Scanning Transmission Electron Microscope

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Scanning Transmission Electron Microscope - STEM

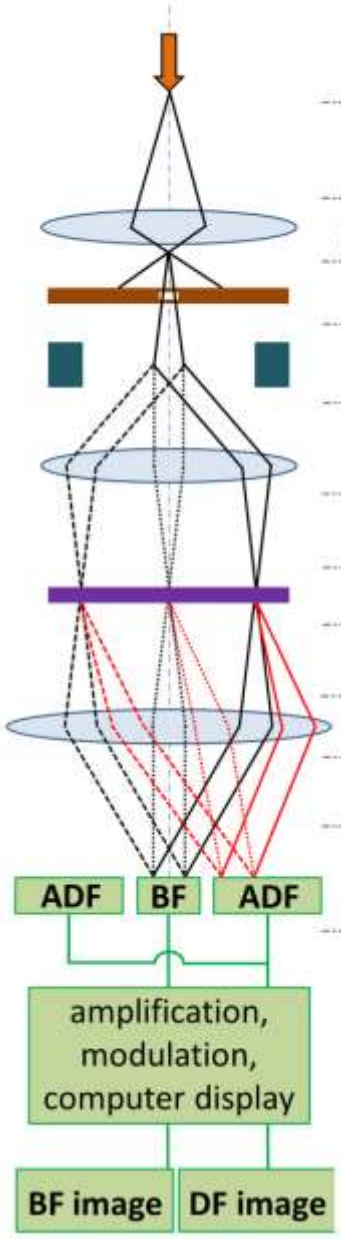
- STEM is similar to TEM.
- While in TEM parallel electron beams are focused perpendicular to the sample plane, in STEM the beam is focused at a large angle and is converged into a focal point.
- The transmitted signal is collected as a function of the beam location as it is rastered across the sample.

There are multiple detectors for STEM imaging:

- BF (bright-field) detector: small angles (<0-10 mrad (milliradian)). These images are similar to the bright-field images obtained using TEM.
- ADF (annular dark-field) detector: larger angles (10-50 mrad)
- HAADF (high-angle annular dark-field) detector: Angles > 50mrad

- None of the elastically scattered electrons reach the detector, so it only images from inelastically scattered electrons.
- This is also known as Z-contrast imaging because there is a direct correlation between the local contrast and local mass-thickness, which depends on the atomic number Z.
- HAADF imaging allows for enhanced contrast, especially at lower atomic numbers, compared to TEM.

STEM mode



Electron Gun

System of condenser lenses

Condenser aperture

Deflection scan coils

Upper-objective polepiece

Specimen

Lower-objective polepiece

STEM detector
(back focal plane of objective lens)

CONTRIBUTIONS

1. Antonie van Leeuwenhoek
2. John Tyndall

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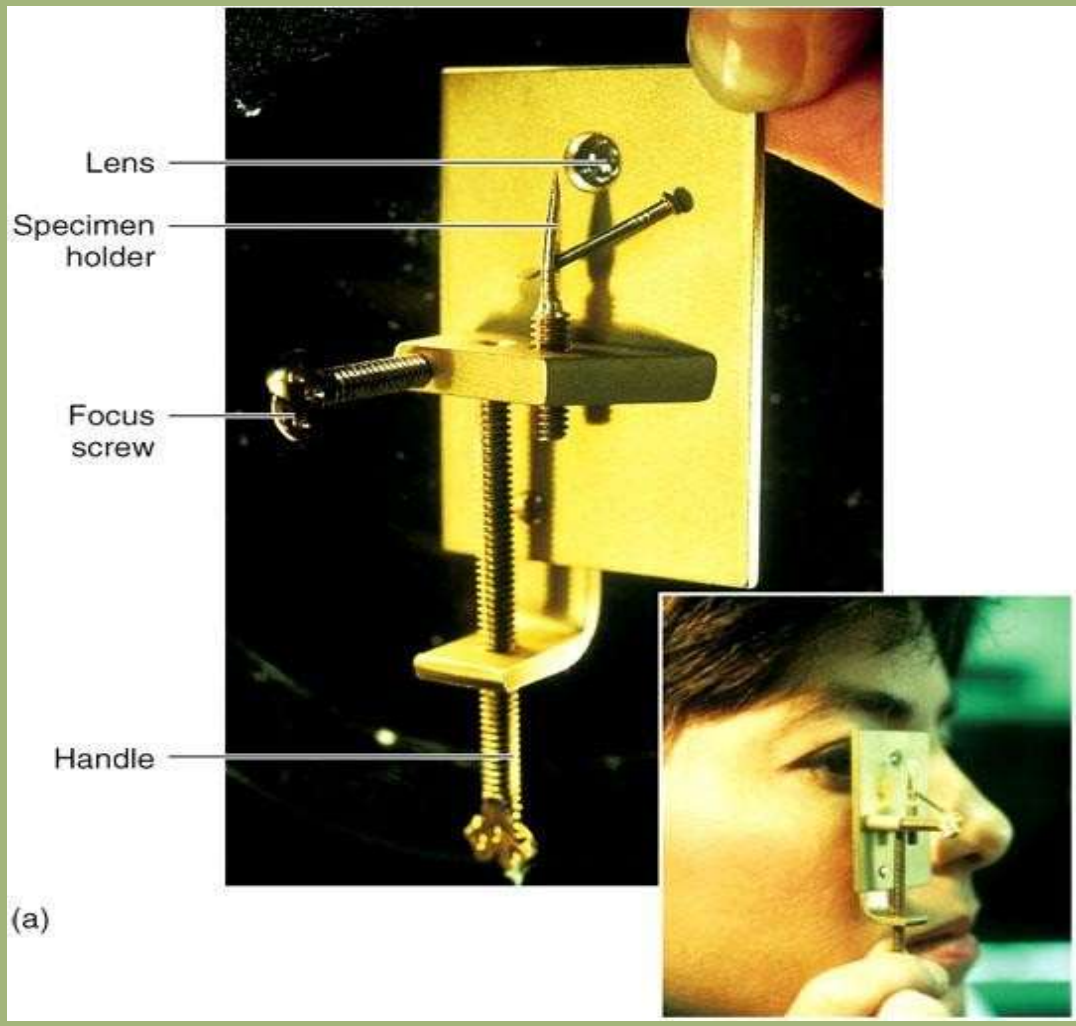
Antonie van Leeuwenhoek

- **Leeuwenhoek** (1632 –1723) was a Dutch tradesman and scientist.
- He is commonly known as "the Father of Microbiology", and considered to be the first microbiologist.
- He is best known for his work on the improvement of the microscope and for his contributions towards the establishment of microbiology.
- Using his handcrafted microscopes, he was the first to observe and describe single-celled organisms. which he originally referred to as *animalcules*, and which we now refer to as microorganisms.

Antonie van Leeuwenhoek

In his youth van Leeuwenhoek was apprenticed to a draper. Later, a civil service position allowed him to give time to his hobby: grinding lenses and using them to study tiny objects.

He was also the first to record microscopic observations of muscle fibers, bacteria, spermatozoa and blood flow in small blood vessels.



In 1676, van Leeuwenhoek observed water closely and was surprised to see tiny organisms - the first bacteria observed by man. His letter announcing this discovery caused widespread doubt at the Royal Society but Robert Hooke later repeated the experiment and was able to confirm his discoveries.

Van Leeuwenhoek did not write books, but sent letters to the Royal Society in London.

The letters were published in the Royal Society's journal *Philosophical Transactions of the Royal Society*.

His simple microscopes were skillfully ground, powerful single lenses capable of high image quality.

He looked at protozoa in rainwater, pond water and well water. He also looked at bacteria in the human mouth and intestine.

In 1677, he first described the spermatozoa of insects, dogs, and humans.

His observations laid the foundations for the sciences of bacteriology and protozoology. He was the first to see bacteria, protists, spermatozoa, the cell vacuole, blood corpuscles, capillaries, and the structure of muscles and nerves.

A brief account of his chief discoveries is presented below.

1674	The Infusoria - (Protist class in modern Zoology)
1676	The Bacteria (Genus Selenomonas - crescent shaped bacteria from human mouth)
1677	The Spermatozoa
1682	The banded pattern of muscular fibers
1687	Research on the coffee beans

John Tyndall

John Tyndall, (born August 2, 1820, Leighlinbridge, County Carlow, Ireland—died December 4, 1893, Hindhead, Surrey, England), Irish experimental physicist who, during his long residence in England, was an avid promoter of science in the Victorian era. After a thorough basic education he worked as a surveyor in Ireland and England (1839–47).

When his ambitions turned from engineering to science, Tyndall spent his savings on gaining a Ph.D. from the University of Marburg, Germany (1848–50), but then struggled to find employment.

An outstanding experimenter, particularly in atmospheric physics, Tyndall examined the transmission of both radiant heat and light through various gases and vapours.

He discovered that water vapour and carbon dioxide absorb much more radiant heat than the gases of the atmosphere and argued the consequent importance of those gases in moderating Earth's climate—that is, in the natural greenhouse effect.

Tyndall also studied the diffusion of light by large molecules and dust, known as the Tyndall effect, and he performed experiments demonstrating that the sky's blue colour results from the scattering of the Sun's rays by molecules in the atmosphere.

Thank you

BACTERIAL STAINING TECHNIQUES

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INTRODUCTION:

- **Microbial Staining – giving colour to microbes.**

- **Because microbes are colourless and highly transparent structures.**

- **Staining – process in which microbes are stained.**

INTRODUCTION - STAINS

- **Stains/dyes - organic compounds which carries either positive charges or negative charges or both.**

- **Based on the charges:**
- **Basic stain/dyes – stain with +ve charge.**
- **Acidic stain/dyes – stain with –ve charge.**
- **Neutral stain/dyes – stain with both charges.**

- **Based on function of stain:**

1. **Simple staining – only one dye is used- differentiation among bacteria is impossible- Eg. Simple Staining.**

2. **Differential staining- more than one dye is used- Differentiation among bacteria is possible- Eg. Gram's staining, Acid-fast staining.**

3. **Special staining – more than one dye used - Special structures are seen.**

Eg. Capsule staining, Spore staining.

Principle of staining:

- Each staining methods have own principles but the following steps may be common:

- Basic stain(+ve charge) –
- To stain -ve charged molecules of bacteria
- Mostly used because cell surface is –ve charge.

- Acidic Stain(-ve charge)

To stain +ve charged molecules of bacteria. Used to stain the bacterial capsules.

- As cell surface is –ve charged- Basic dyes mostly used.

Basic requirements for staining:

- Clean grease-free slide.
- Bacteria tobe stained.
- Inoculating loops- to transfer bacterial suspension to slide.
- Bunsen burner – to sterilise inoculating loops before and after smear preparation.
- Pencil marker – to mark (particularly central portion of slide) where bacterial smear is applied

Basic initial steps before staining:

- ***Smear preparation:***
- **Putting of bacterial suspension (bacteria in liquid) tobe stained on the central portion of slide in a circular fashion, air-dried, heat-fixed, the resultant preparation called *bacterial smear*- appears dull white.**

SIMPLE STAINING:

- **Simple to perform- only one basic stain used.**

Eg. Crystal violet, Methylene blue, Basic fuschin, Malachite green etc.,

Principle:

-All bacteria in smear takes stain and appears in colour of stain.

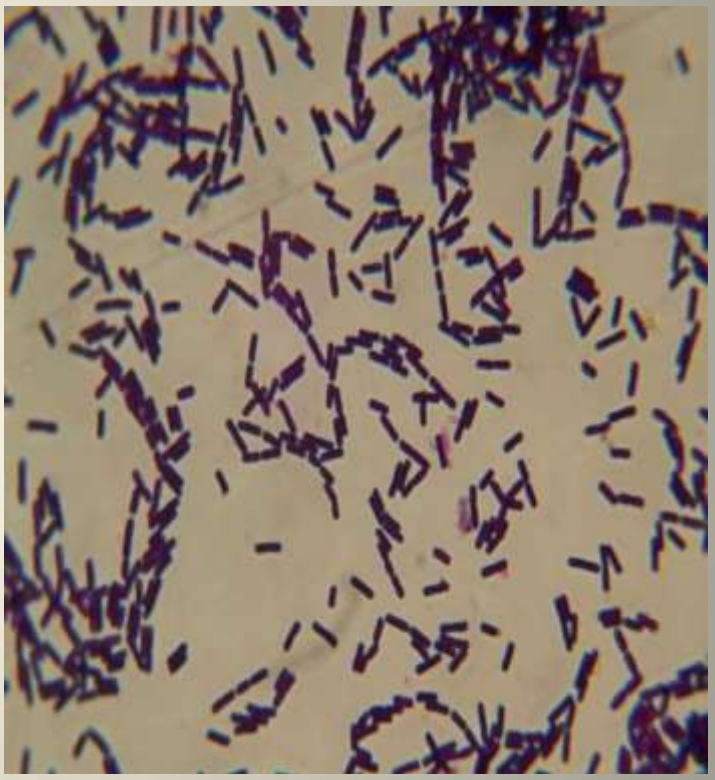
-Basic stain more affinity towards bacterial surface & stains the bacteria.

Uses:

To study morphology and arrangement of bacteria.

Procedure:

- A bacterial smear is prepared, air-dried and heat-fixed.
- A Heat-fixed smear is flooded with either one of the basic stain and allowed to react for 1-2 minutes and then washed under running tap water.
- Air dried and focussed with 10x,45x & 100x.



Results:

- Morphology – spherical / rod.
- Arrangement – cocci – clusters/chains.

GRAM STAINING :

- **DANISH BACTERIOLOGIST HANS CHRISTIAN GRAM (1880)**
- **Based on this reaction, bacteria classified into Gram positive and Gram negative bacteria.**
- **The cell wall compostion differences makes difference.**

REQUIREMENTS – STAINING REAGENTS:

1. Crystal violet – Primary stain
2. Gram's iodine- mordant/fixative
3. Acetone (95%)- decoloriser
4. Safranine/dilute carbol fuchsin – counterstain

PRINCIPLE:

1.Crystal violet - all bacteria take crystal violet- so all appears violet.

2.Iodine – Crystal Violet-iodine(CV-I) complex is formed.

3.Acetone- bacteria with high lipid content loose CV-I complex(appear colouless) but bacteria with less lipid content retains CV-I complex (appear violet).

4.Safranine/ dilute carbol fuchsin – only colouless bacteria takes – appear pink.

RESULT:

Colour:

Purple colored bacteria – Gram positive

Pink colored bacteria – Gram negative

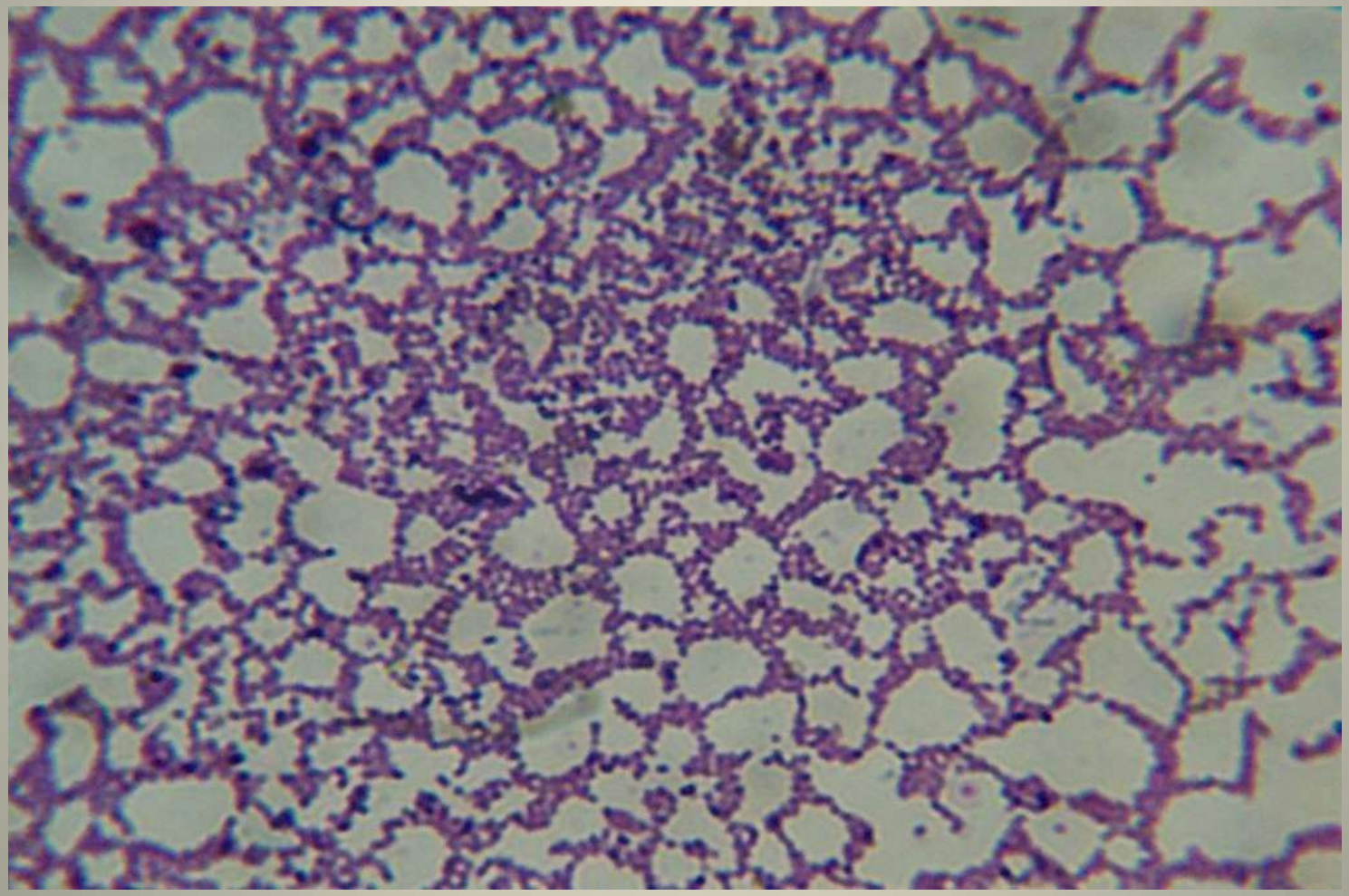
Shape:

Spherical – cocci Rod – bacilli

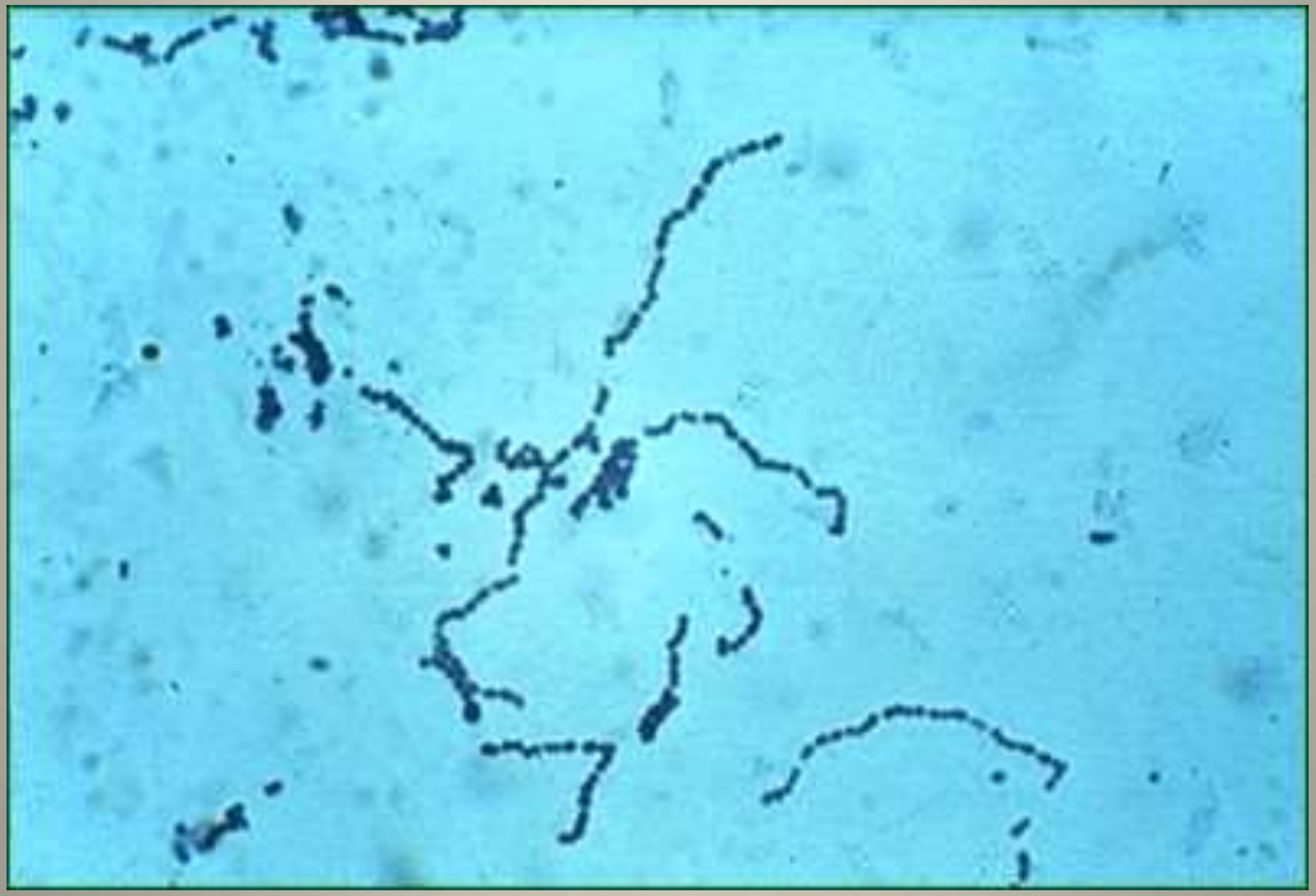
Arrangement

Cocci in clusters – staphylococci Cocci in chains - streptococci

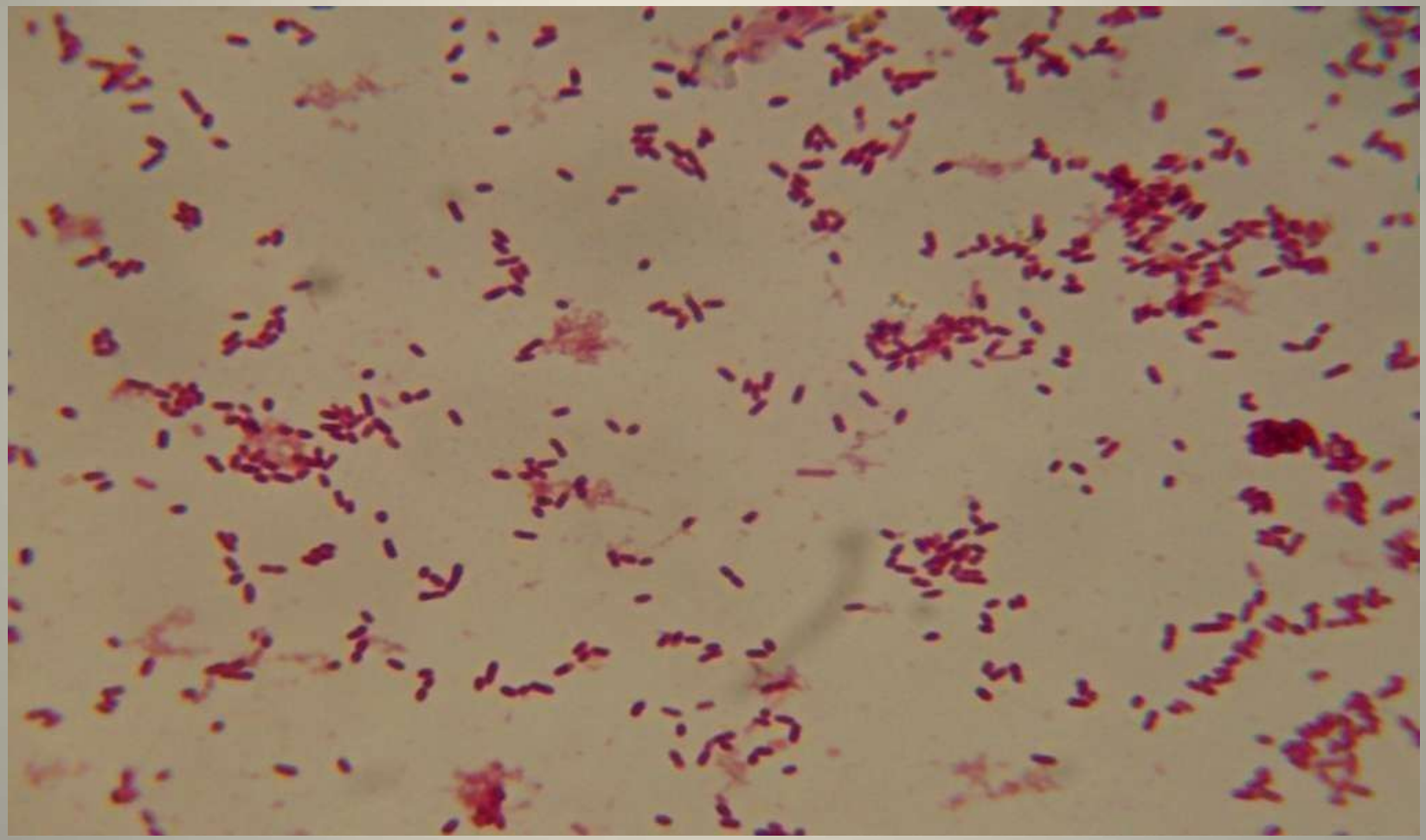
GRAM POSITIVE COCCI IN CLUSTERS



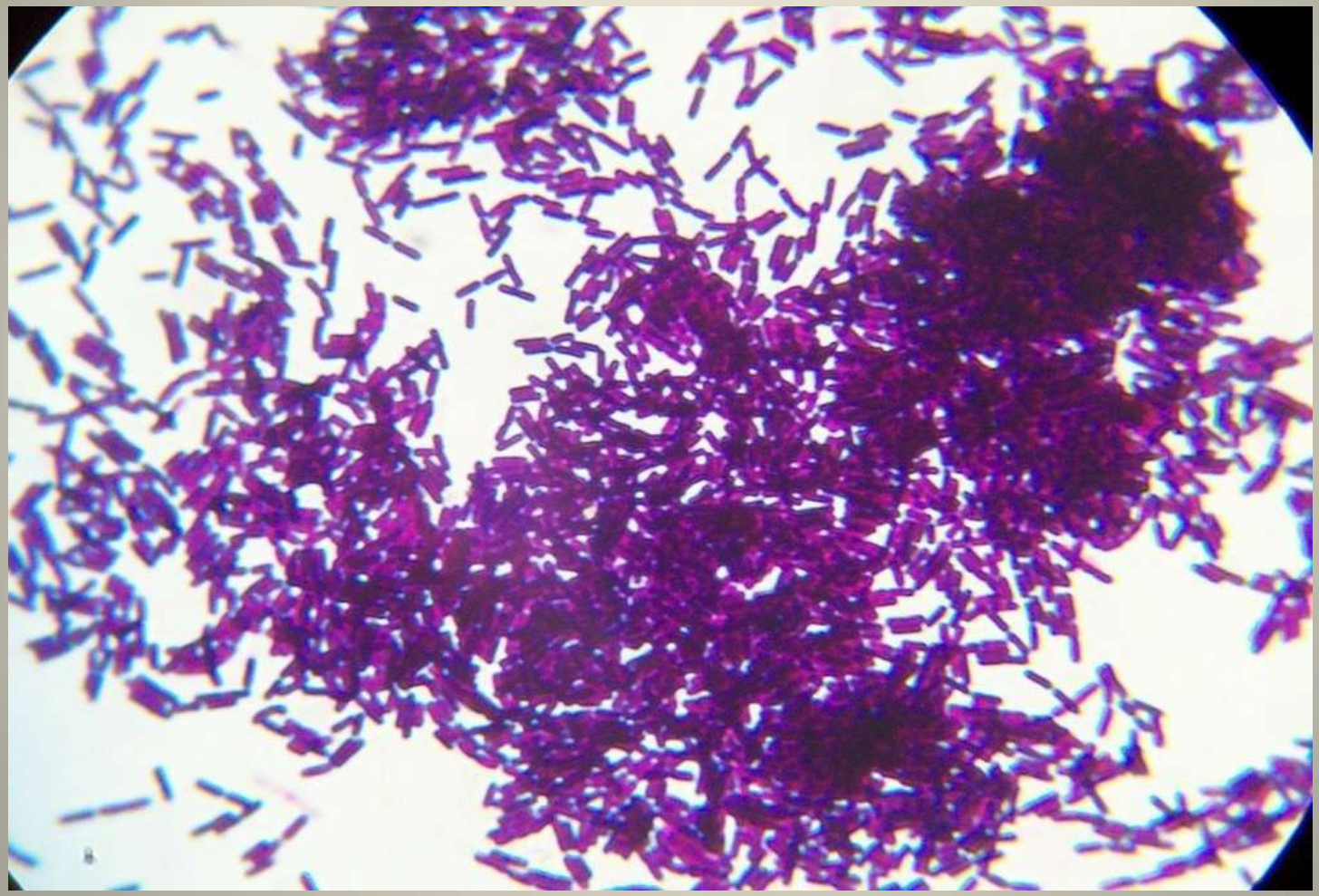
GRAM POSITIVE COCCI IN CHAINS



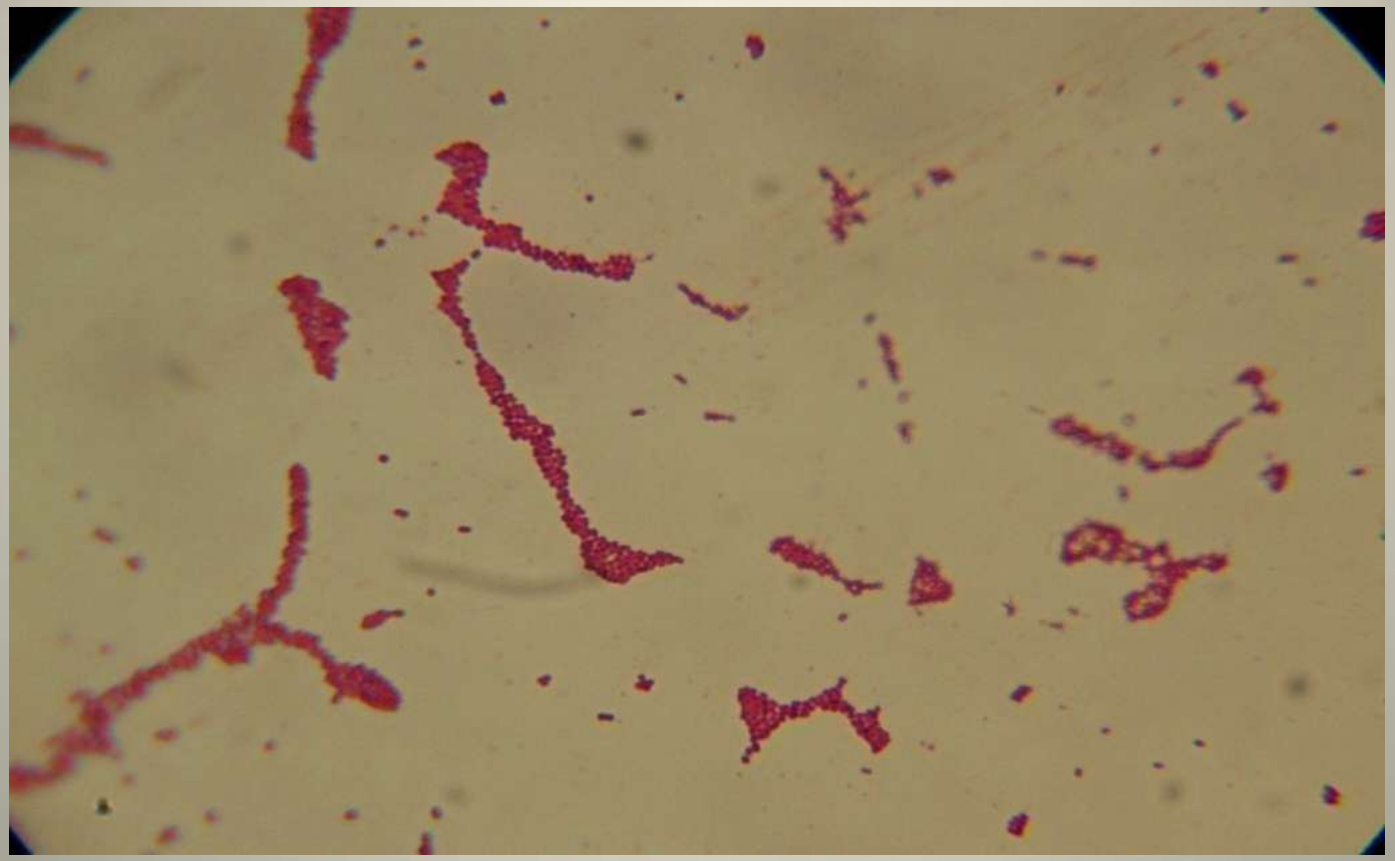
GRAM NEGATIVE BACILLI



GRAM POSITIVE BACILLI



GRAM NEGATIVE COCCI

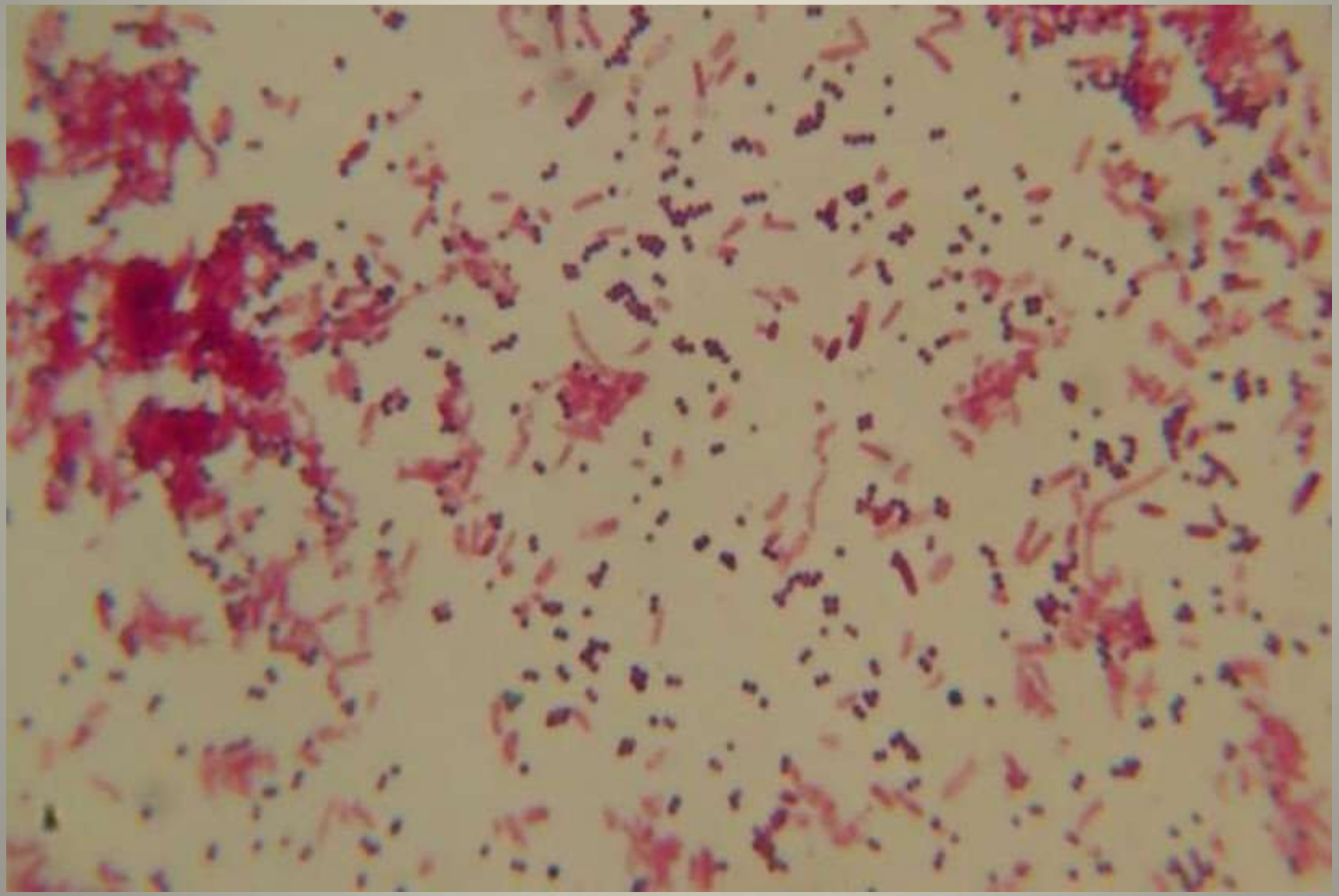


PROCEDURE:

- Crystal violet – 1 min - wash.
- Iodine – 1 min – wash.
- Acetone add drop by drop and watch out colour comes out – wash immediately.
- Safarnine/dilute carbol fuchsin – 1 min- wash.
- Allow to dry – examine under microscope.

Note: Results should be confirmed only with 100x.

Gram stain (Mixed)



EXAMPLES:

- **Gram positive cocci in clusters:**
- 1. Staphylococci species.
- **Gram negative cocci in chains:**
- 1. Streptococci species.
- **Gram negative cocci:**
- 1. Neisseria species.
- **Gram negative bacilli:**
- 1. Escherichia coli
- 2. Klebsiella pneumoniae

- **Gram positive bacilli:**

- 1. Clostridium species.
- 2. Corynebacterium species.
- 3. Bacillus anthracis.

ACID-FAST STAINING: (Ziehl-Neelsen stain)

- To stain Mycobacterium species especially *M.tuberculosis*.
- High lipid content – makes decolorisation very difficult –extraordinary property.
- **Principle:**
- ***Acid fast(resist)*** – Property of Mycobacterium species - once this bacteria stained with primary dye – difficult to decolorise with acid.
- This property due to Mycolic acid in cell wall.
- *M.tuberculosis* – also ***Alcohol fast***

Staining reagents:

1.Strong carbol fuchsin – primary stain
2.20% sulphuric acid/3% Hcl – decoloriser – acid-fast property.
3.95% alcohol- decoloriser- alcohol – fast property
4.Methylene blue/ Malachite green- counterstain.

Note:

5% sulphuric acid – for *M.leprae.*
1% sulphuric acid – for Nocardia species.

Procedure:

1. Strong carbol fucshin-heat till steam rises – allow 5-10 min to act (alternately leave it 10-15 min – cold staining method) – wash.

2. Decolorise with acid-alcohol mixture till get a faint pink colour in the smear (take 3-5 min) – wash.

3. Methylene blue/Malachite green – 2 min – wash.

4. Allow to dry and focuss under microscope.

Result:

- **Pink bacilli – Acid fast bacteria/bacilli**

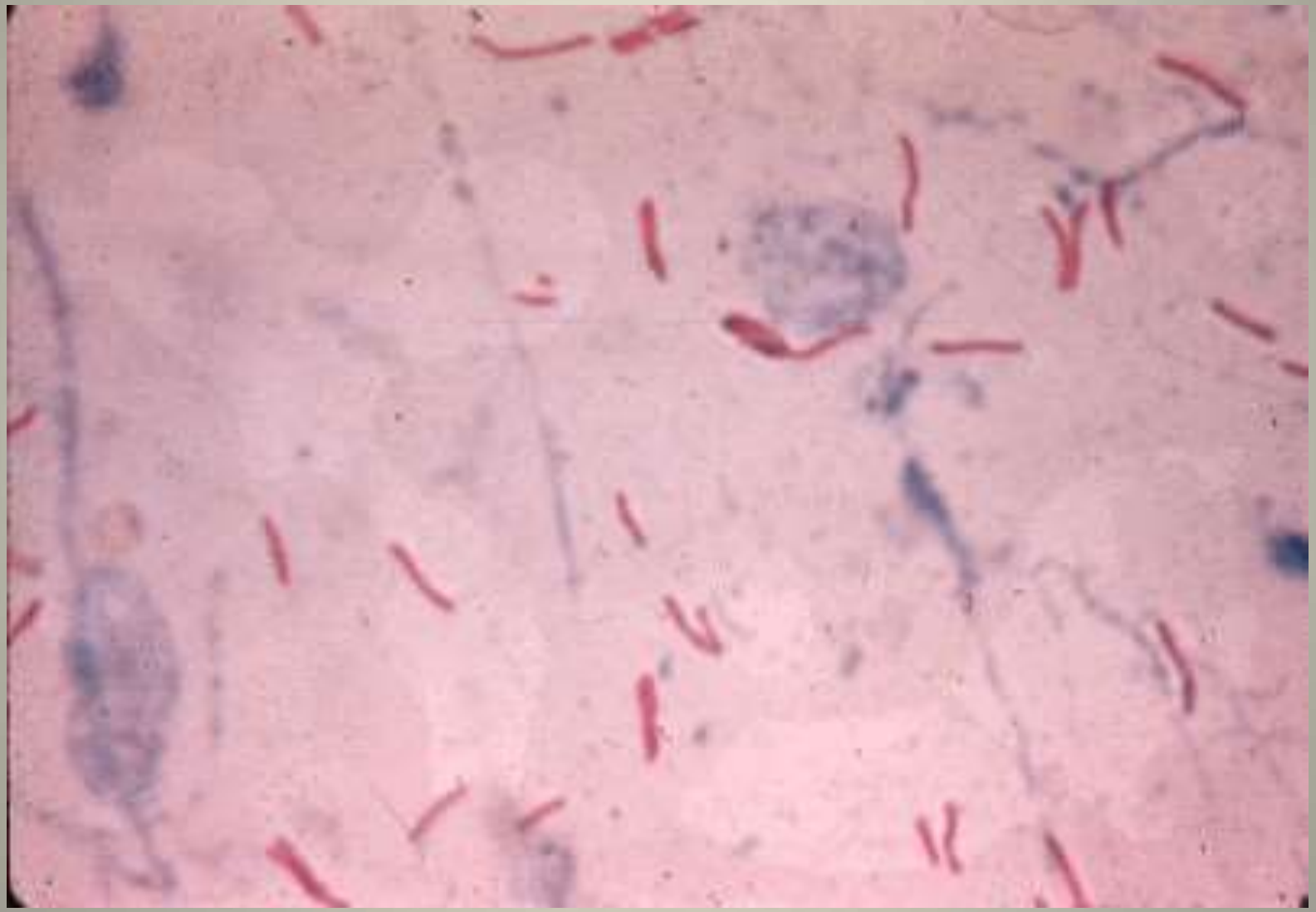
Eg., M.tuberculosis – long slender bacilli.

