The Biophysicist

A Microscope Medley for High School Students

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ABSTRACT Microscopy is crucial to much of biophysics. The variety of approaches to imaging exemplified by contemporary microscopes is remarkable, yet this breadth is generally unknown to students, limiting perceptions of biology, physics, and related fields and of potential career paths. We therefore created and implemented an outreach activity as part of a day camp that especially targeted low-income high school students. The students engaged with 3 very different microscopes: a simple transmitted light microscope; a light sheet fluorescence microscope; and a newly invented multicamera array microscope. With these instruments, we examined subjects such as transgenic zebrafish larvae with fluorescent immune cells, contrasting the various instruments' capabilities, including resolution and field of view. Pre- and postactivity questions showed that the activity succeeded in expanding students' understanding and appreciation of the varied aims and abilities of modern microscopes and moreover led to discussions of model organisms, biophysics, and science funding. Additional activities briefly illustrated the nature of digital images and mathematic manipulation. I describe here the activities and goals, as well as ways they can be generalized and implemented at other institutions with access to different sorts of imaging tools.

KEY WORDS microscopy; outreach; imaging

I. INTRODUCTION

The awe-inspiring ability of microscopes to make visible the world of very small things is well known. In contrast, the variety of approaches to microscopy, and even the variety of aims of microscopy, are not well known either by the public in general or by secondary school (high school) students in particular. In addition to constraining the appreciation of contemporary science and technology, this lack of awareness restricts students' views of careers and paths of study. The relevance of microscopy to biophysics has been evident since Antonie van Leeuwenhoek's revolutionary observations of living, moving, "wee animalcules," and the development of various microscopes has gone hand in hand with advances in biophysics. To broaden students' perspectives on microscopy, to enhance understanding of biophysical concepts, such as the sizes of cells, and to highlight topics at the intersection of biology and physics that may influence future paths of study, I designed an activity for high school students that involved working with 3 very different microscopes and discussing topics such as optical trade-offs, model organisms, digital images, and even science funding. I, with others, implemented the activity in the 2023 day camp that targets a diverse range of high school students, especially low-income students. Here, I describe the activity and its learning goals, with generalizations and variations that may help similar efforts undertaken elsewhere and assessments of how it went.

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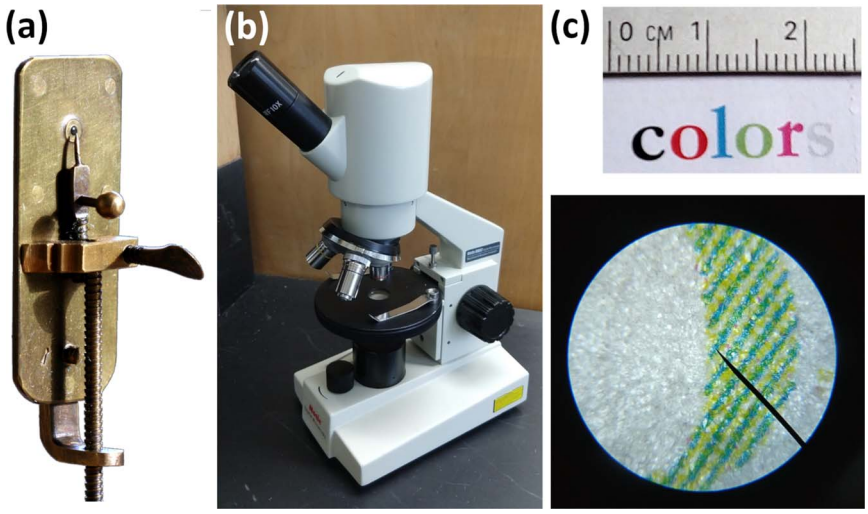


Fig 1. (a) One of Antonie van Leeuwenhoek's early microscopes (photo by Jacopo Werther, licensed under the Creative Commons Attribution-ShareAlike 3.0 Unported license; https://en.m.wikipedia.org/wiki/File:Leeuwenhoek_Microscope.png). (b) A simple transmitted-light compound microscope. (c) Color-printed letters with a ruler for scale, as viewed through the microscope. The yellow and cyan inks making up the green "o" are evident.

The main learning objective of the activity was to understand some of the varied goals and approaches of microscopy. In addition, we sought to convey the sizes of typical eukaryotic cells and to introduce the idea that digital images are arrays of numbers that can be manipulated mathematically.

