Three Dimensional Motion of Caenorhabditis Elegans with Photon Stimulation

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ABSTRACT

The simplicity of C. elegans makes it ideal for quantitative study of motion. The natural state of C. elegans is to freely move in a 3D environment, not to crawl in a constrained 2D system. Therefore, in order to capture the entire set of behaviors that it is capable of exhibiting, we have to observe and quantify the motions of C. elegans in a 3D system. To do this, we have placed three high resolution cameras in three orthogonal directions, imaging a moving sample suspended in a fixed water chamber to correct for optical distortion. After 3D skeletonization of data from three dimensions by NEMO3D, we fit 3D sine and 3D helix functions to define C. elegans motion states, namely, planar sine mode and helix mode in MATLAB. Additionally, we are performing 3D photostimulation with light of varying intensities and categorizing novel avoidance behavior due to a greater degree of freedom of motion.

INTRODUCTION

- The study of C. elegans has traditionally utilized 2 dimensional surfaces like agar to make inferences about their behavior
- However, the natural environment in witch the worm lives 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 ↑: Sample data from previous paper by Kwon, et al.

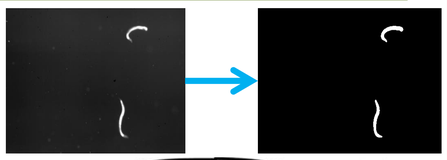
OBJECTIVES

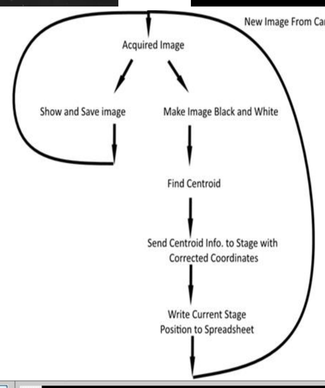
- Observe behavior of C. elegans in 3 dimensions in high definition
- Discover new patterns in motion of C. elegans
- Study the response of the worm's behavior to changes in firmness of surrounding environment (gelatin concentration) and blue-violet (405nm) light intensity when this extra degree of freedom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

ACQUISITION SOFTWARE

Intensity Thresholding →:

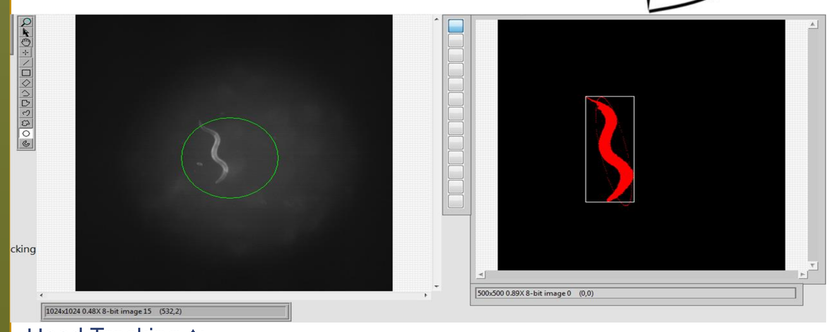
- Greyscale images have pixel values [0, 255]
- Make all pixels with values in a chosen subrange equal to 1 and all others equal 0





Custom LabVIEW Software →:

- Extension of algorithms developed by Steve Mendoza
- Uses intensity threshold-based image processing to determine position of worm in 2 cameras' fields of view then correct stage position to keep worm centered
- Stage coordinates saved in real time and allow for offline analysis



Head Tracking ↑:

- Allows light stimulation of the head during normal forward motion
- Tracks point along major axis of bounding ellipse rather than center of mass
- Ellipse determined by calculating second central moments of binary image and generating the ellipse with the same second central moments

HARDWARE

Materials:

- 2cm cube filled with gelatin containing C. elegans
- 3 Basler NIR cameras with 5X objectives, 100mm tube lenses and adjustable irises
- Top cam has a 405nm violet laser injected 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 imparity