- **M. leprae – short thick bacilli.**

- **Blue colored bacteria – Non-acid fast**

Eg., Epithelial cells, pus cells, other bacteria.

Acid fast stain:



SPECIAL STAIN:

Used to stain special structures of bacteria– capsule, spores, flagella, metachromatic granules.

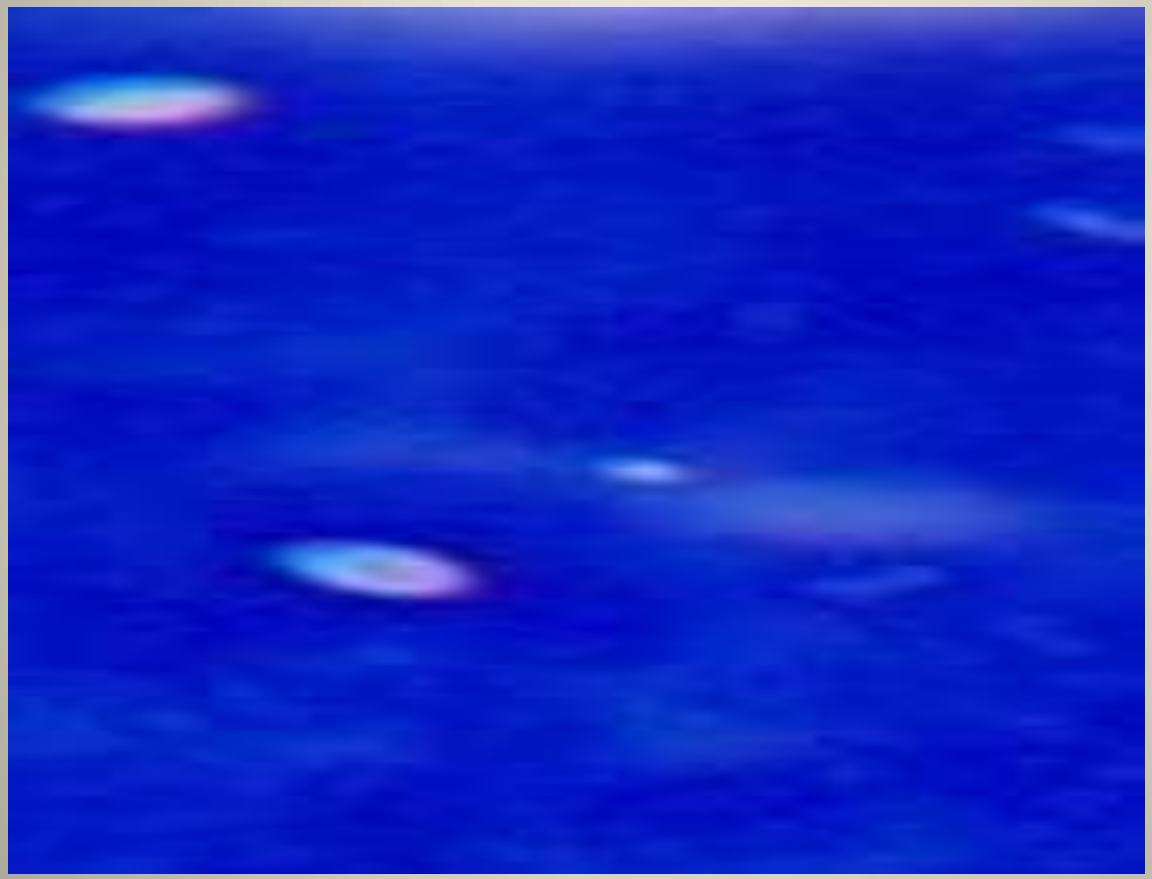
CAPSULE STAIN:

Negative stain:

1. Drop of Nigrosin ink+ indian ink
2. Bacterial culture (1-2 colonies)
3. Spread evenly and air-dry.
4. Look for unstained structures against stained background.

CAPSULE STAIN BY NIGROSIN INK (BLACK)

CAPSULE STAIN- INDIAN INK(BLUE)

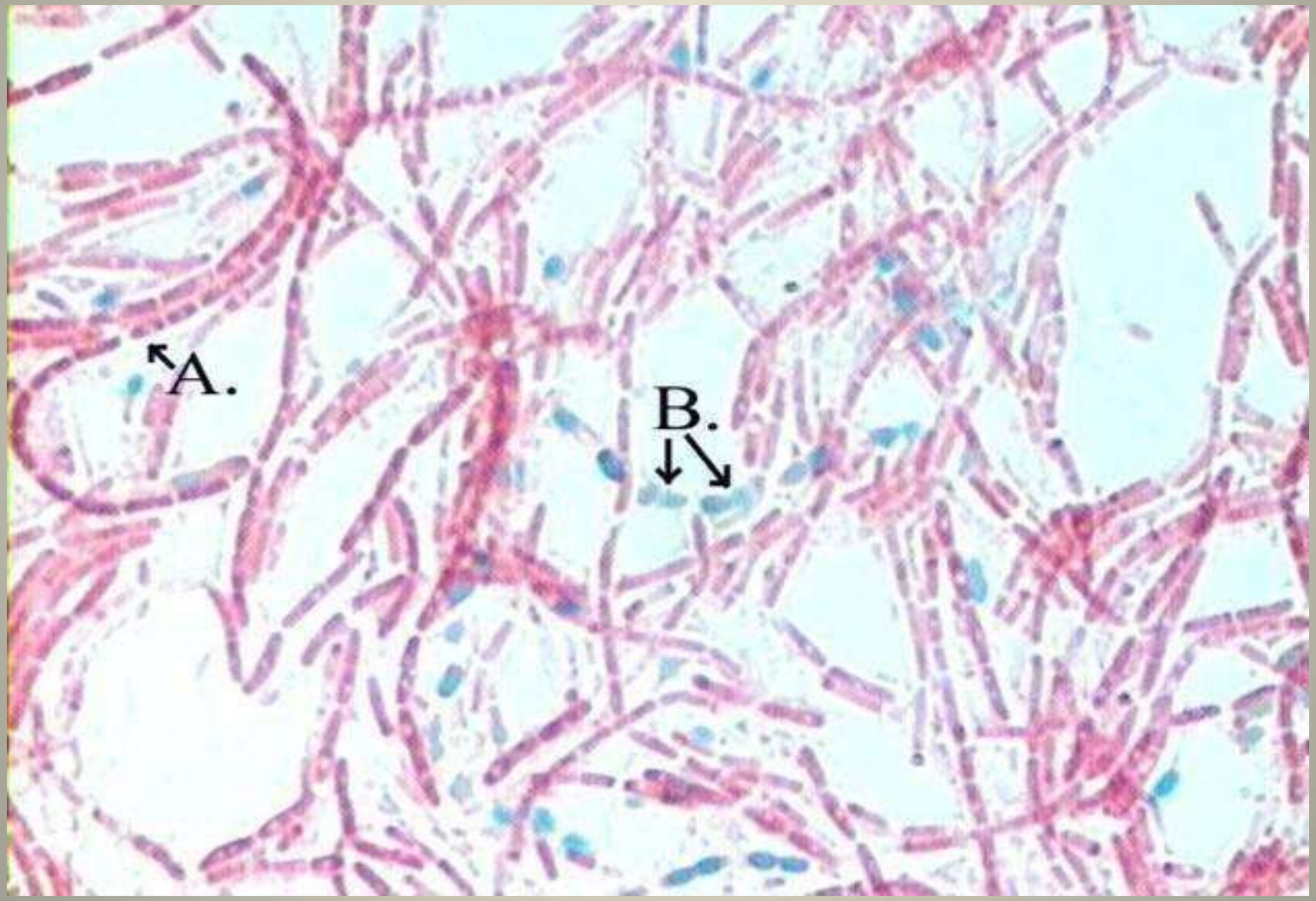


SPORE STAIN:

1. Malachite green- 2 min- heat stain till steam rises -2 min - wash.
2. Counterstain with safranin –1 min- wash.
3. Dry the slide and examine.

Spore forming bacteria:
Eg., Clostridium species.
Bacillus species – Eg. B. anthracis

Spore stain



Louis Pasteur and his contributions

- Louis Pasteur was a French chemist and microbiologist considered the most important founders of Microbiology.
- Microbiology developed as a scientific discipline from the era of Louis Pasteur (1822- 1895) himself.
- He first coined the term "microbiology" for the study of organisms of microscopic size. For his innumerable contributions in the field, he is also known as the **Father of Microbiology**.
- He is renowned for his discoveries of the principles of vaccination, microbial fermentation and pasteurization.
- He is remembered for his remarkable breakthroughs in the causes and prevention of diseases.
- He is regarded as one of the three main founders of bacteriology, together with Ferdinand Cohn and Robert Koch.
- Pasteur's academic positions were numerous, and his scientific accomplishments earned him France's highest decoration, the Legion of Honor, as well as election to the Académie des Sciences and many other distinctions.
- Today there are some 30 institutes and an impressive number of hospitals, schools, buildings, and streets that bear his name- a set of honors bestowed on few scientists.

Major Contributions of Louis Pasteur

The studies on fermentation led Pasteur to take interest to work in microbiology. His contributions to microbiology are as follows:

- He disproved the theory of spontaneous generation of disease and postulated the germ theory of disease: He stated that disease cannot be caused by bad air or vapor but it is produced by the microorganisms present in air.

- The doctrine of spontaneous generation was disapproved by his experiments that showed that without contamination, microorganisms could not develop.
- He proposed the principles of fermentation for preservation of food.
- He introduced the sterilization techniques and developed steam sterilizer, hot air oven and autoclave.
- He described the method of pasteurization of milk and wine.
- He reduced mortality from puerperal fever. He had also contributed for the vaccine development against several diseases, such as anthrax, fowl cholera and rabies.
- Liquid media concept: He used nutrient broth to grow microorganisms.
- He was the founder of the Pasteur Institute, Paris.

Besides in microbiology, Pasteur made significant discoveries in chemistry, most notably on the molecular basis for the asymmetry of certain crystals and racemization.

- Early in his career, his investigation of tartaric acid resulted in the first resolution of what is now called optical isomers.
- His work led the way to the current understanding of a fundamental principle in the structure of organic compounds.

Pasteur's Experiment of the Swan Necked Flask

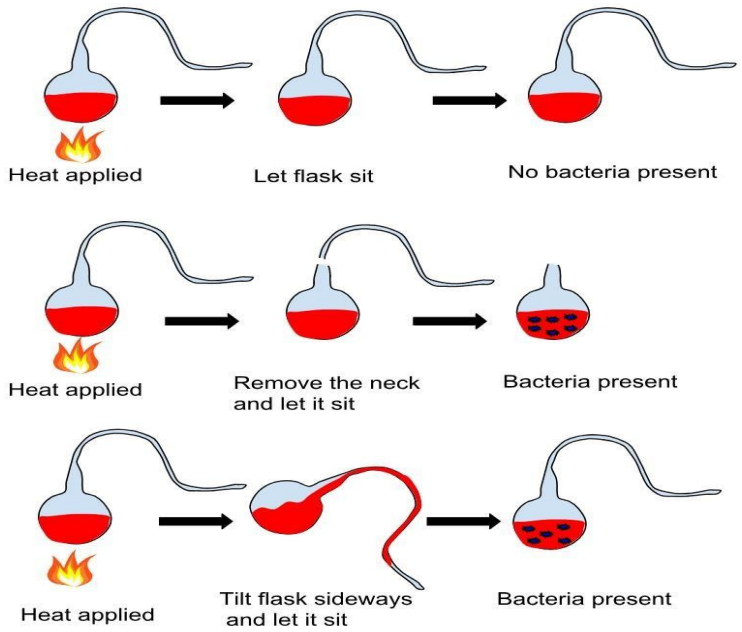


Figure: Louis Pasteur's spontaneous generation experiment illustrates the fact that the spoilage of liquid was caused by particles in the air rather than the air itself. These experiments were important pieces of evidence supporting the idea of germ theory of disease.

- Pasteur first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of cotton was placed in a sterile medium after air had been filtered through it, microbial growth appeared.
- Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere. These flasks looked like the neck of swans hence giving the famous experiment its name.
- Pasteur then boiled the solutions for a few minutes and allowed them to cool.
- No growth took place even though the contents of the flasks were exposed to the air.
- Pasteur pointed out that no growth occurred because dust and germs had been trapped on the walls of the curved necks.
- If the necks were broken, growth commenced immediately.
- By this Pasteur proved that all life even microbes arose only from their like and not de novo (germ theory of disease). Pasteur had not only resolved the controversy of origin of microorganisms but also had shown how to keep solutions sterile with this one single experiment.

Robert Koch and Koch's Postulates

Heinrich Hermann Robert Koch (1843 – 1910) provided remarkable contributions to the field of microbiology. He was a German general practitioner and a famous microbiologist.

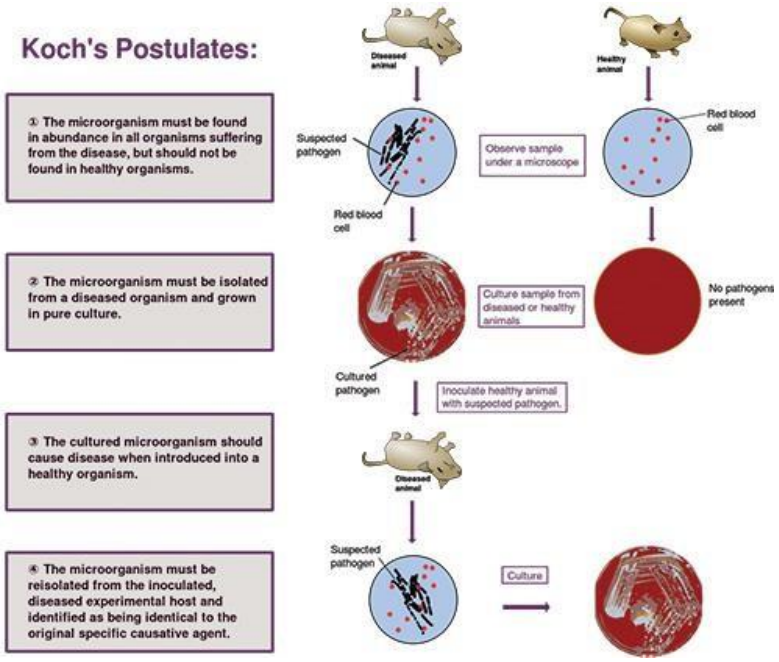
He is credited to be one of the founders of the specific field of modern bacteriology.

As the founder, he identified the specific causative agents of tuberculosis, cholera, and anthrax and gave experimental support for the concept of infectious disease, which included experiments on humans and animals.

For this he is also regarded as a pioneer of public health, aiding legislation and changing prevailing attitudes about hygiene to prevent the spread of various infectious diseases.

For his work on tuberculosis, he was awarded the Nobel Prize in 1905 in Physiology or Medicine.

Robert Koch (1843 - 1910)



Major Contributions of Robert Koch

- He investigated the anthrax disease cycle in 1876, and studied the bacteria that cause tuberculosis in 1882 and cholera in 1883.
- He discovered bacteria such as the anthrax bacilli, tubercle bacilli and cholera bacilli.
- Koch observed the phenomenon of acquired immunity.
- He introduced solid media for culture of bacteria. Koch pioneered the use of agar as a base for culture media. He developed the pour plate method and was the first to use solid culture media for culture of bacteria.

- Koch also developed media suitable for growing bacteria isolated from the body. Because of their similarity to body fluids, meat extracts and protein digests were used as nutrient sources. The result was the development of nutrient broth and nutrient agar media that are still in wide use today.

- He also introduced methods for isolation of bacteria in pure culture.

- He described hanging drop method for testing motility.

- He introduced staining techniques by using aniline dye.

- He invented the hot air oven and steam sterilizer, and also introduced methods to find out the efficacy of antiseptics.

- Koch's Phenomenon: Robert Koch observed that guinea pigs already infected with tubercle bacilli developed a hypersensitivity reaction when injected with tubercle bacilli or its protein. Since then, this observation was called as Koch's phenomenon.

- The medical applications of biotechnology still heavily depend on the Koch's principles of affirming the causes of infectious diseases.

Contribution to the Germ theory

- Building on the early work of Louis Pasteur and the germ theory of disease, Robert Koch established the basic scientific requirements used to demonstrate that each specific disease is caused by a specific microorganism.

- The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax by the German physician.

- These requirements were based on Koch's experiments with anthrax isolated from diseased hosts, and are known as "Koch's Postulates".

Experiment

In the experiment, Koch injected healthy mice with a material from diseased animals, and the mice became ill. After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillus in beef serum. The bacilli grew, reproduced, and produced spores. When the isolated bacilli or spores were injected into mice, anthrax developed.

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens. His criteria for proving the causal relationship between a microorganism and a specific disease are known as Koch's postulates.

Koch's Postulates

- Koch's Postulates consist of the following four rules:
- The microorganism must be identified in all individuals affected by the disease, but not in healthy individuals.
- The microorganism can be isolated from the diseased individual and grown in culture.
- When introduced into a healthy individual, the cultured microorganism should cause disease.
- The microorganism must then be re-isolated from the experimental host, and found to be identical to the original microorganism.

Limitations of Koch's Postulates

- While Koch's Postulates were developed as general guidelines for the identification of infectious causes of disease, there are some inherent limitations that could not be resolved at the time.
- Viruses were not yet able to be cultured during the 1800's. Thus, while it appeared that an infectious agent was responsible for certain diseases, the lack of available techniques to isolate and culture viruses meant that not all Koch's Postulates could be met.
- The third postulate stipulates that the experimental host "should" exhibit disease, not "must". This is because asymptomatic carriers, immunity, and genetic resistance are possible.
- Koch's Postulates do not account for prion diseases and other agents that cannot be grown in culture.
- Most of the human bacterial pathogens satisfy Koch's postulates except for those of *Mycobacterium leprae* and *Treponema pallidum*, the causative agent of leprosy and syphilis, respectively. Both these bacteria are yet to be grown in cell-free culture media.
- Therefore, Koch's Postulates have subsequently been revised to account for recent molecular advances and are no longer an absolute requirement of infectious causality.

Culture Techniques
&
Cultivation of Bacteria

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Cultivation/Culturing of Bacteria

- A microbial culture, is a method of multiplying microorganisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.

- Microbial cultures are used to determine the type of organism, its abundance in the sample being tested, or both.

Purpose of culturing

- Isolation of bacteria.
- Properties of bacteria i.e. culturing bacteria is the initial step in studying its morphology and its identification.
- Maintenance of stock cultures.
- Estimate viable counts.
- To test for antibiotic sensitivity.
- To create antigens for laboratory use.
- Certain genetic studies and manipulations of the cells also need that bacteria to be cultured in vitro.
- Culturing on solid media is another convenient way of separating bacteria in mixture.

Culture Media

An artificial culture media must provide similar environmental and nutritional conditions that exist in the natural habitat of a bacterium.

A culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors.

The pH of the medium must be set accordingly.

Uses:

- ✓ Enrich the number of bacteria.
- ✓ Select for certain bacteria and suppress others.
- ✓ Differentiate among different kinds of bacteria.

Pure culture

- In the laboratory bacteria are isolated and grown in pure culture in order to study the functions of a particular specie.
- A pure culture is a population of cells or growing in the absence of other species or types. A pure culture may originate from a single cell or single organism, in which case the cells are genetic clones of one another.

- Pure cultures are obtained by using variety of special techniques. All glassware, media and instruments must be sterilized i.e. aseptic techniques are used for obtaining pure cultures.
- Basic requirement for obtaining a pure culture are solid medium, a media container that can be maintained in an aseptic condition and a method to separate individual cell.
- A single bacterium, supplied with right nutrients, will multiply on the solid medium in a limited area to form a colony, which is a mass of cells all descended from the original one.

Agar

- Agar, a polysaccharide extracted from marine algae, is used to solidify a specific nutrient solution.
- Unlike other gelling agent, it is not easily degraded by many bacteria.
- It is not easily destroyed at higher temperatures, and therefore it can be sterilized by heating, the process which also liquefies it.
- Once solidified, agar medium will remain solid until

✓ The culture media is contained in a Petri dish, a two part, glass or plastic covered container.

Classification of Culture Media

- Bacterial culture media can be classified in at least three ways.

1. Consistency
2. Nutritional component
3. Functional use

Classification based on consistency

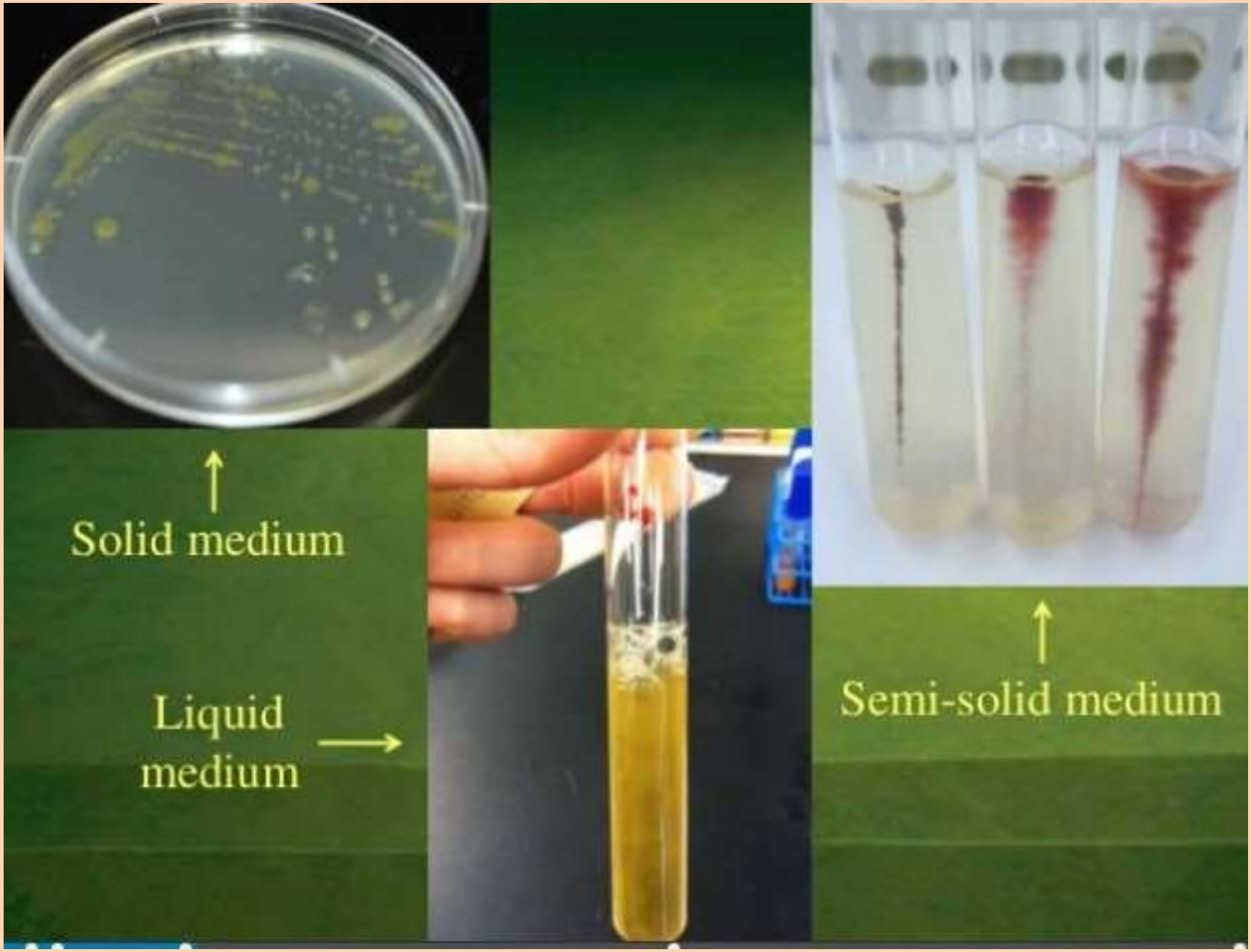
1. Liquid media.
2. Solid media.
3. Semi solid media.

Classification based on consistency:

A. **Liquid media:** These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths” (e.g nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity. No agar is added. Mostly used for inoculums preparation.

B. **Solid media:** An agar plate is a Petri dish that contains a growth medium (typically agar plus nutrients) used to culture microorganisms. 2% of agar is added. Agar is the most commonly used solidifying agent. Colony morphology, pigmentation, hemolysis can be appreciated. Examples include Nutrient agar and Blood agar.

C. **Semi-solid media:** Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains. Examples of Semi-solid media (Hugh & Leifson’s oxidation fermentation). 0.5% agar is added.


Solid medium
Liquid medium
Semi-solid medium

Classification based on Nutritional Components

1. Simple media.
2. Complex media.
3. Synthetic or chemically defined media.

Classification based on Nutritional Components

1. **Simple media:** Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria. It is also called as basal media. Eg: NB, NA. Nutrient Broth consists of peptone, yeast extract and NaCl. When 2% of agar is added to Nutrient Broth it forms Nutrient agar.

2. **Complex media**. Media other than basal media are called complex media. They have special ingredients in them for the growth of microorganisms. These special ingredients like yeast extracts or casein hydrolysate, which consists of a mixture of many chemicals in an unknown proportion.

3. **Synthetic media/Chemically defined media**: Specially prepared media for research purposes where the composition of every component is well known. It is prepared from pure chemical substances. Eg: peptone water (1% peptone + 0.5% NaCl in water).

3.Classification based on Functional Use or Application

1. Enriched media.
2. Selective media.
3. Differential media.
4. Transport media.
5. Indicator media.
6. Anaerobic media.

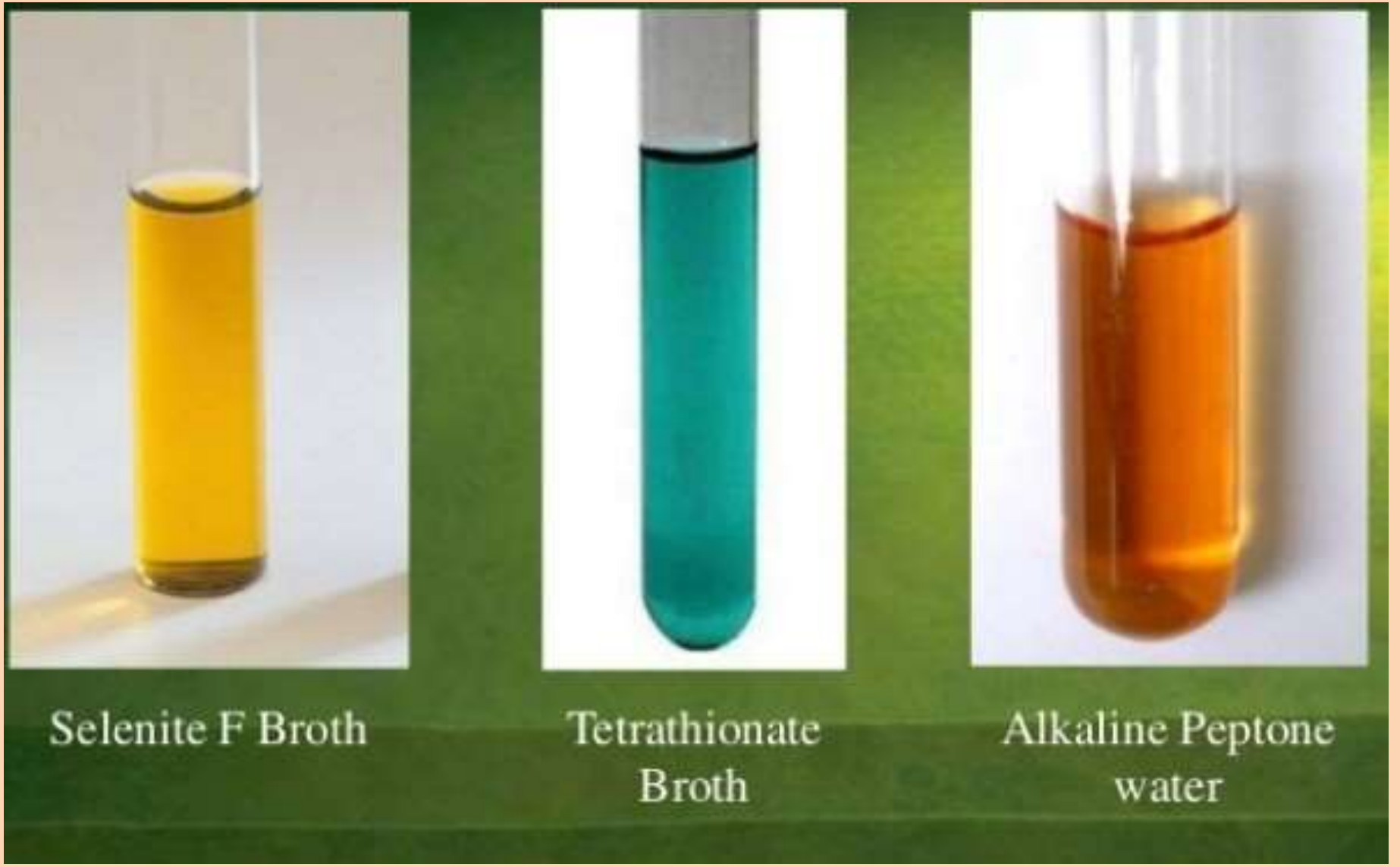
Classification based on Functional Use or Application

1. Enriched media :

- Addition of extra nutrients in the form blood, serum, egg yolk etc to basal medium makes them enriched media.
- Media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium and inhibit growth of unwanted bacterium
- Media is incorporated with inhibitory substances to suppress the unwanted organism, thus increase in numbers of desired bacteria.

Examples of Enriched media: Chocolate agar Blood agar.

- Selenite F Broth – for the isolation of Salmonella, Shigella.
- Tetrathionate Broth – inhibit coliforms .
- Alkaline Peptone Water – for Vibrio cholerae.


Selenite F Broth
Tetrathionate Broth
Alkaline Peptone water

- **Chocolate Agar** • Chocolate agar - is a non-selective, enriched growth medium used for growing fastidious bacteria, such as Haemophilus influenzae .

- **Blood Agar** • Blood agar plate (BAP) Contains mammalian blood (usually sheep or horse), typically at a concentration of 5– 10%. BAP are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity.


Blood
agar
Chocolate
agar

2. Selective media:

- The inhibitory substance is added to a solid media thus causing an increase in number of colonies of desired bacterium.
- Selective media and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria.
- Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen. To make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

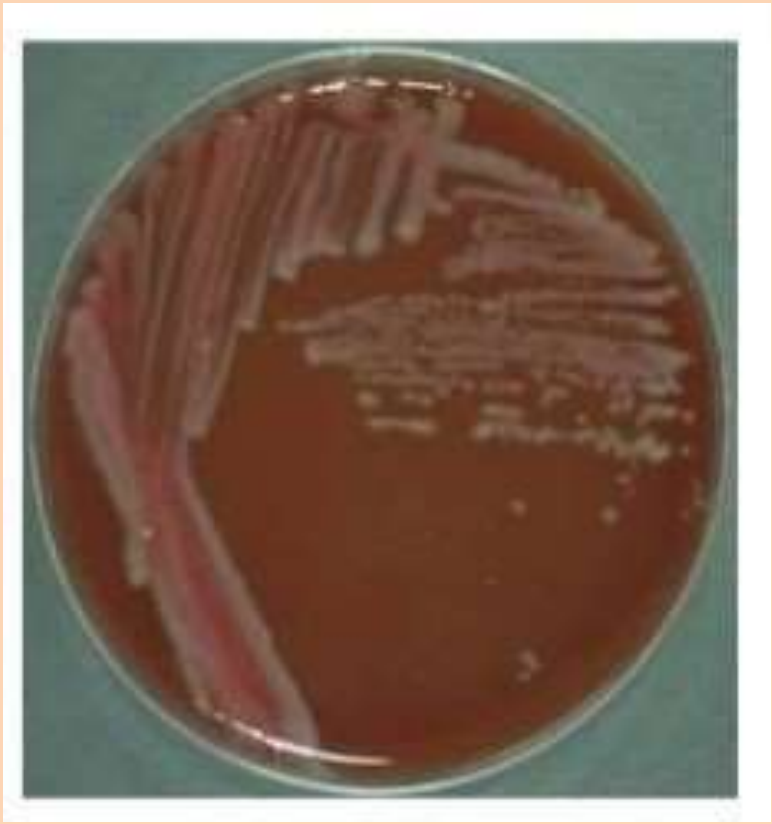
Examples of Selective media :

- Thayer Martin Medium selective for *Neisseria gonorrhoeae*.

- EMB agar is selective for gram-negative bacteria. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria.

- Campylobacter Agar (CAMPY) is used for the selective isolation of *Campylobacter jejuni*.

EMB agar



Campylobacter agar

3. Differential Media

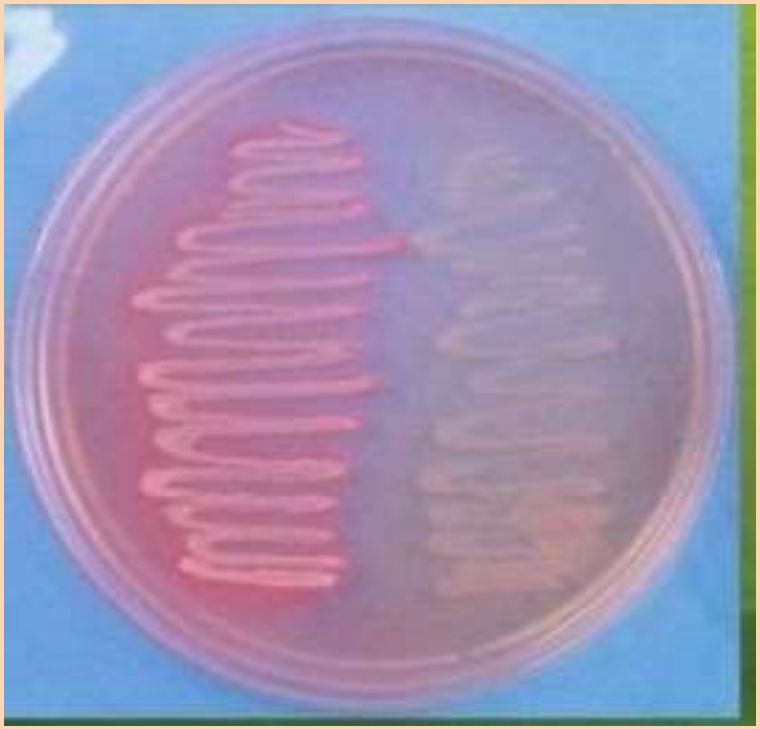
- Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently colored colonies. Substances incorporated in it enable it to distinguish between bacteria.

Example of differential media: MacConkey's agar, CLED agar, XLD agar etc.

- XYLOSE LYSINE DEOXYCHOLATE AGAR • XLD is used as a selective and differential medium for the recovery of Salmonella and Shigella species.

- CYSTEINE LACTOSE ELECTROLYTE DIFFECIENT AGAR • C.L.E.D. Agar is a non selective solid medium for cultivation of pathogens from urine specimens. Lack of salts (electrolytes) inhibits swarming of Proteus spp.

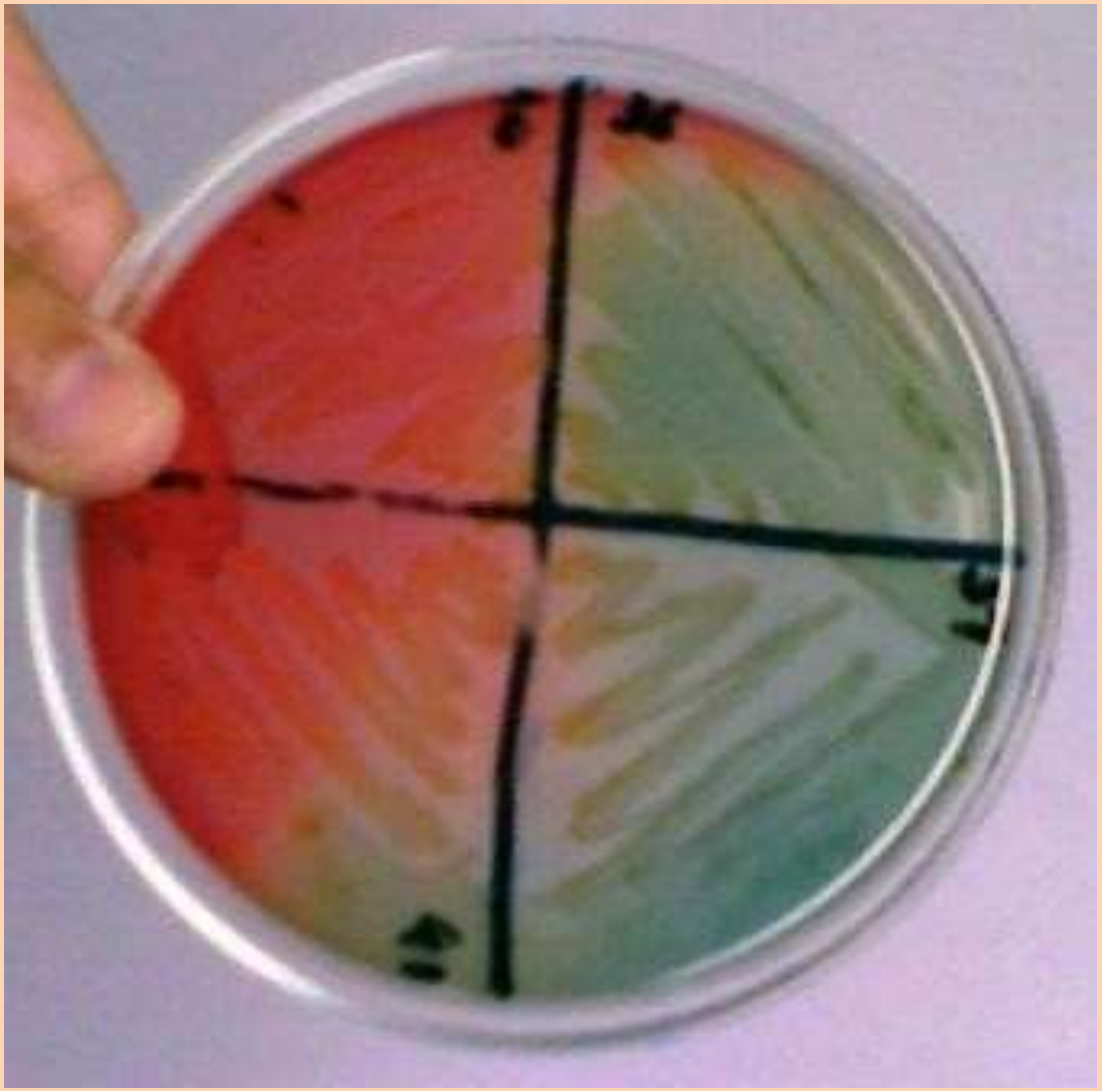
- MacConkey Agar culture medium designed to grow Gram-negative bacteria and differentiate them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria). Lactose fermenters – Pink colonies and Non lactose fermenters – colourless colonies.



Mac’Conkey Agar

C.L.E.D Agar

Growth of pathogenic *E.coli* on CLED agar



4. Transport Media

- Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. Delicate organisms may not survive the time taken for transporting the specimen without a transport media. This can be achieved by using transport media.

Transport media should fulfill the following criteria:

- Temporary storage of specimens being transported to the laboratory for cultivation.
- Maintain the viability of all organisms in the specimen without altering their concentration.
- Contain only buffers and salt.
- Lack of carbon, nitrogen, and organic growth factors so as to prevent microbial multiplication.
- Transport media used in the isolation of anaerobes must be free of molecular oxygen.

Example of Transport media:

- Cary Blair medium for campylobacter species.
- Alkaline peptone water medium for vibrio cholerae.
- Stuart's medium – non nutrient soft agar gel containing a reducing agent & charcoal used for Gonnococci.
- Buffered glycerol saline for enteric bacilli.

5. Indicator Media

- Contains an indicator which changes its color when a bacterium grows in them.

- Eg: Wilson-Blair medium – S. typhi forms black colonies.
- McLeod's medium (Potassium tellurite)– Diphtheria bacilli.
- Urease media: Urea → CO_2 + NH_3.

  NH_3 → Medium turns pink


Wilson-Blair Medium
McLeod's medium


Urease medium

6. Anaerobic media

- Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.
- Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K.
- Boiling the medium serves to expel any dissolved oxygen.

Example of Anaerobic media:

- Thioglycollate medium.

