Al- Mustaqbal University College

First stage.
Department of Optometry(Optics)

جامعة المستقبل الاهلي
مرحلة الاولى
قسم التقنيات البصرية

Lab :1

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is a laboratory instrument used to examine objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures using a microscope. Microscopic means being invisible to the eye unless aided by a microscope.

There are many types of microscopes, and they may be grouped in different ways. One way is to describe the method an instrument uses to interact with a sample and produce images, either by sending a beam of light or electrons through a sample in its optical path, by detecting photon emissions from a sample, or by scanning across and a short distance from the surface of a sample using a probe.

Principle Microscopy is to get a magnified image, in which structures may be resolved which could not be resolved with the help of an unaided eye.

Magnification

•It is the ratio of the size of an object seen under microscope to the actual size observed with unaided eye.

•The total magnification of microscope is calculated by multiplying the magnifying power of the objective lens by that of eye piece.

Resolving power

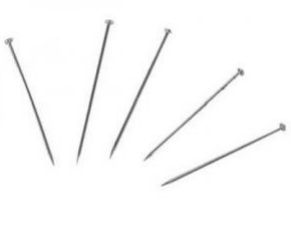
•It is the ability to differentiate two close points as separate.

• The resolving power of human eye is 0.25 mm

• The light microscope can separate dots that are 0.25µm apart.

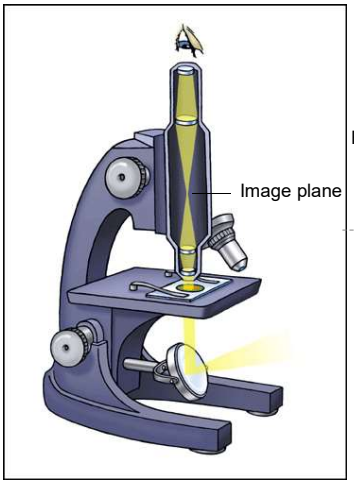
• The electron microscope can separate dots that are 0.5nm

M*icro*- = "small"; -*scope* = "to look at"



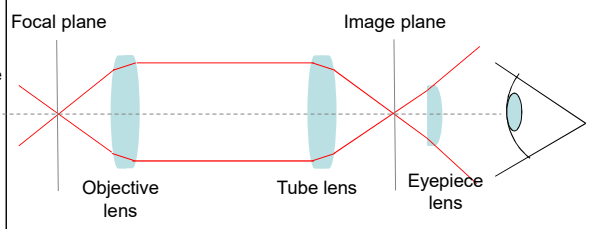
Photographs of cells are taken using a microscope, and these pictures are called **micrographs**.

What is a microscope?



Classic compound microscope

Theoretically a microscope is an array of two lenses.



Light (Optical) Microscopy

- Visible light is uses.
- Glass lens are used
- Advantage: It can often be performed on living cells, so it's possible to watch cells carrying out their normal behaviors (e.g., migrating or dividing) under the microscope.

Principle

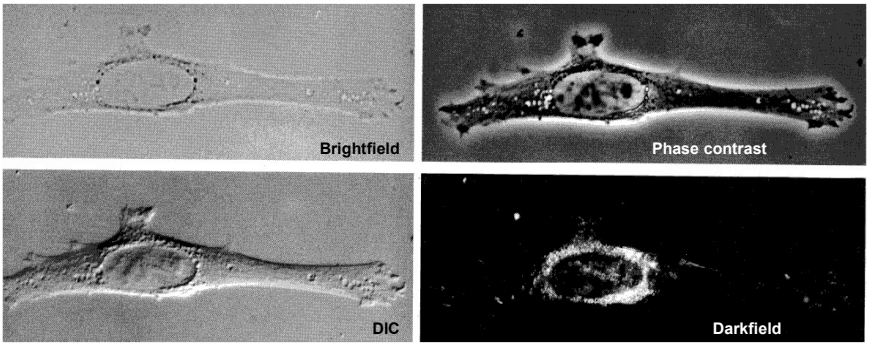
- When a ray of light passes from one medium to another it bends by phenomena called refraction.
- Bending of light slows the speed.
- The bending of light is determined by refractive index of the medium.

