DIY Microscope for Advanced Imaging and Educational Outreach

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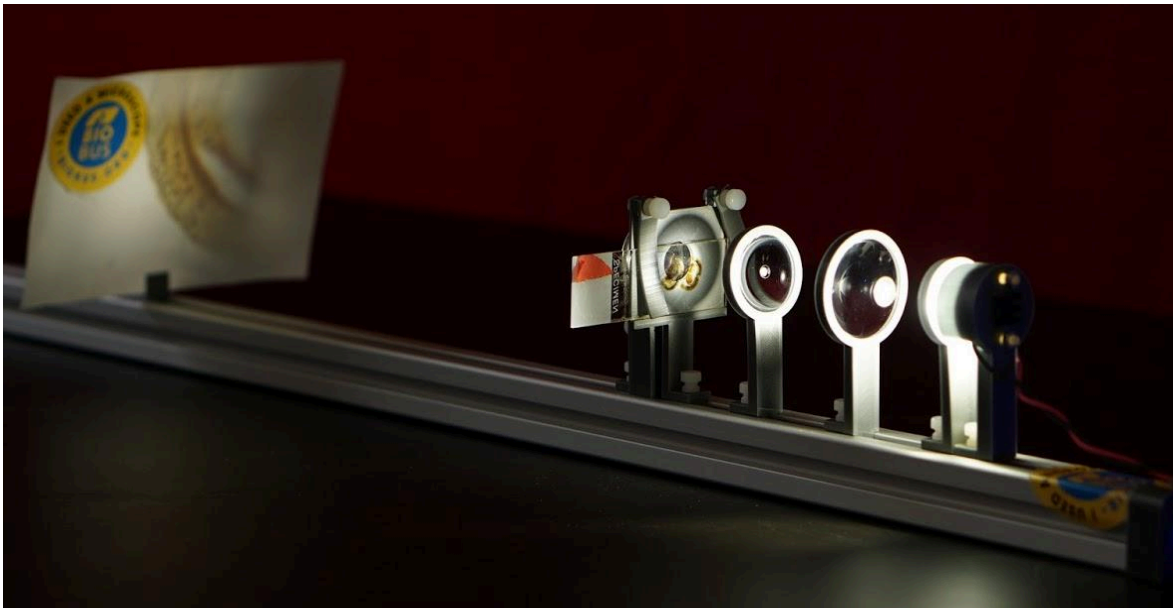

1 INTRODUCTION

Microscopes are among the most versatile and powerful scientific instruments. Their introduction in the 16th century led to an explosion of scientific progress, including the discovery of biological cells and the associated realization that the world is full of organisms and processes that are too small to see by eye[1]. Conventional light microscopy has been overshadowed by the development of techniques with ever finer resolution, including electron microscopy and x-ray microscopy. Recently, however, scientists have developed new approaches to light microscopy that greatly extend its reach. Confocal microscopy, for example, can map structures within living cells. Recently introduced variants of that technique can track individual fluorescent molecules[2] and were recognized with the 2014 Nobel Prize in Chemistry[3]. All of these advanced techniques, however, share the shortcoming that the most advanced instruments are exceptionally expensive closed systems that are not appropriate for classroom use and cannot easily be modified for new applications.

The purpose of this project is to address the need for affordable and flexible instruments for advanced microscopy by developing a modular open-source microscope made with 3D printed parts. The project's joint goals are to make microscopy affordable for science classrooms around the world, and to develop a hackable platform for scientific research. The DIY microscope is affordable enough to be deployed in historically underserved communities. Its open design and construction provide students with a hands-on experience of optics that supports the classroom learning experience. Versions of this instrument, moreover, are

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[1] Croft, William J. *Under the Microscope: A Brief History of Microscopy*. World Scientific, 2006. (https://books.google.com/books/about/Under_the_Microscope.html?id=Cr4_UJkBwYYC. Accessed 19 Nov. 2017.

[2] Sydor, Andrew M., et al. "Super-resolution microscopy: from single molecules to supramolecular assemblies." *Trends in Cell Biology* 25.12 (2015): 730-748.

[3] "The Nobel Prize in Chemistry 2014." https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/. Accessed 19 Nov. 2017.

functional enough to provide the basis for novel microscopy techniques. This was demonstrated by the development of a version of the DIY microscope for fluorescence microscopy and, most recently, by implementing a near-infrared (NIR) microscope that offers new insights into plant metabolism.

2 METHODS AND MATERIALS

2.1 Light microscopy

Optical microscopes use lenses and other optical elements, such as mirrors, prisms and filters, to project magnified images of microscopic objects. Relevant principles of optics are reviewed in standard textbooks[4]. The capabilities and limitations of a particular microscope are determined by the choice of lenses, and how they are arranged. One of the most relevant properties of a lens is its focal length, which is the distance over which it converges a parallel beam of light to a point. Another is the numerical aperture, the ratio of the lens' diameter to its focal length, which determines how much light it collects, and how sharply it focuses the light. This, in turn, helps to determine the resolution of any image that can be obtained with the lens. Figure 1(a) shows two rays from a parallel beam being converged to a point in this way. Similarly, a point source of light located at the focal length from a lens is transformed into a parallel beam of light. This process, known as collimation, is shown in Figure 1(b). If the point source lies on the axis of the lens, also called the optical axis, the collimated beam travels parallel to the axis. If the source is displaced from the axis, the collimated beam travels at an angle that is proportional to the displacement.

