

SpikeTaro Version 1.0

User's Manual

(Manual version 1.0.rev2)

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1 Introduction

SpikeTaro is a program which has noise filtering function, high level analyzing (spike sorting and clustering) functions for neurophysiological data.

SpikeTaro's features include:

- Noise cutoff function by digital filter.
- Separation of single spike for synthesized signal of multi-spike waveform during period of spike duration.
- Spike Sorting based on comparing with spike waveforms by correlation coefficients.
- Clustering by conventional clustering method (Ward Method).
- Producing raster plot.
- Output publication-quality graphics

2 How to use SpikeTaro

2.1 Start up

Important: Login Windows system as administrator to use SpikeTaro.

Click SpikeTaro's shortcut on your desktop of Windows system, or SpikeTaro's icon located in "Windows Start Menu" of [Start] - [CJS] - [SpikeTaro 1.0]. SpikeTaro will start.

At first starting time, inputting of license file (file extension is ".license") is required. Please select license file in "License CD" in [License Authentication] dialog.

2.2 Main menu

FIRst of all, main menu window is opened.

🐰 SpikeTaro 1.0 📃 🗖 🔀	
File Open	[File Open] Button
EIR Filter	[Filtering] Button
Sorting	[Sorting] Button
Clustering	[Clustering] Button
About	[About] Button

Main menu

2.3 Loading physiological data

In order to load physiological data to SpikeTaro, user clicks [File Open] button. After that, [Open data file] dialog opens. By user selecting input file and clicking [OK] button in [Open data file] dialog, [Load Physiological Data] dialog, which is inputted data parameters, opens.

Wav Data Input Physiological Data Data Record Count: 2 Scale Factor for Y: 1,00 Stimulation Unit: Image: Data Includes Stimulation Record Unit: Stim Data Channel: 2 Stim Threshold: 0,000 Disable Stimulation Display Stim Data Column Number: Image: Data Includes Stimulation Record Stim Threshold: Stim Threshold: 0,000 Disable Stimulation Display Stim Data Column Number: Stim Data Column Display Stim Data Stimulation Record) (Hz)) (M

[Load Physiological Data] Dialog

SpikeTaro supports file types of wav and text as input data format.

2.3.1 Wav File

By selecting wav file in [Open data file] dialog, [Wav Data Input] group in the [Load Physiological Data] dialog will be active.

• SpikeTaro supports only PCM (non-compression) type wav file.

Following parameters can set in the [Load Physiological Data] dialog.

• Data Record Count: Input total data channel count in the wav file. In case of stimulation channel is included in the wav file, the count includes stimulation channel.

- Scale Factor for Y: If necessary, input scale factor for physiological data. This factor operates on all channels of physiological data except stimulation data.
- Data Includes Stimulation Record: In case of including stimulation record in input data, check this check box.
- Stim Data Cannel: Input channel number of stimulation channel. Count starts at "1".
- Stim Threshold: SpikeTaro determines to turn stimulation on/off by this threshold.
- Disable Stimulation Display: If you don't want to display stimulation marker in charts and raster plot, check this check box.

2.3.2 Text File

By selecting text file in [Open data file] dialog, [Text Data Input] group in the [Load Physiological Data] dialog will be active. ASCII text type is required for input data.

SpikeTaro accepts following text format;

- Tab separated text data
- Comma separated text data (CVS Format)

[LF] and [CR-LF] is available as Linefeed code.

SpikeTaro requires tabulated data as input data. An example is shown as follows;



Input data format (exsample)

SpikeTaro skips header part of input data.

In [Load Physiological Data] dialog, set up the following parameters.

- Data Includes Time Column: If input data include time column, turn on this check box.
- Time Data Column Number: Input column number of time (count from "1").
- Unit: Choice unit of time. [s], [ms], [µs] is available.
- Recording Rate: In case of data does not include time column, input sampling frequency of input data.
- Scale Factor for Y: If necessary, input scale factor for physiological data. This factor operates on all channels of physiological data except time and stimulation columns.
- Data Includes Stimulation Record: In case of including stimulation record in input data, check this check box.
- Stim data Column Number: Input column number of stimulation record.
- Stim threshold: SpikeTaro determines to turn stimulation on/off by this threshold.
- Disable Stimulation Display: If you don't want to display stimulation marker in charts and raster plot, check this check box.



After clicking [OK] button, the chart of raw data is displayed.



Above figure is a result of loading sample data including program CD (File name: SampleData.txt). Stimulation marker was shown as heavy line under physiological chart. About chart treatment, see section "2.9 Chart treatment".

