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**MATRIC NO.: 16/MHS01/168**

**COURSE: ELECTRON MICROSCOPIC TECHNIQUE AND ULTRASTRUCTURE.**

**COURSE CODE: ANA 402**

**QUESTION:**

1. **WRITE AN ESSAY ON THE HISTORY OF MICROSCOPY**
2. **DIFFERENTIATE BETWEEN THE LIGHT MICROSCOPE AND EKECTRON MICROSCOPE**
3. **DIFEERENTIATE BETWEEN THE SEM AND TEM**

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**MICROSCOPY**

Microscope is an instrument for viewing objects that are too small to be seen by the naked or unaided eye.

**History of Microscopy**

The history of microscopy starts from 710BC, the years and what was invented are stated below;

**710 BC – Nimrud lens:**

The Nimrud lens a piece of rock crystal may have been used as a magnifying glass or as a burning-glass to start fires by concentrating sunlight. It is later unearthed by Austen Henry Layard at the Assyrian palace of Nimrud in modern-day Iraq.

**1000 AD – Reading stone**

The first vision aid, called a reading stone, is invented. It is a glass sphere placed on top of text, which it magnifies to aid readability.

**1021 AD – Book of Optics**

Muslim scholar Ibn al-Haytham writes his Book of Optics. It eventually transforms how light and vision are understood.

**1284 – First eye glasses**

Salvino D’Armate is credited with inventing the first wearable eye glasses.

**1590 – Early microscope**

Zacharias Janssen and his son Hans place multiple lenses in a tube. They observe that viewed objects in front of the tube appear greatly enlarged. This is a forerunner of the compound microscope and the telescope.

**1609 – Compound microscope**

Galileo Galilei develops a compound microscope with a convex and a concave lens.

**1625 – First use of term ‘microscope’**

Giovanni Faber coins the name ‘microscope’ for Galileo Galilei’s compound microscope.

**1665 – First use of term ‘cells’**

English physicist Robert Hooke publishes Micrographia, in which he coins the term ‘cells’ when describing tissue. The book includes drawings of hairs on a nettle and the honeycomb structure of cork. He uses a simple, single-lens microscope illuminated by a candle.

**1676 – Living cells first seen**

Antonie van Leeuwenhoek builds a simple microscope with one lens to examine blood, yeast and insects. He is the first to describe cells and bacteria. He invents new methods for making lenses that allow for magnifications of up to 270 times.

**1830 – Spherical aberration solved**

Joseph Jackson Lister reduces spherical aberration (which produces imperfect images) by using several weak lenses together at certain distances to give good magnification without blurring the image.

**1874 – Abbe equation**

Ernst Abbe writes a mathematical formula that correlates resolving power to the wavelength of light. Abbe’s formula makes it possible to calculate the theoretical maximum resolution of a microscope**.**

**1931 – Transmission electron microscope**

Ernst Ruska and Max Knoll design and build the first transmission electron microscope (TEM), based on an idea of Leo Szilard. The electron microscope depends on electrons, not light, to view an object. Modern TEMs can visualise objects as small as the diameter of an atom.

**1932 – Phase contrast microscope**

Frits Zernike develops phase contrast illumination, which allows the imaging of transparent samples. By using interference rather than absorption of light, transparent samples, such as cells, can be imaged without having to use staining techniques.

**1942 – Scanning electron microscope**

Ernst Ruska builds the first scanning electron microscope (SEM), which transmits a beam of electrons across the surface of a specimen.

**1962 – Green fluorescent protein (GFP) discovered**

Osamu Shimomura, Frank Johnson and Yo Saiga discover green fluorescent protein (GFP) in the jellyfishAequorea victoria. GFP fluoresces bright green when exposed to blue light.

**1972 – First CAT scanner**

Godfrey Hounsfield and Allan Cormack develop the computerised axial tomography (CAT) scanner. With the help of a computer, the device combines many X-ray images to generate cross-sectional views as well as three-dimensional images of internal organs and structures.

**1973 – Electron backscatter patterns observed**

John Venables and CJ Harland observe electron backscatter patterns (EBSP) in the scanning electron microscope. EBSP provide quantitative microstructural information about the crystallographic nature of metals, minerals, semiconductors and ceramics.

**1978 – Confocal laser scanning microscope**

Thomas and Christoph Cremer develop the first practical confocal laser scanning microscope, which scans an object using a focused laser beam.

