An introduction to data collection theory

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Introduction to data collection theory

n The lecture will cover:

- Crystals, lattices, unit cells, the 7 crystal systems, lattice types, Bravais lattices, point groups (very compact!)
- Lattice to crystal structure, contents of a unit cell
- Symmetry
- Diffraction
- Reciprocal space
- The Ewald construction and its use
- What limits Resolution?
- Scattering of X-rays by electrons, atoms, molecules, crystals
- Scattering and Intensity
- Data reduction

X-ray……crystallography……..Why?

n **Why Xrays?**

- We want to locate atoms, preferably as separate items
- The distances between atoms are 0.9~2.3 x 10^{-10}m (1Å is 10^{-10}m)
- Abbé principle: what you can see (resolve) is limited by the wavelength of the light you use:
  - D_{min} = λ/2
- This is X-rays! (λ is *ca.* 0.5 – 4Å)

n **Why crystals?**

- **We have no way to focus X-rays…no lenses**
- Therefore must use **diffraction**
- Diffraction requires **ORDER** (system) in 1, 2 or all 3 directions
- A crystal consists of ions, atoms or molecules stacked in a repetitive, 3-D pattern
- Therefore a crystal will diffract light if the wavelength is right
- **Result is X-RAY DIFFRACTION**

NORSTRUCT

Googles for "What are crystals?"

n   A crystal is a **homogenous solid formed by a repeating three-dimensional pattern** of atoms, ions, or molecules and having fixed distances between constituent parts....

n   Crystals are materials which has its *(sic!)* **molecules arranged in a specific, highly ordered internal pattern**. This pattern is reflected in the **crystal's external structure which typically has symmetrical planar surfaces**. Many common substances, from salt to sugar, from diamonds to quartz form crystals. They can be shaped so that they will vibrate at a specific frequency and are widely used in radio communications and computing devices. New Agers believe that crystals posses healing energy. See New Age

n   **Attractive rock formations of natural or psychological power that should on no account be buried at ancient sites.** People who advocate this do not know the problems they cause archaeologically and otherwise, and are advocating wilful vandalism on a par to lighting fires, writing graffiti, etc., within a sacred area.

Crystals

- A crystal consists of millions of molecules which are stacked together systematically in all three spatial directions.

- **The repeating unit** usually contains a number of molecules.

- The repeating unit is called the UNIT CELL

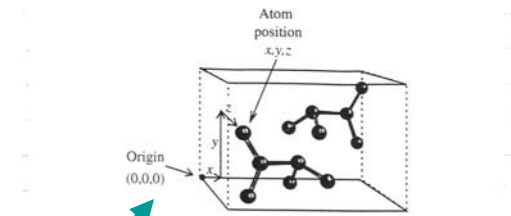- **It is frequently not obvious!**

- Calcium carbonate – hyperlink

Figure 2.4 One unit cell from Fig. 2.3. The position of an atom in the unit cell can be specified by a set of spatial coordinates x,y,z.
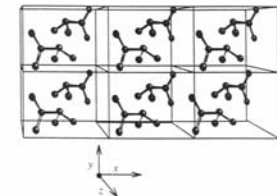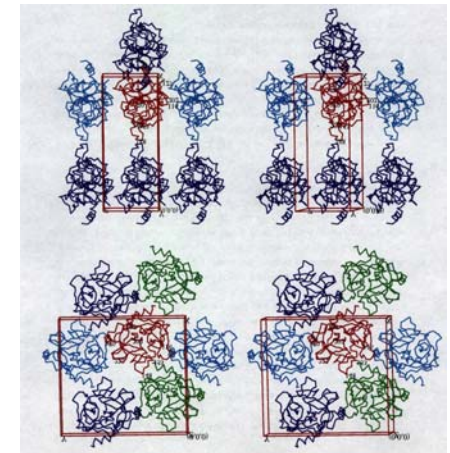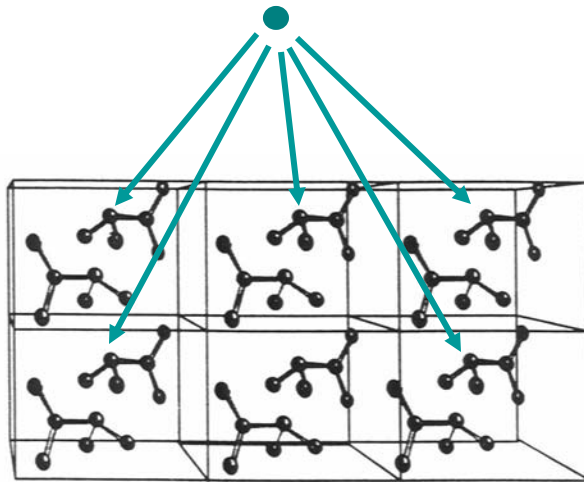
6 Unit cells

Figure 2.3 Six unit cells in a crystalline lattice. Each unit cell contains two molecules of alanine (hydrogen atoms not shown) in different orientations.

A protein crystal

Structure to lattice

If we place a point at the SAME SITE in each unit cell we end up with an array of points which contains all the information needed to describe the REPETITION in the crystal.

This is called the crystal LATTICE

The Lattice

A 3-D unit cell contains 1, 2 or 4 lattice points

NB! Each *corner* lattice point is *shared* with 8 other unit cells

The Unit Cell

- **The repeating unit is a "rhomboid" – a "box" with 6 faces, 8 edges and 3 angles**

- This can be described by three "axes" and 3 angles, a,b,c, α, β, γ……..

- ……..or can be described as three **VECTORS a, b, c**

- Unit cells have to stack together continuously and without gaps

- **This leads to 7 combinations of a, b, c, α, β and γ**

- **The so-called 7 CRYSTAL SYSTEMS**

The 7 crystal systems:

Crystal system	Conditions imposed on cell geometry	Minimum point group symmetry
Triclinic	None	1
Monoclinic	$\alpha = \gamma = 90°$ (b is the unique axis; for proteins this is a 2-fold axis or screw axis) or: $\alpha = \beta = 90°$ (c is unique axis; for proteins this is a 2-fold axis or screw axis)	2
Orthorhombic	$\alpha = \beta = \gamma = 90°$	222
Tetragonal	$a = b; \alpha = \beta = \gamma = 90°$	4
Trigonal	$a = b; \alpha = \beta = 90°; \gamma = 120°$ (hexagonal axes) or: $a = b = c; \alpha = \beta = \gamma$ (rhombohedral axes)	3
Hexagonal	$a = b; \alpha = \beta = 90°; \gamma = 120°$	6
Cubic	$a = b = c; \alpha = \beta = \gamma = 90°$	23

n **After selection of a suitable crystal the first stage in data collection is identification of the CORRECT crystal system**

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Choosing the correct/best lattice

n **1) Right handed** axis system:

n **2) a**, **b** & **c** are chosen to include possible **symmetry** in the lattice

n **3) a**, **b** & **c** are chosen as **short** as possible

n **4)** The chosen unit cell should be the smallest that satisfies 2). This can result in choice of a cell which **also contains lattice points in the centre of one or all of the faces** or **in the centre of the cell** rather than a *Primitive* lattice i.e. one which only contains 1/8 of a lattice point in each corner.

n **5)** The axes are named so that **a<b<c** but within condition 2)

n **6)** All the angles between **a**, **b** & **c** are either <90º or $\geq$ 90º

n **Which is correct here?**
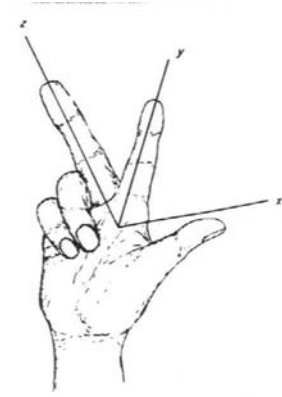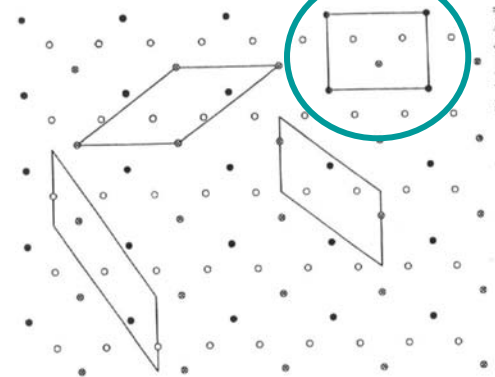

Figure 2.4. Right-hand rule.

This one!

Fig. 1.14 A two-dimensional pattern and some possible choices of unit cell.

Lattice types

- THE 4TH "RULE ABOVE STATED:
  - The chosen unit cell should be the smallest that satisfies 2 above
  - This can result in choice of a cell which **also contains lattice points in the centre of one or all of the faces or in the centre of the cell** rather than a *Primitive* lattice i.e. one which only contains a lattice point in each corner (note that there is actually 1/8th of a lattice point in each corner)
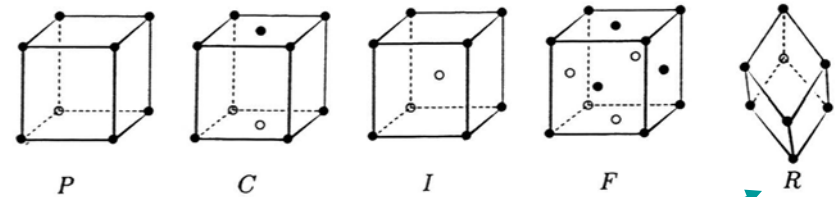
- They are called:
  - **Primitive** – **1 lattice point in the cell – symbol P**
  - **C-face centred** - **2 lattice points in the cell – symbol C**
  - **Body centred** – **2 lattice points in the cell – symbol I**
  - **All-face centred** – **4 lattice points in the cell – symbol F**
  - **Rhombohedral** – **1 lattice point in the cell – symbol R**

- *Combining this* information *with the seven crystal systems* **gives us 14 possibilities……**

………..the 14 so-called Bravais Lattices

NORSTRUCT

Choosing the correct/best Bravais lattice

Lattice	Metric tensor distortion index	Best cell (symmetrized) Best cell (without symmetry restrains)					
primitive cubic	20.51%	83.56	171.29	170.91	89.85	89.66	89.62
		141.92	141.92	141.92	90.00	90.00	90.00
I centred cubic	32.70%	189.79	190.09	242.30	50.59	129.27	100.99
		207.39	207.39	207.39	90.00	90.00	90.00
F centred cubic	32.84%	255.65	255.73	255.59	95.87	96.08	141.85
		255.66	255.66	255.66	90.00	90.00	90.00
primitive rhombohedral	18.39%	255.65	170.91	171.29	90.15	131.76	131.97
		199.29	199.29	199.29	117.96	117.96	117.96
		391.18	391.18	83.56	90.00	90.00	120.00
primitive hexagonal	13.55%	170.91	171.29	83.56	89.62	90.34	90.15
		171.10	171.10	83.56	90.00	90.00	120.00
primitive tetragonal	0.23%	171.29	170.91	83.56	89.66	89.62	89.85
		171.10	171.10	83.56	90.00	90.00	90.00
I centred tetragonal	7.92%	241.65	255.59	83.56	108.57	89.97	89.89
		248.62	248.62	83.56	90.00	90.00	90.00
primitive orthorhombic	0.22%	83.56	170.91	171.29	89.85	90.38	90.34
		83.56	170.91	171.29	90.00	90.00	90.00
C centred orthorhombic	0.22%	241.65	242.30	83.56	89.49	89.97	89.87
		241.65	242.30	83.56	90.00	90.00	90.00
I centred orthorhombic	7.92%	83.56	241.65	255.59	89.89	108.57	89.97
		83.56	241.65	255.59	90.00	90.00	90.00
F centred orthorhombic	7.92%	83.56	351.40	352.09	86.79	76.65	76.59
		83.56	351.40	352.09	90.00	90.00	90.00
primitive monoclinic	0.15%	83.56	170.91	171.29	89.85	90.38	90.34
		83.56	170.91	171.29	90.00	90.38	90.00
C centred monoclinic	0.05%	242.30	241.65	83.56	89.97	90.51	90.13
		242.30	241.65	83.56	90.00	90.51	90.00
primitive triclinic	0.00%	83.56	170.91	171.29	89.85	89.62	89.66

