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#### QUESTIONS

1. Write an essay on the history of microscopy
2. Differentiate between the light and electron microscopy.
3. Differentiate between the SEM and TEM.

## 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.

### **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.

### **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 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 Binnig and Heinrich Rohrer.

Later Binnig 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.

### **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 Zernike'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**.

**Origin:** The origin of the word microscope according to the Online Etymology Dictionary is as follows: 1656, from Mod.L. *microscopium*, lit. "an instrument for viewing what is small," from Gk. *micro-* (q.v.) + *-skopion*. "means of viewing," from *skopein* "look at." Microscopic "of minute size" is attested from 1760s.



### *History of the Compound Microscope*

Just as the Greeks had a fully functioning radiant heating system operating two thousand years before those only now being introduced in the US, so the origins of the compound light microscope appear to be traced, not to Holland, England or France - but to China which is perhaps appropriate given the present predominance of China in supplying compound light microscopes!

### *The Water Microscope*

According to an ancient Chinese text, the Chinese viewed magnified specimens through a lens at the end of a tube, which tube was filled with varying levels of water according to the degree of magnification they wished to achieve. Ingenious, effective and repeatable in the home, today. That this occurred some 4,000 years ago in the Chow-Foo dynasty and more than 3,500 years before the "father of modern microscopy" was born is quite remarkable.



That these Chinese ancients achieved magnification levels of 150 times today's standard, or 100 moou, is breath-taking. It is as if they developed a town car that achieved Mach II. If they did build such a car, no reference to it has ever been found. Similarly, there is no further known reference to such a compound microscope device until we come back to the Greeks again.

No less a person than **Aristotle** describes the workings of a microscope in some detail. The Greeks certainly made good use of curved lenses, which are an essential component of any stereo or compound microscope. Ancient Greek boys probably shared every American boy's sense of triumph of using a curved lens, or magnifying glass, to start a fire. The Greeks, however, also used it for surgical procedures, not on ants as little boys are wont to do, but on people - to cauterize wounds and lesions caused by leprosy and so forth.

Ancient Egyptians and Romans also used various curved lenses although no reference to a compound microscope has been found. The Greeks did, however, give us the word "microscope." It comes from two Greek words, "uikpos," small and "okottew," view. However, while Ancient Chinese, Greeks and Romans all applied their infinite wisdom to the issue, there is no known reference to either the use of artificial light or to multiple lenses. In other words, we can give great credit to the Ancients for their foresight and achievements, but we have to look elsewhere to uncover both the first light and compound microscope.

Incredibly, the next historical references with anything at all to do with microscopes, or more accurately, optics is 1,200 years after Rome was sacked and, even then, the references are only to the use of lenses in the invention of spectacles. Put another way round, some of the smartest people the planet has ever produced, played and worked with single lenses for several thousand years without taking it further.

### *Spectacles*

Then, within just a few short years in Tuscany, Italy, two men claimed to have independently invented spectacles. The evidence? Their tombstones! One, **Salvano d'Aramento degli Amati** died in 1284 in Florence and claimed to have kept the process secret. The other, **Alessandro della Spina** died in 1317 and claimed to have revealed his process. Pisa and Florence are but a short gallop away. Coincidence? You decide.

In any event, a local monk, **Girodina da Rivalta** gave a sermon in 1306 in which he enthusiastically endorsed spectacles as a terrific invention and in passing, indicated that they had been in use for about 20 years. Finally, in 1289, another local from the **Popozo family** bemoaned that "I am so debilitated by age that without the glasses known as spectacles, I would no longer be able to read or write."

## *Telescopes*

At about the same time, it appears that lenses were being used in early telescopes. In the 13th century, the Englishman, **Roger Bacon** discusses them at length. Both spectacles and microscopes are relevant to microscopes because they trace the increasingly sophisticated use of lenses - the essential optical component of any microscope.

