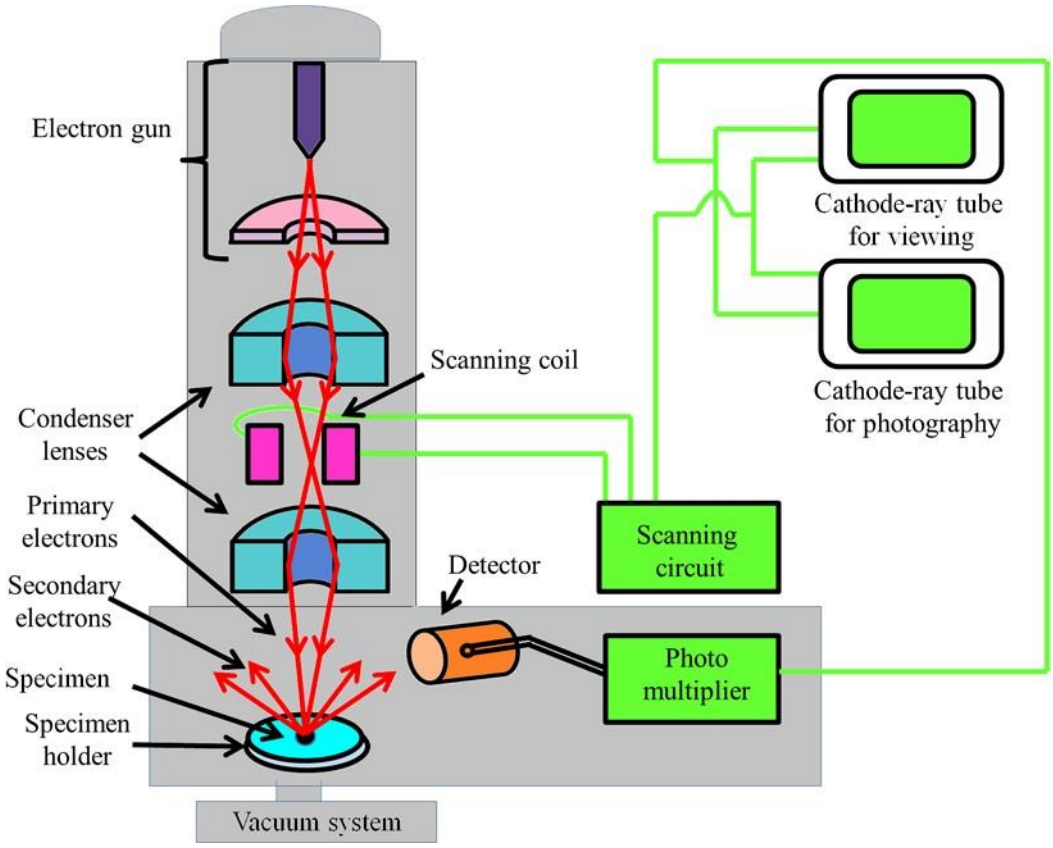
Scanning electron microscope

There are two basic models of the electron microscopes: Scanning electron microscopes (SEM) and transmission electron microscopes (TEM). In a SEM, the secondary electrons produced by the specimen are detected to generate an image that contains topological features of the specimen. The image in a TEM, on the other hand, is generated by the electrons that have transmitted through a thin specimen. Let us see how these two microscopes work and what kind of information they can provide:Figure 18.1 shows a simplified schematic diagram of a SEM. The electrons produced by the electron gun are guided and focused by the magnetic lenses on the specimen.



The focused beam of electrons is then scanned across the surface in a raster fashion (Figure 18.2). This scanning is achieved by moving the electron beam across the specimen surface by using deflection/scanning coils. The number of secondary electrons produced by the specimen at each scanned point are plotted to give a two dimensional image

