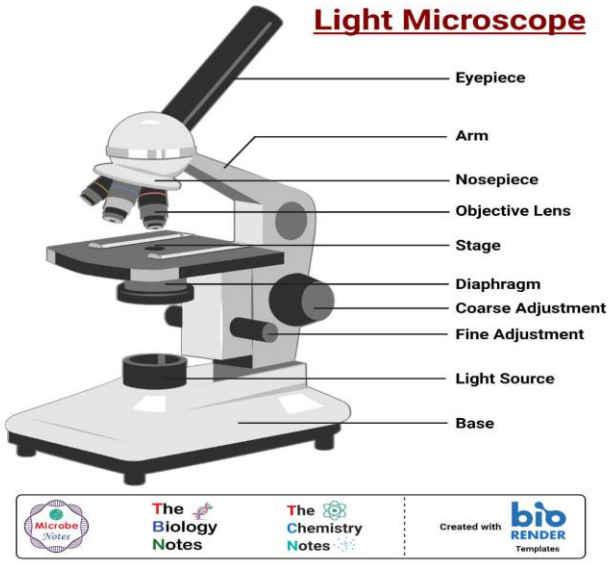
Microscopy



https://microbenotes.com/light-microscope/

Light microscopy in which magnifications is obtained by a system of optical lenses using light waves.

Microscope contains a single lens mounted in a metal frame which is the simple form of microscope- a magnifying lens.

Light microscope always uses sun or ambient indoor light as a source of illuminations.

Because of the traveling of light through the specimens, this instrument is also called as transmission light microscope.

The light microscope obtains a magnified image of specimen which is based on the principles of absorption, transmission, diffraction and refraction of light waves.
All modern light microscopes are mainly made up of more than one glass in combinations in which the major components are the eyepiece lens or ocular lens, the objective lens, and the condenser lens, and instruments of such combinations are therefore called compound microscopes

Condenser lens focuses the light at the specimen from the light source.

The one facing the object is called objective lens and the one close to the eye is called the eyepiece.

It Is objective lens that is responsible for producing magnified images.

Objective lens is available in different magnifications such as 4x, 10x, 20x, 40x, 60x, 100x.

Eyepiece have larger aperture and larger focal length than the objective lens.

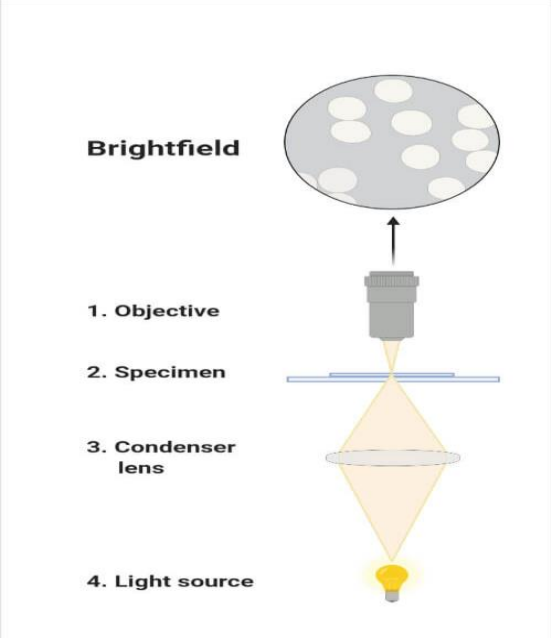
Objective lens and eyepiece both work in combination to magnify the objects.

A compound microscope with single eyepiece is termed as monocular whereas, with to eyepiece is termed as binocular.

The part of the microscope that holds all of the components firmly in position is called the stand.

There are two types of compound microscope stand- an upright or an inverted microscope.

Source is below the condenser lens and the objective lens is above the specimen stage, whereas in inverted microscopes, the condenser lens and the light source is above the specimen stage and objective lens beneath it.