NORSTRUCT

Lattice to structure

What each lattice point represents

n   If we draw the lattice and then replace each lattice point by what it really represents, we get the molecular structure of the crystal:

Lattice

n   Mathematically this is called **_Convolution_**
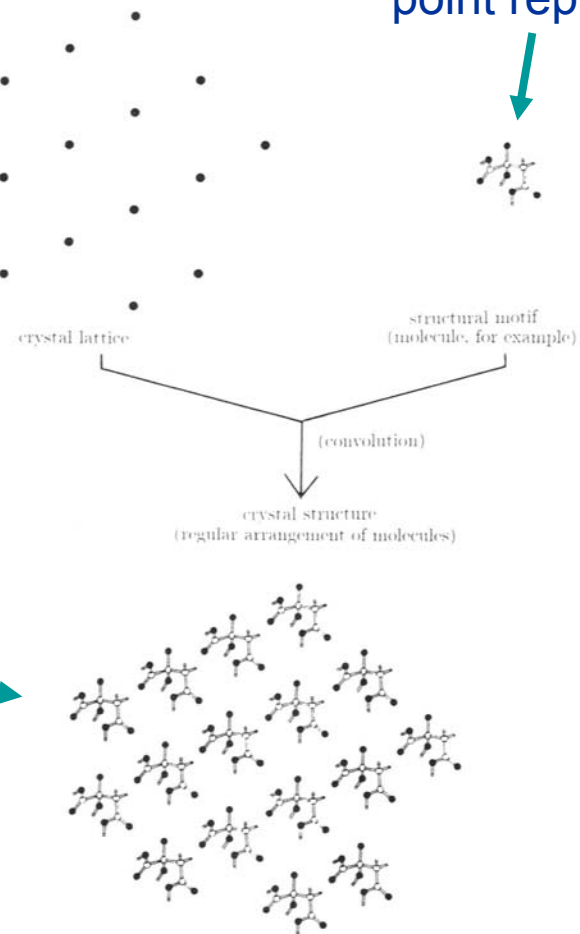
Crystal structure

crystal lattice

structural motif
(molecule, for example)

(convolution)

crystal structure
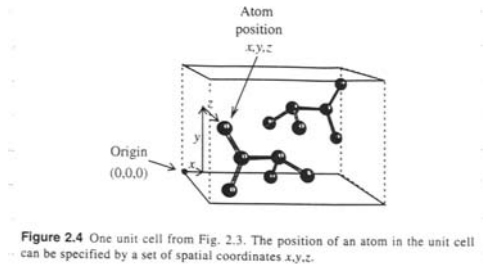(regular arrangement of molecules)

FIGURE 2.16. The crystal structure is composed of a crystal lattice (represented by points) and a structural motif (the atomic contents of one unit cell). The convolution of these two items will give the entire crystal structure.
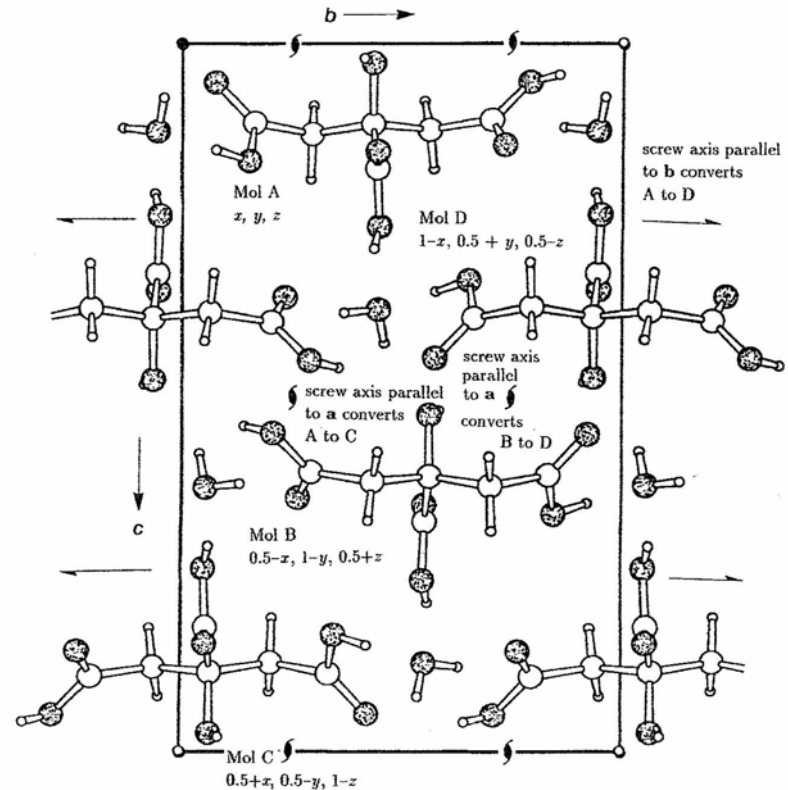
What is in a unit cell?

n   This ranges from one to very many atoms, ions (maintaining neutrality) or molecules.

n   *E.g.*:

Atom position
x, y, z

Origin (0,0,0)

Figure 2.4 One unit cell from Fig. 2.3. The position of an atom in the unit cell can be specified by a set of spatial coordinates x, y, z.

or:

Mol A
x, y, z

Mol D
$1-x, 0.5 + y, 0.5-z$

screw axis parallel to b converts A to D

screw axis parallel to a converts A to C

screw axis parallel to a converts B to D

Mol B
$0.5-x, 1-y, 0.5+z$

Mol C
$0.5+x, 0.5-y, 1-z$

n   All this is represented as one, two, or four points when making a lattice.

n   **What is the relationship between the apparently identical molecules in this unit cell?**

n   **SYMMETRY!**

Symmetry elements, point groups, space groups (I)

- **Symmetry operation**:
  - ….a geometrical movement that leaves a physical object in a configuration indistinguishable from its original configuration.
- **Symmetry elements**
  - **These can be**
    - **A line** (rotation axis)
    - **A plane** (mirror)
    - **A point** (centre)
    - **A rotatory Inversion** centre (*e.g.* in a tetrahedron)

- **Two further operations** are possible *between* symmetry related objects:
  - Screw axis (combination of rotation and *translation*)
  - Glide plane (combination of mirror and *translation*)

- **Point groups**
  - A **POINT GROUP** is a symbol which describes the symmetry **WITHIN** an object
  - **E.g. A Cube, point group 432**
  - **There are 32 of these**

twofold axis
threefold axis
fourfold axis

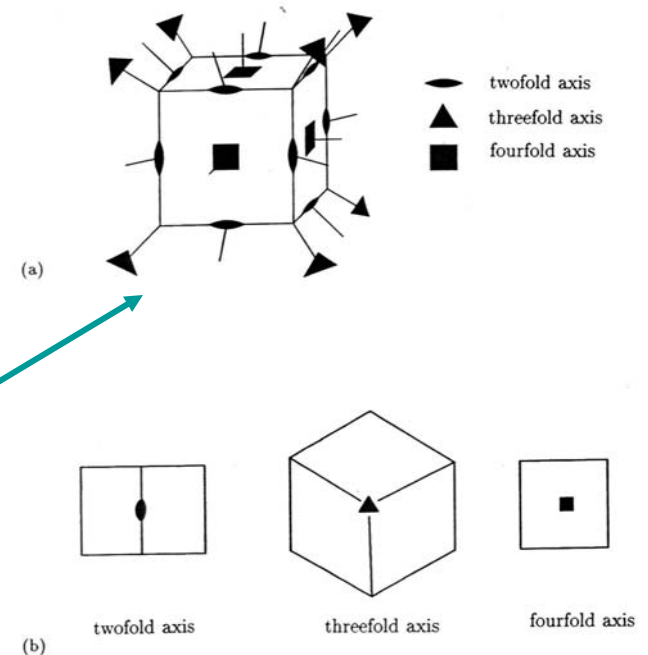twofold axis   threefold axis   fourfold axis

FIGURE 4.7. The symmetry of a cube. (a) The various rotation axes and (b) view down one of each type of rotation axis. The reader is encouraged to pick up a cube and examine its symmetry.

Symmetry elements, point groups, space groups (II)

- When we **replace the lattice points** in the **14 Bravais lattices** by each of the possible **32 point groups** we get 14 x 32 possible **SPACE GROUPS**

- This gives 448, but fortunately quite a few of these are alternative ways of describing the same result (degenerate) so we end up with **230 UNIQUE** space groups
- These are described in detail in International Tables for X-ray Crystallography

- **BUT:**
  - **symmetry involving mirroring or inversion is not possible for proteins – it removes chirality**

- 165 Space Groups **contain mirror and/or inversion symmetry**
- **This would remove chirality**

- **This leaves 65 space groups which are possible in molecular biology**

Edward Hough
February 2009

Diffraction

- von Laue proved that X-rays were light (e/m radiation) by demonstrating diffraction using a crystal of $CuSO_4.6H_2O$

- Parallel light beam through a pinhole:
    - Expect a circle with sharp edges
   - Get a set of concentric rings

- How come?
    - Light is "scattered" by the edge of the circular hole
    - The scattered waves interact
    - In some directions they add….
        - ….in others they cancel

- This is called *constructive* and *destructive* interference
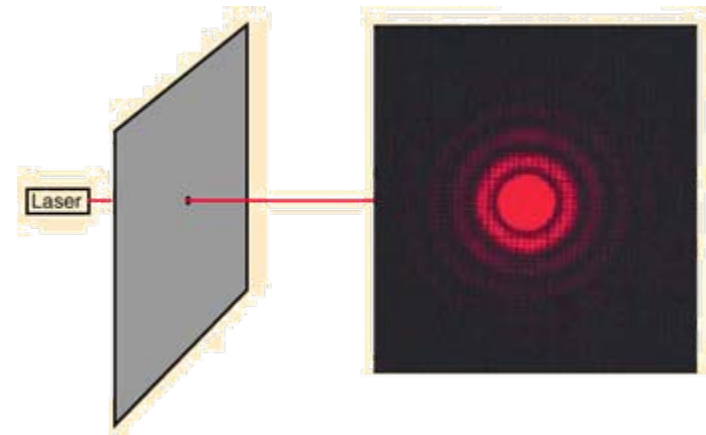
Waves

n An electromagnetic wave is a periodic phenomenon

n It radiates spherically from its source

n It moves through space with a constant velocity (c = 10^{10}m/s for electromagnetic waves in a vacuum)

n The "shape" of a simple wave is that of a Sine or Cosine curve
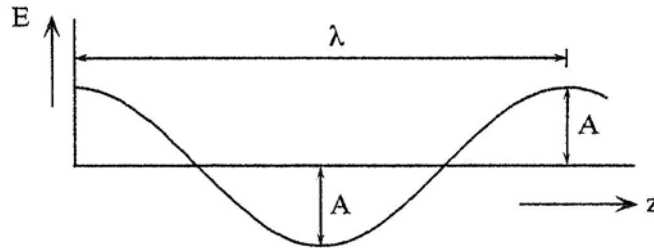
Two waves

