Microscopy

Microscopy: *Technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye)*

Microscope: *An optical instrument that uses a lens or an arrangement of lenses to magnify an object*

Microscopes are instruments designed to produce magnified visual or photographic images of small objects. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image, and render the details visible to the human eye or camera.

- from Ancient Greek word “mikrós” meaning 'small’ and “skopéō” meaning 'to look at or examine’

- **Robert Hooke** is credited to be one of the first two scientists to discover microorganisms in 1665 using a compound microscope that he built himself, the other scientist being Antonie van Leeuwenhoek in 1674.
- The father of microscopy, **Antonie van Leeuwenhoek** developed a very high magnification simple microscope in the 1670s.
- **Applications:**
  - tissue analysis
  - examination of forensic evidence
  - studying the role of protein within the cell
  - the study of atomic structure.

Microscopy

Magnification : The process of enlarging the apparent size, not physical size, of an object. This enlargement is quantified by a size ratio called optical magnification.

- Magnification is a ratio of two sizes - the size of your sample (usually small, perhaps tens of microns) and the size of the image you produce
- Multiplying the power of eye piece and objective lens, for eg. the most common powers being 4X, 10X, 20X, 40X and 100X.

Resolution : Minimum distance between 2 distinct points of a specimen where they can still be seen by the observer or microscope as separate entities. OR the ability of a microscope to distinguish details of a specimen or sample.

- The resolution of a human eye is 200 μm
- The resolution of a light microscope is 0.2 μm

Types of Microscopy

- **Optical microscopy**
  - Bright field microscopy
  - Dark field microscopy
  - Phase contrast microscopy
  - Fluorescence microscopy
  - Confocal microscopy
- **Electron microscopy**
  - Transmission Electron Microscopy (TEM)
  - Scanning Electron Microscopy (SEM)
- **Scanning probe microscopy**
  - Atomic Force Microscope (AFM)
  - Scanning Tunnelling Microscope

Optical microscopy

Also referred to as a **light microscopy**. It is a type of microscopy that commonly uses visible light and a system of lenses to generate magnified images of small objects.

- There are two basic types of optical microscopes:
- **Parts of the microscope:**

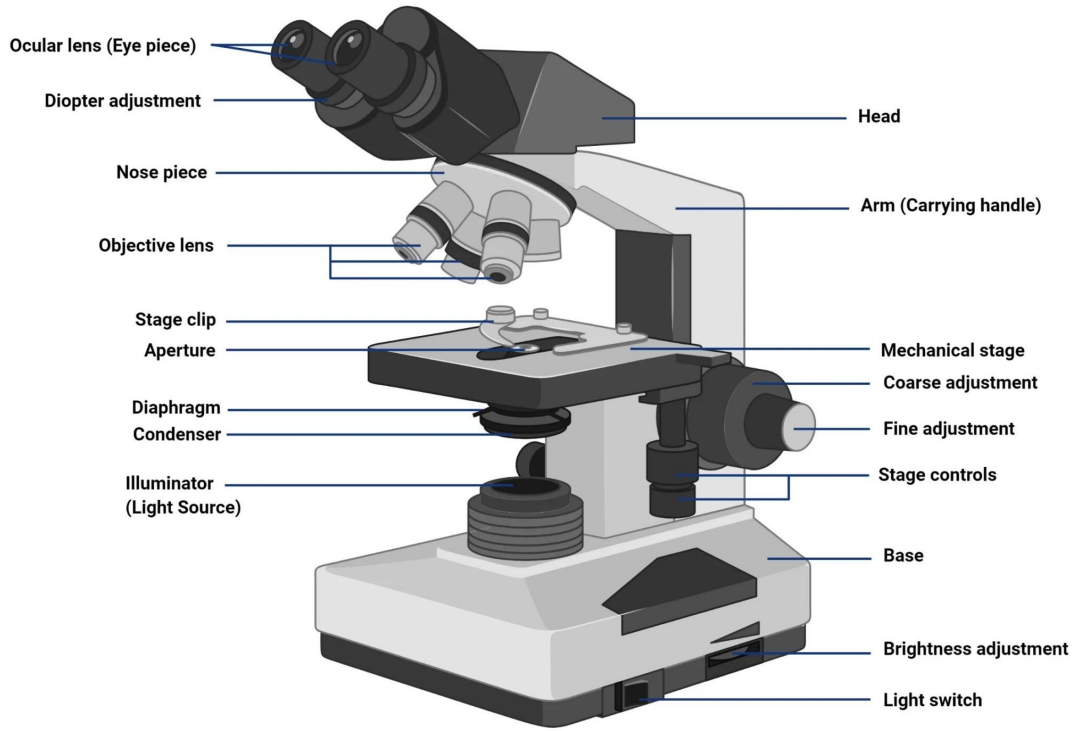
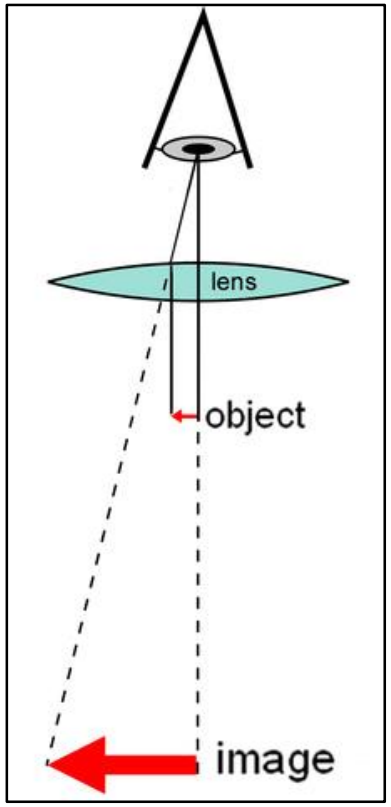
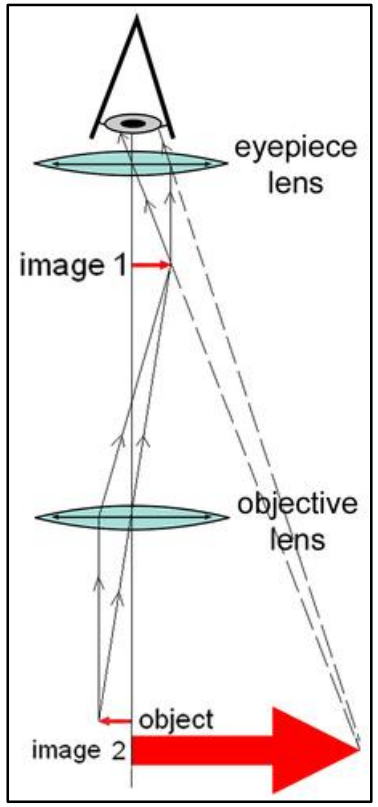


