BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biomedical Science

Course Title : Microbiology

Course Code : BM24AC4

Unit-I

Microscopy

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DEFINITION

- A microscope (Greek: *micron* = small and *scopos* = aim).

- MICROSCOPE - An instrument for viewing objects that are too small to be seen by the naked or unaided eye.

- MICROSCOPY - The science of investigating small objects using such an instrument is called microscopy

Timeline of Microscope Technology

- 167 BCE - The Chinese use water microscopes made of a lens and a water-filled tube to visualize the unseen.

- 1590 - Dutch spectacle-makers Hans Jansen and his son Zacharias Jansen, claimed by later writers to have invented a compound microscope.
- 1609 - Galileo Galilei develops a compound microscope with a convex and a concave lens.
- 1619 - Cornelius Drebbel (1572 – 1633) presents, in London, a compound microscope with two convex lenses.
- 1665 - Robert Hooke publishes Micrographia, a collection of biological micrographs. He coins the word cell for the structures he discovers in cork bark.
- 1674 - Anton van Leeuwenhoek improves on a simple microscope for viewing biological specimens.

HISTORICAL BACKGROUND

1590 - Hans Janssen developed first microscope.

1609 - Galileo Galilei developed first compound microscope

1620 - Christian Huygens, another Dutchman, developed a simple 2-lens ocular system .

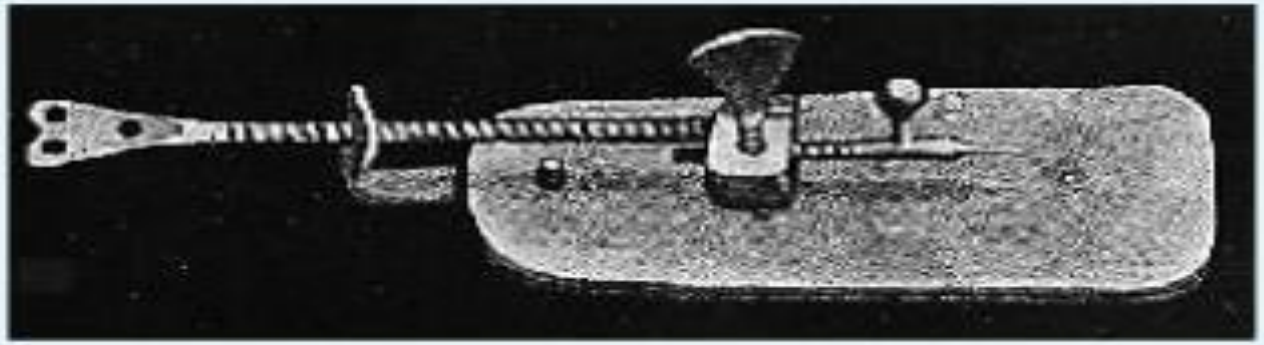
- 1931 - Ernst Ruska starts to build the first electron microscope. It is a Transmission electron microscope (TEM)
- 1938 - James Hillier builds another TEM
- 1953 - Zernike, professor of theoretical physics, receives the Nobel Prize in Physics for his invention of the phase contrast microscope.
- 1955 - George Nomarski, professor of microscopy, published the theoretical basis of Differential interference contrast microscopy.
- 1981 - Gerd Binnig and Heinrich Rohrer develop the scanning tunneling microscope (STM).
- 1986 - Gerd Binnig, Quate, and Gerber invent the Atomic force microscope (AFM

- 1988 - Kingo Itaya invents the Electrochemical scanning tunneling microscope
- 1991 - Kelvin probe force microscope invented.

Anton van Leeuwenhoek
(1632-1723)

- Anton van Leeuwenhoek is generally credited with bringing the microscope to the attention of biologists.
- 1661 - He discovered bacteria, free-living and parasitic microscopic protists, sperm cells, blood cells, microscopic nematodes etc.
- He is the father of Microscopy

Anton von Leeuwenhoek



Microscope used by Anton von Leeuwenhoek

Simple Microscope

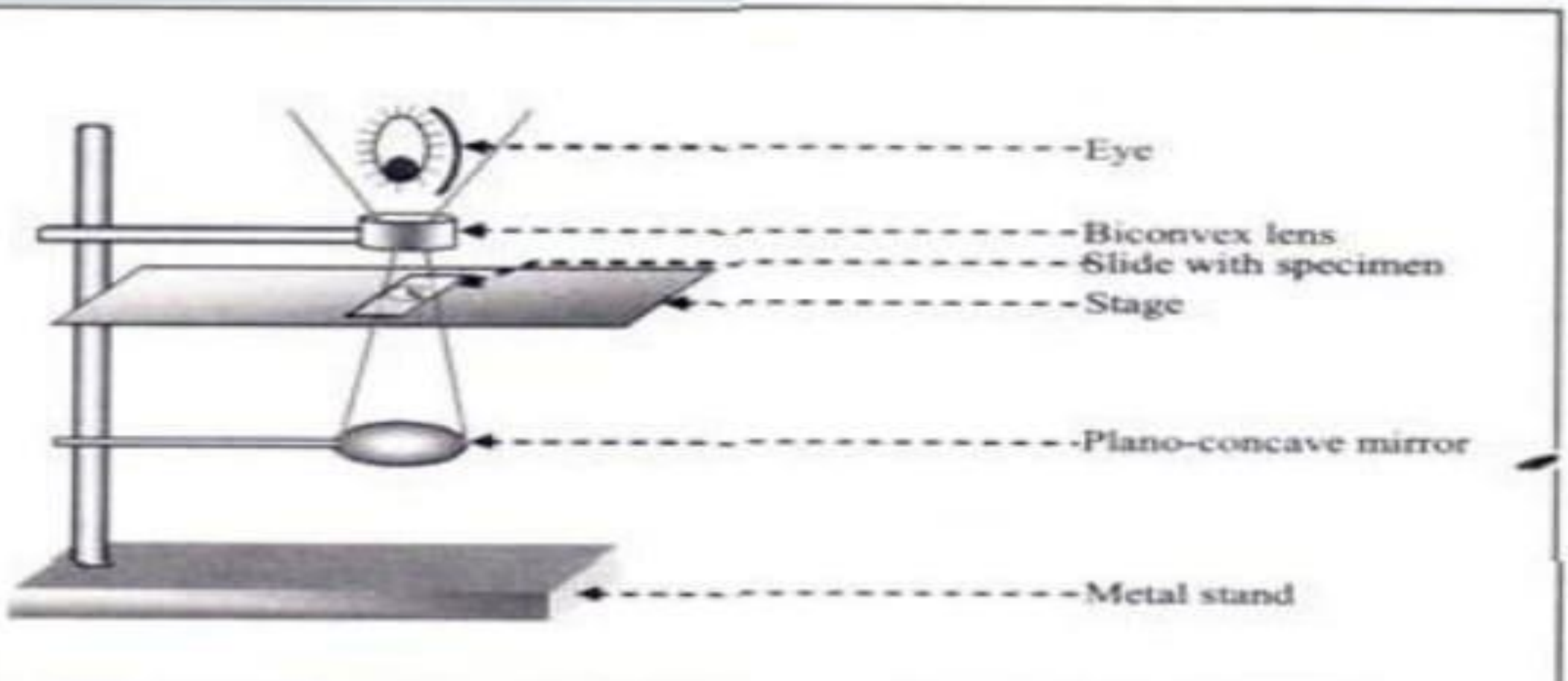


Figure 4.3: A simple microscope

Principle:

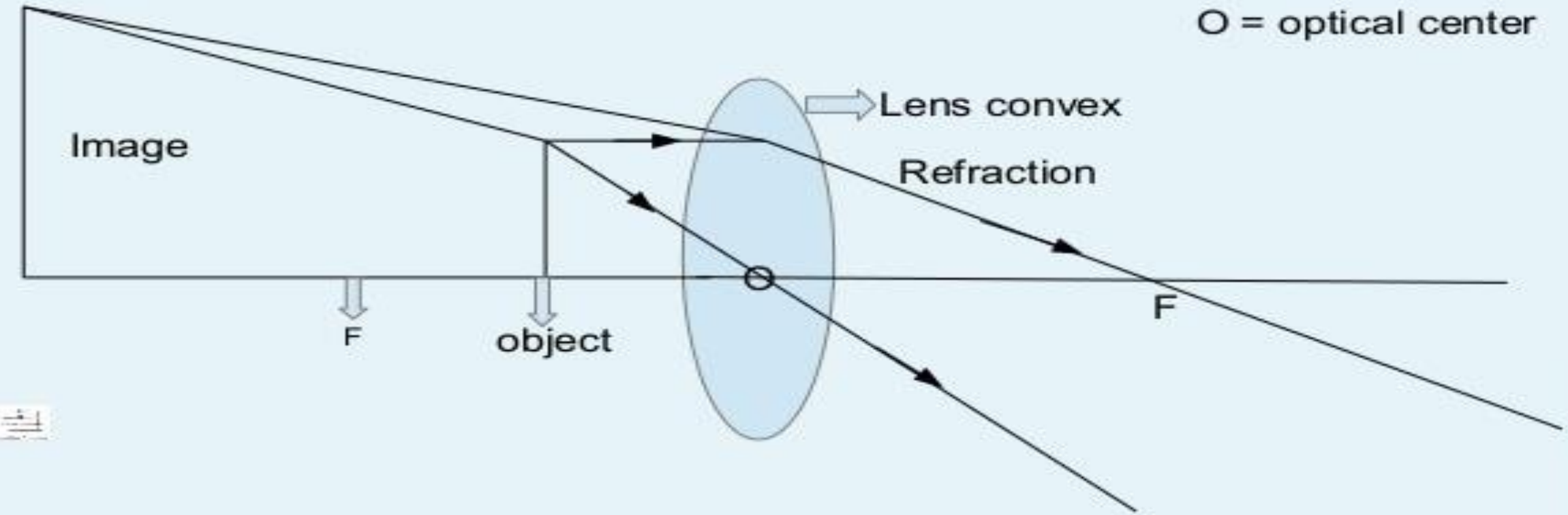
- A simple microscope is used to obtain small magnifications. It is usually to used for study of microscopic algae, fungi and biological specimen.

