DIRECT EXAMINATION

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Learning objectives:

- Take a look on the direct examination methods which used in microbiology.
- Learn the principle and some details of procedure for these methods.

Methods of direct examinations

- **Light Microscopy:**

Direct examination of stained or unstained preparations by **light (bright-field) microscopy** is particularly useful for detection of bacteria, fungi, and parasites.

1. The Gram Stain
2. The Acid-Fast Stain
3. Fungal and Parasitic Stains

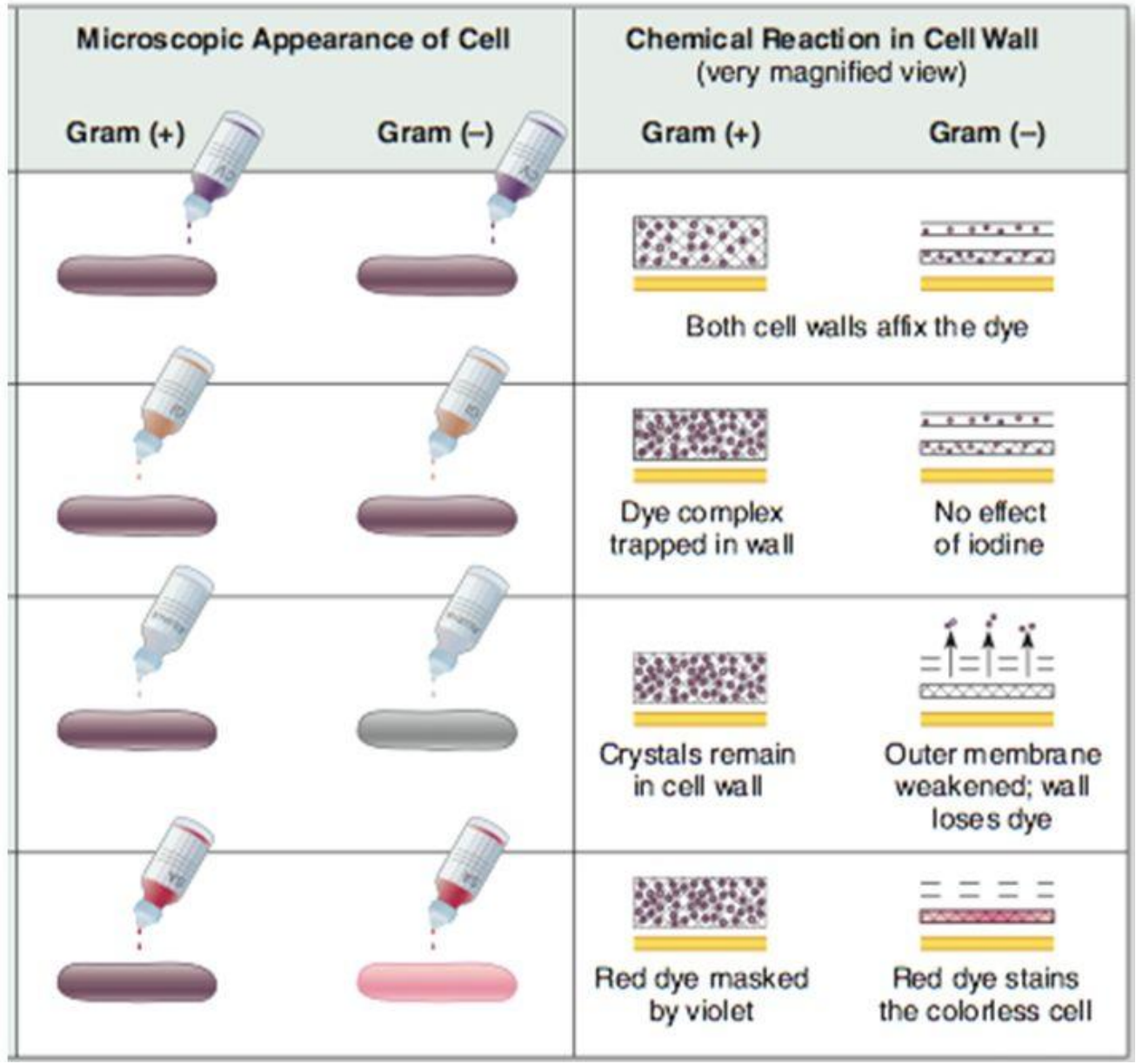
GRAM STAIN PROCEDURE

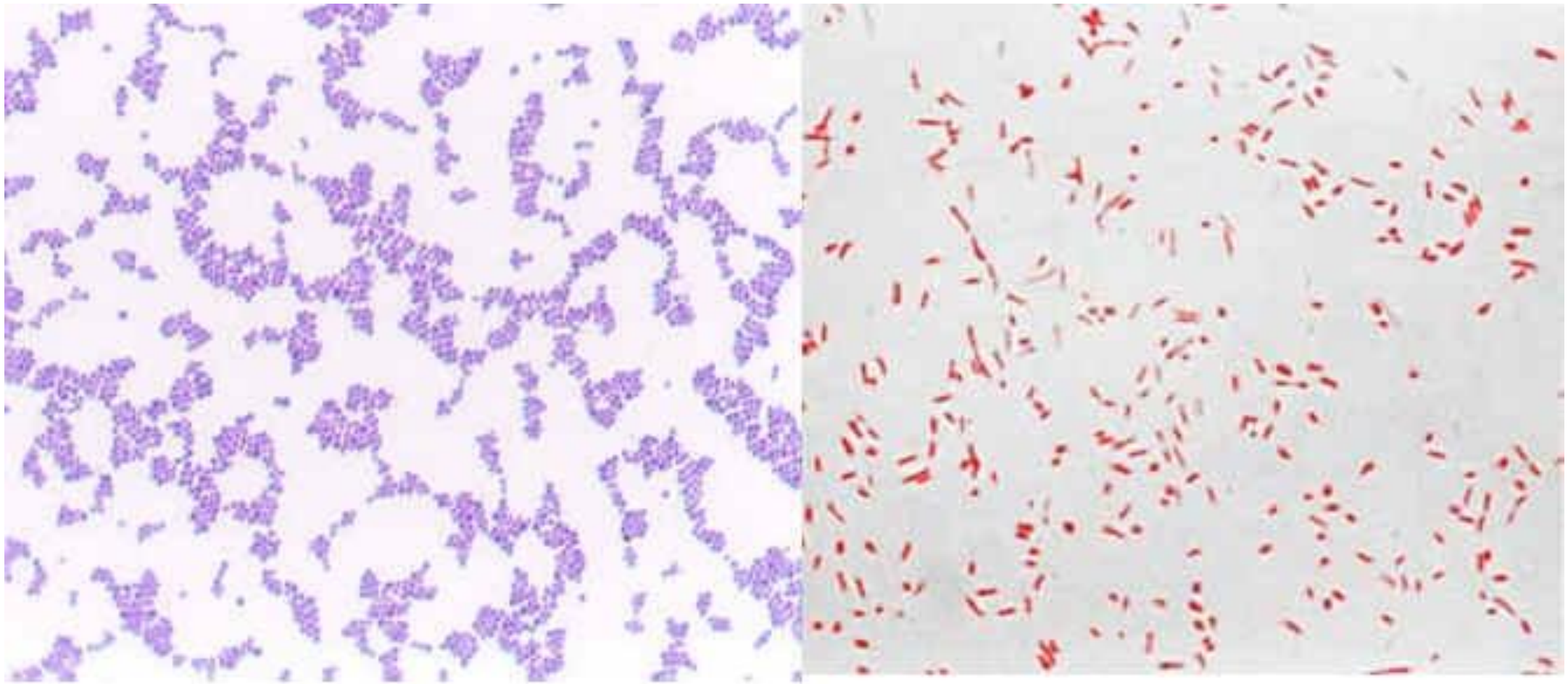
1. Flood with crystal violet for 1 minute. Rinse

