

BIO 101 (BASIC PRINCIPLES OF BIOLOGY)

TOPIC:

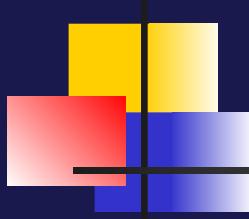
MICROSCOPY



Section taken by:

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Faculty of Science, LASU, Ojo.**

DEFINITION OF MICROSCOPE

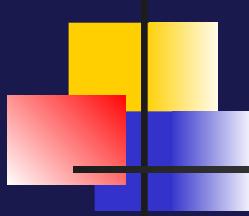


- Microscope is an instrument which provides an enlarged image of minute objects such as; sub cellular structures, and many more that are generally not visible to the naked eyes.

The word “**microscope**” is formed of two Greek words: “***micros***”- small and ‘***skipein***’-to look. ☺



The complexity of microscopes has however since its invention, increased to many folds from simple lens to complex scanning electron microscope



CONCEPT OF MICROSCOPY

Microscopy is the science of investigating small objects and structures using such an instrument.

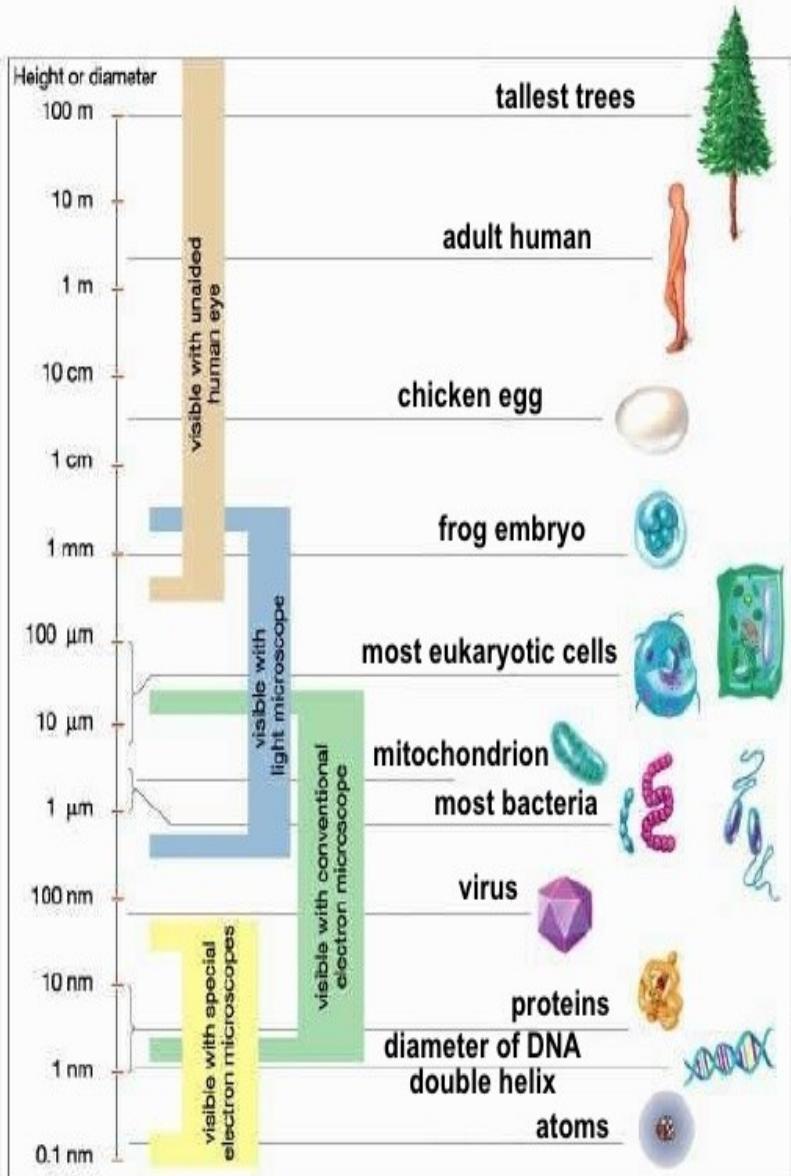
→ In microscopy, the microscope must accomplish three tasks. It must:

1. Produce a magnified image of the specimen,
2. Separate the details in the image and,
3. Render the details visible to the human eye or camera.



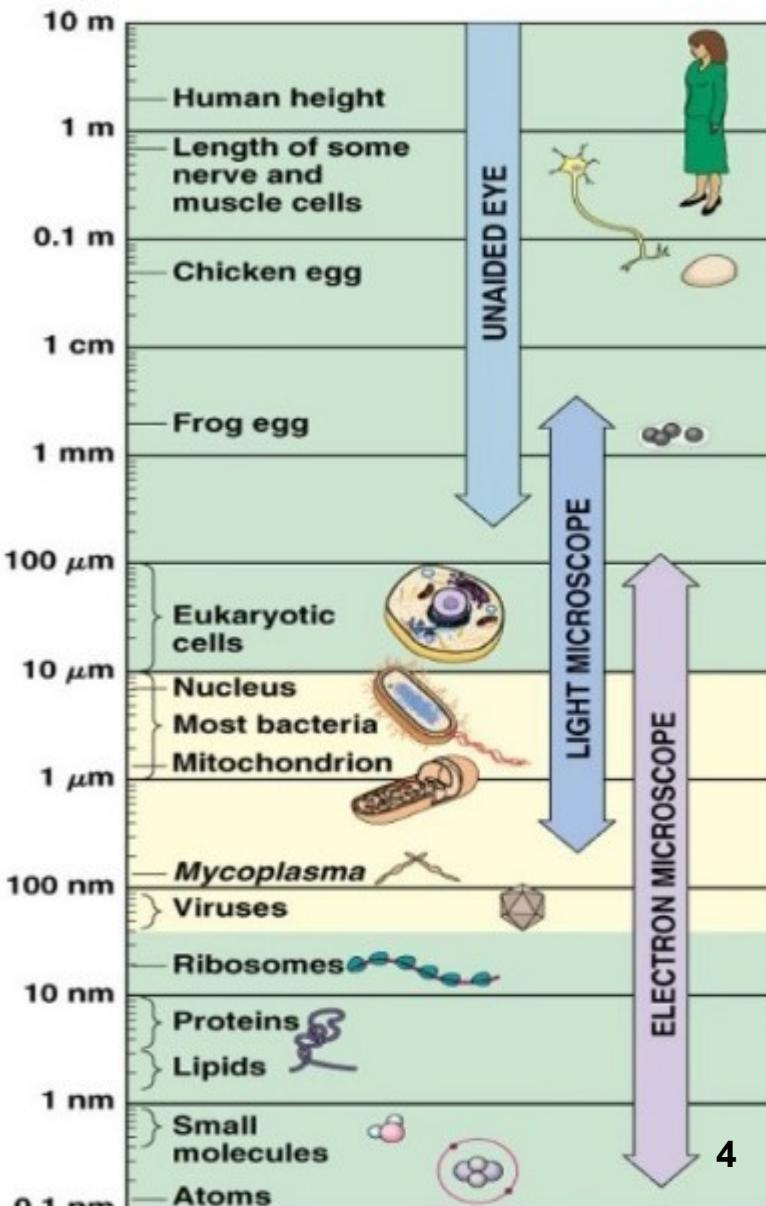
CONCEPT OF MICROSCOPY

SCALE:



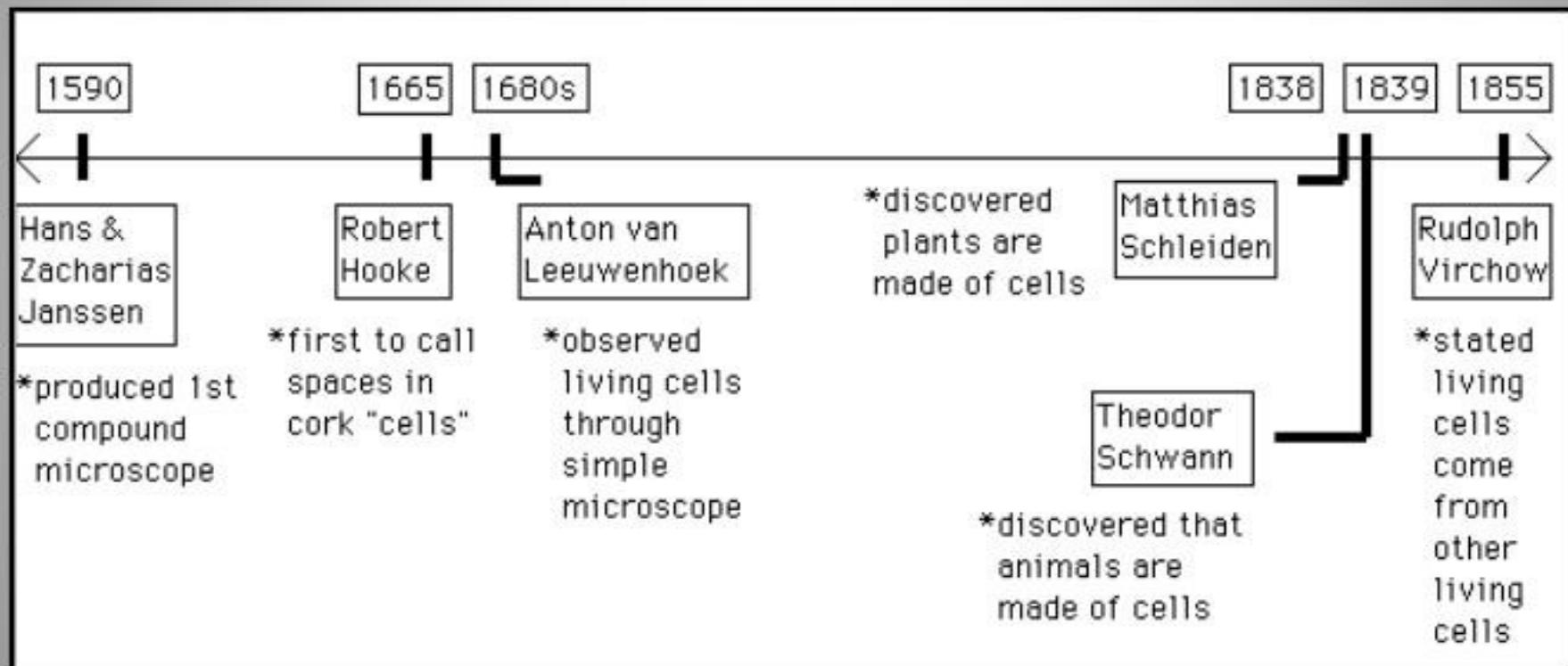
Units of measurement:

- 1 meter (m) = 39.37 inches
- 1 centimeter (cm) = 1/100 m
- 1 millimeter (mm) = 1/1000 m
- 1 micrometer (μm) = 1/1,000,000 m
- 1 nanometer (nm) = 1/1,000,000,000 m



THE HISTORY OF MICROSCOPE

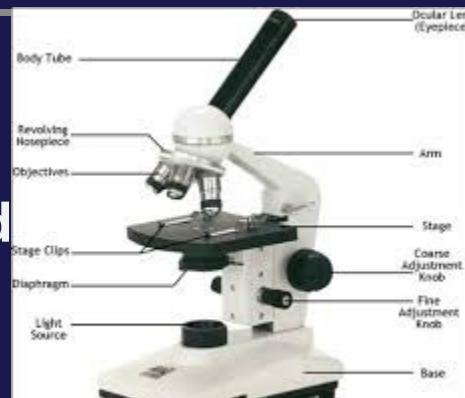
The Cell Theory Timeline



HISTORY OF MICROSCOPE Contd.

PRE 1600:

Zoocharia Jansen in 1590 and his brother Hans in 1595 used second lens that enlarged imaged formed by first lens by 50-100X.



Zacharias Jansen

1580-1638

(together with his father Hans Jansen)



The History

Hans and Zacharias Jansen of Holland in the 1590's created the "first" compound microscope



Zacharias Jansen
1588-1631



HISTORY

- The microscope was invented in the late 1500s by Dutch spectacle-maker Zacharias Jansen.



- It magnified objects 3 to 9 times its size.



HANS LIPPERSHEY



ZACHARIAJ JANSSEN

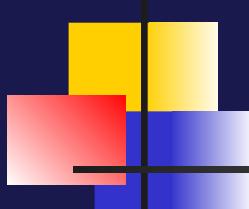


The First Compound Microscope (circa 1595)

History of the Microscope

- Invented by Zach Jansen.
- Zach Jansen was a person who made eye-glasses.
- Came up with and invented the Compound Microscope around 1590.

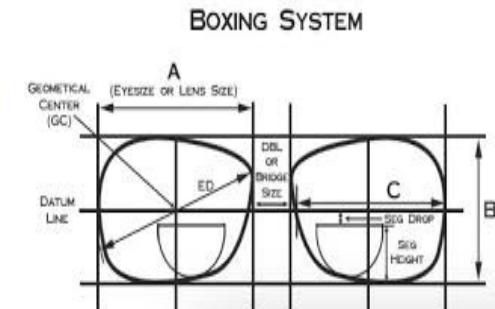




HISTORY OF MICROSCOPE Contd.

PRE 1660:

- ✓ In 11th century , the Arab Alhazan described the use and characteristics of glass lenses
- ✓ Roger Bacon was familiar with lenses and eye glasses , however weren't invented until late 1200s ↗

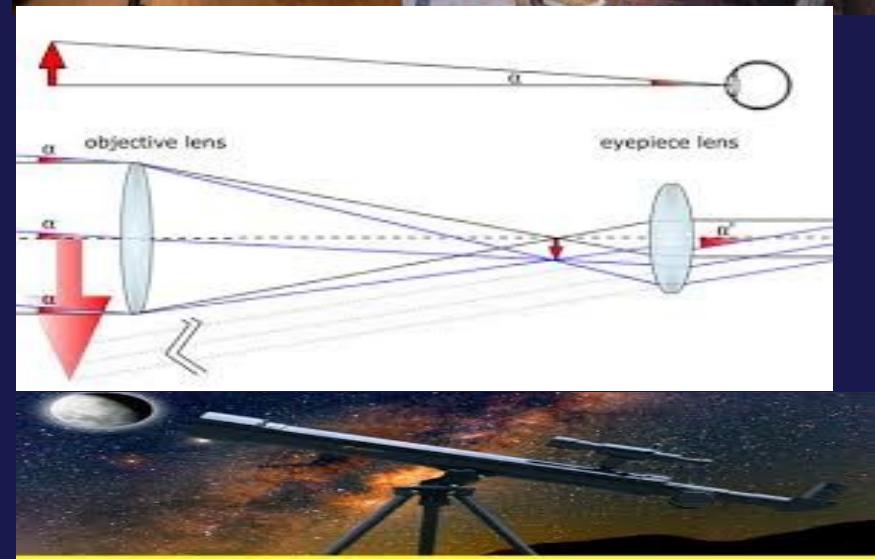




HISTORY OF MICROSCOPE Contd.

In the early & mid 1660s:

- ✓ In 1608 Telescope was invented , with Galileo improving upon it with his own models
- ✓ Around 1600 microscope was invented possibly by Hans and Zacharias Jansen. But lens quality was poor.
- ✓ The first known image of a microscope is a drawing by Isaac Beeckman in 1631.
- ✓ FIRST BIG MICROSCOPE (with 200X maximum magnification): came in 1665 when Robert Hooke published the book "MICROGRAPHIA "

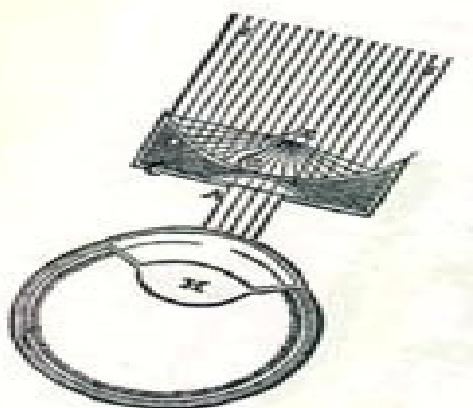


Galileo's Telescope

HISTORY OF MICROSCOPE Contd.

In the late 1660s:

- ✓ Anton van Leeuwenhoek began to grind his own lens and make simple microscope.
- ✓ He discovered nematode and rotifers.



The first microscope



Hooke Microscope
(circa 1670)





HISTORY OF MICROSCOPE Contd.

In the late 1660s:

- ✓ Anton van Leeuwenhoek in 1673, provided improved microscope and was first to observe unicellular animal. He is called “Father Of Microbiology”.

Anton van Leeuwenhoek

- Discoveries:

- 1673: He looked at pond scum under the microscope and discovered small organisms he called *animalcules* or little animals (Protists)
- 1676: discovered bacteria



http://www.kent.k12.wa.us/staff/ThimLynch/hs_d_lassic/he20lesson_protista/Protista_Lesson.html#Algae



Anton Van Leeuwenhoek

Pictures of Leeuwenhoek

Quotes

"I observed I could tell anything withal, I have thought I may truly examine my discovery in paper, so that all ingenues might longer be deceived thereby."

"My work, which I have had during these years performed, or undertaken again the present, I have always had entirely therein a concern; after Antonie van Leeuwenhoek, I have made greater noise than all the physicians have."

Health Care Contribution

During his practice, the following cuts were discovered:
- Infants
- Diseases
- Diseases of Almond Throat
- Blood Cells

Historical Background

Timeline

- 1673 - Letters to the Royal Society of London
- 1674 - Illustrations of plant anatomy and animal microscopists
- 1680 - Member of the Royal Society of London
- 1681 - Member of the French Academy of Sciences
- 1680-1723 - Many publications are written
- 1680 - Antonie van Leeuwenhoek was named "The Father of Microbiology"
- 1680 - Disease named after Leeuwenhoek called "Van Leeuwenhoek's Disease"



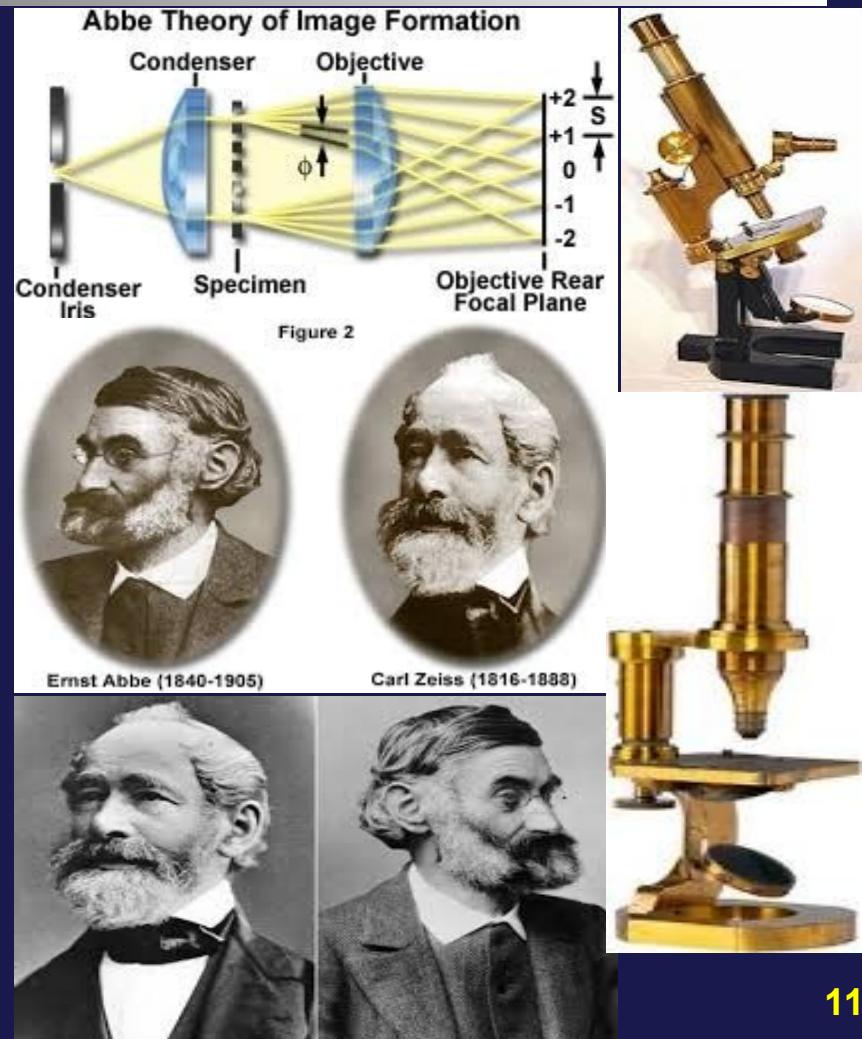
"In the year of 1657 I discovered very small living creatures in rain water."

Antonie van Leeuwenhoek

HISTORY OF MICROSCOPE Contd.

Between 1700 & 1800s:

- ✓ Not much change in basic microscope but certain problems were resolved in this era like color distortion and poor image resolution.
- ✓ Ernst Abbe : A German Physicist opined that oil emersion lens can prevent length distortion .





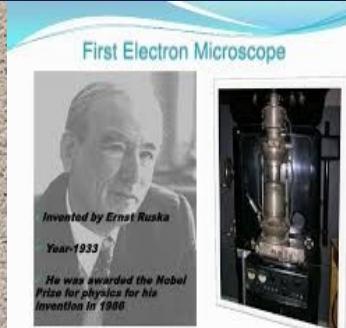
HISTORY OF MICROSCOPE Contd.

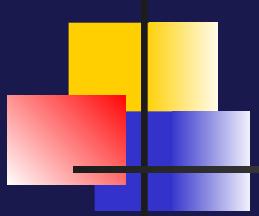
1900 till NOW!:

- ✓ In 1931, a German scientist, Ernst Ruska invented electron microscope which can magnify as much as million times.
- ✓ The only drawback is that living cell can not be viewed by using it.



Electron Microscope

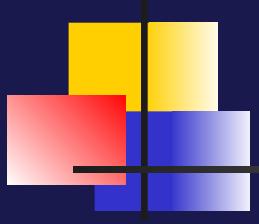




TYPES OF MICROSCOPE

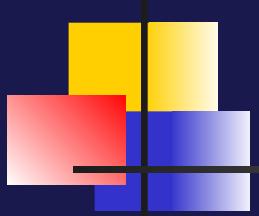
- ✓ Basically there are two types of microscopes:
 1. Light Microscope
 2. Electron Microscope

- ✓ Further classification in these basic microscopes are present. Other microscopes also present such as:
 - Phase contrast Microscopes
 - Fluorescent Microscope
 - Bright field AND Dark field Microscope



TYPES OF MICROSCOPE

- ✓ **Most commonly used microscope.**
- ✓ **Handy in use.**
- ✓ **LM uses light source for illumination of specimen.**
- ✓ **Generally used light sources include sunlight, UV light, laser light, LEDs.**
- ✓ **Types- Simple dissecting microscope, compound microscope, stereomicroscopes.**



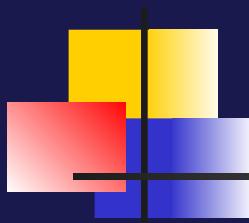
TYPES OF MICROSCOPE contd.

1. Light Microscope (LM):

- ✓ Light Microscope uses sunlight or artificial light. It is the most commonly used microscope.
- ✓ Handy in use.
- ✓ LM uses light source for illumination of specimen.
- ✓ Generally used light sources include sunlight, UV light, laser light, LEDs.
- ✓ LM includes: Simple dissecting microscope, compound microscope, stereomicroscopes. The various other types of light microscope are:

- A.Bright field microscope.
- B.Dark field microscope.
- C.Phase contrast microscope.
- D.Fluorescence microscope.

TYPES OF MICROSCOPE contd.



2. Simple Microscope:

- ✓ Consist of Biconvex lense.
- ✓ Can be moved up and down by adjustment.
- ✓ Object is placed on a platform.
- ✓ Light is focused by concave mirror.

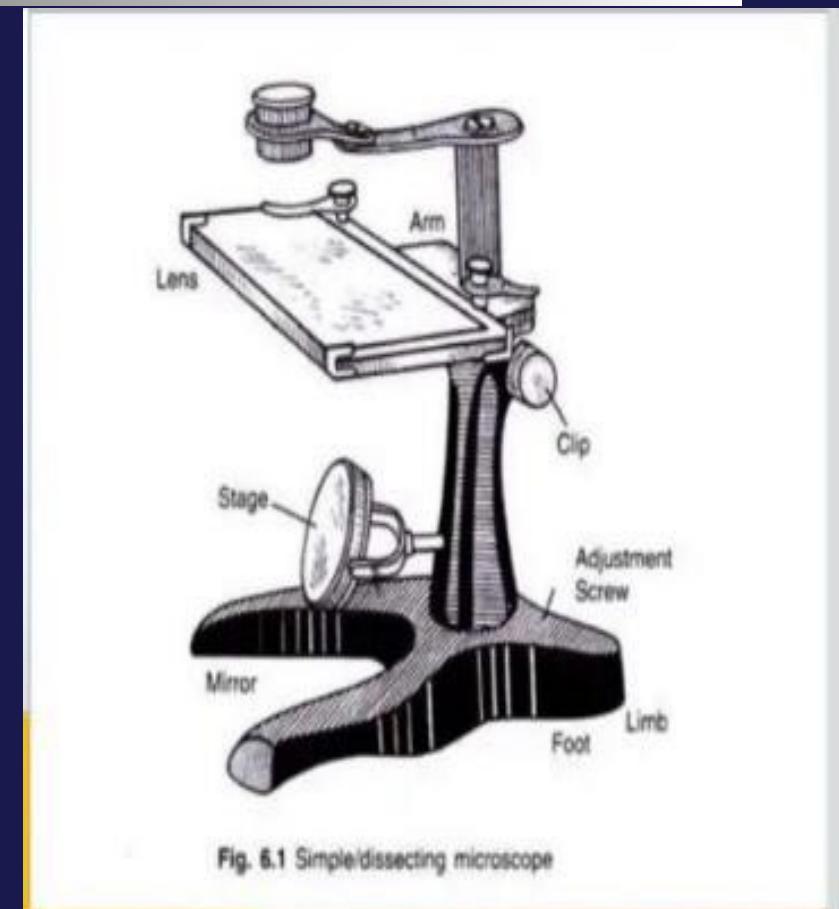
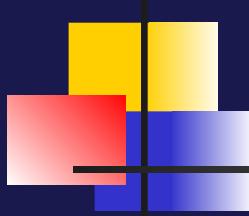


Fig. 6.1 Simple dissecting microscope

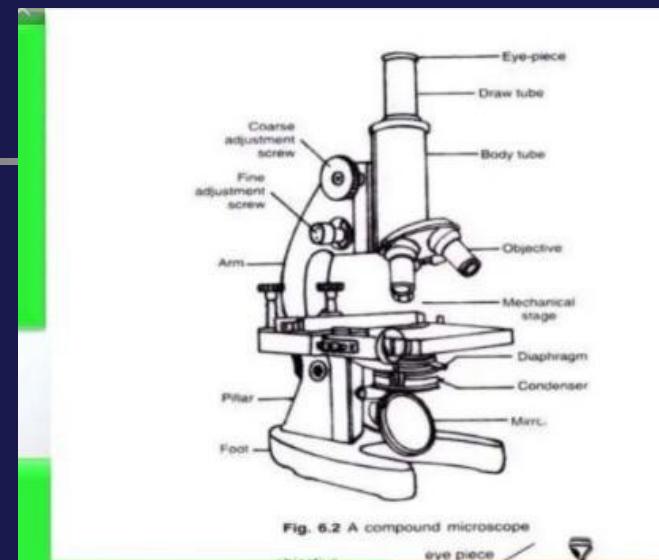
A Simple Dissecting Microscope

TYPES OF MICROSCOPE contd.



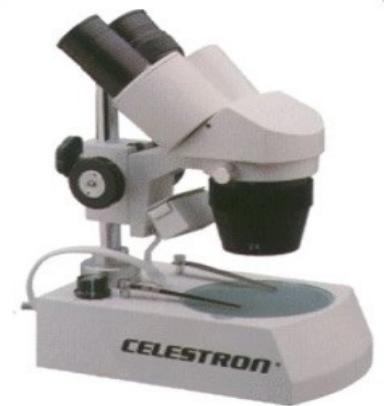
3. Compound Microscope:

- ✓ Has 2-sets of lenses.
- ✓ An Objective lens of short aperture and focal length
- ✓ Another set of lens of larger aperture and focal length facing eye and known as “Eye piece”



Compound Microscopes

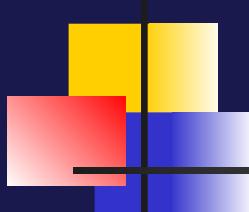
TYPES OF MICROSCOPE contd.



4. Stereo-Microscope:

- ✓ This microscope allows for binocular (two eyes) viewing of larger specimens.
- ✓ ~~This is otherwise known as Dissecting Microscope and serves a different purposes such as:~~
- ✓ Can be used for thicker specimens
- ✓ It produces a three dimensional (3-D) visualization of the sample being examined.
- ✓ It is used for dissections.
- ✓ Its used to study the surfaces of solid specimens or to carryout close work such as: sorting, micro-surgery, watch-making, small circuit board manufacture or inspection, insects and leaves and the likes.
- ✓ Usually magnifies 10x to 20x.

TYPES OF MICROSCOPE contd.



