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សាកលវិទ្យាល័យ ពុទ្ធិសាស្ត្រ

UNIVERSITY OF PUTHISAstra

គោរពខ្លួនឯង

Honor Self

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Respect Others

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Develop Society

FACULTY OF MEDICINE

OBSERVING MICROORGANISMS THROUGH A MICROSCOPE

AN SRIM, MLT, MD

Introduction

- ▶ Microscope is an optical instrument used to magnify (enlarge) minute objects or microorganisms which cannot be seen by naked eye.
- ▶ **Microscopic Methods**
 - ▶ A. Light Microscopy
 - ▶ 1. Brightfield (light) microscopy
 - ▶ 2. Darkfield microscopy
 - ▶ 3. Phase-contrast microscopy
 - ▶ 4. Fluorescent microscopy
 - ▶ 5. Confocal
 - ▶ B. Electron Microscopy
 - ▶ Transmission
 - ▶ Scanning
 - ▶ C. Scanning Probe Microscopes

Microscopy: The Instruments

Ocular lens
(eyepiece)
Remagnifies the image formed by the objective lens

Body tube Transmits the image from the objective lens to the ocular lens

Arm

Objective lenses
Primary lenses that magnify the specimen

Stage Holds the microscope slide in position

Condenser Focuses light through specimen

Diaphragm Controls the amount of light entering the condenser

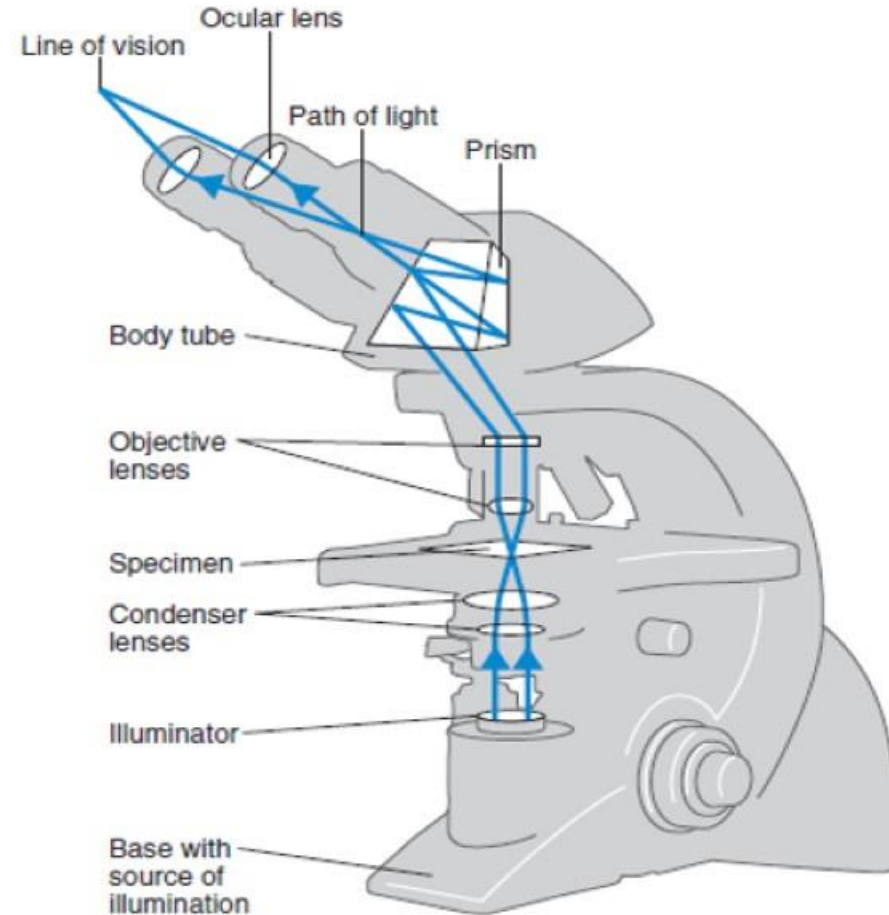
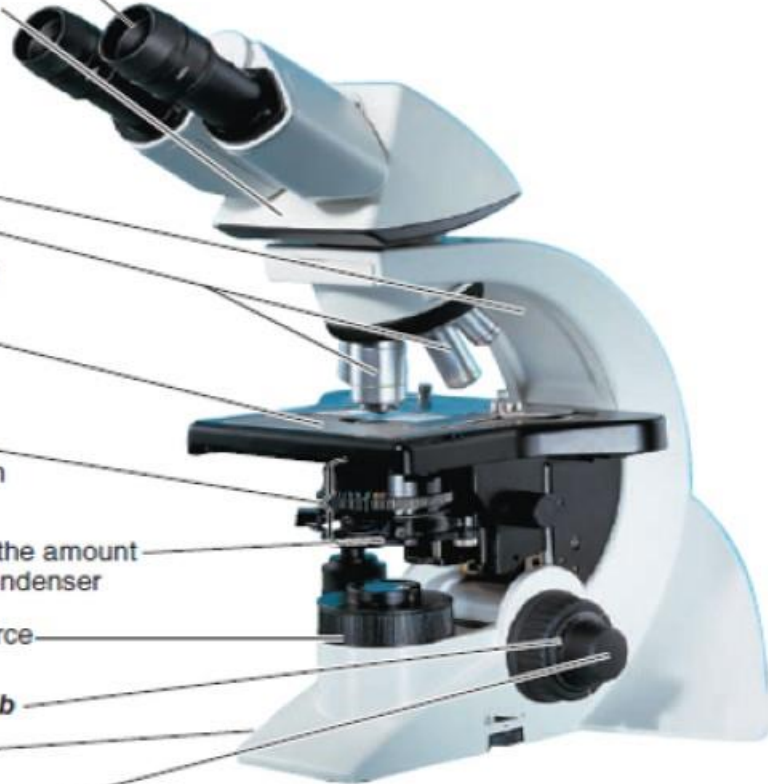
Illuminator Light source

