Phys39

Geometrical optics, Fourier optics, and the Optics of the Microscope

Goals and brief summary:

There are two general goals for this set of experiments. First is to understand how lenses work in the geometrical optics limit, that is, using ray tracing to represent the propagation of light, which is effectively in the limit of infinitely small wavelength of the light. This is the basis for designing and constructing any optical system. Second is to understand wave optics, or Fourier optics, and how the wave structure of light coming from an object is composed of components propagating in different directions, so-called spatial frequency components, which must be "reassembled" into an image of the object. This reassembly of an image is necessarily imperfect, giving limits on image quality and information content. The appreciation of this process is essential for understanding high resolution imaging in microscopy. Based on this introduction you will develop a basic understanding of the compound microscope.

Laser Safety:

Start by reading the **Brandeis manual on laser safety**, and the **memo for use of low power visible lasers**. You MUST do this before starting the experiment. This laser beam is about 1 to 2 milliwatts in intensity. It is less intense than a common laser pointer, just at the threshold of intensity that can cause eye damage, but not skin damage. NEVER look into a laser beam, or allow a beam to be reflected into your eyes or the eyes of someone else in the lab. Before working with any optics in the laser beam, THINK about what you are doing and where the beam might be aimed by what you are doing.

Basics of Handling Optics:

NEVER touch the optical surface of any optical component, lens, mirror, etc. Only handle things by their outer edges or by their mountings. NEVER lay lenses or mirrors on the bench with the optical surface touching the bench. DO NOT leave optical components lying horizontally and uncovered for a long time; they gather dust which can be hard to remove. If you see dirt or a finger print on an optical component, DO NOT try to rub it off with a tissue. Ask for help from the instructor on how to clean things. DO NOT wash optical components with solvents like alcohol or acetone; you may severely damage them.

Preparatory Reading:

Required: Read the attached notes: "RobPhilips_Caltech_notes.pdf."

Starting with refraction and Snell's law, follow the derivation of the "thin lens" law for imaging. Pay attention to the ways of using ray diagrams to understand imaging, and the use of the sign conventions for "real" and "virtual" images. This is the basis of all Geometric Optics and imaging. Pay particular attention to the discussion of lens aberration and the design of achromatic lenses.

If you want more, review the optics book "**Hecht. Optics. 5th ed**", which is at the level of an upper division undergrad physics student. Go through the chapter 10 on diffraction, relating the spacing of stripes in a grating, and the wavelength of the light, to the angles for the different orders of diffracted rays. This is the basis of all that we will study in wave optics, or physical optics, for our understanding of "diffraction limited" imaging. **Read**

Equipment:

2 x 3 foot optical breadboard 3 foot strip of minirail He-Ne laser in mounts Gradient ND filter to adjust laser intensity Red and green LEDs (low power) with power supply CMOS camera attached to the computer by a USB cable Software: ImageJ, Mathematica, Matlab, ToupView for acquisition 1000/in grid, mounted Mounted lenses: steinheil, 2 each of PCX50, ACH50, DCX25, and ACH100 Microscope objective in mount (20X or 40X) Screen ND filters: 1.0, 2.0 O.D. Mirror in two axis tilt mount Post holders on track carriers Translation stages, x and x-y 2 iris diaphrams 1 iris + DCX25 mounted together Dovetail track carriers

Introduction: These experiments are intended to teach you about the use and the limitations of lenses for imaging. We start with some simple imaging experiments to test the basic "thin lens" imaging equation. This forms the basis of optical system design. Throughout the following experiments we will return to this basic design law many times. Then we will turn to the quantitative evaluation of images formed by general purpose lenses of several types. We will explore the limitations of lenses in forming images. There are two main limitations.

First, the wavelength of light is finite. As you will see, an object diffracts light through a range of angles, relative to its incident direction on the sample. Fine features diffract light rays through large angles, which do not pass through the lens. The information carried by these lost rays means that fine features of the object are lost from the image, limiting the resolution of the image.

Second, due to the materials and design of common general purpose lenses, they have aberrations which prevent some of the light originating from the object and passing through the lens from focusing properly in the image. The out of focus light blurs the image, reducing contrast. Certain focusing errors can be avoided by careful lens alignment, but two aberrations remain, chromatic aberration and spherical aberration. Chromatic aberration arises from the variation of the index of refraction of glass with wavelength, causing different wavelengths to focus at different locations. Spherical aberration arises from the spherical shape of the lens surfaces, combined with the nonlinear form of Snell's law of refraction.

