

علم الأحياء الدقيقة  
**Microbiology**  
*Introduction to Bacteriology*



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مكتب ٢ ب ٤٥



# Methods of studying microorganisms

## I. Preparing a smear

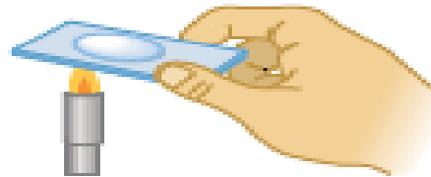


Spread culture in thin film over slide

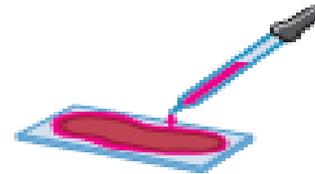


Dry in air

## II. Heat fixing and staining



Pass slide through flame to heat fix



Flood slide with stain; rinse and dry

## III. Microscopy



Slide 100x Oil

Place drop of oil on slide; examine with 100x objective lens

**Figure 2.3** Staining cells for microscopic observation. Stains improve the contrast between cells and their background.

### Step 1



Flood the heat-fixed smear with crystal violet for 1 min

**Result:**  
All cells purple

### Step 2



Add iodine solution for 1 min

**Result:**  
All cells remain purple

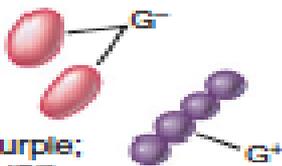
### Step 3



Decolorize with alcohol briefly — about 20 sec

**Result:**  
Gram-positive cells are purple;  
gram-negative cells are colorless

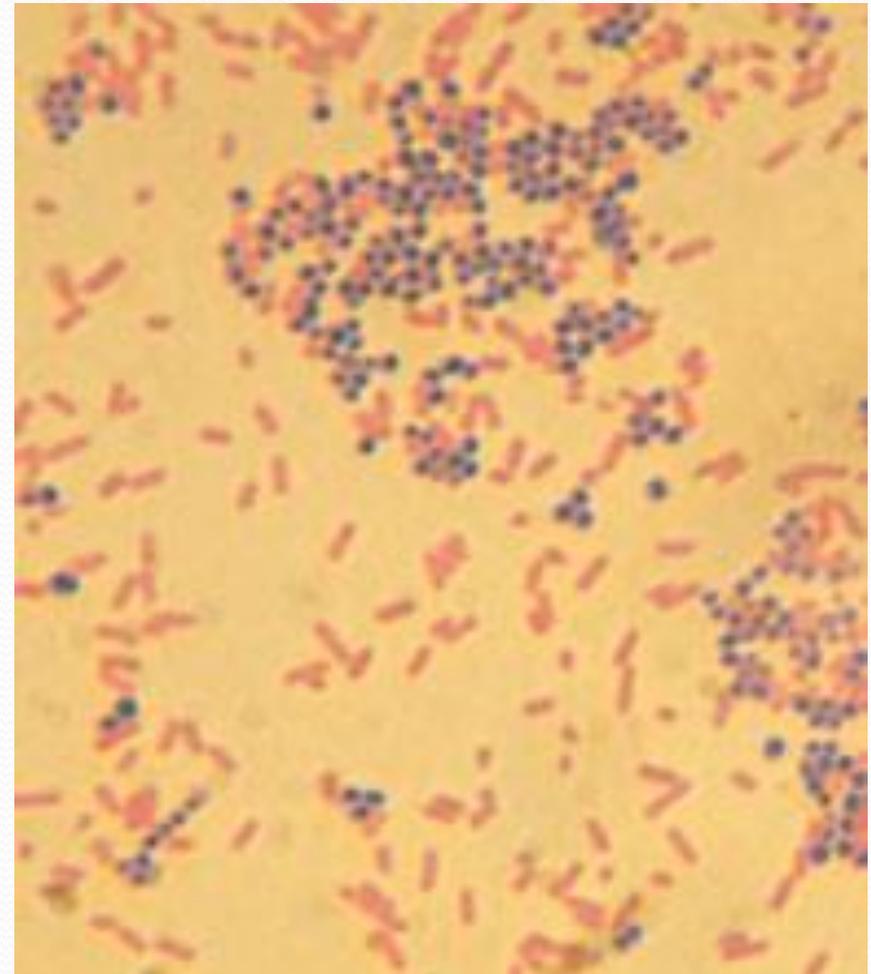
### Step 4



Counterstain with safranin for 1–2 min

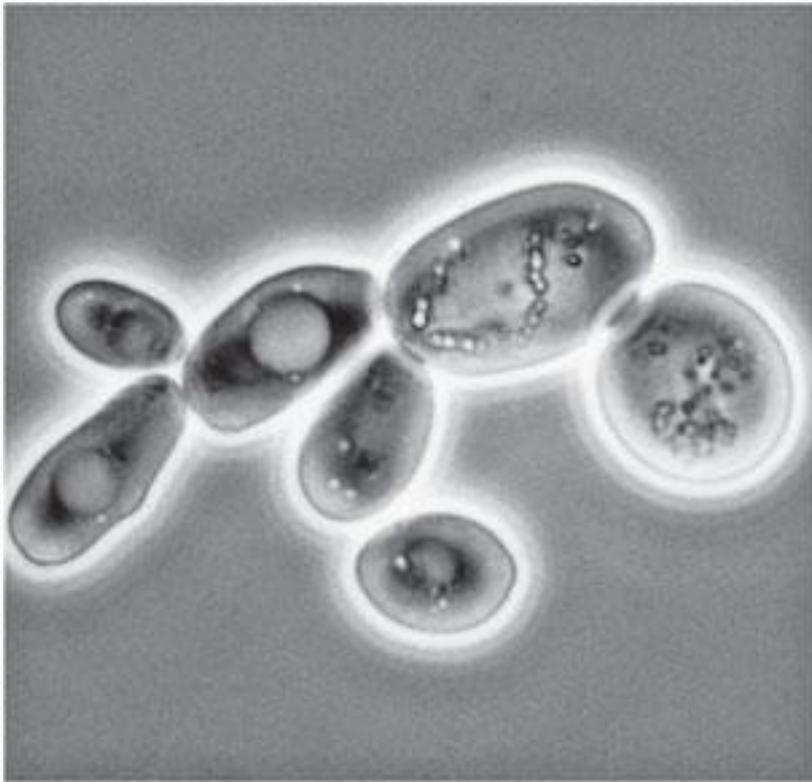
**Result:**  
Gram-positive (G<sup>+</sup>) cells are purple;  
gram-negative (G<sup>-</sup>) cells are pink to red

(8)

