

Theory of X-ray diffraction

A users perspective

Disclaimer: I am not a physicist ...

... but there will be equations!

Acknowledgements:

Slides, ideas & pictures stolen from wherever I could find them

Randy Read, Crystallography course

<http://www-structmed.cimr.cam.ac.uk/course.html>

Books by Drenth, Rupp, etc

Gwyndaf Evans, PhD thesis

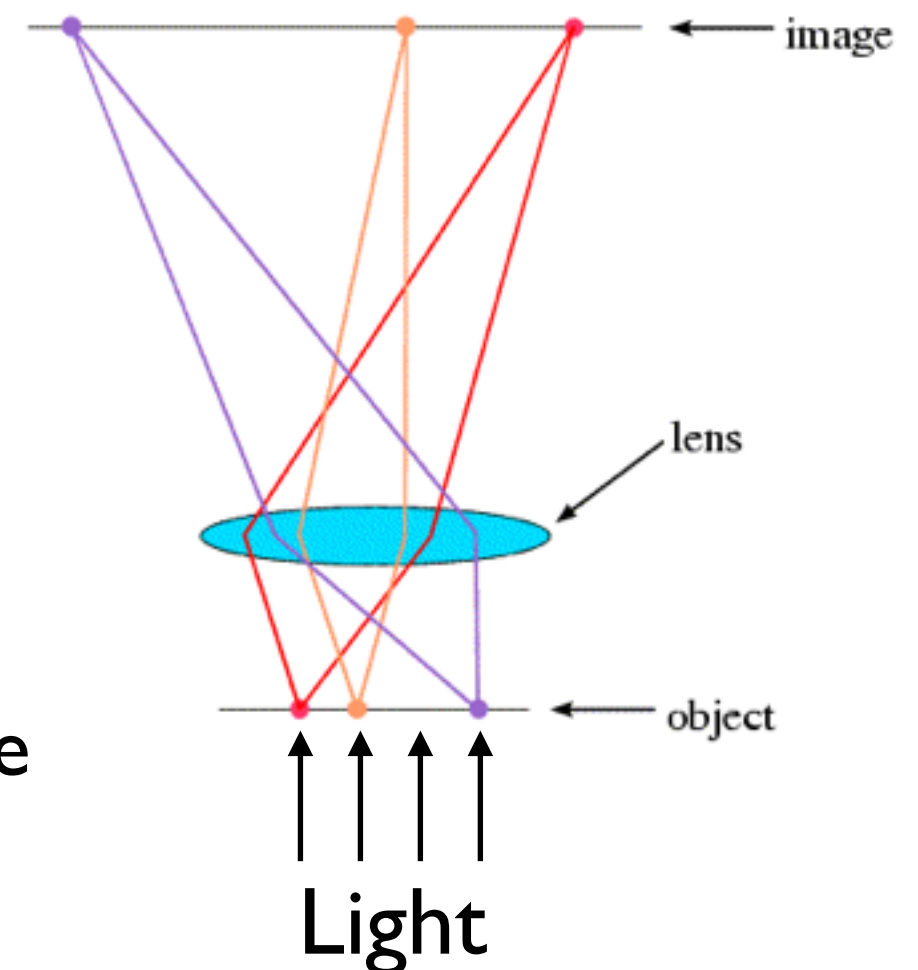
<http://www.gwyndafevans.co.uk/thesis-html/node10.html>

We want to look at molecules to see their structure, in order to understand their function

So we need a **microscope** to see something that is very small (a few nanometers), and we need to use wavelengths of the order of 0.1nm ($= 1\text{\AA}$), i.e. X-rays (or electrons or neutrons)

In a light microscope, light is scattered (diffracted) by the object then the scattered rays are focussed (recombined in phase) by the lens to form an image

BUT for X-rays we cannot make a lens with the accuracy of atomic spacings, so we have to focus the scattered beams in the computer



X-ray scattering

Simple view!

X-rays are scattered by electrons (in atoms)

I. Elastic scattering – no energy loss (Thomson scattering)

This leads to useful diffraction information

The electric field of the X-ray induces reemission of X-rays in all directions, with intensity proportional to $(\text{charge/mass})^2$

A proton is 2000 times as massive as electron, so nuclear scattering is negligible

Ib. Anomalous scattering – incident energy close to an atomic absorption edge

Elastic scattering with a phase change

Useful for phasing

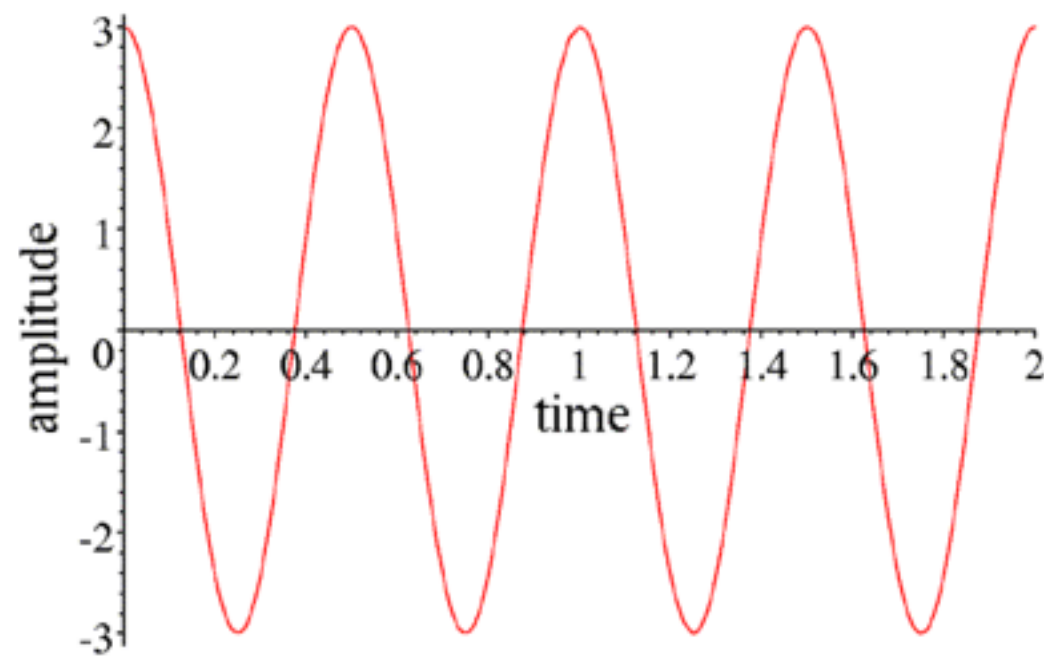
2. Inelastic scattering – transfer of energy to the atom (eg Compton scattering)

This leads to radiation damage and no useful information

Diffraction arises from the interference of **Waves** scattered by different electrons

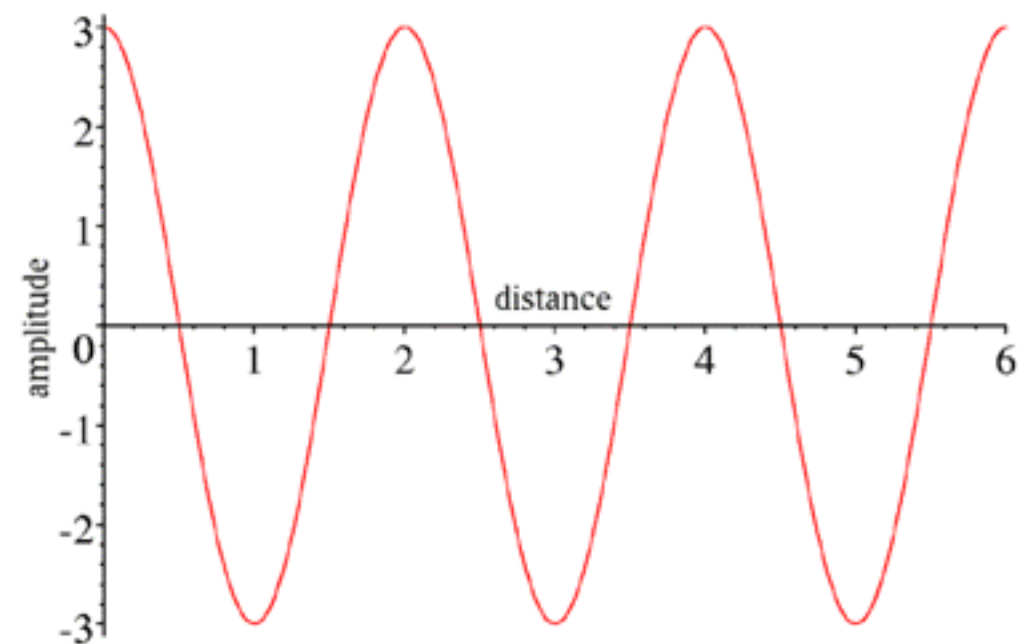
(consider X-rays as waves of their electric field)

so we need to consider the properties of waves, and how they add up



$$A \cos(2\pi \nu t)$$

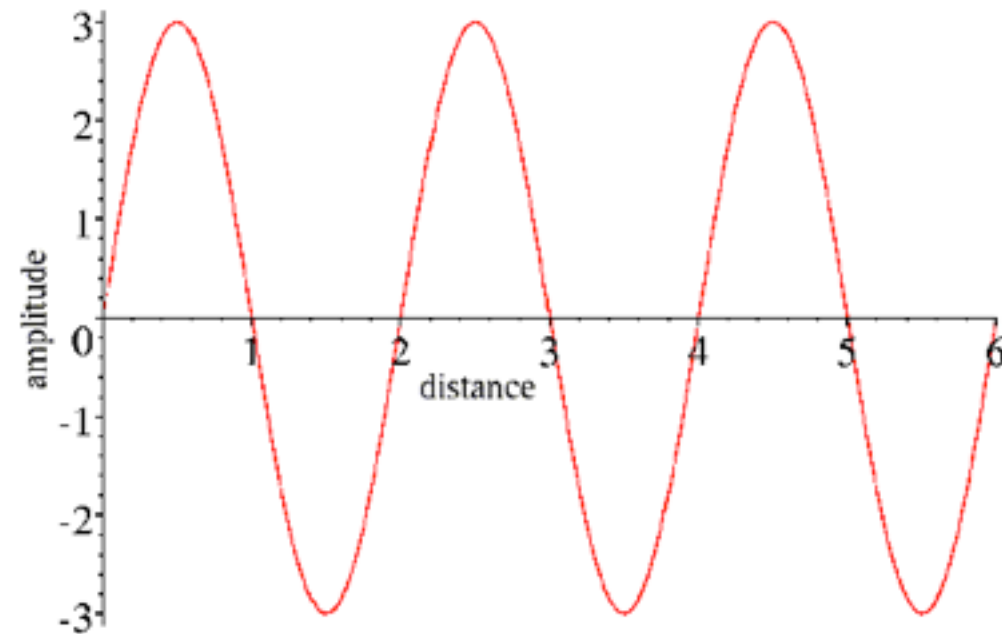
Wave as function of time



$$A \cos(2\pi x / \lambda) = A \cos(-2\pi x / \lambda)$$

Wave as function of position in space

Wave as function of time and space



$$A \cos[2\pi (\nu t - x/\lambda)]$$

A wave is characterised by:-

- amplitude A
- phase Φ – affects the value at time or distance = 0
- frequency ν and wavelength λ

$$\nu \lambda = \text{speed } c$$

We are mainly concerned with waves as a function of position in space (distance) as we will be interested in stationary waves

Frequency ν and wavelength λ

For diffraction, there is no stable interference between waves of different wavelength, but we will need to use the wavelength in understanding the geometry of diffraction

But we can think about the time-scale ...

In a typical diffraction experiment, the X-rays have a wavelength of about $1\text{\AA}$, which is one ten-thousand-millionth of a meter.

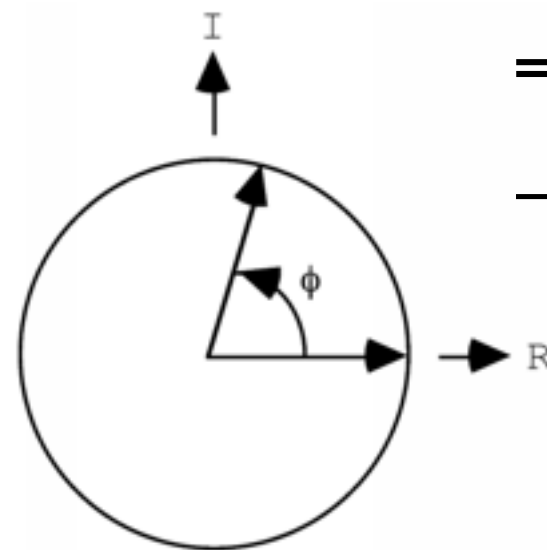
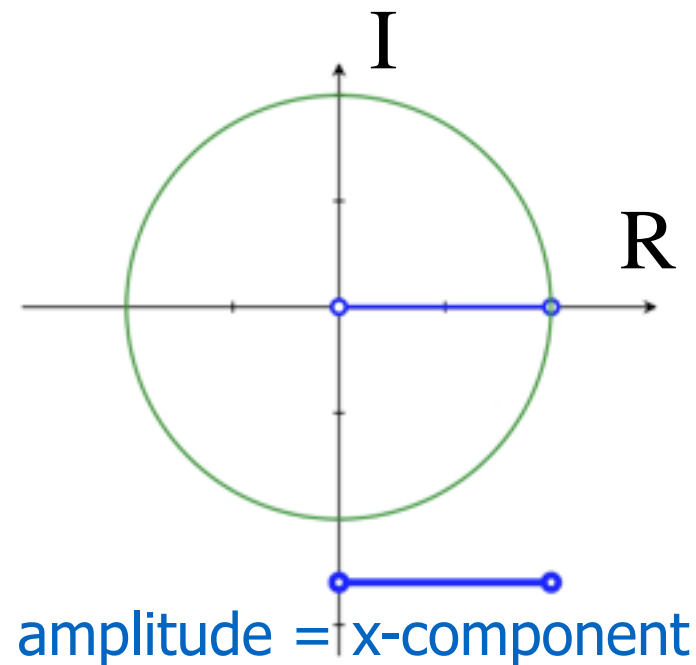
The waves are moving at the speed of light (300 million meters per second), so it takes about 3×10^{-19} second (0.3 attoseconds) for the wave to move from peak to peak.

Obviously it doesn't make much sense to think about measuring the phase of a photon as it strikes a detector! What matters to us in the diffraction pattern is the relative phase of different diffracted rays

Randy Read

Wave as vector (or complex number)

- Represent a wave as the x-component of a rotating vector
- Initial rotation gives initial phase shift Φ (at $t = 0$)



$$\begin{aligned} & A[\cos(\phi + 2\pi\nu t) + i\sin(\phi + 2\pi\nu t)] \\ &= A\exp[i(\phi + 2\pi\nu t)] \\ &\rightarrow A\exp(i\phi) \end{aligned}$$

It is generally convenient to represent a wave as a complex number, a vector in the complex plane, then

$$\exp(i\varphi) = \cos \varphi + i \sin \varphi \quad (\text{Euler's equation})$$

Complex numbers – a reminder

Complex numbers can be represented as a vector in the complex plane (Argand diagram), and (often) behave like vectors

$$z = a + ib = A \exp(i \varphi) = A(\cos \varphi + i \sin \varphi)$$

Amplitude: $|z| = A = \sqrt{a^2 + b^2}$

$$i^2 = -1$$

Phase: $\varphi = \tan^{-1}(b/a)$

Addition: add real and imaginary parts, like vectors

$$z_1 + z_2 = (a_1 + a_2) + i(b_1 + b_2)$$

Complex conjugate

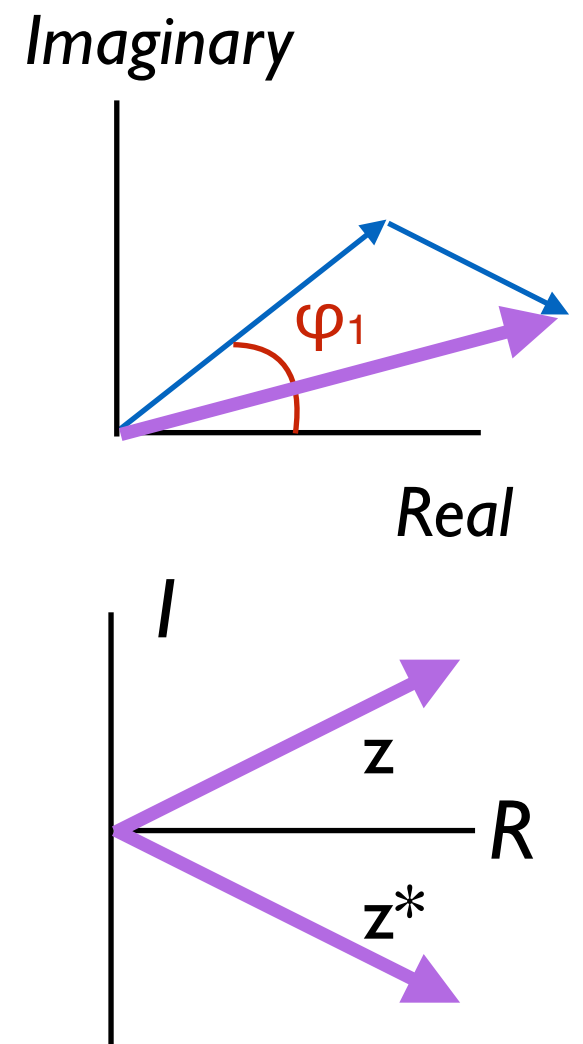
$$z = A \exp(i \varphi) = A (\cos \varphi + i \sin \varphi) = a + ib$$

$$z^* = A \exp(-i \varphi) = A (\cos \varphi - i \sin \varphi) = a - ib \quad \text{complex conjugate}$$

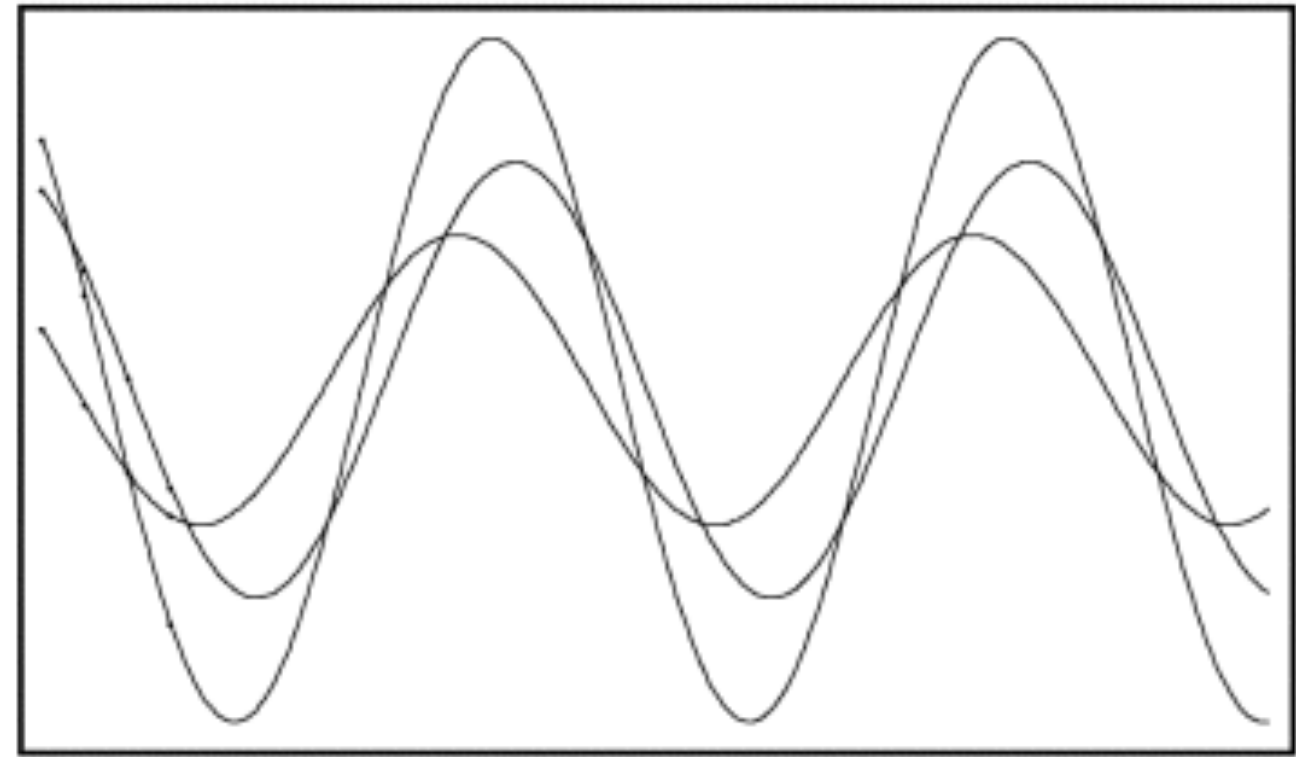
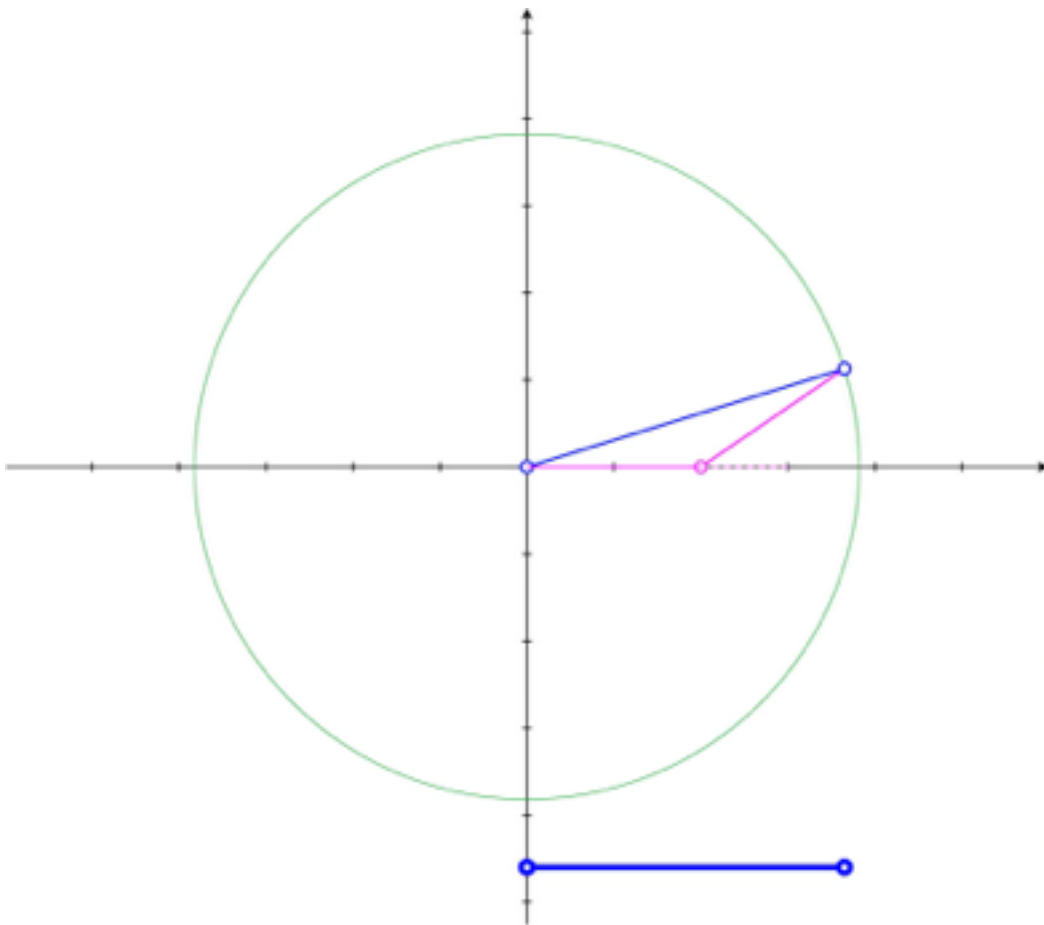
$$z + z^* = 2A \cos \varphi \quad \text{real}$$

$$z z^* = A^2 (\cos^2 \varphi - (i \sin \varphi)^2)$$

$$= A^2 \quad \text{since } i^2 = -1 \text{ and } \cos^2 \varphi + \sin^2 \varphi = 1$$



Adding two waves is equivalent to adding vectors
geometry is easier than trigonometry!



