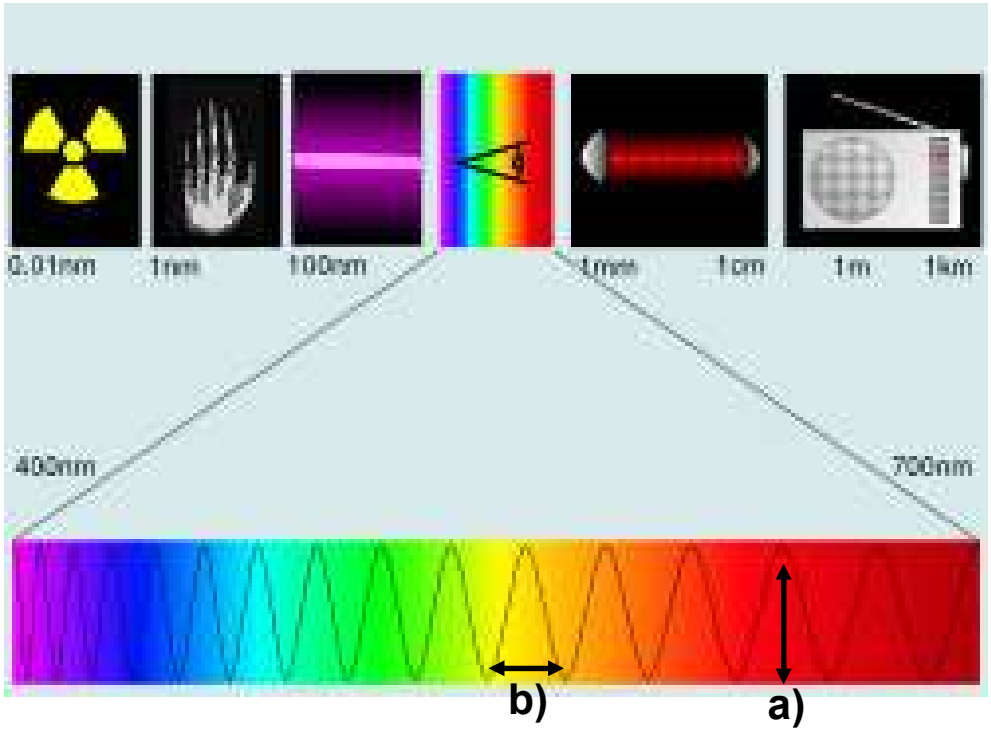
A general introduction to optics and light microscopy

What is light?



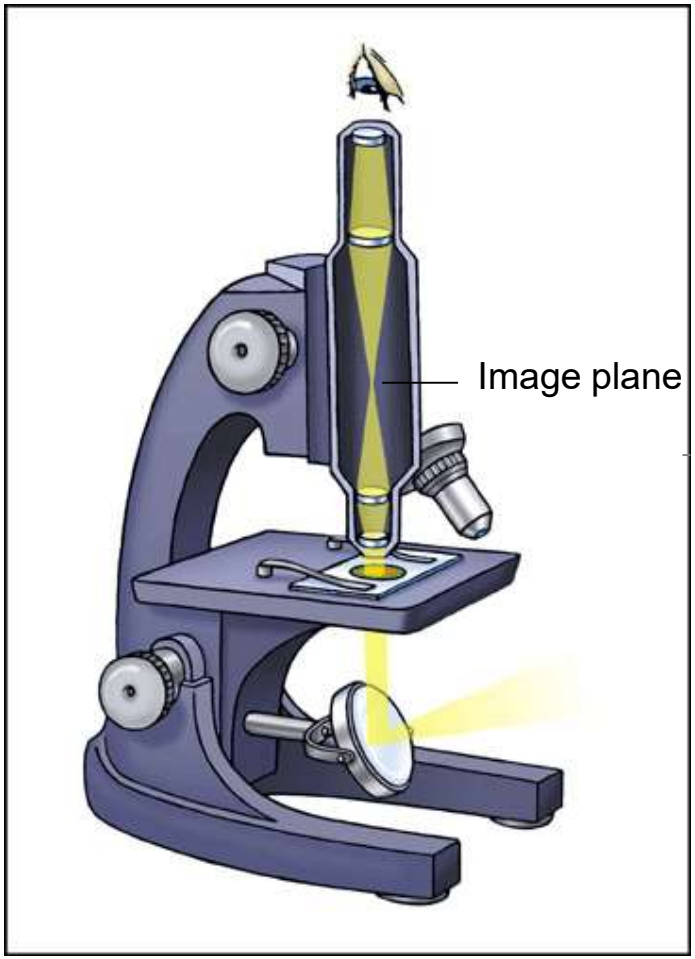
Light is electromagnetic radiation. What we usually describe as light is only the visible spectrum of this radiation with wavelengths between 400nm and 700nm.

The elementary particle that defines light is the photon.

There are 3 basic dimensions of light

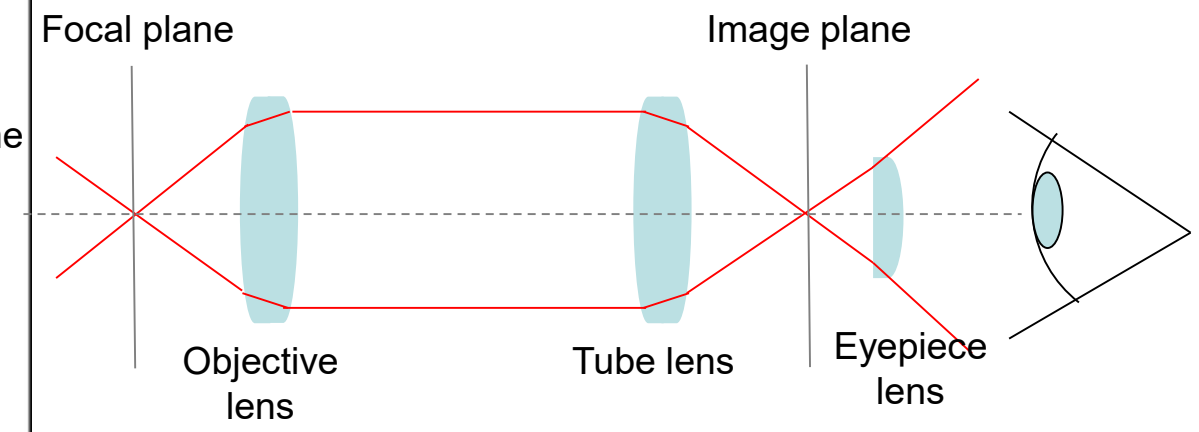
a) Intensity (amplitude) which is related to the perception of brightness
b) Frequency (wavelength), perceived as colour
c) Polarization (angle of vibration) which is not or weakly perceptible to humans

What is a microscope?



Classic compound microscope

Theoretically a microscope is an array of two lenses.



