
EDR4 - Online analyses

Revision History

Date	Version	Description
27/04/2022	1.2	Updated to software version 1.0.19
05/07/2021	1.1	Updated to software version 1.0.9
15/04/2021	1.0	First version of document



Analyses overview

EDR4 is capable of performing several real time analyses while the data is being acquired.

In particular EDR4 offers these predefined online analyses:

- RC estimation;
- Current histogram;
- Current events detection;
- Current spectrum;
- I/V graph;
- Noise report;
- Membrane monitor;
- Voltage automatic controller.

New analyses requests

It is possible to suggest new analyses to be added in the next software releases.

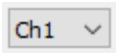


To do so write to support@elements-ic.com and provide:

- A brief explanation of the analysis;
- A minimum set of specifications (e.g., parameters settable by the user, useful noise reductions or spurious data rejection, etc.);
- A sample recording with expected analysis result (e.g., obtained with Python, Matlab, etc.);
- If available, any useful reference is very welcome (e.g., reference to paper/book explaining the analysis, code/pseudocode, etc.).










Analysis common features

Control buttons


Each online analysis has a dedicated widget, and different features, but there are some common controls that apply to most or of the analyses:

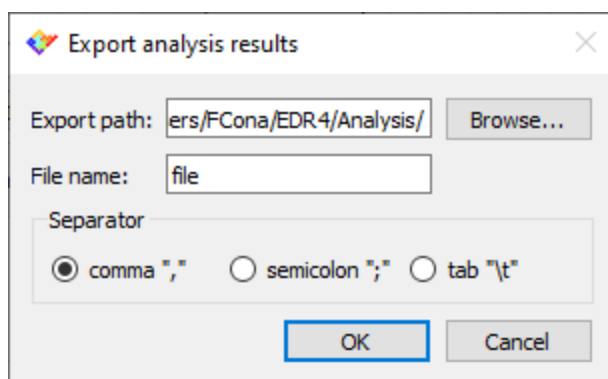
- The channel selector  selects the channel to be analyzed. Analysis that do not display this control usually apply to all channels at once;
- The start button  starts the analysis;
- The reset button  stops and restarts the analysis while resetting the current result;



- The stop button  stops the analysis;
- The lab book button  writes a note on the lab book with some highlights of the analysis results;
- The export analysis button  stores the analysis results in a .csv file;
- The region of interest button  allows the user to perform some actions only on a specific data interval. The exact behaviour depends on the specific analysis;
- The linear , gaussian  and sigmoidal  fit buttons allow the user to fit the analysis results with some basic models. The formula  and table  buttons allow to visualize the fitted models as formulae or tables.

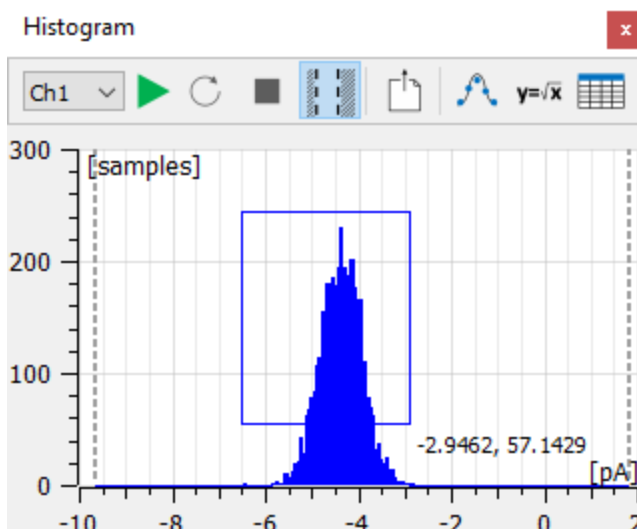
Analysis export

Most analyses' results can be exported to .csv files. In order to do so, click the export button . In the dialog window that will open browse for a location to save the .csv file, select a filename and select the value separator (the comma may create conflicts in some applications depending on your local machine configuration).