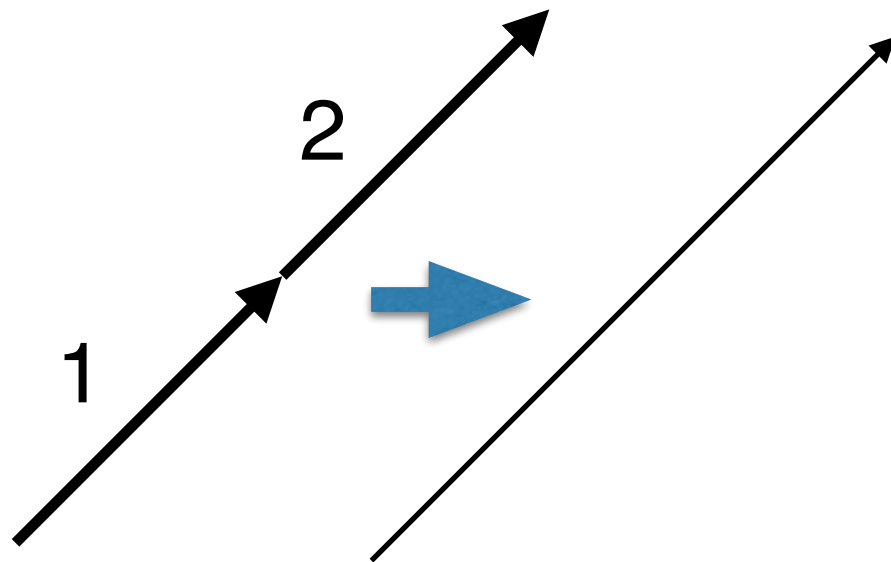
$$A \cos(\alpha + \varphi_1) + B \cos(\alpha + \varphi_2)$$

If the vectors rotate at the same speed (same wave frequency), then the vectors remain in the same relationship to each other and the addition is stable.

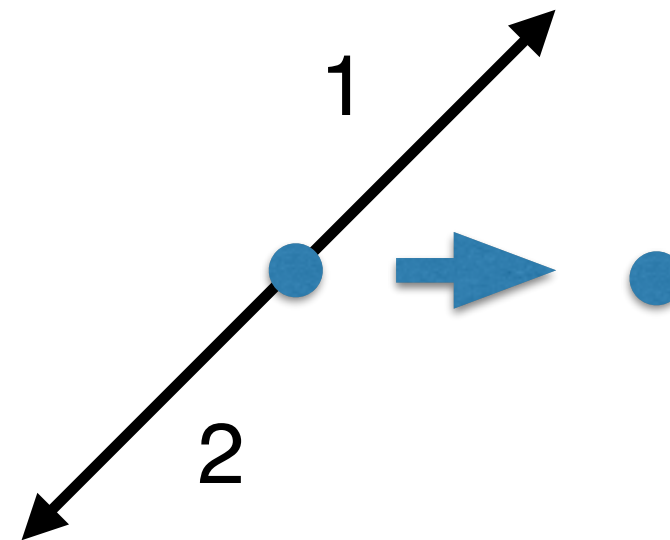
If they have different frequencies their relationship keeps changing

We only get time-stable interference with waves of the same frequency

So now we can add some waves to see if they interfere



In phase
– constructive interference



Out of phase
– destructive interference

... or something in between, depending on their relative phase

Two waves scattered from different points will add up in phase if their path lengths are the same or differ by a multiple of the wavelength

Geometry of diffraction

Vectors – a reminder

As well as complex numbers, we will be using vectors in real and reciprocal space. Vectors in **bold font** e.g. **p**

$$\mathbf{p} = \begin{bmatrix} x \\ y \\ z \end{bmatrix}$$

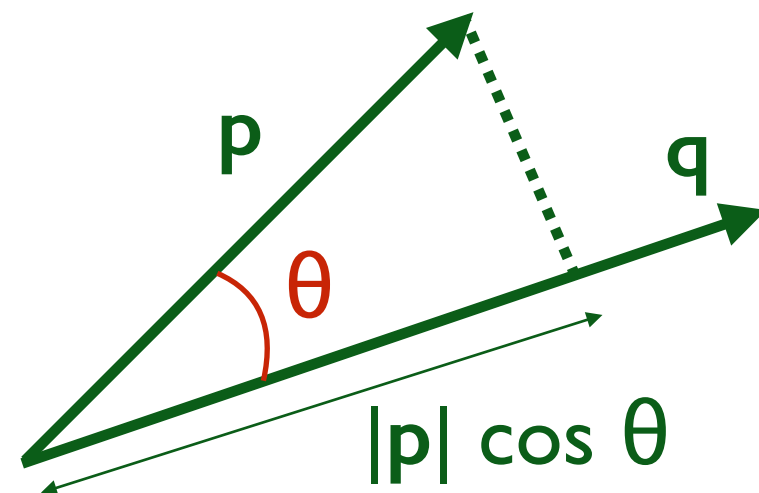
$$\mathbf{p}^T = (x \ y \ z)$$

transpose

for 3D vectors

Scalar product (dot product)

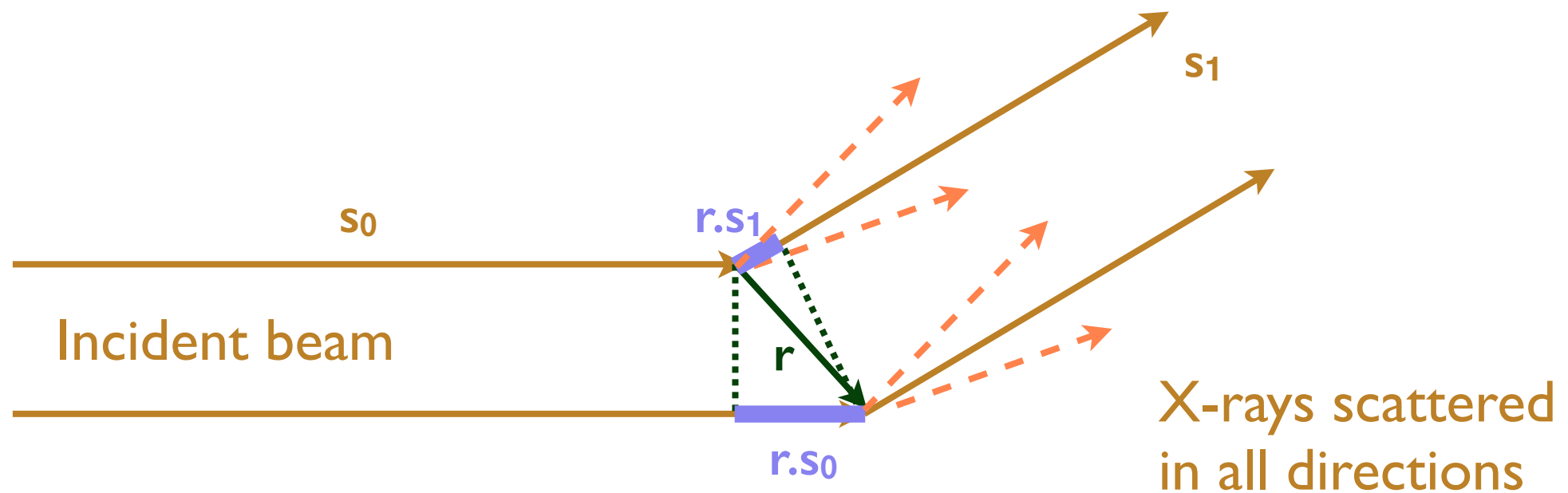
$$\begin{aligned} \mathbf{p} \cdot \mathbf{q} &= |\mathbf{p}| |\mathbf{q}| \cos \theta = \mathbf{q} \cdot \mathbf{p} \\ &= \mathbf{p}^T \mathbf{q} = \mathbf{q}^T \mathbf{p} \\ &= x_p x_q + y_p y_q + z_p z_q \end{aligned}$$



If one vector has unit length, e.g. $|\mathbf{q}| = 1$, then $\mathbf{p} \cdot \mathbf{q}$ is the projection of **p** on to **q**, $= |\mathbf{p}| \cos \theta$

Scattering from two electrons

... using vectors to calculate path difference ($\rightarrow$ phase difference)



Consider two electrons separated by vector r , illuminated by an X-ray beam in direction s_0

X-rays are scattered in all directions, but if we consider just those scattered in direction s_1 , then the path length difference is

$$\delta L = r \cdot s_1 - r \cdot s_0 = r \cdot (s_1 - s_0)$$

(for now with unit vectors $|s_0| = |s_1| = 1$)

Phase shift corresponding to path length difference $\delta L = \mathbf{r} \cdot (\mathbf{s}_1 - \mathbf{s}_0)$ for a wave with wavelength λ

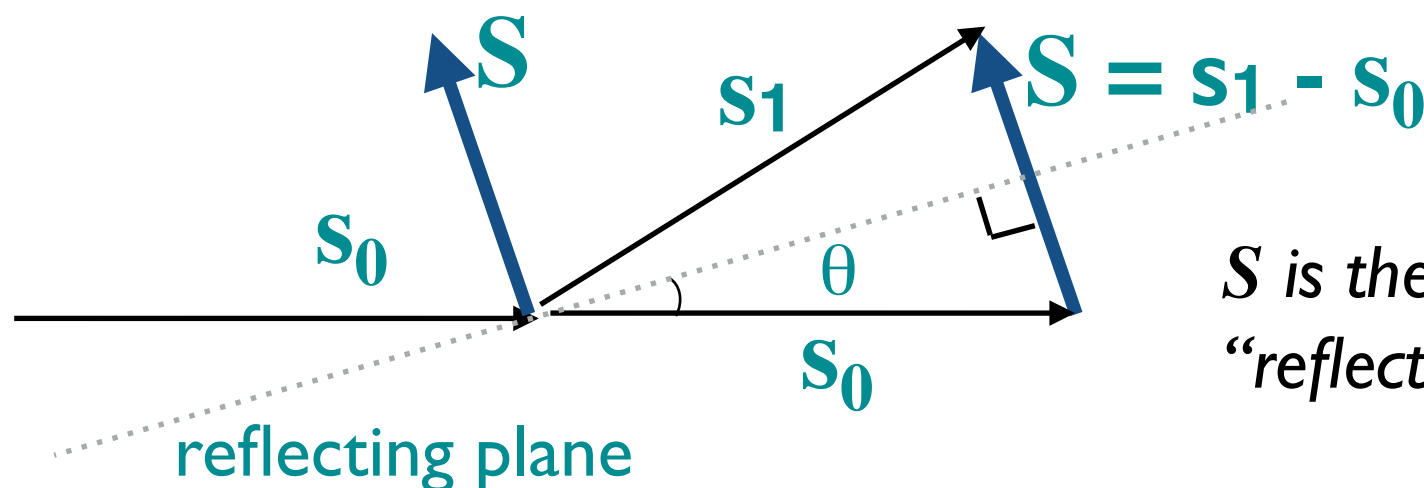
$$= 2\pi \text{ (path difference)} / \lambda$$

$$= 2\pi \delta L / \lambda = 2\pi \mathbf{r} \cdot (\mathbf{s}_1 - \mathbf{s}_0) / \lambda$$

It is convenient to make the length of the wave vectors $\mathbf{s}_0$ and $\mathbf{s}_1 = 1/\lambda$, and $|\mathbf{r}|$ in the same units as λ ie $|\mathbf{s}_0| = |\mathbf{s}_1| = 1/\lambda$

then we can write the phase shift $= 2\pi \mathbf{r} \cdot \mathbf{S}$ (dimensionless) where $\mathbf{S} = \mathbf{s}_1 - \mathbf{s}_0$

resultant wave $= \exp(2\pi i \mathbf{0} \cdot \mathbf{S}) + \exp(2\pi i \mathbf{r} \cdot \mathbf{S})$ ie the sum of scattering from each electron
 $= 1 + \exp(2\pi i \mathbf{r} \cdot \mathbf{S})$ since $\exp(0) = 1$



$\mathbf{S}$ is the perpendicular to an imaginary “reflecting plane” with $|\mathbf{S}| = 2 \sin \theta / \lambda$

$$|\mathbf{S}| = 2 |\mathbf{s}_1| \sin \theta = 2 \sin \theta / \lambda$$

(first hint of Bragg's Law)

Diffraction from many electrons

If we have multiple electrons j at positions $\mathbf{r}_j$ (in the same frame as $\mathbf{s}_0$, relative to an arbitrary origin), then the total scattering in the direction $\mathbf{s}_1 = \mathbf{S} + \mathbf{s}_0$ is

$$F(\mathbf{S}) = \sum_j (1e) \exp(2\pi i \mathbf{r}_j \cdot \mathbf{S})$$

this is the *Structure Factor*, a complex number (ie has amplitude and phase), in units of electrons e , which we often omit and forget

For a continuous distribution of electron density $\rho(\mathbf{r})$ we have an integral

$$F(\mathbf{S}) = \int \rho(\mathbf{r}) \exp(2\pi i \mathbf{r} \cdot \mathbf{S}) d\mathbf{r}$$

This is a Fourier transform, and can be inverted

$$\rho(\mathbf{r}) = \int F(\mathbf{S}) \exp(-2\pi i \mathbf{r} \cdot \mathbf{S}) d\mathbf{S}$$

Position vector $\mathbf{r}$ is in *real space*

Diffraction vector $\mathbf{S}$ is in *reciprocal space (Fourier space)*

$$F(\mathbf{S}) = \int \rho(\mathbf{r}) \exp(2\pi i \mathbf{r} \cdot \mathbf{S}) d\mathbf{r}$$

$$\rho(\mathbf{r}) = \int F(\mathbf{S}) \exp(-2\pi i \mathbf{r} \cdot \mathbf{S}) d\mathbf{S}$$

$F(\mathbf{S})$ for each position in reciprocal space $\mathbf{S}$ depends on $\rho(\mathbf{r})$ for all positions $\mathbf{r}$ in real space

$\rho(\mathbf{r})$ for each position in real space $\mathbf{r}$ depends on $F(\mathbf{S})$ for all positions $\mathbf{S}$ in reciprocal space

If we know the positions of all electrons (atoms) $\rho(\mathbf{r})$, then we can calculate the diffraction pattern $F(\mathbf{S})$

If we know the diffraction pattern $F(\mathbf{S})$, then we can calculate the electron density $\rho(\mathbf{r})$ at all positions

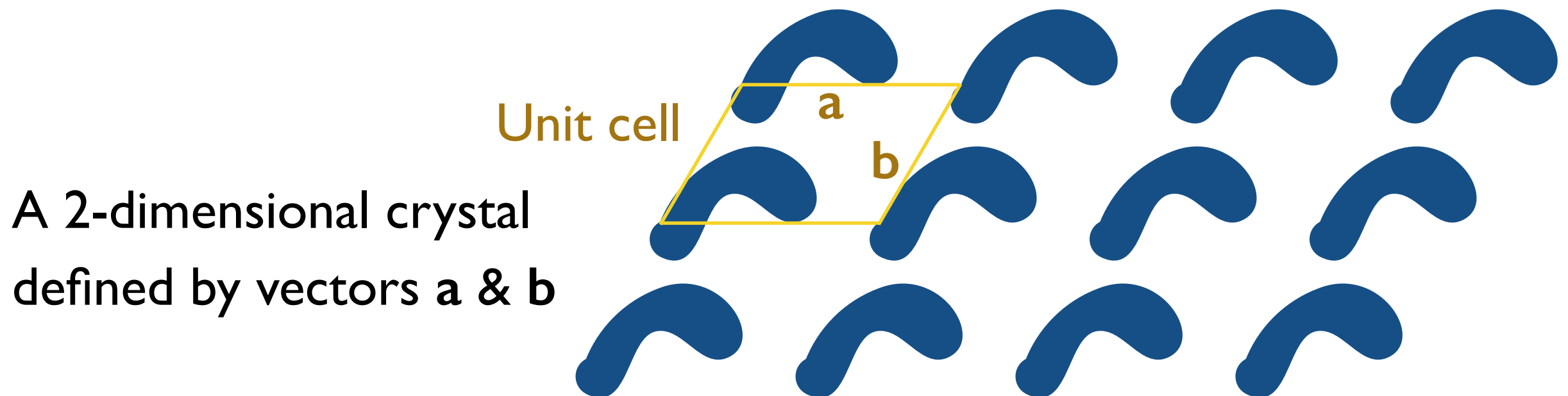
BUT $F(\mathbf{S})$ is complex, and we only measure its amplitude $|F(\mathbf{S})|$ (or actually intensity, $I \propto |F|^2$)

Remember we would need a time resolution of $< 10^{-19}$ seconds to measure phase

Crystals

Crystals

A crystal is a regular (3-dimensional) array of “things” (molecules)



Xrays interact weakly with matter (electrons), and destroy molecules, so we cannot visualise (yet) individual molecules before they are destroyed (ask the free-electron laser people!)

