Instrumentation

B.Sc Botany

Semester I

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

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

Other Uses of Microscope

. Tissue Analysis

. Examining Forensic Evidence

. Studying the Role of a Protein within a Cell

. Studying atomic structures

.etc

Types of Microscopes

The microscopes basically are of two types depending on the lens system or on the basis of lens combination.

Simple Microscope

This type of microscopes consists only a single lens, there is no two or more lens combinati

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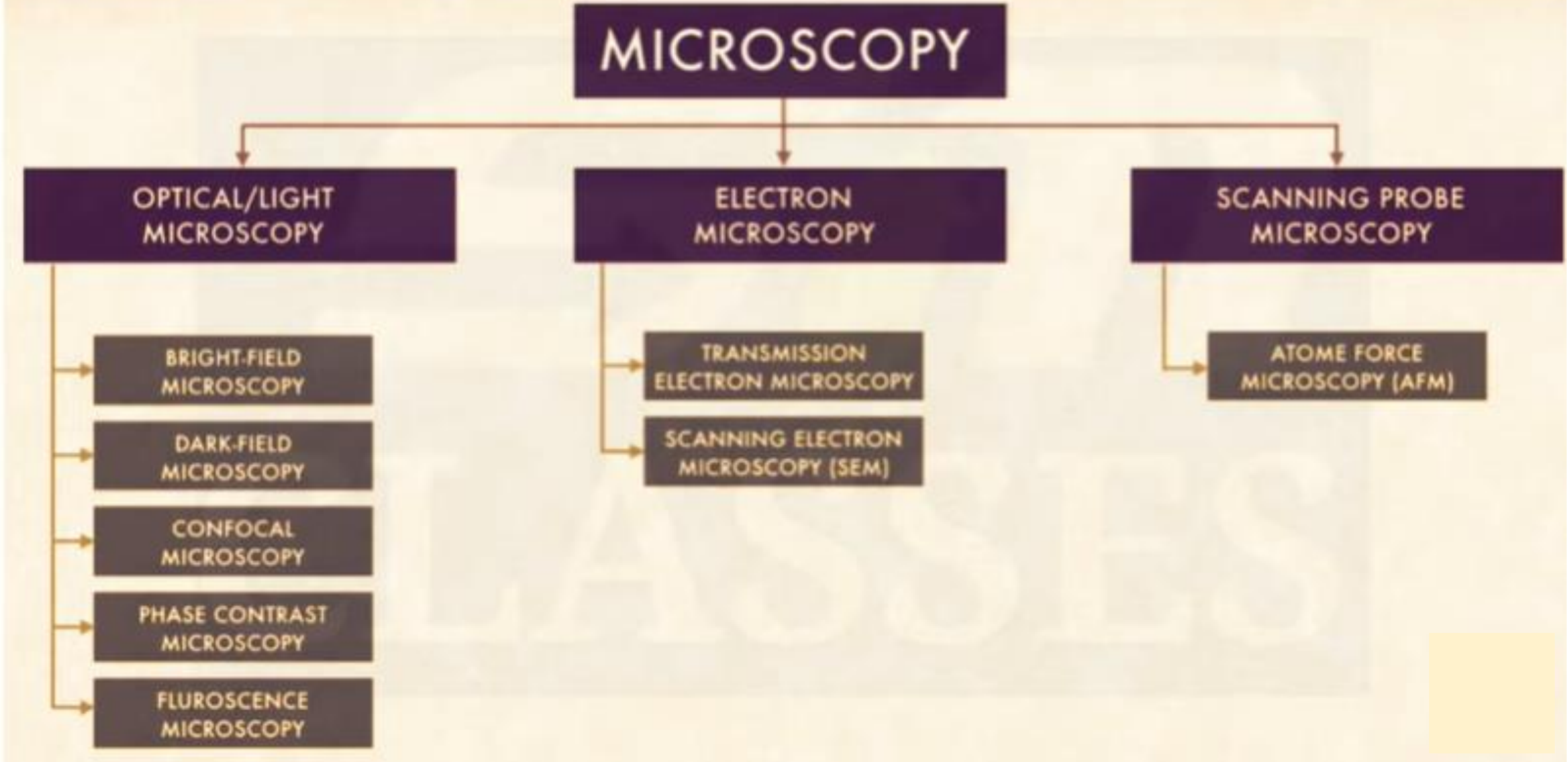
Compound Microscope This type of microscopes consists two or more lens combination.

Depending upon the source of illumination, the microscopes can be classified as



OPTICAL/LIGHT Microscopy

Light is used for visualisation of microorganisms, cellular processes,objects etc.

ELECTRON Microscopy

Electron beam is used for visualisation of microorganisms, cellular processes,objects etc.

SCANNING PROBE Microscopy

Probe is used to scan the specimen surface

Main Components of Microscope

1. Light
2. Lens

Simple Microscope

Compound Microscope

Compound Microscope

The two main salient features of microscopes are

1- Magnification Power

2- Resolution Power

Magnification Power

Magnification Power is the ability to enlarge any object being viewed or it is an ability of the microscope to create a larger view of any object. Magnification power depends on Size and curvature of the lenses.

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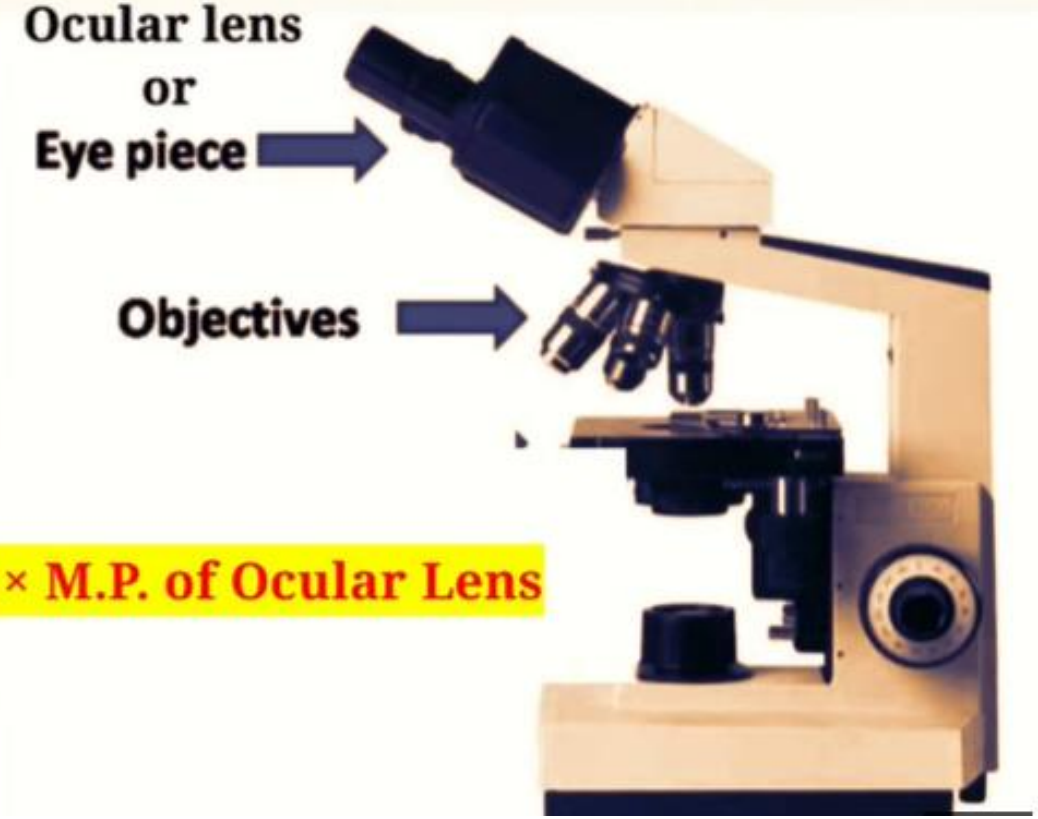
Magnification Power is the ability to enlarge any object being viewed or it is an ability of the microscope to create a larger view of any object. Magnification power depends on Size and curvature of the lenses.

Total magnification of any microscope is calculate by multiplying the magnification power of objective lens to the ocular lens.

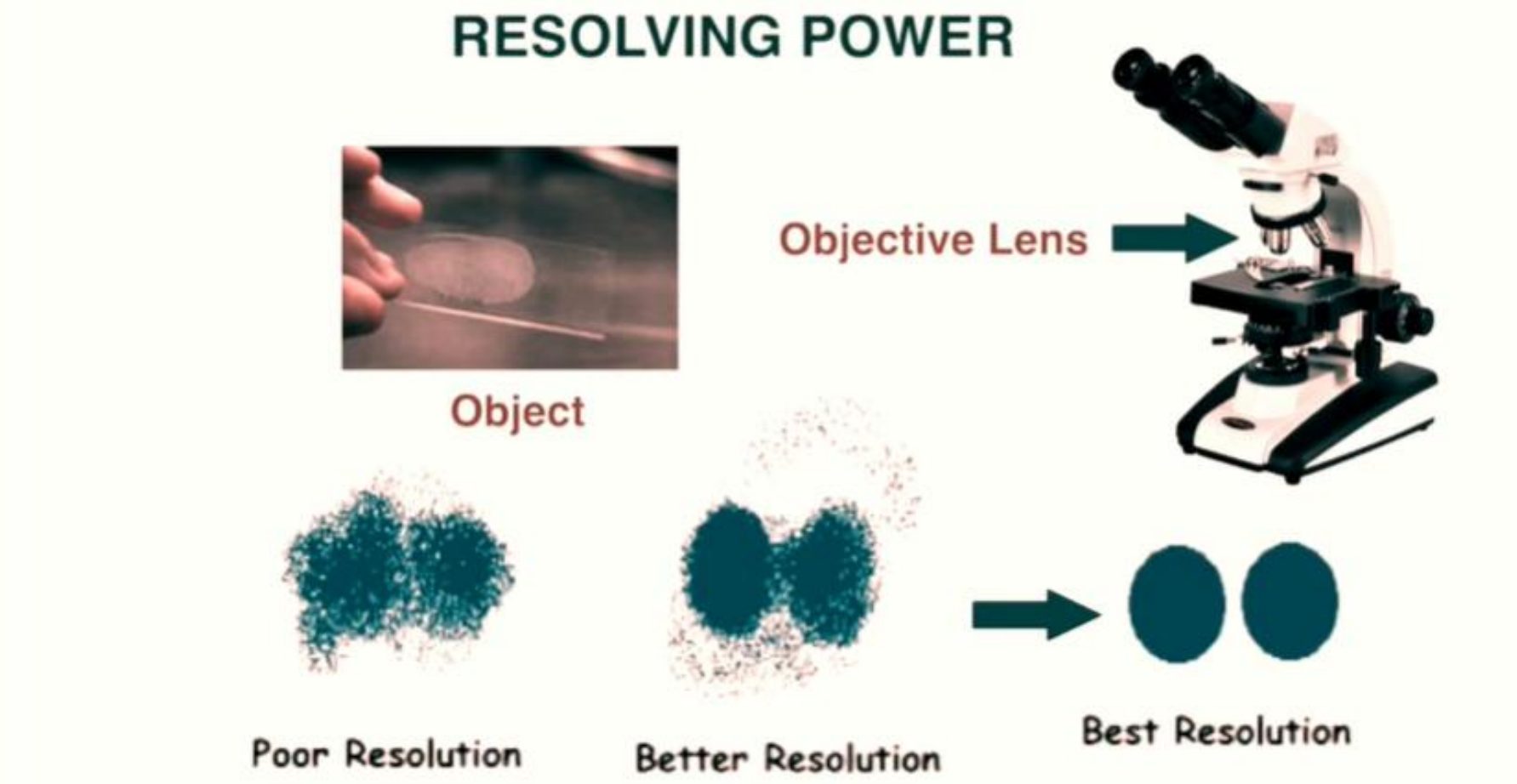


Total Magnification Power = M.P. of Objective Lens × M.P. of Ocular Lens

Resolving Power

Resolution (also called resolving power) is the ability of the lenses to distinguish fine detail and structure

Specifically, it refers to the ability of the lenses to distinguish two points a specified distance apart.



Resolving Power

Resolution (also called resolving power) is the ability of the lenses to distinguish fine detail and structure. Specifically, it refers to the ability of the lenses to distinguish two points a specified distance apart.

For example, if a microscope has a resolving power of 0.4 nm, it can distinguish two points if they are at least 0.4 nm apart.

The resolution power of any microscope is depend on the wavelength of the light or rays used for image formations and on the optical quality of the lenses.

Resolution Power = $0.5\,\lambda / N.A.$

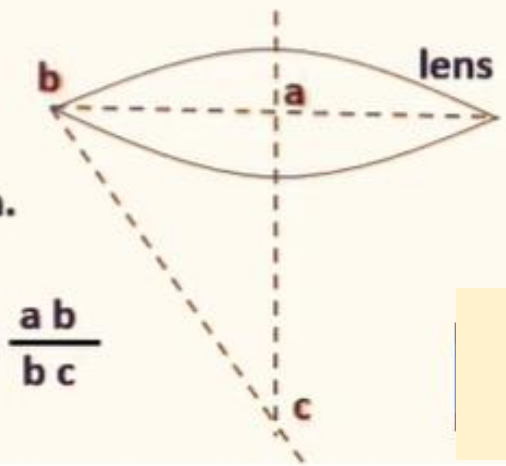
λ = Wavelength of rays

N. A. = Numerical Aperture of Objective lens

Numerical Aperture = $\eta \sin\theta$

η = Refractive index of the medium through which light entering to the lens medium.

Sin θ depends on lense



$$\Theta = \frac{a\,b}{b\,c}$$

Factors Affecting
Limit of Resolution/Resolving Power

. Wavelength of Light

. Refractive Index

. Angular Aperture

Limit of resolution : The smallest separation (linear or angular) between two point objects at which they appear just separated is called the limit of resolution of an optical instrument.

Resolving power : The reciprocal of the limit of resolution of an optical instrument is known as the resolving power of that instrument.

