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Characterizing the Effect of Static Magnetic Fields on *C. elegans* Using Microfluidics

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Abstract

In nature, several organisms possess a *magnetic compass* to navigate or migrate them to desired locations. It is thought that these organisms may use biogenic magnetic matter or light-sensitive photoreceptors to sense and orient themselves in magnetic fields. To unravel the underlying principles of magnetosensitivity and magnetoreception, previous experiments have been conducted on bacteria, vertebrates, crustaceans, and insects. In this study, the model organism, *C. elegans*, is used to test their response and sensitivity to static magnetic fields in the range of 5 milli Tesla to 120 milli Tesla. Single wild-type *C. elegans* are put in microfluidic channels and exposed to permanent magnets for five cycles of thirty-second time intervals. The worm movement is recorded and analyzed with custom software to calculate the average velocity and the percentage of turning and curling. Contrary to some published studies, our results did not show a significant difference compared to control experiments. This suggests that *C. elegans* may not sense static magnetic fields in the range of field strengths that we tested.

Keywords

***C. elegans*, Magnet, Magnetotaxis, Behavior, Microfluidics**

1. Introduction

Over a century ago, it was first postulated that animals possess some sort of a "magnetic compass" to explain the navigation behavior of migratory birds [1]-[3]. Early experiments showed that the navigation of carrier pigeons (*Columbidae livia*) could be disrupted by attaching magnets to their body [4]. Experiments with magnetic coils demonstrated that European robins (*Erithacus rubecula*) orient themselves to artificially generated magnetic fields [5] [6]. The evidence in early studies indicated that some birds may possess a magnetic sense, but the underlying mechanisms behind this sense remain a fascinating and bewildering mystery of nature.

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Even though very few organisms have been identified as being responsive to external magnetic fields, the phenomenon of magnetosensitivity is thought to be much more pervasive in nature. Some examples of magnetosensitivity have been observed in bacteria, vertebrates, crustaceans, insects, and a mollusk species [7]. In one study, migratory lobsters (*Panulirus argus*) were disoriented by an artificially generated magnetic field, which prompted the lobsters to migrate in the opposite direction of their intended destination [8]. In another study, the mole-rate (*Fukomys anselli*) was shown to use magnetic fields to orient their nest construction in a specific direction of a circular arena [9]. When magnetic coils were employed to alter the ambient magnetic field, the mole-rats were found to re-orient the construction of their nests in the direction of the new magnetic field.

To explain the underlying principles of magnetosensitivity, two broad mechanisms of magnetoreception have been proposed. The first proposed mechanism suggests that magnetite (Fe_3O_4) particles, which are naturally found in many organisms, could be integrated into the nervous system of the organism. As supporting evidence, magnetite has been found in the heads of homing pigeons, and the amount of magnetite present could theoretically enable detection of small changes in the magnetic field strength [10]. Brain studies found that the trigeminal brainstem complex in European robins is stimulated by magnetic fields, and an increased neural activity is measured when the birds are exposed to non-uniform magnetic fields [11]. The exact site and distribution of magnetite within the bird's head are less understood, but are hypothesized to be stored in macrophages or in magnetosensitive neurons [12]. In simpler organisms, such as magnetotactic bacteria, the role of magnetite is clearer. Magnetotactic bacteria rely on magnetite-containing organelles to migrate to anaerobic environments that are more favorable for growth [13]. These bacteria interact with and move into alignment each other using magnetic forces produced by the magnetite particles.

The second proposed magnetoreception mechanism suggests that magnetic sensitivity is mediated by the photoreceptor cryptochrome. Studies have shown that the magnetic sense is light-dependent and has been investigated on monarch butterflies (*Danaus plexippus*), fruit flies (*Drosophila melanogaster*), and migrating birds [14]. As an example, it was shown that by filtering light in the ultraviolet-A/blue wavelengths, monarch butterflies did not exhibit the same directional flight compared to in white light [15]. It was hypothesized that the photoreceptor cryptochrome molecules were sensitive to these wavelengths of light, and generated magnetically sensitive radical pair products that eventually enabled magnetosensistivity in the butterflies. In another example, *Drosophila* mutants with cryptochrome deficiency were shown not to display the magnetosensitive response found in wild-type flies, indicating that the photoreceptor cryptochrome was required to produce the magnetosensitive behavior in the flies [16]. A combination of both magnetite-based and photoreceptor-mediated mechanisms mediates and regulates the magnetic sensory system in migrating birds. [17].

