**ANA 402: Electron Microscopic Technique & Ultrastructure**

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**1.1: History of Microscopy**

In view of the immense interest in microscopic investigation evinced during the last fifty years, it is remarkable that no exhaustive history of the instrument has yet appeared. Microscopes let us view an invisible world – the objects around us that are too small to be seen with the naked eye. This timeline provides a look at some of the key advances in microscopy. The origin of the concept of microscopy can be traced as far back as 700 BC with several inventions and discovery leading up to the development of the concept of microscopy.

**1.1.1: Nimrud lens-710 BC**

The Nimrud lens, also called Layard lens, is a 3000-year-old piece of rock crystal, which was unearthed in 1850 by Austen Henry Layard at the Assyrian palace of Nimrud, in modern-day Iraq. It may have been used as a magnifying glass, or as a burning-glass to start fires by concentrating sunlight, or it may have been a piece of decorative inlay. The lens is slightly oval and was roughly ground, perhaps on a lapidary wheel.  It has a focal point about 11 centimeters (4.5 in) from the flat side and a focal length of about 12 cm. This would make it equivalent to a 3× magnifying glass. The surface of the lens has twelve cavities that were opened during grinding, which would have contained naphtha or some other fluid trapped in the raw crystal. The lens is said to be able to focus sunlight although the focus is far from perfect. Because the lens is made from natural rock crystal the material of the lens has not deteriorated significantly over time. The Nimrud lens is on display in the British Museum.

**1.1.2: Reading stone-1000 AD**

A reading stone is an approximately hemispherical lens that can be placed on top of text to magnify the letters so that people with presbyopia can read it more easily. Reading stones were among the earliest common uses of lenses. The regular use of reading stones began around 1000 AD. Early reading stones were manufactured from rock crystal (quartz) or beryl as well as glass, which could be shaped and polished into stones used for viewing. The Swedish Visby lenses, dating from the 11th or 12th century, may have been reading stones. The function of reading stones was replaced by the use of spectacles from the late 13th century onwards, but modern implementations are still used. In their modern form, they can be found as rod-shaped magnifiers, flat on one side, that magnify a line of text at a time, or as large dome magnifiers which magnify a circular area of a page. Larger Fresnel lenses can be placed over an entire page. The modern forms are usually made of plastic.

**1.1.3: Book of Optics-1021 AD**

The Book of Optics is a seven-volume treatise on optics and other fields of study composed by the medieval Arab scholar Ibn al-Haytham, known in the West as Alhazen or Alhacen (965–c. 1040 AD). The Book of Optics presented experimentally founded arguments against the widely held extramission theory of vision (as held by Euclid in his Optica), and proposed the modern intromission theory, the now accepted model that vision takes place by light entering the eye. The book is also noted for its early use of the scientific method, its description of the camera obscura, and its formulation of Alhazen's problem. The book extensively affected the development of optics, physics and mathematics in Europe between the 13th and 17th centuries.

* + 1. **First pair of Eye Glasses-1284**

Salvino D'Armato degli Armati of Florence (1258-1312), which means ''son of Armato'' is credited with inventing the first wearable eyeglasses around 1284 in Italy although there is some doubt to the authenticity of this claim. Leopoldo Del Migliore, in his 1684 history of Florence, wrote that the church of Santa Maria Maggiore contained a memorial honoring D'Armati featured the following inscription: Here lies Salvino degl' Armati, son of Armato of Florence, inventor of eyeglasses. May God forgive his sins? A.D. 1317. Since the church has been rebuilt several times since the 13th century, this tomb no longer exists, and some historians are suspicious of his claims. Armati had injured his own eyes while examining light refraction. Through these experiments he discovered how to increase the appearance of subjects by viewing them through two convex glass pieces.

**1.1.5: Early Microscope-1590**

Sometime about the year 1590, two Dutch spectacle makers, Zaccharias Janssen and his father Hans started experimenting with these lenses. They put several lenses in a tube and made a very important discovery. The object near the end of the tube appeared to be greatly enlarged, much larger than any simple magnifying glass could achieve by itself! They had just invented the compound microscope (which is a microscope that uses two or more lenses). Galileo heard of their experiments and started experimenting on his own. He described the principles of lenses and light rays and improved both the microscope and telescope. He added a focusing device to his microscope and of course went on to explore the heavens with his telescopes. Anthony Leeuwenhoek of Holland became very interested in lenses while working with magnifying glasses in a dry goods store. He used the magnifying glass to count threads in woven cloth. He became so interested that he learned how to make lenses. By grinding and polishing, he was able to make small lenses with great curvatures. These rounder lenses produced greater magnification, and his microscopes were able to magnify up to 270-X.

