**AN ASSIGNMENT**

**ON**

**AN ESSAY ON THE HISTORY OF MICROSCOPY, THE DIFFERENCES BETWEEN LIGHT AND ELECTRON MICROSCOPES AND THE DIFFERENCES BETWEEN SEM AND TEM**

**BY**

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**HISTORY OF MICROSCOPY**

Microscopy is the science of investigating small objects and structures using a microscope.

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.

Although objects resembling lenses date back 4000 years and there are Greek accounts of the optical properties of water-filled spheres (5th century BC) followed by many centuries of writings on optics, the earliest known use of simple microscopes (magnifying glasses) dates back to the widespread use of lenses in eyeglasses in the 13th century.

The earliest known examples of compound microscopes, which combine an objective lens near the specimen with an eyepiece to view a real image, appeared in Europe around 1620.

The inventor is unknown although many claims have been made over the years. Several revolve around the spectacle-making centres in the Netherlands including claims it was invented in 1590 by Zacharias Janssen (claim made by his son) and/or Zacharias' father, Hans Martens, claims it was invented by their neighbour and rival spectacle maker, Hans Lippershey (who applied for the first telescope patent in 1608), and claims it was invented by expatriate Cornelis Drebbel who was noted to have a version in London in 1619.

During the 1st century AD (year 100), glass had been invented and the Romans were looking through the glass and testing it. They experimented with different shapes of clear glass and one of their samples was thick in the middle and thin on the edges. They discovered that if you held one of these “lenses” over an object, the object would look larger.

Someone also discovered that you can focus the rays of the sun with one of these special “glasses” and start a fire. These early lenses were called magnifiers or burning glasses. The word lens by the way, is derived from the Latin word lentil, as they were named because they resembled the shape of a lentil bean (look up lens in a dictionary).

These lenses were not used much until the end of the 13th century when spectacle makers were producing lenses to be worn as glasses.

The early simple “microscopes” which were really only magnifying glasses had one power, usually about 6X - 10X. One thing that was very common and interesting to look at was fleas and other tiny insects. These early magnifiers were hence called “flea glasses”.

Galileo Galilei (also sometimes cited as compound microscope inventor) seems to have found after 1610 that he could close focus his telescope to view small objects and, after seeing a compound microscope built by Drebbel exhibited in Rome in 1624, built his own improved version. Giovanni Faber coined the name microscope for the compound microscope Galileo submitted to the Accademia dei Lincei in 1625 (Galileo had called it the "occhiolino" or "little eye").

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 270X!

Anthony Leeuwenhoek became more involved in science and with his new improved microscope was able to see things that no man had ever seen before. He saw bacteria, yeast, blood cells and many tiny animals swimming about in a drop of water. From his great contributions, many discoveries and research papers, Anthony Leeuwenhoek (1632-1723) has since been called the "Father of Microscopy".

Robert Hooke, an Englishman (who is sometimes called the “English Father of Microscopy”), also spent much of his life working with microscopes and improved their design and capabilities.

Little was done to improve the microscope until the middle of the 19th century when great strides were made and quality instruments like today’s microscope emerged. Companies in Germany like Zeiss and an American company founded by Charles Spencer began producing fine optical instruments.

Because of foreign production, quality microscopes have become affordable for all. Zaccharias Janssen, the inventor of the microscope would marvel at the quality of even the most basic microscopes found in schools today.

The first detailed account of the microscopic anatomy of organic tissue based on the use of a microscope did not appear until 1644, in Giambattista Odierna's L'occhio della mosca, or The Fly's Eye. The microscope was still largely a novelty until the 1660s and 1670s when naturalists in Italy, the Netherlands and England began using them to study biology. Italian scientist Marcello Malpighi, called the father of histology by some historians of biology, began his analysis of biological structures with the lungs. Robert Hooke's Micrographia had a huge impact, largely because of its impressive illustrations.

A significant contribution came from Antonie van Leeuwenhoek who achieved up to 300 times magnification using a simple single lens microscope. He sandwiched a very small glass ball lens between the holes in two metal plates riveted together, and with an adjustable-by-screws needle attached to mount the specimen. Then, Van Leeuwenhoek re-discovered red blood cells (after Jan Swammerdam) and spermatozoa and helped popularise the use of microscopes to view biological ultrastructure.

