



LABORATORY SKILLS

“ 240 MIC ”

LAB 1

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Microbiology Laboratory, Organization and Management



1a. Laboratory Safety Rules



Always wear a laboratory coat when working in the laboratory classroom.

Put nothing in mouth which may have come in contact with infectious material.

Eating and drinking in the laboratory are not permitted at any time.

Keep your workspace free of all unnecessary materials.


Never pipette by mouth. Use the safety pipetting devices which are provided.

1a. Laboratory Safety Rules



- 1. Throw off used pipettes in the appropriate containers.
- 2. Any infectious material which may accidentally fall from pipettes to the laboratory bench or floor should be covered with a disinfectant and reported to any instructor immediately.
- 3. Any spilled or broken containers of culture material should be covered with a disinfectant and then inform instructor.
- 4. Replace caps on reagents, solution bottles, and bacterial cultures after use.
- 5. Do not open Petri dishes in the lab unless absolutely necessary.

1a. Laboratory Safety Rules



Take care of the microscope.

Label everything clearly.

When finished for the day, dispose of all used glassware and cultures in the appropriate containers, clear workbench and wash the top with a disinfectant.

Make sure all burners are turned off at the end of the laboratory period.

Take off your gloves on when you leave the laboratory.

Wash hands thoroughly with soap and water before leaving the laboratory.

Familiarize yourself with the location of safety equipment in the laboratory.

Laboratory Safety Equipment

Eyewash and shower

Sinks

Fire Extinguisher

First Aid Kit

Emergency Gas Valve



Laboratory Safety Equipment

Flammable – Any substance that will burn if exposed to an open flame

Explosive – A substance that may explode if exposed to heat or flame.

Toxic/Poison – A substance that can lead to death if inhaled, ingested, or absorbed by the skin.

Irritant - A substance that causes inflammation upon contact with skin or mucous membranes.

Environmental - Substances that are harmful to the environment. They must be disposed of properly, not washed down the drain



1b. Apparatus and Equipment

1. Microscope: Used to observe very small organisms.



4. Hot Air Oven: It is a dry air type sterilizer. It is used for sterilizing laboratory glass ware. It operates at a temperature of 160 to 180 ° C for one and a half hour.



2. Autoclave: It is a wet air type sterilizer.

- It is used to sterilize culture media, glassware etc.
- Usually autoclave operates at 15 lb/sq. inch steam pressure (121.5 ° C) for 15 min.



5. Laminar Air Flow: It is a chamber which provides a microbe free environment. It is used for transfer of media for culturing bacteria or fungi or any microbes.



3. Incubator: Provide suitable temperature for the growth of organism.



6. Centrifuge: It spins liquid samples to separate their components.



1b. Apparatus and Equipment

7. Spectrophotometer: It is a device which uses a wavelength to determine the concentration of a compound or particles in a solution or suspension.



10. Hot plate: In laboratory, hot plates are generally used to heat glassware or its contents.



8. Micropipettes: Pipettes are used to accurately measure and dispense small volumes of liquid.



11. Weighing scales: These are devices to measure weight or calculate mass.



9. pH meter: It is a device used for measuring the pH, which is either the concentration or the activity of hydrogen ions, of an aqueous solution.



12. Colony counter: Instrument is used for counting of bacterial colonies growing on agar in petri dishes.

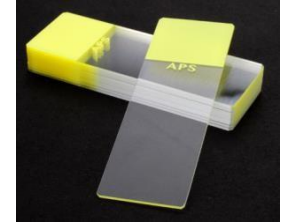


1b. Apparatus and Equipment

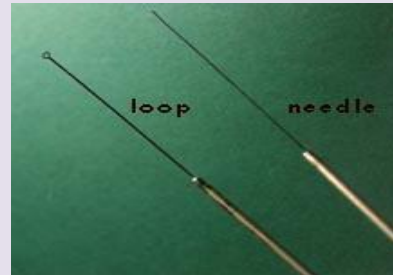
13. Vortex mixer: A vortex mixer, or vortexer, is a simple device used commonly in laboratories to mix small vials of liquid.



16. Slide: Glass support for specimens.



14. Inoculating Loops & Inoculating Needles: Used for inoculating microbes in the liquid media & solid media



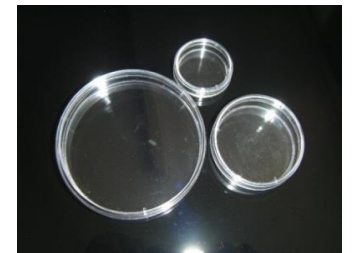
17. Cover Slip: Glass cover for specimens.



