

SCANNING ELECTRON MICROSCOPE

Microbiology – I

BVOC- HCT - 1 semester

Scanning Electron Microscope (SEM)

- TEM images are produced by radiation of electrons that are passed through a specimen.
- **SEM** images are formed by the electrons emitted from the atoms on an object's surface.
- Surfaces of microorganisms are observed in great detail .
- The resolution of 7 nm or less.

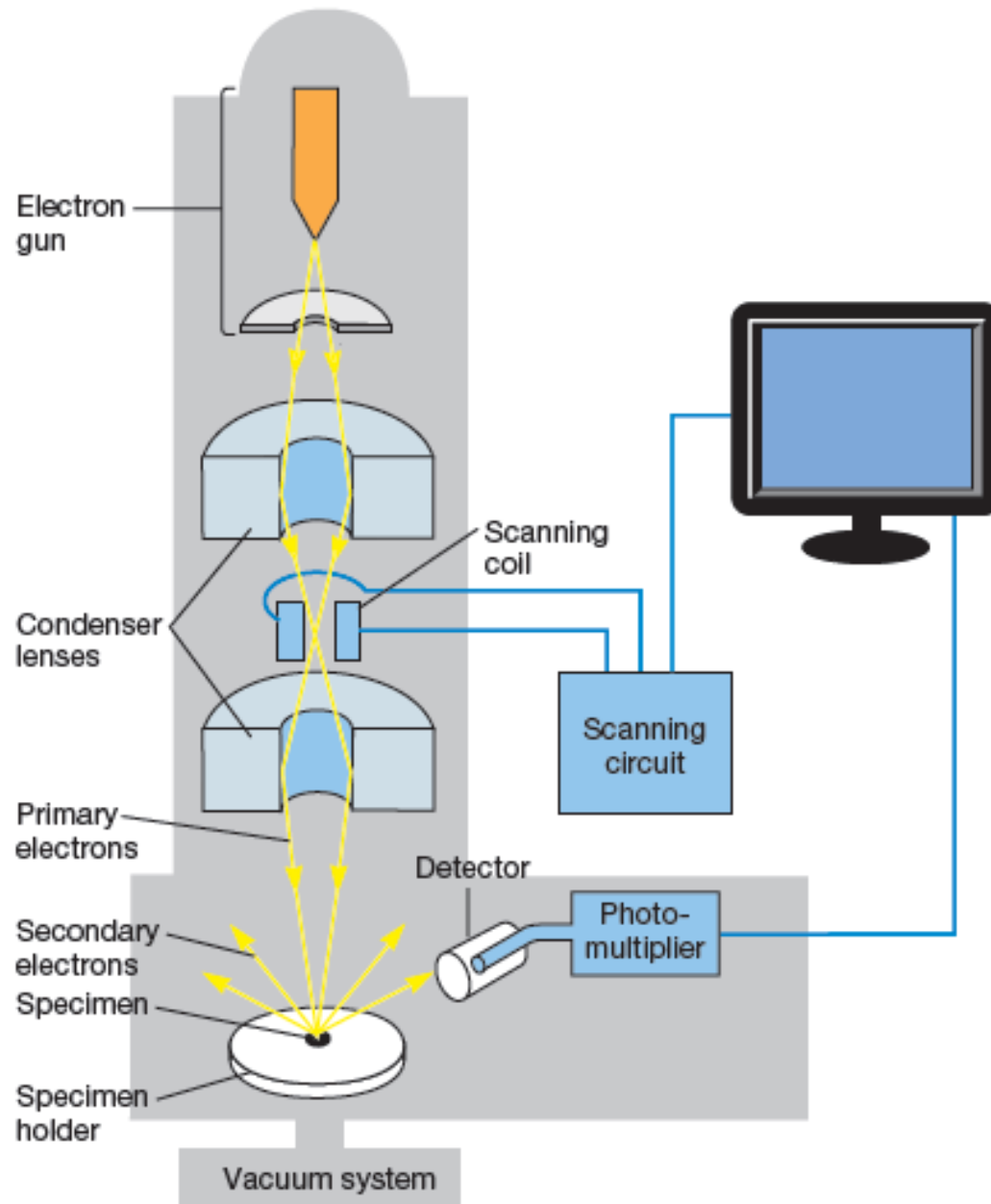


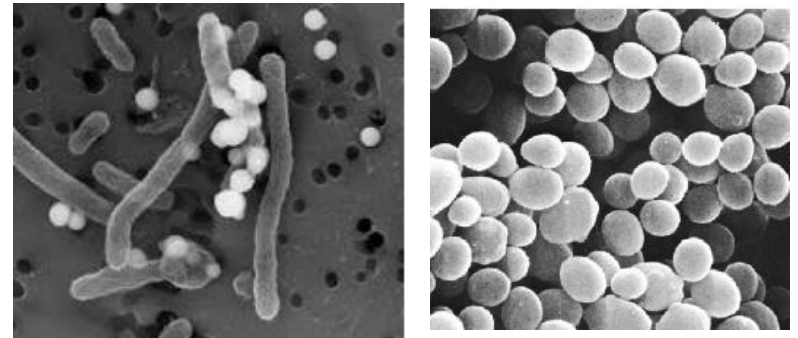
Figure 2.26 The Scanning Electron Microscope.

Image formation in SEM:

- SEM scans are in the form of narrow, tapered electron beam that moves back and forth over the specimen.
- Beam strikes a particular area on the surface atoms discharge a tiny shower of electrons producing **secondary electrons** that are trapped by a specialized detector.
- Secondary electrons strike the scintillator that is placed in the detector that emits light when struck by electrons.
