OPTIX Module 2 – Intermediate

Building sophisticated instrumentation with basic optical elements

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1 Objectives

In this module you will learn

- how to set up a simple brightfield microscope;
- how to establish Köhler illumination in your microscope;
- how to characterize the magnification and resolution of your 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 add additional sheets of paper containing data tables, sketches, or additional notes. Please note that this is *not* your lab report and that you are expected to complete a full lab report written in *LaTeX* after you have completed this module. Keep in mind that this should be a publication style report, which means that it should place a big emphasis on your data and data analysis, and not so much on all the nitty-gritty details of how to assemble the apparatus. Keep this in mind as you work through this module. Your instructor can provide you with more information and will send you a template file that you can use for your report.

2 Tests and assessment:

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` that are posted on our website (`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 class mates and your instructor.

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

For this module you will need the following equipment. You can find it in the box labeled "Module 2" that is located in one of the cabinets in the OPTIX lab. Standard equipment that is used for multiple modules will be located in the cabinets in the OPTIX lab. Please feel free to ask your instructor for help. Also, we have provided you with all the catalog numbers of the components because the Thorlabs and Edmund Optics websites provide more detailed specs about each item. Feel free to use these as a resource.

- 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)
- Objective lenses: Olympus PLN 4x (Edmund Optics, 86-812), Olympus PLN 10x (Edmund Optics, 86-813), Nikon 4x Achromatic Finite Conjugate (59-934), Nikon 10x Achromatic Finite Conjugate (59-935)
- Dual filter holder for holding samples (Thorlabs, FH2D)
- 0.5-inch translation stage (Thorlabs, MT1)

- Mounted standard iris, 25 mm max (Thorlabs, ID25)
- 1/2-inch translation stage with standard micrometer (Thorlabs, MT1)
- Adapter with external SM1 threads and internal RMS threads (Thorlabs, SM1A3)
- Various post-holders (Thorlabs, PH2), posts (Thorlabs, TR2), 1-inch lens mounts (Thorlabs, LMR1), and bases (BA1)
- Slip ring for SM1 lens tubes (Thorlabs, SM1RC)
- 1-inch 1951 USAF test target (Thorlabs, R1DS1P)
- CMOS camera (Thorlabs, DCC1545ML) with ThorCam software

4 Introduction

Microscopes have allowed for the extension of our senses to the far-reaches of the microcosmos. Lenses as we know them were introduced to the West at the end of the 13th century, largely 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 wok of Hans and Zacharias Jenssen [WNHL15].

You will be challenged in this lab to build, characterize, and understand optical microscopes of varying complexity. Our major goal is to move beyond a black-box view of these optical tools. Although many of us have familiarity with commercially built microscopes, by better understanding what is happening inside these systems, one can become better at utilizing these instruments more effectively.

To help you understand these complex lens systems, you will be using the `OpticalRayTracer program`. Please download this software from the website `http://arachnoid.com/OpticalRayTracer/#Downloads`. I would recommend downloading the *Platform-portable Java executable file*. One of the powerful aspects of this program is that it goes beyond the thin lens approximation, and takes into account the non-ideal aspects of lenses that result from the fact that real lenses have thickness. These non-idealities are important to understand when you create and utilize complex multi-lens systems like microscopes.

You will also be analyzing images using `Fiji` image processing software. You may want to download and install the software (`http://fiji.sc/Fiji`), though it will also be on the lab computer.

5 Introduction to plano-convex lenses

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 for the purpose of turning a point source into collimated light or focusing collimated light to a point. These lenses are more effective than bi-convex lenses, which you may have learned about when you were first introduced to lenses. Bi-convex lenses consist of two convex spherical surfaces, typically of the same radius of curvature (but this doesn't have to be the case; it just makes manufacturing them a bit easier). When using the thin lens approximation, these lenses are expected to bring collimated light incident upon them to a single point one focal length away from the lens. In practice, however, the thickness of these lenses results in an effect known as *spherical aberration*, in which not all of the rays come to focus at the same point. Specifically, the rays near the center of the lens do not come to focus at the same point as rays near the edge of the lens (see Figure 5.1).

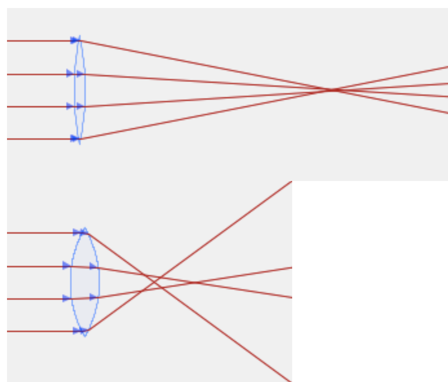


Figure 5.1: *Bi-convex lenses result in spherical aberration, which is more severe in the case thicker lenses with shorter focal lengths.*

A bi-convex lens that has a long focal length is created from surfaces that have a very large radius of curvature. As a result, these lenses can be made quite thin. Conversely, lenses that have a short focal length 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 4-times that of the thicker lens. As you can see, the spherical aberration is much worse for the thicker lens.

