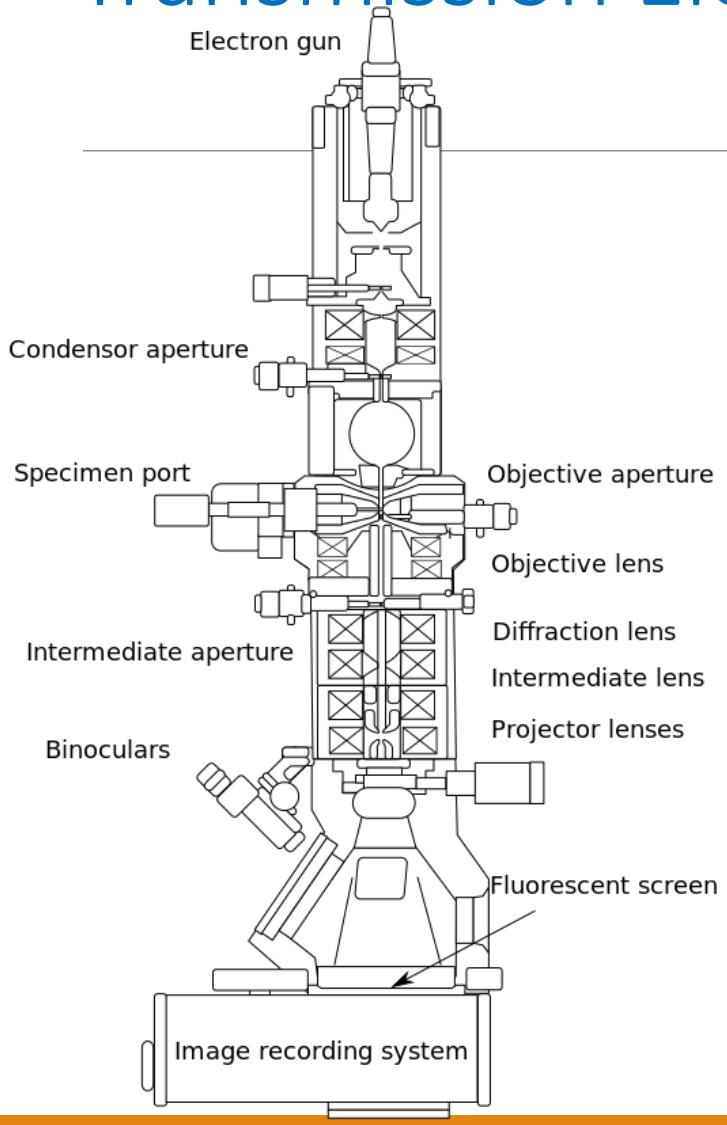
Biological Techniques

DR. A. VINOTH

DEPT. OF BOTANY

ST. XAVIER'S COLLEGE

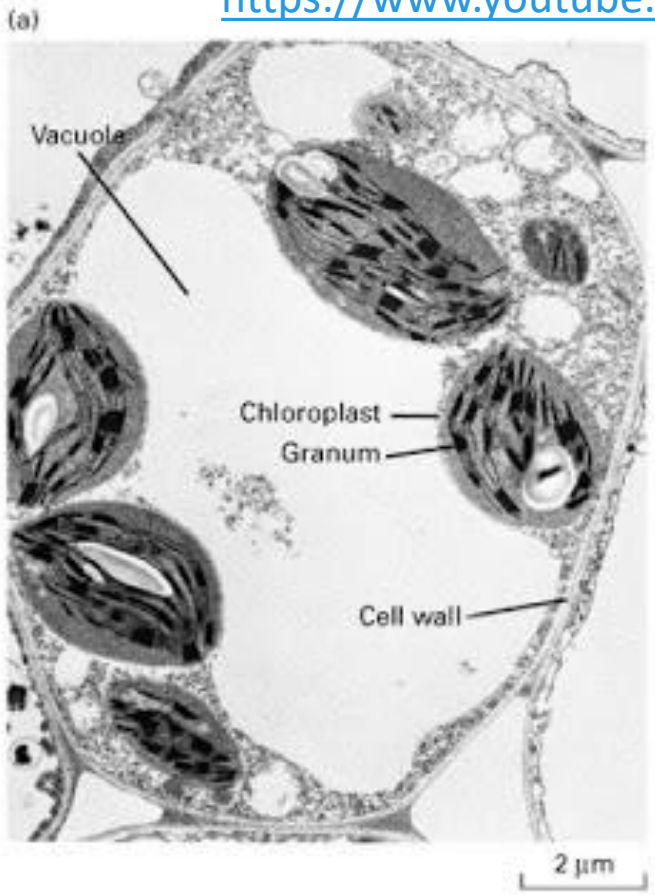
Transmission Electron Microscope

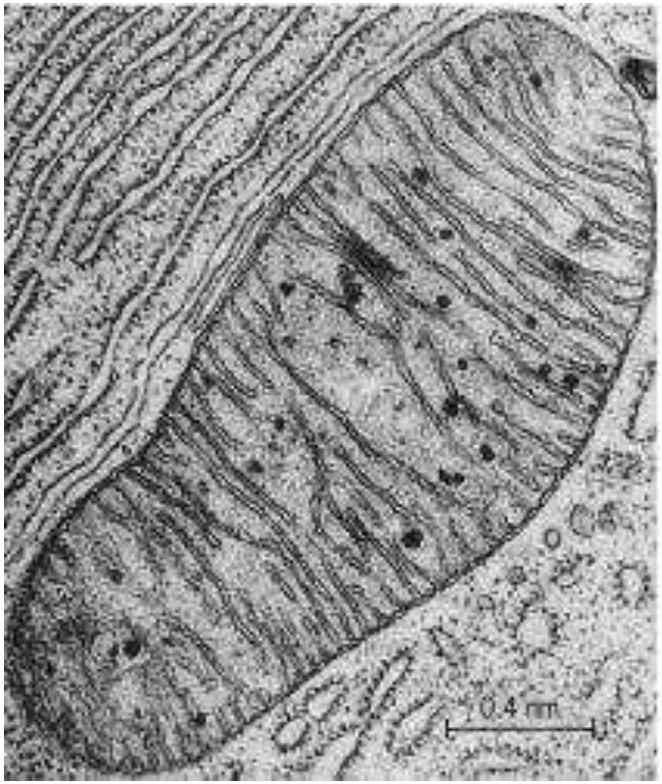


https://www.youtube.com/watch?v=fQJYuTpK8Fs

Transmission Electron Microscope

https://www.youtube.com/watch?v=6CY4Zcw8qIY

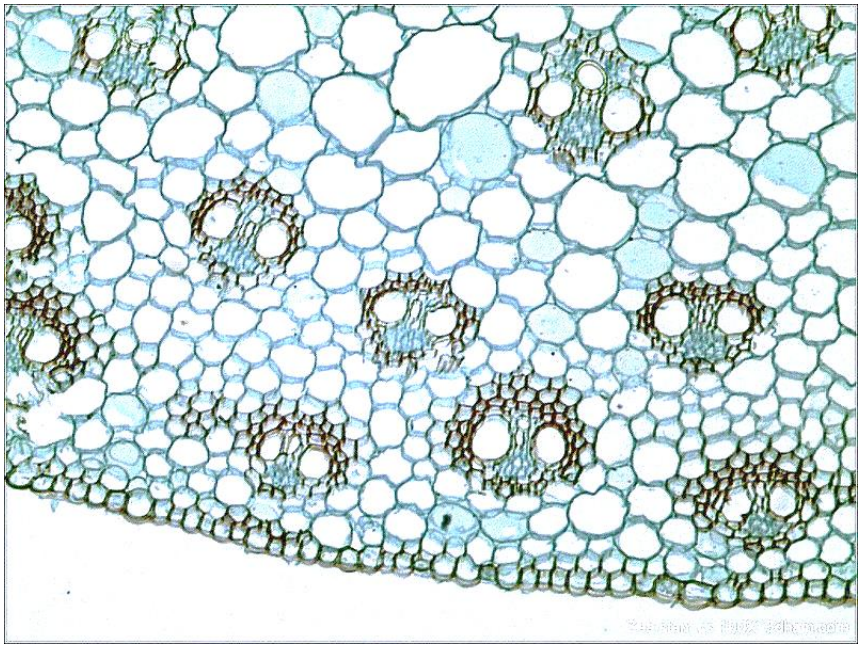




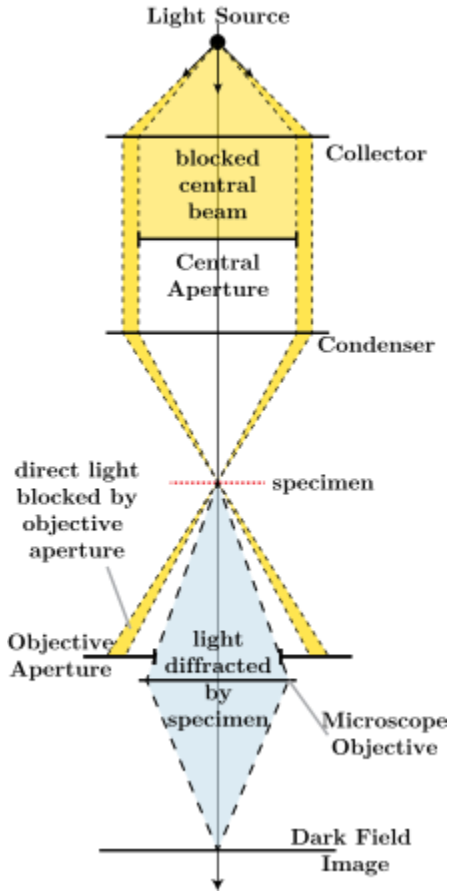
https://www.youtube.com/watch?v=eSKTFXv5rdI

Bright field microscope

- Simplest of all the optical microscopy illumination techniques
- Sample illumination is transmitted white light
