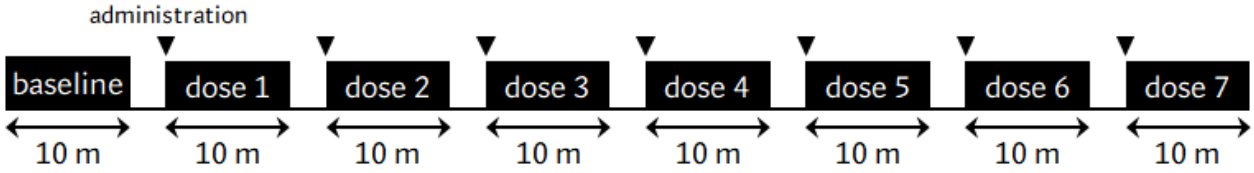
MED64 Burstscope Manual

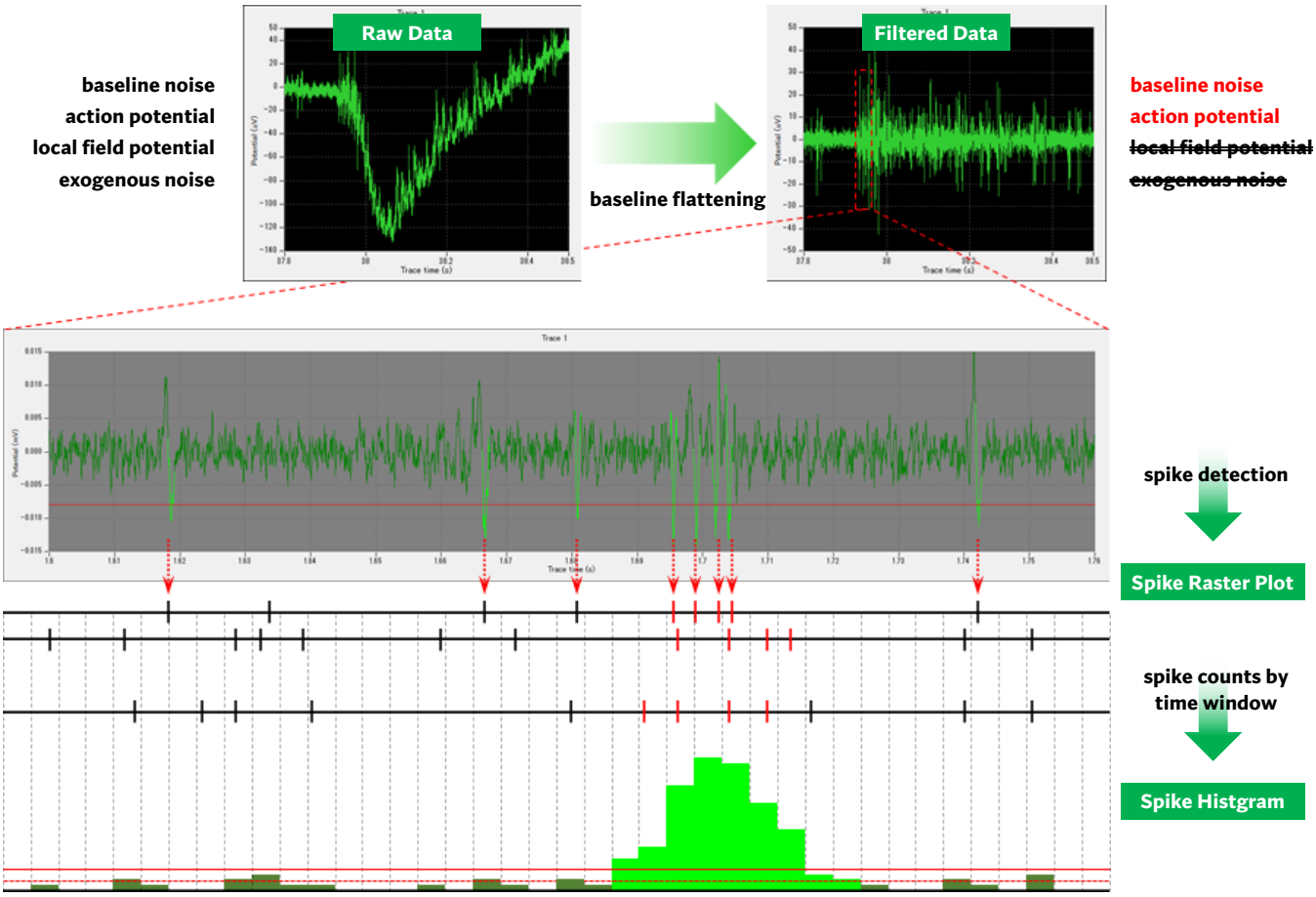
1. Introduction

The MED64 Burstscope is an offline data analysis tool for spontaneous activity exhibited by dissociated neural culture. It is designed for experimental protocols evaluating dose-dependent effects of compounds applied in up to eight escalating doses, and it enables one-click batch processing to obtain results.



Assumed assay protocol. First, the baseline activity is measured for 10 minutes (data acquisition). Then, the process of adding a dose and recording 10 minutes of data is repeated seven times. The data will be a maximum of 8 files in total.

There are many analytical methods for quantitatively evaluating the spontaneous activity of dissociated neural cultures, including different algorithms, and various methods have been developed and are still being devised. However, Burstscope does not employ a method that directly quantifies raw data. Instead, it detects from the raw data the range of action potentials as "spikes," and analyzes the spike trains by focusing on the number of times they are detected and the timing of the detection (see the figure below). This allows the user to obtain information on a series of numerical indicators, such as firing frequency, bursts, and the synchronization of bursts between channels.



Flow chart of spike train analysis in Burstscope.

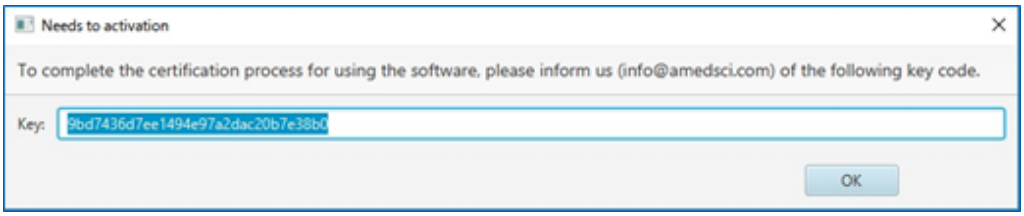
Please note that the analysis does not necessarily require eight data files with eight escalating doses. It is possible to select any 1 through 8 data files with different data recording timings for the batch processing.