2.4 Filtering

SpikeTaro is implemented FIR (finite impulse responses) filter which is liner phase digital filter. By clicking [FIR Filter...] button at main menu, the [FIR Filter Setting] dialog opens.

FIR Filter Setting	
Number of Impulse Responses:	Amplitude Characteristics
Low Cutoff Frequency [Hz]:	
🔲 Hight Pass Filter	
High Cutoff Frequency [Hz]:	
<u>D</u> isplay	
Phase Delay: 0 [points] 0 [ms]	
<u>OK</u> <u>C</u> ancel	

[FIR Filter Setting] dialog

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- Number of Impulse Responses: Choice number of impulse response. In case of choosing lager number, frequency cutoff characteristic becomes to be sharper. However phase delay of filtering data increases.
- Low Cutoff Frequency [Hz]: Input low side of cutoff frequency. If you want to use low pass filter, input "0".
- High Pass Filter: If you want to use high pass filter, check this box.
- High Cutoff Frequency [Hz]: Input high side of cutoff frequency.
- [Display] button: Clicking this button, amplitude characteristic corresponding with setting parameters is shown in chart area. And phase delay information (number of data point and delay time) are displayed under the [Display] button.



[FIR Filter Setting] Dialog (after setting parameters)

Supplemental information

FIR Filter Setting Number of Impulse Responses: Amplitude Characteristics 513 * 10 Low Cutoff Frequency [Hz]: 1 120 😂 Ampulitude Gain [-] 0.1 Hight Pass Filter 0.01 0.001 High Cutoff Frequency [Hz]: 1800 😂 0.0001 1E-05 <u>D</u>isplay 1E-06-Phase Delay: 256 [points] 0 2000 4000 6000 8000 12.8 [ms] Frequency [Hz] <u>C</u>ancel <u>O</u>K FIR Filter Setting Number of Impulse Responses: Amplitude Characteristics 513 💌 10 Low Cutoff Frequency [Hz]: 1 120 😂 Ampultude Gain [-] 0.1 0.01 Hight Pass Filter 0.001 High Cutoff Frequency [Hz]: 0.0001 1800 😂 1E-05 <u>D</u>isplay 1E-06 1489 2289 1089 1289 1689 1889 2089 2489 Phase Delay 256 [points] 12.8 [ms] Frequency [Hz] 0 1 Þ <u>C</u>ancel <u>O</u>K

User can magnify frequency range by dragging chart area.



A slide bar is appeared in magnified chart. To reset scale, click circle shaped button located in left side of the slide bar. [OK] button: By clicking [OK] button, FIR filter is applied to the input data. After finishing filter process, SpikeTaro shows FIR filtered result as chart.

Important information

Filtering process executed by improper parameters often lead to incorrect sorting result. If spike height changes significantly comparing with before and after filtering process, not only noise but also spike frequency elements are probably cut off. In such a case, user should better readjust cutoff parameters in [FIR Filter Setting] dialog.



FIR filtered data

About chart treatment, see section "2.9 Chart treatment".

2.5 Sorting

By clicking [Sorting...] button in main menu, the [Sorting and Clustering Setting] dialog opens. Sorting process is applied to most recently processed FIR filtered data.

Sorting and Clustering Setting	
Sorting Parameters BaseLine Threshold: 0.003 Contribution Ratio (r^2): 0.990 Assume the Same Spike with Spike Height Difference: 10	Ignored Spikes The clusters which include spike count of 2 ♀ [%] for total spike count are ignored. The clusters which include less than 2 ♀ spikes are ignored.
Clustering (Ward Method) Number of Cluster: 10	Output Items Sorting Results Raster Plot OK Cancel

[Sorting and Clustering Setting] Dialog

[Sorting and Clustering Setting] dialog consists of several parameter groups.

- (1) [Sorting Parameters] Group
- Baseline Threshold: Signal selection threshold. When signal peak is larger than this threshold, the spike data, which includes raising phase, peak and falling phase, is detected as sorted target.

For example, user sets this value to 5.0 (red horizontal line) against following data, three spike waveforms (red waveforms) are detected as sorted targets.



Baseline threshold ensample

- Contribution Ratio (r²): SpikeTaro sorts spikes by correlation coefficient between two spike waveforms. This is a threshold which two spike waveforms belong in the same spike group (we call cluster below). To increase this value, the judge level that two spikes belong in the same cluster becomes severer.
- Assume the Same Spike with Spike Height Difference [%]: Sometimes, spike height changes during recording even though the spike evokes from the same origin (for example, bursting vs. non bursting condition, changing recording condition depending on time, etc). This parameter indicates tolerating percentage of spike height thought that spikes evoked from the same origin.

