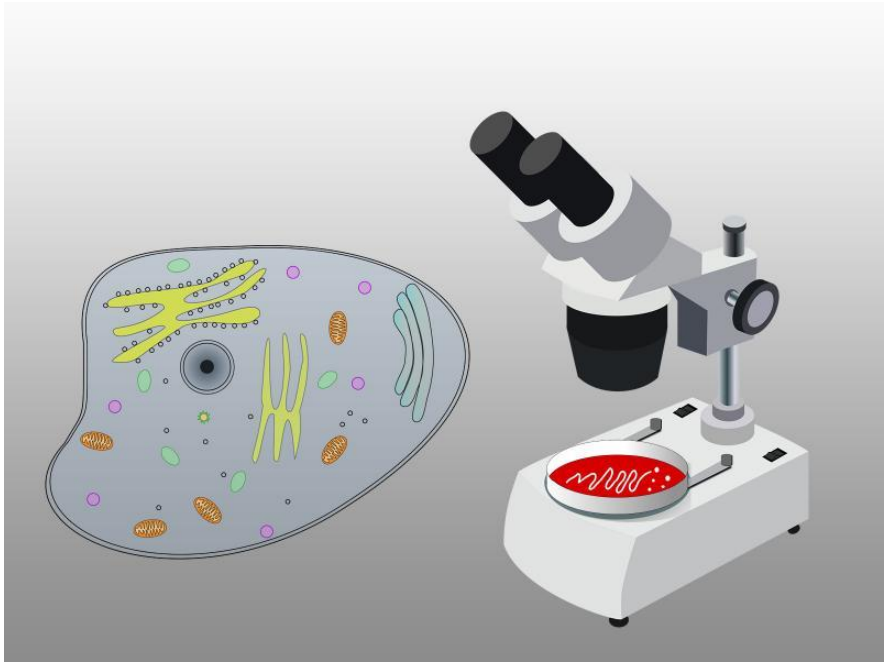
Microscopy & the cell



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MBS 240

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Learning objectives

- Describe the differences between prokaryotic & eukaryotic cells
- Understand the functions of the various components of the cell
- Understand the function of the microscope
- List the different types of microscopes

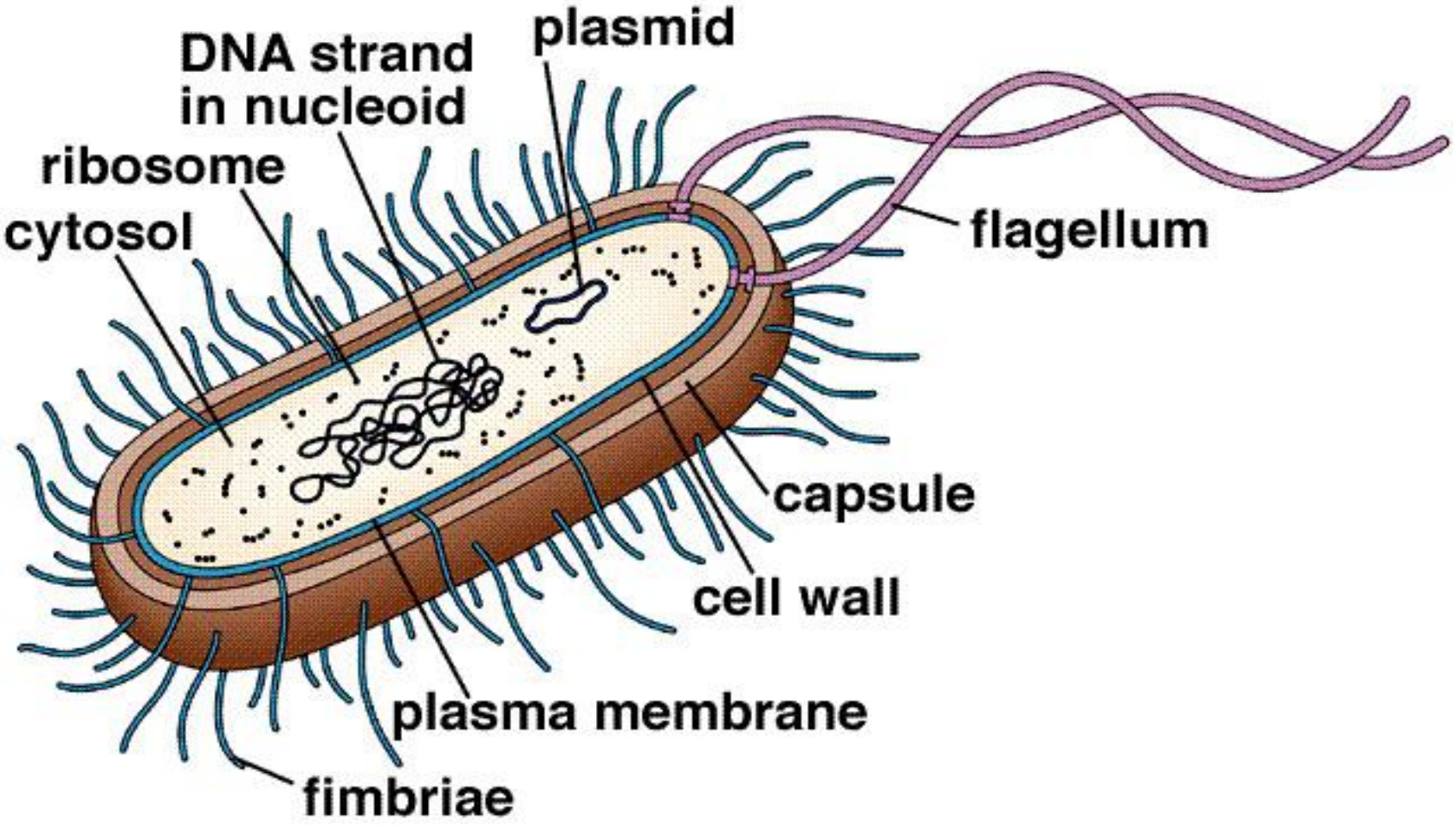
What is a cell?

- The basic structural, functional & biological unit of all living organisms
- Metabolic processes associated with life occur within cells
- Cells arise from pre-existing cells through cell division
- Cells contain hereditary material
  - Passed on to daughter cells during cell division

Prokaryotic cell

- Lack membrane bound organelles
  - Mitochondrion, Golgi bodies etc.
- No true nucleus
- Most are single-celled (unicellular) organisms
  - E.g. bacterial species

Generalized structure of a prokaryote



Eukaryotic cell

- Has several internal structures (organelles)
- Membrane/enveloped nucleus
- Either unicellular or multicellular
  - unicellular example: yeast
  - multicellular examples: plants and animals

Eukaryotic Organelles and Their Functions

General Function: Manufacture	
Nucleus	DNA synthesis; RNA synthesis; assembly of ribosomal subunits (in nucleolus)
Ribosomes	Polypeptide (protein) synthesis
Rough ER	Synthesis of membrane proteins, secretory proteins, and hydrolytic enzymes; formation of transport vesicles
Smooth ER	Lipid synthesis; carbohydrate metabolism in liver cells; detoxification in liver cells; calcium ion storage
Golgi apparatus	Modification, temporary storage, and transport of macromolecules; formation of lysosomes and transport vesicles
General Function: Breakdown	
Lysosomes	Digestion of nutrients, bacteria, and damaged organelles; destruction of certain cells during embryonic development
Peroxisomes	Diverse metabolic processes, with breakdown of H_2O_2 by-product
Vacuoles	Digestion (like lysosomes); storage of chemicals; cell enlargement; water balance
General Function: Energy Processing	
Chloroplasts (in plants and some protists)	Conversion of light energy to chemical energy of sugars
Mitochondria	Conversion of chemical energy of food to chemical energy of ATP
General Functions: Support, Movement, and Communication Between Cells	
Cytoskeleton (including cilia, flagella, and centrioles in animal cells)	Maintenance of cell shape; anchorage for organelles; movement of organelles within cells; cell movement; mechanical transmission of signals from exterior of cell to interior
Cell walls (in plants, fungi, and some protists)	Maintenance of cell shape and skeletal support; surface protection; binding of cells in tissues
Extracellular matrix (in animals)	Binding of cells in tissues; surface protection; regulation of cellular activities
Cell junctions	Communication between cells; binding of cells in tissues

