Developing a software for analysis of Calcium Imaging

A PROJECT REPORT

Submitted by,

Mr. Gagan M Shetty – 20211LCA0002

Under the guidance of,

Dr. Komalavalli C
Dr. Hsien Sung Huang (NTU)

in partial fulfillment for the award of the degree of

BACHELOR OF TECHNOLOGY

IN

SCHOOL OF COMPUTER SCIENCE AND ENGINEERING

At

PRESIDENCY UNIVERSITY

BENGALURU

JANUARY 2024

PRESIDENCY UNIVERSITY

SCHOOL OF COMPUTER SCIENCE ENGINEERING & INFORMATION SCIENCE

CERTIFICATE

This is to certify that the Project report **"Analysis of calcium imaging"** being submitted by "Gagan M Shetty" bearing roll number(s) "20211LCA0002" in partial fulfilment of requirement for the award of degree of Bachelor of Technology in school of Computer Science and Engineering is a bonafide work carried out under my supervision.

Dr Komalavalli C
Professor
School of CSE&IS
Presidency University

Dr. Zafar Ali Khan
Professor & HoD
School of CSE&IS
Presidency University

Dr. C. KALAIARASAN
Associate Dean
School of CSE&IS
Presidency University

Dr. L. SHAKKEERA
Associate Dean
School of CSE&IS
Presidency University

Dr. SAMEERUDDIN KHAN
Dean
School of CSE&IS
Presidency University

PRESIDENCY UNIVERSITY

SCHOOL OF COMPUTER SCIENCE ENGINEERING & INFORMATION SCIENCE

DECLARATION

We hereby declare that the work, which is being presented in the project report entitled **Software for analysis of calcium imaging** in partial fulfilment for the award of Degree of **Bachelor of Technology** in **Computer Science and Engineering**, is a record of our own investigations carried under the guidance of **Hsien Sung Huang Associate professor (NTU), School of Computer Science Engineering, Presidency University, Bengaluru.**

We have not submitted the matter presented in this report anywhere for the award of any other Degree.

Gagan M Shetty
20211LCA0002
7CAI02

ABSTRACT

Calcium imaging is done to identify the activity of Brain Neurons of freely moving animal for observing the behavior of that animal before and after injecting diseases.

In this project I am developing a software to read the video raw data collected over a time using this video data several methods are implemented to identify neurons and traces are drawn for each Neurons individually and analyzed.

ACKNOWLEDGEMENT

First of all, we indebted to the **GOD ALMIGHTY** for giving me an opportunity to excel in our efforts to complete this project on time.

We express our sincere thanks to our respected dean **Dr. Md. Sameeruddin Khan**, Dean, School of Computer Science Engineering & Information Science, Presidency University for getting us permission to undergo the project.

We record our heartfelt gratitude to our beloved Associate Deans **Dr. Kalaiarasan C and Dr. Shakkeera L,** School of Computer Science Engineering & Information Science, Presidency University and **Dr. Zafar Ali Khan**, Head of the Department, School of Computer Science Engineering & Information Science, Presidency University for rendering timely help for the successful completion of this project.

We are greatly indebted to our guide **Dr. Komalavalli C, Professor**, School of Computer Science Engineering & Information Science, Presidency University for his/her inspirational guidance, and valuable suggestions and for providing us a chance to express our technical capabilities in every respect for the completion of the project work.

We would like to convey our gratitude and heartfelt thanks to the University Project-II Coordinators **Dr. Sanjeev P Kaulgud, Dr. Mrutyunjaya MS** and also the department Project Coordinators **Dr. Murali**.

We thank our family and friends for the strong support and inspiration they have provided us in bringing out this project.

Gagan M Shetty

20211LCA0002

LIST OF FIGURES

TABLE OF CONTENTS

CHAPTER-1

INTRODUCTION

Calcium imaging was first demonstrated in the mid 1970's. This calcium imaging is done to visualize and monitor the cells particularly neurons by measuring intracellular changes in calcium levels. This calcium ions are small molecules these dyes are used with the chelator of carboxyl group masked acetoxymethyl esters in order to enter easily in a cell and this calcium dye will help to see glowing or excitation state of neurons calcium dynamics provides insights into the functional activity of cells and is especially helps in study activity of neurons.

Calcium imaging is also known as calcium mapping (neuron identifying) this is a technique used in isolated mouse, mice etc… this below figure will show you how miniscope is connected into mice brain for analyzing the behavior of freely moving animal.

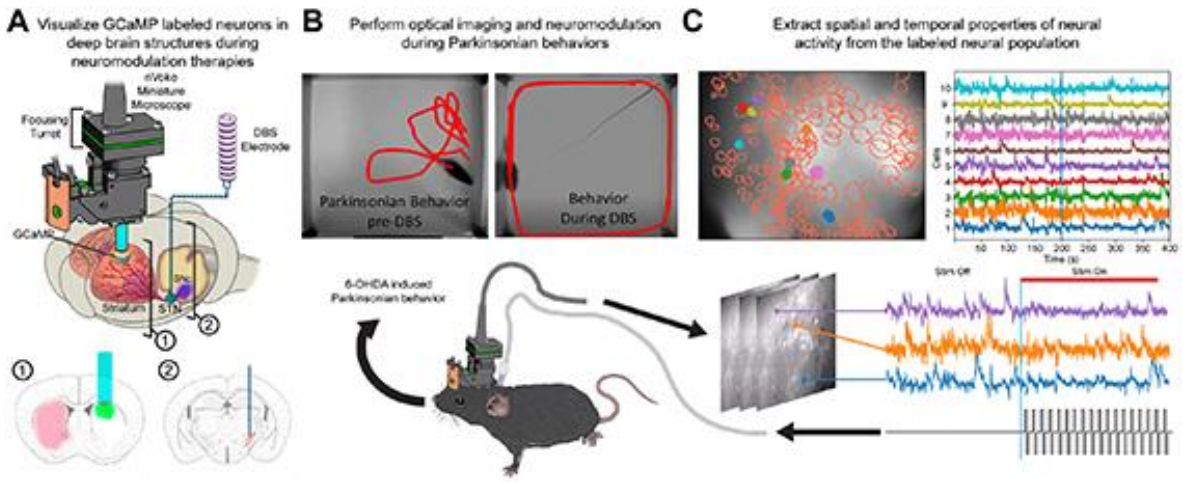


Figure1.1 mice brain connected to miniscope and traces are extracted in the form of line graph.

In above figure' A' you can see the miniscope connected to brain and in figure' B' you can see the behavior of freely moving mice. In figure' C' you can see glowing neurons which are marked manually and traces are identified for each neuron based on those traces further study is carried out is there is any wrong mapping in neuron it will give the bad result so there is no perfect algorithm to map neurons still so it was identified by manually mapping for each neuron which was time consuming and also very hard to deal with large amount of data.

Calcium imaging is commonly a noisy method due to high spatiotemporal information and this noise will be also recorded from animal crash to path and some external noise that occurred externally by

experiments the below figure show the animal path and how experiment will be carried out. Even this mice are so sensitive to the odor so their should not be sensitive smells that should be present because this will impact on experiment being conducted.

Calcium indicators are molecules that emit fluorescence when they bind to calcium ions. This calcium indicators include Fluo-4,Fura-2 and GCaMP(genetically encoded calcium indicator). The loaded cells are then placed under fluorescence microscope equipped with camera to capture emitted fluorescence. The fluorescence emitted by calcium indicator is captured over time generating a series of images that represent changes in calcium levels within the cells. This data is collected and analyzed using a software for a period of time.

The below figure will show you how experiment is done and how miniscope is connected to mice to record the brain neuron activity.

