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ADVANCES IN PHYSIOLOGY EDUCATION

LABORATORY SOURCEBOOK

The construction of high-magnification homemade lenses for a simple microscope: an easy "DIY" tool for biological and interdisciplinary education

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Abstract

The rise of microscopy in the seventeenth century allowed scientists to discover a new world of microorganisms and achieve great physiological advances. One of the first microscopes of the epoch was Antonie van Leeuwenhoek's microscope, a deceptively simple device that contains a single ball lens housed in a metal plate allowing the observation of samples at up to ×250 magnification. Such magnification was much greater than that achieved by rudimentary compound microscopes of the era, allowing for the discovery of microscopic, single-celled life, an achievement that marked the study of biology up to the nineteenth century. Since Leeuwenhoek's design uses a single ball lens, it is possible to fabricate variations for educational activities in physics and biology university and high school classrooms. A fundamental problem, however, with home-built microscopes is that it is difficult to work with glass. We developed a simple protocol to make ball lenses of glass and gelatin with high magnification that can be done in a university/high school classroom, and we designed an optimized support for focusing and taking photographs with a smartphone. The protocol details a simple, easily accessible, low-cost, and effective tool for the observation of microscopic samples, possible to perform anywhere without the need for a laboratory or complex tools. Our protocol has been implemented in classrooms in Chile to a favorable reception.

ball lens; "do it yourself"; glass; Leeuwenhoek; optics

INTRODUCTION

Objectives and Overview

Microscopy is fundamental to physiology—millions of microscopes are used daily around the world by doctors and researchers to investigate fundamental life processes and diagnose afflictions. Although a microscope is considered a universal symbol and tool for the biological sciences, teaching how microscopes work, because of the mathematics and physics involved, is a challenge for high school and university classrooms. As machining glass and manipulating optical tools is out of the scope of most teaching laboratories, students can never build a microscope "from first principles" as electrical engineers can do in circuit design and computer programmers can do with code. We aimed to ameliorate this problem by developing a protocol for students to build simple microscopes by melting and manipulating small pieces of glass in a replica exercise of the famous microscope of Antonie van Leeuwenhoek. Students following this protocol will build their first microscope from scratch, learn about the history of microscopy and progress in biological understanding, and learn the first principles of magnification and optics.

Background

The diversity of life existing on Earth is not restricted to what we can observe with the naked eye. A much greater diversity of microscopic organisms have sizes so small that their study requires optical magnification tools. The development of microscopy, whose beginnings date to the seventeenth century, has allowed scientists to discover countless organisms and study the structural components of life's organization. Microscopy is the fundamental tool for biology, and Antonie van Leeuwenhoek of Delft, The Netherlands (1632–1723) was the pioneer who invented the first high-power microscope (1). His microscope is an elegant device that contains *1*) a single ball lens housed in a metal plate, *2*) a specimen pin where a capillary glass containing the sample in water can be inserted, and *3*) a series of associated screws to allow the positioning and focusing of the sample (Fig. 1). Unlike previous compound microscopes developed, such as Galileo Galilei's (1564–1642), Zacharias Janssen's (1585–1632), and Robert Hooke's (1635–1703), Leeuwenhoek's use of a small, single ball lens with high magnification diminished the visual aberrations typical of multiple-lens compound microscopes, allowing clearer observations (4). Leeuwenhoek was able to make immense advances, discovering species of protozoa and bacteria, earning him the title of father of microbiology.

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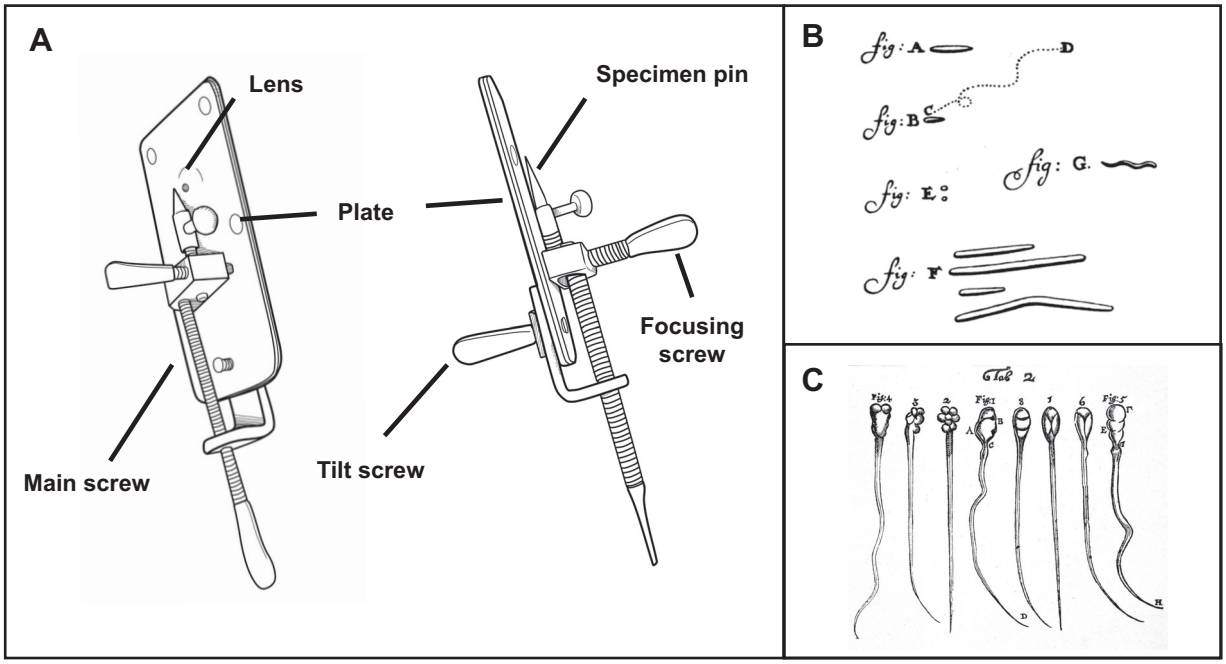


Figure 1. *A*: diagram of the microscope constructed by Antonie van Leeuwenhoek in the 17th century. The microscope's important parts are specified. *B*: Leeuwenhoek's drawings of bacteria (reprinted from Ref. 2 with permission). C: Leeuwenhoek's drawings of different animal sperm (reprinted from Ref. 3 with permission).

