FirstHope Notes | B.Pharm Semester 3

Introduction to Microbiology

Introduction to Microbiology

- Microbiology is the branch of science that deals with the study of microorganisms, which are microscopic organisms that exist as unicellular, multicellular, or cell clusters.
- These organisms include bacteria, archaea, viruses, fungi, prions, protozoa, and algae.
- Microbiology encompasses numerous sub-disciplines including virology, bacteriology, protistology, mycology, immunology, and parasitology.

History of Microbiology

A. Early Observations

- **Antonie van Leeuwenhoek (1632-1723):** Often considered the father of microbiology, Leeuwenhoek was the first to observe and describe microorganisms using a single-lens microscope of his own design. He referred to them as "animalcules."

B. The Golden Age of Microbiology (1850s-1910s)

- **Louis Pasteur (1822-1895):** Demonstrated that microorganisms cause fermentation and disease; disproved the theory of spontaneous generation; developed pasteurization.
- **Robert Koch (1843-1910):** Established Koch's postulates, criteria designed to establish a causative relationship between a microbe and a disease.

C. Modern Microbiology

- **Alexander Fleming (1881-1955):** Discovered penicillin, the first true antibiotic.
- **Development of Molecular Microbiology:** The advent of molecular biology techniques in the mid-20th century allowed for deeper understanding of microbial genetics and metabolism.

Branches of Microbiology

1. **Bacteriology**: Study of bacteria.
2. **Virology**: Study of viruses.
3. **Mycology**: Study of fungi.
4. **Parasitology**: Study of parasites.
5. **Phycology (Algology)**: Study of algae.
6. **Protozoology**: Study of protozoa.
7. **Immunology**: Study of the immune system.
8. **Microbial Ecology**: Study of microbes in their environment.

9. **Industrial Microbiology**: Application of microbes in industry.
10. **Medical Microbiology**: Study of pathogenic microbes and diseases.

Scope and Importance of Microbiology

1. Medical Applications

- **Disease Diagnosis and Treatment:** Identification of pathogens and development of antibiotics and vaccines.
- **Epidemiology:** Understanding the spread and control of diseases.

2. Environmental Impact

- **Biogeochemical Cycles:** Microorganisms play a crucial role in nutrient cycling, including carbon and nitrogen cycles.
- **Bioremediation:** Use of microorganisms to degrade environmental contaminants.

3. Industrial Uses

- **Fermentation Technology:** Production of alcohols, acids, and other chemicals.
- **Biotechnology:** Genetic engineering of microbes for the production of insulin, growth hormones, and other therapeutics.

4. Agricultural Benefits

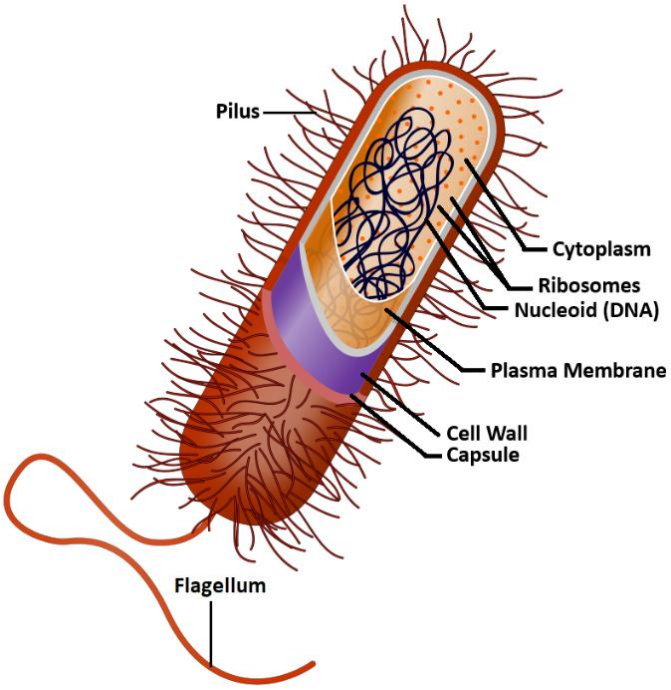
- **Soil Fertility:** Nitrogen-fixing bacteria improve soil health.
- **Biocontrol Agents:** Use of microbes to control pests and diseases.

5. Scientific Research

- **Model Organisms:** Microbes like *Escherichia coli* are used extensively in genetic and molecular biology research.

Introduction to Prokaryotes

- **Prokaryotes** are unicellular organisms that lack a defined nucleus and membrane-bound organelles.
- They are simpler and smaller than eukaryotic cells.



Characteristics of Prokaryotes:

Cell Structure:

1. **Nucleus:** No true nucleus; instead, they have a nucleoid region where the DNA is located.
2. **DNA:** Single, circular chromosome. Plasmids (small circular DNA molecules) may also be present.
3. **Cell Membrane:** Surrounded by a plasma membrane and often a rigid cell wall.
4. **Ribosomes:** Smaller (70S) than those in eukaryotic cells.
5. **Organelles:** Lack membrane-bound organelles. Functions are carried out in the cytoplasm or at the cell membrane.
6. **Size:** Smaller, typically 0.1-5.0 µm in diameter.

Reproduction:

- **Binary Fission:** Asexual reproduction where the cell divides into two genetically identical cells.

Examples:

- Bacteria (e.g., Escherichia coli, Streptococcus)
- Archaea (e.g., Methanogens, Halophiles)

Eukaryotes

- **Eukaryotes** are organisms whose cells contain a true nucleus and membrane-bound organelles.
- They can be unicellular or multicellular, and they exhibit a higher level of complexity than prokaryotic cells.

Characteristics of Eukaryotes:

1. Cell Structure:

1. **Nucleus:** Defined, membrane-bound nucleus containing the cell's genetic material.
2. **DNA:** Linear chromosomes within the nucleus.

3. **Cell Membrane:** Plasma membrane encloses the cell. In plants, fungi, and some protists, there is also a rigid cell wall.
4. **Ribosomes:** Larger (80S) than those in prokaryotic cells.
5. **Organelles:** Presence of membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and (in plants and some protists) chloroplasts.
6. **Size:** Larger, typically 10-100 µm in diameter.

2. Reproduction:

- **Mitosis and Meiosis:** Mitosis for asexual reproduction and growth; meiosis for sexual reproduction leading to genetic diversity.

3. Examples:

- Animals (e.g., Humans, Insects)
- Plants (e.g., Trees, Flowers)
- Fungi (e.g., Yeasts, Mushrooms)
- Protists (e.g., Amoeba, Algae)

Key Differences Between Prokaryotes and Eukaryotes

Feature	Prokaryotes	Eukaryotes
Nucleus	No true nucleus (nucleoid region)	Membrane-bound nucleus
DNA	Circular DNA	Linear chromosomes
Cell Size	Smaller (0.1-5.0 µm)	Larger (10-100 µm)
Cell Division	Binary fission	Mitosis and meiosis
Organelles	No membrane-bound organelles	Membrane-bound organelles present
Ribosomes	Smaller (70S)	Larger (80S)
Cell Wall	Present in most (made of peptidoglycan)	Present in plants and fungi (cellulose or chitin)
Complexity	Simpler structure	More complex structure
Reproduction	Asexual reproduction (binary fission)	Asexual (mitosis) and sexual (meiosis)
Examples	Bacteria, Archaea	Animals, Plants, Fungi, Protists

Ultra-structure of Bacteria

- Bacteria are single-celled, prokaryotic microorganisms that are found in virtually every environment on Earth.
- They belong to the domain Bacteria, one of the two domains of prokaryotes, the other being Archaea.
- Bacteria are among the earliest forms of life, appearing about 3.5 billion years ago.
- The ultra-structure of bacteria refers to the detailed structure of bacterial cells as observed under an electron microscope.
- This allows for the visualization of cellular components that are not visible with a light microscope.