- Contrast in the sample is caused by attenuation of the transmitted light in dense areas of the sample



Dark field microscope



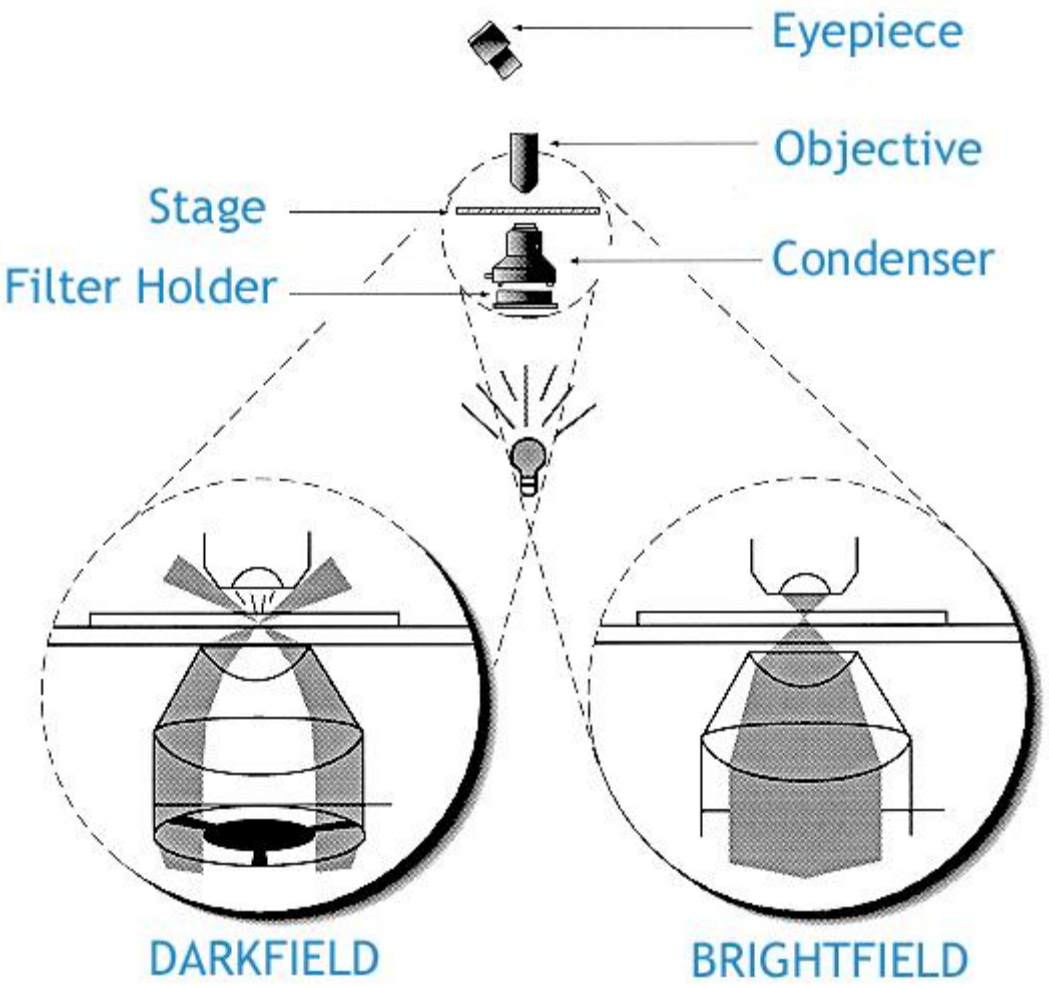
https://en.wikipedia.org/wiki/Dark-field_microscopy

Dark field microscope

- Dark-field is an illumination technique used to enhance the contrast in unstained samples.
- It works by illuminating the sample with light that will not be collected by the objective lens and thus will not form part of the image.
- This produces the classic appearance of a dark, almost black, background with bright objects on it.

