Biomolecular structure

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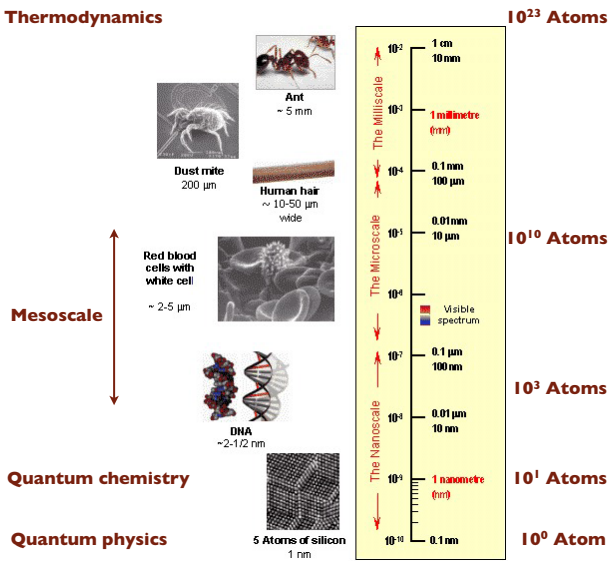
Why structure...?



Outline

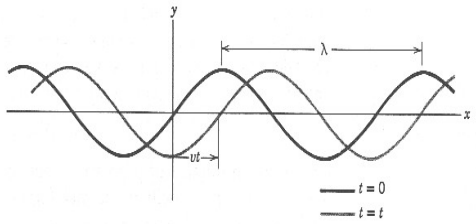
- Diffraction, interference
- X-ray diffraction, X-ray crystallography
- Diffraction-limited microscopies
- Beating the diffraction limit
- Polarization; CD spectroscopy
- Mass spectrometry

We access structure via interaction with electromagnetic waves

Properties of electromagnetic waves

Amplitude (A) - Intensity ~A^2
Oscillation time (**period**): duration of a single oscillation ("T").
Frequency: inverse of period (f).
The wave propagates with a given **velocity** ("phase velocity", "v" or "c")
Distance between points of identical phase: "**wavelength**" (λ)
Phase difference (φ): difference (in time or space) between identical points of waves

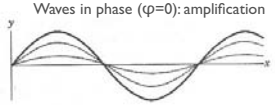
$$\lambda = cT = \frac{c}{f}$$



Diffraction and interference

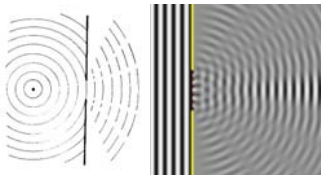
Interaction of waves:
constructive or desctructive interference (amplification versus cancellation)

Waves in phase (φ=0): amplification

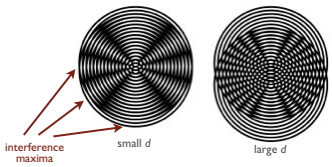


If φ=π : cancellation

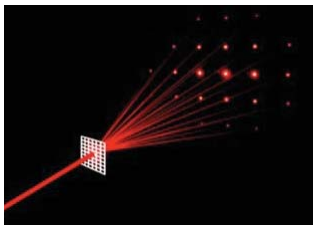
Diffraction through slits with a distance comparable to wavelength (=pointlike slits separated by distance *d*, where *d*~λ)

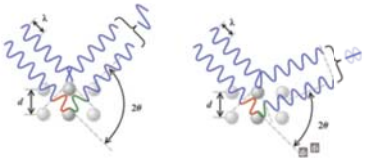


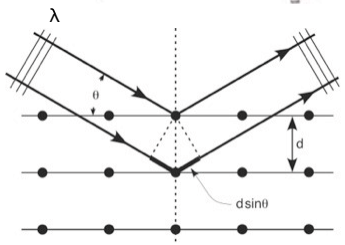
Interference pattern depends on distance (*d*) separating the wave sources



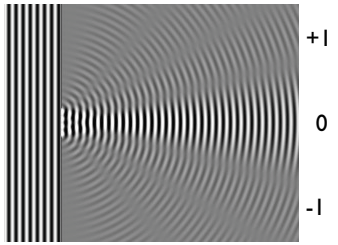
Interference pattern of a 2-dimensional grating



