The Theory of X-ray Diffraction

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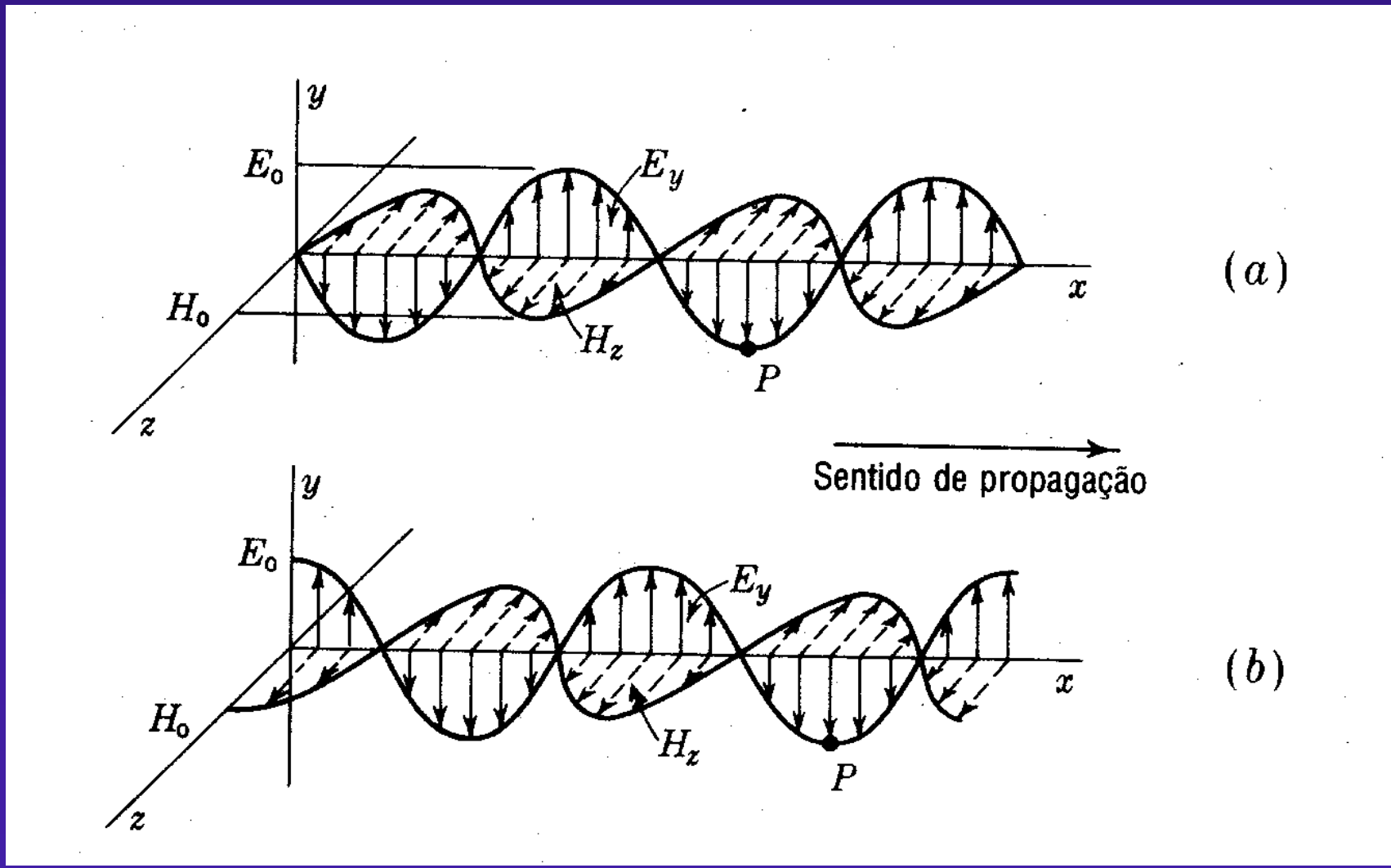
Form and Function at the Roots of Biology

Visible light in the description of nature

Taxonomy

Interaction of Electromagnetic Radiation with matter



Interaction of Electromagnetic Radiation with matter

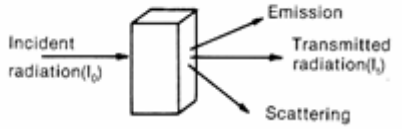


Figure 2.9 Electromagnetic radiation incident on a sample can give rise to absorption, emission, and scattering.

Biological information that can de obtained:

- Structure
- Dynamics
- Energetics
- Analytical

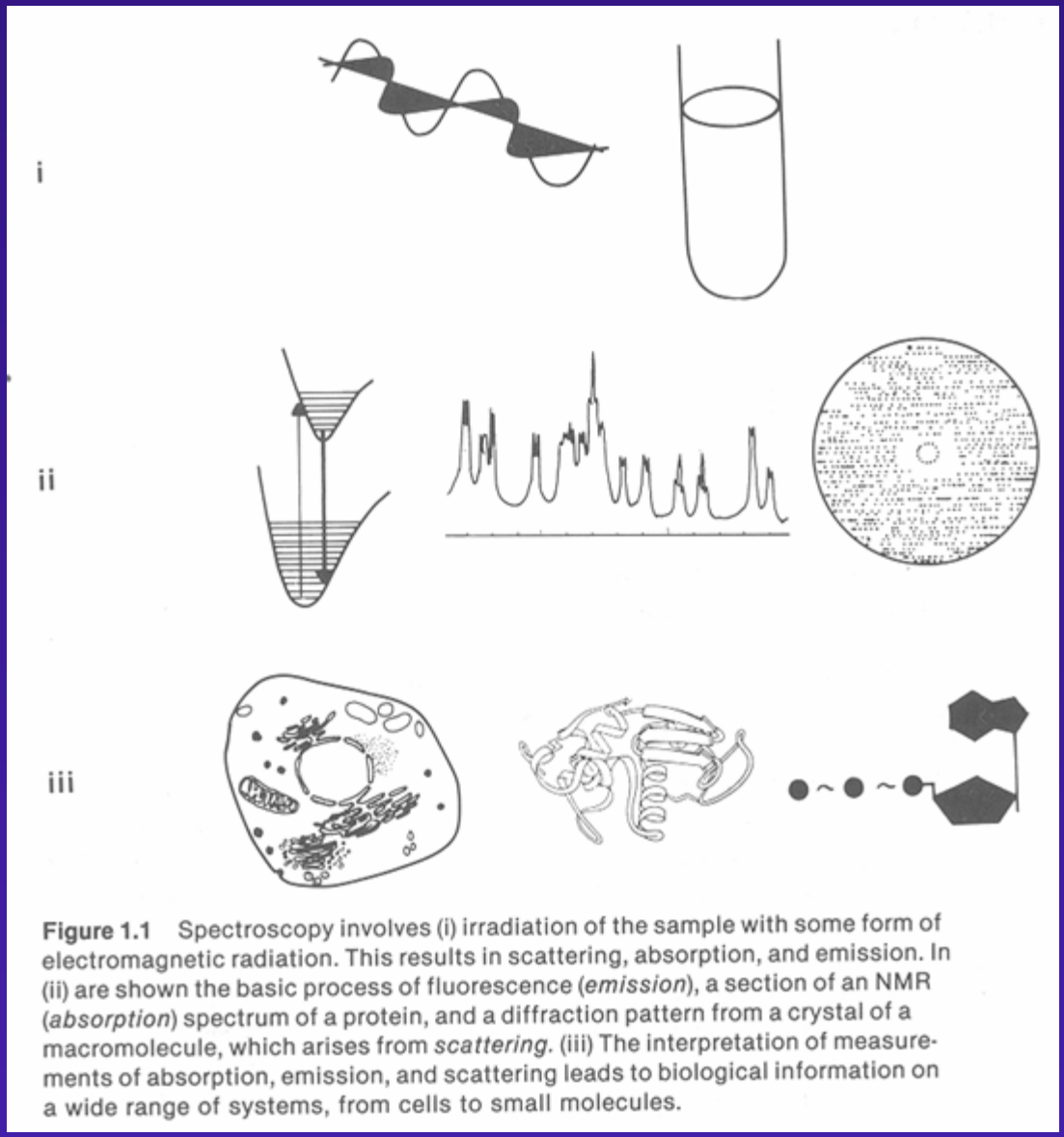
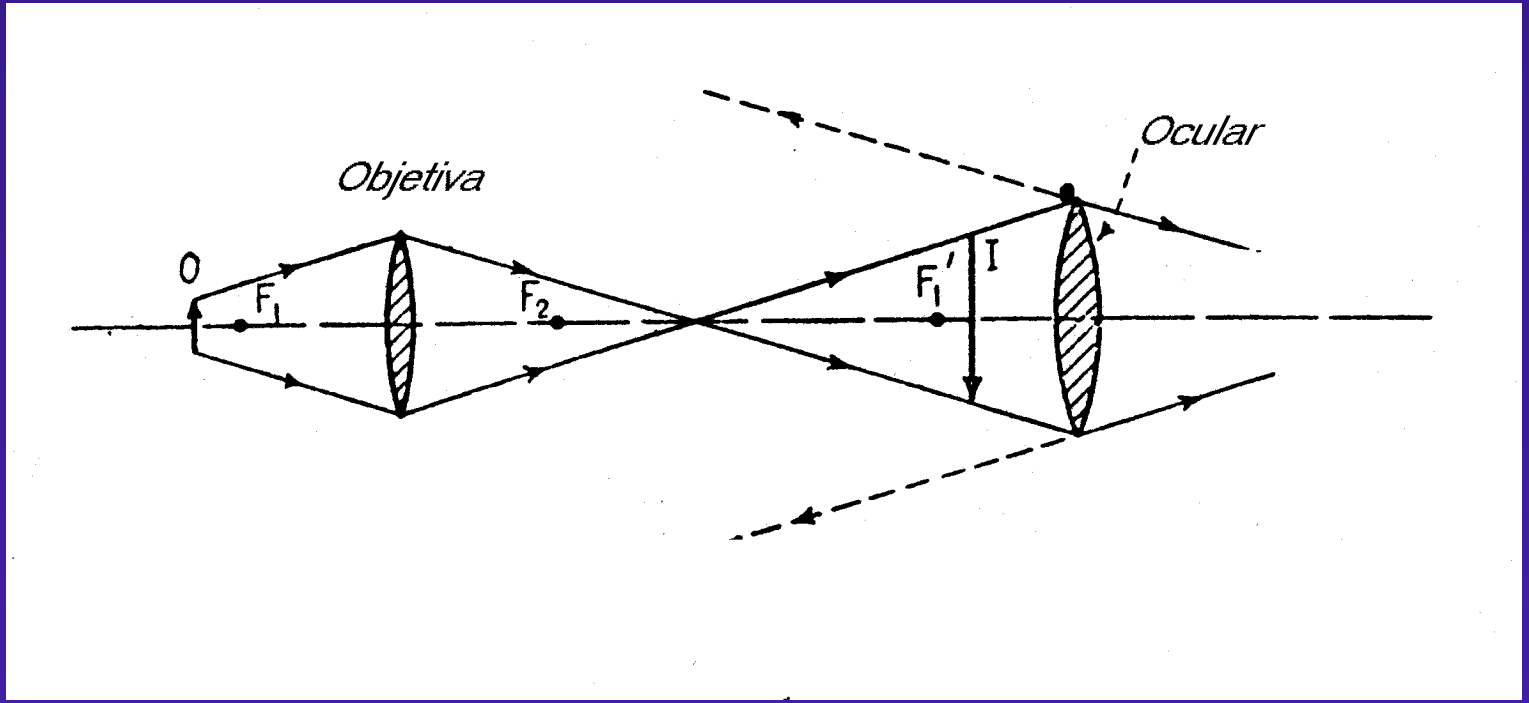
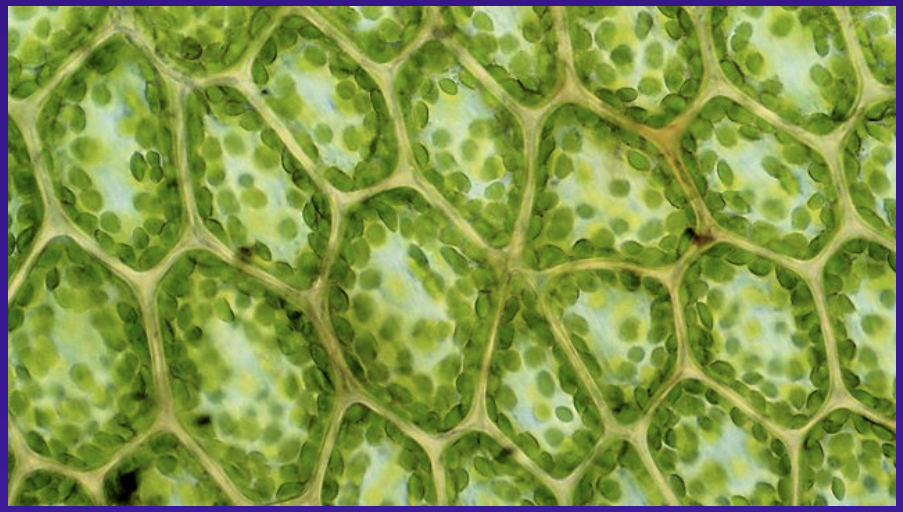