The participants were 16 high school students (6 sophomores, 5 juniors, and 5 seniors), enrolled in a week-long physics and human physiology day camp that is part of a broader outreach program at the University of Oregon: the Student Academy to Inspire Learning (SAIL; 1). SAIL aims to increase the likelihood of pursuing higher education among low-income and underrepresented groups by providing summer camps that combine subject-specific activities and information about college, as well as providing mentoring during the school year. The program began in 2006 with one summer camp, run by faculty in the Department of Economics, and has since expanded to 18 camps serving ~500 students. In summer 2023, ~50% of SAIL students had family incomes <US$50,000, and >50% were from underrepresented groups. I initiated the physics and human physiology camp and have been jointly responsible for it since 2009. The microscopy activity described here is newly designed and implemented for the first time in August 2023. In addition to the 16 high school students, 2 Japanese college students visiting from Nagoya University were present.

II. METHODS

A. A microscopy activity

The prelude to the activity consisted of a brief introduction and some questions. I began with a photo of one of van Leeuwenhoek's microscopes (Fig 1a) and asked if anyone could identify it (one student knew that it was a microscope) .This led to the observation that microscopes the students may be familiar with from school look much different than van Leeuwenhoek's, and as they would soon see, there are contemporary microscopes that are just as different from each other. I then asked the question, "What might it mean for a microscope to be *better* than another?" and the students wrote down their thoughts. I collected the responses, but we did not discuss the answers until after the microscopy activity. We then split into 3 groups and rotated between 3 very different microscopes, each manned by 2 people from my research group, including myself. The microscopy activity overall took 1 h. All experiments with zebrafish were performed in accordance with protocols approved by the University of Oregon Institutional Animal Care and Use Committee and by following standard protocols. The study was reviewed and approved by the University of Oregon Institutional Review Board (STUDY00001191).

The microscopes spanned a wide range of designs and capabilities, especially highlighting trade-offs between magnification and field of view. One was a simple transmitted-light compound microscope (Motic DM52, Motic USA, San Antonio, TX), which was the type likely seen in a middle or high school science classroom. Another was one of my lab's home-built (2, 3) light sheet fluorescence microscopes (4–7), capable of high-resolution 3-dimensional (3D) imaging over a few hundred micron fields of view. The third was a very new multicamera array microscope on loan from Ramona Optics (Durham, NC) that consists of a grid of 48 cameras and lenses that together span a field of view (6 × 8 cm) with 5-μm resolution, returning ~450 MP images at up to 13 frames per second. The resulting image scale, ~3 μm/pixel, is roughly half the resolution, consistent with the Nyquist sampling limit. The differences between these instruments were highlighted by viewing the same subject on each: live zebrafish larvae, from a transgenic line engineered to have its neutrophils, cells of the innate immune system, expressing green fluorescent protein (GFP; Tg [mpx:gfp]) (8). We also examined other objects with each microscope.

A familiar compound microscope (Fig 1b) can convey the idea of magnification. We first looked at numbers printed on paper in a series of decreasing typeface (font) sizes and color-printed letters, and the cyan, magenta, yellow, and black constituent inks were visible (Fig 1c). Considering zebrafish, the few millimeter-long larvae are simply specks by eye, but through the microscope eyepiece, one can easily discern many aspects of the anatomy (e.g., large eyes). However, the frequent darting motion makes it nearly impossible to follow individual animals, and even finding one in the subcentimeter field of view takes some luck (immobilizing larvae in agarose or methylcellulose would address this). A further limitation is the lack of fluorescence imaging; there is no way to discern GFP-expressing neutrophils, even if the magnification were adequate.

Students at the light sheet fluorescence microscope first learned the meaning of *fluorescence*, simply that some materials, illuminated by light of one color, emit light of another color. This quite minimal description sufficed for our learning aims, given the time available, and avoided jargon. We demonstrated fluorescence with a macroscopic example: blue–violet light shining through a bottle of olive oil, with the beam turning a beautiful red (9). In canola oil, for contrast, the beam remains blue–violet (a green laser pointer also works well for demonstrating olive oil fluorescence). The students were then introduced to the light sheet microscope, which looks strikingly dissimilar from the familiar conception of a microscope. The home-built instrument (Fig 2) consists of lasers, lenses, and mirrors on an optical table, with a 3D-printed, water-filled chamber in which larval zebrafish are suspended in plugs of agar gel. The excitation light is shaped into a thin sheet, providing optical sectioning, and by scanning the sheet, 3D imaging. With this microscope, one can watch the rapidly beating heart using transmitted light, and switching to a fluorescence channel, fluorescent neutrophils scattered throughout the body of a transgenic fish (Fig 3). Students were able to move the specimen using joystick controls to pan in all 3 dimensions. The resolution, a few hundred nanometers in the sheet plane and a few microns perpendicular, easily suffices to discern the cells' amoeboid shapes and fingerlike protrusions. The field of view is smaller than the simple compound microscope, however, being about 0.4 mm or roughly one-tenth the length of the fish.