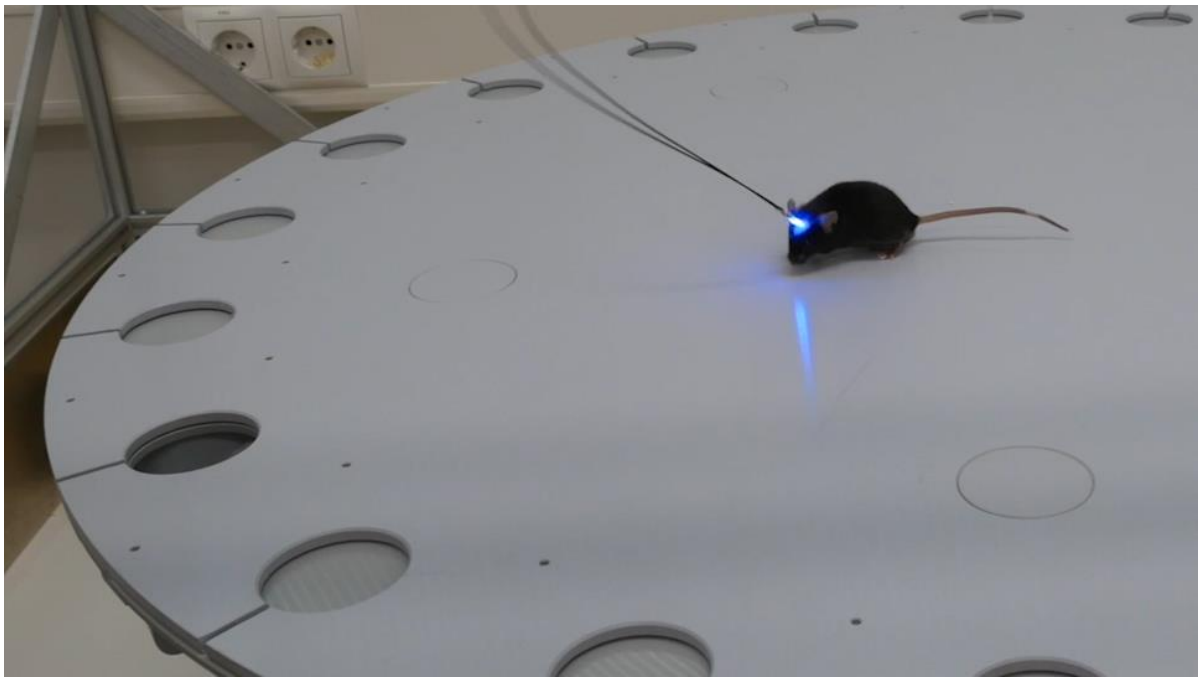


Figure 1.2 freely moving mice and recording neural activity.

The above figure shows the freely moving mice where miniscope is connected to mice brain this miniscope is very less in weight so that mice can carry it easily and this miniscope is placed on a mice before 4 months of conducting experiment so that mice will get used to it from 4 months and then experiment is conducted over a time and also external moving of mice is also recorded in order to see the movement of mice at same time and neuron state this will help researchers to their study.

In order to have a clear data we need to do some analysis tasks required those are :

1. **motion correction**: addressing any motion artifacts in the data to enhance accuracy.

2. **spike detection**: identifying and isolating the spikes within collected data.

3. **memory mapping**: organizing and mapping the data in a systematic and efficient manner for analysis.

4. **identifying neurons**: clearly distinguishing and labeling neurons within the dataset.

5. **Deconvolving**: unraveling the complex signals by separating overlapping or intertwined elements within the data.

Introduction to libraries:

1. **caiman** is a package developed by Giovannucci et al. to analyze brain images on large scale to test the package in a real time Giovannucci et al asked several trained annotators to identify active neurons that were round and donut-shaped in several sets of imaging data from mouse brains each set of data was analyzed by three or more researches then discussed to conclude then they employed using caiman package and later they analyzed it was similar to human identified active neurons in brain image. Has caiman is a open source platform so many laboratories are using it now and it can be customized according to requirements of lab for further usage.

2. **CNMFE** is a package also known as constrained non negative matrix factorization this is a combination of auto sklearn to identify the spatial and temporal traces of neurons from a large scale of data but this package is used for micro endoscopic data.

3. **Carignan:** this is also a fully automated software tool for analysis of calcium imaging in large scale data analysis but this is a software which is developed by combination of caiman, cnmfe and auto-sklearn used for both 1p and 2p miniscope data but this software implementation is very difficult has auto-sklearn is not supported for both windows and macOS it only works with docker.

4. **Auto-Sklearn:** Auto- sklearn is a machine learning tool that is designed to streamline and automate the process of applying machine learning to real world problems. This algorithm will help us to choose best algorithm approach and it also gives all the analysis parameters with accuracy, F1 score etc..

This calcium imaging is done for a good and healthy mouse first and this mice is injected with a disease and analyzed a behavior of mice this will help to study on the related field this neuron activity is done in order to study ADHD disease so this neuron activity and experiment is conducted for so many diseases this ADHD is one of the example that were conducted in our lab.

The calcium imaging is also done by giving anesthesia to mice this mice behavior will be analyzed by this treatment and this will help for further research.

CHAPTER-2

LITERATURE SURVEY

Calcium imaging involves reviewing key scientific papers, articles and research studies that have contributed to the understanding of calcium imaging techniques, applications, and findings in various fields.

Imaging neural activity in mice with improved GCaMP calcium indicators published on (2013).

This papers discusses the development of genetically encoded calcium indicators for imaging neural activity in different model organisms, providing insights into the advancement in molecular tools for calcium imaging.

Calcium imaging analysis -how far have we come? Published on national library of medicine published on august (2021)

MRC Laboratory of Molecular Biology, Cambridge, UK

Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, UK

Contributed equally.

This is a software developed by this team for analysis of calcium imaging they used various techniques NoRMcorre and is available in both python and MATLAB they use denoising motion correction methods for their analysis.

Inscopix is a US based company they use built in package called CNMFE and they implemented their own solution for this is not a open source code they have licensed it and they sell it along with inscopix miniscope machine but its very difficult to analyse the code and results and this software cannot be modified according to the lab requirements.

Automated curation of CNMFE extracted spatial footprints and calcium traces using open source AutoML tools published on (15 july 2020).

This is a software based on CNMFE package which is used for motion correction and it also uses AutoML tool for predicting a best results this will produce a F1 score for analysis and it will be trained using manual marking of ROI and given to machine to learn the features and

machine will produce or mark ROI for new data (supervised technique) is applied in this method.

Calcium peak toolbox for analysis of in vitro calcium imaging data published on BMC neuroscience on (30 November 2022).
This is a automated ca2+ imaging analysis pipeline capture detects neurons, classifies and quantifies spontaneous activity, quantifies synchrony metrics, and generates cell- and network-specific metrics that facilitate phenotypic discovery.

Open source software for real-time calcium imaging and synchronized neuron firing detection published on IEEE (2021).
This is a software in the name of Carignan which uses combination of caiman CNMFE and AutoML tools and trained using manual supervised technique this software was developed in python.
Caiman an open source tool for scalable calcium imaging data analysis published on national library of medicine on(2019).
This is a software for scalable analysis developed both in MATLAB and python this tools will help researchers to make their work easily by automating their neuron identification it is 95% accurate and tested and used in several labs this software was developed by Andrea Giovannucci. This software achieves nearest to human analysis.

CHAPTER-3

RESEARCH GAPS OF EXISTING METHODS

Identifying research gaps in existing methods is crucial for advancing scientific knowledge and improving experimental techniques in the context of calcium imaging several areas may present opportunities for further research and development.

- **Temporal Resolution Improvement:**

Many calcium imaging methods have limitations in capturing rapid changes in neural activity. There is a need for techniques that can enhance temporal resolution, allowing researchers to investigate faster neuronal processes and interactions.

- **Spatial Resolution Enhancement:**

Improving the spatial resolution of calcium imaging can provide a more detailed view of cellular and subcellular structures. Research could focus on developing methods that enable high-resolution imaging without sacrificing other important aspects like signal-to-noise ratio.

- **Simultaneous Multimodal Imaging:**

Integrating calcium imaging with other imaging modalities, such as functional magnetic resonance imaging (fMRI) or optogenetics, can provide a more comprehensive understanding of neural activity. Research is needed to develop methods that allow simultaneous multimodal imaging with high precision.

- **Long-Term Imaging Stability:**

Long-term calcium imaging poses challenges related to phototoxicity, photobleaching, and stability of indicators over extended recording periods. Investigating methods to improve the stability of calcium indicators and minimize potential side effects would be valuable.

- **Improved Calcium Indicators:**

While genetically encoded calcium indicators (GCaMPs) have revolutionized calcium imaging, there is room for improvement in terms of sensitivity, dynamic range, and selectivity. Developing new indicators or optimizing existing ones can enhance the overall performance of calcium imaging techniques.

- **In Vivo Deep Tissue Imaging:**

In vivo calcium imaging in deep brain regions can be challenging due to light scattering and absorption. Research efforts could focus on developing techniques that enable high-resolution imaging in deep brain structures with minimal tissue damage.

