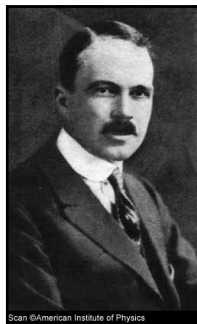




The Phase Problem

Peter Müller

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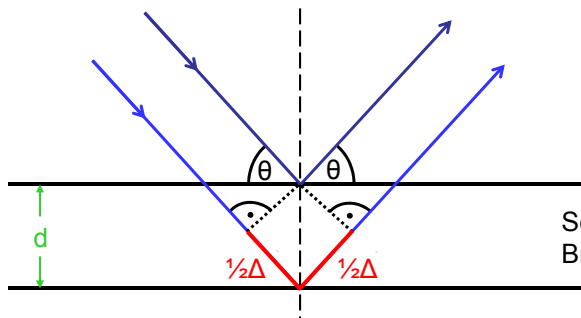


Scan ©American Institute of Physics

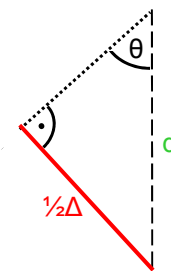
Reflection on Lattice Planes

$$\sin \theta = \frac{\frac{1}{2}\Delta}{d} = \frac{\Delta}{2d}$$

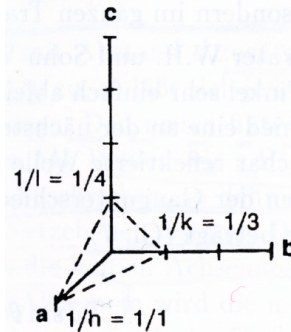
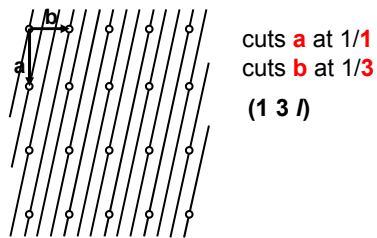
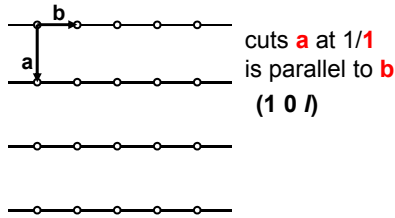
Bragg's Law: $\Delta = 2d \sin \theta = n\lambda$



Set of parallel planes:
Bragg planes

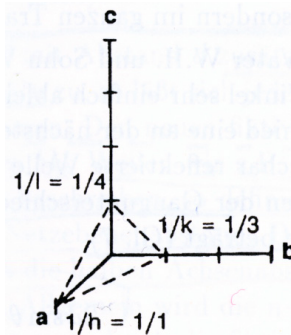
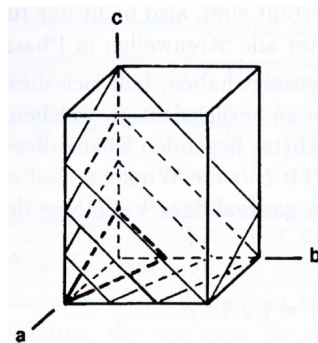


3D Bragg Planes: Miller Indices (h, k, l)



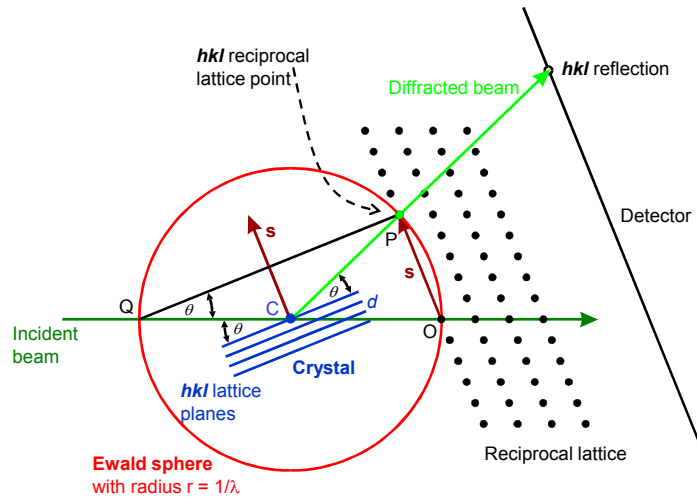
cuts **a** at $1/1$ **(1 3 4)**
cuts **b** at $1/3$
cuts **c** at $1/4$

Real Space \rightarrow Reciprocal Space



Between the points of a crystal lattice in real space, we have Bragg planes.
 Each set of Bragg planes corresponds to one reflection.
 Each set of Bragg planes corresponds to one set of Miller indices.
 Each reflection is identified by the corresponding Miller indices (h, k, l) .
 The reflections form another lattice, the reciprocal lattice.
 The vector d is perpendicular to a set of Bragg planes.
 Its length is equivalent to the distance between two Bragg planes.
 Each reflection (h, k, l) marks the endpoint of the vector $d^* = 1/d = s$.
 The length of s is inversely related to the distance between the Bragg planes.

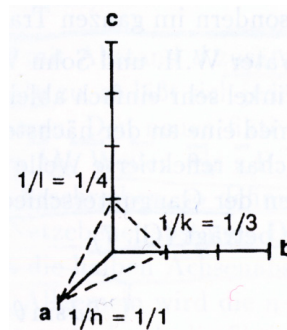
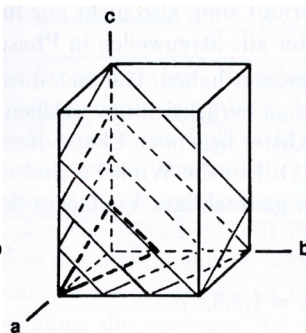
The Reciprocal Lattice: Ewald Construction



Reciprocal Space

The reflection h, k, l is generated by diffraction of the X-ray beam at the Bragg plane set h, k, l , which intersects the three edges of the unit cell at $1/h, 1/k$ and $1/l$.

