A Novel Worm Tracking Calcium Imaging System Utilizing a Mobile Microscope

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Introduction/Objectives

- Our main objective is to create a microscope platform that can track *Caenorhabditis elegans* in real time under a variety of stimulations, to observe their neural behavior. We hope to run novel experiments under various conditions such that we learn more about how *C. elegans* behaves and its corresponding neural network during those behaviors.
- In addition, we are interested in the relationship between neural activity and locomotion of *C. elegans* in a variety of conditions, such as temperature.
- Our microscope is unique because the microscope itself moves on an x-y platform as opposed to other systems where the sample platform is moved. This eliminates artifacts due to stage movement.

Specifications and Design

- Setup consists of three cameras all synchronized to 15 fps via an external trigger signal. The entire tracking program is controlled via LabView.
- Two florescence cameras capture neural images of CFP and YFP, for cameleon data taking
- A third camera captures the entire worm body, for behavioral analysis.
- A white halogen lamp is filtered to only allow wavelength of 433nm to illuminate the sample.
- Image is magnified by a long working distance 20X objective lens.

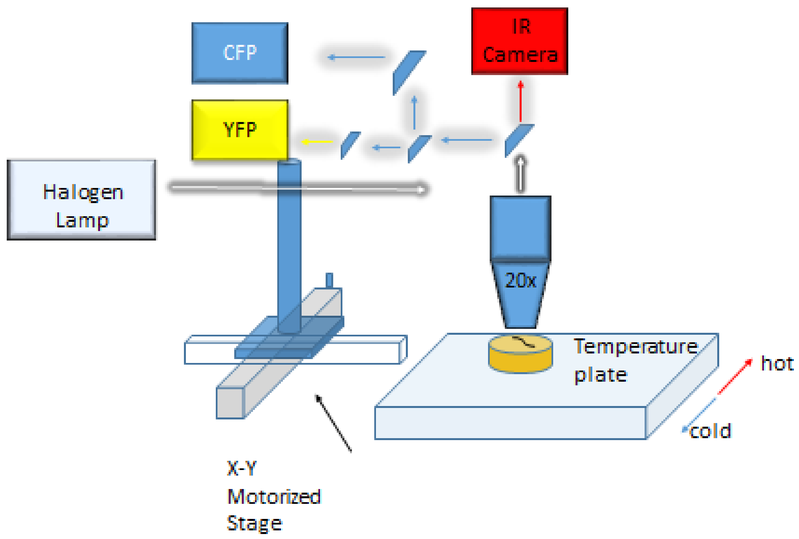

Figure 1: Schematic of Worm tracking microscope showing respective cameras and optical path. The light travels from the halogen lamp, passing through a filter of 433nm, then going into the 20x objective. The light reflecting off the worm and the fluorescence signal go through the same objective, travelling through a variety of filters until they reach the camera each with their respective wavelengths. All optical components are mounted to a motorized x-y stage, allowing the microscope to track the worm without moving the sample stage. Schematic is also shown with a temperature plate setup.

Figures and Results

Our microscope is capable of taking a 0.66mm x 0.66 mm field of view with 20x magnification, enabling us to visualize the soma and dendrite of a single neuron. Show below are images of AIY interneuron of Cameleon labeled *C. elegans*.

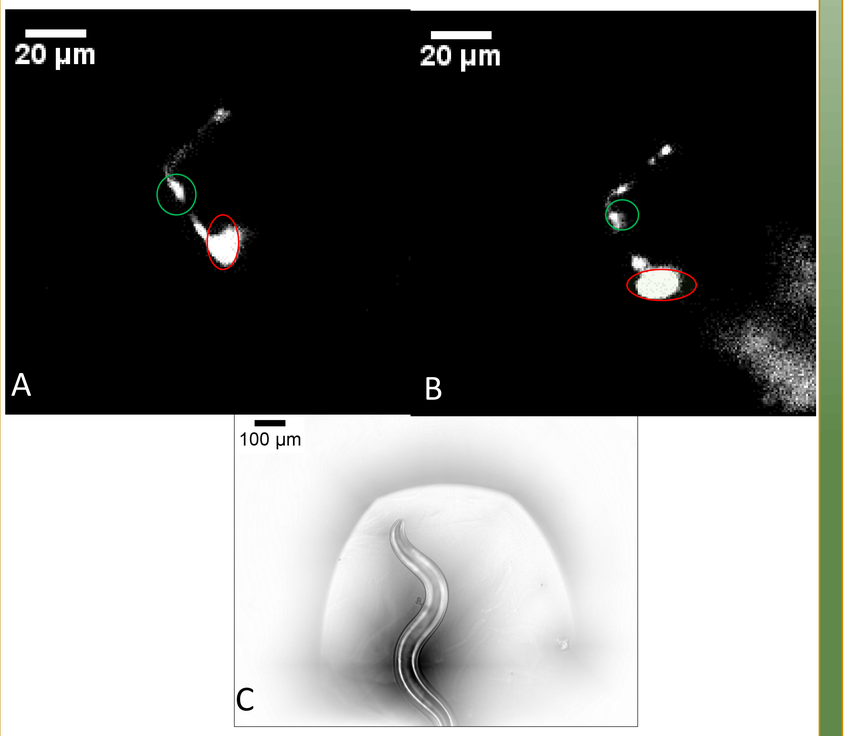

Figure 2: (A-C) Three pictures from each of the three cameras used in the real time worm tracker. A.) CFP channel image. B.) YFP channel image . C.) IR camera image. All three pictures were post processed to maximize contrast. All three are of the same worm taken at the same time. Red indicates soma, green the dendrite.