[4] Hecht, Eugene, *Optics.* Pearson, 2012. https://books.google.com/books/about/Optics.html?id=7aG6QgAACAAJ. Accessed 19 Nov. 2017.

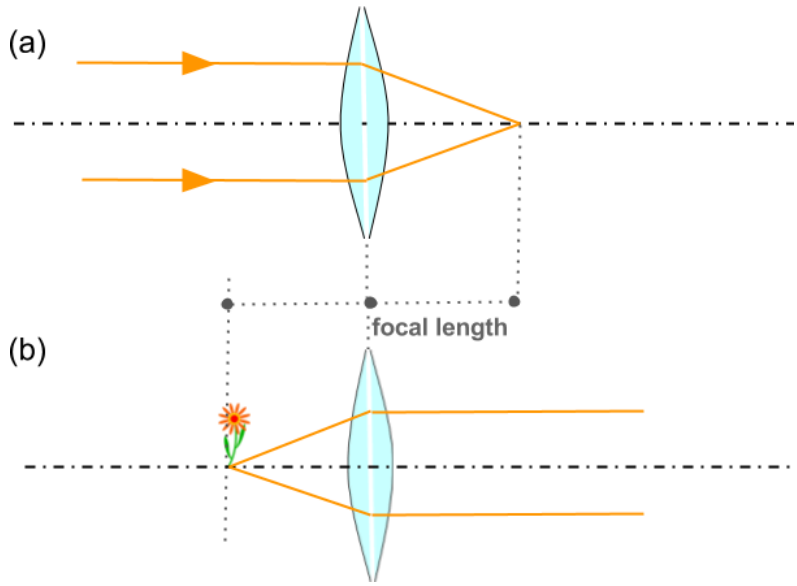

Figure 1. (a) Focusing by a converging lens. (b) Collimation by a converging lens.

The classic configuration for a microscope is made from two lenses, one of which collects light from the sample and collimates it, and the other of which converges the collimated light onto a photodetector. This configuration is depicted schematically in Figure 2. Separating the two lenses by the sum of their focal lengths ensures that a point source in the front focal plane converges to a point in the back focal plane, and collimated light emerges collimated. Obtaining positive magnification requires the objective lens, which is closest to the object, to have a shorter focal length than the imaging lens, which is variously called the ocular or tube lens.

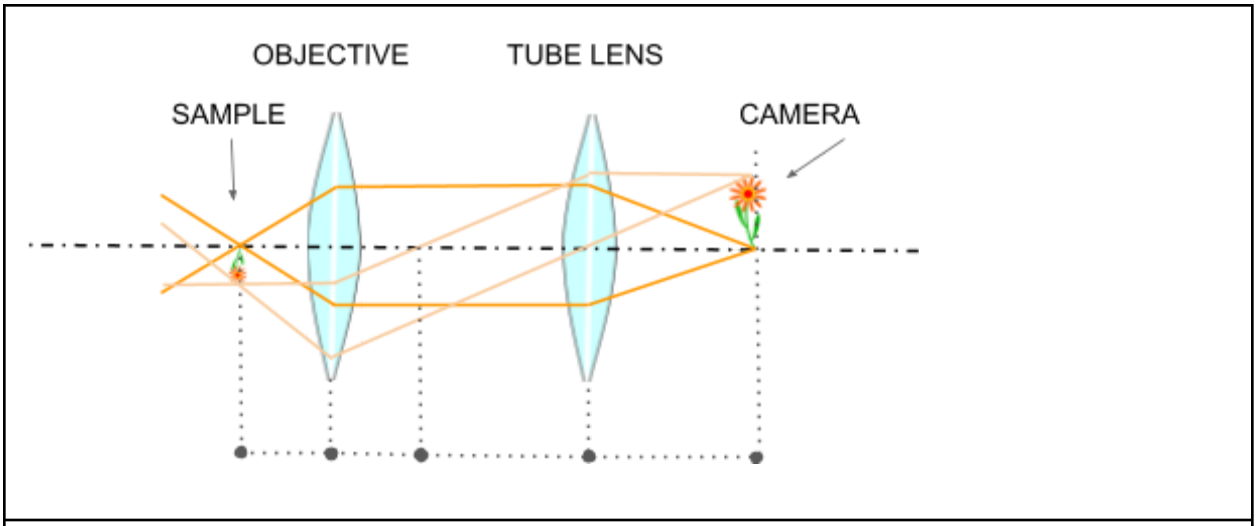

Figure 2. Basic microscope configuration. Light rays originating from the sample are collimated by the objective lens before being brought to a focus on the imaging plane by the tube lens. The lateral magnification is the ratio of the two lenses' focal lengths.

From a practical viewpoint, even this simple combination of optical elements poses practical challenges. The sample, the two lenses, and the detector all have to be arranged along the optical axis in the correct three-dimensional positions and orientations. Departures from the ideal configuration degrade the microscope's performance by introducing aberrations that blur fine features. Commercial microscopes address this challenge by mounting optical elements in rigid monolithic frames. This construction yields excellent performance, but makes modification costly and difficult. The DIY microscope addresses the same challenge with off-the-shelf building materials and desktop additive manufacturing (3D printing). This approach reduces costs dramatically and promotes experimentation.

