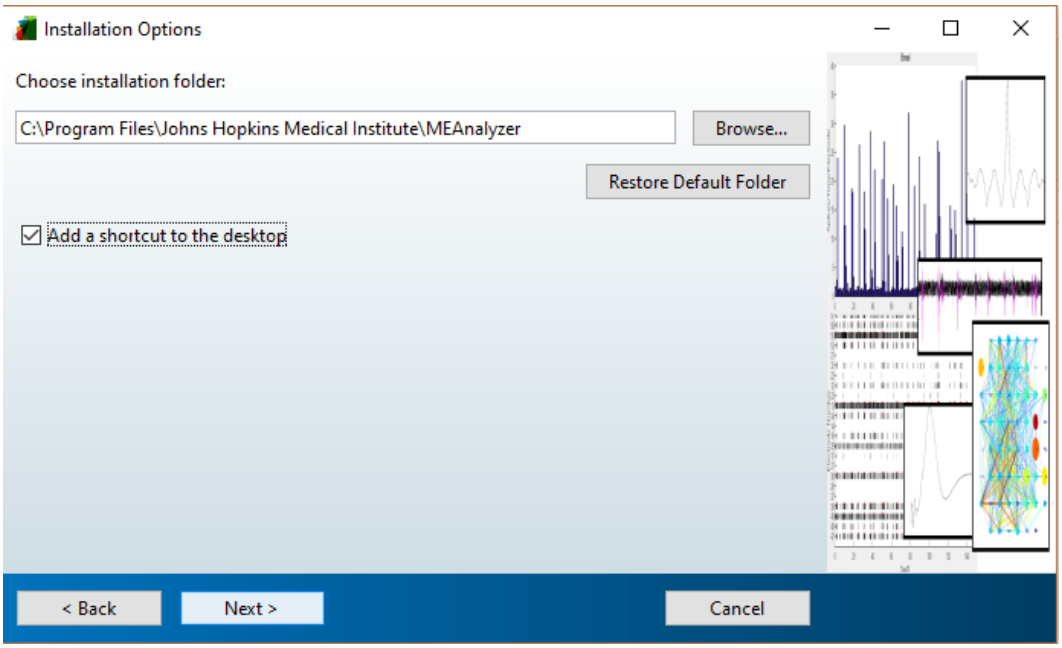
MEAnalyzer User Manual

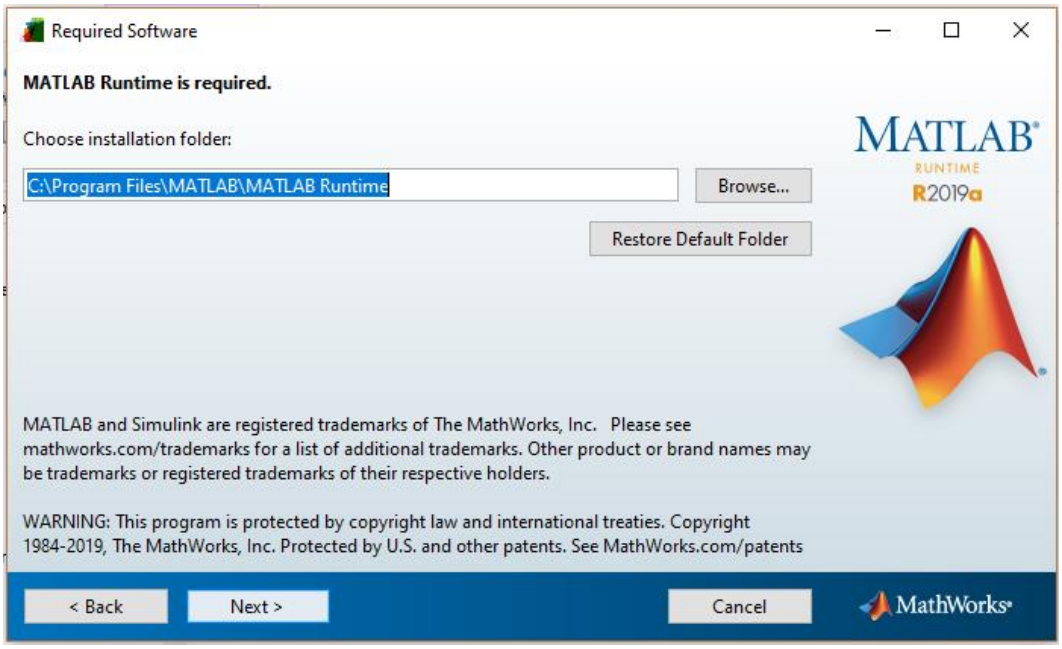
Contents

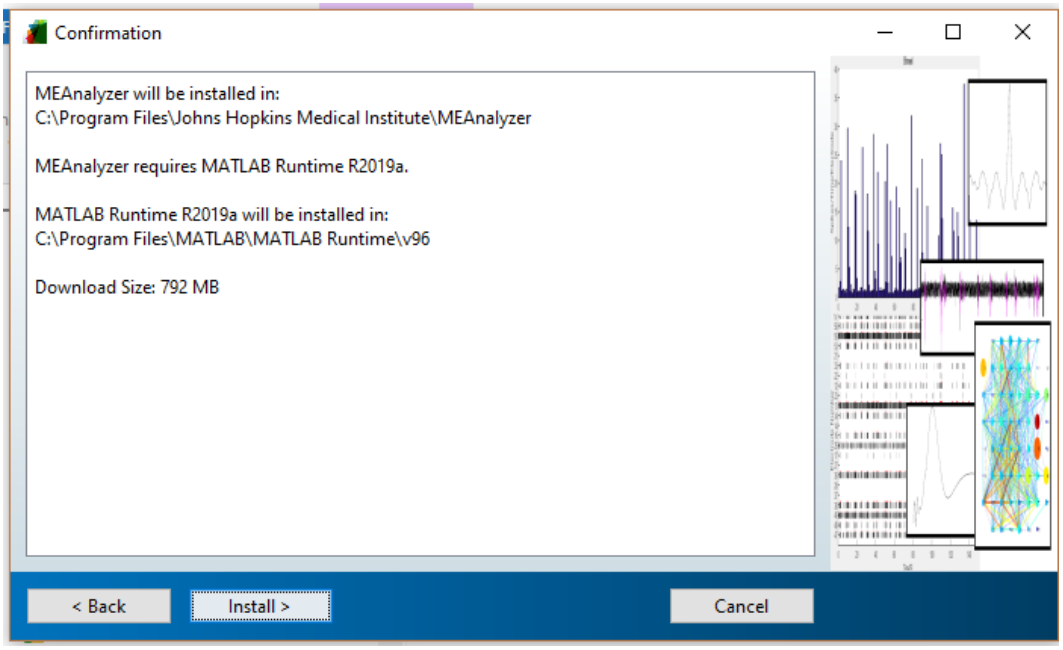
Getting Started

Installation

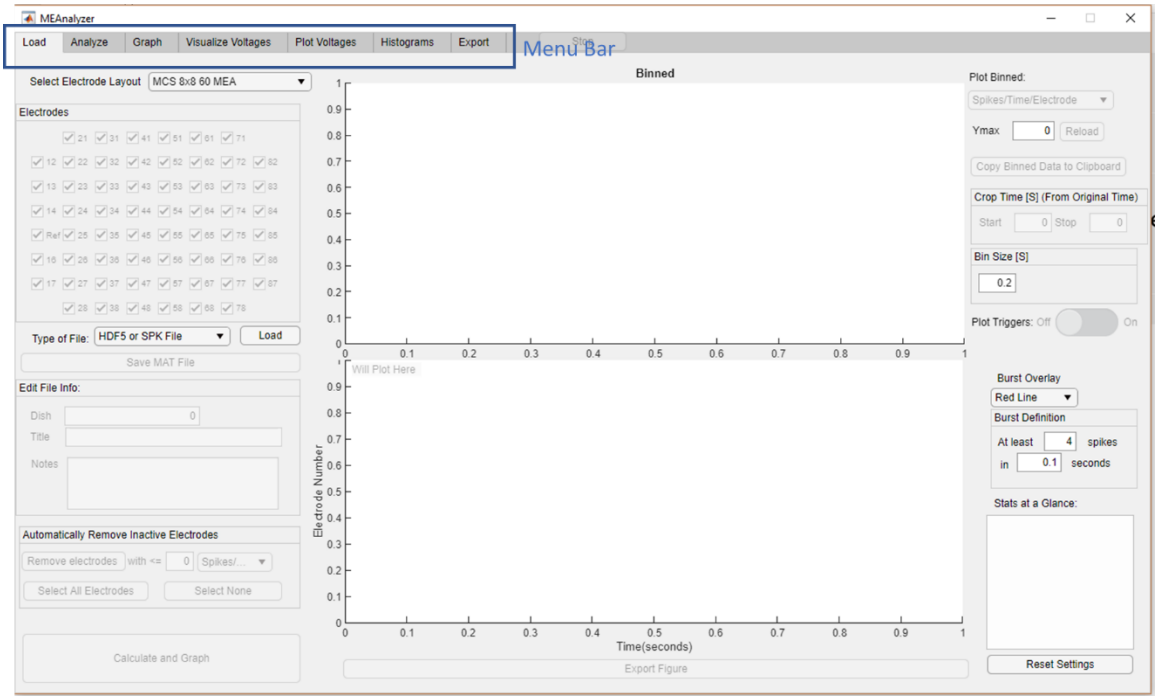
MEAnalyzer was compiled on MATLAB 2019a and requires 64-bit Windows with at least 8GB RAM. The first time installing MEAnalyzer will require prior installation of MATLAB runtime, a standalone set of shared libraries that enables the execution of compiled MATLAB applications without MATLAB or a MATLAB 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. If the runtime does not install properly, you can also access it here: https://www.mathworks.com/products/compiler/matlab-runtime.html







Navigating MEAnalyzer



MEAnalyzer has six main tabs: Load, Analyze, Graph, and Visualize Voltages, Plot Voltages, Histograms, and Export. 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 or SPK file exported from MC_Rack, Experimenter, or Axion, 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, including Autocorrelation and Welch's periodogram that can be calculated based on Network Bursts, Binned Spike Rate, or Binned Bursts.

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.

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.

Plot Voltages

The Plot Voltages tab is optional if voltage data is loaded (not recommended). It is similar to the load tab that displays binned plots and raster plots, but can be color coded to correspond to voltages identified at spike times.

Export

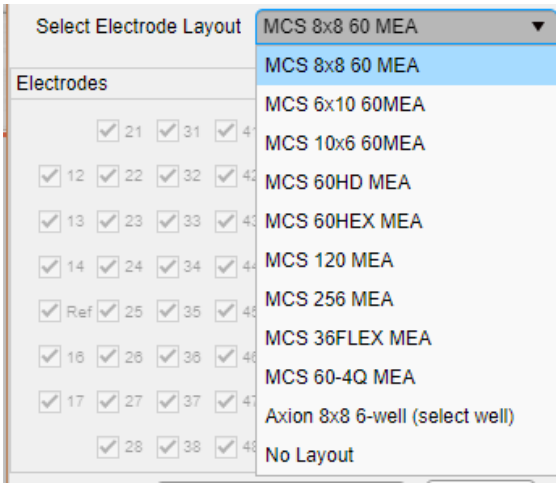
