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**COURSE CODE: ANA 402**

**COURSE TITLE: ELECTRON MICROSCOPIC TECHNIQUE AND ULTRASTUCTURE**

**LECTURER: DR OGEDENGBE OLUWATOSIN**

1. **Write an essay on the history of electron microscopy**

The history of the electron microscope dates back to early twentieth century when the first electromagnetic lens was developed. This opened the door of possibility to use the principles of the lens to invent a microscope that could examine the structure of samples with greater detail. This had the potential to exceed the capabilities of the optical microscope, which was the first type of microscope and only alternative option at the time. The term *microscope* is derived from the Greek works *mikros*and *skopeo*, which mean *small* and *look at*, respectively. Throughout the history of science, there has been a lasting interest in viewing the intricate details of the world in increasing magnifications**.**

**Timeline of electron microscopy**

In 710 BC:

* 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.

In 1000 AD:

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

In 1021 AD:

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

In 1284:

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

In 1590:

* 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.

In 1609:

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

In 1625:

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

In 1665:

* 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.

In 1676:

* 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.

In 1830:

* 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.

In 1874:

* 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.

In 1931:

* 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.

In 1932:

* 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.

In 1942:

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

In 1962:

* 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.

In 1972:

* 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.

In 1973:

* 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.

In 1978:

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

In 1981:

* 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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In 1986:

* 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).

In 1992:

* 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).

From 1993-1996:

* 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.

In 2010:

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

In 2014:

* 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.

Electron microscopes have surpassed many of the limitations of optical microscopes, with improved resolution that makes it possible to view microscopic objects such as atoms. However, enhancements to the electron microscope continue to be made to this day. For example, an environmental-scanning electron microscope that maintains a low vacuum in the sample chamber to view specimen with moisture is currently under development.

1. **Differentiate between light microscope and electron microscope.**

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| **S/N** | **FEATURES** | **LIGHT MICROSCOPE** | **ELECTRON MICROSCOPE** |
| **1** | Lenses | Glass | Magnets |
| **2** | Interior | Air-filled | Vacuum |
| **3** | Fixation | Formaldehyde | Glutaraldehyde |
| **4** | Sectioning | Microtome(slices-2000nm)Whole cells visible | Ultramicrotome(slices-50nm)Parts of cells visible |
| **5** | Embedding | Wax | Resin |
| **6** | Maximum magnification | x1000 - x1500 | x 5,000,000 |
| **7** | Radiation source | Tungsten or quartz halogen lamp | High voltage (50kv) tungsten filament. |
| **8** | Focus  | Lens is movable | Rigidly fixed, adjust lens currents. |
| **9** | Stains | Water soluble dyes | Heavy metals |
| **10** | Electromagnetic spectrum used | Visible light 390nm(red) – 760nm | Electrons app. 4nm |

1. **Differentiate between Scanning electron microscope (SEM) and Transmission electron microscope (TEM).**
2. SEM is based on scattered electrons while TEM is based on transmitted electrons.
3. SEM takes less time to create images than TEM .
4. The sample in TEM has to be cut thinner where as there is no such need with SEM sample.
5. TEM requires extensive sample preparation. The thickness of the specimens to be examined under TEM should be less than 100nm, while the preparation technique of SEM is quite easy.
6. In TEM, pictures are shown on fluorescent screens whereas in SEM, picture is shown on monitor.
7. 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.
8. 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
9. SEM also provides a 3-dimensional image while TEM provides a 2-dimensional picture.
10. TEM has much higher resolution than SEM.
11. SEM is used for surfaces, powders, polished & etched microstructures, chips, chemical segregation whereas TEM is used for imaging of dislocations, tiny precipitates, grain boundaries and other defect structures in solids