microPublication BIOLOGY

12/7/2020 - *Open Access*

Affordable *C. elegans* tracking system for classroom use

Nicholas Leonard[1], Andrés G. Vidal-Gadea[2§]

[1]Normal University High School, Normal, Illinois

[2]School of Biological Sciences, Illinois State University, Normal, Illinois

[§]To whom correspondence should be addressed: avidal@ilstu.edu

Abstract

For decades the nematode *C. elegans* has served as an outstanding research organism owing to its unsurpassed experimental amenability. This advantage has also made this tiny worm an attractive vehicle for science instruction across higher learning institutions. However, the prohibitive cost associated with the automated behavioral assessment of these animals remains an obstacle preventing their full adoption in undergraduate and high school settings. To improve this situation, we developed an inexpensive worm tracking system for use by high school interns and undergraduate students. Over the past two years this tracker has been successfully used by undergraduate students in our introductory Cell and Molecular lab (BSC220) at Illinois State University. Here we describe and demonstrate the use of our inexpensive worm tracking system.

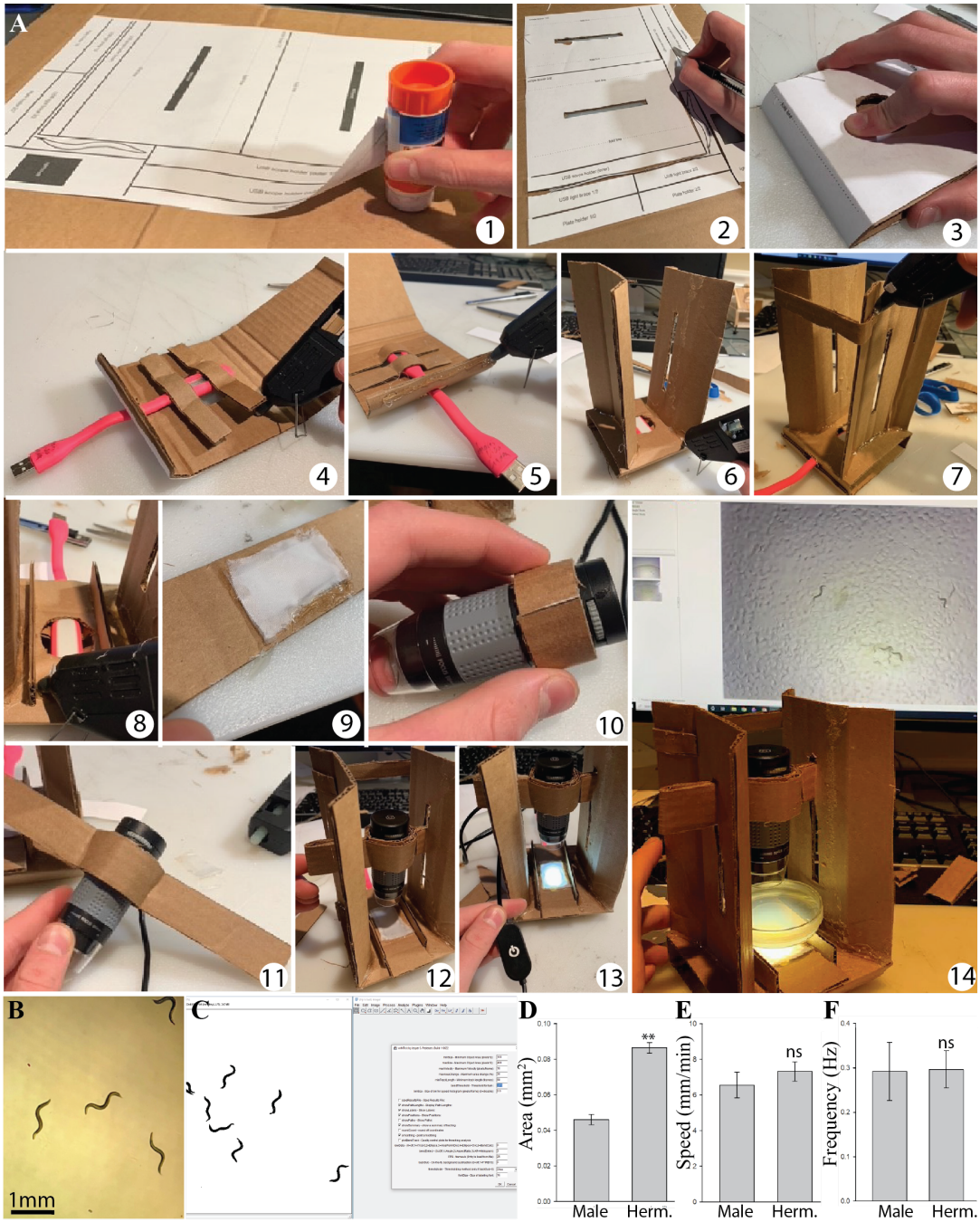

Figure 1.

Construction and use of low cost *C. elegans* tracking system.

A) The assembly of our worm tracker takes under one hour (1-14). See build instructions below for step by step instructions. **B)** The Worm Tracker is used to film day one adult hermaphrodite and male worms. **C)** Tracking of male and hermaphrodite worms using the wrMTrck plugin in imageJ. **D)** The wrMTrck reveals hermaphrodite worms have a larger area than males (t-test, **p<0.001). However, males and hermaphrodites did not differ significantly in their speed (**E**), or body bend frequencies (**F**).

Description

From the beginning of *C. elegans*' inception as a genetic model organism by Sydney Brenner (Brenner 1974), the ability to measure and quantify behavior in these nematodes led to numerous and powerful insights (Apfeld *et al.* 2018). The experimental amenability of worms makes them not just superb research subjects but also useful pedagogical tools. While excellent classroom additions to illustrate many biological processes, the educational potential of worms has lagged due to the expensive equipment required for their study. In most but the wealthiest educational settings lack of equipment discourages individual exploration and falls short of the promise owed to young and driven students. One of the places where this is felt strongly is in the automated quantification of animal behavior. While many systems have been developed

