MED64 Offline Toolkit Manual

1. Introduction

The MED64 Offline Toolkit (MOT) software is designed to supplement the offline analysis feature of Mobius, a MED64 system control software program. MOT consists of multiple independent analysis tools, and files with a unique file format exported by Mobius are entered into MOT. Files with a file format unique to MED64-Entry are also compatible with MOT, and MOT is positioned as the standard software for data analysis for the users of MED64-Entry. User requests and feedback are reflected in the analysis tools and we continuously add new tools and improve features. The latest version can be downloaded from our website (https://alphamedsci.com/english/download/).

1.1. Recommended computer operating environment

The recommended computer operating environment is as follows.

OS   **: Windows 10 64 bit (32-bit version is not supported.)**
CPU   **: Intel Core i7 equivalent or better**
RAM   **: 16 GB**
Space required   **: 64 MB**
Display resolution : 1280 x 1024 or more

1.2. Installing and upgrading

MOT can be installed by double clicking the MOT installer. After installation, start MOT from the start menu to open the launcher window, and select an analysis tool you are going to use from the Application pull-down menu. Paid tools have a padlock symbol and require activation after a 45-day demo period following the installation. If you have already purchased an MOT license, Mobius Spike Sorter, or QT package, please inform us your hardware serial number, which can be found on the Activation window by selecting Activation... under Help on the menu bar. We will send you an applicable key file. Free tools can be used without activation even after the demo period.
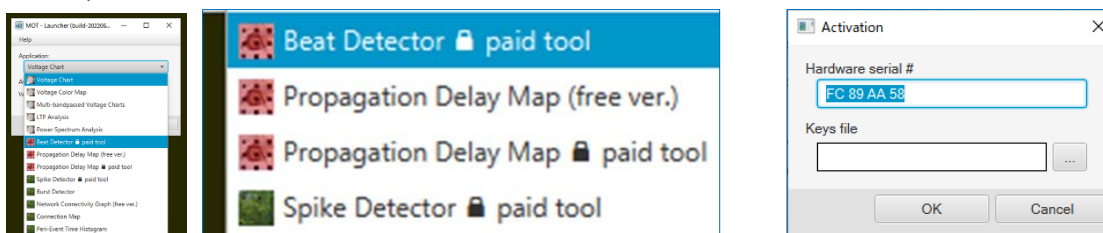
If you have already installed and activated the 20201211 or earlier version of MOT, please upgrade your MOT by following the steps below:
1) Move the activation file from the location shown below to the desktop or another location:
   C/Users/Username/AppData/Local/MED64 Offline Toolkit/app
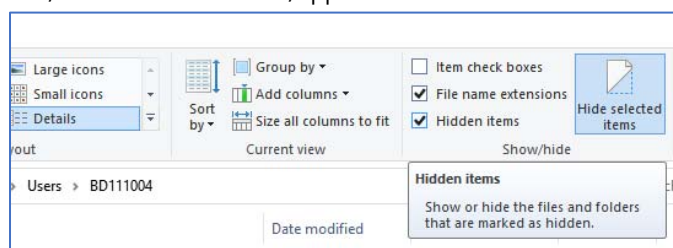
AppData is a hidden item and must be displayed by checking the "Hidden items" checkbox in the "View" tab of the folder settings.

2) Select and delete MED64 Offline Toolkit in Programs and Features on the Control Panel, and delete the MED64 Offline Toolkit folder in "C/Users/Username/AppData/Local/."

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3) Install the new version of MOT and move the activation file from step 1 to the folder shown below:

C/Users/Username/AppData/Local/MED64 Offline Toolkit/app
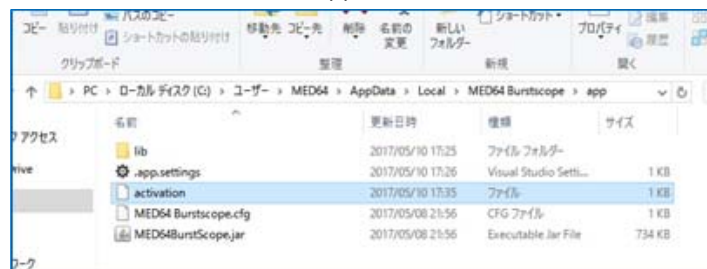
If the activation file is sent as an attached file, it will be named "activation.dat" with the extension.

You can not activate the Peakmap as it is, so please delete the extension ".dat" and move it to the app folder.

1.3. Starting up

1) Start MED64 Offline Toolkit by selecting the toolkit in the AlphaMED Scientific folder under the Start menu.
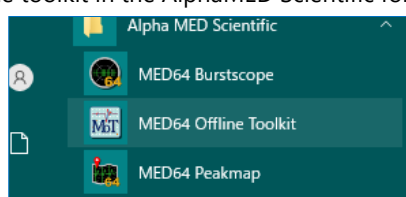
2) After starting up the launcher for selecting an analysis tool, specify the analysis tool you are going to use and the window size, and then click Start. To quit, click Exit.
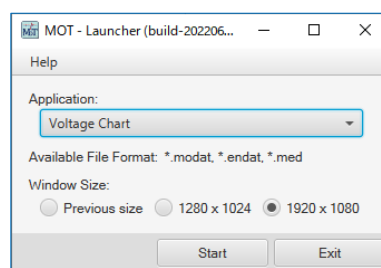
1.4. Last one memory design

MOT saves the setting information that the user specified and/or selected before quitting, and the settings are called and applied at the subsequent activation.

1.5. Change allocation of memory used

MED64 Offline Toolkit is configured by default to use up to 6 GB of memory (assuming a 8 GB PC, leaving 2 GB free for other processing). If you have a PC with sufficient memory size, you can increase the limit to improve processing speed.

Open the MED64 Peakmap.cfg file in a text editor at
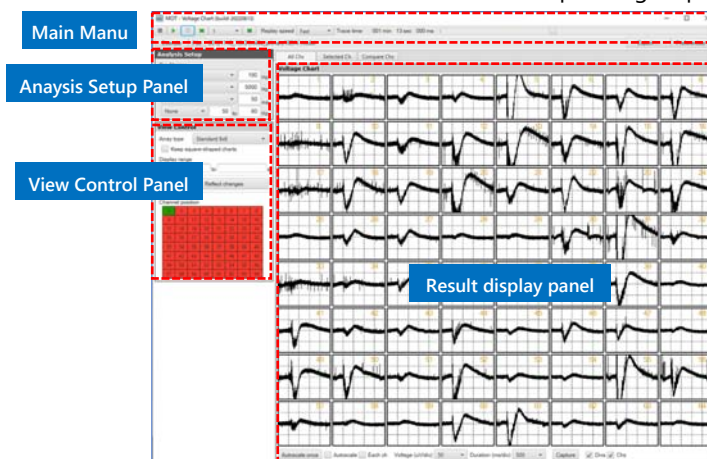
C/user/user name/AppData/Local/MED64 Peakmap/app

Correct the below (e.g., Xmx6000m → Xmx12000m for 6 → 12 GB) and save it to change the memory used.

[JVMOptions]
-Xmx6000m

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1.6. Basics of analysis tools

The analysis tools that constitute MOT have a common screen structure and operating steps.

1.6.1. Main Menu and Analysis Setup panel

1) Click the File button and select the data file to be analyzed. 2) Set the analysis conditions on the Analysis Setup panel. 3) Start the analysis process by clicking the Execute button. Once the process is completed, the analysis results are shown on the result display panels. While there are some exceptions, these three steps are the basic operation of MOT. You can replay the results in a time chart format with time on the horizontal line using buttons: ▶ (Play), ■ (Stop), and ‖ (Pause). For a data file that consists of multiple traces, change the trace number for which you wish to display the results using the Trace button. Some analysis tools provide an Export button to export result files and a Preference button to customize the appearance of graphs and charts.
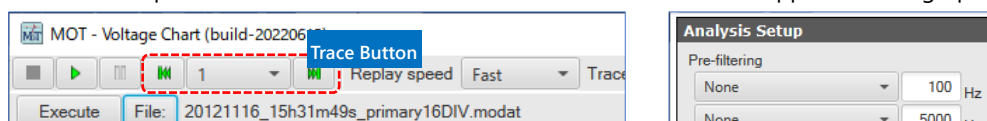

Main menu (left) and Analysis Setup panel (right).

The colors of the title bar and background of the Analysis Setup panel are different from other panels.

1.6.2. View Control panel

After the analysis process is completed, specify the electrodes, time range, and other parameters to display the results on the result display panel. Some analysis tools do not have this panel and the contents of the panel are different depending on the analysis tool. Although you can change the settings on this panel after selecting a data file and before starting the analysis, this panel is basically used to make changes after the analysis process is completed.
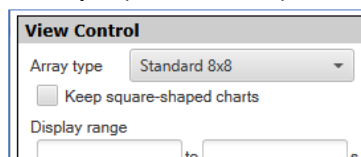
View Control panel in the Voltage Chart tool.

1.6.3. Result Display panel

Depending on the analysis tool, the panel name, panel type, number of panels, and panel features vary on the result display panel.
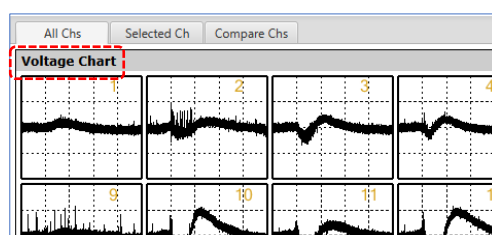

Voltage Chart panel in the Voltage Chart tool.

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Voltage Chart

1. Introduction

Voltage Chart is a dedicated tool for displaying waveforms of raw data. It can display longer data intervals than Mobius and has filtering and file conversion features. Voltage Chart can also export texts of numerical data within the displayed interval and display optional electrode comparison. The result display panel consists of three tab windows. The All Chs tab allows you to replay the waveforms in 64 electrode screens while the Selected Ch tab shows the waveforms of one electrode before and after filtering is applied. The display range for replaying the time axis on the All Chs tab is limited to a maximum of 5,000 ms/div due to limited memory space, but there is no limit on the display range for still waveforms, allowing you to view the entire length of the loaded data file. The Compare Chs tab displays comparison of waveforms of specified intervals for the electrodes selected on the Selected Ch tab in the 1×n electrode layout.
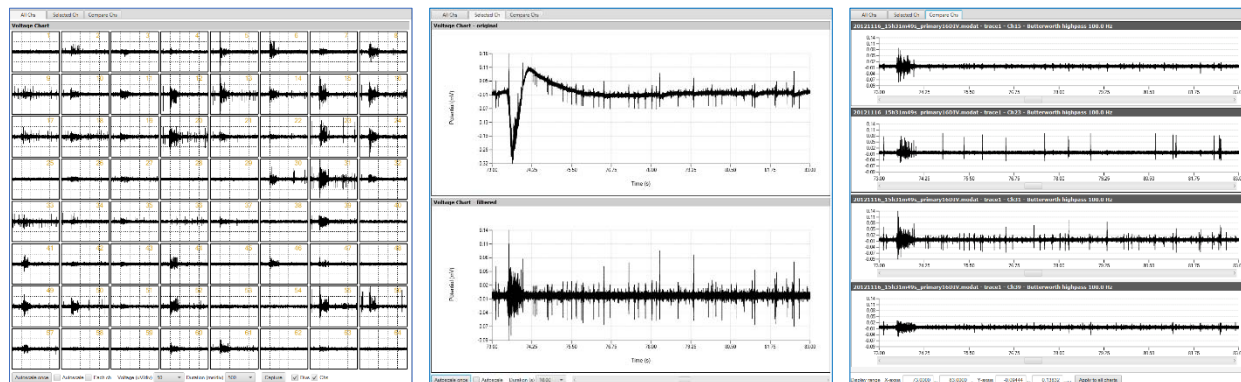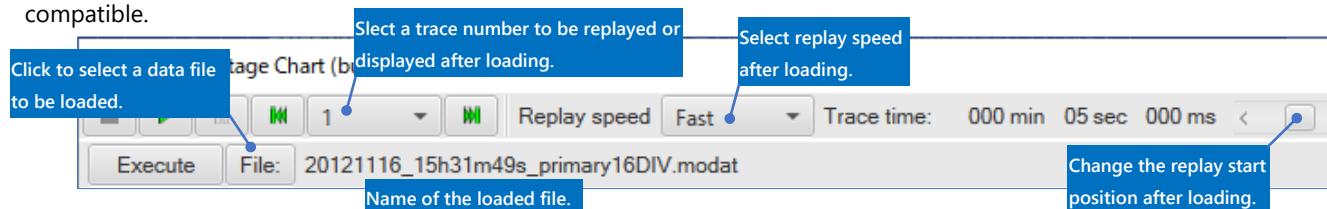

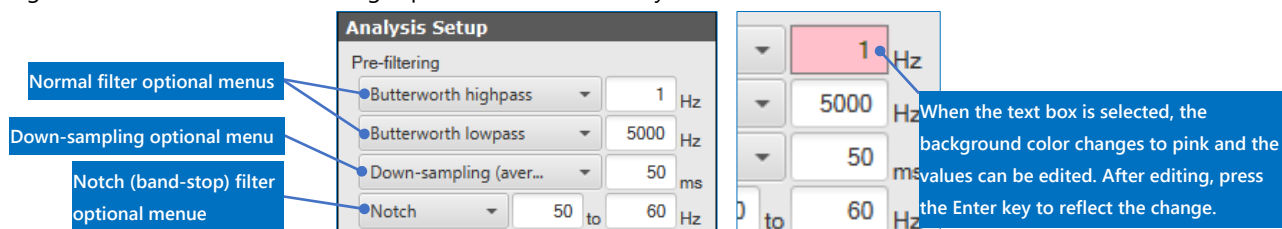**From left to right: All Chs, Selected Chs, and Compare Chs tabs.**

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible.

2.2. Analysis Setup panel

Apply filtering to the raw waveform to be displayed. Click the Execute button to start filtering. The same number of progress windows as the number of set filters are added and the process takes accordingly more time to finish. Filtering is always applied to the original data file and even if filtering is processed consecutively, a filtered data file cannot be re-filtered.

Down-sampling options

Down-sampling (average) or **Down-sampling (median):** This divides the time axis into the specified time windows and calculates the average or median in each interval to use it as the data point of the interval. For example, the data is a discrete value taken every 0.05 ms, so if you enter "50," it is 50 / 0.05 = 1,000, which indicates down-sampling of 20,000 Hz to 20 Hz.

Down-sampling: This divides the time axis into each time window of the specified data point (if 50 is entered, the time axis is divided at intervals of 2.5 ms) and replaces the data of the central time (the 25th point for 50) as the data point of the interval (for unprocessed modat file, it is down-sampling of 20,000 / 50 = 400 Hz).

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2.2. View Control panel

After the data file is loaded or filtered, you can change the settings for displaying waveforms on the View Control panel as needed. Use Array type to specify the format for displaying the electrode array on the All Chs tab, and select the Keep square-shaped charts option to display electrode charts in squares in the Standard 8×8 display. In Display range, you can specify any range for displaying still waveforms, and by enabling Show as a drop chart, you can display the charts with only data points, reducing the processing load related to plotting. For both, the changes will be reflected by clicking the Reflect changes button. In Channel position, you can specify the electrode numbers to be displayed on the Selected Ch tab.

The Rat hippocampal display (center) and the Standard 8×8 display with Keep square-shaped charts selected (right)
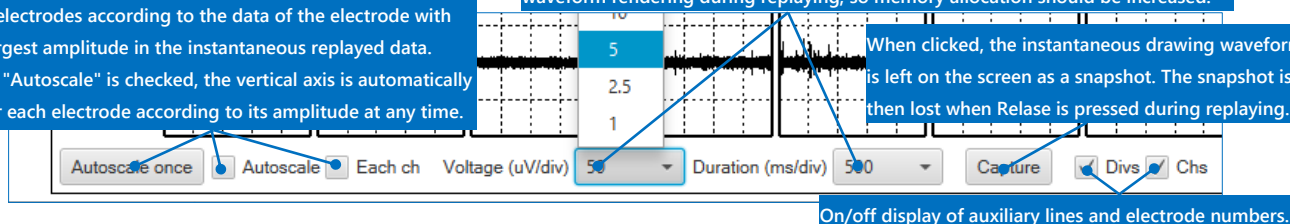
2.3. All Chs tab

After the data file is loaded or filtered, replay the data using the toolbar on the main menu. Settings for the display range and others are provided at the bottom part of the tab.

Clicking Autoscale once automatically ranges the vertical axis of all electrodes according to the data of the electrode with the largest amplitude in the instantaneous replayed data. When "Autoscale" is checked, the vertical axis is automatically set for each electrode according to its amplitude at any time.

Specify the display range of Y-axis (Voltage) and X-axis (Duration) in one section separated by auxiliary lines. 2500 and 5000 ms/div require a large load for waveform rendering during replaying, so memory allocation should be increased.

When clicked, the instantaneous drawing waveform is left on the screen as a snapshot. The snapshot is then lost when Relase is pressed during replaying.

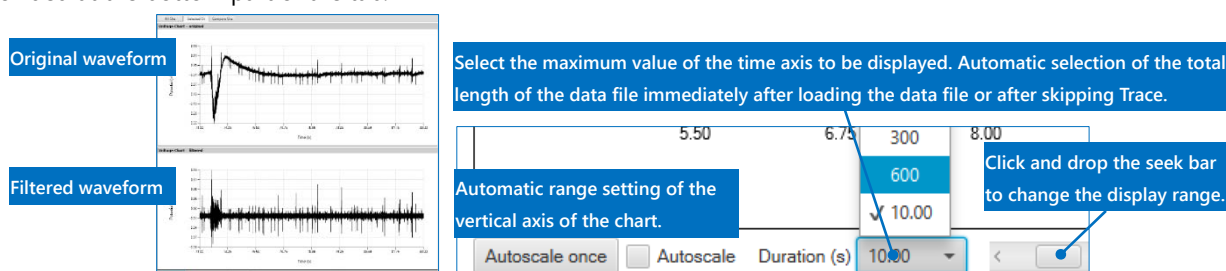On/off display of auxiliary lines and electrode numbers.

Right click on the All Chs tab to see the right-click menu for saving an image of the 64-electrode screen, and select Capture Image to save the 64-electrode screen without the toolbar in the clipboard.
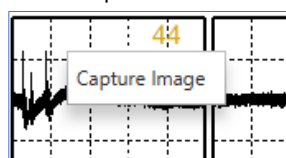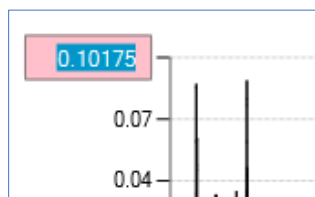

2.4. Selected Ch tab

After the data file is loaded, click an electrode number in Channel position on the View Control panel to display the entire length of the waveform for the data file of the corresponding electrode. The raw waveform before filtering is displayed on the top and the waveform after filtering is displayed on the bottom. The settings for specifying the display range for vertical and horizontal axes are provided at the bottom part of the tab.