2.2 The DIY microscope

The frame for the DIY microscope consists of a length of extruded aluminum rail, a standard low-cost building material that can be purchased at hardware stores. This is shown in the SolidWorks rendering in Figure 3(a) and in the working instrument in Figure 3(b). The extruded

rail features T-shaped slots along its length that can be used to attach objects. The drawing in Figure 3(a) features the entire library of DIY microscope parts that can be affixed to the rail using bolts passing through their bases into threaded inserts within the T slot. Each of these parts is a custom-designed component of the DIY microscope that can be printed out using a 3D printer. The idea of a 3D-printed microscope was originally developed by Francesca Anselmi (BioBus, Inc.) and Joshua Sanders (https://www.sanworks.io/), and founded by BioBus, Inc.

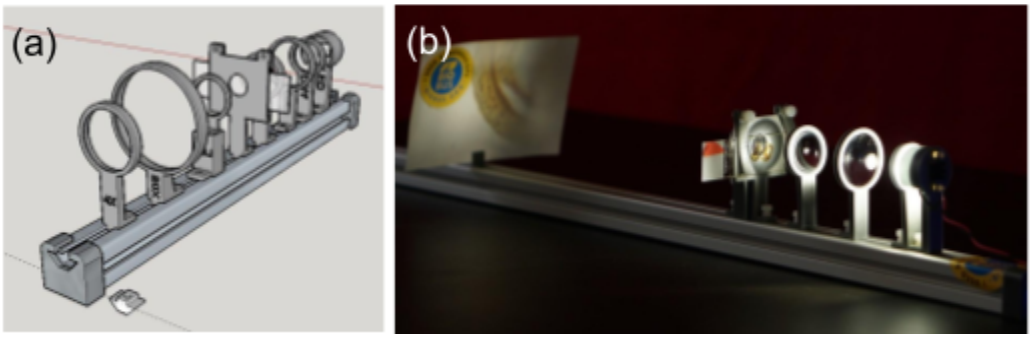

Figure 3. (a) Digital microscope parts file. (b) Working DIY microscope.

Despite rapid advances, 3D printing technology is not yet able to craft optical-quality lenses[5]. The design shown in Figure 3 therefore relies on low-cost aftermarket lenses purchased online from discount suppliers (Edmund Optics). The design for the mount then must be adjusted to match the diameter of the available lens before the mount is printed. This would have been a laborious process in the days before 3D printing. Now, a custom-fit part can be produced in under an hour.

[5] "Creating Camera Lenses with Stereolithography | Formlabs." https://formlabs.com/blog/creating-camera-lenses-with-stereolithography/. Accessed 19 Nov. 2017.

Configuring the DIY system as described in this paper produces an instrument that achieves up to 20x magnification, which is comparable to a research-grade compound microscope. A cell phone camera can then be used to capture magnified images of almost any transparent or translucent sample formed by the microscope. Examples include plant cells, skeletal muscle tissue, the freshwater crustacean *Daphnia magna*, and mouse neurons. These typical results demonstrate that a person without expensive equipment or extensive specialized training can learn to assemble and use this microscope kit to collect research-quality data.

The DIY microscope has been field-tested in STEM outreach activities at the BioBase, a not-for-profit Harlem-based STEM enrichment center, and at the Johns Hopkins Center for Talented Youth. In both of these contexts, students ranging in age from 9 to 18 years old have assembled DIY microscopes and used them to create images of samples.

2.3 Conventional bright-field imaging

A conventional bright-field microscope makes use of illumination strategies to achieve the best possible brightness, contrast and resolution. The goal of these techniques is to illuminate the sample with an ideally defocused image of the light source so that the image will appear uniformly bright. One of these arrangements, called critical illumination, is shown in Figure 4. The collector and condenser lenses are arranged to focus the light source onto the sample plane and therefore onto the image plane. This would yield very undesirable variations in illumination, were it not for a translucent diffuser placed between the light source and the collector. When viewed through a pair of apertures, this diffuse source travels as a semi-collimated beam through the microscope and illuminates the sample uniformly. The diagram in Figure 4 therefore presents a complete layout for the DIY microscope in the configuration intended for viewing by eye.

