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Tips & Tools

Laboratory Activity Using Accessible Microfluidics to Study Nematode Behavior in an Electrical Field †

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INTRODUCTION

Microfluidics enables researchers to shrink experiments to a small scale and overcome limitations of cost, accessibility, and time (1). Unfortunately, most commercial microfluidic devices are too expensive for classrooms, and DIY techniques to create them are imprecise or require materials that are uncommon in classrooms, such as pressure-sensitive adhesives or transparent silicone casts of channel molds (1–3). We present a quick, simple, and low-cost technique for creating machine-cut, reusable microfluidic devices for a variety of classroom activities using readily available craft supplies found in many schools.

In creating this approach, we focused on materials and techniques to quickly create and test microfluidic device designs. We created microfluidic devices by sandwiching double-sided adhesive craft paper containing patterns of channels between two flat sheets of plastic transparency. Using open source vector graphic design software, we created layouts of microfluidic channels for different experiments and cut them into the adhesive craft paper using a desktop craft cutter. Cutting and assembling a single device takes about five minutes. Students tested the microfluidic devices in outreach activities that included observing the laminar flow of fluids and the behavior of microorganisms in an electric field. We successfully cleaned and reused these microfluidic devices multiple times. This approach creates opportunities for K–12 teachers to use microfluidic devices in a wide range of classroom investigations, including physics (e.g., studies of fluid flows at low Reynold's numbers), chemistry (e.g., chemical gradients), and biology (e.g., behavior of microorganisms) (4).

We demonstrate how microfluidic devices can be used to study the behavior of nematodes. Nematodes are a terrific organism for studying biology in the classroom, as they are not dangerous and are inexpensive, easy to acquire, easily visualized with low-magnification microscopes, and display easily observable responses to many environmental stimuli. Using the devices to study nematode behavior is an excellent way to explore key biological concepts, such as biological systems and models and making predictions about organism behavior (Next Generation Science Standards [NGSS] 4). Here, we use *Steinernema* nematodes, which are entomopathogenic (insect eating). Entomopathogenic nematodes are important in agriculture and are used as a safe, biological pesticide that can help reduce or eliminate the use of chemical pesticides (5–7). *Steinernema* nematodes and those of other genera kill a variety of insect pests, including cutworms, fungus gnats, and Japanese beetles (8).

Steinernema nematodes use environmental cues to find food, such as volatile organic compounds released by prey insects (8, 9). Many nematodes also display electrotaxis—movement in response to an electrical field (10, 11). We use microfluidic devices to study the electrotaxis of *Steinernema feltiae*, soil-dwelling nematodes that can be purchased online. The techniques presented in this paper demonstrate how students can use simple and reusable microfluidic devices to study nematode behavior in electrical fields. As the reason for the movement of nematodes in electric fields is not currently known, this activity provides an opportunity for students to propose hypotheses and potentially test them in subsequent experiments.

PROCEDURES

Safety

Steinernema nematodes are not harmful to humans. Care should be taken with hot agar to avoid burns.

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Materials

A detailed list of materials can be found in Appendix 1.

Experiment

Teachers prepare devices ahead of time and load them with nematodes suspended in a solution of agar at time of activity (see Appendix 1). Students place a loaded device under a microscope, and tape the device to the microscope stage to keep it in place. Students count the number of nematodes in each zone in the channels at time zero (Fig. 1); students should choose how to count nematodes that may be positioned on the lines between zones, deciding to define them as either belonging to the zone to the right or to the left of the line, and using this counting approach consistently throughout experiments. Next, students attach the titanium electrodes to a 9V alkaline battery using alligator clips (Fig. 2). Be sure to attach the right lead to the negative terminal, and the left lead to the positive terminal. After connecting the battery, students immediately start a timer and count and record the number of nematodes in each zone every minute for a total of five to ten minutes (see Nematode Data Collection Worksheet in Appendix 3). Depending on grade level, students may be able to perform some of the preparation steps (see Activity Concept Map, Fig. 3, also Appendix 5).

Notes

1. We measured the voltage across the channels as 1.7 to 4.0 V with electrodes connected to a 9V battery and channels filled with agar as described in Appendix 1.
2. Bubbles which may accumulate at the end of the channel connected to the negative terminal of the battery may disrupt the electric field and alter the directional movement of nematodes.