Equations

- ↑ Limit of resolution $= \dfrac{0.61\lambda \uparrow}{n \times \sin\alpha}$

λ = Wavelength of Light

n = Refractive Index

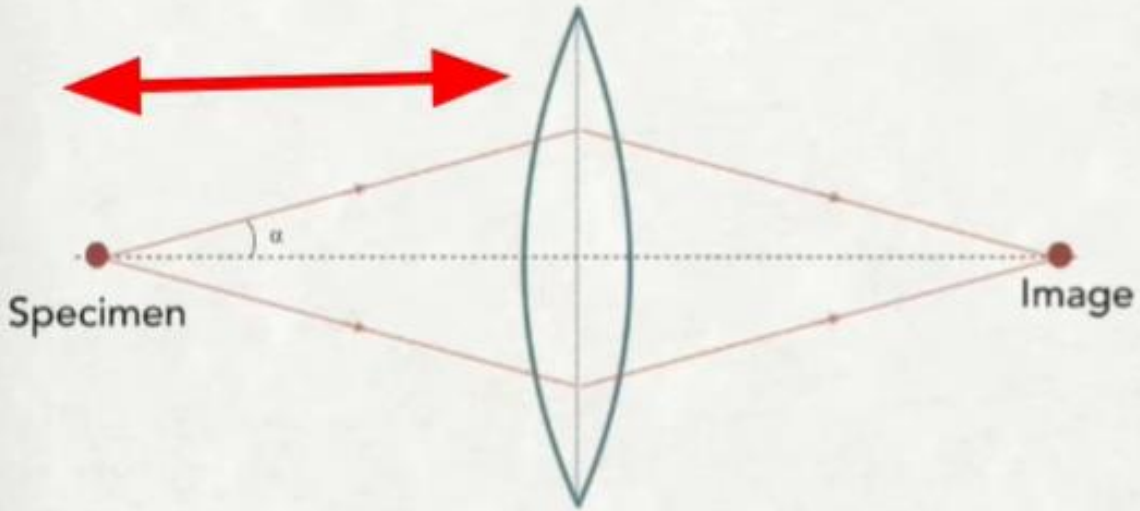
$\sin\alpha$ = Angular Aperture

- ↓ Resolving Power $\propto \dfrac{1}{\text{Limit of Resolution} \uparrow}$

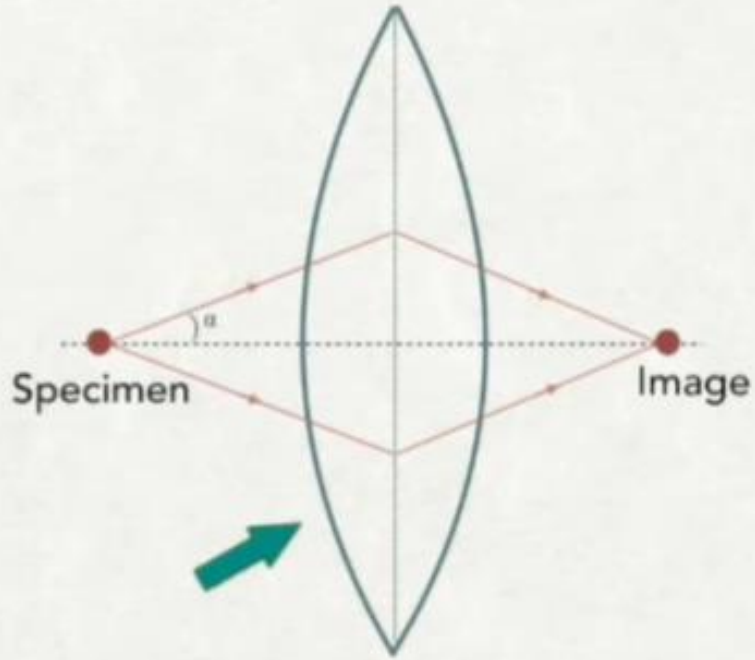
Note:

- n x $\sin\alpha$ is called Numerical Aperture (NA)
- NA is a measure of the ability of a lens to collect light from the specimen
- Lenses with low NA collect less light than Lenses with high NA

RESOLVING POWER



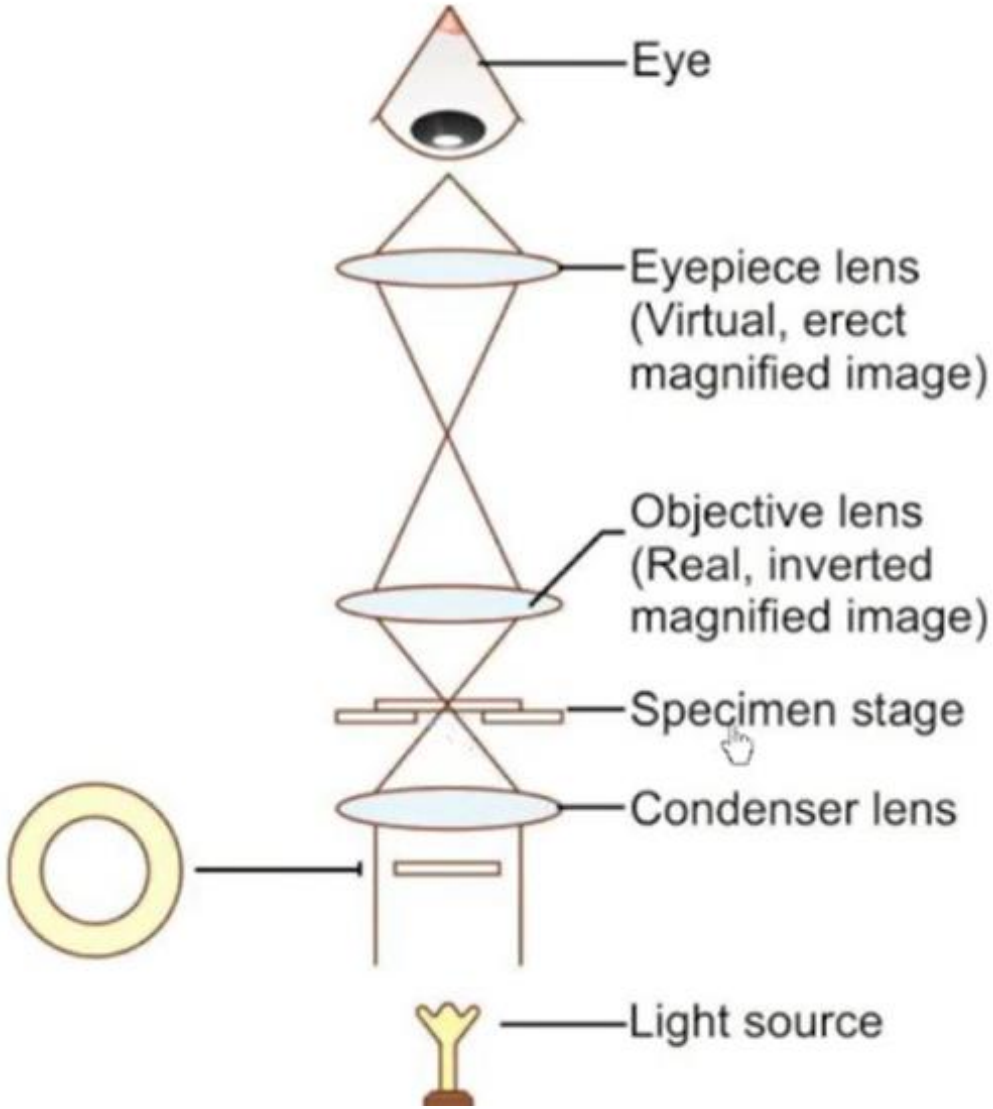
Objective Lens, *Low Aperture*

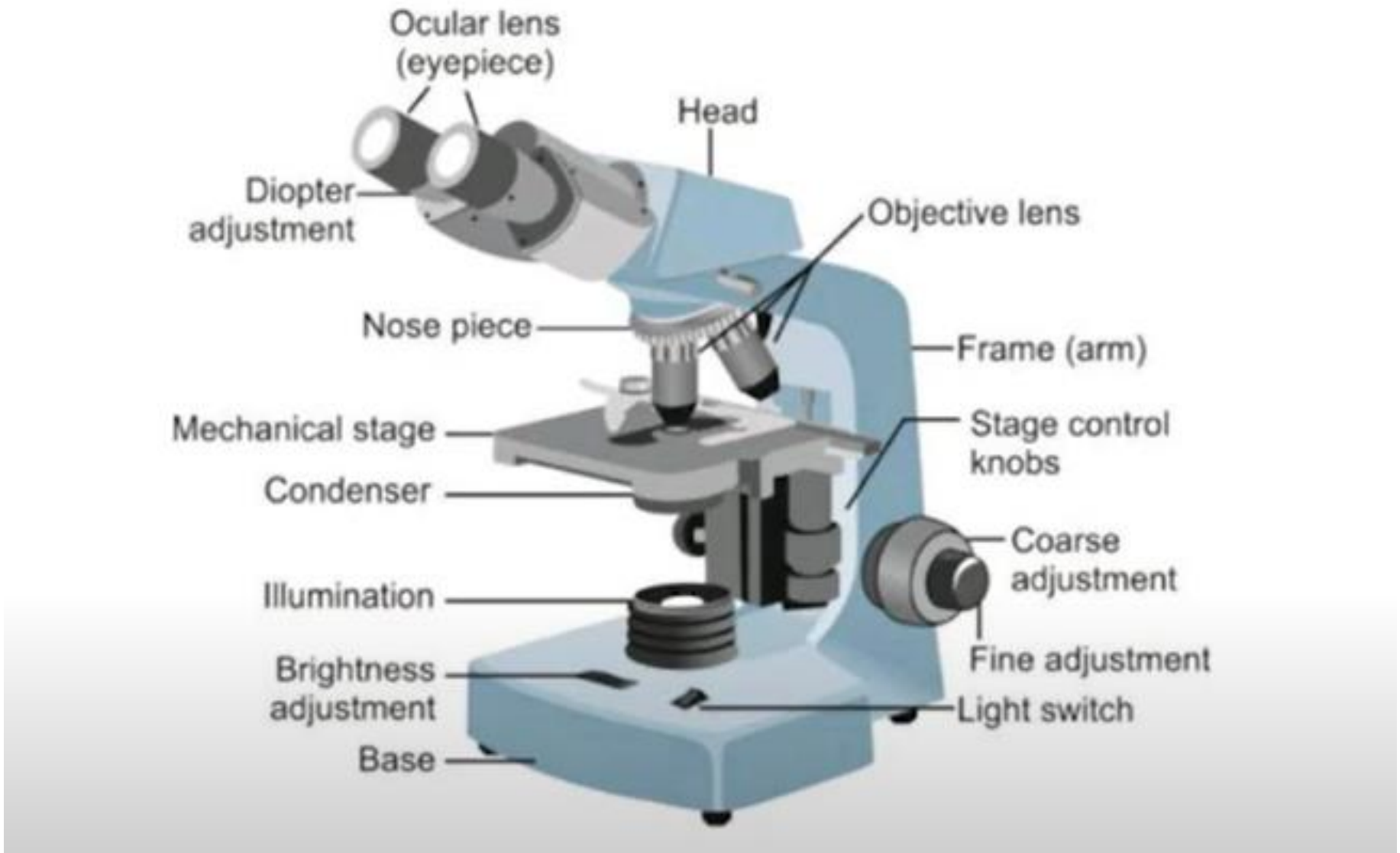


Objective Lens, *High Aperture*

LIGHT MICROSCOPY


1
2
3
9
6
4
8
5
7


Eye
Eyepiece lens
(Virtual, erect
magnified image)
Objective lens
(Real, inverted
magnified image)
Specimen stage
Condenser lens
Light source


Ocular lens
(eyepiece)
Head
Diopter
adjustment
Objective lens
Nose piece
Frame (arm)
Mechanical stage
Stage control
knobs
Condenser
Coarse
adjustment
Illumination
Fine adjustment
Brightness
adjustment
Light switch
Base

