The Biophysicist

Quantifying Planarian Behavior as an Introduction to Object Tracking and Signal Processing

Nicole C. Stowell[1], Tapan Goel[2], Vir Shetty[3], Jocelyne Noveral[1], Eva-Maria S. Collins[1,2,*]

[1]Department of Biology, Swarthmore College, Swarthmore, PA 19081, USA
[2]Department of Physics, University of California San Diego, La Jolla, CA 92093, USA
[3]Department of Physics, Swarthmore College, Swarthmore, PA 19081, USA

ABSTRACT Answers to mechanistic questions about biological phenomena require fluency in a variety of molecular biology techniques and physical concepts. Here, we present an interdisciplinary approach to introducing undergraduate students to an important problem in the areas of animal behavior and neuroscience—the neuronal control of animal behavior. In this lab module, students explore planarian behavior by quantitative image and data analysis with freely available software and low-cost resources. Planarians are ∼1–2-cm-long aquatic free-living flatworms famous for their regeneration abilities. They are inexpensive and easy to maintain, handle, and perturb, and their fairly large size allows for image acquisition with a webcam, which makes this lab module accessible and scalable. Our lab module integrates basic physical concepts such as center of mass, velocity and speed, periodic signals, and time series analysis in the context of a biological system. The module is designed to attract students with diverse disciplinary backgrounds. It challenges the students to form hypotheses about behavior and equips them with a basic but broadly applicable toolkit to achieve this quantitatively. We give a detailed description of the necessary resources and show how to implement the module. We also provide suggestions for advanced exercises and possible extensions. Finally, we provide student feedback from a pilot implementation.

KEY WORDS image analysis; center of mass tracking; animal behavior; hands-on research; inquiry-based learning; undergraduate

I. INTRODUCTION

Active learning opportunities for undergraduate students are a necessary vehicle to integrate knowledge across disciplines. They foster the development of citizens equipped to solve the complex problems of the 21st century, which do not fall into discrete disciplinary compartments (1–3). Acquisition of foundational knowledge specific to each discipline is essential. However, students tend naturally to compartmentalize their knowledge, struggling to incorporate concepts and skills that they have developed across disciplines. In our experience, this is especially challenging for students in the introductory biological teaching laboratory context. Moreover, premajors and non–science, technology, engineering, and mathematics (STEM) majors may not embrace physics and mathematics as core skills required for the study of biology (4–6). Additionally, despite the daily use of computers and smartphones as

"*" corresponding author

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a way of accessing information, students broadly lack the experience of using computational tools to generate and analyze data that allow us to characterize and quantify biological phenomena. On the other hand, students who gain interdisciplinary experience develop an ability to integrate and apply knowledge across subject areas (2). This skill uniquely positions students to succeed in their future academic and professional careers. As educators, it is our responsibility to embrace a multidisciplinary approach to inquiry-based learning in biology and develop students' awareness of the value of integrating across multiple scientific fields (5, 7).

Here, we present a 2-wk laboratory module aimed at premajor undergraduate—freshman and sophomore level—students that requires minimal resources. We use freshwater planarian behavior as a system to teach object tracking and time series analysis as tools for quantifying behavior and dissecting its molecular basis with the use of basic computational skills and inexpensive equipment. Planarians are invertebrates, a few centimeters long, allowing for behavioral observation with the unaided eye. They are famous for their ability to regenerate and a popular model for stem cell research (reviewed, e.g., in Ref 8). In contrast to existing publicly available planarian lab modules, which are qualitative and center around regeneration outcomes (9–11), this laboratory module is centered around quantifying animal behavior and dissecting its molecular mechanism. The module incorporates physical concepts such as center of mass, velocity and speed, and periodic functions. For more advanced students, we provide ideas for introducing more advanced concepts such as Fourier transforms, the Nyquist sampling theorem, and mean square displacement to characterize motion.

A. Scientific and pedagogical background

Experiments in biological physics frequently involve the detection and center of mass tracking of agents, cells, or whole organisms with image data (5, 12–23). Classroom activities that teach undergraduate students basic concepts in object detection and center of mass tracking of isolated agents—without requiring advanced computational image analysis—equip students with the necessary foundation for thinking about quantifying motion without overwhelming them with technical difficulties. While the tracking of (fluorescent) microbeads is a popular laboratory module for teaching image analysis and Brownian motion in introductory physics courses (5, 24–27), it requires specialized and expensive equipment—camera-equipped compound microscopes (ideally with fluorescence capability)—and may be less attractive to life sciences majors.

Instead, tracking the motion of small invertebrates, with or without external stimuli, provides an accessible and low-cost alternative to expose undergraduates to object detection, signal processing, and motion quantification and does not require approval by the Institutional Animal Care and Use Committee. Furthermore, neuroethology—how animal behavior is controlled by the brain—has emerged as an active interdisciplinary research area intersecting neuroscience, ethology, and computer vision (28, 29). Its understanding requires the integration of skills and knowledge across disciplines. Therefore, quantification of behavior serves as a great introduction to interdisciplinary research for students, familiarizing them with biological concepts, while demonstrating how methods from other disciplines contribute to the advancement of biological and medical research. Locomotion in freshwater planarians is an example of such an accessible system to introduce students to the quantification of behavior. The ventral side of a planarian is covered with cilia that enable it to move with a smooth gliding motion (30, 31). Planarians cannot swim in the bulk of a fluid: they either move on the surface, taking advantage of the fluid's surface tension, much like water striders, or they glide on the bottom of the container (30). If cilia beating is impaired, planarians switch to a musculature-based gait termed peristalsis (21, 32). In response to a noxious stimulus, such as amputation, extreme heat, or exposure to certain chemicals, a planarian will exhibit a distinct inchworm-like

behavior of asymmetric contraction-elongation cycles called scrunching (21, 33). Scrunching is not inching, because it does not extend into the third dimension (21). Thus, planarians have 3 distinct gaits that can activate in specific circumstances, can be triggered externally, and can be distinguished by quantitative behavioral analysis (21, 33).

In this lab module, students quantitatively study and perturb planarian gliding behavior and compare it to the scrunching gait. They learn how to handle planarians, imaging techniques, image processing with open source tools such as Fiji (34), a distribution of the ImageJ Java-based image processing program, and data analysis with Microsoft Excel, Google Sheets, OpenOffice, or other freeware alternatives. They use pharmacological and genetic manipulations to probe the molecular mechanisms of behavior and to assess the effects of these perturbations on neuronal pathways by quantifying alterations in behavioral readouts. Students can then develop hypotheses regarding the application of this new knowledge in the context of medical intervention. The "low-tech" nature of the experiments allows the students to engage directly with the scientific and technical concepts instead of working through a series of operations on a black box. As a result, students get hands-on experience with the scientific method, gain experience with animals in research, work collaboratively with their peers, and see how the practice of science transcends disciplinary boundaries.

