2D Worm Tracking Line-Confocal Microscope With Virtual Reality

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ABSTRACT

Calcium dynamic imaging and motion tracking coupled with external stimulations allows for in depth analysis of *C. elegans* behavior. We present an integrated platform to investigate *C. elegans* under a variety of external stimulations, including thermal, electrical, and photo. This platform combines rapid volumetric (20 volume/s) with diffraction limited dual line-confocal microscopy (0.5 um x 1 um x 5 um voxel) to determine the neural pathways different external stimuli induce. External stimuli are controlled with < 10 ms resolution for precise spatio-temporal synchronization. Linear and circular temperature gradients were created with thermal fluctuations of less than 0.05 °C. Virtual thermal stimulation was applied via a 1490 nm infrared laser synchronized with head motion. Infrared laser stimulation allows *C. elegans*' thermoreceptor (AFD neuron) to perceive temperature fluctuations exclusively in the time domain, allowing for the complete virtual manipulation of the nematode's thermal environment. Electrical responses were induced by applying a linear or spatially alternating electrical field (4 to 14 V/cm). Photo stimulation was applied using a 405 nm laser with intensities ranging from 0 to 10 mW/mm^2. Volumetric Calcium imaging of QW1217 strain has also allowed for the complete mapping of the neurons responsible for each of the aforementioned stimuli. The microscope accommodates multiple simultaneous stimuli applications.

OBJECTIVES

- View the neural network of C. elegans in three dimensions while tracked in 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
- 120mW 405nm and 140mW 1490nm lasers.
  - TTL signal modulation
  - Laser diode analog current modulation
- Detachable linear temperature gradient plate.
  - Peltier effect cooling and heating.
- Create linear and rotating electric fields.

LINE-CONFOCAL DESIGN

- 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.

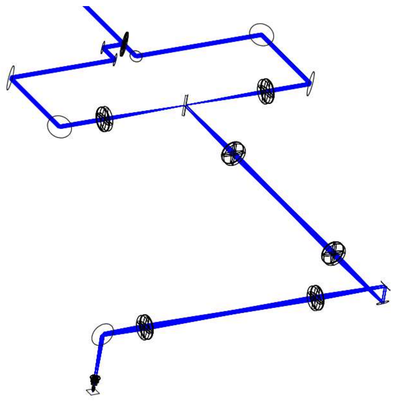


Figure 1: Zemax simulation of cylindrical light sheet beam path. Starting at cylindrical lens after original 473nm laser beam produced by a Coherent Sapphire OEM laser module has been aligned and expanded. Located immediately after is a pair of 100mm relay lenses to re-collimate the cylindrically alter 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.

MICROSCOPE RESULTS

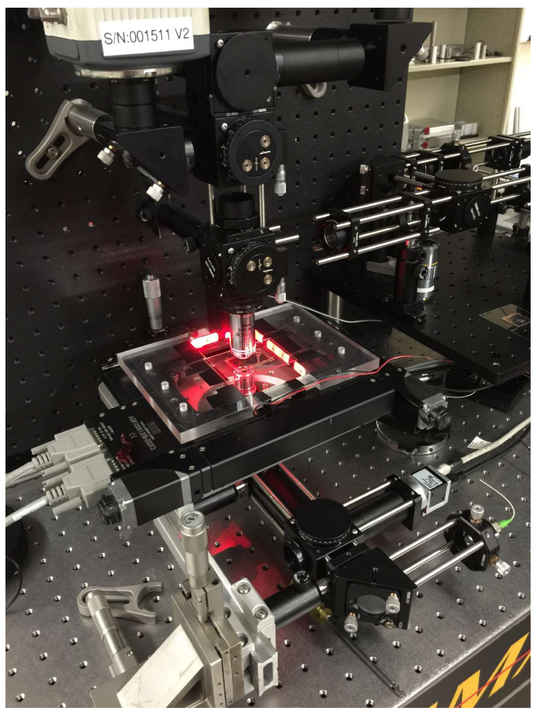
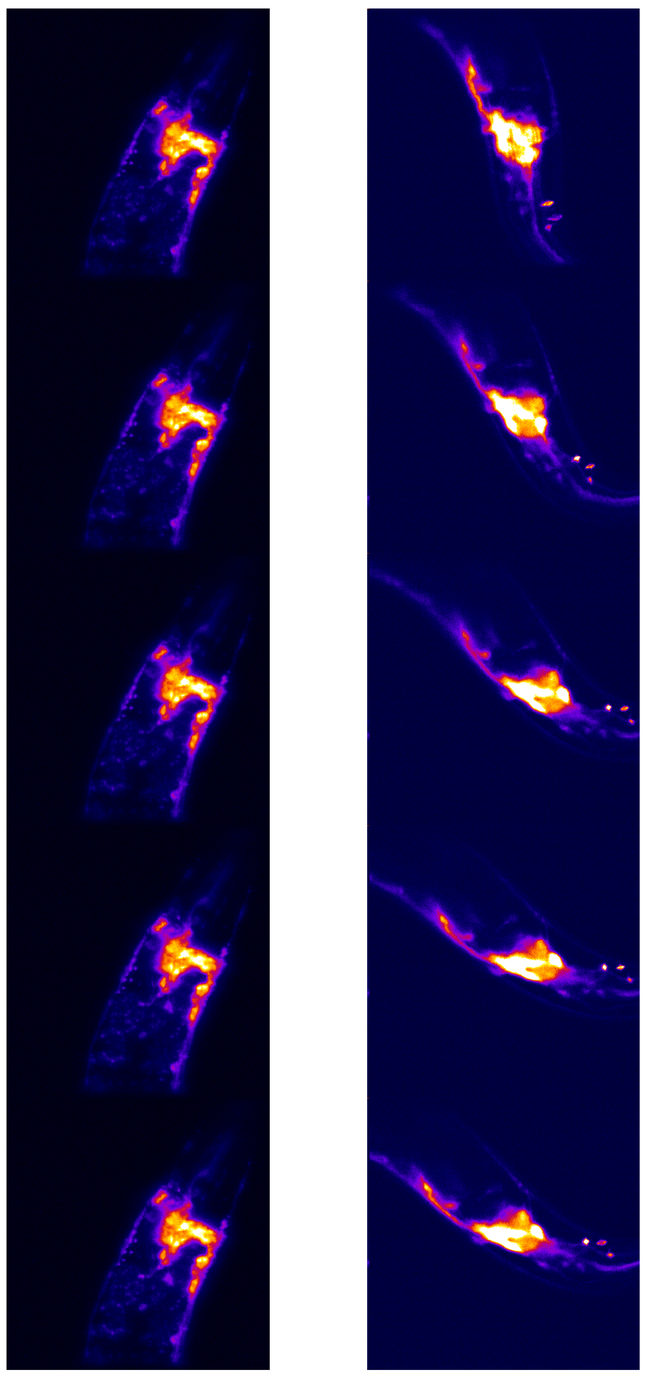