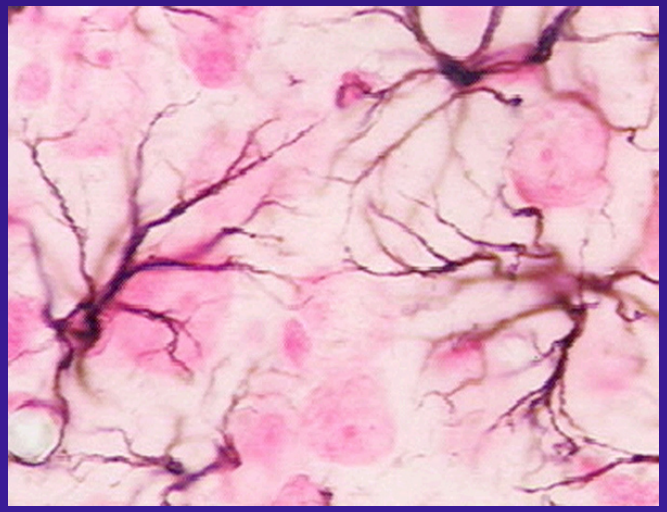
Figure 1.1 Spectroscopy involves (i) irradiation of the sample with some form of electromagnetic radiation. This results in scattering, absorption, and emission. In (ii) are shown the basic process of fluorescence (*emission*), a section of an NMR (*absorption*) spectrum of a protein, and a diffraction pattern from a crystal of a macromolecule, which arises from *scattering*. (iii) The interpretation of measurements of absorption, emission, and scattering leads to biological information on a wide range of systems, from cells to small molecules.

Optical Microscope

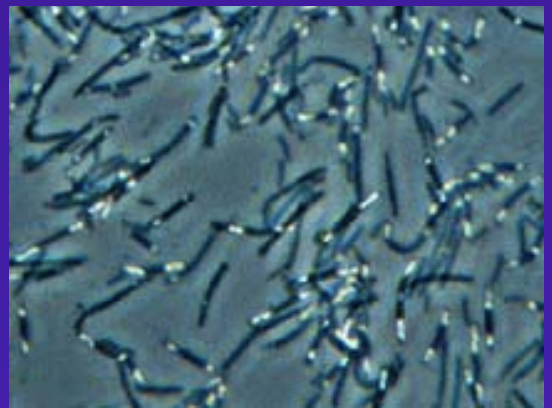


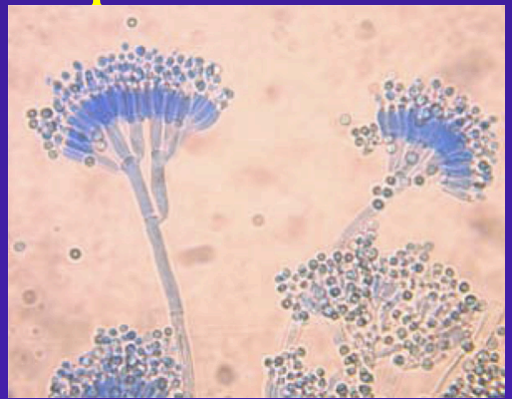
With the optical microscope it was possible to elucidate the cellular structure of living organisms

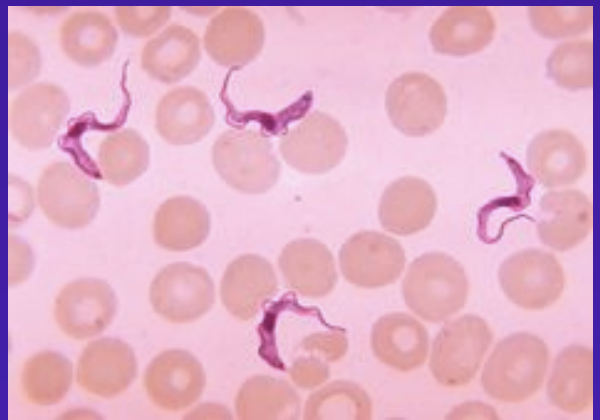




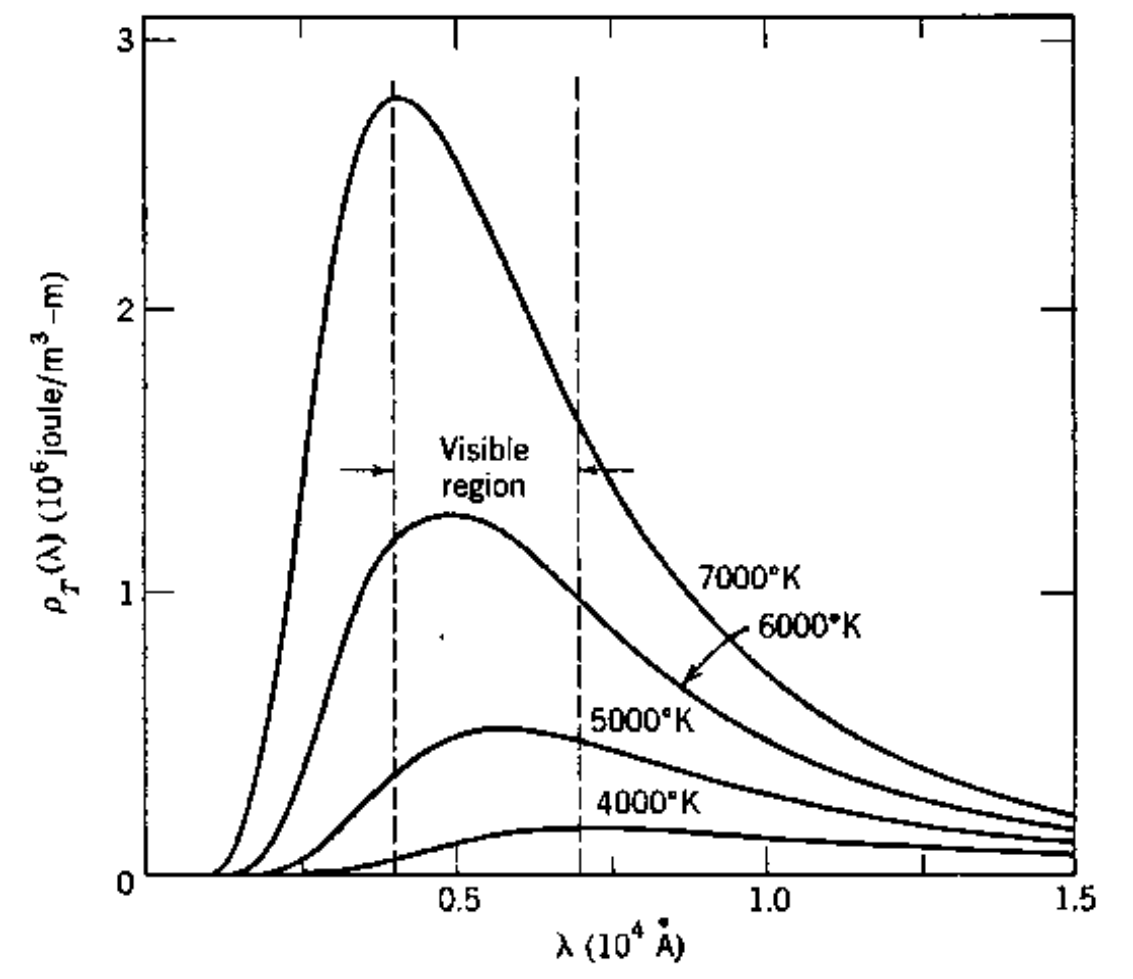
and to identify microbes as bacteria, fungi and parasites







The transtion from Classical to Modern Physics



Max Planck: the black-body radiation

Classical Mechanics → Newton's Laws

$$F = m \cdot a = m \frac{dx^2}{dt^2}$$

Quantum Mechanics → Schröedinger's Equation

$$\frac{\hbar^2}{2m} \cdot \frac{\partial^2 \psi(x,t)}{\partial x^2} + V(x,t)\psi(x,t) = i\hbar \frac{\partial \psi(x,t)}{\partial t}$$

