An Adaptable Machine Vision Package for Whole Brain Cellular Resolution Neural Activity Analysis in Freely Navigating Caenorhabditis Elegans

SUYING JIN and Katsushi Arisaka

UCLA, *Elegant Mind Club* @ Department of Physics and Astronomy

http://www.elegantmind.org

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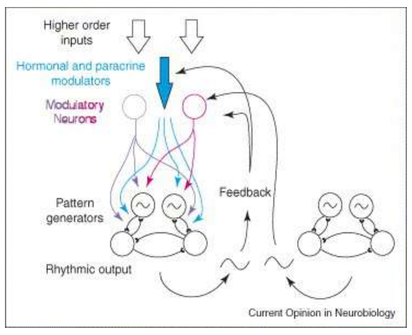
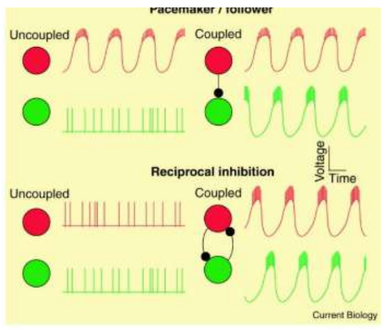
ABSTRACT

- Recent advances in bio-imaging have made large scale recordings of neural activity at cellular resolution possible; providing the unprecedented opportunity to observe the dynamic activity of the entire nervous system in *Caenorhabditis elegans*.
- Calcium imaging data has in the past been limited to immobilized animals. In order to study the full scope of the complex transformation of sensory inputs to appropriate behaviors, the sample must be allowed to freely behave under a variety of controlled stimulations, at which point the challenge of detecting of moving objects is encountered--an active field in computer vision research.
- We have developed a lightweight software package capable of detecting and quantifying calcium dynamics in freely navigating *C. elegans taking* a global optimization approach to motion tracking.
- We make no presumption on the hardware setup of the user with the hopes of increasing accessibility, compatibility and applicability for any future neurodynamic investigations.

SCIENTIFIC MOTIVATION

- How consciousness emerges from the electrical activity of networks of neurons is one of nature's greatest mysteries. The Arisaka Lab takes a physicist's approach to neuroscience by seeking fundamental principles of nervous system function. For this approach, the utility of C. Elegans is unparalled as the simplest possible nervous system exhibiting proto-conscious behavior.
- We are in particular interested in the neural mechanism by which sensory signals are converted by the brain into a perception of external space that informs appropriate motor decisions. This is highly nontrivial, considering sensory signals are functions of time carrying no inherent spatial information.



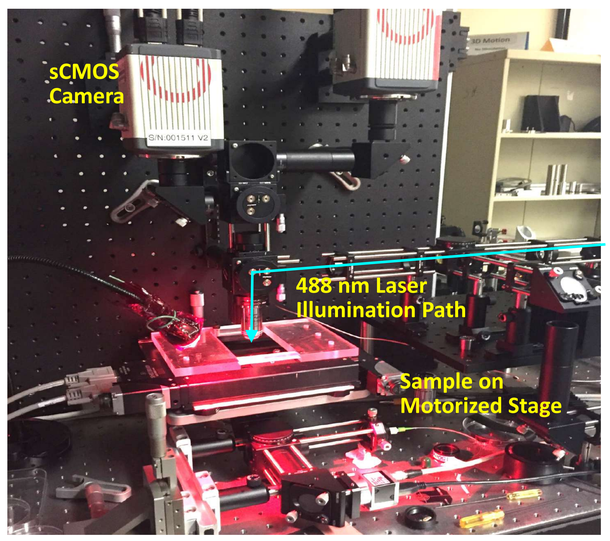


Left: Schematic of the function of a central pattern generator circuit, taken from [4] Right: Two possible forms for a CPG. Taken from [5]

- We believe the fundamental mechanism by which such sensorimotor integration occurs is by utilizing inherently oscillatory networks of cells called CPGs to synchronize and coordinate incoming sensory signals with spatial context provided by the animal's motor actions (corollary discharge).
- Our investigations of the connectome have revealed candidate neuron groups which have the requisite connections, and it remains to observe this neural signal and explore the dynamics of its role in organizing the transformation of sensory signals into appropriate motor actions by watching the neural activity of C. Elegans under controlled stimulation and free response behavior.

EXPERIMENTAL APPROACH

- Neural activity produces rapid changes in calcium ion concentrations within the cell, and this fact is exploited by inserting a gene which produces a fluorescent protein calcium indicator, and this fluorescence is what is picked up by a microscope.
- Strain QW1217 expressing GCAMP6 and RFP (static nuclear markers) is used. Experimental setup pictured below.

