

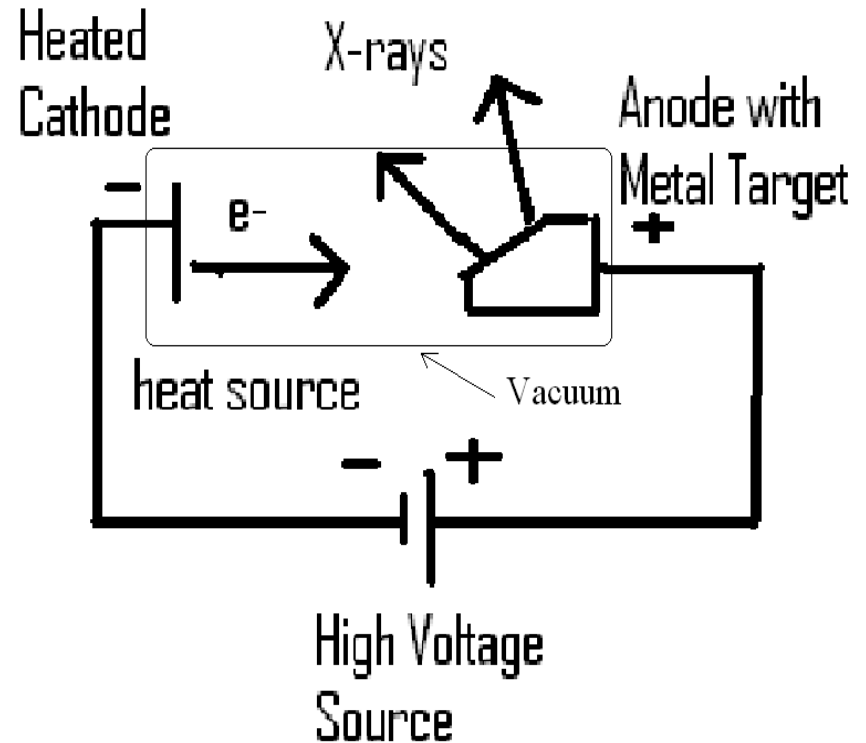
X-ray diffraction

- X-rays discovered in 1895 – 1 week later first image of hand.
- X-rays have $\lambda \sim 0.1$ – few Å
- No lenses yet developed for x-rays – so no possibility of an x-ray camera or microscope. Instead, the diffraction pattern has all the information on amplitudes and phases to reconstruct an image, even w/o a lens to reform the x-rays – we need a method to analyze the diffraction pattern



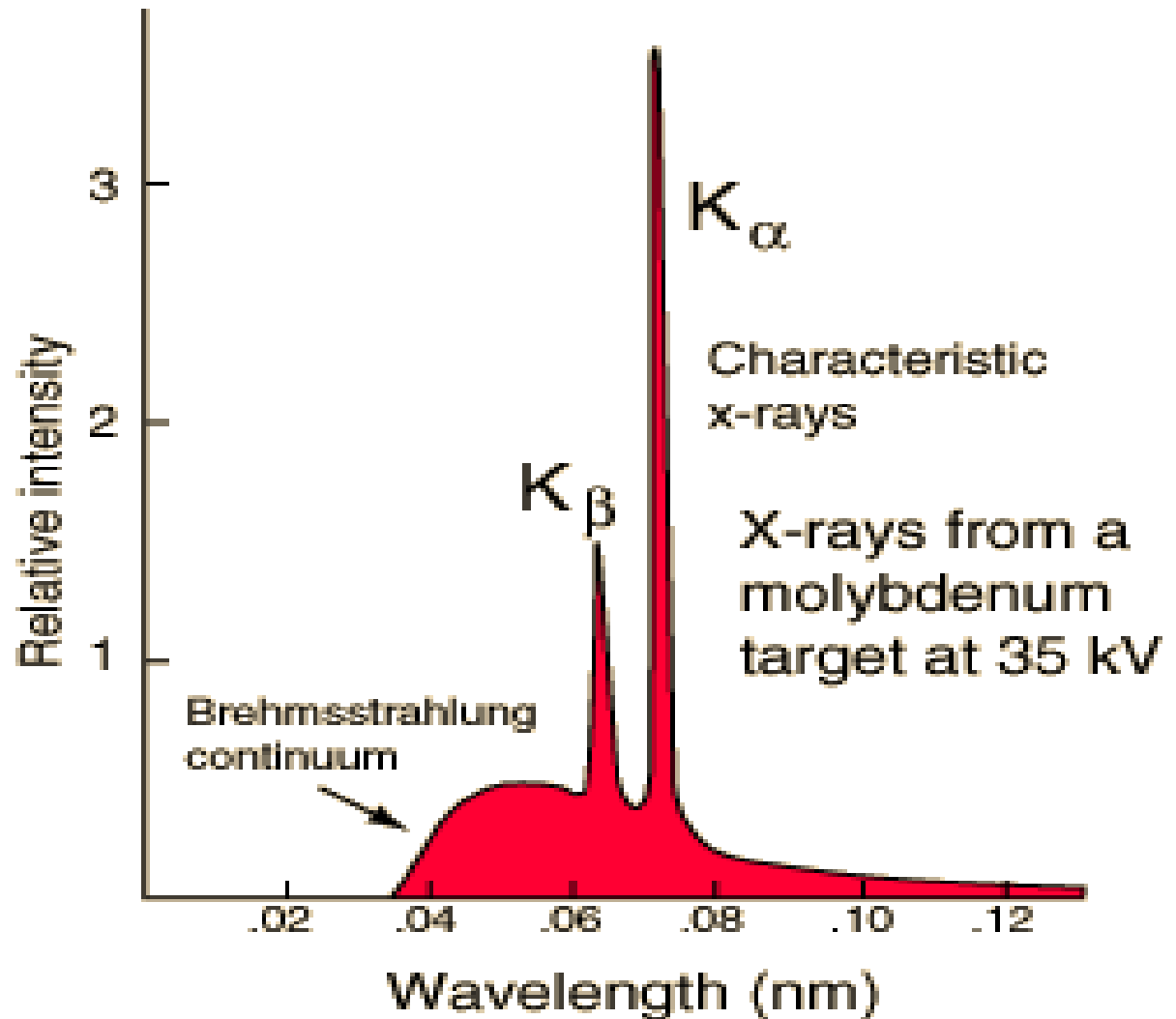
How do we get X-rays?

- The cathode is heated by a heat source to create an electron beam.
- The beam of electrons is then accelerated by the high voltage source, allowing them to collide with the metal target (usually Tungsten)
- X-rays are produced when the electrons are suddenly decelerated upon collision with the metal target (Brehmsstrahlung)
- If the bombarding electrons have sufficient energy, they can knock an electron out of an inner shell of the target metal atoms. Then electrons from higher states drop down to fill the vacancy, emitting x-ray photons (characteristic x-rays)



X-ray production Spectrum

- The characteristic x-rays, shown as two sharp peaks in the illustration occur when vacancies are produced in the $n=1$ or K-shell of the atom
- The x-rays produced by transitions from the $n=2$ to $n=1$ levels are called K-alpha x-rays
- The x-rays produced in the transition from $n=3 \rightarrow n=1$ are called K-beta x-rays.



Synchrotron Radiation

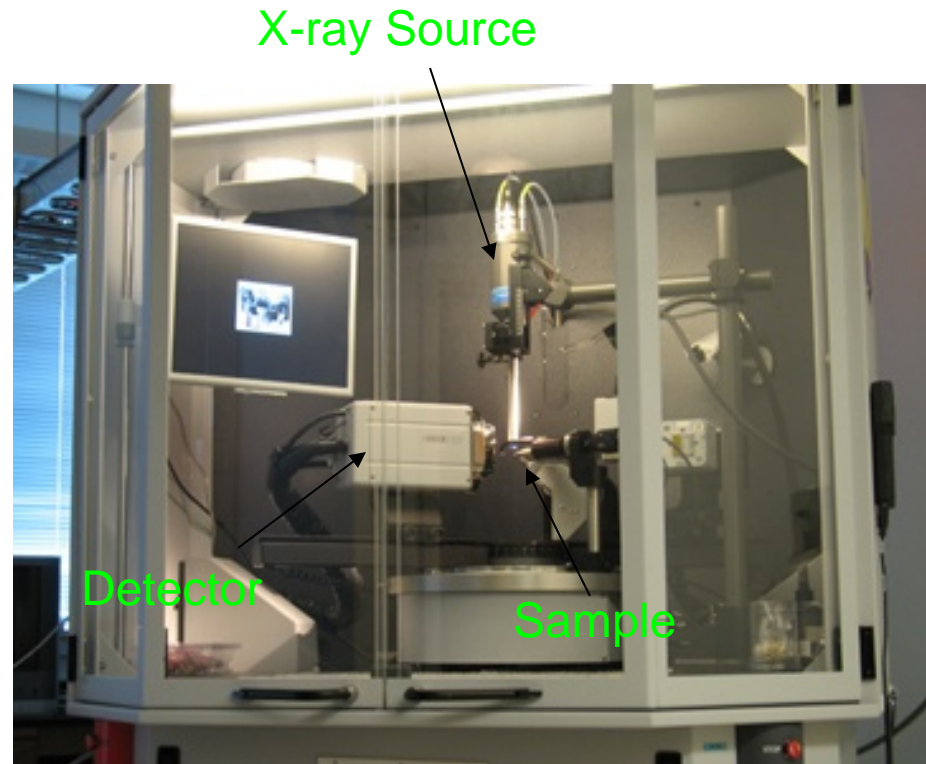
- most devices can only generate one type of electromagnetic radiation
- light globes emit visible light, heat lamps emit infrared light and X-ray tubes emit X-rays.
- Synchrotron radiation extends over a broad range, from the infrared to X-rays, and different parts of this broad spectrum can be used for different purposes.
- the intensity of light being produced is a million times brighter than sunlight and a billion times greater than the radiation from a typical laboratory X-ray source
- The emerging beams are extremely fine – just a few thousandths of a millimeter across – and are emitted in extremely short pulses, typically 10-100 picoseconds in length

Detectors

- The simplest x-ray detector is x-ray sensitive film (cameras)
 - Cameras can direct reflections to films in useful arrangements, allowing determination of indices and intensities for thousands of reflections on a single film
- Scintillation counters
 - Count x-ray photons and give accurate intensities over a wide range
 - consists of a transparent crystal (usually phosphor), and plastic containing anthracene, that fluoresces when struck by ionizing radiation
 - A photomultiplier tube (PMT) measures the light from the crystal and is attached to an electronic amplifier and other electronic equipment to count and possibly quantify the amplitude of the signals produced by the photomultiplier