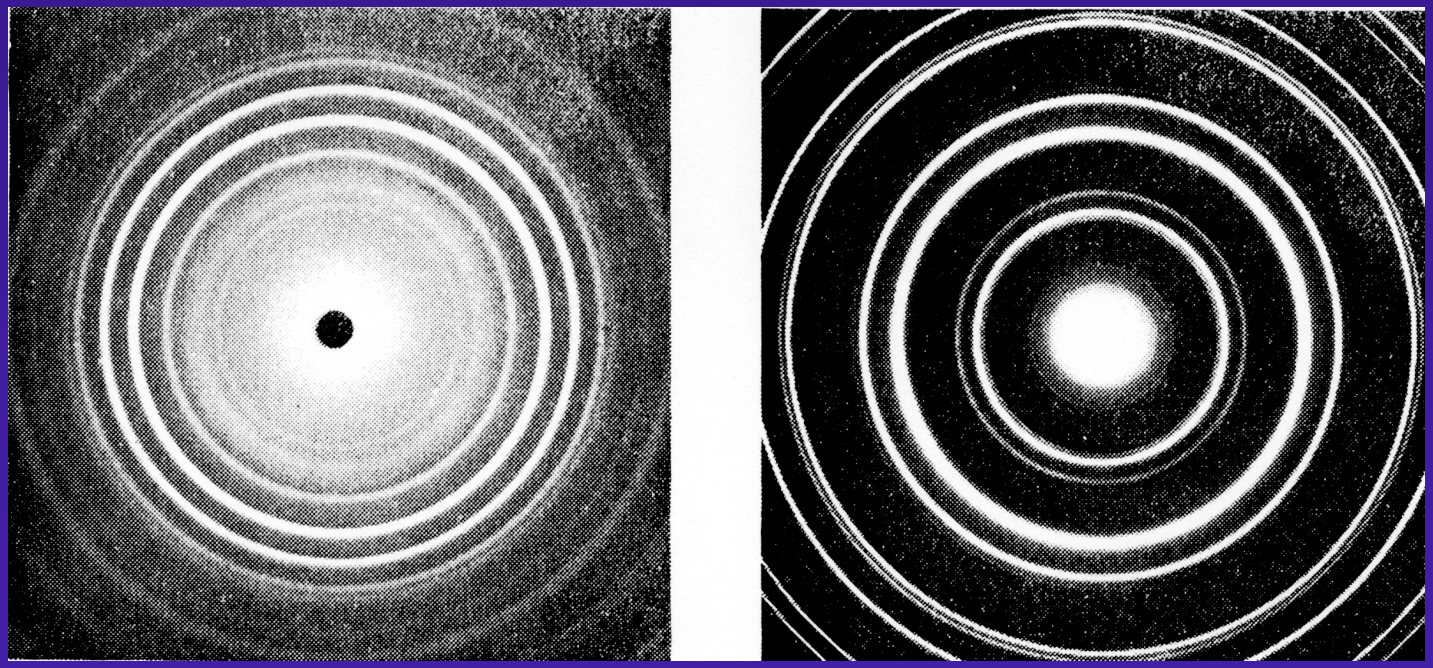
Particle-Wave Duality

Einstein: $E = h\nu$

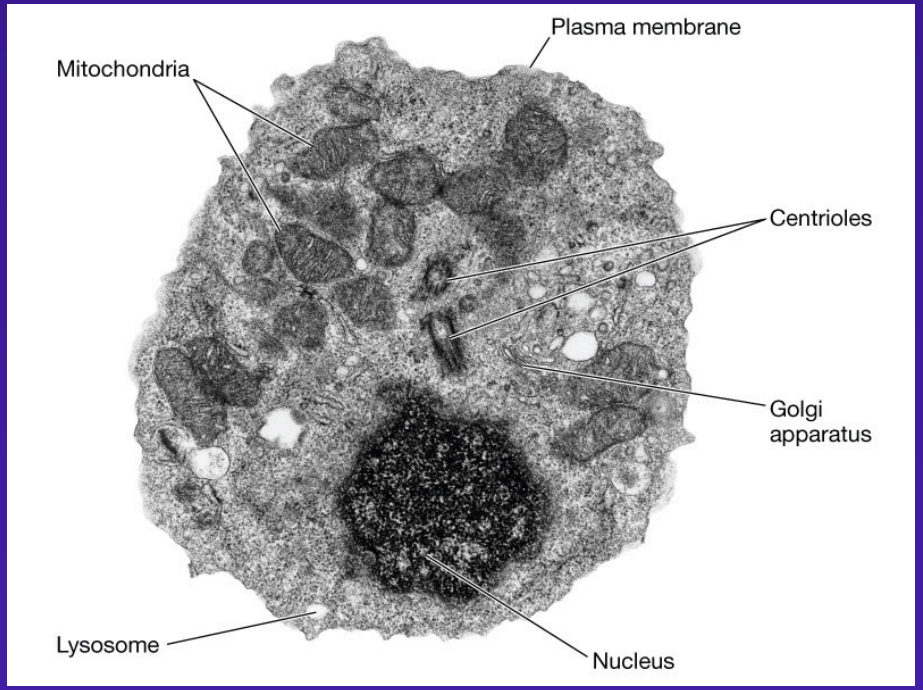
de Broglie: $p = \frac{h}{\lambda}$

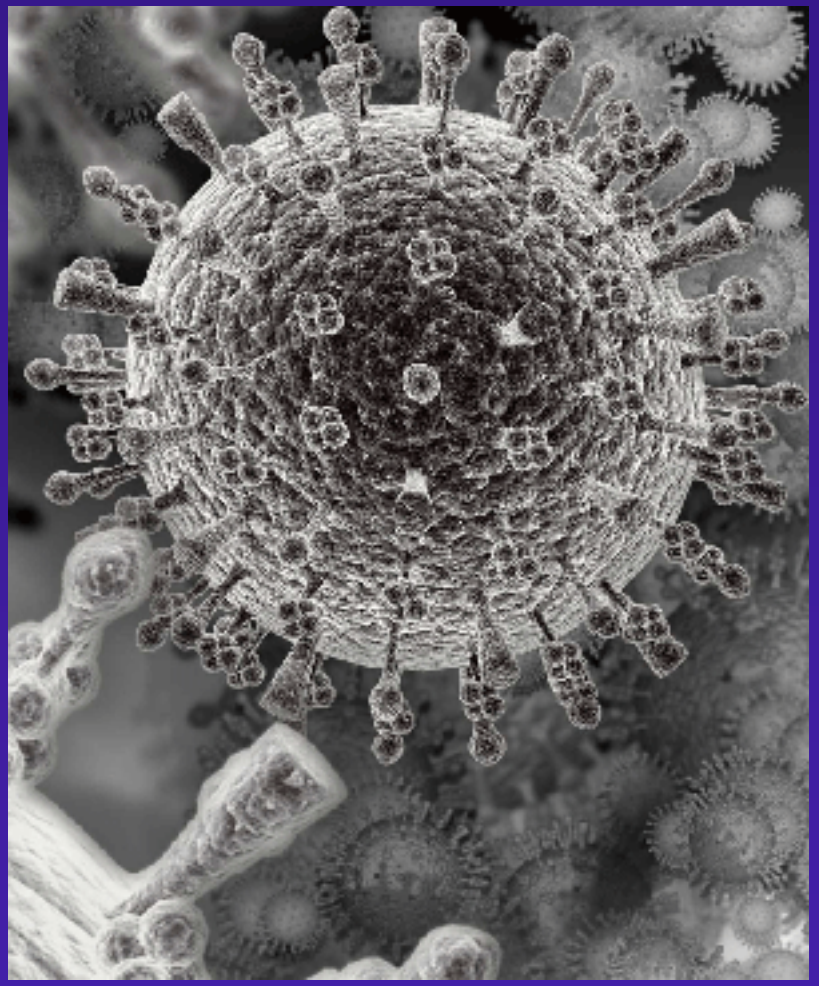
X-rays

Electrons

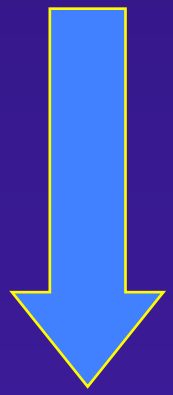


Electron microscopes (scanning and transmission) showed the subcellular structure of cells and also the structure of viruses



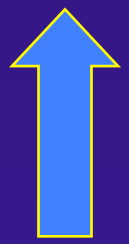


Modern Biology = Molecular Biology

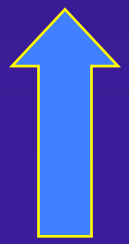


All the events associated with Life occur at the molecular level

Biological Function

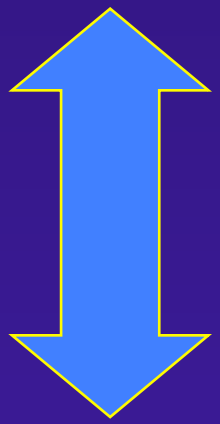


Macromolecular Structure



- Electromagnetic radiation (X-rays, radio-frequencies, UV-Vis, infrared)
- Partícles (electrons, neutrons)
- Microscopies (visible, electrons, atomic force)

Structural Molecular Biology



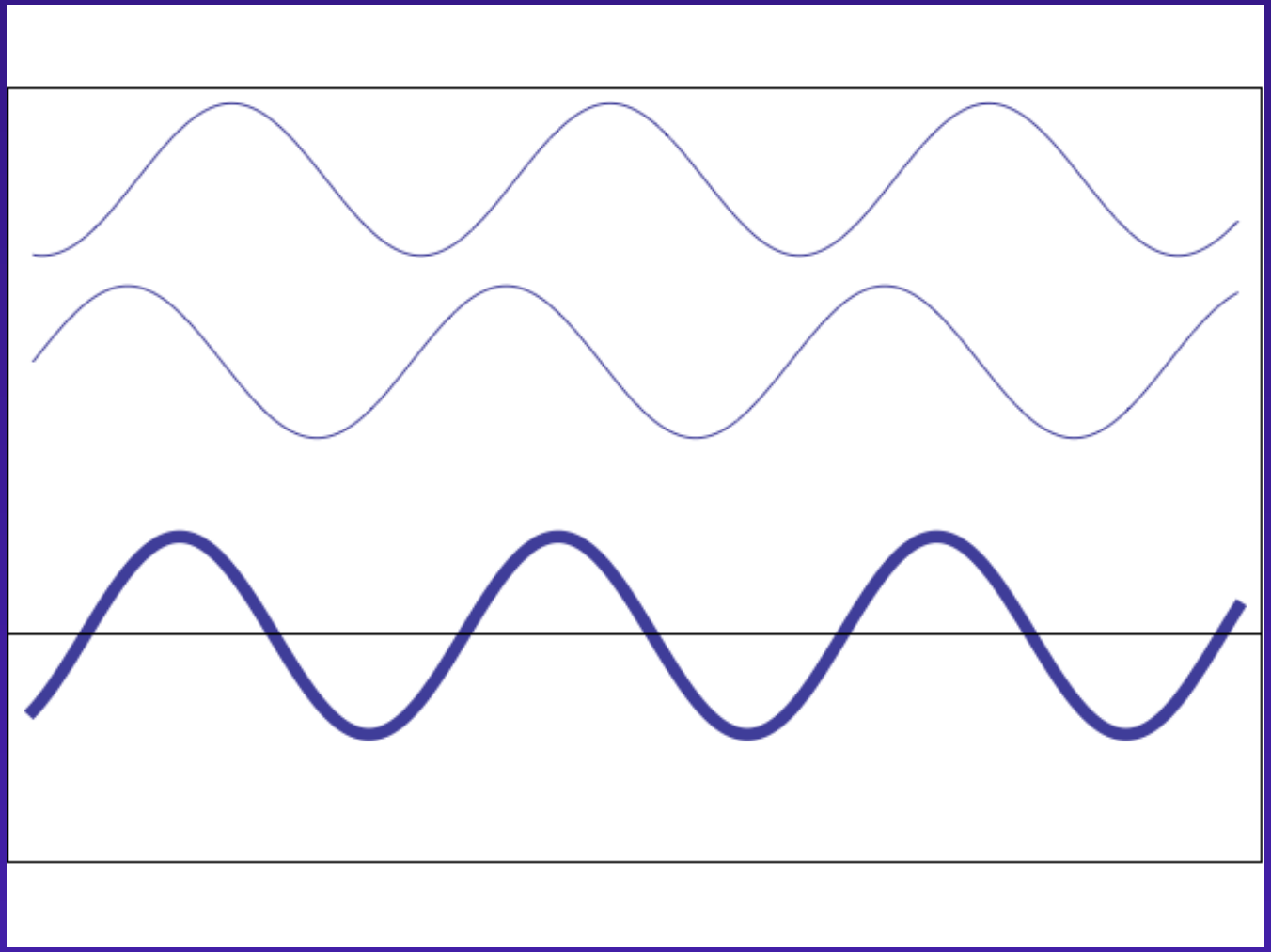
Structural studies of biological moleculas through phisical and chemical techniques and molecular biology

Structure, dinamycs, stability

Structural Biology

- High resolution experimental structures:
  - Protein Crystallography
  - NMR
  - Cryo EM
- Theoretical approaches:
  - Molecular Modeling
  - Molecular dynamics simulations
- Biophysical techniques
  - CD, Fluorescence, FTIR, Raman, XAFS, SAXS, MS, EPR, ...