The export tab allows the user to select desired quantitative calculations and export them to excel spreadsheets. It also allows for the creation of movies that represent spike rates or connectivity graphs of overlapping bursts.

Histograms

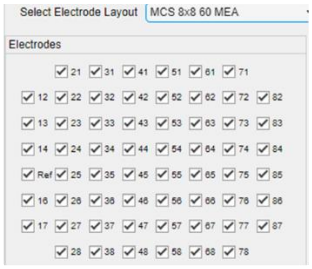
The histogram tab displays fully customizable histograms of inter-spike intervals, inter-burst intervals, burst lengths, and the number of spikes in bursts.

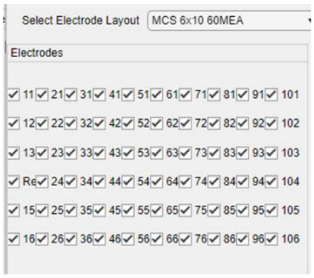
Loading and Saving Spike Trains

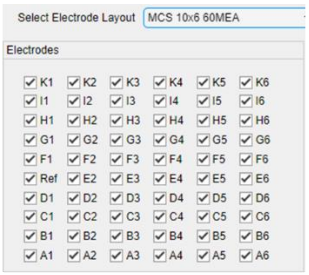
Before loading a file, select the Electrode layout that matches your experiment:

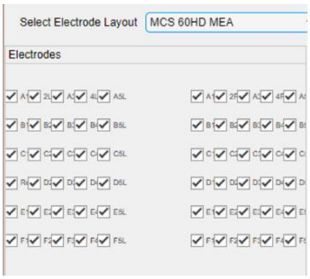


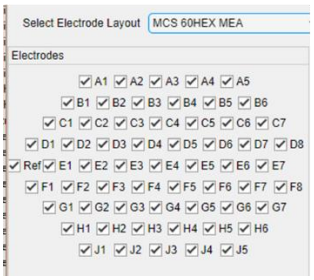
MEAnalyzer is equipped with a variety of electrode layouts to match your desired experiment:

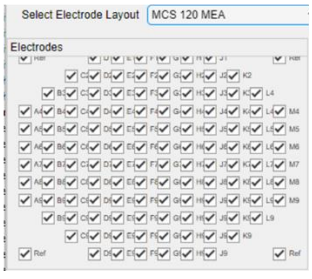


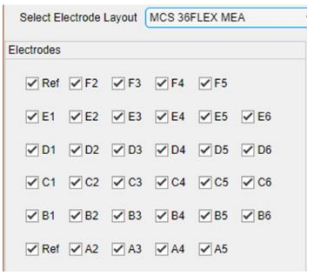


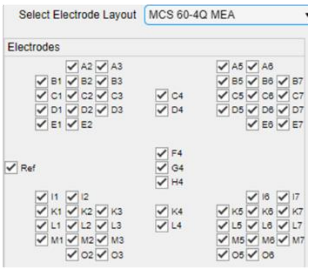




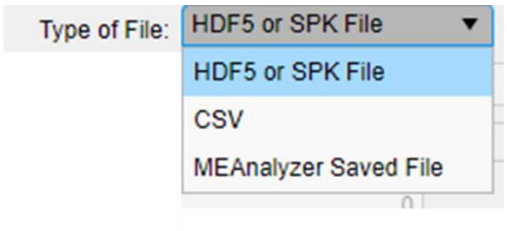






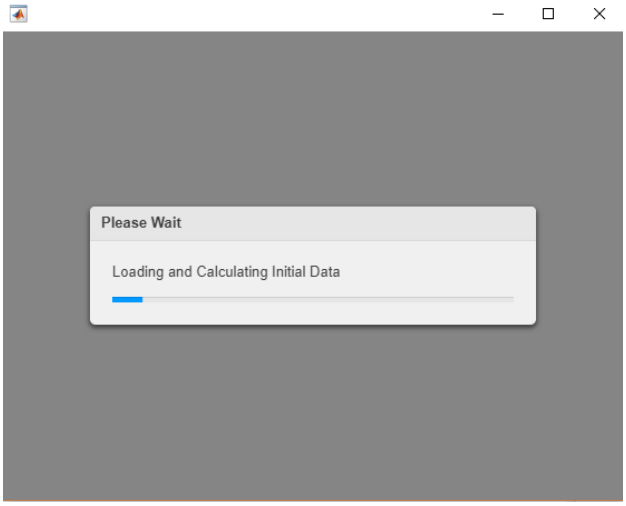


MEAnalyzer allows data to be imported from an HDF5 file exported from an MC_Rack or Experimenter file, a csv file containing spike trains, or a file previously analyzed and saved with MEAnalyzer:



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

As this initial loading may take some time, MEAnalyzer will display popups to let you know that it is still calculating



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.

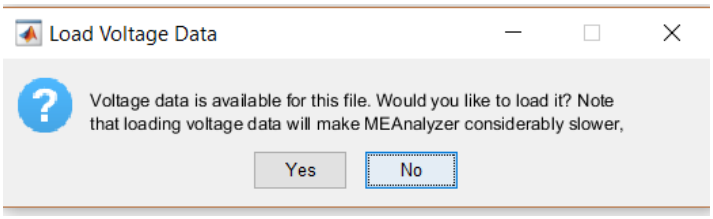
Export to HDF5 from Data Manager

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.