Modern microscope with ICS
(Infinity Colour corrected System)

Your friend - the objective

Objectives can be classified into transmitted light and reflected-light (Epi) versions.

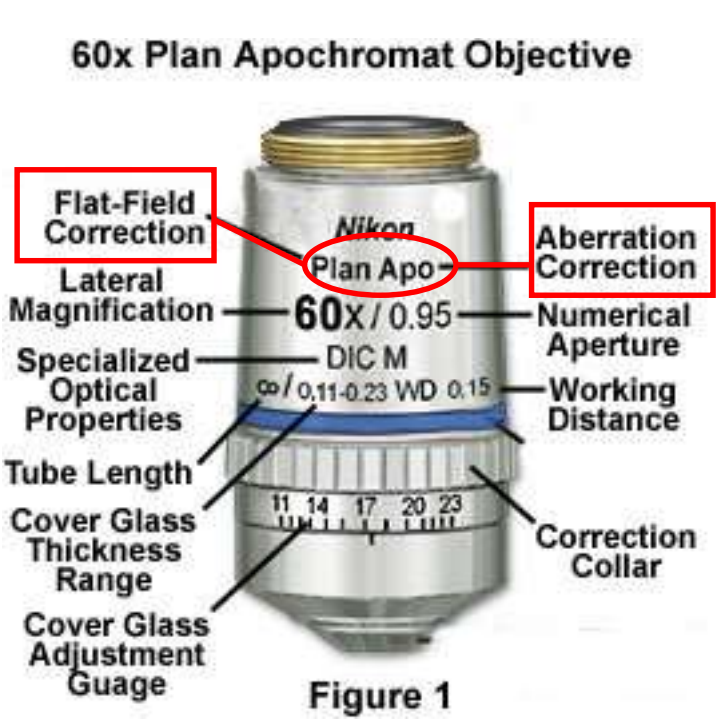


Figure 1

Figure 1

Still your friend - the objective

Figure 1

Figure 1

Microscope magnification

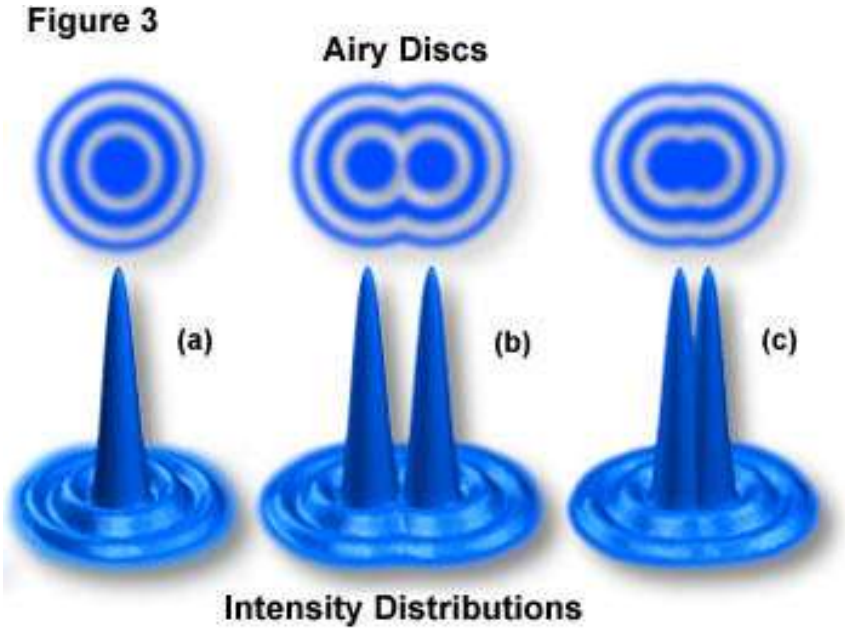
Total magnification is the observed dimension/real dimension and is determined by two components:

magnification by the objective
x
the magnification by eyepiece

BUT maximum magnification does not mean maximum resolution!

Resolution

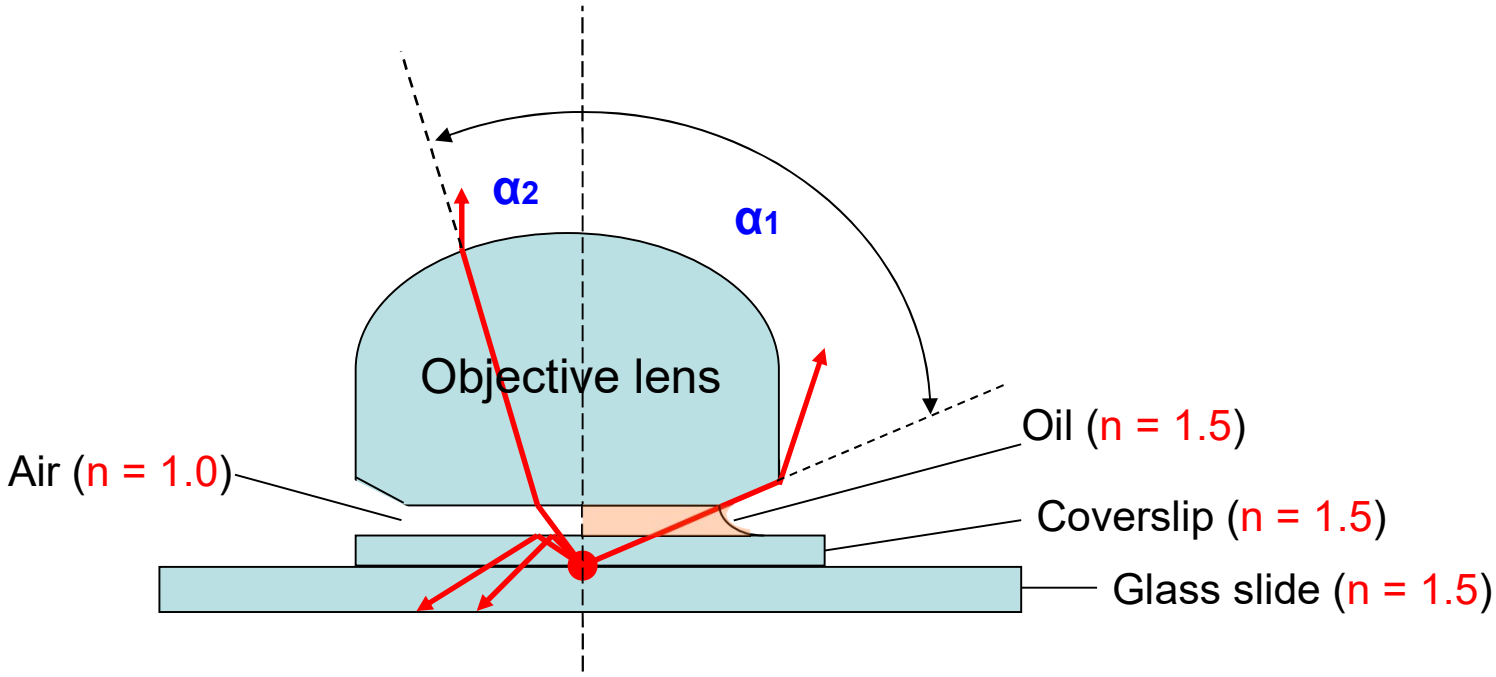
Resolution describes the minimal distance of two points that can be distinguished.



