# Chapter 4
Optics

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A few optical concepts are necessary to understand why the light microscope is constructed as it is and how it produces the images that it does. Also necessary is a concept of how the microscope's optics affect light as it travels through them. A fundamental understanding of the microscope can be achieved using simple metaphors to express these concepts.

Basic Optical Concepts

Light Rays

Rays of light can be pictured as straight lines emerging normal to the surface of a light source (fig. 4.1). Imagine the Sun with emerging light rays. Close to the sun the rays all diverge from one another because the Sun's surface is curved. Very far away from the Sun however any rays reaching an observer would be very nearly parallel to one another. We will use light rays to describe the path of light through the lens systems of the microscope. This form of description is called **geometric optics**.

Figure 4.1
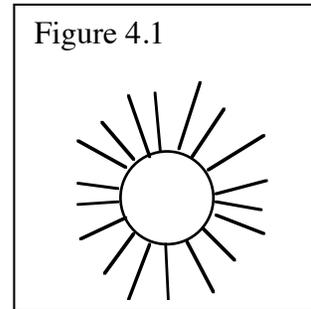
Light Waves

Waves of light can be pictured as waves of water spreading out from a point of origin (fig. 4.2). Imagine ripples on a smooth water surface formed by dropping in a stone. Near the point of origin the waves are very curved. Very far away from the origin, where only a small section of a wave can be seen, the wave appears straight and parallel to the next wave behind it. We will use light waves to explain resolving power and how interference contrast is produced. This form of description is called **wave optics**.

Figure 4.2
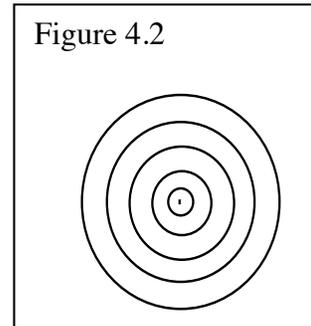
Light Photons

Light originates with an electron transition from an outer to an inner orbital shell of an atom. The electron gives up energy in very discrete amounts during this transition and some of this energy is in the form of visible light. This packet of light energy can be thought of as a **wavetrain** or quantum or photon. A wavetrain has special characteristics that are important in polarized light microscopy. This form of description is called **quantum optics**.

Simple Converging Lens

Figure 4.3 illustrates a simple converging lens that has **parallel light rays** entering from the left. The rays are being focused to a **focal point** at the right on the **lens axis**. The distance from the center of the lens to the focal point is the lenses **focal length**. A plane drawn perpendicular to the lens axis at the focal point is the **focal plane**. A **ray diagram** like this can be interpreted in both directions. If you put a point light source one focal length from a converging lens, parallel light will emerge from the other side. The two sides of a lens are distinguished as **object side** or **front side**, the side where the object is, and **image side** or **back side**, the side where the image is.

Figure 4.3
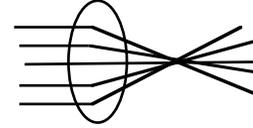
Front or Object Side

The side of a lens where an object to be imaged is placed is called the object side or front side of the lens. The plane at one focal length is the **front focal plane**. The front focal plane of the microscope's objective lens is on the side that faces the specimen. The front focal plane of the condenser is the side that faces the field iris and the illuminator. The field iris is the condenser's "object". The front focal plane of the eyepiece is the side inside the microscope.

Figure 4.4

Object Side   Image Side
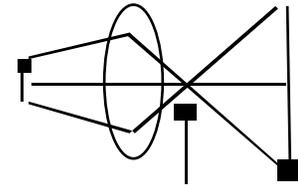
Back or Image Side

The side of a lens where an image is formed is called the image side or back side of the lens. The plane at one focal length on this side is the **back focal plane**. The back focal plane of the objective lens is on the inside of the microscope. Front side and back side of a lens are illustrated in figure 4.4.

Many important images and objects are located at the objective's back focal plane: the Fraunhofer diffraction plane, the Fourier transform of the image, the image of the filament, and the image of the aperture iris. The phase plate of phase contrast and the Wollaston prism of differential interference contrast are placed in the objective's back focal plane.