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	**: 1920 x 1080**

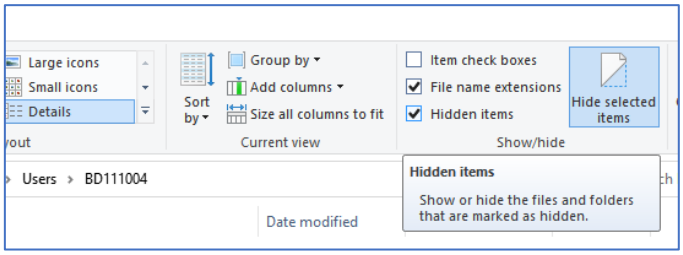
2. Installation

2-1. After double-clicking the installer to install the software, you can use the trial version for 30 days. To continue using the application, you will need to activate it, so please provide us with the Key ID unique to your PC that appears in the pop-up window, and we will send you an activation file that matches the Key ID (for Mobius Spike Sorter users or Burstscope users only).

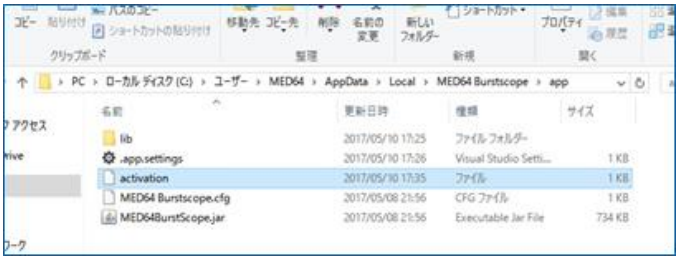


2-2. Move the activation file to the following folder under the C drive.

C:¥Users¥User name¥AppData¥Local¥MED64 Burstscope¥app



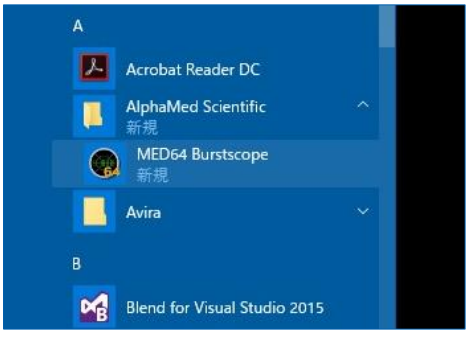
AppData is a hidden item and must be displayed by checking the "Hidden items" checkbox in the "View" tab of the folder settings.



If the activation file is sent as an attached file, it will be named "activation.dat" with the extension.

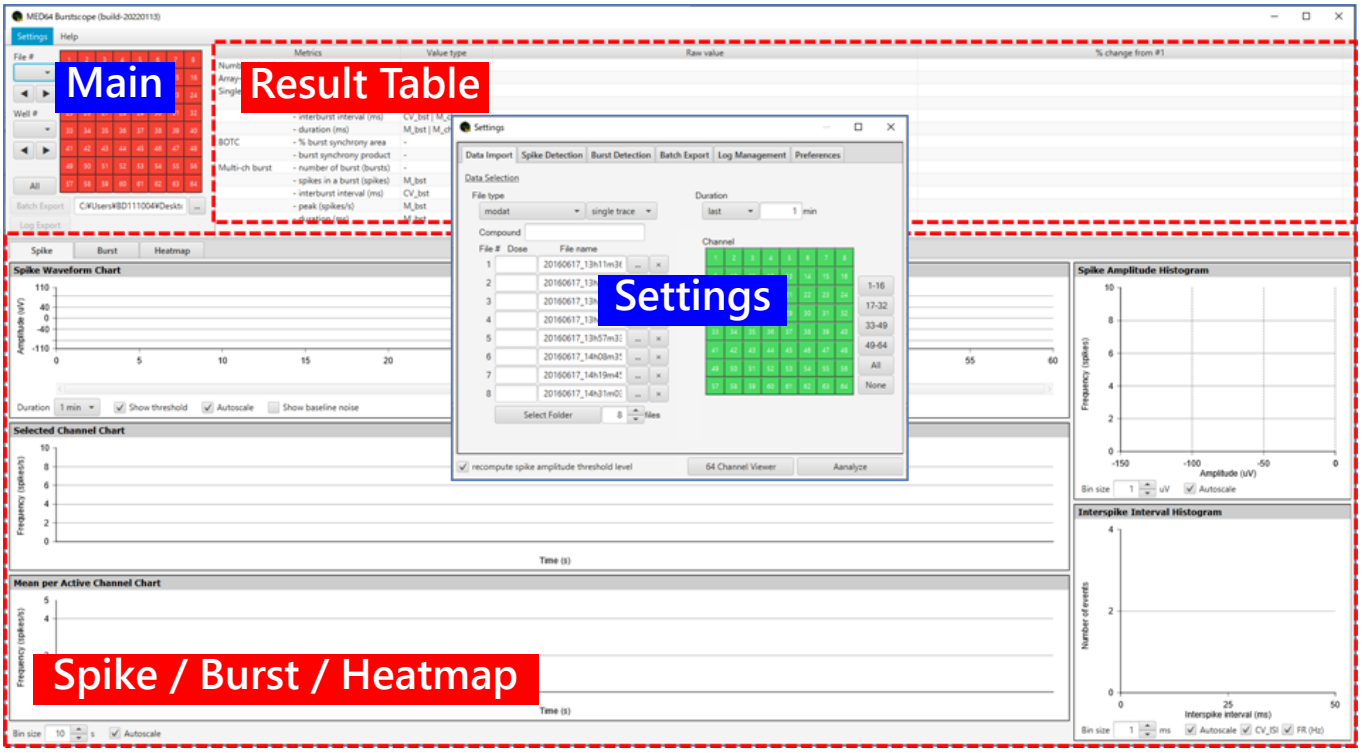
You can not activate the Burstscope as it is, so please delete the extension ".dat" and move it to the app folder.

2-3. Burstscope can be launched by selecting "MED64 Burstscope" from the "Alpha MED Scientific" folder in the "Start" menu.



3. Screen Configuration

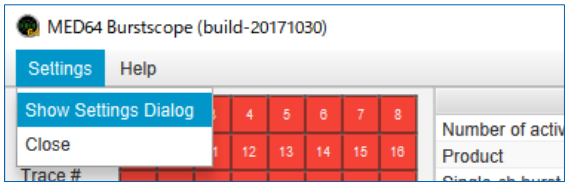
When Burstscope is launched, two windows, Settings and Main, appear as shown in the figure below. In the Settings window, you can enter the analysis conditions, such as an algorithm and threshold values for spike detection, and then click the "Analyze" button to execute the series of processes. The Main window displays the results of the analysis.



The Settings window consists of the following six tabs.

