MEAmapper User Guide

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Getting started

MEAmapper is a tool for creating hierarchically clustered heatmaps from multi-electrode array recordings. Currently, output from Axion Biosystems' Maestro system is supported. There is a specific workflow to generating the input files to MEAmapper that will be covered in the next section. Once data is uploaded through the CrED portal and the job is queued up on the server, the app may be launched and data processing can begin. There are 3 data processing steps, after which heatmaps will be generated and can be downloaded directly from the app. These can also be customized with various user-supplied input files, all optional.

Input Files

Recording using the Axion Maestro system will produce a .raw and a .spk file. MEAmapper will use the first part of the filename to label experiments (separated with an underscore), so name the file according to how you would like treatments to display in the heatmaps. For example, if you would like to label the experiment as "P1", naming the experiment file "P1_20190405_20190502_baseline(000).spk" will accomplish this. No other part of the filename other than the first part is used, so feel free to disregard the rest and label however makes sense for organization's sake. In the heatmap, a treatment of "Drug1" with concentration "2uM" for this plate would display as "Drug1_2uM_P1."

Within AxIS software, double-click the platemap in the top left, which should open a new window. Create one platemap per experiment. For the control being used (whether it is a compound like DMSO or a control cell line), check the "Control" checkbox. This will make later steps easier. Once the platemap is finished, click "Export…" and save the .platemap file.

.Spk files should be loaded into the NeuralMetric Tool for processing into "_neuralMetrics" csv's. These files will have quantified metrics calculated from the .spk file. Set desired parameters for recording length, Burst Parameters and Synchrony Parameters, then File > Batch Process Multiple Files. This will bring up a new window where one can add multiple .spk files at once. Generally, it's best to keep recording lengths the same, so select a desired duration in the Analysis Parameters section. It's suggested under Export Options to select "Export Supplemental Metrics to CSV". This will export all 73 metrics, rather than the "Recommended" 54 metrics, offering more choice in how to display the data. Next, click "Run Batch", and the NeuralMetric csv's will be generated.

The next step uses Axion's "AxIS Metric Plotting Tool". This final step combines recording timepoints and calculates a percent change from baseline. First, click on the "Baseline…" button and upload the baseline NeuralMetric csv file. Next, select the "Comparison…" button and select all other timepoints for that experiment. If the ordering of the files is not correct by default (see the Display Name next to File), click on the field and rename it appropriately. Feel free to use Axion's C# system, or create your own (e.g. 5min, 30 min, 2hWO, etc.). Next, upload the .platemap file with the treatment information created in AxIS by clicking the "Plate Map…" button. There should now be a display of data in the center panel separated by treatment if everything up until this point was performed correctly. To export the MachineReadable files that MEAmapper accepts as input, go to File > Export > Export Statistics Format and save the MachineReadable csv. Once all MachineReadable files have been created (one per independent experiment), MEAmapper may be used to generate custom hierarchically clustered heatmaps.

Optional Files

MEAmapper accepts optional input files that can be used to further customize the look of the heatmaps. On the left side-panel, there is a button for “Metrics Table”, “Inclusions File”, “Exclusions File” and “Annotations Sheet”. The metrics table is a list of metrics that the app uses to subset what metrics are included in the heatmap (only metric names included in the metrics table will show up in the heatmaps). The inclusions file works similarly for treatment names. Use this if you only want to include a few specific treatments/conditions. Exclusions works oppositely of inclusions, so list out treatments/conditions that you do not want to see in the heatmaps. This approach would be preferable if there were only a few to exclude, since the list would be shorter. Either approach can produce the same result, however. The annotations sheet will add a heatmap annotation on the right side of the figure, useful to visualize grouping of similar treatments/conditions. All files should be saved as .csv files (not .xlsx).

The metrics table should be the names of metrics that should be included in the heatmaps. The first row should be a header, followed by the metric names. This is so that metrics can be separated by category, since Axion offers various metric classes including “Activity Metrics”, “Electrode Burst Metrics”, “Network Burst Metrics”, and “Synchrony Metrics”. If you would like your heatmaps to be organized by metric category, create one column per metric category with a header, listing the metrics in that category down the column.

