CHARACTERIZATION AND TEST MATERIAL ENGINEERING

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METALLOGRAPHY

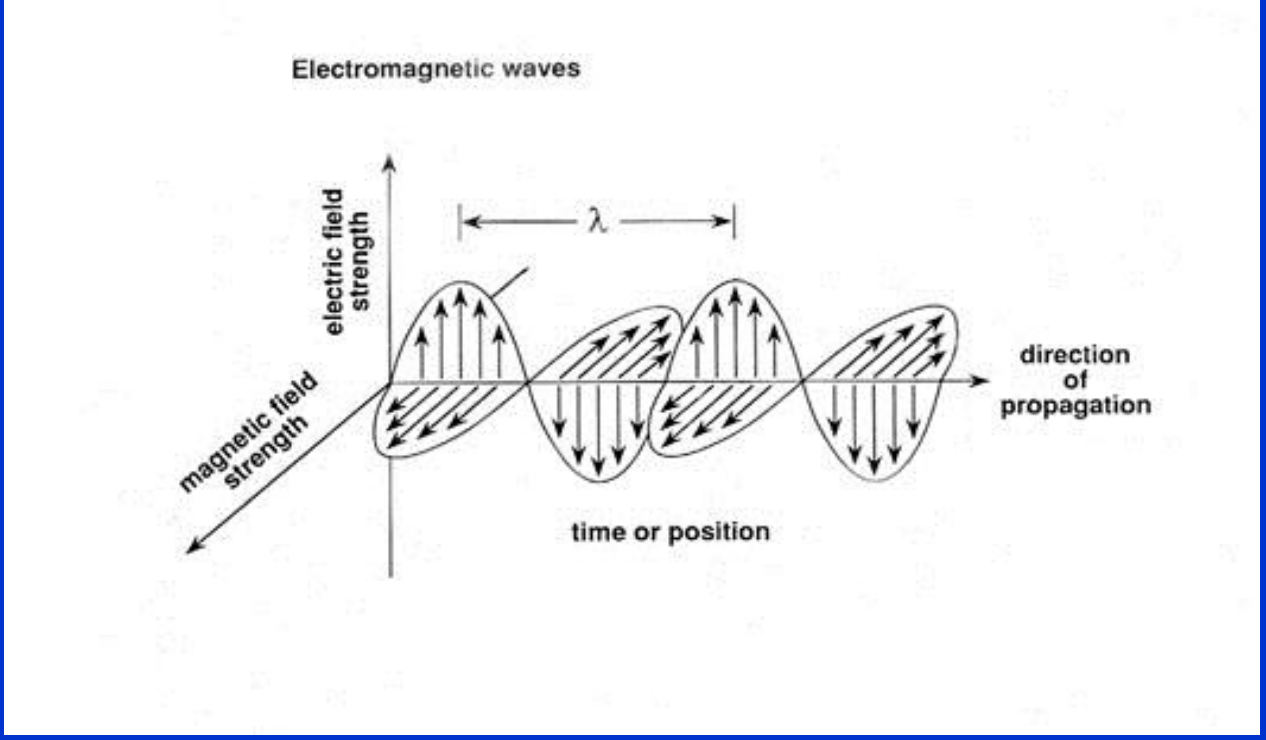
is the study of the physical structure and components of metals (is a solid material (an element, compound, or alloy) that is typically hard, shiny, and features good electrical and thermal conductivity

typically using microscopy.

Ceramic and polymeric materials may also be prepared using metallographic techniques, hence the terms (**Ceramography):** is the art and science of preparation, examination and evaluation of ceramic microstructures. Ceramography can be thought of as the Metallography of ceramics.

The microstructure is the structure level of approximately 0.1 to 100 μm, between the minimum wavelength of visible light and the resolution limit of the naked eye The microstructure includes most grains, secondary phases, grain boundaries, pores, micro-cracks and hardness microindentions. Most bulk mechanical, optical, thermal, electrical and magnetic properties are significantly affected by the microstructure

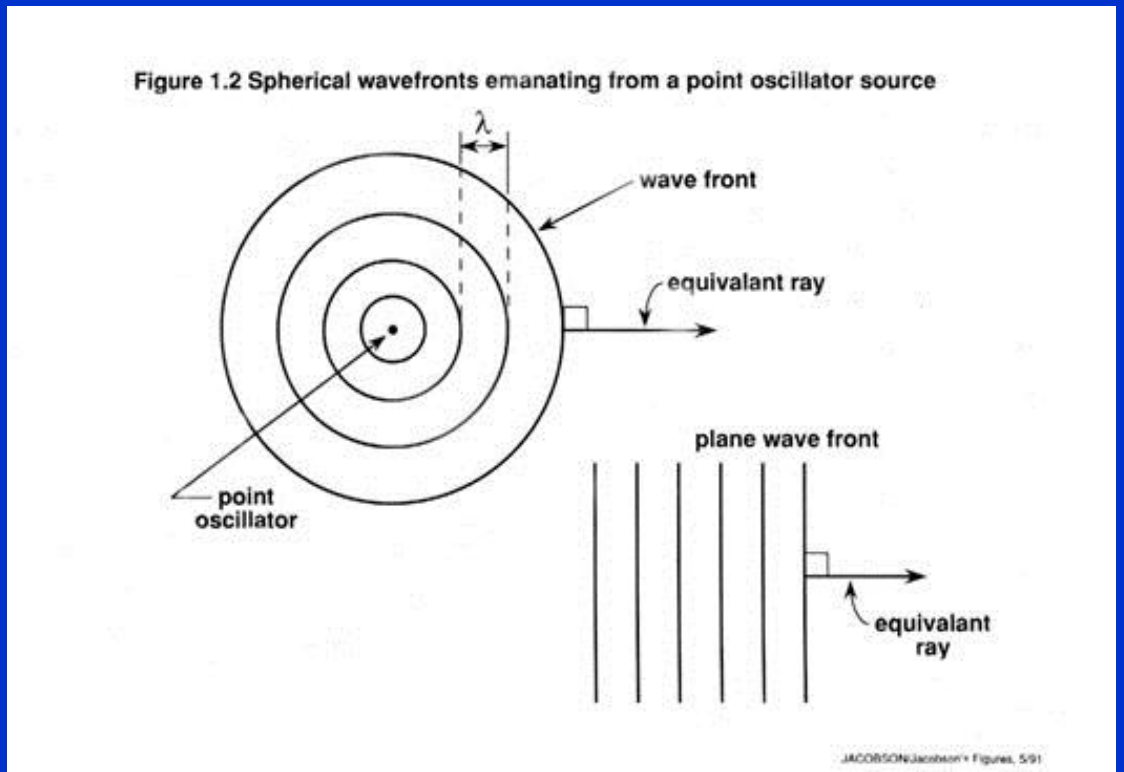
Some Important properties of light for microscopy applications

