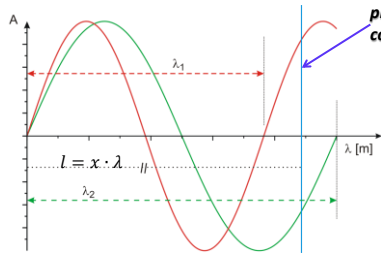
Biomolecular structure. Diffraction, X-ray crystallography, light- and electron microscopy. CD spectroscopy, mass spectrometry



phase difference at a given distance — constructive/destructive interference

$\lambda_2 > \lambda_1$;

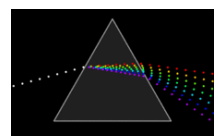
$$f = \frac{c_o}{\lambda_o} = \frac{c_2}{\lambda_2} = \frac{c_1}{\lambda_1}$$

$$\frac{\lambda_2}{\lambda_1} = \frac{c_2}{c_1} > 1;\ c_2 > c_1$$

$c_2 > c_1 \Rightarrow n_m = \frac{c_o}{c_m}$

$n_{m,1} = \frac{c_o}{c_{m,1}}; n_{m,2} = \frac{c_o}{c_{m,2}}$

$c_m = f(\nu) \rightarrow n_m(\nu) = \frac{c_o}{c_m(\nu)}$



Dispersion

Dispersion: A physical quantity possesses frequency dependence.

Physical properties of light (electromagnetic waves)

Intensity ~ A^2 (E,B) — wave property;
Phase — φ — wave property;
Frequency — ***f*** or ν — wave property;
Energy of a single photon ε = h·f — particle property;

Propagation speed — c (c_{vacuum} ~ 3.0 ·10^8m/s.)

- refractive index: $n = \frac{c_{vacuum}}{c_{medium}}$ — how much is the light slower in a medium than in vacuum

Law of ***energy*** conservation: $\varepsilon_{light} = h \cdot f = h \cdot \frac{c_{vacuum}}{\lambda_{vacuum}} = h \cdot \frac{c_{medium}}{\lambda_{medium}}$

$$n = \frac{c_{vacuum}}{c_{medium}} = \frac{\lambda_{vacuum}}{\lambda_{medium}}$$

$n_{21} = \frac{c_2}{c_1} = \frac{\lambda_2}{\lambda_1}$ consequence: if $c_2 > c_1$ then $\lambda_2 > \lambda_1$;

Polarized light — observations

unpolarized

polarized

combined

ctenophore plankton. Almost transparent to normal vision (left), it acquires good contrast between crossed polarizers (center), and even better with combined processing (right).

squid observed in polarized light

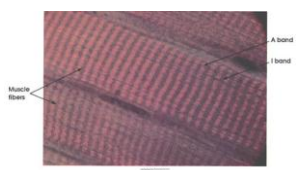
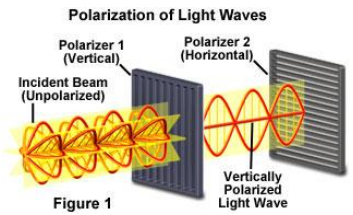
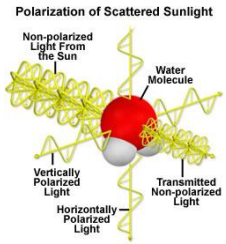


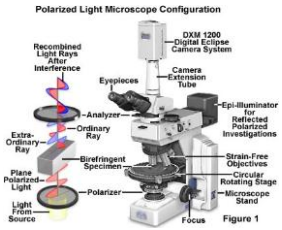
image with !!polarized light!!

The names given to the two major transverse striations of skeletal and cardiac muscle are derived from the studies with routine light microscopic techniques; Alternating dark and light bands are seen within striated muscle fibers, Brücke* (1858).
Polarization microscopy reverses the appearance of the dark band, which becomes bright, and the light band, which appears dark. (measured with crossed analysator and polarisator)

Polarization of light

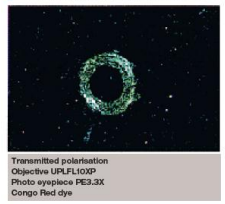






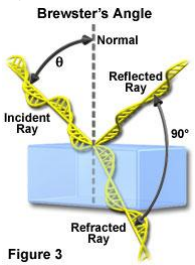
1-3 Renal vein with deposited amyloid (brightfield image)

1-4 Renal vein with deposited amyloids (polarised light image)



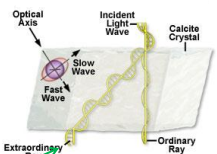
crossed polarisator and analysator !

