

MED 64 The most sensitive microelectrode array system for *in vitro* extracellular electrophysiology

Mobius Offline Toolkit Tutorial

ALPHA ME

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Introduction

Mobius Offline Toolkit (MOT) is an offline software package for the MED64 Mobius. It allows you to perform advanced analysis using spike data recorded from neuron cultures or brain slices using the MED64 System.

Available analysis are:

• Single-Channel Burst Analysis:

The [Time Stamp] data saved by Mobius is used. Detect a set of high-frequency spikes in a channel as a "burst" and perform analysis for the burst. The user defines the parameters for burst detection.

• Multi-Channel Burst Analysis:

The [Time Stamp] data saved by Mobius is used. Detect a set of high-frequency spikes across 64 channels as a "Synchronized burst" and perform analysis for the "Synchronized burst".

• FFT Analysis:

Power Spectrum Density analysis is performed using the Mobius raw data (.modat). The raw data is transformed using Fast Fourier Transform (FFT).

1-1. Running Mobius Offline Toolkit

Upon opening the Mobius Offline Toolkit, a window appears asking which application you would like to run. The options are Single-Channel Burst Analysis, Multi-Channel Burst Analysis and FFT Analysis. First, select the application you would like to run, select the screen resolution that is best for your machine (1280 x 1024 pixels or 1920 x 1080 pixels) and click "Start" to begin the analysis.

Apprication.		
Single-Channel	BurstAnalysis	
Window Size:		
🔘 1280 x 1024	I920 x 1080	

Figure 1-1. Mobius Offline Toolkit initialization window. Select the application you wish to run, the best screen resolution and click the start button.

2. Single-Channel Burst Analysis

The Single-Channel Burst Analysis allows you to detect burst events based on pre-determined settings per channel. The "Time Stamp" data is used as an input and the program will compute and graph:

- Total number of bursts.
- Number of spikes in a burst.
- Inter-burst-intervals.
- Duration of a burst.

All analyses are performed at each channel, and the channel to be displayed is selected with the "Channel Position" in the [Settings] window.



Figure 2-1. Single-Channel Burst Analysis.

2-1. Saving the Time Stamp data with the Mobius

The Single-Channel Burst analysis application is able to load "Time stamp" data saved by the Mobius program. This Time stamp data is saved as a .csv file and can be created in Mobius by using the [Save Spikes] module. Refer to page 88, Data Output in the Mobius Tutorial for outputting the Time Stamp data.

Filename modifier example	Data type	Spike Downsampling
Enable storage	Time stamps + spikes	1/ 1 -

Figure 2-2. The [Save spikes] module from Mobius can export .csv files for use with the Single- and Multi-Channel Burst analyses. To export, you must click the box labeled "Enable storage," select the "Time stamps only" option and click the Green-Red button to execute.

2-2. Setting the analysis parameters

The "Time Stamp" data can be opened by clicking [File] > [Open File], and then selecting a desired .csv file. Once a data is loaded, the file name appears under the menu bar and the time stamp data is displayed in the [Spike Events] window (Figure 2-3).

Single-Channel Burst Analysis Ve	r 1.4.5	R11 9424908		· 36 ·			
File Edit Help							
File: Axol_Bicuculline.modat+Tutoria	al_graphic+analysis1+Save Spikes1+graphic.csv						
Settings	Spike events	-					
Trace:		Ď:10:000	04:00:00:20:00	»	04:00:00 30:000	04 00 00 40 000	
	Number of bursts in a trace				Inter-burst interva	l	

Figure 2-3. When the time stamp data is loaded, the file name appears under the menu bar and time stamp data is displayed in the [Spike events] window.

Select a channel to display analysis by the "Channel Position" panel in the [Settings] window. You can navigate through the different channels by clicking in this window. The selected channel will be highlighted by turning red.



Figure 2-4. Channel Position panel allows you to navigate through the spikes collected at different recording electrodes. In this example, Channel 44 has been selected and is highlighted in red.

2. Single-Channel Burst Analysis

To begin a single-channel analysis, fill the desired values into the fields in the [Settings] window and click "Execute" at the bottom of the window. Analysis for the selected channel are displayed in the remaining windows.

When any of the settings window's parameters are adjusted, you will see the correspoding box turn pink (Figure 2-6). You must push "enter" on your keyboard to accept changes (the box will change back to the normal color).

All fields are controlled independently at each channel. When fields are changed in the [Settings] box, the new parameter is applied only to the current channel. In order to apply changes to all other channels, it is necessary to push "Apply to All" near the bottom of the window. You can also reset all value to the default values by pushing "All Reset."



Figure 2-5. (left) The [Settings] window controls the input parameters for the analysis. To return to the default values, click the "All Reset" button. To apply changes made in the settings values to all channels, click the "Apply to All" button. The execute button will run the burst analysis.

Figure 2-6. (right) Settings window with recent changes made to the "MAX. interval to start burst" and "MAX. interval to end burst" fields. To accept the changes, it is necessary to push enter on the keyboard so the parameter field returns to the white background.

Parameters in the [Settings] window



The figure 2-7 shows schematic representation of the parameters in this window.

Figure 2-7. Schematic representation of the parameters controlled by the [Settings] window.

• MAX. Interval to start burst (ms):

The maximum time duration, in milliseconds, in which more than one spike must have occurred in order to mark the first spike as the beginning of a burst.

• MAX. interval to end burst (ms):

The maximum time duration, in milliseconds, in which the end of a burst is marked because no more spikes have occurred. The max. interval to end burst must be longer in duration than the max. interval to start burst.

• MIN. number of spikes in a burst:

The minimum number of spikes that define a burst. Bursts identified with a less spikes than the minimum are ignored.

• MIN. duration of a burst (ms):

The minimum duration in milliseconds that define a burst. Bursts identified with a duration shorter than the minimum value are ignored.

• MIN. interval between bursts (ms):

The minimum time between bursts (from the end of a burst to the beginning of the next burst) in milliseconds. Bursts identified that have an interval between bursts less than the minimum value will be ignored.

