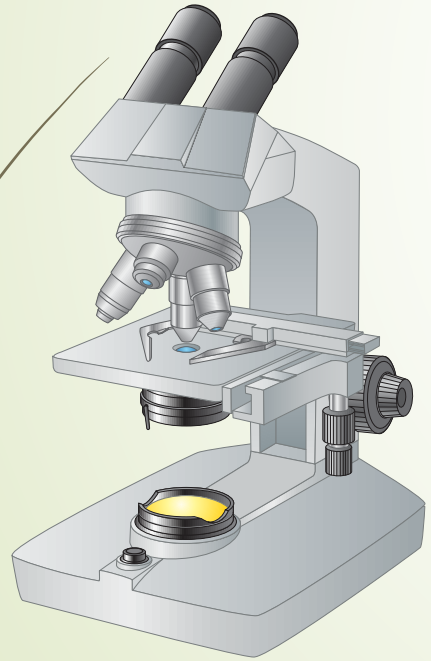
Lecture (4)



Microscopy and Staining

Objectives

1. Identify basic parts of the light microscope and describe the function of each part.
2. Define and calculate microscope magnification
3. Define Microscope resolving power
4. Enumerate some other types of microscopes beside the light microscope.

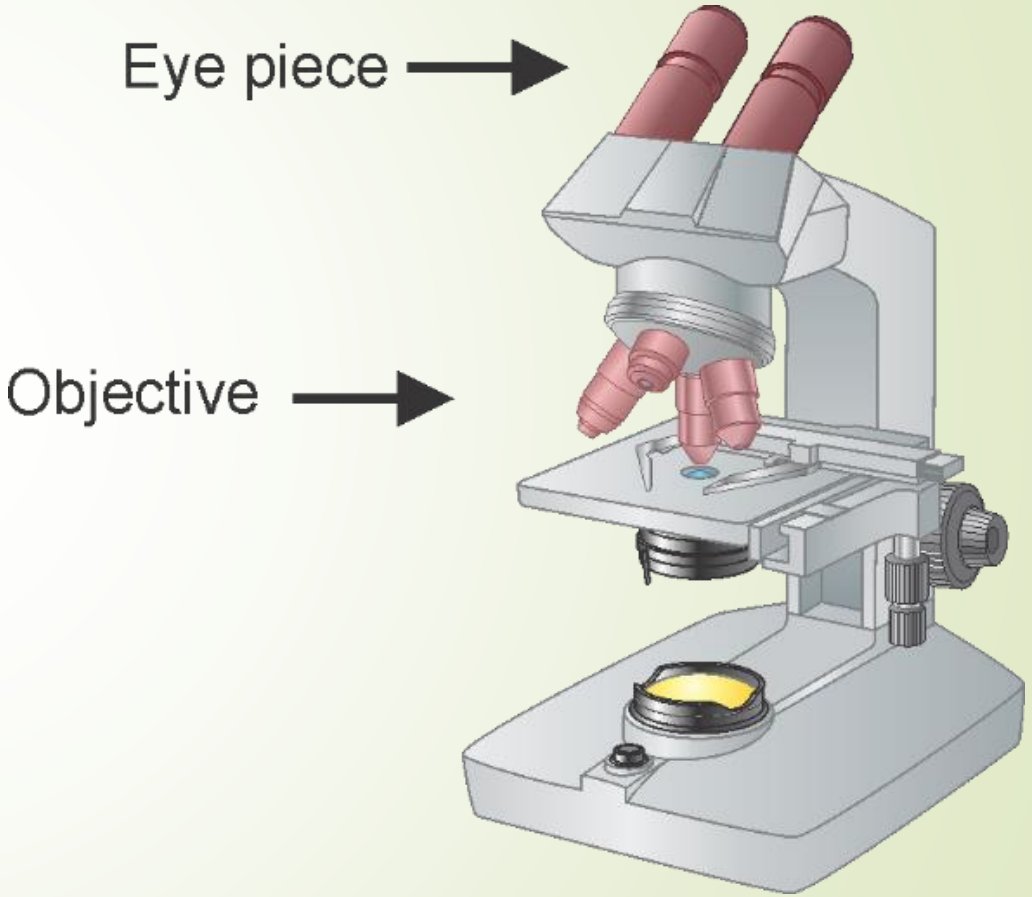
5. Explain the value of staining microorganisms.
6. Compare simple and differential types stains.
7. Describe principle and applications of gram stain and acid-fast stains.

8. Describe basic bacterial morphology [size, shape, arrangement, staining, capsule, spore, and motility].

Light Microscope

Light microscopes are known as **compound** microscopes because there are **two** magnifying lenses in the microscope:

1. **Ocular** lens (eye piece).
2. **Objective** lens.

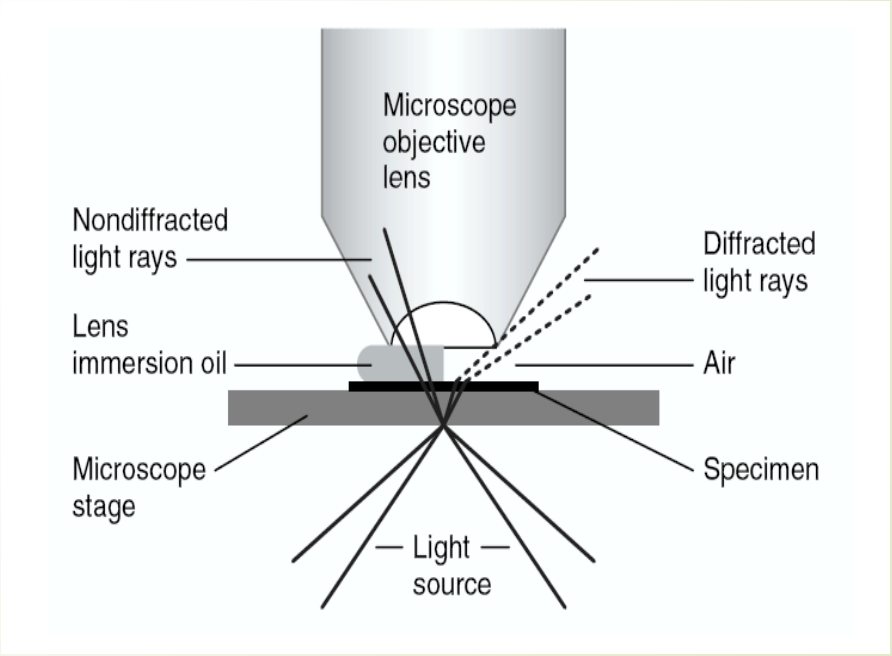


Objective Lens

Most microscopes have three or four different objectives, giving a range of magnifications, typically from 10× to 100×.

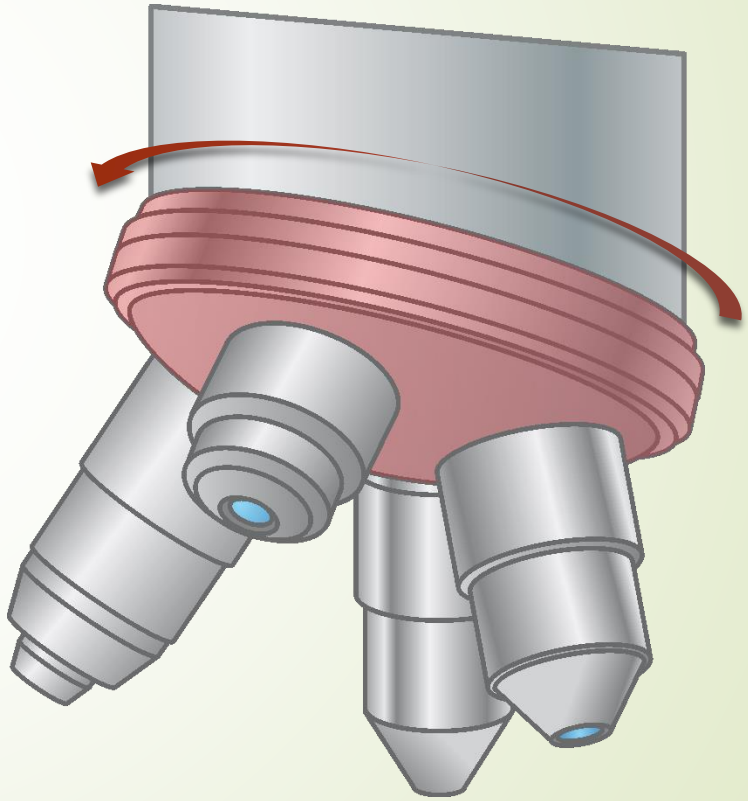
Oil immersion Lens x100

- The 100X objective is called oil immersion lens because this lens is immersed in oil.
- Oil has the same refractive index as glass.
- This allow more light rays to enter the objective lens by minimizing refracted and reflected rays.



Nosepiece

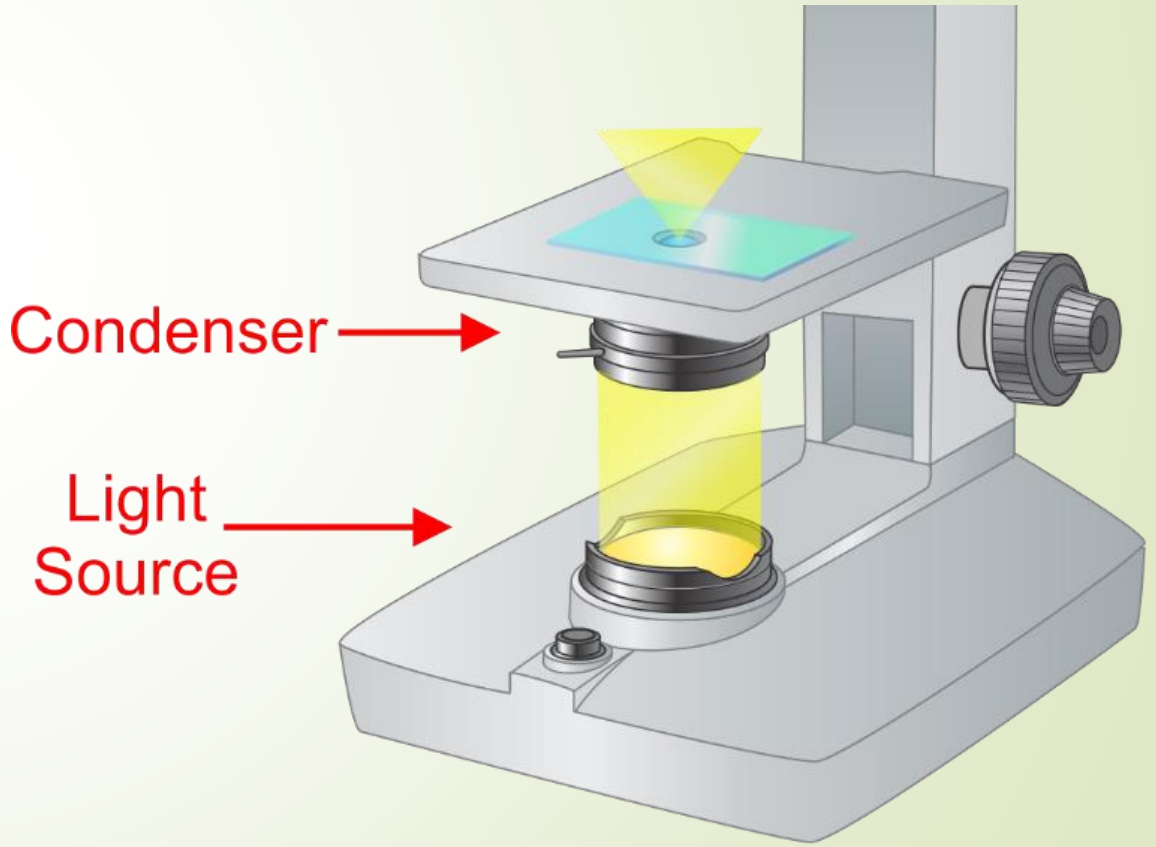
The **nosepiece** rotates allowing the objectives to change and thus change the magnification of the microscope.