Data Import	Setting of data files, channels, etc., to be analyzed (p.4).
Spike Detection	Setting of spike detection, its pre-processing filters, etc., (p. 5).
Burst Detection	Settings of burst detection (p.6).
Batch Export	Batch output of various results obtained (p.11)。
Log Management	Saving and recalling the analysis result (p. 11).
Preferences	Customizing settings for chart displays (p. 12).

The Main window consists of three areas: a control area such as display channels, a tally table of analysis results, and a switching tab for displaying the results of a Spike/Burst/Heatmap analysis. The basic operation of Burstscope is to set the analysis conditions in the Settings window, execute the process, and check the results in the Main window. Therefore, the most important thing when using Burstscope is to have a good understanding of the analysis methods and the algorithms provided in Burstscope. This enables you to obtain results with a single click without having to learn any complicated operations. The Settings window is automatically closed after the analysis, but it can be recalled from the menu bar of the Main window. You can also enter the analysis conditions again to generate the analysis results.



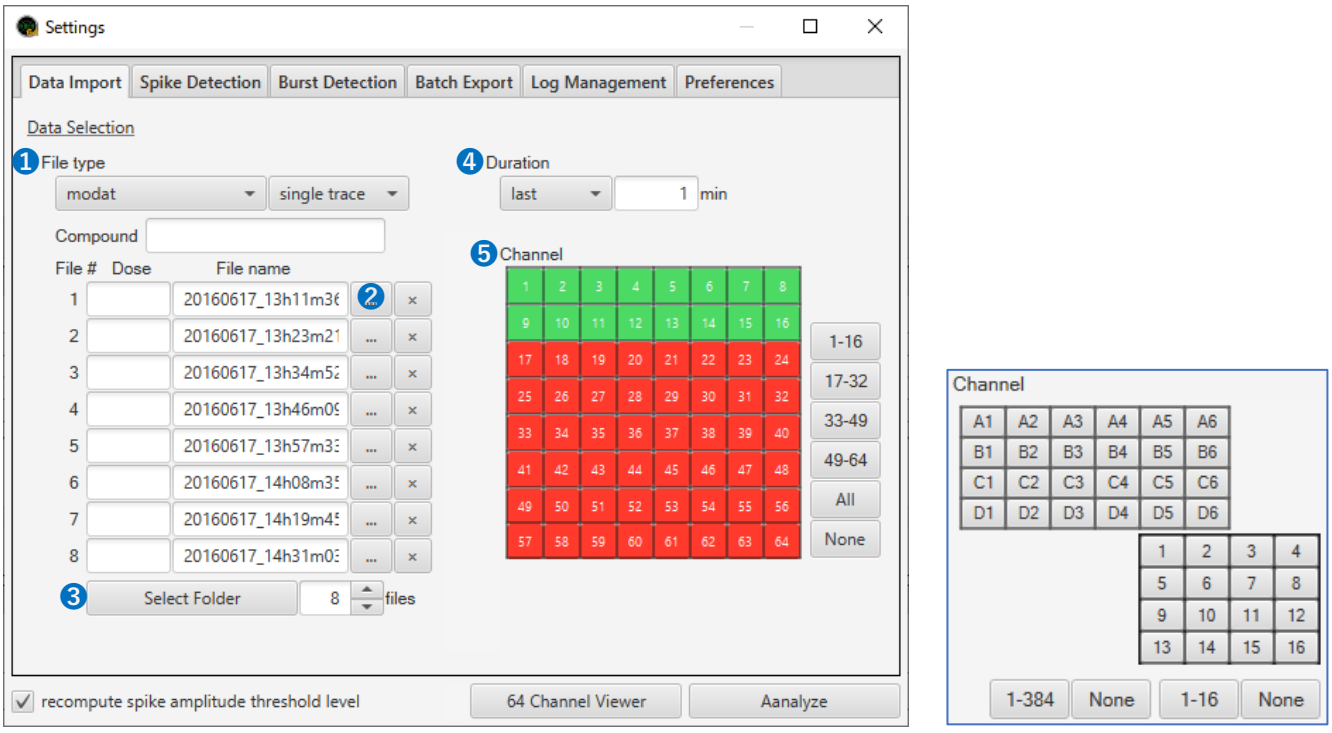
Bring up the Settings window from the Settings → Show Settings Dialog.

4. Data File Selection (Data Import Tab)

The data file formats that can be processed for analysis are as follows.

modat	Original data format of the MED64 system (MED64 Mobius).
csv	Mobius's spike time stamp data format exported by Mobius or Burstscope.
modax	Original data format of MED64-Presto (MED64 Symphony).

Select (limit) the files to be analyzed by clicking ❶ Data Type in the figure below, then click ❷ to select the files individually. If the files to be batch processed are all in one folder, it is also possible to batch select the files in the folder by name. In this case, click ❸ to select the appropriate folder. You do not necessarily need to enter a value in the text box for the name of the compound or dose to be applied (this is to provide a means of capturing labeling information, but as of December 2018, this function has not been implemented).

