Phase-Contrast Microscope

Application

- Unpigmented living cells are not clearly visible in the brightfield microscope because there is little difference in contrast between the cells and water.
- Thus microorganisms often must be fixed and stained before observation to increase contrast and create variations in color between cell structures.
- A **phase-contrast microscope converts slight differences in refractive** index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells.

Working

- The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light.
- As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about 1/4 wavelength.
- The deviated light is focused to form an image of the object.
- Undeviated light rays strike a phase ring in the phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate.
- If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by 1/4 wavelength, the deviated and undeviated waves will be about 1/2 wavelength out of phase and will cancel each other when they come together to form an image (**figure 2.10).**
- **The background, formed by undeviated light, is** bright, while the unstained object appears dark and well-defined.
- This type of microscopy is called **dark-phase-contrast microscopy.**
- Color filters often are used to improve the image.

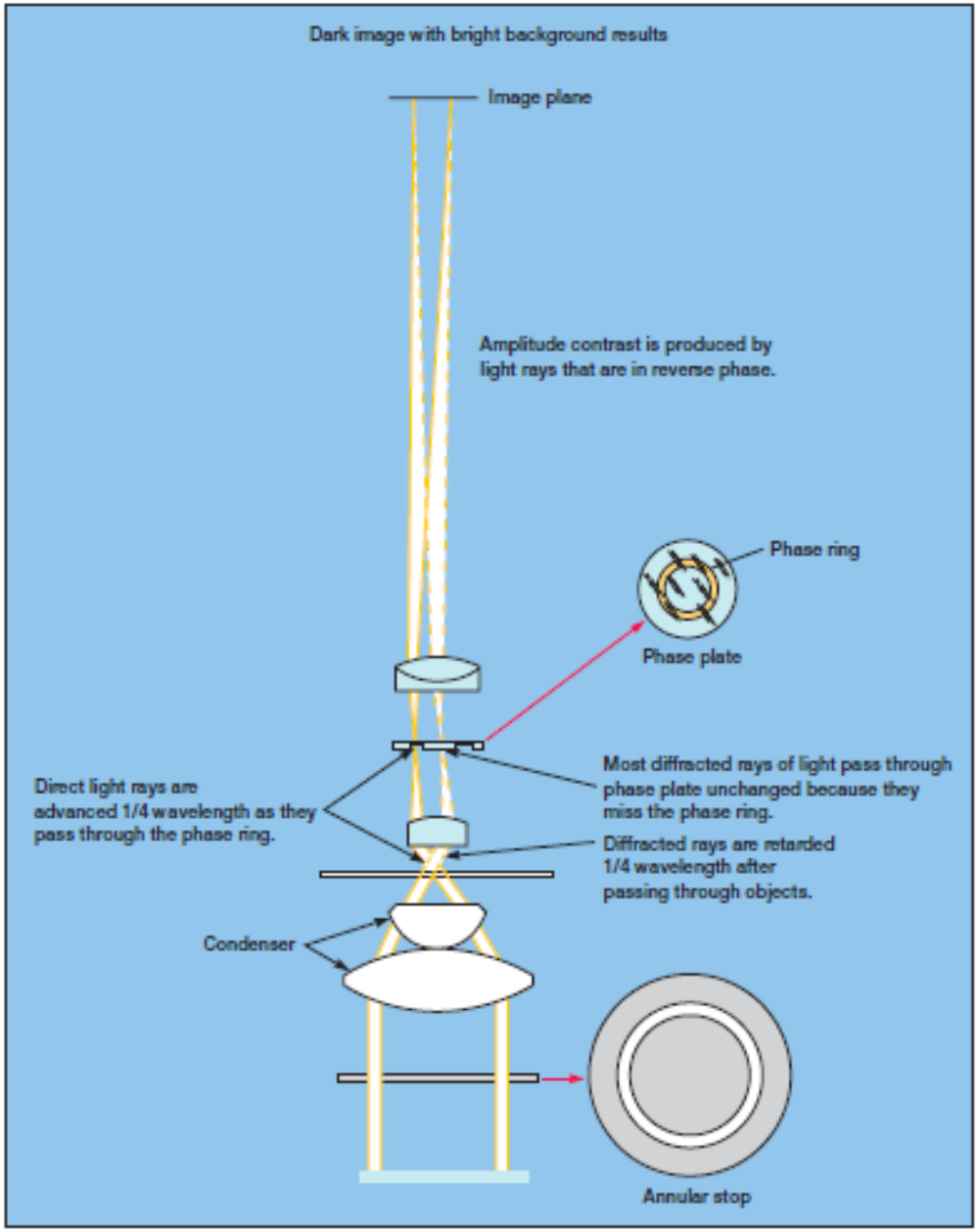

Figure 2.9 **Phase-Contrast Microscopy.** The optics of a dark-phase-contrast microscope.