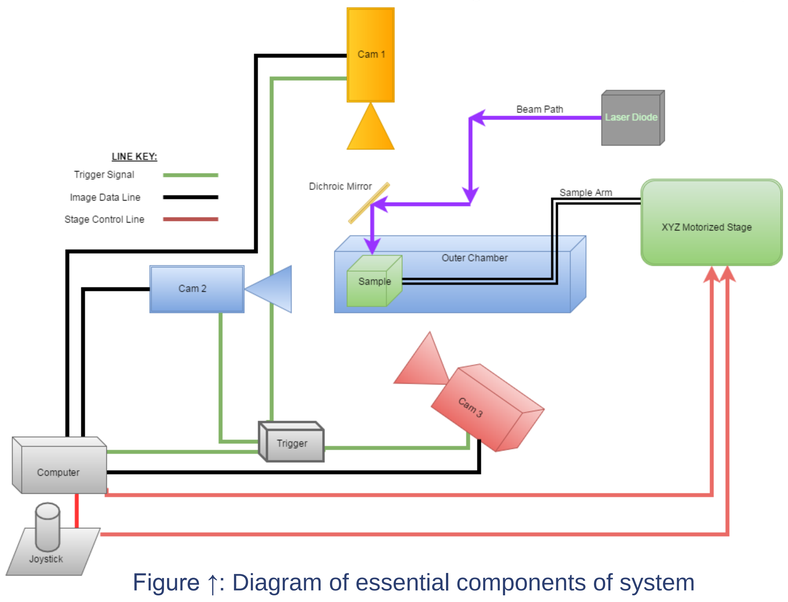
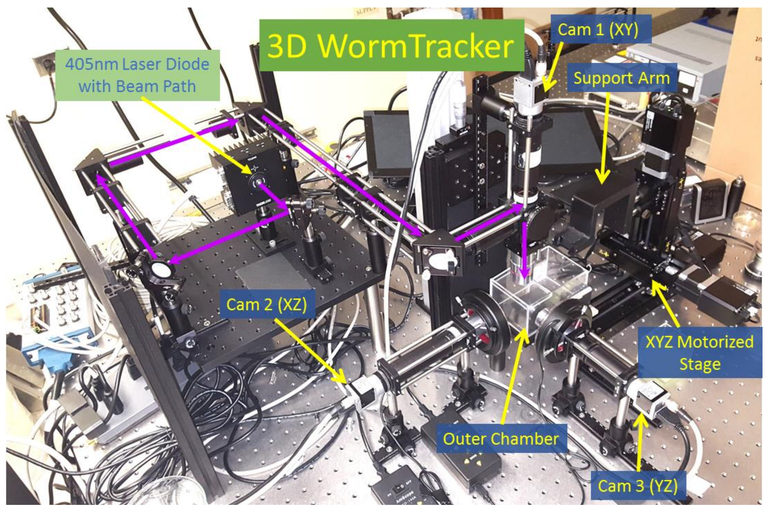
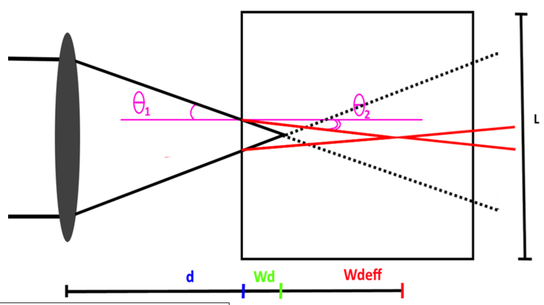


Figure ↑: Diagram of essential components of system





$$Wd_{eff} = f \times (Wd) + d \times (1 - f)$$

$$f = \frac{\sqrt{(Wd)^2 + (R)^2 \times (1 - N^2)}}{(Wd) \times (N)} \qquad N = {}^{n_{air}}/_{n_{water}}$$

Figure ↖: Index of refraction problem illustrated and solved with double chamber

CONCLUSIONS

Discoveries:

- 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 percentage
- Increased complexity in behavior in response to laser stimulation (preliminary)