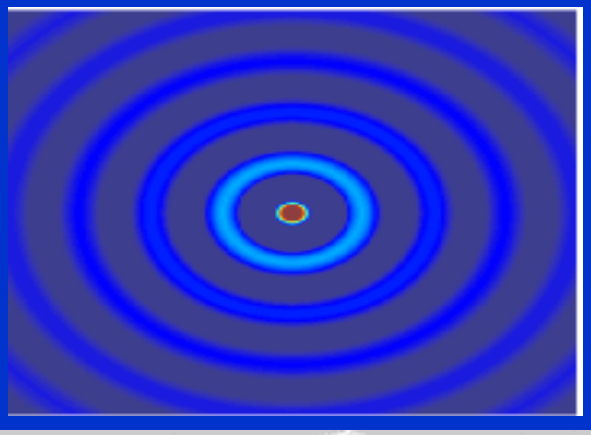


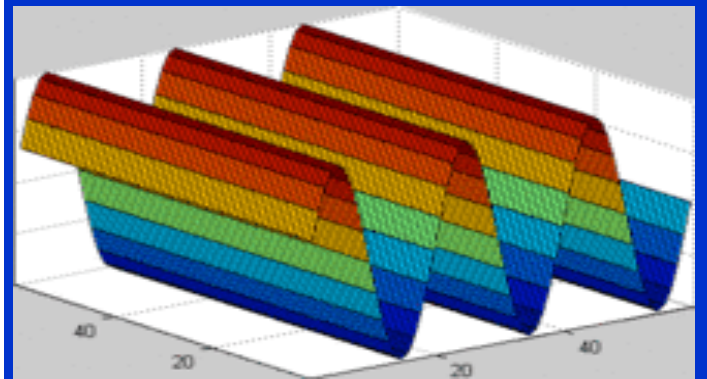
Light as electromagnetic wave with mutually perpendicular E, B components characterized by wavelength,λ, and frequency, ν, in cycles/s. Wave velocity = ν x λ. [λ=500nm--> $\nu=6\times10^{14}$ cycles/s]
Light: is electromagnetic radiation that is visible to the human eye, and is responsible for the sense of sight. Visible light has a wavelength in the range of about 380 nanometres (nm), or 380×10^{-9} m, to about 740 nanometres – between the invisible infrared, with longer wavelengths and the invisible ultraviolet, with shorter wavelengths.

wave front: is the area of points having the same phase: a line or curve in 2d, or a surface for a wave propagating in 3d .

1- **plane wave:** is a constant-frequency wave whose wavefronts (surfaces of constant phase) are infinite parallel planes of constant peak-to-peak amplitude normal to the phase velocity vector.
2-sphrical wave front: Spherical waves coming from a point source. And any point on wave fronts are source of small waves.







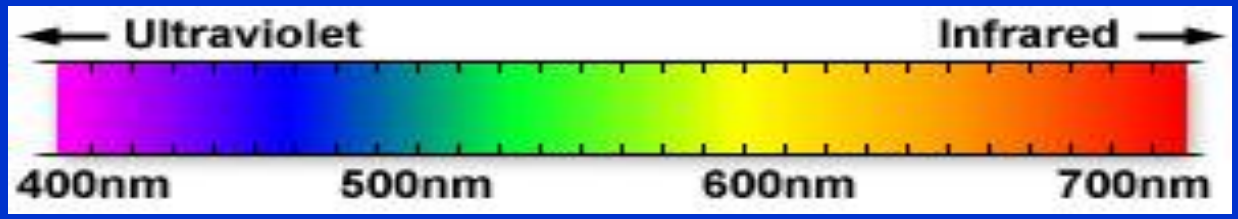
Velocity of light in different media

Index of refraction, n =c/v

C=speed of light in vacuum=$3x10^8$ m/s, v= velocity in media

Light travels slower in more dense media

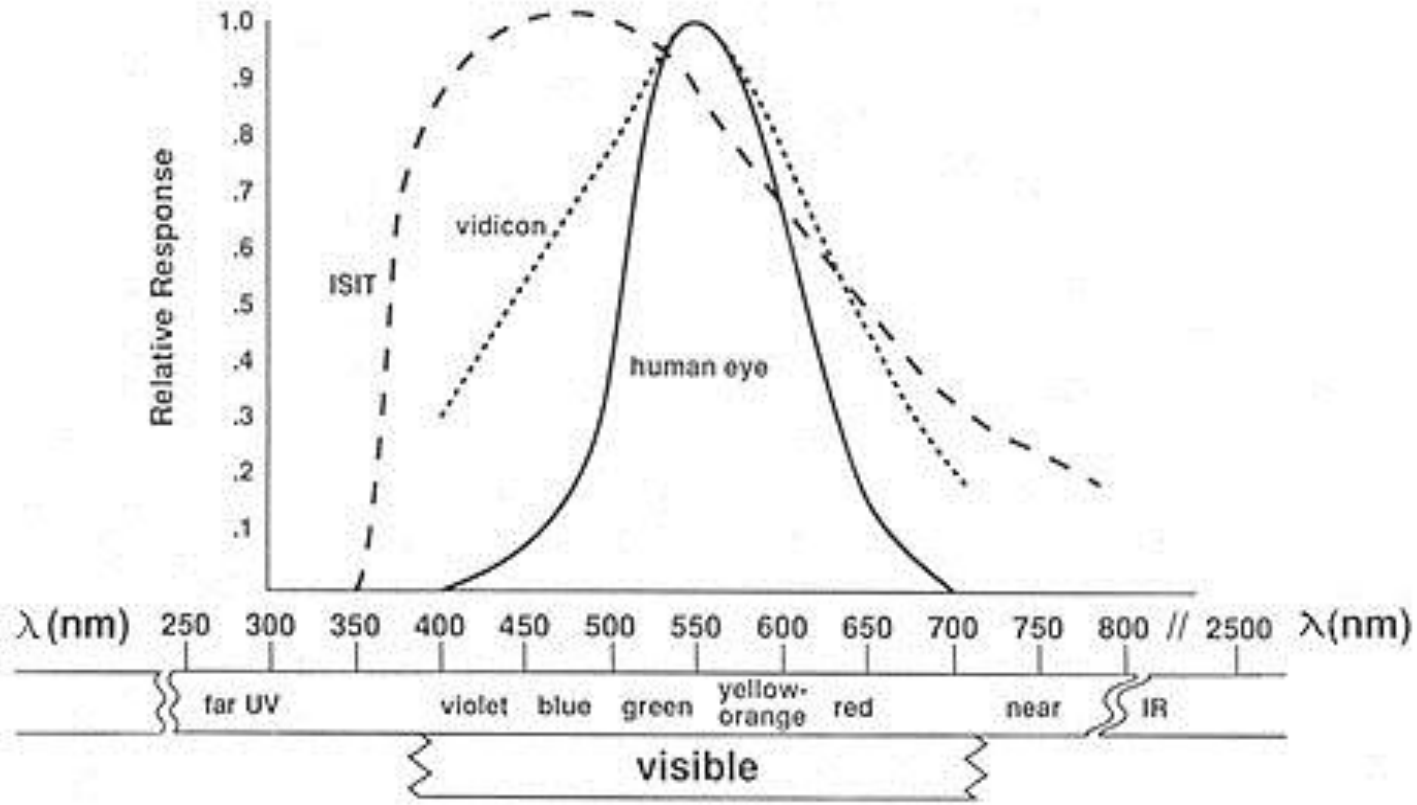
Index of refraction for different media at 546 nm