Converging Lenses Affect Parallel, Diverging, and Converging Rays Differently

All three of the above ray patterns are utilized by the light microscope. Figures 4.5 - 4.7 illustrate the affect of a simple converging lens on each ray pattern.

Parallel Rays (Figure 4.5)

In microscopy, the illuminating light consists of rays or waves that are <u>nearly</u> parallel to one another. This quality of light is called **partially coherent light**. The ultimate resolving power of the microscope depends on the use of partially coherent illumination. The condenser in Köhler illumination is used to create and focus this light onto the specimen.
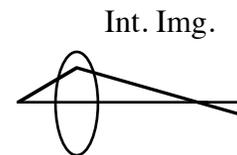
Figure 4.5
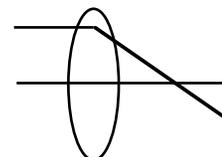
Diverging Rays (Figure 4.6)

Rays of light leaving a point in the specimen diverge due to diffraction, refraction, and reflection. The objective lens forms a real, magnified, intermediate image of these diverging rays inside the microscope at a position near the eyepiece. The objective lens also forms diverging rays from the specimen into a diffraction image in its back focal plane. The microscope's eyepiece presents diverging rays from the intermediate image to the microscopist's eye lens.

Figure 4.6

Int. Img.

Converging rays (Figure 4.7)

Converging rays entering a converging lens cannot be brought to focus by the lens. This is an important optical principle in the microscope. The microscope's eyepiece causes rays from the light source to converge on the microscopist's eye lens thus causing the light source to be out of focus. See the ray diagram in Ch 5, fig 5.1.

Figure 4.7
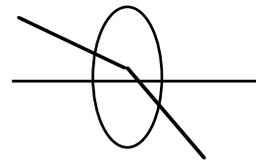
Aberrations

The curved surface of a lens affects how much a ray of light will be deflected. This is called refraction. For example take two parallel rays of light, one hitting the center of the lens normal to the lens surface and the other hitting the edge of the lens. The center ray will not be refracted by the lens, but the peripheral ray will be maximally refracted because the ray is striking the lens at an angle far from the normal do to the curvature of the lens. We will discuss Snell's law of refraction later. A second ray hitting the lens somewhere between the first two will be refracted more than the center ray but less than the peripheral ray. This phenomenon is known as **spherical aberration**.

The color of a light ray also affects how much a lens refracts it. Bluer light is refracted more strongly than redder light. This phenomenon is known as **chromatic aberration**.

There are many other aberrations, some of which we will encounter later on. I point these two out now to make you aware of them and because they are the major ones that lens makers have to correct.

Figure 4.14

Leuwenhook

Lens Types

Magnifying lenses are classified as simple or compound.

Simple Lenses

Simple lenses consist of only a single lens element. Antony van Leuwenhook (figure 4.14) in Holland in the late

1600's invented microscopes that used single simple lenses of his own design. Some of his lenses had a magnification in the range of 400X! Using his microscopes, Leuwenhook made the first observations of many simple organisms.

Figure 4.15

Compound Lenses

There are four basic lens shapes: double convex, double concave, plano convex and plano concave. In the 1830's, the solution to chromatic aberrations was found by cementing two lenses made of different glasses (one double convex and one plano concave) together into an Achromatic Doublet to make a compound lens (see Appendix A for more details). Today, compound lens consists of many lens elements as shown in figure 4.15. These elements are necessary for magnification and correction of various lens aberrations. Modern microscopes use compound lenses. Even so, in considering the optical system of the compound microscope we can think of the various lenses as if they were simple lenses.
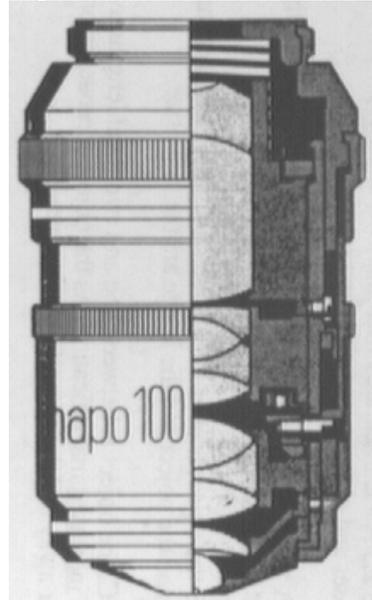
Image Formation by a Converging Lens