over the years and are currently used across the world, automatic quantification of behavior in *C. elegans* has (until recently) remained in the realm of a few wealthy or specialized labs. However, recent advances streamlined and made affordable the automated study and quantification of behavior in *C. elegans* and other organisms. For example, the Haspel lab at NJIT made use of the recently developed TIERPSY behavioral software (Avelino *et al*. 2018) to build numerous worm tracking systems that are at once user friendly, and able to achieve levels of kinematic analysis that previously required considerably more expensive setups (Deng *et al*. 2020).

After building and witnessing the ease of use and power of the setup described above, we decided to reverse engineer a similar worm tracker system for classroom deployment. Our intention was to develop a bare-bones system that allowed as many students as possible to engage in individualized research projects. We implemented this worm tracker in an introductory Cellular and Molecular Laboratory course at Illinois State University (BSC220). In this class, small groups of (three) students built and regularly used the trackers to film and quantify the behavioral phenotypes of *C. elegans* worms illustrating different cellular or genetic manipulations. Because lab fees associated with molecular labs can be restrictive, a key consideration in our approach was cost reduction (along with ease of build and use). Here we describe an affordable (~$30) worm tracking system whose construction and use is amenable to high school and undergraduate students. We provide a list of materials, step by step assembly instructions, and illustrate its ease of construction and use to measure anatomical and kinematic differences between male and hermaphrodite *C. elegans* worms.

The process of filming and analyzing animal behavior involves three distinct components. First is the construction of a worm tracking system which is the focus of this manuscript. The worm tracking system needs to be able to image nematodes at sufficient magnification and contrast to allow machine vision algorithms to locate and track individual animals. While inexpensive USB microscopes are available for purchase (and indeed are used in this build), one key feature of useful worm trackers is their ability to provide optimal underneath illumination in order to produce homogeneous, high-contrast, images. The second component of the system consists of an image acquisition program used to film the animals. There are several free programs available (such as micro-manager), as well as programs freely distributed with the USB microscopes used in the tracker. The third and final component of the ensemble is a worm tracking program able to detect and track individual nematodes from a movie or series of images. Presently TIERPSY is an outstanding free kinematic package with widespread use among worm labs (Javert et al. 2018). However, because of its system requirements we opted for the more limited, but easier to install (and use), imageJ plugin named wrMTrck (Nussbaum-Krammer *et al.* 2015). The wrMTrck plugin is able to extract many useful parameters such as animal size, velocity, and body bend frequency.

