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Designing tools for automated and real-time analysis of multi-electrode arrays for the purpose of testing novel designs

Jansdam, Sebastiaan

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Group Lüttge

Full Name	Student ID
Sebastiaan Jansdam	1358510

BEP Coach: Dr. R. Luttge
Lab Tutor and Coach: Msc. R. Sabahi-Kaviani

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Abstract

Eindhoven University of Technology
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Designing tools for automated and real-time analysis of multi-electrode arrays for the purpose of testing novel designs

by Sebastiaan Jansdam

Neurodegenerative diseases are conditions that affect the brain and nervous system, causing a gradual and irreversible decline in function over time. Examples include Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS).
These conditions can impact people in many ways, including cognitive impairment, memory loss, movement difficulties, and loss of independence. They can also cause emotional and behavioral changes, such as depression and anxiety. To support research into neurodegenerative diseases Brain-on-Chip (BoC) projects offer a practical and accessible method of conducting in vitro research. One type of BoC uses cultured neurons and micro-electrode array technology. Multi-channel Arrays (MEAs) are used for their high temporal and spatial resolution. Allowing research to be done on many neurons simultaneously and allowing the capture of the dynamics of neuronal networks in real time. They also provide the ability to be used for long-term recordings of neuronal activity, which allows for the study of neural plasticity and the effects of long-term interventions.
These accurate recordings generate large amounts of data that require sophisticated analysis to extract meaningful information, which can be time-consuming and challenging. And MEA recordings only provide information on electrical activity, so it can be difficult to interpret the underlying neural processes that generate this activity.
This project set out to tackle both of these problems by automating them in a user-friendly way. But the primary target was the automation of ingesting and analyzing the data coming in from the MEAs for near real-time analysis. This was achieved by using the tools from the data ingestion software, which provides excellent analysis, but limited preprocessing tools. The goal of achieving this by using automation software to export the ingested data and then further preprocess and analyze this data in an automated fashion has not been realized.
This would not only allow for faster analysis of the current MEAs but would also provide a way for faster analysis of novel MEA designs. These novel designs could produce more accurate and faster results in medical research, so their development is to be provided with the tools to make that development faster and easier.

Acknowledgements

I would like to thank Rahman Sabahi Kaviani for the challenges and data he provided me. Furthermore, I would like to thank him for guiding me through the lab and teaching me the procedures used for both the acquisition and analysis of the MEA data. Finally, I would like to thank Regina Lüttge for her support and guidance in this project.

Contents

1 | Project background

The central nervous system (CNS), which includes the brain and the spinal cord, is fundamental to what makes us human. It is composed of highly specialized cells that form intricate networks responsible for our thoughts, emotions, movements, and bodily functions. However, despite its remarkable complexity and resilience, the CNS is also vulnerable to damage and disease.
One of the most significant challenges facing our society is the prevalence of neurodegenerative diseases. These diseases cause a gradual loss of nerve cells and their connections, leading to a range of symptoms such as memory loss, impaired movement, and altered behavior. Examples of neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS). Neurodegenerative diseases not only affect individuals and their families but also have a significant impact on society as a whole. They place a heavy burden on healthcare systems, reduce productivity and quality of life, and strain social and economic resources. As a result, there is an urgent need to develop new strategies to prevent, diagnose, and treat these devastating conditions.
In recent years, a novel in vitro concept has emerged as a promising tool for studying the brain and neurodegenerative diseases. This concept is called Brain-on-Chip (BoC) and involves growing human brain cells on a miniature device that mimics the structure and function of the brain. BoCs allow researchers to study the interactions between different types of brain cells, test the effects of drugs and environmental factors, and model neurodegenerative processes in a controlled and ethical manner.

1.1 | Project challenge

The project described in this report is designed to support the research into novel MEA designs but is applicable in the larger MEA analysis space and the Brain-on-Chip framework in general. Brain-on-chip studies are a type of in vitro research, meaning "in glass" in Latin. These are experiments that are conducted outside of the living organism, in a controlled environment. In the case of brain-on-chips, scientists can grow different types of brain cells on a chip, such as neurons, and create a simplified version of the brain that can be studied in detail. One of the advantages of in vitro studies is that they allow researchers to study individual parameters of neuronal processes and their interactions without the vast complexity of the whole brain. By isolating specific aspects of brain function, scientists can analyze them in a controlled and defined environment. In contrast, studying the brain in vivo, meaning "within the living" in Latin, is more complex and can be influenced by many factors that are difficult to control.
The essence of neuronal communication is the transfer and processing of electrical signals, which is what makes the brain work. Researchers can use techniques such as micro-electrode arrays to measure the electrical activity of neurons in a network. Micro-electrode arrays are small devices that consist of multiple electrodes that are placed on the surface of a culture dish containing neurons. By recording the electrical activity of the neurons, researchers can study how they communicate with each other and how they form networks. The main challenge in this project is to develop a method that can automatically analyze the data collected from the micro-electrode array recordings in real time. This would allow researchers to harvest meaningful data about the neural activity in the cultured neuronal cell networks in a fast and efficient way. By analyzing this data, researchers can gain insights into the behavior of neuronal networks and improve our understanding of the brain. This information can be used to develop new MEAs and treatments for neurodegenerative diseases without the need for long downtime between experiments that were previously needed for this analysis.