After executing the analysis, the results will be displayed on the right side of the display. There are six different windows which summarize the data.

2-3. Obtained burst analysis

Once "Execute" in [Settings] is clicked, the following analyses are obtained for all channels. The analyses displayed are for the individual channel selected at the "Channel Position", and clicking on a different channel will update the display. The time scale (X axis) is displayed in the following format:

0d : 01 : 00 : 00:000 day:hours:min:sec:msec

All graphs and charts can be saved as .csv files by right-clicking anywhere in the graph/chart and then selecting "Save as CSV" (Figure 2-8). All graphs can be saved as image files by selecting "Save Chart Image". Selecting "Setting" will allow you to modify the graph.



Figure 2-8. All graphs/charts can be saved by right-clicking anywhere in the graph/chart, and then selecting "Save As CSV".

Spike Events

The [Spike events] window displays a vertical line which represents a spike at each time point for which a spike was detected during the spike analysis by Mobius. After execution of the analysis, the Mobius Offline Toolkit will highlight spikes included in a burst in red. The red-triangle indicates "beginning of a burst".



Figure 2-9. The spike events window marks all time points where a spike occurred in the recording with a vertical line. Spikes in red have been assigned to a burst. To zoom in, you can use the mouse to click and drag over the area of interest.

The size for time window can be set either for 1sec, 10sec, 1min or 10min by right-clicking anywhere in the chart, and selecting from the choices in the menu (Figure 2-10). Data can be scrolled using the scroll bar on the bottom. Selecting [Save Chart Image] allows you to save "displayed chart" as a graphic file.





Number of bursts in a trace

The total number of bursts, as defined by the settings menu, are displayed for each trace.



Figure 2-11. The [Number of bursts in a trace] window displays a graph summary of the total number of detected bursts per trace of the recording.

Inter-burst interval

The time from the beginning of a burst to the beginning of the next burst is calculated and displayed as a function of time. The data are represented as a frequency (Hz) using the formula below.



Figure 2-12. The time from the beginning of a burst to that of the next burst at a single trace are plotted in terms of frequency (left). Definition and formula for the Inter-burst interval (right).

Number of spikes in a burst

The number of spikes per burst are displayed as a function of time.





Duration of a burst

The length of time (in milliseconds) over which a burst lasts is displayed as a function of time.



Figure 2-14. Burst durations are plotted against time for a single trace of the recording.

Information

This window displays a chart that contains all the data for each individual burst detected and summarizes:

- 1. trace #: Trace number.
- 2. ch: Channel number.
- 3. start time (ms): Time (ms) of a burst to start.
- 4. interburst interval (ms): Time from the beginning of a burst to the beginning of the next burst in ms.
- 5. spikes in a burst (spikes): Number of spikes in a burst
- 6. duration (ms): Total duration of a burst.

The data from this chart are the source data for all of the other windows in this application.

The data can be saved as a .csv file by right-clicking anywhere in the chart and selecting [Save Results]. Right clicking and selecting [Copy Results] allows you to copy the selected data into a spreadsheet program.

nformation	1 1	2	3	4	5	6
tr	ace #	ch	start time (ms)	interburst interval (ms)	spikes in a burst (spikes)	duration (ms)
	1	1	22782.85		3	228.10
	1	1	28376.40	5593.55	3	89.95
	1	1	30921.25	2544.85	3	339.15
	1	1	44382.25	13461.00	3	399.80
	1	1	55456.15	11073.90	3	84.35
	1	1	56844.35	1388.20	3	365.05
	1	2	815.75		3	519.05
	1	2	3669.75	2854.00	9	1112.40
	1	2	5720.80	2051.05	10	388.20
	2		0005 55	A		0070.05

Figure 2-15. The data summarized in the Information window contains all values which are used to generate the various graphics. The table can easily be copied and pasted for use in other spreadsheet programs.

2-4. Data export

All analysis results can be exported as a .csv file. The program allows you to export all results or multiple selected analysis results at one time; data export that can be performed by following procedure using "Batch Export" button in the [Settings] window.

- 1. Click [Edit] > [Preferences], and then select "Batch export".
- 2. Select the analysis result you desire to export. Click OK (Figure 2-16, left).
- 3. Identify the folder in which you desire to export the .csv files, and then click "Batch export" (Figure 2-16, right).

ttings For All Charts Batch Export		
Export Trace Single All (in each)	All (in one)	
Chart Image		ł
Format: 💿 emf 🔘 bmp 🔘	png	ł
Spike events	Spike events	
Number of bursts in a trace	Number of bursts in a trace	T
Number of spikes in a burst	Number of spikes in a burst	1
Inter-burst interval	Inter-burst interval	
Duration of a burst	Duration of a burst	
Chart CSV		
Number of bursts in a trace	Number of bursts in a trace	
Number of spikes in a burst	Number of spikes in a burst	
Inter-burst interval	Inter-burst interval	Export folder:
Duration of a burst	Duration of a burst	D:\2012\Technical issue \Offline toolkit\Offline toolkit
Information CSV		\sample data
All	All	
		Batch Export

Figure 2-16. "Preferences" panel (left). Select the analysis result you desire to export, and then click OK. Click [Batch Export] in the [Settings] window to export the selected analysis result.

Data export can be also performed by right-clicking anywhere in the chart and selecting "Save as CSV". (Figure 2-8, page 6)

2-5. Menu bar controls

File:

[Open file...] opens a dialog box to choose a new .csv file for analysis.

[Exit] closes the program.

Edit:

[Preferences] opens a dialog box for "Setting For All Charts" which allows for customization of the charts displayed and "Batch export" options. In the "Setting for All Charts" option, font properties for axis titles and labels are controlled by clicking the corresponding "Font..." button. Line color and width, as well as marker color and width, can be adjusted for the "Inter-burst interval," "Number of spikes in a burst" and "Duration of a burst" windows. Column color and width apply only to the "Number of bursts in a trace" bar graph. The background color will change the color of the plot areas for "Number of bursts in a trace," "Inter-burst interval," "Number of spikes in a burst" burst interval," "Number of bursts in a trace," "Inter-burst interval," "Number of spikes in a burst" and "Duration of a burst" graphs. Clicking the "Set Default" button at the bottom of the window will reset the values to their default.

