LAB 2: UNDERSTANDING HOW MICROSCOPES WORK

LEARNING GOALS

After completing this laboratory, you should be able to:

- Use ray diagrams and equations to explain how real optical instruments work, including microscopes designed in two different ways.
- Explain why microscope objectives are labeled with a magnification, rather than a focal length, and what determines the magnification produced by a single lens and by a multi-lens instrument.
- Explain how virtual images are used in optical instruments.

PREPARATION

Before coming to lab, answer the following questions in your lab book:

1. Suppose you want to use a single converging lens to form a highly magnified real image of a small object. Where should the lens be positioned, relative to the object? Answer using both a ray diagram and the lens equation.
Ray diagrams should include the lens and its axis, the lens's two focal points, the object, two principal rays, and the image. (It's convenient to represent the object with an arrow).

2. If you move the lens slightly farther away from the object, does the image move closer to or farther from the lens, or stay in the same place? Does the image get smaller or larger? You can explain with either the lens equation or a ray diagram, whichever you prefer.

3. Could you use a single diverging lens to form a real image of an object? Why or why not?

Before coming to lab you should be able to:

- Use the thin lens equation to calculate the relationship between object position, image position, and the focal length of the lens.
- Use the relationship between magnification, object distance, and image distance to calculate image properties.
- Draw a ray diagram to locate the image formed by an object and either a converging or a diverging lens.

INTRODUCTION: MAGNIFICATION AND FOCAL LENGTH

When imaging small objects with a microscope, the easiest way to begin is by examining the object (usually called the sample) under relatively low magnification, because it is easier to locate the region of interest of the sample at low magnification, and then increase the magnification. To facilitate this, microscopes are typically equipped with two or three objective lenses. These lenses are mounted on a rotating turret so that they can be exchanged without moving the sample. The whole microscope is designed so that when the lenses are exchanged, the image remains very close to in focus.

To keep the image in focus, when changing objectives to change magnification, the position of the image formed by the objective lens must stay the same. Because the point is to keep the sample in the same place, the sample location also must stay the same. However, the position of the lens can change, so the distance from the sample to the lens (called the "working distance"[1]), and the distance from the image to the lens, can change.

So far, you have learned in this course that lenses are characterized by a focal length. One of your goals in this lab is to understand why lenses on a microscope are labeled with a magnification, not a focal length, and what determines the magnification produced by a lens. The other is to understand how different designs of microscopes form different types of images.

MEASUREMENT 1: DETERMINING THE FOCAL LENGTH OF A LENS

Throughout the following measurements, the light source with the crosshair pattern will serve as your object ("sample" in microscopy). Keep it positioned with its bright surface at the zero marking on the optical rail, and move the lens and/or the screen as needed.

Summary of measurements to be made:
Determine the focal length of the approximately 10 cm focal length converging lens using a graph with at least five data points.
Prepare a data table including your five points that also includes magnification.

Instructions:
With your lab table, devise a strategy for measuring the focal length of this lens, using a **graph** of data from at least five images of different magnification formed by that lens. Your result should include an estimate of the uncertainty. Check your strategy with your lab instructor and then measure the focal length. Distances should be measured to the nearest millimeter.

As you make your measurements, also measure and record the magnification of each image. Check the consistency of at least one data point using the relationship between magnification, object distance, and image distance.

Tips for measuring magnification: The crosshair pattern on the light source is marked with its actual size (like the measurement scale provided on some microscope objectives). Measure the size of a feature in the image with the calipers, taking care not to scratch the screen (you may want to mark the size on a piece of paper held up to the screen, then lay the paper flat on the lab table and measure with the calipers). Select a feature large enough that you can measure it accurately, but not so large that it will be distorted in the more highly magnified images. You may measure different features for different magnifications.

[1] If the object is a biological specimen that is mounted on a glass microscope slide and sealed with a cover slip, the working distance is defined as the distance from the objective lens to the surface of the cover slip.

MEASUREMENT 2: RELATIONSHIP BETWEEN FOCAL LENGTH AND MAGNIFICATION

Summary of measurements:
Set up a single lens to produce a focused image of an object, as a model for how the objective lens of a microscope produces an image of the sample. On a diagram in your lab book, record the positions of all the parts and the magnification of the image.
Then substitute a different lens in a manner that imitates changing objectives in a microscope, again recording the arrangement of all the parts and the magnification.

