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COURSE CODE: ANA 402 (ELECTRON MICROSCOPIC TECHNIQUES AND ULTRASTRUCTURE

1. Write an essay on the history of microscopy?

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

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.

1. Differentiate between light microscope and electron microscope

BASIS FOR COMPARISION LIGHT MICROSCOPE ELECTRON MICROSCOPE

Source to view the object Visible light source Beam of charged particles i.e. electrons.

Lenses 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. (LM) High voltage electric current is required (around 50,000 volts and above). (EM)

Cooling system There is no requirement of cooling system. (LM) It has high cooling system in order to move out the heat generated by high voltage electric current. (EM)

Preparation Preparation of sample is quick and simple. (LM) Complex preparation. (EM)

Filament No filament used. (LM) Tungsten filament is used. (EM)

Radiation leakage No radiation risk. (LM) There is the risk of radiation leakage. (EM)

Availability Easily available and cheaper in rate. (LM) Not easily available and expensive. (EM)

Visibility Living, as well as the dead sample, can be viewed. (LM) Only dead (fixed) organisms can be viewed. (EM)

Studying the detailed structure of an organism is difficult. (LM) 3D structure is obtained due to which it is easy to study the structural and other details of organisms. (EM)

The natural colour of specimen is obtained. (LM) Only black and white image is obtained. (EM)

The image can be seen directly. (LM) Image is seen only on fluorescent screen. (EM)

1. Differentiate between SEM and TEM

The main difference between SEM and TEM is that SEM creates an image by detecting reflected or knocked-off electrons while TEM uses transmitted electrons (electrons which are passing through the sample) to create an image. As a result, TEM offers valuable information on the inner structure of the sample, such as crystal structure, morphology and stress state information, while SEM provides information on the sample’s surface and its composition.

SEMs use a specific set of coils to scan the beam in a raster-like pattern and collect the scattered electrons.

The transmission electron microscopy (TEM) principle, as the name suggests, is to use the transmitted electrons; the electrons which are passing through the sample before they are collected. As a result, TEM offers invaluable information on the inner structure of the sample, such as crystal structure, morphology and stress state information, while SEM provides information on the sample’s surface and its composition.

Moreover, one of the most pronounced differences between the two methods is the optimal spatial resolution that they can achieve; SEM resolution is limited to ~0.5 nm, while with the recent development in aberration-corrected TEMs, images with spatial resolution of even less than 50 pm have been reported.

Which electron microscopy technique is best for your analysis?

This all depends on what type of analysis you want to perform. For example, if you want to get information on the surface of your sample, like roughness or contamination detection, then you should choose a SEM. On the other hand, if you would like to know what the crystal structure of your sample is, or if you want to look for possible structural defects or impurities, then using a TEM is the only way to do so.

SEMs provide a 3D image of the surface of the sample whereas TEM images are 2D projections of the sample, which in some cases makes the interpretation of the results more difficult for the operator.

Due to the requirement for transmitted electrons, TEM samples must be very thin, generally below 150 nm, and in cases that high-resolution imaging is required, even below 30 nm, whereas for SEM imaging there is no such specific requirement.

This reveals one more major difference between the two techniques; sample preparation. SEM samples require little or no effort for sample preparation and can be directly imaged by mounting them on an aluminum stub.

In contrast, TEM sample preparation is a quite complex and tedious procedure that only trained and experienced users can follow successfully. The samples need to be very thin, as flat as possible, and the preparation technique should not induce any artefacts (such as precipitates or amorphization) to the sample. Many methods have been developed, including electropolishing, mechanical polishing and focused ion beam milling. Dedicated grids and holders are used to mount the TEM samples.

SEM vs TEM: differences in operation

The two EM systems also differ in the way they are operated. SEMs usually use acceleration voltages up to 30 kV, while TEM users can set it in the range of 60 – 300kV.

The magnifications that TEMs offer are also much higher compared to SEMs: TEM users can magnify their samples by more than 50 million times, while for the SEM this is limited up to 1-2 million times.

However, the maximum Field of View (FOV) that SEMs can achieve is far larger than TEMs, which users can only use to image a very small part of their sample. Similarly, the depth of field of SEM systems are much higher than in TEM systems.

In addition, the way images are created are different in the two systems. In SEMs, samples are positioned at the bottom of the electron column and the scattered electrons (back-scattered or secondary) are captured by electron detectors. Photomultipliers are then used to convert this signal into a voltage signal, which is amplified and gives rise to the image on a PC screen.

In a TEM microscope, the sample is located in the middle of the column. The transmitted electrons pass through it, and through a series of lenses below the sample (intermediate and projector lenses). An image is directly shown on a fluorescent screen or via a charge-coupled device (CCD) camera, onto a PC screen.

 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

SEM: Electrons are captured and counted by detectors, image on PC screen

TEM: Direct imaging on fluorescent screen or PC screen with CCD

Operation

SEM: Little or no sample preparation, easy to use

TEM: Laborious sample preparation, trained users required