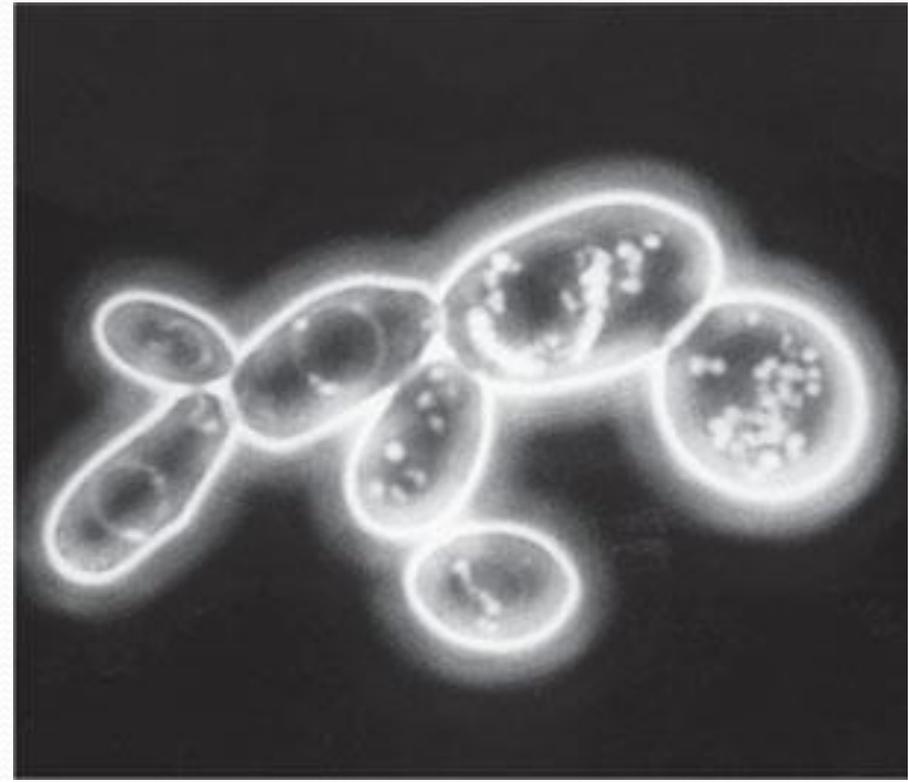


# Methods of studying microorganisms

Phase-Contrast Microscopy

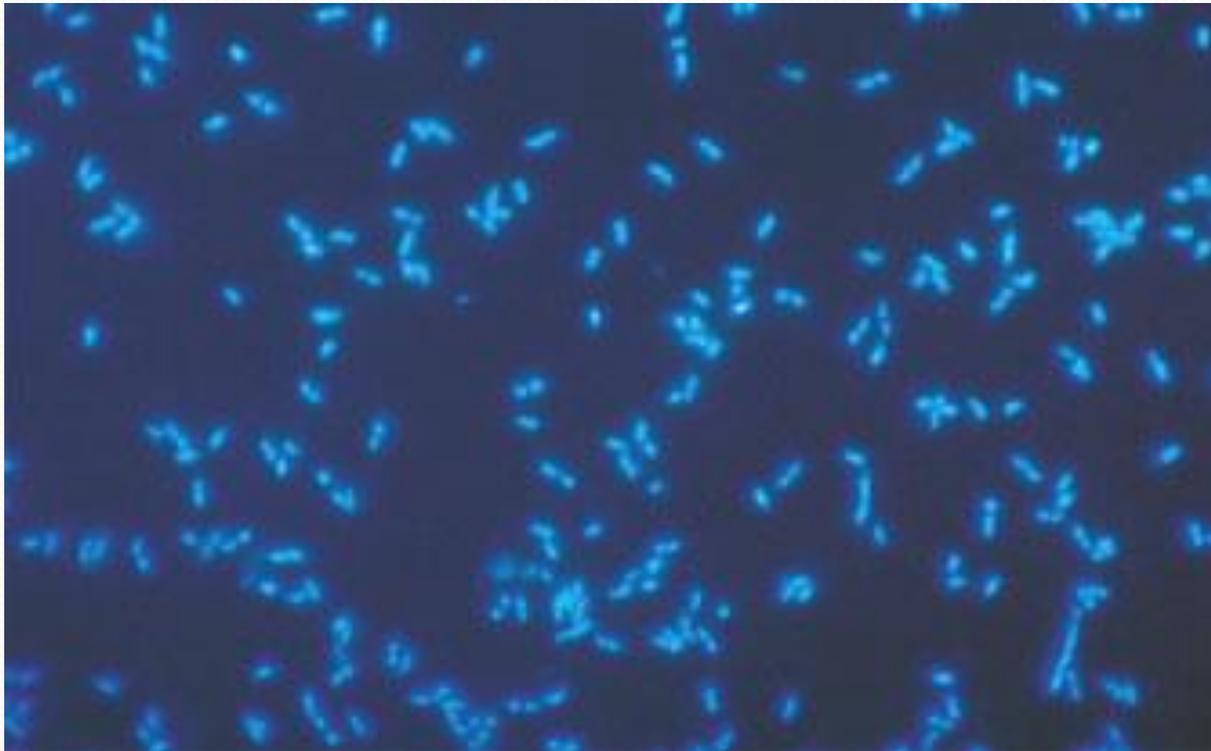


Dark-Field Microscopy