Bright Field Microscope

↓

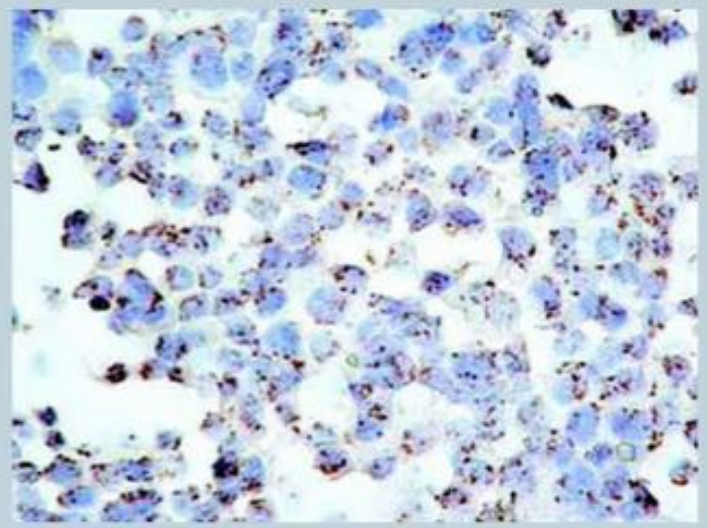
Transparent Specimen

↓

Stain

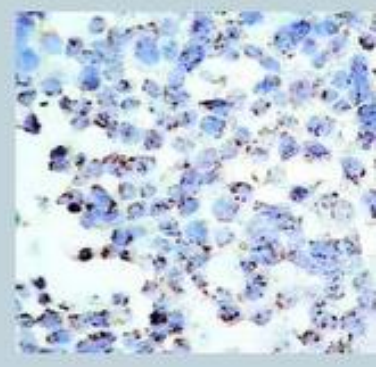
↓

Toxic → Kill



Phase Contrast Microscopy

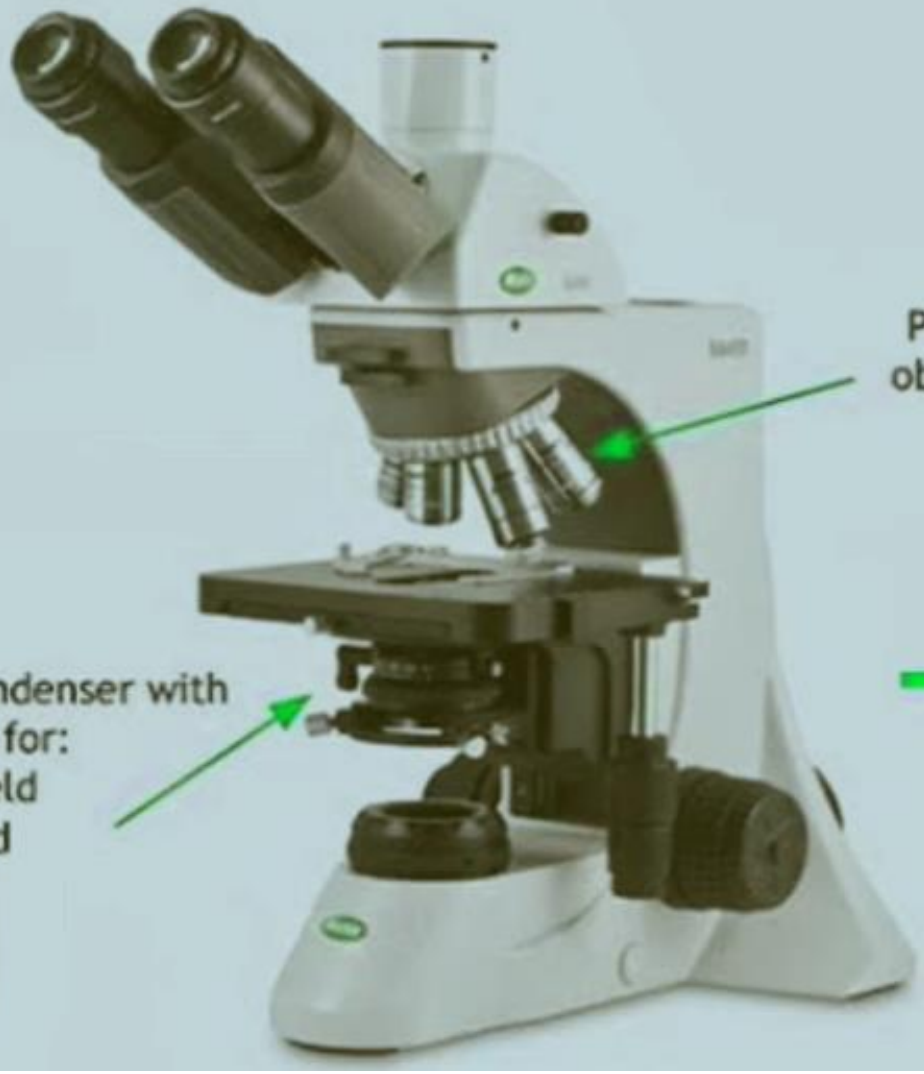
Transparent Specimen



No Stain Required

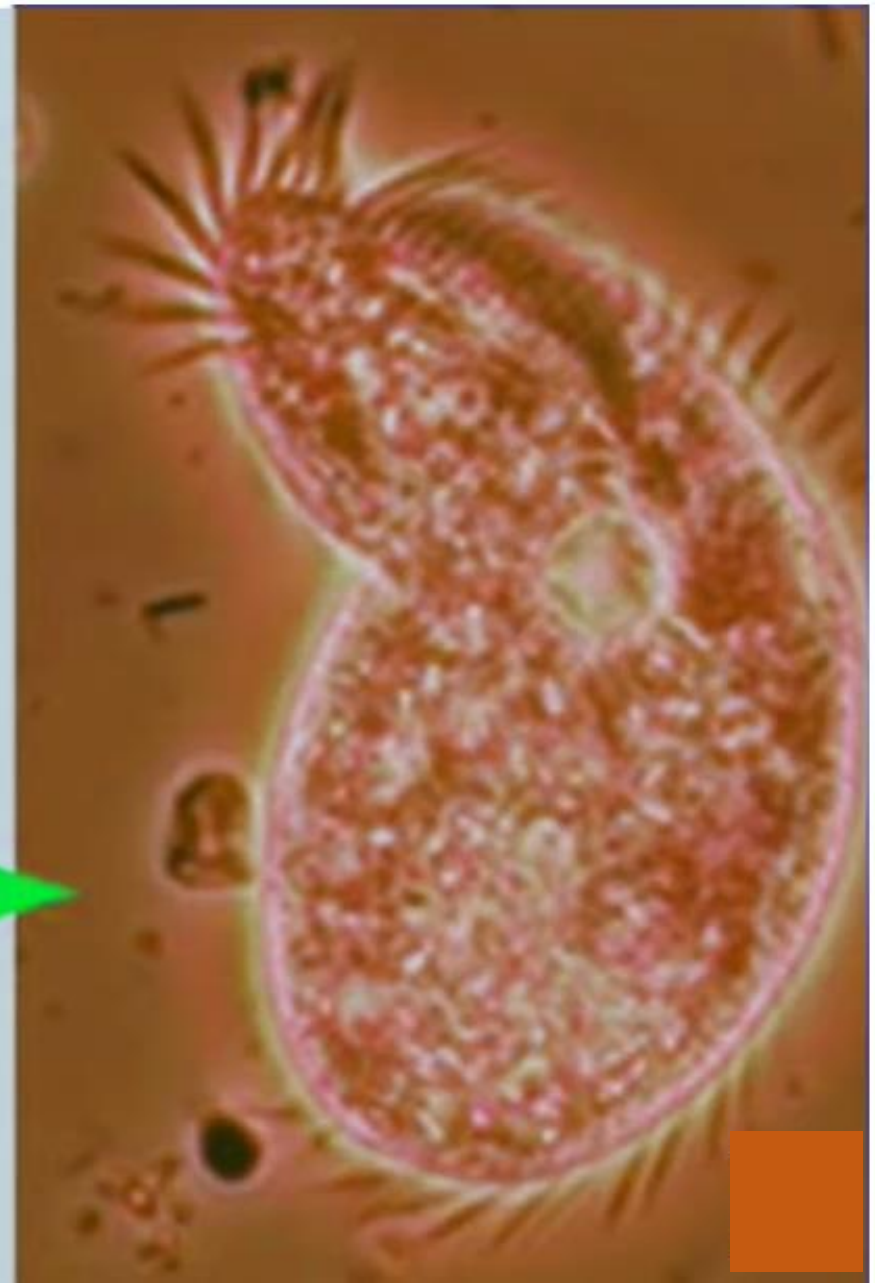
Light Intensity Variation

Phase-contrast Microscopy



Phase contrast objective lenses

Phase condenser with positions for:
-Brightfield
-Darkfield
-Phase 1
-Phase 2
-Phase 3

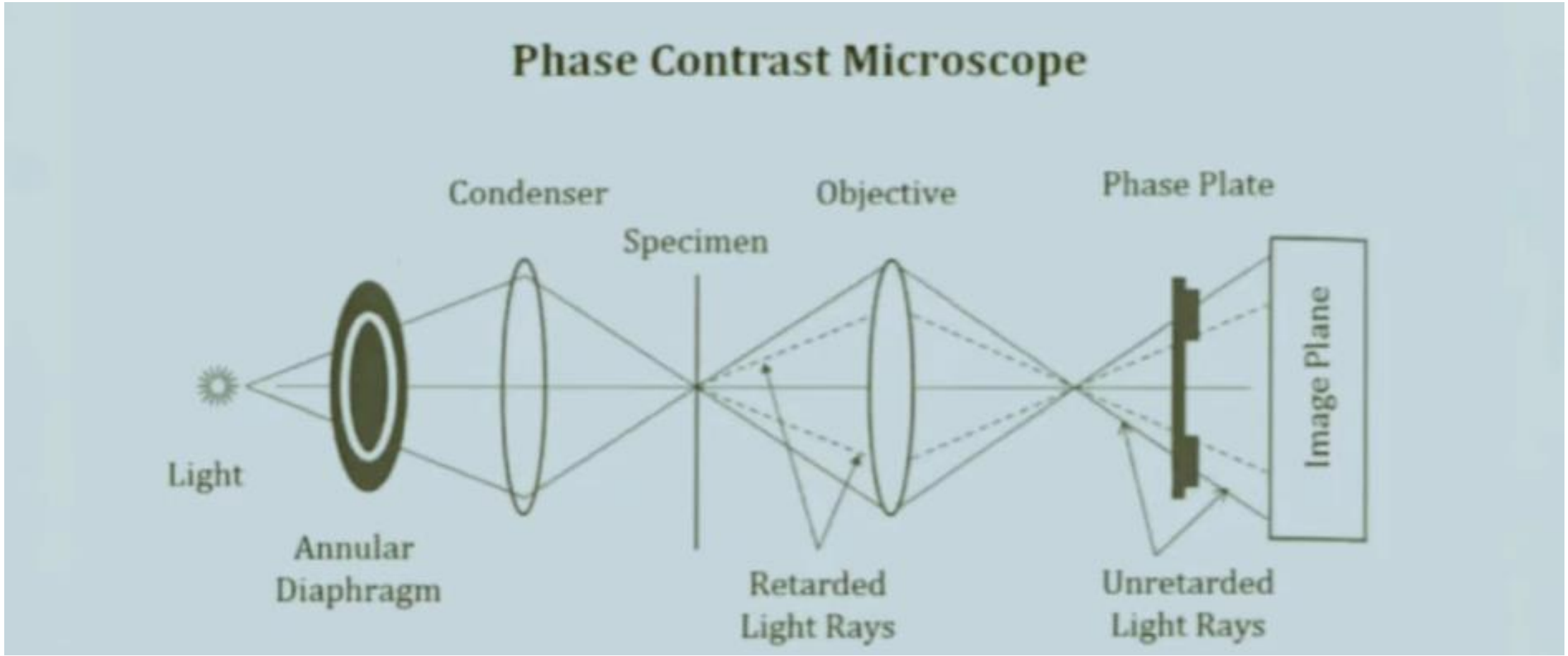


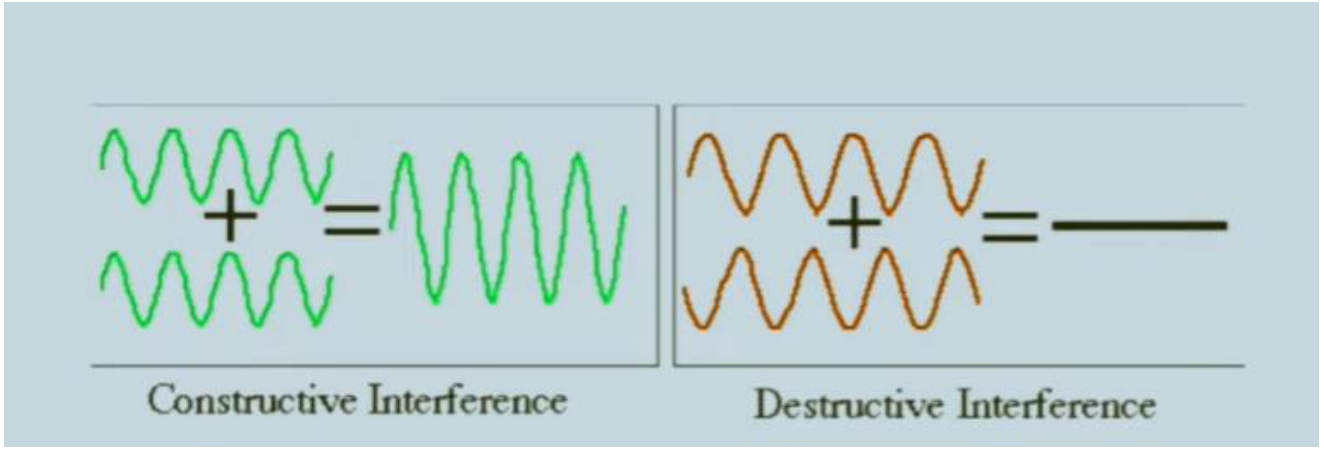
Applications of Phase-contrast Microscopy

To produce high-contrast images of transparent specimens, such as

- living cells (usually in culture),
- microorganisms,
- thin tissue slices,
- subcellular particles (including nuclei and other organelles).

The dynamic motility of mitochondria, mitotic chromosomes & vacuoles.
and how they proliferate through cell division


Phase Contrast Microscope
Condenser
Objective
Phase Plate
Specimen
Image Plane
Light
Annular Diaphragm
Retarded Light Rays
Unretarded Light Rays


Constructive Interference
Destructive Interference

Principle of Phase-contrast Microscopy

phase contrast microscopy is based on the principle that small phase changes in the light rays, induced by differences in the thickness and refractive index of the different parts of an object, can be transformed into differences in brightness or light intensity.

Fluorescent microscope

- Fluorescent microscope is an optical microscope that uses fluorescence instead of scattering, reflecting, attenuation and absorption to study the property of specimen.

Fluorescence :

- The proton absorbed energy at one wavelength and released at different wavelength in higher wavelength of energy.

History :

- British scientist sir George G. stroke first described fluorescence in 1852.

- He observed that fluorescence emission always occurred at a longer wavelength than the excitation light.

- This Shift towards longer wavelength is known as Stoke shif.

Principle :

- The specimen is illuminated with of a specific wavelength.

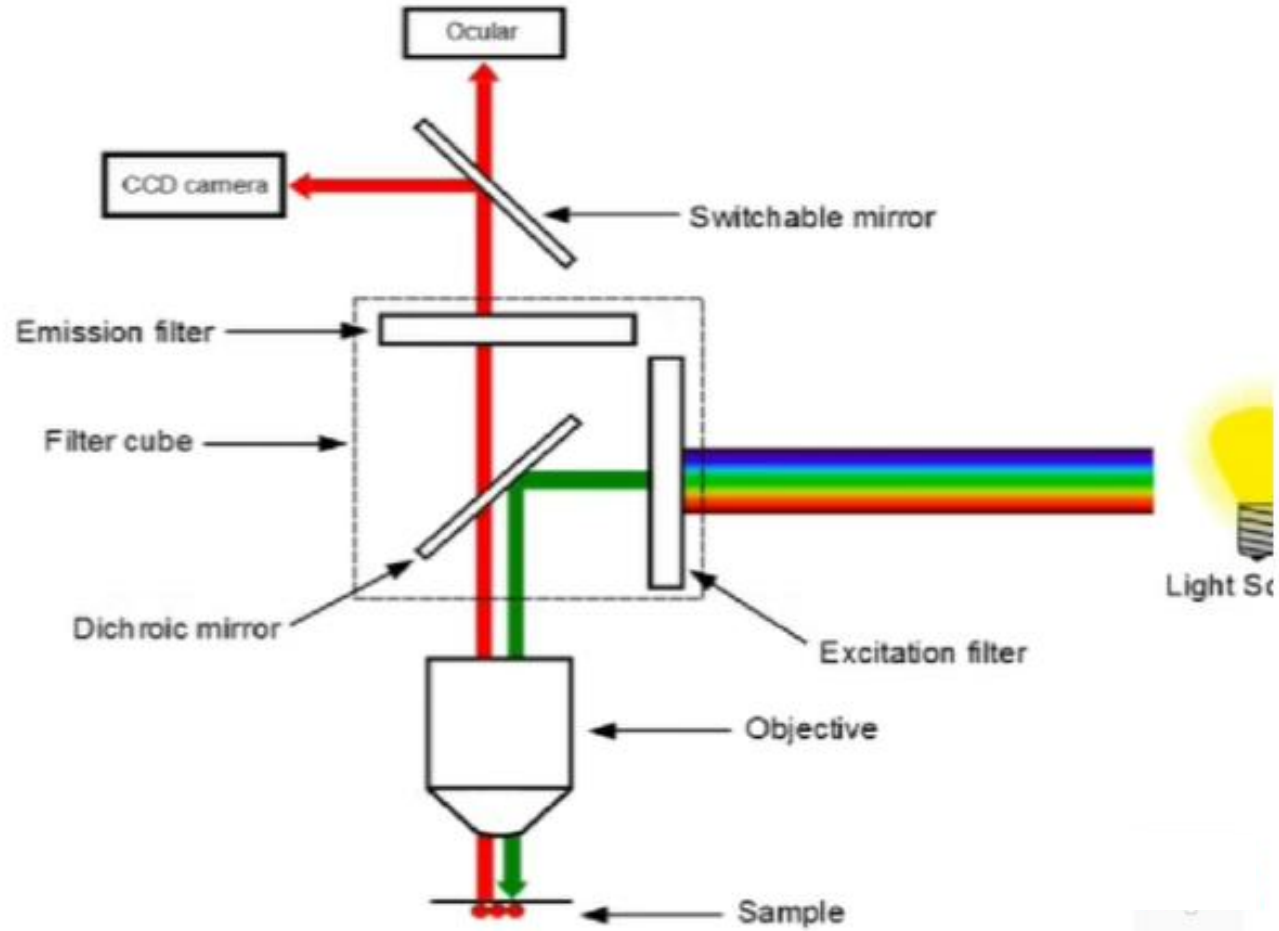
- Which is observed by the fluorophores (substances that can emit light after excitation) causing them light of longer wavelength.

- We can separate the weak and strong emitted fluorescence with the help of spectral emission filter.

Instrumentation :

- Fluorescent microscope uses Mercury and Xenon lamp to produce UV light.

- The light comes into the microscope and hits the mirror, mirror that reflects only one range of wavelength and other wavelength is passed through. It reflects UV light upon the specimen.


Ocular
CCD camera
Switchable mirror
Emission filter
Filter cube
Dichroic mirror
Excitation filter
Objective
Sample

- The UV light ecicites fluorescence within the molecule with the specimen → objective lens collect the fluorescent wavelength light produced.

- This fluorescence light passes through the dichroic mirror and a barrier filter that eliminates the wavelength other than fluorescent making it to the eyepiece to form the image.

Biological fluorescent stains :

- Main fluorescent stains have been designed of range of biological molecules.

- They bind biological material and give the fluorescent.

example - DAPI and hoechst.

Application :

Immunology, cell & molecular biology. Trace elements and in serology.