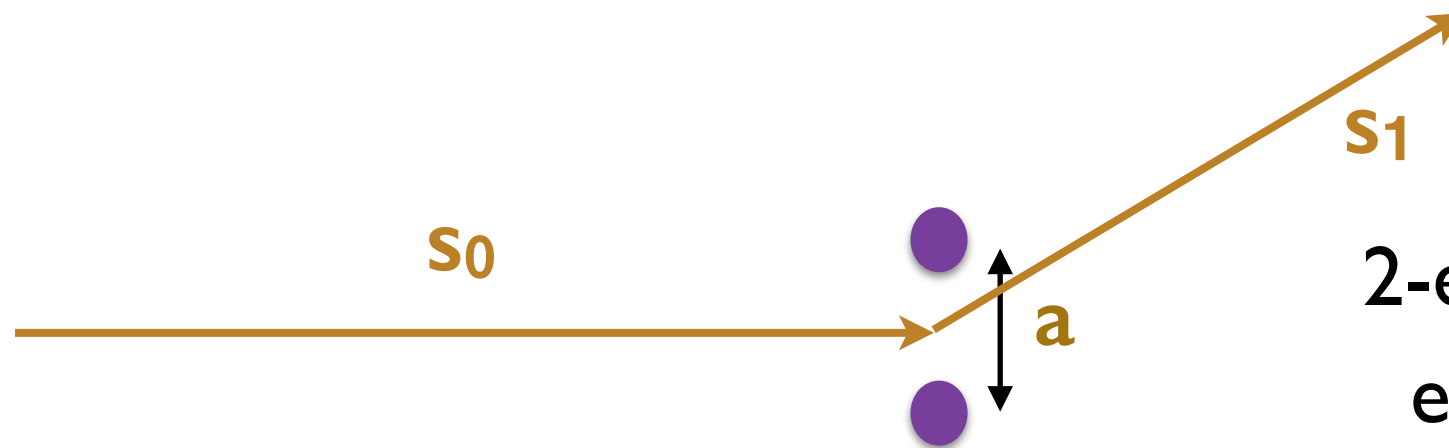
(electrons interact more strongly, so in cryo-EM we can almost visualise single molecules, but need to average many to get good images)

A crystal is an amplifier to make the signal/noise useful

eg crystal $100\text{ }\mu\text{m}$ (10^{-4} M) cube, cell $100\text{\AA}$ ($10\text{nm} = 10^{-8}\text{ M}$)

contains 10^{12} unit cells

Diffraction from a 1-dimensional crystal of single electrons



Same as scattering from 2 electrons, as before

2-electron case (Young's slits)
electrons at positions
 $r - a/2$ and $r + a/2$

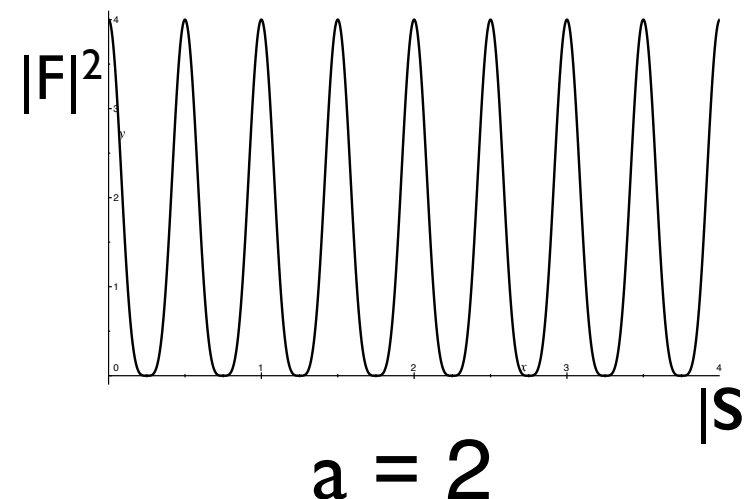
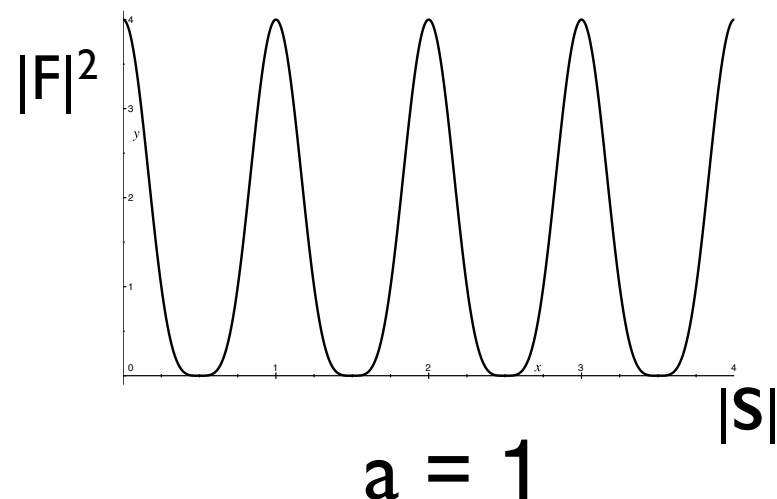
$$F(\mathbf{S} = \mathbf{s}_1 - \mathbf{s}_0) = \exp(2\pi i (\mathbf{r} - a/2) \cdot \mathbf{S}) + \exp(2\pi i (\mathbf{r} + a/2) \cdot \mathbf{S})$$

replace vectors by scalars, $r = 0$, $a = a$, $\mathbf{S} = |\mathbf{S}|$ (for 1-dimensional case)

$$F(\mathbf{S}) = \exp(2\pi i -a/2 |\mathbf{S}|) + \exp(2\pi i a/2 |\mathbf{S}|) = 2 \cos(2\pi a/2 |\mathbf{S}|) / 2$$

$$\text{Intensity} \propto |F|^2 = F F^* = \cos^2(2\pi a/2 |\mathbf{S}|) \quad (\text{imaginary sin terms disappear})$$

$$|\mathbf{S}| = 2 \sin \theta / \lambda$$



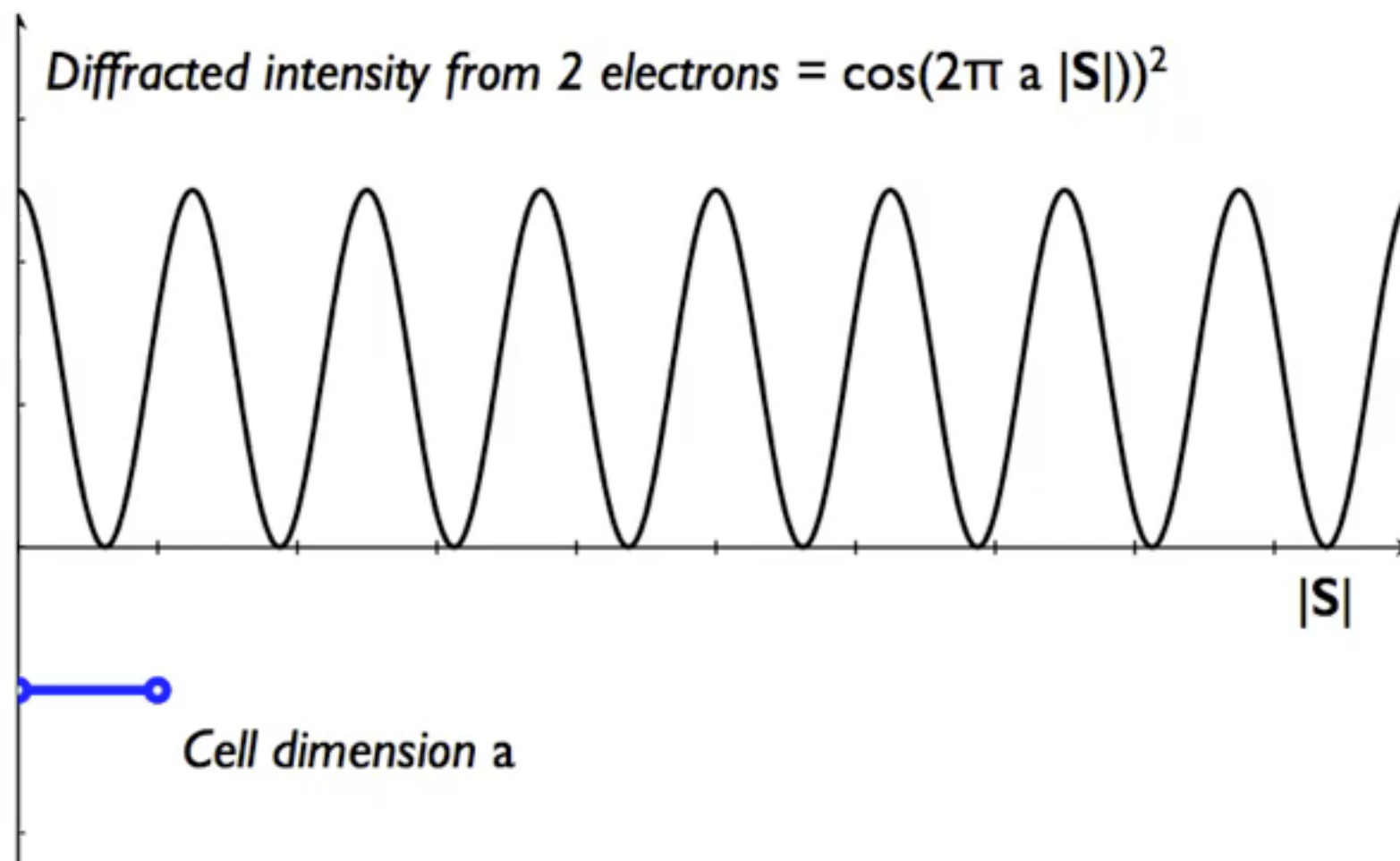
as the cell length a increases,
the spacing of the peaks on $|\mathbf{S}|$
decreases

$\mathbf{S}$ is in *reciprocal space*

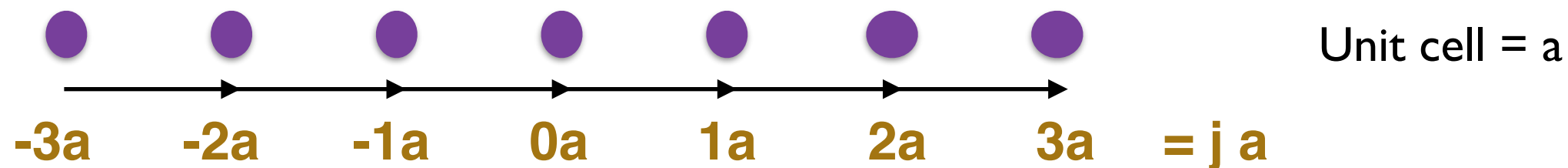
Diffraction from a 1-dimensional crystal of single electrons

$$\text{Intensity} \propto |F|^2 = F F^* = 4 \cos^2(2\pi a |S|)$$

As the cell length a increases, the spacing of the peaks on $|S|$ decreases
 S is in *reciprocal space*

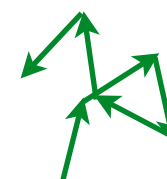


1D crystal (diffraction grating)



$$F(\mathbf{S}) = \sum_{j=-n}^n \exp(2\pi i (j a |\mathbf{S}|)) \quad \text{Structure factor for } 2n+1 \text{ unit cells}$$

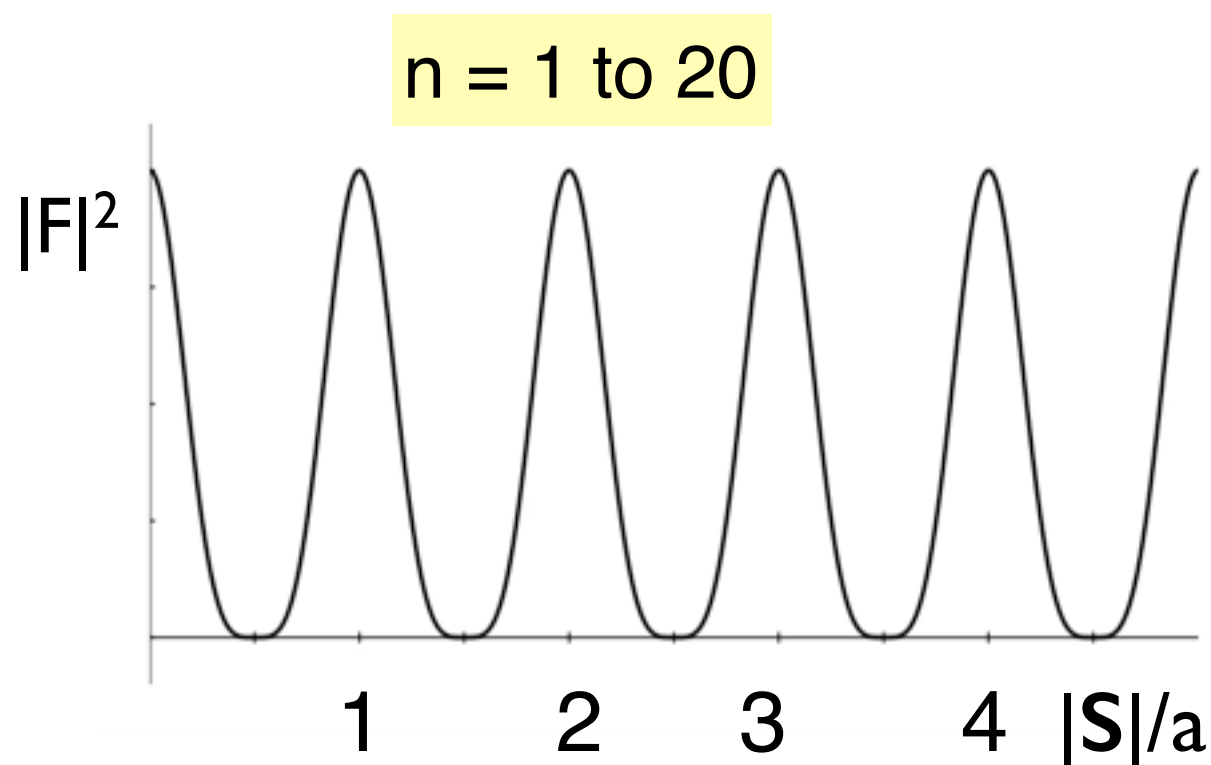
$$\text{Intensity} = |F|^2 = F F^* = \left(\sum_{j=-n}^n \cos(2\pi j a |\mathbf{S}|) \right)^2 / (2n+1)^2$$



Random



In phase



As number of unit cells increases, intensity becomes more concentrated where $a \cdot |\mathbf{S}|$ is an integer where the contributions add in phase

$$h = a \cdot \mathbf{S}$$

$$\mathbf{S} = h \mathbf{a}^*$$

$\mathbf{a}^*$ defines a *reciprocal lattice*

$$\mathbf{a} \cdot \mathbf{a}^* = 1$$

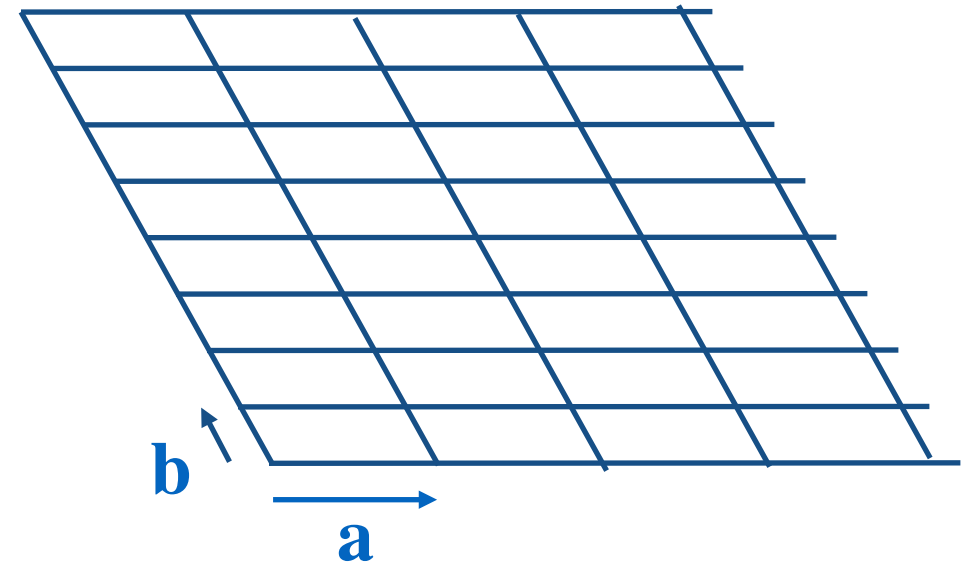
$$|\mathbf{a}^*| = 1/|\mathbf{a}| \quad (\text{for e.g. 1D case})$$

Three dimensional crystals

A 3-dimensional crystal is defined by three vectors, $\mathbf{a}$, $\mathbf{b}$, $\mathbf{c}$

The coordinates of a general unit cell relative to a fixed origin is given by

$\mathbf{r}_{uvw} = u \mathbf{a} + v \mathbf{b} + w \mathbf{c}$ where u, v, w are integers



so total scattering from one point in each cell is given by

$$\begin{aligned} \mathbf{F}(\mathbf{S}) &= \sum_u \sum_v \sum_w \exp(2\pi i \mathbf{r}_{uvw} \cdot \mathbf{S}) \\ &= \sum_u \exp(2\pi i u \mathbf{a} \cdot \mathbf{S}) \sum_v \exp(2\pi i v \mathbf{b} \cdot \mathbf{S}) \sum_w \exp(2\pi i w \mathbf{c} \cdot \mathbf{S}) \end{aligned}$$

As in the 1-dimensional case, $|\mathbf{F}(\mathbf{S})|$ is only non-zero for large numbers of unit cells if $\mathbf{a} \cdot \mathbf{S}$, $\mathbf{b} \cdot \mathbf{S}$, and $\mathbf{c} \cdot \mathbf{S}$ are all integral

ie $\mathbf{a} \cdot \mathbf{S} = h$ $\mathbf{b} \cdot \mathbf{S} = k$ $\mathbf{c} \cdot \mathbf{S} = l$

These are the Laue equations

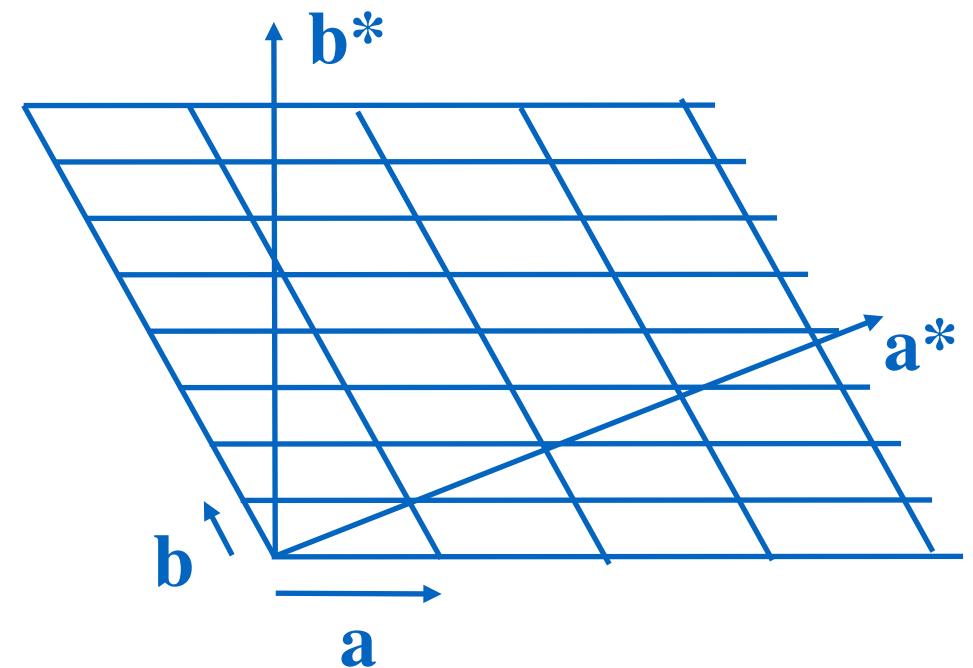
The Laue equations define a **reciprocal lattice** of allowed values of **S**, based on the “reciprocal” vectors **a***, **b***, **c***

$$\mathbf{S} = h \mathbf{a}^* + k \mathbf{b}^* + l \mathbf{c}^*$$

$$\mathbf{a} \cdot \mathbf{a}^* = 1 \quad \mathbf{a} \cdot \mathbf{b}^* = 0$$

$$\mathbf{b} \cdot \mathbf{b}^* = 1 \quad \mathbf{b} \cdot \mathbf{c}^* = 0$$

$$\mathbf{c} \cdot \mathbf{c}^* = 1 \quad \mathbf{c} \cdot \mathbf{a}^* = 0$$



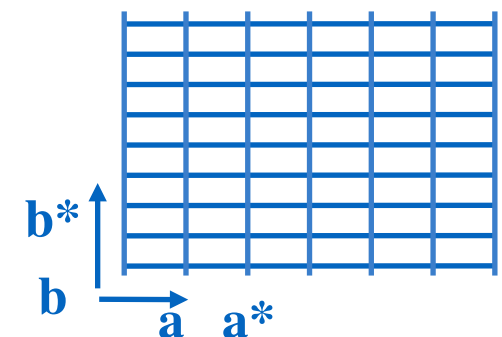
The reciprocal lattice vectors are perpendicular to the *planes* of the real crystal lattice, since the scattering can be considered as coming from reflecting planes (the Bragg planes)

$$\mathbf{a}^* = (\mathbf{b} \times \mathbf{c})/V \quad \mathbf{b}^* = (\mathbf{c} \times \mathbf{a})/V \quad \mathbf{c}^* = (\mathbf{a} \times \mathbf{b})/V \quad V = \text{cell volume}$$