Designing experiments to isolate the exact role of magnetic fields in the navigation or migration of organisms is challenging. This is because the response to magnetic fields is often influenced by several biological and environmental factors that work in unison to produce a behavioral trait. For instance, birds and insects can use other senses to navigate, making it difficult to isolate the response to magnetic fields. Pigeons, for example, use the position of the sun to navigate during migration periods [7]. Early magnetic experiments found that navigation of pigeons was only disrupted on overcast days when magnets were attached to the birds [4]. Birds and higher vertebrates have a large and complex nervous system that can be difficult to interrogate during experiments. As such, there is an increasing interest to study magnetoreception in model organisms, such as *Drosophila* and *Caenorhabditis elegans*, which have a simple nervous system and a well-established genetic model.

The *C. elegans* is a model nematode worm (around 1.3 mm in length at the adult stage) with a short lifespan of 21 days, tractable genome, a simple nervous system comprising 302 neurons, and the relative ease of culture on agarose plates. This worm has been a great candidate for studies on behavior, development, toxicity screening, and neuronal signaling [18]-[20]. In lines with the earlier discussion on the first mechanism of magnetoreception, traces of biogenic magnetite were found in adult *C. elegans* using a superconducting quantum interference device (SQUID) and transmission electron microscopy [21]. The thermal decay of remanence (TDR) measurements confirmed magnetite as the particle of interest near the edges of the nematode's body. Subsequently, several studies examined the (detrimental) effects of magnetic fields on nematodes. In one study, *C. elegans* were exposed to a 1.7 tesla magnetic field at 60 hertz for 84 hours to investigate biohazardous effects of magnetic fields over the lifespan of the worm. The high-power, alternating magnetic fields were shown to disrupt locomotion and reproduction in *C. elegans* [22] [23]. Besides alternating magnetic fields, static magnetic fields also can affect the locomotion and lifespan in *C. elegans*. Hung *et al.* reported reduced crawling speeds when worms were exposed to static magnetic fields [24] [25]. Investigations regarding the directional ability of

worms in magnetic fields have been less conclusive. Using a 3-axis coil system, Pichler studied the response of different species of worms to static magnetic fields of varying intensity and direction. No significant effect was found in turning frequency, velocity, or average direction upon exposing the worms to magnetic fields of up to 3.4 Gauss [26]. In a similar experiment using magnetic coils, Garcia *et al.* tested if the average direction of worm movement was affected by magnetic field strength and direction. At field strengths up to a 10 Gauss, no significant effect was found in average direction of movement [27].

In this paper, we attempt to verify whether static magnetic fields affect the crawling speed or any other movement traits in the wild-type *C. elegans*. A microfluidic chip is designed and a stage is built to apply static magnetic fields using permanent magnets. Here worms are confined to a channel to restrict their movement to two directions, in contrast to being unconstrained on an open agar plate. This eliminates interaction among multiple worms and reduces the field of view for imaging worm behavior. Measurements of worm position are recorded over time under different field strengths. Using custom software, two movement parameters, average velocity and percentage of turning and curling, are calculated. Both parameters did not show a significant change upon exposure to static magnetic fields of up to 120 milli Tesla (mT).