Nose Piece

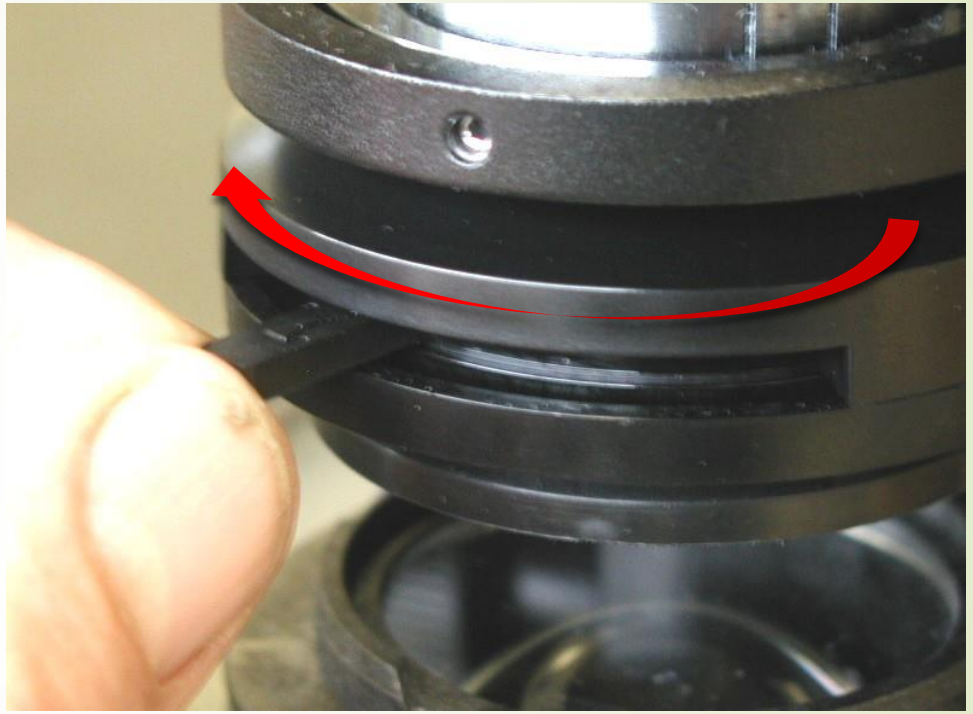
Stage - Illumination

- The **stage** is where the slide is placed.
- **Light source (Illuminator)** provides the illumination for the specimen.
- **Condenser**. Focuses the light through the specimen.



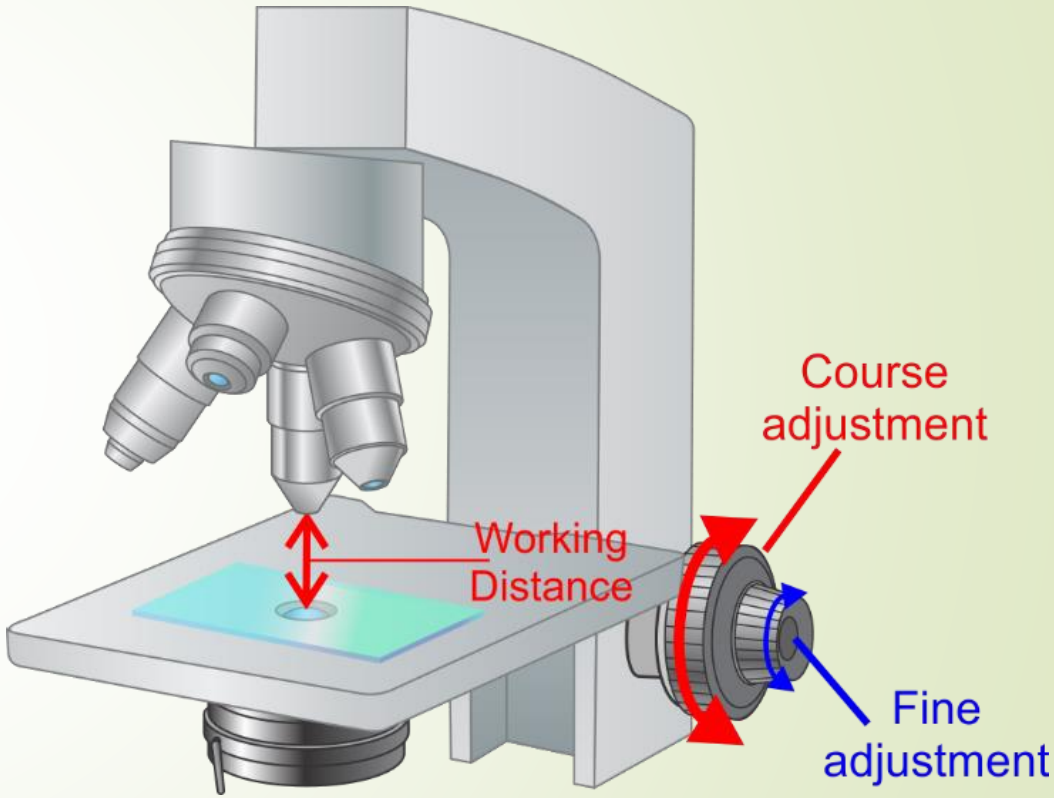
Iris diaphragm

- **<u>Iris diaphragm:</u>** an adjustable opening under the condenser lens that controls the amount of light passing through to the specimen.



Focus Adjustment

- The **coarse and fine adjustment knobs** are used to focus the lenses on the specimen.
- Rotating the **coarse adjustment knob** brings the image into rough focus, while the **fine adjustment knob** is for final, fine focusing.



Magnification

Microscope magnification is determined by **multiplying** the magnification of the objective by the magnification of the ocular lens.

Microscope Magnification =

objective magnification $\times$ ocular magnification

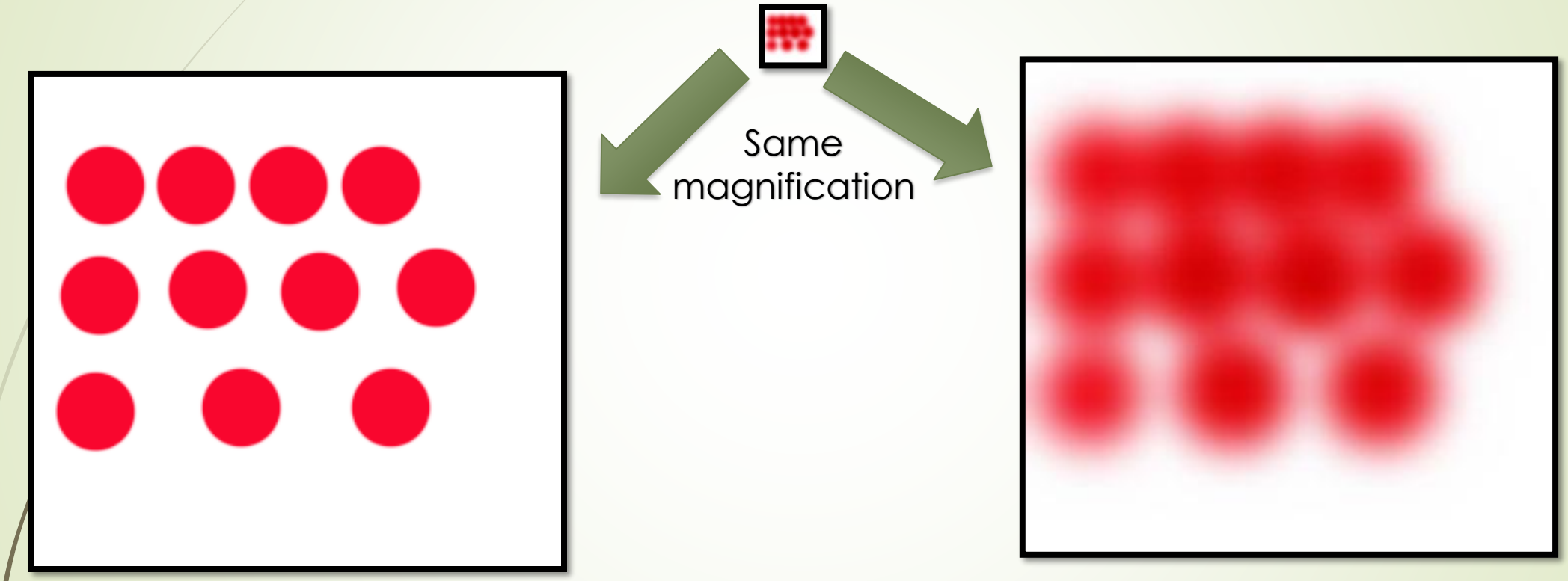
Resolving power

Resolution

- The ability to see things using a microscope is limited by the **resolving power** of the microscope.
- The resolving power of a microscope is **the distance two objects must be apart and still be seen as separate and distinct**.
- The resolving power is of the light microscope 0.2 µm.
- Objects closer together than 0.2 µm will not be distinctly seen. Increasing the magnification will not make the objects more distinct, just bigger.