# Methods of studying microorganisms

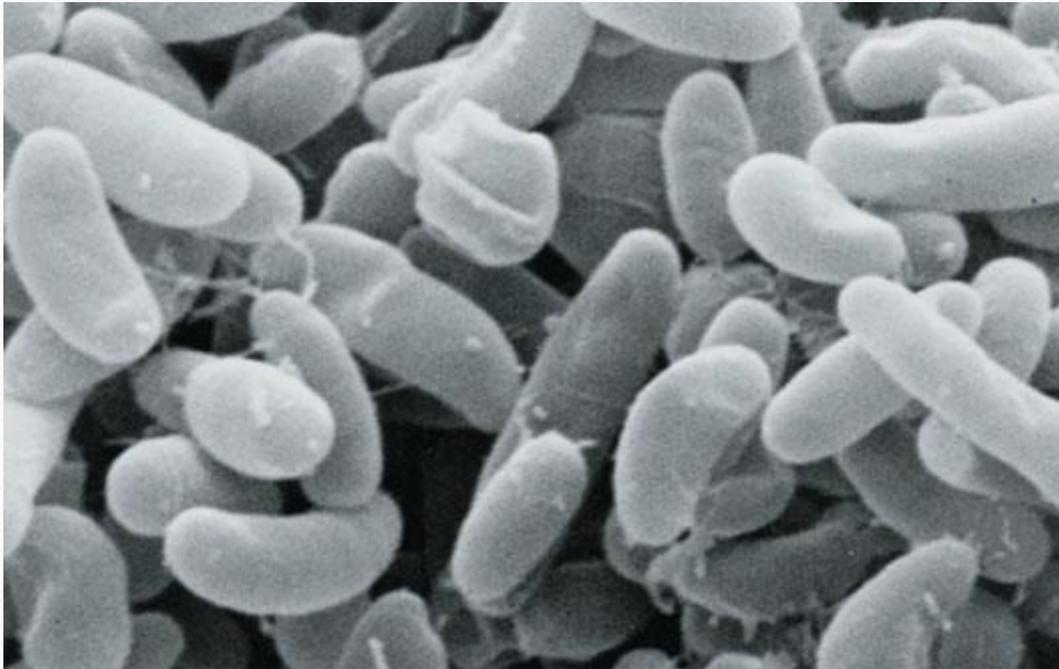
## Fluorescence Microscopy



# Methods of studying microorganisms

## The Electron Microscope:

- 1- Transmission Electron Microscopy