Coarse focusing knob

Base

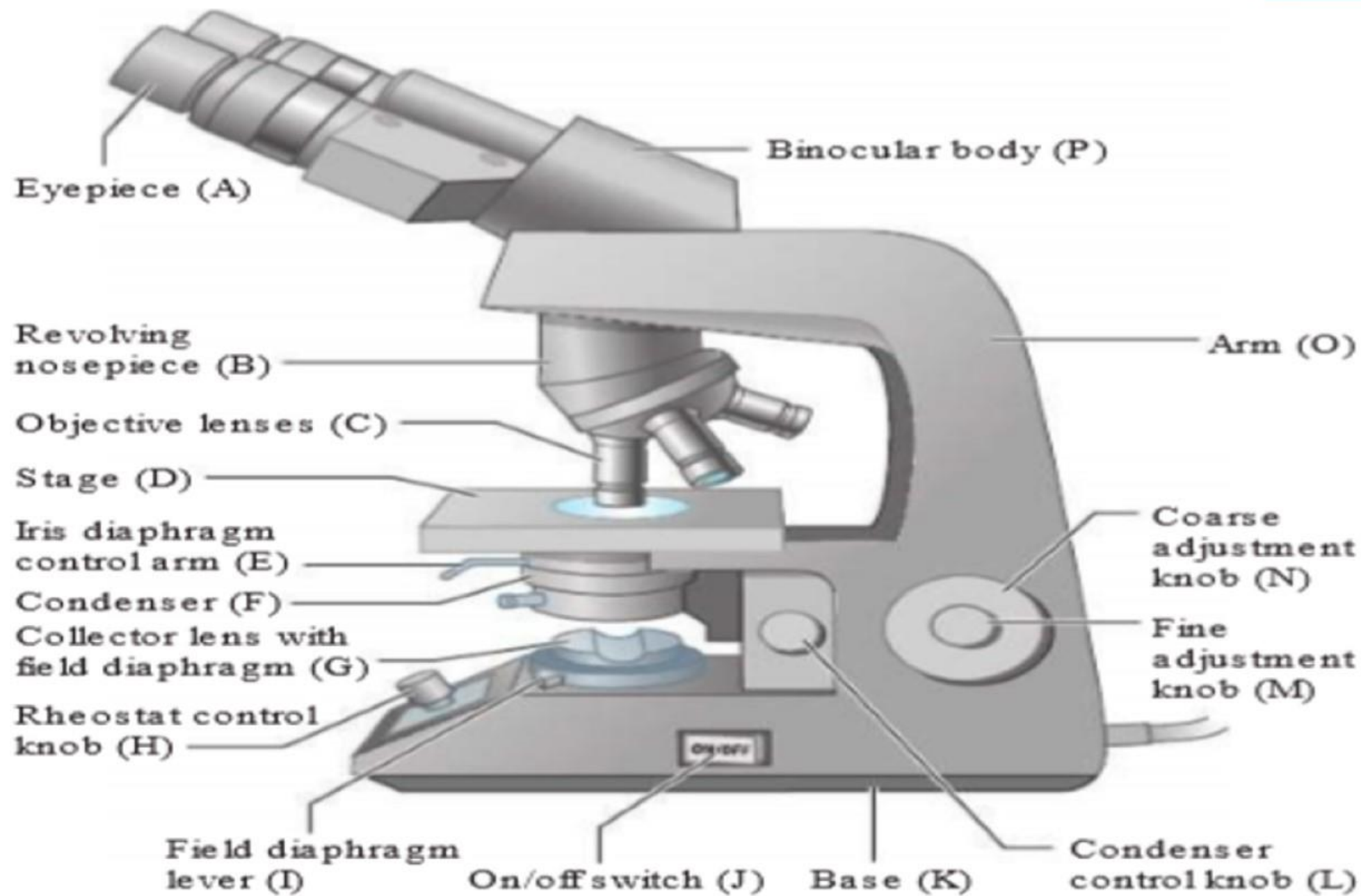
Fine focusing knob

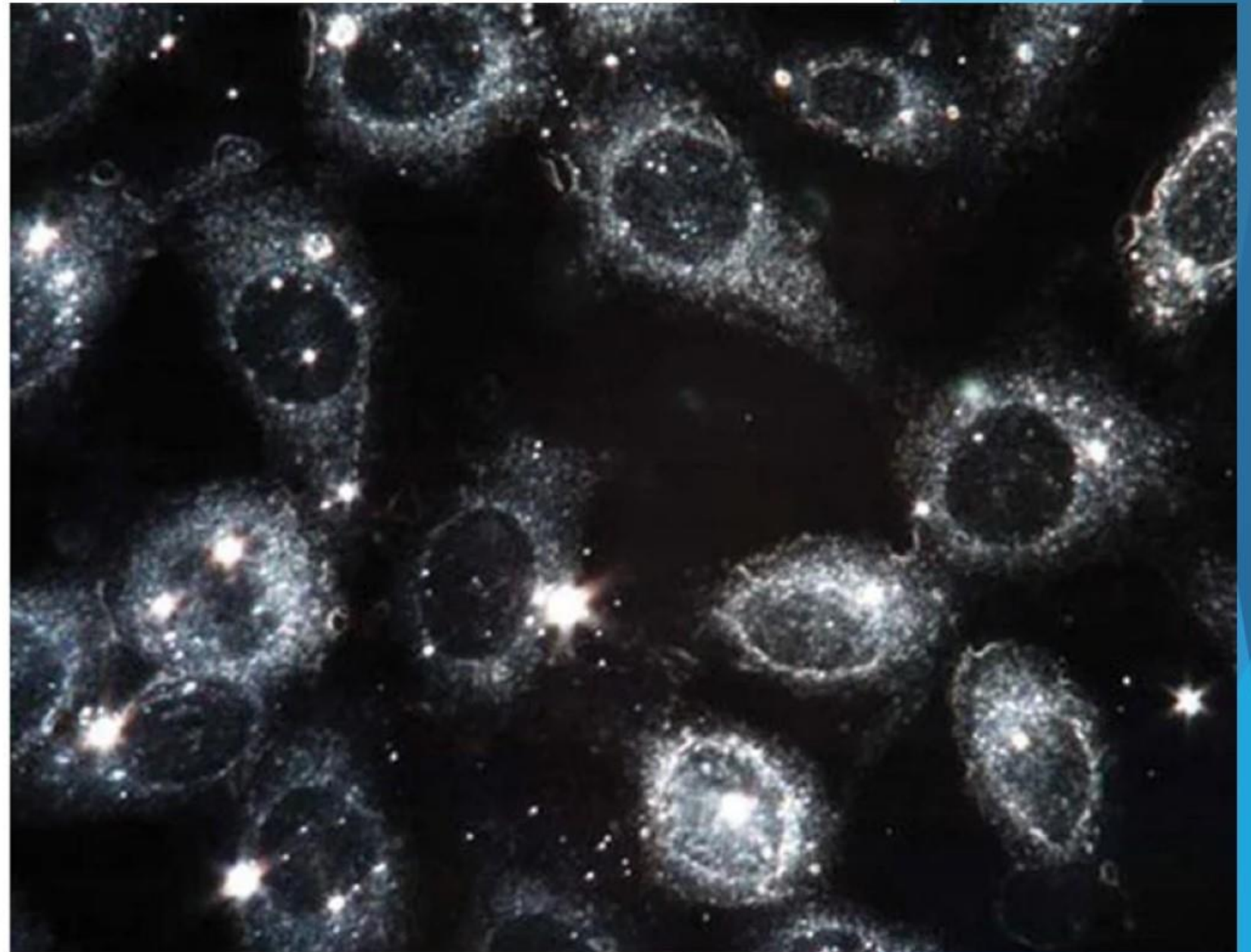
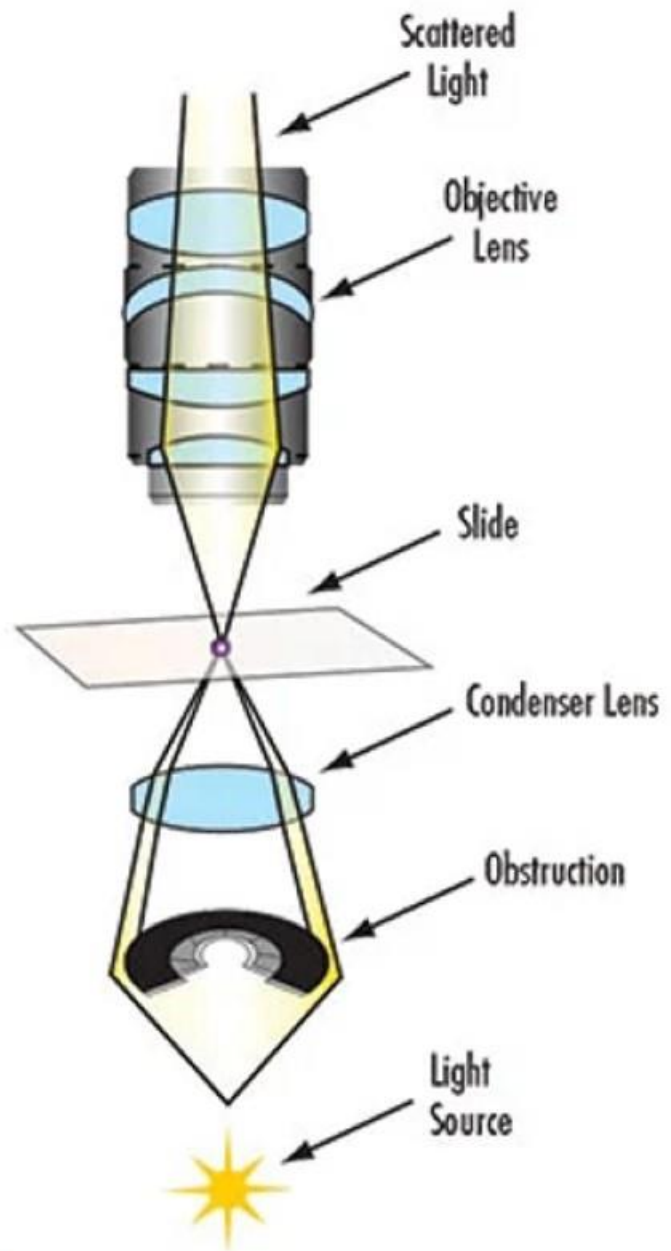
(a) Principal parts and functions



(b) The path of light (bottom to top)

Component	Location	Function
(A) Ocular lens (also known as an eyepiece); a monocular microscope has one; a binocular microscope (such as shown in Figure 2-5) has two	At the top of the microscope	The ocular lens is a 3-10 magnifying lens
(B) Revolving nosepiece	Above the stage	Holds the objective lenses
(C) Objective lenses	Held in place above the stage by the revolving nosepiece	Used to magnify objects placed on the stage
(D) Stage	Directly beneath the nosepiece and objective lenses	Flat surface on which the specimen is placed
Stage adjustment knobs (not shown in Figure 2-5)	Beneath the stage	Used to move the stage and microscope slide
(E) Iris diaphragm control arm	On the condenser	Used to adjust the amount of light passing through the condenser
(F) Condenser	Beneath the stage	Contains a lens system that focuses light onto the specimen
(G) Collector lens with field diaphragm	Beneath the condenser	Controls the amount of light entering the condenser
(H) Rheostat control knob	Front side of the base	Controls the amount of light emitted from the light source
(I) Field diaphragm lever	Attached to the field diaphragm	Used to adjust the amount of light passing through the collector lens
(J) On/off switch	On the side of the base	Turns the light source on and off
(K) Base		Contains the light source
(L) Condenser control knob	Beneath and behind the condenser	Used to adjust the height of the condenser
(M and N) Fine and coarse adjustment knobs	On the arm of the microscope near the base	Used to focus the objective lenses





