

FE 204 Experiment 6
Microscope for the Study of
Microbes
Hanging Drop and Wet-Mount
Techniques

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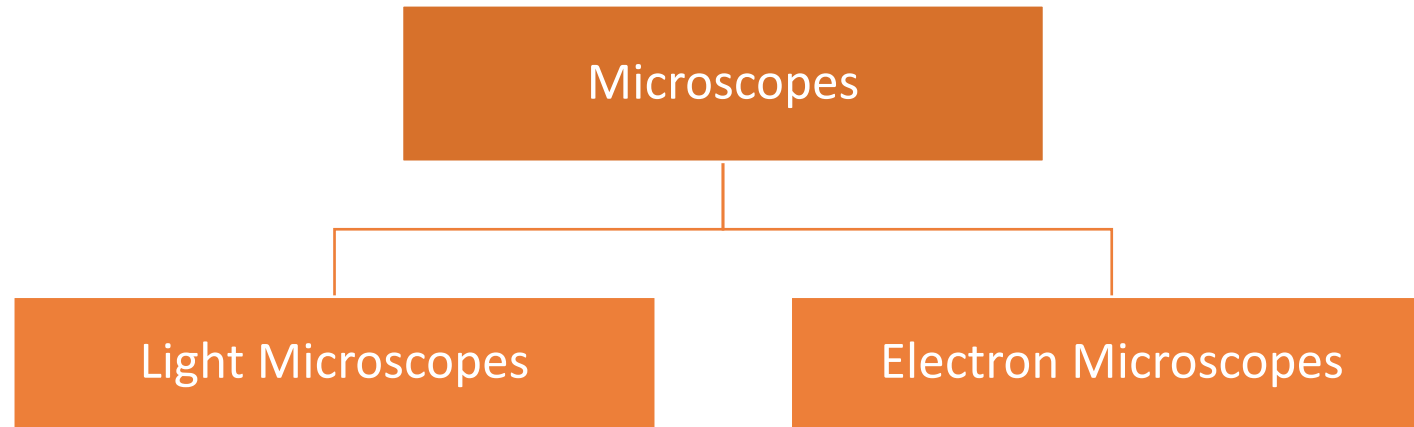
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Microscope

- **Definition:** The microscope is the standard instrument used for the magnification of the microorganism or microstructure.
- First microscope was found by Leuwenhoek in 1660s.



Microscope Types



- Bright field microscope
- Dark field microscope
- Phase contrast microscope
- Polarization microscope
- Interference microscope
- Inverted microscope

- Ultraviolet microscope
- Fluorescence microscope
- Scanning electron microscope
- Transmission electron microscope

Light Microscope

- **Definition:** A microscope that allows light rays to pass directly through to eye without being reflected by an intervening opaque plate in the condenser is called a light microscope.



Parts of Microscope

- **Framework:** Include arm and base.
- **Stage:** Horizontal platform that supports microscope slide.
- **Lens system:** Include oculars, objectives and condenser
- **Light source:** Lamp should have a voltage control to vary intensity of light