Leeuwenhoek's microscope marked the development and technological vanguard of biology for at least two centuries until the improvement of optics technology in Germany during the nineteenth century made possible more sophisticated compound microscopes (5).

Since the Leeuwenhoek microscope is based on a ball lens, its reconstruction is possible in a classroom setting. In this article, we show how to build a Leeuwenhoek-style microscope from scratch, and we also present a protocol to build a low-cost laser-cut acrylic assembly for the lens that allows for smartphone photography. Because of the smartphone revolution, many optics exercises use the power of cell phones combined with additional simple optics to view the microworld (6–9), but directly manipulating glass in the classroom to build microscopes is done by very few (10, 11). Unlike electronics, whereby one can buy resistors, capacitors, chips, and transistors for inexpensively building do-it-yourself (DIY) electronics for biology (12, 13), building optics equipment for the amateur microscopy enthusiast remains an expensive and technically demanding challenge.

The microscope is also a fundamental tool for neurophysiology. In our previous work on the conduction velocity and cable theory of nerves in earthworms (14), there are two nerve fiber systems of two different diameters that have two different conduction velocities. When doing this exercise, we often show a prepared slide of an earthworm cross section that students can examine to see the nerves they are studying electrophysiologically. Additionally, showing the difference in Golgi-stained neurons (in which the axons and dendrites are visualized) versus Nissl-stained neurons (typically only showing cell bodies) can be useful in teaching the history of neuroscience and the emergence of the neuron doctrine. In classes, we often show slides of the neuromuscular junction, the spinal cord, and various types of neurons (Fig. 2). Students often enjoy seeing the slides themselves through microscopes rather than just seeing them on presentations and projections.

As microscopes are pervasive in the high school and university laboratory, the microscope can just be seen as a generic tool, but when a microscope is actually built by hand by a student a new-found appreciation for the science and art of tool building can be brought to students' minds, working as new didactic tools to strengthen scientific learning.

In the protocol shown here we allow students to do just that. We have built a series of optics exercises working with raw materials such as glass and gelatin that students can replicate to build their own microscopes, strengthening interdisciplinary learning in different areas such as biology, physics, optics, engineering, tool building, and the history of technology development (Fig. 3). This activity can also serve as a bridge to learning about more advanced microscopy theories and techniques such as understanding Ernst Abbe's "optical resolution limit" equation (15, 16) and learning how other types of microscopy work, such as DIC-Nomarski microscopy, epifluorescent microscopy, confocal microscopy, and superresolution microscopy (17).

Comparison with Previous Work

The Keeling research group at the University of British Columbia has previously developed a protocol for melting filaments of glass into ball lenses as a Leeuwenhoek's microscope replica exercise for undergraduate students (10, 11). Some differences exist between their work and ours, which are *1*) our use of a variety of materials to prepare the lenses, such as borosilicate glass, common drinking glass, crystal glass, and agarose, and *2*) our design of a smartphone mount

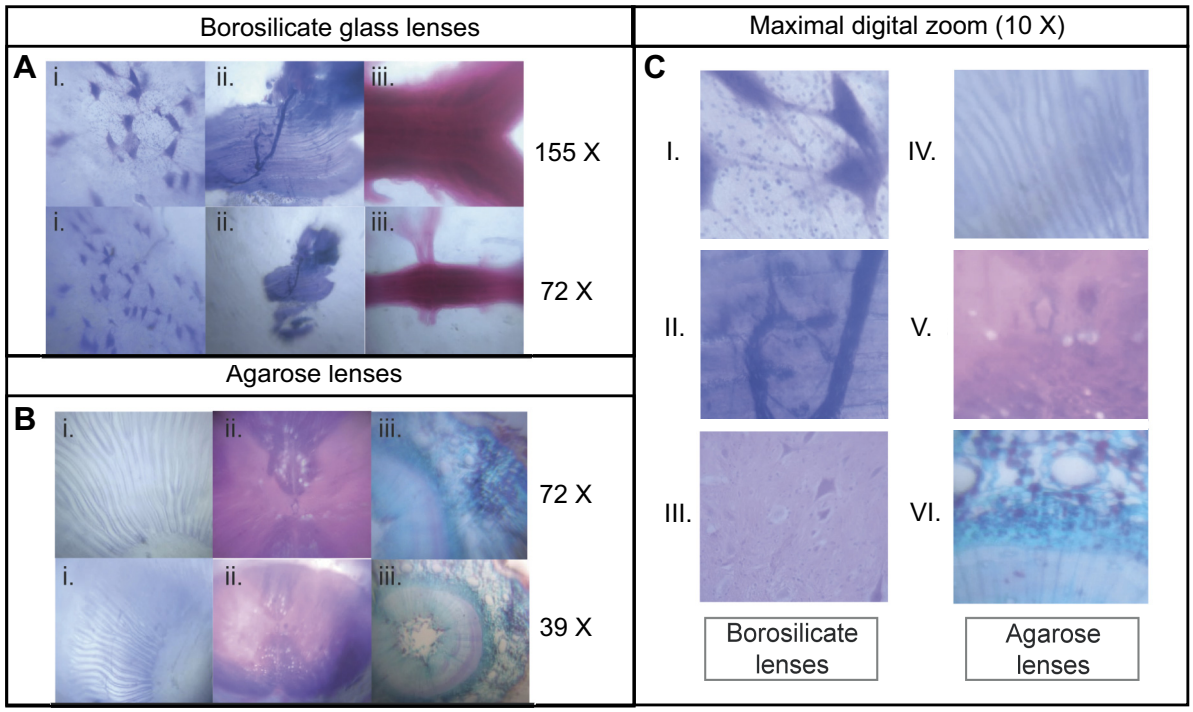


Figure 2. *A*: magnification of mammal motor neurons (*i*), motor nerve ending plates (*ii*), and earthworm nervous system samples (*iii*). Higher magnification (*top*) and lower magnification (*bottom*) are specified. *B*: magnification of mushroom (*i*), rabbit spinal cord (*ii*), and pine stem samples (*iii*). *C*: maximal digital zoom of ×155 borosilicate glass lenses [mammal motor neurons (*I*), motor nerve ending plates (*II*), mammal spinal cord neurons (*III*)] and ×72 agarose lenses [mushroom (*IV*), rabbit spinal cord (*V*), pine stem (*VI*)]. Maximal digital zoom is ×10 of the lens magnification using an android smartphone. Magnification is calculated by the equation of Fig. 4*B* for each lens. Index of refraction (n) of glass is considered 1.52, and index of refraction (n) of agarose is considered 1.34.

to allow easy taking of pictures through the ball lens. Our design of a smartphone mount, inspired by the current zeitgeist of turning smartphones into microscopes (6, 7, 9), makes our design durable for different uses (for example, easy field visualization on scientific and academic field trips) and also allows many people at the same time to view the sample through the smartphone screen.