- Light from a light source (mirror) passes through a thin transparent object. A biconvex lens magnifies the size of the object to get an enlarged virtual image..

- **Parts of a Simple Microscope:**
- The parts of a simple microscope are of two categories as follows:
- (i) Mechanical parts
- (ii) Optical parts

(i) **Mechanical parts:**

These parts support the optical parts and help in their adjustment for focusing the object. Object between the optical center and focus of a lens.The large image front of the lens.They include the following components (Figure).



- Metal Stand:

It has a heavy base plate and a vertical rod fitted to it, which provide support and stability to other parts of the microscope.

- Stage:

It is a rectangular metal plate fitted to the vertical rod. It has a central hole for light to pass from below. Slide with specimen to be observed is kept on the stage, in such a way that, the specimen remains just on the central hole. Some microscopes have a pair of slanting wings projecting from the both the sides of the stage. They provide support to hand for manipulating the object.

(ii) **Optical parts;**

These parts are involved in passing the light through the object (specimen) and magnifying its size.

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.

COMPOUND MICROSCOPE

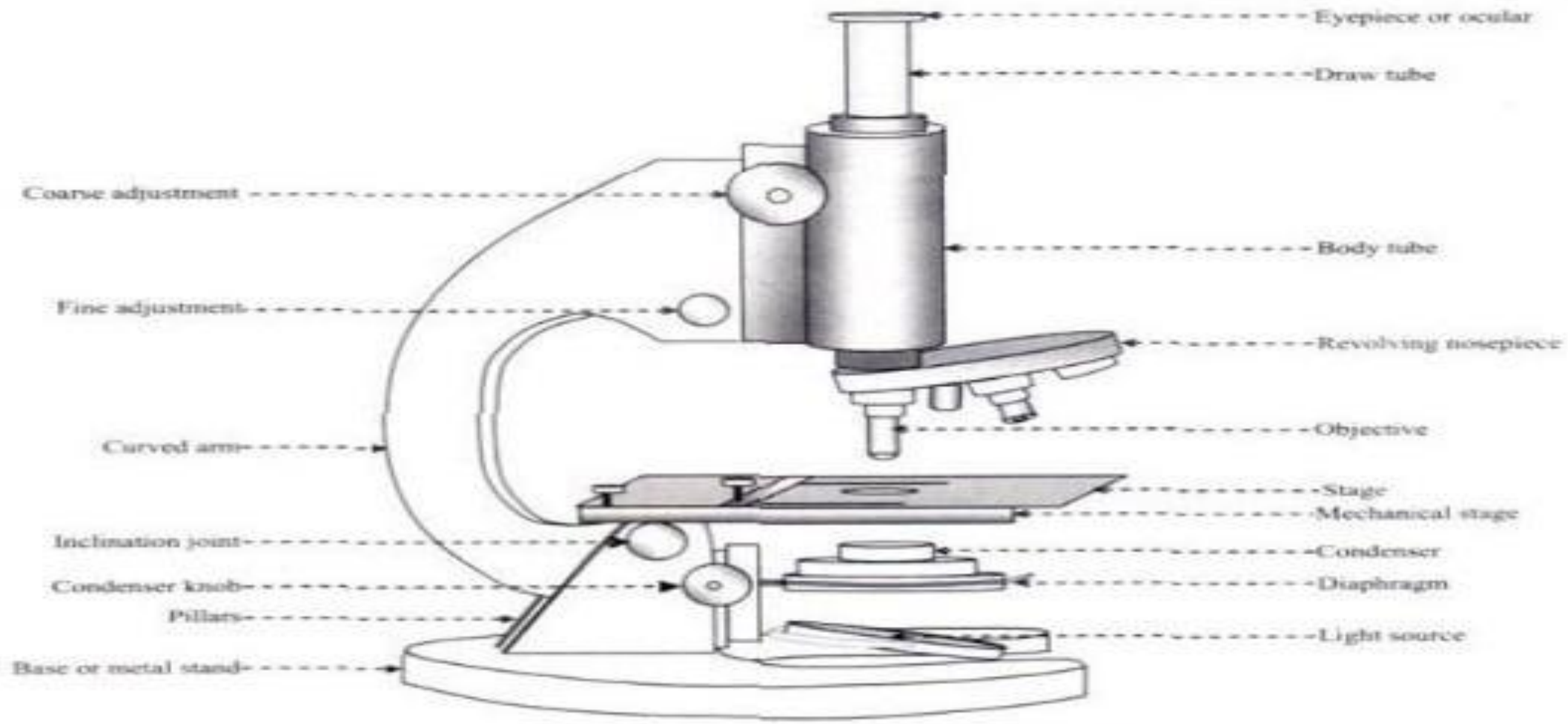


Figure 4.6: A compound microscope

Principle

A compound microscope works on the principle that when a tiny object to be magnified is placed just beyond the focus of its objective lens, a virtual, inverted and highly magnified image of the object is formed at the least distance of distinct vision from the eye held close to the eye piece.

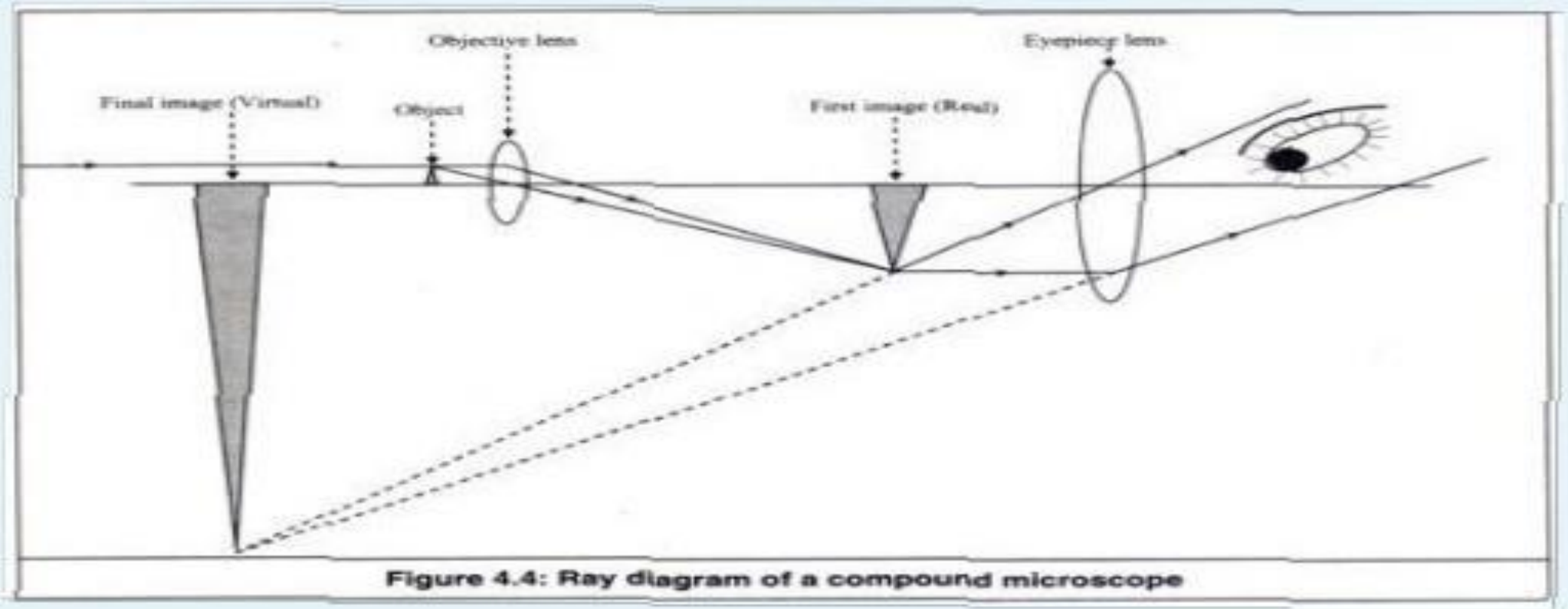
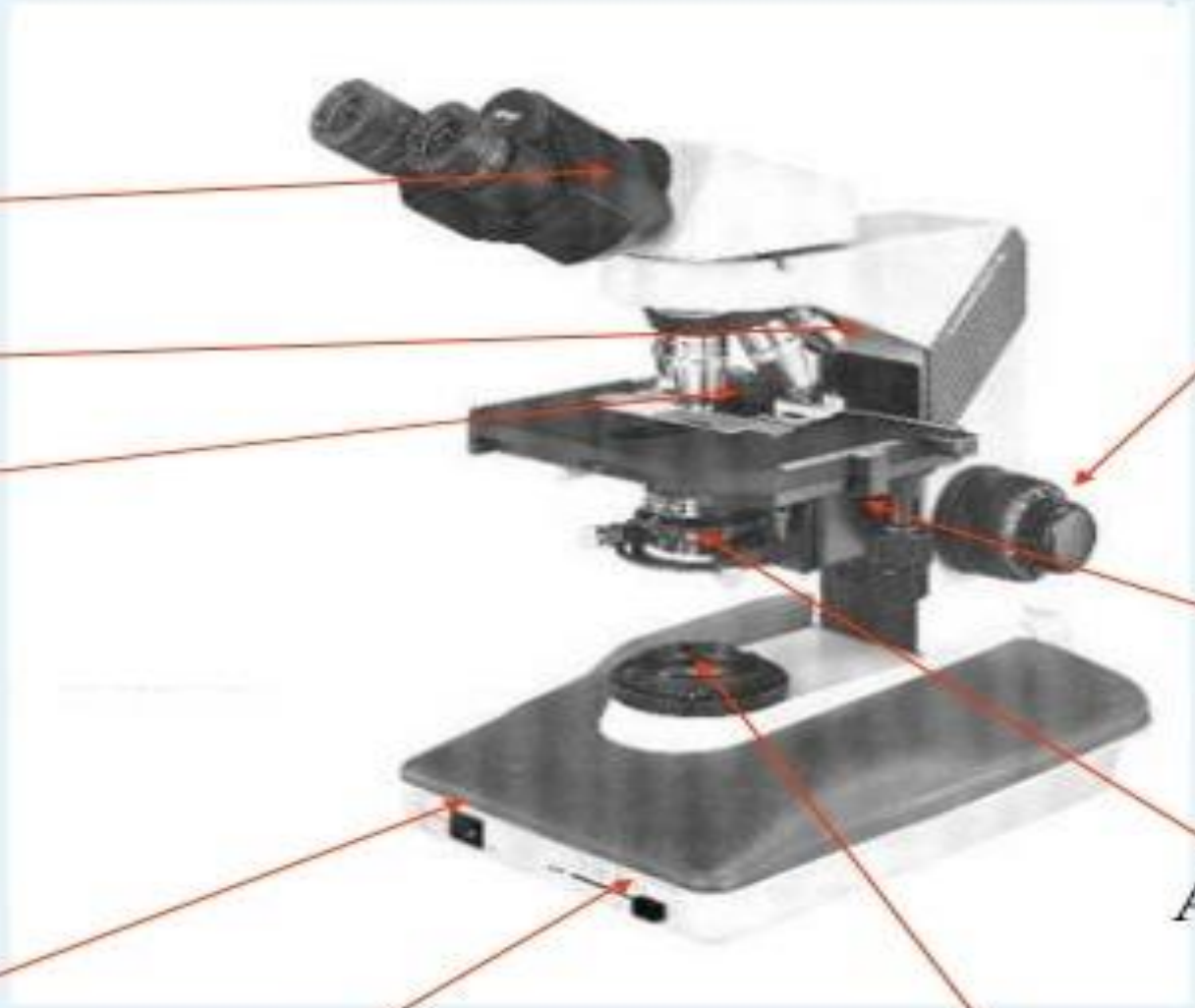