Plano-convex lenses must be oriented appropriately in order to reduce the effects of spherical aberration. Determine the correct orientation using the program **`OpticalRayTracer`**. You will want to look at a single lens bringing the rays of light to focus. Play around with the different parameters, focusing especially on the **`Sphere Radius`** for the left and right sides of your lens, which can be adjusted in the **`Design`** tab. You may also want to increase the number of rays you are looking at, which you can adjust in the **`Configure`** tab. You should observe that the incorrect orientation of the lens actually makes the spherical aberration worse. Sketch below the correct orientation of a plano-convex lens that is being used to focus a collimated beam, and include a printout of the images that allowed you to determine this.

6 Setting up a microscope

We will now build a simple microscopes. A related optical system, the telescope, has many similarities with microscopes, but the two major differences are that the sample imaged by a microscope is much smaller, and that it is brought much closer to the optical system. However, both systems are used to magnify the object and improve our resolution of details in the imaged object. If you'd like to learn more about telescopes, we encourage you to complete `MODULE 4 - INTERMEDIATE`.

A major difference between a telescope and a microscopes is the role of illumination in a microscope. When imaging a star, the telescope acts like a "light bucket" that collects light from the imaged object. We have no control over the amount of light that is emitted, though we can always make our bucket bigger with a larger diameter objective lens (up to the current technological limit). For a 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.

6.1 Microscope version 0

Although we will not be building the microscope described in this first subsection, it is worth discussing how it works. This system 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 6.1, we see an example of 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 Figure 6.1, use the 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.

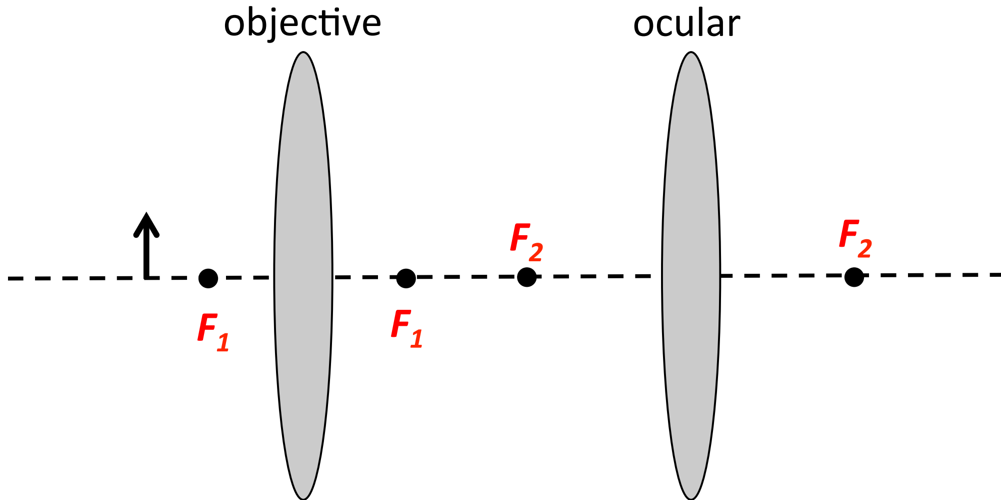


Figure 6.1: *An example of a simple compound microscope made of two biconvex lenses. The focal lengths of the objective and eyepiece lenses are F_1 and F_2, respectively, and the object to be imaged is represented by an arrow.*

6.2 Microscope version 1: The objective lens

The general principles described in the previous section pertain to the microscope you will be building in this section. However, the objective lenses you will be using (and the lenses used in most microscopes) are typically multi-lens systems. For example, Figure 6.2 shows an objective made by Nikon.

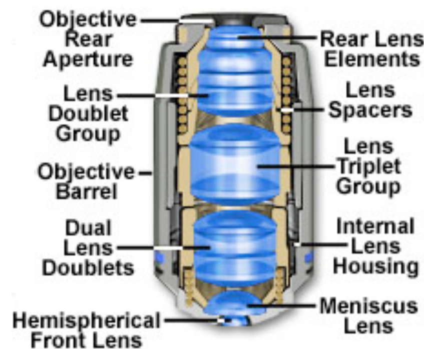


Figure 6.2: *An example of a complex, multi-lens objective [U16].*

We will begin our exploration of microscope objective lenses by considering *finite conjugate objectives*. The meaning of "finite conjugate" will become clear over the next few sections. Though these lenses also serve the purpose of forming a magnified, real image, they have been made more complex with the addition of multiple optical components so as to "feature excellent optical characteristics under a wide spectrum of illumination conditions and provide various degrees of correction for the primary optical aberrations" [Zei16b].