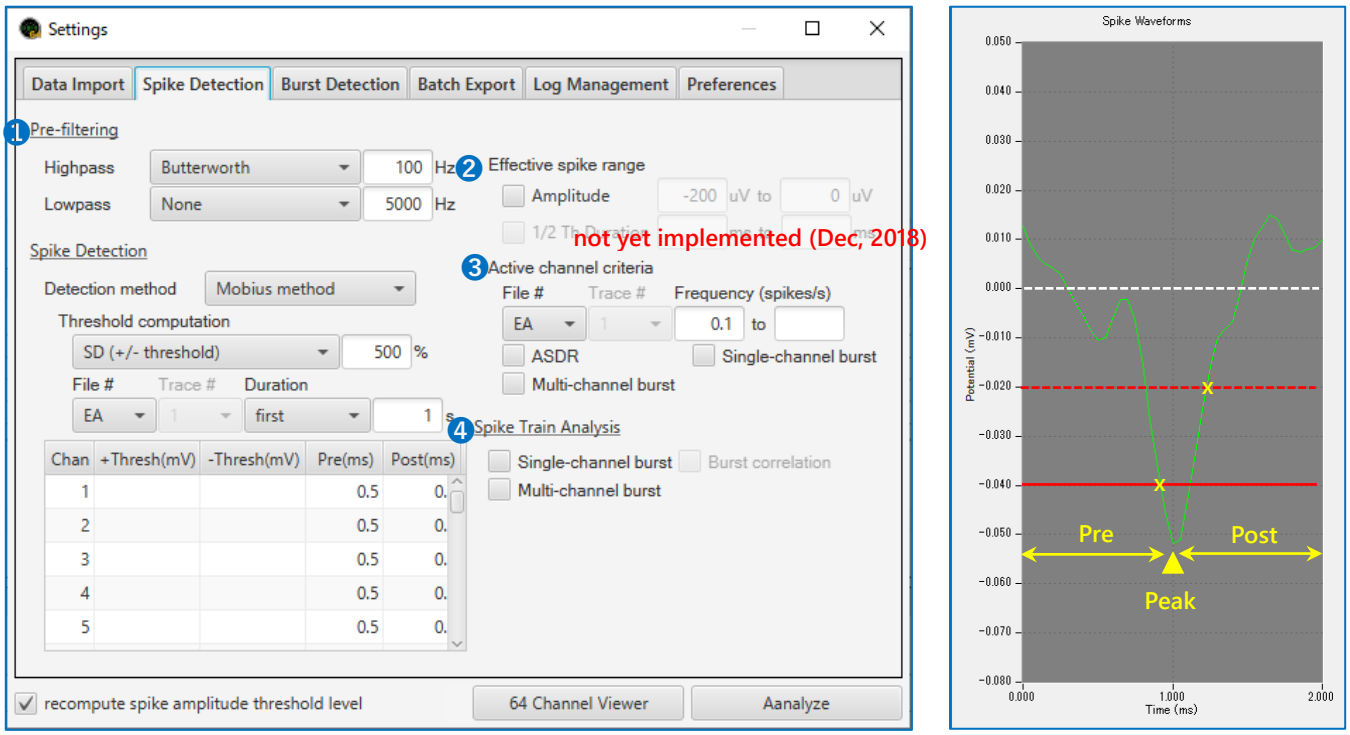


Channel layout will be changed when selected modax.

If you want to analyze only the last minute of a stable response after a compound dosage, for example instead of the entire file length, limit the analysis range with ❹ Duration (the same condition applies to all files processed in batch). If you do not want to analyze all 64 channels obtained in MED64-Quad II, use ❺ Channel to select the target channels (green: channels to analyze, red: channels not to analyze).

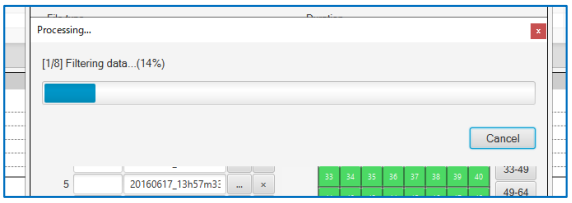
A similar selection and input are required for the other tabs, but since the default values have been entered beforehand, the analysis process can be executed by clicking the "Analyze" button after the file is selected. Burstscope has a "last state preservation specification," which means that all the information you selected and entered is saved when you close the program and is recalled when you start the program again.

5. Spike Detection (Spike Detection Tab)



Spike detection based on the amplitude threshold using the Mobius method: 1. The peak point after crossing the threshold and before reaching the 1/2 threshold is the time stamp point. 2. The specified range before and after the peak point is considered a spike. 3. The next spike detection (threshold judgment) is not performed in the Post range after crossing the threshold.

❶ The spike detection algorithm of Burstscope is identical to that of Mobius, which uses the amplitude threshold (as of December 2018). Filtering can be applied as a preprocessing step for spike detection, but there is one restriction, which is that the upper limit for the low-bass filter is limited to 5000 Hz. Since filtering is very time consuming, there is a function to save the analysis result in the log, and the user can recall it (p. 13).

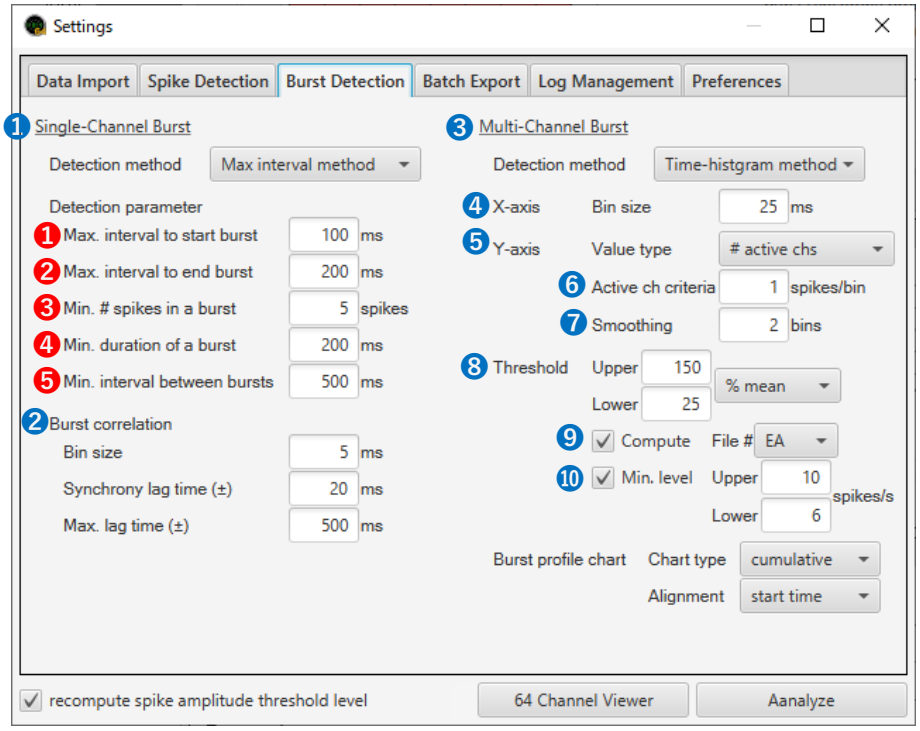


HPF 300 Hz	LFP 5000 Hz	processing time
YES	YES	22m04s
NO	YES	6m15s
YES	NO	22m18s
NO	NO	1m49s

Difference in processing time for 8 files × 10 minutes under identical conditions on a PC that meets the recommended operating environment. Processing time varies by a few minutes depending on the background processing, PC performance, firing frequency, etc.

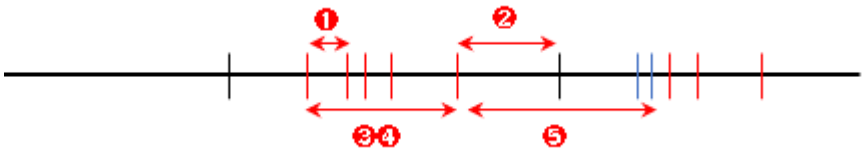
If artifacts with a large amplitude are included in the detected spikes, ❷ the Effective spike range can be used to set an amplitude limit on the waveform to sort the waveform recognized as a spike (lower limit ≤ actual value ≤ upper limit). If there is no signal source (cell) on the electrode and you want to exclude the channel from the analysis, you can set the criteria with ❸ an Active channel criteria (lower limit ≤ actual value ≤ upper limit). The active ch can be decided based on the specific file to be batch processed. You can also apply the decision to all other files (select one of 1–8 from File#), or make an active ch decision for each file (select EA from File#). After the spike detection, ASDR calculation and two types of burst analysis can be performed. For these processes, you can use a check box to indicate whether or not the active channel decision should be reflected for each process. The spike train analysis itself can also be selected in ❹ the Spike Train Analysis.

