1
Light Microscopy

Light or optical microscopy is the primary means for scientists and engineers to examine the microstructure of materials. The history of using a light microscope for microstructural examination of materials can be traced back to the 1880s. Since then, light microscopy has been widely used by metallurgists to examine metallic materials. Light microscopy for metallurgists became a special field named *metallography*. The basic techniques developed in metallography are not only used for examining metals, but also are used for examining ceramics and polymers. In this chapter, light microscopy is introduced as a basic tool for microstructural examination of materials including metals, ceramics, and polymers.

1.1
Optical Principles

1.1.1
Image Formation

Reviewing the optical principles of microscopes should be the first step to understanding light microscopy. The optical principles of microscopes include image formation, magnification, and resolution. Image formation can be illustrated by the behavior of a light path in a compound light microscope as shown in Figure 1.1. A specimen (*object*) is placed at position A where it is between one and two focal lengths from an *objective lens*. Light rays from the object first converge at the objective lens and are then focused at position B to form a magnified inverted image. The light rays from the image are further converged by the second lens (*projector lens*) to form a final magnified image of an object at C.

The light path shown in Figure 1.1 generates the real image at C on a screen or camera film, which is not what we see with our eyes. Only a real image can be formed on a screen and photographed. When we examine microstructure with our eyes, the light path in a microscope goes through an *eyepiece* instead of projector lens to form a *virtual image* on the human eye retina, as shown in Figure 1.2. The virtual image is inverted with respect to the object. The virtual image is often adjusted to be located as the minimum distance of eye focus, which is conventionally taken

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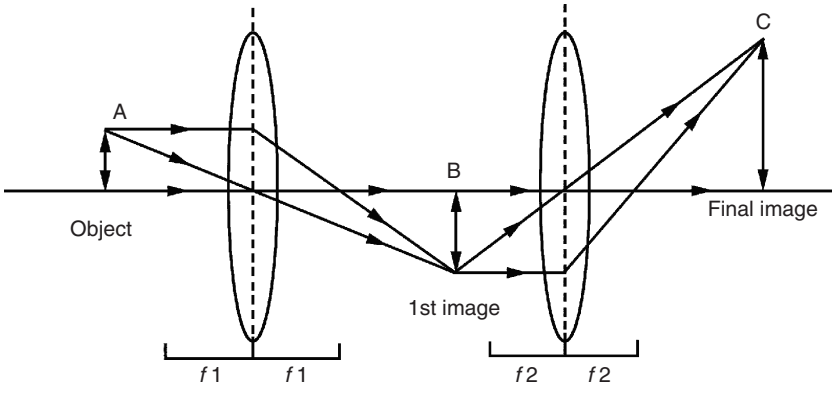

Figure 1.1 Principles of magnification in a microscope.

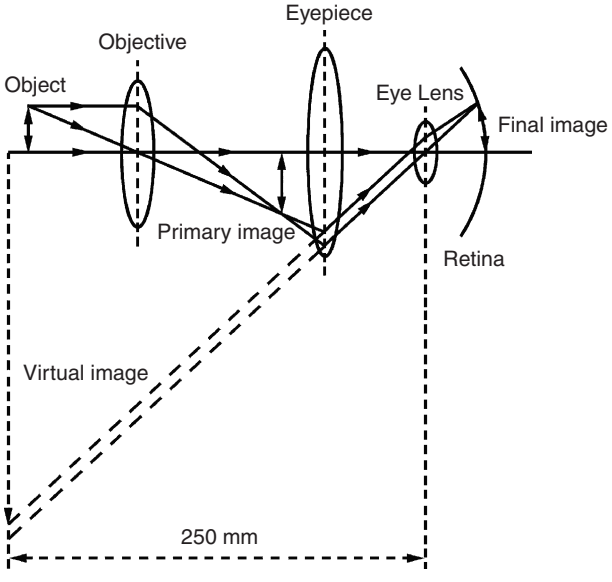

Figure 1.2 Schematic path of light in a microscope with an eyepiece. The virtual image is reviewed by a human eye composed of the eye lens and retina.

as 250 mm from the eyepiece. A modern microscope is commonly equipped with a device to switch from eyepiece to projector lens for either recording images on photographic film or sending images to a computer screen.

Advanced microscopes made since 1980 have a more complicated optical arrangement called "*infinity-corrected*" optics. The objective lens of these microscopes generates parallel beams from a point on the object. A tube lens is added between the objective and eyepiece to focus the parallel beams to form an image on a plane, which is further viewed and enlarged by the eyepiece.

The magnification of a microscope can be calculated by linear optics, which tells us the magnification of a convergent lens, M:

$$M = \frac{v - f}{f} \tag{1.1}$$

where f is the focal length of the lens and v is the distance between the image and lens. A higher magnification lens has a shorter focal length, as indicated by Eq. (1.1). The total magnification of a compound microscope as shown in Figure 1.1 should be the magnification of the objective lens multiplied by that of the projector lens.

$$M = M_1 M_2 \frac{(v_1 - f_1)(v_2 - f_2)}{f_1 f_2} \tag{1.2}$$

When an eyepiece is used, the total magnification should be the objective lens magnification multiplied by the eyepiece magnification.

1.1.2 Resolution

We naturally ask whether there is any limitation for magnification in light microscopes because Eq. (1.2) suggests there is no limitation. However, meaningful magnification of a light microscope is limited by its *resolution*. Resolution refers to the minimum distance between two points at which they can be visibly distinguished as two points. The resolution of a microscope is theoretically controlled by the diffraction of light.

Light diffraction controlling the resolution of microscope can be illustrated with the images of two self-luminous point objects. When the point object is magnified, its image is a central spot (the *Airy disk*) surrounded by a series of diffraction rings (Figure 1.3), not a single spot. To distinguish between two such point objects separated by a short distance, the Airy disks should not severely overlap each other. Thus, controlling the size of the Airy disk is the key to controlling resolution. The size of the Airy disk (d) is related to the wavelength of light (λ) and the angle of light coming into the lens. The *resolution of a microscope* (R) is defined as the minimum distance between two Airy disks that can be distinguished (Figure 1.4). Resolution is a function of microscope parameters as shown in the following equation:

$$R = \frac{d}{2} = \frac{0.61\lambda}{\mu \sin\alpha} \tag{1.3}$$

where μ is the refractive index of the medium between the object and objective lens and α is the half-angle of the cone of light entering the objective lens (Figure 1.5). The product, $\mu \sin\alpha$, is called the *numerical aperture* (NA).

According to Eq. (1.3), to achieve higher resolution we should use shorter-wavelength light and larger NA. The shortest wavelength of visible light is about 400 nm, while the NA of the lens depends on α and the medium between the

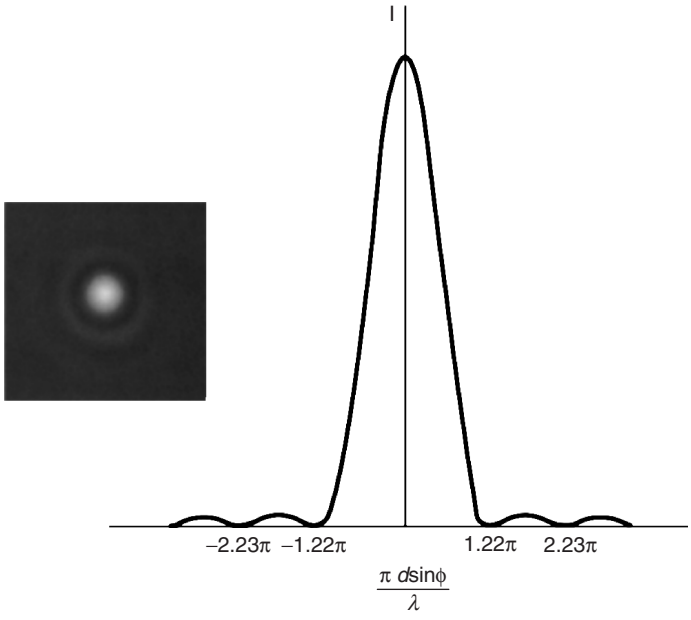

Figure 1.3 A self-luminous point object and the light-intensity distribution along a line passing through its center.

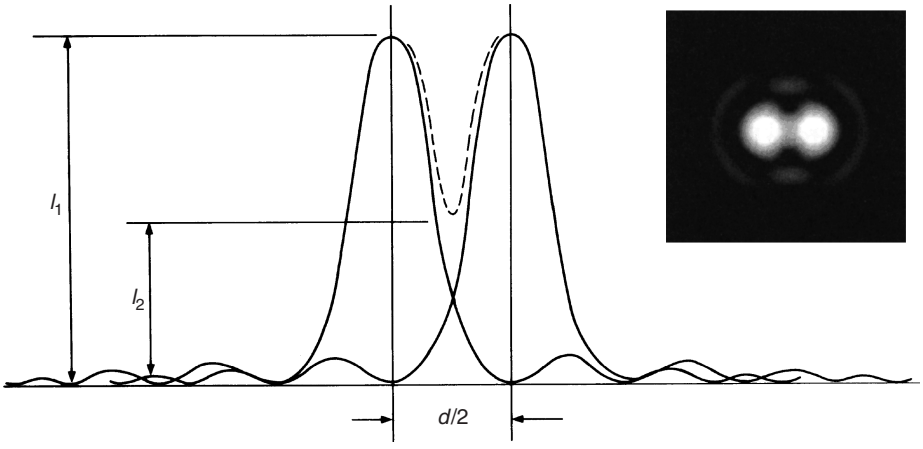

Figure 1.4 Intensity distribution of two airy disks with a distance $d/2$. I_1 indicates the maximum intensity of each point and I_2 represents the overlap intensity.

lens and object. Two media between object and objective lens are commonly used: either air for which $\mu = 1$, or oil for which $\mu \approx 1.5$. Thus, the maximum value of NA is about 1.5. We estimate the best resolution of a light microscope from Eq. (1.3) as about 0.2 μm.

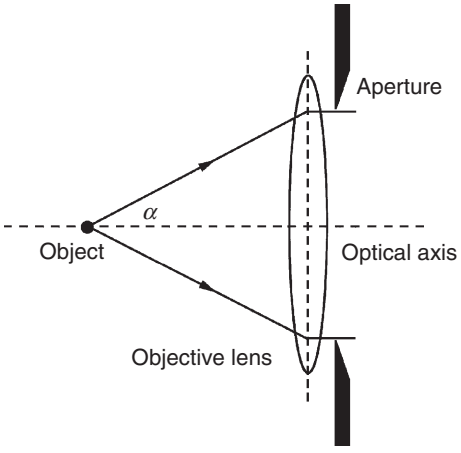

Figure 1.5 The cone of light entering an objective lens showing α is the half-angle.

1.1.2.1 **Effective Magnification**

Magnification is meaningful only in so far as the human eye can see the features resolved by the microscope. Meaningful magnification is the magnification that is sufficient to allow the eyes to see the microscopic features resolved by the microscope. A microscope should enlarge features to about 0.2 mm, the resolution level of the human eye. This means that the microscope resolution multiplying the effective magnification should be equal to the eye resolution. Thus, the *effective magnification* of a light microscope should approximately be $M_{\text{eff}} = 0.2 \div 0.2 \times 10^3 = 1.0 \times 10^3$.

A higher magnification than the effective magnification only makes the image bigger, may make eyes more comfortable during observation, but does not provide more detail in an image.

1.1.2.2 **Brightness and Contrast**

To make a microscale object in a material specimen visible, high magnification is not sufficient. A microscope should also generate sufficient *brightness* and *contrast* of light from the object. Brightness refers to the intensity of light. In a transmission light microscope the brightness is related to the numerical aperture (NA) and magnification (M).

$$\text{Brightness} = \frac{(\text{NA})^2}{M^2} \tag{1.4}$$

In a reflected-light microscope the brightness is more highly dependent on NA.

$$\text{Brightness} = \frac{(\text{NA})^4}{M^2} \tag{1.5}$$

These relationships indicate that the brightness decreases rapidly with increasing magnification, and controlling NA is not only important for resolution but also for brightness, particularly in a reflected-light microscope.

Contrast is defined as the relative change in light intensity (I) between an object and its background.

$$\text{Contrast} = \frac{I_{\text{object}} - I_{\text{background}}}{I_{\text{background}}} \tag{1.6}$$

Visibility requires that the contrast of an object exceeds a critical value called the *contrast threshold*. The contrast threshold of an object is not constant for all images but varies with image brightness. In bright light, the threshold can be as low as about 3%, while in dim light the threshold is greater than 200%.

1.1.3 Depth of Field

Depth of field is an important concept when photographing an image. It refers to the range of position for an object in which image sharpness does not change. As illustrated in Figure 1.6, an object image is only accurately in focus when the object lies in a plane within a certain distance from the objective lens. The image is out of focus when the object lies either closer to or farther from the lens. Since the diffraction effect limits the resolution R, it does not make any difference to the sharpness of the image if the object is within the range of D_f shown in Figure 1.6. Thus, the depth of field can be calculated.

$$D_f = \frac{d}{\tan\alpha} = \frac{2R}{\tan\alpha} = \frac{1.22\lambda}{\mu \sin\alpha \tan\alpha} \tag{1.7}$$

Equation (1.7) indicates that a large depth of field and high resolution cannot be obtained simultaneously; thus, a larger D_f means a larger R and worse resolution.

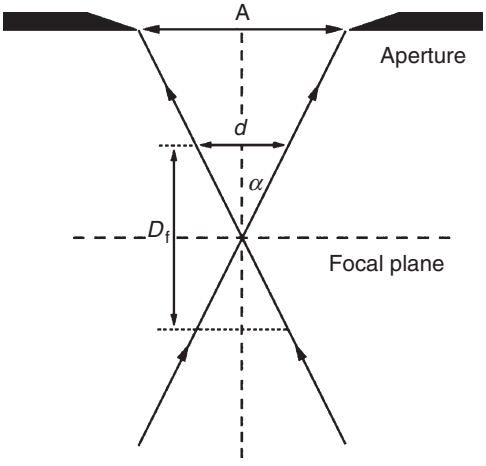

Figure 1.6 Geometric relation among the depth of field (D_f), the half-angle entering the objective lens (α), and the size of the Airy disk (d).

