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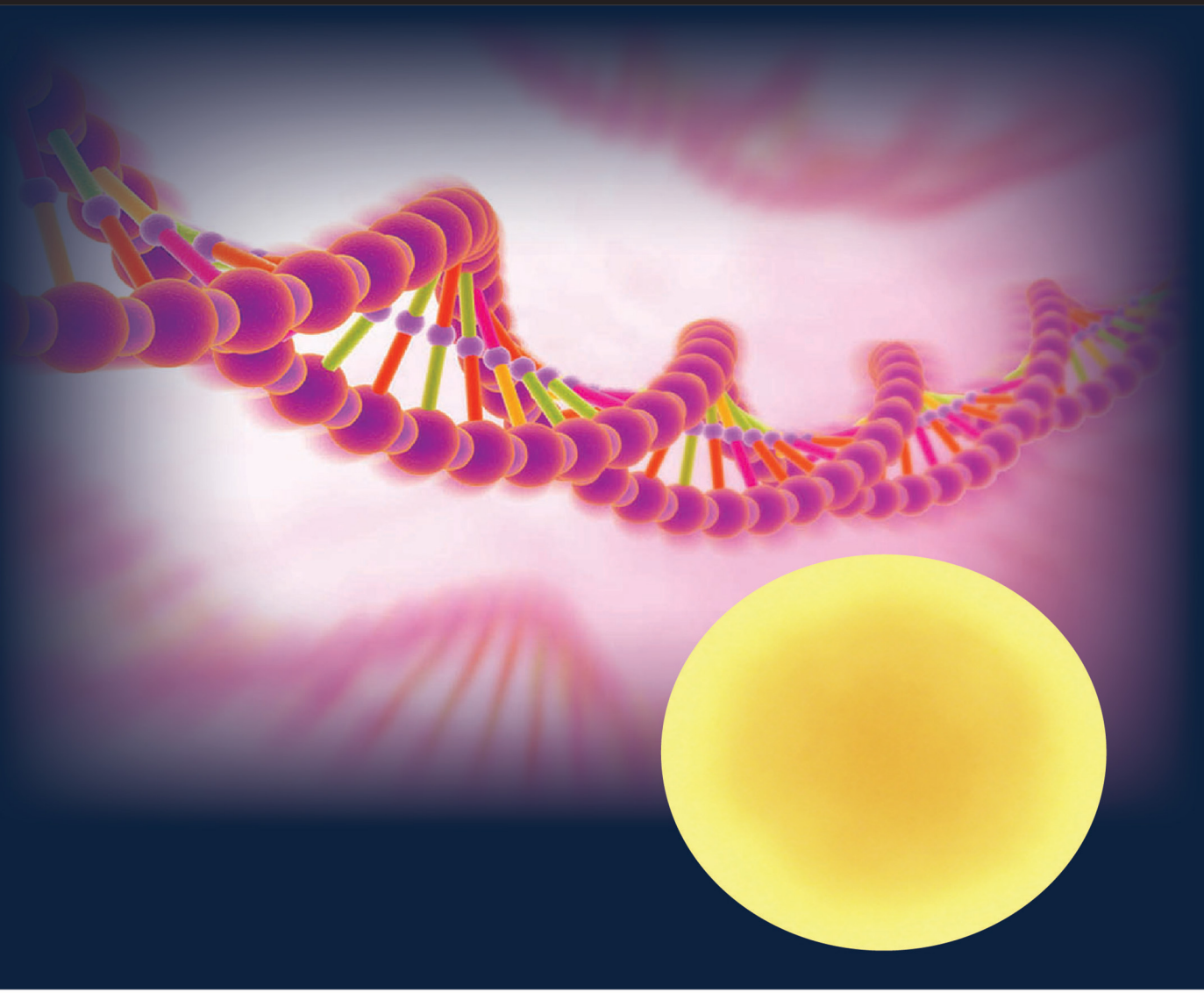
Essentials of MICROBIOLOGY



Surinder Kumar

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2 Chapter

Microscopy

LEARNING OBJECTIVES

After reading and studying this chapter, you should be able to:

- Discuss microscopic methods
- Explain the principles and describe the uses of the following: darkfield microscopy; phase-contrast; microscopy; fluorescent microscopy and electron microscopy.