- Flashes of light produce electrical current which is amplified by the photomultiplier.
- signal are passed to the cathode-ray tube where the image is digitized and seen in the computer that is viewed and captured (photograph).

Secondary electrons – image:

- No. of secondary electrons – detector – nature of the specimen's surface.
- Raised area → large number
- Depression → fewer electrons
- Image appearance on screen:
 - raised areas - lighter
 - depressions - darker.



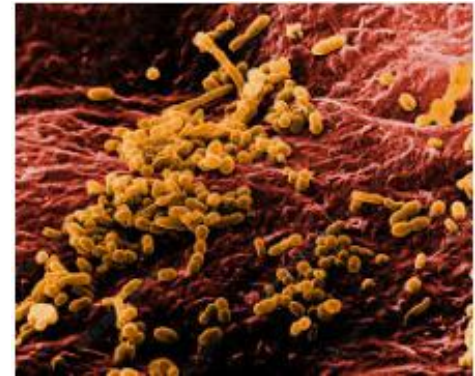
SEM image - bacteria

Specimen preparation for SEM

- It is relatively easy.
- air-dried material - examined directly.
- Microorganisms are fixed, dehydrated, and dried
 - It preserves surface structure
 - prevents collapse of the cells
 - As it is exposed to vacuum.
- Mounted and coated with a thin layer of metal (gold)
 - Prevents the buildup of an electrical charge on the surface
 - Gives better image.

