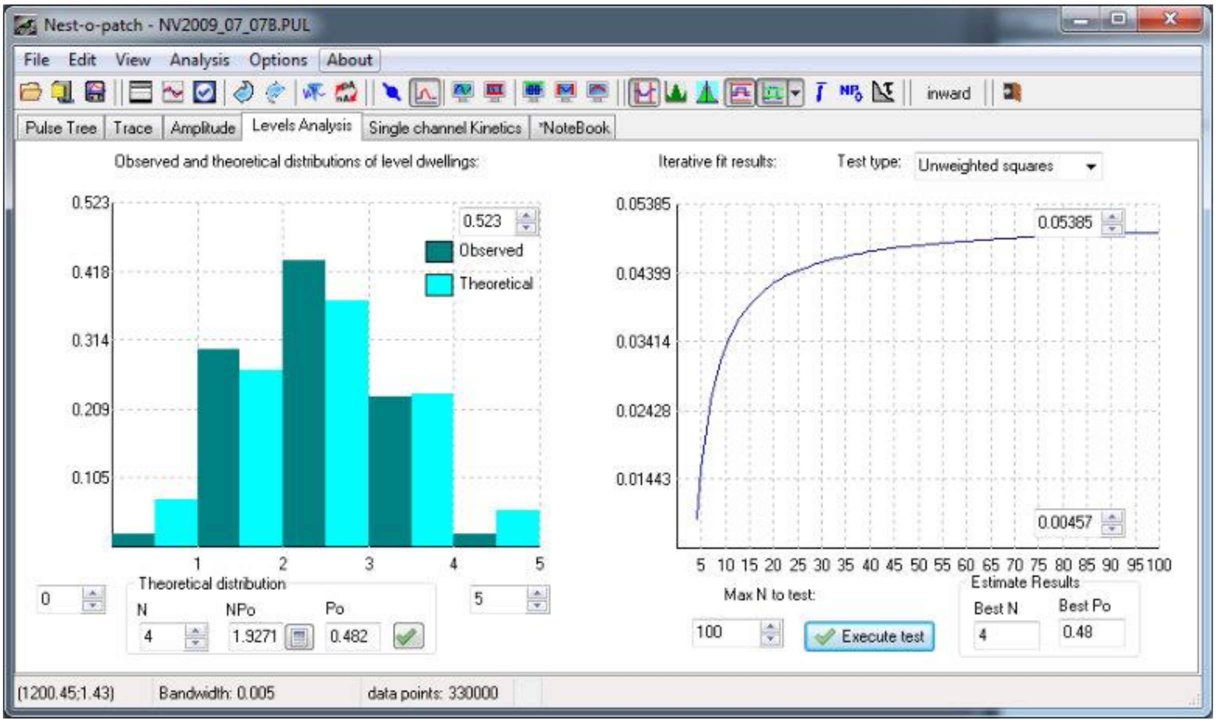


Figure 25. Levels Analysis tab after the execution of least weighted squares test.

Simultaneously, table of the best theoretical distribution and values of best N and Po were sent to the notebook, which now looks like this (text in brackets was added manually; you may edit and comment freely in the notebook):

Pulse File opened: NV2009_07_07b.pul
Data filter. Freq: 80.000
Baseline drift corrected.
Alalysis range: 1156.912 to 1214.706, length 57.79 (appears after time interval selected).
Amplitude histogram calculated.
0 -2.40592 (This is table of levels. "0" is all closed)
1 -2.68891 (Each line shows level number and corresponding current, pA)
2 -2.96802 (The table appeared here immediately after the user defined Levels)
3 -3.25294 (Comments in brackets are added manually, during a preparation of)
4 -3.54756 (the user manual)
Single channel current: -0.28541 (calculated as (i4-i0)/4
Levels: 4

(Next part of the text appeared after the user pressed "NPo" button)
Mean current: -0.5536
NPo as Mean Current / Single Current: 1.940 (single current was found previously)
Events: 609 (events detected by border crossing)
Events Frequency: 10.537 (as events number divided by duration (see "alanysis range" above)
Level dwellings distribution:
0 ; 0.0204 (this table shows total dwelling time at each level divided by time of analysis).
1 ; 0.2927 (which gives probablility to be found at each level).
2 ; 0.4316 (Sum is equal to 1).
3 ; 0.2363
4 ; 0.0189
NPo from Level dwellings: 1.941 (see equation 3. Note good agreement between 2 formulas to find NPo)

(Next part of the text appeared after "Execute Test" button was pressed at the "Levels analysis" tab.)
Best theoretical distribution:
0; 0.0702 (Bar diagrams of both empirical distribution, found above)
1; 0.2648 (and this theoretical, are shown in "Levels Analysis" tab.)

