

# OPTIX Module 1 – Basic

## Basic Microscopes

David Altman, Michaela Kleinert

### 1 Objectives:

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In this module you will learn about

- how to measure the magnification of a simple lens using a CCD camera;
- how to turn this single lens into a simple microscope;
- how to characterize the magnification of this simple microscope.

Use this manual as you work through the module to keep track of your notes and thoughts. In addition, you will have to add a few printouts or refer to data tables or additional notes in your lab notebook. Make sure to add everything to the folder in which you keep this manual. Please note that this lab has no formal lab report. Instead, you will turn in and be graded on your notes in this manual.

### 2 Tests and assessment:

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In preparation for this module, read through the whole manual and answer the questions that are marked with a \*. You should also watch the [VIDEOS](http://www.willamette.edu/cla/physics/info/NSF-OPTIX) that are posted on our website ([www.willamette.edu/cla/physics/info/NSF-OPTIX](http://www.willamette.edu/cla/physics/info/NSF-OPTIX)). They are meant to accompany this manual and will show you some critical steps of the module. When you come to lab, be prepared to discuss your answers to these questions with your classmates and your instructor.

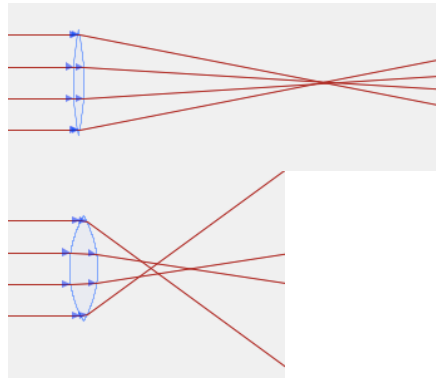
In addition, in order to assess the success of this module, you will take a short assessment test before you start (“pre-assessment”), and another one after you have successfully completed this module (“post-assessment”). At this point you will also have the opportunity to provide us with feedback about the module that we will use to improve it for the next student generation. Thank you for your support!

### 3 Equipment:

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You can find most of the equipment for this module in the box labeled ‘HeNe Optics’ that is located in one of the cabinets in the OPTIX lab. Standard equipment that is used in multiple modules will be located in other cabinets in the OPTIX lab. In addition, you will use some of the equipment in the boxes labeled ‘Module 2’ and ‘Module 4’. Please feel free to also ask your instructor for help. You will need:

- CMOS camera (Thorlabs, DCC1545ML) with ThorCam software
- Mounted plano-convex lenses of various focal lengths ranging from 50-400 mm (Thorlabs, LAXXXX-A-ML), all of which are coated with anti-reflective coating for wavelengths ranging from 350-700 nm (these can be found in the HeNe Optics box).
- White LED light source (Thorlabs, MCWHL5) with a driver (Thorlabs, LEDD1B), and power supply (Thorlabs KPS101)
- Various post-holders (Thorlabs, PH2), posts (Thorlabs, TR2), 1-inch lens mounts (Thorlabs, LMR1), and bases (BA1)
- 1-inch 1951 USAF test target (Thorlabs, R1DS1P)



**Figure 5.1:** Bi-convex lenses result in spherical aberration, which is more severe in the case thicker lenses with shorter focal lengths.

## 4 Introduction:

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Telescopes and microscopes have allowed for the extension of our senses to the far-reaches of both the cosmos and the microcosmos. Lenses as we know them were introduced to the West at the end of the 13th century, largely as the result of cheap high quality glass and the development of advanced techniques in grinding and polishing (they may have also been developed independently in China earlier) [VH16]. However, it took a few more hundred years before lenses were *combined* to form complex optical instruments. Microscopes first appeared in the 16th century with the work of Hans and Zacharias Jansen [WNHL15], and the first working telescope, credited to Hans Lippershey, was reported in 1608 [Wik16b].

