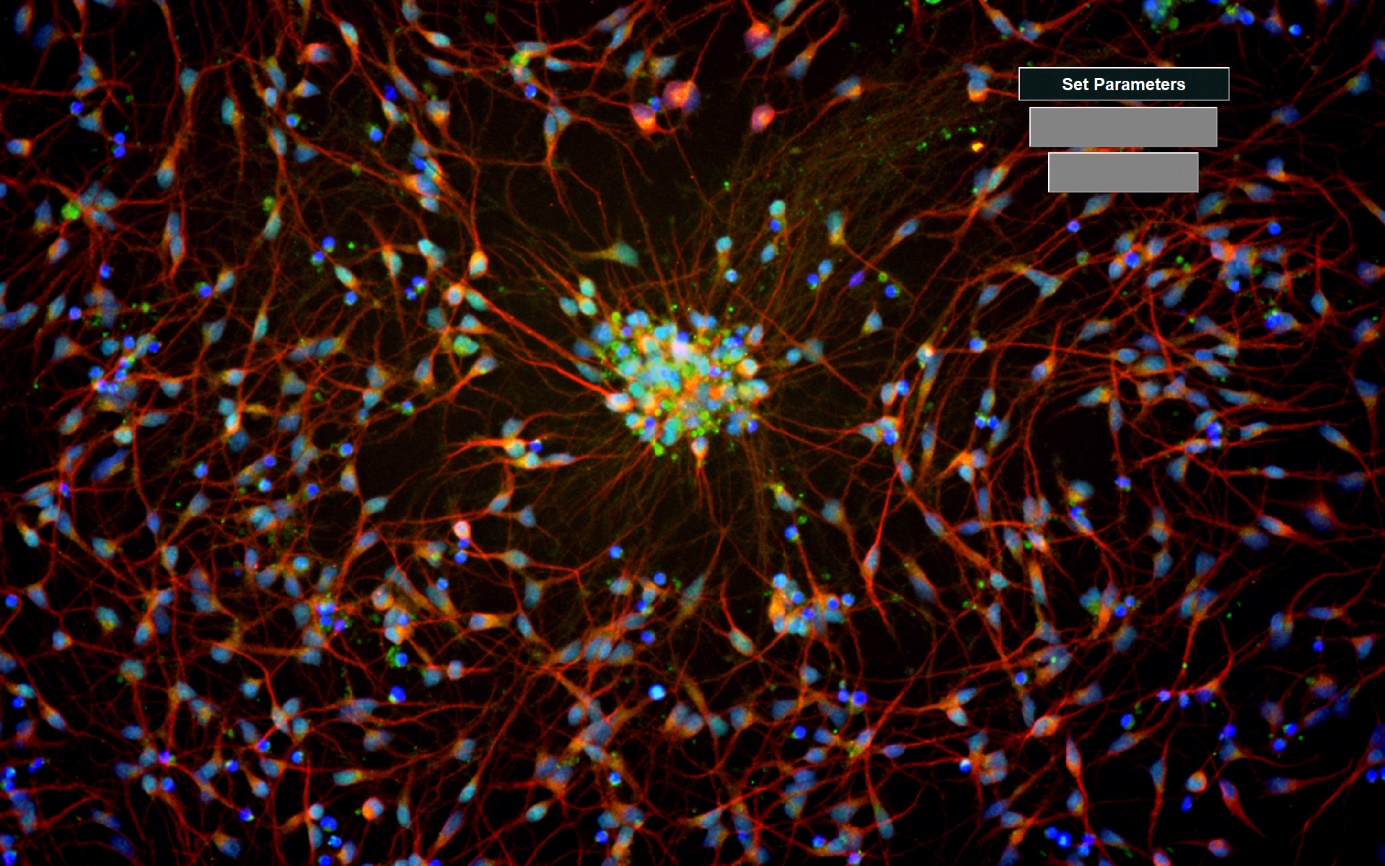
**MEAtoolbox Manual**

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**1. How to get started**  
 **1.1 Installation**

  
  
  
  
  
**1.2 How to use the MEAtoolbox**After the installation is finished the toolbox can be started by double clicking on the icon installed n your desktop. The user will be presented with the start screen as can be seen in figure 1. There is only one button available in the beginning. The correct parameters have to be set before the other 2 other buttons will become available.