The Keeling group focused on optical theory and physics in greater detail than we did in our study, measuring exact magnification of each ball lens with a laser pointer and a printed grid array placed in front of the lens. We did not delve as deeply into the optics theory in our classroom workshops, but we offered the students a "first principles" introduction by explaining the effective focal length and back focal length equations (Fig. 4*B*) at the high school level; we reinforced this knowledge with some more advanced optical theory at the university level.

Another well-known DIY microscope is the "foldscope" (8) from Manu Prakash's design group at Stanford University. The foldscope has a structure made of card stock and an included ball lens. It is notable for its simplicity of design and low cost, and we have used these foldscopes in our outreach to introduce students to ball lens microscopes and alternative designs (along with a replica of the Leeuwenhoek microscope we purchased from the Boerhaave Museum in Leiden, The Netherlands). From these demo units we then proceed to explain the procedure for how to build ball lenses from scratch by melting glass.

Learning Objectives

After completing this activity, the students will be able to

1) Explain the basic principles and equations of how ball lenses work
2) Know how to fabricate their own lenses from molten glass
3) Explain the historical origins of high-power microscopy
4) Observe single-cell life and multicellular life/tissues
5) Gain an appreciation for building a scientific tool from scratch

Activity Level

This activity is suitable for high school students, university students, and lifelong learners.

Prerequisite Student Knowledge or Skills

Before doing this activity, students should have a basic understanding of

1) The cellular basis of life
2) Basic algebra and trigonometry

Students should know how to

1) Manipulate simple tools like forceps and probes with sufficient hand-eye coordination
2) Maintain a safe distance while working with fire

Time Required

50–70 min

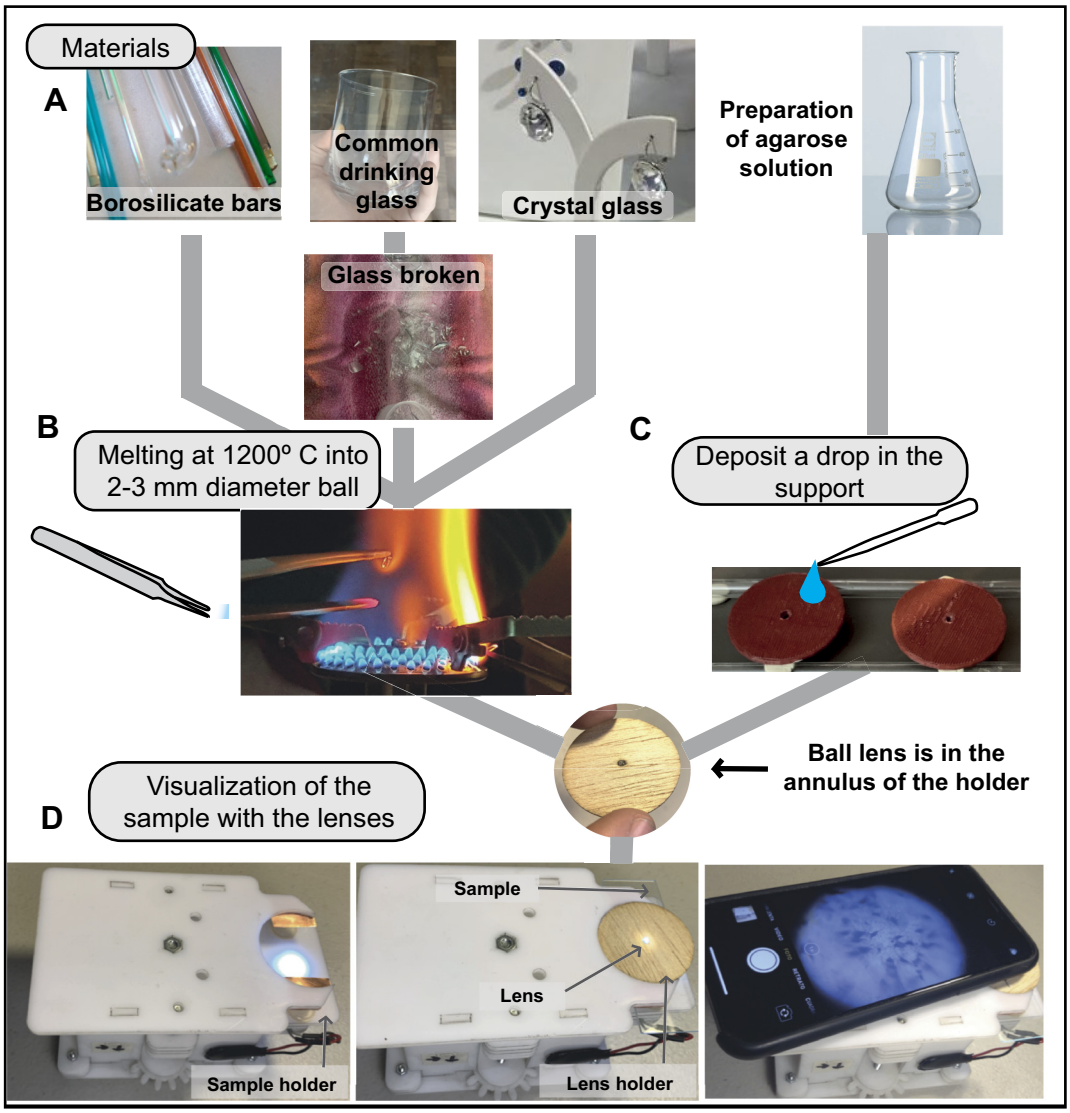


Figure 3. Protocol to fabricate lenses. *A*: diagram of the materials used to fabricate lenses. *B*: glass was melted directly with a fire source such as a butane-propane gas stove or after being crushed to generate smaller pieces of glass. *C*: other lenses based on agarose were generated by depositing 1 or 2 drops of agarose in the hole of the holder support. *D*: visualization of a sample with the lens, using the lens holder, the microscope support, and the smartphone imaging, showing a slide of motor neurons magnified through the fabricated lens.