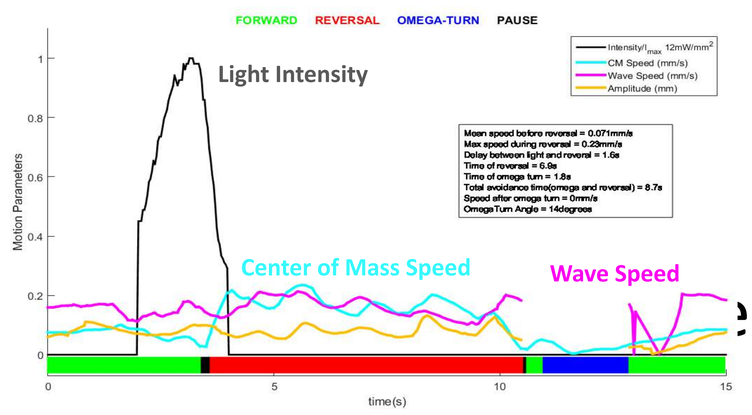
Figure 2: Fully assembled Line-Confocal Microscope. Detection arm uses a Nikon 40x 0.8NA water immersion objective seated on a PI Piezoelectric z-scanner for depth focusing. Also on the detection arm are 2 Hamamatsu Flash 4.0 V2 which will be used to image the neurons in 4MP resolution at 100fps. The speed of cameras can be increased by using a region of interest. Through this same axis, the 405nm laser is inserted. Below the 2D motorized stage is the lower magnification tracking microscope used for behavioral analysis of *C. elegans*. It operators using longer wavelength light as to not disrupt the worm. Also inserted from below is the 1490nm laser for infrared stimulation.



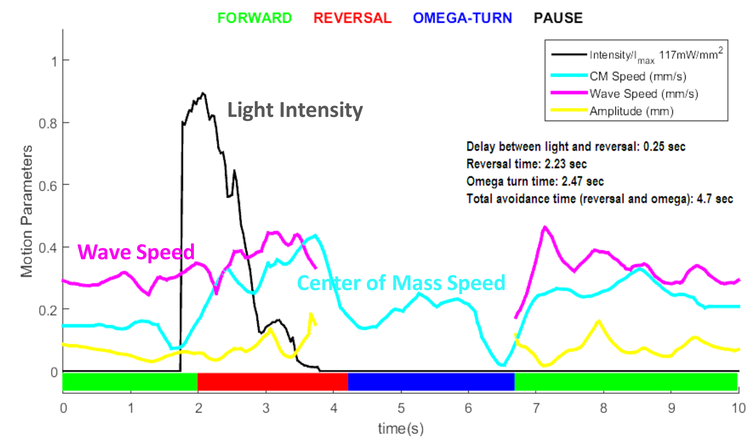
Figures 3 & 4: Line confocal imaging results. On the left is time sequence of an immobilized *C. elegans*. The volumetric imaging is fast enough at 10 volumes person to show signal propagation indicated by sequential firing of nearby neurons circled in red. On the right are preliminary free motion tracks of *C. elegans*. Due to the slow updating rate of the 2D motorized stages, the image quality is slightly compromised.

MICROSCOPE RESULTS Cont.

Response to Blue Light (405 nm), 2% Gelatin Medium



Response to Infrared Light (1490 nm), 2% Gelatin Medium



CONCLUSIONS

Tracking freely moving *C. elegans* with rapid volumetric imaging is a great challenge that has only been achieved by a select few groups. Preliminary results show that this microscope is indeed capable of accomplishing the task and all that is left it improve the image processing algorithms required to control the hardware in the most effective manner.

REFERENCES

- E. Baumgart and U. Kubitscheck, "Scanned light sheet microscopy with confocal slit detection," Opt. Express **20**, 21805-21814 (2012).
- MEI, E., FOMITCHOV, P.A., GRAVES, R. and CAMPION, M. (2012), A line scanning confocal fluorescent microscope using a CMOS rolling shutter as an adjustable aperture. Journal of Microscopy, 247: 269–276. doi:10.1111/j.1365-2818.2012.03642.x
- Greg J. Stephens , Bethany Johnson-Kerner, William Bialek, William S. Ryu , "Dimensionality and Dynamics in the Behavior of C. elegans."

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