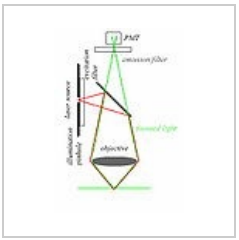
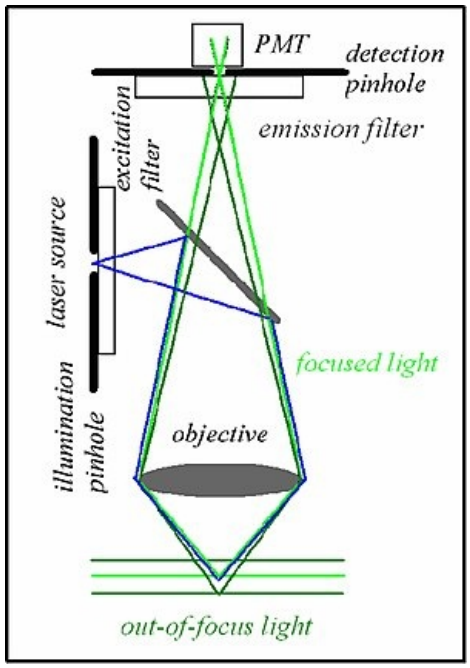
Optical microscopy





Optical microscopy

Optical microscope system

An optical (or light) microscope is a device that we use to view objects that we can not spot by a naked eye. Those are objects that are less than 0.2 mm in size. Its distinctiveness is 0.2 mm - 0.2 µm. In general, we could say that where ends the distinctiveness of an eye, starts a distinctiveness of a optical microscope and where the distinctiveness of a optical microscope ends, starts a distinctiveness of an electron microscope.

History

Already in antiquity, all kind of lenses were used to observe objects that were too small for the naked eye. The first great discovery is however dated back to the 17th century when Anton van Leeuwenhoek invented a device consisting of a screw, a metal plate, grooves for fixing the object and a spherical lens. This device could not in every possible way match today's microscopes, but it is admirable that it achieved an awesome magnification, up to 250-500x. Robert Hook came to the next breakthrough, when he started to work with light in microscopy. Thanks to need for this technology, the microscope was gradually optimised to the form we know today.

Optical microscope system

The optical system of the microscope consists of three connected systems - the lens, the eyepiece and the condenser (the lighting system).

The Lens

The lens displays an object located at a distance <2f and> than f, the image is magnified, overturned and real.

The eyepiece

Using the eyepiece, we view the object bigger (as if we use magnifying glass) (the image is at a distance <f) and a definitive image y" is created.

The condenser

A condenser is a system of lenses between a light source and a preparation to ensure its proper illumination. We can also call it a lighting system that serves to illuminate the subject into the plane.

Magnification of the microscope

The maximum magnification of the optical microscope can not exceed 2000. Greater magnification is considered to be blank because the object is larger but we can not distinguish the details and the object is blurry.

Transverse lens enlargement:

Δ = lens distance and eyepiece distance (optical range), f_{ob} = focal length of lens

$$Z = Z_{ob} \cdot Z_{ok} = \frac{\Delta}{f_{ob}} \cdot \frac{250}{f_{ok}}$$

Numerical aperture:

n = Refractive index of the medium between the preparation and the lens, u = 1/2 of the angle at which the beams from the preparation enter the lens

Microscope resolution:

$$A = n \cdot sin\alpha$$

Distinguishing ability is the ability of the microscope to distinguish two points apart. In general, we denote it by the letter R and can be calculated from the formula R = 1 / dmin (where dmin is the resolution limit, which represents the minimum distance of two points that we are able to distinguish from each other).

$$a = 0,61 \cdot \frac{\lambda}{A} = \frac{0,61 \cdot \lambda}{n \cdot \sin\alpha}$$

where λ = wavelength of the light used

This topic involves following sub-topics:

- Resolution of human eye
- Imaging principle of optical microscope
- Construction and function of optical microscope
- Abbe’s theory
- Limit of resolution of optical microscope
- Magnification of optical microscope
- Depth of sharpness of optical microscope
- Contrast of optical microscope
- Microscopic techniques:
  - Transmitted light microscopy
  - Reflected light microscopy
  - Phase microscopy
  - Inverse microscope
  - Polarizing microscopy
  - Interference microscopy