LS1102

Part I
OPTICAL MICROSCOPY

AND

OBSERVATION

OF

PROKARYOTIC AND EUKARYOTIC CELLS

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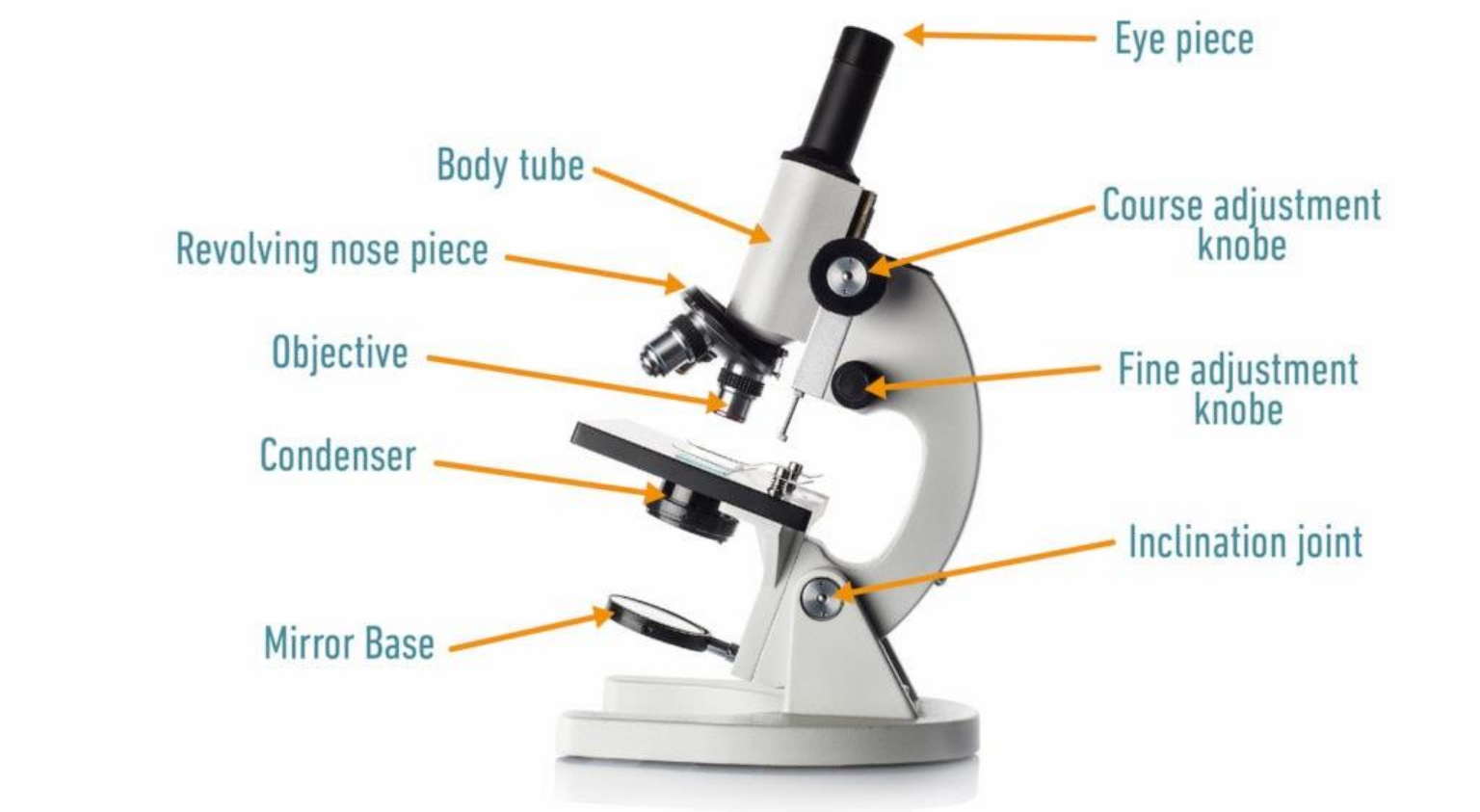
Simple Microscope