You will be using a camera to view images formed by the optical systems you will build in the next few sections. As we learned in the last section, the objective lens of a compound microscope forms a magnified real image. We opt to leave out the eyepiece lens, and directly image the Objective Lens Image using the camera. It is worth noting that, when cameras are incorporated into a microscope (as they often are), this approach of imaging the Objective Lens Image is frequently used.

Let's put together a simple microscope using the 10x magnification Nikon objective. Attach the Nikon objective to the LMR1 lens mount using the "Objective to SM1 adapter." *NOTE*: the mounted objective lens can fall over easily. Please rotate the base so that it lines up with the length of the objective to increase the stability of this element.

You will use this objective to image the *Resolution Target* (which you can find in the blue box labeled 'Module 2'). To illuminate this sample, you will use the bright LED light source. To create an even field of illumination, first collimate the white light source with one of the plano-convex lenses. Arrange the five optical elements (the light source, a collimating lens, the Resolution Target, the objective lens, and the camera) in a straight line on the optics table. The front of the objective,

which goes closest to the resolution target, is the side furthest from the mount. **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.** In other words, you would like the light to pass through the center of and be perpendicular to each lens. **Putting in extra effort now will save you time later.** 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).

Play around with the distances between each element until you have a clear, bright image of the Resolution Target on the camera. In the box below, sketch your system, indicating the distances between each element. Also, take a picture of your system and send it to your instructor.

Capture a jpg-image of the pattern. To interpret this image, we need to know a little bit more about the target you are imaging. This is a standard pattern with black and white bars of known thickness that is often used to test the resolution of optical systems (Figure 6.3). Please familiarize yourself with how to use read this pattern by reading over the wikipedia page [Wik16]. Specifically, make sure you know how to look up the [line pairs / mm] for a given pattern from the table below (Figure 6.4).

Figure 6.3: *1951 USAF resolution test chart from Thorlabs [Wik16]. Please note, the Thorlabs target only has groups from +2 to +7.*

In *Fiji*, 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 μm, 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*: um. 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

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 6.4: *1951 USAF resolution test chart table [Opt16].*

length of the line pair (using the table in Figure 6.4). Write down your work in the box that follows. How does the magnification compare to the reported 10x magnification of the objective?

Unless you were very lucky, you probably measured a magnification that was different from 10x. The reason for this is that, as with a simple biconvex lens, you can place your object at various distances from the objective lens, and as long as you move the camera to the appropriate location on the other side of the lens, you will collect a focused image of your object. However, as you change the distance between your sample and objective, you will also change the magnification of your image. In other words: To get the reported magnification, you must use an objective lens in a particular way.

In Figure 6.5, we see the markings on a nice objective lens. Two important markings on the objective above are the *magnification* (60x) and the *working distance* (0.21). The working distance is the distance between the objective front lens (the lens nearest to the sample) that will lead to the magnification that is marked on the objective. It is in units of millimeters. To gain better control of the position of the sample relative to the objective, we will use a 1/2-inch translation stage. Attach the stage to the optics table so that the micrometer moves the stage along the direction of the optical axis of your system. Then, attach the mounted sample to the translation stage using a post-assembly.

Now, carefully adjust the distance between the sample and the objective lens to match the working distance written on the objective (this is the number next to the letters WD). Then, adjust the position of the camera to bring the image into focus. Measure the magnification of your microscope; include your work and results in the box below. Is your magnification closer to what you expected?

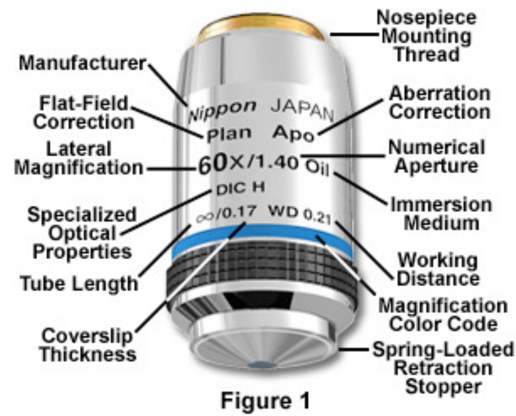


Figure 6.5: *Markings on an objective help the user understand the optical properties and capabilities of their objective [Exp16].*

Measure the distance between the camera and the back of the objective lens, and record it below.