- n If we now add a second wave with the same amplitude, wavelength and frequency but which is displaced from the first wave by distance Z:

    - The first wave is "out of phase" with the second by Z/λ of a complete wave…

        ..or Z/λ x 360° (one cycle is 360°)

- n The sum of the two waves is:

- n Changing Z, *i.e. changing the phase* of the new wave with respect to the original will change the sum.
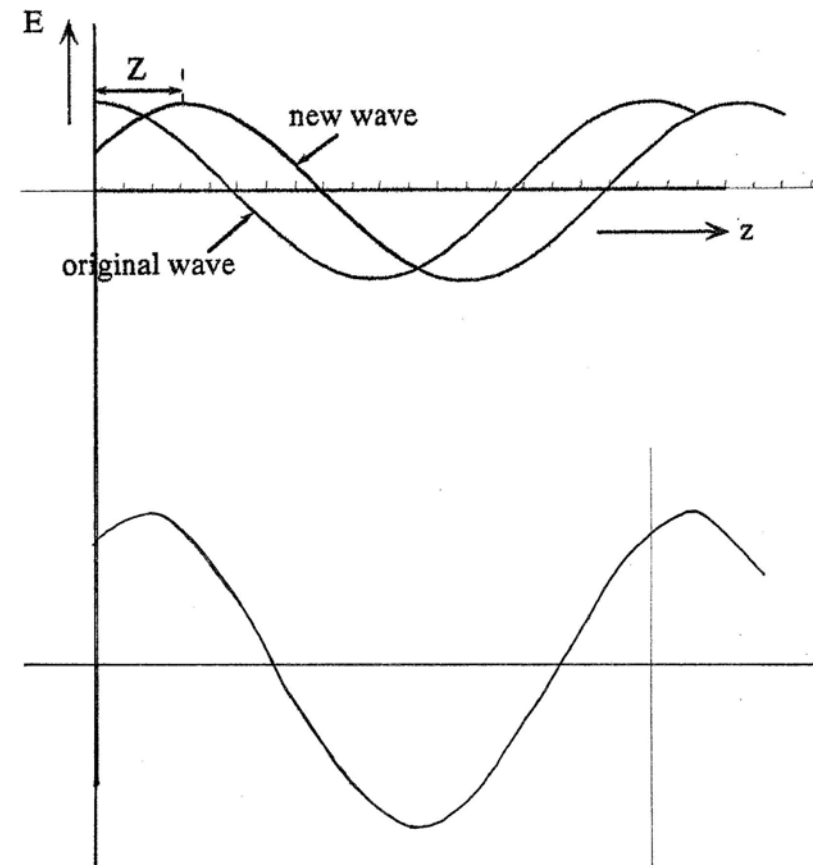
Circular motion and waves

n **Waves –**
http://jjgifford.com/expressions/geometry/harmonic.html

n **A wave:**

- can be resolved into Cosine and Sine components
- has an amplitude (maximum height), A, given by $A = \sqrt{(Cos^2 + Sin^2)}$
- a phase (at any instant) given by $\alpha = Tan^{-1}(Sin/Cos)$
- a frequency, ν
- at time t the displacement, a_t, is related to the amplitude, A, by:
  - $a_t = A Cos\ 2\pi tc/\lambda$
  - $a_t = A Cos\ \omega t$
    - where c/λ is the frequency, ν, and $\omega = 2\pi c/\lambda$
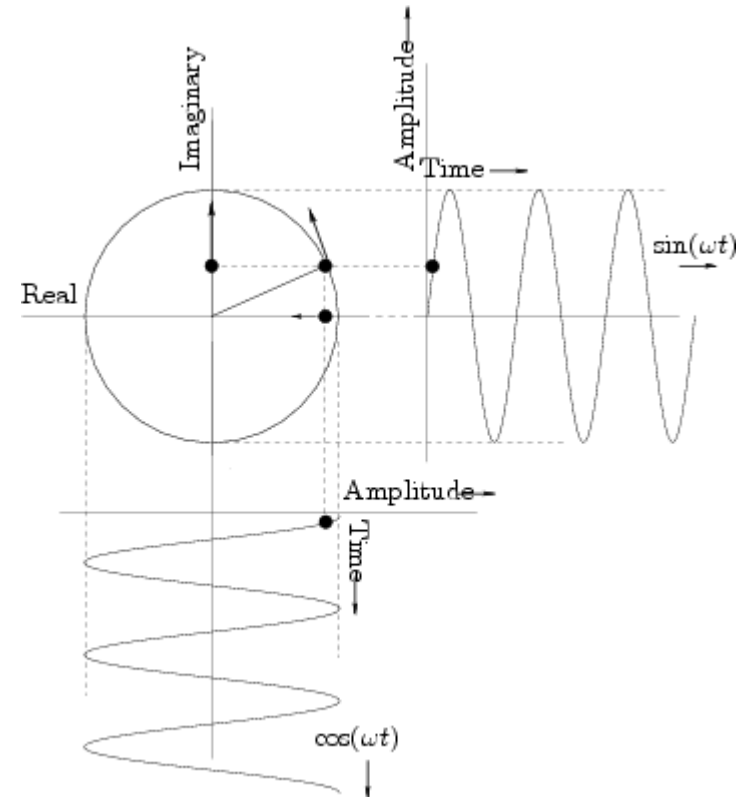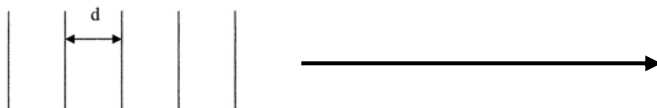
Diffraction grating

n **Demonstration with grating**

n **Theory**

- **Waves add in some directions, cancel in others**
- **Bragg's law**
    - **$n\lambda = 2d\sin\theta$ where:**
        - **n is the diffraction order**
        - **d is the distance between the lines**
        - **λ is the wavelength of the light**
        - **2θ is the angle between the primary beam and the diffracted beam**

n **One dimensional diffraction grating (set of fine parallel lines on a glass plate**

gives this diffraction pattern:

Diffraction pattern (not exact!):

	X		X		X	X	X		X		X

Order(n):

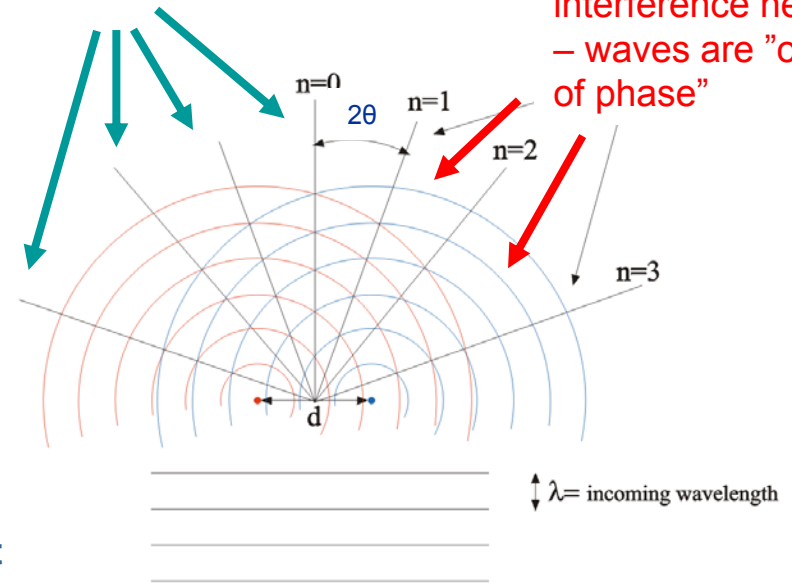|	-3		-2		-1	0	+1		+2		+3

Diffraction grating

n **Explanation**

- **Waves add in some directions, cancel in others**

- **Bragg's law**

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

  - **where:**

    - **n is the diffraction order**
    - **d is the distance between the lines**
    - **λ is the wavelength of the light**
    - **2θ is the angle between the primary beam and the diffracted beam**

Constructive interference here - waves are "in phase"

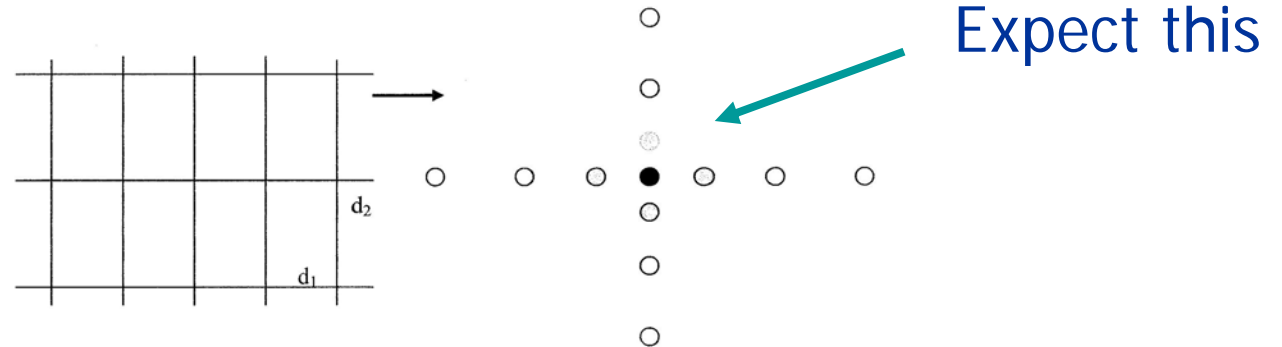Destructive interference here – waves are "out of phase"

n=0   n=1   n=2   n=3

2θ

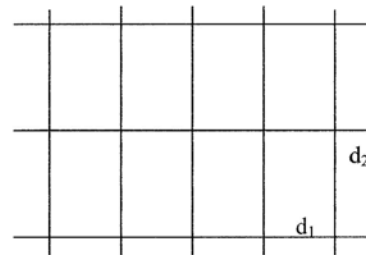λ = incoming wavelength

Bragg's Law : $n\lambda = d\sin\theta$

d is constant
n is a variable integer

Ripple Animation - http://www.surendranath.org/Applets/Waves/RippleTank/RipTankApplet.html
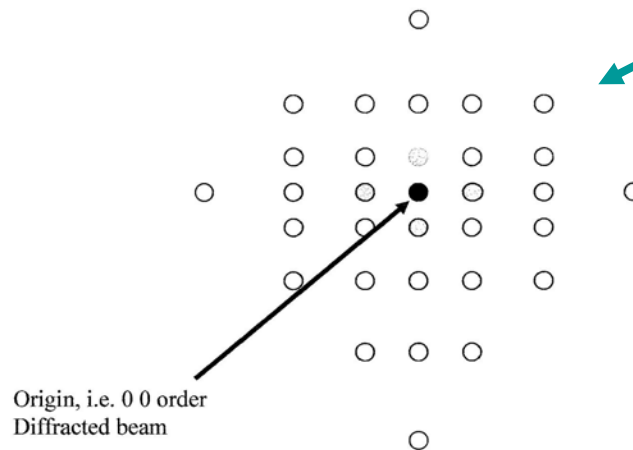
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2 gratings crossed at 90º

Expect this

2 gratings crossed at 90°

d_2

d_1

but

Get this!