Importing with No Electrode Layout

Data may also be imported from any spike train not associated with an electrode layout. Some MEAnalyzer functionality that is dependent on topography, such as the 3-D spike videos. However, as everything else is a mathematical calculation, they will all work the same. Connectivity graphs will be displayed with an arbitrary layout, with all the same visualization options. For this type of format, the csv file must contain spike timestamps in 10^6s units. Each column will represent one spike train. The first row will be interpreted as node labels.

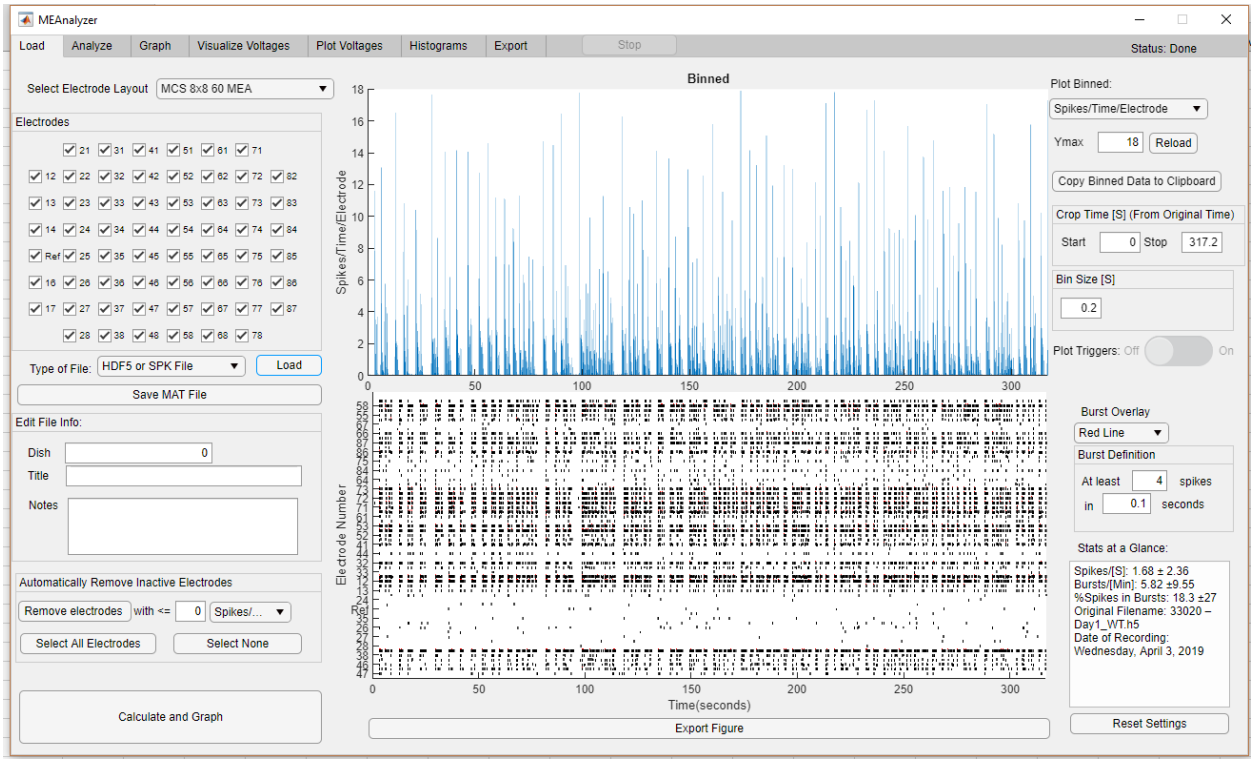
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	1	2	3	4	5	6	7	8	9	Ref	11	12	13	14	15	16	17	18
2	670560	844880	1009520	4760	947560	2622480	81160	2828640	2996880		670560	844880	1009520	4760	947560	5487320	3511520	6380760
3	1392880	1411360	1743360	18680	2817800	2649320	743360	2906360	3593760		1392880	1411360	1743360	18680	2817800	5552240	3570640	7987240
4	2565160	1421240	1811040	32320	2996880	2666320	750000	3511520	3641400		2565160	1421240	1811040	32320	2996880	5604280	3612120	8009920
5	2628360	1666760	2610280	81160	3593760	2701560	752360	3570640	5370280		2628360	1666760	2610280	81160	3593760	5735400	5487320	8045240
6	4266640	1705400	2626480	743360	3641400	3423000	782720	3612120	5379040		4266640	1705400	2626480	743360	3641400	6010840	5552240	8295440
7	6670040	1718200	2630760	750000	5370280	3447200	806040	5487320			6670040	1718200	2630760	750000	5370280	6315760	5604280	
8	9648600	1746520	2663040	752360	5379040	3466440	834520	5552240			9648600	1746520	2663040	752360	5379040	6380760	5735400	
9	9669200	2299720	2683960	782720	8066400	3497280	1425080	5604280			9669200	2299720	2683960	782720	8066400	7987240	6010840	
10	9693640	2364040	2792160	806040	9832120	3544080		5735400			9693640	2364040	2792160	806040	9832120	8009920	6315760	
11	9707760	2541760	2828640	834520	9843160	3671680		6010840			9707760	2541760	2828640	834520	9843160	8045240	6380760	
12		2561160	2906360	1425080	9883600	3825160						2561160	2906360	1425080	9883600	8295440	7987240	
13		2604640	3511520	1429320	9895160							2604640	3511520	1429320	9895160	8727240		
14		2622480	3570640	1468200								2622480	3570640	1468200		8778000		
15		2649320	3612120	2609320								2649320	3612120	2609320		9680600		
16		2666320	5487320	2615200								2666320	5487320	2615200				
17		2701560	5552240	2621640								2701560	5552240	2621640				
18		3423000	5604280	2625440								3423000	5604280	2625440				
19		3447200	5735400	2632120								3447200	5735400	2632120				
20		3466440	6010840	2637880								3466440	6010840	2637880				
21		3497280	6315760	2655440								3497280	6315760	2655440				
22		3544080	6380760	2674400								3544080	6380760	2674400				
23		3671680	7987240	2683120								3671680	7987240	2683120				
24		3825160	8009920	2695320								3825160	8009920	2695320				
25		4240120	8045240	2703480								4240120	8045240	2703480				
26		4261680	8295440	2734120								4261680	8295440	2734120				
27		4317840	8727240	3024600								4317840	8727240	3024600				
28			8778000	3050880									8778000	3050880				
29			9680600	3057480									9703080	3416320				
30			9703080	3416320									9707080	3458640				
31			9707080	3458640									9728240	3469280				
32			9728240	3469280									9767200	3503840				
33			9767200	3503840									9792880	4304400				
34			9792880	4304400									9897680	4318680				
35			9897680	4318680														

Please note that under this setting, there is no option to remove electrodes from analysis, so this should be done before importing the CSV file.

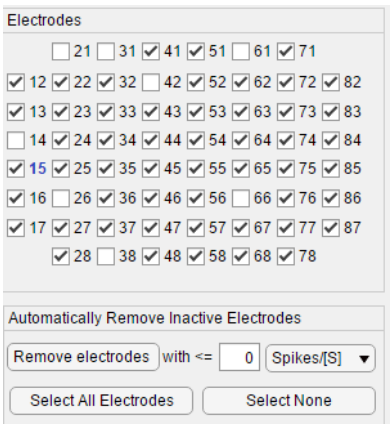
Opening file Saved by MEAnalyzer

Files previously saved from MEAnalyzer as a .MAT file may also be opened to the same settings

Editing Parameters



Electrode Removal

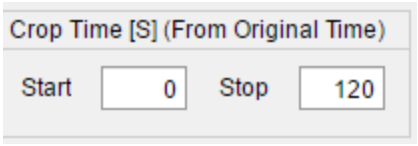


The orientation of the electrode layouts is displayed so you can easily select the electrodes to include or remove. Reasons for excluding electrodes from analysis may include that the electrode has high noise levels, where there is no activity, or where the user is aware that spike detection was erroneous.