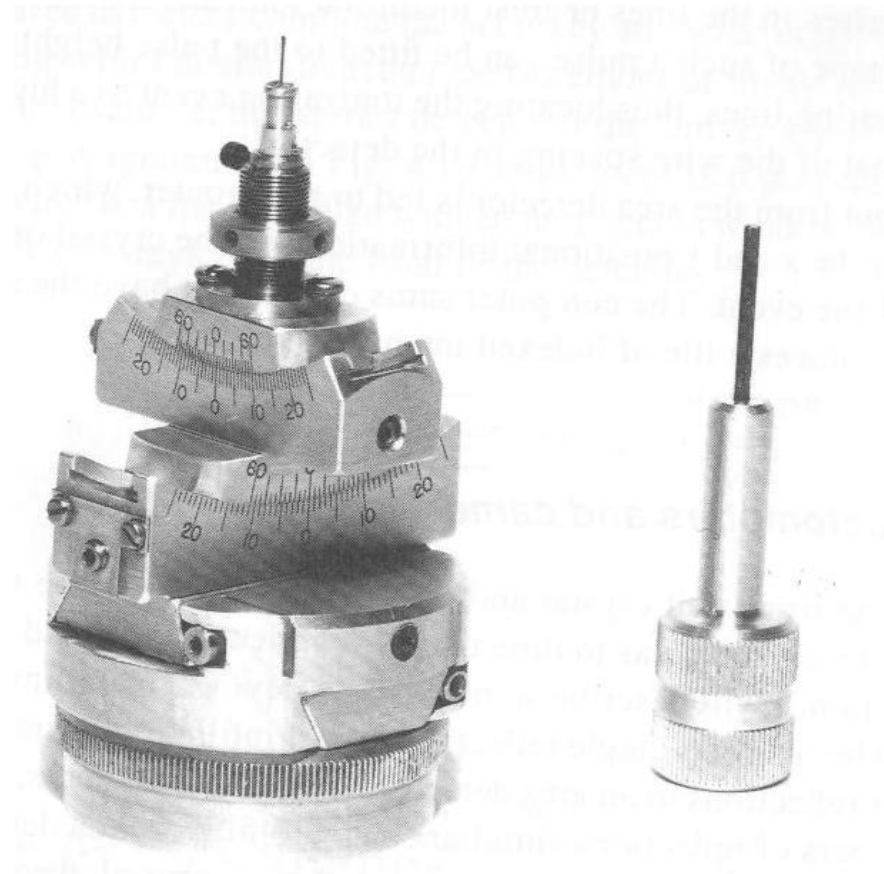
Diffractometer

- A diffractometer usually consists of:
 - a source of radiation
 - a monochromator to choose the wavelength
 - slits to adjust the shape of the beam
 - a sample and a detector



Goniometer

- In all types of data collection, the crystal is placed on a goniometer head, which allows the crystal orientation to be set
- The head consists of:
 - a holder for a capillary tube- which contains the crystal
 - two arcs-for rotating the crystal 40° in each perpendicular plane
 - and two dovetail sledges- which allow translation of the arcs to center the crystal on the axis of rotation

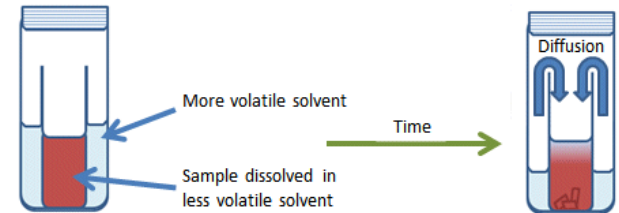


Crystal Growth II

- Methods for growing crystals:

- Vapor Diffusion

- preferred method because it involves straightforward setup and the crystals are harvested with ease
- drop of the protein is equilibrated with a large solution and then volatile species such as water, ions and certain solutes will diffuse between the drop and the solution until equilibrium is reached
- Hope is that an increase in protein concentration will bring the protein to within the crystallization phase

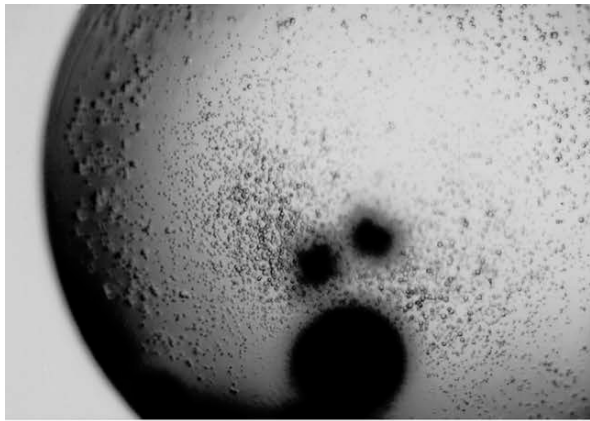


- Dialysis

- protein solution is dialyzed with a crystallization solution, in which the protein concentration is kept constant

- Seeding

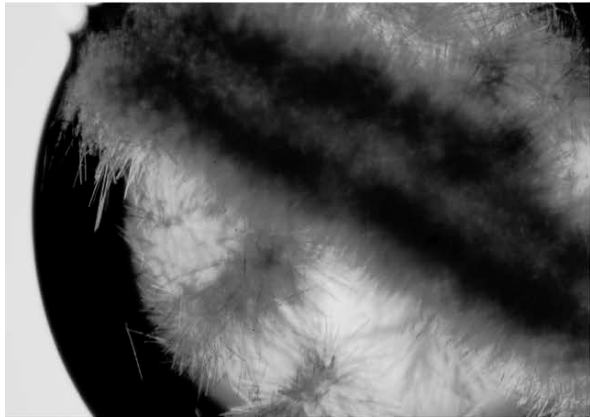
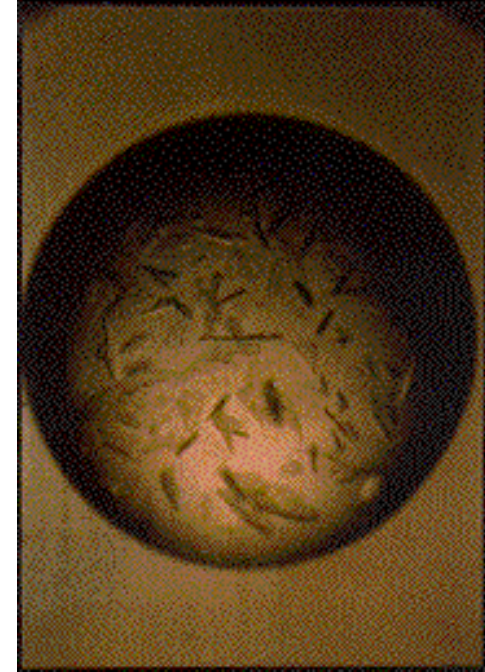
- used when the crystals are too small for x-ray diffraction.
- The crystal is added to a new drop of protein solution, and the crystal then acts as a nucleus for the larger crystal to grow



(a)

(a) Thin crystals obtained without seeding.

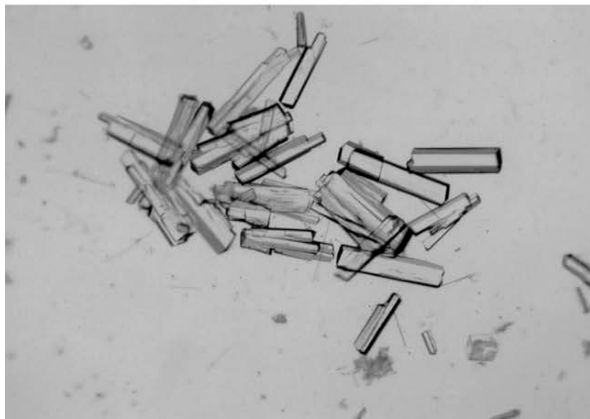
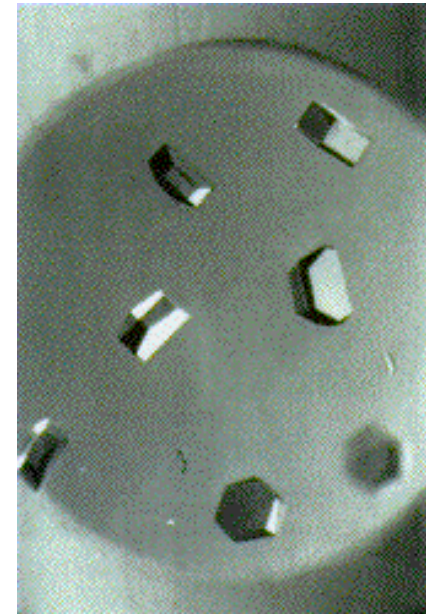
Crystals grown by normal vapor diffusion without microseeding. Conditions are the same as lower image. →



(b)

(b) Crystals after the first microseeding procedure of reduced concentrations of the precipitant sodium citrate.

Crystals produced in vapor diffusion using microseeding →



(c)

(c) Crystals suitable for X-ray analysis (size approximately $0.15 \times 0.05 \times 0.05$ mm) after a second microseeding procedure with the crystals shown in (b).

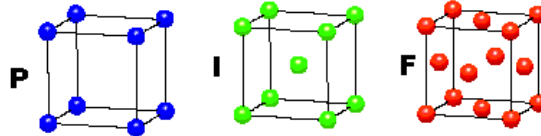
Bravais Lattices

- 14 basic types of crystal structures
- 3 fundamental repeat vectors a , b , c

CUBIC

$$a = b = c$$

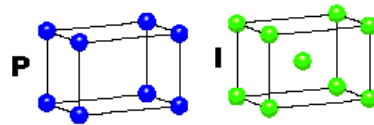
$$\alpha = \beta = \gamma = 90^\circ$$



TETRAGONAL

$$a = b \neq c$$

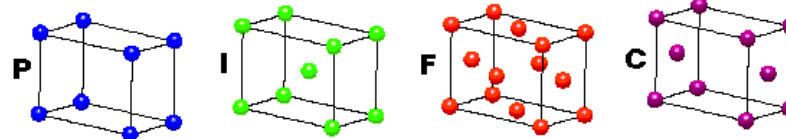
$$\alpha = \beta = \gamma = 90^\circ$$



ORTHORHOMBIC

$$a \neq b \neq c$$

$$\alpha = \beta = \gamma = 90^\circ$$

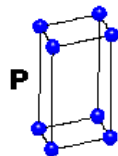


HEXAGONAL

$$a = b \neq c$$

$$\alpha = \beta = 90^\circ$$

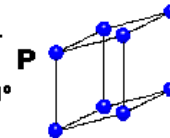
$$\gamma = 120^\circ$$



TRIGONAL

$$a = b = c$$

$$\alpha = \beta = \gamma \neq 90^\circ$$

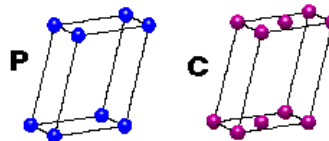


MONOCLINIC

$$a \neq b \neq c$$

$$\alpha = \gamma = 90^\circ$$

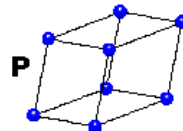
$$\beta \neq 120^\circ$$



TRICLINIC

$$a \neq b \neq c$$

$$\alpha \neq \beta \neq \gamma \neq 90^\circ$$



4 Types of Unit Cell

P = Primitive

I = Body-Centred

F = Face-Centred

C = Side-Centred

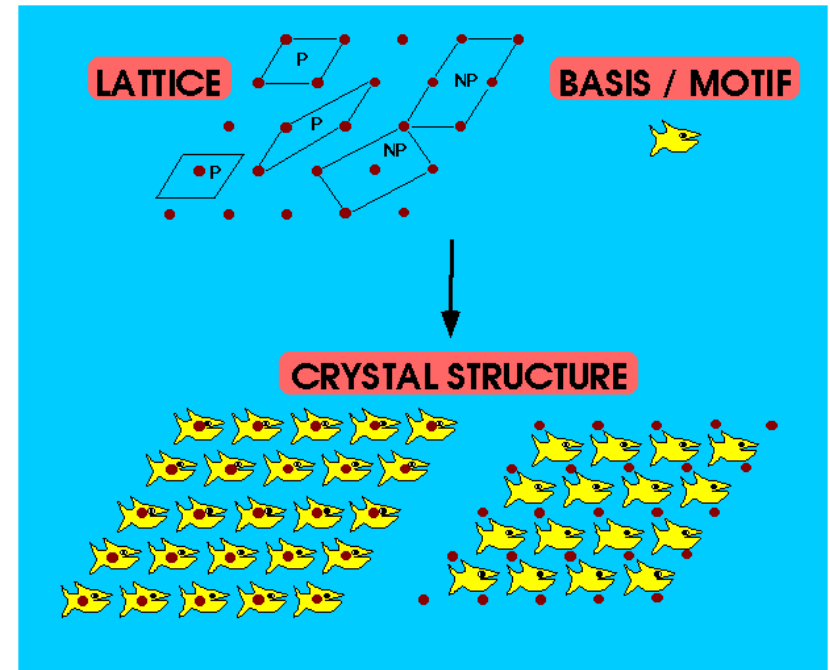
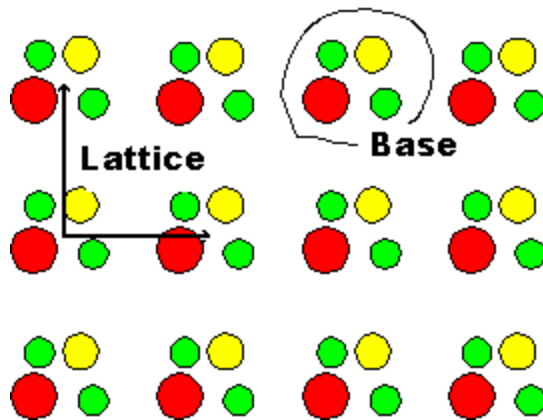
+