Just as telescopes and microscopes have revolutionized our ability to observe the very far away and the very small, respectively, digital cameras (or *Charged Coupled Devices (CCDs)*), which are about 70 times more sensitive to light than film cameras, have revolutionized the way we take and process images. Unlike photographs, digital images can easily be analyzed with a computer, making CCD cameras one of the most versatile and sensitive light detectors in any optics laboratory and in everyday life.

In a CCD camera, light is focused onto a semiconductor *chip* that consists of a 2D array of photon detectors called *pixels*. You can think of them as little buckets full of electrons. An incoming photon can knock one of those electrons into the conduction band of the semiconductor through the photo-electric effect, which reduces the number of electrons in the bucket. At the same time, the removed electrons now contribute to a current in the conduction band of the semiconductor that can be measured electronically. The digital image that is created in the process is a 2D array of pixel values, with each pixel value corresponding to the photon count in a particular detector over a fixed time, called the *exposure time*. High counts correspond to bright pixels and low counts to dim pixels, thus forming a black and white image. Note that CCD images count all photons (almost) equally and do not distinguish between photons of different frequency. However, they typically have different sensitivities for the different wavelengths. While the overall sensitivity is pretty constant over the visible range, it drops off sharply toward smaller or larger wavelengths. In this module, we will only be working with gray scale images taken by a black and white CCD camera. To create color images, the pixels are divided into clusters of three pixels each. A filter that let through only one color of light (red, green, or blue) is applied to each of the three members of a cluster. Thus, each cluster contains one pixel that counts only red photons, one pixel that counts only blue photons, and one pixel that counts only green photons. The color counts of these three individual pixels are read out individually and then combined to create a color image.

In this module you will start by investigating the magnifying properties of a single converging lens (a magnifying glass). You will then increase the complexity of this optical system by adding a second lens and forming a simple microscope. We will use an *external* source of illumination over which we will have more control. Controlling and optimizing our illumination will be a major focus of your work building a microscope.

## 5 Introduction to plano-convex lenses

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Most of the lenses we will be using in this lab are plano-convex, meaning they have one convex spherical surface and one flat surface. These lenses are particularly effective at either turning a point source into collimated light or focusing collimated light to a point. In fact, they are more effective than bi-convex lenses that consist of two convex spherical surfaces, typically of the same radius of curvature, because by design they are thinner. The thickness of a lens affects the degree to which incident collimated light can be focused by the lens. Ideally, all incident parallel rays should meet a focal length away from the lens. In practice, however, the thickness of these lenses results in an effect known as *spherical aberration*, in which rays that hit the lens at different heights come to a focus at different points on the optics axis (the focal “point” gets smeared-out), see Figure 5.1.

Lenses with long focal lengths can be either plano-convex or bi-convex, because a long focal length means that the radius of

curvature of the lens surface is also large, and thus these lenses can be quite thin. Shorter focal length lenses, however, tend to be thicker and thus suffer from more striking spherical aberration. For example, in Figure 5.1, the thinner lens has a radius of curvature that is four times that of the thicker lens. As you can see, the spherical aberration is much worse for the thicker (and thus shorter focal length) lens.

Plano-convex lenses must be oriented appropriately in order to reduce the effects of spherical aberration as you learned in [MODULE 1 - BASIC \(Optics Basics - I\) and INTERMEDIATE](#): The curved side of the lens should always face the (more) collimated side of the laser beam. Sketch the correct orientation of a plano-convex lens that is being used to (a) focus a collimated beam and (b) collimate a strongly diverging beam in the box on the next page.

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## 6 Setting up the observer and the object:

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Before we set up any optical systems we will first create an artificial observer to mimic our own eyes. This observer will consist of the provided CMOS camera (representing the observer's retina) and a single plano-convex lens (representing the eye's lens). Note that this model will not be to scale. While the curvature of the lens of our eyes can change as the ciliary muscles contract or relax, allowing us to focus light from different distances onto the retina, here we will use only a fixed focal length lens. Take the  $f = 100$  mm mounted lens out of the box labeled 'HeNe Optics' and mount it in a lens holder that is connected to a post of appropriate length. Connect a base to a post holder, and slide the post into the post holder. Set up the camera and lens near the corner of the table. We will tweak this alignment in a moment. Start the live feed of the camera using the ThorCam software.