Air	1.0
Water	1.3333
Cytoplasm	1.38
Glycerol	1.46
Crown Glass	1.52
Immersion Oil	1.515
Protein	1.51-1.53
Flint Glass	1.67

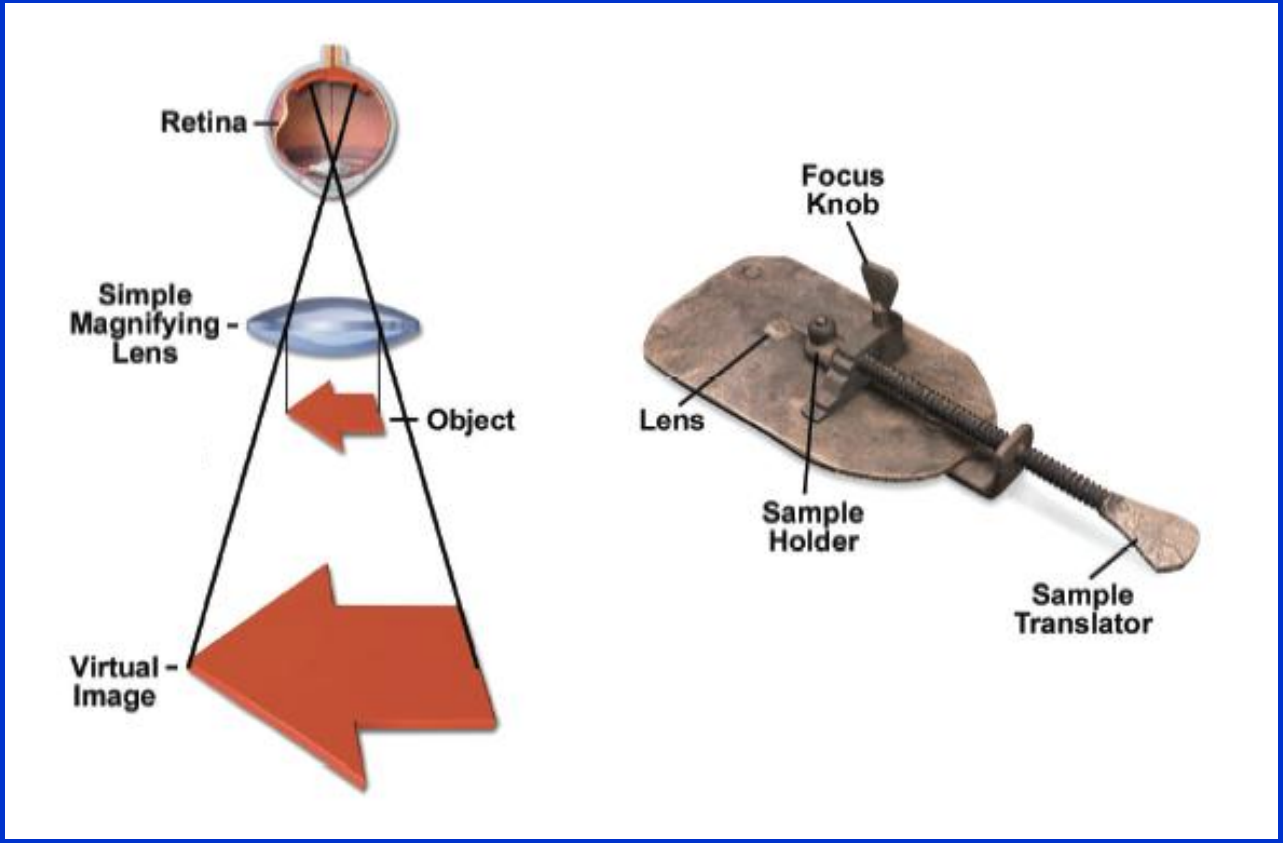
n increases with decreasing λ

UV–Visible–IR portion of optical spectrum; Spectral response of eye and electronic image detectors