Superposição de ondas



Waves o

Structural Biology

- High resolution experimental structures:
  - Protein Crystallography
  - NMR
- Theoretical approaches:
  - Molecular Modeling
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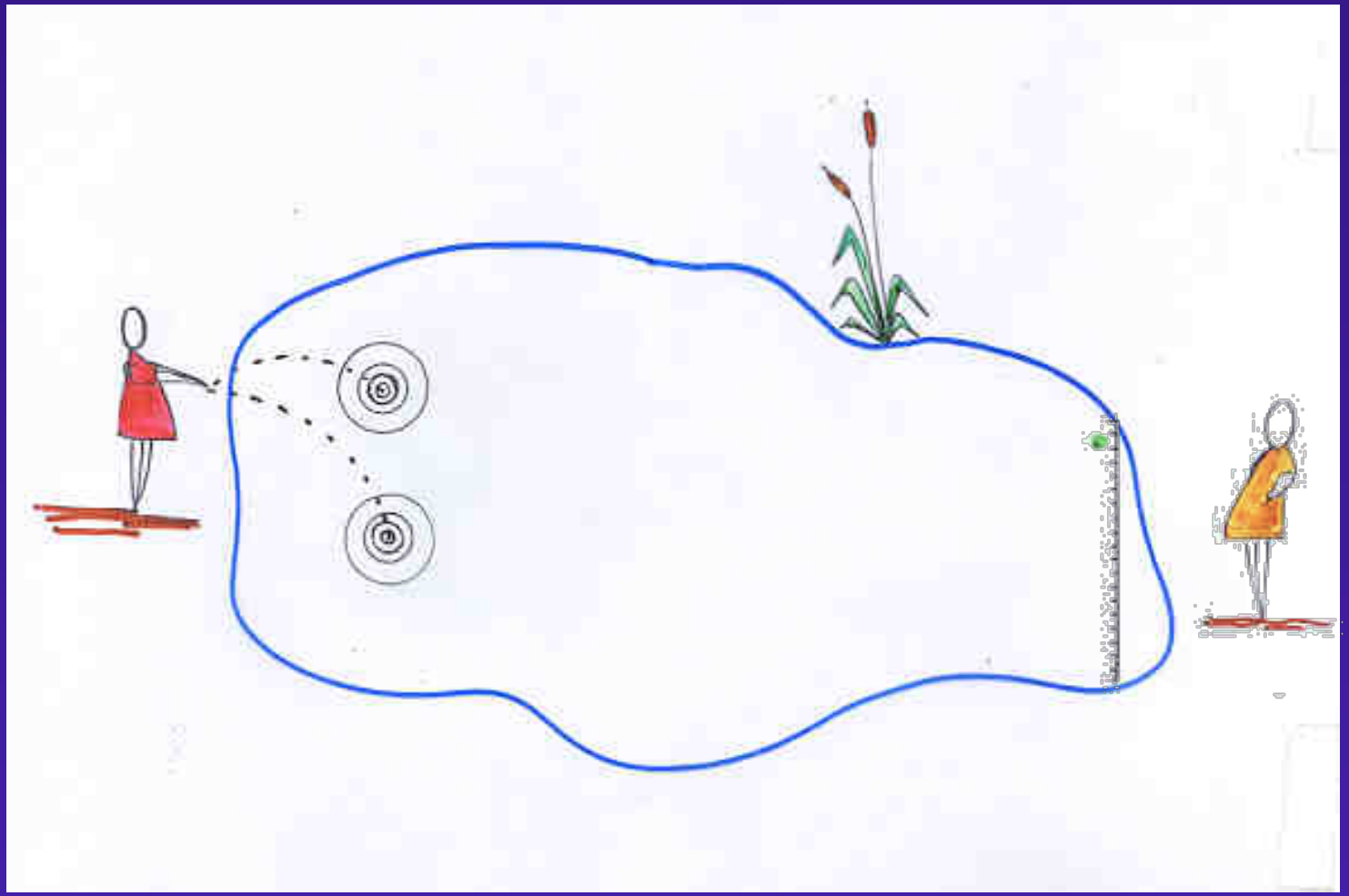
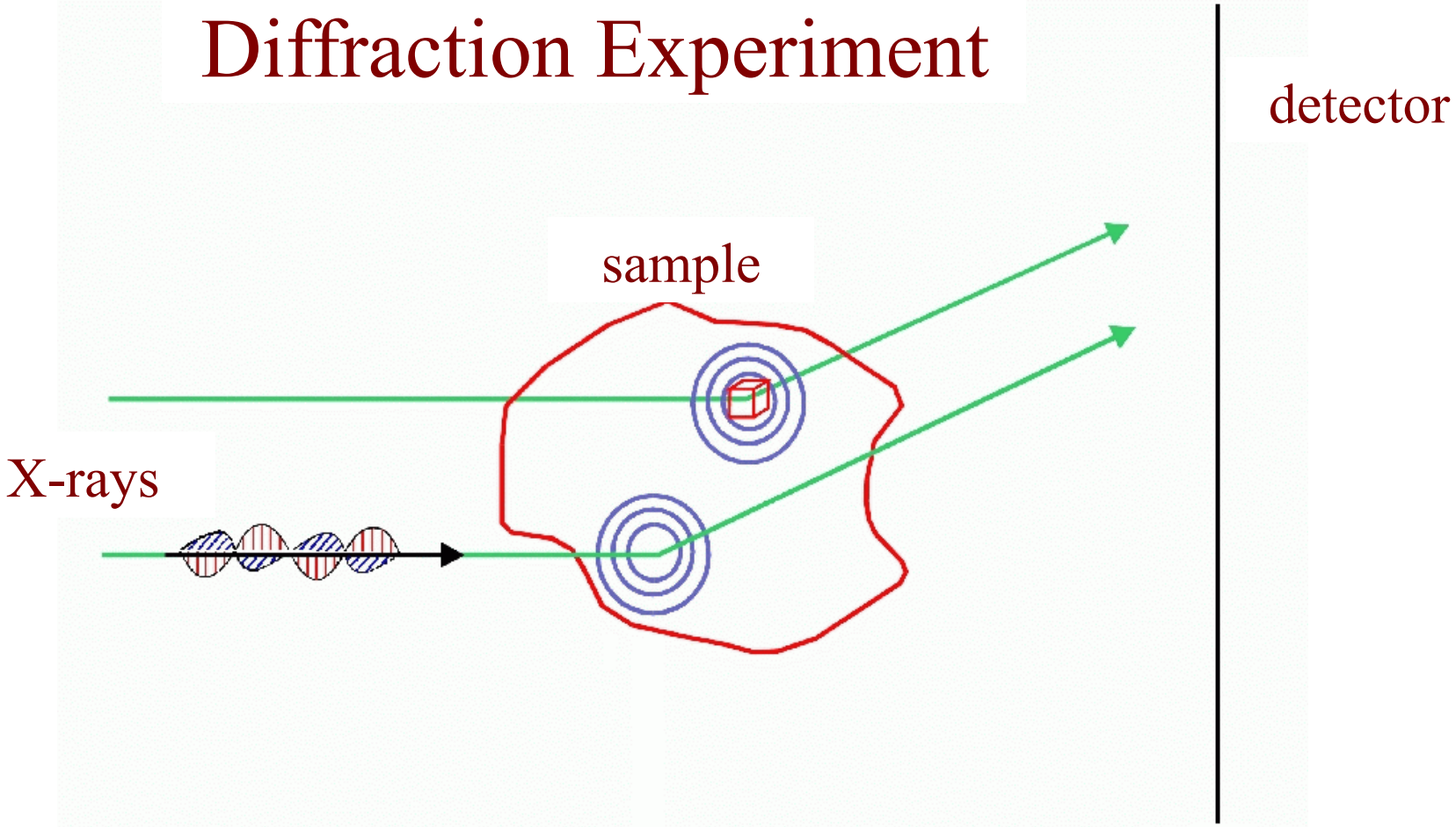
- Biophysical techniques
  - CD, Fluorescence, FTIR, Raman, XAFS, SAXS, MS, EPR, Cryo EM, ...

Difraction: “the lake experiment”



Diffraction Experiment

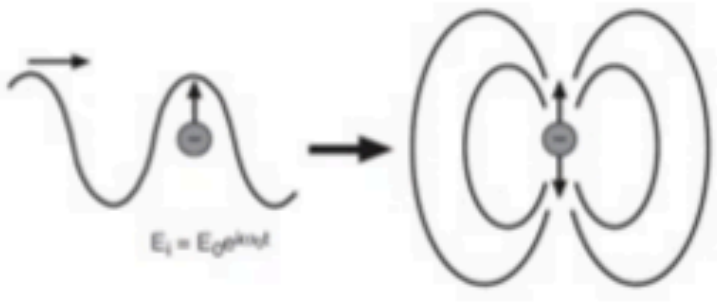


$$\rho(\vec{r}) \xrightarrow{\text{F.T.}} G(\vec{S}) = \int \rho(\vec{r}) \cdot e^{2\pi i \vec{S} \cdot \vec{r}} \, dV$$

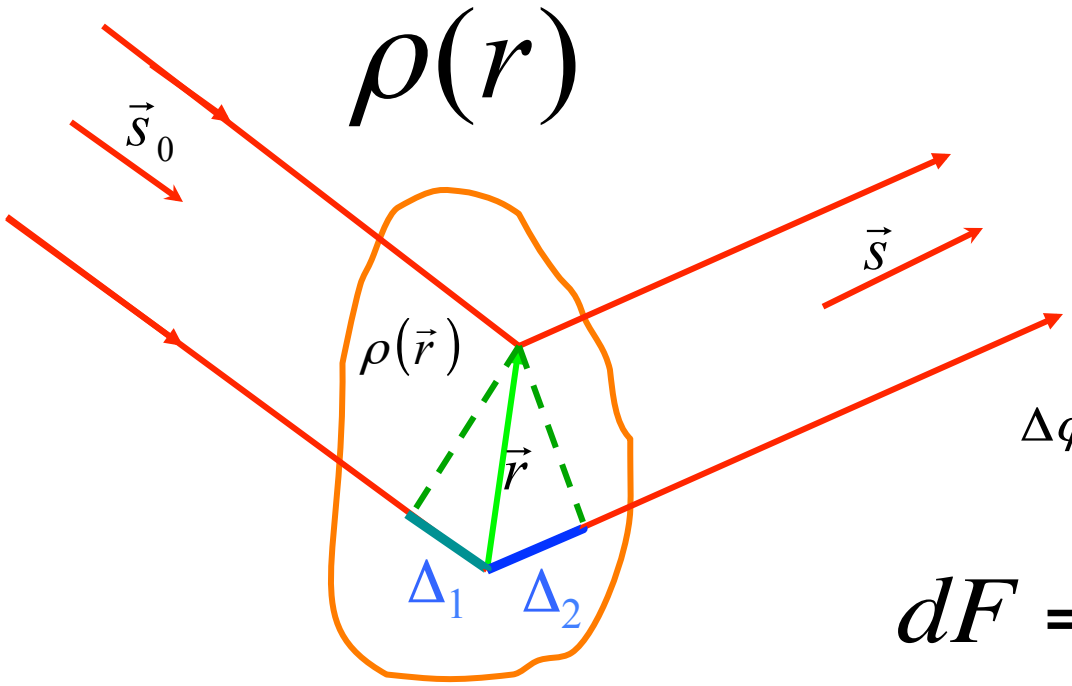
Electron density

Scatterred wave

Thomson Scattering.