Generalized structure of eukaryote

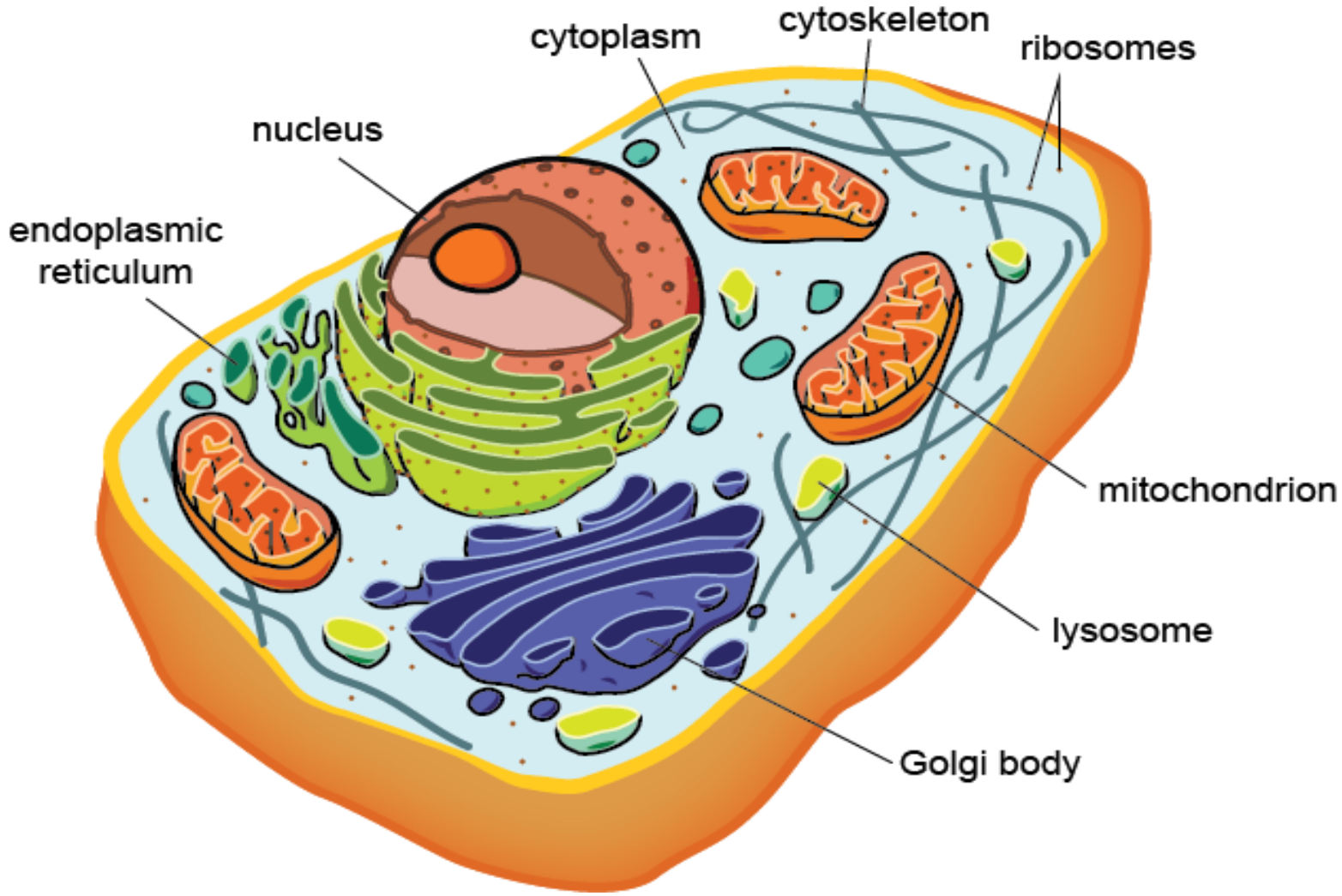
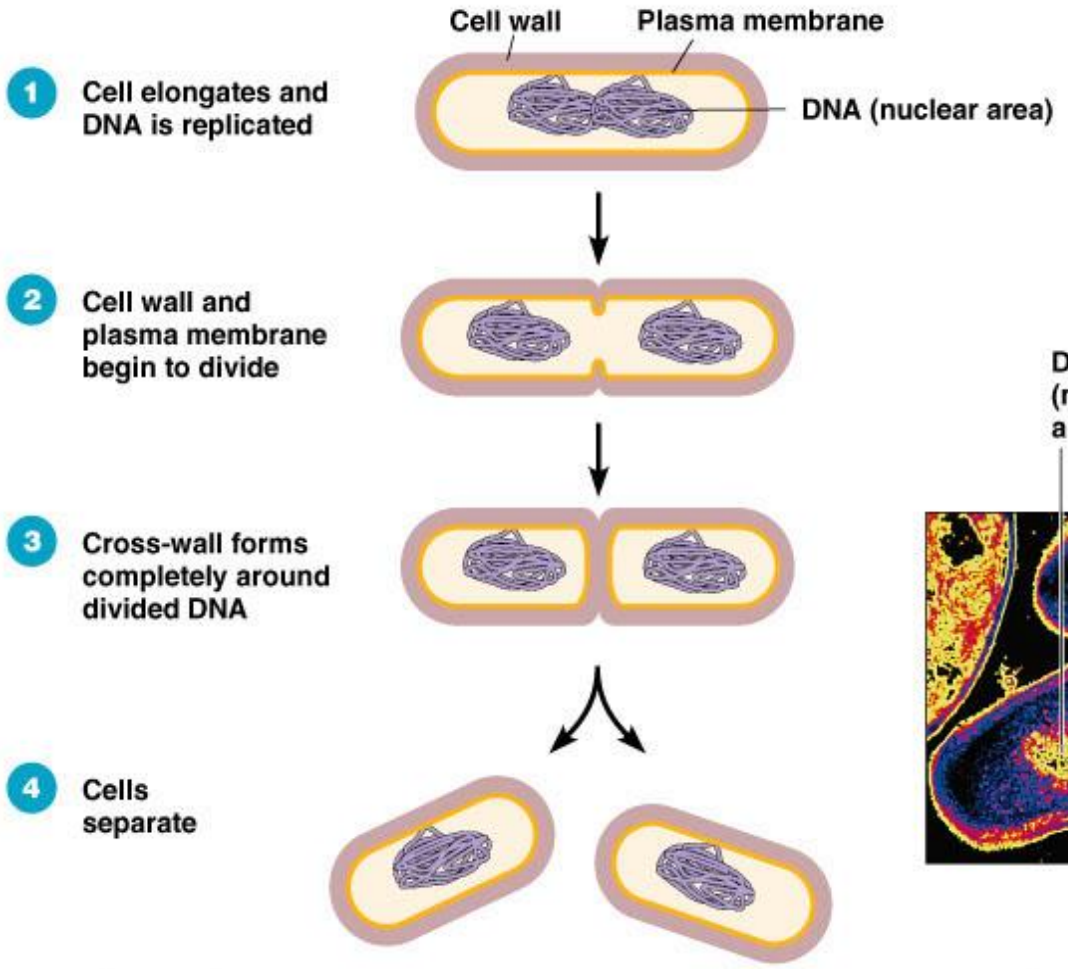


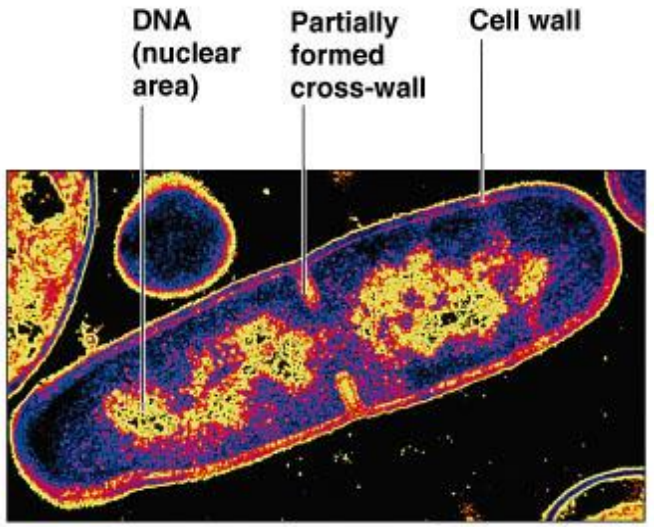
TABLE 4.2 **Principal Differences Between Prokaryotic and Eukaryotic Cells**

Characteristic	Prokaryotic	Eukaryotic
		
Size of cell	Typically 0.2–2.0 μm in diameter	Typically 10–100 μm in diameter
Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA)	Single circular chromosome; lacks histones	Multiple linear chromosomes with histones arrangement
Cell division	Binary fission	Mitosis
Sexual reproduction	No meiosis; transfer of DNA fragments only	Involves meiosis

Prokaryotic cell division- binary fission



(a) A diagram of the sequence of cell division.