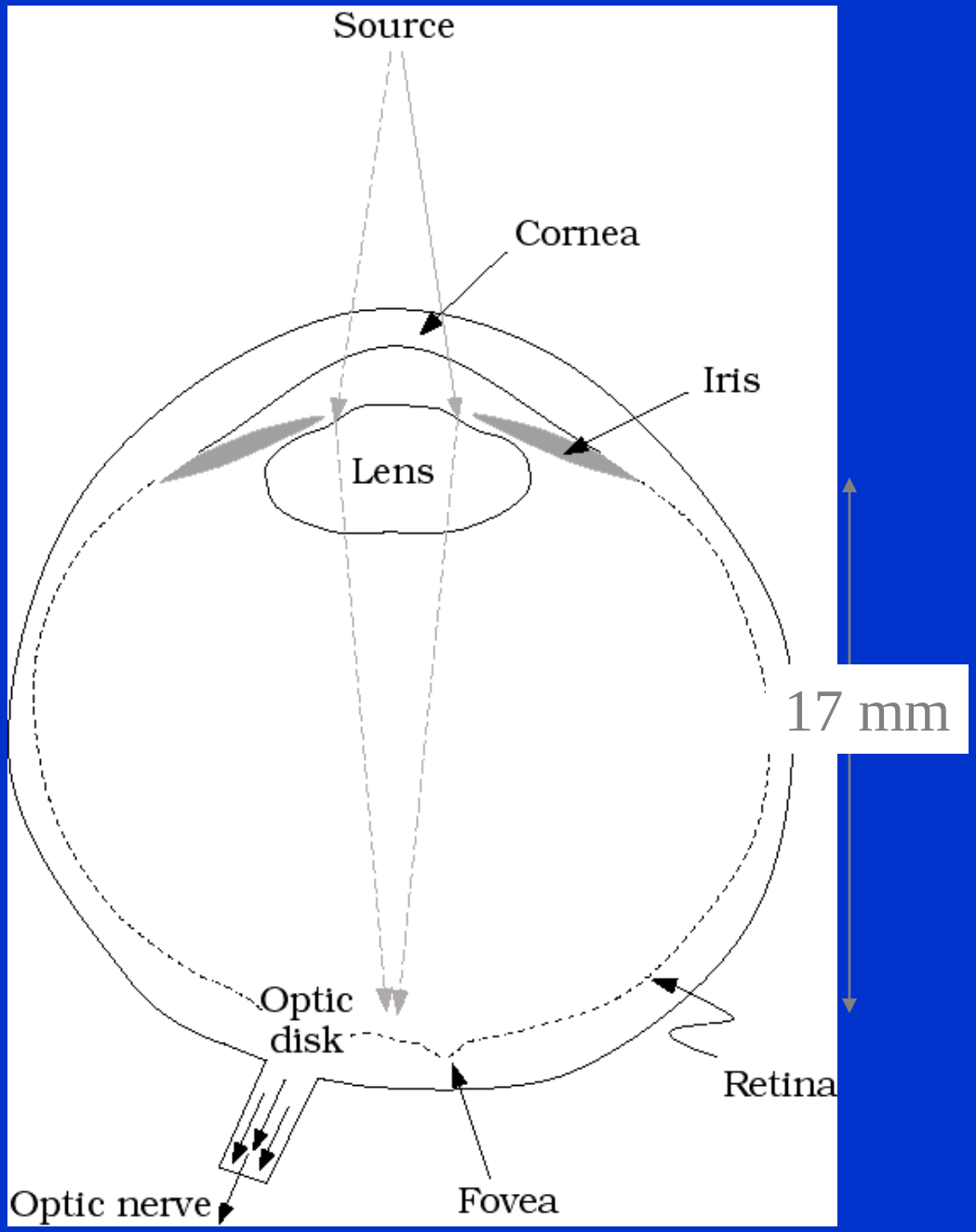


Note: electronic cameras do not have same spectral response as eyes

The simplest microscope: a magnifier



The Cornea and Lens Focus the Image on The Retina



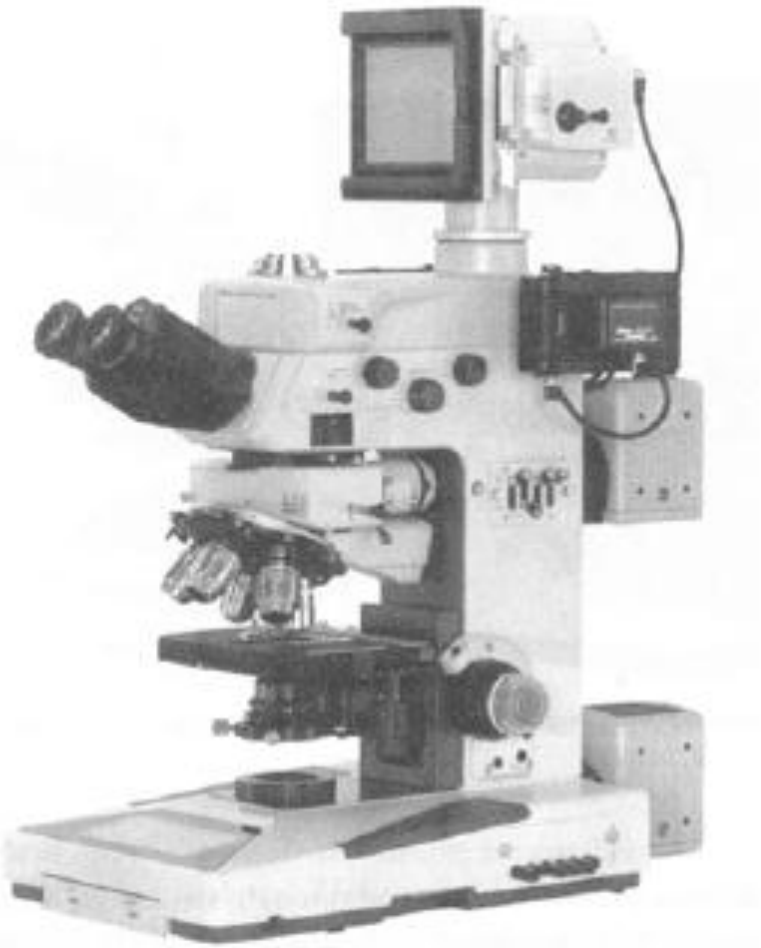
Microscope: is a scientific instrument that makes things normally too small to see look bigger, so they can be seen better and examined correctly. People who use microscopes commonly in their jobs include doctors and scientists.
The earliest microscopes had only 1 lens and are called *simple microscopes*. *Compound microscopes* have at least 2 lenses.
In a compound microscope, the lens closer to the eye is called the *eyepiece*. The lens at the other end is called the *objective*.

The purpose of the microscope is to create **magnification** so that structures can be resolved by eye and to create **contrast** to make objects visible.

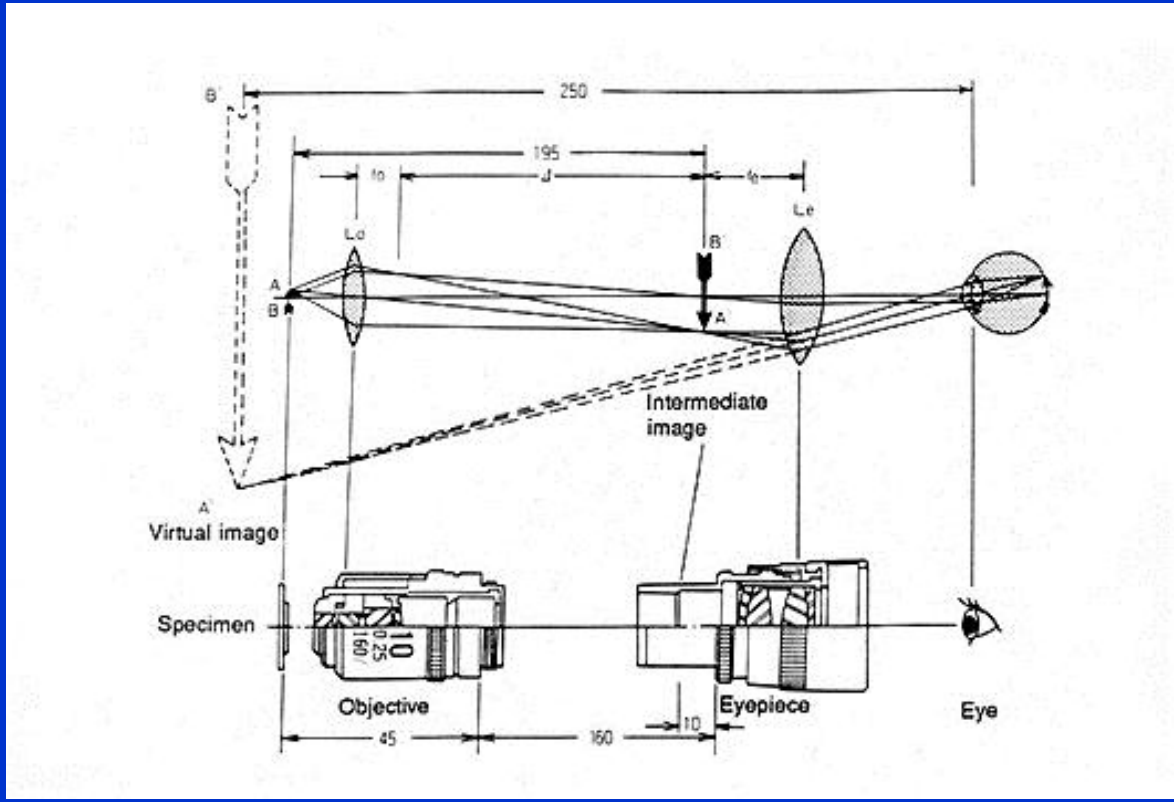
Instrumentation

Several important features are visible:

- Lenses
- Eyepieces (oculars)
- Light source
- Camera



In the compound microscope, the objective forms a real, inverted image at the eyepiece front focal plane (the primary image plane)



The optical tube length (OTL), typically 160mm, is the distance between the rear focal plane of the objective and the intermediate image plane

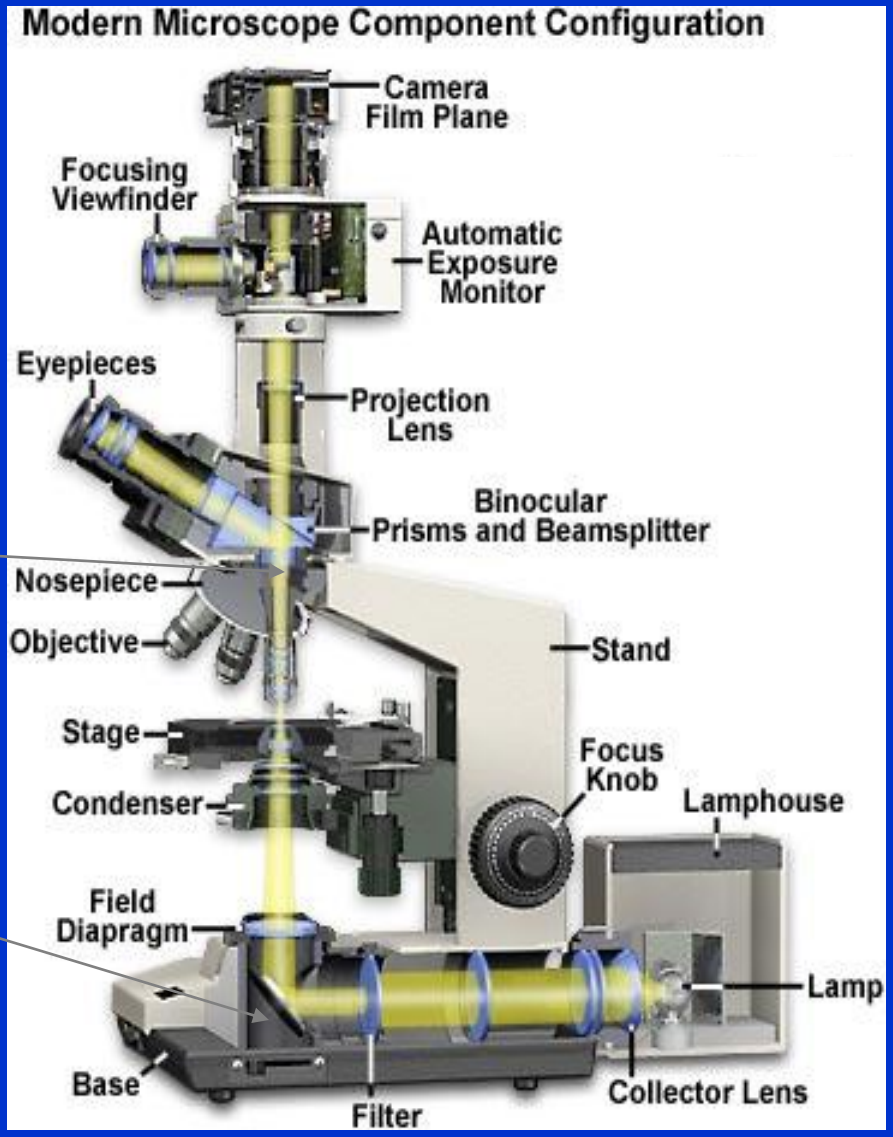
Calculating the magnification on a compound microscope

The compound microscope uses two lenses at once: the eye-piece lens and one of the objective lenses. The magnification of the microscope is the product of the magnifying power of these two lenses. This sounds complicated but it is very easy to calculate because the magnification is written on each of the lenses.

For example: look on top of the eye-piece lens and you will probably find X10 written on it. The three objective lenses are usually: X4 (low power), X 10 (medium power) and X40 (high power).
If you have a X 10 eye-piece and you are using a X4 objective lens (low power) the total magnification will be: 10x4=X40
If you now turn to a X 10 objective lens (medium power) the total magnification will be: 10x10=X100

Modern microscope component identification

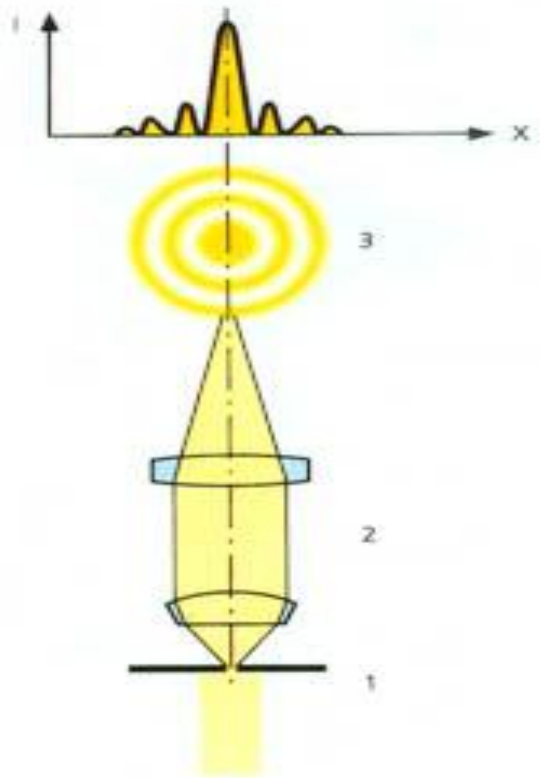
Prisms Used to Re-Direct Light In Imaging Path While Mirrors Are Used in Illumination Path



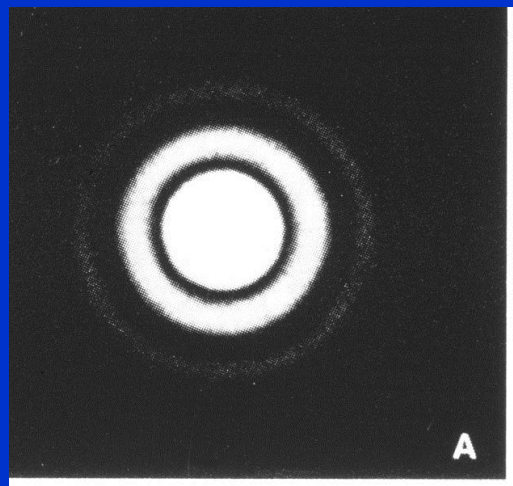
Airy Disk Formation by Finite Objective Aperture

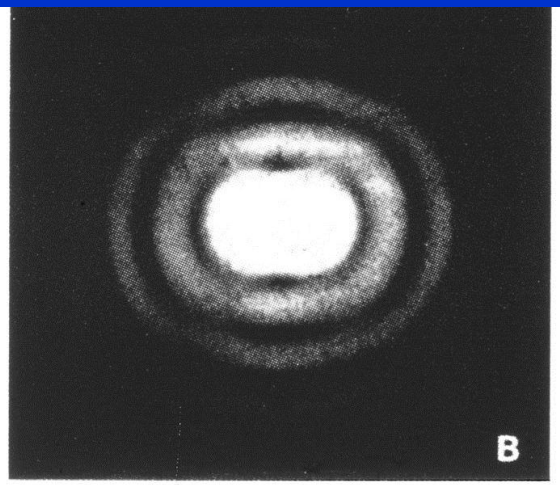
AIRY DISCS: are descriptions of the best focused spot of light that a perfect lens with a circular aperture can make, limited by the diffraction of light.