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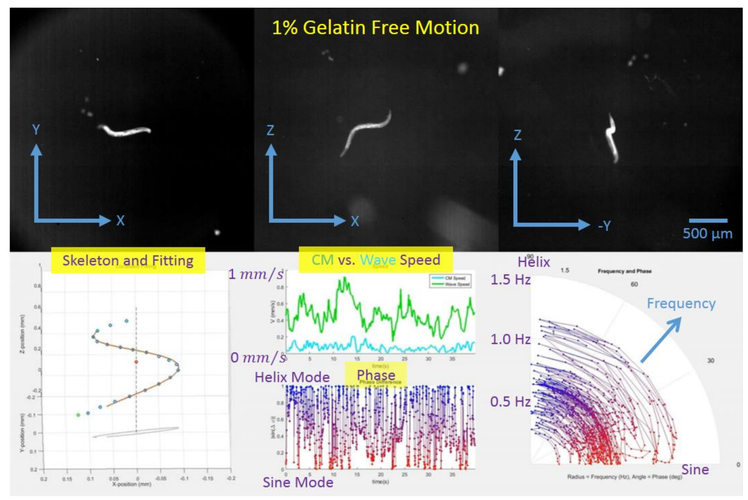
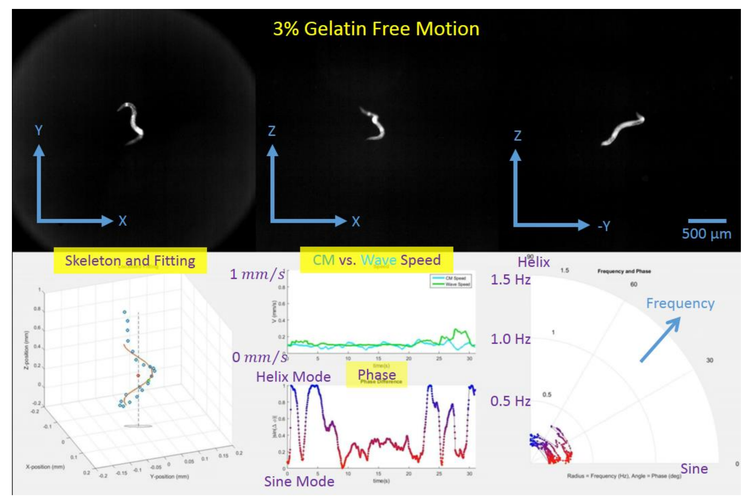
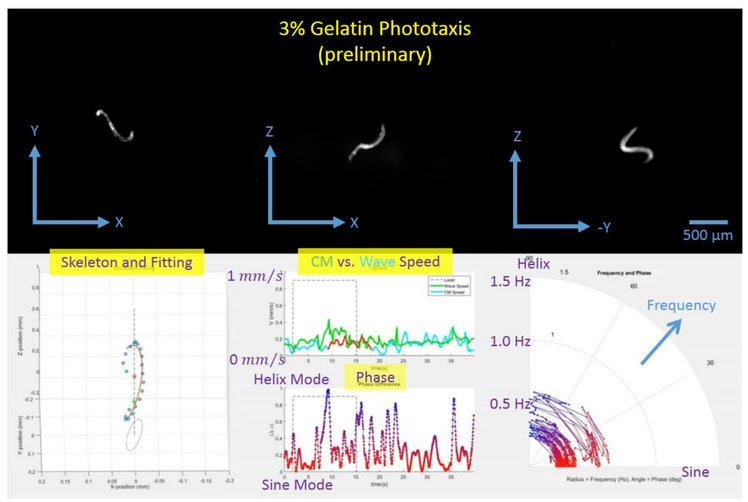
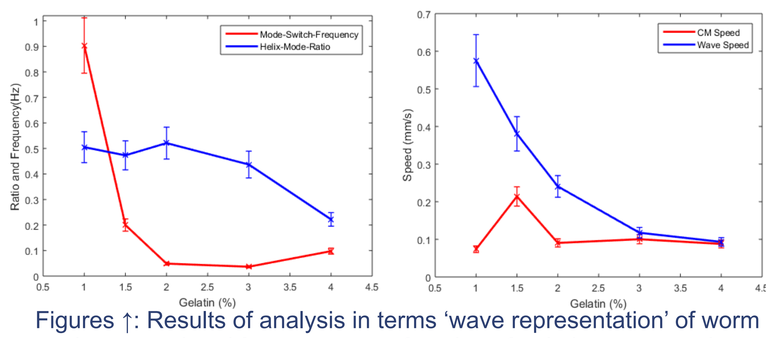
RESULTS









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

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