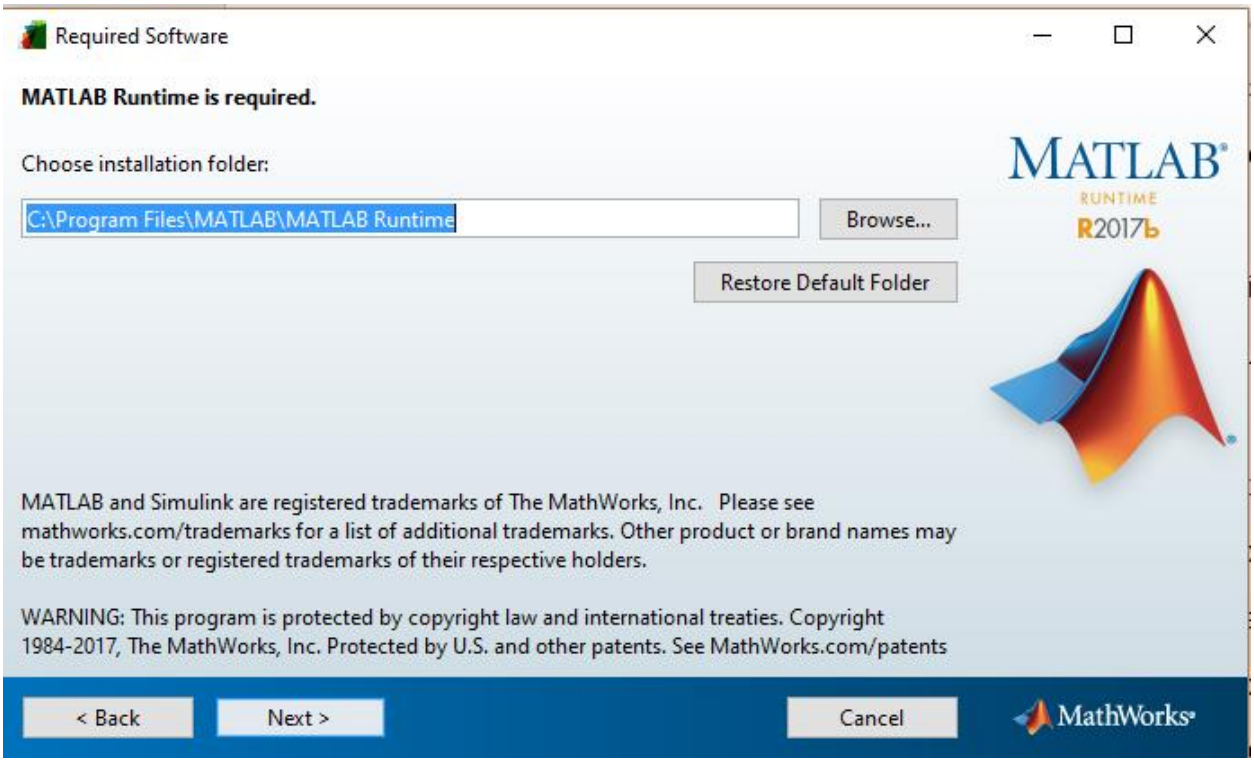
MEAnalyzer User Manual

Contents

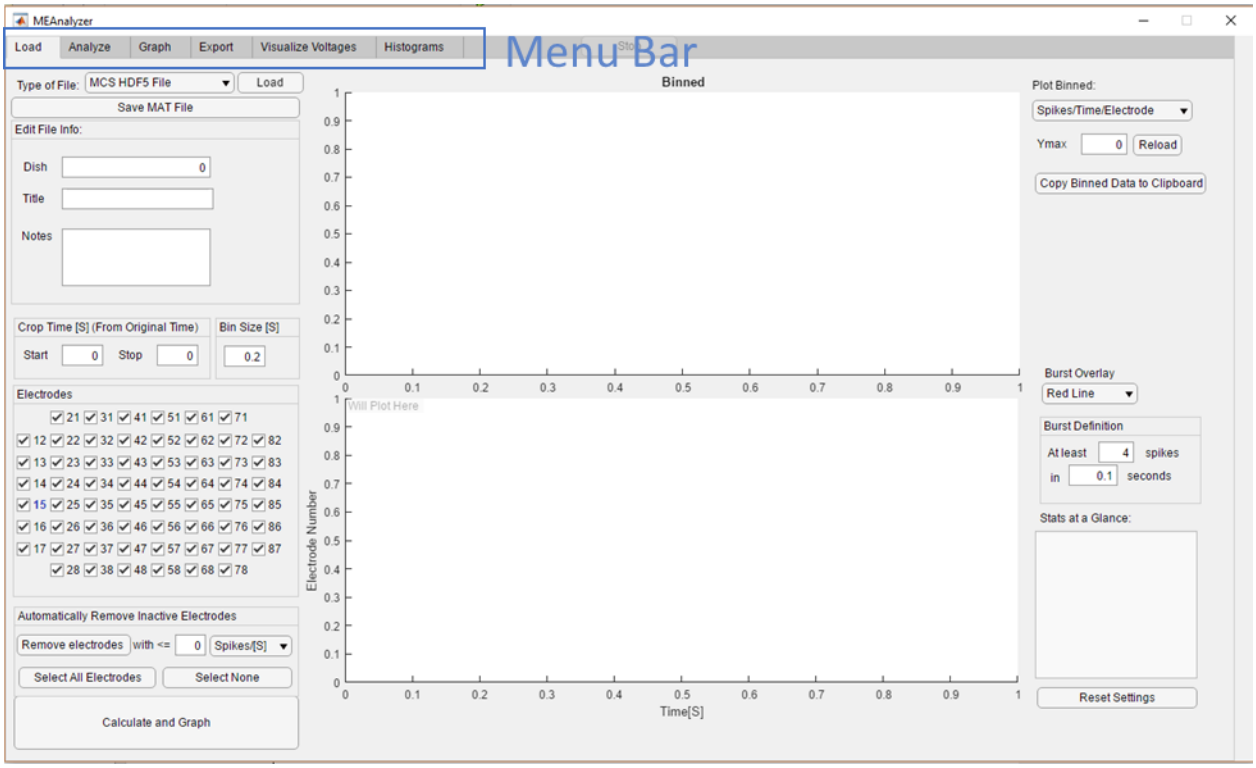
Getting Started

Installation

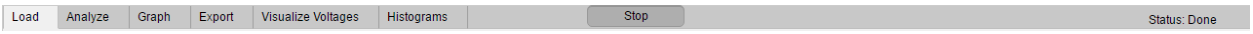
MEAnalyzer was compiled on MATLAB 2017b and requires 64-bit Windows with at least 8GB RAM. The first time installing MEAnalyzer will require prior installation of MATLAB runtime, a standalone set of shared libraries that enables the execution of compiled MATLAB applications without MATLAB or a MATLAB license. If MATLAB runtime is not present, MEAnalyzer will automatically download and install it. Future updates and installations of MEAnalyzer will not repeat this step.