CONCLUSION

We present a simple activity for students to learn about nematode behavior through active learning. We tested this activity successfully in several informal science lab activities for middle and high school students through Discovery Outreach Programs at the Discovery Building at the University of Wisconsin-Madison. We used a customized version of the Science, Technology, Engineering, & Math (STEM) Learning Activation Lab Survey (www.activationlab.org/), which assesses STEM learning activation in youth (Fig. 4). Following the activity, select student comments mentioned nematode behavior, e.g. "I learned what nematodes are and how they react to certain variables," and "I learned about the biology of a specific parasite." Our activity is well suited for teachers to make connections to ecology-focused objectives in science curricula.

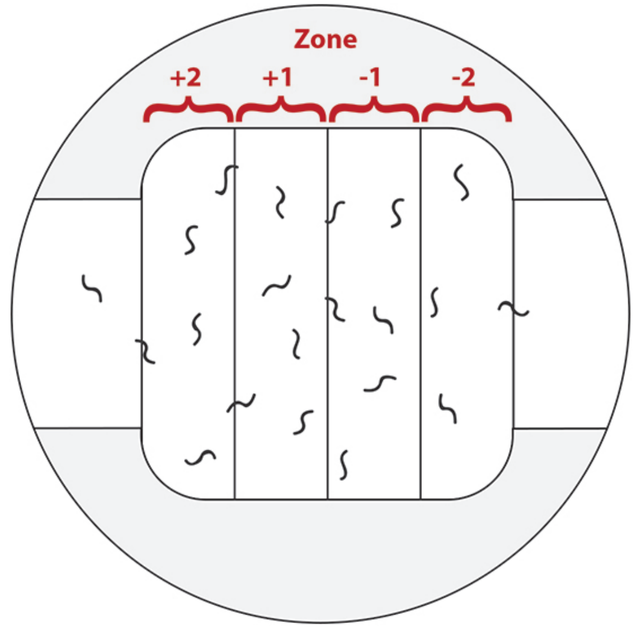

FIGURE 1. Diagram of nematodes in zones under microscope. Nematodes respond to an applied electric field by moving through different zones of a microfluidic channel.

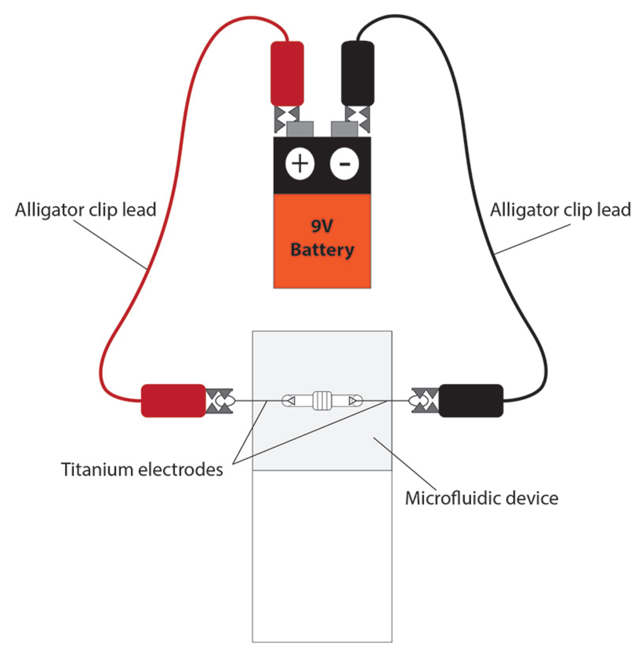

FIGURE 2. Microfluidic setup for the electrotaxis activity. A 9V battery supplies an electric field across a microfluidic channel.

SUPPLEMENTAL MATERIALS

Appendix 1: Device assembly and activity preparations
Appendix 2: Nematode life cycle
Appendix 3: Nematode electrotaxis data collection worksheet
Appendix 4: Predicting laminar flow in microfluidic devices