Phase Contrast Microscope Configuration

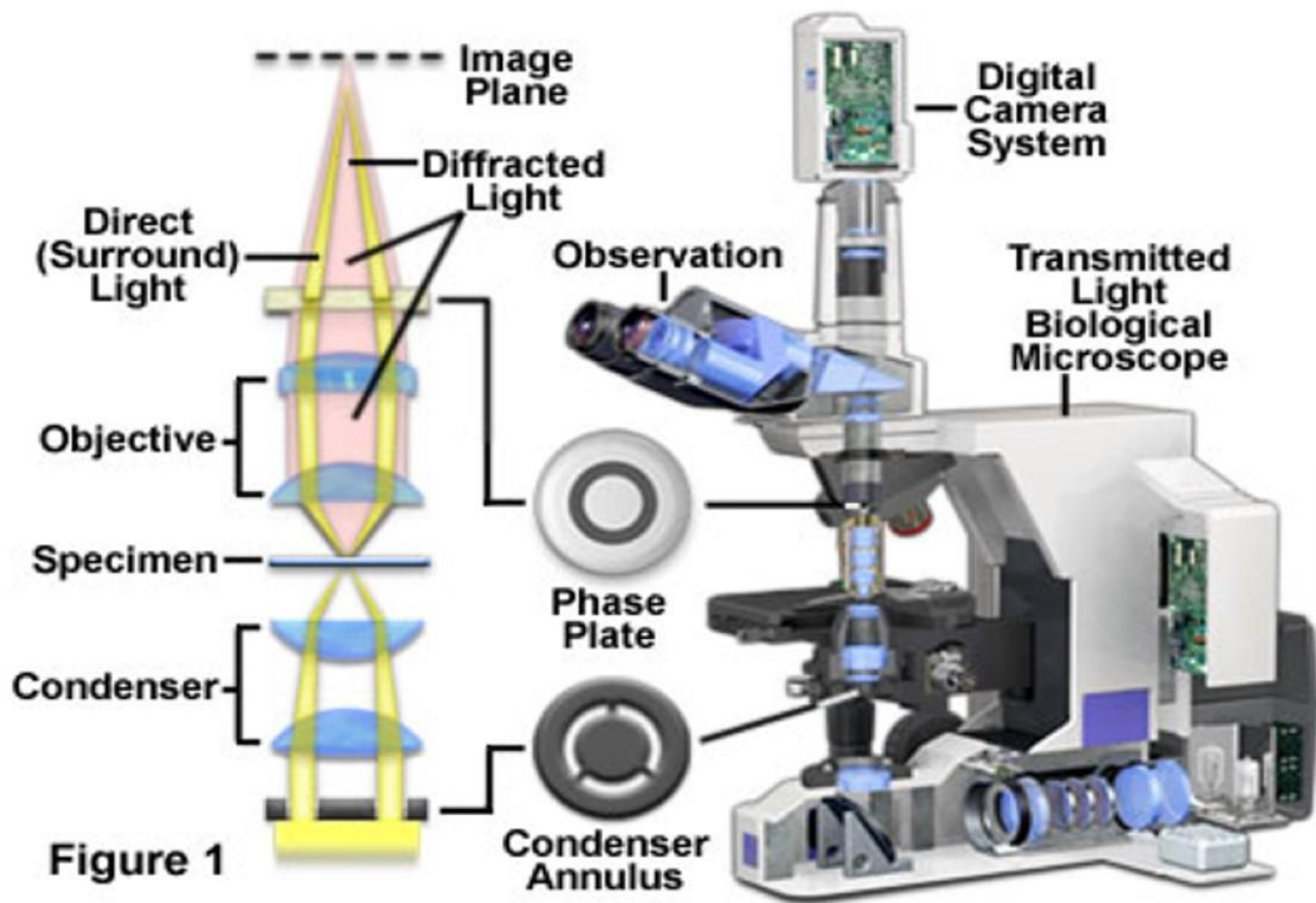
