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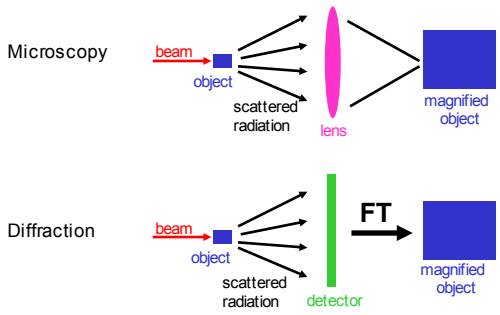
Macromolecular crystallography - Part 1

Macromolecular crystallography (MX)

Often simply called "protein crystallography (PX)" even if the same technique can be applied to nucleic acids, or protein-nucleic acid complexes.

- Single crystal X-ray diffraction: why?
- A "hint" of diffraction theory
- How to solve the phase problem
- Model building and refinement
- Practical considerations (protein production, crystallization, data collection)

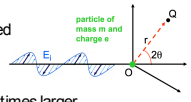
Microscopy vs diffraction



Interaction X-rays/matter

- Thomson scattering: some photons are deflected without loss of energy: the scattered radiation has the same λ of the incident radiation and a fixed phase relationship -- COHERENT
- Compton scattering: some photons are scattered with a small loss of energy: slightly longer λ
- Absorption/Anomalous dispersion: at the right wavelength some photons can be absorbed by atoms in the sample

Thomson showed you can calculate the diffracted intensity and it is $\propto e^4/m^2$

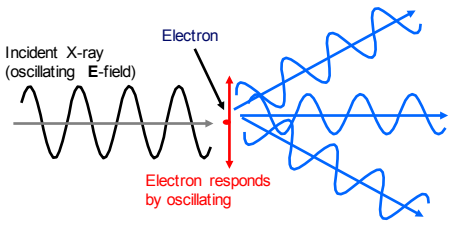


- neutrons: e=0
- protons : same charge as electrons but m is 1837 times larger
  $\mapsto e^4/m^2$ is $(1/1837)^2=2.96\times10^{-7}$ less than for electrons

➡ the scattering of x-rays is mostly due to electrons!!

X-ray scattering by a single electron

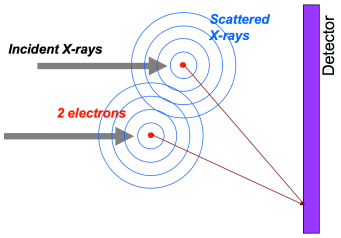
➡ the scattering of X-rays is mostly due to electrons



The electron is said to "scatter" or "diffract" the X-ray

Oscillating electron emits X-rays over a wide angle

X-ray scattering by two electrons



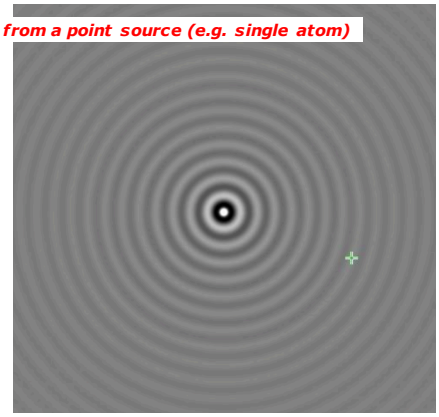
Each electron in the "**structure**" becomes a source of X-rays (a centre of scattering).

The diffraction pattern (that can be observed on a detector) is the resultant of **adding** the scattered X-ray waves.

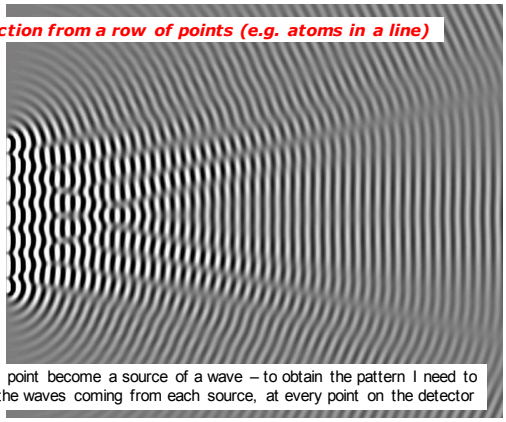
To understand diffraction, we need to know **how to add waves**, that is how waves **interfere** with each other.