Preferences	1.00.01	11 1	
Settings For All Charts Batch Ex	port		
Axis Titles		Chart Type	
X Axis Title:	Font	Line Color:	Green -
Arial (9)		Line Width:	1 •
Y Axis Title: Arial (9)	Font	Marker Color:	Green -
		Marker Width:	4
Axis Labels	East	Column Color:	Green -
Arial (8)	FUIL	Column Width:	20 🗸
Y Axis Label Font Setting:	Font	Background	M/b 2-
Arial (8)		Color:	white
	Set Def	fault Ok	Cancel
	0.901		

Figure 2-17. The chart properties dialog box allows you to customize the appearance of the graphs generated by the analysis application.

Help:

[Single-Channel Burst Analysis] opens the help file for the Single-Channel application.

[Multi-Channel Burst Analysis] opens the help file for the Multi-Channel application.

[FFT Analysis] opens the help file for the FFT application.

3. Multi-Channel Burst Analysis

The Multi-Channel Burst Analysis application allows you to detect and analyze "synchronized burst activity", burst events across 64 channels. The program graphs "time course of spike frequencies across 64 channels" (Array-wide Spike Detection Rate, ASDR) and shows distribution of spike timing across 64 channels in a Raster plot. You will define and detect a set of high-frequency spikes across 64 channels as a "Synchronized burst" using those graphs, and obtain information for the "Synchronized burst" in charts.



Figure 3-1. Window for Multi-Channel Burst analysis.

3-1. Saving the Time Stamp data with the Mobius

The Multi-channel burst analysis application is able to load data saved as spike "Time stamp" from the Mobius program. These time stamp data are saved as a .csv file and can be created in Mobius by using the "save spikes" module. Refer to page 88, Data Output in the Mobius Tutorial for outputting the Time Stamp data.

Save Spikes		
Filename modifier example	Data type	Spike Downsampling
Enable storage	Time stamps + spikes	1/ 1 -

Figure 3-2. The [Save Spikes] module from Mobius can export .csv files for use with the single- and multi-channel burst analyses. To export, you must click the box labeled "Enable storage," select the "Time stamps only" option and click the Green-Red play button to execute.

3-1. Array-wide Spike Detection Rate and Raster Plot chart

The Time stamp data saved with Mobius can be opened by clicking [File] > [Open File]. Once the data is loaded, the "Array-wide Spike Detection Rate" (Top) and the "Raster Plot" analysis (bottom) for the selected trace will be displayed in the [Raster Plot and Spike Detection Rate] window.

The "Array-wide Spike Detection Rate" displays spike frequencies (number of spikes/second) across all 64 channels as a function of time. The size of the binning window are changed by changging the "Bin size (msec)" in the [Settings] window on the left (see the next section for details).

The Raster Plot shows the timing of spikes coming across all 64 channels. Each vertical line in the graph represents the timing of spikes recorded at each of the 64 electrodes, and appearance of long vertical lines across 64 channels indicates synchronized burst. The frequencies are also color coded (e.g., Red: highest frequency, Purple: lowest frequency). The precise definition of synchronized burst activity (array-wide spikes) will be defined by the user (see description below).

The window displays the analysis only for one trace. To navigate through the other traces, it is necessary to change the value of the field "Trace" in the [Settings] window.



Figure 3-3. Graph for Array-wide Spike Detection Rate (ASDR) (top) and Raster Plot (bottom).

To zoom in both charts, you can click and drag over the area of interest while holding the [Shift] button. The chart can be modified by right-clicking anywhere in the window and selecting [Modify Chart] on the chart can be saved by selecting [Save Chart Image]. The time scale (X axis) is displaed as:

0d : 01 :00 : 00:000 day:hour:min:sec:msec

pike Detection Rate Chart Raster P	Pot Batch Export
Marker Presentation	Axis Title Font
Black (default)	X.Axis Title: Font
Orayscale	Arial (9)
Color	Y Axis Tite: Font
	Ana (2)
Raster plot presentation maximum value (spikes/s);	Axis Label Font
1920	X Axis Label Font Setting: Font
	Arial (8)
Background White Color	Y Axis Label Font Setting: Font Arial (8)
Axis Range	
Y Axis Range Min:	1 Max: 64 🔄 Auto
E Show data-containing chan	nels only
XAxis Range Mit: 0000	0.00.00.000 MBC 00.00.0000 V Auto
X Axis Range Min: 0d.00	



Figure 3-4. Modifying the [Raster Plot and Spike Detection Rate] (left). The windows pop-up by right-clicking anywhere in the chart, and then selecting "Modify chart".

The bin size for the ASDR and Raster Plot parameters can be changed in the "Bin size (ms)" in the [Settings] window (right).

3-3. Detecting "Synchronized bursts"

An Event where spike frequencies (number of incoming spikes per second) across 64 channels surpasses a defined threshold, set in the [Settings] window, are detected as "Synchronized burst". Set the binning window size (msec) and all parameters in the [Settings] window, and then click [Detect Bursts] button to detect "Synchronized bursts".



Figure 3-4. Schematic representation of the "Synchronized burst" detection. In this example, events where spike frequencies (spikes/1 second) go over 5000 are detected as "Synchronized bursts".

The thresholds set in the [Settings] window are shown in the ASDR chart and the detected "Synchronized bursts" are indicated with black bars under the ASDR chart (Figure 3-5).



Figure 3-5. Thresholds are shown in the ASDR graph and "Synchronized bursts" are indicated with black bars once "Synchronized bursts" are detected.

When any of the [Settings] window's parameters are adjusted, you will see the box turn pink (Figure 3-6). You must push "enter" on your keyboard to accept changes before the box will change back to the normal color.

Window size (msec):	500
Threshold for burst detection (spikes/sec):	0400
6	Detect Burst

Figure 3-6.

