Lesson Plan: Light Microscopy

Background

Microscopes are used by scientists to see objects that are too small to see with the unaided eye. When natural light – sometimes called "white light" – is used to view the object, the methods are referred to as **light microscopy**. The simplest microscopes consist of a lens and a stage onto which small objects can be placed. A gear that allows the stage to be moved up and down *focuses* the object – focusing means that the distance from the object to the lens is adjusted until the object appears sharp to the person looking at it.

The first microscopes were in use by the late 16th or early 17th century. These microscopes used natural or white light to view objects or specimens. Many changes were made by early scientists to improve the ability to see details of biological specimens clearly. Changes were first made to the microscope lenses and ways of preparing specimens. Improvements included the addition of lenses, the development of higher magnification lenses, and the use of methods to fix and stain specimens with different chemicals or dyes so that cellular components could be more easily seen. Later, devices were added to microscopes to aid viewers in accurately drawing features of the objects being observed. More recently, the addition of cameras and other recording devices to microscopes has allowed images of the specimens to be captured and reproduced. These devices, along with the microscopes themselves, are still undergoing amazing advances today, greatly increasing the ability of scientists to see details of cellular components.

Light microscopes, as well as microscopes that use other types of illumination, are extremely important tools used by biophysicists and other scientists, enabling them to make discoveries about cells and how they work. This lesson plan demonstrates the use of a light microscope to observe readily available biological specimens.

Objectives & Grade Level

Demonstrate the use of a microscope for viewing details of objects that cannot be clearly seen with the unaided eye. Appropriate for grade school to advanced high school science classes; see notes for advanced students.

Materials & Equipment

- Light microscope
  A simple microscope can be used with this lesson plan, e.g., the small wooden microscope available at http://echo-labs.com/woodenscope
- Microscope slides
  Rectangles of transparent plastic may be substituted – they should be sturdy enough to support the specimens without bending and transparent to allow light onto the specimens (e.g., 1" x 3" rectangles cut from plastic bags or sheets)
- Coverslips
  Coverslips are optional for large specimens but may be needed for liquid specimens such as pond water
- Specimens
  Plant leaves, Lichens, Moss, Insects, Pond water, Feathers, etc.

Microscope Assembly

1. We used a small wooden microscope for this lesson plan, available at http://echo-labs.com/woodenscope (see **Note 1**). The microscope was assembled from the starting template (**Figure 1 A**) using the video instructions at the site. Assembling the microscope required only a few minutes after releasing the pieces from the wooden template, which was aided by a small flat spatula. A pair of forceps helped with the assembly.

2. After assembly, make sure that all the parts fit securely into their slots. The microscope should be sturdy and stay together when you move it – if not, try gently pressing the sides together again, along with the top and bottom. The stage should move up and down when you turn the focusing wheel and stay fixed in place at intermediate positions.

3. The assembled microscope has a moveable stage positioned under a single lens with a gear that allows you to focus on the specimen (**Figure 1 B,** see **Note 2**) . The lens magnifies the object less than ~2-3 fold, but photos taken with a cell phone can produce magnifications of ~25X, or more (see **Note 3**).

4. Test your new microscope by looking at a United States penny. The penny we examined has an image of the Lincoln Memorial on the back or "tail" side of the coin (**Figure 2**). If you look carefully at the coin under your microscope, you should be able to see a small figure seated between the columns – *who do you think this is?*

If you can see the figure, you have already learned how to use your microscope! Now you can follow the Procedure below to look at different specimens.