Types of Light Microscopes

1. Bright field Light Microscope
2. Phase Contrast Light Microscope
3. Dark-Field Light Microscope
4. Fluorescence Light Microscope

Contrasting techniques

Fibroblast grown in culture

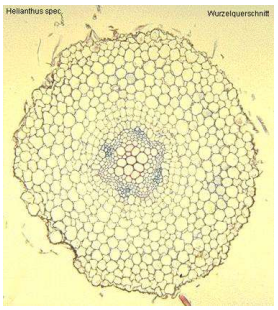


Taken from: http://fig.cox.miami.edu/~cmallery/150/Fallsyll.htm

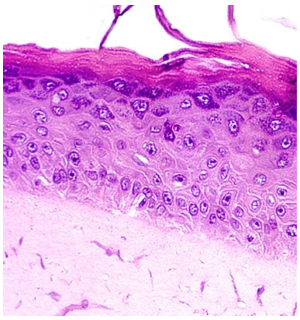
Brightfield

Principle: Light is transmitted through the sample and absorbed by it.

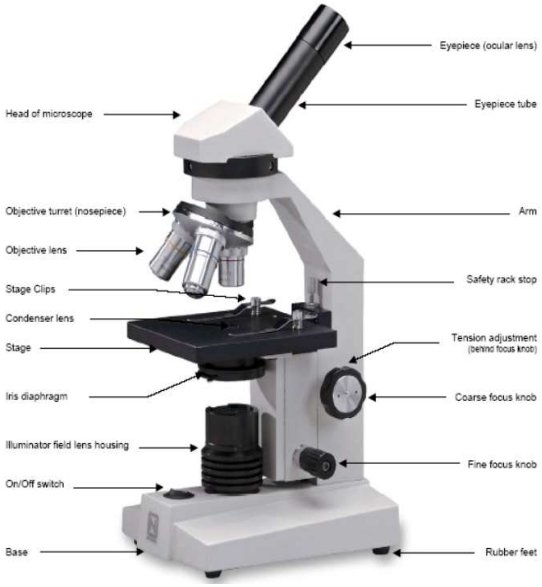
Application: Only useful for specimens that can be contrasted via dyes.
Very little contrast in unstained specimens.
With a bright background, the human eye requires local intensity fluctuations of at least 10 to 20% to be able to recognize objects.



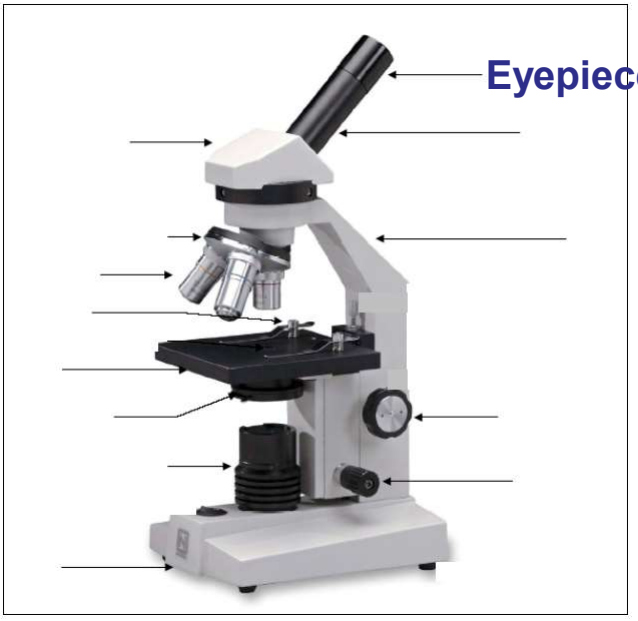
Cross section of sunflower root
(http://www.zum.de/Faecher/Materialien/beck/12/bs12-5.htm)



Piece of artificially grown skin
(www.igb.fhg.de/.../dt/PI_BioTechnica2001.dt.html)