A.) Polarization due to reflection



this ray is completely polarized at Brewster's angle

B.) Birefringent crystal



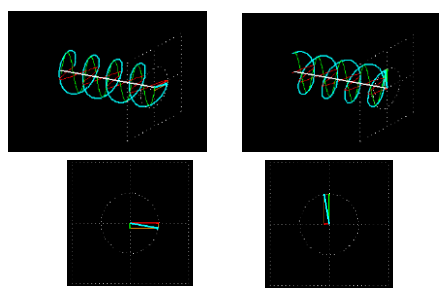
In some crystals, substances:
$c_m = f$ (polarization plane)

this direction is faster, than the other, for a ray of such a polarization plane

Optical birefringence: refractive index (~ speed of light) depends on polarization plane of a ***linear polarized light***

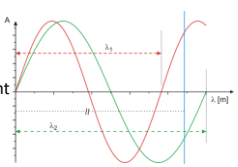
Fermat's principle: or the principle of least time is the principle that the path taken between two points by a ray of light is the path that can be traversed in the least time.

Circular polarized light (left, right)



In some substances:
$c_m = f$ (rotation sense of circular polarization; L/D)

circular birefringence: refractive index (~ speed of light) depends on ***polarization sense of a circular*** polarized light

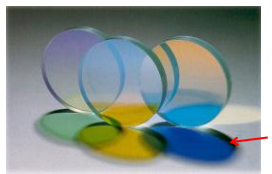


Dichroism

1.) Certain wavelengths of light ***either pass through or are reflected*** from a material surface; (see pendant from dichroic glass — dichoric filters for microscopes)

2.) Refers to the phenomenon that light ***in different polarization states*** travelling through a material ***are absorbed by different amounts***.

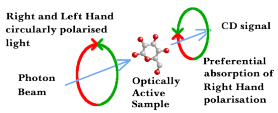
ad. 1.:

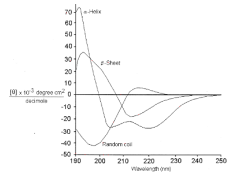


dichroic filters used e.g. in fluorescence microscopes as beam splitters

colors of reflected light

ad. 2.:





Linear-, circular dichroism

- linear — one of the plane polarized light is more absorbed than the other
- circular — one of the circular polarized light (L/D) is more absorbed than the other (CD)

recall:

Dispersion: A physical quantity possesses frequency dependence.

CD dispersion or Optical Rotary Dispersion (ORD) — a method for resolving structural properties of molecules

Summary

$$c_m = f\left(\nu, \underbrace{polarization\ plane(\nu), polarization\ sense(\nu)}_{\text{polarization microscopy, polarimetry, CD(ORD)}}\right)$$

refractometry

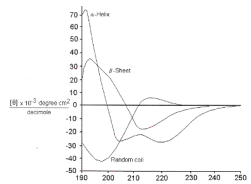
Circular dichroism — a method for investigating biomolecular structures

Principle: wavelength dependent circular dichroism

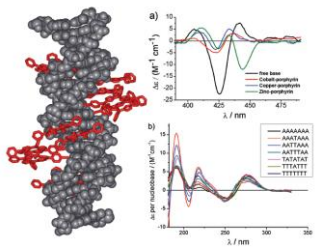
- ✓ absorption of circular polarized light depends on polarization sense: left- or right;

AND

- ✓ absorption of circular polarized light depends on frequency (wavelength) — dispersion



protein CD-spectra

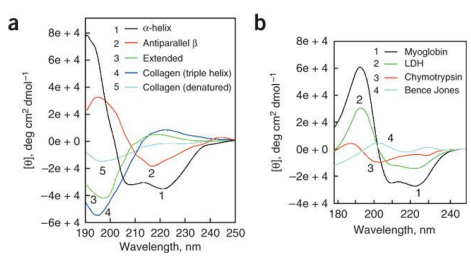


CD-spectra of DNA

What is a CD-spectrum?

x-axis: wavelength or frequency (mostly wavelength)
y-axis: difference in molar extinction coefficients of the left- and right circular polarized lights (or a quantity proportional to this coefficient)

How to determine secondary structure of a protein?



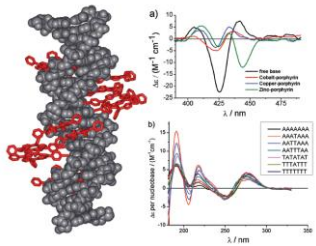
Types of secondary structures of proteins:
- ✓ ***α***-helical
- ✓ ***β***-sheet
- ✓ ***β***-turn
- ✓ random coil

Theory: a signal amplitude, at a given wavelength, is a sum of all the possible structures

Tasks:
- 1. measure a spectrum for each distinct secondary structure;
- 2. combine these distinct spectra with appropriate weighting factors to get the observed one

Results:
relative contributions from each conformation = weighting factors

Types of secondary structures of DNA:
A-DNA
B-DNA
Z-DNA

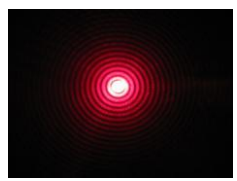


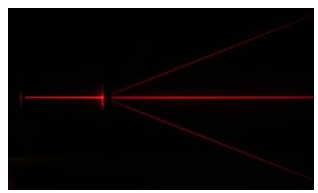
CD-spectra of DNA

What is diffraction?