2.Flood with iodine for 1 minute. Rinse

3.Decolorize with alcohol until no more purple comes off the slide. Rinse

4. Flood with safranin. Rinse and dry.

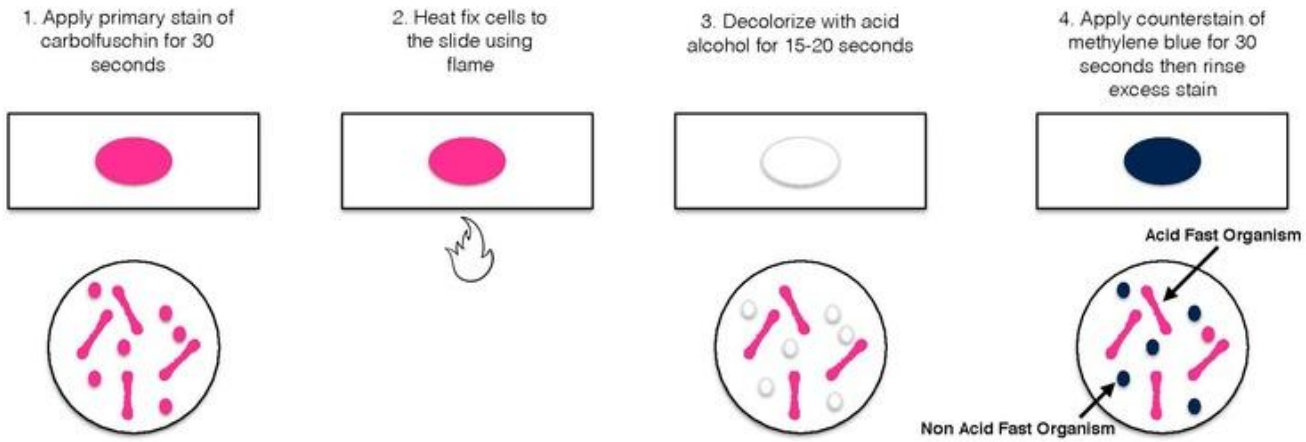


