Development of a Three Dimensional Fluorescence Tracking Microscope for the Motional Analysis and Behavioral Stimulation of C. elegans

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http://www.elegantmind.org

UCLA Science Poster Day on May 23, 2017

ABSTRACT

- Behavioral models of *C. elegans* in 2D impose constraints on the worm's motion, restricting the set of possible natural behavioral states it can occupy.
- Addressing limitations encountered by previous efforts in 3D imaging, we designed and built a microscope capable of tracking the motion of freely moving *C. elegans* via a set of motorized stages.
- The variation of gelatin concentration and the utilization of temporally controlled ultraviolet stimulation were also incorporated into the system. The addition of a refractive index mismatch correction chamber and fluorescence detection enable novel opportunities for observation and categorization of motion.
- A model of motion based on sinusoidal wave propagation was applied to *C. elegans'* forward locomotion, categorizing a set of three 3D motional states: sinusoidal in the worm's ventrodorsal plane, sinusoidal in their lateral plane, and a third state that is helical in shape.

INTRODUCTION

- The study of *C. elegans* has traditionally utilized 2 dimensional surfaces like agar to make inferences about their behavior; however, the natural worm environment is three dimensional, begging the questions:
  - how does this restriction to two dimensions affect the fundamental motion of C. elegans? Is this restriction justified?
  - How does motion change when the worm is given different stimuli?

Figure 1. Sample data from a previous paper by Kwon, et al.

OBJECTIVES

- Observe behavior of *C. elegans* in 3 dimensions in high definition
- Discover new patterns in motion of *C. elegans*
- Study the response of the worm's behavior to changes in firmness of surrounding environment (gelatin concentration) and blue-violet (405nm) light intensity

OUR MODEL

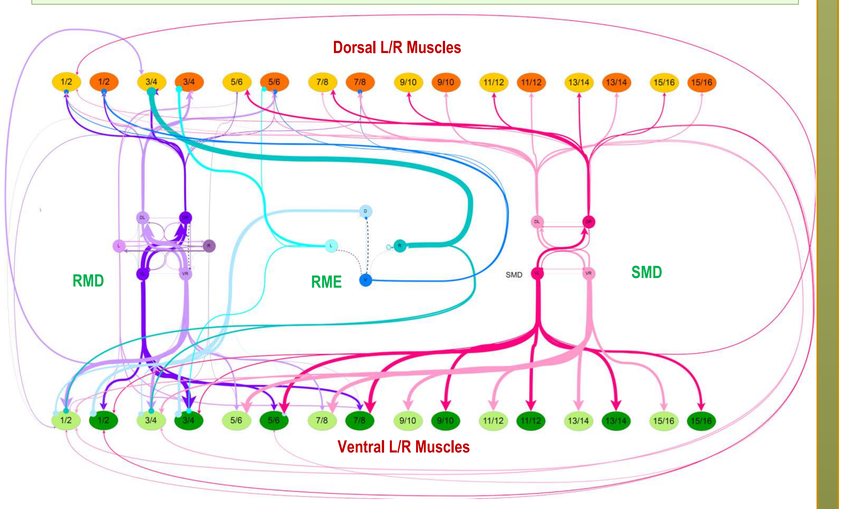


Figure 2. Rhythmic oscillations in central pattern generating (CPG) neurons RMD and SMD control head and body motion respectively

SMD Neuron

$S_L(t) = A_1 \sin(\omega_1 t + \phi_1)$ $S_R(t) = A_2 \sin(\omega_2 t + \phi_2)$

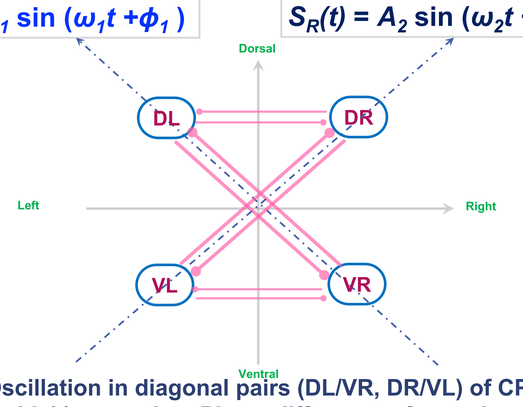


Figure 3. Oscillation in diagonal pairs (DL/VR, DR/VL) of CPG neurons drive sinusoidal locomotion. Phase difference of superimposed waves determines motional state: LR plane, DV plane, or 3D helical motion

HARDWARE

Materials:

- 3 cm cube filled with gelatin containing *C. elegans*
- 3 Basler NIR cameras with 5X objectives, 100mm tube lenses and adjustable irises
- Top camera has a 405nm violet laser to provide photon stimulation
- 3 Zaber linear stages at 90° to each other to keep *C. elegans* at center of field of view in each camera
- Outer water tank fixes distortion from air-gelatin refractive index disparity
- 472nm laser to fluorescently image ventral nerve cord in ST2 worms