- Thomson scattering is a process by which the energy of an electromagnetic wave is partly scattered in various directions by a free electron.
- Incident EM wave accelerates each particle it encounters, particles then re-radiate an EM wave.
- Because of the large mass of the ions their scattering is negligible.



$$\left|\vec{s}_0\right| = \left|\vec{s}\right| = 1$$

$$\Delta\varphi = \frac{2\pi}{\lambda}\left(\Delta_1 + \Delta_2\right) = \frac{2\pi}{\lambda}\,\vec{r}\cdot\left(\vec{s} - \vec{s}_0\right)$$

$$dF = \rho(\vec{r})dV$$

$$\vec{S} = \frac{\left(\vec{s} - \vec{s}_0\right)}{\lambda}$$

$$F\left(\vec{S}\right) = \int \rho\left(\vec{r}\right) e^{2\pi i \vec{r}\cdot\vec{S}} d^3 r$$

$$\rho\left(\vec{r}\right) = \int F\left(\vec{S}\right) e^{-2\pi i \vec{r}\cdot\vec{S}} d^3 S$$

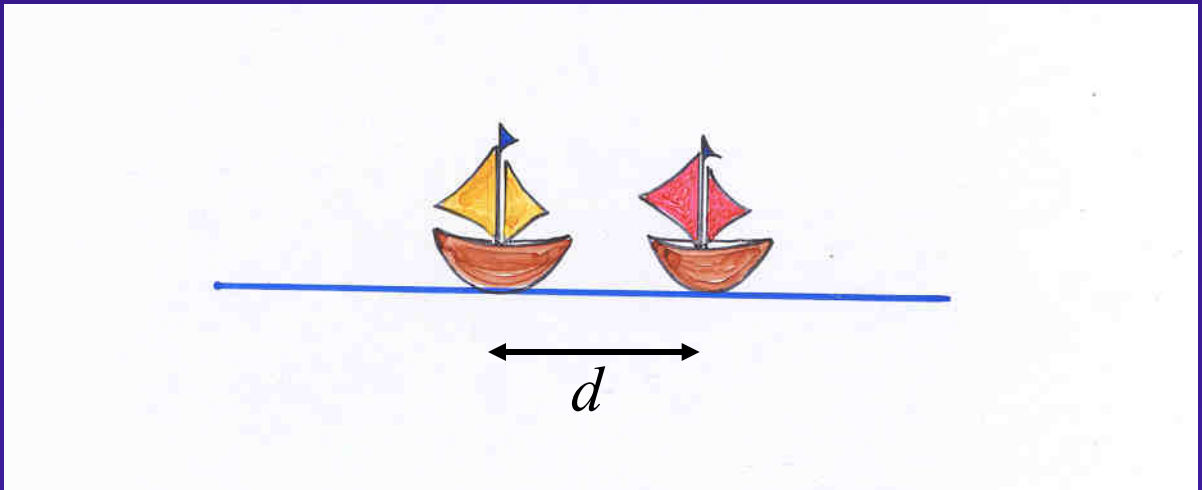
The Phase Problem

$$\rho(\vec{r}) \quad \xleftrightarrow{\text{Fourier Transform}} \quad F\left(\vec{h}\right) = \left|F\left(\vec{h}\right)\right| \cdot e^{i\varphi\left(\vec{h}\right)}$$

Diffraction Experiment $\Rightarrow$ the amplitudes $\left|F\left(\vec{h}\right)\right|^2$ are measured

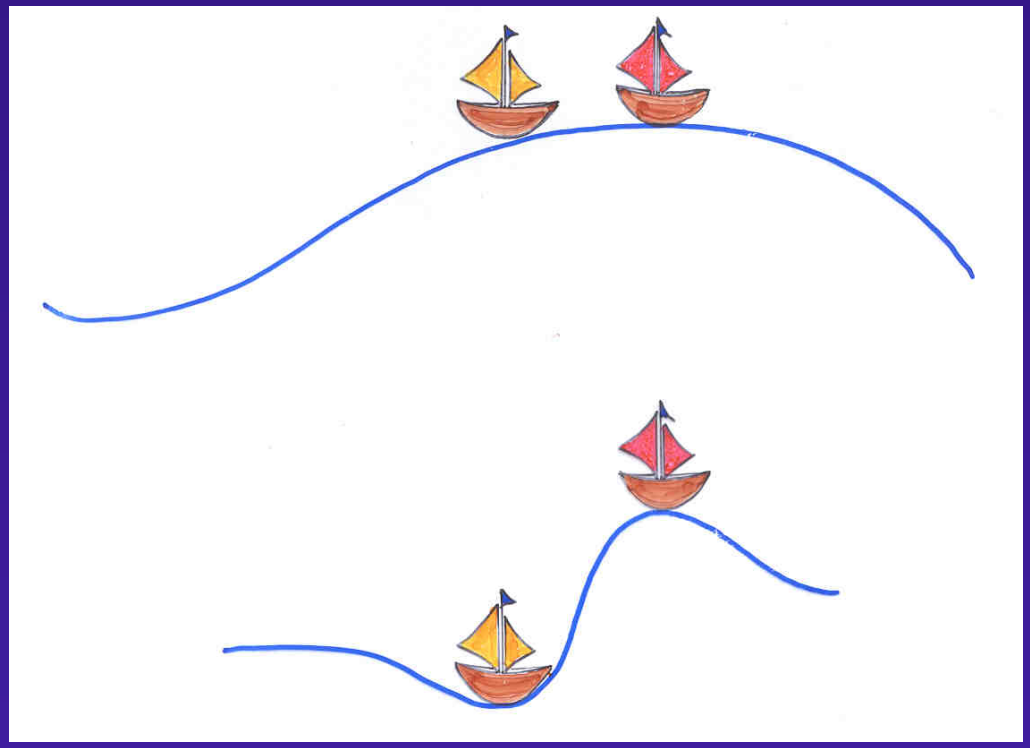
Problem $\Rightarrow$ the phases $\varphi\left(\vec{h}\right)$ are unknown

Why X-rays ?
“the boats example”



How to measure the separation *d* between two boats in the water, given that we are far away from them?

Using water waves, with wavelength λ



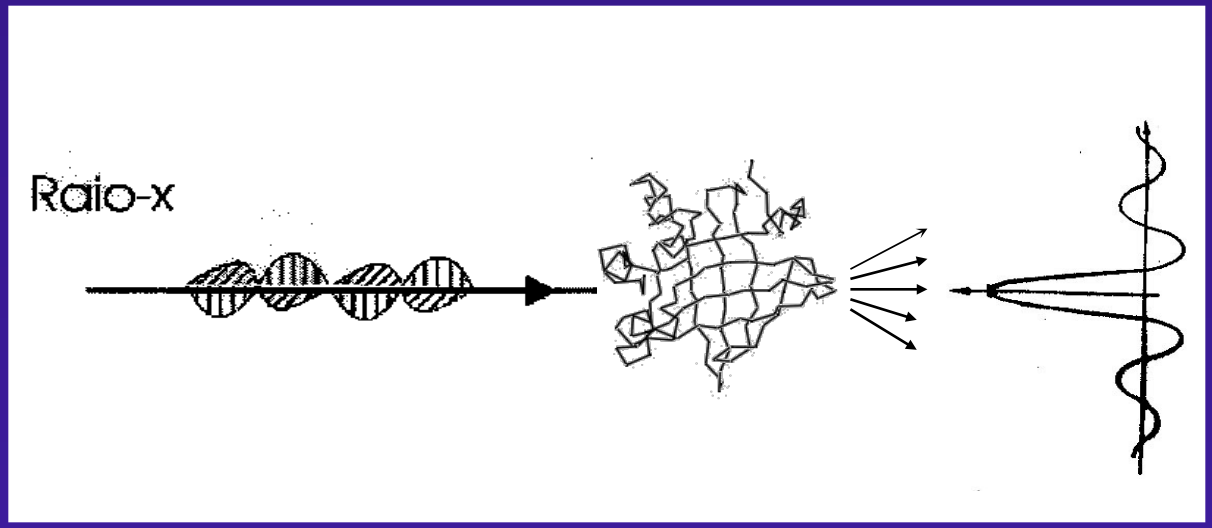
$\lambda >> d$

$\lambda \approx d$

In molecules, the separation between atoms is of the order of 1 to 2Å, therefore we have to use electromagnetic radiation with wavelengths of this magnitude, => X-rays

Possible X-ray scattering/diffraction experiments to elucidate the structure of a protein

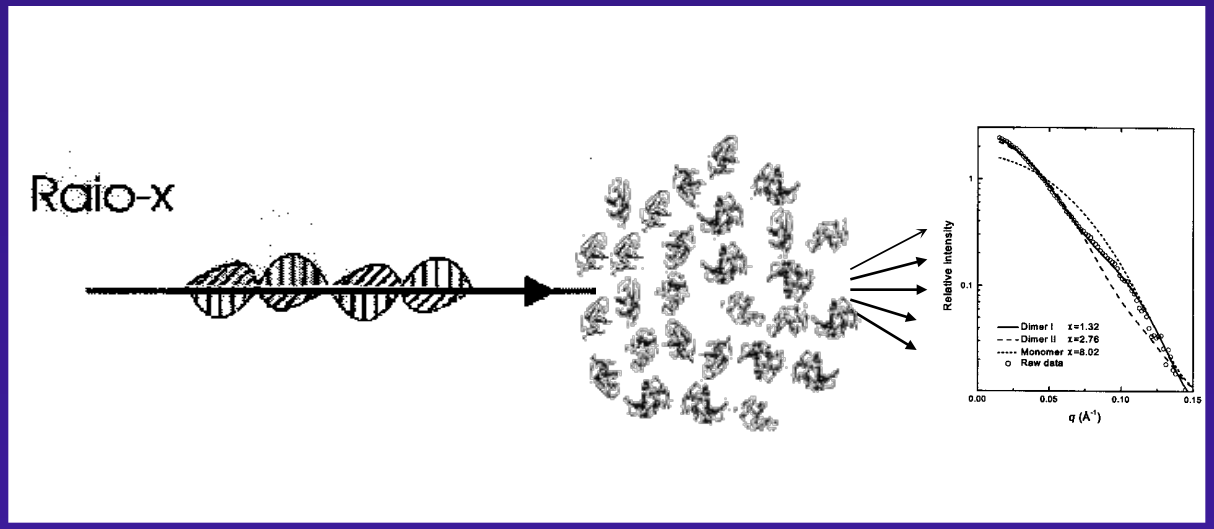
1) Sample is an isolated single molecule



- Very difficult to isolate and immobilize a single molecule
- resulting scattering is continuous and the amplitudes are so small that it may be extremely difficult to measure

Possible X-ray scattering/diffraction experiments to elucidate the structure of a protein

