Tracked Volumetric Neural Imaging of Caenorhabditis elegans Under Two Dimensional Free Motion And Photon Stimulated Conditions

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ABSTRACT

Understanding the neural networks of complex organisms, such as a human's, must began with a complete interpretation of the dynamics exhibited by a simpler organism like *C. elegans*. Through the design of a line-confocal microscope, we aim to observe *C. elegans* traverse a two dimensional plane atop agar and gelatin based plates with varying concentrations. After observing free motion behavior, we will take our study further and stimulate *C. elegans'* ASJ photoreceptor and AIY thermoreceptor with 405nm and 1490nm laser beams, respectively. Coupled with the line-confocal system, two dimensional free motion worm tracking should allow for the better understanding of the neural patterns of *C. elegan.*

INTRODUCTION

With 302 neurons, *C. elegans* is a very simple organism compared to say mice or humans. Therefore, if we aim to understand with full capacity what is consciousness and for what reasons it arises in nature, we must begin an organism like *C. elegans.* To study this worm, we have independently constructed three system, a line-confocal microscope, a two dimensional worm tracker equipped with 405nm and 1490nm photon stimulation lasers, and a thermal gradient temperature plate. When combined this scientific tool will be powerful enough to observe all 302 neurons of while the worm is subject to external stimulations.

OBJECTIVES

- View the neural network of C. elegans in three dimensions while in tracked two dimensional free motion.
- Use offline data analysis to prove that C. elegans is indeed a conscious being that exhibits more than biased random locomotion.
- Simulate virtual realities using 1490nm and 405nm lasers and observe neural pattern differences from free-motion behavior.
- Observe isothermal behavior employing a linear temperature gradient plate.

MATERIALS & METHODS

- Line-confocal microscope with sub 500nm resolution for neuron observation.
- Two-dimensional worm tracking hardware/software.
  - XY translation Zaber Stage.
  - Automatic and manual operation using LabVIEW
- Throlabs 40mW 405nm and 140mW 1490m lasers OEMs.
  - TTL signal modulation
  - Laser diode analog current modulation
- Detachable linear temperature gradient plate.
  - Peltier effect cooling and heating.

LINE-CONFOCAL MICROSCOPE

- Light sheet is produced by scanning a focused line produced by a cylindrical lens at the focal plane of an objective.
- Laser line scanning is performed by galvo and synchronized with Hamamastu Flash 4.0 V2 rolling shutter camera.
- Piezo objective controller is utilized to scan in detection axis to produce three dimensional image.

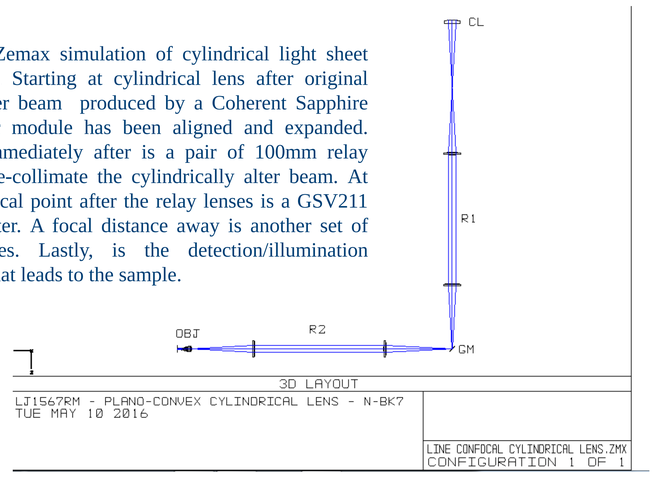

Figure 1: Zemax simulation of cylindrical light sheet beam path. Starting at cylindrical lens after original 488nm laser beam produced by a Coherent Sapphire OEM laser module has been aligned and expanded. Located immediately after is a pair of 100mm relay lenses to re-collimate the cylindrically alter beam. At the back focal point after the relay lenses is a GSV211 golvonameter. A focal distance away is another set of relay lenses. Lastly, is the detection/illumination objective that leads to the sample.