- **Cell-Type Specific Imaging:**

Achieving cell-type specificity in calcium imaging is essential for dissecting the functional roles of different cell populations within neural circuits. Developing methods to selectively image specific cell types or subcellular compartments can enhance the precision of experimental observations.

- **Standardization and Reproducibility:**

Standardizing protocols and methodologies in calcium imaging can improve the reproducibility of results across different laboratories. Research could focus on establishing best practices and guidelines for experimental design, data acquisition, and analysis.

- **Quantitative Analysis Tools:**

Advanced quantitative analysis tools for calcium imaging data are essential for extracting meaningful information. Developing new algorithms and software for more accurate and efficient analysis of large-scale calcium imaging datasets could be an area of research.

- **Human-Specific Challenges:**

Translating calcium imaging techniques to human studies presents unique challenges, including ethical considerations and technical limitations. Research could explore methods for conducting ethically sound and technically feasible calcium imaging experiments in humans.

CHAPTER-4

PROPOSED MOTHODOLOGY

Methodology for conducting experiment on calcium imaging we use inscopix miniature microscope this will help to fit easily on mice brain and due to its less weight. mice will not feel it heavy and due to extension of connections for this microscope will help mice to be freely move in a path.

Figure4.1 inscopix miniature microscope

This above image shows the miniature microscope this microscope is attached on mice brain and recording is taken through this small tiny miniscope the connector is called miniscope cable which transmit the data this cable is very sensitive if any damage or fold is present in cable then it will transmit noisy data this cables and miniscope should be handled very carefully according to the instruction any scratch in lens will also effect the data and further study this data collection will help in the principle of optics.

There are 3 types of miniscope:

nVista, nVoke, nVue.

The nVista miniaturized microscopy system is a type of epifluorescence microscope which offers recording of neural circuit activity with high speed imaging of GCaMP calcium indicator fluorescence. The light source is a single wavelength LED which illuminates GCaMP-expressing brain cells with 475 nm blue light for system specifications.

The nVoke system offers optogenetic manipulation of neural circuit activity concurrently with high speed imaging of GCaMP calcium indicator fluorescence within the same field of view. The temporal resolution and high brightness of the optogenetic irradiance enable a wide range

of excitation and inhibition protocols. The optical design of the nVoke system virtually eliminates optical cross-talk and ensures high optical coupling efficiency. It integrates two LED light sources to allow for simultaneous or sequential cellular-resolution imaging and optogenetic manipulation within the same field of view in freely behaving mice. There are two LED light sources: the **EX-LED** and **OG-LED**.

- The EX-LED excites GCaMP fluorescence with 455 ± 8 nm blue light.
- The OG LED activates red-shifted opsins with 620 ± 30 nm amber light.

The nVue system allows for high speed imaging of both green (e.g., GCaMP) **and** red (e.g., RCaMP, mCherry) fluorescent indicators, thereby enabling users to image more than one neuronal population. Imaging of these indicators may occur independently or concurrently within the same field of view. The nVue system provides two light sources: (1) a 446-471 nm wavelength LED for imaging green fluorescent indicators (e.g., GCaMP) and (2) a 492-529 nm wavelength LED for imaging red fluorescent indicators (e.g., RCaMP). Please refer to for the system specifications.

Figure 4.2. recording DAQ box

This is a inscopix DAQ box for acquisition of data and store the data this will help to conduct experiment very long time.

Recording cameras are setup to record the external behaviour of animal and In miniature their will be camera to record internal neurons.

CHAPTER-5

OBJECTIVES

The objectives of calcium imaging in the context of neuroscience and cell biology are diverse, reflecting the broad range of questions that researchers aim to address. Here are some common objectives of calcium imaging experiments:

- **Study Neuronal Activity:**

Investigate the patterns and dynamics of neuronal activity in response to various stimuli, such as sensory input, synaptic transmission, or behavioural tasks.

- **Examine Synaptic Transmission:**

Understand the dynamics of synaptic transmission by monitoring calcium changes in presynaptic terminals and postsynaptic neurons. This helps unravel the mechanisms underlying communication between neurons.

- **Explore Neural Circuit Function:**

Uncover the organization and function of neural circuits by simultaneously imaging the activity of multiple neurons within a network. This is crucial for understanding information processing in the brain.

- **Investigate Developmental Processes:**

Examine changes in calcium dynamics during developmental processes, such as neurogenesis, synaptogenesis, and circuit maturation. Calcium imaging can provide insights into how neural circuits form and refine over time.

- **Assess the Impact of Neuromodulators:**

Study the effects of neuromodulators e.g., dopamine, serotonin on calcium signalling to understand their role in modulating neuronal activity and behaviour.

- **Characterize Cellular Responses to Stimuli:**

Investigate how different types of cells, including neurons and glial cells, respond to specific stimuli or pathological conditions. This is important for understanding cellular function in health and disease.

- **Evaluate Plasticity and Learning:**

Examine calcium dynamics associated with synaptic plasticity and learning processes. This helps uncover the cellular mechanisms underlying memory formation and adaptive behaviours.

- **Examine Cellular Pathophysiology:**

Investigate aberrant calcium signalling in disease states, such as neurodegenerative disorders or psychiatric conditions. Calcium imaging can provide insights into cellular dysfunction associated with various pathologies.

- **Screen and Validate Drug Compounds:**

Screen for potential therapeutic compounds by assessing their impact on calcium dynamics. Calcium imaging can be used to validate the efficacy of drugs targeting specific pathways or cellular processes.

- **Optogenetic Integration:**

Combine calcium imaging with optogenetics to manipulate and study specific neuronal populations. This allows for a more comprehensive understanding of the causal relationships between neuronal activity and behavior.

- **Functional Connectivity Mapping:**

Map functional connectivity by assessing synchronized calcium activity across different brain regions. This is essential for understanding how information is transmitted and integrated within the brain.

- **Investigate Cellular Communication in Non-Neuronal Cells:**

Explore calcium signalling in non-neuronal cells, such as immune cells or cardiac cells, to understand cellular communication in diverse physiological contexts.

- **Advance Neurotechnologies:**

Contribute to the development of new neurotechnology and imaging tools, including improved calcium indicators, imaging modalities, and data analysis techniques.

CHAPTER-6

SYSTEM DESIGN & IMPLEMENTATION

1.1 Requirements Analysis:

In this project data is collected through external devices and this data should be analyzed and preprocessed using some techniques. The data is a video data and this data should be processed with deep learning approaches for eg. CNN methods

1.2 System Architecture Design:

In this we use Anaconda Navigator has a platform and we create a new environment in order to use a built in packages and auto ml is also used but this need Linux environment and it was very difficult approach in order to implement so anaconda was best method to achieve our outcomes

1.3 Software Design:

The programming language was Python and MATLAB, this was well suited for our analysis and we used Bokeh plot for our visualization techniques this was very best approach in order to get a each traces for each neurons and this was easy to analyses.

Approaches taken for implementation of software:

1. The machine learning- based software tools mentioned above are integral for the analysis of extensive calcium imaging datasets facilitating in-depth studies. In our laboratory I specifically employed the caiman library for implementation and analysis, leveraging its package description to enhance research capabilities.
2. Carignan, while potentially valuable, posed implementation challenges. Especially considering limited timeframe available, additionally, its development requires individual efforts for each package and need more man force in order to understand and implement it more OS knowledge is also required.
3. Caiman is a package used for both offline processing analysis and online analysis (offline analysis means after experiment data is collected and analyzed using software and online is while conducting experiment itself it will help us to analyze but it has its own challenges too).
4. In this caiman software the first step involves to remove motion artifacts from the given data subsequently the active neurons are extracted as individual pairs of a spatial footprint that describes the shape of each component that projected to the image. Temporal traces that collects its fluorescence activity finally neural activity of each fluorescence is deconvolved from dynamics of calcium indicator.
5. Moving on to next step motion correction caiman used NoRMCoree algorithm that corrects non rigid motion artifacts by estimating motion vectors with subpixel resolution over a set of overlapping patches.
6. Deconvolution is done using a sparse non negative deconvolution and implemented using a near-online OASIS algorithm this algorithm is competitive for its state of art.
7. Online approach is done using a CNN based component detection leading to a major performance improvement.
8. Caiman uses greedy approach to detect and mark ROI that detect neuron based on localized spatiotemporal activity.
9. To gain a deeper understanding of caiman and its implementation, you can refer to the research paper published in National Library of Medicine and paper is caiman an open source tool for scalable calcium imaging data analysis and for codes if require for further studies https://github.com/flatironinstitute/CaImAn here is a GitHub link for further code requirements.

