Lecture-6

Microscopes

Microscopes

- Microscopes and microbiology → linking advance
- The microscope is the microbiologist's most basic tool .
- Microscopes use lenses to magnify object's images.
- There are many types of microscopes (look at the next slide) but the most common include two types:- :
  - **Light microscopes (5 types)**
  - **Electron microscope (2 types)**
- Light microscope used to examine cells at relatively low magnifications and electron microscope used to lock at cells and cell structure at very high magnification.

Microscope Type

- 1-Light
  - 1-Brightfield
  - 2-Darkfield
  - 3-Phase-contrast
  - 4-Differential interference contrast (DIC)
  - 5-Fluorescence
- 2-Confocal → Uses a single photon to illuminate one plane of a specimen at a time
- 3-Two-Photon → Uses two photons to illuminate a specimen.
- 4-Scanning Acoustic → Uses a sound wave of specific frequency that travels through the specimen with a portion being reflected when it hits an interface within the material.
- 5-Electron
  - 1-Transmission (TEM)
  - 2-Scanning (SEM)
- 6-Scanned-Probe
  - 1-Scanning tunneling →
    - ✓ Uses a thin metal probe that scans a specimen and produces an image.
    - ✓ Resolving power is much greater than that of an electron microscope.
    - ✓ No special preparation required.
  - 2-Atomic force →
    - ✓ Uses a metal-and-diamond probe.
    - ✓ Produces a three-dimensional image.
    - ✓ No special preparation required.

Some Principles of Light Microscopy

- Compound light microscope uses **visible light** to illuminate cells

- **Many different types of light microscopy**:

1-Brightfield

2-Darkfield

3-Phase-contrast

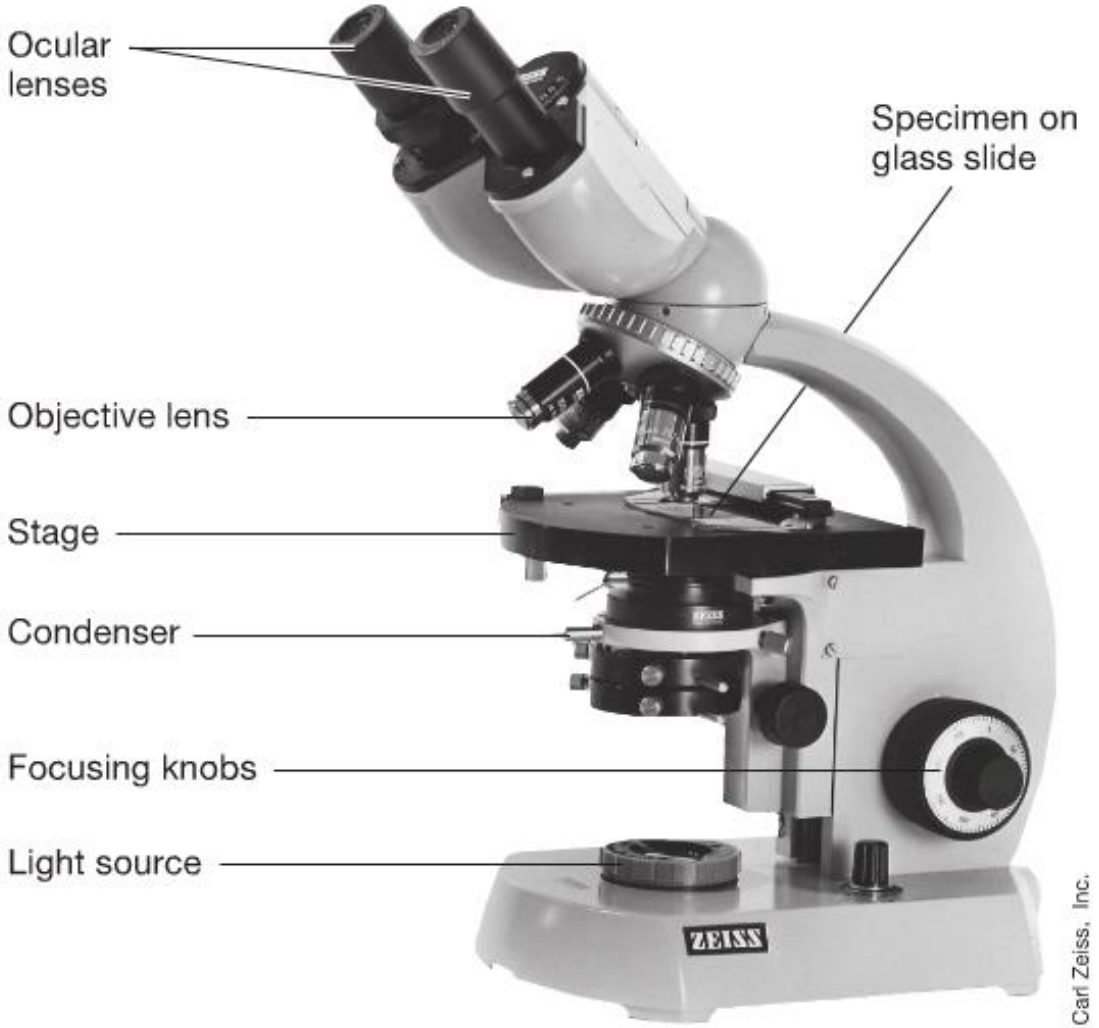
4-Differential interference contrast (DIC)

