TRANSMISSION ELECTRON CRYO-MICROSCOPY (Cryo-TEM)

Instrumental technique

Jayoti Roy

25-05-2019

Cryogenic transmission electron microscopy

Transmission electron cryomicroscopy(cryo-TEM) is an electron microscopy (EM) technique applied on samples cooled to cryogenic temperatures and embedded in an environment of vitreous water.

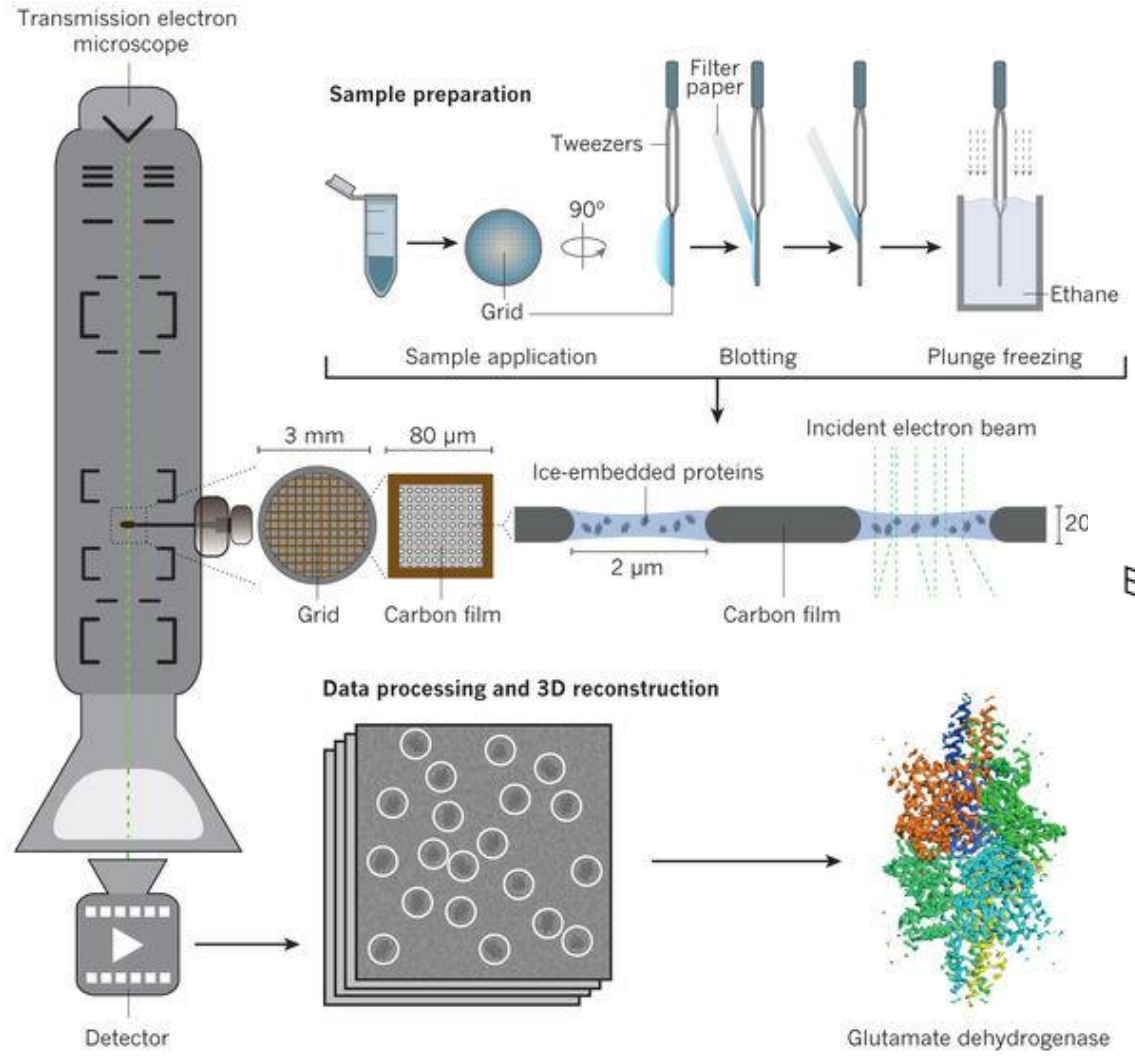
History of Cryo-EM

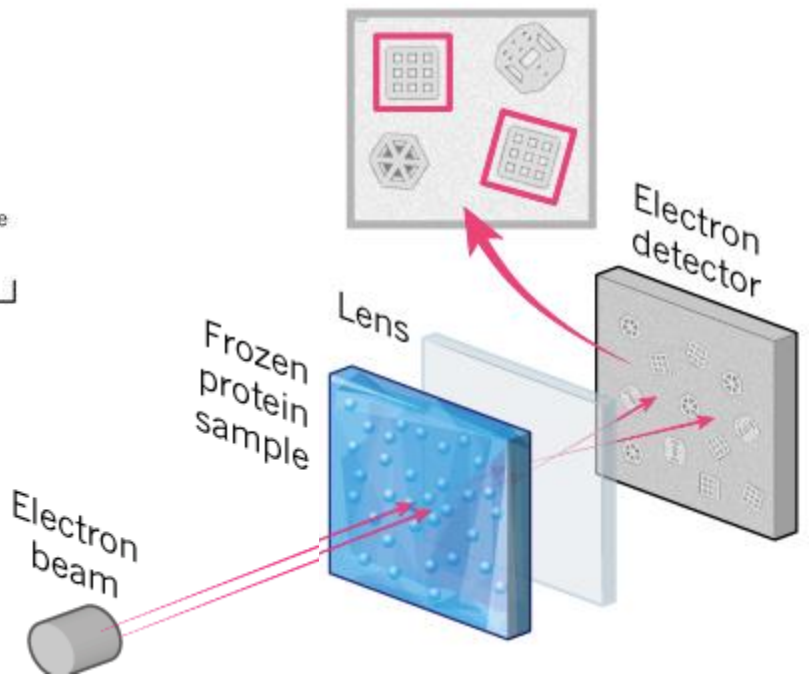
- In the 1960s, scientists were faced with the issue of structure determination methods using electron microscopy damaging the specimen due to high energy electron beams.