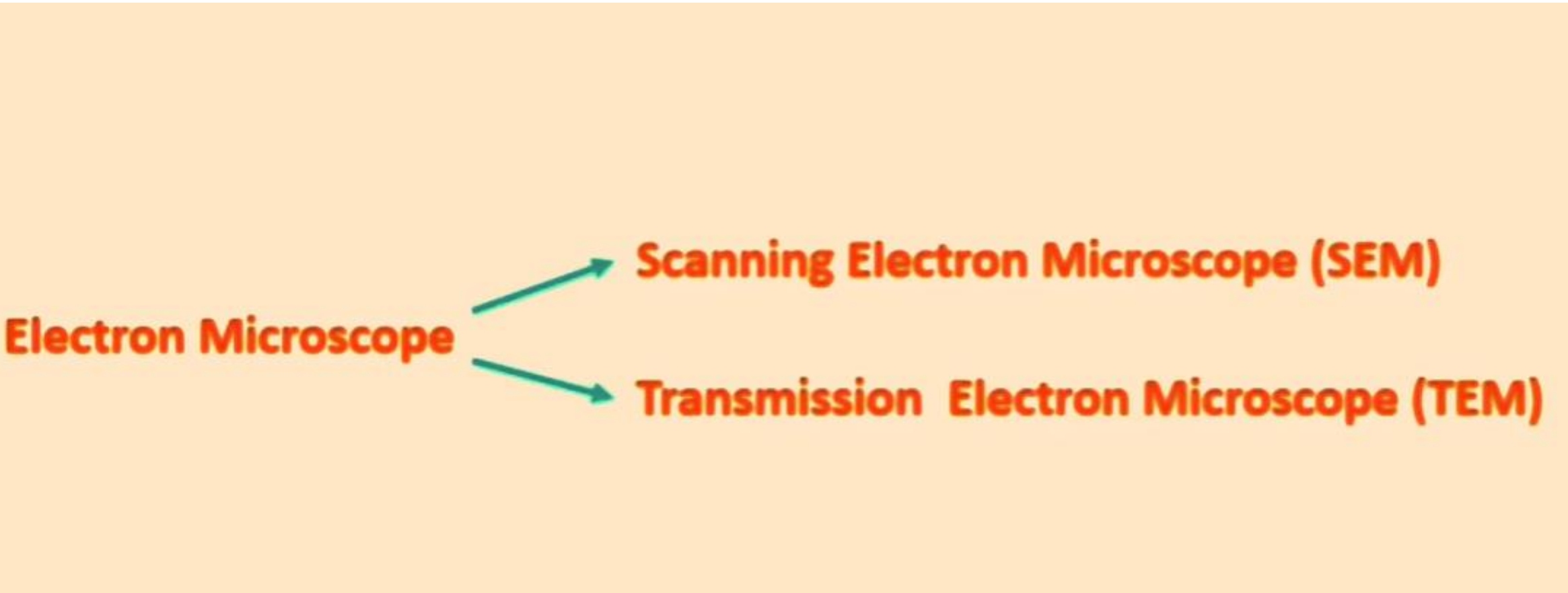
Introduction to Electron Microscope:

In electron microscope, high-speed electron beam is used instead of light waves, which are used in optical microscope. Like light, the stream of electrons has a corpuscular and vibratory character. Electron microscope gives very high magnification and incredibly high resolution.

The first transmission electron microscope was developed by Ernst Ruska and Max Knoll of Germany in 1931. EM is a remarkable research tool of twentieth century. It opened up sub-cellular structures, which were unknown to biologists. It can magnify an object upto 1000000X (one million times). The photomicrographs can be further enlarged and studied by using modern photographic techniques and computer aided techniques.

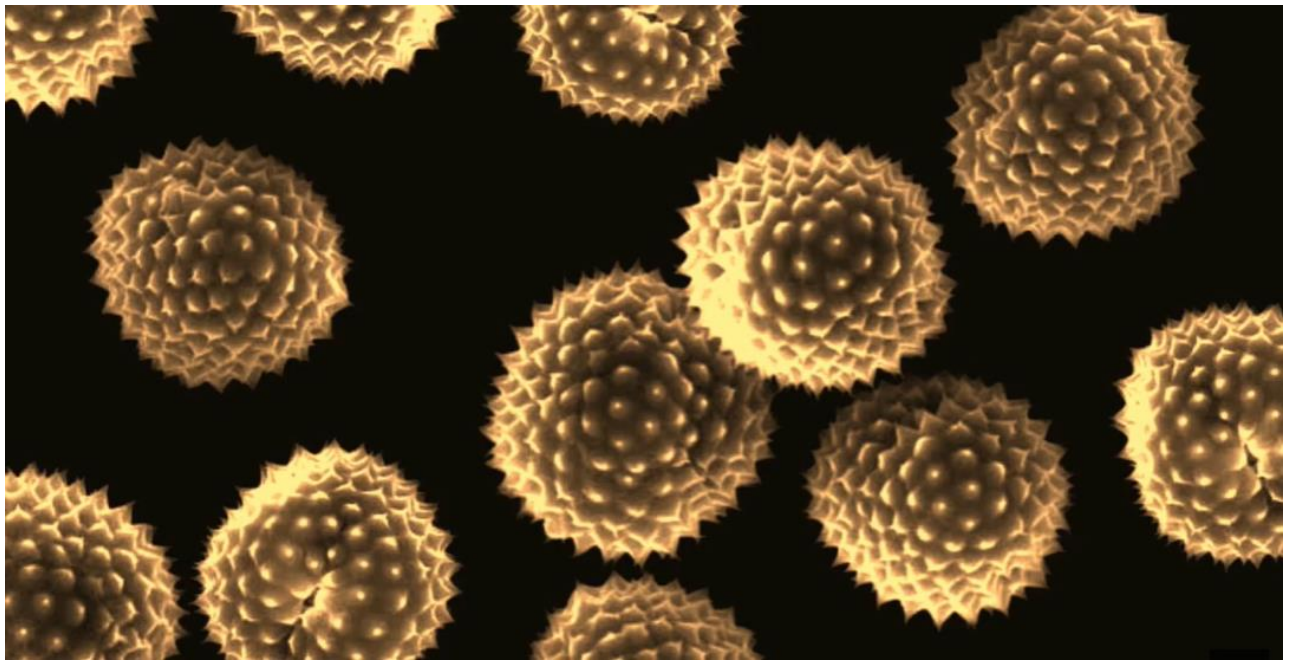
Principle of Electron Microscope:

Electrons are subatomic particles, which orbit around the atomic nucleus. When atoms of a metal are excited by heat energy, electrons fly off from the atom. In electron microscope, tungston is heated by applying a high voltage current, electrons form a continuous stream, which is used like a light beam.

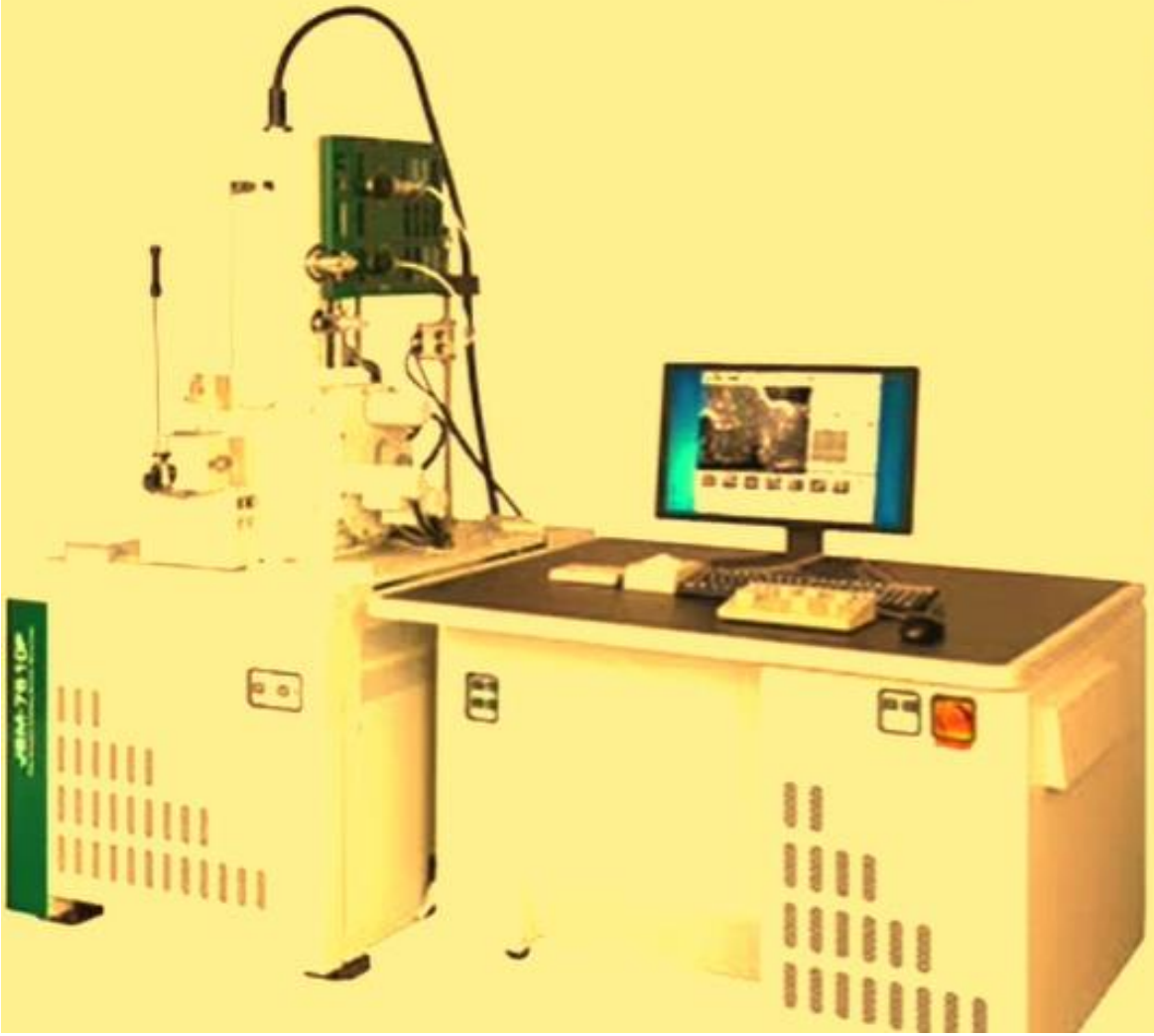

Electron Microscope
Scanning Electron Microscope (SEM)
Transmission Electron Microscope (TEM)

Scanning Electron Microscope (SEM)

- It is a type of electron Microscope
- uses electrons to illuminate a specimen and create an enlarged image



Scanning Electron Microscope (SEM)

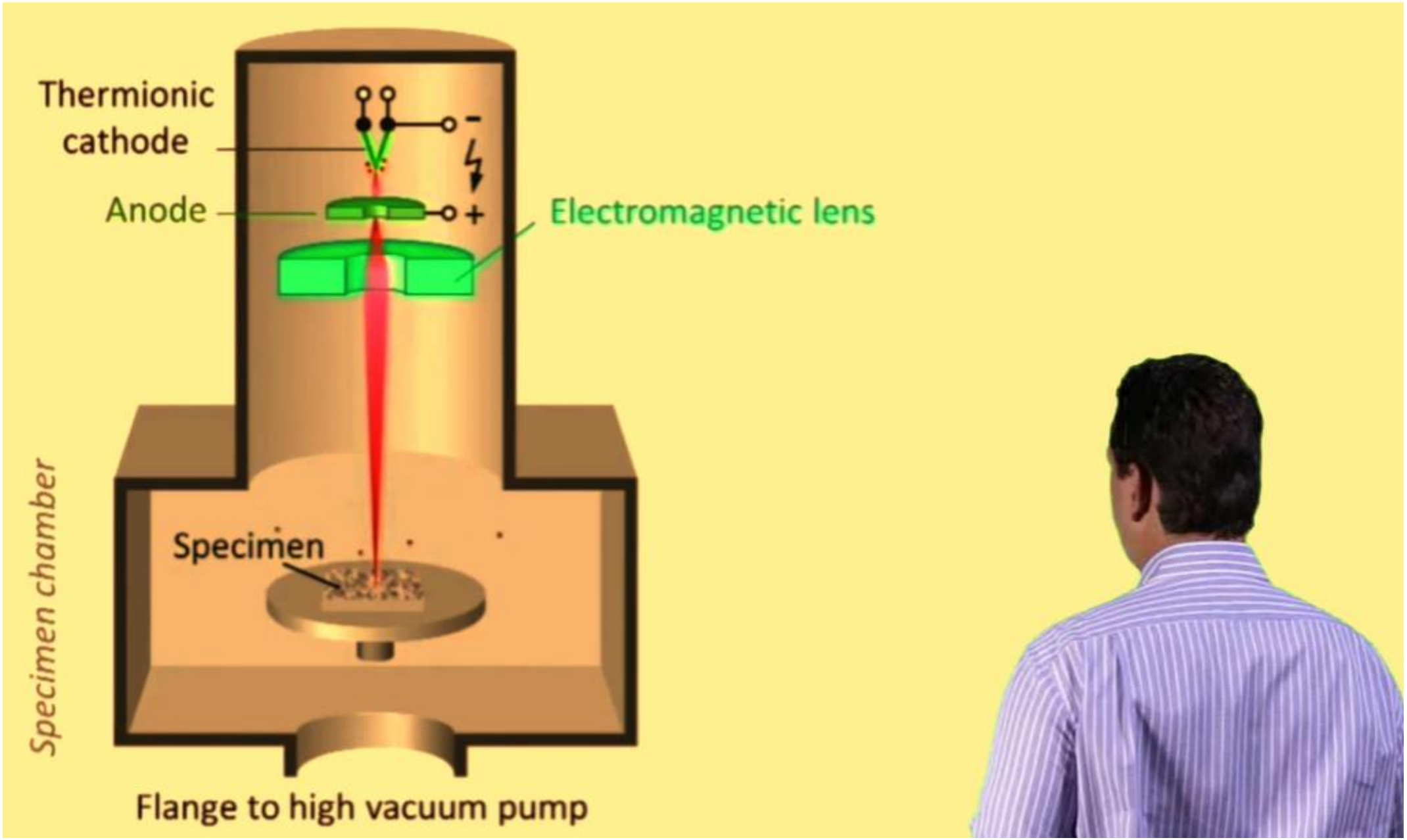


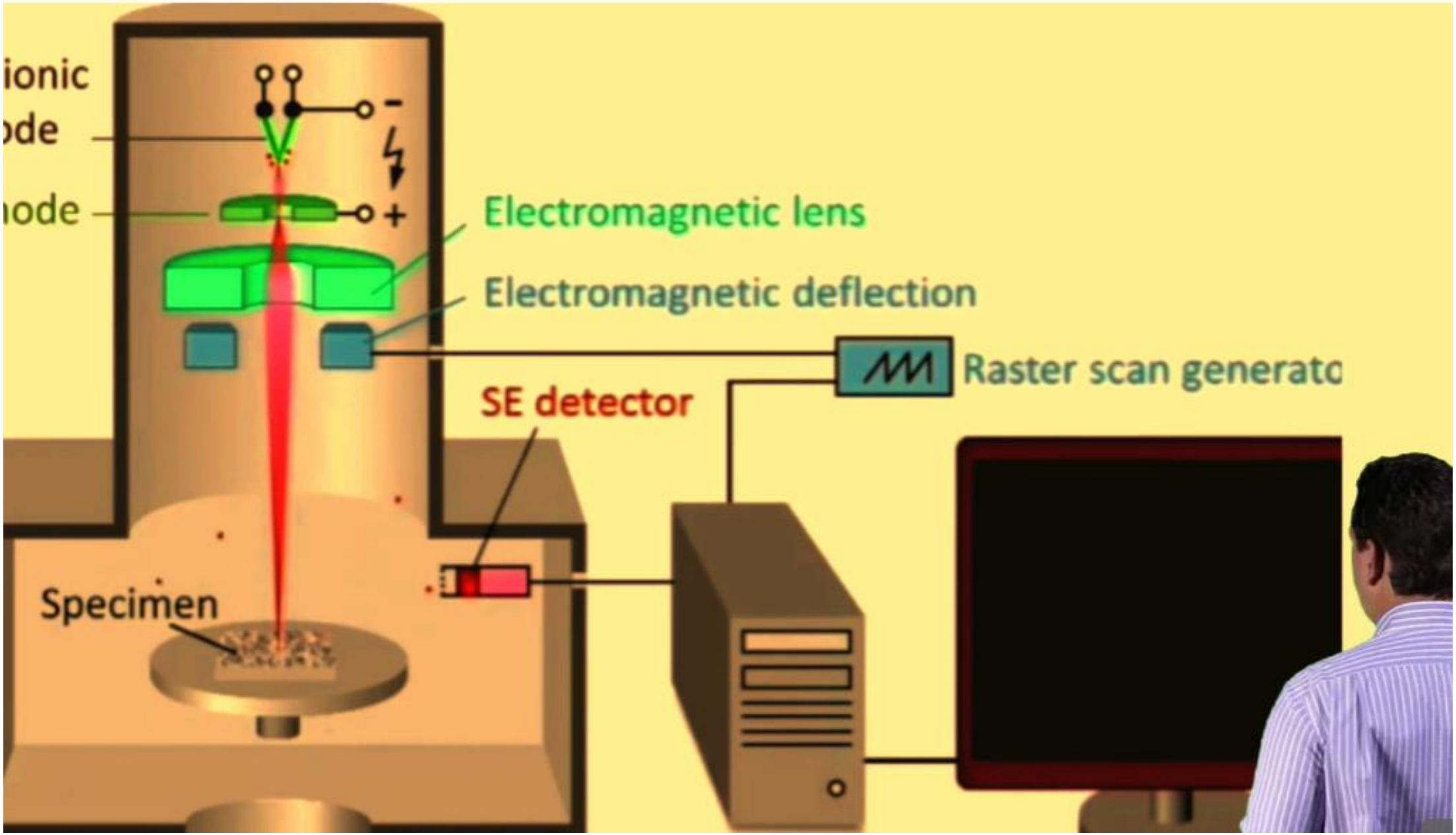
Sample preparation
Cleaning
Fixation
Dehydration
Dry