On 9 October 1676, van Leeuwenhoek reported the discovery of micro-organisms. The performance of a light microscope depends on the quality and correct use of the condensor lens system to focus light on the specimen and the objective lens to capture the light from the specimen and form an image. Early instruments were limited until this principle was fully appreciated and developed from the late 19th to very early 20th century, and until electric lamps were available as light sources. In 1893 August Köhler developed a key principle of sample illumination, Köhler illumination, which is central to achieving the theoretical limits of resolution for the light microscope.

This method of sample illumination produces even lighting and overcomes the limited contrast and resolution imposed by early techniques of sample illumination. Further developments in sample illumination came from the discovery of phase contrast by Frits Zernike in 1953, and differential interference contrast illumination by Georges Nomarski in 1955; both of which allow imaging of unstained, transparent samples.

Electron microscopes

In the early 20th century a significant alternative to the light microscope was developed, an instrument that uses a beam of electrons rather than light to generate an image. The German physicist, Ernst Ruska, working with electrical engineer Max Knoll, developed the first prototype electron microscope in 1931, a transmission electron microscope (TEM). The transmission electron microscope works on similar principles to an optical microscope but uses electrons in the place of light and electromagnets in the place of glass lenses. Use of electrons, instead of light, allows for much higher resolution.

Development of the transmission electron microscope was quickly followed in 1935 by the development of the scanning electron microscope by Max Knoll. Although TEMs were being used for research before WWII, and became popular afterwards, the SEM was not commercially available until 1965. Transmission electron microscopes became popular following the Second World War.

Ernst Ruska, working at Siemens, developed the first commercial transmission electron microscope and, in the 1950s, major scientific conferences on electron microscopy started being held. In 1965, the first commercial scanning electron microscope was developed by Professor Sir Charles Oatley and his postgraduate student Gary Stewart and marketed by the Cambridge Instrument Company as the "Stereoscan". One of the latest discoveries made about using an electron microscope is the ability to identify a virus. Since this microscope produces a visible, clear image of small organelles, in an electron microscope there is no need for reagents to see the virus or harmful cells, resulting in a more efficient way to detect pathogens.

 Scanning probe microscopes

From 1981 to 1983 Gerd Binnig and Heinrich Rohrer worked at IBM in Zurich, Switzerland to study the quantum tunnelling phenomenon. They created a practical instrument, a scanning probe microscope from quantum tunnelling theory, that read very small forces exchanged between a probe and the surface of a sample. The probe approaches the surface so closely that electrons can flow continuously between probe and sample, making a current from surface to probe.

The microscope was not initially well received due to the complex nature of the underlying theoretical explanations. In 1984, Jerry Tersoff and D.R. Hamann, while at AT&T's Bell Laboratories in Murray Hill, New Jersey began publishing articles that tied theory to the experimental results obtained by the instrument. This was closely followed in 1985 with functioning commercial instruments, and in 1986 with Gerd Binnig, Quate, and Gerber's invention of the atomic force microscope, then Binnig's and Rohrer's Nobel Prize in Physics for the SPM. New types of scanning probe microscope have continued to be developed as the ability to machine ultra-fine probes and tips has advanced.

Fluorescence microscopes

The most recent developments in light microscope largely centre on the rise of fluorescence microscopy in biology. During the last decades of the 20th century, particularly in the post-genomic era, many techniques for fluorescent staining of cellular structures were developed. The main groups of techniques involve targeted chemical staining of particular cell structures, for example, the chemical compound DAPI to label DNA, use of antibodies conjugated to fluorescent reporters, see immunofluorescence, and fluorescent proteins, such as green fluorescent protein.

These techniques use these different fluorophores for analysis of cell structure at a molecular level in both live and fixed samples. The rise of fluorescence microscopy drove the development of a major modern microscope design, the confocal microscope. The principle was patented in 1957 by Marvin Minsky, although laser technology limited practical application of the technique. It was not until 1978 when Thomas and Christoph Cremer developed the first practical confocal laser scanning microscope and the technique rapidly gained popularity through the 1980s.

**DIFFERENCES BETWEEN LIGHT AND ELECTRON MICROSCOPES**

Magnification and resolving power are the key differences between Light Microscope and Electron Microscope which is about 1000X of the magnification with resolving power of 0.2um in Light Microscope and that of Electron Microscope is 10,00,000X magnification with resolving power of 0.5nm or even less.

Electron Microscope was developed by Ernst Ruska and Max Knoll, with the use of ‘electrons’ in the microscope instead of visible light which helped in increasing the resolution of the lens along with more magnified and cleared image of an organism.