METHODS

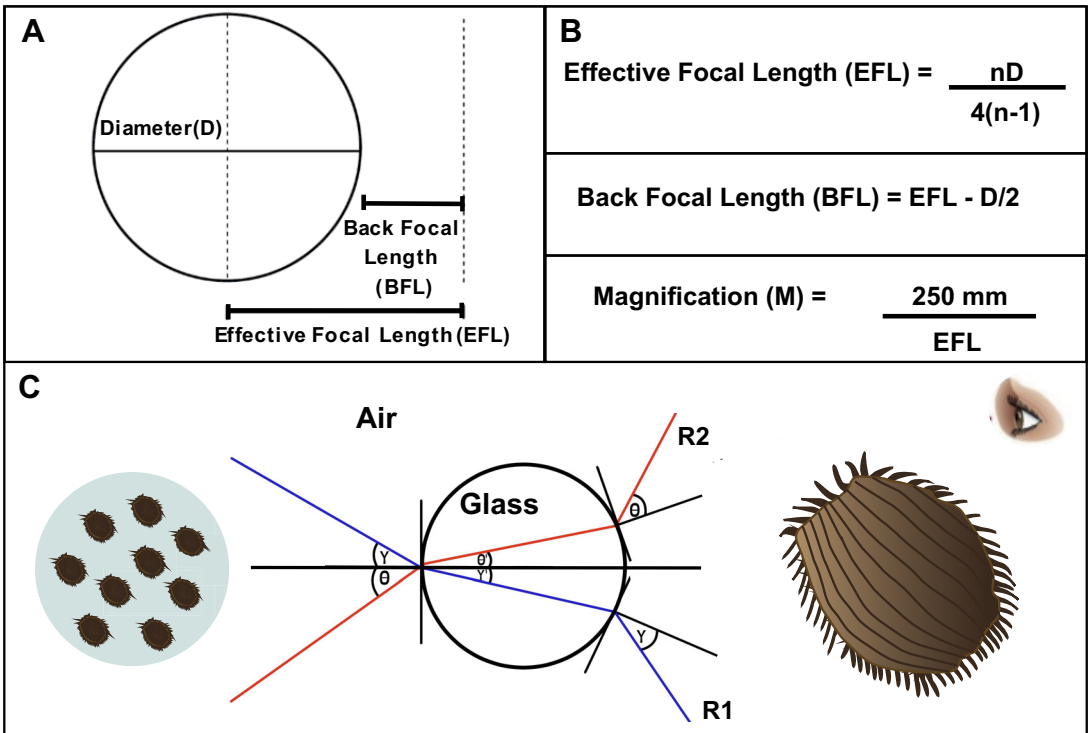
Equipment and Supplies/Instructions

Glass lenses.

To make the glass ball lenses, we used three types of accessible glass: borosilicate glass (used in laboratory glassware), soda-lime glass (common drinking glass), and crystal glass (used in adornments and jewelry). Borosilicate glass and soda-lime glass have similar indices of refraction at ~1.5, whereas crystal glass has an index of refraction of ~1.7 (18) due to the trace amounts of lead added. The capacity of a lens to curve rays of light depends on its index of refraction (n). Thus the higher the index of refraction, the greater capacity of magnification the lens will have. The borosilicate glass we used was 12-in. glass rods we bought specifically for these experiments (part no. 8496K1, McMaster-Carr); the soda-lime glass was a random common kitchen drinking glass; and the crystal glass was extracted from small Swarovski earrings purchased from a jewelry store for ~US $25.

Gelatin lenses.

To build the gelatin lenses with a refraction index of ~1.3 (19), we used powdered commercial agarose added at 2% concentration to heated water (for example, 1 g in 50 mL of water). After the agarose was completely dissolved, we deposited one to three drops of agarose solution in small holes of three-dimensional (3-D) printed annuli with 1.14- and 2-mm diameters [the 3-D printed annulus files are available in Supplemental Material S1A and S1B (all Supplemental Material is available at https://doi.org/10.6084/m9.figshare.13168868)].



D	Diameter (D)	EFL	BFL	Magnification
Agarose	2 mm	1.97	0.97	126 X
n = 1.34	3 mm	2.95	1.45	84 X
	4 mm	3.94	1.94	63 X
Borosilicate glass	2 mm	1.46	0.46	171 X
n = 1.52	3 mm	2.19	0.69	114 X
	4 mm	2.92	0.92	86 X
Crystal glass	2 mm	1.20	0.20	208 X
n = 1.70	3 mm	1.81	0.31	138 X
	4 mm	3.41	1.41	104 X
Diamond	2 mm	0.85	-0.15	NA
n = 2.41	3 mm	1.28	-0.22	NA
	4 mm	1.71	-0.29	NA

Figure 4. *A*: diagram of a ball lens, showing the effective focal length (EFL) and the back focal length (BFL). *B*: equations to calculate EFL, BFL, and magnification. *C*: theoretical diagram showing how light rays are bent from a ball lens and how magnification works. *Center*: the paths of 2 rays of light are represented by colors blue [*ray 1* (R1)] and red [*ray 2* (R2)]. Gamma (γ) and theta (θ) represent the angles that are generated with respect to the normal (perpendicular) of the lens surface. *Left*: a representation of an animalcule (Leeuwenhoek's term for microscopic life) without magnification. *Right*: magnification of an animalcule. *D*: hypothetical parameters calculated for different diameters in agarose, borosilicate glass, crystal glass, and diamond. EFL and BFL are described in millimeters (mm). n, Index of refraction; NA, not applicable.

Because of surface tension the agarose drops take the shape of a ball and solidify in a few minutes (Fig. 3*C*).

Heat source.

We used either a butane culinary blowtorch (often used to make crème brûlée) or a small isobutane-propane camping stove (such as MSR PocketRocket).

Metal tools.