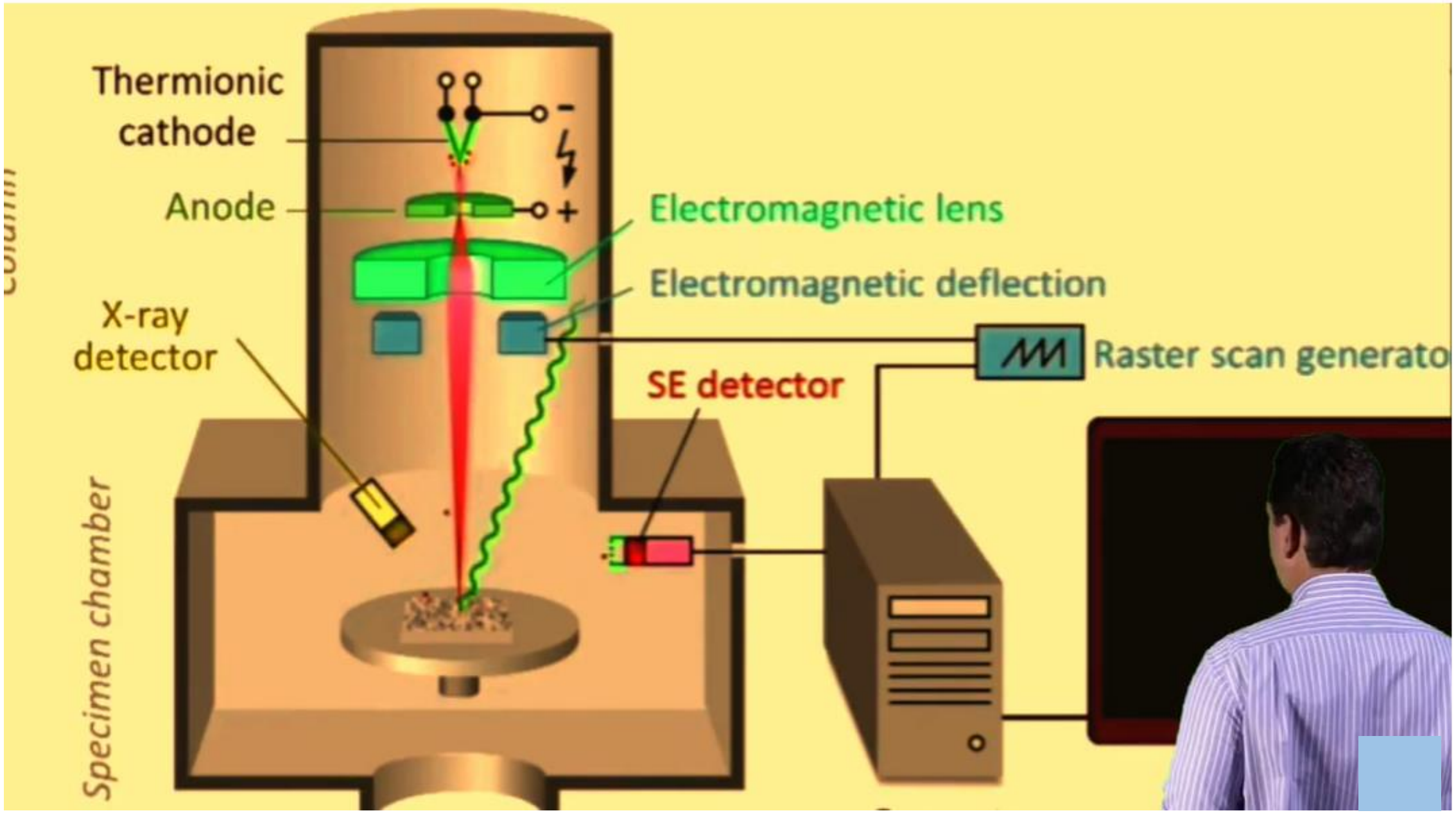
Scanning Electron Microscope (SEM)

- these microscopes cannot be used to image living cells

- produces images of a sample by scanning the surface


Thermionic cathode
Anode
Electromagnetic lens
Specimen
Specimen chamber
Flange to high vacuum pump

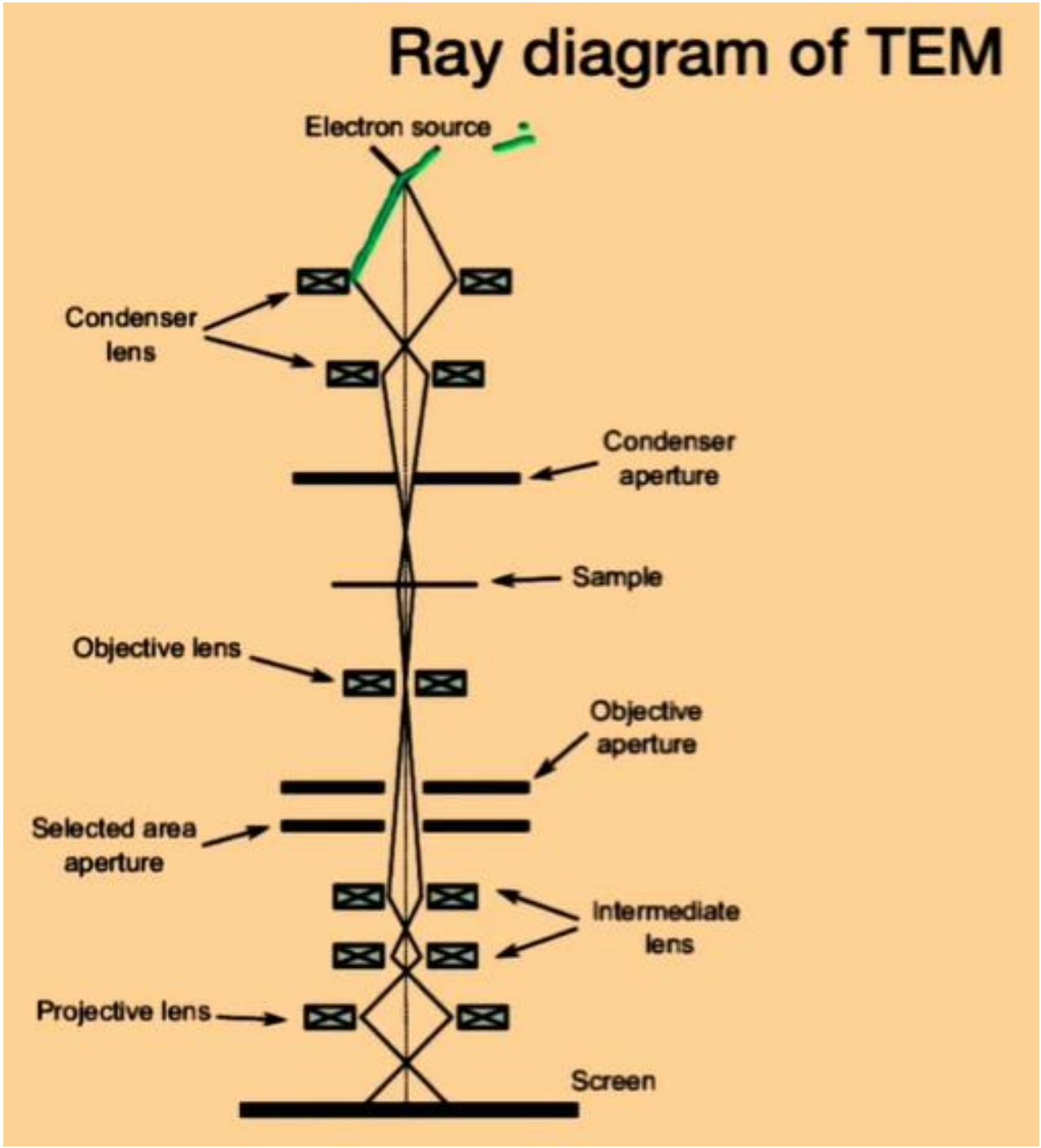

ionic
ode
node
Electromagnetic lens
Electromagnetic deflection
Raster scan generato
SE detector
Specimen


Thermionic cathode
Anode
Electromagnetic lens
Electromagnetic deflection
X-ray detector
SE detector
Raster scan generato
Specimen chamber

APPLICATIONS OF SEM

- For investigation of virus structure
- 3D tissue imaging
- Insect, spore, other microorganism, or cellular component visualise
- Geologist often use SEM to learn more about crystalline structure
- Industries including microelectronics, medical devices, food processing, all use SEM as a way to examine the surface composition of component and products.

Transmission Electron Microscopy


Ray diagram of TEM
Electron source
Condenser lens
Condenser aperture
Sample
Objective lens
Objective aperture
Selected area aperture
Intermediate lens
Projective lens
Screen

Transmission Electron Microscopy

Sample Preparation

Tissue sectioning : thinned to less than 100 nm on an **ultramicrotome**.

Sample staining :

TEM samples of biological tissues need high atomic number stains to enhance contrast. Compounds of heavy metals such as osmium, lead, uranium or gold (in immunogold labelling) may be used in or on the sample in desired cellular or protein region. This process requires an understanding of how heavy metals bind to specific biological tissues and cellular structures

Transmission Electron Microscopy

Sample Preparation

Fixation - fixed with chemical products (e.g. glutaraldehyde)

Rinsing and 'staining' - treated with heavy metal compounds.

Dehydration - washing with increasing ethanol concentration,
followed by final wash in another a polar substance like propylene oxide.

Applications of TEM

- TEMs provide topographical, morphological, compositional and crystalline information.
- The images allow researchers to view samples on a molecular level, making it possible to analyze structure and texture.

- Cancer research - studies of tumor cell ultrastructure

Transmission Electron Microscopy

Advantages

- TEMs offer very powerful magnification and resolution.
- 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.
- TEMs require special housing and maintenance.
- Images are black and white.

Introduction :-

Colorimetry

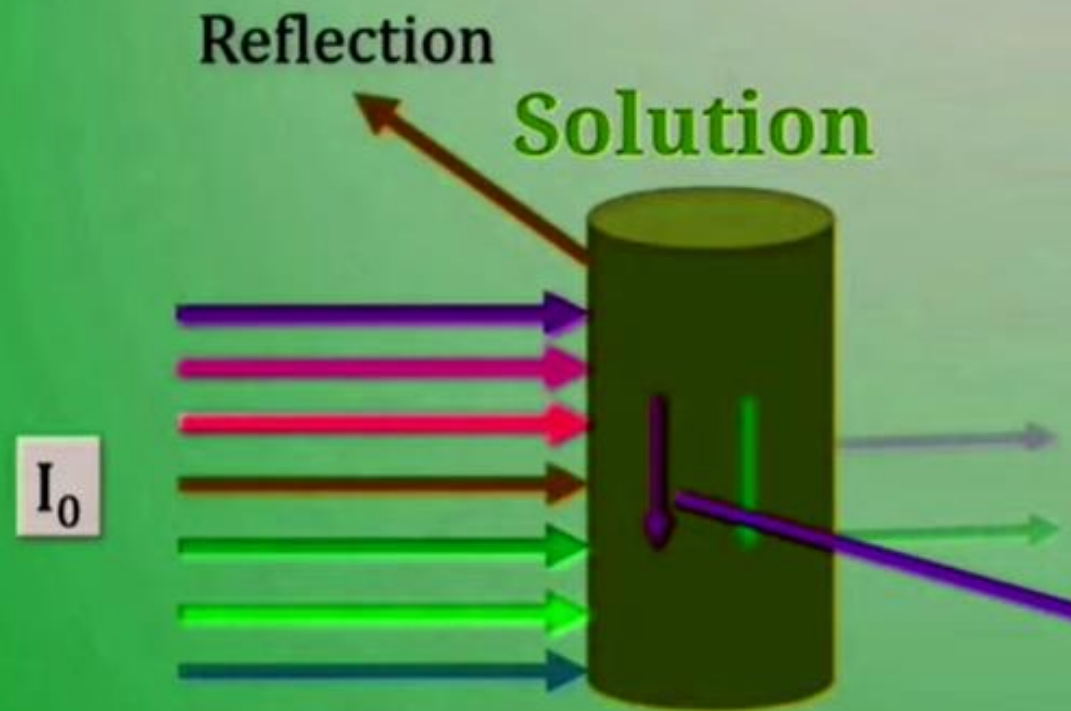
- In this technique , the concentration of compound is determined by measuring the intensity of color.