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Simple microscope



Compound microscope



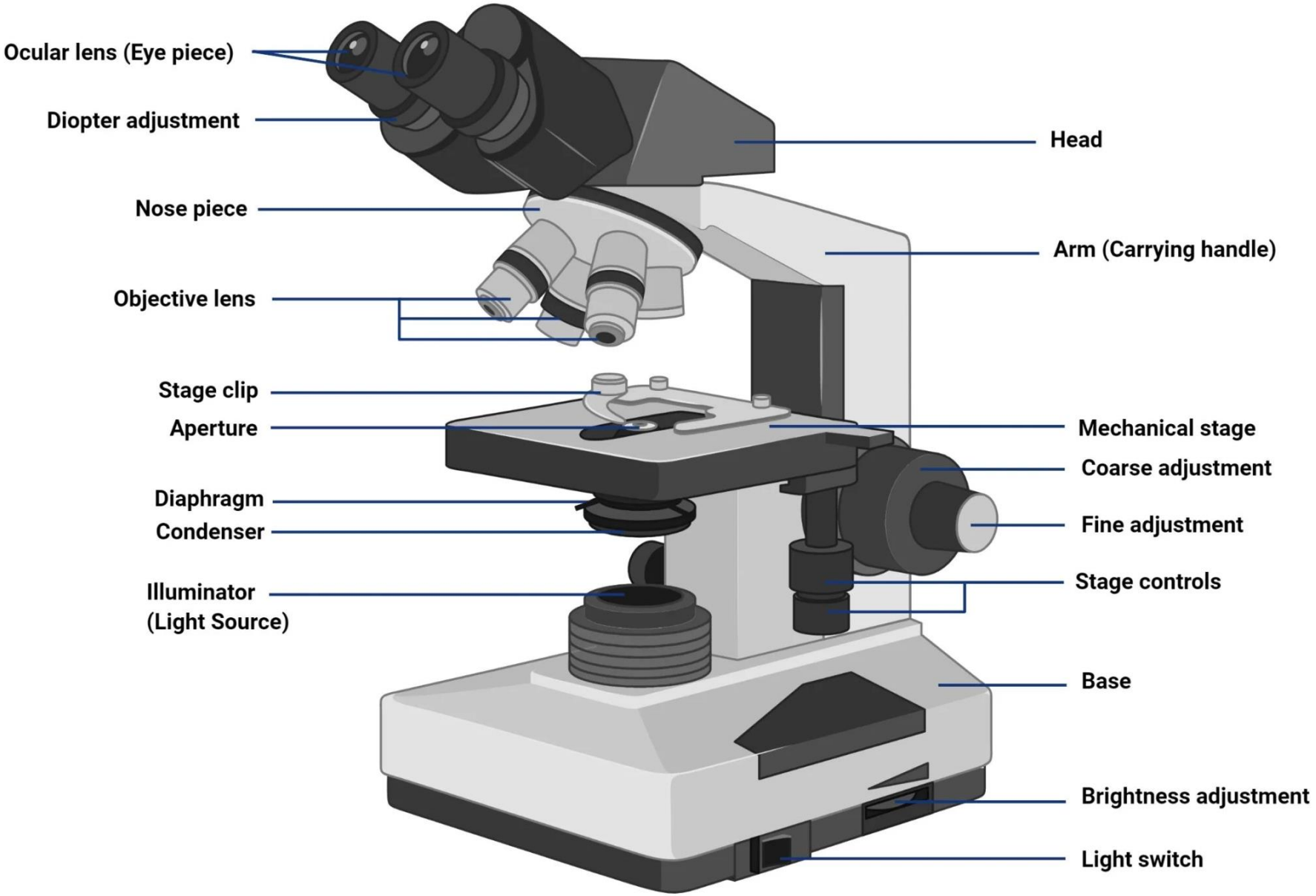


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

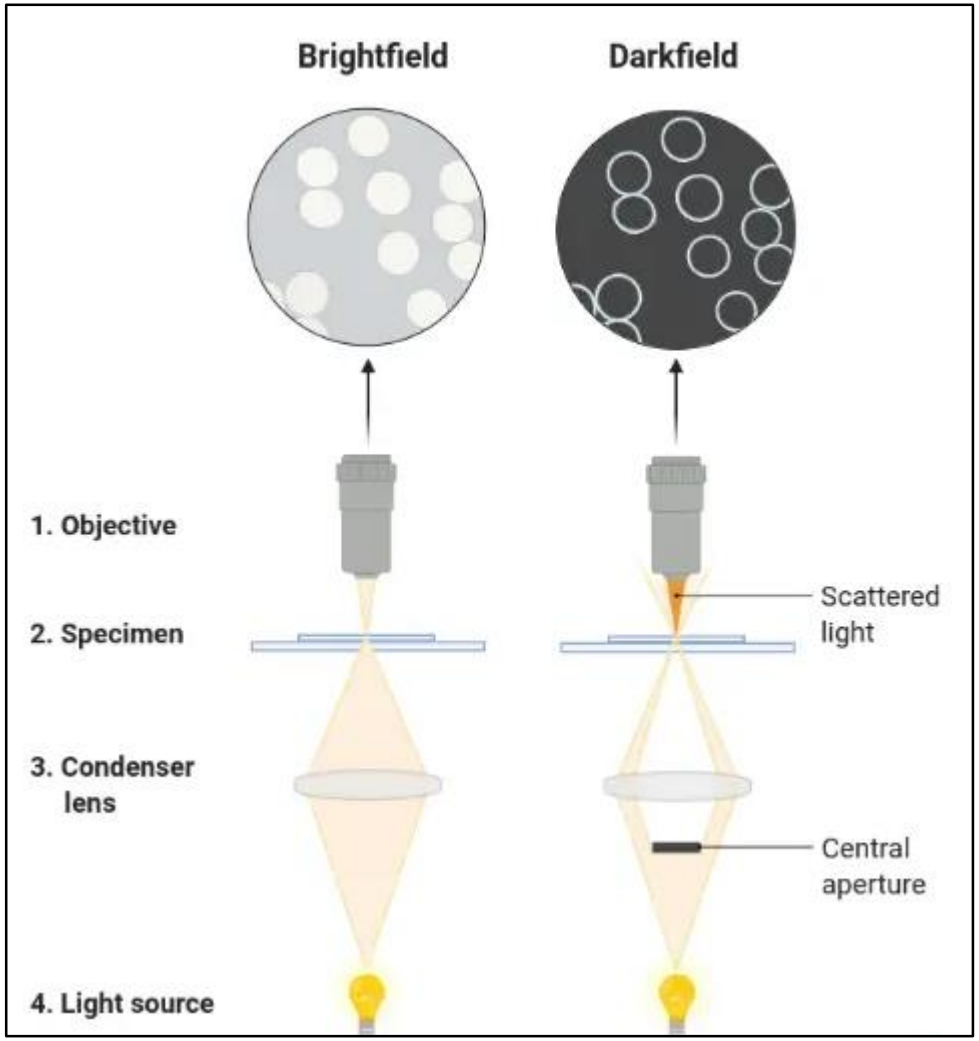
Optical microscopy

Bright-field Microscope:

- This is the simplest and most common type of optical microscope.
- It uses a bright light source to illuminate the sample, and produces a dark image on a bright background.
- It is used to observe the structure and morphology of a wide range of specimens, such as cells, tissues, and microorganisms.

