Light Avoidance Reflex of Adult C. elegans in a 3D Environment

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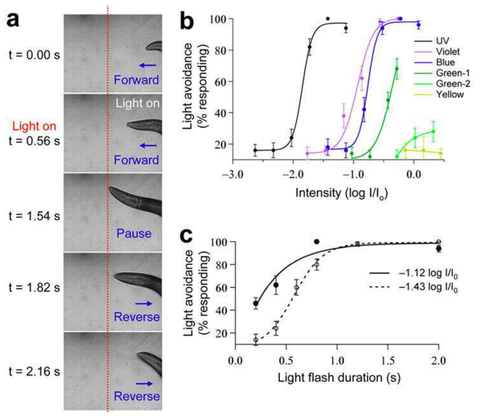
ABSTRACT

- Although C. elegans lives and moves in 3D, the study of their light avoidance reflex in a 3-dimensional (3D) environment is poorly understood.
- We are investigating the light avoidance behavior of C. elegans in 3D as a function of beam intensity using a 3 camera setup, worm-tracking platform, and analysis software that we developed for this experiment.
- In our preliminary data we see worms in 3D will overlap their head and tail after the reversal avoidance in an alpha shape because they have more freedom of movement.
- Observing the behavior of C. elegans under light stimulation in 3D is important because it more accurately resembles their natural environmental conditions. Investigating C. elegans behavior in more natural conditions is essential to better understanding their neuronal pathways and behavior because restricted movement could otherwise restrain their natural function.

INTRODUCTION

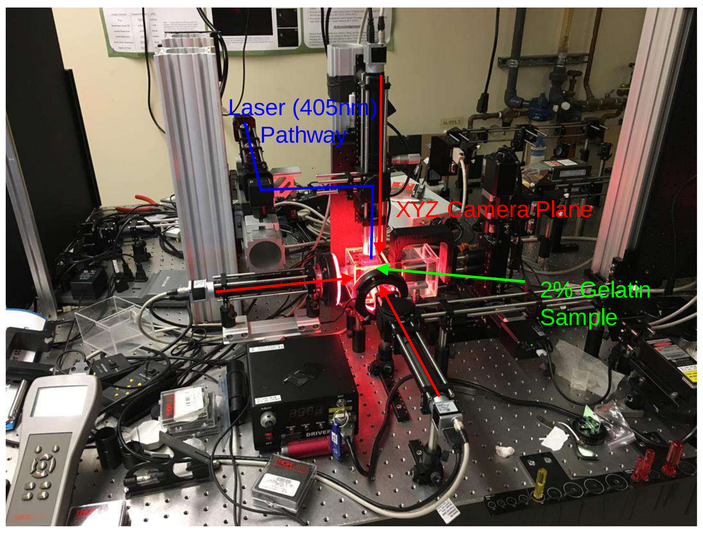


Our 3 Dimensional phototaxis experiment was largely based off of the findings of Ward et al. Their experiment proved that C. elegans, previously believed to have no phototaxis response, does in fact respond to certain light stimuli. Ward et al. found that C. elegans phototaxis behavior is mediated by light-sensitive neurons and requires cGMP/CNG channel - dependent phototransduction.
The original experiment observed C. elegans phototaxis responses in 2-dimensions.
Our experiment will attempt to mimic the procedure and results of Ward et al. in a 3-D environment. Similarly to the experiment cited above, our group's objectives are in the hopes of studying neuronal networks and sensory systems and provide other groups and future experiments with preliminary data for 3-D responses of C. elegans.

HYPOTHESIS

We hypothesize that we will see light avoidance behavior similar to that in 2-dimensions response to blue light. We believe that the movement following the reversal reflex will be an alpha shaped turn (overlapping head and tail).