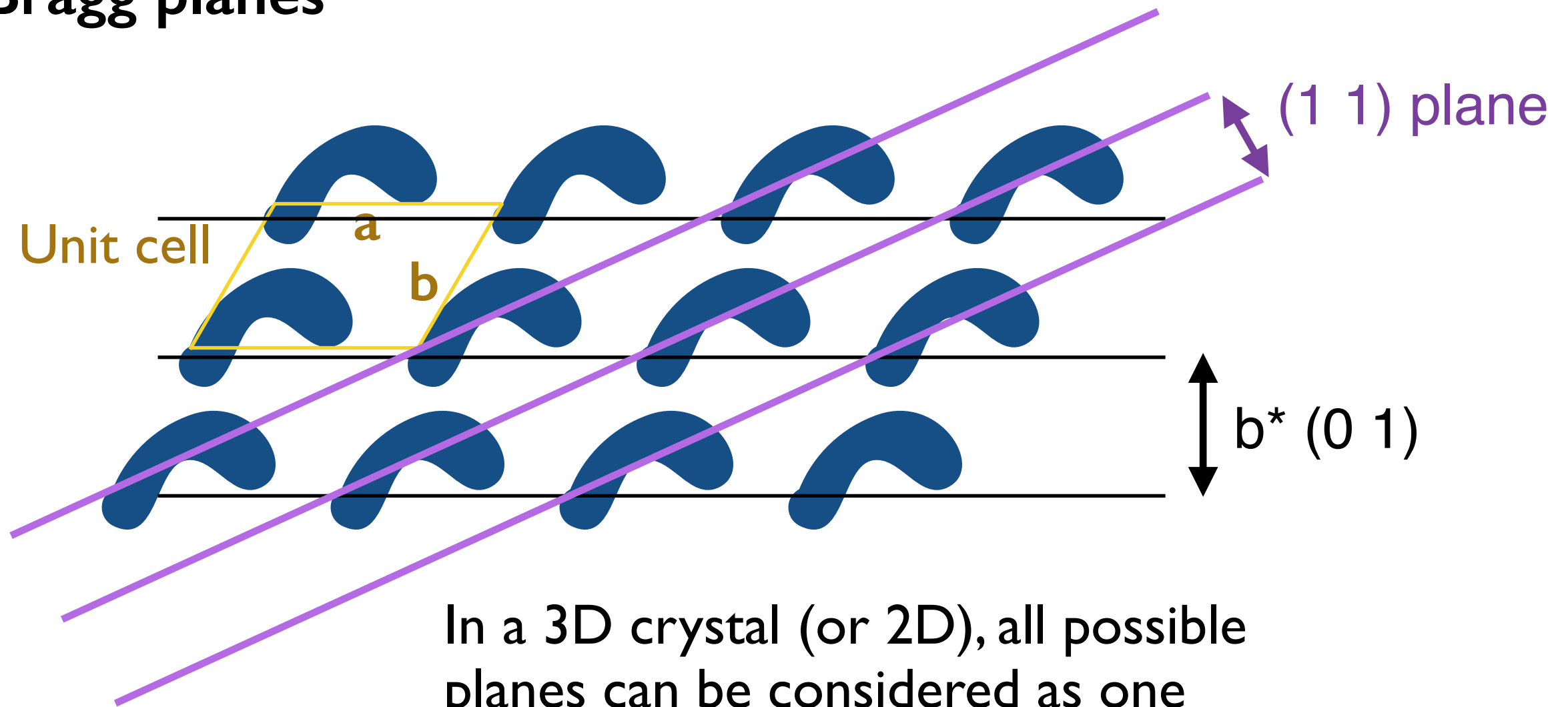
$|\mathbf{a}^*|$ is the spacing between the (bc) planes, $|\mathbf{b}^*|$ between the (ca) planes, $|\mathbf{c}^*|$ between the (ab) planes

For the special case of an orthogonal lattice (only),

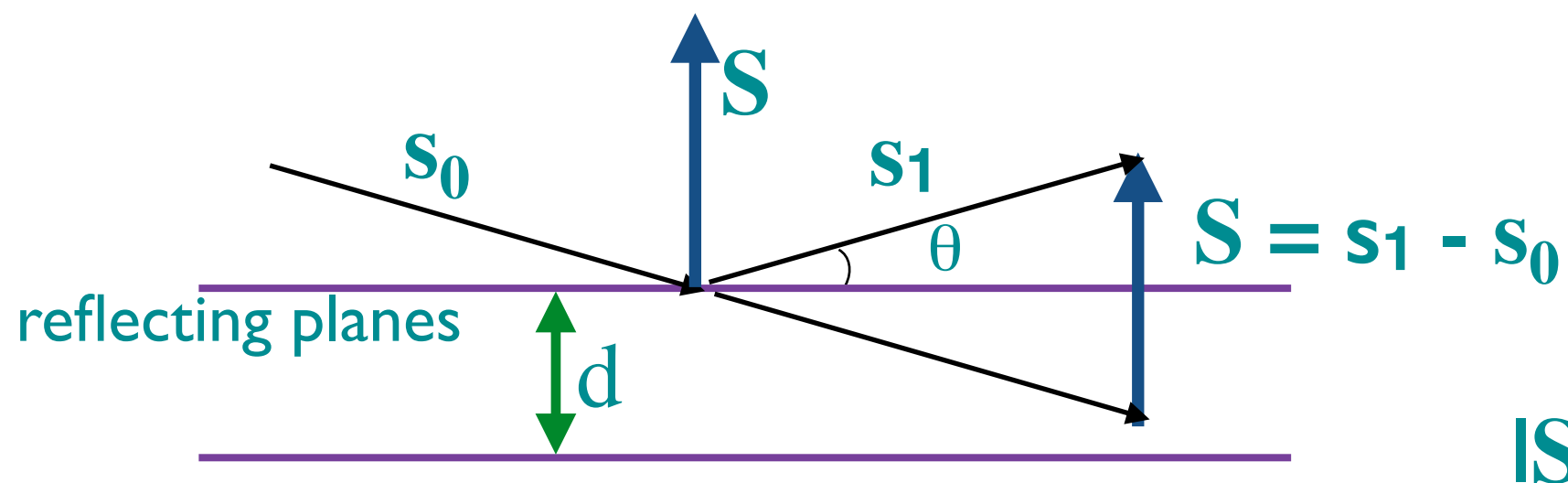
$$|\mathbf{a}^*| = 1/|\mathbf{a}|, \quad |\mathbf{b}^*| = 1/|\mathbf{b}|, \quad |\mathbf{c}^*| = 1/|\mathbf{c}|$$



Bragg planes



In a 3D crystal (or 2D), all possible planes can be considered as one dimensional diffraction gratings



Bragg equation:

$$|S| = 1/d = 2\sin\theta/\lambda$$

$$|S| = 1/d$$

Diffraction from a molecular crystal

For a continuous distribution of electron density $\rho(\mathbf{r})$ we have an integral

$$F(\mathbf{S}) = \int \rho(\mathbf{r}) \exp(2\pi i \mathbf{S} \cdot \mathbf{r}) d\mathbf{r}$$

but in a crystal we know that $\rho(\mathbf{r})$ repeats on a real space lattice defined by $\mathbf{a}, \mathbf{b}, \mathbf{c}$, and $\mathbf{S}$ may only take values defined by the reciprocal space lattice $\mathbf{a}^*, \mathbf{b}^*, \mathbf{c}^*$, so we only need to sum over the contents of one unit cell

It is convenient to write the positions $\mathbf{r}$ as fractions of the unit cell vectors and the reciprocal space vector with the Miller indices hkl and the reciprocal space vectors

$$\mathbf{r} = x\mathbf{a} + y\mathbf{b} + z\mathbf{c}$$

$$\mathbf{S} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$$

$$\text{then } \mathbf{S} \cdot \mathbf{r} = hx (\mathbf{a} \cdot \mathbf{a}^*) + ky (\mathbf{b} \cdot \mathbf{b}^*) + lz (\mathbf{c} \cdot \mathbf{c}^*)$$

$$= hx + ky + lz = h.x \quad \text{since } \mathbf{a} \cdot \mathbf{a}^* = 1 \text{ etc}$$

$$F(\mathbf{h}) = \int \rho(\mathbf{x}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}) d\mathbf{x}$$

Diffraction from a molecular crystal

$$F(\mathbf{h}) = \int \rho(\mathbf{x}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}) d\mathbf{x}$$

Summing over atoms:

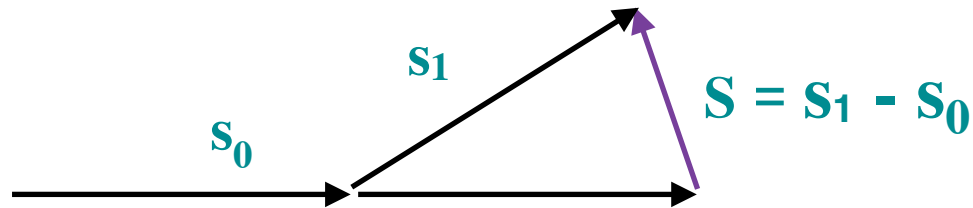
each atom has an approximately spherical distribution of electrons, so we can substitute values of $\rho(\mathbf{x}_{\text{atom}})$ into $\rho(\mathbf{x})$, then integrate across each atom and sum over atoms at positions $\mathbf{x}_j$

We then end up separating the diffraction of each atom type from its position $\mathbf{x}_j$

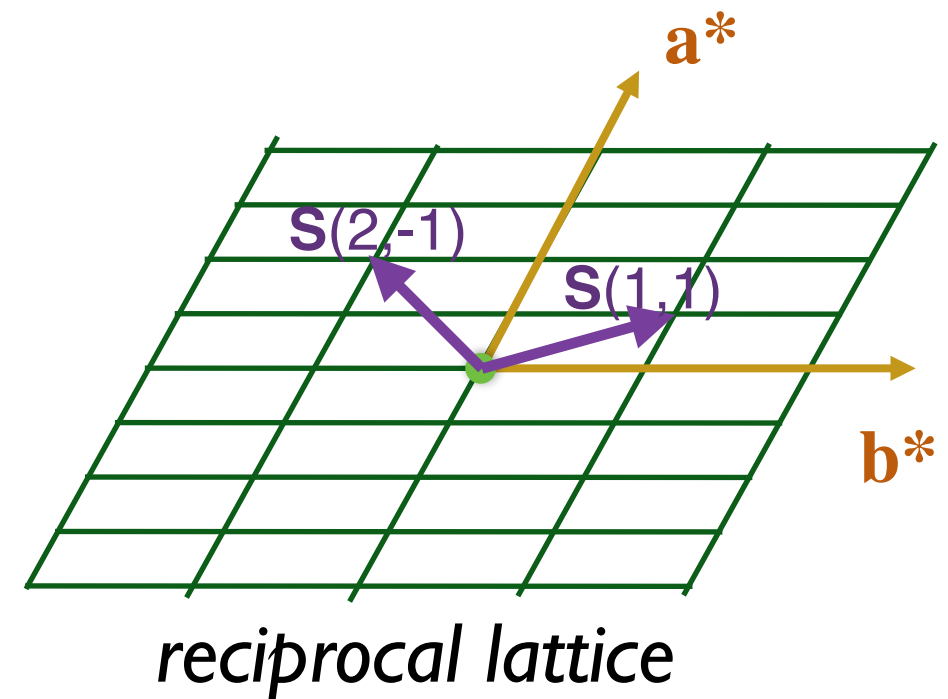
$$F(\mathbf{h}) = \sum_j f_j(|\mathbf{S}|) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j)$$

where $f_j(|\mathbf{S}|)$ expresses the scattering for all the electrons in atom j (usually tabulated or recalculated for each atom type, known as *form factors*)

Reciprocal Lattice and the Ewald Sphere Construction

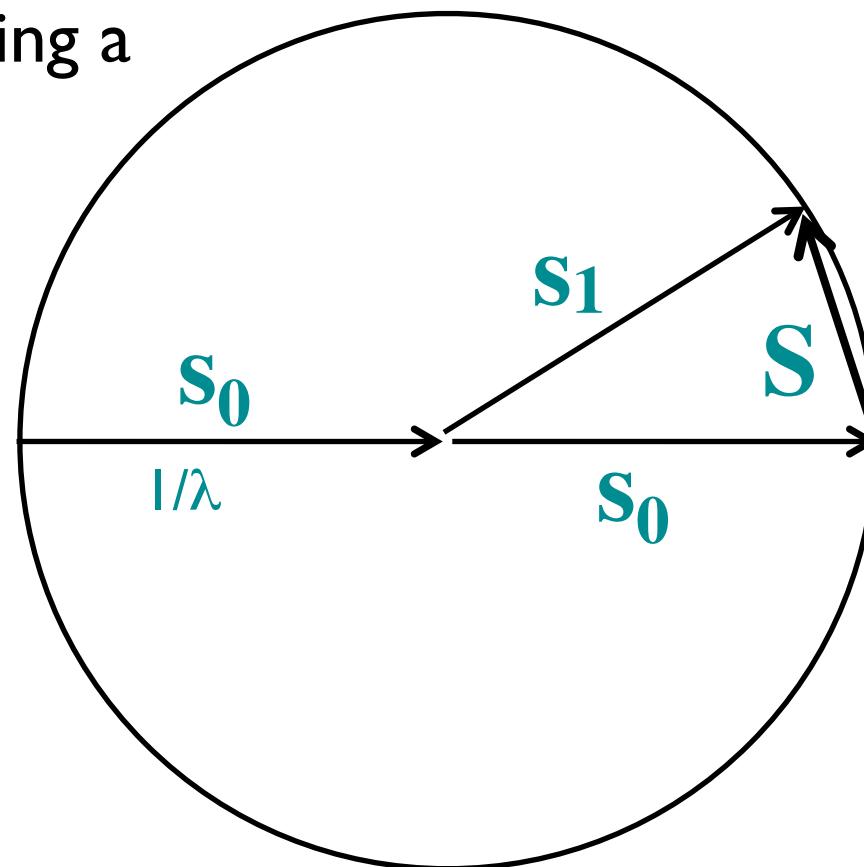


The possible values of S delineate the *reciprocal lattice*

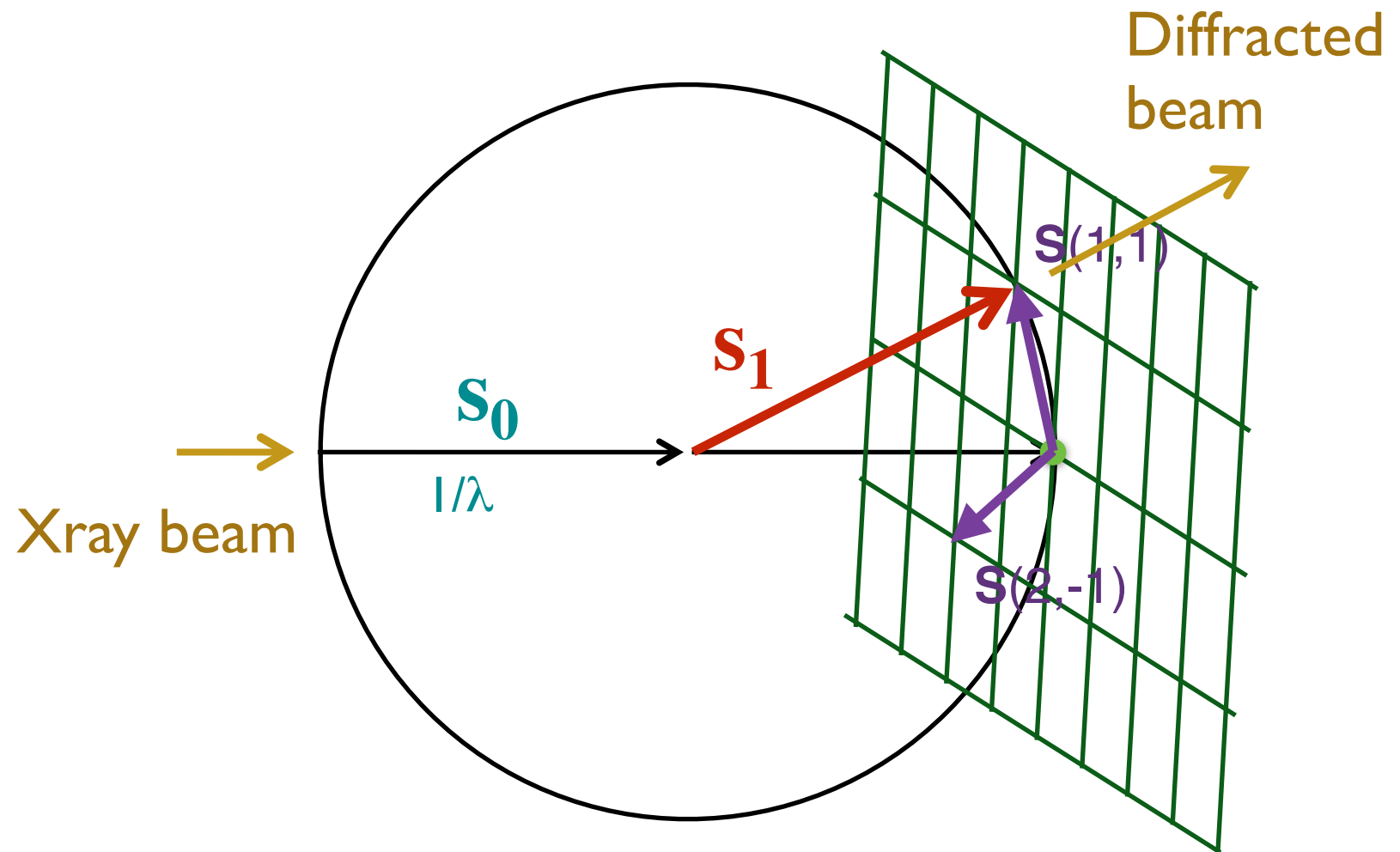


$$S = h a^* + k b^* + l c^*$$

Because s_0 and s_1 have the same length ($1/\lambda$), we can generalise this diagram by drawing a sphere of radius $|s_0| = |s_1| = 1/\lambda$



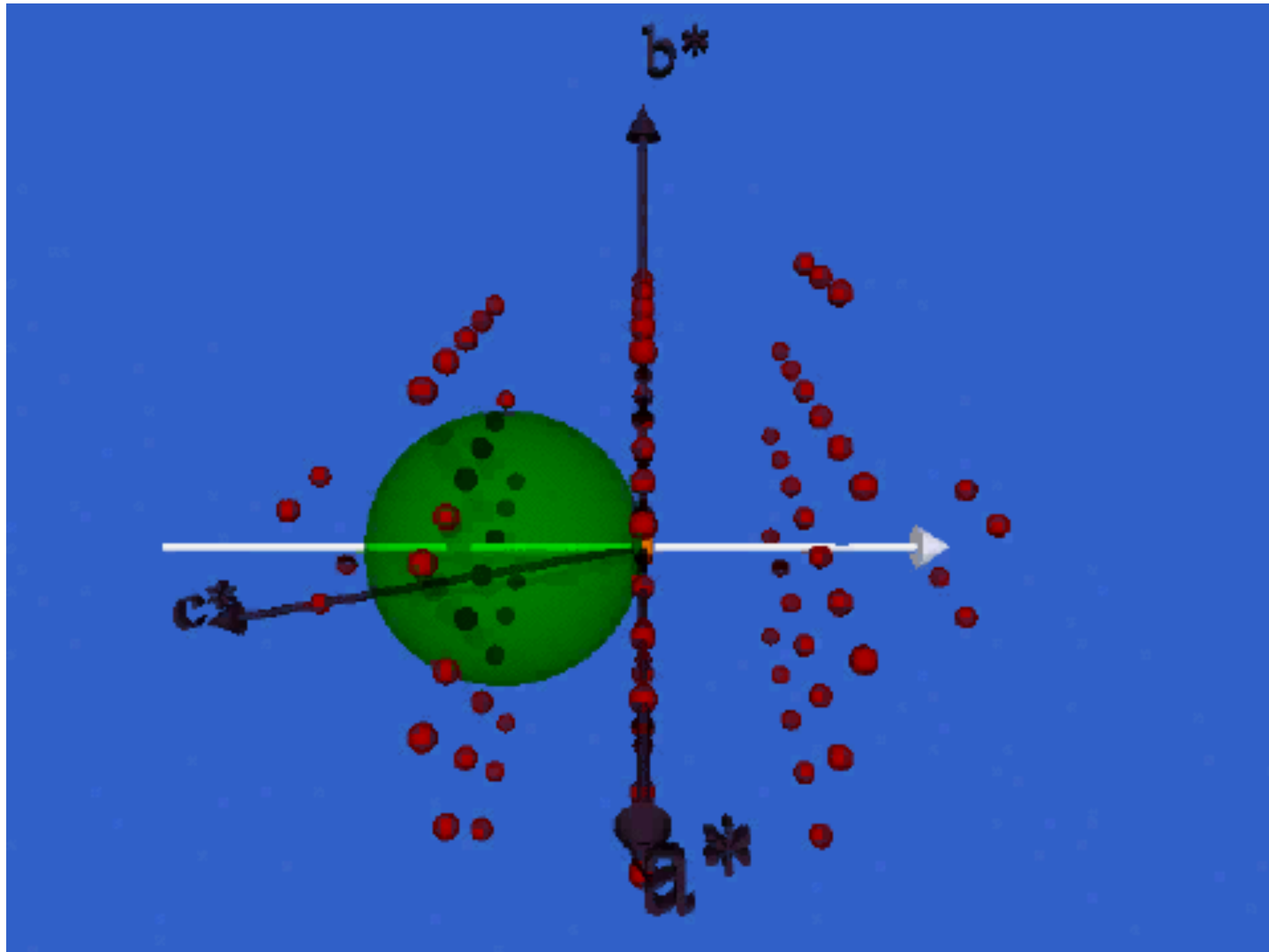
As we rotate the crystal, the reciprocal lattice rotates in the same way, since the diffraction vectors $\mathbf{S}$ are normals to the diffracting planes



