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**ASSIGNMENT TITLE: ELECTRON MICROSCOPY**

**COURSE LECTURER: DR. OGEDENGBE**

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**Question 1: Write an essay on the history of microscopy**

**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.

**Ancient History:**

From ancient times, man has wanted to see things far smaller than could be perceived with the naked eye. Although the first use of a lens is a bit of a mystery, it’s now believed that use of lenses is more modern than previously thought. 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. These early lenses were called magnifiers or burning glasses. The word lens is actually derived from the Latin word lentil, as they were named because they resembled the shape of a lentil bean.

At the same time, Seneca described actual magnification by a globe of water. “Letters, however small and indistinct, are seen enlarged and more clearly through a globe of glass filled with water.” The lenses were not used much until the end of the 13th century when spectacle makers were producing lenses to be worn as glasses. Then, around 1600, it was discovered that optical instruments could be made by combining lenses.

**The First Microscopes:**

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

Sometime, during the 1590’s, 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.

Their first microscopes were more of a novelty than a scientific tool since maximum magnification was only around 9x and the images were somewhat blurry. Although no Jansen microscopes survived, an instrument made for Dutch royalty was described as being composed of “3 sliding tubes, measuring 18 inches long when fully extended, and two inches in diameter”. The microscope was said to have a magnification of 3x when fully closed, and 9x when fully extended.

It was Antonie Van Leeuwenhoek (1632-1723), a Dutch draper and scientist, and one of the pioneers of microscopy who in the late 17th century became the first man to make and use a real microscope, and is often referred to as the father of microscopy. He made his own simple microscopes, which had a single lens and were hand-held. Van Leeuwenhoek achieved greater success than his contemporaries by developing ways to make superior lenses, grinding and polishing a small glass ball into a lens with a magnification of 270x, the finest known at that time (other microscopes of the time were lucky to achieve 50x magnification). He used this lens to make the world’s first practical microscope.

Leeuwenhoek’s microscope used a single convex glass lens attached to a metal holder and was focused using screws. 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. People did not realize that magnification might reveal structures that had never been seen before.

**Fig 1: Image of Leeuwenhoek’s microscope.**

In England, Robert Hooke re-confirmed Leeuwenhoek's discoveries of tiny living organisms in a drop of water. He replicated Leeuwenhoek's light microscope and proceeded to improve upon its design. Hooke was also an avid student of fossils and geology. As the first person to examine fossils with a microscope, he observed close similarities between the structures of fossil shells and petrified wood, as well as living wood and living mollusk shells. His examinations proved that dead wood could be turned to stone by the action of water that is rich in dissolved minerals. As the water seeped into the wood, the saturated solution would slowly deposit minerals throughout the wood. In his book Micrographia, published in 1665, Hooke concluded that the shell-like fossils he studied actually represented organisms that no longer existed on Earth. Two and a half centuries before Darwin, Hooke realized that the fossil record proves there is transformation among the life forms on the planet, and that species have both materialized and disappeared throughout the history of life on earth.

Another breakthrough, in the 18th Century, was the invention of the Achromat lens system by Chester Moor Hall. Around the year 1733, he managed to build the first achromatic objective, consisting of a combination of a convex crown glass and a concave flint glass. Hall tried to keep this a secret by having one type of glass manufactured by one company and the other by another company. Unknowing to him, both companies used the same lens shop which realized, of course, that the final customer was one and the same person. It took another 25 years until John Dollond picked up on the idea and got a patent for "a new method of making the object glasses of refracting telescopes by compounding mediums of different refractive qualities".

During the 19th Century, the exacting natural sciences experienced an enormous upswing in activity. In the 1820s and 1830s, the science of light and the theory of optical imaging were placed on a sound foundation. One of the most successful researchers in this field was Joseph von Fraunhofer (1787-1826). Astronomy was the main field of Fraunhofer's activities, and his most noted achievement is the first description of the dark lines of the solar spectrum as reference points for the measurement of refraction indexes. But most important to the history of microscopy, Fraunhofer also refined the achromat by using scientific and precise manufacturing methods, creating what is now known as the achromat system, the most commonly deployed optical lens system with chromatic aberration correction, and for basic advancement of knowledge on the diffraction of light. Early microscopists were hampered by optical aberration, blurred images, and poor lens design, which hampered high-resolution observations until late in the 1800s. Aberrations were partially corrected by the mid-19th century with the introduction of Lister and Amici achromatic objectives that reduced chromatic aberration and raised numerical apertures to around 0.65 for dry objectives and up to 1.25 for homogeneous immersion objectives.