	A	B	C	D
1	Activity/ Salient Features	Bursts	Network Bursts	Synchrony
2	Number of Spikes	Number of Bursts	Number of Network Bursts	Area Under Normalized Cross-Correlation
3	Number of Active Electrodes	Burst Duration (Median) - Avg (sec)	Network Burst Duration - Median (sec)	Full Width at Half Height of Normalized Cross-Correlation
4	Weighted Mean Firing Rate (Hz)	Number of Spikes per Burst (Median) - Avg	Number of Spikes per Network Burst - Median	Synchrony Index
5		Median ISI within Burst - Avg (sec)	Median ISI within Network Burst - Avg (sec)	
6		Burst Percentage - Avg	Number of Elecs Participating in Burst - Median	
7			Network Burst Percentage	
8			Network IBI Coefficient of Variation	
9				
10				

The inclusions/exclusions file is a list of treatments to either include or exclude. These should be listed down the first column (no header needed)

	A
1	Drug1
2	Drug2
3	List more drug names down this column to include them
4	
5	
6	

The annotations sheet should be a table of drug names (or conditions/ cell lines) and descriptors. The first column will be the drug names (no concentration/ plate number), and the 2nd and beyond columns

should contain some information about it. The table should have a header row, very similar in format to the metrics sheet.

	A	B	C
1	Drug Names	Putative Target	Action
2	Drug1	Cholinergic	agonist
3	Drug2	Glutamatergic	antagonist
4	Drug3	Dopaminergic	antagonist
5	Pos.Ctrl	GABAergic	antagonist
6			

Using the Tool

After experiments have been processed into MachineReadable files and all optional input files have been created, MEAmapper is ready to start creating heatmaps. The first step is to select the analysis directory by clicking on the "Folder select" button. Select the appropriate folder (make sure all of your files are shown in the right-hand content display) and click Select. Now the processing buttons should activate. Before starting with the data preprocessing, it is a good idea to get any optional input files loaded in first. Select your metrics table by using the Metrics Table select button and do the same for the remaining input files.

It is important that the tool correctly picks up the control condition. Previously, if when you created your platemap, you selected the Control checkbox for the control conditions, the default Control compound label will pick it up fine. It is also possible to enter your own regular expression in the text box – just make sure that the control compounds have some consistent naming scheme across all experiments to ensure that none are missed.

All data processing steps should be carried out in order unless your data folder contains saved processed files from a previous analysis using the tool. First time users will want to select the 1. Preprocess Data button which should display a notification when the preprocessing starts and when it finishes. Next, statistical processing can be carried out by selecting the 2. Run Statistics button. This step will likely take the longest, so please be patient (and try not to click it more than once!). Finally, the 3. Generate Heatmaps button will generate the hierarchically clustered heatmaps. This button will generate one heatmap per timepoint, per combination of clustering/ distance algorithm, along with accompanying .csv matrix files that contain the numerical data displayed in the maps. The 3C. Generate Heatmaps – Combined Timepoints option will allow users to select more than one timepoint to be combined into single heatmaps by using the check boxes and clicking OK. This will produce one heatmap and matrix file per clustering/distance algorithm combination.

There is a text field for "Base name for heat maps" which will append to the name of the heatmap and matrix files. This allows users to generate multiple sets of heatmaps without overwriting previous versions (for example if different Optional Files are used), or just to name things for organization's sake. The "Include dendrogram in matrix files?" checkbox will add clustering information into the heatmap matrix files, very useful for tracking dendrogram branches and looking at specific clusters.

The Clustering Algorithm and Distance Algorithm drop-downs and timepoint drop down can be used to alter the front-end display heatmap. Select the desired parameters and click on the Refresh heat map button to refresh the display with those settings. This does not affect the analysis performed or anything about the data that can be downloaded, it is just for visualization purposes.

Select the Download Heatmaps button to save all files uploaded and generated by the tool into a zip folder. This button can be used at any time after the 3. Generate Heatmaps button has finished. Feel free to download files, preview them, and continue to use the tool to generate more sets of heatmaps.

Once all analysis is complete and files have been downloaded, click the Exit button in the top right to kill the app.

For any further questions, I can be reached via email at awooten@sbpdiscovery.org. I also welcome any feedback!