Methods for detecting "Synchronized bursts"

2 different methods are available for detecting "Synchronized bursts" allowing you to detect the burst as you desire.

Burst detector:						
Normal						
Schmitt trigger						
Threshold - high (spikes/s):						
0700						
Threshold - low (spikes/s):						
0300						
Figure. 3-7.						

Normal:

Events where spike frequencies (ASDR) go above a threshold set in the "Threshold-high" (spikes/s) field are detected as the start of a "synchronized burst". When the ASDR falls below the "Threshold-high" value, the end of the "synchronized burst" is detected (Threshold-low will be inactivated with this option).

Schmitt trigger:

Events where spike frequencies (ASDR) go above the threshold set in the "Threshold-high" are detected as the start of a "synchronized burst", however, end of the burst is NOT detected as far as the ASDR go below the threshold set in the "Threshold-low".

The Figure 3-8 highlights how these 2 methods can differentiate your burst detection. In this example, the "Normal" method detects inappropriate or incomplete "synchronized bursts.". Setting threshold at 600 breaks a single "synchronized burst" into 2 different bursts (one of shorter duration and one of longer duration, top left). Schmitt allows to detect a series of high-frequency spieks as a single "synchronized burst".

Normal: Threshold-high (600)







Schmitt: Threshold-high (600), Threshold-low (400)



Figure 3-8 Comparison for the "Normal" and "Schmittt" method for burst detection.

Smoothing

Smoothing also helps you to detect "Synchronized bursts" as desired by detect "Synchronized bursts" based on smoothed data.

Inputting larger number than 0 at the "Smoothing (bin)" will smooth the data by computing moving average at the selected bin size. The smoothing (moving average) is performed with binning size both for pre and post. (e.g., If 5 is selected, smoothing is performed with 11 data points (5 for pre, 5 for post). Smoothed data is shown with red in the ASDR graph. Clicking "Detect burst" button will detect bursts based on this red smoothed data.

Note:

The smoothed data is used only for detecting "Synchronized burst" and defining its onset and offset time. For frequencies analysis, original data is used.



Figure 3-9. Detecting "Synchronized burst" based on smoothed data. The red in the ASDR chart shows smoothed data.

Setting threshold with median percentage

The "Median threshold" option will allow you to detect "Synchronized burst" with the median percentage "percentage for mediam(|x| / 0.6745)". Inputting number (e.g. 200% = x2) in the "Median threshold" and clicking [Apply] will set values in the Threshold-high/low fileds automatically. Clicking detect bursts will detect "Synchronized burst" with the automatically set thresholds.



Figure 3-10. Threshold setting by Median percentage. Inputting number at the "Median threshold (%)", and then click "Apply" will set numbers at the Threshold-high (and -low). Clicking "Detect Bursts" will detect the "Synchronized burst" based on the set thresholds.

3-4. Analysis for "Synchronized bursts"

Once the "Detect Bursts" button in the [Setting] window is clicked, two new windows will appear that display data summaries. The left [Information (summary values)] provides a summary for all "Synchronized bursts" in an each trace. Similarly, the [Information (individual values)] provides information for each individual "Synchronized burst".

Information (summar	y values)				Infor	mation (ir	idividual value	es)			
trace #	ASDR (spikes/s)	total spikes in bursts (spikes)	trace	# burst #	start time	interburst	peak (spikes/s)	duration	spikes in a		
total	100.87	39	3.90	26370			(110)	intervar(ind)	(0)110010)	(11.2)	All
1	78.15	12	6.00	4491		1 1	100.00		1490.00	400.00	447
2	214.98	21	10.50	17749		1 2	10500.00	10400.00	1500.00	300.00	305
3	115.58	6	3.00	4130		1 3	14000.00	3500.00	1780.00	400.00	461
4	88.48	0	0.00			1 4	27500.00	13500.00	1460.00	300.00	328
5	7.15	0	0.00			1 5	39000.00	11500.00	1520.00	300.00	360
interburst interval (ms)						1 6	48200.00	9200.00	1450.00	300.00	370
trace #	mean	SD	SEM	median		1 7	62700.00	14500.00	1370.00	300.00	369
total	8088 89	5739.08	956.51	9000 00		1 8	66300.00	3600.00	1820.00	300.00	389
1	10681.82	4144.35	1249.57	11500.00		1 9	83400.00	17100.00	1570.00	300.00	356
2	5525.00	4231 57	946 21	4600.00		1 10	93800.00	10400.00	1530.00	300.00	342
2	12640.00	9108 13	4073.28	12400.00		1 11	105300.00	11500.00	1630.00	300.00	327
	12040.00	5100.15	4013.20	12400.00		1 12	117600.00	12300.00	1370.00	300.00	337
nesk (enikee/e)						2 1	362419.00		3060.00	500.00	933
peak (spikes/s)		CD	CEN	medien		2 2	363619.00	1200.00	1990.00	400.00	596
trace #	mean	50	SEM	median		2 3	374619.00	11000.00	2860.00	500.00	914
total	2120.26	627.22	100.44	1980.00		2 4	375719.00	1100.00	2030.00	400.00	592
1	1540.83	142.03	41.00	1510.00		2 5	385819.00	10100.00	2880.00	600.00	982
2	2620.00	404.97	88.37	2750.00		2 6	387019.00	1200.00	2130.00	400.00	578
3	1530.00	129.46	52.85	1515.00		2 7	397419.00	10400.00	2750.00	500.00	924
4						2 8	398619.00	1200.00	1980.00	500.00	661
5						2 9	408819.00	10200.00	2990.00	600.00	1016
duration (ms)						2 10	410119.00	1300.00	2200.00	400.00	592
trace #	mean	SD	SEM	median		2 11	422119.00	12000.00	3200.00	600.00	1057

Figure 3-11 Analysis obtained for "Synchronized bursts". The left [Information (summary values)] provides summary for all "Synchronized bursts" per trace while the right [Information (individual values)] provides information for each individual "Synchronized burst".

Both charts can be saved as .csv file by right-clicking anywhere in the chart, and then selecting "Save Results" (Figure 3-12). Selecting "Copy results" will copy selected cells for for pasting into a spreadsheet.