6. Burst Detection (Burst Detection Tab)



❶ Single Channel Burst

A burst is a phenomenon in which action potentials occur transiently and continuously. The interval of occurrence is recognized based on the scarcity of action potentials before and after the occurrence of the burst. Generally, a burst detection is assumed to be the detection of the relevant interval on the potential chart of a single signal source (a single neuron and a single unit), but in MEA, action potentials of multiple signal sources overlap on a single-channel potential chart. Burstscope does not perform clustering, although it may classify (cluster) detected spikes into individual signal sources based on their shapes before the burst detection. The burst detection is performed based on spike trains obtained from 1-channel potential charts. There are many algorithms available to define burst intervals, but Burstscope offers the Max Interval method (Nextechnologies) as an option (as of December 2018). This algorithm is used relatively frequently and its high detection accuracy has been reported (J Neurophysiol, 116, 306-321). For individual ch raster plots, the range that satisfies the conditional statement shown below is detected as bursts.



❶ 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).

❷ 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).

❸ 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

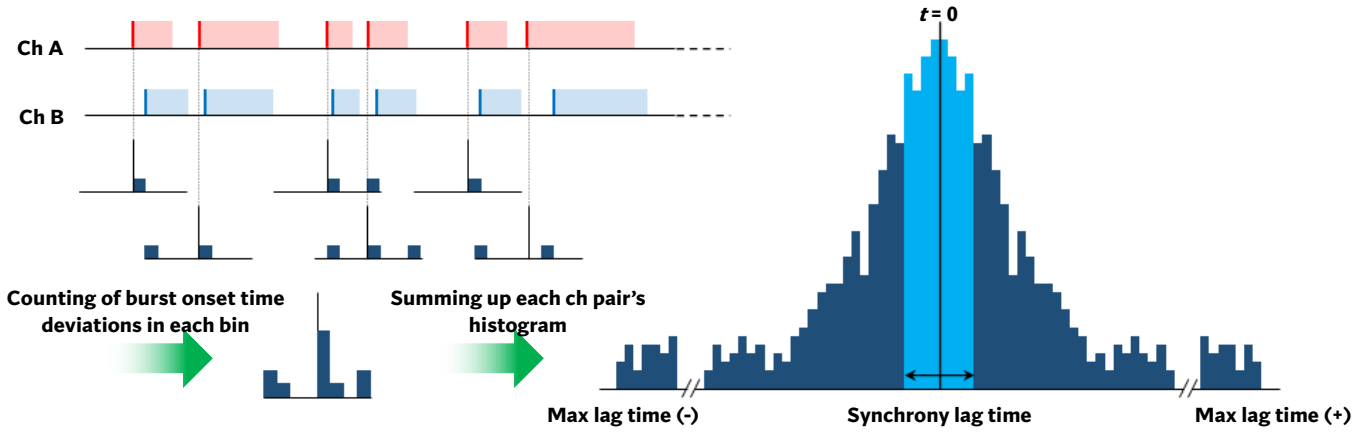
❹ Min. duration of a burst: if the interval from the start spike to the end spike is greater than or equal to the set value (≥), it is judged to be a burst.

❺ 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).

Please note that there are no standardized setting conditions and they vary from report to report. Please set the conditions according to the data you have acquired.

❷ Burst Synchrony

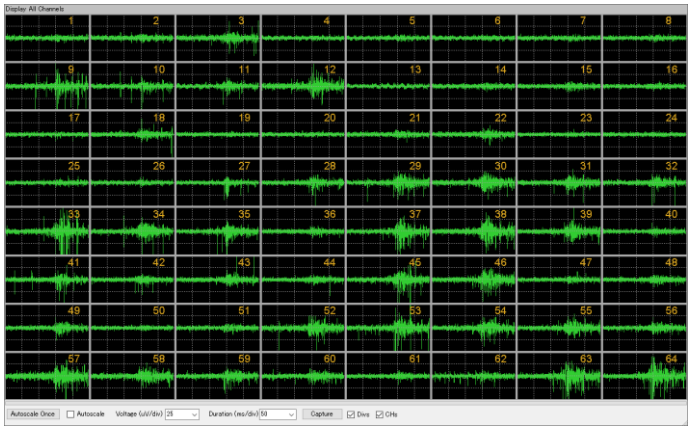
The burst onset time cross-correlogram is an index for evaluating the synchrony of bursts that occur synchronously in the entire array (each channel) and this was originally conceived and implemented in Burstscope. The onset time of a single channel burst detected in each channel is treated as an event sequence, and a cross-correlation histogram is calculated between two different channels. The total of the cross-correlation histograms obtained from a total of 4,032 possible channel pairs (A→B and B→A are treated as different pairs) is totaled to obtain the burst onset time cross-correlogram. If the burst onset times of a channel pair are close to each other, the central area of the histogram will be a large proportion of the total area, and the value obtained by dividing the central area by the total area is used as the synchrony index.



A histogram of the burst onset time of ch B is created by defining the burst onset time of ch A as time 0.
This is done for all bursts of ch A to create a histogram of all the combined bursts.

❸ Multi Channel Burst

In contrast to so-called single-unit bursts, there are bursts that are unique to multi-electrode measurements such as MEA. These bursts that occur simultaneously in the entire array (multiple channels) are referred to here as synchronized bursts (see figure below).



64-channel raw waveform chart when synchronized bursts occur

Although several names and detection algorithms have been devised, Burstscope uses a detection method of ❹ separating the horizontal axis by an arbitrary fixed time (bin) and ❺ creating a histogram that adds together the total number of spikes detected in all channels and the number of active channels on the vertical axis. This method detects synchronized bursts in a range exceeding the threshold (see next page). The vertical axis can be selected from the following options.

ASDR The ch mean of the firing frequency (spike/s), which is the total number of spikes in the active ch of each bin divided by the bin size, further divided by the number of active ch.