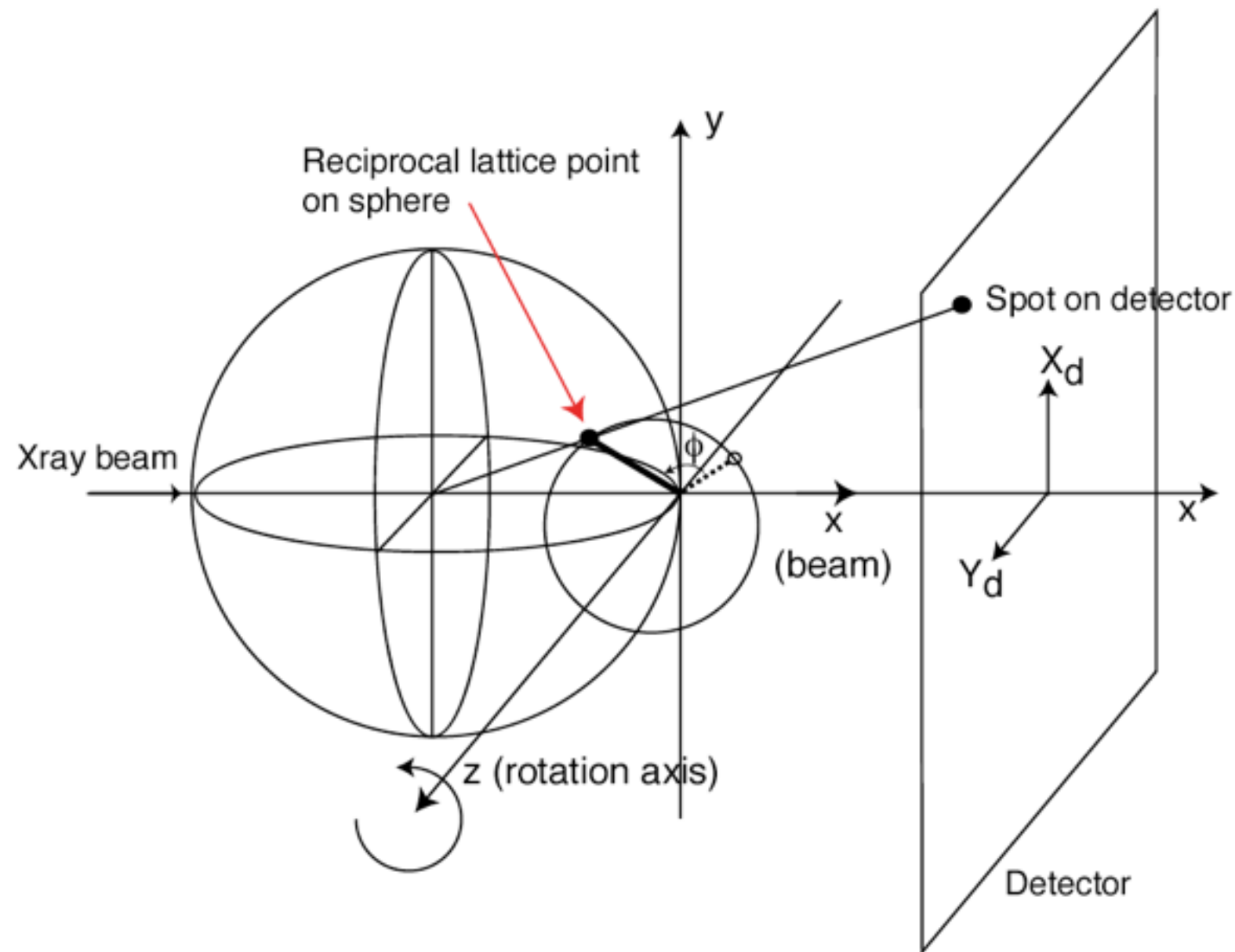
Whenever a reciprocal lattice point passes through the Ewald sphere, a diffracted beam is produced

As the crystal rotates, so does the the reciprocal lattice

As a reciprocal lattice point passes through the Ewald sphere, a diffracted beam is observed along the line from the sphere centre to the reciprocal lattice point



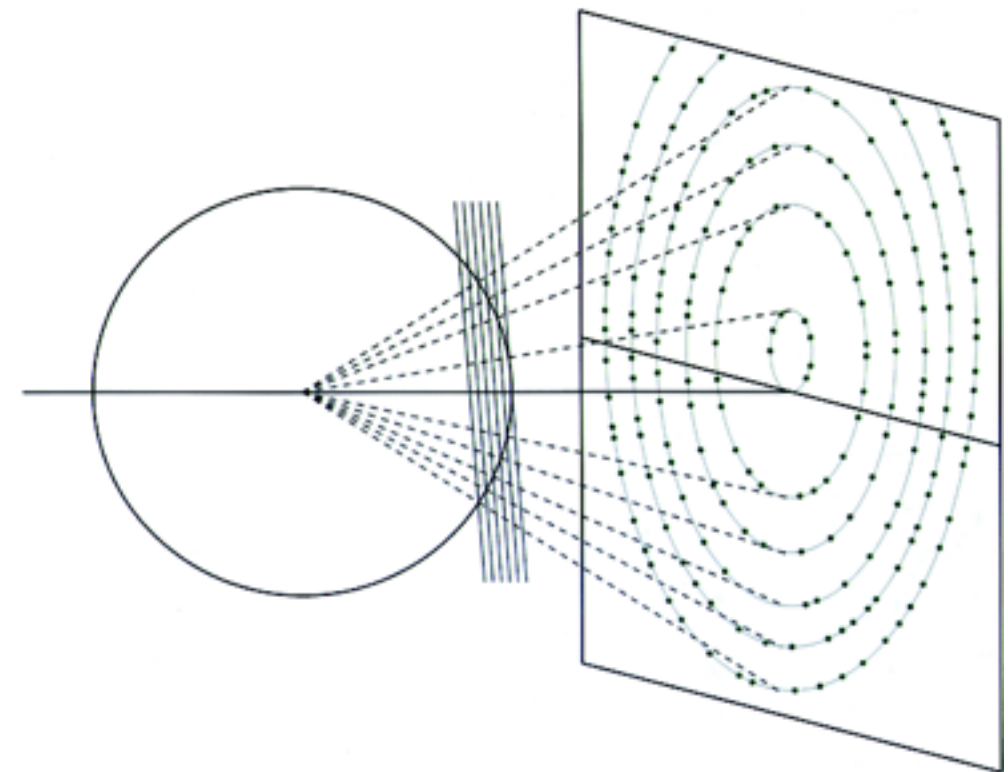
The part of the reciprocal lattice which intersects the sphere is projected on to the detector



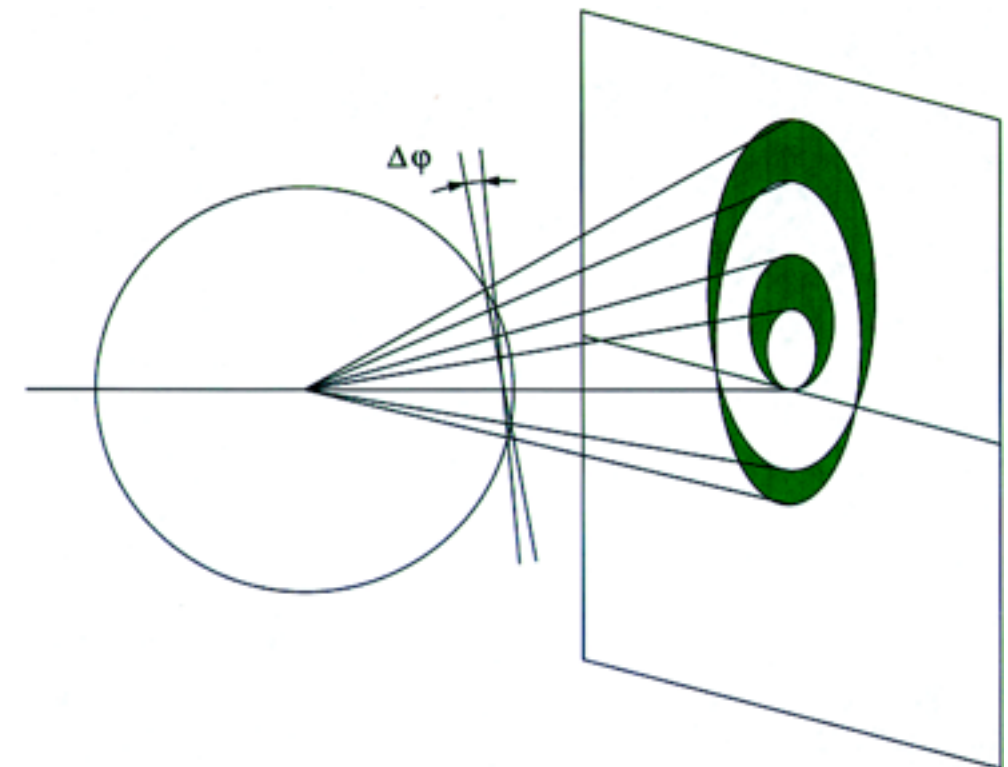
As the crystal rotates, each lattice point in turn passes through the sphere, and a spot is recorded on the detector

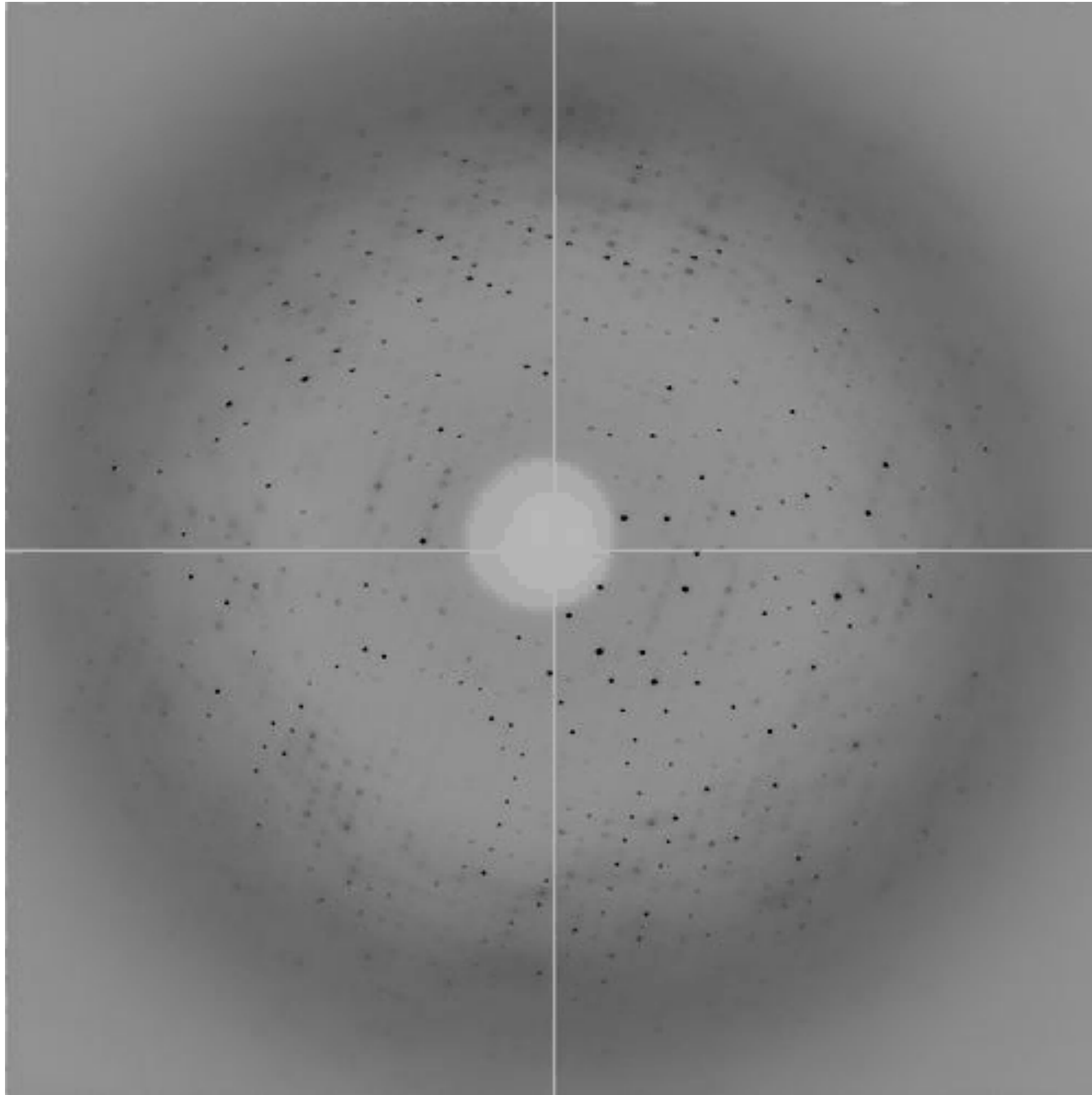
The appearance of diffraction images

Reciprocal lattice points lie in layers (planes). Each plane intersects the sphere in a circle, and the spots projected on the detector lie in ellipses



If the crystal is rotated through a small angle, each circle is broadened into a *lune*. All the spots in a lune belong to one plane of the reciprocal lattice (not necessarily a principal plane)





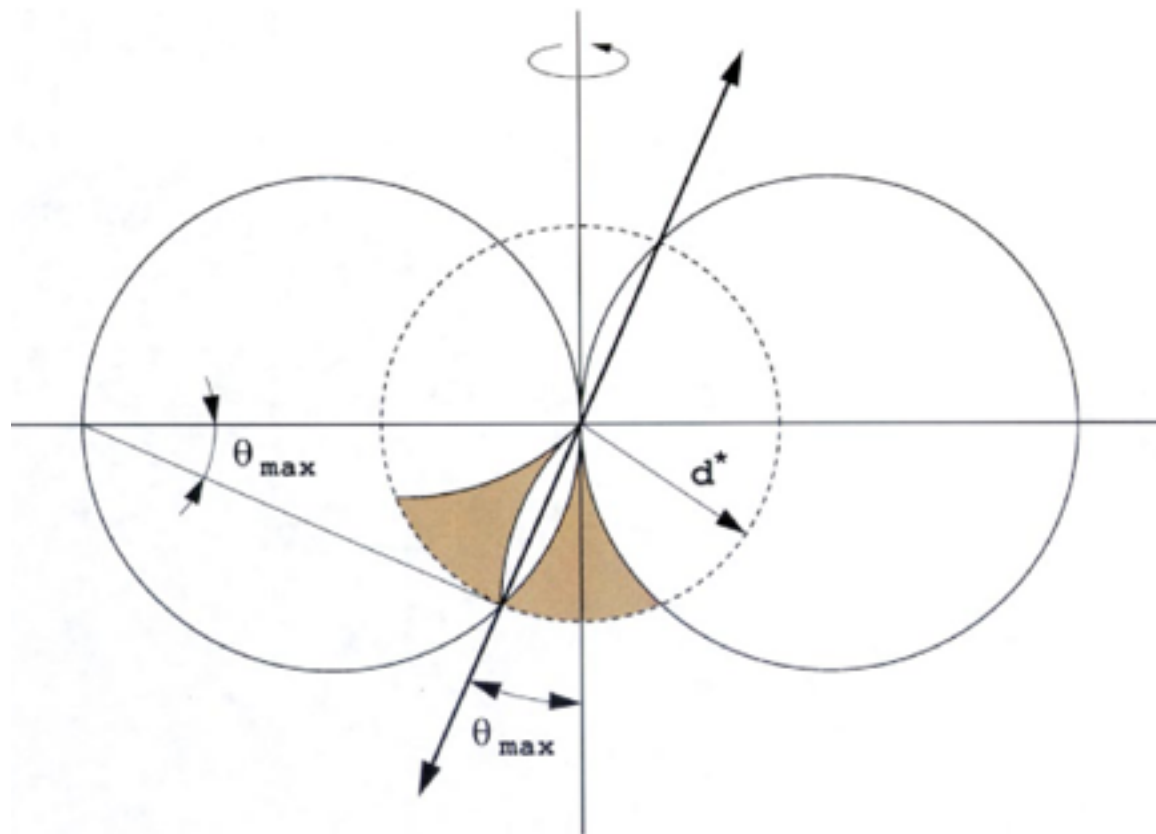
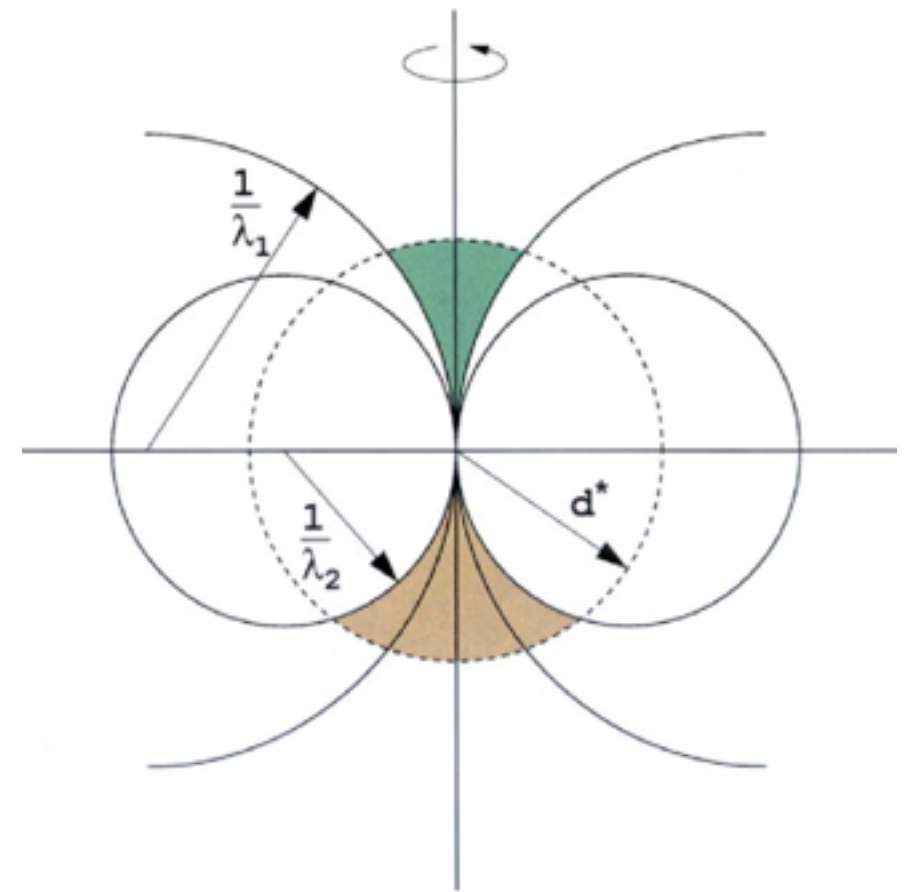
The full diffraction pattern (ie the reciprocal lattice) is 3-dimensional, and we want to measure the whole sphere to the maximum resolution (radius) available.

We slice the 3D reciprocal space into narrow rotation ranges to sample it (typically around 0.1° with modern detectors)

The blind region

Diffraction vectors close to the rotation axis will never pass through the sphere, even in a 360° rotation

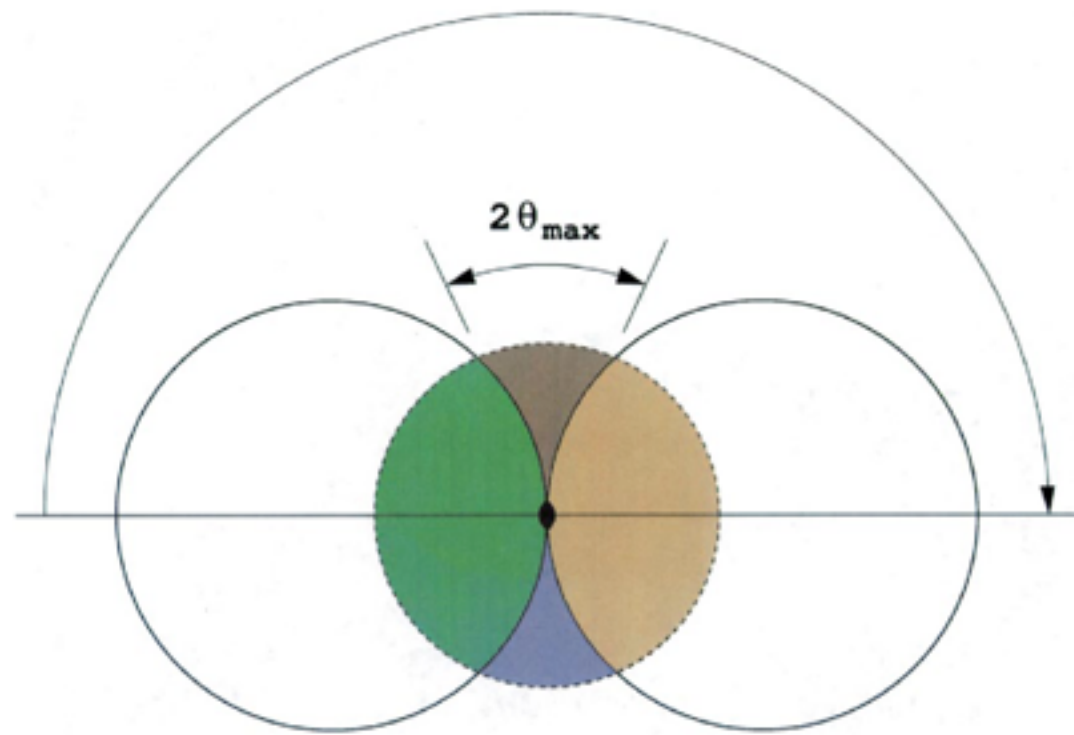
The blind region is smaller for short wavelengths, as the Ewald sphere is flatter



The blind region may be filled in by collecting a second set of data, offsetting the crystal by at least $\theta_{\max}$ or by symmetry (except in P1)

If there is symmetry, offsetting from an axis can remove or reduce the blind region for a single setting

Completeness: total rotation range and the blind region



In a rotation of 180° above, the lower boundary of the initial sphere sweeps out the volume coloured green & the upper boundary the light brown part. The dark brown part is measured twice, and the blue part not at all

To use the Ewald sphere construction to understand which parts of reciprocal space are measured, it is easier to fix the “resolution sphere” of all reciprocal lattice points within a maximum resolution, and to rotate the Ewald sphere. The region collected is the volume swept out by the leading and trailing surfaces of the sphere

Because of Friedel’s law, this dataset is complete (apart from the *blind region*), but if complete anomalous differences are required, then $180^\circ + 2\theta_{\max}$ is required (unless there is symmetry)