ASDR (spikes/s)		number of bursts (bursts)	
	100.97 C	opy Results	9 2
	S	ave Results	1
	115.58		6

Figure 3-12. Exporting the analysis results. Selecting "Copy Results" copy and paste "selected cells" into an excel sheet.

Information (summary values)

This chart shows information for all "Synchronized bursts" pre trace.

	1	2	3	4
Informat	tion (summa	ry valu <mark>e</mark> s)		
trace #	ASDR (spikes/s)	number of bursts (bursts)	burst rate (bursts/min)	total spikes in bursts (spikes)
total	100.87	39	3.9	0 26370
1	78.15	12	6.0	0 4491
2	214.98	21	10.5	0 17749
3	115.58	6	3.0	4130
4	88.48	0	0.0)
5	7.15	0	0.0	٥ ا
interburst i	interval (ms)	5		
trace #	mean	SD	SEM	median
total	8088.89	5739.08	956.5	1 9000.00
1	10681.82	4144.35	1249.5	7 11500.00
2	5525.00	4231.57	946.2	1 4600.00
3	12640.00	9108.13	4073.2	3 12400.00
4				
5				
peak (spik	es/s)	6		
trace #	mean	SD	SEM	median
total	2120.26	627.22	100.44	4 1980.00
1	1540.83	142.03	41.0) 1510.00
2	2620.00	404.97	88.3	7 2750.00
3	1530.00	129.46	52.8	5 1515.00
4				
5		_		
duration (n	ns)	/		
trace #	mean	SD	SEM	median
total	461.54	120.56	19.3	500.00
1	316.67	38.92	11.24	4 300.00
2	509.52	76.84	16.7	7 500.00
3	583.33	75.28	30.73	3 600.00
4				
5	hund ()	9		
spikes in a	burst (spikes)	0	0511	
trace #	mean	SD	SEM	median
total	6/6.15	250.08	40.0	661.00
	3/4.25	47.10	13.6	364.50
2	845.19	1/6.76	38.5	r 914.00
3	688.33	80.71	32.9	о 999.00
4				
I 51		- I		1

Figure 3-13. Information (summary values) chart.

- ASDR (spikes/s): The mean for ASDR (spike frequencies across 64 channels per second).
- number of bursts (bursts): Number of "Synchronized burst" in an entire trace.
- burst rate (bursts /min): Frequencies for "Synchronized burst" per minute.
- total spikes in bursts (spikes): Total number of spikes (across 64 channels) in all "Synchronized bursts".
- Interburst interval (ms): Mean, Standard Deviation (SD), SEM, and median for the time from the beginning of a "Synchronized burst" to the beginning of the next syncrhonized burst.
- peak (spikes/s): Mean, SD, SEM, and median for peak frequencies (number of spikes per second) of all "Synchronized bursts".
- duration (ms): Mean, SD, SEM, and median for duration of all "Array-wide spikes".
- spikes in a burst (spikes): Mean, SD, SEM, and median for number of spikes in an "Synchronized burst".

Information (individual values)

This chart shows information for each individual "Synchronized burst".