Parts of Microscope



Parts of Microscope

Monocular



Binocular

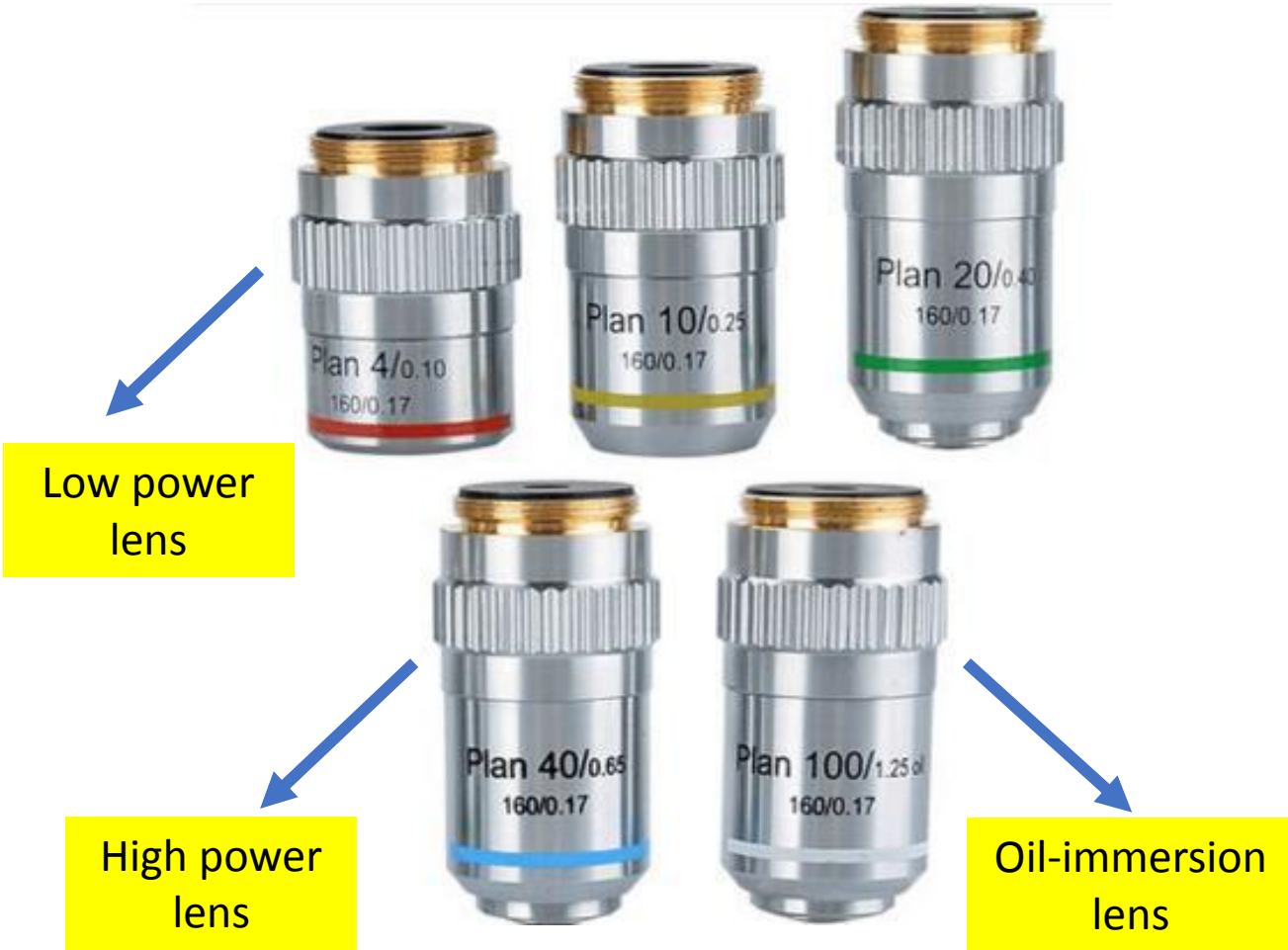


Trinocular



Types of Microscope Head

Parts of Microscope



Total Magnification on Microscope

Low power lens: The lens with the less magnification

High power lens: The lens with the higher magnification

Oil immersion lens: A special lens with oil placed between the lens and the object being visualized (usually 100 x objective)

Ocular		Objective		Magnification at eyepoint
10x	x	10x	=	100x
10x	x	44x	=	440x
10x	x	100x	=	1000x

Care of the Microscope

1. Never touch lenses. If lenses become dirty, wipe gently with lens paper.
2. Never leave a slide on microscope.
3. Always remove oil from the oil-immersion objective after its use.
4. Keep the stage of the microscope clean and dry.
5. Do not tilt the microscope when working it.
6. When the microscope is not in use, keep it covered.
7. Use both hands when holding the microscope.