Resolving power

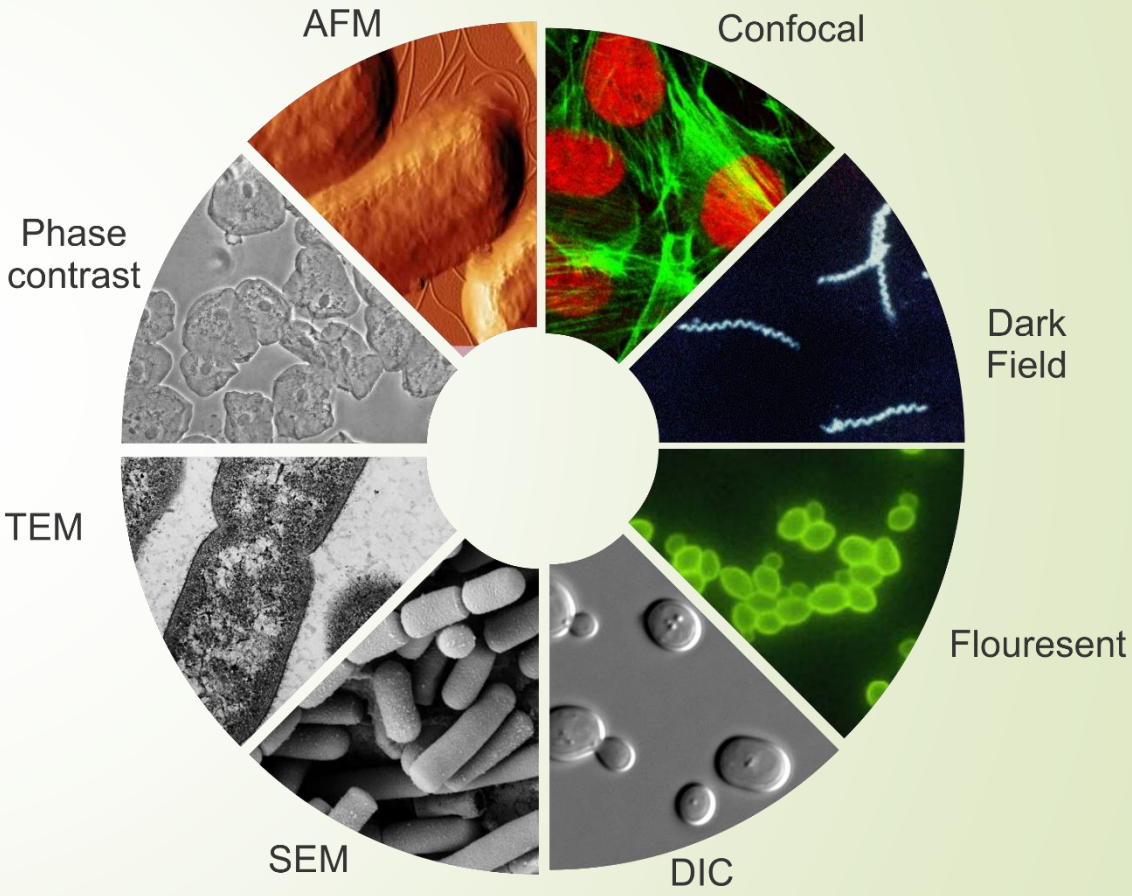
Resolution



Higher resolution

Lower resolution

Other types of Microscopes

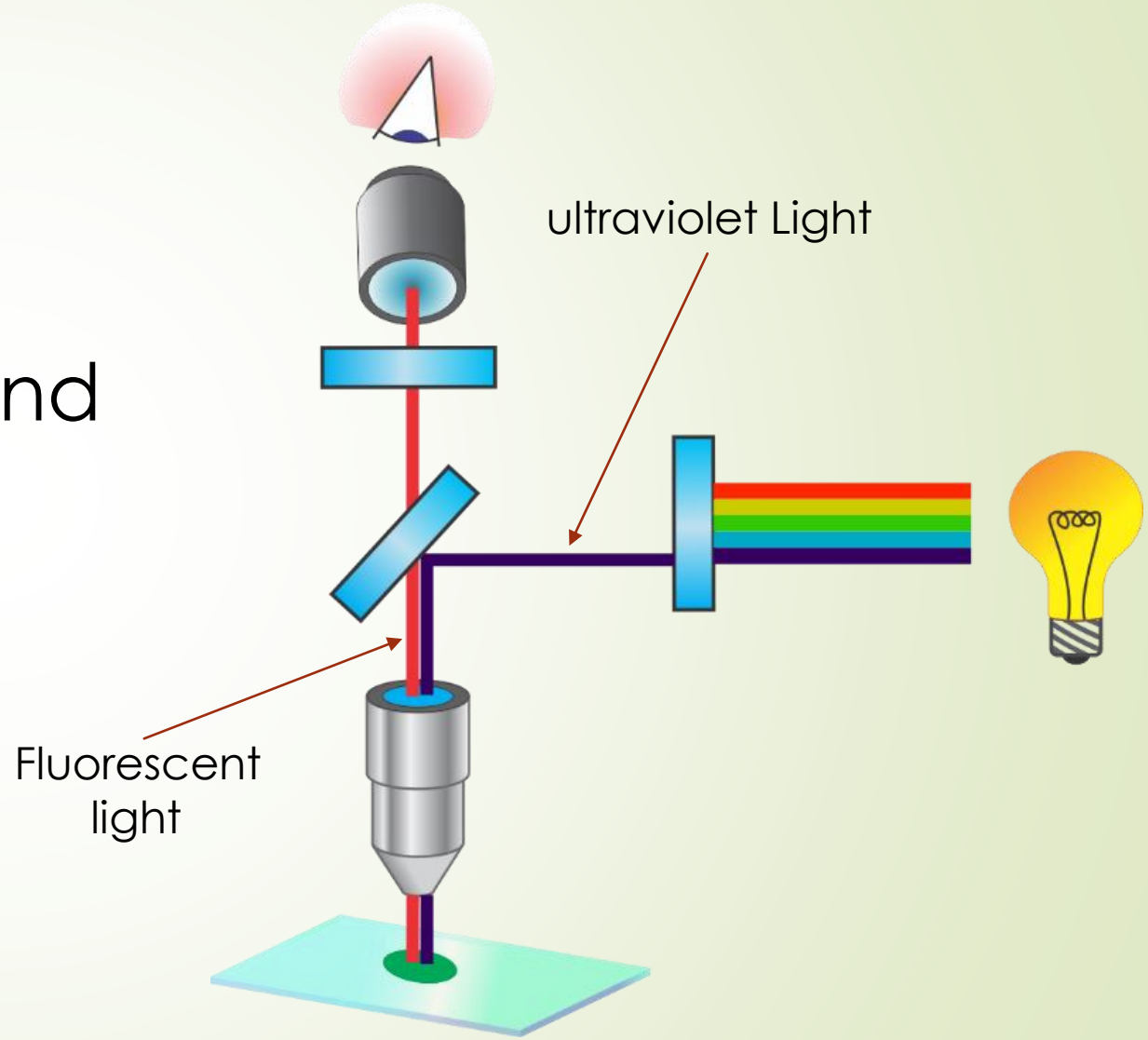


Fluorescence microscope

- In this microscope, the source of illumination is **ultraviolet (U.V) rays**.
- Specimens are stained with a **fluorescent dyes**.
- Fluorescent dyes absorb the UV rays and then re-emit it at a longer visible wavelength (visible light).

Fluorescence microscope

The fluorescence microscope **exposes** a specimen to ultraviolet, and the image is formed with the **resulting** fluorescent light.



Fluorescence microscope

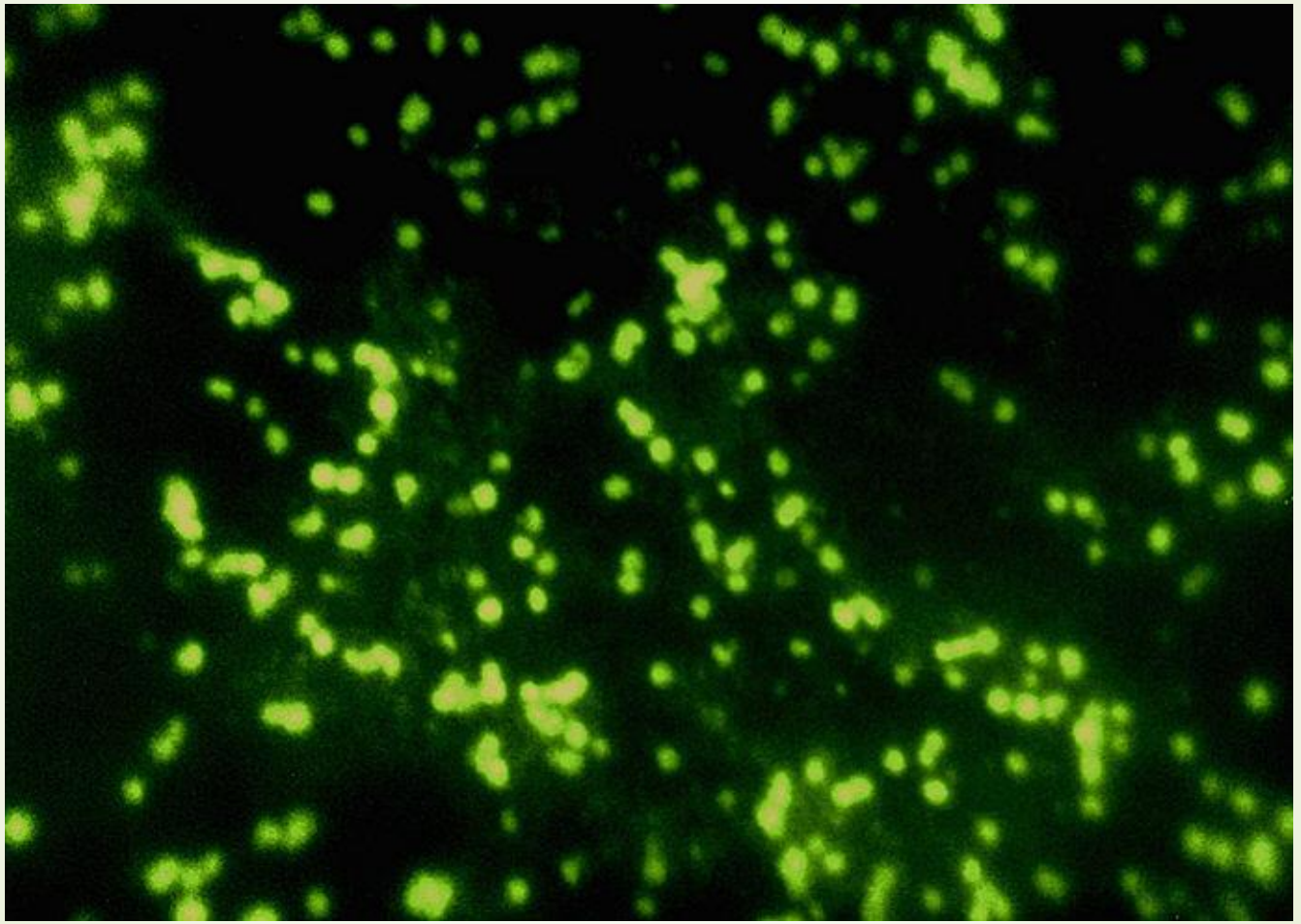
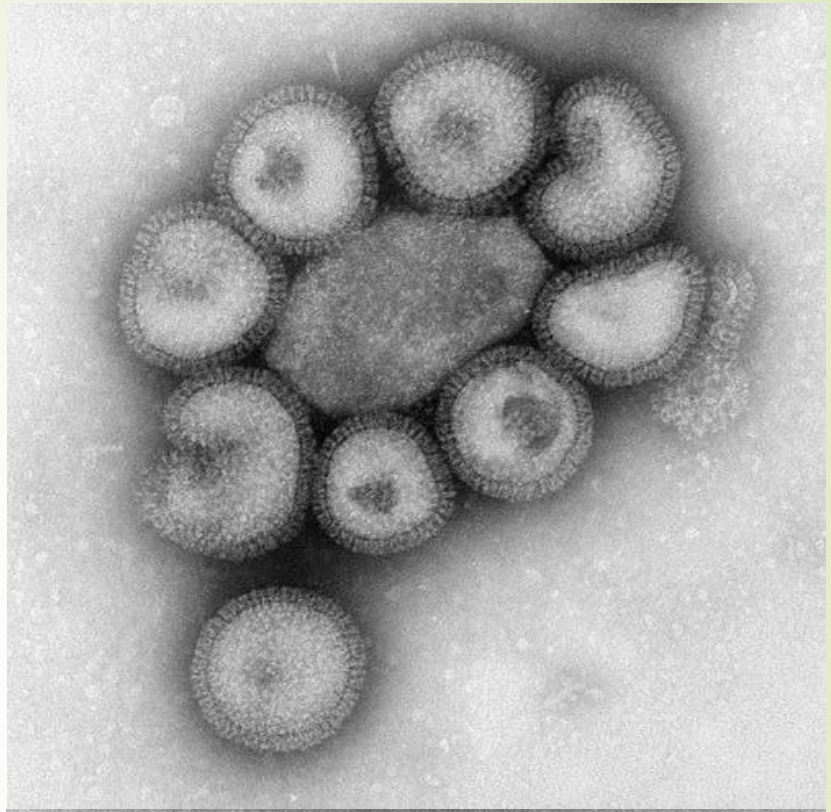
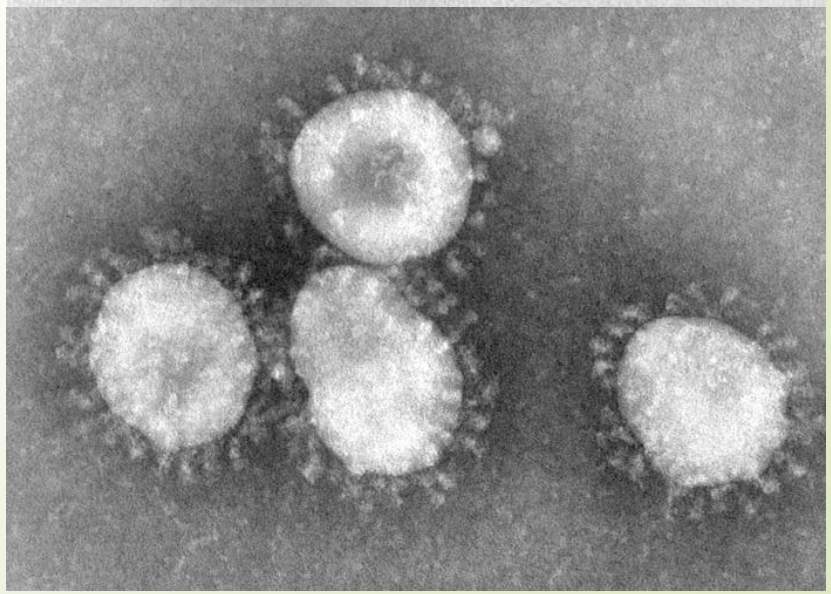