Existing lab modules studying invertebrate behavior are often qualitative and do not incorporate computational image analysis (35–39), or they require more expensive equipment such as microscopes to track *Caenorhabditis elegans* (40) or *Daphnia* (41) and may use specialized tracking software (42, 43), which makes it challenging to implement such modules in a large introductory biology class. Furthermore, the use of specialized tracking software may require some amount of programming literacy, making them more suitable for advanced courses. In contrast, for the lab module described here, we chose freely available tools whose usage requires no prior training, which ensures that the students can follow and understand the process from the raw image data to the calculated parameters that they use to quantify the observed motion. This allows students with diverse backgrounds to start on an "even playing field." Moreover, the tools we are using here are widely used in biology. ImageJ is frequently used to quantify electrophoresis gels and fluorescence in immunohistochemistry images, and spreadsheets are also used in STEM and non-STEM disciplines. This broad software relevance helps to get student buy-in because they can imagine future usage and thus appreciate the value of learning how to work with these programs (44–46). The module can be further customized through the addition of more sophisticated experimental and computational components, depending on availability of time, resources, and student preparation.

B. Course context and assessment

This laboratory module was developed as part of a semester-long introductory biology course taught at Swarthmore College, a highly competitive small residential liberal arts college, for a student audience with diverse disciplinary backgrounds. The racial and ethnic identity of the Swarthmore student population is 6% African American, 11% International, 17% Asian, 13% Hispanic, and 42% White (47). The majority of the introductory biology class is composed of premajors (70%–80%) (48) (Fig 1A). The rest of the class includes both prospective science majors and nonmajors (Fig 1B). The introductory biology class has no prerequisites, and students come with varying levels of preparation. Most students had completed high school–level biology and physics. Eight percent of the students had earned credit for advanced placement (AP) physics, 42% had earned credit for AP calculus and 18% for AP biology. Before the lab module, the students self-reported negligible experience with planarian work and image analysis (Fiji or ImageJ), as well as moderate experience with Excel (Fig 1C).

Each laboratory section contains up to 24 students and is team-taught by a faculty

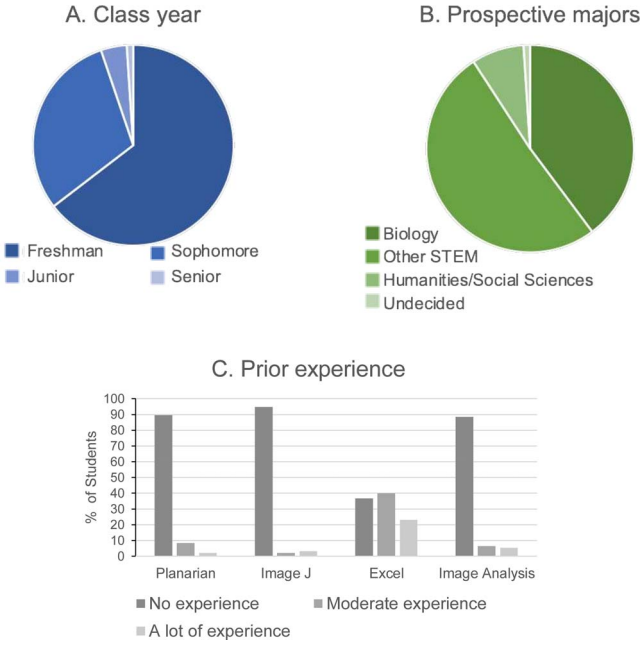

Fig 1. Student demographic data for (A) class year and (B) prospective majors were collected from $n = 96$ students. (C) Students rated their prior experience handling planarians and using the software packages; $n = 95$ responses.

member and a professional laboratory instructor or a pair of laboratory instructors. Students generally work in groups of 4 at designated workstations, and each laboratory session is about 3 h long. This lab module extended over 2 laboratory sessions and was placed in the curriculum at a time when students had already learned about the central dogma, transcription and translation, transcriptional regulation, and genotype-phenotype relationships. This module provides them with a hands-on activity to apply their knowledge and use RNA interference (RNAi) to study the genotype-phenotype connection. We assessed the students' learning experience through an in-class worksheet that was collected and graded at the completion of the laboratory module. At the end of the semester, we anonymously surveyed the students with a 90% response rate. (The survey is provided in Supplemental Material S7.)

C. Lab module goals

Because the challenges of the 21st century require creativity and innovation across disciplinary boundaries, we need to train our students with an inquiry-based approach that

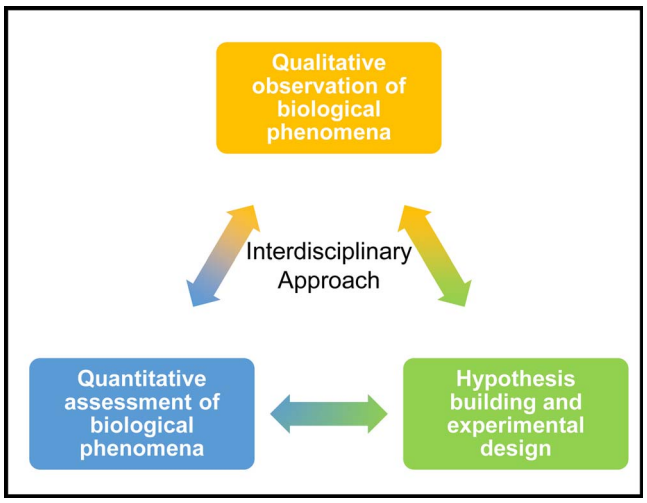

Fig 2. The cycle of qualitative observation, hypothesis generation, and quantitative experiment.

integrates skills and ideas across disciplines and equips them to solve problems on both local and global scales (49). We therefore designed this laboratory module to develop the students' appreciation for the scientific process, which cycles through qualitative observations, hypothesis formulation, and experimental design incorporating quantitative assessment, leading to an understanding that each part of the process lends itself to an interdisciplinary approach (Fig 2). First, the students observe planarian behavior with and without noxious stimulus (scrunching and gliding, respectively). Once students have made their initial observations, they are asked to reflect on (a) what distinguishes gliding and scrunching and (b) what kinds of tools and skills would be required to capture the behaviors quantitatively. Then, the students are prompted to formulate a hypothesis about the behaviors and design an experiment to test it. They conduct the experiment and use quantitative analysis to assess their hypothesis. This lab module promotes deliberate and rich discussions about how each step in the process interacts with both the preceding and following step. The students go back and forth between steps, refining their approaches on the basis of new observations and their hypotheses on the basis of new quantitative and qualitative information. Such discussions should go beyond the lab introduction and accompany the lab work

as it proceeds, with the help of informal interactions between the instructors and individual student groups or more formally by requiring students to associate their work with the 3 steps of the cycle. Students progress through the cycle at least once and are primed to reflect on their own progression through the process. They ultimately gain an understanding that the scientific process is iterative and that the evidence-based study of life exists on a continuum that requires an interdisciplinary approach.