5-Fluorescence

Some Principles of Light Microscopy

Bright-field microscope

- Specimens are visualized because of differences in contrast (density) between specimen and surroundings.
- Contrast differences arise because cells absorb or scatter light to varying degrees.
- Two sets of lenses form the image
  - **Objective lens and ocular lens (compound)**
  - Total magnification = objective magnification × ocular magnification
  - Maximum magnification is ~2,000×


Ocular lenses
Specimen on glass slide
Objective lens
Stage
Condenser
Focusing knobs
Light source
ZEISS
Carl Zeiss, Inc.

(a)

Some Principles of Light Microscopy

- Magnification is not the limiting factor in the ability of seeing small things but also we need a good resolution which is the ability to distinguish two adjacent objects .
- Magnification can be increased with out limit but resolution cannot because it is the function of physical properties of light.
- Light microscopy limits resolution is about 0.2µm , electron microscope resolution is greater than of light microscope .

Some Principles of Light Microscopy

- ***Resolution:*** the ability to distinguish two adjacent objects as separate and distinct
  - Resolution is determined by the wavelength of light used and numerical aperture of lens

Methods to Improve contrast to generate a better final image?

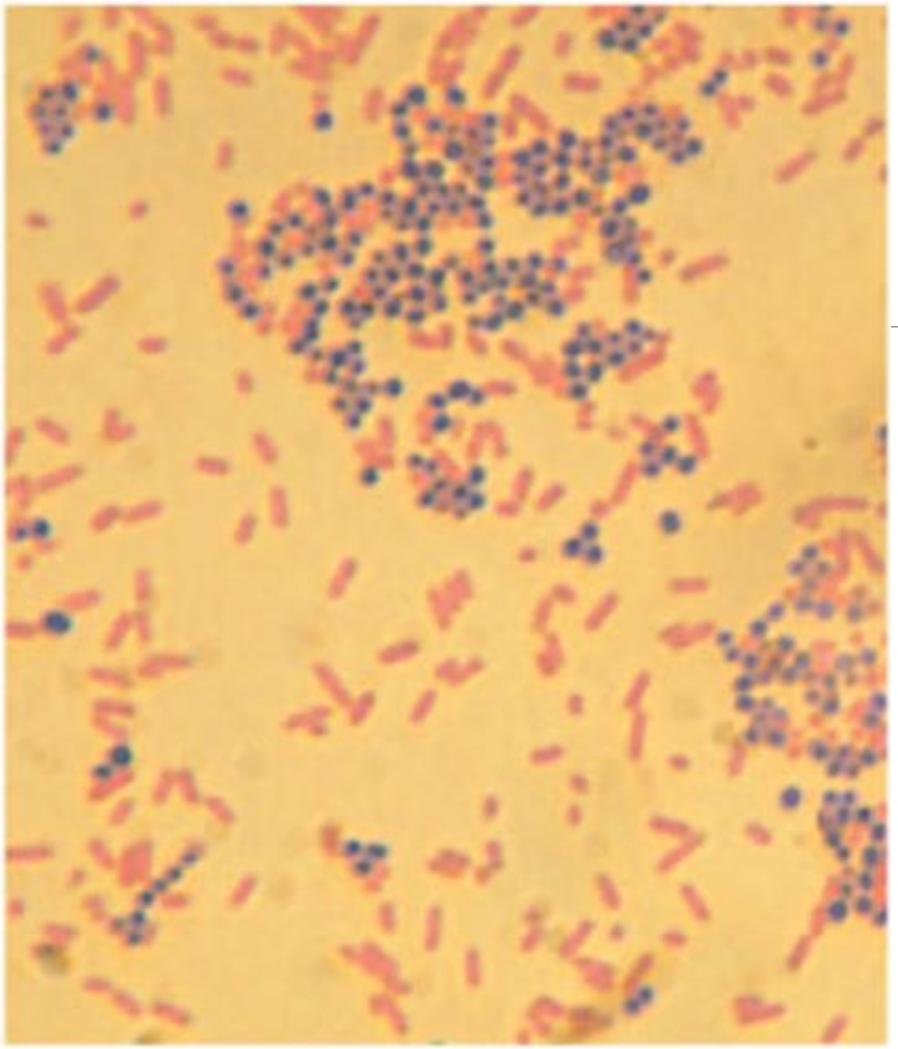
Staining

- Dyes are organic compounds that bind to specific cellular materials
- Examples of common stains are **methylene blue, safranin, and crystal violet**

Differential stains: the *Gram stain*

- Differential stains separate bacteria into groups

  Bacteria can be divided into two major groups: gram-positive and gram-negative
- Gram-positive bacteria appear purple and gram-negative bacteria appear red after staining



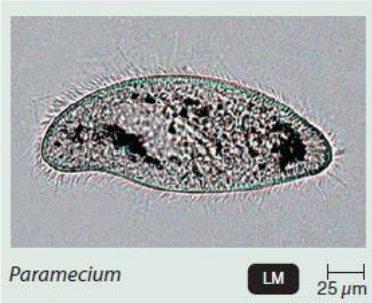
Microscopic observation of gram-positive (purple) and gram- negative (red) bacteria .

Phase-contrast and Dark-felid Microscopy

- Two form of light microscopy improve image with out using staining.
- Phase –contrast microscopy is based on the principle that cells differ in refractive index from their surroundings .
