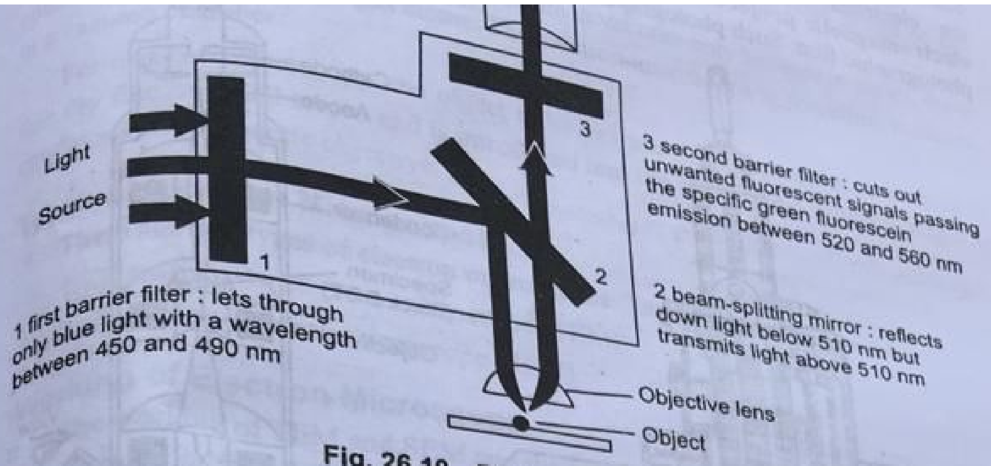

Fig. 26.10 Fluorescent microscope.

SPECTROPHOTOMETRY

In spectrophotometry, microscope with high resolving power is combined with spectroscopic devices for measuring the absorption of spectra of single cell preparation. The ultraviolet microscope, equipped with quartz lenses proves to be extremely useful in the study of nucleic acids because these absorb short ultraviolet rays in a very characteristic and selective fashion.

ELECTRON MICROSCOPE

The elecron microscope was designed by **Knoll** and **Ruska** of Germany in 1932, **Marton** of Belgium in 1934 and **Prebus** and **Miller** of Canada in 1934. It has a very high resolving power. **Seimens** produced first commercial transmission electron microscope. The present limit of resolution of **EM** is about 3 Å. An Angstron (Å) is equal to 1/10,000 of a micron (μ) or 1/2,54,000,000 of an inch.

Principle

In electron microscope, the high speed electrons of short wavelength ($\lambda = 0.50$ Å) are used as illuminating agents instead of light rays. The wavelength of electrons is determined by the voltage at which these are generated. At 50,000 volts the electrons have a wavelength of about 0.50 Å. Thus, the resolving power of electron microscope will be half of 0.50 Å, *i.e.*, 0.25 Å. However, due to technical difficulties, the resolving power of EM is maintained at 10–20 Å.

Principle of Working

In electron microscope, the electromagnetic field acte upon electrons, as glass lenses act upon light rays. Thus, an **electromagnetic condenser lens** collects and condenses the electrons on the object and an **electromagnetic objective lens** focuses the electrons reflected by the object and forms the first image. Another **electromagnetic projector lens** forms the final image on the photographic screen or photographic film. Such photographs are known as **electron micrographs.**