Picture taken from http://microscopy.fsu.edu/primer/anatomy/numaperture.html

Numerical aperture

NA is an estimate of how much light from the sample is collected by the objective

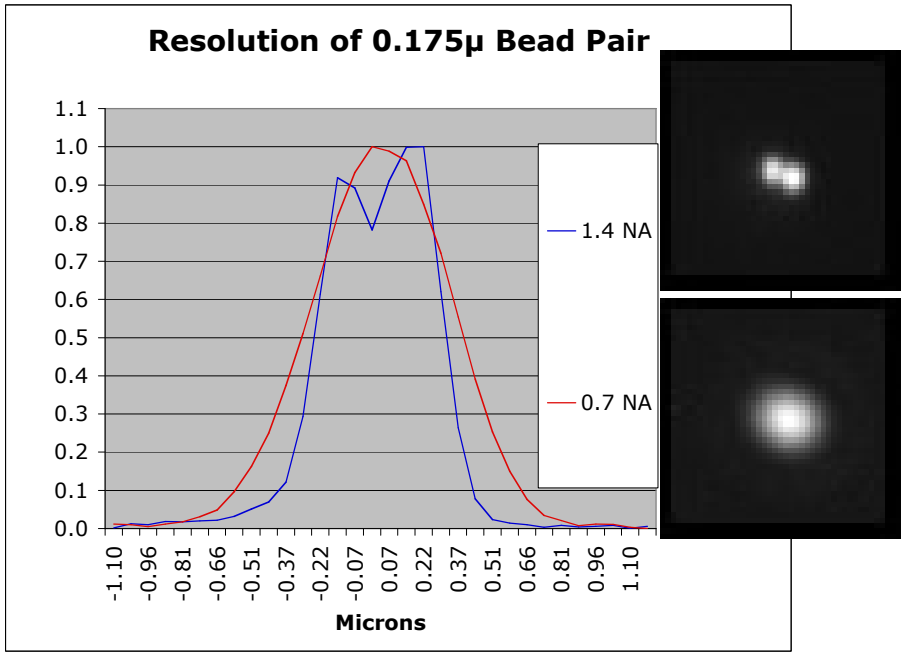


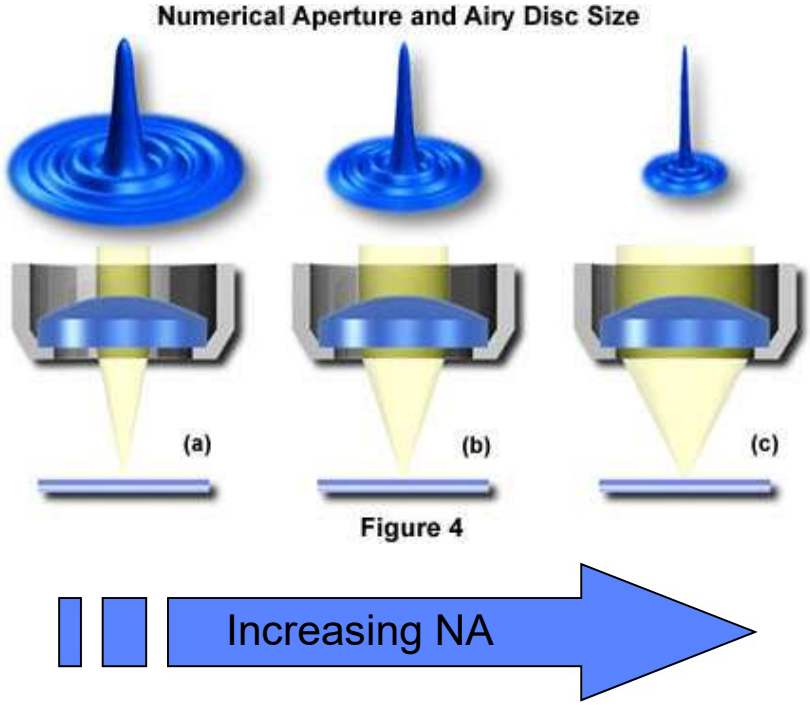
$$NA = n \sin \alpha$$

n = refractive index

α = angle of incident illumination

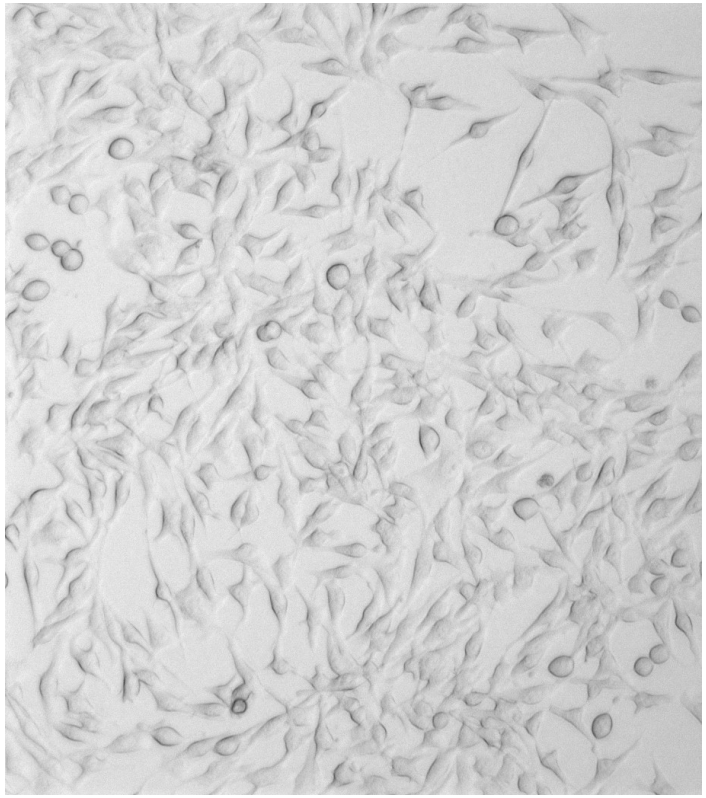
Numerical aperture, NOT magnification determines resolution!





A lens with a larger NA will be able to visualize finer details and will also collect more light and give a brighter image than a lens with lower NA.

How can we use the properties of light to create contrast?



Which properties can be used?