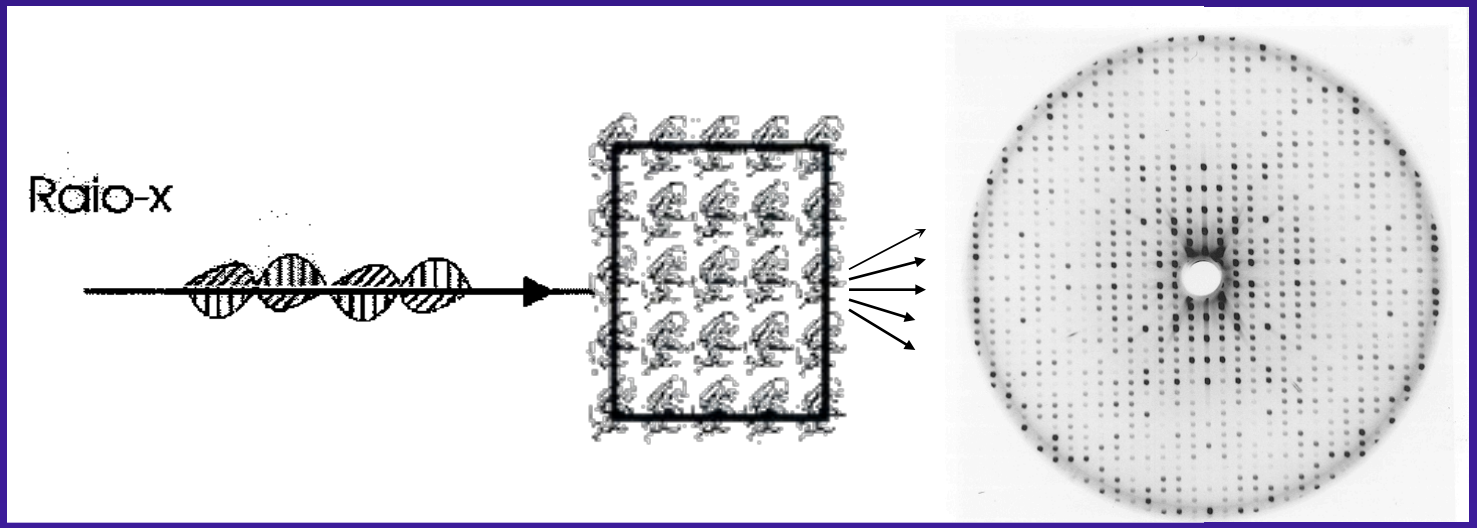
2) Many molecules in solution



- as ther is no order, interference is globally destructive and we can only observe scattering at very low angles
- The result is a Small-Angle X-ray Scattering experiment (SAXS)
- It is very useful to get global inormation about your protein, as shape, size and oligomerization state

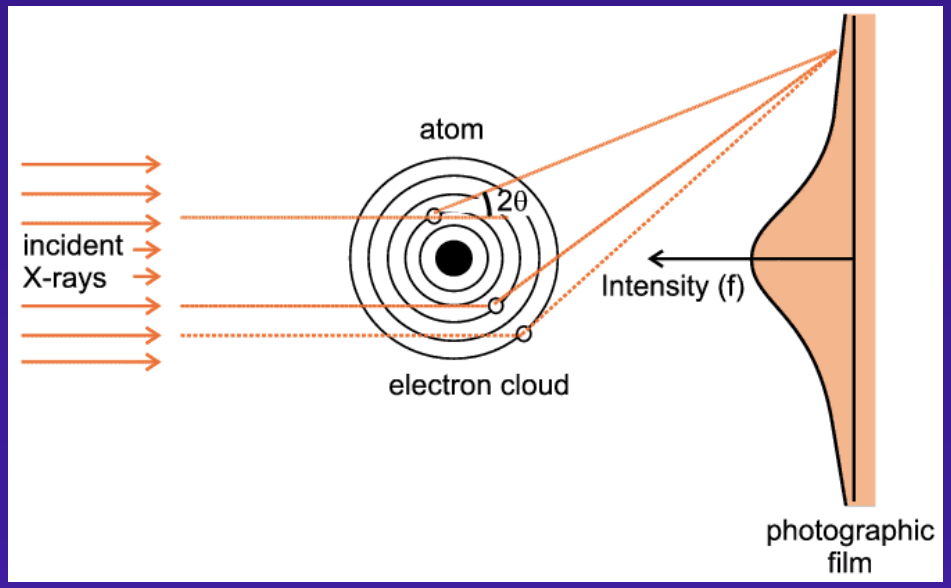
Possible X-ray scattering/diffraction experiments to elucidate the structure of a protein

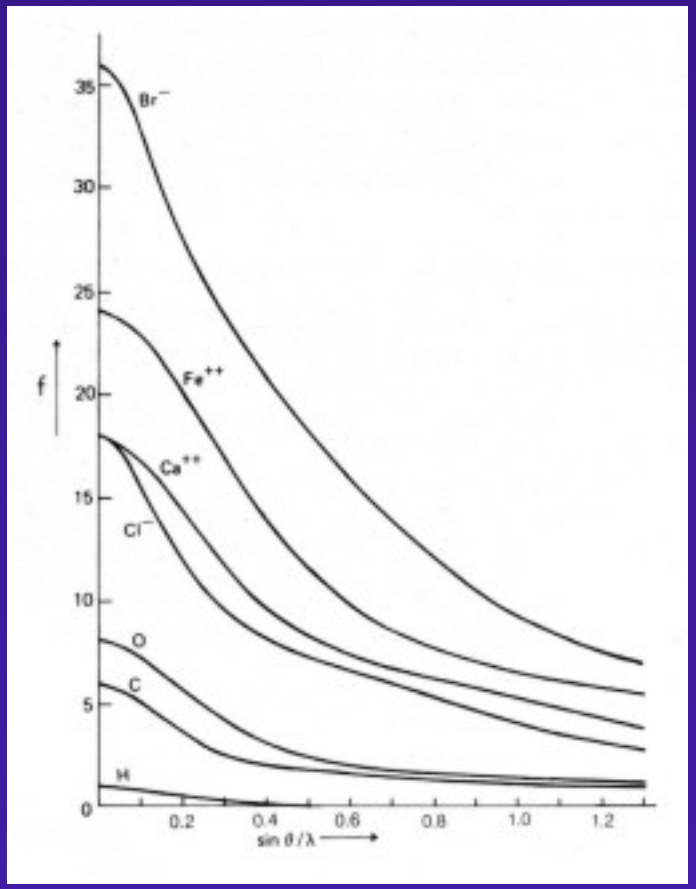
3) Molecules are in ordered in a crystal lattice



- Scattering is discrete and at high resolution : diffraction
- Scattered waves are measurable
- From the diffraction pattern we can determine with precision the atomic positions of all ordered atoms that constitute the crystal
- Problem: we measure the amplitudes of the scattered waves but all phase information is lost -> the Phase Problem

X-ray scattering by an atom





Atomic Scattering Factors

Scattering by a group of N atoms

$$F(\vec{S}) = FT\{\rho(\vec{r})\} = \int_V \rho(\vec{r}) e^{2\pi i \vec{r}\cdot\vec{S}} dV$$

If the electron density ρ(r) is a group of
N atoms, each one defined by its atomic type and position
(x_i, y_i, z_i), with i=1, 2, …,N

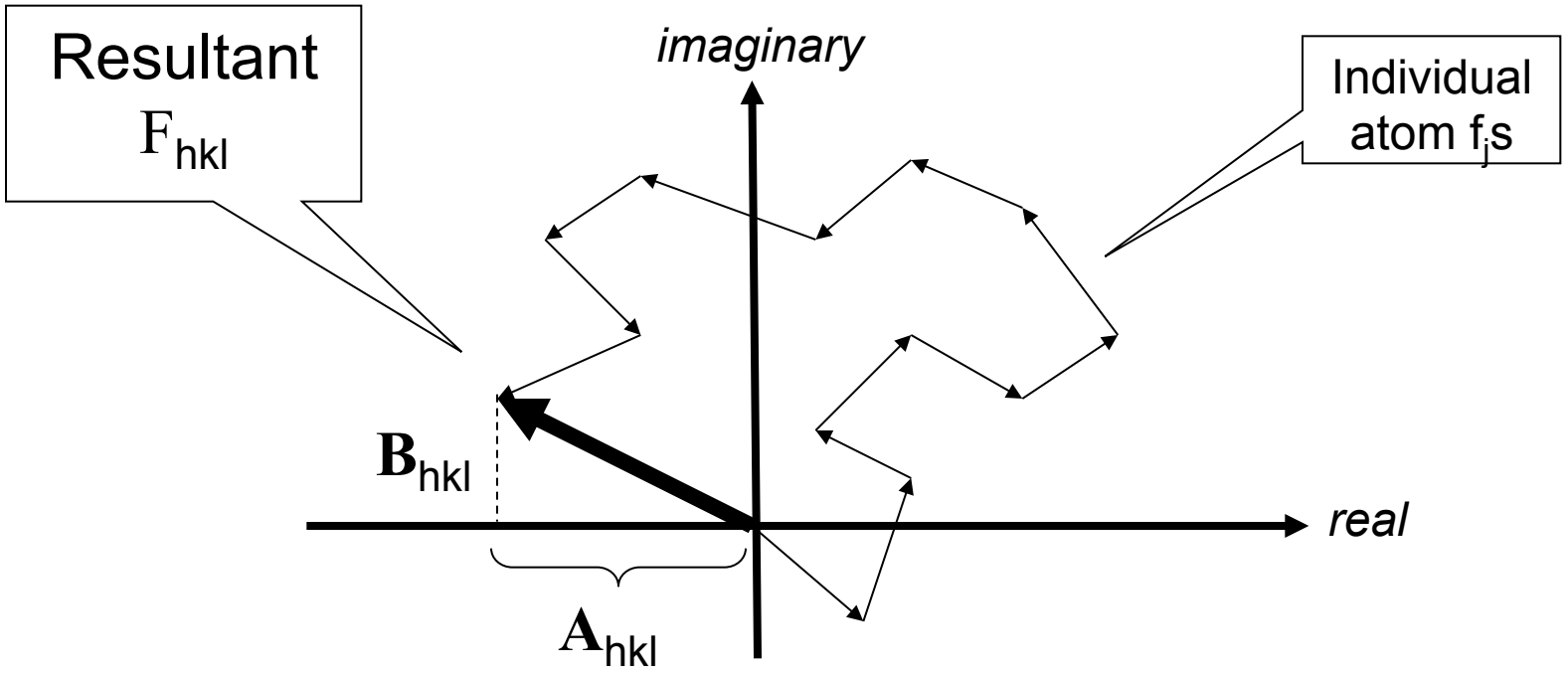
$$F(hkl) = \sum_{i=1}^{N} f_i \, e^{2\pi i(hx_i + ky_i + lz_i)}$$

Structure Factor

Atomic Scattering Factor

The Structure Factor

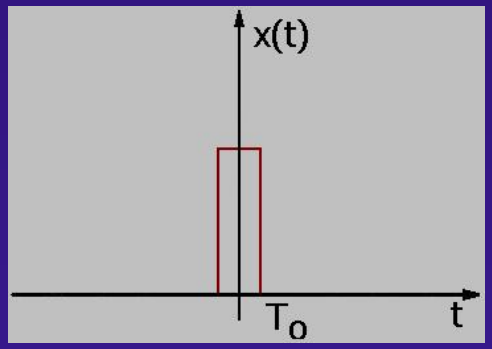
Sum of all individual atom contributions

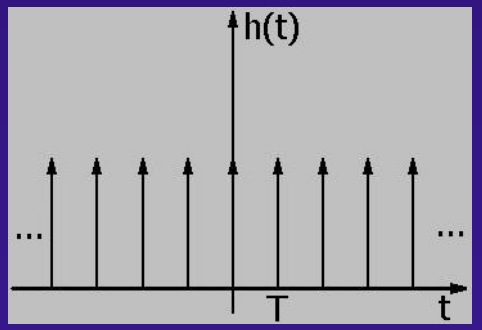


$$\phi_j = 2\pi(hx_j + ky_j + \ell z_j)$$

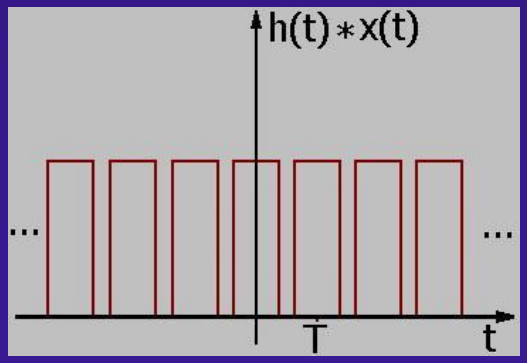
$$\mathbf{F}_{hk\ell} = \sum_{j=1}^{N} f_j e^{2\pi i(hx_j + ky_j + \ell z_j)} = \sum_{j=1}^{N} f_j e^{i\phi_j}$$