2. Materials and Methods

2.1. Fabrication of Microfluidic Chip

A microfluidic chip is designed to study the behavior of *C. elegans* upon exposure to static magnetic fields. (**Figure 1(a)**). Each microfluidic chip comprises eight straight channels (length: 1 cm, width: 250 μm, depth = 40 μm) (**Figure 1(b)**). The channel length is optimized to be within the field of view of a Leica MZ16^{TM} transmission stereozoom microscope during the entire length of the experiment. The channel width and depth are chosen to permit unrestricted sinusoidal movement of individual worms (**Figure 1(c)**). To further avoid interaction between worms, only one worm is used in one channel. The physical layout of the microfluidic chip is drawn in AutoCAD and the mask is printed on a transparency sheet. Afterwards, SU-8 is spin-coated on a silicon wafer (diameter: 3 inches, thickness: 300 μm) and the patterns on the mask are transferred on the SU-8 polymer by exposure by ultraviolet light and subsequent developing in a SU-8 developer. A polydimethylsiloxane (PDMS) polymer is poured on the SU-8 mold and allowed to harden in a low-pressure chamber. The PDMS layer is peeled, punched with holes for inlet/outlet ports, exposed to air plasma, and irreversibly sealed onto a standard glass slide (**Figure 1(b)**).

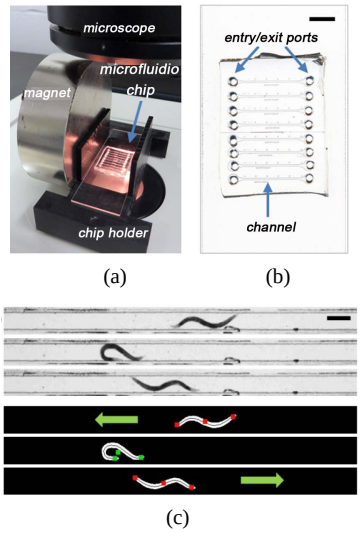


Figure 1. (a) Snapshot of the experimental setup showing the microfluidic chip, chip holder, and a permanent magnet; (b) Image of our fabricated microfluidic chip having eight parallel, straight channels; each with its port for worm entry and exit; (c) The top panel shows three lanes of channels as recorded by the camera. The bottom panel shows the processed images from the custom software that removes the background and identifies the centroid, head, and tail (indicated by dots). The green arrows indicate the direction of worm movement. As the worm reverses direction, the software detects that the spacing between the head and tail is decreased, and records a turning event (indicated by green dots on the worm).

2.2. Chip Filling with Agarose Gel

Agarose is a reliable medium for observing worm movement within microfluidic devices. With liquid media, there is an internal fluid pressure in the channels that can influence the worms' natural movement. The chip is filled with 0.7% agarose in M9 buffer. Previously, *C. elegans* were grown at 20˚C on standard Nutrient Growth Media (NGM) agarose plates with *Escherichia coli* OP50 food [28]. For the magnetotaxis experiments, single L4-stage and young adult worms are picked with a sterilized worm picker and dropped at the chosen entry ports of the microfluidic channels. A custom chip holder houses the microfluidic chip and holds the magnet at fixed distances from the entry ports. All experiments are performed at 22˚C. To nullify any effects of gravity, the chip holder is placed on the flat, horizontal stage of the microscope. For every experiment of a specific magnetic field, around 3 to 5 separate chips are tested with around 3 to 5 worms in every chip.

2.3. Steps for Exposure to Static Magnetic Fields

Figure 2 illustrates the procedure for performing the magnetotaxis experiment. After dropping in the entry port, the worms enter the channels within 5 minutes. The movement of all worms in the channels is recorded simultaneously by a QICam™ 24-bit Color digital camera interfaced with QCapture PRO™ software. Once the worms appear in their respective channels, image frames are captured every one second for the pre-decided length of the experiment and stitched together to create a video file. The initial worm movement is recorded for over one minute to ensure that the worms are healthy. Unhealthy or inactive worms are ignored in the image recording step. Next, we wanted to test whether the natural worm movement is affected by the presence of static magnetic fields. To test this, the magnetic field is applied by placing the magnet at a chosen distance from the microfluidic chip for definite time duration (*i.e.* 30 seconds). Conversely, the magnetic field is removed by moving the magnet at least 24 inches away from the microfluidic device for definite time duration (*i.e.* 30 seconds). We also tried applying and removing the magnetic field multiple times (*i.e.* 5 times) to test if the observed effect in worm behavior is repeatable and/or cumulative. After an experiment, the worms are visually inspected to see if they appear healthy and then the microfluidic chip is discarded.