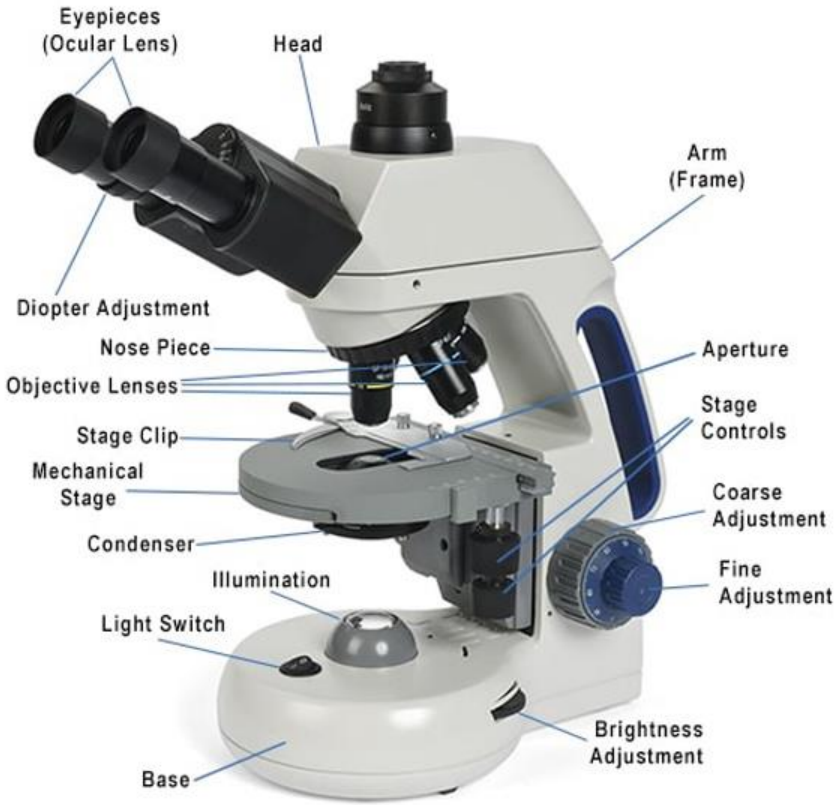
Logan's Simple Microscope (circa 1871)