Original waveform

Filtered waveform

Select the maximum value of the time axis to be displayed. Automatic selection of the total length of the data file immediately after loading the data file or after skipping Trace.

Automatic range setting of the vertical axis of the chart.

Click and drop the seek bar to change the display range.
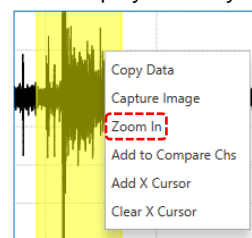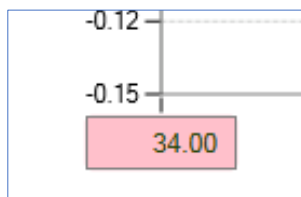
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In addition to specifying a value for the x-axis in Duration (s) on the toolbar, you can change the display range by clicking the maximum or minimum value on the numerical axis to directly edit the range. Furthermore, specifying a range with a drag-and-drop adds Zoom in option in the right-click menu that appears by right-clicking on a chart, and selecting Zoom in enables you to enlarge the specified range for the x-axis. After that, you can select Zoom out to return to the display before you zoomed in.

After clicking and editing the numerical axis, press the Enter key.　　The right-click menu of the Selected Ch tab.

Other right-click menu items

Copy Data: Saves the numerical data of the displayed interval to the clipboard. You can paste the saved data in Excel and other spreadsheets.

Capture Image: Saves the chart panel as an image to the clipboard. You can paste the image in Paint and other image processing software.

Add to Compare Chs: Inserts the selected charts to the Compare Chs tab.

Add X measure: Displays a cursor that shows the x-axis time information on charts. You can move the cursor by dragging and dropping the numerical value of the cursor position.

Clear X measure: Clears the displayed cursor.

2.5. Compare Chs tab

This tab displays the comparison of charts selected in Add to Compare Chs on the right-click menu of the Selected Ch tab. The added charts remain even after the data file is changed, so you can compare charts in different data files. The information of the added data (file name - trace number - electrode number - filtered or not filtered) is displayed on the title bar.
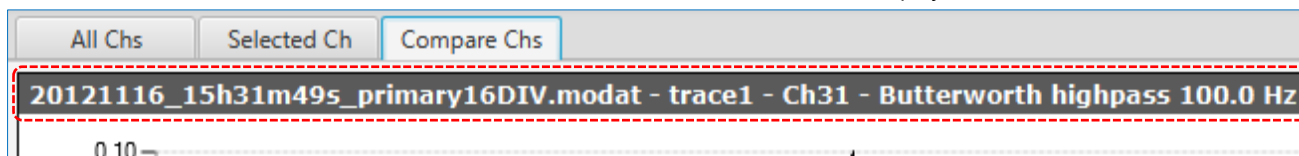
You cannot change the individual axis of each chart. Axes can only be changed together using the settings at the bottom part of the tab.
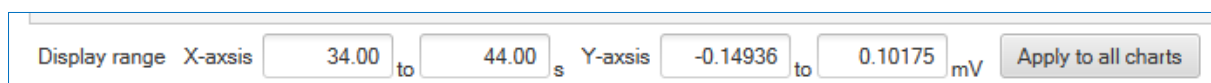

You can also save images, delete inserted charts, and switch image positions using the right-click menu.
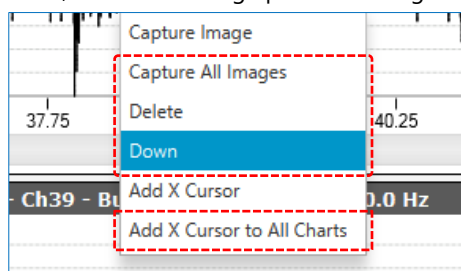
2.6. File export

Filtered waveforms can be exported in modat format (64 chs) or csv format. Click Export... at the upper right of the main window to display the Export window, specify a folder to export to, and then click Export to export the file.
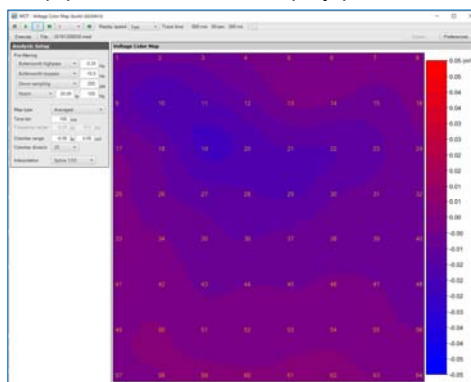
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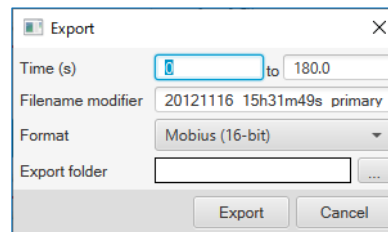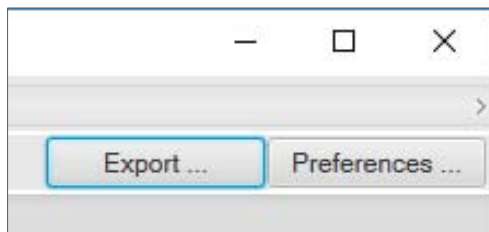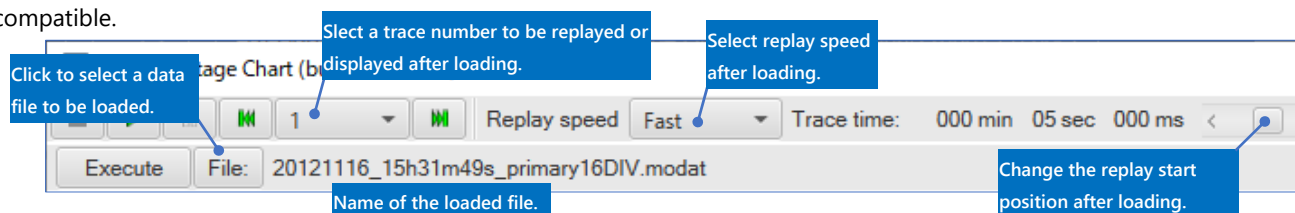
Voltage Color Map

1. Introduction

Voltage Color Map is a tool that expresses voltage levels of raw data as a color map. The distance to the signal source and density vary by electrode in MEA, so the obtained voltage levels change. For that reason, expressing a signal that is traveling from one electrode to another in a color map requires standardizing the voltage level of each electrode. This tool standardizes voltage levels using linear spectral in any frequency band, based on a report by Iwata N et al. (Biosens. Bioelectron., 94, 312–320, 2017). The main window of this tool consists of an Analysis Setup panel and a result display panel, which shows a Voltage Color Map.
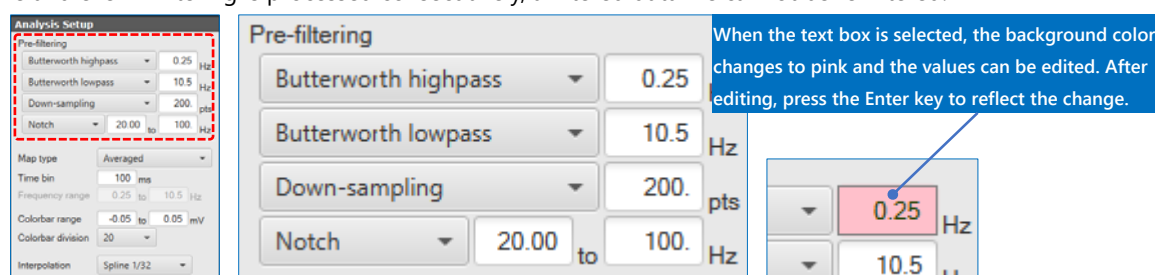

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible.

Click to select a data file to be loaded.

Slect a trace number to be replayed or displayed after loading.

Select replay speed after loading.

Name of the loaded file.

Change the replay start position after loading.

2.2. Analysis Setup panel

Apply filtering to the raw waveform to be displayed. Click the Execute button to start filtering. The same number of progress windows as the number of set filters are added and the process takes accordingly more time to finish. Filtering is always applied to the original data file and even if filtering is processed consecutively, a filtered data file cannot be re-filtered.

When the text box is selected, the background color changes to pink and the values can be edited. After editing, press the Enter key to reflect the change.

Down-sampling options

Down-sampling (average) or **Down-sampling (median):** This divides the time axis into the specified time windows and calculates the average or median in each interval to use it as the data point of the interval. For example, the data is a discrete value taken every 0.05 ms, so if you enter "50," it is 50 / 0.05 = 1,000, which indicates down-sampling of 20,000 Hz to 20 Hz.
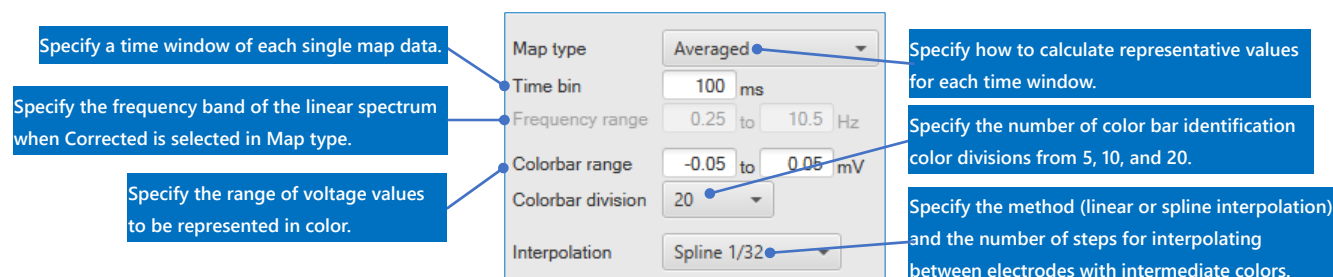
Down-sampling: This divides the time axis into each time window of the specified data point (if 50 is entered, the time axis is divided at intervals of 2.5 ms) and replaces the data of the central time (the 25th point for 50) as the data point of the interval (for unprocessed modat file, it is down-sampling of 20,000 / 50 = 400 Hz).

After the data file is loaded, specify the display conditions of the color map. The map type can be specified as Averaged, Integrated, or Corrected. With Averaged, the average voltage level of each interval specified in Time bin is calculated; with

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Integrated, the total is calculated and the resulting values are expressed in color; and with Corrected, the value corrected using the following formula is expressed in color based on the total linear spectral in the frequency band specified in Frequency range.

$$Average\ voltage\ in\ the\ interval\ of\ the\ electrode \times \frac{Average\ potential\ in\ total\ linear\ spectral\ in\ the\ specified\ frequency\ band\ of\ all\ electrodes}{Total\ linear\ spectral\ in\ the\ specified\ frequency\ band\ of\ the\ relevant\ electrode}$$

With the FFT size for calculating linear spectral being 8192 (fixed), after applying the short-time Fourier transform in the entire length of the trace, the resulting average of each spectral frequency is applied to the equation above.

Specify a time window of each single map data.

Specify the frequency band of the linear spectrum when Corrected is selected in Map type.

Specify the range of voltage values to be represented in color.

Specify how to calculate representative values for each time window.

Specify the number of color bar identification color divisions from 5, 10, and 20.

Specify the method (linear or spline interpolation) and the number of steps for interpolating between electrodes with intermediate colors.

Map type	Averaged
Time bin	100 ms
Frequency range	0.25 to 10.5 Hz
Colorbar range	-0.05 to 0.05 mV
Colorbar division	20
Interpolation	Spline 1/32

After setting conditions, click Execute to generate a color map. To export the results after modifying the conditions, click Execute each time to reprocess.

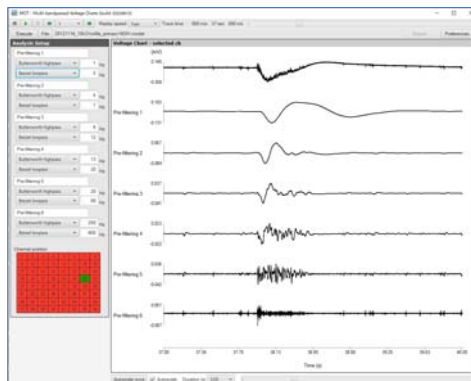## 2.3. Voltage Color Map panel

This panel displays the results based on the analysis conditions set on the Analysis Settings panel. Click the play button on the main menu to display color maps consecutively at the specified play speed. By selecting Capture Image from the right-click menu, you can save the color map of that moment to the clipboard and paste the map in an image processing software program. When MED64-Entry data file is loaded, you can select the corresponding electrode array from Array Type. You can also select View Line Chart to display the value of each time window as a chart, instead of a color map.

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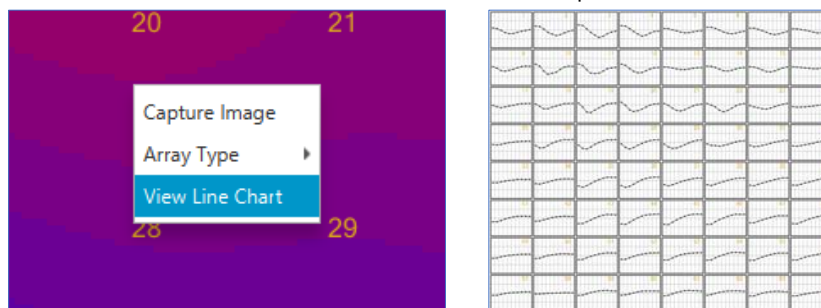
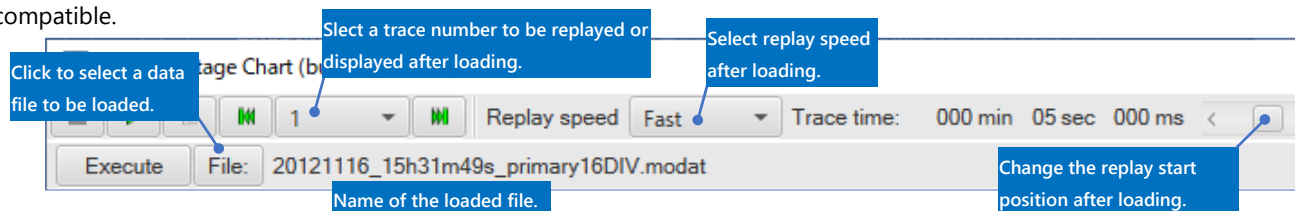# Multi-bandpassed Voltage Charts

1. Introduction

Multi-bandpassed Voltage Charts is a tool to display up to six filtered waveforms for one data file simultaneously for comparison. Filtering is a high-load process, but by narrowing the number of electrodes to be analyzed to one, you can simultaneously compare waveforms that have been filtered in multiple different bandwidths. The main window consists of an Analysis Setup panel and a result display panel, which shows a Voltage Color Map.


2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible.

Slect a trace number to be replayed or displayed after loading.

Select replay speed after loading.

Click to select a data file to be loaded.

Change the replay start position after loading.

Name of the loaded file.

2.2. Analysis Setup panel

Up to six patterns of filtering can be applied. The electrode numbers to be filtered should be selected in Channel position before starting. Click the Execute button to start filtering. The same number of progress windows as the number of set filters appear and the process takes more time accordingly. Filtering is always applied to the original data file, and even if filtering is processed consecutively, a filtered data file cannot be re-filtered.
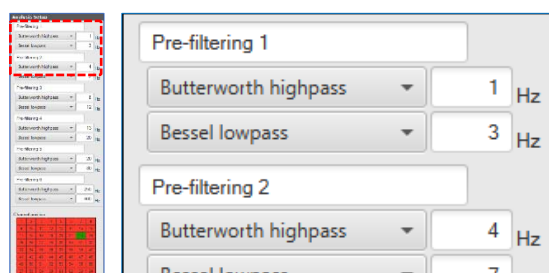
### 2.3. Voltage Chart – selected ch panel

You can copy the displayed waveform image to the clipboard by selecting Capture Image from the right-click menu. The display ranges of the vertical axes of individual waveform charts and the common horizontal axis can be changed by clicking the maximum or minimum value to directly edit the range. The settings for the display range are also provided at the bottom part of the panel.

Select the maximum value of the time axis to be displayed. Automatic selection of the total length of the data file immediately after loading the data file or after skipping Trace.

Automatic range setting of the vertical axis of the chart.

Click and drop the seek bar to change the display range.

