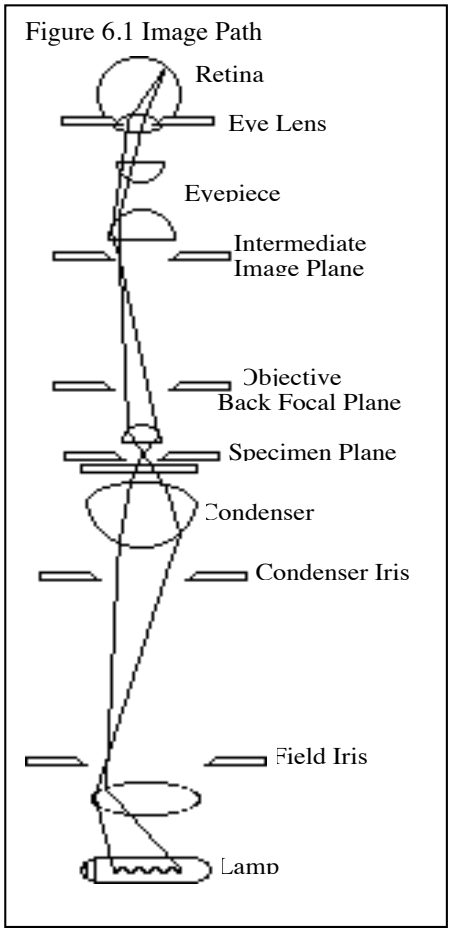
Chapter 6
Image Optical Path

Image Forming Light Path and Image Set

The objective lens forms its own light path and image set that incorporates the ocular, and the microscopist's eye lens. This light path is illustrated in figure 6.1. Figure 6.5 illustrates the ray diagram of image formation.

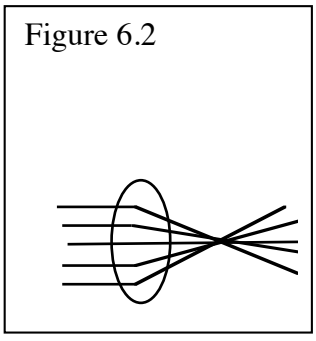


Specimen Plane

Each point in the specimen causes some light to leave in the form of diverging rays. This light is referred to as diffracted light. Some of the light striking the specimen remains unaffected and passes on as nearly parallel rays. This light is referred to as direct light.

Objective Back Focal Plane

The objective lens will focus rays of parallel light in its back focal plane as illustrated in figure 6.2. Important optical elements are placed in the condenser's front focal plane where their image will be passed on toward the objective lens as parallel rays. These include the lamp's filament image, the condenser iris, the dark field annulus, the phase annulus, and the lower Wollaston prism of DIC. In fact, any object placed in the condenser's front focal plane will form a real image in the objective lenses back focal plane. Light from the specimen does not form a recognizable image at this principal plane. Instead, the objective lens forms a diffraction image of the specimen in its back focal plane. This image is referred to as the **Fraunhofer diffraction pattern** and the optical **Fourier transform** of the specimen image. The distribution of light in space at this plane makes all the contrast mechanisms of light microscopy possible.



To see the images in the objective's back focal plane you must look directly down the tube without an eyepiece. If your microscope has a **Bertrand lens**, use it to focus on the objective's back focal plane. To use the Bertrand lens, leave the eyepieces in place. Rotate the Bertrand lens into position and focus it using its focus mechanism (usually a ring or lever associated with the lens) while looking through the eyepieces. If you have no Bertrand lens, perhaps you have a **phase telescope**. This is a special eyepiece that comes with phase contrast equipment. Replace a regular

eyepiece with the phase telescope and slide the inner part of the telescope in and out to focus. If you have neither of these, remove an eyepiece, cover the hole with some aluminum foil and punch a pinhole in the center. This will make a crude, but effective, pinhole camera that will magnify and focus the objective's back focal plane when you look through the pinhole.

Intermediate Image Plane

The intermediate image plane exists inside the microscope at a location that is less than one focal length from the eyepiece (fig. 6.5). A real, magnified image of the specimen exists in the intermediate image plane. This image is used as an object for further magnification by the eyepiece lens. As shown in figure 6.3, when the specimen is between one and two focal lengths from the objective lens a magnified **intermediate image** of the specimen is formed in the image plane. The exact distance form the objective lens to the specimen is the lenses **working distance**. This distance must be far enough to place the intermediate image at the microscope's **tube length**. As shown in figure 6.4, when the object is at exactly one focal length the image focuses at infinity. Many modern objective lenses use this technique called **infinity corrected optics**. These lenses require that an **auxiliary lens** be placed before the eyepiece to create the intermediate image. All other types of objective lenses require that the object be between one and two focal lengths away from the objective lens. Because the intermediate image is closer than one focal length from the eyepiece, a virtual image of the specimen is formed by the eyepiece outside the microscope. This image requires the lens in the microscopist's eye plus her retina plus her brain to be seen.