Thioglycollate medium

Culture Methods

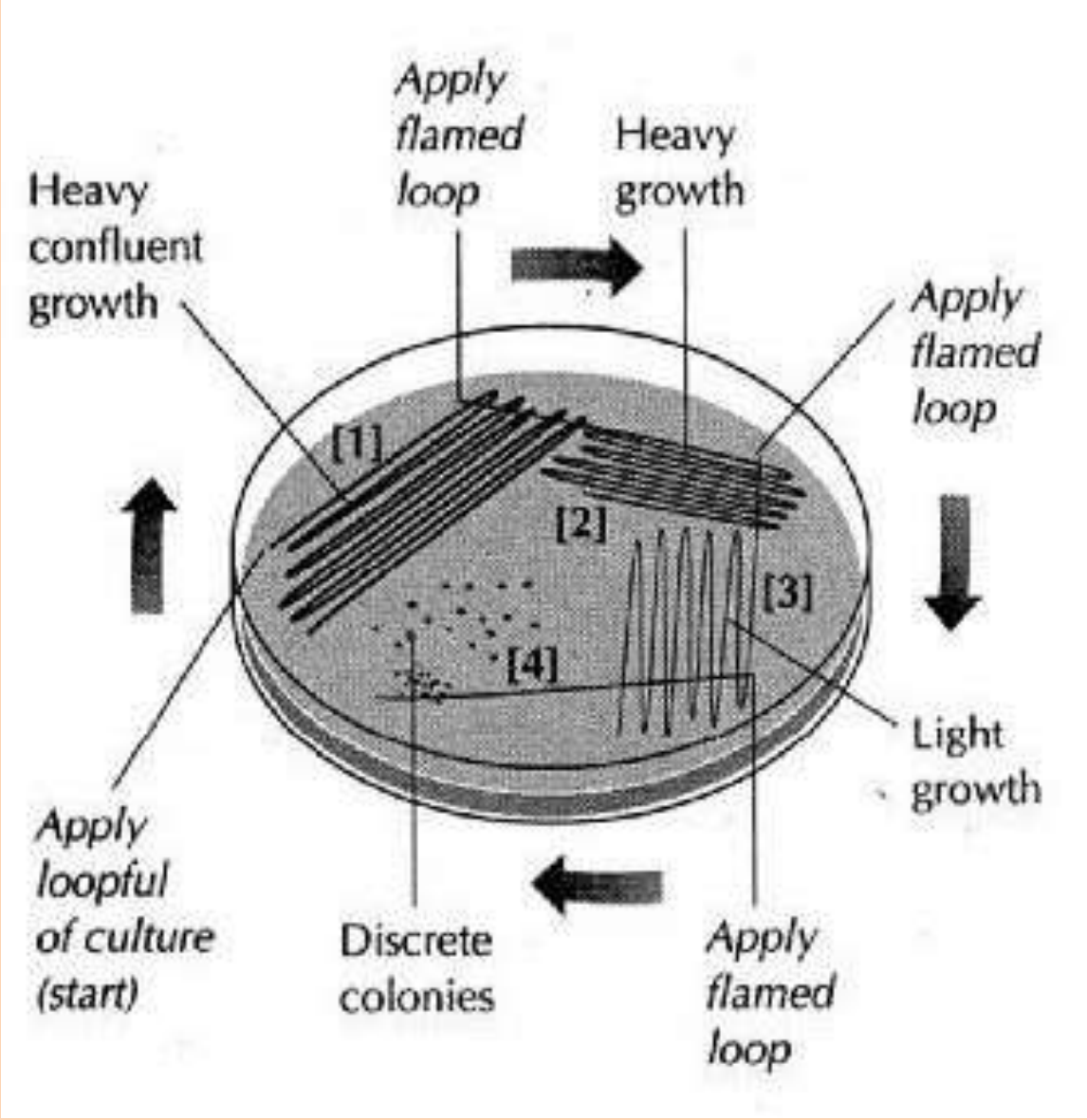
Culture Methods

- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method

Streak culture

Used for the isolation of bacteria in pure culture from clinical specimens.

- Platinum wire is used.
- One loop full of the specimen is transferred onto the surface of a well dried plate.
- Spread over a small area at the periphery.
- The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
- On incubation, separated colonies are obtained over the last series of streaks.


Apply flamed loop
Heavy growth
Heavy confluent growth
Apply flamed loop
[1]
[2]
[3]
[4]
Light growth
Apply loopful of culture (start)
Discrete colonies
Apply flamed loop

Lawn Culture

- Provides a uniform surface growth of the bacterium.
  - Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium
  - Uses
- For bacteriophage typing.
- Antibiotic sensitivity testing.
- In the preparation of bacterial antigens and vaccines.

Antibiotic sensitivity testing

Stroke Culture

- Stroke culture is made in tubes containing agar slope / slant.

Uses:

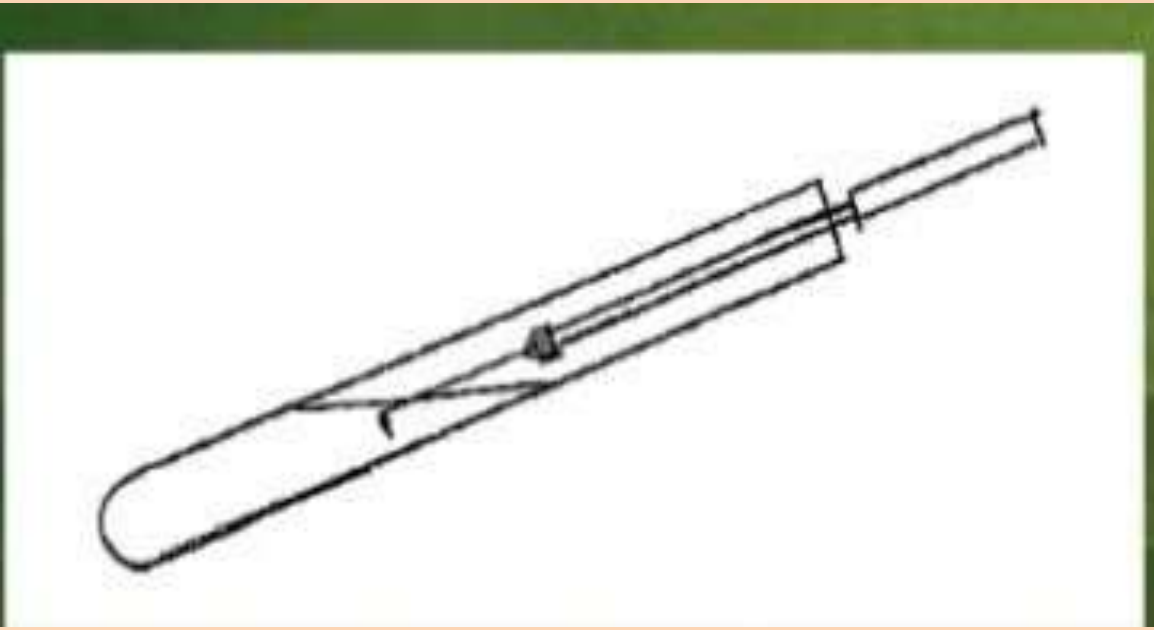
Provides a pure growth of bacterium for slide agglutination and other diagnostic tests.

Stab Culture

Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.

Uses

- Demonstration of gelatin liquefaction.
- Oxygen requirements of the bacterium under study.
- Maintenance of stock cultures.



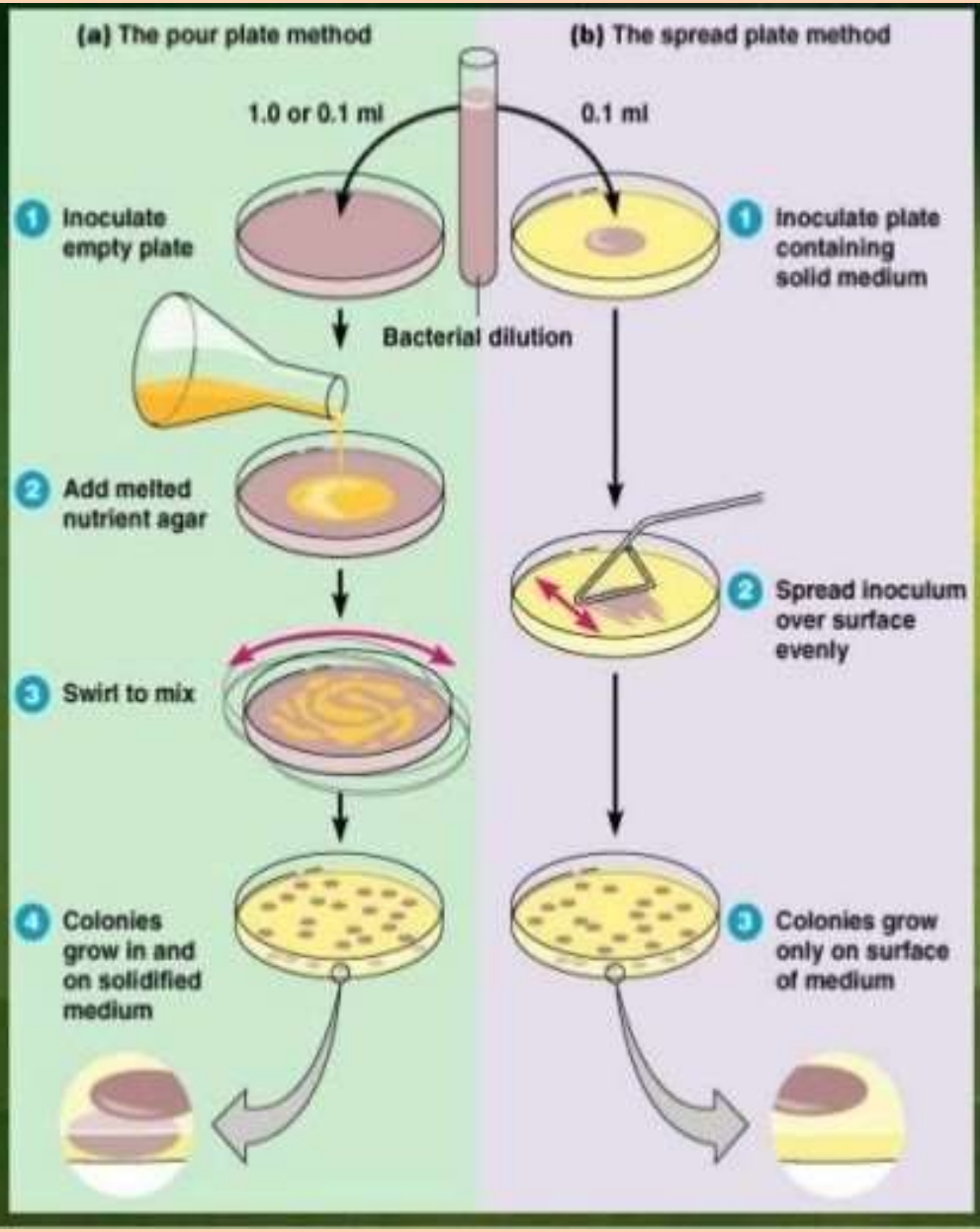
Pour Plate Culture

- 1 ml of the innoculum is added to the molten agar.
- Mix well and pour to a sterile Petri dish.
- Allow it to set.
- depth of the medium.

Uses:

– Gives an estimate of the viable bacterial count in a suspension.

– For the quantitative urine cultures.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
1 Inoculate plate containing solid medium
Bacterial dilution
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

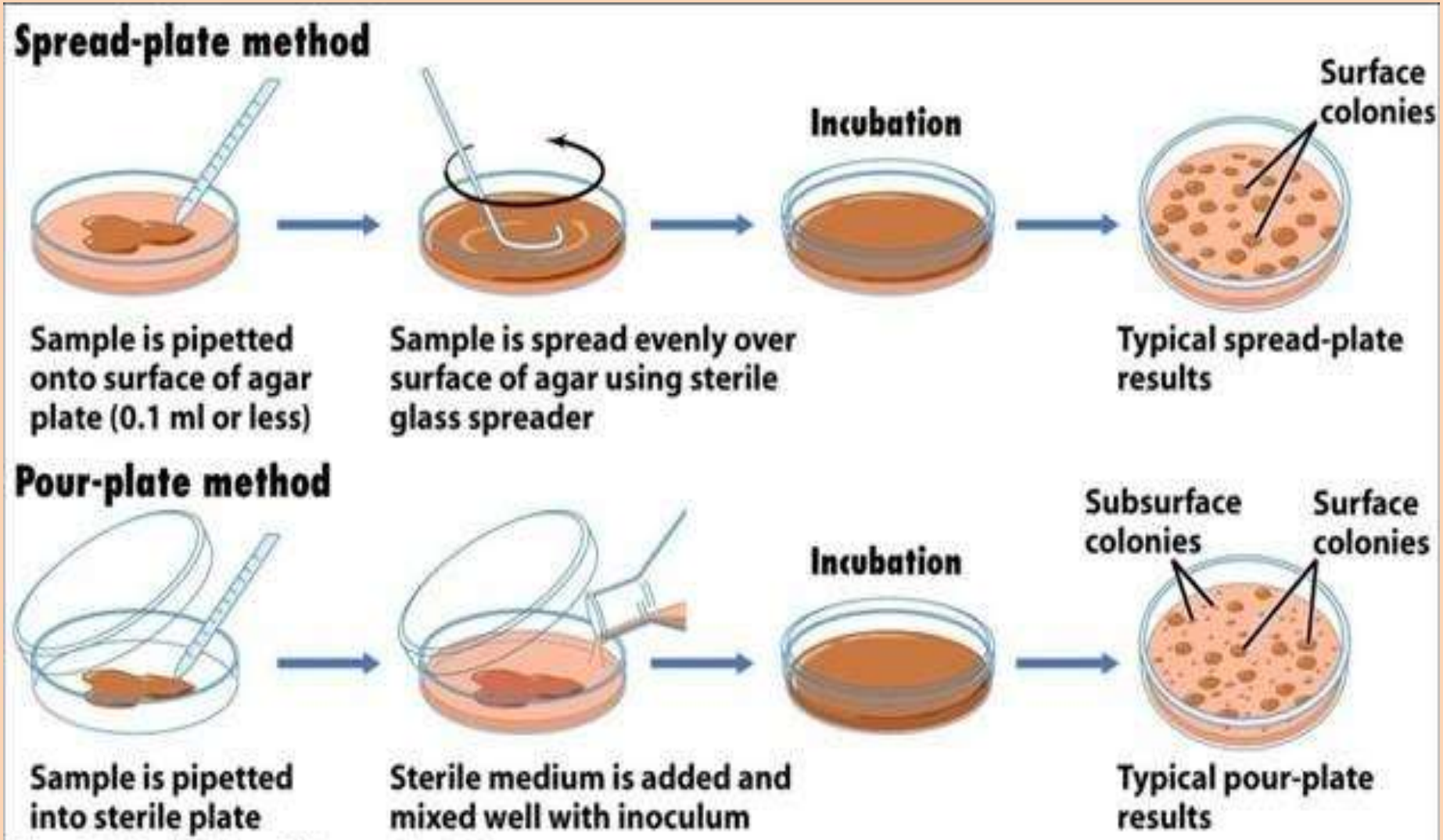
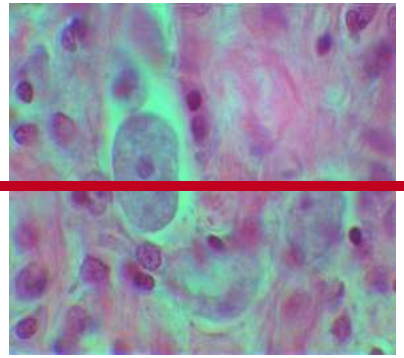


Figure 6-10 Brock Biology of Microorganisms 11/e

THANK YOU

PARASITOLOGY and MEDICAL ENTOMOLOGY



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G.B. NAGAR, KALAVAI.

Parasites of the Digestive system: intestinal protozoa

Entamoeba histolytica

Classification

Phylum :- Sarcomastigophora
Subphylum :- Sarcodina
Superclass:-Rhizopoda
Class:- Lobosea
Orders:- Euamoebida
Genus:- *Entamoeba*
Species:-*histolytica*

Entamoeba histolytica

First described by Losch in 1875 after being isolated in Russia from a patient with dysenteric stool

Geographical distribution

- World wide
- Worldwide amoebiasis causes 40,000-100,000 deaths every year

Habitat

Large intestine of man : Trophozite Forms : Mucous and submucous layer

Morphology

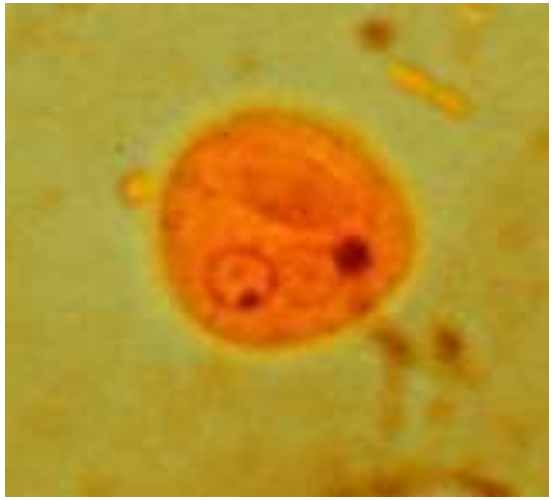
The parasite exists in three morphological forms:

- Tropozoite
- Precyst
- Cyst

Trophozite

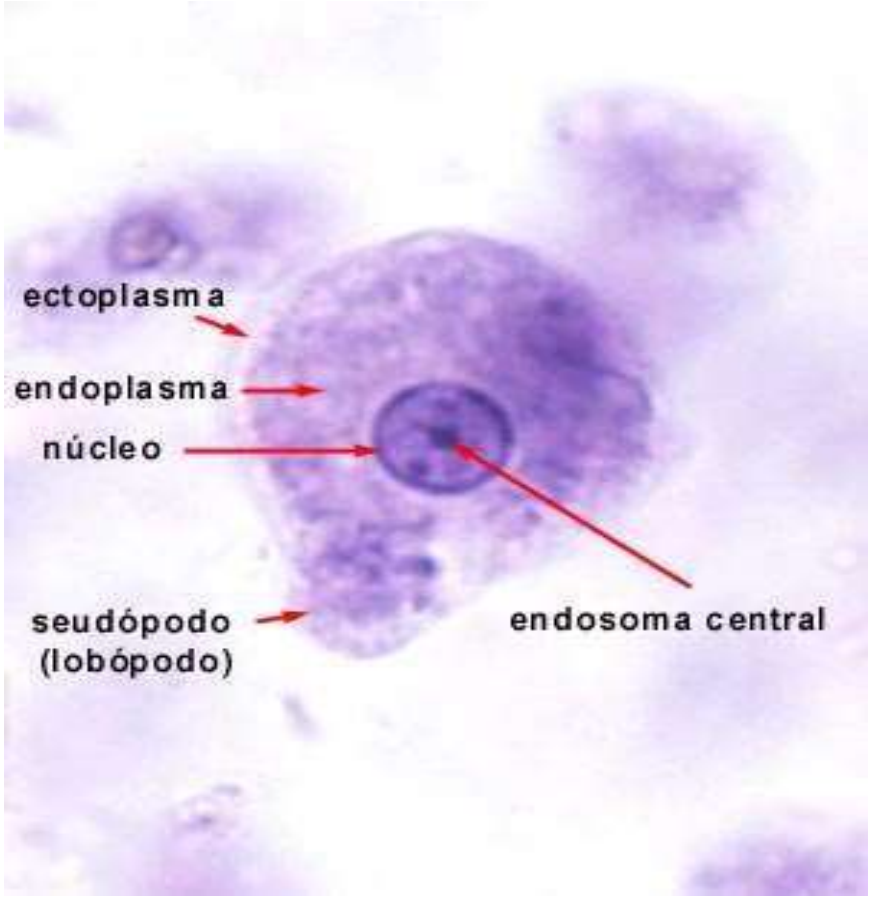
- Up to 60µm in diameter
- Endoplasm granular
- Food vacuoles: RBCs, leucocytes and tissue debris
- Motile
- Blunt single Pseudopodia
- Single Large nucleus
- Only Trophozite present in the tissues

Precyst:-

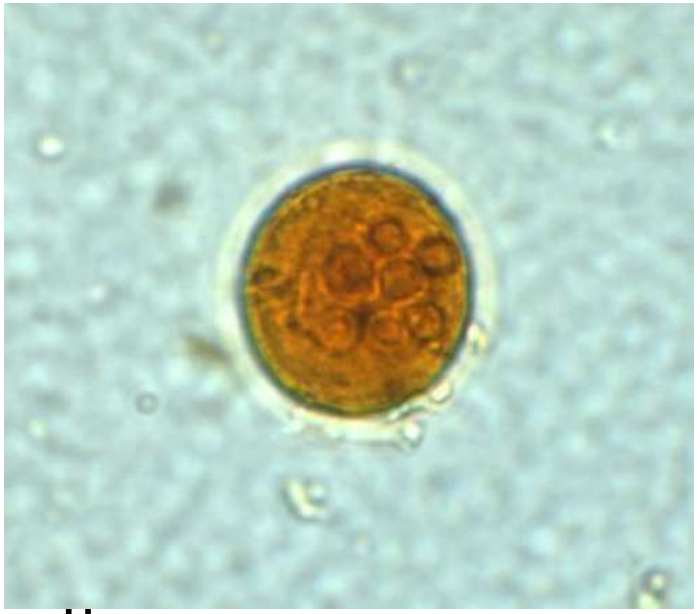


- **S**maller in size
- 10-20µm in diameter
- Oval with a blunt pseudopodium
- Food vacuoles disappear
- Characteristics nucleus

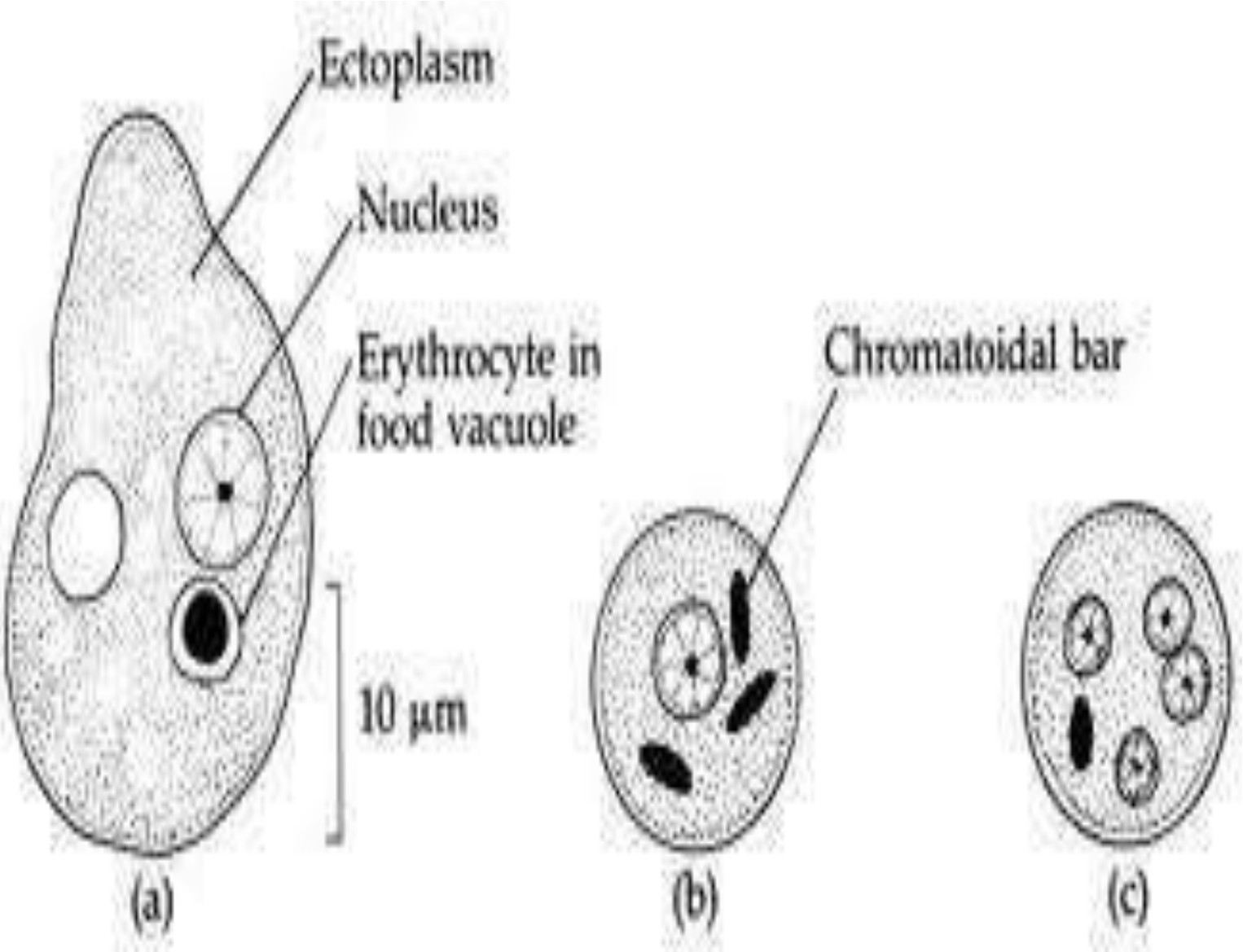
TROPHOZOITE



Cyst

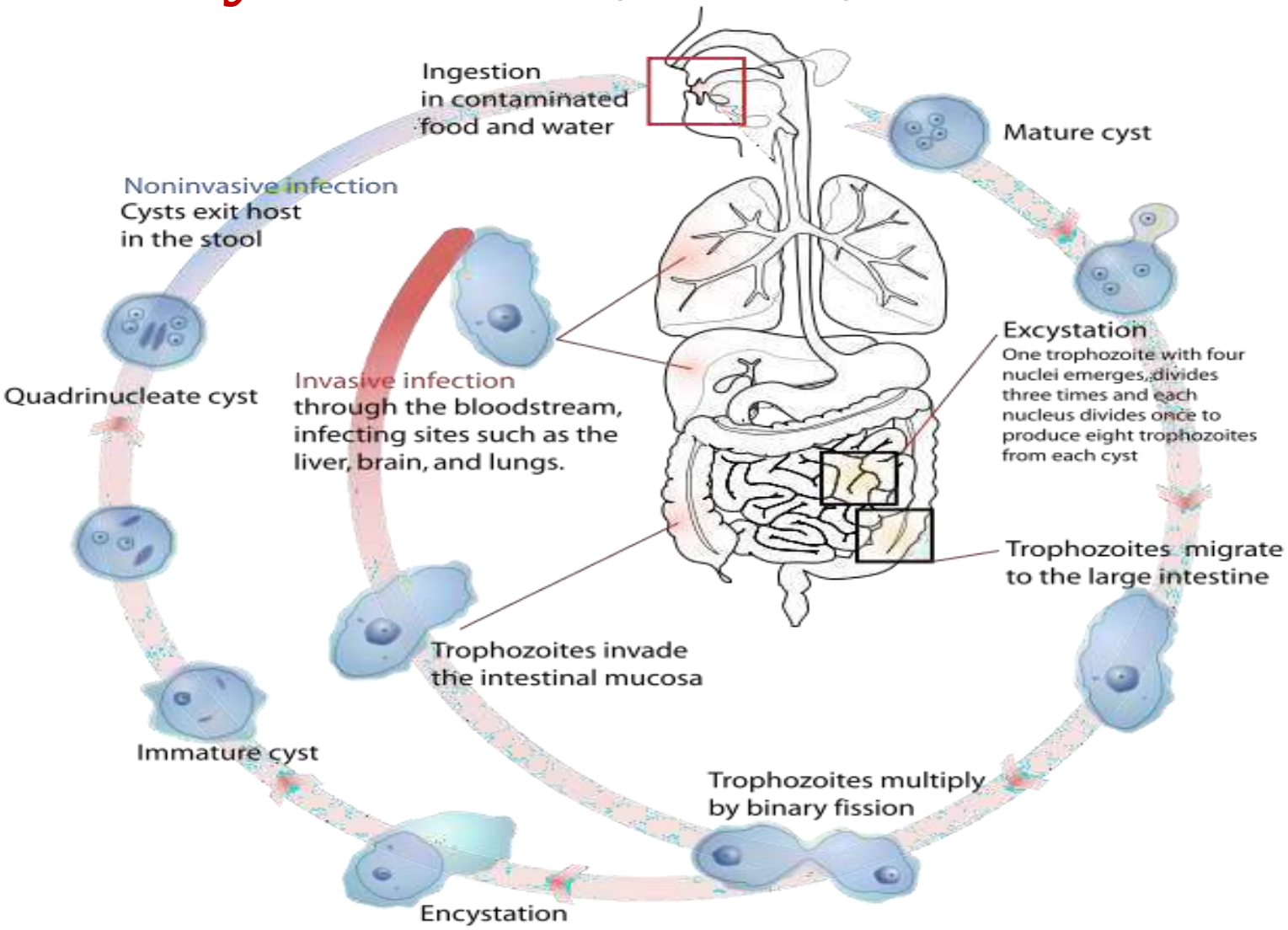


- Spherical, 1-15 µm in diameter
- Surrounded by a thick chitinous wall
- Uni nucleated → Bi nucleated → tetra nucleated
- Cyst are present only in the lumen of the colon and in formed faeces



Tropozoite **Precyst** **Cyst**

Life cycle: In life cycle in only one host: man

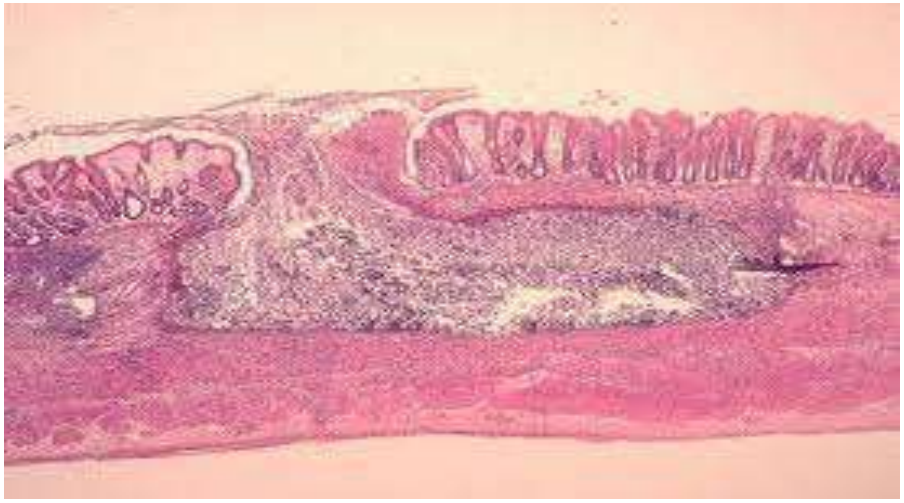


Pathogenicity

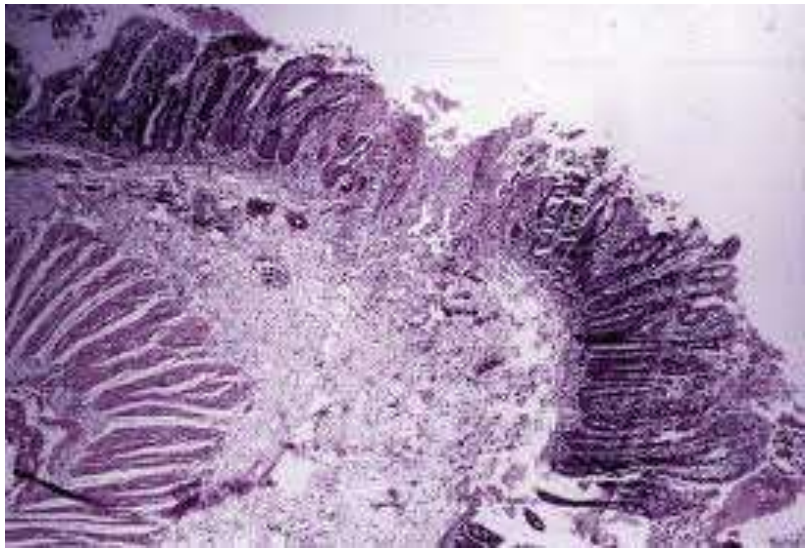
Mode of Transmission: Feco-Oral Route: By Ingestion of contaminated food and Drinking water

Intestinal amoebiasis :

- Intestinal amoebiasis indicate that organism are confined to gastrointestinal tract.
- Incubation period :1-4 weeks
- The amoebae invade the colonic mucosa , producing characteristic ulcerative flask shaped lesions and a profuse bloody diarrhea (amoebic dysentery).



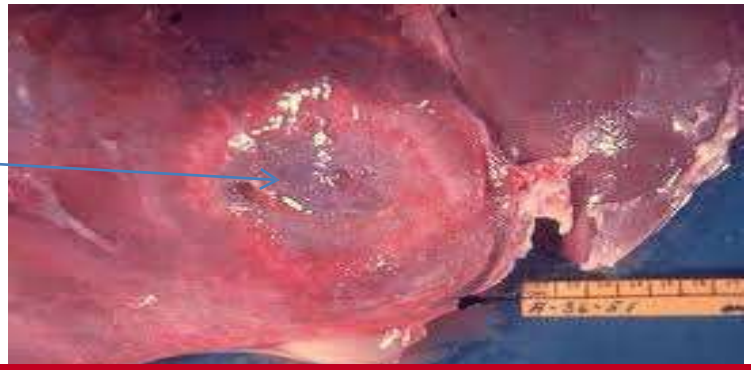
FLASK SAHPED ULCER

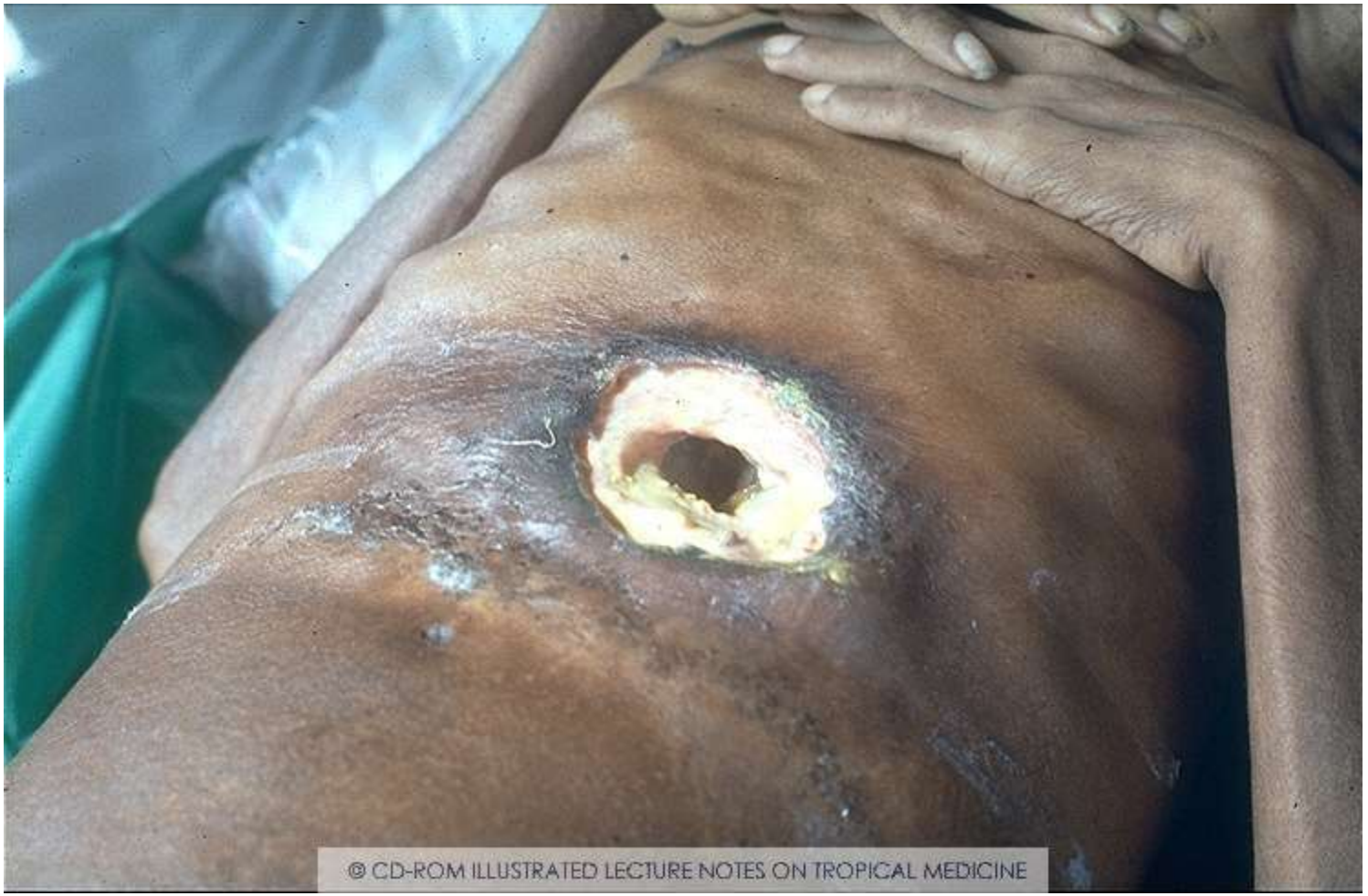


PERFORATED INTESTINE

Extra intestinal amoebiasis:-

- About 5% individuals
- 1. Hepatic amoebasis: Acute Liver Abscess: Develop after 1-3 Months
- Transmit through portal veins from intestine to Liver
- **Pus of liver abscess:** Anchovy Sauce appearance: Contain few Pus cells

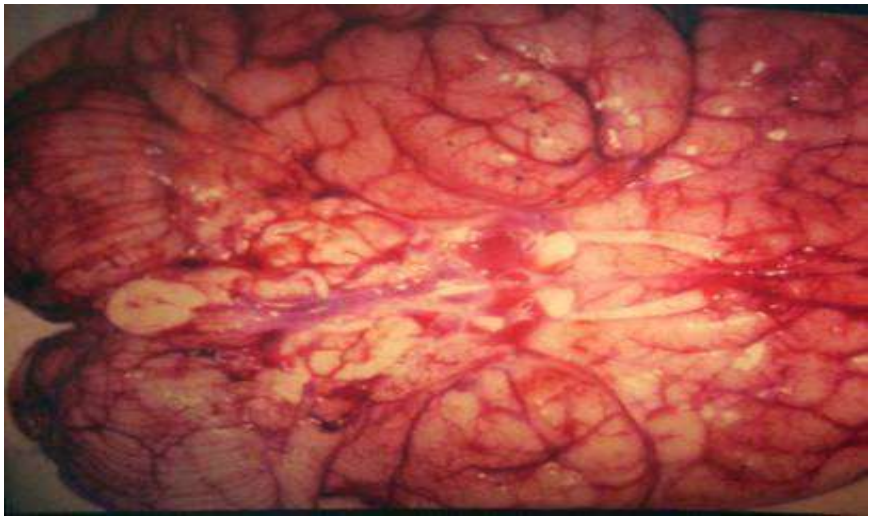




Patient with amoebiasis liver abscess, with perforation of abscess through abdominal skin.

2. Pulmonary Amoebiasis: Transmitted from Liver and develop pulmonary Lesions

3. Cerebral Amoebiasis: Transmitted from Liver to heart then Brain and develop cerebral lesion



Cerebral Amoebiasis

Mild symptoms include:

- Loose stools/diarrhoea, including slimy diarrhoea with pus (which is often foul smelling) and painful passage of stools (tenesmus)
- Stomach pain
- Stomach cramps (colic)
- Nausea

Severe symptoms include:

- Amoebic dysentery (associated with severe abdominal pain, bloody stools, and fever)
- Profuse diarrhoea (patients may pass about 10-12 stools during an acute episode, and still constantly feel an urgency to pass stools)
- Liver abscess
- Severe ulceration
- Severe gastric distention of the bowel
- Peritonitis (inflammation of the intestinal wall and its lining) or colitis (inflammation of the colon, specifically)
- Megacolon (very rare, in 0.5% of the cases)
- Ameboma (which results from formation of annular colonic granulation tissue and may mimic carcinoma of the colon)

Laboratory diagnosis

Intestinal amoebiasis

- **Stool examination :-**In acute amoebiasis, stool or colonic scraping from ulcerated areas are examined by macroscopic and microscopic examination .
- **Blood examination :-** It shown moderate leucocytosis.

Serological tests:-

- These are negatives in early cases however, in later stages of invasive intestinal amoebasis antibodies appear and serological testes become positive
- These test inculde indirect haemagglutination(IHA), indirect fluorescent antibody (IFA) test and enzymes –linked immunosorbent assay (ELISA)

Hepatic amoebiasis

Diagnostic aspiration :- Trophozoites of *E. histolytica* may be demonstrated by microscopy of the pus aspirated by puncture of amoebic liver abscess in less than 15% cases

Liver biopsy :-Trophozite of *E.histolytica* can be demonstrated in the specimens of liver biopsy from the cases of amoebic hepatitis or the wall of the liver abscess

Blood examination:-

It shows leucocytosis with total leukocyte count of 15,000- 30,000µl of which 70-75% are polymorphonuclear leucocytes.

Stool examination:-

In less than 15% cases of amoebic hepatitis , cysts of E.histolytica can be demonstrated in the stool . This indicates persistence of intestinal infection.

Serological tests :-

- IHA,
- IFA,
- ELISA,
- Slide agglutination test,
- Co agglutination test.

Molecular methods :-

- DNA probes
- PCR

TREATMENT

Treatment of amoebiasis is based on the use of amoebicides drugs

Amoebicides with luminal action

- Di-iodohydroxyquin
- Diloxanide furoate
- Paromomycin

Amoebicides effective in the liver, intestinal wall and other tissues

- Emetine
- Dehydroemetine

Amoebicides effective only in the liver

- chloroquine

Amoebicides effective in both tissues and the intestinal lumen

- Metronidazole
- Nitroimidazole

Prevention

- The amoebic infection can be prevented by avoiding faecal contamination of food and water
- There should be proper disposal of human faces through proper drainage system
- Contamination may result from discharge of sewage into rivers. Purified water should be distributed through pipelines to avoid contamination . Boiled water is safe.