This lab module has 4 main objectives:

(a) To provide an environment where students *engage with the scientific method*. At the module onset, students observe planarian behavior qualitatively, developing an intuition for animal behavior and the qualitative differences in movement between "gliding" and "scrunching." Students are then challenged to formulate a hypothesis about whether scrunching, a musculature-driven escape gait, is faster than ciliary gliding (the planarians' default gait) and to explore other ideas of why scrunching could be an advantageous gait in response to noxious stimuli.

(b) Students *learn basic quantitative image and data analysis skills* to test their hypothesis, performing center of mass and worm body-length tracking and calculating the speed of gliding and scrunching for the same planarians. Students then perform a comparative analysis with their newly acquired skills. Because it is impossible to tell qualitatively which behavior is faster, and it depends on the species used (21, 50), this module teaches students the importance of quantification as a necessary tool to answer these kinds of questions.

(c) Through this inquiry-based approach, students *achieve a deeper understanding of fundamental physical and biological concepts with hands-on research-based learning*. They investigate the mechanism of chemically induced scrunching by using molecular approaches—namely RNAi—which allows them to block scrunching from occurring in response to a specific chemical trigger. This inquiry leads the students to associate molecular pathways with phenotypic readouts.

(d) Because students work in small and diverse groups, they naturally *engage in scientific discussions with their peers*. Additionally, because planarian scrunching has been well studied, this topic also allows students *to engage with the primary literature* and compare their data to published values.

We provide a detailed description of the necessary resources and show one possible way of implementing this lab module in a medium-sized teaching laboratory setting (24 students). To facilitate the adaptation to different teaching contexts and classroom settings, we also provide suggestions for alternative and advanced exercises. Finally, we present student feedback from a pilot implementation of the original version of this module in a mixed student population (science majors and nonmajors), consisting largely of freshman and sophomore students.

II. MATERIALS AND METHODS

A. Materials and module preparation

The research was reviewed and approved by the Swarthmore College Institutional Review Board (IRB no. 1718098).

A major strength of this laboratory module is the minimal cost and necessary training associated with the required equipment and biological resources. Table 1 lists the required materials and vendors. Because scrunching is a conserved gait across various planarian species (21), the instructor has some flexibility on which planarian species to use. *Girardia tigrina* can be acquired commercially from Ward's Science (catalog no. 470176) or Carolina Biologicals (no. 132954) if long-term maintenance is not an option. Both vendors also provide a simple guide for planarian maintenance. If manipulation by RNAi is desired, however, the species *Schmidtea mediterranea* should be requested from the Stowers Institute. For this species, well-established protocols for maintenance and molecular work exist (e.g., Refs 51–59), and RNAi can be administered effectively via feeding (31, 60, 61).

Table 1. Materials for each group of 2–3 students.

	Item	Vendor[a]	Part no.
Technology	Microsoft LifeCam Studio for Business	Microsoft via Amazon	5WH-0002
	LED light panel	ME456 Inc via Amazon	ME456 A4
	Computer with Windows operating system[b]		
	Freeware image acquisition	VirtualDub	http://www.virtualdub.org/index.html
	Freeware image processing	Fiji	https://imagej.net/Fiji
	Data processing	Excel	
		Google Sheets	
Reusable materials	Ring stand	Fisher Scientific	14-675AQ
	Clamp	Fisher Scientific	05-769-8Q
	Petri dish	Fisher Scientific	FB0875713
	6-well plate	Fisher Scientific	07-200-80
	Depression spot plate[c]	Corning Pyrex	7220-85
	Dissection microscope[d]		
Consumables	691transfer pipet	Fisher Scientific	13-711-5B
	Plastic cover slip	Fisher Scientific	12-547
	15-ml tubes	Fisher Scientific	12-565-268
Reagents	Allyl isothiocyanate	Sigma-Aldrich	377430
	Instant Ocean salts in deionized (DI) water (0.5g/L)	Spectrum Brands	
House services	DI water		
	Waste collection		

[a] Vendor and part numbers are provided for convenience. Equipment may vary depending on availability.
[b] Any computer that has several USB ports and fulfills the software requirements is sufficient.
[c] We used contact lens containers generously donated by Wöhlk Contactlinsen GmbH, but spot plates or other concave containers can be used as an alternative. Because the containers only come in contact with water, they can be reused.
[d] Not required but nice for students to get a closer look at the planarians.

Student workstations are set up so that each table has the necessary materials listed in Table 1.

Planarians were distributed at the beginning of the laboratory module and reused between laboratory periods. The students saved "their" worms in a 6-well plate—1 plate per group. Each student analyzed at least 1 planarian over the 2-wk module, which allowed students to compare the gliding and scrunching behaviors for the same animal, enabling them to evaluate biological variability across individual planarians. Each group was also provided with a standard petri dish containing extra planarians to practice setting up the experiment and data collection. These planarians were collected at the end of the laboratory session, saved in a "recovery" container, and maintained in the dark before being reintroduced into the stock population.

To induce scrunching, we used a solution of 100 μM allyl isothiocyanate (AITC) (33). Because AITC is a hazardous chemical, laboratory instructors distributed the AITC solution at the appropriate concentration in a 15-ml tube. Other chemical and physical triggers can also be used to induce scrunching (21, 33, 50, 62). The students who handled the planarians wore appropriate personal protective equipment while working with AITC. Groups designated 1–2 students who handled the chemical and worms and 1–2 students responsible for touching the computer keyboard and mouse who did not wear gloves. Students left everything on their benches when finished, and the teaching staff disposed of AITC-contaminated materials appropriately.

B. Biological background

To prepare the students for the laboratory module—allowing for the in-class time to focus on the experimental methods and theoretical concepts—students were assigned relevant biological background material and a video showing a planarian gliding and scrunching (provided as Supplemental Material S4 and S5) ahead of time. They were required to answer prelab questions to ensure that they had engaged with the material. Depending on the student population and overall learning goals,

Table 2. Example prelab questions and learning goals.

Question	Learning goal
After qualitatively observing the planarian gliding and scrunching behaviors in the movie, what are your key observations? Summarize in 2–3 sentences.	Develop skills in careful observation and use descriptive language to capture qualitative differences.
We consider scrunching an escape gait in planarians. Think about what might characterize an escape gait and what you could measure experimentally to determine whether scrunching is an escape gait. Summarize your ideas in a maximum of 2–3 sentences.	Engagement with the scientific question and experimental design.
The planarian needs to sense a stimulus in order to respond to it. How do you think knock-down of a protein involved in sensing will affect the response to the stimulus? Formulate a hypothesis and briefly explain in 2–3 sentences how you would test it experimentally. What controls do you need for your experiments?	Engagement with basic biological concepts and phenotypic readouts, as well as experimental design principles.
Make a histogram of all the instantaneous speeds you recorded in week 1. What can you say about the distribution? Why does it look the way it looks? Summarize your findings in maximum 3–4 sentences.	Engagement with data analysis, visualization, and interpretation.

the content of background material is flexible. Some examples for short reading material can be found online for basic background in RNAi (63–66) and planarian biology (67–70). The Howard Hughes Medical Institute website (71) has an excellent short movie on regeneration, as well.

