بنام خدا

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Viewing the Microbial World

- How tiny are microbes?

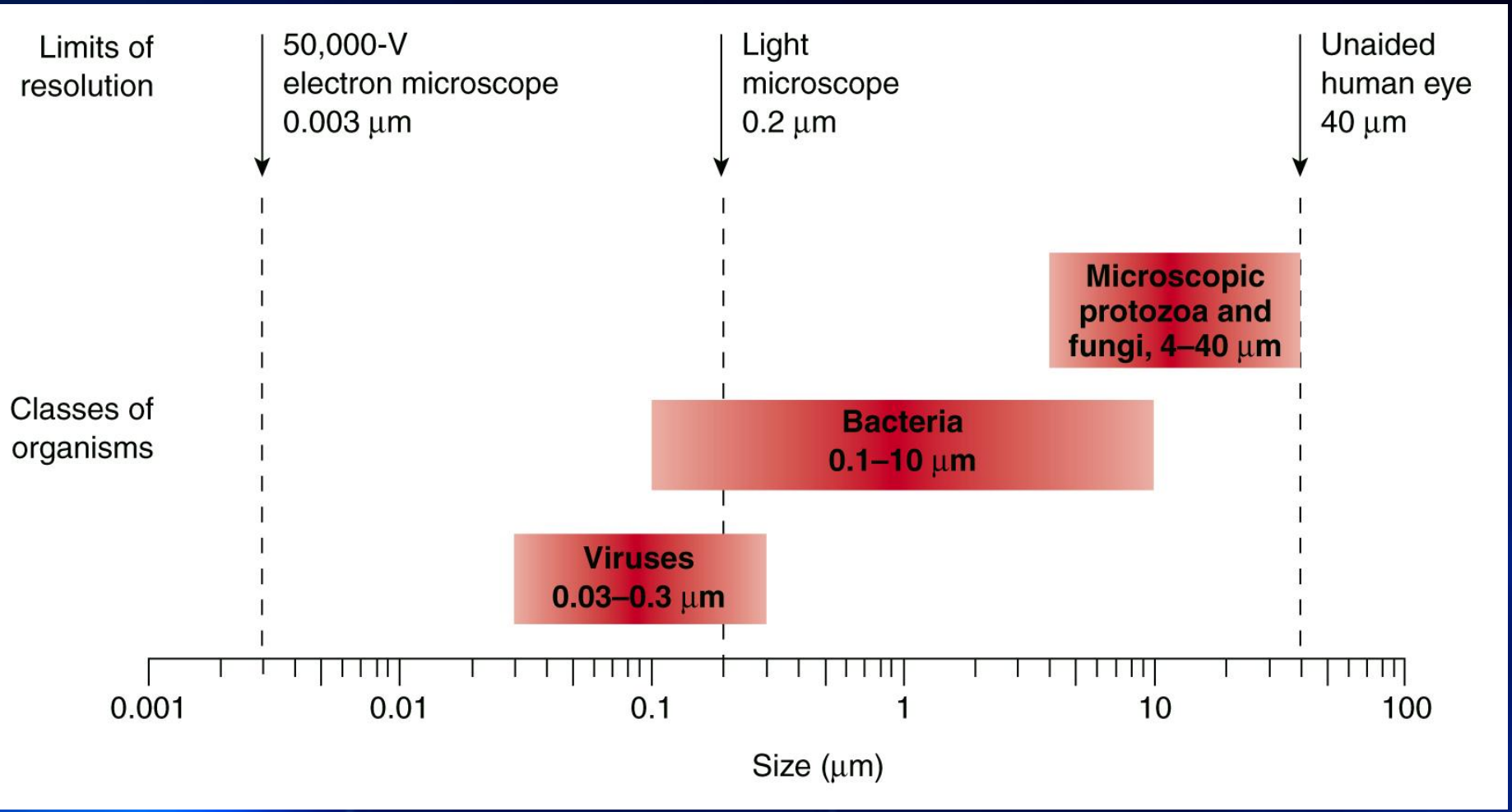
- To express the sizes of microbes, metric system is used

- μm (μ) → bacteria, protozoa

- nm (mμ) → viruses

- Most viruses causing human diseases: about 10-300 nm

Relative sizes of microorganisms



Increasing size & complexity:

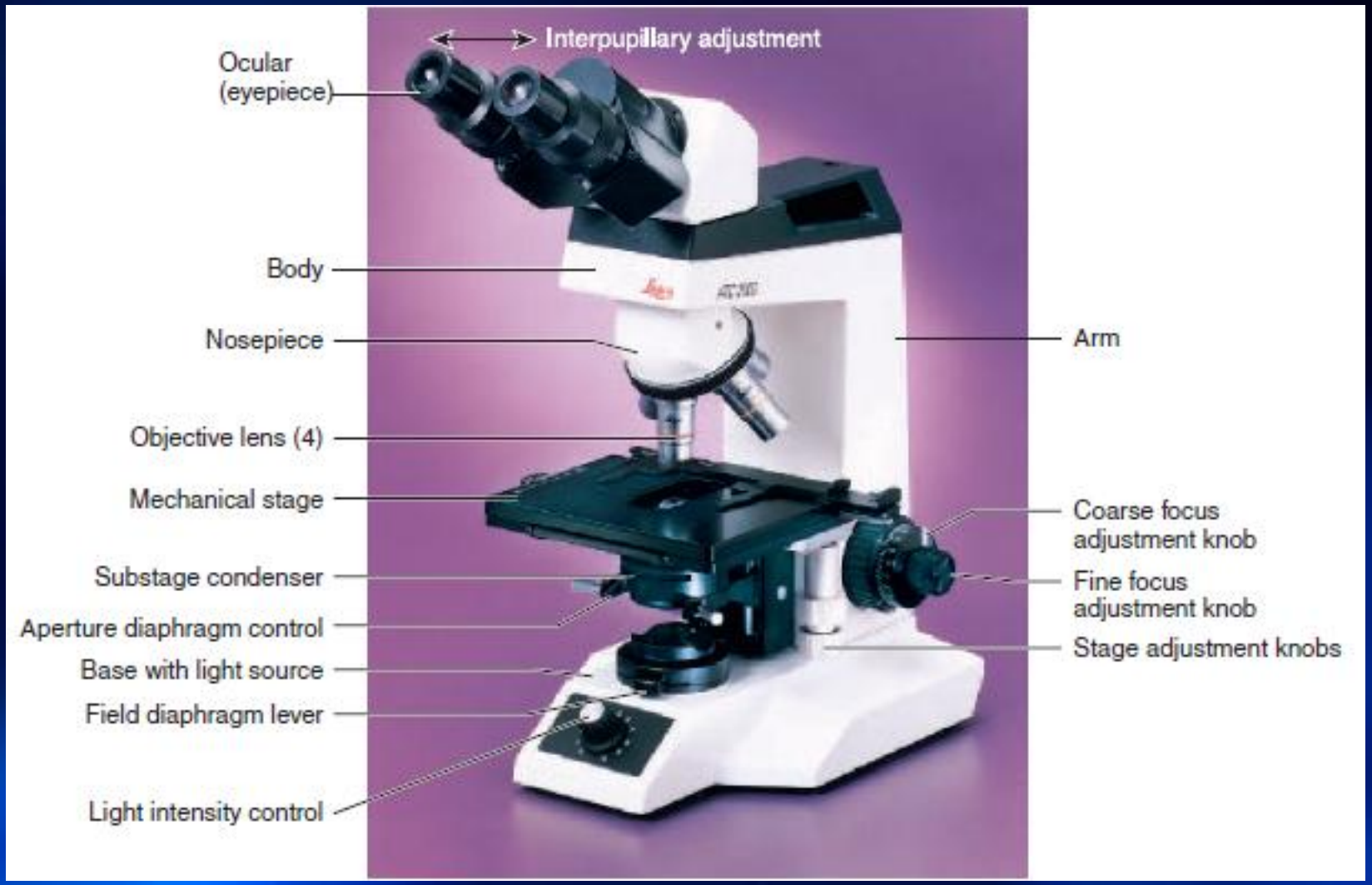
Viruses → Bacteria → Fungi → Parasites

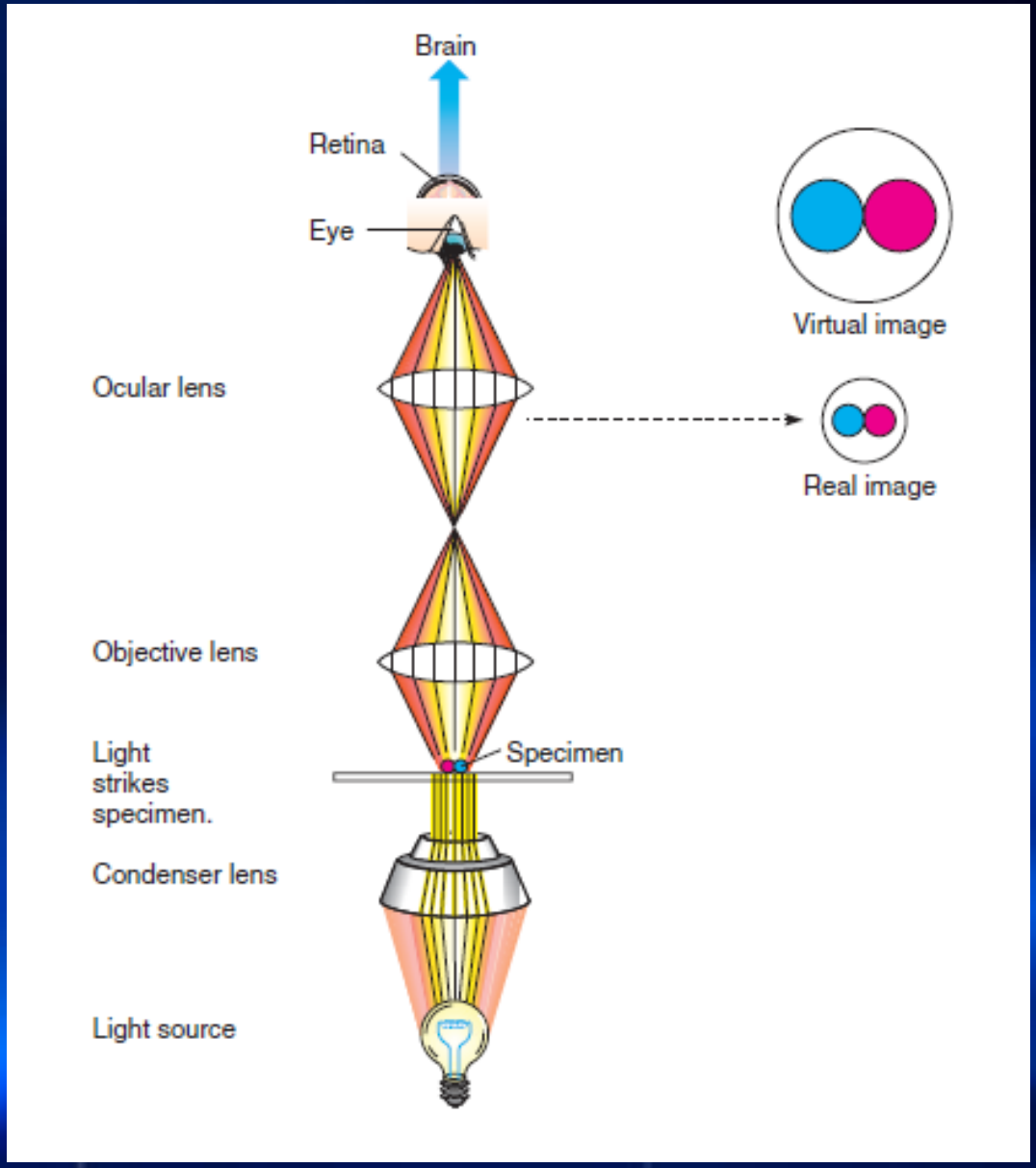
Microscopes

- Human eye, telescope, magnifying glass, microscope

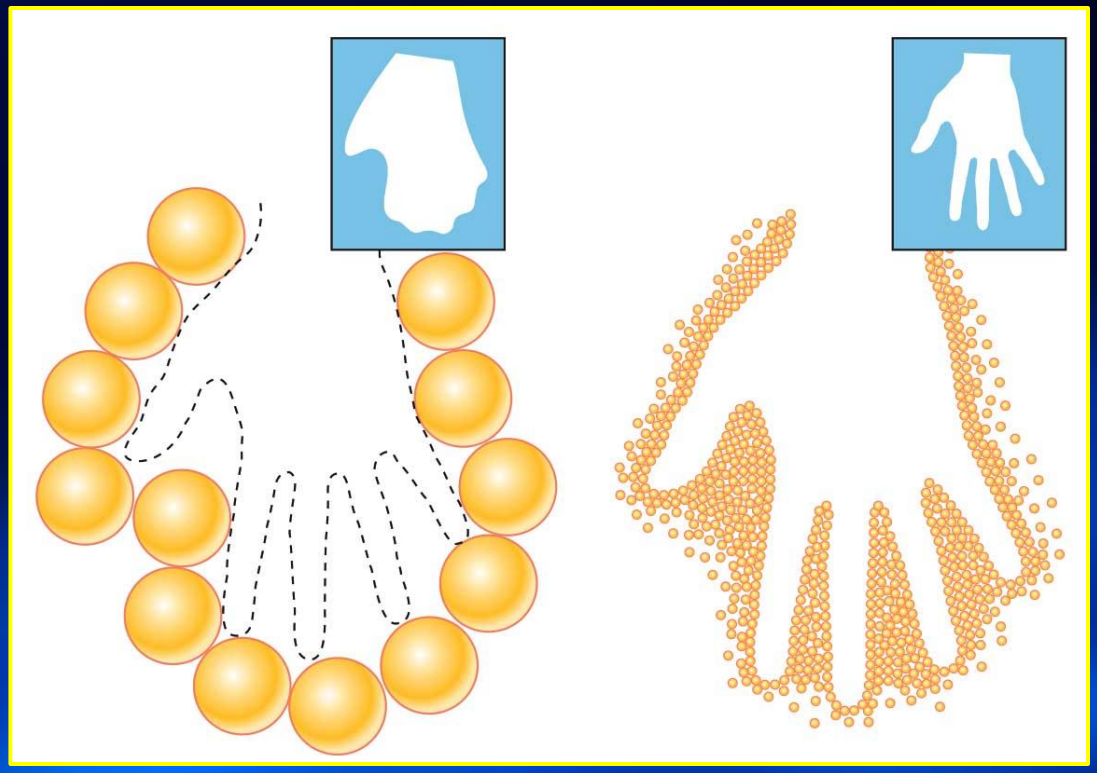
- Resolving power or resolution

- Magnifying power

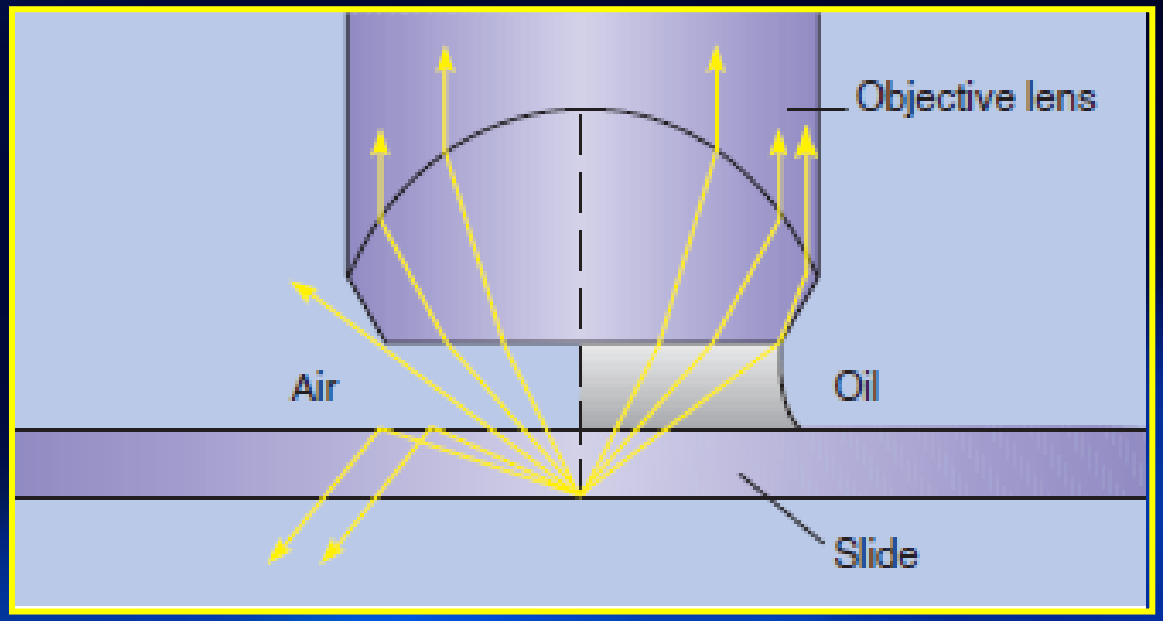

Interpupillary adjustment
Ocular (eyepiece)
Body
Nosepiece
Objective lens (4)
Mechanical stage
Substage condenser
Aperture diaphragm control
Base with light source
Field diaphragm lever
Light intensity control
Arm
Coarse focus adjustment knob
Fine focus adjustment knob
Stage adjustment knobs


Brain
Retina
Eye
Virtual image
Ocular lens
Real image
Objective lens
Light
strikes
specimen.
Specimen
Condenser lens
Light source

Effect of Wavelength on Resolution



Ocular Lens



Condenser Lens

TABLE 2-2 Characteristics of Various Types of Microscopes

TYPE	RESOLVING POWER	USEFUL MAGNIFICATION	CHARACTERISTICS
Brightfield	0.2000 μm	×1,000	Used to observe morphology of microorganisms such as bacteria, protozoa, fungi, and algae in living (unstained) and nonliving (stained) state Cannot observe microbes less than 0.2 μm in diameter or thickness, such as spirochetes and viruses
Darkfield	0.2000 μm	×1,000	Unstained organisms are observed against a dark background Useful for examining thin spirochetes Slightly more difficult to operate than brightfield
