Increasing Student Understanding of Microscope Optics by Building and Testing the Limits of Simple, Hand-Made Model Microscopes

Kevin Drace1*, Brett Couch2, and Patrick J. Keeling2

1Department of Biology, Mercer University, Macon, GA 31207
2Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T1Z4

The ability to effectively use a microscope to observe microorganisms is a crucial skill required for many disciplines within biology, especially general microbiology and cell biology. A basic understanding of the optical properties of light microscopes is required for students to use microscopes effectively, but this subject can also be a challenge to make personally interesting to students. To explore basic optical principles of magnification and resolving power in a more engaging and hands-on fashion, students constructed hand-made lenses and microscopes based on Antony van Leeuwenhoek's design using simple materials—paper, staples, glass, and adhesive putty. Students determined the power of their lenses using a green laser pointer to magnify a copper grid of known size, which also allowed students to examine variables affecting the power and resolution of a lens such as diameter, working distance, and wavelength of light. To assess the effectiveness of the laboratory's learning objectives, four sections of a general microbiology course were given a brief pre-activity assessment quiz to determine their background knowledge on the subject. One week after the laboratory activity, students were given the same quiz (unannounced) under similar conditions. Students showed significant gains in their understanding of microscope optics.

INTRODUCTION

Antony van Leeuwenhoek (1632–1723) is credited with the first discovery of single celled microorganisms. His remarkable contributions to the field of microbiology came about through his exceptional ability to create single lens microscopes capable of achieving magnification power of at least 250 times. The few remaining van Leeuwenhoek microscopes are constructed of brass or silver and utilize several moving parts. They are elegant devices of considerable complexity. However, the fundamental principles of his technology can be duplicated in the laboratory relatively quickly and inexpensively using simple materials (1).

This laboratory exercise was designed to enhance student understanding about microscope optics and design with the aim of improving student ability to more effectively use modern compound microscopes, while at the same time giving them some historical context, as well as a personal interest in microscopes and microscopy. During a two-hour laboratory period, students were given the opportunity to construct their own microscope based on van Leeuwenhoek's general design using simple materials and techniques readily available in any high school or university laboratory environments (2, 3). Once completed, students explored the optical properties of their lenses, the basic principles of magnification, focal length, working distance, and resolving power, and used their hand-made microscopes to make observations of various objects including microorganisms.

Intended audience

This activity was designed for Microbiology and Biology majors, but is broadly applicable to any course that requires students to understand basic microscopy principles and concepts, including high school science classes. Working in pairs, students collect basic data on the lenses they construct. The class data is pooled, and students graph and analyze the class data to describe the relationship between lens size and the magnification power of their lenses.

Learning time

In this version, one laboratory period of two hours is sufficient to build and determine the power and resolution capabilities of the microscope. However, additional time would allow for inclusion of other activities.

Prerequisite knowledge

Laboratory safety guidelines and minimal laboratory experience is required. In this version, the concepts of
magnification and resolution were introduced in lecture and through assigned readings prior to the lab activity; however, this is an optional component. This laboratory can be a stand-alone module or complement lectures and readings, at the discretion of the instructor. Additional sources for background information include the following websites: www.micro.magnet.fsu.edu, www.zeiss.de, www.microscopyu.com, and www.olympusmicro.com.

Learning objectives

After successful completion of this exercise, students will be able to:
1. Describe how a simple lens allows for sample magnification by bending light.
2. Explain the effect of lens diameter and focal length on the magnification power of a simple lens.
3. Demonstrate and explain the effect of working distance on the resolution of a simple lens.
4. Define and calculate the numerical aperture of a homemade lens.
5. Predict the effect of using different wavelengths of light on the resolution of a lens.

PROCEDURE

Materials and equipment

To construct the microscope, students need access to a stapler, Bunsen burner or other source of flame (e.g., a portable plumber's torch), adhesive putty, and two pieces of cardstock paper pre-drilled with a 1/16 inch hole. To determine magnification power, students need access to a laser pointer with a grid of known size fixed over the beam.

For each microscope the following materials are needed:
1 glass Pasteur pipette or capillary tube
5 × 10 cm piece of 1mm poster board (thick side)
5 × 10 cm piece of cardstock (thin side)
1 small dab (about 1 ml) of adhesive putty
1 cover slip

Also needed for measurements:
1 caliper
1 ruler
1 laser pointer (available from online retailers and office supply stores)
1 small grid like those used for mounting electron microscopy samples (e.g., Pelco product 1GC100)

To make the lens, a Pasteur pipette is heated with a portable torch (Fig. 1(A)) and stretched very thin (Fig. 1(B)). The pulled pipette is broken somewhere along this thin portion and slowly fed into the flame, forming a small, glass sphere that can be used as a lens (Fig. 1(C)). The diameter of this newly formed lens is measured and then mounted between the poster board and cardstock, aligning the lens with the pre-drilled holes. Staples hold the final microscope together (Fig. 1(D)) and adhesive putty is used to mount samples for observation (Fig. 1(E) and (F)). Detailed instructions can be found in Appendix 1 or at www.botany.ubc.ca/keeling/resources.html.