Figure 4.4: Ray diagram of a compound microscope

TERMS RELATED TO MICROSCOPE

- ***MAGNIFICATION***
  - **Degree of enlargement**
  - **No of times the length, breadth or diameter, of an object is multiplied**
  - ***RESOLUTION*** **– Ability to reveal closely adjacent structural details as separate and distinct**
  - ***LIMIT OF RESOLUTION (LR)*** **– The min distance between two visible bodies at which they can be seen as separate and not in contact with each other**

BRIGHT FIELD MICROSCOPE



Eyepiece lens

Objectives

Stage

Course and fine adjustments knobs

Stage motion control knobs

Aperture diaphragm

Power switch

Light intensity control

Field diaphragm ring

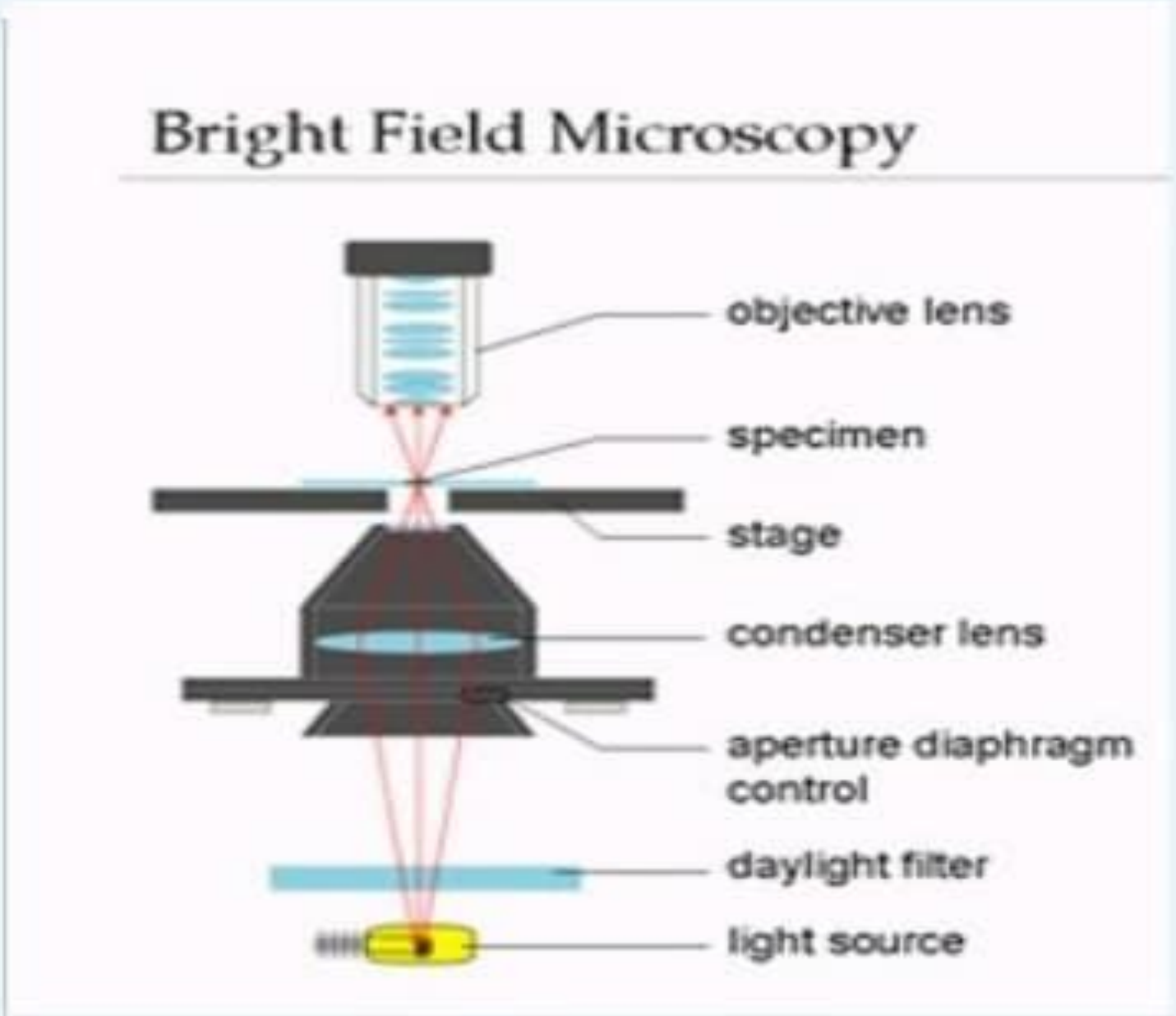
PRINCIPLE

- In bright-field microscopy a specimen is placed on the stage of the microscope and the microscope's light source is aimed at a lens beneath the specimen. This lens is called a condenser.
- The condeser usually contains an aperture diaphragm to control and focuslight on the specimen; light passes through the specimen and the is collected by an objective lens situated in a turret above the stage.

- The objective magnifies the light and transmits it to an oracular lens or eyepiece and into the user's eyes. Some of the light is absorbed by stains, pigmentation, or dense areas of the sample and this contrast allows you to see the specimen.

- For good results with this microscopic technique, the microscope should have a light source that can provide intense illumination necessary at high magnifications and lower light levels for lower magnifications

RAY DIAGRAM OF BRIGHT FIELD MICROSCOPE



ADVANTAGES

- Bright field microscopy is very simple to use with fewer adjustments needed to be made to view specimens.
- Some specimens can be viewed without staining and the optics used in the bright-field technique don't alter the colour of the specimen.
- It is adaptable with new technology and optional pieces of equipment can be implemented .