We may reduce angle α to obtain a better depth of field only at the expense of resolution. For a light microscope, α is around 45° and the depth of field is about the same as its resolution.

We should not confuse *depth of field* with *depth of focus*. Depth of focus refers to the range of image plane positions at which the image can be viewed without appearing out of focus for a fixed position of the object. In other words, it is the range of screen positions in which and images can be projected in focus. The depth of focus is M^2 times larger than the depth of field.

1.1.4 Aberrations

The aforementioned calculations of resolution and depth of field are based on the assumptions that all components of the microscope are perfect, and that light rays from any point on an object focus on a correspondingly unique point in the image. Unfortunately, this is almost impossible due to image distortions by the lens called *lens aberrations*. Some aberrations affect the whole field of the image (*chromatic* and *spherical aberrations*), while others affect only off-axis points of the image (*astigmatism* and *curvature of field*). The true resolution and depth of field can be severely diminished by lens aberrations. Thus, it is important for us to have a basic knowledge of aberrations in optical lenses.

Chromatic aberration is caused by the variation in the refractive index of the lens in the range of light wavelengths (*light dispersion*). The refractive index of lens glass is greater for shorter wavelengths (for example, blue) than for longer wavelengths (for example, red). Thus, the degree of light deflection by a lens depends on the wavelength of light. Because a range of wavelengths is present in ordinary light (white light), light cannot be focused at a single point. This phenomenon is illustrated in Figure 1.7.

Spherical aberration is caused by the spherical curvature of a lens. Light rays from a point on the object on the optical axis enter a lens at different angles and cannot

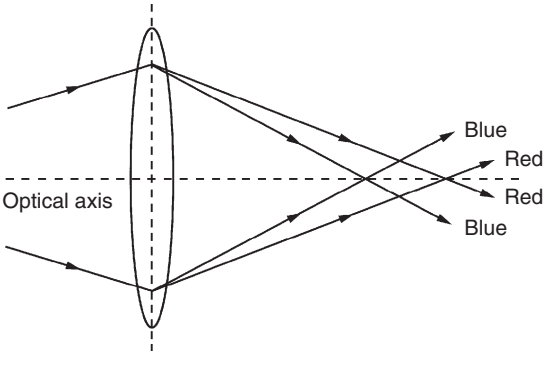

Figure 1.7 Paths of rays in white light illustrating chromatic aberration.

be focused at a single point, as shown in Figure 1.8. The portion of the lens farthest from the optical axis brings the rays to a focus nearer the lens than does the central portion of the lens.

Astigmatism results when the rays passing through vertical diameters of the lens are not focused on the same image plane as rays passing through horizontal diameters, as shown in Figure 1.9. In this case, the image of a point becomes an elliptical streak at either side of the best focal plane. Astigmatism can be severe in a lens with asymmetric curvature.

Curvature of field is an off-axis aberration. It occurs because the focal plane of an image is not flat but has a concave spherical surface, as shown in Figure 1.10. This aberration is especially troublesome with a high magnification lens with a short focal length. It may cause unsatisfactory photography.

There are a number of ways to compensate for and/or reduce lens aberrations. For example, combining lenses with different shapes and refractive indices corrects chromatic and spherical aberrations. Selecting single-wavelength illumination by the use of filters helps eliminate chromatic aberrations. We expect that the extent to which lens aberrations have been corrected is reflected in the cost of the lens. It is a reason that we see huge price variation in microscopes.

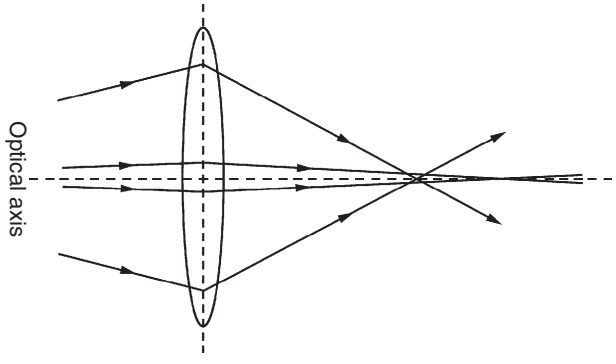

Figure 1.8 Spherical aberration.

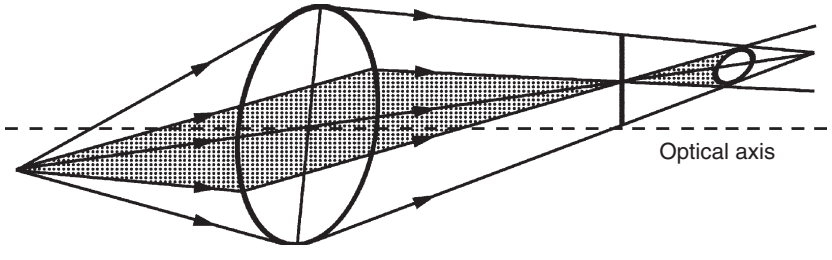

Figure 1.9 Astigmatism is an off-axis aberration.

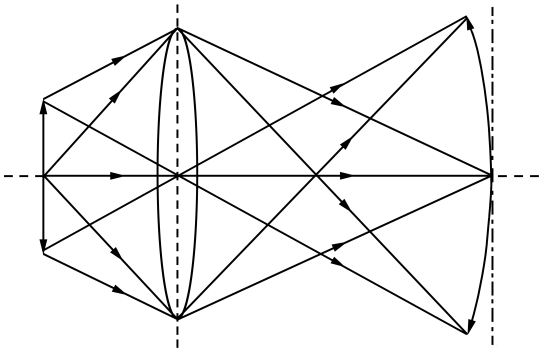

Figure 1.10 Curvature of field is an off-axis aberration.

1.2 Instrumentation

A light microscope includes the following main components:

- illumination system;
- objective lens;
- eyepiece;
- photomicrographic system; and
- specimen stage.

A light microscope for examining material microstructure can use either transmitted or reflected light for illumination. *Reflected-light microscopes* are the most commonly used for metallography, while *transmitted-light microscopes* are typically used to examine transparent or semitransparent materials, such as certain types of polymers. Figure 1.11 illustrates the structure of a light microscope for materials examination.

1.2.1 Illumination System

The illumination system of a microscope provides visible light by which a specimen is observed. There are three main types of electric lamps used in light microscopes:

1) low-voltage tungsten filament bulbs;
2) tungsten–halogen bulbs; and
3) gas-discharge tubes.

Tungsten bulbs provide light of a continuous wavelength spectrum from about 300 to 1500 nm. Their *color temperature* of the light, which is important for color photography, is relatively low. Low color temperature implies warmer (more yellow–red) light while high color temperature implies colder (more blue) light. Tungsten–halogen bulbs, like ordinary tungsten bulbs, provide a continuous

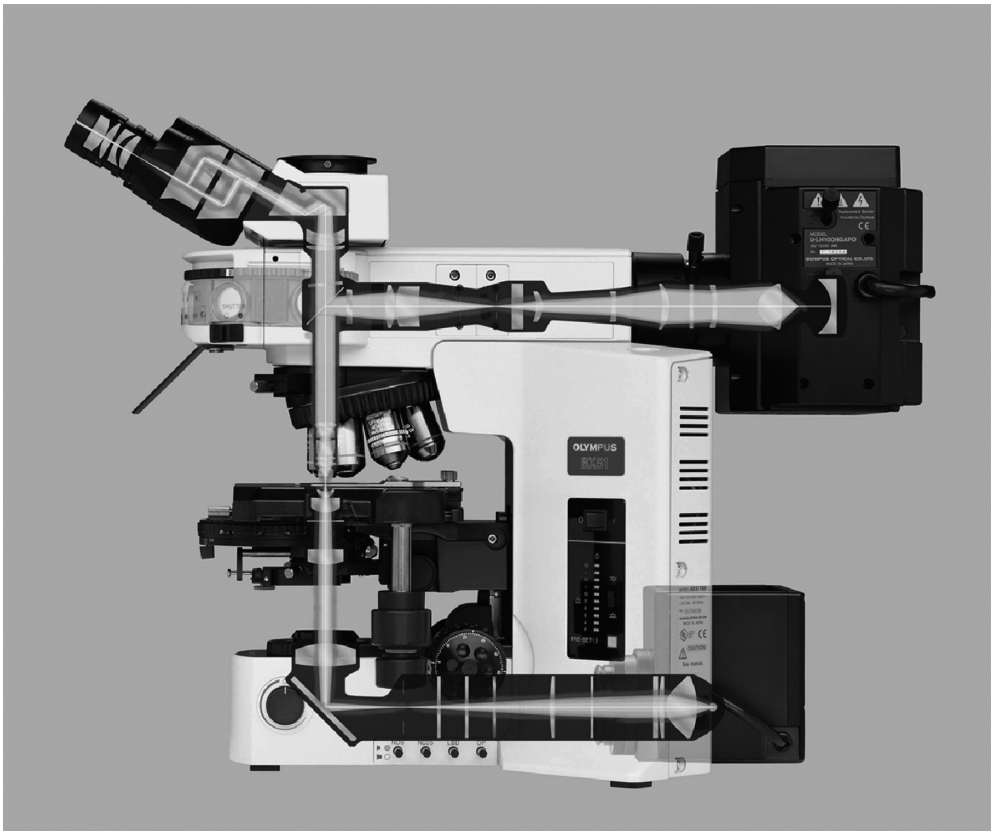

Figure 1.11 An Olympus light microscope used for material examination. The microscope includes transmission- and reflection-illumination systems. (This image is courtesy of Olympus Corporation.)

spectrum. Their light is brighter and the color temperature is significantly higher than ordinary tungsten bulbs. The high filament temperature of tungsten–halogen bulbs, however, needs a heat filter in the light path and good ventilation. Gas-discharge tubes filled with pressurized mercury or xenon vapor provide extremely high brightness. The more commonly used tubes are filled with mercury, of which the arc has a discontinuous spectrum. Xenon has a continuous spectrum and very high color temperature. As with tungsten–halogen bulbs, cooling is required for gas-discharge tubes.

In a modern microscope, the illumination system is composed of a light lamp (commonly a tungsten–halogen bulb), a *collector lens* and a *condenser lens* to provide integral illumination; such a system is known as the *Köhler system*. The main feature of the Köhler system is that the light from the filament of a lamp is first focused at the front focal plane of the condenser lens by a collector lens. Then, the condenser lens collects the light diverging from the source and directs it at a small area of the specimen be examined. The Köhler system provides uniform

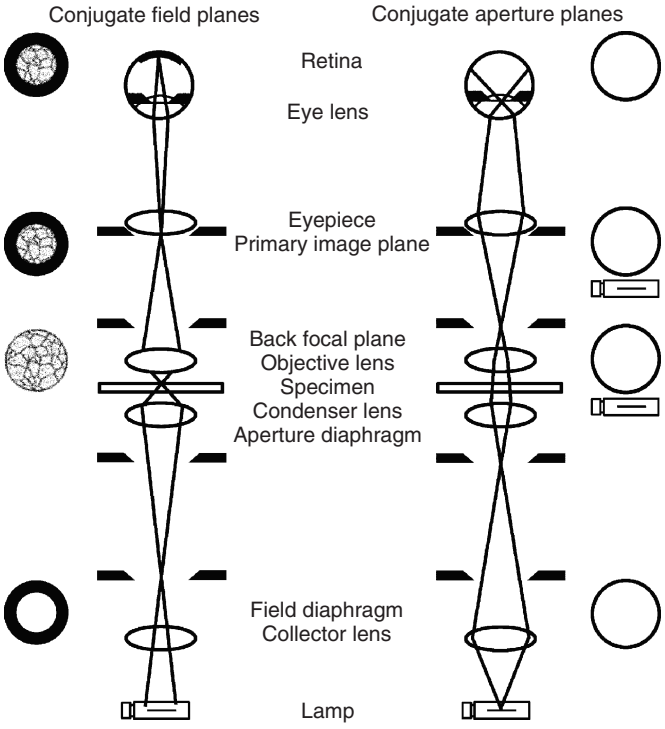

Figure 1.12 Two sets of conjugate focal planes in the Köhler system illustrated in a transmitted-light microscope. Image-forming rays focus on the field planes and illuminating rays focus on the aperture planes. The far left-hand and far right-hand parts of the diagram illustrate the images formed by image-forming rays and illuminating rays, respectively. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

intensity of illumination on the area of specimen. The system generates two sets of *conjugate focal planes* along the optic axis of a microscope as shown in Figure 1.12. One set of focal planes is for illuminating rays; these are known as the *conjugate aperture planes*. Another set comprises the image-forming rays called the *conjugate field planes*. During normal microscope operation, we see only the image-forming rays through the eyepiece. We can use the illuminating rays to check the alignment of the optical system of the microscope.

There are two important controllable diaphragms in the Köhler system: the *field diaphragm* and the *aperture diaphragm*. The field diaphragm is placed at a focal plane for the image-formation rays. Its function is to alter the diameter of the illuminated area of the specimen. When the condenser is focused and centered, we see a sharp image of the field diaphragm with the image of specimen (Figure 1.13). The field diaphragm restricts the area of view and blocks scattering light that could cause glare and image degradation if they entered the objective lens and eyepiece. The aperture diaphragm is placed at a focus plane of the illuminating rays. Its function is to control α, and thus affect the image resolution and depth of field

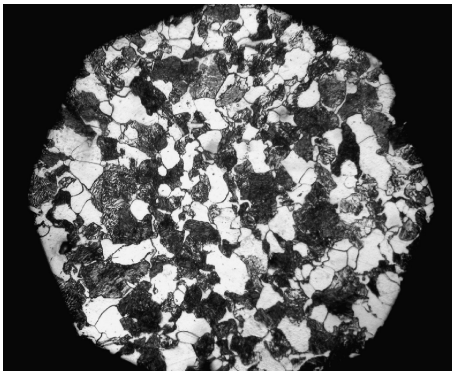

Figure 1.13 Image of the field diaphragm with an image of the specimen. Magnification 100×.

