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**16/MHS03/031**

**HUMAN ANATOMY 400L**

**ELECTRON MICROSCOPY (ANA402)**

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The history of the microscope spans centuries. Roman philosophers mentioned “burning glasses" in their writings but the first primitive microscope was not made until the late 1300’s. Two lenses were placed at opposite ends of a tube. This simple magnifying tube gave birth to the modern microscope.

First Microscope

vintage microscope

Grinding glass to use for spectacles and magnifying glasses was commonplace during the 13th century. In the late 16th century several Dutch lens makers designed devices that magnified objects, but in 1609 Galileo Galilei perfected the first device known as a microscope. Dutch spectacle makers Zaccharias Janssen and Hans Lipperhey are noted as the first men to develop the concept of the compound microscope. By placing different types and sizes of lenses in opposite ends of tubes, they discovered that small objects were enlarged.

Lens Improvement

Later in the 16th century, Anton van Leeuwenhoek began polishing and grinding lenses when he discovered that certain shaped lenses increased an image’s size. The glass lenses that he created could enlarge an object many times. The quality of his lenses allowed him, for the first in history, to see the many microscopic animals, bacteria and intricate detail of common objects. Leeuwenhoek is considered the founder of the study of microscopy and an played a vital role in the development of cell theory.

Achromatic Lens

The microscope was in use for over 100 years before the next major improvement was developed. Using early microscopes was difficult. Light refracted when passing through the lenses and altered what the image looked like. When the achromatic lens was developed for use in eyeglasses by Chester Moore Hall in 1729, the quality of microscopes improved. Using these special lenses, many people would continue to improve the visual acuity of the microscope.

Mechanical Improvements

During the 18th and 19th centuries, many changes occurred in both the housing design and the quality of microscopes. Microscopes became more stable and smaller. Lens improvements solved many of the optical problems that were common in earlier versions. The history of the microscope widens and expands from this point with people from around the world working on similar upgrades and lens technology at the same time.

August Kohler is credited with inventing a way to provide uniform microscope illumination that allowed specimens to be photographed.

Ernst Leitz devised a way to allow for different magnifications using one microscope by putting multiple lenses on a movable turret at the end of the lens tube. Looking for a way to allow more light-spectrum colors to be visible, Ernst Abbe designed a microscope that in a few years would provide Zeiss with the tools to develop the ultraviolet microscope.

Modern Technology Improving Microscopy

The invention of the microscope allowed scientists and scholars to study the microscopic creatures in the world around them. When learning about the history of the microscope it is important to understand that until these microscopic creatures were discovered, the causes of illness and disease were theorized but still a mystery. The microscope allowed human beings to step out of the world controlled by things unseen and into a world where the agents that caused disease were visible, named and, over time, prevented. Charles Spencer demonstrated that light affected how images were seen. It took over one hundred years to develop a microscope that worked without light. The first electron microscope was developed in the 1930’s by Max Knoll and Ernst Ruska.

Electron microscopes can provide pictures of the smallest particles but they cannot be used to study living things. Its magnification and resolution is unmatched by a light microscope. However, to study live specimens you need a standard microscope. Scanning probe microscopy allows specimens to be viewed at the atomic level which began first with the scanning tunneling microscope invented in 1981 by Gerd Bennig and Heinrich Rohrer. Later Bennig and his colleagues, in 1986, went on to invent the atomic force microscope bringing about a true era of nanoresearch.

The history of the microscope spans centuries, however Leeuwenhoek’s first design has remained unchanged since the 1600’s.

Timeline of the Microscope

14th century: spectacles first made in Italy

1590: Two Dutch spectacle-makers and father-and-son team, Hans and Zacharias Janssen, create the first microscope.

1667: Robert Hooke's famous "Micrographia" is published, which outlines Hooke's various studies using the microscope.

1675: Enter Anton van Leeuwenhoek, who used a microscope with one lens to observe insects and other specimen. Leeuwenhoek was the first to observe bacteria. 18th century: As technology improved, microscopy became more popular among scientists. Part of this was due to the discovery that combining two types of glass reduced the chromatic effect.

1830: Joseph Jackson Lister discovers that using weak lenses together at various distances provided clear magnification.

1878: A mathematical theory linking resolution to light wavelength is invented by Ernst Abbe.

1903: Richard Zsigmondy invents the ultramicroscope, which allows for observation of specimens below the wavelength of light.

1932: Transparent biological materials are studied for the first time using Frits Xernike's invention of the phase-contrast microscope.

1938: Just six years after the invention of the phase contrast microscope comes the electron microscope, developed by Ernst Ruska, who realized that using electrons in microscopy enhanced resolution.

1981: 3-D specimen images possible with the invention of the scanning tunneling microscope by Gerd Binnig and Heinrich Rohrer.

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| Differences between Light Microscope and Electron Microscope |
| Light Microscope | Electron Microscope |
| Illuminating source is the Light. | Illuminating source is the beam of electrons. |
| Specimen preparation takes usually few minutes to hours. | Specimen preparation takes usually takes few days. |
| Live or Dead specimen may be seen. | Only Dead or Dried specimens are seen. |
| Condenser, Objective and eye piece lenses are made up of glasses. | All lenses are electromagnetic. |
| It has low resolving power (0.25µm to 0.3µm). | It has high resolving power (0.001µm), about 250 times higher  than light microscope. |
| It has a magnification of of 500X to 1500X. | It has a magnification of 100,000X to 300,000X. |
| The object is 5µm or thicker. | The object is 0.1µm or thinner. |
| Image is Colored. | Image is Black and White. |
| Vacuum is not required. | Vacuum is essential for its operation. |
| There is no need of high voltage electricity. | High voltage electric current is required (50,000 Volts and above). |
| There is no cooling system. | It has a cooling system to take out heat generated by high electric current. |
| Filament is not used. | Tungsten filament is used to produce electrons. |
| Radiation risk is absent. | There is risk of radiation leakage. |
| Specimen is stained by colored dyes. | Specimen is coated with heavy metals in order to reflect electrons. |
| Image is seen by eyes through ocular lens. | Image is received in Zinc Sulphate Fluorescent Screen or Photographic Plate. |
| It is used for the study of detailed gross internal structure. | It is used in the study of external surface, ultra structure of cell and very small organisms. |

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The major 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). |