Image of *Neisseria meningitidis seen under* Fluorescence *microscope*

Electron Microscope

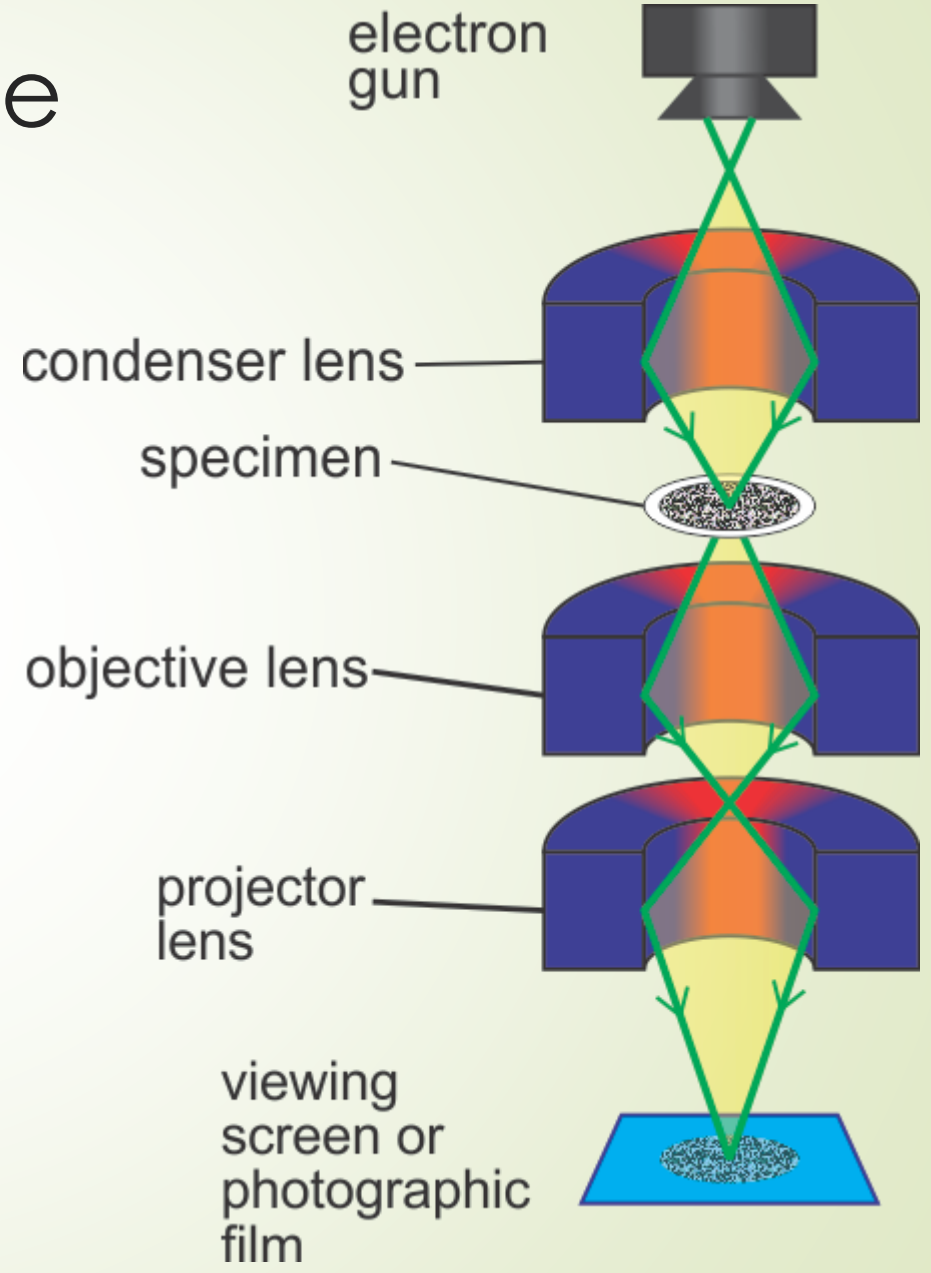
The electron microscope is the only means to see the **viruses** & the fine structure of bacteria & tissue cells.





Electron Microscope

- The source of illumination in this microscope is **electron-gun** which produces an **electron beam.**
- As the electrons strike the specimen, they are differentially ***scattered*** by the number and mass of atoms in the specimen.
- A final image of the object is formed by **electromagnetic lenses** on a **fluorescent screen**.



Staining

Staining

- In their natural state, most of the cells and microorganisms that we observe under the microscope are almost colorless.
- It is necessary to stain bacteria before they can be viewed with the light microscope.

Types of Stains

There are two types of Stains:

1. Simple stains
2. Differential stains

Simple stain

- A simple stain has a single basic dye.
- A Simple stain will give the **same color** to all types of organisms.
- It is used to show shapes of cells and arrangement of bacteria.
- **Example** → Methylene blue stain.

Differential stain

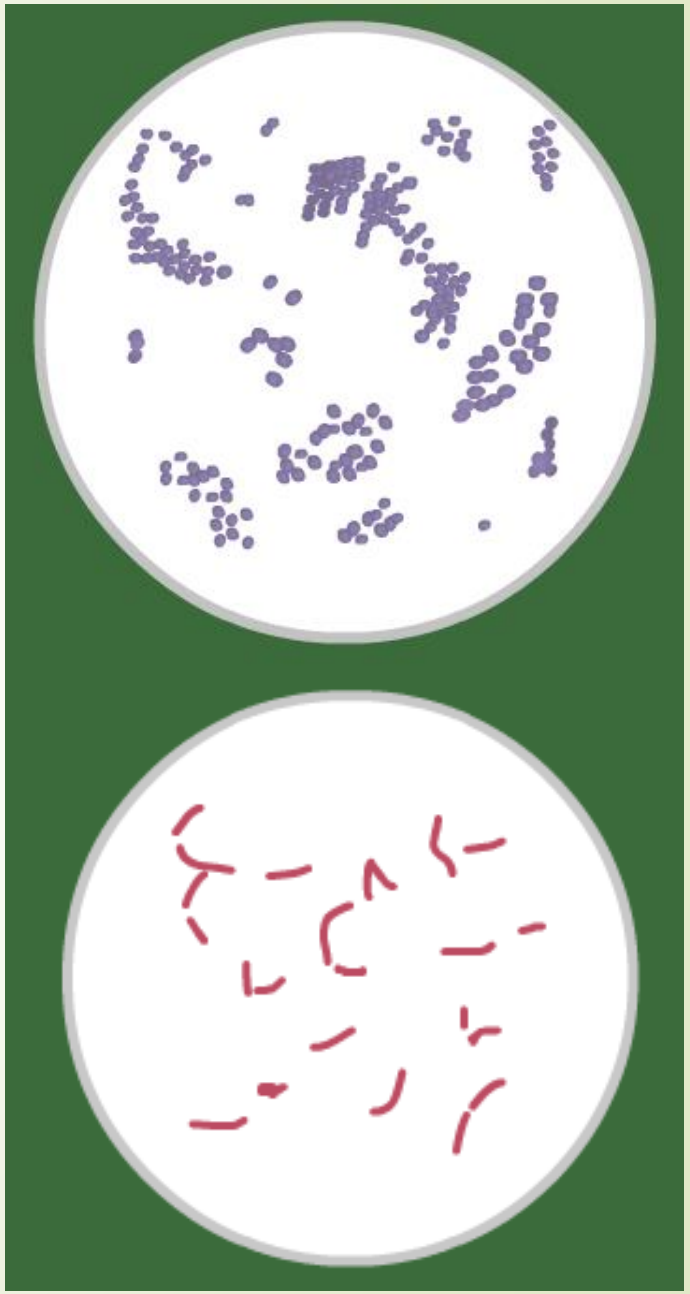
- A differential stain consists of two or more dyes.
- Differential staining procedure helps to **identify** bacteria based on their staining characteristics.
- **Examples** → Gram stain and acid fast-stain.