2.4. Image Analysis to Compute Worm Movement

A custom object tracking software is developed to process the recorded videos of worm movement and to extract the x- and y-coordinates of the central skeleton of worm body (*i.e.* head, tail, and centroid) (**Figure 1(c)**). Briefly, the software converts the video frames into grayscale and identifies the background. The background image is calculated by averaging all the frames of the video together. The difference between the background image and a current frame gives a foreground image containing only the moving worm from that frame. Thereafter, the software performs segmentation and recognizes the boundaries of the worm body, which are used to calculate the coordinates of the central skeleton. Average velocity is estimated by computing the instantaneous

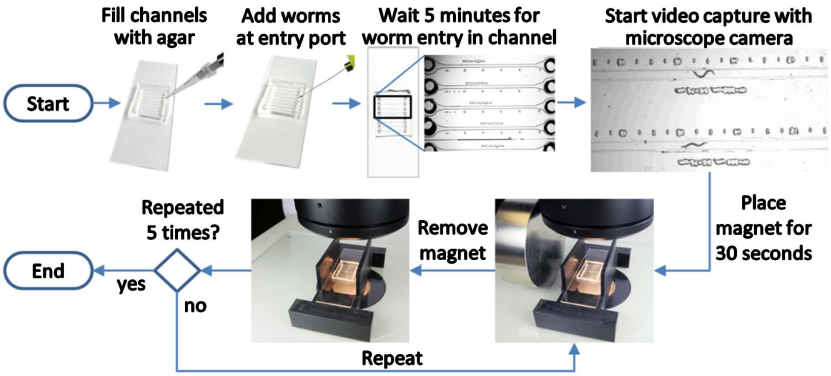


Figure 2. Steps involved in conducting the magnetic field experiment are depicted in the flowchart. The channels are filled with 0.7% agarose and worms are added to the entry port. After the worms enter the channels, the video recording is started. The permanent magnet is placed at a specified distance from the chip holder for 30 seconds and removed. The process is done once or repeated five times. Later the chip is discarded and the video is analyzed.

velocities using the worm centroid locations in successive frames and then averaging these instantaneous velocities over the length of the video [28]. The information from the central skeleton's head and tail locations is used to identify the time instances when the body curls. Both the average velocity and curling/turning behavior are important parameters of worm movement, and the developed software alleviates the trouble of manual image analysis. Statistical analysis of the movement data is performed using GraphPad Prism™ software.

3. Results

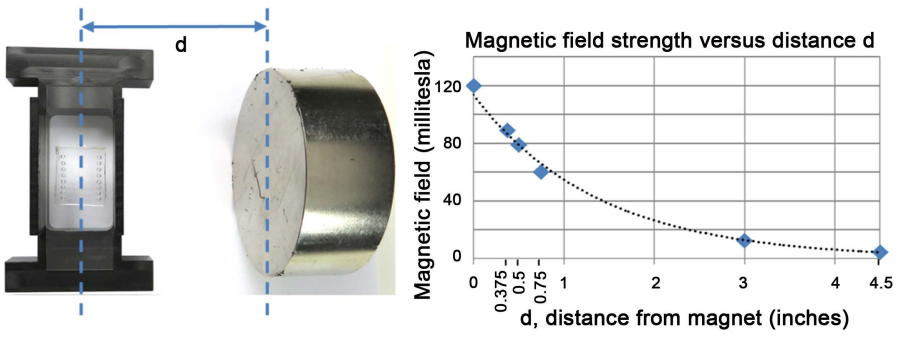
3.1. Characterization of Magnetic Field Strengths