- The amount of chlorine normally used to purify water is insufficient to kill cysts , higher levels of chlorine are effective but the water thus treated must be dechlorinated before use.
- Vegetables that are usually eaten raw should be cleaned with uncontaminated runing water and treated with 5% acetic acid before consuming

DARK FIELD MICROSCOPE

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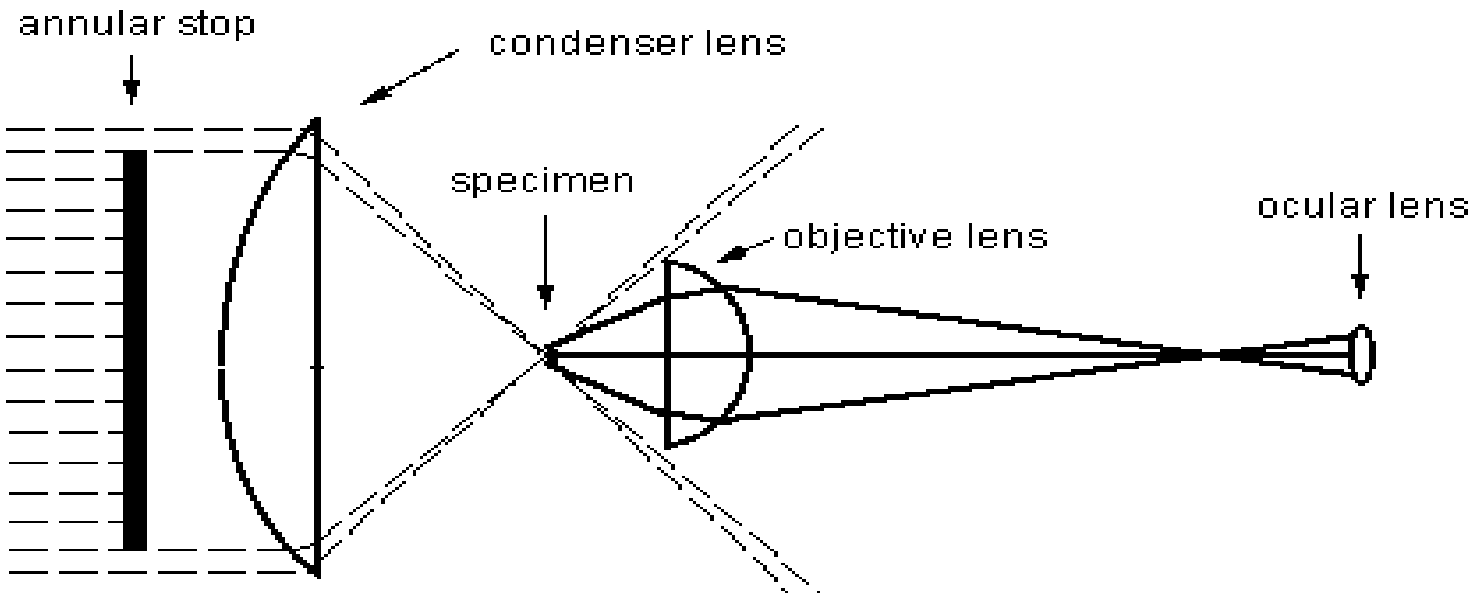
G.B. NAGAR, KALAVAI.

Dark-field microscopy produces an image with a dark background

How it works


annular stop
condenser lens
specimen
objective lens
ocular lens

DEFINATION

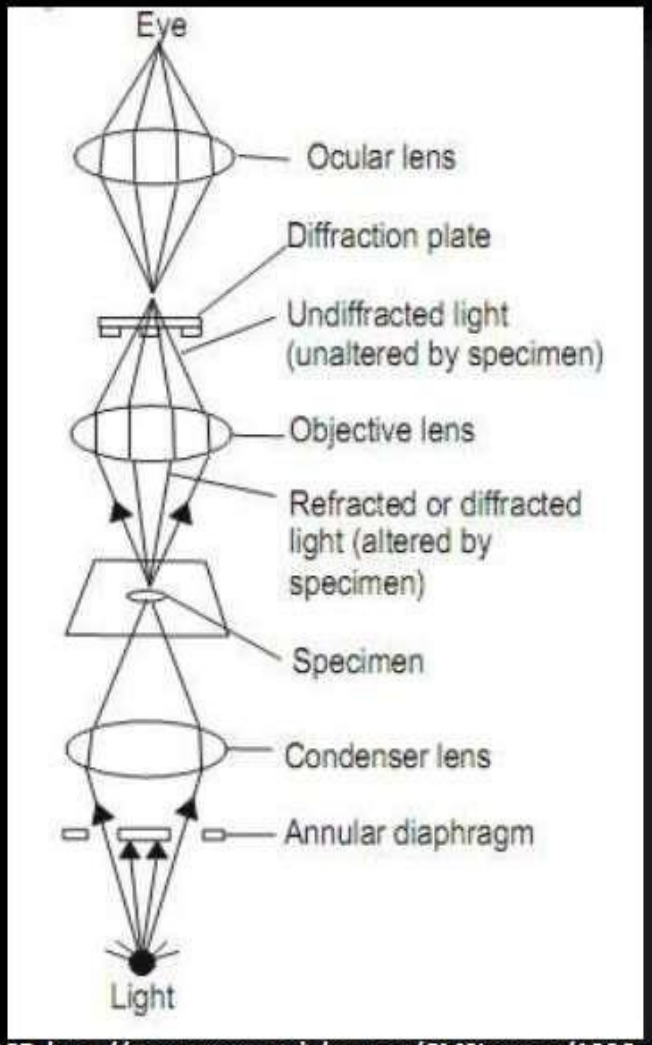
Dark Field Microscopy is a technique used to observe unstained samples causing them to appear brightly lit against a dark, almost purely black, background.

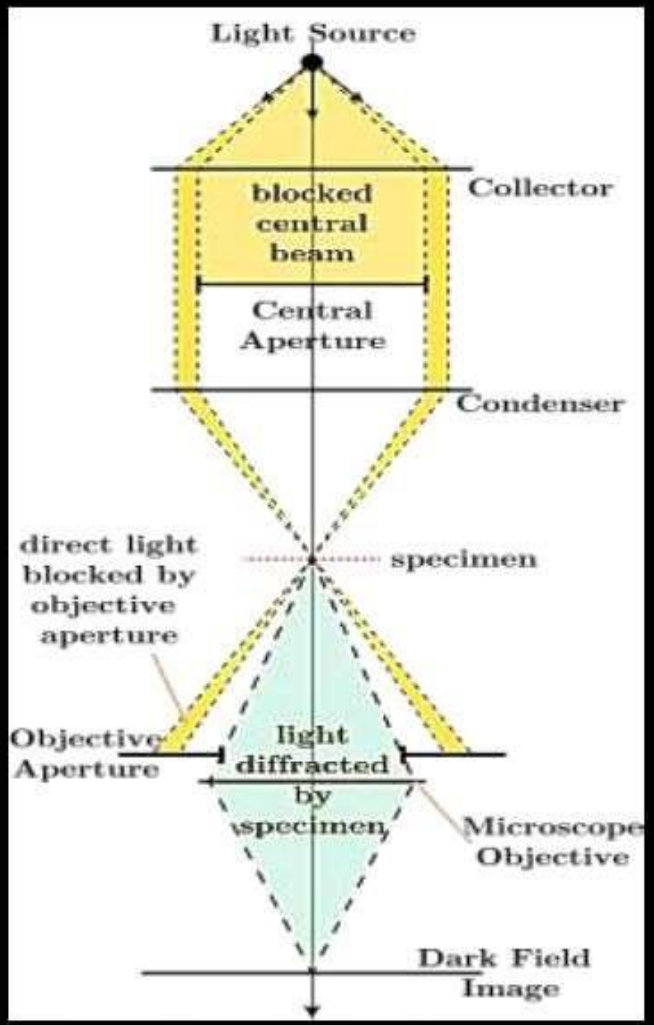
- A dark field microscopy is used to examine live microorganisms that either invisible in the ordinary light microscope, cannot be stained by standard methods, or are so distorted by staining that their characteristics then cannot be identified.

Contd…

- Instead of normal condenser, dark field microscope uses dark field condenser that contain a opaque disc. The disc blocks light that would enter the lens directly, only the light is reflected off the specimen enters the objective lens. Because there is no background light, the specimen appears light against black background- the dark field.

LIGHT DIAGRAMS.





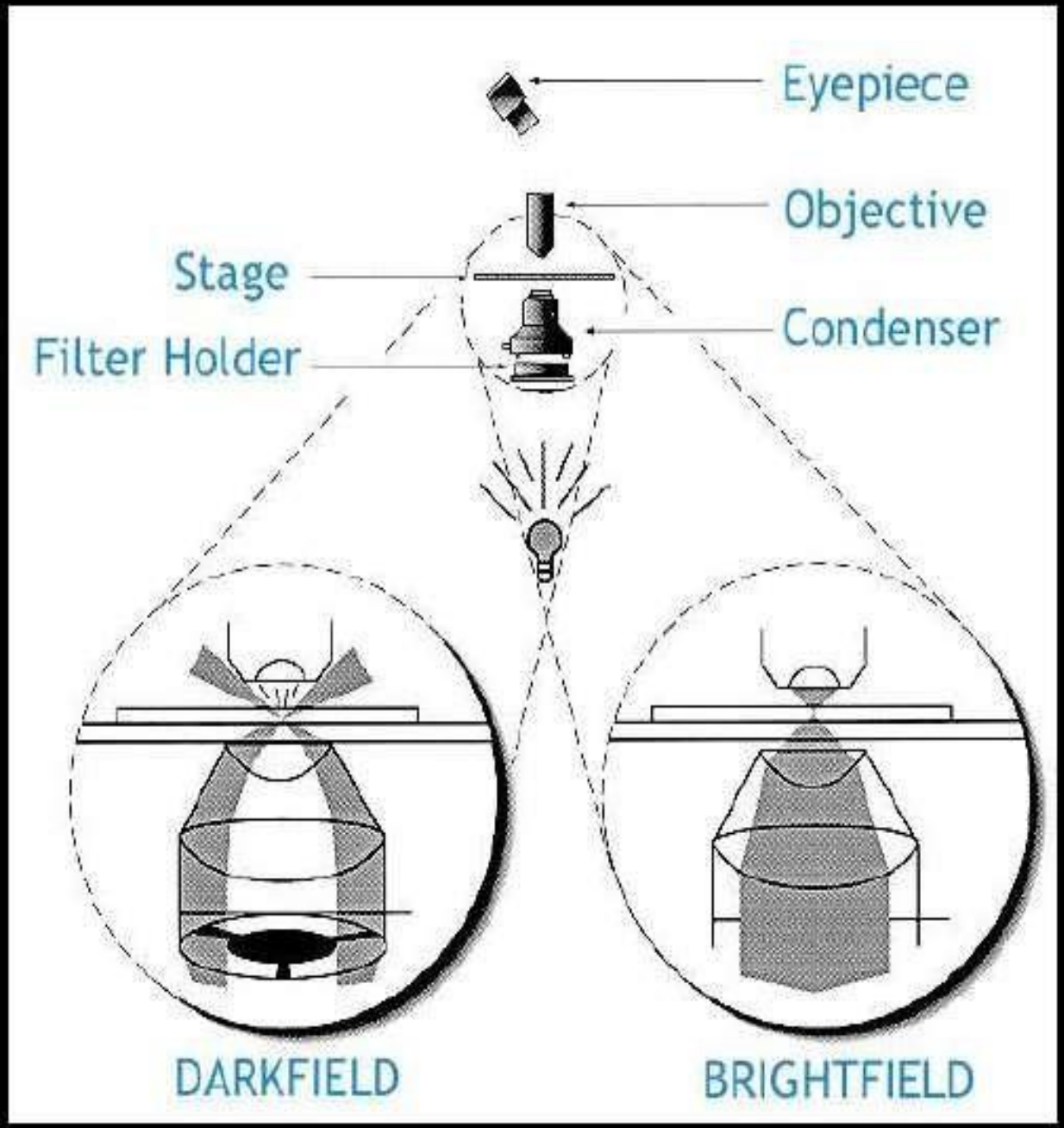
SOURCE: http://secure.tutorsglobe.com/CMSImages/1006_phase%20contrast%20microscope.jpg

PRINCIPLE

- The dark ground microscope creates a contrast between object and surrounding field, such that, the background is dark and the object is bright
- The objective and the ocular lenses used in the dark ground microscope are same as that of ordinary light microscope.

Contd..

- Special condenser is used, which prevents the transmitted light from directly illuminating the specimen
- Only oblique scattered light reaches the specimen and passes onto the lens system causing the object to appear bright against a dark background.


Eyepiece
Objective
Stage
Condenser
Filter Holder
DARKFIELD
BRIGHTFIELD

Uses of Dark field microscopy

- Diagnosis of Syphilis
- (Treponema pallidum).
- Viewing blood cells.
- Viewing bacteria.
- Viewing different types of algae.
- Viewing hairline metal fracture.
- Viewing diamonds and other precious stones.
- Viewing shrimp or other invertebrates

Advantages

- A dark field microscope is ideal for viewing unstained object, 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.

Contd..

- Dark field m/s used in research of live bacterium, as well as mounted cells and tissues.
- It is useful in examining external details, such as outlines, edges and surface defects than internal structures.
- Dark field is used study marine organisms such as algae ,plankton, diatoms, insects, as well as some minerals and crystals, thin polymers and some ceramics.

Contd..

- Dark field has regained its popularity when combined with other illumination techniques, such as fluorescence, which widens its possible employment in certain fields.
- It is useful in examining external details, such as outlines, edges and surface defects than internal structure. .

Disadvantages,

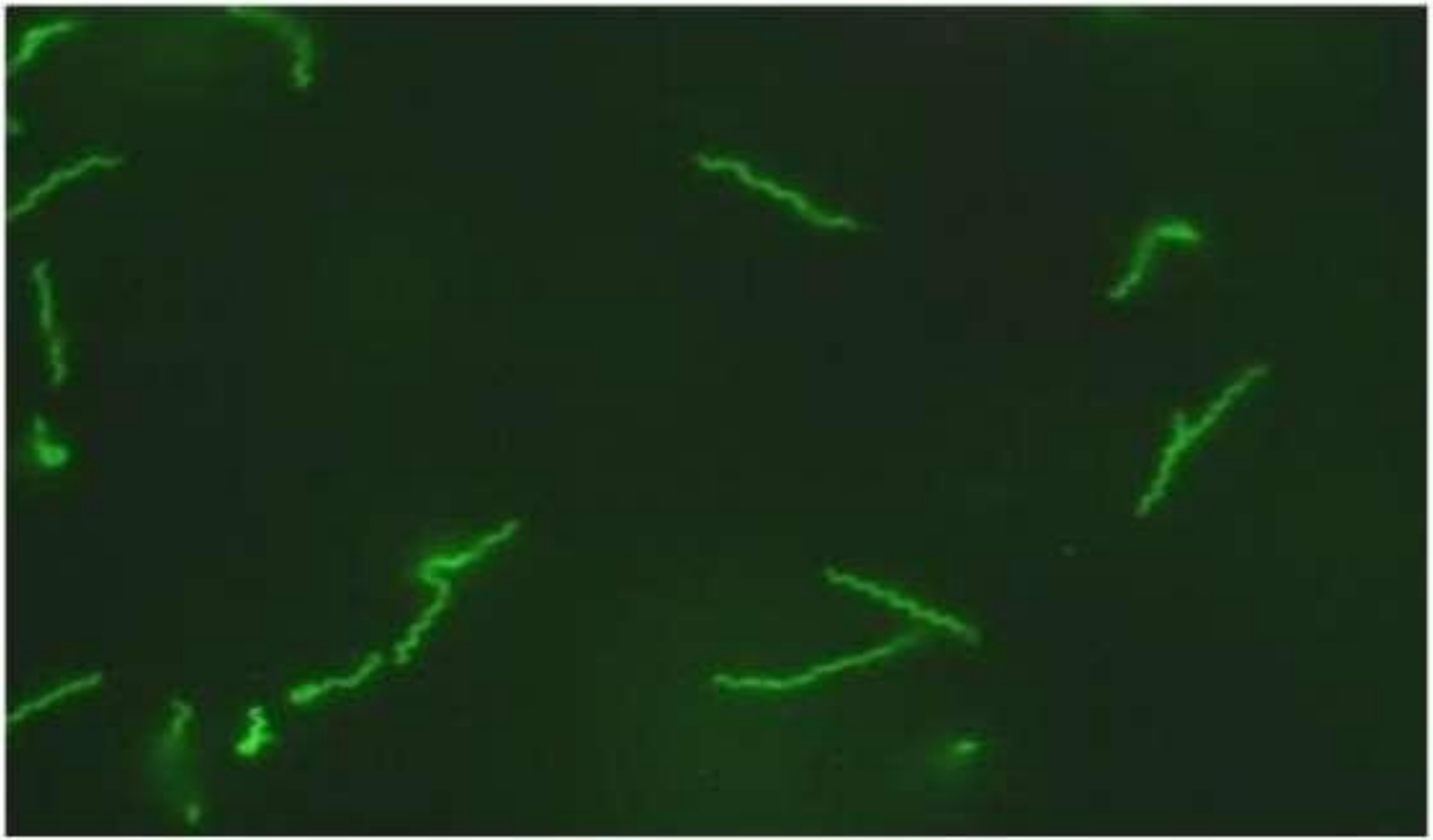
- A specimen that is not thin enough or its density differs across the slide, may appear to have artefacts throughout the image.
- The preparation and quality of the slides can grossly affect the contrast and accuracy of a dark field image.
- You need to take special care that the slide, stage, nose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

Contd…

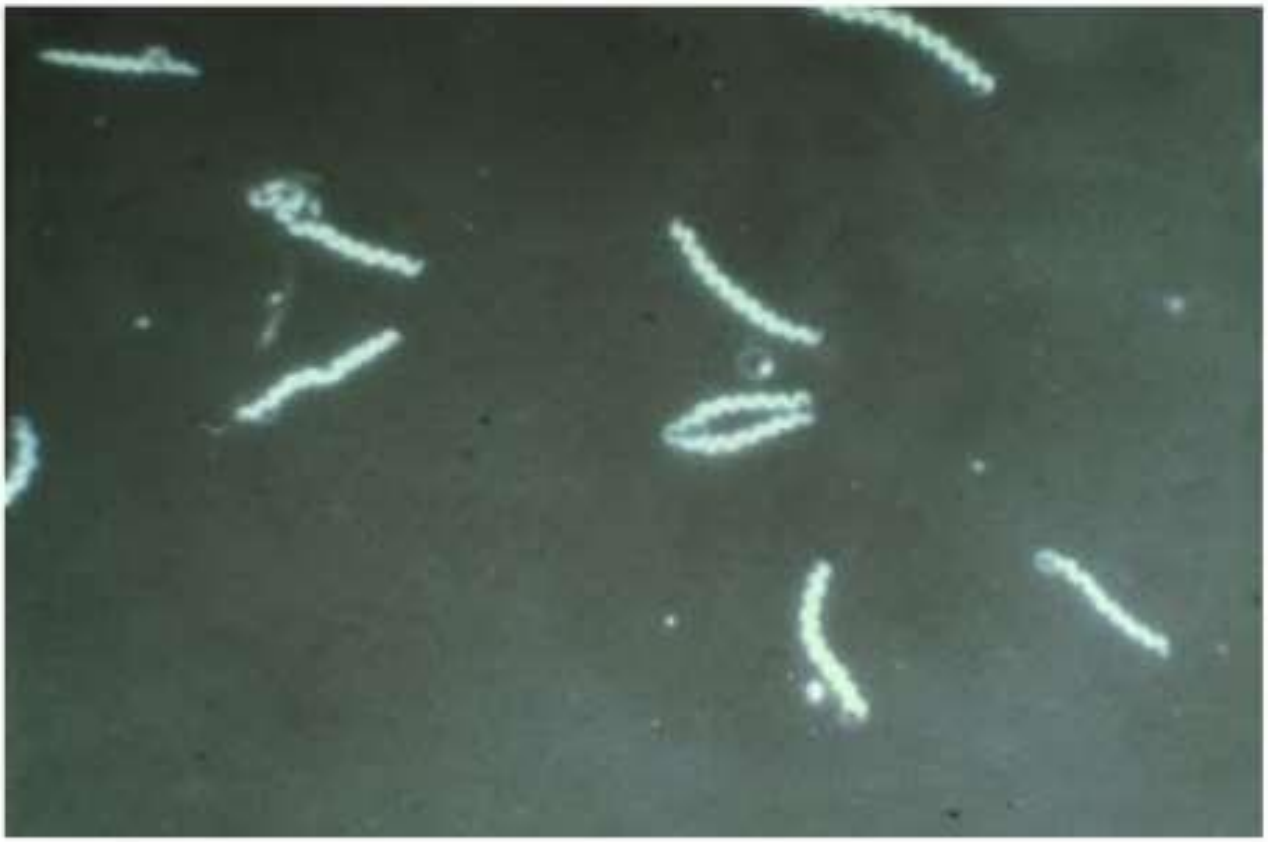
- You need to take special care that the slide, stage, nose and light source are free from dust, as these will appear as part of the image.
- Similarly, if you need to use oil or water on the condenser or slide, it is impossible to avoid air bubbles.
- These liquid bubbles will cause image degradation, and distortion .

Image of sugar crystals using Dark field microscope

Treponema denticola – wet mount, dark field microscopy + fluorescent dye staining



Spirochetes – wet mount by dark field microscopy



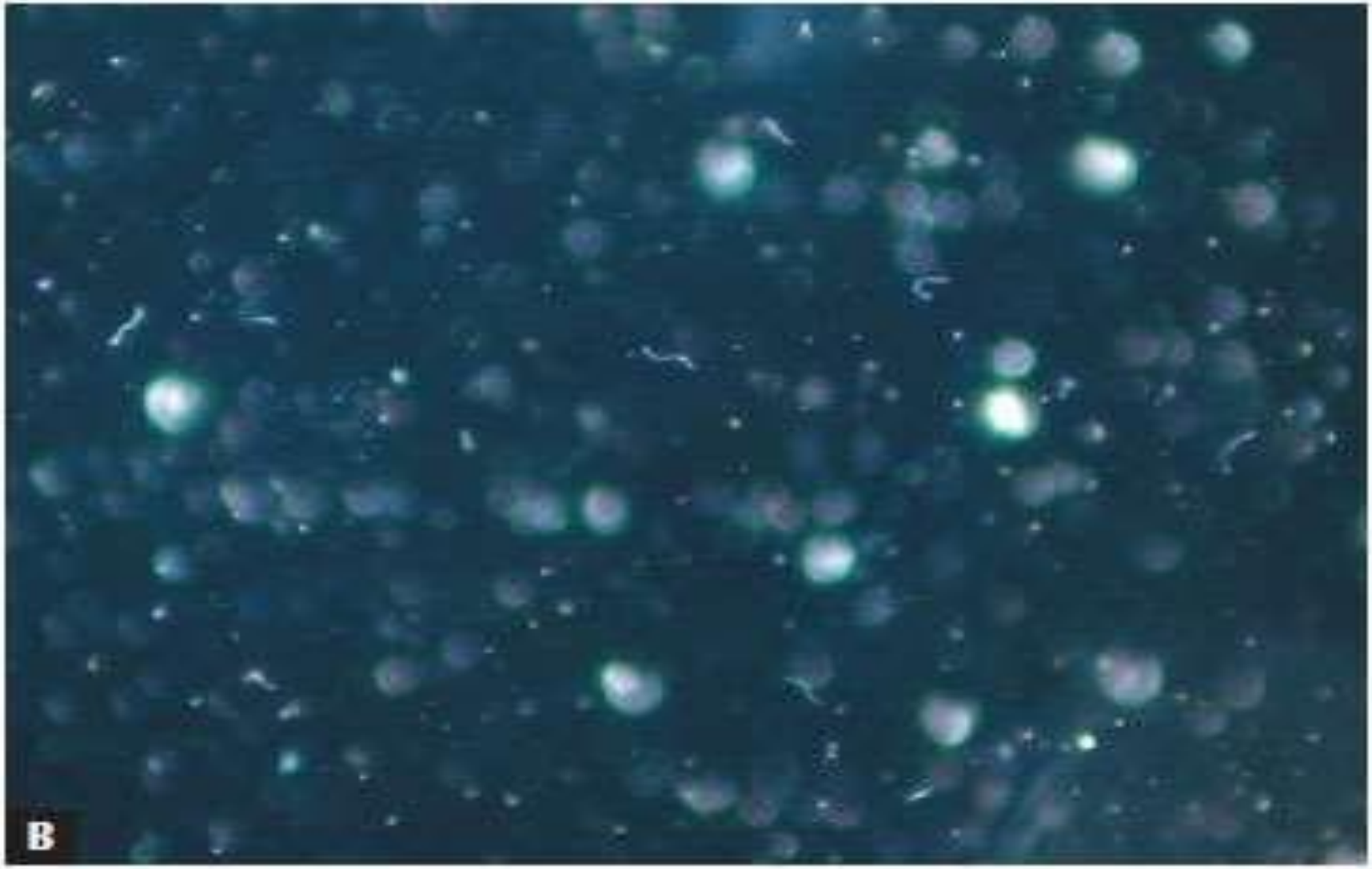
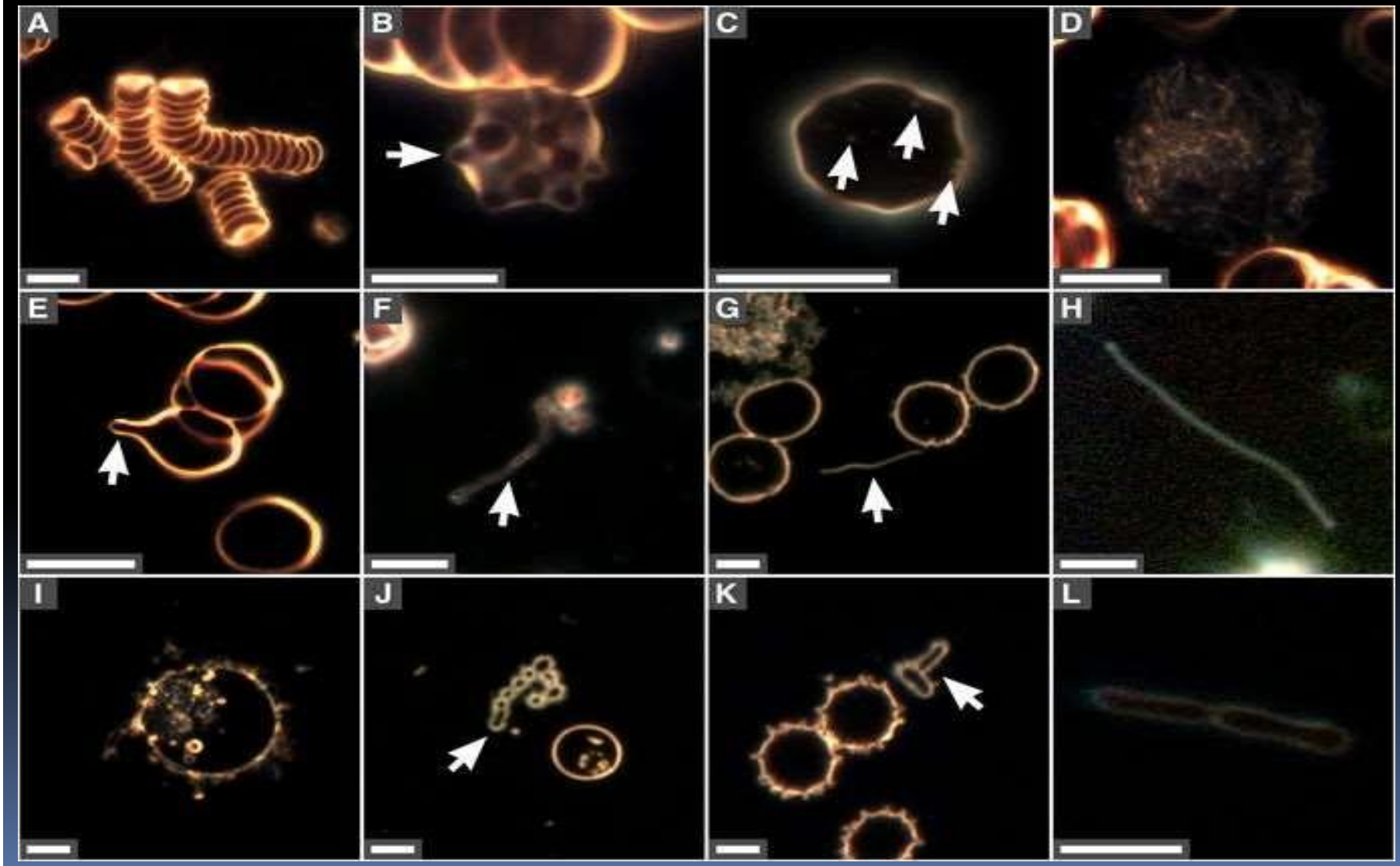


Figure 2. A) Dark-field microscopy showing spirochetal structures on the peripheral blood of a Vivarium employee; 1,000x. B) Blood culture of a patient with BYS in SP4 medium, which is adequate for the growth of Spiroplasmas, showing the growth of spirochetal structures; 400x.

Bacteria like structure in human blood



Thank you

Electron Microscope

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Electron microscope

- An **electron microscope** is a microscope that uses a beam of **accelerated electrons** as a source of illumination.
- The wavelength of an electron can be up to **100,000** times shorter than that of visible light photons.
- Electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects.

- It offers unique possibilities to gain insight into

1. Structure
2. Topology
3. Morphology
4. Composition of materials.

Advantages Of Electron Microscopy

- To study objects of >0.2 micrometer.
- For analysis of subcellular structure.
- For study of intracellular pathogens & viruses.
- For cell metabolism
- For study of minute structure in nature.

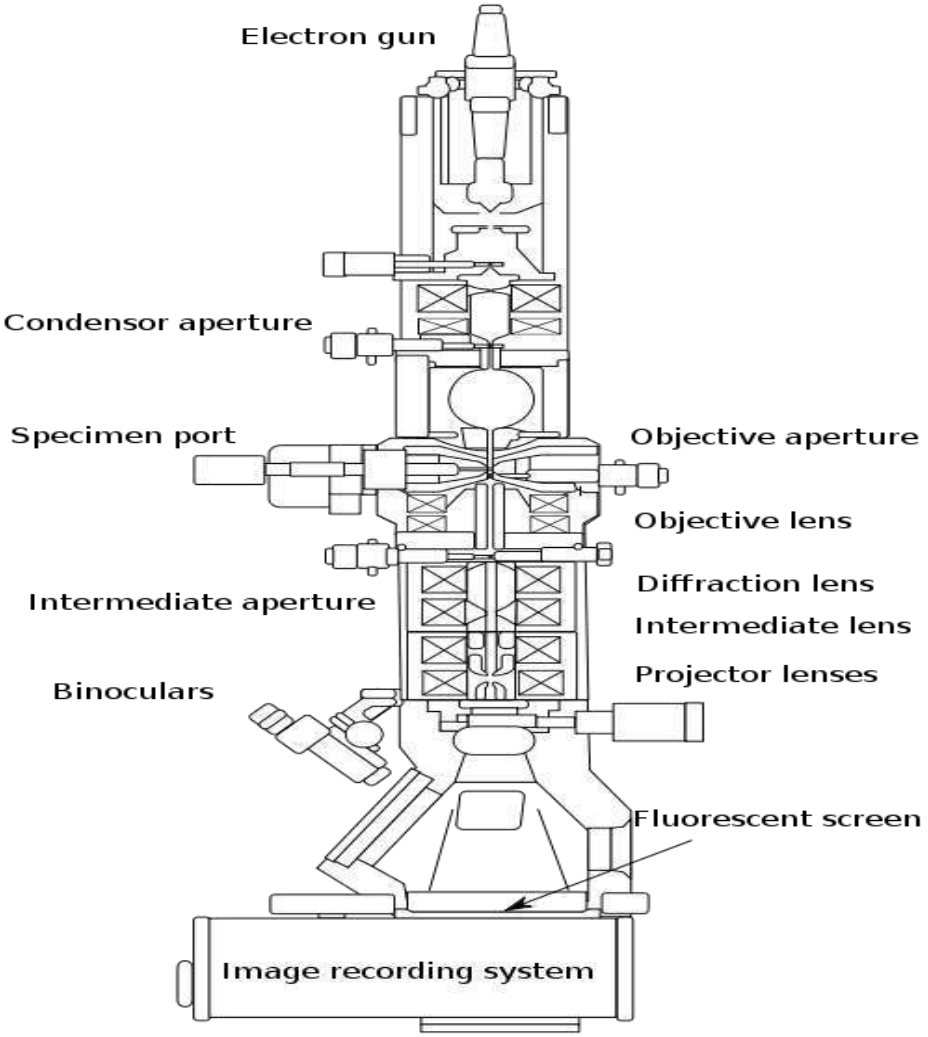
Types of Electron Microscope:

- There are basic 4 types of Electron Microscope:

1. Analytical Electron Microscopy (AEM)
2. Scanning Transmission Electron Microscope (STEM)
3. Scanning Electron Microscope (SEM)
4. Transmission Electron Microscope (TEM)

Transmission Electron microscope

- Is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through it.
- A transmission electron microscope can achieve better than 50 pm resolution and magnifications of up to about 10,000,000x


Electron gun
Condensor aperture
Specimen port
Objective aperture
Objective lens
Diffraction lens
Intermediate aperture
Intermediate lens
Projector lenses
Binoculars
Fluorescent screen
Image recording system

Types of TEM:

- Bright Field (BF) – BFTEM
- Dark field (DF) – DFTEM
- High Resolution TEM – HRTEM
- Energy Filtered TEM – EFTEM
- Electron Diffraction – ED

Techniques Of TEM:

- There are 4 techniques of TEM:
  - Negative staining
  - Shadow casting
  - Freeze fracture replication
  - Freeze etching.

Applications Of TEM

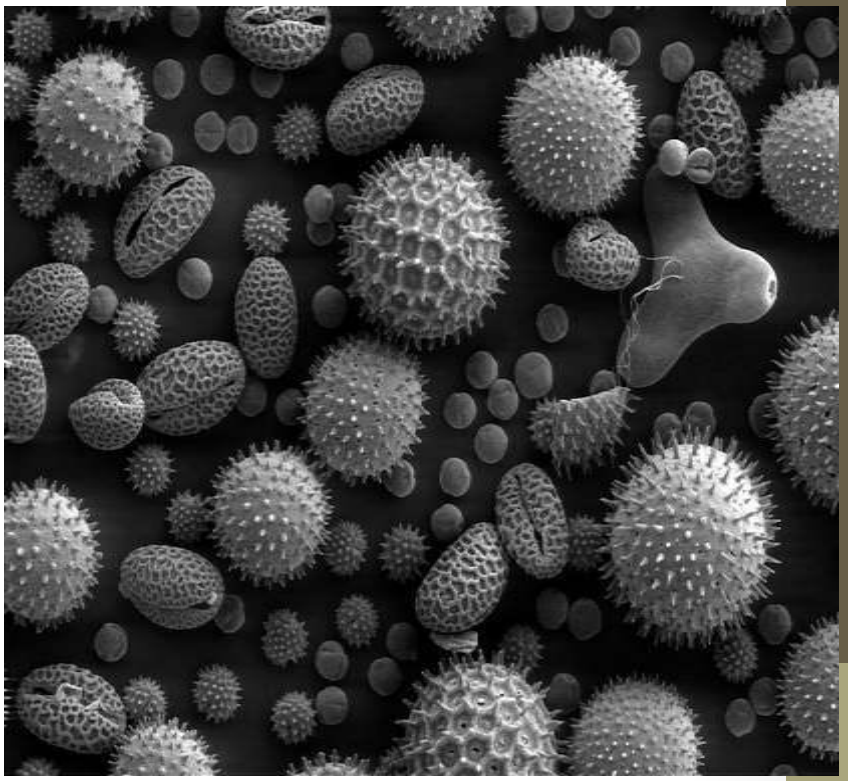
- It can be used for the study of:
  - To examine a small column of atoms.
  - Cancer research
  - Virology
  - Pollution Nanotechnology
  - Semiconductor research
  - Chemical Identity
  - Crystal orientation
  - Electronic structure

Limitations of TEM:

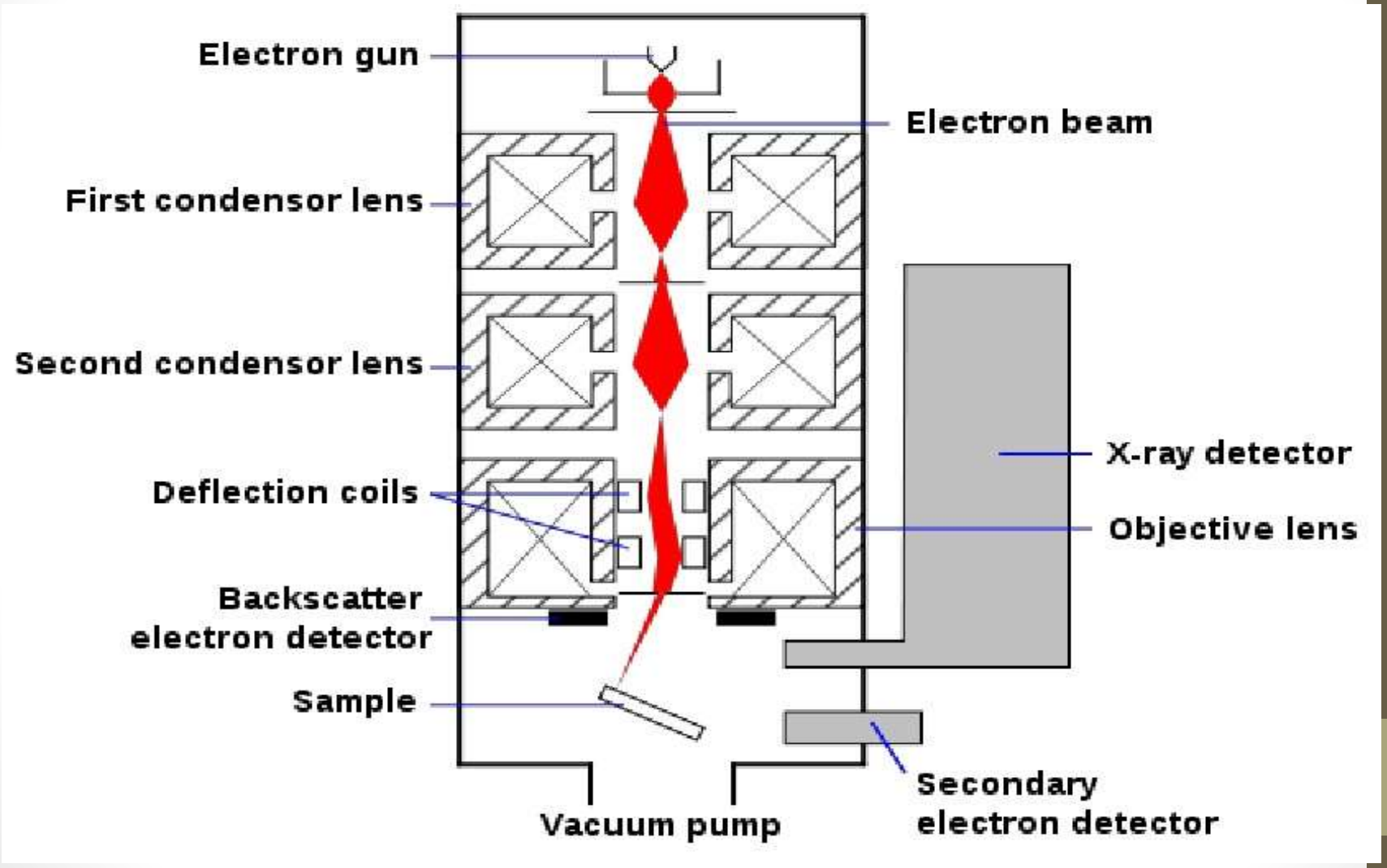
- It is difficult to produce thin sample.
- Relatively time consuming process with a low throughput of samples.
- The structure of the sample may be change during the preparation process.
- Small field of view may not give conclusion result of the whole sample.

Scanning Electron Microscope (SEM)

- A **scanning electron microscope** (**SEM**) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons.
- The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition.



- Types of signals produce by a SEM include:
  - Secondary electrons
  - Back scattered electrons (BSE)
  - X-rays
  - Light rays
- A standard SEM uses secondary electrons & back scattered electrons.


Electron gun
Electron beam
First condensor lens
Second condensor lens
X-ray detector
Deflection coils
Objective lens
Backscatter
electron detector
Sample
Secondary
electron detector
Vacuum pump

Advantages of SEM:

- Better resolution.
- Work with low voltages.
- High brightness source.
- Fast imaging.
- High count rates in X-ray analysis.
- Easy to operate.

Applications of SEM:

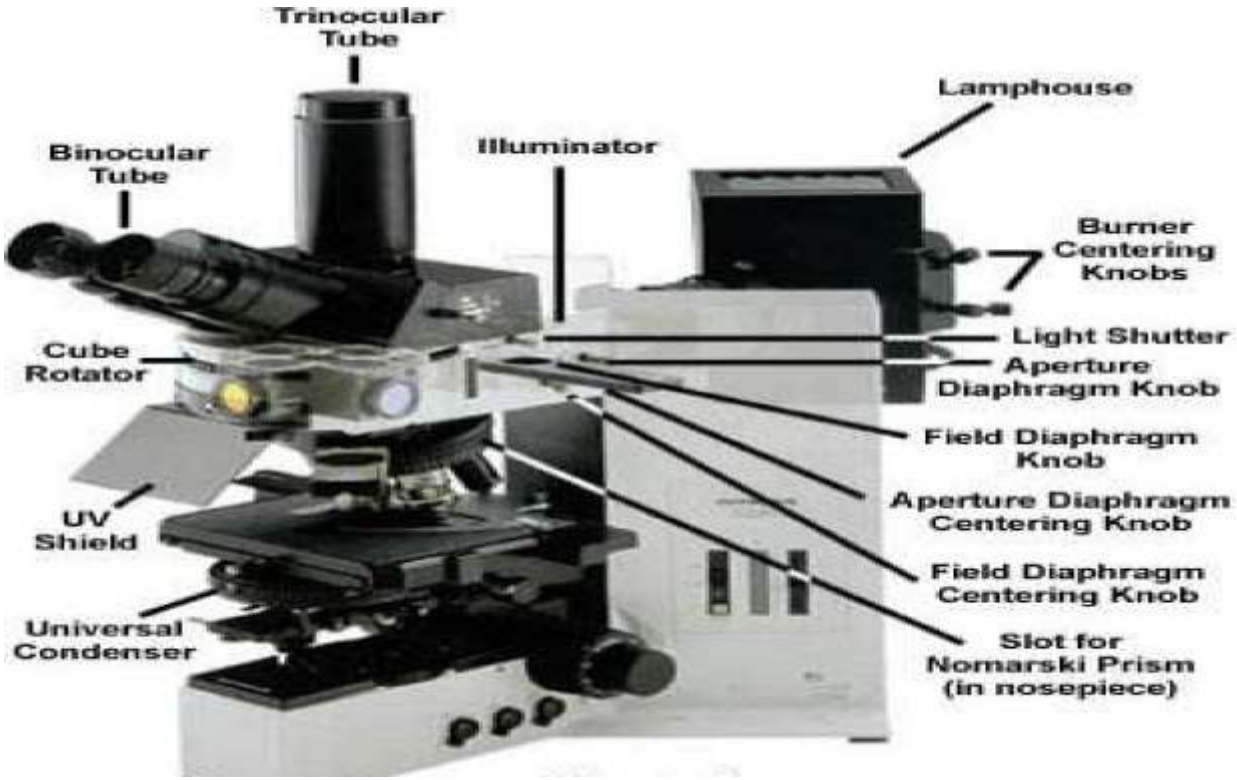
- Use in ultra high vacuum, air, water & various liquid environment.
- Use for the live specimen examination.
- Use for the visualization of intra cellular changes.