Uses

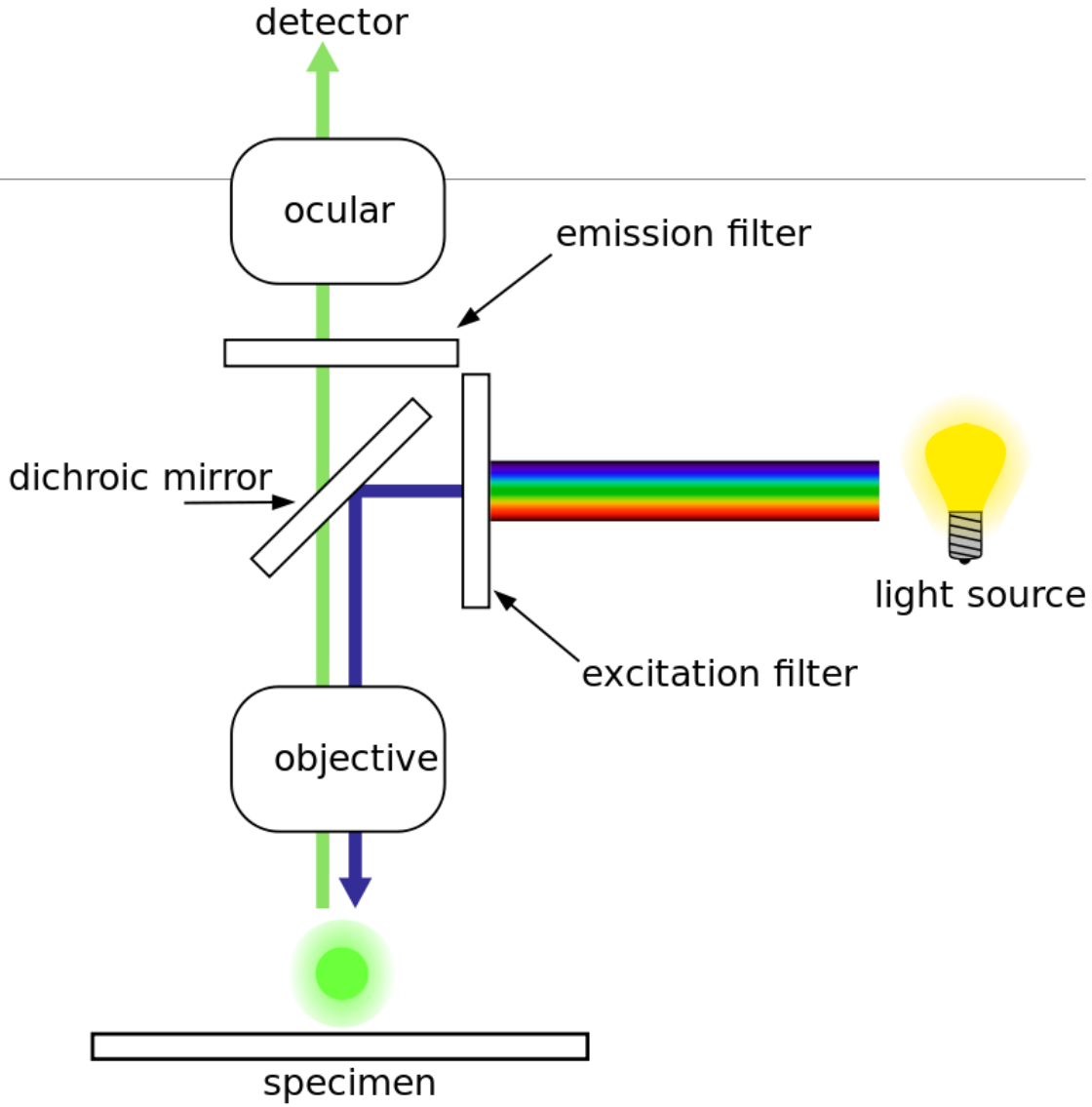
- Suitable for viewing live and unstained biological samples, such as a smear from a tissue culture or individual, water-borne, single-celled organisms.
- The main limitation of dark-field microscopy is the low light levels seen in the final image.
- Strongly illumination causes damage to the sample


Eyepiece
Objective
Stage
Condenser
Filter Holder
DARKFIELD
BRIGHTFIELD

Fluorescence microscope

- Optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances
- epifluorescence microscope
- confocal microscope

Design


detector
ocular
emission filter
dichroic mirror
light source
excitation filter
objective
specimen