The finished microscope with lens is shown in Figure 2(A). The easiest way to make the measurements is to tape the electron microscopy grid onto the laser, put the microscope lens over the grid and point it at the wall. We made a simple device to make this easier to hold steady (Fig. 2(B) and (C)), but it is possible to do with nothing at all.

Student instructions

After a brief introduction to the laboratory exercise, students are given the laboratory handout (Appendix 2, which also gives complete instructions on building the microscope), shown how to use the tools, and given a demonstration on how to pull glass safely and effectively for making a lens. Before finishing the microscope, students measure the diameter of their lens so that they can make observations as a class about the relationship between lens diameter and magnification power.

FIGURE 1. Brief instructions on making a microscope. To make the lens, first melt (A) and stretch (B) a section of glass (most easily the thin end of a Pasteur pipette), making sure to remove it from the flame before stretching it. Break the stretched glass and slowly feed one end into the flame to re-melt the glass, resulting in a near-spherical lens attached to the end of the stretched glass (C). Break the lens from the stretched glass and sandwich it between the two sheets of cardstock, making sure to center the lens between the two holes, and staple them together (D). Attach the sample to be viewed to the adhesive putty, and then attach the adhesive putty to the thin paper side of the microscope with the sample positioned over the lens (E). To view (F), place a finger on the putty and hold the microscope close to one eye and pointed towards a mild light source (for example overhead lighting or a window, not directly at a strong light source). Focusing is achieved by pushing the putty up or drawing it down, pivoting the sample towards or away from the lens, respectively.
Magnification calculations

To determine magnification power, the laser pointer (most effectively a green 532 nm pointer) is used to project an image of the grid onto a wall. This is achieved by mounting the grid onto the end of the laser, and placing the microscope directly over the grid so that the beam is aligned with the lens. One dimension from the projected image of the grid can be measured, and from this and the distance of the lens to the wall, various measurements can be made (Fig. 2(D)).

There are two equations students will use to calculate the power of their lenses. By convention, the power of a lens is calculated from a standard distance of 250 mm from the lens. The first equation converts projected size of an object at an arbitrary distance from the lens to the projected size at 250 mm. The second equation is the ratio of the projected size of the grid at 250 mm to the actual size of the grid. This number is the magnification power achieved by the lens.

The following equations determine the magnification of your lens.

Equation 1: \((X / d) \times 250 \text{ mm} = D\)
Equation 2: \(\left(\frac{D}{A}\right) = P\)

where:
- \(d\) = distance between lens and projected image
- \(A\) = actual size of grid hole (0.204 mm)
- \(X\) = measured size of grid hole
- \(P\) = power of lens
- \(D\) = size of projected grid hole at 250 mm

Theoretical resolution calculations

Resolving power is the ability to perceive adjacent objects or structures as distinct. The theoretical resolution of a lens is related to light-gathering ability of a lens, and is calculated using the Abbe equation \((d = 0.5\lambda / \text{numerical aperture})\) where:

- \(d\) = resolving power
- \(\lambda\) = wavelength of light (dependent on the light source used; 532 nm for the green laser pointer)
- Numerical aperture = \(n(\sin \theta)\)
- \(n\) = refractive index (for air, \(n = 1\); for immersion oil, \(n = 1.51\))
- \(\theta\) = the half-angle of the maximum cone of light that can enter the lens = \(\tan A = a/b\)

For the purpose of this activity, since the lens is touching the grid it is assumed that, \(a = \) one-half of the lens diameter, and that the lens is perfectly spherical; therefore, \(a = b\). For microscope objectives, \(b\) is the focal length of the lens (i.e., the working distance) and \(a\) is one-half of the lens diameter.

Once these calculations are made, the class data is combined and students graph magnification power as a function of lens diameter from combined class data. Students can then use the class data to test their hypotheses regarding the relationship between lens diameter and magnification. Most students assume the larger the diameter, the greater the magnification of the lens, and are surprised when the data refute their assumption.

