

Sample preparation in Electron Microscopy

1. Sample preparation

Chemical Fixation for biological specimens aims to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde and glutaraldehyde, and lipids with osmium tetroxide.

2. Cryofixation – freezing a specimen so rapidly, to liquid nitrogen or even liquid helium temperatures, that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot of its solution state.

3. Dehydration – freeze drying, or replacement of water with organic solvents such as ethanol or acetone, followed by critical point drying or infiltration with embedding resins.

4. Embedding, biological specimens – after dehydration, tissue for observation in the transmission electron microscope is embedded so it can be sectioned ready for viewing. To do this the tissue is passed through a 'transition solvent' such as epoxy propane and then infiltrated with a resin such as Araldite epoxy resin; tissues may also be embedded directly in water-miscible acrylic resin. After the resin has been polymerised (hardened) the sample is thin sectioned (ultrathin sections) and stained - it is then ready for viewing.

5. Sectioning – produces thin slices of specimen, semitransparent to electrons. These can be cut on an ultramicrotome with a diamond knife to produce ultrathin slices about **60-90nm thick**. Disposable glass knives are also used because they can be made in the lab and are much cheaper.

6. Staining – uses heavy metals such as **lead, uranium or tungsten** to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens are can be stained "en bloc" before embedding and also later after sectioning. Typically thin sections are stained for several minutes with an **aqueous or alcoholic solution of uranyl acetate** followed by **aqueous lead citrate**.