Instructions:
Your instructor will show you how light travels through a compound microscope. If you are not familiar with using a compound microscope, your instructor will show you how to examine an object under low magnification and then change the objective to increase the magnification.

Using the optical rail, object, screen, and one of the convex lenses provided, **design and assemble a model of how the objective lens in the microscope produces a focused image of the object**. Do not include the eyepiece lenses in this model; restrict your model to the sample and objective lens only. Keep in mind that you want to produce a highly magnified image, and place the parts of the model accordingly (you may want to look back at your previous table of measurements and magnifications for insight). *Record the positions of all of the parts and the magnification of the resulting image.*

Now prepare to modify your model to put in a different lens, in analogy to how a microscopist can change the objective lens in a microscope. With your lab table discuss the following (and as needed test out your ideas with the compound microscope provided):

- To be equivalent to changing lenses in a microscope, which parts of your model (object, lens, image) must be fixed in place? Which part(s) move(s)?
- When you put in a different lens, how do you expect the magnification of the image will change?

Discuss your plan with your lab instructor and **then build and record the arrangement of the parts of your model using a different lens.** Also record the magnification of the image formed this way.

Conclusions:
In your lab book, summarize your findings, including answering these questions

- What observations demonstrate that your model is consistent with the compound microscope provided?
- What do you conclude about the relationship between the focal length of a lens and the magnification of an image produced by that lens? (What other parameters are involved in determining that relationship?)
- For the image formed by the objective lens in this model, which of the parameters in the thin lens equation are fixed, and which are variable?
- Why is it reasonable that microscope lenses are simply marked with magnification?

INTRODUCTION, PART 2: MICROSCOPE DESIGN

Suppose you have been hired to run the optical microscopy lab at the local hospital. On your first day at work, you discover that the microscopes in the lab, although of very high quality, are quite old and are not equipped to digitally capture the image on a CCD camera. You therefore go to the director of your division with a proposal to purchase new, suitably equipped microscopes. Your director frowns on seeing the amount of money required and says, "Just attach CCD cameras to an eyepiece in place of your eye." Will this do the job, assuming you could find a way to attach the CCD cameras to the eyepieces? To figure this out, in this lab you will construct a simple model of a microscope.

Based on the first part of this lab, you may be wondering why microscopes have two lenses — it may seem like a single lens can work perfectly well as a microscope! In fact, it is possible to capture the image produced by the objective directly to a CCD camera without using any eyepiece lens. However, that is workable because CCD cameras can capture very tiny images with high resolution, and then magnify them electronically. The image produced by a 60x microscope objective is still too small for the unaided human eye to see all the details.

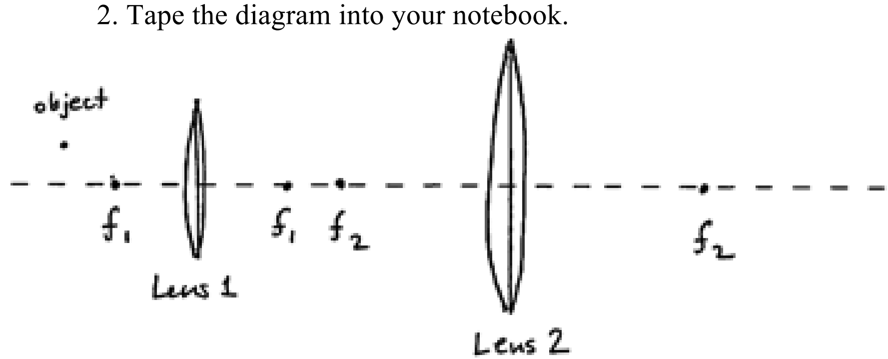
Prior to digital camera technology, using an eyepiece lens offered two advantages, both to enlarge the image further, and also to make it easy for a scientist to reliably view it when sitting at the microscope. Even with digital cameras, it's usually convenient to be able to view the image directly, so most research microscopes allow the user to switch between viewing the image through an eyepiece and using a different second lens to send the image to a camera.

GOAL

To design and construct two different designs of microscope, one that produces a real image on a screen and one that produces a virtual image that must be viewed through the eyepiece lens.