Similar to the light sheet fluorescence microscope, the Ramona Optics array microscope is radically unfamiliar in its design. Rather than 1 or 2 recognizable lenses, a grid of lenses and sensors sits above the sample (Fig 4), covering a field of view of 6 × 8 cm. Because the entire field is imaged simultaneously, with continuous 13 frames per second acquisition of ~25,000 × 18,000 pixel images, one can "zoom in" anywhere. The students observed zebrafish larvae swimming freely in a dish and selected regions at will to obtain a magnified view. Switching

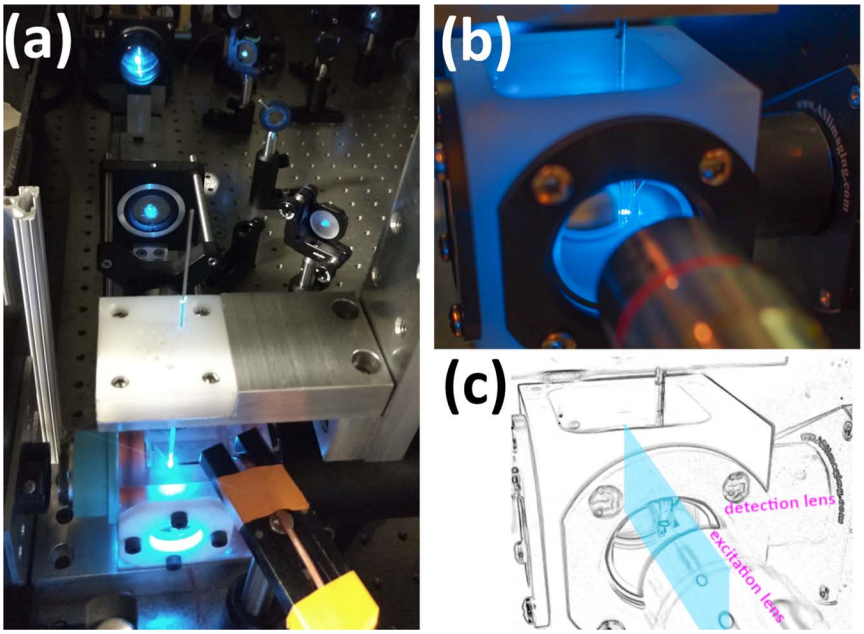


Fig 2. A home-built light sheet fluorescence microscope. (a) Lenses, mirrors, and other optical elements. Laser illumination (blue) is shaped into a thin sheet and directed to the specimen chamber, near the bottom of the image. (b) The specimen chamber. Larval zebrafish are held in plugs of agar gel suspended from the ends of glass capillaries. (c) Illustration of the laser sheet orientation (blue) based on the image in (b). The sheet is not drawn to scale; its vertical extent is roughly 0.4 mm to match the detection field of view.

to the fluorescence channel, immune cells were evident, as was the heterogeneity across larvae, though the few micron resolution does not permit discernment of cellular shapes. Students were able to pan and magnify the live image using the microscope software. We also looked at fabrics, comparing machine-woven and hand-woven cloth; students guessed which was which, generally correctly.

B. Discussion and digital images

A follow-up session the next day elaborated on topics introduced by the microscopy experiences. This lasted for 2 h, with a 15-min break in the middle. Intentionally, to hook students via hands-on activities rather than words, we did not previously explain questions such as "Why study zebrafish?" and "Why would a *physicist* study zebrafish?" These questions were discussed at the second day's meeting, which took place at the University of Oregon's Visualization Lab, an array of screens that include a total of 50 million pixels (Fig 5). The questions noted previously lead naturally to discussions of model organisms and model systems in general, and to the nature of biophysics, a field whose existence is unfamiliar to most high school students.