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.

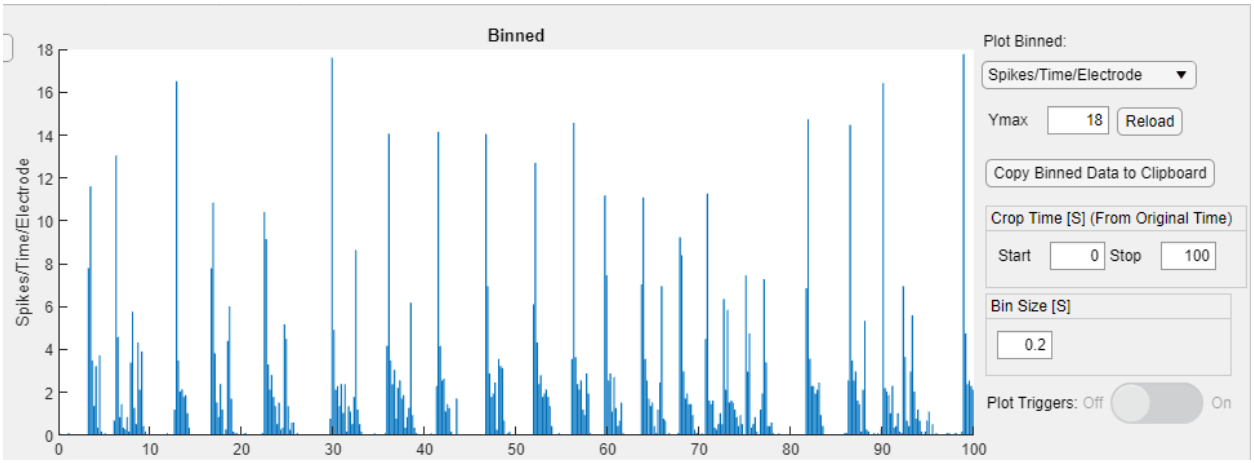
Cropping Time Segment

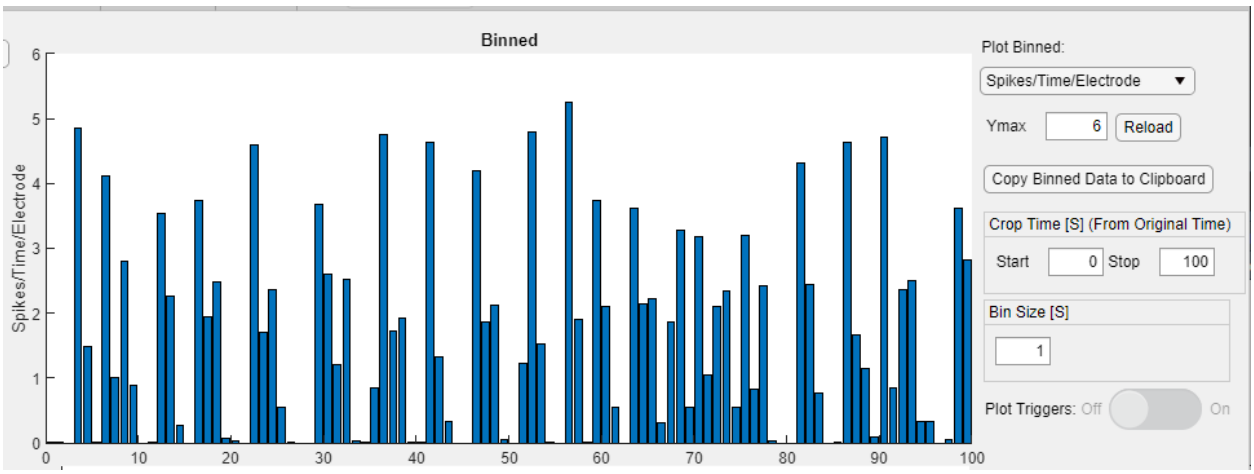
The user may type in the time segment of the original recording that they wish to analyze.



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.





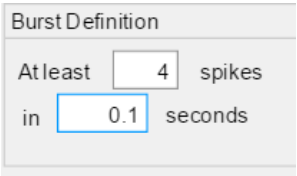
Insert Metadata

For experimental purposes you may want to type in metadata 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

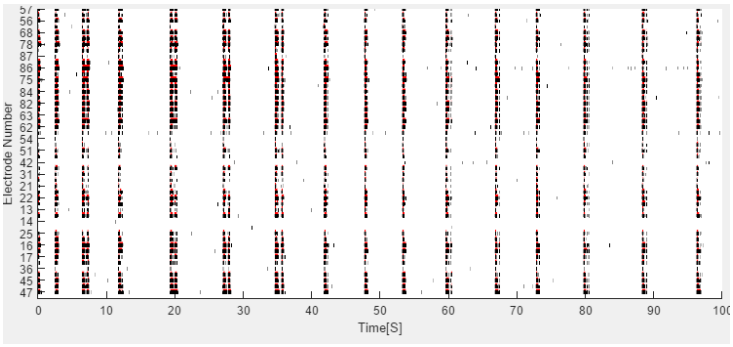


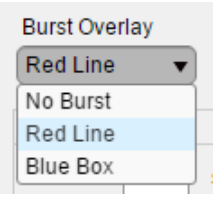
Bursts are calculated by user-defined parameters of the minimum number of spikes in a duration of time

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.

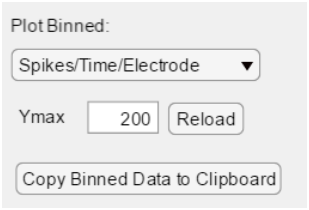
Note that that if many electrodes are included, not all electrode labels will be shown in order to prevent overcrowding.





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.

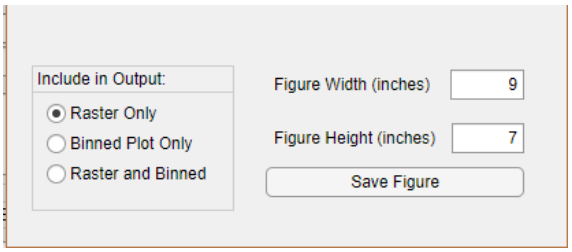
Binned Plot



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.

Exporting Binned Raster Plot

The Binned Spike and Raster plot can easily be exported in publication-ready format by clicking the “Export Figure” button.



You can then select which part of the figure you want to save and in what dimension.

Spike Calculations

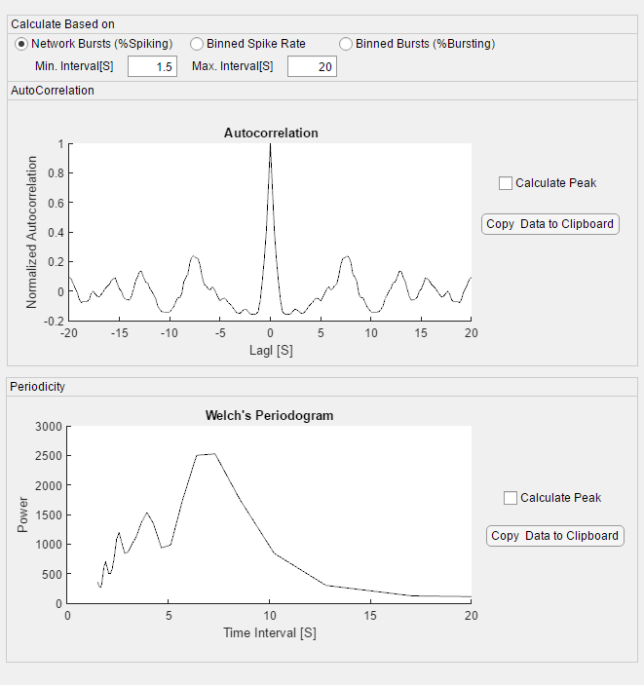
Spike calculations will be displayed on the analyze tab. This allows for a quick scan to aid in the inclusion or removal of electrodes from analysis.

