MICROSCOPES

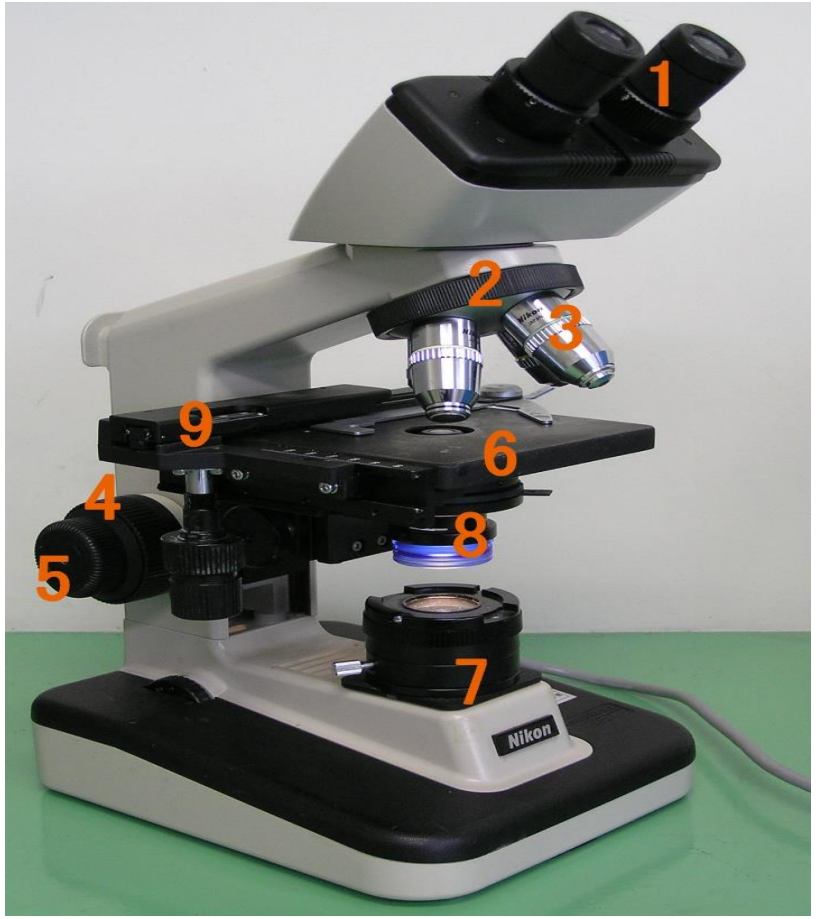
DR. NUPUR MONDAL

Units of Measure

- μm - Micrometer
  - 1,000,000 micrometers = 1 meter
    - Strand of hair has a diameter of ~ 20-180 μm
    - 10^6
- nm - Nanometer
  - 1,000,000,000 nanometers = 1 meter
    - 10^9
    - Wavelength of visible light (400-700 nm)
- Å - Angstrom
  - 10,000,000,000 Angstroms = 1 meter
    - 10^{10}
    - Used to measure the size of atoms/bond lengths
    - Length of a C-H bond in methane is ~1 Angstrom