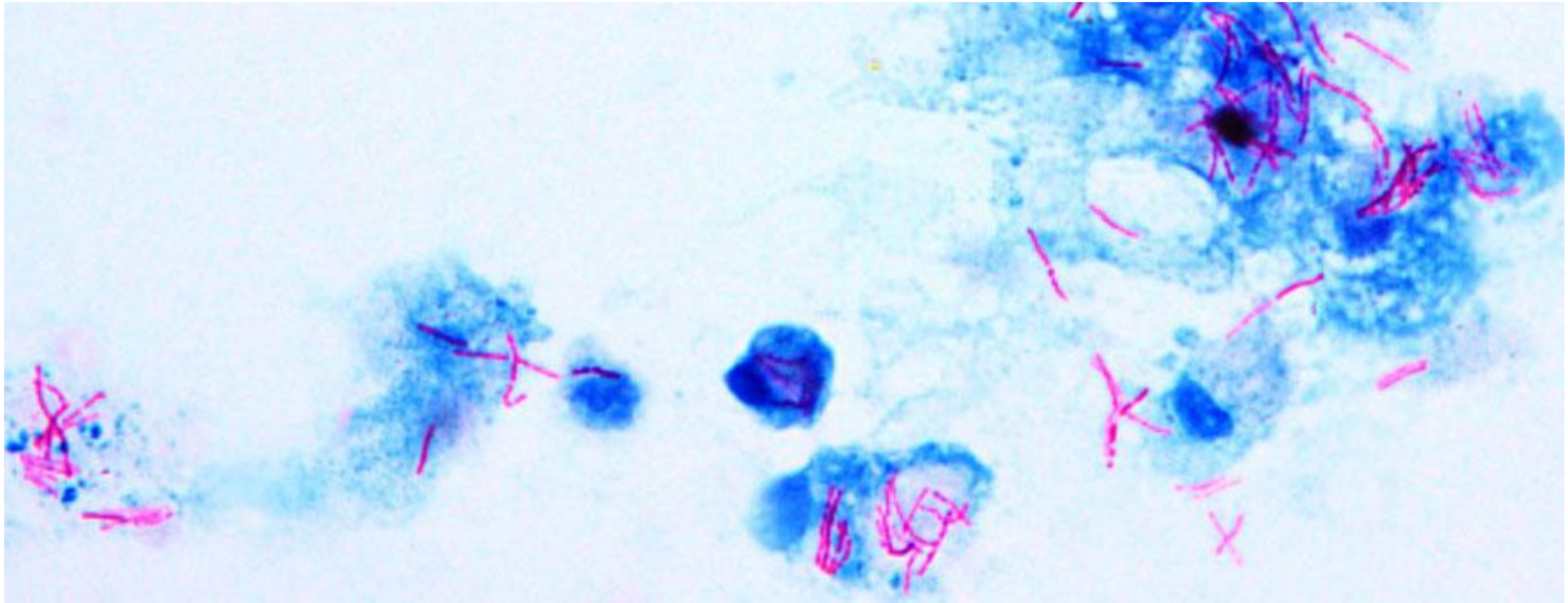


Gram +ve Bacteria

Gram -ve Bacteria

Acid Fast Stain

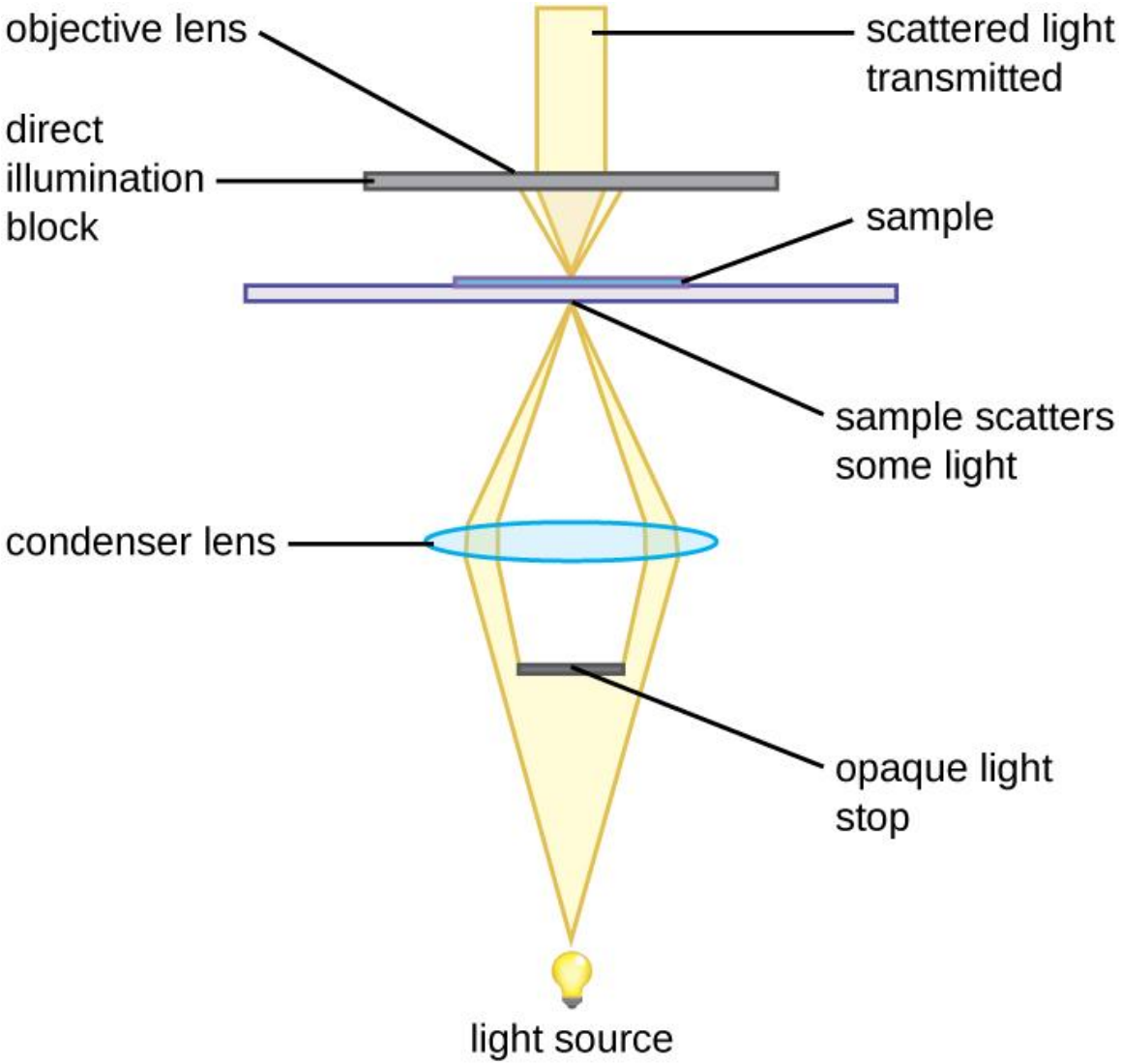


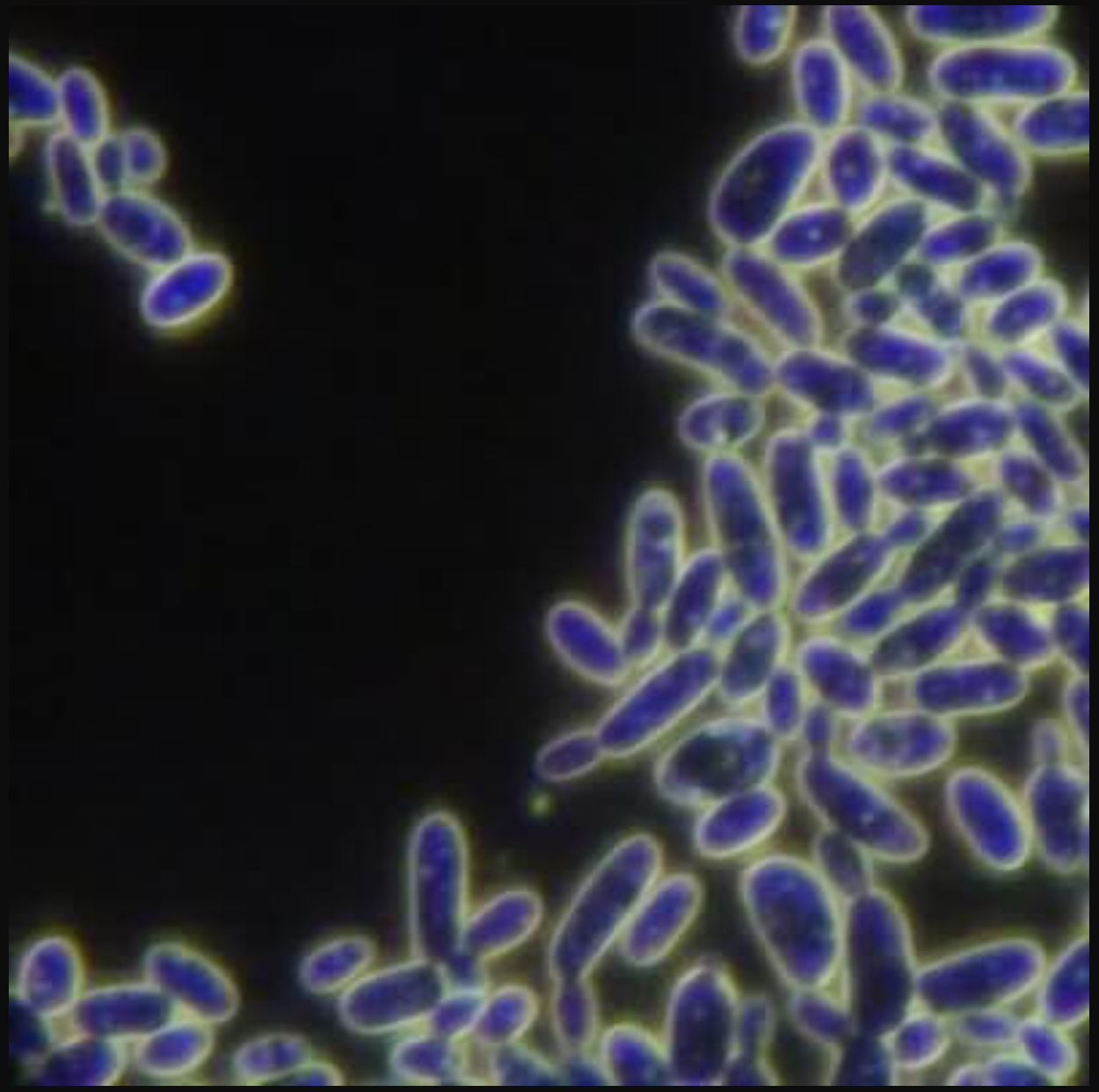