- 2- Scanning Electron Microscopy



# Imaging Cells in Three Dimensions

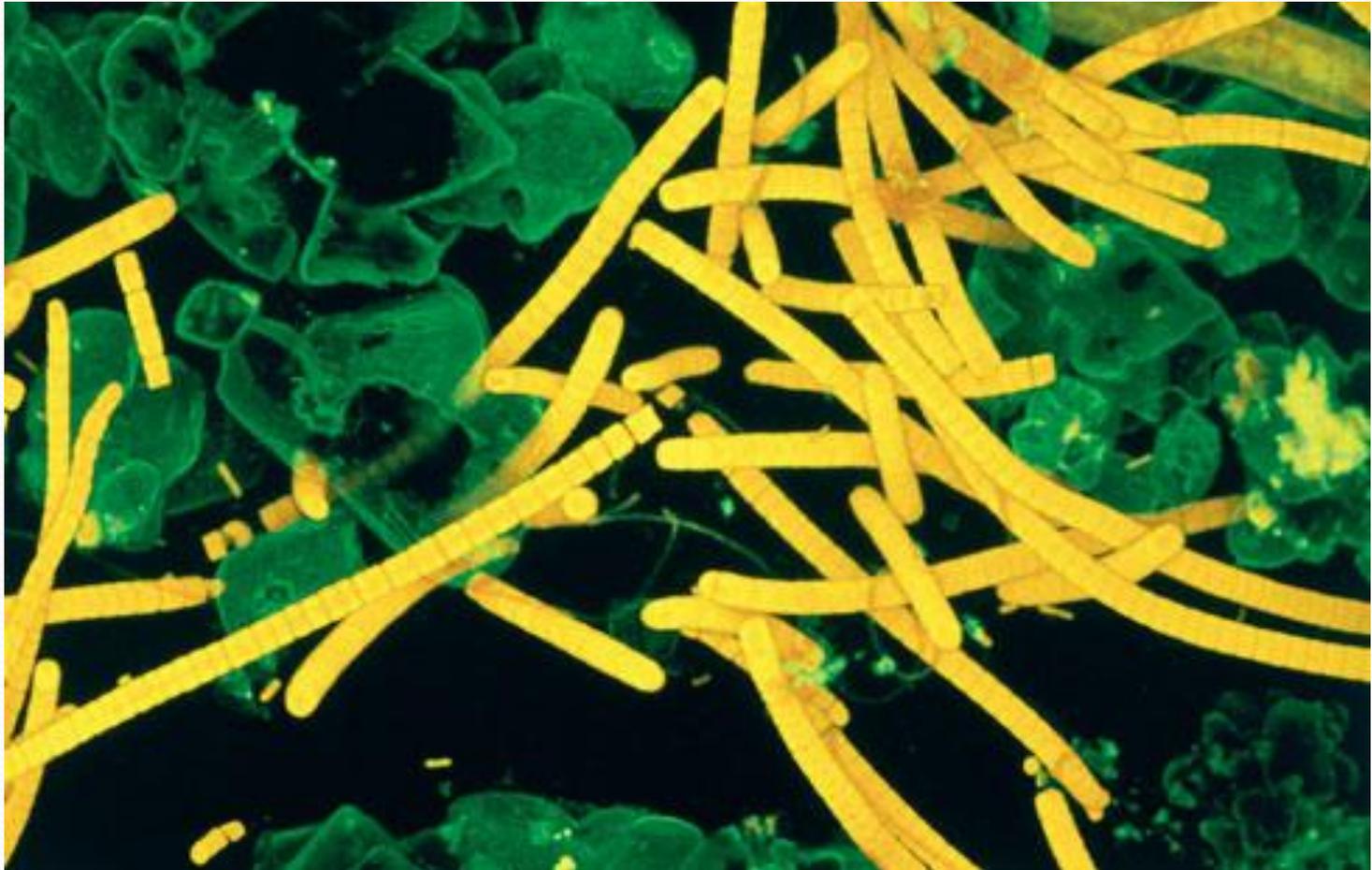
Differential Interference  
Contrast Microscopy