We designed a simple method to apply the static magnetic fields at fixed distances from the microfluidic chip using a permanent magnet. A plastic chip holder is built where the fabricated chip can be placed in. An adhesive tape is used to firmly hold the chip and chip holder on the microscope stage. Because the permanent magnet is heavy, extra caution is required to move the magnet and to ensure that the chip orientation is not changed during moving the magnet. **Figure 3(a)** shows the configuration of the magnet and chip holder. Using wooden blocks as spacers, the magnet is spaced between 0 and 4.5 inches from the chip holder housing the microfluidic chip. We use a cylindrical neodymium permanent magnet (diameter: 3 inches, thickness: 1 inch). With the magnet placed at a specific distance, the field strength at the center of the chip holder is measured with a gaussmeter and a Hall probe (Model 410, Lake Shore Cryotronics™). The measured field strengths at the six positions used in our experiments are shown in **Figure 3(b)**. When the magnet is held closest to the chip holder, the field strength is around 120 milli Tesla (mT). When the magnet is 4.5 inches away from the chip holder, the field strength reduces to nearly zero.

3.2. Effect on the Average Velocity

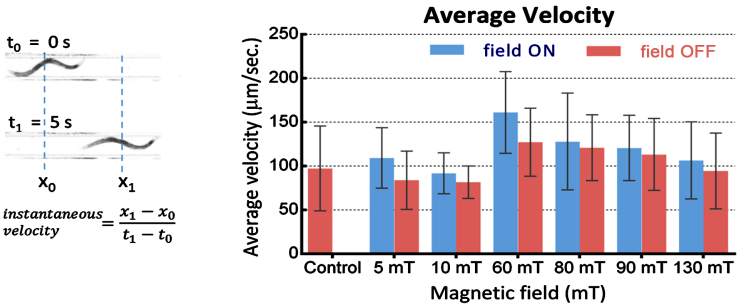
After recording videos of each experiment, the movement of each worm is analyzed separately. The centroid coordinates and the body skeleton in each image frame (image capture speed: 1 frame per second) are extracted using our imaging software. This geometric information is used to identify changes from the worm's natural sinusoidal movement. In general, a worm may exhibit a relative increase or decrease in velocity when exposed to external stimuli. The worm body may turn away from a directional repellant or may curl up to avoid exposure to the repellant. Such curling and omega turns are often observed when worms are exposed to drug chemicals and toxins, such as potassium cyanide [28]. However, in the presence of physical stimuli without any chemicals, such as magnetic and electric fields, the behavioral response of these organisms may be subtle and difficult to quantify by direct observations. As such, we employed our imaging software to automatically extract the average velocities and the percentage of curling and turns.

The average velocity is calculated by taking the mean of all instantaneous velocities (*i.e.* ratio of net displacement to time interval) between successive image frames (**Figure 4(a)**). In **Figure 4(b)**, we plot the average velocities from the different magnetic field experiments. We conducted experiments on the effects of the following field strengths: 5 mT, 10 mT, 60 mT, 80 mT, 90 mT, and 130 mT. In a specific experiment, the average



(a) (b)

Figure 3. (a) The permanent magnet is placed at a specific distance (labeled as "d") from the center of the chip holder; (b) The strength of the magnetic field is measured at the center of the chip holder and plotted in the graph.



(a) (b)

Figure 4. (a) The instantaneous worm velocity is calculated as the ratio of net displacement to the time interval. All the instantaneous velocities are averaged to produce the average velocity of an individual worm; (b) The average velocities of all worms for the different magnetic field strengths are plotted; both during the periods of placing the magnet (*i.e.* field ON) and removing the magnet (*i.e.* field OFF).

velocity of every worm is further averaged to obtain an overall average velocity. In the control experiment, worms are allowed to move in their respective channels in the absence of external magnetic field, and the average velocity is 97.38 ± 48.3 µm/second. For experiments with magnetic fields, worms are allowed to enter the channel and the permanent magnet is placed at a definite distance as shown by **Figure 3**. The magnet is placed for 30 seconds (*i.e.* field ON) and removed for 30 seconds (*i.e.* field OFF). The process is repeated for 5 cycles. The recorded videos are partitioned into two sections: image frames where the field is ON (blue bars, **Figure 4(b)**) and image frames where the field is OFF (red bars, **Figure 4(b)**). The average velocities of the two sections are calculated and shown in **Figure 4(b)**. In all experiments with magnetic field exposure, the range of average velocities is between 84 µm/second to 128 µm/second. The average velocity at 60 mT (with field ON) is statistically significant ($p < 0.05$) compared to others in the group with magnetic field exposure. There is no significant difference amongst the other average velocities. Interestingly, in all experiments, the average velocities with field OFF (red bars, **Figure 4(b)**) are lower than the average velocities with field ON (blue bars, **Figure 4(b)**), even though the relative decrease in velocity (7 µm/second to 30 µm/second) is not significant.

