Electron Microscopy (SEM/TEM)

Advanced Imaging Techniques for Nanoscale Visualization

Electron sources

Wavelength ~0.004 nm vs light ~500 nm

Sample preparation

Fixation, dehydration, coating

Contrast mechanisms

Electron density differences

Cryo-EM revolution

Near-atomic resolution of proteins

Correlative microscopy

Combining light and electron microscopy

Detailed Explanations

1 Electron Sources

Electron microscopy achieves dramatically higher resolution than light microscopy due to the much shorter wavelength of electrons. The de Broglie wavelength of accelerated electrons is approximately **0.004 nm** (at 100 keV), compared to visible light's wavelength of **~500 nm**.

Key Advantages:

- **Resolution limit:** Can resolve features down to 0.05 nm (atomic scale)
- **Magnification:** Up to 50 million times, far exceeding light microscopy's ~2000x practical limit
- **Depth of field:** Much greater than optical microscopy

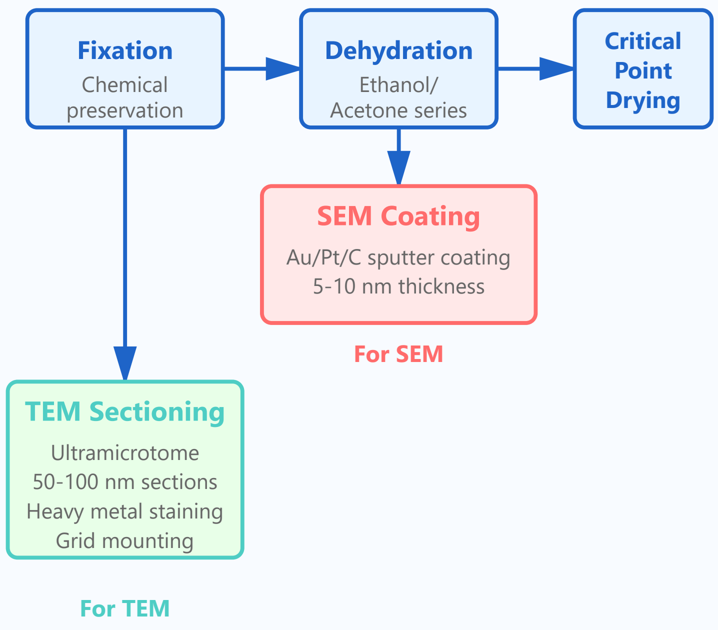
Types of Electron Sources:

- **Thermionic:** Tungsten filament (economical, broad energy spread)
- **LaB_6:** Lanthanum hexaboride (brighter, longer lifetime)
- **Field Emission (FEG):** Highest brightness and coherence for best resolution

Wavelength comparison: Electron vs. Light microscopy

2 Sample Preparation

Proper sample preparation is critical for electron microscopy as samples must withstand high vacuum and electron beam exposure. The preparation process varies between SEM and TEM.



Sample preparation workflow for SEM and TEM

SEM Sample Preparation:

- **Fixation:** Chemical fixation (glutaraldehyde, formaldehyde) to preserve structure
- **Dehydration:** Graded ethanol or acetone series to remove water
- **Critical point drying:** Prevents collapse of delicate structures
- **Coating:** Thin layer of gold, platinum, or carbon (5-10 nm) for conductivity

TEM Sample Preparation:

- **Ultrathin sectioning:** 50-100 nm thick sections using ultramicrotome
- **Staining:** Heavy metal stains (uranyl acetate, lead citrate) for contrast
- **Grid mounting:** Copper grids with support film
- **Alternative methods:** Negative staining, freeze-fracture, immunolabeling

3 Contrast Mechanisms

Electron microscopy generates contrast through interactions between electrons and the sample, based primarily on differences in electron density and atomic number.

SEM Contrast Mechanisms:

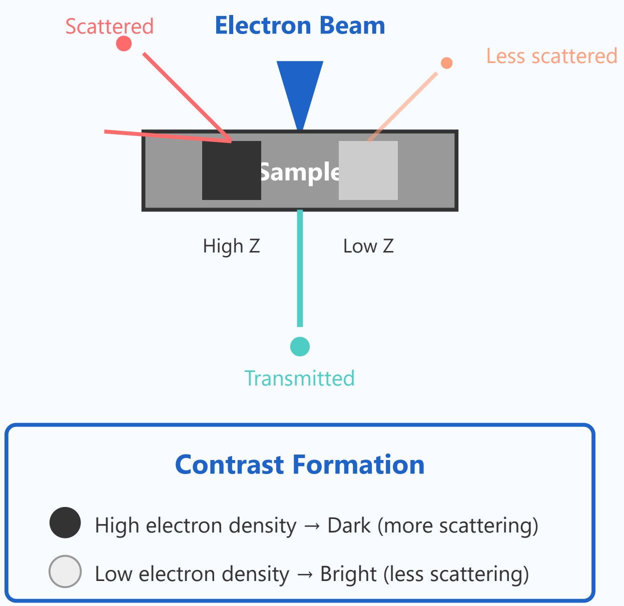
- **Secondary electrons (SE):** Topographical contrast from surface features
- **Backscattered electrons (BSE):** Compositional contrast (atomic number dependent)
- **Characteristic X-rays:** Elemental analysis (EDX/EDS)

