Electron microscopy (EM) techniques

In *transmission electron microscopy* the diffraction and adsorption of electrons as the electron beam passes normally through the specimen is imaged to provide information on the specimen.

In *scanning electron microscopy* an electron beam falls at a non-normal angle on the specimen and the image is derived from the scattered and reflected electrons.

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Transmission electron microscopy

**Advantages:**

See image -Focus electrons
Small amount of sample (1-2ul, ~1-10mg/ml)
Large specimens (>1000Å) - that might not crystallize
Resolution
Sample in solution

**Disadvantages:**

Uses optical system – lens aberration
Vibration of stage
Biological molecules don’t like high energy
EM Methods:

Routine method:

**Negative stain** – involves staining sample

More technically demanding methods:

**2D electron diffraction** – involves 2D crystals

**Cryo** – uses sample vitrification (or rapid freezing)

Transmission electron microscope
Sample grid:
Small (several millimeters) copper discs called grids cast with a fine mesh. This mesh can vary a lot depending on the intended application, but is usually about 15 squares per millimeter (400 squares per inch).

Negative staining is a simple technique for routine examination of structure. It does not allow for high resolution examination of samples.

Coating sample with an electron dense molecule

Enhances the contrast of otherwise “invisible” biological molecule

Examples 1% to 3% solution of are:
Aqueous Uranyl Acetate
Neutral Phosphotungstic Acid
Ammonium Molybdate
Negative staining results:

Gives gross morphology of the specimen

Only reveals the surface of the specimen

It has been possible to “see” protein α-helices

Widely used extremely valuable

2D electron diffraction:

involves 2D crystals

Unstained crystalline samples are embedded in a thin layer (e.g., Glucose) and cooled to Liquid nitrogen temperature.

Collect diffraction
(just like 3D X-ray crystallography)

Tilt sample for 3D

Get phases directly from image

Result:

Membrane proteins

E.g. Photosynthetic reaction center of purple bacterium
Cryo-EM:

Samples vitrified in a thin layer of water at liquid nitrogen temperatures.

This “frozen-hydrated” preparation technique is now the method of examining unstrained non-crystalline specimens.

Result:

Problem image contrast

Solution. Take pictures slightly out of focus

Lose resolution

CTF correction
Image reconstruction:
Assign each image center of gravity and orientation

Image reconstruction:
Add all the 2D images together to make 3D reconstruction
2D Fourier projections in different orientations

added together

create a 3D image

Estimate errors of reconstruction:

Phase residual

\[ \Delta(t) = \sqrt{\frac{\sum (|F_1| + |F_2|) \cdot (\theta \theta)^2}{\sum |F_1| + |F_2|}} \]

FSC

\[ \text{FSC} = \frac{\sum (F_1 \cdot F_2^*)}{\sqrt{(|F_1|)^2 \cdot (|F_2|)^2}} \]

R-factor_{AB}

\[ R = \frac{\sum |F_1 - F_2|}{0.5 \cdot \sum |F_1| + |F_2|} \]

J. A. Lake, 1972
Marriage of cryo-EM and crystallography:

Fit the crystal structures of individual proteins into the electron density of the EM reconstruction of the multi-protein complex.