Phase-contrast	0.2000 μm	×1,000	Can be used to observe unstained living microorganisms
Fluorescence	0.2000 μm	×1,000	Fluorescent dye attached to organism Primarily an immunodiagnostic technique (immunofluorescence) Used to detect microbes in cells, tissues, and clinical specimens
Transmission electron microscope (TEM)	0.0002 μm (0.2 nm)	×200,000	Specimen is viewed on a screen Excellent resolution Allows examination of cellular and viral ultrastructure Specimen is nonliving Reveals internal features of thin specimens
Scanning electron microscope (SEM)	0.0200 μm (20 nm)	×10,000	Specimen is viewed on a screen Gives the illusion of depth (three-dimensions) Useful for examining surface features of cells and viruses Specimen is nonliving Resolution is less than that of TEM

Figure 2.1 Transparent living microorganisms, such as the syphilis spirochaete, can be seen much more easily when observed in a dark field.

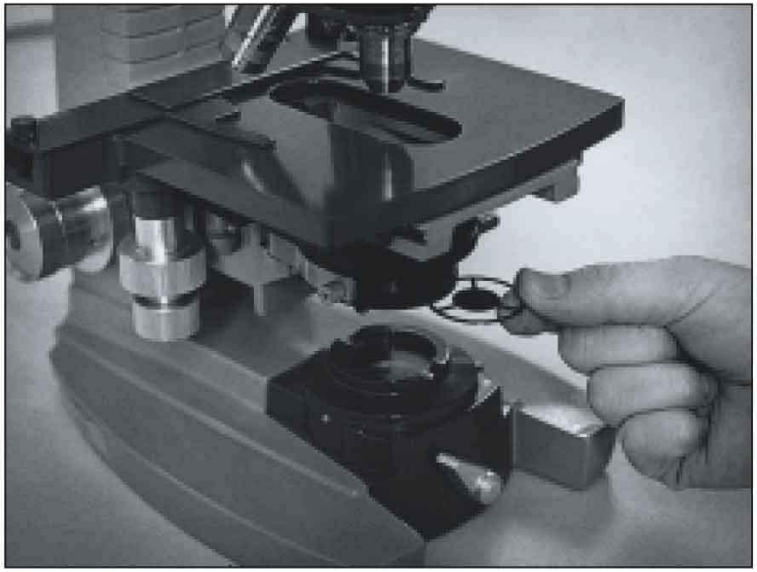


Figure 2.2 The insertion of a star diaphragm into the filter slot of the condenser will produce a dark field suitable for low magnifications.

Figure 3.1 Comparison of brightfield and phase-contrast images

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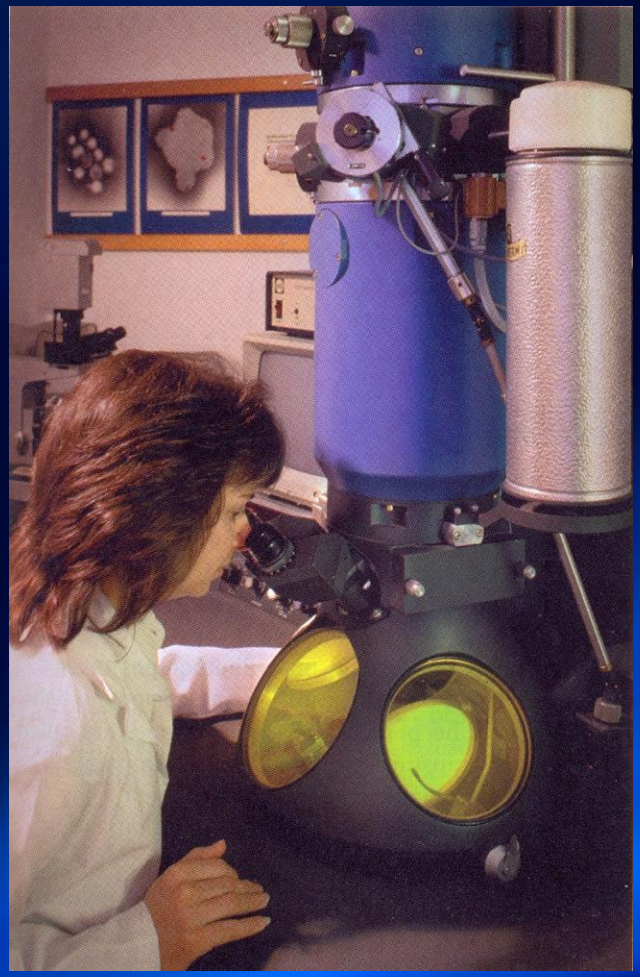
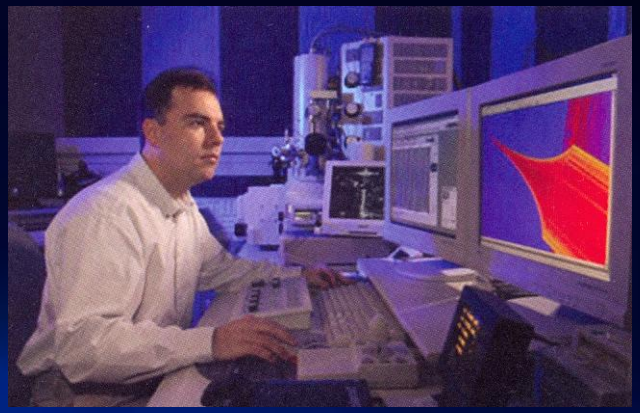
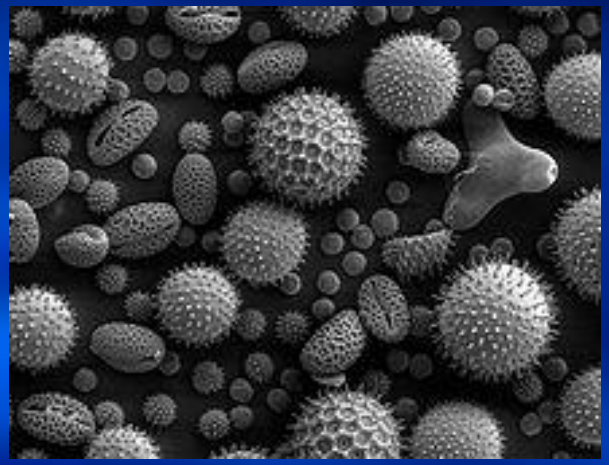
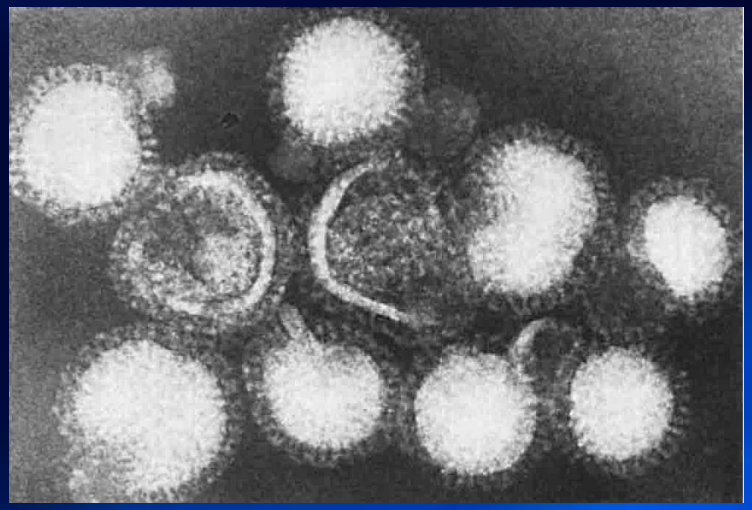