Importance of Bacteria

1. Ecological Role:

- Bacteria play crucial roles in nutrient cycling, such as nitrogen fixation, decomposition, and bioremediation.

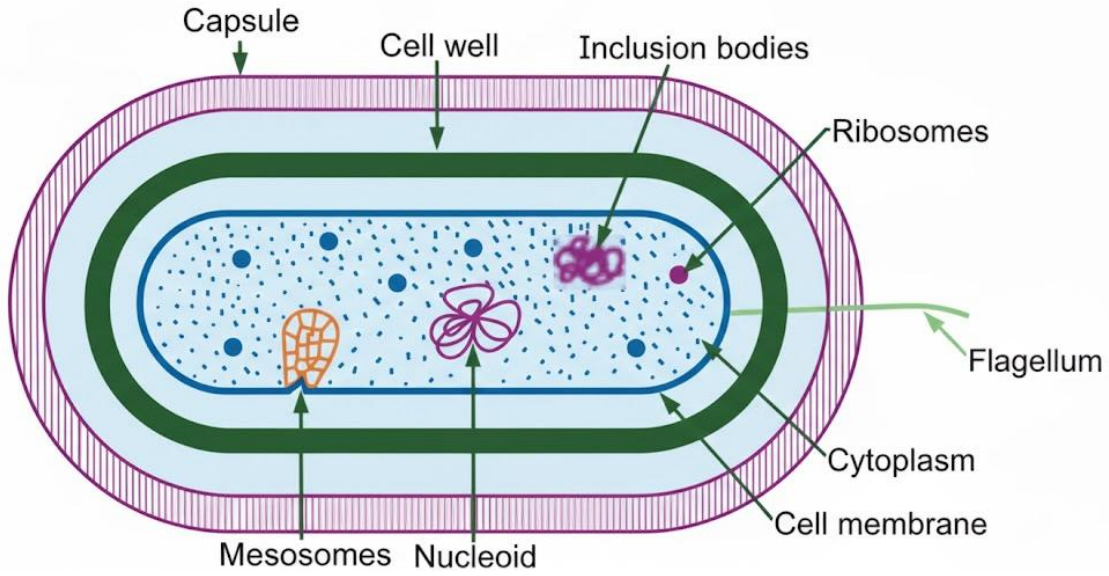
2. Human Health:

- While some bacteria cause diseases, many are beneficial for human health, aiding in digestion and synthesizing essential vitamins.

3. Industrial Applications:

- Bacteria are used in the production of antibiotics, fermentation processes (like yogurt and cheese making), and biotechnology (such as genetic engineering).

Key Components of Bacterial Ultra-structure:



1. Cell Envelope:

A. Cell Wall: Provides shape and protection. Made of peptidoglycan in most bacteria.

i. **Gram-positive bacteria:** Thick peptidoglycan layer with teichoic acids.

ii. **Gram-negative bacteria:** Thin peptidoglycan layer, outer membrane with lipopolysaccharides (LPS).

B. **Cell Membrane (Plasma Membrane):** Phospholipid bilayer that controls the passage of substances in and out of the cell.

2. Cytoplasm:

A. **Nucleoid:** Region where the bacterial chromosome (circular DNA) is located.

B. **Ribosomes:** Sites of protein synthesis, smaller than eukaryotic ribosomes (70S).

C. **Plasmids:** Small, circular DNA molecules that replicate independently of the chromosome and can carry genes for antibiotic resistance, virulence factors, etc.

D. **Inclusion Bodies:** Storage granules for nutrients, waste products, or other substances.

3. Surface Structures:

A. **Capsule:** Gelatinous layer outside the cell wall, protects against phagocytosis and desiccation.

B. **Flagella:** Long, whip-like structures used for locomotion.

C. **Pili (Fimbriae):** Hair-like projections used for attachment to surfaces or other cells and in some cases for conjugation (transfer of genetic material between bacteria).

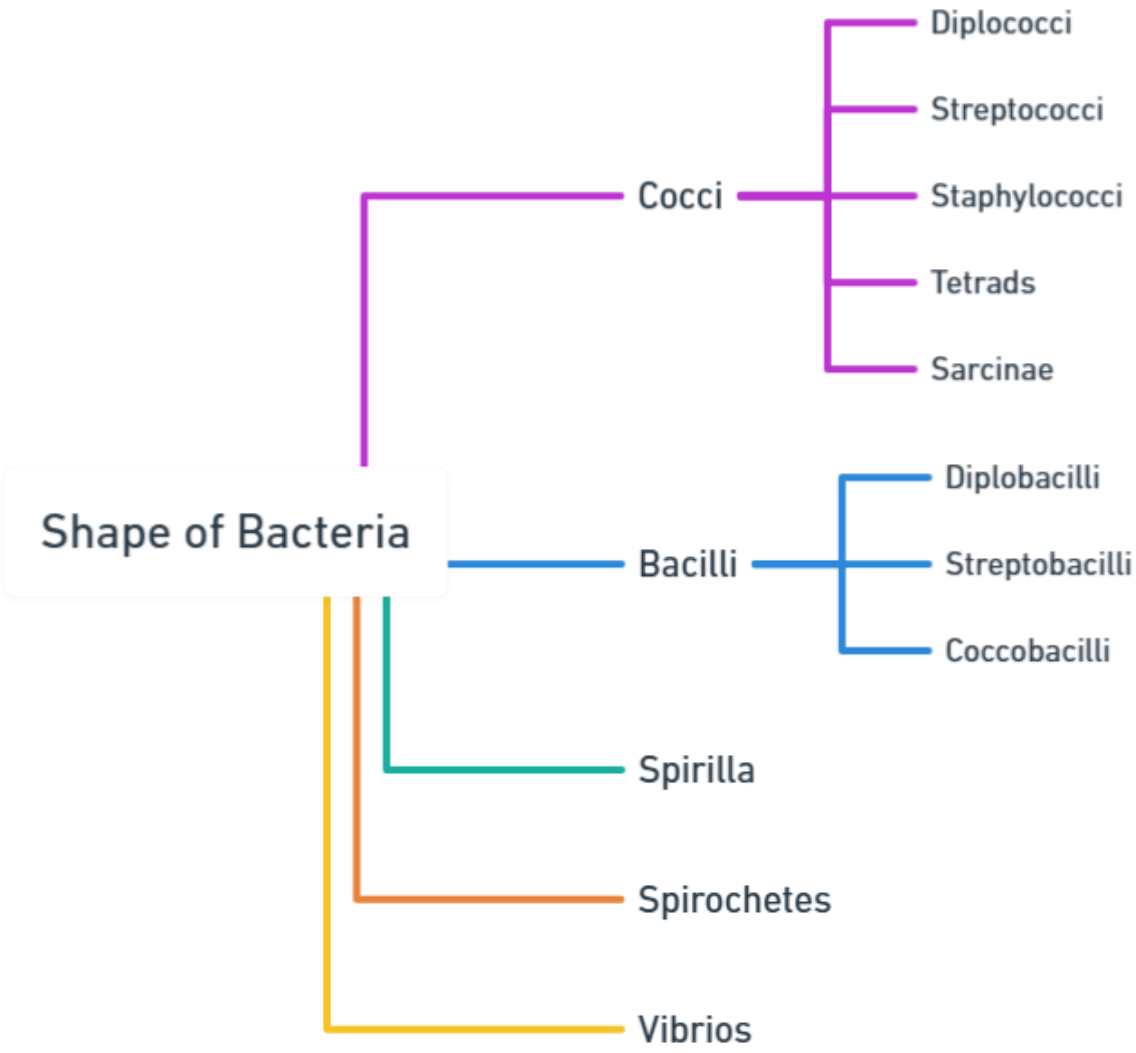
4. Endospores:

➢ Highly resistant structures formed by some Gram-positive bacteria (e.g., Bacillus, Clostridium) under adverse conditions to ensure survival.