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Magnification:

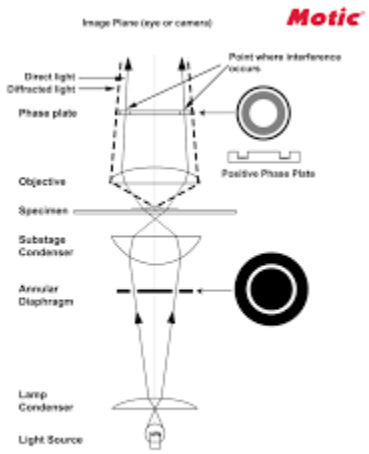
The magnification or linear magnification is defined as the ratio of the image size to the object size.
Both magnification and magnifying power is the different terms, magnifying power or angular magnification is the angle subtended by object and the image.
Magnification of objective lens is called the linear magnification, while magnification of the eyepiece is called the angular magnification. So, the total magnification of the compound microscope is the is product of both the linear magnification of the objective lens and the angular magnification of the eyepiece

Types of light microscope

Phase contrast microscope

Fluorescence microscope

Phase contrast microscope



https://moticmicroscopes.com/blogs/articles/phase-contrast-by-motic

Working way of phase contrast microscope:

This technique is generally based on the fact that light passing from one material and into another material of a slightly different refractive index and/or thickness will undergo in change in phase.
It is most often, used for testing cell organelle preparations for lysis and for viewing unstained cells growing in tissue culture
This method of microscopy images differences in the refractive index of cellular structures. Light which passes from the thicker parts of the cell is held up relative to the light that passes from thinner parts of the cytoplasm.

Applications of Phase Contrast Microscopy

Living cells (usually in culture),

Microorganisms,

Thin tissue slices,

Lithographic patterns,

Fibres,

Latex dispersions,

Glass fragments, and

Subcellular particles (including nuclei and other organelles).

Advantages of Phase Contrast Microscopy

Living cells can be observed in their natural state without previous fixation or labelling.

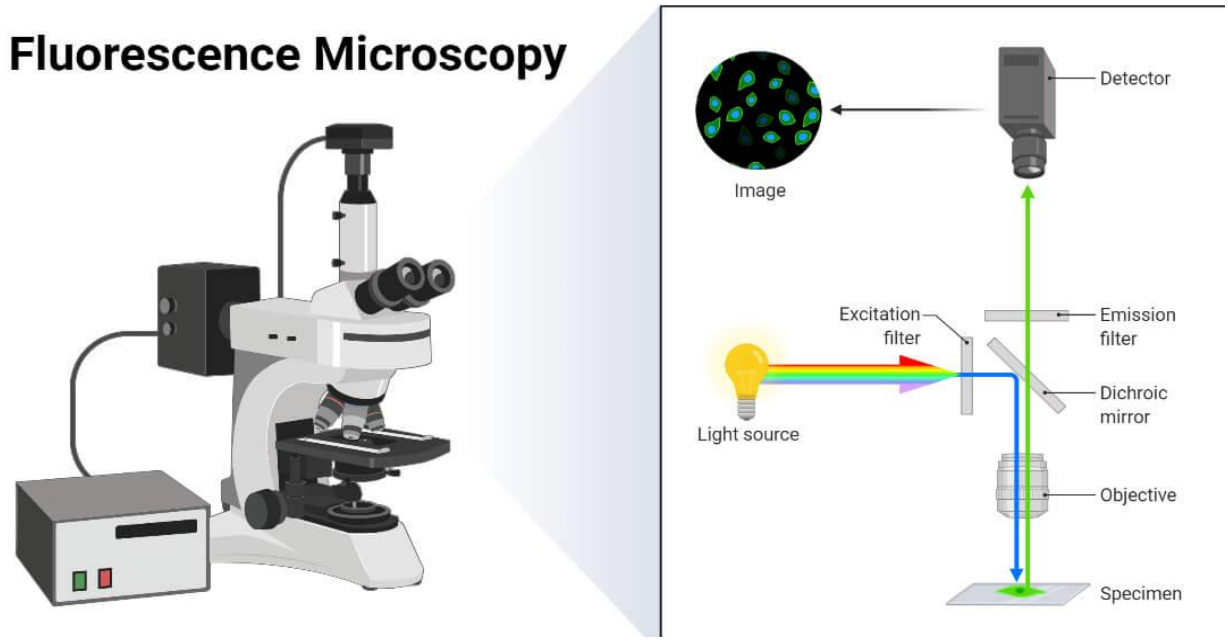
It makes a highly transparent object more visible.