5. Interference Microscope:

- ✓ It is used for quantitative studies of macromolecules of the cell components

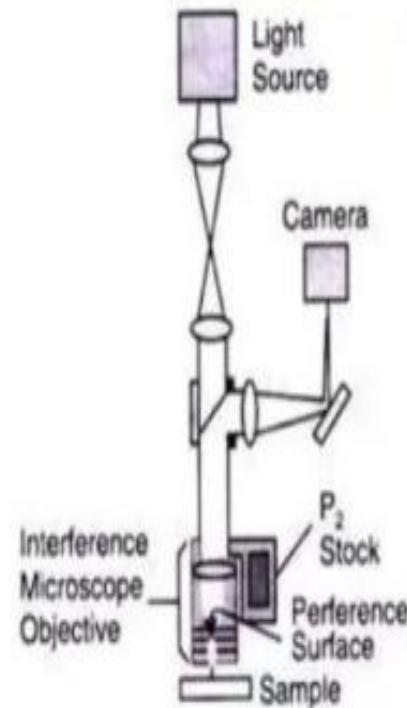
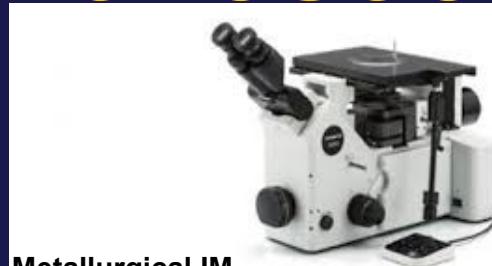
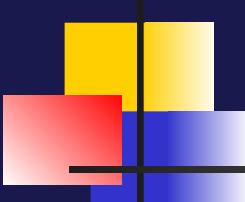


Fig. 6.5 Interference microscope



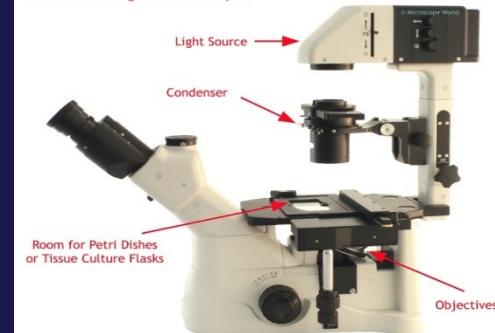
TYPES OF MICROSCOPE contd.



Metallurgical IM



Inverted Biological Microscope



Biological IM



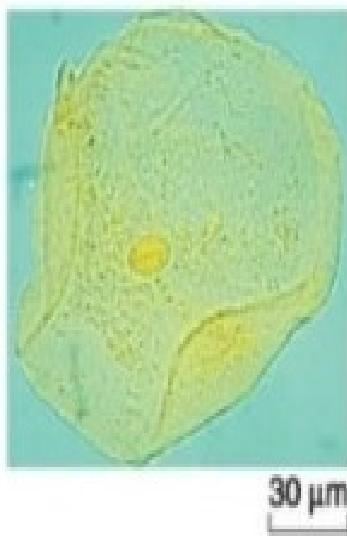
Inverted Microscopes

6. Inverted Microscope (IM):

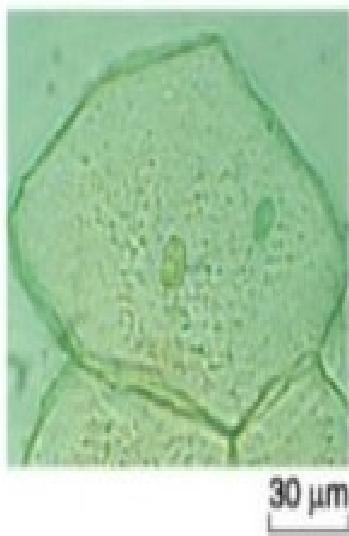
- ✓ It has both the light source and condenser set up high above the stage and pointing down toward the stage.
- ✓ The objectives and objective turret are located beneath the stage pointing up using reflecting objectives.
- ✓ The two basic types of inverted microscopes include biological inverted (BIM) and metallurgical inverted (MIM) microscopes.
- ✓ IM is used in micro-manipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath.
- ✓ The BIM is used to observe cell morphology.

PRINCIPLES OF MICROSCOPY

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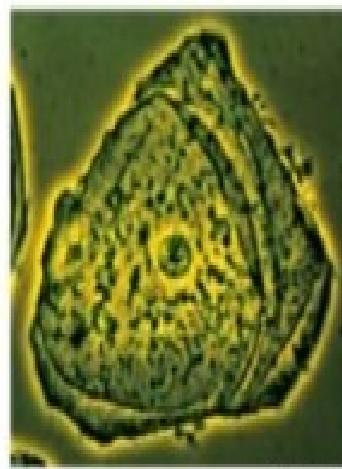
30 µm



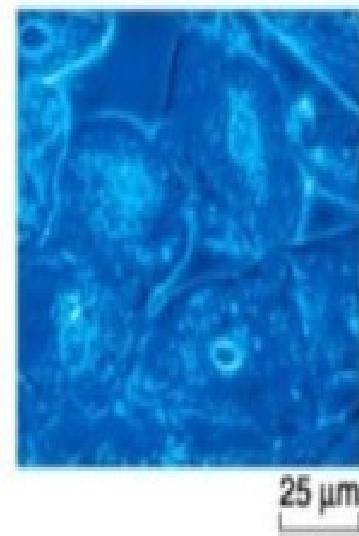
30 µm



25 µm



25 µm



25 µm

Bright-field. Light passing through the specimen is brought directly into focus. Usually, the low level of contrast within the specimen interferes with viewing all but its largest components.

Bright-field (stained). Dyes are used to stain the specimen. Certain components take up the dye more than other components, and therefore contrast is enhanced.

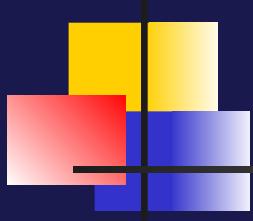
Differential interference contrast. Optical methods are used to enhance density differences within the specimen so that certain regions appear brighter than others. This technique is used to view living cells, chromosomes, and organelle masses.

Phase contrast. Density differences in the specimen cause light rays to come out of "phase." The microscope enhances these phase differences so that some regions of the specimen appear brighter or darker than others. The technique is widely used to observe living cells and organelles.

Dark-field. Light is passed through the specimen at an oblique angle so that the objective lens receives only light diffracted and scattered by the object. This technique is used to view organelles, which appear quite bright against a dark field.

IMAGES AS SEEN BY DIFFERENT MICROSCOPES

TYPES OF MICROSCOPE contd.

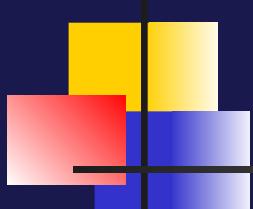


7. Electron microscope (EM or e-microscope):

- ✓ It uses electron. The various type of e-microscopes are:
 - ✓ 1. Transmission e-microscope.
 - ✓ 2. Scanning e-microscope
- ❑ In 1931, a German scientist, Ernst Ruska invented electron microscope which can magnify as much as million times.
- ❑ The only drawback is that living cell can not be viewed by using it.



PARTS OF MICROSCOPE



Parts of simple Microscope

Basically, the microscope is divided into two(2)major parts:

- 1.Mechanical parts
- 2.Optical parts

Mechanical Part:

- 1.This is composed of the body frame which could be unscrewed and serviced.
- 2.Optical Part: These parts are involved in passing the light through the object (specimen) and magnifying its size.



PARTS OF MICROSCOPE

Parts of simple Microscope

The components of the optical parts are as follows:

Mirror:

- A **Plano-convex mirror** is fitted below the stage to the vertical rod by means of a frame.
- It focuses the surrounding light on the object to be observed.

Lens:

- A **biconvex lens** is fitted above the stage, to the vertical rod, by means of a frame.
- It magnifies the size of the object and the enlarged virtual image formed is observed by keeping the eye above it.
- For proper focusing, the lens can be moved up and down by the frame.



PARTS OF MICROSCOPE

Parts of simple Microscope

Eyepiece: The lens the viewer looks through to see the specimen. The eyepiece usually contains a 10X or 15X power lens.

Diopter Adjustment: Useful as a means to change focus on one eyepiece so as to correct for any difference in vision between your two eyes.

Body tube (Head): The body tube connects the eyepiece to the objective lenses.

Arm: The arm connects the body tube to the base of the microscope.

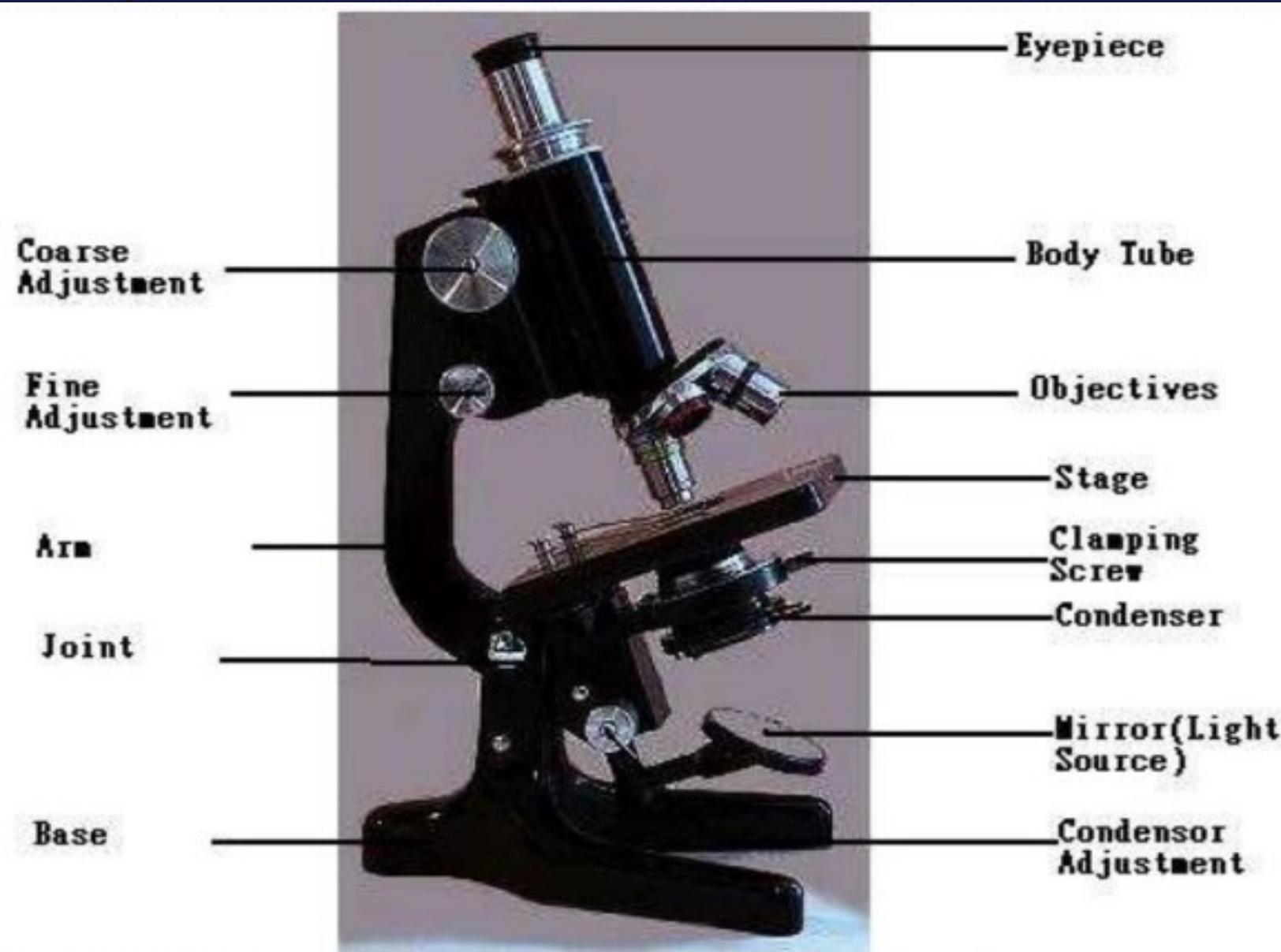
Coarse adjustment: Brings the specimen into general focus.

Fine adjustment: Fine tunes the focus and increases the detail of the specimen.



PARTS OF MICROSCOPE

Parts of a Simple Microscope



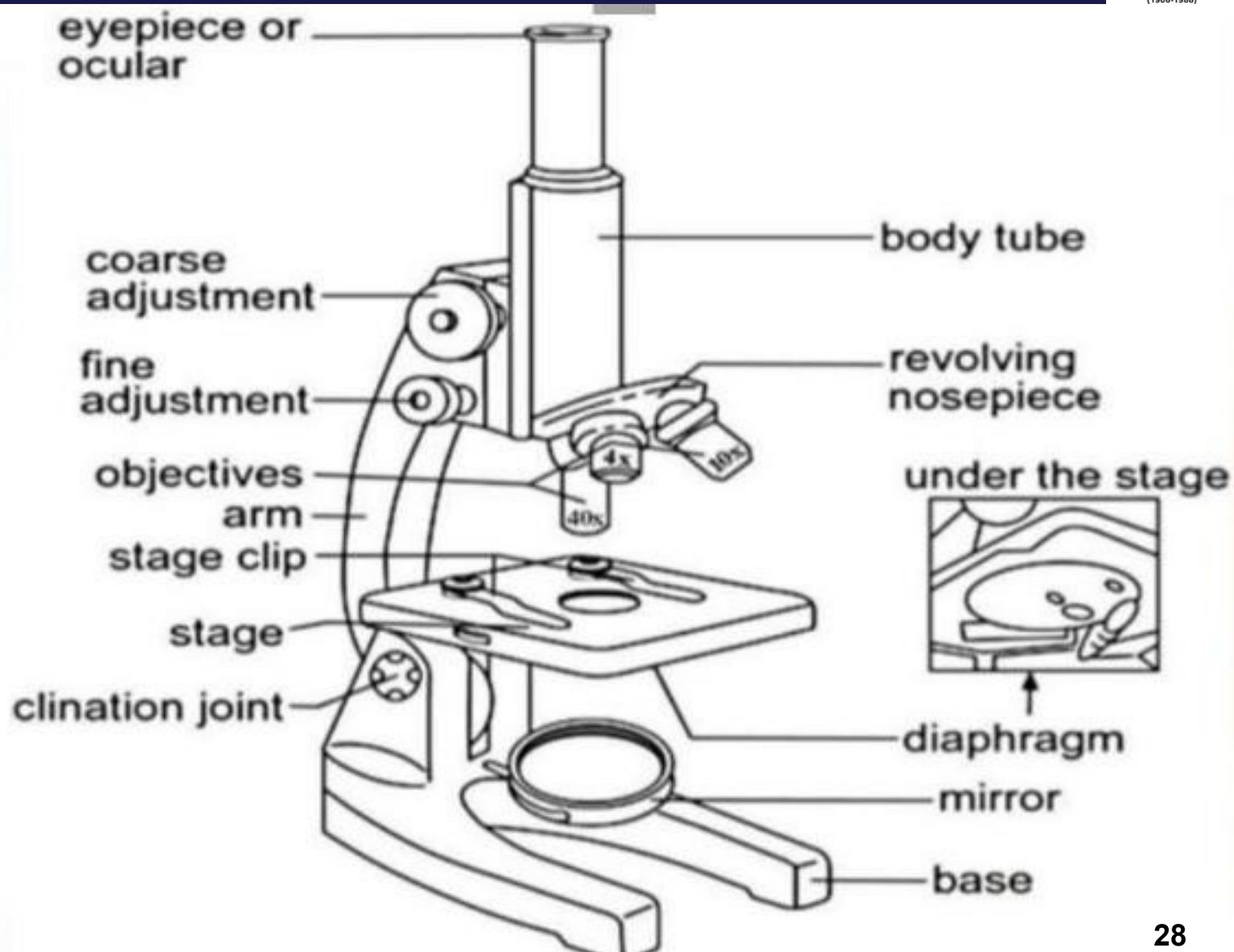


PARTS OF MICROSCOPE

How Does The Microscope Work

- ✓ All of the parts of a microscope work together.
- ✓ The light from the illuminator passes through the aperture, through the slide, and through the objective lens, where the image of the specimen is magnified.
- ✓ The then magnified image continues up through the body tube of the microscope to the eyepiece, which further magnifies the image the viewer then sees.
- ✓ Learning to use and adjust your compound microscope is the next important step.
- ✓ It's also imperative to know and understand the best practices of cleaning your microscope.

PARTS OF MICROSCOPE





PARTS OF MICROSCOPE

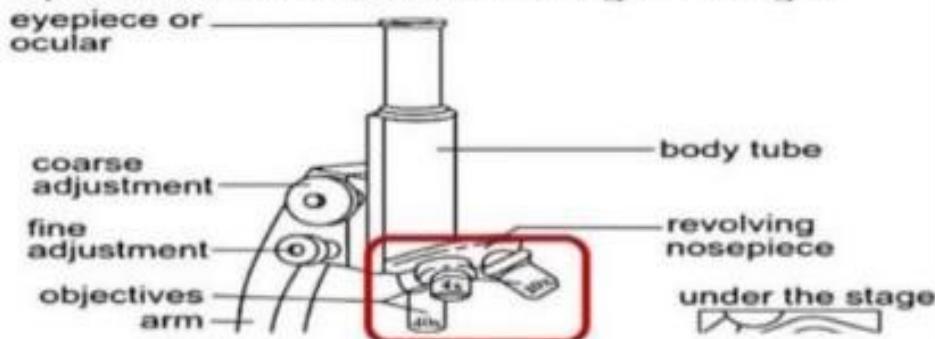
Eyepiece / Ocular Lens

- Magnifies the specimen image
- It is where you look through to view the object placed on the stage.



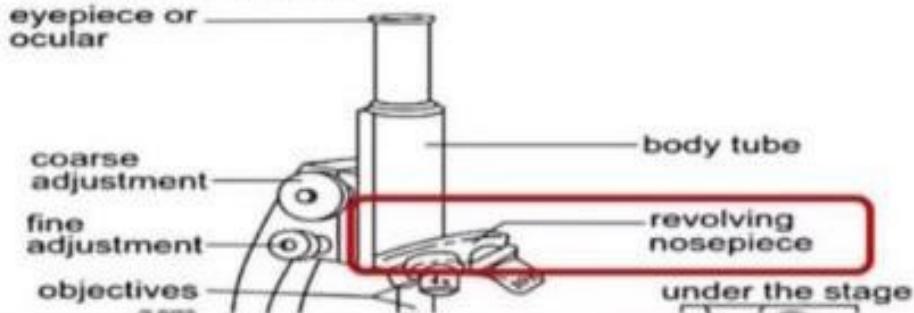
Objective Lenses

- It is used to magnify the images of the specimen to form an enlarged image.



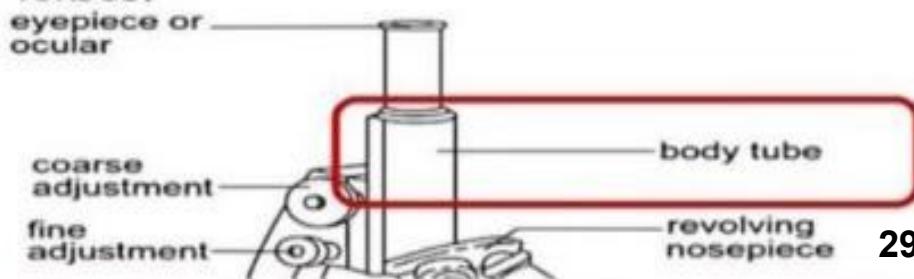
Nose Piece

- It holds the objective lenses and can be turned to increase the magnification.



Body Tube

- It supports the eyepiece and lenses.
- It also maintains the proper distance between the eyepiece and the objective lenses.





PARTS OF MICROSCOPE

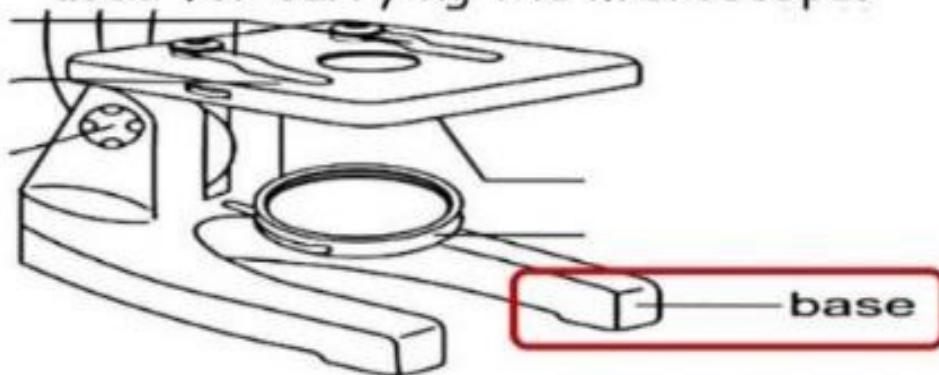
Stage

- Supports the slide/specimen



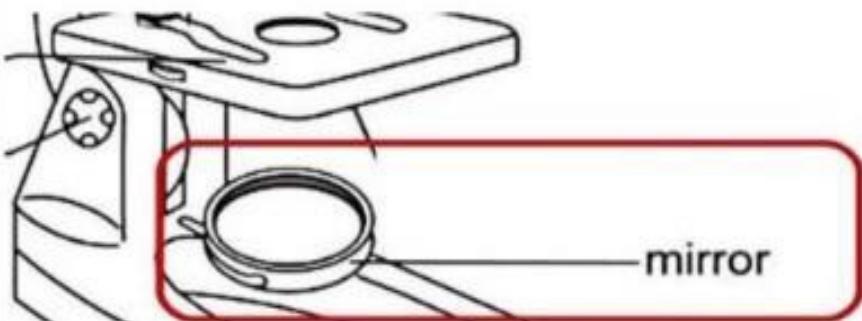
Base

- Supports the microscope and is also used for carrying the microscope.



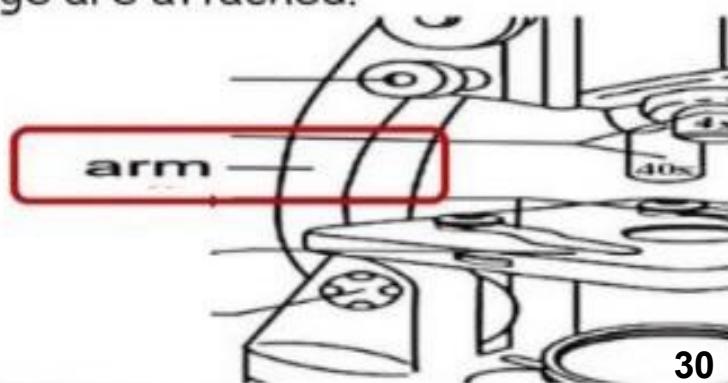
Mirror

- It reflects light to the lens of the microscope.



Arm

- It is the frame to which the base, body and stage are attached.

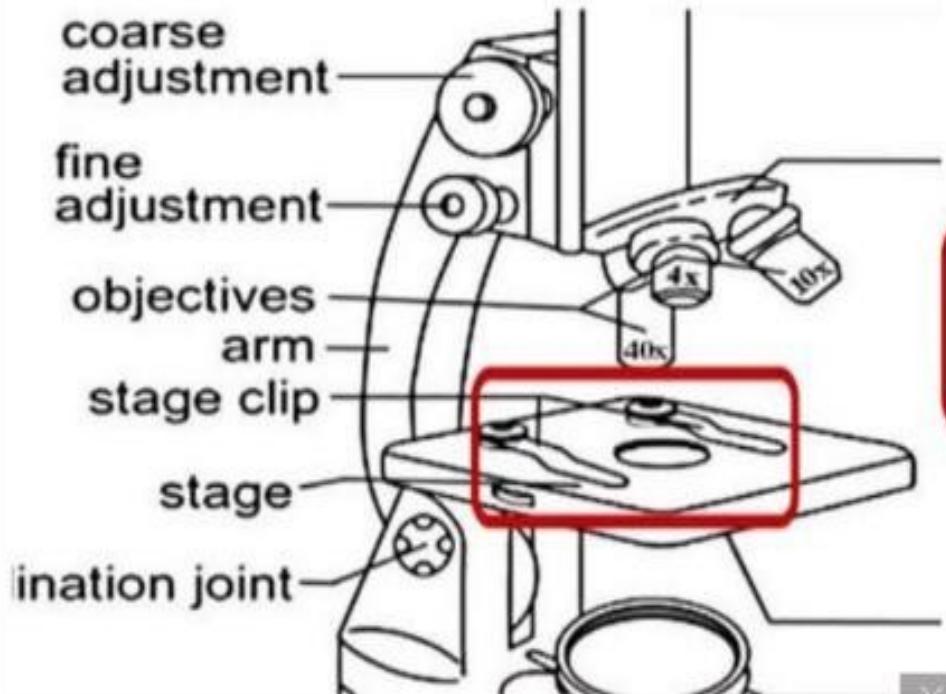


PARTS OF MICROSCOPE



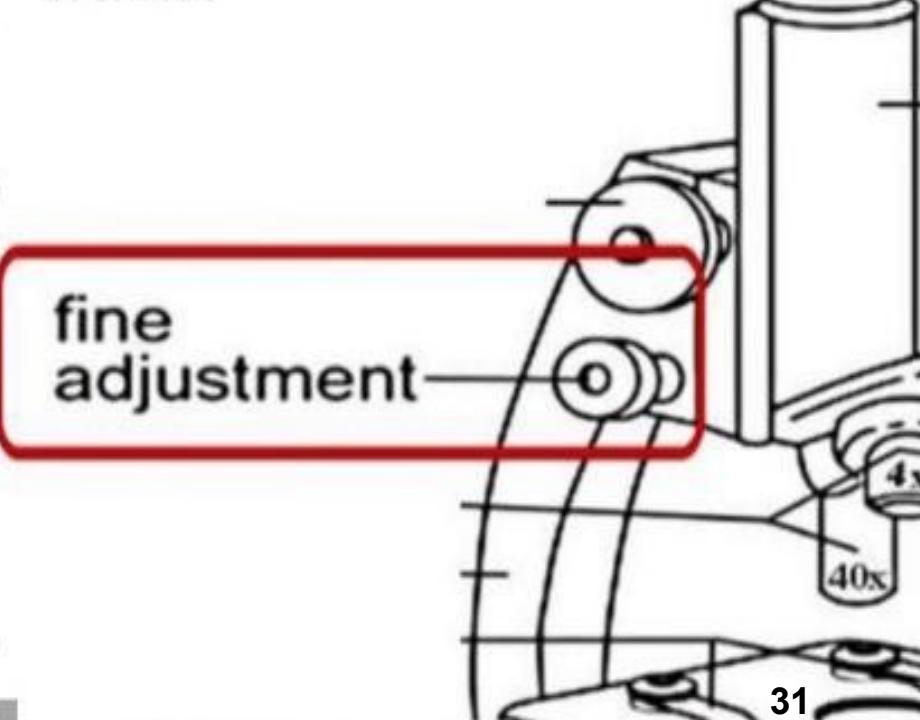
Stage Clips

- These 2 clips hold the slide/specimen in place on the stage.