Both spherical and chromatic aberration can be limited by combining simple lenses in pairs or even triplets, called achromats, made of glasses with different indices of refraction. See the accompanying CalTech notes "RobPhilips_Caltech_notes.pdf." on these aberrations and the design of achromatic lenses. For any very specific imaging task, custom designed lenses can be constructed to essentially eliminate aberrations, leaving only the limitations due to diffraction, and the finite wavelength of light. Microscope objectives are the prime example of such lenses. Such highly engineered lenses tend to be quite expensive.

Our experiments start with making images of a light source, a light emitting diode (LED), with a singlet lens and an achromat, to become familiar with the basics of imaging, as dictated by the thin lens imaging equation.

We will see qualitative evidence of the limitations of simple lenses, and how they are improved on by achromats. We then move on to experiments to make a series of quantitative comparisons of different lens types. They focus on analysis of imaging directly, rather than more indirect tests of lens quality. In addition, the experiments develop the design and use of the microscope for high resolution imaging. We will see how proper illumination of the sample is necessary for achieving the potential resolution and image quality that can be produced by a carefully designed microscope objective. The sample studied is an extremely thin metal foil with a grid of square holes, with periods of 2000 lines per inch in perpendicular directions. It is characterized by sharp edges separating transparent from completely opaque areas. The test of the image is to see how well this property of the sample is reproduced, in sharpness and contrast.

In all that follows, a central concept is the "numerical aperture" of a lens. This is a representation of the maximum cone angle of light that the lens can focus to a point. It is defined as the index of refraction of the medium in which the lens is operating times the Sine of the maximum cone angle. That cone angle is the one whose Tangent is the radius of the lens divided by its focal length. Note that in cameras, one uses the "f" number, which is the focal length of the lens divided by its diameter. High numerical aperture, or low f number, gives high resolution, while low numerical aperture, or high f number, gives low resolution, as you will see in experiments below.



Experiments:

- For all these experiments keep a detailed lab notebook of your results.
- You can keep images and other data in a directory you set up on the computer, under My documents.
- To run the camera use the standalone ToupView / ToupLite program. The software will display live video or snap a image that you can then save as a file in your data directory. Needless to say, you will have to keep records of what image name has what meaning for your experiments.
- For image analysis, use ImageJ. You can keep this open and rapidly switch from taking images to analysis. Explore the menus of ImageJ they are a bit overwhelming at first. For example, to plot an intensity profile along a line on an image, Open the image, select the line in the menu strip of ImageJ, with the cursor draw your line, open the Analysis menu, and select Plot Profile. ImageJ is a very powerful program with a huge variety of things you can do with images. It is worth learning parts as needed. It is an open source, free program (download from http://rsb.info.nih.gov/ij/) that you can install on your own computer to analyze your saved images later.

Do steps 1 – 10.

- 1) Set up the laser beam as an **optical axis** reference at a height of exactly 12 cm above the top of the minirail. All the optics of this system should be exactly centered on the optical axis. This height is most useful for the variety of components we use. Place the mirror at the end of the rail, at an angle to direct the laser beam down the center of the rail. Use an adjustable aperture, closed to its smallest opening, in a mount on a dovetail carrier. Center the aperture opening over the center of the minirail, by alternatively adjusting the lateral position of the laser beam and the aperture opening, until both are centered, testing this by rotating the whole carrier holding the aperture 180 degrees (remove from the track, rotate, and remount), until the opening is centered on the laser beam for both orientations. Slide the aperture down the rail, confirming that the laser beam passes through the opening at all positions; use the fine screw adjustments on the mirror to steer the beam. See last page for layout.
- 2) Thin lens equation: Verify image, object relationships.

It is critical for image clarity that all optical components are aligned on a single **optical axis**. Set a height on the rail that works for all the components in your instrument. For example, set the center of the lenses and LED at a height of 12 cm above the top of the minirail. All the optics of this system should be exactly centered on the optical axis. This height is most useful for the variety of components we use. Use an adjustable aperture, closed to its smallest opening, in a mount on a dovetail carrier. Center the aperture opening over the center of the minirail, by alternatively adjusting the heights of the LED, lens and the aperture opening, until all are centered. Slide the aperture down the rail, confirming that the LED remains centered.