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

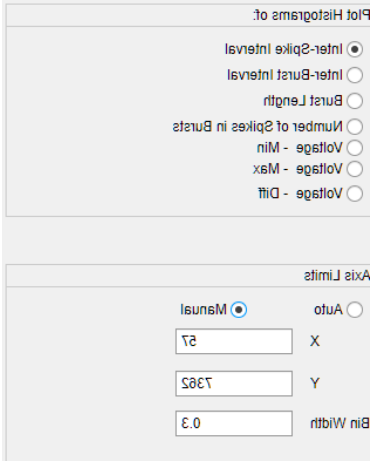
Welch's power spectral density estimates correction instead of a standard power spectrum. This approach reduces the variance of the periodogram by breaking the time series into overlapping segments, computes a modified periodogram for each segment,

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 Periodicity Peaks

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.

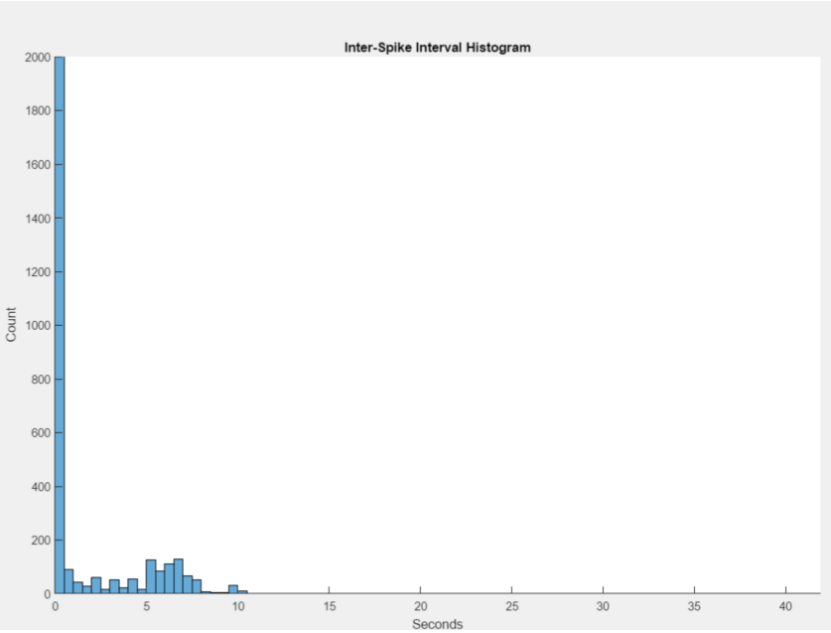
Histograms



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. If voltage segment data is available, histograms can also be created of various voltage parameters at identified spikes. 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.

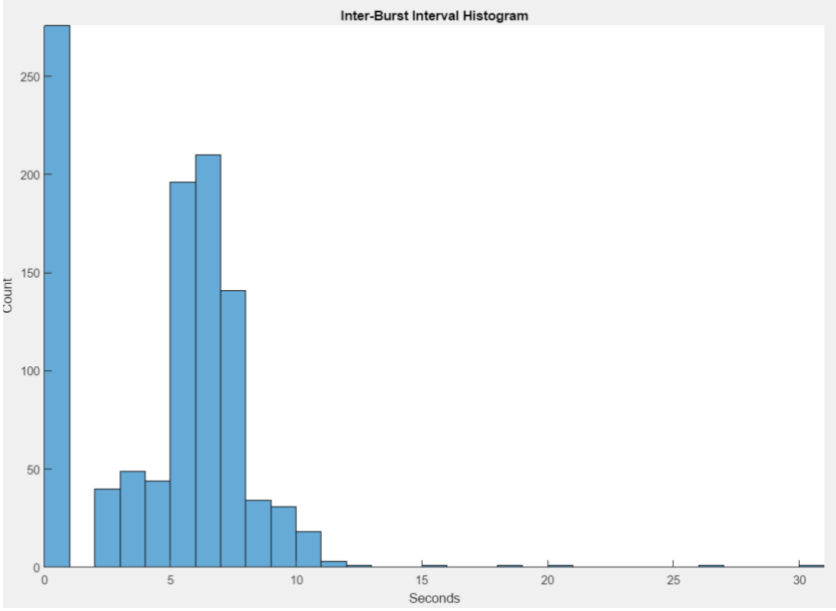
The inter-spike interval

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



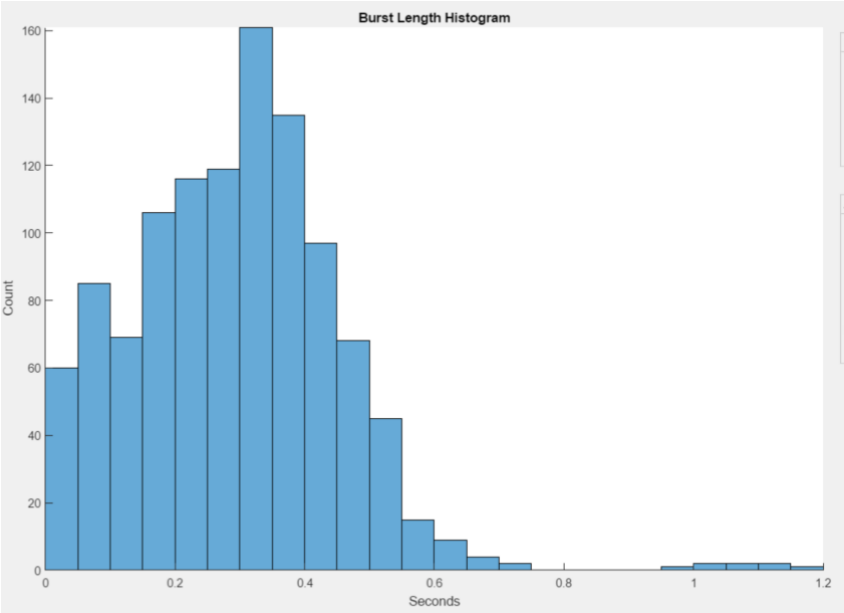
Inter-burst Interval

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.



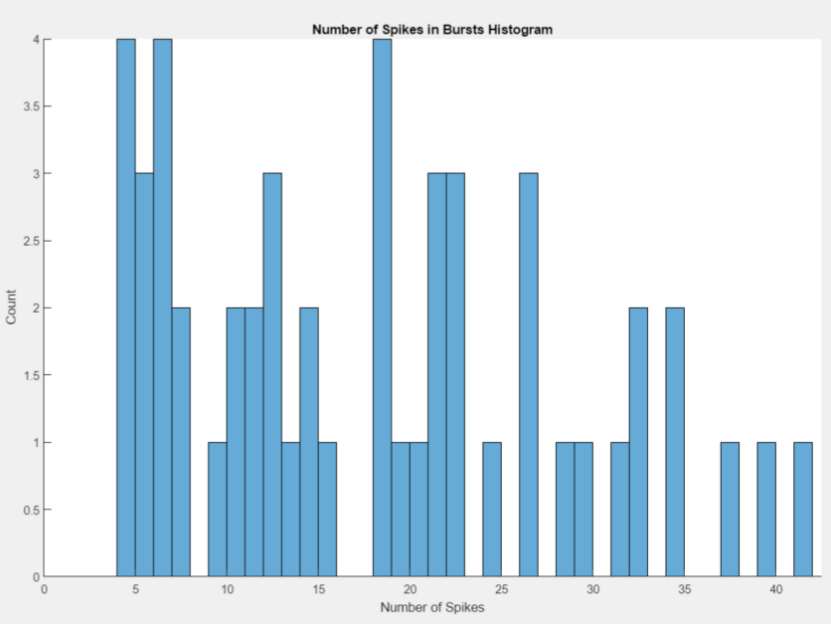
Burst Length

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.