Then, a mere 200-300 years later, we find a plethora of references and hard evidence of both telescopes and microscopes. The Renaissance had arrived and with it, an abundant flowering in the arts and sciences. Most importantly, with the invention of the printing press, ideas and developments could be spread easily and rapidly. As a result, **Thomas Digges'** work on the telescope in England in the mid-16th century and **Hans Lippershey's** work which included applying for a telescope patent were transmitted to others, including no less a genius than **Galileo**.

Galileo immediately began to work with lenses. In a short timeframe, he developed an improved telescope with a focusing device and went on to conquer the stars. That said, we should also pay tribute to **Sir Isaac Newton** who around the same time in the UK, invented the reflecting telescope.

## *Compound Microscopes*

But what of microscopes? Well, the same **Hans Lippershey** and his son, **Zaccharias Hanssen** was experimenting with a variety of lenses. In the late 1590's, they used several lenses in a tube and were amazed to see that the object at the end of the tube was magnified significantly beyond the capability of a magnifying glass. They had just invented the compound microscope. That is to say, they had discovered that an image magnified by a single lens can be further magnified by a second or more lenses.

Then, in the mid 17th century, an Englishman, **Robert Hooke** and a Dutchman, **Anthony Van Leeuwenhoek** took the microscope to new levels. Hooke was a sickly genius who loved to experiment. He did so across a huge range of scientific fields of study and with prolific success. He invented the universal joint, the iris diaphragm (another key component of many modern light microscopes), a respirator, an anchor escapement and balance spring for clocks.

He also worked out the correct theory of combustion; devised an equation describing elasticity that is still used today ("**Hooke's Law**") and invented or improved meteorological instruments such as the barometer, anemometer, and hygrometer; and so on. Most of all, however, he is known for **Micrographia**, his

studies with a microscope, published in 1665. *Micrographia* became an overnight sensation not just for what he described but for the superb drawings that he made.

He described a new world alongside exquisite drawings of the stinging hairs on a nettle, a flea and, most famously of all, the honeycomb structure or "cells" of a cork. It was Hooke who coined the term "cells" when describing living tissue. Interestingly, while Hooke did use a compound microscope, he found that it much strained and weakened his sight. For his *Micrographia*, he preferred to use a simple, single lens microscope made of gold and leather and illuminated by a candle. Perhaps the first light microscope?

### ***Antonie van Leeuwenhoek - the Father of the Microscope***

It was Leeuwenhoek, however, who lived at the same time as Hooke and drew on Hooke's work to take microscope design to new levels of sophistication. As a draper, he used a simple microscope to examine cloth. As a scientist, he began to experiment with new ways of grinding lenses in order to improve the optical quality. In total, he ground some 550 lenses, some of which had a linear magnifying power of 500 and a resolving power of one-millionth of an inch - an astounding achievement.

Leeuwenhoek detailed these achievements in almost 200 letters to The Royal Society in London where no less a person than Robert Hooke validated them. The result of all this work was a simple, single lens, hand-held microscope. The specimen was mounted on the top of the pointer, above which lay a convex lens attached to a metal holder. The specimen was then viewed through a hole on the other side of the microscope and was focused using a screw.

Perhaps his most famous experiment came in 1674 when he viewed some lake water:

*"I now saw very plainly that these were little eels, or worms, lying all huddled up together and wriggling just as if you saw, with thenaked eye, a whole tubful of little eels and water, with the eels squirming among one another; and the whole water seemed to be alive with these multifarious animalcules.*

*This was for me, among all the marvels that I have discovered in nature, the most marvelous of all; and I must say, for my part, that no more pleasant sight has every yet come before my eyes that these many thousand of living creatures seen all alive in a little drop of water, moving among one another, each several creature having its own proper motion."*

**He had discovered bacteria.** He had earned his title of the Father of the Microscope. Interestingly, it took until 1839, nearly two hundred years later, before cells were finally acknowledged as the basic units of life.