Uses of Microscope

- When working with a microscope, you should first use low power lens. Then, you can switch to objective for high power lenses.
1. Position the slide on the stage with the material to be studied on the upper surface of the slide.
 2. Turn on the light source using a minimum amount of voltage or light, reposition the slide, check the condenser.

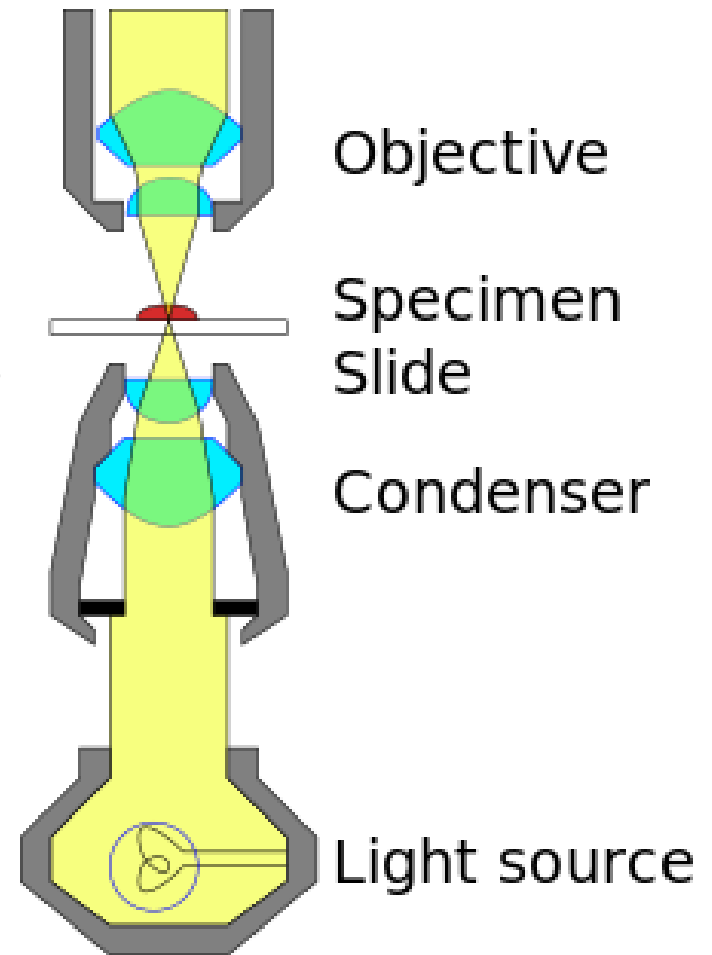
Uses of Microscope

3. If the low-power objective is not directly over the center of the stage, rotate it into position.



Uses of Microscope

4. While looking down through the oculars, bring the object into focus by turning coarse adjustment. After finding microbial cells in the slide, for the better illustration using the fine adjustment knob.
5. Manipulate the diaphragm lever to reduce or increase the light intensity to produce the clearest, sharpest image.



Uses of Microscope

7. Once an image is visible, move the slide about to search out what you are looking for.
8. Once you have identified the structures under low power objective and wish to increase the magnification, you can proceed to either high-power or oil immersion magnification.

<https://www.youtube.com/watch?v=SUo2fHZaZCU>

MICROSCOPIC EXEMINATION TECHNIQUES

Microscopic Examination Techniques

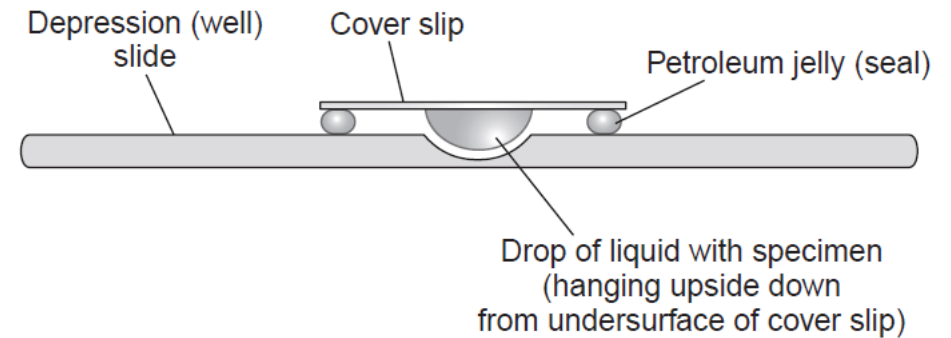
- Examination of microorganisms with microscope provides observation of;
 - The size of individual microorganism,
 - The natural shape of microorganism,
 - The characteristic arrangement,
 - Difference among microbial cells.
 - Mobility of microorganisms