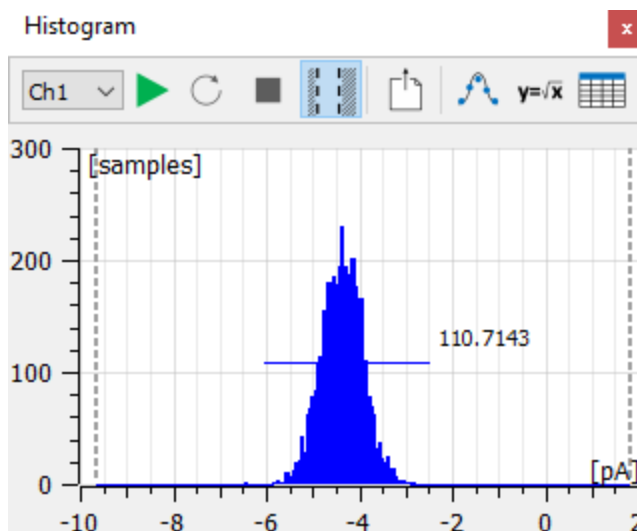


Analysis zoom

All the plots of the analyses can be zoomed. To do it simply left click in one corner of the rectangular area you want to zoom, drag the mouse cursor to the opposite corner of the area and finally release the left button of the mouse.



In order to zoom only along the x-axis or y-axis draw a thin rectangle, elongated along the horizontal or vertical axes respectively. When done properly the rectangle will become a horizontal or vertical line.



To go back to the previous zoom setting right click anywhere within the plot areas.

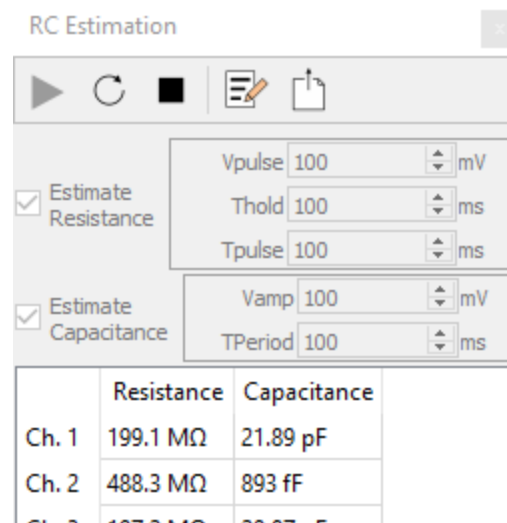


Analyses description

RC estimation

To use the RC estimator select the corresponding entry from the Analysis menu.

To estimate Resistance and Capacitance of the DUT (considering a simple RC parallel model) check both the checkboxes and click the start button. EDR4 will start to apply alternatively triangular waves and square wave protocols to estimate respectively capacitance and resistance values.



The parameters of the triangular wave and of square wave can be set independently of the parameters set in the voltage stimulus widget.

The algorithm estimates the Resistance ($R = \Delta V / \Delta I$) value considering the average current and voltage values.

Capacitance is estimated considering the equation $C = \Delta I / \Delta(dV/dt)$, so considering the current jump corresponding to the voltage slope change when the triangular wave changes direction.

When the “-” is shown instead of the estimated value it means that EDR4 algorithm cannot properly calculate, e.g. if the denominator of an equation is negative for resistance estimation. Some strategies to improve the signal quality when the RC estimation fails include:

- If the current noise amplitude is comparable to the current signal amplitude increase the protocol duration and reduce the sampling rate, in order to have the same amount of samples with lower noise, increase the protocol amplitude to increase the signal to noise ratio, and use a lower current range for a better signal resolution;
- If the current is saturating reduce the protocol amplitude and/or increase the current range.

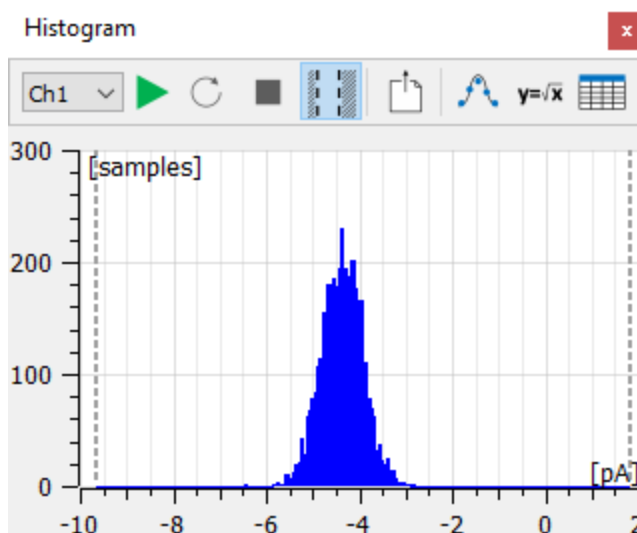


Current histogram

To compute a current histogram select the corresponding entry from the Analysis menu.