DISADVANTAGES

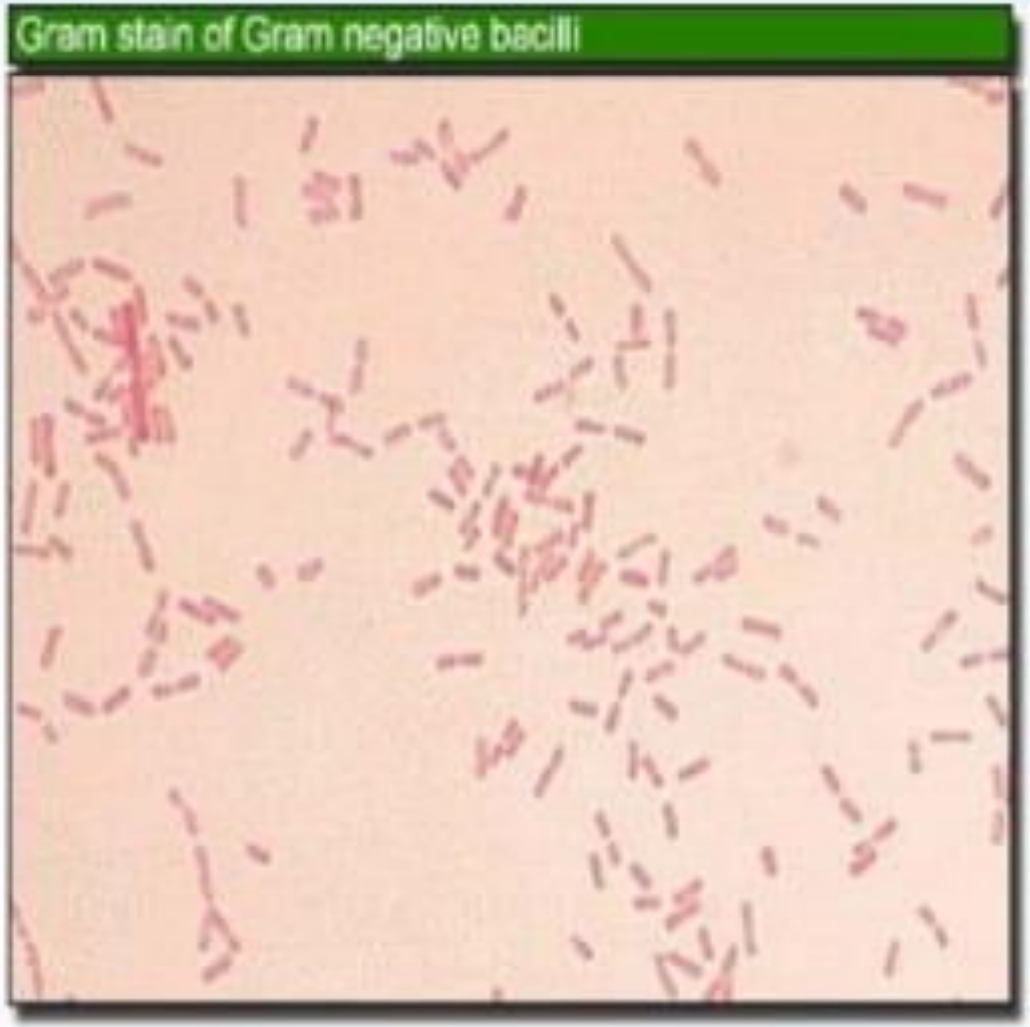
- Certain disadvantages are inherent in any optical imaging technique.
- By using an aperture diaphragm for contrast, past a certain point, greater contrast adds distortion. However, employing an iris diaphragm will help compensate for this problem.
- Bright-field microscopy can't be used to observe living specimens of bacteria, although when using fixed specimens, bacteria have an optimum viewing magnification of 1000x.

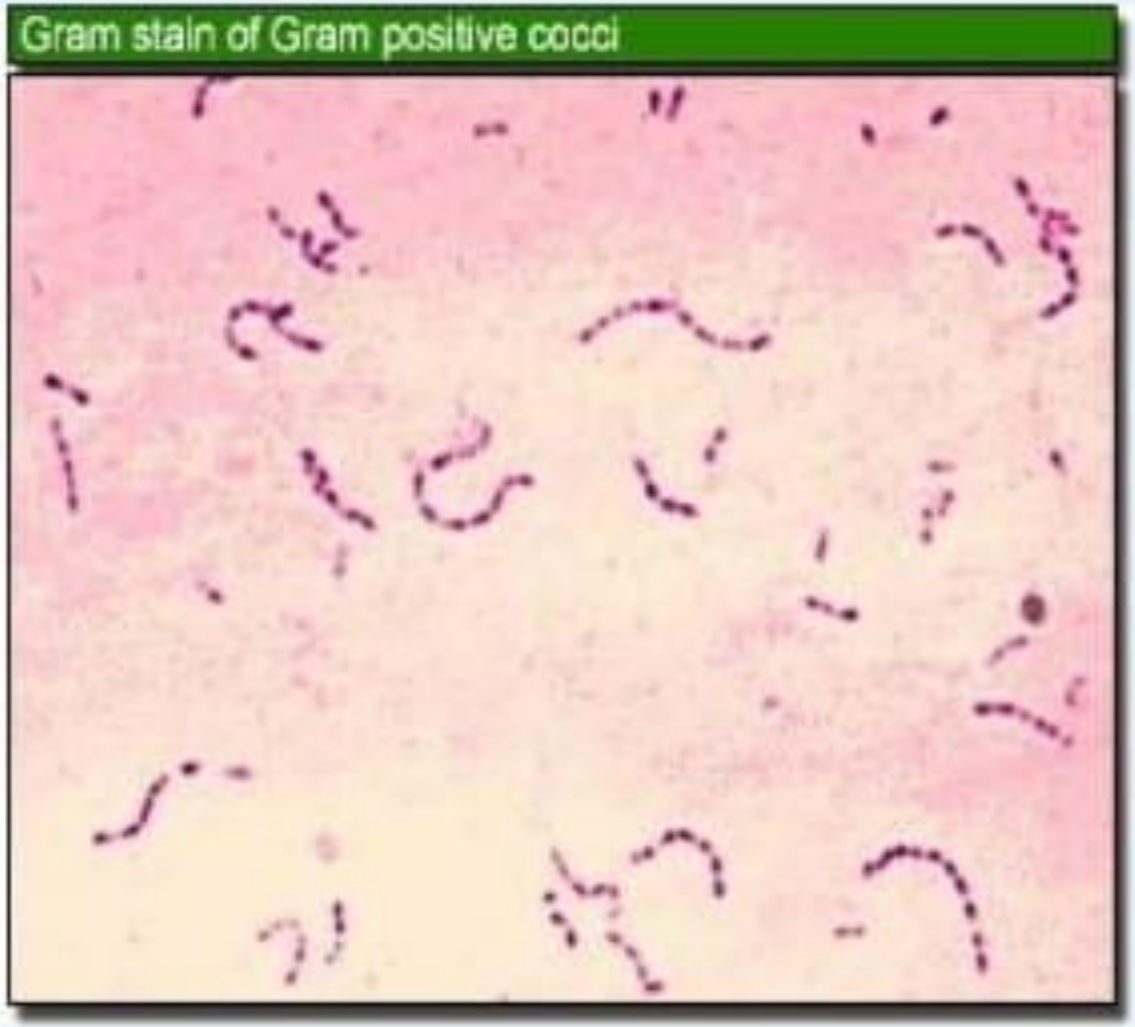
- Bright-field microscopy has very low contrast and most cells absolutely have to be stained to be seen; staining may introduce extraneous details into the specimen that should not be present.

- Also, the user will need to be knowledgeable in proper staining techniques.

- Lastly, this method requires a strong light source for high magnification applications and intense lighting can produce heat that will damage specimens or kill living microorganisms.