Navigating MEAnalyzer



MEAnalyzer has six main tabs: Load, Analyze, Graph, Export, and Visualize Voltages, and Histograms. Due to the orientation of graphical displays in MEAnalyzer, window sizes are not adjustable. If the software is not fully viewable on your monitor, you may need to increase your screen resolution.



To the right of the tabs is a status bar that indicates the current task that MEAnalyzer is performing. In the center is a stop button that will allow you to interrupt ongoing functions.

Outline of Tabs and Functions

Load

The load tab allows for the loading, and customization of spike train data. Spike train time stamps can be loaded from an HDF5 file exported from MC_Rack, a csv file, or a file previously loaded and saved by MEAnalyzer. On this tab, file metadata can be viewed and edited. The time segment of the experiment can be adjusted, as well as the Bin Size for visualizations and subsequent analyses. Labeled electrodes are displayed in their relative locations on the array, and any number of individual electrodes can be removed from analysis. This tab also displays two plots: A raster plot of the selected data and a binned plot.

Analyze

The Analyze tab includes a spreadsheet of electrode-specific calculations (spike rate, burst rate, percent of spikes in bursts) that can easily be copied to the clipboard and pasted into any spread sheet or statistical software. It also contains panels for periodicity analysis.

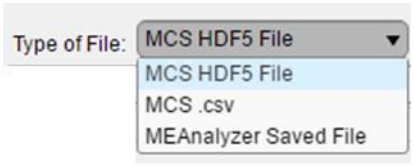
Graph
The Graph tab contains functional connectivity analysis options as well as a topographically correct display of electrode locations and multiple calculation and visualization options.

Export
The export tab allows the user to select desired quantitative calculations and export them to excel spreadsheets. It also allows for the creation of movies that represent spike rates or connectivity graphs of overlapping bursts.

Visualize Voltages
The visualize voltages tab is optional if voltage data is loaded (not recommended). It allows the user to visualize voltages and corresponding spikes.

Histograms
The histogram tab displays fully customizable histograms of inter-spike intervals, inter-burst intervals, burst lengths, and the number of spikes in bursts.

Loading and Saving Spike Trains
MEAnalyzer allows data to be imported from an HDF5 file exported from an MC_Rack file, a csv file containing spike trains, or a file previously analyzed and saved with MEAnalyzer:



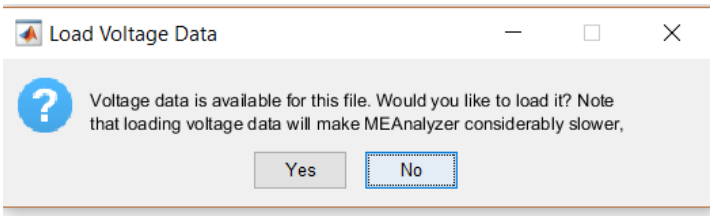
After loading a file, MEAnalyzer will display the full raster plot and binned spikes.

Note that on some systems the GUI popup for opening a file may hide behind the main MEAnalyzer window instead of layering on top. If you do not see a popup, please check behind this window.

Export to HDF5 from Data Manager
To open a file from MC_Rack you will need to convert it to an HDF5 file. You can do this using MCS DataManager, which is available free of charge at:
http://www.multichannelsystems.com/software/multi-channel-datamanager

Make sure to export the Spike Time Stamps. Exporting voltage data is optional and will make the file size considerably larger. Plotting Voltage data will also slow down MEAnalyzer, but the option is there if desired.

Opening .H5 file
MEAnalyzer will display a dialog box where you can select the file you want to load. If Voltage data is attached, MEAnalyzer will present you with the option of loading the data. Remember that loading Voltage Data does not affect spike analysis, and will slow down MEAnalyzer.



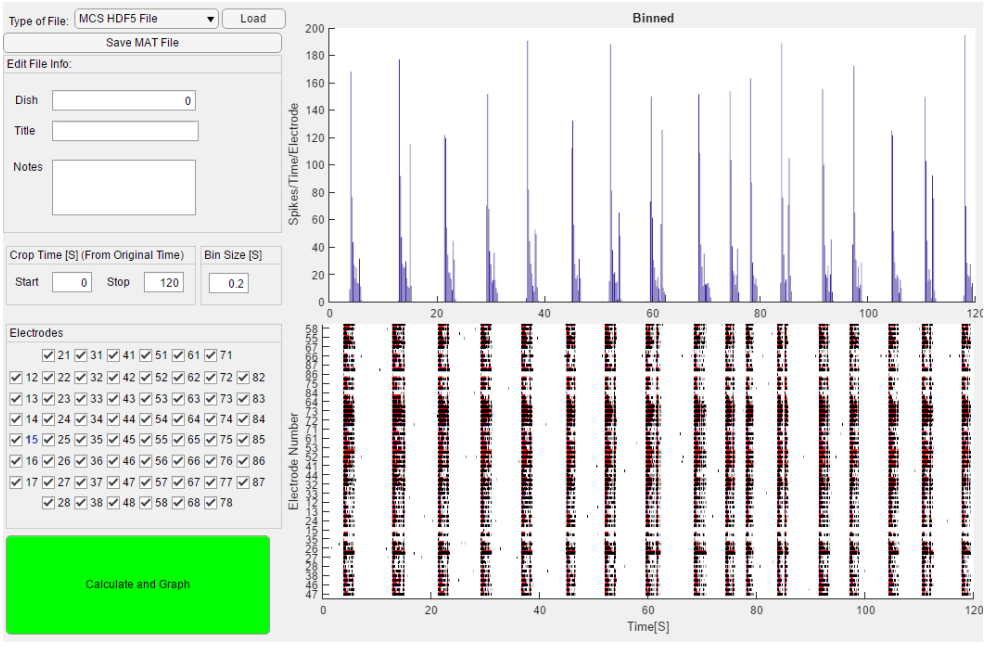
Importing Data from CSV

Spike Trains may be imported either from a CSV file exported from Data Manager. CSV files may also be loaded from other programs as along as the format is the same. Data should start on the 7th row, with the 7th row corresponding to electrode labels and each column containing the time (in µs) of each detected spike.