**Figure 1. Start screen**

**Figure 2. Setting the correct parameters for data analysis.**

**1.2.1 Set Parameters**Before starting the analysis procedure it is important to verify that the settings such as the sampling frequency is set correctly. A new window will appear for the user that highlights the options for adjustments (figure 2). Each value can be altered by clicking on the number and typing in the desired values.

**1.2.1.1 Filtering parameters**

The analysis procedure uses a high pass Butterworth filter to filter the raw data to remove low frequency components and make the spike detection procedure easier.   
  
*-Cuttoff Frequency (Hz):* Frequency that the filter operates at. For example, a cut-off of 200 Hz indicates that all frequency above 200 Hz are allowed through the filter while any frequencies below this cut-off frequency are attenuated.  
*-Order of fit (n):* The order of a fit determines how well the filter performs. The higher the order the better the filtering process but the downside is an increase in complexity leading to longer processing time  
*-Sampling Frequency (Hz):* The frequency at which data is sampled within one second. For example, if 20000 data points are sampled within one second, the sampling frequency is 20000 Hertz.

**1.2.1.2 Baseline Noise Detection**  
Before spike detection can take place the analysis procedure has to determine the baseline noise so it can set a threshold without manual intervention.  
*-Baseline noise time window (ms):* This parameter represents the size of each bin when the data is split in smaller windows to determine which section of the recording is pure noise.  
*-Pure noise time window (s):* This parameter indicates the minimum duration of the time window that is used to establish the baseline noise.  
  
**1.2.1.3 Spike Detection**

*-Min. Spike interval (ms):* The minimum time between spikes. If there are any spikes detected that have a time interval between spikes of less than this parameter, it will be removed.   
*-Min. Spike amplitude (uV):* This parameter represents the minimum voltage that a spike should be. Any detected spikes that have a peak value of less than this parameter will be removed.  
*-Spike Detection threshold (rms):* This parameter represents how strict the threshold is for spike detection. The higher this number is, the stricter the threshold.  
*-Minimum Fire frequency (Hz):* The minimum firing rate a channel should have to be included in the analysis.

**1.2.1.4 Single Channel Burst Detection (Max Interval)**

*-Start Interval (s):* The maximum time interval between the first two spikes of a burst  
*-Spike number (n):* The minimum number of spikes that a burst should comprise of.  
*-Inter Burst Interval (s):* The maximum time interval between detected bursts. If the time interval is smaller than this parameter than the bursts will be combined.  
*-Intra Burst Interval (s):* After finding the start of the burst using the Start Interval parameter, the algorithm will determine if the following spikes after belong to the burst based on this parameter. The interval between the spikes after the 2 initial spikes are found, should be less than this parameter.   
*-Minimum Burst Duration (s):* The minimum duration a burst should be.

**1.2.1.5 Single Channel Burst Detection (Log ISI)**

-Void Threshold (%): The threshold for determining if the peaks detected in the log ISI histogram are separated well enough. If the calculated value is higher than this parameter, the peaks are separated well enough.

-Spike Number (n): The minimum number of spikes that a burst should comprise of.

**1.2.1.6 Network Burst Detection**

*-Synchronized time window (s):* the maximum time window that the analysis procedure will look in the data to determine if bursts are firing synchronously. If bursts from different channels are firing within this time window, these bursts are considered as synchronized bursts.  
*-Min. Synchronized burst number (n):* the minimum amount of synchronized needed to be considered a network burst. *-Min. Channel Participation (%):* the minimum amount of channels that have synchronized bursts that should be participating in the network burst.

**1.2.1.7 Single Channel Bursts Detection Method**

The user has the option to change the method that is used to detect single channel bursts and which will also impact the network burst detection. As these single channel bursts are used to detect the network bursts.

**1.2.1.8 Data format**  
The MEAtoolbox is also compatible with HDF5 files that only contain spike times by ticking the checkbox. The other option is if the user is more experienced in MATLAB to turn your MAT file into a hdf5 file that is always compatible with the MEAtoolbox. In order to achieve this, the user has to create a M x N matrix in which the M represents the channels and the N represents the recorded samples of each channel (figure 3A). When pressing the button the user will be asked to select the data file ,name the new file and indicate several parameters that will stored in the newly created hdf5 file. Of importance is that the manner the locations of the channels is saved is based on the layout of the MEA’s from Multi Channel Systems (figure 3B) and the locations are interpreted by the toolbox as seen in figure 3C.