Now replace the 10x objective with the 4x objective, being careful to maintain the same distance between the objective mount and the camera. Adjust the position of the sample using the translation stage until the image comes into focus on the camera. Measure the working distance and determine the magnification of the image. Include your results below. How do your results compare to what is printed on the side of the objective?

What you have hopefully observed is that finite conjugate objective lenses are designed to create images at a fixed distance from the objective, even for objectives with different magnifications. This is desirable, because it means you can change objectives without having to realign your imaging optics. However, it is worth noting that objectives from different companies may have different fixed distances between the back of the objective and the Objective Lens Image, and so different companies' objectives are not typically interchangeable.

6.3 Microscope version 2: Infinity-corrected optics

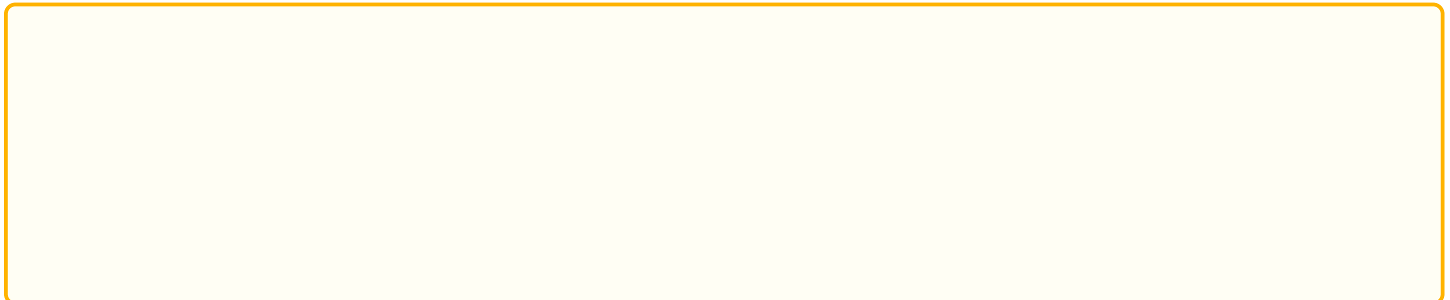
It is often useful to put optical elements between the objective and the camera to manipulate the light collected by the objective. However, the fixed distance between the objective and image limits what elements can be introduced into the microscope. In the last few decades, *infinity-corrected* objectives have become much more commonplace. Unlike finite conjugate objectives, which produce an image at a finite distance from the objective, infinity-corrected objectives produce an image at infinity. In other words, when the distance between the sample and objective is equal to the working distance, the light from the sample that exits the objective is collimated.

As a result, a second lens is required to focus the collimated light exiting the objective. This is referred to as the *tube lens*. Together, the infinity-corrected objective and tube lens form a compound lens system that creates a real image, just like our finite conjugate objective. However, because the light between the two lenses is parallel, the distance between the objective and tube lens is not fixed. This means that there is more space to add complex optical components, and these elements can be more easily added without modification of the objective working distance.

We will modify your setup by incorporating a 10x infinity-corrected objective and a 175-mm focal length plano-convex lens for the tube lens. The working distance, which is not written on this objective, is 10.6 mm. It is important to note that, as with the finite conjugate objectives, you must use this objective correctly to get the reported magnification. In particular, an infinity-corrected objective must be used with a tube lens with the correct focal length. For Olympus objectives, this focal length is 180 mm. Thus, because we only have a 175-mm focal length lens, the magnification will not exactly match the reported 10x.

In the box below, sketch your modified system, indicating the distances between each element. Also, please take a picture of your system, and include a picture of the setup on the next page.

Set up your system, and collect an image of the Resolution Target. Using this image, calculate the magnification of your microscope, and write your results below. How does it compare to the 10x magnification reported for the objective?



Although it would appear that the space between the objective and the tube lens (also known as the "infinity space") can be made arbitrarily long, there are limitations for how long you will want to make this distance. To see this, collect an image of the Resolution Target for which you have made the infinity space very short (less than 50 mm), and compare this to an image of the Resolution Target for a very long (more than 300 mm) distance between the lenses. Include these images on the next pages. In the box below, explain what is different about the images.

As you increase the length of the infinity space, you begin to lose rays at more extreme angles relative to the optical axis. This results in vignetting of your image, in which your image appears brighter in the middle and darker toward the edges. Before continuing on, return to a configuration in which the length of the infinity space is not causing a large amount of vignetting.