Dark-field Microscope:

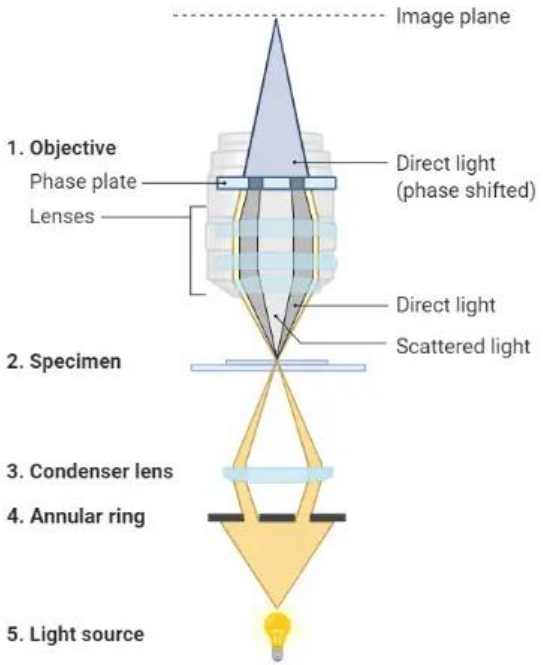
- This type of microscope uses a special condenser lens that directs light at an oblique angle onto the sample, causing the sample to appear bright on a dark background.
- It is used to observe samples that do not absorb or transmit much light, such as bacteria, viruses, and nanoparticles.



Optical Microscopy

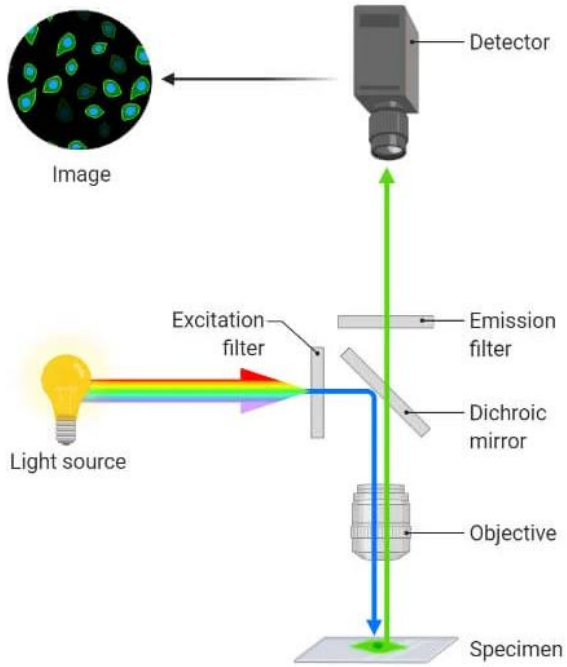
Phase-contrast Microscope:

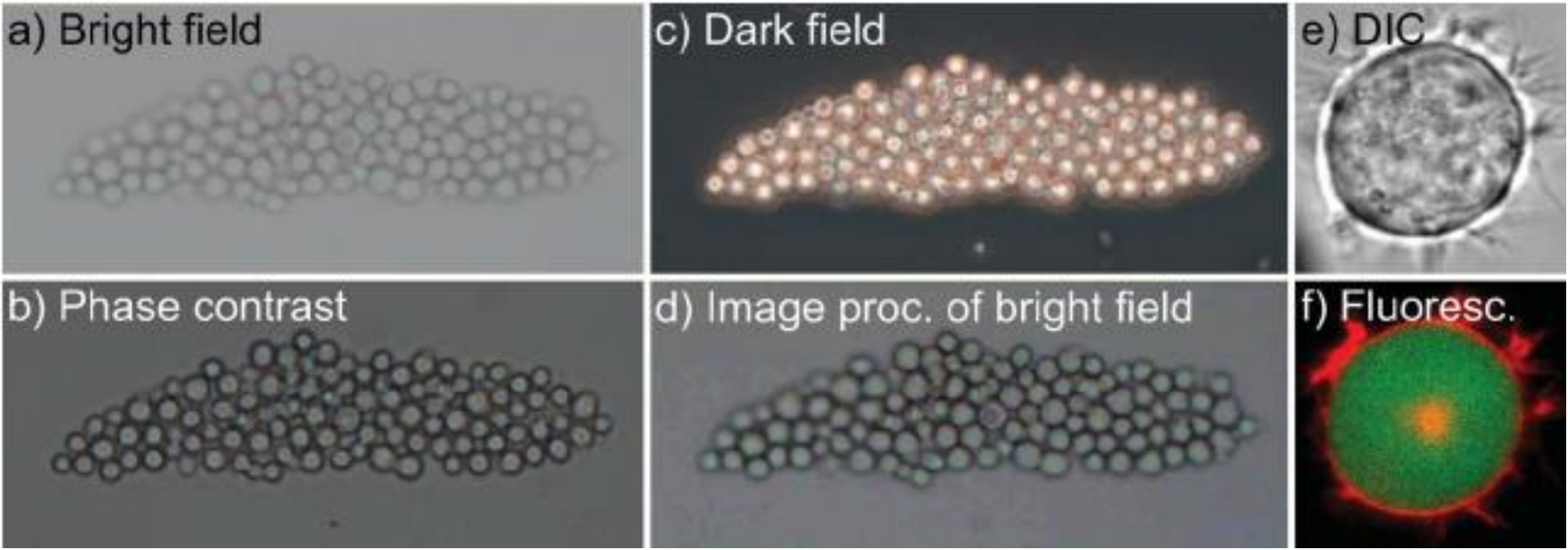
- This type of microscope is used to observe transparent samples, such as living cells or bacteria, which are difficult to see using traditional bright-field microscopy.
- It produces images by detecting the differences in the refractive index of the sample, rather than differences in light absorption.

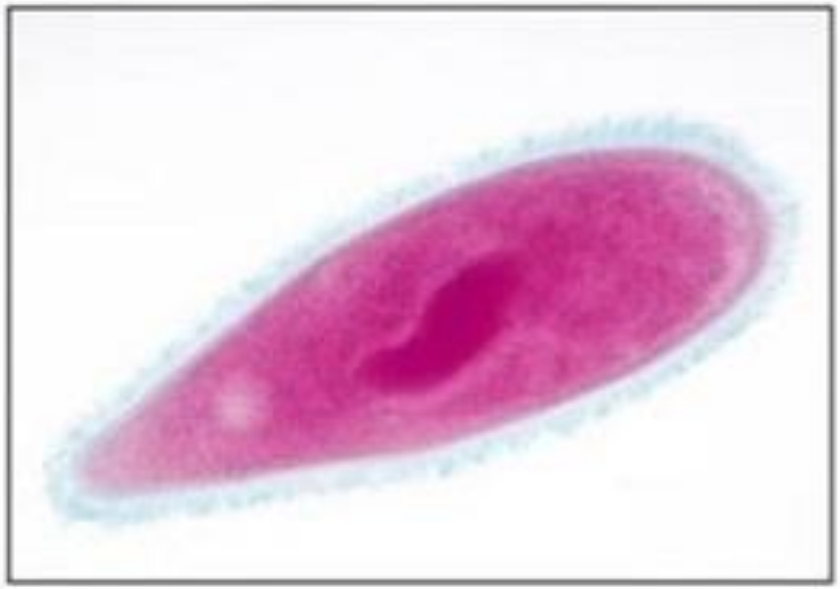


Fluorescence Microscope:

- This type of microscope uses a light source that excites fluorescent molecules within the sample, causing them to emit light of a different color.
- It is used to observe samples that have been labeled with fluorescent dyes or antibodies, such as cells and tissues.




a) Bright field
c) Dark field
e) DIC
b) Phase contrast
d) Image proc. of bright field
f) Fluoresc.