Limitation of SEM:

- It cannot detect very light element (H, He & Li).
- It cannot detect elements with atomic numbers less than 11.

THANK YOU

Fluorescence Microscopy



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What is Fluorescence Microscopy..?

- A **fluorescence microscope** is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances.

- **Fluorescence-**

  Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation.

- **Phosphorescence**

  Phosphorescence is a specific type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs.

Discovery

- British scientist **Sir George G. Stokes first described fluorescence in 1852.**
- He observed that the mineral fluorspar emitted red light when it was illuminated by ultraviolet excitation.
- Stokes noted that fluorescence emission always occurred at a longer wavelength than of the excitation light.
- **This shift towards longer wavelength is known as Stokes Shift.**

- The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope, or
- A more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image.

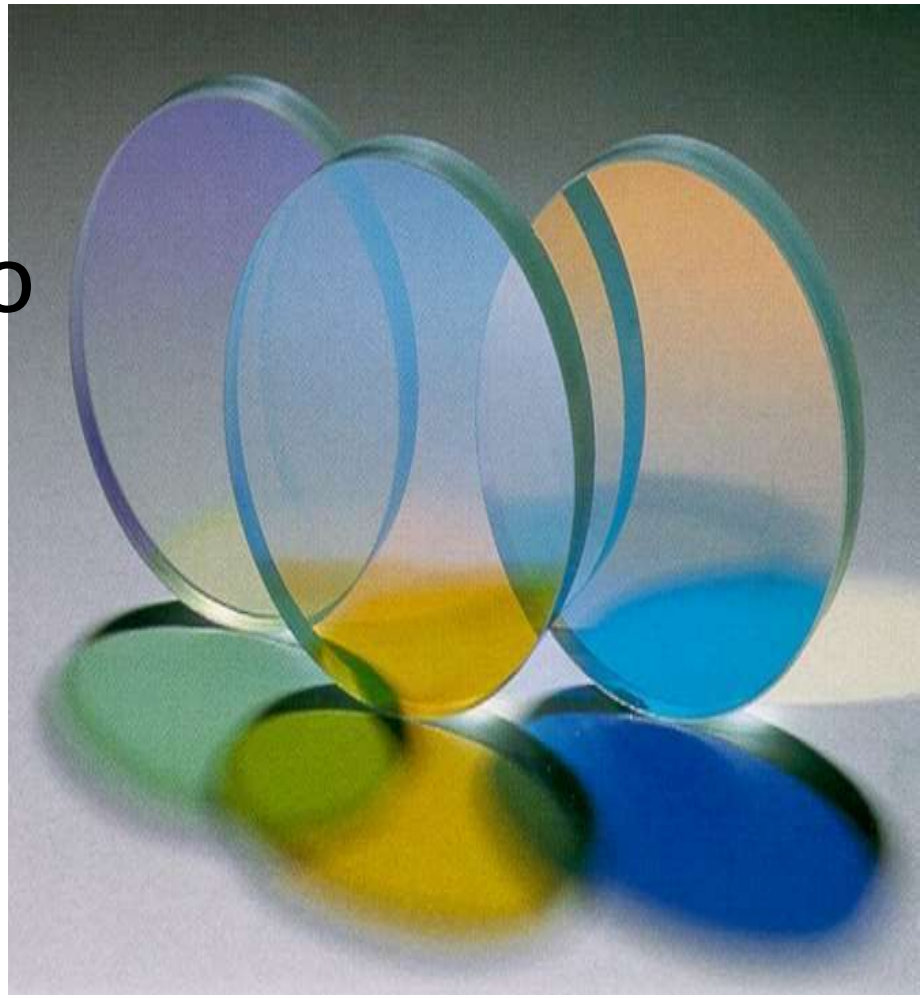
- **On 8 October 2014,** the **Nobel Prize in Chemistry** was awarded to **Eric Betzig, William Moerner** and **Stefan Hell** for **"the development of super-resolved fluorescence microscopy,"** which brings **"optical microscopy into the nanodimension".**

Principle

- The specimen is illuminated with light of a specific wavelength.
- Which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a Principle different color than the absorbed light).
- The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter.

Dichroic filter

- A **dichroic filter** or **thin-film filter**, is a very accurate color filter used to selectively pass light of a small range of colors while reflecting other colors.



Fluorophore

- A **fluorophore** is a fluorescent chemical compound that can re-emit light upon light excitation. Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds.

- Typical components of a fluorescence microscope are a light source, (xenon arc lamp or mercury-vapor lamp or high-power LEDs and lasers), the excitation filter, the dichroic mirror and the emission filter.

- Most fluorescence microscopes in use are epifluorescence microscopes, where excitation of the fluorophore and detection of the fluorescence are done through the same light path (i.e. through the objective).
- These microscopes are widely used in biology and are the basis for more advanced microscope designs.

Epifluorescence microscopy

- The majority of fluorescence microscopes, especially those used in the life sciences, are of the epifluorescence design shown in the diagram.
- Light of the excitation wavelength is focused on the specimen through the objective lens.
- The fluorescence emitted by the specimen is focused to the detector by the objective.
- Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light.

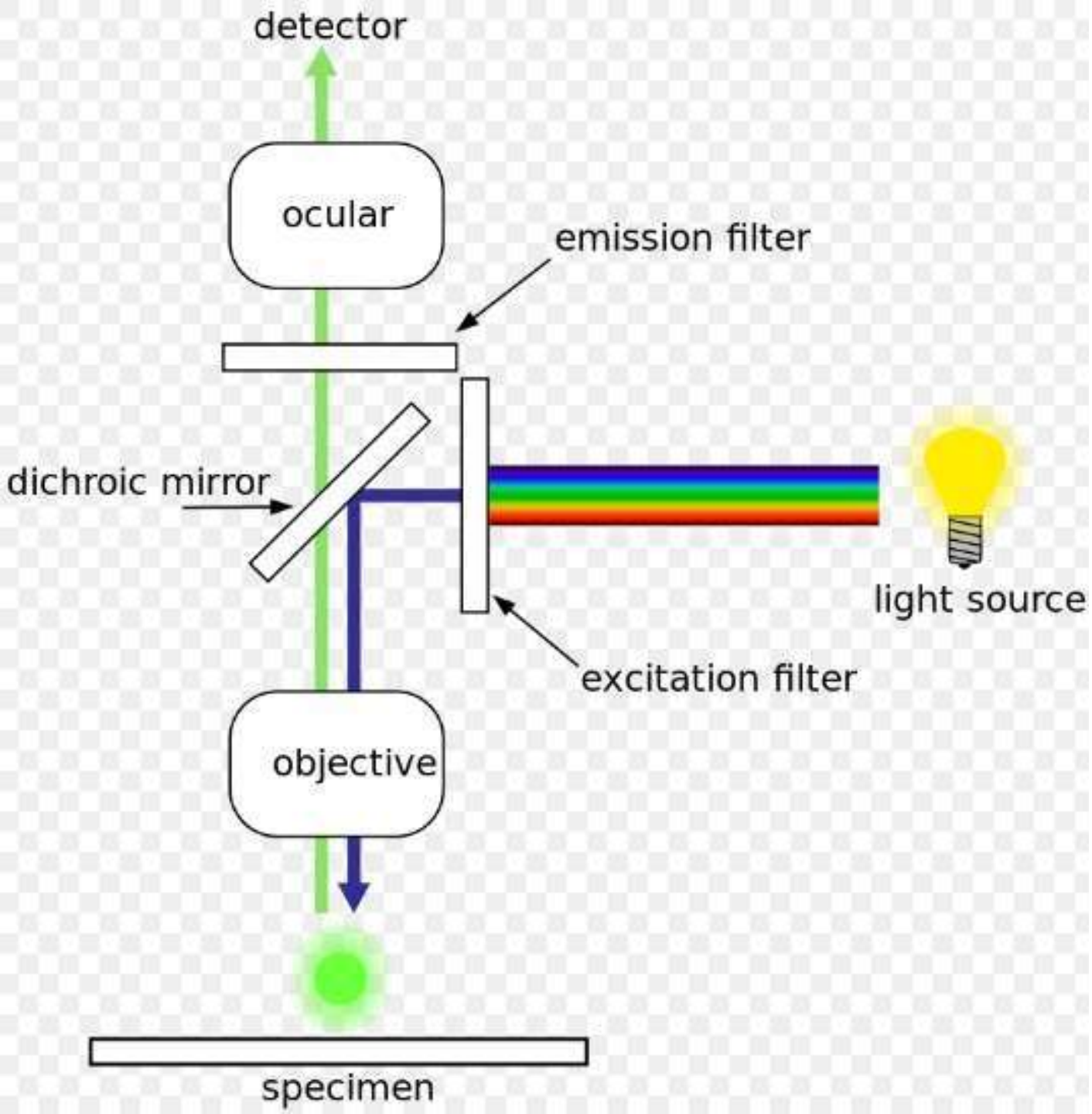

detector
ocular
emission filter
dichroic mirror
light source
excitation filter
objective
specimen

Figure 1 - Epi-Fluorescence Microscope

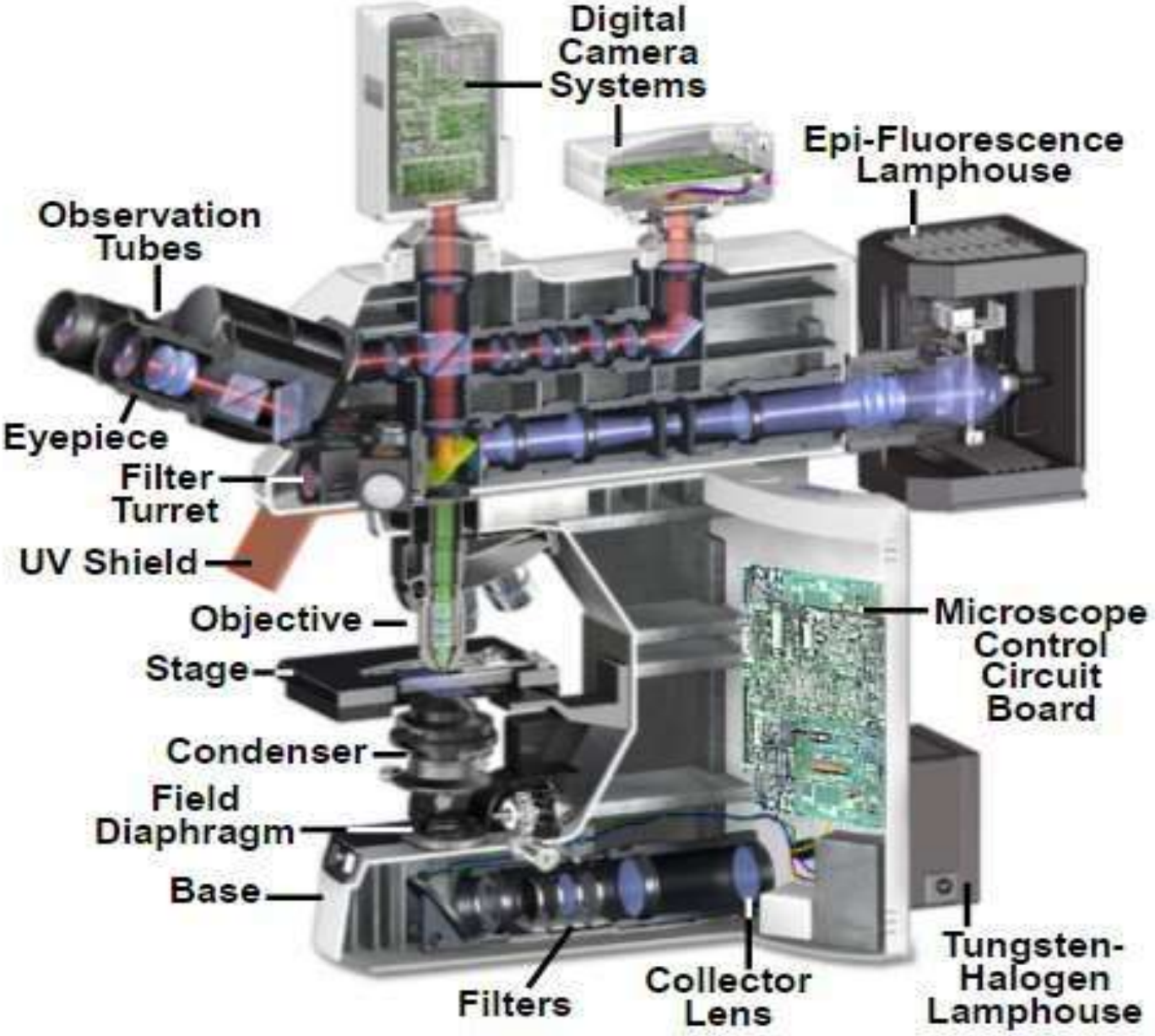
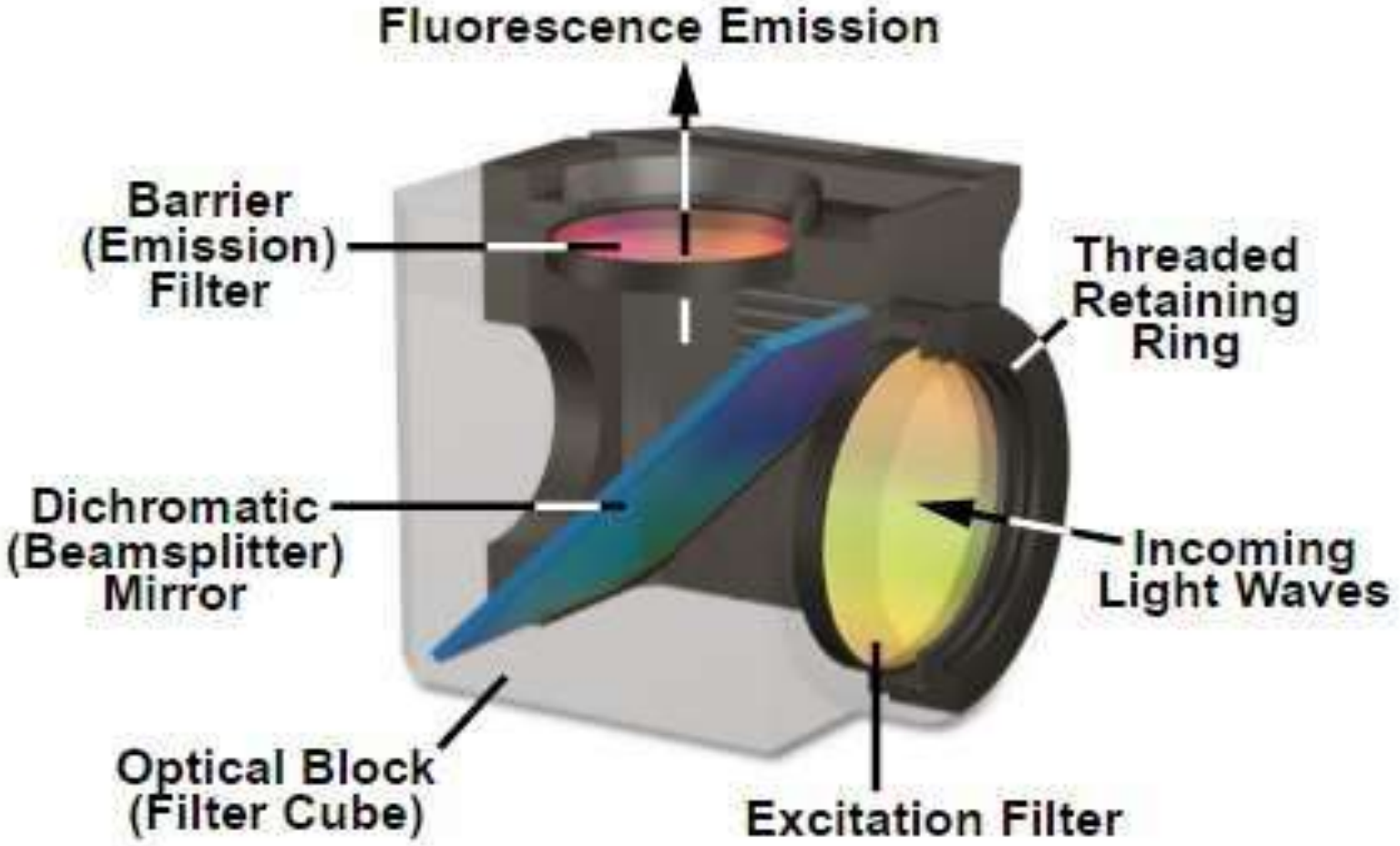


Figure 2 - Fluorescence Filters



Light Source

- Four main types of light source are used, including xenon arc lamps or mercury-vapor lamps with an excitation filter, lasers, and high-power LEDs.
- Lasers are mostly used for complex fluorescence microscopy techniques, while xenon lamps, and mercury lamps, and LEDs with a dichroic excitation filter are commonly used for wide field epifluorescence microscopes.

Xenon arc Lamp

- A **xenon arc lamp** is a specialized type of gas discharge lamp, an electric light that produces light by passing electricity through ionized xenon gas at high pressure.
- It produces a bright white light that closely mimics natural sunlight.

Biological fluorescent stains

- Many fluorescent stains have been designed for a range of biological molecules.
- Some of these are small molecules which are intrinsically fluorescent and bind a biological molecule of interest. Major examples of these are nucleic acid stains like DAPI and Hoechst.

- **DAPI (4',6-diamidino-2-phenylindole)** is a fluorescent stain that binds strongly to A-T rich regions in DNA and Hoechst.
- **Hoechst stains** are part of a family of blue fluorescent dyes used to stain DNA

- A major example of fluorescent stain is phalloidin which is used to stain actin fibres in mammalian cells.

Thank You

Growth curves of micro-organisms

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- Bacteria divide by **Binary Fission**.
- This is a form of **asexual reproduction**.
- Under ideal conditions it can take place every 20 minutes!
- In this way huge numbers of bacteria can be produced very rapidly.

- Because of this we use a special scale called the logarithmic scale to represent their numbers.

- In a logarithmic scale each division represents a unit increase in the value of x in the term 10^x.

- **Thus:**

$10^0 = 0$

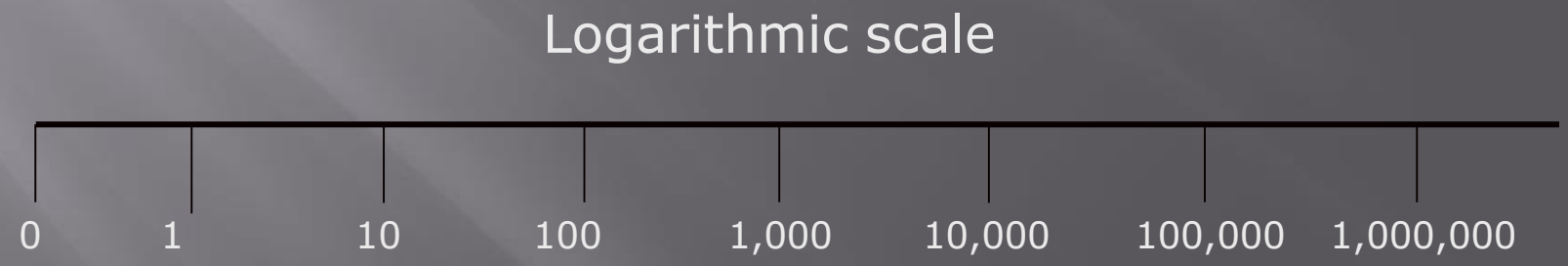
$10^1 = 10$

$10^2 = 100$

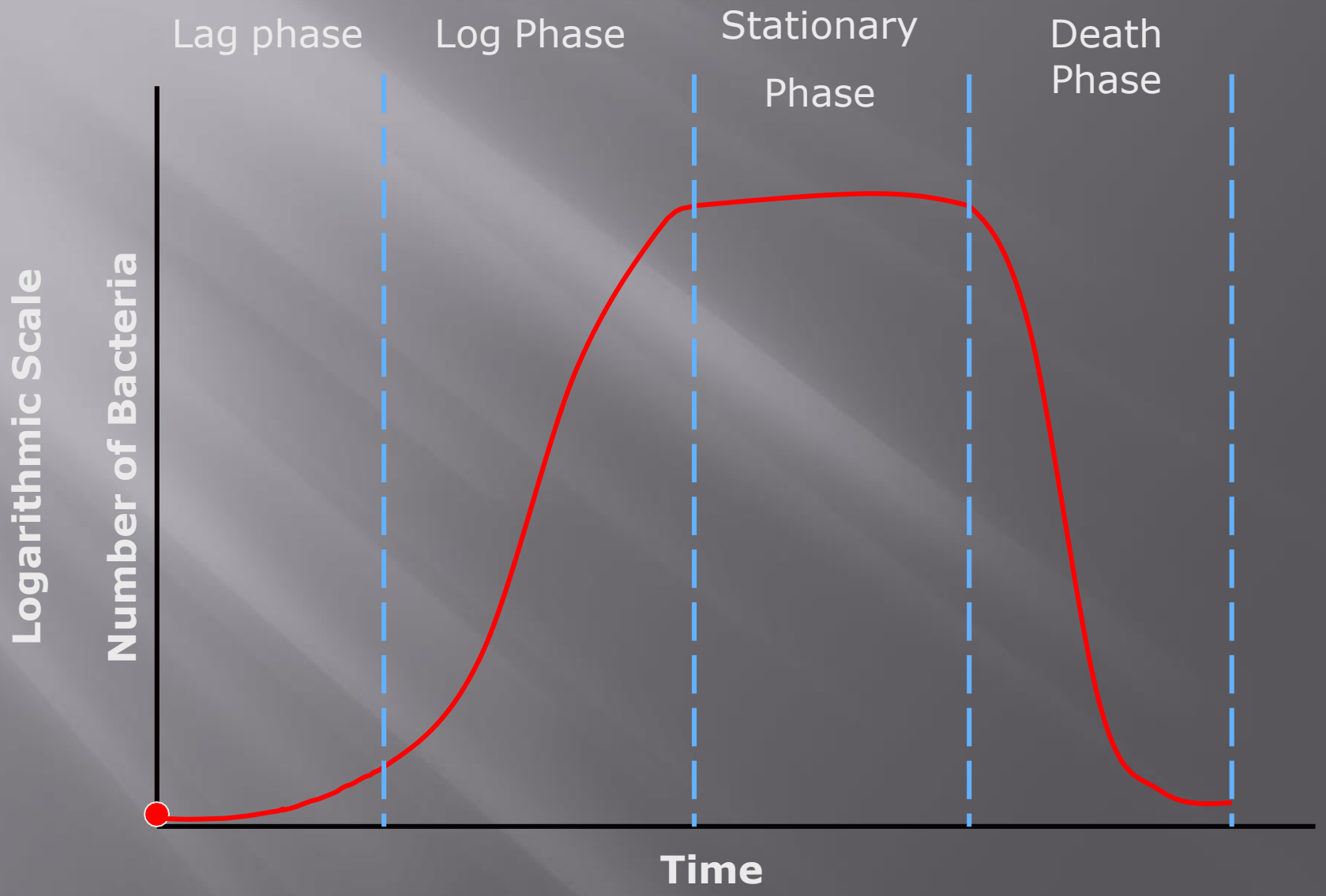
$10^3 = 1000$

$10^4 = 10{,}000$

$10^5 = 100{,}000$ etc



Growth curve for Bacteria



The Lag Phase

- After inoculation there is normally a brief period of adaptation by the cells to the new conditions.
- Bacteria are producing the enzymes necessary to digest the nutrients.
- The rate of growth begins to increase towards the end of this phase.

The Log (Logarithmic / Exponential) phase

- There is a rapid period of growth during this phase due to the fact that:
- Bacteria have developed the necessary enzymes and there are plenty of nutrients.
- There are few waste products being produced.
- The rate of cell division is currently at its maximum with the number of bacteria doubling as often as every 20 minutes.

Limitation of log or exponential phase

- Exhaustion of nutrition
- Accumulation of toxic metabolites end products
- Rise in cell density
- Change in PH

[Log phase is the time when cells are most active metabolically and is preferred for industrial purpose]

The Stationary Phase

- The rate of growth levels stop during this period.
- This is because:
  - The nutrients are becoming used up.
  - The amount of waste produced by the bacteria themselves is increasing.
  - The rate at which new cells are produced is equal to the rate at which other cells are dying.

The Death (Decline) Phase

During this phase more bacteria are dying than are being produced. This is because:

- Very few nutrients are left.
- Many bacteria are poisoned by the waste produced by such large numbers
- Finally, after certain time period all the cells die and culture becomes sterile.

Association of Growth and Cell Changes

- Lag Phase- maximum cell size towards end of phage
- Log Phase- cells are smaller and stain uniformly
- Stationary Phase- cells are Gram variable and irregular staining due to presence of intracellular granules, Sporulation occurs
- Decline Phase- involution forms are common

Batch and Continuous Flow Culture

A bioreactor is a vessel in which biological reactions take place

Food processing

- Modern bio-processing methods involve the use of bacteria (and other organisms) to produce a wide range of products.
- These include dairy products e.g. yoghurts and cheeses, artificial sweeteners, flavourings, vitamins and alcohol products such as wines and beers.

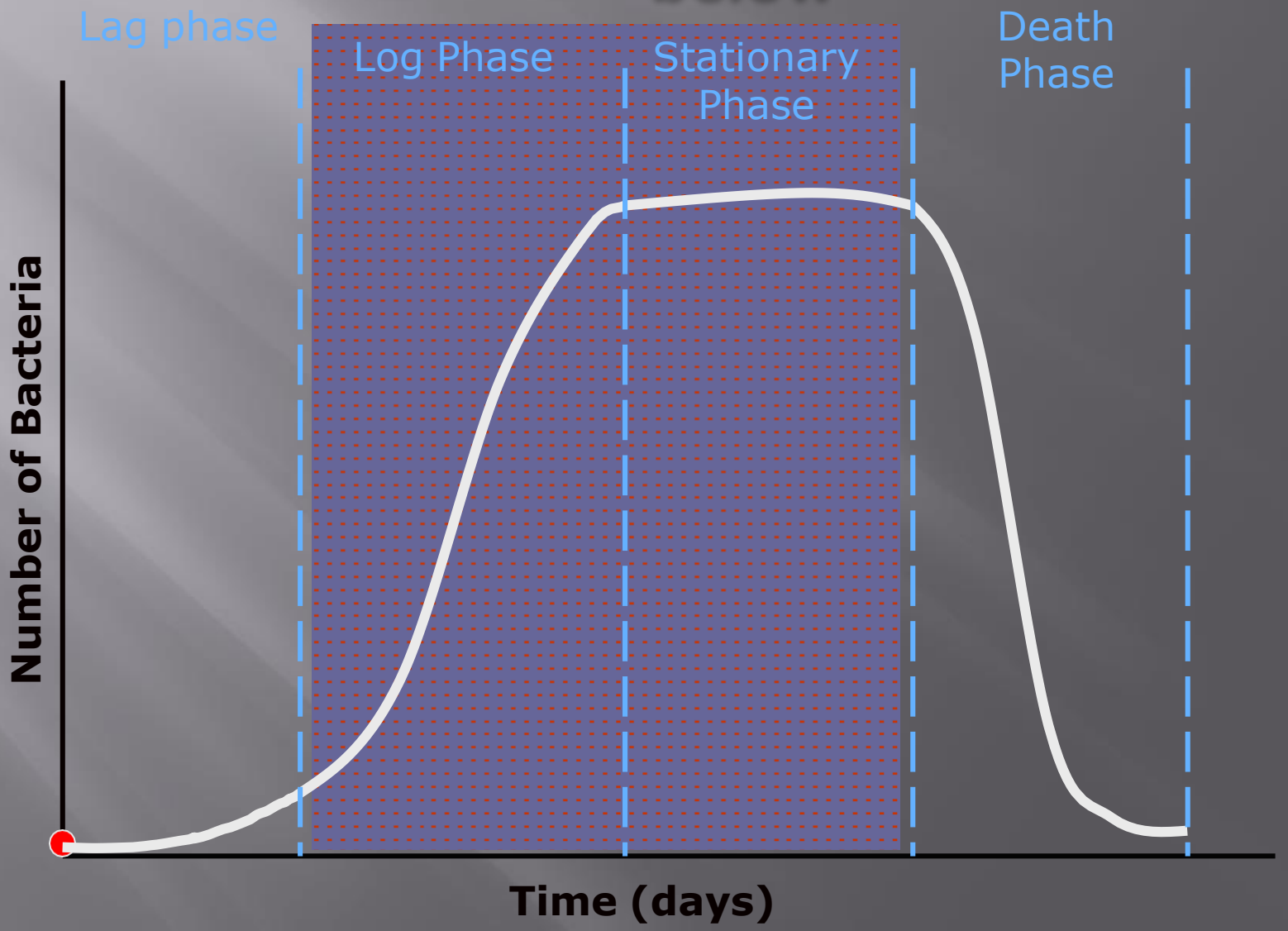
Food processing

- There are two main methods of food processing:
- Batch culture
- Continuous culture

Batch Culture

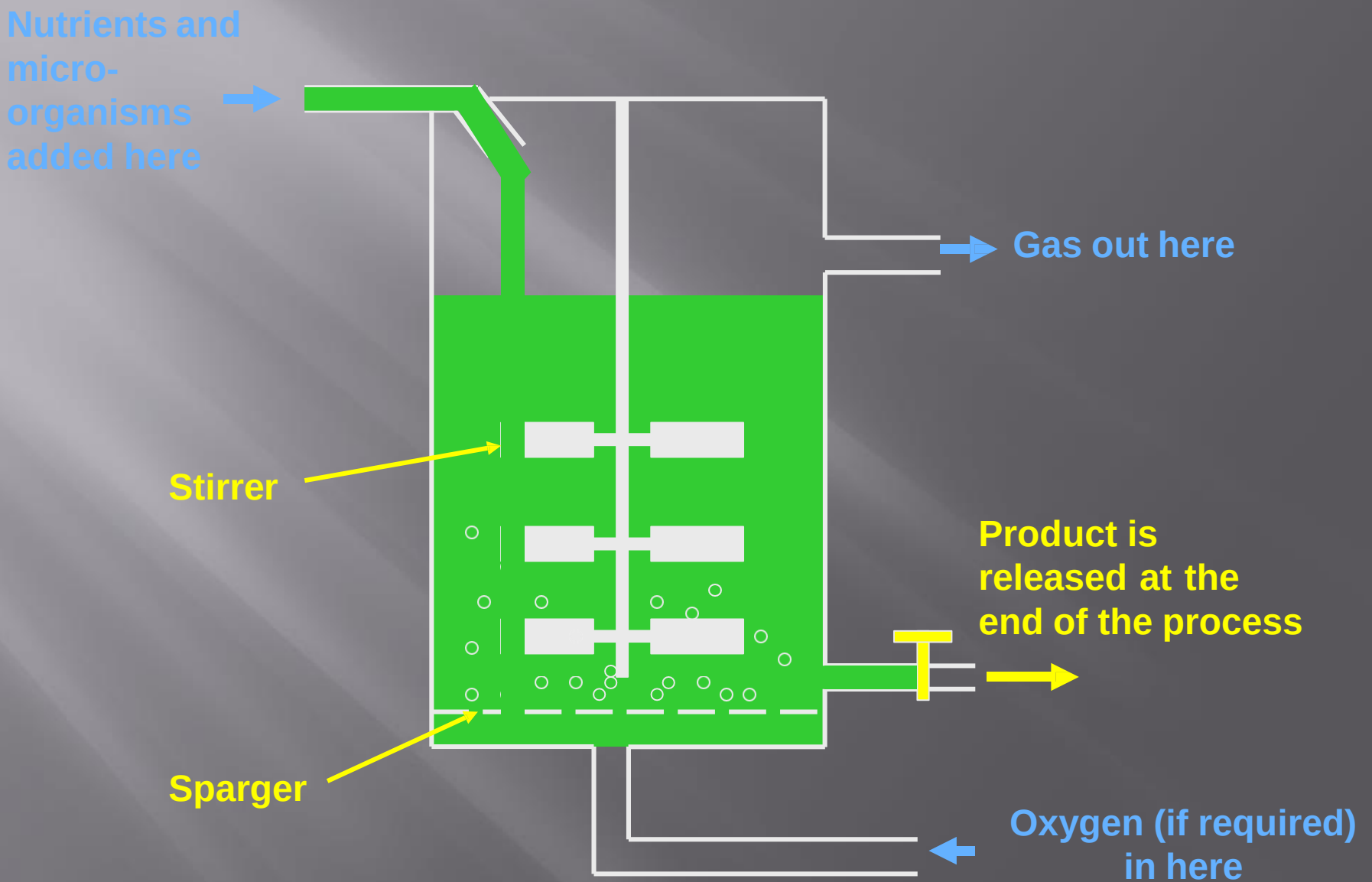
- In batch food processing a fixed amount of sterile nutrient is added to the micro-organisms in the bioreactor.
- The micro-organisms go through the stages of a typical growth curve
- i.e. The Lag, Log, Stationary and Death stages
- Although the reaction may be stopped before the death stage as very little product will be formed at this stage.

In Batch Processing most of the product is formed during the stages highlighted below



- At the end of production the bioreactor is cleared out. The product is separated from the rest of the solution and is purified.
- The bioreactor is cleaned and re-sterilised.
- The process can then be repeated.

Bioreactor for Batch Culture



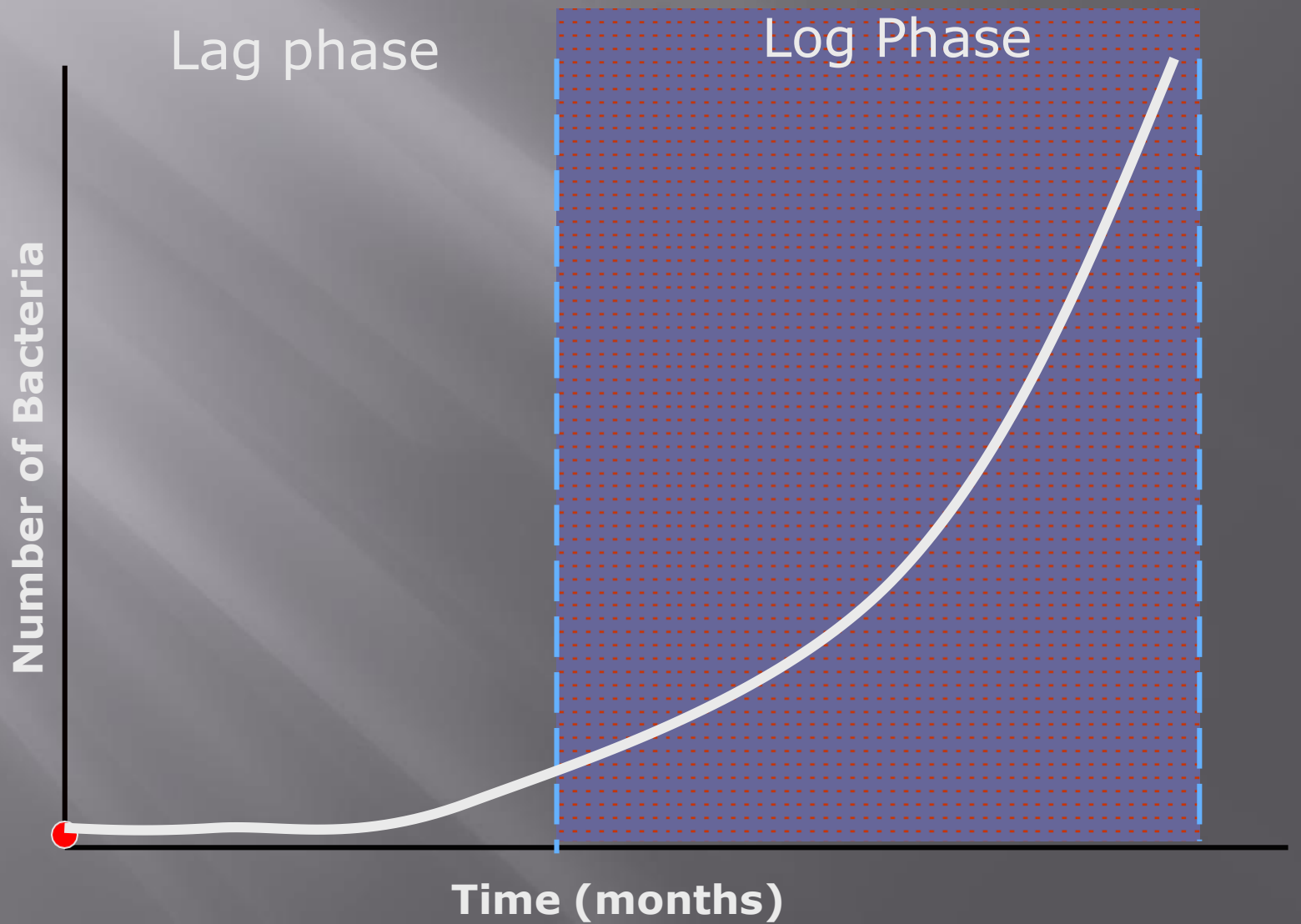
Continuous Culture

- In continuous culture nutrients are continuously fed into the bioreactor.
- At the same time the culture medium (containing some micro-organisms) is continually withdrawn.
- In this method of food processing micro-organisms are maintained in the Log phase of growth and the process can continue uninterrupted for weeks, even months.

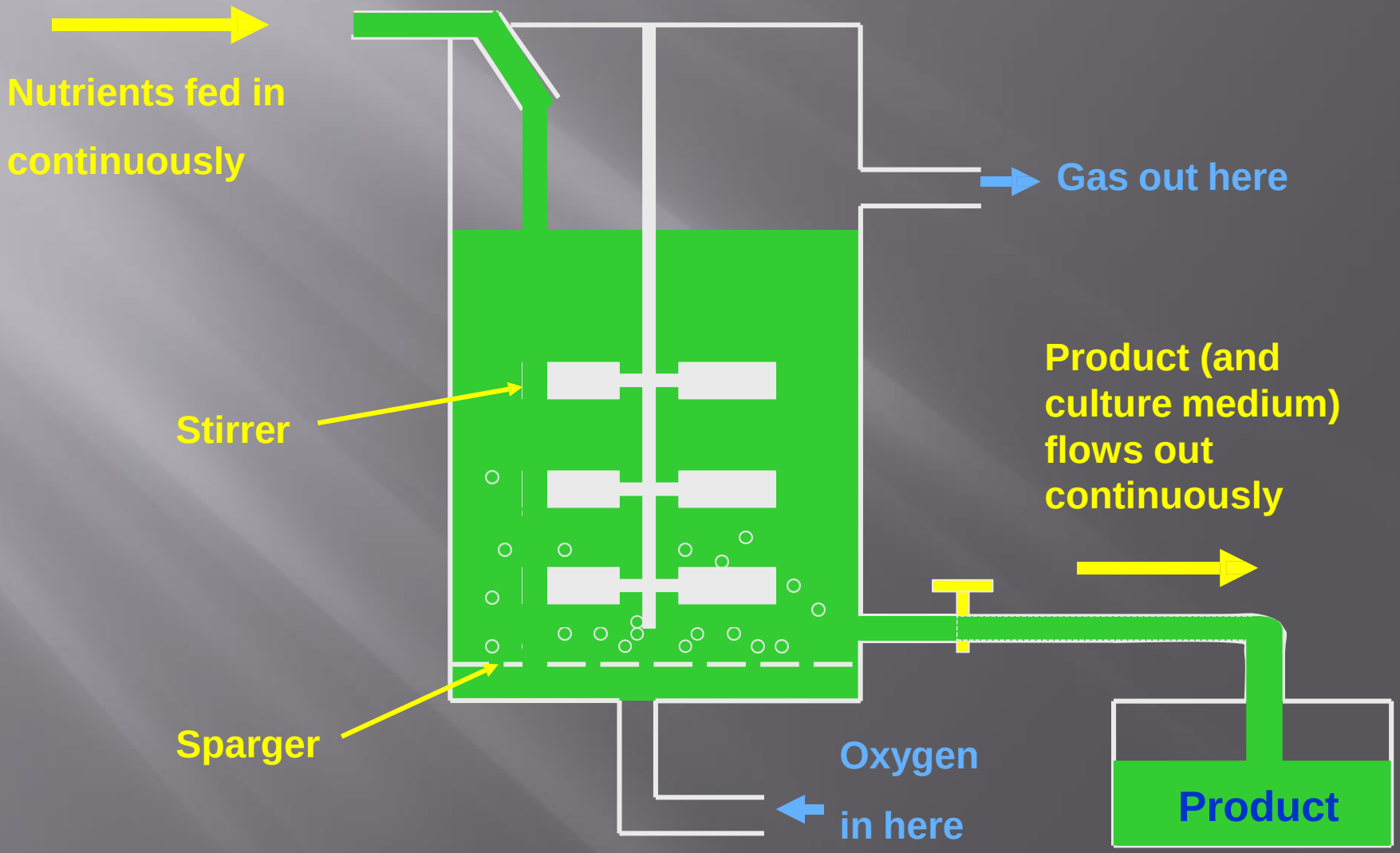
Continuous flow food processing

- In continuous flow bioreactors factors such as temperature, pH, rate of stirring, concentration of nutrients, oxygen and waste products are constantly monitored in order to maintain growth in the Log phase and therefore produce the maximum yield.