7 Crystal Classes

→ 14 Bravais Lattices

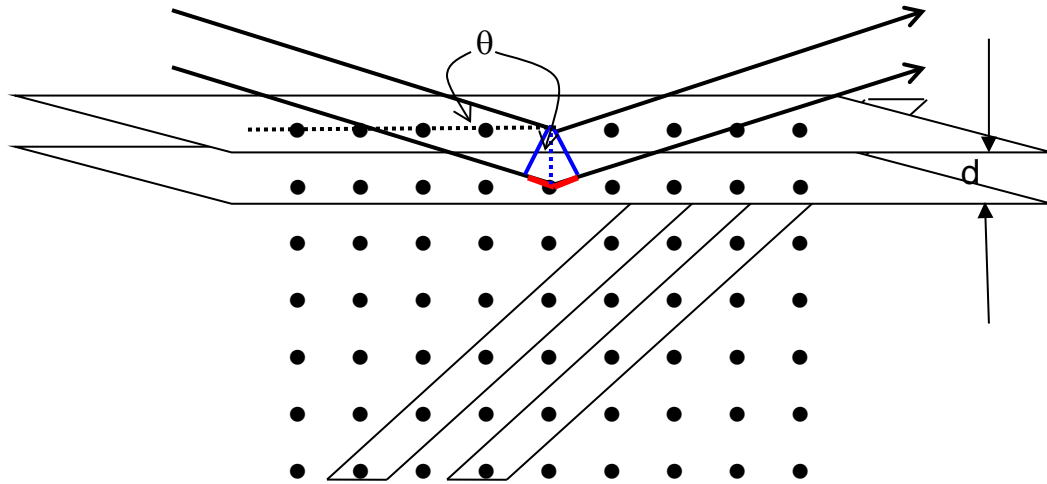
Biological Crystals

- Crystals have a repeating structure on the lattice which is not a single atom, but a single macromolecule



Crystal Diffraction

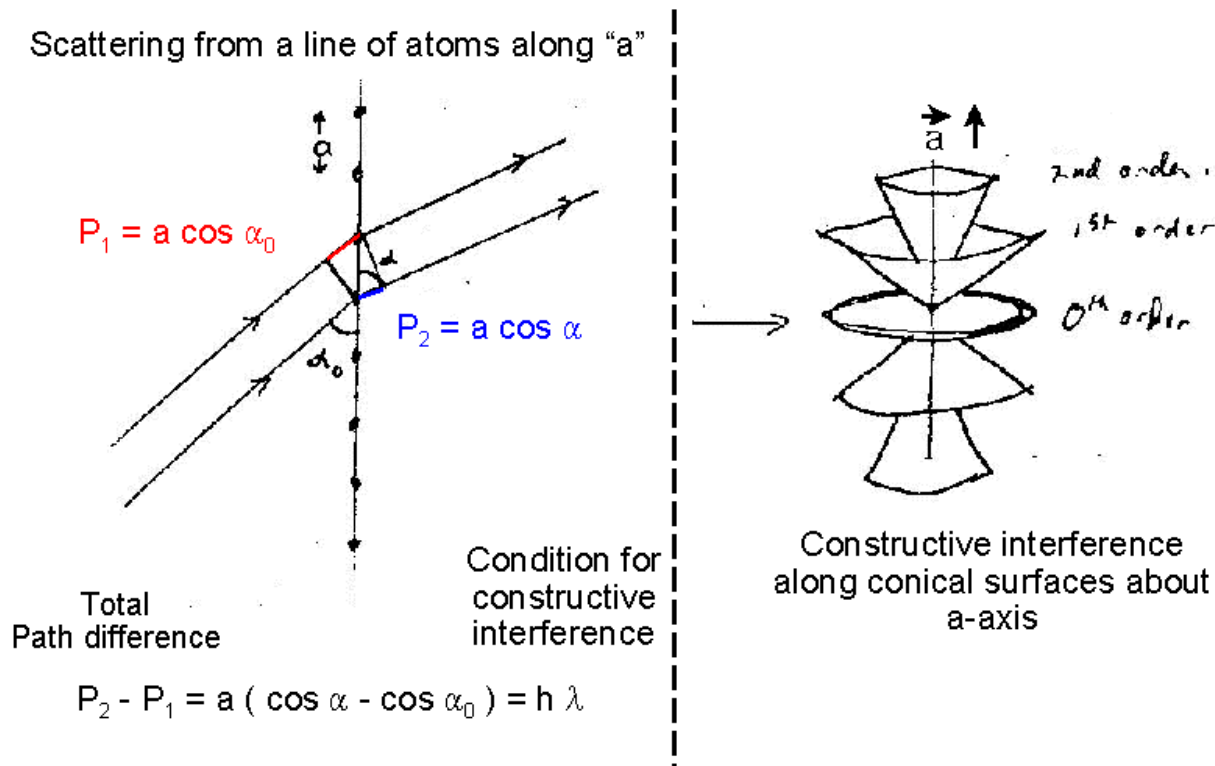
- Bragg diffraction from crystal planes



- Constructive interference when
 $n\lambda = 2d \sin \theta$ – note θ defined above

Von Laue Equations

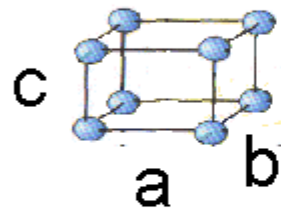
- In a 1-D crystal, with spacing a , we can write (when angle in \neq angle out) that for constructive interference $PD = a(\cos\alpha - \cos\alpha_0) = h\lambda$ – with $\alpha_0 = 90^\circ$ get conical surfaces for max. so on a piece of film get hyperbolas = layer lines



von Laue Equation

For a crystal with cell parameters

$\vec{a}, \vec{b}, \vec{c}$



We have three von Laue equations

$$\begin{aligned} a (\cos \alpha - \cos \alpha_0) &= h \lambda \\ b (\cos \beta - \cos \beta_0) &= k \lambda \\ c (\cos \gamma - \cos \gamma_0) &= l \lambda \end{aligned} \quad \textcircled{1}$$

Where $\cos \alpha_0$, $\cos \beta_0$, $\cos \gamma_0$ are the direction cosines of the incident ray and $\cos \alpha$, $\cos \beta$, $\cos \gamma$ are the direction cosines of the reflected ray in the crystal axis.

So, we must also satisfy

$$\begin{aligned} \cos^2 \alpha_0 + \cos^2 \beta_0 + \cos^2 \gamma_0 &= 1 \\ \cos^2 \alpha + \cos^2 \beta + \cos^2 \gamma &= 1 \end{aligned} \quad \textcircled{2}$$

Using that the angle between the incident and reflected ray is 2θ

$$\textcircled{3} \quad \cos 2\theta = \cos \alpha \cos \alpha_0 + \cos \beta \cos \beta_0 + \cos \gamma \cos \gamma_0$$

von Laue Equation

We square the von Laue Equations

$$\frac{h^2 \lambda^2}{a^2} = \cos^2 \alpha - 2 \cos \alpha \cos \alpha_0 + \cos^2 \alpha_0$$

①²

$$\frac{k^2 \lambda^2}{b^2} = \cos^2 \beta - 2 \cos \beta \cos \beta_0 + \cos^2 \beta_0$$

$$\frac{l^2 \lambda^2}{c^2} = \cos^2 \gamma - 2 \cos \gamma \cos \gamma_0 + \cos^2 \gamma_0$$

$$\left(\frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2} \right) \lambda^2 = 1 - 2(\cos \alpha \cos \alpha_0 + \cos \beta \cos \beta_0 + \cos \gamma \cos \gamma_0) + 1$$

Using eq. ③

$$\left(\frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2} \right) \lambda^2 = 2(1 - \cos 2\theta)$$

Trig. Identity

$$\left(\frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2} \right) \lambda^2 = 4 \sin^2 \theta$$

Generalized Bragg Equation

- Result is $\lambda \left[\frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2} \right]^{1/2} = 2 \sin \theta$
- Compare to Bragg equation in 1 dim:

$$n\lambda = 2d \sin \theta$$

- So, only at certain locations will there be constructive interference – get a set of bright spots in the diffraction pattern and, with lots of patience and effort, can use them to deduce the crystal lattice structure of the molecule being studied

Miller Indices – Bragg Planes

Rules for Miller Indices:

1. Determine the intercepts of the face along the crystallographic axes, *in terms of unit cell dimensions*.

2. Take the reciprocals

3. Clear fractions & reduce to lowest terms

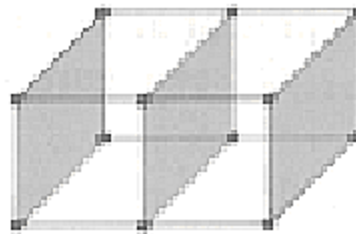
For example, if the x-, y-, and z- intercepts are 2, 1, and 3, the Miller indices are calculated as:

Take reciprocals: $1/2$, $1/1$, $1/3$

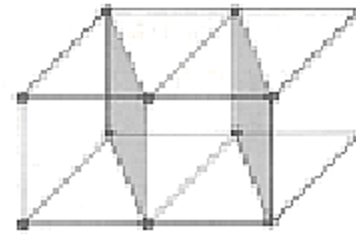
Clear fractions (multiply by 6): 3, 6, 2

Reduce to lowest terms (already there)