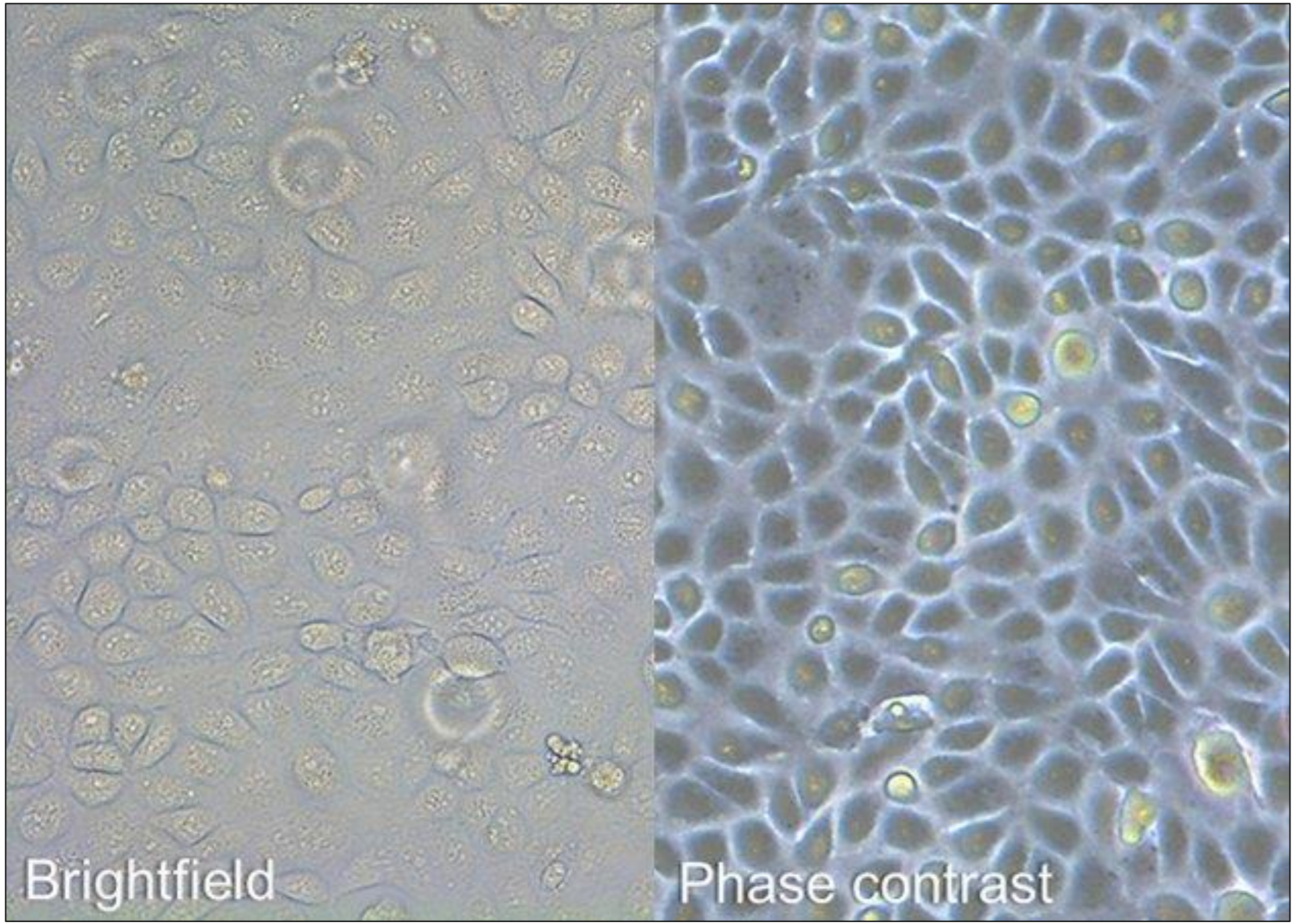


Brightfield

Darkfield

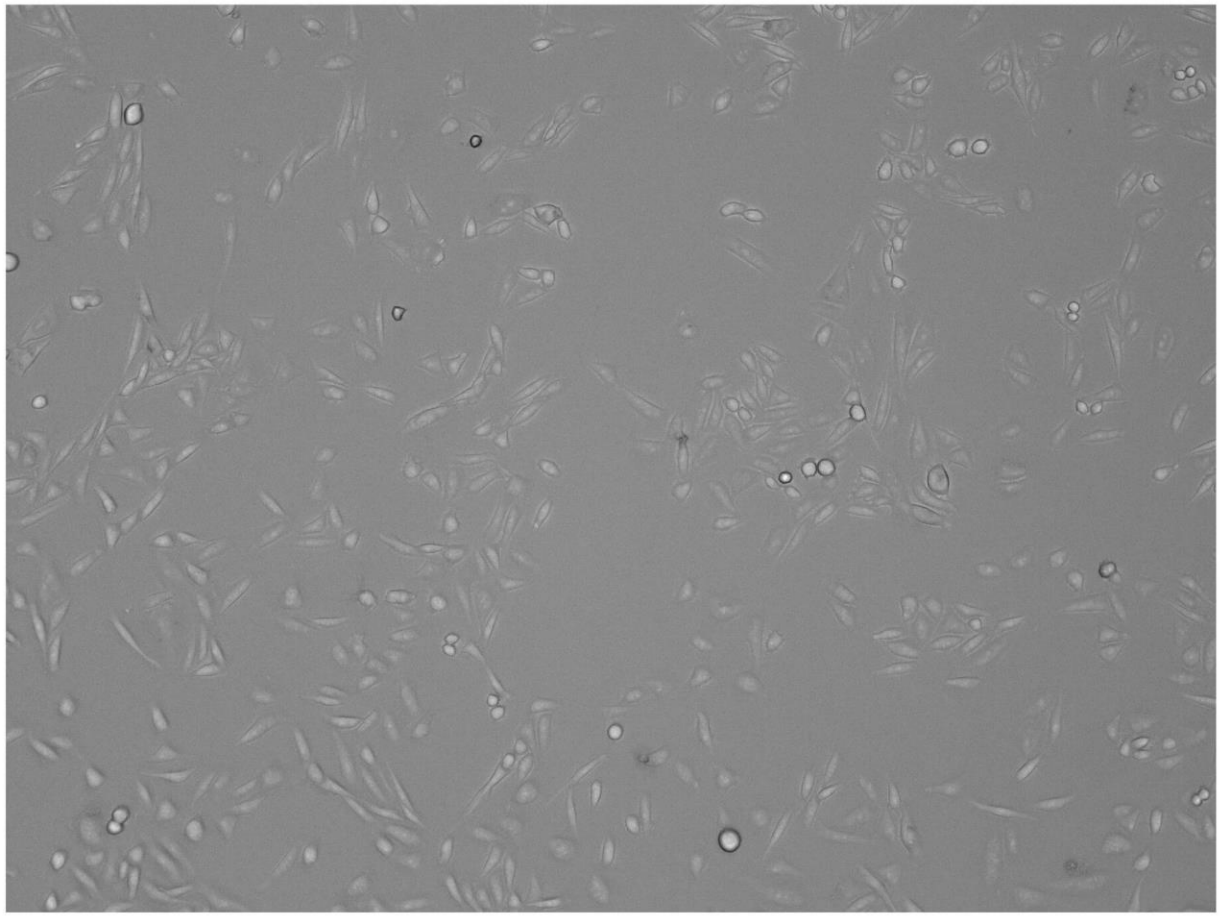
Phase-contrast

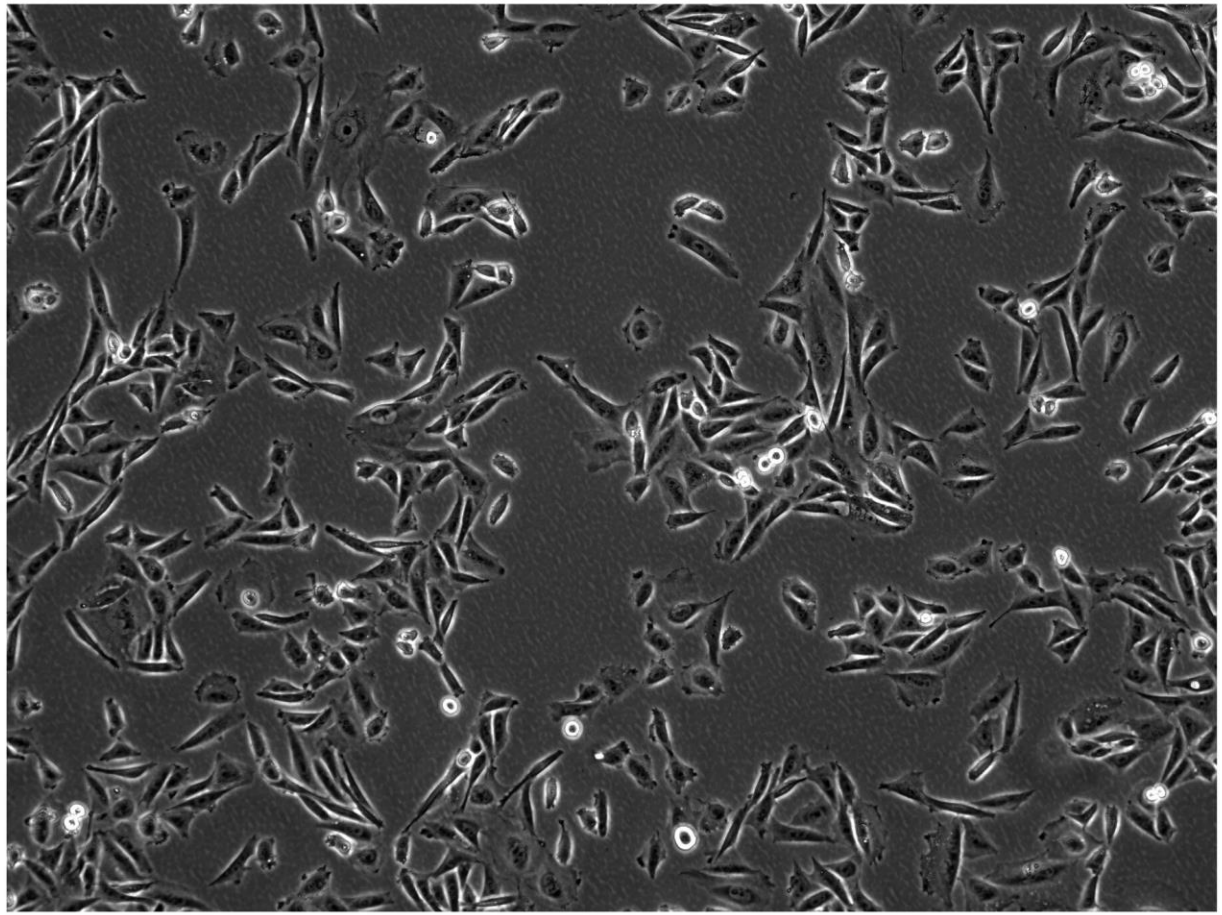
BT-312 - Analytical Biotechnology Laboratory