For the metal tools, we used blunt 5.5-in. tissue and wound dressing forceps (Amazon UPC 767056257368) and metal straight probe tools (Amazon UPC 018018517502). Over-the-counter metallic dental probe picks also work, as well as metallic wax carving tools. We occasionally used needle-nose pliers in lieu of forceps. As the tools can get hot, only metal

material tools (tools without plastic handles that might get soft or melt) should be used.

Human or Animal Subjects

Students typically looked at preprepared slides or easily prepared slides like onion cells, and they collected samples from the field, such as the classic experiment of the microscopic life of pond water, which can contain animal multicellular organisms like the *Daphnia pulex* water flea crustacean and the nematode *Caenorhabditis elegans*. Observations of microscopic multicellular invertebrate animals are typically free of regulatory considerations. When taking ecological samples in the field, however, common practice should be taken to minimize contamination, respect the environment, and "leave no trace" of human activity.

In some of our classes, students took a sample of the epithelial cells from their inside cheek with a cotton swab, put the sample on a microscopic slide, and examined the cells with the microscope lenses they built. We told participants that the experiment was not of notable risk, that it was voluntary, and that they could take the samples themselves. We note that adopters of this activity are responsible for obtaining appropriate permission from the home institution where this activity takes place.

Instructions

Manufacture of ball lenses.

To transform the borosilicate rod glass into ball lenses, we melted the center of the 3-mm-diameter rods in a flame from the butane culinary blowtorch or isobutane-propane camping stove. We pulled the borosilicate rod apart under the flame to get two segments ending in two thin points. We then took one of the two halves and inserted the tapered end into the flame until it formed a small comet-shaped end. With forceps we pulled the comet-shaped end away from the remaining rod over the flame, trying to keep the piece of glass in the tip of the forceps, leaving us with a small comet-shaped "spheroid." We then placed the spheroid on the tip of a metal probe over the flame. With time and patience, the piece of melted glass began to acquire a more ball-like shape because of the surface tension of its fluidlike state under heat. We tried to make lenses as round as possible, of no more than 2–3 mm in diameter, without any bubbles, and with minimal embedded black carbon residue combustion by-product. A video of the protocol is available in Supplemental Material S2.

For the crystal glass, we pried the glass jewelry cubes out of the metal housings of the Swarovski earrings with pliers. Using an additional set of pliers, we then grabbed the crystal glass cube on two sides, held it over the flame, and then pulled the crystal glass apart, resulting in two comet-shaped long filaments. We then worked the filaments into balls in a similar manner as the borosilicate glass rods. For the soda-lime glass, we broke the glass of a common drinking glass into small pieces (Fig. 3*A*) by wrapping the unbroken glass in a towel and breaking it with a hammer. We selected and melted a small piece in the fire, following the same steps as described above for the crystal glass with two pliers.

After we attained a ball lens, we released it from the tip of the metal probe with metal forceps and let it cool in a metal or ceramic bowl. We then put the lens in a circular 3-D printed plastic or laser-cut wooden support, both with a hole in the center. This enabled us to both easily store and use the ball lenses. With both of these supports, we had two hole sizes, 1.14-mm diameter and 2-mm diameter. The 1.14-mm diameter is just an arbitrary diameter due to the limits of the 3-D printer we were using (MakerBot Replicator 1st generation). If lenses were too big, the hole could be easily enlarged with a small scissor blade.

After having made the lenses and putting them in the annuli, the students could observe a sample by bringing the embedded ball lens up to the eye and looking through the lens at a sample in the direction of a light source such as a lamp. As the back focal length of the lenses is very short, 0.3–1.0 mm away from the lens, the sample is in focus only when very close to the lens.

Checking the focal distance and the magnification of lenses.

Using a vernier caliper, we measured the diameter of the fabricated ball lenses. It is then possible to calculate the effective focal length (EFL) and the back focal length (BFL) of the ball lens with the equations shown in Fig. 4*B*. For example, borosilicate glass normally has an index of refraction of 1.52; thus for a 2-mm-diameter lens, the EFL calculates to 1.46 mm, or 0.46 mm away from the edge of the lens (BFL). It is then possible to calculate the magnification with the EFL, again using the equations shown in Fig. 4*B* (20–22); 250 mm is considered a convention, as the focusing average of a healthy eye has a minimum distance of ~250 mm (23), and in this way the approximate magnification M by the lens is calculated by dividing 250 by the EFL (23). Magnifications calculated for different diameters and materials of ball lenses are shown in Fig. 4*D*. It is worth noting that in any material with an index of refraction >2, the focal point will actually be inside the lens (as in the case of diamond, with a refraction index of 2.4), making the lens nonfunctional.

Physical parameters of ball lenses.

Different refraction indices are shown in Fig. 4*D*, and the equation to calculate how a transparent material bends light (Snell's law) is shown in Supplemental Material S3. Lenses are transparent and curved materials, so the light rays bend as a result of the refraction index of the material and the curvature of its surface. With just the refraction index and diameter it is possible to calculate how ball lenses behave by the equations of EFL and magnification (20–22). These equations were used to estimate the magnification of hypothetical lenses manufactured with different materials and diameters (Fig. 4*D*) and to estimate the magnification of the lenses made with the materials used in the activities with students, mainly with borosilicate glass and agarose.

Figure 4*C* contains a light ray bending diagram visually explaining how ball lenses magnify images that students may find useful. The magnification in ball lenses occurs because the light rays that hit the lens with a specific angle (γ) with respect to the "normal" (imaginary line perpendicular to the surface of the lens) are refracted with a new angle (γ') into the lens due to the change in the index of refraction with respect to air. The light beam then exits the glass with the same original angle (γ) with respect to the normal.

Because of the curvature of the lens, the angle changes of the light rays going into and out of the lens result in magnification.

Acrylic support for smartphone image taking.

We developed an acrylic support system that could hold the lenses and allow for sample viewing through a smartphone and subsequent photograph taking for qualitative and quantitative observations. See Fig. 5 for construction. The support system was designed with a mixture of both Google SketchUp and Rhino design software. The two-dimensional (2-D) design was exported to a .dxf file that was then sent to local laser cutting services to cut at ⅛-in. (3 mm) thickness in acrylic (see Supplemental Material S4A and S4B).