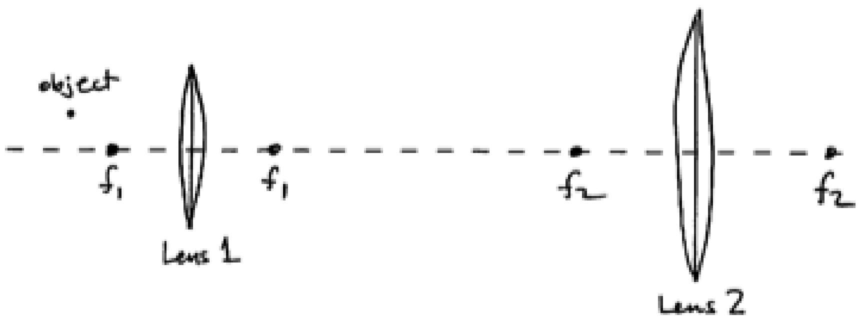
PLANNING[2]: Work through the following exercises with your lab partner or table; each of you should record your answers and tape your diagrams into your lab notebook.

1. The diagram provided below shows an arrangement of a small object and two thin convex lenses analogous to that used in a typical compound microscope. A copy of this diagram is on the last page of this document. Print it out, and on it construct rays showing the image formed by lens 1, then use that image as the object for lens 2 and construct rays showing the image formed by lens 2. Tape the diagram into your notebook.



[2] Questions 1 and 3 inspired by *Tutorials in Introductory Physics*, McDermott, Shaffer, and the University of Washington Physics Education Group, "Convex Lenses" homework, p. HW-140 (Prentice Hall, 2003).

2. Think of lens 1 as the objective lens and lens 2 as the eyepiece lens of a microscope. Does the eyepiece lens form an image that could be detected by a CCD camera or cast on a screen? If so, where should the camera's sensor be placed? If not, is it possible to adjust lens 2 so that its image could be cast on a screen?
3. The diagram below shows another arrangement of a small object and two thin convex lenses. On your printed copy of the diagram, construct rays showing the image formed by lens 1, then use that image as the object for lens 2 and construct rays showing the image formed by lens 2. Tape the diagram into your notebook.



4. Think of lens 1 as the objective lens and lens 2 as the eyepiece lens of a microscope. Does the eyepiece lens form an image that could be viewed on a screen? If so, where should the screen be placed? If not, is it possible to adjust lens 2 so that its image could be viewed on a screen?
5. For this lab you have available a +10 cm lens and a +6 cm lens to use for the two lenses (eyepiece and objective). You want to keep the microscope as compact as possible (it is easier to use and also less stray light from other sources gets in). To keep the distance from sample to image as short as possible, while also obtaining maximum magnification from the objective, which should you use for the objective lens?

Measurements

Design and build an arrangement of two lenses in which the second lens produces a highly magnified real image on a screen, with most of the magnification coming from the first ("objective") lens.

Begin by working out a design with your lab partner or table. (The preparation exercises are intended to help you think about how to arrange the lenses.) Then assemble it, and if you need to, adjust the positions of the lenses to improve the magnified image. Finally, record your arrangement with a careful diagram in your lab book:

<u>In your lab book, draw a neat diagram showing:</u>
- the arrangement of the source, the lenses (labeled with focal lengths), and your eye
- all the measured distances between the parts
- the focal points of both lenses
- the images formed by each lens
- the distances from each lens to the image it forms

(You do not need to include rays unless you wish.)

Also in your notebook:
Record whether the final image is upright or inverted
Calculate the expected magnification. Measure and record the actual magnification, and compare. It may be hard to measure this accurately as the images may be distorted; if so do your best without undue effort.

Then design an arrangement of two lenses in which the second lens produces a virtual image that can be viewed by a person's eye, and build it and look through it. Record the arrangement in your lab notebook as previously, as well as whether you observe the final image as upright or inverted.

Note that it is more difficult to build this design effectively due to the limitations of the types of lenses we have. Don't try for the maximum possible magnification, just substantial magnification. Also you do not need to try to estimate the magnification, as that depends on all kinds of psychological factors as well. A model is set up elsewhere in the lab that you can look at.