Select the current channel you want to apply the analysis to (for multichannel devices). The bin size is automatically computed so that 200 bins cover the whole current range. In alternative rag the vertical dashed lines to select a custom current range and click the “region of interest” button.

Click Start to actually perform the analysis.



Current event detection

To detect current events select the corresponding entry from the Analysis menu.

Select the current channel you want to apply the analysis to (for multichannel devices) and set the analysis' parameters in order to correctly detect the events you are interested in and reject spurious events, and click the “Apply parameters” button.

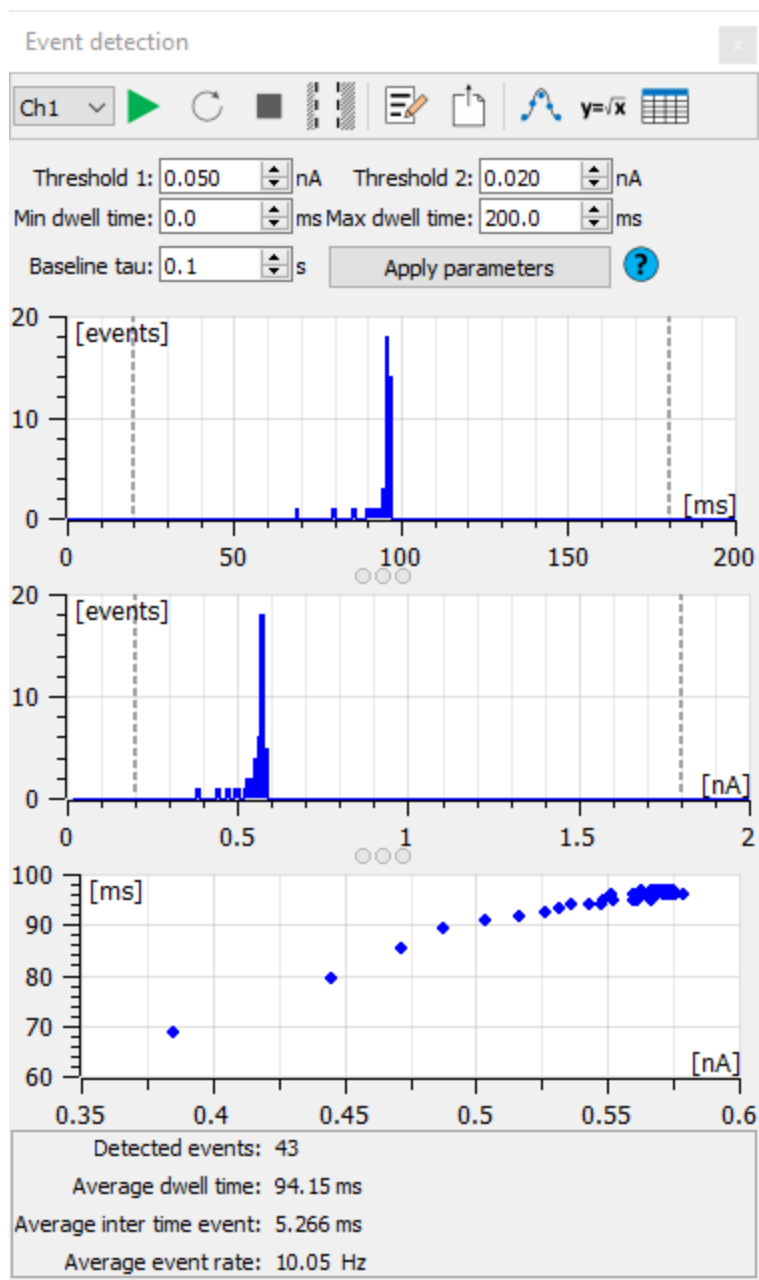
Click Start to actually perform the analysis. This analysis will track the signal baseline and identify as events those intervals that comply with the following rules:

- The event is higher than the baseline if Threshold1 is positive, lower otherwise;
- The event starts when the current deflects more than Threshold1 from the baseline;
- The event ends when the current deflects less than Threshold2 from the baseline;
- Events outside the duration range defined are rejected.