Richard Feynman said that:
"no-one has ever been able to define the difference between interference and diffraction satisfactorily. It is just a question of usage, and there is no specific, important physical difference between them."

He suggested that when there are only a few sources, say two, we call it interference, (as in Young's slits)***, but with a large number of sources, the process be labeled diffraction.***



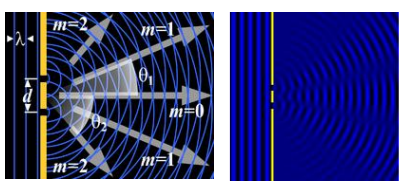


Diffraction of waves as a mean for imaging

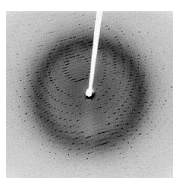
Image: a usually two-dimensional information transferring and storage medium

There is no image when no information is present; If a physical quantity, a signal, does not contain information, it could not result in an image

However, a wave which does not bear information can still inevitably be required to be able to produce an image (without the central maximum ray, no resolved microscopic image is formed)

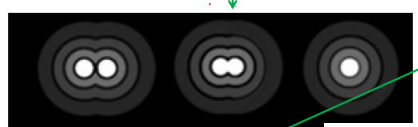


Young's slits

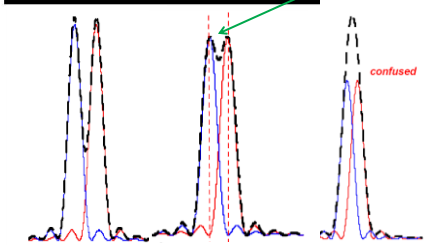


X-ray diffraction pattern

Rayleigh Criterion : Two light sources must be separated by at least the diameter of first dark band.

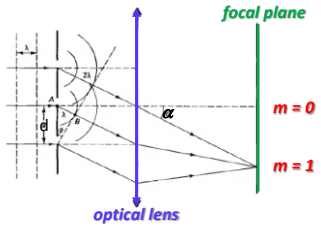


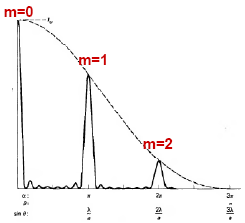
$$x_{min} = d = 0.61 \cdot \frac{\lambda}{n \cdot \sin\alpha}$$



Diffraction grating equation

Number of slits is very high (e.g. cells under a microscope)





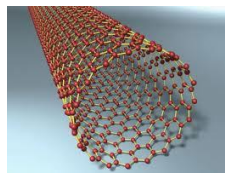
$$\sin \alpha_m = const. \cdot \frac{m \cdot \lambda}{d}$$

$$\sin \alpha_m = 0.61 \cdot \frac{m \cdot \lambda}{n \cdot d}$$

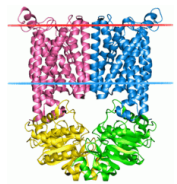
relationship between minimum resolvable distance ***d*** and wavelength ***λ;***
the central maximum (m=0) does not contain information about the grating characteristics

Lower limit of usual optical resolution ~ 200 nm

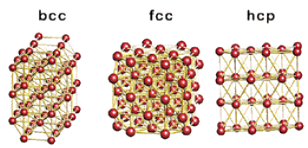
How to resolve structures (gratings) below 200 nm?



carbon nanotube



ATP-binding cassette transporters (ABC-transporter)

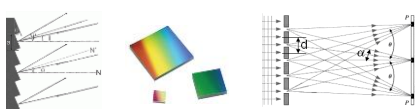


different types of iron lattices

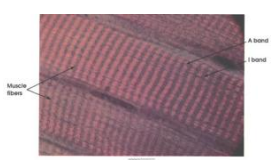
What is a grating?

In most general meaning: a grating is a construction which consists of a periodically repeated physical property, creating a periodic structure.

optical component with a periodic structure



physical property:
transparency (transmission amplitude gratings);
reflectance (reflection amplitude gratings);
refractive index (phase gratings);



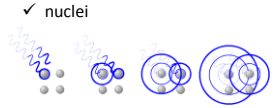
degree of polarization

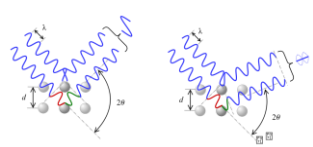
Decrease of wavelength? Method I.