Returning to images, the large screen is not necessary but is captivating for displaying the array microscope images, which contain a few hundred million pixels. Staring at the screen, one has a sense of the constituent pixels, as well as the overall extent. Calling back to the previous day's mention of van Leeuwenhoek's microscopes, we noted that van Leeuwenhoek drew pictures of the bacteria he saw. This was the only available method for recording images for centuries, and through it, we learned a lot. Photographic film then followed and next digital sensors, leading us to an explanation of the nature of digital images as arrays of numbers and the consequence that we can perform mathematic operations on these arrays. To demonstrate this, I showed the numbers corresponding to a small piece of one of the images, about 20 × 20 pixels, with an inset of that part of the image, and the result of thresholding that array to make a binary image (black and white). To illustrate the concept of smoothing, we assigned numbers to a row of people, the left half being 9s and the right half being 3s, and then asked each student to take the average of their and their 2 neighbors' numbers, giving a 6 and a 3 at the boundary and therefore a smoother edge. I then showed and applied Photoshop filters to an image (Adobe Creative Cloud, v. 6.0), highlighting that every such operation, and every Instagram filter, is similarly a mathematic operation applied to an array of numbers.

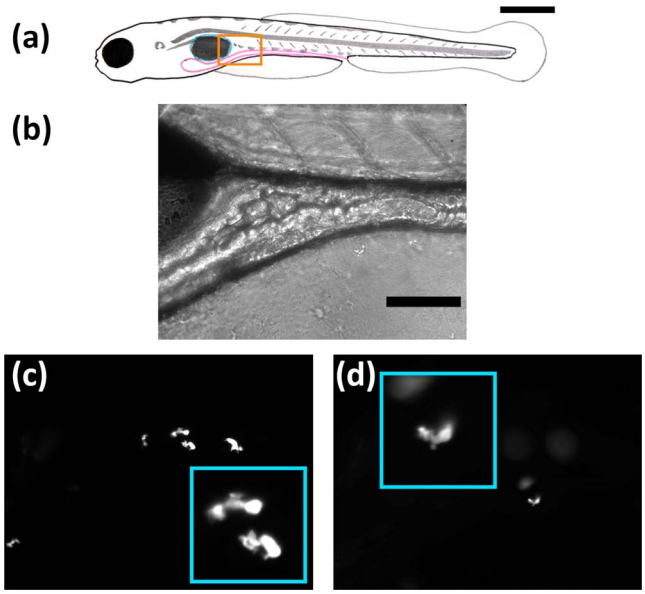


Fig 3. Example light sheet microscope images of a larval zebrafish with fluorescent immune cells. (a) Schematic illustration of a 6-d postfertilization zebrafish larva. The swim bladder is outlined in cyan, and the gut is outlined in magenta. The orange box indicates the region captured by the field of view in the subsequent panels. Scale bar: 0.5 mm. (b) Bright-field image of part of a larval zebrafish. Scale bar: 0.1 mm. (c and d) The 2 light sheet fluorescence microscope images, from planes separated by 23 μm and from the same fish and the same field of view as in (b), showing fluorescent neutrophils in this transgenic fish. Insets are expanded by 3×. The neutrophil in the plane shown in (d), located in a fin, can be discerned in the bright-field image, but the neutrophils in (c), situated in thick, dense tissue near the gut, cannot.

III. EVALUATION

A. Assessment

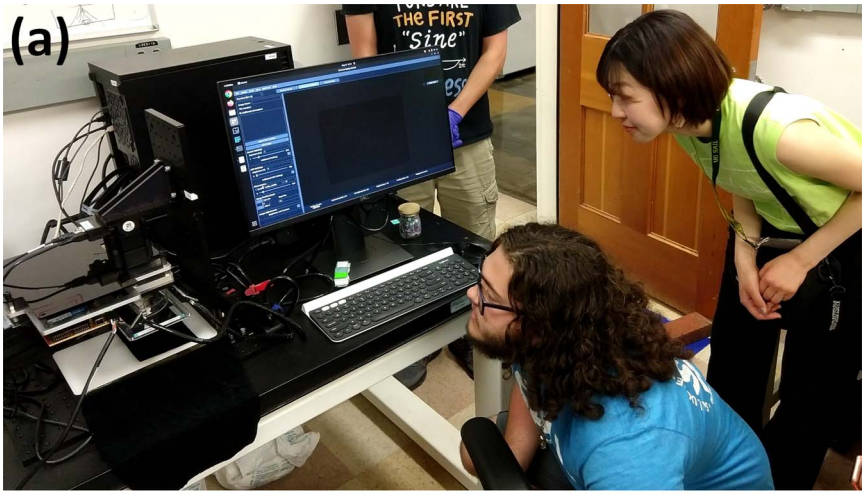
Students found all 3 microscope activities enjoyable and engaging. The combination of hands-on tasks, real-time decisions of regions to look at, and prompts for observations of features such as immune cells were appealing, and there were lots of comments and questions. The only significant flaw was insufficient time: 15 min per activity, so each set of 6 students could cycle through the full set in 45 min.