TEM

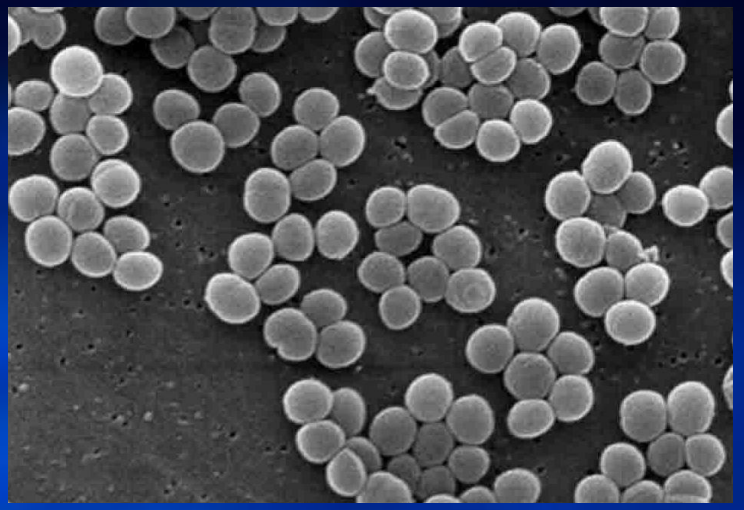




SEM



TEM



SEM

Classification of Bacteria:

- as prokaryotes

- by size and shape

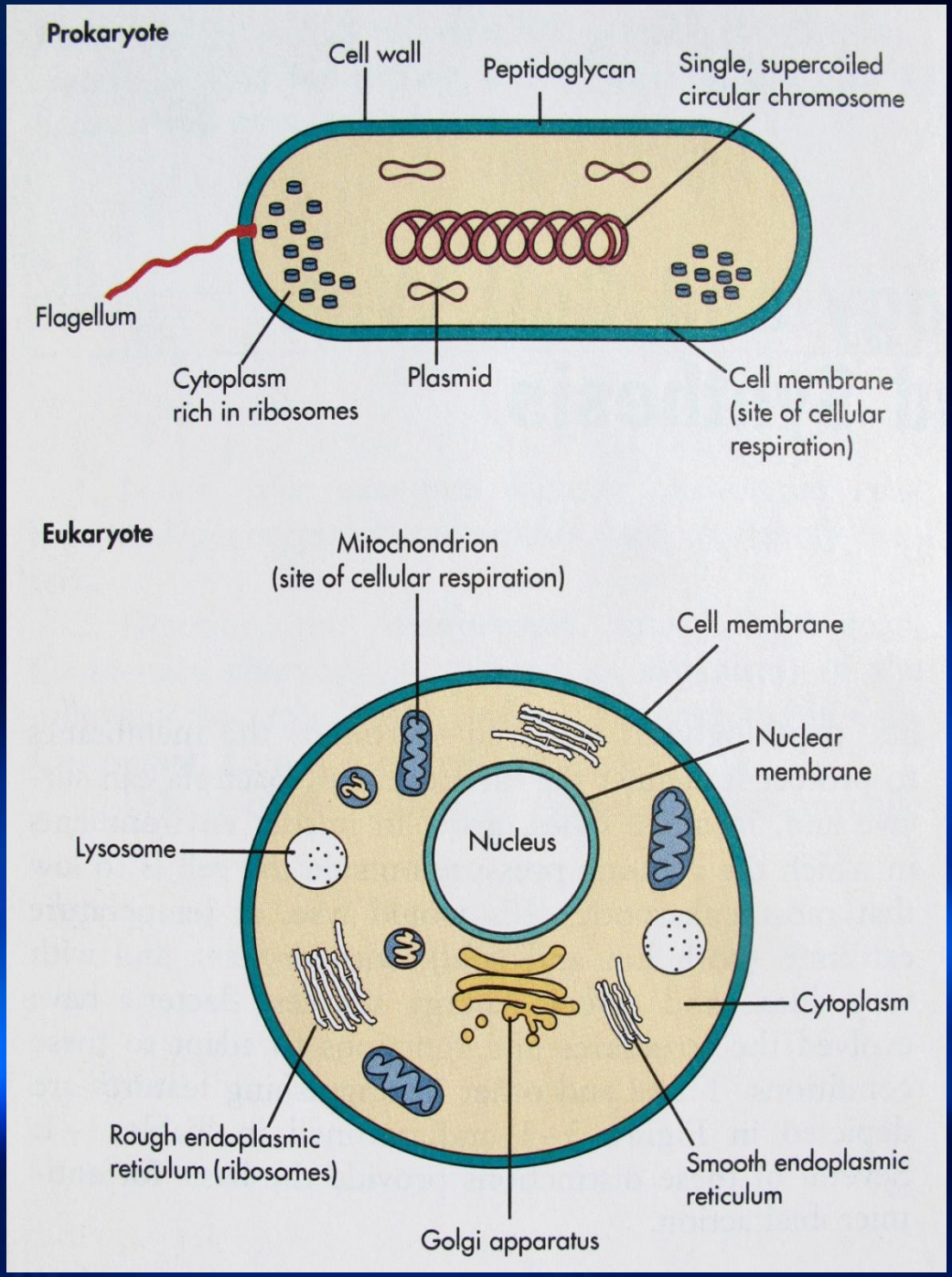
- by Gram staining characteristics

- by genus and species

Classification as Prokaryotes

Distinctive Features of Prokaryotic and Eukaryotic Cells

Cell Component	Prokaryotes	Eukaryotes


Prokaryote
Cell wall
Peptidoglycan
Single, supercoiled circular chromosome
Flagellum
Cytoplasm rich in ribosomes
Plasmid
Cell membrane (site of cellular respiration)
Eukaryote
Mitochondrion (site of cellular respiration)
Cell membrane
Nuclear membrane
Lysosome
Nucleus
Cytoplasm
Rough endoplasmic reticulum (ribosomes)
Smooth endoplasmic reticulum
Golgi apparatus