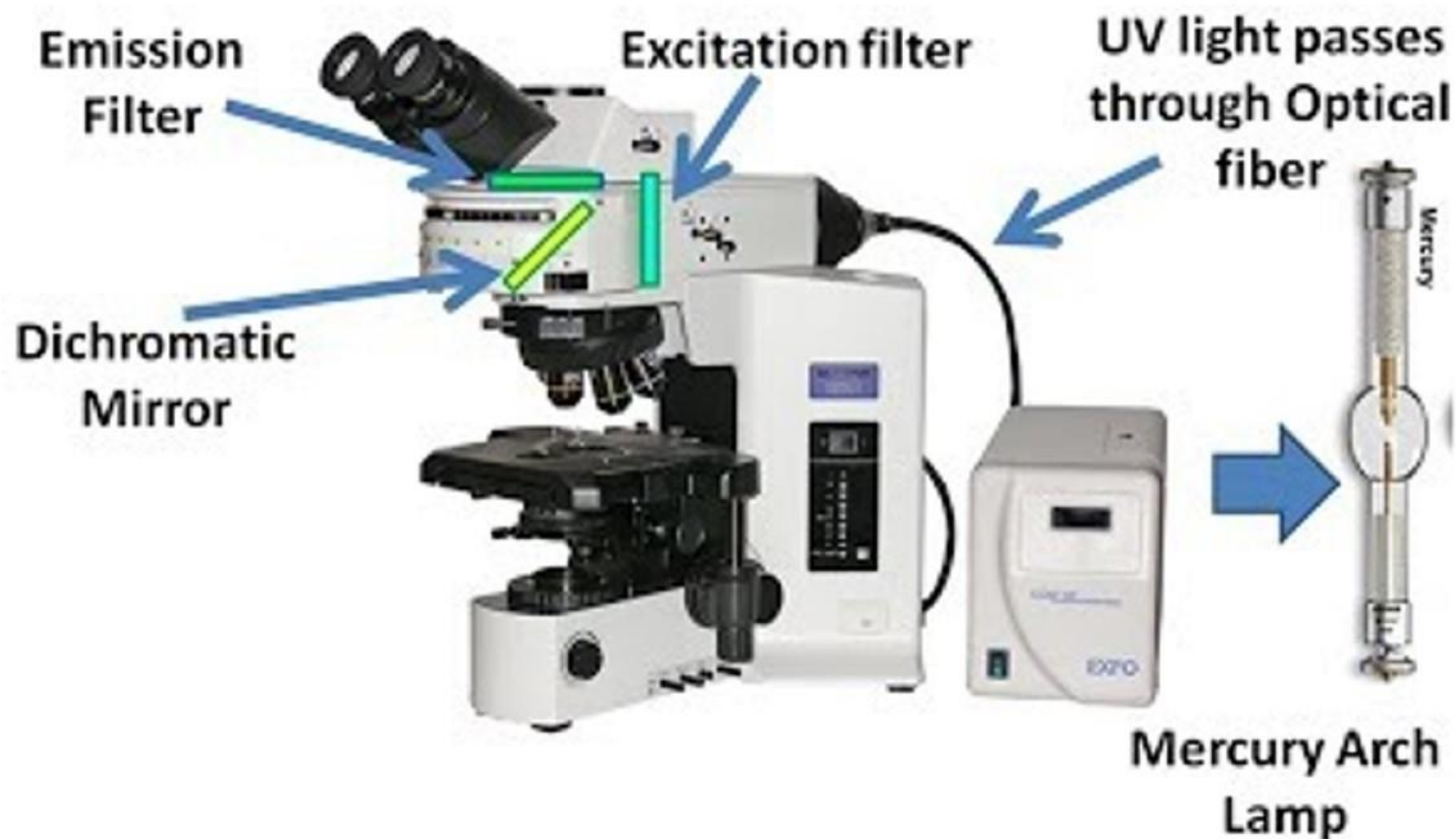
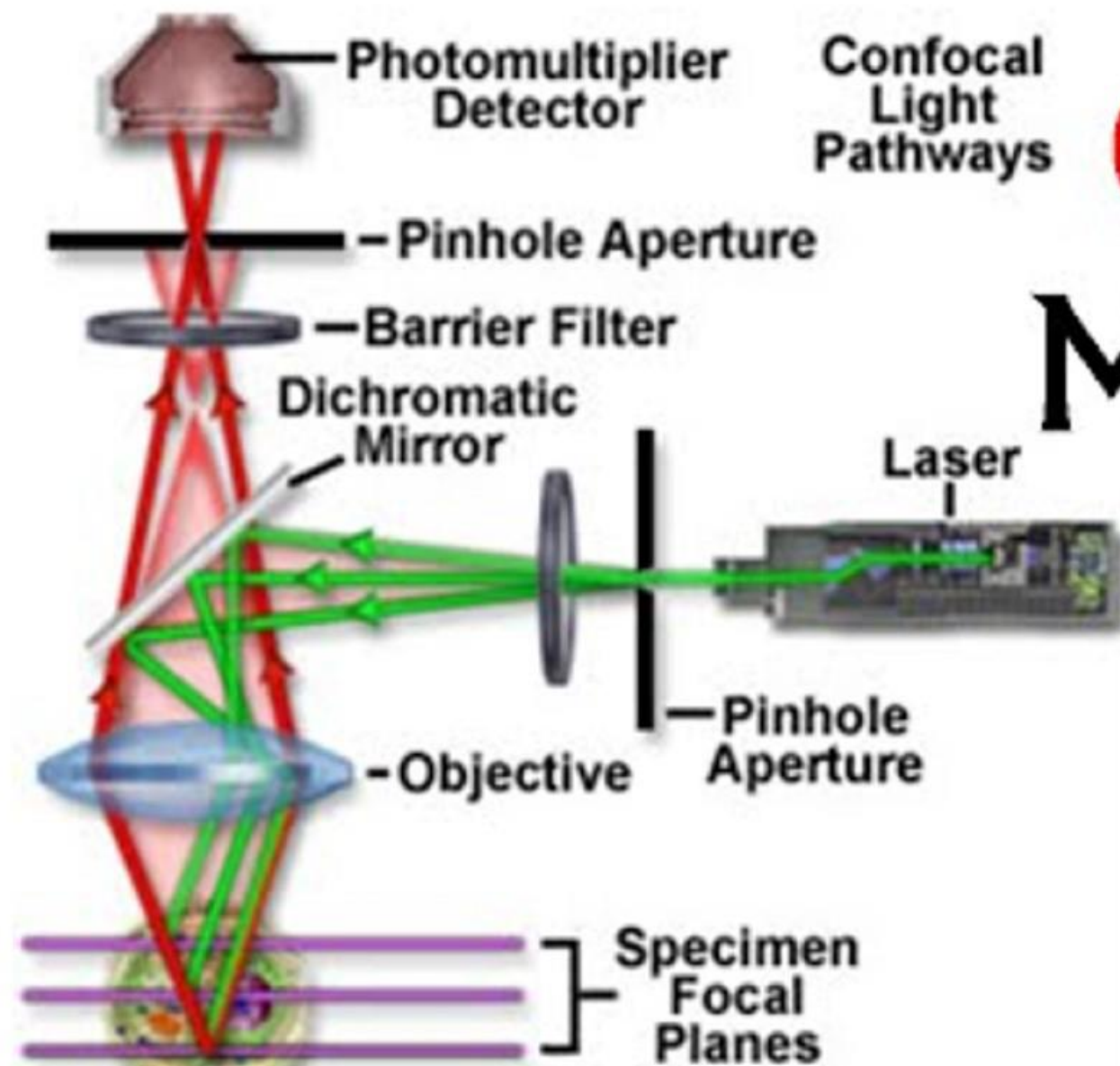