Morphological Classification of Bacteria

- Bacteria are classified based on their morphology (shape), arrangement, staining properties, and other characteristics.
- Here are the main types of morphological classification:

Based on Shape:



I. **Cocci:** Spherical bacteria.
   - **Diplococci:** Pairs of cocci (e.g., Neisseria).
   - **Streptococci:** Chains of cocci (e.g., Streptococcus).
   - **Staphylococci:** Clusters of cocci (e.g., Staphylococcus).
   - **Tetrads:** Groups of four cocci (e.g., Micrococcus).
   - **Sarcinae:** Cubic configuration of eight cocci.

II. **Bacilli:** Rod-shaped bacteria.
   - **Diplobacilli:** Pairs of bacilli.
   - **Streptobacilli:** Chains of bacilli.
   - **Coccobacilli:** Oval and similar to cocci.

III. **Spirilla:** Spiral-shaped bacteria with rigid bodies.

IV. **Spirochetes:** Spiral-shaped bacteria with flexible bodies.

V. **Vibrios:** Comma-shaped bacteria (curved rods).

Based on Arrangement:

- **Diplococci:** Pairs of cocci.

- **Streptococci:** Chains of cocci.
- **Staphylococci:** Grape-like clusters.
- **Tetrads:** Groups of four cocci in a square.
- **Sarcinae:** Cubic configuration of eight cocci.

Based on Staining Properties:

A. Gram Stain:

- Differentiates bacteria into Gram-positive (purple) and Gram-negative (pink) based on cell wall composition.

B. Acid-fast Stain:

- Identifies acid-fast bacteria (e.g., Mycobacterium) that have waxy cell walls resistant to Gram staining.

C. Endospore Stain:

- Highlights the presence of endospores in some bacteria.

Based on Other Morphological Features:

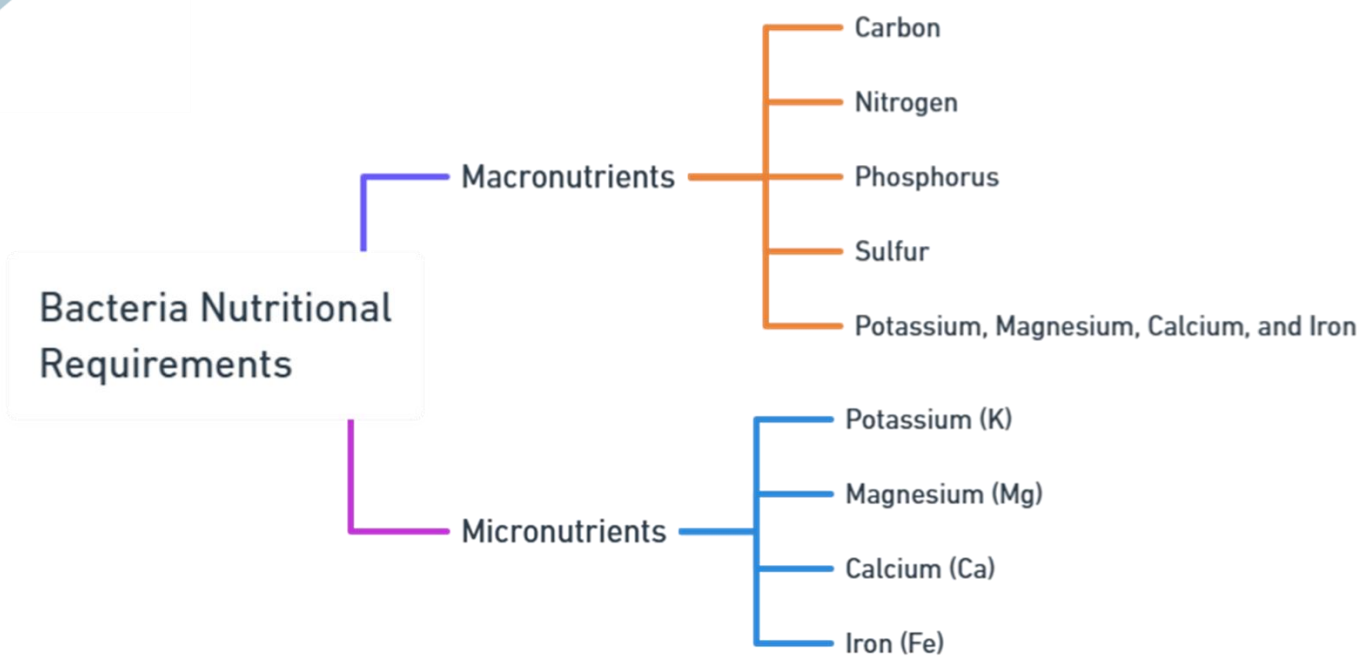
A. **Size:** Varies significantly between species.

B. **Motility:** Presence and arrangement of flagella (monotrichous, lophotrichous, amphitrichous, peritrichous).

C. **Colony Morphology:** Appearance of bacterial colonies on agar plates, including size, shape, color, texture, and hemolysis on blood agar.

Nutritional Requirements of Bacteria

- Bacteria, like all living organisms, require a variety of nutrients to grow and reproduce.
- These nutrients can be categorized into macronutrients and micronutrients.



Macronutrients:

A. Carbon:

- Fundamental for cellular structure and energy. Sources include carbohydrates, lipids, proteins, and carbon dioxide.

B. Nitrogen:

- Essential for amino acids, nucleotides, and other cellular constituents. Sources include ammonia, nitrate, nitrogen gas (for nitrogen-fixing bacteria), and organic nitrogen compounds.

C. Phosphorus:

- Required for nucleic acids, phospholipids, ATP. Common source is phosphate.

D. Sulfur:

- Important for amino acids (cysteine and methionine) and coenzymes. Sources include sulfate and sulfur-containing amino acids.

E. Potassium, Magnesium, Calcium, and Iron:

- Serve various roles in enzyme activation, cellular structure, and electron transport.

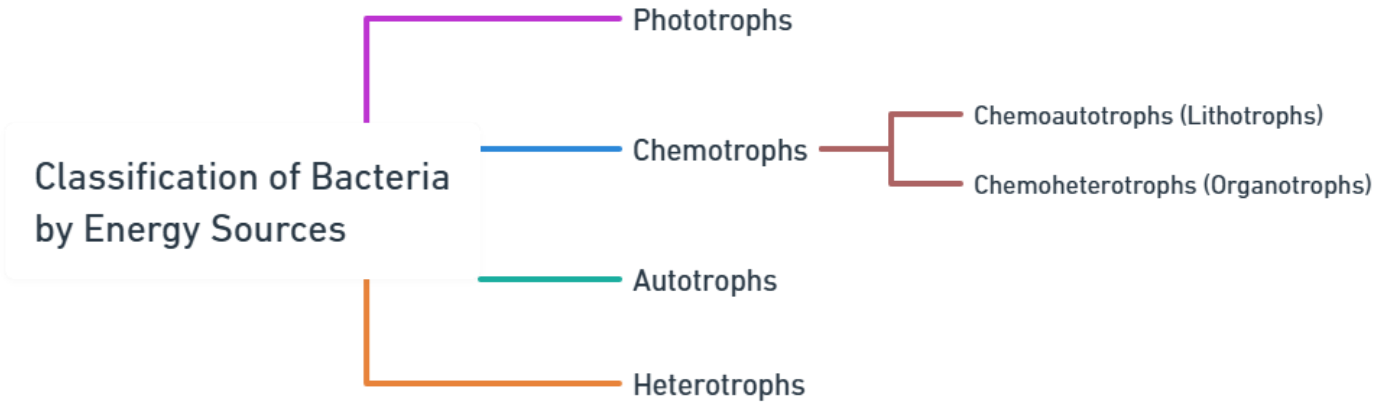
Micronutrients

1. **Potassium (K)**: Enzyme activation and cellular functions.
2. **Magnesium (Mg)**: Stabilizes ribosomes, membranes, and nucleic acids; involved in enzyme reactions.
3. **Calcium (Ca)**: Important for cell wall stability and spore formation.
4. **Iron (Fe)**: Essential for electron transport proteins and enzymes.

