Observation of the Behaviors of C. elegans in Response to Chemical Stimuli Under a Three-Dimensional Imaging System

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ABSTRACT

- *Caenorhabditis elegans* is a model organism that has been studied extensively in neuroscience and other fields to gain insight into mechanisms of locomotion in response to stimuli.
- Most previous behavioral experiments conducted have observed the motion and behaviors of the worm on two-dimensional agar surfaces with chemoattractants despite the fact the natural environment for the soil-dwelling *C. elegans* allows them to move in three dimensions.
- To characterize the chemotaxis behavior in a three-dimensional environment, we observed the motions of worms in a cube cuvette that is filled with gelatin and has a salt gradient. It is expected that when the sodium gradient is established, the *C. elegans* will exhibit biased motion towards the areas with high concentration compared to control trials where there is no gradient.
- By extending classic behavioral chemotaxis assays into three dimensions and quantitatively analyzing the data, we aim to reach a more robust understanding of *C. elegans* behavior.

MATERIALS AND METHODS

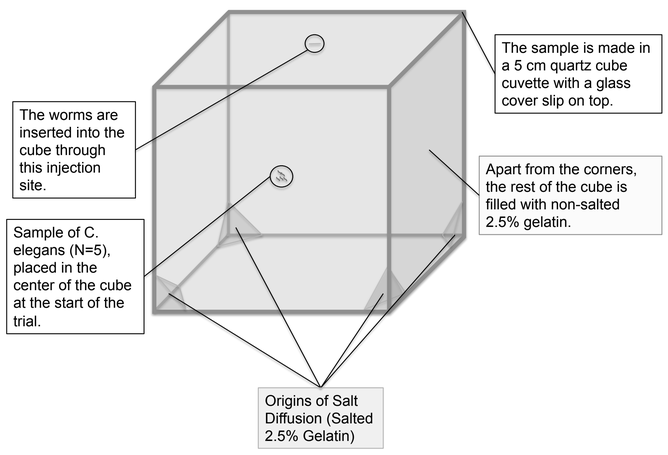


Figure 5. Sample Preparation.
The four corners of a 5 cm cube cuvette is filled with 2.5% gelatin that has 400 mM of NaCl. Then the rest of the cube is filled with 2.5% gelatin that has no salt. The cube is left in a 15℃ incubator for approximately 17 hours so that it may set. After the cube has solidified, a sample of 5 N2 *C. elegans* is injected into the center of the cube and a coverslip is placed on top. Once the cover slip is set in place, the cube is cleaned and taken to the 3D imaging apparatus.

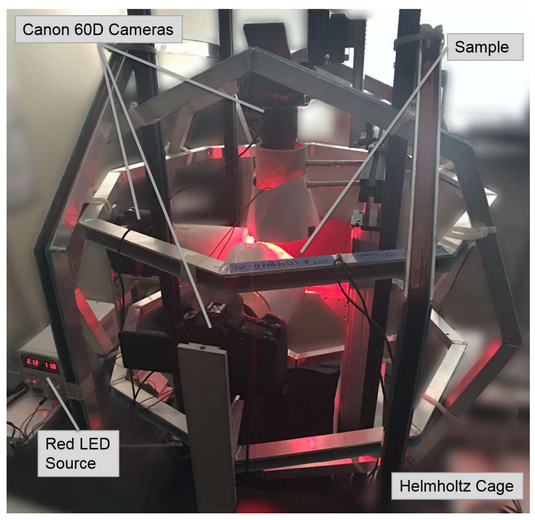


Figure 6. 3D Imaging Setup.
The 5 cm cube cuvette is placed in the center of the apparatus. There are 3 Canon 60D cameras setup around the cube to capture the three faces, or planes, of the gelatin cube – XY, XZ, YZ planes. The red LED lights are turned on while the room's lights are turn off during the trial.

INTRODUCTION

- Previous two-dimensional studies have observed *C. elegans* exhibiting biased motion in reaction to concentration gradients of chemoattractants such as salt.
- Researchers have characterized two types of biased motion:
  - *Biased random walk*: Frequency of worm reversals is lower when the chemoattractant concentration is high.
  - *Klinotaxis*: Worms curve toward chemoattractants during forward motion toward concentration peaks.
- These studies were conducted on two-dimensional agar plates. The goal of the project was to replicate these findings and to quantitatively characterize the locomotion of *C. elegans* in response to chemoattractants in a more realistic three-dimensional environment.

Figure 1. A pirouette consists of a reversal followed by an ω turn (Iino, 2009).

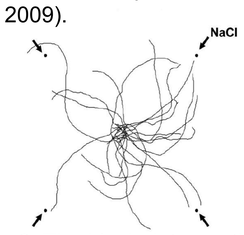


Figure 2. The black dots are the NaCl spots. The tracks of the worms indicate that the worm seems to be slowly curving towards the dots from the center of the grid (Iino, 2009).

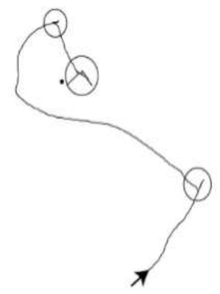


Figure 3. This is a track from previous experiments where the worms were observed in a 2D plane. The black dot is the NaCl spot. The circles are pirouettes. The rest is the run (Iino, 2009).

