Ministry of Higher Education
& Scientific Research
University of Sawah
Dep. Of laboratories

Microbiological methods for identification of microorganisms

Grade: 4

Lecture : 5

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Microbiological methods for identification of microorganisms

- Various methods used to identify organisms cultivated from patient specimens, associated with bacterial identification. The procedures to diagnosis of infectious diseases is as follows:
- 1. Direct examination of patient specimens
- 2. Growth and cultivation of the agents from the specimens
- 3. Analysis of the cultivated organisms to establish their identification and other pertinent characteristics such as susceptibility to antimicrobial agents.

Macroscopic Observation

It provide useful information to both the microbiologist and the physician the macroscopic observation should include the following:

- Swab or aspirate
- Stool consistency (formed or liquid)
- Blood or mucus present
- Volume of specimen
- Fluid—clear or cloudy

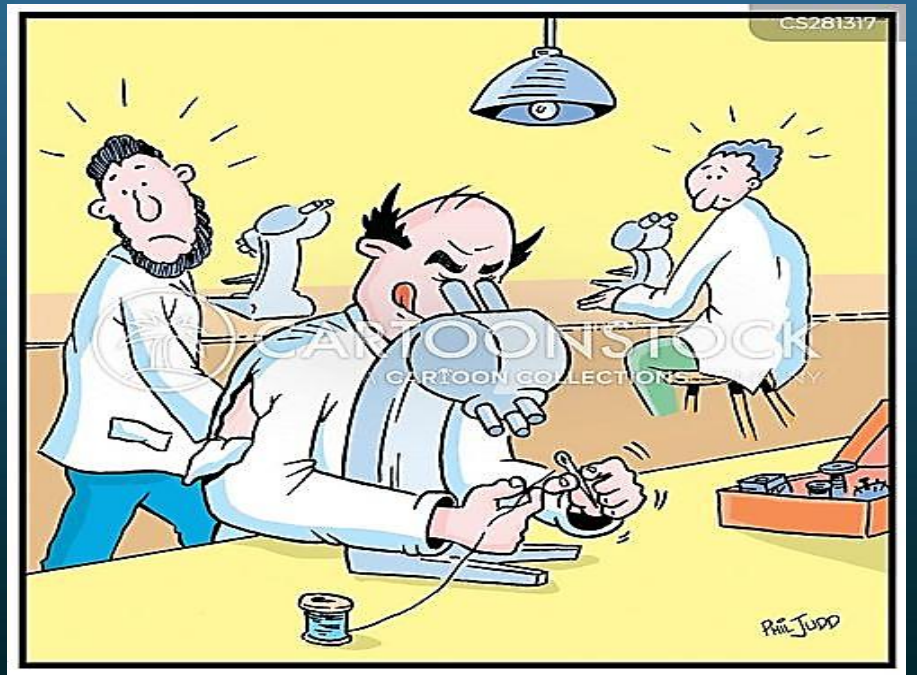
The gross examination also allows the processor to determine the adequacy of the specimen and the need for special processing. Areas of blood and mucus are selected for culture and direct microscopic examination. Anaerobic cultures may be indicated if gas, foul smell, or sulfur granules are present.

Microscopic Observation

Microscopy is defined as the use of a microscope to magnify (i.e., visually enlarge) Because most infectious agents cannot be detected with the unaided eye, microscopy plays a pivotal role in the laboratory.

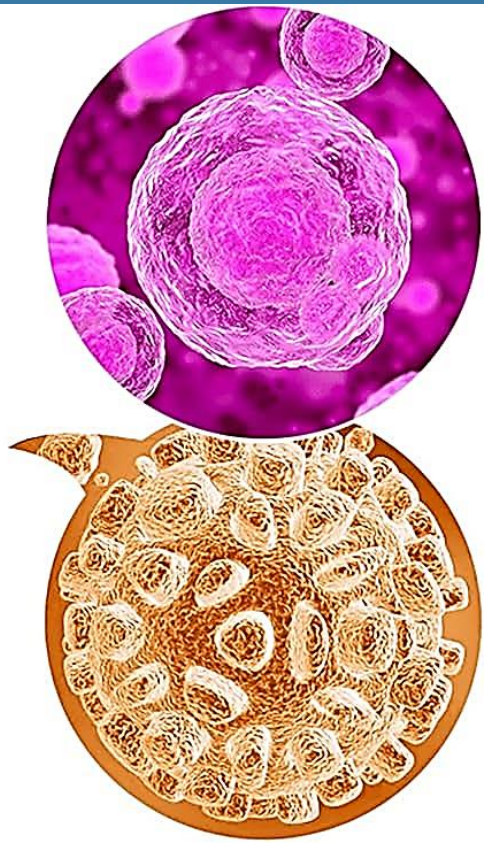
Types of Microscope are:

- Bright-field microscopy
- The phase-contrast microscope
- Dark-field
- Fluorescence microscopy
- Differential Interference Contrast Microscope
- The Electron Microscope

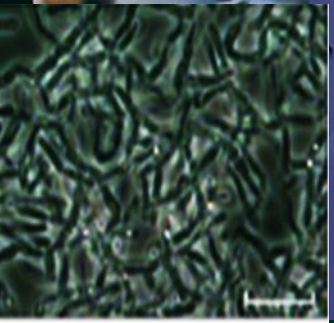


Bright-field microscopy (also known as light microscopy)

Many bacteria are difficult to see well because of their lack of contrast with the surrounding medium. Dyes (stains) can be used to stain cells or their organelles and increase their contrast so that they can be more easily seen in the bright-field microscope

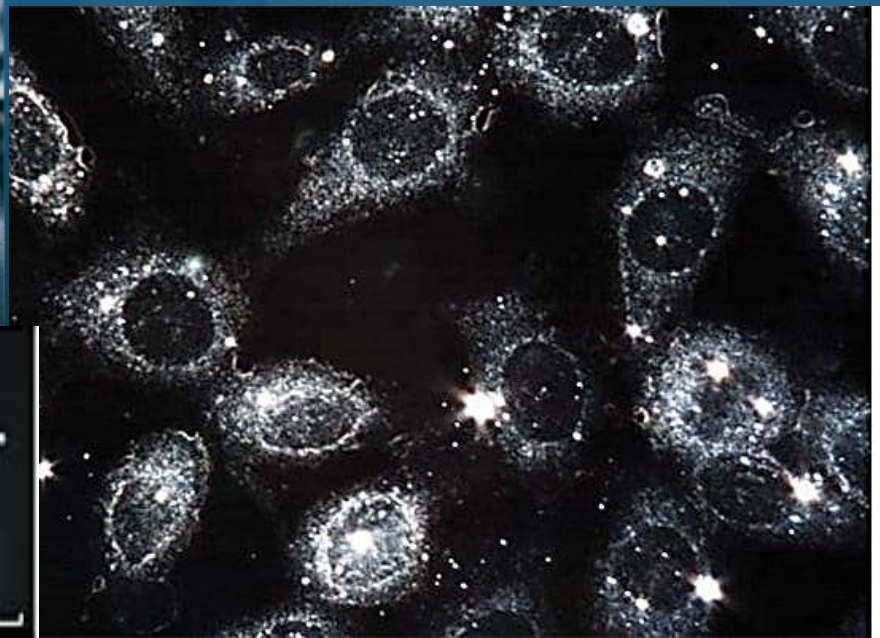


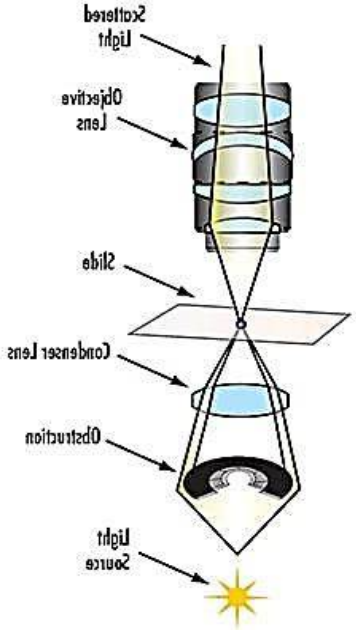
The phase-contrast microscope was developed to improve contrast differences between cells and the surrounding medium, making it possible to see living cells without staining them; with **bright-field microscopes, killed and stained preparations must be used.**