A summary of the analysis is always available as a tooltip by hovering the mouse over the “?”.



Detect current events. A valid event starts when the current surpasses Threshold 1 and then goes below Threshold 2 in a time in the range [Min dwell time; Max dwell time]. The thresholds are computed with respect to the current baseline. The baseline is tracked with a first order filter with time constant Baseline tau. Set Threshold 1 negative to detect negative events (below baseline).



The analysis result will include the following:

- A histogram of the duration of the detected events;

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- A histogram of the amplitude of the detected events
- A dwell time/amplitude scatter plot that shows the relation between the duration and the mean amplitude of each event;
- A report including the average inter event time, the average event duration and the average events rate.

A gaussian fit of the histograms can be performed. Drag the vertical dashed lines and click the “Region of interest” button to fit only the selected portion of the histogram.

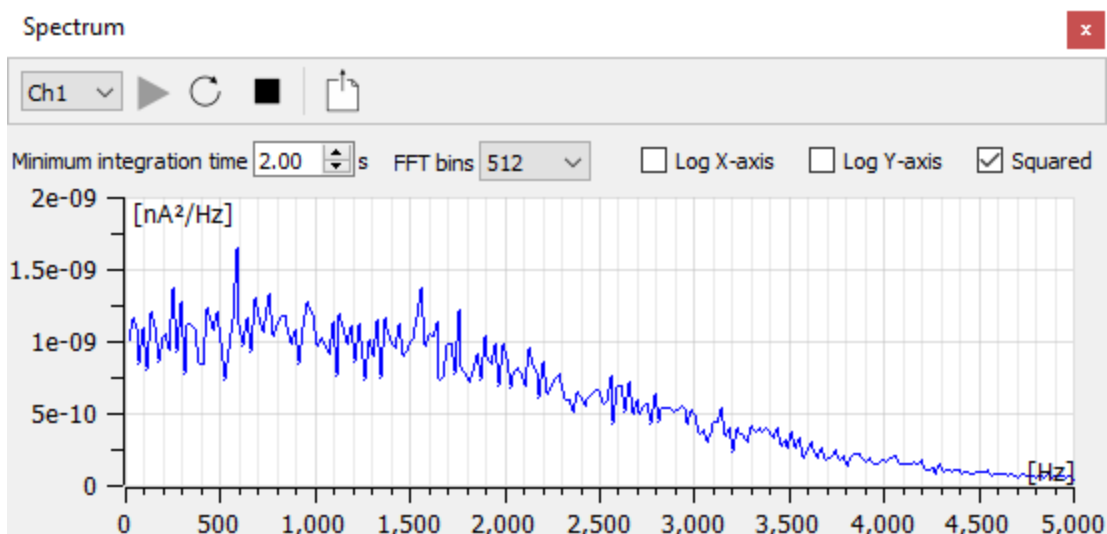
Current spectrum

To compute a current spectrum select the corresponding entry from the Analysis menu.

Select the current channel you want to apply the analysis to (for multichannel devices), the minimum integration period and the number of frequency bins, i.e. the number of points between 0Hz and SR/2: increasing the number of bins increases the resolution and the variance of the spectrum.

Click Start to actually perform the analysis. The spectrum is computed by computing the FFT over windows of N bins current samples, keeping only the first half of the spectrum. The FFT are collected for a duration greater than “minimum integration time” and finally the root mean square of the modulus of the computed FFTs is used as an estimate.

This analysis can optionally visualize the power spectrum or set logarithmic axis.



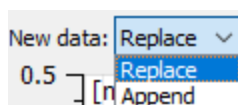
I/V graph

To compute an I/V graph select the corresponding entry from the Analysis menu.