- In 1980, Erwin Knapek and Jacques Dubochet had worked on “beam damage to organic Material” at cryogenic temperatures and had noticed that a thin crystals mounted on carbon film were found to be from 30 to 300 times more beam-resistant at 4 K than at room temperature.

- In 2017, three scientists, Jacques Dubochet, Joachim Frank and Richard Henderson, were awarded the Nobel Prize in Chemistry for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution".

Determination of high-resolution structure through image processing

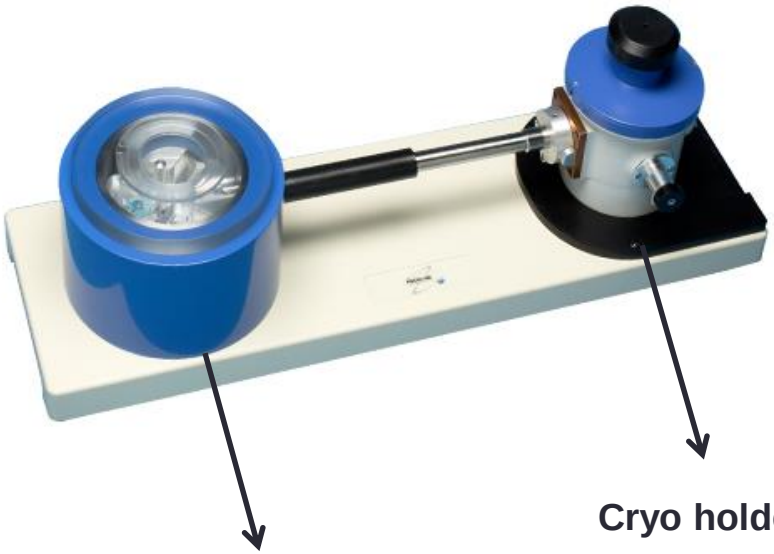




CRYO-ELECTRON MICROSCOPY
A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.