Sets of planes in real space (with spacing d) correspond to points in reciprocal space (distance d^* from the origin).



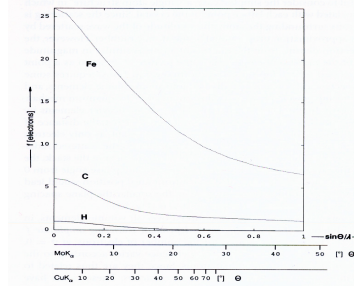
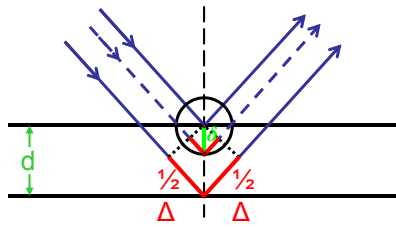
The vector d^* is perpendicular to the Bragg planes and has the length $|d^*| = 2\sin\theta/\lambda$. A reflection is visible when the corresponding set of Bragg planes is in reflex position, that is when Bragg's law is fulfilled. In an alternative description: a reflection is visible when the corresponding scattering vector $s = d^*$ intersects with the Ewald sphere.

The reflections are positioned on a lattice in reciprocal space. Reciprocal unit cell: a^*, b^*, c^* . The dimensions and angles of the reciprocal cell are inversely proportional to the real space cell: if the unit cell doubles, the space between the X-ray reflections will be reduced by factor two.

Intensities of the Reflections

With the help of Bragg's law and the Ewald construction, we can calculate the place of a reflection on the detector, provided we know the unit cell dimensions. Indeed, the position of a spot is determined alone by the metric symmetry of the unit cell. The intensity of a spot, however, depends on the contents of the unit cell (and, of course, on exposure time, crystal size, *etc.*).

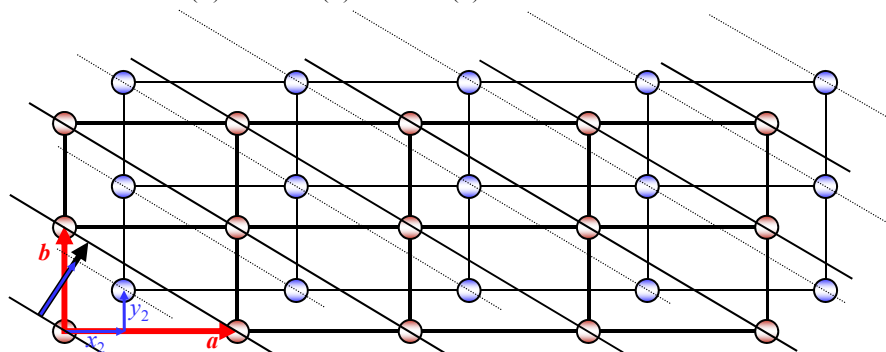
Reflections are weakened by the radius of the atoms (atomic form factor) and the thermal motion of the atoms (temperature factor U). Both these effects are stronger at high resolution.



Structure Factors

Every atom in the unit cell contributes to every reflection according to its chemical nature and its relative position. Owing to this shift in position relative to the other atoms, the photons contributed by each atom in the unit cell have a phase shift relative to those from other atoms.

$$\Phi_i = \Delta\Phi_{i(a)} + \Delta\Phi_{i(b)} + \Delta\Phi_{i(c)} = 2\pi(hx_i + ky_i + lz_i)$$



$$0 \leq \Delta\Phi \leq 2\pi$$

Structure Factors

$$\Phi_i = \Delta\Phi_{i(a)} + \Delta\Phi_{i(b)} + \Delta\Phi_{i(c)} = 2\pi(hx_i + ky_i + lz_i)$$

This makes the structure factor a complex number:

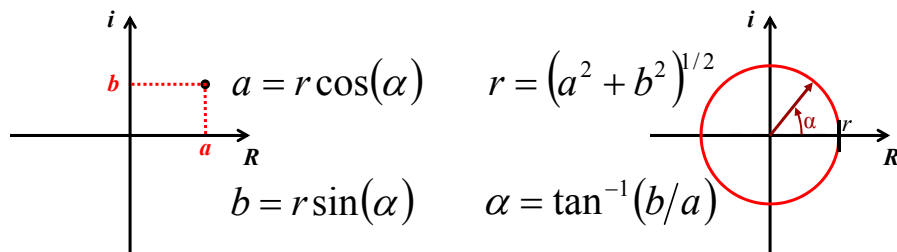
$$F_i = f_i \cdot \exp[i\Phi_i] = f_i(\cos\Phi_i + i\sin\Phi_i)$$

Every atom i in the unit cell contributes to every structure factor $F(hkl)$ (that is reflection) according to its position in the cell and its chemical nature (different values for f_i !):

$$F(hkl) = \sum_i f_i [\cos 2\pi(hx_i + ky_i + lz_i) + i \sin 2\pi(hx_i + ky_i + lz_i)]$$

Complex Numbers and the Argand Diagram

A given complex number may be specified by either its real and imaginary components (a and b) or its magnitude and phase (r and α), with the following relationships between them:

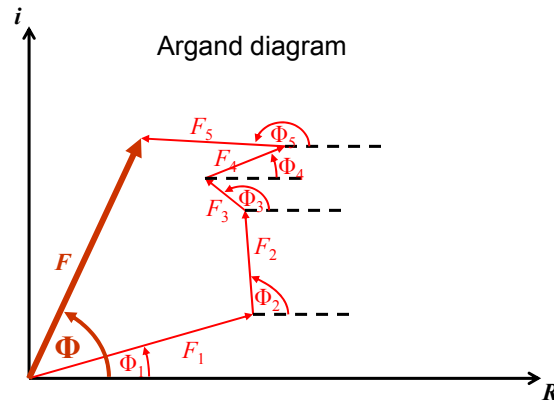


Structure factors $\vec{F}(hkl)$ are complex quantities. If the magnitude of a particular reflection is measured but the phase has not been determined yet, the possible values of that structure factor can be represented by a circle of radius $|\vec{F}|$ in the complex plane.