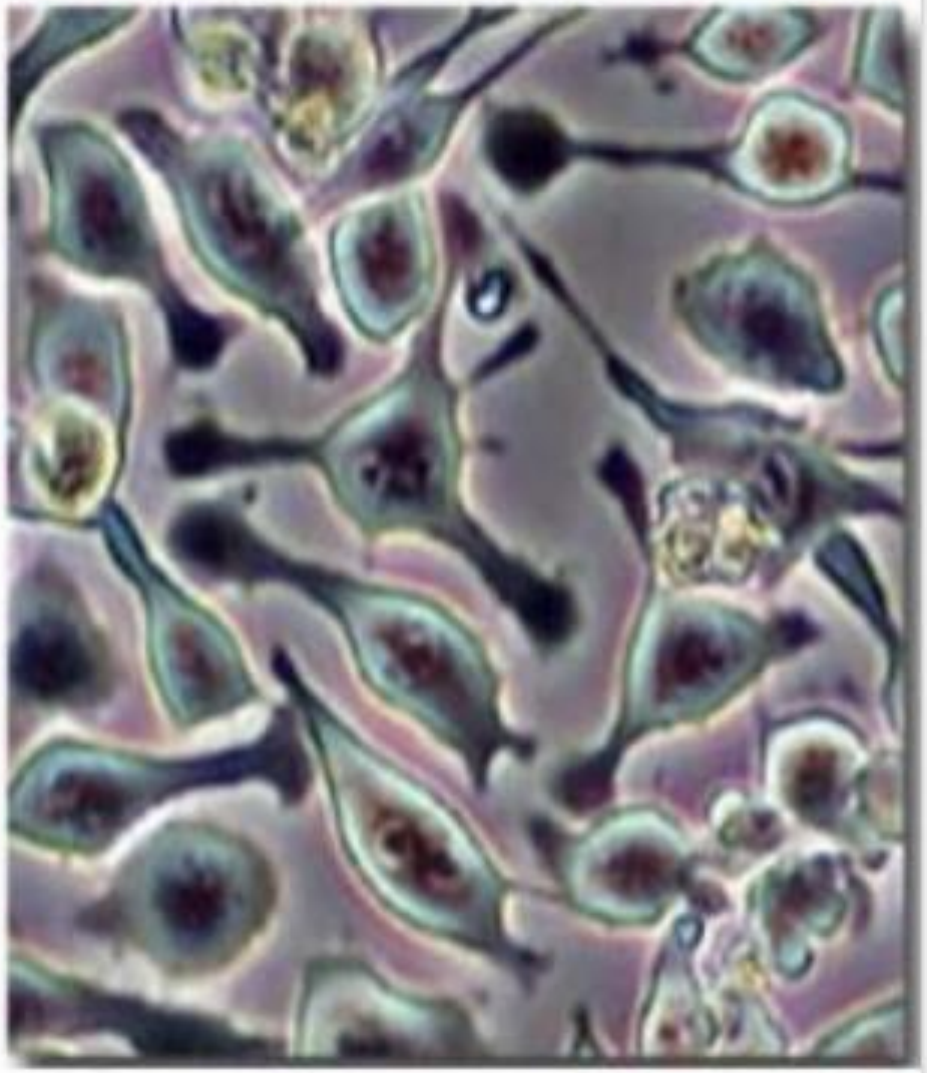
Optical Microscope

1. Ocular lens
2. Objective turret
3. Objective
4. Coarse Adjustment
5. Fine Adjustment
6. Stage
7. Light source
8. Condenser
9. X-Y Control