Notes on assembly

a) Parts #1-6 form the wheel-like gear that moves the stage up and down to focus the specimen: parts #2, 3, 4 & 5 fit onto the end of part #1 to form the gear and part #6 holds it on the end of part #1

b) Parts #7-12 form the stage (part #10) and back (part #7) into which the focusing gear fits; parts #8 and #9 attach the stage to the back; the small parts #11 and #12 position the bottom of the stage at the sides – they fit in the indents below the square holes, facing inwards

c) Parts #13 and #19 are the sides; after positioning the focusing gear, stage, and lens holder (part #14) into the slots in part #13, carefully position and fit part #19 into place

Note that the rounded side of the lens should face down when it is properly positioned in the lens holder

d) Turn the microscope upright and fit part #15 into place over the lens

e) Part #16 fits across the top next to part #15 and part #18 fits across the top at the back to hold the sides together

f) Part #17 fits across the bottom to hold the sides together

Procedure

1. Many different specimens, when examined with the aid of a lens that increases or *magnifies* them in size, show visible features that go unnoticed when viewed with the unaided eye. This lesson plan demonstrates this with different specimens. First, assemble your specimens – these can be small leaves, insects, a feather, or other small objects.

2. Now take one of your specimens, such as a leaf, and look at it without your microscope. Note the coloring of the leaf. The leaf that we examined has areas of green, pink and pink-white (**Figure 3 A,B**). Leaves that show different colors in irregular patches like the one in **Figure 3 A** are called "variegated". Variegated coloring can be observed in leaves of many different plants.

Now put the leaf on the stage of your microscope and look carefully through the lens at the variegated areas where they change from green to pink or pink-white. Focus the microscope by adjusting the focusing gear. *Why does moving the stage up and down make the leaf appear clear or blurry? (see **Note 2** below)*

You can see small pink and pink-white cells in the green regions, as shown in **Figure 3 B** – the cells in the green regions are not all green! Now look carefully at the edge of the leaf under the microscope, where you can see tiny hairs. These hairs cover the entire surface of the leaf and are very difficult to see without a microscope.

*Other plant leaves have different-shaped hairs or bristles on their surfaces or edges (**Figure 3 C,D**) and some plants have spines or thorns on their stems – look at leaves and stems of different plants to see these strikingly different structures on their surfaces.*

Keep notes and write down (or sketch) your observations so you have a record of what you've observed. You can also take photos of your specimens using a cell phone. First, take a photo of the leaf without the microscope – include a ruler in the photo so you can calculate the magnification of the leaf taken under the microscope. To take a photo through the lens of the microscope, rest your cell phone on the top of the microscope, positioning the camera over the lens of the microscope so you can photograph the features you are observing. Before you take the photo, focus the microscope by adjusting the focusing gear. Make sure that you can see the leaf clearly through the lens and that the leaf is in focus in your cell phone camera viewer, then take the photo.

3. Another specimen that shows characteristics that are not easily observed with the unaided eye is a bird's feather. First, examine the feather carefully without a microscope and write down (or draw) the features you observe.

*You should be able to see the thick branch-like structures or "barbs" that are attached to the central shaft and make up a feather (**Figure 4 A**, magenta arrow). If you look closely at a feather without a microscope, you may be able to see the tiny hairs or "barbules" that extend from the barbs, forming a fine fringe (**Figure 4 B**, purple arrow).*

The overall structure of a feather is clear when you examine the feather under a microscope. You can see the barbs and barbules, and you can also see the interlocking of the hair-like barbules on adjacent barbs. This interlocking holds the barbs together so the feather appears to be a single structure, rather than made up of many barbs.

4. Finally, examine an insect, such as a fruit fly. First, capture a fruit fly in a small vial or bottle using a banana or apple as bait, then put the vial or bottle into a refrigerator or on ice for 5-10 minutes to gently anesthetize the fly. The fly will stay still for several minutes after you remove it from the cold. Look carefully at the fly without a microscope and write down your observations. Then place the fly under the lens of the microscope and write down (or draw) what you see.

Take photos with your cell phone with and without the microscope.

Does the fly appear different under the microscope and in the photos from your cell phone, compared to what you can see by eye?

