Microscopy

For Ages: 10-18

1. Description/Learning Objective

The goal is to learn about the physics and historical context of microscopy. The student will explore the phenomenon of light refraction in different media and see how light can be manipulated with lenses to produce magnified images, reliving some of the challenges of first microscopists.

2. Materials Needed

- Foldscope Classroom Kit: https://www.foldscope.com/
- Clear drinking glass or another glass container
- Access to a tap or a bottle of water
- Smartphone with a camera

3. Background and Misconceptions

Have you ever tried to look at something so small you could barely see it? Try looking at your skin, even though it looks smooth at first it is actually built of billions and billions of cells, which you can't see because of how small they are. Finding out about the world on a very small scale can help us understand it better and through that solve more of our problems, like disease, famine or even human behavior. It can also help us engineer wonderful things such as smartphones and space crafts which help us be more connected with each other and the world around us.

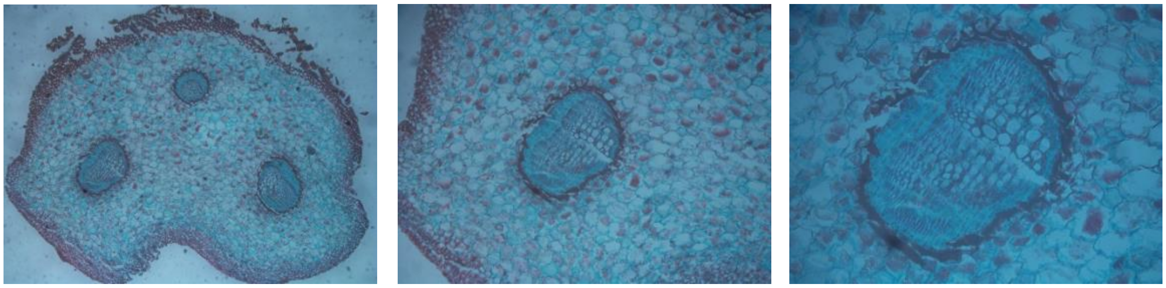

Fig.1. Magnified images of a fern rhizome. Magnification increases from left to right.

We will use light to magnify our tiny objects to large images, but before we do we need to understand how light can help us do that.

Refraction:

Let's think of light as straight rays that travel from left to right side of this sheet of paper. In the diagram below you can see that as light travels from air to glass it slows down. This slowing down causes it to change the direction in which it is travelling. We can measure by how much light has bent after entering a material and work out how quickly it travels in it. This can be quantified by Snell's law that tells us how much the light bends.

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{n_1}{n_2}$$

Where θ_1 is the incidence angle with respect to the normal*, θ_2 is the angle to the normal after refraction and n_1 and n_2 are the refractive indices of air and glass respectively

To make life easier, for our calculations we can use the refractive index to express the change in the speed of light in a different medium. It is defined as

$$n = \frac{speed\ of\ light\ in\ a\ vacuum}{speed\ of\ light\ in\ material\ of\ interest}$$

*Normal defined as an imaginary line perpendicular to the surface where a light ray encounters a new medium.