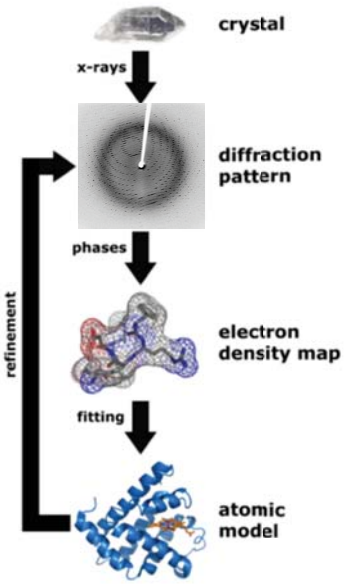




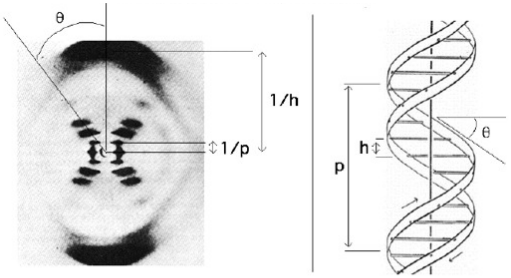
Condition of constructive interference: $2d\sin\theta = n\lambda$



X-ray crystallography



Solving DNA structure with X-ray crystallography

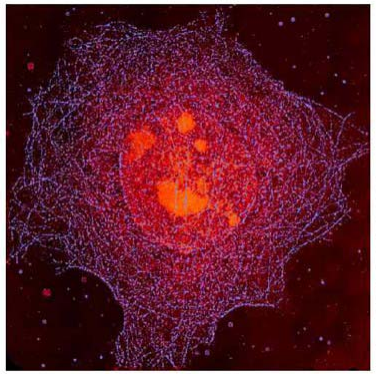


θ - tilt of helix (angle from perpendicular to long axis)

h = 3.4 Å (Distance between bases)

p = 34 Å (Distance for one complete turn of helix; Repeat unit of the helix)

Image formation with X-ray



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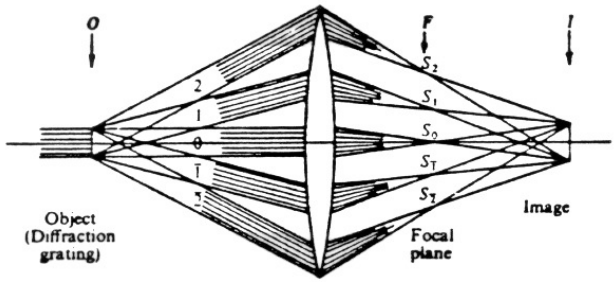
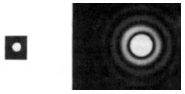
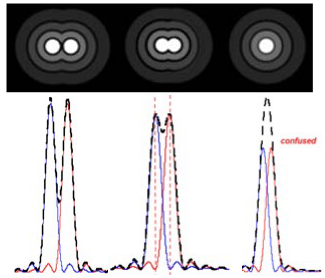
Imaging with waves



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



Due to diffraction: image of a point object is an Airy disk



Smallest resolved distance (Abbé):

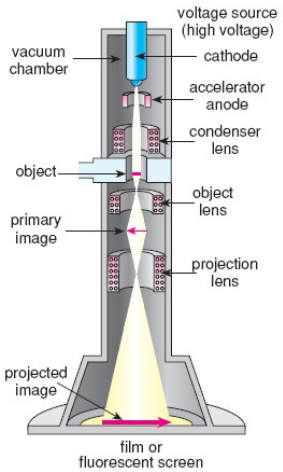
$$d = \frac{0.61\lambda}{n\sin\alpha}$$

How to beat the Abbé formula?

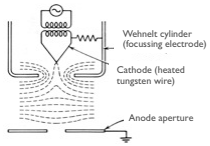
- Improve equation parameters (decrease wavelength, increase numerical aperture)
- Convert resolution problem into position-determination problem
- Use non-diffraction-limited imaging

Image with electron waves: the electron microscope

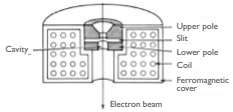
Transmission electron microscope (TEM)



Ray source: electron gun



Focussing: diverting the electron with magnetic lens



$$F = eBV_e \sin\alpha$$

F=force on the electron; e=electron's charge; B=magnetic field; V_e=electron's speed; α=angle between the optical axis and the direction of the magnetic field

Resolution: $$d = \frac{\lambda}{\alpha}$$

d=smallest resolved distance
λ="de Broglie" wavelength
α=angle between the optical axis and the direction of the magnetic field

Based on the de Broglie wavelength the theoretical resolution is: d~ 0,005 nm (=5 pm).