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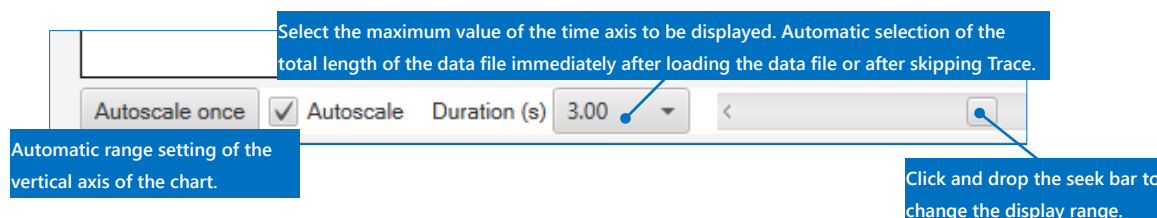
LTP Analysis

1. Introduction

LTP Analysis is an analysis tool that measures the amplitude and the falling slope in the specified range for evoked potential waveforms of hippocampal slice, and displays the measurements on a time chart. Data files that have repeated tens to hundreds of traces where 100-ms data is acquired for each trace are expected to be used for analysis, and the values calculated for each trace are plotted as a time chart. The main window consists of a View Control panel and a result display panel, which shows a Raw Waveform Chart and a Measurement Chart.
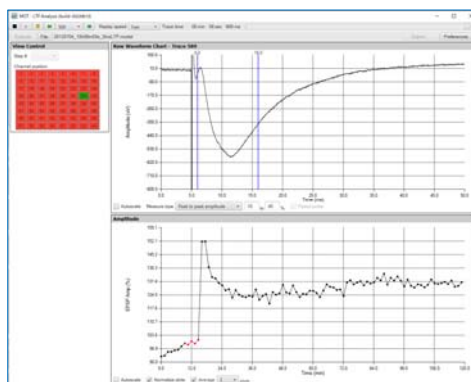
2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible. Set the measurements to be calculated on the Raw Waveform Chart panel and click ▶ (Play) to replay the results on the Measurement Chart panel.
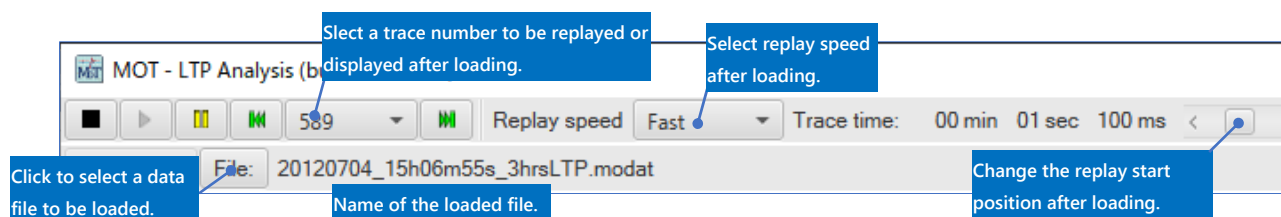
2.2. Raw Waveform Chart panel

This panel displays an evoked potential waveform for each trace and allows you to specify the measure type and the range of the calculation.
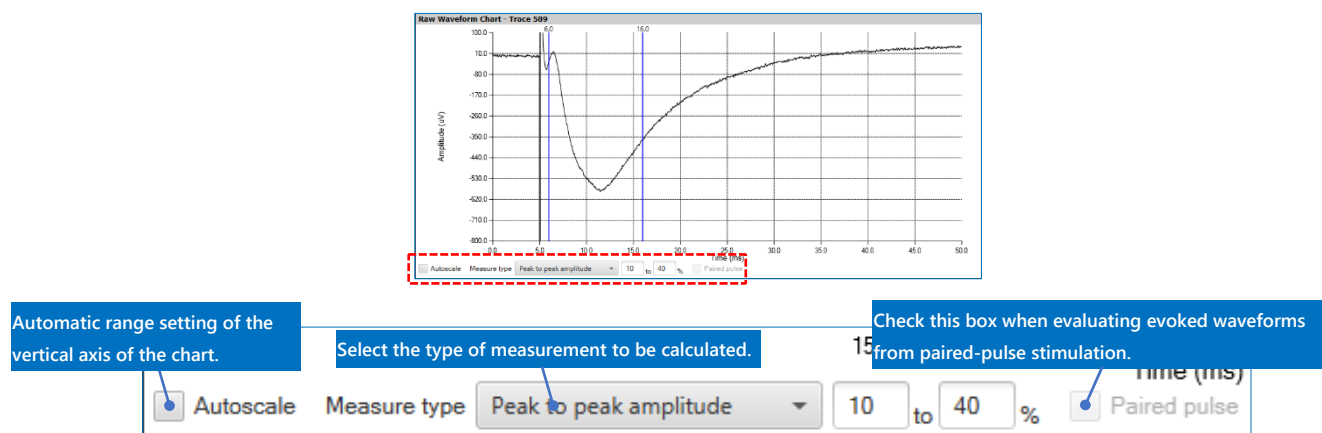
<u>Measure type</u>

Peak to peak amplitude: Calculates the peak-to-peak amplitude between two cursors.

Slope: Calculates the slope of a tangent for the data points between the maximum and minimum values within two cursors using the method of least squares.

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Pop spike amplitude: Finds the intersection of a straight line that passes the maximum value of section 1 (S1) and 2 (S2) and the vertical line that passes the minimum value of section 2, and calculates the amplitude between the intersection and the minimum value of scope 2.

Input-Output curve: Calculates the slope of a tangent of the data points between the minimum value of section 1 (S1), and the maximum and minimum values of section 2 (S2) using the method of least squares.
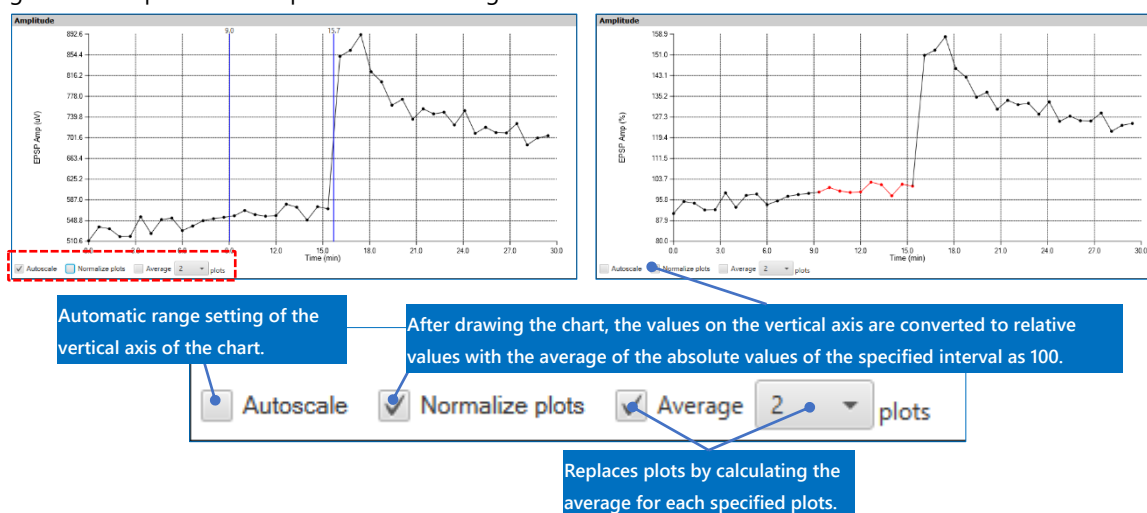

Peak to peak amplitude | Slope | Pop spike amplitude | Input-Output curve
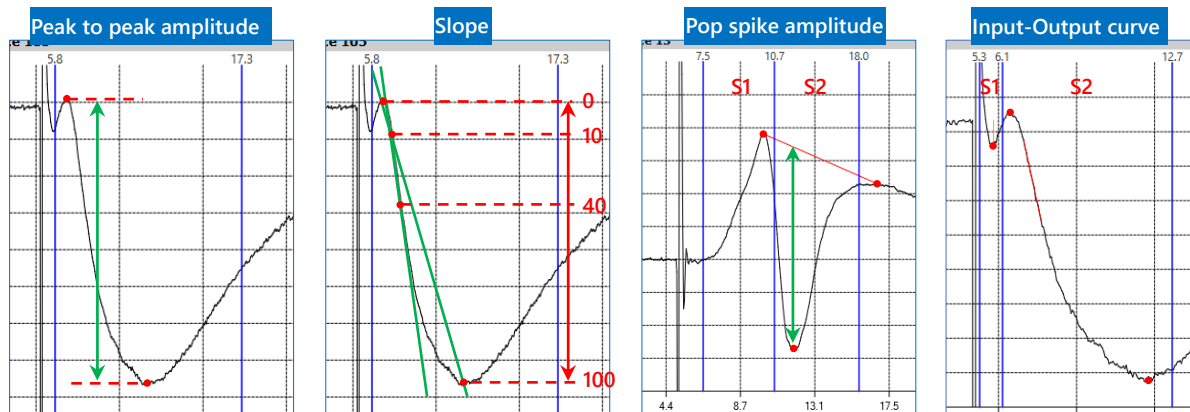
You can copy the image or the numerical data of the raw waveform in the display range to the clipboard from the right-click menu.
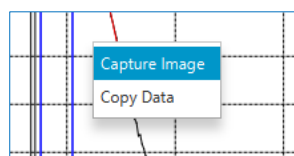
2.3. Measurement Chart panel (Amplitude、Slope、Pop Spike Amplitude、Input-Output Curve)

The measurements calculated on the Raw Waveform Chart panel is displayed as a time chart with the measurements on the vertical axis and time on the horizontal axis. The Input-Output curve is displayed as a chart with the slope on the vertical axis and the amplitude on the horizontal axis. Similar to the Raw Waveform Chart panel, the image or the numerical data of the chart in the display range can be copied to the clipboard from the right-click menu.

Automatic range setting of the vertical axis of the chart.

After drawing the chart, the values on the vertical axis are converted to relative values with the average of the absolute values of the specified interval as 100.

Replaces plots by calculating the average for each specified plots.

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Power Spectrum Analysis

1. Introduction

Power Spectrum Analysis is a tool that applies the short-time Fourier transform to raw data waveforms to generate a power spectrum density (PSD) graph for each electrode, and displays its spectrogram. It offers an optional post hoc analysis, which calculates the total and maximum value of the obtained PSD within the specified frequency range. The main window consists of an Analysis Setup panel, a View Control panel, and three result display panels: Power Spectrum Density, Spectrogram, and Post Hoc Analysis.
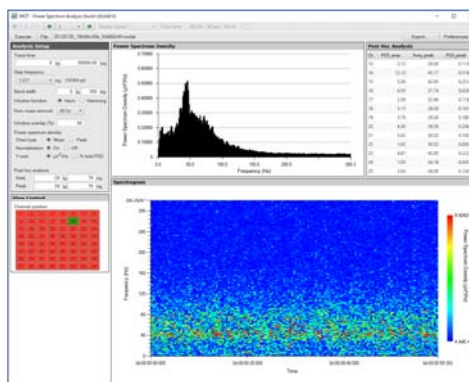
2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible. Replays of raw waveform charts and results are not available with this tool.
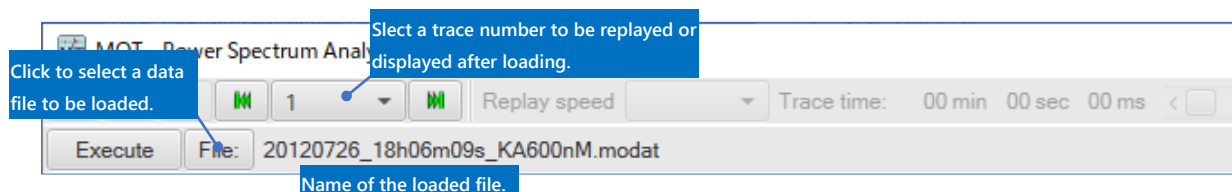
2.2. Analysis Setup panel

After the data file is loaded, set the analysis conditions. Select a text box by clicking on the text box. When the background color turns pink, it means that the text box is available for editing. After editing, press the Enter key to reflect the change (the background color turns back to white).
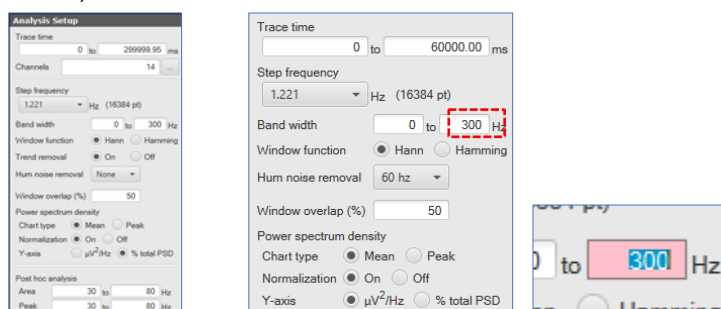
Changes are not reflected until the Enter key is pressed after editing.

Analysis conditions

Trace time (ms): Allows you to specify the interval of the data file to be analyzed. The residual interval that cannot be divided with the FFT size at the end of the interval to be analyzed is excluded from the analysis.

Channels: Allows you to specify the electrodes of the data file to be analyzed. You can reduce the processing load by narrowing down the number of the electrodes.

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Step frequency (Hz): Allows you to specify the frequency resolution of the PSD to be obtained. In other words, this is the same as specifying any FFT size (FFT frame) between 2^5 and 2^{21}. A modat file that has not been downsampled is a discrete value for every 0.05 ms, so for example, for 2^{14} (16,384), the fundamental cycle is 16,384 × 0.05 = 819.2 ms, which means that 1.221 Hz is the fundamental frequency.

Band width (Hz): Allows you to specify the frequency band of PSD.

Window function: Allows you to specify a window function to apply to the FFT frame.

Hum nose removal: This is an option to remove 50 Hz or 60 Hz of power spectrum density.

Window overlap (%): Allows you to specify a rate to overlap an FFT frame with the adjacent FFT frame between 0% and 50%.

Power Spectrum Density

Chart type: Allows you to specify whether to display the average or the maximum value of the obtained PSD in each time window as the result on the PSD chart.
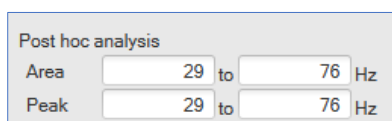
Normalization: Allows you to specify whether to normalize PSD or not. If On is selected, PSD would be the value that is divided by the square of the FFT size, facilitating the comparison of PSDs between data files with different sampling frequencies.

Y-axis: Allows you to specify the display format of PSD. When % of total PSD is selected, the PSD of each frequency bin is indicated as a percentage to all PSDs in the bandwidth specified in Band width (Hz) (On and Off for Normalization does not affect PSD values if the % of total PSD format is selected).

Note: This tool removes the trend where the value derived from applying linear regression (y = ax + b) to a set of data is subtracted from the data, and then applies the Fourier transform.

Post hoc analysis

Specifying the frequency range and conducting an analysis will automatically calculate the total PSD (Area), maximum PSD, and its frequency (Peak). When you enter an integer in the text box, it will be replaced with a multiple of the Step frequency (Hz) nearest to the integer.
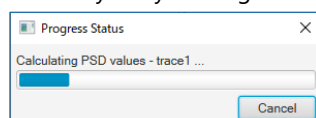

After specifying the analysis conditions, click Execute on the main menu to start the analysis. A progress bar that indicates the progress of the analysis appears, but you can stop the analysis by clicking Cancel.


2.3. Power Spectrum Density panel

After the analysis is complete, the power spectrogram of the electrodes selected in Channel position on the View Control panel is displayed. If the data file consists of multiple traces, click the Skip button on the main menu to change the trace to display the results. To change the display range, directly edit the maximum and minimum values of the axis or change the range on the PSD Chart Format tab on the Preferences window, which can be opened by selecting Modify PSD Chart from the right-click menu on the PSD chart.
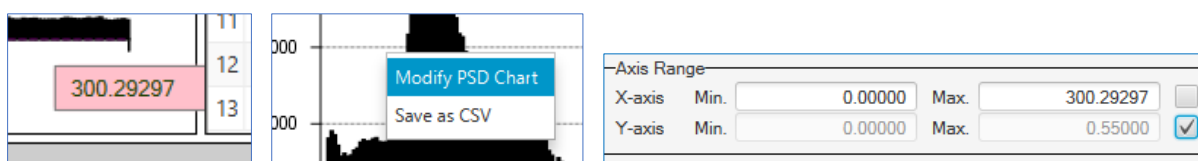
In addition, selecting Save As CSV from the right-click menu will export a csv file that shows the PSD summary of each FFT frame of the selected electrodes.

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FFT frame average of PSD for each frequency

Maximum PSD value in the all FFT frames for each frequency

PSD for each frequency in the 1st, 2nd, 3rd ... th FFT frame

2.4. Spectrogram panel

After the analysis is complete, the spectrogram of the electrodes selected in Channel position on the View Control panel is displayed. If the data file consists of multiple traces, click the Skip button on the pull-down menu or on the toolbar to change the trace to display the results. To change the display range, directly edit the maximum and minimum values of the axis or change the range on the Spectrogram Format tab on the Preferences window, which can be opened by selecting Modify PSD Chart from the right-click menu.
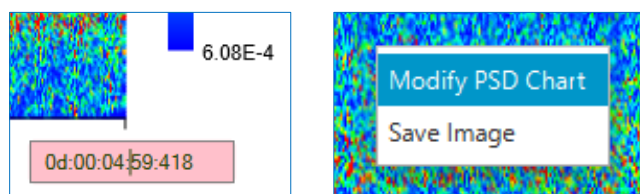
In addition, selecting Save Image from the right-click menu will export the currently displayed spectrogram to a file in emf, bmp, or png format.

Specifying colors

The shades of spectrograms indicate the size of PSD, and spectrograms are displayed in 256 shades of colors (0–255) by default. Selecting Auto in the PSD Range for Quantization field on the Spectrogram Format tab will automatically assign the minimum value (0) and the maximum value (255) from all PSDs obtained through the analysis. Selecting Show lines in PSD chart will also display dots that indicate the minimum and maximum values on the PSD chart.

When selected, the minimum and maximum values are automatically set based on the analysis result.

2.5. Batch export of analysis results

You can batch export csv files and image files using the right-click menu on the PSD chart or the spectrogram. To export files, select the target items on the Batch Export tab on the Preference window, click Export ... on the main menu to specify the export location, and click the Export button.

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Settings on the Batch Export tab

Export Trace: Allows you to specify traces to export the results. With Single, only the currently displayed trace is exported; with All (in each), all traces are exported to individual files; and with All (in one), all traces are exported to one file.

When All (in one) is specified, the results of the subsequent trace is listed following an empty row.

290	993.6523	2791398	30593784	35937.
291	994.873	2788417	31876025	11974.
292	996.0937	2782918	31886287	6716.3
293	997.3145	2733955	32438032	7671.2
294	998.5352	2725548	31769845	7974
295	999.7559	2714779	29077167	1982.8
296				
297	Frequecy	Power Spectrum Density		
298	(Hz)	(uV^2/Hz)		
299		mean	peak	
300	0	3.04E+08	3.17E+09	4.37E+
301	1.220703	5.41E+08	5.25E+09	1.16E+
302	2.441406	4.55E+08	8.25E+09	3.16E+
303	3.662109	1.7E+08	6E+09	83690
304	4.882812	1.06E+08	4.52E+09	613855
305	6.103516	82264091	3.11E+09	32278

Chart Image: This exports the spectrograms of the currently selected electrodes. You can specify emf, bmp, or png format. Even if All (in one) is specified for Export Trace, charts will be individually exported for each trace just like for All (in each). Additionally, the texts entered in the text boxes will be added to the end of the exported file name in the format of "Read file name + texts" (this is the same with Chart CSV).

Chart CSV: Exports a csv file that shows the PSD summary of each FFT frame of the selected electrodes.

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# Discrete Wavelet Transform

1. Introduction

This applies the Gabor wavelet transform to raw waveforms. The main window consists of an Analysis Setup panel, a View Control panel, and two result display panels: All Chs and Selected Ch.
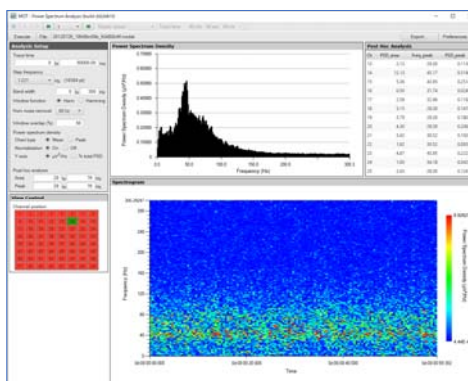

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible.
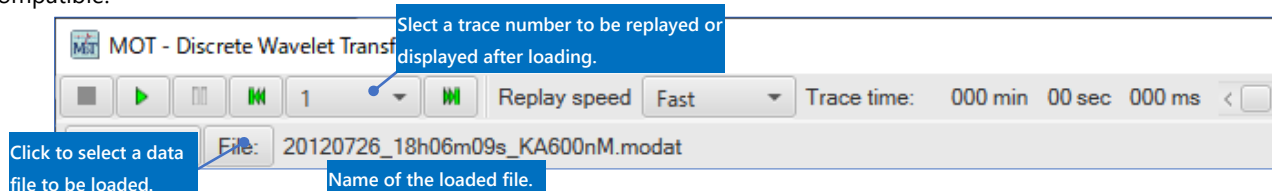
2.2. Analysis Setup panel

After the data file is loaded, set the analysis conditions. This computation takes a significantly high load, so it takes some time to complete the analysis. For that reason, the interval and electrodes to be analyzed should be narrowed down by specifying values in Trace time and Channels.