Mount the lenses you need for the following experiments in post holders. The lenses should appear to be oriented exactly perpendicular to the optical minirail, as you look

down on it. You can also look at where the lens directs the transmitted beam, and keep it centered on the optical axis, which you have marked on a screen further down the rail. Using a simple post-holder mount, we can only adjust the height of the lens and rotate it about a vertical axis. For a well-made lens and holder, this is usually enough, since it should be centered in the holder and oriented vertically. If it is far off laterally, one can use a dovetail carrier to allow for lateral adjustment of the lens; this is not necessary for very small deviations.

For our first experiments, mount the LED near the end of the track. Make a screen from a piece of 8.5" x 10" piece of paper. Place a double-convex lens of focal length (*f*) 25 mm (labeled DCX25) on the track between the LED and the screen, near the LED, and move it until you see an image of the LED on the screen. Measure the object distance (*o*) from the lens to the LED and the image distance (*i*) from the lens to the screen and see if they satisfy the thin lens law: 1/o + 1/i = 1/f. Distances are positive if they are on opposite sides of the lens. Measure the dimensions of the image and using the magnification law, M = -i/o, calculate the dimensions of the LED. Negative magnification means the image is inverted.



Move the screen to various positions, and repeat the above steps. In particular, experimentally determine the object and image distances for when M = -4 and for when M = -1? What values of *o* and *i* did you meaure? How do these compare with theory? How close can you place the screen to the LED and still make an image? What is the magnification (this may be hard to measure, but symmetry should provide the answer)? As the screen is moved very far away, to what distance from the LED does the position of the lens converge? Form an image of the LED of size about 1 cm and place the variable aperture next to the lens. Close the aperture to its smallest opening and observe the image quality. Slowly open the aperture and notice changes that occur after a certain aperture size - lots of stray light, which reduces the contrast in the image. This is spherical aberration. The outer edges of the lens are not focusing light properly on the image. Now, leaving its carrier in place, replace the green LED with the red one. To get a focused image, you must move the lens or the LED. This demonstrates chromatic aberration. Return to the green LED. Now replace the DCX25 lens with an achromat, ACH50, correctly oriented with its flatter side toward the LED. Get a focused image, and repeat the experiments with the variable aperture. Do you see an improvement over the DXC25? These qualitative observations are made more quantitative in the following experiments.

3) Mount a microscope 10X objective in the specially assembled carrier with x-y translation stage at its base. Position it at the correct distance from the CMOS sensor in the camera. The sensor is 17.5 mm back from the front of the threaded lens mount (which we don't use) on the opening in the camera. From the sensor to the face of the mount into which the microscope objective is screwed should 180 mm – 200 mm. This distance is called the "optical tube length" and different lens manufactures use different values, but in the range of 150mm – 200mm. Keeping the optical axis aligned requires delicate adjustments both horizontally and vertically. Additionally, the objective and other lenses must be carefully oriented exactly parallel to the optical axis. Later, when setting up lens systems, remember to check that the image formed by the objective lens is still centered on the camera. Small lens misalignments will displace this image a lot.



Köhler illumination (see illustrations on p. 11):

The above diagram illustrates a simple arrangement of lenses and diaphragms to achieve the desired properties of Kohler illumination. Note: it is important that all 3 of the following criteria be simultaneously satisfied:

- a) The light source (LED) is imaged on the aperture diaphragm by the collector lens. Adjust the magnification such that the image of the filament on the aperture diaphragm is about 1cm. We want the LED image at the aperture diaphragm to be magnified enough to fill the maximum aperture of the microscope objective.
- b) The field diaphragm is imaged on the sample by the condenser lens. The image of the field diaphragm is demagnified at the sample by 1:4 or 1:5, i.e. enough to fall within the normal field of view of the 10X objective lens. To calculate the positions of the condenser lens and the field diaphragm, assume M = -i/o = -1/5 and use the thin lens formula to solve for *o* and *i*. The negative sign is because the image is inverted.
- c) Place the aperture diaphragm one focal length from the condenser lens (25 mm). How do you test if you did this step correctly?