Types of Stains

Type	Simple stains	Differential stains
Number of Dyes Used	Uses a single dye	Uses two or more dyes
Observed details	Size, shape, and arrangement of cells	Size, shape, and arrangement of cells + Distinguish different types of bacteria
Examples	Methylene blue stain	Gram stain

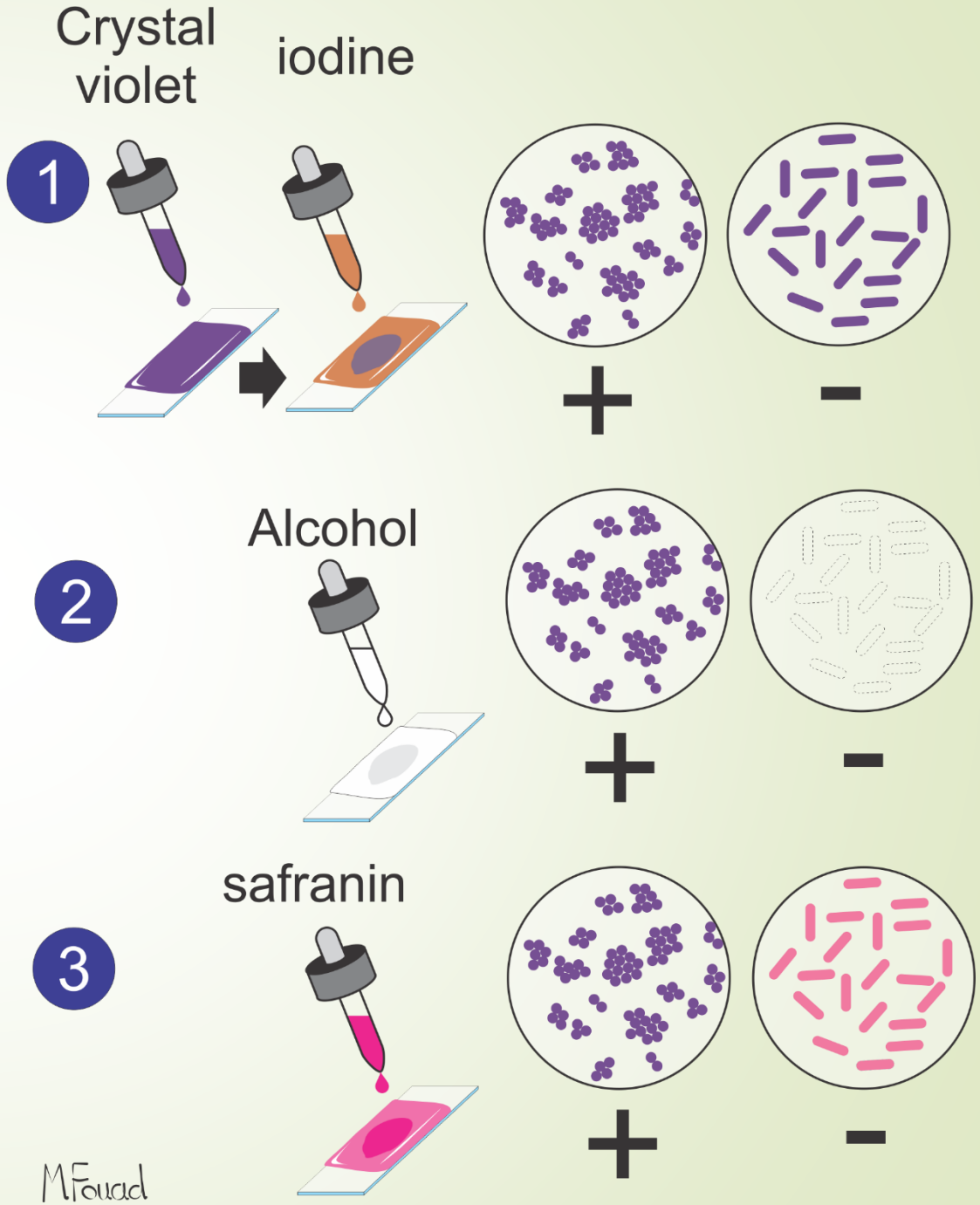
Gram Stain

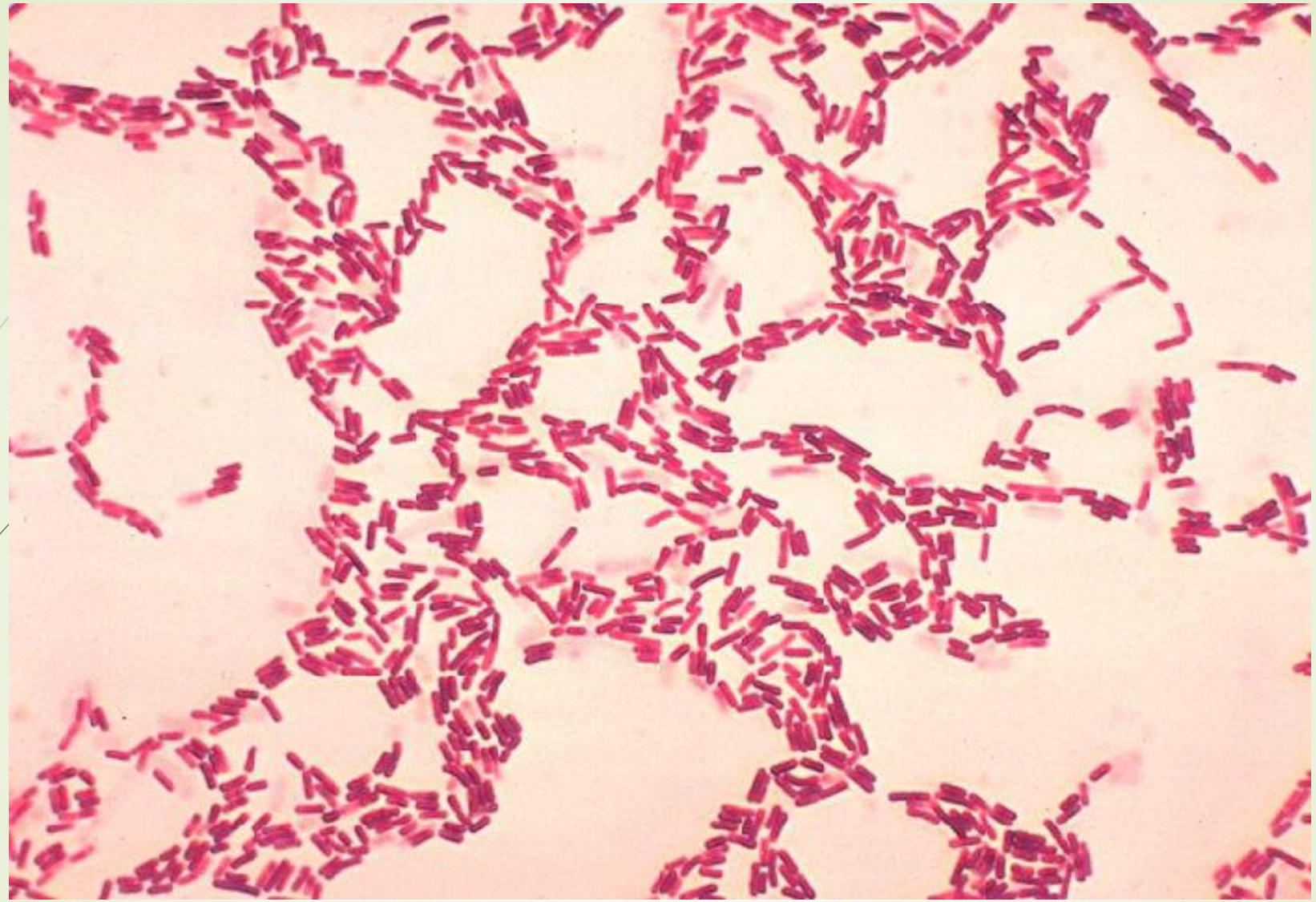
- The gram stain is most commonly used staining procedure.
- It separates bacteria into two classifications according to the composition of their cell walls:
  1. **Gram positive** which take blue or violet color.
  2. Gram negative which take pink color.



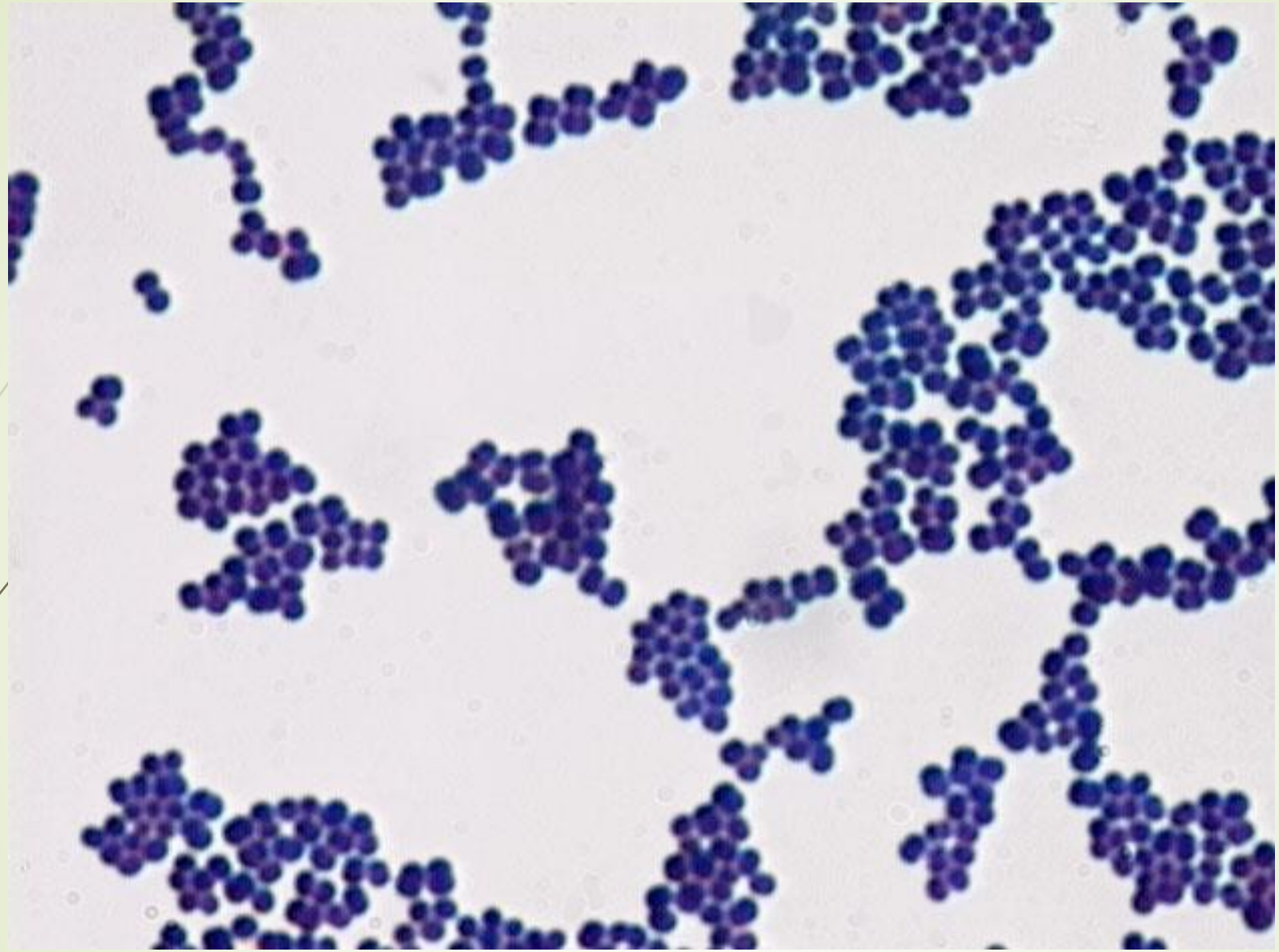
Steps of gram stain

- **Crystal violet** is applied first. It stain all cells blue.
- **Iodine** binds to crystal violet and traps it in the cell.
- **Ethanol**, extracts the blue dye from gram-negative bacteria; the gram-positive bacteria remain blue.
- **Safranin** stains the decolorized gram-negative cells pink; the gram-positive cells remain blue.