Design

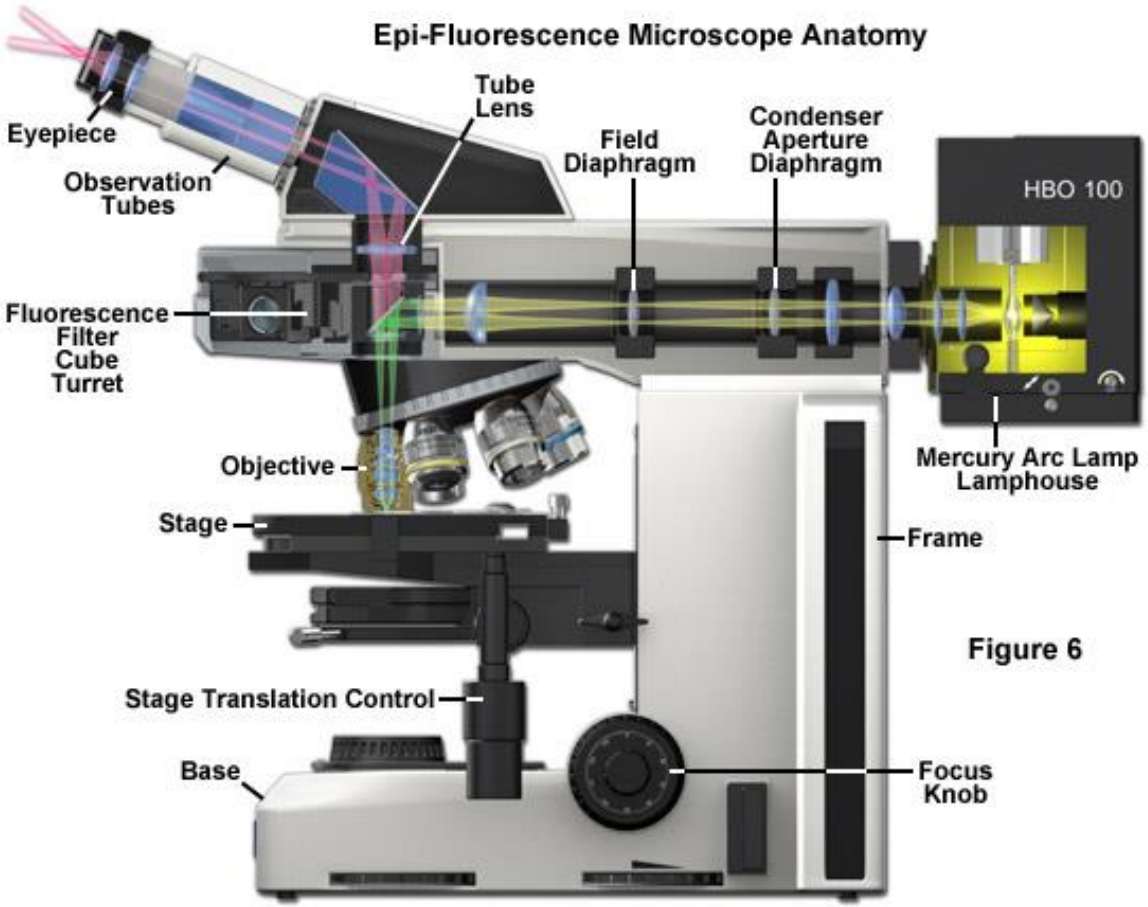


Figure 6

Principle

- Illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths
- Typical components of a fluorescence microscope are a light source (xenon arc lamp or mercury-vapor lamp are common; more advanced forms are high-power LEDs and lasers), the excitation filter, the dichroic mirror (or dichroic beamsplitter), and the emission filter
- Multi-color images of several types of fluorophores must be composed by combining several single-color images

Sample preparation

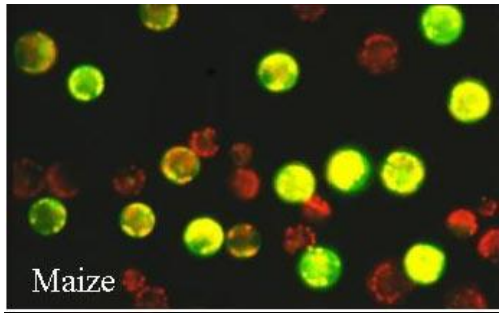
- Sample to be suitable for fluorescence microscopy it must be fluorescent

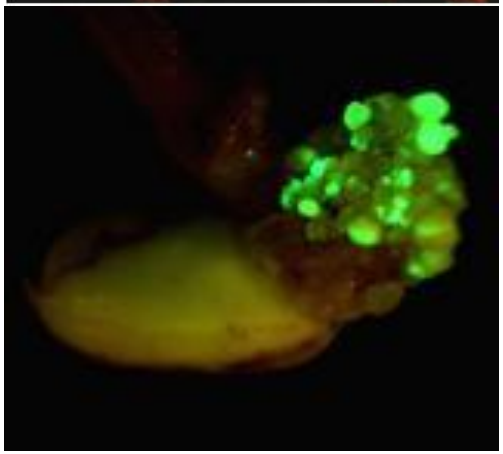
- Labelling with fluorescent stains or, in the case of biological samples, expression of a fluorescent protein.

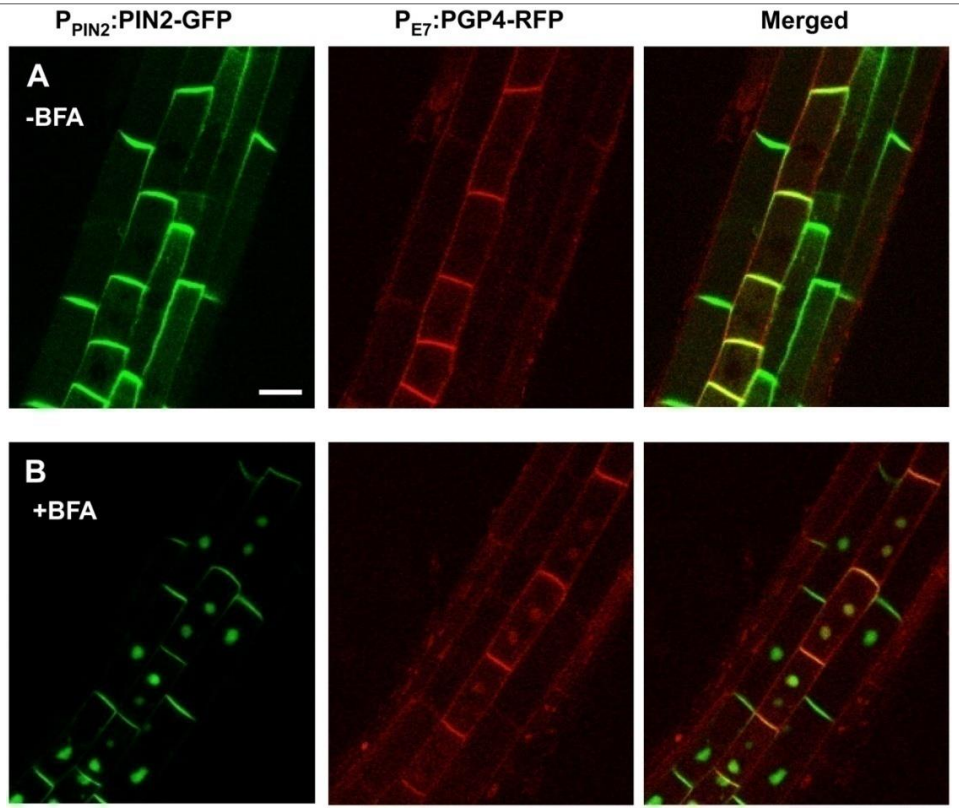
- Allows the specific and sensitive staining of a specimen in order to detect the distribution of proteins or other molecules of interest.

Fluorescein diacetate – protoplast staining

GFP, RFP, CFP, YFP







Limitations

- Fluorophores lose their ability to fluoresce as they are illuminated in a process called photobleaching.
- Fluorescent molecules have a tendency to generate reactive chemical species when under illumination which enhances the phototoxic effect.
- Fluorescence microscopy only allows observation of the specific structures which have been labeled for fluorescence.