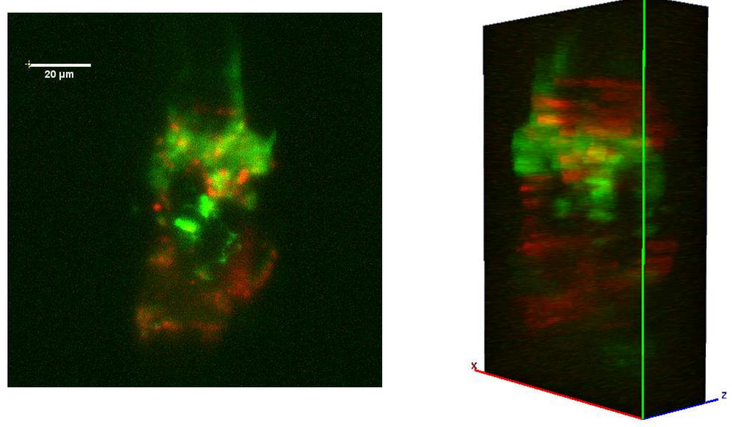


EXTRACTION OF NEURAL ACTIVITY

Raw Data Examples and Detection Demonstration

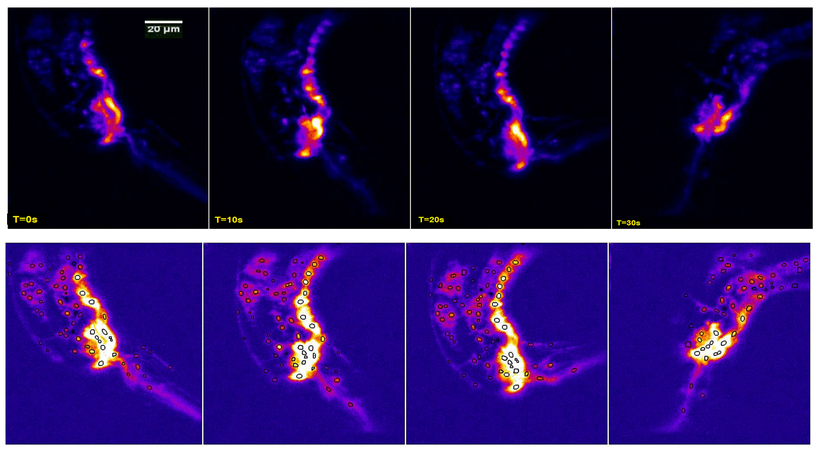
SLM BB system: 2-channel, volume scanning data, no tracking

Left: Maximum intensity projection, Right: Volume rendered



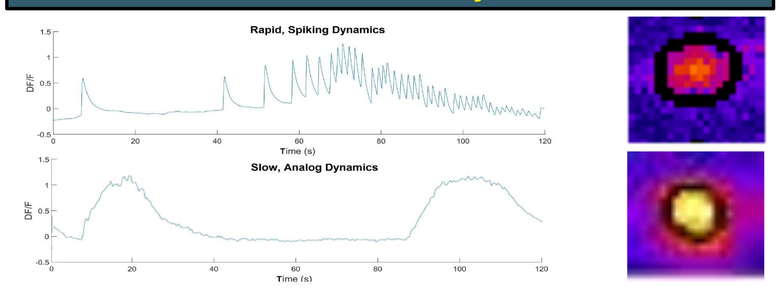
Line Confocal System: volume scanning data, with motion tracking.