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Microscopy

Microscopic principles & applications

- Microscopy is used for two basic principles:
  - Initial detection of microbes
  - Definitive identification
- Allows for the morphological properties of an organism to be identified

Microscopic methods

- 5 general methods used

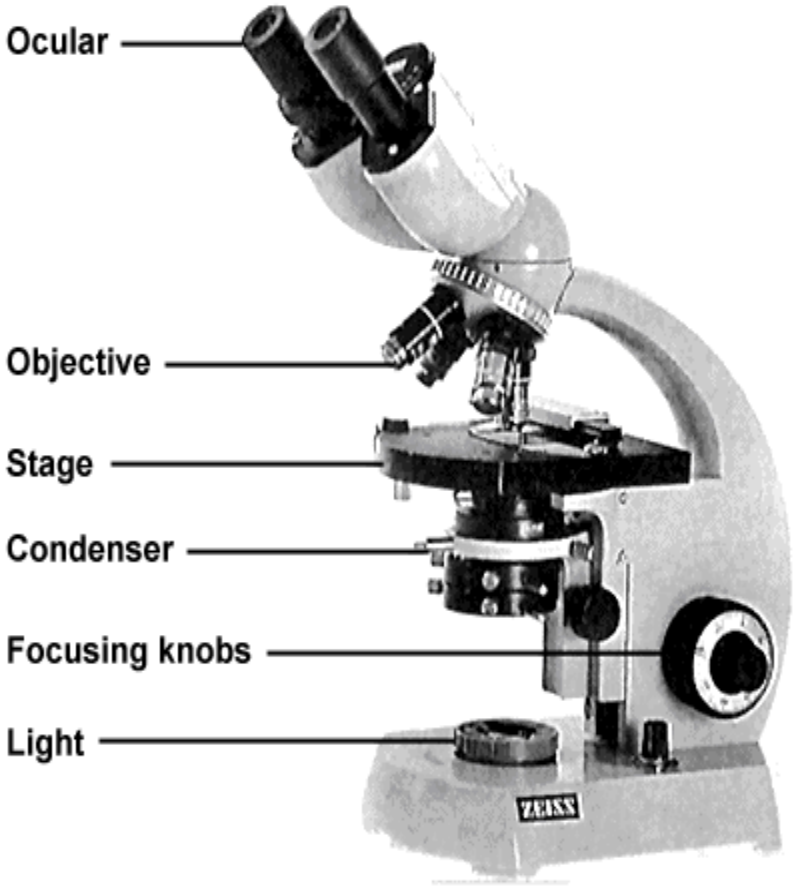
1. Brightfield (light) microscopy
2. Darkfield microscopy
3. Phase contrast microscopy
4. Fluorescence microscopy
5. Electron microscopy

Brightfield (light) microscopy

- Basic components consists of:
  - light source, specimen stage, a condenser, two lens system
- Specimen visualized by transillumination
  - To improve resolution
    - Organisms must be stained with a dye

Components of a brightfield

- Light source: illuminates specimen
- A condenser: used to focus light on specimen
- Objective & ocular lens: used to magnify image of the specimen
  - Oil immersion reduces dispersion of light



Advantages

- Cheap to set up
- Ideal for resource limited settings

Limitations

- Poor resolving poor
  - Low sensitivity
- Requires stains to improve resolution

Darkfield microscopy

- Objective & ocular lenses similar to brightfield
- Special condenser is used
  - Prevents transmitted light from directly illuminating specimen
  - Only oblique scattered light reaches specimens
    - Causes specimen to be brightly illuminated against black background
- Resolving power is significantly improved

T. pallidum (bacterium that causes syphilis)

Advantages

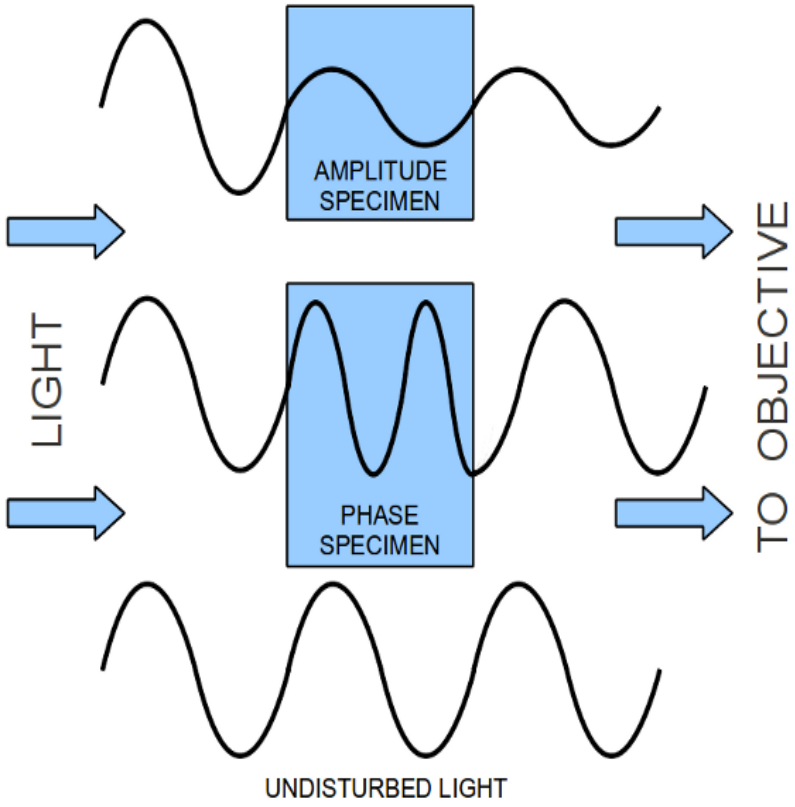
- Simple and effective
- Ideal for unstained biological samples