2; 0.3743
3; 0.2351
4; 0.0554
Best N: 4; BestPo: 0.49
Minimal weighted squares value:0.0710

Now you may want to save results of the analysis.

button saves the Trace when Trace tab is active; when Amplitude tab is active, it looks like and saves current amplitude as csv file; looks like and saves notebook when Notebook tab is active; curve of iterative tests (from the right graphical panel) when Levels analysis tab is active and table of openings and of closures, when Single channel kinetic tab is active. Besides that, you can use "Save…" menu commands, from submenu File or Analysis | save results.

3. ANALYSIS OF SINGLE CHANNEL KINETICS.

As was mentioned in **VI.7**, for the analysis of single channel, the channel must be single. That is, there should be 2 levels ("all closed" and 1 open) and 3 borders. Preconditioning and marking of the recording is not different from the analysis of small ensemble, which was described above. So, let's look at the following recording (Figure 26):

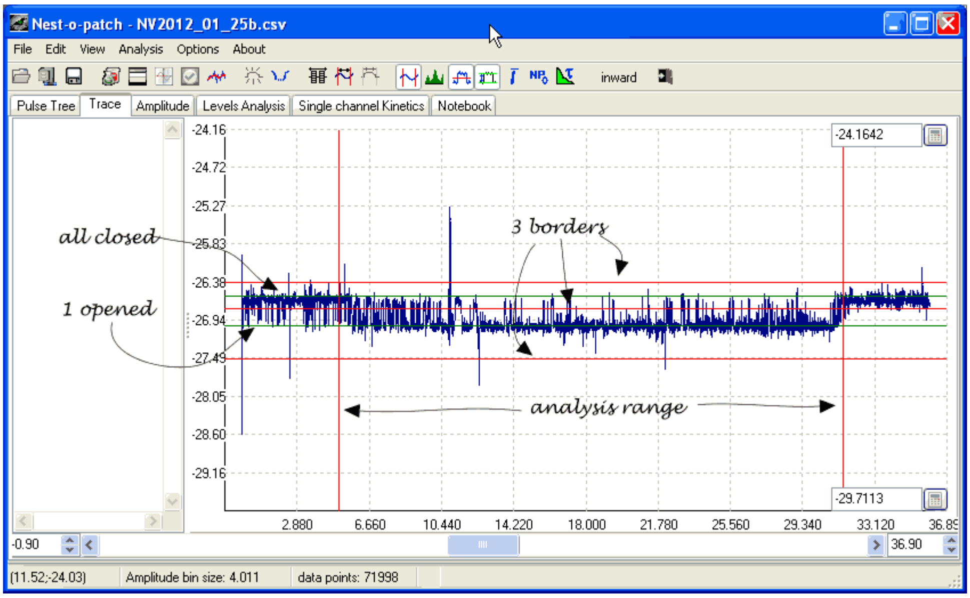


Figure 26. Recording with a single level and high Po. Two levels and 3 borders are defined.

Now press , Single channel Kinetics tab is opened (Figure 27).

Events are detected by border crossing, where border is the middle of three Borders. Table of all events is on the left. First 2 columns describe openings, second two – closures. **Time** is moment when the channel was opened or closed, Duration – how long was it in the open state before was closed again, or in the closed state, before was opened.
Indeed, first Closure in the Figure 27 is registered at 5.077 s; if you look at the Figure 26, you can see that it is shortly after the beginning of analyzed interval, judging by the position of left range line. First Opening is at 5.10150 s, that is, 0.0245 s later. Indeed, duration of the first closure is 0.0245 s, as you can see in the 4th column.