Data collection: For my project we collect data using the inscopix miniscope, which is mounted on mice head and connected to brain region analyzed the behavior of mice for over a time. Subsequently experiment are conducted by allowing a mice in a path and then connected to a device to record the neuron activities of moving mice and this data is then used for analysis of neuron activities so this analysis software is a caiman software that has been developed. Image data is given to the software and this software will automatically gives some required graphs to learn with individual neurons.

Following these steps, a behavior test is conducted to analyze the mice behavior in conjunction with neuron activity over a specific period this recording is external recording of mice parallel to brain recording. This tests provide insights into the correlation between the observed behaviors and corresponding neuronal responses over a time.

Data Analysis: data analysis is an integral component of calcium imaging. Primarily aimed at comprehending spikes or neuron activity. This analysis is crucial for understanding the correlation between individual neurons. By employing data analysis we can generate graphical representation of our experiment facilitating visual understanding. This in turn, enables us to make informed decisions for the progression of further research based on insights derived from analyzed data.

MATLAB based Software: Their also exists MATLAB software completely GUI based, designed for motion correction, ROI detection and visualization of data in graphical representation. With this tools users can simply provide input data and software will correspondingly give output results.

This software is also working in system very beautifully so its more convenient to use both MATLAB based and Machine learning based software.

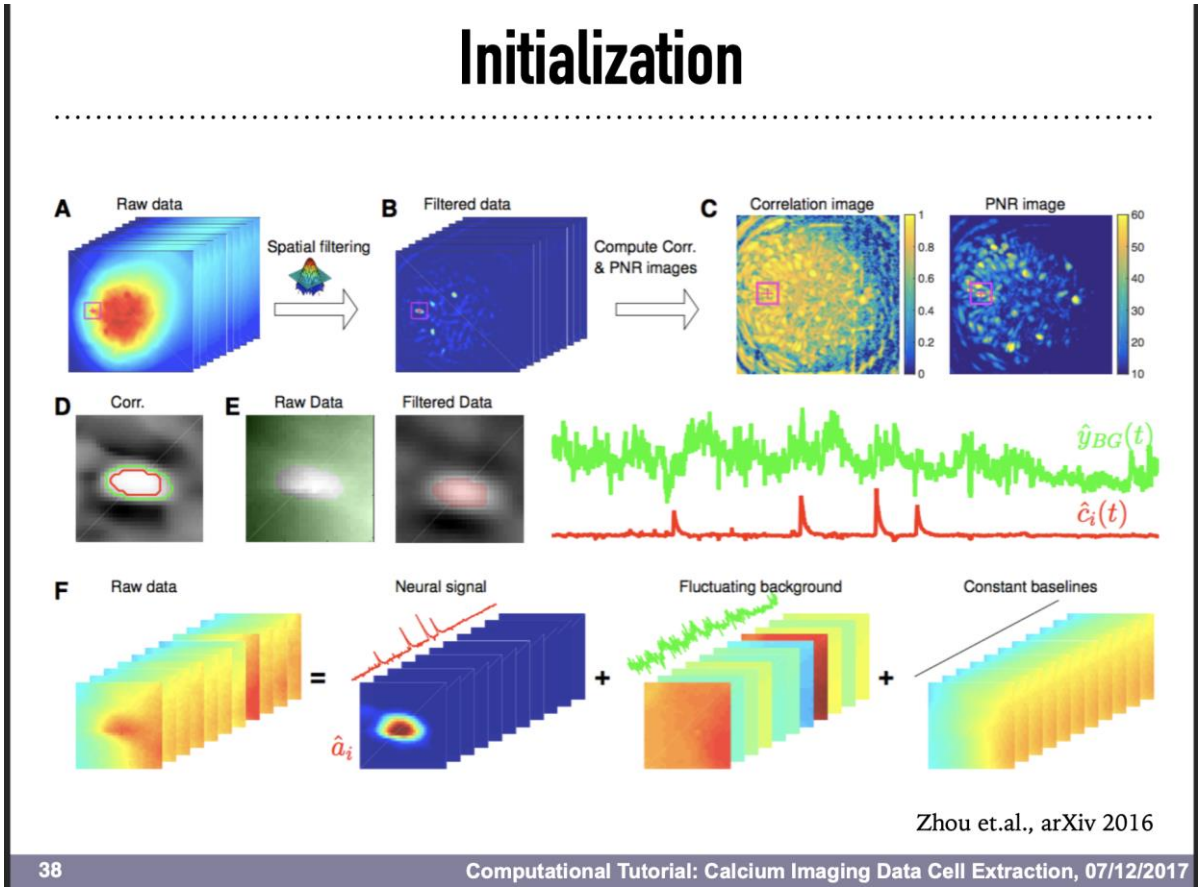


Figure 6.1 CNN based image processing for calcium imaging.

CHAPTER-7

TIMELINE FOR EXECUTION OF PROJECT

(GANTT CHART)

Week 1 : understanding of calcium imaging by reading research papers and through videos and learning it by lab mates.

Week 2: understanding of inscopix approach to do calcium imaging and analyzing the inscopix software by giving different input and collecting results here we got to know that our requirements was different from this software.

Week 3: figuring out new techniques for developing software using python but it was very difficult to understand the parameters so their was lack of information and their was no supportive files to start coding.

Week 4: we found matlab software for motion correction and it was working beautifully but we were doing manual ROI which was time consuming so we decided to develop code to mark this ROI using machine learning method (supervised learning).

Week 5: This packages was developed and their was no proper guidance to install into host system it took very long to install the packages and setting up the system for our needs.

Week 6: Through several research paper and by different labs approach we found some python packages those are pre trained using machine learning by their own data for eg. Caiman was developed in one of laboratory they used 1000 manual marked ROI to train and 240 raw data to test and they done a behavior test and they found it similar to human manual ROI markings and they tested it using different data and approach still they were getting best results so we used that package in our lab to test our data and we found 95% accuracy for 10 raw data.

Week 7: The collected data is analyzed for entire week for different types of data and this data was used for behavior tests and plotted some graphs like heatmap and neuron analysis for spike detection over a time it was working good and decided to improve the existing software based on lab requirements.

Week 8: Further procedure was just conducting research on different packages like CNMFE and AutoML but our system was failed due to absence of linux configuration and system configuration so we dropped those apporahes for time being and just focused with existing software and with further experiment new data is collect and testing to be done to know software approach still it was needed to upgrade basically but requirements was not clear because of experiment time was very slow.

CHAPTER-8

OUTCOMES

CaImAn (Calcium Imaging Analysis) is an open-source software package designed for the analysis of calcium imaging data. It is widely used in the neuroscience community for processing and extracting meaningful information from large-scale calcium imaging datasets. The outcomes of calcium imaging analysis using CaImAn include several key aspects:

- **Spatial Footprints:**

CaImAn identifies spatial footprints, representing the regions of interest (ROIs), corresponding to individual cells or cellular structures in the imaging data. These footprints provide information about the location and size of active regions within the field of view.

- **Temporal Traces:**

For each identified spatial footprint, CaImAn extracts the corresponding temporal trace, representing the calcium dynamics over time. These traces depict the changes in fluorescence intensity associated with cellular activity.

- **Cell Activity Metrics:**

CaImAn calculates various metrics related to cell activity, including spike rates, calcium event detection, and other parameters characterizing the temporal dynamics of individual cells. These metrics help researchers quantify and compare neuronal or cellular responses.

- **Deconvolution of Calcium Signals:**

CaImAn includes algorithms for deconvolving the observed calcium fluorescence signals to estimate the underlying spiking activity of neurons. This deconvolution process aims to provide a more accurate representation of the neural activity.

- **Motion Correction:**

Calcium imaging data may suffer from motion artifacts due to the movement of the specimen or tissue. CaImAn implements motion correction algorithms to align frames, compensating for any shifts or distortions during the imaging process.

- **Automated Analysis and Batch Processing:**

CaImAn is designed for automated analysis, making it suitable for large-scale datasets. It allows for batch processing of multiple imaging sessions, facilitating efficient and consistent analysis across experiments.

- **Interactive Visualization:**

The software provides tools for the interactive visualization of spatial footprints, temporal traces, and other analysis results. This aids researchers in exploring and validating the outcomes of the analysis.

- **Integration with Other Tools:**

CaImAn is often used in conjunction with other neuroscientific tools and software packages. It may integrate with data visualization platforms, statistical analysis tools, and other resources commonly employed in calcium imaging research.