Absorption

Scattering

Refraction

Phase

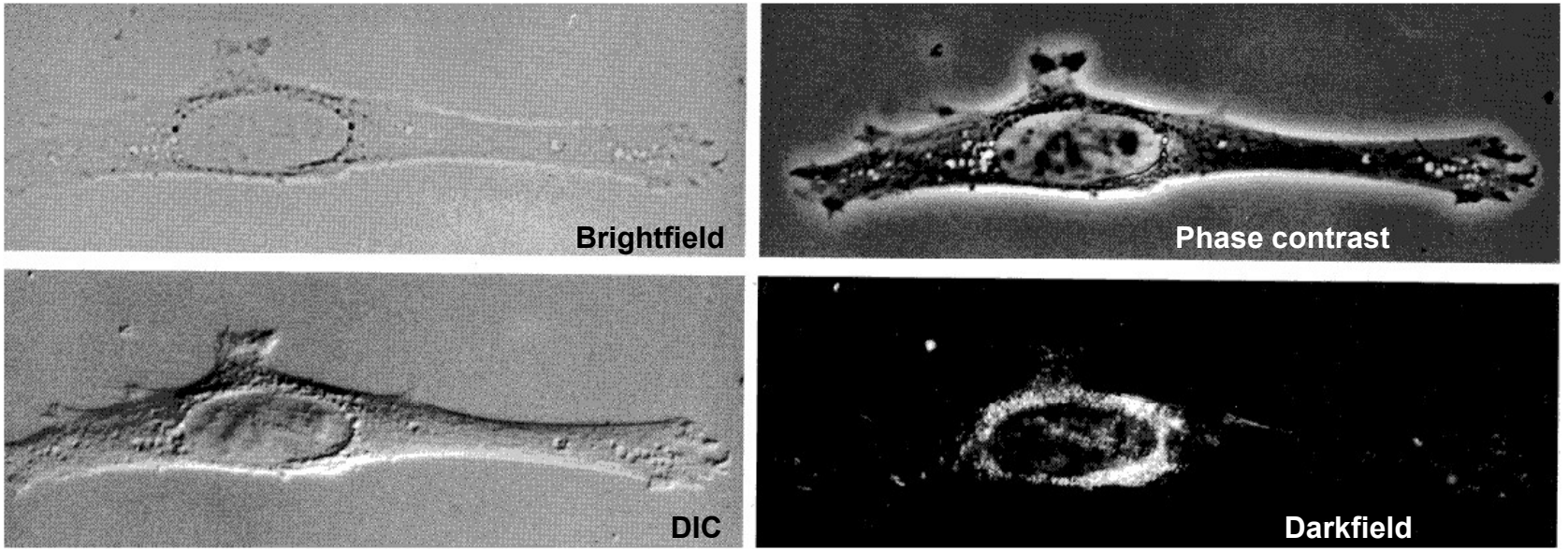
Polarization

Contrast

Contrast

Contrast

Contrasting techniques



Taken from: http://fig.cox.miami.edu/~cmallery/150/Fallsyll.htm

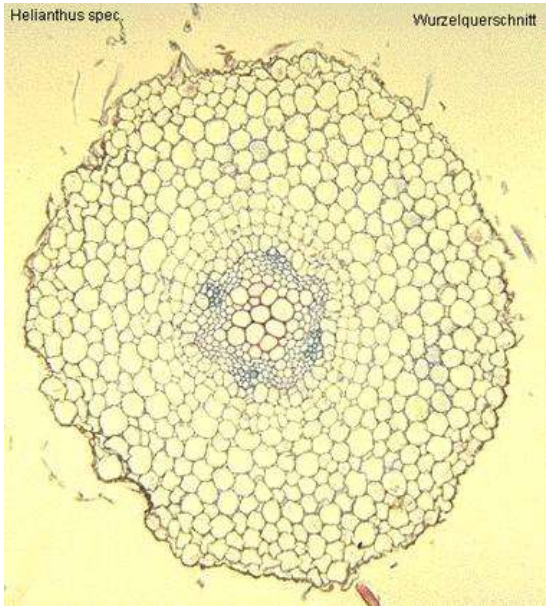
Contrasting techniques

- Brightfield
- Darkfield
- Phase Contrast
- Polarization Contrast
- Differential Interference Contrast (DIC)

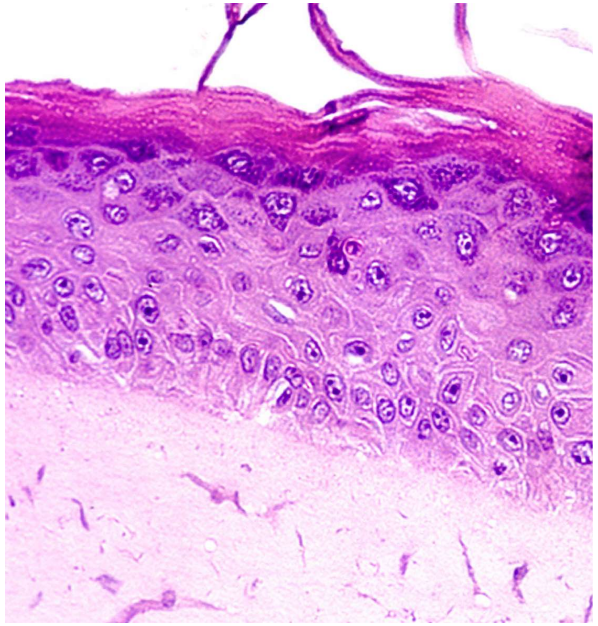
Brightfield

Principle: Light is transmitted through the sample and absorbed by it.

Application: Only useful for specimens that can be contrasted via dyes. Very little contrast in unstained specimens. With a bright background, the human eye requires local intensity fluctuations of at least 10 to 20% to be able to recognize objects.



Cross section of sunflower root
(http://www.zum.de/Faecher/Materialien/beck/12/bs12-5.htm)



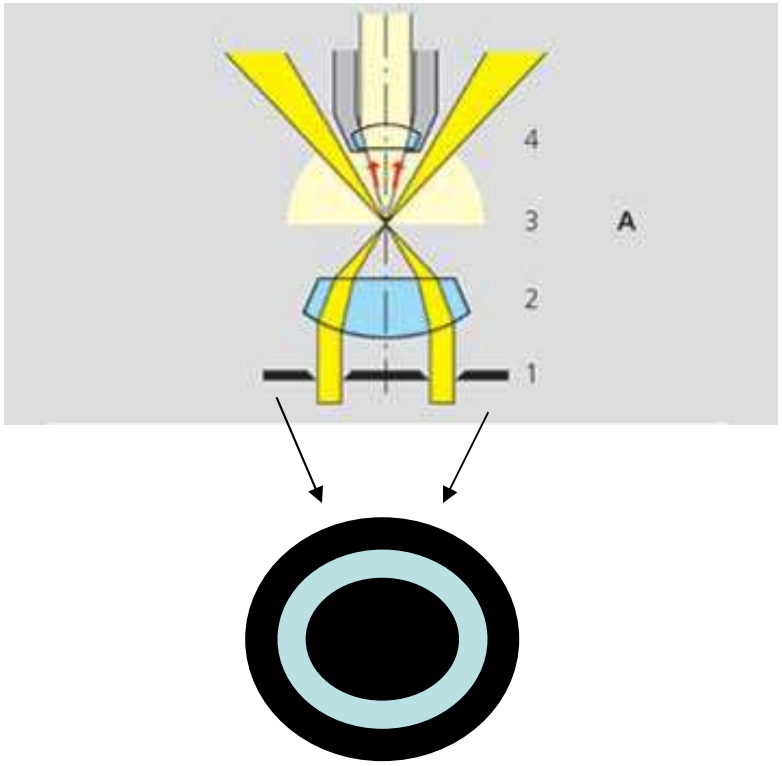
Piece of artificially grown skin
(www.igb.fhg.de/.../dt/PI_BioTechnica2001.dt.html)