Introduction :-

Colorimeters

- They are simple instrument which measure the intensity of transmitted light through color solution

Term Related to Colorimetry :

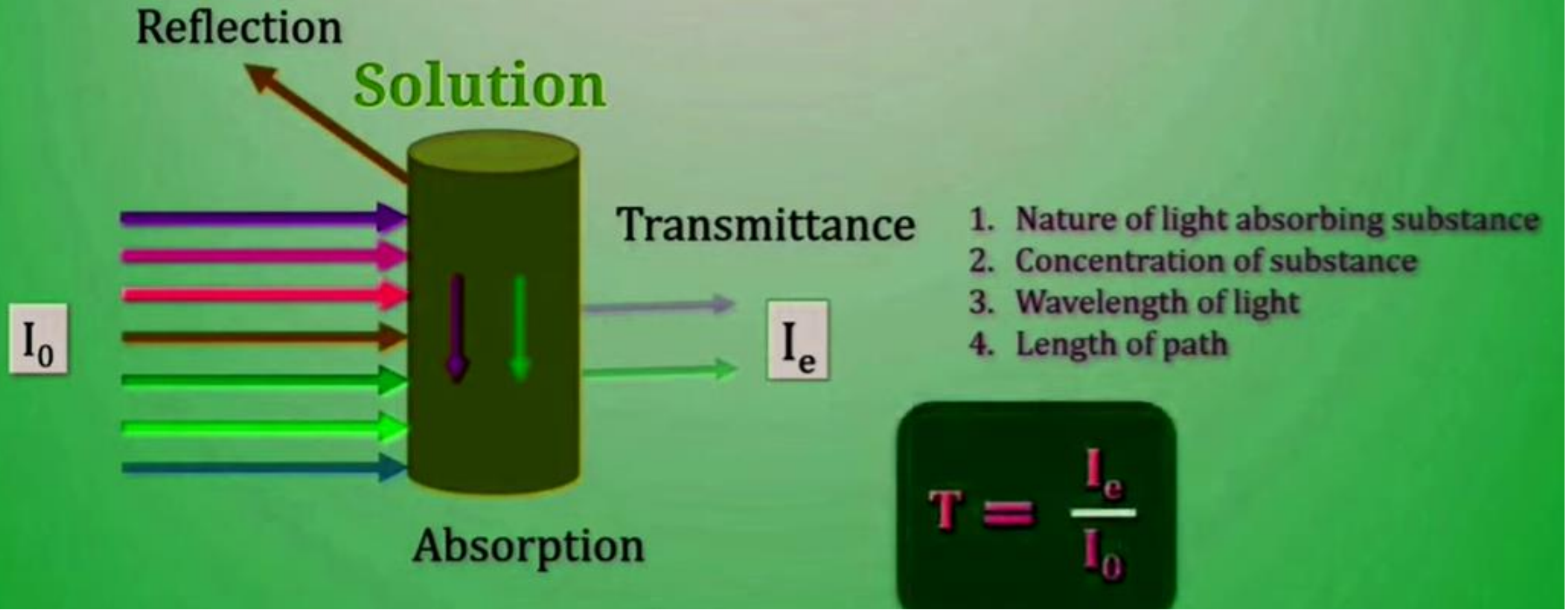


Absorbance (A)

or

Optical Density (OD)

Term Related to Colorimetry :-



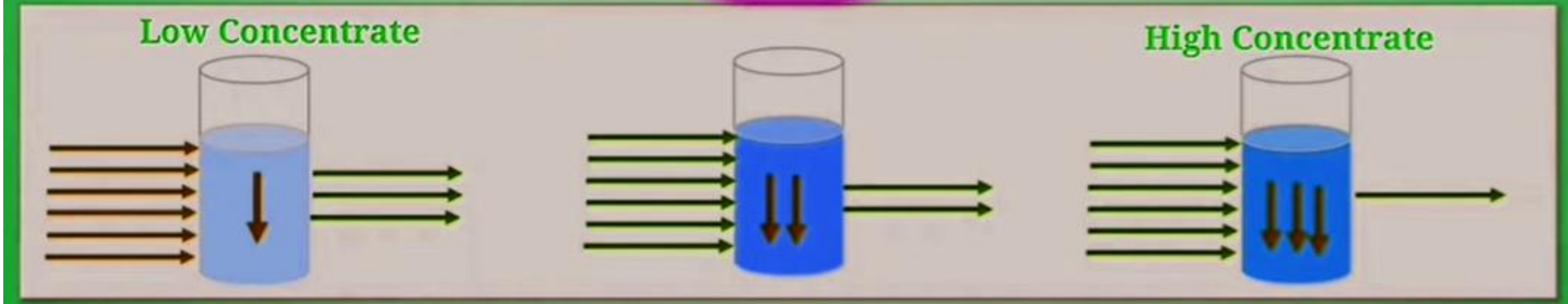
1. Nature of light absorbing substance
2. Concentration of substance
3. Wavelength of light
4. Length of path

$$T = \frac{I_e}{I_0}$$

Principle of Colorimetry :-

Colour Intensity $\propto$ Concentration of Substance

Beer's Law



Colour Intensity	↑ ↑
Concentration	↑ ↑
Absorbance	↑ ↑
Transmittance	↓ ↓

Concentration of Substance $\propto$ Absorbance $\propto \frac{1}{\text{Transmittance}}$

Beer's law

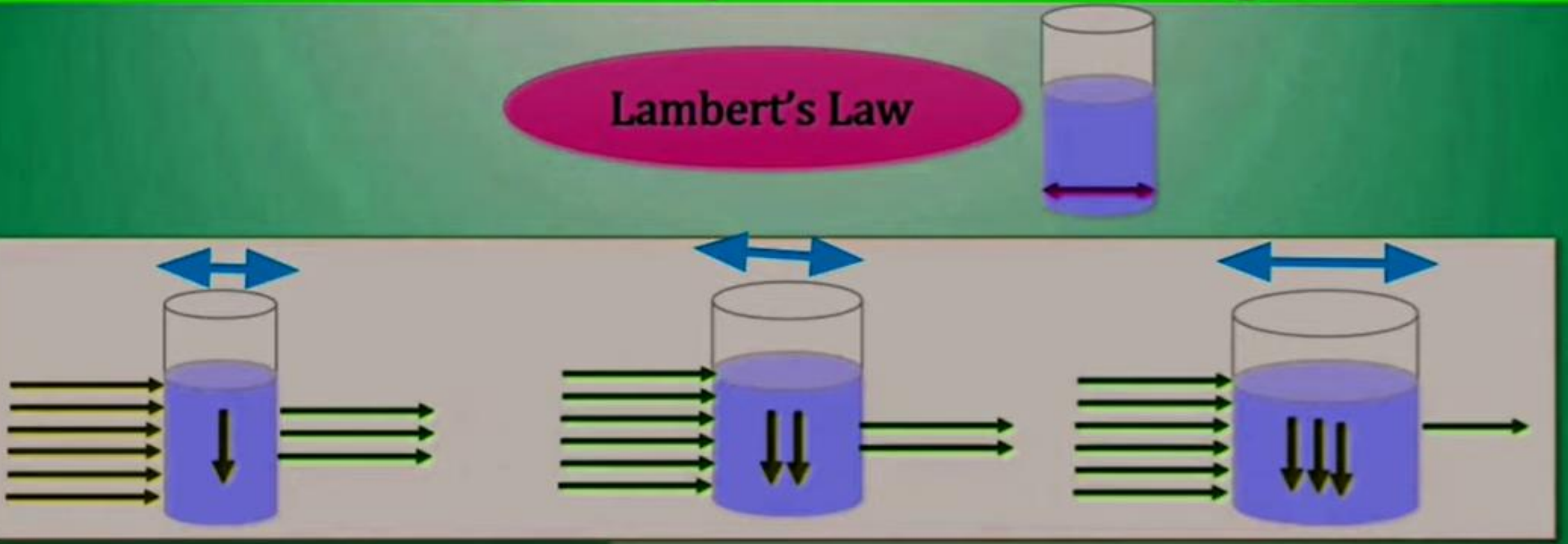
When a parallel beam of monochromatic light is passed through a solution, the absorbance (A) of the solution is directly proportional to concentration (C) of the substance in solution

$$A = KC$$

K is a proportionality constant

Principal of Colorimetry

Lambert's Law



Colour Intensity	SAME	SAME
Concentration	SAME	SAME
Length of Path	↑	↑
Absorbance	↑	↑
Transmittance	↓	↓

Length of Path $\propto$ **Absorbance** $\propto \frac{1}{\text{Transmittance}}$

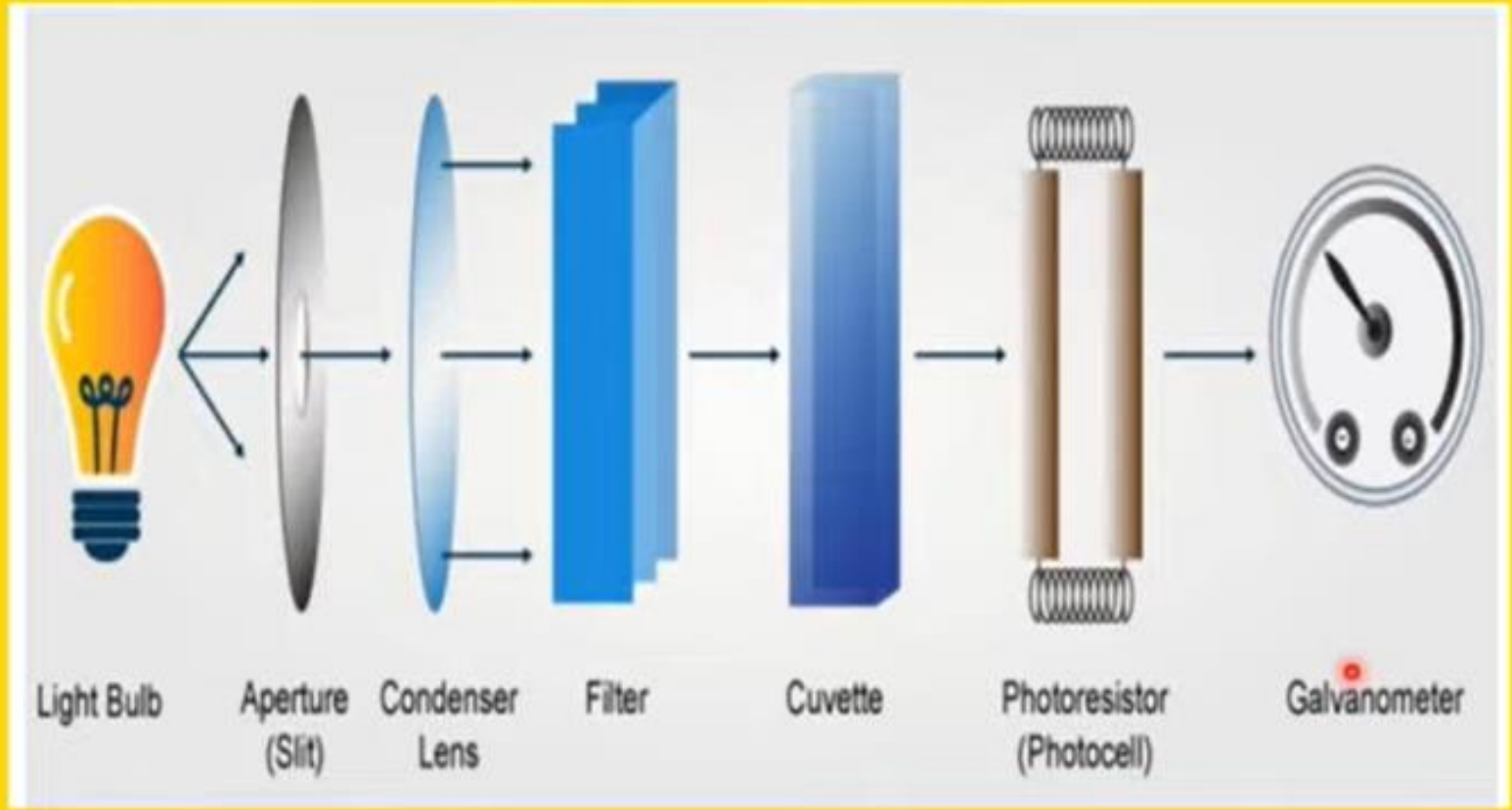
Lambert's Law

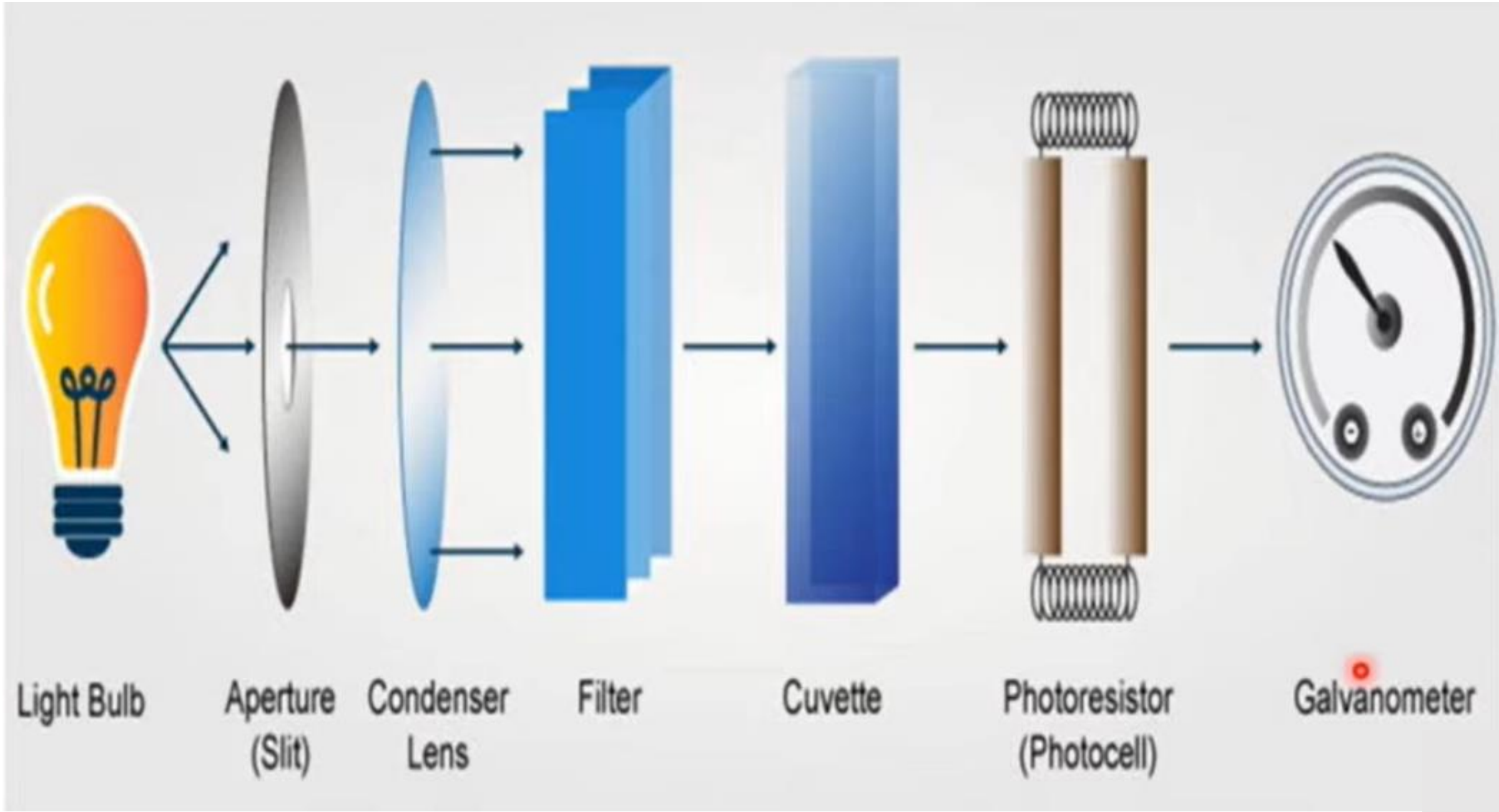
When a parallel beam of monochromatic light is passed through a solution, the absorbance (A) of the solution is directly proportional to length of the light path (l)