Growth Factors

- **Vitamins**: Often function as coenzymes in metabolic pathways.
- **Amino Acids**: Required for protein synthesis.
- **Purines and Pyrimidines**: Needed for nucleic acid synthesis.

Classification of Bacteria by Energy Sources



1. Phototrophs:

- **Energy Source:** Light
- **Example:** Cyanobacteria (photosynthetic bacteria that use light energy to produce organic compounds).

2. Chemotrophs:

- **Energy Source:** Chemical compounds
- **Subtypes:**

A. Chemoautotrophs (Lithotrophs):

- **Energy Source:** Inorganic chemicals (e.g., hydrogen sulfide, ammonia)
- **Example:** Nitrosomonas (oxidizes ammonia to nitrite)

B. Chemoheterotrophs (Organotrophs):

- **Energy Source:** Organic compounds
- **Example:** Escherichia coli (uses glucose and other organic molecules for energy).

3. Autotrophs:

- **Carbon Source:** Carbon dioxide
- **Example:** Nitrifying bacteria (convert ammonia into nitrates, using carbon dioxide for their carbon source).

4. Heterotrophs:

- **Carbon Source:** Organic compounds
- **Example:** Lactobacillus (uses organic molecules like lactose as both carbon and energy sources).

Type	Energy Source	Carbon Source	Example
Phototrophs	Light	CO2 or organic	Cyanobacteria (photoautotroph)
Chemotrophs	Inorganic/Organic chemicals	CO2 or organic	Nitrosomonas (chemoautotroph)
Chemoautotrophs	Inorganic chemicals	CO2	Thiobacillus
Chemoheterotrophs	Organic compounds	Organic compounds	Escherichia coli
Photoautotrophs	Light	CO2	Cyanobacteria
Photoheterotrophs	Light	Organic compounds	Purple non-sulfur bacteria

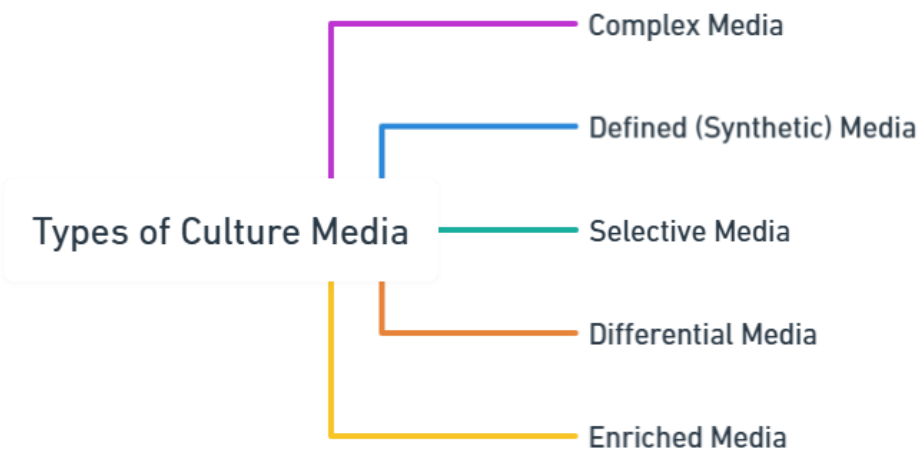
Raw Materials Used for Culture Media

- Culture media are essential for growing, isolating, and identifying microorganisms in the laboratory.
- The composition of these media can vary depending on the specific requirements of the microorganisms being cultured.
- Here are some of the common raw materials used in the preparation of culture media:

Basic Components:

1. **Peptones:** Hydrolyzed proteins providing amino acids and peptides.
2. **Yeast Extract:** Supplies vitamins, nitrogen, and other growth factors.
3. **Beef Extract:** Provides amino acids, peptides, nucleotides, organic acids, vitamins, and minerals.
4. **Agar:** A polysaccharide used as a solidifying agent in solid media.
5. **Carbohydrates:** Provide carbon and energy sources (e.g., glucose, lactose).
6. **Mineral Salts:** Provide essential ions (e.g., NaCl, K2HPO4, MgSO4).

Types of Culture Media:

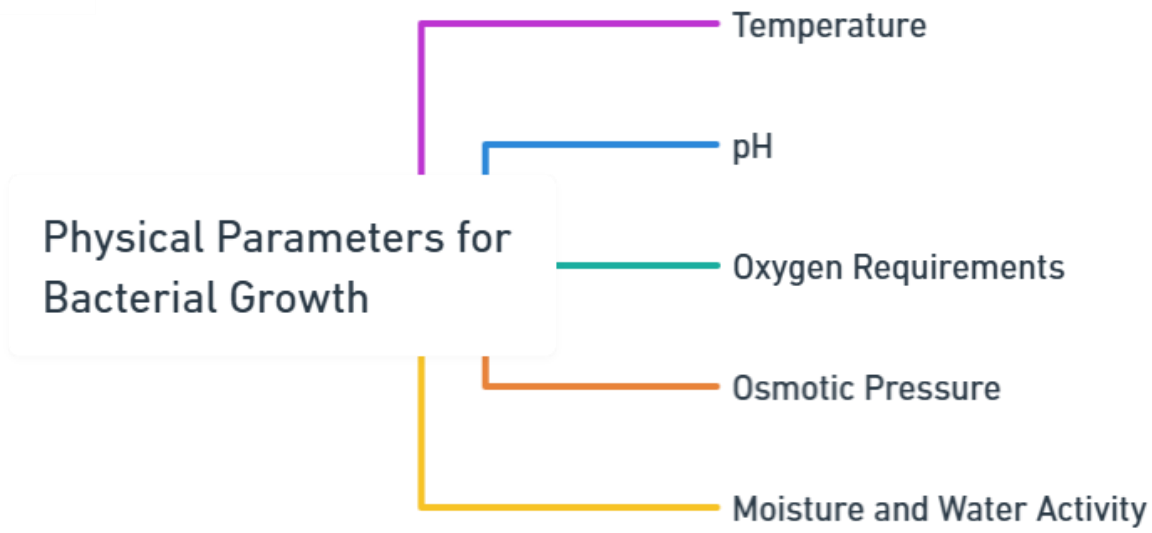


1. **Complex Media:**
   - **Description:** Contains a variety of ingredients like peptones, meat extracts, and yeast extracts, with unknown exact composition.
   - **Example:** Nutrient Agar, Tryptic Soy Broth.
2. **Defined (Synthetic) Media:**
   - **Description:** Composed of precise amounts of pure chemicals, with a known exact composition.
   - **Example:** Minimal Salt Media.
3. **Selective Media:**
   - **Description:** Contains specific agents to inhibit the growth of certain microbes while allowing others to grow.
   - **Example:** MacConkey Agar (selects for Gram-negative bacteria).
4. **Differential Media:**
   - **Description:** Includes indicators to differentiate between microbial species based on biological characteristics.
   - **Example:** Blood Agar (differentiates based on hemolysis).
5. **Enriched Media:**
   - **Description:** Supplemented with special nutrients to support the growth of fastidious organisms.
   - **Example:** Chocolate Agar.

Uses of Culture Media:

- **Isolation and Identification:** To isolate and identify microorganisms from samples.
- **Enumeration:** To count the number of microorganisms in a sample.
- **Sensitivity Testing:** To determine the susceptibility of microorganisms to antibiotics.
- **Research:** To study the physiology, genetics, and biochemical properties of microorganisms.
- **Industrial Production:** For the production of antibiotics, vaccines, and other microbial products.