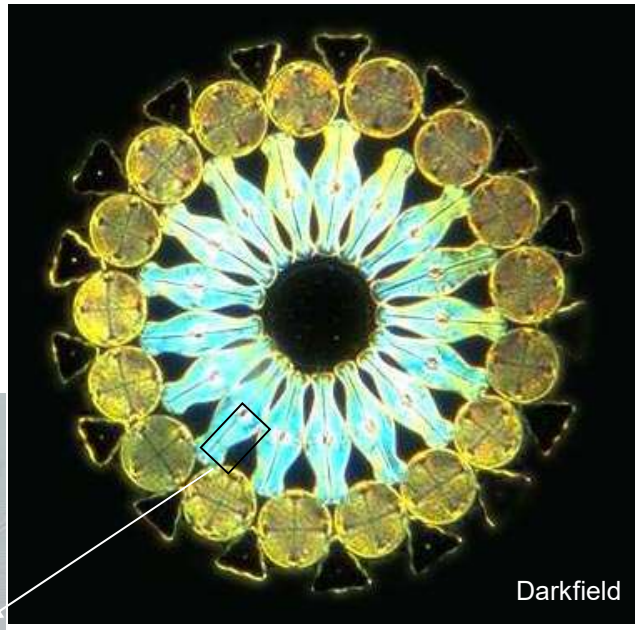
→ all our microscopes can be used for brightfield

Darkfield

Principle: The illuminating rays of light are directed through the sample from the side by putting a dark disk into the condenser that hinders the main light beam to enter the objective. Only light that is scattered by structures in the sample enters the objective.

Application: People use it a lot to look at Diatoms and other unstained/colourless specimens





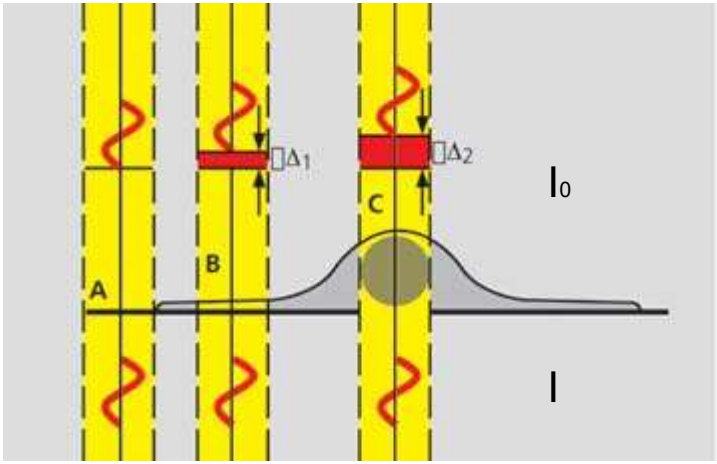
Symbiotic Diatom colony

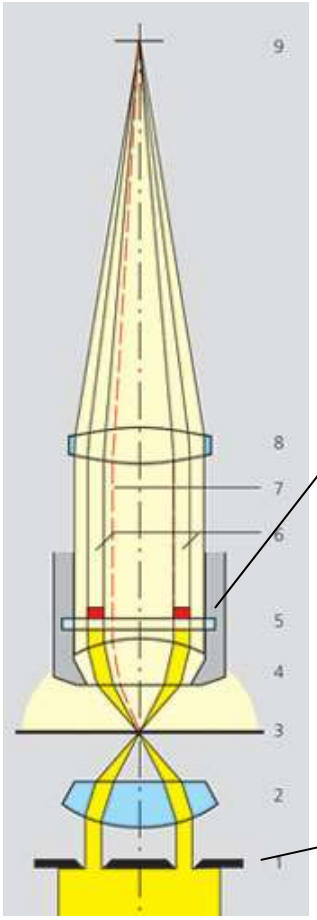
(www1.tip.nl/~t936927/making.html)

→ we do not have microscopes set up for darkfield

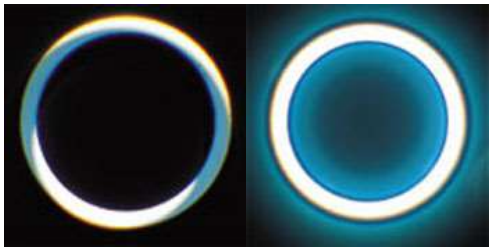
Phase contrast in theory

Principle: Incident light [Io] is out of phase with transmitted light [I] as it was slowed down while passing through different parts of the sample and when the phases of the light are synchronized by an interference lens, a new image with greater contrast is seen.