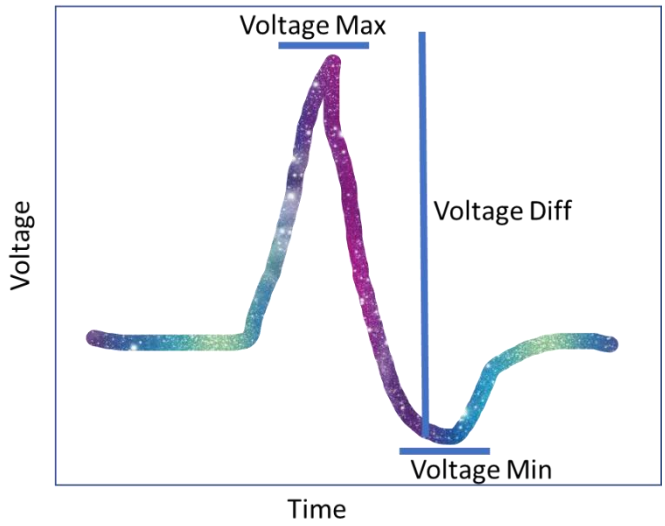


Number of Spikes in Bursts

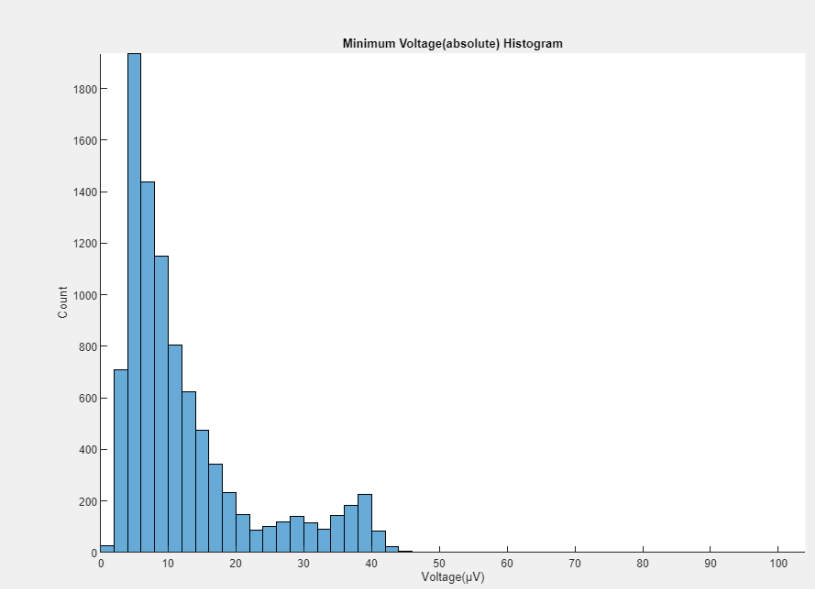
The histogram of the number of spikes in each burst can be used to inform burst detection parameters or to compare changes in activity patterns.



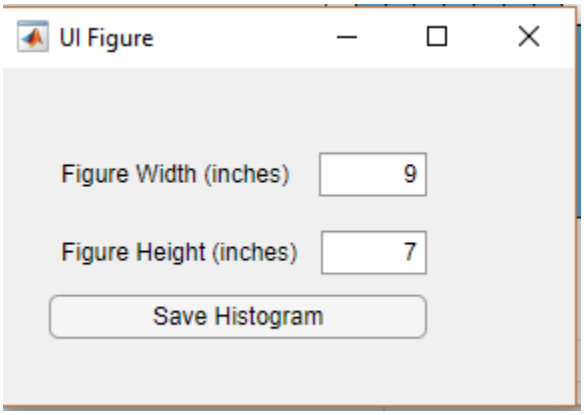
Voltage Histograms



If voltage segment data is available for the spike trains, MEAnalyzer can calculate a histogram for the maximum voltages, the absolute value of the minimum voltages, or the difference between the two.



Exporting Histograms

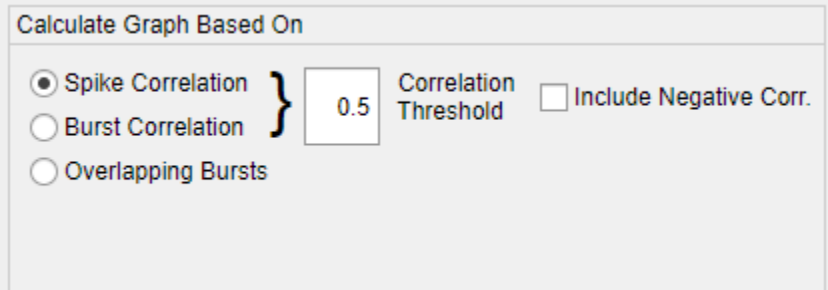


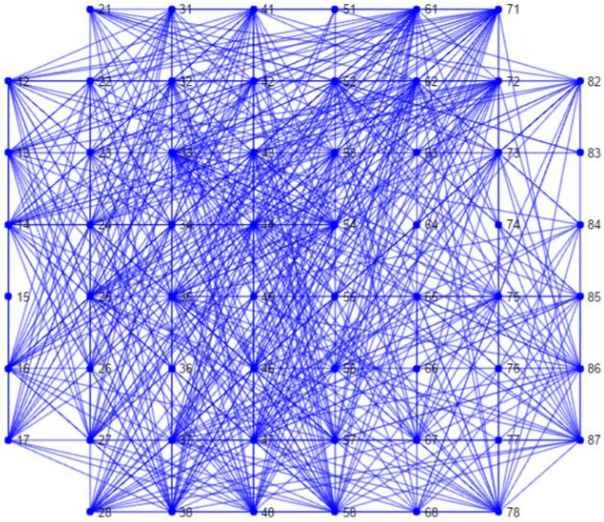
Similar to exporting the binned spike and raster plot, Histograms may be exported according to user-defined dimensions

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.

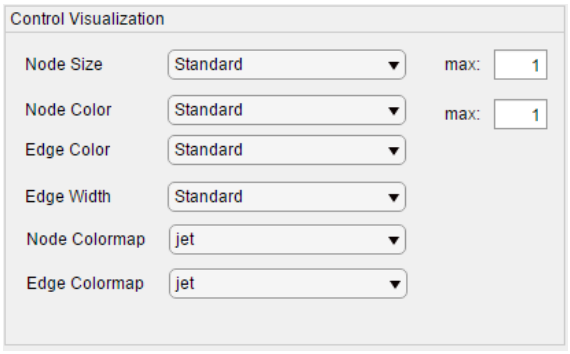




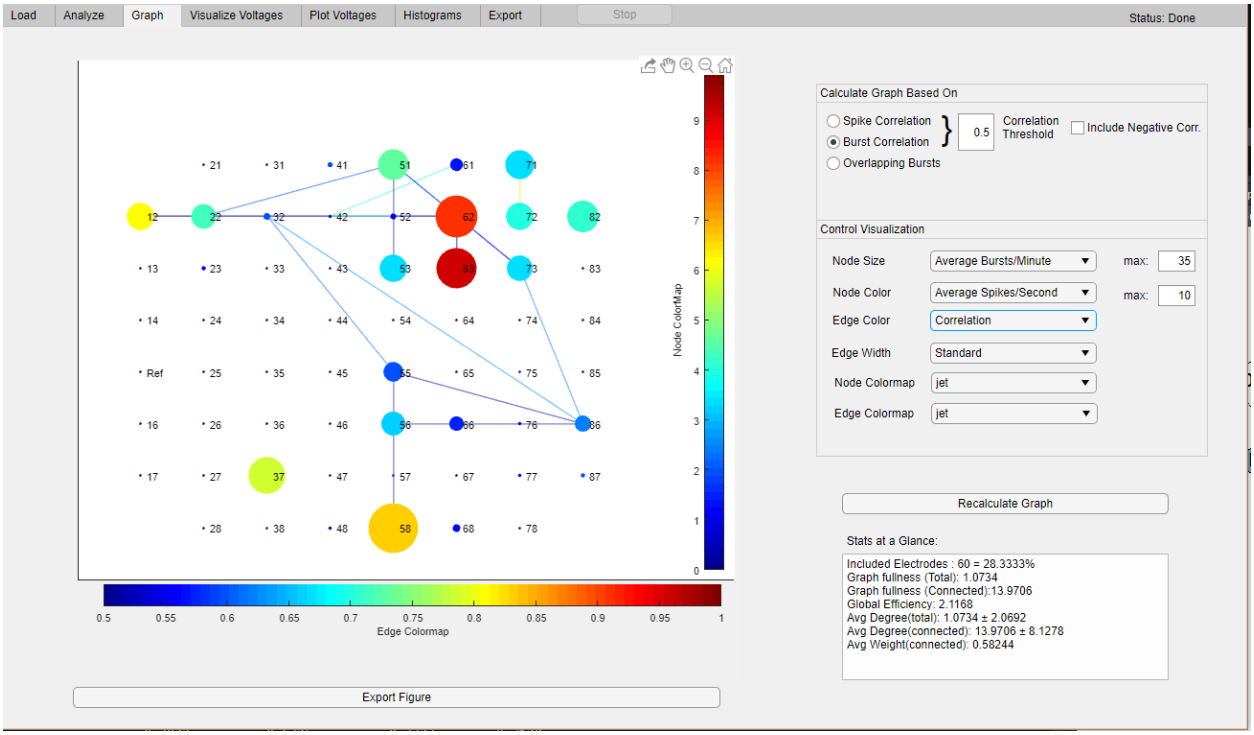
Spike Correlation, and Burst Correlation look at the cross-correlation between two electrodes at the time lag of 0 based on either the binned spikes or binned bursts. An edge is created between two electrodes if their mean cross-correlation at a time lag of 0 exceeds the user-defined threshold. As an 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.