**1.1.6: Compound Microscope-1609**

 In 1609, Galileo Galilei made a microscope by converting one of his telescopes. It had a diverging lens as an eyepiece and a converging lens as an objective. An early microscope made of two converging lenses was presented around 1620 by the astronomer Cornelius Drebbel. However, it was apparently not Drebbel’s own idea but that of Johannes Kepler. Later on in 1625, Giovanni Faber coined the name microscope for Galileo’s invention.

**1.1.7: First use of the Term “Cells”-1665**

The cell was first discovered and named by Robert Hooke in 1665. He remarked that it looked strangely similar to cellula or small rooms which monks inhabited, thus deriving the name. However what Hooke actually saw was the dead cell walls of plant cells (cork) as it appeared under the microscope. Hooke’s description of these cells was published in *Micrographia*. The cell walls observed by Hooke gave no indication of the nucleus and other organelles found in most living cells. The first man to witness a live cell under a microscope was Anton van Leeuwenhoek, who in 1674 described the algae Spirogyra. Van Leeuwenhoek probably also saw bacteria.

**1.1.8: Spherical Aberration Solved-1830**

Spherical aberration is a type of aberration found in optical systems that use elements with spherical surfaces. Lenses and curved mirrors are most often made with surfaces that are spherical, because this shape is easier to form than non-spherical curved surfaces. Light rays that strike a spherical surface off-center are refracted or reflected more or less than those that strike close to the center. This deviation reduces the quality of images produced by optical systems. 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.

**1.1.9: Abbe’s Equation-1874**

In Abbe's 1874 paper, titled "A Contribution to the Theory of the Microscope and the nature of Microscopic Vision. Abbe states that the resolution of a microscope is inversely dependent on its aperture, but without proposing a formula for the resolution limit of a microscope. Ernst Abbe writes a mathematical formula that correlates resolving power to the wavelength. The distance between two successive points of a wave (from one peak or crest of a wave and the next peak or crest). Usually refers to an electromagnetic wave, measured in nanometers (nm) of light. Abbe’s formula makes it possible to calculate the theoretical maximum resolution of a microscope.

**1.1.10: TEM (Transmission Electron Microscopes)-1931**

The first electron microscope was developed by Max Knoll and Ersnt Ruska in Germany, 1931. At the time, regular microscopes (also called "light microscopes") were placing limitations on scientists and their research. Scientists were beginning to want to view objects even closer up than the magnification (approximately 1000x) and resolution of the light microscope allowed for. Thus, the electron microscope was developed in order to see fine details of objects and the interior structures of the cell (e.g. nucleus, mitochondria, etc.).

**1.1.11: Phase contrast microscope**

Frits Zernike, a Dutch physicist and mathematician, built the first phase contrast microscope in 1938. It took some time before the scientific community recognized the potential of Zernike’s discovery; he won the Nobel Prize in 1953 and the German-based company Zeiss began manufacturing his phase contrast microscope during World War II. Zernike experimented with the speed of the light path directed onto a specimen; he discovered interference patterns resulting in the image appearing darker or lighter. He developed a system using annuli or rings, placed in the lens and beneath the lower condenser of a compound microscope, to cause interference in light patterns. Zernike manipulated the rings and light source, ultimately reducing the light wavelength by a ½ phase. Each magnification setting, whether 10x or 100x, needs an analogous annulus ring in the light condenser.

**1.1.12: Scanning electron Microscope-1942**

The invention of the SEM principle cannot be pinpointed to only one contributor in history. However, it was the German scientist Max Knoll who built the first “scanning microscope” in 1935. Manfred von Ardenne laid a further foundation for the SEM as well as for the transmission electron microscope (TEM). He described the theoretical principles in a 1938 published paper (von Ardenne 1938). The first true SEM with a resolution of 50 nm and magnification of 8,000 was a contribution from the USAmerican scientists Zworykin, Hillier, and Sniyder in 1942 who still could not convince their fellow scientists of its usefulness (Zworykin et al. 1942). Charles Oatley, in his two-decade long process of researching the SEM, finally achieved the acceptance of the SEM as “one of the most powerful and productive methods of microscopy yet invented” (Oatley 1982).

