1 An Introduction to Electron Microscopy (EM)

1.1 Imaging Methods in Electron Microscopy

1.1.1 Conventional Transmission Electron Microscopy (TEM)

A beam of electrons can interact with an object in a conventional transmission electron microscope in one of two ways. Usually elastic scattering takes place, whereby the electrons change their path in the specimen without a loss of energy. Inelastic scattering can also occur, resulting in a loss of energy due to an interaction of the impinging electrons with the orbital electrons surrounding the nucleus of each atom in the object. Those electrons which are not or hardly scattered contribute positively to the image. Those which are considerably deflected are prevented from doing so by apertures in the optical path. As a result differences in light intensity (contrast) are created in the final image, which relate to areas in the object with different scattering potentials. This fact can be deduced from the following formula of Rutherford (adaptation of Coulomb’s law), which describes the deflection potential of an atom:

\[ k = \frac{-e \cdot eZ}{r^2}, \]

in which \( k \) = deflection potential, \( e \) = electron charge, \( Z \) = positive charge of the nucleus (atomic number), \( r \) = distance from electron to nucleus.

1.1.1.1 Bright Field Electron Microscopy

The electron beam is hardly deflected by those elements (e.g. C, O, H, N, S, P) which are present in biological material. Thus biological objects have little or no inherent contrast when viewed in the electron microscope. Instead, contrast has to be obtained artificially by introducing elements with higher atomic number (e.g. Os, Mn, U, Pb, W etc.) into the object. This can be achieved during the fixation (e.g. with OsO₄, see Chap. 2.1.1.2.1 or KMnO₄, see Chap. 2.1.1.2.3) or through special staining (see Chap. 2.3.1.3 and 2.6.5) techniques.

An aggregation of atoms with low atomic number can sometimes be seen in the TEM without staining. The mass thickness or density of the atoms at the region in question is then obviously such that a scattering of the electrons in the
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beam does, in fact, occur. Clearly such an effect will take place more and more frequently the thicker the object becomes. As a result the maximal thickness of a biological specimen in a TEM, operated at normal (100 kV or less) accelerating voltages, lies around 500 nm. At greater thicknesses the electron beam will penetrate but cannot effectively leave the object. Hence in order to produce meaningful micrographs a biological object in a TEM should not exceed 100 nm in thickness. This fact constitutes one of the major problems in object preparation for biological TEM.

Contrast is modulated by two other factors. One is the size of the objective aperture: the smaller the opening becomes, the greater is the contrast achieved (at a loss, however, of beam intensity and resolution). The second factor is the accelerating voltage which is applied between the cathode and anode: the greater the voltage becomes, the greater is the kinetic energy of the electrons. This in turn causes a reduction in the angle of their deflection and therefore a reduction in contrast.

Literature


1.1.1.2 Low Dose Transmission Electron Microscopy

Under routine conditions in normal TEM the specimen is exposed to an electron beam intensity equivalent to several hundred to several thousand electrons per Å². The total dosis is composed of the individual doses which correspond to searching, focussing and exposing the specimen. One can, however, avoid beam damage to the specimen by reducing this total dose. Some EMs have built-in devices for this to be done on a regular basis. The procedure is as follows: scan the specimen at the lowest possible magnification and beam intensity; the dose will be around $10^{-2}$e per Å² s⁻¹; focus at the desired magnification, but not at the position which is to be exposed; move the specimen to the position required (during this step one should try to shield the specimen from the beam); finally expose the specimen with the first electrons which impinge on the region in question at the desired magnification. Done in this manner, negatives can be obtained from a specimen, or part of it, which has been exposed to only 6–10 electrons per Å² as the total dose. Naturally in order to obtain the maximum of information one should use a special film emulsion and an appropriate developer. As a check on the quality and correctness of information obtained one usually takes a second picture of the same region but under routine conditions (the focus remaining unchanged).