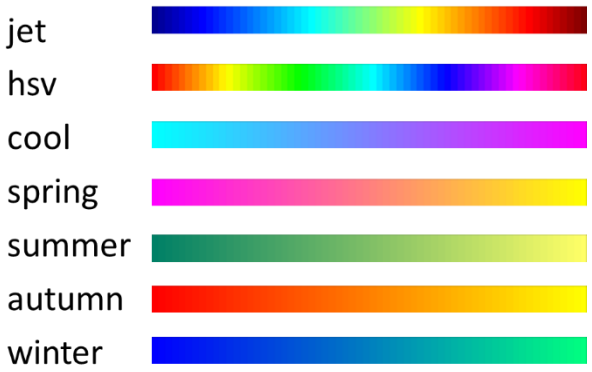


Node Size and color can be changed to represent standard graph math metrics. For example, they can be set to represent node degree or the cluster coefficient. Node size and color can also be changed to represent spike train metrics, such as spike rate or burst rate.



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

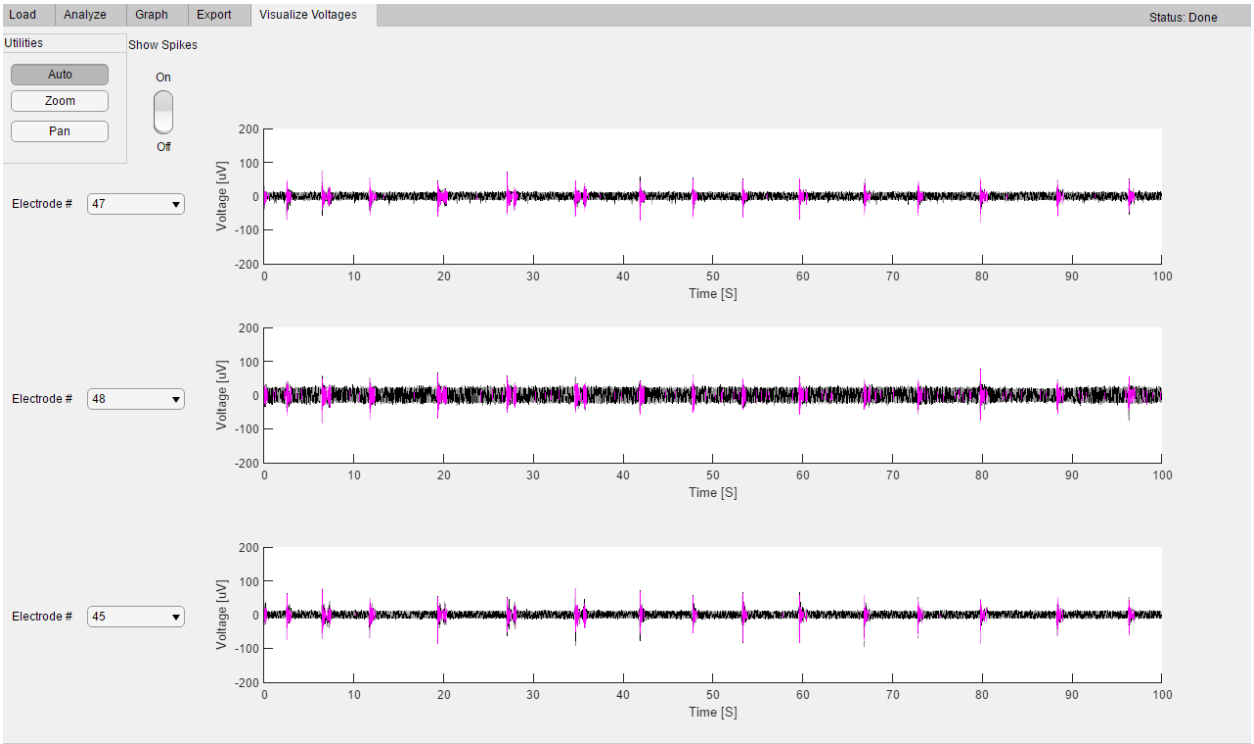
In Graph Theory, node degree represents the number of other nodes that a node is connected to. In MEAnalyzer , degree is normalized so that it represents the percentage of other nodes that an individual node is connected to.

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.

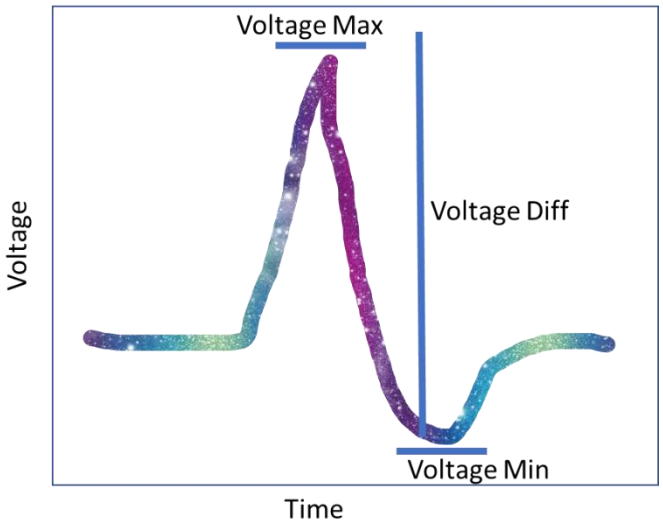
Visualizing Voltages

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.