Fine Adjustment Knob

- This knob moves the stage slightly to sharpen the image for PRECISION

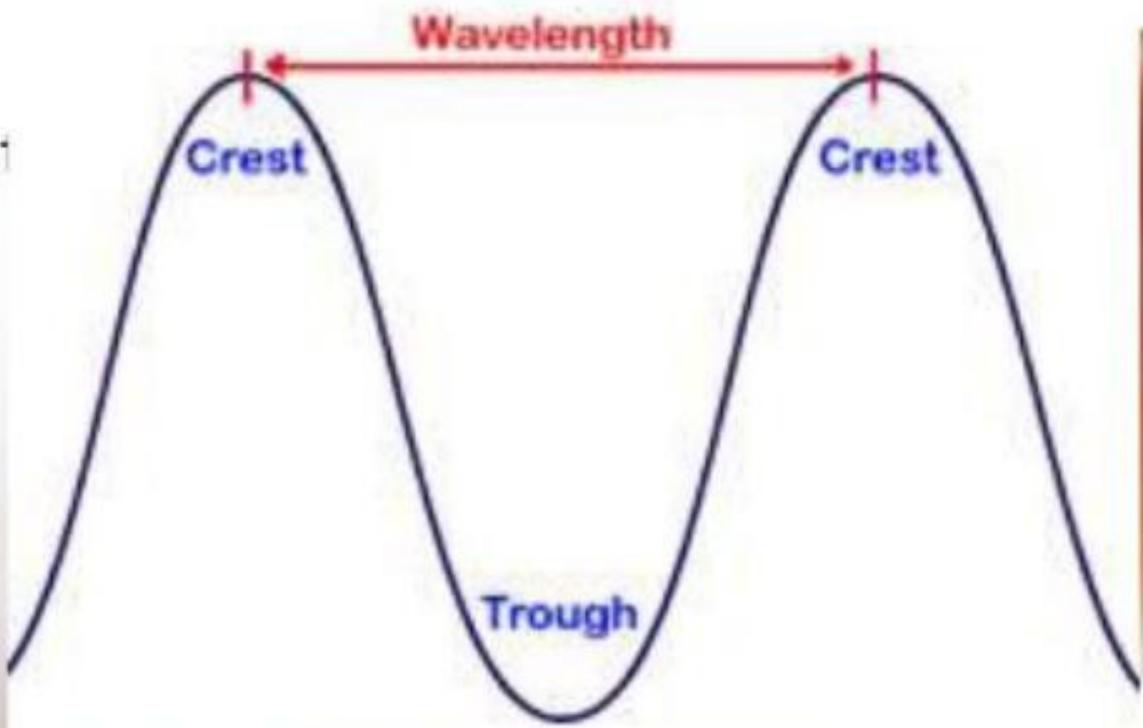


PARTS OF MICROSCOPE



WAVELENGTH

- Wavelength is a physical length from one point of the wave to the same point on the next wave

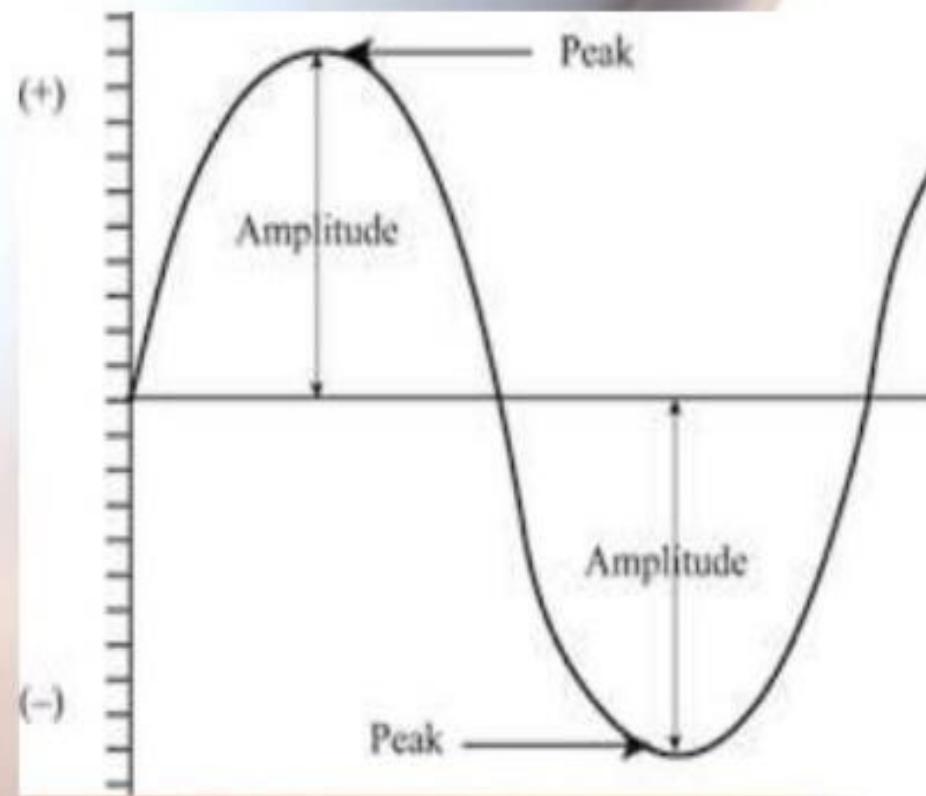


PARTS OF MICROSCOPE



AMPLITUDE

- The maximum extent of a vibration or oscillation measured from the position of equilibrium is called amplitude.



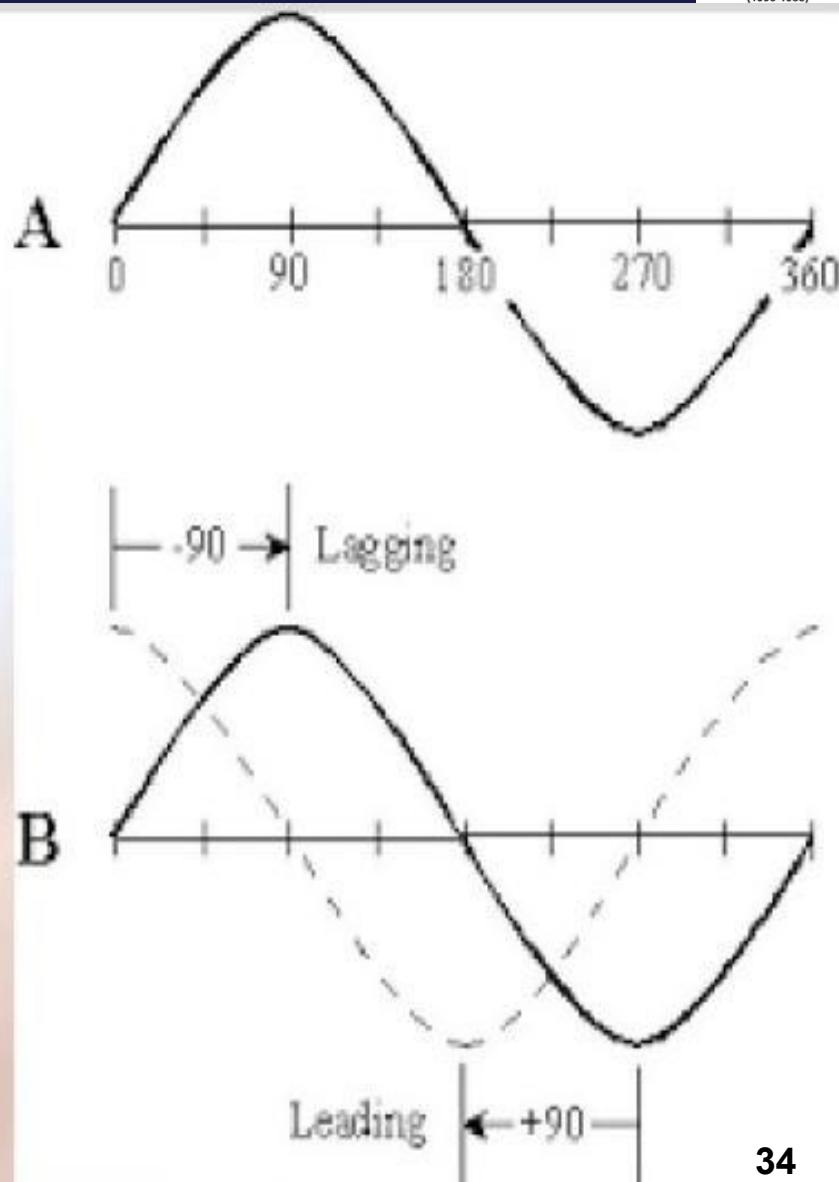
PARTS OF MICROSCOPE



Ernst Ruska
(1906-1988)

PHASE

- It is a position of a point in time on a waveform cycle .



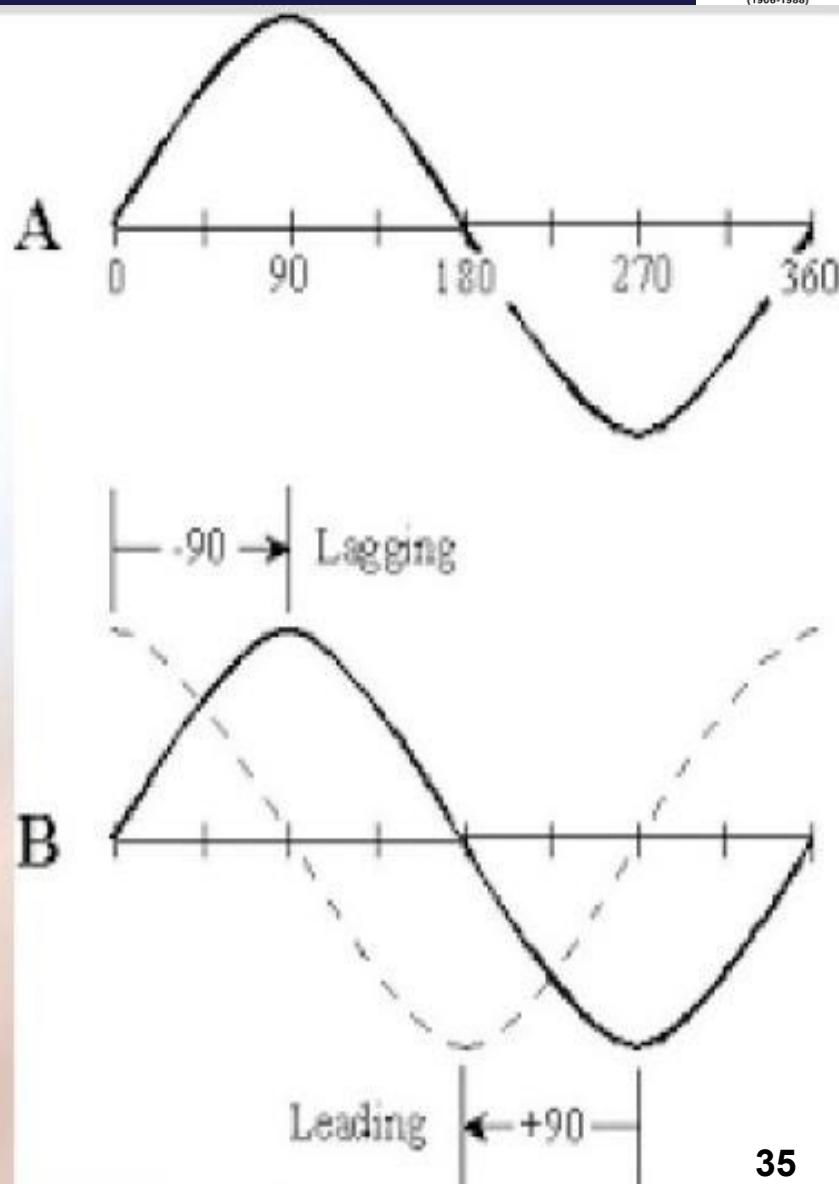
PARTS OF MICROSCOPE

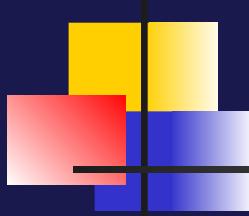


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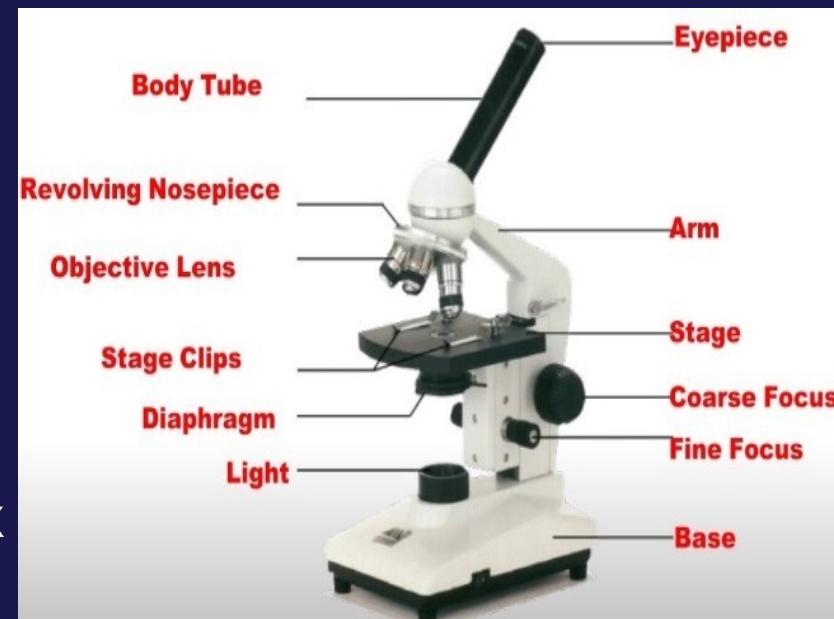


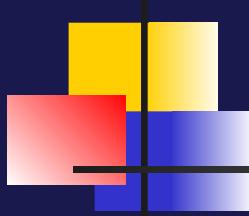
MAGNIFICATION IN MICROSCOPY

MAGNIFICATION

Your microscope has 3 magnifications:

- Scanning,
- Low and
- High.
- Each objective will have written the magnification.
- In addition to this, the ocular lens (eyepiece) has a magnification.
- The total magnification is the ocular x objective

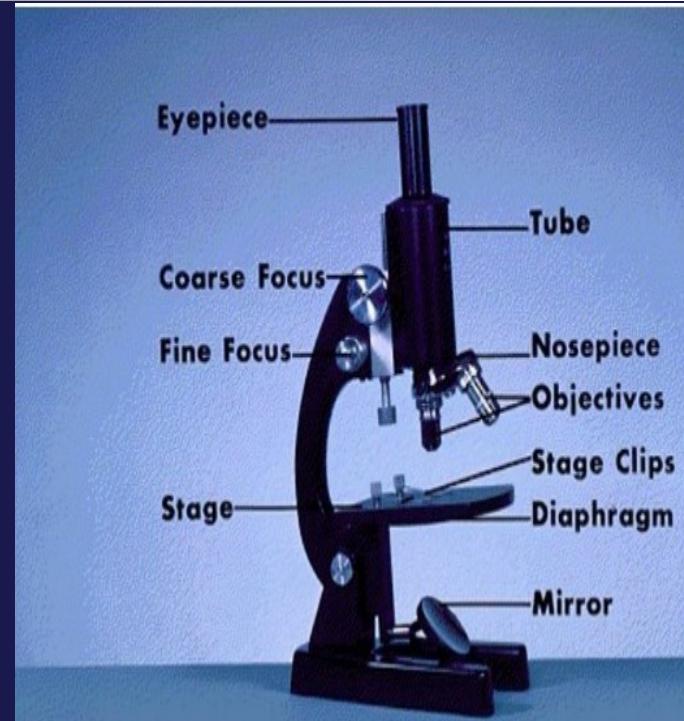


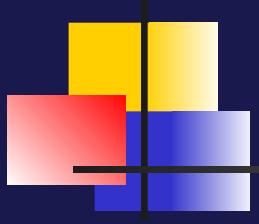


PRINCIPLES OF MICROSCOPY

LIGHT MICROSCOPY:

- ✓ Microscopes are of great importance in the study of microorganisms and biomolecules.
- ✓ Light microscopes are simplest of all microscopes.
- ✓ In light microscopy, light typically passes through a specimen and then through a series of magnifying lenses
- ✓ Light microscopes use lenses to bend and focus light rays to produce enlarged images of small objects.



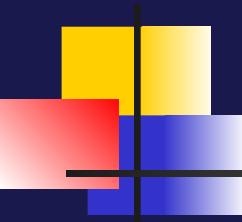


PRINCIPLES OF MICROSCOPY

Workings of Light Microscope (LM):

- ✓ The specimen is mounted on slide and positioned in specimen stage.
- ✓ Beam of light is focused on specimen by condenser.
- ✓ Objective lens picks up light transmitted by specimen and produce first magnified image.
- ✓ This image is further magnified by eyepiece lens.
- ✓ Eyepiece only magnifies image and brings no change in resolution.

TYPES OF MICROSCOPE contd.



APPLICATIONS OF LIGHT MICROSCOPE (LM)

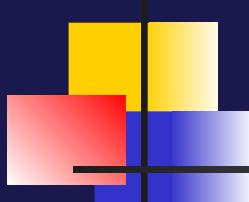
- 1. LM is used to study preserved minute specimen.**

- 2. Used to study activities inside the cell.**

- 3. Used in identifying macromolecules of cell.**

- 4. Used in Medical diagnosis.**

- 5. Used in histopathological studies.**



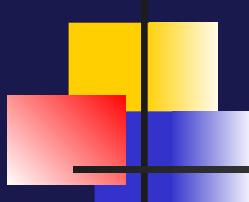
PRINCIPLES OF MICROSCOPY

WORKINGS OF COMPOUND MICROSCOPE:

- ✓ Light is transmitted and focused by mirror and condenser.
- ✓ Focused light illuminate the object or specimen.
- ✓ The refracted light is collected by an objective where primary image of the object is formed, it is real, inverted enlarged image of the object.
- ✓ The eyepiece further magnifies this primary image into virtual, erect enlarged image, this is the final image that lies above the stage.

Magnification

➤ Total magnification of specimen is by multiplying the *objective lens magnification power* by the *ocular lens magnification power*.
Low power X10,
High power X40 and Oil Immersion X100

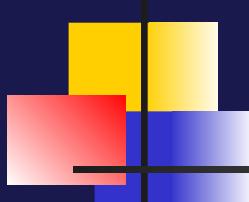


PRINCIPLES OF MICROSCOPY

WORKINGS OF COMPOUND MICROSCOPE contd:

Resolution:

- ✓ Is also called resolving power of image.
- ✓ This means, the ability to distinguish that two objects are separate and not one.
- ✓ Resolving power of a microscope is determined by the wave length of light entering the objective lens.
- ✓ A general principle of microscopy is that the shorter the wave length of light used in the instrument, the greater the resolution.

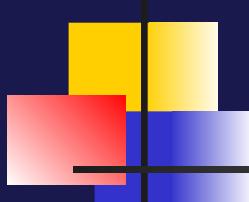


PRINCIPLES OF MICROSCOPY

WORKINGS OF COMPOUND MICROSCOPE contd:

Oil Immersion:

- ✓ The white light used in a compound light microscope has relatively long wave length and cannot resolve structures smaller than about 0.2 μm .
- ✓ Immersion oil is placed between the glass and objective lens. ↗ The immersion oil has the same refractive index as glass of the microscope.
- ✓ The oil enhances the resolution by preventing light rays from dispersing and changing wave length after passing through the specimens.

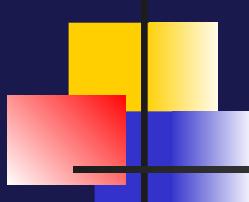


PRINCIPLES OF MICROSCOPY

WORKINGS OF COMPOUND MICROSCOPE contd:

Applications:

- ✓ Observation of morphology of microorganisms.
- ✓ Detection of cell structures.
- ✓ Observation of intracellular structures.
- ✓ Observation of motility.
- ✓ Measurement of size.
- ✓ Observation of blood smears.

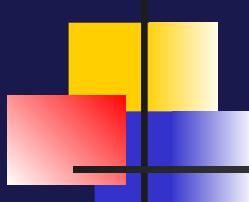


PRINCIPLES OF MICROSCOPY

WORKINGS OF BRIGHT-FIELD MICROSCOPE:

A. Bright-Field Microscopy:

- ✓ The ordinary microscope is also referred to as Bright-Field microscope.
- ✓ It forms dark image against bright background.
- ✓ The useful magnification of Light microscope is limited by its resolving power.
- ✓ The resolving power is limited by wavelength of illuminating beam.
- ✓ Resolution is determined by certain physical parameters like wavelength of light and light generating power of the objective & condenser lens.



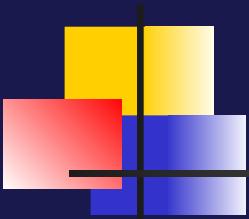
PRINCIPLES OF MICROSCOPY

WORKINGS OF BRIGHT-FIELD MICROSCOPE:

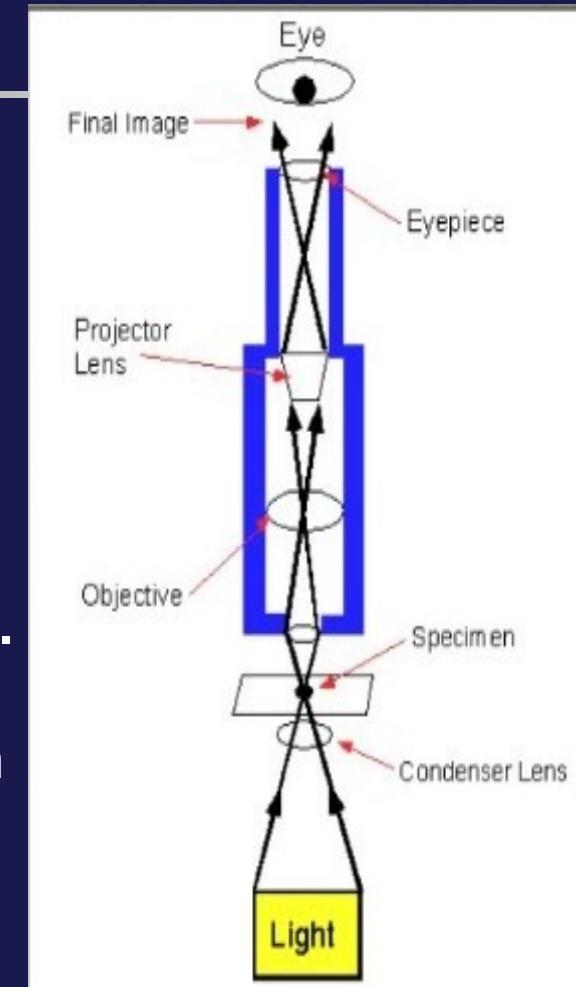
- ✓ In bright field, microscope field appears bright whereas microorganism appears dark as they absorb light.
- ✓ Normally micro-organism do not absorb light but absorbing ability increases due to staining.

PRINCIPLES OF MICROSCOPY

HOW IMAGE IS FORMED IN BRIGHT-FIELD MICROSCOPE:



- **Image is created by objective and ocular lenses working together.**
- **Light from illuminated specimen is focused by the objective lens creating enlarged image within the microscope.**
- **The ocular lens further modifies the primary image.**
- **Total magnification is calculated by magnification by objective multiply by magnification by eyepiece.**
E.g. : $45x \times 10x = 450x$

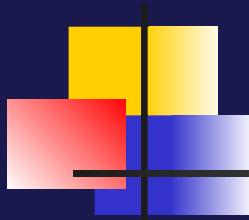


PRINCIPLES OF MICROSCOPY

IMAGE FORMED IN BRIGHT-FIELD MICROSCOPE:



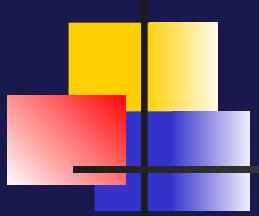
PRINCIPLES OF MICROSCOPY



ADVANTAGES OF BRIGHT-FIELD MICROSCOPE:

- ✓ Bright field compound microscopes are commonly used to view live and immobile specimens such as bacteria, cells, and tissues.
- ✓ For transparent or colorless specimens, however, it is important that they be stained first so that they can be properly viewed under this type of a microscope.
- ✓ Staining is achieved with the use of a chemical dye. By applying it, the specimen would be able to adapt the color of the dye. Therefore, the light won't simply pass through the body of the specimen showing nothing on the microscope's view field

PRINCIPLES OF MICROSCOPY

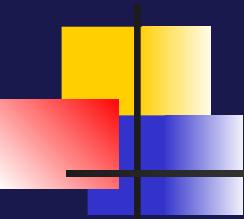


WORKINGS OF DARK-FIELD MICROSCOPE:

B. Dark-Field Microscopy:

- ✓ Dark field microscopy is frequently performed on the same microscope on which bright-field microscopy is performed.
- ✓ Instead of the normal condenser that contains an opaque disk.
- ✓ The disk blocks light that would enter the objective lens directly.
- ✓ Only light that has been reflected or refracted by the specimen forms the image.
- ✓ The field surrounding specimen appears dark while the object brightly illuminated

PRINCIPLES OF MICROSCOPY



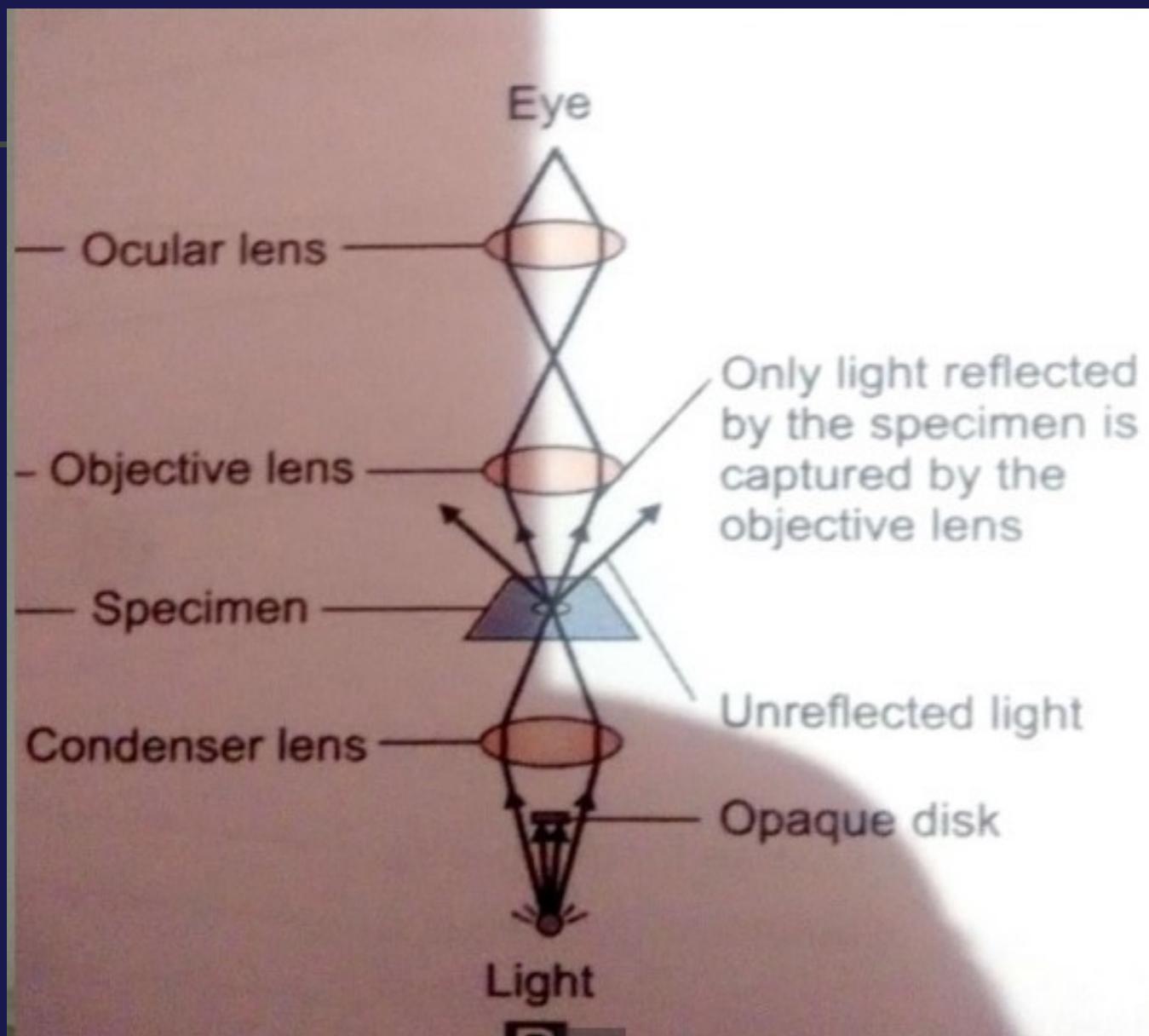
WORKINGS OF DARK-FIELD MICROSCOPE:

- ✓ In dark field microscope, a dark background is established against a brightly illuminated object.

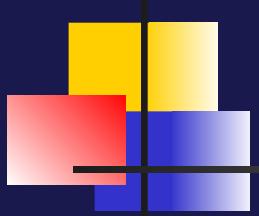
- ✓ Dark field microscopy requires additional dark field condenser and dark field object lens.

PRINCIPLES OF MICROSCOPY

HOW IMAGE IS FORMED IN DARK-FIELD MICROSCOPE:



PRINCIPLES OF MICROSCOPY

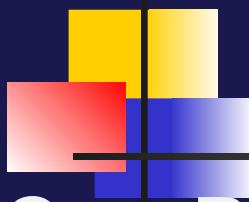


ADVANTAGES OF DARK-FIELD MICROSCOPE:

- ✓ The advantage of darkfield microscopy also becomes its disadvantage: not only the specimen, but dust and other particles scatter the light and are easily observed.
- ✓ For example, not only the cheek cells but the bacteria in saliva are evident.
- ✓ The dark field microscopes divert illumination and light rays thus, making the details of the specimen appear luminous.
- ✓ Dark field light microscopes provide good results, especially through the examination of live blood samples.
- ✓ It can yield high magnifications of living bacteria and low magnifications of the tissues and cells of certain organisms.
- ✓ Certain bacteria and fungi can be studied with the use of dark field microscopes.

PRINCIPLES OF MICROSCOPY

WORKINGS OF PHASE CONTRAST MICROSCOPE:

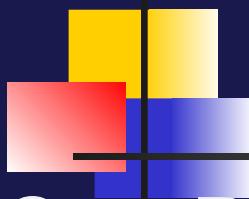


C. PHASE CONTRAST Microscopy:

- ✓ Phase contrast microscope was developed by Fritz Zernike, and was awarded Nobel prize in Physics in 1953.
- ✓ By this, microscopy organism can be seen alive, without staining.
- ✓ It requires additional specialized structure annular diaphragm and phase contrast ring.
- ✓ The images differentiates in refractive index of cellular structure.
- ✓ Light passes through thicker parts of cell is held up relative to the light that passes through thinner parts of cytoplasm.

PRINCIPLES OF MICROSCOPY

WORKINGS OF PHASE CONTRAST MICROSCOPE:

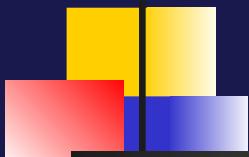


C. PHASE CONTRAST Microscopy:

- ✓ The principle of phase-contrast microscopy is based on the wave nature of light rays and the fact that light rays can be in phase or out of phase.
- ✓ In a phase-contrast microscope, one set of light rays comes directly from the light sources.
- ✓ The other set comes from light that is reflected or diffracted from a particular structure in the specimen (diffraction is the scattering of light rays-direct rays and reflected or diffracted rays are brought together) together).