- **Dark-Field and Fluorescence Microscopy**

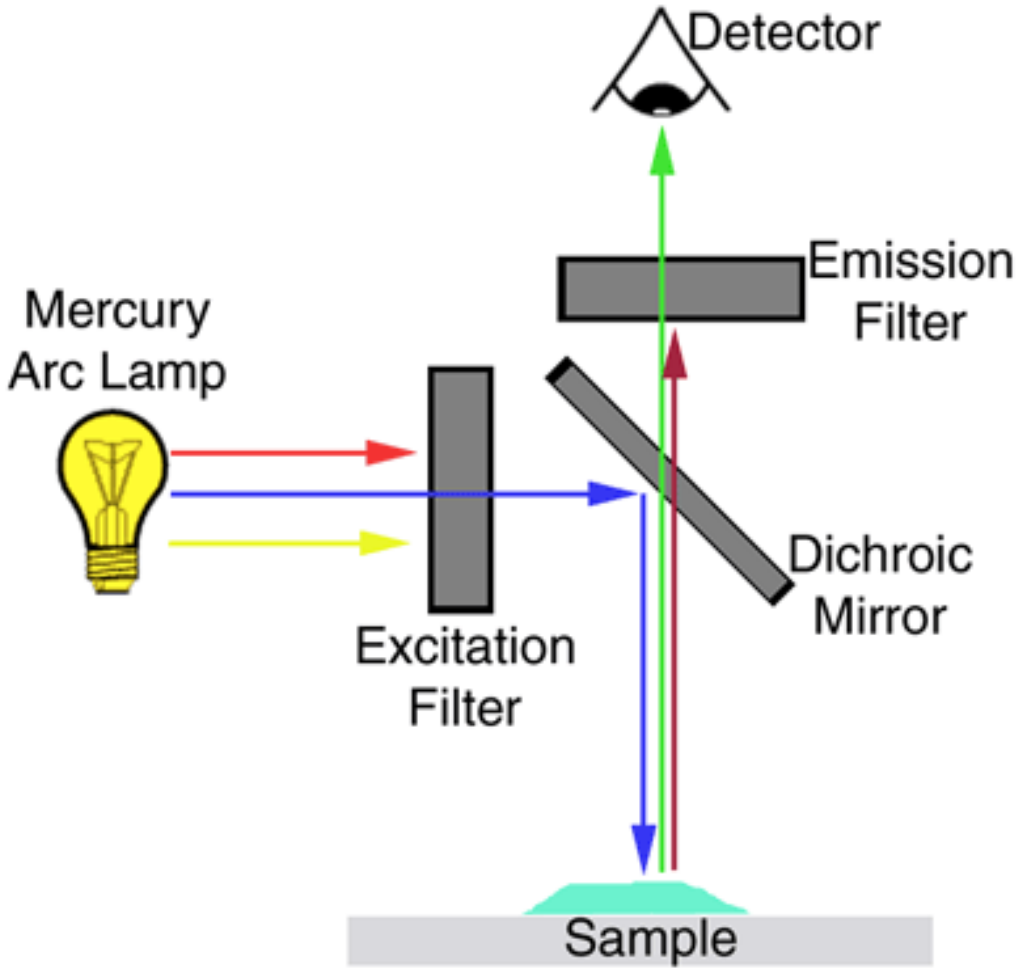
a condenser focuses light diagonally on the specimen in such a way that only light reflected from particulate matter such as bacteria reaches the eyepiece. The angles of incident and reflected light are such that the organisms are surrounded by a bright halo against a black background.