6.4 Microscope Resolution

Resolution describes the ability of the microscope to distinguish nearby objects or to distinguish small details of an object. There are two factors we can consider when talking about the resolution of your telescope. The first is magnification. When two nearby objects are imaged, but the collected light falls on the same pixel, they obviously cannot be distinguished from one another. However, if the image is magnified such that the light collected from each falls on different pixels, it may now be possible to resolve them. Thus, resolution can be improved by increasing magnification of the image (or alternatively by decreasing the size of the camera's pixels).

However, there is a limit to how much you can improve your resolution through magnification. Diffraction of light which occurs at both the sample and the back aperture of the objective limits the resolution of the image [Zei16a]. Though we will not be deriving this effect, in the 19th century, Ernst Abbe determined that the resolution limit in either the x- or y-axis (the axes perpendicular to the optical axis of the microscope) is given by the relation

$$d_o = \frac{\lambda}{2nsin(\alpha)} \tag{6.1}$$

where λ is the wavelength of the light, n is the refractive index of the medium in front of the objective, and α is the objective aperture angle (defined in Figure 6.6), which is the limit of the angle of light that can enter the objective.

In the denominator is a term denoted the numerical aperture, which is defined as

$$NA = nsin(\alpha) \tag{6.2}$$

Understanding the numerical aperture of your microscope is important because it allows you to understand the resolution of your instrument. Let's think about how we can improve the numerical aperture, and thus the resolution, of a microscope.

In general, will increasing the magnification of your microscope increase its resolution? To help you answer the question, sketch in the box on the next page lenses representing your 4x and a 10x objectives, each of which is at the working distance from the sample. The working distances of of the 10x and 4x lenses are 10.6 and 18.5 mm, respectively. By *carefully* measuring the diameter of the front lenses of the objectives (without actually touching the lenses), determine the angle α for each lens.

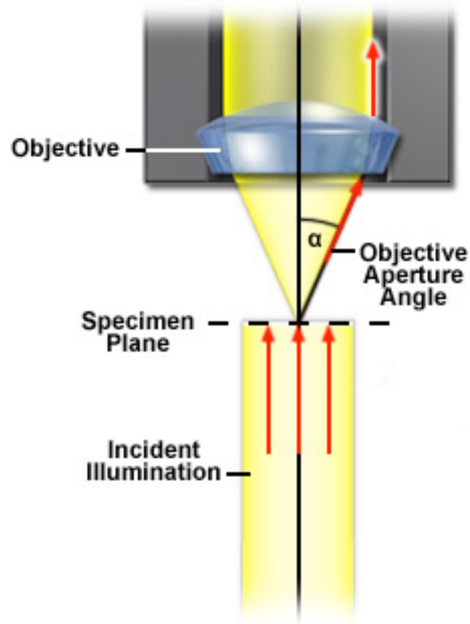
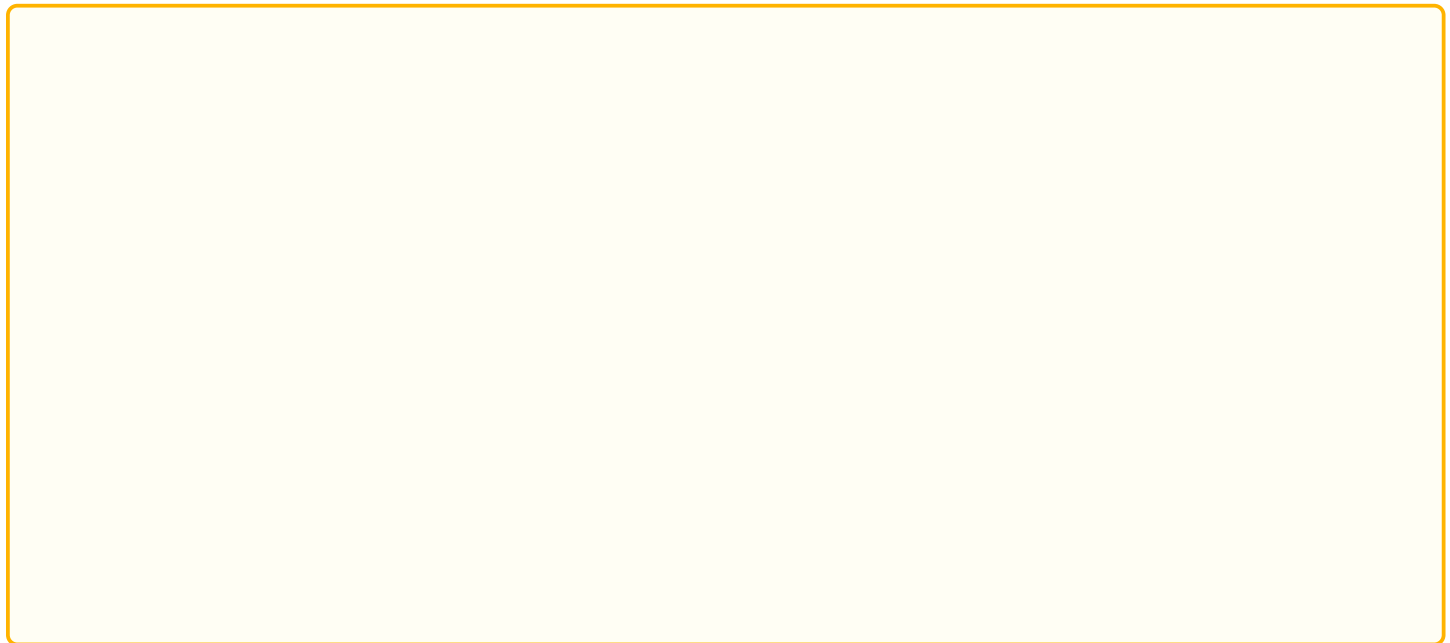
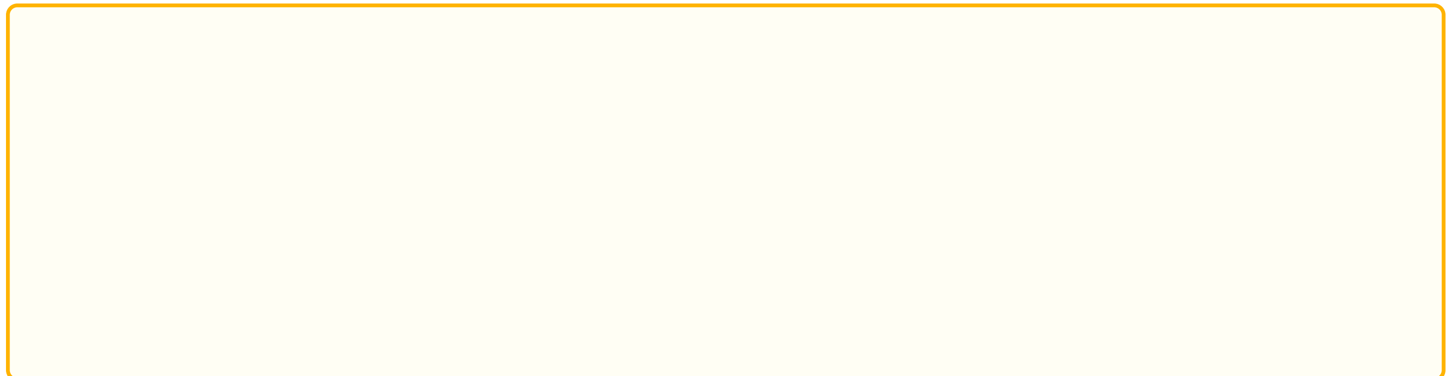