III. RESULTS AND DISCUSSION

A. Laboratory module

As students arrived in the teaching laboratory, prelab questions based on the assigned background material were collected and briefly checked for completion to ensure that students were prepared for the module. Examples of prelab questions we have used in our implementation of this laboratory module are listed in Table 2, some of which build on course material the students previously encountered; thus, a brief introduction to these concepts may be necessary for these questions to be useful.

Students were instructed to share their responses to the prelab questions with their lab partners and compare their answers. Additionally, each group was provided with planarians for qualitative observations and Benchtop Questions (see Supplemental Material S6) to further expand on the prelab questions. Lab instructors circulated to facilitate the conversations and reinforce the concepts by highlighting where students should expand their thinking. They helped students recognize the value of broadening their answers—and subsequently their knowledge—as they collaborated with their peers. This process was followed by a class discussion of the theoretical and technical concepts at the board (20–30 min), allowing for ample time for interruptions and student questions.

If this laboratory module is part of a larger class with multiple separate laboratory sections taught by different instructors, as is the case in our introductory course, the blackboard discussion may be synchronized between laboratory sections, sharing written materials or prerecorded introductions that have been prepared by one of the instructors. We have employed both approaches in the past and found that either works well if all the instructors have reviewed the material together and are provided with ample room for tailoring the introduction to the specific student environment.

For the first week of this module, it is important to cover basic physical and image analysis concepts, providing the students with the adequate background to engage fully with the quantitative applications. While students were encouraged to explore their individual hypotheses, we asked everyone to test the hypothesis that scrunching—the escape gait—was faster than gliding.

Because the planarian motion is 2-dimensional motion, the instructor first provided a basic review of the concepts of velocity and

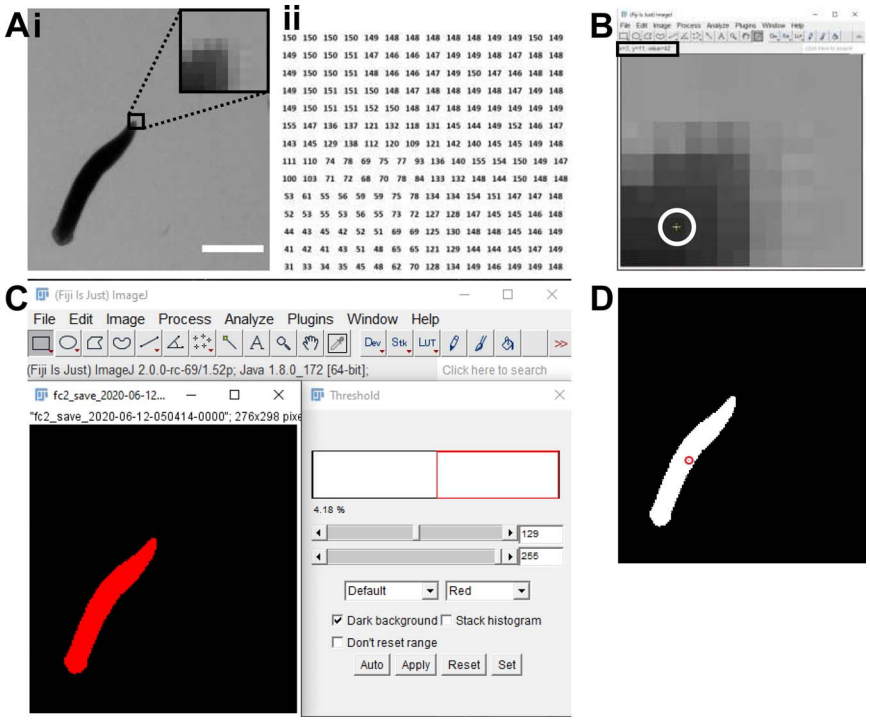

Fig 3. Example images to explain image data and basic image analysis. (A) Image as a matrix. (i) The image of the planarian. Scale bar: 5 mm. (ii) The matrix of intensity values corresponding to inset in (i). (B) The point tool in Fiji can be used to display coordinates and intensity values of a pixel (shows that the planarian is darker than its environment). (C) Thresholding isolates the planarian from the background with the intensity information. (D) Binary image obtained after thresholding shows the isolated planarian (in white). The center of mass of the planarian is the center of the red circle.

speed, including a brief review of vectors. These concepts are best introduced with real-life examples—students running, a car going around a curve—because they allow students from all levels of preparation to derive these concepts. Apart from the notion of direction, it is important to discuss that the magnitudes of the 2 quantities can be different depending on the type of motion and the time interval over which the quantities are measured (Supplemental Material S6). The distinction of instantaneous versus average velocities and speeds can be further expanded during the subsequent discussion of measurement noise and resolution.

Next, we engaged students in designing an experiment that measures an object's speed from images of the object at specific time points, which naturally led to a discussion of (a) what images are and (b) what values are necessary to identify the trajectory of an object. It is important that the instructor explains, with the use of example images that resemble the data the students will acquire in this module, that (a) images are matrices, (b) every position has an *x*- and a *y*-coordinate, and (c) each coordinate has an intensity value (Fig 3). The students will then understand that the planarian image can be isolated from the background with the process of thresholding, because the worm is darker than the background given the specific imaging conditions. At this point, the instructor showed students how to threshold an image with the Fiji software and track the planarian's center of mass (COM) over time with the built-in particle tracking function (50). If the students are provided with a practice image sequence (e.g., Supplemental Material S4), they can follow along in real time.

The discussion then focused on the question of converting these COM coordinates obtained from the images to meaningful speed values. This process required instructors to introduce the concepts of converting scales (pixels to millimeters), extracting sampling rates (frames per second), and converting frames to seconds.

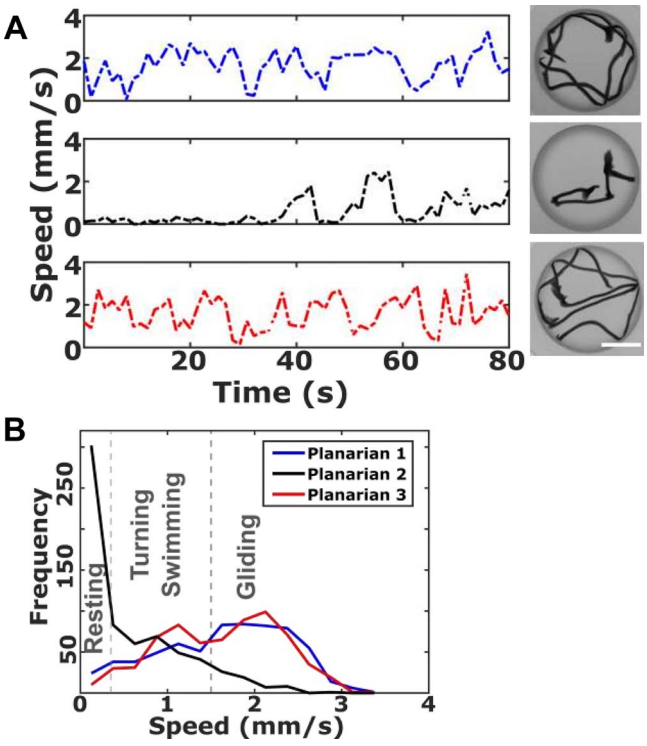

Fig 4. Instantaneous speed. (A) Instantaneous speed plotted as a function of time for 3 different planarians tracked over 80 seconds. Corresponding minimum intensity projections (MIPs) of the planarian motion show the planarian trajectories. The middle trajectory is short, reflecting extended resting at the beginning of the recording. Scale bar: 10 mm. (B) Histograms of the instantaneous speeds for the worms in panel A. At speeds below 0.25 mm/s, the worms are resting in place and center of mass motion is only due to shape changes of the planarian. Higher speeds, up to 1.5 mm/s, are associated with turning and swimming behaviors. Speeds $>$ 1.5 mm/s are due to worms gliding on the substrate. These speed cutoffs are not absolute and were defined based on the observed motion of the worms.