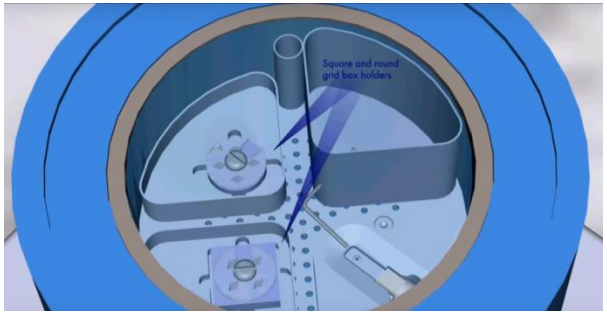
M.Dutta *et al*.,J. Indian Inst. Sci.|VOL 98:3|231–245 September 2018

Sample holder in Cryo-TEM



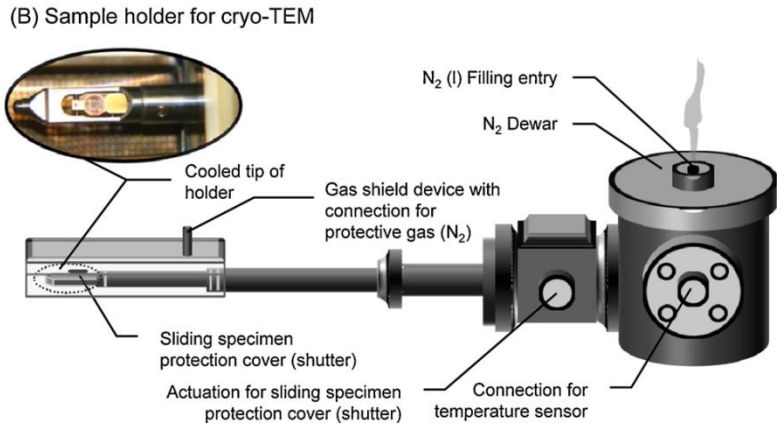
Cryo holder

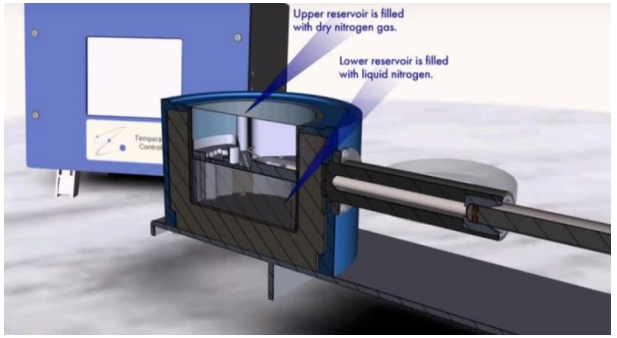
Cryo workstation



Grid box holder

Perforated tray surface





Reservoir

J. Kuntsche et al. International Journal of Pharmaceutics *417 (2011) 120– 137*

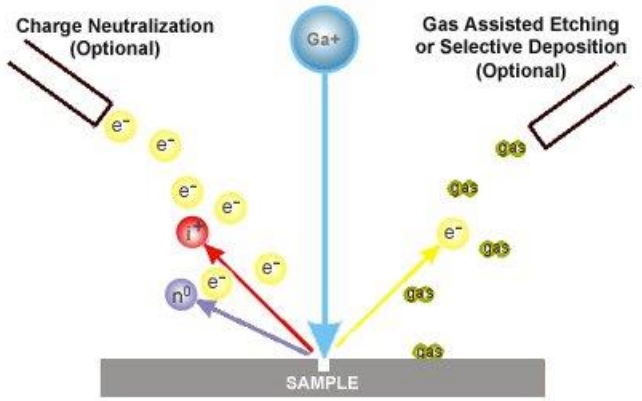
Different methods of sample preparation

1. Cryo-sectioning or cryo-fixation :-

- Samples for electron cryotomography **(**Cryo-ET) (typically small cells e.g. Bacteria, viruses) are prepared in standard aqueous media and applied to an TEM grid.
- The grid is then plunged into a cryogen (typically ethane), so efficient that water molecules do not have time to rearrange into a crystalline lattice. The resulting water state is called "vitreous ice" and preserves native cellular structures.
- They can then be cut in thin sections (40 to 200 nm thick) with a diamond knife in a cryo-ultramicrotome at temperatures lower than -135 °C (devitrification temperature).