Opening file Saved by MEAnalyzer

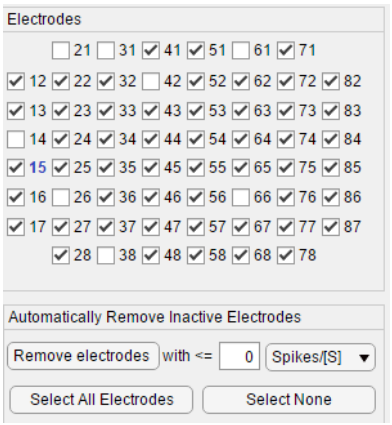
Files previously saved from MEAnalyzer as a .MAT file may also be opened. MEAnalyzer will load the .MAT file and search for the SpikeData saved in variable “x”

Editing Parameters



Electrode Removal

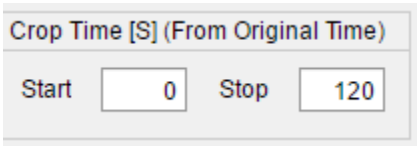
MEAnalyzer is currently set up for an 8x8 multichannel array plate from MultiChannel Systems. The orientation of the electrodes is displayed so you can easily select the electrodes to include or remove. Reasons for excluding electrodes from analysis may include that the electrode has high noise levels, where there is no activity, or where the user is aware that spike detection was erroneous.



The user may also use the “Remove electrodes” button to automatically remove inactive electrodes, as defined by electrodes that do not exceed the threshold of spikes or bursts desired to be considered active.

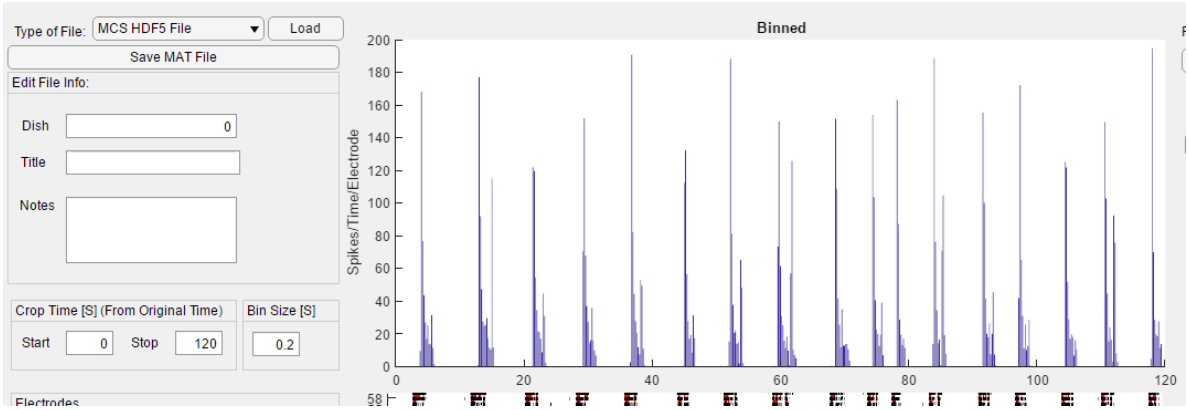
Cropping Time Segment

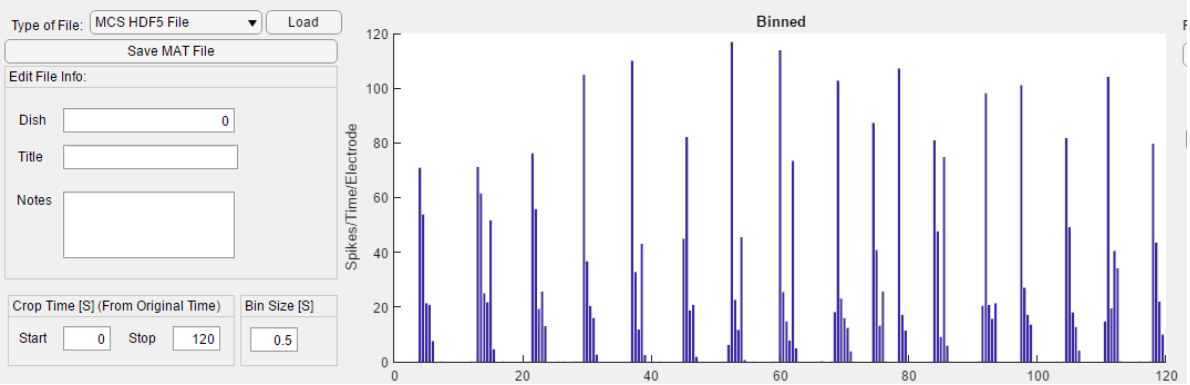
The user may type in the time segment of the original recording that they wish to analyze.



Adjusting BinSize

BinSize defines the length of time over which spikes are summed in the Binned plot. Binsize is also used on other tabs to calculate periodicity and network connectivity.





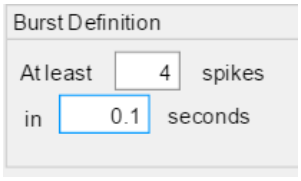
Insert Metadata

For experimental purposes you may want to type in meta data such as the number of the MEA plate, the title or experimental condition, and any notes about the experiment. When saving the MEAnalyzer file or exporting results to excel these Metadata will also be included.

Spike Train Calculations

After changing spike parameters it will be necessary to redo all spike calculations by clicking on the “Calculate and Graph” button. All calculations will be computed with the previously defined parameters and electrodes.