Definition

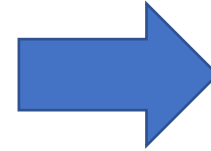
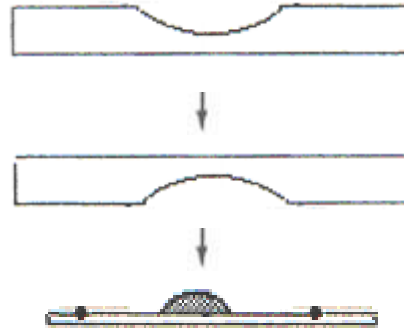
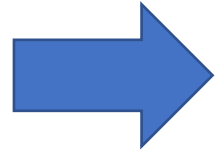
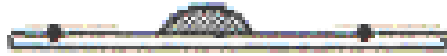
- **Hanging drop technique:** Employs a glass slide with a circular concavity in the centre into which a drop of fluid, containing the 'microorganisms', hangs from a coverslip.
- **Wet mount technique:** The sample is placed in a drop of water or other liquid held between the slide and the cover slip by surface tension.

Material Used in LAB

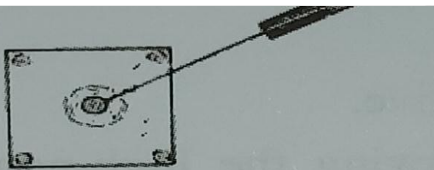
- Microscope
- Sterile distilled water
- Depression slide
- Microscope slides
- Cover slips
- Inoculating loop
- Vaseline



Hanging Drop Technique



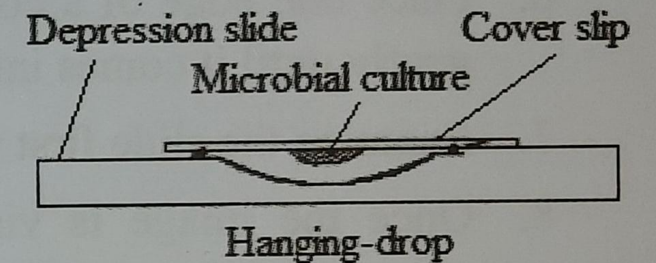
1. Apply a small amount of vaseline to edges of the cover slip
2. Place three or four loopfuls of microbial suspension on the center of cover slip.



1. Transfer least 3 loops of culture onto center of a cover glass.

A depression slide is lowered in an inverted position on to the cover slip and slightly apply press on to the slide.