A converging lens forms an image of an object at a distance from its back focal plane. The image's location and magnification depend upon the object's location. Figures 4.8 - 4.12 illustrate what happens as an object, illuminated by parallel light, is brought closer and closer to a converging lens.

Figure 4.8

Greater Than Two Focal Lengths

In figure 4.8 the object is greater than two focal lengths from the lens. The lens forms a **real image** of reduced magnification. For example, the condenser forms a reduced magnification image of the field iris in the specimen plane.
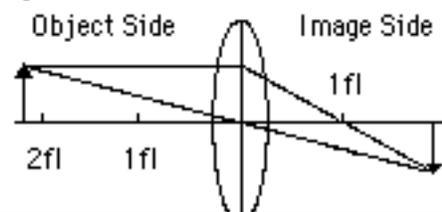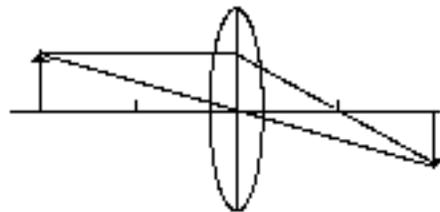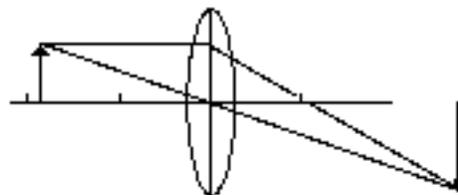
Figure 4.9

Exactly Two Focal Lengths

In figure 4.9 the object is at exactly two focal lengths. Here the image is also at exactly two focal lengths and the object and the image are the same size. A 1X objective does this.
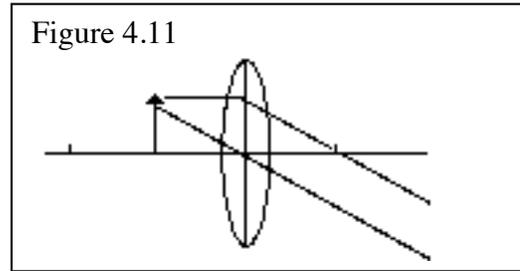
Figure 4.10

Between One And Two Focal Lengths

In figure 4.10, the object is between one and two focal lengths away. The lens produces a real image of increased magnification. As the object approaches one focal length, the image moves farther away and the

magnification increases. In the microscope, the objective forms the intermediate image this way (figure 4.13).

Exactly One Focal Length
In figure 4.11, the object is at exactly one focal length and the image focuses at <u>infinity</u>. Infinity corrected objectives function this way.

Figure 4.11

Closer Than One Focal Length
In figure 4.12 the object is closer to the lens than one focal length, the lens cannot form a real image of the object. However, if another lens, such as your eye, is placed in the image plane, a **virtual image** appears in the **object** plane at a magnification equal to the objective times the eyepiece. In the microscope, the eyepiece functions this way (figure 4.13).

Figure 4.12

Virtual Image
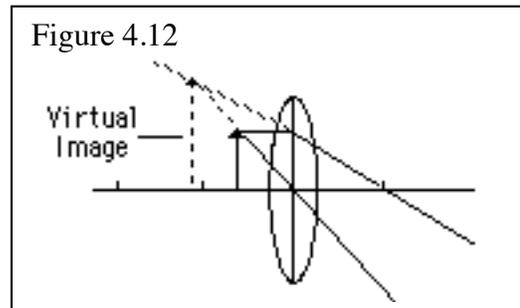
Rules of Geometric Optics
Figures 4.8 through 4.12 are drawn according to the rules of geometric optics. For these figures the rules are: 1) starting at a point on the specimen draw a vector parallel to the lens axis to the center line of the lens, then continue this vector through the focal point of the lens on the lens axis; 2) starting at the same point on the specimen, draw a vector from the point through the center of the lens on the lens axis.