RESULTS

Figure 7. Worm Trajectory in Control.
This is the 3D trajectory of the free motion of a worm in a controlled space. The green "x" is where the worm starts and the blue circle is the end of the track

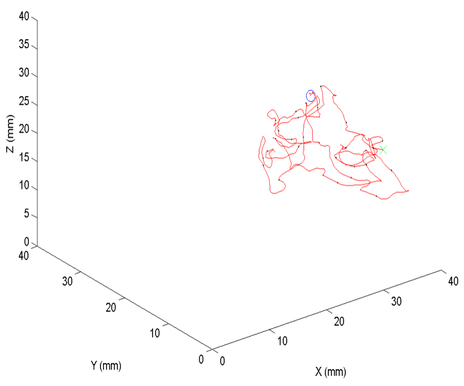


Figure 9. Salt Gradient.
After letting the cube set and the salt to diffuse, this is the estimated salt gradient at each point in the cube when the trial starts.

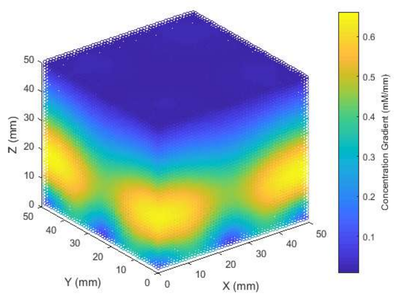


Figure 10. Salt Concentration.
After letting the cube set and the salt to diffuse, this is the estimated salt concentration at each point in the cube when the trial starts.

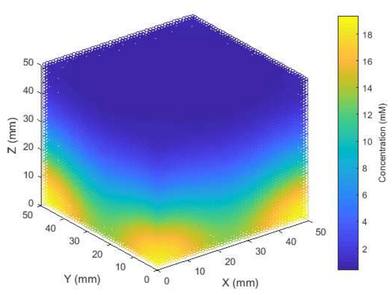


Figure 8. Worm Trajectory in Salt Gradient.
This is the 3D trajectory of a sample of worms in an established salt gradient. The green points are where the worms starts and the red point are the end of the track.

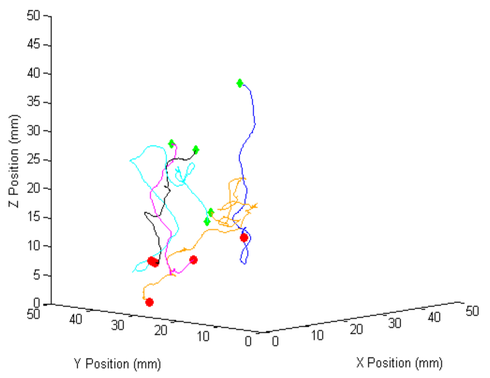


Figure 11. Angular Frequency of Control.
A view of free motions as a function of angular frequency.

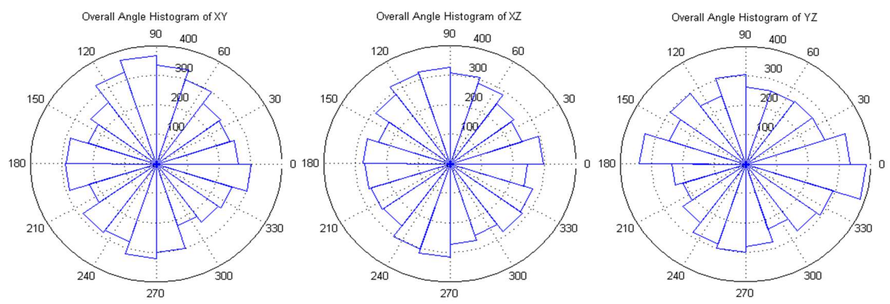
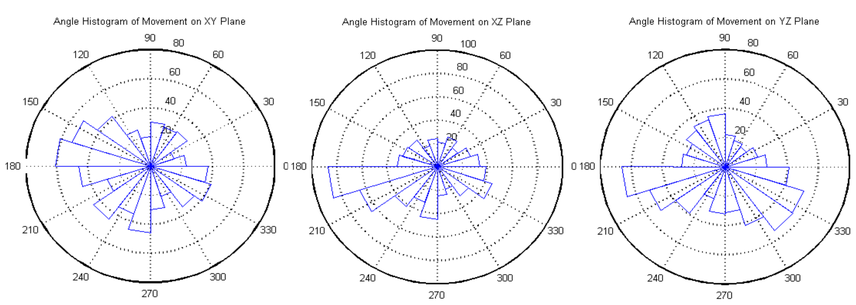


Figure 12. Angular Frequency of a Worm in Salt Gradient.
A view of chemotaxis as a function of angular frequency.



CONCLUSION

- In the trajectory of the control (Figure 9), there is no clear directionality in the worm's movement. It randomly moves around in the area that it was deposited.
- In the trajectories of the worms in a salt gradient (Figure 10), there is a directionality to them. The worms all seem to head down the cube, to area of higher salt concentrations.
- Worms in a 3D environment experience less biased random walk than those in a 2D environment. The decrease in biased random walk is most likely due to the increase in degrees of freedom. The worms on a 2D agar plate are only able to move left and right. In a 3D gelatin cube, the worms are able to not only move in those direction but also up, down, and diagonal.

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