IMAGES FORMED BY BRIGHT FIELD MICROSCOPE





Dark Field Microscope

DARK FIELD MICROSCOPE

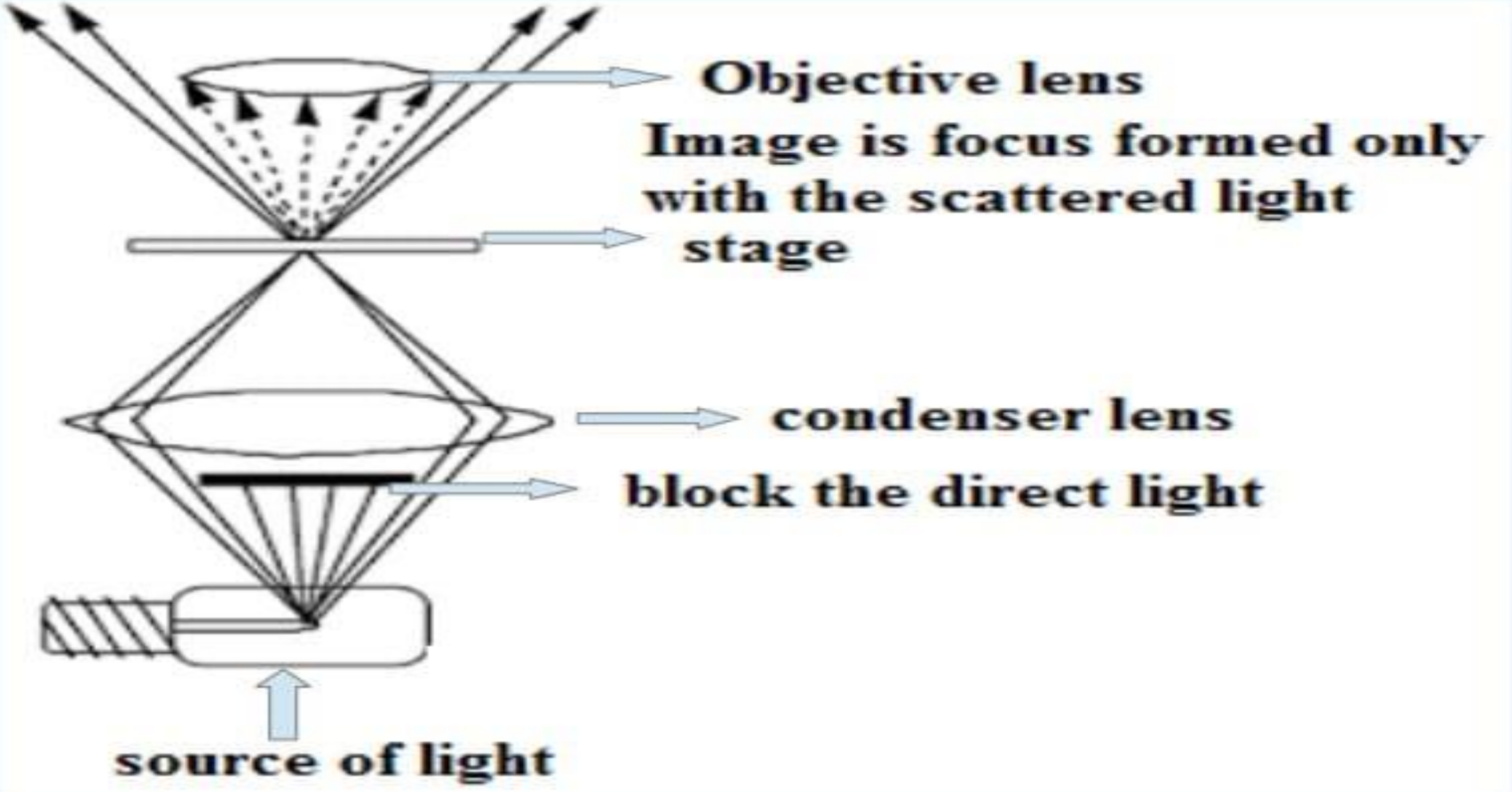
WORKING PRINCIPLE

- When observing unstained/living cells, such specimens & their components have refractive indices close to that of the medium in which they are suspended & are thus difficult to see by bright field techniques, due to their lack of contrast.

- Dark field microscopy overcomes these problems by preventing direct light from entering the front of the objective & the only light gathered is the reflected or diffracted by structures within the specimen.

- This causes the specimen to appear as a bright image on a dark background, the contrast being reversed & increased.
- Dark field permits the detection of particles smaller than the optical resolution that would be obtained in bright field, due to high contrast of scattered light.
- In the microscope, oblique light is created by using a modified or special condenser that form a hollow cone of direct light which will pass through the specimen but outside the objective.

RAY DIAGRAM OF DARK FIELDMICROSCOPE



APPLICATIONS

- Blood cells (biological dark field microscope, combined with phase contrast)
- Bacteria (biological dark field microscope, often combined with phase contrast)
- Different types of algae (biological dark field microscope)
- Hairline metal fractures (metallurgical dark field microscope)
- Diamonds and other precious stones (gemological microscope or stereo dark field microscope)
- Shrimp or other invertebrates (stereo dark field microscope)

ADVANTAGES

- A dark field microscope is ideal for viewing objects that are unstained, transparent and absorb little or no light.
- These specimens often have similar refractive indices as their surroundings, making them hard to distinguish with other illumination techniques.
- You can use dark field to study marine organisms such as algae and plankton, diatoms, insects, fibers, hairs, yeast and protozoa as well as some minerals and crystals, thin polymers and some ceramics.

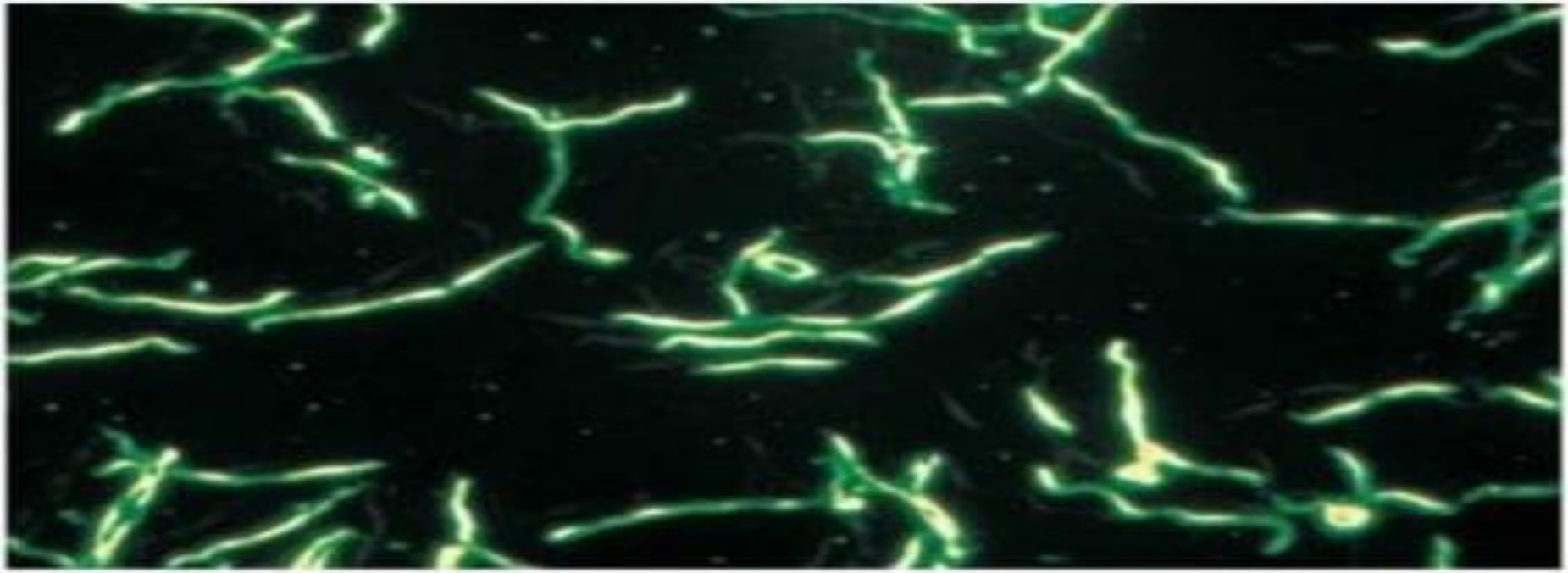
- You can also use dark field in the research of live bacterium, as well as mounted cells and tissues.
- It is more useful in examining external details, such as outlines, edges, grain boundaries and surface defects than internal structure.
- Dark field microscopy is often dismissed for more modern observation techniques such as phase contrast , which provide more accurate, higher contrasted images and can be used to observe a greater number of specimens.
- Recently, dark field has regained some of its popularity when combined with other illumination techniques, such as fluorescence, which widens its possible employment in certain fields.

DISADVANTAGES

- First, dark field images are prone to degradation.
- A specimen that is not thin enough or its density differs across the slide, may appear to have artifacts throughout the image.
- The preparation and quality of the slides can grossly affect the contrast and accuracy of a dark field image.
- You need to take special care that the slide, stage, nose and light source are free from small particles such as dust, as these will appear as part of the image.

- Similarly, if you need to use oil or water on the condenser and/or slide, it is almost impossible to avoid all air bubbles.
- These liquid bubbles will cause images degradation, flare and distortion and even decrease the contrast and details of the specimen.
- Dark field needs an intense amount of light to work. This, coupled with the fact that it relies exclusively on scattered light rays, can cause glare and distortion.
- It is not a reliable tool to obtain accurate measurements of specimens.
- Finally, numerous problems can arise when adapting and using a dark field microscope. The amount and intensity of light, the position, size and placement of the condenser and stop need to be correct to avoid any aberrations.