- **Scalability:**

CaImAn is designed to handle large datasets efficiently, making it suitable for experiments with a high density of cells or extended recording durations. This scalability is important for accommodating the growing complexity of calcium imaging studies.

- **Community Support and Documentation:**

CaImAn benefits from an active user community, and ongoing development is supported by regular updates. Documentation and user forums contribute to the accessibility and usability of the software.

CHAPTER-9

RESULTS AND DISCUSSIONS

Discussion:

1. **Interpretation of Results:**

Provide interpretations of the observed spatial footprints and temporal traces. Discuss the implications of identified cellular activity patterns in the context of the experimental conditions and research objectives.

2. **Functional Significance:**

Relate the calcium dynamics metrics to the functional significance of cellular activity. Discuss how the observed patterns contribute to the understanding of neural function, synaptic transmission, or cellular responses to specific stimuli.

3. **Comparison with Literature:**

Compare your findings with existing literature, highlighting similarities or differences in calcium dynamics. Discuss how your results contribute to or challenge current theories or models in the field.

4. **Impact of Experimental Variables:**

Discuss the impact of experimental variables such as stimulation protocols, imaging parameters, or cell types on the observed calcium dynamics. Consider how variations in these variables may influence the results.

5. **Biological Insights:**

Provide biological insights gained from the analysis. Discuss the relevance of the identified cellular activity patterns to the broader understanding of neuronal circuits, cellular communication, or specific physiological processes.

6. **Limitations and Challenges:**

Acknowledge any limitations or challenges encountered during the calcium imaging analysis. Discuss potential sources of variability, experimental constraints, or aspects that may require further investigation.

7. **Future Directions:**

Suggest avenues for future research based on the outcomes of the calcium imaging analysis. Identify unanswered questions, propose additional experiments, or recommend improvements to the methodology.

CHAPTER-10

CONCLUSION

- **Spatial Footprints and Cellular Identities:**

The identification of spatial footprints has allowed us to pinpoint the locations and sizes of active cellular regions within the imaging field. This comprehensive mapping provides a foundation for understanding the spatial organization of neural activity.

- **Temporal Dynamics and Calcium Metrics:**

The analysis of temporal traces has revealed intricate patterns of calcium dynamics over time. Metrics such as spike rates and calcium event detection have quantified the temporal aspects of cellular responses, contributing to a nuanced characterization of cellular activity.

- **Deconvolution and Enhanced Activity Representation:**

Utilizing the deconvolution capabilities of CaImAn has significantly improved the accuracy of representing spiking activity from the observed calcium signals. This enhancement offers a more faithful depiction of the underlying neural activity, providing a clearer understanding of cellular responses.

- **Motion Correction and Data Quality:**

Implementation of robust motion correction algorithms has effectively mitigated motion artifacts, ensuring the integrity of spatial and temporal information. This enhancement is pivotal for maintaining data quality and preserving the accuracy of our imaging results.

REFERENCES

- **Pnevmatikakis, E. A., & Giovannucci, A. (2017). NoRMCorre: An online algorithm for piecewise rigid motion correction of calcium imaging data. Journal of Neuroscience Methods, 291, 83-94.**

- **Chen, T. W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., ... & Kim, D. S. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature, 499(7458), 295-300.**

- **Kaifosh, P., & Losonczy, A. (2016). The sharp wave-ripple in the rodent hippocampus. Journal of Neuroscience, 36(35), 10851-10861.**

- **Mukamel, E. A., Nimmerjahn, A., & Schnitzer, M. J. (2009). Automated analysis of cellular signals from large-scale calcium imaging data. Neuron, 63(6), 747-760.**

- **Dombeck, D. A., Khabbaz, A. N., Collman, F., Adelman, T. L., & Tank, D. W. (2007). Imaging large-scale neural activity with cellular resolution in awake, mobile mice. Neuron, 56(1), 43-57.**

- **Pnevmatikakis, E. A., Soudry, D., Gao, Y., Machado, T. A., Merel, J., Pfau, D., ... & Paninski, L. (2016). Simultaneous denoising, deconvolution, and demixing of calcium imaging data. Neuron, 89(2), 285-299.**

- **Giovannucci, A., Friedrich, J., & Gunn, P. (2019). CaImAn as an open-source tool for scalable calcium imaging data analysis. eLife, 8, e38173.**

- **Pachitariu, M., Stringer, C., Schröder, S., Dipoppa, M., Rossi, L. F., Dalgleish, H., ... & Harris, K. D. (2017). Suite2p: beyond 10,000 neurons with standard two-photon microscopy. BioRxiv.**

- **Chen, X., Mu, Y., Hu, Y., Kuan, A. T., Nikitchenko, M., Randlett, O., ... & Ahrens, M. B. (2018). Brain-wide organization of neuronal activity and convergent sensorimotor transformations in larval zebrafish. Neuron, 100(4), 876-890.**

- **Zhuang, J., Ng, L., Williams, D., Valley, M., Li, Y., Garrett, M., ... & Ng, P. (2017). An extended retinotopic map of mouse cortex. Elife, 6, e18372.**

- **Pnevmatikakis, E. A., & Giovannucci, A. (2017). NoRMCorre: An online algorithm for piecewise rigid motion correction of calcium imaging data. Journal of Neuroscience Methods, 291, 83-94.**

- **Chen, T. W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., ... & Kim, D. S. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature, 499(7458), 295-300.**

- **Kaifosh, P., & Losonczy, A. (2016). The sharp wave-ripple in the rodent hippocampus. Journal of Neuroscience, 36(35), 10851-10861.**

- **Mukamel, E. A., Nimmerjahn, A., & Schnitzer, M. J. (2009). Automated analysis of cellular signals from large-scale calcium imaging data. Neuron, 63(6), 747-760.**

- **Dombeck, D. A., Khabbaz, A. N., Collman, F., Adelman, T. L., & Tank, D. W. (2007). Imaging large-scale neural activity with cellular resolution in awake, mobile mice. Neuron, 56(1), 43-57.**

- **Pnevmatikakis, E. A., Soudry, D., Gao, Y., Machado, T. A., Merel, J., Pfau, D., ... & Paninski, L. (2016). Simultaneous denoising, deconvolution, and demixing of calcium imaging data. Neuron, 89(2), 285-299.**

- **Giovannucci, A., Friedrich, J., & Gunn, P. (2019). CaImAn as an open-source tool for scalable calcium imaging data analysis. eLife, 8, e38173.**

- **Pachitariu, M., Stringer, C., Schröder, S., Dipoppa, M., Rossi, L. F., Dalgleish, H., ... & Harris, K. D. (2017). Suite2p: beyond 10,000 neurons with standard two-photon microscopy. BioRxiv.**

APPENDIX-A

PSUEDOCODE

Calcium imaging analysis software

```
import bokeh.plotting as bpl
import cv2
import glob
from IPython import get_ipython
import logging
import matplotlib.pyplot as plt
import numpy as np
import os

try:
    cv2.setNumThreads(0)
except():
    pass

try:
    if __IPYTHON__:
        get_ipython().run_line_magic('load_ext', 'autoreload')
        get_ipython().run_line_magic('autoreload', '2')
except NameError:
    pass

import caiman as cm
from caiman.motion_correction import MotionCorrect
from caiman.source_extraction.cnmf import cnmf as cnmf
from caiman.source_extraction.cnmf import params as params
from caiman.utils.utils import download_demo
from caiman.utils.visualization import plot_contours, nb_view_patches, nb_plot_contour

bpl.output_notebook()
```

```
logging.basicConfig(format=
                    "%(relativeCreated)12d [%(filename)s:%(funcName)20s():%(lineno)s]
[%(process)d] %(message)s",
                # filename="/tmp/caiman.log",
                level=logging.WARNING)
fnames = ['Sue_2x_3000_40_-46.tif']  # filename to be processed
if fnames[0] in ['Sue_2x_3000_40_-46.tif', 'demoMovie.tif']:
   fnames = [download_demo(fnames[0])]

display_movie = False
if display_movie:
   m_orig = cm.load_movie_chain(fnames)
   ds_ratio = 0.2
   m_orig.resize(1, 1, ds_ratio).play(
      q_max=99.5, fr=30, magnification=2)
# dataset dependent parameters
fr = 30
decay_time = 0.4

strides = (48, 48)
overlaps = (24, 24)
max_shifts = (6,6)
max_deviation_rigid = 3
pw_rigid = True


p = 1
gnb = 2
merge_thr = 0.85
rf = 15
stride_cnmf = 6
K = 4
gSig = [4, 4]
```

```
method_init = 'greedy_roi'
ssub = 1
tsub = 1
min_SNR = 2.0
rval_thr = 0.85
cnn_thr = 0.99
cnn_lowest = 0.1
opts_dict = {'fnames': fnames,
        'fr': fr,
        'decay_time': decay_time,
        'strides': strides,
        'overlaps': overlaps,
        'max_shifts': max_shifts,
        'max_deviation_rigid': max_deviation_rigid,
        'pw_rigid': pw_rigid,
        'p': p,
        'nb': gnb,
        'rf': rf,
        'K': K,
        'gSig': gSig,
        'stride': stride_cnmf,
        'method_init': method_init,
        'rolling_sum': True,
        'only_init': True,
        'ssub': ssub,
        'tsub': tsub,
        'merge_thr': merge_thr,
        'min_SNR': min_SNR,
        'rval_thr': rval_thr,
        'use_cnn': True,
        'min_cnn_thr': cnn_thr,
        'cnn_lowest': cnn_lowest}

opts = params.CNMFParams(params_dict=opts_dict)
```

```
if 'dview' in locals():
    cm.stop_server(dview=dview)
c, dview, n_processes = cm.cluster.setup_cluster(
    backend='multiprocessing', n_processes=None, single_thread=False)
mc = MotionCorrect(fnames, dview=dview, **opts.get_group('motion'))
```