$$A=Kl$$

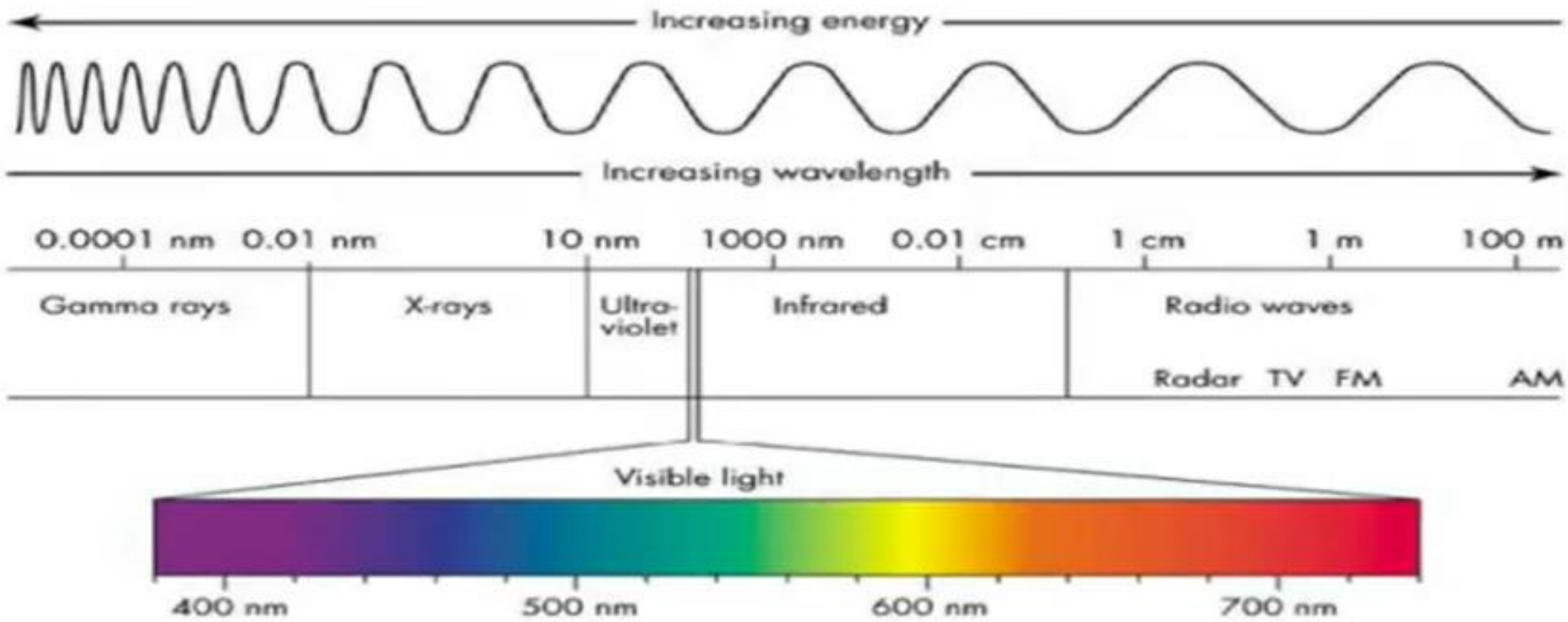
Combining both laws we get A=KCL

Colorimeters




Light Bulb
Aperture (Slit)
Condenser Lens
Filter
Cuvette
Photoresistor (Photocell)
Galvanometer

SPECTROPHOTOMETRY


Increasing energy
Increasing wavelength
0.0001 nm
0.01 nm
10 nm
1000 nm
0.01 cm
1 cm
1 m
100 m
Gamma rays
X-rays
Ultra-violet
Infrared
Radio waves
Radar
TV
FM
AM
Visible light
400 nm
500 nm
600 nm
700 nm

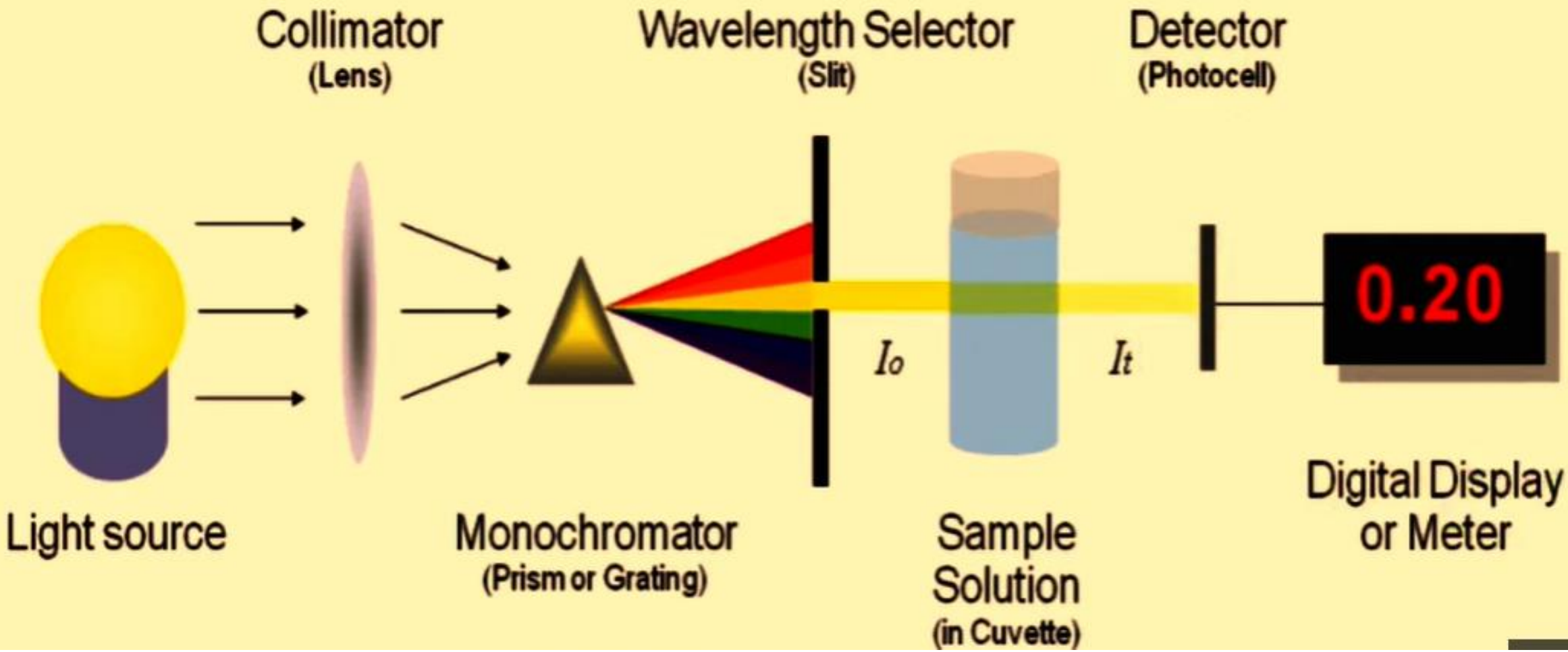
- Spectrophotometer techniques are mostly used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer.
  - ***Spectrophotometry*** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

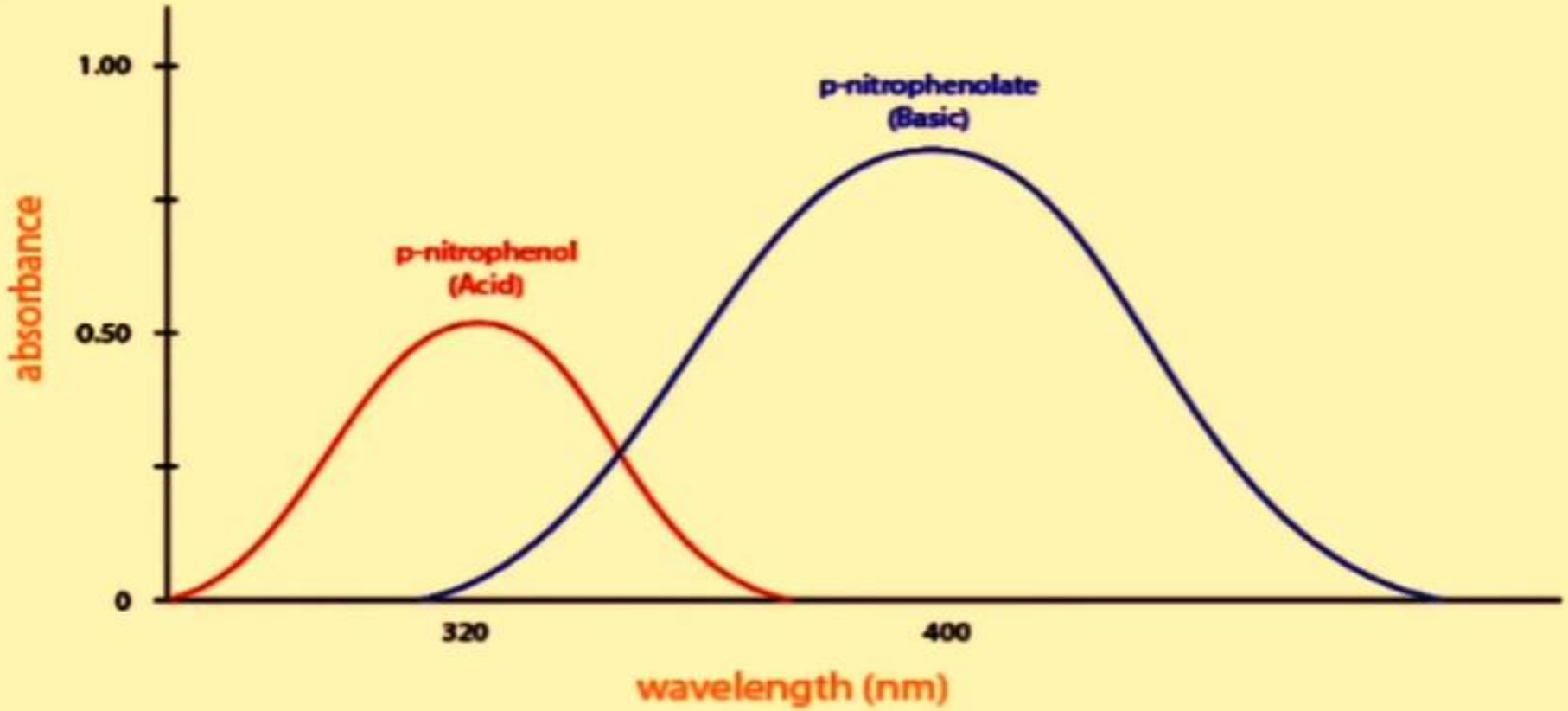
Spectrophotometer

- A is an instrument that measures the amount of light absorbed by a sample.

- **Spectrophotometer is an analytical instrument used to measure intensity as a function of light source wavelength.**

Spectrophotometer Device and it's Mechanism :-





Absorbance of two different compounds

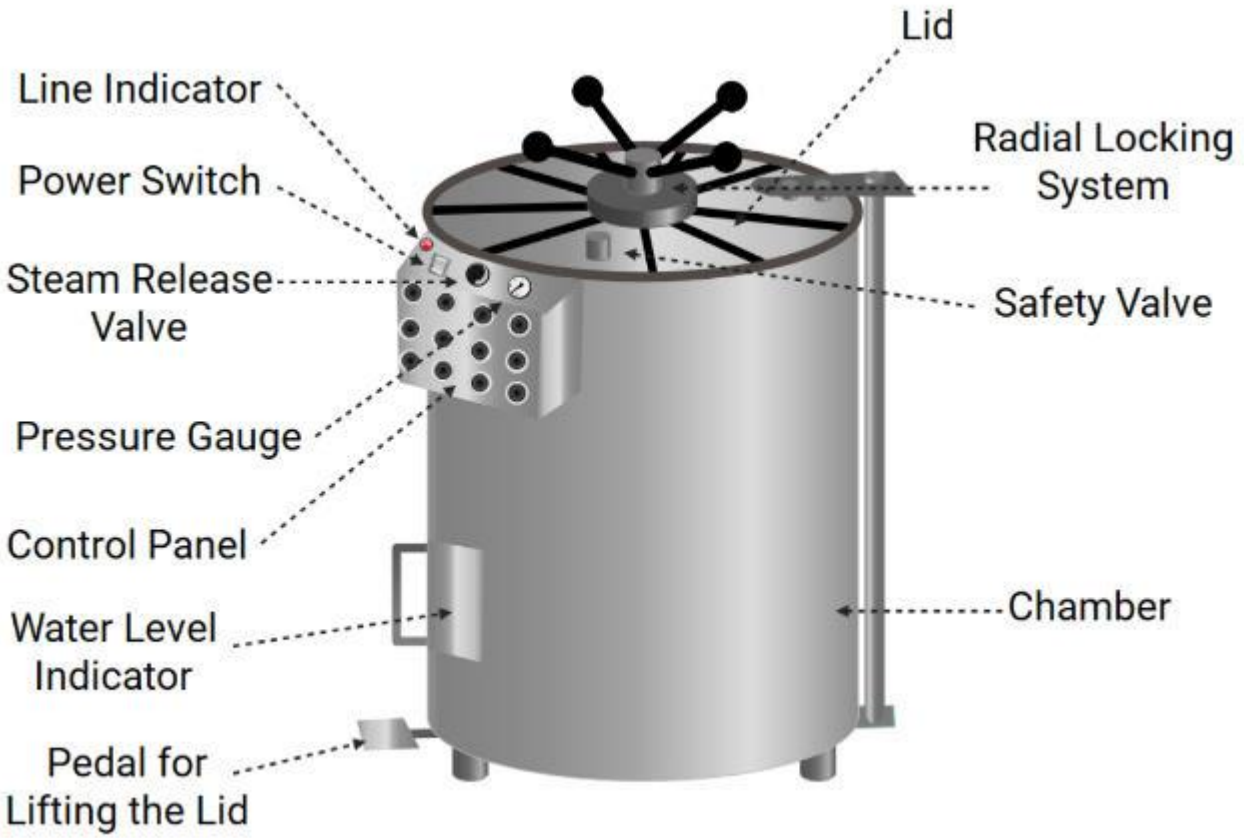
Common Lab Instruments

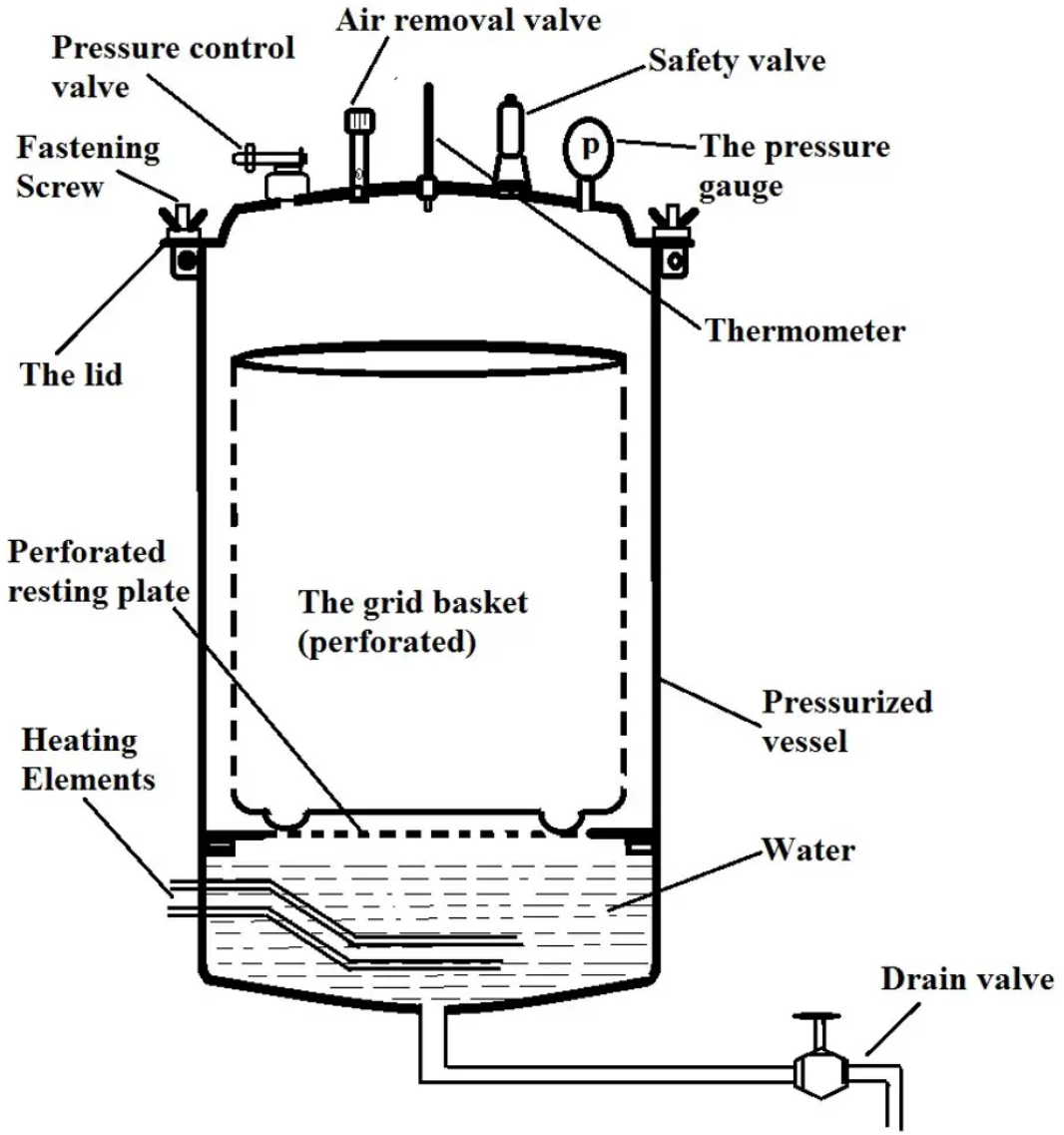
- Autoclave
- Hot Air Oven
- Laminar Air Flow
- Centrifuge

An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure.