PHASE CONTRAST MICROSCOPE

Living Cells in Brightfield and Phase Contrast



Brightfield

Phase contrast

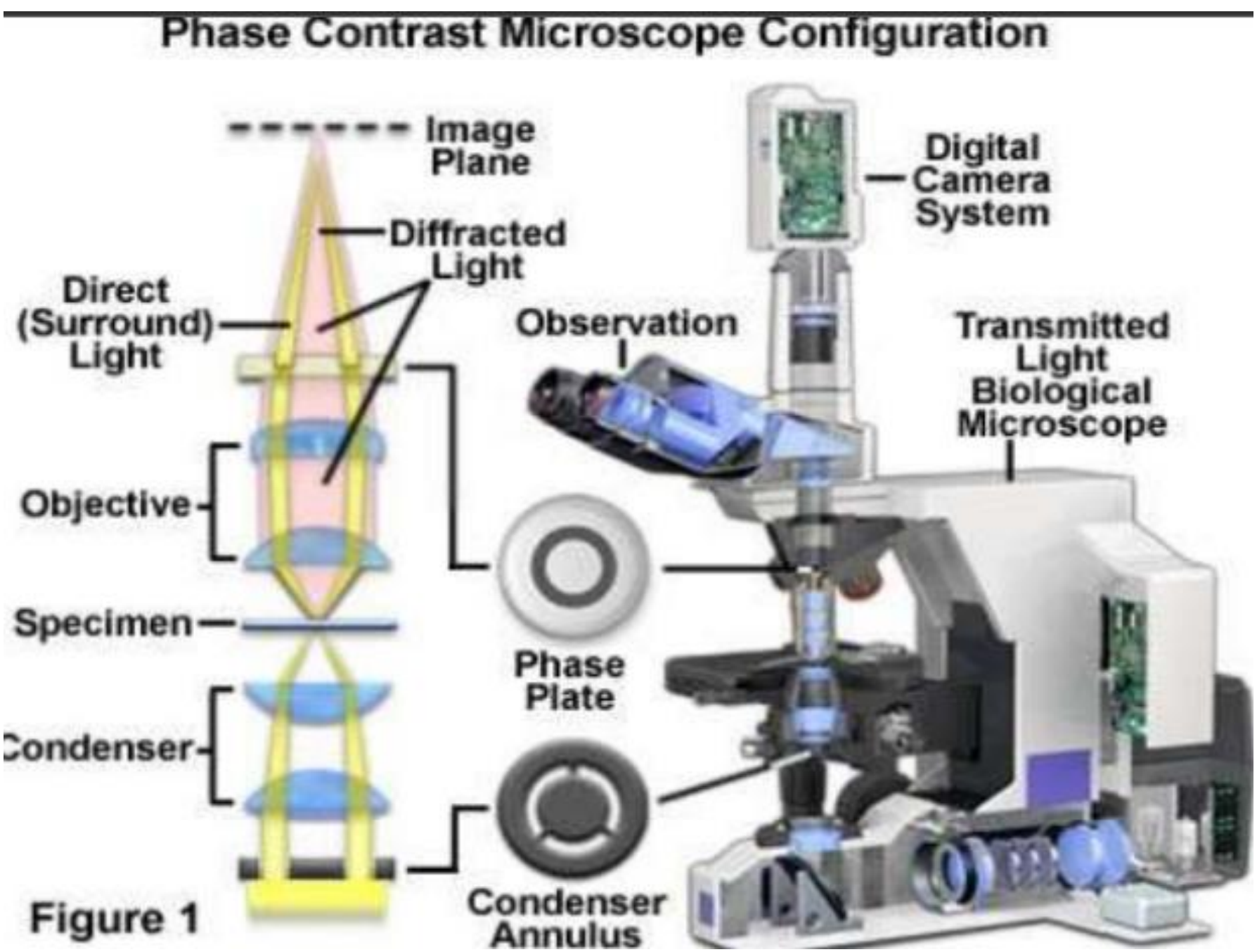
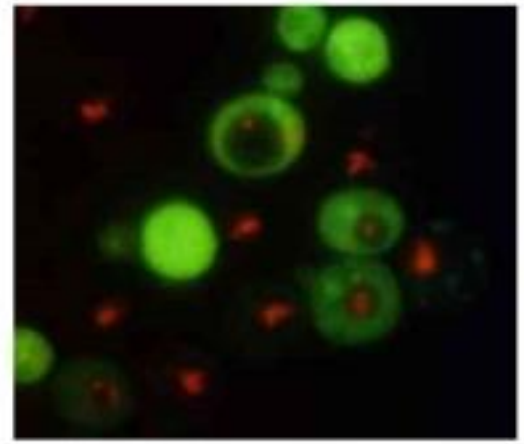

Phase Contrast Microscope Configuration
Image Plane
Diffracted Light
Direct (Surround) Light
Objective
Specimen
Condenser
Phase Plate
Condenser Annulus
Digital Camera System
Observation
Transmitted Light Biological Microscope

Figure 1

FLUORESCENCE MICROSCOPE

TYPES OF FLUOROPHORES USED

- fluorescein,
- DAPI,
- propidium iodide
- green fluorescent protein (GFP)
- Texas Red