**2.1: Differences between Light and Electron Microscope**

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| **S.N.** | **Character** | **Light Microscope** | **Electron Microscope** |
| 1. | Alternatively known as | Optical microscope | Beam microscope |
| 2. | Invented by | It is believed that Dutch spectacles makers Zacharius Jansen and his father Hans were the first to invent the compound microscope in the 16th century. | In 1931 physicist Ernst Ruska and German engineer Max Knoll. |
| 3. | Illuminating source | Uses light (approx wavelength 400-700 nm) to illuminate the objects under view. | Uses a beam of electrons (approx equivalent wavelength 1 nm) to make objects larger for a detailed view. |
| 4. | Principle | The image formed by absorption of light waves. | The image formed by scattering or transmission of electrons. |
| 5. | Structure | Light microscopes are smaller and lighter. | Heavier and larger in size. |
| 6. | Lenses used | Lenses are made of glass. | Lenses are made of electromagnets. |
| 7. | Vacuum | Not used under a vacuum | Operates under a high vacuum |
| 8. | Specimen type | Fixed or unfixed, stained or unstained, living or non-living. | Fixed, stained and non-living. |
| 9. | Specimen observed | Both live and dead specimens can be observed. | Only dead specimens are possible to be observed. |
| 10. | Specimen preparation | Less tedious and simple. | It generally involves harsher processes, e.g. using corrosive chemicals. More skill required – both to prepare specimens and to interpret EM images (due to artifacts). |
| 11. | Preparation time | Specimen preparation takes usually a few minutes to hours. | Specimen preparation takes usually takes a few days. |
| 12. | Thickness of specimen | 5 micrometer or thicker | Ultra-thin, 0.1 micrometers or below |
| 13. | Dehydration of Specimen | Specimens need not be dehydrated before viewing. | Only dehydrated specimens are used. |
| 14. | Coating of specimen | Stained by colored dyes for proper visualization. | Coated with heavy metals to reflect electrons. |
| 15. | Mounting of specimen | Mounted on the glass slide. | Mounted on the metallic grid (mostly copper). |
| 16. | Focusing | Done by adjusting the lens position mechanically. | Done by adjusting the power of the electric current to the electromagnetic lenses. |
| 17. | Magnification power | Low magnification of up to 1,500x. | High magnification of up to 1,000,000x. |
| 18. | Resolving power | Low resolving power, usually below 0.30µm. | The high resolving power of up to 0.001µm, about 250 times higher than the light microscope. |
| 19. | Viewing of the image formed | Light microscope images can be viewed directly. Images are viewed by the eyes through the eyepiece. | Images are viewed on a photographic plate or zinc sulfate fluorescent screen. |
| 20. | Nature of Image formed | Poor surface view | Good surface view and internal details |
| 21. | Image Color | Colored images. | Electron microscopes produce grayscale (sometimes called “black and white”) images (except “false-color” electron micrographs). |
| 22. | Image dimension | Image plane “flat” (2D). | 2D only in a Transmission electron microscope (TEM);  Scanning electron microscope (SEM) images give depth information which seems like 3D. |
| 23. | Living processes | Visualization of living processes such as microscopic pond life in action and even cell division is possible. | Living processes cannot be viewed. |
| 24. | Room settings | No special settings required. | It must be used in a room where humidity, pressure, and temperature are controlled. |
| 25. | Simplicity in use | Simple to use | Users require technical skills |
| 26. | Electric Current | No need for high voltage electricity. | High voltage electric current is required (50,000 V or above). |
| 27. | Filaments | No filaments used. | Tungsten filaments used to generate electrons. |
| 28. | Cooling System | Absent | Cooling system present to pacify the heat generated due to high voltage electric current. |
| 29. | Radiation leakage | No radiation risk. | Risk of radiation leakage. |
| 30. | Complexity | Less complex | Complex |
| 31. | Expense | Cheap to buy and has low maintenance costs. | Very expensive to buy as well as to maintain. |
| 32. | Suitability /   Practicality | Suitable for most basic functions, and is very common in schools and other learning institutions. | Limited to specialized use such as research. |
| 33. | Advantages | * Easy to use * Cheap * True color but sometimes require staining * Live specimens | * High resolution * Provide detailed images of surface structures and interior structures * High magnification * 3D images |
| 34. | Disadvantages | * Low resolution due to shorter wavelength of light (0.2nm) * Low magnification * The specimen used is thin. | * Expensive * Requires extensive training * Sample must be dead * Black and white/false-color image |
| 35. | Types/ Variants | * Dark-field microscope * Phase-contrast microscope * Fluorescent microscope Confocal microscope Polarized microscope * Differential interference contrast microscope | * Transmission electron microscope (TEM) * Scanning electron microscope (SEM) |
| 36. | Application | It is used for the study of detailed gross internal structure. | It is used in the study of the external surface, the ultrastructure of cell and very small organisms. |

**3.1: Differences between the SEM and TEM**

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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). |

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