Note: Even with a PC that meets the recommended operating environment, the analysis requires at least 1 minute to process one electrode for 10 seconds only.
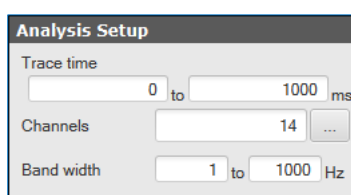
2.3. All Chs tab

After the data file is loaded or analyzed, replay the data using the toolbar on the main menu. You can use this as an auxiliary function when examining the potential waveforms and the electrodes to be analyzed.

2.4. Selected Chs tab

This tab displays the potential waveform chart of the electrodes selected in Channel position and a scalogram. The latter can only be displayed for the electrodes to be analyzed.

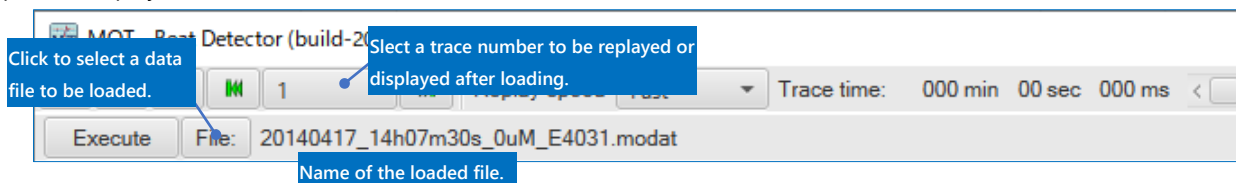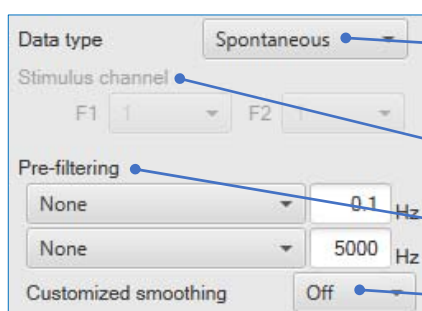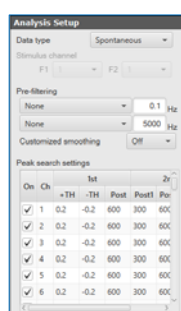アルファメッドサイエンティフィック株式会社
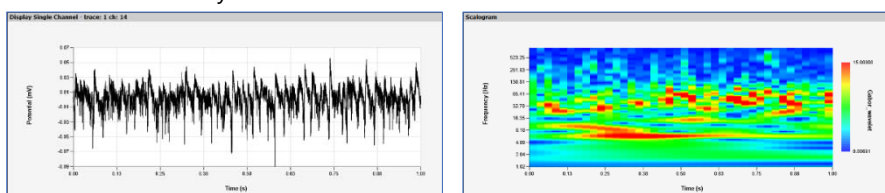
Beat Detector

1. Introduction

Beat Detector is a tool that detects the field potential waveforms for each pulse of myocardium culture specimen to calculate the field potential duration (FPD) and interspike interval (ISI). Mobius exports information concerning the detection time of the field potential waveforms as a timestamp file and you can use the file to generate an excitation propagation map in the Propagation Delay Map free ver. tool. The main window consists of an Analysis Setup panel, a View Control panel, and a result display panel. The result display panel provides the All Chs tab and the Selected Ch tab.

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat, endat, or med (Conductor files) formats are compatible. Replays of raw waveform charts and results are not available with this tool.

Click to select a data file to be loaded.

Slect a trace number to be replayed or displayed after loading.

Name of the loaded file.

2.2. Analysis Setup panel

After the data file is loaded, set the analysis conditions. Select a text box by clicking on the text box. When the background color turns pink, it means that the text box is available for editing. After editing, press the Enter key to reflect the change (the background color turns back to white).

The internal processing is different between autonomic beat and electrical pacing, so the data type needs to be selected.
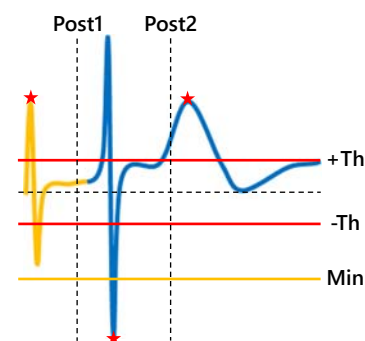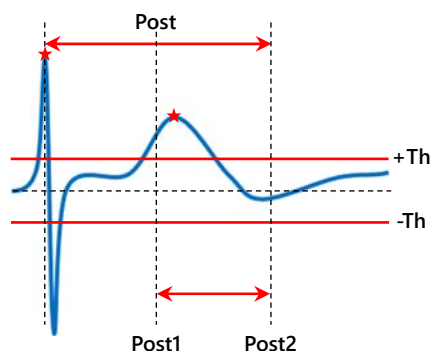
When Paced is specified for Data type, select the number of stimulated electrodes. If only one has been stimulated, specify the number for both F1 and F2.

Select the number of electrodes of the MED probe used.

Select On to search the peaks by flattening the waveforAms of MOT's unique algorithm.

Set the range to detect the first and second peaks in the Peak search settings. If Paced is specified for Data type, the settings are different from when Spontaneous is selected, with the waveform assumed to have stimulation artifacts immediately before the pacing response.

Algorithm when Spontaneous is specified

The peak that appears immediately after the intersection of the signal and the amplitude threshold (+ for +Th and - for -Th) is used as the first peak, and the next peak is not searched for a range between the first peak and the point specified as post 1. Starting from the first peak, the maximum value between posts 1 and 2 is recognized as the second peak.

Algorithm when Paced is specified

The peak that appears immediately after the intersection of the signal and the amplitude threshold (+ for +Th and - for -Th) is recognized as the artifact peak, and the - peak between posts 1 and 2 is searched. If the peak is deeper (smaller) than the minimum value, it is considered as the first peak. Starting from the first peak, the maximum value between posts 1 and 2 is recognized as the second peak.

After specifying the analysis conditions, click Execute on the main menu to start the analysis.

2.3. View Control panel

After the data file is loaded or filtered (after the progress window disappears), you can change the settings for displaying waveforms on the View Control panel as needed. Use the Array type to specify the format for displaying the electrode array on the All Chs tab, and the Channel position to specify the electrode numbers to display potential waveforms on the Voltage Chart panel on the Selected Ch tab.

2.4. All Chs tab

Similar to the Voltage Chart tool on the All Chs tab, this tab displays the potential waveforms of 64 electrodes. You can also replay the waveforms.

2.5. Selected Ch tab

The potential waveforms of the electrodes whose numbers have been selected in Channel position and a cursor at the peak point that was recognized through the analysis are displayed. Blue represents the position of the first peak and red the position of the second peak. Cursor number 1 is assigned to the peak point recognized last in all data intervals and subsequent numbers are assigned backwards. If the data fluctuates partially, causing the peak points to move outside the specified search range, you can change the cursor position to any location by clicking and dropping the number. When doing so, you can specify the range by clicking and dragging on the chart (highlighted in yellow) and then right-click on the number to modify the peak position to the maximum or minimum value of the signal in the specified range.

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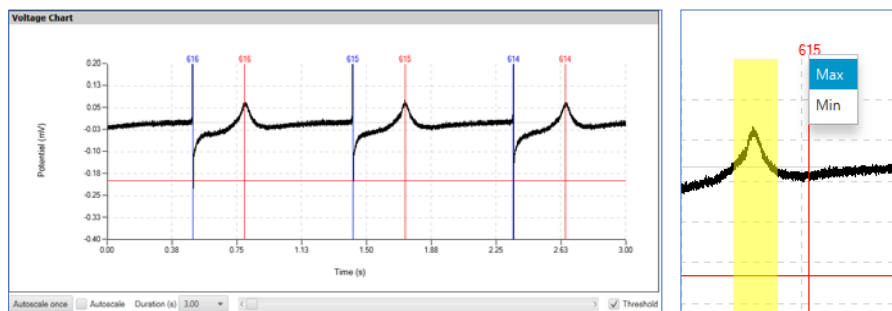
The Beat Parameters panel displays the FPD (time interval between the first and second peaks) and ISI (time interval between the first peak of the previous pulse and the first peak of the current pulse) calculated for all analyzed electrodes and all data intervals, based on the recognized peak position. When the peak position is changed on the Voltage Chart panel, FPD and ISI are also corrected instantaneously based on the changed peak position (highlighted in red). You can copy the numerical values to the clipboard from the right-click menu and paste them in Excel or other spreadsheet.

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Propagation Delay Map (free ver.)

1. Introduction

Propagation Delay Map (free ver.) displays color maps to show the propagation of electric excitation (myocardium FP waveform) via gap junctions for each pulse of myocardium culture specimen. Setting the first electrode that generates excitation among all electrodes as the standard, the difference in the timing of electric excitation generation (delayed time) among electrodes is calculated and displayed in a contour map. The observation point of the generation of electric excitation of each electrode in this tool is the peak time of the first spike of the myocardium FP waveform for each pulse, and the timestamp data file exported after detecting FP waveforms using Mobius can be used as an input file. In addition to autonomic beat, this tool displays a map of beats paced by an electrical pacing. In the latter, the latent time from when the peak point of the waveform appears as an artifact on each excited electrode to the peak time of the first spike is regarded as the difference in the propagation of excitation. Therefore, when using electric pacing data, the results of the latent time calculated using the Extract Spike Measures module and exported using the Save Measures Data module are used as the input file (for more details, see the Mobius tutorial).
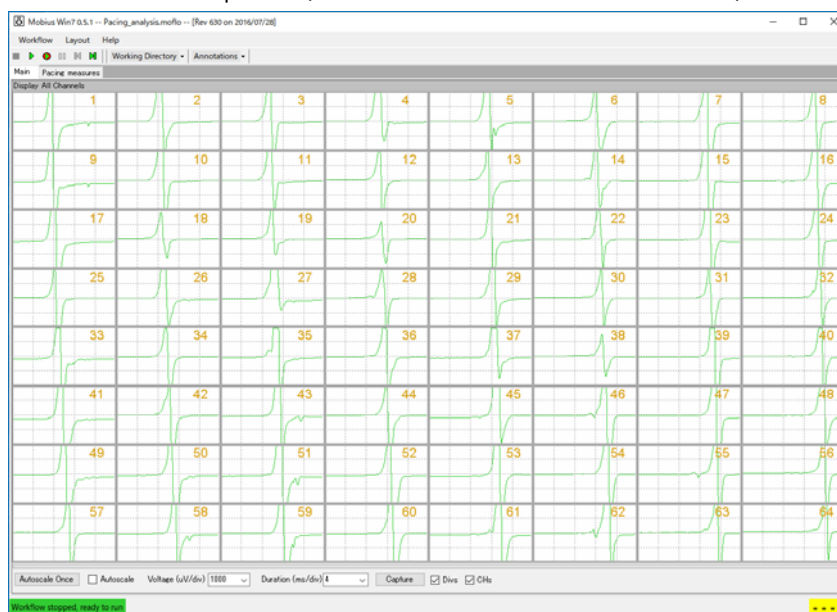

Autonomic beat data. The timing of the appearance of the first spike is gradually delayed from electrode 1 to electrode 64.
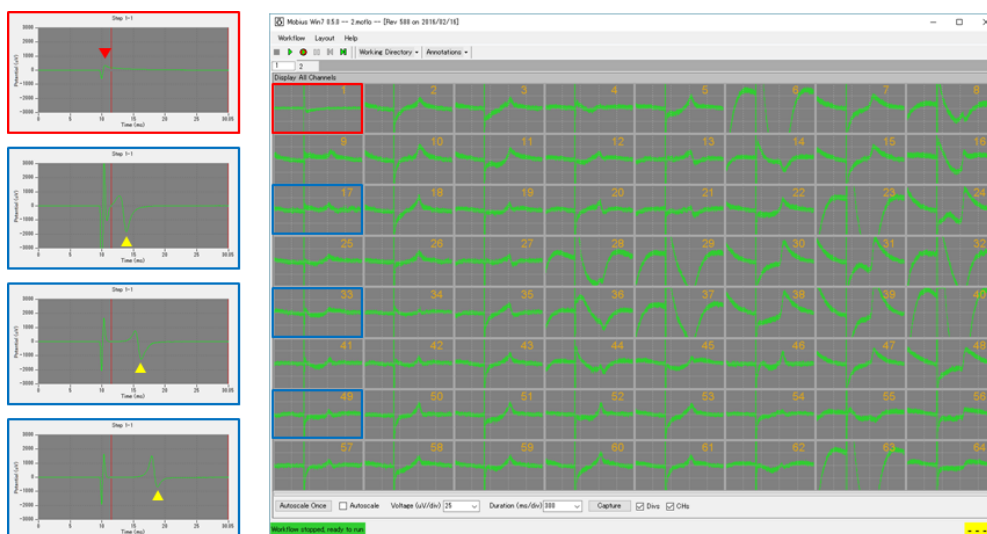
Electrical pacing data. The farther the electrode is, the more delayed the propagation of the excitation (the upward-pointing triangle in the blue box) generated by the stimulation to electrode 1 (the downward-pointing triangle in the red box) is.

The main window consists of an Analysis Setup panel and two result display panels: Propagation Delay Map and Propagation Velocity (um/ms).
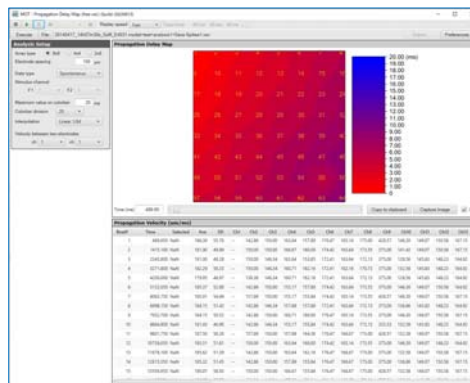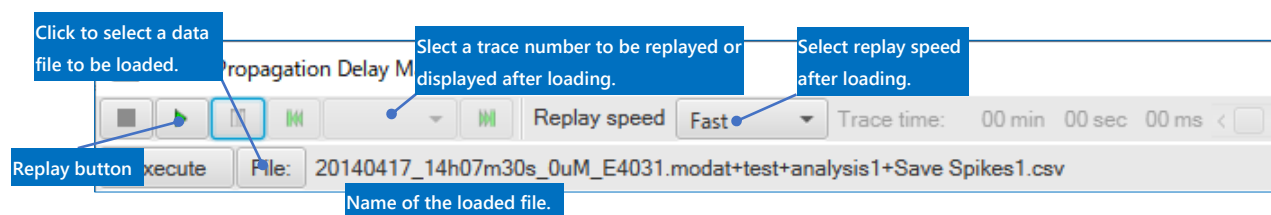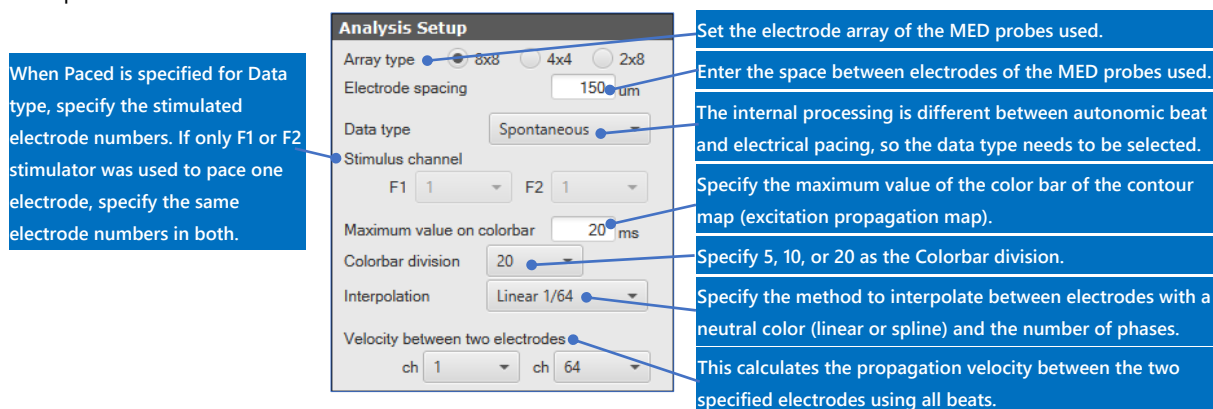
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2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in spike timestamp format (csv format), which is the unique format of Mobius, are compatible. After processing is done, click ▶ (Play) to replay the obtained excitation propagation maps.

2.2. Analysis Setup panel

After the data file is loaded, you can set the analysis conditions. Click Execute to generate an excitation propagation map and to calculate the propagation velocity. To export the results after exporting the results and modifying the conditions, click Execute each time to reprocess.

Internal algorithm when Spontaneous is selected

This algorithm searches an electrode that has the largest total number and a small electrode number from the list of timestamps (FP waveform detection time) contained in the file. Within that electrode, it searches the second timestamp in the range of 600 to 3,000 ms after the first timestamp, and then searches the subsequent timestamps in the same manner as an indication for beat timing. It searches the timestamps for other electrodes in the range of 500 ms before and after the timestamp for each beat in the single electrode obtained as a result, and sets the electrode with the first timestamp as the electrode that generates the first excitation (pacemaker site). This process is repeated for each beat to generate a propagation map.

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2.3. Propagation Delay Map panel

This panel displays an excitation propagation map for each detected beat. Click the Play button on the command bar to display the propagation map in phases at the specified play speed (in animation). Select two electrode numbers on the propagation map by left-clicking and right-clicking to display the propagation velocity calculated based on the time differences between the timestamps of the electrodes. By using the seek bar at the bottom of the panel, you can skip to a propagation map of any time. By clicking the Copy to clipboard button, you can save the time difference between the timestamps of the electrodes, which were used to generate the currently displayed propagation map, as numerical information to the clipboard and paste it in Excel or other spreadsheet. By clicking the Capture Image button, you can save the image to the clipboard and paste it in an image processing software program.

This displays the propagation velocity calculated between the two specified electrodes.