Gram Negative Bacilli



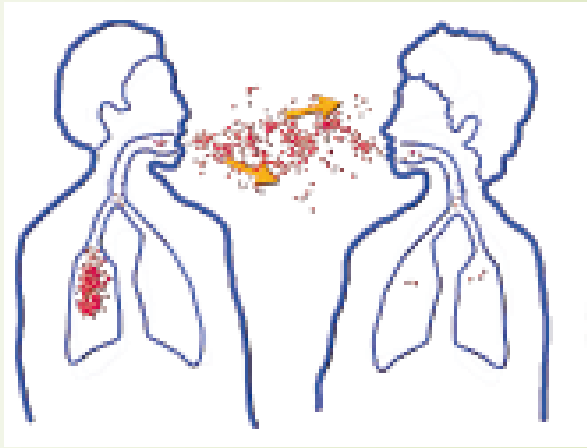
Gram Positive Cocci

Acid Fast stains

- Acid-fast stains are useful in identification of **acid-fast bacteria**.
- Acid fast bacteria are **difficult to stain**, the bacteria takes up stain by prolonged application or by heating.
- However, once stained it **resist decolonization** even with **acidic** solution.

Acid Fast stains

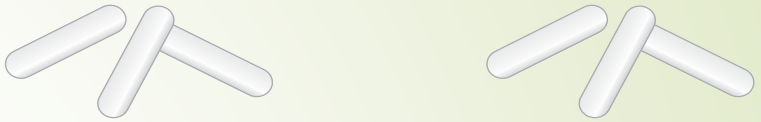
- The cell walls of acid fast bacilli contain mycolic acids (waxy material).
- The most clinically important acid-fast bacterium is *Mycobacterium tuberculosis* the causative agent of tuberculosis (مرض السل).



Acid fast stain → Ziehl-Neelsen stain

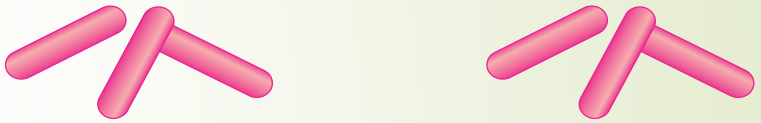
Cells prior to staining are colorless

1



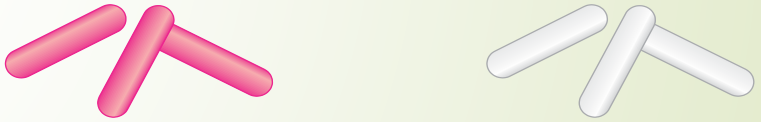
Cells are colored red by hot carbolfuchsin

2



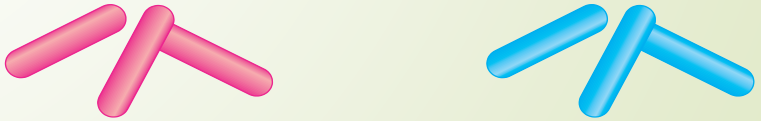
The decolorizing agent, acid-alcohol removes the red color from non acid-fast cells; acid fast cells retain the stain.

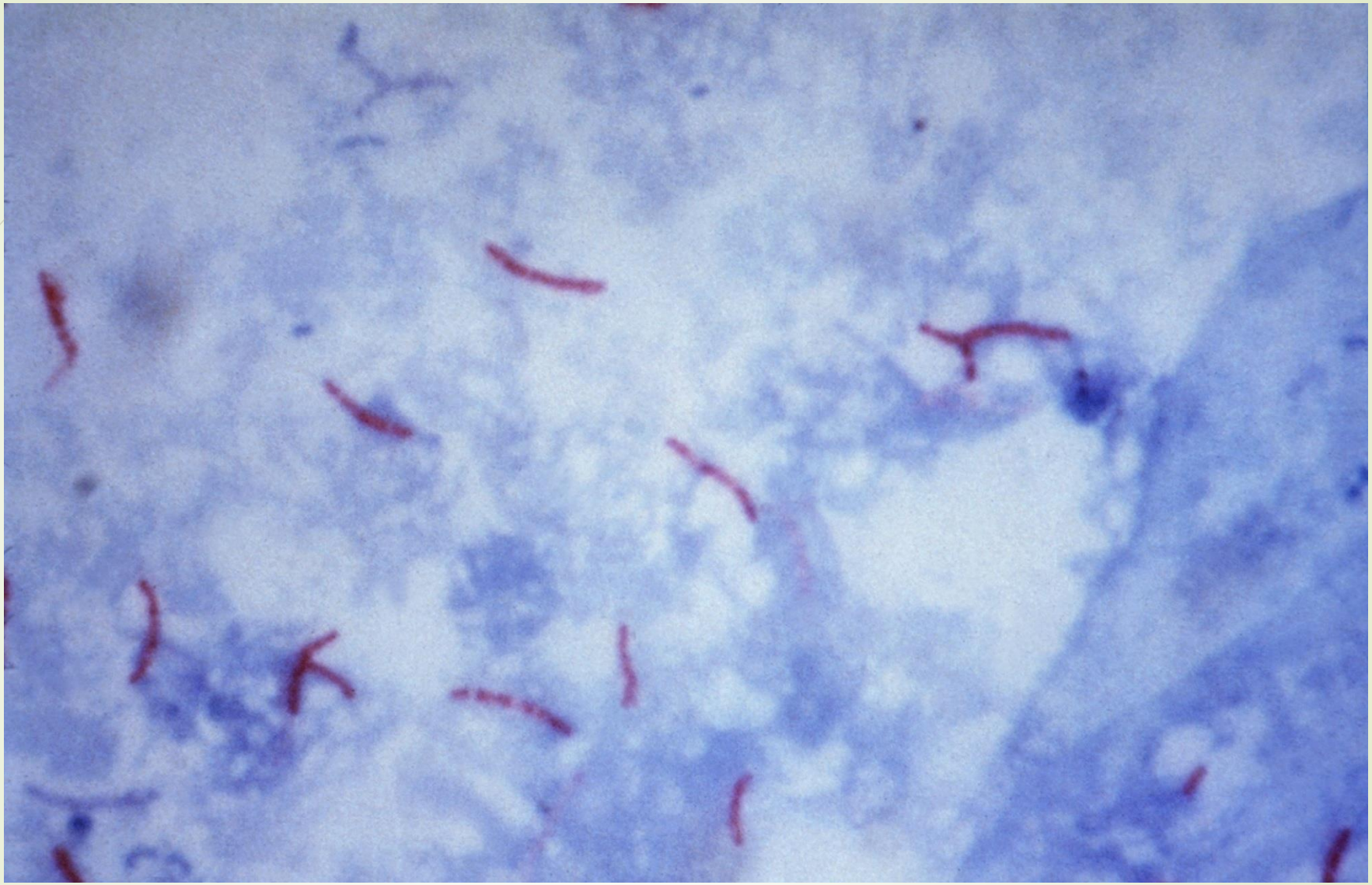
3



Non acid fast cells take up the counter-stain, methylene blue, and are colored blue; acid fast cells remain red.

4

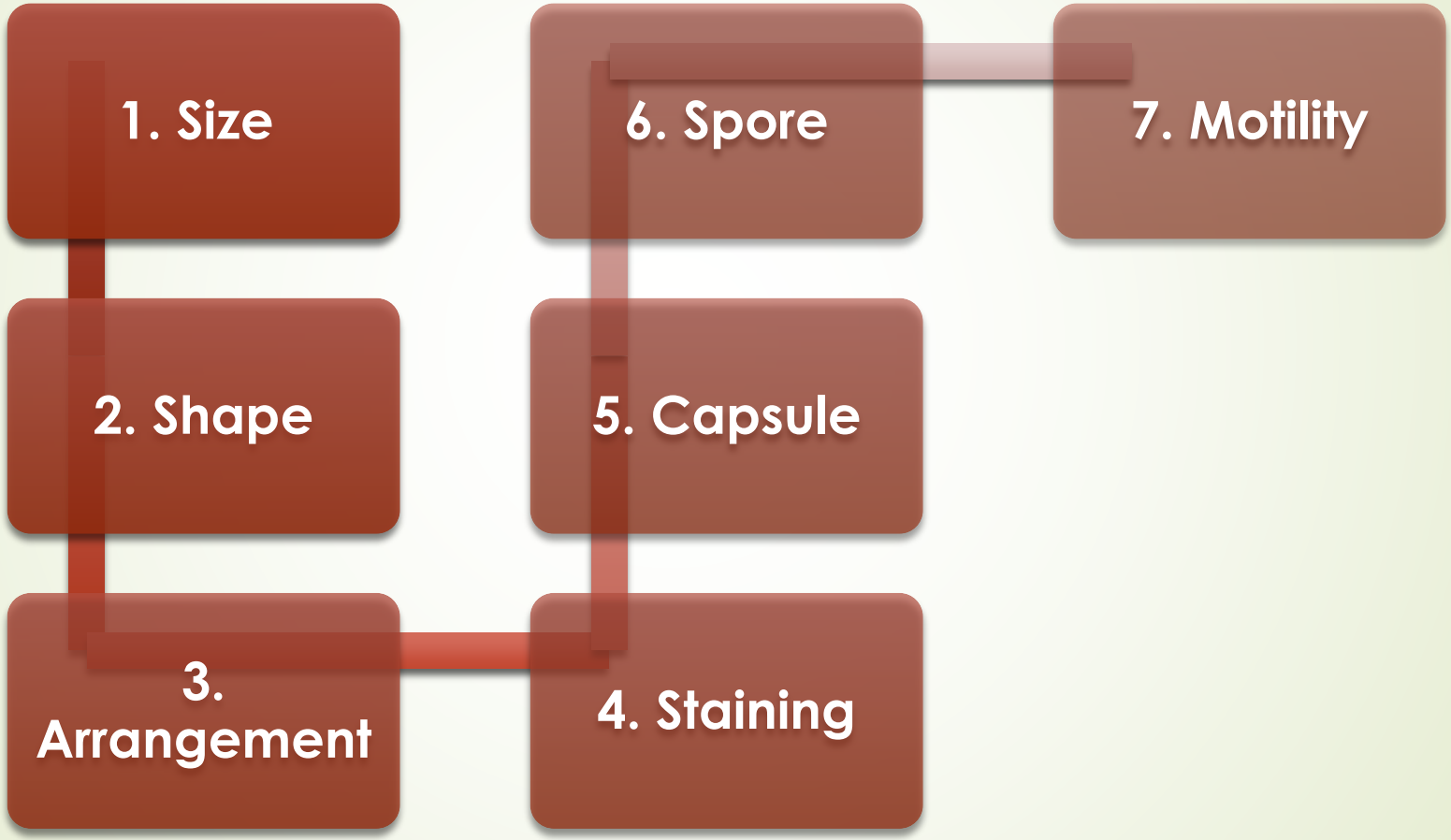




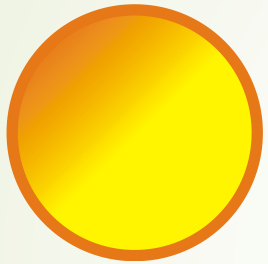
Mycobacterium tuberculosis bacteria stained using acid-fast Ziehl-Neelsen stain.