Origin, i.e. 0 0 order
Diffracted beam

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Where do the extra spots come from?

1. **The light behaves as if the *intersections* also scatter**

n **These form new sets of "lines"**

n **The diffracted spots lie in rows going from the origin in a direction which is perpendicular to the lines involved**

n **The distance from the origin to each spot is proportional to 1/d where d is the distance between the sets of parallel lines involved**

n **the diffraction pattern**

n **is actually a "reciprocal" picture of the crossed gratings**
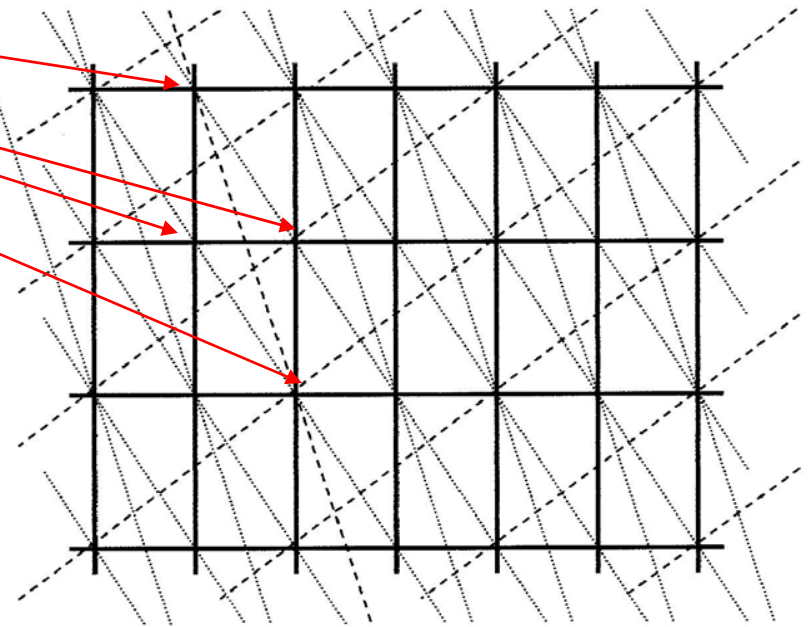
Crystal planes

n **If we send an X-ray beam through a crystal and move the crystal and detector in a very special manner while recording the image, it will look highly systematic and will resemble that with the crossed diffraction gratings :**
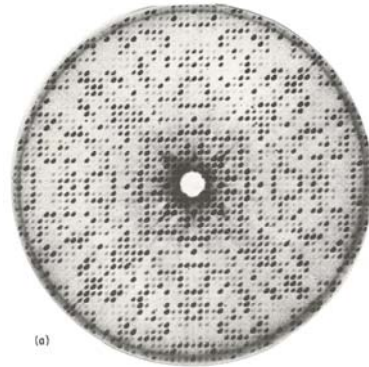
n **However, a quick shot with a stationary crystal (your first check image!) will look much less systematic :**
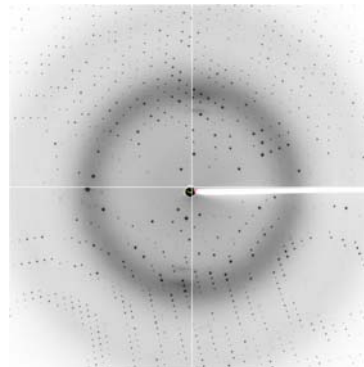
n **The system IS STILL THERE, but is not immediately obvious**

Edward Hough
February 2009

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Crystal planes

n **The ORDER/repetition <u>in a crystal</u> is 3-dimensional so the complete difraction pattern emerges from the crystal spherically**

n **The complete diffraction pattern will contain a large number of sharply defined diffracted beams**

n **Where have these come from?**

- **examining a model of a crystal structure e.g. Calcium carbonate reveals very few obvious planes**

NORSTRUCT

Defining planes in a crystal

- Useful analogy is to think of the unit cell as the room you are sitting in.
- Define one corner as the **ORIGIN** and the three edges from this as the **AXES**, a, b, c.
- What, in relation to these axes, can we call the walls, diagonals, or imaginary walls/**PLANES** dividing the room into ½'s, ¼'s, etc?

- *i.e.* we need an **INDEXING** system which tells us:
  - which axes intercept a given plane
  - how many times they do so across the whole room *in each direction*
  - the latter have to be **whole numbers** (otherwise we would end up in the next room!)

- The real walls will have indices 1 0 0, 0 1 0, 0 0 1 since they:
  - only are intersected by one axis
  - are parallel to the other two axes
- The diagonals will be 1 1 0, 1 0 1, 0 1 1 and the 3-D diagonal will be 1 1 1
- Imaginary walls dividing the room into halves will be 2 0 0, 0 2 0, 0 0 2
- *Etc*!

NORSTRUCT

Miller indices

n Back to the unit cell/crystal

- In crystallography these indices are called **Miller indices**

- Symbols used are: h, k & ℓ

- Once we have identified the correct lattice and unit cell we are able to index the spots in a diffraction pattern *i.e.* relate them back to the crystal planes which caused them

Miller indices

n Example; 15 0 0:

- The 15 0 0 planes are parallel to the b & c axes and there are 15 of them across the room in the a-direction.

- If the the angles in the unit cell are 90°, and they are diffracting, the value of d in Bragg's law will be a/15

- **More generally**: the perpendicular distance, d_{hkl}, between planes, hkl, is a function of the cell parameters a,b,c,α,β,γ *via*:

$$\frac{1}{2\,d_{hkl}} = \frac{\sin\theta}{\lambda} = \tfrac{1}{2}(h^2 a^{*2} + k^2 b^{*2} + l^2 c^{*2} + 2hk\,a^*b^*\cos\gamma^*$$
$$+ 2hl\,a^*c^*\cos\beta^* + 2kl\,b^*c^*\cos\alpha^*)^{1/2}$$

What we need

The notation * means that we are using the parameters for the **RECIPROCAL UNIT CELL**

NORSTRUCT

"Reflections"

n The Bragg approach regards the diffracted beams as if they are **REFLECTED** from the crystal planes in the same way that light is reflected by a mirror

n For this reason the diffracted beam are habitually called **REFLECTIONS**

n **This terminology will be used hereafter!**

What does the diffraction pattern give us?

n **Two types of information:**

- **Geometric,** leading to:
    - **Symmetry** in the diffraction pattern
    - **Crystal System, Lattice type** and **Unit cell dimensions**
    - Information about possible **translational symmetry** between the molecules in the unit cell
    - Combining these gives us the **SPACE GROUP**
    - With this information we can now **INDEX** the individual spots i.e. relate them to the crystal planes which caused them.

- **Intensity,**
    - The intensities of the diffracted beams vary from zero to very strong
    - This information is related to the density of electrons in the planes which caused them
    - i.e. to the **MOLECULAR STRUCTURE**

n **We thus have to:**
- **RECORD** the reflections
- **INDEX** the reflections i.e. identify the Lattice and unit cell
- measure their **INTENSITIES**

NORSTRUCT

# Precession photograph of a crystal of tetragonal HEWL.

We are looking down a 4-fold symmetry axis

There is twofold symmetry across the diagonals

There is also twofold symmetry along the vertical and horizontal "axes"

Looking carefully also shows that some potential reflections are systematically absent.

The **photograph is actually of the RECIPROCAL LATTICE**

(b)

NorStruct
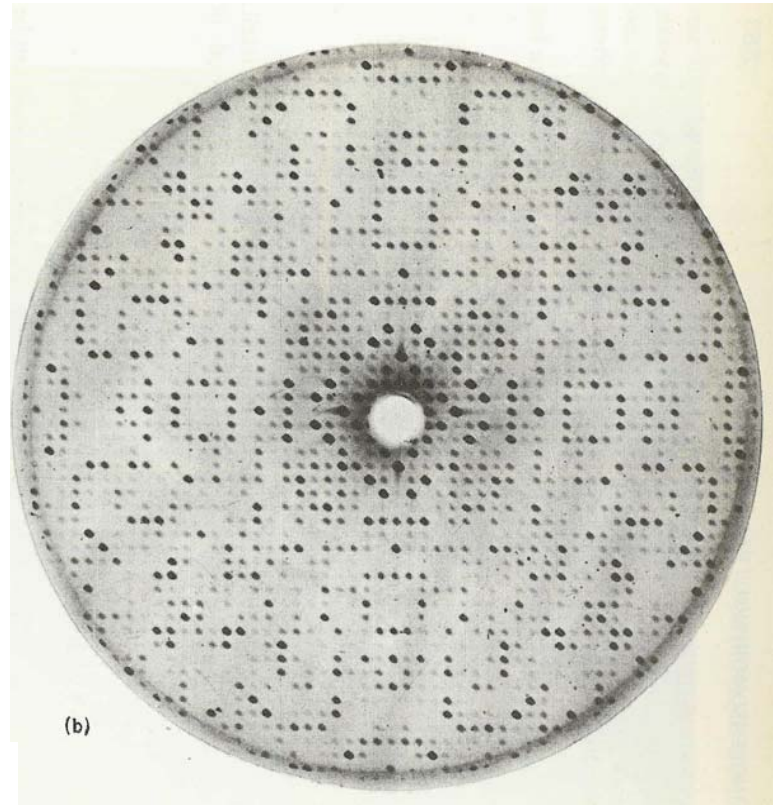
Reciprocal Space

- A crystal in an X-ray beam will diffract X-rays producing a number of sharply defined emerging X-ray beams

- The cross sectional area of these is determined by that of the primary beam and/or the size of the crystal

- The diffracted beams arise from parallel planes in the crystal which **happen to be oriented so that Bragg's law is fulfilled**

The incident beam comes from an X-ray generator or a synchrotron i.e. is
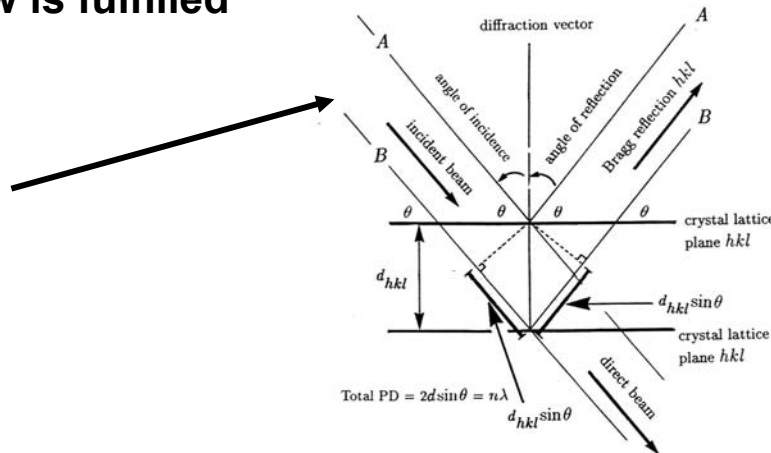**UNMOVABLE!!**

FIGURE 3.10. (b) Geometry of diffraction and its relationship to Bragg's Law. Note the definition of θ.