Methods

Worm Tracker Construction

Refer to Figure 1A for a series of images illustrating the assembly steps described below.

1. Print and glue the stencil (provided in the Supplementary Materials) to a sheet of corrugated cardboard (thicker cardboard is harder to cut but provides a stronger build).
2. Use an x-acto knife (or scalpel) to cut the pieces to be used in the tracker following directions on stencil.
3. Prefold pieces (where indicated), beginning assembly by folding the tracker base.
4. Glue the USB light braces to secure the USB light beneath the tracker making sure the light is aligned with the circular hole on the base, and that the LED faces upwards (through the hole). The USB plug from the LED light should protrude through the rectangular hole cut in the back of the base.
5. Use glue to close the base up.
6. Prefold and glue the scope towers along the longest sides of the base.
7. Glue the tower brace strip to the top of both tower arms to provide structural reinforcement.
8. Cut and glue the worm plate holders onto the tracker base.
9. Cut a piece of diffuser cloth and glue it to the diffuser holder (a kimwipe will serve as a diffuser if fabric is not available).
10. Wrap the Inner microscope holder tightly around the top of the USB scope and glue it onto itself, creating a tight sleeve for the scope. Make sure to leave the USB light dial (above) and the focus dial (below) on the microscope free to rotate.
11. Fold in half each of the outer scope holders gluing the ends to the inner holder.

12. Insert the folded ends of the microscope holders through the cut in the towers; they should be tight but allow adjustment.
13. Connect the USB light to a USB LED dimmer, and this to a USB power outlet. The USB dimmer allows to control the amount of light hitting the setup which is important to obtain homogeneous illumination.
14. Place a plate with nematodes on the plate holder and connect the microscope to a windows PC with preinstalled acquisition software, imageJ, and the wrMTrck plugin to start using the setup.

The instructions above will produce a worm tracking system which, depending on the materials and number built, can cost as little as $30 depending on material selection (see below). We feel that this is a cost that is permissive in the context of most secondary and tertiary educational settings. Several upgrades can be implemented to increase the longevity (and cost) of the tracker. For example, replacing the cardboard with plastic or wood would produce a much stronger and longer lasting device. We felt that for the sake of minimizing difficulty and cost we show here the most inexpensive and simplest build. The tracker described here was successfully constructed and used by our BSC220 students, as well as by high school interns in our lab.

Image Acquisition

Once the worm tracking system is assembled and connected to a windows machine, the acquisition software provided by the microscope manufacturer can be used to film freely behaving animals. For the example presented here we used Digital Viewer (available online, see below) which works with the various brands of USB microscopes we tested. In addition, the $20 USB microscope we tested for this in this manuscript comes with a calibrating ruler, and with an Android phone connector which enables students owning this type of device to record movies without needing a computer. Prior to filming the diffuser slide positioned below the plate of worms. This diffuser ensures that the LED light is evenly distributed throughout the plate. Uniform lighting is of paramount importance during filming. A plate with worms to be filmed is placed above the diffuser. It is best to not attempt filming too many worms at one time, as this increases the likelihood of animals colliding (which complicates tracking). A copper ring can be placed one the plate to keep all the animals within a small area in the field of view (Simoneta and Golombek 2006). We usually film through the lid of the plate but this can also be removed (if reflections appear on its surface). Prior to filming, we turned off the LED lighting that comes onboard with the USB microscopes. This type of illumination is not optimal when filming worms. We used the focus dial on the microscope to ensure animals are in focus and change the amount of underneath LED lighting using the connected LED dimmer. Ideally, plates are devoid of any visible imperfections that might challenge animal detection. To compare worm strains with different locomotor abilities we usually make movies with five to ten animals lasting about 30-60 seconds. Healthy wild type worms crawl at about 1cm/min using body bends with 0.5Hz of frequency (Vidal-Gadea *et al.* 2011), therefore this duration is sufficient for most rudimentary analyses. We recommend making additional movies at this point, as it is relatively easy to make additional movies in anticipation of any possible challenge during the analysis step. Movies of males and hermaphrodite worms used here are available as supplementary materials.