2.4. Propagation Velocity (um/ms) panel

This panel displays the propagation velocity for each beat. The propagation velocity of the electrode that generated excitation first will not be shown (it is indicated with "--"). By selecting Copy to clipboard from the right-click menu, you can save all numerical values on the table to the clipboard in csv format and paste them in Excel or other spreadsheet.

Electrode average and standard deviations of measures

The beat numbers are shown in ascending order (from the end of the data interval).

In this example, ch 1 generated excitation first. The first peak search time of ch 1 was set as the standard and the time difference between that and other electrodes were calculated, so ch 1 (red box) does not show any data.
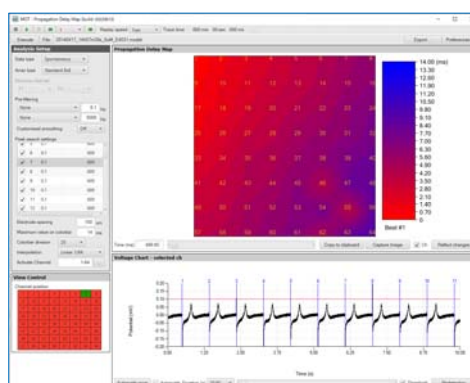
Select Copy Velocities in the Currently Displayed Map to save the calculated propagation velocity of the combination of all electrodes in the currently displayed propagation map to the clipboard.

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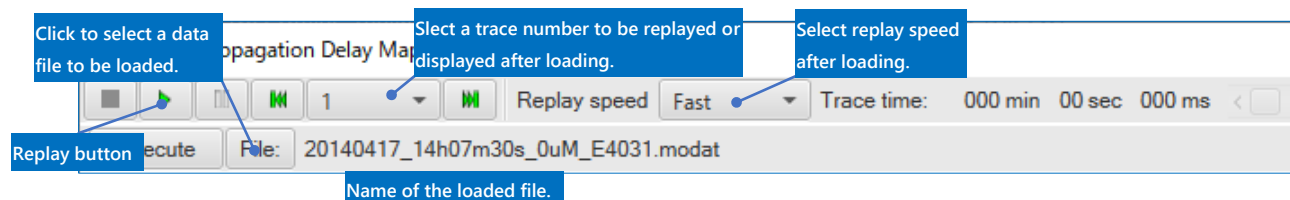
Propagation Delay Map

1. Introduction

Propagation Delay Map is a paid tool of Propagation Delay Map (free ver.). You can directly load modat format data and complete all processes within one tool without having to detect FP waveforms or to export their timestamp (detection time information) data in Mobius. Some functions of Beat Detector and Propagation Delay Map (free ver.) are combined in this tool, and the two result display panels of Propagation Delay Map and Voltage Chart – selected ch have almost the same interface as the panels included in Propagation Delay Map (free ver.) and Beat Detector, respectively. For that reason, please read the explanation of each tool beforehand in order to understand this tool thoroughly. The main feature of this tool is that when the baseline of a potential waveform fluctuates or first peak amplitudes between electrodes vary, resulting in problems in detecting the FP waveform and/or its first peak position, you can manually fix these problems, without reanalyzing, to reflect the results instantaneously in the excitation propagation map.
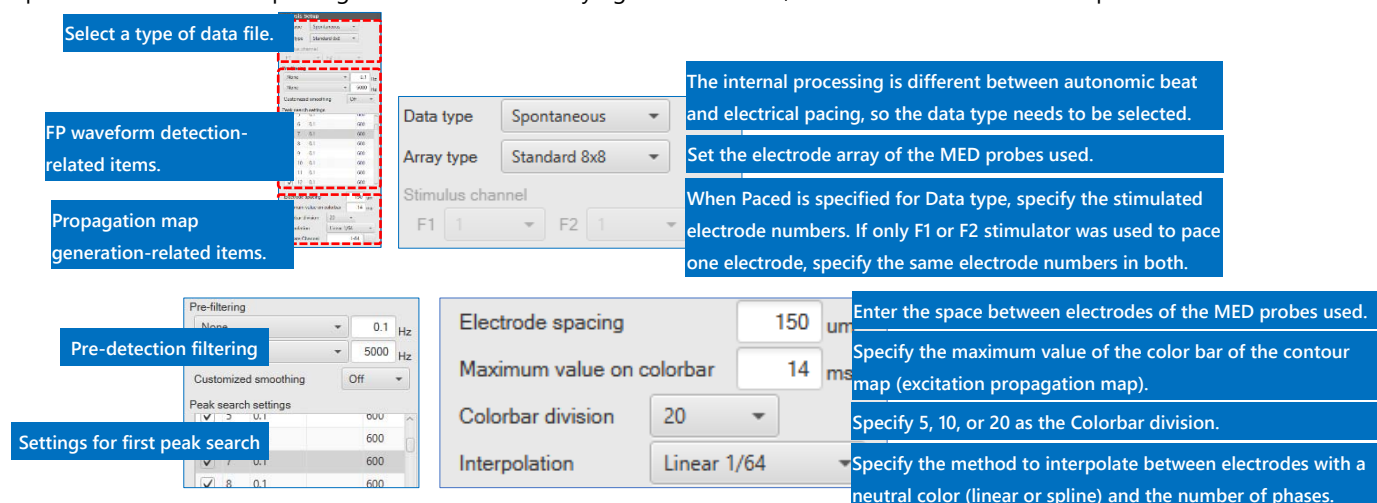

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in spike timestamp format (csv format), which is the unique format of Mobius, are compatible. After processing is done, click ▶ (Play) to replay the obtained excitation propagation maps.

Click to select a data file to be loaded.

Slect a trace number to be replayed or displayed after loading.

Select replay speed after loading.

Replay button

Name of the loaded file.

2.2. Analysis Setup Panel

After the data file is loaded, you can set the analysis conditions. Click Execute to generate an excitation propagation map. To export the results after exporting the results and modifying the conditions, click Execute each time to reprocess.
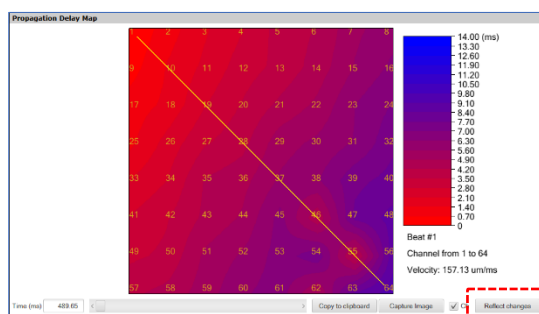

Select a type of data file.

FP waveform detection-related items.

Propagation map generation-related items.

The internal processing is different between autonomic beat and electrical pacing, so the data type needs to be selected.

Set the electrode array of the MED probes used.

When Paced is specified for Data type, specify the stimulated electrode numbers. If only F1 or F2 stimulator was used to pace one electrode, specify the same electrode numbers in both.

Pre-detection filtering

Settings for first peak search

Enter the space between electrodes of the MED probes used.

Specify the maximum value of the color bar of the contour map (excitation propagation map).

Specify 5, 10, or 20 as the Colorbar division.

Specify the method to interpolate between electrodes with a neutral color (linear or spline) and the number of phases.

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<u>Internal algorithm when Spontaneous is selected</u>

This algorithm searches an electrode that has the largest total number and a small electrode number from the list of timestamps (FP waveform detection time) contained in the file. Within that electrode, it searches the second timestamp in the range of 600 to 3,000 ms after the first timestamp, and then searches the subsequent timestamps in the same manner as an indication for beat timing. It searches the timestamps for other electrodes in the range of 500 ms before and after the timestamp for each beat in the single electrode obtained as a result, and sets the electrode with the first timestamp as the electrode that generates the first excitation (pacemaker site). This process is repeated for each beat to generate a propagation map.

2.3. Propagation Delay Map panel

This panel displays an excitation propagation map for each detected beat. Click the Play button on the command bar to display the propagation map in phases at the specified play speed (in animation). Select two electrode numbers on the propagation map by left-clicking and right-clicking to display the propagation velocity calculated based on the time differences between the timestamps of the electrodes. By using the seek bar at the bottom of the panel, you can skip to a propagation map of any time. By clicking the Copy to clipboard button, you can save the time difference between the timestamps of the electrodes, which were used to generate the currently displayed propagation map, as numerical information to the clipboard and paste it in Excel or other spreadsheet. By clicking the Capture Image button, you can save the image to the clipboard and paste it in an image processing software program.

To reflect the changes made on the Voltage Chart – selected ch panel in the excitation propagation map, click Reflect changes.

2.4. Voltage Chart – selected ch panel

The potential waveforms of the electrodes whose numbers have been selected in Channel position and a cursor at the position of the first peak point that was recognized through the analysis are displayed. Unlike Beat Detector, the cursor numbers are assigned in order. By clicking and dropping the number, you can change the cursor position to anywhere. After clicking and dragging the number on the chart to specify the range (highlighted in yellow), you can right-click on the number to change the peak position to the maximum or minimum value of the signal in the specified range.
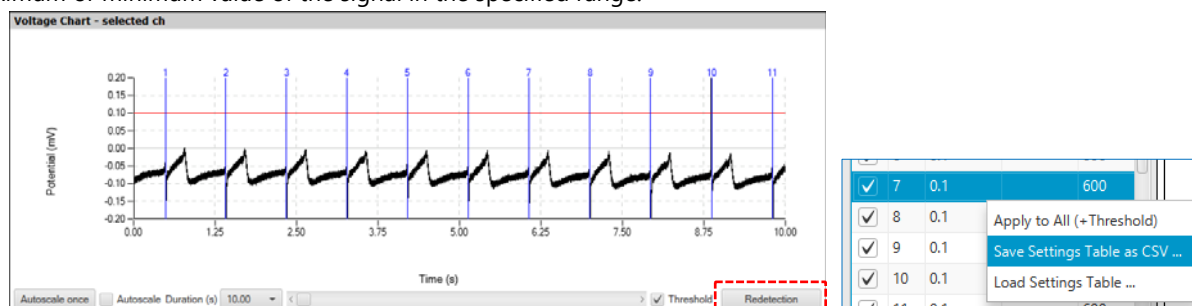

When you have directly edited the threshold in Peak search settings on the Analysis Setup panel, click the Redetection button to redetect the peak position.

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Spike Detector

1. Introduction

Spike Detector is a tool to detect the extracellular potential waveform that originates from the action potential of neural culture as a spike and to visualize the results in various charts. You can export the information related to spike detection time as a timestamp file for burst interval detection in the Burst Detector tool. The main window consists of an Analysis Setup panel, a View Control panel, and 12 result display panels arranged on four tabs.
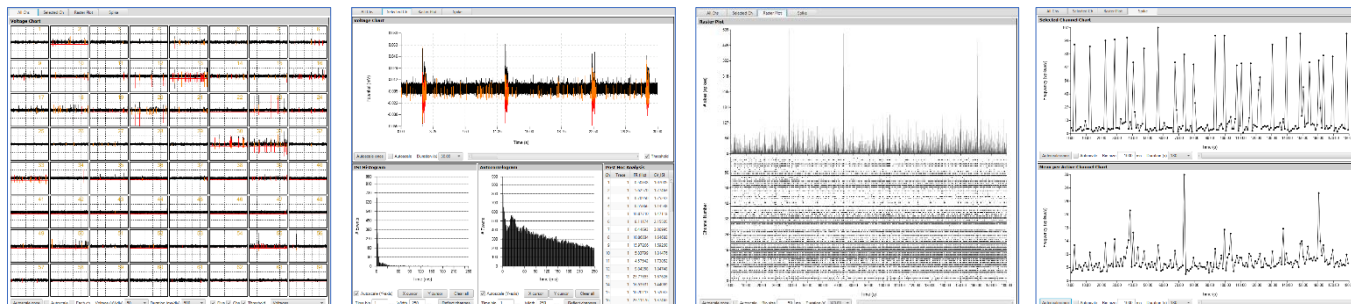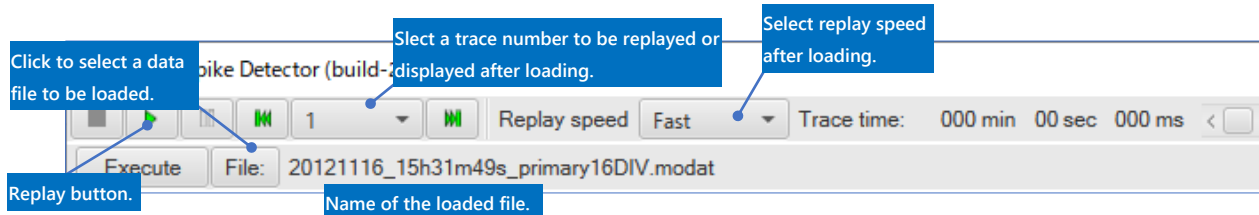

From left to right: All Chs、Selected Ch、Raster Plot、Frequency tabs.

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat and endat formats are compatible.

2.2. Analysis Setup panel

After the data file is loaded, set the analysis conditions. Select a text box by clicking on the text box. When the background color turns pink, it means that the text box is available for editing. After editing, press the Enter key to reflect the change (the background color turns back to white).
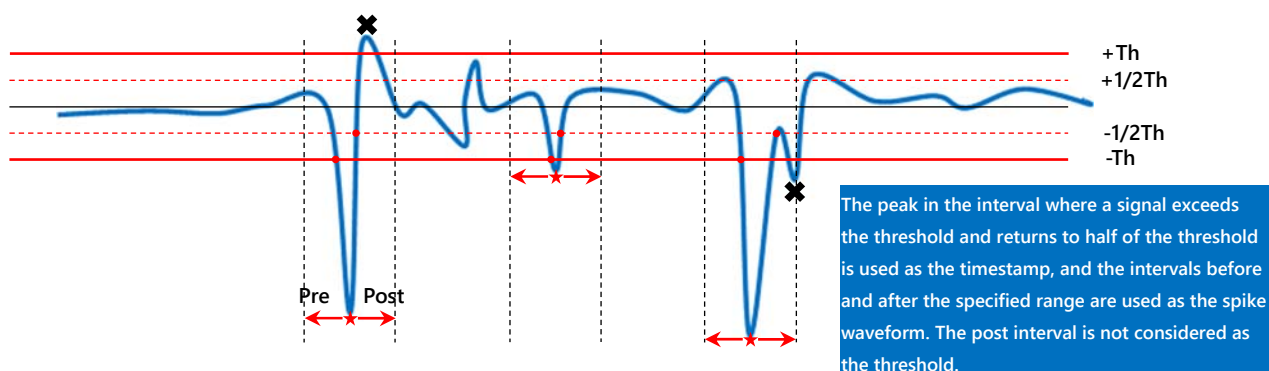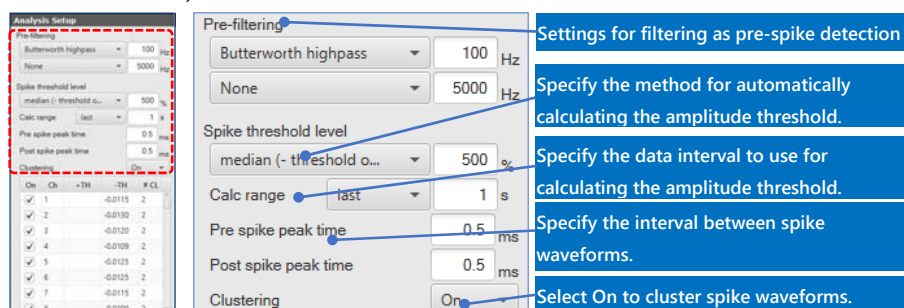
Clustering

Based on k-means clustering, where the spike width (X) at half threshold and spike peak potential (Y) are explanatory variables, the detected spike is divided into the specified number of clusters.

After specifying the analysis conditions, click Execute on the main menu to start the analysis. To export the results after exporting the results and modifying the conditions, click Execute each time to reprocess.

2.3. View Control Panel

After the data file is loaded or filtered (after the progress window disappears), you can change the settings for displaying waveforms on the View Control panel as needed. Use the Array type to specify the format for displaying the electrode array on the All Chs tab, and the Channel position to specify the electrode numbers to display potential waveforms on the Voltage Chart panel on the Selected Ch tab.
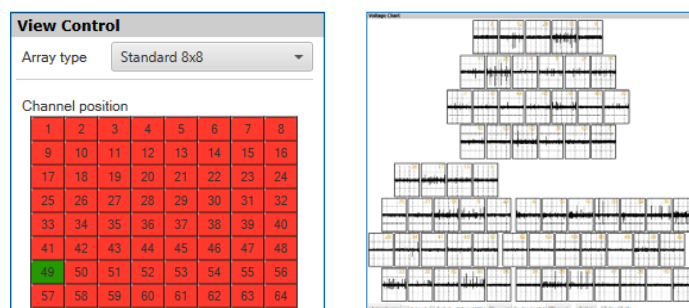
Voltages Chart (right) when Rat hippocampal is specified as the array type.

2.3. All Chs tab

This tab displays the progress of the spike detection and detected spike waveforms for 64 electrodes. Select one tab out of five (display method) to display.
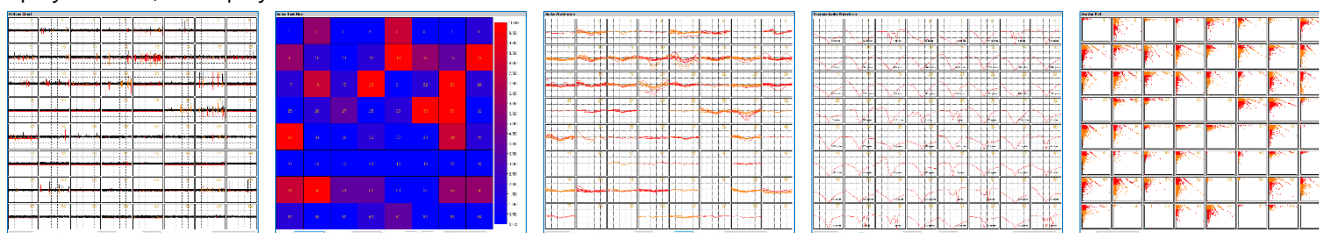
From left to right: Voltage Chart, Spike Heat Map, Spike Waveform, Averaged Spike Waveform, Scatter Plot.

Descriptions of the settings at the bottom of each chart are provided below:

2.3.1 Voltage Chart panel

This panel highlights detected spike waveforms in red.

Clicking Autoscale once automatically ranges the vertical axis of all electrodes according to the data of the electrode with the largest amplitude in the instantaneous replayed data. When "Autoscale" is checked, the vertical axis is automatically set for each electrode according to its amplitude at any time.

Specify the display range of Y-axis (Voltage) and X-axis (Duration) in one section separated by auxiliary lines. 2500 and 5000 ms/div require a large load for waveform rendering during replaying, so memory allocation should be increased.