This combination of requirements is usually satisfied by using two short focal length lenses, in our case, two DCX 25's. As usual, the numerical aperture of the condenser

lens is defined as the Sine of its maximum cone angle (times the index of refraction of the medium in which it operates, usually air, as in our case, but sometimes immersion oil with an index of 1.52). The effective diameter of this lens is determined by the aperture diaphragm, making the numerical aperture of the condenser adjustable; it is normally adjusted to be a little less than the numerical aperture of the objective lens, which is normally printed on the outside of the lens. With this adjustment, and the field diaphragm adjusted to illuminate a little less than the full field of view, one avoids flare due to light striking the insides of the objective lens, while still achieving the maximum resolution that the lens is capable of. Normally, in fact, the aperture diaphragm is adjusted to give the "best looking" image, in terms of subjectively judged contrast and resolution.

Using the diagram above as a guide, and a 10X objective lens, set the condenser lens at about 30mm from the sample, which should be placed in the correct position to be focused on the camera. From this initial setting of the condenser lens, you should be able to calculate the rest of the locations for the other elements.

With the proper arrangement, you should be able to see the focused circle of the field diaphragm in the image, when the diaphragm is closed to its smallest size. You should adjust the position of the field diaphragm to achieve this. Likewise, there should be independent functions of the two diaphragms: the brightness of the illuminated area should not change when changing the field diaphragm, and the diameter of the illuminated area should not depend on the size of the aperture diaphragm.

4) Following the sketch below, examine the image of the 1000 or 2000 line/inch metal foil grid using Köhler illumination. The camera detector plane should be 160 mm – 180 mm behind the back of the objective. Place the sample a couple millimeters in front of the objective lens. Adjust the camera sensitivity to its lowest point by decreasing the frame time in the properties dialog box of the **ToupView** window. It is best to keep all other settings at their default values. Focus the objective lens on the sample, adjusting the LED intensity as necessary so the camera is not saturated, but has a full dynamic range image. Adjust the focus of the LED to get a uniformly illuminated image. It may be helpful to use a paper tube between the camera and the objective to keep stray room light from striking the camera. See p. 11



5) Record an image, and transfer it to ImageJ. Measure the period of the grid in pixels, and determine the pixel size, in microns. Compute the dimensions of the image sensor (Summit SK2-1.3X 1.3 MP PC/MAC Digital Microscope Camera) and verify that it is a "1/3 inch" diagonal sensor.

		Summit SK2-1.3X 1.3MP PC / MAC Compatible Digital Microscope Camera
	General	
	Brand	OptixCam
SUSC STATES	SKU	OCS-SK2-1.3X
	Warranty	1 Year
	Product Weight	2.0000
	Sensor	
	Sensor Type	CMOS
	Sensor Size	1/3 Inch
	Sensor Details	Aptina MT9M111 CMOS
	Color/Monochrome	Color
	Pixel Pitch	3.6 µm x 3.6 µm
	Sensor Color Depth	8bpp / 24bit RGB
	Scan Mode	Progressive
	Signal to Noise Ratio	44 db
	Sensitivity	1.0V/Lux-sec (550nm)
	Dynamic Range	71 db
6 TouriView - 🗆 🗙	A/D Conversion	10-bit Parallel, 8-bit RGB to PC
File Edit View Browse Setun Canture Image Process Lawer Measurements Ontions Window Hein	Shutter	Electronic, Rolling Shutter
😼 🖬 🖉 Micrometer () - 40X - 50% - □ 🕅 👌 ∠ / / ½ ⊥ - □ ⊂ ○ - ◎ P - ৲ T 🔆 🖽 🛱 😘 \Xi		
Camera + 9 x 4 Video (EXCCD01400KPA) P x	Camera	
Camera List - 0 2 4 6 8 10 12 14 16 18 20 22	Camera Mount	C-Mount, Ocular Mount
EXCCD01400KPA		
BL Capture & Baselution	Hesolution	1.3 Megapixel, 1280x1024 pixels
Exposure & Gain	Camera Output Resolutions	1280 X 1024; 640 X 512; 320 X 256
White Balance	FPS @ Max Res	15@1280x1024; 26@640x512; 48@320x256
Color Adjustment	FPS @ Min Res	50fps @ 320 x 256
Prower Prequency (Antu-mcker)	Camera Interface	USB 2.0
Color/Gray	White Balance	Automatic, Manual
A Flip	Software Included	OptixCam K2View
Samping	Driver API Compatibility	DirectX, TWAIN
Mise ·	Power Source	USB Powered
Parameters	File Formats	BMP, JPEG, Own Proprietary, PNG, TGA, TIFF
	Exposure Control	Automatic, Manual
	Exposure Time	2000ms-0.14Ms
	Operating Temperatures	10°C ~ 50°C
	Operating Humidity	30% ~ 80% RH
Camera a Folder & Under State a Mean	Storage Temperature	-20°C ~ 60°C
EXCCD01400KPA Frame Rate: 15.0; Frame: 1766 1360 x 1024 🖙 40X; 628000 Micrometer x10		