Temp	Pressure	Time
115°C	10 Psi	20 min
121°C	15 Psi	15 min
126°C	20 Psi	10 min
134°C	30 Psi	3 min

Autoclave


Lid
Line Indicator
Power Switch
Radial Locking System
Steam Release Valve
Safety Valve
Pressure Gauge
Control Panel
Water Level Indicator
Chamber
Pedal for Lifting the Lid


Air removal valve
Pressure control valve
Safety valve
Fastening Screw
The pressure gauge
Thermometer
The lid
Perforated resting plate
The grid basket (perforated)
Pressurized vessel
Heating Elements
Water
Drain valve

Pressure Cooker Type

Common Laboratory Autoclave

Vertical Autoclave

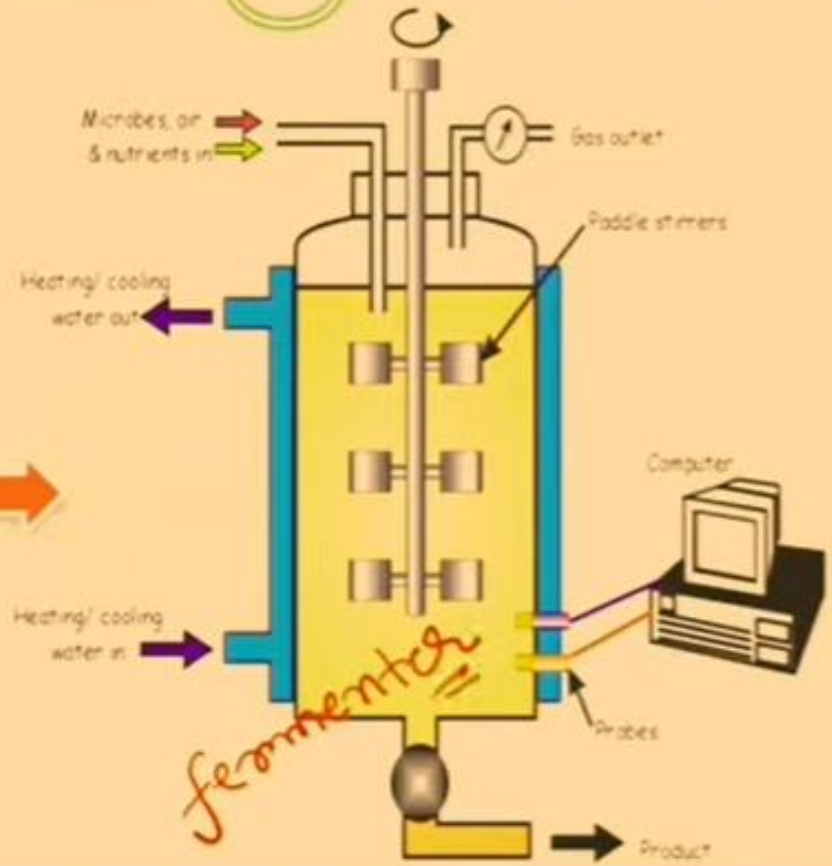
Horizontal Autoclave

Large Automatic Hospital Autoclave

Fermentation

- Fermentation is the process of growing microorganisms in a nutrient media by maintaining physico- chemical conditions and thereby converting feed into a desired end product

FERMENTATION BASIC FLOW CHART



YEAST

SUGAR

FERMENTATION GIVES ALCOHOL

Fermentation

- The basic principle involved in the industrial fermentation technology is that organisms are grown under suitable conditions, by providing raw materials meeting all the necessary requirements such as carbon, nitrogen, salts, trace elements and vitamins.

- The end products formed as a result of their metabolism during their life span are released into the media, which are extracted for use by human being and that have a high commercial value.

Fermentation types

- Fermentations are classified into different types depending on different criterias-
- On the basis of oxygen requirement fermentations are classified into two groups as aerobic and anaerobic.

Aerobic Fermentation

- A large number of industrial processes, although called “fermentations”, are aerobic in nature since they are carried on by aerobic microorganisms.
- In modern day aerobic fermentation processes the fermenter is provided with an aerator (aeration device), through which sterile air is forced in, and agitator (impeller) with which the medium is continuously mixed.
- Thus aerobic conditions can be generated in the closed fermentation vessel with submerged cultures.
- E.g. Acetic acid(vinegar) production, peniciliin production

Anaerobic fermentation

- Anaerobic fermentations are those which are carried out in the absence of oxygen by facultatively anaerobic bacteria or by strictly anaerobic bacteria. The fermenter is designed to operate under anaerobic or microaerophillic conditions is the same as that designed to operate under aerobic conditions, except that the **aeration and agitation devices are not necessary**.
- However, many anaerobic fermentations, especially those involving facultative anaerobes, such as yeast, require mild aeration for inoculum build up, to increase cellnumbers and sufficient agitation for mixing and maintainance of temperature.
- E.g. Acetone-butanol fermentation
- Alcoholic fermentation

Types of Fermentation

•**Homo fermentation:** only one type of product formation
•**Hetero fermentation:** more than one product formed
Based on the end products formed, fermentation can be categorized as follows:

1. Lactic Acid Fermentation

- Lactic acid is formed from pyruvate produced in glycolysis. NAD+ is generated from NADH.
- Enzyme lactate dehydrogenase catalyses this reaction.
- Lactobacillus bacteria prepare curd from milk via this type of fermentation.
- During intense exercise when oxygen supply is inadequate, muscles derive energy by producing lactic acid, which gets accumulated in the cells causing fatigue.
- This method makes sauerkraut, pickles, kimchi, yogurt, and sourdough bread.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Lactate.dehydrogenase]{Lactobacillus} 2CH_3CHOHCOOH(LacticAcid)$$

2. Alcohol Fermentation

This is used in the industrial production of wine, beer, biofuel, etc.
The end-product is alcohol and CO_2. Pyruvic acid breaks down into acetaldehyde and CO_2 is released. In the next step, ethanol is formed from acetaldehyde. NAD+ is also formed from NADH, utilized in glycolysis. Yeast and some bacteria carry out this type of fermentation. Enzyme pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Decarboxylase]{Dehydrogenase} 2CH_3CHO(Acetaldehyde) + 2CO_2 \rightarrow 2C_2H_5OH(Ethanol)$$

3. Acetic acid Fermentation

Vinegar is produced by this process. This is a two-step process.
The first step is the formation of ethyl alcohol from sugar anaerobically using yeast.
In the second step, ethyl alcohol is further oxidized to form acetic acid using acetobacter bacteria.
Microbial oxidation of alcohol to acid is an aerobic process.

$$C_2H_5OH\,(ethanol) + O_2 \xrightarrow{acetobacter} CH_3COOH\,(acetic\ acid) + H_2O$$

4. Butyric acid Fermentation

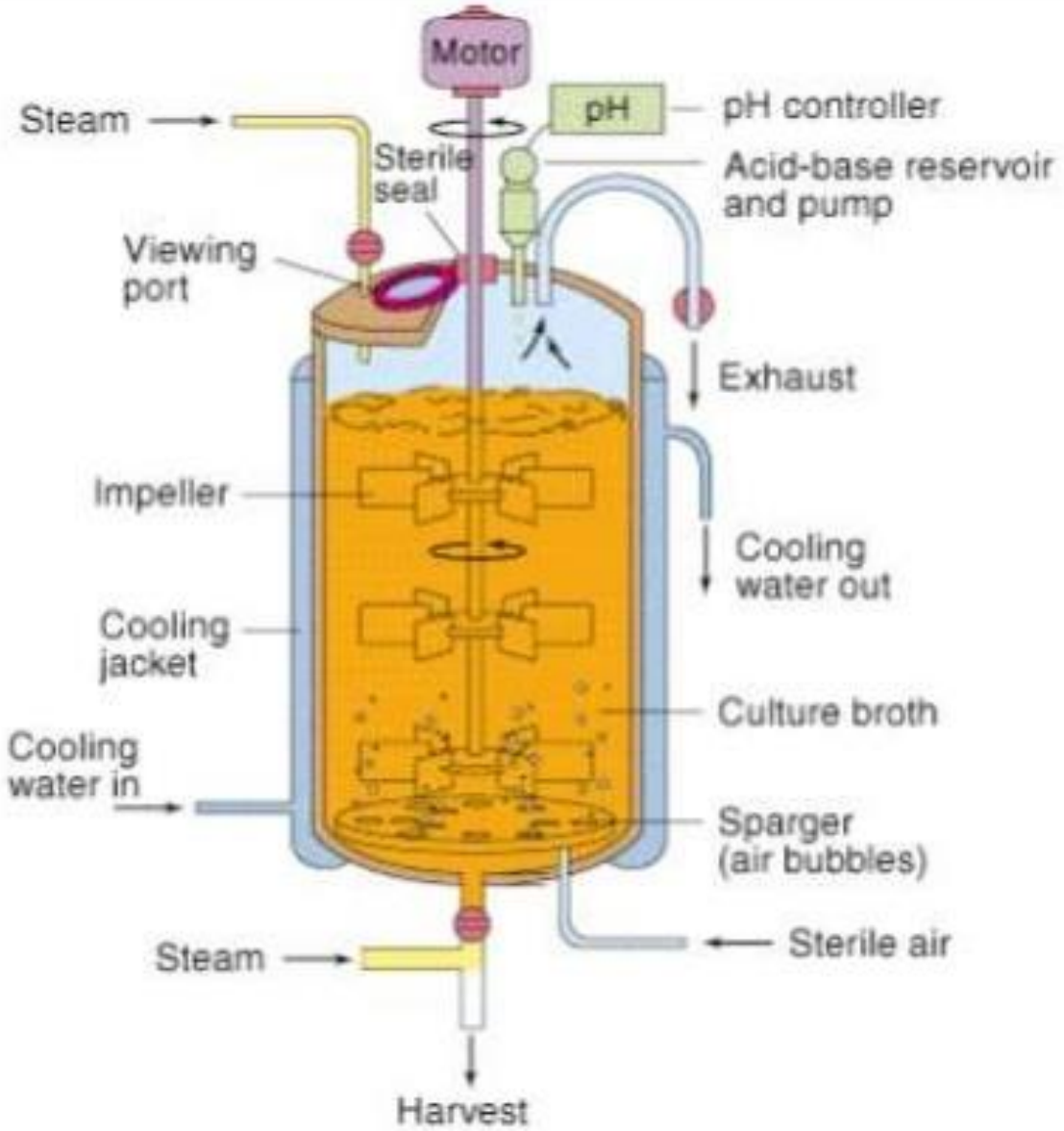
This type of fermentation is characteristic of obligate anaerobic bacteria of genus clostridium. This occurs in retting of jute fibre, rancid butter, tobacco processing and tanning of leather. Butyric acid is produced in the human colon as a product of dietary fibre fermentation. It is an important source of energy for colorectal epithelium. Sugar is first oxidized to pyruvate by the process of glycolysis and then pyruvate is further oxidized to form acetyl-CoA by the oxidoreductase enzyme system with the production of H_2 and CO_2. Acetyl-CoA is further reduced to form butyric acid. This type of fermentation leads to a relatively higher yield of energy. 3 molecules of ATP are formed.

$$C_6H_{12}O_2 \xrightarrow[butyricum]{clostridium} C_4H_8O_2 + 2CO_2 + 2H_2$$

What are Fermentors?

Fermentors, also known as bioreactors, are sterilised and enclosed vessels that are used for the growth of microorganisms under optimal conditions. The microorganisms can be grown in large quantities to produce metabolites for commercial uses. Fermentors are equipped with special components for heating, mixing, and aeration. Its volume can be as big as 500,000 litres for an industrial scale, or as small as 1 litre for laboratory uses.

Typical Fermentor



A bioreactor should provide for the following:
1.Agitation (for mixing of cells and medium),
2.Aeration (aerobic fermentors); for O2 supply,
3.Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, and liquid leveled.
4.Sterilization and maintenance of sterility, and
5.Withdrawal of cells/medium
Bioreactors are used for the production of biomass, metabolites, and antibiotics.

Parts of fermenter

1. Fermenter Vessel

- A fermenter is a large cylinder closed at the top and bottom connected with various pipes and valves.

2. Heating and Cooling Apparatus

3. Aeration System

- It contains two separate aeration devices (sparger and impeller) to ensure proper aeration in a fermentor.

4. Sealing Assembly

- The sealing assembly is used for the sealing of the stirrer shaft to offer proper agitation.

5. Baffles

- The baffles are incorporated into fermenters to prevent a vortex improve aeration in the fermenters.
- It consists of metal strips attached radially to the wall.

6. Impeller

- Impellers are used to provide uniform suspension of microbial cells in different nutrient mediums.

7. Sparger

- A sparger is a system used for introducing sterile air to a fermentation vessel. It helps in providing proper aeration to the vessel.

8. Feed Ports

- They are used to add nutrients and acid/alkali to the fermentor.

9. Foam-Control

- The level of foam in the vessel must be minimized to avoid contamination, this is an important aspect of the fermentor.

10. Valves

- Valves are used in the fermentor to control the movement of liquid in the vessel.

11. Controlling Devices for Environmental Factors

- A variety of devices are utilized to control environmental elements like temperature, oxygen concentration, pH, cell mass, essential nutrient levels, and product concentration.

12. Use of Computer in Fermenter

Types and Applications of bioreactor

Some important applications of the bioreactor are:

Type of bioreactor	Applications
Stirred tank fermenter	Antibiotics, citric acid, Exopolysaccharides, cellulose, Chitinolytic enzymes, Laccase, Xylanase, Pectic, and pectate lyase, Tissue mass culture, Lipase, Polygalacturonases, Succinic acid
Bubble column fermentor	Algal culture, Chitinolytic enzymes
Airlift fermentor	Antibiotics, Chitinolytic enzymes, Exopolysaccharides, Gibberelic acid, Laccase, Cellulase, Lactic acid, Polygalacturonases, Tissue mass culture
Fluid bed fermentor	Laccase
Packed bed fermentor	Laccase, Hydrogen, Organic acids, Mammalian cells,
Photobioreactor	Wastewater treatment, water quality management, remediation of contaminated soil
Membrane bioreactor	Alginate, Antibiotic, Cellulose hydrolysis, Hydrogen production, Water treatment, VOCs treatment

Pulsed field fermentor