



Principle of Microbiology  
( Practical )

## Lecture 2

### Light compound Microscope

Microscopy is a fundamental technique in microbiology, enabling the visualization of microorganisms that are invisible to the naked eye. In food science, microscopes are essential for identifying microbial contaminants, studying foodborne pathogens, and ensuring food safety and quality . The **light compound microscope** is the most commonly used instrument in microbiology laboratories due to its ability to provide detailed images of bacteria, yeasts, molds, and other microorganisms present in food samples

#### Aim of the lecture

##### **The primary aim of this lecture is to:**

1. **Introduce the fundamental principles** of the light compound microscope, including magnification, resolution, and contrast, as applied in food microbiology.
2. **Familiarize students with key microscope components** and their functions to ensure proper handling and optimal usage.
3. **Demonstrate practical applications** in food science, such as microbial detection, quality control, and staining techniques for pathogen identification.
4. **Teach essential sample preparation methods**, including wet mounts, smear techniques, and staining (e.g., Gram staining, methylene blue).
5. **Highlight limitations and best practices** to ensure accurate observations and avoid common microscopy errors.

## Principles of the Light Compound Microscope

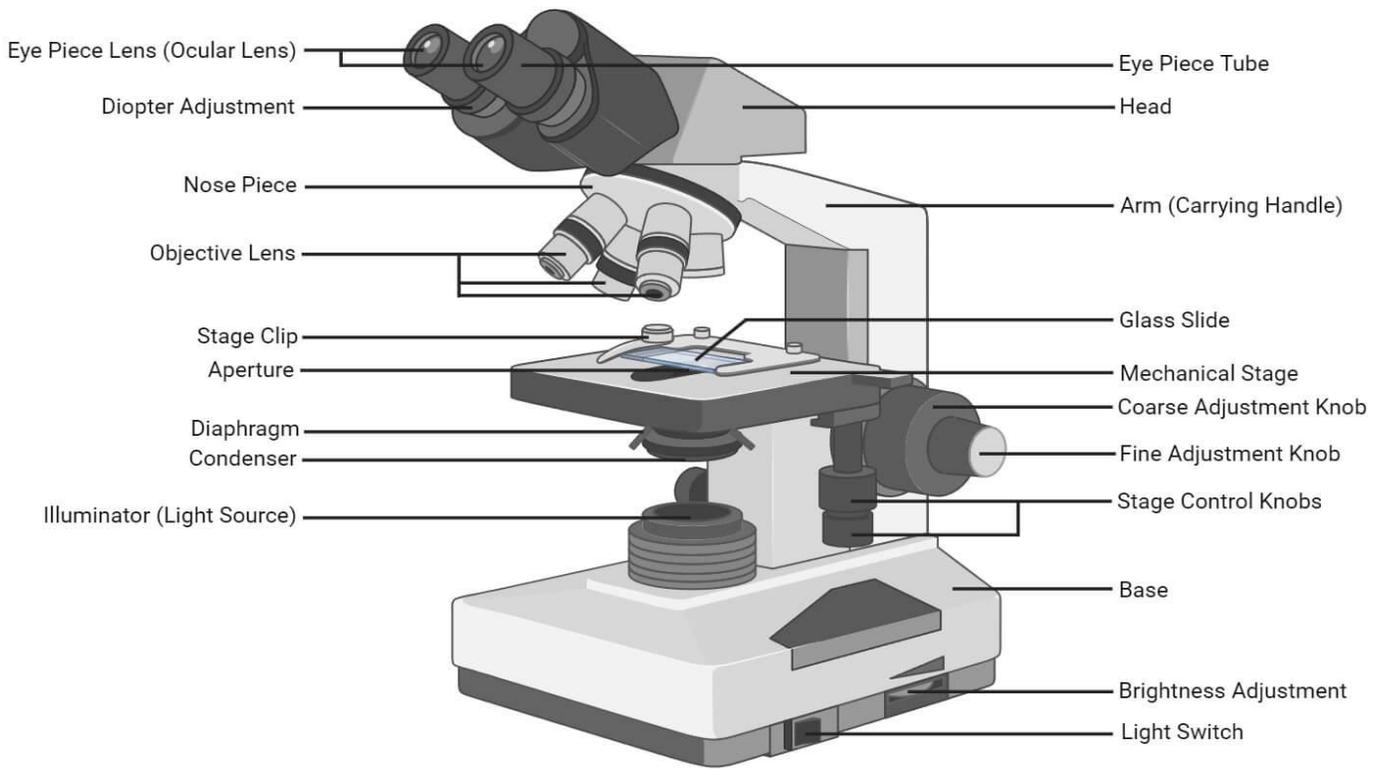
The light compound microscope operates based on the principles of **magnification, resolution, and contrast**:

1. **Magnification** – Achieved through a combination of **objective lenses** (4x, 10x, 40x, 100x) and an **eyepiece** (typically 10x), resulting in total magnifications from 40x to 1000x .
2. **Resolution** – The ability to distinguish two closely spaced objects (typically  $\sim 0.2 \mu\text{m}$  for light microscopes) .
3. **Contrast** – Enhanced using staining techniques (e.g., Gram staining) or specialized microscopy methods (phase contrast, dark field) to improve visibility of transparent specimens

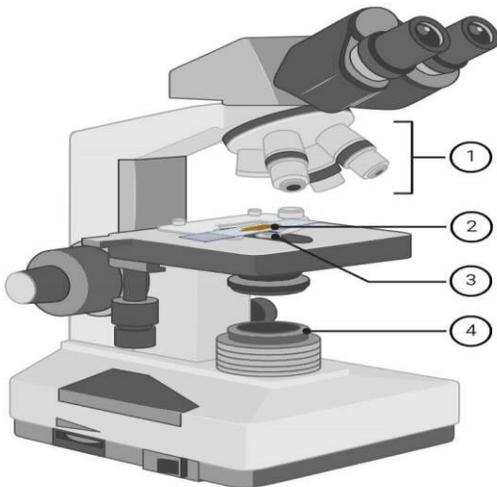
### ❖ Key Components of a Compound Microscope

<b>Component</b>	<b>Function</b>
<b>Eyepiece (Ocular Lens)</b>	Further magnifies the image (10x)
<b>Objective Lenses</b>	Primary magnification (4x, 10x, 40x, 100x oil immersion)
<b>Condenser</b>	Focuses light onto the specimen
<b>Diaphragm</b>	Adjusts light intensity for optimal contrast
<b>Stage &amp; Mechanical Stage</b>	Holds and positions the slide
<b>Coarse &amp; Fine Focus Knobs</b>	Adjusts focus at different magnifications
<b>Light Source</b>	Illuminates the specimen (LED or halogen)

# Parts of a Compound Microscope



## Principle of Compound Microscope

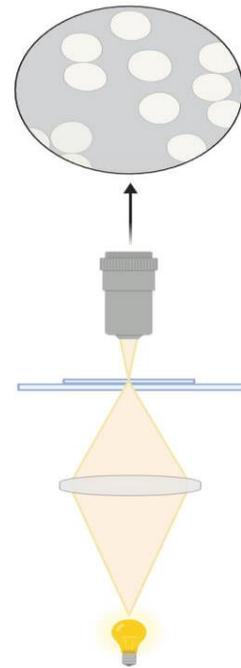


1. Objective

2. Specimen

3. Condenser lens

4. Light source



## - *Practical Applications*

1. **Microbial Detection** – Identifying bacterial contaminants (e.g., *Salmonella*, *E. coli*) in food samples .
2. **Quality Control** – Assessing spoilage microorganisms (molds, yeasts) in dairy, meat, and fermented products .
3. **Staining Techniques** – Gram staining differentiates bacteria (Gram-positive vs. Gram-negative), aiding in pathogen identification .
4. **Direct Observation** – Wet mounts of food suspensions can reveal live microbial motility (e.g., protozoa in water samples)

## - *Sample Preparation Techniques*

1. **Wet Mounts** – Simple method for observing live microbes (e.g., yeast in yogurt).
2. **Smear Preparation** – Spreading a thin layer of sample on a slide, followed by heat fixation.
3. **Staining** – Common stains include:
  - **Gram stain** (crystal violet, iodine, alcohol, safranin)
  - **Methylene blue** (for direct cell observation)
  - **Lactophenol cotton blue** (for fungal structures)
4. **Oil Immersion** – Required for 100x objective to improve resolution by reducing light refraction

## -Limitations & Considerations

- Limited resolution (~0.2  $\mu\text{m}$ ) means viruses and some small bacteria require electron microscopy .
- Specimen thickness – Only thin, translucent samples can be observed.
- clearly Artifacts – Improper staining or fixation can distort microbial structures.

## -Safety & Maintenance

- Clean lenses with lens paper (**never use tissue/cloth**).
- Store properly with the lowest objective in place.
- Dispose of slides in biohazard containers if contaminated.

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## **References:**

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