Physical Parameters for Bacterial Growth



1. Temperature:

- Bacteria have different optimal temperature ranges for growth:
  - **Psychrophiles:** Grow at low temperatures (0-20°C).
  - **Mesophiles:** Grow at moderate temperatures (20-45°C), including most human pathogens.
  - **Thermophiles:** Grow at high temperatures (45-80°C).
  - **Hyperthermophiles:** Grow at extremely high temperatures (above 80°C).

2. pH:

- The pH of the environment affects bacterial enzyme activity and overall metabolism:
  - **Acidophiles:** Thrive in acidic environments (pH < 5.5).
  - **Neutrophiles:** Prefer neutral pH (5.5-8.0).
  - **Alkaliphiles:** Grow best in alkaline conditions (pH > 8.0).

3. Oxygen Requirements:

- Different bacteria have varying oxygen requirements for growth:
  - **Obligate Aerobes:** Require oxygen for growth.
  - **Facultative Anaerobes:** Grow with or without oxygen.
  - **Obligate Anaerobes:** Oxygen is toxic; grow in its absence.
  - **Microaerophiles:** Require reduced oxygen levels.
  - **Aerotolerant Anaerobes:** Do not use oxygen but can tolerate its presence.

4. Osmotic Pressure:

- Bacteria require specific osmotic conditions for optimal growth:
- **Halophiles:** Require high salt concentrations.

- **Osmotolerant:** Can tolerate a range of osmotic pressures.

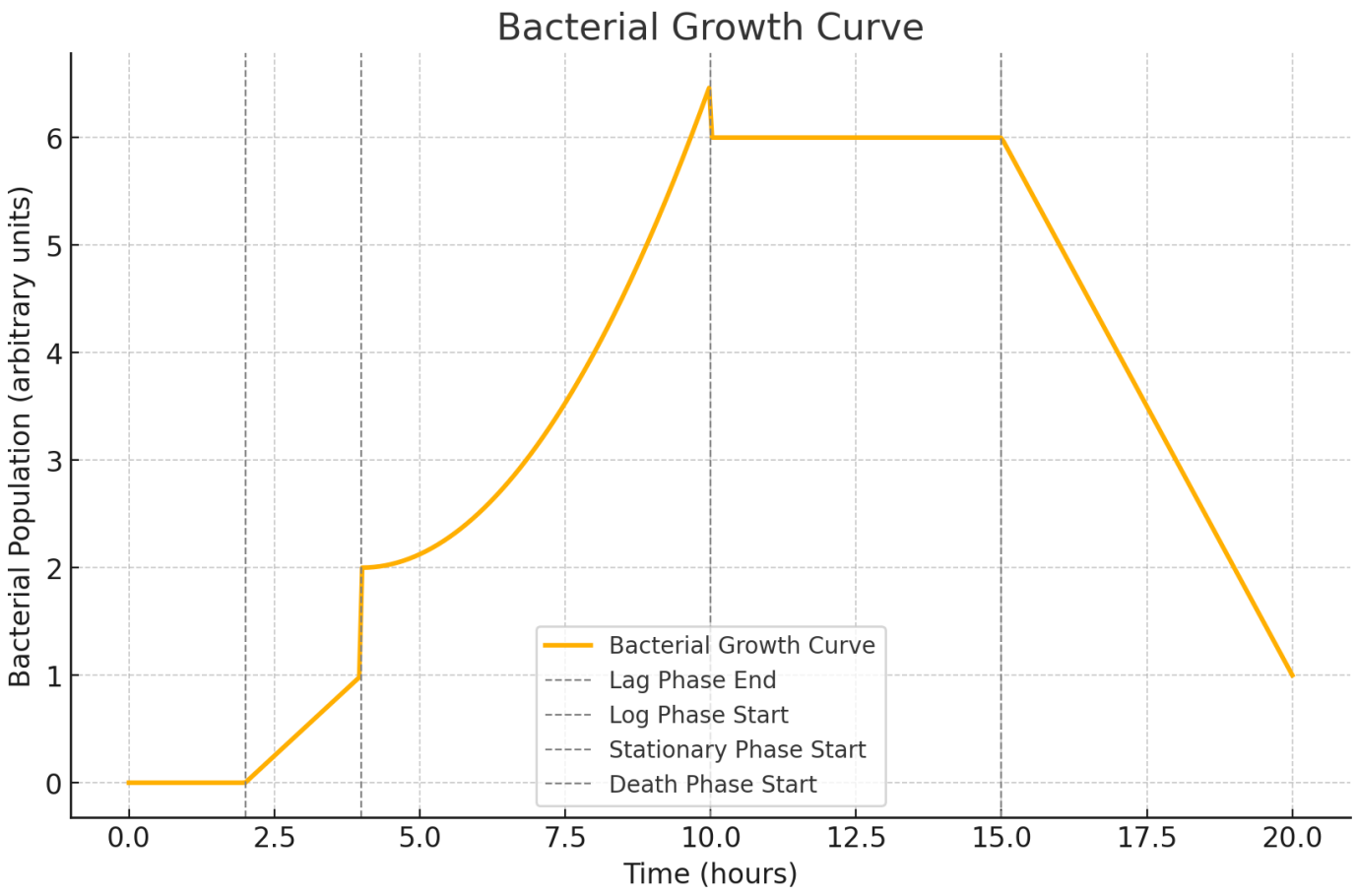
5. **Moisture and Water Activity:**
   - Water is essential for bacterial growth:
     - Bacteria need adequate moisture for nutrient uptake and waste elimination.
     - Desiccation can inhibit bacterial growth or cause dormancy.

Bacterial Growth Curve

- The bacterial growth curve represents the growth of a bacterial population over time in a closed system (batch culture).

It has four distinct phases:

1. **Lag Phase:**
   - Bacteria adapt to new environment.
   - Metabolic activity is high, but cell division is minimal.
   - Synthesis of enzymes, proteins, and other molecules needed for growth.
2. **Log (Exponential) Phase:**
   - Rapid cell division and exponential growth.
   - Population size doubles at a constant rate.
   - Nutrients are abundant, and metabolic activity is at its peak.
   - Generation time (time for the population to double) is calculated in this phase.
3. **Stationary Phase:**
   - Growth rate slows as nutrient depletion and waste accumulation occur.
   - Number of new cells equals the number of dying cells.
   - Metabolic activity continues, but at a reduced rate.
   - Secondary metabolites like antibiotics may be produced.
4. **Death (Decline) Phase:**
   - Nutrients are exhausted, and toxic waste products accumulate.
   - The number of dying cells exceeds the number of new cells formed.
   - The population declines at an exponential rate.



- The graph above illustrates the bacterial growth curve, showing the distinct phases:
    1. **Lag Phase**: Initial period where bacteria adapt to the environment.
    2. **Log (Exponential) Phase**: Rapid increase in population as cells divide at a constant rate.
    3. **Stationary Phase**: Growth rate slows as nutrients deplete and waste accumulates.
    4. **Death (Decline) Phase**: Bacterial cells die at an exponential rate due to unfavorable conditions.
- This representation highlights the dynamics of bacterial population growth in a closed system over time.

Isolation Methods for Pure Cultures

- Isolation of pure cultures involves separating a single species of microorganism from a mixed population.