Figure 5 *shows an image of a fruit fly taken without a microscope and through the lens of a small wooden microscope. The fly's red eye, which is typical of wild-type fruit flies, is easy to see. The black color at the end of the abdomen shows that this is a male fly. The yellow coloring of the abdomen (yellow arrow) is due to the Malpighian tubules, the organs of fruit flies that function like the kidneys of other organisms, which appear yellowish through the wall of the abdomen. Small bristles can be seen on the edge of the thorax of the fly (yellow arrow) and the wing veins can also be clearly seen (yellow arrow).*

5. Determine the magnification of the specimens in your photos by measuring the length that they appear in the photo taken through the lens of the microscope and their actual length without the microscope. The magnification due to the microscope and the photo can be calculated as

$$M = S_F / S_A$$

where

- M is the magnification
- S_F is the final size of the specimen in the photo taken using the microscope
- S_A is the actual size of the specimen without the microscope, either measured from life or from the photo taken without the microscope. If measured from the photo taken without the microscope,

  S_A = specimen size in the photo divided by the length of 1 cm of the ruler measured from the photo

Notes

1. A simple light microscope consists of a lens, a specimen holder or stage, and a focusing mechanism – your small wooden microscope is a *simple* light microscope. A *compound* light microscope has an additional lens (or lenses) in the eyepieces that magnify the image from the lens or set of lenses near the specimen. The different parts or components of a compound light microscope have names that include *eyepieces* or *oculars*, *objectives*, *condenser*, and *light source*. If you use a microscope often, you will want to learn the names of the microscope parts. A guide to the components of simple and compound light microscopes can be found at https://en.wikipedia.org/wiki/Optical_microscope

2. Light microscopes use a lens to enlarge or magnify an object. Lenses focus light to a point, which is a given distance or ***focal length*** from the lens. If you place an object at the focal point of the lens, the object will appear sharp when you view it through the lens. *Focusing* a specimen under a microscope means to move it into a position close to the focal

point of the lens. For the small wooden microscope, moving the stage up and down will focus the specimen by changing the distance of the specimen from the lens until its height is close to the focal length of the lens.

3. *Magnification* refers to the enlargement of the object by a lens. Your small wooden microscope has a lens that magnifies objects by less than ~2-3 fold. If you examine an object by placing your cell phone over the microscope lens and look at the object through the camera viewer, you are using the lens in your cell phone camera to magnify the objects further. This converts the simple wooden microscope into a compound microscope! The total magnification is the product of the magnification of the lenses and is given by

$$M = mn$$

- M is the total magnification of the object
- m is the magnification of the wooden microscope lens, and
- n is the magnification of your cell phone camera lens

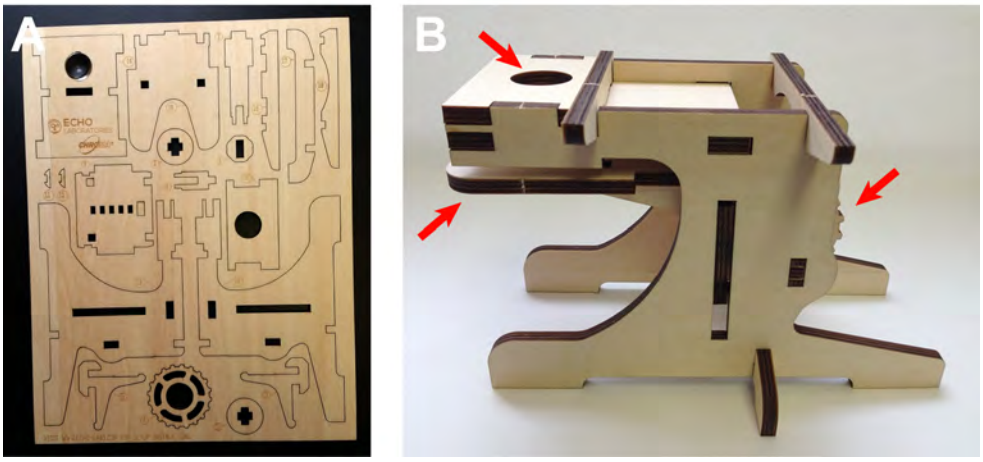
4. **Advanced topic 1**: early lenses for microscopes consisted of glass or quartz and were curved or spherical. Because the lenses were not flat, light passing through the edges was bent at different angles relative to light passing through the center. This results in an image that is distorted at the at the edges, an effect that is referred to as ***spherical aberration***. If you look carefully at a specimen through your small wooden microscope, you can see a difference in the focus and size of the specimen at the edges and center of the lens – this is caused by spherical aberration.