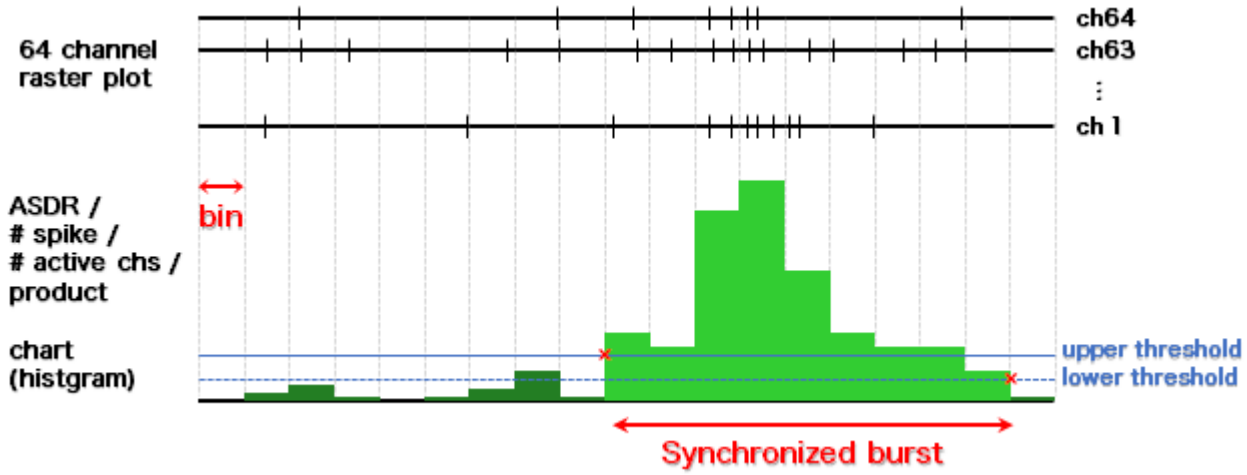
 # spikes Total number of spikes for each bin.

 # active chs Number of active chs in each bin. The base firing frequency can be specified (❻).

product Total number of spikes in each bin × Number of active chs.

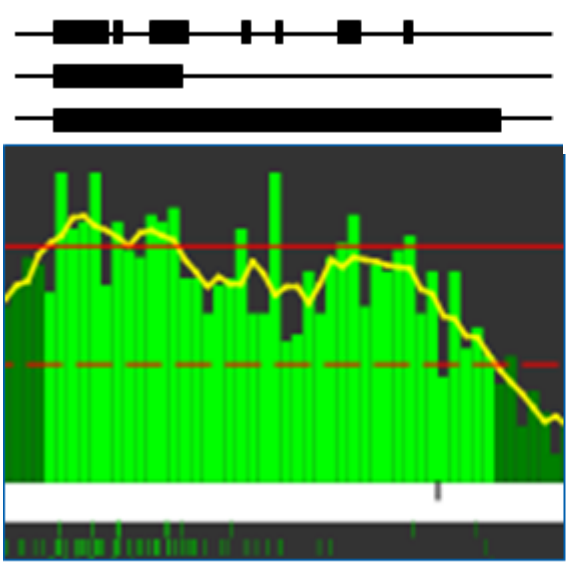
This time histogram-based detection method is from Mukai et al., in "Analysis of spontaneous activity transition processes during neural network formation (2002)" (at the time # spikes is selected) and Pelt J et al., in "Long-term characterization of firing dynamics of spontaneous bursts in cultured neural networks (2004)" (product selected, active ch criteria set to 1 spikes/bin).

*The interface has been changed since the 181217 version. The rate threshold in the detection algorithm of the previous version corresponds to ASDR on the vertical axis, and the network burst corresponds to the product on the vertical axis (the active ch criteria is 1 spikes/bin). In the previous version, the lower threshold was set to 50% of the automatically calculated threshold (fixed).



The algorithm for detecting synchronized bursts. Note that the information is processed and compressed from raw data to raster plots and then to ASDR charts. The correct idea is to find the synchronized burst interval by looking at the raster plot (which involves the subjectivity of the researcher), and then to find the algorithm and analysis conditions to successfully detect the range of the raster plot. The detection range of the synchronized bursts starts from the point where the value exceeds the upper threshold (set value < actual value) and ends where it reaches the lower threshold (set value ≥ actual value).

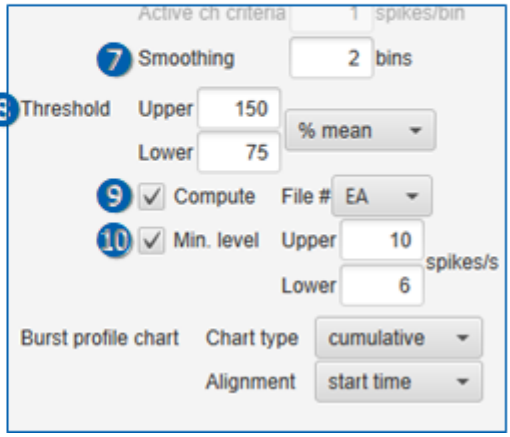
Burstscope has the option of ❼ smoothing the time histogram when determining the threshold, and the option of ❽ applying two different threshold levels at the start and end of detection, similar to the spike detection in Mobius.



Threshold determination with a histogram

Threshold determination with a smoothed histogram

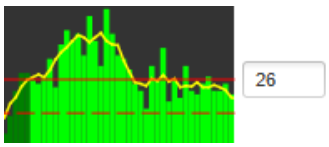
Threshold determination with a smoothed histogram and two threshold levels



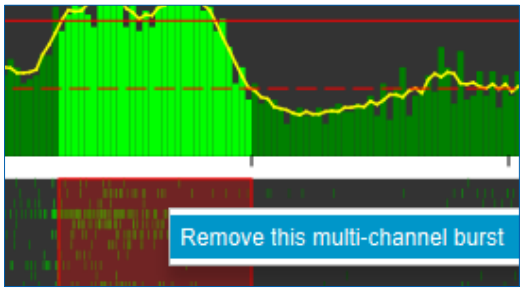
The yellow broken line is the smoothed histogram.

The threshold can also be ❾ automatically calculated based on the value of the histogram, but if the total number of spikes detected is scarce, the threshold may be set at an extremely low level. To avoid this, a ❿ minimum threshold can also be set. For data files for which the automatically calculated threshold is below the minimum threshold, the minimum threshold will be set as the threshold.

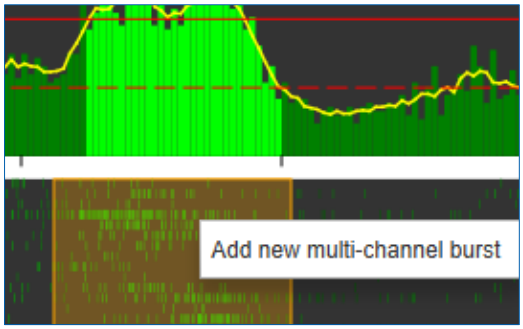
The threshold can be manually changed on the time histogram, but for a time histogram recalled from the analysis log, the threshold cannot be manually changed. If the upper threshold is changed to lower than the lower threshold, the burst duration is determined only by the upper threshold.