Note that tiled detectors lose completeness in the gaps
~8% for *Pilatus 6M*

Symmetry of diffraction: Friedel's law

In the absence of *anomalous scattering*, the electron density $\rho(\mathbf{r})$ is real, not complex

$$z = \exp(i \varphi) = \cos \varphi + i \sin \varphi$$

$$z^* = \exp(-i \varphi) = \cos \varphi - i \sin \varphi \quad \text{complex conjugate}$$

$$z + z^* = 2 \cos \varphi \quad \text{real}$$

$$F(\mathbf{h}) = \sum_j f_j(\mathbf{h}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j) \quad F(-\mathbf{h}) = \sum_j f_j(\mathbf{h}) \exp(2\pi i -\mathbf{h} \cdot \mathbf{x}_j)$$

$$\text{ie } F(-\mathbf{h}) = F(\mathbf{h})^*$$

$F(\mathbf{h})$ and $F(-\mathbf{h})$ are related by a centre of symmetry

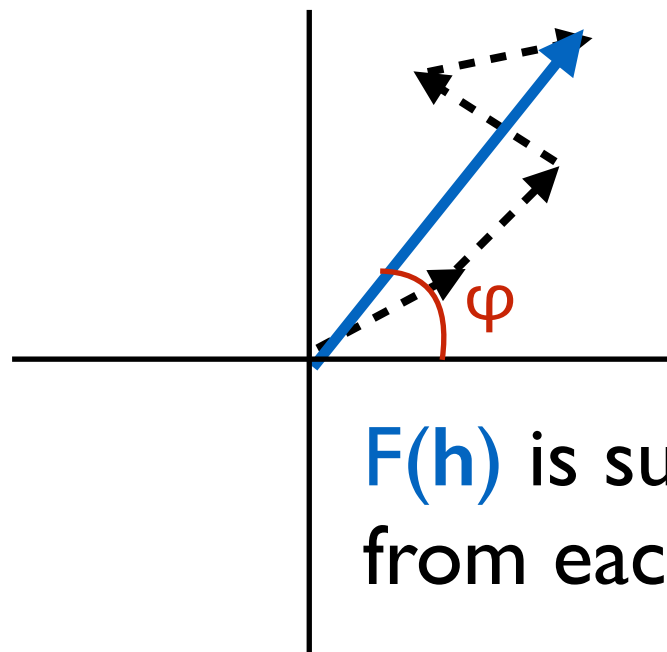
they have the same amplitude, but phases φ and $-\varphi$ respectively

$$\rho(\mathbf{x}) = 1/V \sum_{\text{all } \mathbf{h}} F(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x})$$

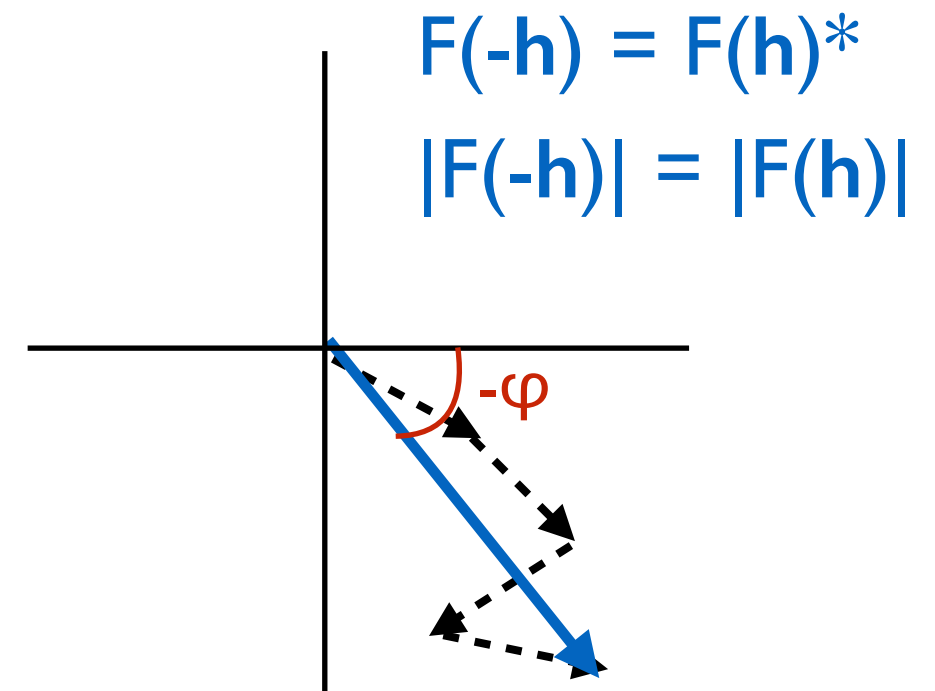
$$= 1/V \sum_{\text{half } \mathbf{h}} [F(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x}) + F(-\mathbf{h}) \exp(-2\pi i -\mathbf{h} \cdot \mathbf{x})]$$

$$= 1/V \sum_{\text{half } \mathbf{h}} 2 F(\mathbf{h}) \cos(-2\pi \mathbf{h} \cdot \mathbf{x}) \quad \text{real}$$

No anomalous scattering



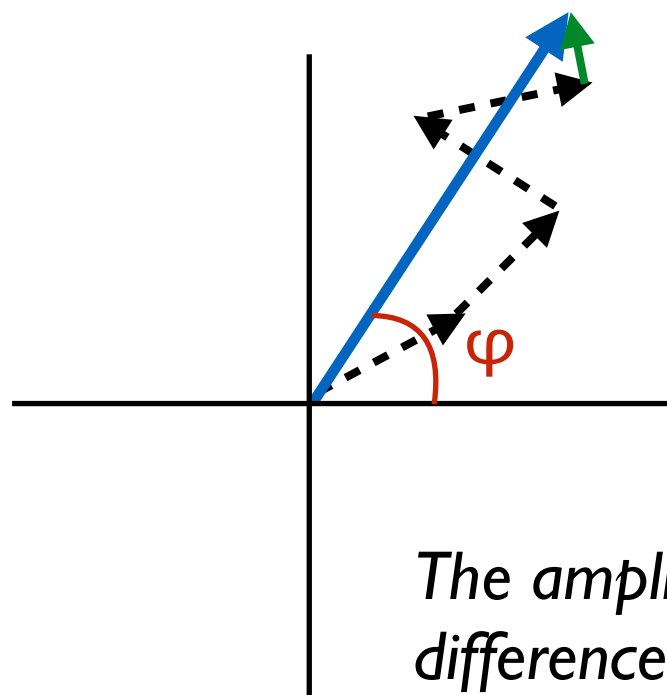
$F(h)$ is sum of contributions from each atom



$$F(-h) = F(h)^*$$

$$|F(-h)| = |F(h)|$$

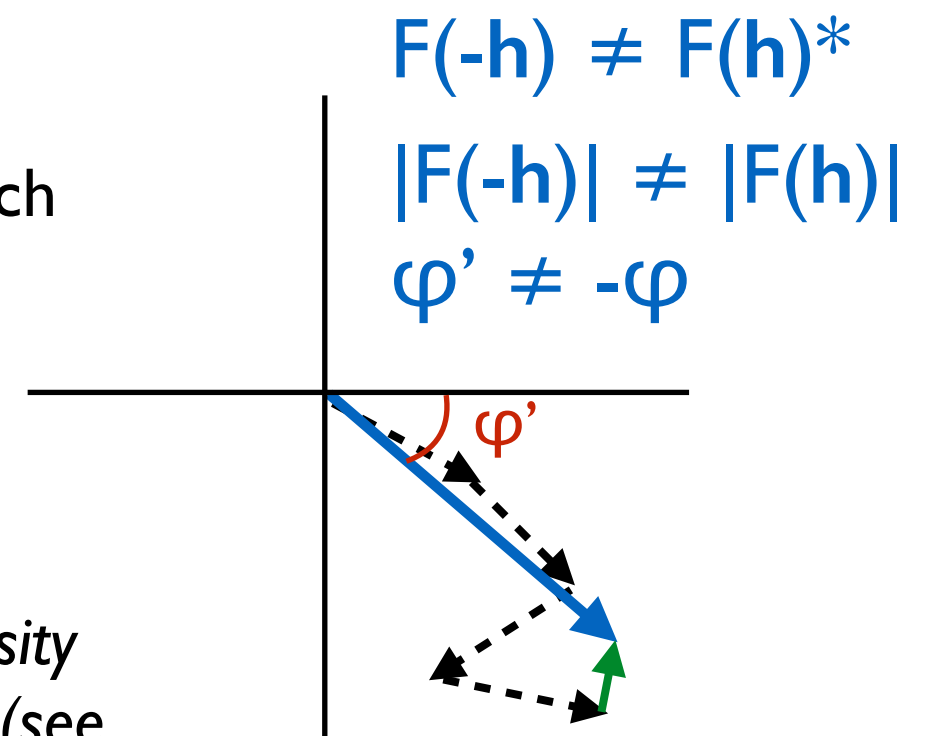
Anomalous scattering



Scattered X-rays from an atom close to its absorption edge undergo a $+90^\circ$ phase shift, which breaks the Friedel symmetry

Scattering electron density is complex

The amplitude difference inferred from the intensity difference $I(h) - I(-h)$ can give phase information (see later lecture)



$$F(-h) \neq F(h)^*$$

$$|F(-h)| \neq |F(h)|$$

$$\varphi' \neq -\varphi$$

Symmetry of diffraction

The symmetry of the crystal is reflected in the symmetry of the diffraction pattern

$$F(\mathbf{h}) = \sum_j f_j(\mathbf{h}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j)$$

For each unique atom $\mathbf{x}_j$ there may be other atoms related by symmetry operators ($k = 1, m$), working on fractional coordinates

$$\mathbf{x}_{jk} = R_k \mathbf{x}_j + \mathbf{t}_k$$

$$\begin{aligned} F(\mathbf{h}) &= \sum_j \sum_k f_j \exp(2\pi i \mathbf{h} \cdot (R_k \mathbf{x}_j + \mathbf{t}_k)) \\ &= \sum_k \exp(2\pi i \mathbf{h} \cdot \mathbf{t}_k) \sum_j f_j \exp(2\pi i \mathbf{h} \cdot (R_k \mathbf{x}_j)) \end{aligned}$$

phase change *Rotation*

Consider a symmetry-related reflection, Replace $\mathbf{h}$ by $\mathbf{h}'_m = \mathbf{h}^T R_m$

$$F(\mathbf{h}'_m) = \sum_k \exp(2\pi i \mathbf{h}'_m \cdot \mathbf{t}_k) \sum_j f_j \exp(2\pi i \mathbf{h}'_m \cdot (R_m R_k \mathbf{x}_j))$$

but since the operators form a group, $R_m R_k = R_i$, another symmetry operator, so $|F(\mathbf{h}'_m)| = |F(\mathbf{h})|$, i.e. the intensity pattern follows the group rotational symmetry, plus an inversion from Friedel's law $|F(-\mathbf{h})| = |F(\mathbf{h})|$ and reflections have predictably-related phases

Example: space group $P 2_1$, operators x, y, z ; $-x, y + \frac{1}{2}, -z$

Confusing at first glance!

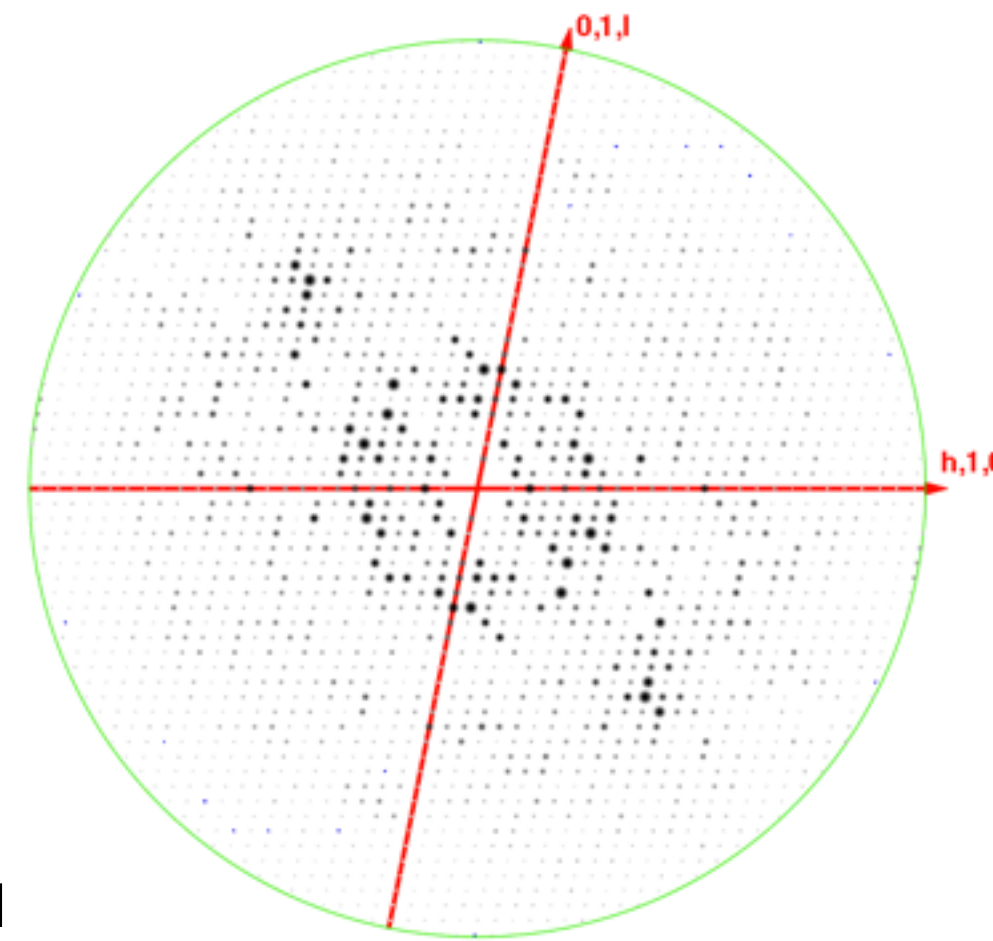
$$\begin{aligned}
 F(hkl) &= \sum_j f_j \{ \exp(2\pi i (h k l) \cdot (x_j y_j z_j)) + \\
 &\quad \exp(2\pi i (h k l) \cdot (-x_j y_j -z_j)) \exp(2\pi i (h k l) \cdot (0 \frac{1}{2} 0)) \} \\
 &= \sum_j f_j \{ \exp(2\pi i (h k l) \cdot (x_j y_j z_j)) + \\
 &\quad \exp(2\pi i (h k l) \cdot (-x_j y_j -z_j)) \exp(2\pi i k/2) \} \\
 &= \sum_j f_j \{ S_1 + S_2 \}
 \end{aligned}$$

$$\begin{aligned}
 \text{NB } (h k l) \cdot (-x y -z) \\
 = (-h k -l) \cdot (x y z)
 \end{aligned}$$

$$\begin{aligned}
 F(-hk-l) &= \sum_j f_j \{ \exp(2\pi i (-h k -l) \cdot (x_j y_j z_j)) + \\
 &\quad \exp(2\pi i (-h k -l) \cdot (-x_j y_j -z_j)) \exp(2\pi i k/2) \} \\
 &= \sum_j f_j \{ \exp(2\pi i (-h k -l) \cdot (x_j y_j z_j)) \exp(2\pi i k/2) + \\
 &\quad \exp(2\pi i (h k l) \cdot (x_j y_j z_j)) \} \exp(-2\pi i k/2) \\
 &= \exp(-2\pi i k/2) \sum_j f_j \{ S_1 + S_2 \}
 \end{aligned}$$

$$F(-hk-l) = \exp(-i\varphi) F(hkl) \quad \text{where } \varphi = 2\pi k/2$$

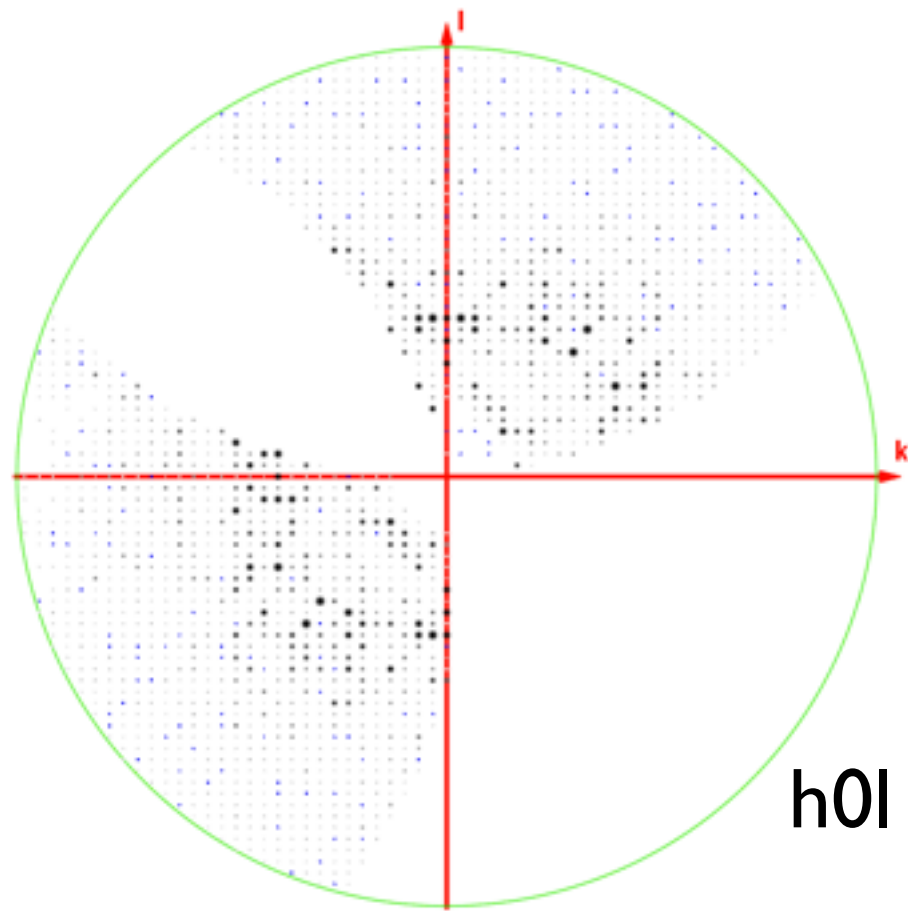
$$|F(-hk-l)| = |F(hkl)| \quad \text{same amplitude, different related}$$