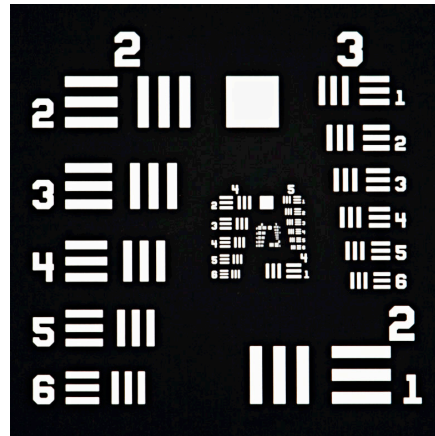
Our object will be illuminated by the bright white LED source you can find in the box labeled 'Module 2'. Connect it to its power supply and mount it to the opposite corner of the optics table, facing the "observer". The light from the LED diverges rapidly. Take another short focal length lens, mount it, and place it between the LED source and the observer such that the light from the LED is collimated. In the box below, describe how you oriented the lens. Also record the distances between the lens and the LED, and the lens and the observer and explain why/how you picked these distances.

Check that all four optical elements are arranged in a straight line (otherwise your camera won't be able to record any useful data!), and that you can see the light from the LED on your camera. **It is important that all of your optics share the same optical axis to ensure that the remainder of the lab goes as smoothly as possible.** Here are a few hints to help you: you can orient the base of your post-mounts 90 degrees to the optical axis and push the base all the way against the screw that mounts the base to the table. To ensure the height of all the lenses is the same, use the same height posts and post-holders, and mount everything such that the posts are as far in as possible (sitting at the bottom of the post holder). At this time, also confirm that all lenses are nicely centered, just as you learned in [MODULE 1 - BASIC \(Optics Basics - I\) or INTERMEDIATE](#), and also watch the [VIDEO](#) called [HOW TO ALIGN A LENS](#) if you need a refresher.

## 7 A simple magnifying glass:

We will begin with the simplest possible optical system: A single converging lens. Take the  $f = 50$  mm mounted lens out of the box labeled 'HeNe Optics' and mount it in a lens holder that is connected to a post of appropriate length. Connect a base to a post holder, and slide the post into the post holder.

You will use this lens to image the *Resolution Target* (you can find it in the blue box labeled 'Module 2'), which will act as our object (see figure 7.1).



**Figure 7.1:** 1951 USAF resolution test chart from Thorlabs [Wik16a]. Please note, the Thorlabs target only has groups from +2 to +7.

This target contains a standard pattern of black and white bars of known thickness that is often used to test the resolution of optical systems. Please familiarize yourself with how to interpret these pattern by reading over the wikipedia page [Wik16a]. Specifically, make sure you know how to look up the [line pairs / mm] for a given pattern from the table below (Figure 7.2).

Number of Line Pairs / mm in USAF Resolving Power Test Target 1951												
Element	Group Number										For High Res only	
	-2	-1	0	1	2	3	4	5	6	7	8	9
1	0.250	0.500	1.00	2.00	4.00	8.00	16.00	32.0	64.0	128.0	256.0	512.0
2	0.280	0.561	1.12	2.24	4.49	8.98	17.95	36.0	71.8	144.0	287.0	575.0
3	0.315	0.630	1.26	2.52	5.04	10.10	20.16	40.3	80.6	161.0	323.0	645.0
4	0.353	0.707	1.41	2.83	5.66	11.30	22.62	45.3	90.5	181.0	362.0	—
5	0.397	0.793	1.59	3.17	6.35	12.70	25.39	50.8	102.0	203.0	406.0	—
6	0.445	0.891	1.78	3.56	7.13	14.30	28.50	57.0	114.0	228.0	456.0	—

**Figure 7.2:** 1951 USAF resolution test chart table [Opt16].

In this first exercise we want to use a single lens as a magnifying glass. Discuss with your group members typical situations in which you'd use a magnifying glass and decide what typical distances between the eye and the magnifier and between the magnifier and the object are. Record a summary of your discussion in the box below. Also add a sketch in which you define the distances.