Instruction Let Start Intro S	1	2	3 	4	5 	6 				7								8 I										
Variation Instruction	form	ationali	individual	3)														↓_										
bitol interim	-	- V-	start time	interhurst	nank	duration	aoikea in	a burat (a	oikes)	-								· · · · ·										
1 1	trace #	burst #	(ms)	interval (ms)	(spikes/s)	(ms)	AL	ch1	ch2	ch3	ch4	ch5	ch8	ch7	ch8	interval t	between 1	st and last	spike (ms)									
1 2 165000 160000 160000 30000 300 30000 <t< td=""><td>1</td><td></td><td>1 100.00</td><td></td><td>1490.00</td><td>400.00</td><td>447</td><td>12</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>ch1</td><td>ch2</td><td>ch3</td><td>ch4</td><td>ch5</td><td>ch6</td><td>ch7</td><td>ch8</td><td>ch9</td><td>ch10</td><td>ch11</td><td>ch12</td><td>ch</td></t<>	1		1 100.00		1490.00	400.00	447	12	1	0	0	0	0	0	0	ch1	ch2	ch3	ch4	ch5	ch6	ch7	ch8	ch9	ch10	ch11	ch12	ch
1 3 140000 30000 170000 44000 30000 440000 30000 420000 300000 440000 300000 320 6 0	1		2 10500.00	10400.00	1500.00	300.00	385	8	0	0	0	0	0	0	0	183.70						_		357.40	384.60	206.25		-
1 1 275000 145000 146000 3000 1 0<	1		14000.00	3500.00	1780.00	400.00	481	14	0	0	0	0	0	0	0	251.90								280.45	295.95	285.35	<u> </u>	-
1 5 398000 115000 15200 30000 30000 30000 30000 30000 30000 30000 30000 30000 30000 300000	1	4	4 27500.00	13500.00	1460.00	300.00	328	6	0	1	0	0	0	0	0	297.95								381.80	357.15	306.55		-
1 6 44306 0 5000 0 1446 00 1460 0 1 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 1 0	1		5 39000.00	11500.00	1520.00	300.00	360	16	0	0	0	0	0	0	0	159.15								283.90	281.55	283.70		-
1 7 42700 0 14600 0 12700 0 2000 0	1		48200.00	9200.00	1450.00	300.00	370	10	1	0	0	0	0	1	0	244.15								288.80	292.35	255.10	<u> </u>	-
1 8 66000 0 36000 0 18200 0 2000 0 190 0 0	1	1	62700.00	14500.00	1370.00	300.00	369	8	0	0	0	0	0	0	0	265.30						-		291.60	294.00	263.25		-
s g 834000 177000 177000 177000 177000 177000 177000 177000 177000 177000 177000 177000 2200 177000 2200	1	1	66300.00	3600.00	1820.00	300.00	389	17	0	0	0	0	0	0	0	184.30								285.90	288.35	197.40		-
1 1 900000 104000 15000 30000 342 7 1 1 0	1	5	83400.00	17100.00	1570.00	300.00	356	7	0	1	0	0	0	0	0	262.15								290.95	280.65	265.30		-
1 1 1050000 115	1	10	93800.00	10400.00	1530.00	300.00	342	7	1	1	0	0	0	0	0	229.90								292.20	267.85	251.85		-
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	1	11	1 105300.00	11500.00	1630.00	300.00	327	8	0	0	1	0	0	0	0	198.90								282.65	284.20	272.85		-
2 1 3624190 1 36600 5600 683 34 0	1	13	117600.00	12300.00	1370.00	300.00	337	7	0	1	0	0	0	0	0	200.75								267.45	275.05	175.30		-
2 1 33241800 306000 90000 833 34 0 0 0 1 0 2 0 1212 40715 44064 417.0 412.00 2 2 3061500 1200.00 11600.00 560.00 560.00 10 0														-		135.55	•							285.30	292.50	230.25		-
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	2	1	1 362419.00		3060.00	500.00	933	34	0	0	0	1	0	2				-										-
2 3 37281900 110000 28000 9000 944 43 0 1 0 <td>2</td> <td></td> <td>2 363619.00</td> <td>1200.00</td> <td>1990.00</td> <td>400.00</td> <td>596</td> <td>25</td> <td>0</td> <td>0</td> <td>0</td> <td>٥</td> <td>0</td> <td>0</td> <td>0</td> <td>352.00</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>121.25</td> <td></td> <td>469.65</td> <td>437.90</td> <td>412.90</td> <td></td> <td>-</td>	2		2 363619.00	1200.00	1990.00	400.00	596	25	0	0	0	٥	0	0	0	352.00						121.25		469.65	437.90	412.90		-
2 4 3757460 11000 20000 40000 502 18 1 0 2 0 0 6 5 5 1 0 2 0 0 0 5 55 1 0 1 0 0 0 1 0 0 1 0 0 1 0 0 0 1 0 0 0 0 1 0	2	1	3 374619.00	11000.00	2860.00	500.00	914	43	0	1	0	0	0	0	0	378.85	•					-		382.50	375.10	268.85		-
2 5 3551900 101000 28800 60000 982 35 0 1 0 0 0 2430 2430 0 20560 20560 20560 20560 20560 20560 20560 20560 20560 20570	2	4	4 375719.00	1100.00	2030.00	400.00	592	18	1	0	0	2	0	0	0	435.55		-				-		491.30	456.90	426.35		-
2 6 307190 2130.0 2130.00 570.0 570.0 570.0 2 7 387418.00 1230.00 2130.00 570.00 570.	2		5 385819.00	10100.00	2880.00	600.00	982	35	0	1	0	1	0	0	0	357.05				24.20				354.00	394.40	369.75		-
2 7 397498.00 10400.00 2750.00 500.00 600.0 1 0 0 2 1 46.16 1 0.0 974.0 </td <td>2</td> <td></td> <td>387019.00</td> <td>1200.00</td> <td>2130.00</td> <td>400.00</td> <td>578</td> <td>17</td> <td>1</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>430.45</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>-</td> <td></td> <td>305.20</td> <td>309.30</td> <td>514.70</td> <td></td> <td>-</td>	2		387019.00	1200.00	2130.00	400.00	578	17	1	0	0	0	0	0	0	430.45						-		305.20	309.30	514.70		-
2 8 39881800 1200.00 1980.00 600.00 661 22 0 0 1 0 0 0 0 1 0 0 0 1 0 0 0 1 0	2	1	7 397419.00	10400.00	2750.00	500.00	924	38	0	1	0	0	0	2	1	340.00						440.70		373.40	3/9.45	370.10		-
2 9 40819 00 16200 00 2896 00 600 00 106 44 2 1 0 0 2 0 400 0 600 00 400 0 500 00	2	1	398619.00	1200.00	1980.00	500.00	661	22	0	0	0	1	0	0	0	404.10	۲					140.75		490.40	402.00	431.40		+
2 10 41011900 120000 22000 4000 552 22 0 1 0 <td>2</td> <td>5</td> <td>408819.00</td> <td>10200.00</td> <td>2990.00</td> <td>600.00</td> <td>1016</td> <td>44</td> <td>2</td> <td>1</td> <td>0</td> <td>0</td> <td>0</td> <td>2</td> <td>. 0</td> <td>301.04</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>1.00</td> <td></td> <td>e0e.10</td> <td>403.00</td> <td>459.90</td> <td><u> </u></td> <td>-</td>	2	5	408819.00	10200.00	2990.00	600.00	1016	44	2	1	0	0	0	2	. 0	301.04						1.00		e0e.10	403.00	459.90	<u> </u>	-
2 11 422109 0 12000.00 3200.00 6400.00 1557 38 1 1 0 1 0 0 367.6 3	2	10	410119.00	1300.00	2200.00	400.00	592	22	0	1	0	1	0	0	0	940.04	110.46	·				4.30		396.10	305.05	900.00	<u> </u>	-
2 12 42321900 110000 197000 40000 541 17 0 1 0 2 0 1 1 335.60 15700 507.80 509.66 <td>2</td> <td>11</td> <td>422119.00</td> <td>12000.00</td> <td>3200.00</td> <td>600.00</td> <td>1057</td> <td>38</td> <td>1</td> <td>1</td> <td>0</td> <td>1</td> <td>1</td> <td>0</td> <td>0</td> <td>342.25</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>585.10</td> <td>549.70</td> <td>200.40</td> <td></td> <td>-</td>	2	11	422119.00	12000.00	3200.00	600.00	1057	38	1	1	0	1	1	0	0	342.25								585.10	549.70	200.40		-
2 15 4301900 6600.00 2800.00 6000.00 999 42 0 1 0 2 0 1 105.00 105.00 500.00 2	2	12	423219.00	1100.00	1970.00	400.00	541	17	0	1	0	2	0	1	1	201.00				105.70				278.00	200.00	200.00	-	+-
2 14 41241960 2300.00 2730.00 500.00 841 36 0 0 3 0 1 0 24.35 44149.00 200.00 <th2< td=""><td>2</td><td>13</td><td>3 430119.00</td><td>6900.00</td><td>2800.00</td><td>600.00</td><td>999</td><td>42</td><td>0</td><td>1</td><td>0</td><td>2</td><td>0</td><td>0</td><td>1</td><td>420.50</td><td></td><td>-</td><td></td><td>364.36</td><td></td><td>-</td><td></td><td>570.00</td><td>300.000</td><td>309.65</td><td>-</td><td>+</td></th2<>	2	13	3 430119.00	6900.00	2800.00	600.00	999	42	0	1	0	2	0	0	1	420.50		-		364.36		-		570.00	300.000	309.65	-	+
2 15 44119 00 6000.00 2000.00 6000.00 1011 46 0 0 1 0 0 6 500.75 2 400.10 500.75 500.00 500.75 500.00 500.75 500.00 500.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 500.00 600.10 200.75 200.75 200.75 200.75 200.75 200.75 200.75 200.75 200.75 200.75 200.75 200.75 200.	2	14	432419.00	2300.00	2730.00	500.00	841	36	0	0	0	3	0	1	0	463.50		-		240.60		-		407.46	400.00	200.00	\vdash	+
2 16 443219.00 1800.00 2570.00 500.00 654 40 3 1 0 2 0 <td< td=""><td>2</td><td>15</td><td>441419.00</td><td>9000.00</td><td>3000.00</td><td>600.00</td><td>1031</td><td>45</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>403.04</td><td></td><td>-</td><td>-</td><td>a-10.00</td><td>-</td><td>-</td><td></td><td>677.75</td><td>536.20</td><td>161.00</td><td></td><td>+</td></td<>	2	15	441419.00	9000.00	3000.00	600.00	1031	45	0	0	0	1	0	0	0	403.04		-	-	a-10.00	-	-		677.75	536.20	161.00		+
400.55 442.20 40.15 402.25	2	16	443219.00	1800.00	2570.00	500.00	834	40	3	1	0	2	0	0	0	000.75			-	45.45	-	-		479.40	200.20	000.000	-	+
2 17 452219.00 9000.00 2900.00 600.00 1022 40 0 0 0 3 0 0 0 483.95 999.5 999.5 599.50	2	17	452219.00	9000.00	2980.00	600.00	1022	40	0	0	0	3	0	0	0	400.00	-46.65	-	-	38.96		-		593.90	677.76	478.00	-	+
2 18 454319.00 2100.00 2520.00 500.00 856 40 1 0 0 2 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1	2	18	454319.00	2100.00	2520.00	500.00	856	40	1	0	0	2	0	0	0	407.00	-	-		166.46	-	-		400.00	400.00	405.00		+