Animal Tracking

Once the animals have been filmed and avi (or TIFF) files generated, they can be imported into the analysis software in order to track the worms and generate descriptive statistics. As we mentioned above there are many available tracking packages, including a few freely available ones. For use in a research lab setting, TIERPSY is likely the best option providing a plethora of useful parameters that include centroid-based metrics (e.g. velocity), and posture based metrics (e.g. body curvature). An alternative that is more amenable to a high school or undergraduate lab setting is the wrMTrck imageJ plugin written by Jesper Pedersen (see below, Nussbaum-Krammer *et al.* 2015). Among the useful parameters measured by this program are body bend frequency, speed, and animal size. These metrics are sufficient to separate populations of animals that perform differently due to a plethora of possible experimental manipulations.

Sample Application

We illustrate the use of the worm tracking system by comparing day one adult male and hermaphrodite N2 *C. elegans* worms. Five to ten Day 1 adult male or hermaphrodite worms were moved to an unseeded NGM plate inside of a copper ring to prevent animals from exiting the field of view (Simoneta and Golombek 2006). After two minutes of acclimation we used the worm tracker to film the animals for 30 seconds at 25fps using our worm tracking system (Figure 1B). Movies were exported as uncompressed AVI files (UYVY codec), uploaded to imageJ and analyzed using the wrMTrck plugin (Figure 1C). Hermaphrodite animals were significantly larger (Figure 1D), but neither faster (Figure 1E), nor did they have greater body bend frequency than adult males (Figure 1F).

Reagents

Organisms

We used ten males and ten hermaphrodite *C. elegans* worms of the N2 strain obtained from the Caenorhabditis Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure (P40 OD010440).

Statistics

We used SigmaPlot 11(Systat) to compare the metrics from both populations using two tailed t-tests.

Hardware

The following hardware was used to construct the worm tracking system:

A Windows computer may be required for filming, and it is required for analysis (not included in cost calculation).

USB microscope. $20. Can be obtained from Amazon (ASIN: B08BFJZJ6N)

USB LED light. $9. One needed (but comes in 8-pack) from Amazon (ASIN: B088KPZYS9)

USB LED dimmer. $9. Suggested. Can be obtained from Amazon (ASIN: B01I17OHVQ)

Diffuser fabric. $8. Enough for many setups. Can be obtained from Amazon (ASIN: B01JGY8U66)

Total build $46 ($31/ea if eight units are built)

Software

For use with the USB microscope there are many freely available softwares. We use Plugable Digital Viewer (although micro-manager should also work). Digital Viewer: https://plugable.com/drivers/microscope/

To run the worm tracking software, it is necessary to first install imageJ which is an open source Java image processing program found here: https://imagej.net/Fiji/Downloads

Worm tracking in imageJ is accomplished by the wrMTrck plugin created by Jesper Pedersen available here: http://www.phage.dk/plugins/wrmtrck.html. A useful set of written and video tutorials are available online here: (http://www.phage.dk/plugins/download/wrMTrck.pdf and here: https://www.youtube.com/watch?v=V9M0s0E-0uI respectively.

Acknowledgments: We thank the Caenorhabditis Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure (P40 OD010440), for providing strains.

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Funding: Funding was provided by NIH Grant 1R15AR068583-01A1, and NSF Grant 1818140 to A.G.V.-G.

12/7/2020 - *Open Access*

Author Contributions: Nicholas Leonard: data curation, investigation, methodology. Andrés G. Vidal-Gadea: conceptualization, methodology, funding acquisition.

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Citation: Leonard, N; Vidal-Gadea, AG. (2020), Affordable *C. elegans* tracking system for classroom use. microPublication Biology.