In [ ]:

```
%%capture
#%% Run piecewise-rigid motion correction using NoRMCorre
mc.motion_correct(save_movie=True)
m_els = cm.load(mc.fname_tot_els)
border_to_0 = 0 if mc.border_nan == 'copy' else mc.border_to_0


#%% compare with original movie
display_movie = False
if display_movie:
    m_orig = cm.load_movie_chain(fnames)
    ds_ratio = 0.2
    cm.concatenate([m_orig.resize(1, 1, ds_ratio) - mc.min_mov*mc.nonneg_movie,
                    m_els.resize(1, 1, ds_ratio)],
                   axis=2).play(fr=60, gain=15, magnification=2, offset=0)
```

Memory mapping

```
#%% MEMORY MAPPING
# memory map the file in order 'C'
fname_new = cm.save_memmap(mc.mmap_file, base_name='memmap_', order='C',
                           border_to_0=border_to_0, dview=dview) # exclude borders

# now load the file
Yr, dims, T = cm.load_memmap(fname_new)
images = np.reshape(Yr.T, [T] + list(dims), order='F')
    #load frames in python format (T x X x Y)
```

Now restart the cluster to clean up memory

```
#%% restart cluster to clean up memory
cm.stop_server(dview=dview)
c, dview, n_processes = cm.cluster.setup_cluster(
    backend='multiprocessing', n_processes=None, single_thread=False)
cnm = cnmf.CNMF(n_processes, params=opts, dview=dview)
cnm = cnm.fit(images)

# cnm1 = cnmf.CNMF(n_processes, params=opts, dview=dview)
# cnm1.fit_file(motion_correct=True)

#%% plot contours of found components
Cn = cm.local_correlations(images.transpose(1,2,0))
Cn[np.isnan(Cn)] = 0
cnm.estimates.plot_contours_nb(img=Cn)
%%capture
#%% RE-RUN seeded CNMF on accepted patches to refine and perform deconvolution
cnm2 = cnm.refit(images, dview=dview)
```

Component Evaluation

In [ ]:

```
cnm2.estimates.evaluate_components(images, cnm2.params, dview=dview)
```

Plot contours of selected and rejected components

In [ ]:

```
#%% PLOT COMPONENTS
cnm2.estimates.plot_contours_nb(img=Cn, idx=cnm2.estimates.idx_components);

# accepted components
cnm2.estimates.nb_view_components(img=Cn, idx=cnm2.estimates.idx_components);

# rejected components
if len(cnm2.estimates.idx_components_bad) > 0:
    cnm2.estimates.nb_view_components(img=Cn,
idx=cnm2.estimates.idx_components_bad)
```

```
else:
    print("No components were rejected.")
```

Extract DF/F values

```
#%% Extract DF/F values
cnm2.estimates.detrend_df_f(quantileMin=8, frames_window=250);
```

Select only high quality components

```
cnm2.estimates.select_components(use_object=True);
```

Display final results

```
cnm2.estimates.nb_view_components(img=Cn, denoised_color='red')
print('you may need to change the data rate to generate this one: use jupyter notebook --
NotebookApp.iopub_data_rate_limit=1.0e10 before opening jupyter notebook')
```

Closing, saving, and creating denoised version

You can save an hdf5 file with all the fields of the cnmf object

```
save_results = False
if save_results:
    cnm2.save('analysis_results.hdf5')
```

Stop cluster and clean up log files

```
#%% STOP CLUSTER and clean up log files
cm.stop_server(dview=dview)
log_files = glob.glob('*_LOG_*')
for log_file in log_files:
    os.remove(log_file)
```

View movie with the results

We can inspect the denoised results by reconstructing the movie and playing alongside the original data and the resulting (amplified) residual movie

```
cnm2.estimates.play_movie(images, q_max=99.9, gain_res=2,
                          magnification=2,
```

```
                    bpx=border_to_0,
                    include_bck=False);
```

The denoised movie can also be explicitly constructed using:

```
#%% reconstruct denoised movie
denoised = cm.movie(cnm2.estimates.A.dot(cnm2.estimates.C) + \
                cnm2.estimates.b.dot(cnm2.estimates.f)).reshape(dims + (-1,),
order='F').transpose([2, 0, 1])
```

This is the basic code for calcium imaging analysis and this was deployed directly into host system so this is basic copy of code the original changes and requirements are done and deployed directly into host system.

APPENDIX-B

SCREENSHOTS

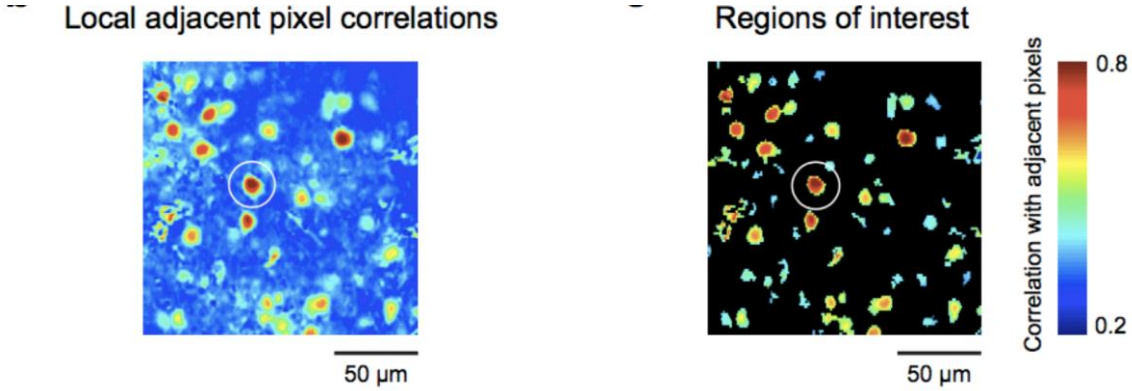


Figure B.1 ROI for basic analysis

The above image shows the ROI marked for whole and then best neurons are selected from the image the below images also show how each neurons are marked for whole image and this mapping will be used for further analysis which you can see in below figure