1.2 | Current methods

Currently at the TU/e MEA research is done using equipment from Multi Channel Systems GmbH (MCS), a division of Harvard Bioscience, Inc., which makes off the shelve tools for MEA analysis. The tools used include the 120 electrode 120pMEA100/30iR-Ti-pr MEA chip [1] and the MEA2100-HS120 system [14] to read the data off of this chip.

Figure 1.1: MCS 120pMEA100/30iR-Ti-pr MEA chip [1]

These chips contain 120 electrodes that can be used to receive or send electrical signals. As the cells find themselves on and around the electrodes their inter-cellular communication is measured as an electrical potential. These 120 measurements are then stored in MCS's proprietary format to be analyzed using MCS's software.

1.3 | Neural Networks

To understand the research goal it is necessary to have a better understanding of neural networks and how scientists have come to understand them. Without this, it is not possible to analyze even the most basic functionality of neural networks and discuss the research results.

1.3.1 | Neuronal behavior

The human brain is a complex organ that contains billions of neurons, each of which communicates with other neurons to perform a variety of functions. Neuronal communication is characterized by electrical signals known as action potentials (AP), spikes, discharges, or nerve impulses. The fluid within and around neuronal cells consists mostly of water, with an asymmetric distribution of electrical charge. At rest, there is a negative electrical potential inside the cell, approximately -65µV compared to the outside [6]. When stimulated, ion-permeable channels in the cell membrane open, causing the membrane to depolarize and generate an AP. When an AP is large enough to pass a certain threshold of about -55µV it creates a spike. This electrical signal travels down the axon of the neuron and is transmitted from the axon terminal where it triggers the release of neurotransmitters into the synaptic cleft, which is the small gap between the presynaptic and postsynaptic neurons. The neurotransmitters then bind to receptors on the postsynaptic neuron, which generates a small electrical signal called a postsynaptic potential.

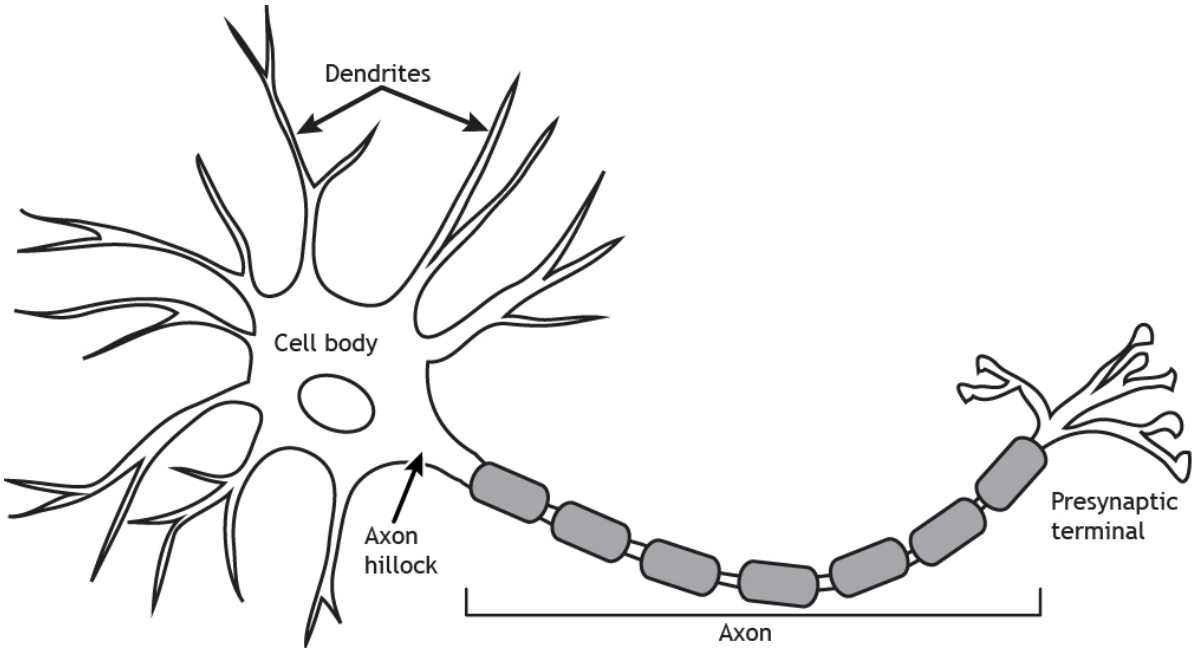

Figure 1.2: Neuron as depicted by Casey Henley [6]