(2) [Ignored Spikes] Group

- The clusters which include spike count of X% for total spike count are ignored:
- The clusters which include less than X spikes are ignored:

Above two parameters indicate setting condition that clusters of sorting results are ignored. Now, if sorting process output a lot of clusters and in each cluster includes few spikes, user can ignore such the clusters using those parameters.

For example,

- Input data includes 1000 spikes.
- After sorting, 20 clusters were created.

- In those 20 clusters, 5 clusters include over 150 spikes.
- Remaining 15 clusters include less or equal to10 spikes.

In above situation, this parameter set value at 1% or 10, SpikeTaro ignores the 15 clusters which include less or equal to 10 spikes.

(3) [Output Items] Group

- Sorting Results: To check this box, SpikeTaro outputs sorting result as waveform.
- Raster Plot: To check this box, SpikeTaro outputs raster plots of each separated cluster.

To click [OK] button in [Sorting and Clustering Setting] dialog, sorting process starts. After finishing the process, sorting results are shown on your display.

Sorting Result 1: Cluster information

🐺 Sorting Results Summary	
+++++++++ channel 1 ++++++++++	~
Detected Spike Count = 548 Number of spikes including Cluster1 = 15 Number of spikes including Cluster2 = 57 Number of spikes including Cluster3 = 53 Number of spikes including Cluster4 = 18 Number of spikes including Cluster5 = 15 Number of spikes including Cluster6 = 13 Number of spikes including Cluster7 = 60 Number of spikes including Cluster8 = 11 Number of spikes including Cluster9 = 22 Number of spikes including Cluster10 = 16 Number of spikes including Cluster11 = 12 Number of spikes including Cluster12 = 15 Ignored Spike Count = 241	
E	~ 1:

Cluster Information window

This window indicates follows information

- Total spike count detected by SpikeTaro.
- Spike count in each cluster.
- Detected but ignored spike count.

By drag lower right side of the window, user can change the window size.

Sorting Result2: Waveform of each cluster

SpikeTaro outputs waveform of each cluster. Title bar in each waveform window shows cluster id which correspond with above Cluster Information.



Waveform in each cluster

About chart treatment, see section 2.9 Chart treatment.

Sorting Result3: Raster plot

SpikeTaro also outputs raster plot separated by each cluster id.





About the plot treatment, see section 2.9 Chart treatment.

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2.6 Clustering

SpikeTaro can execute Ward method of conventional clustering method. This process is independence on above spike sorting process. In this process, SpikeTaro uses spike height and spike duration as clustering parameters.

By clicking [Clustering...] button in main form, the "Process Parameters" dialog is opened again. Clustering process is applied to FIR filtered data.

10 📚 [%]	iclude less than 🛛 2 📚
Clustering (Ward Method) Number of Cluster:	

[Sorting and Clustering Setting] Dialog

- (1) Clustering (Ward Method)
- Number of Cluster: Set number of cluster separated by Ward method. Default is cluster count gotten by previous sorting process.



By clicking [OK] button, clustering result is displayed.

Clustering result

About the plot treatment, see section 2.9 Chart treatment.

2.7 Information about SpikeTaro

By clicking [About] button in main menu, SpikeTaro's version number, license information and contact information are displayed.

2.8 Exit SpikeTaro

To Exit SpikeTaro, Click " \times " button in title bar of the main menu.

2.9 Chart treatment

2.9.1 Time charts

In this section, we explain treatment methods of time charts which are "Raw Data" chart, "FIR Filtered Data" chart and "Sorting Results" chart. For above charts, the treatment methods are common. Now we explain the treatment methods using "Sorting Results" chart.

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(1) Change window size

Time chart is displayed by following format that outer window includes respective time charts. Size of the outer window can be changed by dragging size grip mark located in right bottom of outer window.

Obsetter ID Sorti Image: Construction ID Image: Cons	C C	er ID 3 Sorti	Cluster 10 4 Sorti
Obstater ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 6 Sorti Image: Charter ID 6 Sorti 1 Image: Charter ID 10 Sorti Image: Charter ID 10 Sorti 1 Image: Charter ID 10 Sorti Image: Charter ID 10 Sorti	111	Time	00 06 13 19 26 32 13 63 13 63 14 64 Time
Cluster ID 9 Sorti	Clust	er D 7 Sorti .	Cluster 10 8 Sorti
a - 3500-003 + + + + + + + + + + + + + + + + + +	💶 🗖 🔀 Clust	er ID 11 Sort	Cluster ID 12 Sort

Size grip mark

After change outer window size, [Windows Control] - [Tile] button is appeared by right mouse button clicking at gray region of outer window. When user selects [Tile], respective time charts are rewritten corresponding with size of outer window.