The resulting scattering (diffraction) pattern very much depends on how these scattering centres are arrayed, i.e. on the structure

Diffraction from a point source (e.g. single atom)



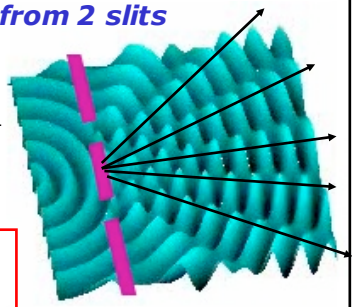
Diffraction from a row of points (e.g. atoms in a line)



Each point become a source of a wave – to obtain the pattern I need to add the waves coming from each source, at every point on the detector

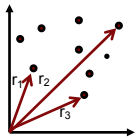
Water wave example of diffraction from 2 slits

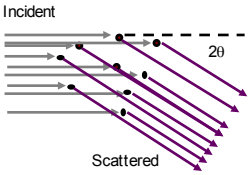
Diffraction of water waves through slits gives a pattern of peaks and troughs. This pattern **depends** on the slit structure (width, separation) and the wavelength of the waves.

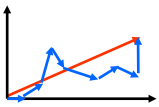


Key point:
If we can work out this relationship, we can measure the diffraction pattern and figure out the structure that gives rise to it.

Scattering from many electrons (i.e. a molecule):



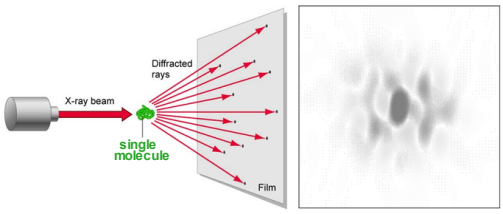




It is like adding many waves/vectors each representing a wave.

I can consider all the electrons in one atom as a "block" – I sum together the contributions of the atoms.

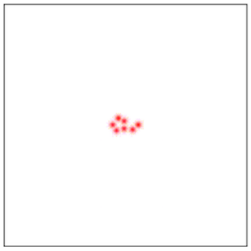
X-ray diffraction with single objects?



The intensity of the X-ray radiation diffracted by a single molecules is very small:

➡ impossible to measure with current technology.

Molecules & diffraction patterns

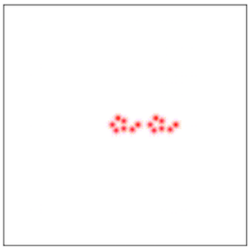


Single molecule

Diffraction pattern related to structure but too weak to detect in practice

Images from Kevin Cowtan Book of Fourier

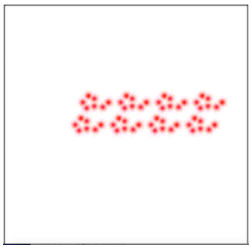
Crystals & diffraction patterns

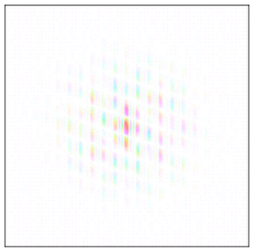


Two molecules

- similar to single molecule
- modulated by a vertical fringe function
- modulation depends on spacing between molecules
- still too weak

Crystals & diffraction patterns

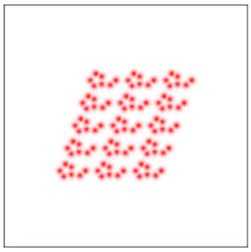


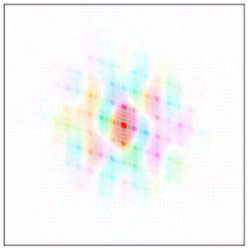


Eight molecules

- similar to single molecule
- modulated by a vertical and horizontal fringe function
- modulation depends on spacing between molecules

Crystals & diffraction patterns





Fifteen molecules

- similar to single molecule
- modulation function gets sharper
- intensity proportional to number of molecules