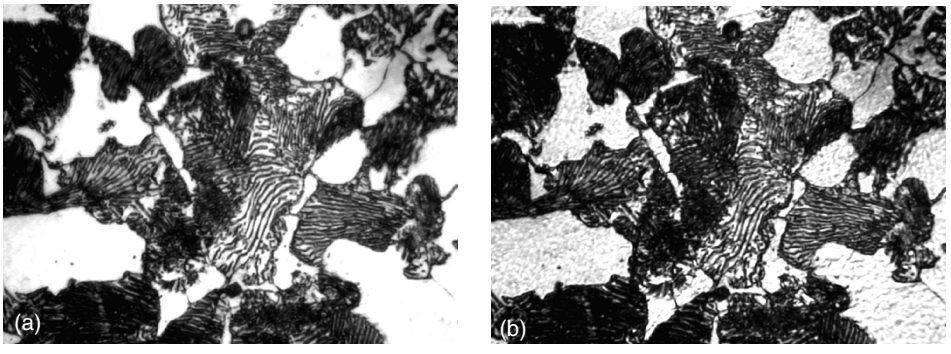

Figure 1.14 Effect of aperture diaphragm on specimen image when: (a) the aperture is large and (b) the aperture is small. Magnification 500×.

(Sections 1.1.2 and 1.1.3). We cannot see the aperture diaphragm with the image of specimen. Figure 1.14 illustrates the influence of the aperture diaphragm on the image of a specimen.

The main difference between transmitted-light and reflected-light microscopes is the illumination system. The Köhler system of reflected light illumination (*epi-illumination*) is illustrated in Figure 1.15 in which a *relay lens* is included. The illuminating rays are reflected by a semitransparent reflector to illuminate the specimen through an objective lens. There is no difference in how reflected and transmitted-light microscopes direct light rays after the rays leave the specimen. There may be a difference in the relative position of the field and aperture diaphragms (Figure 1.12). However, the field diaphragm is always on the focal plane of the image-forming rays while the aperture diaphragm is on a focal plane of the illuminating rays.

Light filters are often included in the light path of illumination systems, even though they are not shown in Figures 1.12 and 1.15. Light filters are used to control the wavelengths and intensity of illumination in microscopes in order to

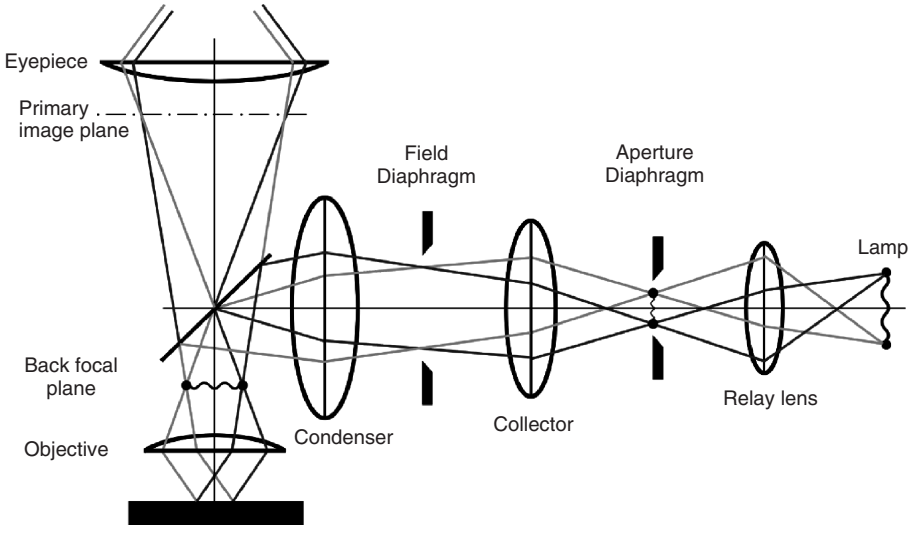

Figure 1.15 Illumination system of a reflected-light microscope with illuminating rays.

achieve optimum visual examination for photomicrography. *Neutral density* (*ND*) *filters* can regulate light intensity without changing wavelength. *Colored filters* and *interference filters* are used to isolate specific colors or bands of wavelength. The colored filters are commonly used to produce a broad band of color, while the interference filters offer sharply defined bandwidths. Colored filters are used to match the color temperature of the light to that required by photographic films. Selected filters can also increase contrast between specimen areas with different colors. *Heat filters* absorb much of the infrared radiation that causes heating of specimen when a tungsten–halogen bulb is used as light source.

1.2.2 Objective Lens and Eyepiece

The objective lens is the most important optical component of a light microscope. The magnification of the objective lens determines the total magnification of the microscope because eyepieces commonly have a fixed magnification of 10×. The objective lens generates the primary image of the specimen, and its resolution determines the final resolution of the image. The numerical aperture (NA) of the objective lens varies from 0.16 to 1.40, depending on the type of lens. A lens with a high magnification has a higher NA. The highest NA for a dry lens (where the medium between the lens and specimen is air) is about 0.95. Further increase in NA can be achieved by using a lens immersed in an oil medium. The oil-immersion lens is often used for examining microstructure greater than 1000× magnification.

Classification of the objective lens is based on its aberration-correction capabilities, mainly chromatic aberration. The following lenses are shown from low to high capability.

- achromat;
- semiachromat (also called ''fluorite''); and
- apochromat.

The achromatic lens corrects chromatic aberration for two wavelengths (red and blue). It requires green illumination to achieve satisfactory results for visual observation of black and white photography. The semiachromatic lens improves correction of chromatic aberration. Its NA is larger than that of an achromatic lens with the same magnification and produces a brighter image and higher resolution of detail. The apochromatic lens provides the highest degree of aberration correction. It almost completely eliminates chromatic aberration. It also provides correction of spherical aberration for two colors. Its NA is even larger than that of a semiachromatic lens. Improvement in quality requires a substantial increase in the complexity of the lens structure, and costs. For example, an apochromatic lens may contain 12 or more optical elements.

The characteristics of an objective lens are engraved on the barrel as shown in Figure 1.16. Engraved markings may include the following abbreviations.

- ''FL,'' ''FLUOR,'' or ''NEOFLUOR'' stands for ''fluorite'' and indicates the lens is semiachromatic;
- ''APO'' indicates that the lens is apochromatic;
- If neither of the above markings appears, then the lens is achromatic;
- ''PLAN'' or ''PL'' stands for ''planar'' and means the lens is corrected for curvature of field, and thus generates a flat field of image;

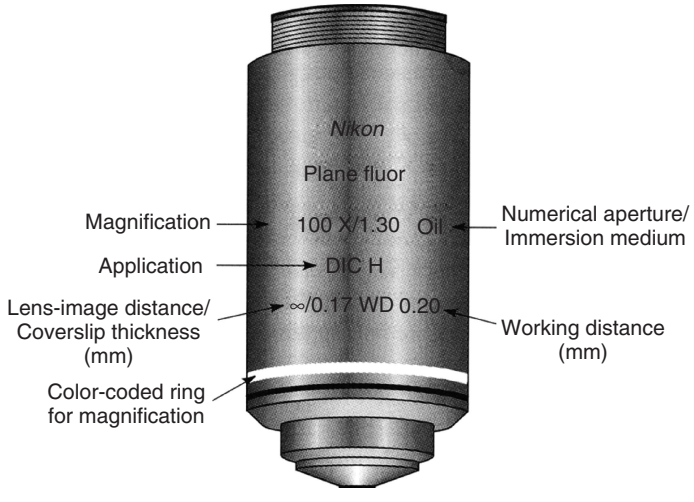

Figure 1.16 Engraved markings on the barrel of an objective lens. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

- "DIC" means the lens includes a Wollaston prism for *differential interference contrast* (Section 1.4.4);
- "PH" or "PHACO" means the lens has a phase ring for phase-contrast microscopy (Section 1.4.2); and
- "number/number" indicates *magnification/numerical aperture*. Thus, "40/0.75" means the lens has a magnification of 40× and a numerical aperture of 0.75.

The eyepiece is used to view the real primary image formed by the objective lens. In some cases it also completes the correction of aberrations. The eyepiece allows a glass disc with an etched graticule to be inserted into the optical path. The graticule serves as a micrometer for measurement. The eyepiece has either a helical thread or a sliding mount as a focusing mechanism. Importantly, the focusing mechanism of an eyepiece provides a "parfocal" adjustment of the optics so that the same focal plane examined by the eye will be in focus on the film plane of the camera mounted on the microscope. Thus, focusing the eyepiece is a necessary step before photographing images in a microscope.

We can summarize the methods for achieving optimum resolution and depth of field in light microscopy. While both resolution and depth of field are crucial for achieving high-quality images, one often is achieved at the expense of the other. Thus, compromises must be made while using good judgment.

1.2.2.1 Steps for Optimum Resolution

- use an objective lens with the highest NA possible;
- use high magnification;
- use an eyepiece compatible with the chosen objective lens;
- use the shortest possible wavelength light;
- keep the light system properly centered;
- use oil immersion lenses if available;
- adjust the field diaphragm for maximum contrast and the aperture diaphragm for maximum resolution and contrast; and
- adjust brightness for best resolution.

1.2.2.2 Steps to Improve Depth of Field

- reduce NA by closing the aperture diaphragm, or use an objective lens with lower NA;
- lower the magnification for a given NA;
- use a high-power eyepiece with a low-power, high- NA objective lens; and
- use the longest possible wavelength light.

1.3 Specimen Preparation

The microstructure of a material can only be viewed in a light microscope after a specimen has been properly prepared. Metallurgists have developed extensive

techniques and accumulated knowledge of metal specimen preparations for over a century. In principle, we can use these techniques to examine not only metallic materials but also ceramics and polymers; in practice, certain modifications are needed and a certain degree of caution must be exercised. The main steps of specimen preparation for light microscopy include the following.

- sectioning;
- mounting;
- grinding;
- polishing; and
- etching.

1.3.1 Sectioning

Sectioning serves two purposes: generating a cross section of the specimen to be examined; and reducing the size of a specimen to be placed on a stage of a light microscope, or reducing the size of a specimen to be embedded in mounting media for further preparation processes. The main methods of sectioning are abrasive cutting, electric discharge machining, and microtomy that is mainly for polymer specimens.

1.3.1.1 Cutting

Abrasive cutting is the most commonly used method for sectioning materials. Specimens are sectioned by a thin rotating disc in which abrasive particles are supported by suitable media. The abrasive cutoff machine is commonly used for sectioning a large sample. The machine sections the sample with a rapidly rotating wheel made of an abrasive material, such as silicon carbide, and bonding materials such as resin and rubber. The wheels are consumed in the sectioning process. Abrasive cutting requires cooling media in order to reduce friction heat. Friction heat can damage specimens and generate artifacts in the microstructure. Commonly used cooling media consist of water-soluble oil and rust-inhibiting chemicals. The abrasive cutoff machine can section large specimens quickly but with poor precision.

More precise cutting can be achieved by a diamond saw or electric discharge machine (EDM) (Figure 1.17). The diamond saw is a precision abrasive cutting machine. It sections specimens with a cutting wheel made of tiny diamond particles bonded to a metallic substrate. A cooling medium is also necessary for diamond saw cutting. Electrically conductive materials can be sectioned by an EDM. Cutting is accomplished by an electric discharge between an electrode and the specimen submerged in a dielectric fluid. EDM is particularly useful for materials that are difficult to section by abrasive cutting. EDM may produce significant changes at the machined surface because the electric discharge melts the solid in the cutting path. The solidified material along a machining path must be carefully removed during further preparation processes.

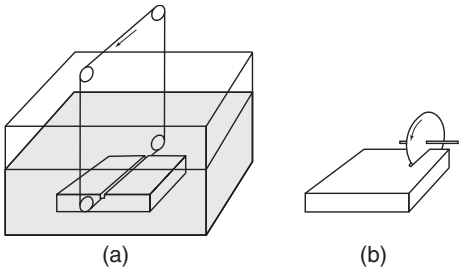

Figure 1.17 Specimen sectioning by: (a) wire cutting with electric discharging and (b) diamond saw sectioning.

1.3.1.2 **Microtomy**

Microtomy refers to sectioning materials with a knife. It is a common technique in biological specimen preparation. It is also used to prepare soft materials such as polymers and soft metals. Tool steel, tungsten carbide, glass, and diamond are used as knife materials. A similar technique, *ultramicrotomy*, is widely used for the preparation of biological and polymer specimens in transmission electron microscopy. This topic is discussed in Chapter 3.

1.3.2 **Mounting**

Mounting refers to embedding specimens in mounting materials (commonly thermosetting polymers) to give them a regular shape for further processing. Mounting is not necessary for bulky specimens, but it is required for specimens that are too small or oddly shaped to be handled or when the edge of a specimen needs to be examined in transverse section. Mounting is popular now because most automatic grinding and polishing machines require specimens to have a cylindrical shape. There are two main types of mounting techniques: *hot mounting* and *cold mounting*.

Hot mounting uses a hot-press equipment as shown in Figure 1.18. A specimen is placed in the cylinder of a press and embedded in polymeric powder. The surface

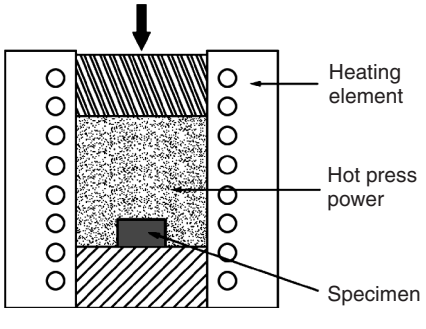

Figure 1.18 Internal arrangement of a hot mounting press.

to be examined faces the bottom of the cylinder. Then, the specimen and powder are heated at about 150 °C under constant pressure for tens of minutes. Heat and pressure enable the powder to bond with the specimen to form a cylinder. Phenolic (bakelite) is the most widely used polymeric powder for hot mounting. Hot mounting is suitable for most metal specimens. However, if the microstructure of the material changes at the mounting temperature, cold mounting should be used.

In cold mounting, a polymer resin, commonly epoxy, is used to cast a mold with the specimen at ambient temperature. Figure 1.19a shows a typical mold and specimens for cold mounting. Figure 1.19b demonstrates the casting of epoxy resin into the mold in which the specimen surface to be examined is facing the bottom. A cold mounting medium has two constituents: a fluid resin and a powder hardener. The resin and hardener should be carefully mixed in proportion following the instructions provided. Curing times for mounting materials vary from tens of minutes to several hours, depending on the resin type. Figure 1.20 shows the specimens after being cold mounted in various resins.