**1981 - Scanning tunnelling microscope**

Gerd Binnig and Heinrich Rohrer invent the scanning tunnelling microscope (STM). The STM ‘sees’ by measuring interactions between atoms, rather than by using light or electrons. It can visualise individual atoms within materials.

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**1986 – Nobel Prize for microscopy**

The Nobel Prize in Physics is awarded jointly to Ernst Ruska (for his work on the electron microscope) and to Gerd Binnig and Heinrich Rohrer (for the scanning tunnelling microscope).

**1992 – Green fluorescent protein (GFP) cloned**

Douglas Prasher reports the cloning of GFP. This opens the way to widespread use of GFP and its derivatives as labels for fluorescence microscopy (particularly confocal laser scanning fluorescence microscopy).

**1993–1996 – Super-resolution microscopy**

Stefan Hell pioneers a new optical microscope technology that allows the capture of images with a higher resolution than was previously thought possible. This results in a wide array of high-resolution optical methodologies, collectively termed super-resolution microscopy.

**2010 – Atoms of a virus seen**

Researchers at UCLA use a cryoelectron microscope to see the atoms of a virus.

**2014 – Chemistry Nobel prize for super microscopes**

Nobel Prize in Chemistry awarded to Eric Betzig, Stefan Hell and William Moerner for the development of super-resolved fluorescence microscopy which allows microscopes to now ‘see’ matter smaller than 0.2 micrometres.

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|  **Difference Between Electron Microscope And Light Microscope**  |
| **Light Microscope** | **Electron Microscope** |
| Uses light ( approx 400-700 nm) as an illuminating source | Uses electron beams (approx 1 nm) as an illuminating source. |
| Lower magnification than an electron microscope | Higher magnification |
| No risk of radiation leakageRadiation source: Tungsten or quartz halogen lamp | Risk of radiation leakage Radiation source: high voltage tungsten voltage |
| Specimen preparation takes about a few minutes or an hour | Specimen preparation takes several days |
| Both live and dead specimen can be seen | Only dead and the dried specimen can be seen |
| The image formation depends upon the light absorption from the different zones of the specimen | The image formation depends upon the electron scattering |
| The image is seen through the ocular lens. No screen needed | The image is received on a zinc sulphate fluorescent screen |
| Useful magnification of 500x to 1500x | Direct magnification as high as 16000x and photographic magnification as high as 1000000 x |
| Low resolution | High resolution |
| Inexpensive and requires low maintenance cost | Expensive and high maintenance |
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**Differences between SEM and TEM are as follows:**

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| **Properties** | **Scanning Electron Microscopy  (SEM)** | **Transmission Electron Microscopy (TEM)** |
| Light Source | SEM is based on scattered electrons, i.e. electrons emitted from the surface of a specimen. It is the EM analog of a stereo light microscope. | Electrons are used as “light source”. TEM is based on **transmitted electrons** and operates on the same basic principles as the light microscope. |
| Purpose | SEM provides detailed images of the surfaces of cells.  SEM focuses on the sample’s surface and its composition, so SEM shows only the morphology of samples. | Transmission electron microscope is used to view thin specimens (tissue sections, molecules, etc). TEM can show many characteristics of the sample, such as internal composition, morphology, crystallization, etc. |
| Sample Preparation | Sample is coated with a thin layer of heavy metal such as gold or palladium. | The sample in TEM has to be cut thinner (**70-90 nm**) because electrons cannot penetrate very far into materials. |
| Resolution | SEM can resolve objects as close as 20 nm. | TEM has a much higher resolution than SEM. **It can resolve objects as close as 1 nm**i.e. down to near-atomic levels. |
| Magnification | The magnifying power of SEM is up to 50,000X. | The **magnifying power** of TEM is up to **2 million times.** |
| Processing of sample (s) | SEM allows for a large amount of sample to be analyzed at a time | With TEM only a small amount of samples can be analyzed at a time. |
| Image formation | Secondary or backscattered electrons arising from the interaction of electron beam and metal-coated specimen are collected and the resulting image is displayed on a computer screen. | Transmitted electrons hit a **fluorescent screen** giving rise to a “shadow image” of the specimen with its different parts displayed in varied darkness according to their density. The image can be studied directly by the operator or photographed with a camera. |
| 3D picture | SEM  provides a 3-dimensional image | TEM provides a 2-dimensional picture. |
| Current Applications | To study topography and atomic composition of specimens, process control and also, for example, the surface distribution of immuno-labels | To image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture). |