Introduction to Electron Microscopy
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Instrumentation and Image Formation

The types of electron microscopes

Transmission electron microscope (TEM)  Scanning electron microscope (SEM)
The types of electron microscopes

Transmission electron microscope (TEM)

- Electron beam
- Specimen: ~100 nm
- Projection

Scanning electron microscope (SEM)

- Electron beam
- Specimen
- Surface

Examples TEM

Mouse intestine

- Actin filaments
- Junction
- Glycocalix
- Microvilli

Specimens courtesy of Bärbel Stecher, Institute of Microbiology, ETH Zurich
Examples TEM

Mouse intestine

Membrane (lipid bilayer)

Actin filaments

500 nm

Elektronenmikroskopie ETH Zürich

Examples TEM

Immunolabelling: Localization of proteins

H/K-ATPase in cimetidine-treated resting gastric parietal cells (rabbit).

Pseudomonas aeruginosa

Examples SEM

Properties of electrons

- Very similar to photons:
  - Wave-particle duality
  - Optical properties (Diffraction, chromatic aberration, spherical aberration, astigmatism etc.)
  - Resolution depends on aperture and wavelength (Diffraction limited resolution)

Abbe’s equation \( d = 0.61 \frac{\lambda}{NA} \)

\[ NA = n \cdot \sin \alpha \]
Resolution of electron microscopes

The higher the energy of the electrons, the lower the wavelength, the higher the resolution.

TEM: 40 – 300 kV
Effective instrument resolution TEM: ≈ 0.5 nm (120 kV)

SEM: 0.5 – 30 kV
Effective instrument resolution SEM: ≈ 1 nm

Resolution of biological objects limited by specimen preparation:
Practical resolution: > 1 nm

Transmission electron microscope vs. Widefield light microscope

Transmission electron microscope

- Illumination
- Condenser lens
- Specimen
- Objective lens
- Projector lens
- Final image

Widefield light microscope
Electron microscopes are high vacuum systems

Example: Transmission electron microscope

- **Cathode**
- **Specimen holder**
- **Viewing screen**

**Atmosphere:** 1000 mbar

**10^-7 - 10^-10 mbar**
- Ion getter pump
- Turbo molecular pump
- Oil diffusion pump

**10^-5 - 10^-7 mbar**
- Turbo molecular pump
- Oil diffusion pump

**10^-0 - 10^-2 mbar**
- Rotary pump

**Atmosphere:** 1000 mbar

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**Scanning electron microscope vs. Confocal laser scanning microscope**

**Scanning electron microscope**
- Illumination
- Detector
- Lens system
- Beam scanner
- Lens system
- Specimen

**Confocal laser scanning microscope**
- Illumination
- Detector
- Lens system
- Specimen

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**Confocal laser scanning microscope**

- **Specimen**
- **Illumination**
- **Detector**
- **Lens system**
- **Beam scanner**
- **Specimen**
Electron source

- Equivalent lens action

High voltage (e.g. 120 kV)

Electromagnetic lenses

Electromagnetic lens of a transmission electron microscope

- Polepieces
- Vacuum seal
- Water cooling
- Soft iron
- Windings

The focal length can be changed by changing the current:

No movement or exchange of the lens is required for focusing or changing magnification!
Chromatic aberration
Due to energy difference of electrons (wavelength)

1. Electron (98 kV)
2. Electron (100 kV)
3. Electron (102 kV)

Curvature and distortion of field

Field curvature
Barrel distortion
Pincushion distortion

Spherical aberrations

Axial astigmatism - confusion of the image

Cellulose filter paper imaged in SEM

With astigmatism

Under focused image elliptic deformation
Focus circle of least confusion
Over focused image elliptic deformation

Without astigmatism

Focus, corrected astigmatism circle of confusion minimized
Electromagnetic lenses

Axial astigmatism - confusion of the image

Reasons:
• Inhomogenities of the lens
• Contamination of lenses and apertures
• Charging of specimen