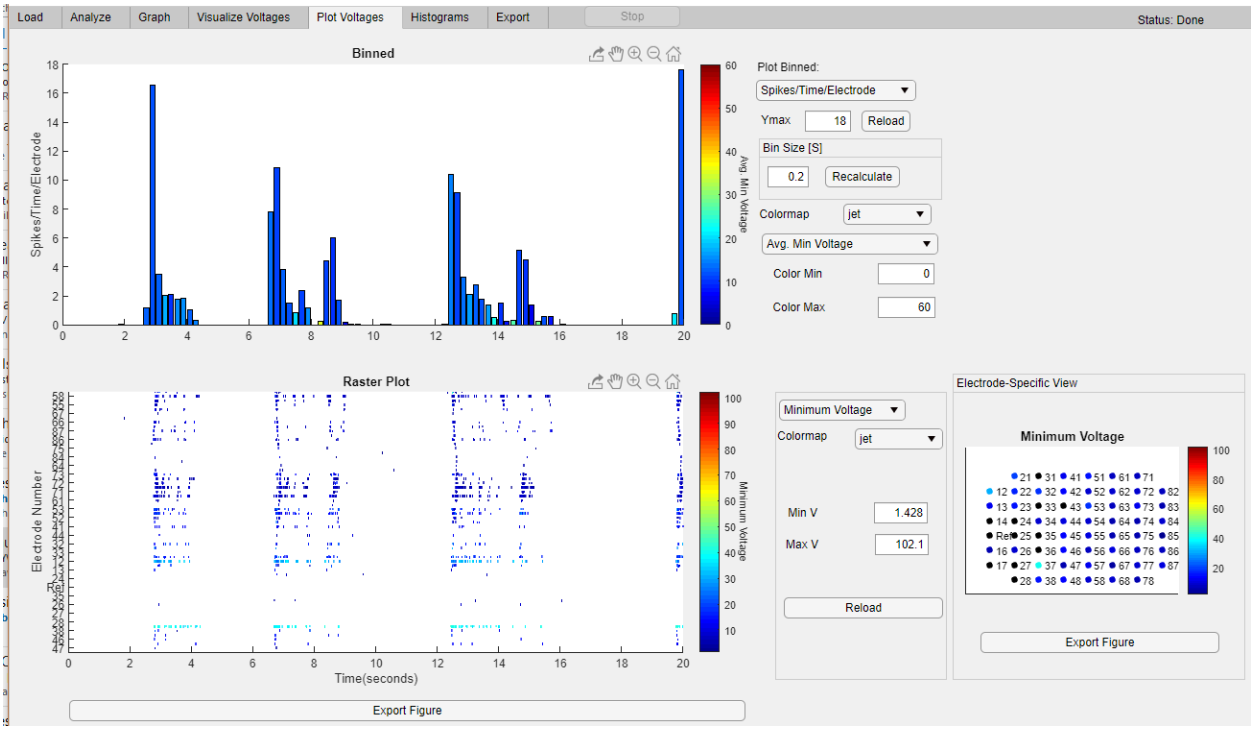


Please note, it is often standard practice when saving MEA data during recording to save the raw voltage data and spike time stamps and segments. As spikes are often calculated based on filtered data, there may be some mismatch between spike segments that have been stored and the raw voltage data. For this reason, MEAnalyzer has increased the time span usually included in spike segments and highlights them in the raw voltage.

Plotting Voltages



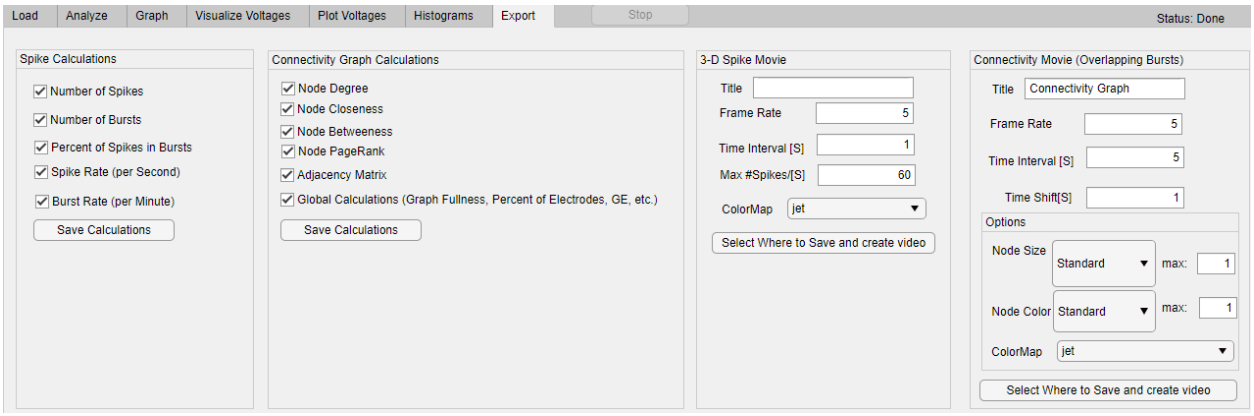
If spike segment data is available, MEAnalyzer offers additional visualization options for the voltages. This is the same data used in the histograms tab. This tab allows you to plot pseudo-colored raster plots and binned values where the color corresponds to the voltage value of your choosing. Additionally, an electrode specific view is available to display average electrode activity. Please note that these data represent the desired metric from spike waveforms and are not an average of all voltage measurements.



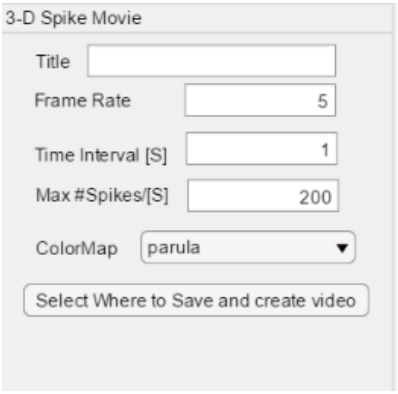
Exporting Data

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.

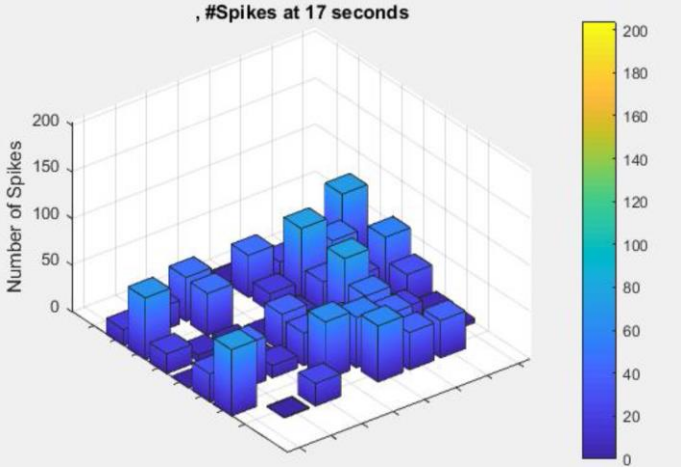


4D Spike Visualizations

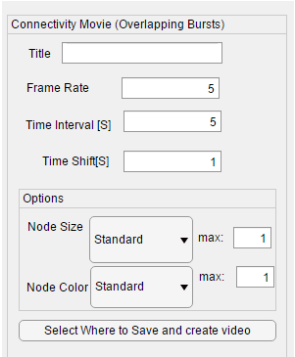


Exporting the data as a 4D Spike Visualization creates a movie file where each frame consists of a 3D bar graph.

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.

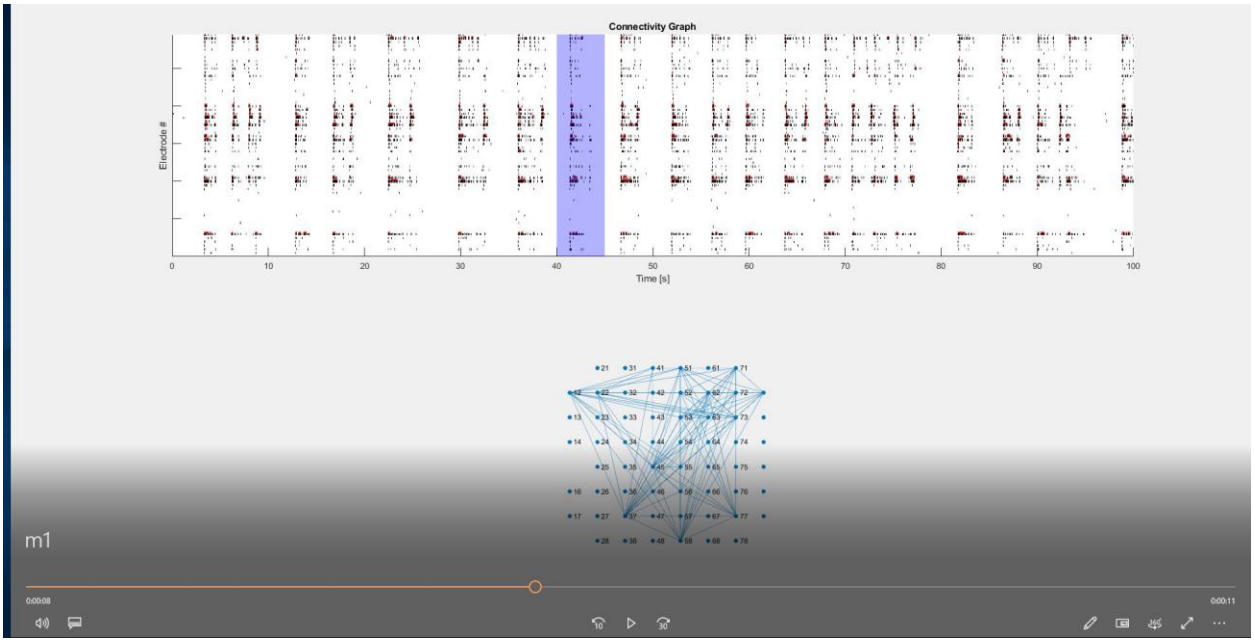


Connectivity Graph Movie



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.


Connectivity Graph
Electrode #
Time [s]
m1
0:00:08
0:00:11