15. Bunsen Burner:
Source of flame.



18. Petri Dishes: Petri dishes are often used to make agar plates for microbiology studies.



1c. Introduction to Microscopy

"Micro" refers to **tiny**, "scope" refers to **view or look at**.

Microscopes are tools used to enlarge images of small objects so as they can be studied.

Light Microscope

The compound light microscope is an instrument containing **two lenses**, which magnifies the picture.

Because it uses more than one lens, it is sometimes called the **compound microscope**.

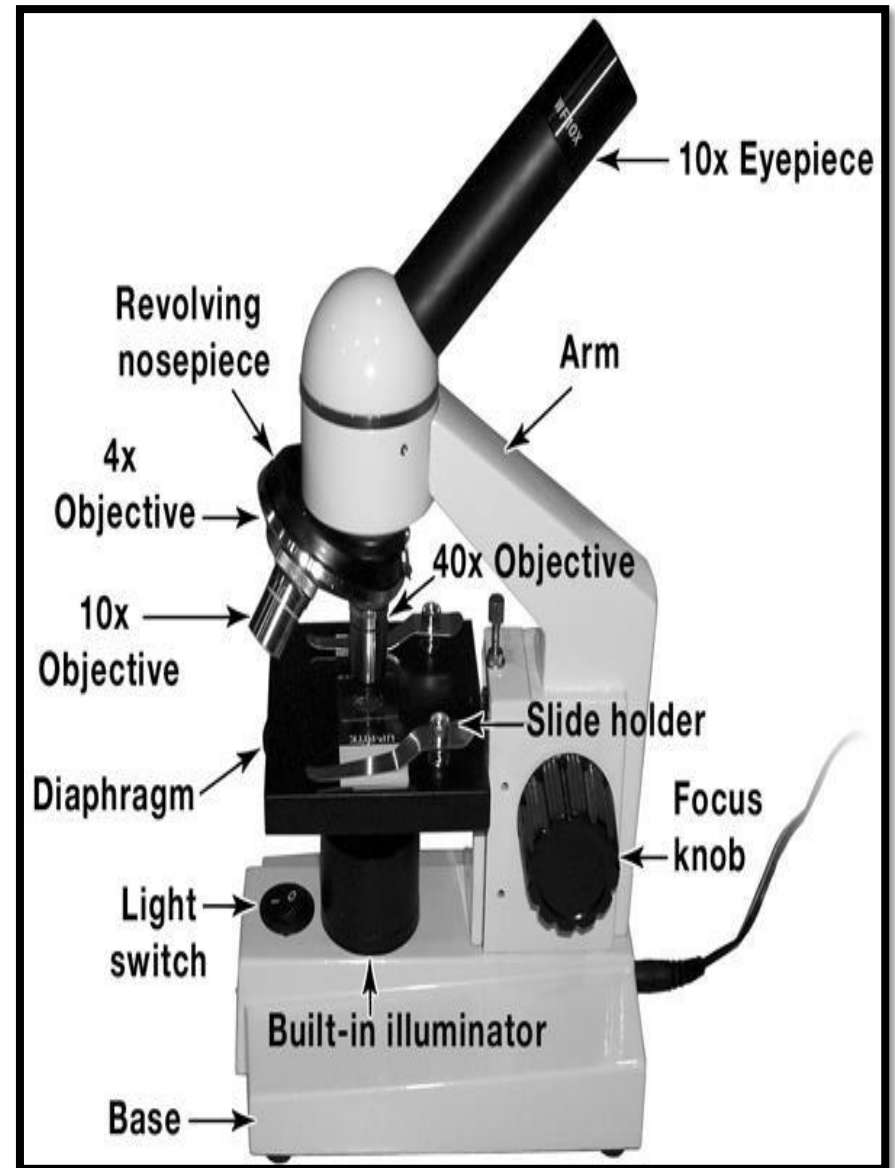


Fig. 1 Light microscope

Microscope parts

- **Eyeiece or Ocular (10x magnification):** One (monocular) or two (binocular) lenses that you look through to see the image
- **Arm:** Supports the upper half of the microscope
- **Objective Lenses**
 - 4x Objective (Red band):** Low power scanning objective
 - 10x Objective(yellow band):** Medium power objective
 - 40x Objective (blue band):** High power objective
 - 100x Objective(black and a white band):** Oil Immersion Objective Lens



