**DEPARTMENT OF HUMAN ANATOMY**

**AFE BABALOLA UNIVERSITY, ADO EKITI STATE**

**COURSE TITLE:** ELECTRON MICROSCOPIC TECHNIQUE AND ULTRASTRUCTURE.

**COURSE CODE:** ANA 402

AN ASSIGNMENT ON ELECTRON MICROSCOPY

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QUESTION 1

Write an essay on the history of microscopy

The history of microscopy begins in the Middle Ages. As far back as the 11th century, plano-convex lenses made of polished beryl were used in the Arab world as reading stones to magnify manuscripts. However, the further development of these lenses into the first microscopes cannot be attributed to any one person. It took the ideas and designs of many scientists and scholars to produce instruments capable of strong magnification.

Although the idea of magnifying objects with two glass lenses positioned one in front of the other originated as early as the beginning of the 16th century, it was some time before such an instrument was built. The Dutch spectacle maker Hans Janssen and his son Zacharias are generally credited with creating these compound microscopes. The two of them built what was probably the first compound microscope in the last decade of the 16th century. It had a magnification that could be adjusted between 3 and 9x.

But other scientists applied themselves to building these magnifying instruments, too. 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.

Hooke’s Micrographia

It was the English universal scholar Robert Hooke who really put the relatively new science of microscopy on the map. In 1667 he was the first to publish a fundamental work on the subject called "Micrographia". The drawings it contained of his observations with the microscope made the microcosm accessible to a wider public. Some of the pictures he presented are 50x magnifications. Hooke obtained his microscopes from the instrument maker Christopher Cock from London. He improved them by combining the customary oil lamp illumination with a cobbler’s ball, a glass flask filled with water that focused the light on the specimen to illuminate it more homogeneously. However, Hooke had major problems with image aberrations that became even more pronounced when two lenses were used.

Living cells observed for the first time by a draper

The highest microscope magnifications for quite a time to come were achieved by the Dutchman Antonie van Leeuwenhoek, who lived from 1632 to 1723. The cloth merchant, for whom microscopy was only a hobby at first, managed to magnify objects more than 200 times with his microscopes. Instead of working with compound microscopes like Hooke, however, he used microscopes that only had one single lens. He thus avoided the problem Hooke had had with the additive effects of aberrations of two lenses. However, one of the disadvantages of only using one lens was that the instrument had to be held up close to the eye.

Even today, no one really knows exactly how Leeuwenhoek managed to make such good lenses that were almost free of surface inclusions and defects and allowed such high magnifications. When he died at the age of 91 in 1723, his knowledge of lens making died with him. Thanks to the good magnification of his microscopes, Leeuwenhoek was the first to observe living and moving cells such as bacteria and spermatozoa.

As microscopes were primarily considered a pastime for the rich and their potential value for science was not recognized, several decades passed without any further pioneering technical improvements. Not until later in the 18th century were achromatic microscope objectives built that prevented major chromatic aberrations, thereby reducing the loss in resolving power caused by lens imperfections. One of the reasons it took so long for achromatic objectives to be invented was a statement by Isaac Newton. Newton, already a famous natural scientist, had erroneously maintained in 1666 that achromatization was not possible.

Abbe’s law of diffraction and Koehler illumination

At the beginning of the 19th century, Joseph von Fraunhofer was able to reduce image aberrations even further by making considerable improvements to the quality of glass used for microscope optics. In the mid 19th century, the Italian Giovanni Amici pressed ahead with the development of water and oil immersion objectives. Finally, in 1873, Ernst Abbe provided the scientific basis for producing powerful microscopes in series. Before that, microscope lenses had always been made by trial and error and not, as from now on, by calculated optics. At the same time, however, Abbe predicted that the maximum resolving power of light microscopes was limited to 200 nm at the most. As well as the basis for calculating microscope optics, Abbe also came up with improved oil immersion objectives and an optimized illumination device over the next few years. Only a few years later, August Koehler developed "Koehler illumination", which standardized microscope illumination and remains a generally accepted illumination procedure to this day.

Question 2

Differentiate between light microscope and electron microscope

The Difference Between Light Microscope and Electron Microscope in Tabular Form

|  |  |  |
| --- | --- | --- |
| **Basis of Comparison** | **Light Microscope** | **Electron Microscope** |
| **Illuminating Source** | Uses visible light to illuminate the specimen. | Uses a beam of electrons (radiations) to illuminate the specimen. |
| **Specimen Preparation** | Preparation of specimen to be viewed under a light microscope takes a few minutes to hours. | Preparation of specimen to be viewed under electron microscope often takes several days. |
| **Size of the Instrument** | The size of a light microscope is relatively smaller and can be operated as a desktop instrument. | The size of an electron microscope is relatively larger due to separate systems such as cooling system, image processing system, vacuum system etc. |
| **Lens** | Condenser, objective lens and eye piece lenses are made of glass. | All lenses are electromagnetic. |
| **Image Formation** | The image formation depends upon light absorption in different zones of the object. | Image formation depends upon the scattering of electron beams by different regions of the object due to heavy metal staining. |
| **Image View** | Image is seen by eyes through ocular lenses (eye piece). No screen is needed. | The image is received on zinc sulphate fluorescent screen or photographic plate. |
| **Magnification** | Has a magnification of 500X to 1500X. | Has a magnification of 100000X to 500000X. |
| **Nature of Specimen** | Fixed, unfixed, stained or unstained living or non-living specimen can be observed under a light microscope. | Only fixed and stained non-living specimen can be observed under an electron microscope. |
| **Specimen Mounting** | Specimen is usually mounted on glass slide. | Specimen is mounted on metallic grid (usually copper). |
| **How Magnification is changed** | Magnification is changed by changing the objective or eye piece lenses. | Magnification is changed by adjusting the power of electric current to the electromagnetic lenses. |
|  |  |  |
| **Stains** | Color imparting dyes are used for staining to provide contrast and differentiation. | Heavy metals are used as stains, which deflect the electron rays to produce the image. |
| **Limitation** | Live cell imaging is possible and hence the living cellular processes can be visualized | Live cell imaging is not possible and hence the living cellular processes cannot be visualized. |
| **Color Visualization** | It is possible to visualize the natural color of the specimen under a light microscope. | It is not possible to visualize the natural color of specimen under electron microscope. |
| **Vacuum Condition** | Vacuum condition is not required for the working of a light microscope. | Vacuum condition is essential for its working because the electron beams have a shorter wave length and can easily be destroyed or deflected by molecules in the air. |
| **Resolving Power** | Has a relatively low resolving power of about 200 nm. | Has a resolving power of 0.1 nm. |
| **Filament** | Does not use filaments anywhere in its operation. | Uses tungsten filament to produce electrons. |

Question 3

Differences between the SEM and TEM

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| --- | --- | --- |
|  | **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 |

Generally, TEMs are more complex to operate. TEM users require intensive training before being able to operate them. Special procedures need to be performed before every use, with several steps included that ensure that the electron beam is perfectly aligned.

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