Brightfield/Trans

Phase contrast



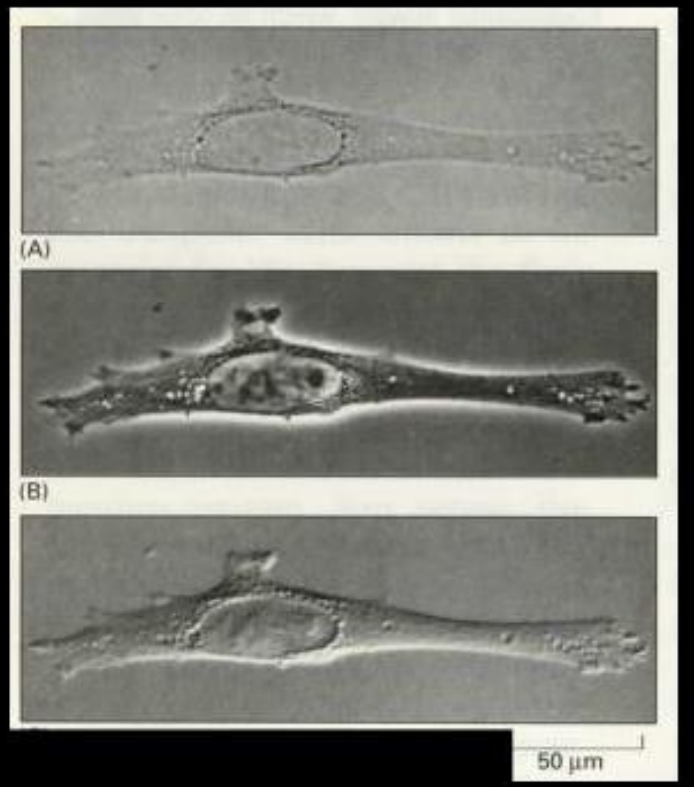


10x FL Ph ∞/1.0 mm

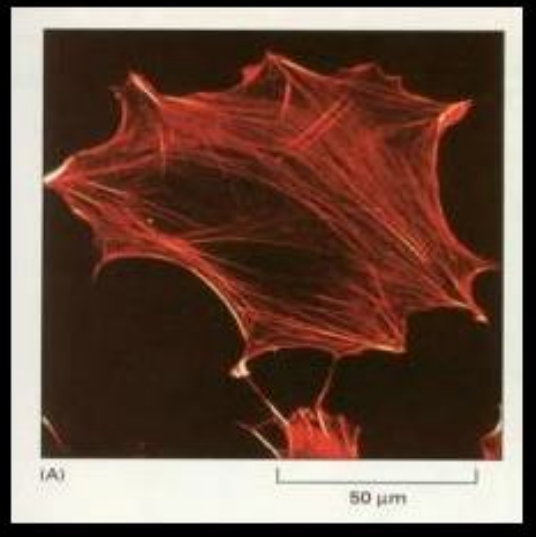
Bright Field

Phase-Contrast

Differential Interference Contrast (DIC)