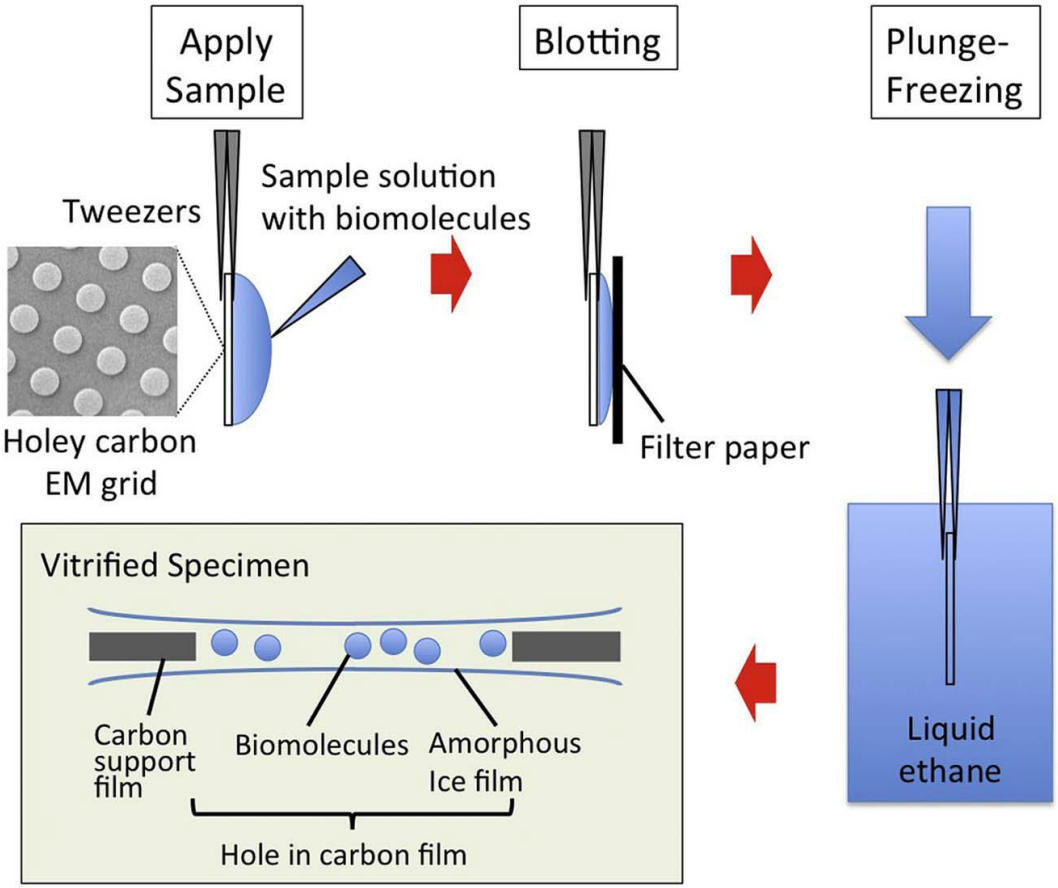
2. Focused ion beam (FIB) milling :-

In FIB-milling, plunge-frozen samples are exposed to a focused beam of ions, typically gallium, that precisely whittle away material from the top and bottom of a sample, leaving a thin lamella suitable for ECT imaging.



. Focused ion beam

Cryofixation



“Classic” specimen preparation by plunge-freezing. Aqueous sample solution is applied onto glow-discharged (hydrophilic) holey carbon film supported by EM grid. Excess solution is removed with filter paper from one or both sides. Blotted grid is rapidly plunged into a cryogen precooled at liquid nitrogen temperature. Biomolecules embedded in thin amorphous ice film are observed in cryo-electron microscope.