Burst Calculations



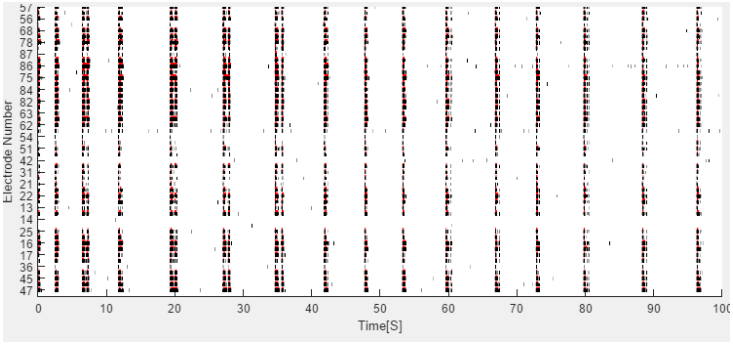
Bursts are calculated by user-defined parameters of the minimum number of spikes in a duration of time

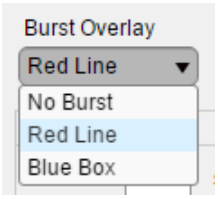
Raster Plot

The raster plot is presented as vertical black lines. The x-axis corresponds to the electrodes in the order they were loaded and they-axis corresponds to the time. For each electrode a vertical

black line will be plotted at each time point where a spike was detected. The time axis will be adjusted to start at 0.

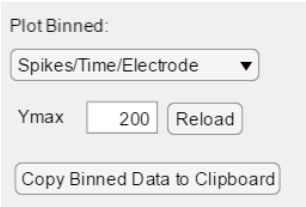
Note that that if many electrodes are included, not all electrode labels will be shown in order to prevent overcrowding.





The bursts may also be shown on the raster plot if desired. It may be displayed as a red line on top of the spikes or a blue box overlaying the spikes.

Binned Plot



The binned plot is displayed on top of the raster plot, and is calculated according to the BinSize as defined by the user. During the first data plotting the y-axis will be chosen automatically. However, you can adjust the y-axis as well as the type of data that is plotted. The binned data plotted can be: Spikes/Time/Electrode, Percent of Electrodes Spiking, and Percent of Electrodes Bursting. Traditionally, the percent of electrodes spiking can be interpreted as network bursts if they exceed a certain percentage. The data from the selected binned plot can be copied to the clipboard for easy transfer to spreadsheet software.

Spike Calculations

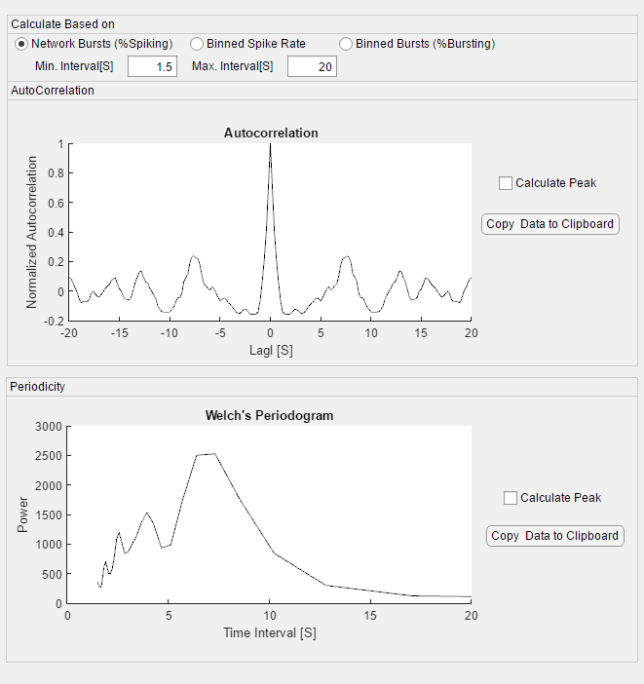
Spike calculations will be displayed on the analyze tab. This allows for a quick scan to aid in the inclusion or removal of electrodes from analysis.

Electrode Label	Spikes/Second	Bursts/Minute	Percent of Spikes in Bursts
12	11.4300	13.8000	97.2003
13	0.9700	0.6000	5.1546
14	15.6100	13.2000	98.2703
16	17.1400	12	98.5998
17	15.5800	9.6000	98.4596
21	6.1400	11.4000	93.6482
22	14.0300	13.8000	97.8617
23	15.4000	12.6000	97.9870
24	15.0600	9.6000	98.6056
25	14.6000	12	98.2192
26	18.6100	11.4000	98.7104
27	8.2900	12	94.0893
28	5.3000	9.6000	91.1321
31	16.9600	10.8000	98.4670
32	18.1900	13.8000	98.1858
33	8.1000	12.6000	95.3086
34	15.8900	10.8000	98.4267
35	0.3800	0	0
36	2.4700	8.4000	79.7571
37	1.1100	4.8000	50.4505
38	14.2600	13.2000	96.9846
41	11.9500	13.2000	96.9038
42	3.5400	12.6000	78.8136
43	10.2900	13.8000	96.6958
44	10.9100	12	90.0092
45	11.0500	15	96.3801
46	10.8500	13.2000	97.1429
47	9.7000	12	94.7423
48	13.8300	13.2000	96.4570
51	15.3500	11.4000	98.3713
52	0.0300	0	0

Copy Selected Data To Clipboard

Periodicity Analysis

Periodicity analysis can be used to identify repetitive oscillating behavior. Periodicity is shown on the analyze tab and can be calculated using autocorrelation or periodicity. The user can select the type of binned data that is used for the calculations: Network bursts (% of electrodes spiking), binned spike rate, or binned bursts (% bursting).



All plots can be copied to the clipboard for easy transfer to spreadsheet software and further analysis. Quick rudimentary analysis options attempt to identify the peak, and the associated periodic interval by finding the highest local maxima that is greater than 2 standard deviations of the mean.

Autocorrelation

Autocorrelation measures the similarity between a signal and a copy of itself that has been shifted by a time lag. If the signal demonstrates periodic behavior there will be a peak at the lag that represents the cycle length. The normalized autocorrelation function $r(\tau)$ measures the probability that the next event will occur at time $t + \tau$.

$$r(\tau) = \frac{\sum_{t=1}^{T-\tau}(y_t - \bar{y})(y_{t+\tau} - \bar{y})}{(T-1)Var(y)}; \tau = 0, \pm 1 * fs, , \pm 2 * fs, \ldots.$$

where fs is the sampling frequency (binSize-1) and τ is the time lag.