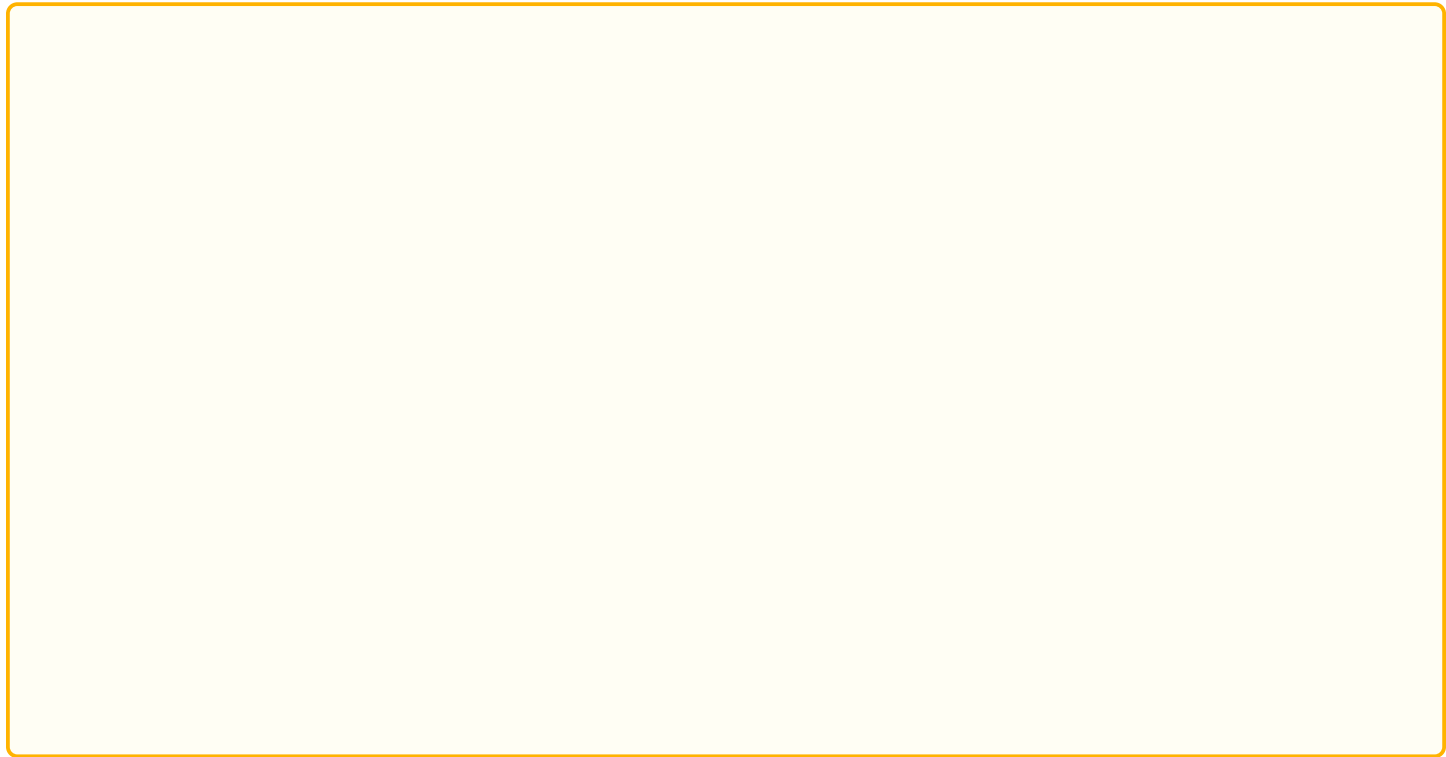
Then insert the object at your proposed distance. Add the magnifier lens between the object and the observer and adjust it until you get a clear, bright image of the Resolution Target on the camera. Again make sure that the lens is nicely centered and that it is on the same line as the other optical elements. Measure the final distances and record them, together with a sketch of your system, in the box below.