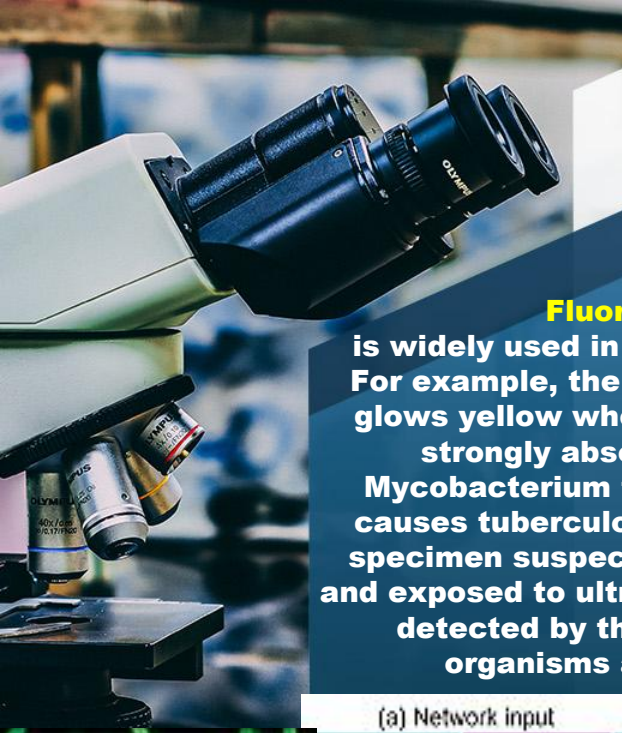


Dark-field

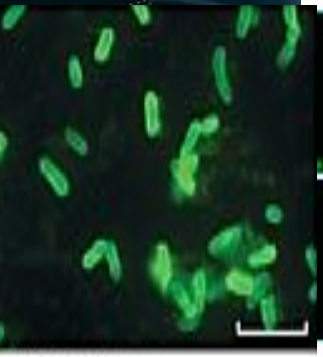
this technique has been particularly useful for observing organisms such as Treponema pallidum, a spirochete that is smaller than 0.2 µm in diameter and therefore cannot be observed with a bright field or phase-contrast microscope

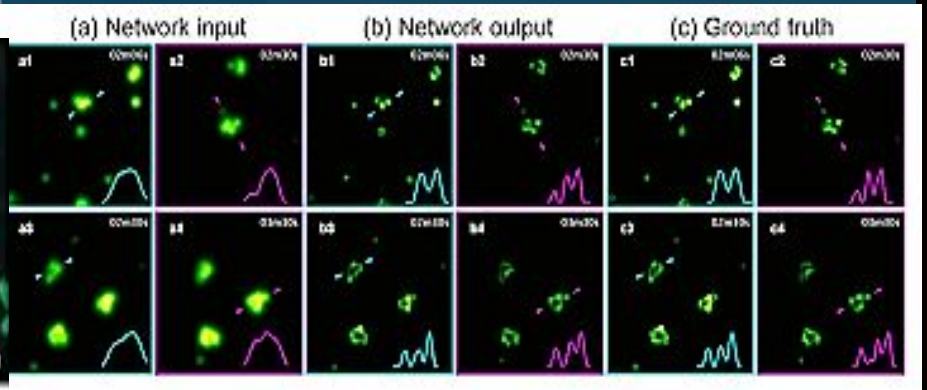






Fluorescence microscopy is widely used in clinical diagnostic microbiology. For example, the fluorochrome auramine O, which glows yellow when exposed to ultraviolet light, is strongly absorbed by the cell envelope of Mycobacterium tuberculosis, the bacterium that causes tuberculosis .When the dye is applied to a specimen suspected of containing M. tuberculosis and exposed to ultraviolet light, the bacterium can be detected by the appearance of bright yellow organisms against a dark background.





Differential Interference Contrast Microscope Structures

such as spores, vacuoles, and granules, appear three dimensional. DIC microscopy is particularly useful for observing unstained cells because of its ability to generate images that reveal internal cell structures that are less apparent by bright-field techniques.

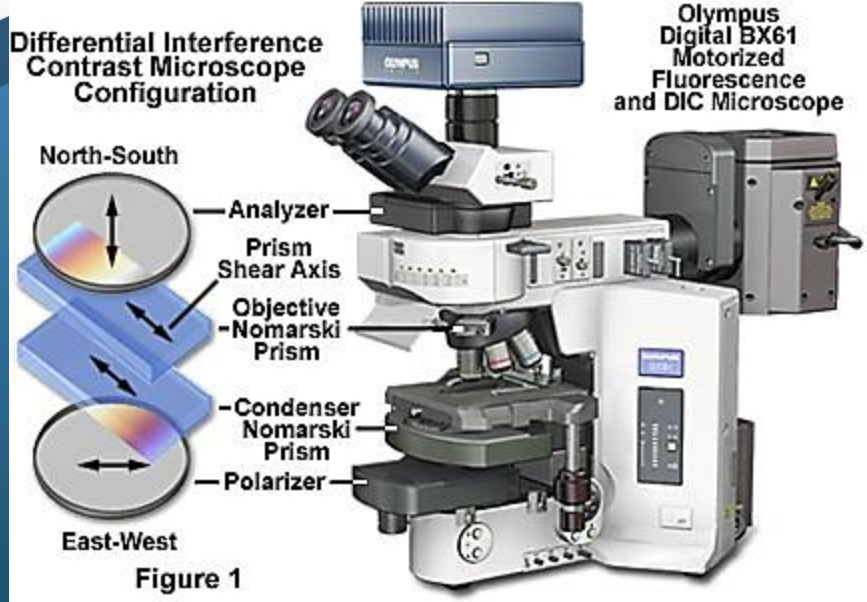
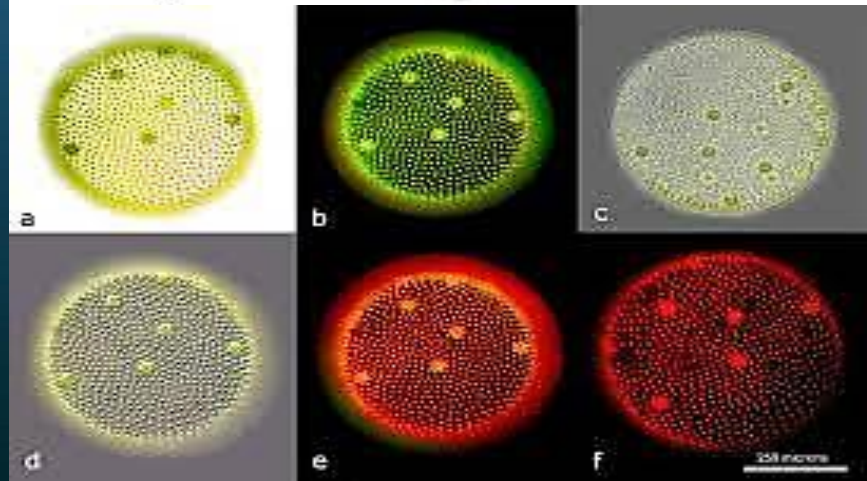