**Microscopes go into Large Scale Production:**

Within this period (the 1800s) of innumerable technical advances, a mechanic named Carl Zeiss began his own business in the German university town of Jena, Thuringia, with the goal of providing researchers with high-quality instruments. Between 1846 and 1866, microscopes of uniformly high quality were built in Zeiss' workshop in accordance with very strict rules of craftsmanship. In the beginning, these were very simple instruments that were used as dissection microscopes, but in 1857 the Zeiss workshop produced the first genuine compound microscope (equipped with an eyepiece and an objective). The new instrument was called the Stativ 1, which combined practical functionality with the skilled optical refinement provided by a craftsman.

After almost 20 years, Zeiss was employing about 20 qualified staff members and took great pride in what had become a prosperous business. He knew that his instruments were good, but he refused to accept the trial and error method used at the time for the production of optics. Zeiss also was aware that competition from other microscope manufacturers would eventually bypass his accomplishments if he failed to continue to produce innovations. With the ultimate goal of creating reproducible products, Zeiss acknowledged that his manufacturing procedure had to be based on precise rules and strict guidelines, or as he once said: "The working hand should have no other function than to precisely implement the shapes and dimensions of all the design components determined beforehand by computation." For assistance in this endeavor, Zeiss formed a partnership with Dr. Ernst Abbe, a brilliant physicist and mathematician. Abbe was appointed as the research director of Zeiss Optical Works in late 1866. For the next six years, the team worked intensively to lay the scientific foundations for the design and fabrication of advanced optical systems. In 1869, they introduced a new illumination apparatus that was designed to improve the performance of microscope illumination. Three years later, in 1872, Abbe formulated his wave theory of microscopic imaging and defined what would become known as the Abbe Sine Condition. Several years later, Zeiss was producing a line of 17 different objectives, including three immersion systems, all featuring a level of image quality unknown until then. The construction of microscopes on a sound theoretical basis was possible at last, and still is today.

The Zeiss enterprise continued to push onward in the late 1800s. Abbe became an equal partner, and forward-thinking intelligence became the inherent capital of the young company. In his later years, Abbe became equally famous as a social reformist. Several problems still remained for Zeiss Optical Works however, since the quality of optical glass produced during the period was not sufficient to provide the theoretical resolution that was dictated by Abbe's sine condition. The glass used in the construction of microscope lenses was not homogeneous and it tended to undergo a phase separation during cooling, which led to a varying refractive index throughout the glass, and therefore, light waves passing through these lenses were refracted unpredictably. As a matter of fact, first-rate resolution was unattainable with the poor quality glass.

Abbe first met Otto Schott, a glass chemist, in 1881. Over the next several years, Abbe and Schott developed several new glass formulas and made adjustments to the mixing and annealing process to eliminate internal defects and produce optical-grade glass with a uniform refractive index. In 1884, Schott, Abbe, and Zeiss formed a new company known as "Jenaer Glaswerk Schott und Genossen". Continued experimentation with glass recipes and preparation techniques yielded highly successful results, and in 1886, they introduced a new type of objective, the apochromat. By this time, an incredible 44 different types of optical glass were being produced. The creation of the apochromat objectives (with and without immersion media) eliminated color aberrations, which greatly assists bacteriologists in identifying infectious bacteria, and brought the resolving power of the microscope to the limit known today. The progress made in the development of objectives led to fields of view larger than anything ever achieved before. In the course of time, it also became evident that more attention would have to be paid to illumination. Professor August Köhler (1866-1948) became an early member of staff at Carl Zeiss in Jena, and in 1893 he published guidelines for an innovative scheme to illuminate microscope specimens. Köhler cleverly devised a microscope illumination system that made it possible to use the entire resolving power of Abbe's objectives. The Köhler illumination system provides homogeneously illuminated images. By incorporating a field iris diaphragm into the microscope illumination beam path, stray light was minimized and a simple procedure ensured proper positioning of the condenser for highest resolution and desired contrast (it is particularly beneficial that the aperture diaphragm in the condenser allows the image contrast and the resolving power to be balanced against each other without any loss in the consistency of the image brightness). Köhler's innovation was important in microphotography at the time of its development, and has since become a highly significant method for virtually all forms of optical microscopy. Knowledge and observance of Köhler's rules and the associated settings of the microscope (whether automatically via personal computer and motorized functions or manually) are still essential today. Early in the twentieth century, microscope manufacturers began par focalizing objectives, allowing the image to remain in focus when the microscopist exchanged objectives on the rotating nosepiece. In 1924, Zeiss introduced a LeChatelier-style metallograph with infinity-corrected optics, but this method of correction would not see widespread application for another 60 years. Shortly before World War II, Zeiss created several prototype phase contrast microscopes based on optical principles advanced by Frits Zernike. Several years later the same microscopes were modified to produce the first time-lapse cinematography of cell division photographed with phase contrast optics. This contrast-enhancing technique did not become universally recognized until the 1950s and is still one of the methods of choice for many cell biologists today. Physicist Georges Nomarski introduced improvements in Wollaston prism design for another powerful contrast generating microscopy theory in 1955. This technique is commonly referred to as Nomarski interference or differential interference contrast (DIC) microscopy and, along with phase contrast, has allowed scientists to explore many new arenas in biology using living cells or unstained tissues. Robert Hoffman introduced another method of increasing contrast in living material by taking advantage of phase gradients near cell membranes. This technique is now termed Hoffman Modulation Contrast, and is available as optional equipment on most modern microscopes.