Faculty instructions

This exercise was performed during a two-hour laboratory period with approximately twenty students in pairs constructing each microscope, but can be modified to suit the objectives of the instructor. As originally designed, students are allowed to proceed at their own pace when constructing the microscopes, while the instructor oversees progress. Logistically, it may be easier to have students rotate through stations for each step of construction and observation. In smaller groups, students can be encouraged to make two lenses (one large and one small), to think more directly about the relationship they expect to find between lens size and power, and to increase the sample size of class data. Once the lens is made and the diameter measured, microscope construction only takes a few minutes. Each group is directed to begin observations of various samples: onionskin, large protozoa, hair or feathers. One by one, each group is allowed to mount their microscope onto the laser to determine magnification power. This is best performed in a low-light environment and typically takes less than ten minutes. After each group rotates through this station, they are instructed to write their results on the board so that the entire class can collect and analyze the data. Refer to Appendix 1 for details and tips for microscope construction and sample observations.
DISCUSSION

Field testing and evidence of student learning

During the first week of class in the spring and fall semesters of 2011, students in four separate sections (approximately 20 students per section; 73 students total) of General Microbiology (BIO 303) at Mercer University were introduced to the fundamentals of microscopy during lecture and then participated in the laboratory activity.

To evaluate learning gains, students were given a pre- and post-activity assessment which covered the five learning objectives for the laboratory activity. Immediately prior to the activity, students were given an unannounced quiz (Appendix 4 and 5) to determine their background level of knowledge about basic microscopy concepts. One week after the activity, students were given the same assessment along with an evaluation questionnaire for the exercise. Each of the questions was designed to assess the learning objectives.

Pre- and post-assessment questions

Question 1. In your own words, explain how a lens is used to magnify an image. Draw a diagram demonstrating how light from the sample travels through the lens and reaches the viewer. (Objective 1)

Question 2. What effect, if any, does the diameter of the lens have on magnification power? (Objective 2)

Question 3. Define focal length and explain how it affects magnification power. (Objective 2)

Question 4. How does the working distance of a lens affect the numerical aperture? (Objective 3)

Question 5. What effect does the numerical aperture have on resolution? (Objective 4)

Question 6. Would shortening the wavelength of light used in a microscope increase or decrease resolution? (Objective 5)

Combining all four sections, students significantly increased their average score of 42% on the pre-activity assessment quiz to an overall average of 71% on the post-activity assessment quiz ($p$ value < 0.001 using a paired, two-tailed t-test). A closer look at each individual question (Fig. 4) demonstrates an increase in learning for each objective. The results obtained from the spring and fall semester classes are similar with regards to the level background knowledge students brought to the course, as well as the level of knowledge gained through the activity. One difference between the two semesters is the ability to “explain how a lens is used to magnify an image” and to “draw a diagram demonstrating how light from the

Sample data

An example of a completed microscope, the laser mounting device, and the magnified electron microscope grid is shown in Figure 2. The magnification power of each microscope is related to the diameter of the lens used. As an example, student data from Fall 2011 demonstrates that as the diameter of the lens increases in size, the magnification power decreases (Fig. 3).

Safety issues

During the exercise, students are instructed to heat and pull glass pipettes over a Bunsen burner. Proper laboratory safety guidelines should be observed, including wearing eye protection and proper clothing and footwear, and by following proper glass disposal procedures for broken glass. Students operate a laser, which has the potential to cause eye damage and students should avoid looking directly into the pointer or pointing the laser at reflective surfaces (4). Instructors should read the warnings associated with their specific laser pointers and avoid high-powered pointers.

FIGURE 3. Class-generated data comparing the lens diameter with the magnification power of each microscope.
sample travels through the lens and reaches the viewer.” The fall 2011 class had much more trouble answering this question; however, it appears that students overall gained some benefit from the activity as the percentage of correct answers increased from the pre-activity assessment quiz to the post-activity assessment quiz.

During the post-activity assessment quiz, students were asked to evaluate the exercise on a scale from 0 to 5, where 0 indicated complete disagreement with the statement and 5 indicated complete agreement (Fig. 5). In general, students found the activity to be enjoyable and instructional. Most indicated that that their favorite part was being able to make their own microscope and test the limits of their own creation. The major obstacles students had during the activity were with melting the pipettes and observing living organisms. At all times, students appeared very engaged with the material and some became competitive with each other, wanting to make the lens with the best magnifying power.

Overall, our analysis indicates an increase in student understanding of microscope optics by building and testing the limits of simple, hand-made model microscopes. The pre-activity assessment quiz, laboratory exercise, and post-activity assessment quiz were performed after the microscopy lecture in an attempt to isolate the gain in student understanding from the activity itself. Our results indicate that this exercise met all of the stated objectives.

Possible modifications

The concepts of optical aberrations, contrast, field of view, and focal length can also be explored and could serve as useful additions or variations on the basic laboratory outline for instructors wishing to emphasize other microscopy concepts. An alternative approach to calculation of lens power based on focal length is presented in Appendix 6.

SUPPLEMENTAL MATERIALS

Appendix 1: Microscope Construction and Sample Observation
Appendix 2: Student Laboratory Handout
Appendix 3: Student Laboratory Handout Answer Key
Appendix 4: Pre- and Post-Activity Assessment
Appendix 5: Pre- and Post-Activity Assessment Answer Key
Appendix 6: Alternative Laboratory Exercise

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REFERENCES