Examine the compound microscope at your lab station, and identify each part that corresponds to the parts of your model microscope. Also explain to your lab partner why the path followed by the light from the objective lens to the eyepieces is enclosed in a light-tight box. (How might this improve the images you can see with the microscope?)

Conclusions

For each of the two microscope designs:

• Explain why the image viewed by your eye is real or virtual, based on the location of the image formed by the objective lens.

• Explain why the image formed by the eyepiece lens and viewed by your eye is upright or inverted.

Also answer the following:

Which design of a microscope is required to project the microscope's image onto the detector of a CCD camera? For the microscopes at the hypothetical lab described in the introduction, explain what modifications would need to made, and whether buying new microscopes is needed.

The following are optional additional activities and information for those who are interested, but not required!

FOLLOW-UP: FOCUSING IMAGES

Turn on the illumination for the compound microscope nearest your station, and place one of the samples provided on the microscope stage. Be very cautious in focusing just to be sure to avoid damaging the objectives.

Position the lowest magnification objective above the sample and turn the focusing knob so that you can see a sharp image of the sample. Then turn the focusing knob gently, to gain a sense of how rapidly the image goes in and out of focus. Then switch to the highest magnification. Does the focus disappear more or less rapidly at higher magnification?

How far the objective lens can be moved before the image goes out of focus depends on two properties of imaging systems known as depth of field and depth of focus, which we will not have time to study in great detail (though if you are a photographer you may already know about these). The lenses we used in lab to build the model microscope have long focal lengths, in order to allow us to easily work with this equipment. Ordinarily, long focal length lenses also have long depths of field and focus. For this reason, in your model, you could move the lens a significant distance while the image grew gradually more blurry, and you may have found it difficult to identify the position giving the most focused image. On a real microscope, all the lenses involved have much shorter focal lengths and so the depth of field and focus are much shorter.

FOLLOW-UP NOTES ON DIGITAL MICROSCOPY

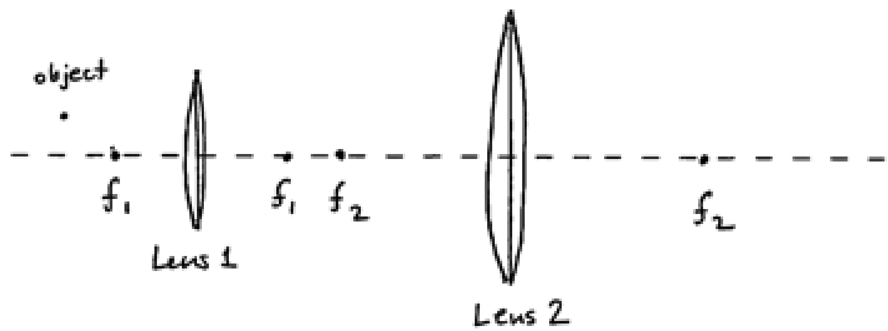
Modern microscopy is typically done using CCD cameras to record images. Unlike cameras for photography, ordinarily CCD cameras do not have lenses in front of the detector chip— the chip is simply protected with a transparent window. In addition, the size of the chip and the individual pixels of the CCD camera are such that the magnification provided by the microscope objective is the right amount of magnification; further magnification by a second lens would make the image too large for the chip.

For this reason, microscopes are designed with ports to which the CCD camera can attach, so that the image produced by the objective will fall directly on the detector. (For a high-quality microscope equipped with what is called an "infinity-corrected objective", the story is a little more complicated and actually does involve a second lens which does not provide any further magnification, but for the purposes of understanding the basics, you can think of this as the image produced by the objective alone.) Then there is a mirror (or a prism operated in total internal reflection mode, as in last week's lab) that can be positioned to direct the rays coming from the objective either toward the CCD camera or toward the eyepiece.

Before CCD cameras, microscopes were sometimes provided with so-called "photoeyepieces" which could produce a real image on the film plane in a photographic camera with the camera's lens removed. A microscopist could thus view the sample looking through a normal eyepiece, then remove one eyepiece and replace it with a photoeyepiece with camera attached, and take a picture of the sample. So the lab director's idea is not so crazy ... but has been rendered obsolete in most modern microscope technology.

Diagrams for Lab 2, Part 2 Planning

Planning question 1



Planning question 2