BACTERIAL MORPHOLOGY

BACTERIAL MORPHOLOGY

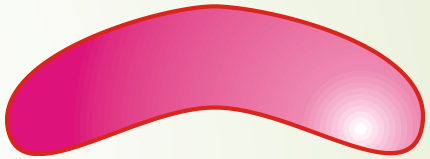


2. Bacterial shapes

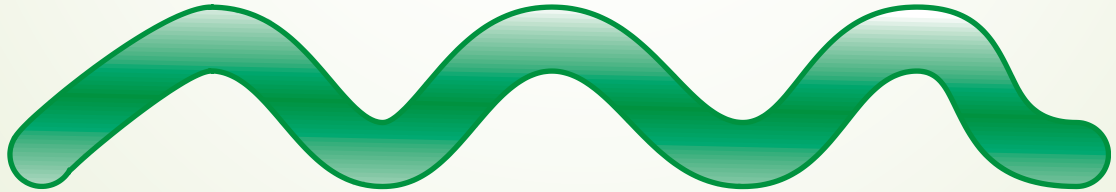


Coccus

Bacillus

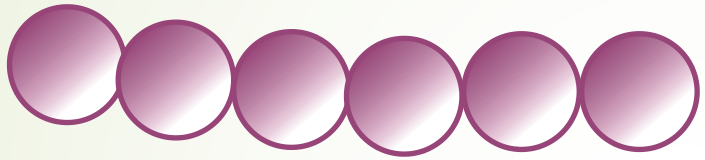


Vibrio

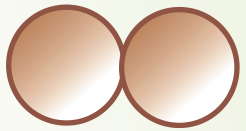


Spirochete

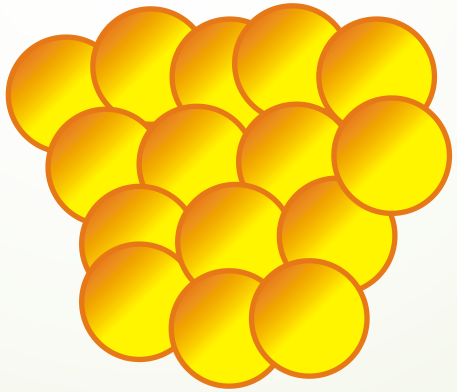
3. Bacterial arrangement



Streptococci
(Chain)



Diplococci
(Pair)

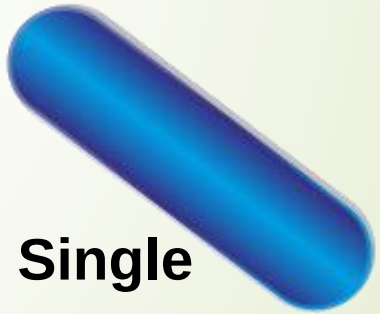


Staphylococci
(Cluster)

3. Bacterial arrangement

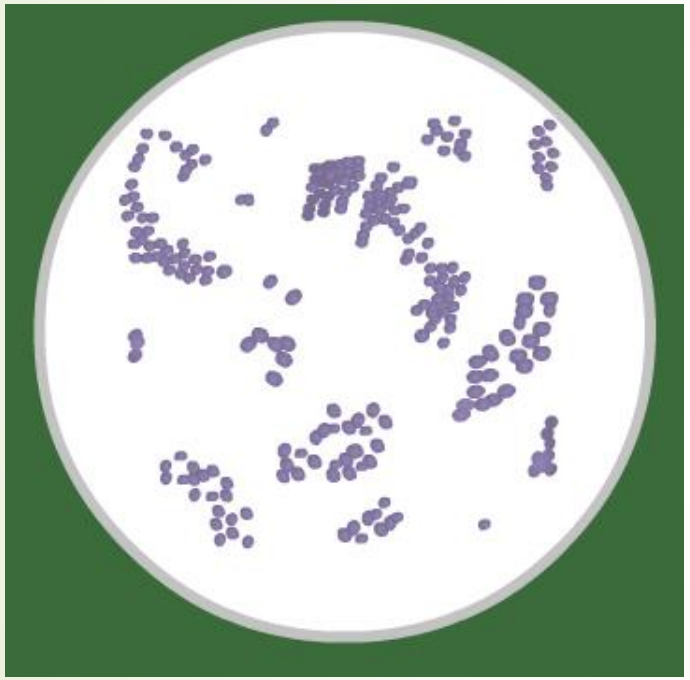
Chain

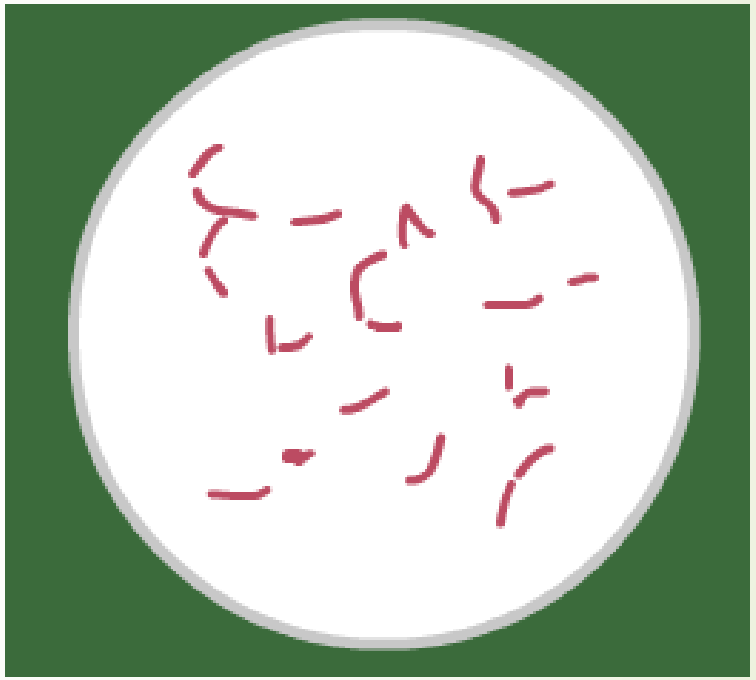
Palisade



Single

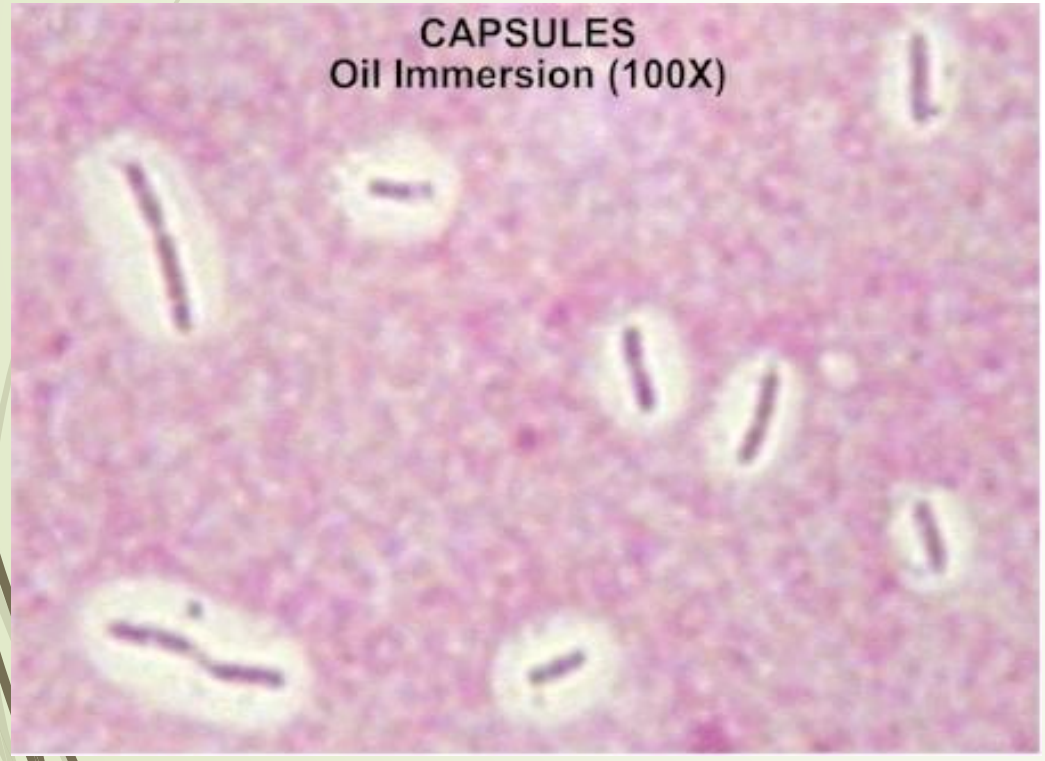
4. Staining

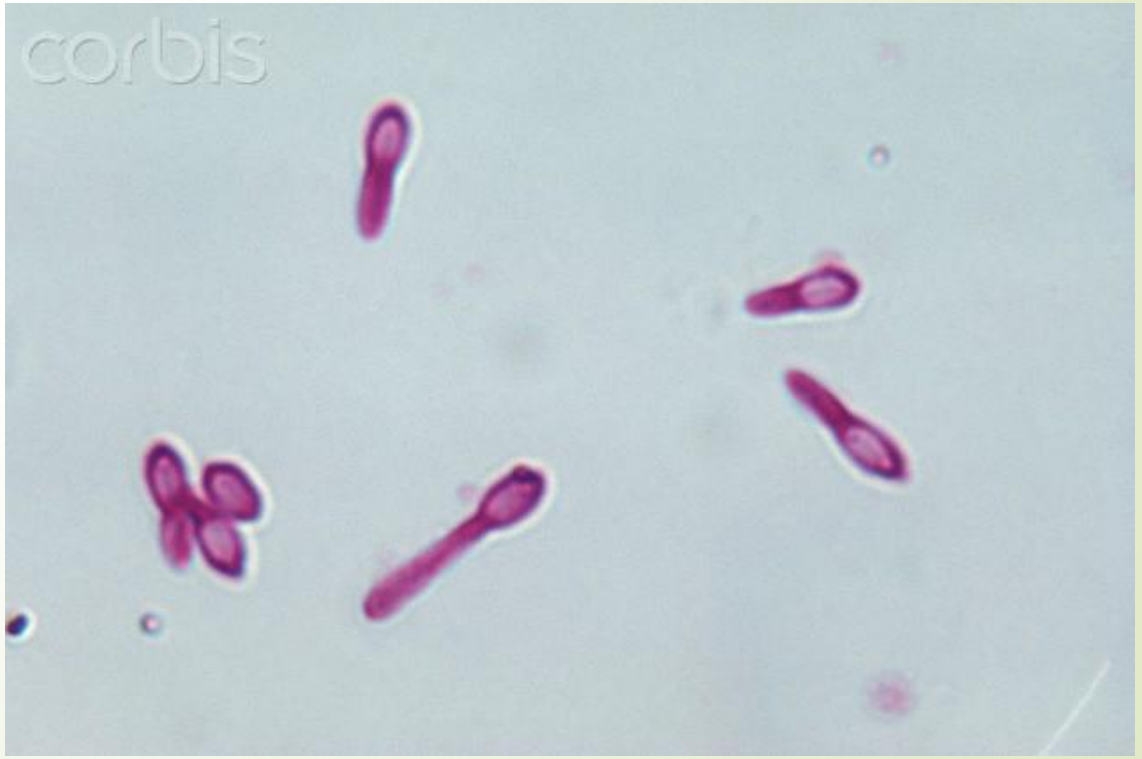




Gram positive → Blue or violet color
Gram negative → Red color.