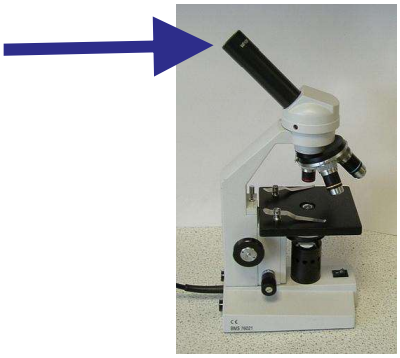


Typical Classroom Microscope

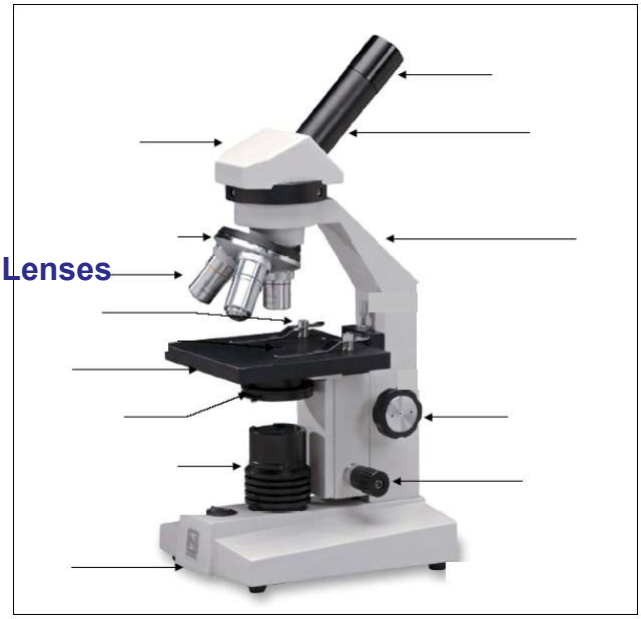


Eyepiece

- Also known as the ocular
- Contains the first lens you look through - usually a magnification of 10x
- Located on the top of the body tube