This signal can either be excitatory or inhibitory, depending on the type of neurotransmitter and receptor involved. Excitatory signals make it more likely that the postsynaptic neuron will generate an action potential, while inhibitory signals make it less likely. Neurons can communicate with each other through different types of synapses, including chemical and electrical synapses. Chemical synapses are the most common type of synapse, and they use neurotransmitters to transmit signals between neurons. Electrical synapses, on the other hand, use gap junctions to allow the flow of electrical current directly between neurons. Neuronal communication is not limited to a single neuron, but it can also occur between networks of neurons. As the original statement suggests, a larger system of a few neurons connected in circuitry can form a higher functional network. These networks can perform complex functions and behaviors, such as processing sensory information, controlling movement, and generating thoughts and emotions. Understanding neuronal communication is crucial to understanding the function of the brain. While a single neuron may not behave like a network, considering the behavior of individual neurons is necessary to understand neuronal communication. However, to understand complex brain functions and behaviors, it is essential to analyze neural networks and their organization at various levels of detail.
While whole-brain analysis is out of the scope of MEA research, there are three levels of scope that are interesting to us:

- Intra-cellular behavior concerns the inner workings of a cell
- Inter-cellular behavior concerns how cells communicate with each other. This is what the MEA system measures.
- Network-wide behavior concerns the whole network. Two cells are a network, but they would not be functional. The network-wide behavior is an important performance indicator.

1.4 | Neurodegenerative diseases

Neurodegenerative diseases are a group of disorders characterized by the progressive loss of neurons and their functions in the brain and nervous system. These diseases are often chronic and have no known cure. Examples of neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS), among others.
One way that a neuron might deteriorate in a neurodegenerative disease is through the accumulation of abnormal proteins in the cell. For example, in Alzheimer's disease, amyloid beta protein accumulates in the spaces between neurons, forming plaques, and tau protein accumulates inside the neurons, forming tangles. These protein accumulations can disrupt communication between neurons and lead to cell death. This causes problems with short-term memory, language, and disorientation
In Parkinson's disease, the neurons that produce dopamine, a neurotransmitter important for movement,

deteriorate and die. The loss of these neurons leads to the characteristic motor symptoms of Parkinson's, such as tremors, rigidity, and slowness of movement.
In Huntington's disease, a genetic mutation causes an abnormal protein called huntingtin to accumulate in neurons in the brain. This accumulation causes the neurons to become damaged and eventually die, leading to a range of symptoms including movement problems, cognitive decline, and psychiatric symptoms.
Sadly a cure has remained elusive for all of these diseases. But we know that neural networks in Parkinson's patients show a clear difference from a normal brain in MEA research [20]. This means that MEAs could also be valuable tools in the development of new treatments for diseases with similar degeneration like Alzheimer's.

1.5 | Project goal

The goal of this Bachor End Project (BEP) is to develop tools for automated real-time data analysis and data ingestion of MEAs to ease the development of novel MEA chip designs. This would allow for faster data analysis and removing the need for downtime between experiments and allowing for insight to be gained during said experiments.

1.6 | Project plan and outline

To develop these tools we need to understand what is being analyzed. The information in chapter 1 provides a background to the nature of this data. The specifics of the data ingestion using the McS equipment will be discussed in chapter 2. There the nature of the problems with the proprietary file formats are introduced, and in chapter 3 these issues will be further addressed and a case study introduced. In chapter 4 the automation problems will be introduced, several solutions will be weighed, and a recommendation and implementation will be made. These form part of the case study that will be rounded off in chapter 5. After which a summary of conclusions and recommendations will be given in chapter 6 where an outlook is also given. Finally, the code for the proposed solutions will be found in Appendix A.

2 | Data Ingestion

Data ingestion from MEAs is done by using the Multi Channel Experimenter. The MEA data is captured as analog signals and needs to be digitized before analysis. The Multi-channel experimenter (MCE) software is used for the acquisition and analysis of these digital signals. The MEA data ingestion process starts with the connection of the MEA device to the MCE software. The MCE software captures the analog signals from the MEA device and digitizes them. The digitized signals are then sent to the MCE data acquisition module, where they are stored in a buffer. The MCE data acquisition module is responsible for managing the data buffer and ensuring that the data is stored correctly. The data buffer is divided into chunks, and the data is continuously transferred from the buffer to the analysis module. The MEA data is then analyzed using various analysis tools and techniques available in the MCE software. The MCE software allows researchers to filter, segment, and visualize the MEA data to extract meaningful information. The data can be further analyzed using statistical techniques to understand the behavior of the neurons under different experimental conditions. In summary, MEA data ingestion with the Multi-channel experimenter software involves capturing and digitizing the analog signals from the MEA device, storing the digitized data in a buffer, and analyzing the data using various analysis tools and techniques available in the MCE software.
In practice, this would look like figure 2.1.