- Has only one magnifying lens
- No objectives

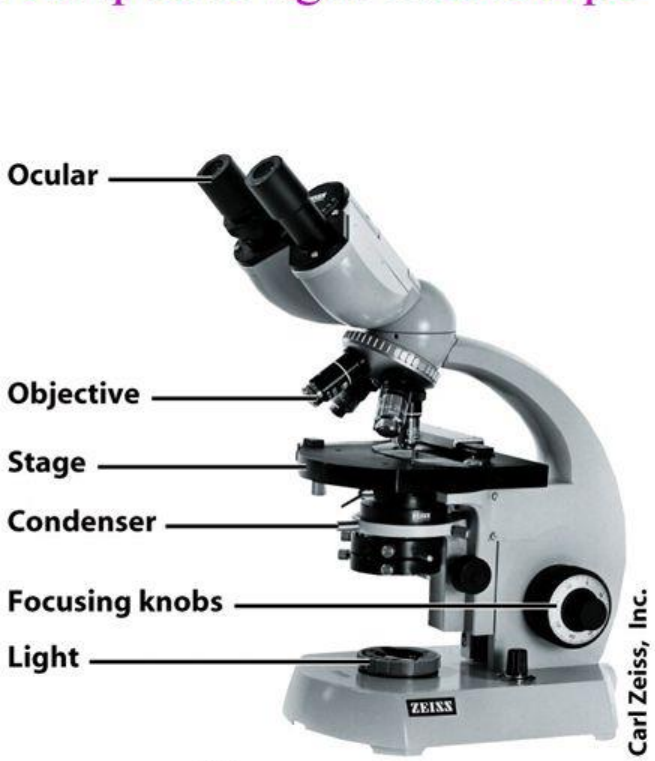
Compound Microscope

- Has two magnifying lenses
  - Oculars / eyepiece
  - Objectives

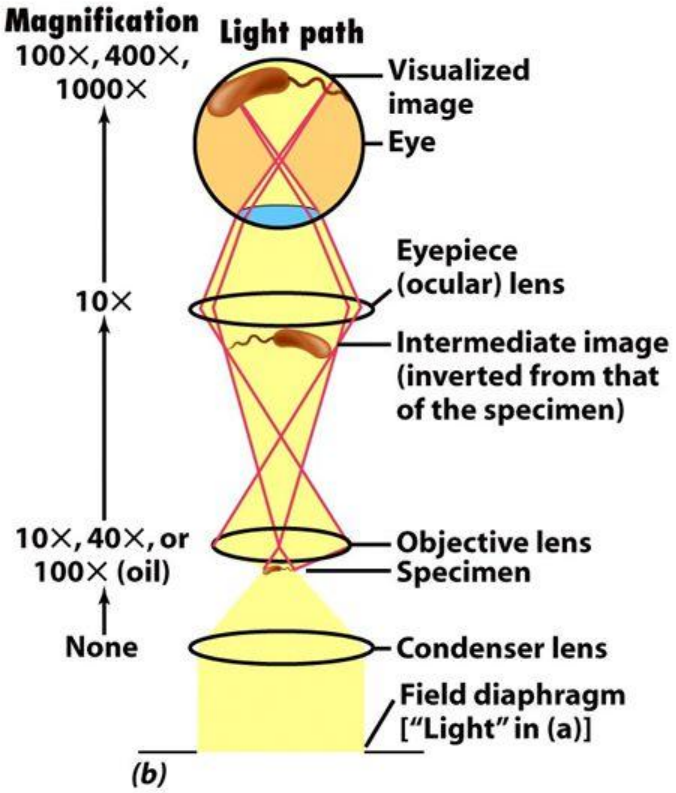

Eye piece
Body tube
Course adjustment knobe
Revolving nose piece
Objective
Fine adjustment knobe
Condenser
Inclination joint
Mirror Base


Eyepieces
(Ocular Lens)
Head
Arm
(Frame)
Diopter Adjustment
Nose Piece
Aperture
Objective Lenses
Stage
Controls
Stage Clip
Mechanical
Stage
Coarse
Adjustment
Condenser
Fine
Adjustment
Illumination
Light Switch
Brightness
Adjustment
Base

A compound light microscope



(a)



(b)

Figure 4-1 Brock Biology of Microorganisms 11/e


OLYMPUS
OLYMPUS

1. Resolution:

The resolution of an optical microscope can be defined as:

“The smallest distance between two points on a specimen that can still be distinguished as two separate entities”.

2. Magnification:

in physical terms is defined as "a measure of the ability of a lens or other optical instruments to magnify, expressed as the ratio of the size of the image to that of the object". This means, that an object of any size is magnified to form an enlarged image.

The magnification required to produce the visible image can be calculated using the formula:

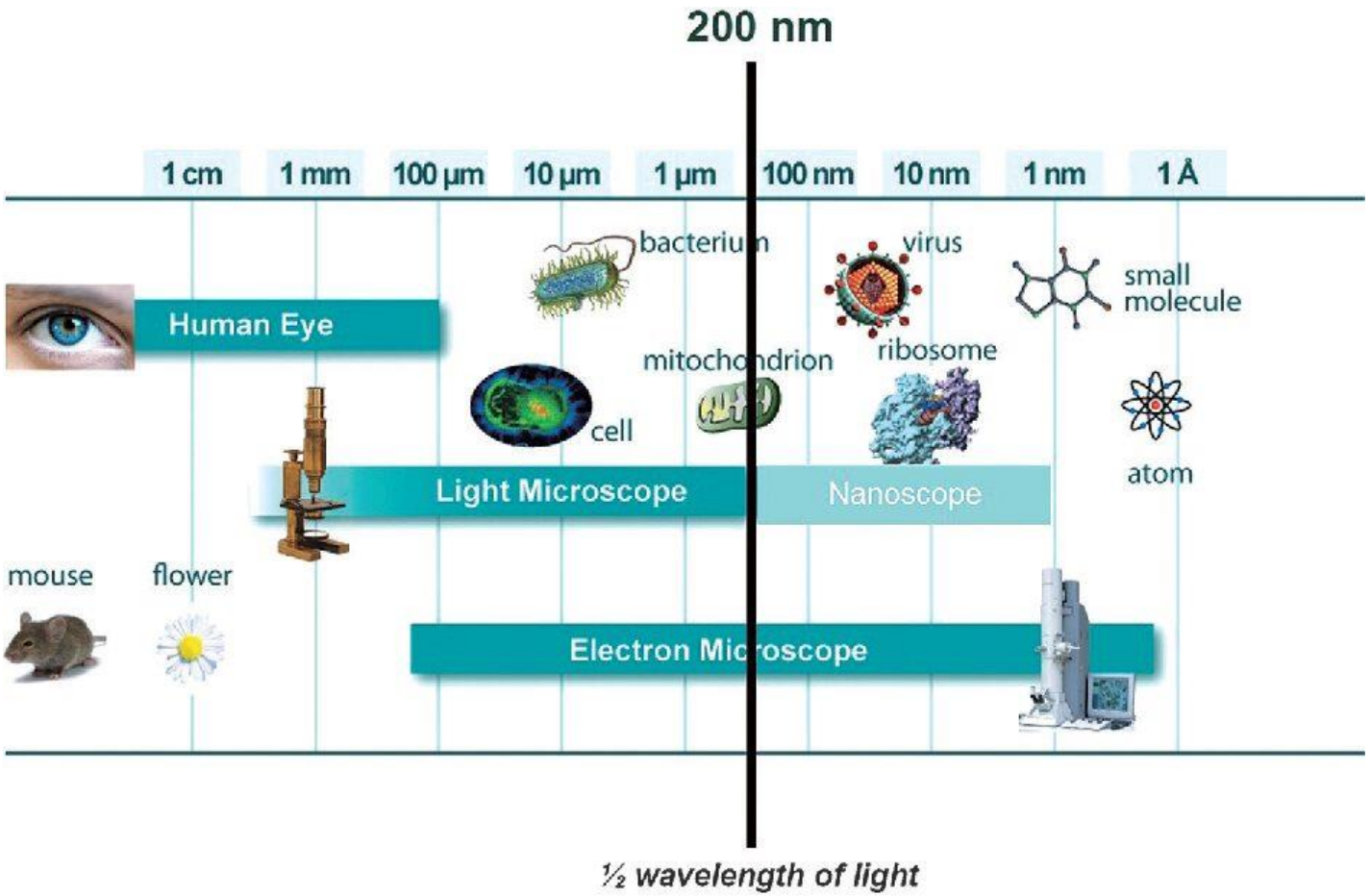
Magnification = Image ÷ Object

It is important that the all the units are in unison so that the final answer obtained is correct.

Eye piece 10X; Lenses 4X, 10X, 40X, 100X = Actual magnifications: 4X, 100X, 400X, 1000X

3. Empty magnification

No improvement in resolution


200 nm
1 cm
1 mm
100 µm
10 µm
1 µm
100 nm
10 nm
1 nm
1 Å
bacterium
virus
small molecule
Human Eye
mitochondrion
ribosome
cell
atom
Light Microscope
Nanoscope
mouse
flower
Electron Microscope
½ wavelength of light

2nd Day

Gram staining

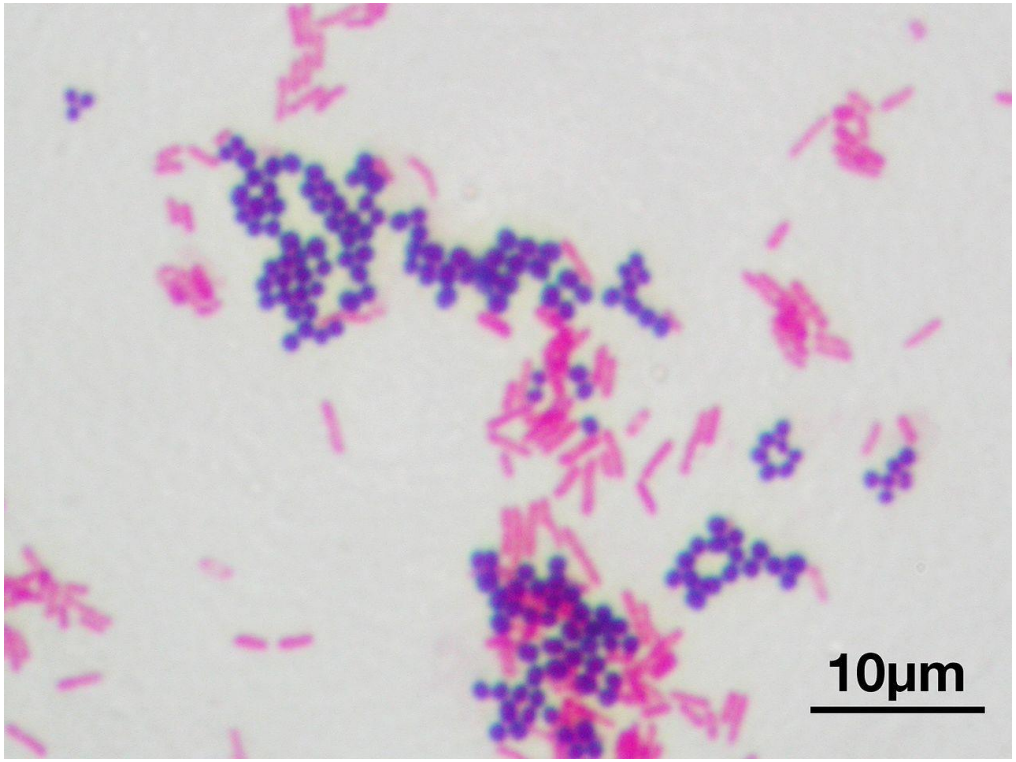
To classify bacterial species into two large groups: Gram-positive bacteria and Gram-negative bacteria.