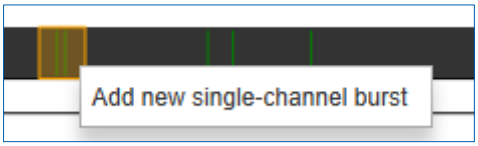
In addition, you can visually determine the synchronized burst duration and manually make adjustments.



Move the mouse over a burst duration on a 64ch raster plot and delete it using the right-click menu.



Click-and-drag on the 64ch raster plot to add a synchronized burst duration using the right-click menu.



For a single channel burst, the burst duration can also be changed on the raster plot.

7. Main Window

7-1. Control Area and Result Table

In the control area, you can select the file number and ch to display, execute a batch output of detailed results, or manually create an analysis log file. In the result table, you can use the drag-and-drop menu to select a range, and select "Copy to Clipboard" from the right-click menu to copy and paste the range into Excel or another spreadsheet.

Result Table

Number of active channel　Total number of chs that exceed the set firing frequency

Array-wide spike detection rate　ch mean of mean firing frequency (per unit time) of active ch

Single ch burst - number of bursts　[ch mean/SD/median] of the total number of bursts within the measurement time.

- spikes in a burst　[ch mean/SD/median] of the burst mean of the number of spikes per each burst
- interburst interval　[ch mean/SD/median] of the mean value of each interburst interval
- burst duration　[ch mean/SD/median] of the burst mean for each burst length

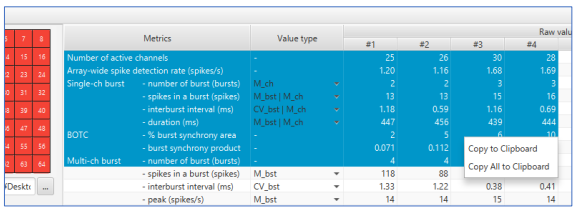
BOTC - %synchrony area　Central area of cross-correlation/total area

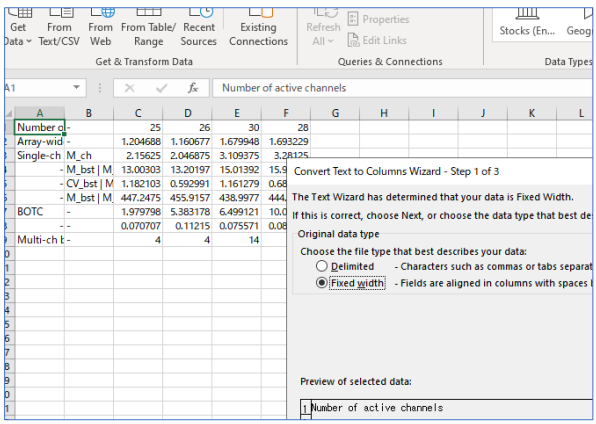
- synchrony product　Central area of cross-correlation^2/Total area

Multi ch burst - number of bursts　Total number of bursts within the measurement time

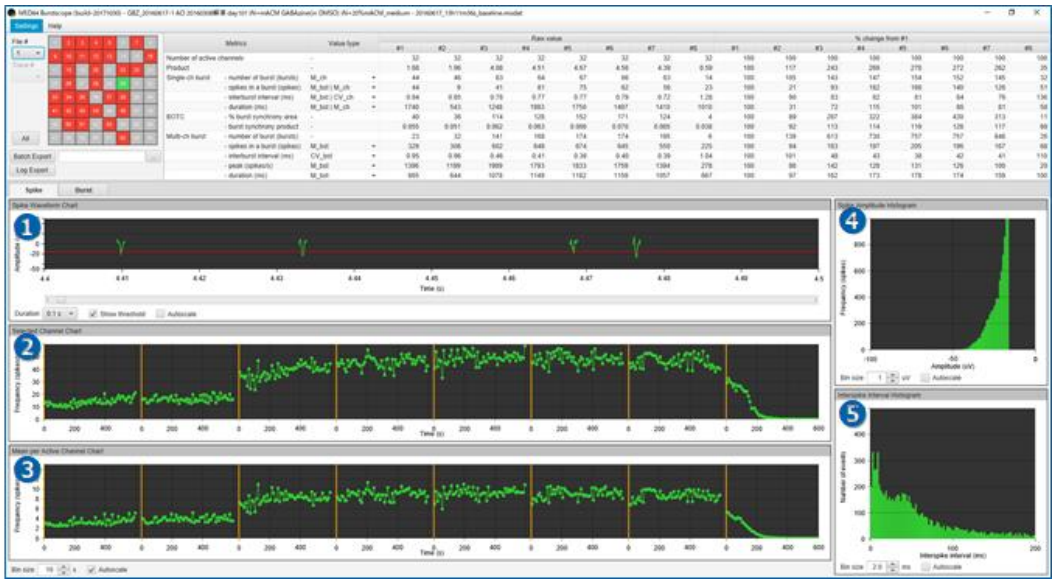
- spikes in a burst　[Burst mean/SD/median] of spikes per each burst
- interburst interval　[Mean/SD/median] of each interburst interval
- burst peak　[Mean/SD/median] of each burst peak
- burst duration　[Mean/SD/median] of each burst length

The parameters obtained for the detected spikes and bursts are shown above. In this case, there is options to choose how to calculate the representative value to be aggregated as a parameter. For a single channel burst, after calculating the [Mean/Median/Standard Deviation/Coefficient of Variation] of bursts in each channel, the [Mean/Median/Standard Deviation/Coefficient of Variation] between those channels is also calculated. Burstscope performs aggregation on all of these combinations, but only one combination can be displayed in the result table. You can use the drag-and-drop menu to select a range in the result table, and select "Copy to Clipboard" from the right-click menu to copy and paste the range into Excel or another spreadsheet. To copy the entire area, select "Copy All to Clipboard." If the data is displayed in a single column in Excel, select "Text to Columns" from the "Data" tab and display the data as comma-delimited cells to create the same cell positions as in Burstscope.