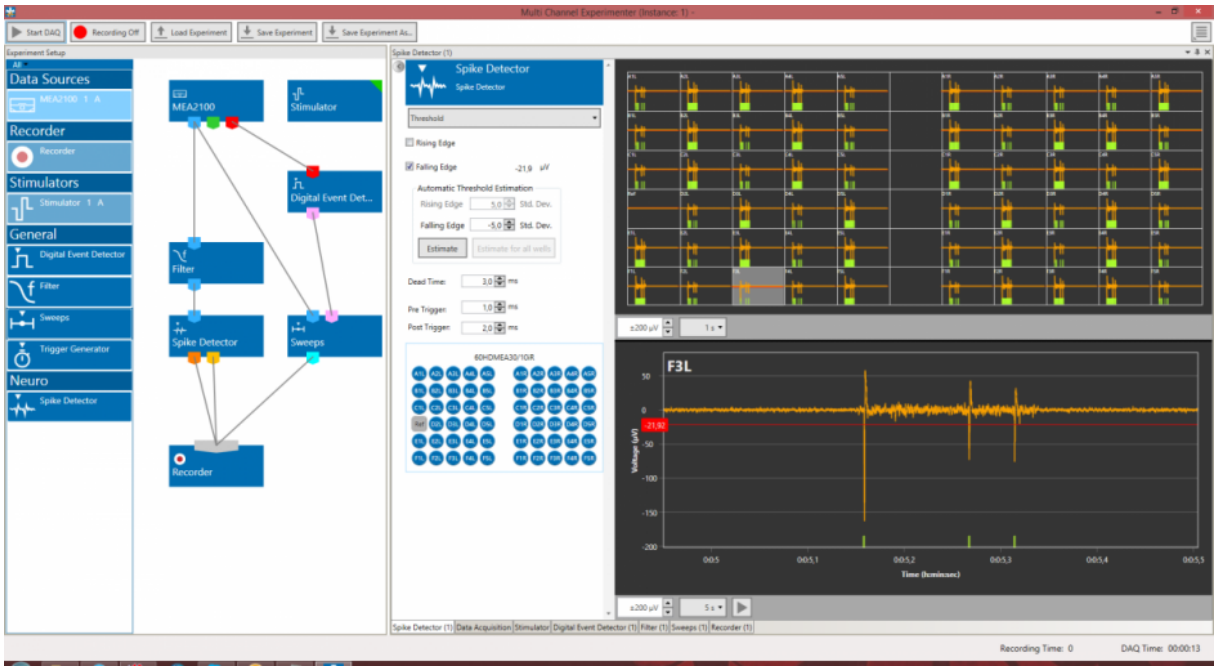

Figure 2.1: Multi-channel experimenter screenshot by MCS

Where on the left we have our available tools, in the middle the tools that are in use, and on the right a full view of the output and settings of each tool.
The tools that are used depend on the type of analysis that needs to be done, but the most basic setup would be:

- Data source tool

  This tool allows for the software to interface with the Multi-channel systems MEA2100 system. Here you would also change setting like polling rate (20kHz is standard practice) and disable/enable certain electrodes.

- Recorder tool

  This tool allows for any data generated by another tool to be recorded and exported. This data is then stored as a ".msrd" file. This is a proprietary file format that is used within the Multi-channel systems suite of tools.

Other tools are available in the MCE. However, these are not relevant to the data ingestion process used.

Along with the exported data, a number of metadata files are exported. These files contain the metadata for all used tools and equipment. This includes their settings and properties in XML format. These settings may include the type, order, and point at which a filter is set to operate in the MCE, or settings such as the polling rate of the equipment itself.
The exported data is formatted in MCS's proprietary .msrd file format. This file format can not be used outside of the MCS suite of tools. This is because MCS has made the decision to make its software freely available. This makes it unnecessary to take out licenses for the tools that their hardware runs on. Making it easier for end users to share data and collaborate. But to keep the software from being used on other tools MCS allows it software to only use data in .msrd format, which is only generated by their equipment. This normally does not pose any issues, but if preprocessing of the data is needed it is necessary to use the multi channel Data Manager (MCDM) to export the data in a different format.

3 | Data Analysis

Data analysis of MEAs is essential for understanding and interpreting neural activity. MEAs are a set of electrodes that a culture is grown upon or are implanted into living tissues or cells, such as the brain or heart, to record electrical signals. These signals represent the firing of individual neurons, and analyzing them allows researchers to study the dynamics of neural circuits, and their role in brain function, development, and disease.
The data generated by MEAs is complex and requires specialized analytical tools and methods to extract meaningful information. This data analysis involves the detection and sorting of individual spikes, the identification of patterns of activity, the calculation of firing rates and correlations, and the construction of network models. Through data analysis, researchers can also investigate the effects of experimental manipulations, such as novel MEA designs and drug treatments, and determine how they affect neural activity.
The primary tool for analyzing data is the multi channel Analyzer (MCA). This tool is however limited by the necessity to use the proprietary .msrd file format. This severely limits preprocessing of the data, thus a different tool would be used when preprocessing is necessary. For this project, the MEA-Toolbox [11] from the Leiden University Medical Centre (LUMC) and the Netherlands Organ on Chip Initiative (NOCI) [16] is therefore used as an additional analysis tool.

3.1 | Multi Channel Analyzer

The multi channel Analyzer is the standard tool for analyzing MEA data produced by MCS equipment. It is fast, stable, and easy to use with a drag-and-drop interface that is identical to that of the MCE. Here many tools, called instruments, are available to perform the analysis. For the analysis of the novel MEA designs the following instruments are the most important. More extensive explanations of these instruments and their interaction are available in the documentation [15].

- Raw Data explorer

  Here previously exported data and metadata from the MCE are imported.