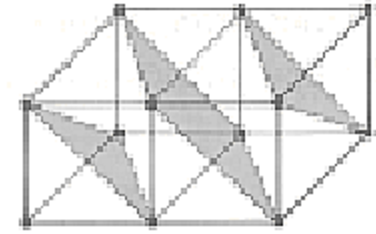
Primitive cubic lattice



100 planes

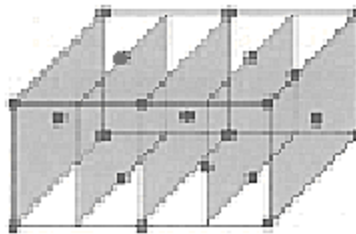


110 planes

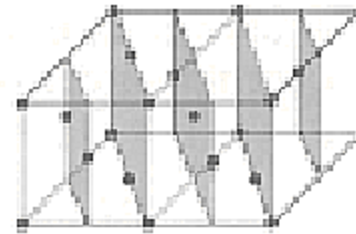


111 planes

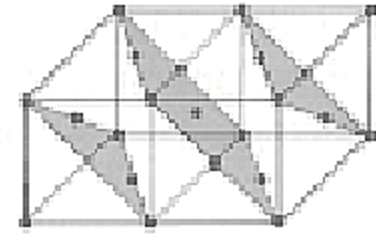
Face-centred cubic lattice



200 planes

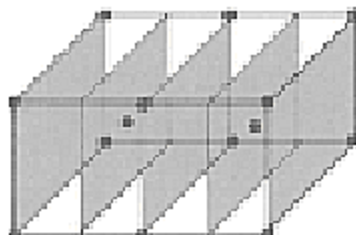


220 planes

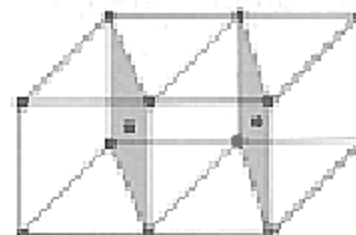


111 planes

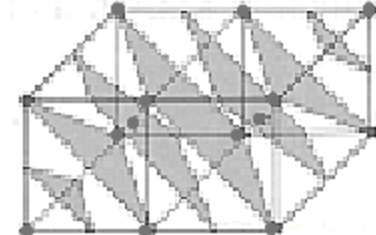
Body-centred cubic lattice



200 planes



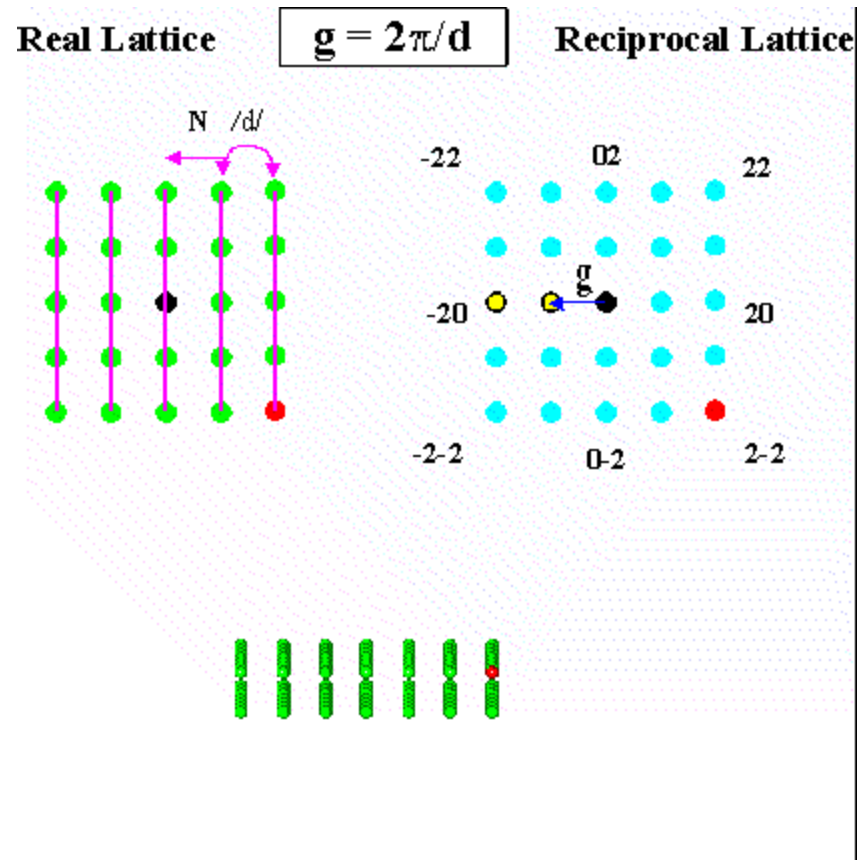
110 planes



222 planes

Miller indices for three types of cubic lattices.

Reciprocal (Fourier) Space

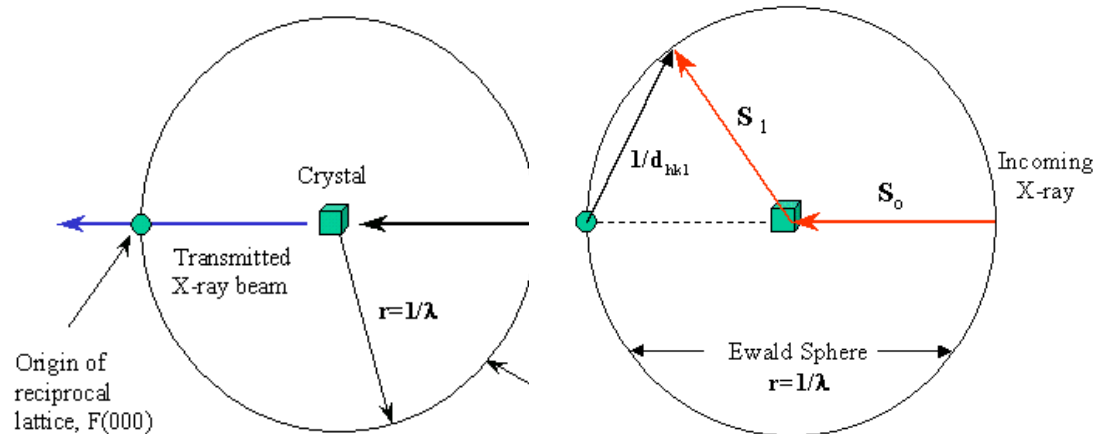


How can we observe diffraction?

- We want to get as many spots as possible so we need to rotate/oscillate the crystal being studied to get all possible “Bragg planes” giving rise to interference spots
- Or – we can use a “powder” of small crystallites – with randomly oriented axes – to see an average = powder diffraction pattern (but lose lots of detailed information)

Ewald Sphere construction

- [Animation link](#)

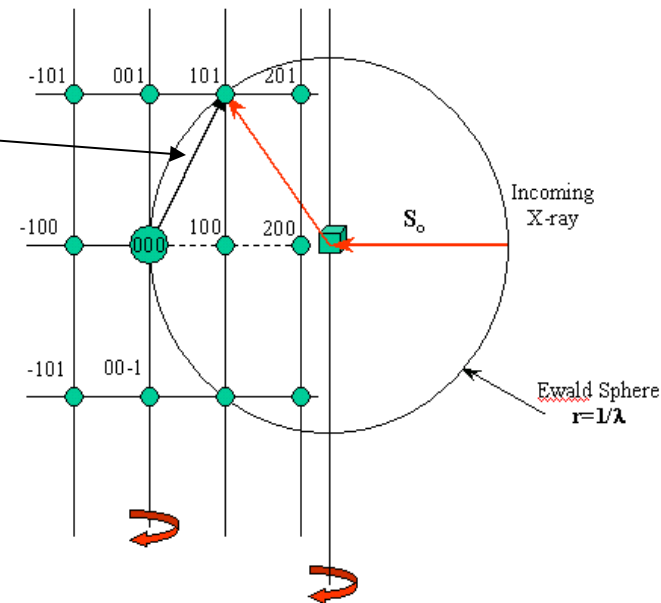


- [Second Link](#)

- Matching $(1/d_{\text{plane}})$ with

$$|\vec{s}| = |\vec{s} - \vec{s}_o| = \frac{2}{\lambda} \sin \theta$$

leads to a diffraction spot



Real Problem 1

- Up till now, we have treated the problem as if there is only 1 identical point scatterer at each lattice site
- If the scatterers are not points, but real macromolecules, then there is a “structure factor” accounting for interference within the macromolecule (like $P(\theta)$ in light scattering)

$$F = \frac{E_{\text{scattered}}}{E_{\text{scattered by } 1e^- \text{ at origin in each unit cell}}}$$

Real Problem II

- We can show that

$$F = \iiint \rho(\vec{r}) e^{2\pi i \vec{r} \cdot \vec{s}} d^3 r$$

where ρ is the electron density in the macromolecule and s is the reciprocal vector:

$$|\vec{s}| = \frac{\hat{s}}{\lambda} - \frac{\hat{s}_o}{\lambda} = \frac{2}{\lambda} \sin \theta$$

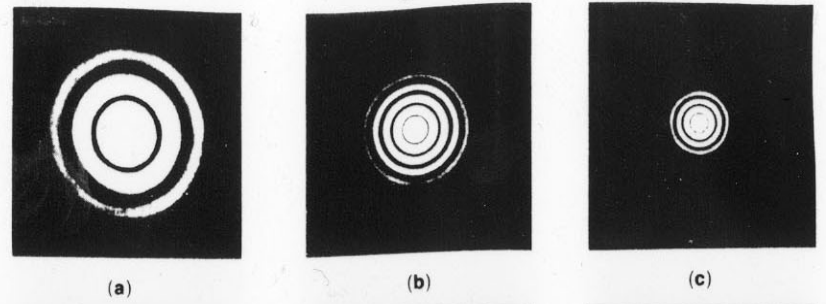
- Mathematically F is the Fourier Transform of ρ or $F = \text{FT}[\rho]$ –
- If we can invert this, we can find ρ :
 $\rho = \text{FT}^{-1}[F]$ which gives us the structure