5. Capsule
6. Spore
7. Motility





Quizzes

1. What is the total magnification when using 40× objective of a compound light microscope equipped with a 10× ocular lens?

A. 40×

B. 50×

C. 400×

D. 4000×

2. Viruses can be seen by _____?

A. Fluorescent microscope

B. Dark-ground microscope

C. Electron microscope

D. Fluorescent microscope

3. The part of the microscope that used to <u>focus</u> the lenses on the specimen is called _____?

A. The stage
B. The coarse and fine adjustment knobs
C. The light source
D. The condenser

4. The part of the microscope where the slide is put is called:

A. The stage
B. The coarse and fine adjustment knobs
C. The light source
D. The condenser

5. Simple stain:

A. Use two or more dyes
B. Distinguish different types of bacteria
C. Show bacterial size, shape and arrangement
D. None of the above

6. Gram stain is

A. Simple stain
B. Differential stain

7. In Fluorescence microscope the source illumination is:

A. Visible light

B. Ultraviolet rays

C. Electron gun

8. Example of Simple stain

A. Gram stain
B. Acid Fast stain
C. Methylene blue stain

9. Gram stain separate the bacteria into two categories:

A. Gram **positive** that take **red** color and gram **negative** that take **blue** color

B. Gram **positive** that take **blue** color and gram negative that are red color

C. Gram **positive** that take **green** color and gram **negative** that take **blue** color

D. Gram **positive** that take **red** color and gram **negative** that take **green** color

10. In Ziehl-Neelsen stain Acid fast bacteria appear as:

A. Red against blue background
B. Blue against red background
C. Blue against green background

11. In Gram stain we use the following dyes in order:

A. Crystal violet → Ethanol → Iodine → Safranin.

B. Safranin → Crystal violet → Iodine → Ethanol.

C. Iodine → Crystal violet → Ethanol → Safranin.

D. Crystal violet → Iodine → Ethanol → Safranin.

12. Fill in the spaces:

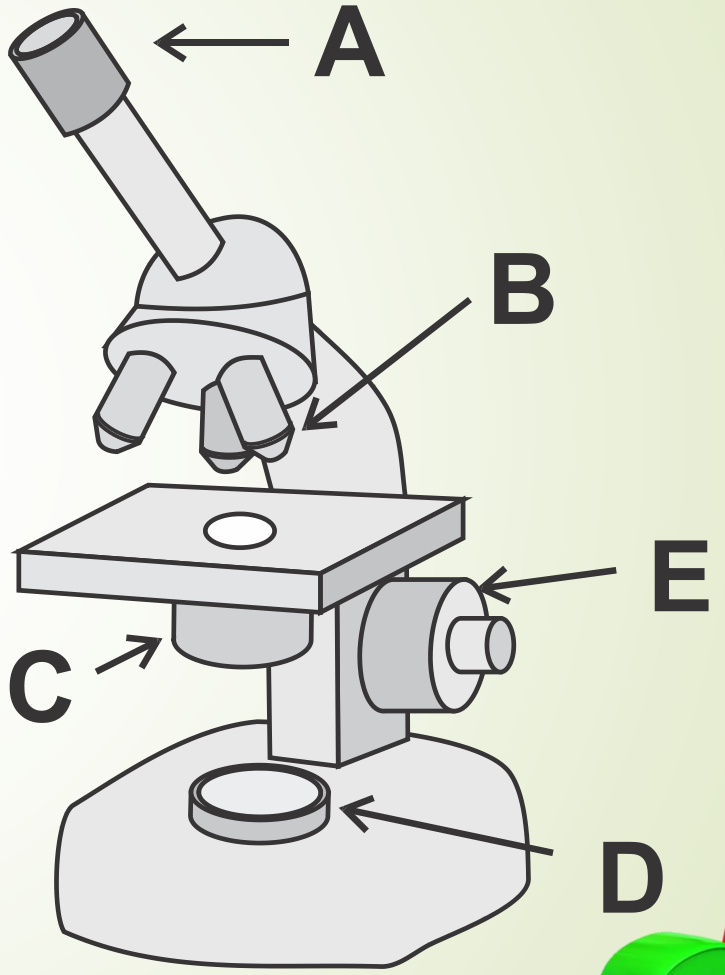
A:……

B:……

C:……

D:……

E:……



13. Reorder the steps of gram stain: (Ethanol, Iodine, Crystal violet, Safranin)

A:……

B: ……

C:……

D:……

14. Reorder the steps of Ziehl-Neelsen stain

[....] Acid alcohol

[....] Methylene blue

[....] Hot Carbol-fuchsin

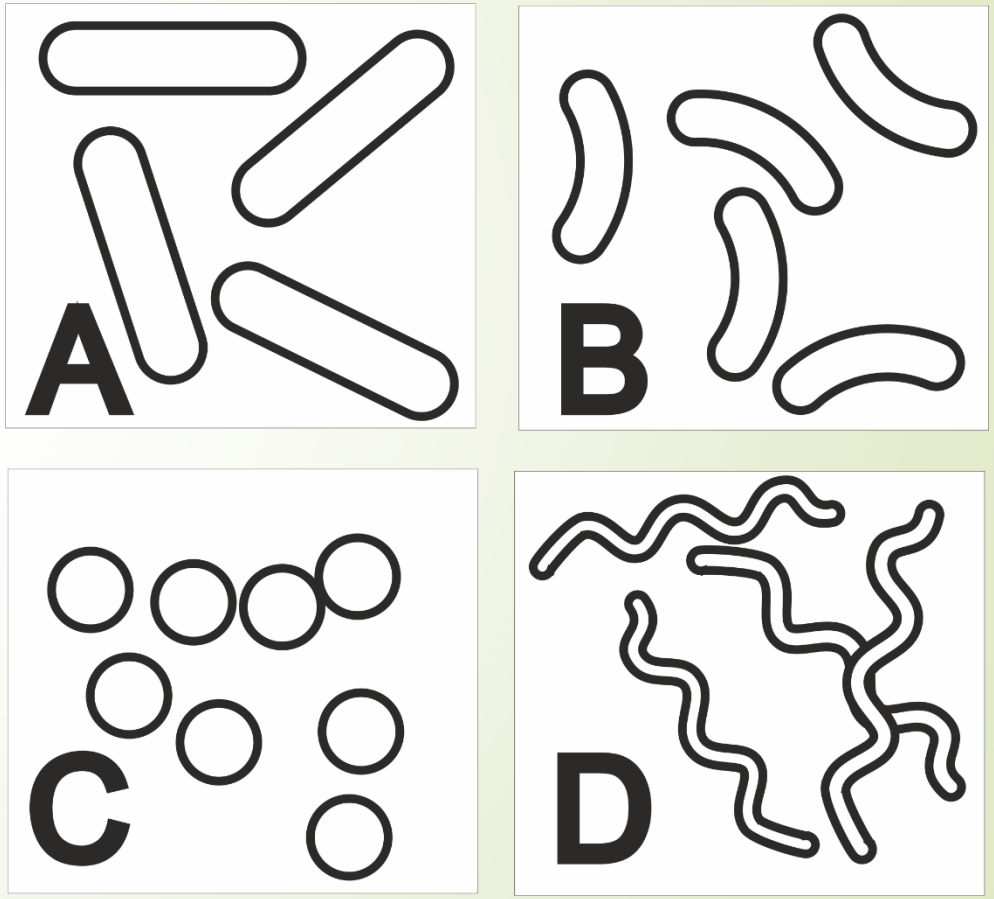
15. Fill in the spaces:

A:.....

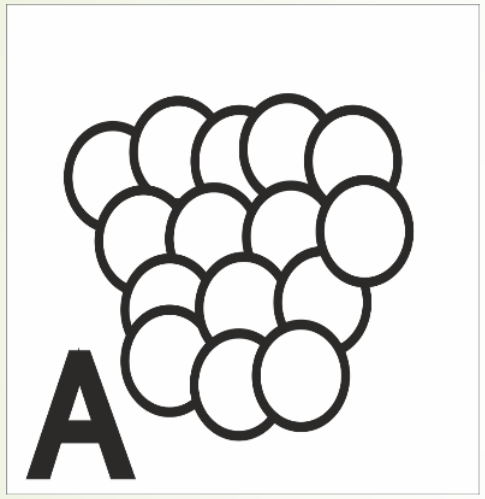
B:.....

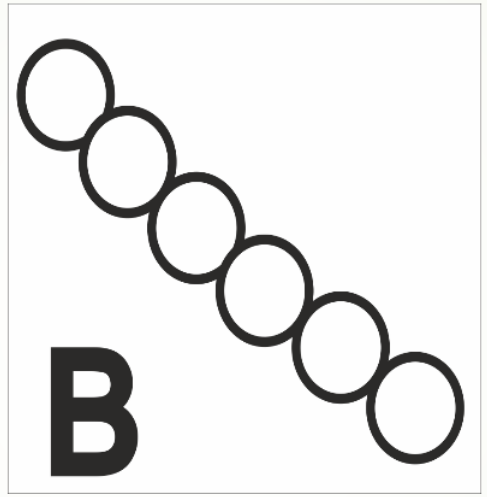
C:.....

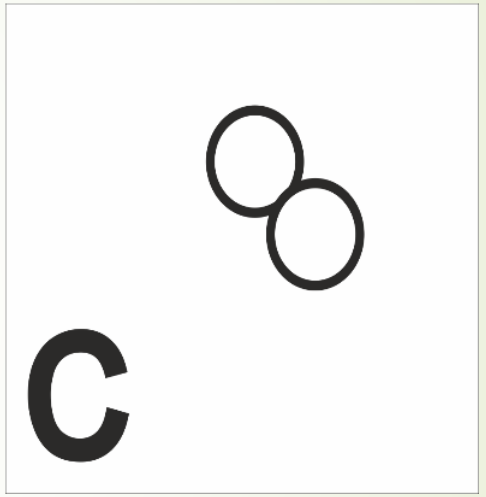
D:.....



Study the diagram and choose the correct answer:







16. Shape (A) is:

A. Diplococci
B. Streptococci
C. Staphylococci

17. Shape (B) is:

A. Diplococci
B. Streptococci
C. Staphylococci

18. Shape (C) is:

A. Diplococci
B. Streptococci
C. Staphylococci