- We need to record diffracted beams from all possible sets of planes in our crystal

- This means that we have to **MOVE the crystal** (**and maybe the detector**) under data collection

- **How can we predict this?**

NORSTRUCT

Construction of a Reciprocal Lattice from a 2-D Unit Cell

- **Draw the unit cell**
- **Define a lattice point as the origin**
- **Draw a normal to the selected plane which passes through the origin**
- **Measure the perpendicular distance, d, between the plane and its nearest neighbour**
- **Plot a point along the normal which 1/d from the origin – good idea to multiply all of these by a scale factor!**
- **Done for a sufficient number of planes sets this will result in a**

RECIPROCAL LATTICE

- **Each POINT in a reciprocal lattice represents a set of parallel planes that go through the whole crystal**
- **The vector from the origin to any RL-point is:**
  - **Perpendicular/Normal to the actual planes**
  - **Its length is 1/d**
- **It is also the "DIFFRACTION VECTOR" – comes later!**
- **ANIMATION**

NORSTRUCT

The Ewald construction

n **Graphically**

- Draw a reciprocal lattice
- Draw a **sphere** (**Ewald Sphere**) with its origin on the primary beam and radius $1/\lambda$.
- Place this sphere so that the point (O) where the primary beam cuts the sphere is on the origin of the R-L
- Any reciprocal lattice point which lies on the sphere then satisfies Bragg's law and DIFFRACTS
- The direction of diffracted beam is from the centre of the sphere through the RL-point (MP in the figure)

n Can you spot any other diffracting planes?
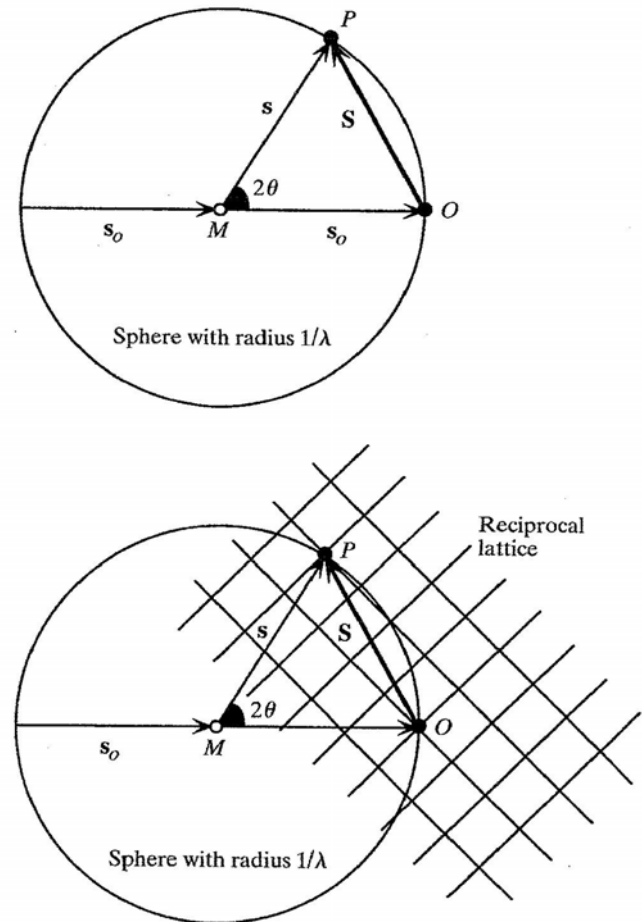
n The construction is valid for all types of diffraction

Fig. 2.1.5.2. The circle is, in fact, a sphere with radius $1/\lambda$. s_O indicates the direction of the incident beam and has a length $1/\lambda$. The diffracted beam is indicated by vector **s**, which also has a length $1/\lambda$. Only reciprocal lattice points on the surface of the sphere are in a reflecting position Reproduced with permission from Drenth (1999). Copyright (1999 Springer-Verlag.
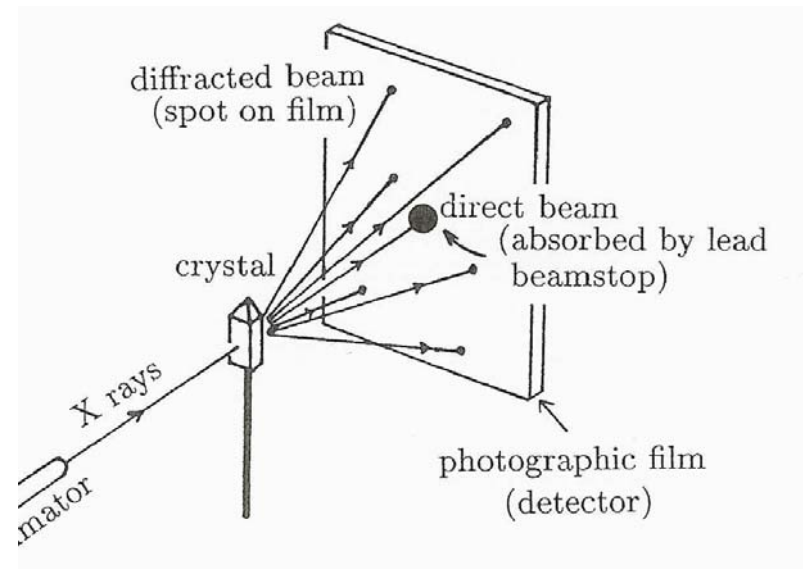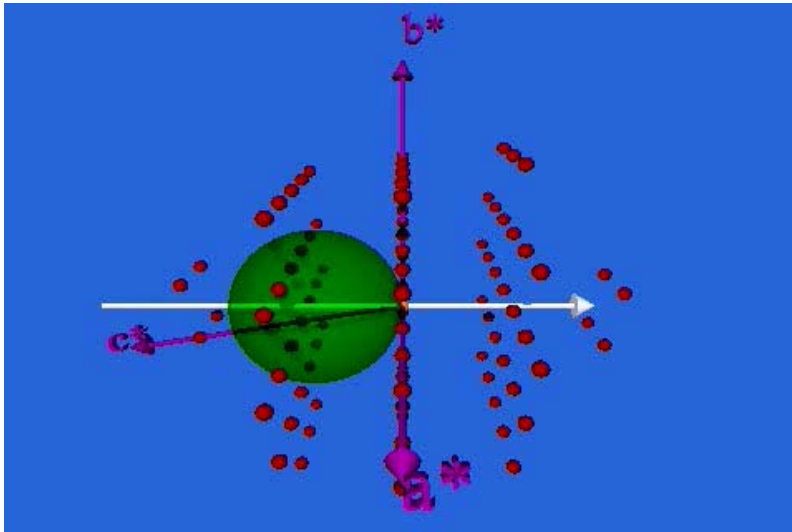
Ewald in action

http://marie.epfl.ch/x-ray/
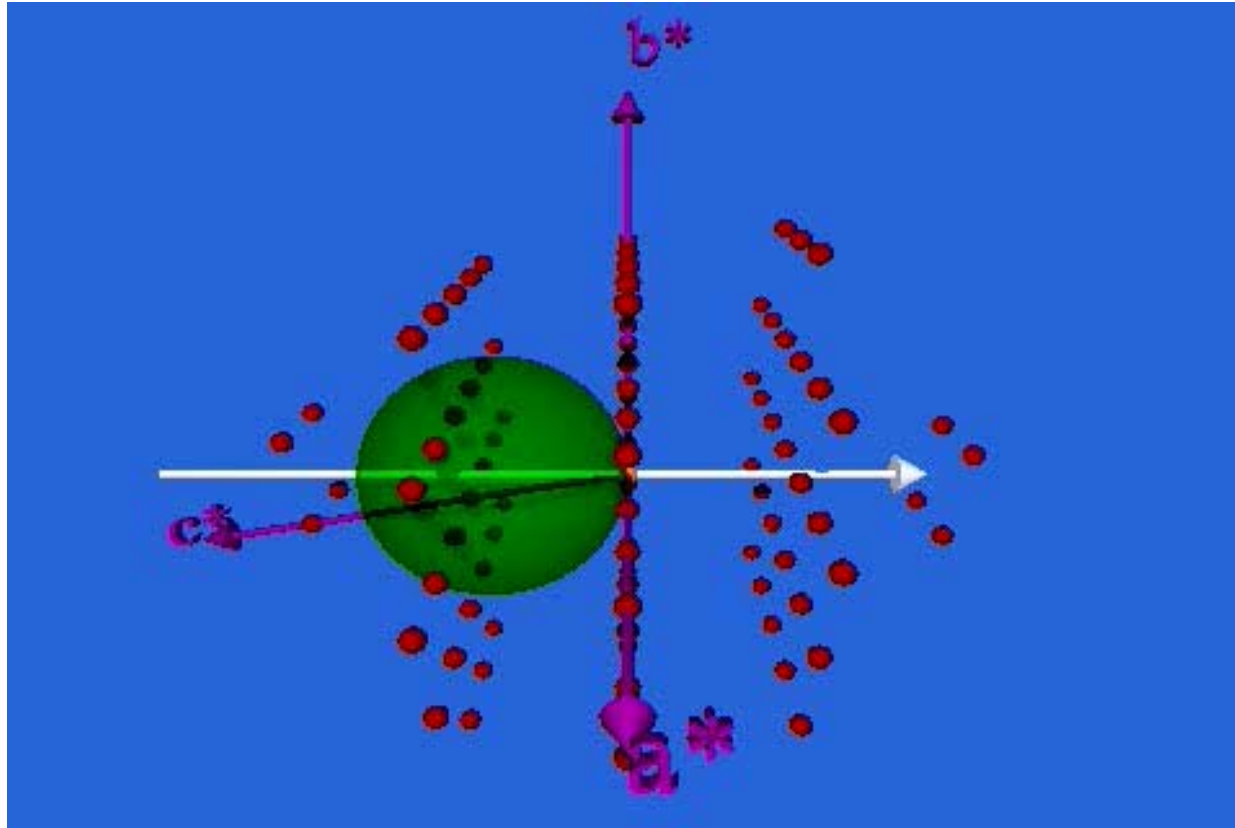
Ewald in action

We record **PART** of the diffraction pattern, usually on a **FLAT** detector

http://marie.epfl.ch/x-ray/

Value of the Ewald construction (I)

n **The construction tells us:**

- **How the reciprocal lattice** (and thereby the crystal) **must be placed in the primary beam** in order to obtain diffraction from (a) given plane(s).

- The **direction(s)** of the diffracted beam(s).

- That we can bring other reflections into diffracting positions by **rotating the reciprocal lattice i.e. the crystal**. This is precisely what happens when you rotate the crystal in steps (e.g. in 0.5º) under a data collection.