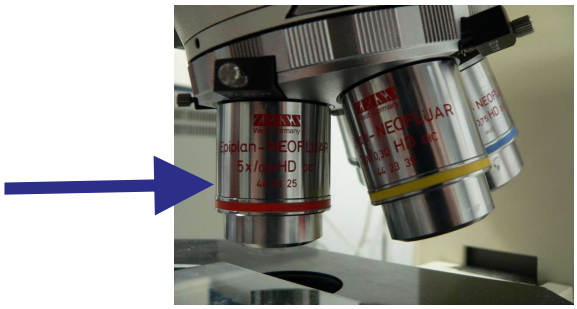


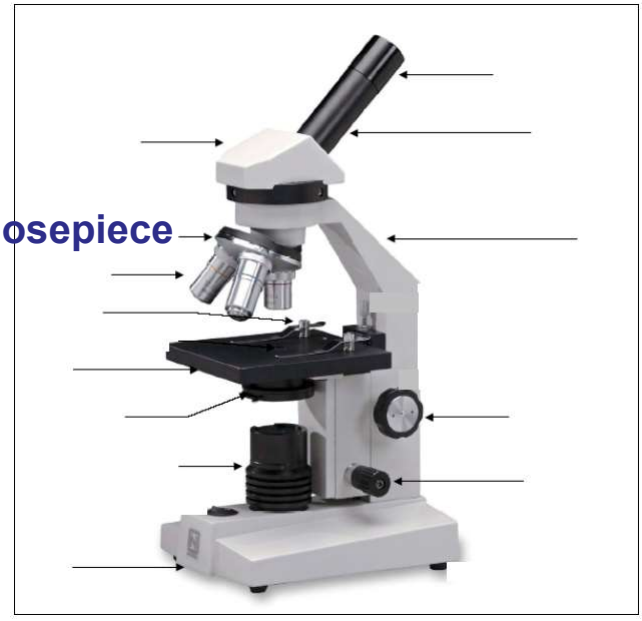
Objective Lenses



Objective Lenses

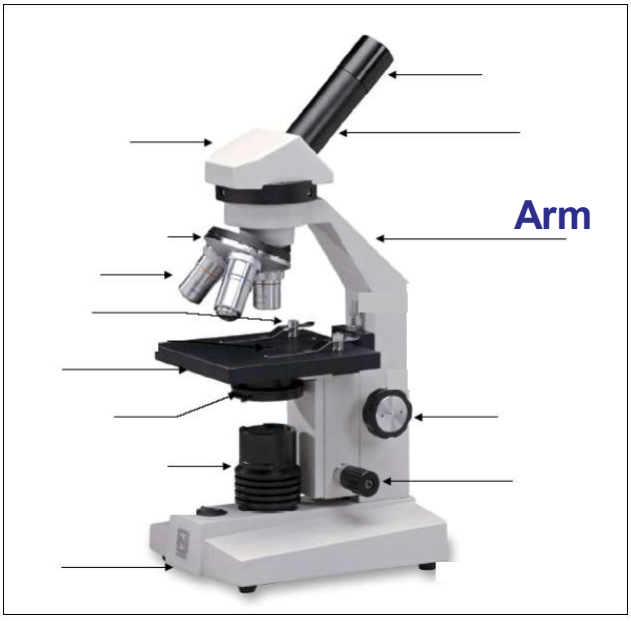
- Used in combination with the eyepiece to provide a range of magnification
- Magnification ranges from 40x to 400x
- Located on the nose-piece at the bottom of the body tube





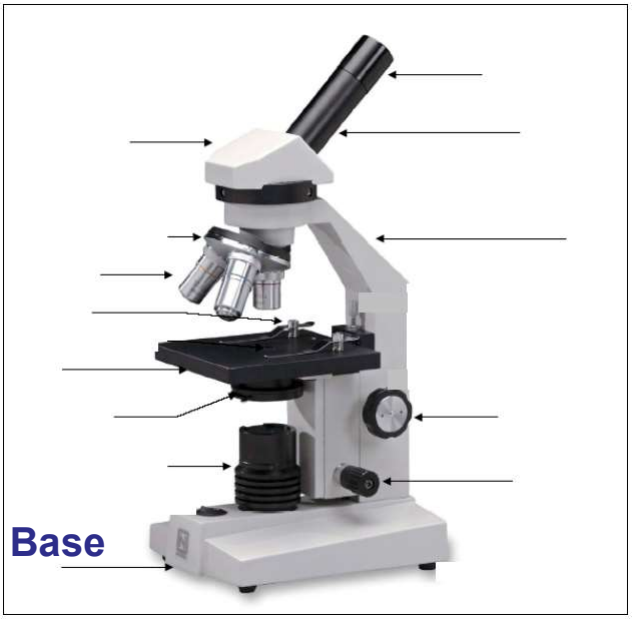
Nosepiece

- Holds the objective lenses
- Rotates to enable magnification
- Located at the bottom of the body tube



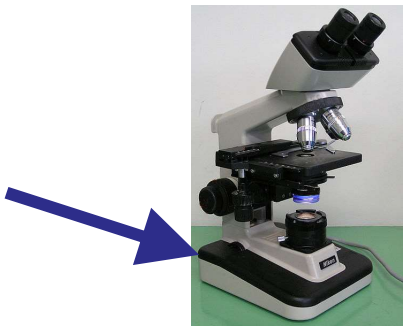
Arm

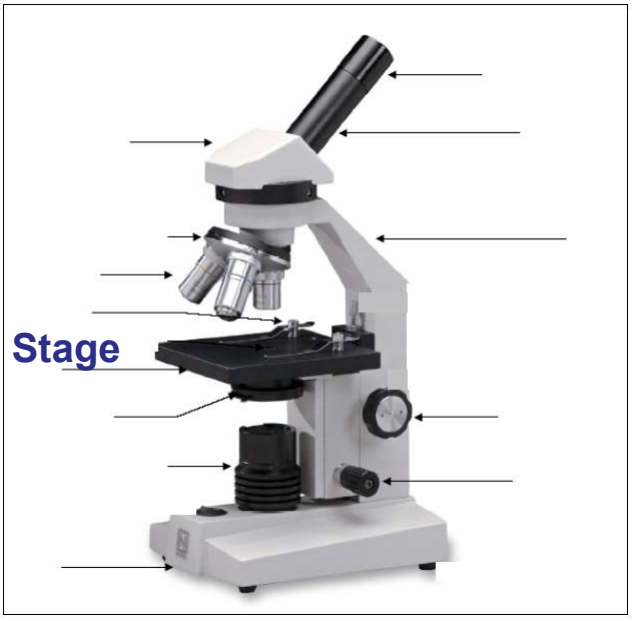
- Supports the upper parts of the microscope
- Used to carry the microscope
- When carrying a microscope, always have one hand on the arm and one hand on the base. **Use two hands!!**