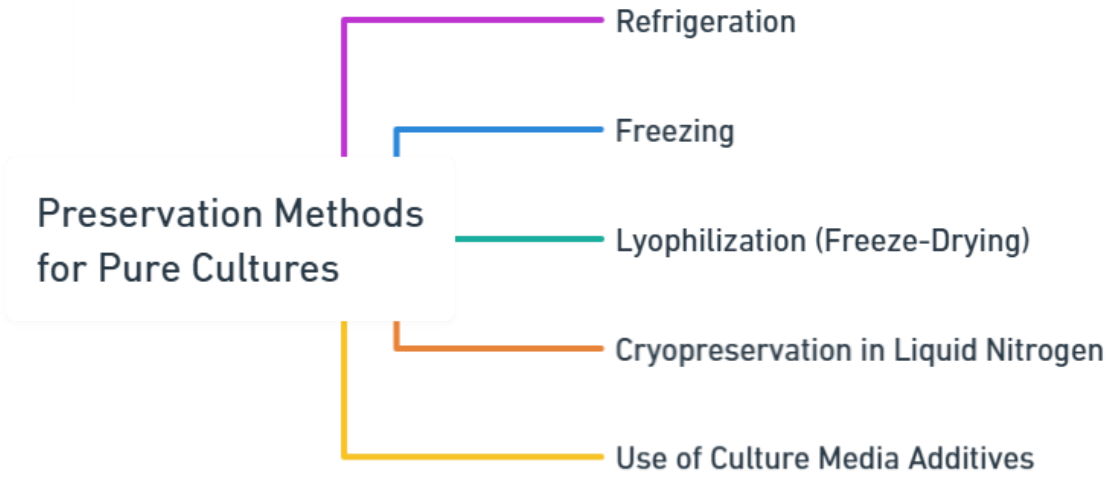
Common methods include:

1. **Streak Plate Method:**
   - **Procedure:** A sterile inoculating loop is used to spread a diluted sample over the surface of an agar plate in a series of streaks.
   - **Purpose:** To separate individual cells to form isolated colonies.
   - **Advantage:** Simple and effective for isolating bacteria from mixtures.
2. **Pour Plate Method:**
   - **Procedure:** A diluted microbial sample is mixed with molten agar and poured into a Petri dish. After solidification, colonies develop both on and within the agar.
   - **Purpose:** To isolate colonies from a mixed culture.
   - **Advantage:** Useful for counting colonies and isolating pure cultures.
3. **Spread Plate Method:**
   - **Procedure:** A diluted microbial sample is spread evenly across the surface of an agar plate using a sterile spreader.
   - **Purpose:** To obtain isolated colonies.
   - **Advantage:** Effective for quantifying and isolating microorganisms.
4. **Serial Dilution:**
   - **Procedure:** The microbial sample is serially diluted in a series of dilution blanks, and aliquots from each dilution are plated.
   - **Purpose:** To reduce the number of organisms and obtain isolated colonies.
   - **Advantage:** Reduces the concentration of bacteria to isolate pure cultures.

Preservation Methods for Pure Cultures

- Preserving pure cultures is essential for maintaining bacterial viability and genetic stability over extended periods.

Common preservation methods include:



1. Refrigeration

- **Temperature**: 4°C
- **Duration**: Short-term storage (weeks to months).
- **Procedure**: Cultures are stored on agar slants or in broth media in a refrigerator.
- **Advantages**: Simple and easy for short-term use.

2. Freezing

- **Temperature**: -20°C to -80°C
- **Duration**: Long-term storage (years).
- **Procedure**: Cultures are mixed with a cryoprotectant (e.g., glycerol) to prevent ice crystal formation and stored in a deep freezer.
- **Advantages**: Effective for long-term preservation with high viability.

3. Lyophilization (Freeze-Drying)

- **Procedure**: Cultures are frozen and then subjected to a vacuum to remove water by sublimation, leaving a dry powder.
- **Storage**: The powder is sealed in ampoules or vials and stored at room temperature or in a refrigerator.
- **Advantages**: Very long-term storage (decades), maintains viability and genetic stability.

4. Cryopreservation in Liquid Nitrogen

- **Temperature**: -196°C
- **Duration**: Very long-term storage (decades).
- **Procedure**: Cultures are suspended in a cryoprotectant and stored in liquid nitrogen.
- **Advantages**: Maintains high viability and genetic stability over very long periods.

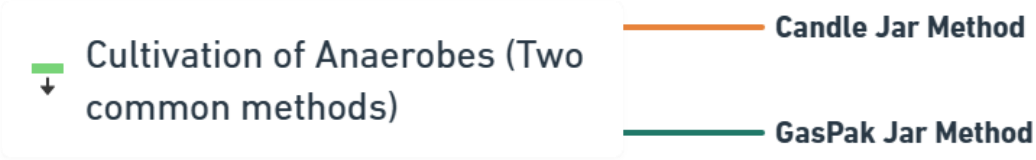
5. Use of Culture Media Additives

- **Procedure**: Addition of substances like glycerol, dimethyl sulfoxide (DMSO), or skim milk as protectants during freezing.

- **Goal**: Enhance survival rates during storage and thawing.
- **Advantages**: Increases the effectiveness of freezing methods.

Cultivation of Anaerobes

- Anaerobes are microorganisms that grow in the absence of oxygen.
- Cultivating anaerobes requires special techniques to create and maintain oxygen-free environments.
- Two common methods for cultivating anaerobes are the candle jar method and the GasPak jar method.



1. Candle Jar Method

- **Description:** A simple and inexpensive method to create a microaerophilic (low oxygen) environment.
- **Procedure:**
  - Place culture plates inside a large, airtight jar.
  - Add a lit candle to the jar and seal the lid.
  - The candle burns, consuming most of the oxygen and increasing the levels of carbon dioxide until the flame extinguishes due to oxygen depletion.
- **Applications:** Suitable for growing microaerophilic bacteria and some facultative anaerobes that require reduced oxygen levels but not complete anaerobiosis.
- **Advantages:** Easy to set up and inexpensive.
- **Disadvantages:** Does not achieve complete anaerobic conditions; only reduces oxygen levels.

2. GasPak Jar Method

- **Description:** A widely used method for creating an anaerobic environment using chemical reactions.
- **Procedure:**
  - Place culture plates or tubes inside the GasPak jar.
  - Add a GasPak sachet to the jar, which contains chemicals that, when activated, generate hydrogen and carbon dioxide gases.
  - Seal the jar tightly. The hydrogen gas reacts with oxygen in the presence of a palladium catalyst to form water, thus removing oxygen and creating an anaerobic atmosphere.

- An indicator strip is often used to confirm the absence of oxygen.

➢ **Applications:** Suitable for the cultivation of obligate anaerobes, facultative anaerobes, and microaerophiles.

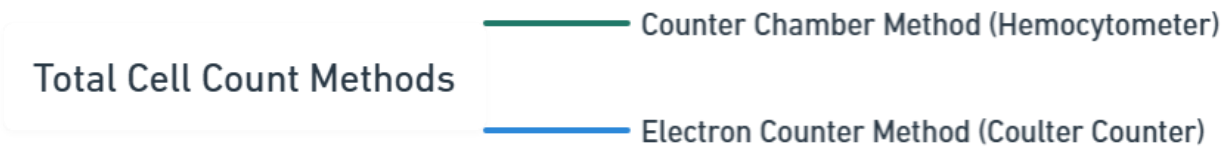
➢ **Advantages:** Reliable and produces a true anaerobic environment.

➢ **Disadvantages:** Requires the purchase of GasPak sachets and a specialized jar.

Quantitative Measurement of Bacterial Growth

- Quantifying bacterial growth involves measuring either the total cell count or the viable cell count.

Total Cell Count Methods



A. Counter Chamber Method (Hemocytometer)

➢ **Description**: A manual counting method using a specialized slide with a grid.

➢ **Procedure**:

1. A known volume of bacterial suspension is placed on the hemocytometer grid.
2. The grid is viewed under a microscope.
3. Cells within the grid areas are counted.
4. The total cell concentration is calculated based on the grid volume and dilution factor.

➢ **Advantages**: Simple, inexpensive, provides immediate results.