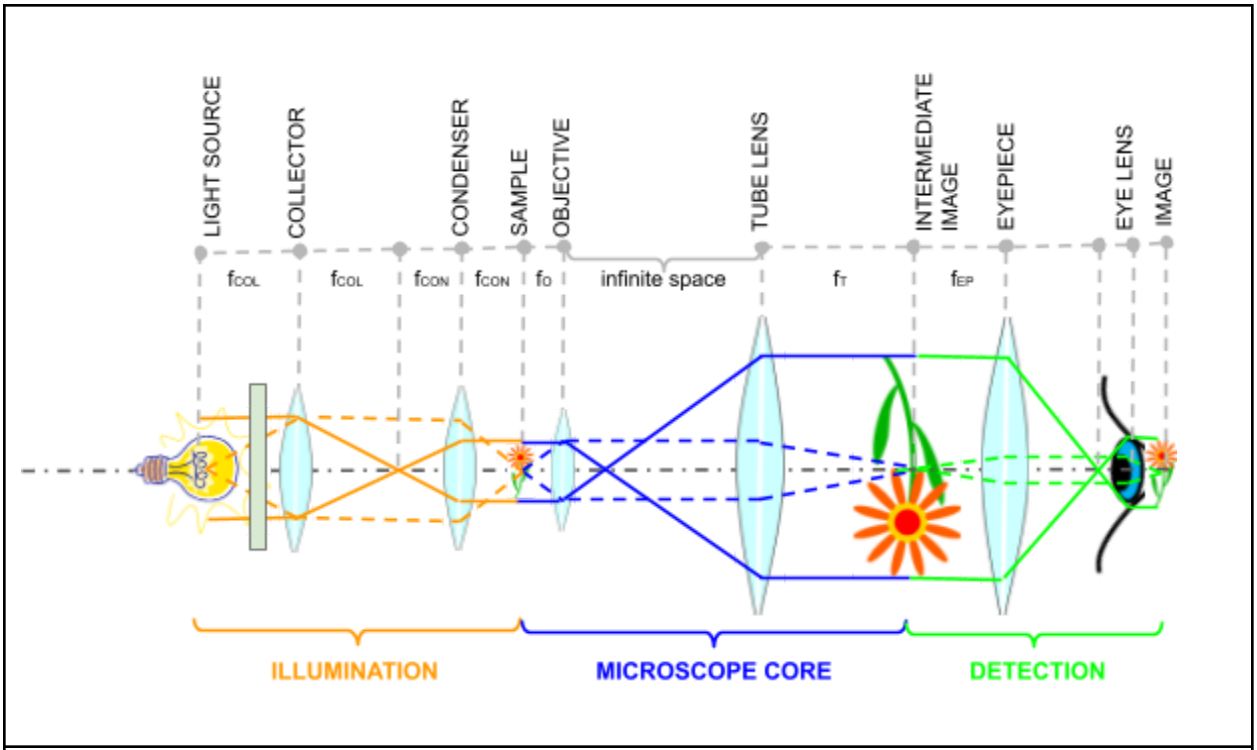

Figure 4. Diagram of complete DIY microscope configuration. The Köhler illumination system provides uniformly bright collimated light to the sample plane. A standard microscope magnifies the image and projects it onto the intermediate image plane. When the image is to be viewed by eye, an additional eyepiece collimates the light from the intermediate plane in preparation for focusing by the eye's lens onto the retina.

When the microscope is intended for use with a camera, the eyepiece is removed and the camera's sensor is centered in the intermediate image plane. The bright-field light source for the DIY microscope is a high-intensity white LED. In this mode of operation, the continuum of wavelengths contained in the white light spectrum contribute to image formation.

2.4 Fluorescence microscopy

Some materials have a property called fluorescence, which means that they absorb light of one wavelength and emit light of another wavelength. Typical fluorescent materials absorb short-wavelength (blue) light and emit longer wavelength (green) light. Placing a filter after the sample to block the short wavelength illumination then permits only the fluorescence to pass

through to the camera and form an image. The resulting image provides a detailed view of just those regions of the sample that fluoresce.

Fluorescence microscopy is particularly useful when interesting structures within samples can be deliberately labeled with fluorescent dyes. Traditionally, this has required chemical stains, most of which are lethal to biological samples. Recently, however, advances in genetic engineering have enabled scientists to modify organisms so that they naturally express fluorescent proteins associated with specific internal structures[6]. This means that specific structures within living cells can be viewed in real time through fluorescence microscopy.

The DIY microscope can be modified for fluorescent imaging by replacing the white LED in the illuminator with a blue LED, and introducing a band-pass green filter between the tube lens and the camera plane.

2.5 Infrared imaging

One of the downsides to white light imaging is blurring and distortion of the image due to chromatic aberration, an effect caused by the unequal bending of different wavelengths of light by the lenses. One method for avoiding this problem is to illuminate the sample with a narrower range of wavelengths, in this case by using an infrared LED as the light source. This nearly monochromatic illumination makes it possible to capture much sharper images at high magnifications without the use of more expensive achromatic lenses.

Because IR light is not visible to the human eye, samples can only be viewed through a camera when illuminated in this way. It is important to note that most commercial cameras come with built-in IR-blocking filters. However, cameras intended for use at night or in low-light conditions use IR and so do not have an IR filter.

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[6] Remington, S. James. "Green fluorescent protein: a perspective." *Protein Science* 20.9 (2011): 1509-1519.

The DIY microscope can be modified for infrared microscopy by using a low-cost IR LED for illumination and acquiring images with a Raspberry Pi single-board computer outfitted with an infrared camera module. This setup can be used to demonstrate the benefits of single-color microscopy for high-resolution imaging.

2.6 NDVI imaging

With recent advances in affordable imaging technology, including high-quality cameras with infrared sensitivity, maker communities have been able to implement new research and improve on it like never before. One technique which has benefitted from this exposure is Normalized Difference Vegetation Index, or NDVI, analysis. This is a method of assessing photosynthetic plant health through comparison of visible and infrared light radiation. Originally used by NASA satellites to make images of the Earth with multi-spectral scanners (MSS), this has been adapted by botanists and farmers to assess plant health.