Base

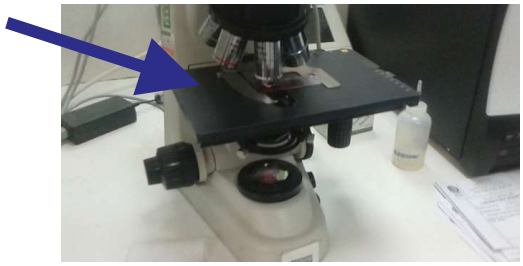
- Supports the whole microscope
- Used to carry the microscope
- When carrying a microscope, always have one hand on the arm and one hand on the base. **Use two hands!!**

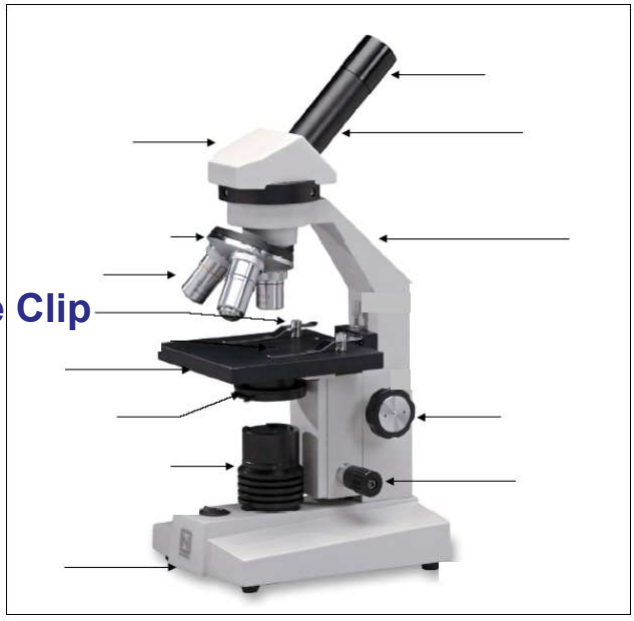




Stage

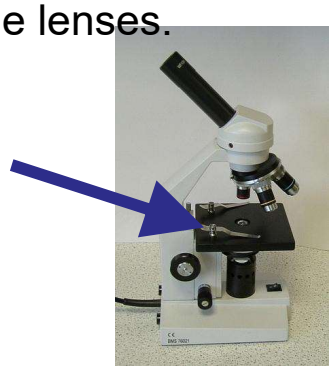
- Supports the slide
- The slide contains the specimen or object that you are viewing with the microscope.





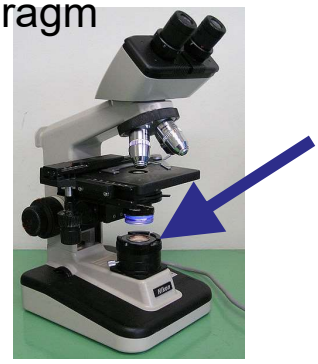
Stage Clip

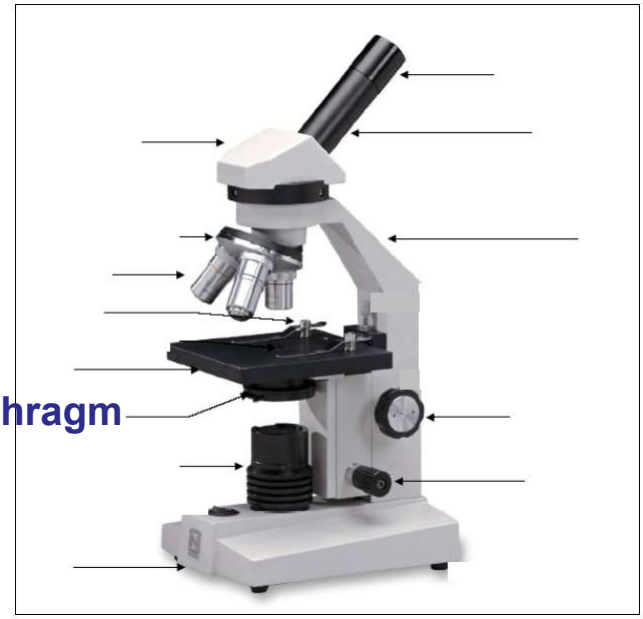
- Helps to hold the slide in place
- Usually one on each side of the hole (stage opening) = 2 stage clips
- The stage opening allows light to pass from the light source to the lenses.