Atomic force Microscopy



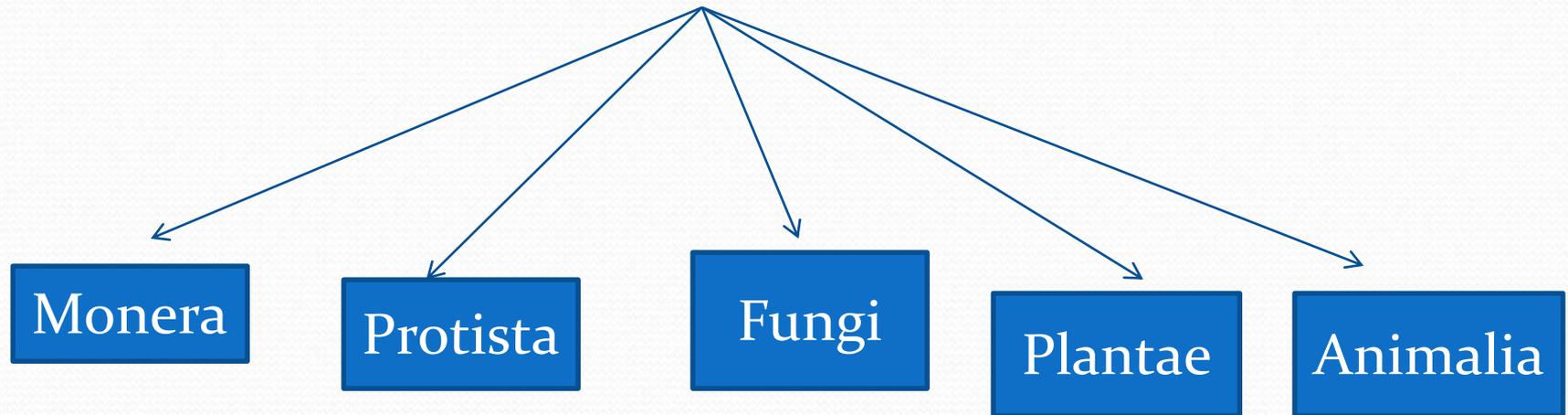
# Confocal Scanning Laser Microscopy



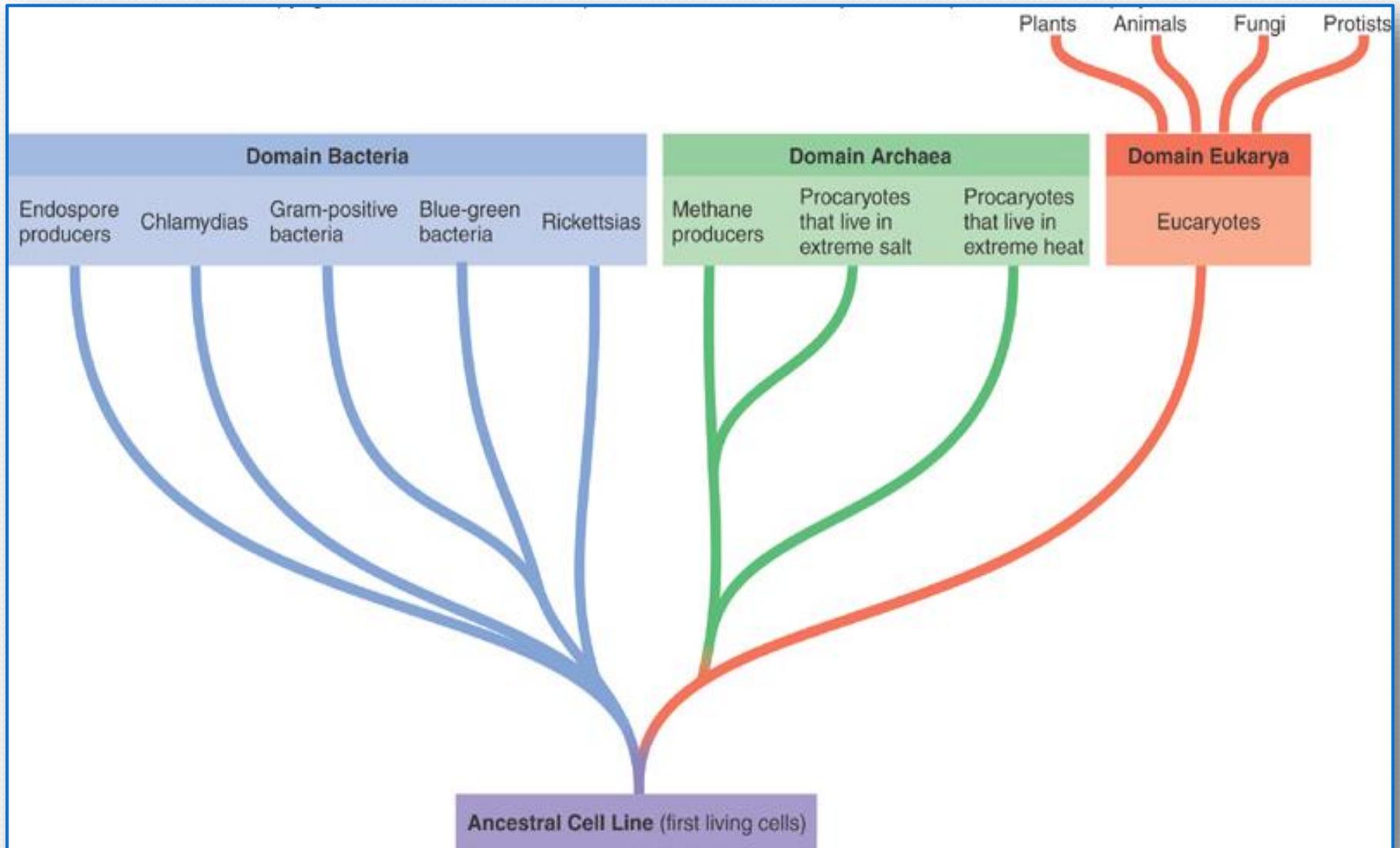
# Classification of living organisms

The modern classification of five Kingdoms system of living organisms, according to Whittaker (1969), classify the living organisms to five kingdoms:

Living Organisms



# Classification of living organisms



# Microorganisms

```
graph TD; A([Microorganisms]) --> B[Prokaryotic cells]; A --> C[Eukaryotic cells]; B --> D["Cyanobacteria<br/>Bacteria<br/>Archaea"]; C --> E["Fungi (Molds and Yeast)<br/>Algae<br/>Protozoa<br/>(Parasitic worms) Helminths"];
```

The diagram is a hierarchical flowchart. At the top is an oval labeled 'Microorganisms'. Two arrows point down from this oval to two rounded rectangular boxes: 'Prokaryotic cells' on the left and 'Eukaryotic cells' on the right. From 'Prokaryotic cells', an arrow points down to a larger rounded rectangular box containing a list of three items: 'Cyanobacteria', 'Bacteria', and 'Archaea'. From 'Eukaryotic cells', an arrow points down to a larger rounded rectangular box containing a list of five items: 'Fungi (Molds and Yeast)', 'Algae', 'Protozoa', '(Parasitic worms) Helminths'.