- Filter

  Highpass- and notch-filters allow the system to filter out most noise from the measuring equipment. This is done by having a notch filter at the power cycle frequency (50Hz in Europe). And by setting a highpass filter to 200Hz to exclude changes in electrical potential that are not caused by spiking activity.

- Spike detector

  The spike detector can extract spikes from a raw data stream by different methods and generates time stamp data for these spikes.

- Spike analyzer

  The spike analyzer takes the time stamp data from the spike detector and relays it to the user in a clear and concise way.

- Burst analyzer

  The burst analyzer takes the time stamp data from the spike detector and detects bursts using a maximum interval method. It then displays these bursts and associated network bursts.

These tools are also available in the MCE, where they are run in real-time. As such running the analysis in the MCE environment might be better if this real-time analysis is a priority. MCE however only allows for online analysis, which necessitates the equipment and culture to be connected to the computer, and as such analysis at a later time, or at another location must be done in the MCA.
One limitation of the MCA tool is a lack of preprocessing possibilities. This is a problem because preprocessing is necessary when artifacts appear that can not be filtered out by the filter instruments, and are detected as spikes. These artifacts can come from various different sources like local field potentials [13], which are not relevant for the analysis, or interfacing noise from the equipment used, which may come from issues as trivial as a faulty USB cable.

Because the measurements taken during the development of the novel MEAs showed such artifacts regularly they would be detected as spikes, and those spikes would in turn be detected as bursts, there would be no reliable correlation between these detected indicators of activity [17, 21] and actual neuronal activity. As such preprocessing of the recorded data was deemed necessary.

3.2 | Preprocessing

To preprocess the recorded data it needs to be in a format that could be read and written by software suitable for preprocessing the recorded data. Since MCS supports exporting recordings as HDF5 files through the MCDM that format was chosen. HDF5 is a file format that is designed for large data structures with metadata and is often used for the analysis of MEA data [9, 11].
Because the HDF5 file can not be converted back into msrd after the preprocessing, the MEA-toolbox [11] will be used to analyze the data. Since this toolbox is written for MATLAB and a seamless user experience was desirable the preprocessing script was also written in MATLAB. That way the script could be adjusted to call for functions from the MEA-toolbox and thus run the analysis in one click.
The code to the preprocessing script can be found in Appendix A and works as follows:

- When run, the program opens a dialog box where the user can select a folder. All hdf5 files in the selected folder will then be run through the program.
- The program will then take the selected files in alphabetical order and read the first 30 seconds of data into memory.
- For every electrode it will then find any abnormally large voltage values. Where normal spikes fall in the 30-100 μV range, the anomalies exceed 4000 μV and have been recorded to be as high as $4V$.
- When such an anomaly is found its value is set to zero. This way the analysis tools will not record it as a spike.
- When all the anomalies have been corrected the file is saved in the same folder under the same name as the original file, but with the word ”filtered” appended to the file name.
- This is repeated for all selected files and takes some twelve seconds per file.

These files are then ready to be analyzed in MEA-toolbox.

3.3 | MEA-toolbox

MEA-toolbox is a MATLAB toolbox for MEA analysis developed by Hu, M et al [11] and can be downloaded on GitHub [10] where documentation can also be found.
All tools present in the MCA can also be found in the MEA-toolbox. It actually contains more tools that are intuitively laid out in a ribbon-style interface. It is however slow to run and less stable than MCA or MCE. This makes it unsuitable for real-time use, where MCE shines, but the ability to take HDF5 files makes the toolbox most suitable for our use-case. To use the MEA-toolbox first the parameters must be set for analysis. For all analyses, the default values were used as provided by the MEA-toolbox. After these settings have been confirmed and saved the files may be analyzed and loaded. This analysis and loading takes twice as long as the length of the recording at 20kHz i.e. a sixty-second recording would take one-hundred-and-twenty seconds to be analyzed and loaded. After loading the following is displayed: a heatmap of activity on the MEA 3.1 and a raster plot.

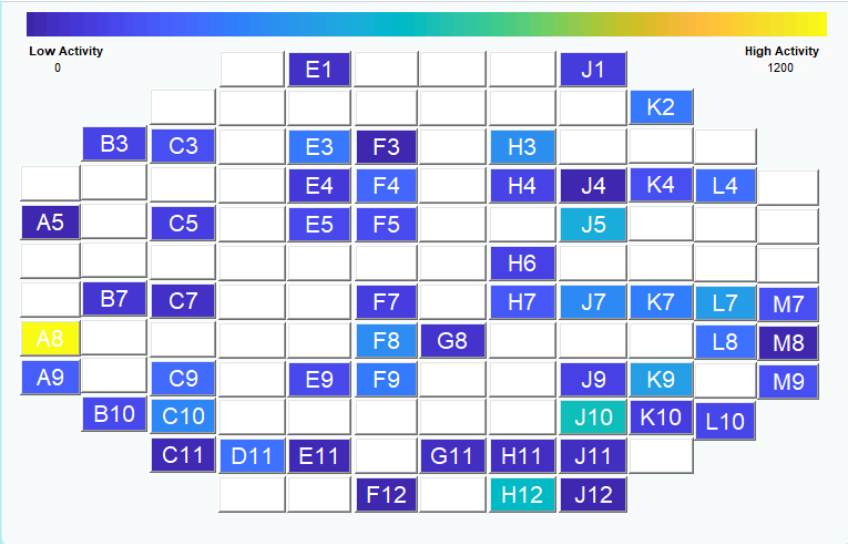

Figure 3.1: MEA-toolbox Heatmap

This raster plot is a fast way to compare neuron behavior. Below we compare the two sets of data.

