Negative Staining in Electron Microscopy

Negative Staining

In electron microscopy, staining is usually done with heavy metal salts commonly derived from molybdenum, uranium, or tungsten. Heavy ions are used since they will readily interact with the electron beam and produce phase contrast. A small drop of the sample is deposited on the carbon coated grid, allowed to settle for approximately one minute, blotted dry if necessary, and then covered with a small drop of the stain (for example 2% uranyl acetate). After a few seconds, this drop is also blotted dry, and the sample is ready for viewing.

Freeze-fracture or freeze-etch – a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly (cryofixed), then fractured by simply breaking or by using a microtome while maintained at liquid nitrogen temperature. The cold fractured surface (sometimes "etched" by increasing the temperature to about -100°C for several minutes to let some ice sublime) is then shadowed with evaporated platinum or gold at an average angle of 45° in a high vacuum evaporator.

Conductive Coating – An ultra thin coating of electrically-conducting material, deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done to prevent the accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. Such coatings include gold, gold/palladium, platinum, tungsten, graphite etc. and are especially important for the study of specimens with the scanning electron microscope.