Scattering from a 3D crystal

scattering from a crystal

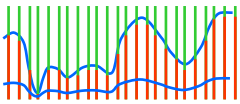
$$F_{3D}(s) = F(s) \left[\frac{\sin(2N+1)\pi(a\cdot s)}{\sin\pi(a\cdot s)}\right]\left[\frac{\sin(2N+1)\pi(b\cdot s)}{\sin\pi(b\cdot s)}\right]\left[\frac{\sin(2N+1)\pi(c\cdot s)}{\sin\pi(c\cdot s)}\right]$$

scattering from one molecule

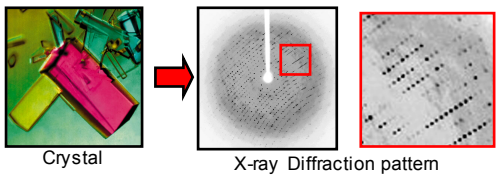
3D fringe function that depends on the lattice spacing **a, b, c**

Don't worry about the math! The fringe function has two effects:
- makes the pattern "discrete"
- amplifies the signal

The diffraction pattern from a crystal is the diffraction from the molecule **sampled and amplified** according to the fringe function



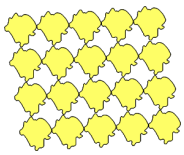
We can use X-rays with crystals



Crystal

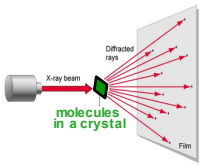
X-ray Diffraction pattern

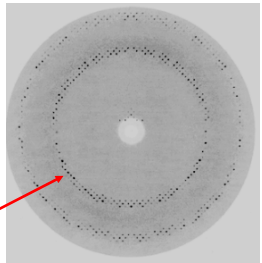
Crystal: an ordered array of molecules



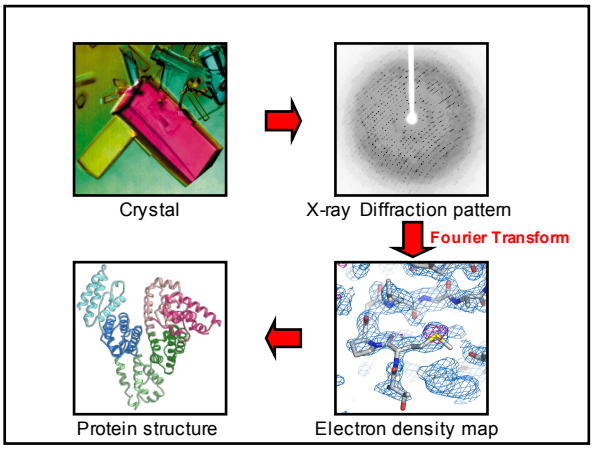
Two effects on diffraction:
- constructive interference in some directions, so that all molecules scatter in phase: strong signal
- destructive interference in most directions, so that the diffraction pattern is non-zero only at a few specific position: discrete spots

Scattering from a 3D crystal





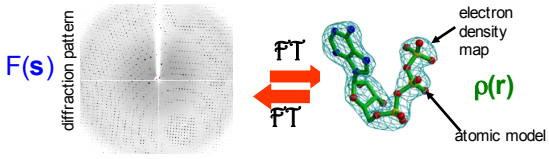
Each of this points (*reflection*) corresponds to a particular direction.



Diffraction and Fourier synthesis

$F(\mathbf{s})$ = Fourier Transform $[\rho(\mathbf{r})]$ the diffraction pattern is the Fourier Transform of the electron density

$\rho(\mathbf{r})$ = Fourier Transform $[F(\mathbf{s})]$ the electron density is the Fourier Transform of the diffraction pattern



We can always go back and forward using Fourier transforms:

- if we know the electron density we can calculate the diffraction pattern
- if we know the diffraction pattern we can calculate the electron density

The electron density equation

$\rho(\mathbf{r})$ = FT $[F(\mathbf{s})]$ the electron density is the FT of the diffraction pattern

If we know the structure factors **F(s)** (i/e the diffracted beam) *in modulus and phase*, for all the directions (all the **s**) we can calculate the electron density distribution $\rho(\mathbf{r})$, that is we can determine the position of all the atoms in the molecule.