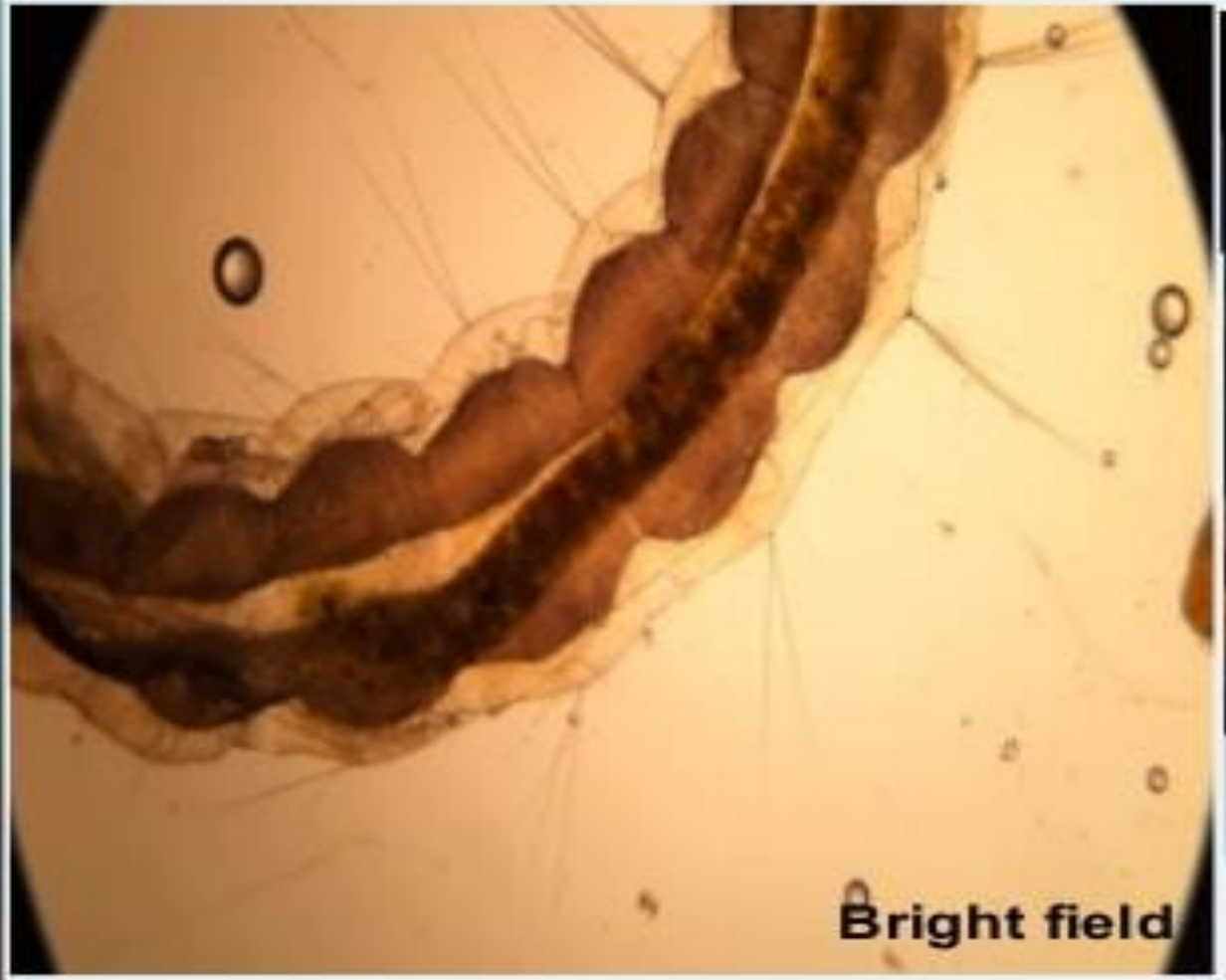
IMAGES FORMED BY DARK FIELD MICROSCOPE



(a) *T. pallidum*

Treponema vincenti

Different between Bright and Dark fielf Microscopy



ELECTRON MICROSCOPE

Electron microscope is a type of microscope that uses a particle beam of electrons to illuminate a specimen & create a highly-magnified image.

Electron microscopes have much greater resolving power than light microscopes & can obtain much higher magnifications up to 2 million times, while the best light microscopes are limited to magnifications of 2000 times.

Two Type

- Transmission Electron Microscope
- Scanning Electron Microscope

Cont.....

TRANSMISSION ELECTRON MICROSCOPE (TEM)

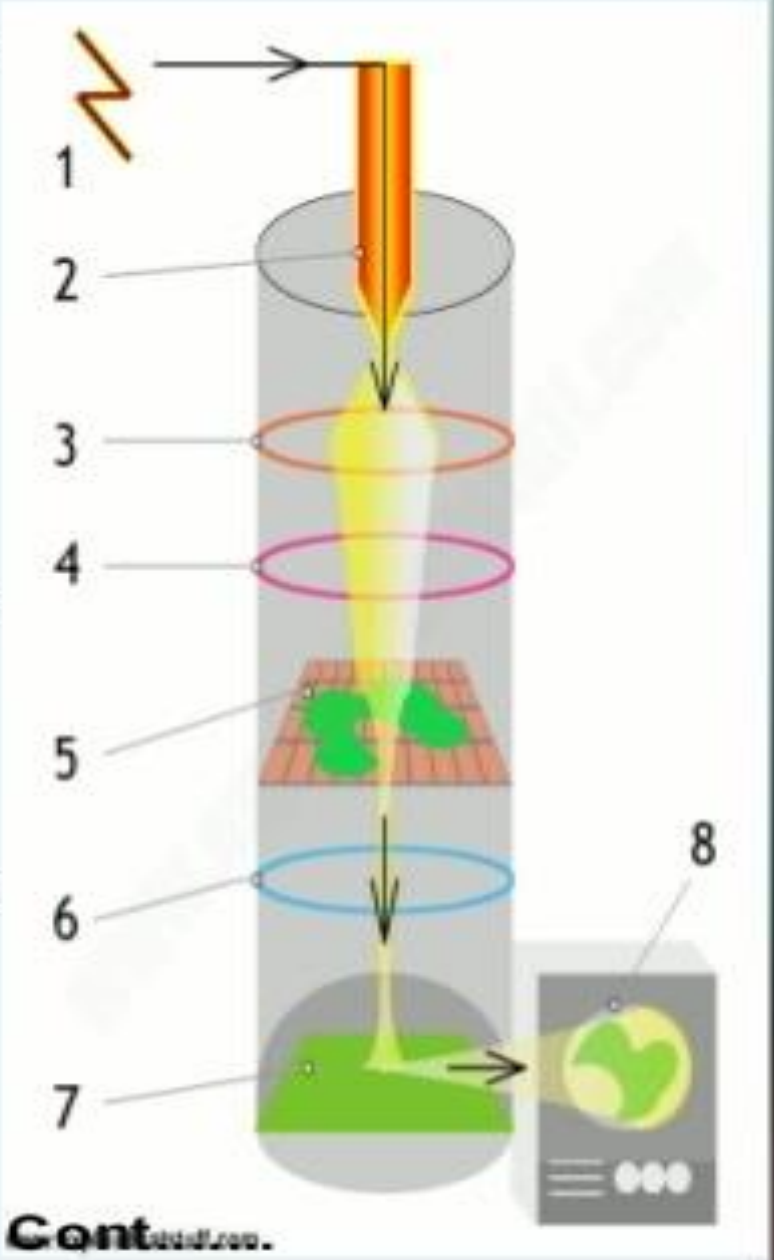
WORKING PRINCIPLE

- Electrons possess a wave like character.
- Electrons emitted into vacuum from a heated filament with increased accelerating potential will have small wavelength.
- Such higher-energy electrons can penetrate distances of several microns into a solid.
- If these transmitted electrons could be focused -images with much better resolution.
- Focusing relies on the fact that, electrons also behave as negatively charged particles and are therefore deflected by electric or magnetic fields.

TRANSMISSION ELECTRON MICROSCOPE WORK

A transmission electron microscope fires a beam of electrons through a specimen to produce a magnified image of an object.

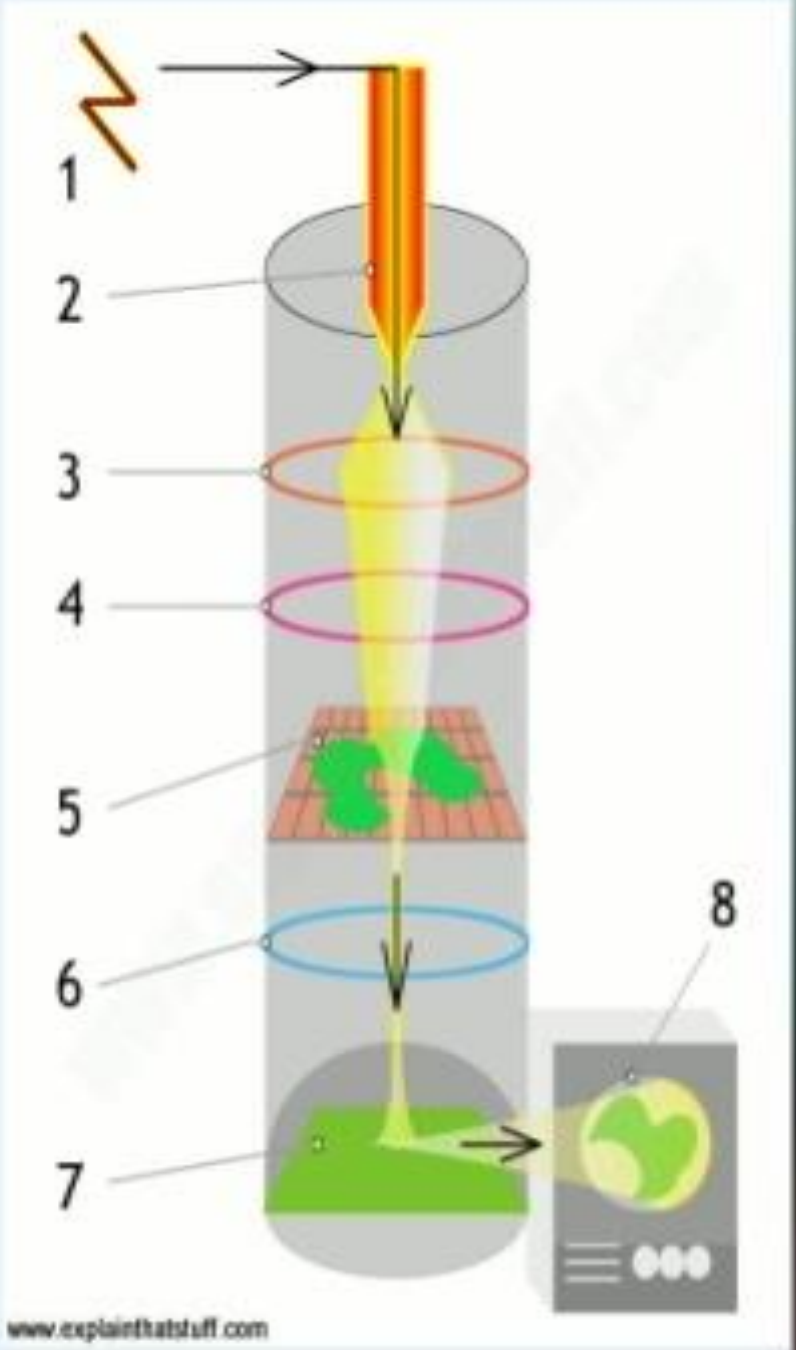
1) A transmission electron microscope fires a beam of electrons through a specimen to produce a magnified image of an object.
2) A high-voltage electricity supply powers the cathode.
3) The cathode is a heated filament, a bit like the electron gun in an old-fashioned cathode- ray tube (CRT) TV. It generates a beam of electrons that works in an analogous way to the beam of light in an optical microscope.
4) An electromagnetic coil (the first lens) concentrates the electrons into a more powerful beam.
5) Another electromagnetic coil (the second lens) focuses the beam onto a certain part of the specimen.



