Course: PG-Pathshala

Paper 10: Techniques in Biophysics II (Based on Spectroscopy)

Module 4: Scanning Electron microscopy (SEM)

Content Writer: Dr. M. R. Rajeswari, AIIMS, New Delhi

Objectives:

- **Introduction of Scanning electron microscope**
- **To know the working principle of Scanning electron microscope**
- **To elucidate the instrumentation and specimen preparation for transmission electron microscopy.**
- **To explore the application of SEM in surface details of micro-organisms, cells and tissues.**
- **To know the main differences between the TEM and SEM is based on their basic function**

SCANNING ELECTRON MICROSCOPY :

1 Introduction

The Scanning Electron Microscope, which is utilized in various fields such as medical, biological, metals, semiconductors and ceramics, is broadening its application frontier. With abundant attachments and devices being combined, its capability is expanding. SEM is regarded as one of the most powerful tools being used at R&D institutes and quality control inspection sites all over the world.

The scanning electron microscope is mainly used for examining the **surface details of micro-organisms, cells and tissues. Therefore we can call as t**he champion of micro surface observation. The first step of a scientific evaluation is to thoroughly and in depth analysis to observe the form of the material. For this purpose, we have a magnifying glass or an optical microscope. But, as long as light is used, we can't see anything smaller than the wavelength of light and therefore observing a nano structure is extremely difficult. The Scanning Electron Microscope (hereinafter "SEM") enables a clear observation of very small surface structures, which is not possible with an optical microscope (hereinafter "OM"). Moreover, as it can provide images with deeper focal depth, it enables observations of 3-dimensional images, with a similar sense as when we look at a substance with the naked-eye, by enlarging the specimen surface which has a rough structure.

The complex interaction between the accelerated electrons and the specimen results in various physical products such as elastically scattered electrons, secondary electrons, X-rays, etc. SEM utilizes electrons to show an enlarged image of a specimen. The Scanning Electron Microscope (SEM) introduced here utilizes an electron beam whose wavelength is shorter than that of light and therefore observing a structure down to several nm in scale becomes possible.

1nm = billionth = 10-9m

2. Different types of Ems

Based upon these various physics products, different kinds of electron microscopes have been developed. Some of the commonly used electron microscopes are:

1. **Transmission electron microscope (TEM) (which we discussed in previous module 3)**
2. **High voltage electron microscope (HVEM)**
3. **Scanning electron microscope (SEM)**
4. **Scanning transmission electron microscope (STEM) (will not be discussed here)**

3. Limitations of TEM and evolution of SEM

There are following drawbacks of TEM technique.

1.) Many materials require extensive sample preparation to produce a sample thin enough to be electron transparent, which makes TEM analysis a relatively time consuming process with a low throughput of samples.

2.) Graphene, a carbon nanomaterial, relatively transparent, very hard and just one atom thick, is currently being used as a platform on which the materials to be examined are placed. Being almost transparent to electrons, a graphene substrate has been able to show single hydrogen atom and hydrocarbons.

3.) The structure of the sample may also be changed during the preparation process. Also the field of view is relatively small, raising the possibility that the region analyzed may not be characteristic of the whole sample.

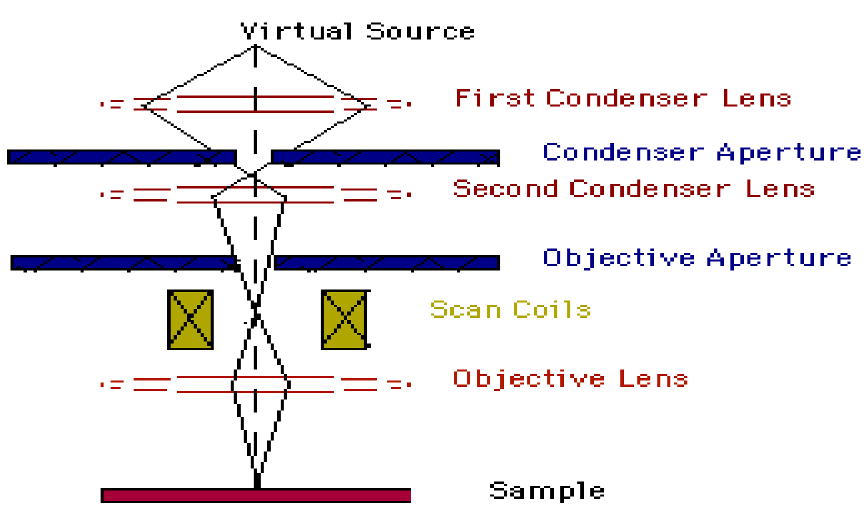
4.) There is potential that the sample may be damaged by the electron beam, particularly in the case of biological materials.