An important issue in the selection of a mounting material is hardness compatibility with the specimen. Generally, plastics used for embedding are not as hard as the specimen, particularly when the specimens are of metallic or ceramic. Too great a difference in hardness can cause inhomogeneous grinding and polishing, which in turn may generate a rough, rather than sharp edge on the specimen. A solution to this problem is to embed metal beads with a specimen to ensure that the grinding surface has a more uniform hardness.

There are a number of other mounting techniques available but they are less widely used. The simplest is *mechanical clamping*, in which a thin sheet of the specimen is clamped in place with a mechanical device. *Adhesive mounting* is glueing a specimen to a large holder. *Vacuum impregnation* is a useful mounting method for porous specimens and ceramics. It removes air from the pores, crevices, and cracks of specimens, and then replaces such empty space in the specimen with epoxy resin. First, a specimen is ground with grit paper to flatten the surface to

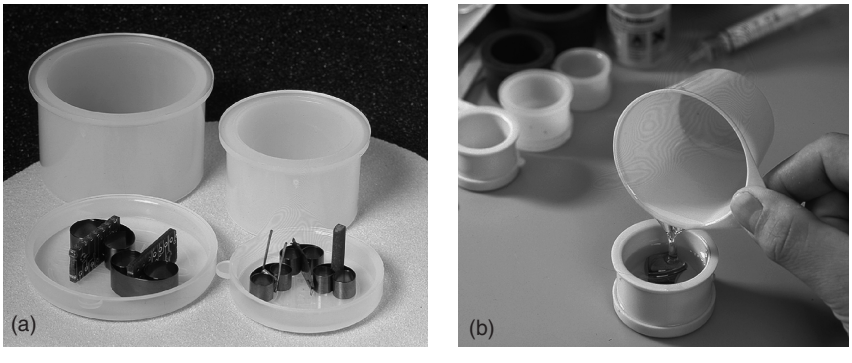

Figure 1.19 Cold mounting of specimens: (a) place specimens on the bottom of molds supported by clamps and (b) cast resin into the mold. (Reproduced with permission of Struers A/S.)

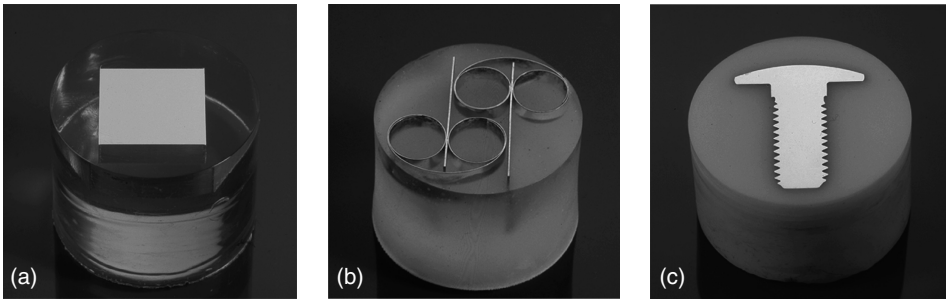

Figure 1.20 Cold mounted specimens: (a) mounted with polyester; (b) mounted with acrylic; and (c) mounted with acrylic and mineral fillers. (Reproduced with permission of Struers A/S.)

be examined. The specimen is placed with the surface uppermost inside the mold in a vacuum chamber. Then, the chamber is evacuated for several minutes before filling the mold with epoxy. The vacuum is maintained for a few minutes and then air is allowed to enter the chamber for a curing period.

1.3.3 Grinding and Polishing

Grinding refers to flattening the surface to be examined and removing any damage caused by sectioning. The specimen surface to be examined is abraded using a graded sequence of abrasives, starting with a coarse grit. Commonly, abrasives (such as silicon carbide) are bonded to abrasive paper. Abrasive paper is graded according to particle size of abrasives such as 120-, 240-, 320-, 400-, and 600-grit paper. The starting grit size depends on the surface roughness and depth of damage from sectioning. Usually, the starting grade is 240 or 320 grit after sectioning with a diamond saw or EDM. Both *hand grinding* and *machine grinding* are commonly used.

1.3.3.1 Grinding

We can perform hand grinding with a simple device in which four belts of abrasive paper (240-, 320-, 400-, and 600-grit) are mounted in parallel as shown in Figure 1.21. Running water is supplied to cool specimen surfaces during hand grinding. Grinding produces damage that must be minimized by subsequent grinding with finer abrasives. The procedure is illustrated in Figure 1.22. In particular, two procedures must be followed to ensure optimal results. First, specimens are rinsed with running water to remove surface debris before switching grinding belts; and secondly, specimens are rotated 90° from the previous orientation. Rotation ensures that grinding damage generated by a coarse grit is completely removed by a subsequent finer grit. Thus, at the end of any grinding step, the only grinding damage present must be from that grinding step. Damage from the final grinding step is removed by polishing.

Figure 1.21 Hand grinding using a simple hand grinding device. (Reproduced with permission of Buehler Ltd.)

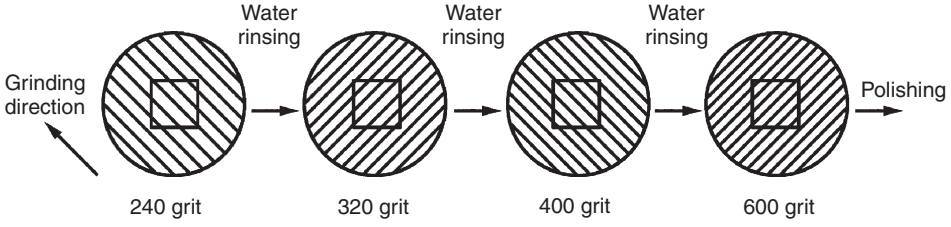

Figure 1.22 Hand grinding procedure.

Automatic grinding machines have become very popular because they reduce tedious work and are able to grind multiple specimens simultaneously. Also, machine grinding produces more consistent results. A disc of abrasive paper is held on the surface of a motor-driven wheel. Specimens are fixed on a holder that also rotates during grinding. Modern grinding machines control the speeds of the grinding wheel and the specimen holder independently. The direction of rotation of the holder and the compressive force between specimen and grinding wheel can also be altered. The machines usually use running water as the cooling medium during grinding to avoid friction heat and to remove loose abrasives that are produced continuously during grinding.

1.3.3.2 **Polishing**

Polishing is the last step in producing a flat, scratch-free surface. After being ground to a 600-grit finish, the specimen should be further polished to remove all visible scratches from grinding. Effects of grinding and polishing a specimen surface are shown in Figure 1.23. Polishing generates a mirror-like finish on the specimen

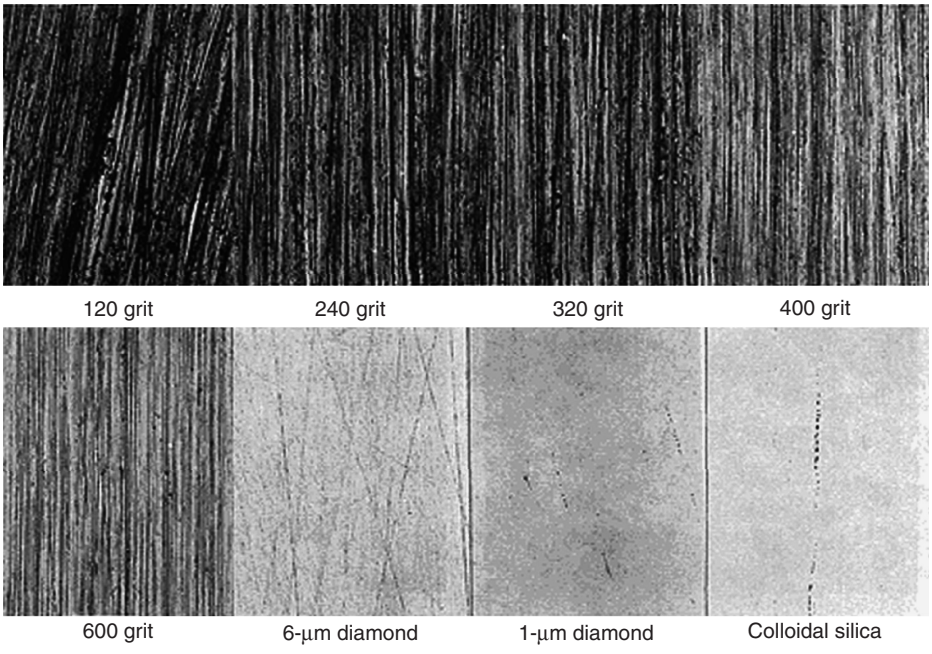

Figure 1.23 Sample of specimen surfaces after grinding and polishing with abrasives of different grits and size. (Reproduced with permission of ASM International®. All Rights Reserved. *www.asminternational.org*. Ref. [2]. © 1984 ASM International®.)

surface to be examined. Polishing is commonly conducted by placing the specimen surface against a rotating wheel either by hand or by a motor-driven specimen holder (Figure 1.24). Abrasives for polishing are usually diamond paste, alumina, or other metal-oxide slurries. Polishing includes coarse and fine polishing. Coarse polishing uses abrasives with a grit size in the range from 3 to 30 μm; 6-μm diamond paste is the most popular. The abrasive size for fine polishing is usually less than 1 μm. Alumina slurries provide a wide range of abrasive size, ranging down to 0.05 μm.

A *polishing cloth* covers a polishing wheel to hold the abrasives against the specimen during polishing. Polishing cloths must not contain any foreign matter that may scratch specimen surfaces. Polishing cloths should also be able to retain abrasives so that abrasives are not easily thrown out from the wheel. For coarse polishing, canvas, nylon, and silk are commonly used as they have little or no nap. For fine polishing, medium- or high-nap cloths are often used; one popular type consists of densely packed, vertical synthetic fibers.

When hand polishing using a polishing wheel, we should not push the specimen too hard against the wheel as excessive force will generate plastic deformation in the top layer of a polished surface. We should also rotate the specimen against the rotation direction of wheel. Without rotation, artifacts of comet tailing will appear on the polished surfaces as shown in Figure 1.25. After each polishing step,

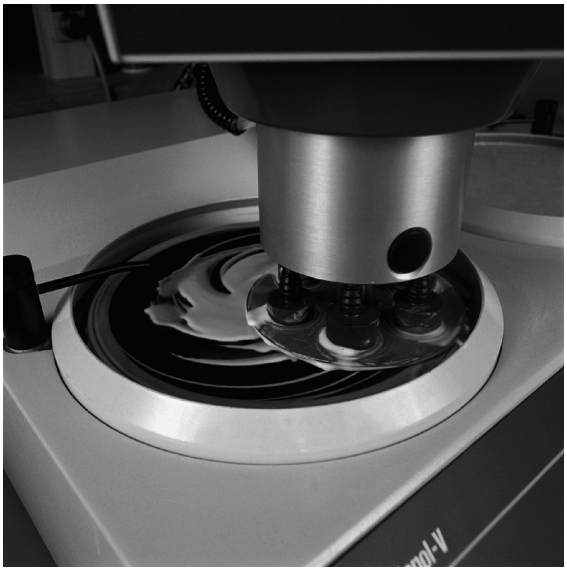

Figure 1.24 Polishing on a rotating wheel with a mechanical sample holder. (Reproduced with permission of Struers A/S.)

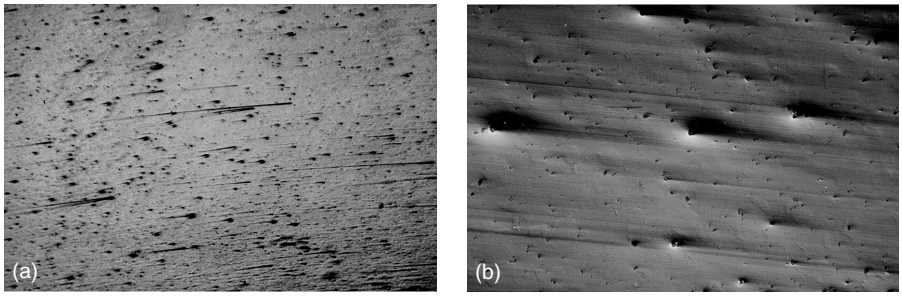

Figure 1.25 Comet tailing generated by polishing on specimen surface: (a) bright-field image and (b) Nomarski contrast image. (Reproduced with permission of Struers A/S.)

the surface should be cleaned in running water with cotton or tissue, followed by alcohol or hot-air drying. Alcohol provides fast drying of surfaces without staining.

Electrolytic polishing is an alternative method of polishing metallic materials. A metal specimen serves as the anode in an electrochemical cell containing an appropriate electrolyte. The surface is smoothed and brightened by the anodic reaction in an electrochemical cell when the correct combination of bath temperature, voltage, current density, and time are used. The advantage of this method over conventional polishing is that there is no chance of plastic deformation during the polishing surface. Plastic deformation in the surface layer of specimens can be generated

by compression and shear forces arising from conventional polishing methods. Plastic deformation from polishing may generate artifacts in microstructures of materials.

The aforementioned methods of specimen preparation, except microtomy, are regarded as an important part of metallography. These methods are also used for nonmetallic materials such as ceramics, composites, and polymers. However, various precautions must be taken in consideration of each material's particular characteristics. For example, ceramic materials are brittle. To avoid fracture they should be mounted and sectioned with a slow-speed diamond saw. Composite materials may exhibit significant differences in mechanical properties between the reinforcement and matrix. These specimens require light pressure and copious cooling during grinding and polishing. Polymeric materials can be examined by either reflected or transmitted-light microscopes. For reflected-light microscopy, specimen preparation is similar to that of metals. For transmitted-light microscopy, a thin-section is required. Both surfaces of the thin section should be ground and polished. This double-sided grinding and polishing can be done by mounting the specimen in epoxy, preparing one surface, mounting that polished surface on a glass slide, and finally grinding and polishing the other side.

