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COURSE TITLE: THE ELECTRON MICROSCOPIC TECHNIQUE AND  
ULTRASTRUCTURE.

## HISTORY OF MICROSCOPE.

### ***History of Microscopy***

#### ***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 jellyfish *Aequorea 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.*

### **DIFFERENCES BETWEEN LIGHT MICROSCOPE AND ELECTRON MICROSCOPE.**

FEATURES	LIGHT MICROSCOPE	ELECTRON MICROSCOPE
Electromagnetic spectrum used	Visible light 390nm(red )-760nm	Electrons App. 4nm
Maximum resolution power	app. 200nm or 0.2 microns	0.14nm
Maximum magnification	X 1000- X 1500	X 5,000,000
Radiation source	Tungsten or quartz Halogen lamp	High voltage (50v) Tungsten filament
Lens	glass	magnet
Interior	Air-filled	vacuum
Focussing screen	Human eye (retina), Photographic film	Fluorescent screen, Photographic film

Fixation	formaldehyde	Glutaraldehyde, OSO <sub>4</sub>
Embedding	wax	resin
Sectioning	Microtome Slices- 20 000nm Whole cells visible	Ultramicrotome Slices – 50nm Parts of cells visible
Stains	Water soluble dye	Heavy metals
Support	Glass slide	Copper grid

## DIFFERENCE BETWEEN SCANING ELECTRON MICROSCOPE AND TRANSMISSION ELECTRON MICROSCOPE.

### ***SEM VERSUS TEM***

1. *SEM is based on scattered electrons while TEM is based on transmitted electrons.*
2. *SEM focuses on the sample's surface and its composition whereas TEM provides the details about internal composition. Therefore TEM can show many characteristics of the sample, such as morphology, crystallization, stress or even magnetic domains.*
  - *On the other hand, SEM shows only the morphology of samples.*
3. *The sample in TEM has to be cut thinner where as there is no such need with SEM sample.*
4. *TEM has much higher resolution than SEM.*
5. *SEM allows for large amount of sample to be analyzed at a time whereas with TEM only small amount of sample can be analyzed at a time*
6. *SEM is used for surfaces, powders, polished & etched microstructures, ICchips, chemical segregation whereas TEM is used for imaging of dislocations, tiny precipitates, grain boundaries and other defect structures in solids*
7. *In TEM, pictures are shown on fluorescent screens whereas in SEM, picture is shown on monitor.*
8. *SEM also provides a 3-dimensional image while TEM provides a 2-dimensional picture.*
9. *TEM requires extensive sample preparation. The thickness of the specimens to be examined under TEM should be less than 100nm.*