5.) The main difference between the TEM and SEM is based on their basic function. In TEM one can see internal anatomy of the sample while in case of SEM only surface morphology can be examined.

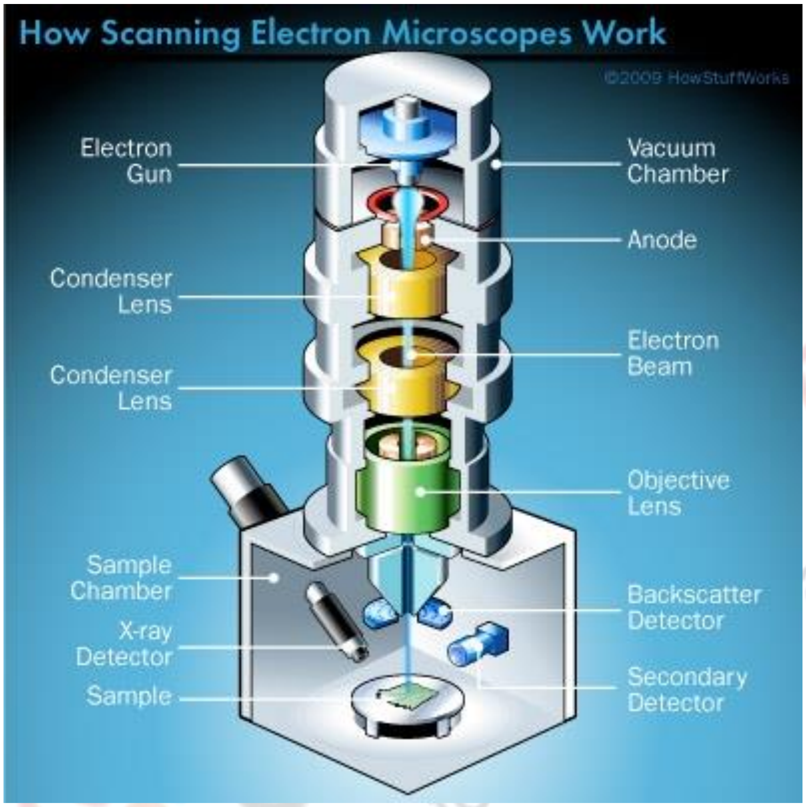
And therefore , the SEM was developed.

4. Instrumentation:

The image in SEM is obtained by scanning the specimen surface with a narrow electron beam, collecting and amplifying the generated signals and feeding them to the cathode ray tube for display