Common Fluorophores in Widefield and Confocal Microscopy

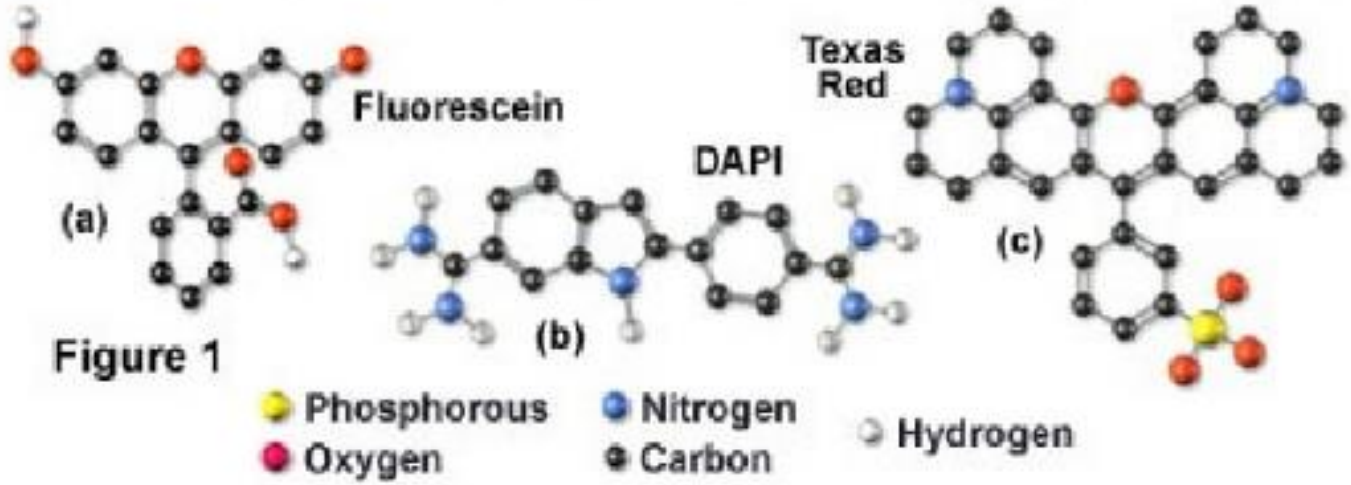


Figure 1

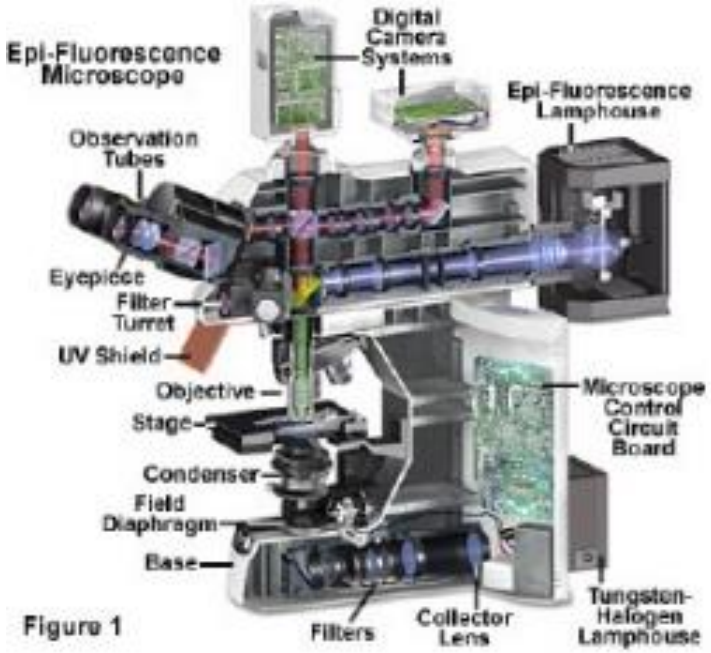

Epi-Fluorescence Microscope
Digital Camera Systems
Epi-Fluorescence Lamphouse
Observation Tubes
Eyepiece
Filter Turret
UV Shield
Objective
Stage
Condenser
Field Diaphragm
Base
Microscope Control Circuit Board
Tungsten-Halogen Lamphouse
Filters
Collector Lens