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**Figure 3. File layout of the hdf5 file format compatible with the MEAtoolbox**

**1.2.1.9 Save Changes**

When the user is satisfied with the parameters then the user has to press the save changes button otherwise the changes will not be saved.The default values for each parameters were taken from the literature. While the MEAtoolbox is analysing the raw data files a progress bar will be shown to indicate the progress of each data file. After the analysis procedure is finished a graphical user interface (GUI) will appear of the last file that was analysed (figure 3).

**1.2.2 Analyse Files**The user will be prompted to select a folder that contains h5 files to start the analysis process.  
The MEAtoolbox will analyse all the h5 files within the selected folder using the same set parameters.  
Important to remember is that the MEAtoolbox will currently **only** accept h5 files that are obtained after being converted using a free converting program from multi-channel system. The software can be obtained at <https://www.multichannelsystems.com/software/multi-channel-datamanager>.Work is in progress to make the MEAtoolbox compatible with more file formats.The end result are analysed .mat files that are put inside a new folder that is created within the selected folder titled   
‘Analysed files’.

**1.2.3 Load Files**The user can open analysed .mat files inside the analysed folder created after the analysis procedure is finished to view the results of the analysis.

**2. Home**

**Figure 4. Home Screen**  
  
**2.1 Overview of all channels**  
Overview of all the channels in the well depending on the layout of the MEA it can change from a single well to a multi well layout. An example is given of a multiwell data set (figure 4). This overview allows for a quick overview of the active and non-active channels represented by their colour. Each channel can be pressed for additional single channel information

**2.1.1 Single Channel information**

When a single channel is pressed, a new screen will appear that will provide additional information about the channel (figure 5).

**Figure 5. Single Channel Information**

**2.1.1.1 Filtered voltage trace**

On the top, the filtered voltage trace is displayed of the selected channel and which part of the voltage trace is used to set the threshold for spike detection together with the thresholds used for spike detection.

**2.1.1.2 Single Channel Spike Raster Plot**

Spike raster plot of a single channel in which each black line represents a detected spike.

**2.1.1.3 Single Channel Firing rate histogram**

Histogram of firing rate over the whole duration of the recording.

**2.1.1.4 ISI histogram**

Histogram of the distribution of the inter spike interval (ISI).

**2.1.1.5 Single Channel Bursts**

Spikes are visualized together with single channel bursts (either max interval or log ISI method).

**2.2 Voltage trace view or most active channels**Depending if it’s a single well or multiwell MEA dataset the panel will be different. In the case of a multiwell dataset the panel will display the voltage traces of all channels in well 1. The voltage view can be changed to display other wells by using the drop box below the panel. This allows for a quick overview of the voltage traces of one well. If the dataset is a single well MEA dataset, the panel will display the 5 most active channels of the dataset. If needed the user can press the button and a bigger display will be given of the most active channels that allows for a more in depth look at the channels.

**2.3 Colour bar**   
The colour bar that highlights the overall activity of the whole well or multiple wells with the number at low activity representing the lowest amount of spikes detected in the whole well in a single channel whereas the number at high activity represents the highest amount of spikes detected in a single channel. The colour bar’s location is different depending if the dataset is from a multiwell or single well.

**2.4 General information**

The name of the data file can be found here and below the name of the file there are the parameters used to analyse the file. Below the information about which parameters are used there is a general data button. The general data button be pressed for a table that contains spike train metrics about each individual channel (for example, ISIs, firing rates and single channel bursts).

**2.5 Whole array spike raster plot and Heatmap**

Depending on the dataset this panel is different. If the dataset is a single well data then there will be two separate panels which contain a heatmap on the left side and a raster plot of the whole array on the right side. The heatmap on the left side is based on the number of spikes in which high amounts of spikes are represented as yellow and low amounts of spikes are in blue. For a more detailed view of the heatmap or if the heatmap needs to be saved, the heatmap button can be pressed. On the right side is the whole array spike raster plot is in which each black line represents a spike. Below the raster plot is a plot containing the array wide firing rate of the whole recording. In the case of a multiwell dataset only the whole array spike raster plot is present as can be seen in figure 4.