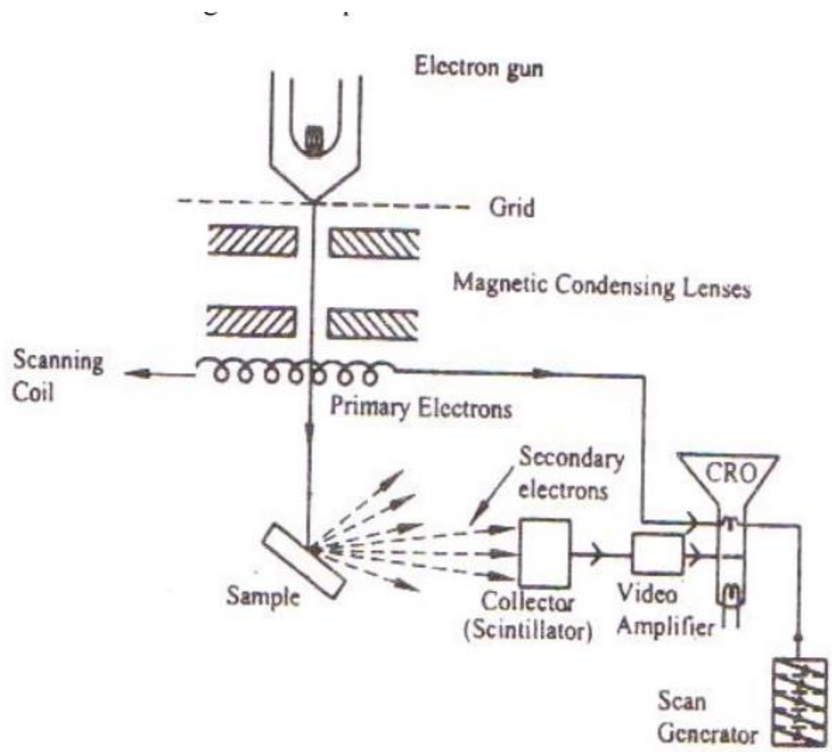
Schematic representation of a Scanning Electron Microscope (SEM).



5 Principle: When the accelerated primary electrons strikes the sample , it produces secondary electrons . these secondary electrons are collected by a positive charged electron detector which in turn gives a 3- dimensional image of the sample.

6 Construction: It consists of an electron gun to produce high energy electron beam. A magnetic condensing lens is used to condense the electron beam and a scanning coil is arranged in-between magnetic condensing lens and the sample.

The electron detector (Scintillator) is used to collect the secondary electrons and can be converted into electrical signal. See below :



These signals can be fed into CRO through video amplifier as shown. Therefore, Scanning electron microscope consists two main parts,

- The probing system
- The display system

The Probing system

The electron beam is generated by an electron gun (or electron probe), which consists of a V-shaped hairpin tungsten filament. When the filament is heated, electrons are emitted and accelerated through the anode.

The display system As the electron beam strikes the specimen, many signals are generated, such as secondary electrons, X-rays, backscattered electrons, auger electrons etc. Different signals give different information about the specimen.

- Secondary electrons provide information on surface morphology
- X-rays are used for elemental analysis
- Backscattered electrons- surface topology

7. How does SEM function ?

Principle and function:

SEM function on the principle of scanning the surface of the specimen and there are two types of electron are formed. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

Primary electron imaging.

When an electron from the beam encounters a nucleus in the sample, the resultant columbic attraction results in the deflection of the electron's path, known as Rutherford elastic scattering. A few of these electrons will be completely **backscattered**, re-emerging from the incident surface of the sample. Since the scattering angle is strongly dependent on the atomic number of the nucleus involved, the primary electrons arriving at a given detector position can be used to yield images containing both topological and compositional information.

Secondary electron imaging.

The high energy incident electrons can also interact with the loosely-bound conduction band electrons in the sample. The amount of energy given to these **secondary** electrons as a result of the interactions is small, and so they have a very limited range in the sample (a few nm). Because of this, only those secondary electrons that are produced within a very short distance of the surface are able to escape from the sample. This detection mode is most widely used of the SEM modes. Secondary electrons emitted by the sample are attracted by a grid (at positive potential) located in front of the detector. The detector collects all the electrons coming towards it. These electrons are accelerated towards a scintillator by applying strong positive voltage (10-12.5 kv). When electrons hit the scintillator, they generate photons, which are guided by the light pipe to the photomultiplier where photoelectrons are produced. These photoelectrons are amplified and fed to the cathode ray tube (display screen).