No special preparation of fixation or staining etc. Is needed to study an object under a phase-contrast microscope which saves a lot of time.

Examining intracellular components of living cells at relatively high resolution. Eg: The dynamic motility of mitochondria, mitotic chromosomes & vacuoles.

Fluorescence microscope

In this the specimens itself acts as a light source, the specimens used to study are either fluorescent materials or stained with fluorescent dye.
The fluorescence microscope is most often similar to the ordinary microscope except that the illuminating light is passed through two sets of filters.
Excitation filter: It filters the light before reaches the specimen.
Barrier or emission filter: It filters the light illuminated from the specimen.
The excitation filter passes only the wavelength that excites the particular fluorescent dye, while the barrier blocks out this light and passes only those wavelengths emitted when the dye fluoresces



https://microbenotes.com/fluorescence-microscope-principle-instrumentation-applications-advantages-limitations/

Principle of Fluorescence Microscope

Fluorescent dyes, also known as fluorophores or fluorochromes, are molecules that absorb excitation light at a given wavelength (generally UV), and after a short delay emit light at a longer wavelength. The delay between absorption and emission is negligible, generally on the order of nanoseconds.

Fluorescence microscopy uses a much higher intensity light to illuminate the sample. This light excites fluorescence species in the sample, which then emits light of a longer wavelength.

The Image produced is based on the second light source or the emission wavelength of the fluorescent species rather than from the light originally used to illuminate, and excite, the sample.

Applications of Fluorescence Microscope

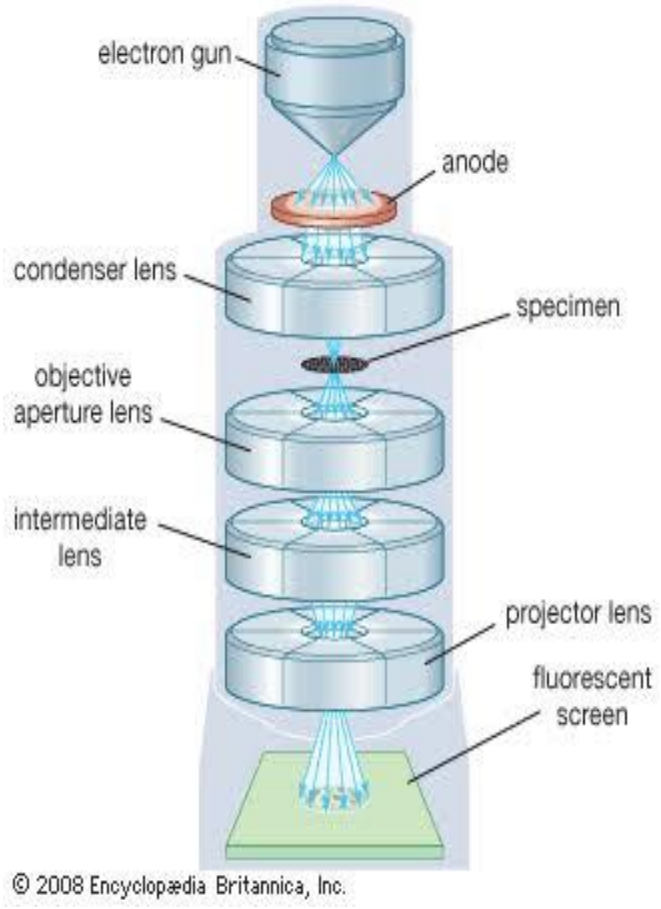
To identify structures in fixed and live biological samples.
Fluorescence microscopy is a common tool for today's life science research because it allows the use of multicolour staining, labelling of structures within cells, and the measurement of the physiological state of a cell.