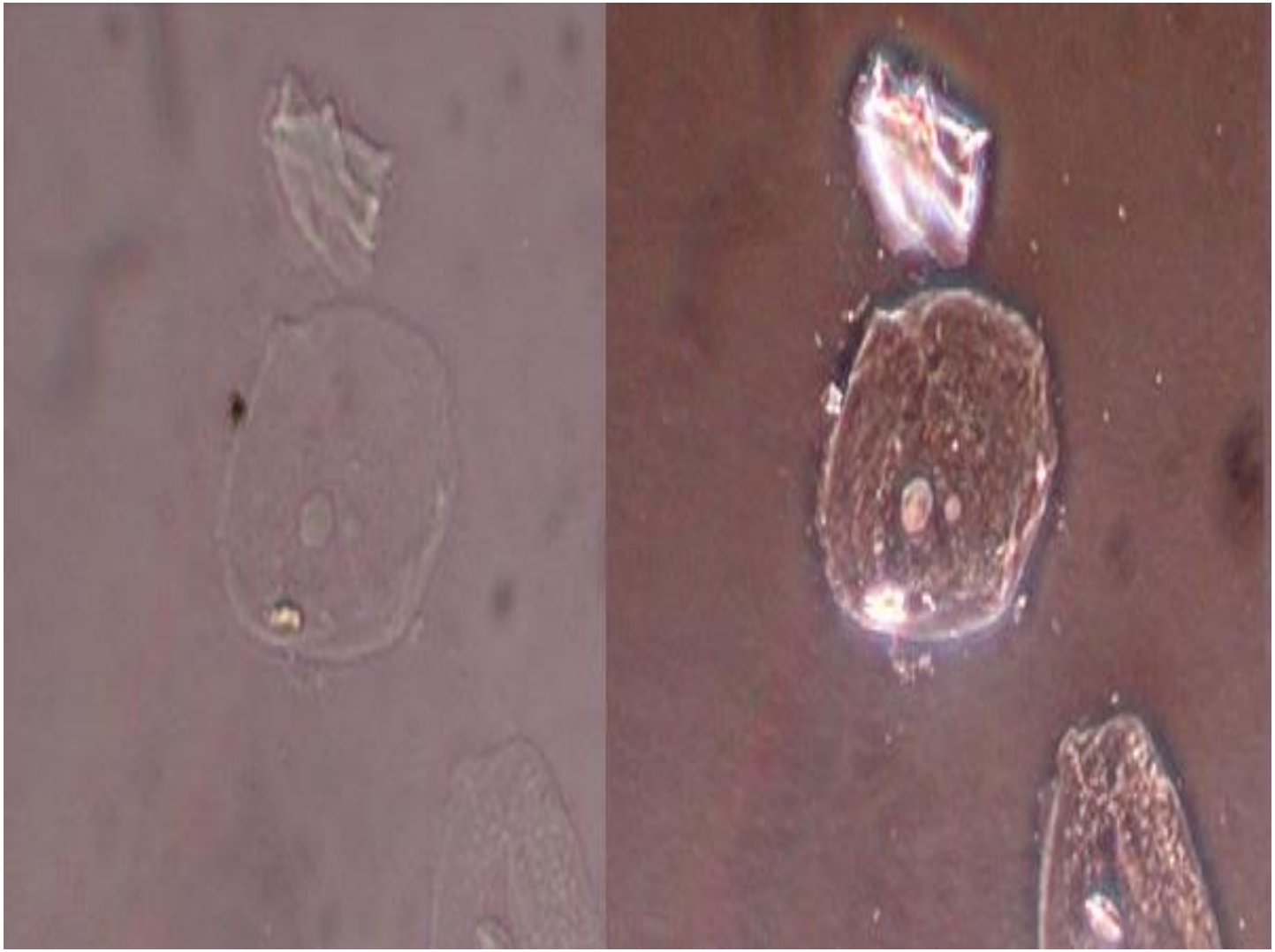
Limitations

- Requires strong illumination of sample
  - May damage sample

Phase contrast microscopy

- Enables internal details of microbes to be examined
- Parallel light beams affected by differences in specimen density
  - Creates 3-D image of specimen

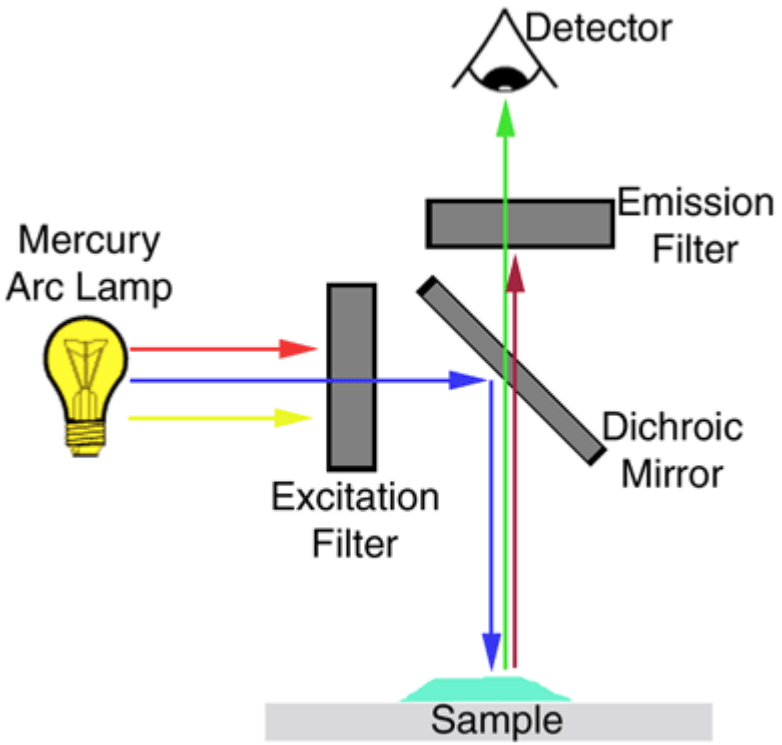




Comparison of cells using bright field microscopy (left) & phase contrast (right)

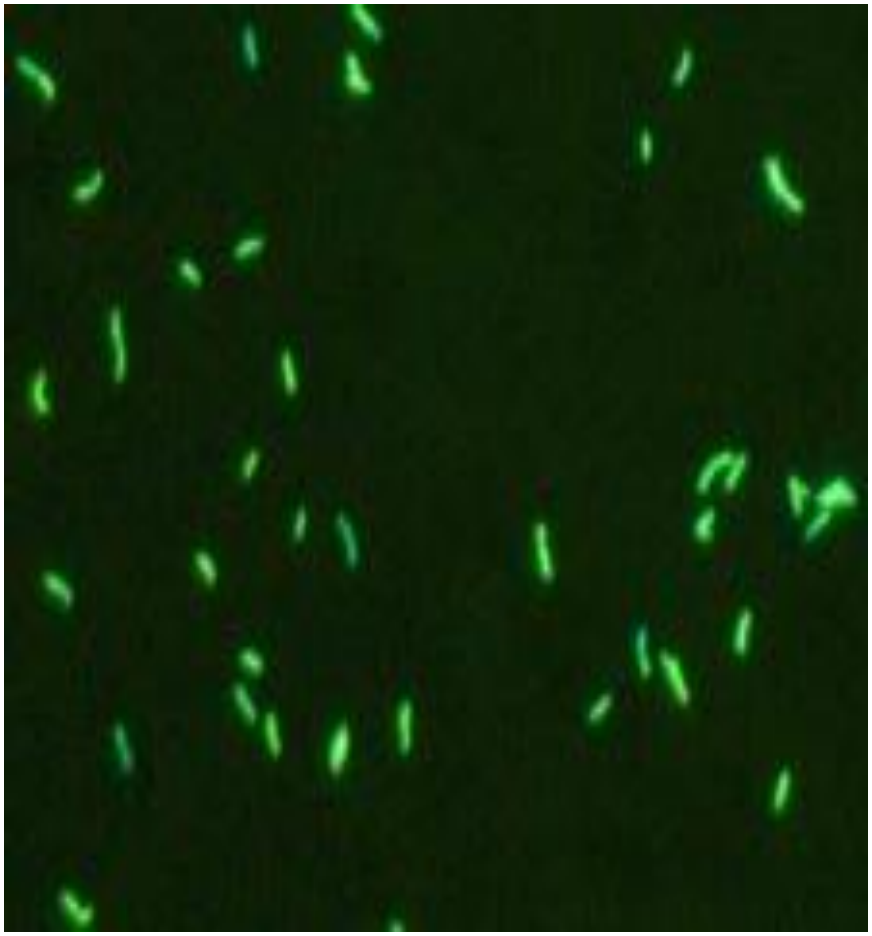
Fluorescence microscopy

- Involves staining microbes with fluorescent dyes
  - Examination with fluorescence microscope
  - Shorter wave length light
- Expensive to set up & run