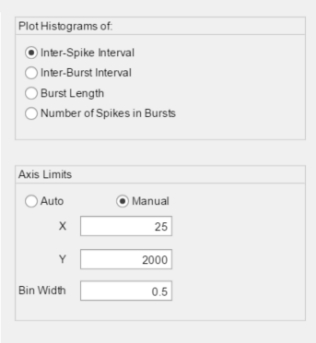
Welch's Periodogram

Welch's power spectral density estimates correction instead of a standard power spectrum. This approach reduces the variance of the periodogram by breaking the time series into overlapping segments, computes a modified periodogram for each segment, and then averages the segments to estimate power spectral density (PSD). The PSD reports how much of expected signal power is at each frequency, and is expressed as a function of the frequency by: $P_{xx}(f) = \frac{1}{f_s}\sum_{m=-\infty}^{\infty} R_{xx}(m)e^{-j2\pi mf/f_s}$. Peaks in the power spectrum correspond to the repeating periodic intervals. Calculating periodicity in this manner allows for identification of multiple periodic frequencies.

Calculating Peak

The data can be copied and pasted into Excel for plotting purposes and for identifying peaks. MEAnalyzer can help with peak detection if the “Calculate Peak” button is selected. Only one peak will be identified, and it will be the highest value between the Min and Max Interval that is at least two standard deviations above the mean in that interval. The data in each plot can be copied directly into excel or other programs for custom peak selection methods.

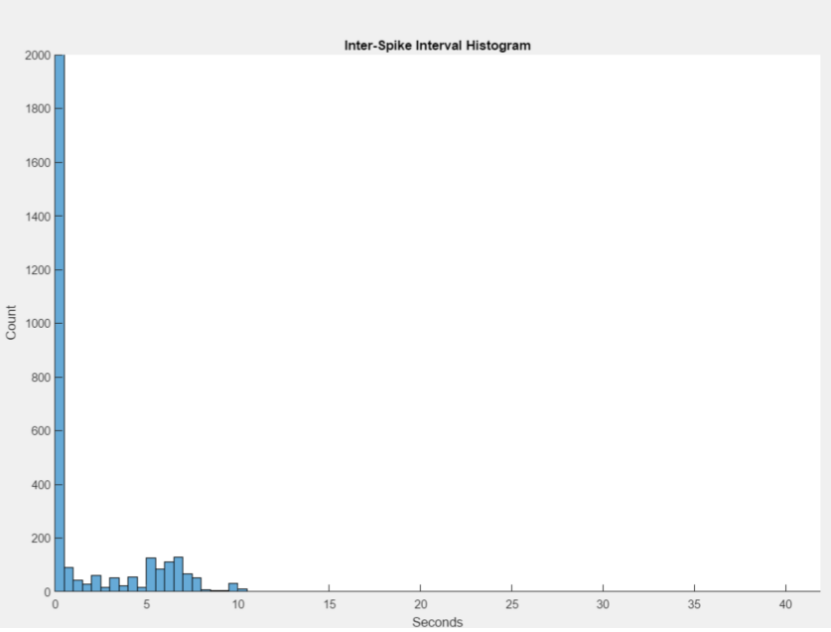
Histograms



The histogram tab allows for the creation of fully customizable histograms of inter-spike intervals, inter-burst intervals, burst lengths, and the number of spikes in bursts. These histograms can be used to analyze data sets, or to inform parameter choice for other analysis methods. Idea axis limits and bin widths can be automatically chosen or can be manually specified.

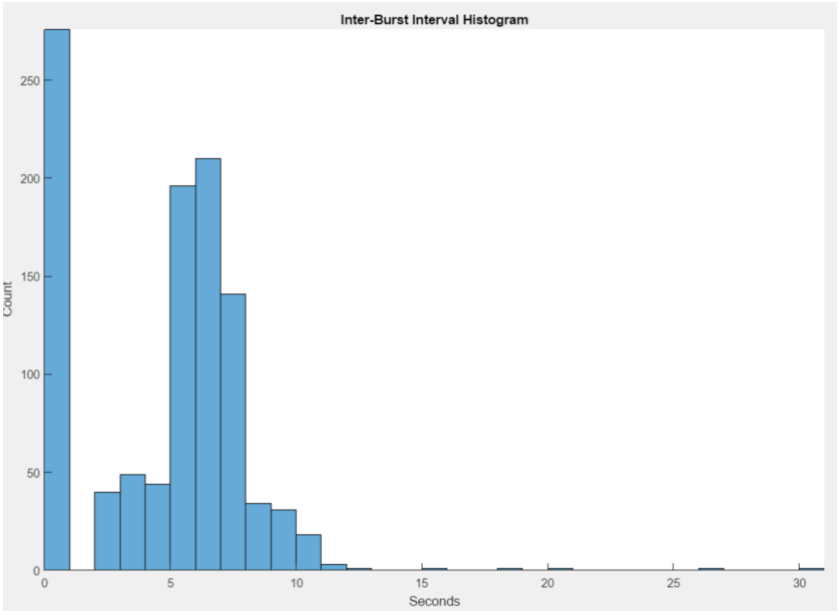
The inter-spike interval

The inter-spike interval (ISI) option allows for the traditional display of time between spikes.



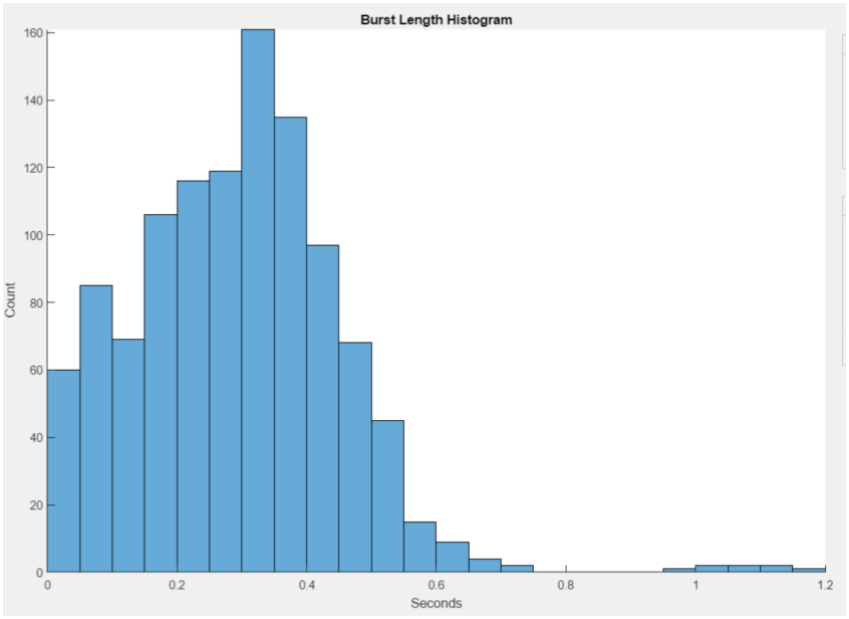
Inter-burst Interval

The inter-burst interval option creates a histogram of the time between when one burst ends, and the following burst begins. While this is one potential option for describing periodicity, please refer to the periodogram or autocorrelation options on the analyze tab for more advanced methods.