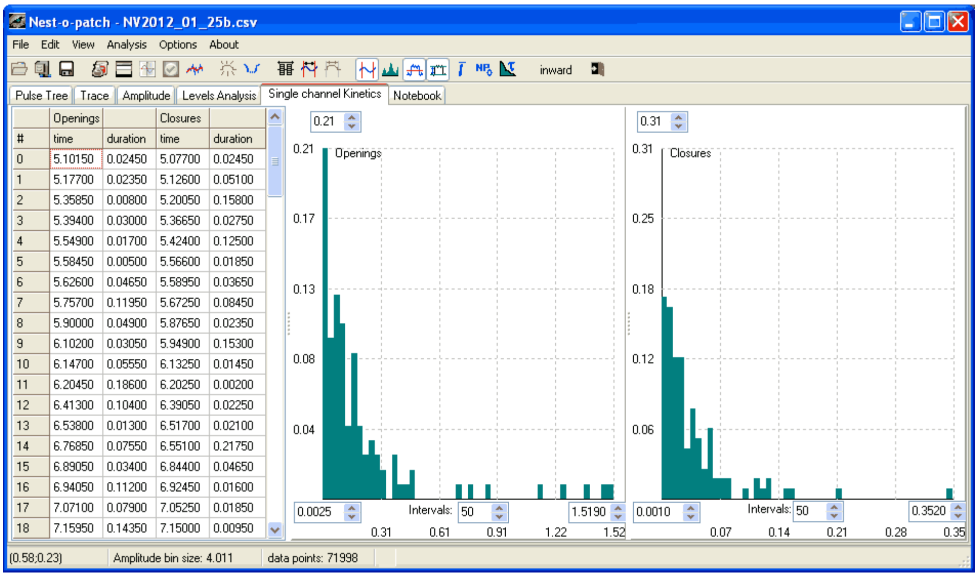


Figure 27. Results of kinetic analysis for the recording shown in Fig. 26 (v.1.1).

Distributions of the opened and closed state durations are shown in the histograms on the right. Theoretically they should follow exponential law (or sum of several exponents, if more than one opened or closed states exist); in this case we have relatively few events and exponential law is not so evident. Nest-o-Patch 1.2 itself does not analyse the distributions, but the table of events and both histograms can be saved as csv files for an analysis elsewhere. Use commands Analysis|save results|Save openings/save closures/save events for this purpose. In addition, following text is generated in the Notebook:

Mean open time: 0.18477
Total open time: 21.61751
Mean closed time: 0.03783
Total closed time: 4.38799
NPo: 0.83127
Events frequency: 8.95964

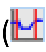
Mean open time is average time of dwelling in opened state; total open time is the cumulative time in the opened state over the analysed interval; closed times are similar. NP_o is calculated using Equation 3. Events frequency calculated as:

$$\mu = \frac{N_o + N_c}{T_o + T_c}$$

Where N_o and N_c are numbers of openings and closures, T_o and T_c – total time in opened state and total time in closed state.

4. USE OF THE PROGRAM FOR THE ANALYSIS OF ENSEMBLE CURRENTS

Nest-o-Patch 1.2 can be used also for the basic analysis of large ensemble currents, for example, ones recorded in whole-cell mode, both in patch-clamp or two-electrode voltage clamp. It is possible and easy to measure time intervals, current values and get I/V tables from pulse protocols. Here follow several tips on this use of the program.

To measure time, passed between two events (for example, stimulus and peak), use "select interval for the analysis" tool ().

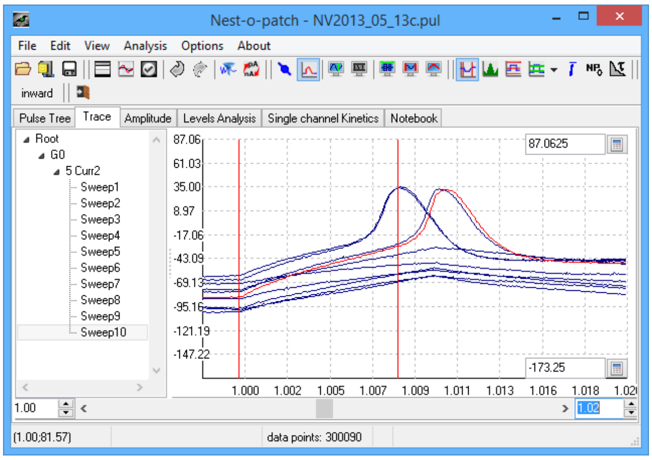


Figure 28. “Analysis range” tool used to measure time between stimulus and peak.

Time coordinates and legth of the interval are sent to the Notebook:

Alalysis range: 1.000 to 1.008. Length: 0.008. So, interval is 8 ms.

To measure value of a current, you can use two different techniques. First, it is possible to set Levels at a baseline and peak current and go to the Notebook to see the value.