Capture a jpeg image and open it in *Fiji*. Then measure the length of a well-resolved line pair. We will want these measurements to be in units of real distance (not pixels). So before making your measurement, go to the **Measurement** pull-down menu, and click **Set Scale**. The vertical and horizontal lengths of a pixel for this camera are both  $5.20 \mu\text{m}$ , and the aspect ratio is defined as the ratio of these. Thus, you will want to fill in the following values: *Distance in pixels*: 1, *Known distance*: 5.20, *Pixel aspect ratio*: 1, and *Unit of length*:  $\mu\text{m}$ . Click the **Global** box so that the scale is set to all images.

Calculate the magnification of your sample by taking the ratio of the length as measured from your snapshot to the actual length of the line pair (using the table in Figure 7.2).

Let's fill in some more theory. Essentially, you just made use of the lens equation,  $\frac{1}{f} = \frac{1}{s} + \frac{1}{s'}$ . Remember that  $f$  is the focal length of your magnifier (50 mm here), and that  $s$  and  $s'$  are the distances between the object and the lens, and the lens and image, respectively. From your measured values of  $s$  and  $s'$  above, calculate  $f$ . Does it agree with the nominal 50 mm?

Are you surprised that the agreement is not good at all? If so, remember that you have another lens in your system: The observer's eye lens! Thus, you actually have a *two-lens system*. Remember that the image of the first lens acts as the object of the second lens. Using the lens equation twice, first find the location of the image of the first lens (assuming  $f_1 = 50 \text{ mm}$ ). Then find the distance between this new object and the observer's lens, and calculate the location of the final image on the camera (assuming  $f_2 = 100 \text{ mm}$ ). (A sketch might help!) Does this distance agree with your measured distance?



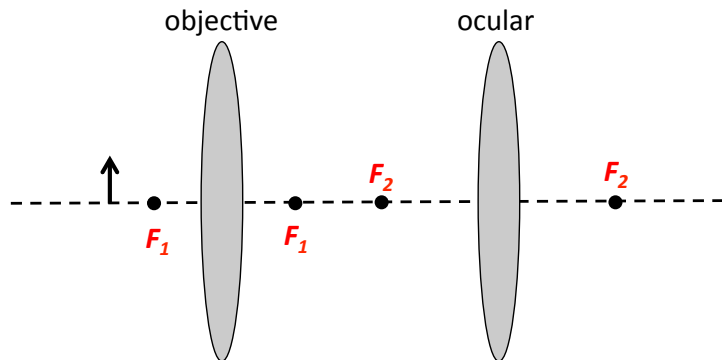
## 8 Setting up a microscope

We will now slightly modify our simple one-lens system and build a simple microscope that consists of two lenses with positive focal lengths, known as the *objective lens* and *eyepiece (or ocular)*. The objective lens has the shorter focal length of the two lenses.

In Figure ??, we see an example of such a compound microscope formed from two biconvex lenses (the term *compound* is used because, unlike a simple system like a magnifying glass, it contains multiple lenses). Here is the two-step process to explain how a magnified image is formed by this microscope:

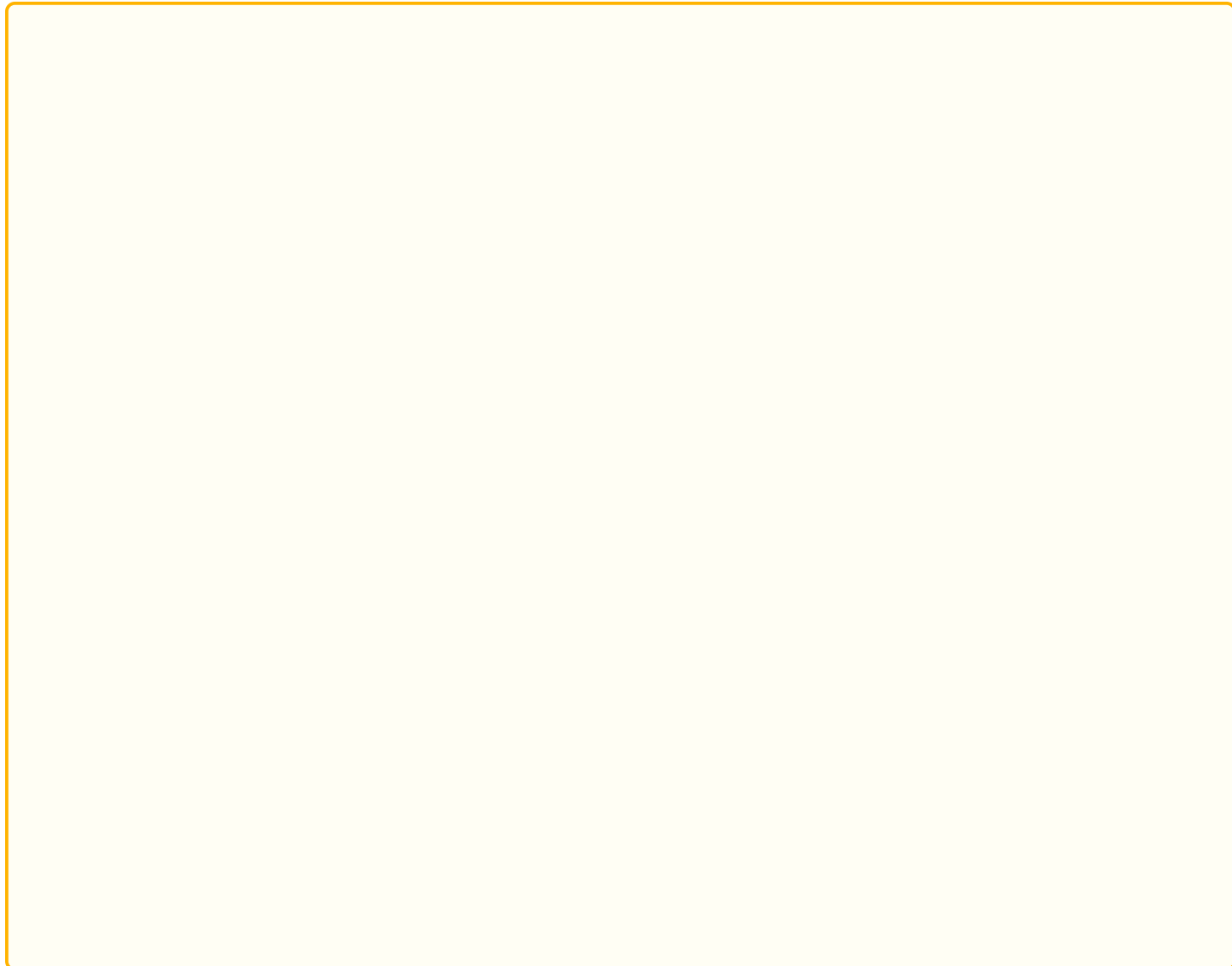
1. The object (the small arrow) is located at a distance from the objective lens that is greater than its focal length. Thus, a *real* image is formed between the two lenses, which we will call the *Objective Lens Image*.
2. The Objective Lens Image is the object for the eyepiece lens. However, this image is closer than one focal length to the eyepiece, and so the eyepiece forms a virtual image, which we will call the *Eyepiece Image*.

On the figure below, use ray diagrams to show how the microscope yields a virtual image. Indicate both the Objective Lens Image and Eyepiece Image in your drawing. The microscope forms a virtual image that is magnified, and the total magnification is calculated as the magnification from the objective multiplied by the magnification from the eyepiece.