LINE-CONFOCAL RESULTS

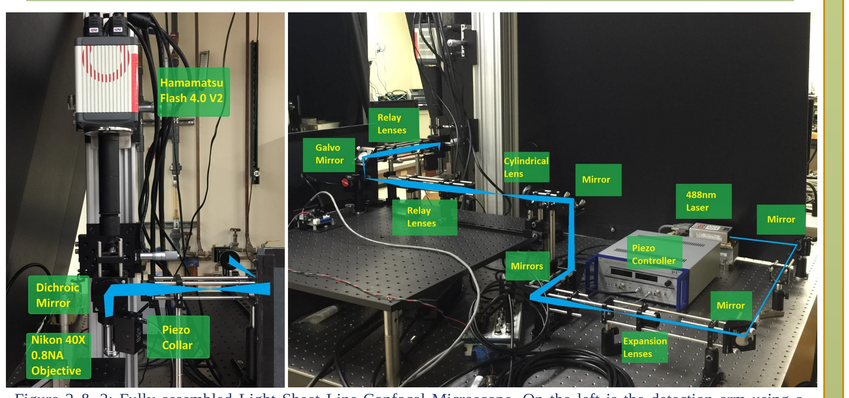

Figure 2 & 3: Fully assembled Light Sheet Line-Confocal Microscope. On the left is the detection arm using a Nikon 40x 0.8NA water immersion objective seated on a PI Piezoelectric z-scanner for depth focusing. Also on the detection arm is the Hamamatsu Flash 4.0 V2 which will be used to image the neurons in 4MP resolution at 100fps.

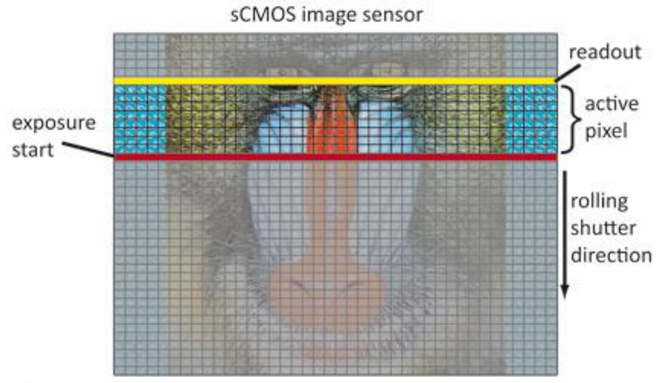

Figure 4: Conceptual diagram of rolling shutter line-confocality. As the rolling shutter of the camera translate across the sensor synchronized with the laser line produced by the cylindrical lens out of focus scattered light is rejected and thus improves the overall resolution of the system.

Figure 5: Line-confocal taken image of paralyzed C. elegans' neural network. Current resolution allows for detection of individual neurons. Combined with WormTracking and photon stimulation, this set-up will allow for exploration of the complex neural dynamics and locomotion of C. elegans that has yet to be investigated in depth.

2D-WORMTRACKER AND STIMULATIONS

- WormTracker software completely automated operational at 30fps.
- Uses a Basler acA2040-90um NIR camera to perform tracking calculations.
  - Illumination provided by ~650nm LEDs.
- 1490nm and 405nm laser diodes with power outputs of 40mW and 10mW, respectively, at the sample.
- Stimulations are controlled using LabVIEW software that modulates the laser duration to be synchronized to the head movements of the worm.

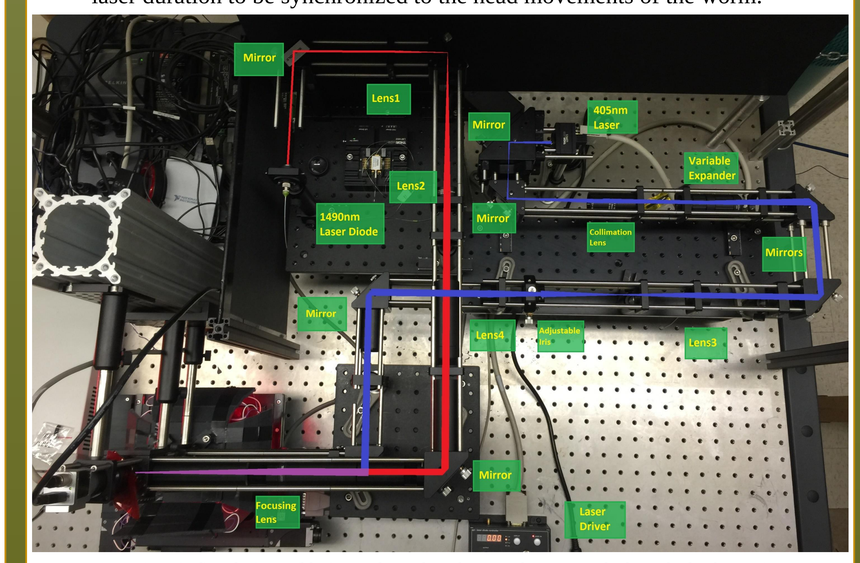

Figure 6: 1490nm (red) and 405nm (blue) stimulation laser beam paths. Exiting the laser diode, the 1490nm enters a mirror alignment. Lens 1 then expands the laser beam in conjunction with lens 2. Lastly, a mirror is place for final beam alignment corrections and the beam is passed through a focusing lens used to focus the beam to the back focal plane of the objective in order for the beam to exit collimated for better beam control.