Phase ring



not aligned aligned

Phase stops

→ most of our microscopes are set up for phase contrast

Phase contrast in theory

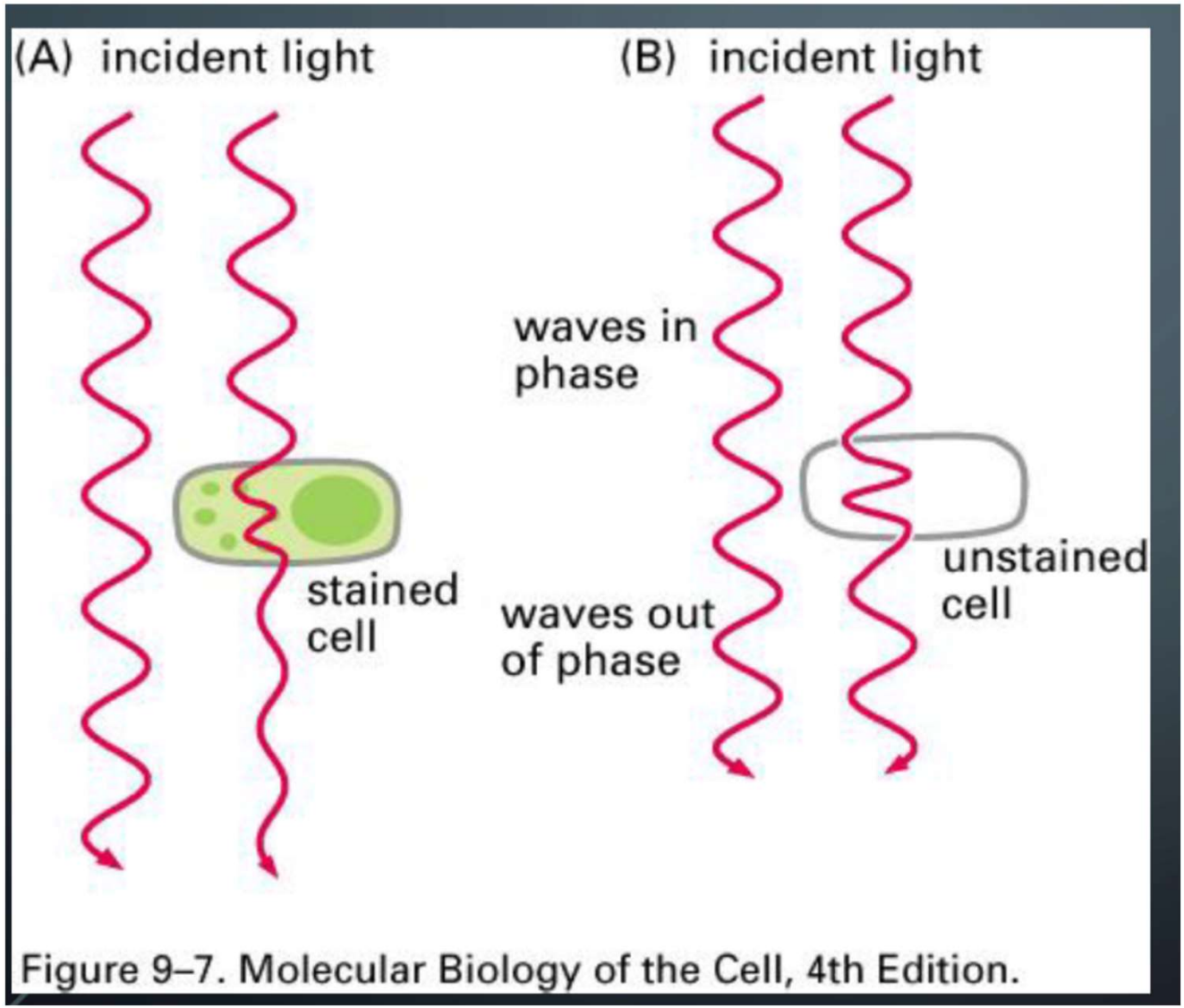
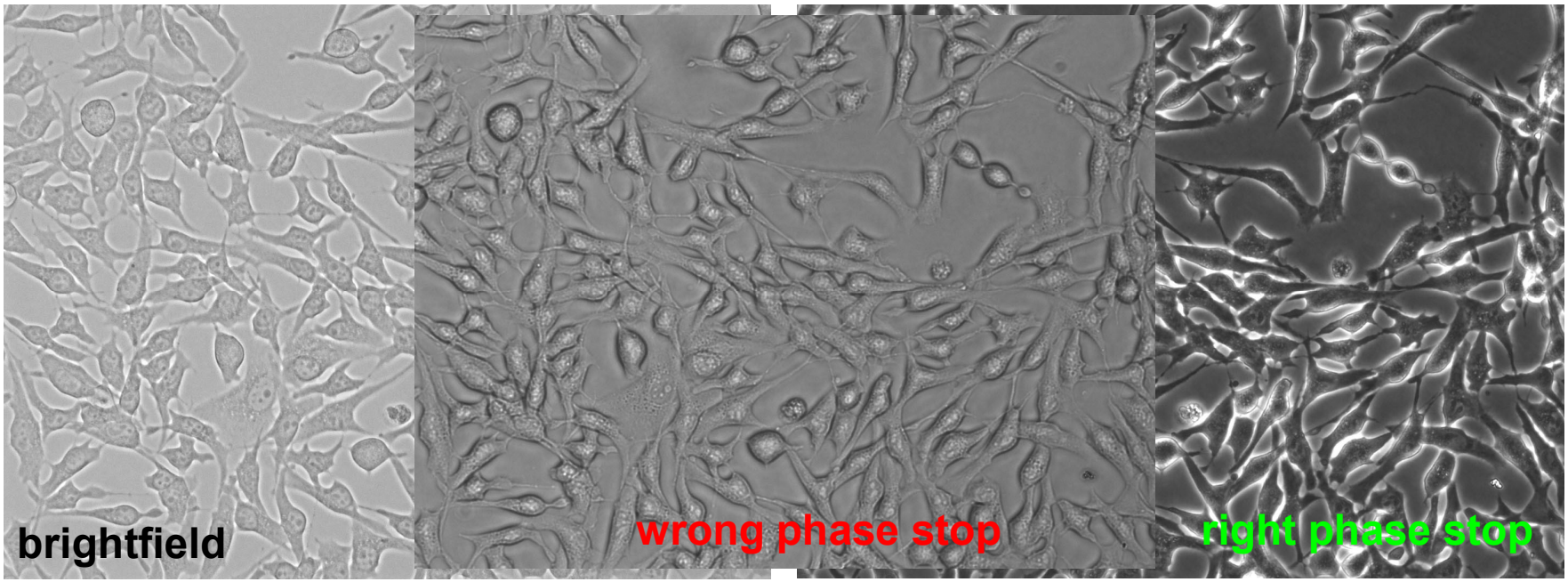


Figure 9–7. Molecular Biology of the Cell, 4th Edition.

Phase contrast in practice

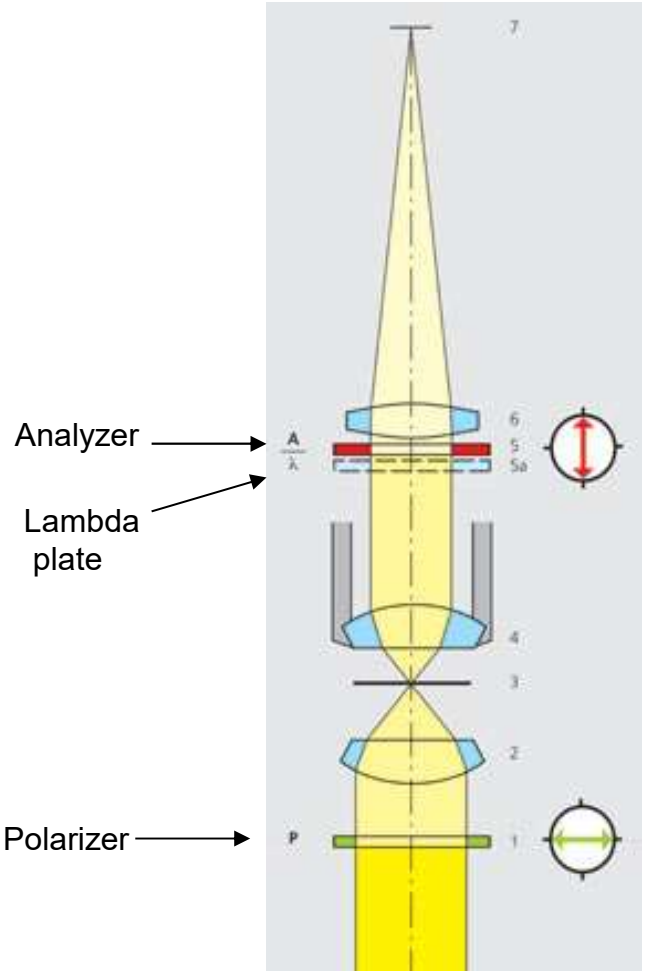
Application: Phase contrast is the most commonly used contrasting technique in this institute. All tissue culture microscopes and the time-lapse microscopes are set up for phase.