Fig. 2 Binocular

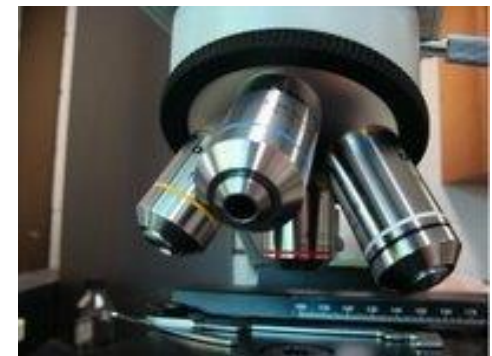


Fig. 3 Objective Lenses

Microscope parts

Name	Characteristics	Magnifying power
Low power scanning	shortest objective, red stripe	4 X
Medium power	next shortest, yellow stripe	10 X
High power	intermediate length, blue stripe	40, 43 or 45 X
Oil Immersion	longest, black stripe	100 X

Revolving nosepiece: Can be rotated to change objectives

•**Stage:** The platform for holding the slide

•**Slide holder:** Clips to hold slide to stage



Fig. 4 Stage & Slide holder

Microscope parts

- **Focus knob:** The knob that adjusts the height of the stage
- **Light switch:** Switch to turn on the built-in illuminator
- **Built-in illuminator:** Light source required to view specimens
- **Base:** Supports entire microscope
- **Power cord:** For recharging batteries to illuminator
- **Diaphragm:** Adjusts amount of light entering specimen
- **Condenser:** It focuses light on an object plane



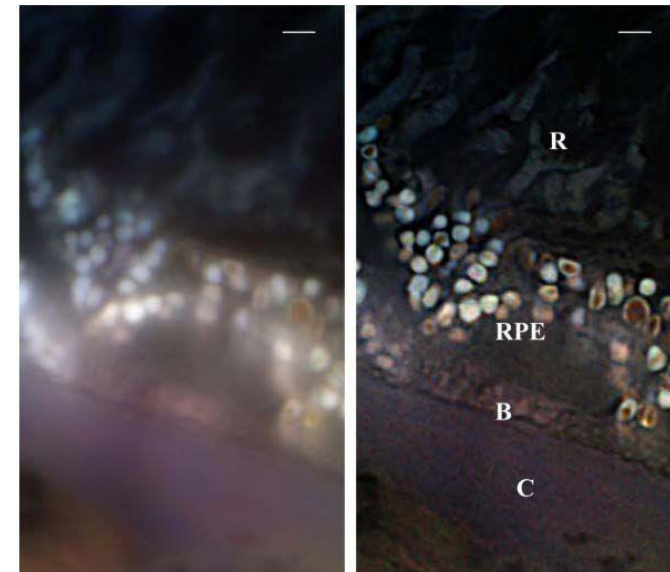
Fig. 5 Knobs

Magnification and Resolution

🕒 **Magnification:** An increase in the apparent size of an object, as under the microscope.

🕒 **Total magnification:** To calculate the total magnification of any specimen being viewed multiply the power of the eyepiece (ocular lens) by the power of the objective lens being used.

For example, if the eyepiece magnifies 10X and the objective lens magnifies 40X, then 10×40 gives a total magnification of 400X.



🕒 **Resolution:** It is the ability to differentiate two objects



General Rules to Remember While Using the Microscope:

- 1. Use light from a microscope lamp unless microscope has internal illumination.**
- 2. Adjust the condenser so that it is flush with, but not above, the stage.**
- 3. Place the specimen to be observed directly over the lens of the condenser.**
- 4. Focus first with low power. Bring down the objective to its lowest point without touching the slide) and observe the slide as the objective is used by rotating the course adjustment knob in a counter-clockwise direction.**



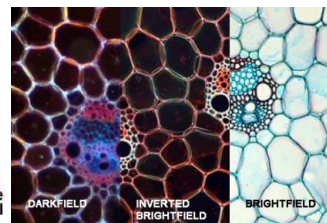
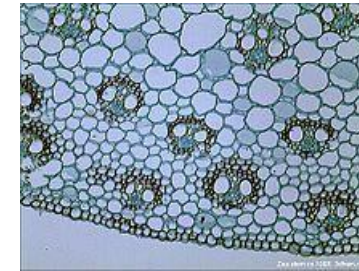
Care of the microscope

1. Never touch the glass with your fingers.
2. Always carry the microscope with two hands. Never turn the microscope upside down or the eyepiece could fall out.
3. Place the microscope on a level surface.
4. Check to make sure the battery operated built-in illuminator is working.
5. Store the microscope with a dust cover to keep it clean.
6. When you are finished using the microscope, switch to the lowest power objective (4x), lower the stage, switch off the power, cover the microscope with a dust cover, and return the microscope to its storage area.