8. Some data SEM.

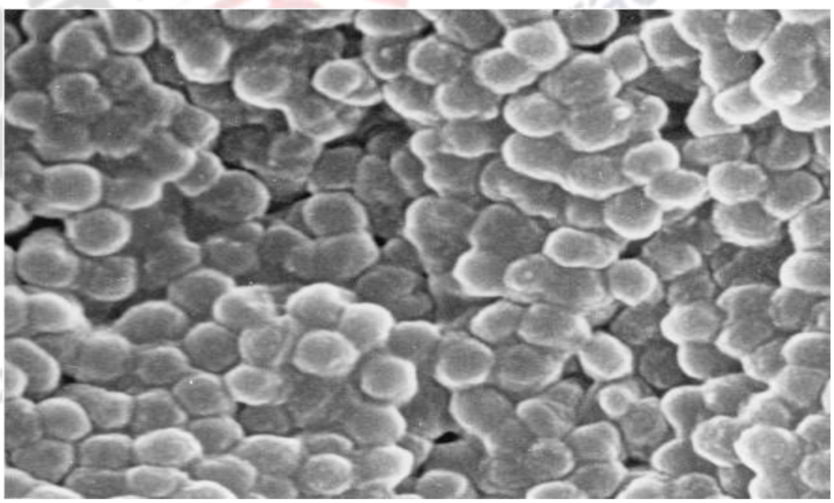


Figure 1 Scanning electron micrograph of *Acinetobacter baumannii* type strain ATCC 19606 (final magnification, 318,000) (Berezin and Towner; Clinical Microbiology Reviews, 1996, 148–165).

Figure 2 The pollen grains taken on an SEM show the characteristic depth of field of SEM micrographs. (see below). Left Pollens (1228x935)

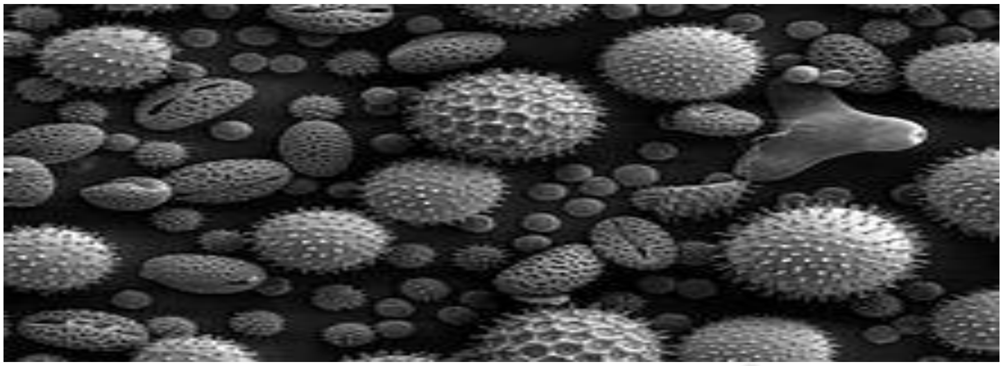
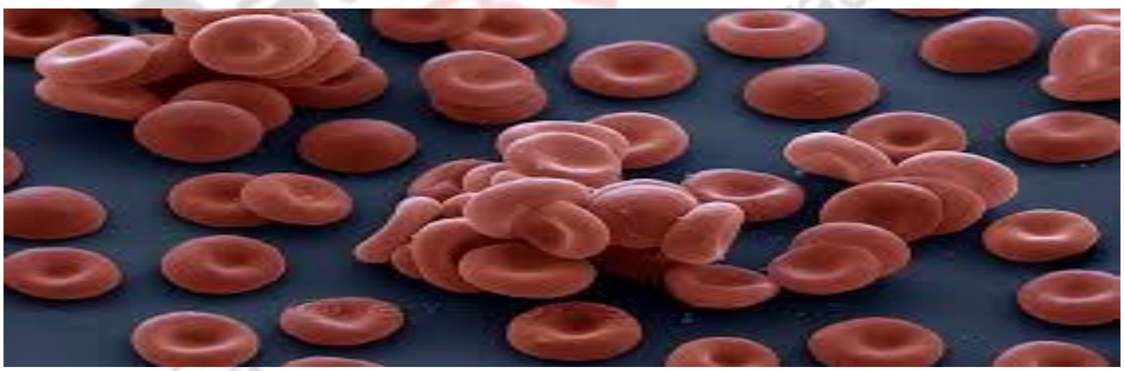


Figure 3 Red blood cells (1421x900)