Figure 1

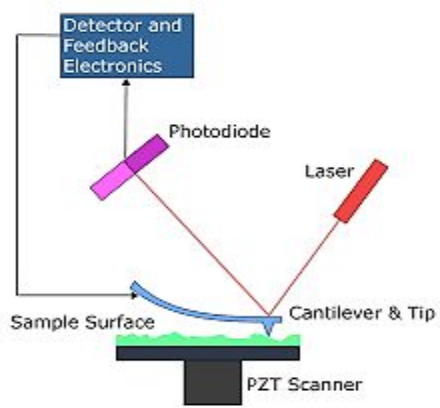


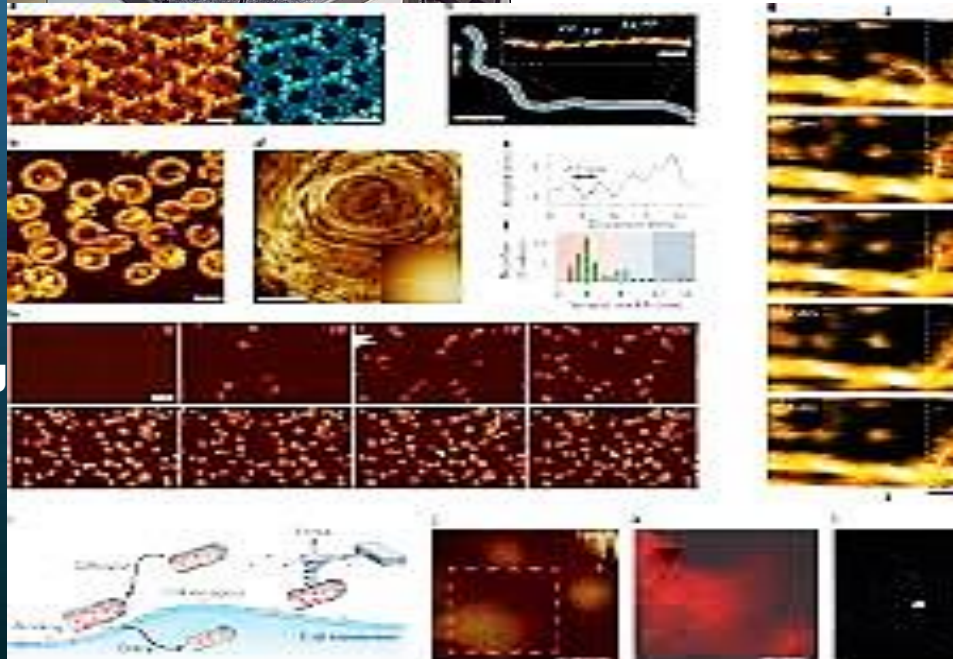
The Electron Microscope

There are two types of electron microscopes in general use: The transmission electron microscope (TEM), which has many features in common with the light microscope

Scanning Probe Microscopes

A new class of microscopes, called scanning probe microscopes, measures surface features by moving a sharp probe over the object's surface. the scanning electron microscope (SEM). is particularly useful for providing three dimensional images of the surface of microscopic objects.



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