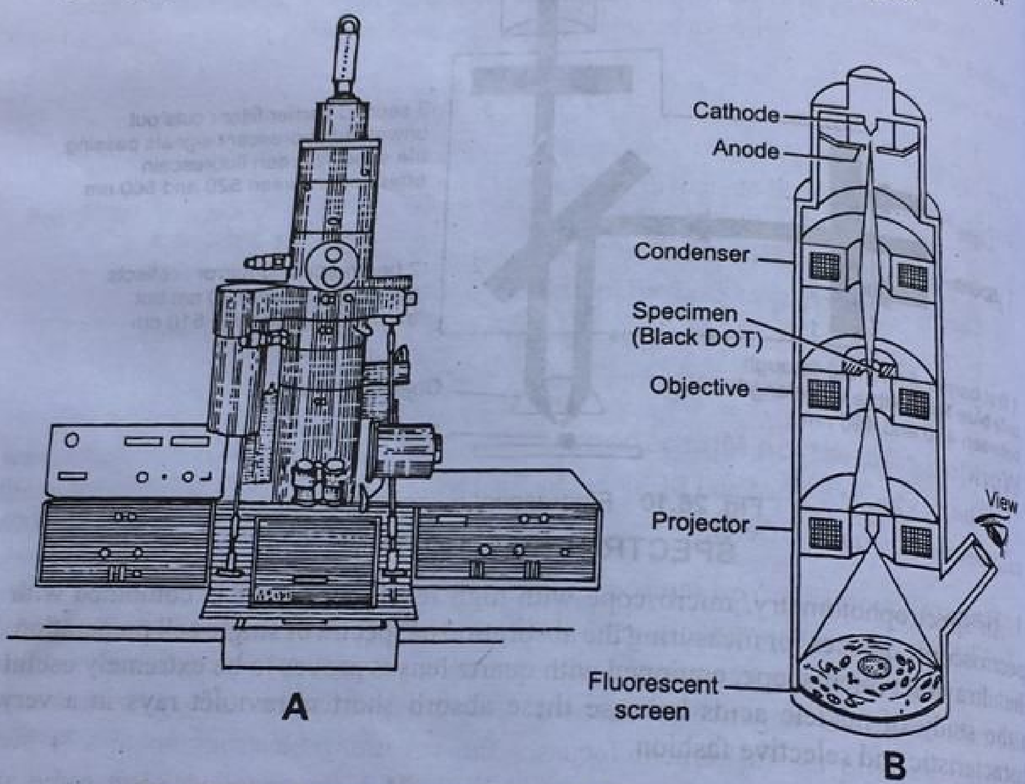

Fig. 26.11 Electron microscope. **A.** External view **B.** In section

Nature of Image

The image formed by the electron microscope does not have colours. The electron micrographs are in the shades of black, gray and white. The colour enhanced micrographs are produced by computer aided shading.

Structure

The electron micrscope comprises the following parts :

1. **Filament** or **cathode** which emits stream of electrons.

2. **Cathode ray tube,** through which electrons travel and reach the condenser lens.
3. **Condenser lens** is the magnetic coil which focuses or condenses the electrons beam in the plane of the object.
4. **Objective lens** is also an electromagnetic coil which produces first magnified image of the object.
5. **Ocular** or **projector lens** is another electromagnetic coil which magnifies the first image formed by the objective lens.
6. **Fluorescent screen** or **photographic plate** receives the final image of the object.

The electrons can travel in the straight line in vacuum only because in the air, these collide with oxygen or nitrogen atoms. The electron microscope is, therefore, enclosed in a vacuum chamber.

For electron microscopy, the object to be examined is prepared as an extremely thin dry film on small screen and is introduced into the microscope between magnetic condenser and magnetic objective.

Types of Electron Microscopes

There are two types of electron microscopes. These are :

1. Transmission electron microscope (TEM).
2. Scanning electron microscope (SEM).

Working of Electron Microscope

The working of TEM and SEM and the image formed by the two types of electron microscopes is quite different.

1. Transmission Electron Microscope (TEM)

TEM is the most commonly used electron microscope. This was built by **Ruska** and his colleagues. In transmission electron microscope, an electric field propels electrons from a negatively charged electrode. Magnetic field created by electromagnets in the instrument focusses the streaming electrons on to a stained specimen. Many of these electrons pass right through the specimen. But some are scattered by the atoms of the metallic stains that have combined with certain atoms of the specimen. The electrons that pass through are focused with electromagnets on to a screen coated with a phosphorescent material. The electrons striking the screen excit the fluorescent material to emit visible light that can be seen directly. Electrons that ar not transmitted by the specimen fall out and are lost from the beam and lea correspondingly dark regions on the viewing plate. The image many be formed or phosphorescent screen or on photographic plate. The image from a TEM is a pattern bright and dark areas corresponding to the areas of greater or lesser electron densit

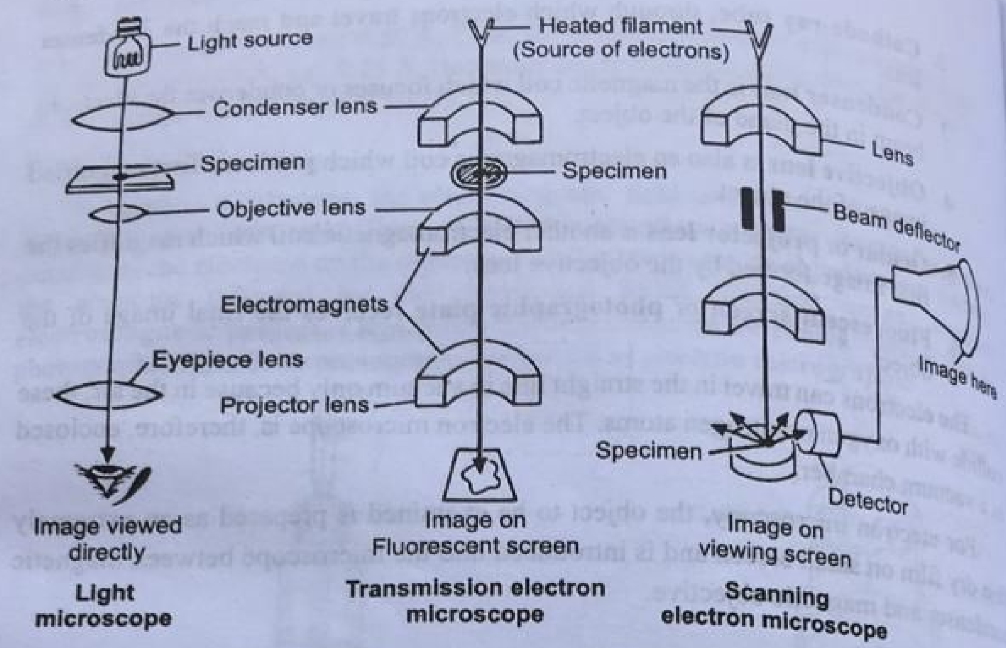

Fig. 26.12 Formation of image **A.** By light microscop, **B.** By transmission electron microscope **C.** By scanning electron microscope.