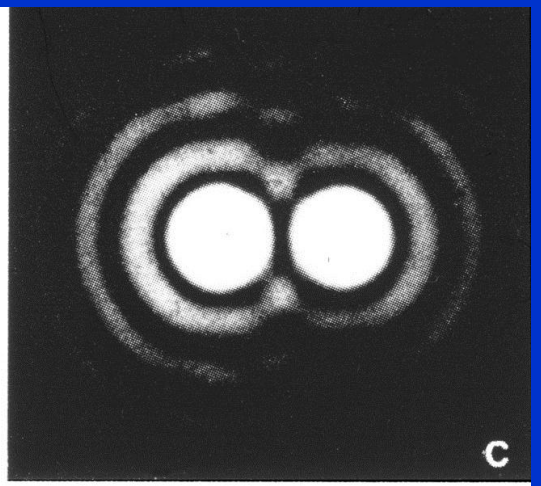
The width of central maximum prop. to λ and inversely prop. to objective aperture

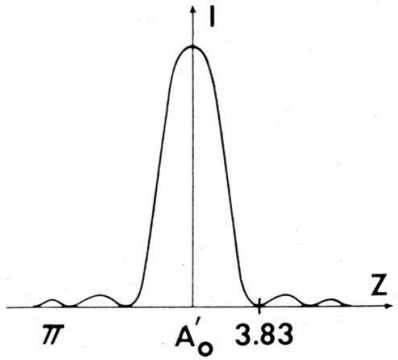


Resolution in Fluorescence Depends on Resolving Overlapping “Airy Disks”

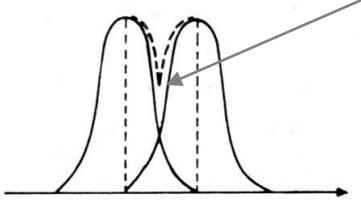


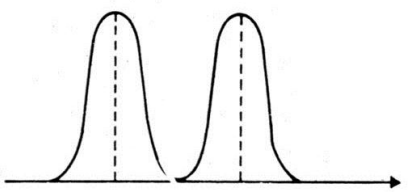






Raleigh Criteria: Overlap by r’, then dip in middle is 26% below Peak intensity





Resolution

- **Maximum resolution:**

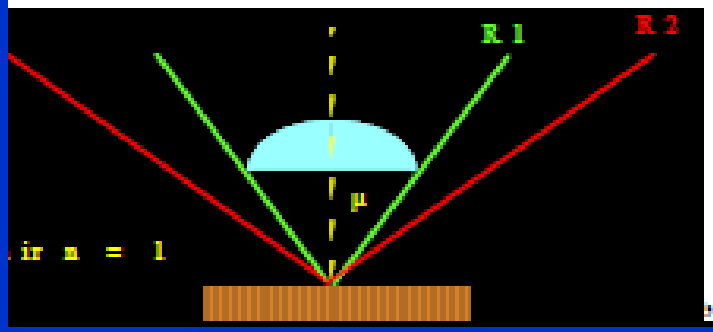
$$R = \frac{(0.61 \bullet \lambda)}{N.A.}$$

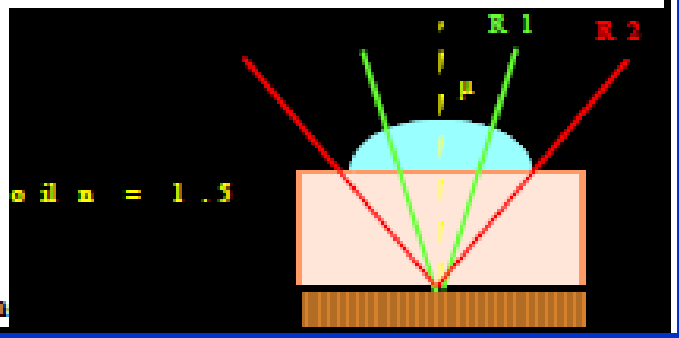
where: 0.61 is a geometrical term, based on the average 20-20 eye, λ = wavelength of illumination, N.A. = Numerical Aperture

The N.A. is a measure of the light gathering capabilities of an objective lens.

N.A. = n sin α where:

n = index of refraction of medium, α = < subtended by the lens





Resolving Power

- Human eye: about 0.2 mm
- Compound Light Microscope: about 0.2 µm
- Transmission Electron Microscope: about 0.2 nm