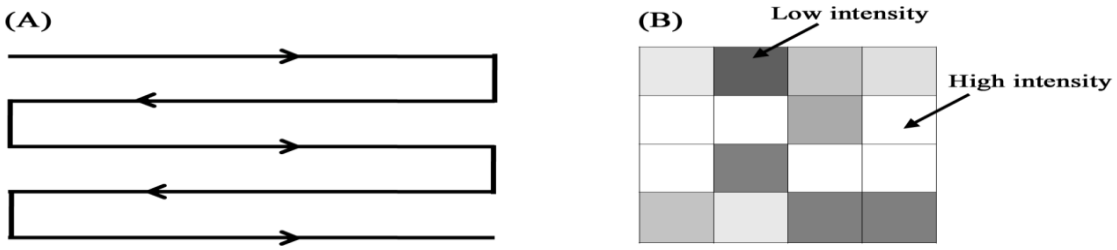


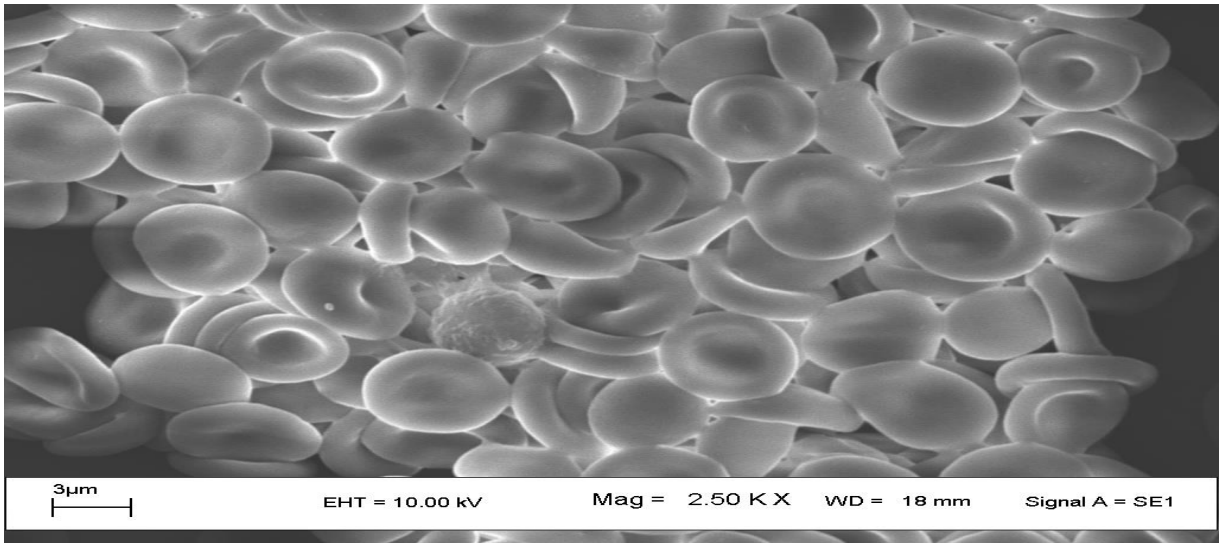
Figure 18.2 A diagrammatic representation of the raster scanning (A) and the intensity plot for the scanned area (B).

In principle, any of the signals generated at the specimen surface can be detected. Most electron microscopes have the detectors for the secondary electrons and the backscattered electrons. Figure 18.3 shows the interaction volume within the specimen showing the regions of secondary electrons (energy < 50 eV) and backscattered electrons.Figure 18.3 Specimen-electron interaction volume within the specimen. Notice the different regions where secondary electrons and backscattered electrons come from.