PRINCIPLES OF MICROSCOPY

WORKINGS OF PHASE CONTRAST MICROSCOPE:

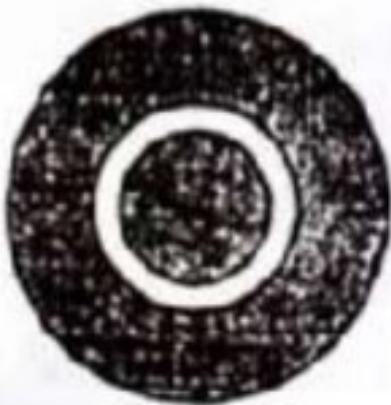
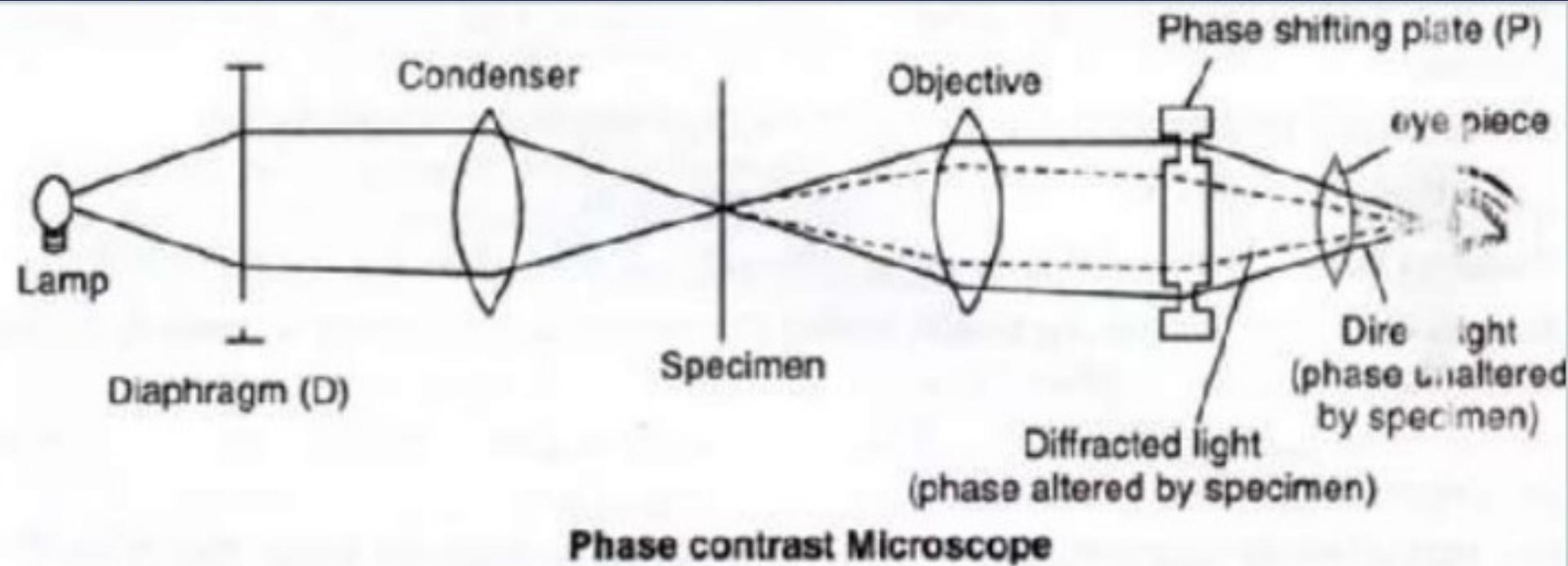


PHASE CONTRAST Microscopy contd.:

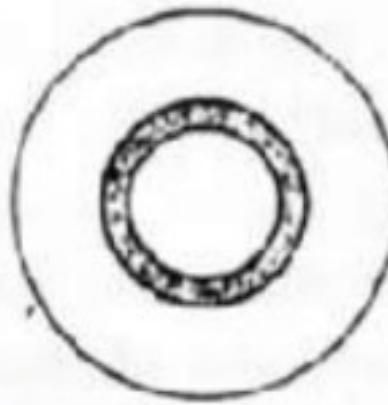
- ✓ Both combined rays form an image of the specimen on the ocular lens, containing areas that are relatively light (in-phase), through shades of gray, to black(out phase).
- ✓ Through the use of annular lens, the differences in phase are amplified so that in-phase light appears brighter than out-of-phase.
- ✓ It is used to study the behaviour of living cells and;
- ✓ To observe nuclear and cytoplasmic changes.

PRINCIPLES OF MICROSCOPY

HOW IMAGE IS FORMED IN PHASE CONTRAST MICROSCOPE:



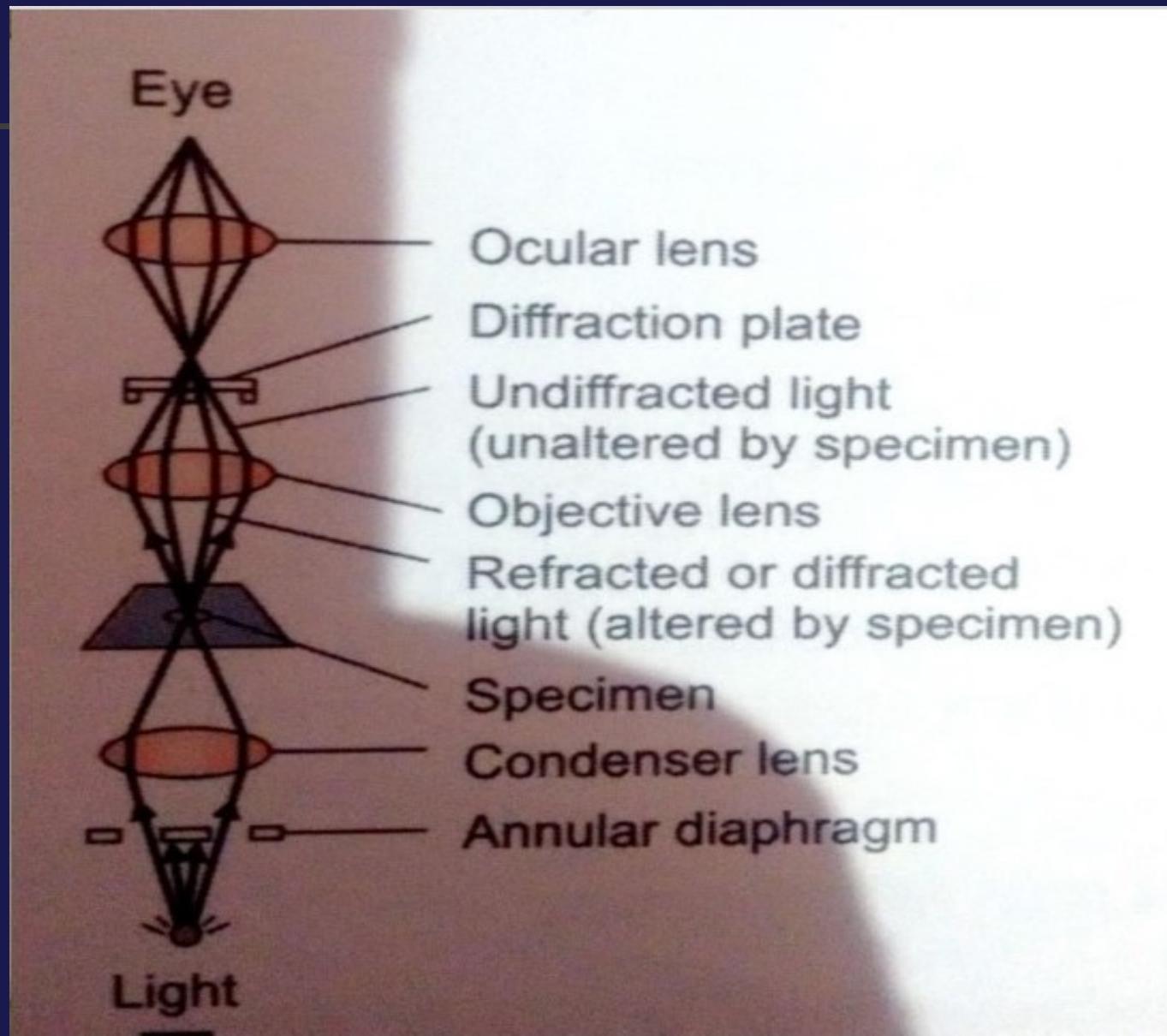
Diaphragm (D)

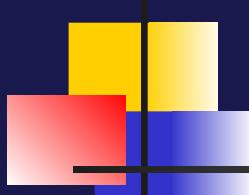


Phase shifting plate

PRINCIPLES OF MICROSCOPY

HOW IMAGE IS FORMED IN PHASE CONTRAST MICROSCOPE:



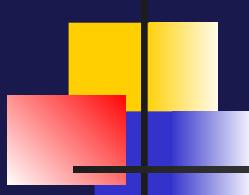


PRINCIPLES OF MICROSCOPY

WORKINGS OF PHASE CONTRAST MICROSCOPE:

Applications:

- ✓ Observation of morphology of microorganisms.
- ✓ Detection of cell structures.
- ✓ Observation of intracellular structures.
- ✓ Observation of motility.
- ✓ Measurement of size.
- ✓ Observation of blood smears.
- ✓ To study unstained living cells.
- ✓ Detailed examination of internal structures In living microorganism living.



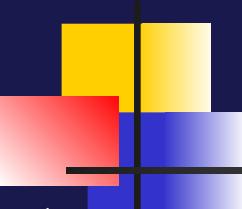
PRINCIPLES OF MICROSCOPY

WORKINGS OF PHASE CONTRAST MICROSCOPE:

Applications contd.:

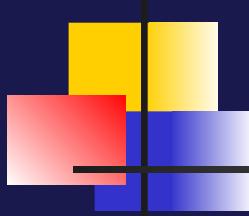
- ✓ To study flagellar movements and motility of bacteria and protozoans. bacteria and protozoans.
- ✓ To study intestinal and other living protozoa such as amoeba and trichomonas.
- ✓ To examine fungi grown in culture
- ✓ Phase contrast microscopy is used in study of living cells and tissues.
- ✓ Microbes and parasites can also be studied.
- ✓ Useful in observing cells cultured in vitro during mitosis.

PRINCIPLES OF MICROSCOPY



FLUORESCENCE MICROSCOPE

- ✓ Popularly used to achieve high labeling of cellular compartments.
- ✓ This microscope additionally requires an excitation filter, a barrier and a dichromatic mirror, fluorescent stain.
- ✓ A specific wavelength of light is used to excite fluorescent molecule in specimen.
- ✓ Light of higher wavelength is then imaged.

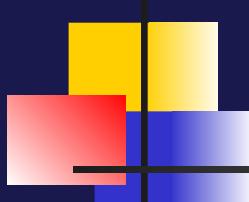


PRINCIPLES OF MICROSCOPY

FLUORESCENCE MICROSCOPE

WHY FLUORESCENCE MICROSCOPE?

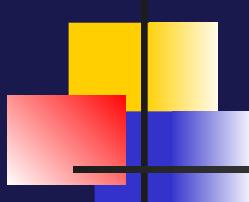
- ✓ In all types of microscopes, cell constituents are not distinguishable, although staining does , but not totally.
- ✓ In fluorescent microscopy, various fluorescent dyes are used which gives property of fluorescence to only specific part of the cell and hence it can be focused..



PRINCIPLES OF MICROSCOPY

THE WORKINGS OF FLUORESCENCE MICROSCOPE

- ✓ When certain compounds are illuminated with high energy light, they then emit light of a different, lower frequency.
- ✓ This effect is known as fluorescence.
- ✓ Often specimens show their own characteristic auto-fluorescence image, based on their chemical makeup.



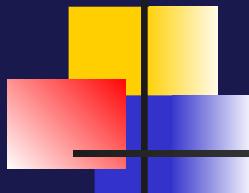
PRINCIPLES OF MICROSCOPY

THE WORKINGS OF FLUORESCENCE MICROSCOPE

- ✓ Many different fluorescent dyes can be used to stain different structures or chemical compounds.
- ✓ One particularly powerful method is the combination of antibodies coupled to a fluorochrome as in immunostaining.
- ✓ Examples of commonly used fluorochromes are fluorescein or rhodamine.

PRINCIPLES OF MICROSCOPY

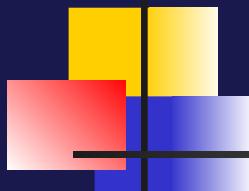
THE WORKINGS OF FLUORESCENCE MICROSCOPE



1. A component of interest in the specimen is specifically labeled with a fluorescent molecule called a fluorophore called a fluorophore
2. The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit longer wavelengths of light (of a different color than the wavelengths of light absorbed).
3. Typical components of a fluorescence microscope are the light source (xenon arc lamp or mercury vapor lamp), the excitation filter, the dichroic vapor lamp), the excitation filter, the dichroic mirror and the emission filter.

PRINCIPLES OF MICROSCOPY

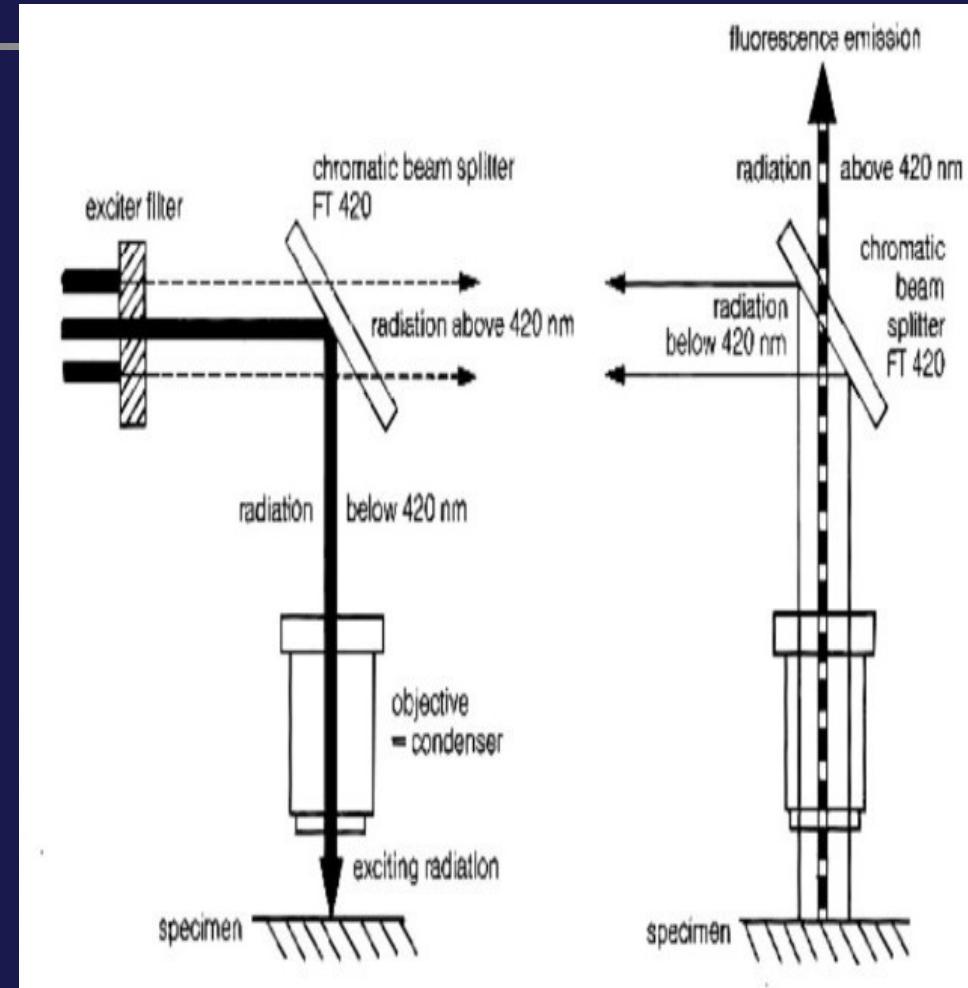
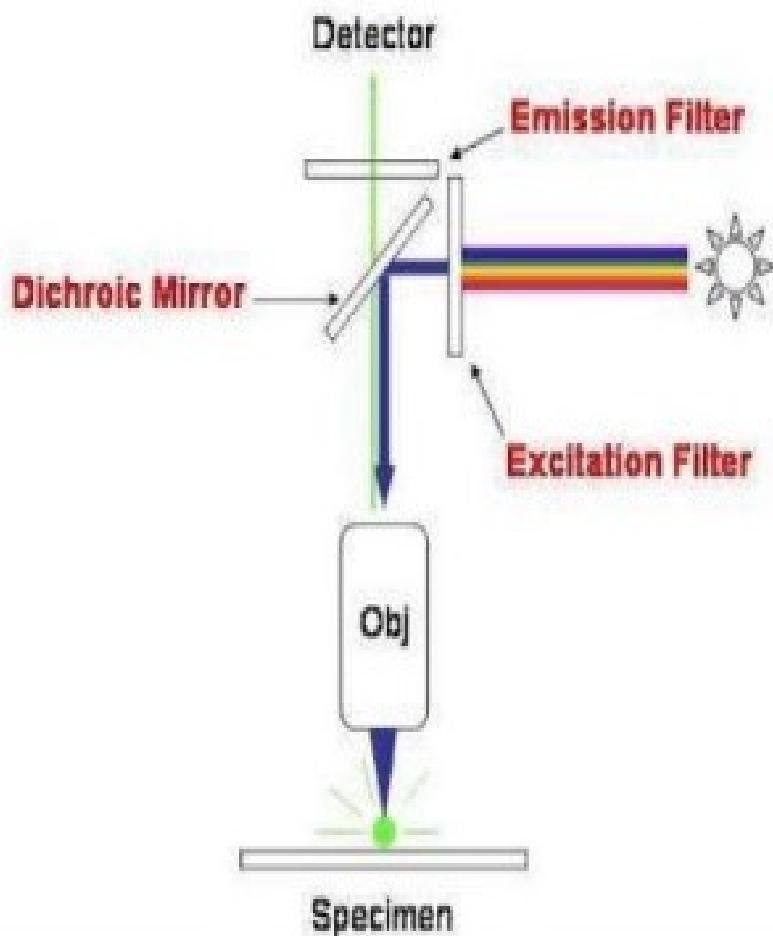
THE WORKINGS OF FLUORESCENCE MICROSCOPE



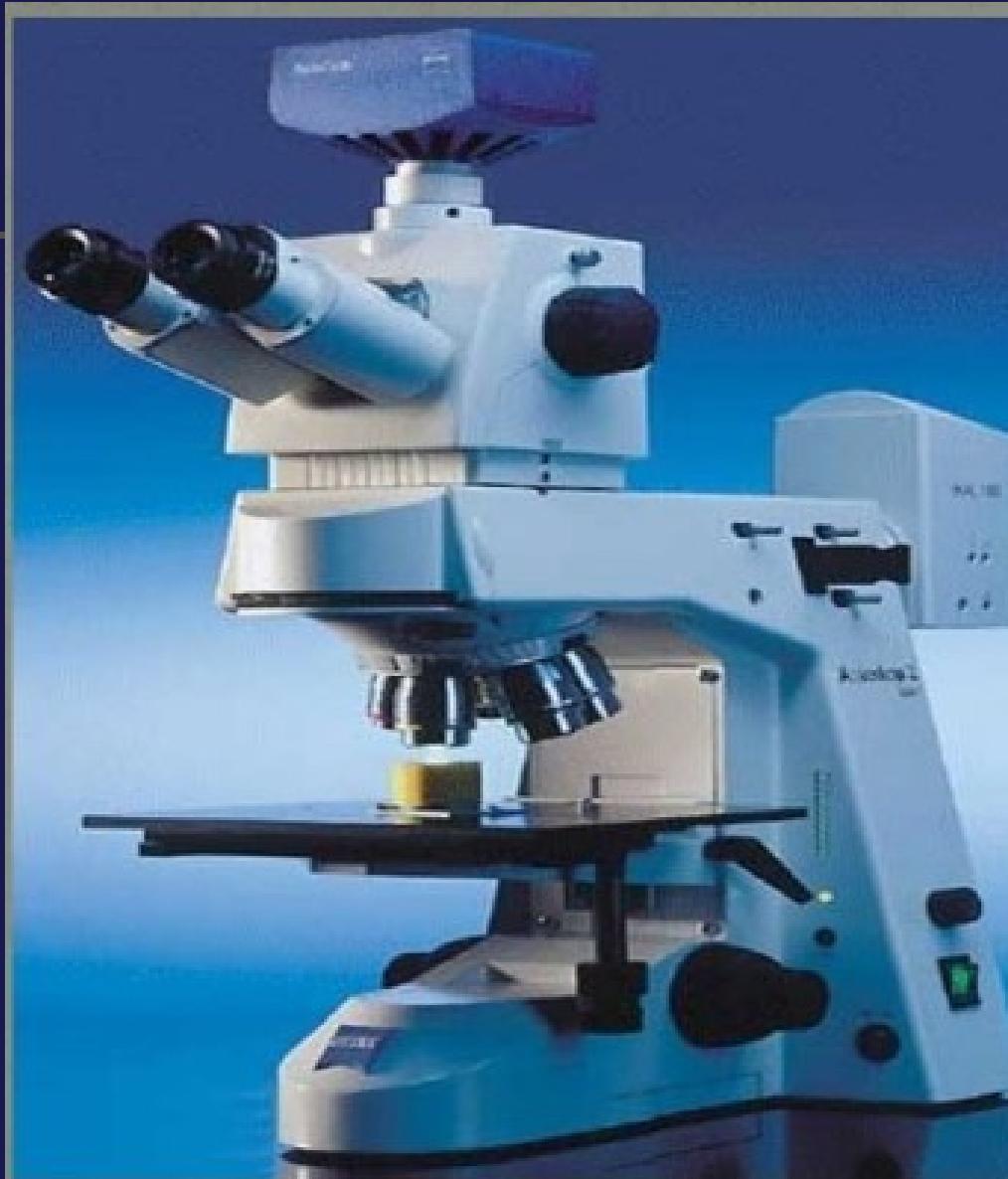
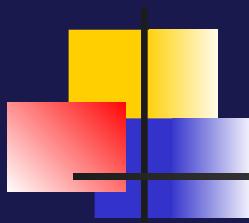
4. The illumination light is separated from the much weaker emitted fluorescence through the use of an emission filter.
5. The filters and the dichroic are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen.
6. In this manner, a single fluorophore (color) is imaged at a time. Multi-color images of several fluorophores must be composed by combining several single-color images.

PRINCIPLES OF MICROSCOPY

THE WORKINGS OF FLUORESCENCE MICROSCOPE



PRINCIPLES OF MICROSCOPY



FLUORESCENCE MICROSCOPE

PRINCIPLES OF MICROSCOPY

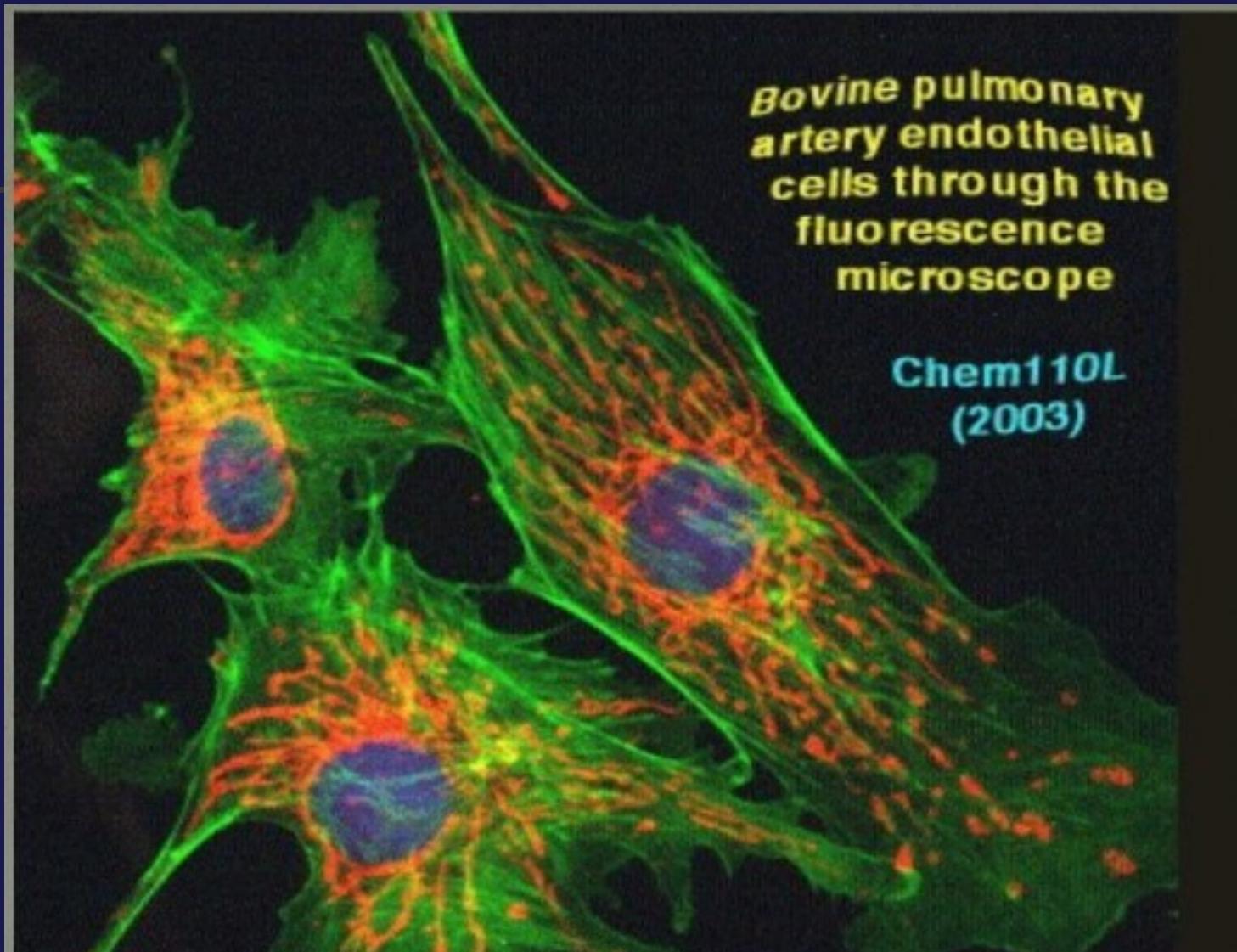
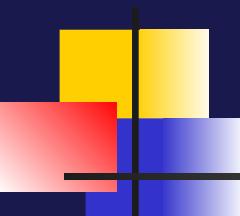
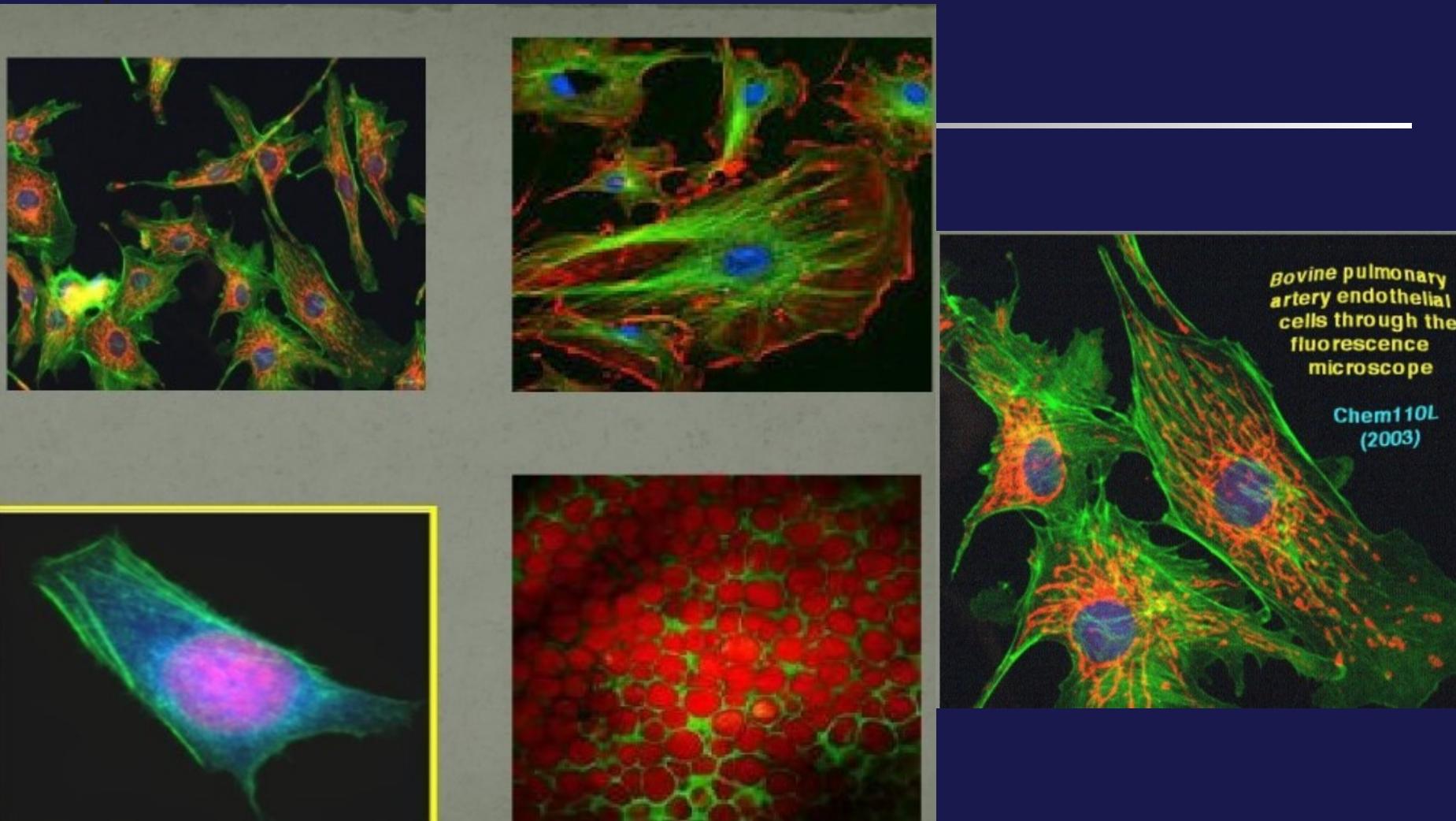
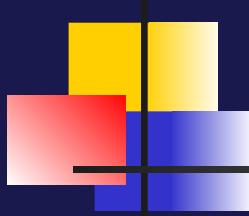


IMAGE AS SEEN BY FLUORESCENCE MICROSCOPE

PRINCIPLES OF MICROSCOPY



IMAGES AS SEEN BY FLUORESCENCE MICROSCOPE



PRINCIPLES OF MICROSCOPY

APPLICATIONS OF FLUORESCENCE MICROSCOPE

Fluorescence microscopy is a critical tool for:

- Academic Research
- Pharmaceutical Research,
- Pathology,
- Clinical Medicine.