**Electron Microscope Appears:**

The use of visible light in microscopy limits the resolution that could be achieved, but this problem was overcome in 1931 when two German scientists Max Knoll and Ernst Ruska discovered that beams of electrons could be used instead of light. The electron microscope could to be used to observe objects that were not visible using light microscopes.

Scientists working for corporations competed to develop the first commercial electron microscope and Ernst Ruska, working for Siemens, eventually achieved this in 1938. By the late 1930s, microscopes had been developed that could achieve resolutions as low as 10nm and by the mid-1940s, resolutions as low as 2nm had been achieved. The main competitors in Europe were Siemens, Philips and Carl Zeiss. In the late 1930s, the scientists in Japan formed the Japan Electron Optics Laboratory that eventually manufactured the greatest variety of electron microscopes among all of the companies.

The early versions of electron microscopes used transmission electron microscopy. The first scanning electron microscope hit the market in 1965, which revolutionized the world of material science.

**Question 2: Differentiate between the light microscope and electron microscope.**

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| **Light Microscope** | **Electron Microscope** |
| Illuminating source is the Light. | Illuminating source is the beam of electrons. |
| 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. |
| The object is 5µm or thicker. | The object is 0.1µm or thinner. |
| Natural color of sample is maintained. | Image is Black and White. |
| 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. |
| Cheap to purchase | Expensive to buy |
| 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, ultrastructure of cell and very small organisms. |
| Small and portable. | Large and requires special rooms. |
| Specimen preparation takes usually few minutes to hours. | Specimen preparation takes usually takes few days. |
| Material rarely distorted by preparation. | Preparation distorts material. |
| Vacuum is not required. | Vacuum is required. |
| Radiation risk is absent. | There is risk of radiation leakage. |
| Magnifies objects only up to 2000 times | Magnifies over 500,000 times. |
| Specimens can be living or dead | Specimens are dead, as they must be fixed in plastic and viewed in a vacuum |
| Stains are often needed to make the cells visible | The electron beam can damage specimens and they must be stained with an electron-dense chemical (usually heavy metals like osmium, lead or gold). |

**Question 3: Differentiate between the SEM and TEM.**

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| **Transmission Electron Microscope (TEM)**  | **Scanning Electron Microscope (SEM)** |
| * TEM creates an image by detecting transmitted electrons.
* TEM analyses the internal structure of a sample.
* The sample in TEM has to be cut thinner (70-90 nm) because electrons cannot penetrate very far into materials.
* With TEM only a small amount of samples can be analyzed at a time.
* The magnifying power of TEM is more than fifty million times.
* The electron beam makes use of transmitted electrons
* This is the most common form of electron microscope and gives a 2-dimensional image.
* 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.
* Laborious sample preparation, trained users required
* It is used 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).

 * TEM has the best resolution which gives about 0.5 angstroms.

Bacterium (TEM) | * SEM creates an image by detecting reflected electrons
* SEM analyzes the surface of a sample
* Sample is coated with a thin layer of heavy metal such as gold or palladium.
* SEM allows for a large amount of sample to be analyzed at a time
* The magnifying power of SEM is up to two million times.
* Makes use of scattered or reflected electrons.

 * This gives excellent 3-dimensional images of surfaces
* 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.
* Little or no sample preparation, easy to use.
* To study topography and atomic composition of specimens, process control and also, for example, the surface distribution of immuno-labels
* SEM has a poorer resolution which is about 0.4 nanometers.

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| A head and the right eye of a fly |

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| Four conodont elements stuck on a pin head (SEM) |

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