INTRODUCTION

Microscope is an optical instrument used to magnify (enlarge) minute objects or micro-organisms which cannot be seen by naked eye.

MICROSCOPY: INSTRUMENTS

Microscopic Methods

A. Light Microscopy
   1. Brightfield (light) microscopy
   2. Darkfield microscopy
   3. Phase-contrast microscopy
   4. Fluorescent microscopy
   5. Confocal

B. Electron Microscopy
   - Transmission
   - Scanning

C. Scanning Probe Microscopes

A. LIGHT MICROSCOPY

Light microscopy refers to the use of any kind of microscope that uses visible light to observe specimens. A modern compound **light microscope (LM)** has a series of lenses and uses visible light as its source of illumination.

Principles of Light Microscopy: The Brightfield Microscopy

In light microscopy, light typically passes through a specimen and then through a series of magnifying lenses. The most common type of light microscope, and the easiest to use, is the **brightfield microscopy,** which evenly illuminates the field of view.

1. Compound Light Microscopy

A series of finely ground lenses forms a clearly focused image that is many times larger than the specimen itself. This magnification is achieved when light rays from an **illuminator**, the **light source** used to illuminate the specimen positioned on a stage, pass through a **condenser**, which has lenses that direct the light rays through the specimen. From here, light rays pass into the **objective lenses**, the lenses closest to the specimen. The image of the specimen is magnified again by the **ocular lens**, **or *eyepiece***. Three different objective lenses are commonly used: **low power (x10); high dry (x40); and oil immersion (x100)**.

Resolution

The limitation of brightfield microscopy is the resolution (also called **resolving power**) of the image (i.e. the ability to distinguish that two objects are separate and not one). The resolving power of a microscope is determined by the wavelength of light used to illuminate the subject and the angle of light entering the objective lens (referred to as the numerical aperture).

Immersion oil: Immersion oil is placed between the glass slide and the oil immersion objective lens. The immersion oil has the same refractive index as glass, so the oil becomes part of the optics of the glass of the microscope. The oil enhances the resolution by preventing light rays from dispersing and changing wavelength after passing through the specimens. A specific objective lens, the oil immersion lens, is designed for use with oil; this lens provides 100x magnification on most light microscopes.

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2. Darkground (Darkfield) Microscopy

Darkfield microscopy is frequently performed on the same microscope on which brightfield microscopy is performed. Instead of the normal condenser, a darkfield microscopy uses a darkfield condenser that contains an opaque disc. The disc blocks light that would enter the objective lens directly. Only light that is reflected off (turned away from) the specimen enters the objective lens. Because there is no direct background light, the specimen appears light against a dark background. This creates a 'dark-field' that contrasts against the highlighted edge of the specimens and results when the oblique rays are reflected from the edge of the specimen upward into the objective of the microscope.

Use

This technique is particularly valuable for observing organisms such as ***Treponema pallidum***, a spirochete which cannot be observed with direct light.

3. Phase-contrast Microscopy

Principle: In a phase-contrast microscope, one set of light rays comes directly from the light source. The other set comes from light that is reflected or diffracted from a particular structure in the specimen. When the two sets of light rays—direct rays and reflected or diffracted rays—are brought together, they form an image of the specimen on the ocular lens, containing areas that are relatively light (in phase), through shades of gray to black (out of phase). Through the use of annular rings in the condenser and the objective lens, the differences in phase are amplified so that in-phase light appears brighter than out-of-phase light. The special phase condenser consists of annular diaphragms on a rotating disk fitted to the bottom of the condenser.

Uses

1. To study unstained living cells.
2. Detailed examination of internal structures in living microorganisms.
3. To study flagellar movements and motility of bacteria and protozoans.
4. To study intestinal and other live protozoa such as amoebae and *Trichomonas*.
5. To examine fungi grown in culture.

Differential Interference Contrast (DIC) Microscopy

Differential interference contrast (DIC) microscopy is similar to phase-contrast microscopy in that it uses differences in refractive indices. The resolution of a DIC microscope is higher than that of a standard phase-contrast microscope.

Fluorescence Microscopy

Fluorescence microscopy takes advantage of fluorescence, the ability of substances to absorb short wavelengths of light (ultraviolet) and give off light at a longer wavelength (visible). If tissues, cells or bacteria are stained with a fluorescent dye and are examined under the microscope with ultra-violet light instead of ordinary visible light, they become luminous and are seen as bright objects against a dark background.