Conventional NDVI analysis takes advantage of the differences in light reflectance by plants performing photosynthesis. During photosynthesis, the chlorophyll molecules in the chloroplasts strongly absorb blue and red light. As a consequence, reflectance in these wavelength regions is very low. . By contrast, plant cells absorb weakly in the near infrared (NIR) and also dispose of waste heat from photosynthesis in these long wavelengths. This means that plants' reflectance in the near infrared (NIR) is comparatively high. This means that by taking a multispectral picture of the plant and doing a ratiometric comparison of the visible and NIR reflectance for each pixel, it is possible to generate a figure that shows areas of photosynthetic productivity. Most frequently, this technique is used to analyze aerial photographs to distinguish photosynthetically active regions from other types of ground cover. We propose to apply NDVI analysis to video microscope images of plants and other biological samples to obtain novel information on metabolic activity. This can be achieved using a color

camera that has lacks an infrared filter in front of its image sensor. The red pixels in such a camera should be sensitive to NIR light. Placing a blue band pass filter in front of the sensor then will block red light. With the red light blocked, the “red” pixels in the RGB camera will capture exclusively NIR signals. The ratio between the blue and red pixel, then, returns an estimate of the chlorophyll content.

We are planning to adapt NDVI to image the chlorophyll level in individual chloroplast in living plant cells with the DIY microscope. This will be a scientific first for the DIY microscope technology, showing that, with basic understanding of optics principles, affordable DIY optical technology can be used to collect functional signals from living samples.

2.7 Hardware

Aluminum rails and mounting hardware were purchased from McMaster Carr. Lenses were purchased as stock-clearance items from Edmund Optics. The design for the DIY parts was modified to accommodate the diameters of the lenses as received. Plastic mounts for the optical elements were printed on an Ultimaker II, whose use for this project was donated by Josh Sanders (sanworks.io) and Spheryx, Inc. Microscope slides were purchased from Carolina Biological or donated by volunteer scientists at BioBus, Inc..

Bright-field images were illuminated with a white LED (Cree XLamp CXA1512) purchased from Newark Electronics. Infrared images were recorded with an IR LED operating at a wavelength of 940 nm that was purchased from Adafruit Industries. Fluorescence images were recorded with a commercial blue source.

All bright-field and fluorescence images were recorded with a cell-phone camera. The model used (Samsung Galaxy S6) records images with a resolution of 1920 x 1080 pixels.

Infrared images were recorded with a Pi-NoIR v2 camera module attached to a Raspberry Pi 3B single-board computer running the Raspbian operating system. This system provides full-color images with a resolution of 3280 x 2464 pixels. The camera differs from a standard cell-phone camera in that it does not have an infrared filter, and thus can record images in infrared light. Both the Raspberry Pi computer and the camera module are open-source products of the Raspberry Pi foundation. Software for image acquisition and image analysis on the Raspberry Pi was written in the python programming language[7] using the OpenCV library of computer vision algorithms[8].

2.8 Samples

Mounted samples of mouse neurons were courtesy of P. Gupta from Cold Spring Harbor Laboratory. Human skeletal muscle fibers were purchased from Carolina. Samples of grass courtesy of Joshua Sanders of Sanworks (www.sanworks.io).

Transgenic fruit flies (*Drosophila melanogaster*) modified to express green fluorescent protein (GFP) were courtesy of Natalie Kolba from Columbia University, and were maintained according to the method of Stocker and Gallant [9].

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[7] Van Rossum, Guido, and Fred L. Drake Jr. *Python Reference Manual*. Amsterdam: Centrum voor Wiskunde en Informatica, 1995.

[8] Bradski, Gary. "The OpenCV Library." *Dr. Dobb's Journal: Software Tools for the Professional Programmer* 25.11 (2000): 120-123.

[9] Stocker, Hugo, and Peter Gallant. "Getting started: an overview on raising and handling *Drosophila*." *Drosophila: Methods and Protocols* (2008): 27-44.

3 RESULTS

3.1 Bright-field imaging

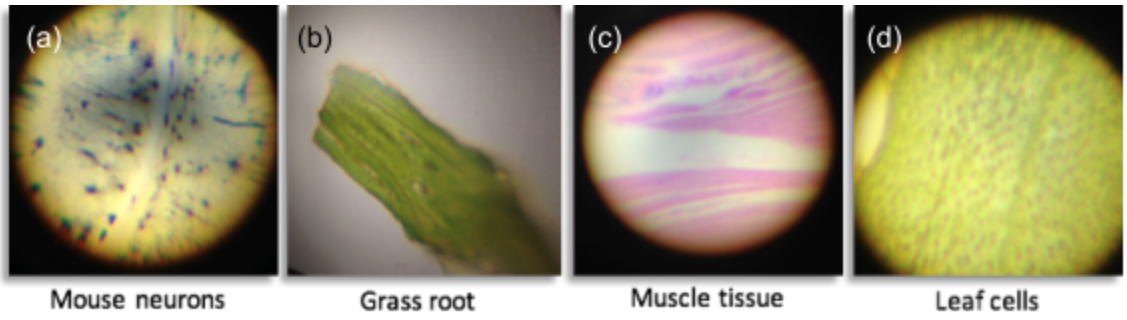

Figure 5. Images of samples captured using the DIY microscope configuration described in Sec. 2.3. (a) Mouse neurons. (b) Section of grass root. (c) Mounted and stained muscle tissue. (d) *Elodea canadensis* leaf.