MASK = STRUCTURE = $\rho(x)$

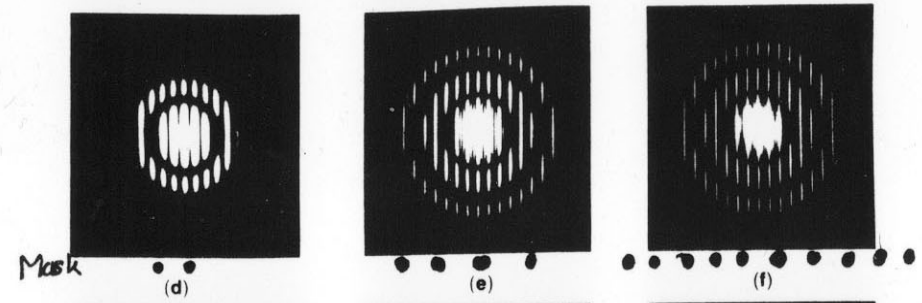
$J_1(x)$
Mask: ↘

Diffraction Pattern = $F(s)$

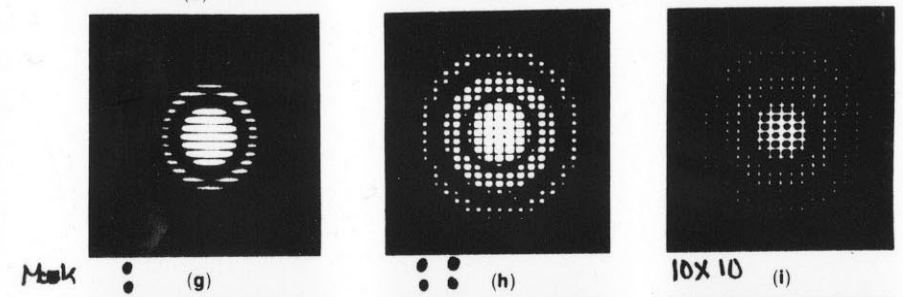
Holes of increasing size



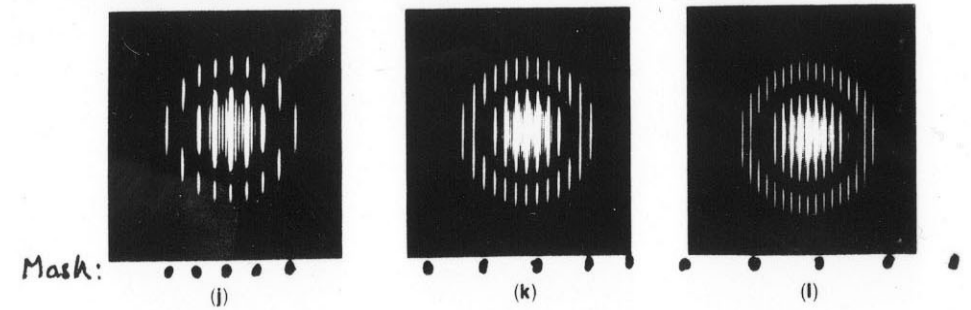
Several equally spaced holes



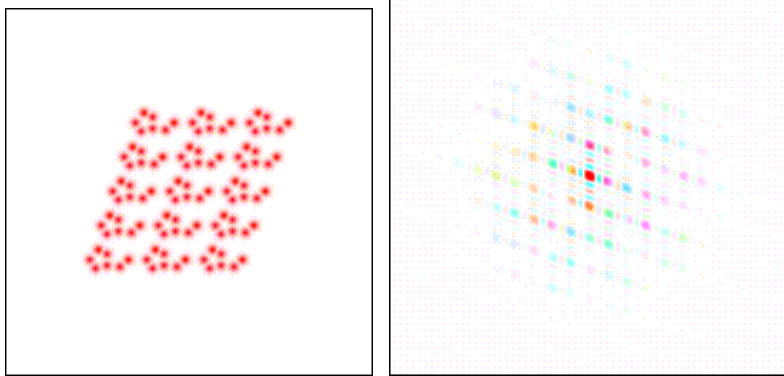
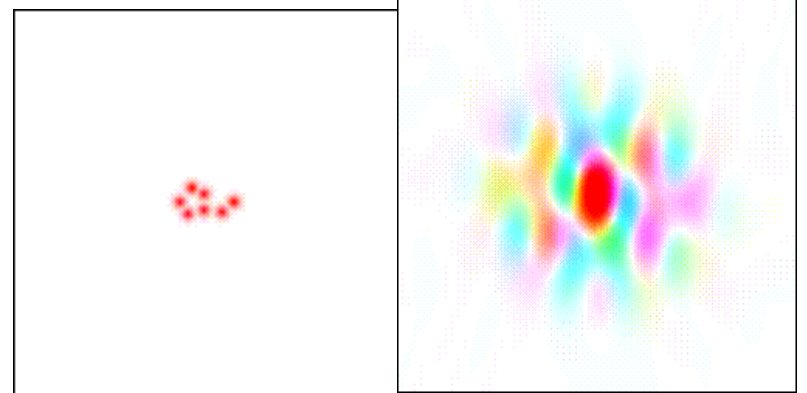
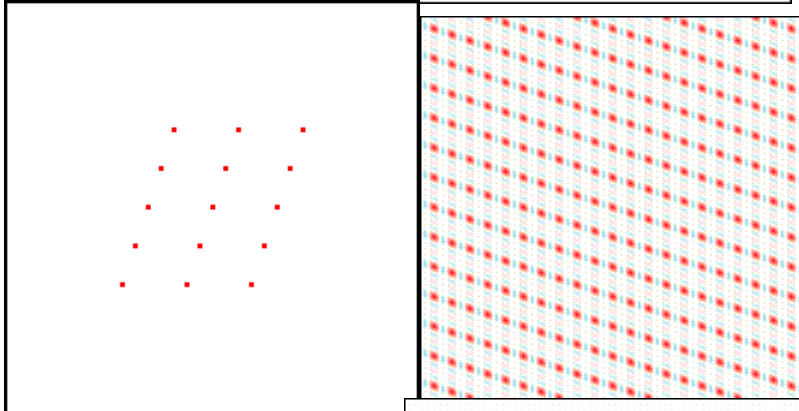
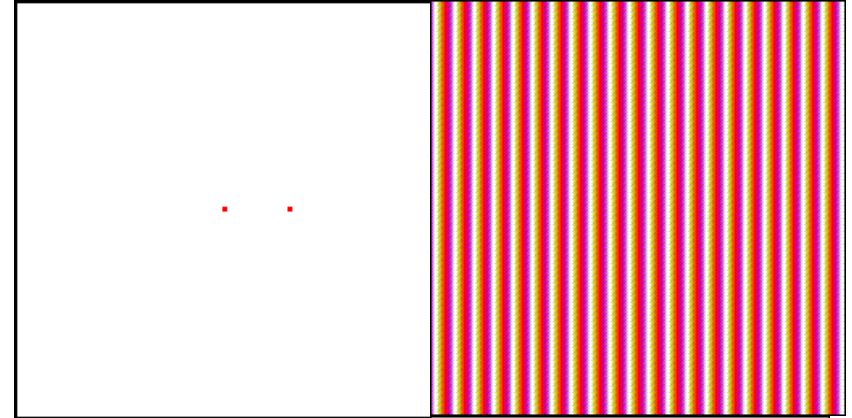
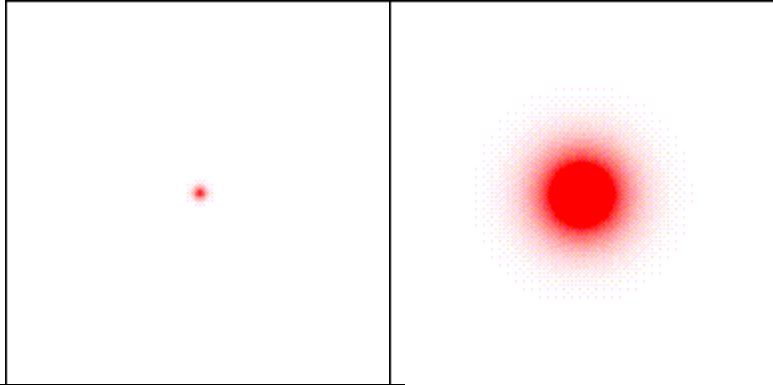
Arrays of holes



5 increasingly spaced holes



Some FT's



Phase Problem

- Unfortunately, what we measure is not F directly, but the intensity I where

$$I \propto |E^2| \propto |F^2|$$

- But $F = (\textit{amplitude})e^{i(\textit{phase})}$ and

$$|F^2| = (\textit{amplitude}^2)$$

so that we lose all the phase information and cannot do the inverse FT exactly

What to do?

- We can measure:
 - Diffraction spot intensities
 - Unit cell dimensions
 - Symmetry group
- We want to find out:
 - Location of each atom in unit cell
 - Type of atom and expected F
 - Phase associated with each atom

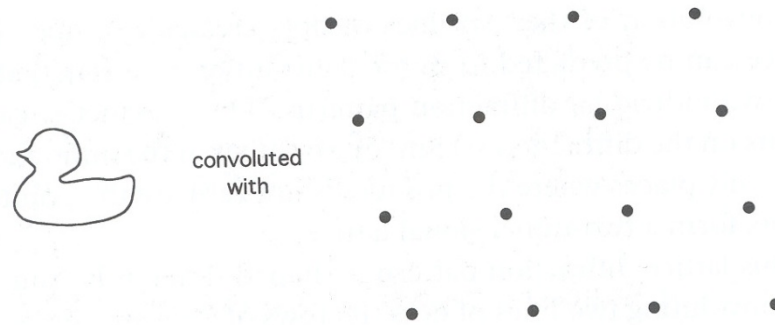
Patterson Function

- From measurements of I we can take its FT: $FT[I] = FT[|F|^2] = FT[F(s)F^*(s)] = P(r)$
= Patterson function – this can be calculated based on the x-ray picture
- But – the convolution theorem says:

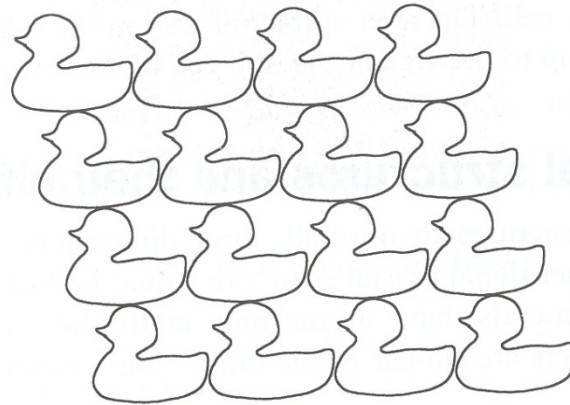
$$P(r) = FT[F(s)] \otimes FT[F^*(s)] = \rho(\vec{r}) \otimes \rho(-\vec{r})$$

- Convolution

Convolution of a duck with a lattice



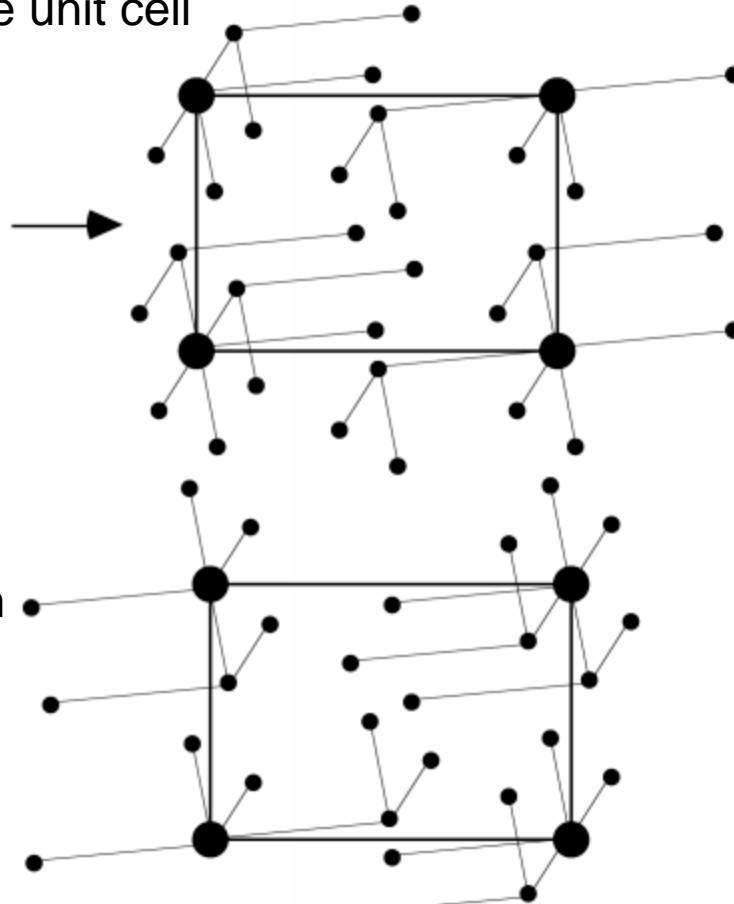
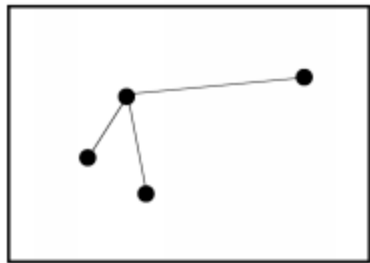
makes