Super-resolved fluorescence microscopy

Stefan Walter Hell

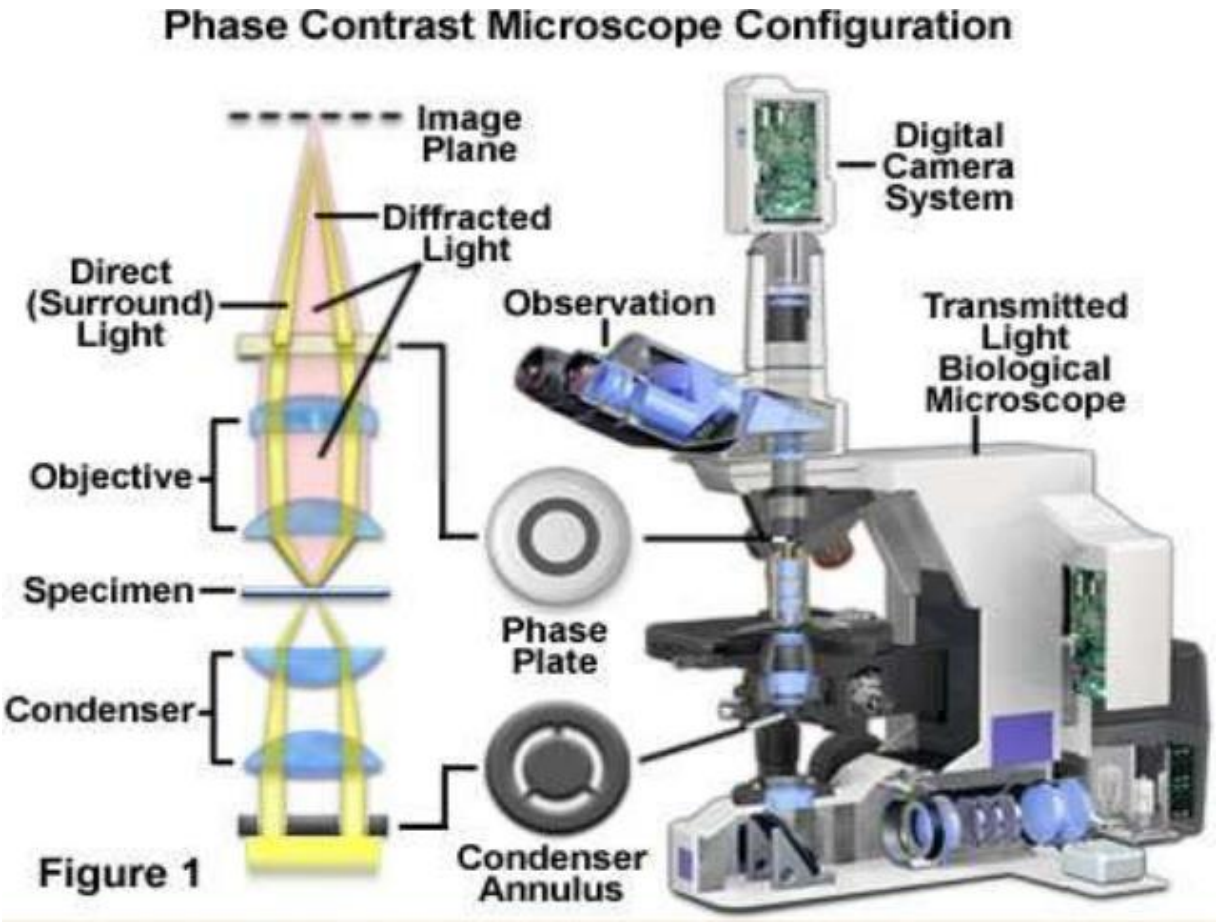
Eric Betzig

William Moerner

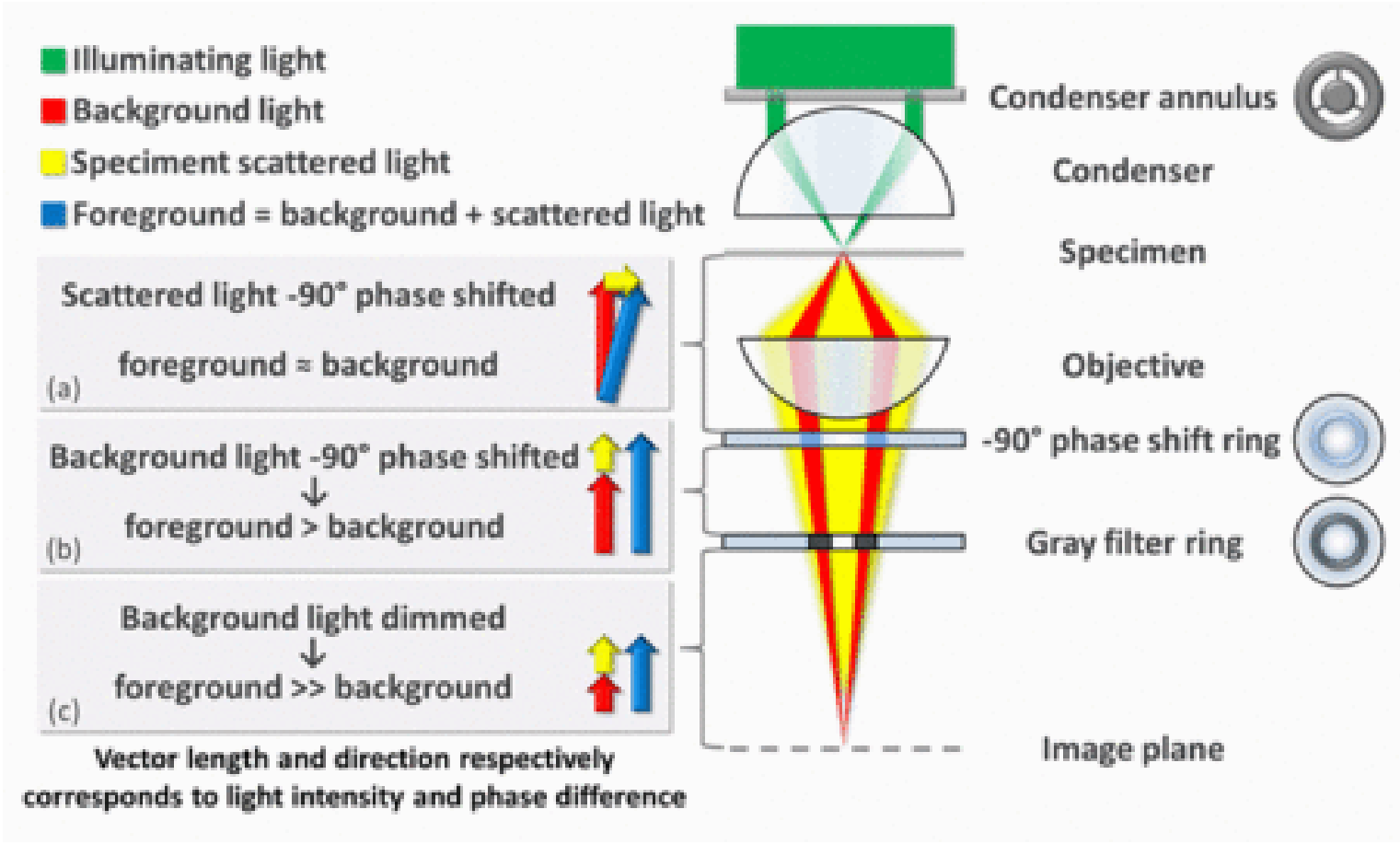
Phase-contrast microscopy

- Converts phase shifts in light passing through a transparent specimen to brightness changes in the image
- Visible when shown as brightness variations.
- Frits Zernike was awarded the Nobel prize (physics) in 1953

Design


Phase Contrast Microscope Configuration
Image Plane
Diffracted Light
Direct (Surround) Light
Objective
Specimen
Condenser
Figure 1
Digital Camera System
Observation
Transmitted Light Biological Microscope
Phase Plate
Condenser Annulus

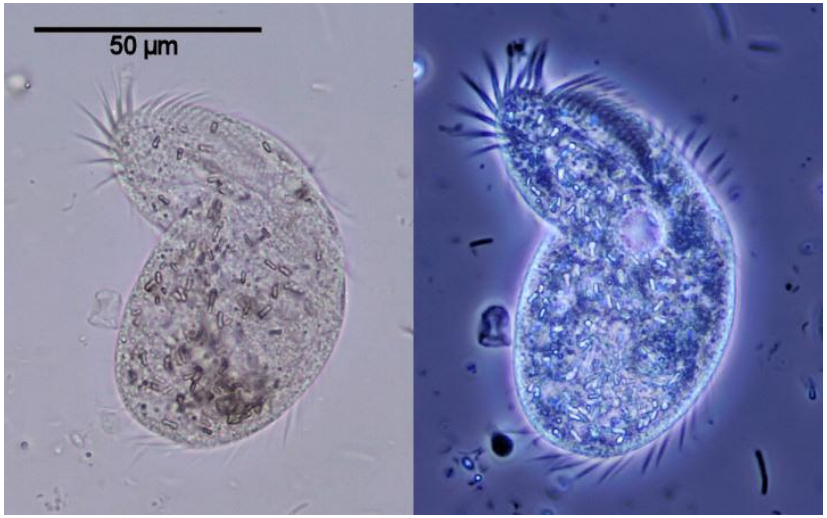
Principle

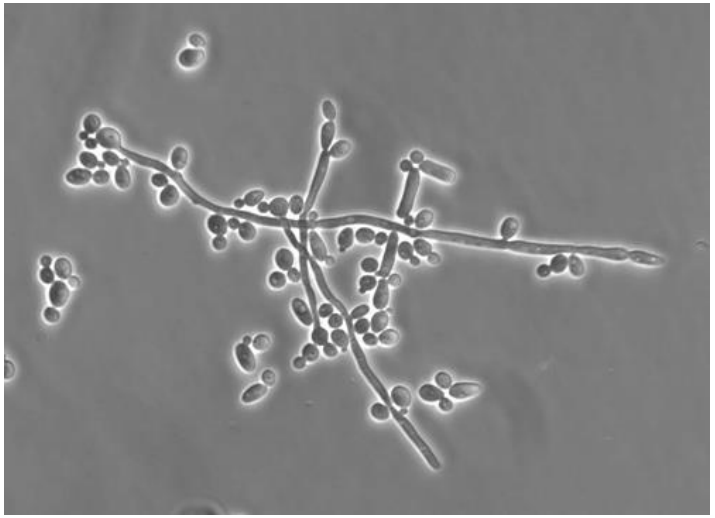

Illuminating light
Background light
Speciment scattered light
Foreground = background + scattered light
Scattered light -90° phase shifted
foreground ≈ background
(a)
Background light -90° phase shifted