Figure 5 shows typical results obtained with the DIY microscope configured for bright-field operation, as depicted in Fig. 4. The image in Figure 5b was projected on a paper screen, positioned in the image plane after the tube-lens. Images in Figure 5(a), (c) and (d) were recorded with a phone camera placed after the eyepiece to accommodate its built-in lens. Circular vignetting reflects the actual path of light through the microscope. This could be removed either by expanding the image to fill the camera's aperture, or by cropping the images. For the purposes of demonstrating the DIY microscope's imaging capabilities, these steps were not necessary.

FIgure 5(a) shows the sagittal cross-section of a mouse brain that has been stained with Golgi protocol to emphasize individual neurons. The neurons' cell bodies are visible as dark elliptical masses approximately 6 x 9 pixels across, which is consistent with a mean diameter of 16 μm

given an estimated magnification of 2 μm/pixel. This is consistent with previously reported sizes of similar structures[10].

The grass root presented in Fig. 5(b) was collected from a private garden in Port Jefferson. The root anatomy is clearly resolved, from the epidermis on the outside to the cortex, the pericycle and the central phloem[11]. Individual cells are not resolved in this image because the sample is not sectioned and stained, but instead is placed on the slide whole.

Figure 5(c) illustrates the benefits of staining for resolving fine features. In this case, a commercial microscope sample of skeletal muscle tissue is stained with H&E dye to clearly distinguish the so-called A bands from other structures, which are not stained. These elongated fibers contract to exert forces that move bones in vertebrates. Once again, individual cells cannot be resolved in this image because the stain emphasizes tissue structure.

Individual cells are clearly resolved in the sample of pondweed (*Elodea canadensis*) shown in Fig. 5(d). This sample was grown in the laboratory at BioBus. This sample is neither sectioned nor stained. The leaf's planar structure naturally lends itself to imaging. Each cell appears as a rectangle with a 2:1 aspect ratio, with a typical length of 50 μm at an estimated magnification of 5 μm/pixel.

3.2 Fluorescence imaging

The images in Fig. 6 were obtained with a larval fruit fly that was dissected along the medial line to expose the brain and mouth hooks. This transgenic organism expresses green fluorescent protein (GFP) in its neurons, permitting detailed imaging of its nervous system. The image in

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[10] https://synapseweb.clm.utexas.edu/dimensions-dendrites; Fiala, John C., Kristen M. Harris, and K. H. Spacek. "Dendrite structure." *Dendrites*. Oxford University Press, 1999. 1-34.
[11] http://www.robinsonlibrary.com/science/botany/anatomy/roots.htm

Fig. 6(a) details the larva's mouth hooks, which are used for olfaction and locomotion, as well as eating. Their bright fluorescence demonstrates that these structures contain a large number of neurons. Each mouth hook measures 100 μm across and is 200 μm long.

Focusing further back on the larva reveals two lobes of the central brain, together nerve fibers emerging from it. Both of these structures appear far brighter in fluorescence than the surrounding tissues.

These images, taken with a low cost camera, demonstrate how low-cost equipment can be used to reproduce imaging techniques normally found only in research labs.

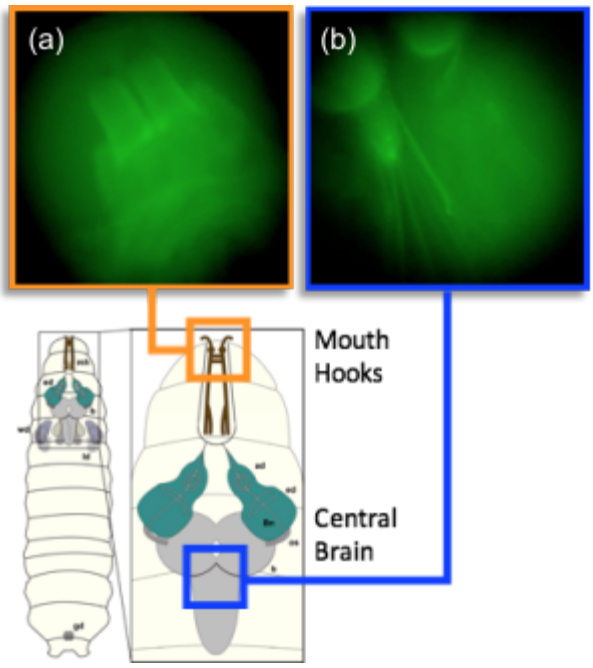

Figure 6. (a) Fluorescence microscope image of larval fruit fly mouth hooks and (b) central brain. Boxes overlaid on the sketch[12] identify the corresponding structures in the organism.

[12] Jeibmann, Astrid, and Werner Paulus. "*Drosophila melanogaster* as a model organism of brain diseases." *International Journal of Molecular Sciences* 10.2 (2009): 407-440.