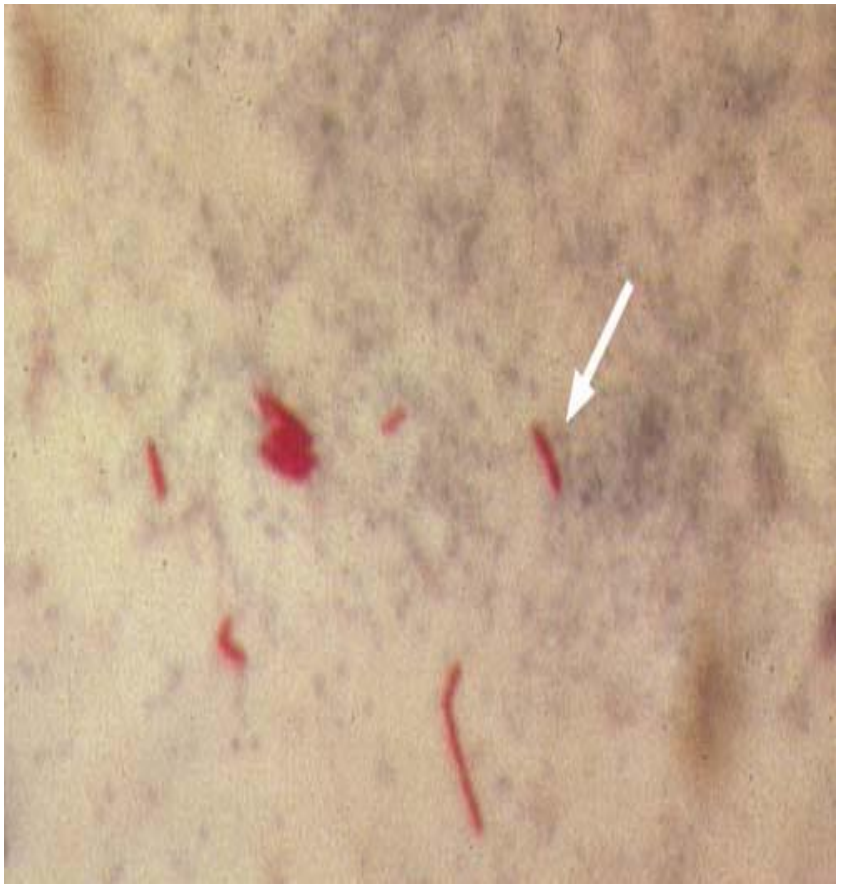


Fluorescence LED microscopy of *Mycobacterium tuberculosis*

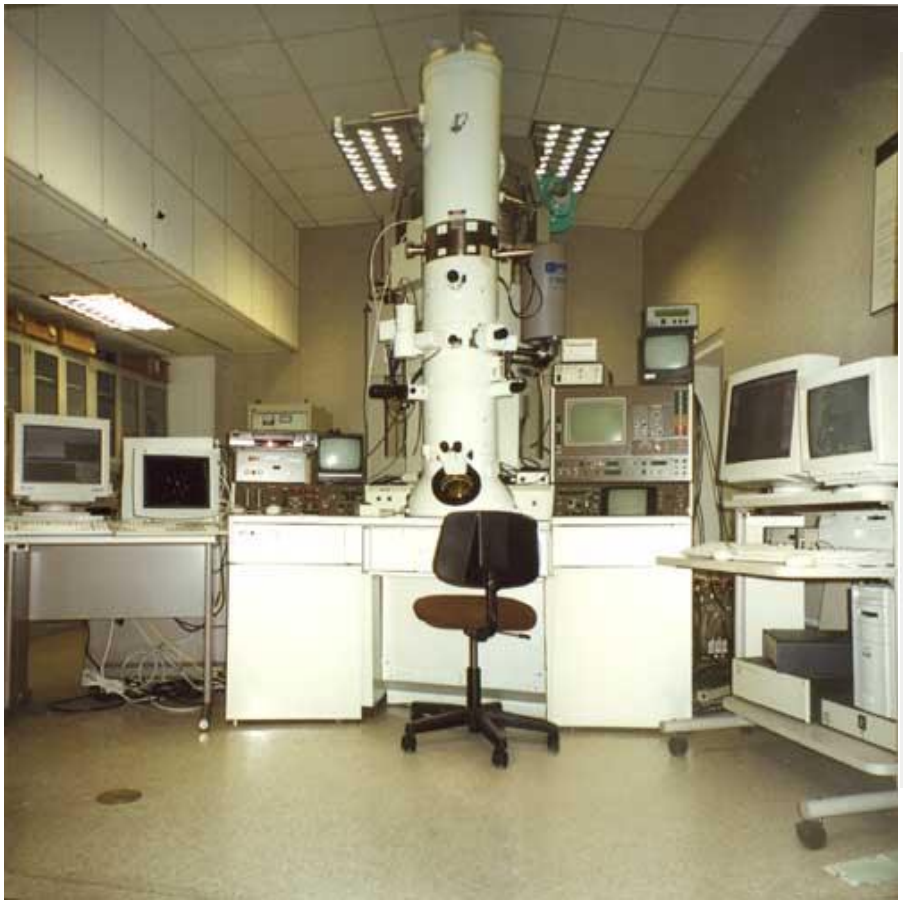
FM image

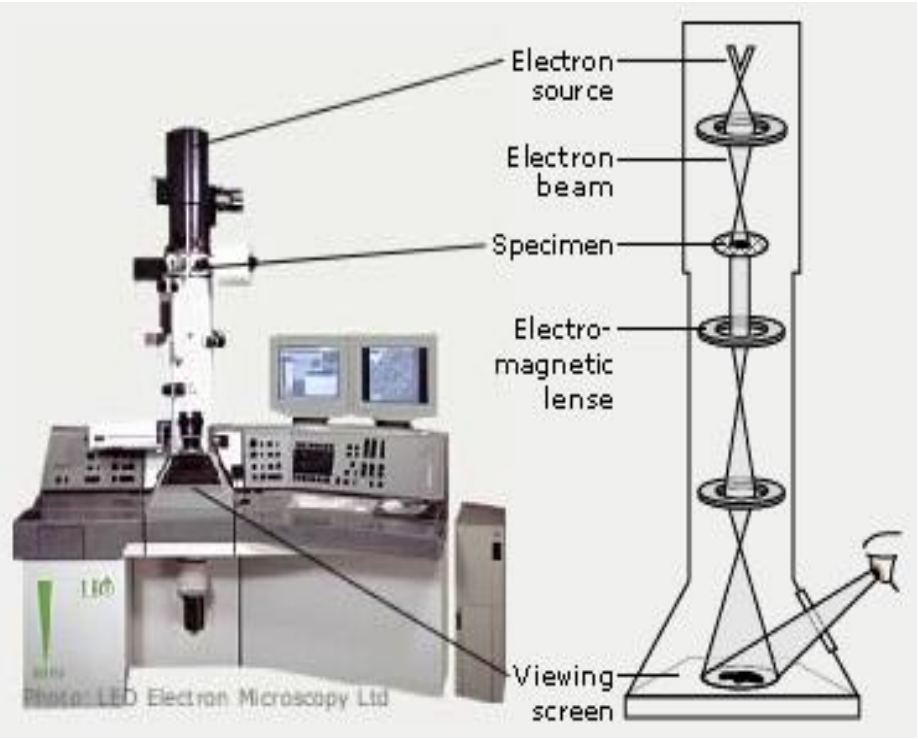


Vs. ZN light microscopy

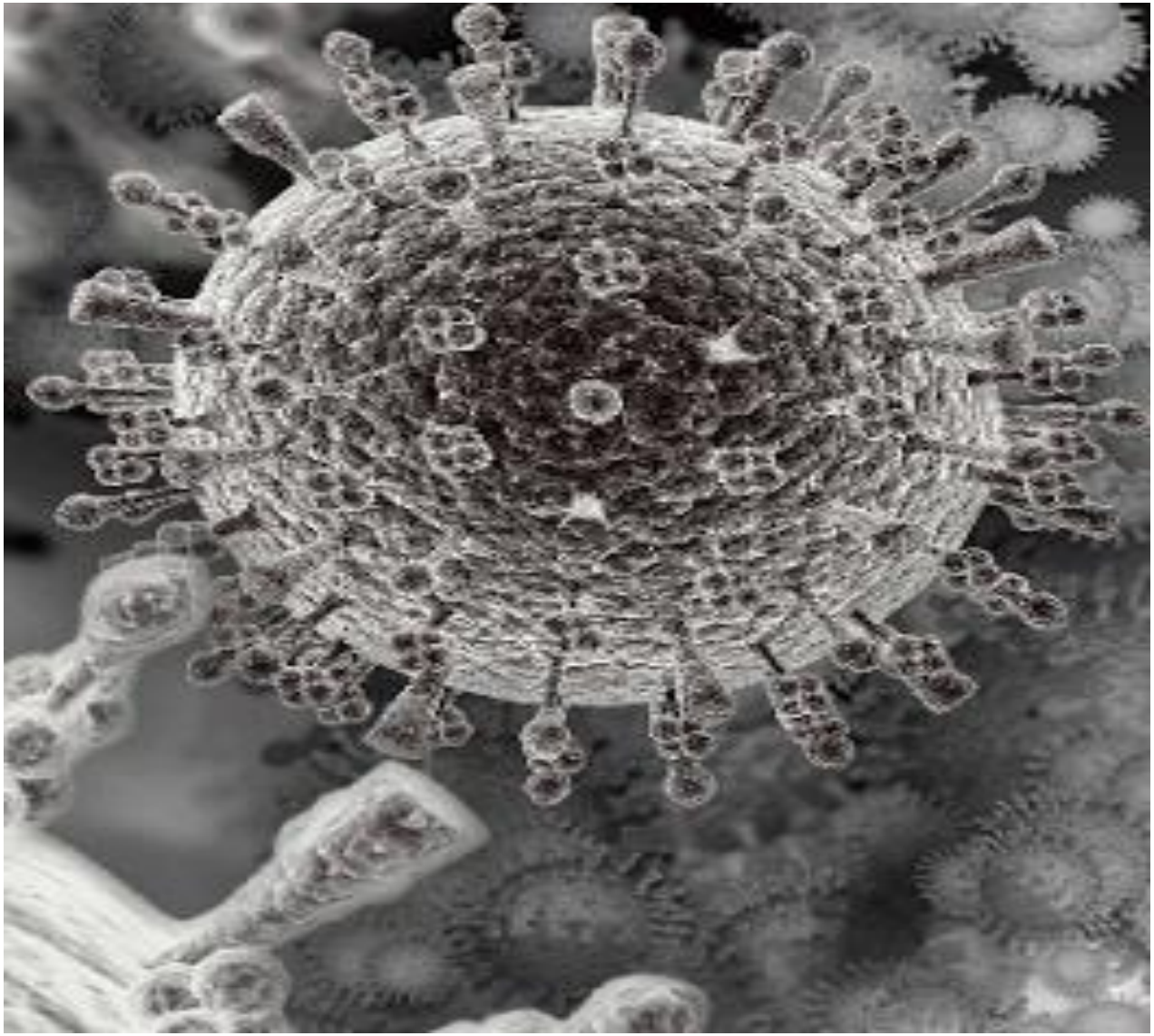


Electron Microscope (EM)