foreground > background
(b)
Background light dimmed
foreground >> background
(c)
Vector length and direction respectively corresponds to light intensity and phase difference
Condenser annulus
Condenser
Specimen
Objective
-90° phase shift ring
Gray filter ring
Image plane

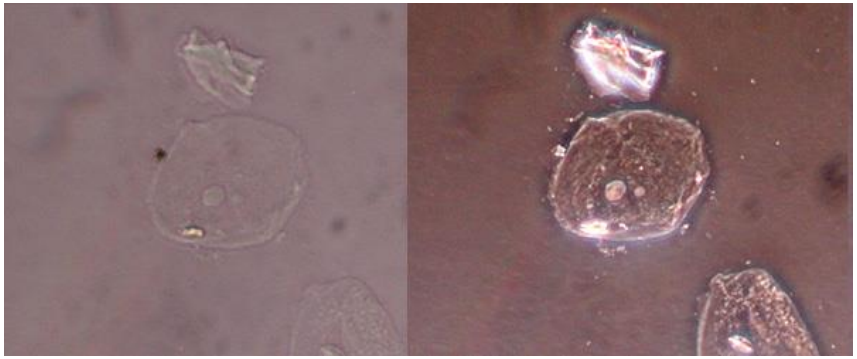
Principle

- Separate the illuminating (background) light from the specimen-scattered light.
- Image contrast is increased in two ways: by generating constructive interference between scattered and background light rays
- The background light is phase-shifted by −90° by passing it through a phase-shift ring, which eliminates the phase difference between the background and the scattered light rays.
- Reducing the amount of background light that reaches the image plane
- Constructively interfere, resulting in an increase in the brightness of these areas
- The background is dimmed ~70-90% by a gray filter ring