3.3 Infrared imaging

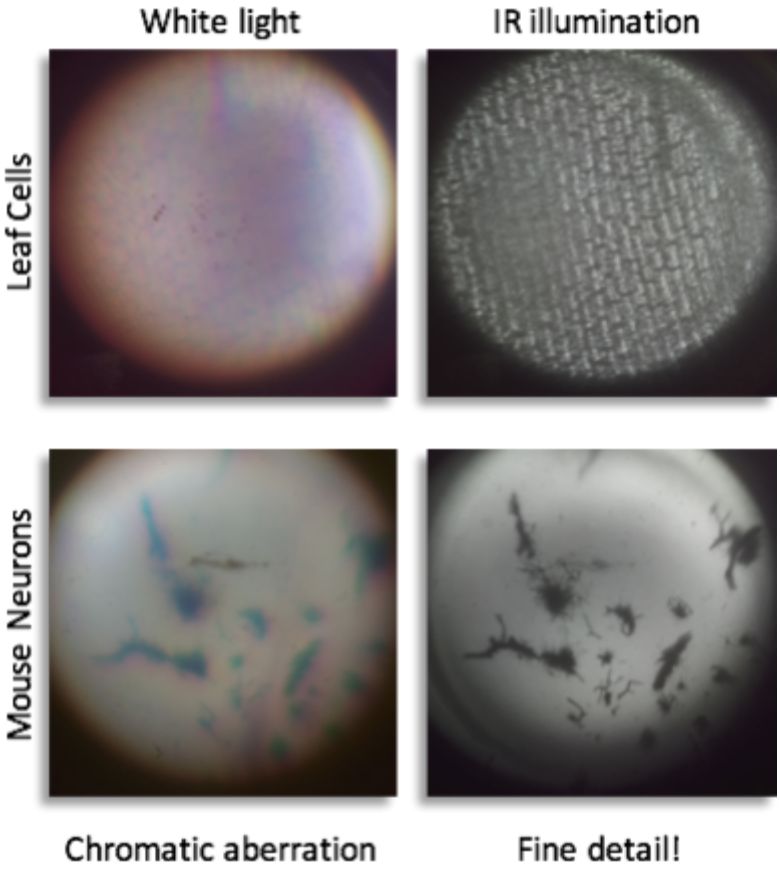

Figure 7. Comparison between imaging in broadband white light and imaging in nearly monochromatic infrared light. Images of *Elodea* leaves and stained mouse neurons recorded with white light suffer from chromatic aberration, and so appear blurred. The samples are sharply resolved in infrared light.

The images in Fig. 7 were captured with the Raspberry Pi NoIR camera using both broadband white light illumination and single-wavelength infrared illumination. The low-cost plano-convex lenses used for the DIY microscope have no corrections for chromatic aberration. Different wavelengths focus to different planes, thereby blurring the images and creating attractive but distracting colored halos around objects.

Replacing the illuminator with a single-wavelength infrared source eliminates chromatic aberration. The resulting monochromatic images are sharply resolved. Most notably, individual cells in the *Elodea* leaf can be seen clearly, as can granuoles and other structures within the cells. Similarly, individual dendrites are resolved in the infrared image of mouse neurons. Still finer structures might be observed if the infrared illumination were replaced with monochromatic light of a shorter wavelength. Infrared illumination is useful, however, for developing modes of imaging that detect metabolic processes within living cells.