**2.6 Analyse files**

The user will be prompted to select a folder that contains h5 files to start the analysis process.  
The MEAtoolbox will analyse all the h5 files within the selected folder using the same set parameters.  
Important to remember is that the MEAtoolbox will currently **only** accept h5 files that are obtained after being converted using a free converting program from multi-channel system. The software can be obtained at <https://www.multichannelsystems.com/software/multi-channel-datamanager>.Work is in progress to make the MEAtoolbox compatible with more file formats.The end result are analysed .mat files that are put inside a new folder that is created within the selected folder titled   
‘Analysed files’. **2.7 Load Files**The user can open analysed .mat files inside the analysed folder created after the analysis procedure is finished.

**2.8 Full voltage trace view**

The user can view the voltage traces of all the channels in one well. This means 120 voltage of a 120 channel MEA however in the case of the multiwell this would mean for example 12 channels if there are 12 electrodes per well. If the button is pressed, the user is requested to put in how long the time window is that will be shown. In the case of a multiwell dataset, the user is also asked to fill in a specific well. Once chosen the individual traces will be shown, the user can move through the recording in time by using the left and right arrow keys on the keys. The y axis that is shown by default is based on the maximum detected voltage value in the data, however the user can change it by using the up and down keys on the keyboard.

**2.9 Remove Channels**

The user can delete channels from further analyses by removing it by pressing this button. The use will be prompted to type in the selected channel for deletion. Important to realise this is that this action is **irreversible** and if the user wants to get the data back, the user will have to re-analyse the file.

**2.10 Top Channels**The user can plot the top most active channel in one go with this button for visual inspection to quickly determine if thespikes were correctly detected.

**2.11 MEA movie activity**The user can create a video of the spiking activity of the whole recording. High activity patterns are indicated in yellow whereas blue represents low activity or none. The video is accompanied with the 5 most active channels on the right side. The video has a dimension of 640x 480 with 10 frames per second.

**2.12 Neuro Endpoints**The Neuro endpoints button allows the user to extract 20 different endpoints that can be used to determine the status of the neuronal cultures grown on top of the MEAs. This can only be performed on analysed data files with the mat extension. The user will be prompted with a question. If all the analysed files of which the 20 endpoints are to be extracted are in one folder already the user can press continue and the user will be prompted to select the correct folder. If the analysed files are not in one folder the user can select terminate and the user will have to create a new folder that will hold all the analysed files. Therefore, the user will be prompted to name the new folder and select in which folder the new folder should be in. In the next prompt the user is to select the folders that contain analysed files that the user wants to move to the new folder. The user can do this repeatedly using the continue button until all the folders are selected. Once finished, select the terminate button and a new prompt will ask for confirmation. When all the analysed files are in one folder, a new popup will appear in which the user has to select folder containing all the analysed files to calculate the neuro endpoints for. After collecting all the data there will be an extra prompt if the data is from a multiwell to ask the user if the toolbox can group certain wells together.   
The user will be asked how many groups there are in the data. Important is that each group has to have equal amounts of wells. For example you have 4 groups in a 24 multiwell data set.

This would mean that each group contains 6 wells. The user can assign which wells belong to each group. After which the user will be asked to name the newly created excel that will contain all the neuro endpoints (figure 6A). A second popup will appear to name a second excel file. This 2nd excel is all the neuro endpoints for all files but without the grouping (figure 6B). If the user does not choose to do the grouping then there will be no extra prompt and a pop up will appear to name an excel file that contains all the neuro endpoints (figure 6B).

**Figure 6. Endpoint measurements**

**2.13 Heatmap**

In case the user is interested to have a different view of the overall activity in the well or multiple wells the user can view the overall activity as a heatmap.

