Types of Microscopy

The earliest microscopes used visible light to create images and were little more than magnifying glasses. Today, more sophisticated compound light microscopes (Figure 3-1) are routinely used in microbiology laboratories. The various types of light microscopy include bright-field, dark-field, fluorescence, and phase contrast microscopy (Figure 3-2). Each method has specific applications and advantages, but the most commonly used in introductory classes and clinical laboratories is bright-field microscopy. In many research applications, electron microscopy is used because of its ability to produce higher quality images of greater magnification.

Light Microscopes

Bright-field microscopy produces an image made from light that is transmitted through a specimen (Figure 3-2A). The specimen restricts light transmission and appears "shadowy" against a bright background (where light enters the microscope unimpeded). Since most biological specimens are transparent, contrast between the specimen and background can be improved with the application of stains to the specimen (see Sections 4 and 5). The price of improved contrast is that the staining process usually kills cells. This is especially true of bacterial staining protocols.

Image formation begins with light coming from an internal or an external light source (Figure 3-3). It passes through the condenser lens, which concentrates the light and makes illumination of the specimen more uniform. Refraction (bending) of light as it passes through the objective lens from the specimen produces a magnified real image. This image is magnified again as it passes through the ocular lens to produce a virtual image that appears below or within the microscope. The amount of magnification produced by each lens is marked on the lens (Figure 3-4A and B). Total magnification of the specimen can be calculated by the following formula:

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\text{Total Magnification} = \frac{\text{Magnification of the Objective Lens}}{\text{Magnification of the Ocular Lens}}
\]
The practical limit to magnification with a light microscope is around 1300X. Although higher magnifications are possible, it becomes increasingly difficult to maintain image clarity as the magnification increases. Clarity of an image is called resolution. The limit of resolution (or resolving power) is an actual measurement of how far apart two points must be in order for the microscope to view them as being separate. Notice that resolution improves as resolving power is made smaller.

The best limit of resolution achieved by a light microscope is about 0.2 μm. (That is, at its absolute best, a light microscope cannot distinguish between two points closer together than 0.2 μm.) For a specific microscope,
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amplitudes) increases. Light waves that are out of phase by exactly one-half wavelength cancel each other and result in no intensity; that is, darkness. Wavelengths that are out of phase by any amount will produce some degree of cancellation and result in brightness less than maximum, but more than darkness. Thus, contrast is provided by differences in light intensity that result from differences in refractive indices in parts of the specimen that put light waves more or less out of phase. As a result, the specimen appears as various levels of darks against a bright background.

Fluorescence microscopy (Figure 3-2D) uses a fluorescent dye that emits fluorescence when illuminated with ultraviolet light. In some cases, specimens possess naturally fluorescing chemicals and no dye is needed.

Figure 3-3. Markings of Magnification and Numerical Aperture on Microscope Components. (A) Three plan apochromatic objective lenses on the nosepiece of a light microscope. Plan means the lens produces a flat field of view. Apochromatic lenses are made in such a way that chromatic aberration is reduced to a minimum. From left to right, the lenses magnify 10X, 20X, and 40X, and have numerical apertures of 0.40, 0.70, and 0.85. The 20X lens has other markings on it. The mechanical tube length is the distance from the nosepiece to the ocular and is usually between 160 to 210 mm. However, this 20X lens has been corrected so the light rays are made parallel, effectively creating an infinitely long mechanical tube length (\(00\)). This allows insertion of accessories into the light path without decreasing image quality. The thickness of cover glass to be used is also given (0.17 ± 0.01 mm). (B) A 10X ocular lens. (C) A condenser (removed from the microscope) with a numerical aperture of 1.25. The lever in the upper right is used to open and close the iris diaphragm and adjust the amount of light entering the specimen.

Numerical aperture is a measure of a lens's ability to "capture" light coming from the specimen and use it to make the image. As with magnification, it is marked on the lens (Figures 3-4A and C). Using immersion oil between the specimen and the objective lens increases its numerical aperture and in turn, makes its limit of resolution smaller. (If necessary, oil may also be placed between the condenser lens and the slide.) The result is better resolution.

The light microscope may be modified to improve its ability to produce images with contrast without staining, which often distorts or kills the specimen. In dark field microscopy (Figure 3-2B), a special condenser is used so that only light reflected off the specimen enters the objective. The appearance is of a brightly lit specimen against a dark background, and often with better resolution than that of the bright field microscope.

Phase contrast microscopy (Figure 3-2C) uses special optical components to exploit subtle differences in the refractive indices of water and cytoplasmic components to produce contrast. Light waves that are in phase (that is, their peaks and valleys exactly coincide) reinforce one another and their total intensity (due to the summed amplitudes) increases. Light waves that are out of phase by exactly one-half wavelength cancel each other and result in no intensity; that is, darkness. Wavelengths that are out of phase by any amount will produce some degree of cancellation and result in brightness less than maximum, but more than darkness. Thus, contrast is provided by differences in light intensity that result from differences in refractive indices in parts of the specimen that put light waves more or less out of phase. As a result, the specimen appears as various levels of darks against a bright background.

Fluorescence microscopy (Figure 3-2D) uses a fluorescent dye that emits fluorescence when illuminated with ultraviolet light. In some cases, specimens possess naturally fluorescing chemicals and no dye is needed.

The Electron Microscope

The electron microscope uses an electron beam to create an image, with electromagnets acting as lenses. The limit of resolution is improved by a factor of 1000 (theoretically down to 0.1 nm, but more realistically down to 2 nm) over the light microscope.

The transmission electron microscope (TEM) (Figure 3-5) produces a two-dimensional image of an ultrathin section by capturing electrons that have passed through the specimen. The degree of interaction between the electrons and the heavy metal stain affects the kinetic energy of the electrons, which are collected by a fluorescent plate. The light of varying intensity produced is directly proportional to the electron's kinetic energy and is used to produce the
image. The TEM is useful for studying a cell's interior, its ultrastructure. A sample transmission electron micrograph is shown in Figure 3-6.

A scanning electron microscope (SEM) (Figure 3-7) is used to make a three-dimensional image of the specimen's surface. In this technique, a beam of electrons is passed over the stained surface of the specimen. Some electrons are reflected (backscatter electrons), whereas other electrons (secondary electrons) are emitted from the metallic stain. These electrons are captured and used to produce the three-dimensional image. A sample scanning electron micrograph is shown in Figure 3-8.