Graphs below represents preliminary data taken from a linear temperature gradient during isothermal behavior. The graphs were generated by taking the ratios of YFP/CFP, indicating neural activities in AIY, and plotted with x-y coordinates of the microscope movement and worm body.

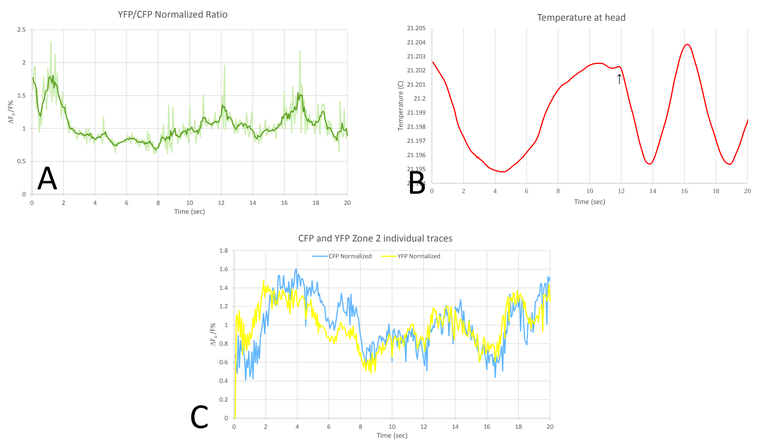

Figure 3: (A-C) Graphs of some preliminary data with AIY. Data show possible relation between temperature sensed and AIY activity. A.) AIY activity graph from dendrite (green area in fluorescence image. B.) Temperature at head location. C.) YFP and CFP individual traces

Current Status and Improvements

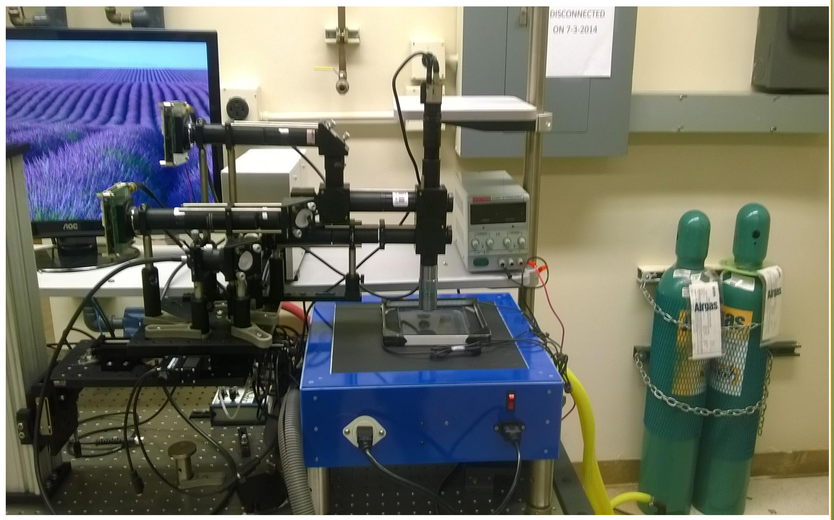

Figure 4: Our worm tracker setup shown with our thermotaxis setup using a linear temperature plate. The current set up is shown with 20x magnification.

- Our current setup can track one neuron at 15 fps, while taking three pictures simultaneously. As long as the microscope is in focus, our current setup can take data for several minutes. Our best data is taken for a time period of 30 seconds.
- Its current FOV with 20x using the fluorescence images is 666mm, with the 2048 resolution it has a resolution of 300 nm in principle.
- Can also be modified to 10x to image the entire worm in fluorescence camera, for future experiments with multiple neurons.
- Current system has manual focus to enable refocusing due to an uneven stage.

Conclusions/Future Directions

- We have completed the first trials of real time worm tracking on a linear temperature gradient without moving the stage, which is an experiment that has not been achieved before.
- Next step is to incorporate concept of worm tracking with advanced fluorescence imaging techniques, such as light field microscope to observe the whole brain of *C elegans* while it is freely navigating.

Reference

- Zhaoyu Li, Jie Liu, Maohua Zheng, X.Z. Shawn Xu, Encoding of Both Analog- and Digital-like Behavioral Outputs by One C. elegans Interneuron, Cell, Volume 159, Issue 4, 6 November 2014, Pages 751-765, ISSN 0092-8674,

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