Maximum intensity projections shown of GCAMP dynamics

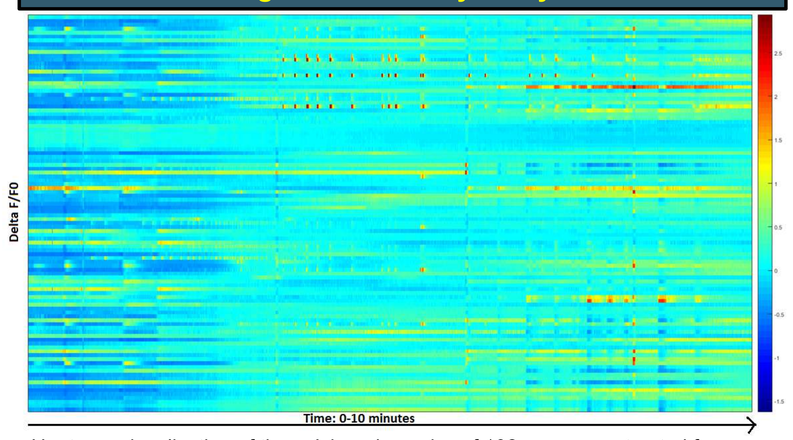


Demonstration of detection algorithm on moving worm

Cellular Resolution Dynamics

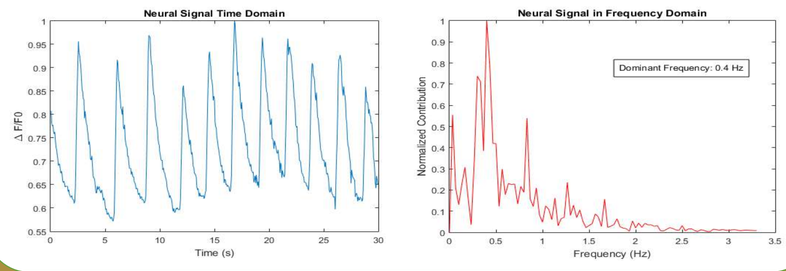


Large Scale Activity Analysis

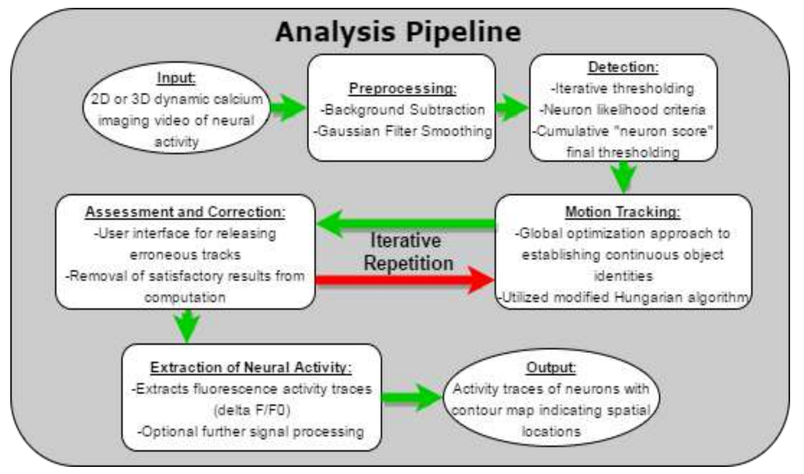


Heatmap visualization of the calcium dynamics of 102 neurons extracted from an immobilized worm.

Spectral Decomposition

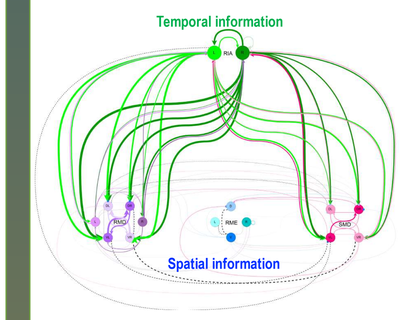


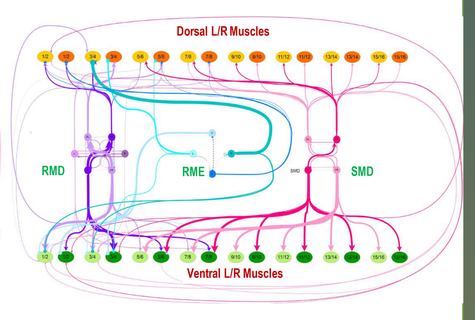
ALGORITHM DESIGN



SCIENTIFIC IMPLICATIONS

- In order to test our theories on the role of the CPG in sensorimotor integration, we must observe the neural dynamics while each part of this process is occurring in C. elegans. That is, we must explicitly observe the entire nervous system while controlled stimulations are delivered, and the animal is allowed to freely respond.
- This required an optical system capable of tracking the worm while it is freely moving, and as presented here, software capable of analyzing the neural dynamics from a freely behavior sample is a key part of opening this scientific possibility





FUTURE DIRECTIONS

- Although the connectome (identities of all 302 neurons and their mutual connections) has been known for decades [5], there is still no efficient way of identifying the neurons from imaging data. The next step is to implement an automated identification procedure based on a similar global optimization approach.
- We aim to apply the software to analyze data taken of the worm while it is responding to well controlled stimuli for which the worm has very characteristic behavioral responses.
- We can then correlate the patterns of neural activity at each known neuron at a given time with the corresponding stimulation input, processing, and response and piece together the story of sensory signals are transformed into appropriate motor action by the mysterious network action of the brain.

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