On/off display of auxiliary lines, electrode numbers, and threshold.
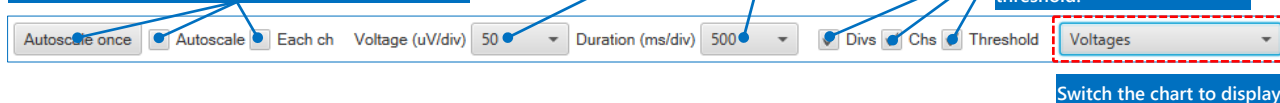
Switch the chart to display.

2.3.2. Spike Heat Map panel

This panel calculates the frequency based on the number of detected spikes every 500 ms and displays them in color.

Specify the method to interpolate between electrodes with a neutral color (linear or spline) and the number of phases.

Specify 5, 10, or 20 as the Colorbar division.

Specify the range of spike detection rate to express in color.

Interpolation None ▼ Colorbar division 20 ▼ Colobar range 0 to 10 Hz Spike Heat Map ▼

2.3.3. Spike Waveform panel

All items except for Display Count are the same as Voltage Chart.

The number of spikes to overlay. When the upper limit is reached, each time a spike is overlaid, the oldest spike waveform is erased.

Autoscale once ☐ Autoscale ☐ Each ch Voltage (uV/div) 50 ▼ Display Count 10 ▼ ☑ Divs ☑ Chs Spike Waveforms ▼

2.3.4. Averaged Spike Waveform panel

All items except for Cluster # and Spike Count are the same as Voltage Chart.

Specify the cluster number of the cluster average waveform to be displayed.

On/off display of number of detected spikes.

Autoscale once ☐ Autoscale ☑ Each ch Voltage (uV/div) 50 ▼ Cluster # 1 ▼ ☑ Divs ☑ Chs ☑ Spike Counts Averaged Spike Wave... ▼

2.3.5. Scatter Plot panel

The Scatter Plot panel does not have individual settings. It displays a scatter diagram of the detected spike with the spike width at half threshold on the x-axis and spike peak potential on the y-axis.

☑ Chs Scatter Plot ▼

2.4. Selected Ch tab

2.4.1. Voltage Chart panel

After the data file is loaded, click an electrode number in Channel position on the View Control panel to display the entire length of the filtered waveform for the data file of the corresponding electrode. Specify the time range to display in Duration. By clicking and directly editing the extreme values on the vertical and horizontal axes, you can change the display range to any range.
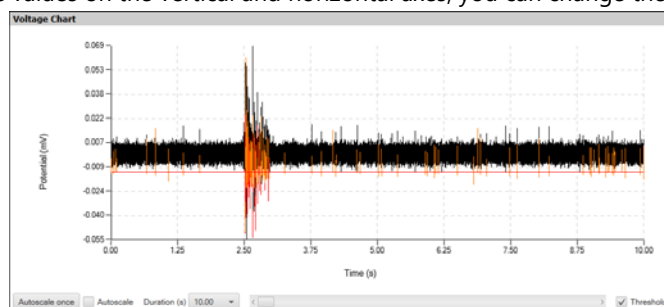

2.4.2. ISI Histogram Panel

This panel displays a histogram of the intervals of detected spikes of the electrodes specified in Channel position. Specify the class width of the histogram in Time bin and the maximum value of the class in Width. Click the X cursor button and the Y cursor button to show cursors on the chart. The cursors remain even after displaying another electrode chart in Channel position, so they can be used as marking to compare peak positions and their times. Select Copy Data from the right-click menu to save the numerical data in the display range and Capture Image to save the image to the clipboard.

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When the text box is selected, the background color changes to pink and the values can be edited. After editing, press the Enter key to reflect the change.
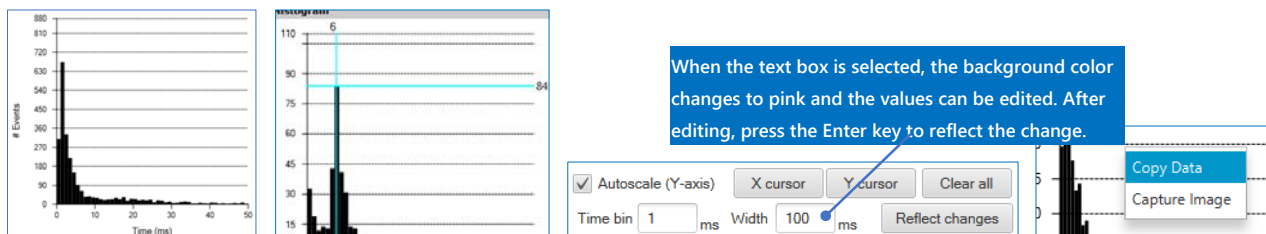
2.4.3. Autocorrelogram panel

This panel displays an autocorrelogram of spikes of the electrodes specified in Channel position. An autocorrelogram is a histogram that sets the detection time of each spike to 0, displays the time difference of the detected spike column of an electrode from other spikes in the specified class of the time window, and displays the spike detection frequency of each class in a bar graph.
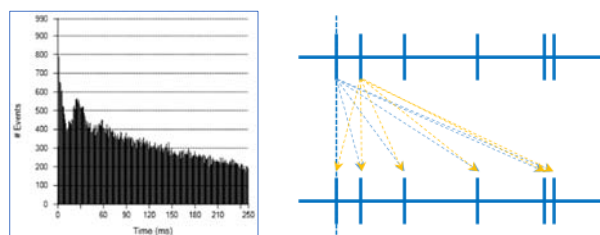
2.4.4. Summary panel

This panel displays the spike detection frequency (FR) for each electrode and coefficient of variation of interspike intervals (CV_ISI) in all data intervals.

Summary			
Ch	Trace	FR (Hz)	CV_ISI
1	1	0.50838	1.07005
2	1	5.34079	1.71467
3	1	0.70391	1.74313
4	1	0.54749	1.11484
5	1	10.48045	1.43128

2.5. Raster Plot tab

This tab displays a time histogram of the total number of detected spikes in all electrodes with the elapsed time being the class on the top and a 64-electrode raster plot using the same time axis on the bottom. Save the image to the clipboard by selecting Capture Image from the right-click menu or the numerical data of the time histogram by selecting Copy Data.
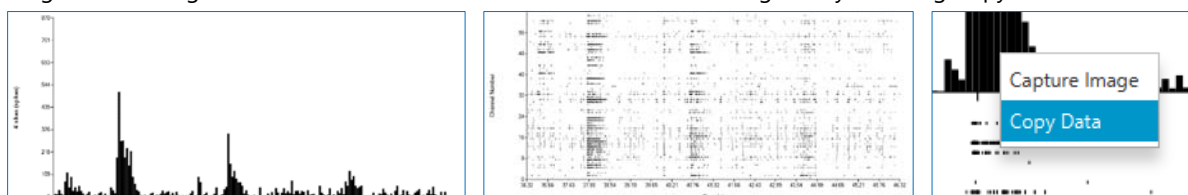
Specify a time window (class width of elapsed time).

2.6. Frequency tab

2.6.1. Selected Channel Chart panel

This panel calculates the number of detected spikes per second for each specified Bin size based on the number of detected spikes for the selected electrodes and plots them as a line graph. Save the image to the clipboard by selecting Capture Image from the right-click menu or the numerical data of the time histogram by selecting Copy Data.

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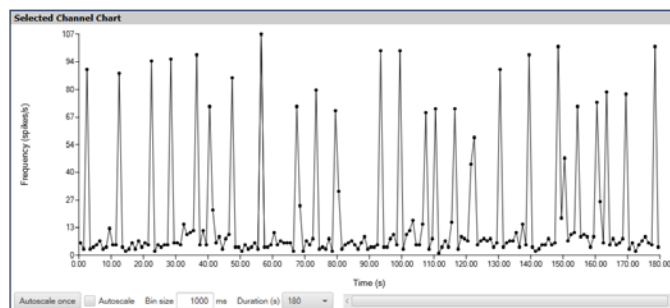
2.6.2 Averaged Channel Chart Panel

This panel plots electrode average chart of the spike detection frequency using only electrodes for which spike detection is enabled.
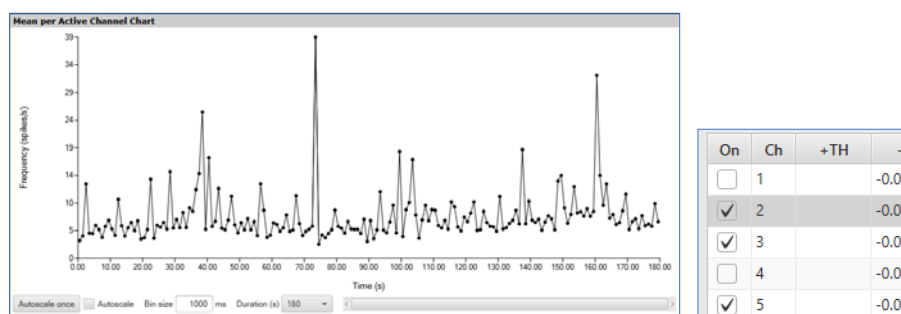

Ch 1 and Ch 4 are not selected and enabled for spike detection, so they are excluded from the calculation of average value (right).

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Burst Detector

1. Introduction

Burst Detector is a tool that detects an interval called a burst firing, in which bursting occurs transiently after a static interval, based on the information of spike detection time obtained through spike detection, and calculates its quantitative index. This tool analyzes two types of bursts that we named Single Channel Burst and Multi Channel Burst. Single Channel Burst is a burst interval in which action potentials occur continuously and transiently on the chart of one electrode. Max Interval method (Nextechnologies) calculates the number of detected bursts, burst width, interval between bursts, and other numerical indicators using algorithms.
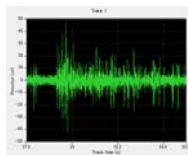
Multi Channel Burst is an interval of a phenomena where burst firing occurs simultaneously in multiple electrodes (synchronized burst), which is found in the dissociated cultured neuron network. The number of detected bursts, synchronized burst width, interval between synchronized bursts, and other numerical indicators are calculated using an algorithm based on a report by Mukai et al. (Elect. Eng. Jpn., 145, 28–37, 2002).
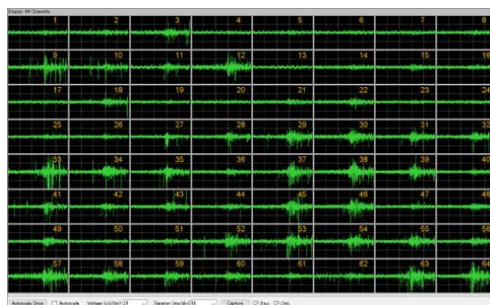
Both bursts require a unique spike timestamp file obtained through spike detection in Mobius as the input file. For Single Channel Burst, the burst interval should generally be detected from only one signal source (one nerve cell, single unit), but in MEA, the action potentials of multiple signal sources are duplicated on a potential chart of one electrode in most cases. Therefore, detecting bursts after clustering spike waveforms into individual signal sources based on the spike waveform shape is close to the original meaning of burst detection, but this tool does not perform clustering. The main window of this tool consists of an Analysis Setup panel, a View Control panel, and two tabs: Single Channel Burst and Multi Channel Burst. The results are displayed on each tab.

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in spike timestamp format (csv format), which is the unique format of Mobius, are compatible. After the data is loaded, the Single Channel Burst tab displays a raster plot for the specified electrode while the Multi Channel Burst tab displays a raster plot for all electrodes and a time histogram for spike detection frequency.
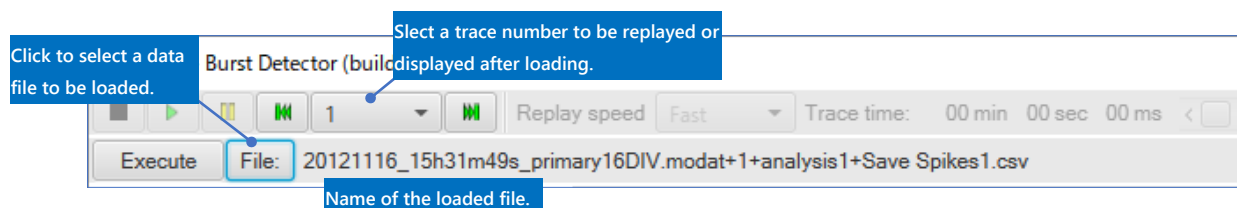

2.2. Analysis Setup Panel

After the data file is loaded, set the analysis conditions. Select a text box by clicking on the text box. When the background color turns pink, it means that the text box is available for editing. After editing, press the Enter key to reflect the change (the background color turns back to white).

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Enter any numerical value in each item. The specified analysis conditions are applied to all traces. For Single Chanel Burst Settings, you can specify conditions for each electrode selected in Channel position and assign the same conditions to other electrodes by clicking Apply to All. Click Restore default to set to default. After setting the analysis conditions, click the Execute button to start analyzing.

Algorithm for single channel burst detection
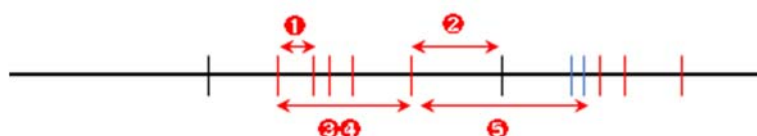
Max. interval to start burst: The preceding spike whose interval between successive spikes is less than the set value (<) is considered the burst start spike (temporary).

Max. interval to end burst: The preceding spike whose interval between successive spikes is longer than the set value (>) is considered to be the burst end spike (temporary).

Min. # spikes in a burst: If the number of spikes in the burst interval (temporary) is greater than or equal to the set value (≥), and

Min. duration of a burst: if the interval from the start spike to the end spike is greater than or equal to the set value (≥), it is judged to be a burst.

Min. interval between bursts: The interval between successive bursts (from the end spike of the preceding burst to the start spike of the following burst) should be greater than or equal to the set value (<) (spike trains within the interval are not used to determine the burst detection).

Algorithm for multi channel burst detection
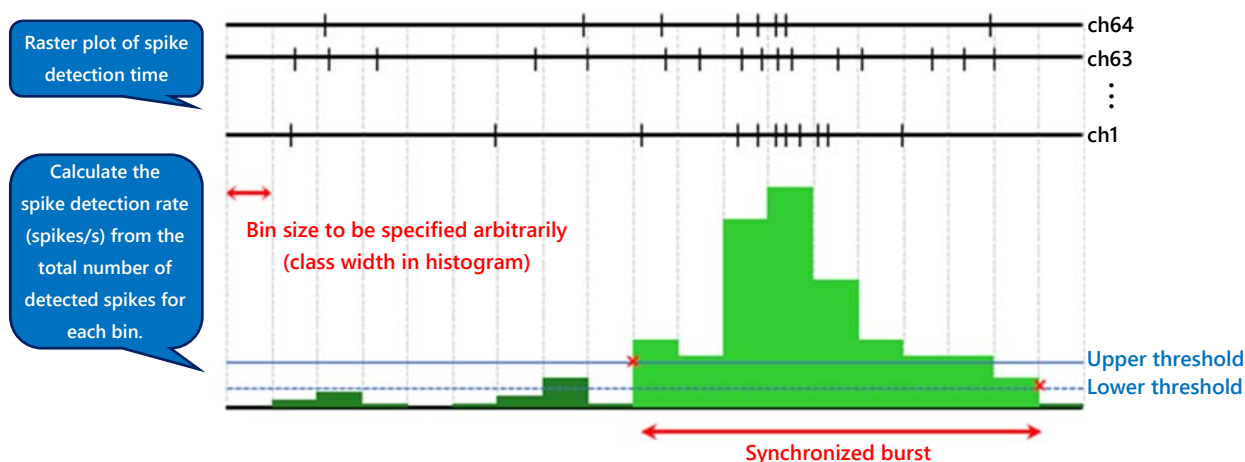
Bin size: Specify a bin size on the x-axis (equivalent to class width in histogram) to generate an ASDR (Array-wide Spike Detection Rate) chart. After clicking to select, the background color turns pink. Press the Enter key after editing the numerical value to reflect the change (the background color turns back to white).

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Smoothing: To smooth the ASDR chart when determining the synchronized burst interval, specify a value larger than 0 (this will provide a value that averages the ASDR of each bin with the ASDR of the bin number before and after the specified ASDR). For example, as shown in the figure below, if the difference between the base firing frequency and the firing frequency in the synchronized burst interval is low using the data obtained with an MED Mini Probe (16-electrodes), an unintended interval with a low threshold set may be detected as a synchronized burst. Additionally, when there is a large fluctuation for each bin in a synchronized burst interval, the interval may be detected multiple times in pieces. In such a case, detecting the threshold with a smooth (red lines) waveform will reduce the rate of false detections.

The threshold is crossed many times, so if the threshold is determined as it is with the green histogram, the intervals are detected in pieces.
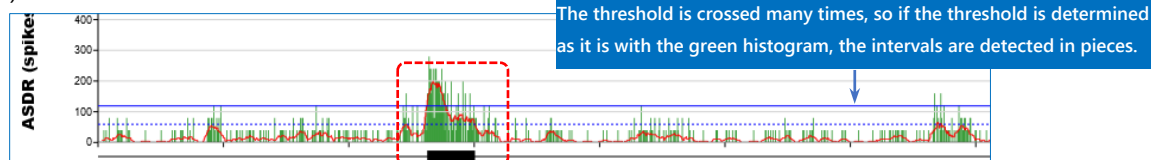
Burst detector: This allows you to specify Normal or Schmitt trigger as the method to determine the threshold. The former determines the start bin and end bin of the burst with a single threshold (Threshold - high). The latter determines the start bin (upper threshold) and end bin (lower threshold) with two thresholds.

Upper threshold: This is the threshold to determine the start bin of a synchronized burst.

Lower threshold: This is the threshold to determine the end bin of a synchronized burst. After calculating the threshold using Threshold calculation, enter 50% of the upper threshold. The value must be smaller than Threshold - high.

Threshold calculation: Use this when calculating the threshold based on the data without specifying it arbitrarily. Select the mean or median of ASDR traces, specify a percentage to multiply (X/100), and then press Apply to automatically set the threshold. You can manually change the threshold even after it is automatically set.