In Continuous culture most of the product is formed during the stage highlighted below



Bioreactor for Continuous Culture



PHASE CONTRAST MICROSCOPY

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Microscope

- A microscope is an instrument used to see objects that are too small for the naked eyes.
- The science of investigating small objects using such an instrument is called microscopy.

TYPES OF MICROSCOPE

- **Optical microscope**:-The most common type of microscope which uses light to image the sample.
- **Electron microscope**:- It uses electron to image the sample.

  Scanning electron microscope (SEM)

  Transmission electron microscope (TEM)
- Ultra microscope
- Scanning probe microscope

LIGHT MICROSCOPE

- It uses visible light to detect small objects.
- It is a well use or well known research tool in biology.
- We can use light microscope to enlarge cytoplasm, nucleus, cell membrane, chloroplast, Vacuoles, cell wall etc.

MAGNIFICATION AND RESOLUTION

Magnification:-

Degree of enlargement

Decrease the number of times in length, breath and diameter of the object is multiplied.

Resolution:-

The minimum distance between two visible bodies at which they can be seen as separate and distinct object and not in contact with each other.

TYPES OF LIGHT MICROSCOPE

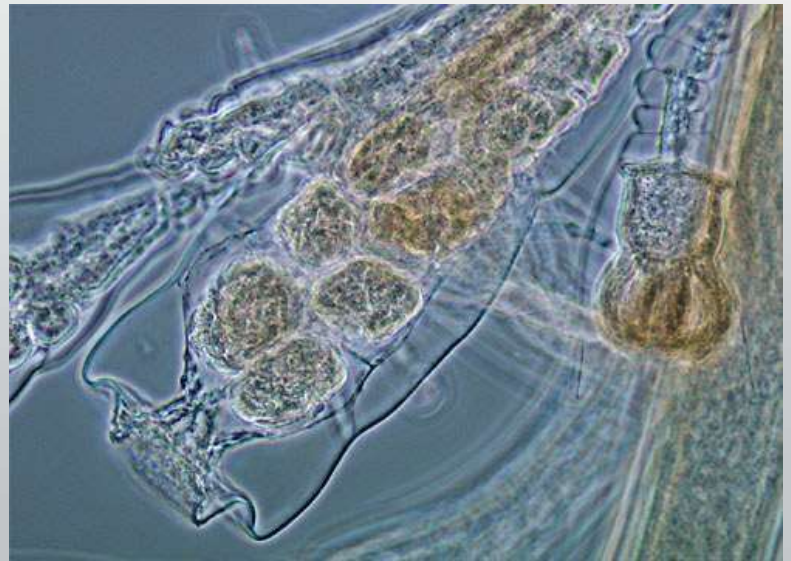
- **Bright field microscope:-** Produces a dark image against a brighter background.
- **Dark field microscope:-** Produces a bright image of the object against a dark background.
- **Phase contrast microscope:-** Enhances the contrast between intracellular structures having slight differences in refractive index.

Comparison of Light Microscopy

Bright Field

Dark Field

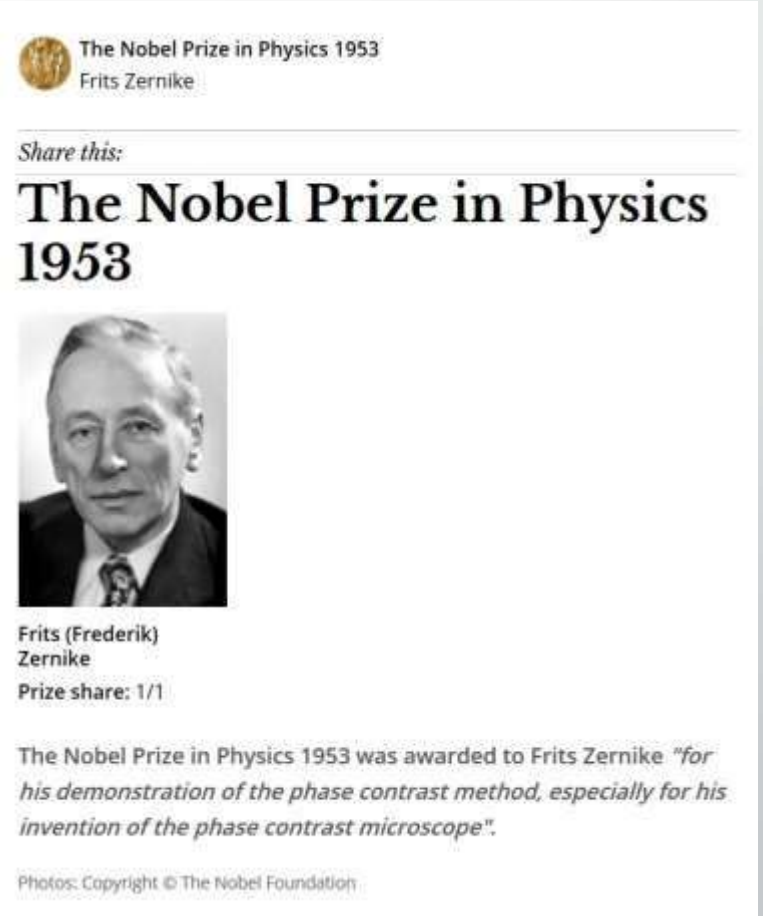
Phase contrast




50 µm

Phase contrast microscopy

- It is the first microscopic method which allow the observation of living cell.
- It was invented by Frits Zernike and was awarded noble prize in 1953.



The Nobel Prize in Physics 1953
Frits Zernike

Share this:

The Nobel Prize in Physics 1953

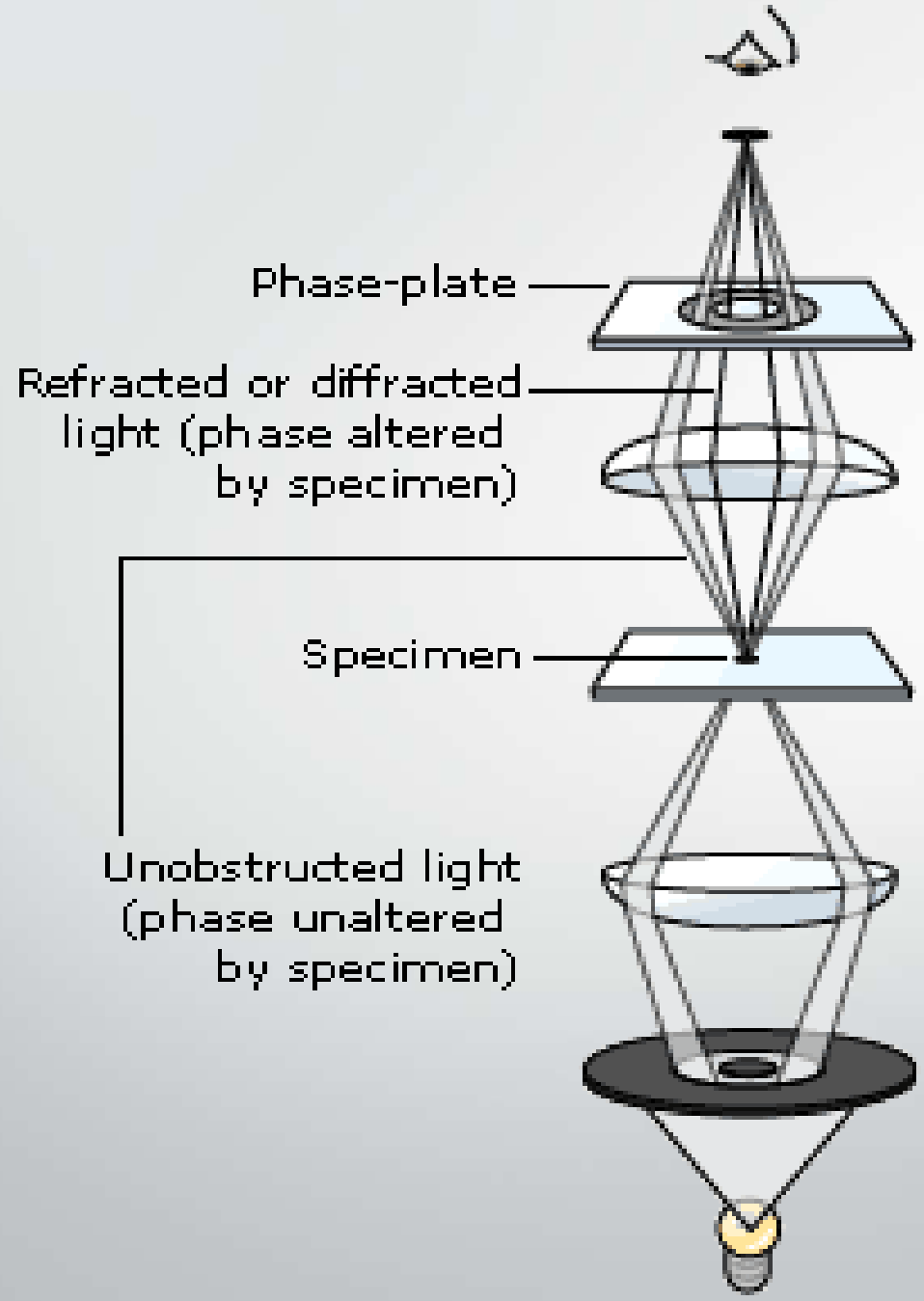
Frits (Frederik) Zernike

Prize share: 1/1

The Nobel Prize in Physics 1953 was awarded to Frits Zernike *"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope".*

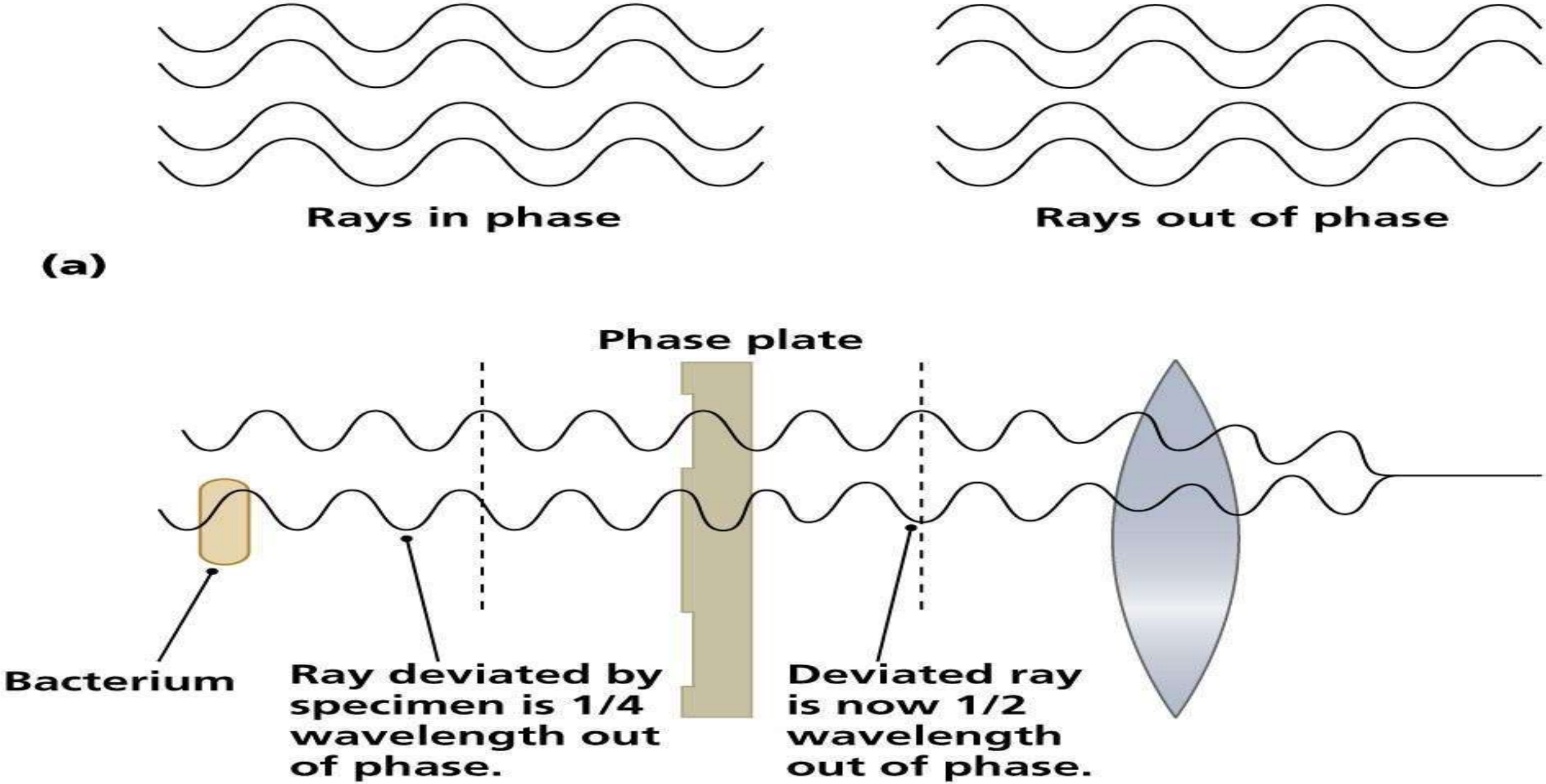
Phase-Contrast Microscopy

- The phase contrast microscopy made it possible to study living cells and how they proliferate through cell division.
- Used to examine living organisms or specimens that would be damaged/altered by attaching them to slides or staining.
- It uses a conventional light microscope fitted with a phase-contrast objective & phase-contrast condenser.
- Light passing through one material & into another material of slightly different refractive index or thickness will undergo a change in phase. This change in are translated into variations in brightness of the structures.


Phase-plate
Refracted or diffracted light (phase altered by specimen)
Specimen
Unobstructed light (phase unaltered by specimen)

PRINCIPLE OF PHASE CONTRAST MICROSCOPY

- Unstained bacteria have constituents of different refractive index .
- Diffraction of light
- Phase contrast microscope employs an optical mechanism to translate minute variations in phase into corresponding changes in intensity of image.



USES OF PHASE CONTRAST MICROSCOPY

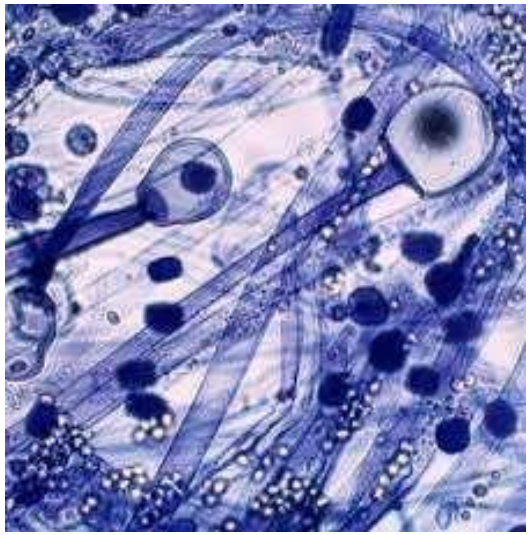
- Phase contrast enables visualization of internal cellular components.
- Diagnosis of tumor cells .
- Examination of growth, dynamics, and behavior of a wide variety of living cells in cell culture

Application

- Applications for phase contrast microscopy equipment range from the study of living biological specimens, medical applications, study of live blood cells, and other biological and science applications
- Most commonly used to provide constrast of transparent specimens such as living cells or small organisms.

THANK YOU

Mucor



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TAXONOMY:

- Kingdom: Fungi
- Division: Zygomycota
- Class: Mucormycotina
- Order: Mucorales
- Family: Mucoraceae
- Genus: *Mucor*

MORPHOLOGY

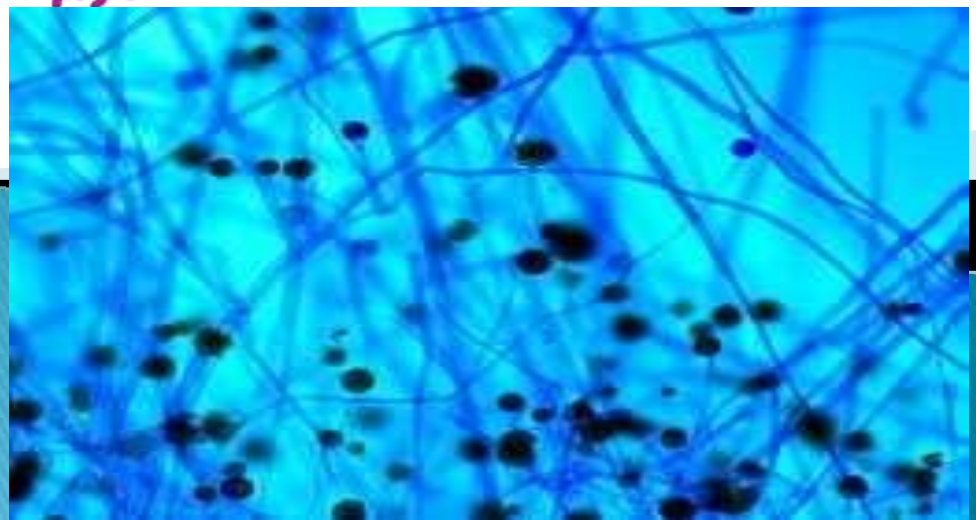
→Common contaminant.

→Colonies are fast-growing and resemble white-to-gray cotton candy.

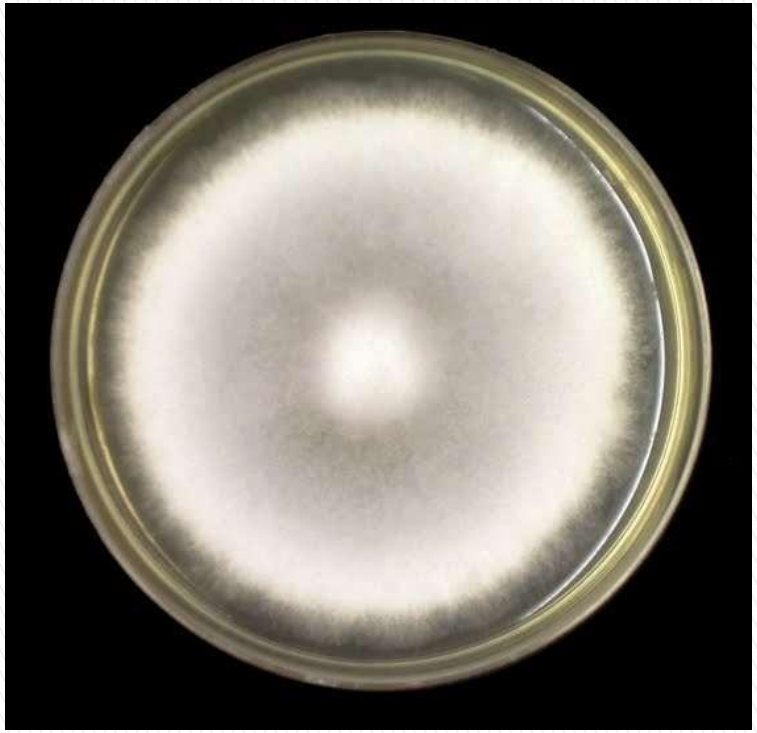
→Hyphae are wide, 6-15µ.

→No rhizoids

→Sporangiophores are long, branch, Large (50-300µ).



culture media:



Sabouraud dextrose agar medium

Potato dextrose agar medium

LIFE CYCLE:

Take place in

*vegetative reproduction

*Sexual reproduction

*Asexual reproduction

Vegetative Reproduction:

- It consists of mother mycelium
- Each fragments can split into many sub-units.
- Each sub-units have a capability to grow like new mycelium.
- Like fragmentation methods.

2) Asexual reproduction:

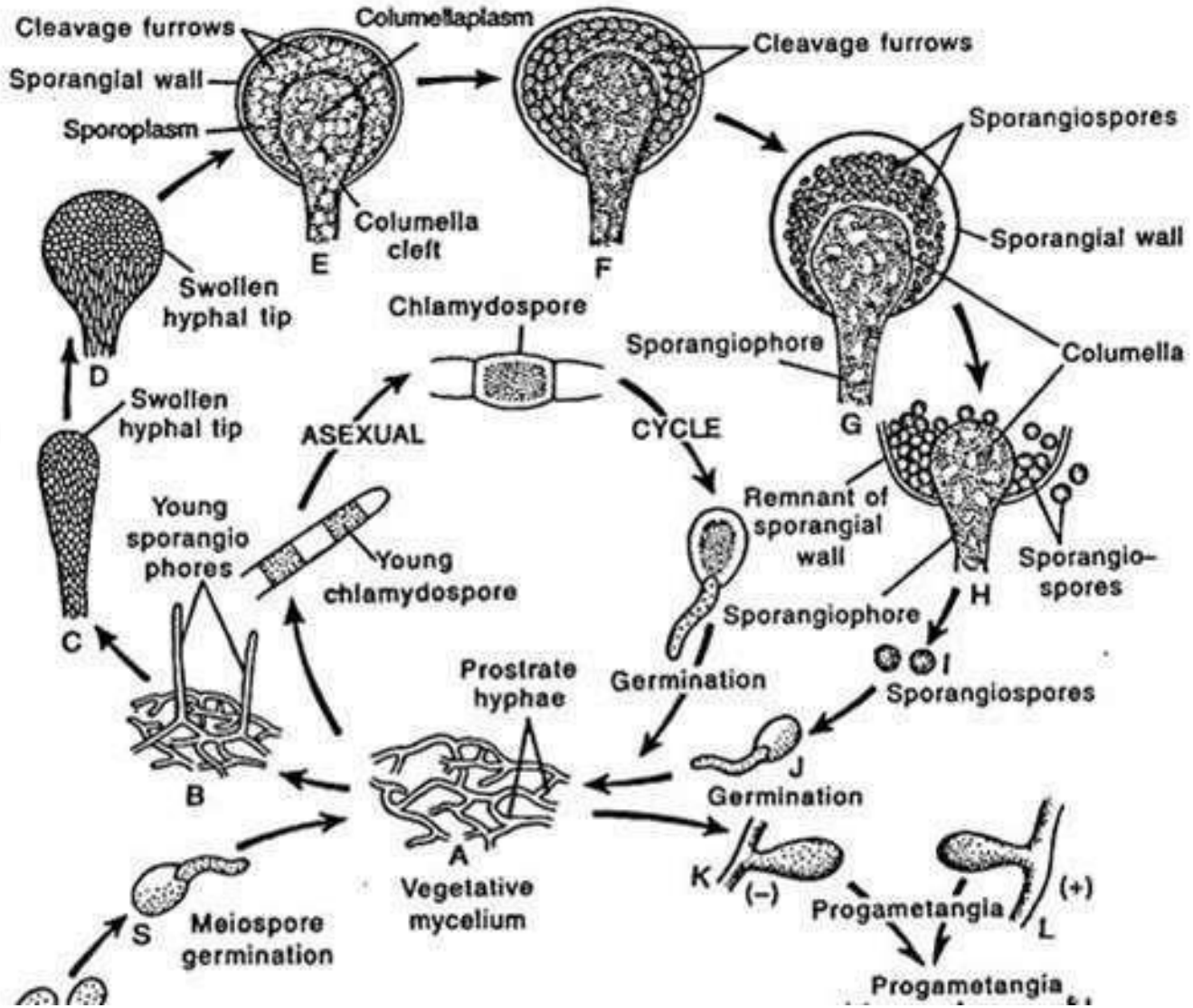
- It mainly takes in three spores.

1) Sporangiospores
2) Chlamydospores
3) Oidio spores.

SPORANGIOSPORES:

It takes place by steps:

sporangiopores englongation
Columenllaplasm
bursting
germination


Cleavage furrows
Columellaplasm
Sporangial wall
Sporoplasm
Columella cleft
E
F
Cleavage furrows
Sporangiospores
Sporangial wall
Columella
G
Swollen hyphal tip
D
Chlamydospore
Sporangiophore
Swollen hyphal tip
ASEXUAL
CYCLE
Young sporangio phores
Young chlamydospore
Remnant of sporangial wall
Sporangio-spores
H
C
Sporangiophore
Prostrate hyphae
Germination
I
Sporangiospores
J
Germination
B
A
Vegetative mycelium
K
(–)
Progametangia
(+)
L
S
Meiospore germination
Progametangia

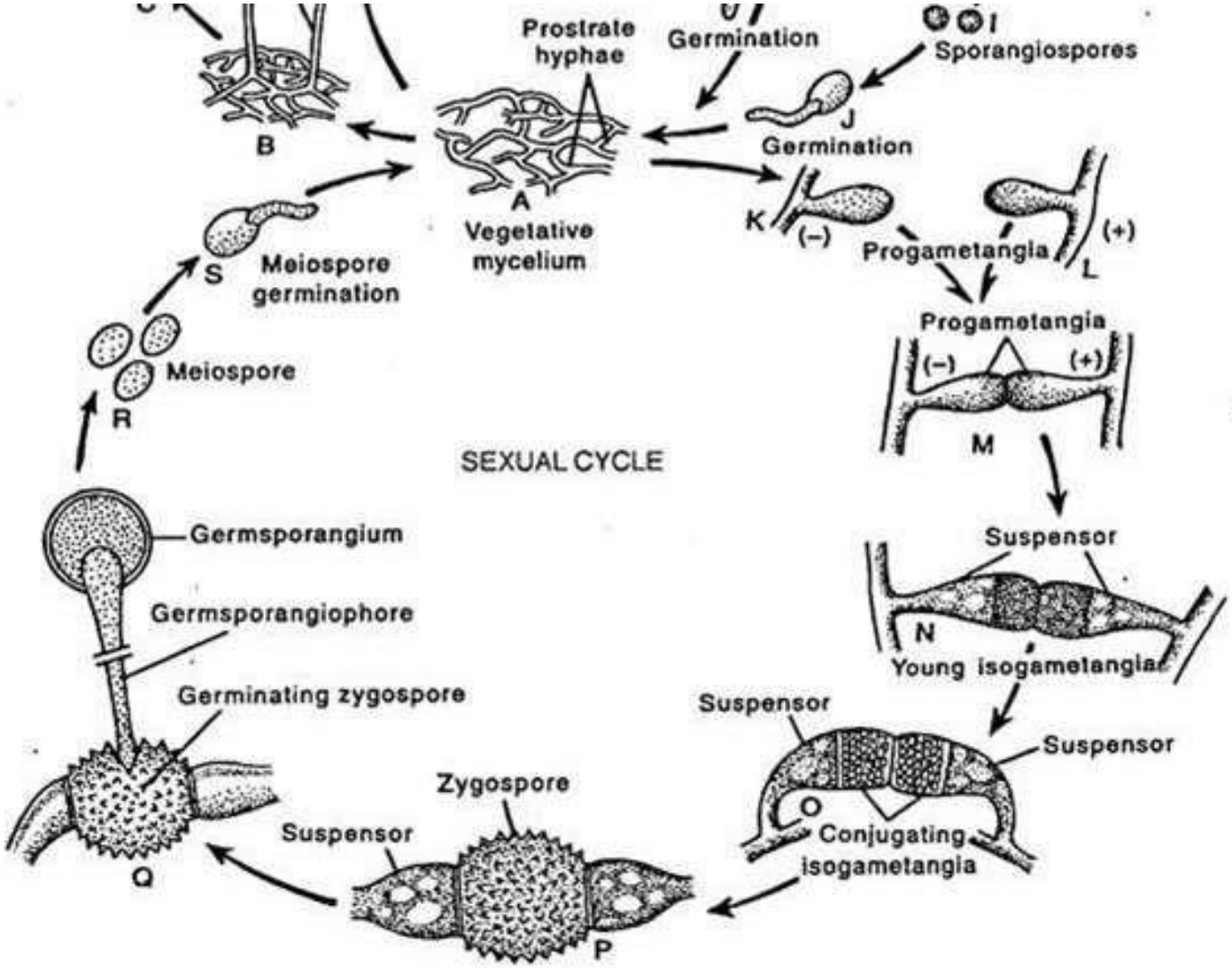
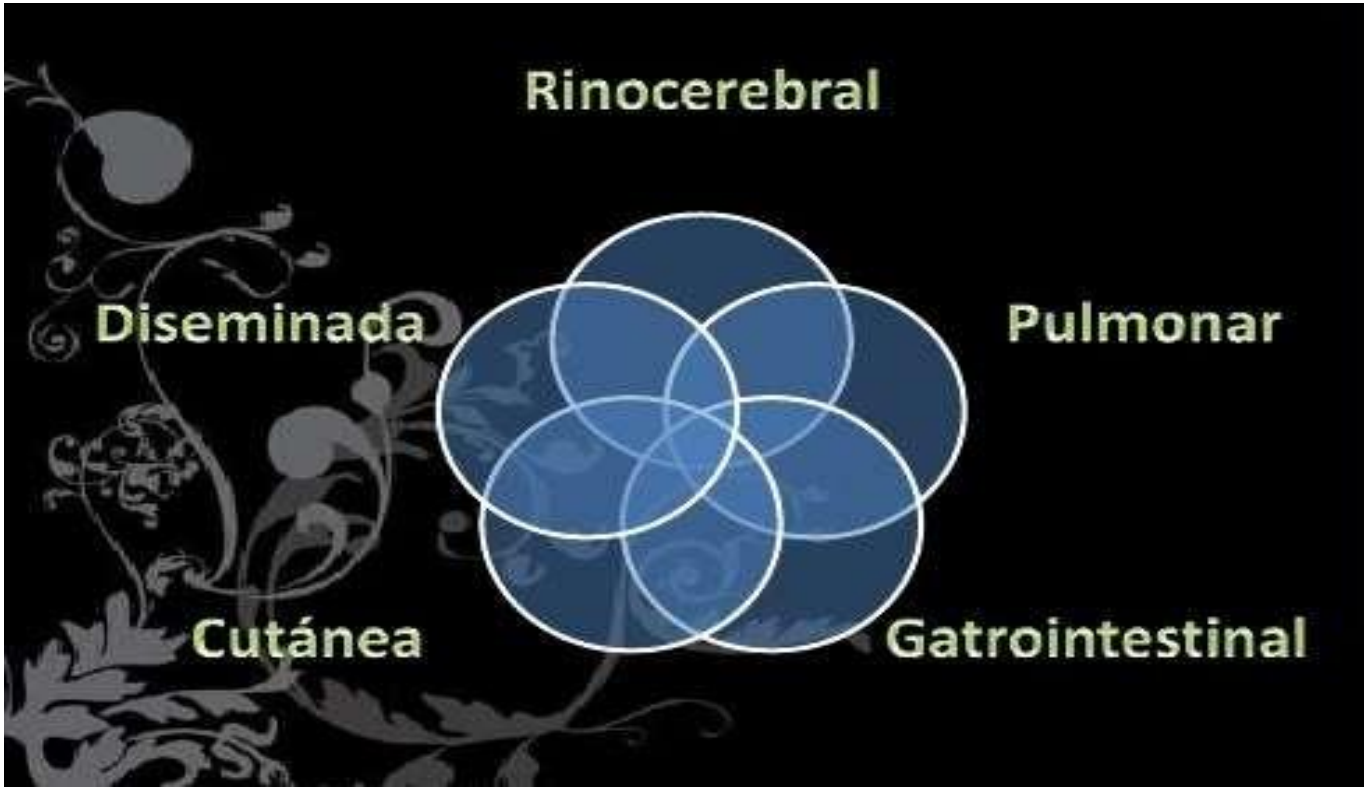


Fig. 4.28 : Life cycle of *Mucor mucedo*

EPIDEMIOLOGY

- INTERNATIONALY 1% PATIENTS WITH LOW IMMUNITY.
- MUCOR MYCOSIS CARRIES A VERY HIGH MORTALITY (50%-85%).
- NO RACIAL FACTORS PREDISPOSE.
- SEX IS NOT LIKELY TO AFFECT.

Clinical feature:



Rhinocerebral :

→Opportunistic infection of sinuses , nasal passages , oral cavity.

→Rapid death.

→Grow rapidly and release air-borne spores.

→Enter through nose, oral mucosa and throat.

→Attack immunelow peoples.

Risk factors:

→Diabetes Mellitus
→Burns
→Iron overload
→Transplantation
→Chemotheraphy
→Intravenous drug use

PULMONARY :

- Rare diseases occur in patients who have prolonged neutropaenia.
- Treatment with deferoxamine
- Indistinguishable from Invasive Pulmonary Aspegillosis.
- **Symptoms**: fever , Cough , dyspnoea, Chest Pain, Pleural effusion.
- ***Species:*** *Mucormycotina*
- Spores -3 to 11 µm.
- Routes: Inhalation , cutaneous …

- Life-threatning diseases.
- Specimen: Nasal Seceations.
- Drug: amphotericin B (sideeffects)
- Treatment: antifungal theraphy.

GASTROINTESTINAL:

- Transmission: Inhalation of spores.
- Common in children.
- Affects Immuno-comprised patients.
- Symptoms varies depend upon the part of the body.
- Common Symptoms: abdominal pain, Bowel Obstruction, Vomiting , Bloody diarrhea,Dyspepsia.
- Prognosis is very poor.

DISEMMINATED:

- Rare, fatal complication disease.
- Multiorgans manifestations.
- Symptoms: mental status changes.
- Occur who are already sick from medical condition.
- Leads to coma.

CUTANEOUS:

*Reddish and swollen skin, skin trauma, ulcer.

*mainly painful patches of skin.

* fungal enter through skin.

*patients who are low Ig A and IgG antibodies.

*skin theraphy , transplantation.

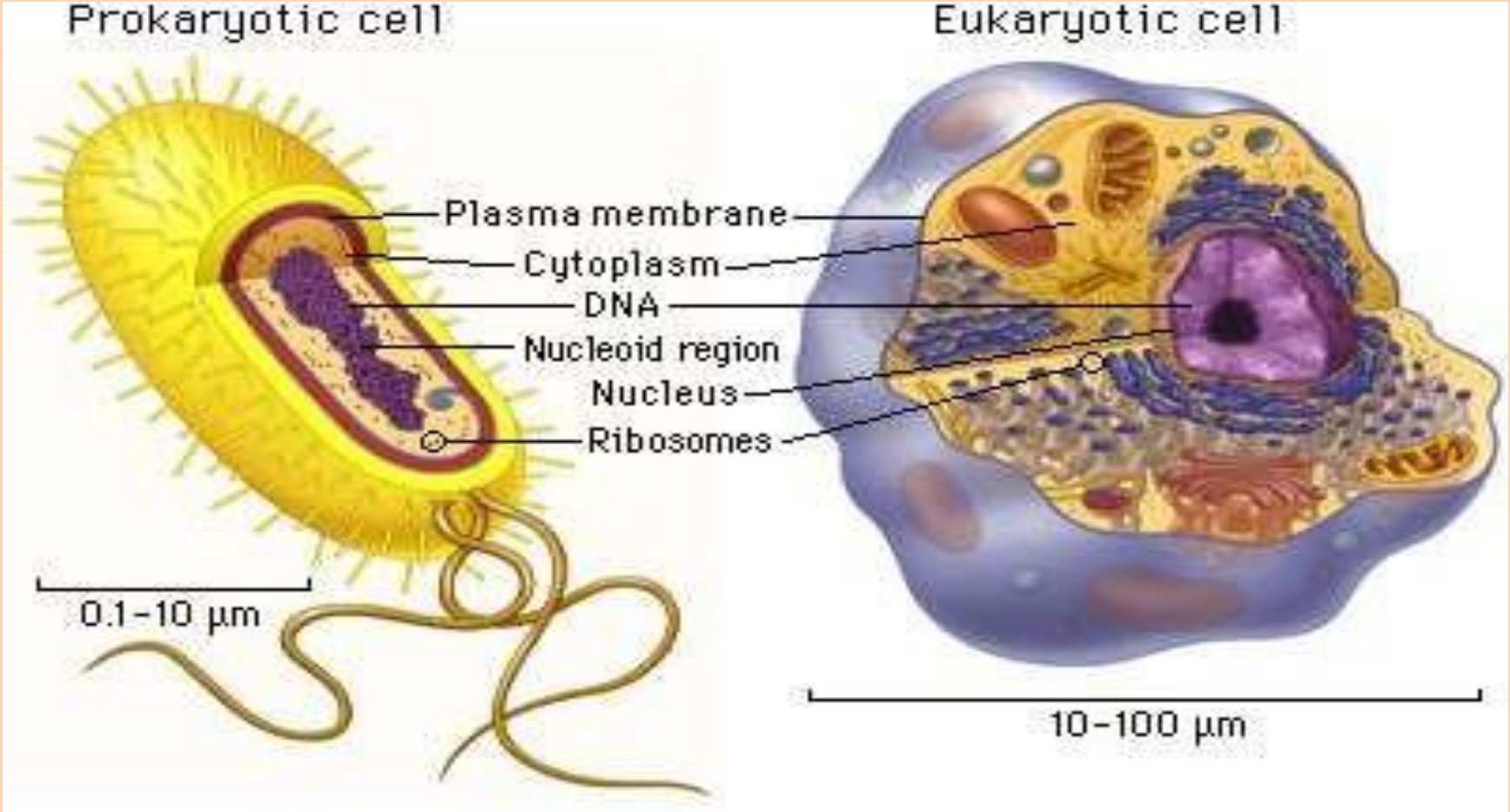
*Very poor
*Surgery can done depend upon area

Treatment:

Antifungal medicine, usually amphotericin B, through a vein.


Thank You

Prokaryote vs Eukaryote



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Cells have evolved two different architectures:

- Prokaryote “style”
- Eukaryote “style”

- **Prokaryotic cells** were here first and for billions of years were the only form of life on Earth. All **prokaryotic** organisms are **unicellular**

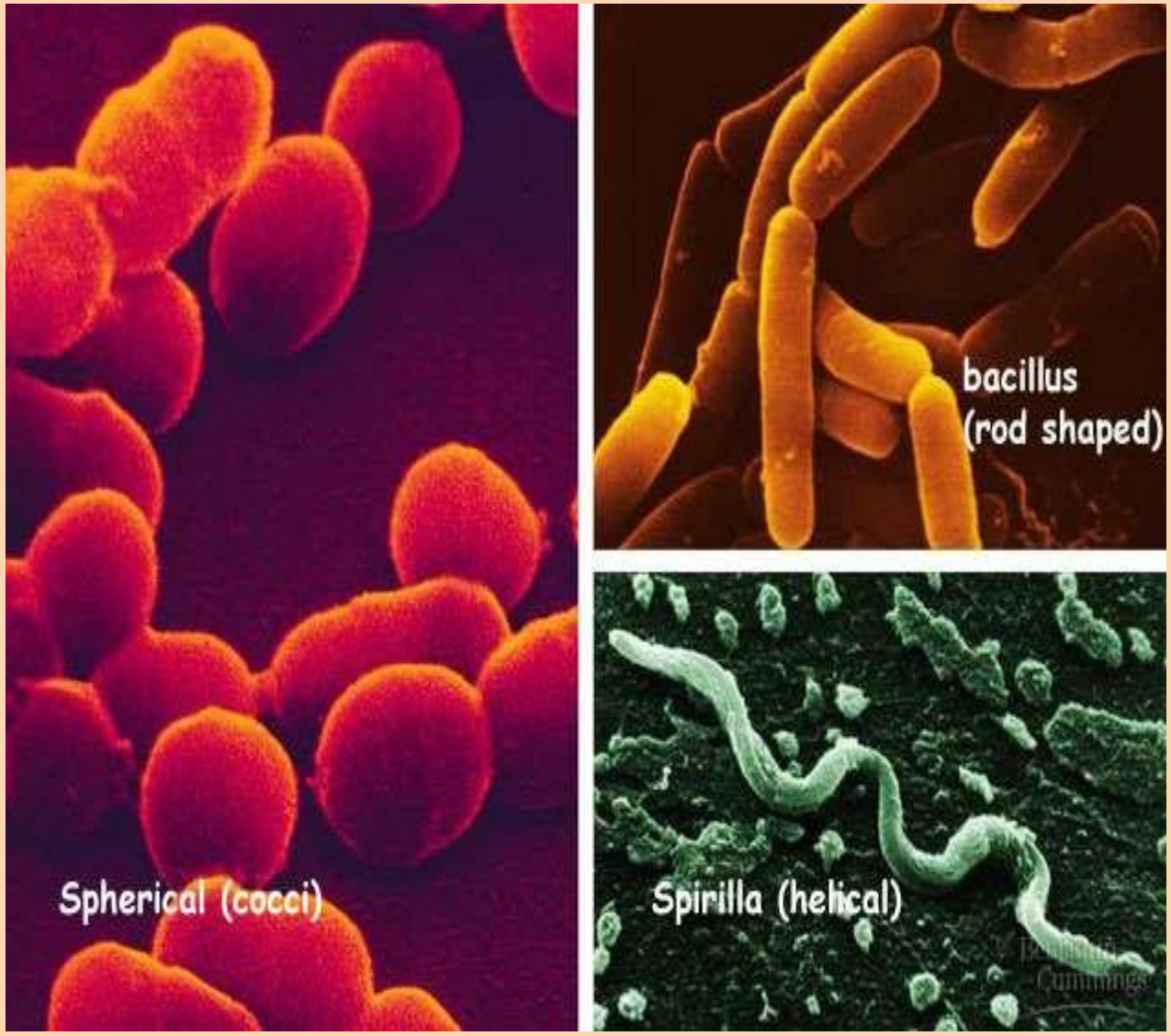
- **Eukaryotic cells** appeared on earth long after **prokaryotic cells** but they are much more advanced. **Eukaryotic** organisms unlike **prokaryotic** can be **unicellular** or **multicellular**.