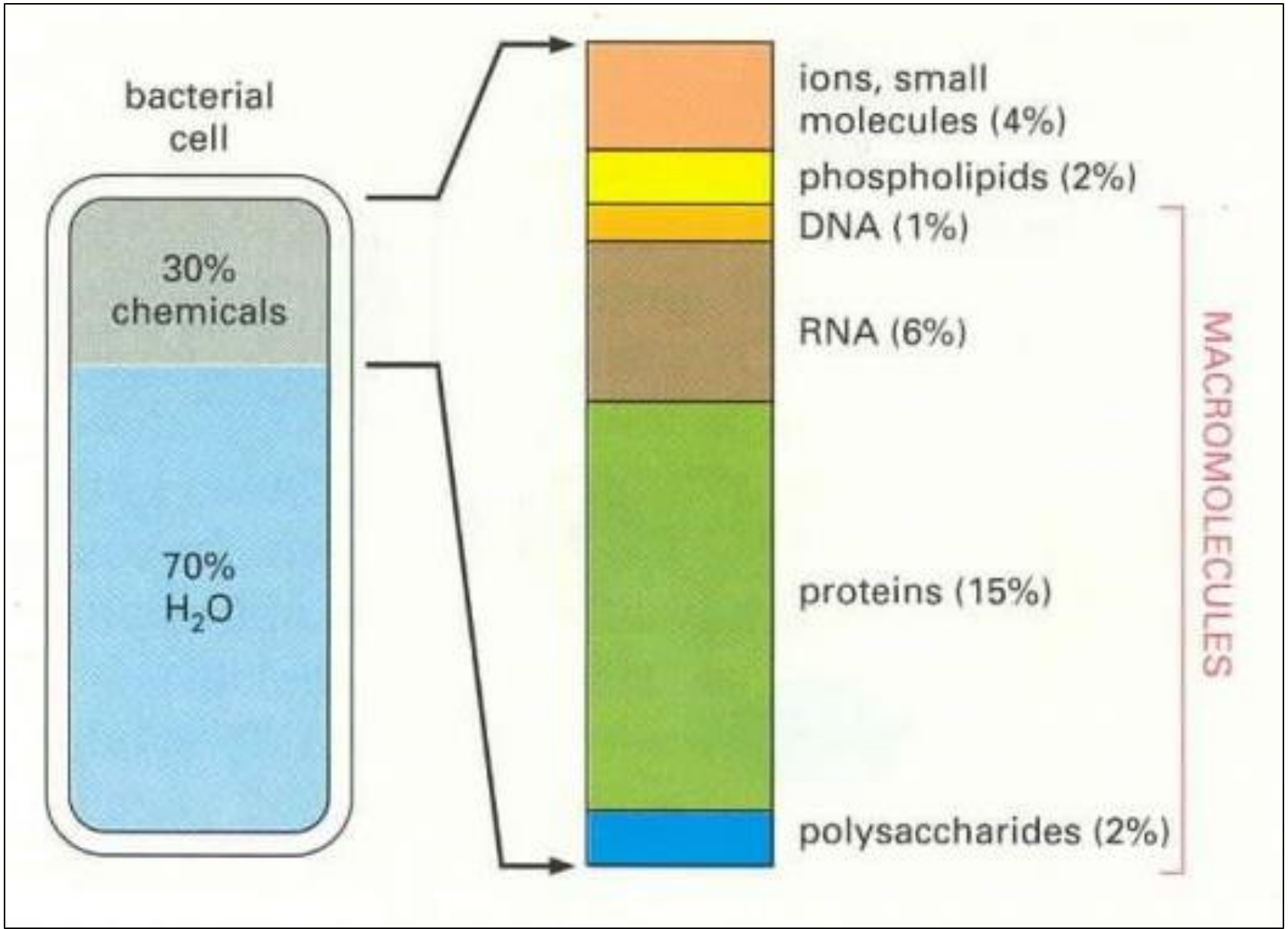
Fluorescence

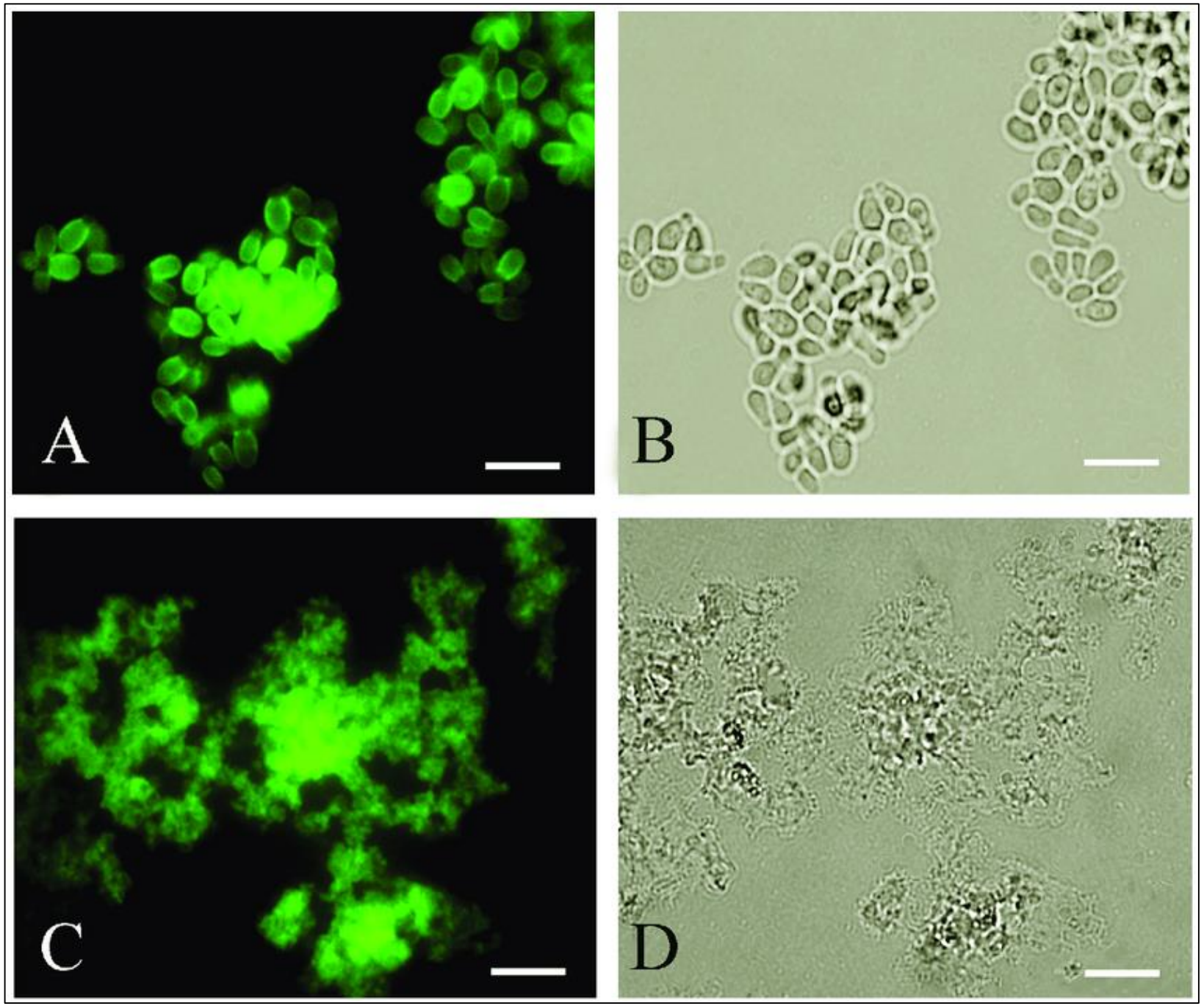


- A dark field microscopy is used to examine live micro-organisms that either invisible in the ordinary light microscope, cannot be stained by standard methods, or are so distorted by staining that their characteristics then cannot be identified.

- Instead of the normal condenser, a dark field microscope uses a dark field condenser that contain a opaque disc. The disc blocks light that would enter the lens directly, only the light is reflected off the specimen enters the objective lens. Because there is no background light, the specimen appears light against black background- the dark field.