$$d_{min} \approx \frac{m \cdot \lambda}{\sin \alpha}$$

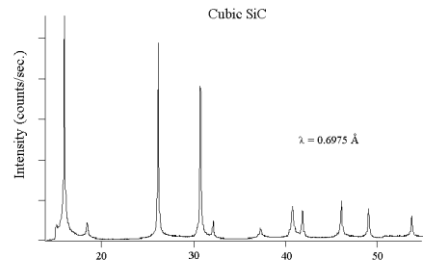
Whether the wavelength can be in pm range?
- X-ray — below optical region
- diffraction angle might be also small (< 1 deg.)
- Image reconstruction is required
- resolution on/below nm scale

Physical property of grating — reflection:
- ✓ electrondensity
- ✓ nuclei



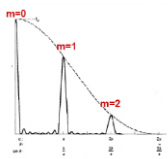


constructive destructive



$$d = \frac{n \cdot \lambda}{2 \cdot \sin\theta}$$

recall: optical grating



$$d_{min} \approx \frac{m \cdot \lambda}{\sin\alpha}$$

Decrease of wavelength? Method II.

Instead of em.w. electron beam

$$\lambda = \frac{h}{m_e v_e}$$

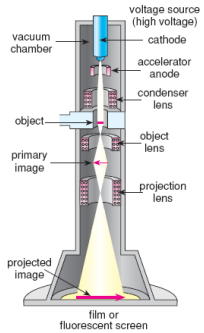
wavelength region ~ pm

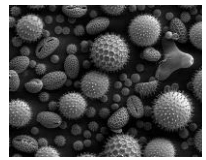
accelerating voltage 40-400 keV

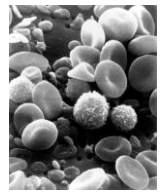
resolution down to ~ 50pm

0.1 nm

TEM

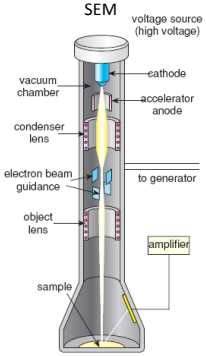


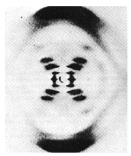




1 nm

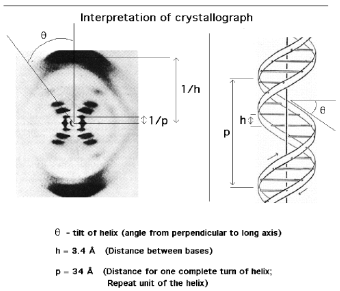
SEM



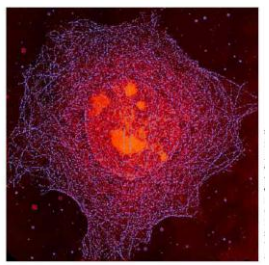


X-ray diffraction pattern from B form of DNA

After Glusker & Trueblood, *Crystal Structure Analysis: A Primer*, Oxford Univ. Press, New York, ©1972, p. 137, Fig. 39(b); found in Tinoco, Sauer & Wang, *Physical Chemistry*, Prentice-Hall, Inc., Englewood Cliffs, N. J., ©1978.



Special techniques allow X-ray imaging!

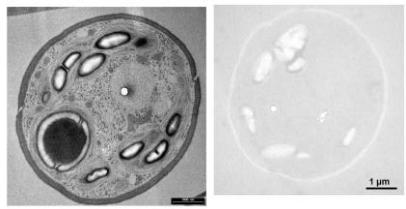


Contrast enhancement

Possible sources of contrast:
Absorption of electrons
Scattering of electrons
(Diffraction and phase contrast)

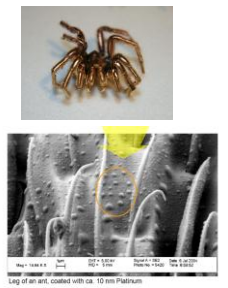
None of them is present in biological tissues

TEM



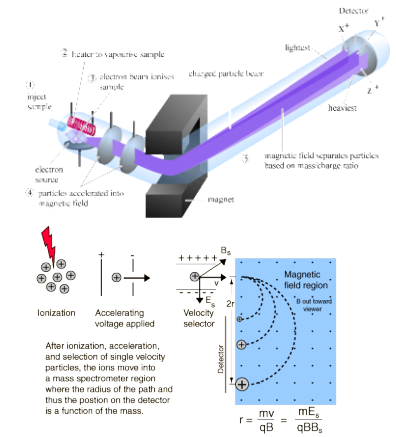
treatment with heavy metals (U, Pb, Os)

SEM



coating with metal vapors

Mass spectrometry



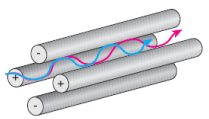


Figure X.51. Illustration of the quadrupole mass analyzer, featuring the paths of an ion that will be selected (red) and an ion with different ratio m/z (blue)