Prokaryotes

1- Bacteria (formerly Eubacteria)

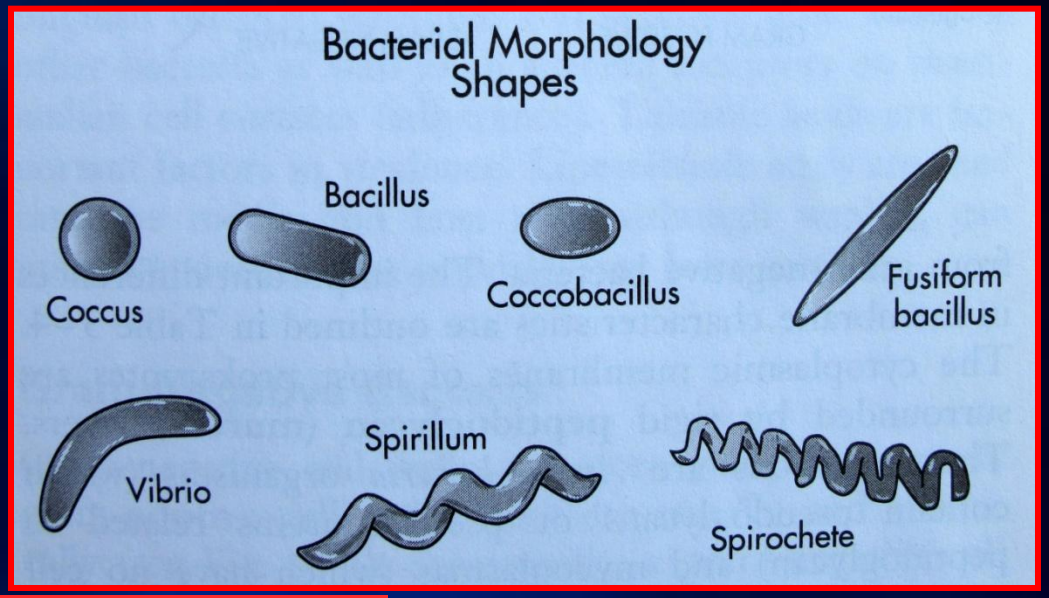
2- Cyanobacteria

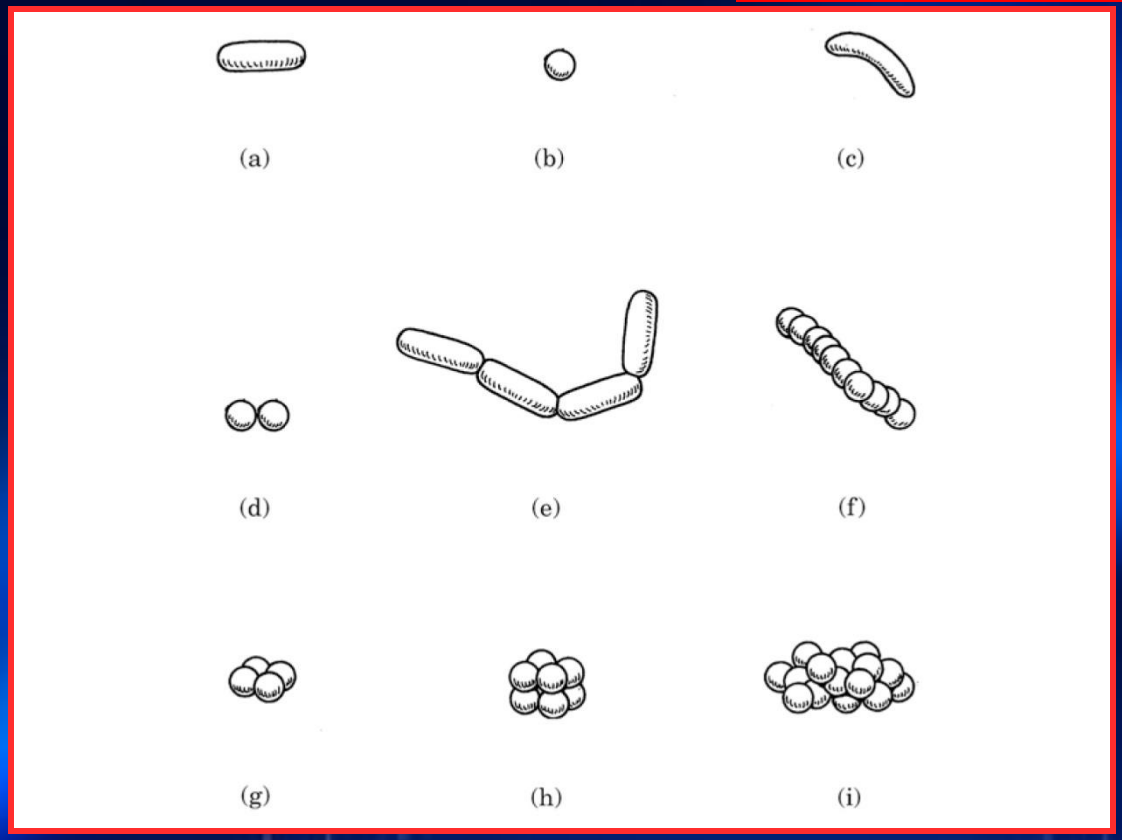
3- Archaea (formerly Archeobacteria)

Classification by size and Shape

- from Spirochetes (up to 250 μm long)

- to Mycoplasma (0.15 μm in diameter)


Bacterial Morphology
Shapes
Bacillus
Coccus
Coccobacillus
Fusiform
bacillus
Vibrio
Spirillum
Spirochete


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)

Classification by Gram Staining Characteristics

- Clinical samples → artificial medium → pure culture → examining the shape and color by staining

- Hans Christian Gram (1880s)

- Crystal violet → W → lugol's solution (3% I_2/KI – mordant) → W → alcohol-acetone mixture → W → safranin or fuschin (counterstain)

- Gram-positive bacteria → blue or purple

- Gram-negative bacteria → red or pink

- Gram-variable bacteria → mixture of blue and pink

Fig. 3(a). Gram staining.

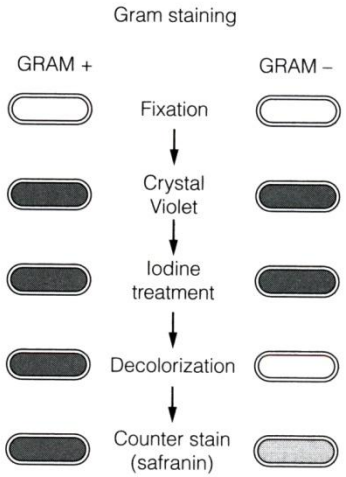
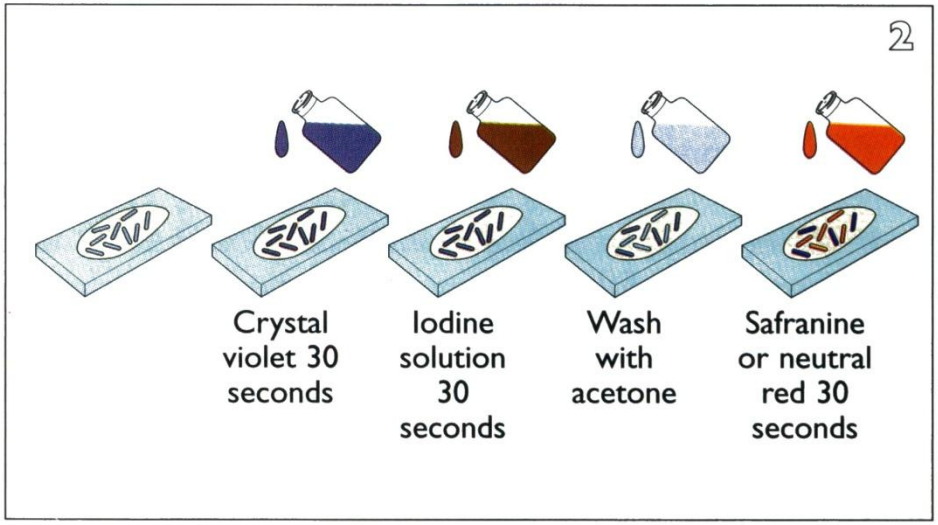
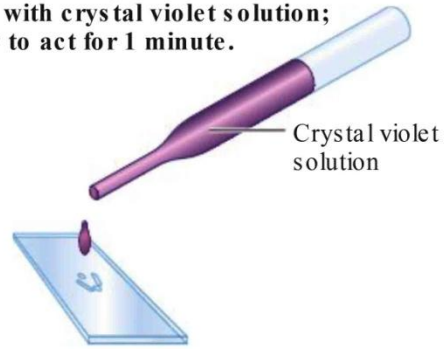