- Remember that it is a **SPHERE** i.e. reflections will also occur "above and below the plane of the page".

- The **number of RL-points** which can diffract will depend on:

  - **the wavelength** used – **shorter** wavelength means **bigger sphere** and **more reflections**

  - **the volume of the reciprocal cell** – proteins have big cells/small reciprocal cells *i.e.* lot of reflections!

Value of the Ewald construction (II)

n **The construction is also useful to explain several factors which limit the quality of our diffraction data**

- **Mosaicity –**
  - **In the construction above each set of planes is reduced to an infinitely small RL-point. This is true for a PERFECT crystal but in real life our crystal will consist of small blocks of perfectly stacked unit cells which are imperfectly stacked against each other:**

  - **Each of these will generate its own reciprocal lattice but in a slightly different orientation.**
  - **The result of this is that our RL-points are diffuse.**
  - **The diffracted beam is also more diffuse**

- **Monochromaticity –**
  - **The Ewald sphere has a radius of $1/\lambda$**
  - **For a perfectly monochromatic beam it will be infinitely thin.**
  - **In reality we are always going to have a wavelength spread so that the sphere has a finite thickness.**

- **Overlap**
  - **As a consequence of the points above each REAL RL-point will be active for some time as the rotation proceeds.**
  - **This means that the diffracted beams will be diffuse**
  - **If the rotation angle/unit cell is "large" we risk that reflections begin to overlap on the detector**

Value of the Ewald construction (III)

- **Partiality**
    - **For a given rotation angle RL-points will traverse the sphere either:**
        - **Completely to be FULLY recorded**
        - **Partially – either starting or completing the traverse.**

- **Symmetry**
    - **The reciprocal lattice contains the symmetry appropriate to the correct lattice type (can see this in the Lysozyme photo!).**
    - **This means that we do not have to rotate the crystal through 360° in order to record the intensities of all accessible unique reflections.**
    - **The rotation range necessary to record a complete unique data set will depend on**
        - **The lattice type**
        - **The way the crystal is oriented on the detector**

- **Strategy**
    - **This can be predicted using a STRATEGY program**

- **Blind zones**
    - **It is clear from the Ewald construction that, for a given orientation of the crystal on the rotation axis, some RL-points will NOT TRAVERSE the sphere**

Resolution *etc.*

- **Bragg's law – nλ = 2dSinθ**
  - **To obtain the highest resolution, i.e. the smallest value of d we can:**

    - **Make a detector which enables us to record reflections at the largest possible diffaction angle, θ**
    - **Use the shortest practically possible wavelength, λ.**

- **Resolution is limited by:**
  - **Mathematics – maximum value for a Sine is 1.000 at 90°. The limiting resolution is thus λ/2.**
  - **The wavelength.**
  - **Mechanics:**
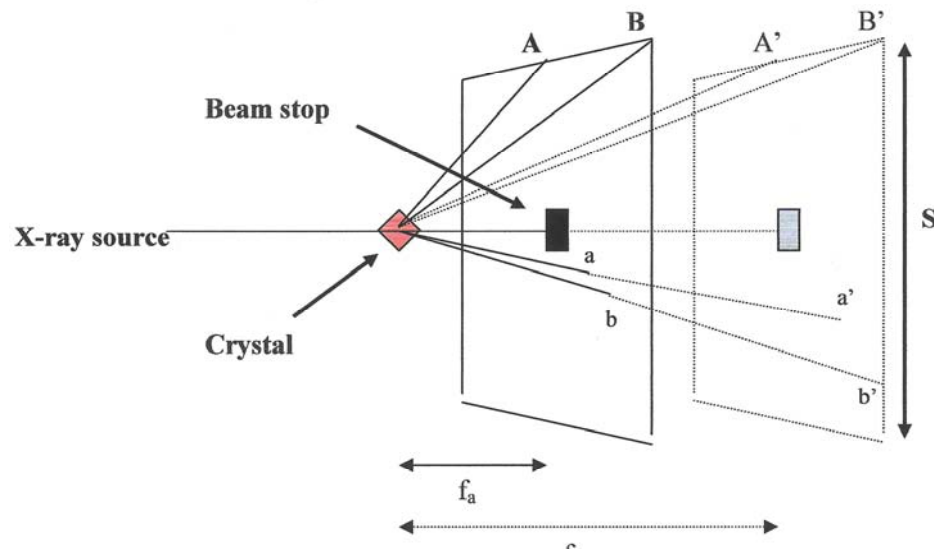
We can increase resolution by:

- Making a **larger detector** (larger S).

- Moving the **detector closer to the crystal** (f_a gives higher 2θ than f_b).

- **Moving the detector** sideways or up/down or even rotating it around an axis that goes
- through the crystal. The latter is better but is more difficult mechanically (it would give us a "two-circle" diffractometer, the spindle or φ-axis is the first-circle).

- Making a **Curved detector** – technically more difficult but actually used e.g. at the Photon Factory in Japan with a so-called Weissenberg detector.

- There are mechanical limits to all of these approaches.

- *N.B.* the **separation of reflections** on the **detector increases with increasing crystal to detector distance**. Reflections a' and b' in the figure are better separated than their equivalents at a and b. This is particularly **important for crystals with large unit-cell dimensions** where reciprocal lattice points and thus reflections are close together.

- *In actual fact the resolution for biological macromolecules is seldom limited by mechanical factors but rather by factors that lie in the molecules and crystals themselves!*
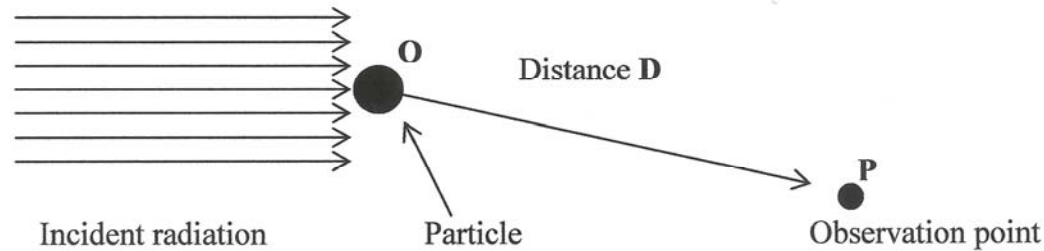
Scattering and absorption

- Our visual awarenes of the world we live in is a result of two phenomena:
    - *The scattering..*
        and..
        absorption….
                of electromagnetic radiation.
- The light falling on objects around us is scattered either in a random manner or by reflection (directional conservation).

- The colours we observe are the result of selective absorption of *all* (black!), *none* (white…if the incident light is white!) or *part* of the visible spectrum of the incident light.

- In all these cases the size of the scattering body is much larger than the wavelength of the incident radiation.

    Etc!

Edward Hough
February 2009

Interaction between X-rays and matter

n   Classical approach:

Incident radiation   Particle   Observation point

(Distance **D**, **O**, **P**)

n   When a charged particle (*eg.* an electron) is exposed to electromagnetic (e/m) radiation the interaction between the electric vector in the light and the charge on the particle may cause the later to oscillate periodically

n   So long as the frequency is reasonably appropriate the particle will oscillate with the same frequency as that of the incident e/m radiation

n   The natural vibration frequency of an electron lies in the X-ray region of the e/m spectrum.