BUT: MOST OF YOU ARE USING IT IN THE WRONG WAY!! Because you do not use the right phase stop with the corresponding objective!



Polarization Contrast

Principle: Polarized light is used for illumination. Only when the vibration direction of the polarized light is altered by a sample placed into the light path, light can pass through the analyzer. The sample appears light against a black background. A lambda plate can be used to convert this contrast into colours.



Application: Polarization contrast is used to look at materials with birefringent properties, in which the refractive index depends on the vibration direction of the incident light, e.g. crystals or polymers.

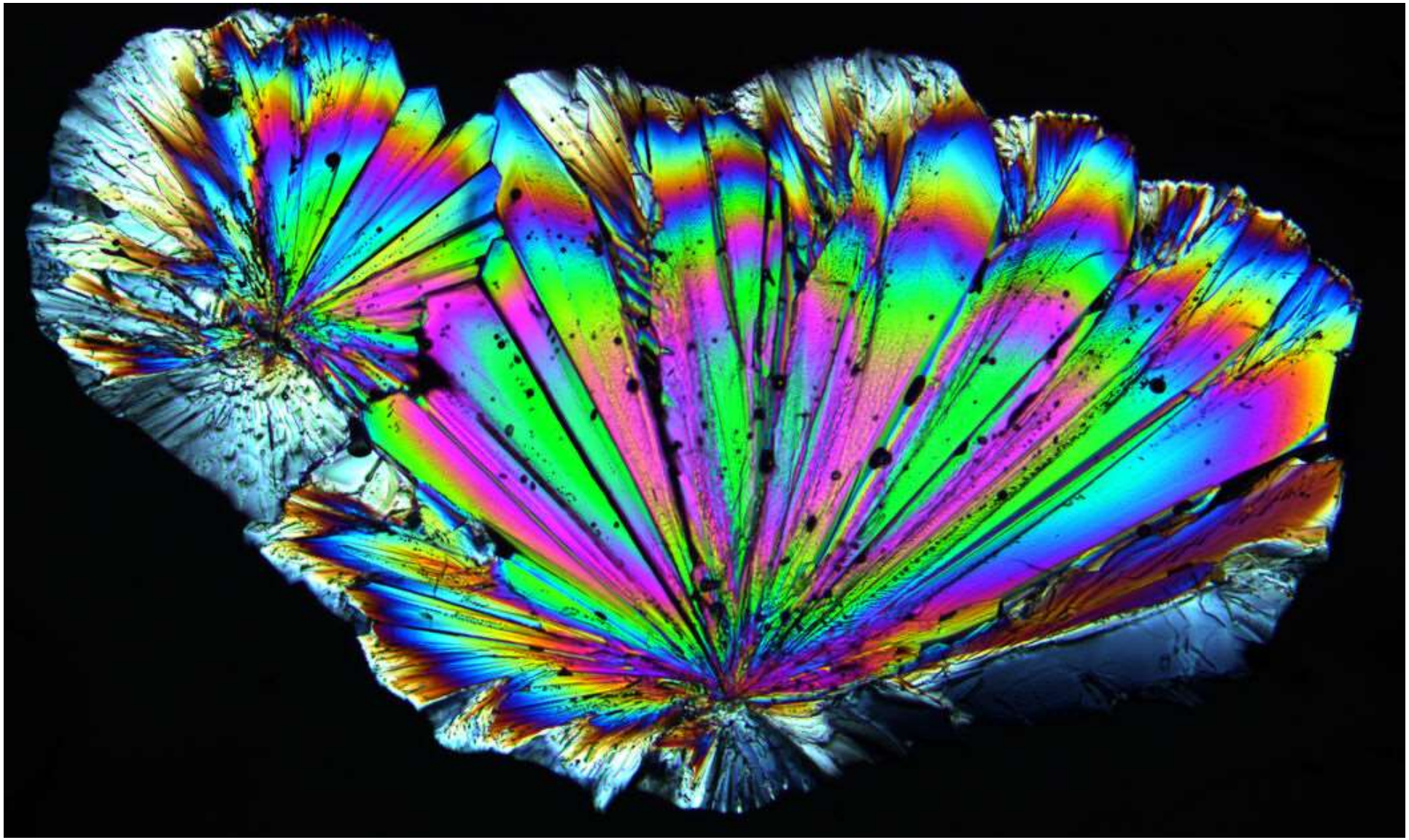
Brightfield

Polarization contrast

Polarization contrast with Lambda plate

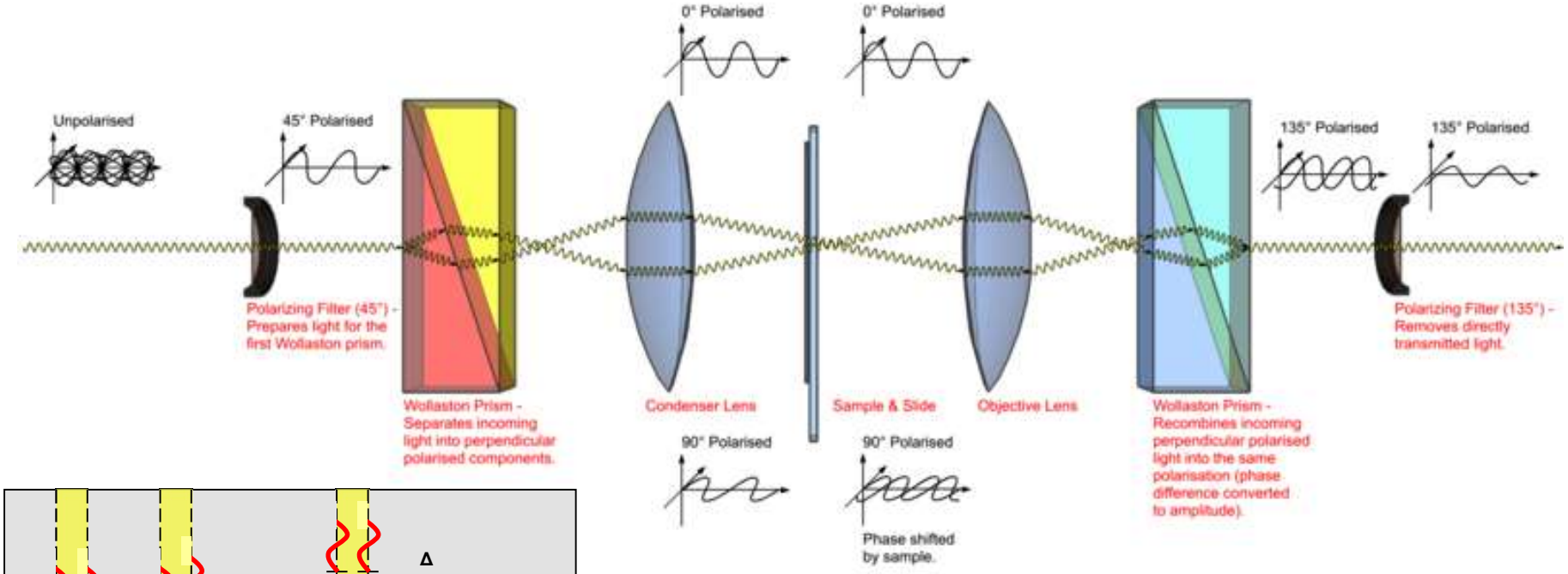
→ *we do not have microscopes set up for polarization contrast*