Convolution ideas

Single molecule per unit cell

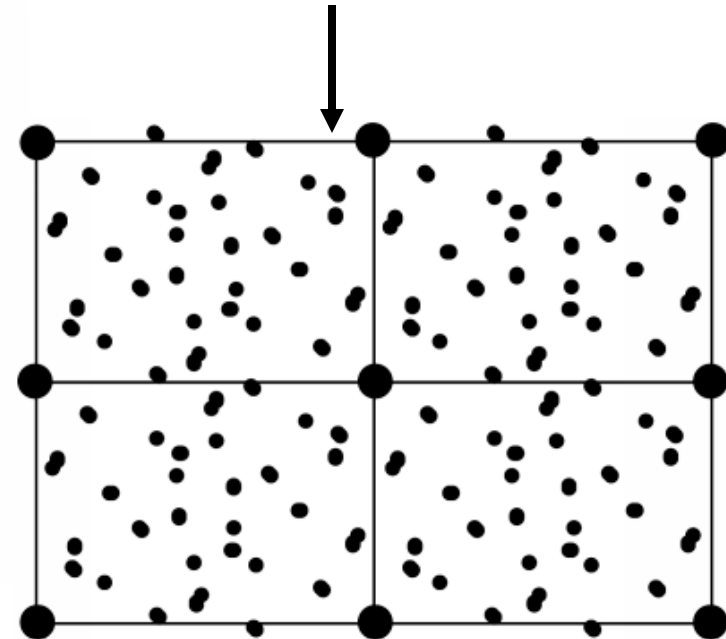
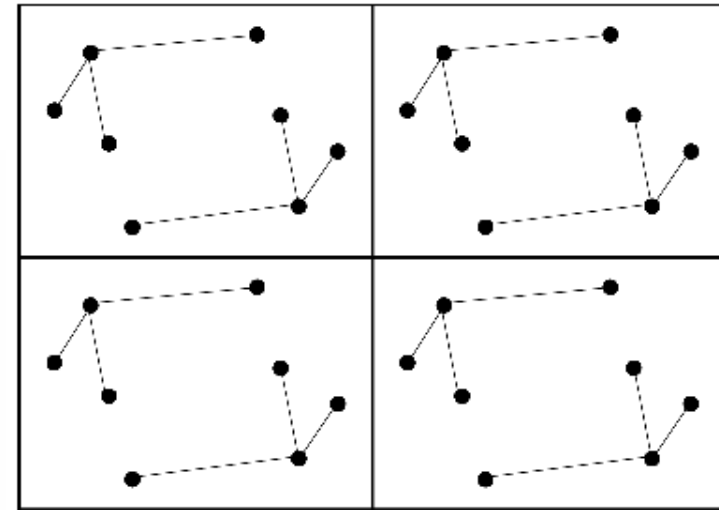
Each atom is placed at each site of the unit cell



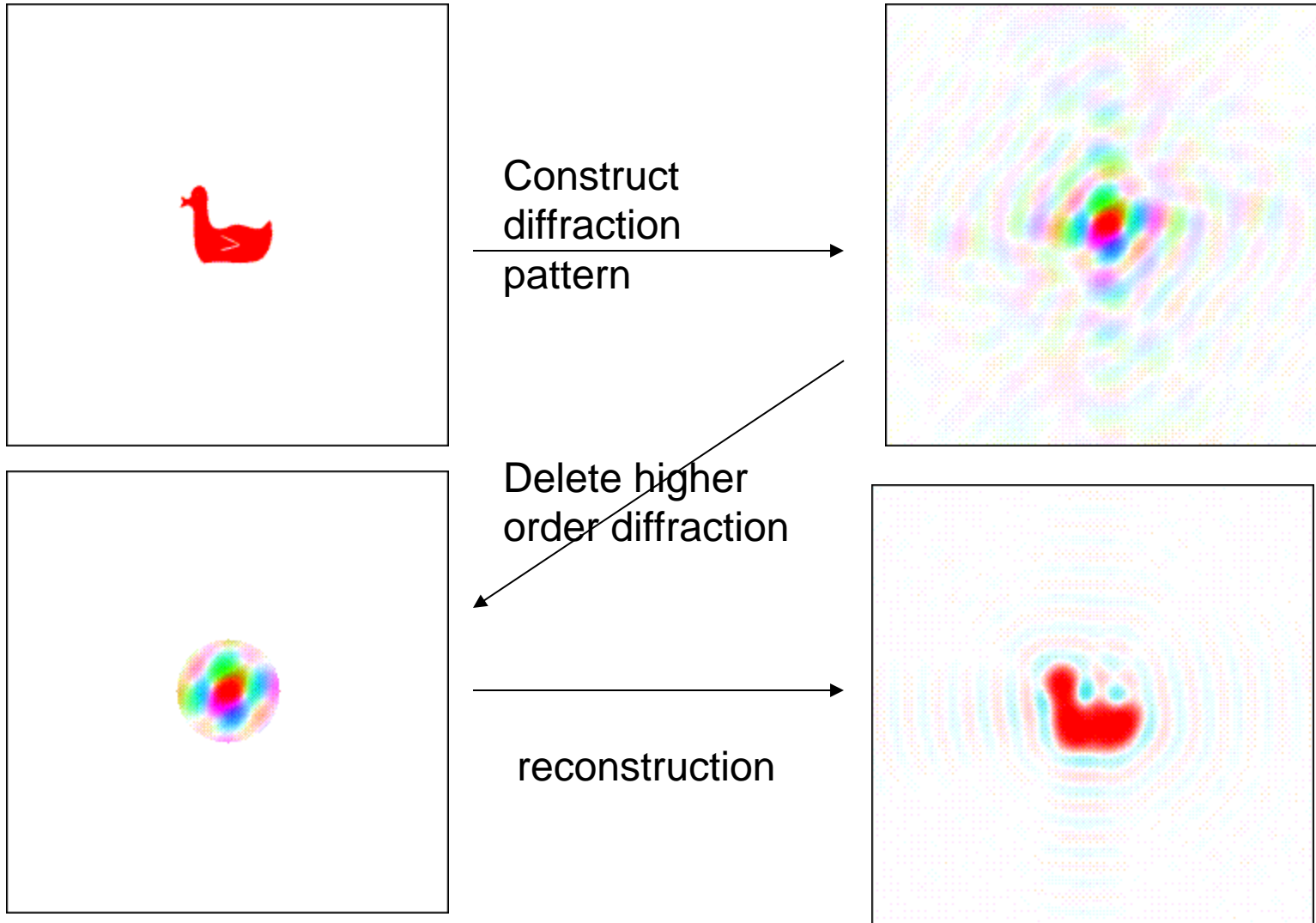
The same set of spots comes from placing the inverse image at each site

Number of spots = N^2 where N = # atoms - gets huge!