Interaction between X-rays and matter
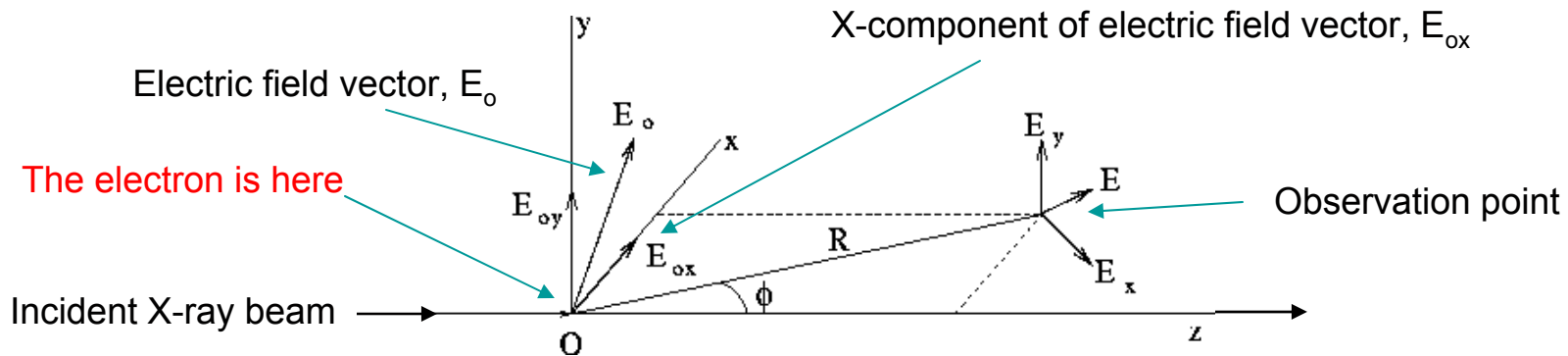
n   *Eg.* Water in an X-ray beam :
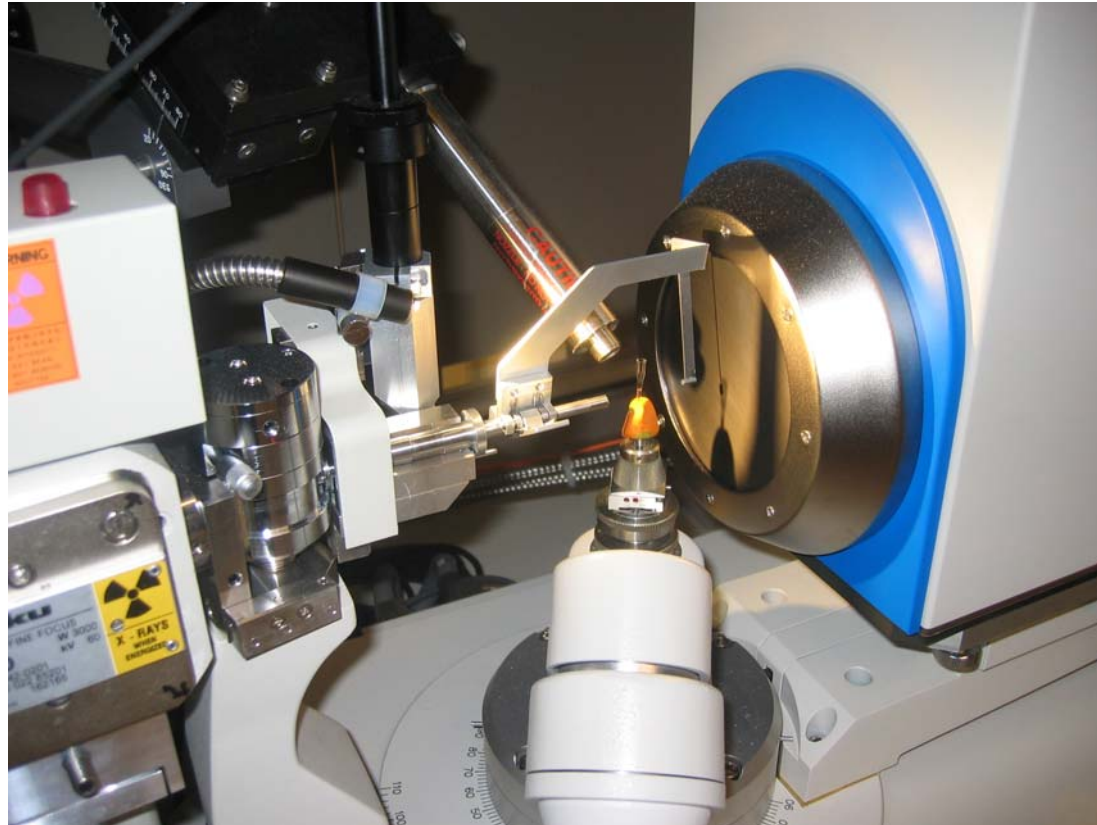
Water in an X-ray beam
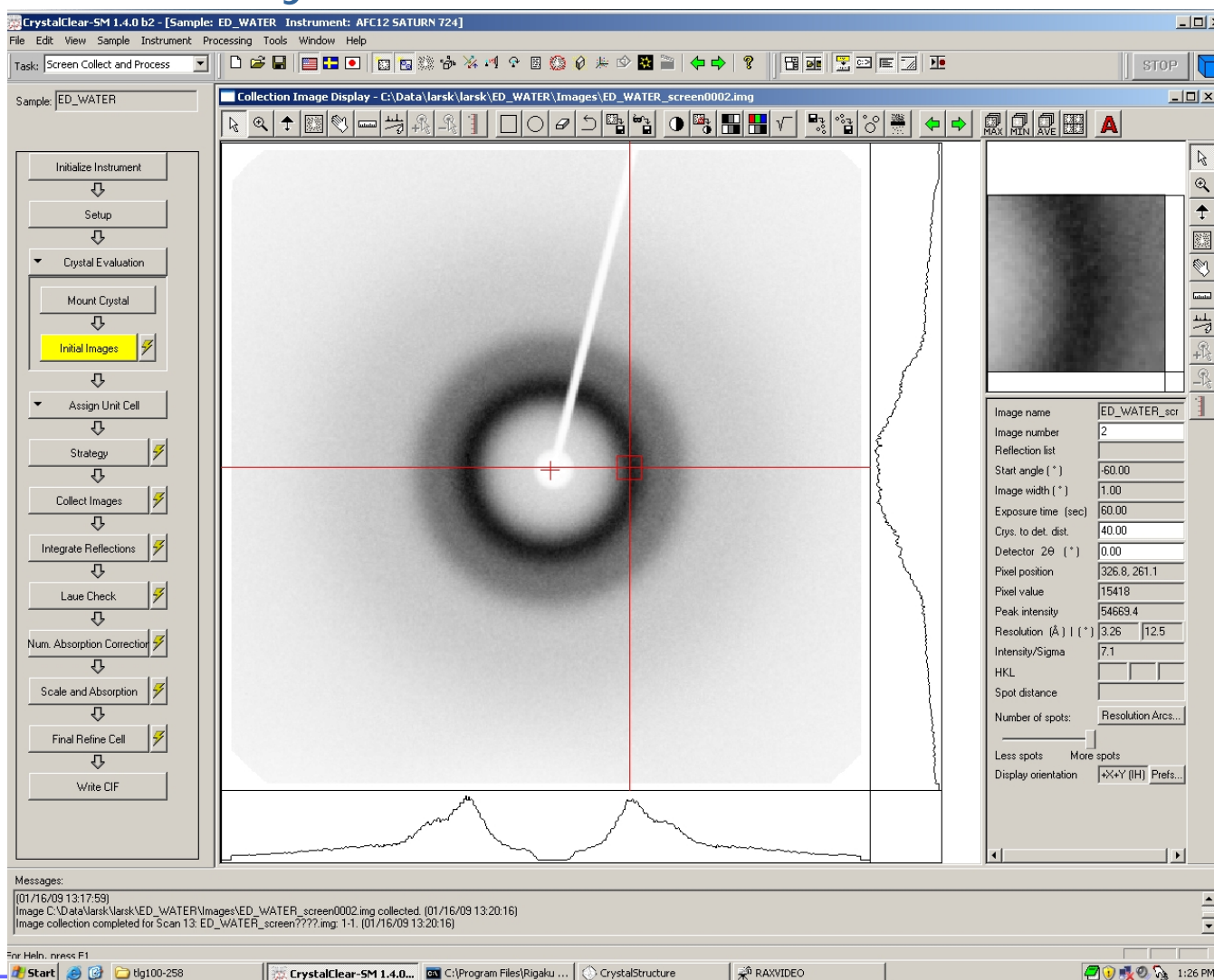
Scattering theory

n   For an electron *at a frozen instant in time*:

Electric field vector, E_o

X-component of electric field vector, E_{ox}

The electron is here

Observation point

Incident X-ray beam

The electric field vector of the incident radiation, Eo, produces a force, eEo, which causes it to accelerate – Newton!

$$\ddot{x} = \frac{eE_{ox}}{m} \exp(i\omega t) \qquad \text{--------- 1}$$

e is the charge on an electron and m its mass

Scattering of radiation by a single charged particle

- From e/m theory an oscillating charged particle will emit e/m radiation spherically and with the same frequency as the exciting radiation:

$$E_x = \frac{-e}{4\pi\epsilon_o c^2 R} \ddot{x}(t - R/c) \cos\phi \qquad \text{-----------------------------} \quad 2$$

- where E_x is the electric field vector of the *scattered* radiation in the xz plane, c is the speed of light, ϵ_o is the permitivity of free space and the (t-R/c) term is the propagation time from the electron to the observation point at angle ϕ from the incident beam

- Combining equations 1 and 2 gives us the amplitude of the scattered radiation due to the **x-component** of the incident radiation as:

$$E_x = \frac{-e^2 E_{ox}}{4\pi\epsilon_o mc^2 R} \cos\phi \qquad \text{-------------------------------------} \quad 3$$

- The **y-component** is similar but independent of ϕ so we can combine the two components using Pythagoras to get:

$$E^2 = \frac{e^4}{(4\pi\epsilon_o)^2 m^2 c^4 R^2}(E_{oy}^2 + E_{ox}^2 \cos\phi) \qquad \text{----------------------------} \quad 4$$

Scattering of radiation by a single charged particle

- Equation 4 applies to a frozen instant in time i.e. for a specific direction for E_o.

- Averaging over **ALL** directions for E_o (in the x-y plane) gives us total electric field at the observation point:

$$< E^2 > = \frac{e^4}{(4\pi\epsilon_o)^2 m^2 c^4 R^2} < E_o^2 > \left(\frac{1 + \cos^2 \phi}{2}\right) \quad \text{-----------------} \quad 5$$

- Since **INTENSITY** is proportional to the **SQUARE** of E we can rewrite equation 5 as:

$$I = I_o \frac{e^4}{(4\pi\epsilon_o)^2 m^2 c^4 R^2} \left(\frac{1 + \cos^2 \phi}{2}\right) \quad \text{-----------------} \quad 6$$

Intensity of scattered beam at observation point

Intensity of incident beam

"Polarisation correction" for an **Unpolarised Incident beam**

NORSTRUCT

Scattering of X-radiation by an ATOM

- Equation 6 gives us the intensity of the beam scattered by a **single electron** which is able to oscillate at the same frequency as the incident X-ray beam.
- What happens when we have several electrons eg. 6 in a carbon atom?
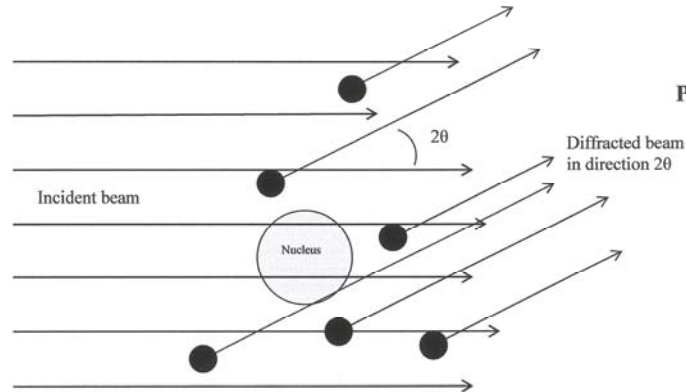
- When ϕ is zero (i.e. in the direction of the primary beam) the electrons will scatter in phase so that the result will be 6 times greater than for a single electron.
- As ϕ increases the scattering from the individual electrons will be increasingly out of phase (destructive interference) so that the total scattered intensity will decline.

Scattering of X-radiation by an ATOM

n   The result can be calculated from the theoretical probability distribution of electrons around their nuclei:

$$f_0 = \sum_{n=1}^{Z} \int_0^{\infty} 4\pi r^2 \rho_n(r) \frac{\sin sr}{sr} dr \qquad \text{where} \qquad s = \frac{4\pi \sin \theta_B}{\lambda},$$

n   This is "summarised" in the so-called **ATOMIC SCATTERING or FORM FACTOR , f_o**

n   Note that we have replaced ϕ by θ and that the wavelength, λ, of the incident beam has turned up!

n   The atomic scattering factor curve for carbon is:
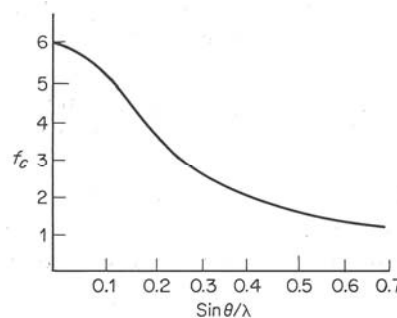
Increasing angle from incident beam

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February 2009

NORSTRUCT

Scattering of X-rays by a *vibrating* atom

- Atomic motion (thermal vibration or "dynamics") makes the electron clouds even more diffuse so that atoms which vibrate scatter less strongly than stationary atoms.