The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls.

Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet.

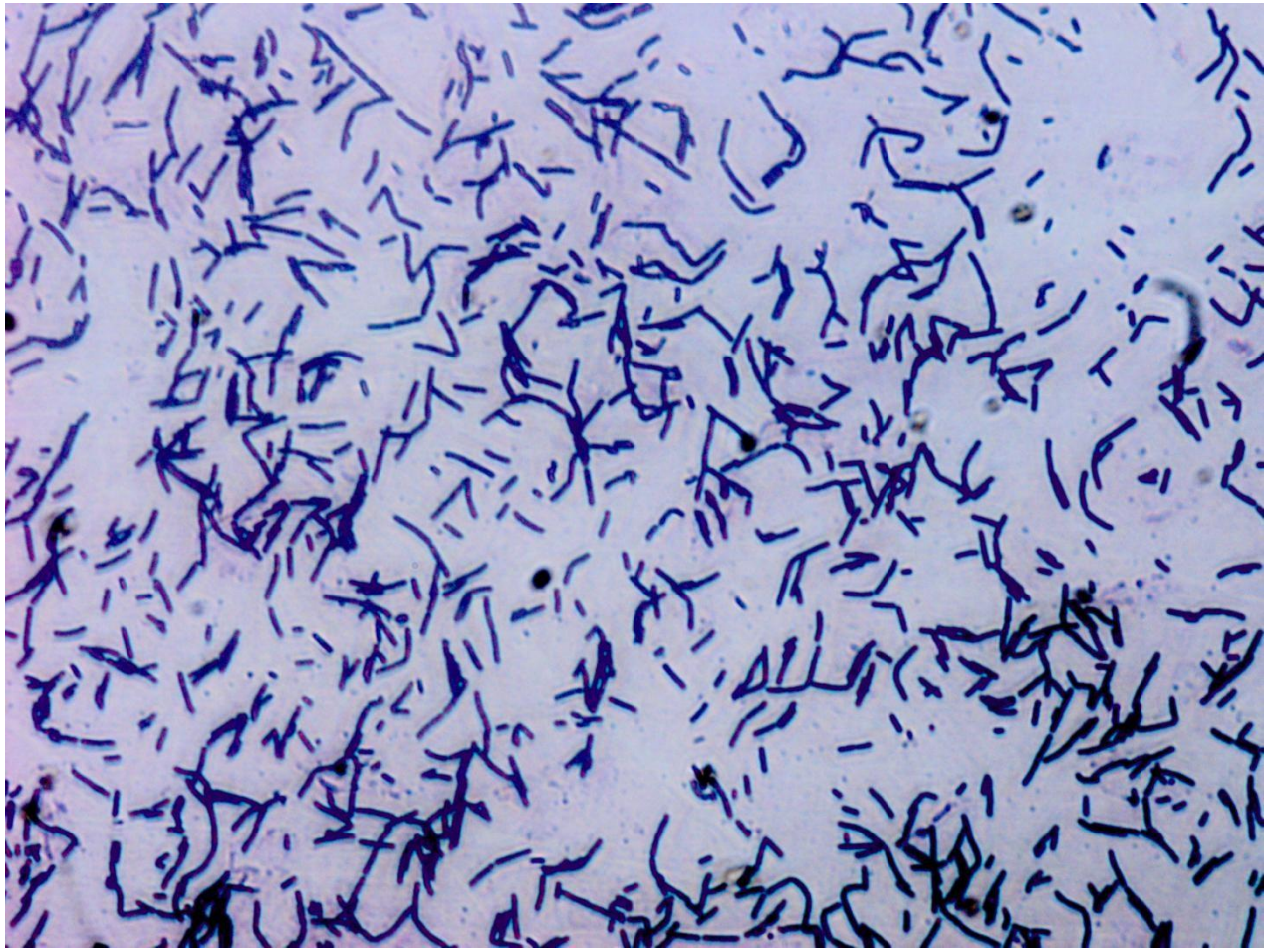
Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol. They are stained pink or red by the counterstain,[2] commonly safranin or fuchsine.