Figure 1

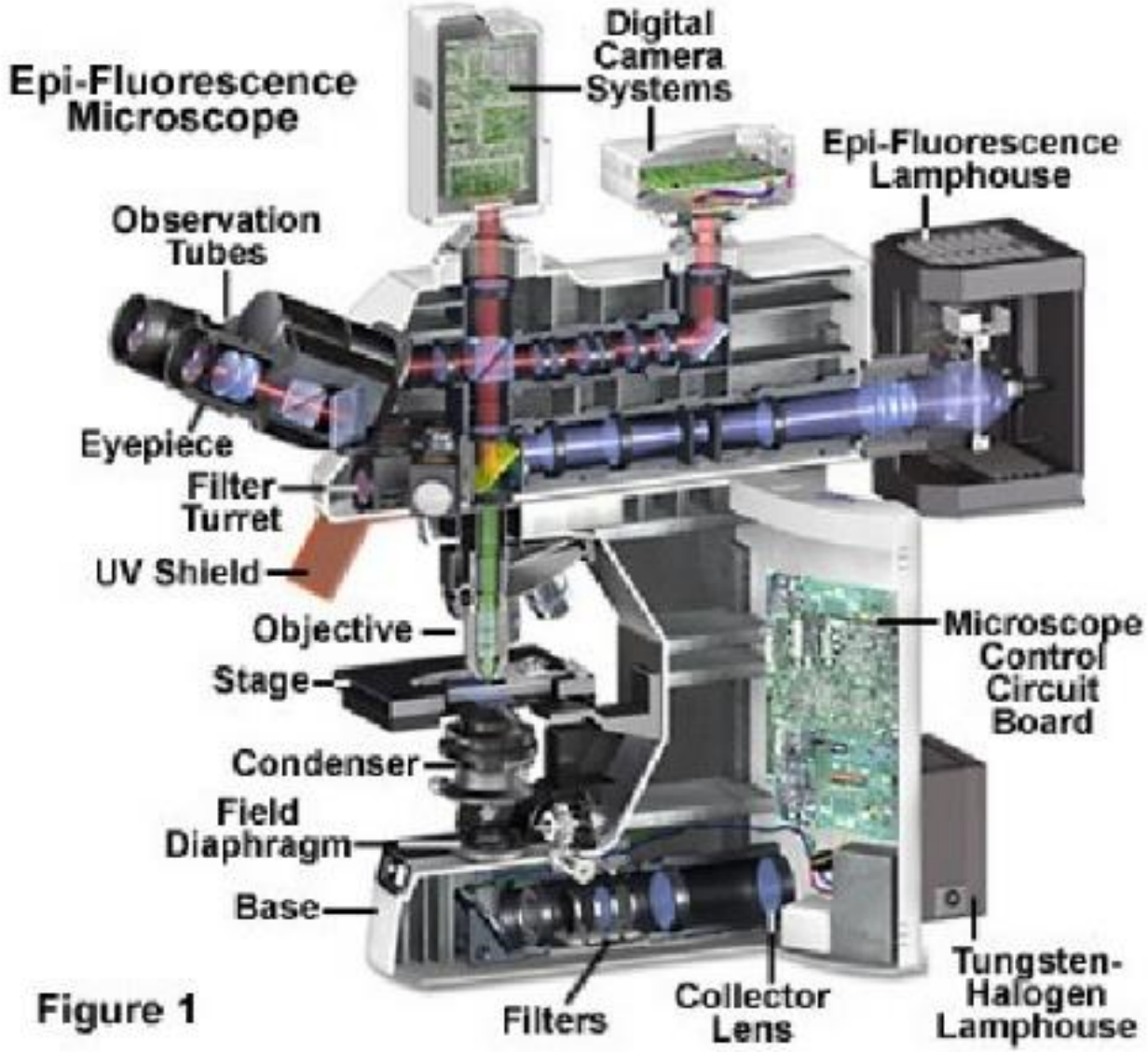