🛃 Oh 1 Sorting Result Data				
🛃 Cluster ID 1 Sorti 🔳 🗖 🔀	🛃 Cluster ID 2 Sorti 🔳 🗖 🗙	🛃 Cluster ID 3 Sorti 🔳 🗖 🗙	🛃 Cluster ID 4 Sorti 🔳 🗖 🗙	
4616-1453 *262-503 *262-503 *100-	00 00 13 19 26 32 13 63 14 63 14 61 4 Time	-11/e-000 00 00 13 19 26 32 19 60 16 14 Time	-3.51e-003 00-005-13-19-20-32 -3.51e-003 00-005-13-19-20-32 13-63-14-64 Time	
🛃 Cluster ID 5 Sorti 🔳 🗖 🔀	🖁 🛃 Cluster ID 6 Sorti 🔳 🔳 🔀	🛃 Cluster ID 7 Sorti 🔳 🖬 🔀	🛃 Cluster ID 8 Sorti 🔳 🗖 🔀	
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Changing outer window size and rewrite respective time chart

- (2) Treatment of respective time charts
- Changing window size

Respective time charts can move freely within the outer window by dragging these title bars. Moreover, user can change the window size by dragging size grip mark (red circle in under figure).



• Magnification display of chart data

User can magnify displayed data in the time char by dragging area of the chart. Vertical and horizontal scroll bars are appeared in magnified chart. To reset scale, click circle shaped button located in top or left side of the slide bars.



• Popup menu

Popup menu is appeared when user clicks time chart area by right button of mouse.





Popup menu includes [Save Graphics...], [Save as Text...] and [Properties...] buttons.

- [Save Graphics...]: Save displaying chart as graphics file. As Saving graphics format, Enhanced Meta File (.emf), Bitmap (.bmp), PNG (.png) are available.
- [Save as Text...]: Save displaying chart as text file. Tab separated text data and comma separated text data (CVS Format) are available.
- [Properties...]: Setting of parameters for chart display. User selects this, [Graph Properties] window will opens.

Graph Properties	
Axis Range X Axis Range Min: Y Axis Range Min:	Max: Auto Max: Auto
Axis Titles X Axis Title: Time Font MS UI Gothic (9) Y Axis Title: Amplitude Font MS UI Gothic (9) Axis Labels X Axis Label Font Setting: Font MS UI Gothic (9) Y Axis Label Font Setting: Font MS UI Gothic (9)	Title and Line Graph Title: Line Color: Black
	<u>QK</u> <u>C</u> ancel

User can set ranges of X, Y axises (maximum, minimum), auto range, titles of axises, font (size, type), chart title, line color and width. By click [OK] button, these parameters are reflected.

2.9.2 Raster plot

(1) Change window size

Size of the raster plot can be changed by dragging size grip mark located in right bottom of raster plot window.

(2) Popup menu

Popup menu is appeared when user clicks raster plot area by right button of mouse. The functions of menu [Save Graphics...], [Properties...] are the same of above popup menu of time chart, except setting parameters of [Marker Color] and [Marker Size].

• When user clicks [Save As Text], spike peak time sequence of each cluster will be output.



2.9.3 Clustering results plot

(1) Change window size

Size of the clustering results plot can be changed by dragging size grip mark located in right bottom of the plot window.

(2) Change marker size

User can change marker size by clicking $[\blacktriangle]$ or $[\blacktriangledown]$ buttons located in right bottom of the window.

(3) Popup menu

Popup menu is appeared when user clicks the plot area by right button of mouse. The functions of menu [Save Graphics...], [Properties...] are the same of previously explained in time chart, except setting parameters of [Marker Color] and [Marker Size].



3 Contact us

Contact information about SpikeTaro is as follows:

SpikeTaro customer support TEL: +81-75-321-7300 FAX: +81-75-321-7305 E-mail: spiketaro@chino-js.com

We also announce new SpikeTaro information in SpikeTaro home pages. Pease visit our home pages.

SpikeTaro home page URL:

http://www.spiketaro.com/en/ (English)

http://www.spiketaro.com/ja/ (Japanese)