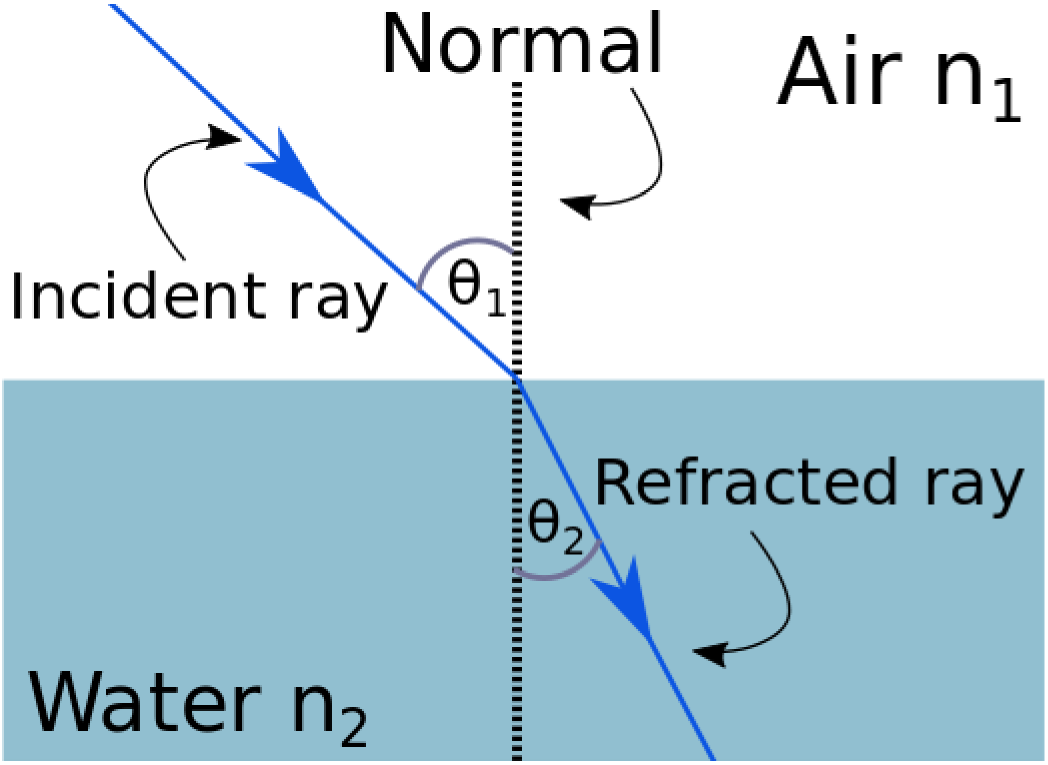

Fig.2. Ray diagram of refraction of light when traveling from air to water. Notice the bending of the ray of light.

Being able to manipulate the direction in which light travels can be extremely powerful when combined with precise engineering of glass shapes. Glass lenses can be used to create images larger than the object we are looking at. In the diagram below we see that the rays carrying information about the object are refracted and change direction when entering the lens. To the observer it seems like the light has come from somewhere else, such as a larger object, which is the magnified image. An image like this, caused by the apparent divergence of light rays is called a virtual image.

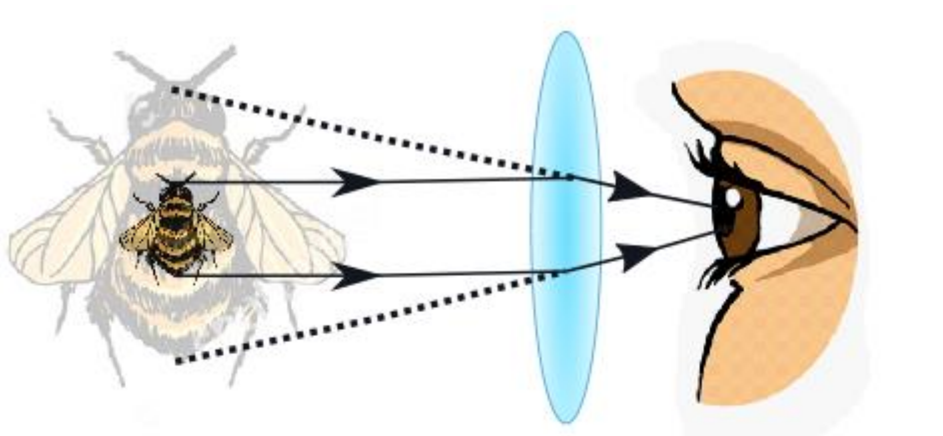

Fig.3. Ray diagram of a simple magnifying lens and a magnified virtual image.

We can quantify magnification simply by the ratio:

$$Magnification = \frac{Size\ of\ image}{Size\ of\ object}$$

We could achieve even better magnification using the first lens to create a magnified image and then magnifying that image with a second lens. Unfortunately making good lenses is difficult and in the early 1700's when microscopy was being developed, two lens system magnification was limited by the quality of lenses.

An ingenious solution to this problem is making a single ball lens. Because of spherical symmetry, many problems that the first hemisphere creates are compensated for in the second hemisphere, resulting in a high-quality lens. The smaller this ball lens is made the larger the magnification.