For example, if we are interested in the size of inhibitor-sensitive current in a following picture:

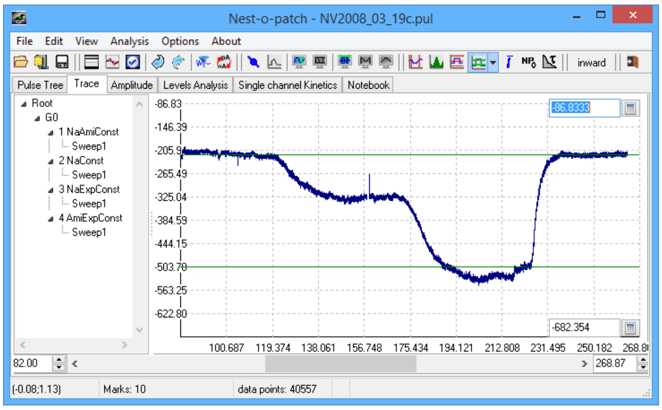


Figure 29. “Levels” tool were used to find the size of the second peak.

“Levels” were set at the current levels before and after application of the inhibitor. The Notebook contains following:

0 -217.38980
1 -503.69791
Single channel current: -286.30811
Levels: 1

Inhibitor-sensitive current is -286 pA (negative velue corresponds to the inward current).

Second, in many cases more accurate, technique is the use of “Mean current” tool ( ). This tool may be useful in two cases. First, for finding an IV plot. If a Trace was loaded in “overlaid sections” view, this tool generates a complete IV table (see Figure 30 and a following fragment of the Notebook).

As a result, Notebook contains following lines:

Alalysis range: 0.493 to 0.626. Length: 0.133.

Group 0; Series 1.

Sweep	Holding	Current	
0	-100.00	-781.58	(Here first column is sweep number)
1	-80.00	-592.54	(Second – holding potential)
2	-60.00	-438.96	(Third – current value, pA)
3	-40.00	-288.22	
4	-20.00	-137.70	
5	0.00	39.21	

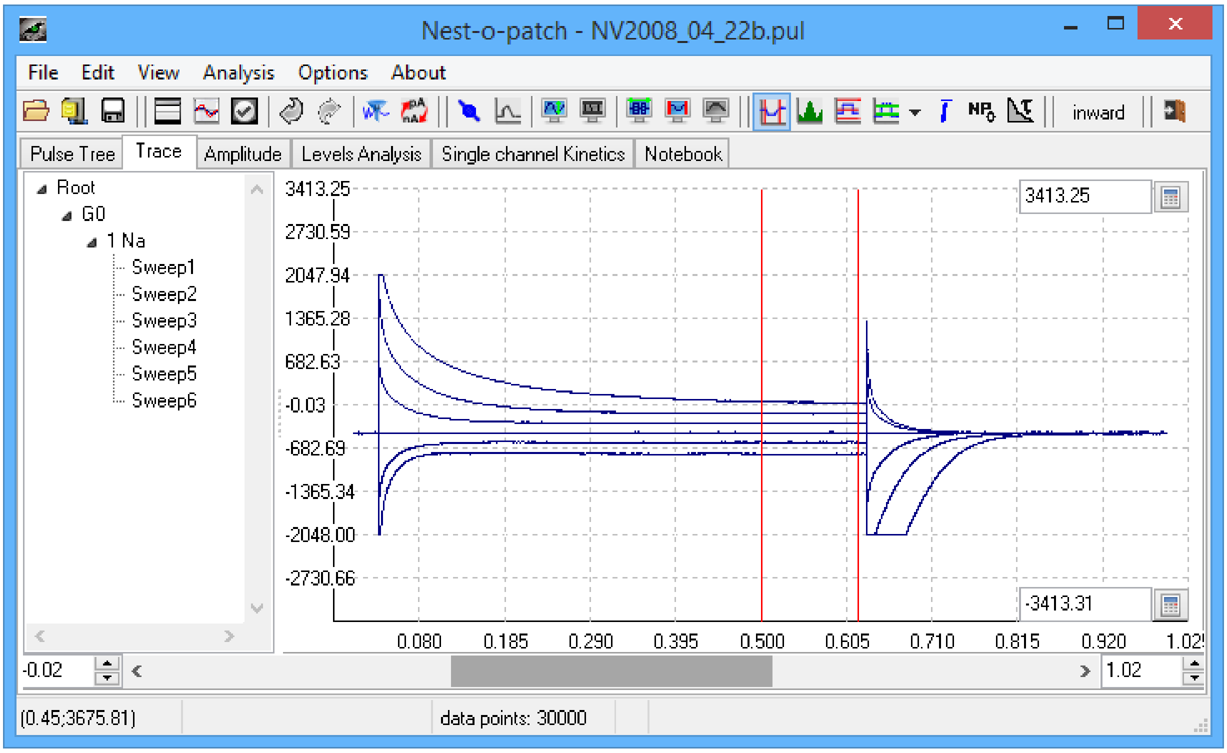


Figure 30. Time interval selected for the Voltage-Current analysis.