Fig. 3(b). Cell wall location: The thick peptidoglycan layer is outermost in Gram-positive bacteria. This layer is much thinner in Gram negatives and is trapped between the outer and inner membranes.

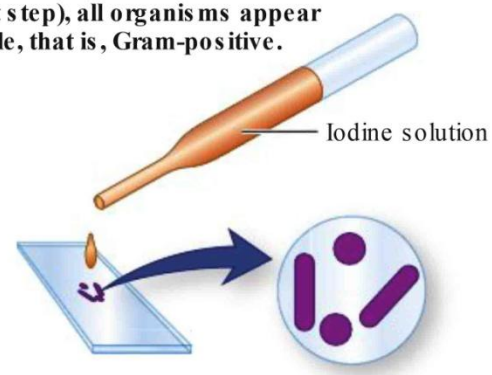


2 The gram stain procedure.

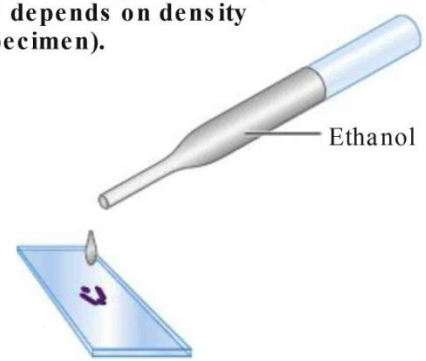
1. Methanol-fix specimen to slide. Flood slide with crystal violet solution; allow to act for 1 minute.



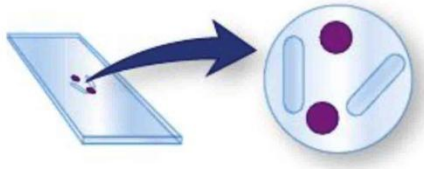
2. Rinse the slide, then flood with iodine solution; allow iodine to act for 1 minute. Before ethanol decolorization (next step), all organisms appear purple, that is, Gram-positive.



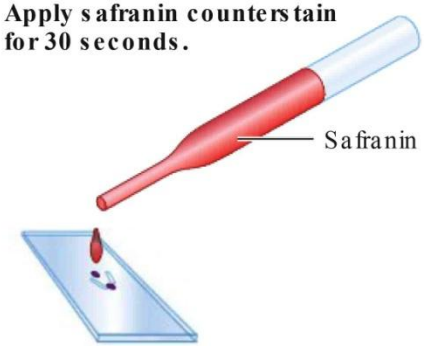
3. Rinse off excess iodine. Decolorize with ethanol, approximately 5 seconds (time depends on density of specimen).



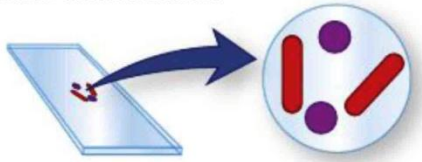
4. Wash slide immediately in water. After ethanol decolorization, those organisms that are Gram-negative are no longer visible.