The top layer of the acrylic layer supporting the smartphone had a thin copper "lip" glued on the underside (Fig. 5, *D* and *F*) to hold the embedded lens annulus (Fig. 3*D*), minimizing structural thickness that would prevent the sample from approaching the lens. The microscope also had hand-turnable gears that permitted the movement of the sample toward or away from the lens to find the focus.

Troubleshooting

With the construction of the lenses, three common problems occurred. First, many times the students made lenses that were too small (<1.8 mm in diameter), so achieving focus was very difficult because of the short back focal lengths approaching the width of the cover glass (0.15–0.2 mm) of prepared slides. Students were encouraged to try to build a second, bigger lens that could focus more easily (higher back focal length). Second, sometimes the tweezers were not cleaned sufficiently before the fabrication of the lens, and contact between the tweezers and the molten glass left combustion and carbonation products inside the lenses that subsequently obscured the magnification. Third, this activity is a protocol to develop ball lenses, and such lenses generate spherical aberration that causes a deformation of the image on the periphery of the visual field (Supplemental Material S5). In the history of microscopy, spherical aberration was surpassed after the fabrication of elliptical lenses in the nineteenth century (21, 22). Although with this activity we cannot avoid spherical aberration, the central regions of the lenses have appropriate visualization and magnification to identify the microscopic details of a sample.

Safety Considerations

Of utmost safety importance in this activity is the use of the high-intensity flame. Students should be accompanied by multiple adults in the room, typically one adult for every six to eight students. Given that glass has a melting point between 550°C and 1,500°C (24), and a typical butane flame can reach these temperatures, students are told to respect and be careful of the fire. Students with long hair are advised to put their hair in ponytails, and the lights are dimmed slightly to enable better visualization of the flame.

Another risk is that as students are heating the glass ball lenses with their forceps, they can accidentally drop the ball lenses on the table or the floor. If the table or floor is made of

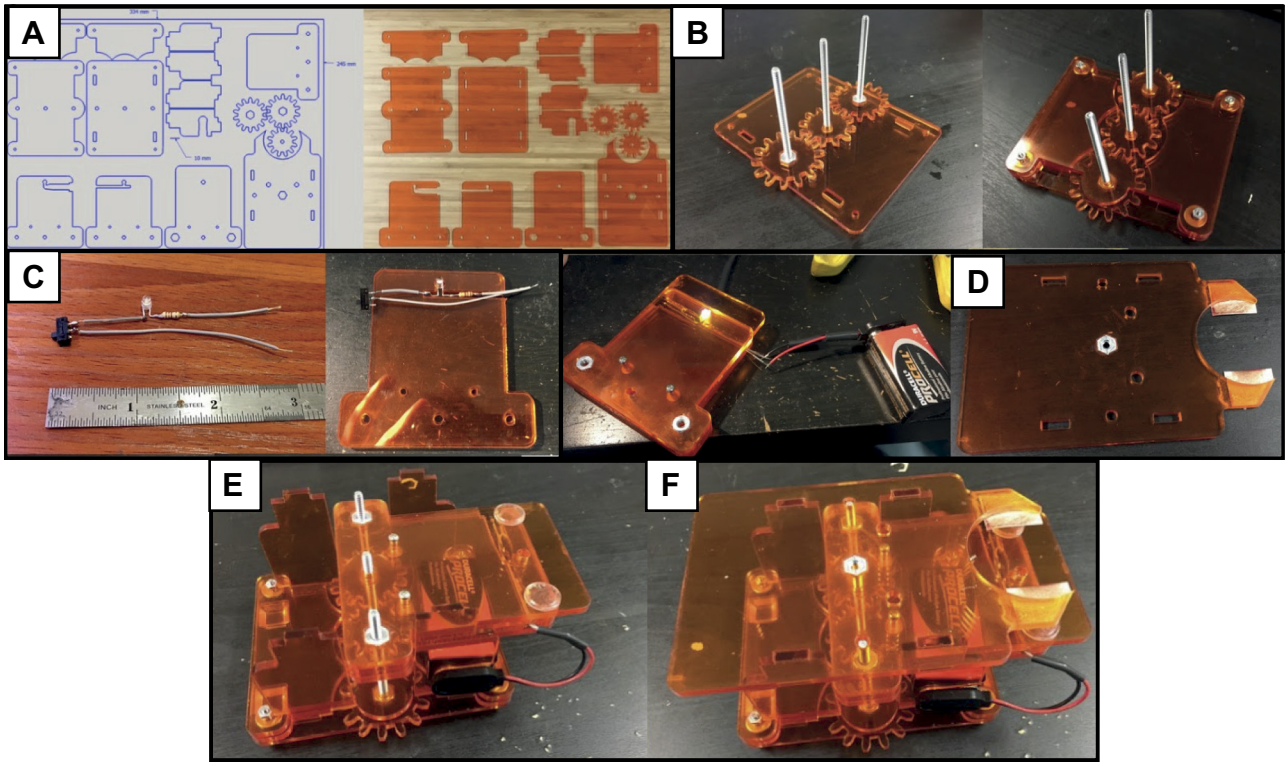


Figure 5. Fabrication of lens holder for smartphone. *A*: the 2-dimensional (2-D) design file (Supplemental Material S4A) of all the components and the laser cut output. *B*: the depiction of the gear turning mechanisms. *C*: the fabrication of the light source and the moving "table" the sample rests on. *D*: the single-layer "top" that supports the smartphone. *E*: partial construction. *F*: full construction. Extensive assembly instructions with more detailed photos are available in Supplemental Material S4B.