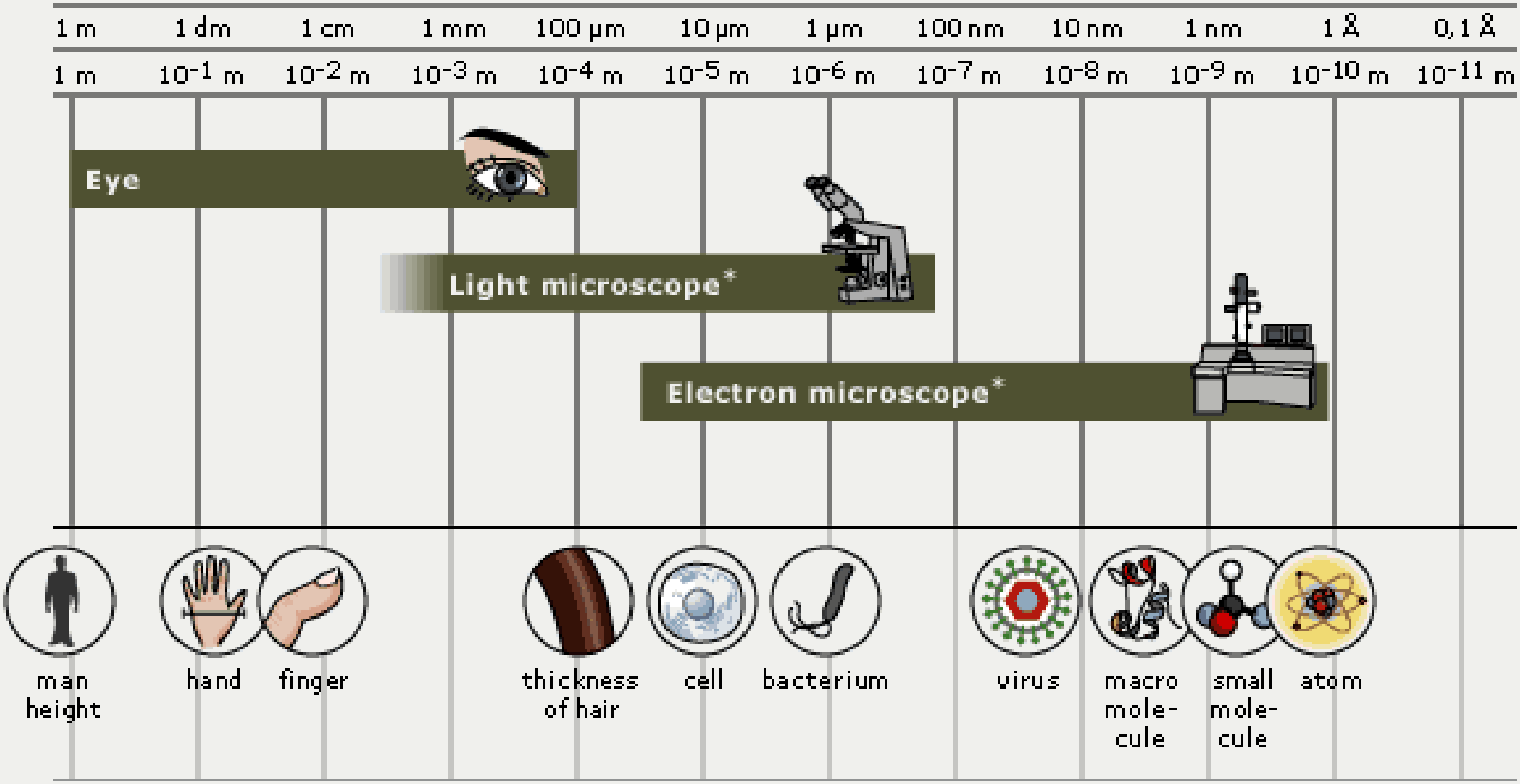
- High resolving power
  - 1000 times more than light microscope
- Beam of electrons focused using electromagnetic lenses
- Commonly used stains
  - Heavy metals e.g. gold & osmium tetroxide
- Disadvantages: cannot view living specimens



Influenza virus under electron microscope

Resolving Power Line

What can you see with the different types of microscopes? The human eye is capable of distinguishing objects down to a fraction of a millimeter. With the use of light and electron microscopes it is possible to see down to an angstrom and study everything from different cells and bacteria to single molecules or even atoms.



* Light microscope includes phase contrast and fluorescence microscopes. Electron microscope includes transmisson electron microscope.

Staining methods

- Used to enhance visualization of structures under a microscope i.e.. cellular components
- Different stains can be used for preferential staining of cell components
  - Such as nucleus, cell wall
- Most stains can be used on fixed cells