5. Apply safranin counterstain for 30 seconds.

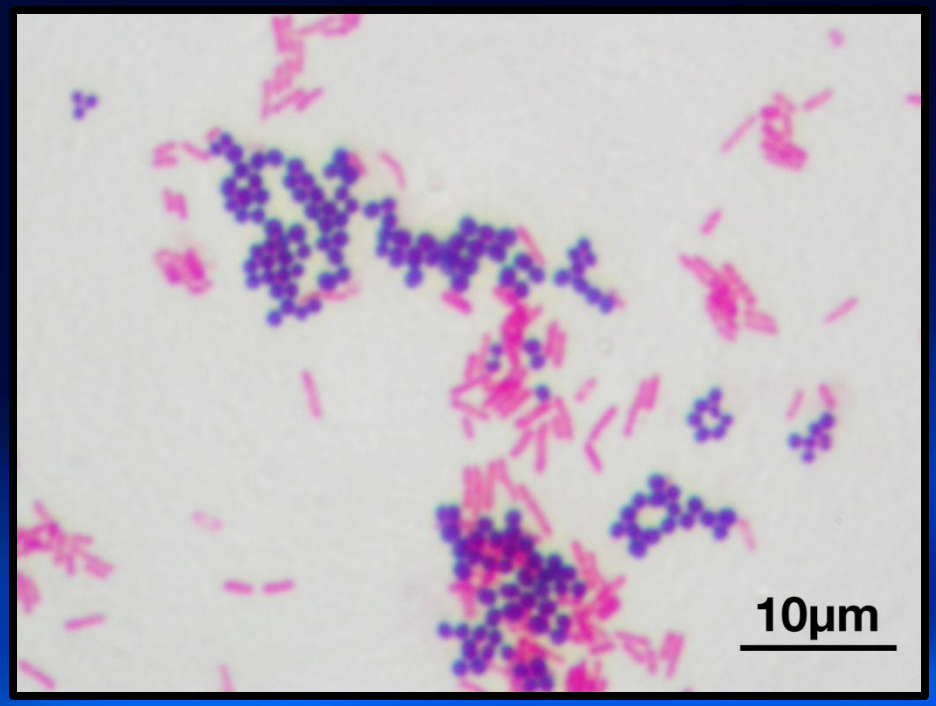


6. Wash in water, blot, and dry in air. Gram-negative organisms are visualized after application of the counterstain.

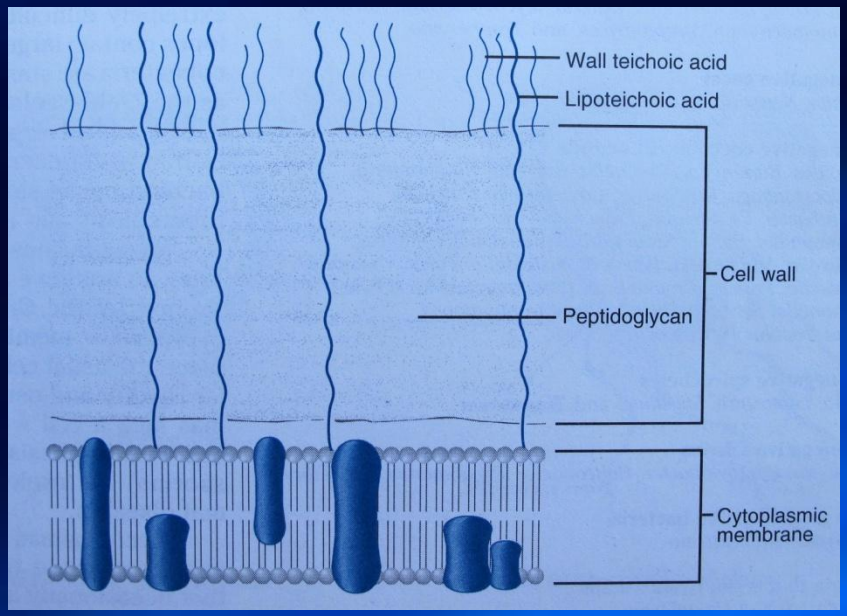


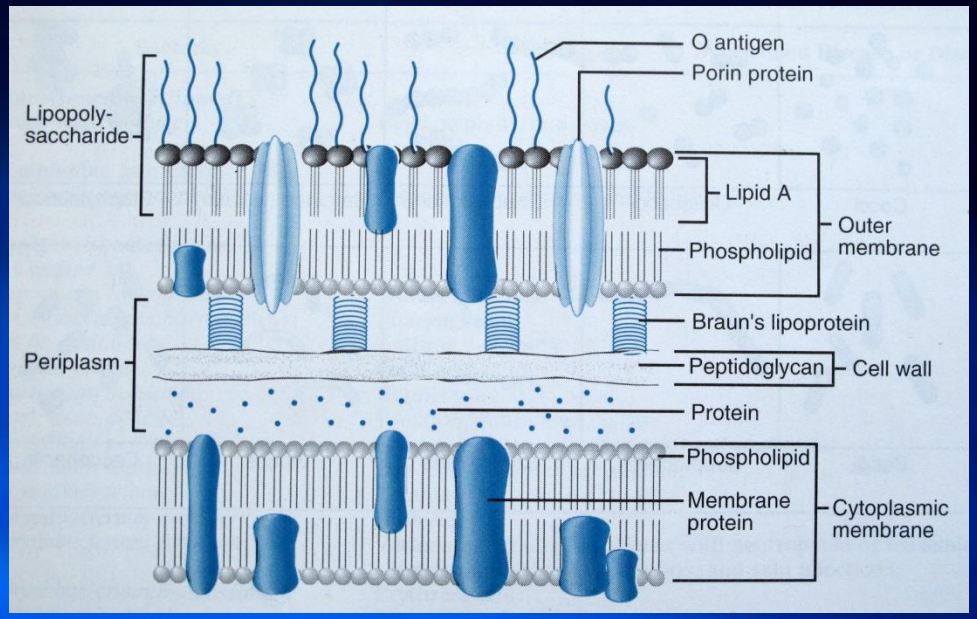
Key

- Gram-positive violet
- Gram-negative red
- Colorless


10µm

Gram-positive vs Gram-negative Bacteria





(Structural differences)

Bacteria that Resist Gram's Stain

- Mycobacteria → Zhiel-Neelsen → acid-fast

- Mycoplasma

Classification by Genus and Species

- Taxonomy: classification, nomenclature, and identification

- Taxon: Kingdom (Domain), Division (Phylum), Class, Order, family, genus, species

- Binomial system of nomenclature (Carolus Linnaeus – 18th century)


Domain (Kingdom)
Division (Phylum)
Class
Order
Family
Genus
Species
Strain
قلمرو
شاخه
رده
راسته
خانواده
جنس
گونه
سویه

TABLE 3-3 Comparison of Human and Bacterial Classification

	HUMAN BEING	*ESCHERICHIA COLI* (A MEDICALLY IMPORTANT GRAM-NEGATIVE BACILLUS)[a]	*STAPHYLOCOCCUS AUREUS* (A MEDICALLY IMPORTANT GRAM-POSITIVE COCCUS)[a]
Kingdom (Domain)	Animalia (*Eucarya*)	Procaryotae (*Bacteria*)	Procaryotae (*Bacteria*)
Phylum	Chordata	Proteobacteria	Firmicutes
Class	Mammalia	Gammaproteobacteria	Bacilli
Order	Primates	Enterobacteriales	Bacillales
Family	*Hominidae*	*Enterobacteriaceae*	*Staphylococcaceae*
Genus	*Homo*	*Escherichia*	*Staphylococcus*
Species (a species has two names; the first name is the genus, and the second name is the specific epithet)	*Homo sapiens*	*Escherichia coli*	*Staphylococcus aureus*

[a]Based on Bergey's Manual of Systematic Bacteriology, vol. 1, 2nd ed. New York: Springer-Verlag, 2001. A bacillus is a rod-shaped bacterium. A coccus is a spherical-shaped bacterium.

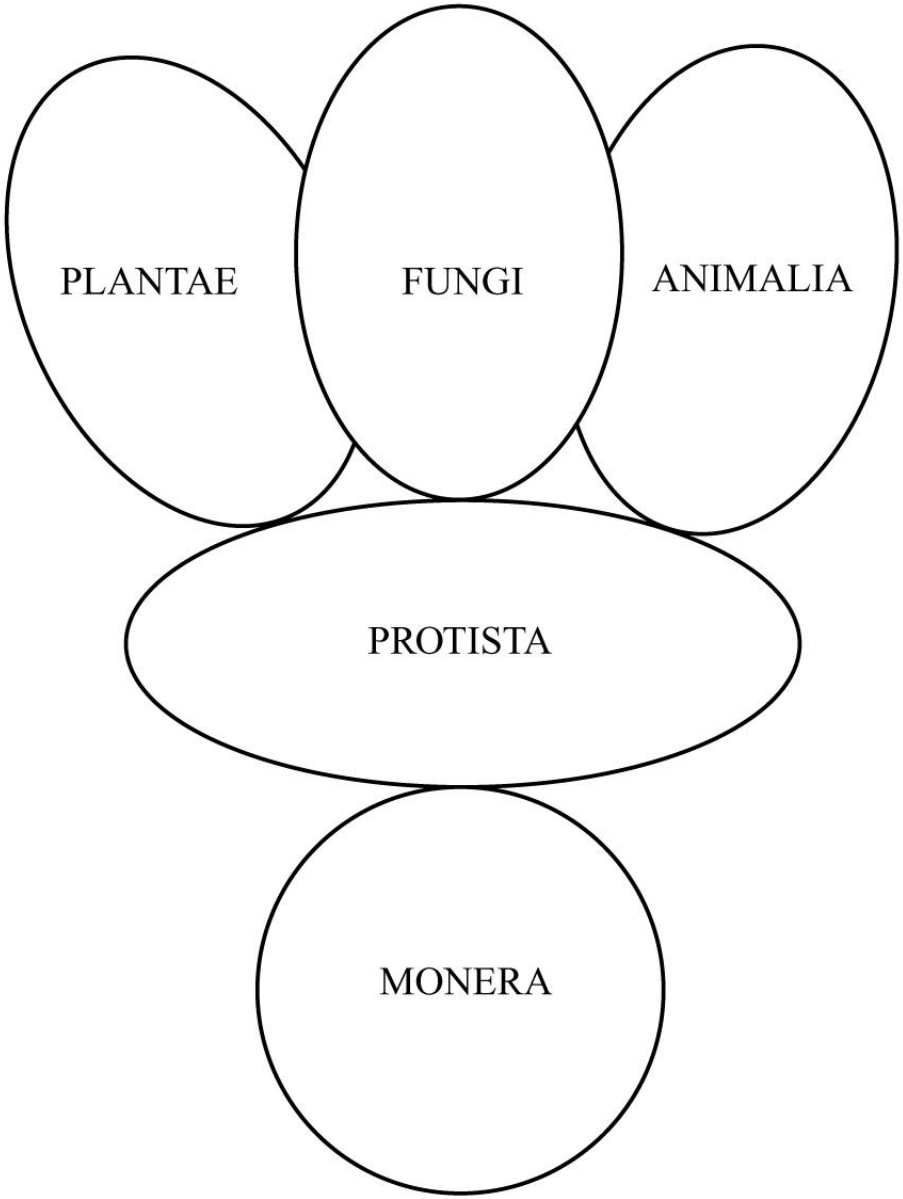
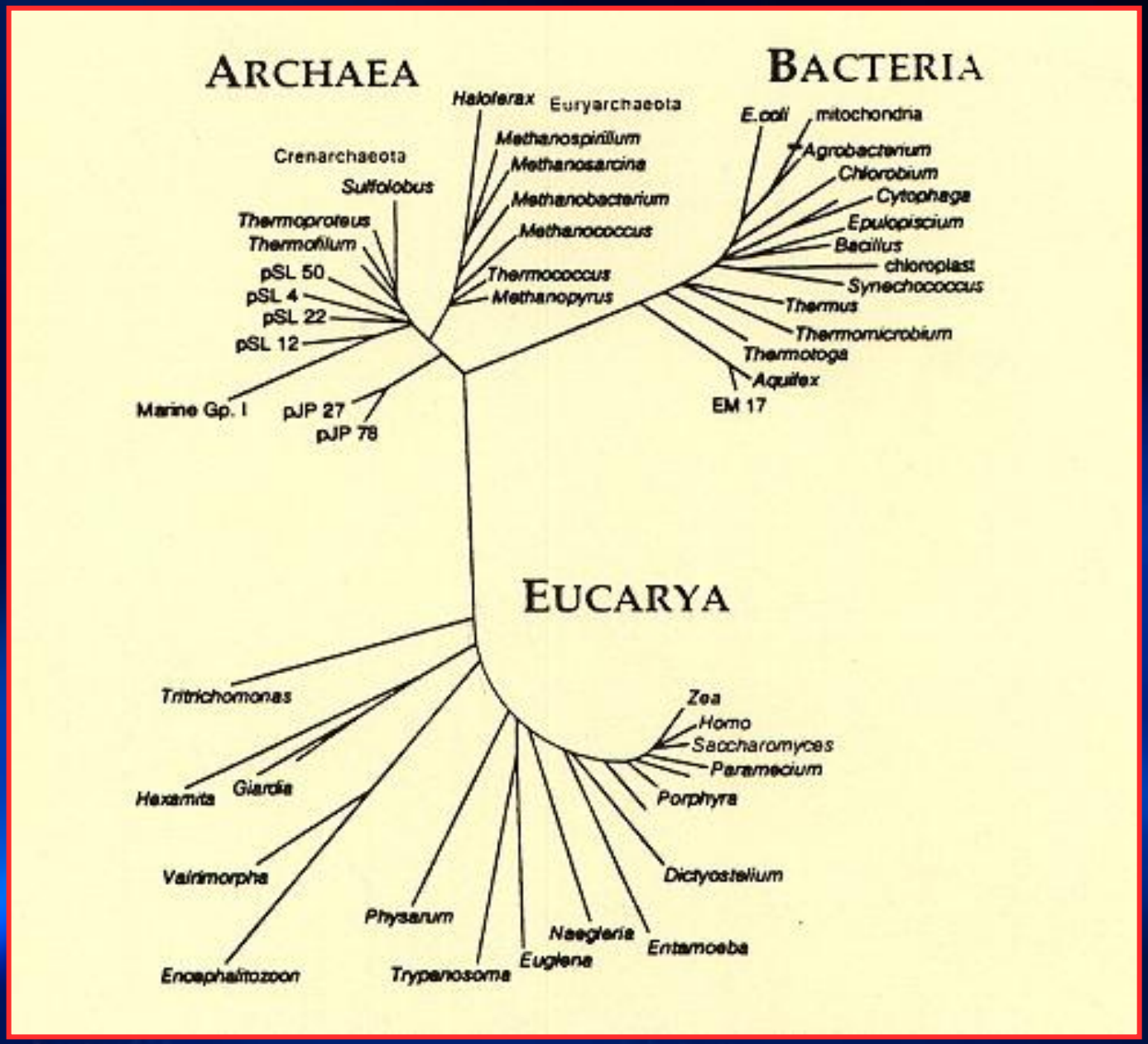