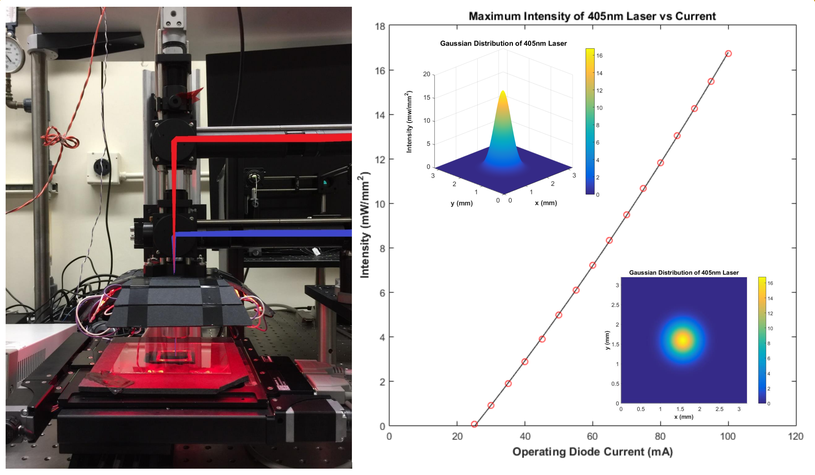

Figure 7 & 8: On the left is the detection arm of the 2D-WormTracker. In line are 2 dichroic mirrors that diver stimulation sources towards the sample. On the right is the calibration data for the stimulation sources. At peak power, the 405nm laser produces ~17mW/mm^2. 2D and 3D beam distributions, modelled as a Gaussian distr.

2D-WORMTRACKER RESULTS

Real-time worm tracking by user created software using LabVIEW allows for precise movement capture of *C. elegans* motion. After tracking its motion, we conduct offline data analysis using MATLAB programs also created by members of the lab. Results show that we have a very controlled system in terms of photon power that reaches the worm and movement tracking. Preliminary data also indicates that *C. elegans* exhibits much faster and stronger reactions towards the 1490nm stimulation.

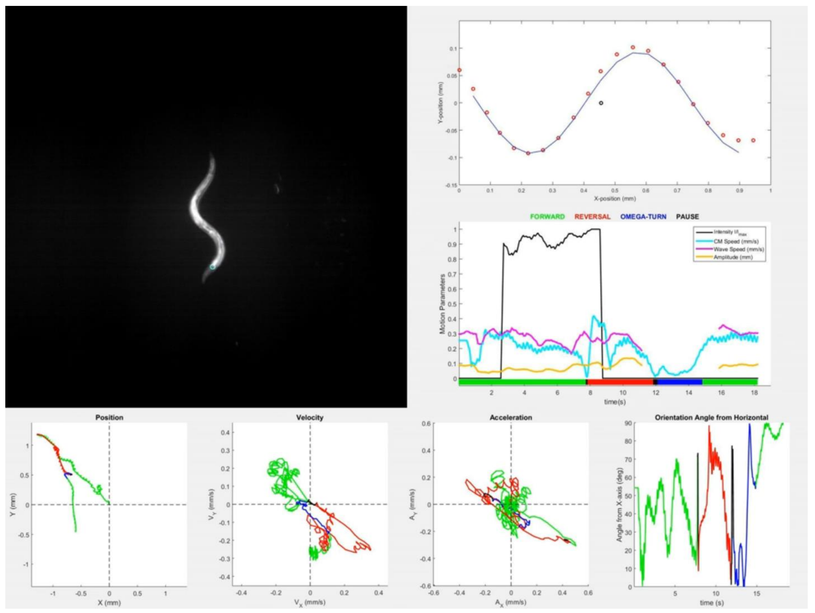

Figure 9: Data analysis run of tracked C. elegans under 405nm photon stimulation. 2D-WormTracker's capabilities allow for power offline data analysis using MATLAB software. Here we show that the tracker can have a complete capture of the movements of *C. elegans* motion under photon stimulated conditions.

CONCLUSIONS

Although our efforts have produced unique systems with open geometry for combination, we still have much work ahead of us. In the near future, we will conclude the integration and will start producing data that will allow for a complete analysis of *C. elegans'* 2D free motion and motion under photon stimulation of their ASJ and AIY neurons. We will also be able to monitor the neural activity to detect pattern differences between free motion and under stimulated conditions.

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