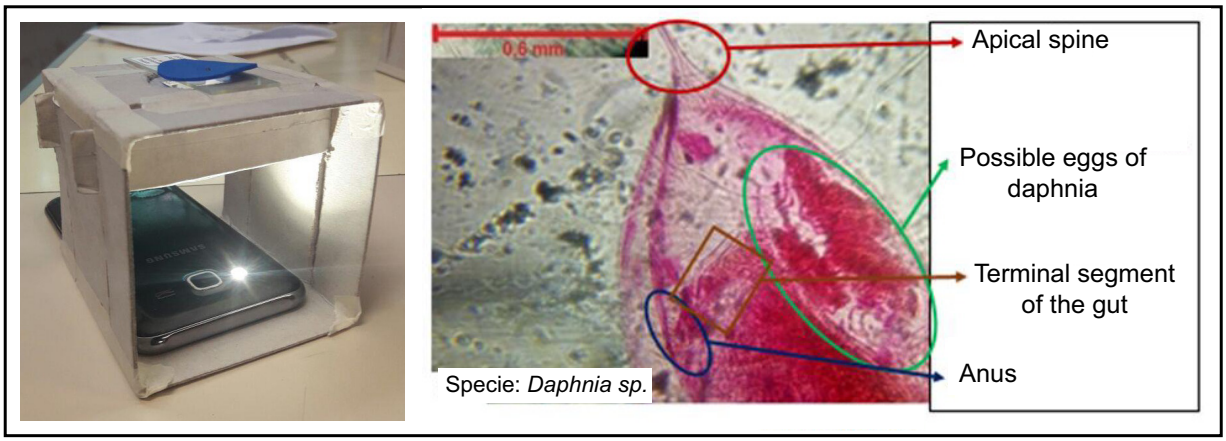


Figure 6. A cardboard device that acts as a support for the microscope designed by the students (*left*) and a daphnia sample, identifying some of its anatomical structures, photographed and annotated by the students (*right*). They estimated the magnification of their borosilicate lens to be ×75.

stone or tile, this is not a problem, but if they are made of wood, light smoldering may occur because of the hot glass. Students need to be reminded that the glass will remain hot for ~1–2 min after being in the fire, so care should be taken before handling them with their fingers. We had metal or ceramic crucibles present that the students could put the ball lenses in while they were cooling. If concerns over high temperatures used in this experiment are pertinent, gelatin lenses can be built instead of glass lenses.

RESULTS

Expected Results

Development of the activity.

We developed this activity in various education institutions in Chile—Alberto Blest Gana High School (15 students) in Santiago, Jorge Alessandri Rodríguez High School (15 students) in Santiago, Luis Cruz Martínez High School (35 students) in Andacollo, Nido de Águilas High School (8 students) in Santiago, and the Faculty of Sciences at the University of Chile (60 students) in Santiago—with a total of 133 students of different ages (10–25 yr old of roughly equal gender mix and various socioeconomic levels, but many in situations of social vulnerability). The activities began with a demonstration by the teacher of how lenses are made. With borosilicate glass rods as the source material, the students then made their own lenses of different diameters and used them for the observation of biological specimens.

There were a variety of activities the students could do. Our most common activity, given our group's focus in neuroscience, was to have preprepared or purchased slides of neurons for students to examine, such as pyramidal cells of the cerebral cortex, the neuromuscular junction, Golgi-stained hippocampal neurons, and cross sections of earthworm nerves. Other activities the students did were to look at epithelial cells swiped off the side of the inner cheek with a cotton swab, seeing the individual cells of an onion by carefully pulling off a piece of "skin" from the onion and placing it on a slide, and looking at preprepared slides of microscope life like *Volvox*, *Daphnia*, *Hydra*, and biological tissues samples such as pine stalks, rabbit marrow, etc. A highly effective exercise was to collect drops of pond water and examine them under their microscopes, replicating the experience and discoveries

Table 1. *Selected answers to the three questions we asked students regarding this activity*

Question	Answer
Why do you think it is important to repeat a methodology developed over 300 years ago?	This activity is important because it shows principles of magnification that are the basis of all current microscopes. This activity arouses curiosity since it is very simple and entertaining, and it requires only a few materials. It is affordable to be able to do it in schools of any means, which allows us to bring science and ambition into the classroom without barriers.
What is the value of this work in the school classroom?	The value of this activity in the classroom is that it directly approaches and promotes the interest of students in science through tool building. Manufacturing your own tools allows focusing knowledge for different ways of learning.
What expected lessons learned from the biology and natural sciences curriculum can be achieved with the tools used in this workshop?	• Students can describe the optics, structure, and operation of elementary microscopes and telescopes. • Students can identify a problem and hypothesize and design various experiments to answer specific biological questions. • Students can describe classical scientific research, recognizing their historical and theoretical importance. • Students gain the ability to identify microscopic biological structures and their main components.

Shown responses to questions represent the most common answers given by students.

Table 2. *Physical parameters that can be calculated by the students*

Physical Parameters			
Magnification	**Field of view**	**Effective Focal Length (EFL)**	**Back Focal Length (BFL)**
The students calculated the magnification using the equation in Fig. 4B and also using a micrometric ruler.	The students measured the fields of view with a micrometric ruler and estimated cell size and the size of some structures.	The students calculated the EFL of their ball lenses using the equation of Fig. 4B.	The students calculated the BFL of their ball lenses using the equation of Fig. 4B. With this they can estimate the distance to focus the sample from the edge of the lens.

By showing the physical parameters that can be calculated by the students to add quantitative rigor to this activity, students can appreciate microscopy in a biological, historical, and physics context.

of Antonie van Leeuwenhoek during the late seventeenth century. A drop of water can contain various forms of life such as algae, amoebas, *Caenorhabditis elegans,* and *Daphnia*.

During observation, students would take photographs through their smartphones and perform qualitative and quantitative observations. They used a micrometric ruler (OMAX 0.01 mm Microscope Camera Calibration Slide; Amazon.com) to calculate the microscope's magnification and consequently estimate the sizes of cells and their structures. A good photograph is easily taken with the smartphone support that we designed in this study, but in some cases when our activities occurred in remote locations where our microscope support structure was not available (or in cases in which they did not have the materials that we suggest for their construction), students could recreate the microscope stand with homemade materials such as cardboard and rubber (see Fig. 6 for an example of student work).

When closing the activity, students addressed different survey questions, such as the ones shown in Tables 1 and 2, which allowed them to assess the activity they just concluded, explain it in a broader educational context, and evaluate their result in a qualitative and quantitative approach. Finally, students who had built "the most perfect lens" were often proud of their creations and would post photos of their magnified images to various social networks.

Misconceptions.

We observed that hand building the ball lenses permits students to quickly understand the counterintuitive nature of the phenomenon of magnification. For example, a student will normally guess that the larger the diameter of a glass ball lens, the higher the magnification, when the physics is actually the reverse: the smaller the diameter, the greater the magnification and the shorter the focal length. Having glass ball lenses available of different diameters for students to examine, from 2 mm to 4 mm, makes this immediately apparent to students.