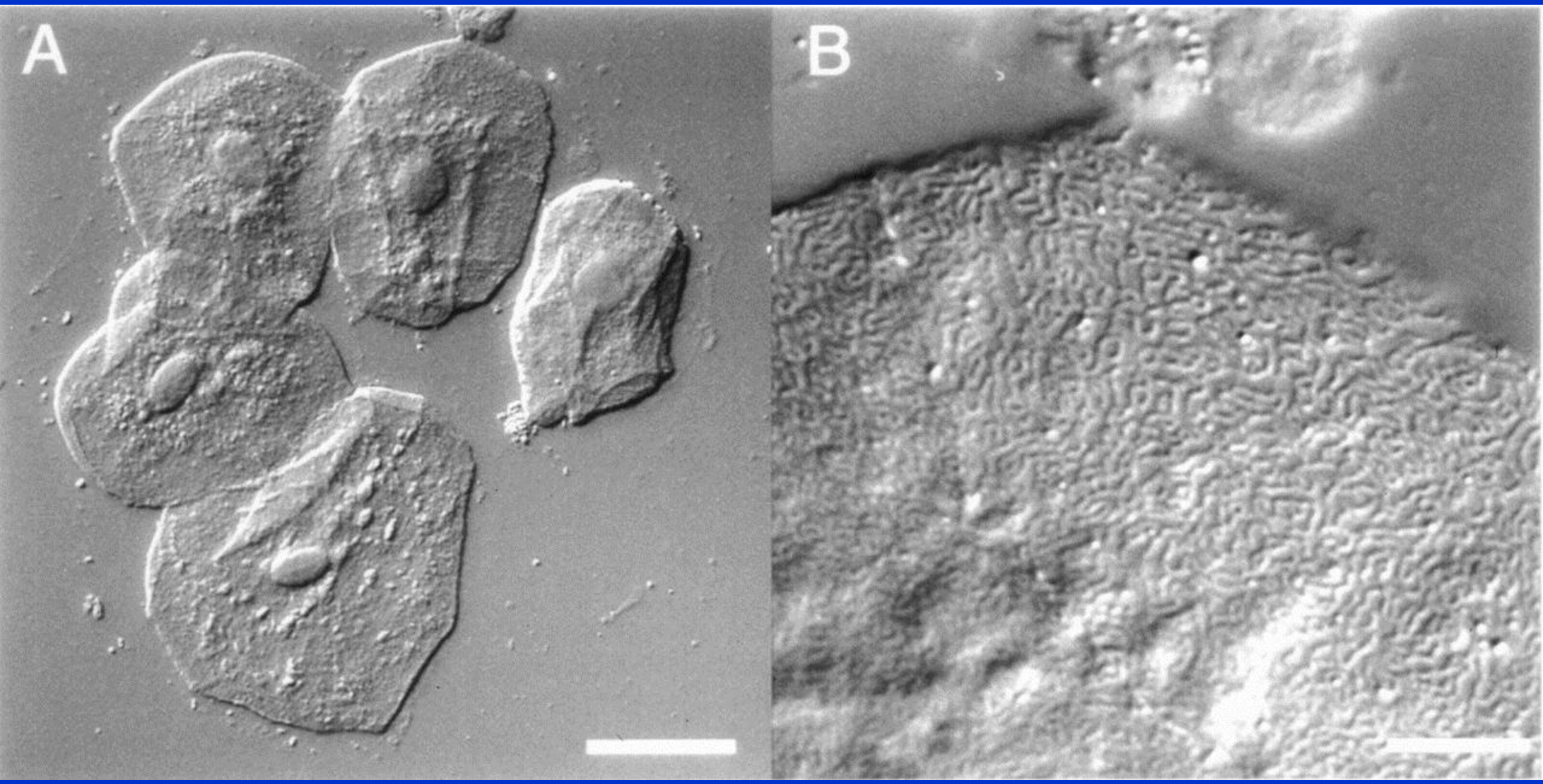
Factors Affecting Resolution

- **Resolution (d_{min}) improves (smaller d_{min}) if $\lambda\downarrow$ or $n\uparrow$ or $\alpha\uparrow$**
- **Assuming that $\sin\alpha = 0.95$ ($\alpha = 71.8°$)**

Wavelength		Air (n= 1)	Oil (n = 1.515)
Red	650 nm	0.42 µm	0.28 µm
Yellow	600 nm	0.39 µm	0.25 µm
Green	550 nm	0.35 µm	0.23 µm
Blue	475 nm	0.31 µm	0.20 µm
Violet	400 nm	0.27 µm	0.17 µm
		Resolution $_{air}$	**Resolution $_{oil}$**

- **(The eye is more sensitive to blue than violet)**

Ridges in The Surface of Cheek Cells for Resolution Tests



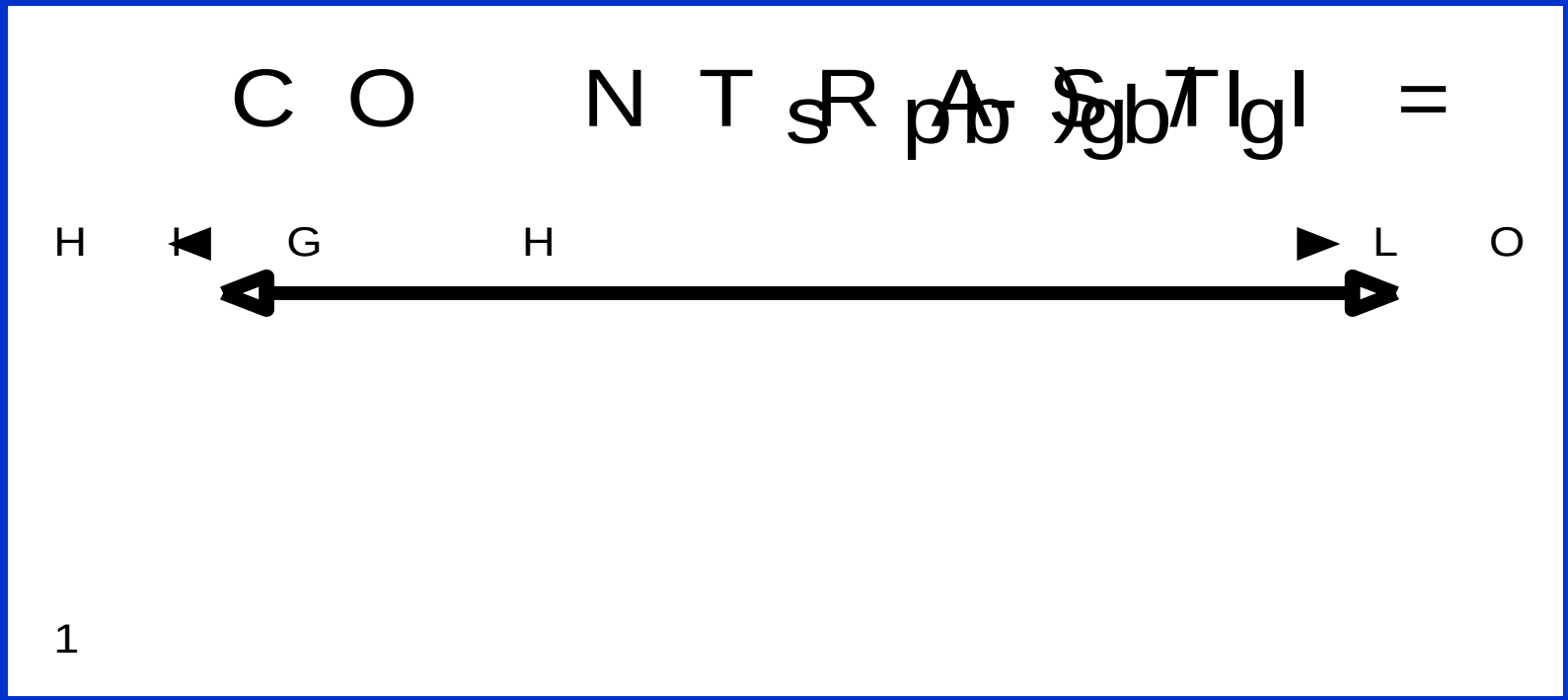
High Resolution DIC Microscopy

Contrast
All the resolution in the world won't do you any good, if there is no contrast to visualize the specimen

Phase contrast microscopy is an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. Phase shifts themselves are invisible, but become visible when shown as brightness variations.