To assess whether the experience changed students' understanding of microscopy, we returned in the second day's session to the previously mentioned question: "What might it mean for a microscope to be better than another?" The preactivity responses were rather limited. Eleven of 18 referred to magnification or resolution; a few noted ease of use; and none commented on field of view. The postactivity responses were broader and more insightful and included customizability, field of view, speed, and more. Students realized that there are many different axes along which one might optimize.

Before the microscopy activity, students were asked to indicate on a logarithmically spaced number line of sizes where they thought immune cells and bacteria would lie. About half the students assigned bacteria a larger size than immune cells. Revisiting this question afterward, all students realized that bacteria are smaller than immune cells. Estimates of size were still not particularly accurate (average $\approx$ 1 μm for immune cells), but I note that we did not explicitly teach anything about the sizes or even orders of magnitude of the objects we viewed; these followed simply from observation. A longer activity could easily also incorporate instruction on size scales.

Discussion of microscopes and uses spurred student thinking along many axes, some of which were unanticipated. Some asked, for example, about cost; I asked all the students to guess the prices of various microscopes. The first guesses for the array microscope were around US$30,000; jaws dropped at the actual price, in the range of US$500,000–1,000,000. The simple compound microscope is around US$300. For the light sheet fluorescence microscope, we noted the differences between home-built systems (such as ours) and commercial microscopes (~US$200,000–500,000). We discussed where the money comes from, with a brief explanation of the unfamiliar world of research grants. Money is an interesting topic, and one that is not often brought up in the context of science.

A postcamp survey administered to students by the SAIL program, included as Supplemental Material, revealed a highly positive assessment of these microscopy and imaging experiences. The activities were tied for the third most frequently listed as favorites among the week 11 items, most of which have been honed by years of practice (the first and second place activities involved the



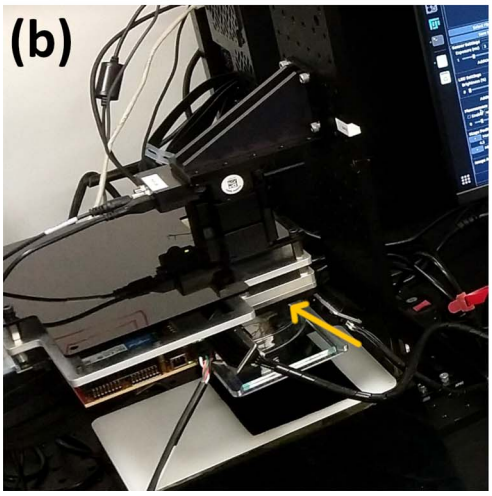


Fig 4. (a) Students with the Ramona Optics multicamera array microscope. (b) An array of 48 cameras and lenses faces downward (orange arrow) over the sample to be imaged, covering an area (6 × 8 cm) at ~3 μm/pixel.

physics of climbing, climbing at the university gymnasium rock wall, and hands-on exploration of human cadavers, whose impact is stunning).

B. Generalizability

Although the microscopy activities described here involve specialized equipment, including expensive or customized microscopes, and specialized materials, including transgenic zebrafish, I believe they can be replicated at most colleges and universities by using other instruments and specimens that similarly illustrate the breadth of contemporary biophysical microscopy. The choice of microscopes highlighted 3 distinct approaches: simple, inexpensive, but relatively featureless imaging, as exemplified by the familiar compound bright-field microscope; high-resolution fluorescence imaging, as exemplified by the light sheet fluorescence microscope; and large field of view microscopy, as exemplified by the Ramona Optics multicamera array microscope. Lacking a light sheet microscope, one could illustrate the same themes using more common confocal microscopes; both are capable of 3D imaging, and the trade-offs between them regarding imaging speed, phototoxicity, and field of view are irrelevant to this activity. Lacking a confocal microscope, a standard epifluorescence microscope would also suffice, given a roughly 2D sample, such as cultured cells or tissue slices. To substitute for the array microscope, slower large-field instruments, such as plate scanners would work. Alternatively, one could tackle a different imaging axis altogether, such as electron microscopy, highlighting greatly enhanced resolution at the expense of working with nonliving specimens.