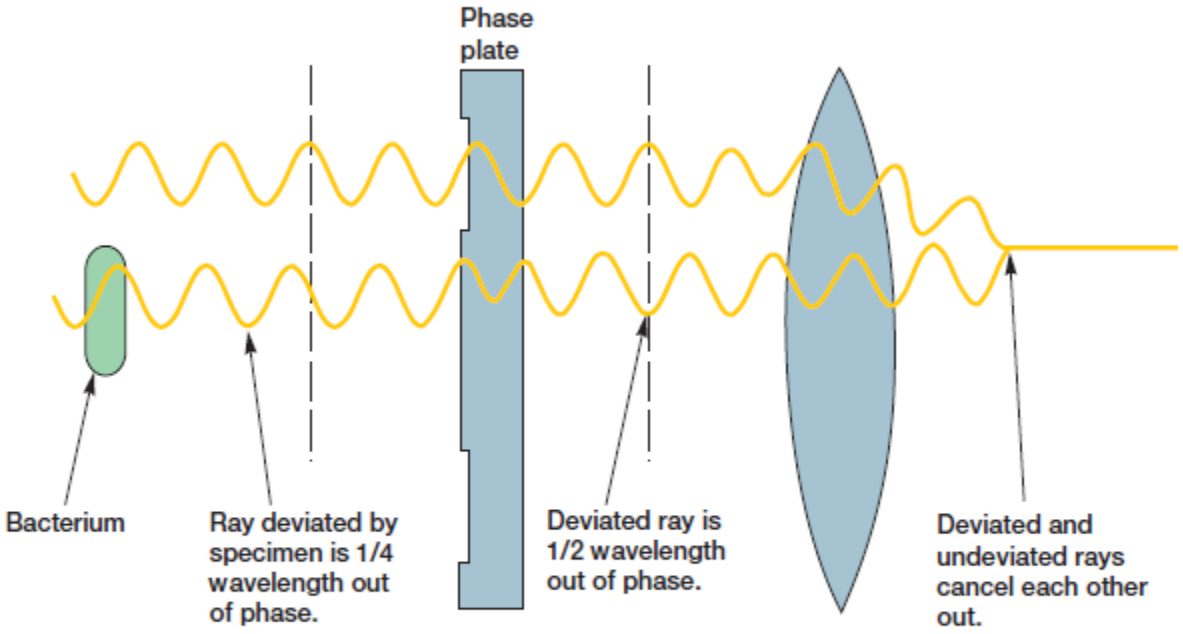

Figure 2.10 The Production of Contrast in Phase Microscopy. The behavior of deviated and undeviated or undiffracted light rays in the dark-phase-contrast microscope. Because the light rays tend to cancel each other out, the image of the specimen will be dark against a brighter background.

- Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies that contain poly--hydroxybutyrate, polymetaphosphate, sulfur, or other substances .
- These are clearly visible *because they have refractive* indexes markedly different from that of water.
- Phasecontrast microscopes also are widely used in studying eucaryotic cells.

THE FLUORESCENCE MICROSCOPE

Principle

- The microscopes thus far considered produce an image from light that passes through a specimen.
- An object also can be seen because it actually emits light, and this is the basis of fluorescence microscopy.
- When some molecules absorb radiant energy, they become excited and later release much of their trapped energy as light.
- Any light emitted by an excited molecule will have a longer wavelength (or be of lower energy) than the radiation originally absorbed.
- **Fluorescent light is emitted very quickly by the excited** molecule as it gives up its trapped energy and returns to a more stable state.

Working

- The **fluorescence microscope (figure 2.12) exposes a specimen** to ultraviolet, violet, or blue light and forms an image of the object with the resulting fluorescent light.
- A mercury vapor arc lamp or other source produces an intense beam, and heat transfer is limited by a special infrared filter.
- The light passes through an exciter filter that transmits only the desired wavelength.
- A darkfield condenser provides a black background against which the fluorescent objects glow (A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective. Only light that has been reflected or refracted by the specimen forms an image**. The field surrounding a specimen** appears black, while the object itself is brightly illuminated).
- Usually the specimens have been stained with dye molecules, called **fluorochromes, that fluoresce** brightly upon exposure to light of a specific wavelength, but some microorganisms are autofluorescing.
- The microscope forms an image of the fluorochrome-labeled microorganisms from the light emitted when they fluoresce (**figure 2.13).**
- **A barrier** filter positioned after the objective lenses removes any remaining ultraviolet light, which could damage the viewer's eyes, or blue and violet light, which would reduce the image's contrast.