Figure Whittaker's five-kingdom system of classification

The Three-Domain System Devised by Carl R. Woese (late 1970s)



How classifying bacteria ?

Bacteria vary in their:

- Shape
- Size
- Structure
- Chemical activities (metabolism)
- Type of required nutritions
- Form of energy they use
- Physical condition they are capable of growth
- Reaction to certain dyes
- Genetics

Phenotype

Genotype

How Do Scientists Determine How Closely Related One Organism Is to Another?

- The most widely technique → rRNA sequencing

- The gene code for SSUrRNA (small subunit rRNA)

- 16S rDNA (1500 nt) or 18S rDNA (2000 nt)

- The less similar the sequences, the less related are the organisms

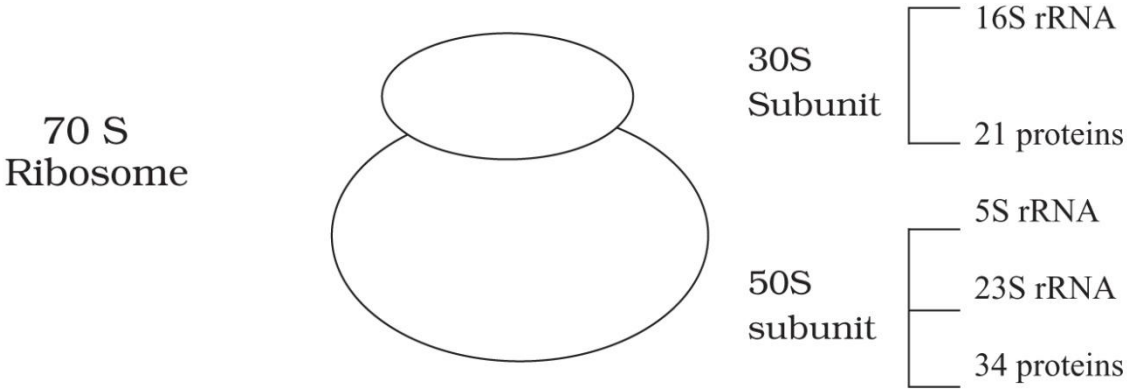


Figure The bacterial ribosome. Each subunit comprises rRNA and proteins. The nucleotide sequence of small subunit (16S) rRNA is widely used in determining the phylogenetic (evolutionary) relationship between bacteria (see Chapter 7)

Table Comparison of procaryotic and eucaryotic ribosomes

	Procaryotic	Eucaryotic
Overall size	70S	80S
Large subunit size	50S	60S
Large subunit RNA	23S & 5S	28S, 5.8S & 5S
Small subunit size	30S	40S
Small subunit RNA	16S	18S

How naming bacteria ?

✓ As in animal & plants → Latin binomial

Escherichia coli

Escherichia coli	→	*E. coli*
Staphylococcus aureus	→	*S. aureus*
Streptococcus pneumoniae	→	*S. pneumoniae*
Helicobacter pylori	→	*H. pylori*

Genus Species

* **Family & Order names:**

✓**Do not print italic**

✓**Capital initial letters**

✓**Standardized endings**

Family → **"– aceae"**
Enterobacteriaceae

Order → **"– ales"**
Actinomycetales

Singular vs plural

bacterium	→	bacteria
coccus	→	cocci
bacillus	→	bacilli
fungus	→	fungi
pseudomonas	→	pseudomonads

Terms & Definition

- ✓ **Infection**
- ✓ **Disease**
- ✓ **Subclinical (asymptomatic or non-apparent) infection**
- ✓ **Clinical infection** → **Symptoms** → **Signs** →
- ✓ **Colonization** → ***Escherichia coli*** **in the bowels**; ***Staphylococcus epidermidis*** **on the skin**
- ✓ **Carrier** → ***Staphylococcus aureus*** **in upper respiratory tract**

Terms & Definition

★ Pathogen → invasive agent, virulent agent

★ Virulence

★ Pathogenicity

★ Opportunistic pathogen
- **Non-pathogenic in immunocompetents**
- **Life-threatening infection in immunocopromised patients**

Terms & Definition

- ★ Bacteraemia
- ★ Septicaemia **(sepsis, septic shock)**

Cloud Gate sculpture by Anish Kapoor, Millennium Park, Chicago, Illinois