2.2. Single Channel Burst Tab
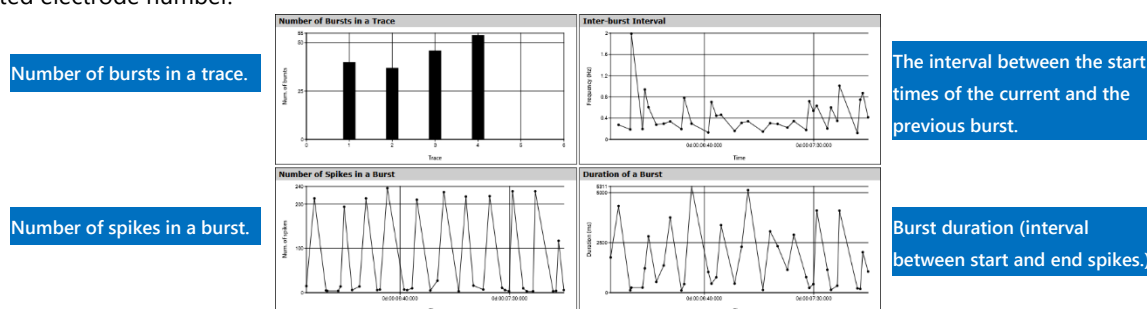
2.2.1. Single Channel Raster Plot Panel

This panel displays a raster plot of the electrode number selected on the View Control panel. The detected burst intervals are indicated in red and the start spike positions are indicated with an upward-pointing triangle. By selecting Display from the right-click menu, you can change the display range to 1 sec, 10 sec, 1 min, or 10 min. Click the ○ button at the left of the seek bar to display all data intervals. Select Save Chart Image to export the chart as an image in emf, bmp, or png format.
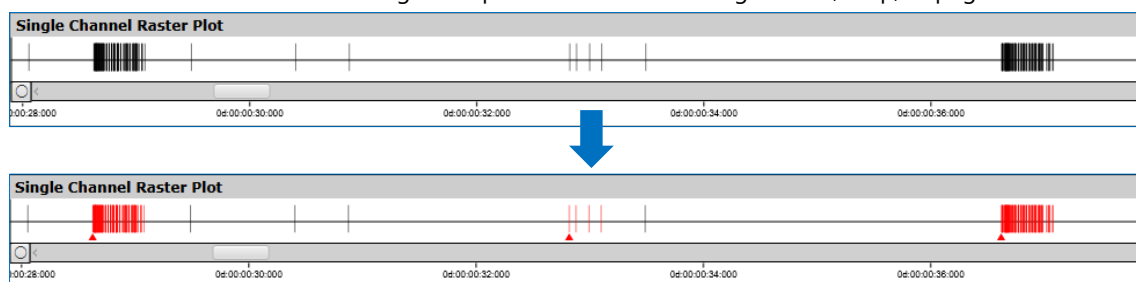
The upward-pointing triangles indicate start spikes of the burst.

2.2.2. Number of Burst in a Trace / Inter-burst Interval / Number of Spikes in a Burst / Duration of a Burst panel

This panel displays the number of detections for each trace and three types of measurement time charts of detected bursts for the selected electrode number.

Number of bursts in a trace.

The interval between the start times of the current and the previous burst.

Number of spikes in a burst.

Burst duration (interval between start and end spikes.)

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The burst intervals on the Inter-burst Interval panel are expressed in frequency (Hz). If the start time of an instant burst is T_i and the start time of the previous burst is T_{i-1}, the interval is expressed as:

$$Inter-burst\ Interval = \frac{1000}{T_i - T_{i-1}}$$

To change the display range on the chart, select Setting... from the right-click menu and open the edit window. You can specify the display range for each trace and electrode. To use the same display range in all electrodes, click Apply to All after editing the display range. In such a case, the specified range is not shared between different traces, even with the same electrode (you need to individually specify it for each trace).
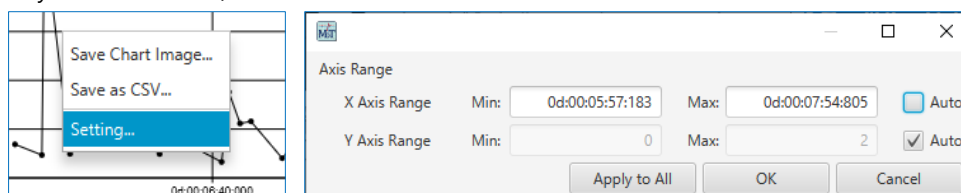
Select Save Chart Image... to select emf, bmp, or png format and export the currently displayed chart image of the selected trace and electrode number. Select Save as CSV... to export the numerical data of the charts of the selected traces and electrode numbers in csv format (this includes the numerical data outside the display range).

2.2.3. Burst Characteristics panel

The numerical indicators of the detected burst are shown on each row.

Select Copy Results from the right-click menu to copy the numerical data in the specified cell and Save Results... to export the entire table in csv format. For the former, you can select consecutive, multiple ranges by first selecting one cell and then another while pressing the Shift key.

2.2.4 Batch export of analysis results

Select a location to export to in the Export window and click the Export button to export all results. Click the Preferences button to open the Preferences window and specify the contents of the export file on the Batch Export tab.

Settings on the Batch Export tab

Export Trace: Allows you to specify the traces to export the results. With Single, only the currently displayed trace is exported; with All (in each), all traces are exported to individual files; and with All (in one), all traces are exported to one file, leaving an empty row between traces.

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An empty row between traces

Chart Image: Allows you to specify the result display panel to export the image and its file format (select emf, bmp, or png). Even if All (in one) is specified, charts will be individually exported for each trace just like for All (in each). The texts entered in the text boxes will be added to the end of the exported file name in the format of "Read file name + texts."

Chart CSV: Exports the real values on the specified result display panel in a csv file. This exports the real values in all intervals, not just the display range.

Information CSV: Exports the table on the Burst Characteristics panel in csv format.

2.3. Multi Channel Burst tab

2.3.1. Array-wide Spike Detection Rate Chart and Multi Channel Raster Plot panel

The specified display ranges of the axes and label font can be modified on the Preferences window, which can be opened by clicking the Preferences button or by selecting Modify Chart... from the right-click menu on the panel. By dragging and dropping it on the chart, you can enlarge the display range of the x-axis to any size, and by clicking the button on the left of the seek bar after enlarging the display range, you can switch the display to show the entire length of the trace.
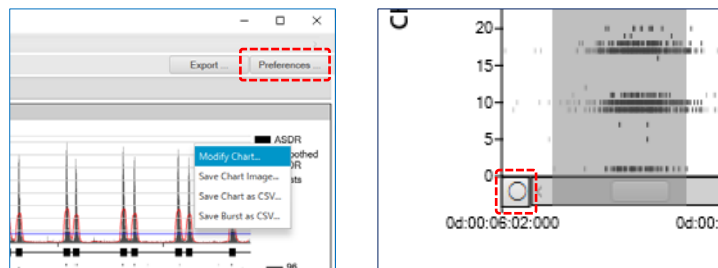
The settings of an ASDR chart and all electrode raster plots can be modified on the ASDR Chart Format tab and the Raster Plot Format tab, respectively. Specify the display color of the raster plot in the Plot Type field on the Raster Plot Format tab. When Black is selected, the raster is displayed in one color of black, and when Grayscale or Color is selected, the raster is displayed in a grayscale or a color scale of five shades for each bin.

Rank of spike detectin rate	Grey scale	Color scale
＞4 (over rank 4)	0 (Black)	Red (255, 0, 0)
4 (maximum spike detection rate specified arbitrary)	0 (Black)	Red (255, 0, 0)
3	(255-min)×0.25	Orange (255, 165, 0)
2	(255-min)×0.50	Green (0, 128, 0)
1	(255-min)×0.75	Blue (0, 0, 255)
0 (minimum spike detectin rate)	255-min※	Purple (128, 0, 128)

Charts are displayed in shades with 55 subtracted,

in order to avoid using a shade close to white for displaying charts for which white is set as the background color.

2.3.2. Burst Characteristics - Individual Values panel

The numerical indicators of the detected synchronized burst are shown on each row. After specifying the range by dragging and dropping, select Copy Results from the right-click menu to save the numerical data of the selected range to the clipboard. You can also select Save Results... to export the entire table in a csv file.
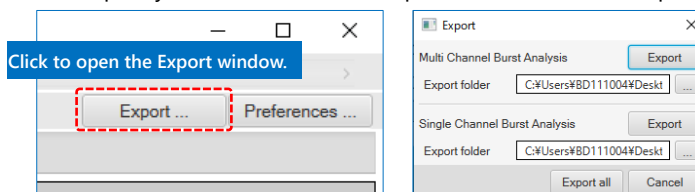
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2.3.3. Burst Characteristics - Summary Values panel

This panel aggregates the numerical indicators of the synchronized burst for each trace and calculates and displays the burst average, standard deviation, standard error, and median of the four numerical indicators. After specifying the range by dragging and dropping, select Copy Results from the right-click menu to save the numerical data of the selected range to the clipboard. You can also select Save Results... to export the entire table as a csv file.

2.3.4 Batch export of analysis results

Select a location to export to in the Export window and click the Export button to export all results. Click the Preferences button to open the Preferences window and specify the contents of the export file on the Batch Export tab.

Settings on the Batch Export tab

Export Trace: Allows you to specify traces to export the results. With Single, only the currently displayed trace is exported; with All (in each), all traces are exported to individual files; and with All (in one), all traces are exported to one file, leaving an empty row between traces.

Chart Image: This allows you to specify bmp or png as the export format for images on the Array-wide Spike Detection Rate Chart and Multi Channel Raster Plot panel. Even if All (in one) is specified, charts will be individually exported for each trace just like for All (in each). The texts entered in the text boxes will be added to the end of the exported file name in the format of "Read file name + texts."

Chart CSV: Exports the numerical data of the ASDR chart. With Chart, the data of the entire trace is exported, and with Burst, only the data of the burst interval is exported. The latter exports data from before and after the range specified in Export range (ms) in Save Burst as CSV on the ASDR Chart Format tab, starting from the synchronized burst start bin (Start time) or the ASDR peak bin (Peak time).

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Information CSV: Summary values and Individual values are two numerical data on the Burst Characteristics panel. Select Save Results… from the right-click menu to modify them to be the same file as the one to be exported. Details shows detailed information on the electrode number and the detection time of the spike included in each burst.

trace #	burst #	ch	time (ms
1	1	43	1038.
1	1	17	1039
1	1	36	10434.
1	1	33	10434.
1	1	49	10438.
1	1	27	1043
1	1	9	10442.
1	1	63	1045(
1	1	41	10450.
1	1	9	10452.
1	1	17	1045
1	1	10	1045
1	1	41	10456.
1	1	10	1046(

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Network Connectivity Graph (free ver.)

1. Introduction

Network Connectivity Graph (free ver.) graphically displays the synchronous activity connection between networks on the multielectrode array based on the spike detection time of each electrode. This is based on a report by Faustino Martins JM et al. (Cell Stem Cell, 27, 498, 2020). The time axis is divided into the specified time window and when one or more spike is found in one time window in multiple electrodes, each pair is considered to have been connected. The total number of connections in all data intervals is displayed on a two-dimensional map, with the strength expressed in the thickness of the lines.
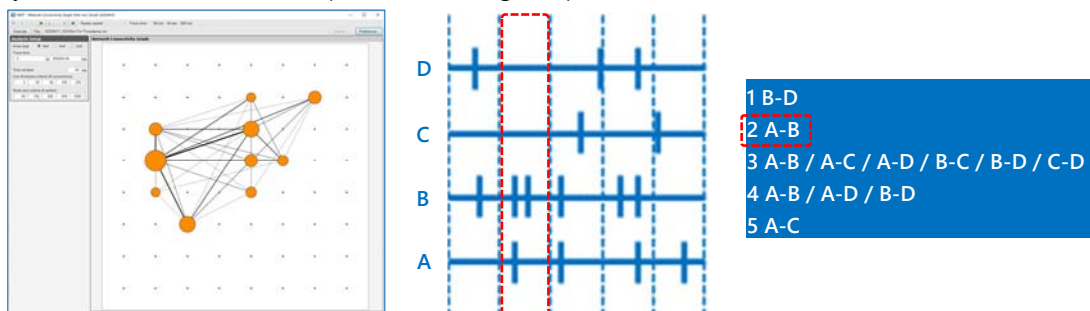
1 B-D
2 A-B
3 A-B / A-C / A-D / B-C / B-D / C-D
4 A-B / A-D / B-D
5 A-C

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in spike timestamp format (csv format), which is the unique format of Mobius, are compatible.

Click to select a data file to be loaded.

Slect a trace number to be replayed or displayed after loading.

Name of the loaded file.
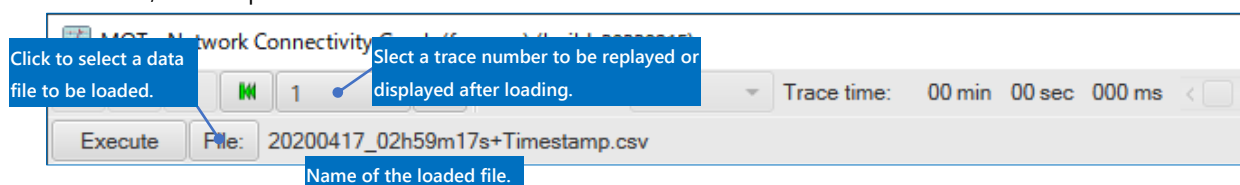
2.2. Analysis Setup panel

After the data file is loaded, you can set the analysis conditions. Click Execute to the process.

Specify array type used.

Specify trace time to be analyzed.

Specify a time window.

Specify the connection strength in five levels.

Specify the level of the number of detected spikes in five levels.
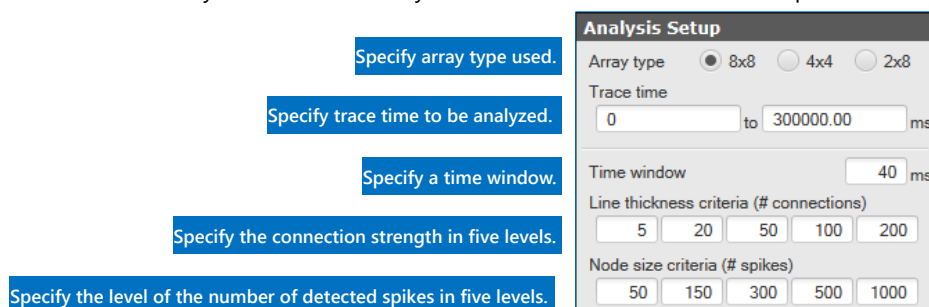

2.3. Network Connectivity Graph panel

Select Copy Image to Clipboard from the right-click menu to save the image to the clipboard. Select Copy Data to Clipboard to save the numerical data of the number of detected spikes for each electrode and the number of connections for each electrode pair to the clipboard.

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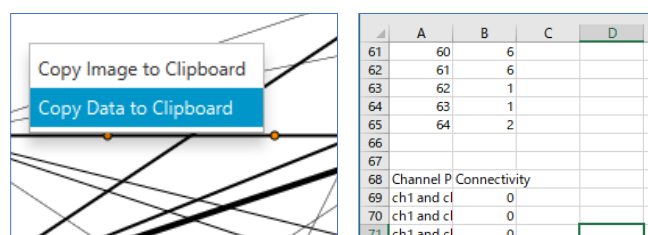
# Connection Map

1. Introduction

Connection Map is a tool to obtain an cross-correlation histogram based on the spike detection time for each electrode, and to graphically display the strength of the functional connection between spikes (signal sources that generated spikes) based on the results. This is based on a report by Kudoh SN et al. (IEEJ Trans. ELS, 129, 1611–1618, 2007). For more details, please see the reference source.
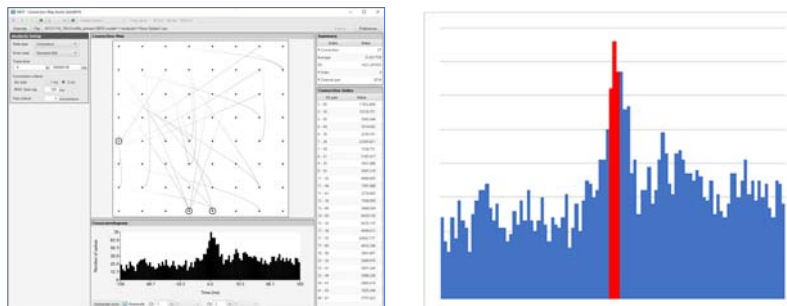
2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in spike timestamp format (csv format), which is the unique format of Mobius, are compatible.
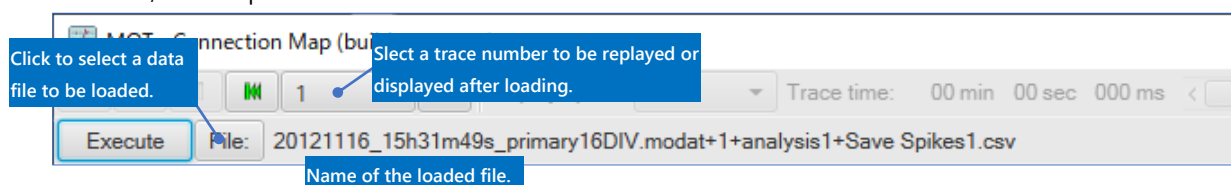
2.2. Analysis Setup panel

Although spikes from multiple signal sources may be detected in one electrode, the spike timestamp files after clustering them based on their shapes will have the cluster ID information as well as the detected electrode number. When analyzing such data files, select Clustered for Data type to reflect the cluster ID in the analysis.

Click the Execute button to start analyzing, generate an cross-correlation histogram for all signal source pairs based on the time window specified in Bin size, and calculate the following indicators:

$$Connection\ index = A_{peak} \times \left(0.01 \times \frac{A_{peak}}{A_{total}} + \frac{1}{\Delta t + 1} + \frac{1}{Kurtosis} \right)$$

A_{peak} indicates the area in 2-ms range around the peak in the cross-correlation histogram, A_{total} indicates the entire area in the cross-correlation histogram, and $Kurtosis$ indicates the degree of peak of the cross-correlation histogram.

2.3. Connection Map panel

This panel graphically displays the strength of the functional connection and the electrodes of the signal sources that form a hub based on the Connection index. Select Copy Image to Clipboard from the right-click menu to save the image to the clipboard. The electrode with a signal source that is determined to be a hub is circled.