1.3.4 Etching

Chemical etching is a method to generate contrast between microstructural features in specimen surfaces. Etching is a controlled corrosion process by electrolytic action between surface areas with differences in electrochemical potential. Electrolytic activity results from local physical or chemical heterogeneities that render some microstructural features anodic and others cathodic under specific etching conditions. During etching, chemicals (*etchants*) selectively dissolve certain areas of the specimen surface because such areas exhibit different electrochemical potentials and will serve as the anode in an electrochemical reaction on the specimen surface. For example, grain boundaries in polycrystalline materials are more severely attacked by etchant, and thus are revealed by light microscopy because they reflect light differently, as illustrated in Figure 1.26a and appear as the dark lines shown in Figure 1.26b. Also, grains are etched at different rates because of differences in grain orientation (certain crystallographic planes are more subject to etching), resulting in crystal faceting. Thus, the grains show different brightness. Etching a specimen that has a multiphase microstructure will result in selective dissolution of the phases.

Many chemical etchants are mixtures of acids with a solvent such as water. Acids oxidize atoms of a specimen surface and change them to cations. Electrons released from atoms of specimen surfaces are combined with hydrogen to form hydrogen gas. For more noble materials, etchants must contain oxidizers (such as nitric acid, chromic acid, iron chloride, and peroxides). Oxidizers release oxygen, which accepts electrons from atoms of the specimen

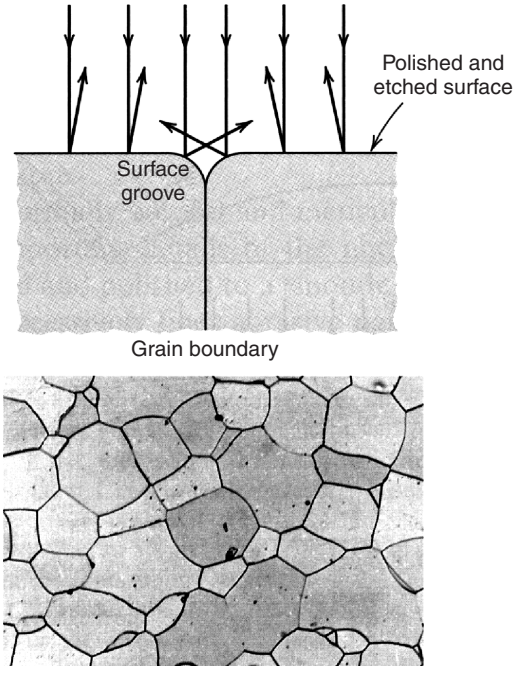

Figure 1.26 Contrast generated by etching grain boundaries in light microscope: (a) reflection from different parts of a surface (Reproduced with permission from Ref. [3]. © 2006 John Wiley & Sons Inc.) and (b) micrograph of grain boundaries that appear as dark lines. (Contribution of the National Institute of Standards and Technology.)

surface. Table 1.1 lists some commonly used etchants, their compositions and applications.

Etching can simply be performed by immersion or swabbing. For immersion etching, the specimen is immersed in a suitable etchant solution for several seconds to several minutes, and then rinsed with running water. The specimen should be gently agitated to eliminate adherent air bubbles during immersion. For swab etching, the polished surface of a specimen is wiped with a soft cotton swab saturated with etchant. Etching can also be assisted with direct electric current, similar to an electrolytic polishing, using the specimen as an anode and an insoluble material (such as platinum) as the cathode in an electrochemical cell filled with electrolyte. The electrochemical reaction on the anode produces selective etching on the specimen surface. Since electrochemical etching is a chemical reaction, besides choosing a suitable etchant and electrolyte, temperature and time are the key parameters to avoiding underetching and overetching of specimens.

We may also use the method of *tint etching* to produce color contrast in microstructures. Tint etchants, usually acidic, are able to deposit a thin (40–500 nm) film such as an oxide or sulfide on specimen surfaces. Tint etching requires a very

Table 1.1 Common etchants for light microscopy.

Materials	Composition[a]	Procedure
Al and alloys	*Keller's reagent* 2.5 ml HNO_3, 1.5 ml HCl 1.0 ml HF, 95 ml water	Immerse 10–20 s
Fe and steels	*Nital* 1–10 ml HNO_3 in 90–99 ml methanol	Immerse few seconds to 1 min
Fe and steels	*Picral* 4–10 g picric acid, 100 ml ethanol	Immerse few seconds to 1 min
Stainless steels	*Vilella's Reagent* 1 g picric acid, 5 ml HCl, 100 ml ethanal	Immerse for up to 1 min
Cu and alloys	2 g $K_2Cr_2O_7$, 8 ml H_2SO_4, 4 drops HCl, 100 ml water	Add the HCl before using; immerse 3–60 s
Ti and alloys	10 ml HF, 5 ml HNO_3, 85 ml water	Swab 3–20 s
Mg and alloys	1 ml HNO_3, 75 ml ethylene glycol, 25 ml water	Swab 3–120 s
Zn and alloys	*Palmerton's Reagent* 40 g CrO_3, 3 g Na_2SO_4, 200 ml water	Immerse up to 3 min; rinse in 20% aq. CrO_3
Co and alloys	15 ml HNO_3, 15 ml acetic acid, 60 ml HCl, 15 ml water	Age 1 h before use; immerse for up to 30 s
Ni and alloys	50 ml HNO_3, 50 ml acetic acid	Immerse or swab 5–30 s; use hood, do not store
Al_2O_3	15 ml water, 85 ml H_3PO_4	Boil 1–5 min
CaO and MgO	Concentrated HCl	Immerse 3 s to a few minutes
CeO_2, $SrTiO_3$, Al_2O_3, and ZrO–ZrC	20 ml water, 20 ml HNO_3, 10 ml HF	Immerse up to 15 min
Si_3N_4	Molten KOH	Immerse 1–8 min
SiC	10 g NaOH, 10 g $K_3Fe(CN)_6$ in 100 ml water at 110 °C	Boil to dryness
Polyethylene (PE)	Xylene	Immerse 1 min at 70 °C
Poly(acrylonitrile butadiene styrene) (ABS); high-impact polystyrene (HIPS); and poly(phenylene oxide) (PPO)	400 ml H_2SO_4, 130 ml H_3PO_4, 125 ml water, 20 g CrO_3	Immerse 15–180 s
Polypropylene (PP)	6 M CrO_3	Immerse 96 h at 70 °C
Phenol formaldehyde	75 ml dimethyl sulfoxide, 25 ml HNO_3	Immerse 4 h at 75–80 °C

[a]The names of reagents are given in italics.

high-quality polished surface for best results. Tint etching can also be done by *heat tinting*, a process by which a specimen is heated to a relatively low temperature in air. As it warms, the polished surface is oxidized. The oxidation rate varies with the phase and chemical composition of the specimen. Thus, differences in the thickness of oxidation films on surfaces generate variations in color. Interference colors are obtained once the film reaches a certain thickness. The effectiveness of heat tinting depends on the material of specimens: it is effective for alloy steels and other nonferrous metals and carbides, but not for carbon or low-alloy steels.

1.4 Imaging Modes

The differences in properties of the light waves reflected from microscopic objects enable us to observe these objects by light microscopy. The light wave changes in either amplitude or phase when it interacts with an object as illustrated in Figure 1.27. The eye can only appreciate amplitude and wavelength differences in light waves, not their phase difference. The most commonly used examination modes, *bright-field* and *dark-field imaging*, are based on contrast due to differences in wave amplitudes. The wave phase differences have to be converted to amplitude differences through special optical arrangements such as in the examination modes of *phase contrast, polarized light*, and *Nomarski contrast*. This section introduces commonly used modes of light microscopy for materials characterization.

1.4.1 Bright-Field and Dark-Field Imaging

Bright-field imaging is the predominant mode for examining microstructure. Dark-field imaging is also widely used to obtain an image with higher contrast than in

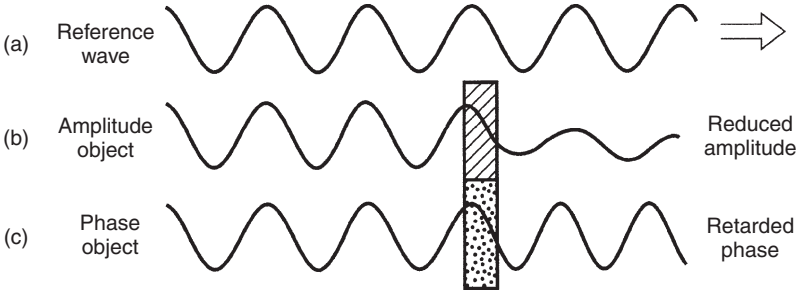

Figure 1.27 (a) Reference wave; (b) amplitude difference; and (c) phase difference generated by objects. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

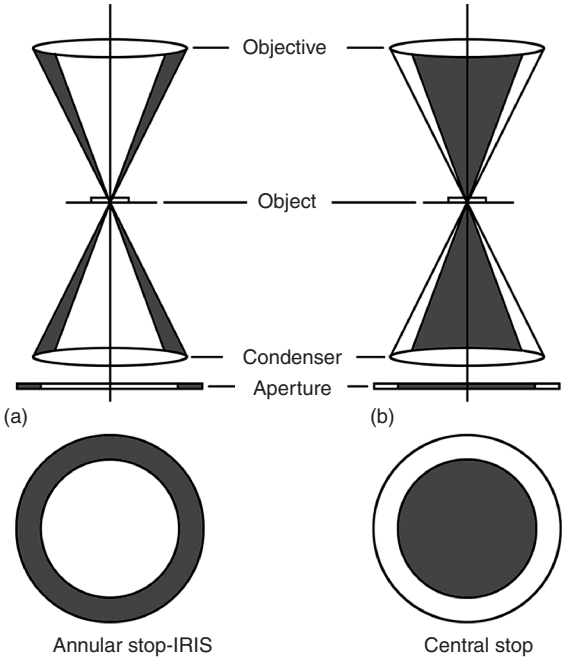

Figure 1.28 (a) Bright-field illumination and (b) dark-field illumination in transmitted mode. Shaded areas indicate where the light is blocked.

bright-field imaging. Figure 1.28 illustrates the difference in optical arrangement between these modes in transmitted illumination. In bright-field imaging, the specimen is evenly illuminated by a light source. Dark-field imaging requires that the specimen is illuminated by oblique light rays. There is a central stop in the light path to block the central portion of light rays from illuminating the specimen directly. Thus, the angle of the light rays illuminating the specimen is so large that light from the specimen cannot enter the objective lens unless it is scattered by microscopic objects. The dark field in reflected illumination is also realized using a central stop in the light path (Figure 1.29), similar to that of transmitted illumination. The light rays in a ring shape will be further reflected in order to illuminate a specimen surface with an oblique angle. Figure 1.30 shows the comparison between bright- and dark-field images of an identical field in a high-carbon steel specimen under a reflected-light microscope. Microscopic features such as grain boundaries and second-phase particles appear self-luminous in the dark-field image, as shown in Figure 1.30.

1.4.2 Phase-Contrast Microscopy

Phase contrast is a useful technique for specimens such as polymers that have little inherent contrast in the bright-field mode. In the technique, a phase change due to

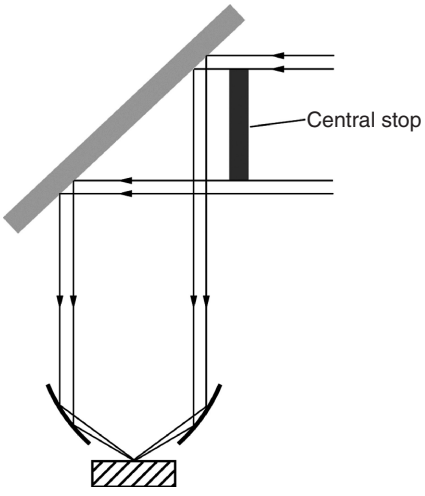

Figure 1.29 Dark-field illumination in a reflected-light microscope.

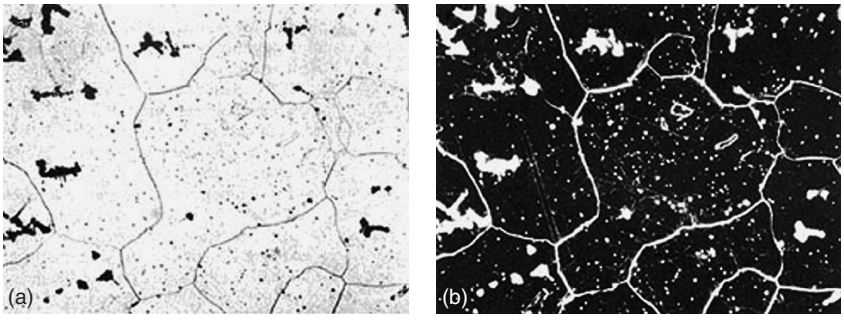

Figure 1.30 Comparison between: (a) bright-field and (b) dark-field images of AISI 1080 high carbon steel. In addition to grain boundaries and oxide particles, annealing twins are revealed in the dark-field image. (Reproduced with permission of ASM International®. All Rights Reserved. *www.asminternational.org*. Ref. [2]. © 1984 ASM International®.)

light diffraction by an object is converted to an amplitude change. This conversion is based on interference phenomenon of light waves as illustrated in Figure 1.31. Constructive interference occurs when combining two same-wavelength waves that do not have a phase difference between them. However, completely destructive interference occurs when combining two waves with a phase difference of a half-wavelength $\left(\frac{\lambda}{2}\right)$.