6) The specimen sits on a copper grid in the middle of the main microscope tube. The beam passes through the specimen and "picks up" an image of it.

7) The image becomes visible when the electron beam hits a fluorescent screen at the base of the machine. This is analogous to the phosphor screen at the front of an old-fashioned TV .

8) The image can be viewed directly (through a viewing portal), through binoculars at the side, or on a TV monitor attached to an image intensifier (which makes weak images easier to see).



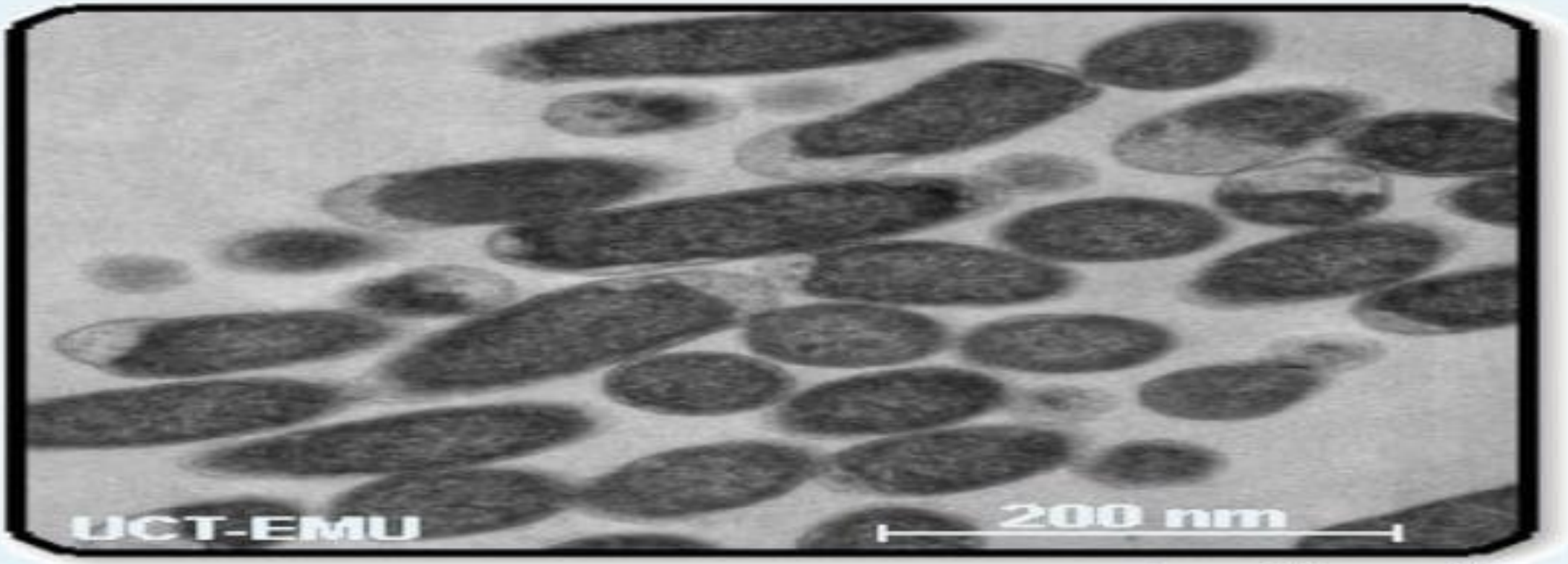
ADVANTAGES

- TEMs offer very powerful magnification and resolution.
- TEMs have a wide-range of applications and can be utilized in a variety of different scientific, educational and industrial fields
- TEMs provide information on element and compound structure .
- Images are high-quality and detailed.

DISADVANTAGES

- TEMs are large and very expensive.
- Laborious sample preparation.
- Operation and analysis requires special training.
- Samples are limited to those that are electron transparent.
- TEMs require special housing and maintenance.
- Images are black and white .

IMAGES FORMED BY TRANSMISSION ELECTRON MICROSCOPE



E. coli bacteria

SCANNING ELECTRON MICROSCOPE (SEM)

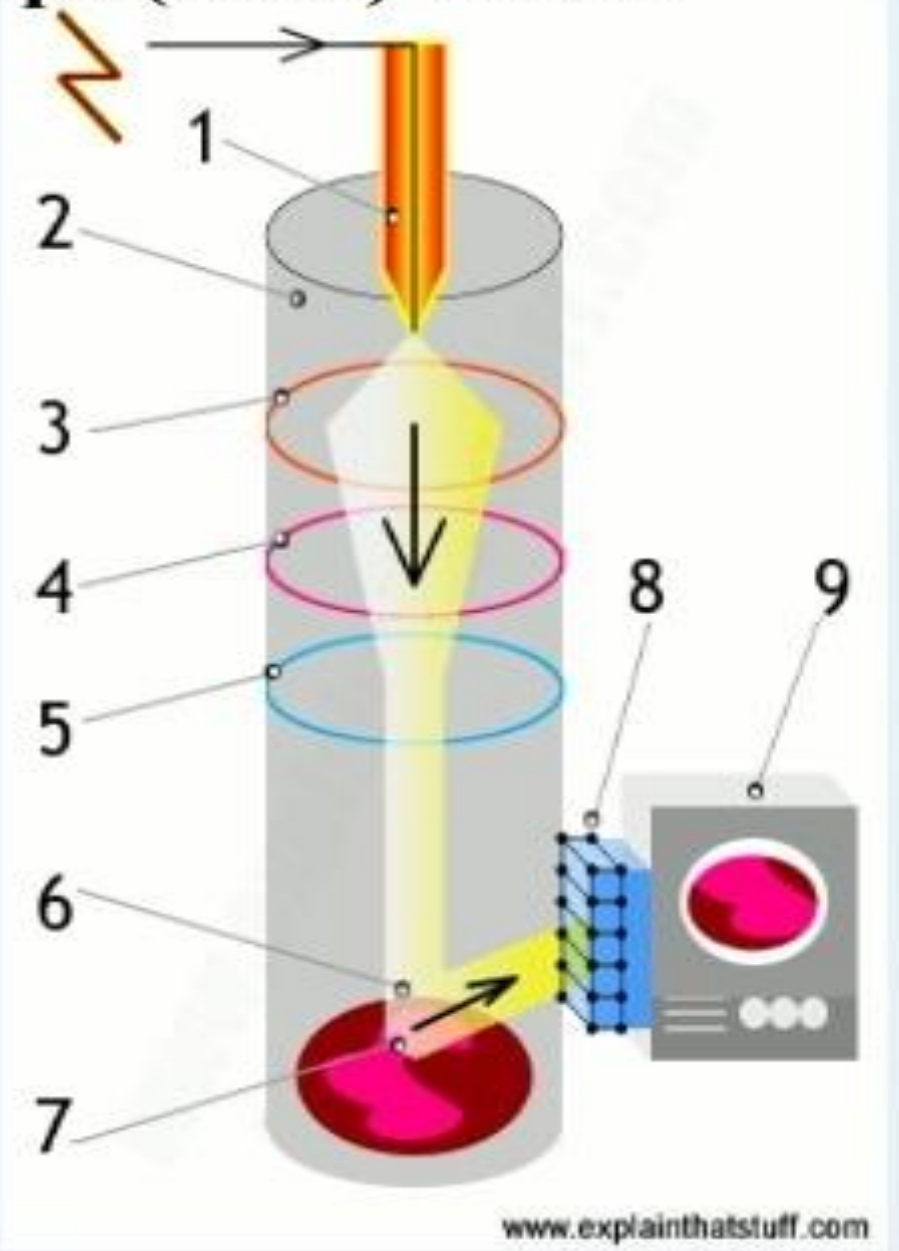
WORKING PRINCIPLE

- Incoming (primary) electrons
  a) can be "reflected" (backscattered) from a bulk specimen.
  b) can release secondary electrons.
- Primary electrons are focused into a small-diameter electron probe that is scanned across the specimen.
- Electrostatic or magnetic fields, applied at right angles to the beam, can be used to change its direction of travel.
- By scanning simultaneously in two perpendicular directions, a square or rectangular area of specimen (known as a raster) can be covered.
- Image of this area can be formed by collecting secondary electrons from each point on the specimen.