Commonly used stains

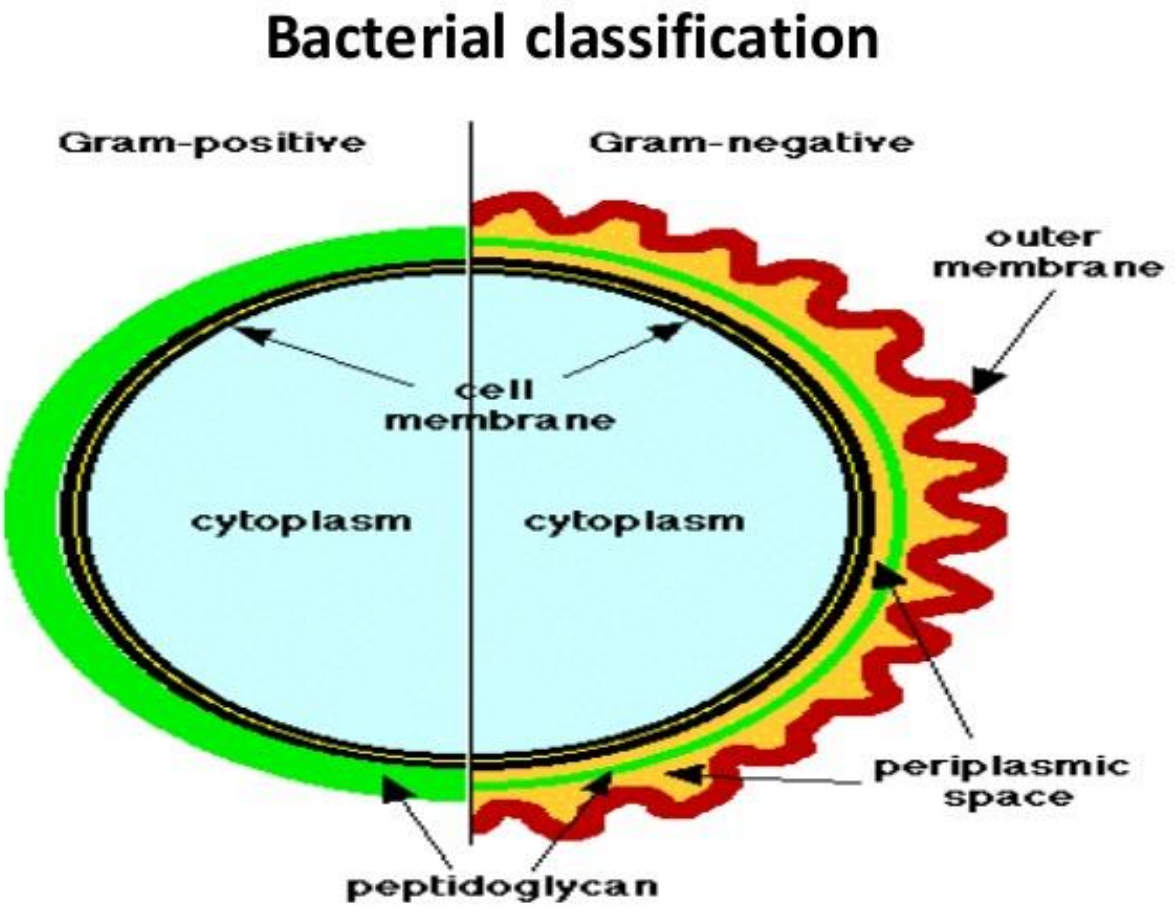
Direct examination

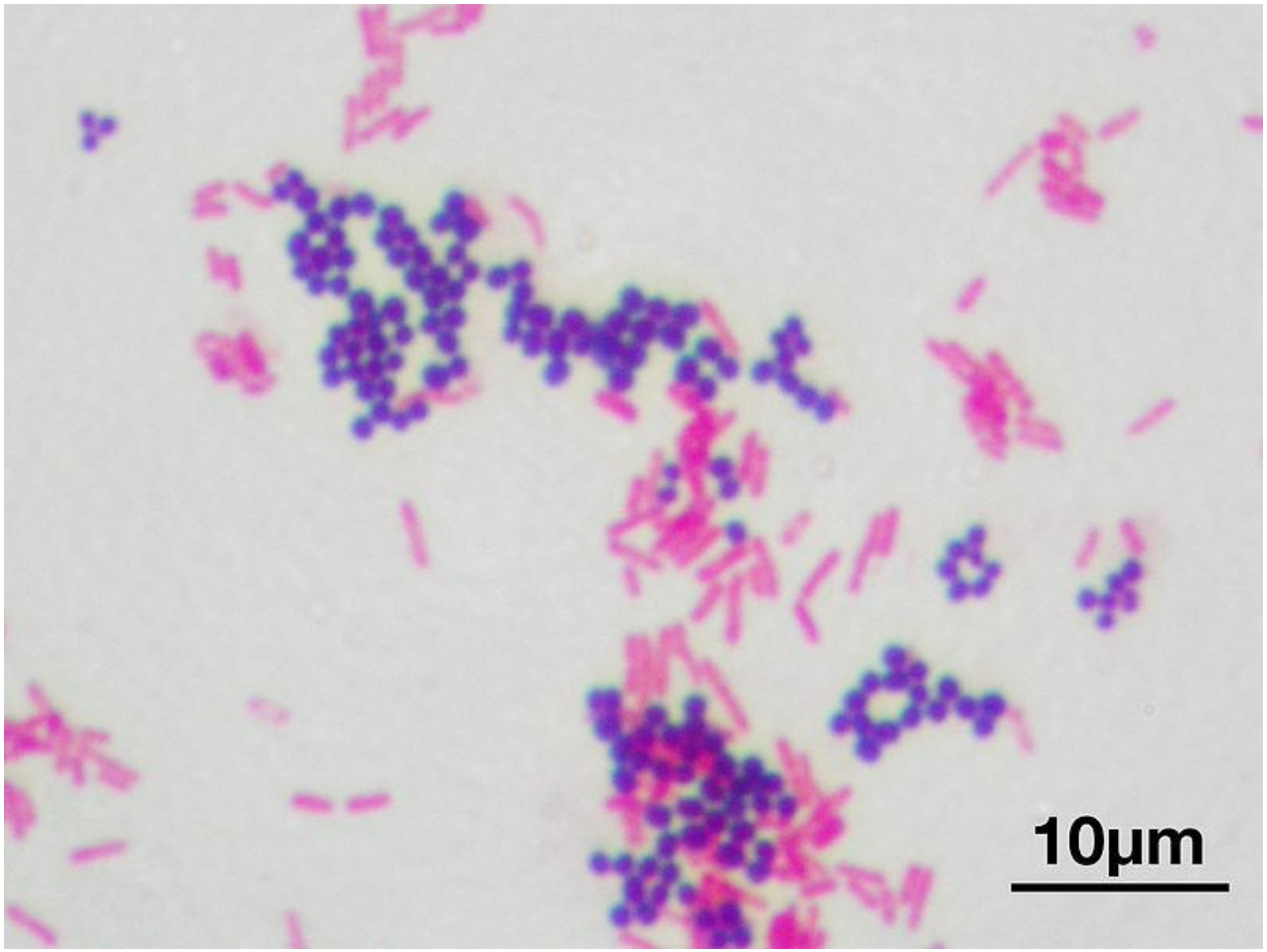
1. KOH
   - Used to dissolve proteinaceous material
   - Ideal for detection of fungal elements
2. India ink
   - Modification of KOH
   - Ink is added as a contrast material

Differential stains

1. Gram stain
   - Most commonly used stain in microbiology lab
   - Separates gram –ve & +ve bacteria
     - Based on cell wall
2. Wright-Giemsa stain
   - Used to detect blood parasites

Cell wall classification





Gram positive cocci (*S. aureus*) and gram negative bacili (*E. coli*)

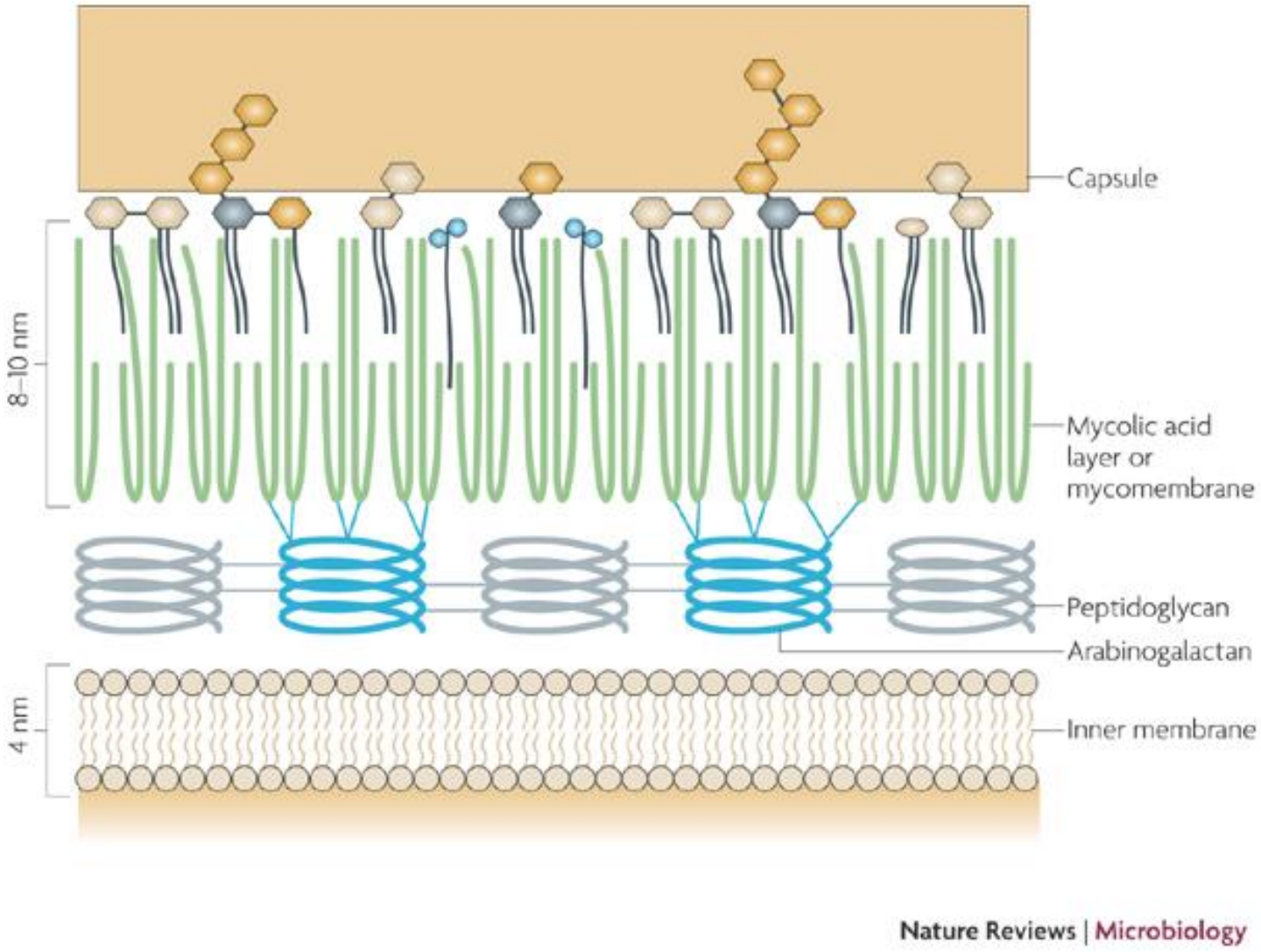
Gram stain specimens under light microscope