EXPERIMENTAL SET-UP



3 Dimensional phototaxis experimental setup:

- Infrared backlight
- Worm tracking cameras
- 405 nm laser diode at I
- XYZ Motorized stage
- sCMOS Camera
- Resolution:
  - 1020 pixels * 1020 pixels

Bio sample preparation:

- 3cm quartz cube (index of refraction similar to water).
- 2% gelatin prepared using m9 buffer (standard solution to use with nematodes)
- N2 adult (egg bearing) C. Elegans inserted into cube using long sterile pick and sealed in with liquid gelatin
- Incubated for approximately 7 minutes to solidify gel at top of cube sealed with thin microscope slide

FREE MOTION RESULTS

Figure 1a: Free Motion Raw Image

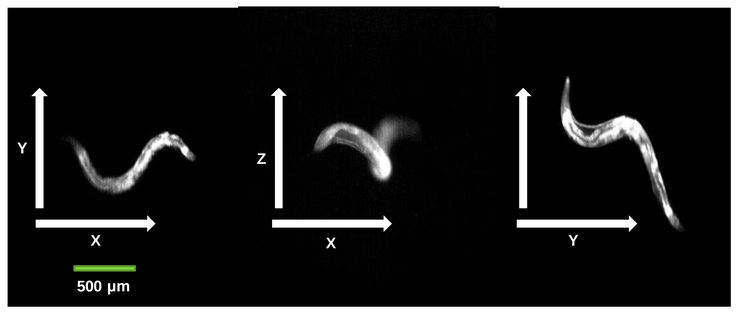


Figure 1b: Free Motion Trajectory

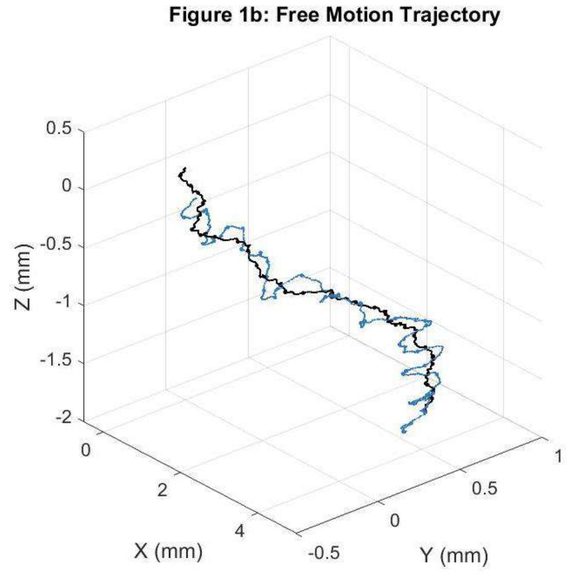
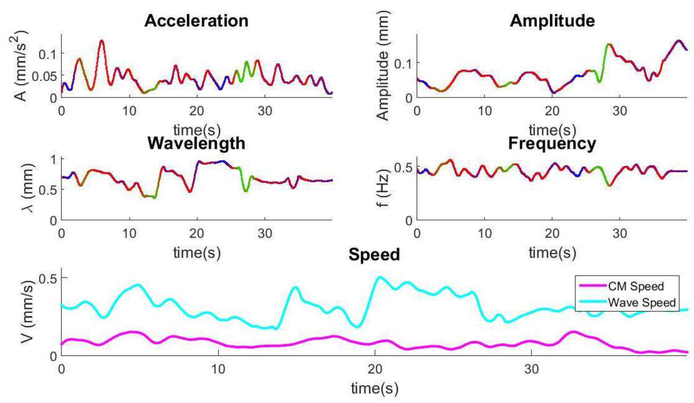