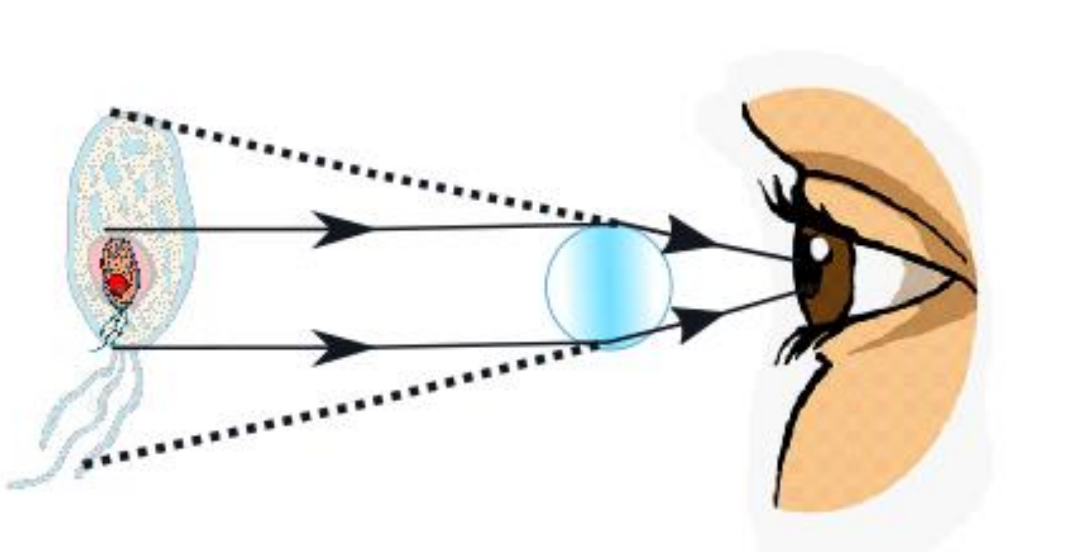

Fig. 4. The ball lens microscope operation principle. You can see that magnification results from the same ray tracing principles we saw in the case of a magnifying glass. The difference here is that the lens is perfectly spherical and very small, only about 1 mm in diameter.

In the early 1700's Antonie van Leeuwenhoek, a self-taught Dutch microscopist, came up with a method of making very small and very high-quality glass ball lenses. The very large magnification compared with other contemporary microscopes allowed Anthony to see microbes for the first time in history. A combination of optics knowledge and craftsmanship elevated van Leeuwenhoek to the position of one of the pioneers of microscopy.

In modern times van Leeuwenhoek's idea has been revived by Manu Prakash who combining it with origami, an ancient Japanese art of folding paper, succeeded in creating a cheap and easy to use microscope which you can now use to discover the secrets of the small scale.

4. Instructions

1. Use the provided instruction manual to put together the foldscope kit. Video tutorials are also available on the Foldscope Blog: http://microcosmos.foldscope.com/

5. Teacher Guided Questions to Inquiry

1. What is the angle of refraction when the ray in air is incident at the glass surface at 40 degrees to the normal?

2. Fill a glass cup with water until it is half full. Submerge a straight object, like a pencil in the water at an angle and look at it from the side to see it both above and below the water.

   a. Does the object seem straight still, or has it started bending?
   b. Explain what is happening.

3. If light were travelling from glass into the air, could there be a certain angle at which the light would be trapped in glass and never pass through to the air? Discuss with the teacher.

4. Use the magnifying glass to look at the microscope slide sample.

   a. Can you see any of its detail? Describe the sample, include size, colour and any other detail you can observe.
   b. Now look at the polymer fibre filters and 0.5 mm grid plastic slide. Can you see any detail at all?

6. Guided Inquiry

1. Use the foldscope to have another look at the 0.5 mm grid and determine how wide an area you are looking at. Hint: the area is a circle and you can characterize it using its diameter, the grid can serve as reference. The area we can see is called the Field of View and is expressed as a diameter of the circle. What size is the field of view?

