**TOSIN FATUNDIMU**

**17/MHS03/031**

**ANA 402**

1. **History of the Microscopy**

**During that historic period known as the Renaissance, after the "dark" Middle Ages, there occurred the inventions of printing, gunpowder and the mariner's compass, followed by the discovery of America. Equally remarkable was the invention of the light microscope: an instrument that enables the human eye, by means of a lens or combinations of lenses, to observe enlarged images of tiny objects.**

**Long before, in the hazy unrecorded past, someone picked up a piece of transparent crystal thicker in the middle than at the edges, looked through it, and discovered that it made things look larger. Someone also found that such a crystal would focus the sun's rays and set fire to a piece of parchment or cloth. Magnifiers and "burning glasses" or "magnifying glasses" are mentioned in the writings of Seneca and Pliny the Elder, Roman philosophers during the first century A. D., but apparently they were not used much until the invention of spectacles, toward the end of the 13th century. They were named lenses because they are shaped like the seeds of a lentil.**

**The earliest simple microscope was merely a tube with a plate for the object at one end and, at the other, a lens which gave a magnification less than ten diameters; ten times the actual size. These excited general wonder when used to view fleas or tiny creeping things and so were dubbed "flea glasses."**

**About 1590, two Dutch spectacle makers, Zaccharias Janssen and his son Hans, while experimenting with several lenses in a tube, discovered that nearby objects appeared greatly enlarged. That was the forerunner of the compound microscope and of the telescope. In 1609, Galileo, father of modern physics and astronomy, heard of these early experiments, worked out the principles of lenses, and made a much better instrument with a focusing device.**

**The father of microscopy, Anton van Leeuwenhoek of Holland, started as an apprentice in a dry goods store where magnifying glasses were used to count the threads in cloth. He taught himself new methods for grinding and polishing tiny lenses of great curvature which gave magnifications up to 270 diameters, the finest known at that time. These led to the building of his microscopes and the biological discoveries for which he is famous. He was the first to see and describe bacteria, yeast plants, the teeming life in a drop of water, and the circulation of blood corpuscles in capillaries. During a long life, he used his lenses to make pioneer studies on an extraordinary variety of things, both living and non-living and reported his findings in over a hundred letters to the Royal Society of England and the French Academy.**

**Robert Hooke, the English father of microscopy, re-confirmed Anton van Leeuwenhoek's discoveries of the existence of tiny living organisms in a drop of water. Hooke made a copy of Leeuwenhoek's light microscope and then improved upon his design.**

**Later, few major improvements were made until the middle of the 19th century. Then several European countries began to manufacture fine optical equipment but none finer than the marvelous instruments built by the American, Charles A. Spencer, and the industry he founded. Present day instruments, changed but little, give magnifications up to 1250 diameters with ordinary light and up to 5000 with blue light.**

**A light microscope, even one with perfect lenses and perfect illumination, simply cannot be used to distinguish objects that are smaller than half the wavelength of light. White light has an average wavelength of 0.55 micrometers, half of which is 0.275 micrometers. (One micrometer is a thousandth of a millimeter, and there are about 25,000 micrometers to an inch. Micrometers are also called microns.) Any two lines that are closer together than 0.275 micrometers will be seen as a single line, and any object with a diameter smaller than 0.275 micrometers will be invisible or, at best, show up as a blur. To see tiny particles under a microscope, scientists must bypass light altogether and use a different sort of "illumination," one with a shorter wavelength.**

**The introduction of the electron microscope in the 1930's filled the bill. Co-invented by Germans, Max Knoll, and Ernst Ruska in 1931, Ernst Ruska was awarded half of the Nobel Prize for Physics in 1986 for his invention. (The other half of the Nobel Prize was divided between Heinrich Rohrer and Gerd Binnig for the STM.)**

**In this kind of microscope, electrons are speeded up in a vacuum until their wavelength is extremely short, only one hundred-thousandth that of white light. Beams of these fast-moving electrons are focused on a cell sample and are absorbed or scattered by the cell's parts so as to form an image on an electron-sensitive photographic plate.**

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

|  |  |  |
| --- | --- | --- |
|  | **Light microscope** | **Electron Microscope** |
| **Source to view the object** | **Visible light source .** | **Beam of charged particles i.e. electrons.** |
| **Lense 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 risk.** | **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.** |

1. **Difference between the SEM and TEM**

|  |  |  |
| --- | --- | --- |
| **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).** |