Kinds of Microscopes

1. **Bright field Microscope:** The specimen is illuminated/enlighten by a beam of tungsten light focused on it by a condenser, in this specimen appears dark against a bright background.

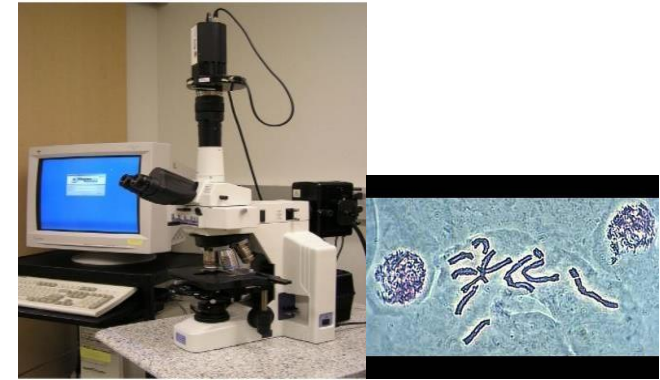
This microscope is used to observe nonviable, stained preparation.



2. **Dark field Microscope:** The specimen is not illuminated directly. Specimen appears bright against a dark background.

Kinds of Microscopes

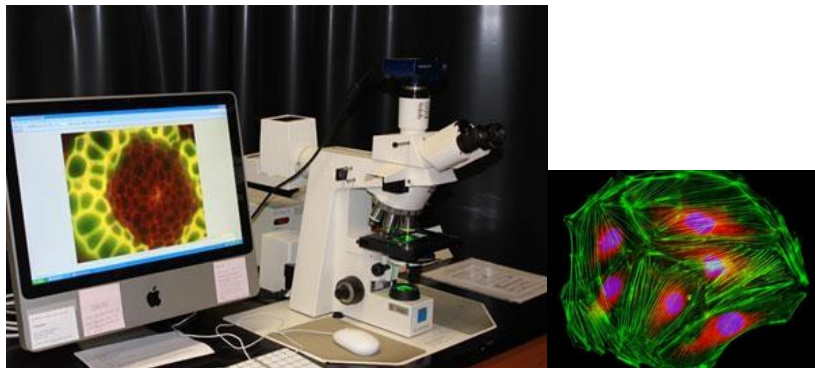
3. Phase-Contrast Microscope: The unstained microorganisms are observed easily. It has special objectives and a condenser. The image of the specimen appears dark against a light background.



4. Fluorescent Microscope: This microscope is used to observe the specimens that are chemically treated with a fluorescent dye.

The specimens are illuminated with an ultraviolet light. This microscope is used for the detection of antigen-antibody reactions.

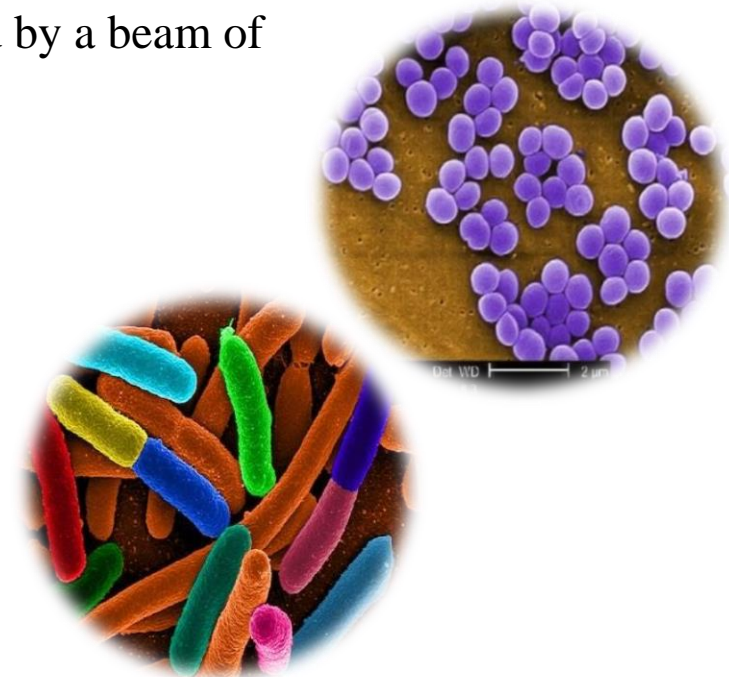
The fluorescent portion of the dye becomes visible against the black background.



Kinds of Microscopes

5. **Electron Microscope:** It produces an electronically-magnified image of a specimen for detailed observation.

It is used to observe submicroscopic cellular particles as well as viral agents. The specimen is illuminated by a beam of electrons rather than light.



The End