Students were shown how to use a ruler, or any object of known size, to obtain the scale conversion. Next, we prompted students to think about tradeoffs that would dictate how their data should be acquired. We discussed image acquisition rates and the need for balancing the amount of data generated with the temporal resolution necessary to measure speeds in a meaningful way (Fig 4 and Supplemental Material S6). Instructors can also discuss tradeoffs between the use of smaller containers and how the container would affect the observed planarian behavior and speed profiles. Smaller containers with 1 planarian/container allow for more worms to be imaged in parallel at the same time and thus speed up data collection, whereas larger containers allow for longer straight-line motion without boundary effects. Again, it is advisable to use various real-life examples and visualizations to introduce these concepts to facilitate understanding in a diverse student population. Once these concepts were explained, the students were ready to think about planarian gliding speeds and make predictions of what speed versus time plots and speed histograms should look like (Fig 4), relating back to their qualitative observations from the beginning of the exercise. Interpreting the histograms in conjunction with the speed versus time plots is a useful exercise because it allows students to relate their quantification to the biological phenomenon. Because the containers are small enough to allow the worm to interact with the wall a few times over the duration of imaging, one would observe slowing down, turning, and speeding up in the speed versus time data of the worm (Fig 4A). This phenomenon is best seen through direct comparison of the trajectories obtained by minimum intensity projections (MIPs), as described in the next paragraph, with the speed versus time plots (Fig 4A). Additionally, planarians can rest for some time—evident as short MIP trajectories and near-zero speeds—or crawl to the water surface and exhibit swimming, which is slower than gliding (72). The speed histograms allow students to determine the frequency with which these different behaviors occurred (Fig 4B). If students are more advanced, the instructor can guide them to think about the change in calculated speed as a function of time interval and discuss the differences between instantaneous or average speed and velocity (Supplemental Material S6).

For data acquisition, we used a webcam and the VirtualDub freeware, version 1.10.4 (Table 2 and Fig 5A,B). We provide step-by-step instructions in the Supplemental Material S6 for this specific setup. Once the data files were acquired with the software, the data were imported into Fiji to show the planarian trajectories with the MIP (Fig 5C). Because the planarian body is dark on a light background, the MIP shows the position of the planarian in

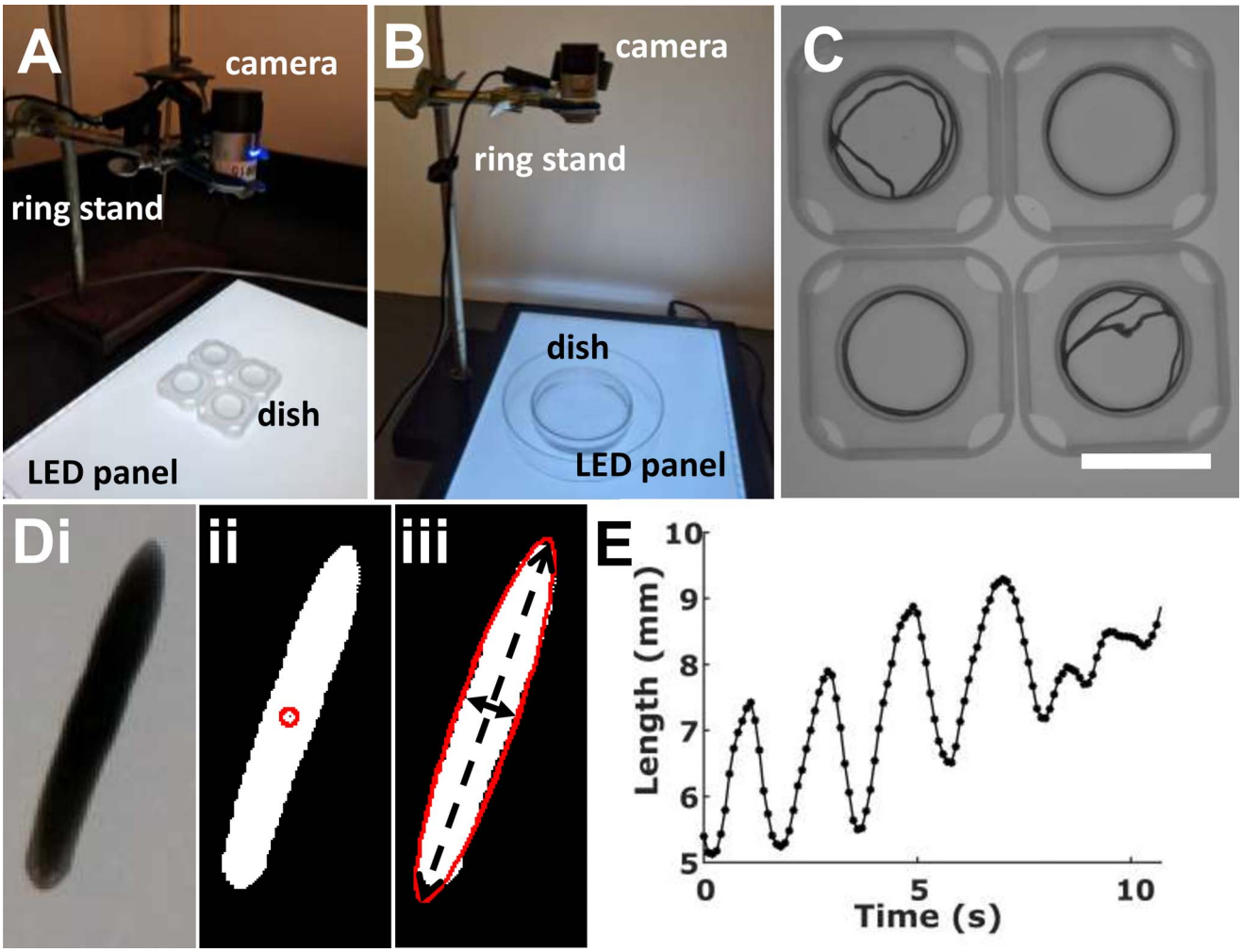

Fig 5. Experimental setup and example data. (A) Setup for acquisition of gliding movies and (B) setup for acquisition of scrunching movies using allyl isothiocyanate. (C) Example trajectories, obtained from a minimum intensity projection in Fiji, for gliding planarians. Scale bar: 25 mm. (D) Example images explain the image analysis for scrunching. (i) Grayscale image of planarian. (ii) Binarized image of the planarian (center of mass of the planarian is the center of the red circle). (ii) An elliptical fit to the binarized image (in red) allows for extraction of the length of the planarian as the major axis length (dashed black line) of the ellipse. (E) Length versus time plot shows the periodic length changes that are characteristic of scrunching, with a sequence of 4 full scrunching cycles.

each frame (i.e., its trajectory). This step enables students to visualize the planarians' behaviors quickly and discuss their observations with their peers. Moreover, it allows them to connect the quantitative analysis that follows to these direct observations, which is helpful for integrating novel interdisciplinary concepts.