Figure 6.6: *Definition of the objective aperture angle for a microscope objective [Zei16a].*



Assuming that the space between the sample and the lens is simply filled with air ($n = 1.00$), what is the NA for each objective? Does this value match the NA reported on the side of each objective?



We see that, because of their shorter working distance of the higher magnification lens, your 10x objective has a higher numerical resolution and thus a smaller resolution limit. Using your resolution target, determine the resolution of your microscope with the 10x infinity-corrected objective and write it below. Include an image of the camera snapshot that allowed you to determine your microscope resolution.

Is your microscope near its theoretical diffraction limited resolution?

6.5 Microscope version 3: Köhler illumination

We will now focus on improving your image by better controlling the sample illumination. We would like to have illumination that is uniform and smooth. By smooth, we mean that any structure in our image should come from the sample, and not from the illumination itself. We would also like to be able to control the intensity and the area of illumination. All of this will be achieved through implementation of Köhler illumination.

6.5.1 Control over the area of illumination

We have achieved smooth and even illumination of the sample by collimating the light coming from the LED. Although the condenser lens alone can be used to create this uniform illumination, we will introduce another lens in order to improve our control over certain aspects of the illumination. This lens, called the *collector*, is put in front of the LED and brings the illumination to a focus. The condenser lens is then used to collimate the light following this focal point (Figure 6.7).