3.3. Effects on the Turning and Curling Behavior

A worm tends to turns away from an aversive environment (e.g. drug chemicals or toxins) or can curl its body to avoid exposure to the environment [28]. However, such turning and curling actions are also a part of their repertoire of behavioral traits in the natural environment. Hence it is important to identify whether the frequency of turning and curling events are significantly different in experimental tests. **Figure 5** shows representative images of the worm's body posture during turning and curling in different magnetic field strengths. Images are grouped in two categories: with field ON and field OFF. The time points of the images are labeled next to individual images and red arrows indicate the direction of worm movement. In all magnetic fields, the changes in body postures and the time taken to accomplish a turn appear similar with no noticeable differences. High magnetic field strengths (90 mT to 120 mT) do not appear to hinder the turning and curling of *C. elegans*.

Figure 6 shows the percentage of worms that turned and curled during the first 30 seconds of placing the magnet or removing it (*i.e.* field ON and field OFF periods, respectively). The imaging software identifies a turn or curl when the head and tail turn inwards such that the net distance between them is less than one half of the body length. The time instances when a turn or curl occurred is outputted by the software and random videos are manually verified for accuracy and consistency of this result. Typically, a worm takes around 7 to 12 seconds to complete a turn. If a worm decides to turn away from the magnet, it would possibly indicate that the worm does not prefer being in the magnetic field. In the control experiment where no magnetic field is applied throughput the video, the average percentage of turns and curls is around 71.5% ± 49%. There is no statistically significant difference between the percentage of curls and turns in experimental and control groups. The result indicates that curling or turning is probably not a clear indication of sensitivity to magnetic fields.

In the earlier discussion, we counted the number of body turns or curls within the first 30 seconds of placing or removing the magnet, which is sufficient time for worms to complete a turn or curl. We wished to confirm whether the worm response was different if the steps of placing and removing the magnet were repeated over

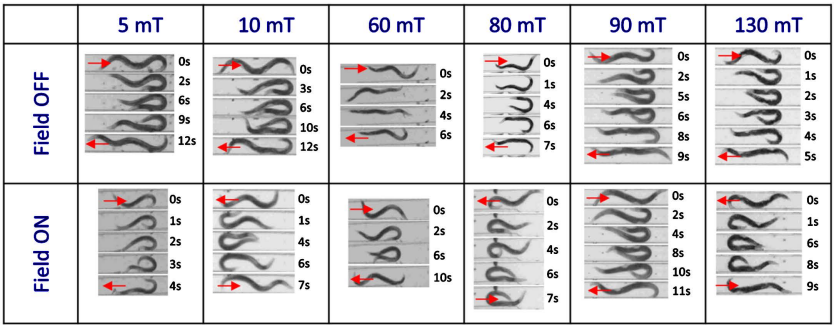


Figure 5. Shown are the time-lapsed images of the worm during the turning behavior and under different magnetic field. Images are grouped into time periods of placing the magnet (*i.e.* field ON) and removing the magnet (*i.e.* field OFF).