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

INTRODUCTION

- ✓ German scientists Knoll and Ruska discovered electron microscope.
- ✓ Electron microscopy (EM) is in some ways comparable to light microscopy.
- ✓ Rather than using glass lenses, visible light, and the eye to observe the specimen, the EM uses electromagnetic lenses, electrons, and a fluorescent screen to produce the magnified image.
- ✓ Electron beam is used as source of illumination.
- ✓ That image can be captured on photographic film to create an electron photo-micrograph.

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

INTRODUCTION CONTD.

- ✓ The superior resolution of the EM is due to the fact that electrons have a much shorter wavelength than the photons of white light.
- ✓ The electron beam is focused by circular electron magnets, which are analogous to the lenses in the light microscope.
- ✓ The object which is held in the path of the beam scatters the electrons and produces an image which is focused on a fluorescent viewing screen.

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

INTRODUCTION CONTD.

- ✓ The wavelength of electrons used in EM is 0.005nm as compared to 500nm with visible light i.e. about 100,000 times shorter than that of ordinary light.
- ✓ Theoretically, if conditions were identical in the optical and EMs, the resolving power of the EM should be 100,000 times (resolution down to 0.0025).
- ✓ However, the numerical aperture of an EM lens is very small and does not approach the width of the objective lens of an optical microscope.

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

INTRODUCTION CONTD.

- ✓ The electron beam is focused by circular electron magnets, which are analogous to the lenses in the light microscope.
- ✓ The object which is held in the path of the beam scatters the electrons and produces an image which is focused on a fluorescent viewing screen.

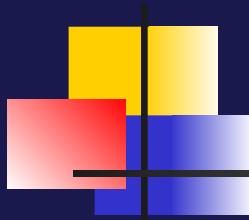
PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

TYPES OF ELECTRON MICROSCOPE

1. Transmission Electron Microscope
2. Scanning Electron Microscope

PRINCIPLES OF MICROSCOPY



ELECTRON MICROSCOPE (EM)

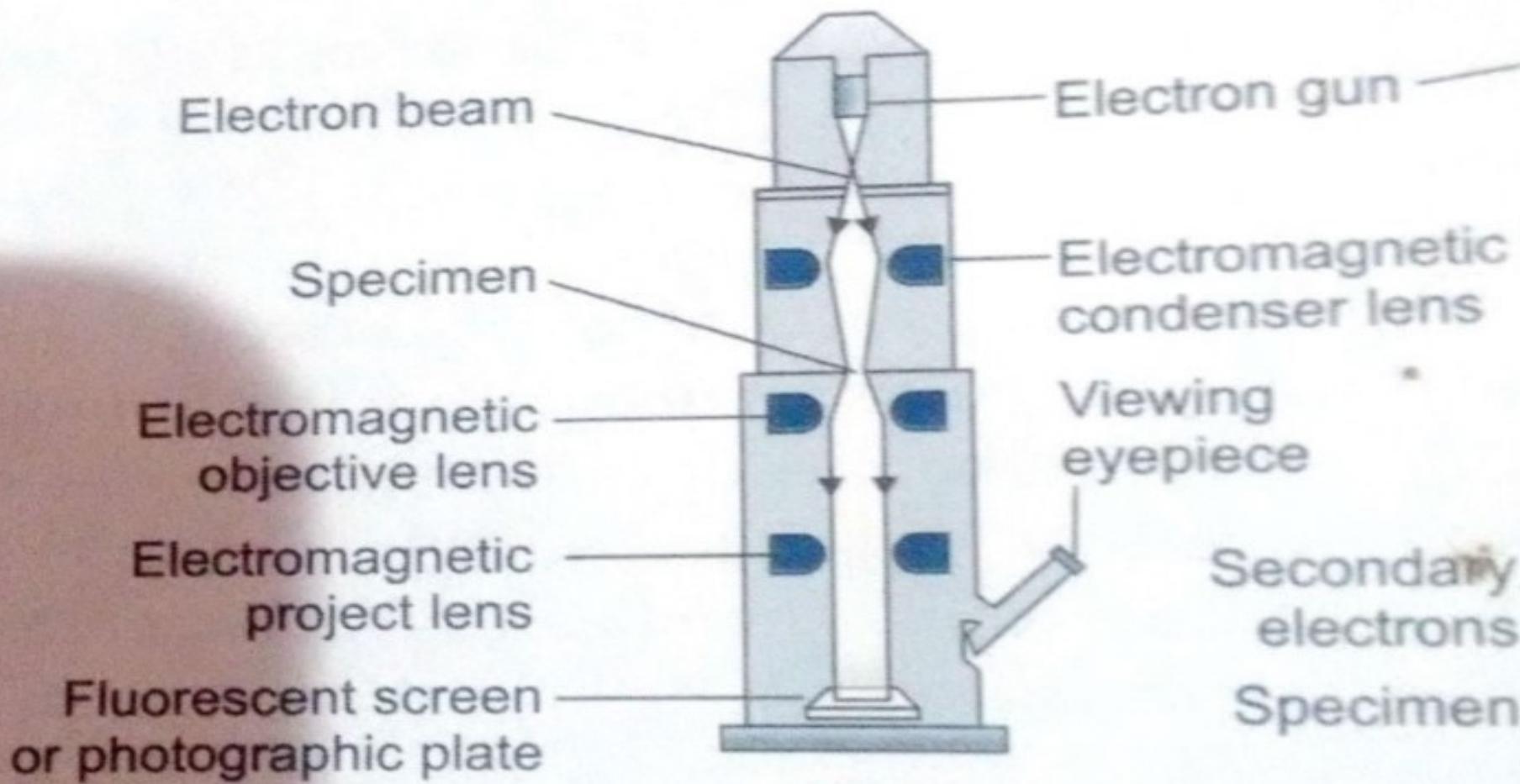
TYPES OF ELECTRON MICROSCOPE

1. TRANSMISSION Electron Microscope (TEM)

In a Transmission e- microscope,

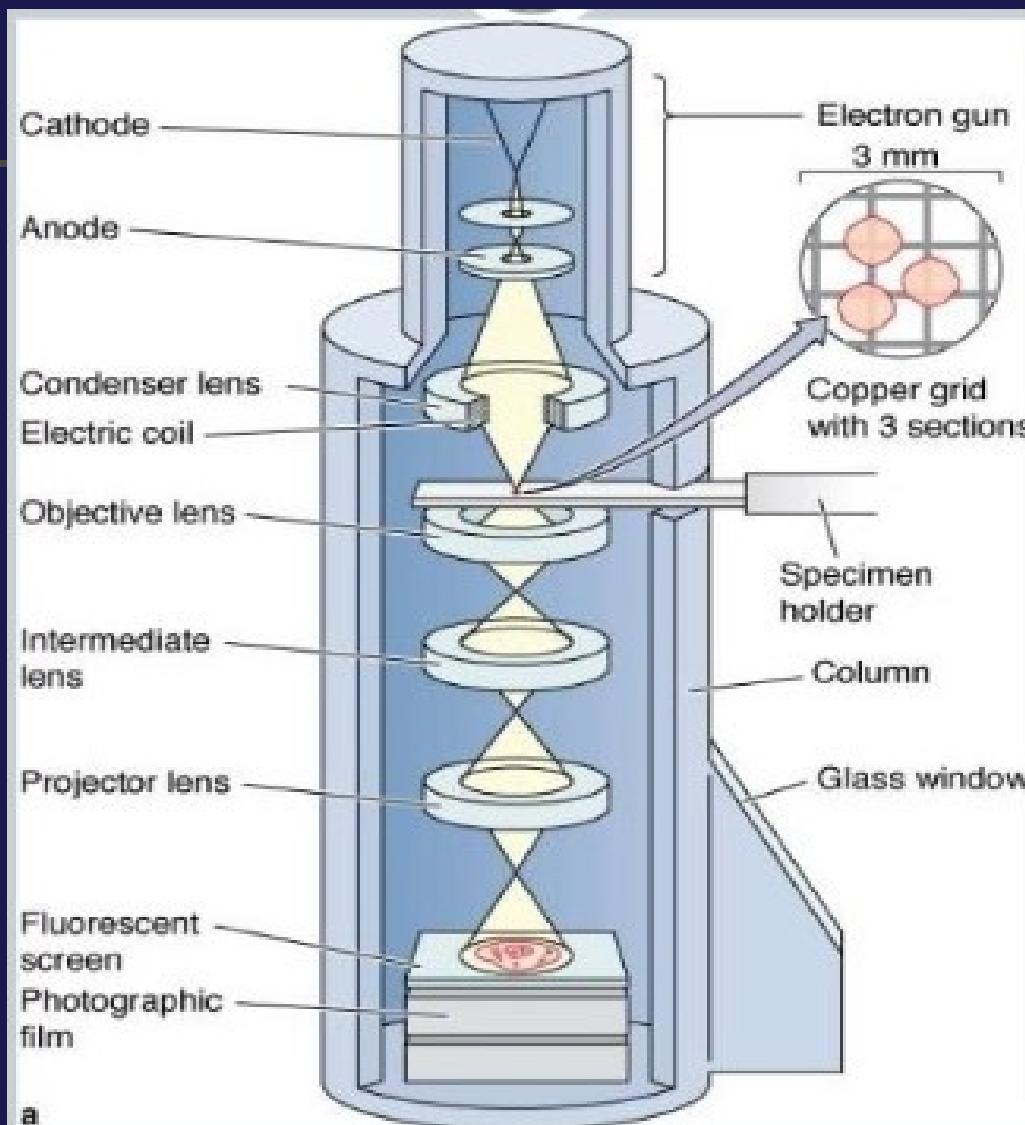
- Electrons pass through the specimen and scattered.
- Magnetic lenses focus the image onto a fluorescent screen or photographic plate.
- Electron light pass directly through the specimen that has been prepared by thin sectioning, freeze fracturing, or freeze etching.
- TEM is used to observe internal and ultra-structures (fine details) of cell structure.
- TEM Magnification is over 20million times.

PRINCIPLES OF MICROSCOPY



TRANSMISSION ELECTRON MICROSCOPE (TEM)

PRINCIPLES OF MICROSCOPY



TRANSMISSION ELECTRON MICROSCOPE (TEM)

PRINCIPLES OF MICROSCOPY



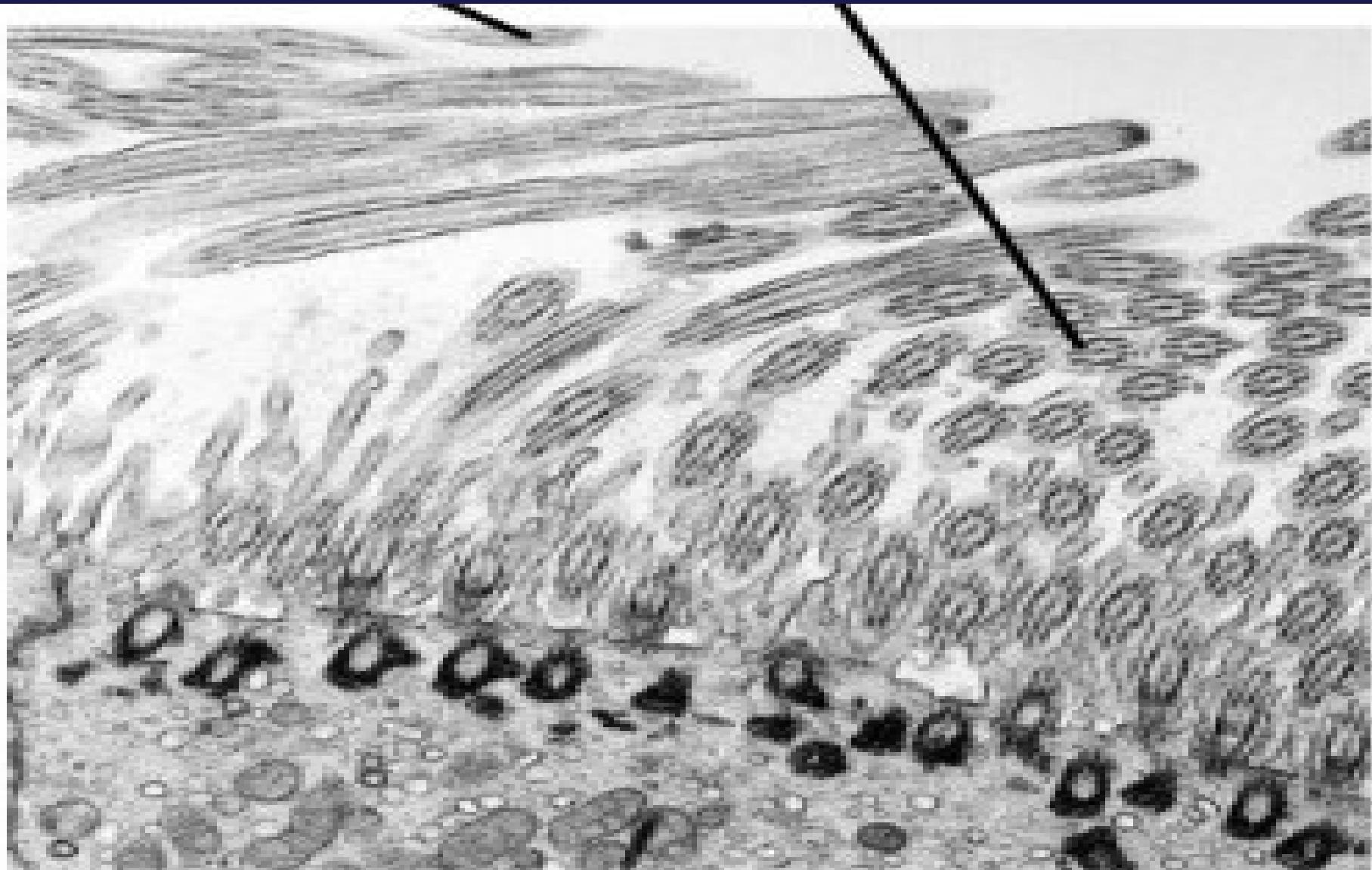
TRANSMISSION ELECTRON MICROSCOPE (TEM)

PRINCIPLES OF MICROSCOPY



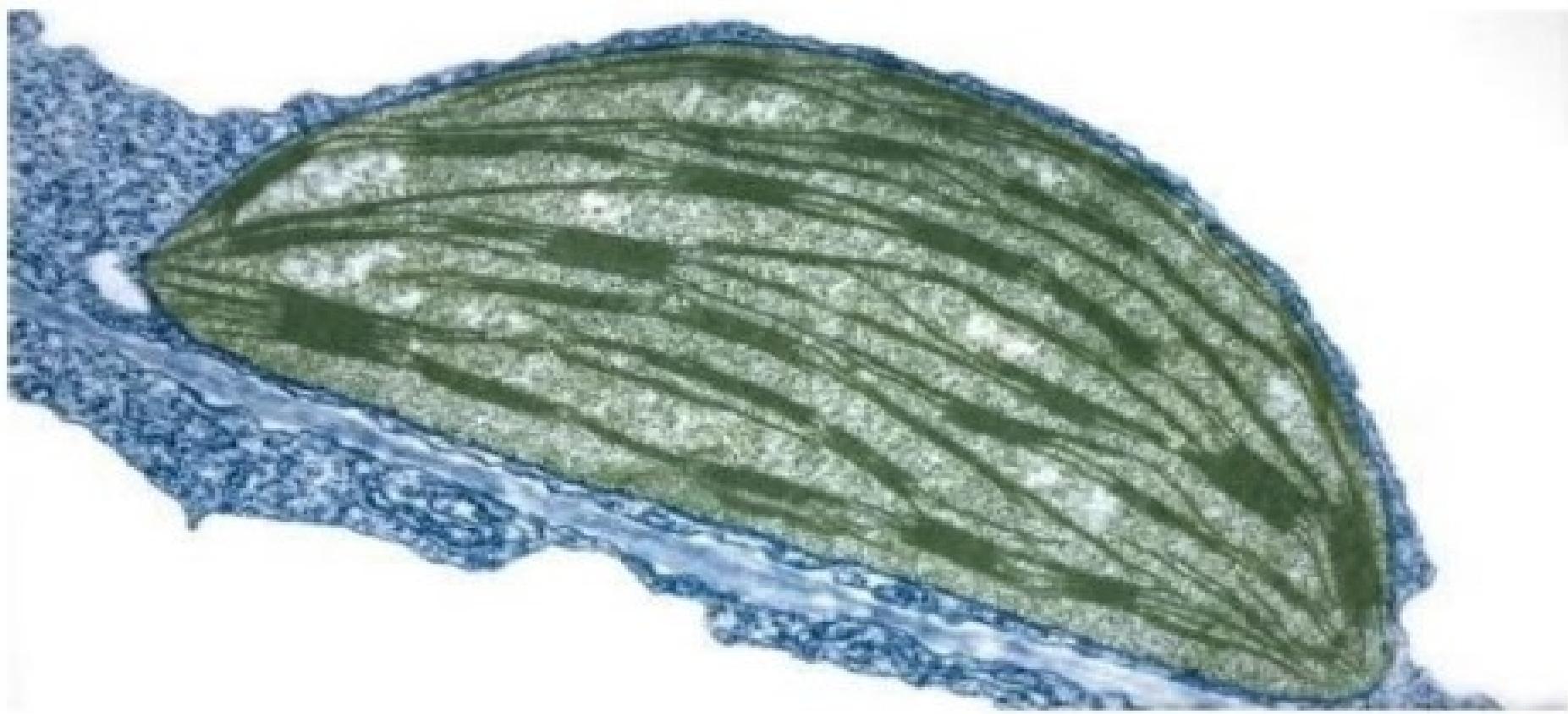
IMAGES AS SEEN USING TEM

PRINCIPLES OF MICROSCOPY



Cilia on Rabbit Lungs AS SEEN BY TEM MICROSCOPE

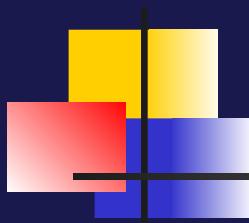
PRINCIPLES OF MICROSCOPY



Chloroplast from a tobacco leaf

Chloroplast from a Tobacco Leaf AS SEEN BY TEM

PRINCIPLES OF MICROSCOPY



H1N1 virus

H1N1 Virus AS SEEN BY TEM

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

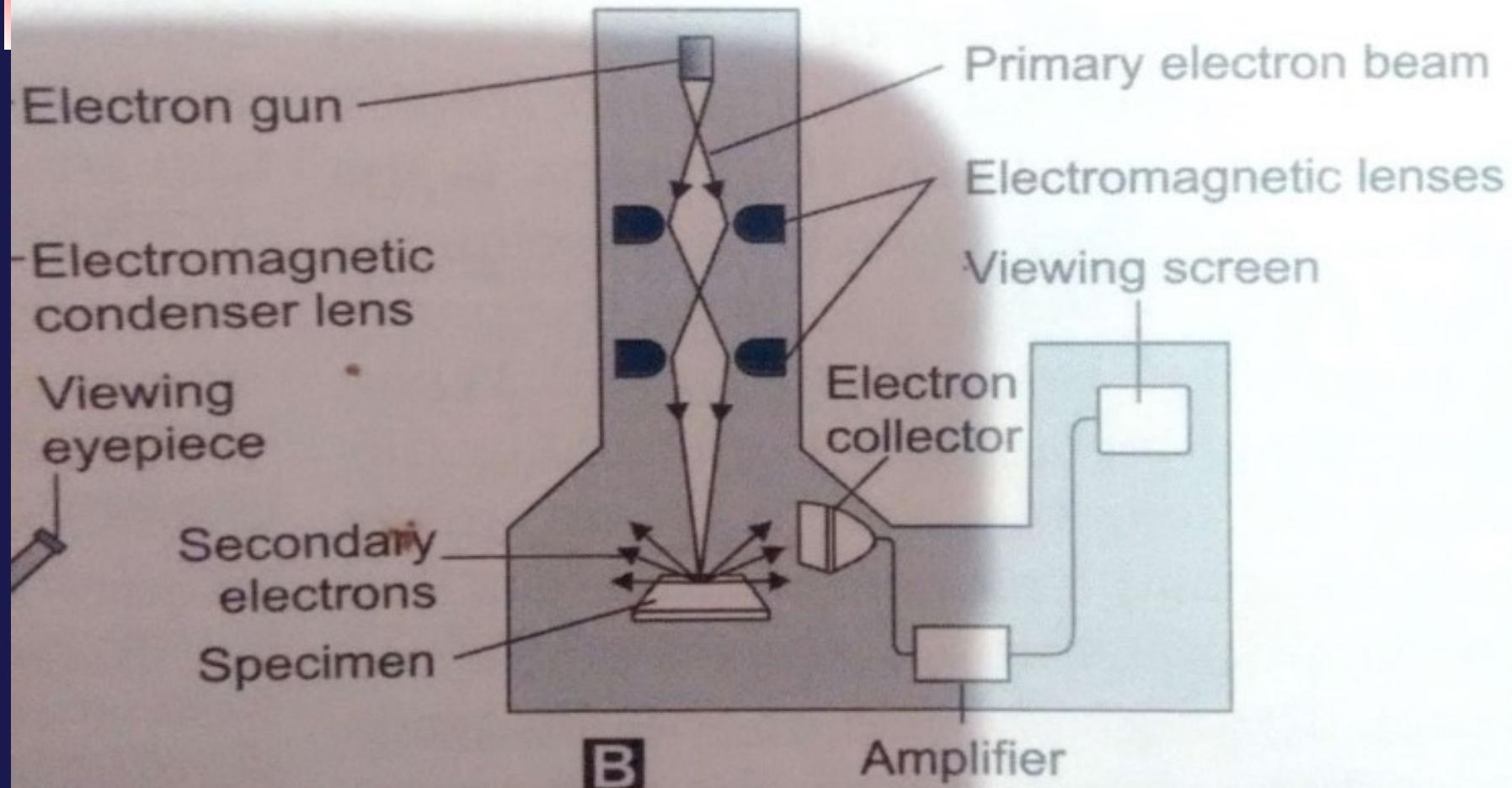
TYPES OF ELECTRON MICROSCOPE

2. SCANNING Electron Microscope (SEM)

In a Scanning e- microscope,

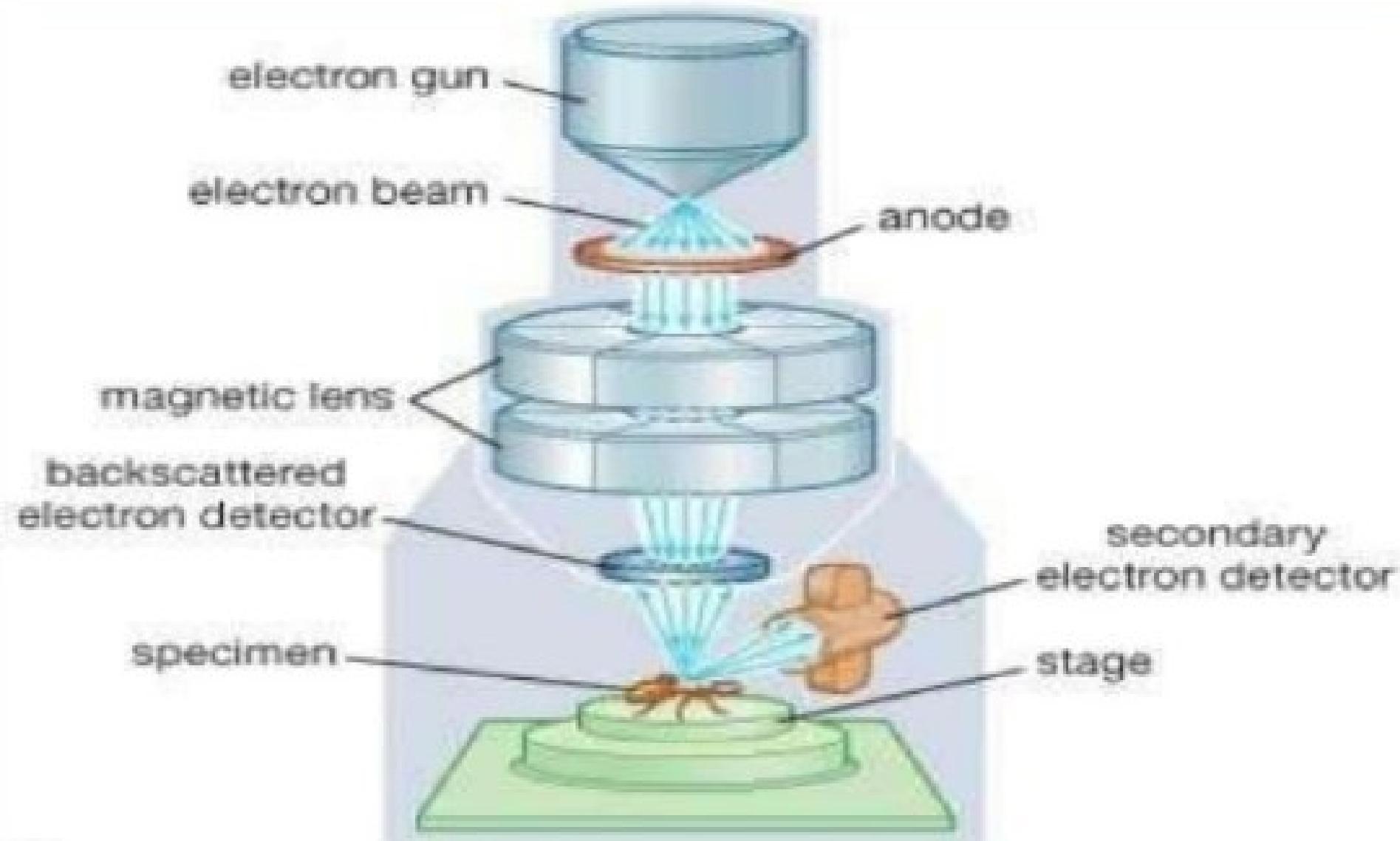
- Primary electron sweep across the specimen and knock electrons from its surface.
- The second electron is picked up by a collector, amplified, and transmitted onto viewing screen or photographic plate.
- It scan a beam of electrons back and forth over the surface of a specimen producing three dimensional views of the surfaces of whole microorganism.
- SEM is use to stufy the surface of cell(s) and organisms.
- SEM Magnification is 20,000 times.