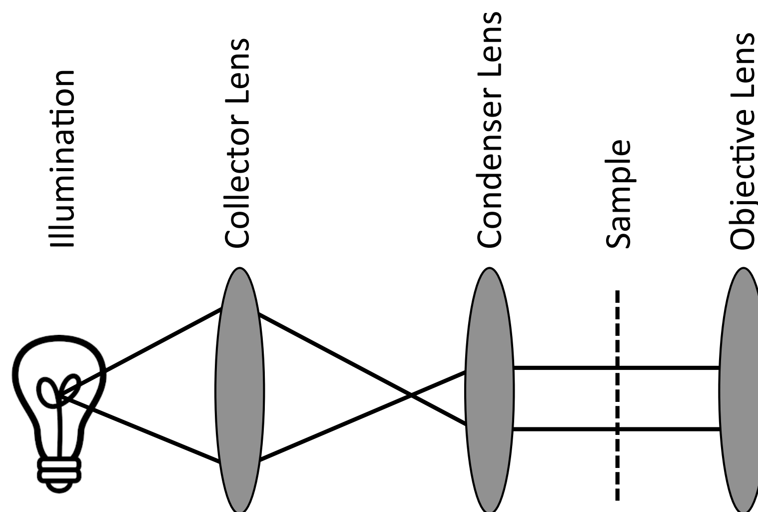
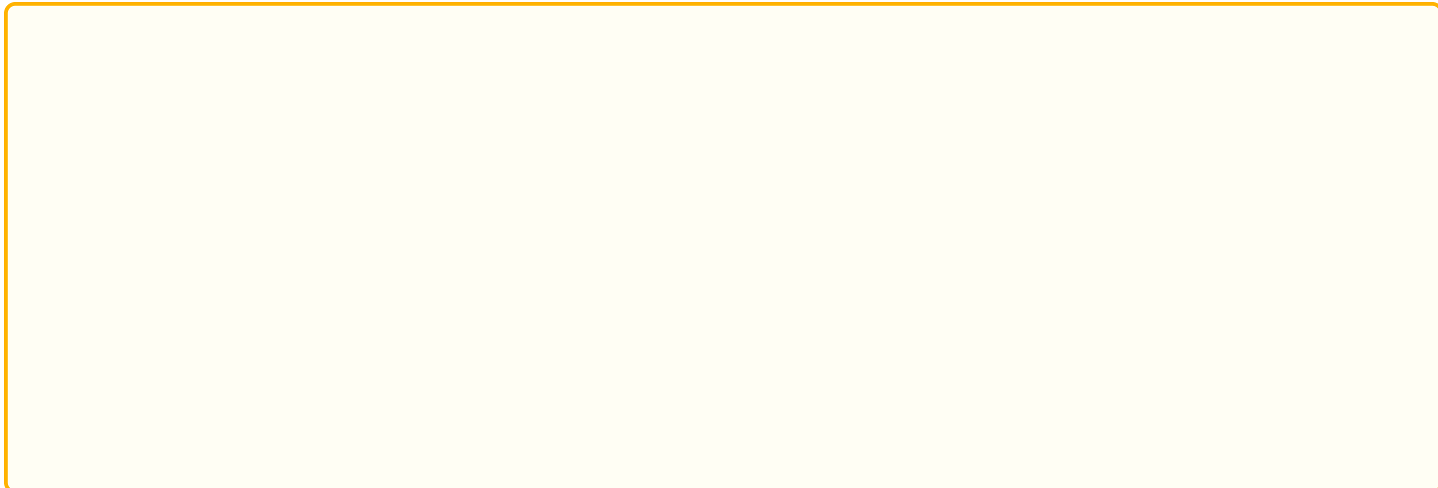


Figure 6.7: *Using a collector and condenser lens to create uniform illumination at the sample.*

Choose a second lens that will serve as your collector lens, and arrange your collector and condenser lenses so that they create uniform illumination at the sample plane. Return your sample (the Resolution Target) to its post-holder, and make sure that

the illumination region is large enough to image the entire sample. If it is not, adjust your lenses to increase the size of your illumination region. In the box below, draw a sketch of your setup, including the focal lengths of your lenses and the distances between all optical elements. Take a picture of your setup, and include it on the next page.



We will now explore how the use of both the collector and condenser lenses gives us more control over the sample illumination. To understand this, we need to first understand a new concept: *conjugate planes*. These are planes in the optical path which can simultaneously be brought into focus. You already identified two conjugate planes earlier in our discussion of microscopes when you imaged the sample plane at the camera. A ray diagram describing the relationship between the sample plane and the imaging plane of the camera is shown below (in this, and many of the figures that follow, a finite-conjugate objective is shown; an infinity corrected objective requires the addition of a tube lens):