Second, this tool is indispensable when you have relatively small ensemble of channels, such that single channel events cannot be resolved, but channel noise is large enough, like shown in the Figure 31. Here, to measure effect of an inhibitor on the current value, mean current was measured in the absence of an inhibitor, then in the presence.

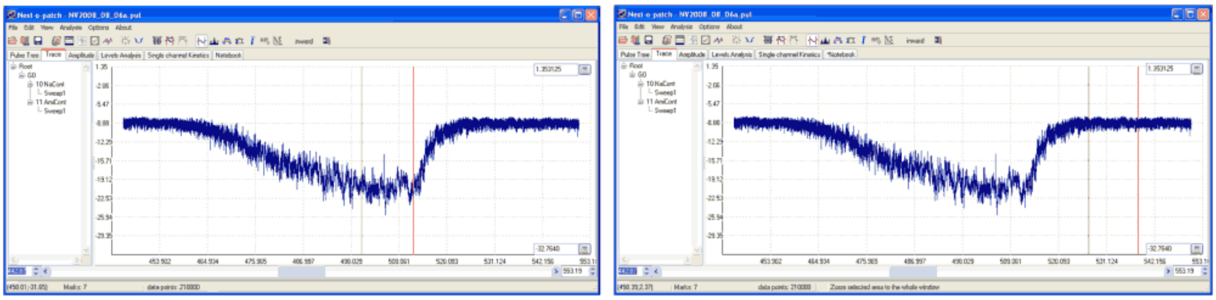


Figure 31. Using Mean current tool to measure value of an ensemble current. Left, plateau current in the absence of an inhibitor selected; right – in the presence. In both cases  button pressed.

Analysis range: 500.466 to 512.396
Group 0:Mean current: -20.58660
Alalysis range: 527.020 to 538.308
Group 0:Mean current: -8.88580

Difference is: -20.5866 – (-8.8858) = -11.7 pA.

Even easier way is to set one ("all closed") level and measure the mean current in an area of interest:

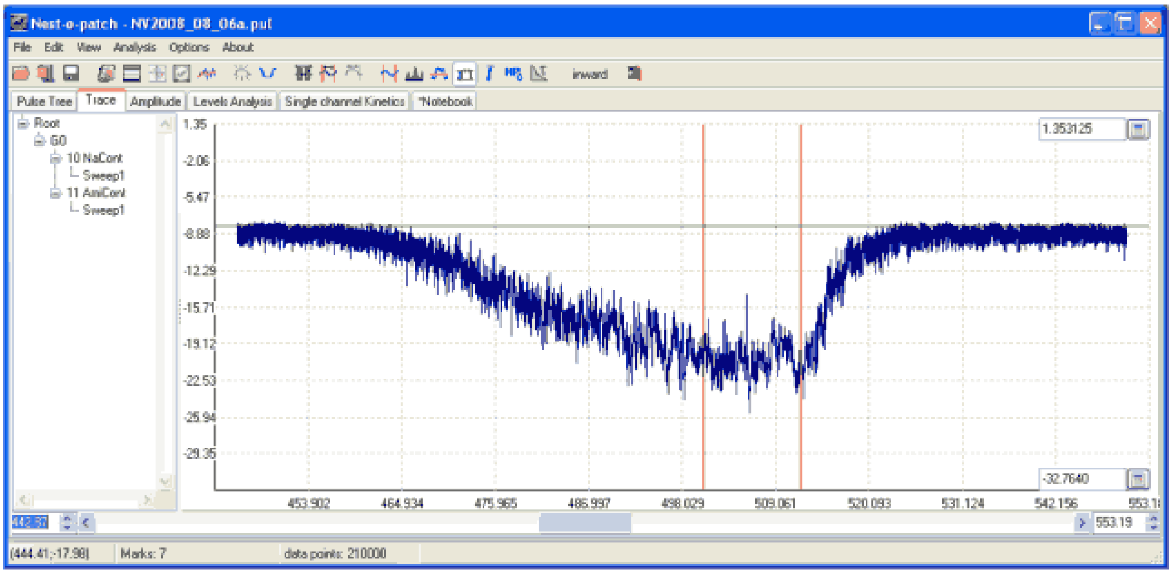


Figure 32. 'All closed" level defined, "Mean current" tool calculates current relative to this level.

This time the notebook contains:

Group 0:Mean current: -12.42336

Result is different from what we calculated before, because inhibition is not 100% and calculation of mean current in the presence of the inhibitor took into account this remaining channel activity.

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