**2.14 Raster plot**

The user can press this button to get a more detailed view of the raster plot and array wide firing rate. Once pressed, a new window will appear, and 2 new options will appear together with a more detailed view of the spike raster plot. The single channel bursts can be displayed in red overlaid with the spike raster plot or the network bursts in blue. The other option will only appear if the dataset is from a multiwell in which case in the lower left corner and pull-down menu will appear that allows for the selection of each individual well together with their spike density function (figure 7A). it is also possible to zoom in as can be seen in figure 7B where the first 200 seconds are shown of the data where the network bursts are displayed in blue.

**Figure 7. Raster plot**

 **3. Bursts**

**Figure 8. Bursts Screen**

**3.1 Select channel**

Selection of a specific channel that allows for a more detailed investigation in burst detection and network burst detection. Before any other options become available the user must select a channel for investigation (figure 8). Once a channel is selected several buttons will become available in the button panel.

**3.2 Switch view**

Switches the panel that displays the voltage trace with the whole array spike raster plot together with the spike density plot. Once this button is selected two different options will become available in the button panel and the other options will be greyed out. The other buttons will be become available again when you press the same button again.

**Figure 9. Rerun Burst Detection**

**3.3 Rerun burst detection**

The button panel will change and will reveal the two available burst detection methods as described in the paper and some additional buttons. After a burst detection method is selected, the user can change the parameters that are used to detect the bursts. The result is a comparison between the detected bursts using the previous parameters versus the newly chosen parameters (figure 9). The toolbox will automatically save the bursts detected using the new parameters.

**3.3.1 Finish burst detection**Once the rerun burst detection button is pressed the name of this button will change and new buttons will appear, and the previous buttons will be greyed out. To get access back to the old buttons the user must press the finish burst detection button.

**3.3.2 Burst detection methods**

The user can choose one of the two detection methods to rerun the burst detection for the selected channel. After a method is selected the individual boxes for that detection method will become accessible and the user can alter the default parameters. The adjustable parameters can be found described in the paper.

**3.3.3 Rerun burst detection buttons**

These 4 buttons are only available when the rerun burst detection is pressed. To rerun the burst detection with the newly changed parameters the user must press the burst detection button. The result is a comparison between the previous selected (default) parameters on the top of the figure versus the result of the newly chosen parameters on the bottom. For the precise numbers, the statistics button can be pressed. The revert to default button can be used to go back to the default settings in case this is needed. An example is given in figure 9 where we changed the minimum amount of spikes that is considered to be a burst from 4 to 2 spikes.

**Figure 10. Rerun Network Burst Detection**

**3.4 Rerun network burst detection**

Similarly to the rerunning burst detection, this button will allow the user to fine tune the network burst detection without having to rerun everything. After the user has changed the parameters used to detect the network bursts the toolbox will plot both the old detected network bursts and the new detected network bursts in blue which will allow for an easier comparison.

**3.4.1 Finish Network Burst Detection**

When the rerun network burst detection button has been pressed several new options will appear but also old buttons when become unavailable. When the user is finished with fine tuning the parameters to detect network bursts, the user has to press the finish network burst detection in order to gain back the old buttons.

**3.4.2 Network Burst Detection Methods**

There are several methods that the user can use to detect network bursts and the user has to select one before the adjustable parameters become available. The adjustable parameters are described in the paper.

**3.4.3 Rerun Network Burst Detection buttons**

The 4 buttons found here are the exact same as the 4 buttons found during rerunning burst detection. To actually rerun the network burst detection with the newly changed parameters the user must press the network burst detection button. The result is a comparison between the previous selected (default) parameters on the top of the figure versus the result of the newly chosen parameters on the bottom. For the precise numbers, the statistics button can be pressed. The revert to default button can be used to go back to the default settings in case this is needed. An example is given in figure 10 where we changed the minimum percentage of channels that has to participate in the network burst to be considered a network burst from 0.25 to 0.8 percent.

**3.5 Zoom in Bursts**

When selected, prompt will be given, and a number will be asked. This number is a burst number. For example, when the number 1 is selected the figure will zoom in on the first detected burst. This option is only available when the voltage trace of the selected channel is displayed.

**3.6 Burst statistics**

A table will be presented that contains all the burst metrics for the selected channel of both the max interval method and log ISI method.