Modern microscopes have lenses that are corrected for spherical aberration by blocking the light at the edges, producing an image only with the center of the lens, by adding another lens that has the opposite effects on the bent light at the edges, bringing it into focus with the light at the center, or by altering the lens at the edges using special lens-grinding methods. The lenses in microscopes that are used today are mounted in tube-shaped cases called ***objectives***. The objectives collect light from the specimen and direct the light to the eyepieces, permitting the viewing of detailed specimens by the user; they are one of the most important parts of a light microscope.

5. **Advanced topic 2**: microscopes that are specialized to perform different types of imaging are referred to by the optical methods that they use. For example, phase contrast microscopes enhance contrast due to differences in the way light is bent by the cellular components, or differences in their ***refractive index***, while fluorescence microscopes are designed to excite fluorescent specimens and collect the fluorescence light the specimens emit. Information about different types of microscopy, including phase contrast microscopy, polarized light microscopy, and electron microscopy can be found in Slayter (1970); the pages at http://www.microscopyu.com also provide information about DIC microscopy, confocal microscopy and different methods of super-resolution microscopy.

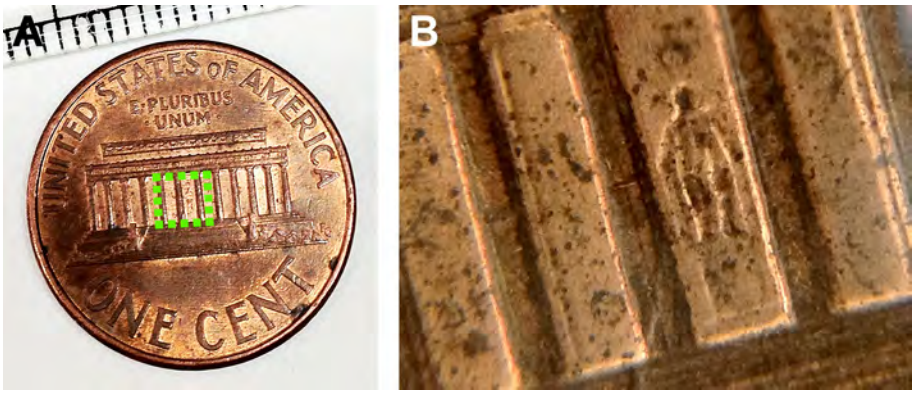
Figures

Figure 1 A Small Wooden Microscope



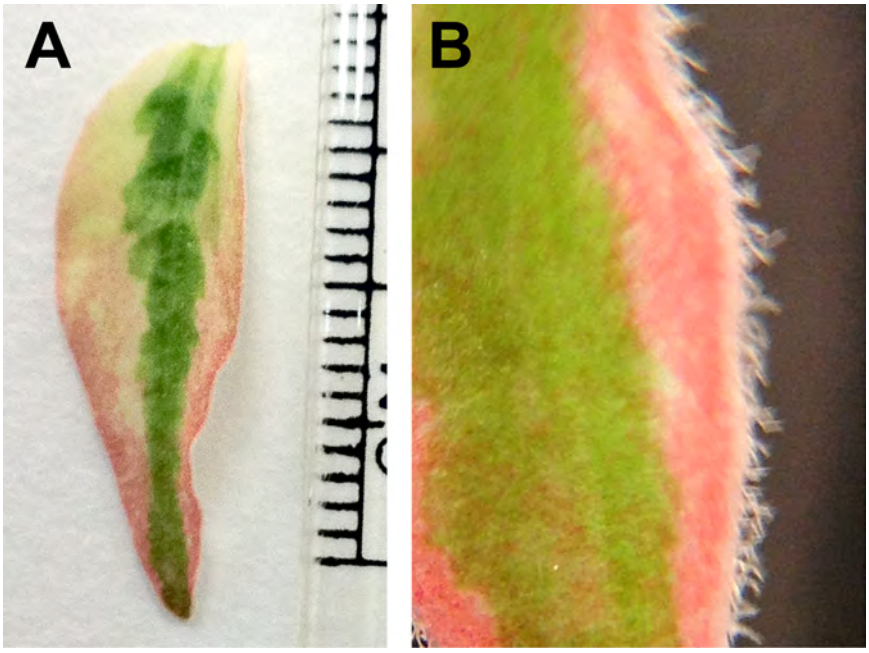
A) Template for a small wooden microscope (a gift of Echo Laboratories and Chroma Technology Corp). **B)** The microscope after assembly. It has a single (recessed) lens (top left, red arrow), a specimen stage (middle left, red arrow) and a focusing gear (right, red arrow). Specimens can be placed directly on the stage or mounted on a microscope slide and the slide placed on the stage. The images shown in **Figures 2-5** were taken with a cell phone camera through the lens of a small wooden microscope. The images above are shown with permission of Echo Laboratories and Chroma Technology Corp.