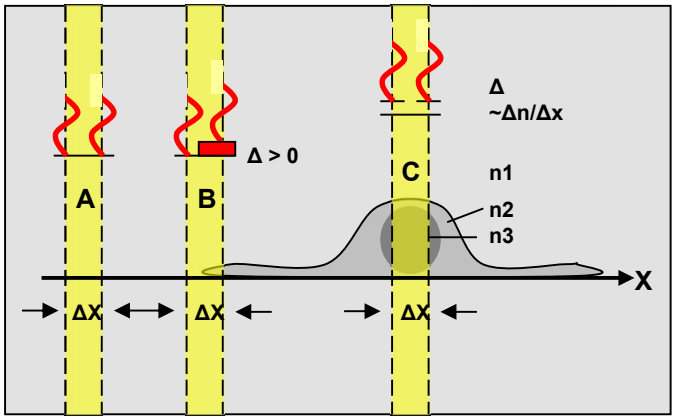
Sugar crystals

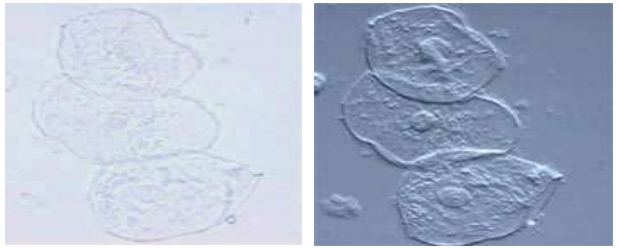


D(ifferential) I(nterference) C(ontrast)

Principle: Also known as Nomarski microscopy. Uses polarized light for illumination. Synchronizing of the different phases of incident and transmitted light is done by a set of prisms and filters introduced into the light path.







→ *most of our microscopes are set up for DIC*

Contrasting techniques - a summary

- Brightfield -absorption

  Light is transmitted through the sample. Only useful for specimens that can be contrasted via dyes. Very little contrast in unstained specimens.

- Darkfield -scattering

  The illuminating rays of light are directed from the side so that only scattered light enters the microscope lenses, consequently the cell appears as an illuminated object against the view.

- Phase Contrast- phase interference

  Incident light [Io] is out of phase with transmitted light [I] and when the phases of the light are synchronized by an interference lens, a new image with greater contrast is seen

- Polarization Contrast -polarization

  Uses polarized light for illumination. Only when the vibration direction of the polarized light is altered by a sample placed into the light path, light can pass through the analyzer. The sample appears light against a black background.

- Differential Interference Contrast (DIC) – polarization + phase interference

  Also known as Nomarski microscopy. Synchronizing of the different phases of incident and transmitted light is done by a set of special condenser lens mounted below the stage of a microscope

- Fluorescence Contrast (->Ireen)

Final words from your friend - the objective

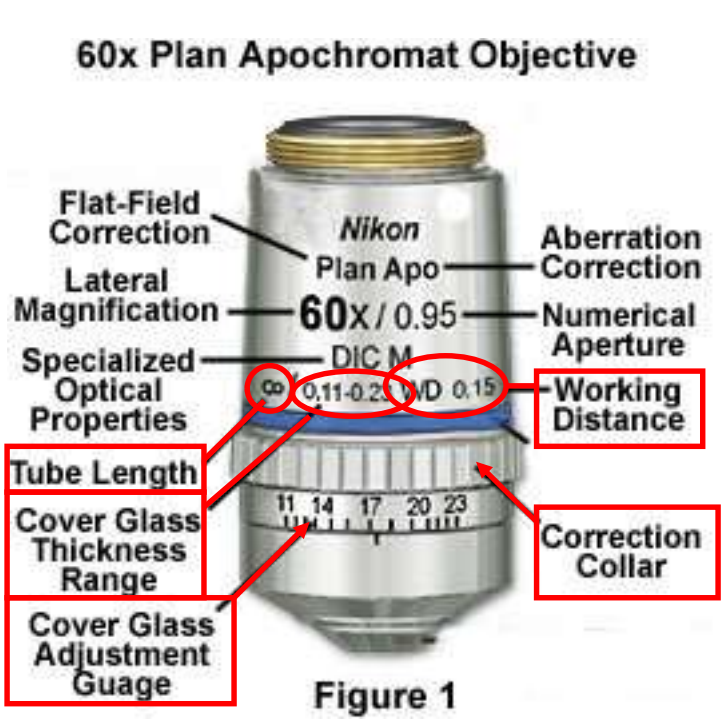
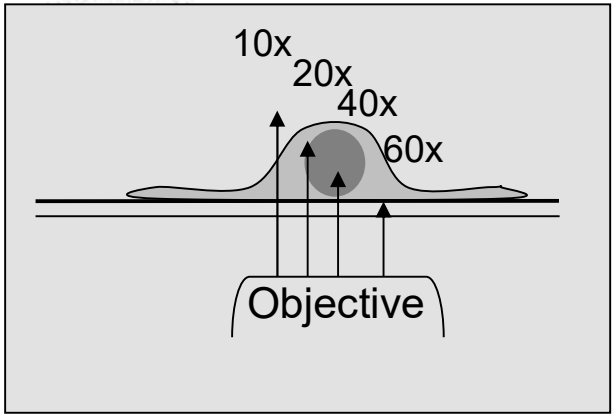


Figure 1

Coverslip-types:	
1:	0.13 - 0.17 mm
1.5:	0.16 - 0.19 mm
2.0:	0.19 - 0.23 mm



Useful links

- Zeiss – Microscopy from the very beginning
  http://www.zeiss.de/C1256B5E0047FF3F?Open
- Molecular Expressions homepage
  http://micro.magnet.fsu.edu/index.html
- Wikipedia
  http://en.wikipedia.org/wiki/Main_Page