➢ **Disadvantages**: Cannot differentiate between live and dead cells, time-consuming, subject to human error.

B. Electron Counter Method (Coulter Counter)

➢ **Description**: An automated device that counts cells by measuring changes in electrical resistance.

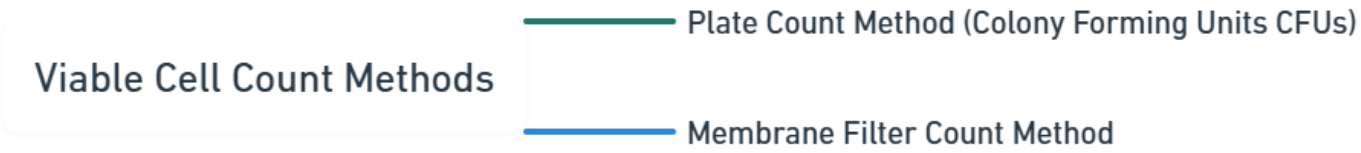
➢ **Procedure**:

1. The bacterial suspension is passed through a small aperture.
2. As cells pass through the aperture, they disrupt an electrical current, causing changes in resistance.
3. Each disruption is counted as a cell.

➢ **Advantages**: Rapid, automated, and precise.

- **Disadvantages**: Expensive equipment, cannot differentiate between live and dead cells, requires maintenance.

Viable Cell Count Methods



A. Plate Count Method (Colony Forming Units - CFUs)

- **Description**: A method to count viable bacteria by growing colonies on agar plates.
- **Procedure**:
  1. A known volume of a diluted bacterial suspension is spread on an agar plate.
  2. The plate is incubated to allow colony formation.
  3. Colonies are counted, and the number of viable cells is calculated based on the dilution factor.
- **Advantages**: Only counts viable cells capable of forming colonies, widely used, relatively simple.
- **Disadvantages**: Time-consuming (requires incubation), may underestimate cell count if cells clump together or are non-culturable under the given conditions.

B. Membrane Filter Count Method

- **Description**: A method to count viable bacteria in liquid samples by filtration.
- **Procedure**:
  1. A known volume of liquid sample is filtered through a membrane with a pore size small enough to retain bacteria.
  2. The membrane is placed on an agar plate or absorbent pad soaked with nutrient medium.
  3. The plate is incubated to allow colony formation.
  4. Colonies on the membrane are counted to calculate the viable cell concentration.
- **Advantages**: Useful for low bacterial concentrations, suitable for large volumes, allows for concentration of bacteria.
- **Disadvantages**: Time-consuming, requires filtration apparatus, may not capture all viable cells if they pass through the membrane or do not grow on the selected medium.

Phase Contrast Microscopy

- Phase contrast microscopy is a technique that enhances the visibility of transparent and colorless specimens by converting subtle differences in the optical path length (caused by variations in refractive index and thickness) into variations in contrast.
- This allows for the observation of live, unstained cells, making internal structures visible without damaging or altering the specimen.

Types of Phase Contrast Microscopy

1. Positive Phase Contrast

- Annular Diaphragm and Phase Plate Configuration:
  - An annular diaphragm creates a hollow cone of light that passes through the specimen.
  - A phase plate in the objective lens retards the direct light by a quarter wavelength.
  - This configuration results in a brighter image of the specimen against a dark background.

2. Negative Phase Contrast

- Reverse Phase Plate Configuration:
  - The phase plate advances the direct light by a quarter wavelength.
  - The specimen appears darker against a bright background, offering an inverse contrast effect.

Procedure for Phase Contrast Microscopy

1. Preparation of the Microscope:

- **Alignment:** Ensure the microscope is properly aligned, following Köhler illumination principles. Adjust the condenser and objective lenses for optimal performance.
- **Phase Contrast Components:** Install phase contrast objectives and matching condenser annuli, ensuring each objective lens corresponds to a specific annular ring in the condenser.

2. Specimen Preparation:

- **Live Cells:** Prepare a wet mount by placing a drop of the culture on a clean glass slide and covering it with a coverslip to create a thin, even layer suitable for observation.
- **Avoiding Stains:** Phase contrast microscopy relies on natural refractive differences, so staining is unnecessary and should be avoided.

3. Microscope Settings:

- **Adjusting the Annular Diaphragm:** Rotate the condenser turret to select the appropriate annulus corresponding to the objective lens in use.

- **Focus Alignment:** Initially focus the specimen using bright field mode, then switch to phase contrast mode and fine-tune the focus.

4. **Observation:**
   - **Image Optimization:** Adjust light intensity for the best image quality and use the fine focus knob to observe different planes within the specimen.
   - **Recording Observations:** Capture images or videos using a camera attached to the microscope if necessary.

Applications

- **Cell Biology:** Observing live cell behaviors, such as mitosis, motility, and intracellular processes.
- **Microbiology:** Studying bacteria, protozoa, and other microorganisms without the need for staining.
- **Medicine:** Analyzing sperm motility, blood cells, and tissue cultures.

Advantages

- Allows observation of live, unstained specimens.
- Enhances contrast without dyes, preserving cell integrity.
- Non-destructive and suitable for dynamic observations.

Limitations

- Halo artifacts may appear around structures, potentially obscuring details.
- Not suitable for thick specimens due to light scattering.
- Requires specialized equipment (phase contrast objectives and condensers).

Dark Field Microscopy

- Dark field microscopy enhances the contrast of transparent and unstained specimens by illuminating them with light that does not enter the objective lens unless it is scattered by the specimen.
- This creates a bright image of the specimen against a dark background, making normally invisible structures visible.

Procedure for Dark Field Microscopy

1. **Preparation of the Microscope:**
   - **Dark Field Condenser:** Install a dark field condenser that directs light obliquely onto the specimen. High-power objectives may require an oil immersion condenser to achieve optimal results.
2. **Specimen Preparation:**

- **Slide Preparation:** Place a drop of the specimen on a clean slide and cover it with a coverslip to create a thin, even layer suitable for observation.
- **Mounting Media:** Use media that do not interfere with the specimen's refractive properties or add background fluorescence.

3. **Microscope Settings:**
   - **Adjusting the Illumination:** Close the iris diaphragm to produce a hollow cone of light and align the condenser to ensure that direct light does not enter the objective.
   - **Objective Selection:** High numerical aperture objectives are necessary to capture scattered light effectively.
4. **Observation:**
   - **Focusing:** Initially focus on the specimen under low magnification, then switch to higher magnification and adjust the fine focus.
   - **Image Enhancement:** Adjust the light intensity to optimize the brightness of the specimen against the dark background.
5. **Documentation:**
   - **Image Capture:** Use a sensitive camera to record images, as the light levels in dark field microscopy are generally low.

Applications

- **Microbiology:** Detecting thin organisms like spirochetes (e.g., *Treponema pallidum*) that are difficult to see under bright field illumination.
- **Cell Biology:** Observing the motility and behavior of live cells and organelles.
- **Aquatic Biology:** Examining plankton and other transparent aquatic organisms.

Advantages

- Visualizes specimens that are invisible under bright field microscopy.
- No need for staining or complex preparation steps.
- Suitable for live specimens, maintaining their natural state.