6) With the object in sharp focus on the camera, place a 50 mm focal length achromat lens (ACH100), flat side toward the camera, at the proper position, between objective and camera, to produce a new image with a magnification of 0.5. The image before the ACH100 is inserted serves as a virtual object for the ACH100. Call the distance between the virtual object and the ACH100, *vo*. The magnification formula states M = -i/vo = +0.5, where *i* is the new location of the camera. The positive sign means that the image and object are on the same side of the lens and consequently the image is erect. Consequently, the image on object have opposite signs. Use the thin-lens formula to solve for *i* and *vo*. Move the camera forward to the position of this new image. You should have doubled the linear dimensions of the field of view. If you have done this correctly, the image will be in focus on the camera without having to change the focus of the objective lens. Record an image and confirm the magnification of 0.5. Then remove this lens for the rest of this lab.

7) Vary the angular aperture controlling the cone angle of the illumination light. You can look at this cone of light by moving a piece of paper in the space from the lens to the objective. As you change the aperture size, you will have to keep adjusting the LED intensity to keep the camera from saturating, which would give an artificially sharp image. Note the changes in the image, as you vary the aperture from a very small cone angle in steps to the largest one possible with this lens. Record images and take traces across them to see quantitatively the sharpness of edges, the contrast, and fringe effects at the sharp edges, in crossing the grid pattern. Keep notes on these measurements. What setting produces the best image, with the sharpest edges and the highest contrast, that is, the best representation of the object?

8) With proper Köhler illumination, you should be able to see the image of the LED in the back focal plane of the objective, which is somewhere inside the objective lens body. To focus this plane on the camera, place a "Bertrand lens" (a ACH50 lens) between the objective and the camera, and adjust its position from the midpoint toward the camera to get this image in focus. With the Bertrand lens in focus, as you close the aperture diaphragm you should see it imaged on the camera. In a microscope with a larger field of view than this camera gives us, one can easily adjust the aperture diaphragm to be something like 80% of the numerical aperture of the objective, in other words, 80% of the diameter of the visible back focal plane. This usually gives the best image.



9) You have seen the best image that this microscope objective can make of this sample, as a reference for the following experiments. Now examine 1-to-1 image transfer from the same object to the object plane of the microscope objective, that is, the objective will be focusing on an image made by a lens system, as shown in the sketches above. For this test of lenses, you will use 4 different lens arrangements, all with nominal 50 mm distance from the object to the first lens, and 50 mm from the last lens to the microscope objective object plane. For 1 - to - 1 imaging, we use a symmetric lens arrangements, which helps limit aberrations. The point of this exercise is to see the effects of two important limitations of lens systems: first is resolution limited due to diffraction, that is the finite wavelength of light, and the geometry of the system, especially its numerical aperture, and second is resolution and contrast limited by spherical aberration (recall the stray light at large aperture in section 3 above). See the CalTech notes for a discussion of spherical aberration. For each lens arrangement, vary the aperture used in the image transfer, and record images, making quantitative measurements of image resolution and contrast, by making traces across images in ImageJ. The four cases to study are sketched above, varying from the worst to the best for off-the-shelf optics. In your experiments, be sure to verify that your illumination of the sample gives a cone of light coming from the sample that fills the full area of the first lens. As you record images, be sure to adjust illumination and camera sensitivity to get full dynamic range, but unsaturated images. For each lens system, after achieving the best

focus, switch to the red LED at the same position as the green one, and see that the focus has changed. Try to estimate the change of focus position of the microscope objective quantitatively, although we have no very good quantitative way to do this. See if for the achromats the change is the least.