Principal Use

The principal use of fluorescence microscopy is a diagnostic technique called the **fluorescent antibody (FA) technique**, or **immunofluorescence** employed for detection of antigen **(direct fluorescent antibody technique)** and antibodies **(indirect fluorescent antibody methods)**.

Epifluorescence Microscopy

A common variation of the standard fluorescence microscope is the epifluorescence microscope, which projects the ultraviolet light through the objective lens and onto the specimen.

Confocal Microscopy

In confocal microscopy, lenses focus a laser beam to illuminate a given point on one vertical plane of a specimen. In effect, this microscope is a miniature CAT scan for cells.

B. ELECTRON MICROSCOPY

- Electron microscopy is in some ways comparable to light microscopy. Rather than using glass lenses, visible light, and the eye to observe the specimen, the electron microscope uses electromagnetic lenses, electrons, and a fluorescent screen to produce the magnified image. That image can be captured on photographic film to create an electron photomicrograph.
- The electron beam is focused by circular electro-magnets, which are analogous to the lenses in the light microscope. The object which is held in the path of the beam scatters the electrons and produces an image which is focused on a fluorescent viewing screen.
- The wavelength of electrons used in an EM is 0.005 nm as compared to 500 nm with visible light, i.e. about 100,000 times shorter than that of ordinary light. Theoretically, the resolving power of the EM should be 100,000 times (reso-lution down to 0.0025 nm). In practice, the best resolution that can be obtained is 0.3–0.5 nm, a hundred times better than that of the light microscope.

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Types of Electron Microscopes

There are two types of electron microscopes in general use:

(i) Transmission Electron Microscope (TEM)

In transmission electron microscope (TEM), electrons like light pass directly through the specimen that has been prepared by thin sectioning, freeze fracturing, or freeze etching. It is used to observe fine details of cell structure.

(ii) Scanning Electron Microscope (SEM)

A scanning electron microscope scans a beam of electrons back and forth over the surface of a specimen producing three-dimensional views of the surfaces of whole microorganisms.

C. SCANNING-PROBE MICROSCOPY

Scanning probe microscopes map the bumps and valleys of a surface on an atomic scale. Their resolving power is much greater than the electron microscope, and the samples do not need special preparation as they do for electron microscopy. Among the new scanned-probe microscopes are:

1. **Scanning tunneling microscopy (STM)**: There are used to provide incredibly detailed views of molecules, such as DNA.
2. **Atomic force microscopy (AFM):** These produce three-dimensional images of the surface of a molecule.

Key Points

- *Microscopy:* A simple microscope consists of one lens; a compound microscope has multiple lenses.

Compound Light Microscopy

- The most common microscope used in microbiology is the compound microscope/bright field microscope/light microscope.
- *Darkfield microscopy:* It is most useful for detecting the presence of extremely small organisms.
- *Phase-contrast microscopy:* It allows the detailed observation of the living organisms.
- *Fluorescence microscopy:* Fluorescence microscopy is used primarily in a diagnostic procedure called fluorescent-antibody (FA) technique, or immunofluorescence.
- *Electron microscopy:* Electron microscopes use electromagnetic lenses instead of glass lenses, electrons and fluorescent screens to produce a magnified image. There are two types (i) Transmission electron microscopes (TEMs); and (ii) Scanning electron microscopes
- *Scanned-probe microscopy:* Their resolving power is much greater than the electron microscope.

IMPORTANT QUESTION

Write short notes on:
a. Darkfield microscopy
b. Fluorescent microscopy
c. Phase-contrast microscopy
d. Electron microscopy.

MULTIPLE CHOICE QUESTIONS (MCQs)

1. Which of the following is not a modification of a compound microscope:
   a. Bright microscopy
   b. Darkfield microscopy
   c. Elecron micrscopy
   d. Phase-contrast microscopy
2. Wavelenth of electrons used in an electron microscopy is:
   a. 0.001 nm
   b. 0.005 nm
   c. 100 nm
   d. 500 nm
3. Theoretically, the resolving power of the electrone microscope is:
   a. 10,000 times
   b. 100,000 times
   c. 5,00,000 times
   d. 1,000 times

ANSWERS (MCQs)
Ans: 1. b; 2. b; 3. b

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ISBN 978-93-5152-380-2