Resolution and contrast in the electron microscope

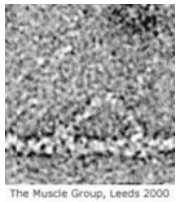
A. Theoretical resolution:
Modified Abbe-formula (for small α angles)
Based on electon velocity (100000 km/s), d=0.005 nm

$$d = \frac{\lambda}{\alpha}$$

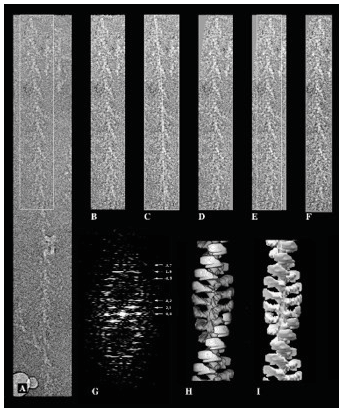
B. Real resolution: limited by small NA, ~0.1 nm.
Because of small NA, depth of focus is large (several μm).

C. Practical resolution in biological samples:
1/10 of section thickness.

D. Contrast mechanism: electron diffraction
Contrast enhancement: by electron dense dyes



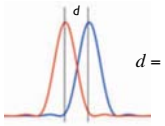
Cryo-electron microscopy, particle-analysis image reconstruction



Super-resolution microscopy

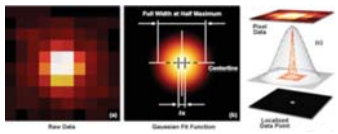
Resolution problem is converted into position-determination problem

Resolution problem (Abbé)

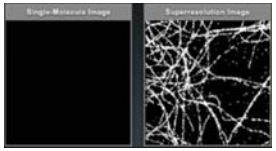


$$d = \frac{0.61\lambda}{n\sin\alpha}$$

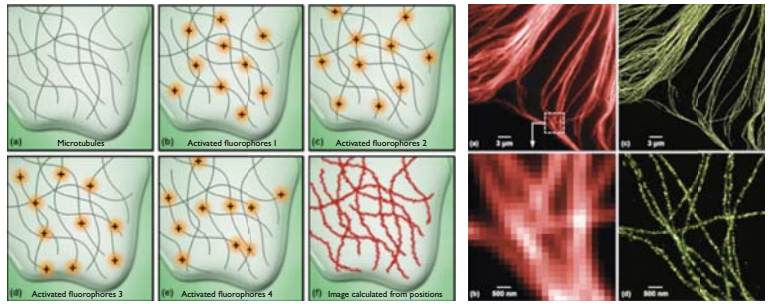
Position determination problem
(precision depends on photon count)



"Stochastic" data collection, single fluorophores



STORM: "stochastic optical reconstruction microscopy"

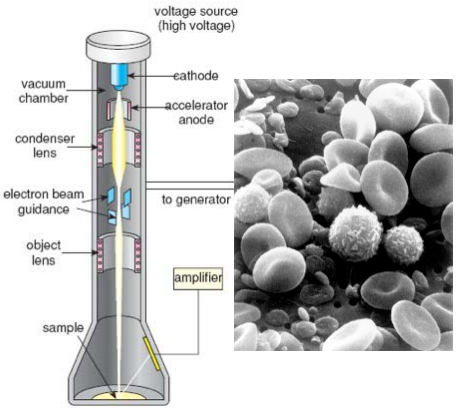


Data collection process

Microtubular system

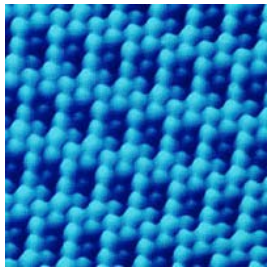
Depart from diffraction-limited imaging

Scanning electron microscope (SEM)



Scanning-probe microscopies (SPM, AFM)

Oxygen atoms on the surface of rhodium crystal



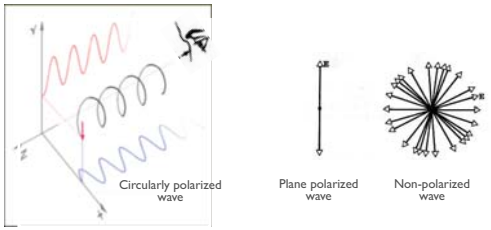
Unit of nanoworld:
1 nanometer

Polarization

- **Polarization:** oscillation is oriented in some preferred direction
- **Birefringence** is related to polarization: anisotropic propagation velocity
- Only **transverse** waves can be polarized.