Characteristics of Prokaryotes

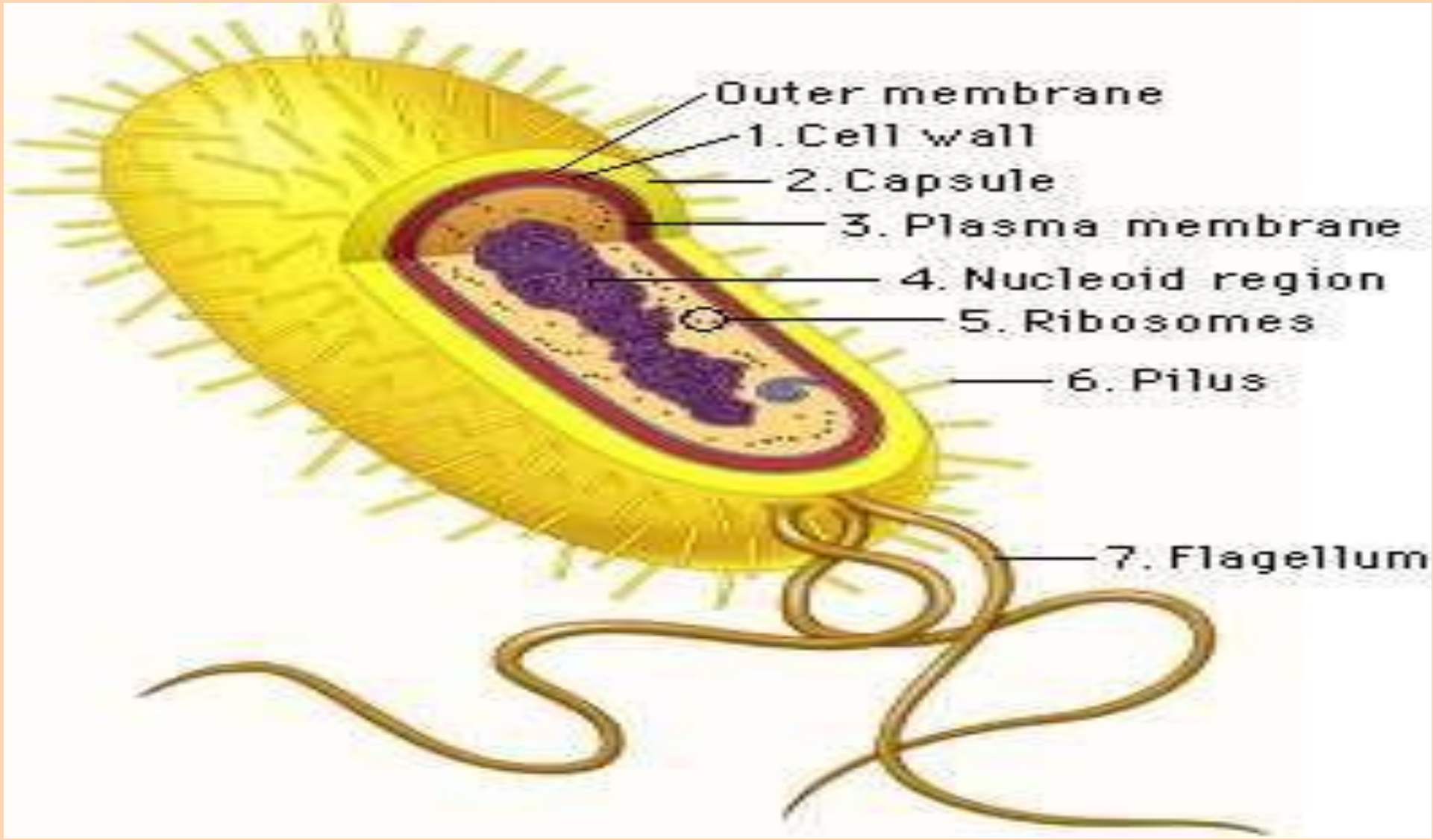
- Prokaryotes are the simplest type of cell.
- Oldest type of cell appeared about four billion years ago.
- Prokaryotes are the largest group of organisms
- Prokaryotes unicellular organisms that are found in all environments.

- Prokaryotes do not have a nuclear membrane. Their circular shaped genetic material dispersed throughout cytoplasm.
- Prokaryotes do not have membrane-bound organelles.
- Prokaryotes have a simple internal structure.
- Prokaryotes are smaller in size when compared to Eukaryotes.

Shapes of Prokaryotes



- Cocci = spherical (round)
- Bacillus = (rod shaped)
- Spirilla = helical (spiral)


Outer membrane
1. Cell wall
2. Capsule
3. Plasma membrane
4. Nucleoid region
5. Ribosomes
6. Pilus
7. Flagellum

Characteristics of eukaryotes

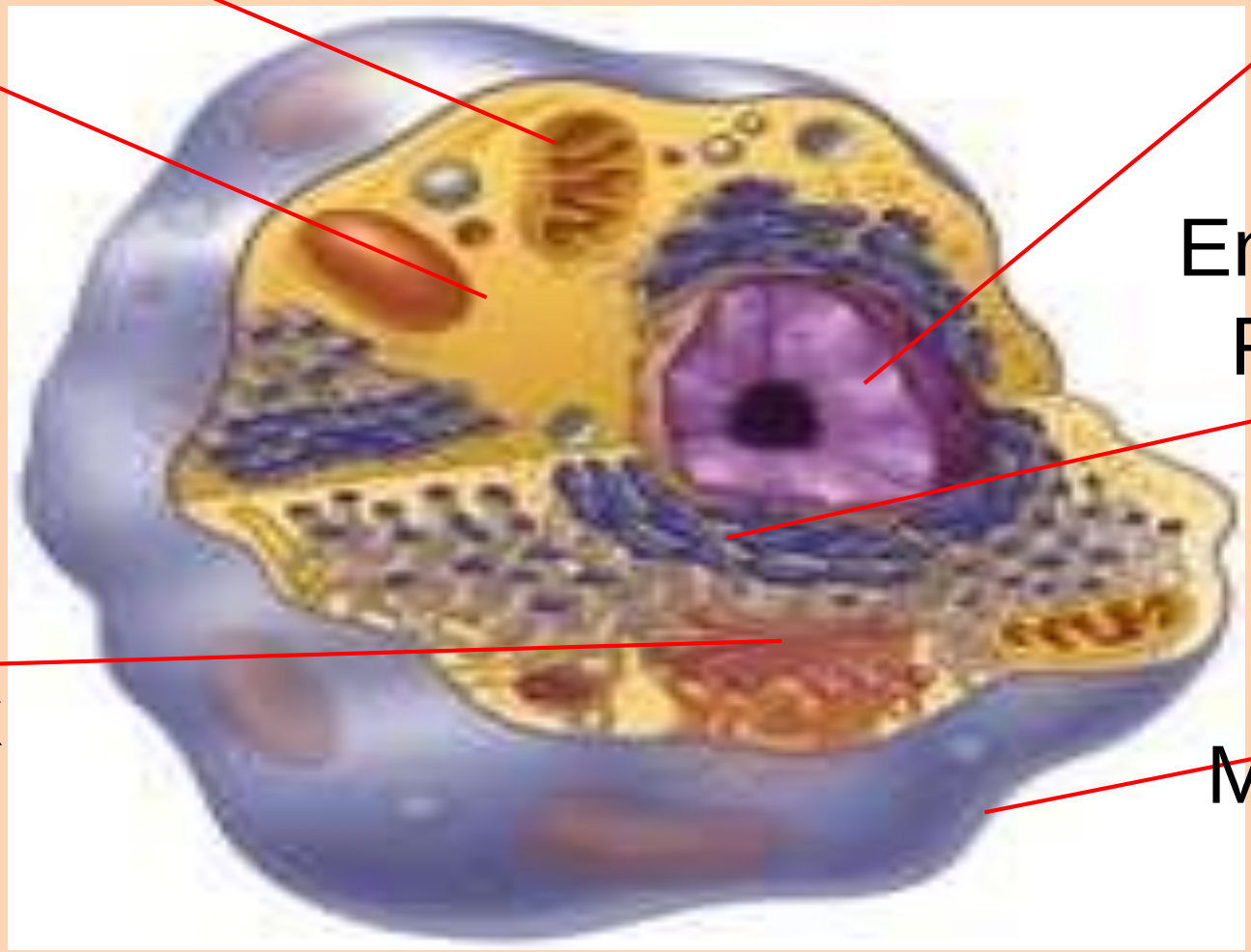
- Eukaryotic cells appeared approximately one billion years ago
- Eukaryotes are generally more advanced than prokaryotes
- Nuclear membrane surrounds linear genetic material (DNA)

- Unlike prokaryotes, eukaryotes have several different parts.
- Prokaryote’s organelles have coverings known as membranes.
- **Eukaryotes** have a complex internal structure.
- Eukaryotes are larger than prokaryotes in size .

Mitochondria

Cytoplasm

Nucleus



Endoplasmic Reticulum

Golgi Complex

Cell Membrane

Differences

Prokaryotes

- Organelles lack a membrane
- Ribosomes are the only organelles
- Genetic material floats in the cytoplasm (DNA and RNA)

Eukaryotes

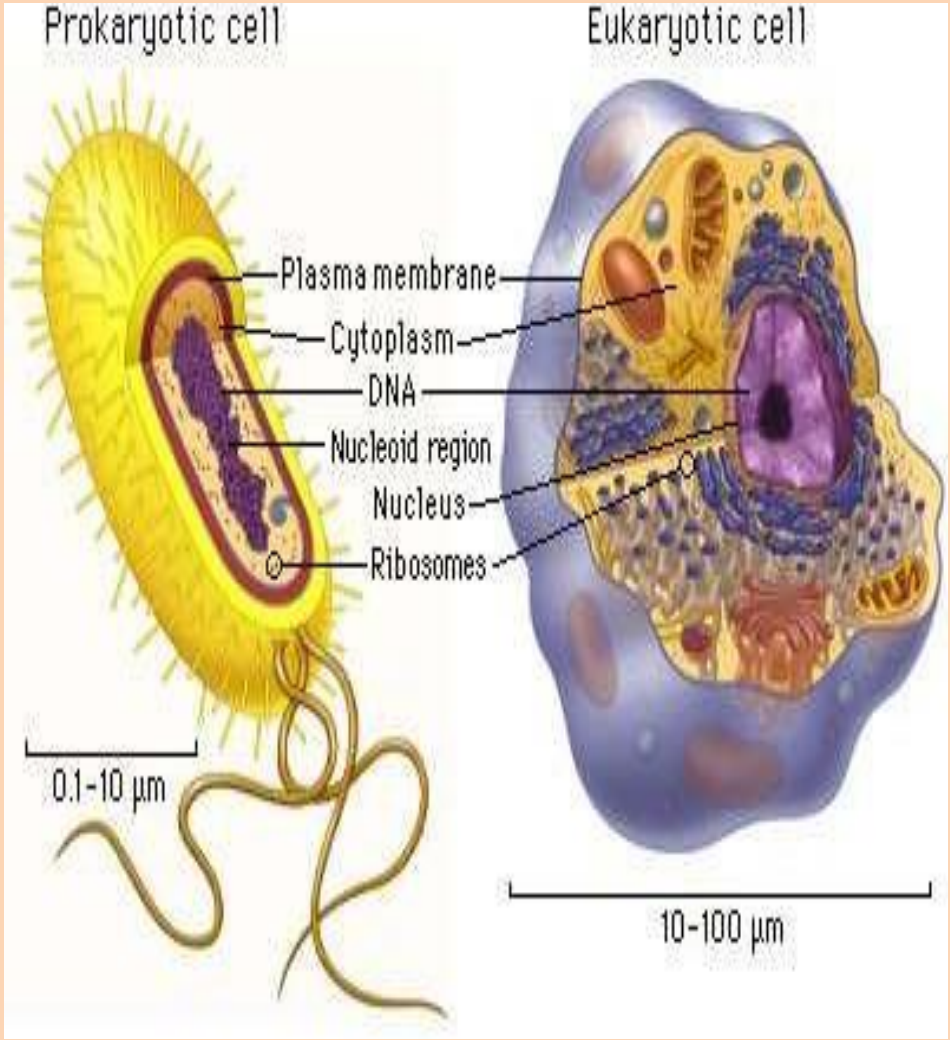
- Organelles covered by a membrane
- Multiple organelles including ribosomes
- Membrane covered Genetic material

Prokaryotes

- Circular DNA
- Unicellular
- Cells are smaller in size
- Has larger number of organisms
- Appeared 4 billion years ago

Eukaryotes

- Linear DNA
- May be multicellular or unicellular
- Cells are larger in size
- Has smaller number of organisms
- Appeared 1 billion years ago

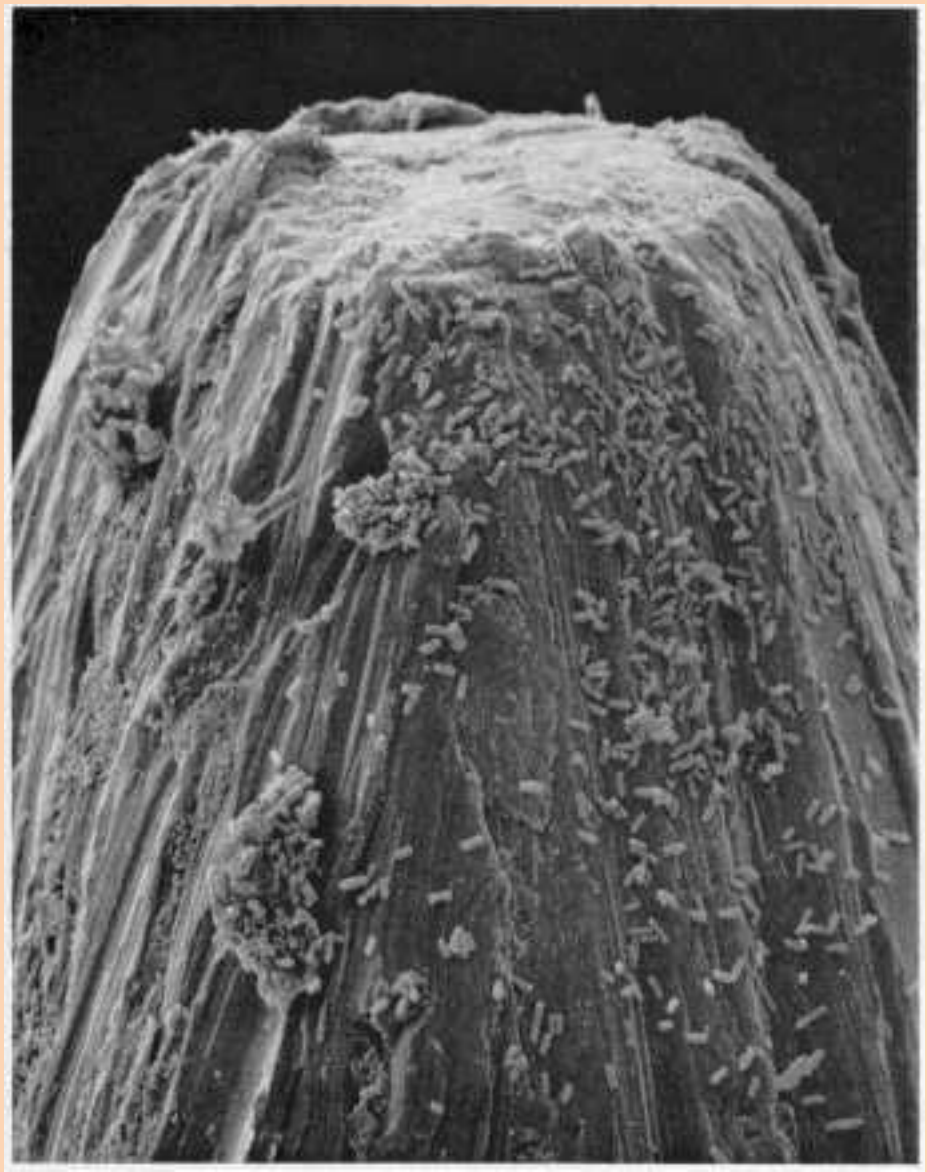


Similarities

- Both types of cells have cell membranes (outer covering of the cell)
- Both types of cells have ribosomes
- Both types of cells have DNA
- Both types of cells have a liquid environment known as the cytoplasm

Prokaryote cells are smaller and simpler

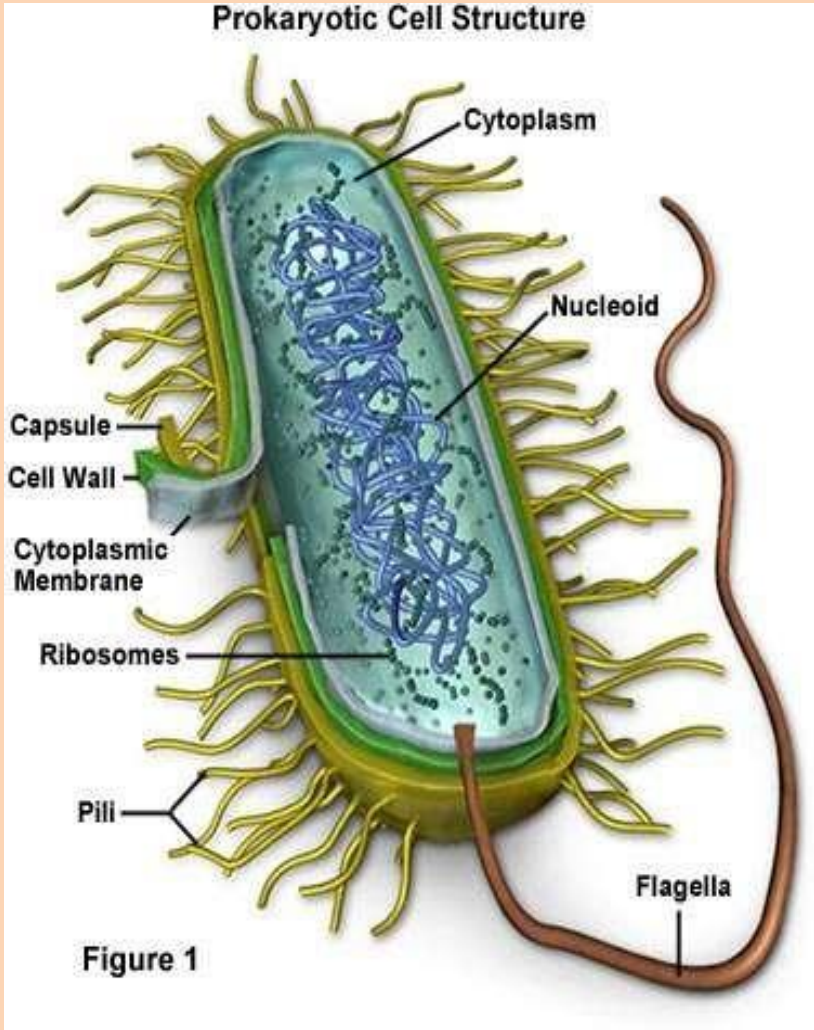
- Commonly known as bacteria
- 10-100 microns in size
- Single-celled (unicellular) or
- Filamentous (strings of single cells)



These are prokaryote E. coli bacteria on the head of a steel pin.

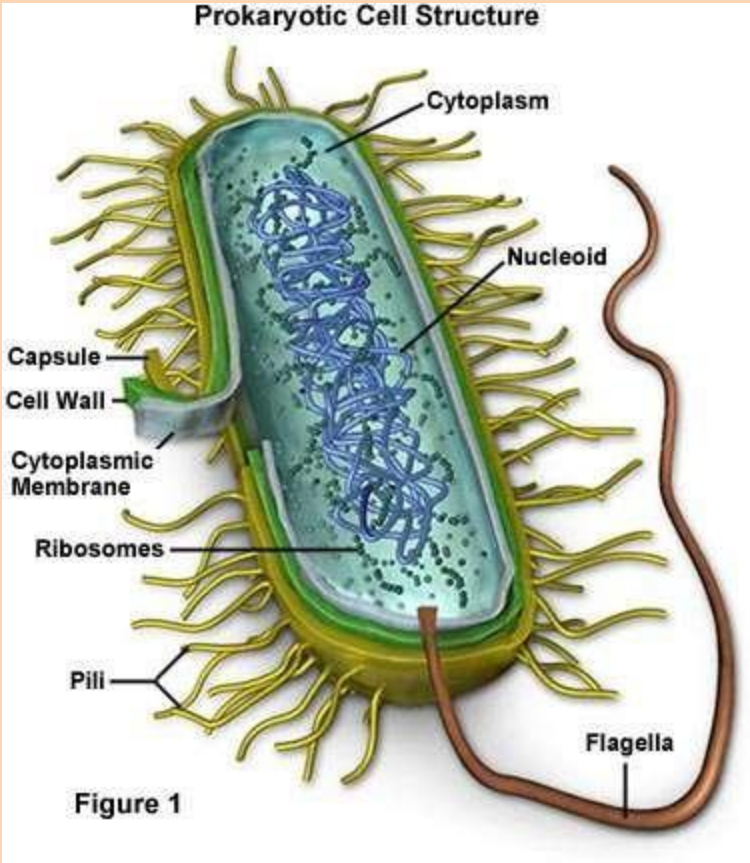
Prokaryote cells are simply built (example: E. coli)

- capsule: slimy outer coating
- cell wall: tougher middle layer
- cell membrane: delicate inner skin



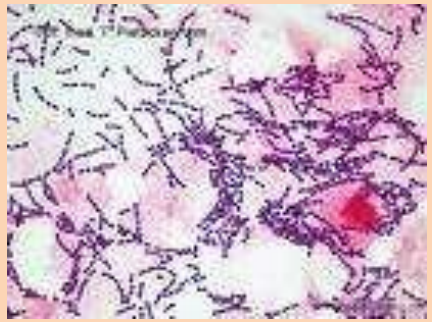
Prokaryote cells are simply built (example: E. coli)

- cytoplasm: inner liquid filling
- DNA in one big loop
- pilli: for sticking to things
- flagella: for swimming
- ribosomes: for building proteins



Prokaryote lifestyle

- unicellular: all alone
- colony: forms a film
- filamentous: forms a chain of cells



Prokaryote Feeding

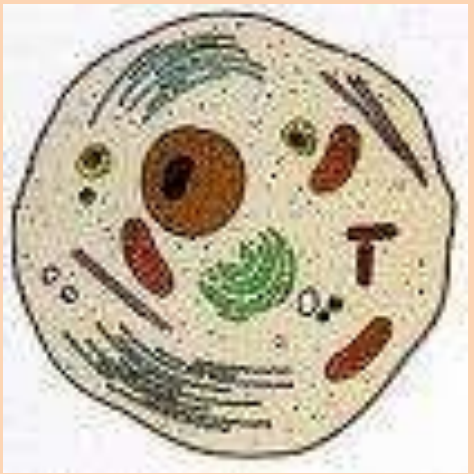
- Photosynthetic: energy from sunlight
- Disease-causing: feed on living things
- Decomposers: feed on dead things

Eukaryotes are bigger and more complicated

- Have **organelles**
- Have **chromosomes**
- can be **multi-cellular**
- include **animal** and **plant** cells

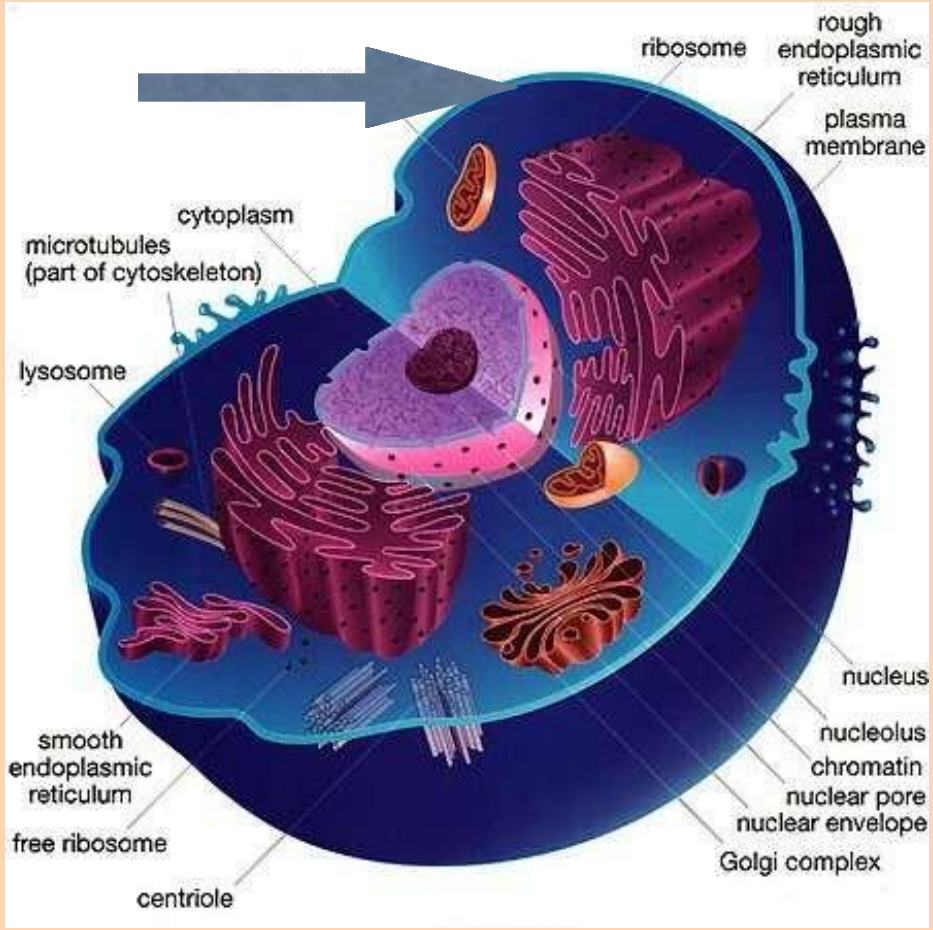
Organelles are membrane-bound cell parts

- Mini “organs” that have unique structures and functions
- Located in cytoplasm

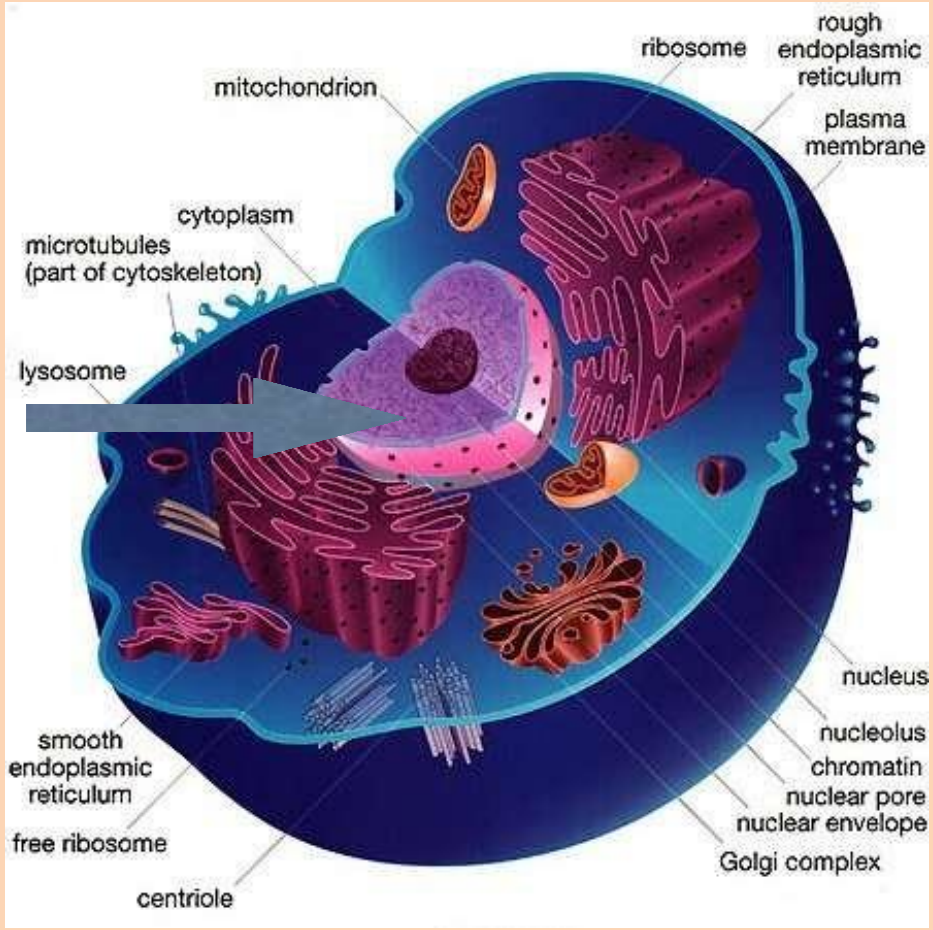


Cell Structures

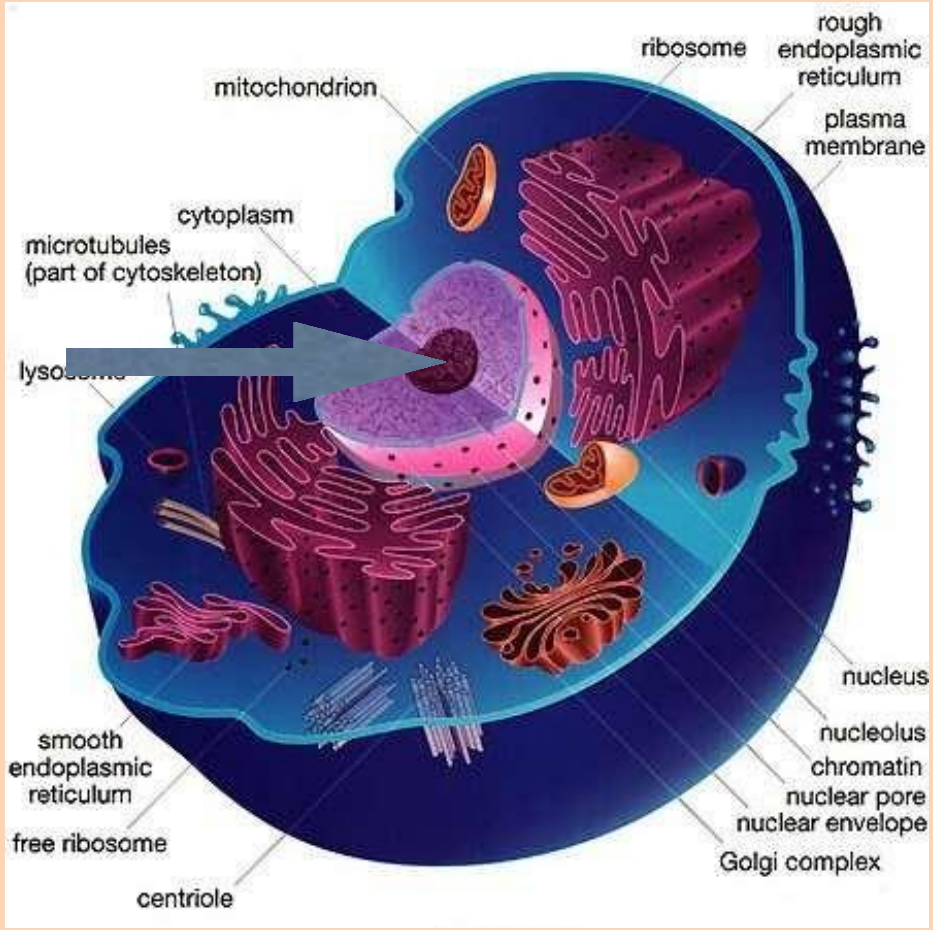
- Cell membrane
  - delicate lipid and protein skin around cytoplasm
  - found in <u>all</u> cells



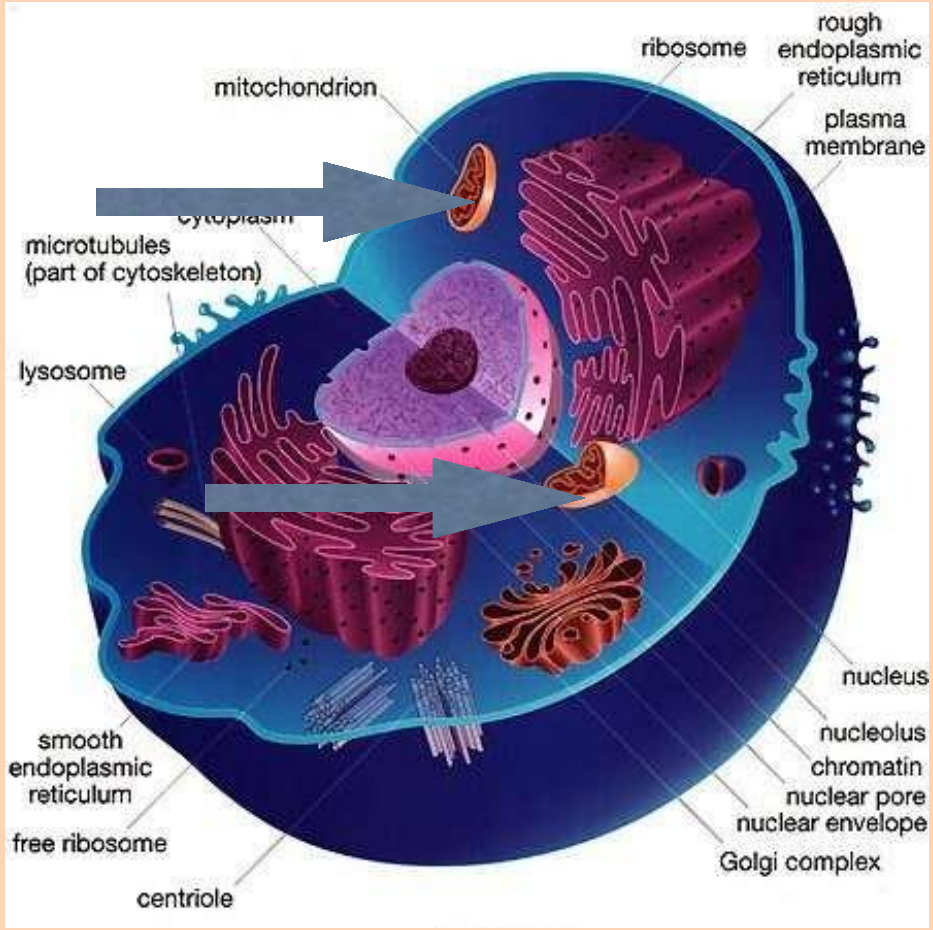
- Nucleus
  - a membrane-bound sac evolved to store the cell's chromosomes(DNA)
  - has pores: holes



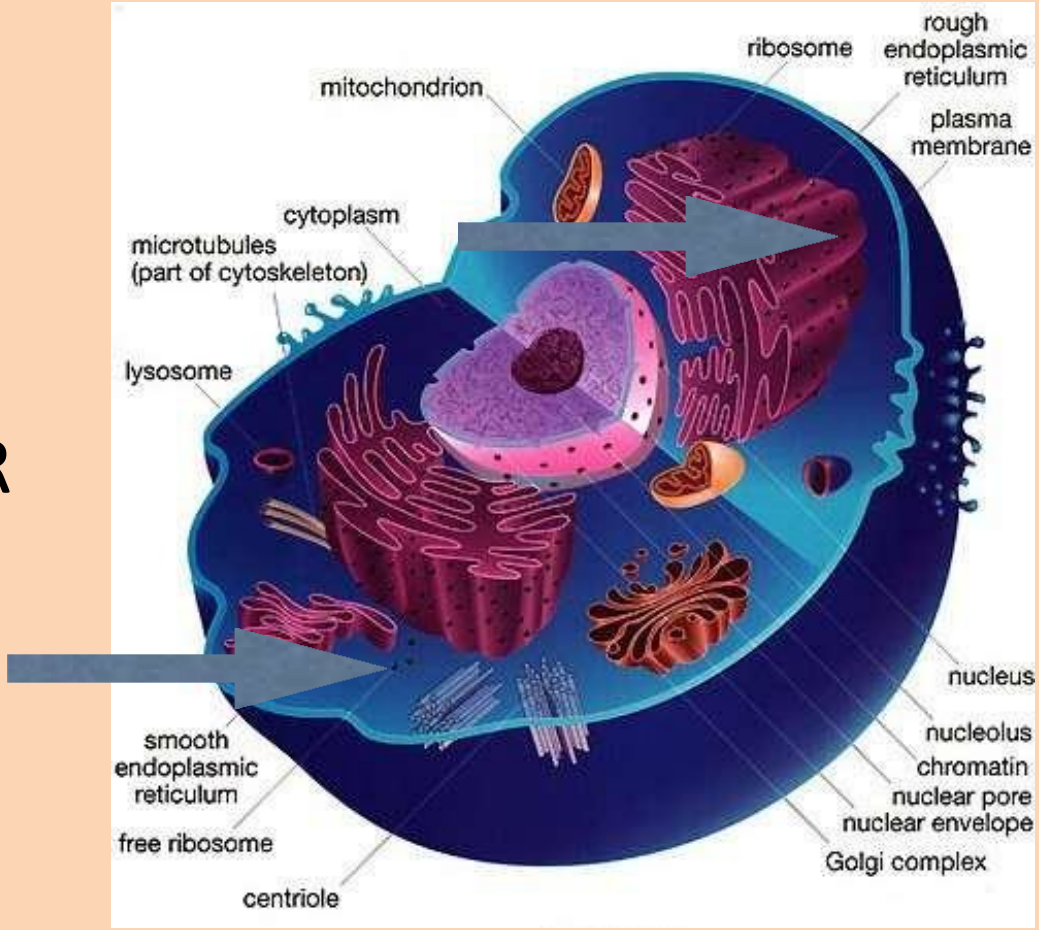
- Nucleolus
  - inside nucleus
  - location of ribosome factory
  - made or RNA



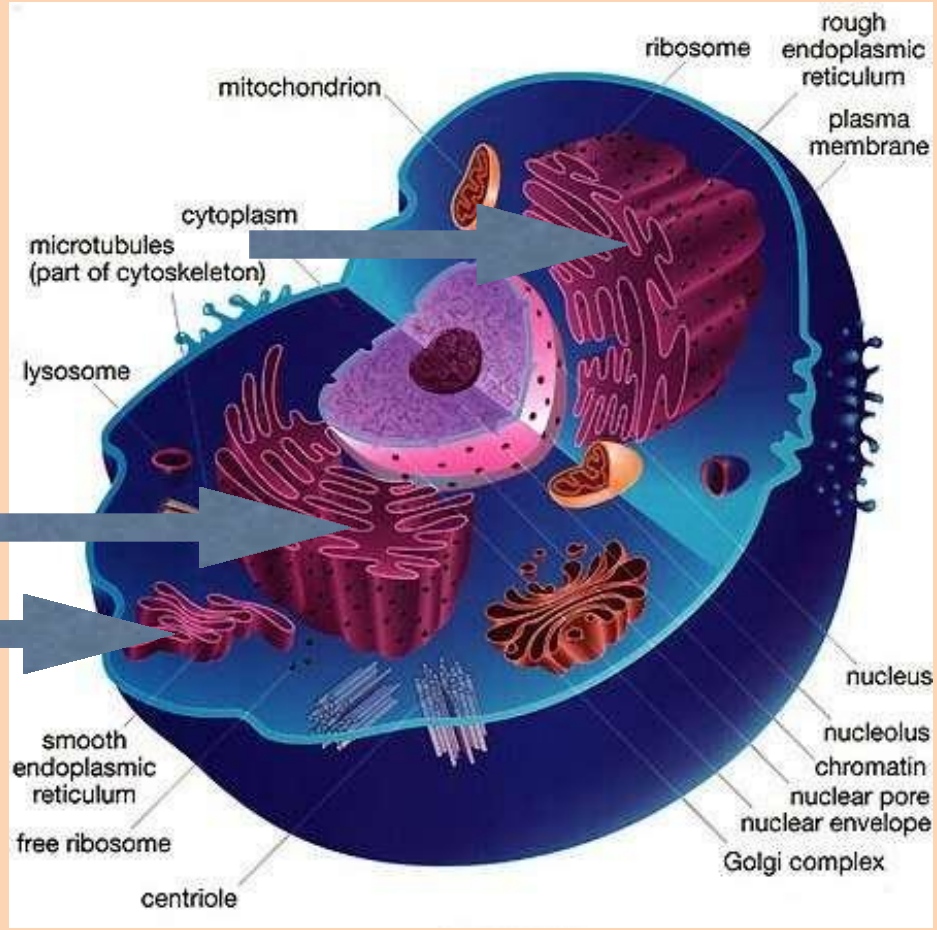
- **Mitochondrion**
  - makes the cell's energy
  - the more energy the cell needs, the more mitochondria it has



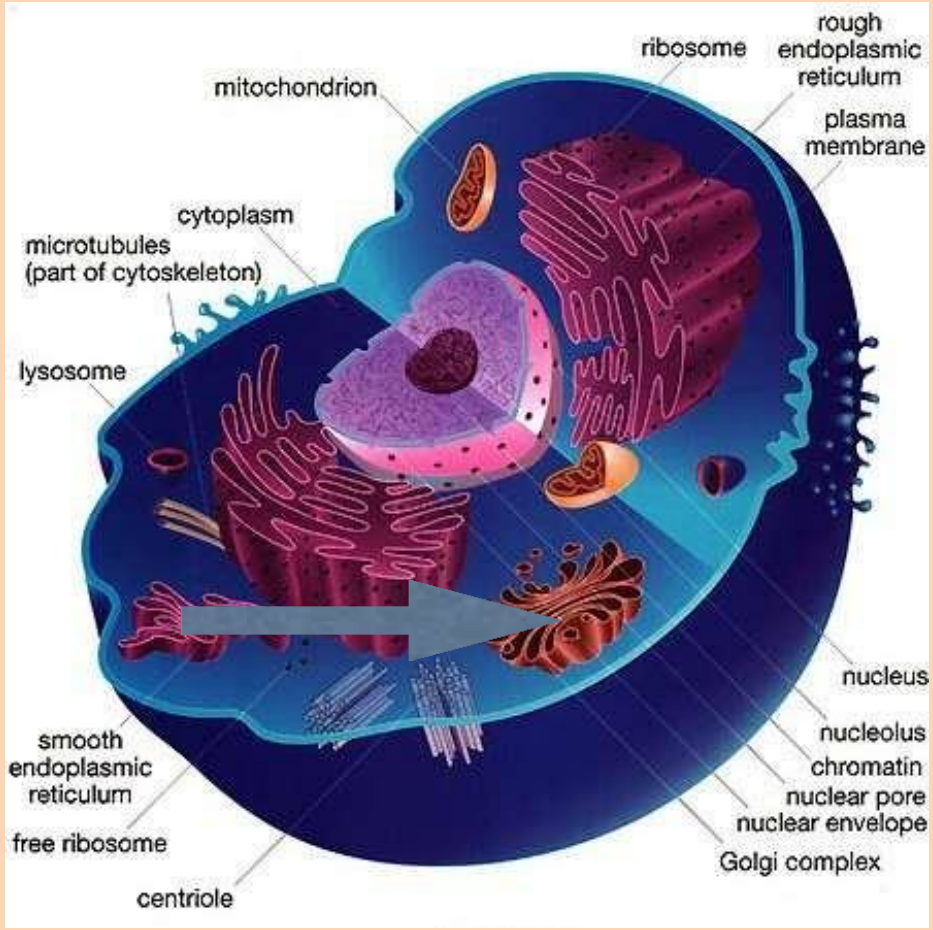
- Ribosomes
  - build proteins from amino acids in cytoplasm
  - may be free-floating, or
  - may be attached to ER
  - made of RNA