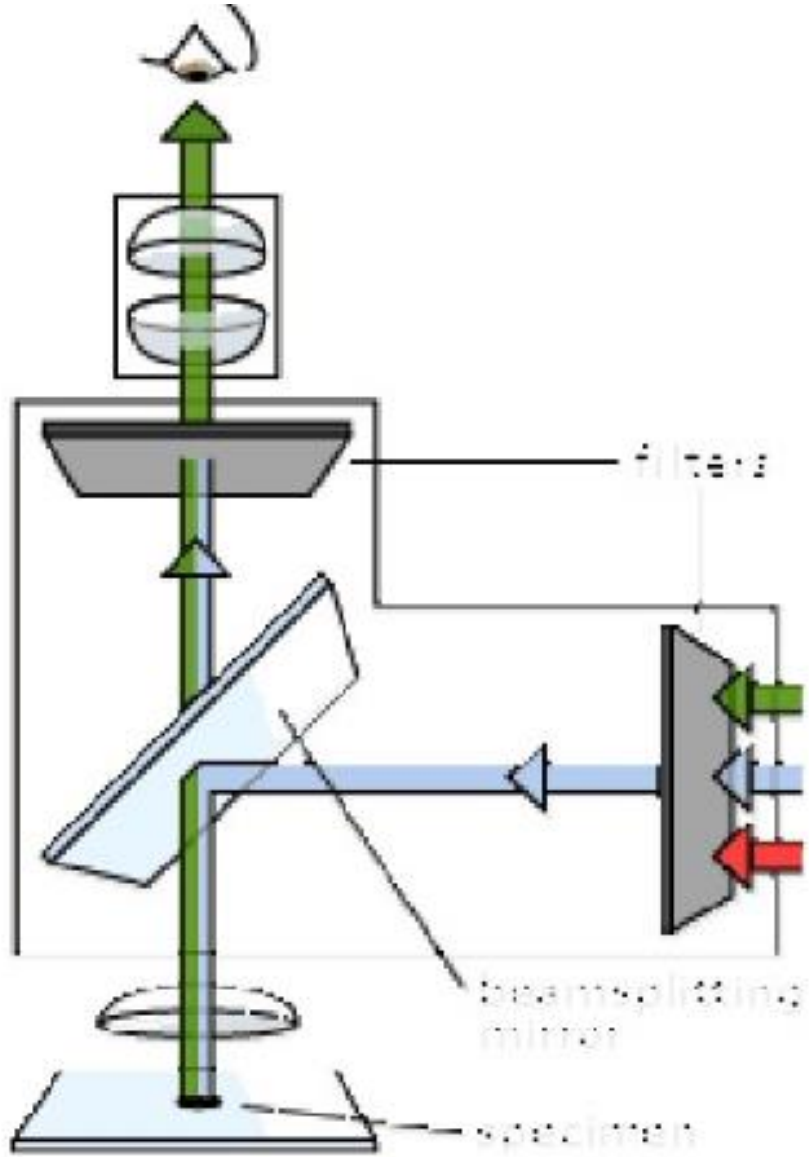