### *18th/19th Centuries*

The next major step in the history of the microscope occurred another 100 years later with the invention of the achromatic lens by **Charles Hall**, in the 1730s. He discovered that by using a second lens of different shape and refracting properties, he could realign colors with minimal impact on the magnification of the first lens.

Then in 1830, Joseph Lister solved the problem of spherical aberration (light bends at different angles depending on where it hits the lens) by placing lenses at precise distances from each other. Combined, these two discoveries contributed towards a marked improvement in the quality of image. Previously, due to the poor quality of glass and imperfect lens, microscopists had been viewing nothing but distorted images - somewhat like the first radios were extremely crackly.

It is worth remembering that up until now, each new stride has been in the quality or application of the lenses. Then, in 1863, one of the several new manufacturers of microscopes, the **Ernst Leitz** company, addressed a mechanical issue with the introduction of the first revolving turret with no less than five objectives.

This improvement was quickly followed in 1866 when **Carl Zeiss** recruited **Ernst Abbe** as his director of research at the Zeiss Optical Works. Abbe laid out the framework of what would become the modern computational optics development approach. He made clear the difference between magnification and resolution and criticized the practice of using eyepieces with too high a magnification as "empty magnification." By 1869, his work produced a new patented illumination device - the Abbe condenser.

**Abbe Condenser:** Abbe's work on a wave theory of microscopic imaging (the Abbe Sine Condition) made possible the development of a new range of seventeen microscope objectives - three of these were the first immersion objectives and all were designed based on mathematical modeling. As Abbe noted, his creations were "based on a precise study of the materials used, the designs concerned are specified by computation to the last detail - every curvature, every thickness, every aperture of a lens - so that any trial and error approach is excluded."

From here on, microscopes were designed based on sound laws of physics rather than the trial and error that had characterized the pioneers. At the same time, a number of companies set up specialized manufacturing plants focused on manufacturing precision microscopes. Research and development continued to bear fruit.



In 1880, the first microtomes began to be used that enabled significantly thinner samples to be prepared in order to improve sample. In 1893, another Zeiss employee, August Kohler figured out an unparalleled illumination system that is still known as Kohler illumination. Using double diaphragms, the system provides triple benefits of a uniformly illuminated specimen, a bright image and minimal glare. In other words, Kohler achieved an almost perfect image.

The mass market for microscopes had arrived at the same time as precision engineering and it is little wonder that a plethora of stunning results were obtained: In 1879, Walter Flemming discovered cell mitosis and chromosomes, an achievement recognized as one of the 100 most important scientific achievements of all time.

### ***20th Century***

At the turn of the 19th/20th centuries **Louis Pasteur** invented pasteurization while **Robert Koch** discovered his famous or infamous postulates: the anthrax bacillus, the tuberculosis bacillus and the cholera vibrio.

**UV and Phase:** By 1900, the theoretic limit of resolution for visible light microscopes (2000 angstroms) had been reached. In 1904, Zeiss overcame this limitation with the introduction the first commercial UV microscope with resolution twice that of a visible light microscope. In 1930 **Fritz**

**Zernike** discovered he could view unstained cells using the phase angle of rays. Spurned by Zeiss, his phase contrast innovation was not introduced until 1941 although he went on to win a Nobel Prize for his work in 1953.

**Electron Microscopes:** In 1931 **Max Knoll** and **Ernst Ruska** invented the first electron microscope that blasted past the optical limitations of the light. Physics dictates that light microscopes are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers.

Knoll and Ruska built a transmission electron microscope (TEM) - one that transmits a beam of electrons (as opposed to light) through the specimen. The subsequent interaction of the beam of electrons with the specimen is recorded and transformed into an image. Then, in 1942, Ruska improved on the TEM by building built the first scanning electron microscope (SEM) that transmits a beam of electrons across the specimen.