Evaluation of Student Work

Generally all of the students, independent of their age (>10 yr) and educational level (>5th grade), could successfully fabricate glass ball lenses in a typical class time of 1.5 h. With patience, a student can readily learn how to fabricate ball lenses of useful sizes between 2 and 3.5 mm in diameter. With a diameter of <2 mm the back focal length is very close to the edge of the lens (Fig. 4D; <0.5 mm), so it requires that the sample be almost touching the lens. On the other hand, building lenses of >3.5 mm in diameter is difficult because it is necessary to spend much more time melting the glass to reach a ball shape because of glass's poor thermal conductivity when working with a small flame. Thus, borosilicate glass ball diameters of 2 mm (×171 magnification) to 3.5 mm (×98) are ideal for viewing individual cells or microscopic life (Fig. 2, Fig. 6, Fig. 7).

Using the equations of Fig. 4*B*, students could make estimates of the magnification and focal length of the lens. In this way it is possible to relate the observation of the samples with an estimated magnification calculated from the diameter. Additionally, by using a micrometric ruler it is possible to measure the field of view of the lens and thereby quantity cell sizes or the sizes of some structures (Fig. 7) as well as calculate the physical parameters of the lenses.

The elaboration and use of the support allowed for easy visualization. Our supports allowed students to learn about

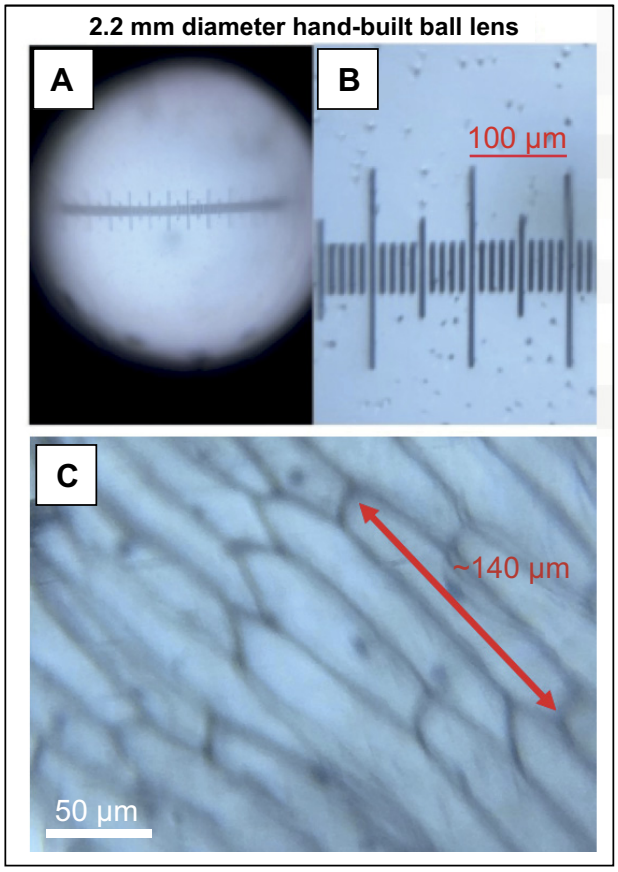


Figure 7. Measuring visual field and cell size. *A*: iPhone 6 at normal setting. *B*: max digital zoom. *C*: onion at max digital zoom, measuring the diameter of a large onion cell.

the basic elements of modern optical microscopes, as the support contains a gear that moves a stage to focus the sample, a light source to illuminate the sample, and a mount for the lens. These elements are analogous to macro- and micrometric gears, the stage, the light source, the condenser, and the objectives in modern microscopes.

Our activity links the experimental approach with lessons learned in school to enhance reflective thinking and critical learning (25). This activity also fits into the basic curricula of high school and university first-year students in biology. For example, the Chilean natural science curriculum of the Ministry of Education involves teaching functional knowledge of the cell and its components, comparison of organisms in relation to their structural characteristics, processes of the natural and technological world using the senses, and planning of experimental scientific research based on instruments suitable for the study (26).

This activity encourages students to understand how the phenomenon of image magnification occurs through simple microscopes, which motivates them to understand the principles of optics and the mathematical equations necessary to enable magnification. This is pertinent in Chile, which is usually positioned below the average of the other countries participating in the Programme for International Student Assessment (PISA) test (http://www.oecd.org/pisa/) and below the level of a developed country. The utilization of these types of interactive activities contributes to the development of STEAM areas in underdeveloped and developing countries.

Self-fabrication of lenses and visualization of biological samples allow the activity to be available to a wide range of ages of students without necessarily a background in mathematics and physics. We have witnessed the cognitive leap that occurs in students when they see microscopic organisms under a lens they themselves built, mirroring the same experience that occurred in the seventeenth century with Antonie van Leeuwenhoek.

Having students build their own science tools that they then use for their own experiments leads to greater appreciation of the scientific method (27), interest in the learning process (28, 29), and promotion of cognitive attention (30). Students have "intellectual ownership" of their science tools and are proud when they achieve good results with the tools they themselves built.

As microscopy is transversal in many areas such as biology, science history, tool building, neuroscience, microbiology, zoology, physics, pedagogy, and science communication, this activity allows many avenues for continued investigation by motivated and curious students (31).

Inquiry Applications

With the use of filters and LEDs of various wavelengths, our protocol can be modified for fluorescence microscopy and dark-field microscopy, allowing for simplification of previous open-source designs (32). Also, although we only tested borosilicate glass, common drinking glass, crystal glass, and agarose in our designs, students could try to make ball lenses of other transparent materials.

As molding glass into large complexly curved shapes is logistically complicated for a high school classroom, students could alternatively build complex shapes with agarose lenses and custom molds with 3-D printers. Future directions of this work include building a digital library of custom molds that students can 3-D print and then fabricate complexly shaped lenses with agarose solutions. Students could then experiment with various shapes to further investigate optic phenomena such as spherical and chromatic aberration.

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DISCLOSURES

T.C.M. is co-owner of the business Backyard Brains, Inc., which has commercialized this open-source activity, having sold versions of the microscope developed here. D.P.F. does not have any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

D.P.F. and T.C.M. conceived and designed research; performed experiments; analyzed data; interpreted results of experiments; prepared figures; drafted manuscript; edited and revised manuscript; and approved final version of manuscript.

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