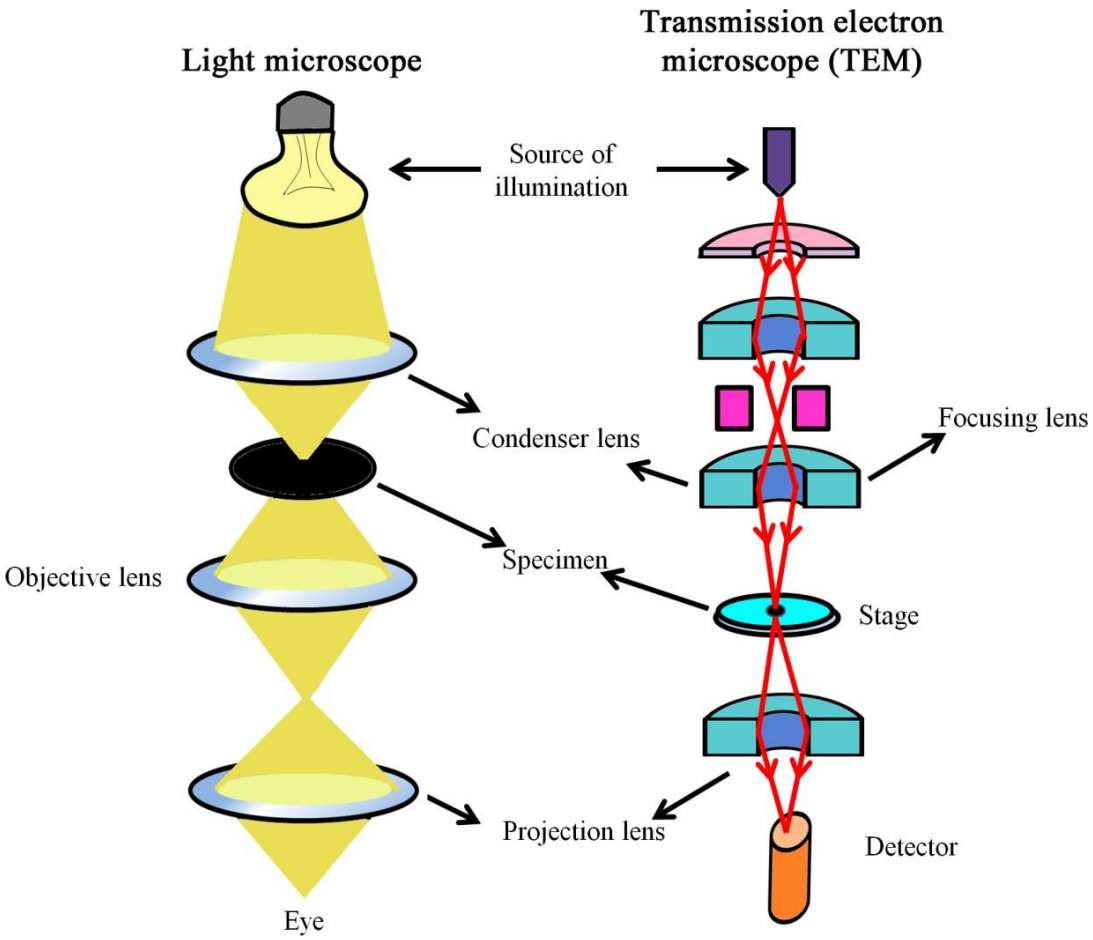
A secondary electron detector is biased with positive potential to attract the low energy secondary electrons. Detector for backscattered electrons is not biased; the high energy backscattered electrons strike the unbiased detector. As backscattered electrons come from a significant depth within the sample (Figure 18.3), they do not provide much information about the specimen topology. However, backscattered electrons can provide useful information about the composition of the sample; materials with higher atomic number produce brighter images.

Sample preparation for SEM:

A specimen to be analyzed by electron microscopy has to be dry which most biological samples are not. As dehydration might lead to structural changes, the specimens are first fixed to preserve their structural features. Fixation is the first step and can be achieved using chemical methods such as fixation with glutaraldehyde or physical methods such as cryofixation in liquid nitrogen. The fixed specimens are then dehydrated usually by exposing them to an increasing gradient of ethanol (up to 100%). The specimens are then dried using critical point method. The dried specimens are then coated with a conducting material usually gold to make the surface conducting and cause it emit more secondary electrons. A SEM image of human erythrocytes coated with gold is shown in figure 18.4



Transmission electron microscopes



Transmission electron microscopes usually have thermionic emission guns and electrons are accelerated anywhere between 40 – 200 kV potential. However, TEM with >1000 kV acceleration potentials have been developed for obtaining higher resolutions. Owing to their brightness and very fine electron beams, field emission guns are becoming more popular as the electron guns.

Sample preparation for TEM: The very first requirement of TEM is that the specimens have to be very thin. As for SEM, the specimens to be used for TEM also need to be fixed and dried. Preparation of specimens for TEM can be a fairly tedious process: The samples are usually fixed using a combination of glutaraldehyde and paraformaldehyde. A secondary staining can be done with OsO4 (Osmium tetroxide). OsO4 fixes the unsaturated lipids and being a heavy metal acts as an electron stain too. The samples are then dehydrated exactly as done for SEM analysis. The dried samples are then sectioned to obtain ultrathin (<100 nm thickness) sections. This is typically achieved by embedding the sample in a plastic mold and cutting the sections. Epoxy and acrylic resins are also used for embedding the samples for sectioning. The sections are then stained with a heavy metal stain such as uranyl acetate and phosphotungstic acid. The stained sample is then deposited on a carbon coated grid

and analyzed by TEM. Figure 18.6 shows a TEM image recorded for a peptide that self-assembled into spherical structures.