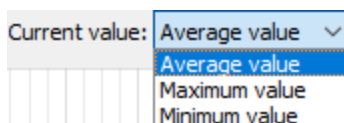


Select the current channel you want to apply the analysis to (for multichannel devices). Set the “Ignore transient” time to eventually neglect capacitive peaks located at the beginning of voltage steps.

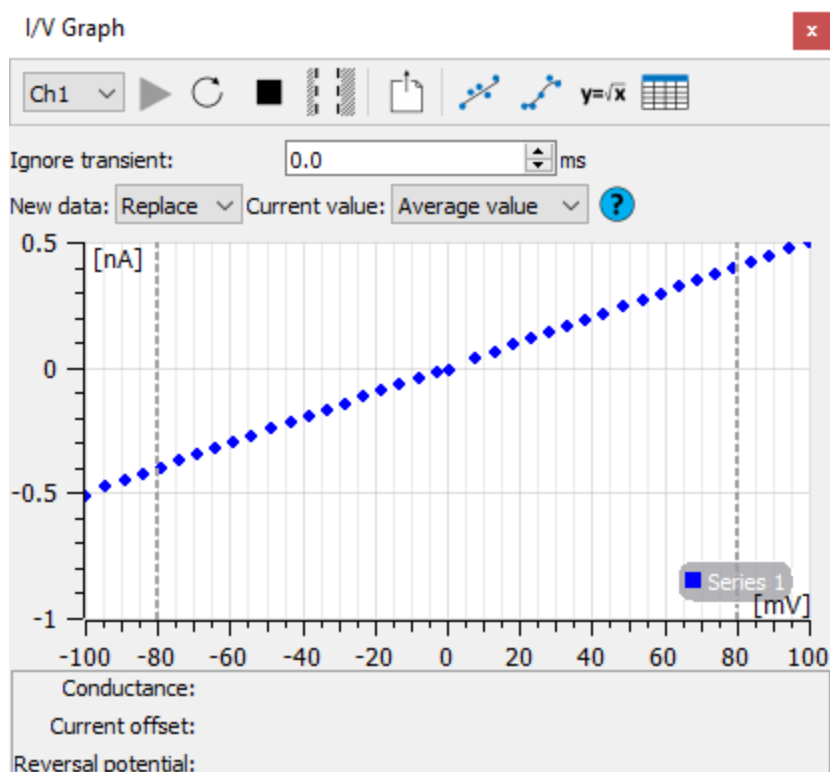
The “New data” combobox can be used to select if data coming from subsequent analysis should be replaced or appended.



The “Current value” combobox can be used to select how the current value of the I/V graph should be computed.



Start a voltage protocol (e.g. Conductance estimation protocol or a Ramp protocol to have a varying voltage) and click the start button to actually perform the analysis.



Clicking the linear or sigmoidal fit buttons a fit of the I/V graph will be performed. Drag the vertical dashed lines and click the “Region of interest button” in order to fit only the points within

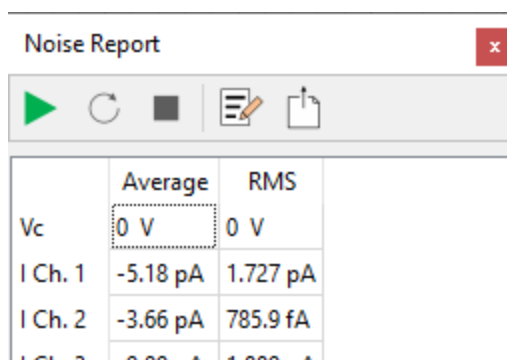


the interval identified. Performing the linear fit provides also a brief report including the estimated conductance, the current offset and the reverse potential.

Noise report

To have an indication of the noise and offset values for every channel select the corresponding entry from the Analysis menu.

The analysis will compute the offset and the RMS (root mean square) of all of the available current channels.