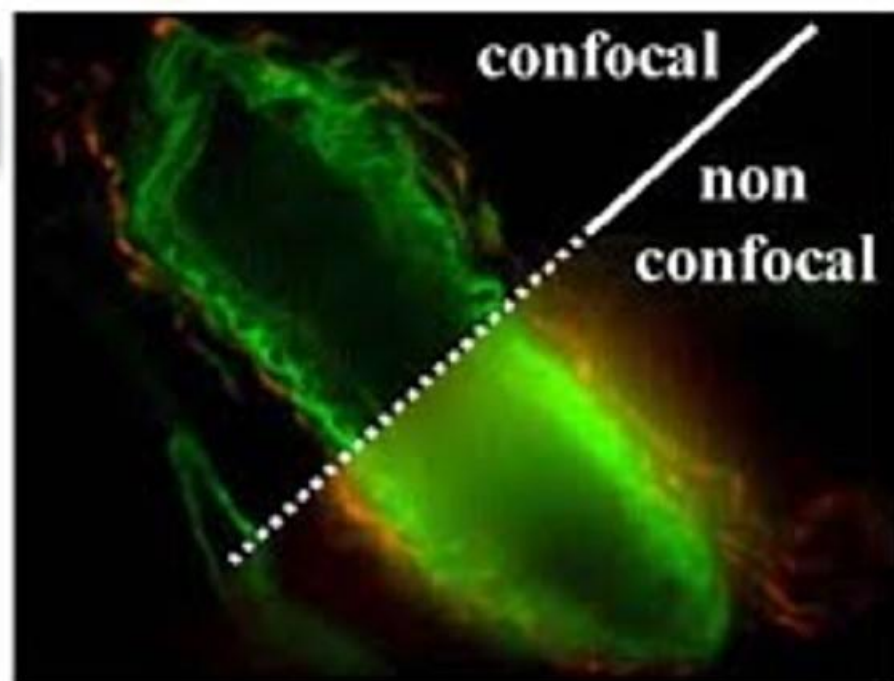
Figure 1

Fluorescence Microscopy





Confocal Microscopy



LIGHT MICROSCOPY

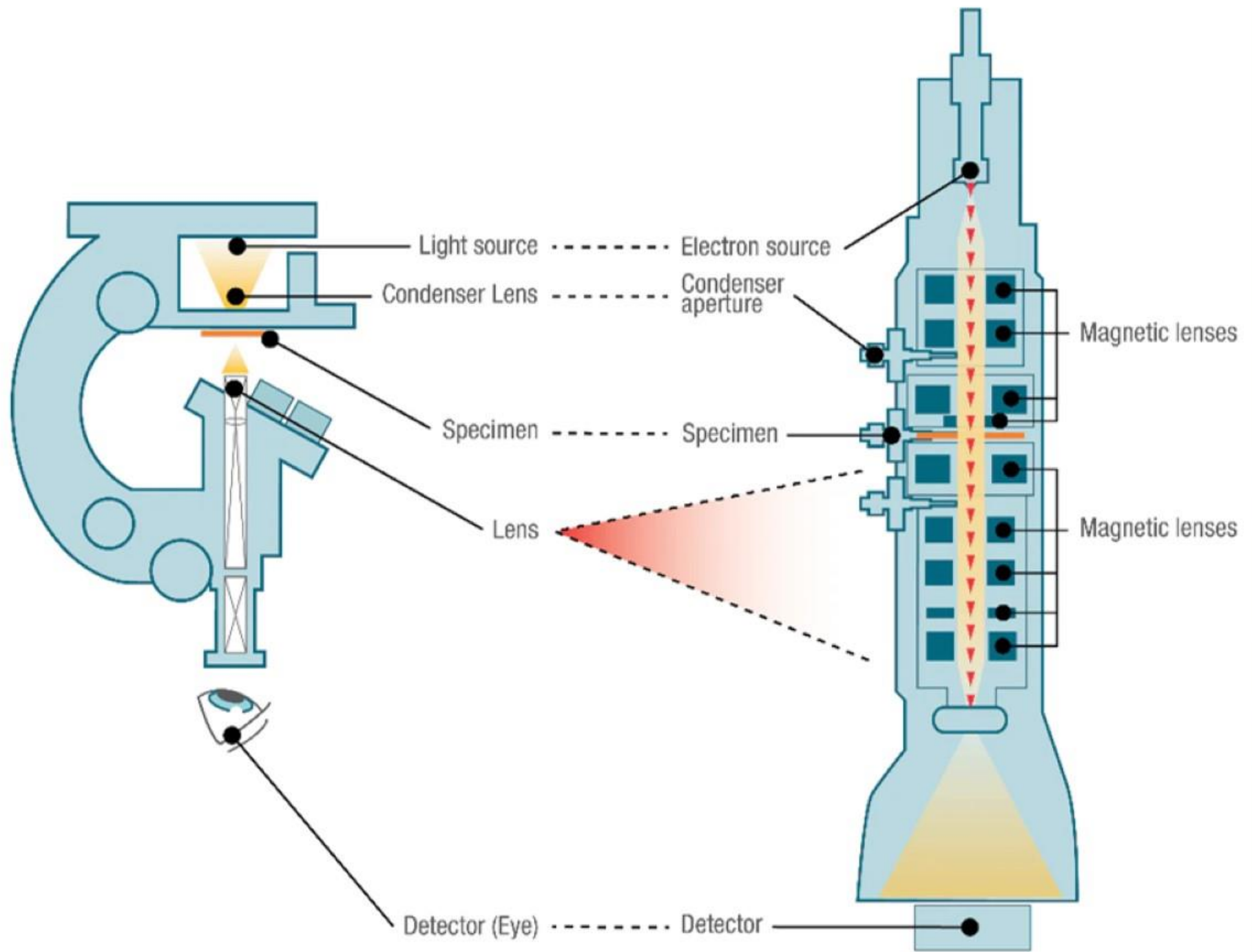
- ▶ Any kind of microscope that uses visible light to observe specimens
- ▶ **Light microscope** has a series of lenses and uses visible light as its source of illumination.
- ▶ Light typically passes through a specimen and then through a series of magnifying lenses.
- ▶ The most common type of light microscope is the **brightfield microscopy**, which evenly illuminates the field of view.

Compound Light Microscopy

- ▶ **Illuminator: light source** used to illuminate the specimen positioned
- ▶ **Condenser:** lenses that direct the light rays through the specimen
- ▶ **objective lenses:** closest to the specimen
- ▶ **ocular lens, or *eyepiece***
- ▶ Three different objective lenses are commonly used:
 - ▶ **low power (x10)**
 - ▶ **high dry (x40)**
 - ▶ **oil immersion (x100)**

