* 25–10 Specialty Microscopes and Contrast

All the resolving power a microscope can attain will be useless if the object to be seen cannot be distinguished from the background. The difference in brightness between the image of an object and the image of the surroundings is called **contrast**. Achieving high contrast is an important problem in microscopy and other forms of imaging. The problem arises in biology, for example, because cells consist largely of water and are almost uniformly transparent to light. We now briefly discuss two special types of microscope that can increase contrast: the interference and phase-contrast microscopes.

PHYSICS APPLIED
Interference microscope

An **interference microscope** makes use of the wave properties of light in a direct way to increase contrast in a transparent object. Consider a transparent object—say, a bacterium in water (Fig. 25–33). Light enters uniformly from the left and is coherent (in phase) at all points such as a and b. If the object is as transparent as the water, the beam leaving at d will be as bright as that at c. There will be no contrast and the object will not be seen. However, if the object's refractive index is slightly different from that of the surrounding medium, the wavelength within the object will be altered as shown. Hence light waves at points c and d will differ in phase, if not in amplitude. The interference microscope changes this difference in phase into a difference of amplitude which our eyes can detect. Light that passes through the sample is superimposed onto a reference beam that does not pass through the object, so that they interfere. One way of doing this is shown in Fig. 25–34. Light from a source is split into two equal beams by a half-silvered mirror, MS_1. One beam passes through the object, and the second (comparison beam) passes through an identical system without the object. The two meet again and are superposed by the half-silvered mirror MS_2 before entering the eyepiece and the eye. The path length (and amplitude) of the comparison beam is adjustable so that the background can be dark; that is, full destructive interference occurs. Light passing through the object (beam bd in Fig. 25–33) will also interfere with the comparison beam. But because of its different phase, the interference will not be completely destructive. Thus it will appear brighter than the background. Where the object varies in thickness, the phase difference between beams ac and bd in Fig. 25–33 will be different, thus affecting the amount of interference. Hence *variation in the thickness of the object will appear as variations in brightness in the image.*

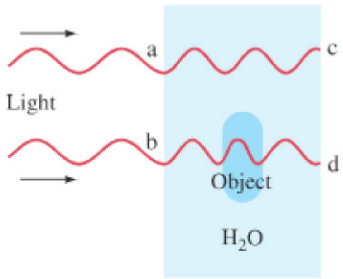

FIGURE 25–33 Object—say, a bacterium—in a water solution.

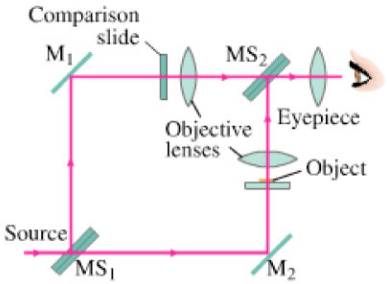

FIGURE 25–34 Diagram of an interference microscope.

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Phase-contrast microscope

A **phase-contrast microscope** also makes use of interference and differences in phase to produce a high-contrast image. Contrast is achieved by a circular glass *phase plate* that has a groove (or a raised portion) in the shape of a ring, positioned so undeviated source rays pass through it, but rays deviated by the object do not pass through this ring. Because the rays deviated by the object travel through a different thickness of glass than the undeviated source rays, the two can be out of phase and can interfere destructively at the object image plane. Thus the image of the object can contrast sharply with the background. Phase-contrast microscope images tend to have "halos" around them (as a result of diffraction from the phase-plate opening), so care must be taken in the interpretation of images.