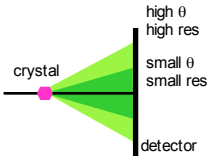
BUT there are two problems with this equation:

- **Problem # 1: resolution limits**
- **Problem # 2: the phase problem**

The electron density equation: problem # 1:resolution limits

Theoretical limit

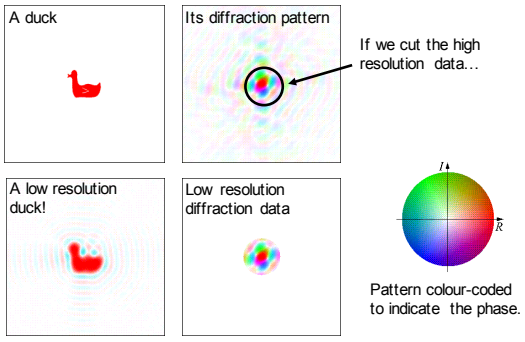
There are theoretical limits that depends on the wavelength of the X-ray radiation. Typically one uses a wavelength of roughly 1 A, which would allow for resolution of 0.5 A.



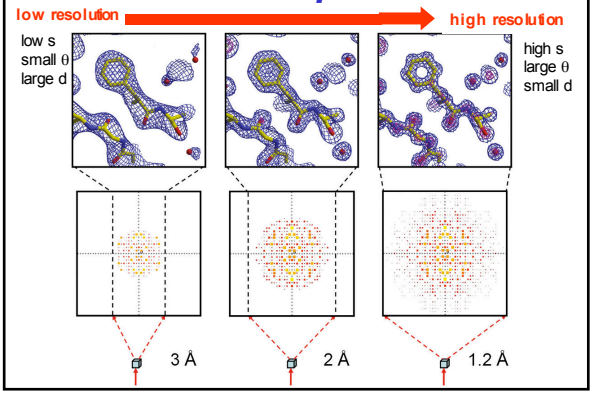
Practical limit

In practice for macromolecules the resolution limit is usually set by the intrinsic degree of order of the crystal typically one sees diffraction to 2.0-3.5 A – this is even more true of membrane protein crystals which tend to be more disordered due to the less directional nature of hydrophobic interactions.

Why is resolution important?

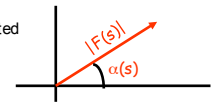


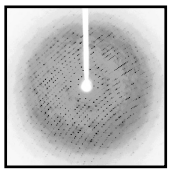
Resolution is important



The electron density equation: problem # 2:the phase problem

- Each diffracted wave can be represented by a vector, with amplitude and phase





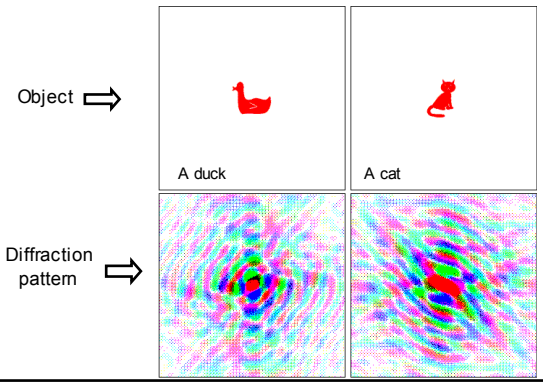
In a diffraction experiment we measure the intensity of each spot I(**s**), which is the square of the amplitude.

We can derive the amplitude F(**s**) but we have lost the information about the relative phase

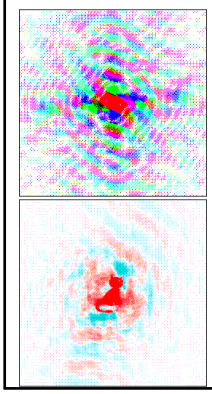
⇒ ***the phase problem!!***

Universal problem in crystallography – also for small molecules.

Phases and amplitudes



Phases are more important than amplitudes



Duck amplitudes
&
Cat phases

A (blurry) cat !!

When we calculate a Fourier transform using the amplitudes from one object and the phases from another we obtain an image that is more similar to the object that supplied the phases than the object that supplied the amplitudes.