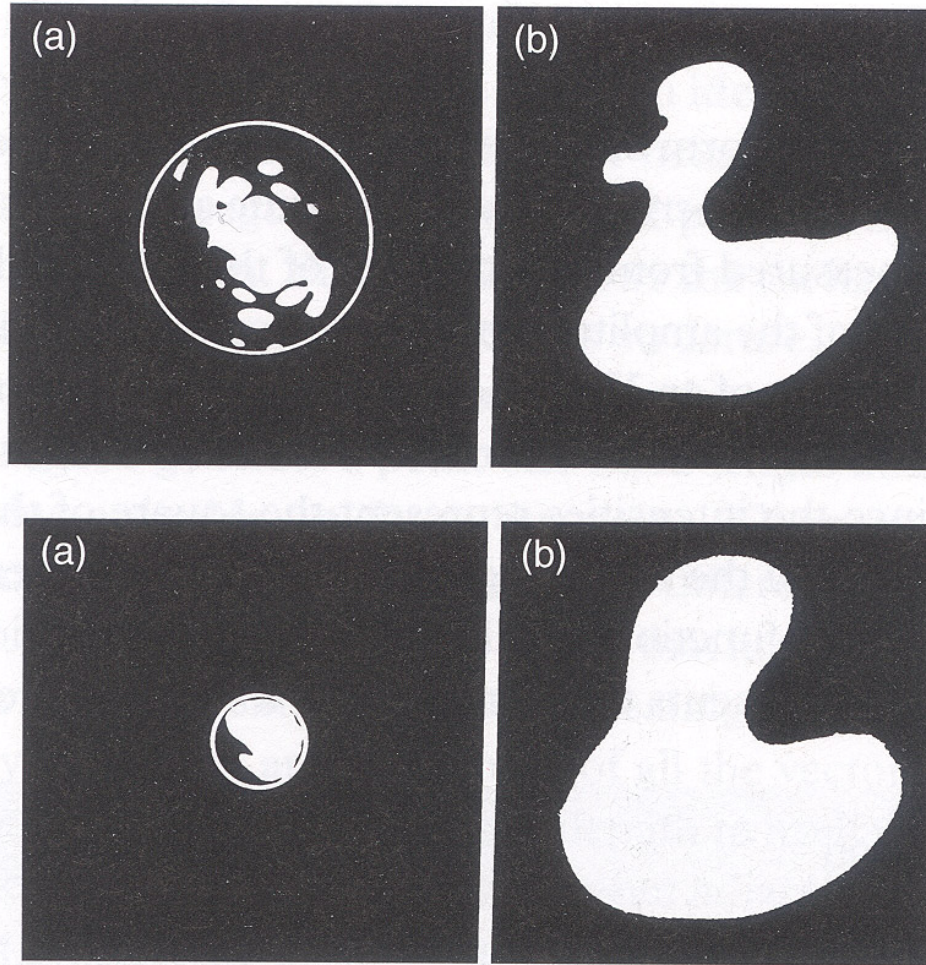
2 molecules/unit cell



Loss of information

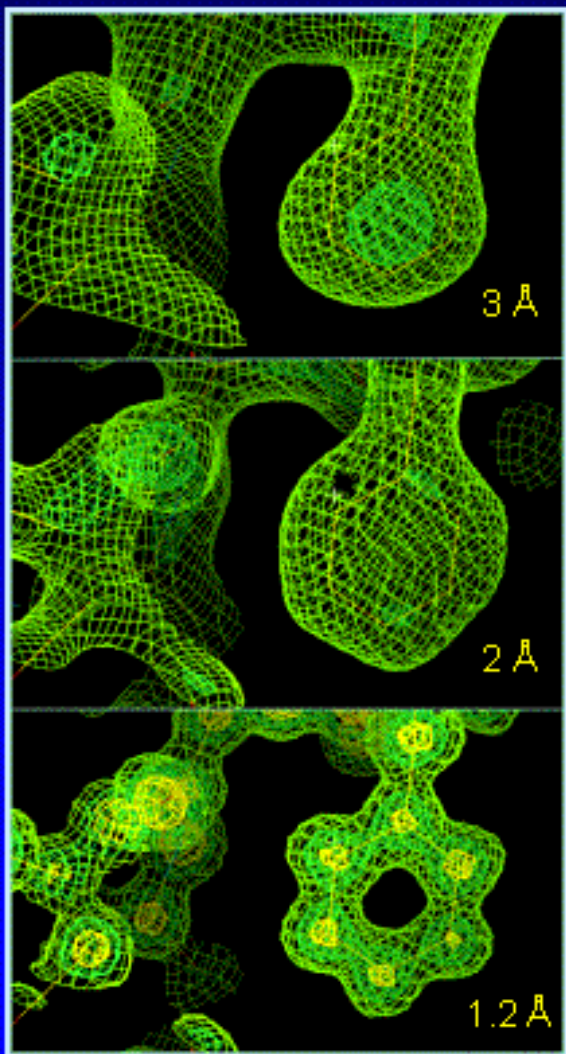


Resolution of the Diffraction Duck

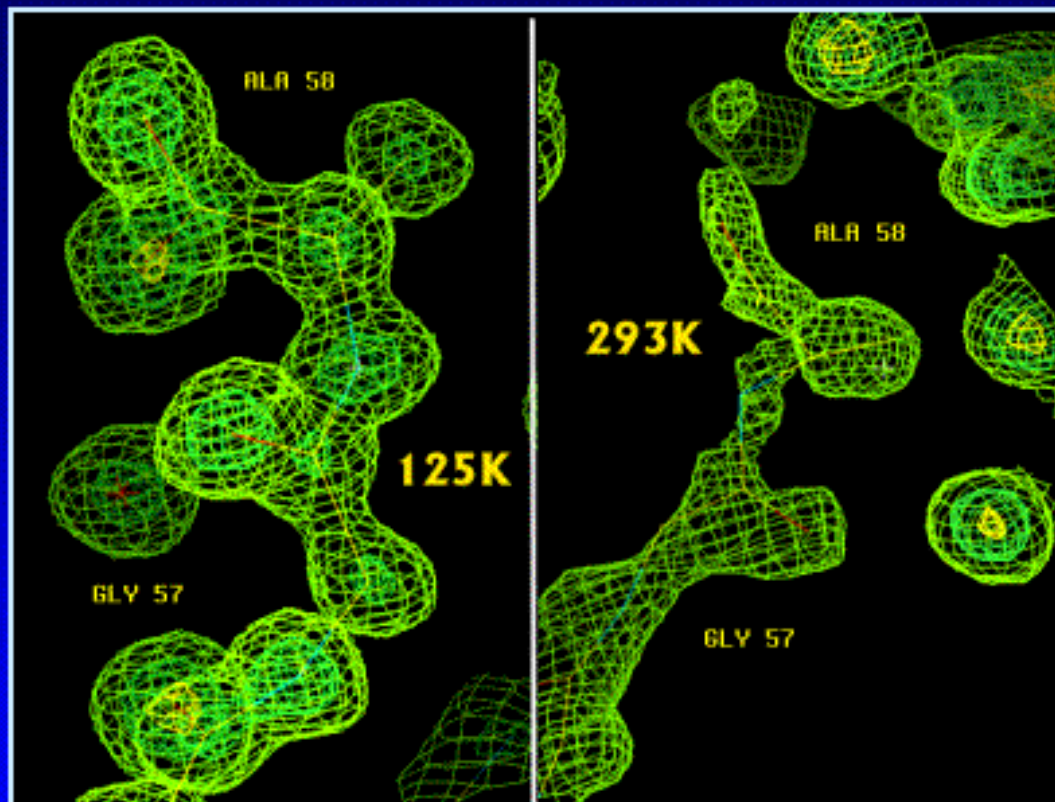




Importance of resolution



Reduced disorder at low temperature



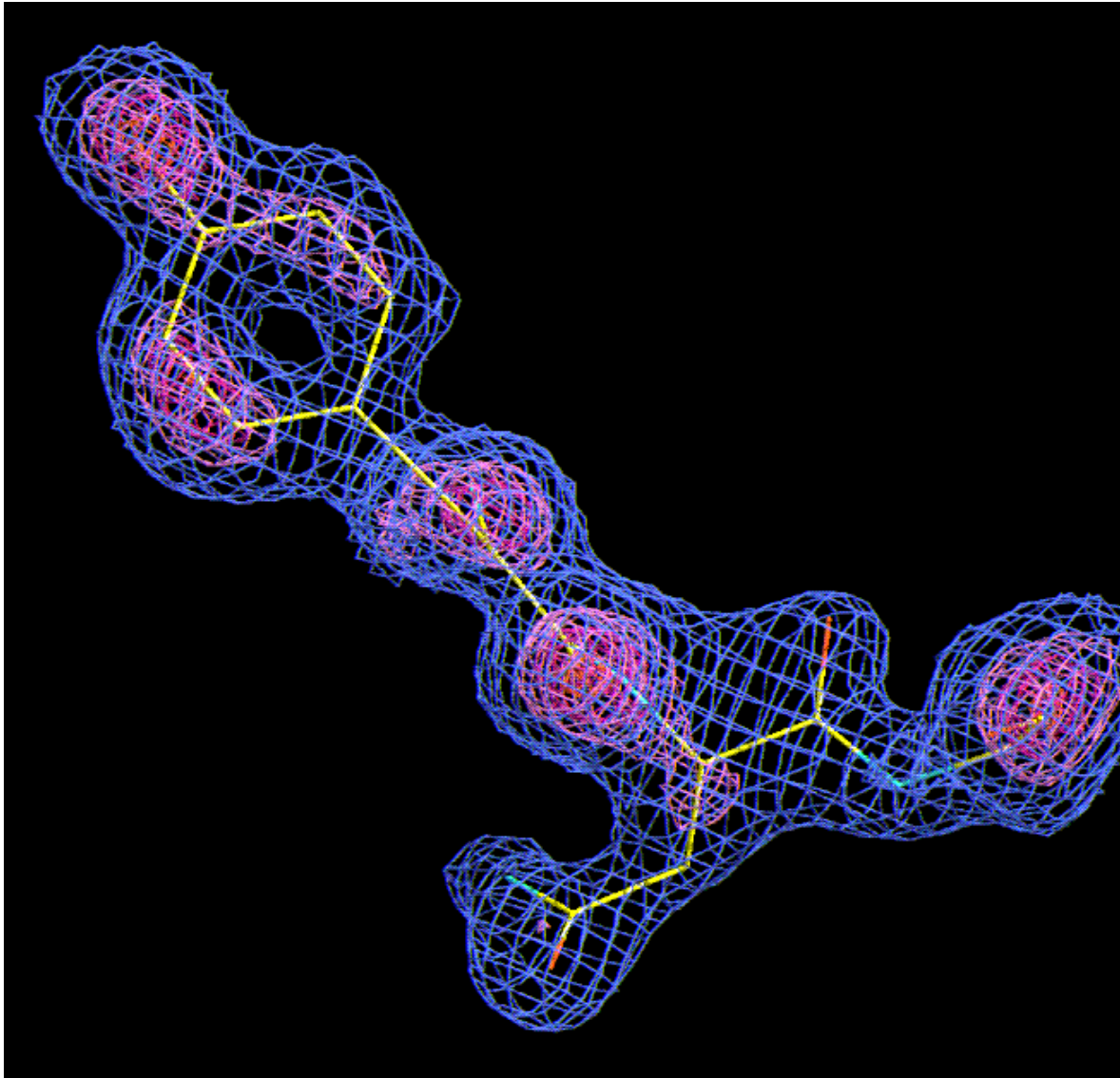
Dramatic improvements in the overall structure are likely to result from better definition of disordered regions regardless of resolution

How to Solve the Phase Problem

- Several tricks used to help identify spots
 - Heavy atom method (if $MW < 1000$ or so)

If the molecule has a heavy atom – it gives rise to lots of intensity and can be used to pick out those spots from Patterson from this atom – use this to help calculate amplitude and phase from this heavy atom – if no heavy atom present – can try to synthesize the molecule with an added specific heavy atom and then make a crystal of it.
 - Multiple Isomorphous Replacement (MIR) –
 - make several heavy atom derivative crystals and use these to solve the phase problem by taking difference Patterson's – in general need at least 2 isomorphous replacements to solve a structure

Great data



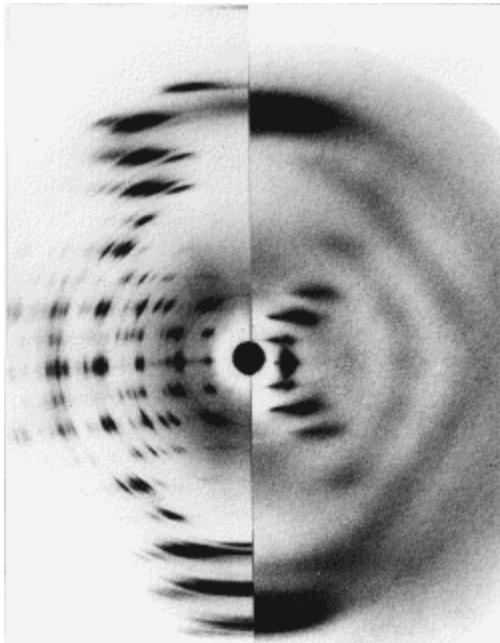
Part of tetanus
C protein –
tyrosine residue
showing ring
hole at high
resolution

Fiber X-ray Diffraction

- Some samples do not form crystals, but can be studied by x-ray diffraction if they can be drawn out into fibers – two types: small crystallites oriented parallel to each other (as in A-DNA) and non-crystalline fibers, with all macromolecules oriented in the same direction, as formed in a capillary tube, for example (as in B-DNA)
- So, these samples need to be elongated – so they can be oriented by flow or other means (B-field, etc.)
- The fiber is put in the x-ray beam with its axis perpendicular to the beam

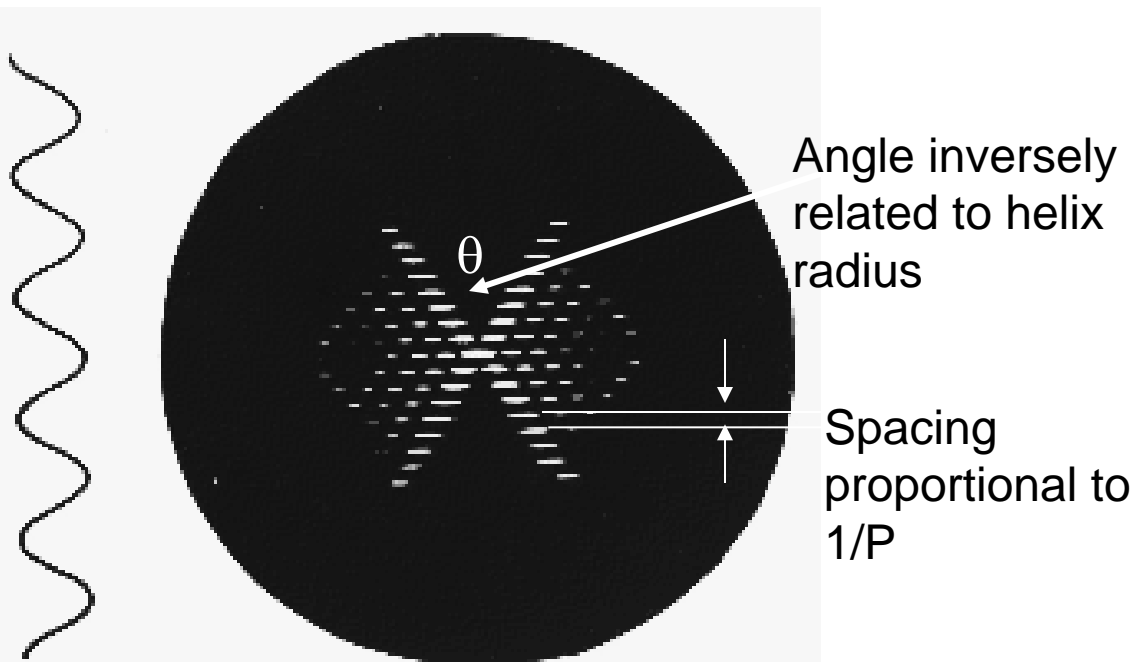
Fiber diffraction patterns

- Fiber diffraction patterns –
Left (A-DNA – oriented crystallites- showing spots); right (B-DNA fiber showing layer line structure due to helices – but without spots along layer lines because the helices are randomly oriented around the helix axis)