- Phase –contrast microscopy resulting a dark image on light background
- The dark-fielded microscopy is a light microscope in which the light reaches the specimen from the sides only . Thus the specimens appears light on a dark background.
- The dark-fielded microscopy used in observed microbial motility .

Fluorescence Microscopy

- Used to visualize specimens that fluoresce – emit light of one color following absorption of light of another color .
- **Cells fluoresce either :**
  - Contain naturally fluorescent substances such as chlorophyll
  - Because cells have satin with fluorescent dye .
- DAPI (4`,6-diamidino-2-phenylindole) is widely used fluorescent dye staining cell`s DNA .



Brightfield Microscope

Uses visible light as a source of illumination; cannot resolve structures smaller than about 0.2 µm; specimen appears against a bright background. Inexpensive and easy to use.

Darkfield Microscope

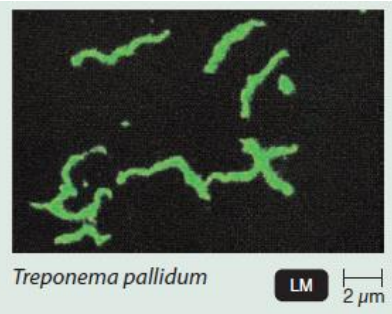
Uses a special condenser with an opaque disk that blocks light from entering the objective lens directly; light reflected by specimen enters the objective lens, and the specimen appears light against a black background

Phase-contrast Microscope

Uses a special condenser containing an annular (ring-shaped) diaphragm. The diaphragm allows direct light to pass through the condenser, focusing light on the specimen and a diffraction plate in the objective lens. Direct and reflected or diffracted light rays are brought together to produce the image.
No staining required

Differential interference contrast (DIC) Microscope

Like phase-contrast, uses differences in refractive indexes to produce images. Uses two beams of light separated by prisms; the specimen appears colored as a result of the prism effect.
No staining required.



Fluorescence Microscope

Uses an ultraviolet or near-ultraviolet source of illumination that causes fluorescent compounds in a specimen to emit light.

Electron Microscopy

- Electron microscopes use **electrons** instead of photons(visible light) to image cells and structures.
- Electromagnets function as lenses in EM , whole system operates in a vacuum.
- EM are fitted with cameras to allow a photograph to be taken