Structure Factors

$$F(hkl) = \sum_i f_i [\cos 2\pi(hx_i + ky_i + lz_i) + i \sin 2\pi(hx_i + ky_i + lz_i)]$$

$$= \sum_i f_i \exp[i\Phi_i]$$



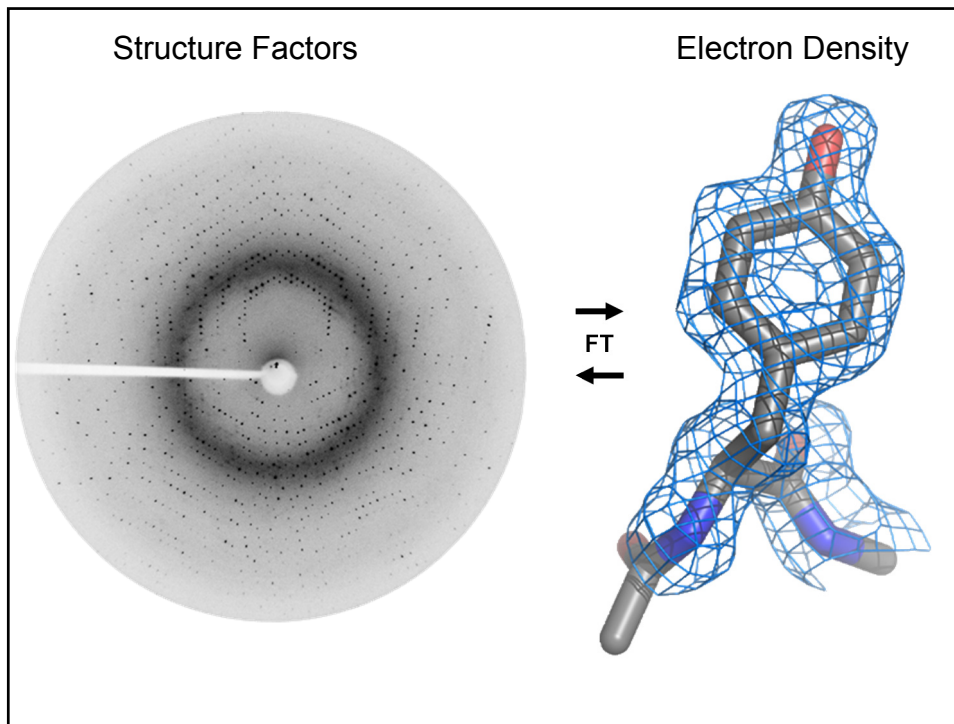
Structure Factors

$$F(hkl) = \sum_i f_i [\cos 2\pi(hx_i + ky_i + lz_i) + i \sin 2\pi(hx_i + ky_i + lz_i)]$$

↓_{FT} ↑

$$\rho_{x,y,z} = \frac{1}{V} \sum_{hkl} F_{hkl} \cdot \exp[-i2\pi(hx + ky + lz)]$$

Electron Density



Structure Factors

$$F(hkl) = \sum_i f_i [\cos 2\pi(hx_i + ky_i + lz_i) + i \sin 2\pi(hx_i + ky_i + lz_i)]$$

↓ FT ↑

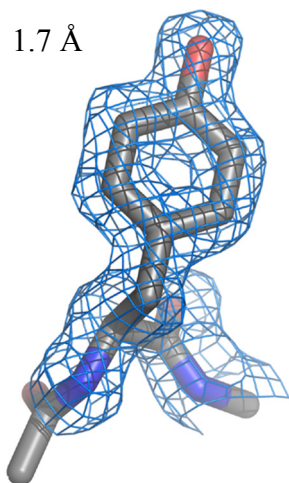
$$\rho_{x,y,z} = \frac{1}{V} \sum_{hkl} F_{hkl} \cdot \exp[-i2\pi(hx + ky + lz)]$$

Electron Density

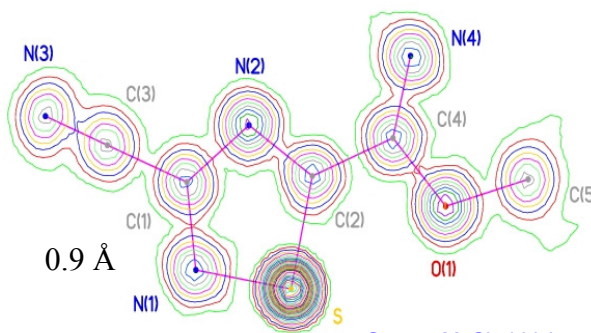
Electron Density

$$\rho_{x,y,z} = \frac{1}{V} \sum_{hkl} F_{hkl} \cdot \exp[-i2\pi(hx + ky + lz)]$$

1.7 Å



It is sufficient to calculate the electron density at a number of grid points within the unit cell and interpolate between the points.



George M. Sheldrick

Calculating Electron Density

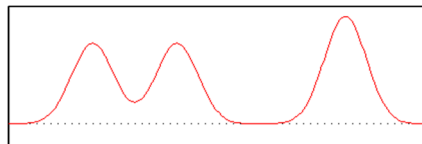
$$\rho_{x,y,z} = \frac{1}{V} \sum_{hkl} F_{hkl} \cdot \exp[-i2\pi(hx + ky + lz)]$$

To make this equation work we need the volume of the unit cell (easy), a dataset with the intensities for h , k and l (also easy) and the phase of every structure factor (not quite so easy).