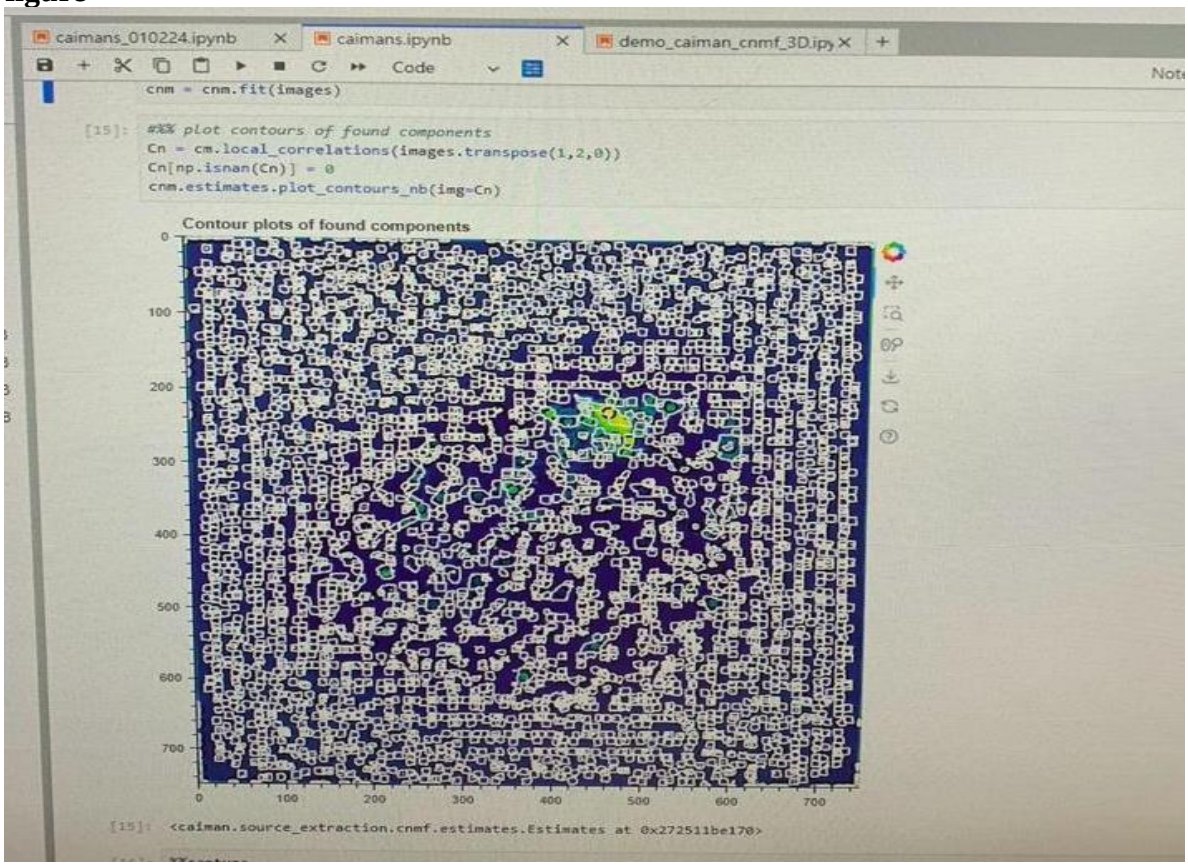
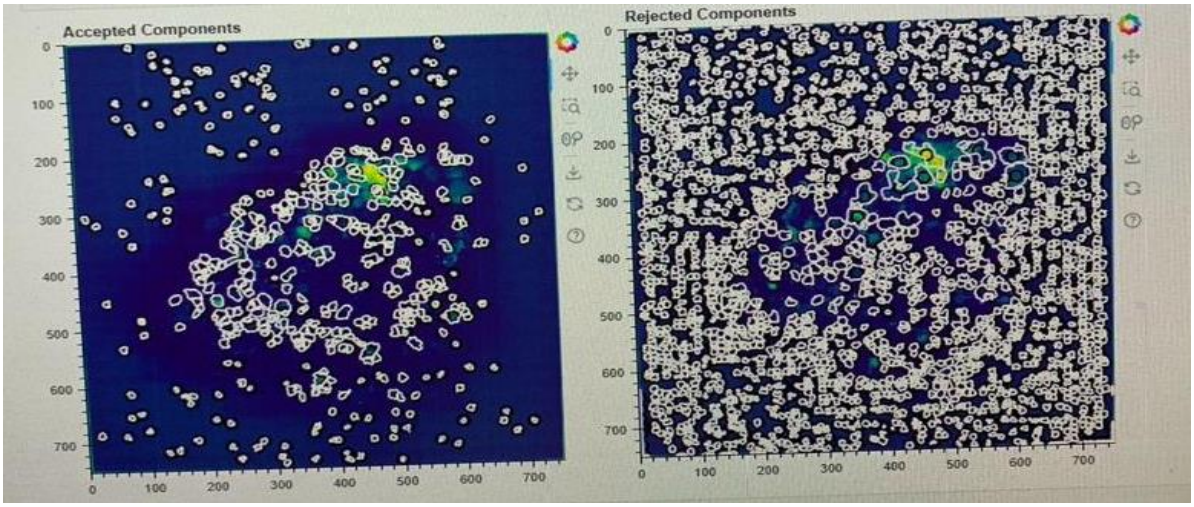
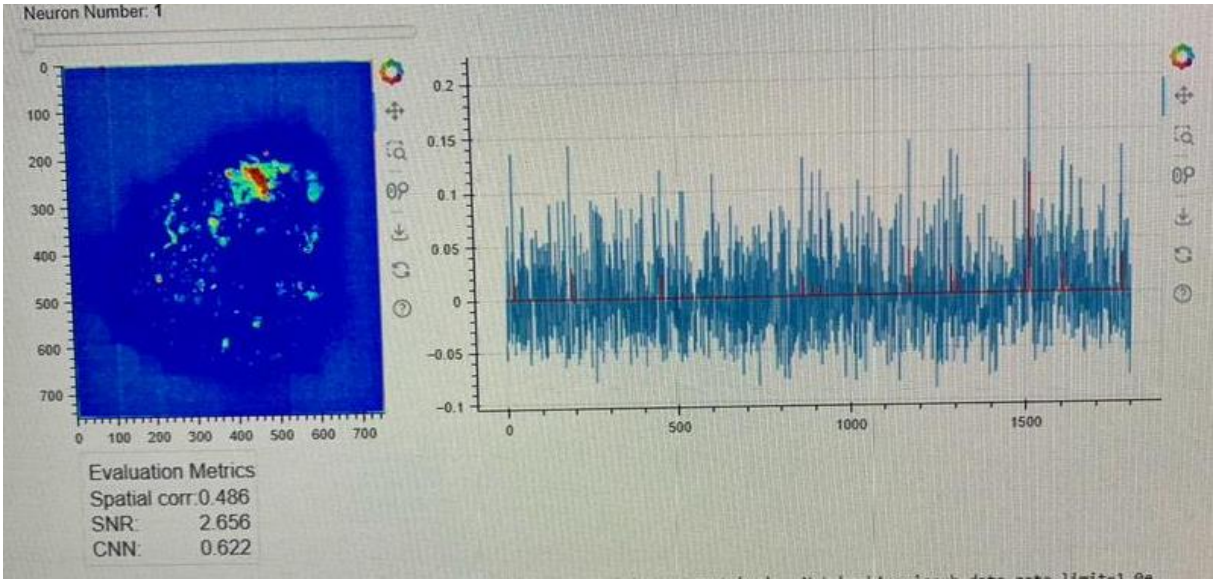


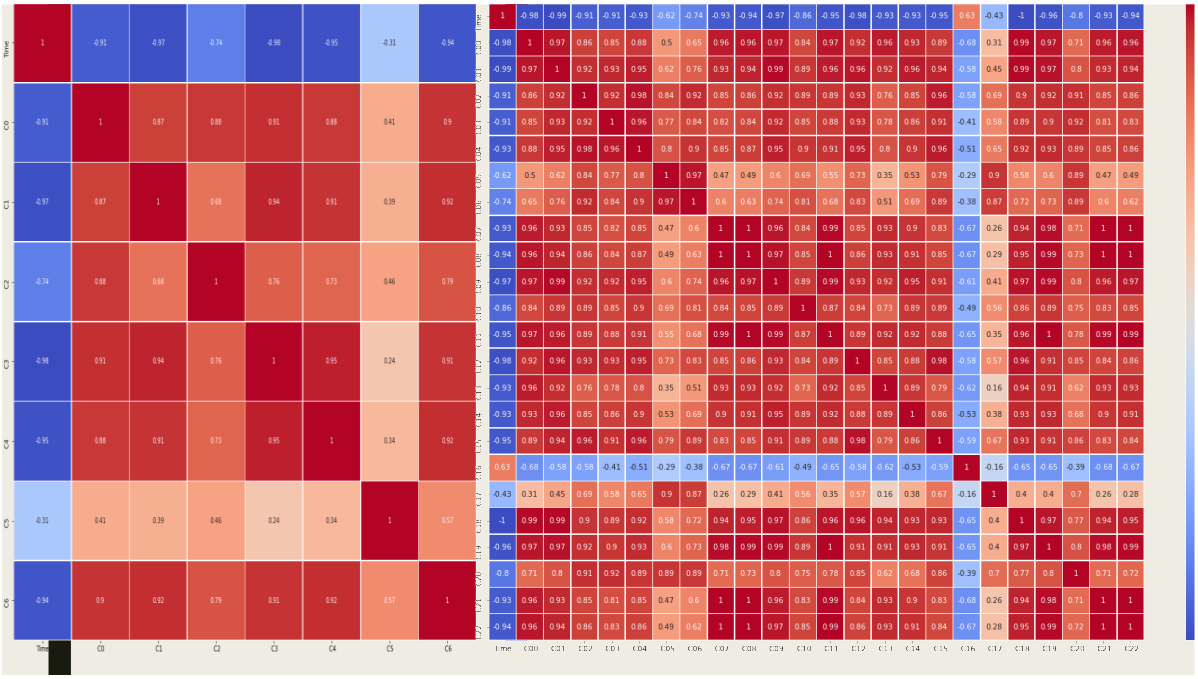
Figure B.2 shows the original image of mapping for data collected in a lab

In below figure shows the accepted and rejected components separately and this data can be downloaded easily this is a beauty of bokeh plotting which helps for research studies.