Figure 6.3

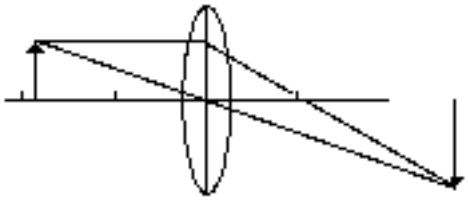
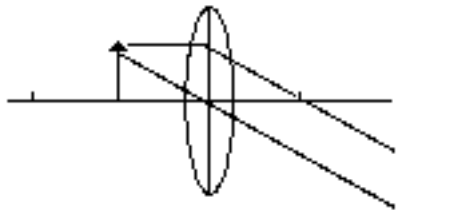


Figure 6.4



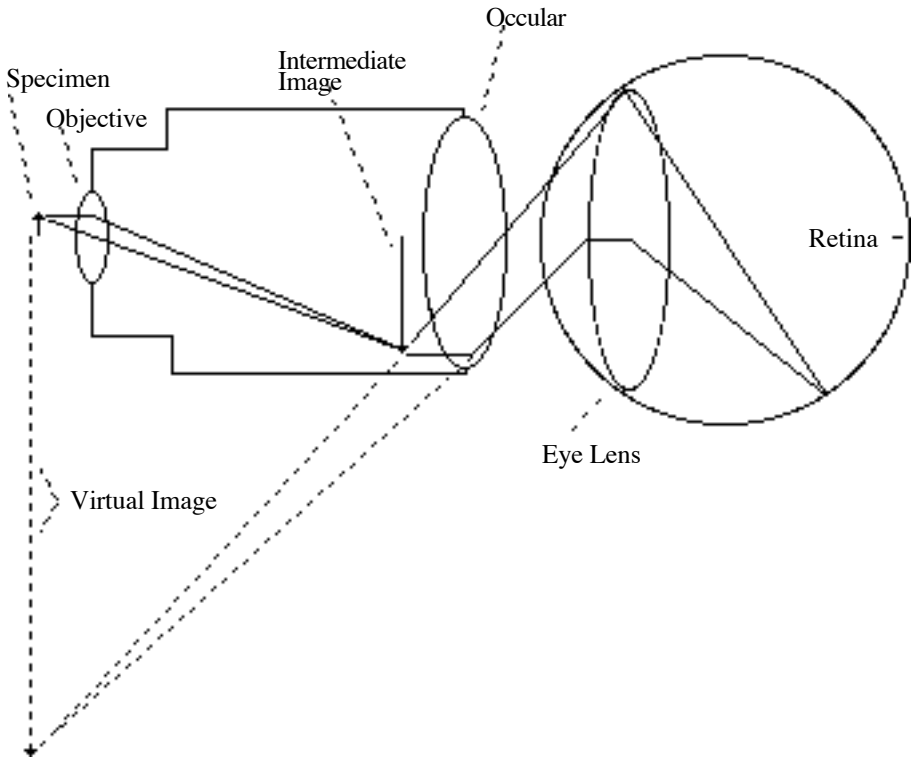
Eyepoint Image Plane

The eyepoint is near the back focal plane of the eyepiece. It is outside the microscope. It is the point where the microscopist's eye lens is placed. All the real images that occur at the objective's back focal plane also occur at the eyepoint: the filament image, the condenser aperture image, the phase annulus image, and the image of any other object placed in the condenser's front focal plane or the objective's back focal plane. When the microscopist looks into the microscope and focuses on the image of the specimen, the eyepoint is less than one focal length from the microscopist's eye lens. Any object that forms a real image at the eyepoint will be completely out of focus on the microscopist's retina. In this way, a light source that consists of a small coiled inhomogeneous filament can completely and evenly illuminate the specimen by Köhler illumination.

Retinal Image Plane

The lens in the eye of the microscopist is also part of the microscope's imaging system. The eyepiece produces a virtual image requiring an additional lens. The microscopist's eye lens serves this purpose (figure 6.5). The virtual image "exists" outside the microscope at the location of the specimen. The lens in the microscopist's eye converts this image into a real image on the retina.

Figure 6.5 Schematic of image formation in the light microscope



Exercises

1) Examine the intermediate image of a specimen in your microscope by focusing on a specimen at low power, removing the eyepiece and placing a screen made from a piece of lens tissue into the tube. Over what distance is this image in focus?

2) Using Köhler illumination, observe a familiar specimen under high power. Close the condenser aperture from fully open to fully closed. What happens to the depth of field; to the contrast; to the intensity of the illumination?