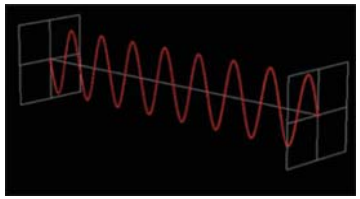
Polarization of Electromagnetic waves

Head-on view of polarization plane:

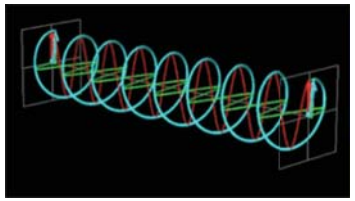


Interaction of polarized light with matter

Linearly (plane) polarized light

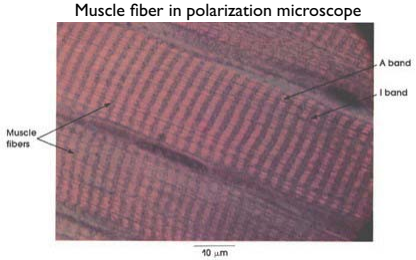


Circularly polarized light

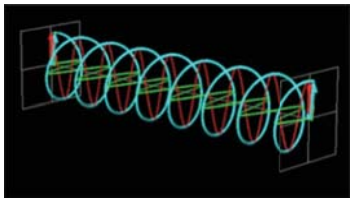


Rightward rotating (D)

Muscle fiber in polarization microscope



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



Leftward rotating (L)

Circular birefringence: refractive index (~speed of light) depends on *rotational direction of circularly* polarized light

Circular dichroism (CD) spectroscopy

Principle: wavelength-dependent differential absorption of L/D circularly polarized light

- Absorption of circularly polarized light depends on rotation direction (L/D) **AND**
- Absorption of circularly polarized light depends on frequency (wavelength)

Dichroism ("two colors"):
1.) Certain wavelengths of light either pass through or are reflected from a material surface
2.) Light in different polarization states travelling through a material are absorbed by different amounts.

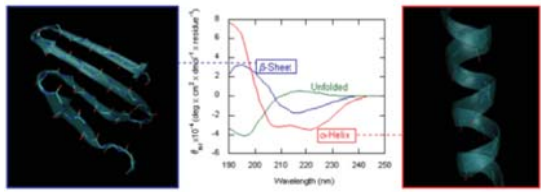
Chiral molecules display strong circular dichroism

CD spectrum:

x-axis: wavelength or frequency (mostly wavelength); **y-axis:** difference in molar extinction coefficients of L and D circular polarized light (or a quantity proportional to this coefficient)

Circular dichroism:
$\Delta A(\lambda) = A(\lambda)LCPL - A(\lambda)RCPL$

λ: wavelength

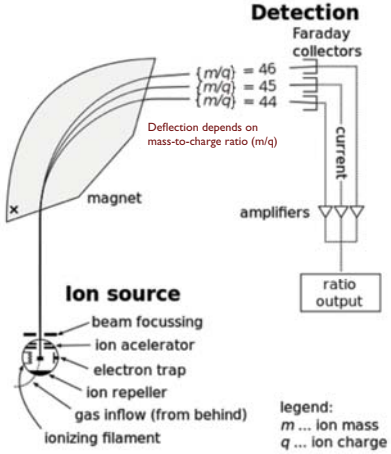


Mass spectrometry

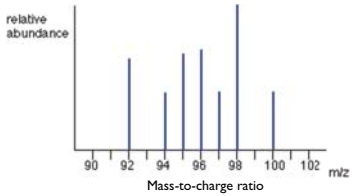
Mass spectrometry (MS): analytical technique producing spectra of the masses of the atoms or molecules in a sample. The spectra are used to determine the elemental or isotopic signature, thereby elucidating the chemical structures of molecules.

Steps:

1. Ionization
2. Acceleration
3. Deflection
4. Detection



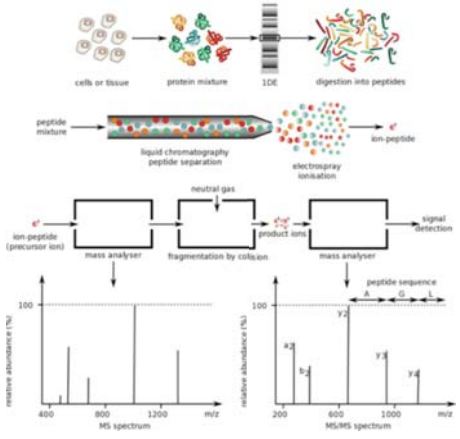
Result: "Stick" diagram



Spectrum is compared with structure database

Mass spectrometry applications

Protein analysis (proteomics)



Real-time tissue analysis ("onco-knife")