Figure 1.32 illustrates generation of phase contrast by a special optical arrangement in a transmitted-light microscope. This optical arrangement creates

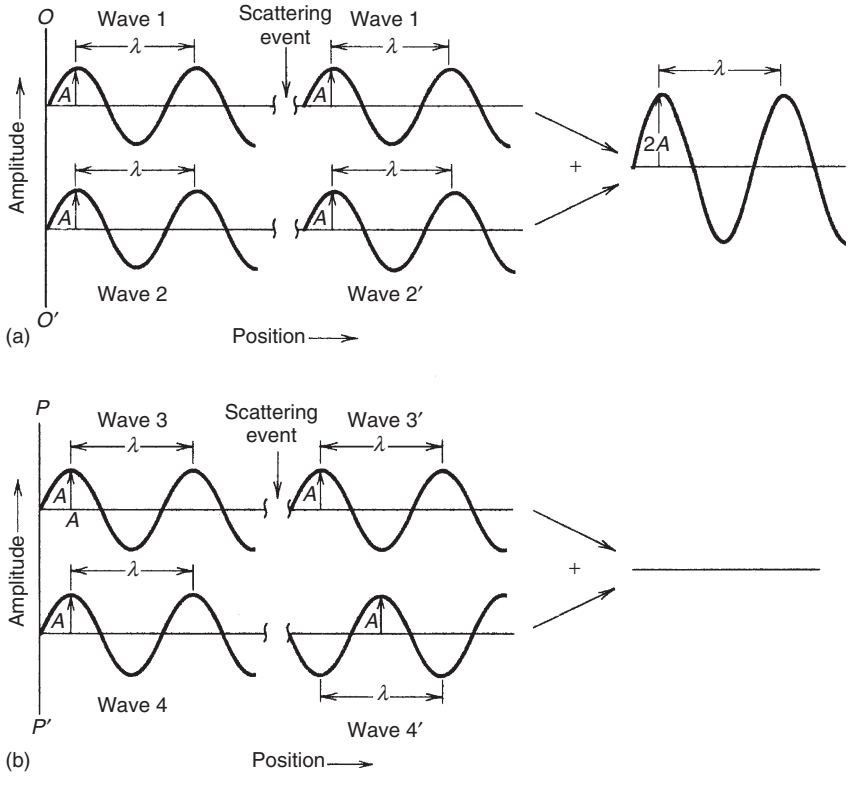

Figure 1.31 Illustration interference between waves: (a) constructive interference and (b) completely destructive interference. (Reproduced with permission from Ref. [3]. © 2006 John Wiley & Sons Inc.)

completely destructive interference when light is diffracted by an object in the specimen. A *condenser annulus*, an opaque black plate with a transparent ring, is placed in the front focal plane of the condenser lens. Thus, the specimen is illuminated by light beams emanating from a ring. The light beam that passes through a specimen without diffraction by an object (the straight-through light beam) will pass the ring of a *phase plate* placed at the back focal plane of the objective lens. The phase plate is a plate of glass with an etched ring of reduced thickness. The ring with reduced thickness in the phase plate enables the waves of the straight-through beam to be advanced by $\frac{\lambda}{4}$. The light beam diffracted by the object in the specimen cannot pass through the ring of the phase plate but only through the other areas of the phase plate. If the diffracted beam is delayed by $\frac{\lambda}{4}$ while passing through the object, a total $\left(\frac{\lambda}{2}\right)$ difference in phase is generated.

When the straight-through beam and diffracted beam recombine at the image plane, completely destructive interference occurs. Thus, we expect a dark image of the object in phase-contrast microscopy. Variation in phase retardation across

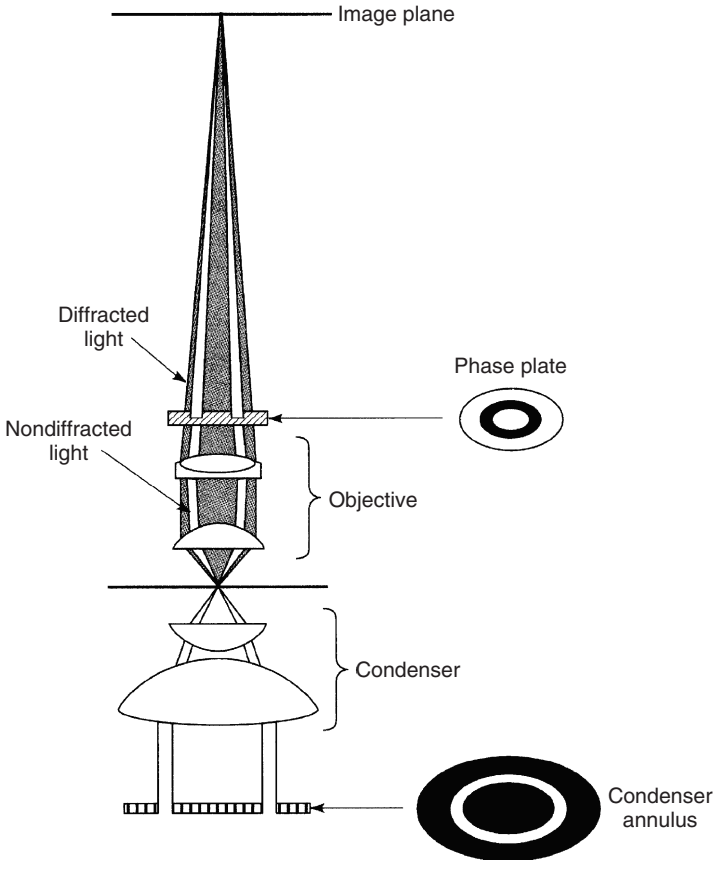

Figure 1.32 Optical arrangement of phase-contrast microscopy. Shading marks the paths of diffracted light. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

the specimen produces variations in contrast. Figure 1.33 shows image differences between bright-field and phase-contrast images of composites in transmitted-light microscopy. In reflected-light microscopy, phase contrast can also be created with a condenser annulus and phase plate similar to those in transmitted-light microscopy.

1.4.3 Polarized-Light Microscopy

Polarized light is used to examine specimens exhibiting optical anisotropy. Optical anisotropy arises when materials transmit or reflect light with different velocities in different directions. Most materials exhibiting optical anisotropy

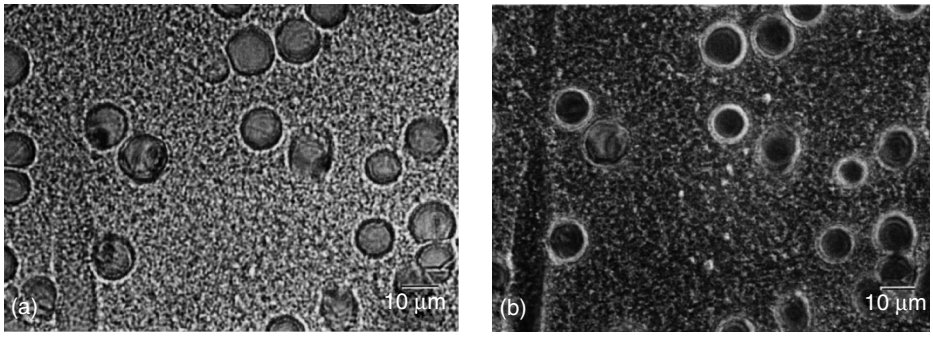

Figure 1.33 Comparison of light transmission images of glass-fiber-reinforced polyamide in: (a) bright-field and (b) phase-contrast modes. (Reproduced with kind permission of Springer Science and Business Media from Ref. [4]. © 1996 Springer Science.)

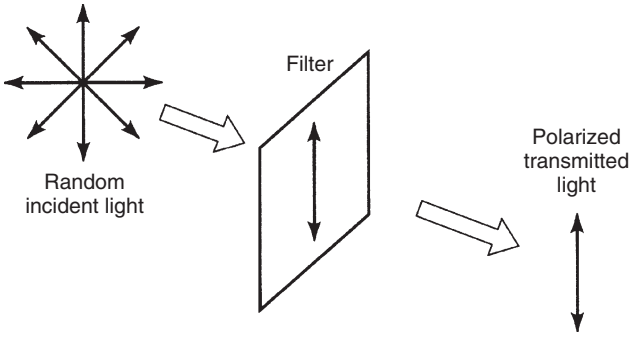

Figure 1.34 Formation of plane-polarized light by a polarizing filter. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

have a noncubic crystal structure. Light, as an electromagnetic wave, vibrates in all directions perpendicular to the direction of propagation. If light waves pass through a polarizing filter, called a *polarizer*, the transmitted wave will vibrate in a single plane as illustrated in Figure 1.34. Such light is referred to as *plane-polarized light*. When polarized light is transmitted or reflected by anisotropic material, the polarized light vibrates in a different plane from the incident plane. Such polarization changes generate the contrast associated with anisotropic materials.

Figure 1.35 illustrates the interaction between polarized light and an anisotropic object. For a transparent crystal, the optical anisotropy is called *double refraction* or *birefringence*, because refractive indices are different in two perpendicular directions of the crystal. When a polarized light ray hits a birefringent crystal, the light ray is split into two polarized light waves (*ordinary wave* and *extraordinary wave*) vibrating in two planes perpendicular to each other. Because there are two refractive indices,

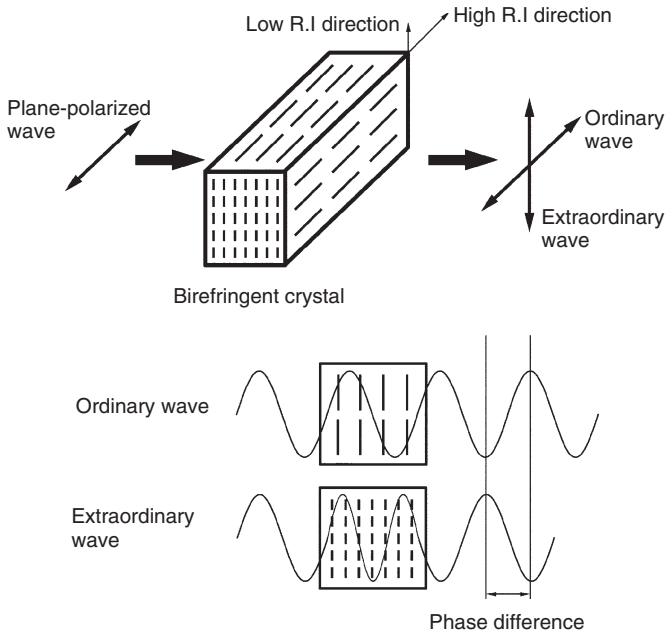

Figure 1.35 Interaction between plane-polarized light and a birefringent object. Two plane-polarized light waves with a phase difference are generated by the materials. RI, refractive index.

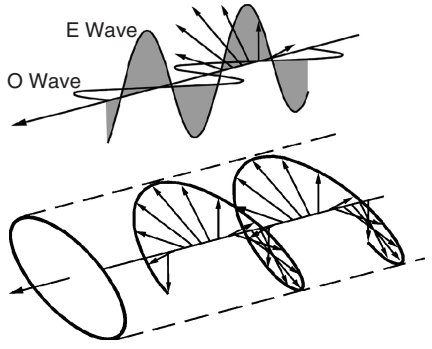

Figure 1.36 Resultant polarized light by vector addition of two plane-polarized light waves from a birefringent object, the ordinary (O) wave and extraordinary (E) wave. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

the two split light rays travel at different velocities, and thus exhibit a phase difference. These two rays, with a phase difference vibrating in two perpendicular planes, produce resultant polarized light because the electric vectors of their waves are added (Figure 1.36). The resultant polarized light is called *elliptically polarized light* because the projection of resultant vectors in a plane is elliptical.

If the two polarized-light waves with equal amplitude have a phase difference of $\frac{\lambda}{4}$, the projection of resultant light is a spiraling circle. If the two polarized-light waves with equal amplitude have a phase difference of $\left(\frac{\lambda}{2}\right)$, the projection of resultant light is linear (45° from two perpendicular directions of polarized-light wave planes). If the two polarized-light waves have another phase difference, the projection of the resultant ray is a spiraling ellipse.

Differences in the resultant light can be detected by another polarizing filter called an *analyzer*. Both polarizer and analyzer can only allow plane-polarized light to be transmitted. The analyzer has a different orientation of polarization plane with respect to that of the polarizer. Figure 1.37 illustrates the amplitude of elliptically polarized light in a two-dimensional plane and the light amplitude passing through an analyzer that is placed 90° with respect to the polarizer (called the *crossed position* of the polarizer and analyzer).

Anisotropic materials are readily identified by exposure to polarized light because their images can be observed with the polarizer and analyzer in the crossed position. Such situations are illustrated in Figure 1.37 as the phase difference between ordinary and extraordinary wave are not equal to zero nor to $m\lambda$ (m is an integer). Understandably, when examining anisotropic materials with polarized light, rotation of the analyzer through 360° will generate two positions of maximum and two positions of minimum light intensity.

Polarized light can enhance the contrast of anisotropic materials, particularly when they are difficult to etch. It can also determine the optical axis, demonstrate *pleochroism* (showing different colors in different directions) and examine the thickness of anisotropic coatings from its cross sections. Figure 1.38 demonstrates

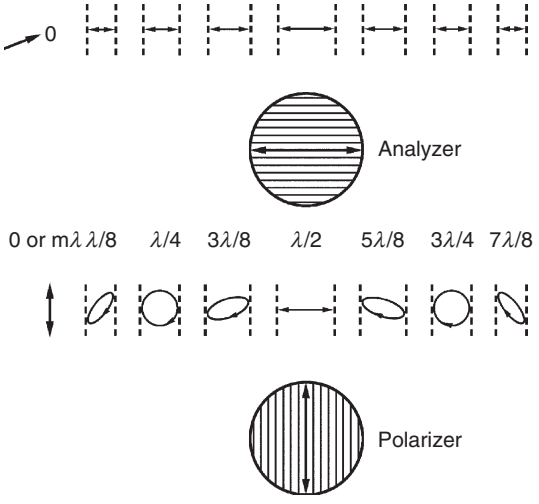

Figure 1.37 Intensity change of polarized light passing through an analyzer when the elliptically polarized light changes. The polarizer and analyzer are in a crossed position. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

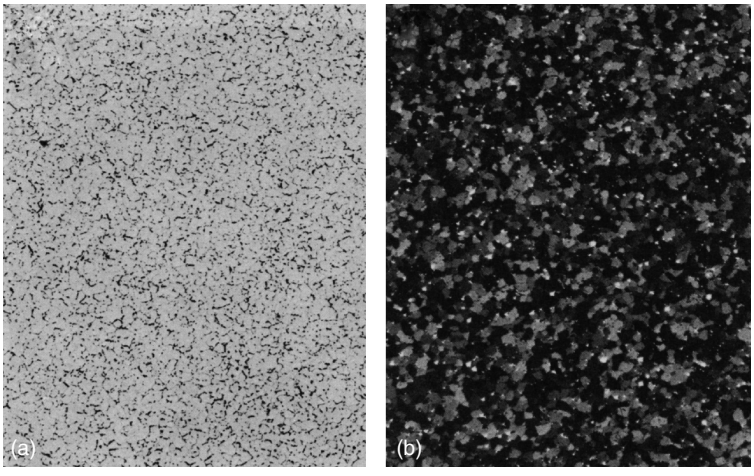

Figure 1.38 Example of using polarized-light microscopy for pure titanium specimen: (a) bright-field image and (b) polarized-light image in which grains are revealed. (Reproduced from Ref. [2]. © N. Gendron, General Electric Co.)

that polarized light reveals the grains of pure titanium, which cannot be seen in the bright-field mode. Figure 1.39 shows a polarized-light micrograph of high-density polyethylene (HDPE) that has been crystallized. Polarized light revealed fine spherulite structures caused by crystallization.