3.4 NDVI imaging

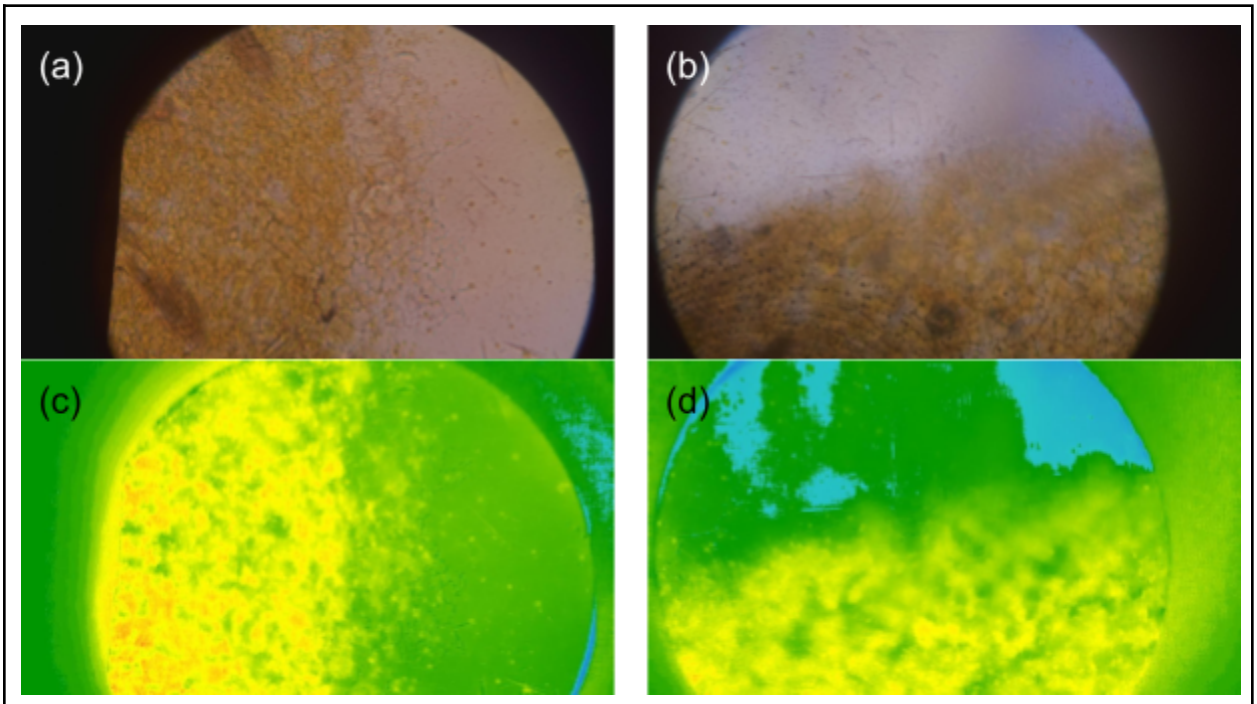

Figure 8. Images of duckweed chloroplasts- (a),(b)- and NDVI photo analysis- (c),(d). Warmer colors represent higher photosynthetic productivity, while cooler colors are “dead zones.”

Wet mounts were made of samples of the freshwater plant duckweed. These were placed under a commercial microscope with conventional halogen illumination for initial proof-of-concept trials of NDVI microscopy. The Pi NoIR camera, outfitted with a blue filter for NDVI imaging, was positioned after the microscope eyepieces with a custom adapter, and the final images were

formed by a telescope including the eyepiece and the built-in camera lens. Figures 8(a) and 8(b) show two typical snapshots of the field of view, rendered as standard full-color images. The field of view is vignetted by the camera's field pupil because the handheld camera is not located at the microscope's true eyepoint. The sample is arranged in each image so that half of the visible field is covered by living duckweed, and the other half is filled with pond water, which presumably does not have a high concentration of chloroplasts.

Figures 8(c) and 8(d) show the same data converted to ratiometric images in the red and blue channels, and rendered with the NDVI color map. Because of the blue filter blocking red light from reaching the sample, the red pixels on the camera are measuring the near infrared (NIR) signal. The heat scale in the map is inversely proportional to the ratio of reflected blue vs. near infrared (NIR) light. Specifically, yellow-red regions in the color map correspond to regions of decreased blue/NIR signal . This is consistent with regions of higher photosynthetic activity because chlorophyll molecules in the chloroplasts absorb blue light and reflect NIR light. The water-rich regions, by contrast, appear green-blue in the color map because water tends to reflect both NIR and blue light (higher blue/NIR ratio). These preliminary results therefore support the proposal that NDVI imaging can be used to identify regions of photosynthetic activity in microscope images. In the future, we plan to test NDVI imaging in the DIY microscope.

4 ANALYSIS

The experiments reported here demonstrate that the DIY microscope platform can be used to perform measurements on biologically-relevant samples using a range of different imaging methods. We have demonstrated bright-field microscopy, fluorescence microscopy, and infrared microscopy, all of which are standard techniques for commercial microscopes. More recently, we have adapted a commercial microscope to perform NDVI imaging. If confirmed, the preliminary results reported here are noteworthy because, to the best of our knowledge, NDVI

microscopy has not been used elsewhere to perform functional images of plant cells[13]s. Our preliminary results show that the NDVI signal is proportional to the concentration of chlorophyll in the plant cells and chloroplasts. This, in turn, can be used as a proxy for photosynthetic activity. Since chlorophyll structure is quickly affected by metabolic stress, as well as stress caused by viral infections and other physical agents, NDVI microscopy could be a useful tool to monitor the health and metabolism of plant tissue at the single cell level. More work needs to be done to confirm these results and to compare them with those obtained by functional fluorescence imaging of chlorophyll, the current standard in the field (Li et al. Sensors 2014). With respect to fluorescence microscopy, our implementation of NDVI microscopy would be simpler and cost-efficient. It would also have the potential to be applied in the field (in the form of a portal microscope) - something that would be much harder for fluorescence microscopy, constituting one of the major limitations of this technique (Li et al. Sensors 2014) .

In its basic form, the DIY microscope also has proven useful for educational outreach. It has been field tested with students ranging in age from 8 years old to 17 years old, and during professional developments for teachers, generating widespread enthusiasm and positive feedback Explaining how to assemble the DIY microscope provides a good basis for teaching students about the principles of optics. The images that the microscope captures are also useful teaching aids for biology and other disciplines. The DIY microscope's low cost and open structure make it both affordable and accessible to schools.

5 CONCLUSION

The DIY microscope composed of standard hardware, discount optics and 3D printed parts has been demonstrated in this project to be a useful platform for research and education. The

[13] Li, Lei, Qin Zhang, and Danfeng Huang. "A review of imaging techniques for plant phenotyping." *Sensors* 14.11 (2014): 20078-20111.

flexible design of this system lends itself to experimentation. So far, we have modified the base design to capture fluorescent images and infrared images. The addition of NDVI imaging capability suggests that the DIY microscope can be used to monitor photosynthesis in plants. It may work also for studying other aspects of metabolism in other types of cells by monitoring local heat output.