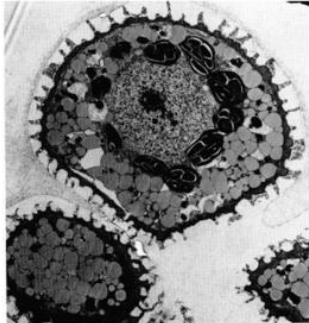
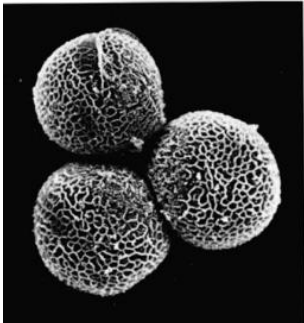
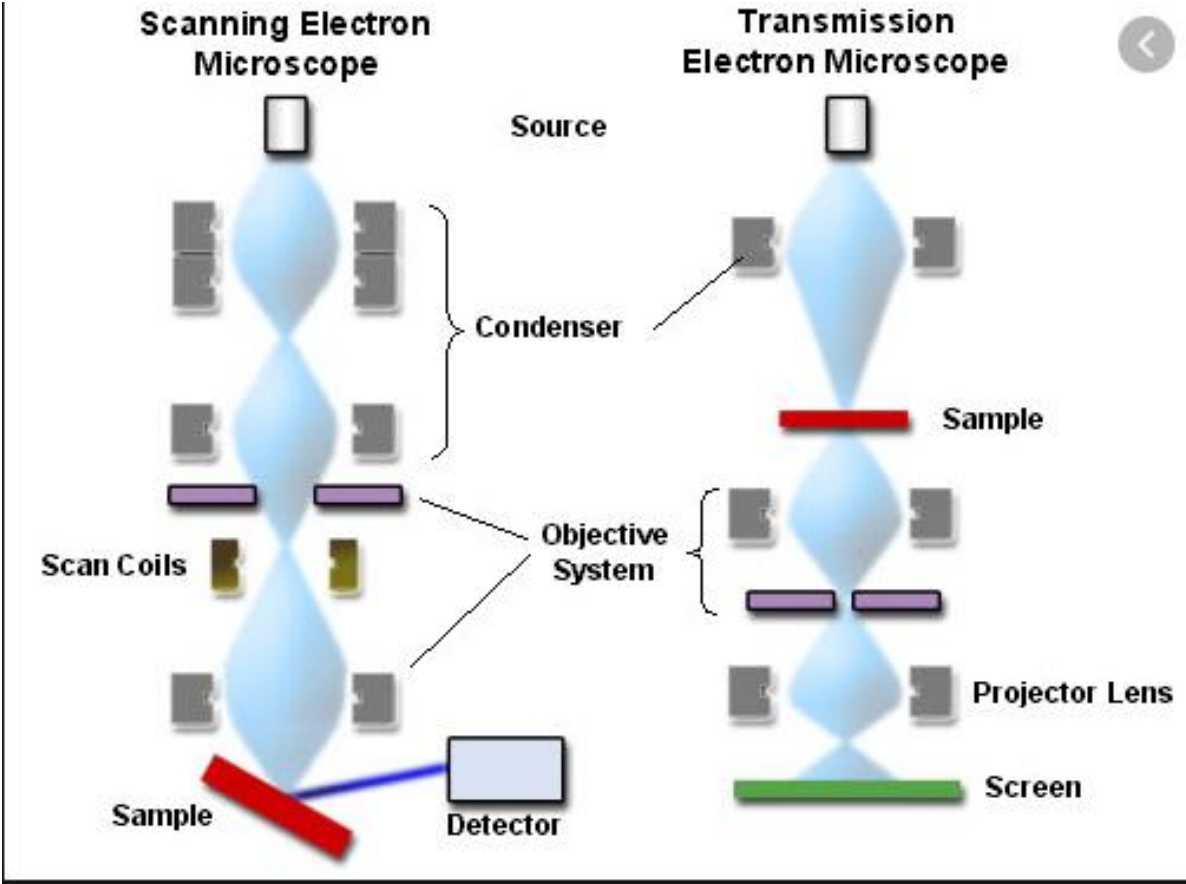
Advantages:

- It gives realistic three-dimensional image of the microorganism's surface
- The actual *in situ* location of microorganisms in ecological niches can be examined
- Ex. human skin and the lining of the gut.



Differences b/w TEM & SEM

| TEM | SEM |
|---|---|
| Electron beam passes through thin sample. | Electron beam scans over surface of sample. |
| Specially prepared thin samples are supported on TEM grids. | Sample can be any thickness and is mounted on an aluminum stub. |
| Specimen stage halfway down column. | Specimen stage in the chamber at the bottom of the column. |
| Image shown on fluorescent screen. | Image shown on TV monitor. |
| Image is a two dimensional projection of the sample. | Image is of the surface of the sample |



SEM vs. TEM image

Limitations of electron microscopy

- Expensive to buy and maintain
- Requires extremely stable high voltage supplies and currents to each electromagnetic lens
- Continuously pumped with high vacuum systems and cooling water supply circulation through the lens and pumps.
- Very sensitive to vibration and external magnetic fields.
- Requires specialized skill training for operation
- Samples have to be viewed in a vacuum.