Optical Microscopy

The optical (or light) microscope is an instrument that magnifies and resolves the structure of objects. Objects may be viewed by either transmitted or reflected light and by a selection of illumination methods. The optical microscope is probably the single most widely used instrument in scientific research, finding applications in diverse disciplines including materials science, mineralogy, chemistry, biology, medicine, particle analysis and forensic science.

1. The Microscope

The most elementary microscope is the simple microscope which employs a single lens (magnifying glass) and is limited to a maximum magnification of about twenty ($20\times$). The compound microscope—with a useful magnification range of a few times to over $2000\times$—consists of four components: a light source, condenser lens, objective lens and eyepiece (ocular) (Fig. 1). The condenser collects light rays from the light source which are focused onto the specimen. The objective lens (the lens nearest the object) forms a real, magnified aerial image of the object in the intermediate image plane. This real image is further magnified by the eyepiece to form the final image, which can be (a) viewed directly in the eyepiece, (b) projected onto a viewing screen or photographic film, or (c) observed by way of a television system.

2. Numerical Aperture and Resolution

Each optical system has a maximum resolving power—or resolution—that is determined by a number of factors. The typical unaided human eye has a resolving power of approximately 150 μm; that is, two discrete object points with a separation of 150 μm or greater can be resolved as two separate and distinct entities. At closer spacing the object points cannot be individually identified by the unaided eye. The maximum resolving power of a compound light microscope is approximately 0.2 μm and is determined by the numerical aperture of the objective lens and the wavelength of illumination.

Numerical aperture (NA) is defined as being equal to $n \sin \theta$, where n is the refractive index of the medium between the objective lens and the object ($n \cong 1$ for air) and θ is half the angular aperture (or acceptance angle of image-forming rays) of the objective lens (Jenkins and White 1957). The use of optical immersion oil ($n = 1.52$) can raise the value of n so that lenses with NA values as high as 1.40 can be routinely offered by the lens manufacturers. NAs as high as 1.60 are attainable with special lenses designed to be used with high-refractive-index liquids such as naphthalene monobromide ($n = 1.66$) (Phillips 1971).

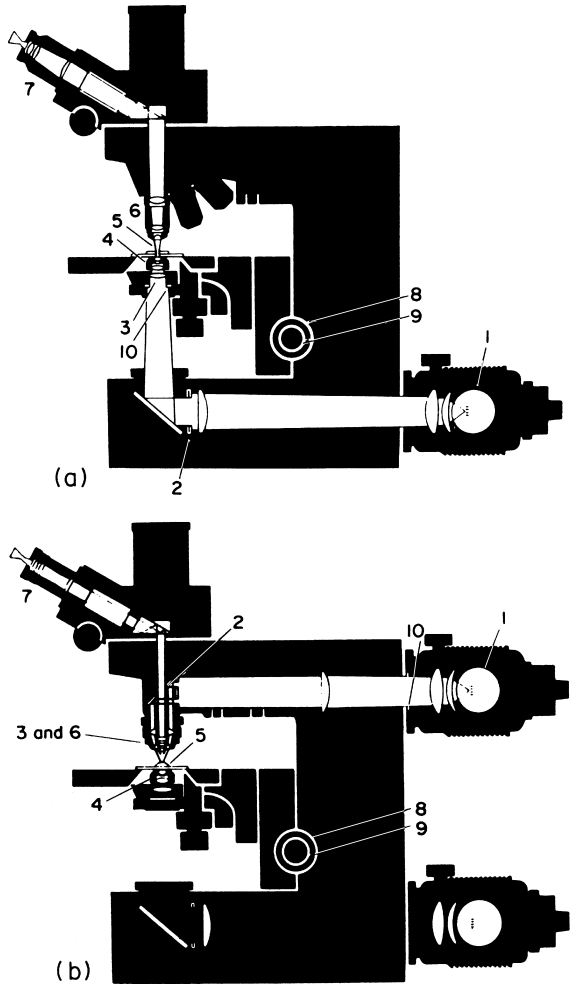

Figure 1
Ray paths and optical component locations for (a) transmitted light, and (b) reflected light microscopes: 1, light source; 2, field diaphragm; 3, condenser lens; 4, substage condenser; 5, object (specimen); 6, objective lens; 7, eyepiece (ocular); 8, coarse focus; 9, fine focus; and 10, aperture diaphragm (courtesy of E. Leitz).

The NA values of individual objective lenses typically vary from less than 0.1 to 1.40. Lenses labelled with NAs of 0.95 or less are usually intended to be used in air only (i.e., dry), whereas lenses of NA of 1.0 or higher must be immersed, usually in an optical oil of $n = 1.52$, as specified by the manufacturer. Immersion lenses are labelled with the immersion media with which they are designed to be used.

The resolution of an optical microscope is given by the relationship

$$d = 0.61\lambda/\mathrm{NA}$$

where d is the smallest resolvable distance in the image