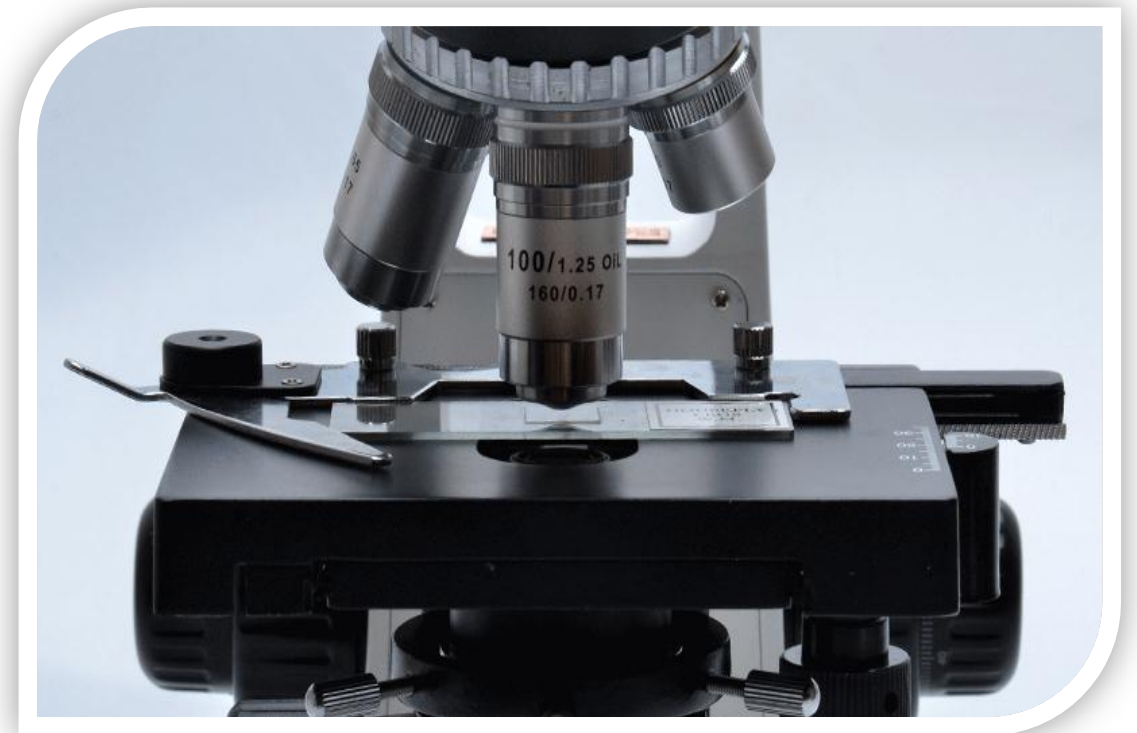
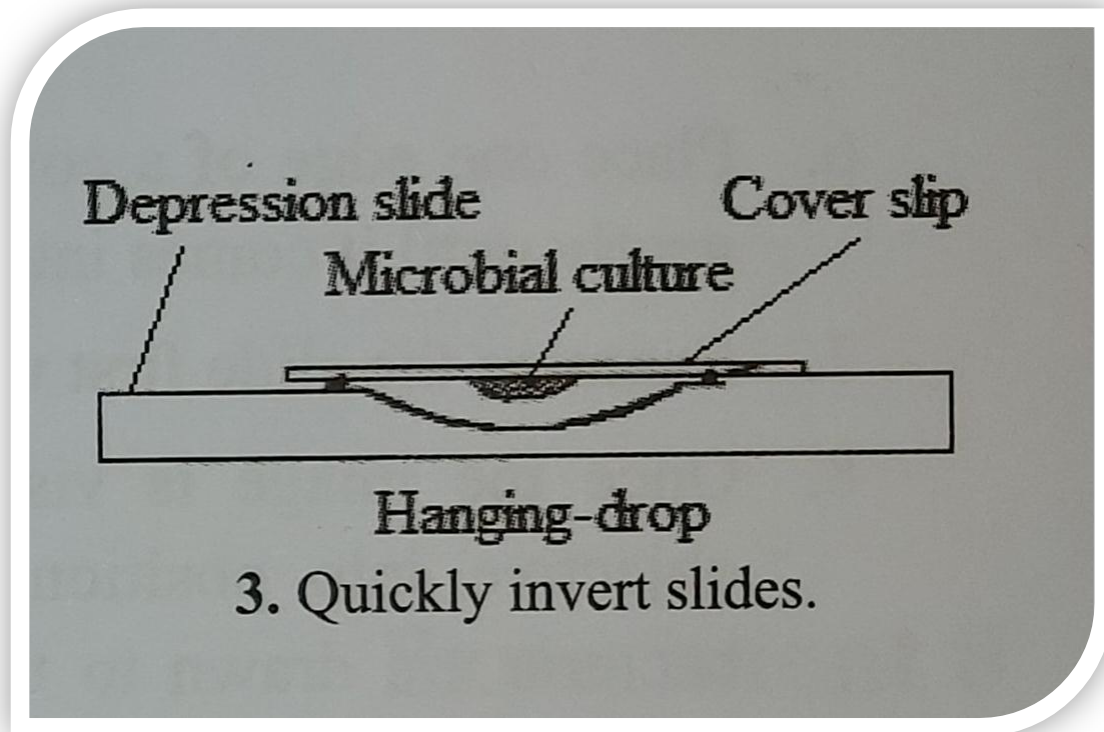
Turn both slides in the right side up readily and see the droplets of bacterial suspension on the hole of depression slide.