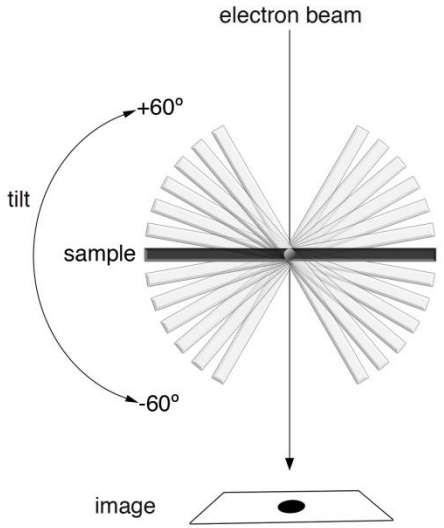
Electron cryotomography

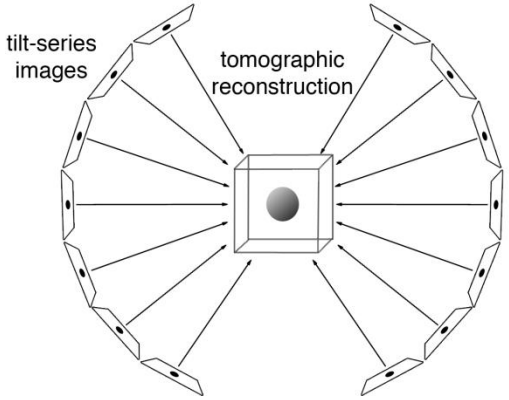
- Cryo-ET is a specialized application of transmission electron cryo-microscopy (Cryo-TEM) in

Which samples are imaged as they are tilted, resulting in a series of 2D images that can be combined to produce a 3D reconstruction.

A) Single particle analysis (SPA)
the images of randomly orientated homogeneous particles are recorded at low electron exposure. Then, particle images selected from digitized images are iteratively aligned against a reference and averaged.

B) Subtomogram averaging (STA).
2D projections of heterogeneous particles are collected by tilting the specimen stage, and 3D tomograms are calculated using weighted back projection or other reconstruction algorithms. Selected subsets of the tomogram (“subtomograms”) containing individual particle volumes are picked, aligned, classified and averaged.





This schematic shows the concept of electron tomography. A sample is imaged in a TEM as it is tilted to different angles, resulting in a "tilt-series" of 2D images (top). This tilt-series is then computationally reconstructed into a 3D "tomogram" (bottom).

Advantages of using cryo-TEM

- Using cryo-TEM, it becomes possible to view cells, cell organelles as well as macromolecules complexes of well over 500 kD.
  Recently, Cryo-Electron Microscopy was also used to determine high resolution structures of 200kD proteins.

- Cryo-TEM offers a great advantage in that it provides high magnification allowing for the specimen to be viewed and studied closely. Moreover, it offers a significant advantage in that through the direct acquisition of the images; the specimen can be statistically analyzed allowing for the reconstruction of the structural information.

- Stains are not necessary which means that the specimen is not distorted through the use of stains and other dyes. Given that low dose methods are commonly used, the electron beam does not cause much damage to the specimen.

- It is possible to distinguish between nucleic acids, proteins and lipids by cryo-TEM.