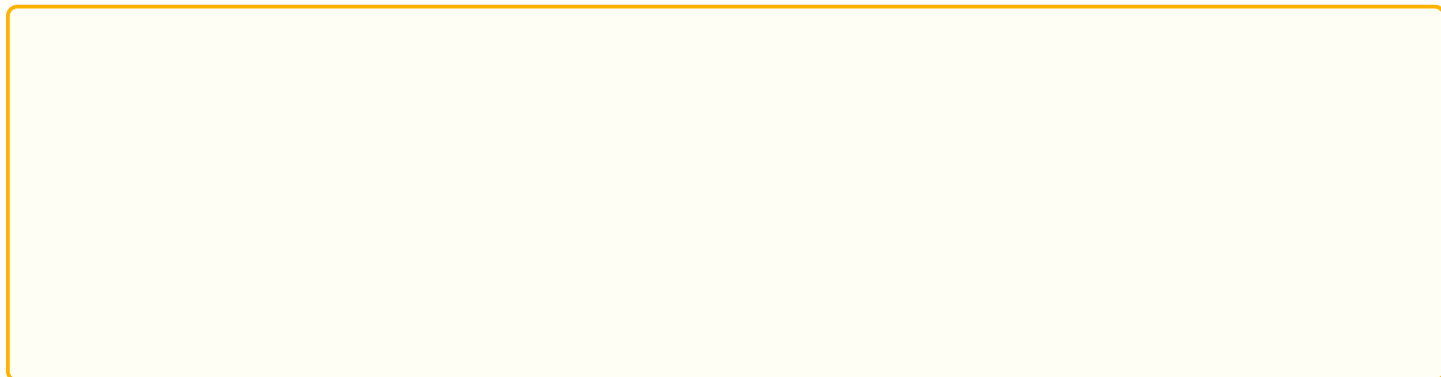


Take another lens out of the 'HeNe Optics' box, mount it, and build this simple microscope. Remember that this is now a three-lens system: Two lenses that form the microscope, and one lens that forms your eye. It will be very helpful to calculate

the expected distances *before* you start moving lenses around! After you have the expected distances, you can place the lenses roughly where they should be and then fine-tweak the alignment until you get a sharp image on your camera. Sketch the final setup below and indicate all distances in this sketch. You can also use this box for your calculations.