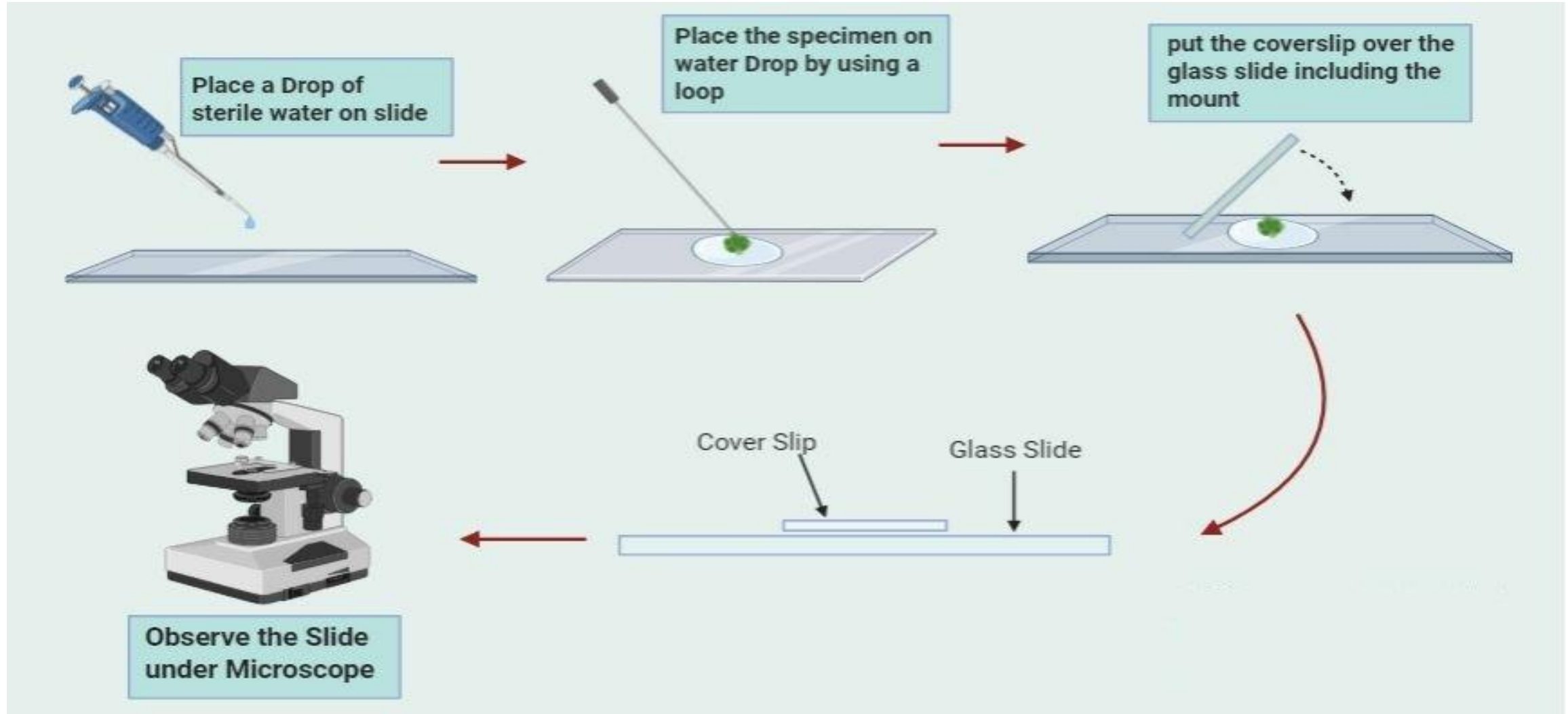
3. Quickly invert slides.

