Bridging In-Vitro Neural Dynamics and Real-World Applications: Experimental, Analytical, and Computational Approaches in Biocomputing

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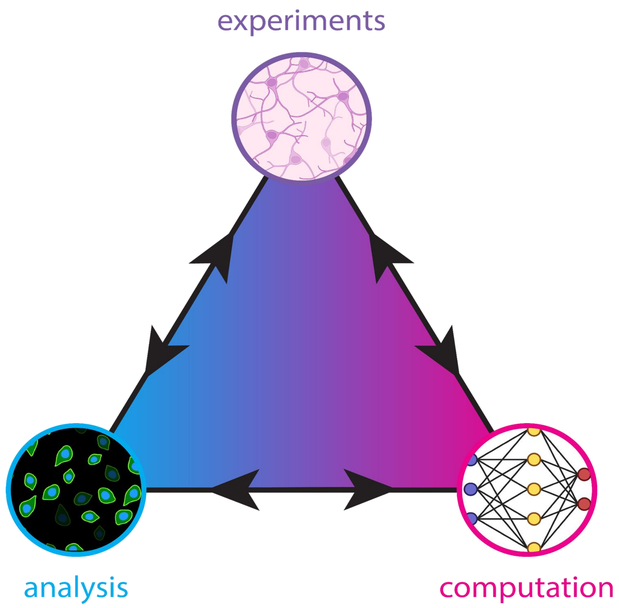
Project Overview

Introduction

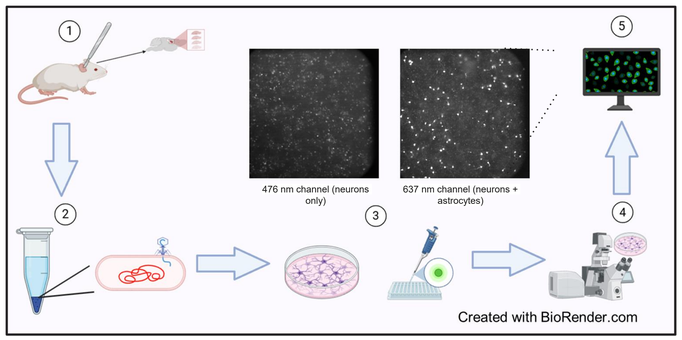
Our project is a collaboration between the Army Research Laboratory and the Losert Laboratory at the University of Maryland, focused on understanding living neural networks in the context of biocomputing. Our project addresses three major aspects of this field- experimentation, analysis, and computation. We developed novel experiments to better understand the impact of astrocytes on living neural network communication, a more robust analysis pipeline for processing experimental data, and worked on developing advanced bio-inspired algorithms for defense applications, based on living neural networks.

Overview

- The overarching **objective** of our project is to further **probe the inner workings of the living neural networks** that make up our Biocomputing Platform.
- We are accomplishing this goal via **experimentation on cell cultures, analysis of the associated data, and further development of a bio-inspired algorithm.**
- We are conducting **stimulation experiments** to leverage the computing power of neurons and astrocytes and **developing methods to** better **analyze experimental data.**
- Once data is captured from the experiments, it will be analyzed by processing software like CaImAn to **break down** the movie **data into** computationally useful formats, like **calcium traces**, obtained **by examining the fluorescence (brightness) of neurons** infected by virus that increase fluorescence when neurons are activated.
- An important aspect of the pipeline is **taking the** raw fluorescence **data and extracting peaks in the data**, which are areas where the neuron has spiked. This can be done through the use of denoising filters and deconvolution.
- Our algorithm development builds on these findings, using ideas from biology to build a high-performing sensor fusion and drift detection model.



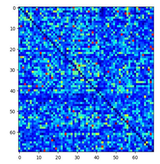
Experimental Methodology



(1) Cells dissected and plated from E18 rat cortex and seeded at 1.5M cells/ dish **(2)** Cells transduced with custom lentiviral vector at 3MOI (multiplicity of infection) on DIV (Days *in virto*) 3-5 for 48 hours **(3)** Cells incubated with 1uL of Calbryte© 630 fluorescent dye for 1 hour **(4)** Cells imaged on Nikon Ti2 microscope and stimulated; stimulation recordings involved taking two videos: a 1 minute video in the 476 nm channel at 20 Hz and an 8 minute video in the 637 nm channel at 20 Hz with a 4 minute global stimulation of 15 milliseconds pulse duration **(5)** Fluorescent data collected as a DMT file; by subtracting the neuron calcium data from the neuron+astrocyte calcium data, astrocyte data is retrieved; subsequent data denoising, bleach correction and deconvolution performed offline

Data Analysis

Understanding Functional Relationships Between Neurons

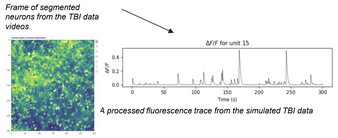


A functional connectivity matrix displaying the relationships between neurons (the axes numbers represent the neuron trace number) where darker blue indicates a strong relationship

We developed a Logistic Regression based method for inferring, from a population of neurons and their activity data, whether (and how strongly) one neuron predicts the activity of another.
Pictured on the left is a matrix of the top 25% of neurons in terms of activity and their relations to each other where a darker blue represents a stronger predictive power. This modeling helps us understand how neuronal systems interact internally (A. Sheikhattar, 2018).