Chemical Composition of Cells

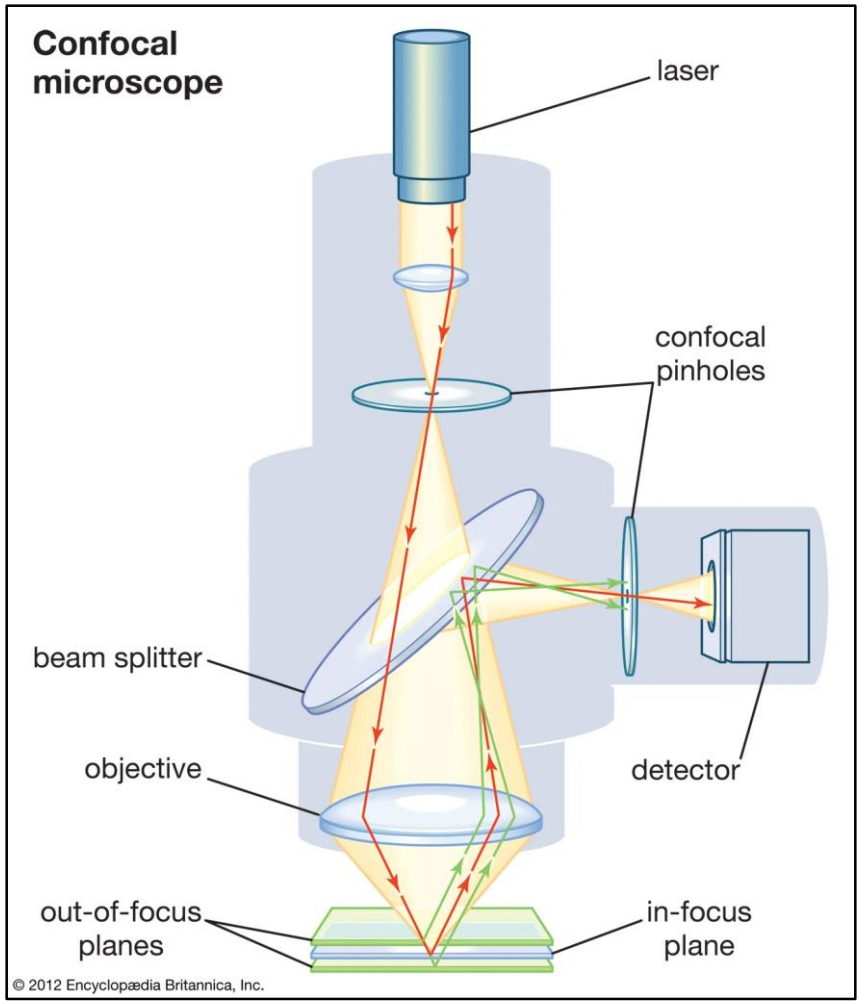



A
B
C
D

Optical Microscopy

Confocal Microscope:

- This type of microscope uses a laser to scan a sample point by point, and creates a 3D image by stacking the individual images together.
- It is used to observe thick or complex samples, such as tissues and embryos, and can produce high-resolution images with minimal background noise.



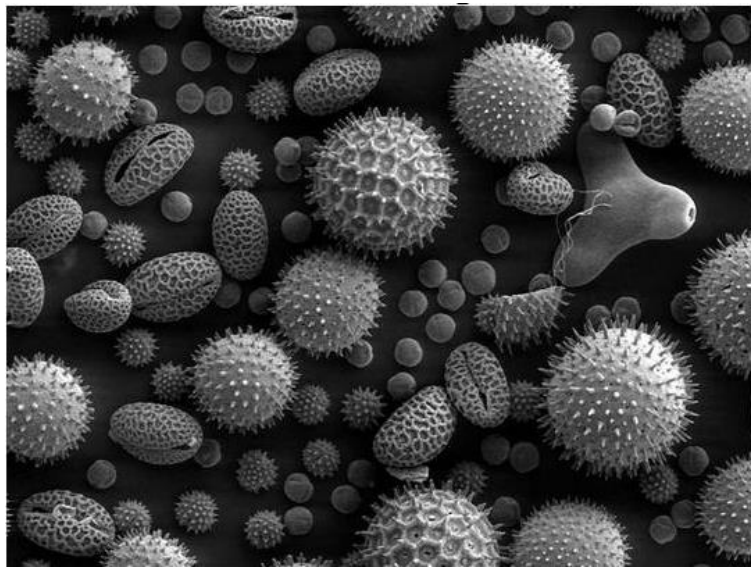
Electron microscopy

Scanning Electron Microscopy (SEM)

- Uses a fine beam of focused electrons to scan a sample's surface.
- Gives insight into a sample's topography and elemental composition.
- SEM has the potential to magnify an image up to 2 million times.

Transmission Electron Microscopy (TEM)

- Uses a broad beam of electrons to create an image of a sample's internal structure
- A beam of electrons is transmitted through a sample, creating an image that details a sample's morphology, composition, and crystal structure



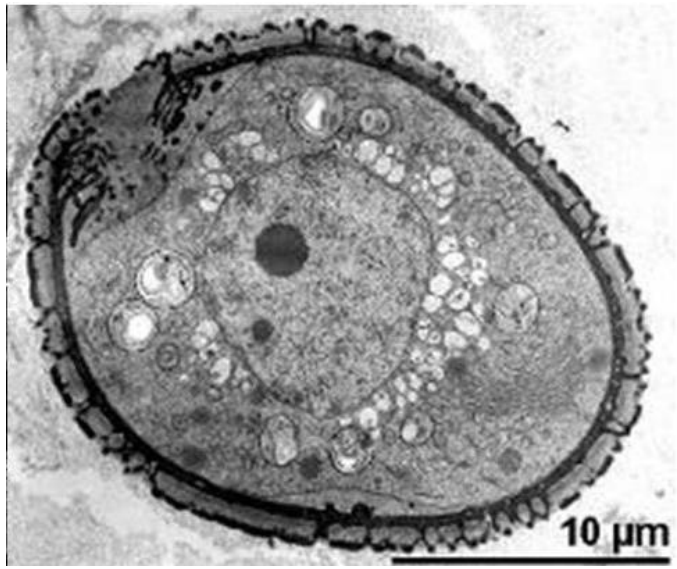


Image of pollen grain taken by SEM vs TEM

Scanning probe microscopy

Atomic Force Microscope (AFM)

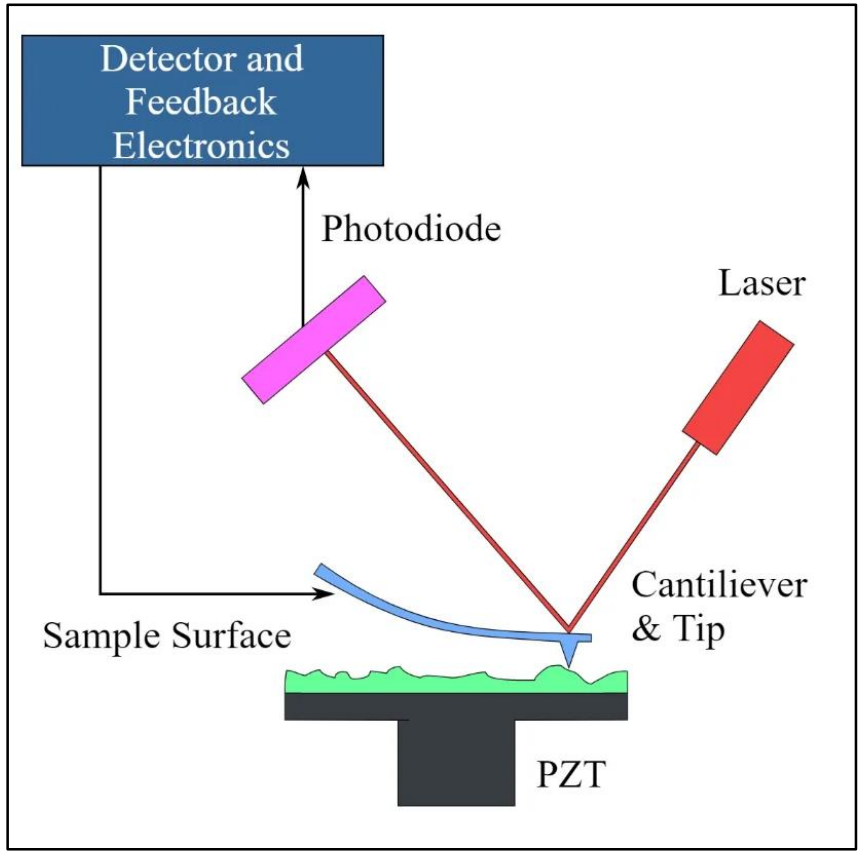
A surface scanning technique that has sub-nanometer scale resolution.