Electron microscope

Working Principle of Electron microscope

- The electron gun generates electrons.
- Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
- To move electrons down the column, an accelerating voltage (mostly between 100 kV-1000 kV) is applied between the tungsten filament and anode.
- The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.
- The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
- The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
- The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
- The ocular lenses then produce the final further magnified image.

Transmission Electron Microscope (TEM)



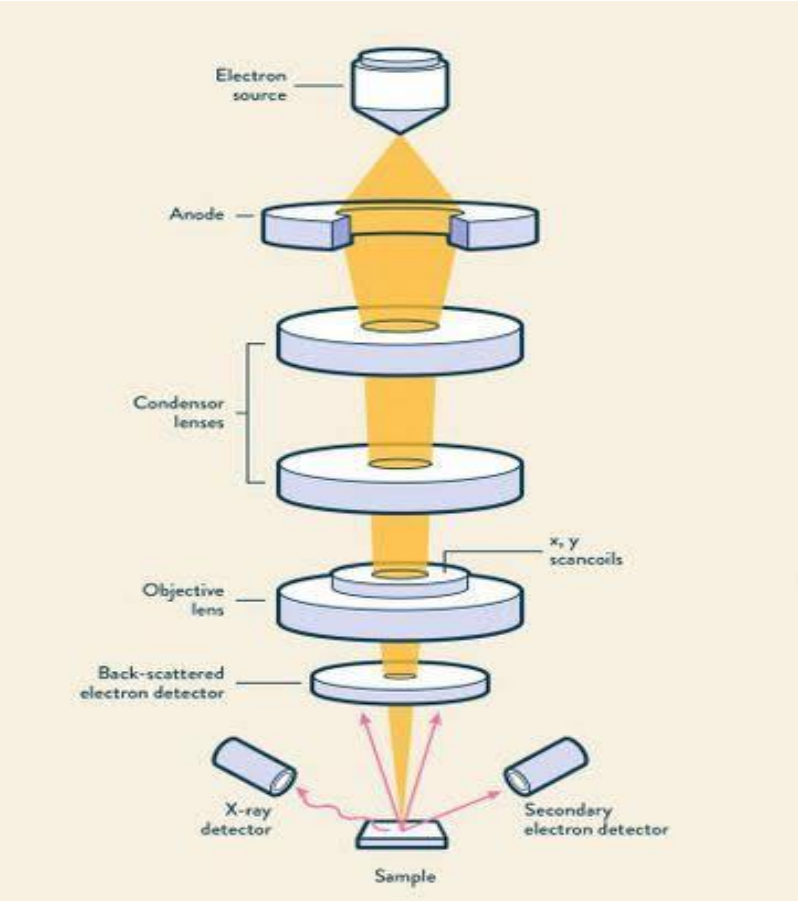
https://www.britannica.com/technology/transmission-electron-microscope

- The transmission electron microscope is used to view thin specimens through which electrons can pass generating a projection image. TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture).

Scanning Electron Microscope (SEM)

- the SEM, electrons which are reflected back from the specimen (secondary electrons) are collected, and the surfaces of specimens are then imaged.
- To produce an image, the Scanning electron microscope (SEM) scans a narrow, tapered beam of electrons back and forth over the specimen.

- When this beam strikes a particular area of the specimen, surface atoms discharge a tiny shower of electrons called secondary electrons, and these are trapped by a special detector.
- Secondary electrons then entering in the detector strike a scintillator which causing it to emit light flashes that a photomultiplier converts to an electrical current and amplifies.
- Then, the signal is sent to a cathode-ray tube and produces an image like a television picture, that can be viewed or photographed. The number of secondary electrons that reaches the detector depends on the nature of the specimen's surface.
- When the beam of electrons strikes a raised area of specimen, a large number of secondary electrons enter into the detector; in contrast, some of the electrons escape a depression in the surface and reach the detector. Thus, the raised areas of specimen are appearing lighter on the screen and depressions are darker.
- A realistic 3D image of the microorganism's surface or specimen results. The actual in situ location of microorganisms in ecological niches such as the lining of the gut and the human skin and can be examined.



https://www.britannica.com/technology/scanning-electron-microscope

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