TEM Contrast Mechanisms:

- **Mass-thickness contrast:** Dense/thick regions appear darker
- **Diffraction contrast:** Crystalline structure and defects
- **Phase contrast:** High-resolution imaging of atomic structure
- **Z-contrast (STEM):** Atomic number sensitive imaging

Enhancing Contrast:

- Heavy metal staining (uranyl acetate, osmium tetroxide)
- Objective aperture selection

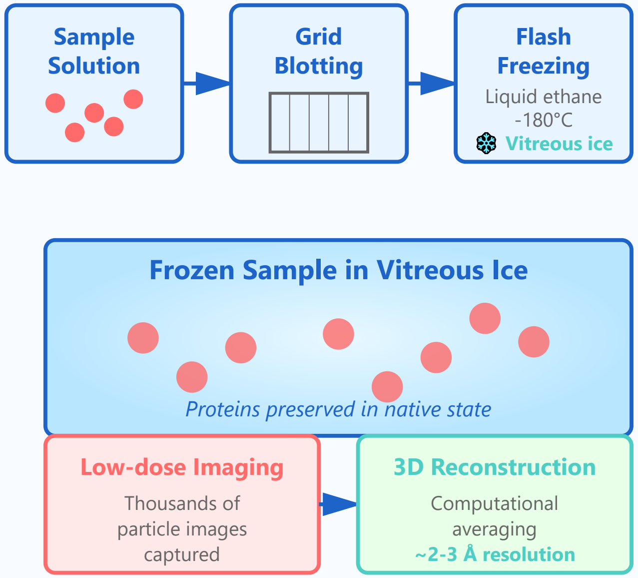


Electron-sample interactions creating contrast in electron microscopy

- Defocus optimization for phase contrast

4 Cryo-EM Revolution

Cryo-electron microscopy (cryo-EM) has revolutionized structural biology by enabling near-atomic resolution imaging of biological macromolecules in their native state, without crystallization. The 2017 Nobel Prize in Chemistry recognized this breakthrough.



Cryo-EM workflow: from sample to near-atomic resolution structure

Key Advantages:

- **No crystallization required:** Study proteins that are difficult or impossible to crystallize
- **Native hydration state:** Samples preserved in vitreous ice
- **Multiple conformations:** Capture dynamic protein states
- **Resolution:** Routinely achieving 2-3 Å resolution, approaching X-ray crystallography

Technique:

- **Flash freezing:** Rapid vitrification in liquid ethane (~-180°C)
- **Low-dose imaging:** Minimize radiation damage

- **Single-particle analysis:** Combine thousands of images computationally
- **Direct electron detectors:** Improved sensitivity and speed

Applications:

- Protein structure determination (ribosomes, ion channels, enzymes)
- Virus structure analysis
- Drug discovery and design
- Understanding disease mechanisms

5 Correlative Microscopy

Correlative Light and Electron Microscopy (CLEM) combines the advantages of both techniques, enabling researchers to precisely locate and study specific features identified in fluorescence microscopy at ultrastructural resolution in electron microscopy.

Advantages of CLEM:

- **Molecular specificity:** Fluorescence labels identify specific proteins or structures

- **Contextual ultrastructure:** EM provides detailed structural information
- **Dynamic to static:** Track living processes, then preserve for detailed analysis
- **Rare event detection:** Use fluorescence to find rare cells/structures for EM study

Workflow Strategies:

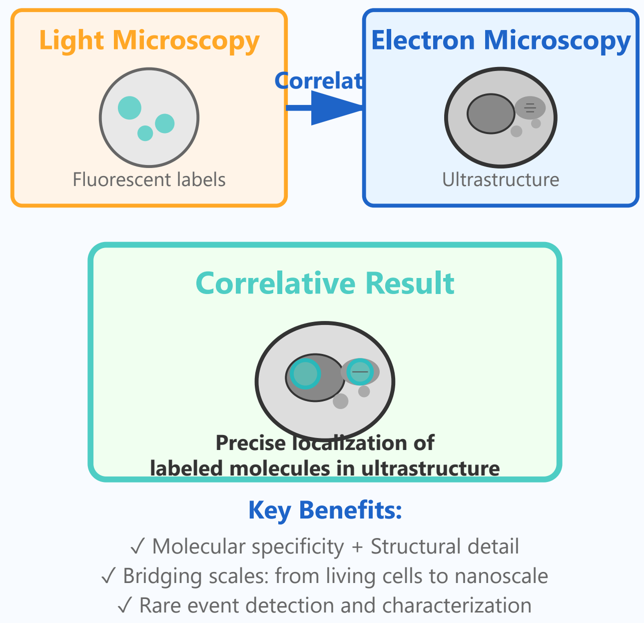
- **Pre-embedding:** Live-cell fluorescence imaging followed by EM preparation
- **Post-embedding:** EM processing first, then fluorescence labeling on sections
- **In-resin:** Imaging fluorescence on resin-embedded samples before sectioning

Technical Considerations:

- Coordinate registration between imaging modalities
- Preservation of fluorescence during EM processing
- Fiducial markers for alignment
- 3D correlative approaches (array tomography, FIB-SEM)

Applications:

- Protein localization at ultrastructural level
- Virus entry and trafficking studies
- Organelle interactions and dynamics



Correlative Light and Electron Microscopy (CLEM) workflow

- Disease pathology research