Figure 3-14. Information (individual values) chart.

- 1. *trace#:* Trace number.
- burst# : ID number for a "Synchronized burst".
- start time (ms): Onset time for a "Synchronized burst".
- *interburst interval (ms)*Time from the beginning of a "Synchronized burst" to the beginning of the next "Synchronized burst".
- peak (spikes/s): Spike frequencies at the peak of an "Synchronized burst".
- duration (ms): Duration for a "Synchronized burst".
- spikes in a burst (spikes): Total number of spikes in a "Synchronized burst". "All" is for all 64 channels. Ch 1-64 for individual channel.
- interval between first and last spike (ms):
 Inverval from the 1st spike to the last spike per channel in a "Synchronized burst".

3-5. Data export

All analysis results can be exported as a .csv file. The program allows you to export all results or multiple selected analyses at one time, which can be performed by following the procedure below:

- 1. Clicking [Edit] > [Preferences], and then select "Batch export".
- 2. Select the analysis results you desire to export. Click OK.
- 3. Identify the folder in which you desire to export the .csv files, and then click "Batch export".

Export Trace Single All (in each) All (in one)	
Format: Format	
Spike events	
Chart CSV	
Chart Chart	
Burst Burst	
Information CSV	
Summary values Summary values	
Individual values Individual values Export folder:	_
Details Details Details Details	
Sample data	
Set Default OK Cancel Batch Export	t

Figure 3-15. "Preferences" panel (left). Select the analysis result you desire to export, and then click OK. Click [Batch Export] in the [Settings] window to export the selected analysis results.

Data export can be also performed by right-clicking anywhere in the chart and select "Save as CSV". (Figure 3-11, page 17)

3-6. Menu bar controls

File:

[Open file...] opens a dialog box to choose a new .csv file for analysis.

[Exit] closes the program.

Edit:

[Preferences] opens a dialog box for customizing the chart displayed or batch export.

Preferences		X							
Spike Detection Rate Chart	Raster Plot	Batch Export							
Marker Presentation		Axis Title Font							
Black (default)		X Axis Title: Font							
Grayscale		Arial (9)							
Color		Y Axis Title: Font							
		Allal (3)							
Raster plot presentation maximum value (spikes/s	s):	Axis Label Font							
1000		X Axis Label Font Setting: Font Arial (8)							
Background White	•	Y Axis Label Font Setting: Font Arial (8)							
Axis Range									
Y Axis Range Min:		1 Max: 64 🔲 Auto							
Show data-containing channels only									
XAxis Range Min: 0d:00:00:53:652 Max: 0d:00:01:03:256 Auto									
Set Default OK Cancel									



Help:

[Single-Channel Burst Analysis] opens the help file for the Single-Channel application.

[Multi-Channel Burst Analysis] opens the help file for the Multi-Channel application.

[FFT Analysis] opens the help file for the FFT application.

4. FFT Analysis

The FFT Analysis application performs a Fast Fourier Transform (FFT) on raw data recorded by Mobius (.modat). An FFT analysis takes a voltage signal in the time domain and transforms the signal into the component sine waves that compose the raw signal. The analysis is obtained with a Power Spectrum Density (PSD) and Spectrogram charts.