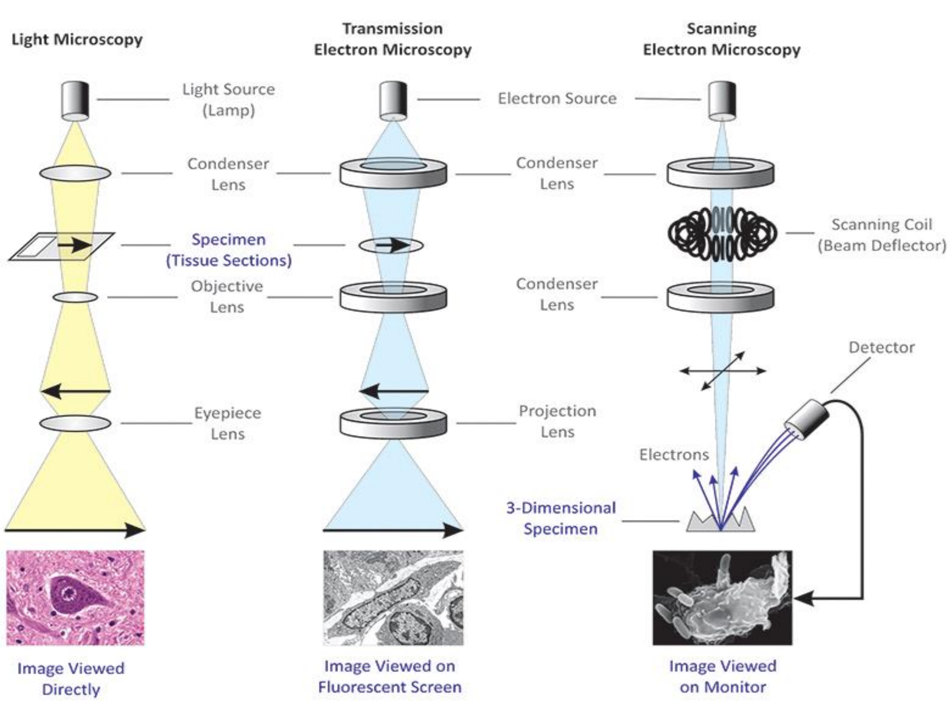


9 Comparison of Transmission electron microscope (TEM) and Scanning electron microscope (SEM).

Factors	TEM	SEM
Operational mode	Only surface (morphology)	Internal study (anatomy)

	See figure 7.30	See figure 7.28b
Source of detection	Transmitted electrons	Reflected and backscattered electrons, X- rays
Voltage applied for electron generation	10-12.5 kv	100kv
Medium	Low Vacuum requirement	Only high vacuum
Resolving power	0.2 nm	0.1 nm

10. Comparison of LM, TEM, SEM



Scanning Electron microscope is also used for Elemental analysis with different operational method

11. SEM Elemental analysis

Scanning Electron microscope is also used for Elemental analysis with different operational method. SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

12. SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

- **External morphology (texture)**
- **Chemical composition (when used with EDS)**
- **Orientation of materials making up the sample**
- **The EDS component of the system is applied in conjunction with SEM analysis to:**
- **Determine elements in or on the surface of the sample for qualitative information**
- **Measure elemental composition for semi-quantitative results**
- **Identify foreign substances that are not organic in nature and coatings on metal**