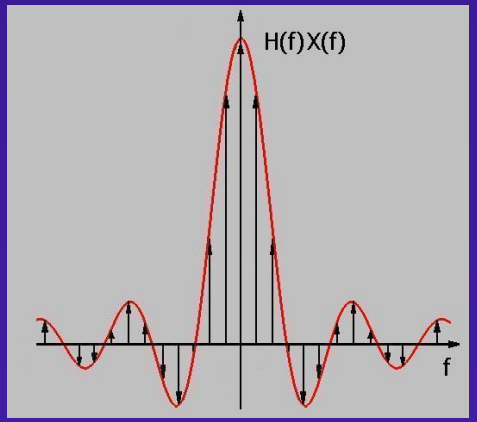
Diffraction is all about Fourier Transforms and Convolution Theorem



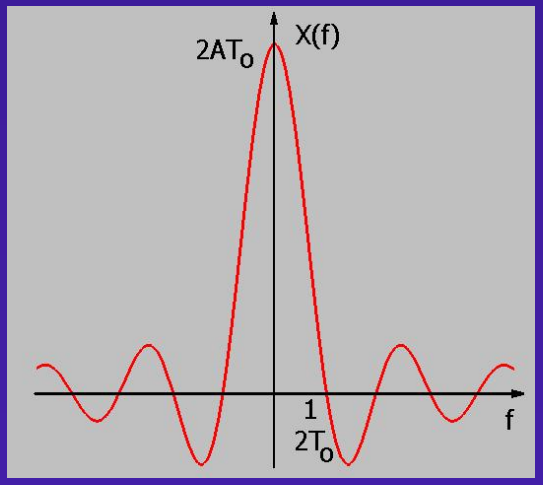


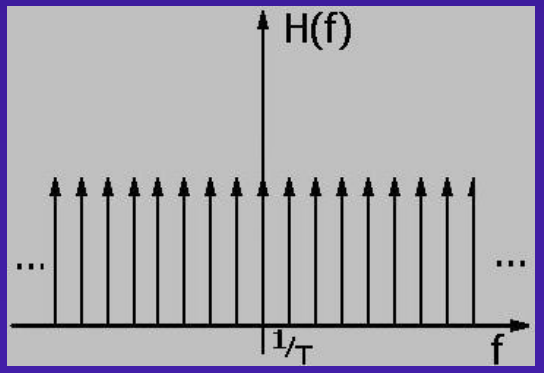
Convolution



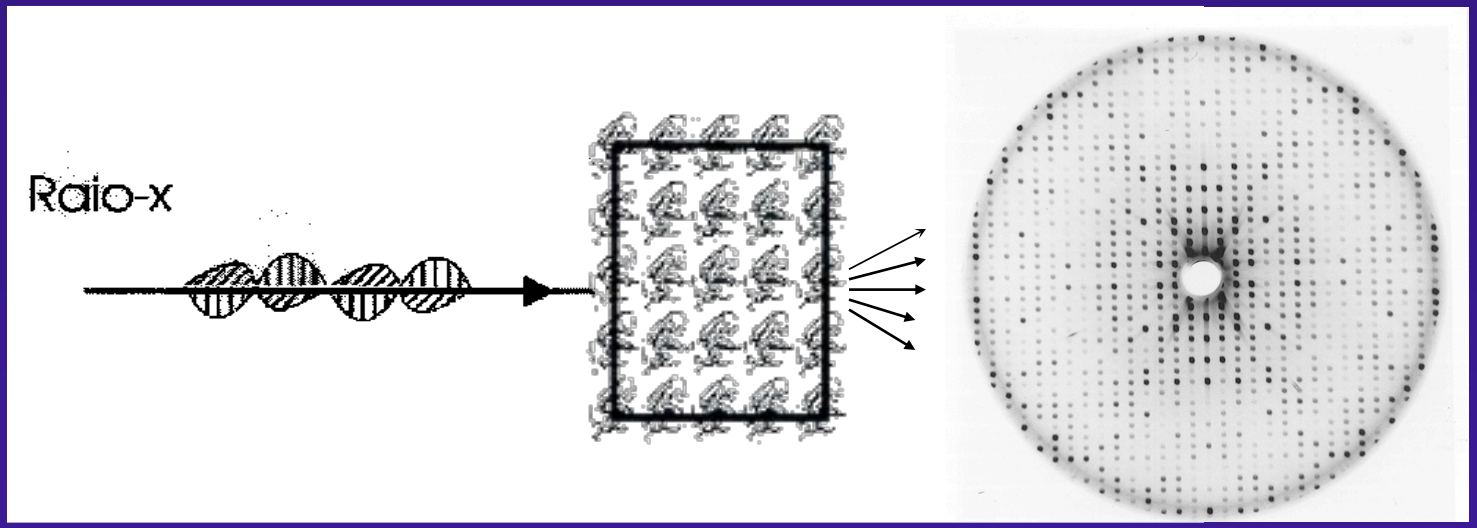


Multiplication



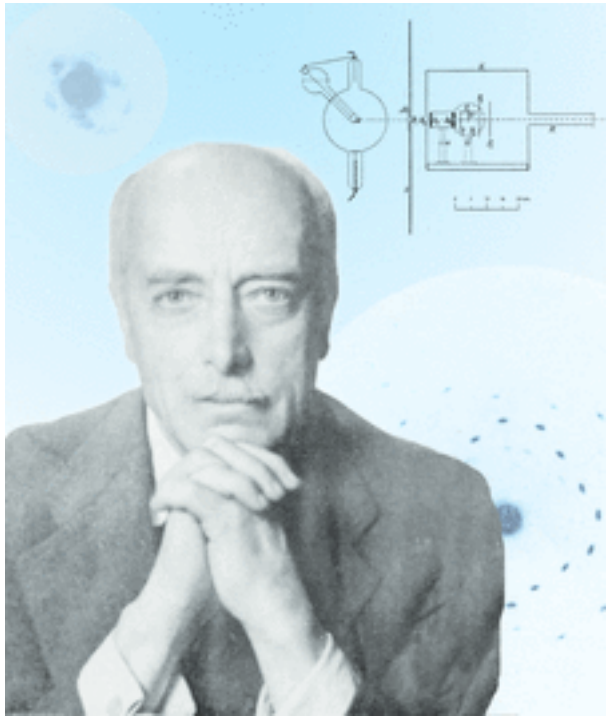


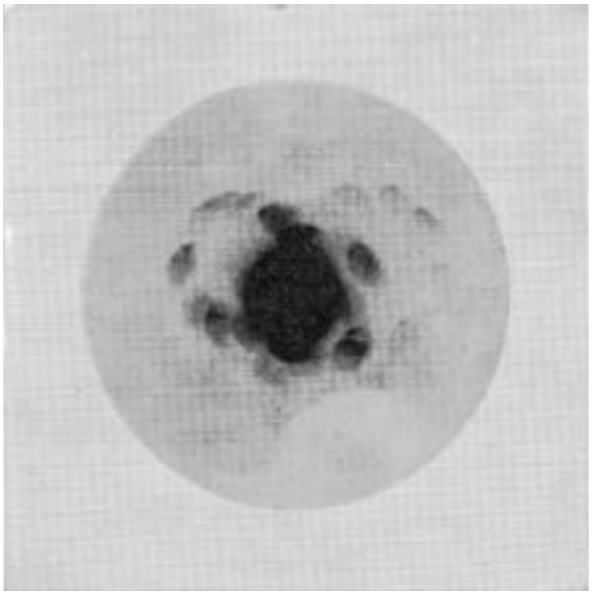
Scattering by a crystal



- A crystal is a repetition of a basic unit (Unit Cell), repeated periodically along the directions of the cell parameters a,b, and c
- Therefore, the SCATTERING OF A CRYSTAL is the same scattering of a Unit Cell multiplied by the number of unit cell in the crystal and sampled at the reciprocal lattice

1912: Laue solves the quest about the nature of X-rays

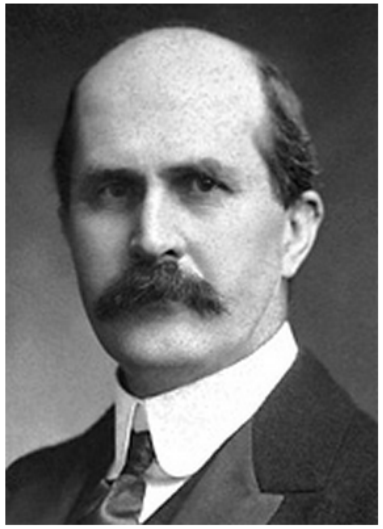




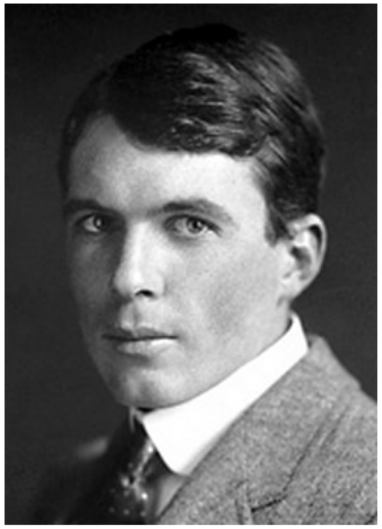
Friedrich W, Knipping P, von Laue M (1912). "Interferenz-Erscheinungen bei Röntgenstrahlen". Sitzungsberichte der Mathematisch-Physikalischen Classe der Königlich-Bayerischen Akademie der Wissenschaften zu München 1912: 303.

2013 - 100 years of the fundamental discovery of Molecular Sciences
The structure of NaCl

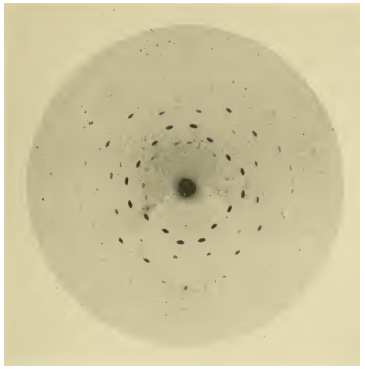
The Nobel Prize in Physics 1915
William Bragg, Lawrence Bragg



Sir William Henry Bragg



William Lawrence Bragg



PROCEEDINGS

OF THE

CAMBRIDGE PHILOSOPHICAL SOCIETY.

Mr Bragg, Diffraction of Short Electromagnetic Waves, etc. 43

The Diffraction of Short Electromagnetic Waves by a Crystal. By W. L. Bragg, B.A., Trinity College. (Communicated by Professor Sir J. J. Thomson.)

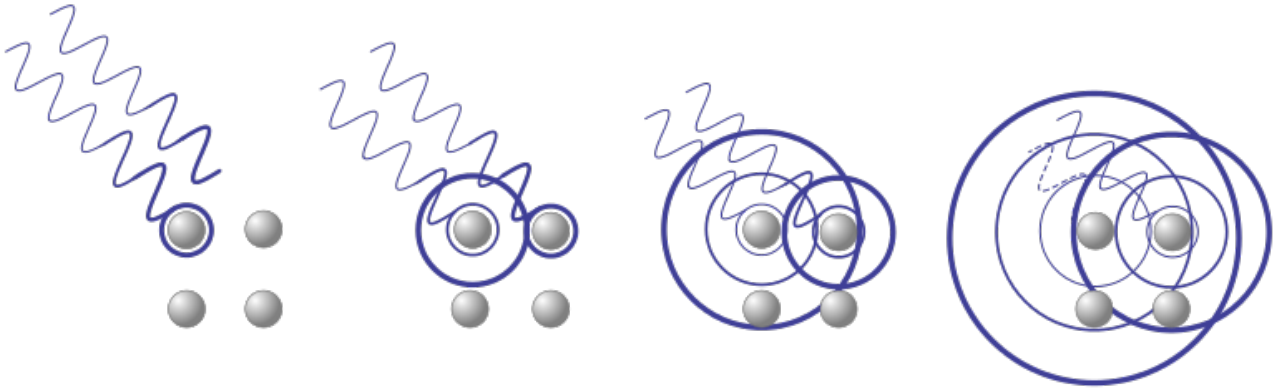
[*Read* 11 November 1912.]