- **The real scattering power of atoms, f, is thus:**

  - proportional to the number of electrons (atomic number)
  - declines with the diffraction angle (2θ)

- **and is REDUCED by the factor $\exp{-B.(Sin\theta/\lambda)^2}$ due to thermal vibration.**

- **B is the Debye-Waller or Temperature factor**
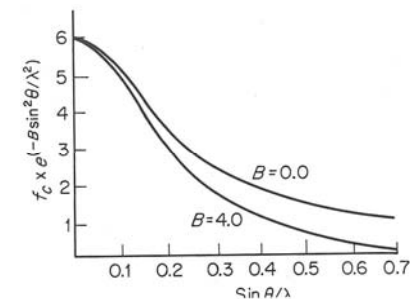
Scattering of X-rays by a vibrating atom

n Scattering factor curve for carbon with
NO VIBRATION
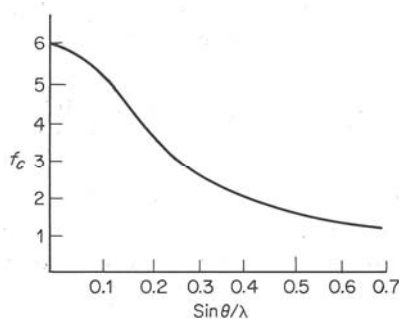
The negative effect
of thermal vibration
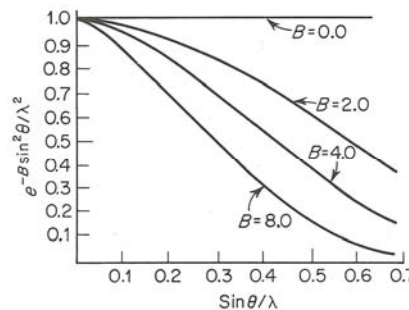
Scattering factor after
correction for thermal
vibration for B = 0.0
(*i.e.* no vibration) and B
= 4.

$$f_0 \quad \times \quad \exp\text{-}B.(Sin\theta/\lambda)^2 \quad = \quad f_j$$

Scattering of X-rays by molecules, unit cells and crystals

n **MOLECULE**

- Scattering by a molecule follows the same logic *i.e.* is the sum of the scattering from each of the atoms
- Once more because the atoms are at different positions in the unit cell the result will be dependant on which direction the scattered radiation is examined
- *i.e.* constructive interference (strong scattering) in some directions and destructive interference (weak scattering) in others.

n **UNIT CELL**

- The other molecules in a unit cell will be in positions which are related to the first molecule by the lattice and symmetry operations for the actual SPACE GROUP
- The total scattering from each other molecule will be the same as that from the first, but they are not in the same place in the cell.
- The total scattering from the UNIT CELL will again be the sum of that from each molecule with adjustment for different location

- This is called the **STRUCTURE FACTOR**

n **CRYSTAL**

- This is simply the scattering from one unit cell multiplied up by the number of unit cells in the crystal

Calculating Structure Factors

n   The formula for this is

$$\mathbf{F}_{hkl} = \sum_{J=1}^{n} fj \{Cos2\pi(hx_j + ky_j + lz_j) + iSin2\pi(hx_j + ky_j + lz_j)\}$$

n   Where the sign Σ means "the sum of" and:

-   the summation is over **all** the N atoms (j=1 to N) **in the unit cell**
-   f_j is the scattering power of atom j at the relevant scattering angle and corrected for its thermal vibration
-   x_j, y_j, z_j are the coordinates of atom j
-   h, k, l are the indices of the reflection we are looking at

n   It looks complicated but in reality it is just doing what we have discussed above

n   Using this formula we can calculate the INTENSITIES of all the reflections in the diffraction pattern…

-   **IF WE KNOW WHERE THE ATOMS ARE**

n   **Although valuable this assumes that we have SOLVED THE STRUCTURE i.e. it is the OPPOSITE OF WHAT WE NEED**

Anomalous dispersion/Flourescence

n   The whole logic above was based on the assumption that the electrons involved in the scattering process behave as if they are **alone in the universe,** so called **Thompson scatterers**.

n   This is obviously untrue since they experience the charge on their atomic nuclei and effects from the other electrons (apart from Hydrogen!)

n   **Fortunately** the assumption is pretty good as a first approximation.

n   **Equally fortunately** it is **NOT TRUE** for the inner electrons in many atoms

n   This means that we have to introduce **two wavelength dependent corrections** to the atomic scattering factor, fj so that it becomes:

$$f_o^{\mathrm{anom}} = f_o + \Delta f' + i\Delta f'' = f' + i\Delta f''$$

n   **Which results in intensity changes which are extremely valuable for**:

-   Determining the **absolute configurations** of chiral molecules
-   Solving the phase problem – **SAD, MAD**

Scattering and Intensity

n   The **structure factor formula**:

$$F_{hkl} = \sum_{J=1}^{n} fj \{Cos2\pi(hx_j + ky_j + lz_j) + iSin2\pi(hx_j + ky_j + lz_j)\}$$

n   This enables us to **calculate** a diffraction pattern as a set of so-called **structure factors** with:

- a scattering **AMPLITUDE, |F$_{hkl}$|**, and
- a **PHASE, α_{hkl}**, which comes out of the equation:
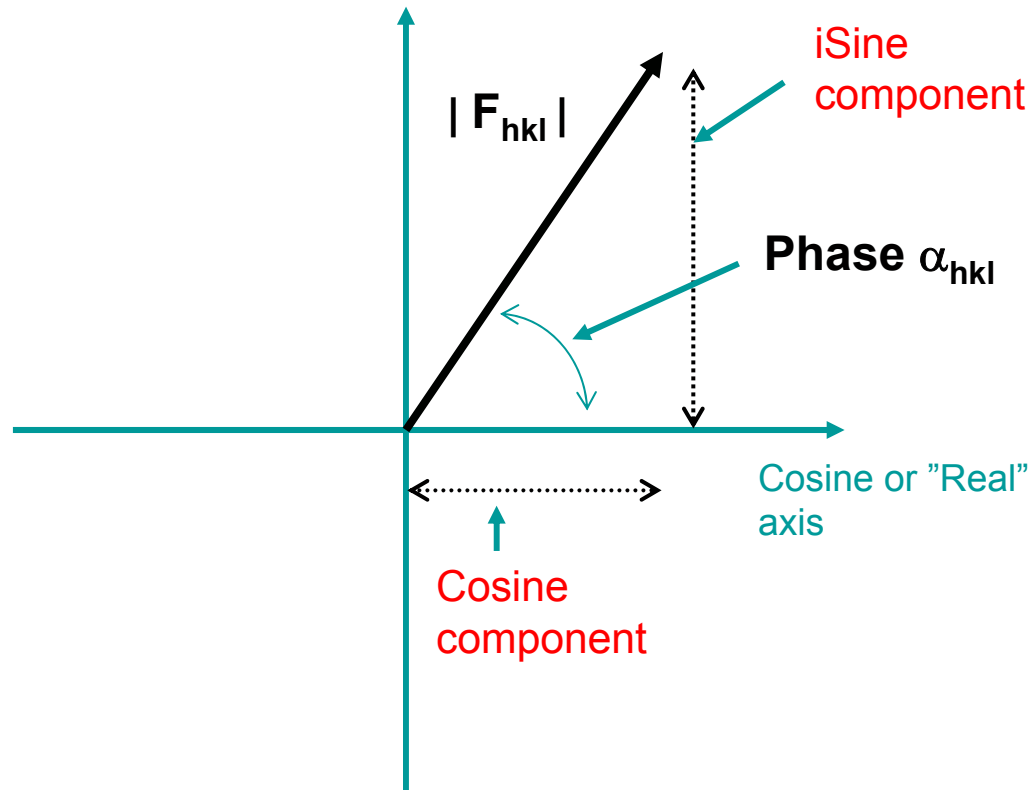
$$\alpha_{hkl} = Tan^{-1}(\{Sin2\pi(hxj + kyj + lzj)/ \{Cos2\pi(hxj + kyj + lzj)\}$$

n   **Although valuable this assumes that we have SOLVED THE STRUCTURE i.e. it is the OPPOSITE OF WHAT WE NEED!**

n   **The structure factor itself can be plotted as a vector in a "Phase/Amplitude" diagram:**

Phase /amplitude (Argand) diagram

Imaginary or iSine axis

$|F_{hkl}|$

iSine
component

Phase α_{hkl}

Cosine or "Real"
axis

Cosine
component

$|F_{hkl}|$ is the **AMPLITUDE** of the scattered wave – its "length"

α_{hkl} is its **PHASE** *relative to all the other scattered waves*

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February 2009

Amplitude and Intensity

- The **structure factor formula** enables us to calculate the **amplitude** of the total wave scattered by a given set of crystal planes *if we know the structure*.

- We can only record the **INTENSITY** of an X-ray wave

- **Intensity** is defined as: **energy/measuring area/time**
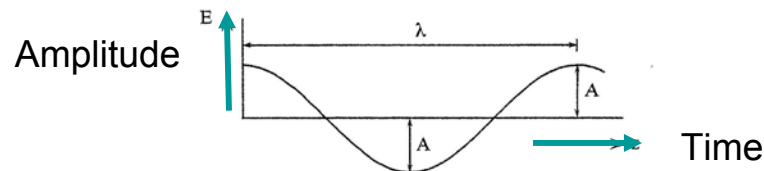 - Where
    - Measuring area is the area of the detector which we are looking at
      - Frequently $100\mu^2$
    - Time is the exposure time for an image or the time that a given sets of planes are able to diffract

- Back to a Sine wave:

  Amplitude

- We are recording **Energy over** an interval of **time**

- This is the Area under the wave curve

- i.e. it is the **Integral** of the wave over the time period
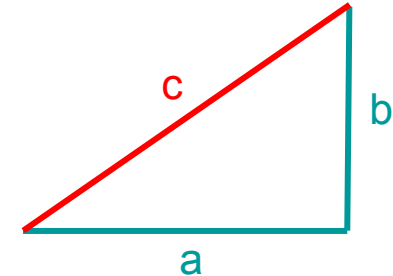
- It is therefore a function of Amplitude^2

The Phase Problem

_n **Because *intensity* is a function of *amplitude^2* we cannot measue the phase α**

- **Pythagoras:**

$$c^2 = a^2 + b^2$$

Knowing c^2 gives **NO INFORMATION** about the relationship between a and b apart from Pythagoras

- **We measure $|\,F_{hkl}\,|^2$ but this gives us no information about the *relative* sizes of the iSine and Cosine components**
- **Thus we have no way to measure the PHASEs, α**
- **No problem right now………**
- **………………………………….but BIG problem later!!**

Data reduction

n   The formula relating structure factors to the crystal structure is:

$$\mathbf{F}_{hkl} = \sum_{J=1}^{n} fj \; \{Cos2\pi(hx_j + ky_j + lz_j) \; + \; iSin2\pi(hx_j + ky_j + lz_j)\}$$

n   The process of converting **MEASURED INTENSITIES** to **OBSERVED STRUCTURE FACTORS** is called……..

n   ……….**DATA REDUCTION**

Data reduction (I)

n In most cases this is basically a simple mathematical operation using the formula:

$$\left| F_{hkl} \right| = \left(\frac{kI_{hkl}}{Lp} \right)^{\frac{1}{2}}$$

n Conversion of the observed **INTENSITIES, I$_{hkl}$,** to the structure factor amplitudes, IF_{hkl}I that we need involves correction for:

- **Polarisation** (p) …we have seen this before!
    - arises whenever reflection or a reflection like process occurs
    - reduces the intensity in a **predictable** manner
- **Lorentz effect (L)**
    - a predictable rotation-speed effect
- **k** is a **scale factor** relating the whole observed data set to what would have been observed from a single unit cell.
- **Absorption** of the diffracted beams by passage through the crystal
    - Reduces some intensities more than others – can be corrected
- **Response characterstics of the detector**
- **Loss of scattering power due to radiation damage**

NORSTRUCT

Data reduction

n **Integration of the observed diffracted beams will also involve effects due to :**

- the size and shape of the crystal
- the size and collimation of the primary beam
- General background effects – eg. scattering by air and the solvent and loop around the crystal
- Possible fluorescence from heavy atoms in the structure/crystal
- Mosaicity of the crystal – no crystals are perfect, this makes the spots diffuse!

n **The set of F_{hkl}'s** are the **OBSERVED STRUCTURE FACTOR AMPLITUDES**

n **The set of F_{hkl}'s are the raw data we need to solve the structure**

Edward Hough
February 2009

NORSTRUCT

In for landing…

…..Sean takes over!!