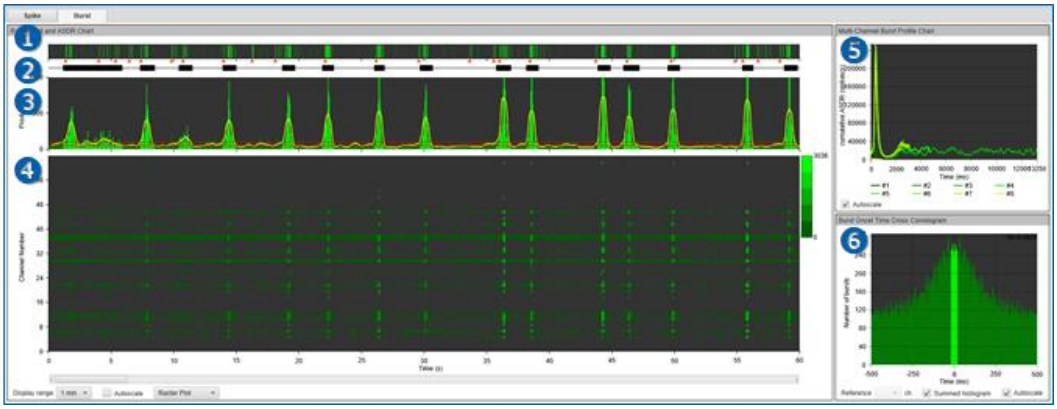


7-2. Spike Tab



❶ Spike waveform
❷ Firing frequency of selected ch
❸ Mean firing frequency of active ch
❹ Spike amplitude histogram
❺ Inter-spike interval histogram

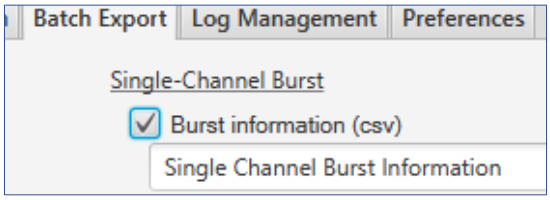
7-3. Burst Tab

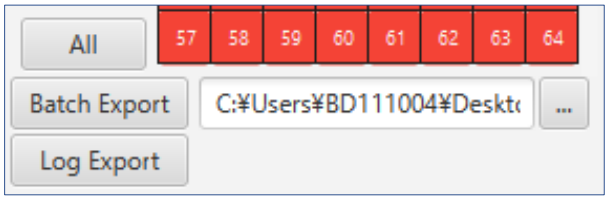


❶ Raster plot of individual ch.
❷ Bar chart of intervals detected as synchronized bursts
❸ Histogram of firing frequency of all active chs separated by bin
❹ Raster plot of all active ch
❺ Accumulated waveform of synchronized bursts
❻ Cross-correlation histogram with single ch burst start time as event

8. Batch Export of Detailed Results -Batch Export Tab-

The channels for spikes and bursts, the detailed data for each burst, and the numerical data for charts can be exported to a file by clicking the "Batch Export" button in the Main window after the analysis is complete. However, by default, nothing is exported, so please check the files to be exported in the "Batch Export" tab of the Settings window. The output file will have the name of the data file with the text entered in the text box.





Spike Detection

Filtered raw data (modat) *Not supported in the 20181217 version.

Spike time stamp (csv) Information on the detection time of each channel and each spike.

Spike frequency chart (csv) Information on the number of spikes detected per second for each channel.

Spike amplitude histogram (csv) Numerical data of the amplitude histogram for each active channel.

Spike ISI histogram (csv) Numerical data of the ISI histogram for each active channel.

Single-Channel Burst

Burst information (csv) Information about each channel, the start time of each burst, the interval from the preceding burst, the number of spikes, and the burst width.

Multi-Channel Burst

ASDR chart (csv) Numerical data for the chart.

Multi-channel burst profile chart (csv) Numerical data for the chart.

Burst information (csv) Information about the start time of each burst, the interval from the preceding burst, the peak amplitude within the burst interval, the number of spikes, and the burst width.

Intra-burst spike time stamp (csv) Spike timestamp information included with each burst.

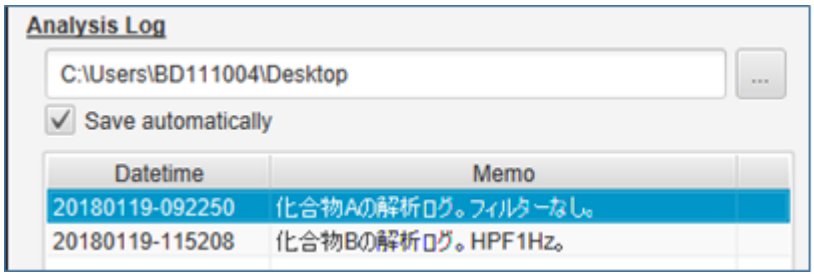
Burst Correlation

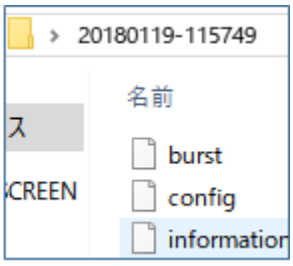
Synchrony index (csv) Synchrony index of all combinations of chs for which data exists.

Burst onset time cross correlogram (csv) Cross-correlation histogram of burst start times (user-specified accumulation/mean).

9. Saving and Recalling Analysis Logs -Log Management Tab-

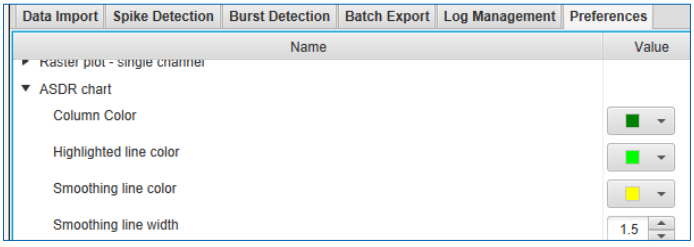
When the results of the analysis such as a tally table are saved, but you want to revisit the post-analysis chart after some time, it takes time to process the chart again if the analysis results include filtering. Therefore, if you check the "Save automatically" checkbox in the "Log Management" tab of the Settings window before analysis, you can generate a log that can immediately recall the analysis results after analysis processing. You can also generate a log by clicking the "Log Export" button in the Main window after analysis. The log is generated as a folder named after the analysis date and time, and it contains several configuration files. Logs can be managed with notes and the analysis results are displayed in the Main window by selecting the log to be recalled and clicking the "Restore" button.





10. Display Customization -Preferences Tab-

The appearance of the chart, such as its line thickness and background color, can be customized to your liking from the "Preferences" tab of the Settings window.



11. Change Allocation of Memory Used

MED64 Burstscope is configured by default to use up to 10 GB of memory (assuming a 12 GB PC, leaving 4 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 Burstscope.cfg file in a text editor at

C/user/user name/AppData/Local/MED64 Burstscope/app

Correct the below (e.g., Xmx12000m → Xmx16000m for 12 → 16 GB) and save it to change the memory used.

[JVMOptions]
-Xmx12000m

Version: 190620

■ Manufacturing
Alpha MED Scientific, Inc.
Saito Bio-Incubator 209, 7-7-15, Saito-asagi, Ibaraki, Osaka, 567-0085, Japan
E-mail: support@med64.com
Website: https://alphamedsci.com/english

■ Sales & Technical Support
SCREEN Holdings Co., Ltd. Bioscience & Pharmaceuticals
Furukawa-cho 322, Hazukashi, Fushimi-ku, Kyoto 612-8486, Japan
Website: https://screen-cell3imager.com/english