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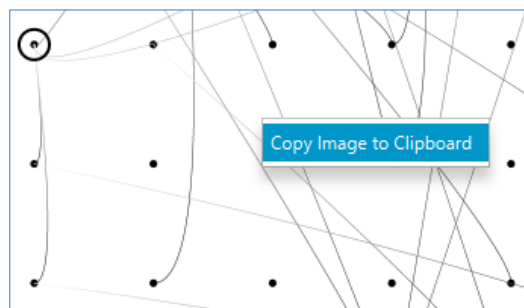
2.4. Crosscorrelogram / Summary / Coonection Index panel

This panel displays the calculated values obtained during the process of generating the Connection Map and their total values. By selecting Remove from the right-click menu on the Connection Index panel, you can manually delete any functional connections.
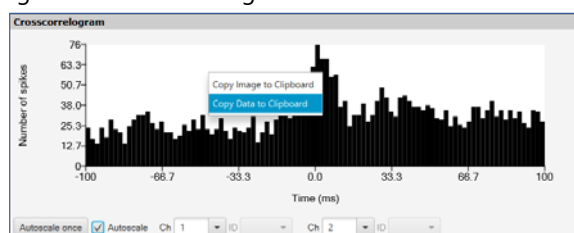
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Peri-Event Time Histogram

1. Introduction

Peri-Event Time Histogram (PETH) is a tool that accumulates the number of detections for each stimulation (event) in the intervals before and after the stimulation for spikes as responses evoked by repeated stimulations, such as illuminations and lights, and displays them as a histogram. The main window of this tool consists of an Analysis Setup panel, a View Control panel, and three tabs: Chart, Raster/Histogram, and All Chs Raster. The results are displayed on each tab.
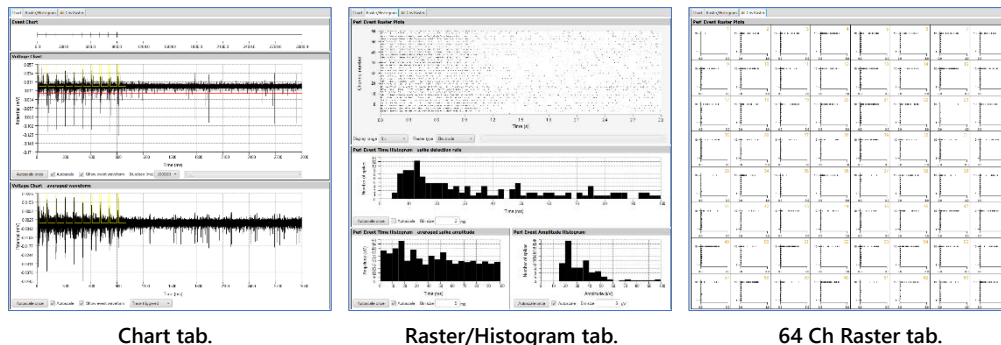
| Chart tab. | Raster/Histogram tab. | 64 Ch Raster tab. |

2. Operation steps

2.1. Load the data file

Click the File button on the main menu and select a data file. Data files in modat and endat formats are compatible.

Click to select a data file to be loaded.

Slect a trace number to be replayed or displayed after loading.

Name of the loaded file.
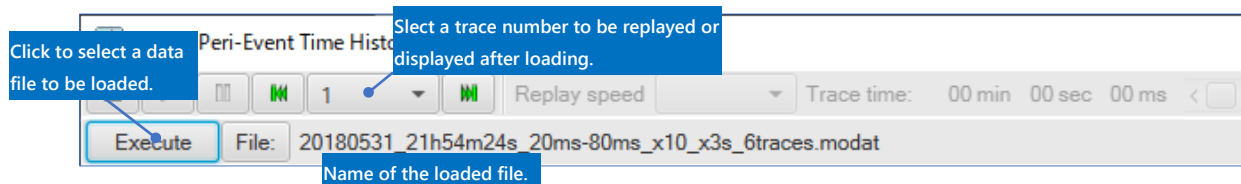
2.2. Analysis Setup Panel

After the data file is loaded, set the analysis conditions. While Prefilter may be applied before generating PETH, this process is not compulsory. Only Butterworth is available for the highpass filter, and Butterworth or Bessel can be selected for the lowpass filter. The cutoff frequency of the lowpass filter is limited to 5,000 Hz. Click Reflect changes to apply the filter to the loaded data file and change the waveforms on the Raw Waveform Chart panel and Raw Waveform Chart - trace averaged panel to the post-filtered waveforms.

When the text box is selected, the background color changes to pink and the values can be edited. After editing, press the Enter key to reflect the change.
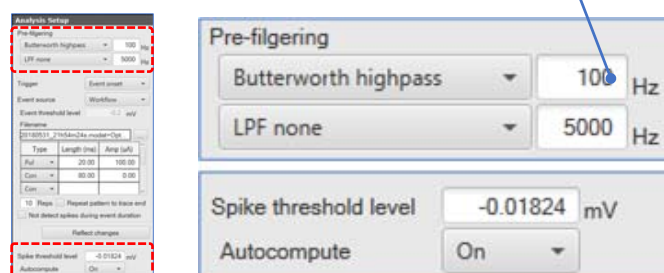
While spike detection is determined based on the amplitude threshold, the threshold can be automatically calculated according to the On/Off selection of Autocompute after the data file is loaded. If On is selected, a different threshold for each electrode is calculated based on the following equation:

$$5 \times median \left\{ \frac{|Amplitude\ value\ of\ each\ data\ point\ within\ 2\ seconds\ after\ starting\ the\ file|}{0.6745} \right\}$$
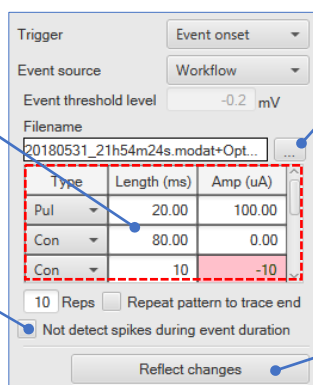
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If Off is selected, all electrodes are automatically set to -0.02 mV. In both cases, consecutive spike detections are not determined within the range of 0.5 ms after the intersection of the threshold and input signal. Similarly to the Schmitt trigger method in Mobius, the spike detection time is the signal peak amplitude time in the interval from the intersection with the threshold to the intersection with the half threshold, and the peak amplitude is the extreme value of the interval. You can manually modify the threshold for each electrode, as long as the modification is made before clicking Execute to generate PETH.

Setting event triggers

To set event triggers required for generating PETH, select Event onset or Trace onset for Trigger. With the former, when multiple events exist in one trace, each event is treated as an independent trigger; and with the latter, when multiple events exist in one trace, the trace start time is treated as the trigger.

Next, select Raw Data or Workflow for Event source. The former sets the amplitude threshold to the loaded data file and the intersection of that threshold and the input signal is used as the event time. To be more specific, considering the electroshock-induced response measurement in the dissociated cultured neuron network, the amplitude of an artifact caused by electrical stimulation is larger than that of an evoked action potential. Therefore, you can adjust the threshold level to detect only artifacts that trigger events. In addition to modifying the threshold level in the Event threshold level field, you can also modify the level by directly dragging and dropping the threshold cursors on the chart. When Workflow is selected, ~+acquisition.moflo that is generated when the data is obtained is loaded, the stimulation pattern information applied is obtained, and the timing in which stimulation pulse occurs is automatically treated as an event trigger. You can also edit the stimulation pattern arbitrarily without loading the workflow (or after it is loaded).
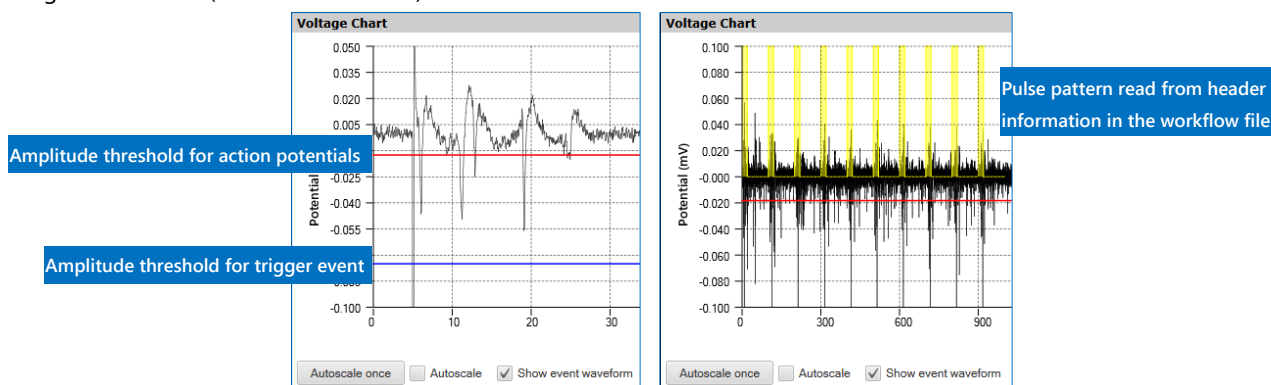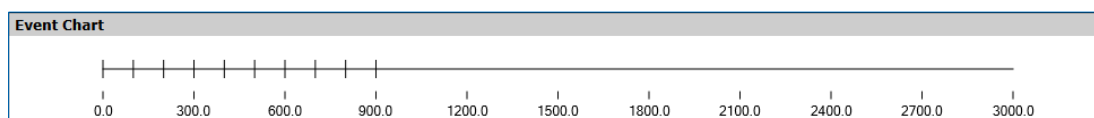

Setting up event triggers with Raw Data (left) and with Workflow (right).

2.3. Chart Tab

2.3.1. Event Chart Panel

This panel displays a time chart after PETH is generated that shows the event occurrence time. You cannot edit the axis and the right-click menu is not available.

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2.3.2. Raw Waveform Chart Panel

This panel displays a raw waveform chart for one trace of the electrodes selected on the View Control panel. The red cursor is the amplitude threshold for spike detection, and the blue cursor is the amplitude threshold for event detection (displayed only when Raw data is selected in Event source). The yellow lines indicate artifacts and by disabling Show event waveform under the chart, you can hide the lines.
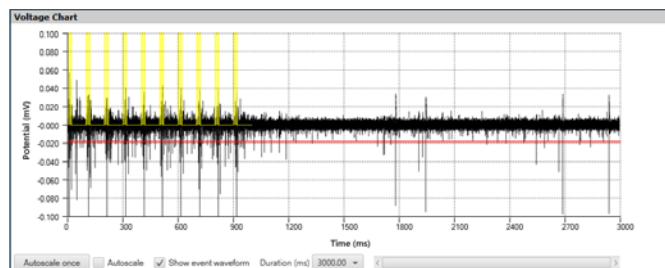
The display ranges of the x- and y-axes can be modified by clicking and editing the maximum or minimum value. Select Capture Image from the right-click menu to save the chart image and select Copy Data to save the numerical data (note: this is not available in ver. 20190701) to the clipboard. Select Apply Threshold to All Channels to apply the currently displayed threshold to all other electrodes.
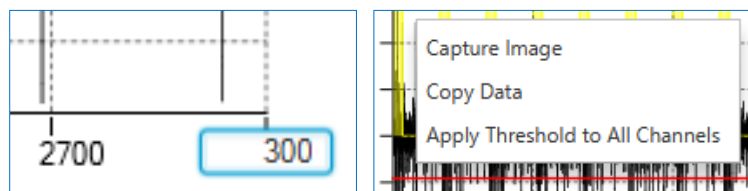
Click Autoscale Once under the chart to automatically adjust the axes according to the maximum and minimum values of the currently displayed y-axis. Select the Autoscale box to automatically adjust the axes each time the electrode number is switched.

2.3.3. Raw Waveform Chart – averaged waveform Panel

This panel displays a raw waveform chart after PETH is generated that arithmetically averages all traces of one electrode. While random noise and spontaneous firing cancel out each other by arithmetical averaging, the overall signal amplitude also becomes small. Similar to the Raw Waveform Chart panel, you can edit the axes and open the right-click menu.

2.4. Raster/Histogram Tab

2.4.1. Peri-Event Raster Plots Panel

Select Electrode from Raster type to display a raster plot for each trace with time on the x-axis and electrode number on the y-axis. Select Event to display a raster plot for each electrode and trace with time on the x-axis and event number on the y-axis. As time 0 is the time an event occurred, you can visualize the response timing after the event and the response reproducibility with repeated events.

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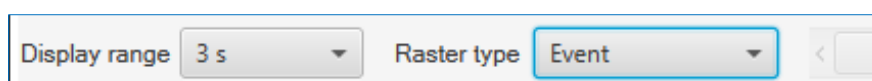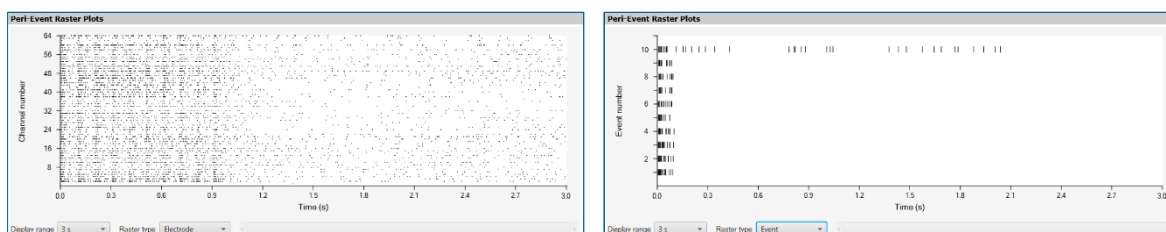
For both raster types, you can specify the display range of one screen using Display range under the chart and modify the range using the slider. By clicking the maximum and minimum values on the axes, you can directly edit numerical values to specify the display range. Select Capture Image from the right-click menu to save the image and select Copy Data to save the numerical data to the clipboard. You can also copy the information of charts not selected in Raster type.
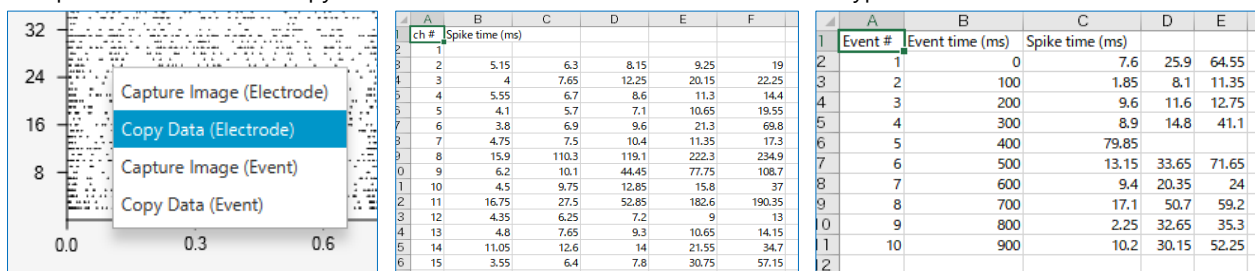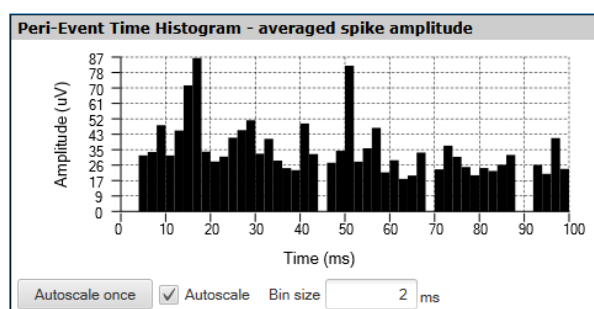

2.4.2. Peri-Event Time Histogram - spike detection rate panel

This panel displays a histogram for each electrode and trace with time on the x-axis and the number of detected spikes on the y-axis. As time 0 is the time an event occurred, if an established response that is evoked by an event exists, the number of detected spikes (frequency) of the bin (class) containing its latent time is larger than other bins and appears as a peak. Specify the resolution of the x-axis (class width in histogram) in Bin size under the chart. Select Copy Image from the right-click menu to save the image in the display range and select Copy Data to save the numerical data to the clipboard.
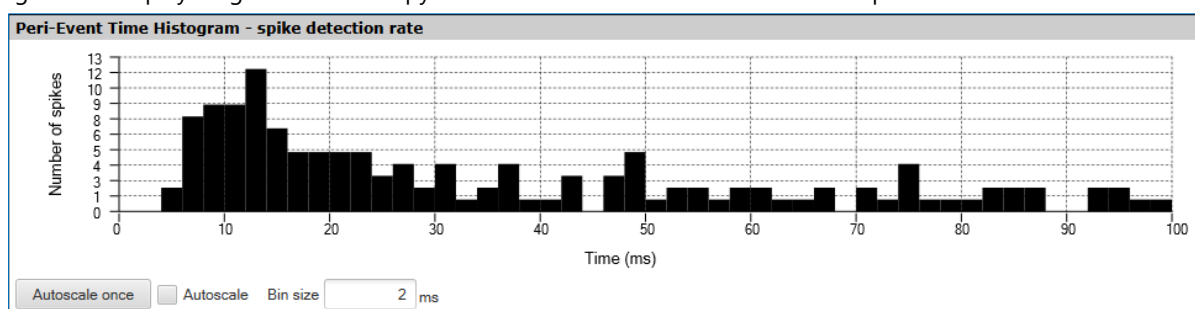

2.4.3. Peri-Event Time Histogram - averaged spike amplitude Panel

This panel displays a histogram for each electrode and trace with time on the x-axis and the average peak amplitude of detected spikes on the y-axis. Specify the resolution of the x-axis (class width in histogram) in Bin size under the chart. Select Copy Image from the right-click menu to save the image data in the display range and select Copy Data to save the numerical data to the clipboard.

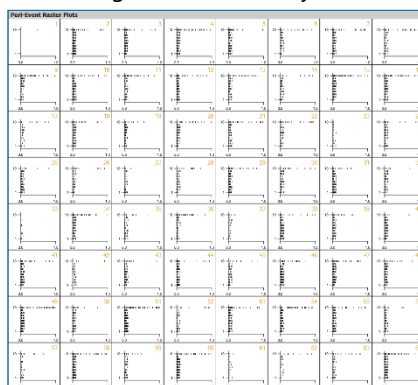### 2.4.4. Peri-Event Amplitude Histogram Panel

This panel displays a histogram for each electrode and trace with peak amplitude of spike on the x-axis and the number of detected spikes on the y-axis. Specify the resolution of the x-axis (class width in histogram) in Bin size under the chart. By selecting Copy Image from the right-click menu, you can save the image data in the display range, and by selecting Copy Data, you can save the numerical data to the clipboard.

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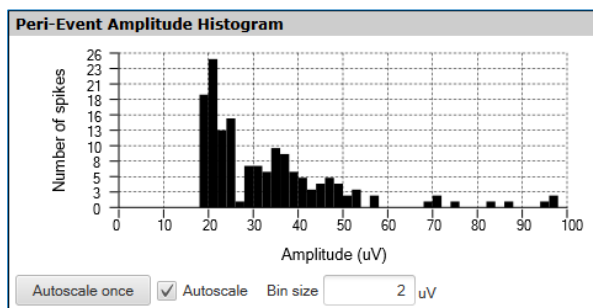
2.5. All Chs Raster Tab

2.5.1. Peri-Event Raster Plots Panel

This panel displays a peri-event time raster plot for all 64 electrodes. The x-axis is linked to one electrode peri-event time raster plot on the Raster/Histogram tab. By selecting Copy Image from the right-click menu, you can save the image, and by selecting Capture all images individually, you can export the image files individually for each electrode.

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