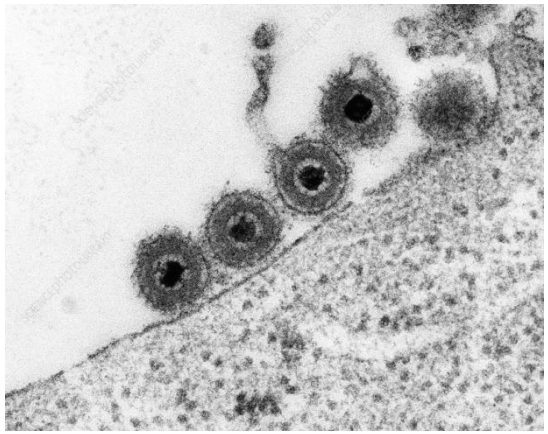


Image of herpes simplex virus by TEM

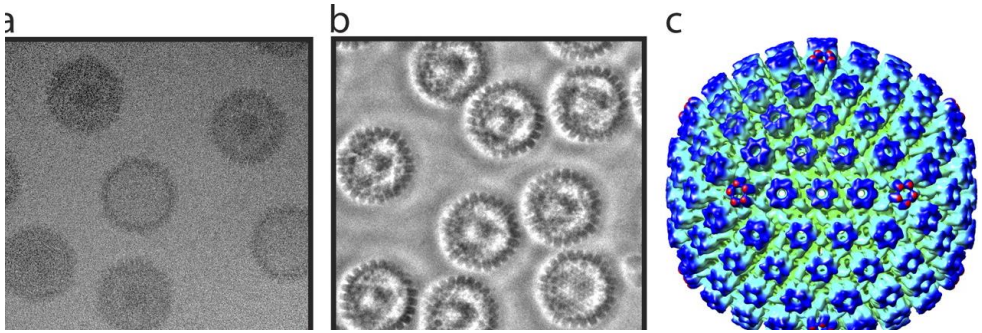
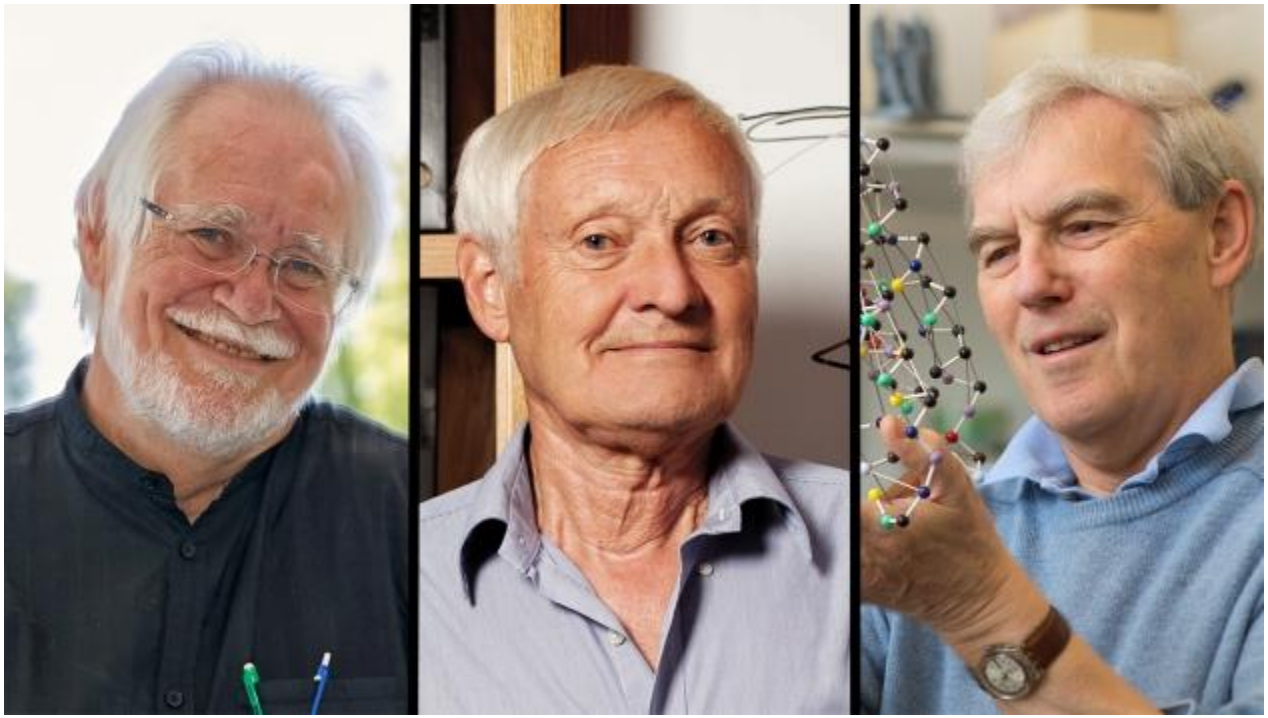


Image of herpes simplex virus by Cryo-TEM (resolutions up to 1.8 Å)

Rochat *et al*.,JOURNAL OF VIROLOGY, Feb. 2011, p. 1871–1874

The Nobel Prize in Chemistry 2017

“For developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution."



Jacques Dubochet Joachim Frank Richard Henderson

Thank You