Ruska's principles still form the basis of modern electron microscopes - microscopes that can achieve magnification levels of up to 2 million times! The second major development for microscopes in the 20th century was the evolution of the mass market. Started in the 19th century when Leitz claimed to have exported 50,000 microscopes to the U.S., this trend accelerated in the 20th century. As a result, a large number of manufacturers sprang up to offer more competitively priced alternatives to established European companies such as Zeiss and Leitz.

**China:** China has become a major supplier of microscopes for everyday use and, with the evolution of their optical manufacturing capability, now supplies optical components to some of the major microscope brands. This market trend has had a beneficial effect on the price of microscopes, enabling the spread of microscopes beyond the realm of the research scientist to everyday commercial and individual use.

New light sources - halogen, fluorescent and LED have all improved or added a greater versatility of the light microscope, while the advent of boom stands have led to extensive commercial inspection applications that cannot be undertaken with a standard pedestal microscope base. The most recent innovation, however, has been the arrival of the digital microscope.

**Digital Microscopes:** Digital microscopes allow for live image transmission to a TV or computer screen and have helped revolutionize microphotography. Digital microscopes simply integrate a digital microscope camera on the trinocular port of a standard microscope. An alternative and more flexible solution is simply to place a digital microscope camera on a trinocular microscope!

**Dino-Lite:** One of the more original innovations in the 21st century has been Dino-Lite Digital microscopes. Dino-Lite are handheld digital microscopes, not much larger than a fat pen. They offer low power zoom capability with

magnification up to 500x. They have had a marked impact on industrial inspection applications.

## DIFFERENCES BETWEEN LIGHT MICROSCOPY AND ELECTRON MICROSCOPY

LIGHT MICROSCOPY	ELECTRON MICROSCOPY
Illuminating source is the Light.	Illuminating source is the beam of electrons.
Specimen preparation takes usually few minutes to hours.	Specimen preparation takes usually takes few days.
Live or Dead specimen may be seen.	Only Dead or Dried specimens are seen.
Condenser, Objective and eye piece lenses are made up of glasses.	All lenses are electromagnetic.
It has low resolving power ( $0.25\mu\text{m}$ to	t has high resolving power ( $0.001\mu\text{m}$ ),

0.3 $\mu$ m).	about 250 times higher than light microscope.
It has a magnification of 500X to 1500X.	It has a magnification of 100,000X to 300,000X.
Image is Colored.	Image is Black and White.

Other differences include;

1. Vacuum is not required for the light microscope while vacuum is required for the electron microscope
2. In the light microscope, There is no need of high voltage electricity while in the electron microscope, High voltage electric current is required (50,000 Volts and above).
3. In the light microscope, Filament is not used while in the electron microscope, Tungsten filament is used to produce electrons.

4. In the light microscope, Specimen is stained by colored dyes while in the electron microscope, Specimen is coated with heavy metals in order to reflect electrons.
5. In the light microscope, Image is seen by eyes through ocular lens while in the electron microscope, Image is received in Zinc Sulphate Fluorescent Screen or Photographic Plate.

#### DIFFERENCES BETWEEN THE 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.

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 while Electrons are used as "light source". TEM is based on transmitted electrons and operates on the same basic principles as the light microscope.

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 while 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 is coated with a thin layer of heavy metal such as gold or palladium while The sample in TEM has to be cut thinner (**70-90 nm**) because electrons cannot penetrate very far into materials.

SEM can resolve objects as close as 20 nm while TEM has a much higher resolution than SEM; It can resolve objects as close as 1 nm i.e. down to near-atomic levels.

The magnifying power of SEM is up to 50,000X while The magnifying power of TEM is up to 2 million times.

SEM allows for a large amount of sample to be analyzed at a time while With TEM only a small amount of samples can be analyzed at a time.

In SEM, 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 while in TEM 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.

SEM provides a 3-dimensional image while TEM provides a 2-dimensional picture.

SEM are used to study topography and atomic composition of specimens, process control and also, for example, the surface distribution of immuno-labels while TEM is used to o 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).