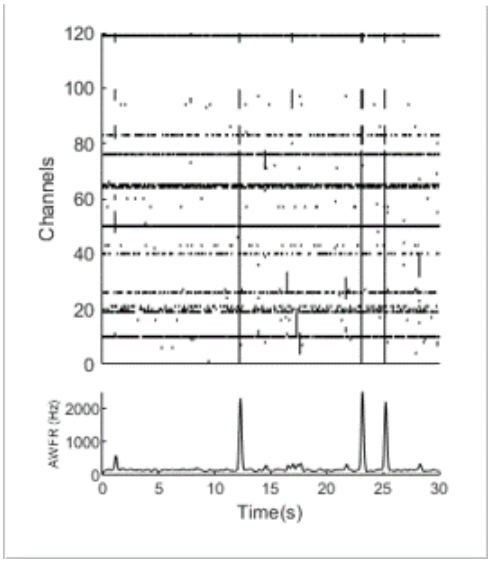

Figure 3.2: Raw Data

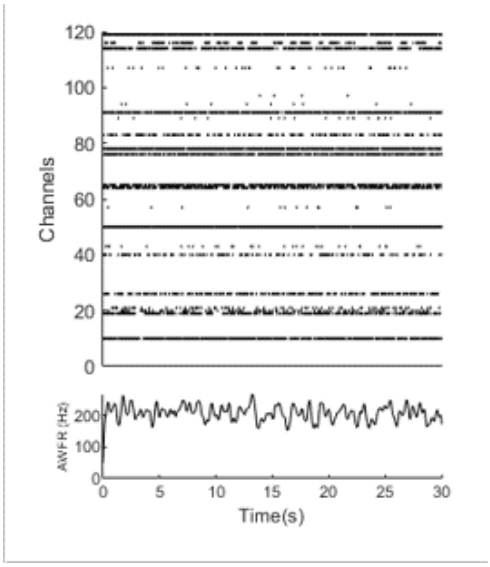

Figure 3.3: Processed Data

In the raw data rasterplot, we find large spikes that are found across almost all channels. This is the arifact that is succesfully filtered out in the processed data.

4 | Automation

A major hurdle to development with MEAs is the long time it takes for analysis and data acquisition. These tasks, while important, should not require someone to sit in front of the computer after setup. Removing this tedious part of the workflow for MEA analysis allows researchers more time, and the ability to do interesting research. If, for example, an experiment needs a researcher to be hands-on with the cell culture during measurements, like applying medicinal compounds to the culture during measurements, they currently need an extra pair of hands, which may not always be available. Automating these tasks would allow the researchers to do such work without the need for extra hands, thus making the research faster and cheaper.

There are two ways to automate the ingestion and analysis of the data.
One way is to build the tools to not need human interaction after setup. This is functionally the best method but is very hard, or even impossible to implement due to the proprietary nature of the existing tools.
The other way is to emulate what the user would normally do. This method can cause significant system overhead but is simple to implement. Even within this option, there are different ways to implement this emulation.
The two explored implementation methods were smart automation and dumb automation.

4.1 | Smart automation

Smart automation of ingestion and analysis would work very simply, but would also be resource intensive. This method would have a script scan the page for text to click, then click those buttons in the right order.

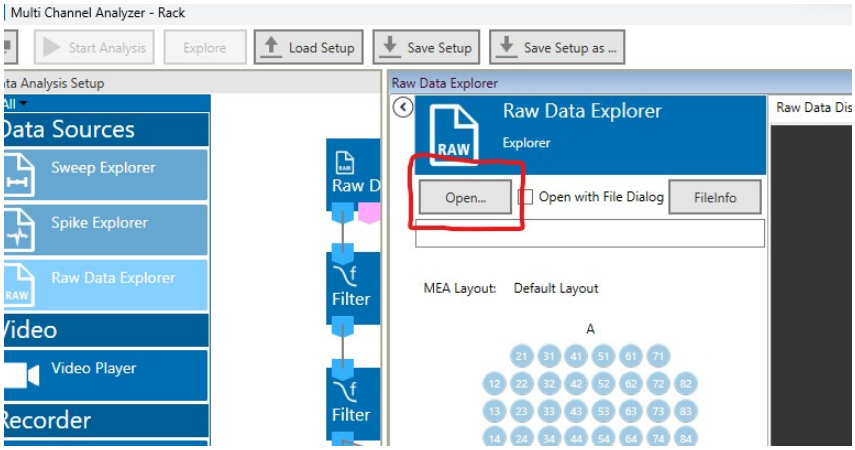

Figure 4.1: The program would look for the word "Open" and click it

This smart automation can be made to react to changing situations and environments. But it does come at the cost of system resources. The running program in takes up 60MB of space in RAM and up to 6% CPU utilization on a modern CPU[1].