To obtain the COM coordinates for each frame, an individual planarian was isolated by cropping and Fiji's built-in *Duplicate* function. To distinguish the planarian from the background more easily, a frame without the planarian can be subtracted by Fiji's image subtraction function (50). A threshold was then applied to the cropped image sequence, and the built-in *Analyze Particle* function was used to determine the COM coordinates in every frame. The *Analyze Particle* function also has a size threshold that allows users to set a minimum size for objects to be recognized. This function can be used to eliminate small objects in the analysis. The results were saved as a TXT or CSV file for further processing. A step-by-step description of this process and how to calculate gliding speeds is provided in Sabry et al (50). Briefly, students choose a short part of the trajectory where the planarian travels a distance of about 2 body lengths

without turning. The speed of gliding is calculated as the mean of the instantaneous speed over this part of the trajectory and normalized to the planarian length. The standard deviation of the normalized instantaneous speed measurements is taken as the uncertainty in the measurement.

In the second week of the module, instructors reviewed the key material from week 1. They reminded students of the initial hypothesis we asked them to test (in addition to their own, if they had a different one)—that the escape gait (scrunching) is faster than gliding and that quantitative data is needed to support or reject this hypothesis. Depending on the planarian species used, gliding can be faster or slower than scrunching (21). To foster active and inclusive participation, key concepts were assigned to the student groups who had 5 minutes to discuss and then briefly (2–3 minutes) report to the entire class. The instructor also reviewed the scrunching gait by showing a length versus time plot (Fig 5E). Students were asked to describe the plot in an interactive discussion and to consider which parameters could be extracted to arrive at the parameters of amplitude, frequency, and period. At this point, it is important to explain the difference between a continuous signal, as drawn, and the actual discrete data that is being collected in the experiment. The instructor asked the student groups to derive how fast pictures need to be acquired (sampling rate) to detect the periodicity of the signal. Depending on the student population, this concept can be further explored with the provided optional exercises on sampling rate and Fourier transforms. Instructors then led students through an example of how scrunching frequency is determined and how frequency is converted to relative speed by the protocol described in Sabry et al (50). In brief, students applied the same thresholding procedure as in week 1, but instead of COM tracking, they used an elliptical fit to extract the length of the planarian in every frame (Fig 5D,E). The length data was then plotted as a function of time, and 3 or more complete consecutive scrunching cycles were extracted by visual inspection (Fig 5E), ensuring that only straight-line motion was used. This exercise allowed students to determine the 4 scrunching parameters (frequency, maximum elongation, asymmetry of elongation and contraction cycles, and relative speed) (21, 50). Because the acquisition of data is similar to week 1, instructors invited students to share their experiences and "lessons learned." Students were then ready to compare the gliding and scrunching speeds of individual planarians. Time allowing, student groups pooled their data to obtain population-level statistics for the gliding and scrunching speeds and assessed the variability across individuals. They compared their individual and population values to the published literature (21, 50). Although we did not introduce statistical tests in this module—they learned about it in a later module in this course—this point in the exercise would be a natural extension.

Depending on how scrunching is induced, instructors should share information about the method (i.e., demonstrating how cutting is accomplished and highlighting the safety considerations of using AITC, or other chemicals, to induce scrunching) (33, 50). It is also important to discuss with the students how RNAi can be used as a tool to determine the mechanism of AITC-induced scrunching *before* starting their experiments. The students can be encouraged to formulate a hypothesis on how *TRPA1(RNAi)* planarians would react upon AITC exposure and which control experiments would be required to convince them of the specificity of the RNAi phenotype.

AITC is the pungent ingredient in mustard and wasabi and is sensed by transient receptor channel ankyrin 1 (TRPA1) in the planarian species *S. mediterranea* and *Dugesia japonica* (33, 73). Primer information for cloning this gene from *S. mediterranea* complementary cDNA and information about controls for RNAi are provided in Supplemental Material S6. If AITC sensation is impaired through RNAi-mediated knockdown, planarians no longer scrunch on AITC exposure; instead, they glide normally as they do in water. Students tested this in our implementation of the module by observing *TRPA1(RNAi)* and *control(RNAi)* pla-

narians in a 100-μM AITC solution. Whereas *control(RNAi)* planarians scrunch upon AITC exposure, *TRPA1(RNAi)* planarians glide, as they do in water. Movies from 4 trials of each condition are provided as Supplemental Material S8 for *D. japonica* planarians. *TRPA1(RNAi)* planarians continue to scrunch when exposed to other TRPA1-independent scrunching inducers. Thus, these RNAi experiments can be used to both discuss the mechanism of scrunching induction and the specificity of the scrunching phenotype (50). In terms of experimental logistics, the setup and data collection for the RNAi planarians is the same as for wildtype (control) planarians. Once students collected and analyzed their data, they were instructed to compare their data with other lab groups, as well as values reported in the literature and to consider how they would report their findings and discuss possible discrepancies between their data. Time permitting, the instructors encouraged student pairs to discuss how they could test alternative hypotheses about scrunching and, time allowing, test these experimentally with their newly acquired skills.

B. Optional advanced exercises

1. Fourier analysis and sampling frequency

Because our course primarily consisted of premajor students, including many non-STEM prospective majors, we performed the scrunching time series analysis manually, as described in Sabry et al (50). However, scrunching is well-suited for an introduction to Fourier analysis for more advanced students in upper level courses. Because the length versus time signal of a scrunching planarian is primarily composed of a single frequency that students can estimate from the time domain plots, this Fourier analysis exercise can be used to introduce students to the basic principles and computational aspects of Fourier analysis.

Fourier analysis is commonly used for the analysis of a variety of temporal and spatial data in biophysics, such as spectra and time series data or quantification of spatial patterns. Most programming languages have packages for Fourier analysis that use Fast Fourier Transform algorithms. It is useful for students to be aware of computational aspects of these algorithms to better understand how their data collection strategies via sampling rate and signal duration can affect the quality of the analysis they can perform.