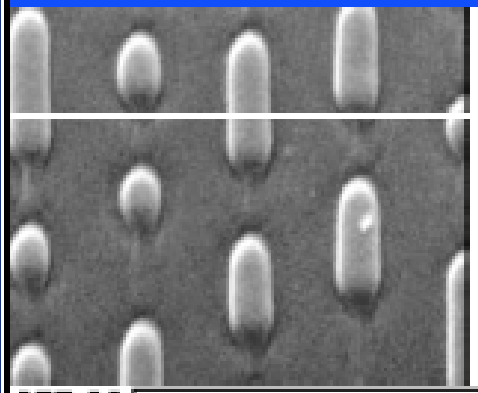
When light waves travels through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the medium. Changes in amplitude (brightness) arise from the scattering and absorption of light, which is often wavelength dependent and may give rise to colors. Photographic equipment and the human eye are only sensitive to amplitude variations. Without special arrangements, phase changes are therefore invisible. Yet, phase changes often carry important information.

Contrast



Contrast

Contrast is defined as the difference in light intensity between the specimen and the adjacent background relative to the overall background intensity.



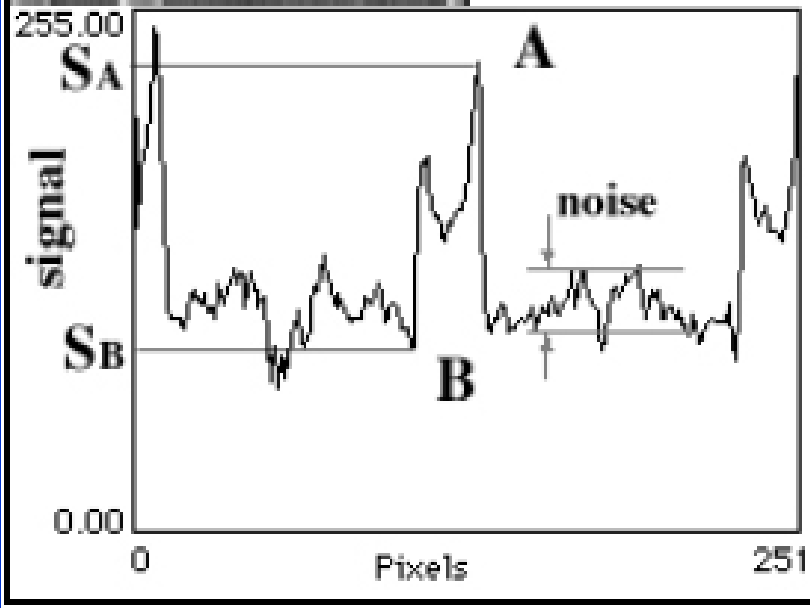


Image contrast, C is defined by

$$C = \frac{(S_{specimen} - S_{background})}{S_{background}}$$

$S_{specimen}$ and $S_{background}$ are intensities measured from the specimen and background, e.g., A and B, in the scanned area.

CONTRAST MODES OF LIGHT MICROSCOPY

MODE	MECHANISM OF CONTRAST
Brightfield	Absorption of light
Phase contrast	Optical path length (index, density)
DIC	Rate of change of optical path
Widefield fluorescence	Absorption of light, quantum yield of fluorophore
Confocal fluorescence	same as fluorescence
Darkfield	light scattering by edges in specimen
Interference reflection contrast	interference between reflections from ventral cell surface and substratum
Polarization	Extinction between crossed polars caused by specimen birefringence

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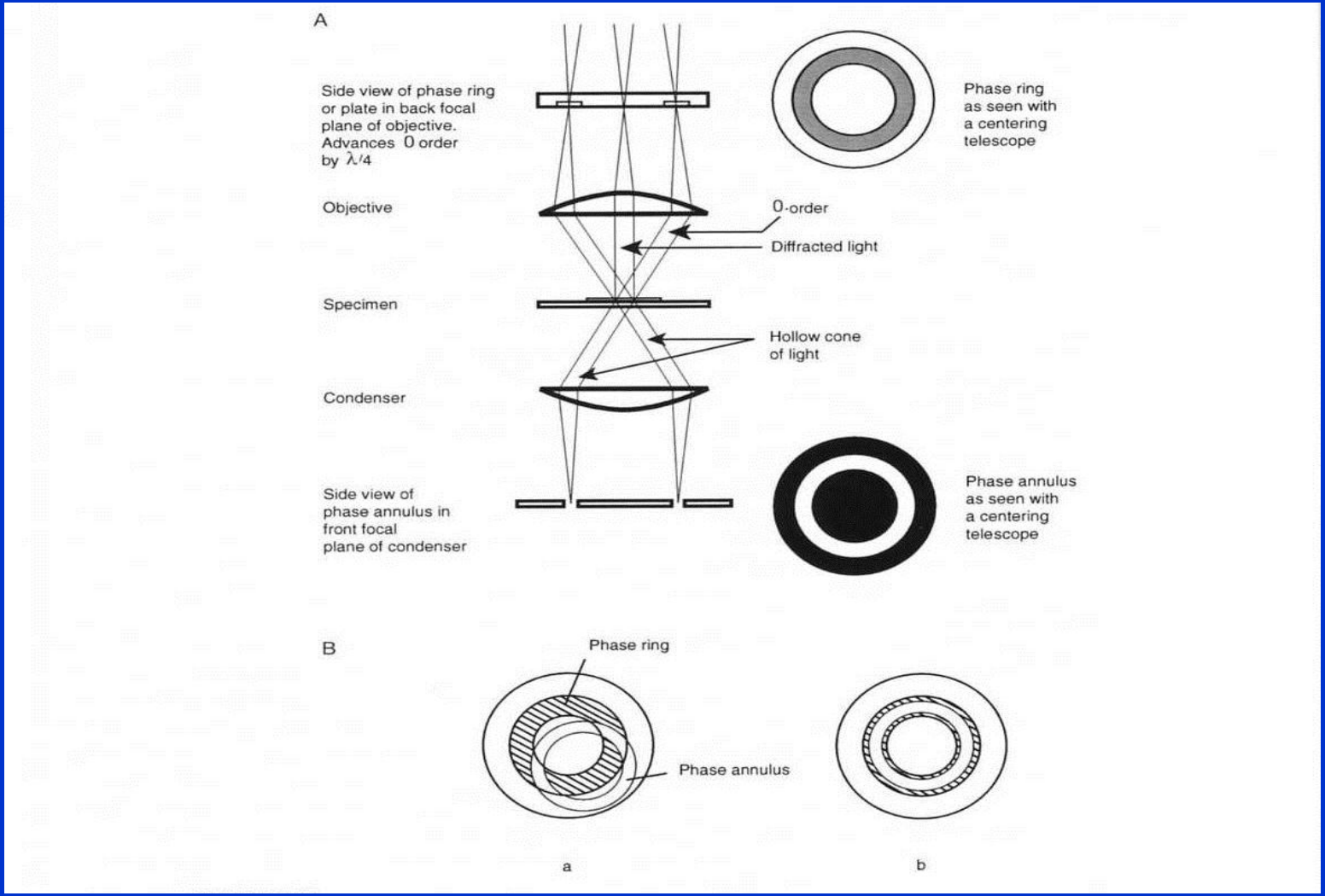

Phase contrast microscopy