

Chapter 5

Illumination Optical Path

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Illumination Path and Image Set

The illumination light path (fig. 5.1) begins with the lamp and progresses through all the optics of the microscope. At certain **principal planes** along the way real images of parts of the illumination system are formed.

Collector Lens

Just in front of the microscope's lamp is a lens that magnifies and projects an image of the lamp's filament toward the condenser. The collector lens concentrates the lamp's light and focuses the filament image at the proper position in the microscope for Köhler illumination.

Field Lens

Some microscopes have an additional lens near the field iris that works in concert with the collector lens to produce a real image of the filament properly placed in the condenser's front focal plane.

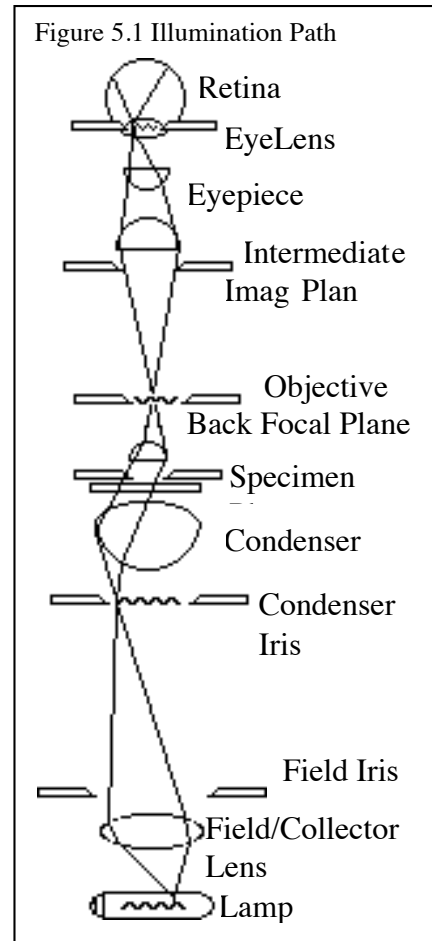
Field Iris

The field iris is positioned greater than two focal lengths from the condenser's front focal plane. The condenser therefore forms a real image of reduced magnification of the field iris in the specimen plane. In setting up Köhler illumination, the field iris is observed along with the specimen. First, the field iris is focused by adjusting the vertical position of the condenser (with the specimen in focus) and then it is opened to just outside the field of view. This iris controls the size of the area of illumination on the specimen i.e. the **field** of illumination.

Tip In bright field microscopy, illuminating only that part of the specimen that the objective lens can see is extremely important. Structures outside the field of view, if illuminated, will scatter light into the field of view causing glare. Glare diminishes both contrast and resolution. When you change magnification you also change the area of the field of view and thus when changing objectives you should adjust the field iris.

Condenser

The condenser is the most important lens in the illumination system. The condenser concentrates light from the filament image into partially coherent light onto the



specimen plane. This light is formed into a cone shape that spreads out from the specimen toward the objective lens. The condenser must be able to form a light cone with a sufficiently large angle to fill the entire back focal plane of the objective lens. If this is not possible the condenser will limit the numerical aperture of the entire microscope. The condenser also forms an image of the field iris in the specimen plane.

Aperture Iris or Condenser Iris

In the Köhler alignment procedure you remove an eyepiece, look down the tube and close the aperture iris about 50%. Köhler places the aperture iris in the optical path so that its image appears in the back focal plane of the objective. This placement is done when you move the condenser up and down until the field iris is in focus. You remove the eyepiece to see the back focal plane of the objective.

The aperture iris functions in the same way as the iris in a photographic lens. Stopping the iris down will reduce the intensity of light, increase the depth of field and depth of focus, increase contrast in the specimen image, and reduce resolution in the specimen image. Closing the condenser iris reduces the angle of the cone of illumination produced by the condenser thus lowering the condenser's numerical aperture. Stopping this iris down too far creates fringes around objects in the image. These fringes are subtle and are often mistaken for part of the specimen's structure. This iris controls the aperture (angle) of illumination.

The principal use of the condenser iris is to adjust the amount of contrast one sees in a specimen. Do this by observing the specimen while stopping the iris down. Do not go too far down or you may see things that are not there. Usually covering 50% of the objective's back focal plane is enough. Do not use this iris to adjust image brightness this is the use of neutral density filters. When you change objective lenses you must readjust the aperture iris setting.

Objective

Since a real image of the filament exists in the condenser's front focal plane, the condenser projects parallel rays of this image toward the objective lens. The objective lens therefore forms a real image of the filament in its back focal plane.

Ocular

The ocular receives diverging rays of light from the real image of the filament in the objective's back focal plane. This image is farther from the ocular than two focal lengths so the ocular forms an image of the filament at reduced magnification at the eyepoint.

Eye Lens

The microscopist's eye lens is placed at the ocular's eyepoint. The real image of the filament at the eyepoint is therefore less than one focal length away and provides diverging rays that cannot be focused on the microscopist's retina. The microscopist can not see an image of the light source but rather an evenly illuminated field.

Exercises

- 1) Set up Köhler illumination. Focus on the frosted end of a slide. Move the objective lens aside and observe the effect of adjusting first the aperture iris and then the field iris on the light at the surface of the slide. Try the same exercise by placing a white card on edge over the center of the condenser. What effect does each iris produce?
- 2) If you can remove the diffuser, look for the image of the filament on the condenser iris. How much depth of field does this image have?
- 3) Look for the various images described in this chapter that occur in the objective's back focal plane. Which ones can you see in your microscope?
- 4) Examine the eyepoint of your microscope by focusing on a specimen, then holding a piece of lens tissue at the normal eye position and adjusting its distance to achieve the smallest spot of light. How far from the eyepiece is the eyepoint? What is the diameter of the eye point? Use a low magnification objective, remove the diffuser, and try to observe the filament image at the eyepoint.