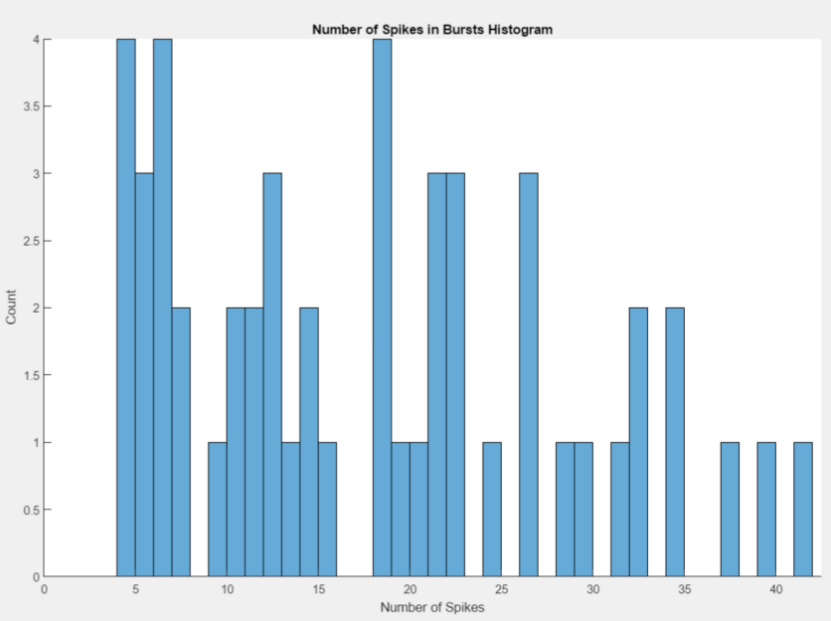
Burst Length

The burst length option creates a histogram of the length (in seconds) of each burst. This can also be used to inform burst detection parameters.



Number of Spikes in Bursts

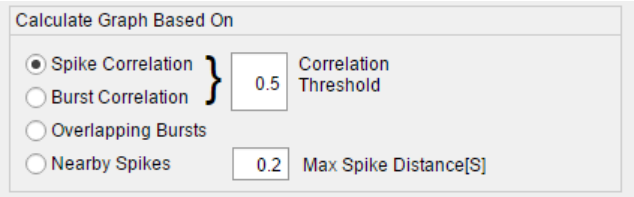
The histogram of the number of spikes in each burst can be used to inform burst detection parameters or to compare changes in activity patterns.

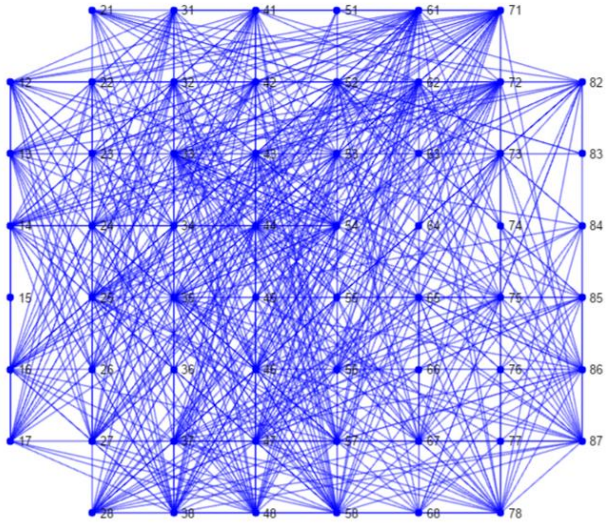


Connectivity Analysis

Graph Selection

MEAnalyzer provides options to create functional connectivity graphs based on a variety of different measures. Each electrode is a node, and an edge is created if it displays correlation based on the selected measure.