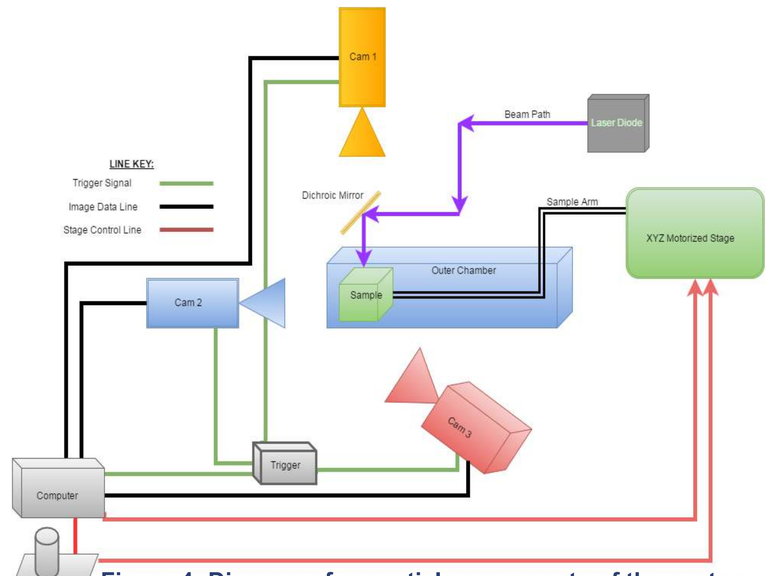


Figure 4: Diagram of essential components of the system

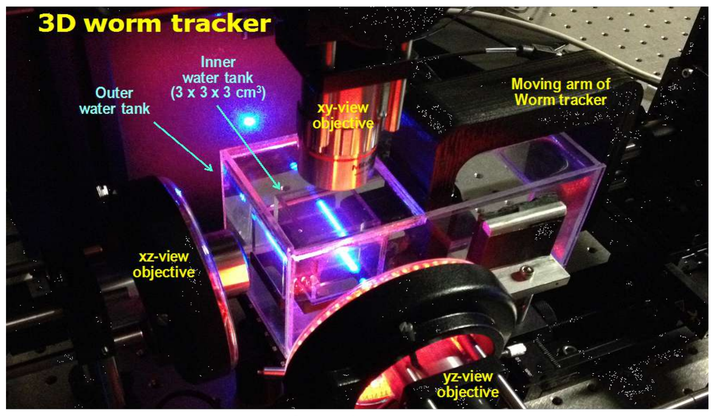


Figure 5: Image of the 3D worm tracking system

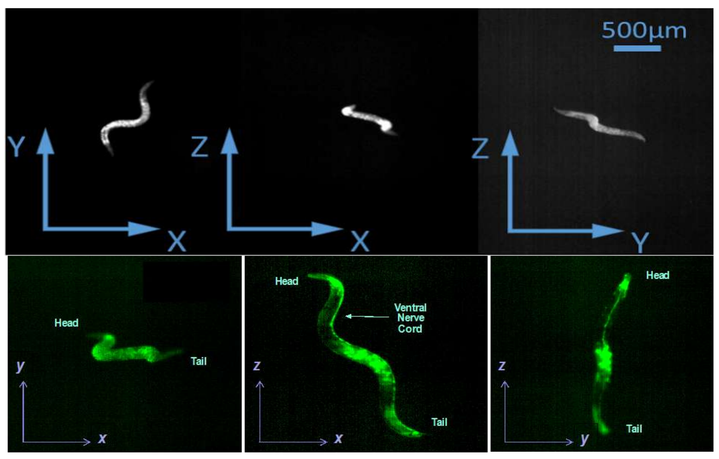


Figure 6.: Images taken using the system using red light (top) and GFP fluorescence (bottom)