[Plate II.]

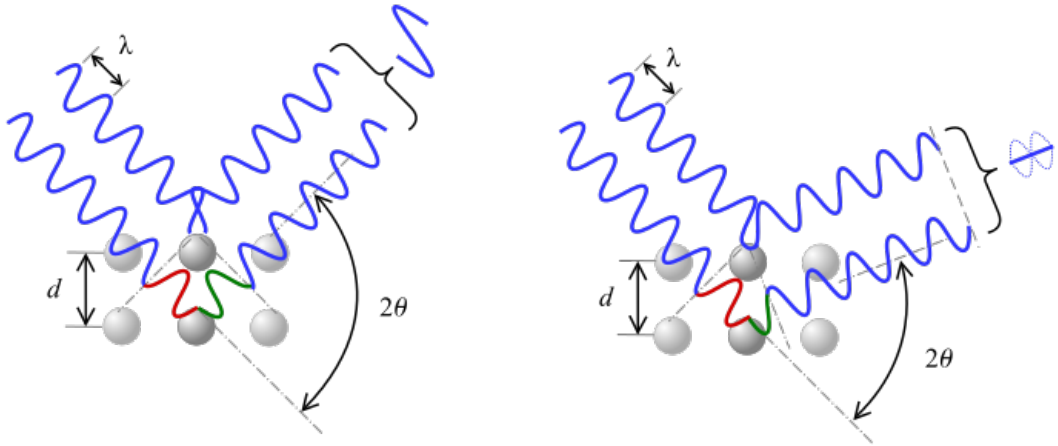
Herren Friedrich, Knipping, and Laue have lately published a paper entitled 'Interference Phenomena with Röntgen Rays*,' the experiments which form the subject of the paper being carried out in the following way. A very narrow pencil of rays from an X-ray bulb is isolated by a series of lead screens pierced with fine holes. In the path of this beam is set a small slip of crystal, and a photographic plate is placed a few centimetres behind the crystal at right angles to the beam. When the plate is developed, there appears on it, as well as the intense spot caused by the undeviated X-rays, a series of fainter spots forming an intricate geometrical pattern. By moving the photographic plate back-

The Nobel Prize in Physics 1915 was awarded jointly to Sir William Henry Bragg and William Lawrence Bragg *"for their services in the analysis of crystal structure by means of X-rays"*

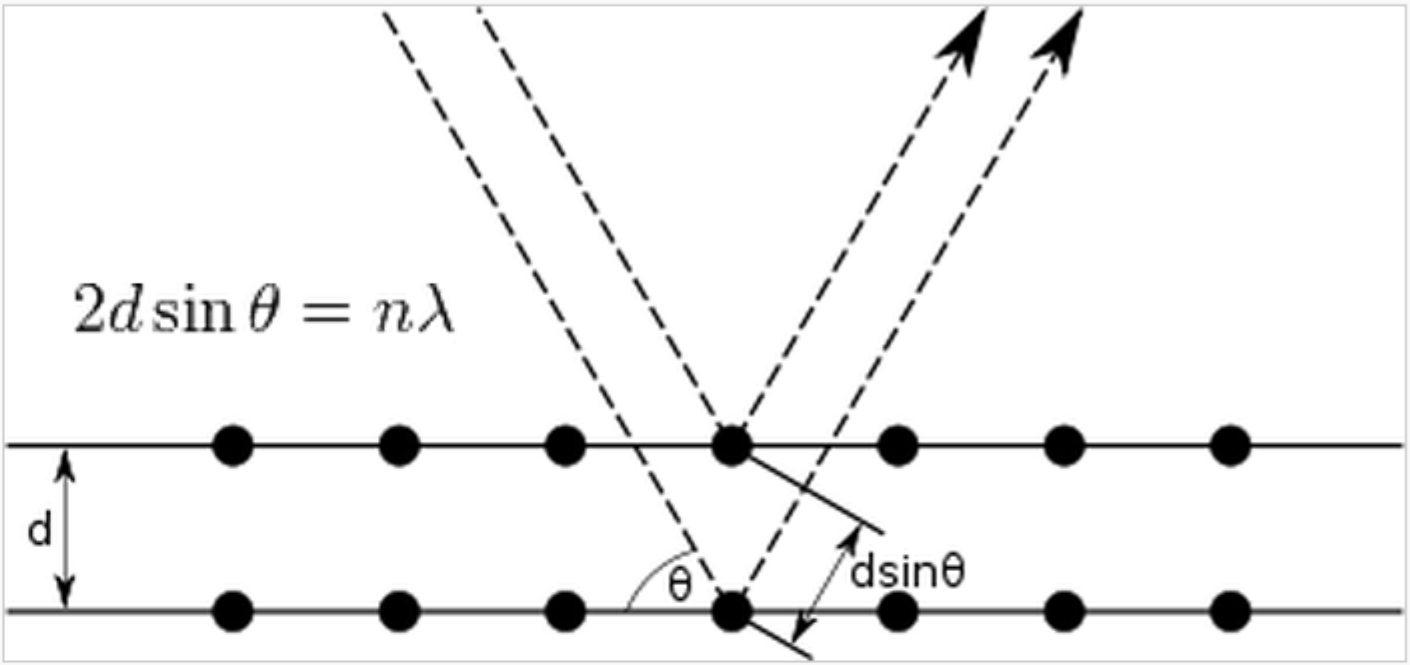
The interaction of X-rays with atoms in a crystal



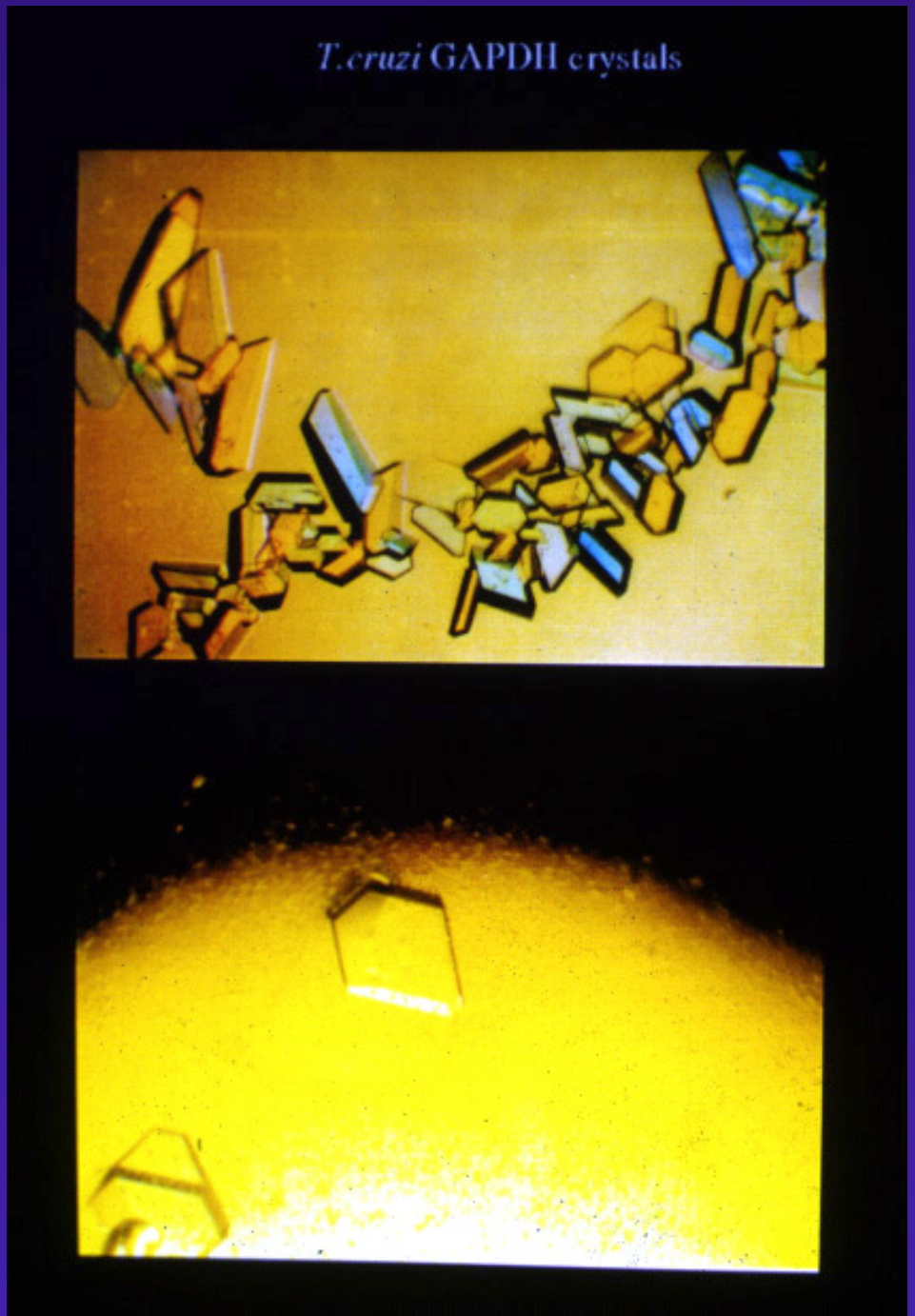
Depending on the 2θ angle, the phase difference os the scattered waves show constructive (left) or destructive (right) interference



Bragg's Law



Bragg diffraction. Two beams with identical wavelength and phase approach a crystalline solid and are scattered off two different atoms within it. The lower beam traverses an extra length of $2d\sin\theta$. Constructive interference occurs when this length is equal to an integer multiple of the wavelength of the radiation.



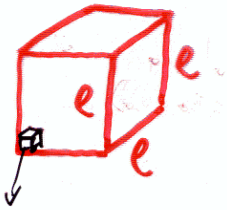
Obtaining adequate protein single crystals is the major bottleneck in the process of elucidating a new structure.

Adequate crystals means:

- highly ordered
- suitable size (used to be 0.1mm, nowadays can be microns or even sub-microns when using XFELs)

Number of protein molecules in a typical protein crystal

cristal de proteína



$\ell = 0{,}1\ mm = 10^{-4}\ m$

$V_{cristal} = \ell^3 = 10^{-12}\ m^3$

"cela unitária" = unidade repetida na rede cristalina

p. ex., se tivermos uma molécula de proteína em cada cela unitária:

um valor típico seria

$a = 46{,}5\ \text{Å}$ p/ uma proteína globular de 36 kDa

$V_{cela} = a^3 \approx 100\,000\ \text{Å}^3 = 10^5 \times (10^{-10})^3 = 10^{-26}\ m^3$

Logo, o número de celas unitárias em um cristal típico

$$N = \frac{V_{cristal}}{V_{cela}} = \frac{10^{-12}\ m^3}{10^{-25}\ m^3} = 10^{13}$$

Steps in the analysis of protein crystals by X-ray diffraction

- Crystallization
- X-ray diffraction data collection
- Solution of the Phase Problem
  - Multiple Isomorphous Replacement
  - Anomalous Dispersion (SAD or MAD)
  - Molecular Replacement
- Electron density map interpretation
- Structure refinement