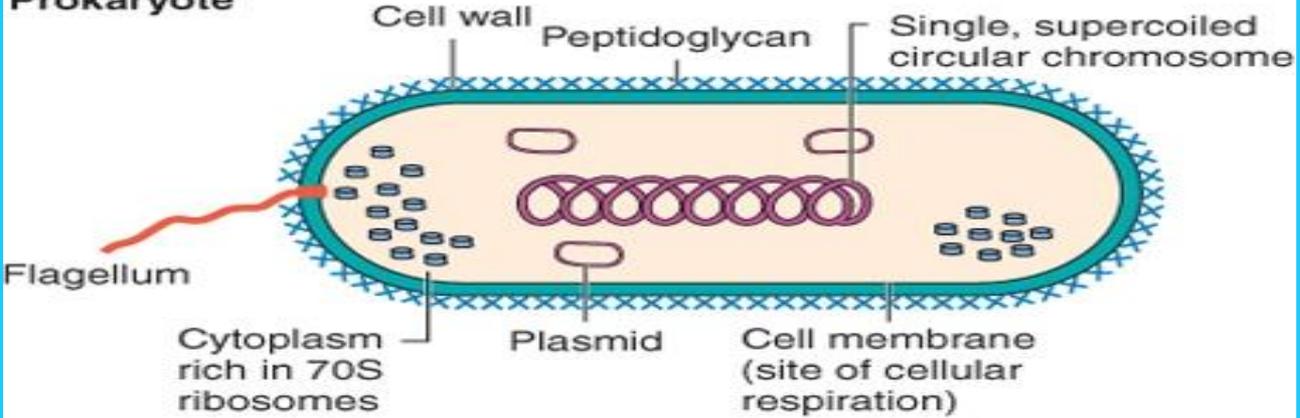
## Prokaryotic cells

Cyanobacteria  
Bacteria  
Archaea

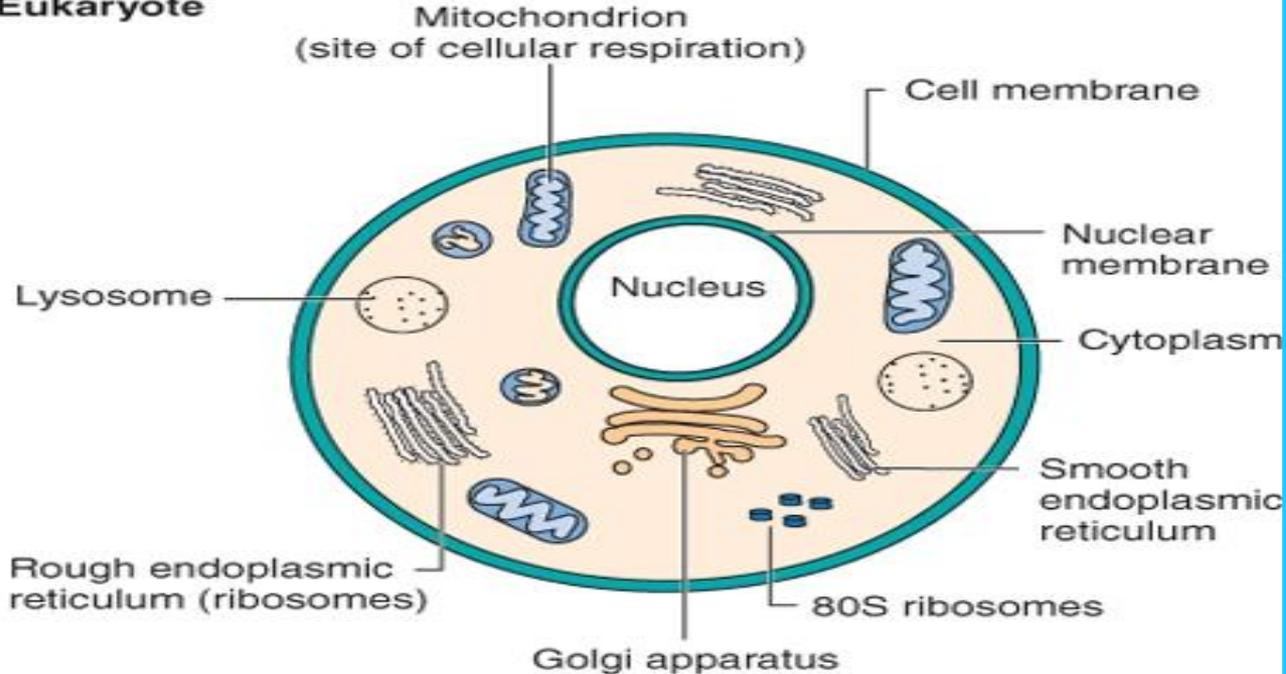
## Eukaryotic cells

Fungi (Molds and Yeast)  
Algae  
Protozoa  
(Parasitic worms) Helminths

**Prokaryote**



**Eukaryote**



# Major Differences between Prokaryotic and Eukaryotic microorganisms

Prokaryotes	Eukaryotes
1- Lack membrane bound nucleoid region	1- Membrane bound nucleus containing DNA
2- DNA-one circular molecule one chromosome	2- DNA-linear molecules arranged to form several chromosomes
3- Haploid-One copy of a gene	2- Diploid-Two copies of a gene
4- Plasma membrane does not contain sterols	4- Plasma membrane contains sterols
5- Reproduction—simple binary fission	5- Reproduction—meiosis and mitosis