Principle:

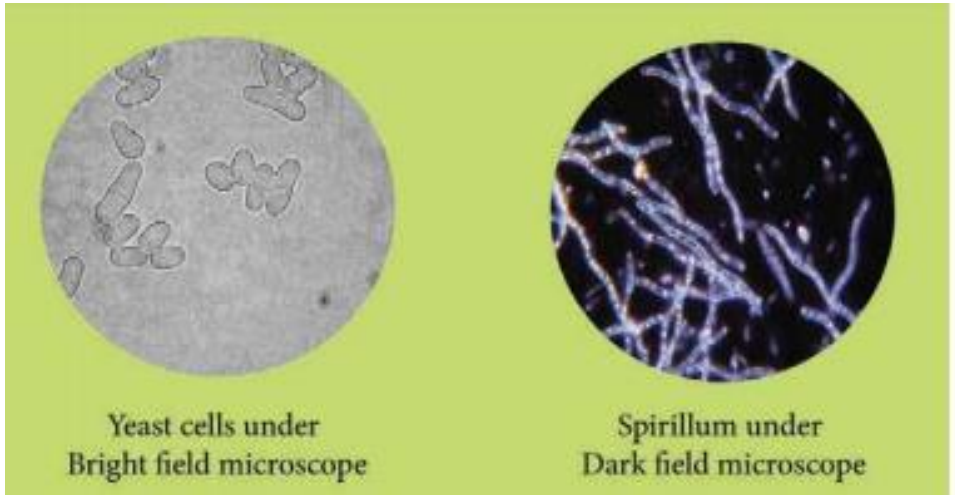
- An AFM generates images by scanning a small cantilever over the surface of a sample. The sharp tip on the end of the cantilever contacts the surface, bending the cantilever and changing the amount of laser light reflected into the photodiode.
- The height of the cantilever is then adjusted to restore the response signal, resulting in the measured cantilever height tracing the surface.

Application:

- used widely to collect data on various mechanical, functional and electrical properties at the nanoscale as well as for topography (surface) studies.

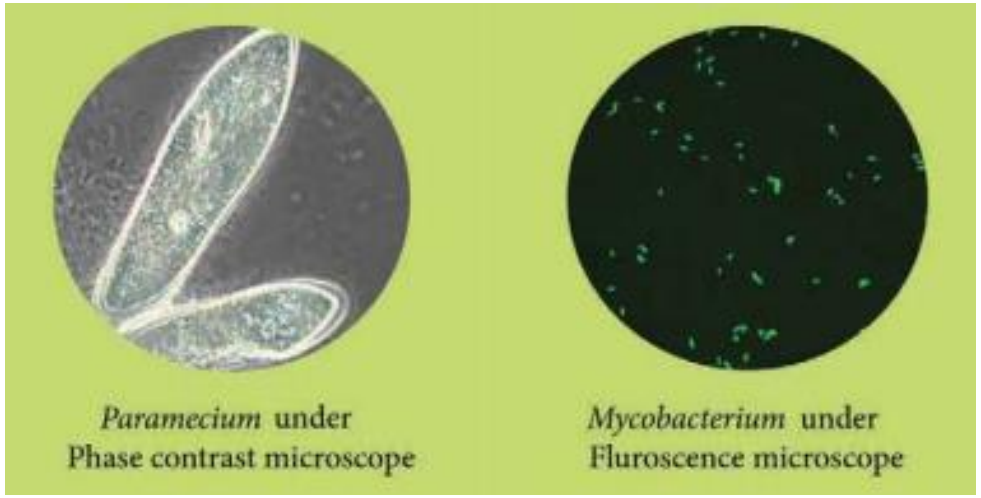


Comparison of different types of Microscopy



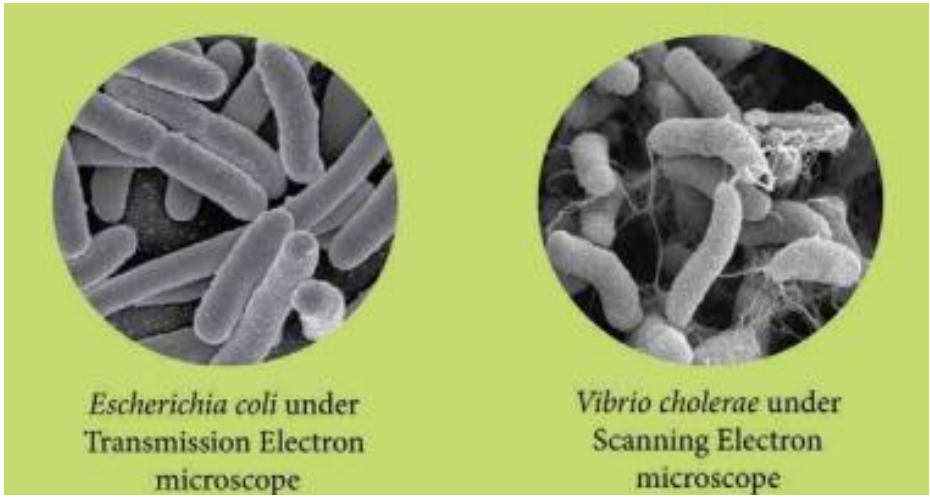
Yeast cells under Bright field microscope

Spirillum under Dark field microscope



Paramecium under Phase contrast microscope

Mycobacterium under Fluroscence microscope



Escherichia coli under Transmission Electron microscope

Vibrio cholerae under Scanning Electron microscope

Important Links

- https://www.thoughtco.com/facts-about-cells-373372
- https://www.thoughtco.com/history-of-the-microscope-1992146
- https://www.thoughtco.com/anton-van-leeuwenhoek-1991633
- https://www.britannica.com/biography/Antonie-van-Leeuwenhoek
- https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/01%3A_Introduction_to_Microbiology/1.01%3A_Introduction_to_Microbiology/1.1B%3A_History_of_Microbiology_-_Hooke_van_Leeuwenhoek_and_Cohn

- **Microscope Parts and Functions | How to Use a Microscope**
- https://www.youtube.com/watch?v=FnOvLEaC4gg
- https://www.youtube.com/watch?v=6gIneqf6pYU
- https://www.youtube.com/watch?v=BHRj8Ni_Tm4
- https://www.microscopemaster.com/cheek-cells-microscope.html
- https://www2.mrc-lmb.cam.ac.uk/microscopes4schools/humancheek.php