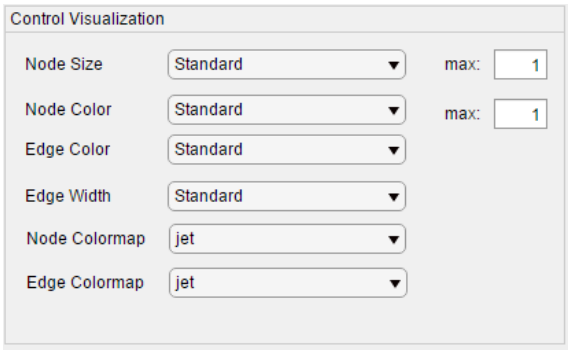




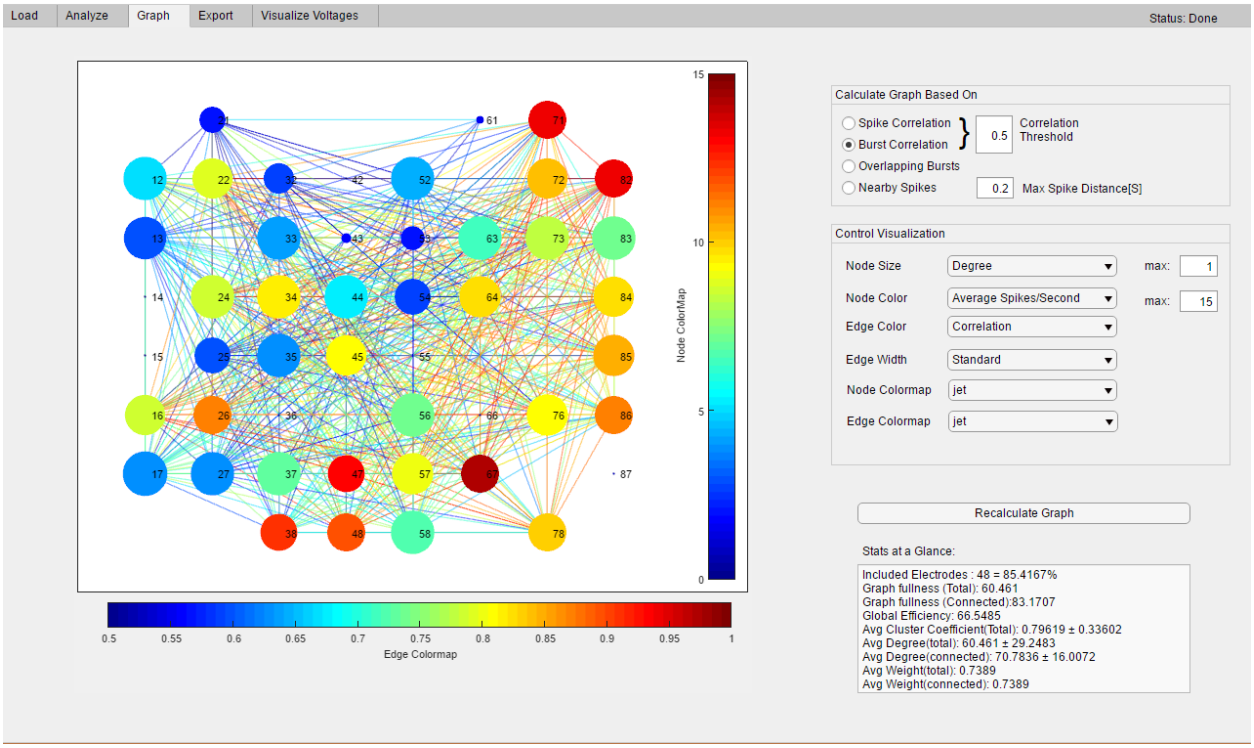
Spike Correlation, and Burst Correlation look at the cross-correlation between two electrodes at the time lag of 0 based on either the binned spikes or binned bursts. An edge is created between two electrodes if their mean cross-correlation at a time lag of 0 exceeds the user-defined threshold. As an example, a Spike Correlation of 0.5 would approximate a 50/50 probability of the two electrodes showing spike activity at the same time.

Graph Visualization

MEAnalyzer includes options to change the size and color of nodes and edges to represent different variables. The Node and Edge size and color can be adjusted, along with their own individual colormaps. Whenever a visualization option is selected the values are scaled to match the range designated by the “max” numerical field.

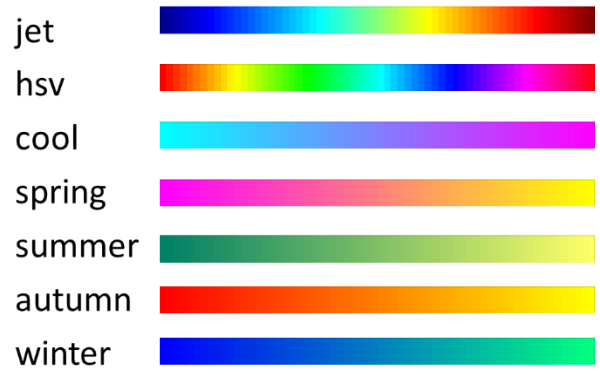


Node Size and color can be changed to represent standard graph math metrics. For example, they can be set to represent node degree or the cluster coefficient. Node size and color can also be changed to represent spike train metrics, such as spike rate or burst rate.



If a cross-correlation method was used to create the graph, then the edges have a weight property that corresponds to the average cross-correlation of two nodes. The visualization can be adjusted to let the color or width of each edge represent the edge weight.

The following color maps are available:



Graph Math

Graph Fullness

Graph fullness is calculated as the percent of possible edges that are connected

$$\frac{n_{edges}}{\frac{(n_{electrodes})(n_{electrodes}-1)}{2}}$$

Degree

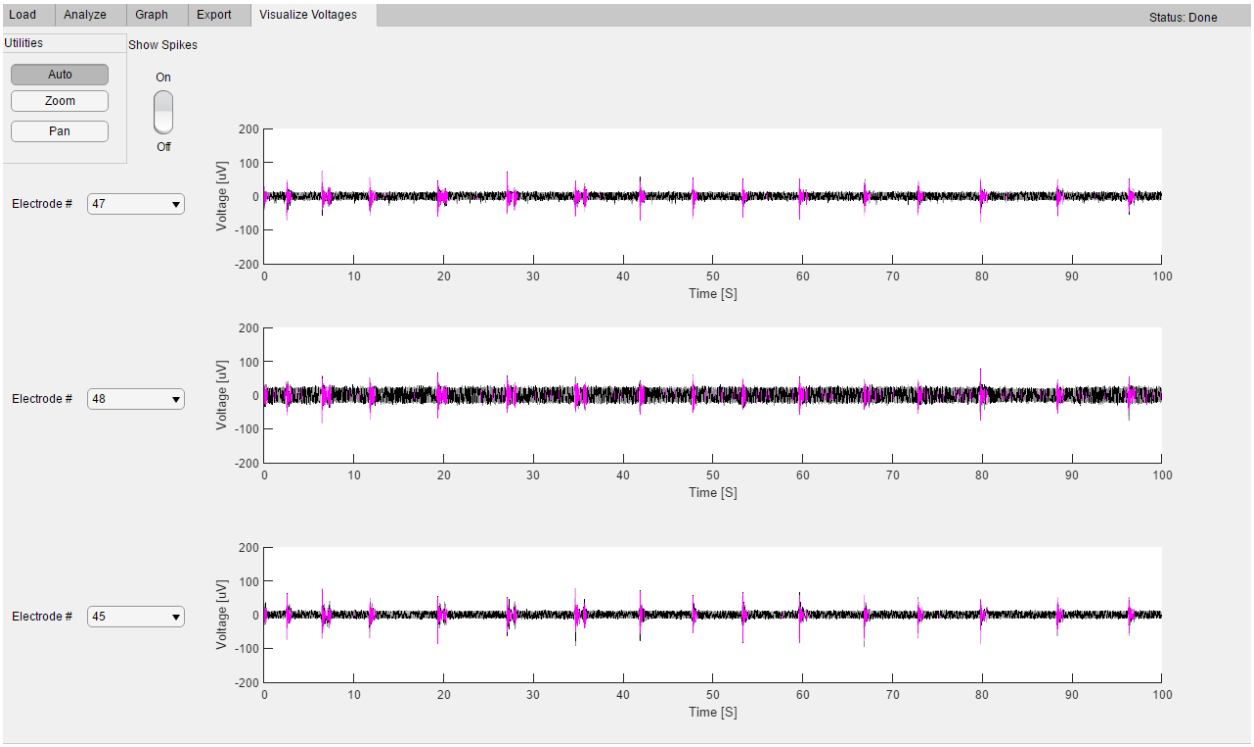
In Graph Theory, node degree represents the number of other nodes that a node is connected to. In MEAnalyzer , degree is normalized so that it represents the percentage of other nodes that an individual node is connected to.