C. albicans

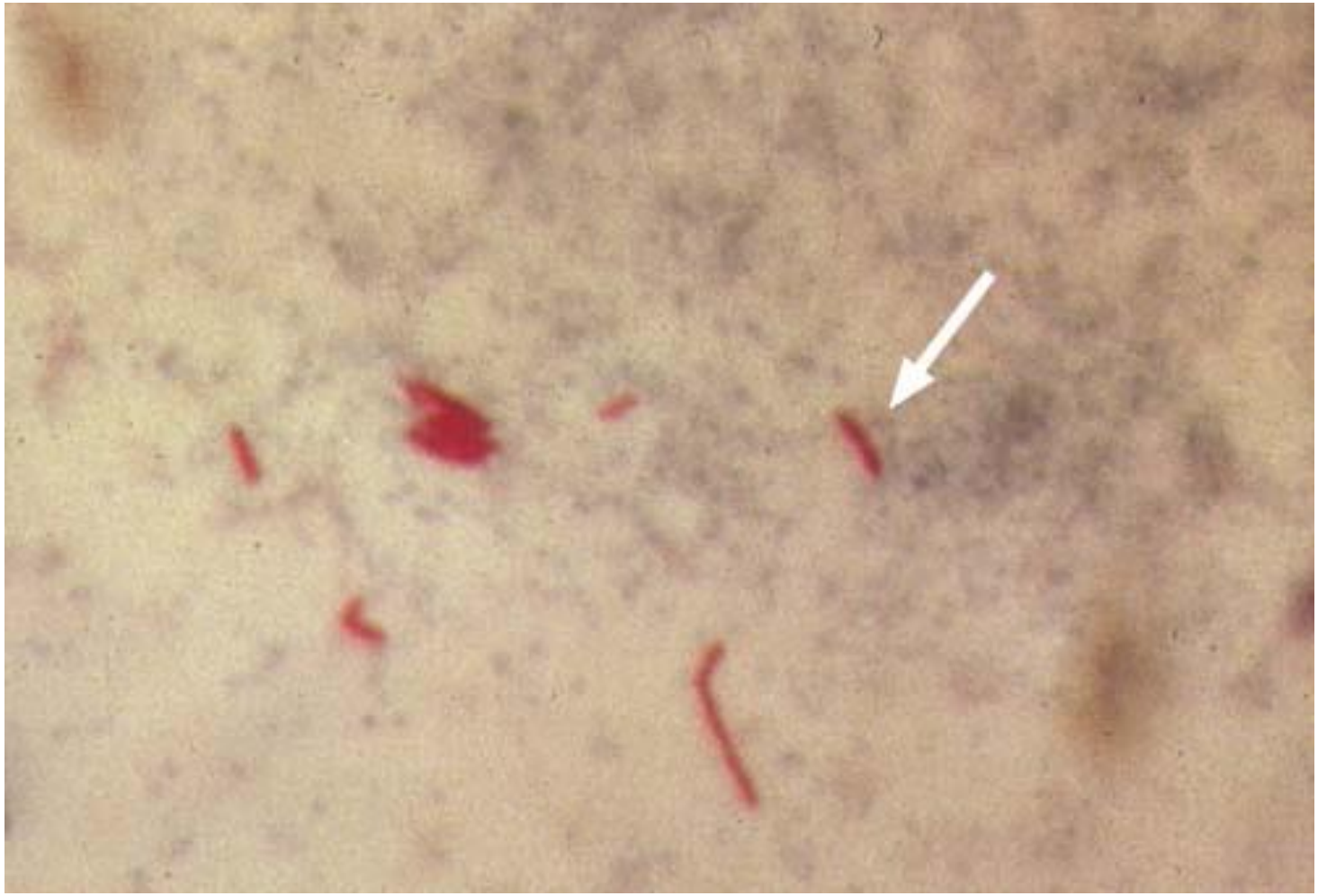
Acid-Fast stains

1. Ziel-Neelsen stain
   - Used to stain Mycobacteria & other acid-fast organisms
2. Auramine-rhodamine
   - Fluorescent dyes that stain acid-fast organisms
3. Modified acid-fast stain
   - Used to stain weak acid-fast organisms
     - E.g. *Norcardia spp.*

Mycobacterial cell wall

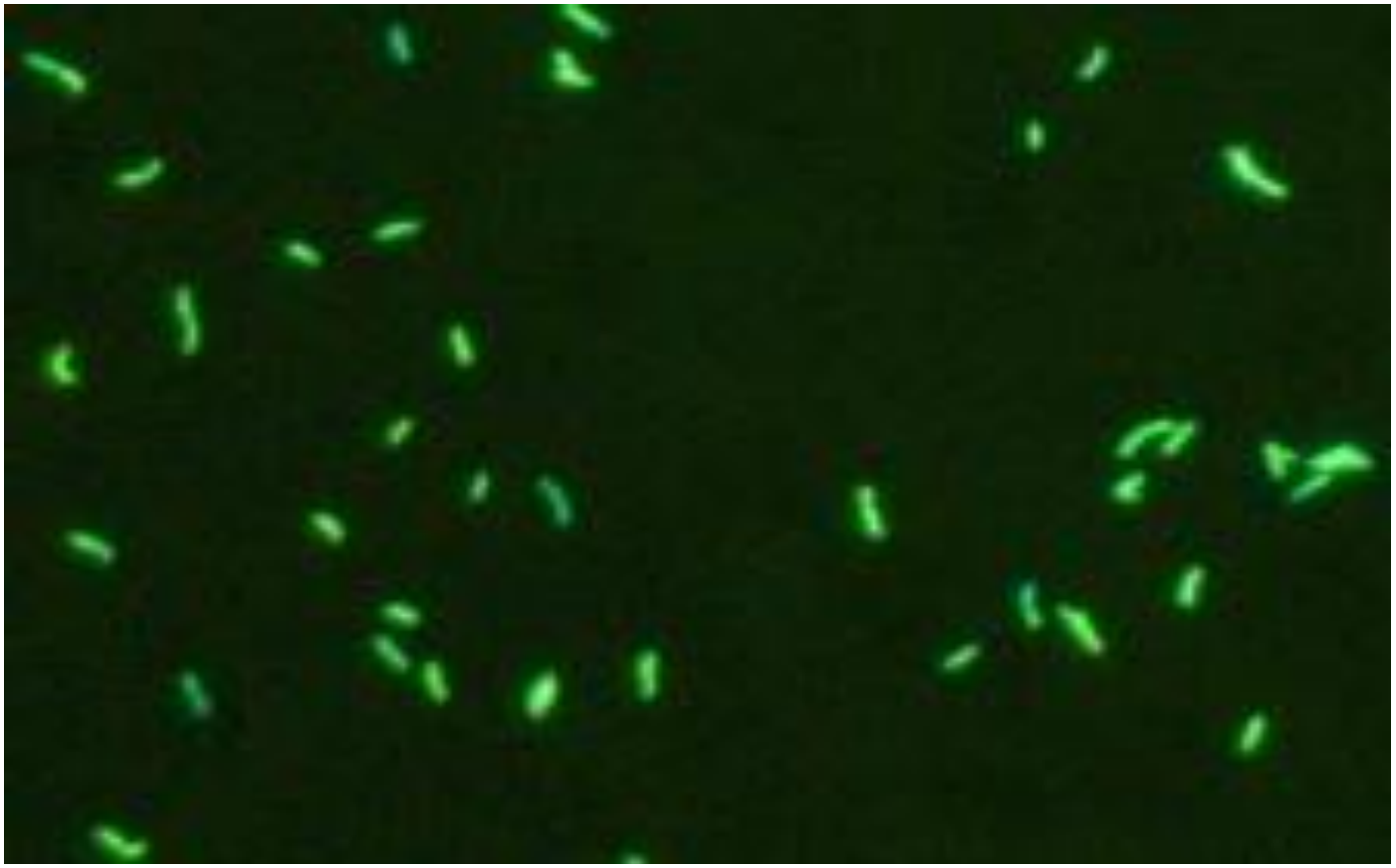


Ziehl-Neelsen stain for AFB



Arrow pointing at Acid Fast Bacilli (*Mycobacterium tuberculosis*)

Auramine-rhodamine-Fluorescence LED microscopy of *Mycobacterium tuberculosis*



Further reading

- **Murray, Medical Microbiology** 6th edition chp. 14 on microscopic principles and applications
- **Mims' Medical Microbiology** 4th Ed chp. 32 on diagnosis of infection (section on non-cultural techniques for the laboratory diagnosis of infections)