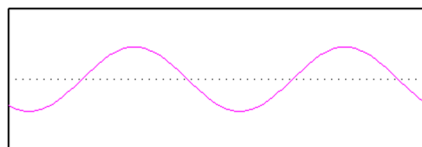
We can measure the intensities and determine the unit cell from the locations of the reflections, but it is very difficult to actually measure the relative phase of the reflections. This is known as the “crystallographic phase problem”.

The Phase Problem: Fourier Synthesis

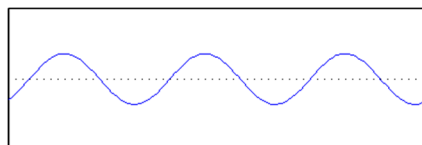
Let's imagine a one dimensional crystal with a three-atomic molecule in the unit cell: two carbons and one oxygen. The electron density in this unit cell looks like this:



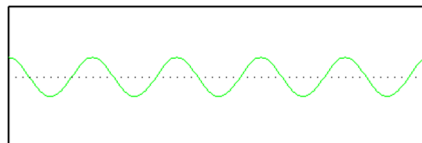
Try to represent this function in terms of sine waves. The first sine wave has a frequency of 2, that is there are two repeats of the wave across the unit cell. One peak represents the oxygen, and the other the two carbons:



The second sine wave has a frequency of 3; three repeats of the wave across the unit cell. It has a different phase, in other words we start at a different place on the wave. The amplitude is also different:

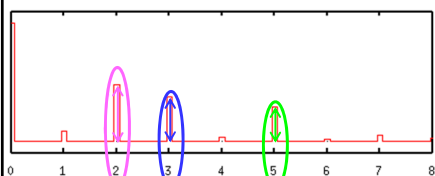


Finally, we introduce a sine wave with a frequency of 5. Two of the peaks of this wave are lined up with the carbon atoms:



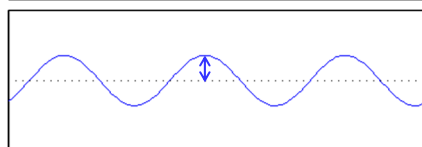
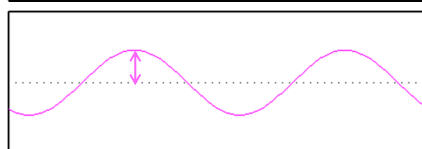
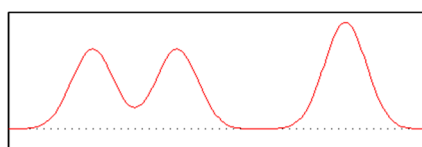
Kevin Cowtan <http://www.ytbl.york.ac.uk/~cowtan/>

The Phase Problem: Fourier Synthesis

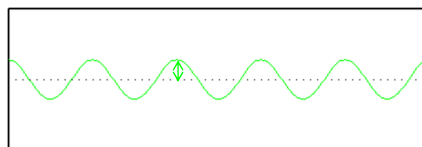
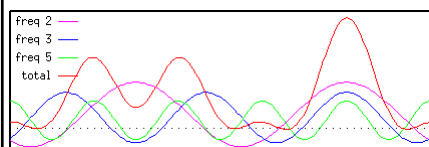


Fourier Transform of the same unit cell

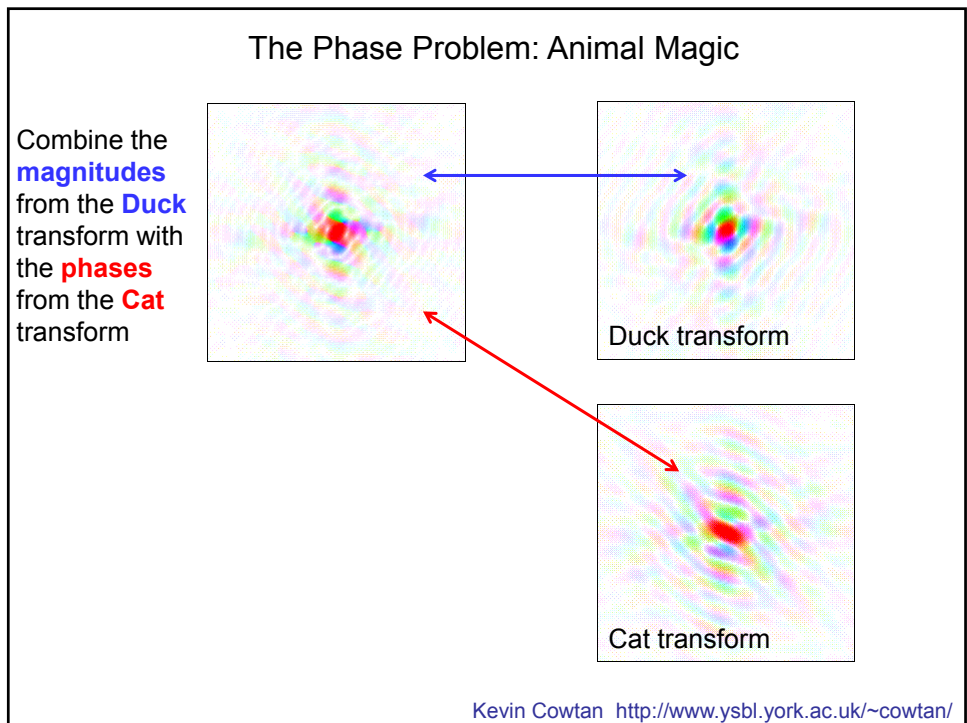
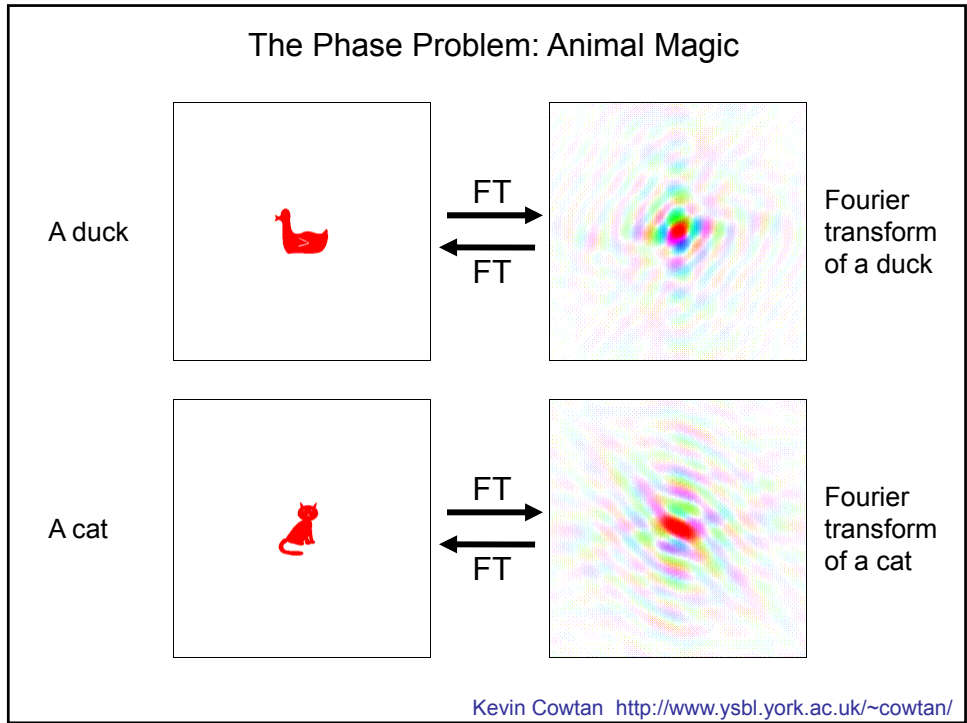
FT
FT