Figure 2 Testing Your Microscope using a US Penny



A) Look at a US one-cent coin, or penny, to test your new microscope. This image, taken without the microscope, shows the Lincoln Memorial on the back of a penny. **B)** An image taken through the lens of a small wooden microscope (region in green dashed box in (**A**) shows a seated figure – Abraham Lincoln! Magnification ~16X.

Figure 3 Observing Plants



A) This variegated plant leaf has regions of green, pink and pink-white, which can be easily seen without a microscope. **B)** This image, taken through the lens of a small wooden microscope, shows that pink and pink-white cells are present in the regions of green cells. There are also tiny hairs along the edge of the leaf that cover the entire surface of the leaf – these cannot be easily seen without a microscope. Magnification, ~25X**.**

C) This lichen, growing on a piece of tree bark, has curly leaves that can be seen without a microscope. **D)** The leaves have bristles at their edges (green arrow), which go unnoticed if the lichen is not examined under a microscope. Magnification, ~4X**.**

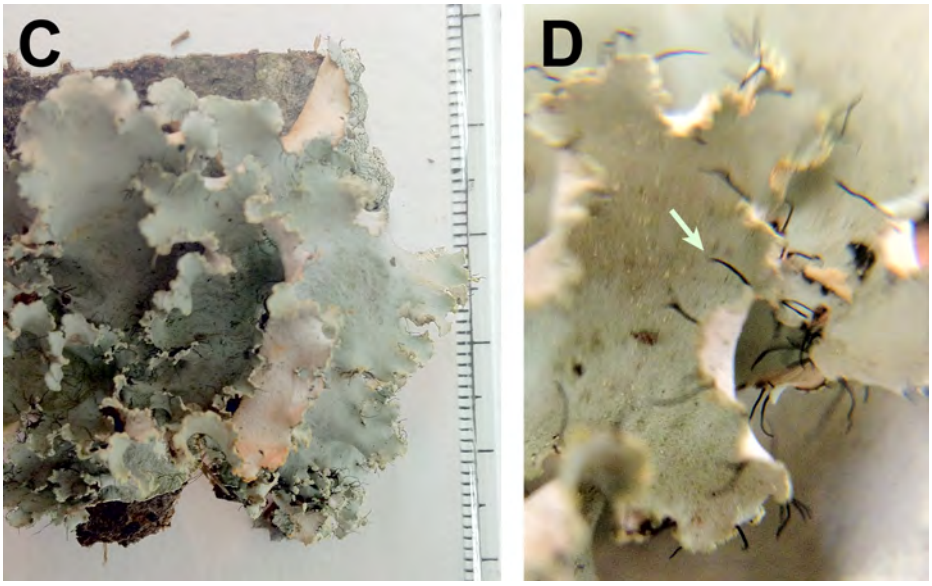
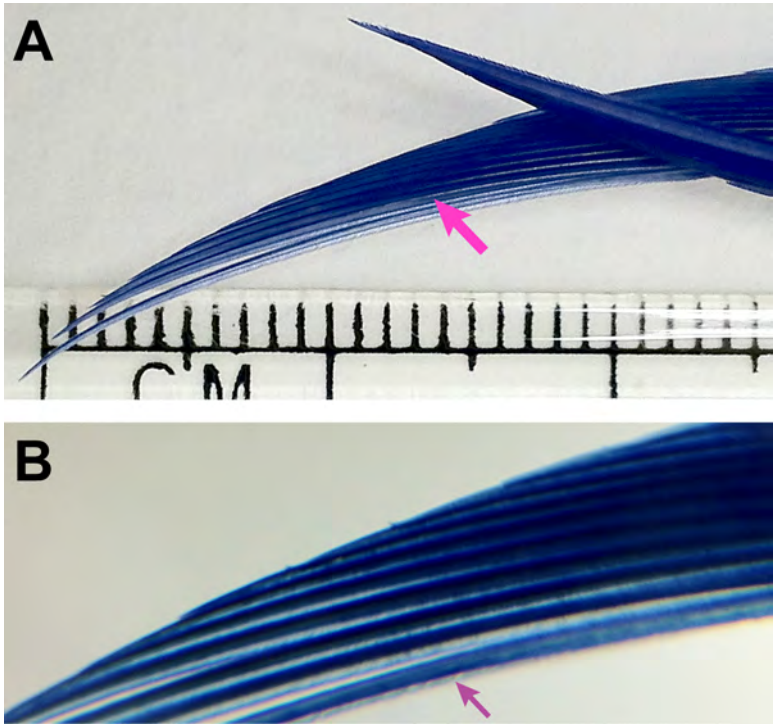
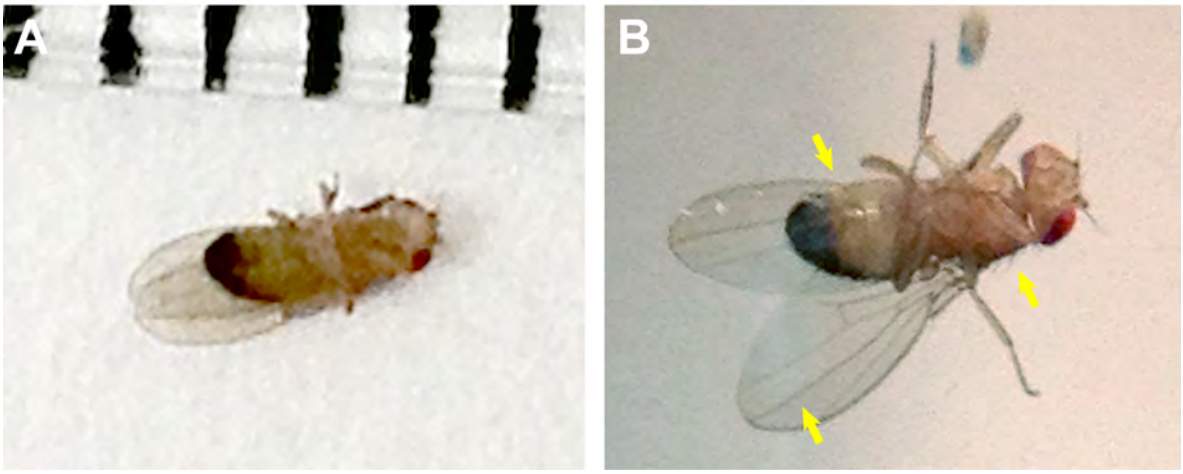


Figure 4 Observing a Feather



A) The thick blue barbs (magenta arrow) that form this bird's feather can be easily seen when viewed without a microscope. **B)** The small thin barbules that form a fine fringe extending from each barb (small purple arrow) are more clearly observed when viewed through the lens of a microscope. Magnification, ~11X.

Figure 5 Observing a Fruit Fly



A) A fruit fly imaged viewed without a microscope and **B)** another fly of the same size visualized through the lens of a small wooden microscope. The red eye and dark abdomen at the end of the fly can be seen in the photo taken without the microscope. They are clearly observed in the photo taken through the lens of the microscope, together with the yellowish color of the abdomen (top, yellow arrow). The thoracic bristles (right, yellow arrow) and wing veins (bottom, yellow arrow) are also clearly visible. Magnification, ~18X**.**

References

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