Light Source

- Provides light necessary for viewing the specimen
- Usually either a mirror or illuminator
- Sends light through the stage opening to the diaphragm



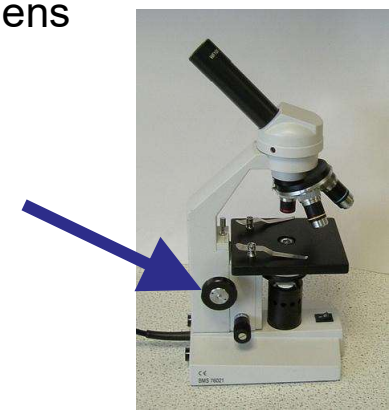


Diaphragm

- Wheel or lever located below the stage opening
- Regulates the amount of light that can enter the lenses
- May need to be adjusted based on the thickness of the specimen being studied

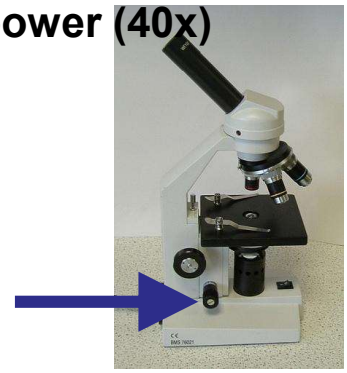
Coarse Adjustment Knob

- Raises and lowers the stage or objective lenses
- Used only when focusing the **low power (4x)** objective lens

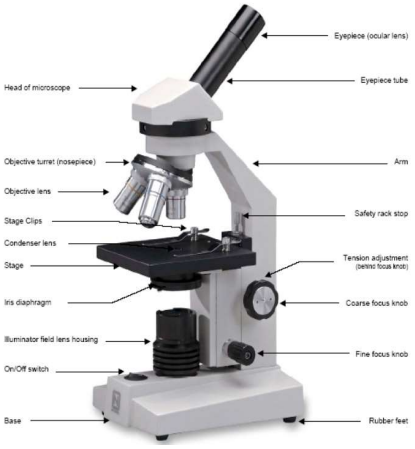


Fine Adjustment Knob

- Raises and lowers the stage or objective lenses a small distance for exact focusing
- Used when focusing the **medium power (10x)** and **high power (40x)** objective lenses

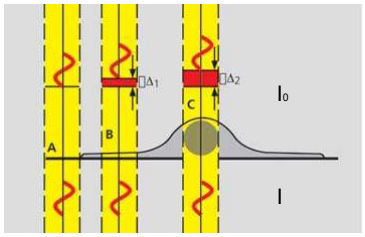


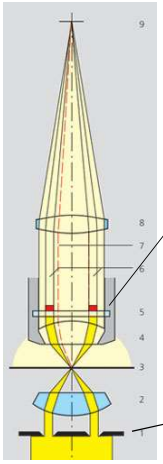
Let's Review...



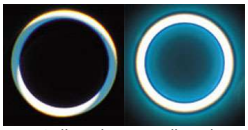
Phase contrast

Principle: Incident light [Io] is out of phase with transmitted light [I] as it was slowed down while passing through different parts of the sample and when the phases of the light are synchronized by an interference lens, a new image with greater contrast is seen.