Now we add them all together:

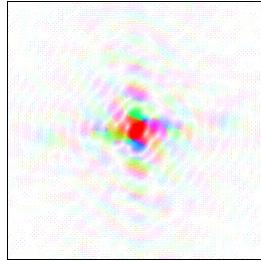


Kevin Cowtan <http://www.ytbl.york.ac.uk/~cowtan/>

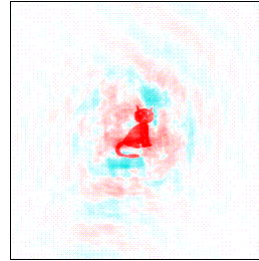


The Phase Problem: Animal Magic

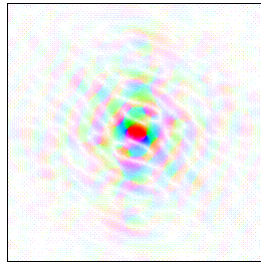
Combine the **magnitudes** from the **Duck** transform with the **phases** from the **Cat** transform



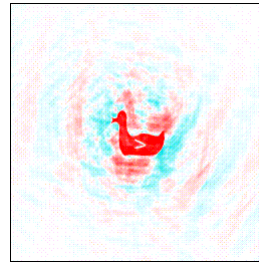
FT
←
FT



Combine the **magnitudes** from the **Cat** transform with the **phases** from the **Duck** transform



FT
←
FT



The phase contains the bulk of the information!

Kevin Cowtan <http://www.yesbl.york.ac.uk/~cowtan/>

Symmetry in Reciprocal Space

Directly or indirectly, most structure solution approaches utilize symmetry in reciprocal space.

Symmetry in the crystal (real space) influences the symmetry in reciprocal space. A twofold in real space causes a twofold in reciprocal space, a mirror, causes a mirror, a fourfold a fourfold, etc. Reciprocal space, however, is centrosymmetric, and translational symmetry has no effect on the symmetry in reciprocal space. That means $P2$, $C2$, Cc , $P2_1$, $P2/m$, $P2_1/m$, $P2_1/c$, etc. all correspond to the same symmetry group in reciprocal space.

The symmetry group in reciprocal space is called Laue group. **The Laue symmetry can be lower than the metric symmetry of the unit cell, but never higher.** (A monoclinic crystal with $\beta = 90^\circ$ is still monoclinic.)

The Patterson Function

We measure intensities I . After applying several corrections, they translate into squared structure factors also known as structure factor amplitudes F^2 .

intensities I :

$$I \longrightarrow F^2 \quad F: \text{structure factors}$$

The Fourier transform of the structure factors (with phases) is the electron density function. The Fourier transform of the squared structure factor amplitudes (as measured, without the phases) is the Patterson function. The unit cell of the Patterson map is the same as that of the crystal structure.

The Patterson function has some very interesting features:

It consists of peaks, that have coordinates (u, v, w) and an intensity

The distance of each peak from the origin corresponds to an interatomic distance in real space.

The height of the peaks is proportional the involved electrons and proportional the multiplicity of the corresponding vector in real space.

These features make it possible to use the Patterson map to solve the phase problem.

The Patterson Function

That means, a peak u, v, w in the Patterson map indicates that atoms exist in the unit cell at x_1, y_1, z_1 and x_2, y_2, z_2 such that

$$u = x_1 - x_2$$

$$v = y_1 - y_2$$

$$w = z_1 - z_2$$

Thus, for N atoms in a unit cell, the Patterson map will contain N^2 peaks. N out of these N^2 peaks will be of zero length, from each atom to itself. All these zero-vectors will fall on top of one another in the origin of the Patterson map, which generates a very high zero-peak.