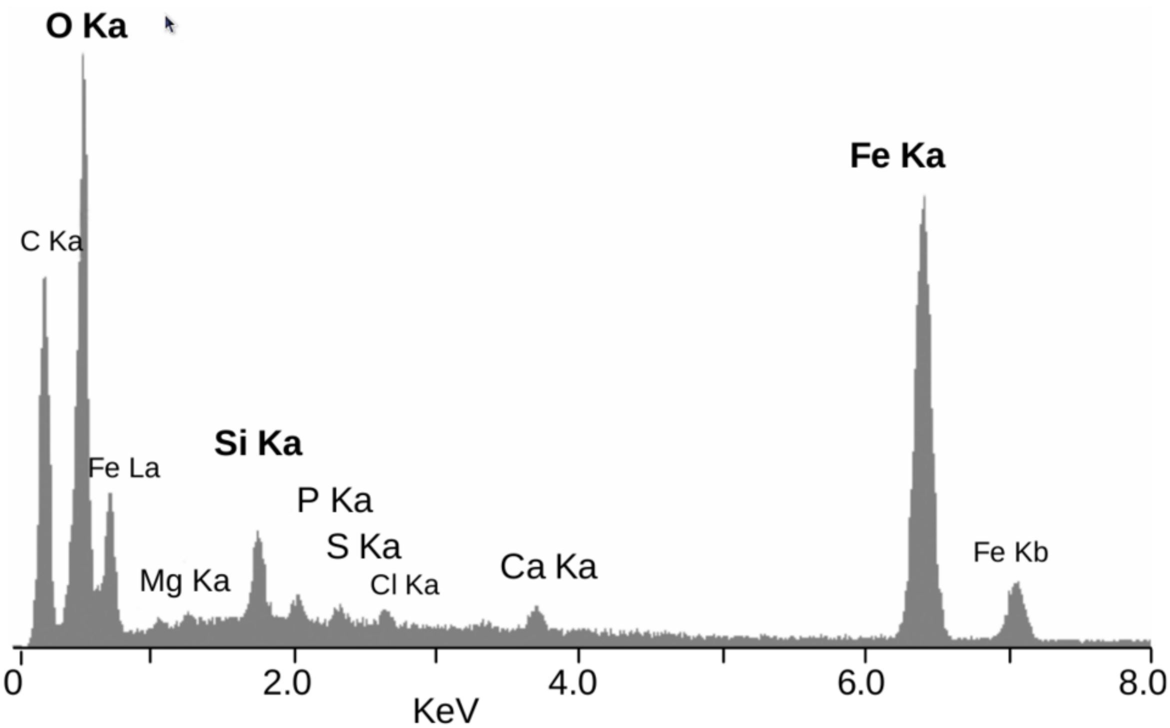
13. Principle :

When incident electron beam hits the atoms of the specimen, X-rays are emitted which are characteristic for the particular atom present in the specimen. The high velocity incident electron beam caused electron of the outer shell having the lowest energy to be ejected. An electron falling from the higher energy orbit, thus loosing energy in transition fills this vacancy. This energy is emitted as X-rays. A special detector mounted in SEM collects these X-rays.

Each element contains electrons in orbits with particular discrete energy levels. X-rays collected by the detector are analyzed for energy. Corresponding to the energy detected the element to which the electron belongs can be identified. If a number of elements are simultaneously irradiated by the electron beam a spectrum of X-ray energy is emitted. Thus information on the types of element, their concentration and distribution can be obtained by analyzing such X-ray spectra. The spectra can be produced with the help of either a wavelength disperse spectrometer (WDS) or

SEM analysis :

EDS spectrum of the mineral crust of the vent shrimp *Rimicaris exoculata*[1] Most of these peaks are X-rays given off as electrons return to the K electron shell.(K-alpha and K-beta lines) One peak is from the L shell of iron.



(Ref CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=767641)

Summary:

1. What is Scanning electron microscopy. The complex interaction between the accelerated electrons and the specimen results in various physical products such as elastically scattered electrons, secondary electrons, X-rays, etc. SEM utilizes electrons to show an enlarged image of a specimen
2. The scanning electron microscope is mainly used for examining the surface details of micro-organisms, cells and tissues.
3. Principle of SEM..When the accelerated primary electrons strikes the sample , it produces secondary electrons and these secondary electrons are collected by a positive charged electron detector which in turn gives a 3- dimensional image of the sample.

4. Differences between TEM and SEM -- The main difference between the TEM and SEM is based on their basic function. In TEM one can see internal anatomy of the sample while in case of SEM only surface morphology can be examined.
5. Examples of SEM in biolgy
6. EDS for to measure elemental composition for semi-quantitative results, and to identify foreign substances that are not organic in nature and coatings on metal Chemical composition
7. Principle of SEM for elememtal analysis ..The primary electrons arriving at a given detector position can be used to yield images containing both topological and compositional information The information on the types of element, their concentration and distribution can be obtained by analyzing such X-ray spectra. The spectra are produced with the help of either a wavelength disperse spectrometer (WDS) or an energy disperse spectrometer (EDS). EDS and its applications of the Scanning Electron Microscope, in various fields such as medical, biological, metals has been elucidated in this module.