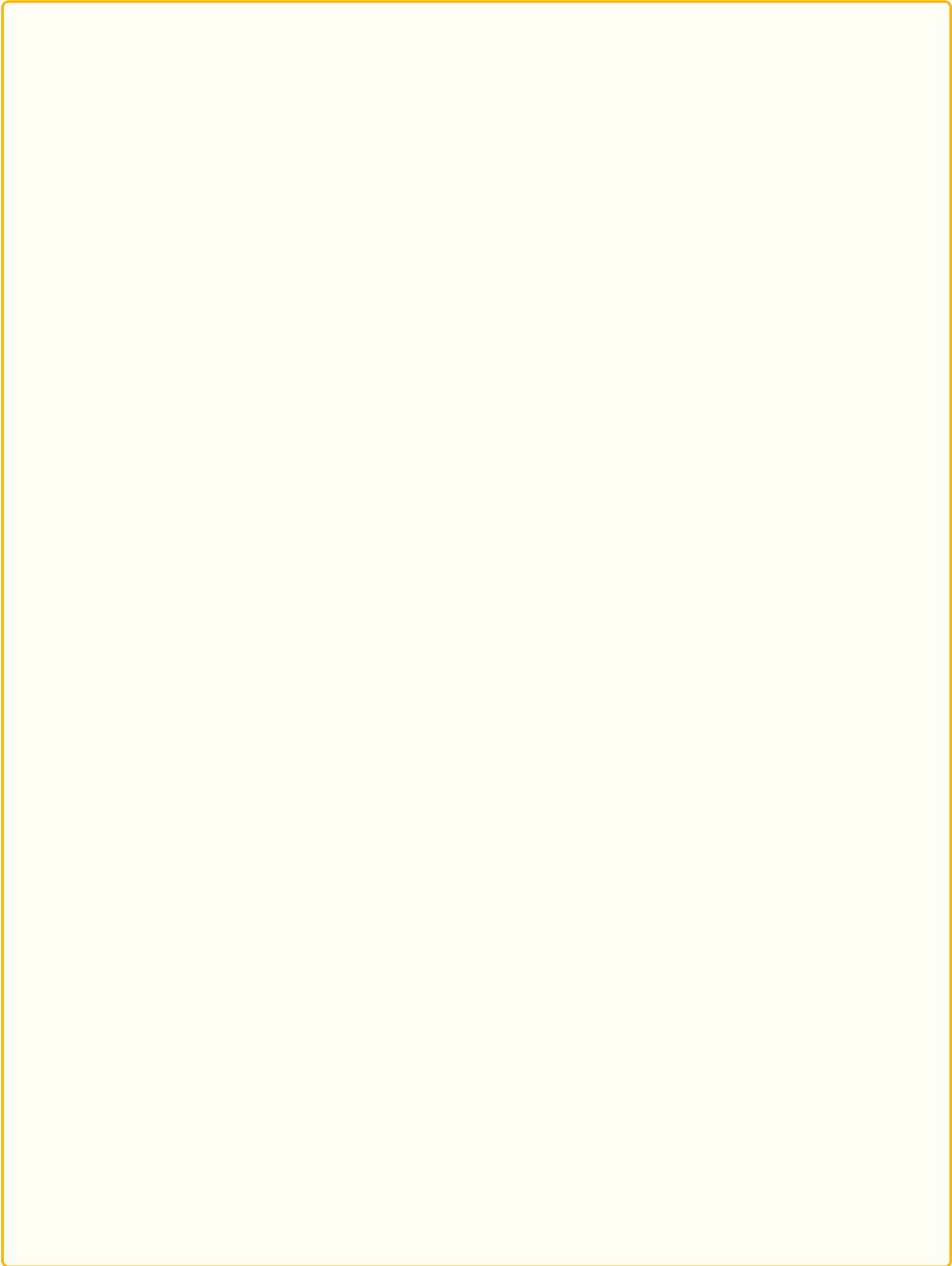


Similar to what you did previously, capture an image and use *Fiji* to measure the magnification of this optical system. Compare it to the magnification of the simple magnifying glass. Also calculate the expected magnification based on the focal lengths and distances you measured above and compare it to the measured one. Are they in agreement?



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And that's it! You made it successfully through the first module and are now qualified to perform the more advanced modules. Please leave us any comments, suggestions, or concerns in the box below, so that we can optimize this module for future student generations. Thanks!





## References

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- [Opt16] Edmund Optics, "<http://www.edmundoptics.com/testing-targets/test-targets/resolution-test-targets/1951-usaf-glass-slide-resolution-targets/1790/>", Feb 2016.
- [VH16] Al Van Helden, *The galileo project*, <http://galileo.rice.edu/sci/instruments/telescope.html#1>, February 2016.
- [Wik16a] Wikipedia, *1951 usaf resolution test chart*, "[https://en.wikipedia.org/wiki/1951\\_USAF\\_resolution\\_test\\_chart](https://en.wikipedia.org/wiki/1951_USAF_resolution_test_chart)", Feb 2016.
- [Wik16b] ———, *History of the telescope*, "[https://en.wikipedia.org/wiki/History\\_of\\_the\\_telescope](https://en.wikipedia.org/wiki/History_of_the_telescope)", Feb 2016.
- [WNHL15] Adam J.M. Wollman, Richard Nudd, Erik G. Hedlund, and Mark C. Leake, *From animaculum to single molecules: 300 years of the light microscope*, *Open Biol.* **5** (2015), 150019.