Taking this into account, the Patterson map contains $N^2 - N + 1$ theoretically distinguishable peaks.

In real life many of those peaks, which are relatively broad, will overlap and be, in fact, not distinguishable from some neighbor peaks. Usually, the strongest peaks, corresponding to vectors between heavy atoms, are well resolved and usable.

One Heavy Atom in $P-1$

For every atom at x, y, z there is a symmetry equivalent atom at $-x, -y, -z$ and hence a Patterson peak at $2x, 2y, 2z$.

The compound $C_{32}H_{24}AuF_5P_2$ crystallizes in $P-1$ with two molecules per unit cell (that makes one per asymmetric unit). The two gold atoms are related by the inversion center. Besides the peak on the origin (height 999) there is a peak with height 374 at $u = 0.318 = 2x$, $v = 0.471 = 2y$, $w = 0.532 = 2z$, which is much higher than all the other peaks (≤ 145). Its height is consistent with the calculated value for a Au-Au vector (377).

To calculate the positions x, y, z of the gold atom in the unit cell, we can divide each of the Patterson coordinates by 2. However, we also need to take into account the fact that there is always a peak at $u+1, v+1$ and $w+1$ (corresponding to next unit cell in the crystal)! Thus:

$$x = 0.318/2 = \mathbf{0.159} \text{ or } 1.318/2 = \mathbf{0.659}$$

$$y = 0.471/2 = \mathbf{0.236} \text{ or } 1.471/2 = \mathbf{0.736}$$

$$z = 0.532/2 = \mathbf{0.266} \text{ or } 1.532/2 = \mathbf{0.766}$$

Those eight truly equivalent solutions correspond to the eight positions of the inversion center in the space group $P-1$. Choice of origin.

George M. Sheldrick

Symmetry of the Patterson Function

For every vector $i \rightarrow j$ there is always a vector $j \rightarrow i$. Thus the Patterson is always centrosymmetric.

In general, the symmetry of the Patterson is determined by the symmetry of the diffraction pattern (also centrosymmetric). Glide planes and screw axes of the space groups correspond to mirror planes and normal rotation axes in reciprocal space. The Patterson has the same symmetry as the Laue group.

Harker Sections

Space group symmetry leads to accumulation of Patterson peaks in certain sections (planes or lines). *E.g.*

$P2_1$: atoms at x, y, z and $-x, \frac{1}{2}+y, -z$. \rightarrow Eigenvectors at $2x, \frac{1}{2}, 2z$;
Harker section at $v = \frac{1}{2}$.

$P2$: atoms at x, y, z and $-x, y, -z$. \rightarrow Eigenvectors at $2x, 0, 2z$;
Harker section at $v = 0$.

Pm : atoms at x, y, z and $x, -y, z$. \rightarrow Eigenvectors at $0, 2y, 0$;
Harker section at $u = 0, w = 0$.

Space groups $P2$ and Pm both have the same systematic absences (none), but they have different Harker sections.

Problems of the Patterson Method

Frequently, localizing a single heavy atom in a structure is enough to find all other atoms by means of iterative difference Fourier synthesis with amplitudes $|F_o - F_c|$ and phases ϕ_c .

Problems arise in non-centrosymmetric space groups when the heavy atom substructure is centrosymmetric (*e.g.* only one heavy atom). In this case, all phases derived from the heavy atom positions are 0° or 180° and the calculated electron density is centrosymmetric, *i.e.* it shows a double-image.

Another problem is the atom-type assignment: Electron density and Patterson peak heights are proportional to atomic number, however only roughly, and isoelectronic species are notoriously difficult to distinguish. This is a problem also with direct methods and sometimes even during structure refinement.

Lack of any heavy atoms is also difficult for Patterson methods.

Direct Methods

Direct methods determine the phases directly from the diffraction pattern without any knowledge about the nature of the sample.

Several statistical equations relate the phase of a reflection to its intensity and the intensity and phase of other reflections in the dataset. This can be done with a certain probability. Many probability relations together with fast computers make it possible to determine the phases of many measured reflections with some accuracy.

Direct methods usually do not use structure factors but operate in E -space. The advantage is, that the intensity of a normalized structure factor does not decline with the resolution; and direct methods assume atoms to be point-scatterers anyway.

Direct methods are predominantly used as “black-box” methods.

E^2-1 Statistics

We measure intensities I

$I \longrightarrow F^2$ F : structure factors

Normalized structure factors E :

$E^2 = F^2 / \langle F^2 \rangle$ $\langle F^2 \rangle$: mean value for reflections at same resolution

$\langle E^2 \rangle = 1$

$\langle |E^2 - 1| \rangle =$ 0.736 for non-centrosymmetric structures
0.968 for centrosymmetric structures

Heavy atoms on special positions and twinning tend to lower this value.
Pseudo translational symmetry tend to increase this value.

Direct methods typically operate in E -space, not F -space.

This is emphasizing the high resolution data and coming close to the point-atom approximation.

Direct Methods

Assumption 1:

Electron density (including the background) is never negative.