Phase Contrast Images





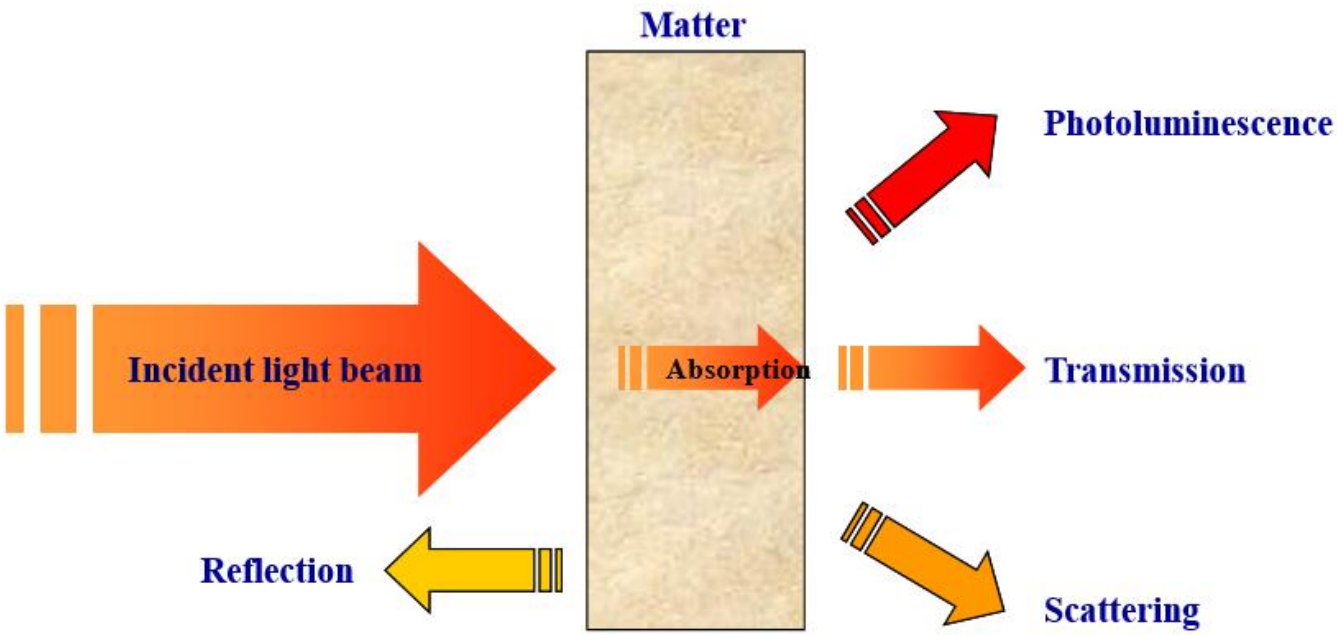


Applications

- Live cell imaging
- To study cell division
- Differential interference contrast (DIC) microscopy

Micrasterias furcata – *fresh water algae*

Spectroscopy



Principle

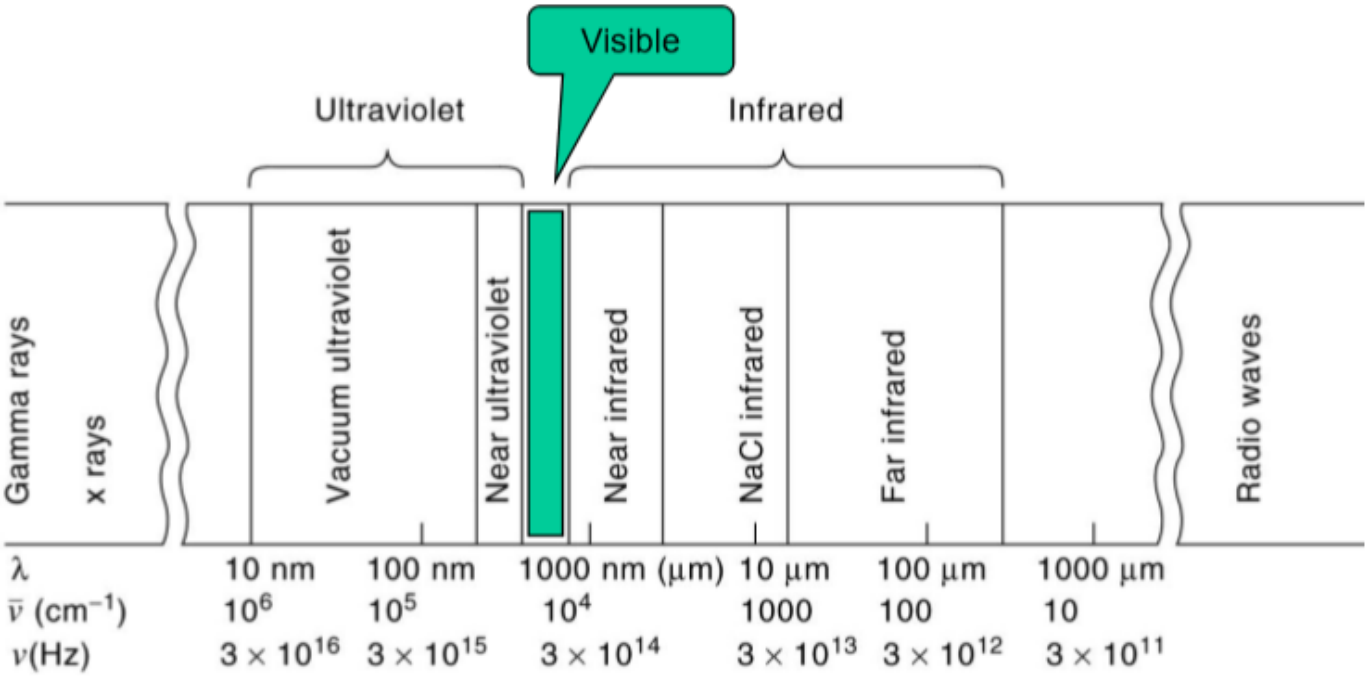
The complement of the absorbed light gets transmitted.

The color of an object we see is due to the wavelengths transmitted or reflected. Other wavelengths are absorbed.

The more absorbed, the darker the color (the more concentrated the solution).

In spectrochemical methods, we measure the absorbed radiation.