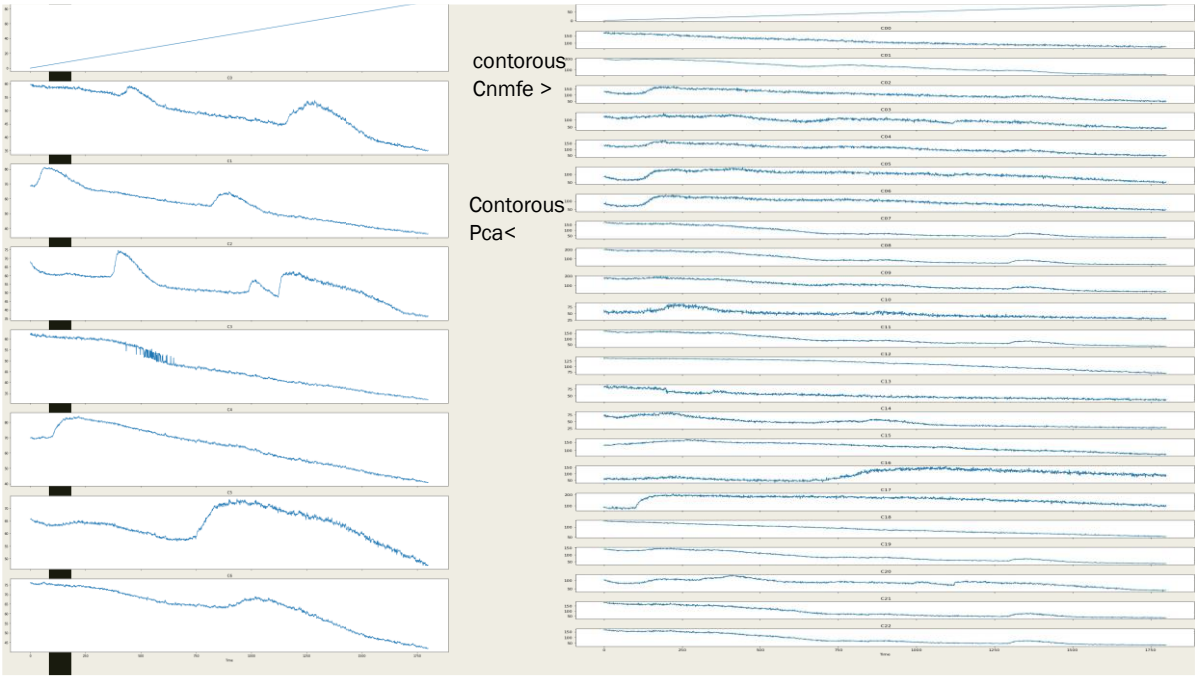




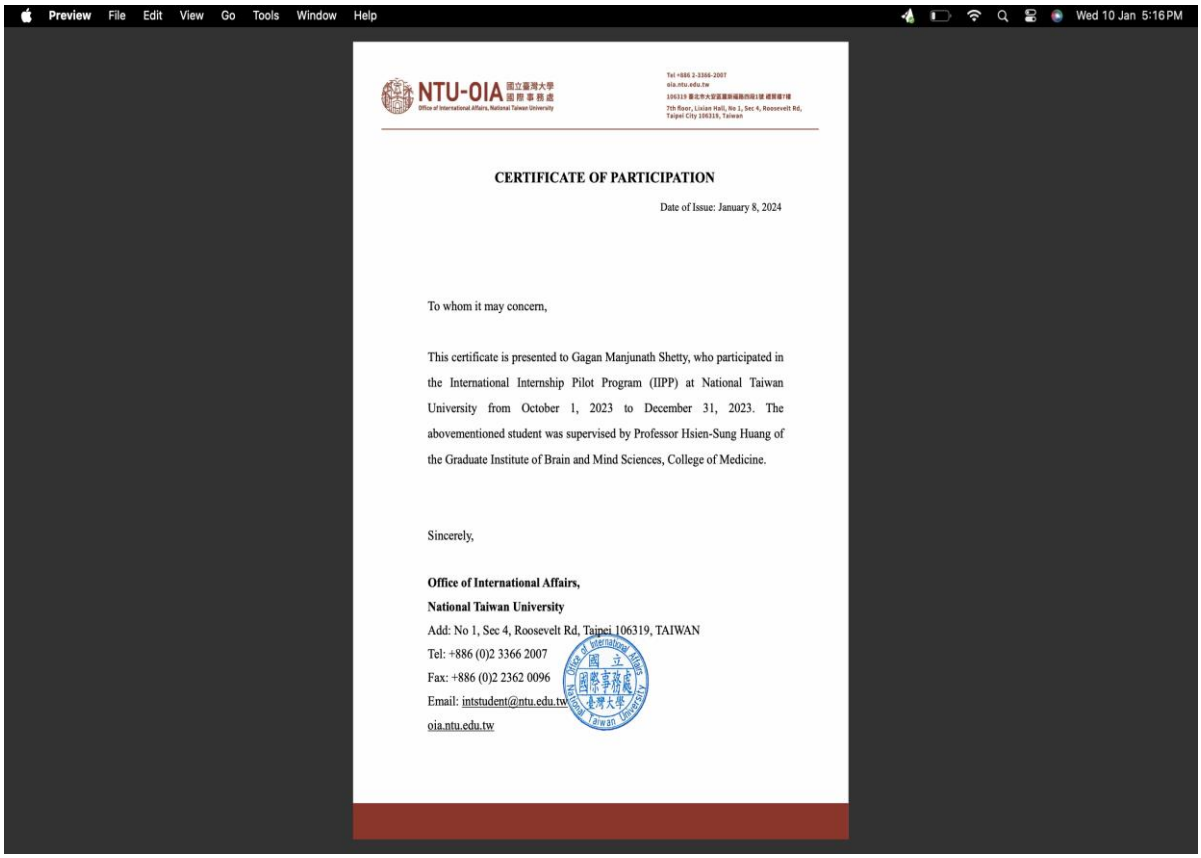
In this figure it shows neuron number 1 and it will also show the graph for neuron behaviour over a period of time this will help to know at what time spike has occurred and this will help to analyse the behavior of mice.



This above figure shows two different data heatmap plotting and below example shows the each neuron behavior for a period of time .



Certificate from National Taiwan university



NTU-OIA 國立臺灣大學 國際事務處
Office of International Affairs, National Taiwan University

Tel:+886 2-3366-2007
oia.ntu.edu.tw
106319 臺北市大安區羅斯福路四段1號 總辦事7樓
7th floor, Linlan Hall, No 1, Sec 4, Roosevelt Rd, Taipei City 106319, Taiwan

CERTIFICATE OF PARTICIPATION

Date of Issue: January 8, 2024

To whom it may concern,

This certificate is presented to Gagan Manjunath Shetty, who participated in the International Internship Pilot Program (IIPP) at National Taiwan University from October 1, 2023 to December 31, 2023. The abovementioned student was supervised by Professor Hsien-Sung Huang of the Graduate Institute of Brain and Mind Sciences, College of Medicine.

Sincerely,

Office of International Affairs,
National Taiwan University
Add: No 1, Sec 4, Roosevelt Rd, Taipei 106319, TAIWAN
Tel: +886 (0)2 3366 2007
Fax: +886 (0)2 2362 0096
Email: intstudent@ntu.edu.tw
oia.ntu.edu.tw