→ Harker-Kasper Inequalities (1948)

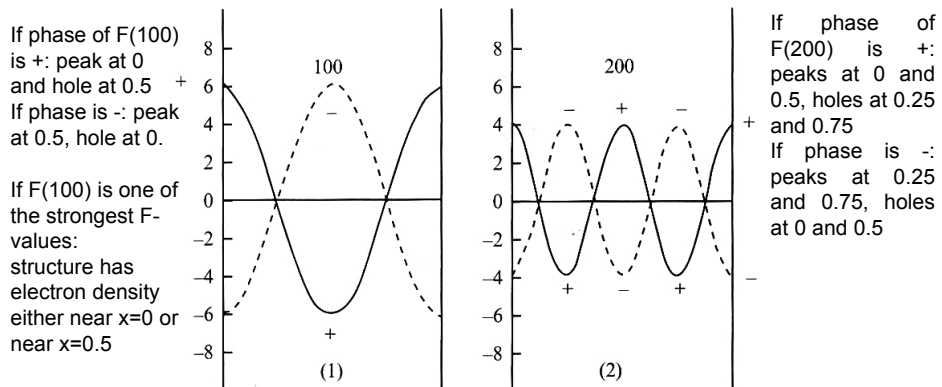
David Harker and John Kasper: Relations between the magnitudes of some structure factors and the signs or phases of others limit the choice of phases.

The electron density map will be dominated by the strong reflections.
Use only the strongest peaks (*E*-values) for phasing.

Direct Methods

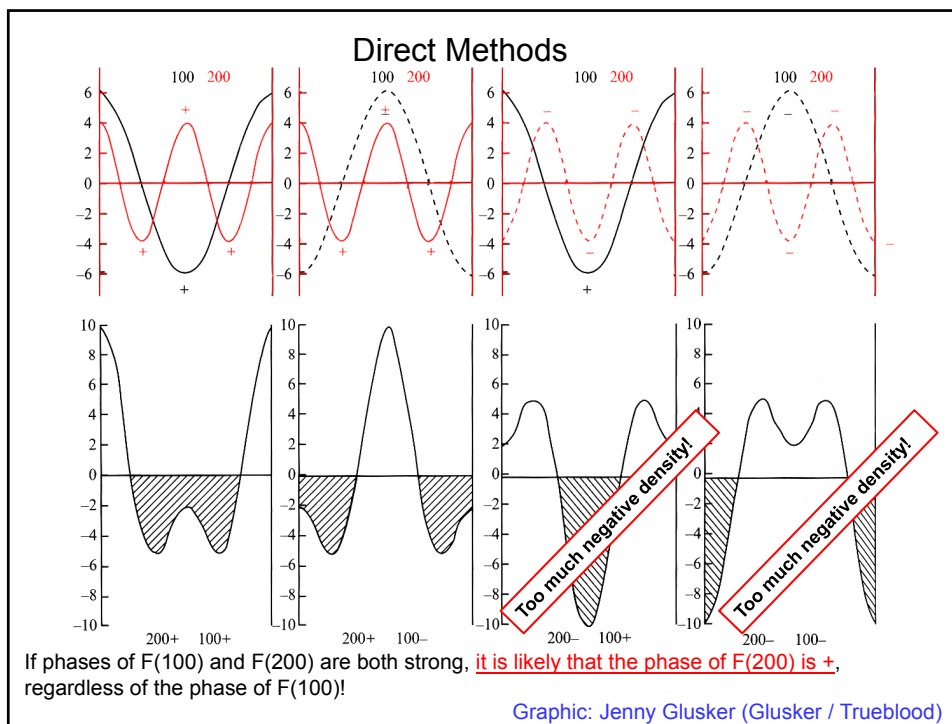
Centrosymmetric Example:

Phases can only be 0° (+) or 180° (-). Reflections (1 0 0) and (2 0 0).



If phases of F(100) and F(200) are both strong, it is likely that the phase of F(200) is +, regardless of the phase of F(100)!

Graphic: Jenny Glusker (Glusker / Trueblood)



Direct Methods

Assumption 1:

Electron density (including the background) is never negative.

→ Harker-Kasper Inequality (1948)

Assumption 2:

Atoms are randomly distributed, are essentially identical, are point atoms and are resolved from one another.


High resolution data required; E -values instead of F .

For randomly distributed equal point atoms, the squared electron density is similar to the electron density itself (times a scaling factor).

$$\rho_x = Q \cdot \rho_x^2$$

The electron density map will be dominated by the strong reflections.

Use only the strongest peaks (E -values) for phasing.



Sayre Equation: 1952
David Sayre (1924 - 2012)

$$\rho_x = Q \cdot \rho_x^2$$

Independently observed by Sayre, Cochran and Zachariasen.

$$\bar{F}_{\bar{h}} = q \sum_{\bar{h}'} \bar{F}_{\bar{h}'} \cdot \bar{F}_{\bar{h}-\bar{h}'}$$

$\bar{h}, \bar{h}', \bar{h}-\bar{h}'$ are reflections $(h,k,l), (h',k',l'), (h-h', k-k', l-l')$
 q is a resolution dependent factor.

$$\phi_{\bar{h}} \approx \phi_{\bar{h}'} + \phi_{\bar{h}-\bar{h}'}$$

Triplet phase relation for $E_{\bar{h}}, E_{\bar{h}'}, E_{\bar{h}-\bar{h}'} > 1$

Centrosymmetric case: ϕ is 0° or 180°

$$\text{sign}_{\bar{h}} \approx \text{sign}_{\bar{h}'} \cdot \text{sign}_{\bar{h}-\bar{h}'}$$

Centrosymmetric example: \bar{h} is $(5\ 0\ 0)$, \bar{h}' is $(2\ 0\ 0)$, $\bar{h}-\bar{h}'$ is $(3\ 0\ 0)$; all strong.

If phase of $F(200)$ is - and phase of $F(300)$ is +, it is likely that the phase of $F(500)$ is -

Direct Methods

<p>$F(200) -, \text{phase } 180^\circ$</p> <p>$F(300) +, \text{phase } 0^\circ$</p> <p>$F(500) -, \text{phase } 180^\circ$</p> <p style="text-align: center;">Sum</p>	<p>$F(200) -, \text{phase } 180^\circ$</p> <p>$F(300) +, \text{phase } 0^\circ$</p> <p>$F(500) +, \text{phase } 0^\circ$</p> <p style="text-align: center;">Sum</p>
--	--

Gives clearest peaks!
correct phases!