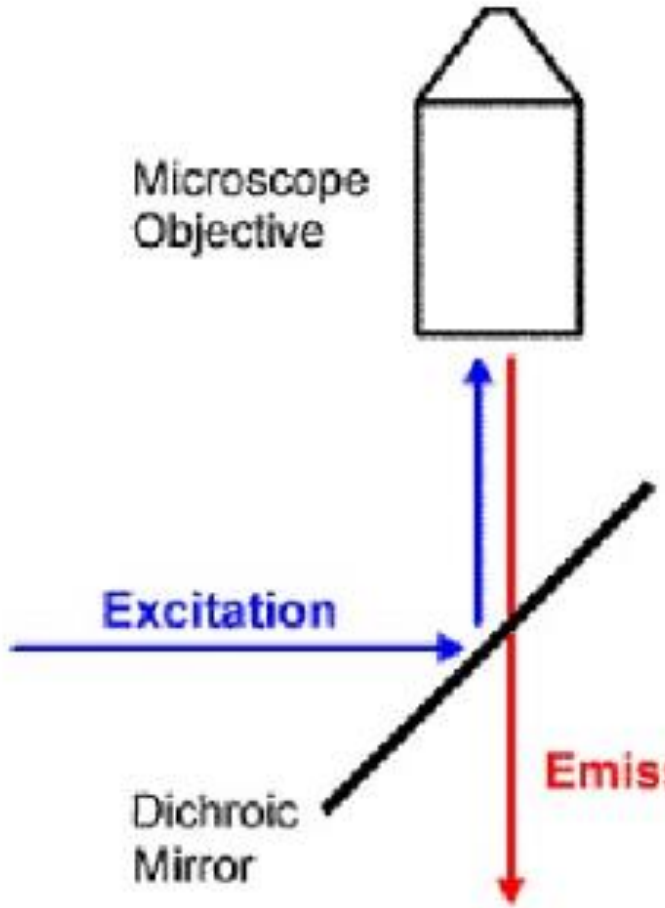
Figure 1

Cutaway diagram of a modern epi-fluorescence microscope



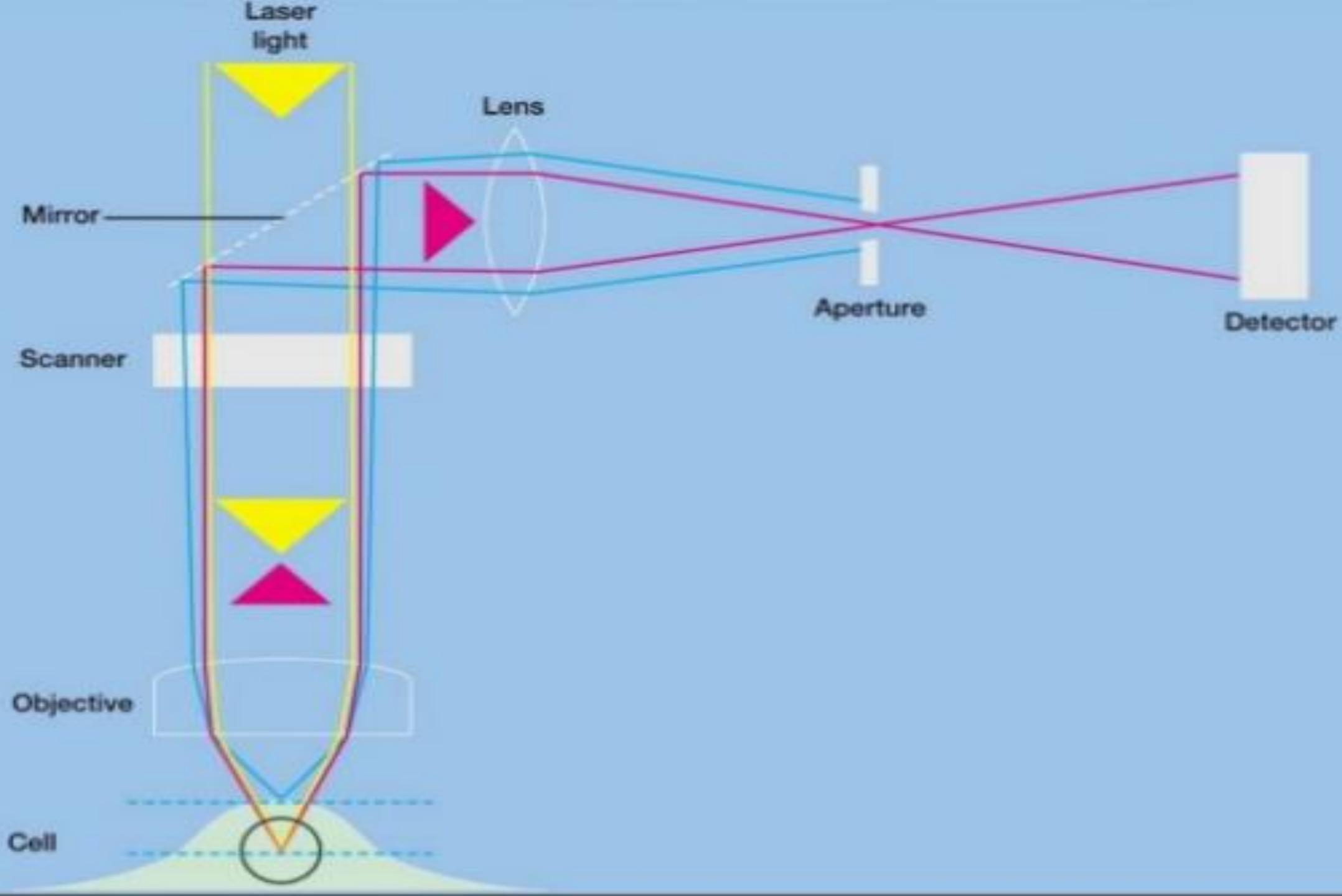
The Dichroic Mirror

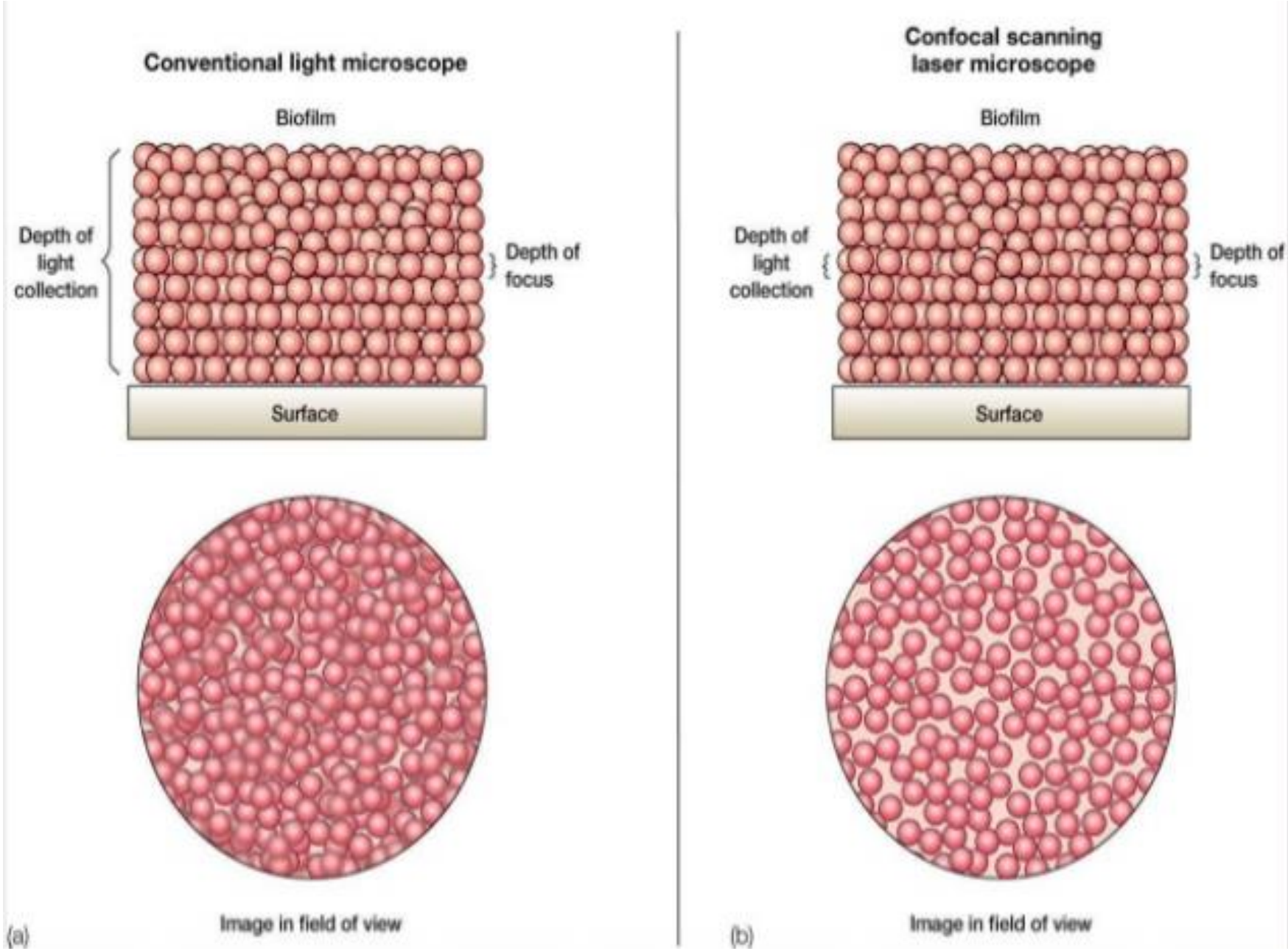
dichroic, two color



- The **excitation** light reflects off the surface of the dichroic mirror into the objective.
- The fluorescence **emission** passes through the dichroic to the eyepiece or detection system.

CONFOCAL MICROSCOPE


Laser light
Lens
Mirror
Aperture
Detector
Scanner
Objective
Cell


Conventional light microscope
Biofilm
Depth of light collection
Depth of focus
Surface
Image in field of view
(a)
Confocal scanning laser microscope
Biofilm
Depth of light collection
Depth of focus
Surface
Image in field of view
(b)