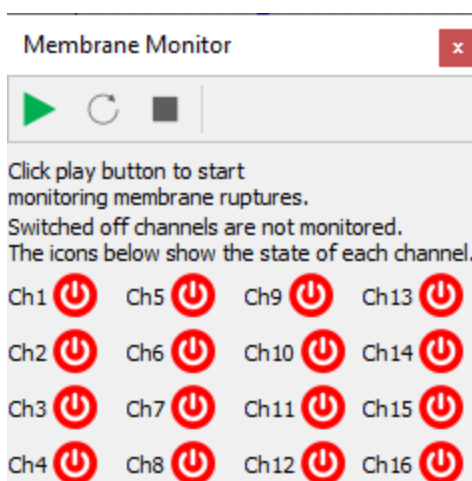


	Average	RMS
Vc	0 V	0 V
I Ch. 1	-5.18 pA	1.727 pA
I Ch. 2	-3.66 pA	785.9 fA
I Ch. 3	0.00 pA	1.000 pA

Membrane monitor

To use the membrane monitor select the corresponding entry from the Analysis menu.




This analysis can be used to automatically switch off saturating current channels when lipid bilayer membranes break. This can be particularly useful in multichannel devices, where a saturating channel can induce interferences on nearby channels, so switching it off as soon as possible can preserve the quality of data acquired in channels connected to intact membranes.



The control has an icon for each current channel. Once the start button is pressed all channels are monitored to detect persisting saturation.

The icons can change in 3 different ways:



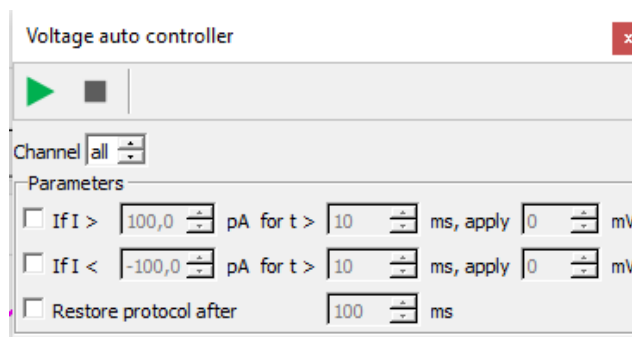
-  Switched on channel: the current channel is working normally and the “Membrane monitor” control is not enabled, so only the user can turn the channels on and off;
-  Monitored channel: the current channel is working normally and the “Membrane monitor” control is enabled, so it can turn channels off if they saturate (the user can switch the channels on and off as well from the controls in the plot window);
-  Switched off channel: the current channel is switched off, so no potential is applied to it and it won't be recorded if a recording starts.


The meaning of the icons can be quickly checked by hovering the mouse over them.

NOTE: Since switched off channels are not recorded, so channels that are switched off before a recording starts can be switched back on before the recording ends, but they won't be recorded nonetheless.

Voltage automatic controller

The “Voltage Automatic Controller” control can be used to automatically apply a specific voltage when the current exceeds a given threshold.



The Channel field can be used to select a specific current channel to monitor, or it can be set to  in order to monitor all channels.

It is possible to set 2 distinct conditions with 2 different voltage levels. The first condition allows setting a specific voltage level if the current gets higher than a given threshold for more than a given amount of time. The time threshold is used to reject spurious current events, but on the other hand it can be set to 0 in order to catch even the shortest current spike above the selected current threshold. The second condition works the same exact way, but it is used to catch events where the current gets lower than a specific threshold. The checkboxes on the left of the conditions must be checked to enable either of the two conditions monitoring.

NOTE: If both conditions monitoring are enabled it is possible to create an unstable oscillation, i.e. if condition 1 sets a low voltage that triggers condition 2, which in turn sets a high voltage that triggers condition 1, and so on.



NOTE: Since the control is performed by the software the application of the selected voltage is not instantaneous, but it can take 50-100ms from the current event to the corresponding voltage application. This delay can increase at sampling rates lower than 10kHz.

The voltage automatically applied by the “Voltage Automatic Controller” control is kept indefinitely, until the user applies another voltage protocol, even if the control is turned off with the stop button. However, it is possible to automatically restore the voltage protocol that was performed before the control intervenes. This can be done by ticking the checkbox in the last row: in this case the voltage protocol being performed is restored after the automatic voltage application, when a given amount of time has elapsed. This feature can be useful in some situations, for example for nanopore declogging: the automatic control can be used to apply a very negative voltage to unclog the nanopore when a low current is detected for a prolonged time, and then the experimental condition can be restored without any intervention of the user.