4.2 | Dumb automation

Dumb automation is very simple in its execution. Instead of finding the places to click itself, coordinates to click are provided to the program. This means that the overhead is much lower, but it comes with the downside that the program can not handle moved UI elements. The position of UI elements is the same in all instances of MCA and MCE for a given resolution and scaling. So as long as the experimenter uses a 1080p monitor at normal scaling in Windows no issues should arise.
This program used 4MB of RAM and ran below 0.4% CPU utilization.

[1] Ryzen 5 5600x@4.2GHz

4.3 | implementation

As the dumb automation method runs faster this has been implemented for MCA and the script can be found in appendix A.
This script was written for AutoHotKey [4]. A lightweight tool made to mimic human input on a computer. The script opens the newest file in the default folder for MCA. It then runs the analyzer and changes tabs to the spike detector. This allows any researcher to gain insight into the activity of the network during the analysis just by looking over. When the analysis is done the results are automatically exported as desired and the cycle starts over again. The program takes a little under 17 seconds to run through a minute of data from the 120 electrode MEA [1] at 10kHz sampling rate.

5 | Application and Interpretation

In chapters 3 and 4 tools are developed for automated ingestion and analysis of the MEA data. In chapter 3 the possibility of a case study is discussed. The case study functions as an evaluation of the performance of the systems.
The new filtering system resulted in an elimination of artifacts in the data as shown in figures 3.2 and 3.3. And caused a decrease of 8711 spikes detected. Making quantitative comparisons to other measurements possible. These measurements are compared on three criteria:

- Spike frequency in spikes per minute
- Burst frequency in bursts per minute
- Network burst frequency in network bursts per minute

These are all criteria that indicate activity and development of neural networks [17, 21]. While there are more possible criteria for this comparative analysis the three listed above are used most commonly in literature with the former two having the strongest correlation to neuron development [17].

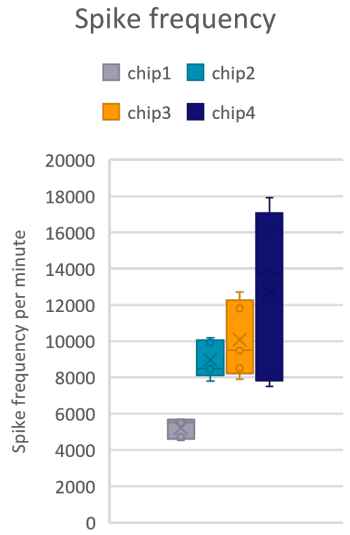

Figure 5.1

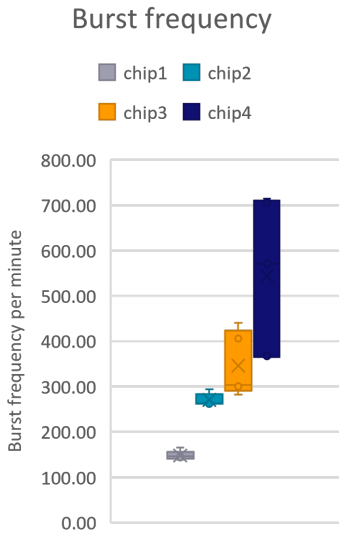

Figure 5.2

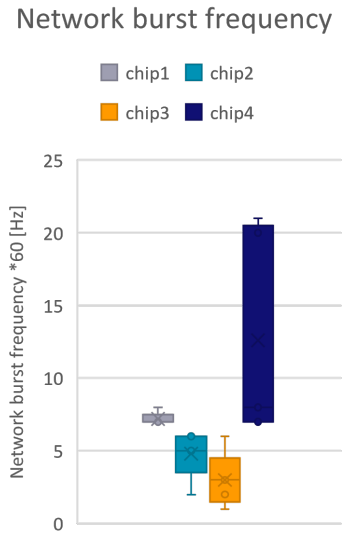

Figure 5.3

Here four different MEA chips are compared. We see from the scale that a difference of 8711 spikes would have had a significant change in the spread of these four chips. And now that the data from these different measurements can be compared, conclusions on the performance of these newly developed chips can be made.
Now using this data the odds of the difference between the chips being due to chance can be calculated. This results in P (two tailed) =0.0001 for chip 2 to be the same as the control chip 1. P=0.001 for chip 3 to be the same as control and P=0.008 for chip 4 to be the same as control. All of these are statistically significant even with the low sample size of 5 samples per chip. It can therefore be concluded that the three newly made chips perform better than control in both spike frequency and burst frequency behaviour. For network burst frequency the new chips do not perform better. This is due to the individual network burst being longer and more continuous and results in higher time spent in network burst than control for all chips.

The case study for the application of the automation tool was already discussed in chapter 4 but bears repeating here. The program works to take away menial tasks from the data ingestion and analysis portions of the MEA analysis. The implementation of the analyzer works well with an analysis time of under 17 seconds whereas manually performing the same task took around 30 seconds.

6 | Conclusion and recommendations

In this last chapter, a summary of all conclusions is given followed by recommendations.