Students can be led through exercises on how signal length and sampling rate affect the quality and resolution of the Fourier transform with interactive Python scripts, which we provide as Supplemental Material S1–S3. Here, students are asked to choose subsets of data to generate the Fourier transform and evaluate how the sharpness of the peak frequency and resolution changes in response to alterations to signal duration, sampling frequency, and noise levels for the same signal (Fig 6). In the first exercise, students are asked to choose a continuous subset of the signal. The script then displays the corresponding Fourier transform along with the Fourier transform of the original signal.

Instructors should point out the change in the resolution of the frequency and the change in the width of the frequency peak. Students can be encouraged to explore the causes of these changes by choosing different subsets of the signal. The exercise can also be used to discuss the reciprocal relationship between time and frequency domain signals: how wide signals in the time domain become narrow in the frequency domain and vice versa.

In the second exercise, students are asked to choose a sampling rate for the signal. The script then displays the original and the modified signal in the time domain as well as the corresponding Fourier transforms. Students should be encouraged to explore how changing the sampling rate affects the position and width of the frequency peak and how lowering the sampling rate below a certain threshold significantly alters the frequency they are able to detect. This exercise would naturally lead to the Nyquist sampling criterion, which states that to obtain the frequency of a signal, the sampling rate must be at least twice that frequency. In the third exercise, students are asked to add various noise levels to the signal

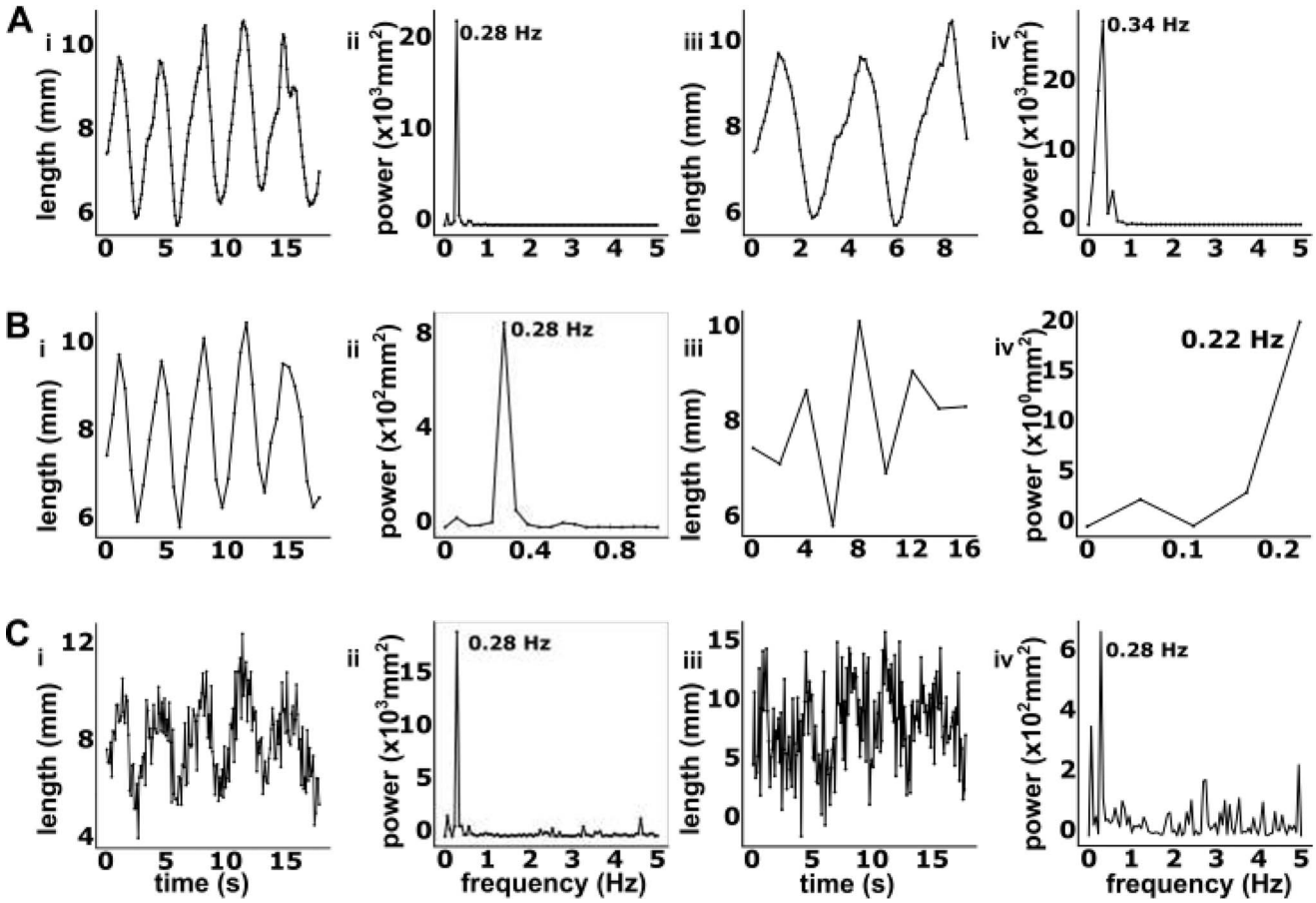

Fig 6. Screenshot of Python module illustrating the time series signals and corresponding Fourier transforms for the 3 exercises described in the main text. (A) Complete (i) length versus time plot and the corresponding (ii) Fourier power spectrum. (iii) Original signal truncated by half. (iv) Truncating the signal by half shifts the peak frequency and broadens the peak. (B) (i) Reducing the sampling frequency, to one-fifth in this case, (ii) reduces the maximum frequency that can be sampled in the Fourier transform. (iii) Reducing the sampling rate below the Nyquist frequency generates a time series very different from the real signal. (iv) The Fourier transform of the signal sampled below the Nyquist frequency does not show a peak at the correct frequency. (C) The primary frequency of the signal can still be extracted from the Fourier transform for a noisy time domain signal. The addition of noise adds high-frequency components. Noise generated from a normal distribution with mean zero and SD = 1 mm for (i) and (ii) and SD = 2 mm for (iii) and (iv).

and explore how noise changes the time signal and its corresponding Fourier transform. This last exercise will illustrate the power of Fourier transforms in extracting the peak frequency of a signal whose periodicity may not be obvious in the time domain because of noise. These exercises should then be connected back to the biological phenomenon of scrunching by using different scrunching inducers (amputation, noxious heat, chemicals) and observing how planarians respond with a mixed phenotype, wherein scrunching may be accompanied by head wiggling or other body shape changes (21, 33) for which manual determination of the frequency may no longer be possible from the time series data.