Real and Virtual Images
Figures 4.8 and 4.12 illustrate the formation of the two types of images in a light microscope: a real image and a virtual image.

Real Image
A real image can be seen by placing a screen in the image path. The real image forms on the side of the lens opposite the object. A slide projector produces a real image on a projection screen. The objective lens produces a real image in the intermediate image plane.

Virtual Image
A virtual image cannot be seen on a screen. A second lens is required to see a virtual image. To a human, the virtual image appears to be on the same side of the lens as the object. The image you see in a compound microscope is a virtual image. Figure 4.12 illustrates the virtual image as a back projection of the geometric optical diagram. In reality a second lens is required to see the virtual image and this would cause the image to be inverted relative to the specimen – see figure 4.13. In upright microscopes the virtual image is inverted. In inverted microscopes the image is made to be upright by optics in the binocular. This is helpful when manipulation of the specimen while observing it under the microscope is required.

Figure 4.13 Schematic of image formation in the light microscope.
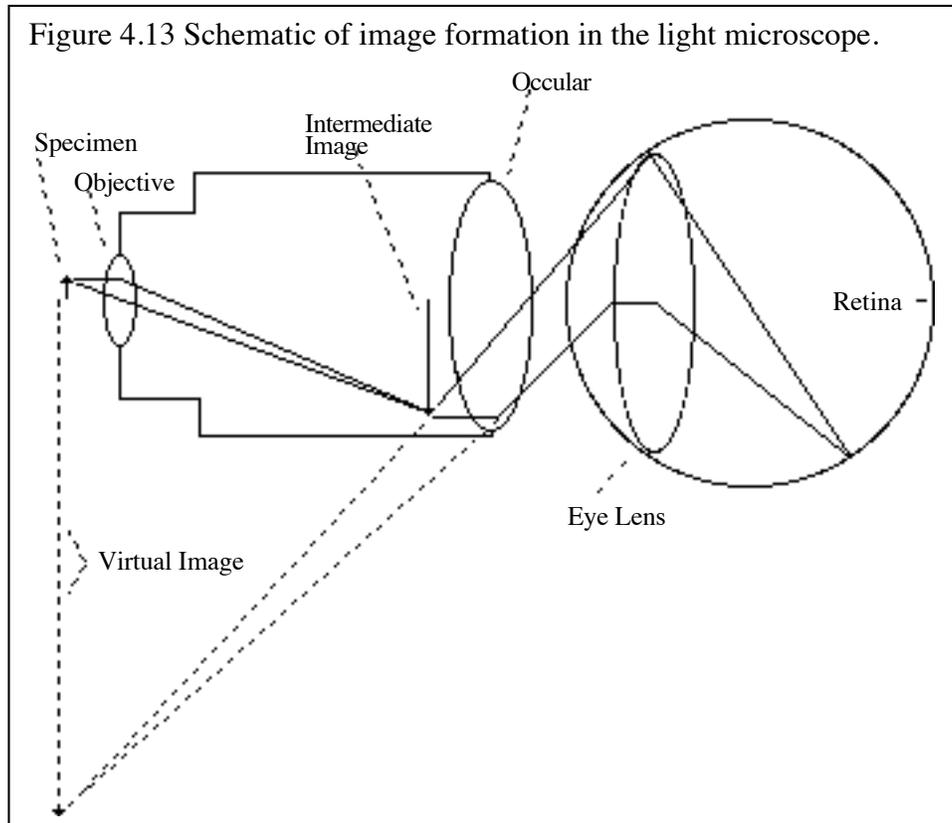
Exercises:

Make a simple sketch of the arrangement of lenses in a microscope indicating the position the front and back focal planes. Include the condenser, objective, eyepiece and eye lens in your sketch.