Light microscope contains an Eyepiece (Ocular lens), tube, coarse focus, fine focus, resolving nose piece, objective, stage clips, diaphragm, mirror, light source, condenser, three or four objective lenses.

The light microscope uses the visible light as the source to view the object, along with glass lenses/transparent lenses and projection screen. As these microscopes are easy to handle and simple & easy in working. They can be commonly seen in schools, colleges labs, doctors, clinic.

The microscope is based on its resolving power, magnification, lenses used, source to view the object. “Resolving power” is the most important, which is the ability to differentiate two very small and closely attached objects clearly. Lesser the distance between the objects, finer will be the result.

Light Microscope also referred as Optical Microscope can be classified as Simple and Compound Microscope. In the simple type single lens like magnifying glass are only used, whereas in compound type several lenses are used to magnify the objects clearly.

An electron microscope is much widely used by scientists and in research labs to get the keen knowledge of even the smallest microorganisms as well as to study all their characteristics in detail. As the name suggests, Electron Microscope uses electrons instead of visible light source to view the objects.

Electrons microscopes are the most advanced type of microscopes. In the year 1920, it was recognized that electrons when moved in a vacuum, they behave like “light”. They travel in straight lines and have wavelike properties, with a wavelength much shorter than that of visible light.

The main difference between light microscope and electron microscope is that an electron microscope uses beams of electrons to magnify the image of an object while light microscope uses rays of visible light to form highly magnified images of tiny areas of materials or biological specimens. More differences between the two are listed below;

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### Comparison Chart

| **Basis for Comparision** | **Light Microscope** | **Electron Microscope** |
| --- | --- | --- |
| 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. |
| Source to view the object | Visible light source. | Beam of charged particles i.e. electrons. |
| Lens used | Glass lenses. | Electromagnetic lenses. |
| Magnification | 1000X. | 10,00,000X. |
| Resolving power | 0.2um. | 0.5nm. |
| Screen | Projection screen. | Fluorescent screen. |
| Voltage | No need of high voltage electricity. | High voltage electric current is required (around 50,000 volts and above). |
| Cooling system | There is no requirement of cooling system.  | It has high cooling system in order to move out the heat generated by high voltage electric current.  |
| Preparation | Preparation of sample is quick and simple. | Complex preparation. |
| Filament  | No filament used. | Tungsten filament is used. |
| Radiation leakage | No radiation risks. | There is the risk of radiation leakage.  |
| Availability | Easily available and cheaper in rate. | Not easily available and expensive. |
| Visibility | Living, as well as the dead sample, can be viewed. | Only dead (fixed) organisms can be viewed. |
|  | Studying the detailed structure of an organism is difficult. | 3D structure is obtained due to which it is easy to study the structural and other details of organisms. |
|  | The natural colour of specimen is obtained. | Only black and white image is obtained. |
|  | The image can be seen directly. | Image is seen only on fluorescent screen. |
|  | It requires low maintenance cost. | It requires high maintenance cost. |

**DIFFERENCES BETWEEN SEM AND TEM**

|  |  |  |
| --- | --- | --- |
| **PROPERTIES** | **SEM** | **TEM** |
| Type of electrons | Scattered, scanning electrons | Transmitted electrons |
| High tension | ~1 – 30 kV | ~60 – 300 kV |
| Specimen thickness | Any | Typically, <150 nm |
| Type of info | 3D image of surface | 2D projection image of inner structure |
| Max. magnification | Up to ~1 – 2 million times | More than 50 million times |
| Max. FOV | Large | Limited |
| Optimal spatial resolution | ~0.5 nm | < 50 pm |
| Image formation | Electrons are captured and counted by detectors, image on PC screen | Direct imaging on fluorescent screen or PC screen with CCD |
| Operation | Little or no sample preparation, easy to use | Laborious sample preparation, trained users required. |
| Purpose  | It provides detailed images of the surfaces of cells. SEM focuses on the sample’s surface and its composition, so it shows only the morphology of samples. | TEM can show many characteristics of the sample, such as internal composition, morphology, crystallization, etc. It is used to view thin specimen. |
| 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. |
| Processing of sample (s) | SEM allows for a large amount of sample to be analysed at a time | With TEM only a small number of samples can be analysed at a time |
| Current Applications | To study topography and atomic composition of specimens, process control and, 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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