PRINCIPLES OF MICROSCOPY



SCANNING ELECTRON MICROSCOPE (TEM)

PRINCIPLES OF MICROSCOPY



SCANNING ELECTRON MICROSCOPE (TEM)

PRINCIPLES OF MICROSCOPY



SCANNING ELECTRON MICROSCOPE (SEM)

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

WORKINGS OF ELECTRON MICROSCOPE

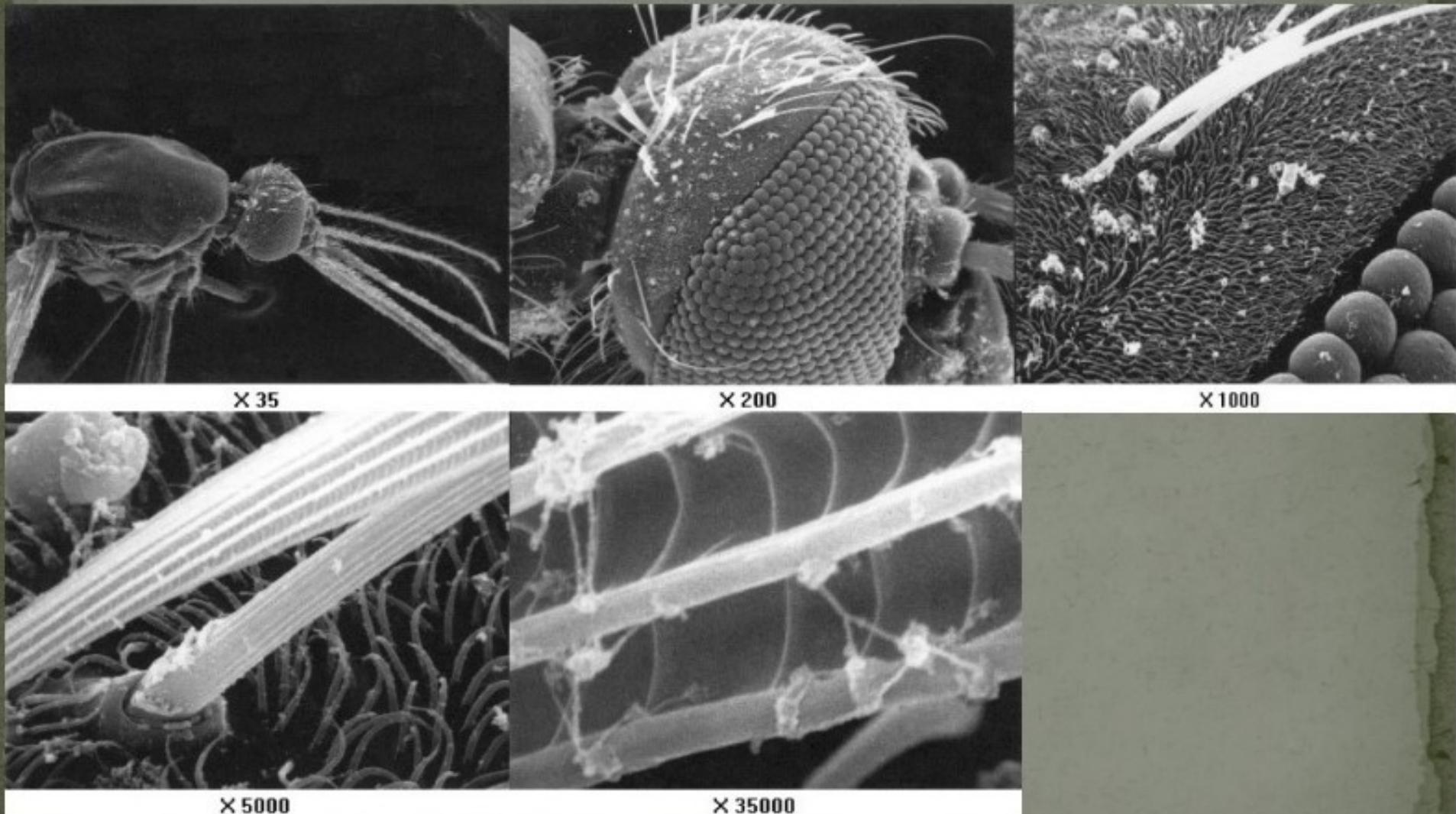
- ✓ Beam of electron travel through column of microscope in vacuum.
- ✓ Different electromagnetic lens focuses electrons into thin beam.
- ✓ Beam travel through specimen. Some electrons may get scattered while others transmitted (TEM), hit fluorescent screen(detector) and forms image.
- ✓ In SEM, reflected electron from specimen forms magnified image.

PRINCIPLES OF MICROSCOPY



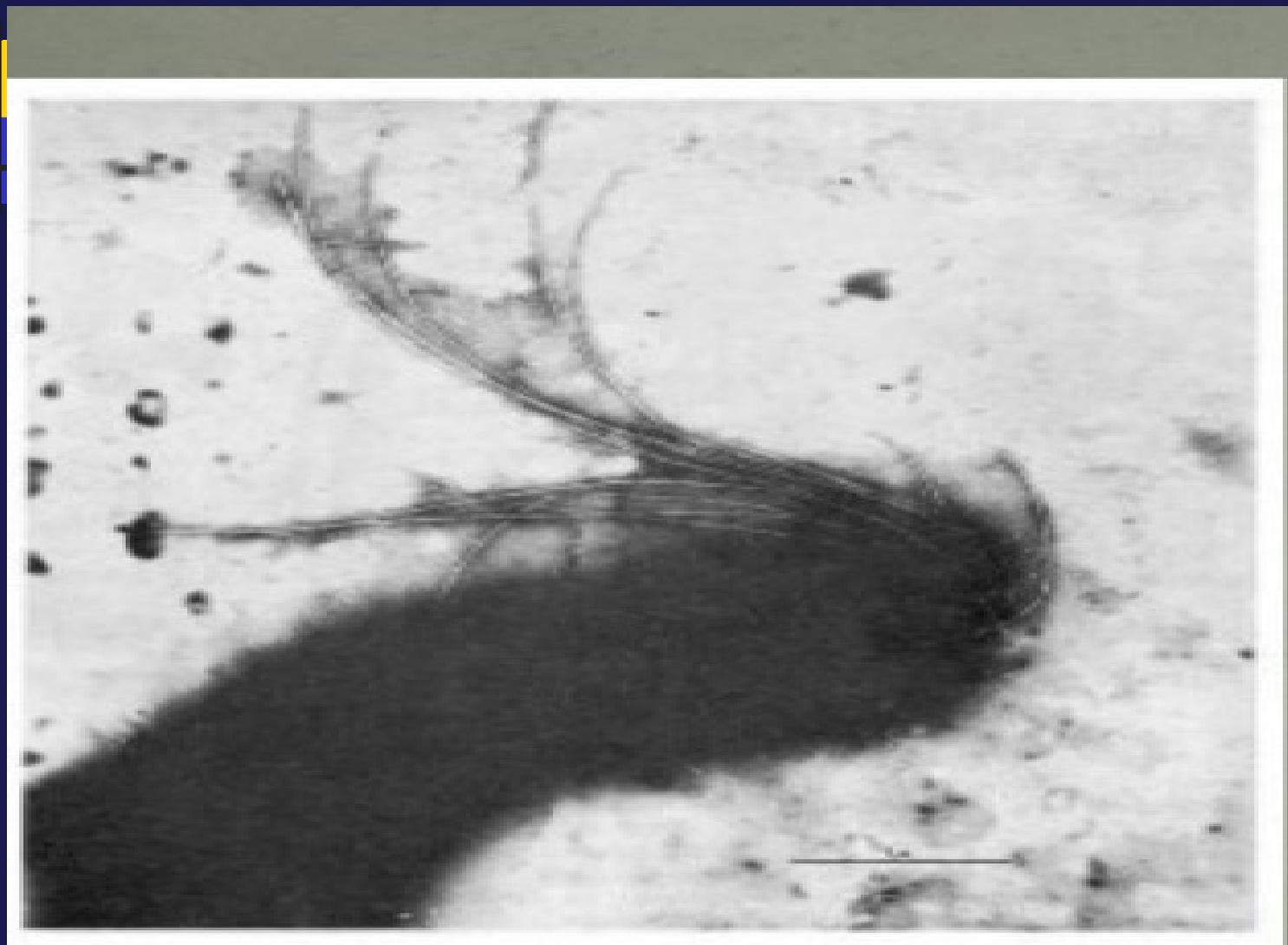
IMAGES AS SEEN USING SEM

PRINCIPLES OF MICROSCOPY



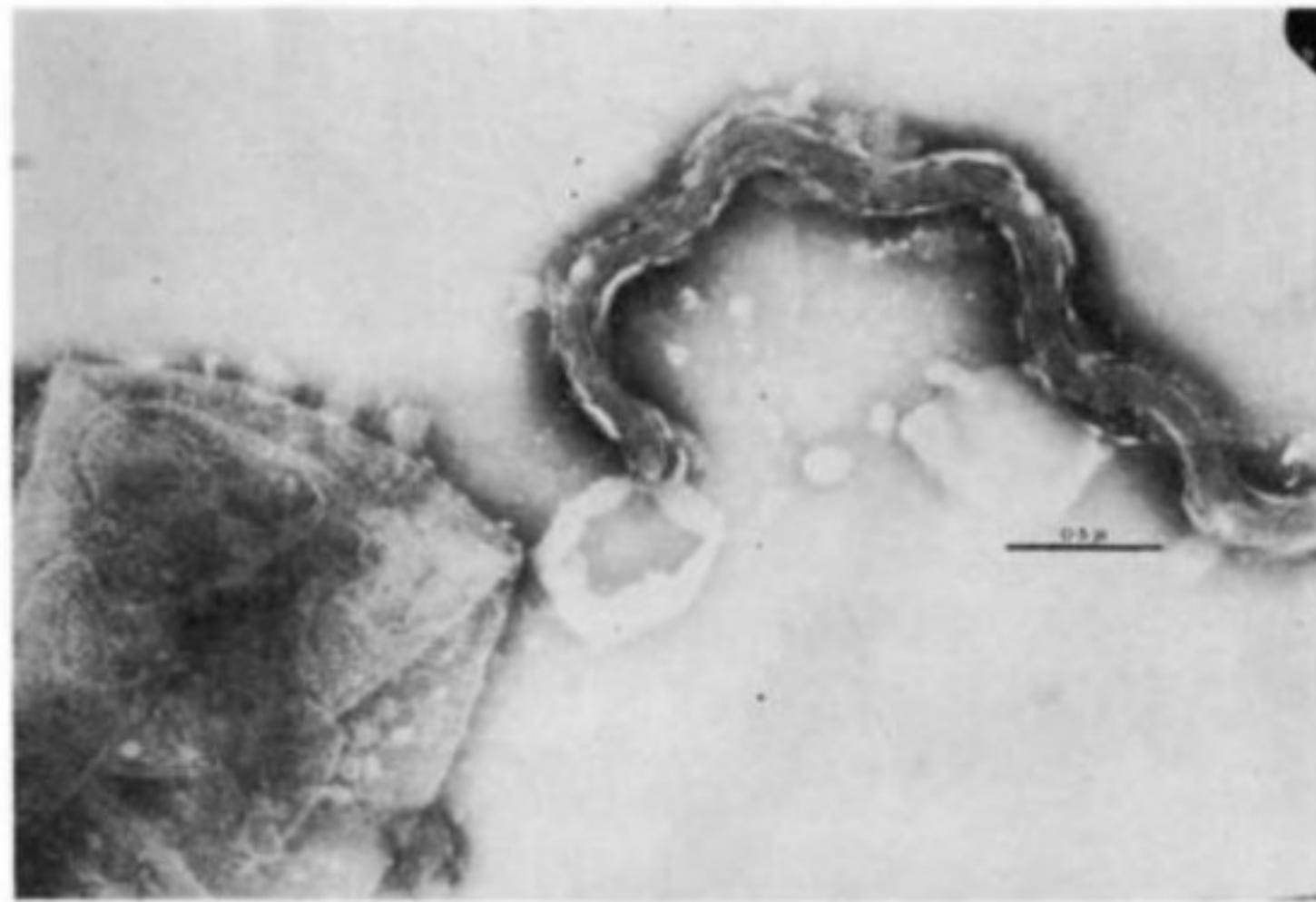
IMAGES AS SEEN BY SCANNING ELECTRON MICROSCOPE (SEM)

PRINCIPLES OF MICROSCOPY



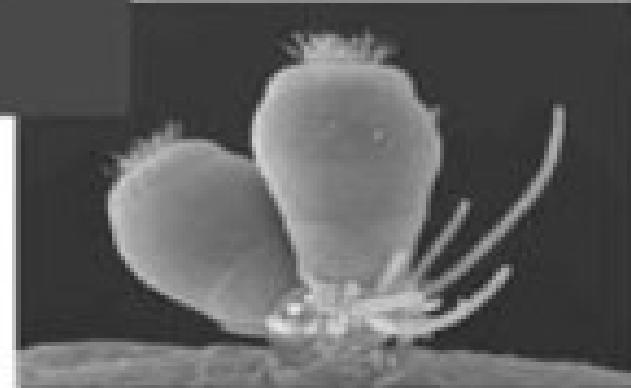
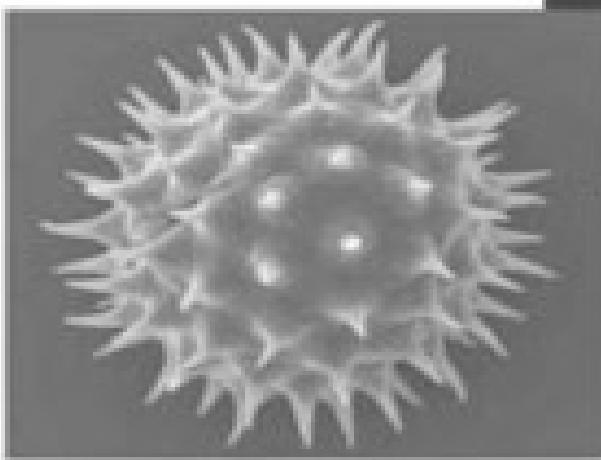
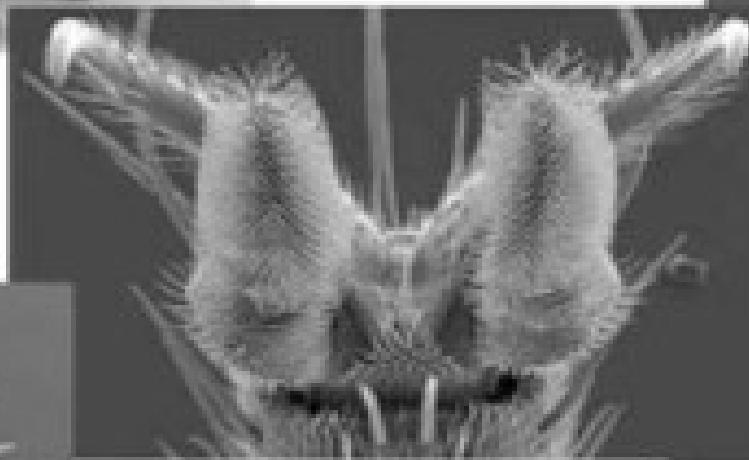
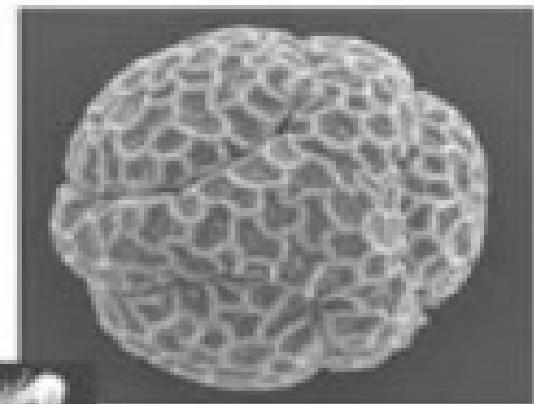
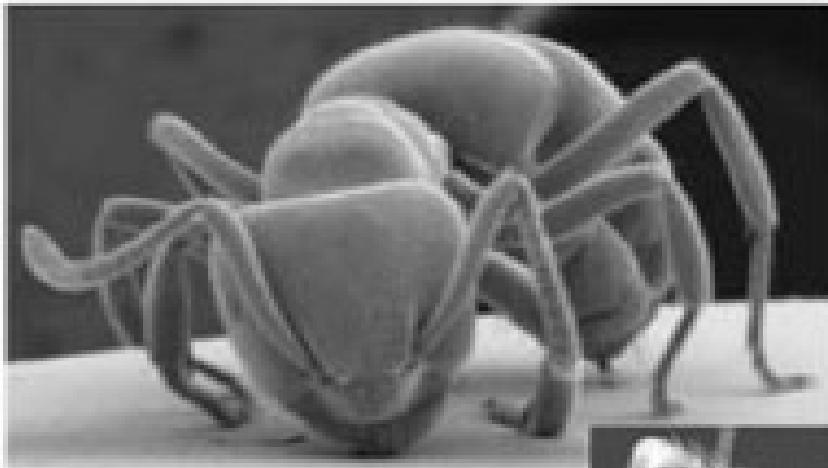
Spirillum volutans --Electron micrograph showing individual flagella ...AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



Leptospira biflexa -- Electron micrograph showing axial filament ...AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



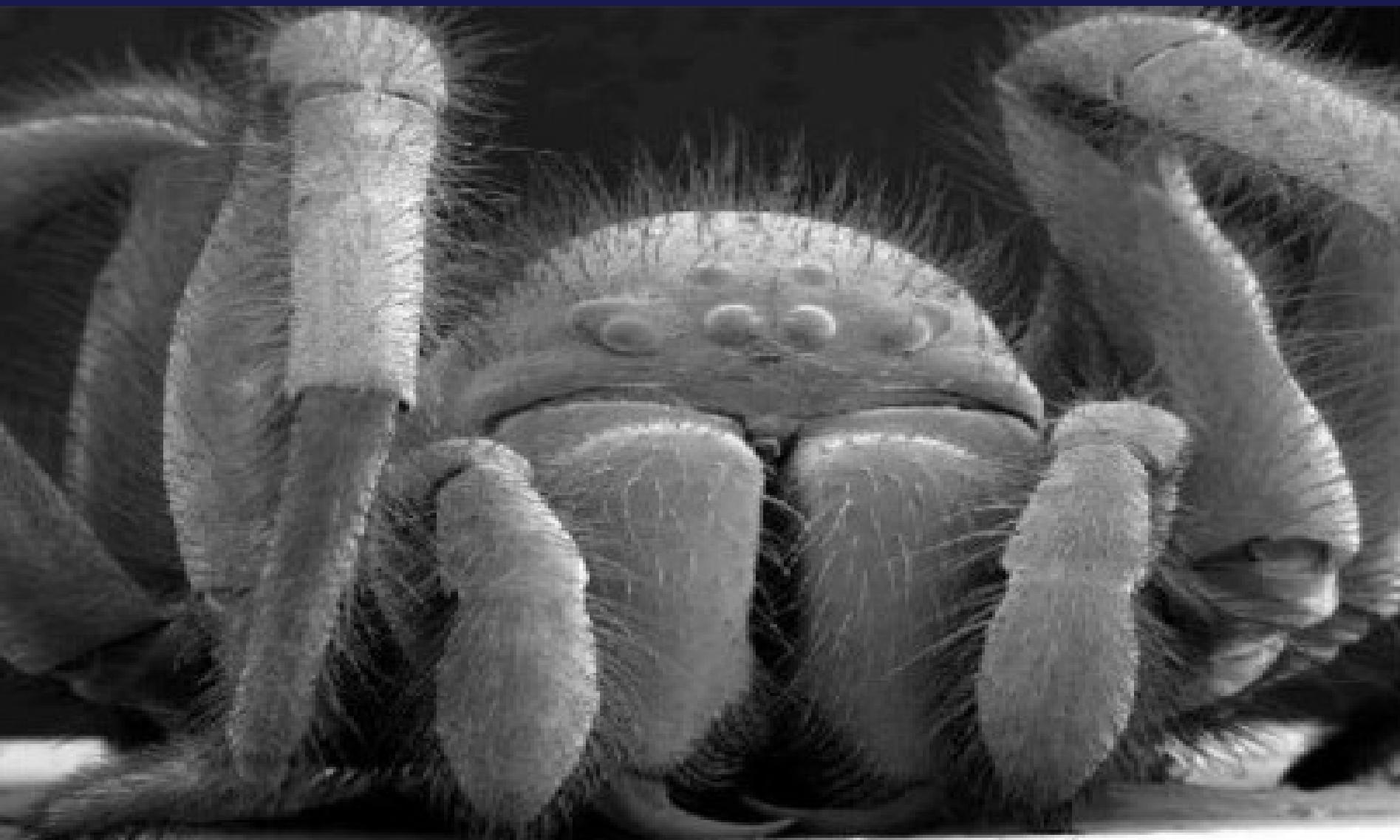
IMAGES AS SEEN USING SEM

PRINCIPLES OF MICROSCOPY



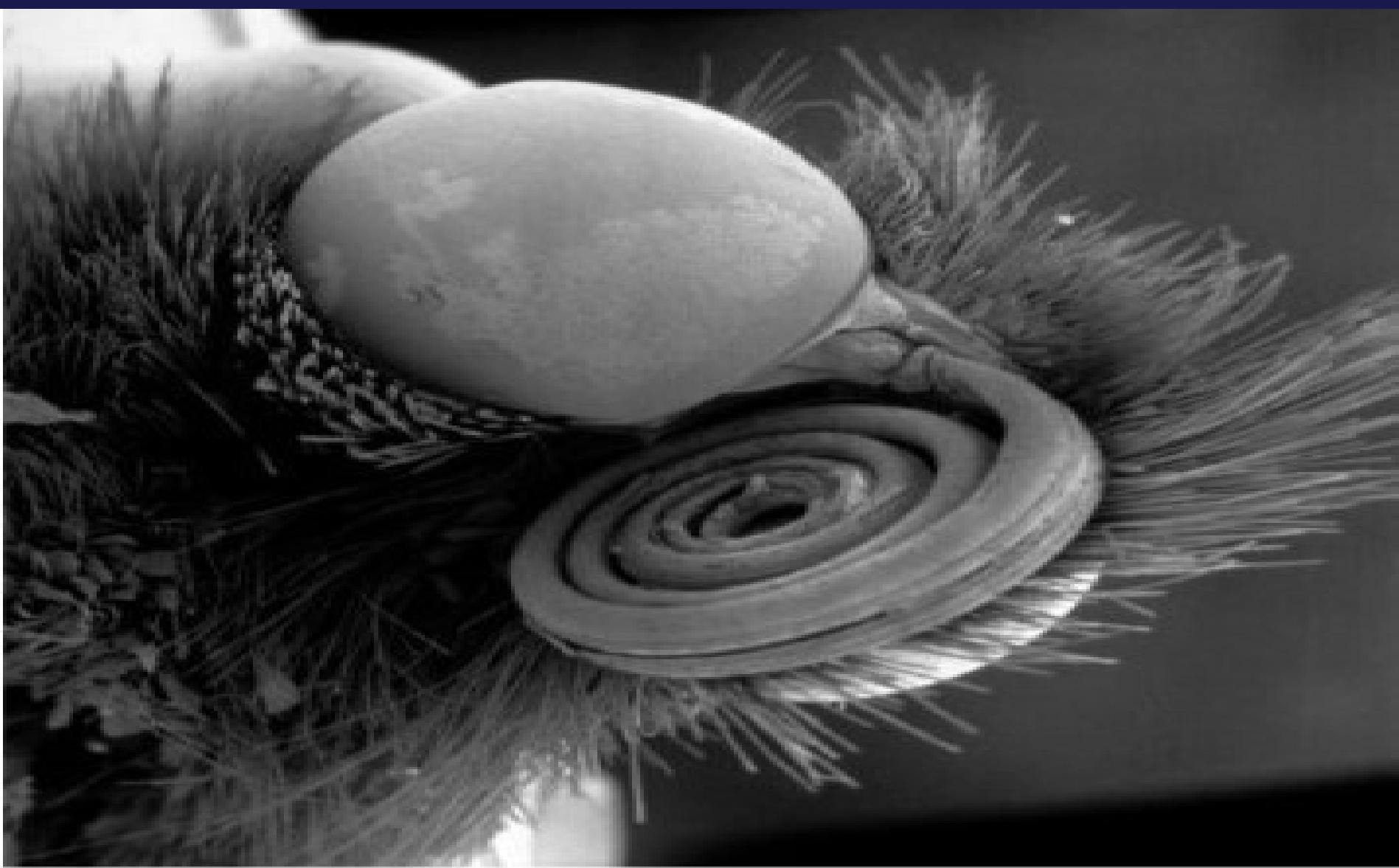
HEAD OF DRAGON FLY AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



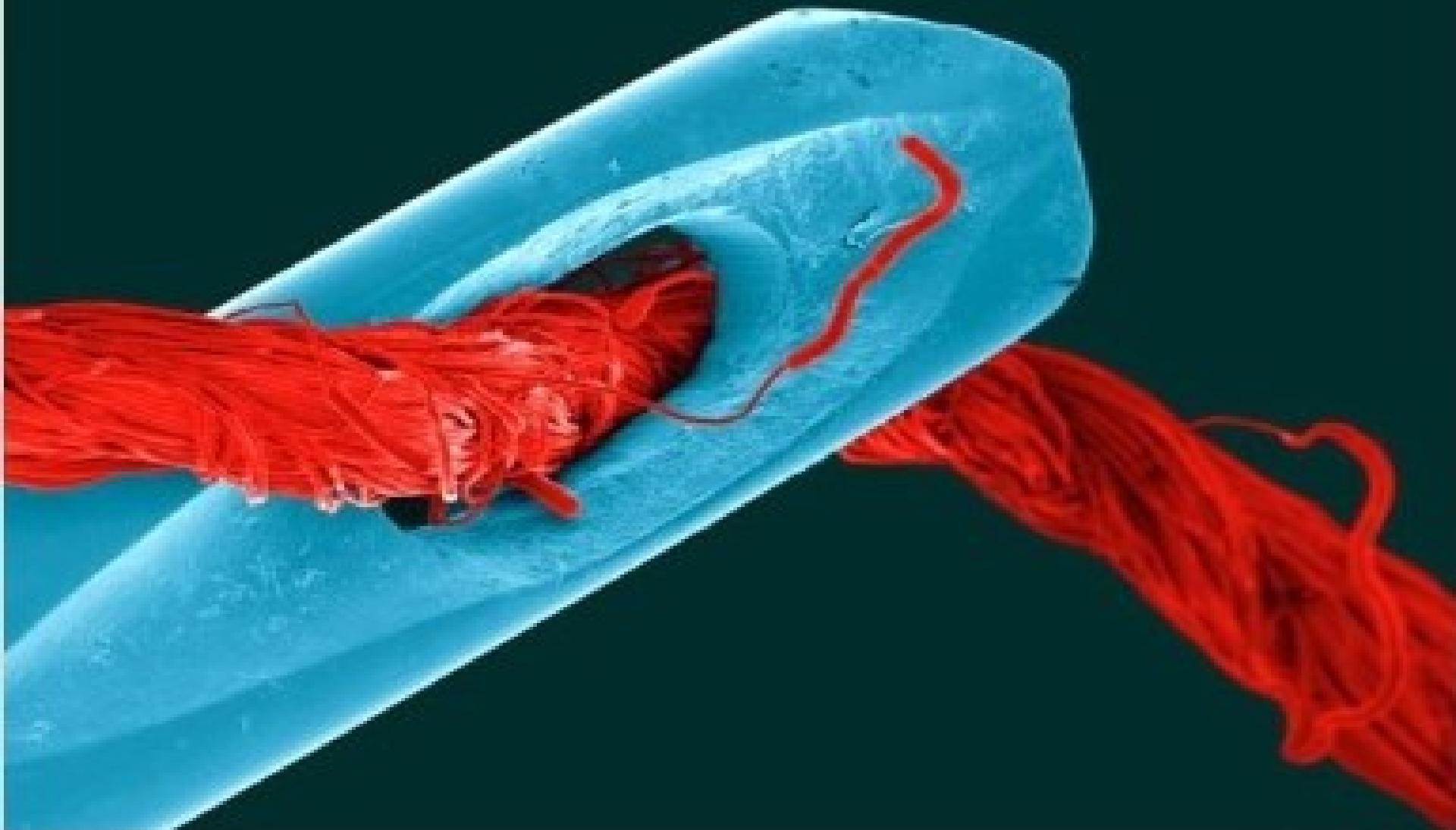
SPIDER AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



HEAD OF BUTTERFLY AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



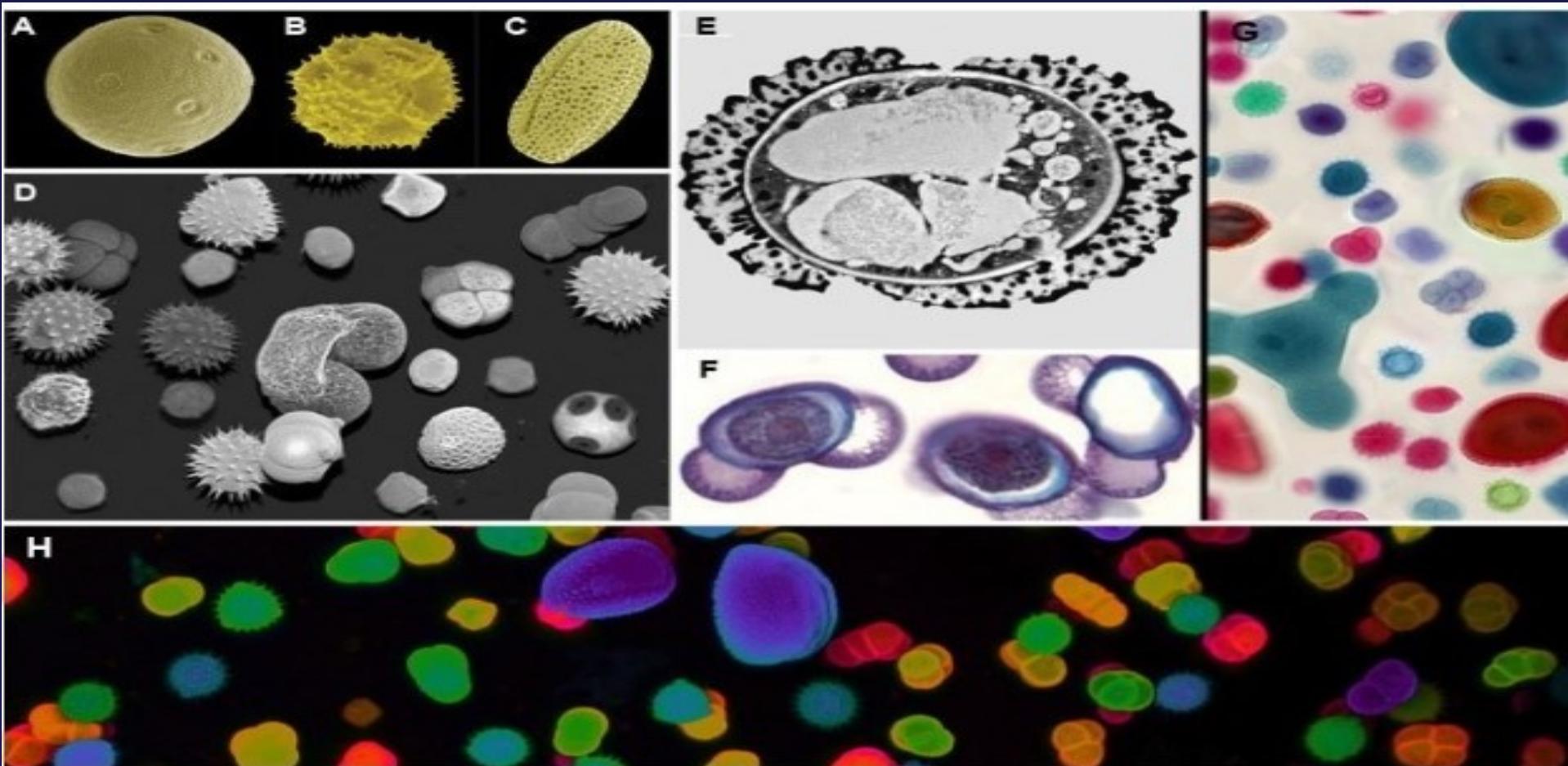
A SPIDER THREAD AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