Limitations

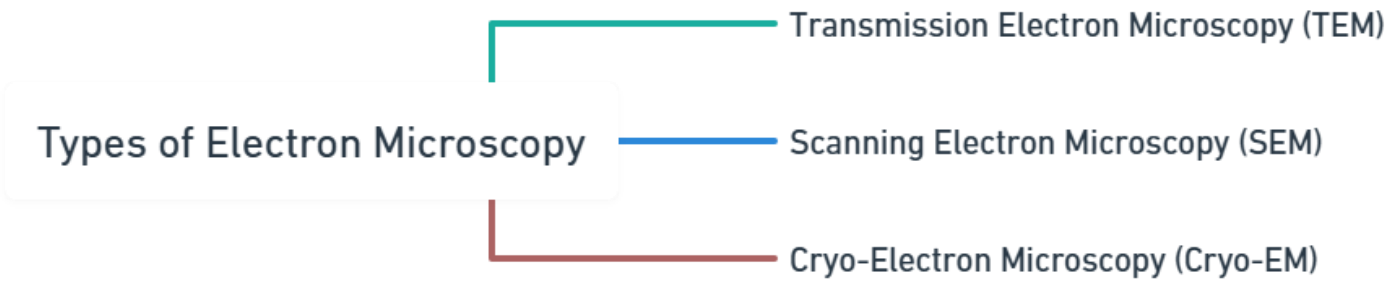
- Low light levels necessitate sensitive detection equipment.
- Not ideal for quantitative measurements due to varying light scattering properties.
- Artifacts, dust, or debris can scatter light, potentially leading to misleading images.

Electron Microscopy

- Electron microscopy offers much higher resolution than light microscopy by using electron beams instead of light.

- There are several types, each with unique principles and advantages.

Types of Electron Microscopy:



1. Transmission Electron Microscopy (TEM):

- Electrons pass through a thin specimen.
- Provides detailed images of internal structures.

2. Scanning Electron Microscopy (SEM):

- Electrons scan the specimen surface.
- Provides detailed 3D images of surfaces.

Applications

- **TEM:** Studying ultrastructure of cells and viruses.
- **SEM:** Examining surface topology and morphology.

Transmission Electron Microscopy (TEM)

Principle

- TEM works by transmitting a beam of electrons through an ultra-thin specimen.
- As electrons interact with the specimen, the transmitted electrons form an image.
- Due to the short wavelength of electrons, TEM can resolve details as small as 0.1 nm, making it suitable for studying cellular ultrastructure and material properties.

Procedure for TEM

1. Specimen Preparation:

- **Fixation:** Chemical fixatives (e.g., glutaraldehyde) are used to preserve the specimen's structure. Osmium tetroxide is applied post-fixation to stabilize lipids and enhance contrast.
- **Dehydration:** The specimen is passed through a graded series of ethanol or acetone to remove water.
- **Embedding:** The specimen is infiltrated with a resin (e.g., epoxy) and polymerized to form a solid block.

- **Sectioning:** Ultra-thin sections (50–100 nm) are cut using an ultramicrotome equipped with a diamond or glass knife.
- **Mounting:** The sections are collected on copper grids coated with a support film.
- **Staining:** Heavy metal stains (e.g., uranyl acetate, lead citrate) are applied to enhance electron contrast.

2. **Operation of the TEM:**

- **Vacuum System:** The microscope's column is kept under a high vacuum to prevent electron scattering by air molecules.
- **Electron Source:** The electron gun (either thermionic or field emission) is activated to generate the electron beam.
- **Alignment:** Electromagnetic lenses are used to align the electron beam.
- **Imaging:** The specimen grid is inserted, and the focus and astigmatism are adjusted for a sharp image.

3. **Image Recording:**

- **Detection:** The image is viewed on a fluorescent screen.
- **Image Capture:** A digital camera or photographic film is used to capture the image.

Applications

- **Cellular Ultrastructure:** Detailed study of organelles like mitochondria and the endoplasmic reticulum.
- **Virology:** Visualization of virus particles.
- **Materials Science:** Analysis of crystal structures and defects.

Advantages

- Extremely high resolution.
- Ability to visualize detailed internal structures.

Limitations

- Specimen preparation is complex and time-consuming.
- Specimens must be ultra-thin and electron-transparent.
- Live specimens cannot be observed.

Scanning Electron Microscopy (SEM)

Principle

- SEM scans a focused electron beam across the specimen's surface.
- Secondary electrons emitted from the specimen surface are collected to form an image.

- SEM provides high-resolution, three-dimensional images that reveal the specimen's surface topology.

Procedure for SEM

1. Specimen Preparation:

- **Fixation:** The specimen is fixed to preserve its structure, similar to TEM.
- **Dehydration:** Critical point drying is used to prevent the collapse of structures caused by surface tension.
- **Mounting:** The specimen is attached to an aluminum stub using conductive adhesives.
- **Coating:** A thin layer of conductive metal (e.g., gold or platinum) is sputter-coated on the specimen to prevent charging and improve signal quality.

2. Operation of the SEM:

- **Vacuum System:** The specimen chamber is evacuated to reduce electron scattering.
- **Electron Source:** An electron beam is generated using a thermionic or field emission gun.
- **Beam Focusing:** Electromagnetic lenses are used to focus the electron beam into a fine spot.
- **Scanning:** The electron beam is raster-scanned over the specimen's surface.

3. Detection and Imaging:

- **Secondary Electron Detector:** Secondary electrons emitted from the specimen are collected.
- **Image Formation:** The intensity of these electrons is used to form a grayscale image displayed on a monitor.

4. Image Processing:

- **Adjustments:** Brightness, contrast, and focus are modified for optimal image quality.
- **Data Capture:** Images are saved digitally for further analysis.

Applications

- **Surface Morphology:** Studying the texture and topography of materials.
- **Biology:** Examining surface structures of cells and tissues.
- **Nanotechnology:** Visualizing nanoparticles and nanostructures.

Advantages

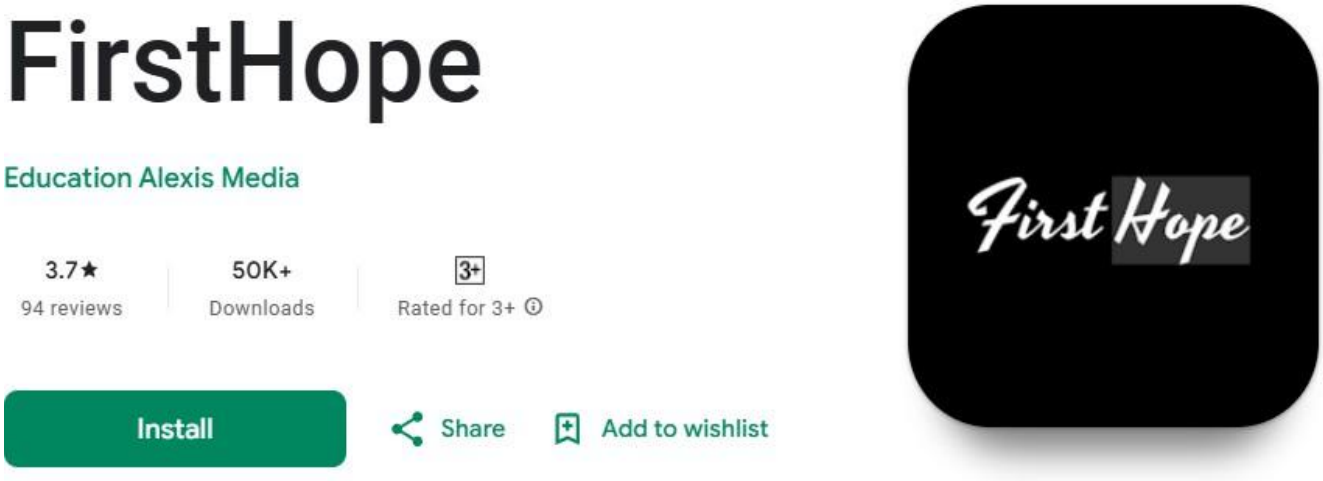
- High-resolution imaging of surfaces.
- Depth of field allows for 3D-like visualization.
- No need for ultra-thin specimens.

Limitations

- Internal structures cannot be viewed.
- Non-conductive specimens require a conductive coating.
- Specimens must withstand vacuum conditions.

First Hope

Easy Short Notes Designed to Help You Score Better



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