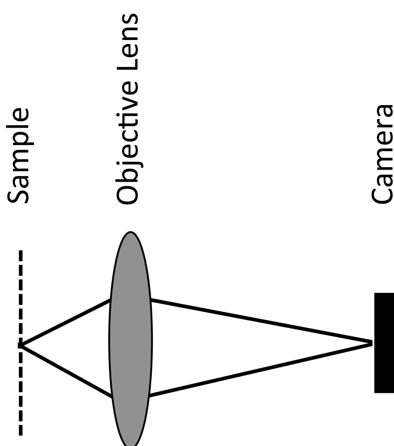


Figure 6.8: *A ray diagram allows us to see the relationship between the sample plane and the imaging plane of the camera.*

Note: these rays *do not* describe the path traversed by light from the illumination source. As we have already discussed, rays from the LED are collimated at the sample (see Figure 6.7). Nonetheless, what Figure 6.8 reveals is that features in focus at the sample plane are also focused at the camera, allowing it to collect an image of the sample. We thus call these two conjugate planes the *imaging-planes* (also known as the *field set of conjugate planes*).

There is an additional plane in your system that is conjugate to both of these planes. To find this plane, consider the image of the sample plane made by the condenser lens. In other words, you can identify this plane by pretending that the light propagates in the opposite direction. Find the image of the sample made by the condenser for this backward propagating light. In the box below, sketch the illumination source, collector lens, condenser lens, sample plane, finite-conjugate objective, and camera. Draw the rays that allow us to identify the location of all three conjugate imaging-planes. (Note: The box is on the next page.)

*

In the box below, derive a formula for the location of the newly identified imaging-plane in terms of the focal length of the condenser lens and the distance from this lens to the sample plane. Then, calculate the location of this plane in your microscope.

It is important for this third conjugate plane to be in the space between the collector and condenser lenses (the reason for this will become clear later). Did the position of the plane that you calculated above satisfy this criterion? If it did, continue on. If not, reposition your illumination and collector and condenser lenses so that (1) they are still creating collimated illumination at the sample and (2) the conjugate plane discussed above is between the two lenses. Once you have completed this task, continue on.

Place an iris on a post, and place the post in a post-holder assembly. Make sure you can adjust the height of the iris so that it is at the same height as the other optical elements in your setup.

Now, place the iris between the collector and condenser lenses. Reduce the size of the iris until it is almost closed. Adjust its position along the optical axis until you can see an image of the iris aperture on top of your sample. Center the aperture on your sample, and fix the post-holder securely to the table. Measure the distance between the condenser lens and this iris, and write the value below. How does this value compare to the location of the field set of conjugate planes?

Hopefully you found that the iris is in a plane that is conjugate with the sample and the camera. You now have an optical element that will let you control the field of illumination of your sample by opening or closing the iris. Because the three imaging-planes are conjugate to one another, both the iris and sample are imaged at the camera. The iris that is located in an imaging-plane and that controls the area of illumination is called the *field diaphragm*.

To achieve the optimum reduction of stray light on the final image, the iris should be closed to only just cover the edges of the

image of the sample on the sensor. In other words, you will want to close the iris until you *just* see it at the edge of your camera image.

6.5.2 Control over the intensity of illumination

Place the second iris on a post, and place this in a post-holder assembly. Adjust the height of the iris so that it is at the same height as the other optical elements, and place it between the condenser and collector lenses. Now, move the iris along the optical axis, and at a variety of positions, open and close the iris. Find a position where opening and closing the iris only adjusts the intensity of the light, and does not affect the image of the sample itself. To do this, you will need to turn off the auto brightness adjust in the settings menu of the ThorCam software. Fix the post-holder securely to the table. Measure the distance between the condenser lens and this iris, and write the value below.

Because the iris is not being imaged, it is not aligned with one of the microscopes imaging-planes. In fact, the iris has been placed in another important set of planes within the microscope. To understand these planes, we have to consider the path that the illumination takes in the microscope. As discussed above, the illumination light is collimated at the sample plane. However, there are planes along the optical path where the illumination light is focused. These are called the illumination-planes (also known as the *aperture set of conjugate planes*).

Let's identify these planes in your microscope. In the box below, sketch the illumination, collector lens, condenser lens, sample plane, finite-conjugate objective, and camera. Consider rays that are collimated at the sample plane. Draw rays going in both directions from the sample plane, and find the locations of three planes that comprise the illumination-planes. Note - one of these should be the location of the illumination source itself. Determine the locations of the other two planes, and describe these locations below.

*

How does the location you determined for the illumination-plane between the collector and condenser lenses compare to the location of your iris?

The iris that is located in an illumination-plane and that controls the intensity of illumination is called the *aperture diaphragm*.

Congratulations - you have built a microscope with Köhler illumination!

Based on your work in this section of the lab, list a number of factors you would take into consideration when designing a microscope. Create a numbered list, and for each factor, explain in detail how it would influence your design of the telescope.

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!

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