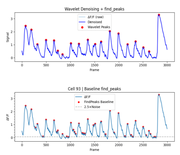
Processing of Simulated TBI Data

We modified existing analysis pipelines to begin processing videos of neurons that have been stretched to simulate traumatic brain injuries (TBIs). The objective is to extract trace activity data from these recordings to understand and quantify the impact of TBIs.



A processed fluorescence trace from the simulated TBI data

Peak Detection



The bottom figure shows peak identification (displayed by the red dots) using the baseline SciPy find_peaks method and the top figure shows peak identification following the application of wavelet denoising, more robust to noise in the data

We experimented with new peak detection methods like template matching (matching expected peak shapes with traces to identify peaks) as well as other algorithms like CASCADE (a deep-learning based method). In an effort to deal with the noise that often accompanies calcium trace data (due to experimental errors) we also applied different types of denoising methods such as Savitzky-Golay smoothing and wavelet-based smoothing to deal with noise in traces. The application of wavelet denoising is shown to the left.

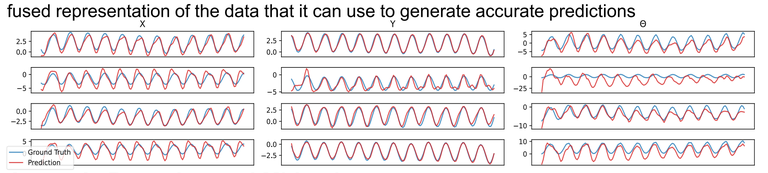
Bio-inspired Computation

Overview

The final line-of-research pursued was bio-inspired algorithm development. We extended the ideas of the rhythmic sharing algorithm in (Kang, 2025) to anomaly detection and sensor fusion, applying it to datasets provided by Lockheed Martin. The datasets are created from a simulation of 4 aircraft tracking a target.

Multi-Sensor Fusion

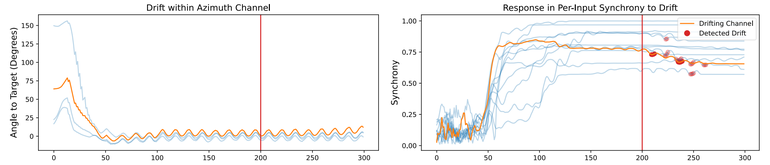
The dataset includes X, Y, and target azimuth data for each of the tracking aircraft. The bio-inspired algorithm learns the underlying dynamics of these channels, internally developing a fused representation of the data that it can use to generate accurate predictions



Anomaly Detection and Mitigation

While (H. Kang, 2025) showed that shifts across all channels can be easily detected with their rhythmic sharing algorithm by tracking the synchronization between nodes, we extended their work to develop a per-channel synchronization metric. This metric is highly sensitive to drifts within each individual channel, shown below.

Given the ability to predict almost-unnoticeable drifts within a single input channel, and our ability to fuse the dynamics of each input channel, the next goal of the project is to mitigate the anomaly by predicting only the anomalous channel using the dynamics of the other channels



Conclusion and Future Directions

Conclusions

The three research lines presented here show how the NSSSIP team tackles questions in biocomputing using three different approaches: experiments, analysis, and computation. Our experimental work focuses on novel techniques to stimulate understudied elements of living neural networks, like astrocyte cells. Our analytical work takes experimental data, like physical simulations of traumatic brain injuries, and uses state-of-the-art techniques to track, monitor, and map the connections between neurons to understand their computational mechanisms. Finally, our computational work combines our experimental and analytical learnings to build novel, neurologically inspired algorithms that solve real-world problems for our partners. By combining these foci, this work shows the role neuroscience research plays in our understanding of the brain and in developing a competitive edge for the warfighter.

Future Directions

Experimentally, the next step is to examine how the “fast” and “slow” nodes of the living neural network work together to accomplish biocomputing tasks. Analytically, we plan to continue analyzing the data on neuronal adaptation to the simulated traumatic brain injuries, applying different algorithms through the lens of the small-world networks found within the full human connectome. Computationally, we plan to improve the detection of changes to the synchronization of the input channels, which is central to our algorithm’s detection of anomalies. We want to continue applying the algorithm to challenging data from Lockheed Martin in addition to academic benchmarks to understand its performance.

FOR FURTHER INFORMATION:

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