**3.7 Button Panel**

After a channel is selected this button panel becomes available. The first four options are only available when the voltage trace is displayed whereas the last 3 options are only available when the switch view button is selected. These buttons allow for the visualization of the used thresholds for spike detection and burst detection

**4. Connectivity**

**Figure 11. Connectivity Screen**

**4.1 Load**  
The data has to be loaded in before the other options become available .

**4.2 Option buttons**

The first option is will add arrowheads to the drawn lines which can be useful for clarity to determine the direction of the connection. The second option limits the amount of connections drawn in the top left panel. Normally all the connections will be drawn however if this option is selected only the top 200 connections will be drawn (with the highest probability).

**4.3 Export figure**

Allows the user to export the figure in HD with 1200 dpi and resolution of 1920x1080.

**4.4 Visualize connections**

Will draw the connections one by one in the panel above the button. Depending on how anu connections are found this step might take a long time therefore it is recommended to always first visualize the strongest 60 connections first

**4.5 Select channel**

This button will prompt to select a channel and will only visualize the connections associated with the selected channel. Either the selected channel is connected to other channels or other channels are connected to the selected channel.

**5. Spikes**

**Figure 12. Spikes sorting screen**

**5.1 Load Spike waveforms**

Click to load in the spike waveforms of the detected spikes in the unsorted waveforms panel. After loading in the waveforms, it is possible to look at the spike waveforms of each channel by using the pull-down menu beneath the panel (figure 12). **5.2 Save Spike sorted waveforms**

After the spike sorting process is finished, the different detected clusters and the associated spike waveforms can be saved.

**5.3 Run sorting**

Starts the spike sorting process for the current selected channel. After the spike sorting process is finished, the different sorted spikes are displayed in the sorted waveforms panel and several new buttons will become available.

**5.4 Run all channels**

Starts the spike sorting process for the all the channels that have spike waveforms. The algorithm will go through each channel one by one therefore this can take a while before it is finished depending on the amount of spikes.

**5.5 Display Wells**

Normally the spike sorting process will take place channel by channel basis. However, ticking this checkbox on all the spike waveforms will be taken together per well and the user can perform spike sorting on all the spikes in a well.

**5.6 Clusters**

Visualizes the detected clusters in the cluster panel which allows for modification of the detected clusters and opens up 3 new buttons (Waveforms (5.13), ReSort(5.14) and Delete(5.15)).

**5.7 Reset**

Removes any modifications done after the sorting.

**5.8 Edit unsorted**

When this button is pressed all the unsorted spike waveform still in the unsorted waveforms panel are considered to be one cluster. This allows the user to combine clusters that should belong to the same cluster. For example after the sorting process was finished the user noticed two cluster that are very similar in the sorted spike waveform panel(5.12)). The user can delete these two clusters by using the del buttons next within the panel and these waveforms will appear in the unsorted waveforms panel (5.11). By using this button the spike waveforms within the panel will be combined to form one cluster.

**5.9 Plot all waveforms**

By default, only up to 1000 waveforms are plotted however all waveforms can be plotted with this button

**5.10 Discard unsorted**

Removes any waveforms that are in the unsorted panel. This action cannot be undone using the reset button.

**5.11 Unsorted Spikes Waveforms**

After loading in the spike waveforms, the waveforms will appear in this panel and the user can view all the spike waveforms for each channel.

**5.12 Sorted Spike Waveforms**

After the sorting process is finished the spike waveforms will disappear from the unsorted waveforms panel (5.11) and appear sorted with unique colours for each cluster. By using the del button next to each spike waveform the user can send the spike waveforms back to the unsorted waveforms panel if the user does not agree with the sorting.

**5.13 Waveforms**

Allows for switching between 3 types of plots in the cluster panel. The default is the spike waveforms, a scatterplot of 2 dimensions in the clustering space and lastly a line plot of all 5 dimensions in the clustering space.

**5.14 Resort**

Clustering of selected clusters is repeated, and the result is a merged model with the previous model which allows the unselected clusters to remain the same. The clustering space does not change.

**5.15 Delete**

Selected clusters’ gaussian are deleted and their associated waveforms are re-distributed to the remaining gaussians following the same rule used during the clustering process. The gaussian with the highest probability determines where the waveforms belong to.

**5.16 Cluster stability**

Displays the peak-to-valley distance of the cluster that is selected together with the firing rate which will allow the user to see the stability of the cluster over time and assess the data.