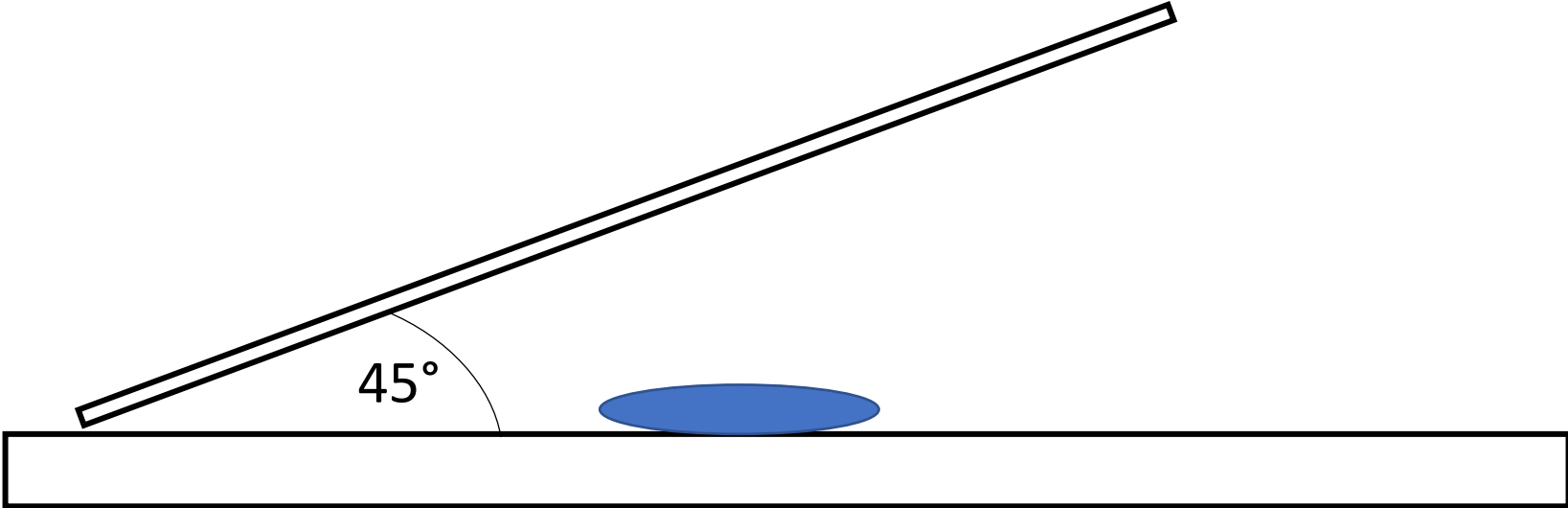
Hanging-Drop Technique

- Once the slide is placed on the microscope stage and examine.