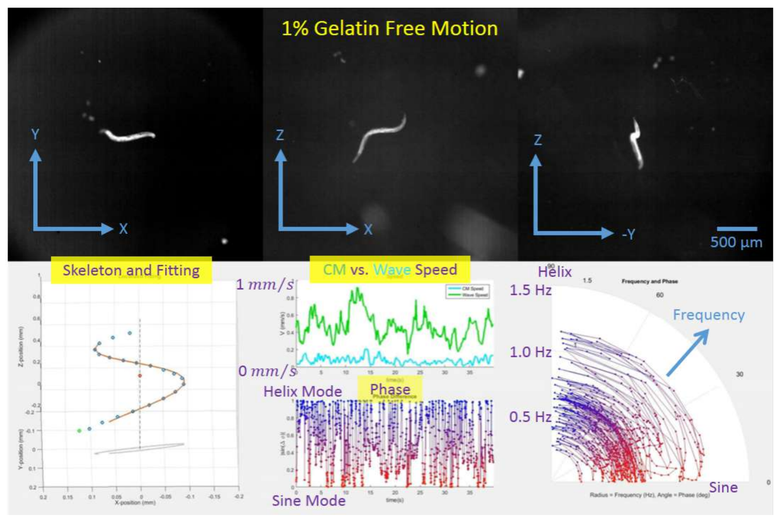
CONCLUSIONS

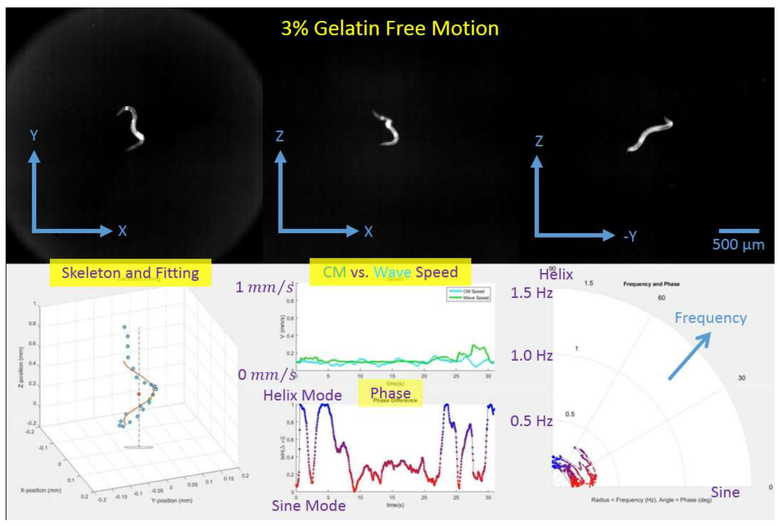
- Planar Sine Mode (PSM) of motion and Helix Mode (HM) of motion
- Transitional states between PSM and HM
- Dependence of PSM, HM and their transitions on gelatin percent
- Increased complexity in behavior in response to laser stimulation

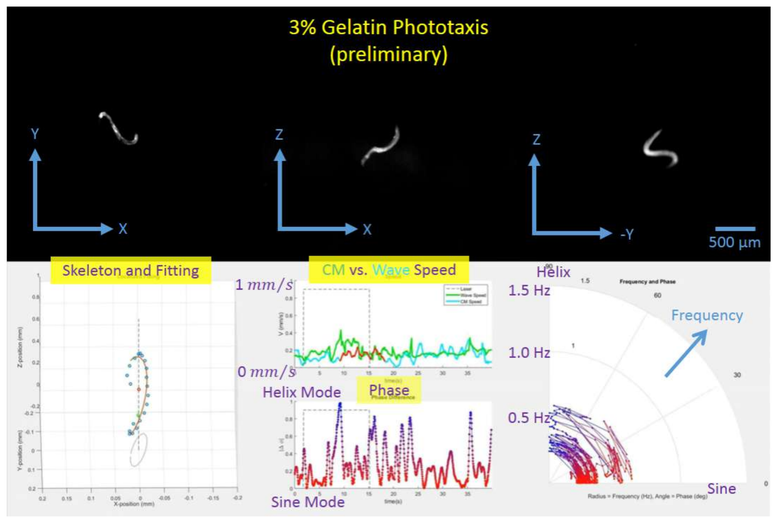
ACKNOWLEDGEMENTS

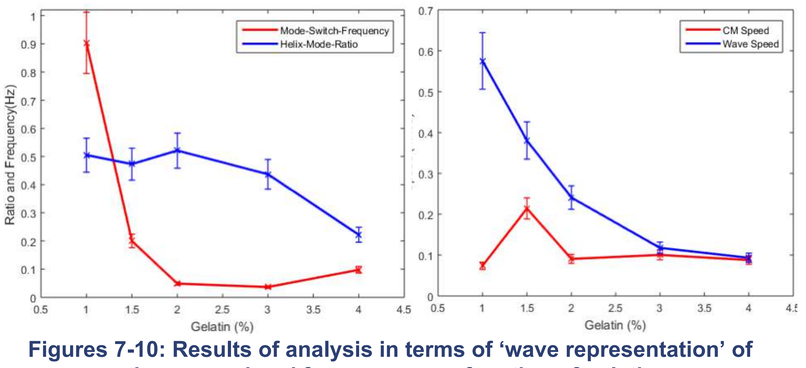
We thank Richa Raghute and Ivy Kuo for sample preparation. We also thank the NSF IDBR program, as well as the UCLA Physics and Astronomy Department, UCLA Dean's office and CNSI for funding.

RESULTS









Figures 7-10: Results of analysis in terms of 'wave representation' of worm motion - speed and frequency as a function of gelatin concentration

REFERENCES

Kwon, N., Hwang, A. B., You, Y., Lee, S. V., & Je, J. H. (2015). Dissection of C. elegans behavioral genetics in 3-D environments. *Sci. Rep. Scientific Reports, 5*, 9564. doi:10.1038/srep09564

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