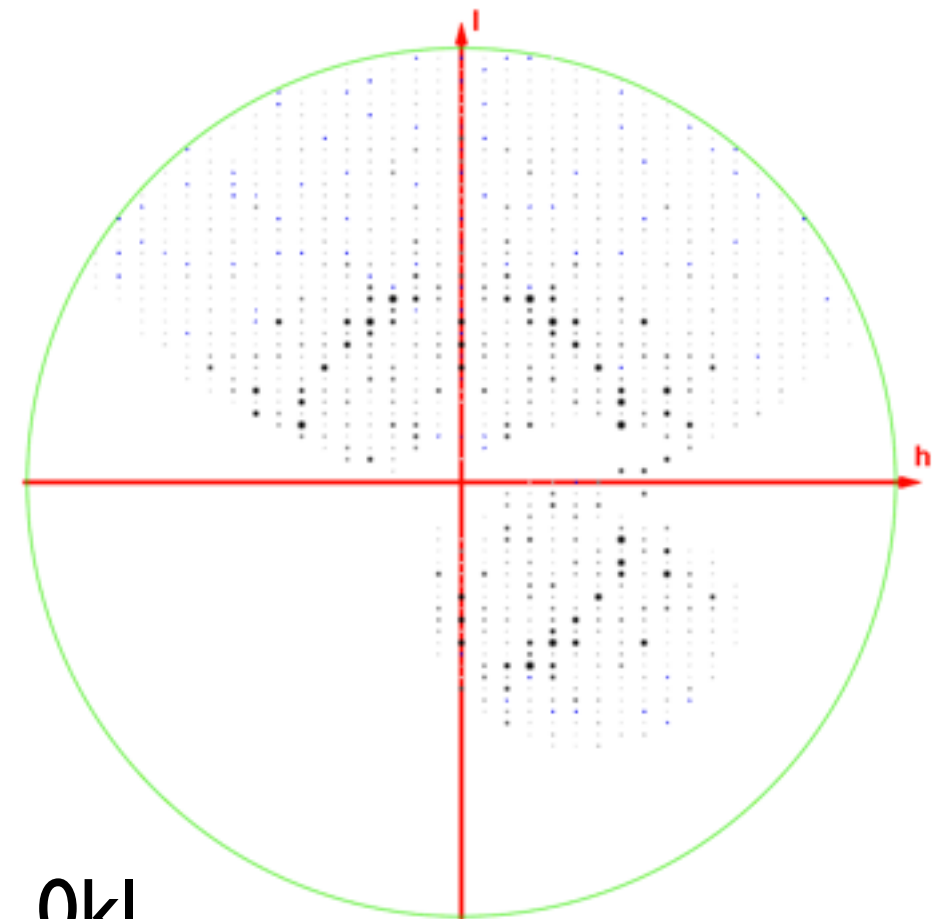
$h1l$ zone

2-fold symmetry

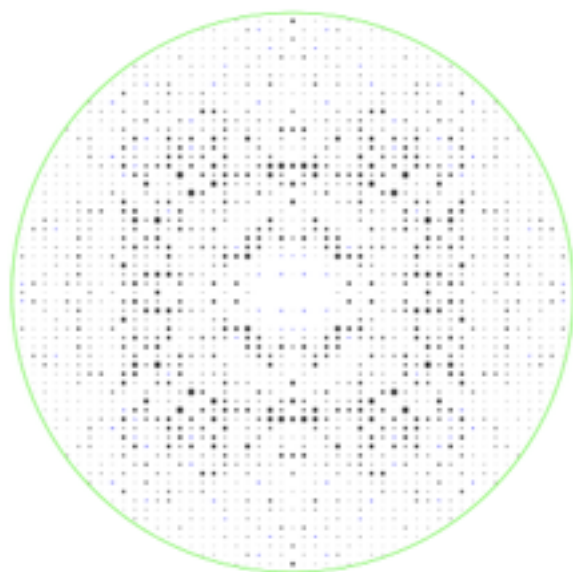
Orthorhombic $P2_12_12_1$ mmm symmetry



$h0l$

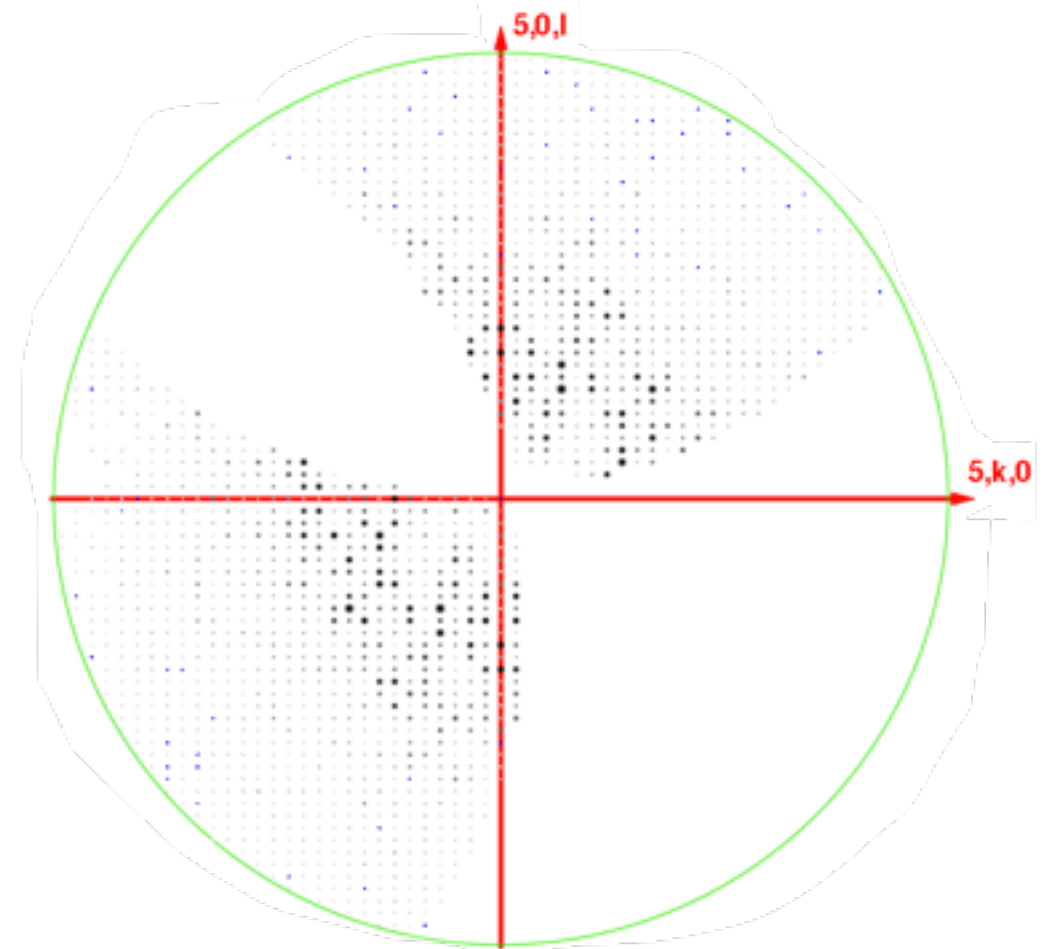


$0kl$



$5kl$

mmm symmetry
from point group
 $222 + \text{inversion}$



Determination of Space Group Symmetry

(see *future lectures*)

By inspection of the observed diffraction pattern, we may be able to infer the crystal symmetry

observed rotational symmetry (2, 3, 4, 6- fold rotations) gives the point group

BUT symmetry related reflections have the same amplitude (and intensity) and we cannot observe their phase, so translational elements of symmetry are harder to determine

e.g. do we have a pure rotation axis $(-x, y, -z)$ or a screw axis $(-x, y + \frac{1}{2}, -z)$

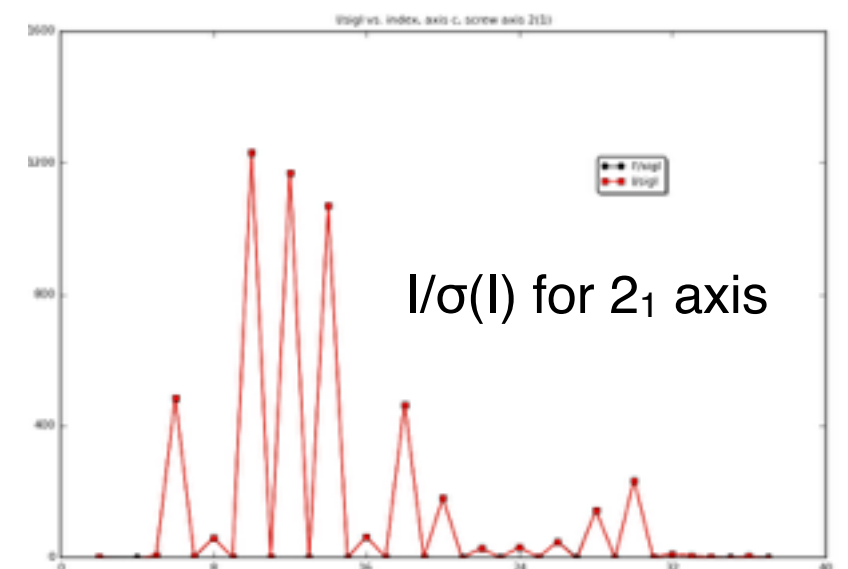
Screw axes give systematic absences along reciprocal lattice axes, which (with any luck) allow us to distinguish screws from rotations

eg $P2_1$ screw axis $(-x, y+1/2, -z)$

$$\begin{aligned} F(0k0) &= \sum_j f_j \{ \exp(2\pi i ky) + \exp(2\pi i ky) \exp(2\pi i \frac{1}{2}k) \} \\ &= 2 \sum_j f_j \exp(2\pi i ky) \quad \text{if } k = 2n \text{ even} \\ &= 0 \quad \text{if } k = 2n+1 \text{ odd} \end{aligned}$$

since $\exp(2\pi i \cdot \frac{1}{2}k) = +1$ if k is even, $= -1$ if k is odd

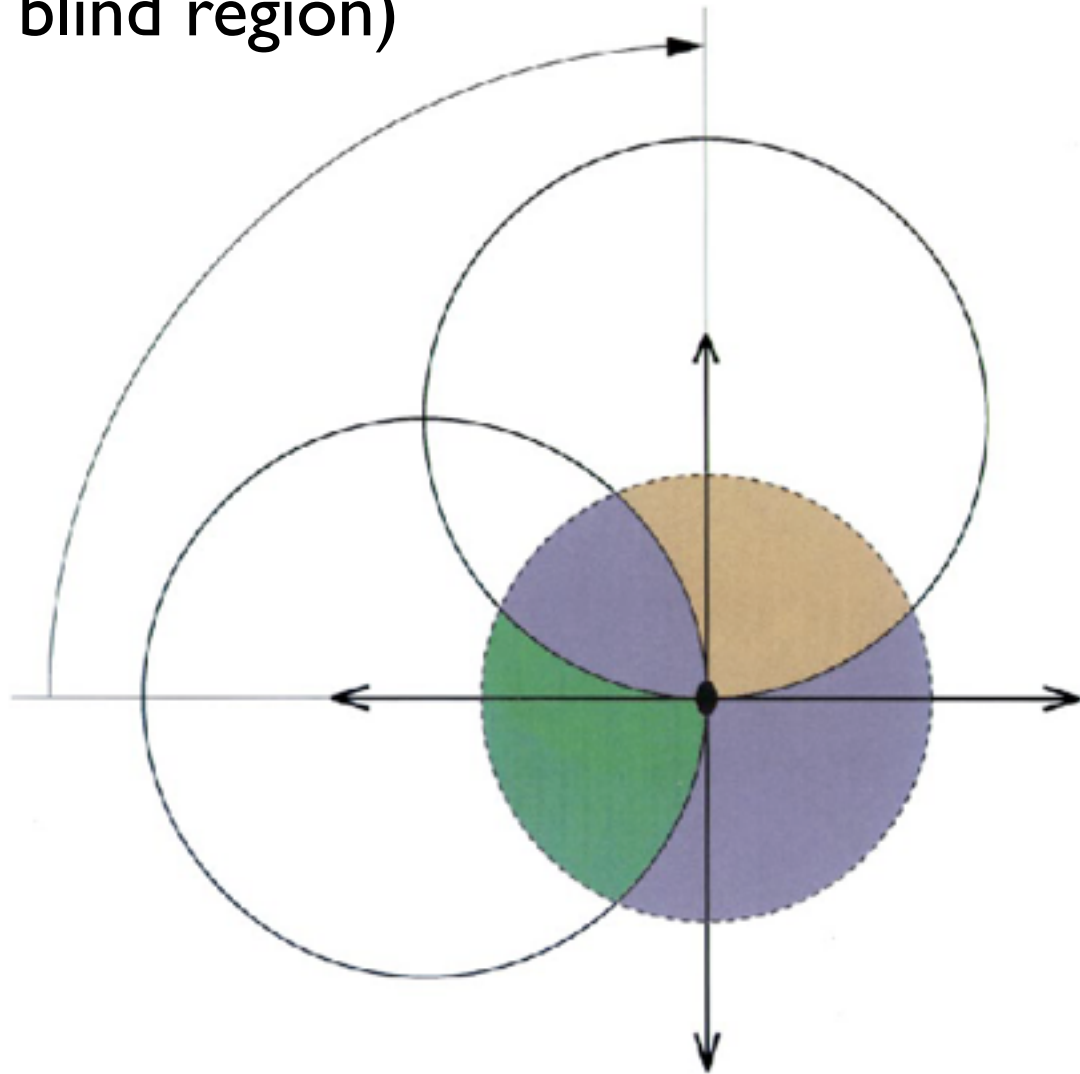
Axial reflections $0k0$ have zero intensity if k is odd



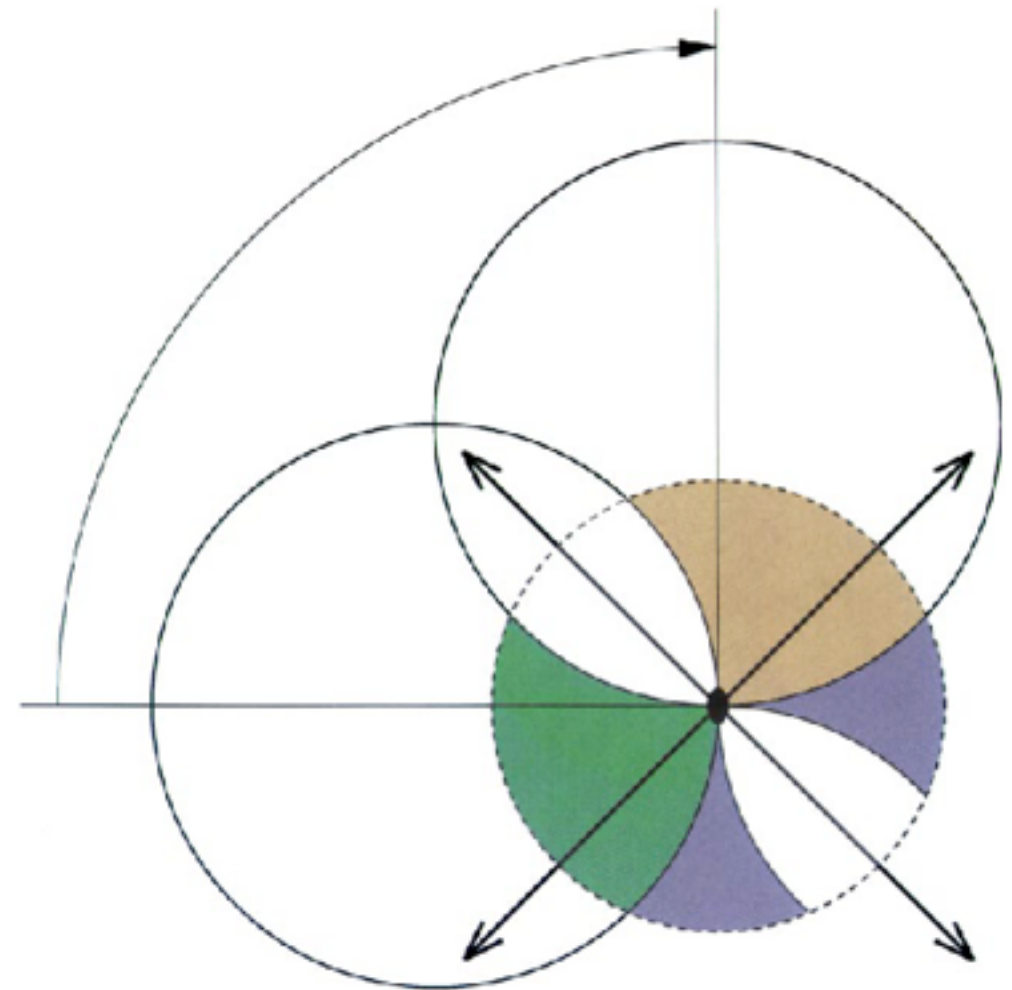
Symmetry and data collection strategy

Total rotation range: an orthorhombic example

Rotation of an orthorhombic crystal by 90° starting from an axis gives a complete dataset (except for the blind region)



A 90° rotation starting at a diagonal collects the same 45° twice, and gives incomplete data



BUT it is probably best to collect 180° or 360° anyway, if you can

Calculation of electron density maps

$$\rho(\mathbf{x}) = 1/V \sum_{\mathbf{h}} F(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x})$$

$F(\mathbf{h})$ in electrons, V in $\text{\AA}^3$, $\rho(\mathbf{x})$ in $\text{e}/\text{\AA}^3$

In order to calculate an electron density map $\rho(\mathbf{x})$, we need the phase of $F(\mathbf{h}) = |F| \exp(i \varphi)$

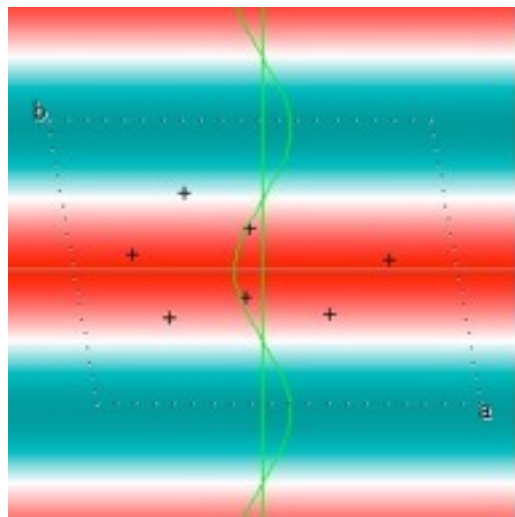
The phase normally comes from a model (refinement or molecular replacement), or from experimental phasing

How is the electron density built up from the summation of the complex structure factors?

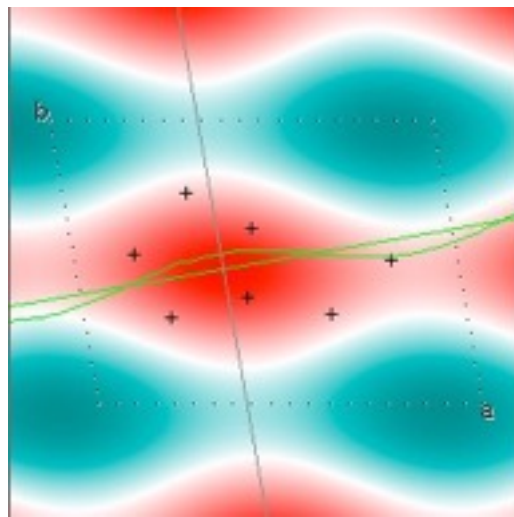
Calculation of electron density map from structure factors (with phases)

$$\rho(\mathbf{x}) = 1/V \sum_{\mathbf{h}} F(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x})$$

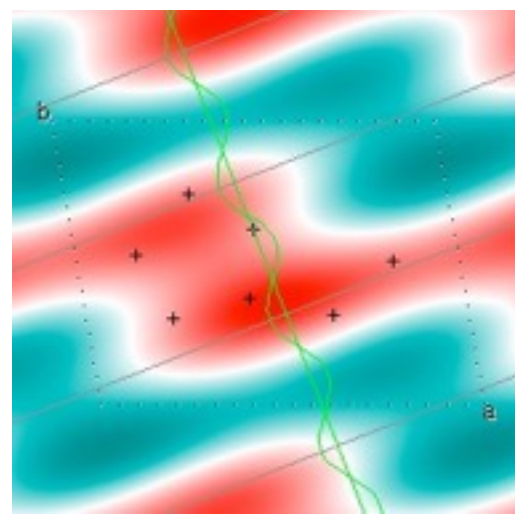
Each structure factor (reflection) contributes a spatial wave, depending on its hkl indices, amplitude and phase. Reflections from the outside of the diffraction pattern (high resolution) give the fine detail, as they are added



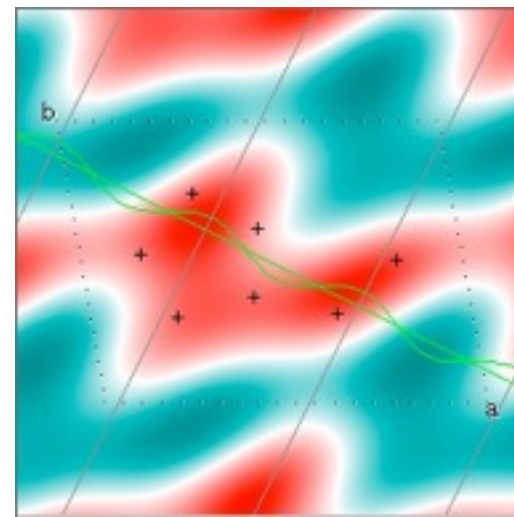
0,1



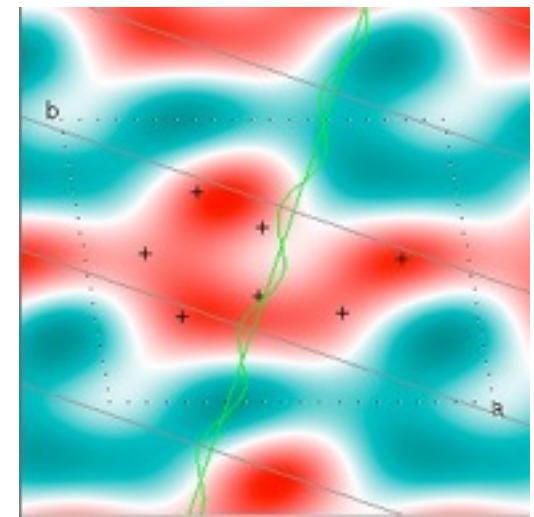
+(1,0)



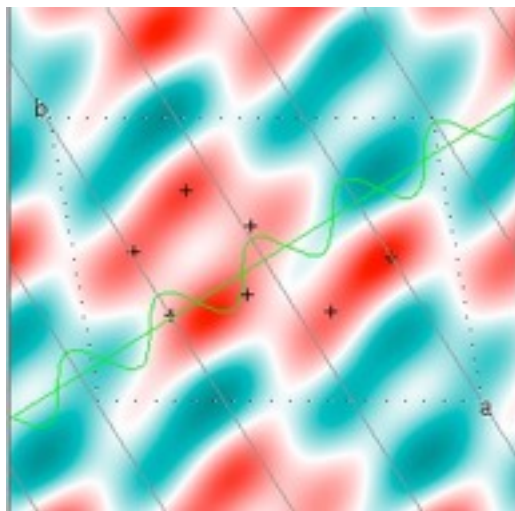
+(-1,2)



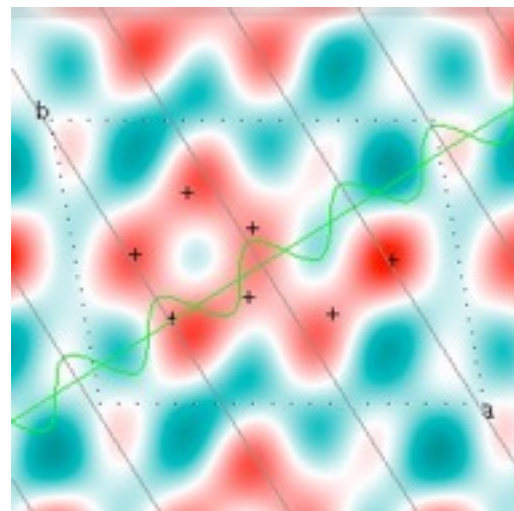
+(-2,1)



+(1,2)



+(3,-2)