The wavelength of a typical electron beam is about 0.005 nanometre produced by using 50,000 volts. It permits resolution of 0.0025 nanometre. Because of difficulties in construction and operation of magnetic lenses, the highest resolution now available is about 0.1 to 0.2 nanometre.

2. Scanning Electron Miscroscopy (SEM)

SEM is used for examining the surface of a specimen, because the image or photograph is formed by the electrons reflected from the surface of an object. Knoll demonstrated the feasibility of scanning electron microscope. A very fine pencil of electrons (about 20 nanometer in diametre) is focused on the specimen and then scanned over the surface. Some of them may be reflected from the surface, or may excite emission of secondary electrons from the surface of specimen at the point where the beam is scanning. These secondary electrons are collected by the positively charged grid, the **collector.** It gives rise to a flash of light in a solid scintillator. The light output is amplified in a **photomultiplier** or **video amplifier.** The signal from the grid is transferred to a television tube which scans and forms the image on the screen. The image formed is three-dimensional.

The resolving power of scanning electron microscope is comparatively less than that of transmission microscope (50 Å).

The transmission electron microscope uses the electrons that have passed through the specimen to form an image and the scanning electron microscope uses electrons that are scattered from the specimen's surface.

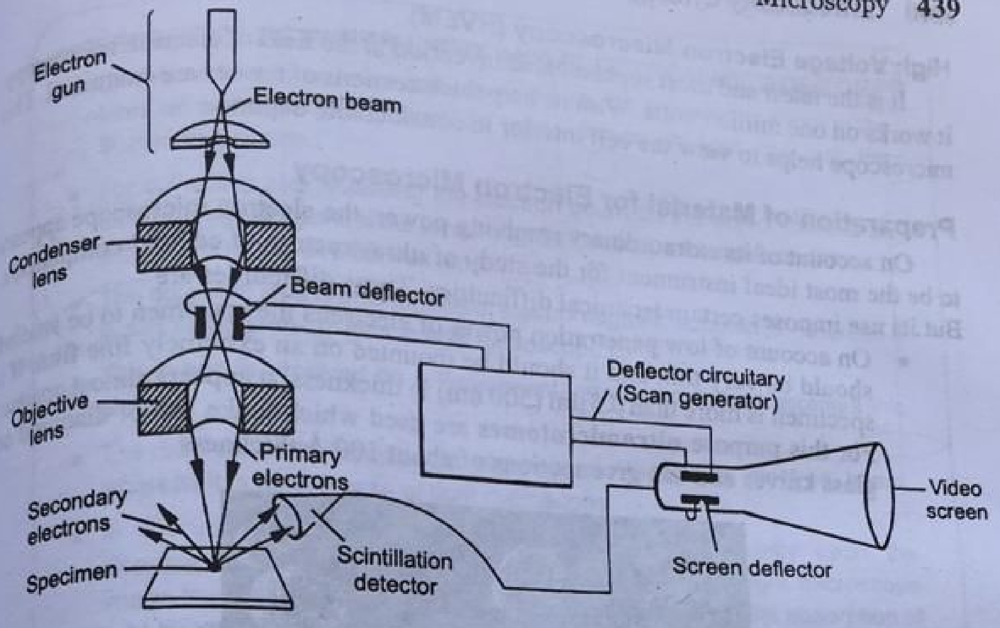

Fig. 26.13 Essential components of scanning electron microscope.

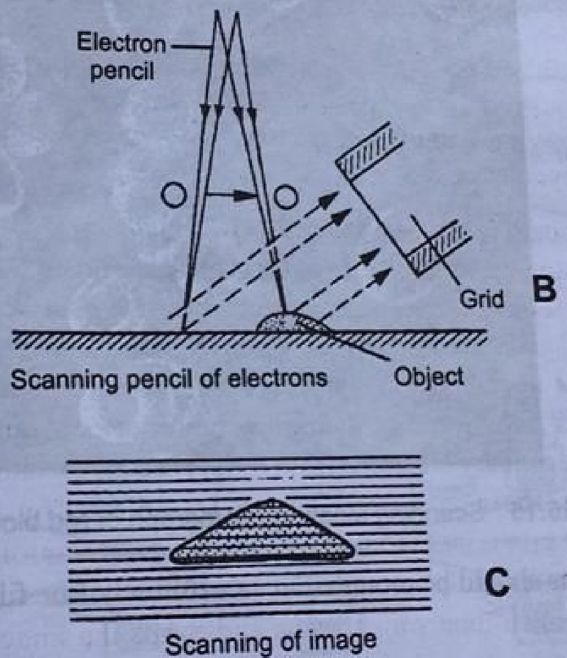

Fig. 26.14 Scanning electron microscope. A. Scanning pencil of electrons which moves across the specimen; B. Scanning of image.