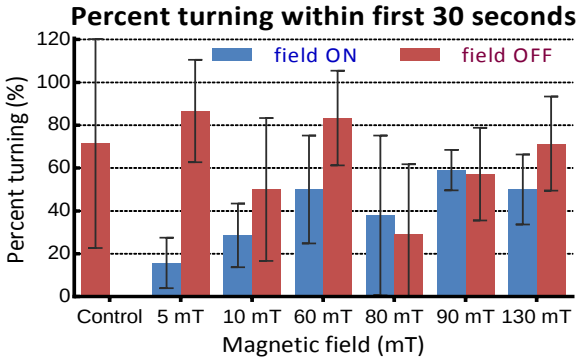


Figure 6. The plot shows the percentage of worms turning or curling within first 30 seconds of placing the magnet (*i.e.* field ON) or removing the magnet (*i.e.* field OFF).

multiple cycles. **Figure 7** shows the percentage of worms that turned or curled during the first 30 seconds of placing or removing the magnet through 5 consecutive cycles. Here the results from all magnetic field experiments are combined with field strengths between 5 mT to 120 mT (**Figure 3**). The plot indicates that percentage of turning or curling is consistently within a fixed range (30% to 70%) over the course of the experiment, and there is no statistically significant difference between the values.

4. Discussion

We reviewed the protocols used in our experimental procedure and tested a number of alternatives to see if the behavioral response of worms changed because of the alterations. A discussion of some alternatives to our previously discussed experimental protocols follows here.

The choice of media for filling the microfluidic channels was 0.7% agarose in M9 buffer. The standard aqueous M9 buffer solution also could be used as the migration media. But we observed significant drifts in the worm movement when an aqueous media was used. In addition, because the liquid is exposed to the outside air through the entry and exit ports, even a small change in liquid pressure is noticeable under the microscope. The liquid pressure also can alter the worm movement and influence the derived results. On the other hand, agarose gel provides a good resistance for worms to push against and attempts to mimic the natural soil environment.

The choice of the permanent magnet was selected based on the available physical sizes and strengths of the magnets. The chosen permanent magnet has a wide diameter to produce a uniform field near and around the chip holder. A gaussmeter was used to check the uniformity of the magnetic field. Magnets with smaller diameter (<2 inches) were seen to produce non-uniform magnetic fields around the chip holder. Permanent magnets with field strengths greater than 120 mT were not readily available. Alternating magnetic fields can be tested with duty cycles around 60 Hz and considerably high power than permanent magnets [22] [23].

Our tests were performed on L4-stage and young adult *C. elegans*. We chose not to use adult worms as they may have eggs within them that could influence their natural movement. Since the literature suggested that adult

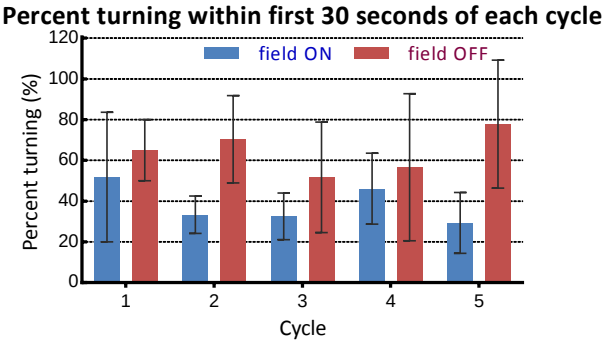


Figure 7. The plot shows the percentage of worms turning or curling within first 30 seconds of placing the magnet or removing the magnet, repeated over five cycles. The results are combined from all magnetic field experiments.

worms have some levels of magnetite particles within their bodies [21], we also tested adult worms in the experimental setup. However, with adult worms, we did not see any noticeable difference in behavior upon exposure to static magnetic fields. Rather, the adult worms appeared more sluggish than young adults and more hesitant to enter the channels, even in control experiments.

To test the effect of field directionality on worm behavior, we tried flipping the magnet and thereby changing the direction of the field. We saw no convincing results to indicate worms prefer the north/south pole or can sense the change in field directions. We further tested putting two magnets on either side of the chip holder and re-orienting them to different directions and distances. No noticeable change in worm movement was observed.

5. Conclusion

In conclusion, the results presented here indicate that effects of static magnetic fields, in the range of 5 mT to 120 mT, on the average velocity and the turning and curling behavior of wild-type *C. elegans*, are not significant. A microfluidic device setup was used to test single worms under different field strengths and an imaging software was used to automate the steps involved in computing worm movement. A number of alterations in the experimental protocol were tested, and our data suggest that static magnetic fields do not produce significant change in movement or direction of *C. elegans* nematodes.

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