2. Mean square displacement and directionality of motion

More advanced students with an interest in physics or mathematics may also be encouraged to explore the data further by calculating the mean square displacement (MSD) from the COM tracking data. The scaling between MSD and time can be used to describe the motion as random (diffusive) or directed (ballistic). Other types of motion are possible, and several great resources are available that can help students dig deeper into these concepts (74–78). This analysis can be achieved in 2 ways, depending

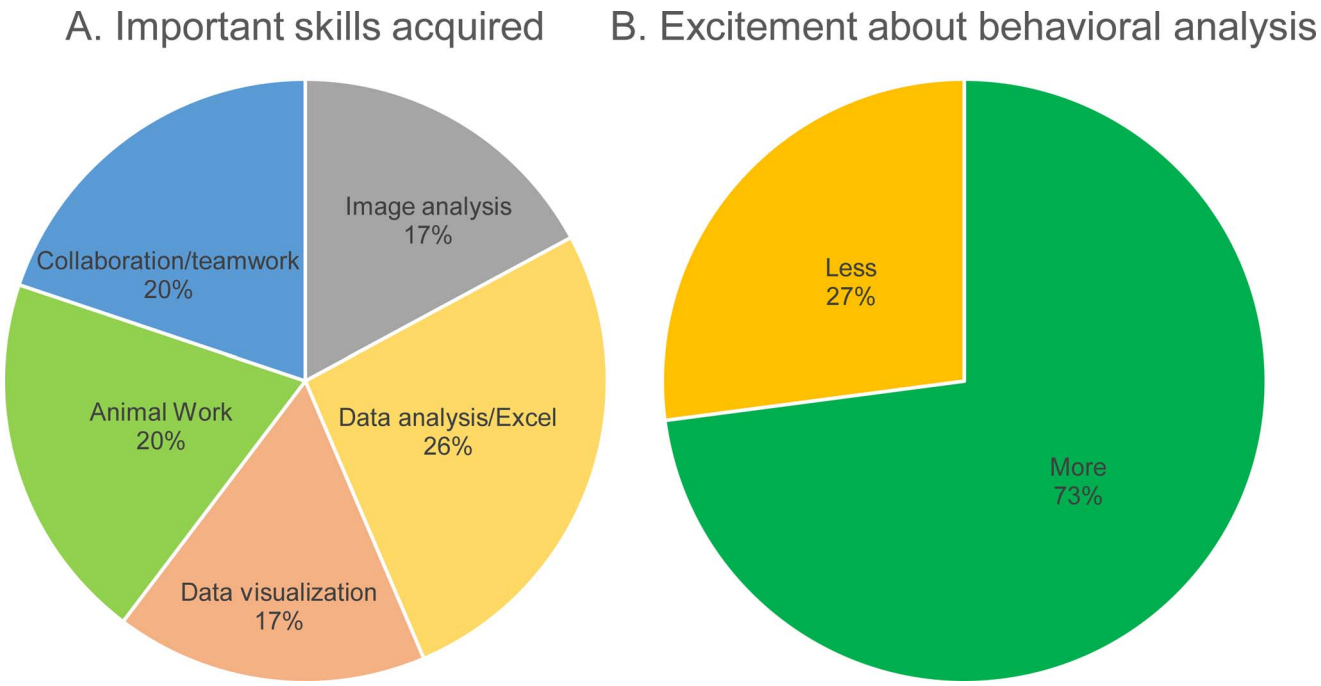

Fig 7. Acquired skills and tools. (A) Students were asked to select the most important skills acquired during the lab module ($n = 257$ responses). Note: students could select more than one skill; therefore, percentages reflect cumulative responses. (B) Students rated their excitement about behavioral analysis as a result of having completed the lab, choosing between "more" and "less" excited; "no change" was not an option ($n = 96$ responses), students selected 1 response.

on the desired learning outcomes. The code (in Python or another language) can be provided by the instructor to allow focus on the content, or the students can write their own code with instructor guidance. MSD analysis in the context of the experiments described above allows the students to quantify whether the directionality of motion differs between gaits and between the RNAi treatment and control groups. This measure also lends itself to the quantification of custom expansions of the lab module, wherein students may choose to study the effect of environmental stimuli (e.g., different wavelength of light, food, temperature gradients) and interactions with the container boundary on behavior more broadly.

C. Student feedback and assessment

At the end of the semester, we anonymously surveyed the students (Fig 7) and received a 90% response rate. The goal of this survey was to assess whether the students felt that they gained the skills we wanted to teach them and whether this module, geared specifically toward quantitative skills, sparked their interest in this area. To establish a baseline, we first asked the students to self-rate their experience level with the key components of the module (Fig 1 and Supplemental Material S7). The vast majority (88%–95%) of students described themselves as inexperienced with both the biological system and the technical aspects of the module. When asked which acquired skills they deemed important—students were allowed to select more than one—and likely to be useful in future courses, the distribution of student responses was fairly equal across the key concepts (Fig 7A), which illustrates that the lab module served the diverse student population well by incorporating biological, physical, and computational concepts and that students valued all of the skills that were incorporated. Additionally, 20% of student responses recognized the value of the collaborative nature of the work. Finally, 73% of students described themselves as more excited about behavioral analysis after having participated in the lab module, demonstrating that using this interdisciplinary approach to teaching object track-

ing and signal processing can engage and excite most students (Fig 7B).

IV. CONCLUSION

This interdisciplinary laboratory module aimed at premajor undergraduates—including prospective non-STEM students—requires minimal resources and develops a student's ability to use basic computational skills to quantify the behavior of freshwater planarians. Students use object tracking and time series analysis to explore the molecular mechanism of animal behavior through a multidisciplinary approach that incorporates basic physical concepts. By adding more advanced hands-on computational and experimental exercises, this module can easily be expanded to upper level and advanced courses. It can also be adapted to tracking other organisms should planarians not be available, because—except for the RNAi experiments and the particular nature of the scrunching gait—the tracking methods and software did not use any specific assumptions about the model system (79, 80). Moreover, this module lends itself well to remote learning, in that image data can be collected by the instructor and distributed electronically to the students for analysis (in addition to the Supplemental Material provided). Because the computational tools are freely available and easily implemented on any computer and no programming skills are required, support of the data analysis is straightforward and can be provided easily by online support sessions (e.g., lab group meetings with an instructor present). On the basis of our recent experience with remote teaching, maintaining some form of synchronous activity, such as small group meetings, is important for student engagement and success. In summary, this laboratory module is both versatile and appealing to a diverse student population and can be executed at minimal cost to the institution.

SUPPLEMENTAL MATERIAL

Supplemental material is available at the following links:

https://doi.org/10.35459/tbp.2020.000159.s1
https://doi.org/10.35459/tbp.2020.000159.s2
https://doi.org/10.35459/tbp.2020.000159.s3
https://doi.org/10.35459/tbp.2020.000159.s4
https://doi.org/10.35459/tbp.2020.000159.s5
https://doi.org/10.35459/tbp.2020.000159.s6
https://doi.org/10.35459/tbp.2020.000159.s7
https://doi.org/10.35459/tbp.2020.000159.s8

AUTHOR CONTRIBUTIONS

E-MSC designed the lab module, JN and NCS tested early versions of the module and helped in the design of the final version. TG and E-MSC designed the optional advanced exercises and VS and TG wrote the Python script and associated user manual. NCS and E-MSC designed and administered the postlab survey and wrote the first manuscript draft, and all authors edited and co-wrote the final version of the manuscript.

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