Figure 1c: Kinematic and Wave Parameters



PHOTOTAXIS RESPONSE RESULTS

Figure 2a: Alpha Turn Raw image

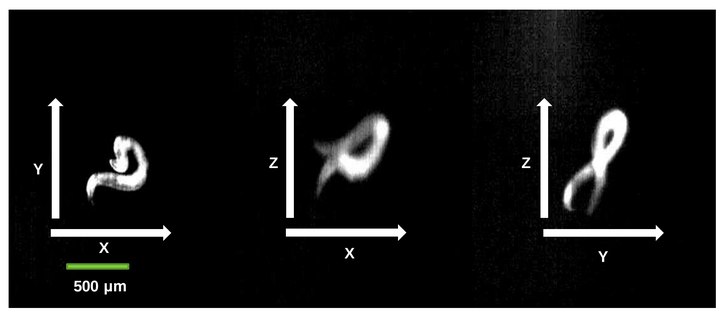


Figure 2b: Phototaxis Response Trajectory

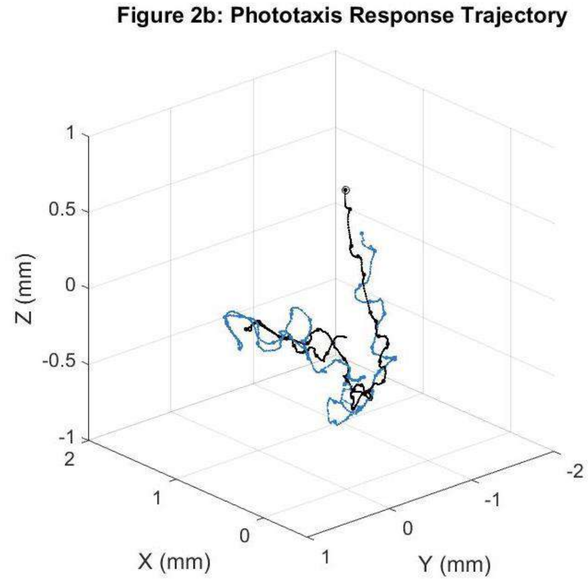
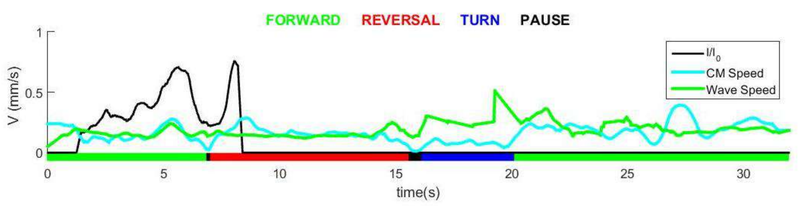


Figure 2c: Velocity and Laser Response



CONCLUSIONS

- We conclude that C. elegans responds to photo stimulation in 3D by reversing and making an alpha shaped turn. This “alpha” turn is similar to the omega turn elegans exhibit in two dimensional environments. However, during the Alpha turn the head and tail overlap, naturally, because of the freedom of motion that is not seen in 2D motions.
- As seen in the phototaxis response image (Figure 2a), the main difference is that in 2D the head and tail are not able to overlap so the C. elegans is restricted in its natural movement.
- By adding an extra degree of movement, we were able to observe C. Elegans respond to the phototaxis by reversing, making an alpha turn, and then continuing in a path less than 180 degrees from the initial direction. The 180 degrees reversal is expected in 2D but not common in 3D as we have observed. Consider Figure 1b in comparison to Figure 2b, C. Elegans responds to the stimulation by making the apha turn and begins to move forward in a direction that is less than 180 degrees from the initial direction. We conclude that this is possible because of the added degree of freedom.
- Observing elegans in a 3D environment allows us to see more dynamic movement and behavior of the organism. The conclusion is significant in showing that the movement observed in this experiment is much more indicative of elegans movement in a natural habitat. There should be more experiments done in 3D to improve the accuracy of future experiments to the natural behaviors of C. Elegans.

REFERENCES

- Ward, Alexander, et al. “Light-Sensitive Neurons and Channels Mediate Phototaxis in C. Elegans: Abstract: Nature Neuroscience.” Nature Neuroscience 11.8 (2008): 916–922. Web. 20 Apr. 2016.
- Ward, Alexander. "Phototaxis and Phototransduction Mechanisms in the Model System C. Elegans." *Deep Blue*. University of Michigan, Jan. 2010. Web.

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