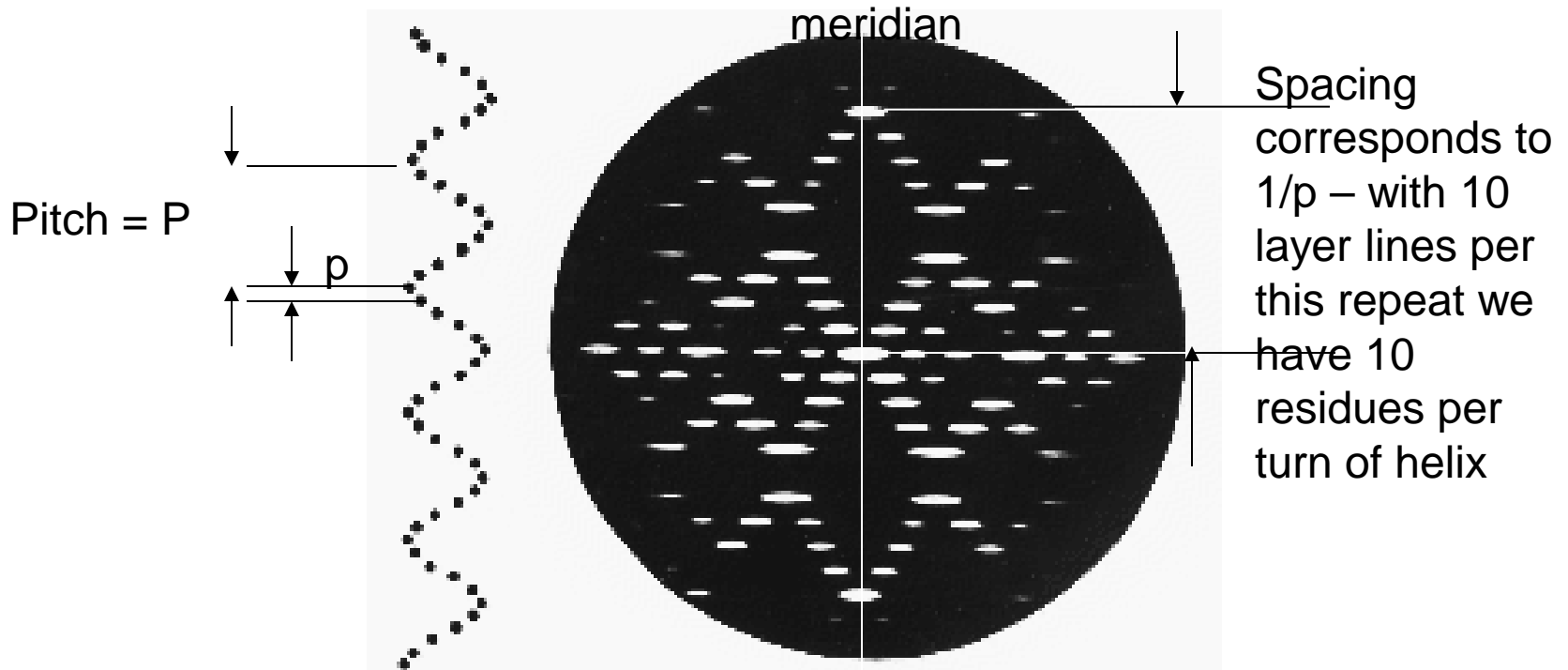
Diffraction from a continuous helix

- A continuous helix is defined by its pitch P and its diameter d – the helix is like a one-dimensional crystal (along z) – and scatters at a set of planes perpendicular to the x -axis – in reciprocal space, these planes are separated by $1/P$ and gives rise to a characteristic X pattern with the angle θ related to the helix diameter

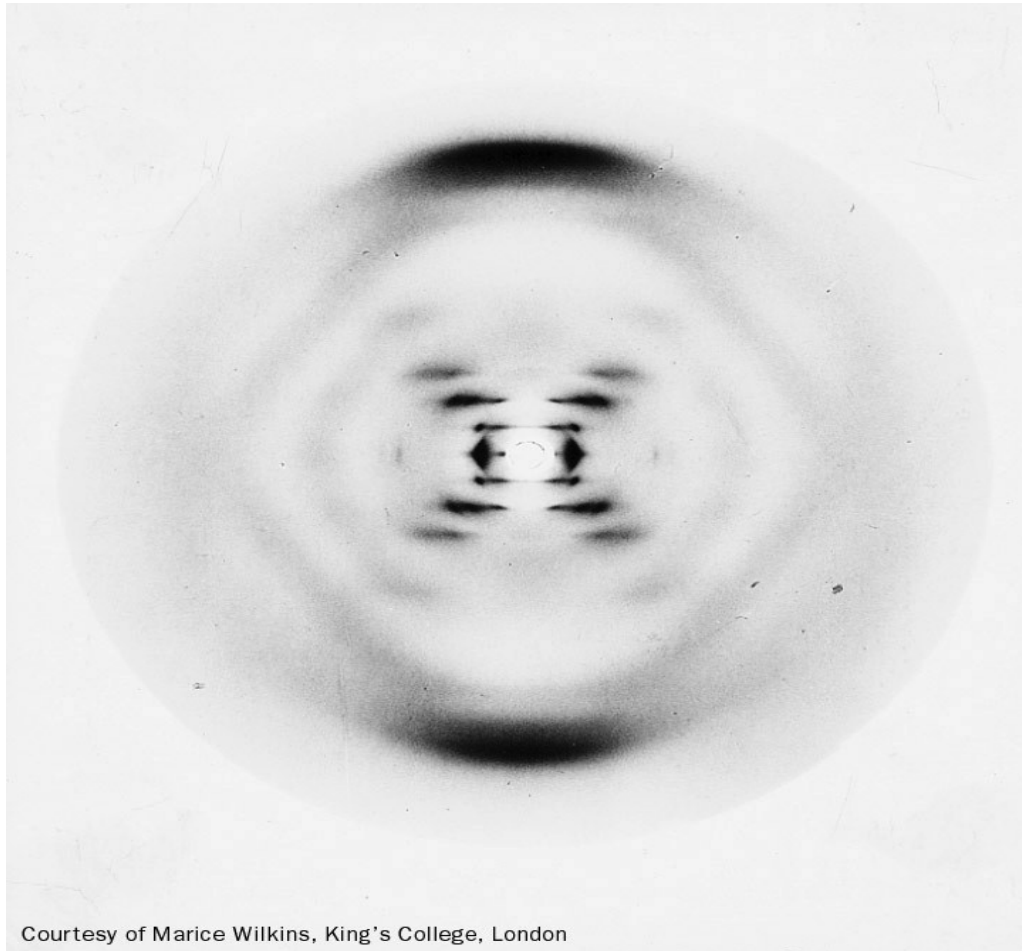


Diffraction from a discrete helix

- For a discrete helix with identical repeating “atom” the diffraction pattern is similar, with layer lines again $\sim 1/P$ and an overall X pattern with angle $\sim 1/d$, but with additional structure – the helix is also described by the axial rise per atom (or residue) = p
- The diffraction pattern spacing of X's along the meridian is related to $1/p$
- Then the number of residues/turn of helix is given by the ratio of P/p
- With different residues each has a “structure factor” that varies intensity

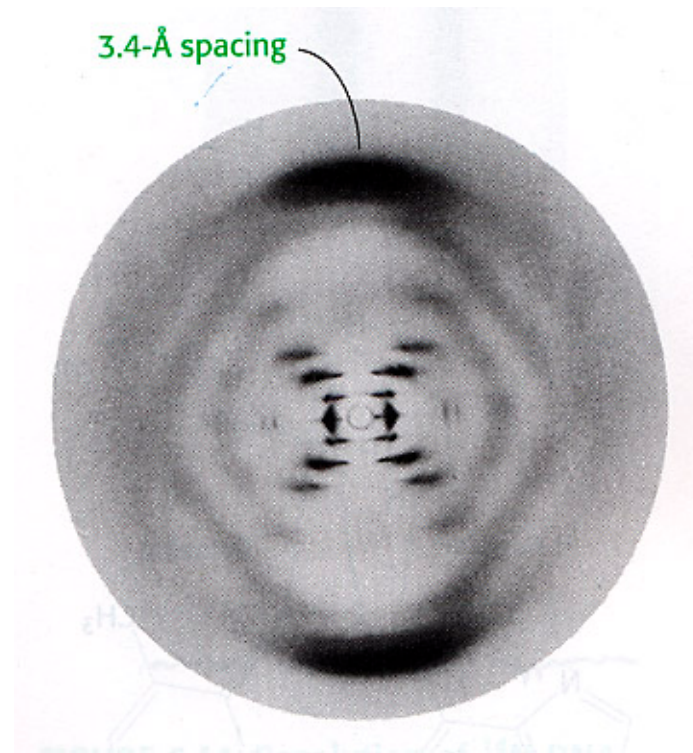


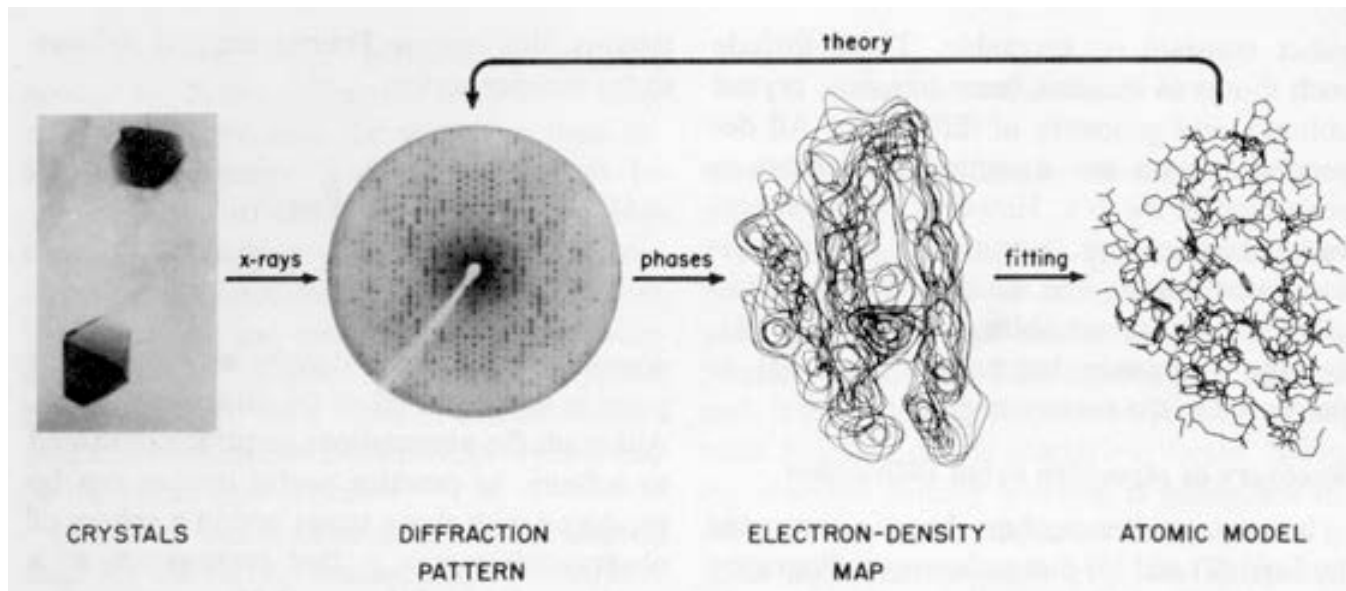
X-ray diffraction photograph of a vertically oriented Na^+ DNA fiber in the B conformation taken by Rosalind Franklin.



Courtesy of Marice Wilkins, King's College, London

X-ray Diffraction Pattern of DNA Fiber





DNA structure