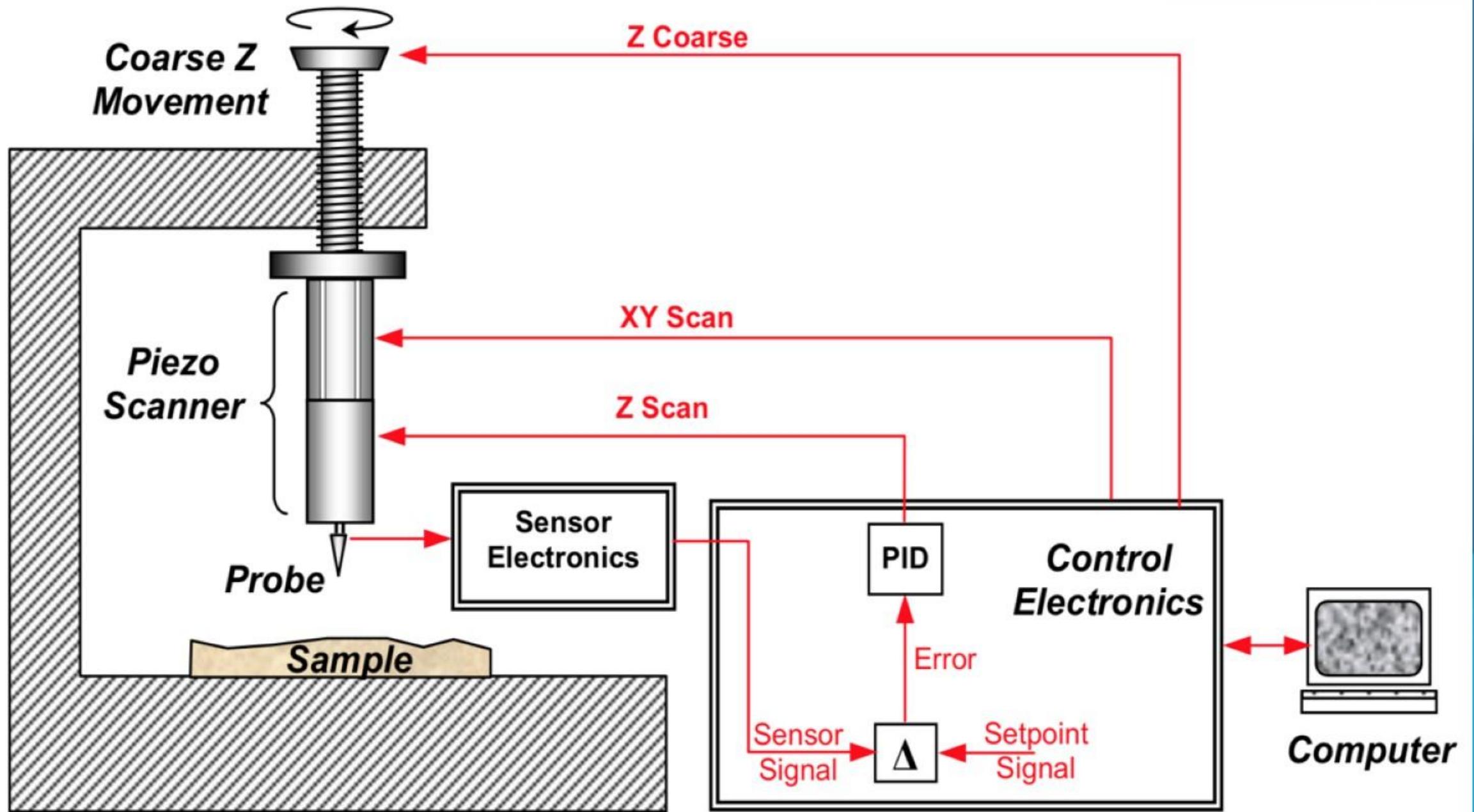
ELECTRON MICROSCOPY

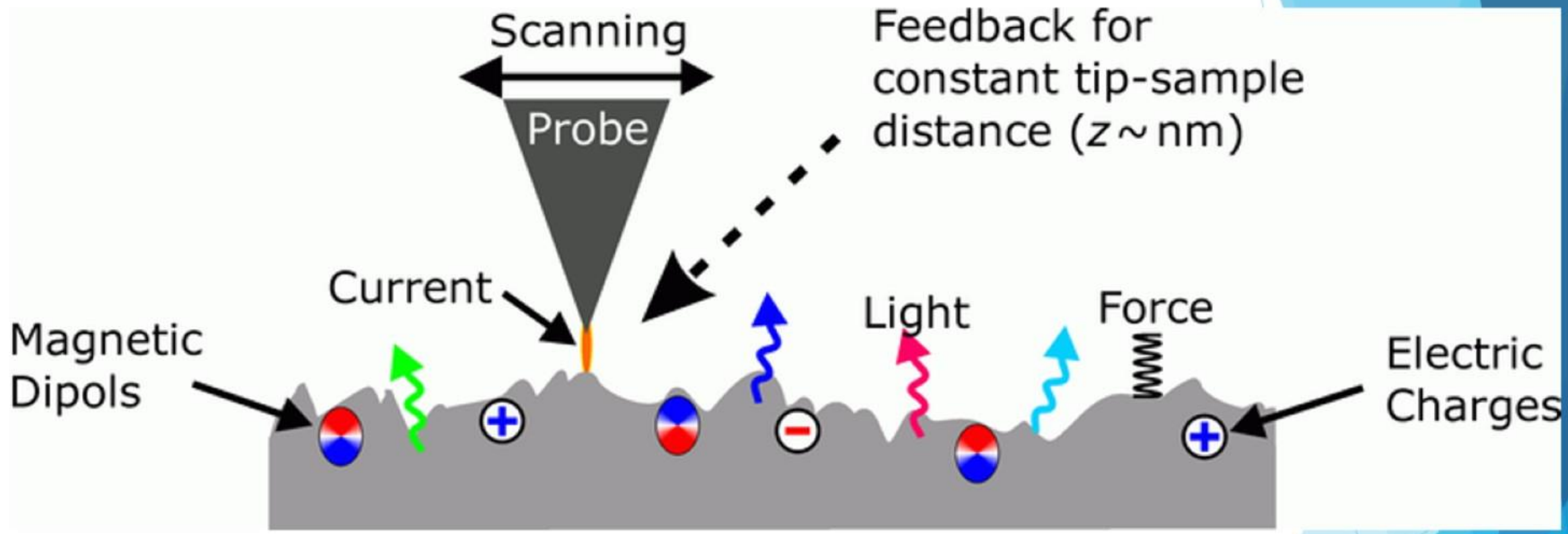
- ▶ Uses electromagnetic lenses, electrons, and a fluorescent screen to produce the magnified image
- ▶ The electron beam is focused by circular electromagnets, which are analogous to the lenses in the light microscope.
- ▶ The wavelength of electrons used in an EM is 0.005 nm
- ▶ Can be obtained is 0.3–0.5 nm
- ▶ Transmission Electron Microscope (TEM): electrons like light pass directly through the specimen that has been prepared by thin sectioning, freeze fracturing, or freeze etching. It is used to observe fine details of cell structure.
- ▶ Scanning Electron Microscope (SEM): a beam of electrons back and forth over the surface of a specimen producing three-dimensional views of the surfaces of whole microorganisms.



SCANNING-PROBE MICROSCOPY

- ▶ Scanning probe microscopes map the bumps and valleys of a surface on an atomic scale.
- ▶ Their resolving power is much greater than the electron microscope, and the samples do not need special preparation as they do for electron microscopy.
- ▶ Among the new scanned-probe microscopes are:
 - ▶ Scanning tunneling microscopy (STM): These are used to provide incredibly detailed views of molecules, such as DNA.
 - ▶ Atomic force microscopy (AFM): These produce three-dimensional images of the surface of a molecule.