Specimen holders and stages

Transmission electron microscope
Specimen holders and stages

Transmission electron microscope

Specimen size:
• 3 mm in diameter
• 100 nm in thickness

Specimen holders and stages

Scanning electron microscope

Specimen size:
• 100 mm in diameter
• 2 cm in z-direction (not electron transparent)
Electron – specimen interactions

Inelastic scattering:

Energy is transferred from the primary electron to the specimen

Emission of electrons and radiation
Electron – specimen interactions

- Primary electrons
- Unscattered electrons
- Inelastically scattered electrons
- Elastically scattered electrons
- Secondary electrons
- Backscattered electrons
- Auger electrons
- Cathode luminescence
- X-rays
- Specimen Interaction volume
- SEM analysis
- TEM analysis

Imaging in the transmission electron microscope

Contrast formation in TEM

- Absorption contrast
- Scattering/phase contrast

NOTE: Mechanisms occur at the same time (superposition)
Contrast formation in TEM

Biological specimen consist of light elements:

- Absorption contrast weak
- Scattering/phase contrast weak

"LOW CONTRAST"

Contrast enhancement required:
- Treatment with heavy metals (Ur, Pb, Os)
- Heavy metals attach differently to different components

Main contrast formation in plastic embedded specimens
- Scattering of electrons through heavy metals
Imaging in the transmission electron microscope

Thin section of alga stained with heavy metals (Ur, Pb)

Thin section of alga without heavy metal staining

1 µm
Contrast enhancement by underfocusing

Thin section of a frozen-hydrated apple leaf ("unstained")

Phase contrast only between H₂O and biological material

Imaging in the transmission electron microscope

The CCD camera for electron microscopy

- Electrons need to be converted to photons (scintillator)
- CCD has to be protected from electron bombardment
- Nowadays direct electron CCD available, no scintillator required (very expensive)
Imaging in the scanning electron microscope

Primary electrons
- Backscattered electrons
- Auger electrons
- Elastically scattered electrons
- Inelastically scattered electrons
- Unscattered electrons

Secondary electrons
- Cathode luminescence
- X-rays

TEM analysis

Specimen Interaction volume

SEM analysis

Imaging in the scanning electron microscope

Scanning and signal detection

PE...Primary electron beam

SE secondary electrons
Signal and detection

Different properties of the different signals

► Specific detectors
► Different/specific information

Secondary electron detector

- Primary electrons
- +200-500V – Collector voltage
- +7-12kV HV
- Photomultiplier
- Electrons → Photons → Electrons
Contrast formation in SEM using SE

- Different number of electrons from different spots of the specimen

Dependent on:
- Topography of the specimen
- Location of the detector
- Acceleration voltage of primary electrons
- Composition of the specimen

Contrast based on SE - topography

PE: Primary electrons
SE: Secondary electrons
R: Excited volume

Specimen
Imaging in the scanning electron microscope

Contrast based on SE

Wing of butterfly

Contrast based on SE – detector position

Virtual light source

Mouse kidney (glomerulus)
Contrast formation in SEM

Biological material (light elements):
- Only few electrons escape from specimen
- Almost no contrast, similar contrast everywhere on specimen
- Unsharp image

Contrast enhancement
- Localization of the signal to the surface
- Coating of biological specimen with thin **heavy** metal layer (a few nm)
- Reducing acceleration voltage

Contrast formation

Uncoated
- Primary electron beam

Coated with 4 nm platinum
- Primary electron beam

Platinum...
### Contrasting based on SE: Non-coating vs. coating with heavy metals

![Uncoated](image1.png) ![Coated with 4 nm platinum](image2.png)

*Freeze-fractured yeast*  

500 nm  

Electron microscopy ETH Zurich

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### Focusing and magnification in TEM and SEM

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| **SEM:**                                      | **Confocal scanning laser microscopy:**     |
| Focusing:                                     | Focusing:                                   |
| Change current in magnetic lenses for focusing (objective lens) | Moving objective or stage in z |
| Move stage in z                               | Magnification:                              |
| **Magnification:**                            | Change scanning field (scan a smaller or larger area with the same number of pixels), pixel size changes. |
| Change scanning field (scan a smaller or larger area with the same number of pixels), pixel size changes. | Changing the whole objective |