6.1 | Conclusions and Recommendations

In chapter 1 background information is given on the state and importance of Multi-electrode arrays. Reasoning for the development of improved MEAs is given and a research goal was set.
"Develop tools for automated real-time data analysis and data ingestion of MEAs to ease the development of novel MEA chip designs". Important reasons to develop these tools are:

- Reducing manual labor
- Allowing more freedom in research setup
- Allowing for faster research
- Allowing for direct comparisons to other chips.

The tools preprocess data to allow for direct comparisons between noise-plagued measurements and automate the analysis of MEA data. However, these preprocessing tools do not run in real-time. While the real-time performance of the multi channel Analyzer is not missed as its tools can be used in real-time through the multi channel experimenter, the preprocessing in its current form can not be run in real-time. The selected case studies have shown what works at this moment, and that those working elements work well.

6.2 | Outlook

The developed tools are ready to be used to make the research around MEAs easier. There are however some clear paths for improvement.

- The preprocessing code can be plugged into the MEA-toolbox by Hu, M et al. The code to both is freely available and their combination would be an improvement to the current situation.
- The script for automating the MCA could be adapted to work on the MCE, this could save some time in the future, but might not be necessary now that the features of MCE are fully used.

There are many ways the systems for MEA research can still be improved, but these are a clear path to continue toward the one-click brain-on-chip.

6.3 | Concluding remarks

I really loved working on this project, maybe a bit too much. MEAs are an exciting area of research that has gained considerable interest in recent years, and it's not difficult to see why. These MEAs are versatile tools used to record the activity of neurons in vitro, enabling researchers to investigate the workings of the brain and its electrical signaling at a cellular level. The interest that helped me through the start of this project is was made it difficult to end. Working with MEAs requires a diverse range of skills, including electronics, computer programming, data analysis, and statistics. As a result, working on this project was both challenging and rewarding, providing opportunities for personal and professional growth. The ability to apply a range of skills to a single project was one of the most satisfying aspects of working on it, even if I worked longer than I should have done, and I believe this versatility will continue to help me for years to come.

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A | Appendix A, scripts

A.1 | Spike preprocessing

```
    %% clearing
% clc
% clear all
%% read h5 function

%% variables
threshhold = 4000;
waita= 0;
%% select file
correctfolder=uigetdir; %lets the user select  a folder for analysis
if correctfolder == 0
    return
else
    cd(correctfolder) ; %change the current folder to the user slected one
    files=[dir('*.h5')];
end

for f = 1:length(files)
file = files(f).name;
path = append(files(f).folder,'\');
waita = waita +1;
waitbar (waita/length(files));
%% import file
matrix=h5read(fullfile(path,file),'/Data/Recording_0/AnalogStream/Stream_0/ChannelData')

%% find errors
ExeptMatrix = matrix > threshhold | matrix < -threshhold;
%totalerr = sum(sum(ExeptMatrix));

%% define area around errors
remMatrix = ExeptMatrix;
for i = 1:width(ExeptMatrix)
    for j = 1:height(ExeptMatrix)
        if ExeptMatrix(j,i) == 1
            for n = 1:15
                if j-n > 0
                    remMatrix(j-n,i) = 1;
                end
                if j+n < width(ExeptMatrix)
                    remMatrix(j+n,i) = 1;
                end

            end
        end
    end
end
%sum(sum(remMatrix)
%% adjust errors
matrix2 = matrix;
matrix2(ExeptMatrix) = 0;

%% check errors were removed
exept2 = matrix2 > threshhold | matrix2 <-threshhold;
totalerr2 = sum(sum(exept2));
```

```
if totalerr2 > 0
    quit
end
%% create file
name = 'filtered';
namepath = append(path,name,file);
copyfile(fullfile(path,file),namepath)
%% write back to file

h5write(namepath,'/Data/Recording_0/AnalogStream/Stream_0/ChannelData',matrix2);
a= matrix2;

end
close all force
```

A.2 | automated analysis AHK code

```
    Loop, 10
{
WinWait, Multi Channel Analyzer - Rack,
IfWinNotActive, Multi Channel Analyzer - Rack, , WinActivate, Multi Channel Analyzer - Rack,
WinWaitActive, Multi Channel Analyzer - Rack,
MouseClick, left,  431,  983
Sleep, 200
MouseClick, left,  461,  197
Sleep, 200
WinWait, Open File,
IfWinNotActive, Open File, , WinActivate, Open File,
WinWaitActive, Open File,
MouseClick, left,  971,  75
Sleep, 200
MouseClick, left,  971,  75
Sleep, 100
MouseClick, left,  833,  142
Sleep, 100
MouseClick, left,  1127,  537
Sleep, 100
WinWait, Multi Channel Analyzer - Rack,
IfWinNotActive, Multi Channel Analyzer - Rack, , WinActivate, Multi Channel Analyzer - Rack,
WinWaitActive, Multi Channel Analyzer - Rack,
MouseClick, left,  143,  54
Sleep, 100
MouseClick, left,  593,  992
Sleep, 100
Sleep, 60000
}
Esc::ExitApp
```