An isotropic material (either with cubic crystal structure or amorphous) cannot change the plane orientation of polarizing light. When a polarized light wave leaves the material and passes through the analyzer in a crossed position, the light will be extinguished. This situation is equivalent to the case of a resultant ray having a phase difference of zero or $m\lambda$. However, isotropic materials can be examined in the polarized-light mode when optical anisotropy is introduced into the materials or on their surfaces. For example, if an isotropic transparent crystal is elastically

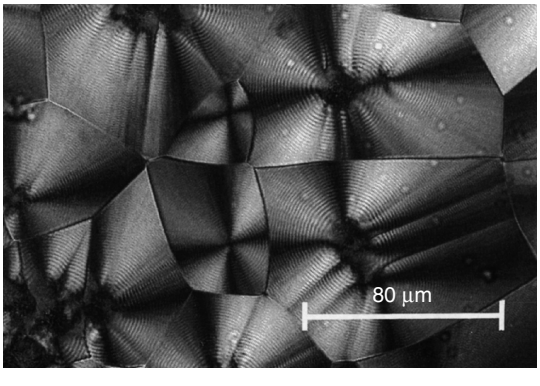

Figure 1.39 Polarized-light micrograph of crystallized high-density polyethylene (HDPE). (Reproduced with permission from Ref. [5]. © 2000 John Wiley & Sons Ltd.)

deformed, it becomes optically anisotropic. A thick oxide film on isotropic metals also makes them sensitive to the direction of polarized light because of double reflection from surface irregularities in the film.

1.4.4 Nomarski Microscopy

Nomarski microscopy is an examination mode using diffraction interference contrast, DIC. The images that DIC produces are deceptively three-dimensional with apparent shadows and a relief-like appearance. Nomarski microscopy also uses polarized light with the polarizer and the analyzer arranged as in the polarized-light mode. In addition, double quartz prisms (*Wollaston prisms* or *DIC prisms*) are used to split polarized light and generate a phase difference.

The working principles of Nomarski microscopy can be illustrated using the light path in a transmitted-light microscope as illustrated in Figure 1.40. The first DIC

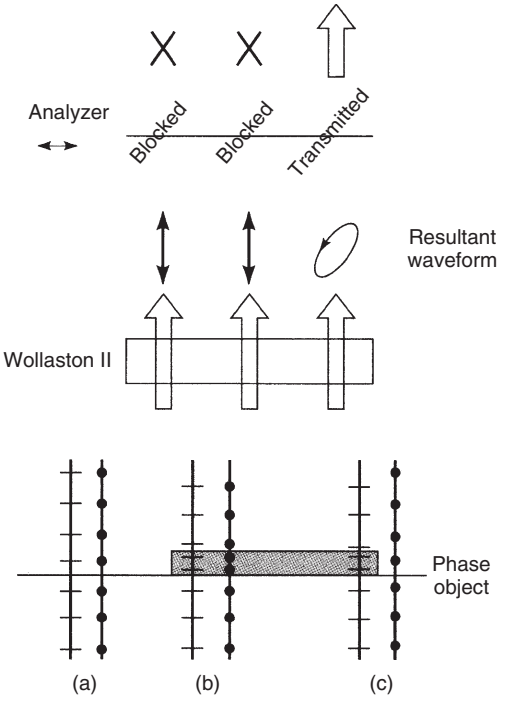

Figure 1.40 Nomarski contrast generation using polarized light. The first differential interference contrast (DIC) prism (not shown) generates two parallel polarized beams illuminating the specimen. The second DIC prism recombines two beams. Elliptically polarized light is generated by the second DIC prism when a phase difference between the two beams is induced by an object: (a) both polarized beams do not pass through a phase object; (b) both beams pass through a phase object; and (c) one of the two beams passes through a phase object. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

prism is placed behind the polarizer and in front of the condenser lens, and the second DIC prism is placed behind the objective lens and in front of the analyzer. The two beams created by the prism interfere coherently in the image plane and produce two slightly displaced images differing in phase, thus producing height contrast. The first DIC prism splits the polarized-light beam from the polarizer into two parallel beams traveling along different physical paths. If a specimen does not generate a path difference between the two parallel beams as shown by two left-side beam pairs in Figure 1.40, the second DIC prism recombines the pairs and produces linearly polarized light with the same polarization plane as it was before it was split by the first DIC prism. Thus, the analyzer in the crossed position with a polarizer will block light transmission. However, if a specimen generates a path difference in a pair of beams, as shown by the right-side beam pair, the recombined pair produced by the second DIC prism will be elliptically polarized light. The analyzer cannot block such light and a bright area will be visible.

The optical arrangement of Nomarski microscopy in a reflected-light microscope is similar to that of a transmitted-light microscope, except that there is only one DIC prism serving both functions of splitting the incident beam and recombining reflected beams as illustrated in Figure 1.41. The DIC prism is commonly integrated in the barrel of the objective lens, indicated by "DIC" marked on the barrel surface (Section 1.2.2).

Figure 1.42 compares bright-field and Nomarski images using a carbon steel specimen as an example. Contrast enhancements are achieved in the Nomarski

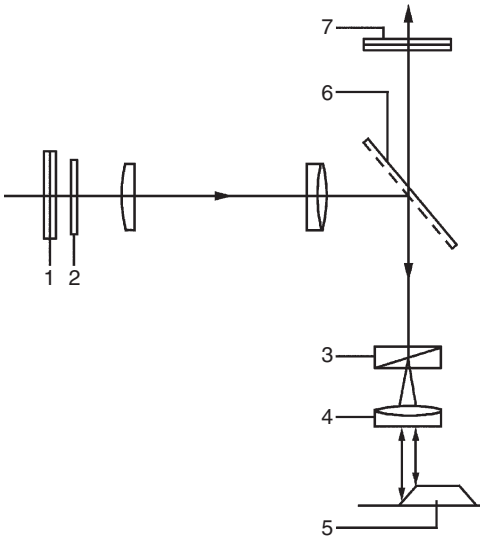

Figure 1.41 Optical arrangement of Nomarski microscopy in reflected light illumination. 1, polarizer; 2, $\frac{\lambda}{2}$-plate; 3, DIC prism; 4, objective lens; 5, specimen; 6, light reflector; and 7, analyzer.

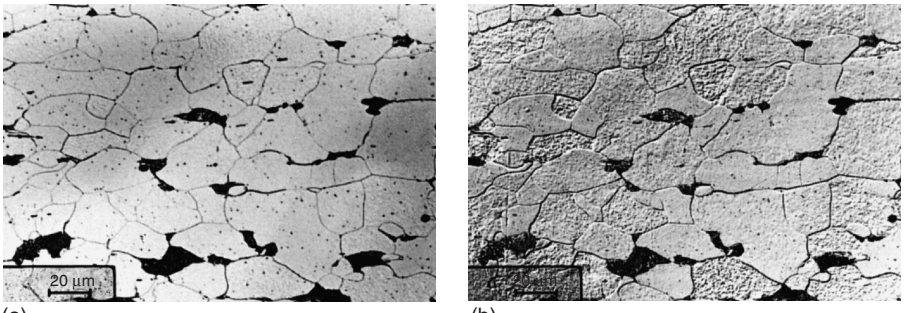

Figure 1.42 Effects of Nomarski contrast on carbon steel micrographs: (a) bright field and (b) Nomarski contrast of the same field. (Reproduced with permission of Gonde Kiessler.)

micrographs. The Nomarski image appears three-dimensional and illuminated by a low-angle light source. However, the image does not necessarily represent real topographic features of a surface because the shadows and highlights that result from phase differences may not correspond to low and high relief on the surface, particularly in transmitted-light microscopy. The reason for this is that the phase differences generated in Nomarski microscopy may result from differences either in the optical path or in refractive index.

1.4.5 Fluorescence Microscopy

Fluorescence microscopy is useful for examining objects that emit fluorescent light. Fluorescence is an optical phenomenon; it occurs when an object emits light of a given wavelength when excited by incident light. The incident light must have sufficient energy, that is, a shorter wavelength than that light emitting from the object, to excite fluorescence. While only a small number of materials exhibit this capability, certain types of materials can be stained with fluorescent dyes (*fluorochromes*). The fluorochromes can selectively dye certain constituents in materials, called *fluorescent labeling*. Fluorescent labeling is widely used for polymeric and biological samples.

Fluorescence microscopy can be performed by either transmitted or reflected illumination (epi-illumination). Reflected light is more commonly used because it entails less loss of excited fluorescence than transmitted light. Figure 1.43 illustrates the optical arrangement for fluorescence microscopy with epi-illumination. A high-pressure mercury or xenon light can be used for generating high-intensity, short-wavelength light. The light source should be ultraviolet, violet, or blue, depending on the types of fluorochromes used in the specimen. A fluorescence filter set, arranged in a cube as shown in Figure 1.43, includes an *exciter filter*, *dichroic mirror*, and a *barrier filter*. The exciter filter selectively transmits a band of short wavelengths for exciting a specific fluorochrome, while blocking other wavelengths. The dichroic mirror reflects short-wavelength light to the objective

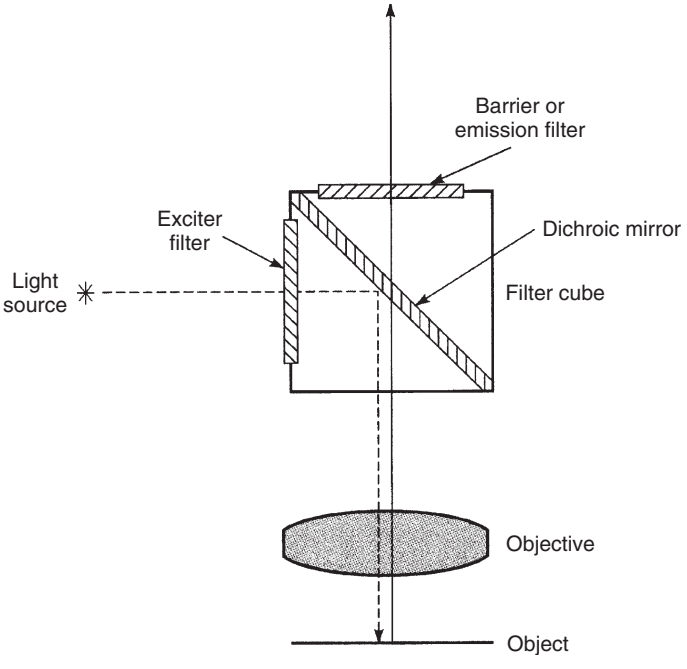

Figure 1.43 Optical arrangement for fluorescence microscopy with epi-illumination. The dotted line indicates the path of excitation light, and the solid line indicates the path of fluorescent light. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

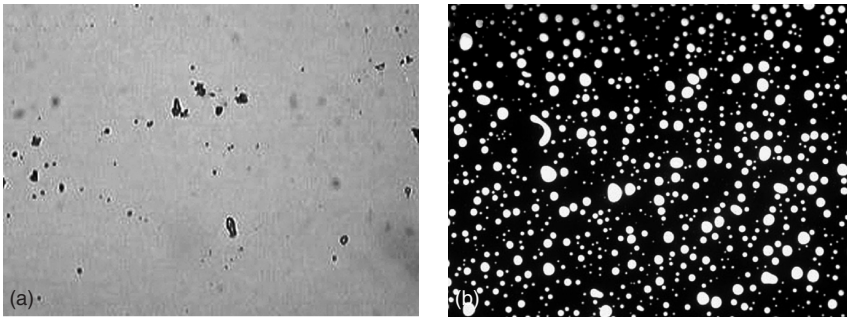

Figure 1.44 Micrographs of asphalt–polyolefin elastomer (POE) blend obtained with transmitted-light microscopy: (a) bright-field image that cannot reveal the two phases in the blend and (b) a fluorescence-labeled image that reveals two-phase morphology. (Reproduced with permission of Jingshen Wu.)

lens and specimen, and also transmits returning fluorescent light toward the barrier filter. The barrier filter transmits excited fluorescent light only, by blocking other short-wavelength light. Figure 1.44 shows an example of fluorescence microscopy used for examining a polymer blend. Fluorescence labeling reveals the dispersed

polymer particles in an asphalt matrix, which cannot be seen in the bright-field image.

1.5 Confocal Microscopy

Confocal microscopy is a related new technique that provides three-dimensional (3D) optical resolution. Image formation in a confocal microscope is significantly different from a conventional light microscope. Compared with a conventional compound microscope, a modern confocal microscope has two distinctive features in its structure: a laser light source and a scanning device. Thus, the confocal microscope is often referred to as the *confocal laser scanning microscope* (CLSM). The laser light provides a high-intensity beam to generate image signals from individual microscopic spots in the specimen. The scanning device moves the beam in a rectangular area of specimen to construct a 3D image on a computer.

1.5.1 Working Principles

The optical principles of confocal microscopy can be understood by examining the CLSM optical path that has reflected illumination as illustrated in Figure 1.45. The laser beam is focused as an intense spot on a certain focal plane of the specimen by a condenser lens, which also serves as an objective lens to collect the reflected beam. A pinhole aperture is placed at a confocal plane in front of the light detector. The reflected beam from the focal plane in a specimen becomes a focused point at the confocal plane. The pinhole aperture blocks the reflected light from the out-of-focal plane from entering the detector. Only the light signals from the focal point in the specimen are recorded each time. Since the pinhole aperture can block a large amount of reflected light, high-intensity laser illumination is necessary to ensure that sufficient signals are received by the detector. The detector is commonly a *photomultiplier tube* (PMT) that converts light signals to electric signals for image processing in a computer.