A Gram stain of mixed *Staphylococcus aureus* (*S. aureus* ATCC 25923, Gram-positive cocci, in purple) and *Escherichia coli* (*E. coli* ATCC 11775, Gram-negative bacilli, in red), the most common Gram stain reference bacteria

Gram positive

Bacillus subtilis

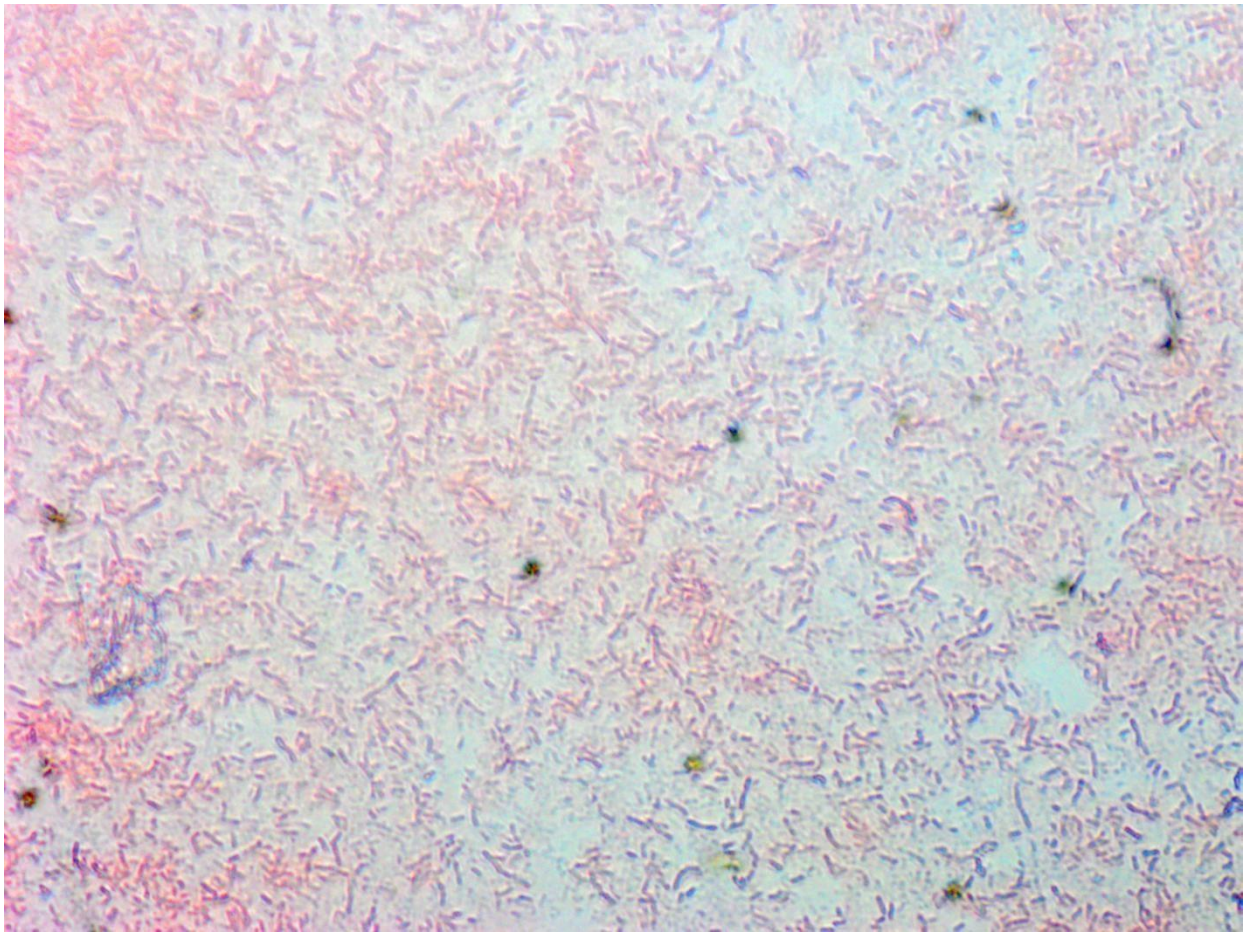


B. subtilis cells are typically rod-shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase.

Ref: Ananya, Rahul, Sudhangshu, Sudip, Lekha, and PPD; IISERK 2019

Gram Negative

Escherichia coli



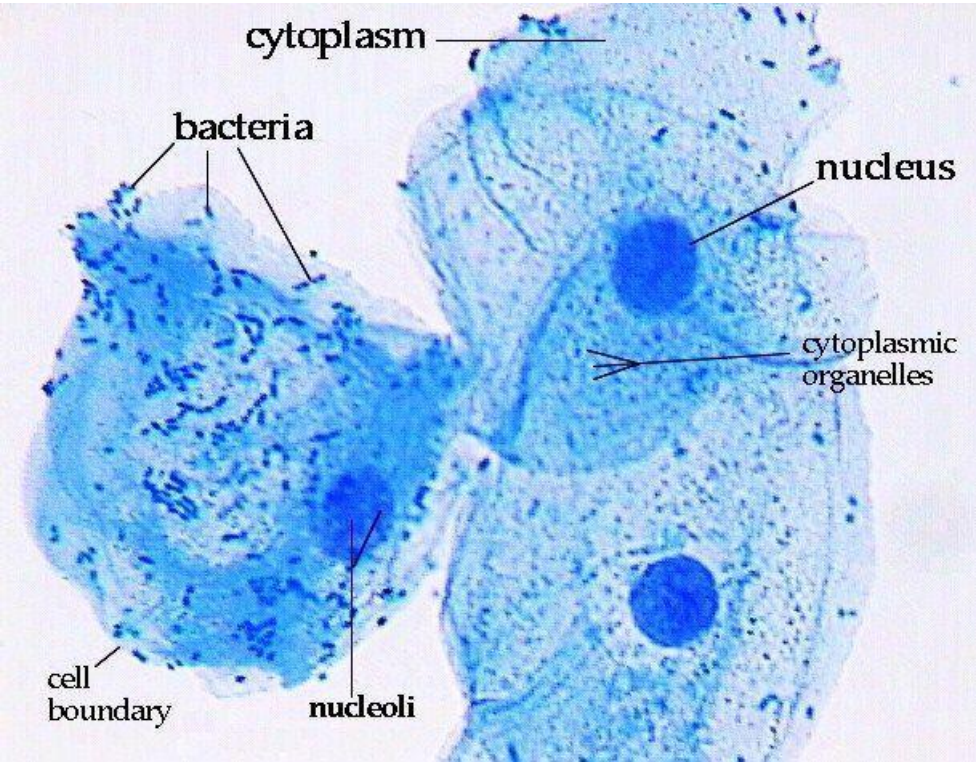
E. coli cells are rod-shaped, and are about 2.0 μm long and 0.25–1.0 μm in diameter, Average cell volume of 0.6–0.7 μm^3.
E. coli is Gram-negative because its cell wall is composed of a thin peptidoglycan layer with an outer membrane

Ref: Ananya, Rahul, Sudhangshu, Sudip, Lekha, and PPD; IISERK 2019

Human Cheek Epithelial Cells

The tissue that lines the inside of the mouth is known as the basal mucosa and is composed of squamous **epithelial cells**.

These cells divide approximately every 24 hours and are constantly shed from the body.



Epithelial cells: stained with methylene blue

Ref: internet

Protocol

Basic Cell Biology: Microscopic observation of cheek epithelial cells

To view cheek cells, gently scrape the inner of your cheek with a toothpick.

Add a drop of water (20-25µl) to the center of a glass slide and gently tap the toothpick to the center. Some of the cheek cells should fall onto the slide

Add a drop of **methylene blue** stain (specific stain) and cover with a cover slip.

Observe the cheek cells under both resolutions: low (10X) and high power (40X) in a microscope.

Draw a diagram of one cheek cell and label its parts.

(At minimum you should observe the cell membrane, nucleus and cytoplasm)