Microscopy

Introduction

main branches: optical, electron and scanning probe microscopy. (+ less used X-ray microscopy)
Optical and electron microscopy involves the diffraction, reflection, or refraction of radiation incident upon the subject of study, and the subsequent collection of this scattered radiation in order to build up an image.
Scanning probe microscopy involves the interaction of a scanning probe with the surface or object of interest.

Optical microscopy

Definition

Optical or light microscopy involves passing visible light transmitted through or reflected from the sample through a single or multiple lenses to allow a magnified view of the sample.

The resulting image can be detected directly by the eye, imaged on a photographic plate or captured digitally.
The single lens with its attachments, or the system of lenses and imaging equipment, along with the appropriate lighting equipment, sample stage and support, makes up the basic light microscope.

Video tutorial

Watch this three YouTube videos

Part 1 (http://www.youtube.com/profile?user=microscopyokiminfo#p/u/2/L6d3zD2LtSI)

Part 2 (http://www.youtube.com/profile?user=microscopyokiminfo#p/u/1/-A9tVEsTi9w)

Part 3 (http://www.youtube.com/profile?user=microscopyokiminfo#p/u/0/XbLD2zJDEzs)

Optical microscopy - limitations

OM can only image dark or strongly refracting objects effectively.
Out of focus light from points outside the focal plane reduces image clarity. Compound optical microscopes are limited in their ability to resolve fine details by the properties of light and the refractive materials used to manufacture lenses. A lens magnifies by bending light. Optical microscopes are restricted in their ability to resolve features by a phenomenon called diffraction which, based on the numerical aperture AN of the optical system and the wavelengths of light used (λ), sets a definite limit (d) to the optical resolution. Assuming that optical aberrations are negligible, the resolution (d) is given by:

$$d = \frac{\lambda}{2A_N}$$

In case of λ = 550 nm (green light), with air as medium, the highest practical AN is 0.95, with oil, up to 1.5.

Due to diffraction, even the best optical microscope is limited to a resolution of around 0.2 micrometres.

Optical microscopy - types

- Optical microscopy techniques
- Bright field optical microscopy
- Oblique illumination
- Dark field optical microscopy
- Phase contrast optical microscopy
- Differential interference contrast microscopy
- Fluorescence microscopy
- Confocal laser scanning microscopy
- Deconvolution microscopy
- Near-field Scanning OM

Electron Microscopy

Definition and types

developed in the 1930s that use electron beams instead of light.
because of the much lower wavelength of the electron beam than of light, resolution is far higher.
TYPES
Transmission electron microscopy (TEM) is principally quite similar to the compound light microscope, by sending an electron beam through a very thin slice of the specimen. The resolution limit (in 2005) is around 0.05 nanometer.

Scanning electron microscopy (SEM) visualizes details on the surfaces of cells and particles and gives a very nice 3D view. The magnification is in the lower range than that of the transmission electron microscope.

Transmission Electron Microscopy (TEM)

beam of electrons is transmitted through a specimen, then an image is formed, magnified and directed to appear either on a fluorescent screen or layer of photographic film or to be detected by a sensor (e.g. charge-coupled device, CCD camera.
involves a high voltage electron beam emitted by a cathode, usually a tungsten filament and focused by electrostatic and electromagnetic lenses.
electron beam that has been transmitted through a specimen that is in part transparent to electrons carries information about the inner structure of the specimen in the electron beam that reaches the imaging system of the microscope.
spatial variation in this information (the "image") is then magnified by a series of electromagnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or CCD camera. The image detected by the CCD may be displayed in real time on a monitor or computer.

Scanning Electron Microscopy (SEM)

type of electron microscope capable of producing high-resolution images of a sample surface.
due to the manner in which the image is created, SEM images have a characteristic 3D appearance and are useful for judging the surface structure of the sample.

Resolution
depends on the size of the electron spot, which in turn depends on the magnetic electron-optical system which produces the scanning beam.
is not high enough to image individual atoms, as is possible in the TEM … so that, it is 1-20 nm

X-ray microscopy

less common,
developed since the late 1940s,
resolution of X-ray microscopy lies between that of light microscopy and the electron microscopy.
X-rays are a form of electromagnetic radiation with a wavelength in the range of 10 to 0.01 nanometers, corresponding to frequencies in the range 30 PHz to 30 EHz.