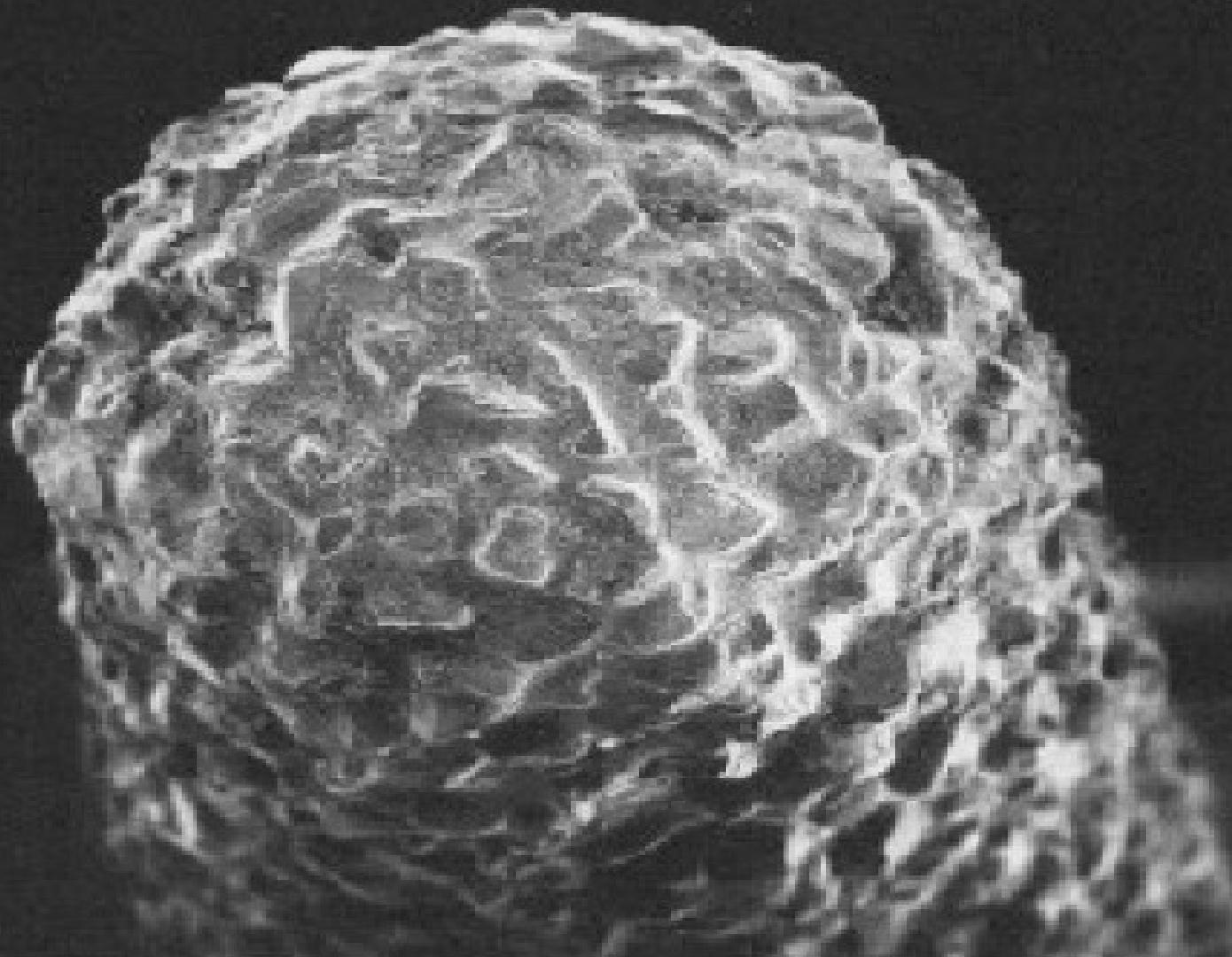
A FLEA AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



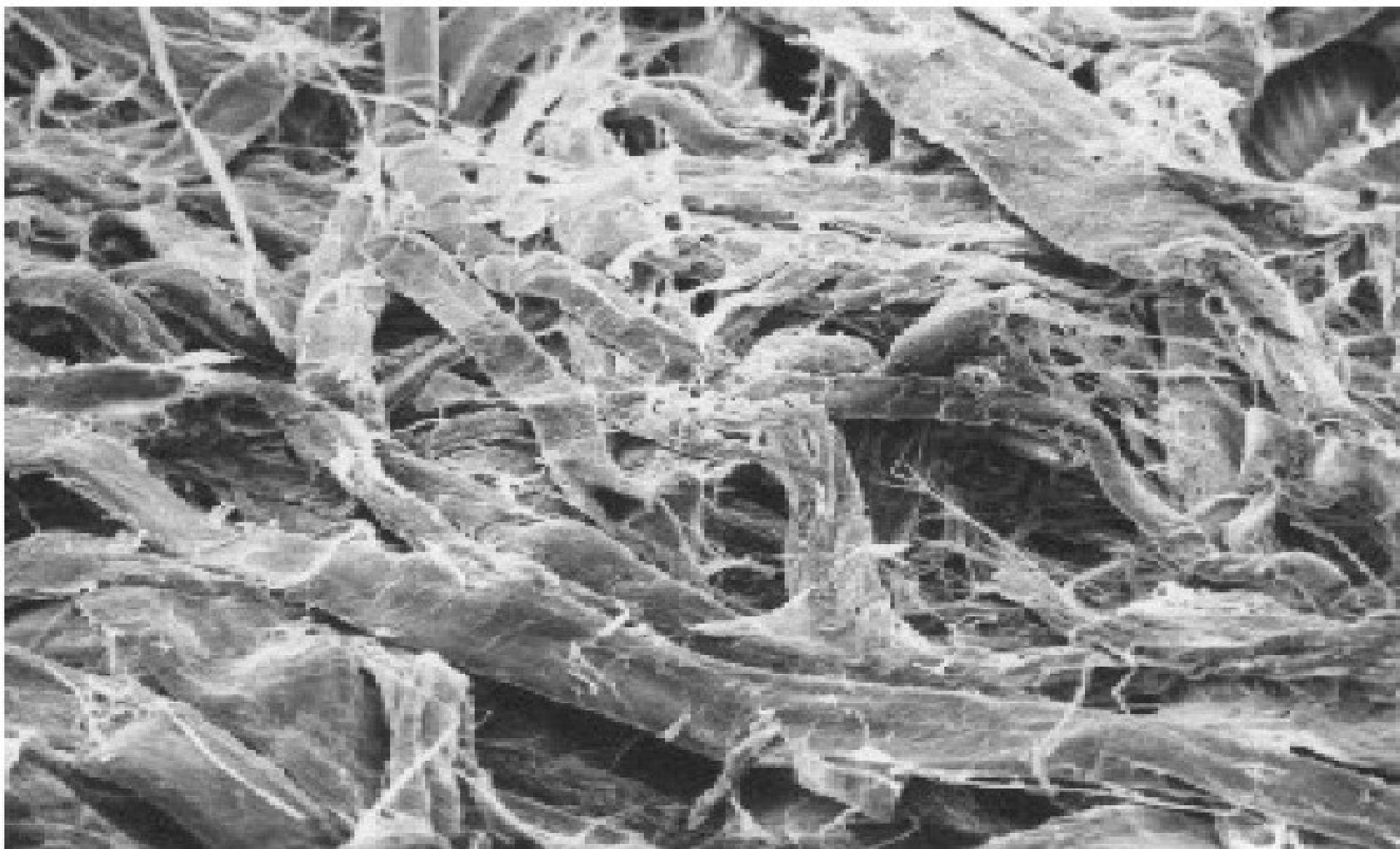
A, B, C pollen grains: Scanning electron microscope D pollen grains: Confocal Laser Scanning Microscope E pollen grains: Transmission electron microscope F pollen grains: Light microscope G Mixed pollen grains (bright field light microscope, stained) H pollen grains confocal laser scanning microscope

PRINCIPLES OF MICROSCOPY



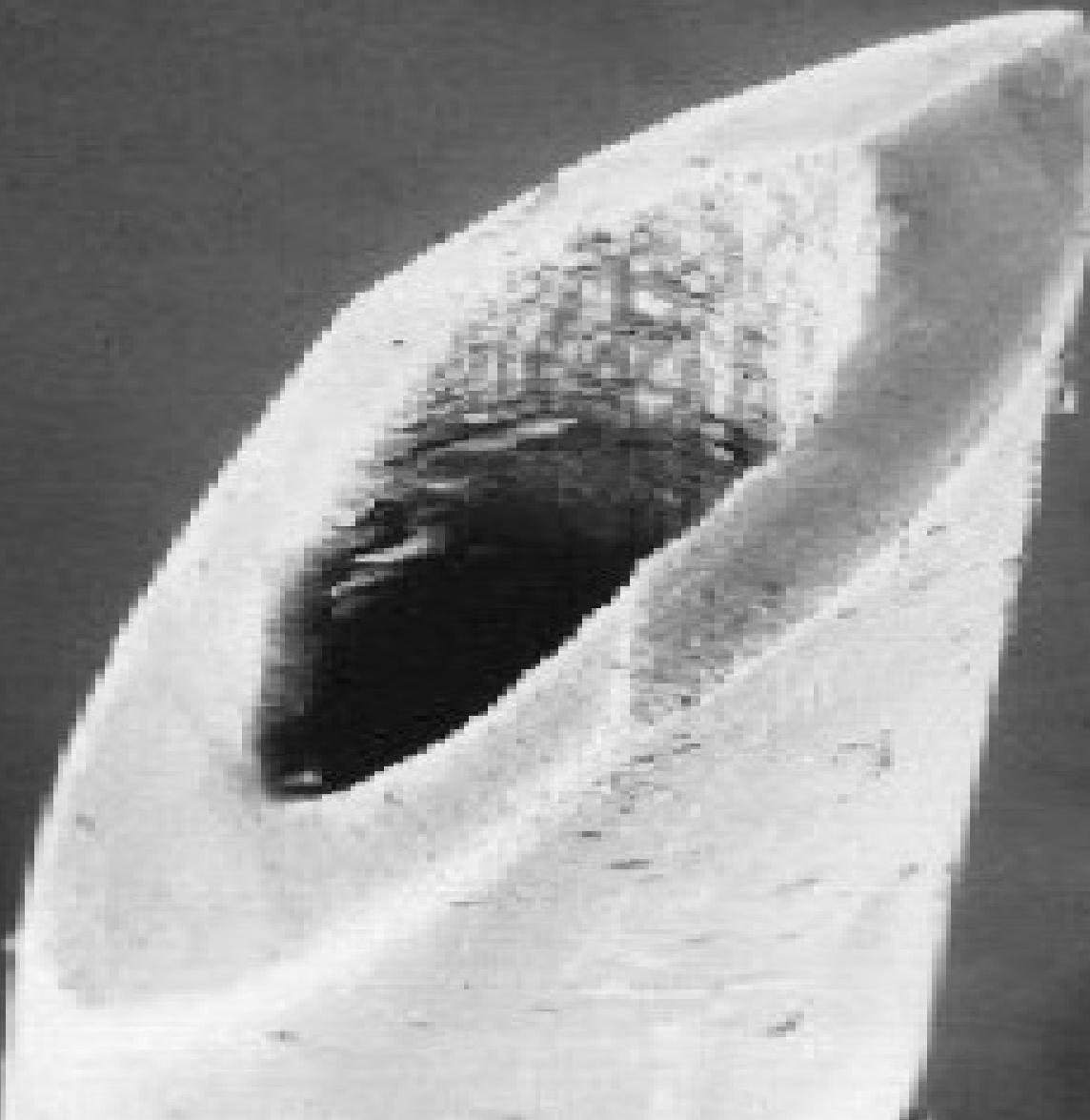
A Dentist Drill AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



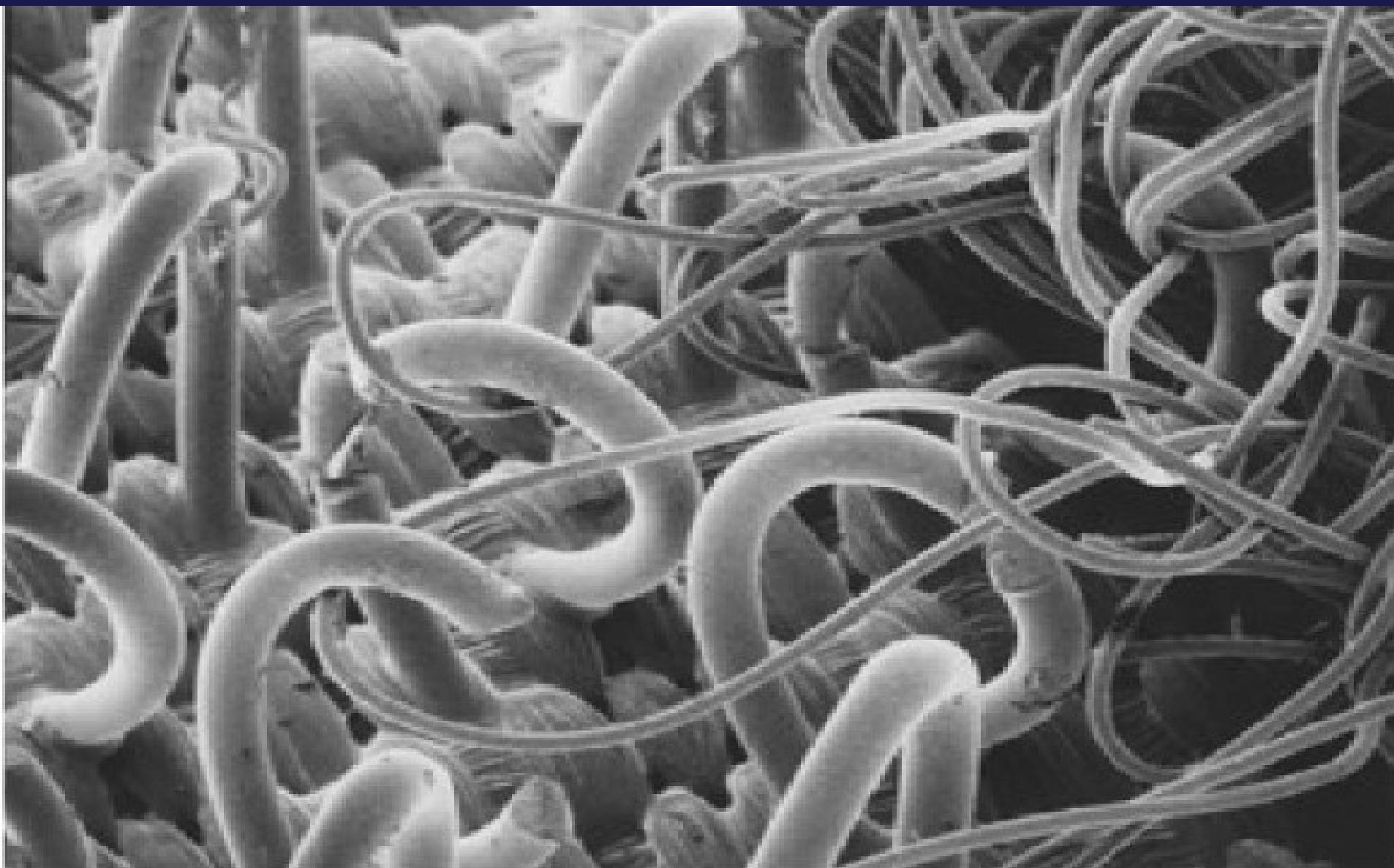
Toilet Paper AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



Hypodermic Needle AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



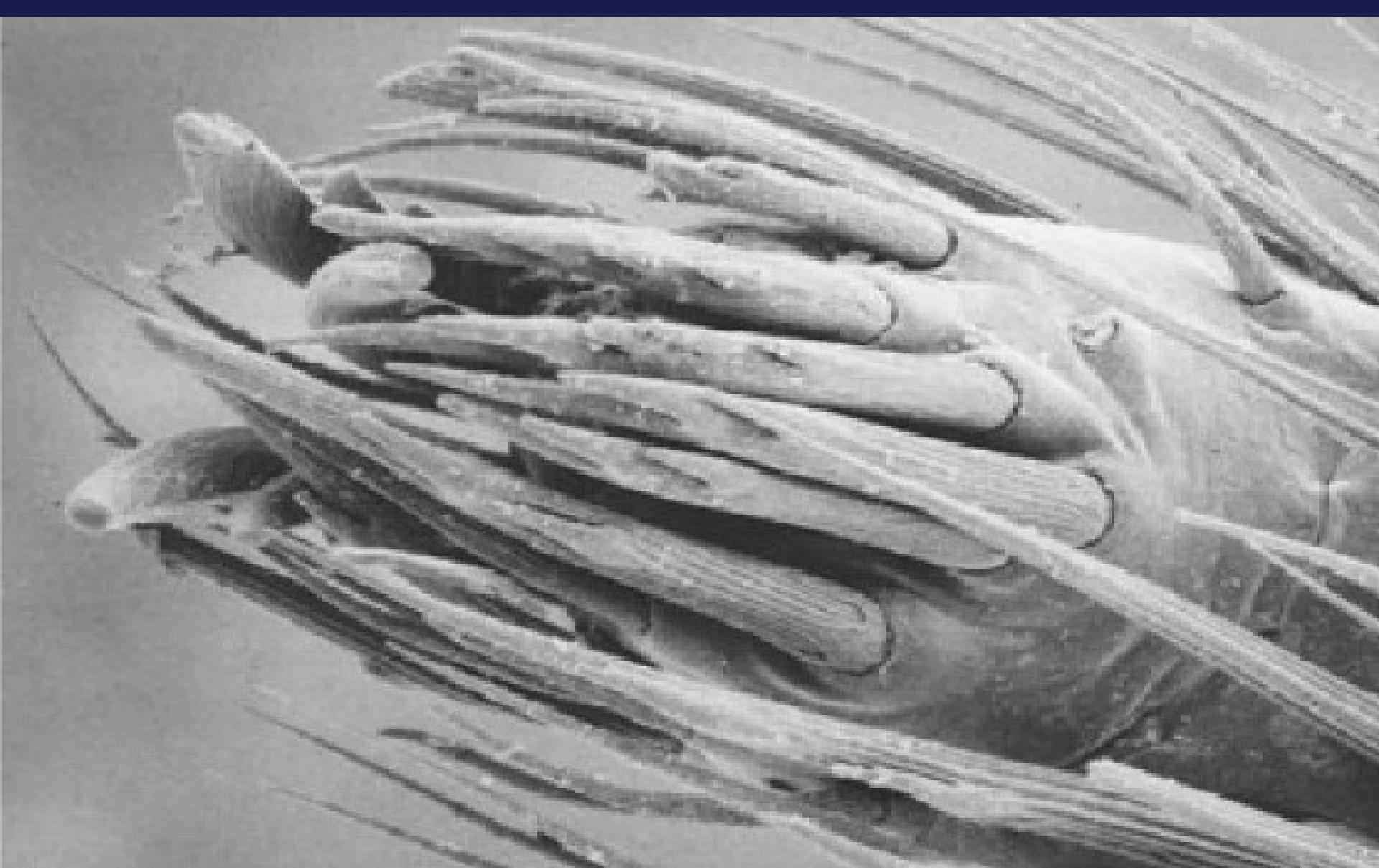
Velcro AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



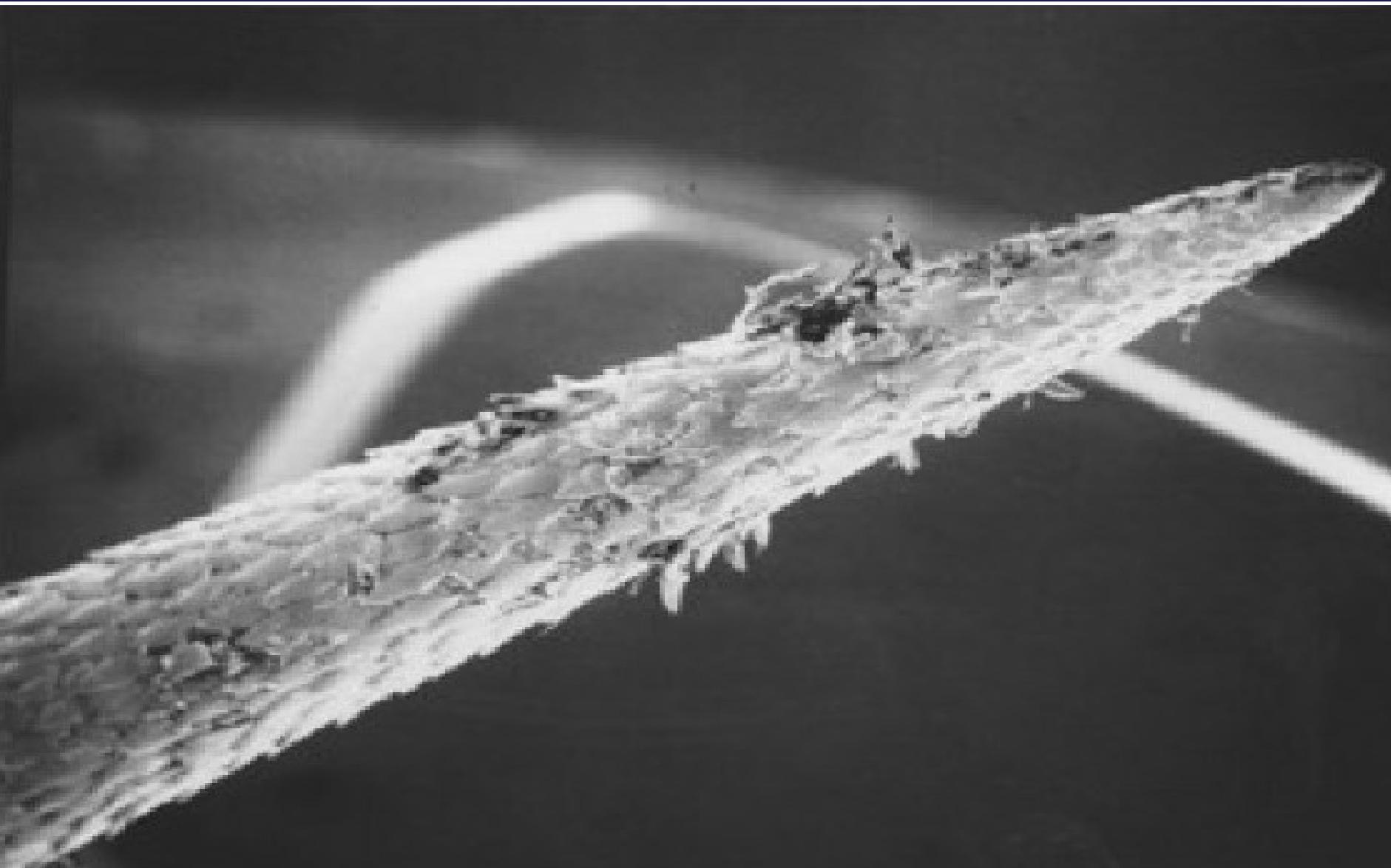
Staple through a paper AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



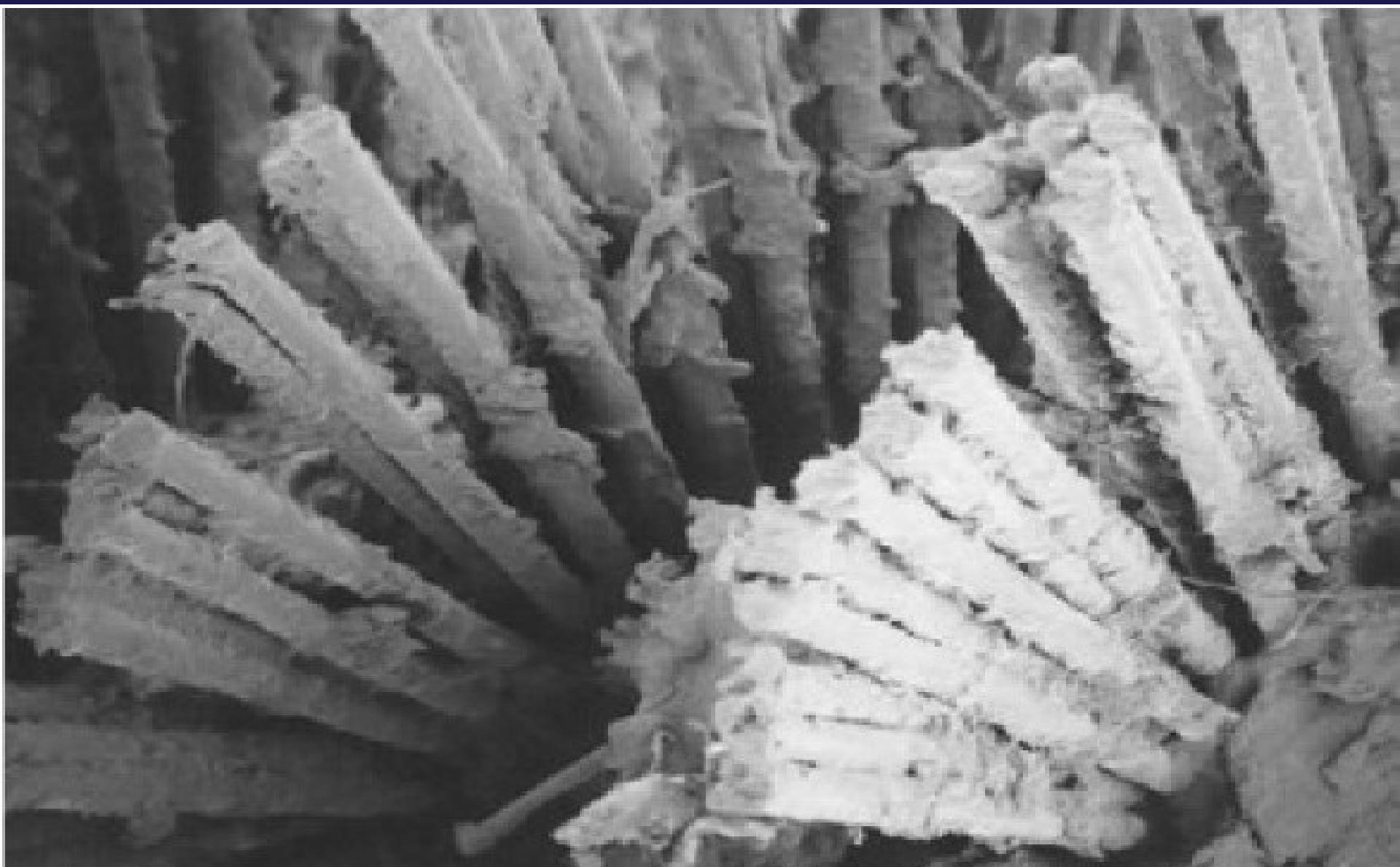
Black Widow Spider Claw AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