Colour chart

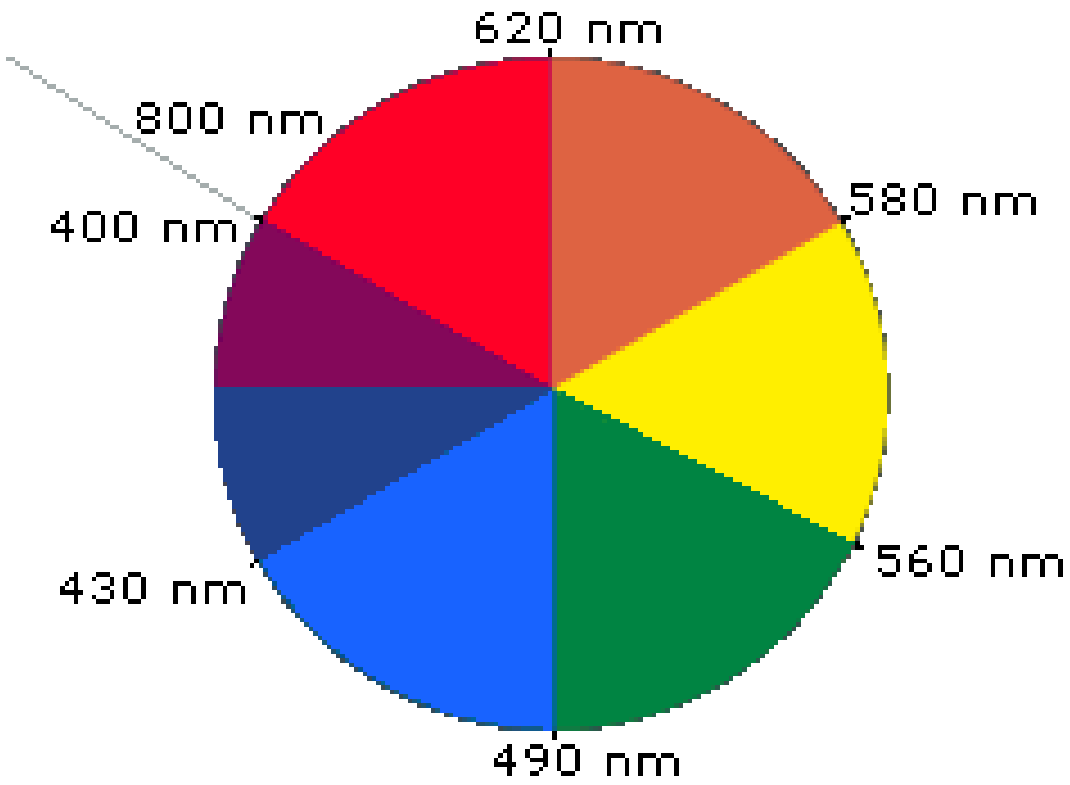


Colour chart

Colors of Different Wavelength Regions

Wavelength Absorbed (nm)	Absorbed Color	Transmitted Color (Complement)
380–450	Violet	Yellow-green
450–495	Blue	Yellow
495–570	Green	Violet
570–590	Yellow	Blue
590–620	Orange	Green-blue
620–750	Red	Blue-green

Colour chart



Principle

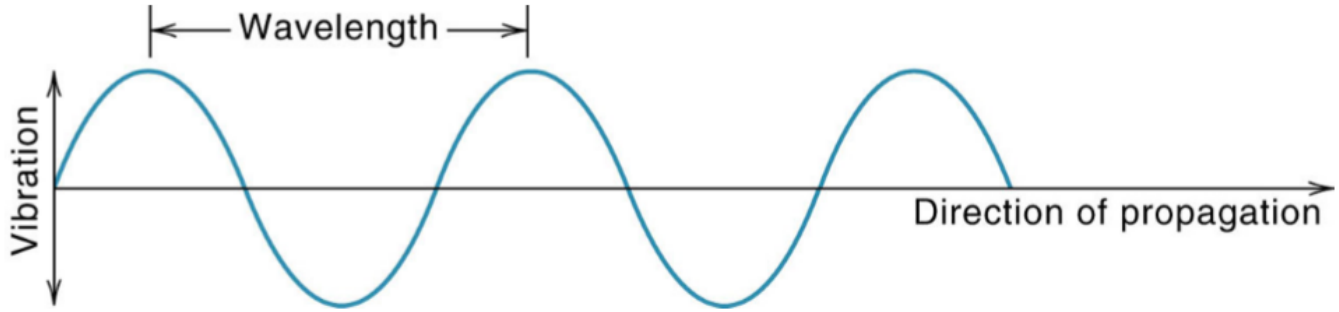
The distance of one cycle is the wavelength (λ).

The frequency (ν) is the number of cycles passing a fixed point per unit time.

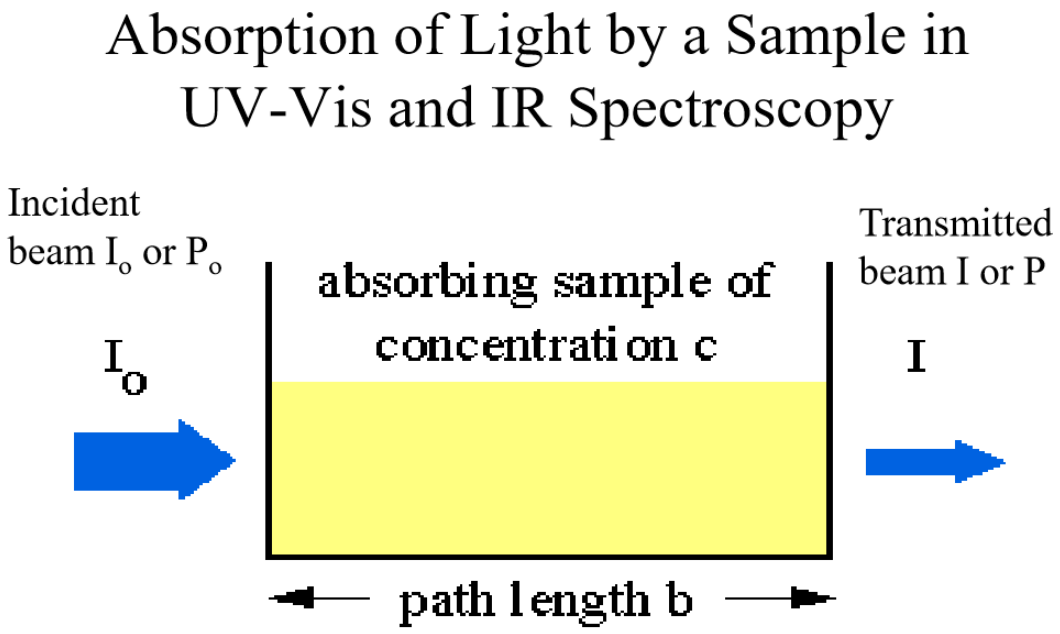
$\lambda = c/\nu$ (c = velocity of light, 3×10^{10} cm s^{-1}).

The shorter the wavelength, the higher the energy: $E = h\nu$

This is why UV radiation from the sun burns you.



Absorption


Absorption of Light by a Sample in
UV-Vis and IR Spectroscopy
Incident
beam I_o or P_o
I_o
absorbing sample of
concentration c
Transmitted
beam I or P
I
path length b

UV Visible Spectroscopy