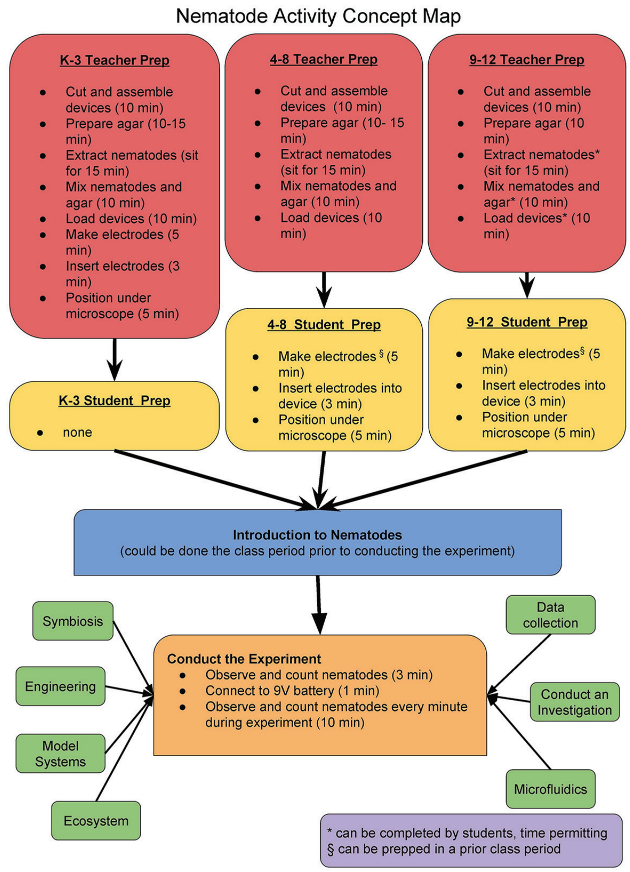

FIGURE 3: Activity Concept Map. An overview of suggested preparation and timing for activity (see Appendix 5 for full size image).

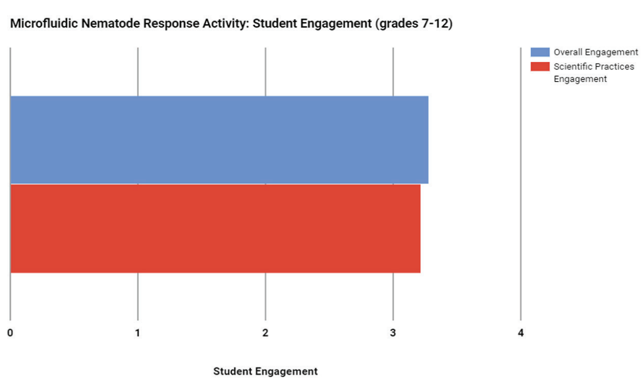

FIGURE 4. Analysis of student engagement (*N*=40). An engagement score of 3 or above is considered to be a high level of engagement based on the validated activation survey.

Appendix 5: Nematode Activity Concept Map
Appendix 6: Basic microfluidic device file (SVG format)
Appendix 7: Electrotaxis microfluidic device file (SVG format)
Appendix 8: Electrotaxis printed backs file (SVG format)

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REFERENCES

1. Whitesides GM. 2006. The origins and the future of microfluidics. Nature 442:368–373
2. Friend J, Yeo L. 2010. Fabrication of microfluidic devices using polydimethylsiloxane. Biomicrofluidics 4(2):026502.
3. Yuen PK, Goral VN. 2010. Low-cost rapid prototyping of flexible microfluidic devices using a desktop digital craft cutter. Lab Chip 10:384–387.
4. Stilwell MD, Nepper JF, Clawson ED, Blair V, Tangen T, Weibel DB. 2017. Exploring predatory nematode chemotaxis using low-cost and easy-to-use microfluidics. Am Biol Teach 44(9):753–762.
5. Shapiro-Ilan DI, Han R, Dolinksi C. 2012. Entomopathogenic nematode production and application technology. J Nematol 44(2):206–217.
6. Griffin CT. 2012. Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. J Nematol 44(2):177–184.
7. Shapiro-Ilan DI, Gaugler R. Nematodes. Biological control: a guide to natural enemies in North America. Cornell University College of Agricultural and Life Sciences, n.d. Web. 25 May 2017. https://biocontrol.entomology.cornell.edu/pathogens/nematodes.php
8. Dillman AR, Guillermin ML, Lee JH, Kim B, Sternberg PW, Hallem EA. 2012. Olfaction shapes host–parasite interactions in parasitic nematodes. Proc Natl Acad Sci 109(35):E2324–E2333.
9. Hallem EA, Dillman AR, Hong AV, Zhang Y, Yano JM, DeMarco SF, Sternberg PW. 2011. A sensory code for host seeking in parasitic nematodes. Curr Biol 21(5):377–383.
10. Ilan T, Kim-Shapiro DB, Bock CH, Shapiro-Ilan DI. 2013. Magnetic and electric fields induce directional responses in Steinernema carpocapsae. Int J Parasitol 43(10):781–784.
11. Shapiro-Ilan DI, Lewis EE, Campbell JF, Kim-Shapiro DB. 2011. Directional movement of entomopathogenic nematodes in response to electrical field: effects of species, magnitude of voltage, and infective juvenile age. J Invertebr Pathol 109:34–40.