PORCUPINE QUILL AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



MASCARA BRUSH AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



ANT AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY

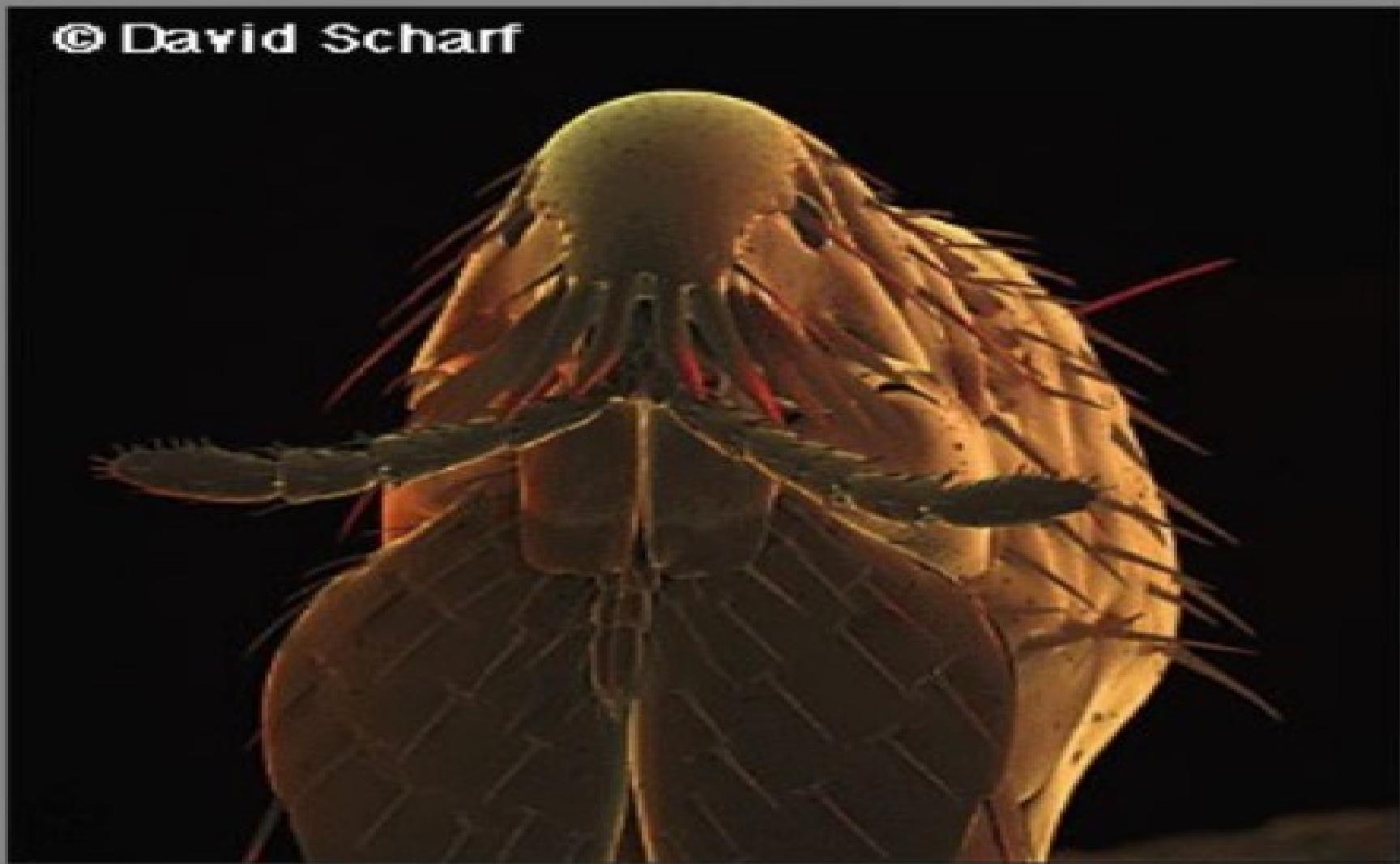
© David Scharf



BLACK FLY AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY

© David Scharf



CAT FLY AS SEEN BY SEM

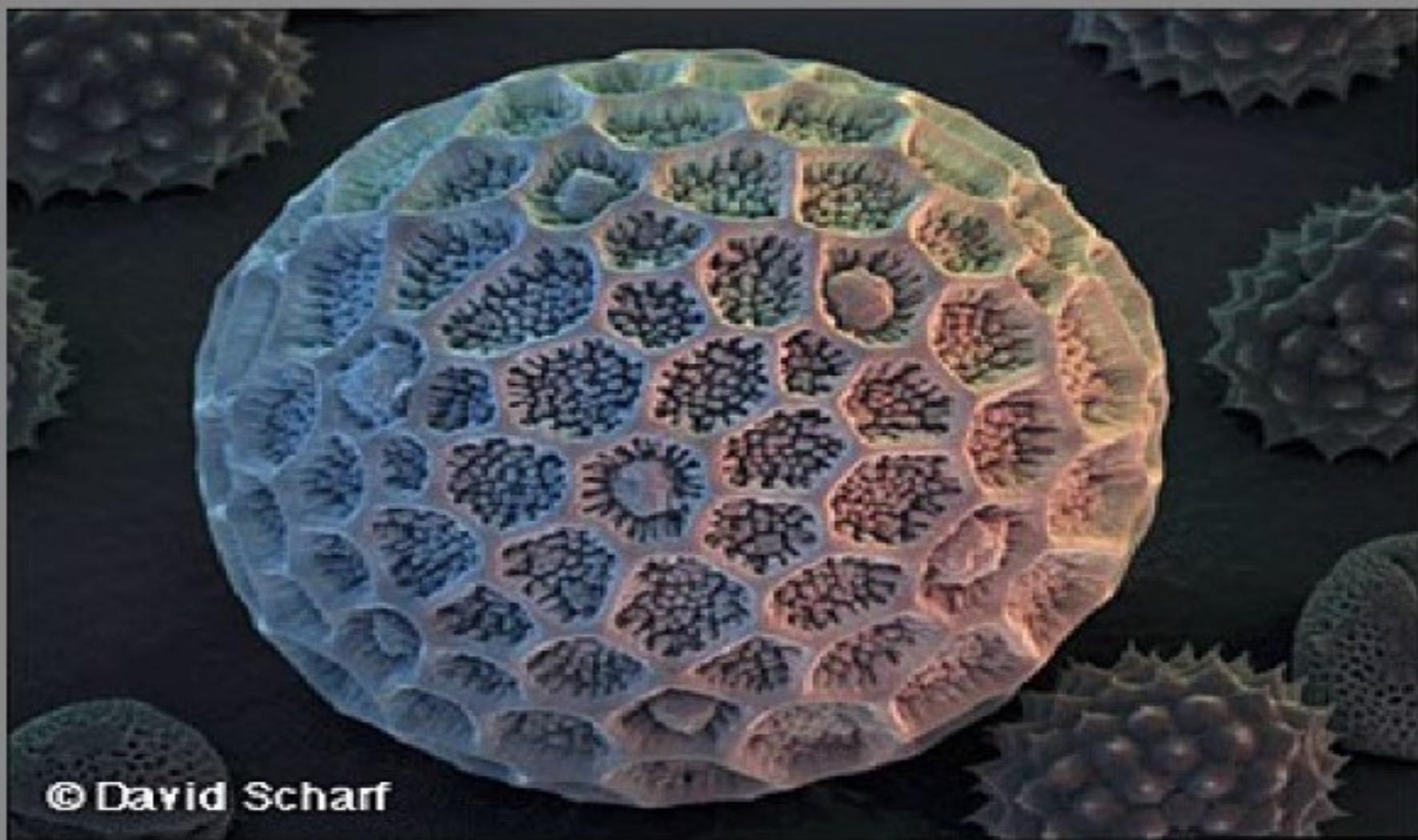
PRINCIPLES OF MICROSCOPY

© David Scharf



MITE FEEDING AS SEEN BY SEM

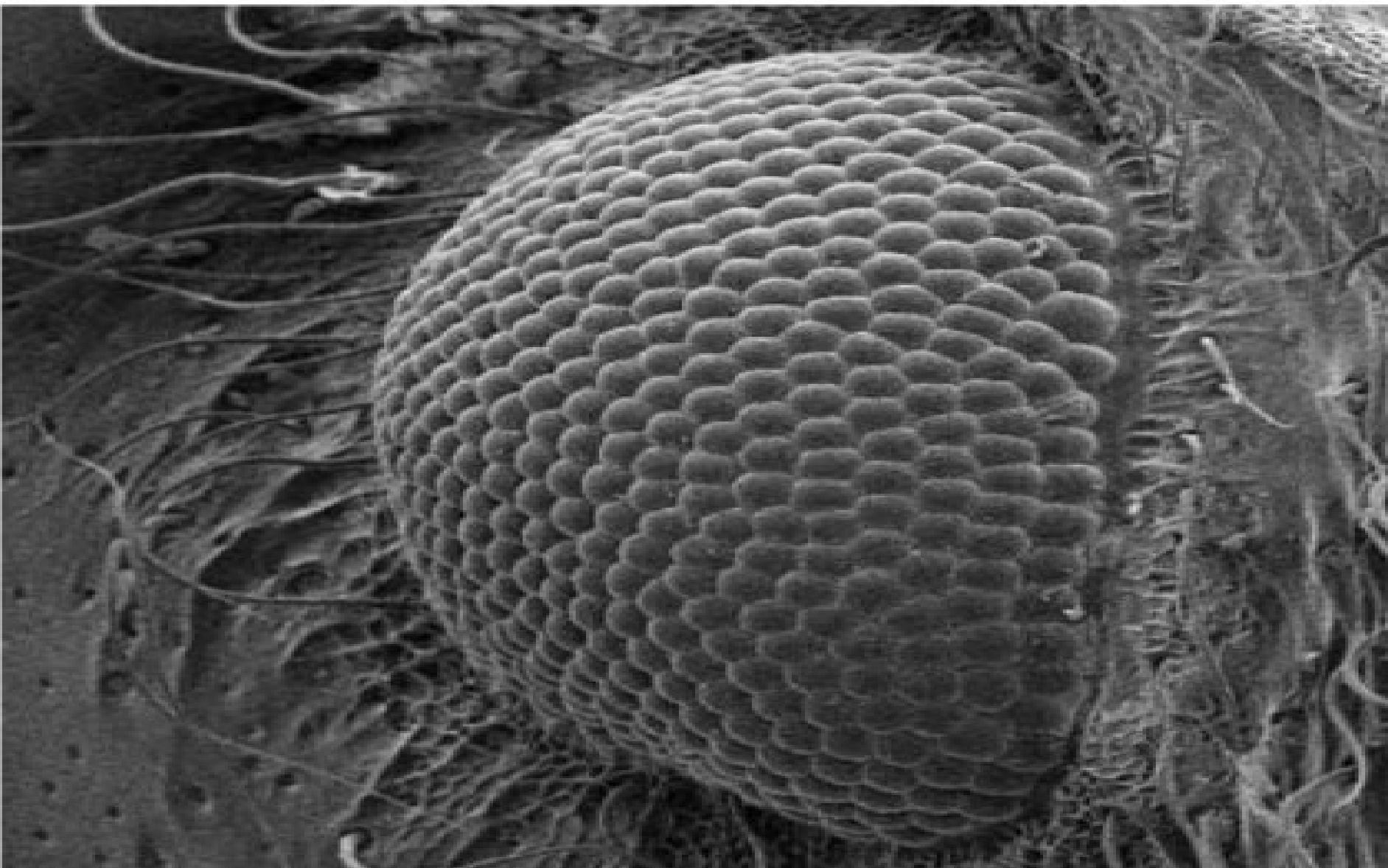
PRINCIPLES OF MICROSCOPY



© David Scharf

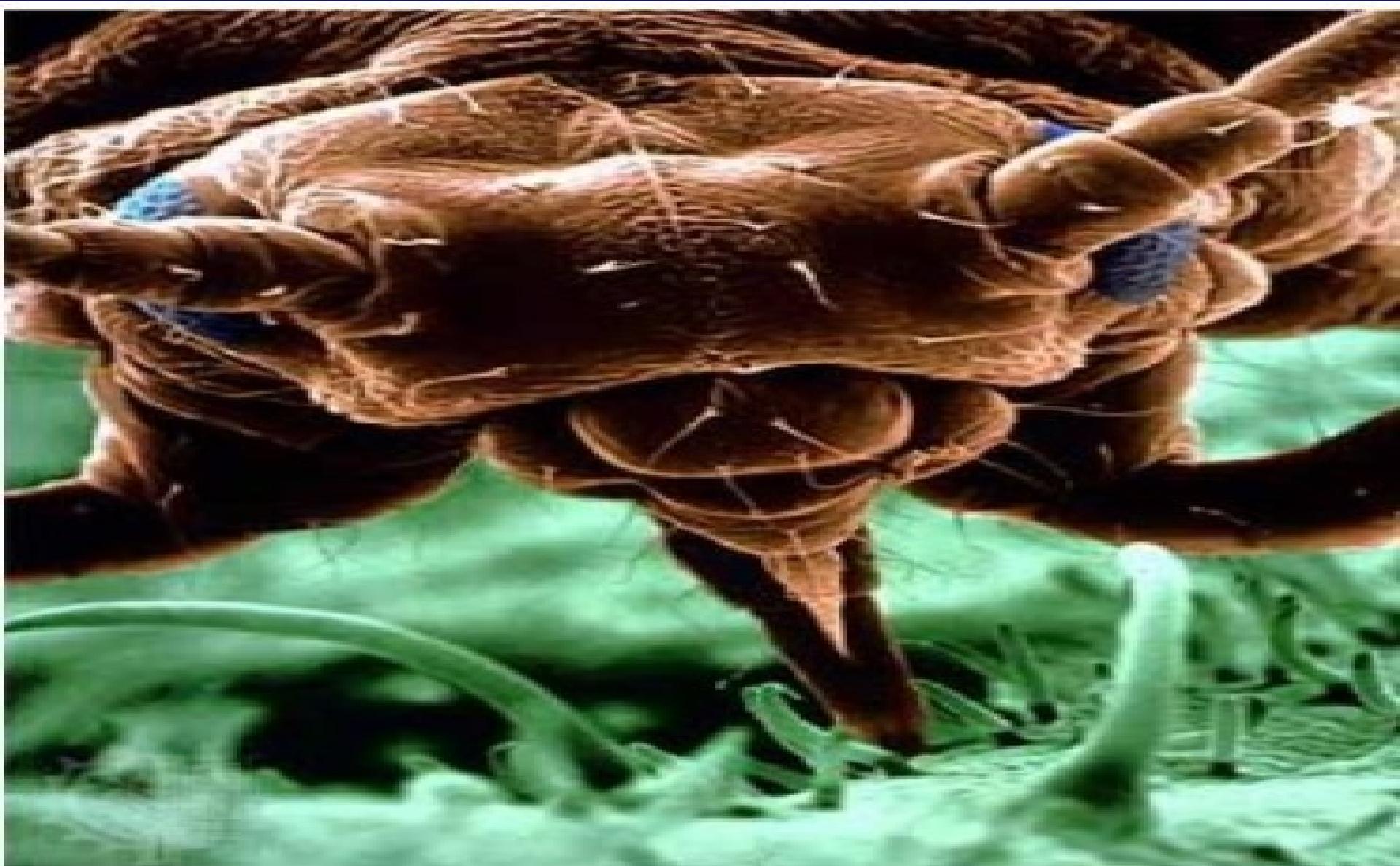
POLLEN GRAIN AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



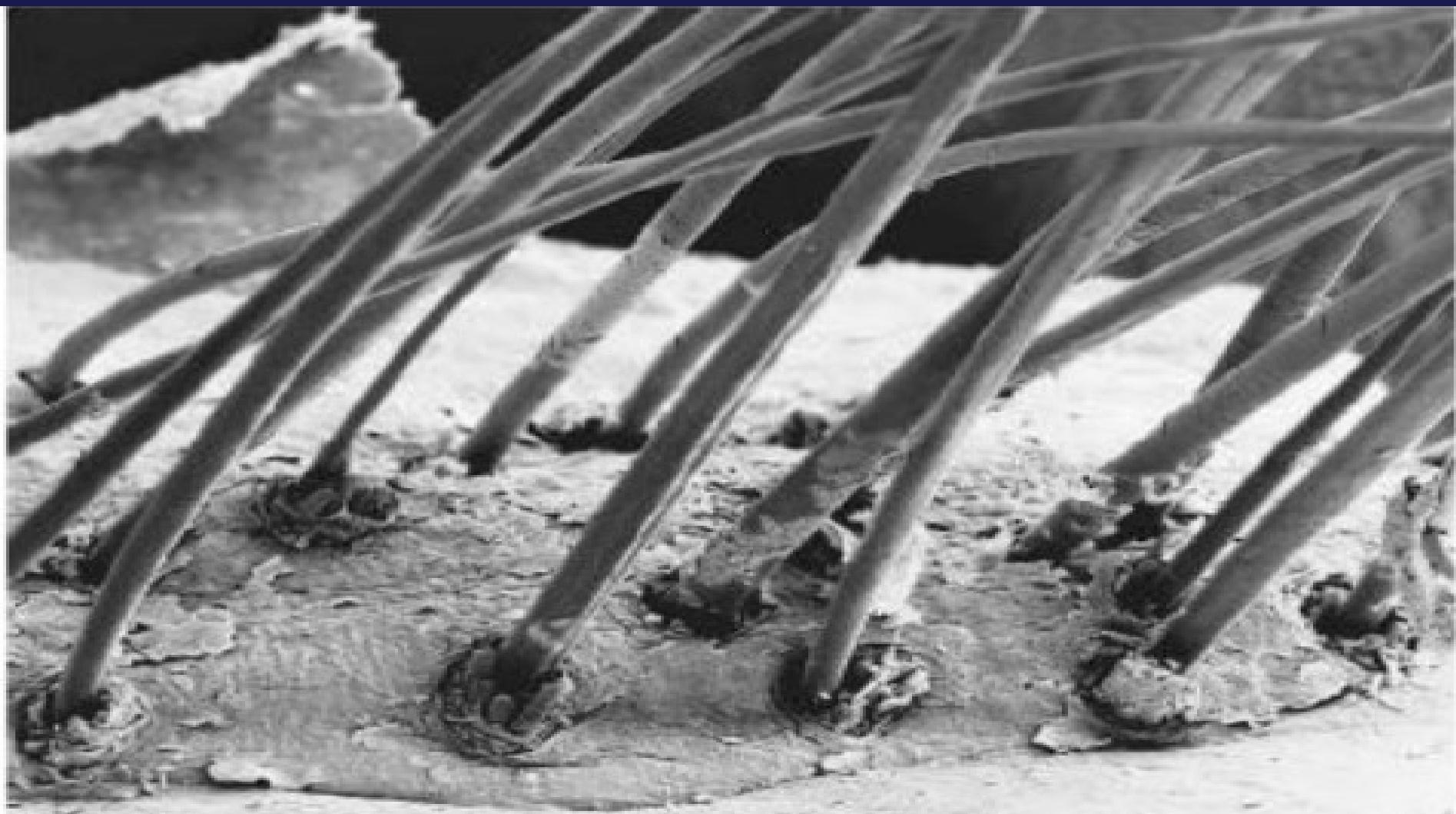
ANT EYE AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



APHID ON A LEAF AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



EYE-LASHES AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



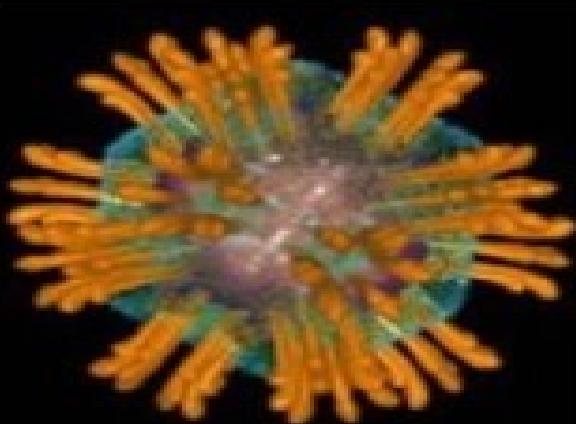
DOG- FLEA AS SEEN BY SEM

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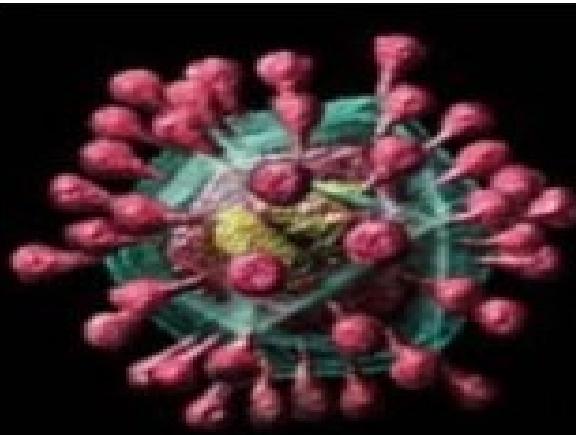
PRINCIPLES OF MICROSCOPY

VIRUS

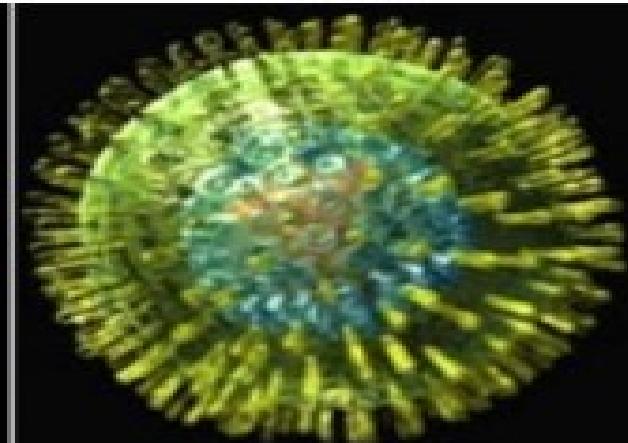
and



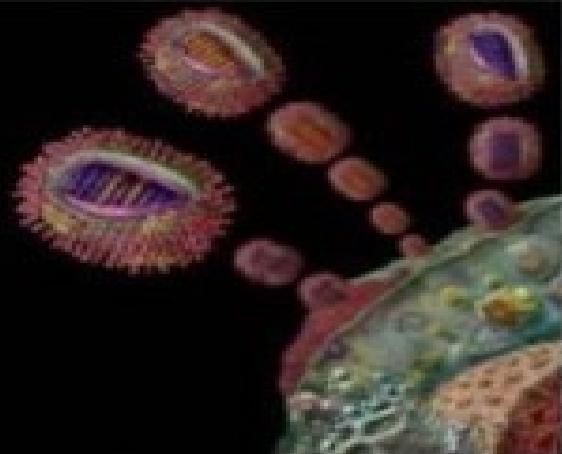
Hepatitis C virus



Coronavirus



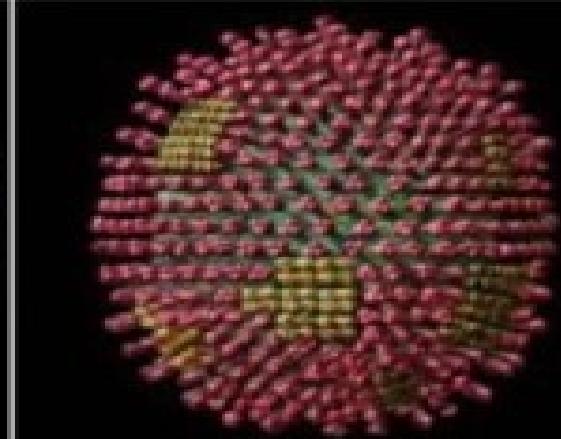
Herpes virus



Bird flu virus



Smallpox virus



Influenza virus

VARIOUS VIRUSES AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY



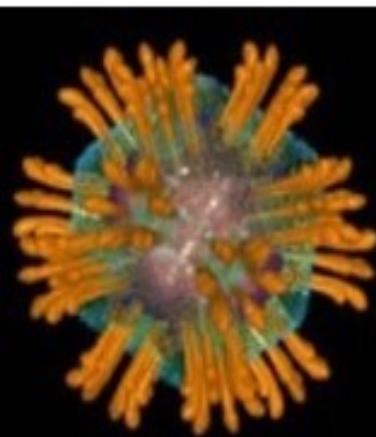
BACTERIA CELLS AS SEEN BY SEM

PRINCIPLES OF MICROSCOPY

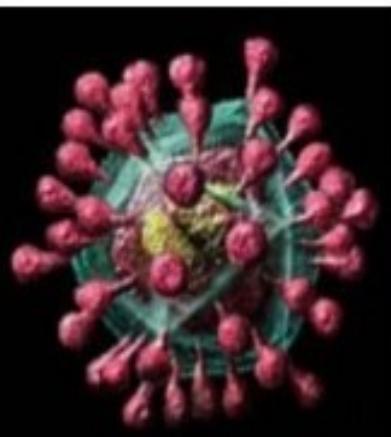
VIRUS

and

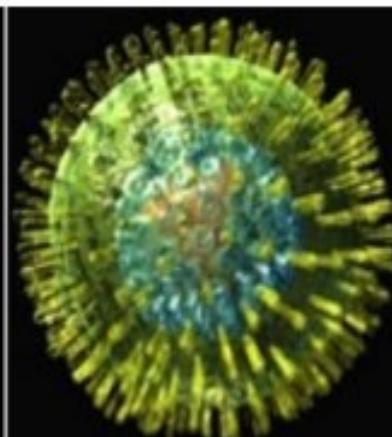
CELL?



Hepatitis C virus



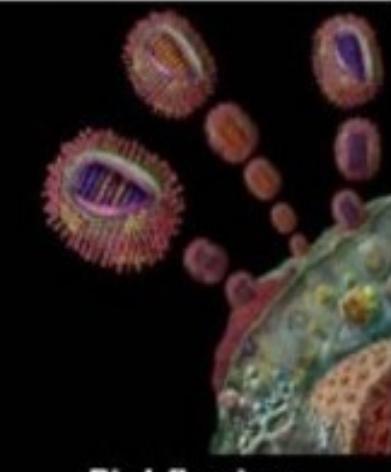
Coronavirus



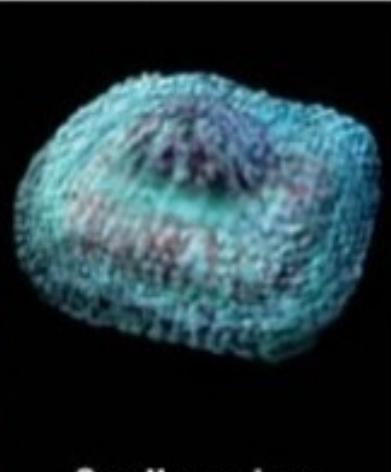
Herpes virus



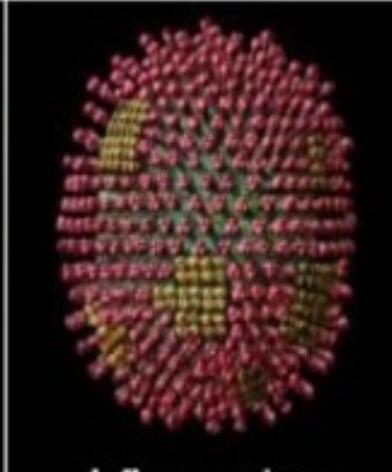
E.coli bacterial cells



Bird flu virus

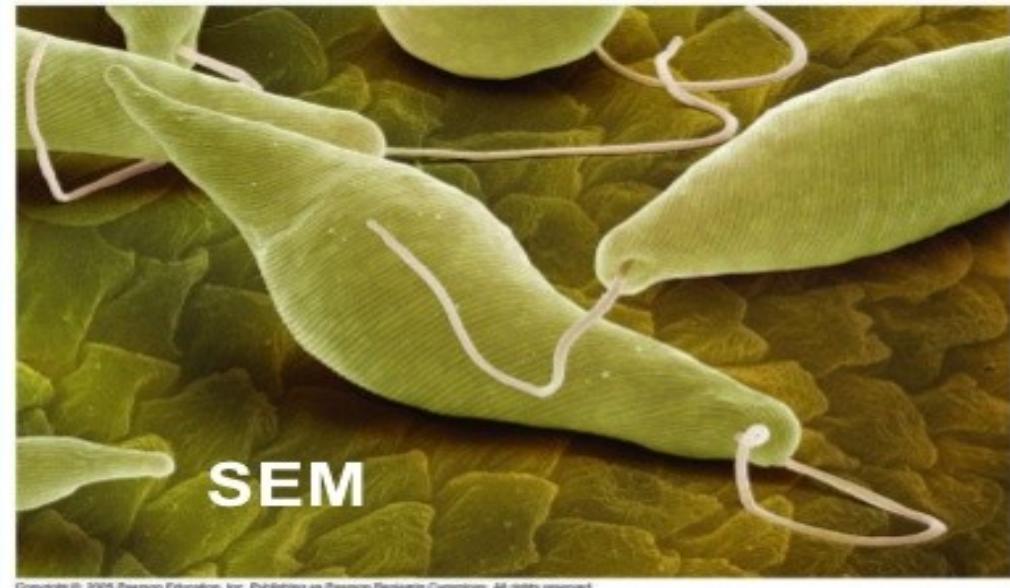


Smallpox virus



Influenza virus

PRINCIPLES OF MICROSCOPY



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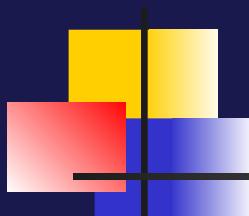
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COMPARISON OF EUGLENA IMAGES AS SEEN BY TEM AND SEM

PRINCIPLES OF MICROSCOPY

ELECTRON MICROSCOPE (EM)

APPLICATIONS OF ELECTRON MICROSCOPE (EM)

- 
- 1. EM is used to study the causes of disease.**
 - 2. Used to study 3D structure of cells .**
 - 3. Analysis of surface fracture or surface contamination of cells.**
 - 4. Important part in production of silicon chips.**
 - 5. Used in industrial search centers.**

THANK YOU!!!