How a Scanning Electron Microscope (SEM) works

A scanning electron microscope scans a beam of electrons over a specimen to produce a magnified image of an object. That's completely different from a TEM, where the beam of electrons goes right through the specimen.

1) Electrons are fired into the machine.
2) The main part of the machine (where the object is scanned) is contained within a sealed vacuum chamber because precise electron beams can't travel effectively through air.
3) A positively charged electrode (anode) attracts the electrons and accelerates them into an energetic beam.
4) An electromagnetic coil brings the electron beam to a very precise focus, much like a lens.



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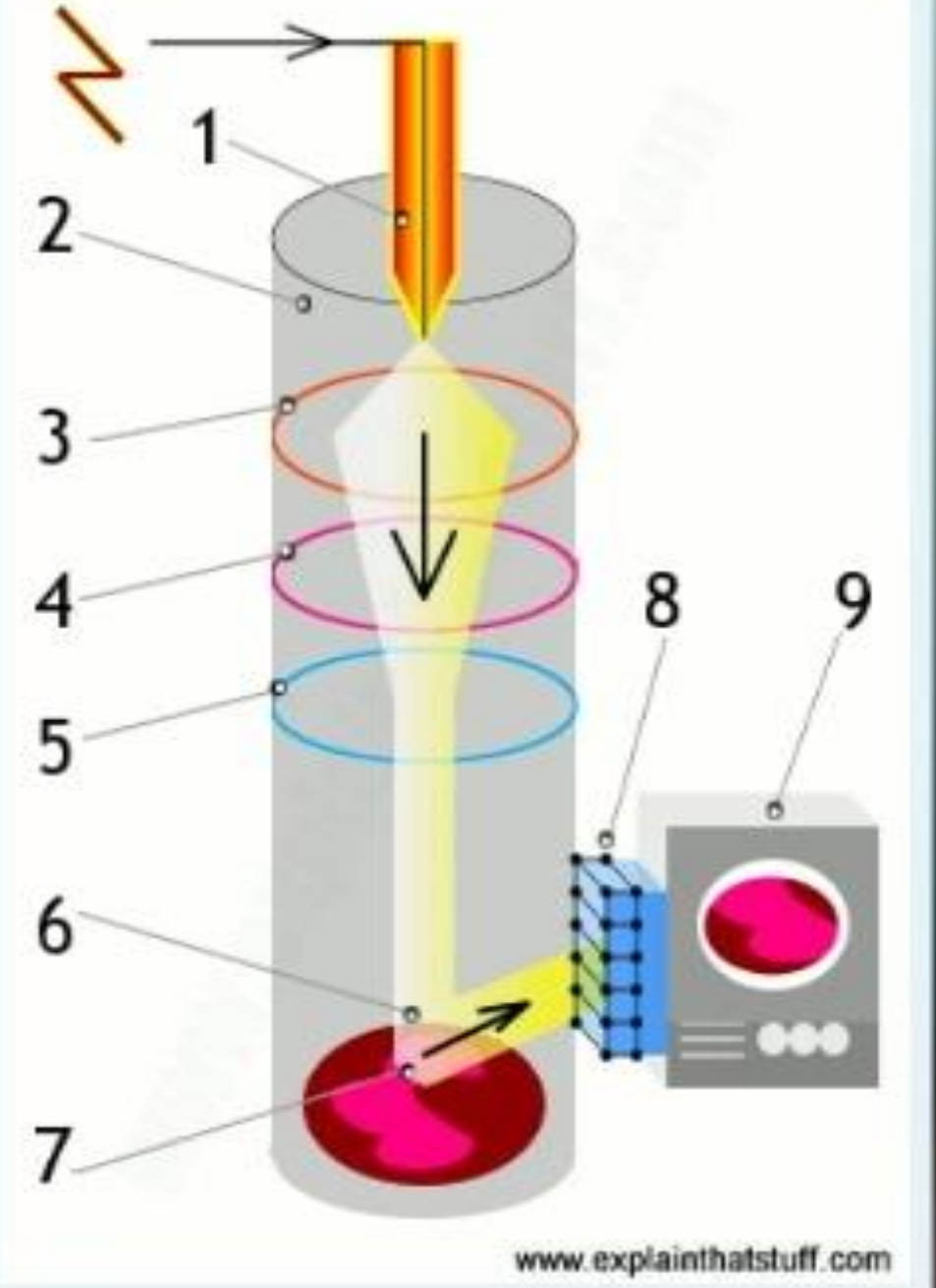
5) Another coil, lower down, steers the electron beam from side to side.

6) The beam systematically scans across the object being viewed.

7) Electrons from the beam hit the surface of the object and bounce off it.

8) A detector registers these scattered electrons and turns them into a picture.

9) A hugely magnified image of the object is displayed on a TV screen. Scanning tunneling microscopes (STMs)



ADVANTAGES

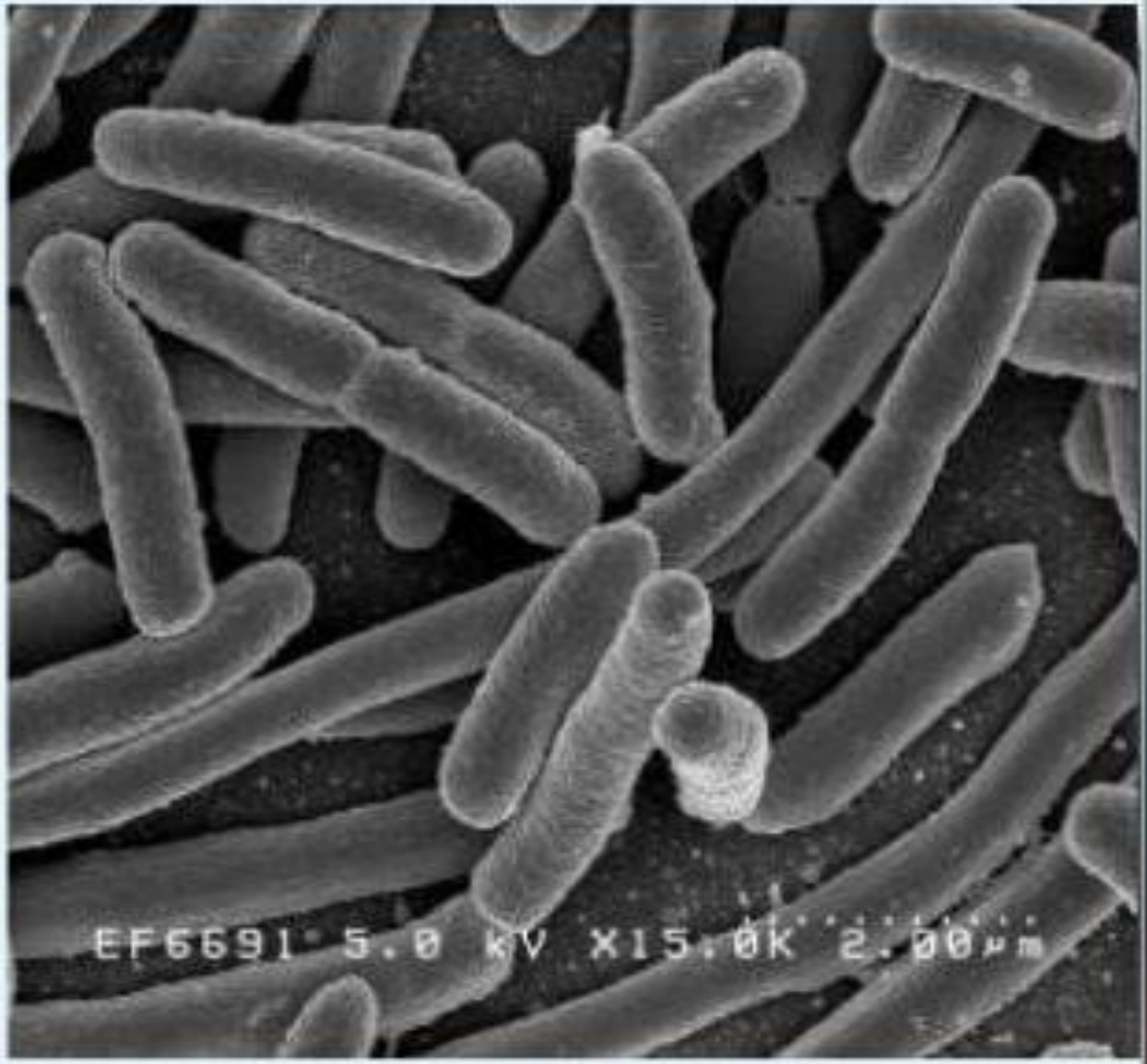
- It gives detailed 3D and topographical imaging and the versatile information garnered from different detectors.
- This instrument works very fast.
- Modern SEMs allow for the generation of data in digital form.
- Can magnify up to 100,000x
- Most SEM samples require minimal preparation actions.

DISADVANTAGES

- SEMs are expensive and large.
- Special training is required to operate an SEM.
- The preparation of samples can result in artifacts.
- SEMs are limited to solid samples.
- SEMs carry a small risk of radiation exposure associated with the electrons that scatter from beneath the sample surface.

IMAGE FORMEDBY SCANNING ELECTRON MICROSCOPE

Salmonella typhimurium (red)



E. coli bacteria

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2. Frazier, W.C. and Westhoff, D.C. (1988). Food Microbiology. 4th Edition.McGraw Hill, NY.

Thank You