Regarding specimens, static specimens can suffice, but living organisms are particularly effective

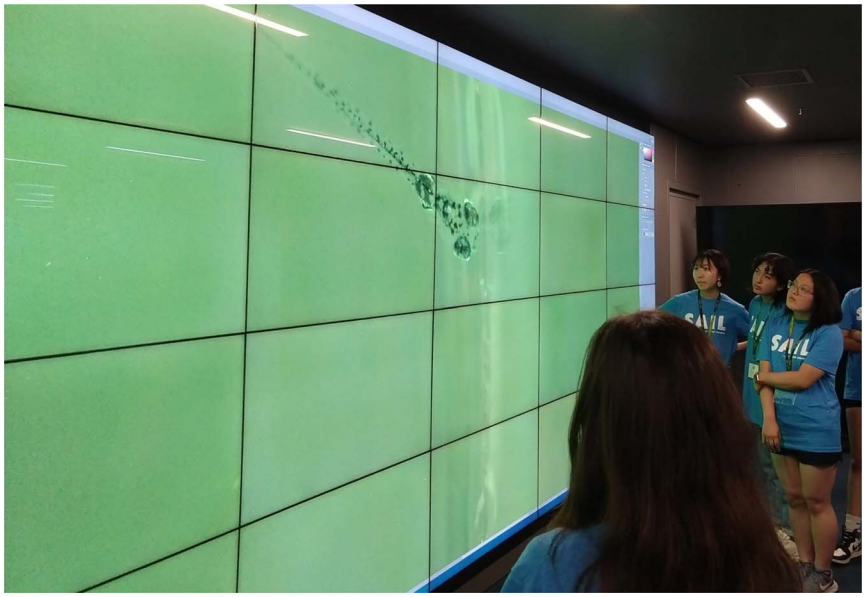


Fig 5. An image of a larval zebrafish taken with the Ramona Optics multicamera array microscope, displayed on a monitor array of 50 million pixels.

at capturing students' attention. Zebrafish (*Danio rerio*) are effective but challenging, requiring expertise, as well as appropriate vertebrate animal use protocols. However, many alternatives are possible. For example, rotifers (*Brachionus plicatilis*) are highly motile zooplankton that are readily commercially available, being commonly used as food for aquarium fish. The bodies are somewhat autofluorescent; moreover, adding fluorescent microparticles to the surroundings can illustrate the remarkable fluid flow they generate to draw material toward them. I speculate that a reason the zebrafish larvae were appealing to students is that they were visible both by eye under the various microscopes, including the simple microscope. In contrast, I have found it difficult in the past to excite students about smaller microorganisms such as are found in pond water, which are apparent only under magnification. The fish larvae are visible as creatures darting around in a dish of water, and one can then zoom into with a microscope. This is also the case for rotifers. Other "model organisms" may also be good subjects. Roundworms (*Caenorhabditis elegans*) and fruit fly larvae (*Drosophila melanogaster*) are common, important to contemporary research, and available as a variety of fluorescent transgenic animals, though one should be aware that roundworms are challenging to see by eye because of their transparency and fruit fly larvae are motionless on easily accessible timescales.

IV. CONCLUSIONS

The activity described here aimed to broaden high school students' conceptions of what microscopy means, introducing them to different types of microscopes and to various factors that might guide scientists and engineers using optical tools. The resulting discussions illuminated many aspects of biophysics and even touched on mechanisms of science funding not usually brought to the attention of students. These activities are adaptable to other institutions with other types of imaging devices. Perhaps most importantly, the activities made apparent to students that physics, biology, engineering, and even computer science all contribute to the design and use of systems that can peer into living things.

SUPPLEMENTAL MATERIAL

Supplemental Data consisting of the SAIL student survey is available at: https://doi.org/10.35459/tbp.2024.000261.S1.

AUTHOR CONTRIBUTIONS

RP designed the activities, led the implementation, collected survey data, and wrote the article.

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The author declares no competing interests.

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