4-1. Opening the Mobius (.modat) data with the FFT Analysis application

- 1. Select the [FFT Analysis] application at the start menu.
- 2. Go to [File] > [Open file].
- 3. Select a file and click OK.
- 4. The file name will appear in the menu bar. Select a Trace to be analyzed.

FFT Analysis Ver 1.4.5
File Edit Help
File: 20120726_18h06m09s_KA600nM.modat
Settings
Trace:
1 💌
Channel position:

Figure 4-1. Mobius data (.modat) is loaded and now ready to be analyzed.

4-2. Setting analysis parameters

Set the analysis parameters in the "Settings" window.

Settings	Trace: Choose the trace to be analyzed
Trace:	Channel position:
Channel position:	Choose a channel for the analysis displayed.
	• <i>Trace time (msec):</i> The trace time allows you to select a particular subset of the data. Define the time window by entering the starting time in the field to the left and the ending time in the field to the right (in msec, maximum window is 300 s).
Trace time (ms):	 PSD chart: Choose between displaying the [Mean] values or the [Peak] values of the Power Spectrum Density (PSD) chart.
0 to 299999.9!	
PSD chart:	 Step frequency (Hz): This parameter defines bin size of the bar graph of the PSD
Step frequency (Hz): 625.0 • (32 pt) Scale:	 Scale: Displays the PSD on a [Linear] or a [Logarithmic] scale.
● Linear ● Log Band width (Hz): 0 to 10000	 Band width (Hz): Defines the frequency range which is displayed in the PSD and Spectrogram charts.
Window function: Hann Hamming Window overlap (%): 0 Hum noise removal: none Execute Export folder: D:2012\Technical issue Voffline toolkit\FFT_data 	 Window function: Choose between Hann and Hamming window functions. When performing an FFT analysis on a finite dataset, the abrupt start and end of the signal introduces noise, called spectral leakage, whereby the peak that corresponds to a particular frequency will become smeared over the frequency domain. Windowing can reduce spectral leakage. The type of window used can reveal different features in the frequency domain. The Hann window function removes any discontinuity from the signal because the window touches zero at both ends. The Hann window function also reduces spectral leakage noise sufficiently for about 95% of applications and is the
	recommend starting point for any FFT analysis. Alternatively, the Hamming window function does not touch zero at both ends of the window and, as a result, performs better at reducing the noise of the nearest neighbor in the frequency domain.

- Window overlap (%): Defines the amount of window overlap, as a percentage, for the analysis. Overlapping the window function during the analysis can increase the time resolution of the spectrogram.
- *Hum noise removal:* Allows for the removal of 50 Hz or 60 Hz noise, when used.

When any of the settings window's parameters are adjusted, you will see the box turn pink. You must push "enter" on your keyboard to accept changes before the box will change back to the normal color.

Settings	
Trace time (msec): 0 to 100000	
PSD chart: 💿 Mean 🔘 Peak	
Step frequency (Hz): 0.610 (32768 pt)	Figure 4-3.

Once all parameters are set, click [Execute] button to perform the analysis. The Power Spectrum Density chart and Spectrogram chart are obtained on the left.



Figure 4-4. Power Spectrum Density chart (left) and "Spectrogram" chart (right).

PSD chart can be exported as a .csv file by right-clicking anywhere in the chart, and selecting "Save As CSV" (Figure 4-5). Power Spectrogram chart can be saved as an image file by right-clicking anywhere in the chart, and then selecting "Save image". Selecting "Modify PSD chart (or Spectrogram) will pop-up [Chart Properties], where both charts can be modified.

Power	Spectrum D	ensity	
	200000000		
₽	18000000 -	Modify PSD Chart	
^2J	16000000 -	Save As CSV	
۲Ę,	14000000 -		
insit	120000000 -		
Ğ	10000000 -	1.00.0	
ctrur	8000000 -	ALL ALL	

Figure 4-5. Exporting the PSD chart as .csv file.

4-3. Data export

All analysis results can be exported as a .csv file. The program allows you to export all results or multiple selected analyses at one time, which can be performed by following the procedure below:

- 1. Clicking [Edit] > [Preferences], and then select "Batch export".
- 2. Select the analysis result you desire to export. Click OK.
- 3. Identify the folder in which you desire to export the .csv files, and then click "Batch export".

Preferences Power Spectral Density Chart Spe	ectrogram Batch Export	
Export Trace Single O All (in each)	All (in one)	
Chart Image Format:	◎ png	
Spectrogram	Spectrogram	
Chart CSV		
		Export folder: D:\2012\Technical issue \Offline toolkit\Offline toolkit
		\sample data
	Set Default OK Cancel	Batch Export

Figure 4-6. "Preference" panel (right). Select the analysis result you desire to export, and then click OK. Click [Batch Export] in the [Settings] window to export the selected analysis result.

Data export can be also performed by right-clicking anywhere in the chart and selecting "Save as CSV". (Figure 4-5, page 23)

4-3. Menu bar controls

File:

[Open file...] opens a dialog box to choose a new binary .modat file for analysis.

[Exit] closes the program.

Edit:

[Preferences] opens a dialog box for customizing the chart displayed or batch export.

Preferences					×
Power Spectral Density Chart	Spectrogram	Batch Export			
Chart Type Chart Type Column		Axis Title Font X Axis Title: Arial (9)		Font	
Line Color: Green	•	Y Axis Title: Arial (9)		Font	
Line Width: 1 Column Color: Green	•	Axis Label Font X Axis Label Font Setting: Arial (8) Y Axis Label Font Setting:		Font	
Column Width: 1	•				
Color	•	Arial (8)			
Axis Range X Axis Range Min:		Max:		V A	uto
Y Axis Range Min:		Max:		V A	uto
Set Default OK Cancel					

Figure 4-7. The chart properties dialog box allows you to customize the appearance of the graphs generated by the analysis application.

Help:

[Single-Channel Burst Analysis] opens the help file for the Single-Channel application.

[Multi-Channel Burst Analysis] opens the help file for the Multi-Channel application.

[FFT Analysis] opens the help file for the FFT application.

4. FFT Analysis

Mobius Offline Toolkit Tutorial

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