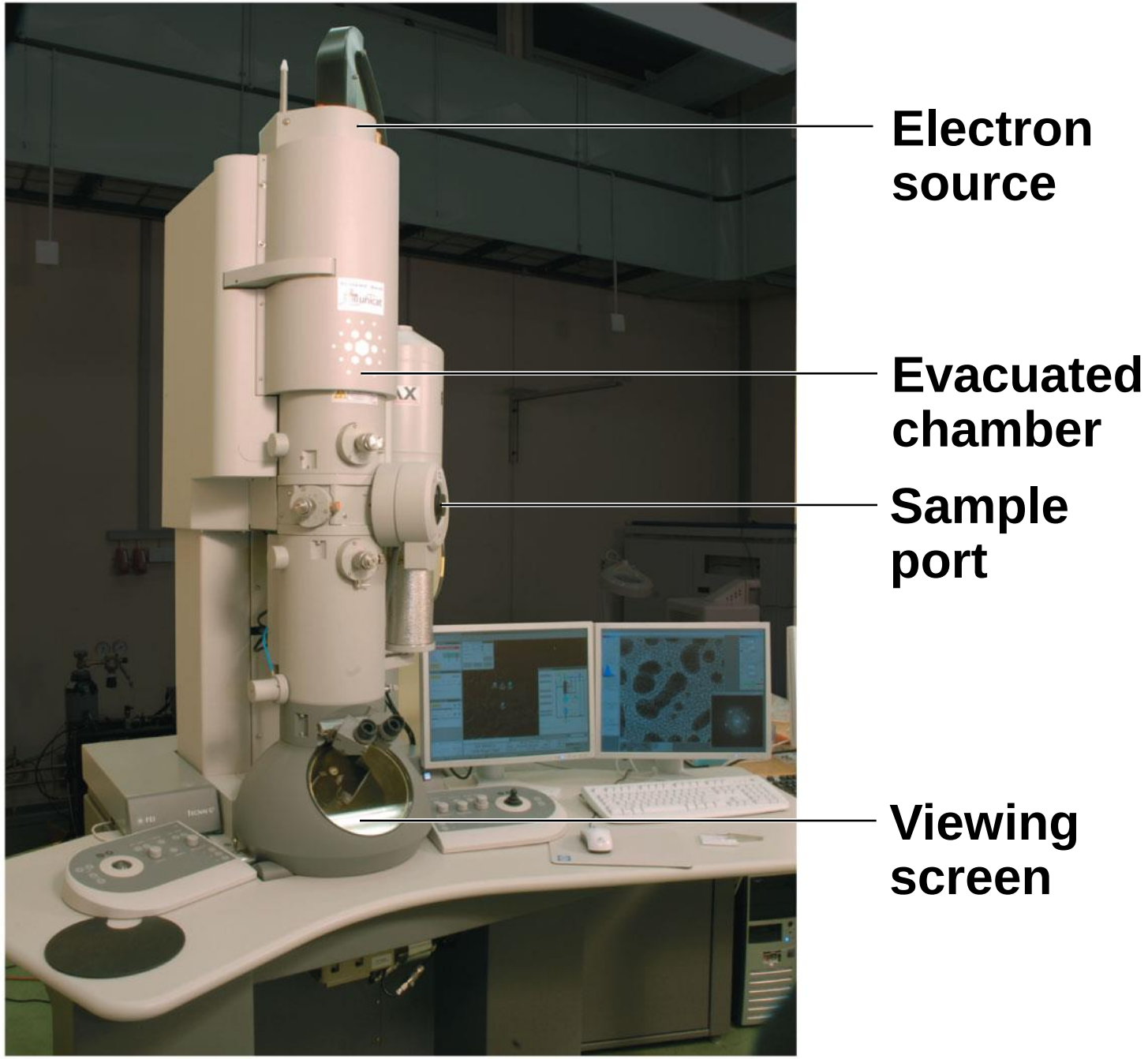
Two types of electron microscopes:

- *Transmission electron microscopes (TEM)*
  - *(need thin section),negative stain*
- *Scanning electron microscopes (SEM)*
  - *Coat with heavy metal*