10) You have seen the best image that this microscope objective can make of this sample, as a reference for the following experiments. Now examine 1- to -1 image transfer from the same object to the object plane of the microscope objective, that is, the objective will be focusing on an image made by a lens system, as shown in the sketches below. Use either a pair of ACH100 or ACH50 lenses. For 1 - to - 1 imaging, we use a symmetric lens arrangements, which helps limit aberrations. The point of this exercise is to see the effects of an important limitation of lens systems: resolution limited due to diffraction, that is the finite wavelength of light, and the geometry of the system, especially its numerical aperture. As you record images, be sure to adjust illumination and camera sensitivity to get full dynamic range, but unsaturated images. See illustrations on p. 11



Set up the 1 - to - 1 imaging system as sketched above, using two ACH50 or ACH100 lenses. Check how the lenses are oriented! The curved sides of the lens should face the AP. Focus the objective on the image, and verify that it is of good quality. Vary the aperture and record the changes in image quality.

- a) Using a piece of paper, look in the plane of the aperture, between the two lenses. This plane, at the focal distance from the first lens, is referred to as the Fourier Transform (FT) plane, since the pattern observed here is in fact a representation of the Fourier transform of the sample. Each point in this plane represents light diffracted by the sample in a particular direction. For this periodic sample, the FT is dominated by the Fourier series due to that periodic structure, which is represented by a set of diffraction orders, making a two dimensional array of bright spots. Each spot represents a term in the Fourier series; its intensity is the square of the amplitude of that term in the series. See Hecht chapter 13.2, especially 13.2.2, Abbe's Theory of Image Formation.
- b) Measure the spacing of the diffraction spots. Using the sample period of 1000 2000 lines/inch, the lens focal length (50 or 100 mm) and the wavelength of the LED (green is 550nm) and justify the spacing of the spots from your knowledge of the rules of diffraction. Referring to the FT of a square wave, which is similar to this grid in one dimension, note the relative intensity of the spots at successively larger distances from the center.
- c) Adjust the LED intensity / exposure time to get a good non-saturated image. Close the aperture to transmit just the central spot in the FT plane. What do you see in the

image? It helps if there is some dirt in the original image; you should see a very blurred image of only the dirt. Now open the aperture to transmit sequentially added diffraction spots, and record the series of images for each successive set of added spots. Compare the resulting images with the Fourier series representation for the corresponding set of terms, using a program like Mathematica. Clearly, even at the largest aperture, the image is only an approximate representation of the square grid of the sample. What is the numerical aperture of this image transfer system, at full aperture opening? Note that the image formed with the full aperture is of lower resolution than that obtained with the red LED, at the best settings of the system. Why is this so?

At this point it should be clear that in addition to the use of a well designed objective lens, proper illumination of the sample is needed to achieve the best image quality in microscopy, meaning diffraction limited resolution, and high contrast. The illumination by a convergent cone of light is essential. Illumination by the narrow laser beam resulted in limited resolution, due to diffraction of light outside the aperture of the lens. However, in illumination by a cone of light, the oblique rays diffract light through large angles that still enter the aperture of the objective lens, as shown in the sketch below. These large angle diffracted rays carry the information representing the finest details of the object to the image. The large range of illumination angles also washes out the diffraction fringes seen with highly collimated illumination, giving the highest contrast as well as the highest resolution.

A final consideration in illumination of the sample is avoiding having light enter the objective lens in such a way that it actually leaves the lens system inside the objective lens, scattering off of interior support structures, leading to "flare" which means stray light randomly reaching the image, and reducing contrast. This requires limiting both the area of the sample illuminated and the maximum angle of the cone of illumination. To achieve these limits, using two independent aperture diaphragms, one for the illuminated field of view and one for the cone of illumination (called the aperture diaphragm, referring to the numerical aperture of the condenser), a scheme of illumination was designed by Köhler, which still bears his name.



QBIO 120 Optics Lab 1 Set-up