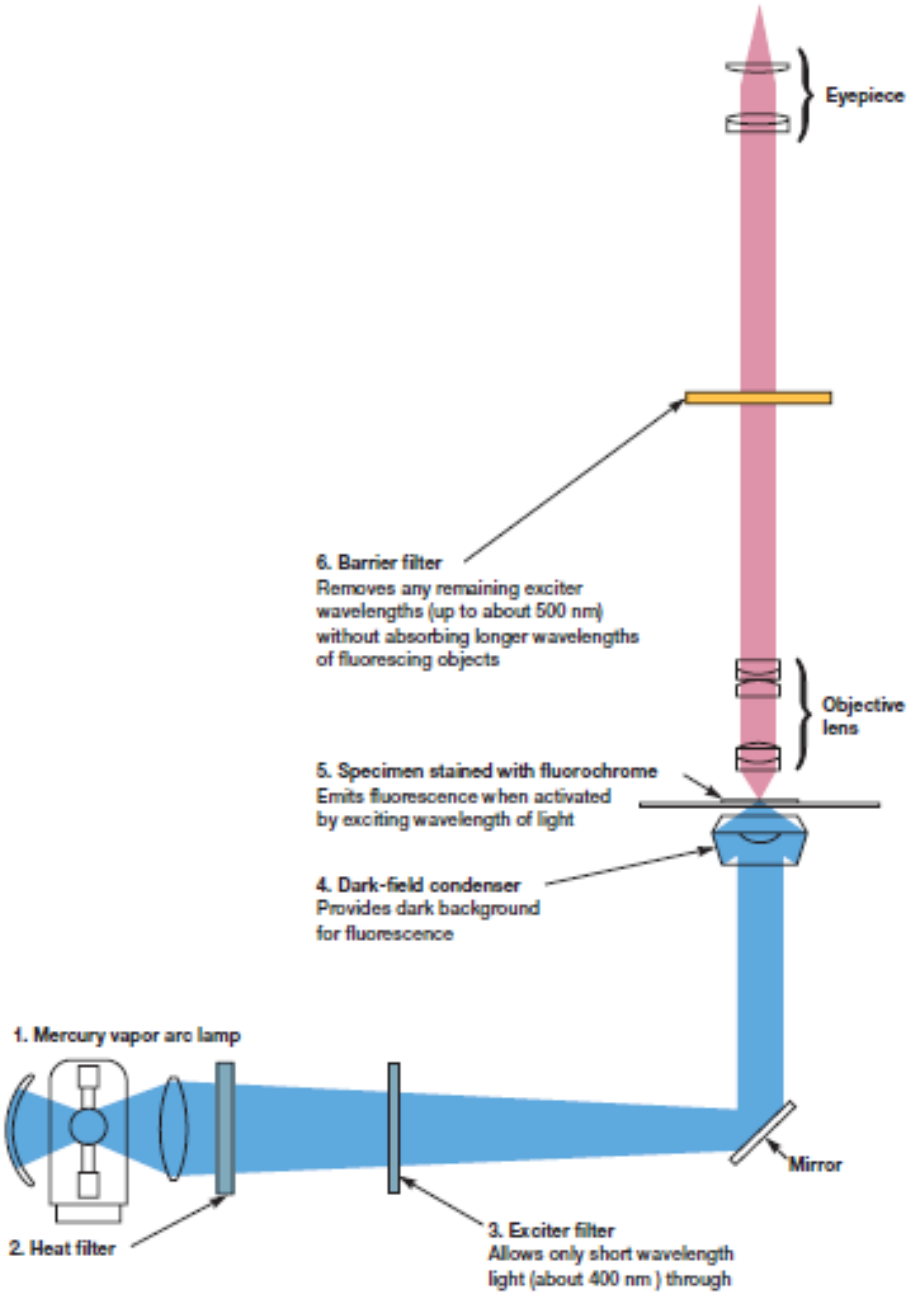

Figure 2.12 **Fluorescence Microscopy.** The principles of operation of a fluorescence microscope.

Applications

- The fluorescence microscope has become an essential tool in medical microbiology and microbial ecology.
- Bacterial pathogens (e.g., *Mycobacterium tuberculosis, the cause of tuberculosis)* can be identified after staining them with fluorochromes or specifically labeling them with fluorescent antibodies using immunofluorescence procedures.
- In ecological studies the fluorescence microscope is used to observe microorganisms stained with fluorochrome-labeled probes or fluorochromes such as acridine orange and DAPI (diamidino-2-phenylindole, a DNA-specific stain).
- The stained organisms will fluoresce orange or green and can be detected even in the midst of other particulate material.
- It is even possible to distinguish live bacteria from dead bacteria by the color they fluoresce after treatment with a special mixture of stains.
- *Thus* the microorganisms can be viewed and directly counted in a relatively undisturbed ecological niche.