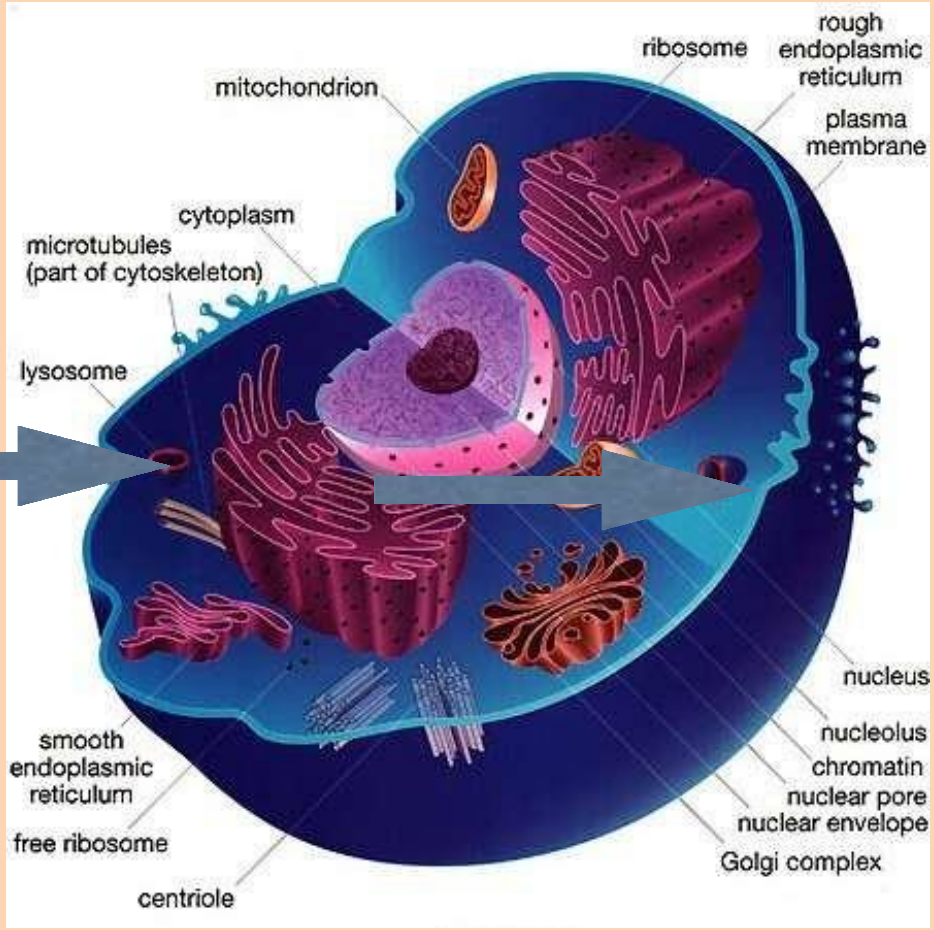
- Endoplasmic reticulum
  - may be smooth: builds lipids and carbohydrates
  - may be rough: stores proteins made by attached ribosomes



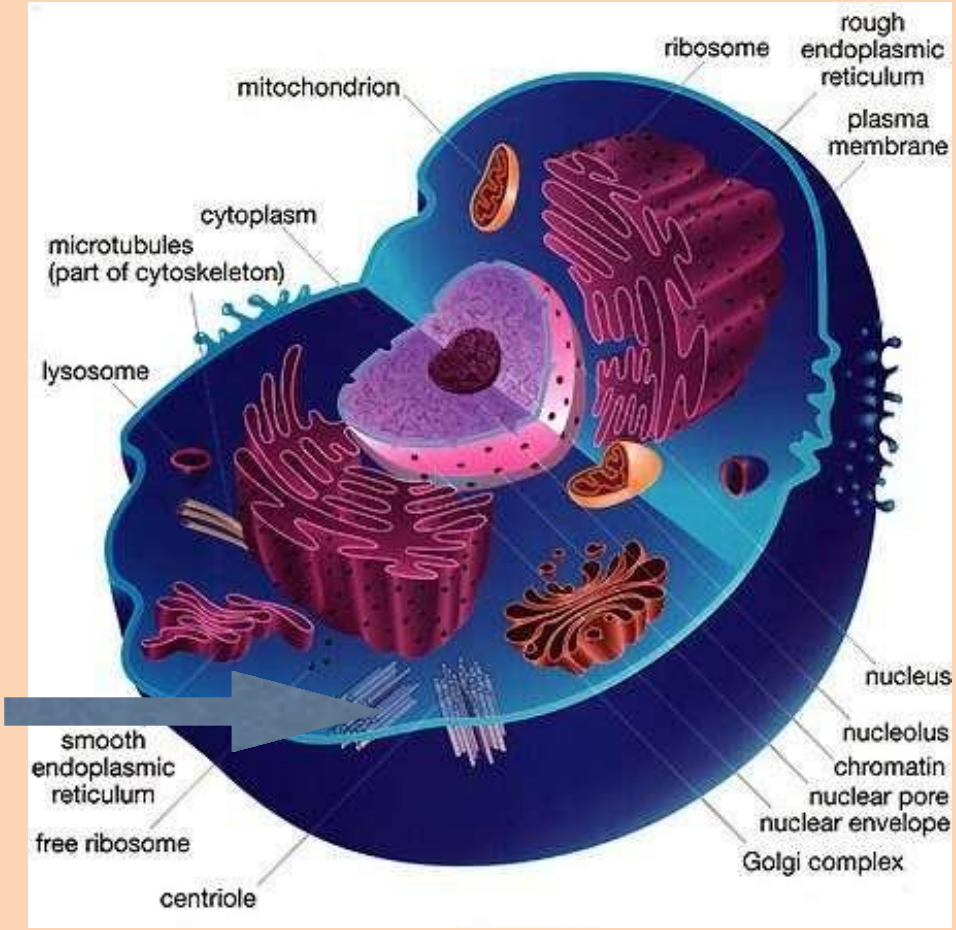
- Golgi Complex
  - takes in sacs of raw material from ER
  - sends out sacs containing finished cell products



- Lysosomes
  - sacs filled with digestive enzymes
  - digest worn out cell parts
  - digest food absorbed by cell

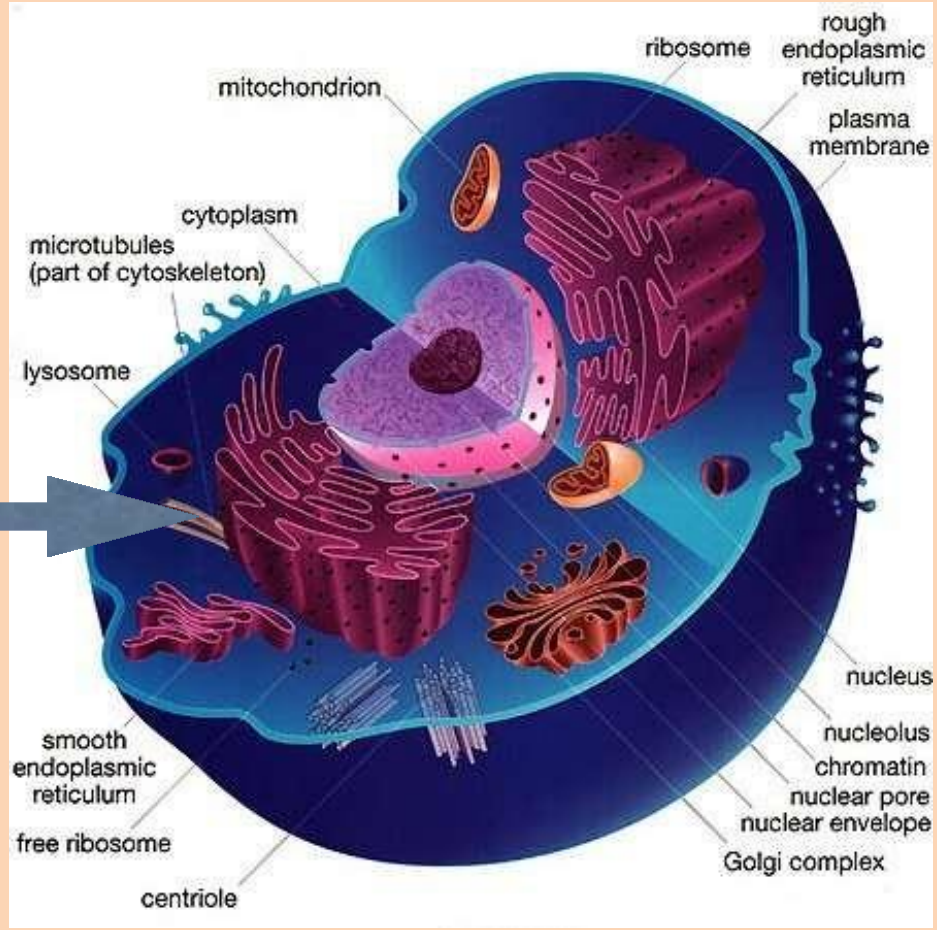


- Centrioles
  - pair of bundled tubes
  - organize cell division



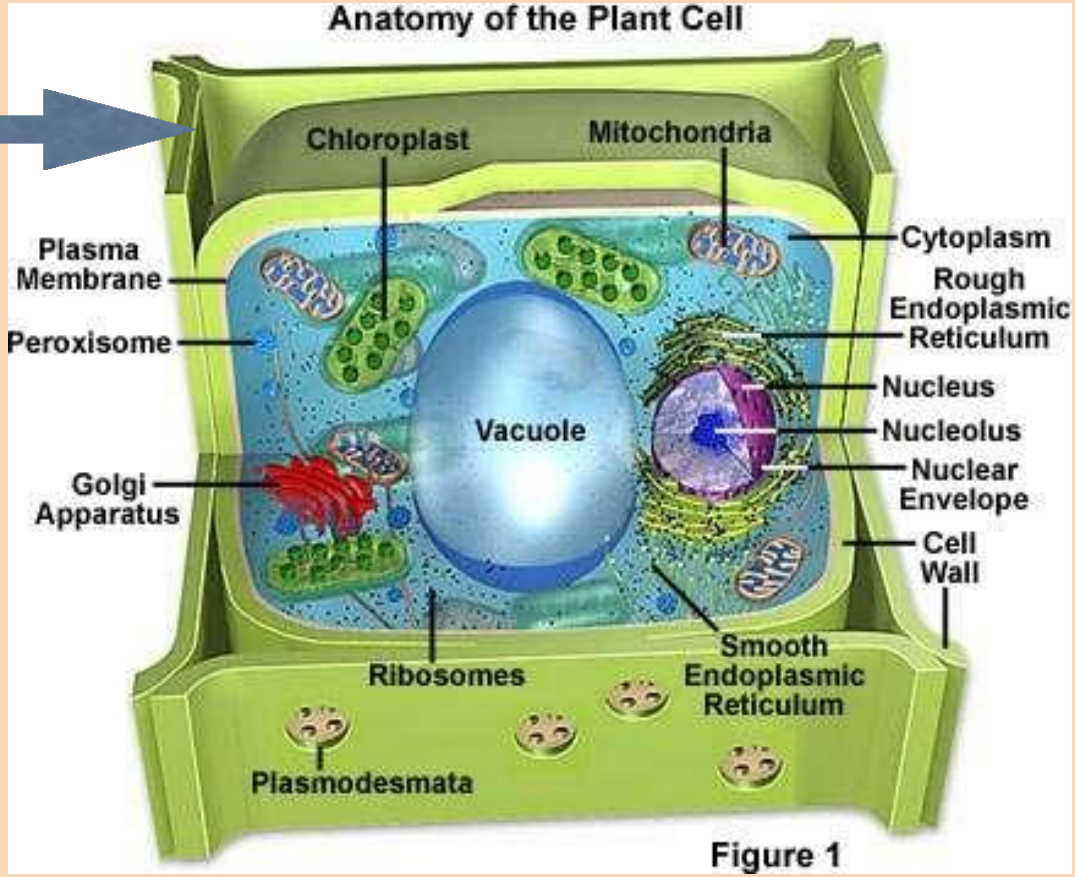
Cytoskeleton

- made of microtubules
- found throughout cytoplasm
- gives shape to cell & moves

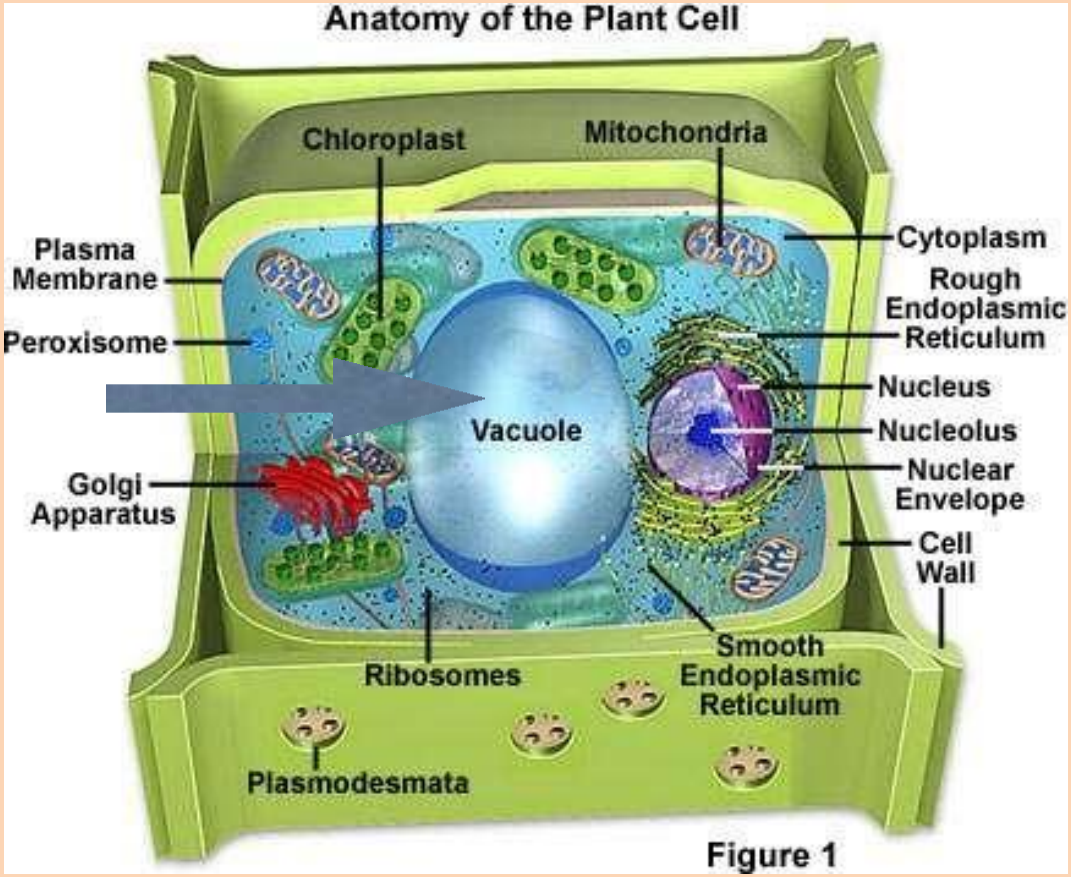


Structures found in plant cells

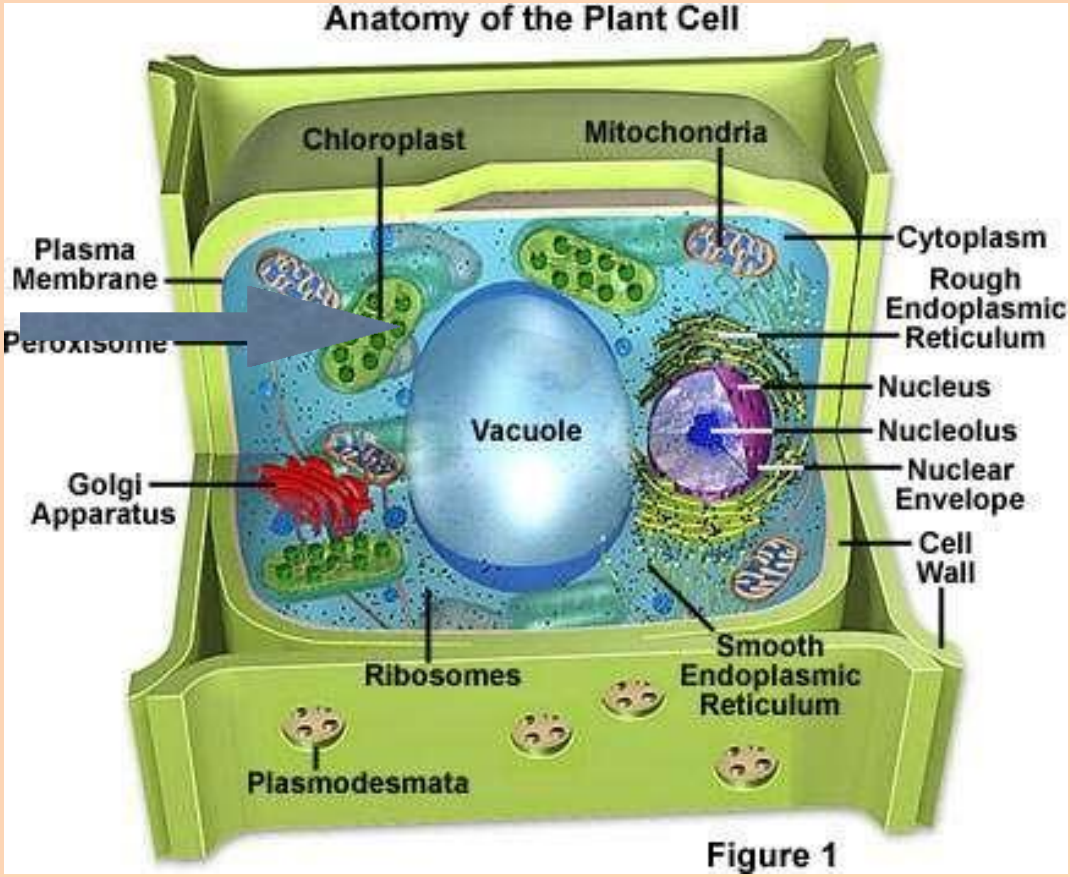
- Cell wall
  - very strong
  - made of cellulose
  - protects cell from rupturing
  - glued to other cells next door



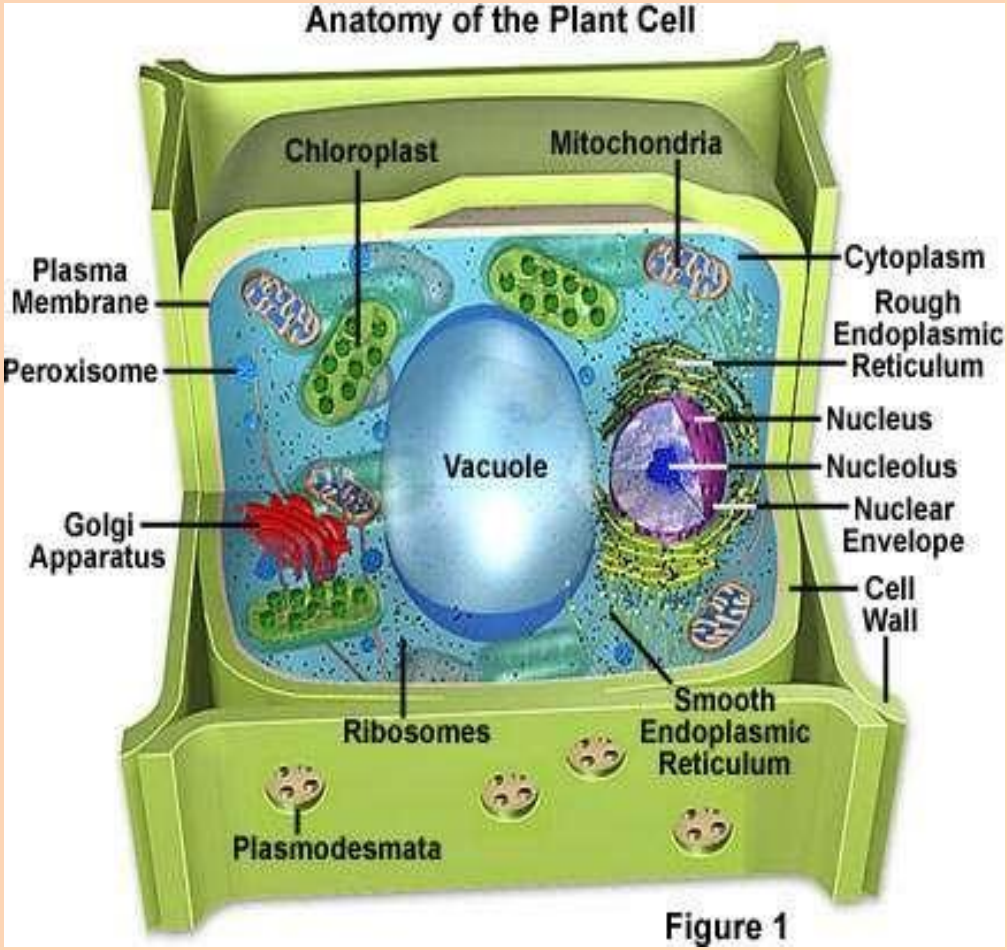
- Vacuole
  - huge water-filled sac
  - keeps cell pressurized
  - stores starch

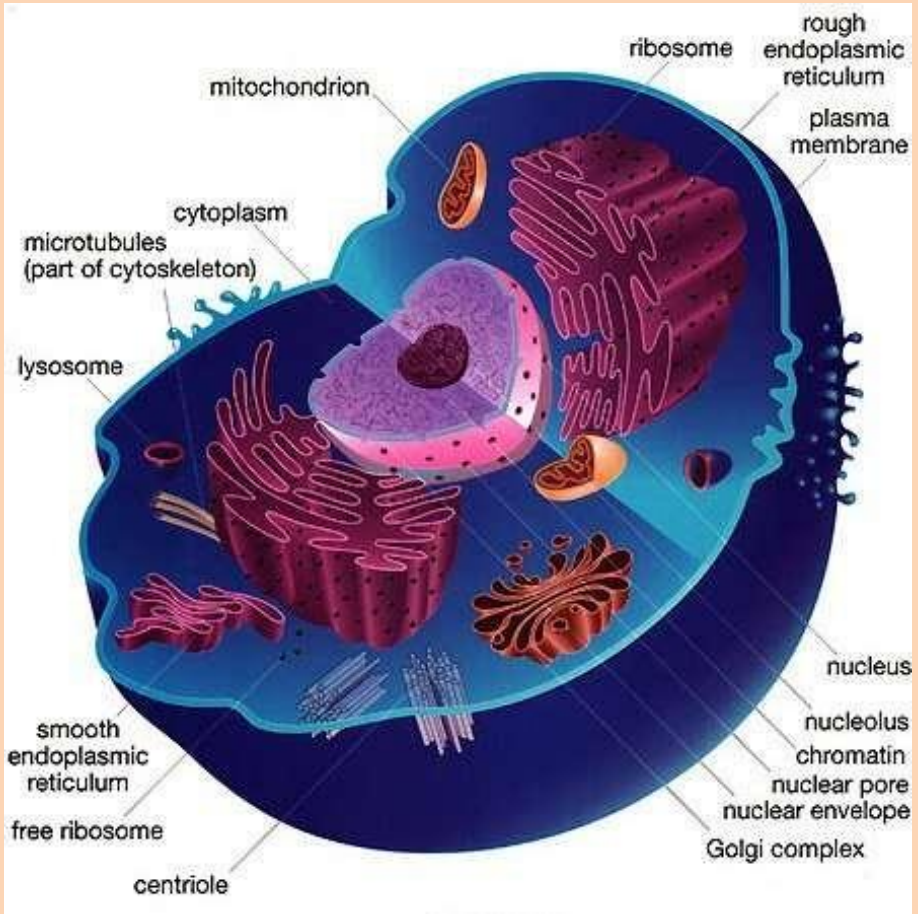


- Chloroplasts
  - filled with chlorophyll
  - turn solar energy into food energy



Difference between Animal & Plant Cell





Structure	Animal cells	Plant cells
cell membrane	Yes	yes
nucleus	Yes	yes
nucleolus	yes	yes
ribosomes	yes	yes
ER	yes	yes
Golgi	yes	yes
centrioles	yes	no
cell wall	no	yes
mitochondria	yes	yes
cholorplasts	no	yes
One big vacuole	no	yes
cytoskeleton	yes	Yes

Eukaryote cells can be multicellular

- The whole cell can be specialized for one job
- cells can work together as tissues
- Tissues can work together as organs

Advantages of each kind of cell architecture

Prokaryotes	Eukaryotes
simple and easy to grow	can specialize
fast reproduction	Multi-cellularity
all the same	can build large bodies

Scope of Microbiology
&
Spontaneous generation

Presented by
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Scope of Microbiology

Microbiology is the study of microscopic organisms that are either single-celled (unicellular), cell colony (multicellular) or acellular (lacking cells).

Microbiology includes many sub-disciplines like virology, mycology, parasitology and bacteriology.

Microbiologists can work in the area of food, pharmacy, agrochemistry biotechnology, biorefinery, environment, pollution control and bioremediation. In the field of agriculture, microbiologists act as environmental and health specialists to study the role of microbes in plant disease, pest control, nutrition and soil fertility.

BRANCHES OF MICROBIOLOGY

- Bacteriology
- Mycology
- Protozoology
- Phycology
- Virology
- Medical microbiology
- Pharmaceutical microbiology
- Industrial microbiology
- Food microbiology
- Agricultural microbiology
- Environmental microbiology
- Aero microbiology

HISTORY

- Antonie von leeuwenhoek -1674 observe microorganisms via microscope
- Ferdinand Cohn described several bacteria
- Louis pasteur –[1822-1895] father of microbiology
- Robrt Koch-[1843- 1910] father of medical microbiology
- Louis desinged vaccines & pasteurization techniques
- Koch is best known for his contributions to the germ theroy of diseases
- Martinus Beijerinck- [1851-1931] work on TMV and developed culturing methods for the cultivation of a wide range of microbes

- Sergal –[1885-1953] develop the concept of chemolithotrophy
- He was responsible for the isolaton & description of both nitrifying & non- nitrogen fixing bacteria
- Edward Jenner – developed vaccination against small pox
- Alexander flemming [1929] discovered penicillin
- F.Griffth demonstrated that the D.pneumoniae could be genetically modified
- In 1944 Avery identified the DNA as the transforming principle
- Bawden & Pirie showed that TMV is made up of nucleic acid and proteins only

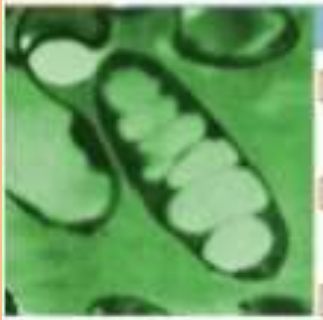
- 1881- Anthrax vaccine by Pasteur.
- 1882- Koch discovered cause of **TB**.
- 1884- **Autoclave** & Gram Stain.
- 1885- **Rabies** vaccine by Pasteur,
- Escherich discovered ***E. coli***
- 1887- Richard Julius Petri
- 1889- Beijerinick isolates root nodule bacteria & in1899- proves virus causes tobacco mosaic disease.

THE GOLDEN AGE
1857-1914

- 1857 - Pasteur described **fermentation.**
- 1861- Disproved spontaneous generation.
- 1867- Lister publishes on antiseptic surgery.
- 1876- Telephone
- 1877- Koch's postulates. (**germ theory**)
- 1879- Bulb
- 1880- Laveran discovered *Plasmodium* (**malaria**)

1903- Aircraft

- 1903- Antibodies
- 1911- Rous (viruses can cause cancer)
- 1914 WORLD WAR I
- 1915-17 -bacterial viruses by D'Herelle & Twort



- 1923: 1st edition of David Bergey's Manual
- 1928: Griffith's transformation
- 1931: Photosynthetic bacteria
- 1933: Ruska's electron microscope
- 1953: DNA double helix
- 1955: F factor plasmid (Jacod & Wollman)
- 1961: lac operon (Jacob & Monad)
- 1970: Amber & Smith (RE)
- 1977: Woese divided Procaryotes into Bacteria & Archaea
- 1980: STM & computers

- 1983-84: Mullis (PCR technique)
- 1995: Chicken pox vaccine approved(USA)
- 1997: Largest bacteria (*Thiomargarita namibiensis*)
- 2006: RNAi
- 2009: GFP

SPONTANIOUS GENERATION CONCEPT

- Proposed by greek philosopher aristotle
- According to him animals originate spontaneously from the soil, plants etc.
- His influence was still strongly felt in the 17 th centaury.
- Pasteur disproved this theory on April 1864. By simply using a flask
- When microbes were identified, this theory is totally eradicated.

Spontaneous Generation

- Before the 17th century, people believed that living things could come from nonliving things.
  - The Cell Theory has not been written.

Example: Rotting meat ➡ Maggot

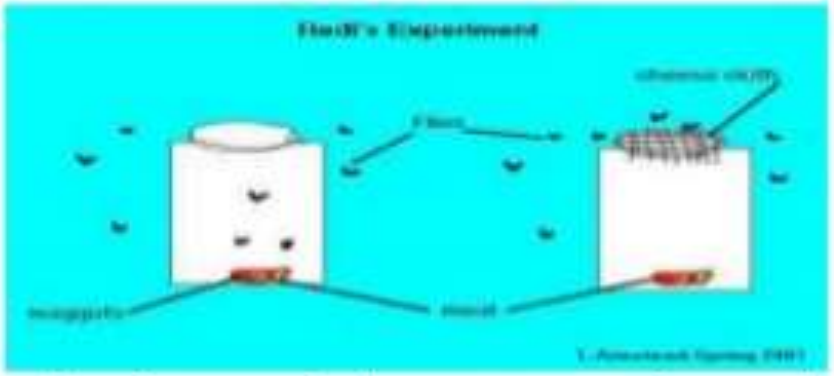
- **Biogenesis** – Living things come from other living things

Spontaneous Generation and Microorganisms

- The invention of microscopes allowed people to discover microorganisms.
- People believed these microorganisms developed spontaneously from the air.
- They believed the air had a life force that could create these bacteria.

Redi's Experiment

- Control group – Uncovered jar that contained meat
- Experimental group – Netting-covered jar
  - Allowed air to enter and prevented flies from landing on meat



- Results: Maggots seen in jar without netting, but not seen in jar with netting

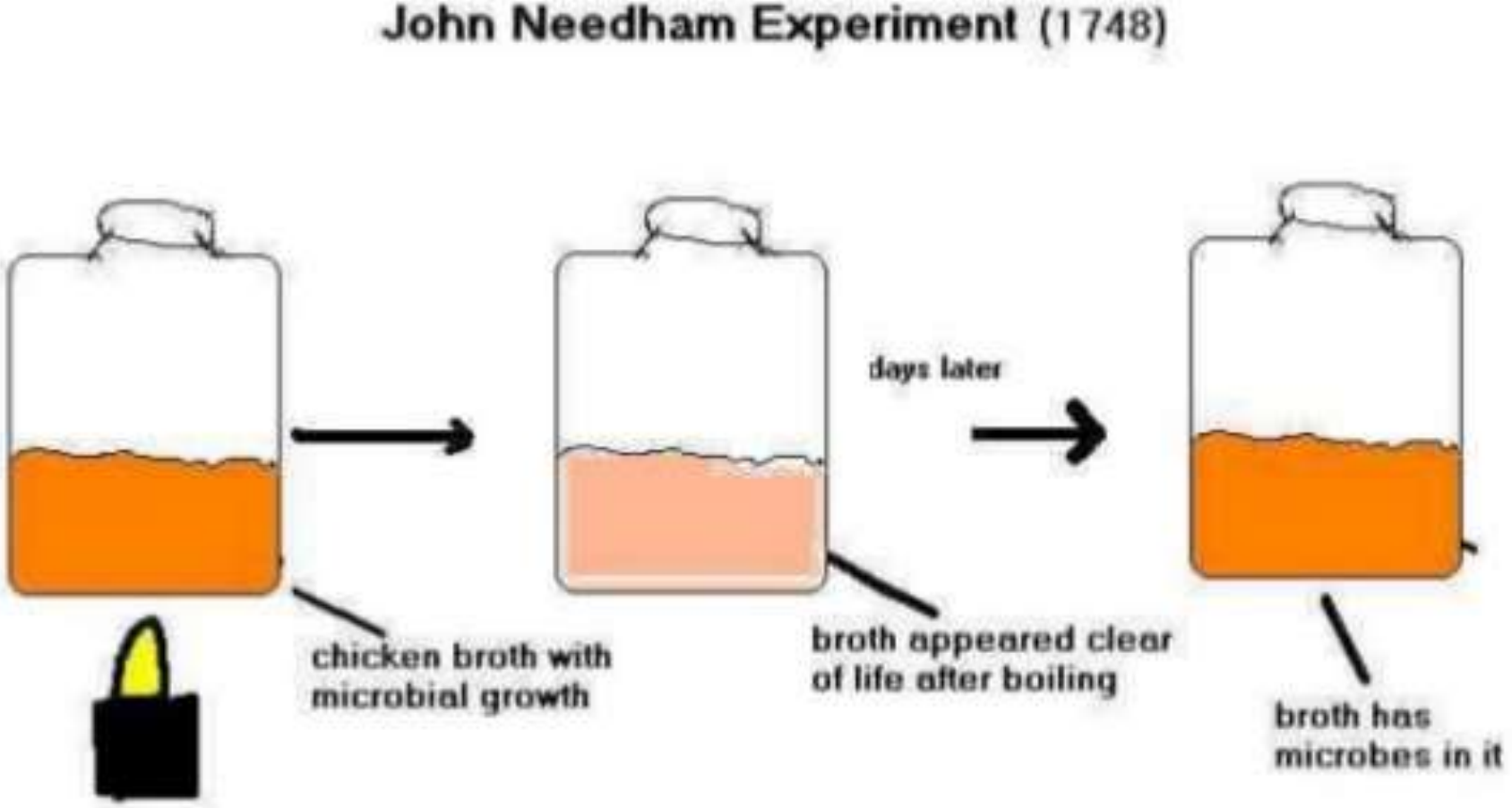
John Needham

- Created an experiment that was believed to **prove spontaneous generation**

Procedure:

- Briefly boiled a broth mixture in an open container and allowed it to cool to room temperature
- He would then later seal the containers
- Microorganisms would begin to appear a few days later, seeming to conclude that spontaneous generation was responsible for their growth

Needham's Experiment

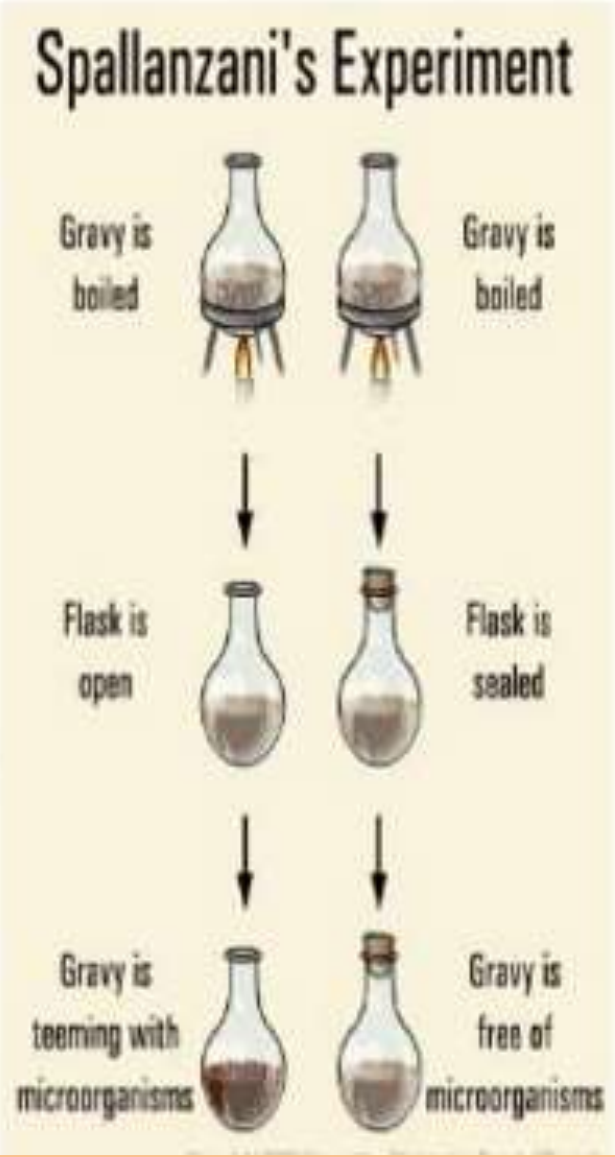


John Needham Continued

- Problem with his experiment:
- He left the flasks open to the air to cool so other bacteria could enter the container after the boiling

Spallanzani's Experiment

- Spallanzani attempted to refine Needham's experiment to disprove spontaneous generation.
- He performed basically the same experiment except with 2 differences: 1) He boiled the broth longer (to kill ALL of the microorganisms) and 2) He immediately capped off one of the containers.

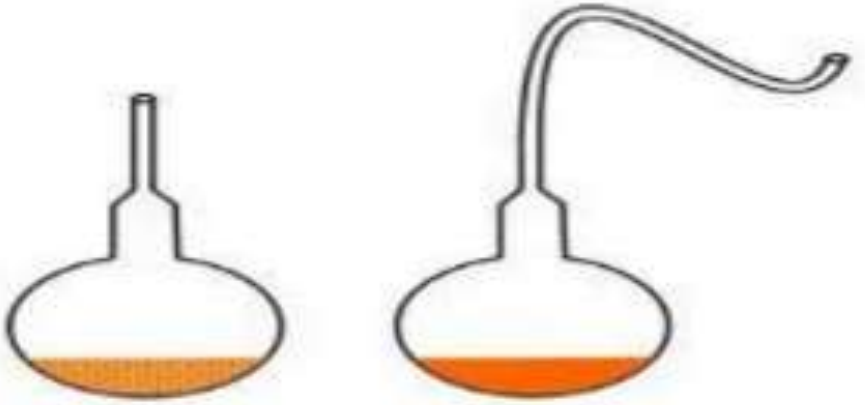


Critics of Spallanzani

- Spallanzani's results suggested that spontaneous generation was false because no bacteria grew in the closed flask while they did appear in the open flask.
- People who disagreed with Spallanzani argued that his experiment did not disprove spontaneous generation because he had "killed the life force" in the air by boiling it and then plugging the flask

Pasteur's Experiment

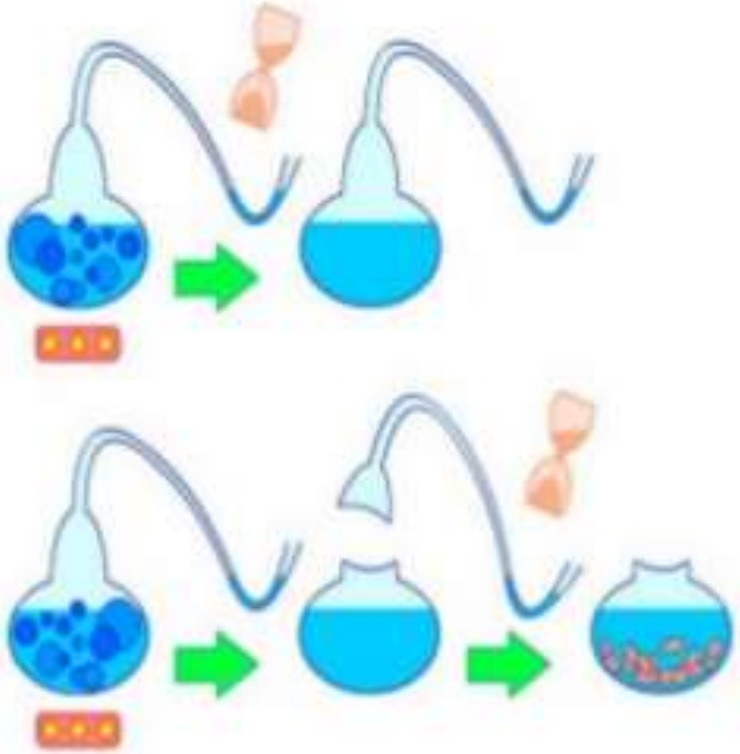
- Louis Pasteur created an experiment where broth from boiled meat was placed in a curve-necked flask.
  - Air could enter through the neck, but solid particles could not. (This was important because the "life force" would still be able to re-enter the flask after being boiled.)



Pasteur's Experiment: Cont'd

Results

- Broth boiled inside the flask remained clear for one year.
- After necks were broken off, broth became cloudy and contaminated with microorganisms after one day.



Conclusion

- Microorganisms come from other microorganisms (NO SPONTANEOUS GENERATION)

Conclusion

- Each of these experiments played a role in debate of spontaneous generation vs. biogenesis (all life comes from other life).
- From these experiments, it was proven that spontaneous generation was false and that biogenesis was true.

Questions

THANK YOU