To acquire an image of the focal plane, the plane has to be scanned in its two lateral directions (x–y directions). To acquire a 3D image of a specimen, the plane images at different vertical positions should also be recorded. A scanning device moves the focal laser spot in the x–y directions on the plane in a regular pattern called a *raster*. After finishing one scanning plane, the focal spot is moved in the vertical direction to scan the next parallel plane.

Figure 1.46 illustrates two different methods of scanning in CLSM: specimen and laser scanning. Specimen scanning was used in early confocal microscopes. The specimen moves with respect to the focal spot and the optical arrangement is kept stationary, as shown in Figure 1.46a. The beam is always located at the

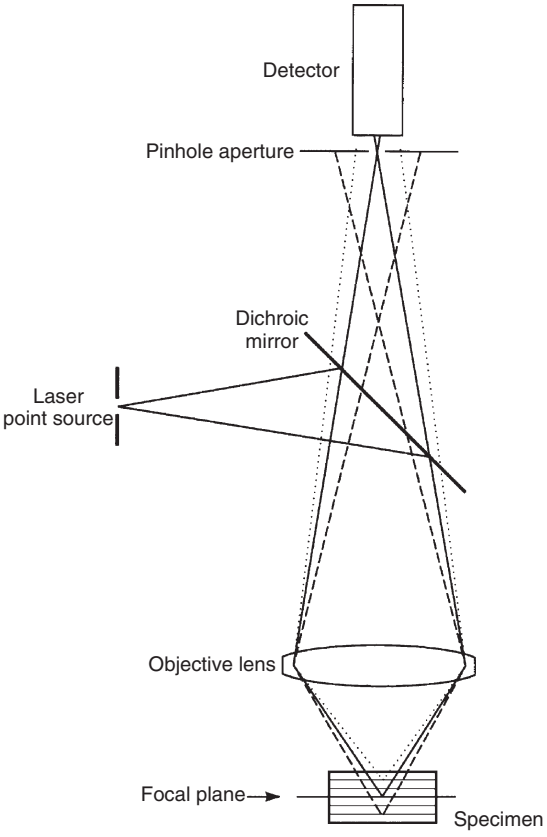

Figure 1.45 Optical path in the confocal microscope. (Reproduced with permission from Ref. [1]. © 2001 John Wiley & Sons Inc.)

optical axis in the microscope so that optical aberration is minimized. The main drawback of this method is the low scanning speed. Laser scanning is realized by two scanning mirrors rotating along mutually perpendicular axes as shown in Figure 1.46b. The scan mirror can move the focal spot in the specimen by sensitively changing the reflecting angle of the mirror. Changing the vertical position of the spot is still achieved by moving the specimen in the laser-scanning method.

The resolution of the confocal microscope is mainly determined by the size of the focal spot of the laser beam. High spatial resolution of about 0.2 μm can be achieved. A 3D image of specimen with thickness of up to 200 μm is also achievable, depending on the opacity of specimen. Most confocal microscopes are the fluorescence type. The microscopic features under the specimen surface are effectively revealed when they are labeled with fluorescent dyes. Therefore, the major use of confocal microscopy is confocal fluorescence microscopy in biology.

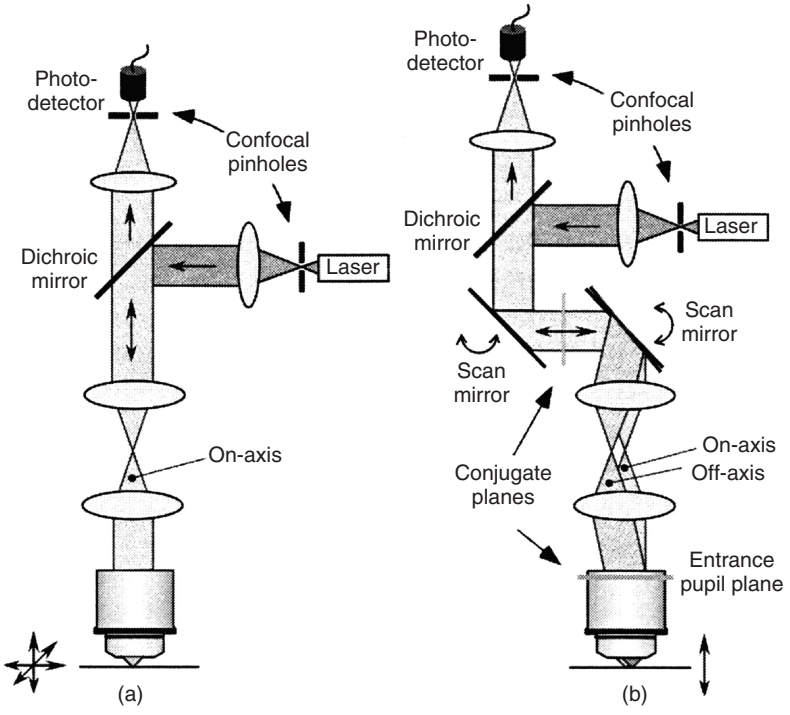

Figure 1.46 Two scanning methods in the confocal microscope: (a) specimen scanning and (b) laser scanning. (Reproduced with permission from Ref. [6]. © 2006 Michiel Müller.)

1.5.2
Three-Dimensional Images

The technique of confocal microscopy can be considered as optical sectioning. A three-dimensional (3D) image is obtained by reconstructing a deck of plane images. Figure 1.47 shows an example of how a 3D image is formed with confocal fluorescence microscopy. A biological specimen, a *Spathiphyllum* pollen grain, was fluorescently labeled with acridine orange. In total, 80 sections of the pollen grain were imaged for 3D reconstruction. The vertical distance between sections is 1.1 μm. Figure 1.47 shows 11 of 80 plane images and the reconstructed 3D image.

Although its major applications are in biology, confocal microscopy can also be used for examining the surface topography and internal structure of semitransparent materials. Figure 1.48 shows an example of using confocal fluorescent microscopy to examine particulate penetration through an air filter made of polymer foam. The particulates were fluorescently labeled and their locations in the polymer foam are revealed against the polymer-foam surfaces.

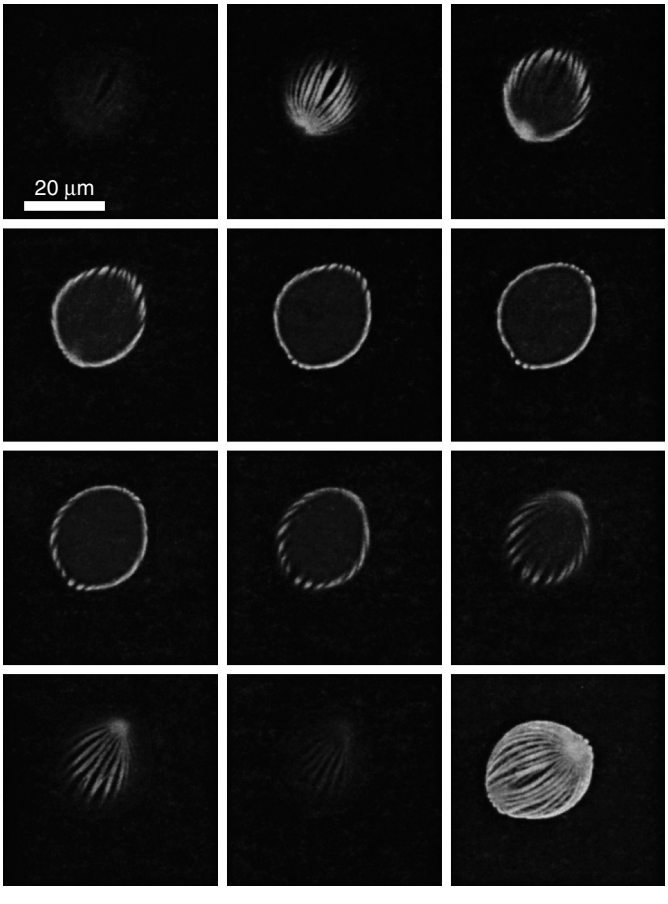

Figure 1.47 Confocal fluorescence micrographs of a *Spathiphyllum* pollen grain. The optical section sequence is from left to right and top to bottom. The bottom right image is obtained by 3D reconstruction. (Reproduced with permission from Ref. [6]. © 2006 Michiel Müller.)

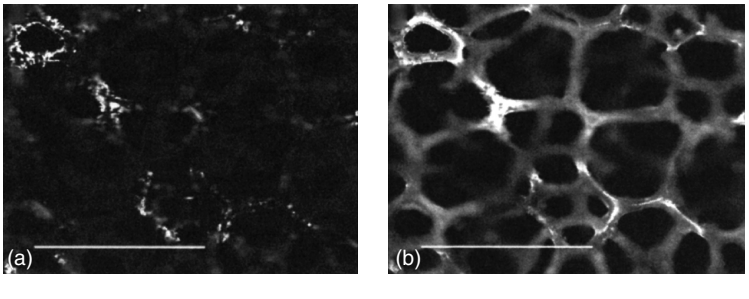

Figure 1.48 Confocal micrographs of polyurethane foam with labeled particulates: (a) highlights of particulates and (b) locations of particulates on the foam surfaces. The scale bar is 1 mm. (Reproduced with permission from Ref. [7]. © 2002 Woodhead Publishing Ltd.)

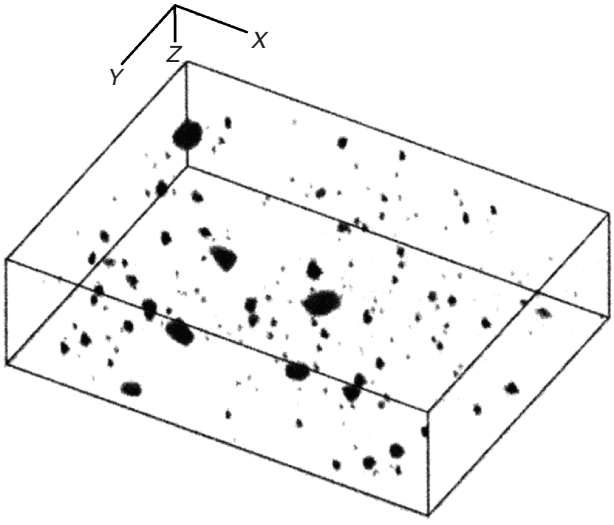

Figure 1.49 3D confocal fluorescent micrograph of silica particles in low-density polyethylene. (Reproduced with permission from Ref. [7]. © 2002 Woodhead Publishing Ltd.)

Figure 1.49 shows an example of using confocal fluorescent microscopy to reveal microscopic features in a specimen. The specimen is low-density polyethylene (LDPE) containing fluorescently labeled silica particles. The particle size and distribution in the polymer matrix can be clearly revealed by 3D confocal microscopy. Thus, confocal microscopy provides us a new dimension in light microscopy for materials characterization, even though its applications in materials science are not as broad as in biology.

Questions

1.1 Three rules govern light path for a simple lens:
1. A light ray passing through the center of a lens is not deviated.
2. A light ray parallel with optic axis will pass through the rear focal point.
3. A ray passing through the front focal point will be refracted in a direction parallel to the axis. Sketch the light paths from object to image in a single-lens system in the following situations.
   a. $a < f$; (b) $a = f$; (c) $2f > a > f$; (d) $a = 2f$; and (e) $a > 2f$, where a is the distance of object from lens and f is the focal length of lens. By this exercise, you may understand that $2f > a > f$ is necessary to obtain a real magnified image.

1.2 The working distance between the specimen and objective lens is determined by the magnifications of the lens. Estimate the difference in the working distance for objective lenses with powers of 5×, 20×, and 50×.

1.3 Calculate the resolution and the depth of field of the objective lenses of a light microscope, listed below. The refractive index of vacuum is 1, and that of air can be treated as 1. Assume blue light is used in the microscope.

Magnification/NA
5×/0.13
10×/0.25
20×/0.40
50×/0.70
100×/0.90.

1.4 To obtain a 400 magnification image we may choose a 40× objective lens with a 10× projector lens, or a 20× objective lens with a 20× projector lens. What are the differences in their image quality?

1.5 We often have a much worse resolution of a lens than the calculated one in Question 1.3. Why?

1.6 Compare the resolution and depth of field of light microscopes and electron microscopes. The wavelength of electrons is 0.0037 nm (100 kV) and the angle α of an electron microscope is 0.1 rad.

1.7 One found that he/she cannot a focused micrograph of a specimen even though he/she has observed a focused image of the specimen by looking through eyepiece. Why?

1.8 We have samples of an annealed Al alloy and an annealed plain carbon steel to be examined. The polishing area of the samples is about 5 mm × 3 mm.

- To avoid plastic deformation in the surface layer, what are the maximum compression forces that should be used for polishing the samples of annealed Al alloy and plain carbon steel, respectively?
- If normal compression force cannot cause plastic deformation, what kind of loading on the samples more likely causes plastic deformation?

Yield strength of annealed Al alloy = 150 MPa
Tensile strength of annealed Al alloy = 400 MPa
Yield strength of annealed plain carbon steel = 400 MPa
Tensile strength of annealed plain steel = 700 MPa.

1.9 Describe a specimen preparation procedure for examining:

a. a cross section of a metal needle;
b. a coating layer on metal substrate;
c. lead–tin solder; and
d. polyethylene blended with another crystalline polymer.

1.10 Which parts of a specimen will be highlighted under dark-field illumination in an optical microscopic study if the specimen is a polycrystalline metal with a few ceramic particles?

1.11 Why do we rotate the analyzer when examining microstructure with polarized light?

1.12 Why do we say that the Nomarski contrast may not provide a real three-dimensional image?

1.13 Suggest an imaging mode to examine a titanium specimen containing both hexagonal close-packed (HCP) and body-centered cubic (BCC) phases, and justify your suggestion.

1.14 Why is fluorescence microscopy more commonly used in biological and polymer specimens than in metals and ceramics?

1.15 Can we examine a metal or a ceramic specimen with confocal microscopy?

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