Phase ring

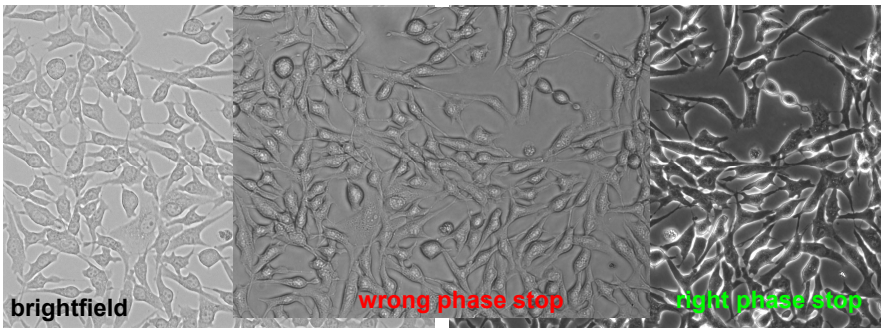


Phase stops

https://www.youtube.com/watch?v=fCZw4X7V5Pw

Phase contrast

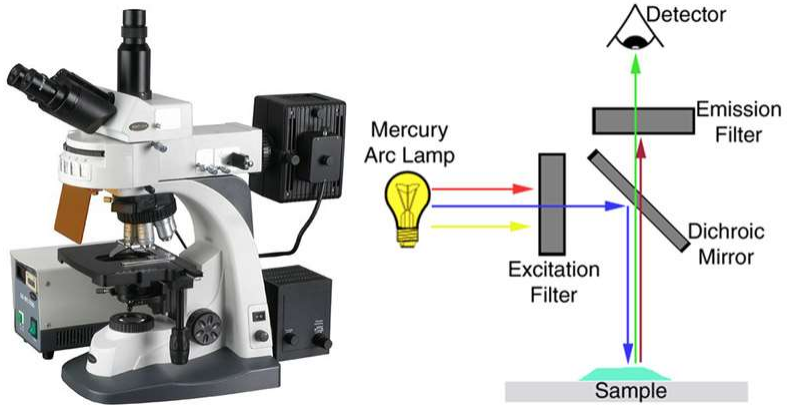
Application: Phase contrast is the most commonly used contrasting technique All tissue culture microscopes and the time-lapse microscopes are set up for phase.



Applications

- Determine morphologies of living cells such as plant and animal cells
- Studying microbial motility and structures of locomotion
- To detect certain microbial elements such as the bacterial endospores

Fluorescence



https://www.youtube.com/watch?v=SfzmW7EMdLE

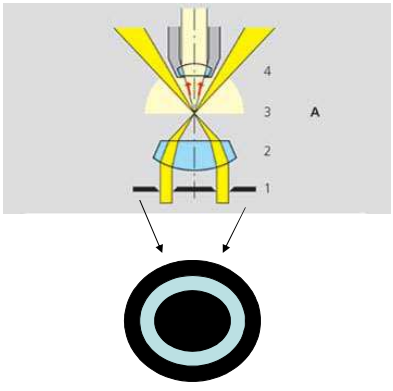
Applications

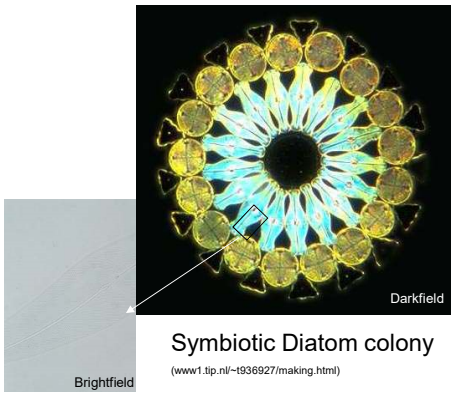
- Used in the visualization of bacterial agents such as *Mycobacterium tuberculosis.*
- Used to identify specific antibodies produced against bacterial antigens/pathogens in immunofluorescence techniques by labeling the antibodies with fluorochromes.
- Used in ecological studies to identify and observe microorganisms labeled by the fluorochromes
- It can also be used to differentiate between dead and live bacteria by the color they emit when treated with special stains

Darkfield

Principle: The illuminating rays of light are directed through the sample from the side by putting a dark disk into the condenser that hinders the main light beam to enter the objective. Only light that is scattered by structures in the sample enters the objective.

Application: People use it a lot to look at Diatoms and other unstained/colourless specimens





Symbiotic Diatom colony

(www1.tip.nl/~t936927/making.html)

Applications

- It is used to visualize the internal organs of larger cells such as the eukaryotic cells
- Identification of bacterial cells with distinctive shapes such as *Treponema pallidum*, a causative agent of syphilis.