Dark Field


objective lens
scattered light transmitted
direct illumination block
sample
sample scatters some light
condenser lens
opaque light stop
light source



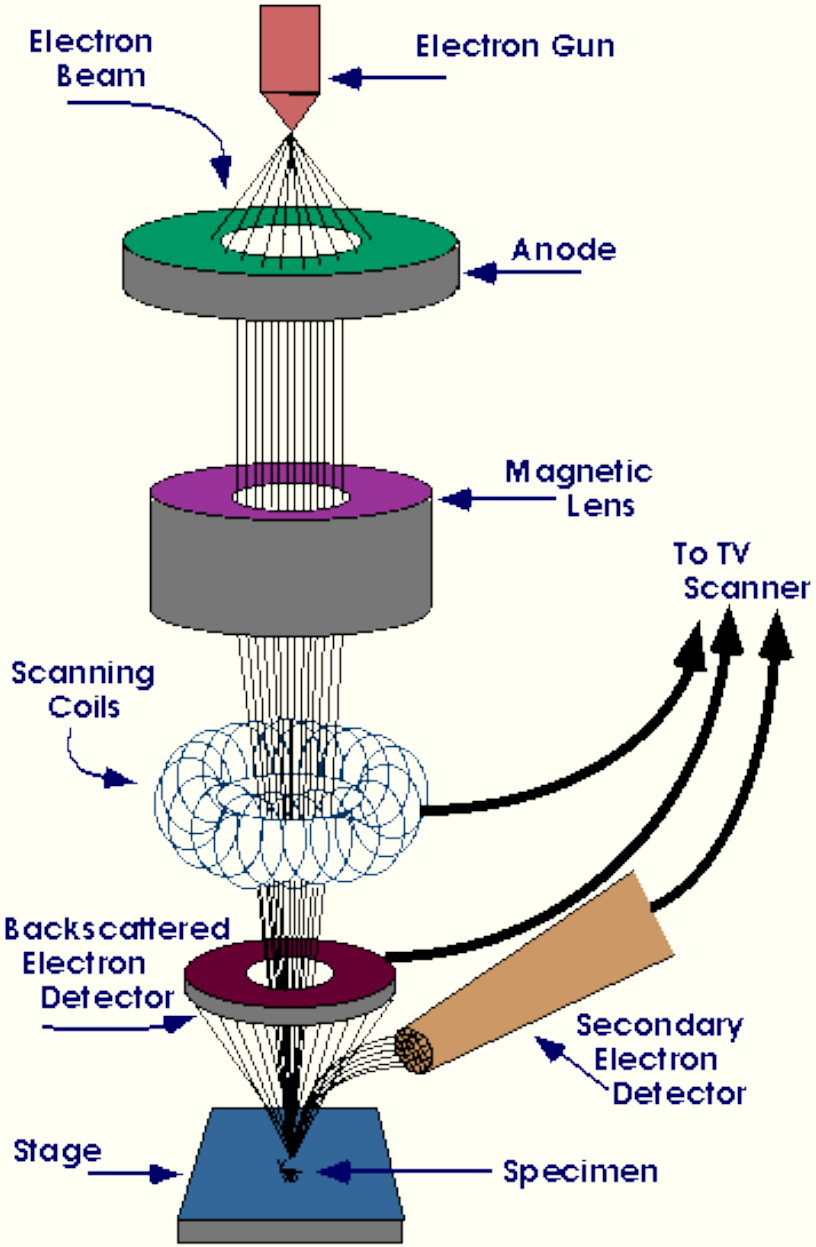
Fluorescence Microscope



- **Electron Microscopy**

Electron microscopy demonstrates structures by transmission of an electron beam and has 10 to 1000 times the resolving power of light microscopic methods. For practical reasons its diagnostic application is limited to virology, where, because of the resolution possible at high magnification, it offers results not possible by any other method. Using negative staining techniques, direct examination of fluids, and tissues from affected body sites enables visualization of viral particles.

EM


Electron Beam
Electron Gun
Anode
Magnetic Lens
To TV Scanner
Scanning Coils
Backscattered Electron Detector
Secondary Electron Detector
Stage
Specimen