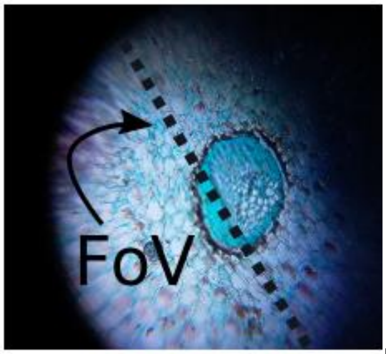

Fig. 5. Illustration of the field of view concept.

2. Take the microscope slide with a sample on it and have a look at it through the foldscope. Now that you have a more powerful magnification try to characterize it again. Measure its size, decide on the color and describe any special features. You can try and draw it too. This is how the first microscopists let the world know about the microbial world!

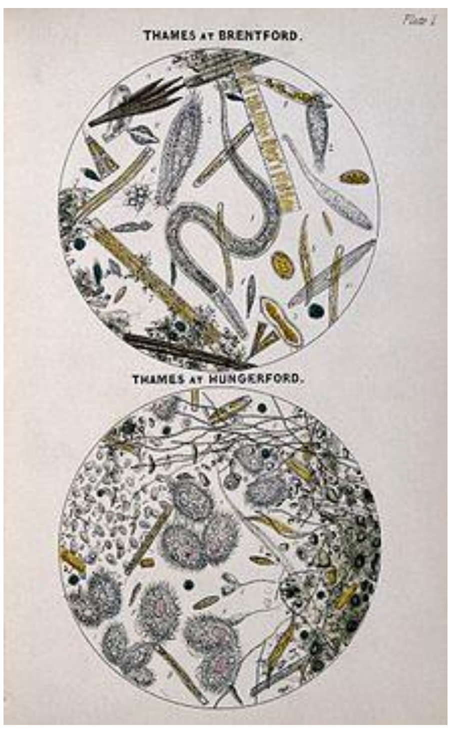

Fig. 6. A drawing by Arthur Hill Hassall of the microbes he saw in two samples of water from the river Thames. (taken from Wikipedia)

3. Using your smartphone take a picture of the sample through the foldscope. Now measure the sample size and calculate the magnification. What is the difference between magnification in the foldscope and zooming in using the digital zoom in your

phone? Discuss with the teacher. (enlarging pixels vs enlarging an image by diverging rays)

7. Analysis Questions

1. What is the role light refraction plays in magnification?

2. How are virtual images produced?

3. What is magnification and why can we not use digital zoom to achieve true magnification?

4. What advantages could there be to taking a video rather than a single static picture?

5. What is the origin of contrast in the examples above? Can you think of any samples we could not see under the bright light microscope?

Answer Key

Teacher Guided Questions to Inquiry

1. Use Snell's law, answer is 25 degrees
2. A) The object will look like it is bending. This is because of refraction of light.
   B) Light changes direction as it enters a medium with a different refractive index. This is perceived by us as a bending of the straight object.
3. This question is about the critical angle and can lead to a discussion about fibre optics. As you go from a medium with a high refractive index to a medium with a low refractive index there will be an angle of incidence at which the refraction from the normal is 90 degrees and more. This is when light is trapped in the higher refractive index material and we call it total internal reflection. It allows us to make fibre optic cables that trap light and therefore allow us to send signals very quickly over large distances.
4. A) Not a lot of detail can be seen
   B) Again, some detail can be seen but not a lot. This is because magnification is too small

Guided Inquiry

1. The grid provides a reference point for size
2. The size can be measured using reference grid
3. Magnification is due to the divergence (real or apparent) of light rays resulting from refraction while digital zoom is the enlargement of the pixels of the photograph or video.

Analysis Questions

1. Causes divergence of light rays that create a larger image of the object
2. Virtual images are formed from the apparent divergence of light rays
3. Enlargement of the image due to divergence of light rays is magnification. Digital zoom is just the enlargement of pixels in an image.
4. Makes it possible to measure time resolved effects such as motion of the microbes to study their behavior.
5. Absorption is the source of contrast here. Transparent samples that do not absorb visible light will not be visible. We could use staining or dark field microscopy to still see the sample.