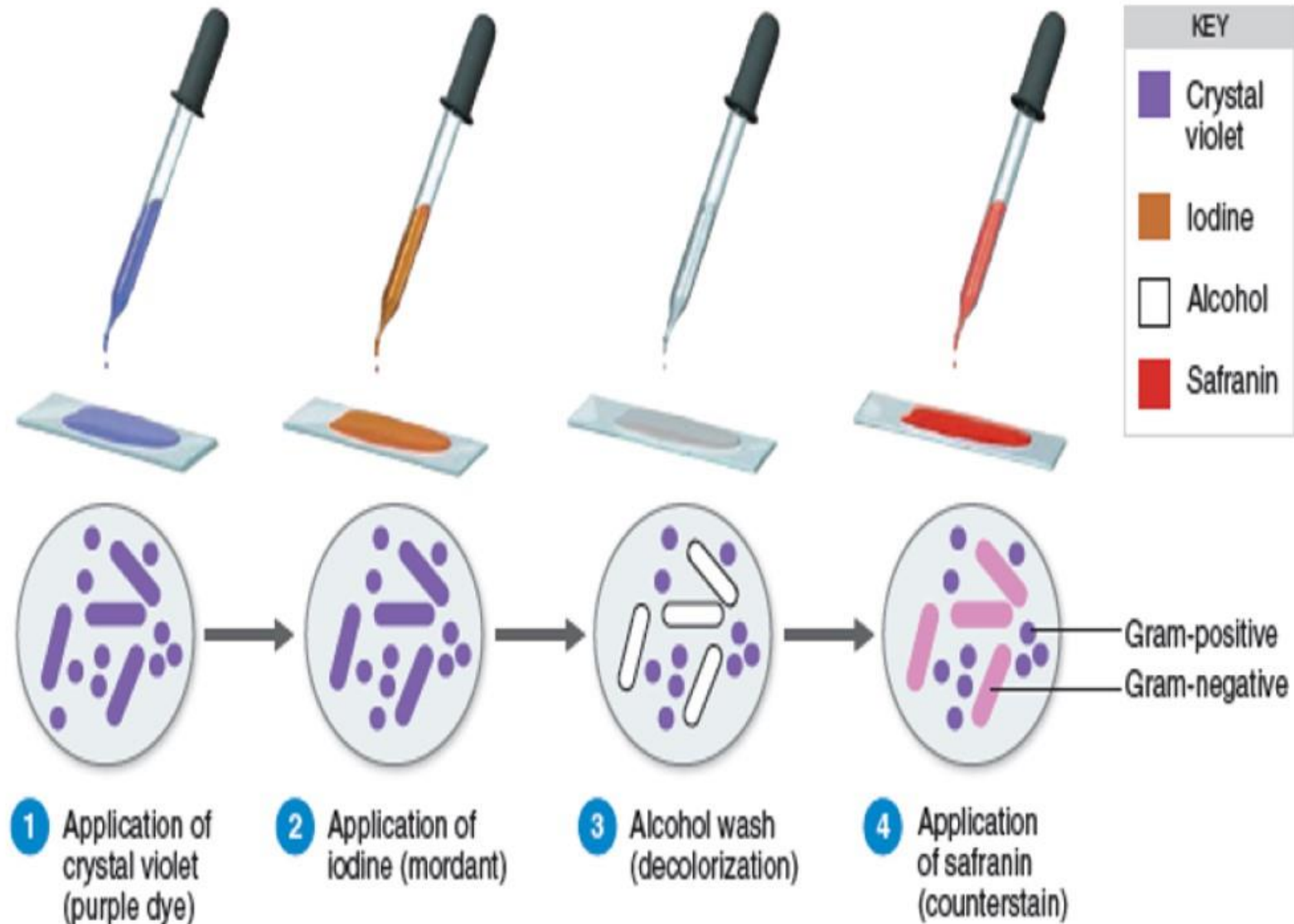




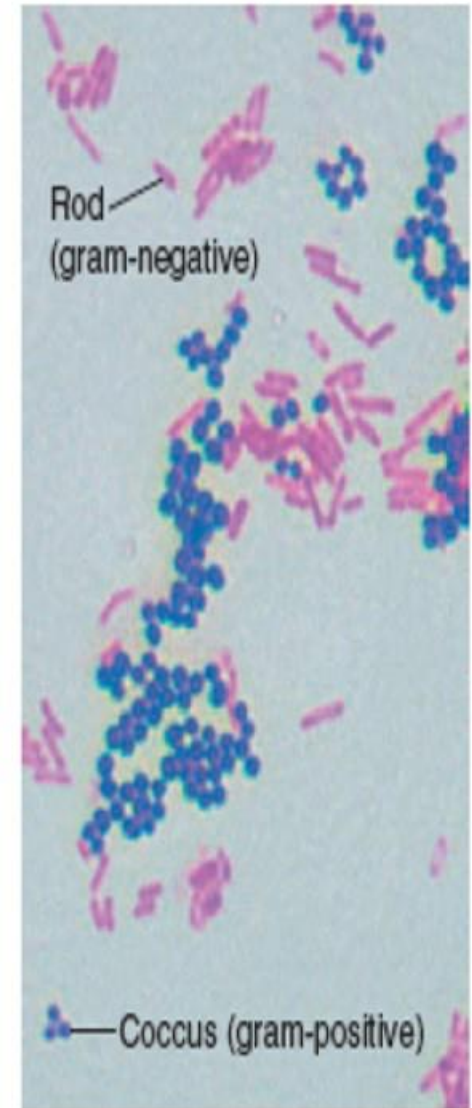
Measurement

Metric Unit	Meaning of Prefix	Metric Equivalent	U.S. Equivalent
1 kilometer (km)	<i>kilo</i> = 1000	1000 m = 10^3 m	3280.84 ft or 0.62 mi; 1 mi = 1.61 km
1 meter (m)		Standard unit of length	39.37 in or 3.28 ft or 1.09 yd
1 decimeter (dm)	<i>deci</i> = 1/10	0.1 m = 10^{-1} m	3.94 in
1 centimeter (cm)	<i>centi</i> = 1/100	0.01 m = 10^{-2} m	0.394 in; 1 in = 2.54 cm
1 millimeter (mm)	<i>milli</i> = 1/1000	0.001 m = 10^{-3} m	
1 micrometer (μ m)	<i>micro</i> = 1/1,000,000	0.000001 m = 10^{-6} m	
1 nanometer (nm)	<i>nano</i> = 1/1,000,000,000	0.000000001 m = 10^{-9} m	
1 picometer (pm)	<i>pico</i> = 1/1,000,000,000,000	0.000000000001 m = 10^{-12} m	

Gram Stain



(a)



(b)

LM | 5 μ m

References

- ▶ Microbiology An Introduction [Tortora] -12E (2016)
- ▶ Burton's Microbiology for the Health Sciences, 10e (Sep 11, 2014)