Too much negative
density!

Graphic: Jenny Glusker (Glusker / Trueblood)

Direct Methods

Tangent Formula (Karle & Hauptman, 1959)

$$\tan(\phi_{\bar{h}}) = \frac{\sum_{\bar{h}'} |E_{\bar{h}'} E_{\bar{h}-\bar{h}'}| \sin(\phi_{\bar{h}'} + \phi_{\bar{h}-\bar{h}'})}{\sum_{\bar{h}'} |E_{\bar{h}'} E_{\bar{h}-\bar{h}'})| \cos(\phi_{\bar{h}'} + \phi_{\bar{h}-\bar{h}'})}$$

Allows expanding the phase set from a known subset of E-factors to further E-factors. "Engine" in most classic direct methods

Start Phases from simple statistical analysis of a small subset of the strongest E-values, from triplet phase relations and, after 1975, negative quartets. Also random start phases (Yao Jia-Xing, 1981).

1985 Nobel prize in Chemistry to Hauptman & Karle

Direct Methods

Triplet phase relation (Sayre): 1952

$$\phi_{\bar{h}_1} \approx \phi_{\bar{h}_2} + \phi_{\bar{h}_3} \quad h_n = (h_n, k_n, l_n) \quad h_1 = h_2 + h_3$$

$$\phi_{\bar{h}_1} + \phi_{\bar{h}_2} + \phi_{\bar{h}_3} \approx 0^\circ \quad h_1 + h_2 + h_3 = 0$$

$$E_{\bar{h}_1} \gg 1; E_{\bar{h}_2} \gg 1; E_{\bar{h}_3} \gg 1$$

Probability to be correct depends on E-values and number of atoms in unit cell (proportional $N^{-1/2}$, $E_{\bar{h}_1}$, $E_{\bar{h}_2}$, $E_{\bar{h}_3}$).

Negative Quartets (Schenk 1973, Hauptman 1975, Giacovazzo 1976):

$$\phi_{\bar{h}_1} + \phi_{\bar{h}_2} + \phi_{\bar{h}_3} + \phi_{\bar{h}_4} \approx 180^\circ \quad h_1 + h_2 + h_3 + h_4 = 0$$

$$E_{\bar{h}_1} \gg 1; E_{\bar{h}_2} \gg 1; E_{\bar{h}_3} \gg 1; E_{\bar{h}_4} \gg 1;$$

$$E_{\bar{h}_1 + \bar{h}_2} \ll 1; E_{\bar{h}_1 + \bar{h}_3} \ll 1; E_{\bar{h}_1 + \bar{h}_4} \ll 1$$

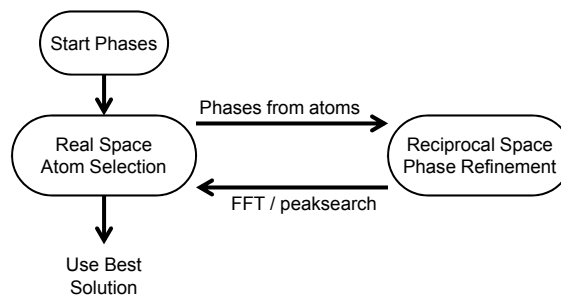
Schenk discovered that the quartet phase sum is close to 180° for four strong primary E-values and small cross-terms.

Hauptman and Giacovazzo independently derived probability formulas for the negative quartets. Giacovazzo's formula is simpler and more accurate.

Probability proportional N^{-1} , $E_{\bar{h}_1}$, $E_{\bar{h}_2}$, $E_{\bar{h}_3}$, $E_{\bar{h}_4}$ and $(2 - E_{\bar{h}_2}^2 - E_{\bar{h}_3}^2 - E_{\bar{h}_4}^2)$

Direct Methods

Dual Space Recycling (Weeks, DeTitta, Hauptman, Sheldrick): 1993 (Shake-n-bake, SHELXD)



First used by Weeks, DeTitta, Hauptman *et al.* in Shake-n-Bake. Later by Sheldrick in SHELXD.

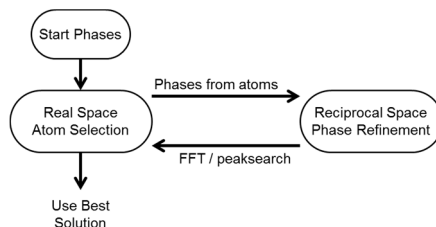
The real space cycle allows for manipulations on the atom level (sensible distances, angles, etc.). Phase refinement may use the Tangent Formula or other methods (e.g. parameter shift).

This approach works well for location of heavy atoms from anomalous diffraction data but also for regular structures, small and large.

The magic is in the details.

Direct Methods

Random Omit Maps (Sheldrick): 1999 (2001) SHELXD



About 30% of the atoms (peaks) are omitted **at random** in the real space step.

The phases calculated from the remaining 70% are refined in reciprocal space .

The next map is calculated from the resulting phases and observed E-values. A new peak search is performed based on this map.

This is repeated many times (20 to 1000).

Interestingly, this even works without any phase refinement in the reciprocal-space cycle!

Direct Methods

Free Lunch Algorithm (Giacovazzo, 2005; also E. Dodson and Sheldrick)

Extrapolate data to higher resolution than what was measured.

Intensities (amplitudes) of unmeasured reflections are estimated by Fourier transformation of the existing electron density (after density modification) and normalized to fit the Wilson plot.

Those “made up” reflections can then be used for phasing.

Charge Flipping (Oszlányi & Sütő, 2004; Palatinus)

Intrinsic Phasing (Sheldrick): 2009 (?)

SIR (Giacovazzo): constantly improving