Wet Mount Technique

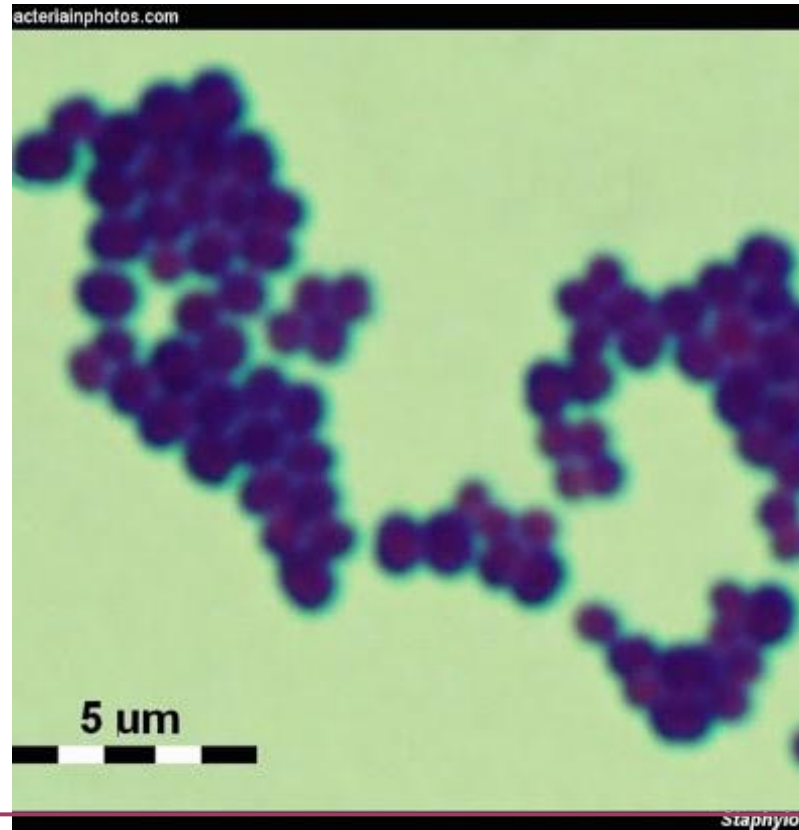




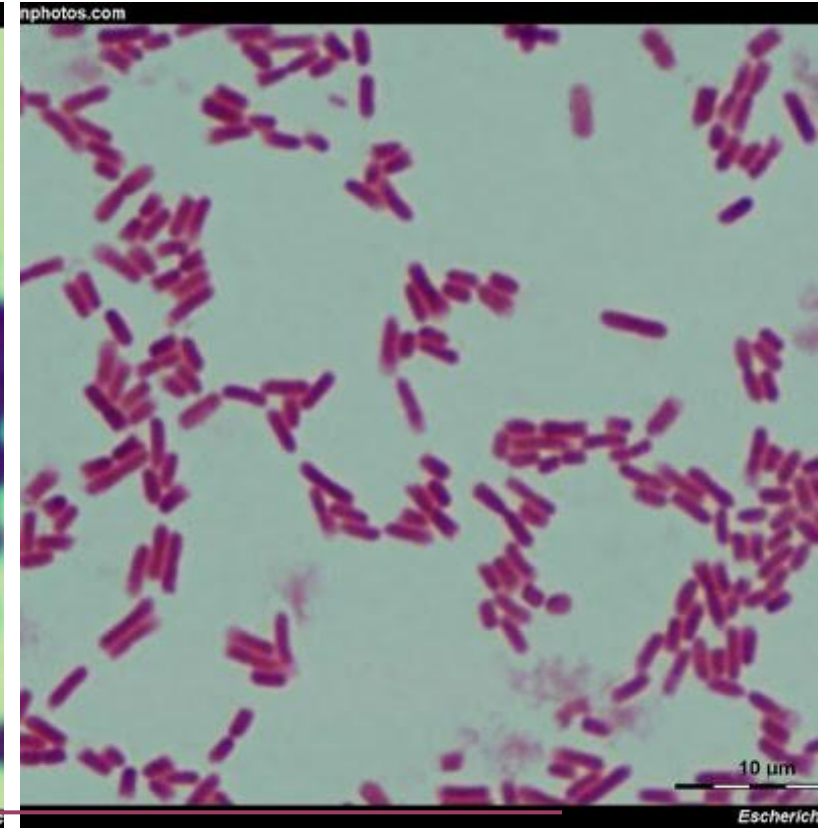
Results



Vibrio cholerae



Staphylococcus aureus



Escherichia coli

Results



Parts of Microscope