Cluster Coefficient

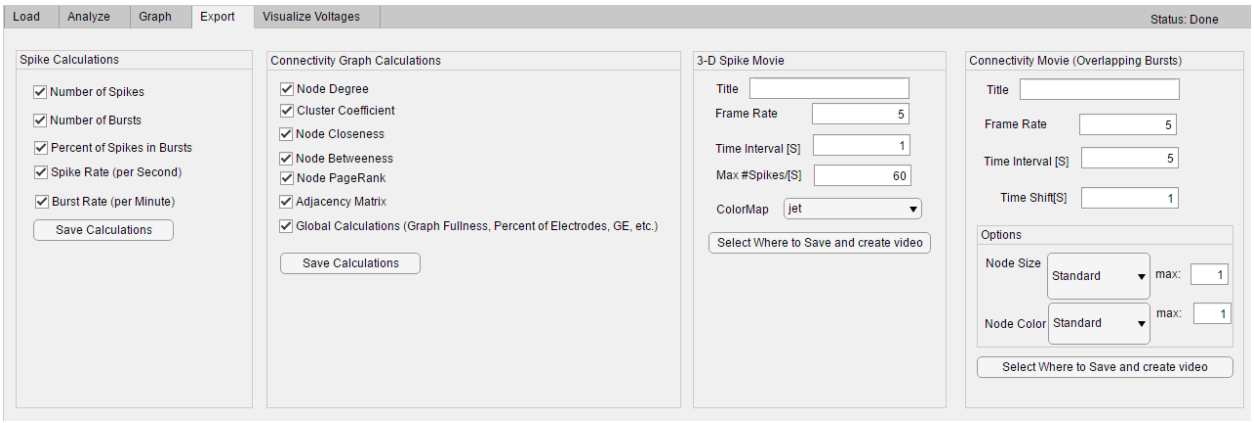
Cluster Coefficient essentially measures the connectivity density of each graph[18] where each node i in graph G is defined as $CC_i = \frac{2n_i}{k_i(k_i-1)}$ and n_i is the number of connections between a node and its topographical neighbors, and k_i is the node degree.

Visualizing Voltages

MEAnalyzer does not perform spike detection, but if Voltage Data is available it may be useful to visualize voltages. This can help the user evaluate which electrodes are appropriate for exclusion from analyses, either because of high electrode noise or inappropriately detected spikes. Three electrode voltages can be visualized at once. The user may decide to display spikes overlaying the voltage plots.



Exporting Data



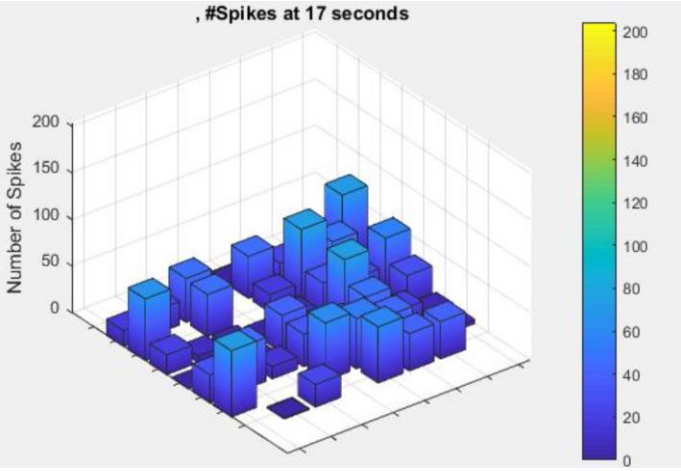
Exporting to Excel

Under “Spike Calculations” and “Connectivity Graph Calculations” the user may select the desired metrics to export. Upon clicking the “Save Calculations” button MEAnalyzer will present a dialog box that allows the user to define the location and file to be created. In each case, an excel file of multiple sheets will be created, and the first sheet will contain the metadata and calculation parameters for the Spike Trains.

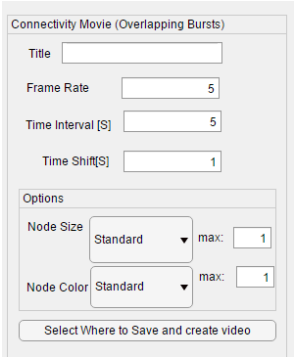
4D Spike Visualizations

Exporting the data as a 4D Spike Visualization creates a movie file where each frame consists of a 3D bar graph.

Any electrodes removed from analysis will not be represented by bars in the movie file. The x-y- location of each bar will correspond to the topographical location of that electrodes on the plate. The height and color of each bar will correspond to the total number of spikes by that electrode in the time interval defined by the user. If a title is given it will be displayed at the top of the video. To be able to create comparable movie files between different plates, the maximum number of spikes can be adjusted to control the graph visualization.



Connectivity Graph Movie



Connectivity graphs based on overlapping bursts may not be appropriate for longer experiments, as longer time lengths will have a higher probability of overlapping bursts and may result in a full graph that would prevent comparisons between different experiments. To represent longer time lengths with the overlapping burst method, the connectivity graph movie option was created.

This will create a movie file where each frame consists of a raster plot and connectivity graph. The connectivity graph will be the graph based on overlapping bursts for a subset of the full time, as defined by the Time Interval. The raster plot will have a shaded region that shows the time interval for which the connectivity is being calculated.