

Microscopy Techniques in Biology

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An organized overview of the main optical and electron microscopy methods, their principles, advantages, limitations, and typical applications.

1. Overview

Technique	Light vs. Electron	Resolution (approx.)	Sample Requirement	Typical Use
Bright-field (BF) microscopy	Light	200 nm	Fixed, stained, thin sections	General histology, routine diagnostics
Phase-contrast (PC) microscopy	Light	200 nm	Live or fixed, unstained	Observation of transparent cells (e.g., bacteria, organelles)
Differential interference contrast (DIC)	Light	200 nm	Live or fixed, unstained	High-contrast 3D-like images of live cells
Fluorescence microscopy (wide-field)	Light	200 nm	Fluorophore-labeled specimens	Localization of proteins, nucleic acids
Confocal laser scanning microscopy (CLSM)	Light	200 nm (laterally) / 500 nm (axially)	Fluorophore-labeled, often fixed or live	Optical sectioning, 3D reconstruction
Super-resolution fluorescence (STED, PALM/STORM)	Light	20–50 nm	Fluorophore-labeled, often fixed	Nanoscale protein distribution
Two-photon microscopy	Light (infrared)	~300 nm	Live tissue, deep penetration	In-vivo imaging of thick specimens
Light-sheet fluorescence microscopy (LSFM)	Light	200 nm	Live or cleared specimens	Fast volumetric imaging of embryos, organoids
Scanning electron microscopy (SEM)	Electron	1–5 nm	Fixed, dehydrated, conductive coating	Surface topography, morphology

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Transmission electron microscopy (TEM)	Electron	0.1–1 nm	Ultra-thin sections (<70 nm), heavy metal staining	Internal ultrastructure, organelle architecture
Cryo-EM (cryo-TEM, cryo-ET)	Electron	3–5 nm (single particle)	Vitrified, unstained specimens	Near-native structures of macromolecules, complexes
Scanning transmission EM (STEM)	Electron	0.5–2 nm	Thin sections, often stained	Combined surface/internal detail
Atomic force microscopy (AFM)	Mechanical probe	0.1–10 nm	Live or fixed, any substrate	Surface topography, mechanical properties

2. Light Microscopy Techniques

2.1 Brightfield (BF)

- **Principle:** Light passes through a stained specimen; contrast arises from absorption.
- **Key Points:** Simple, inexpensive; limited contrast for unstained, transparent samples.

2.2 Phase Contrast (PC)

- **Principle:** Converts phase shifts (caused by refractive index differences) into intensity differences.
- **Advantages:** No staining needed; good for live cells.
- **Limitations:** Halo artifacts; less effective for thick specimens.

2.3 Differential Interference Contrast (DIC)

- **Principle:** Uses polarized light and Wollaston prisms to generate a pseudo-3D image.
- **Strengths:** High contrast, minimal halos, good depth perception.
- **Drawbacks:** Requires specialized optics; image interpretation can be non-intuitive.

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2.4 Fluorescence Microscopy

- **Basic Concept:** Fluorophores absorb light at one wavelength and emit at a