# Major Differences between Prokaryotic and Eukaryotic microorganisms

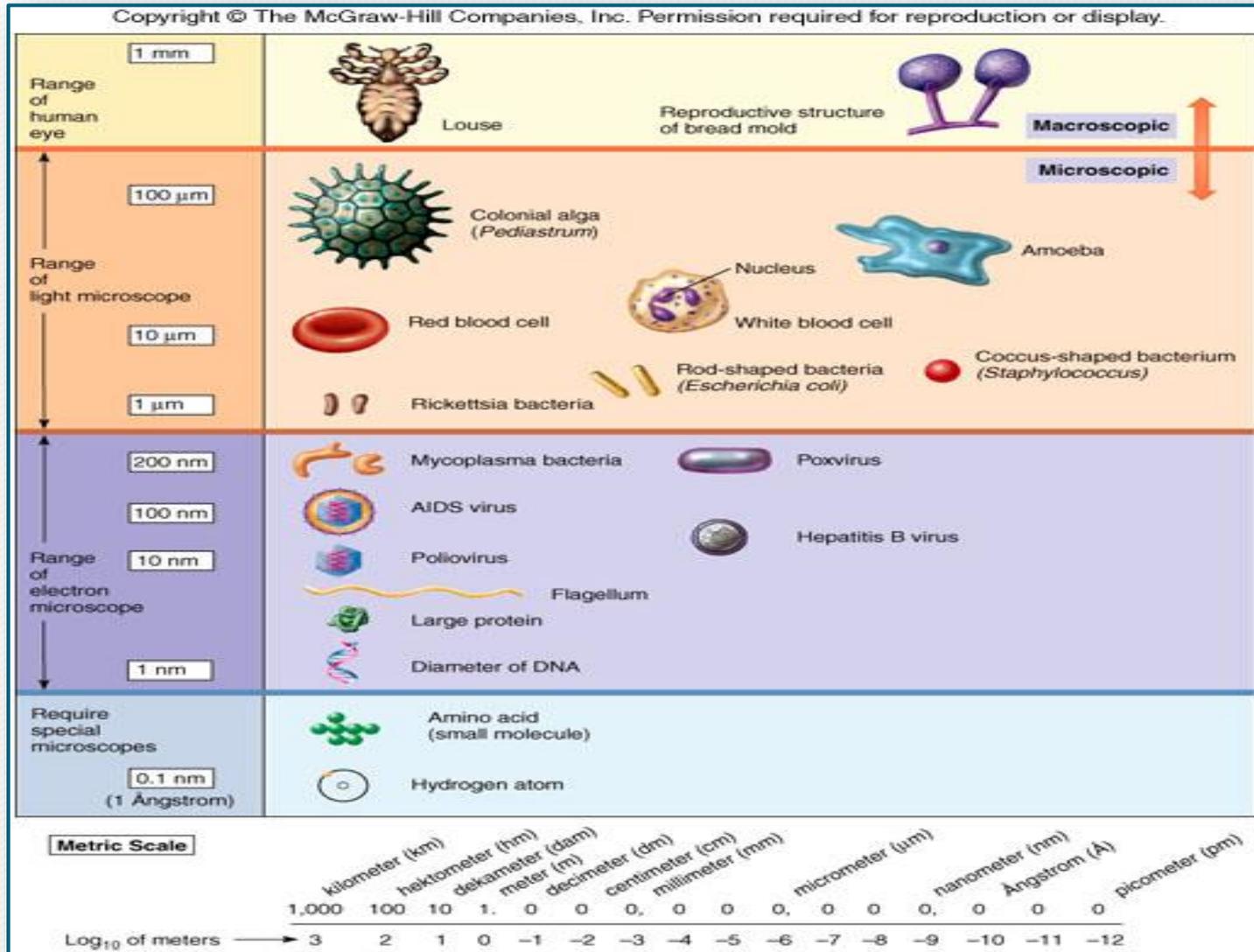
Prokaryotes	Eukaryotes
6- lack membrane bound organelles	6- Presence of membrane bound organelles such as chloroplasts and mitochondria
7- Cell walls almost contain complex polysaccharide Peptidoglycan	7- Cell wall is chemically simple when present

# Size of Microorganisms

Microorganisms vary in size ranging from 10 **nm** (nanometers) to 100 **μm** (micrometers):

- Viruses in **nm** =  $10^{-9}$  **m** (meter)
- Bacteria in **um** =  $10^{-6}$  **m**
- Helminths in **mm** =  $10^{-3}$  **m**

# Size of Microorganisms



# Nomenclature

- **Binomial** (scientific) nomenclature
- **Genus** – *Escherichia*, always **capitalized**
- **Species** - *coli*, lowercase

Both **italicized** or **underlined**: *Escherichia coli* or Escherichia coli.

# Identification

- Morphological characteristics.
- Physiological/Metabolic characteristics.
- Ecological characteristics.
- Genetic characteristics.
- Molecular characteristics.

# QUESTIONS??