Electron Microscopy

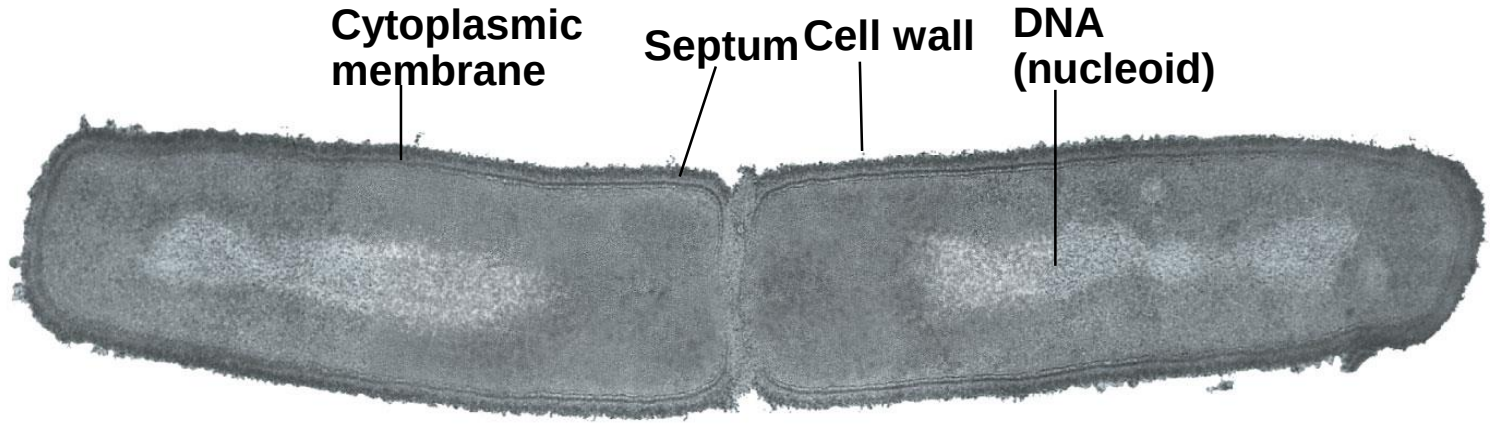
- Transmission electron microscopy is used to examine cells and cell structure at very high magnification and resolution , even enabling one to view structures at the molecular level .
- This is because the wavelength of electrons is much shorter than the wavelength of visible light and wavelength affects resolution .
- Unlike visible light , electron beams can not penetrate very well. So, special techniques of thin sectioning are needed to prepare specimens before observing them .
- To obtain sufficient contrast, the preparation are treated with stains such as osmic acid , permanganate , uranium , lanthanum ; because these substances are composed of atoms of high atomic weight , they scatter electrons well and thus improve contrast.

Figure 2.9



Electron Microscopy

- Scanning electron microscopy used to observed external features of an organisms or cell .
- No need for thin sections
- The specimen is coated with a thin film of a heavy metal such as gold .
- An electron beam then scans back and forth across the specimens. Electrons scattered from the metal coating are collected and activate a viewing screen to produce an image.



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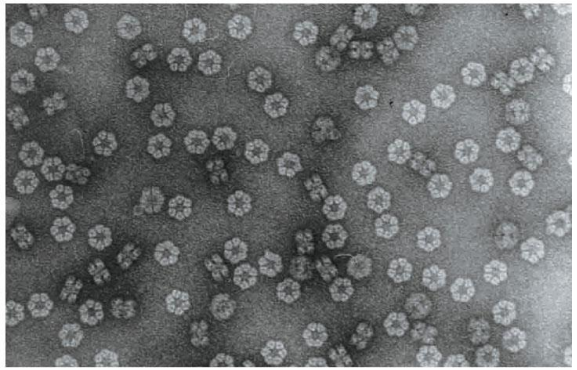

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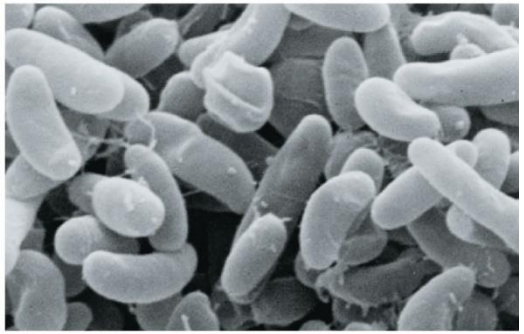

(c)

Note that:-

- ✓ Electron micrographs taken either TEM or SEM are black and white images .
- ✓ Some false color is added to these images to boost their artistic appearance.

Electron micrographs. *(a)* Micrograph of a thin section of a dividing bacterial cell, taken by transmission electron microscopy (TEM). Note the DNA forming the nucleoid. The cell is about 0.8 μm wide. *(b)* TEM of negatively stained molecules of hemoglobin. Each hexagonal-shaped molecule is about 25 nanometers (nm) in diameter and consists of two doughnut-shaped rings, a total of 15 nm wide. *(c)* Scanning electron micrograph of bacterial cells. A single cell is about 0.75 μm wide.

(Brock Biology of Microorganisms 13th Edition 2012)

Home work
Black board

1- Differentiate between the 4 types of light microscopy. Provide examples of images produced by each type.
2- Explain the difference between the 2 types of electron microscope. Provide examples of images produced by each types
3- Is there any other types of microscopies?

REMEMBER

You can always ask questions through our discussion board on
www.lms.ksu.edu.sa