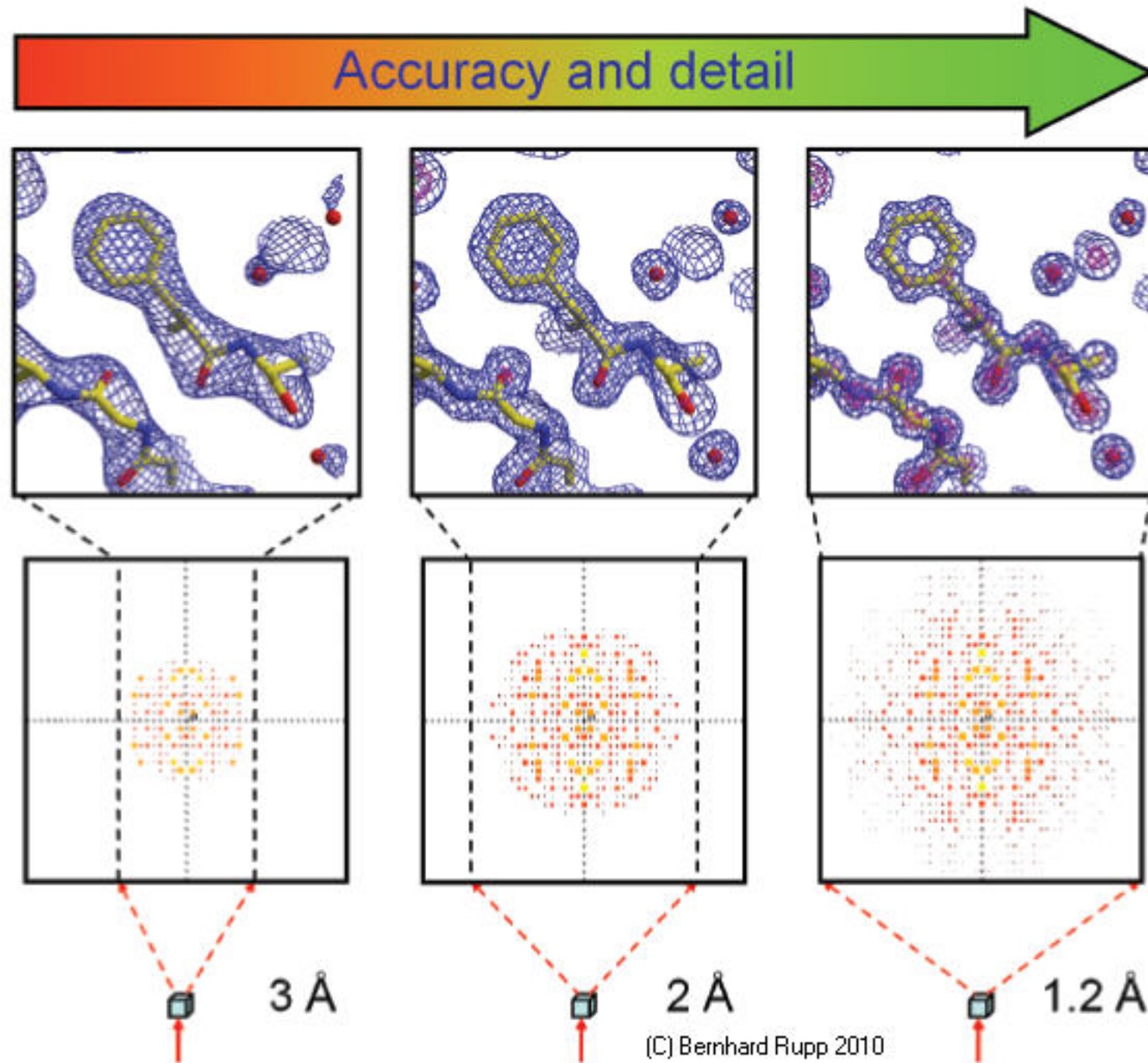


+(3,1)

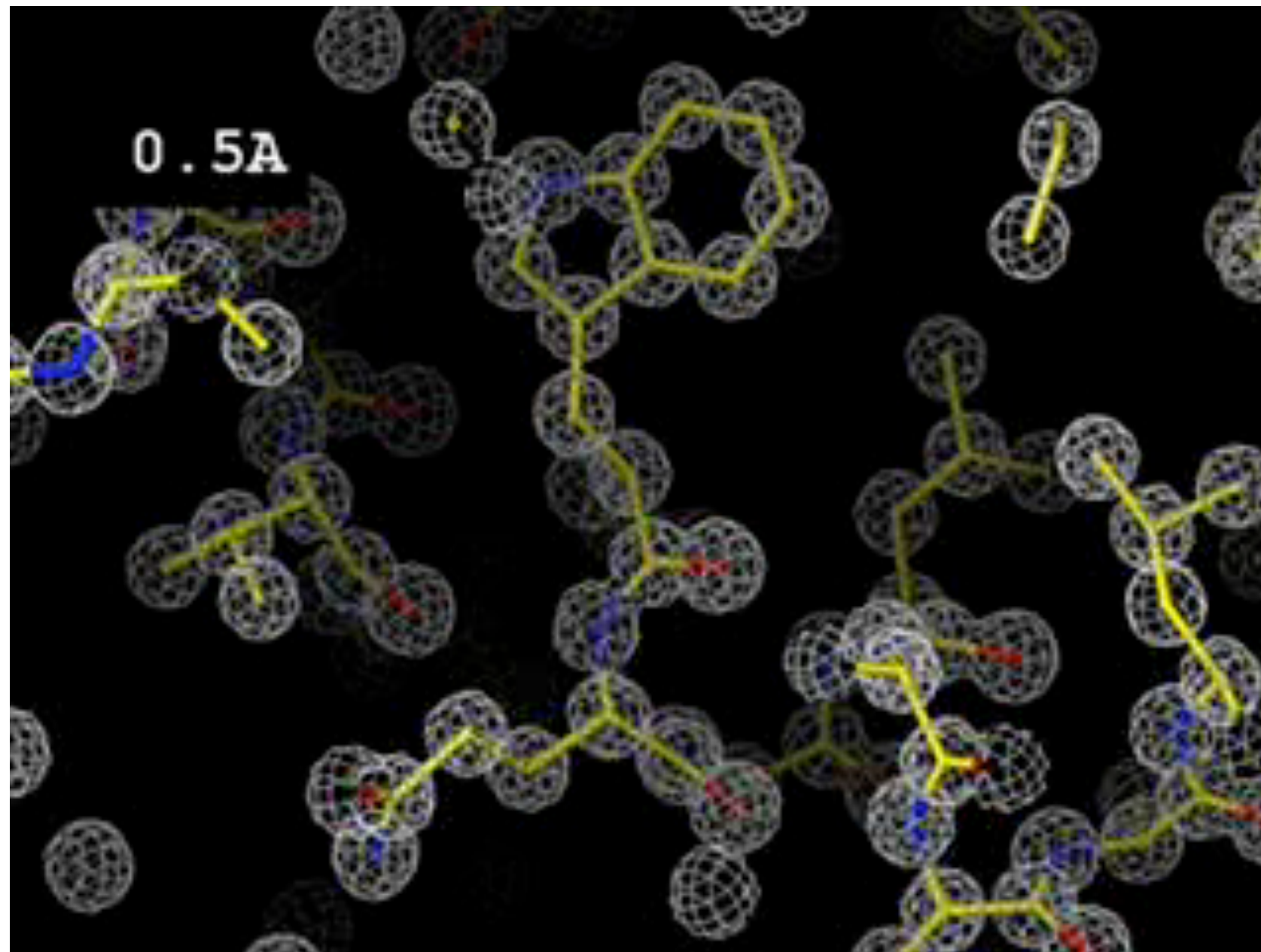
Low resolution reflections from low angle, low indices: these give the broad impression of the structure

$$|S| = 1/d = 2\sin\theta/\lambda$$

Resolution



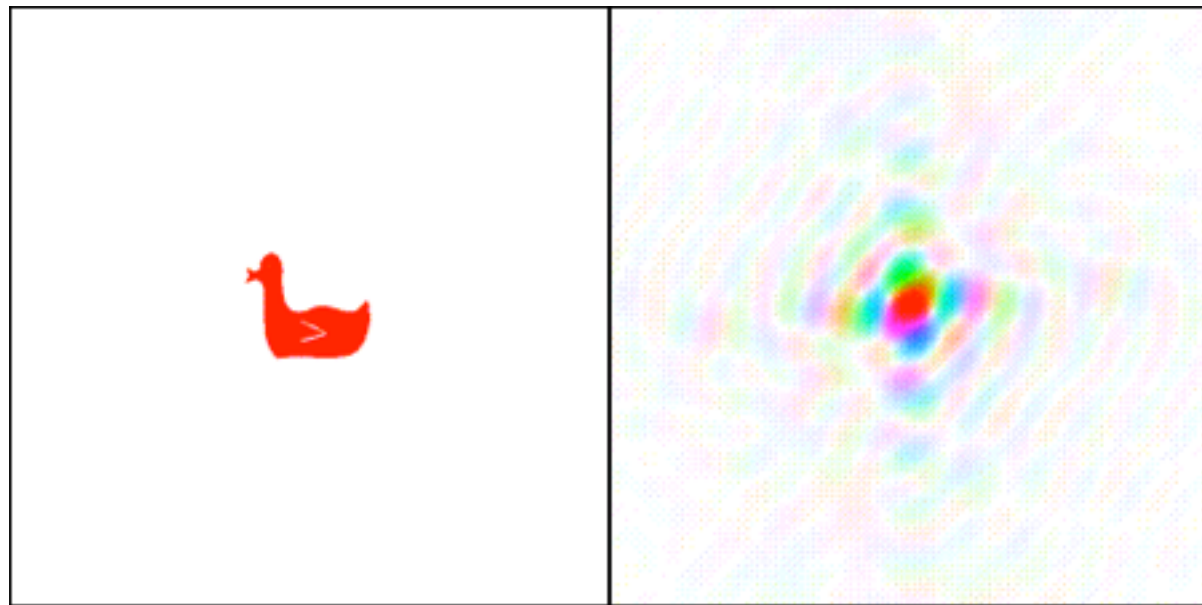
Resolution



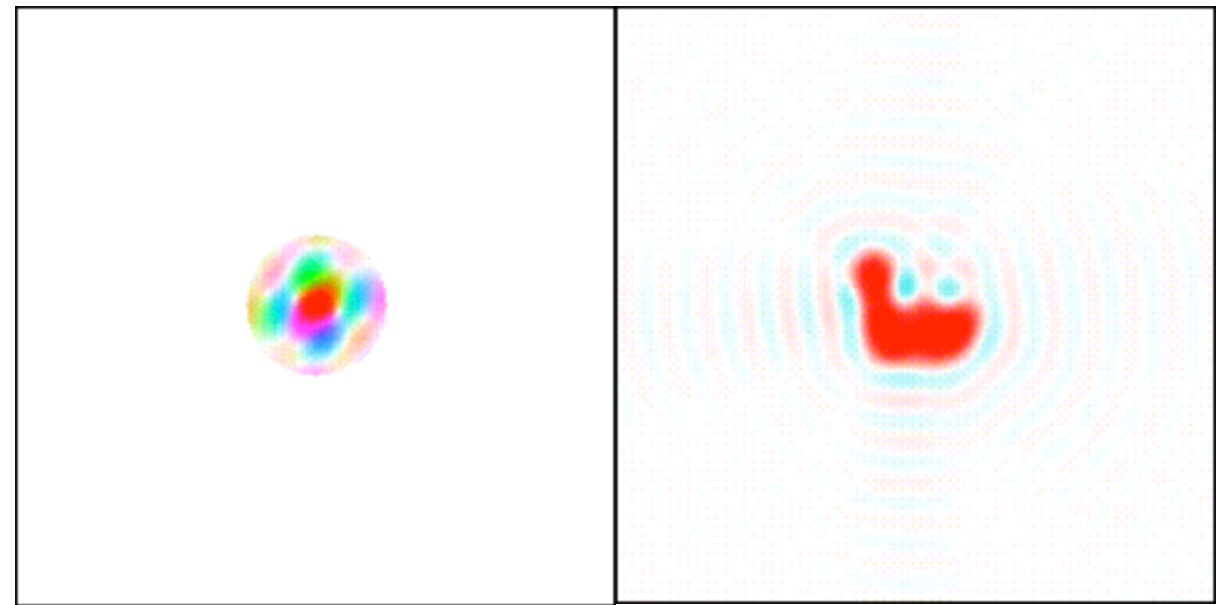
James Holton

The importance of data completeness

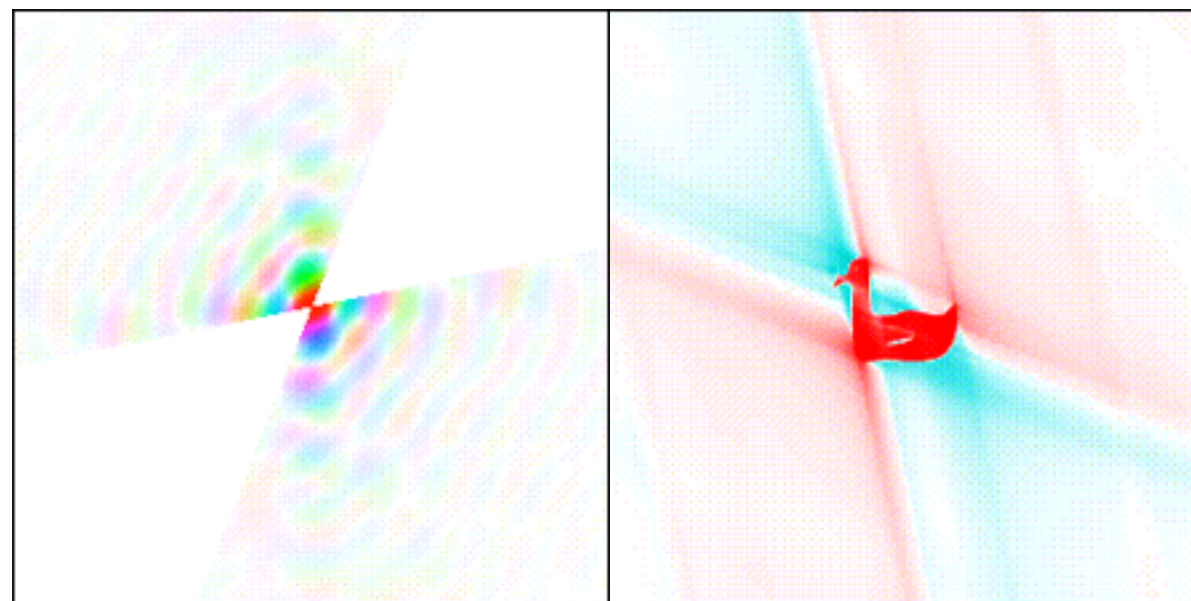
Resolution and missing data



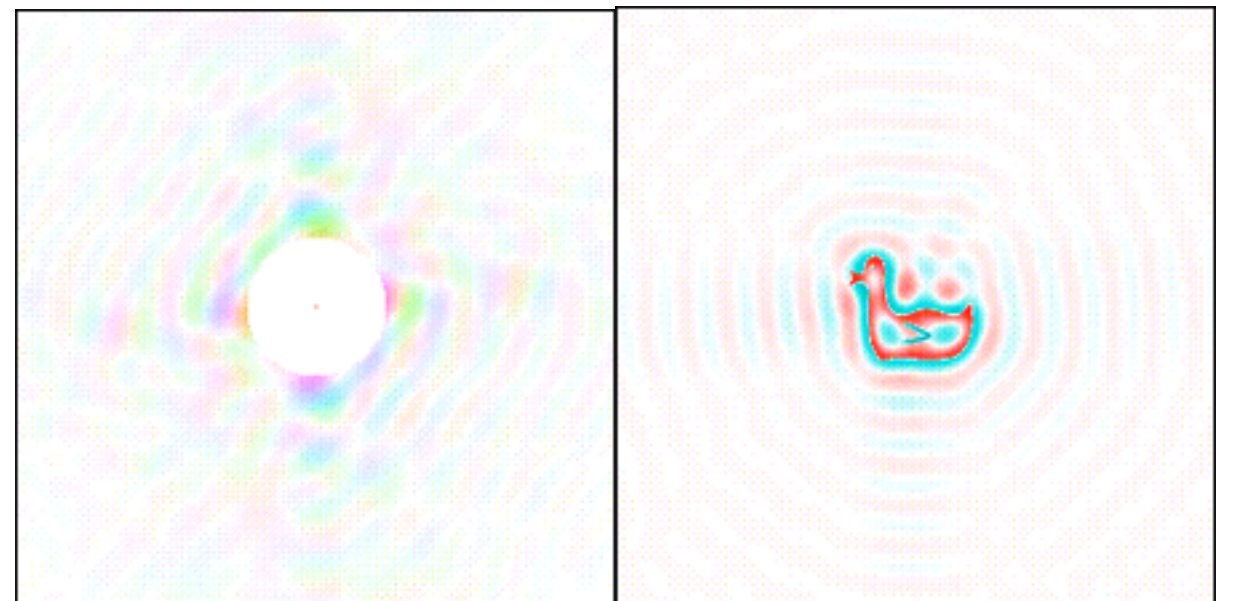
A duck and its Fourier transform



A low-resolution duck



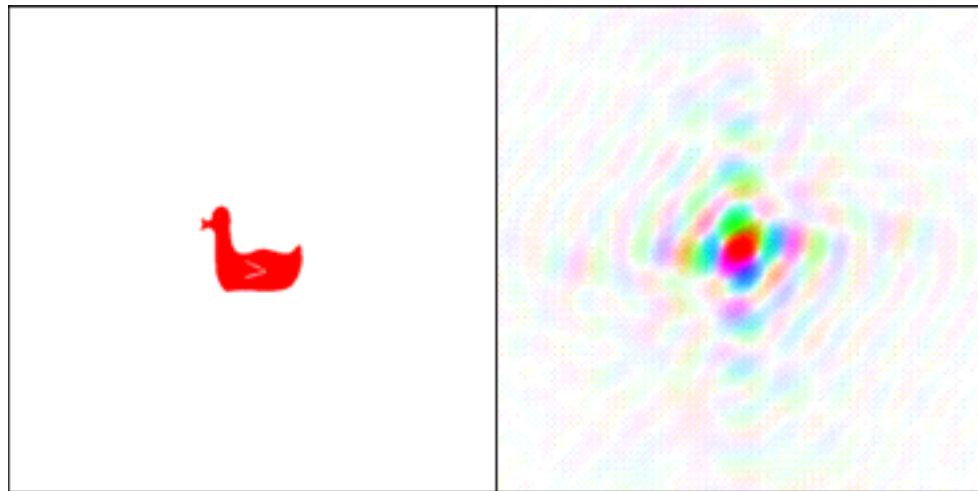
Incomplete data: missing wedge



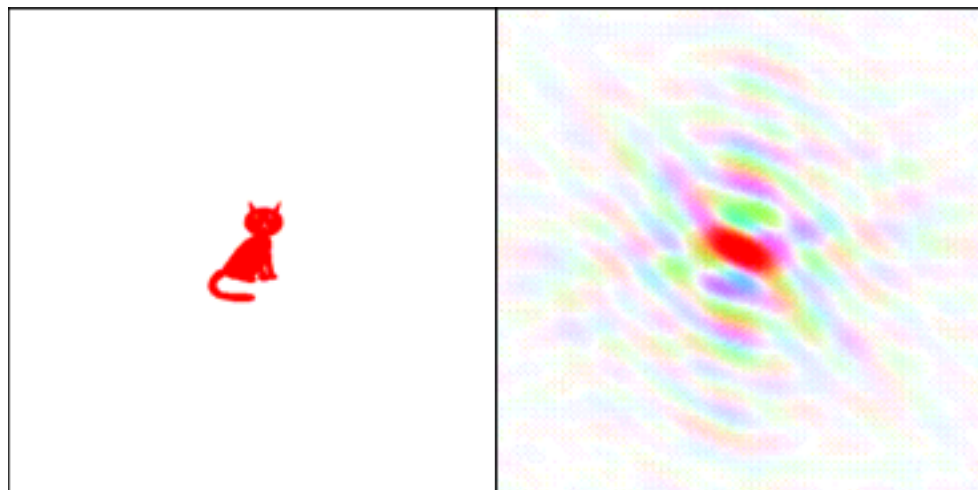
A duck without low-resolution reflections

Fourier transforms: the importance of phases

(phase encoded by colour)



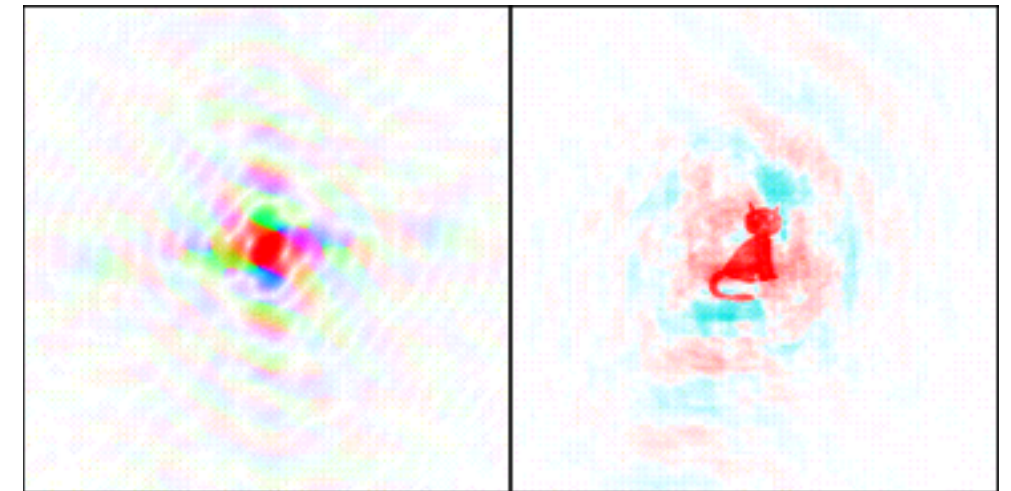
Duck and its transform



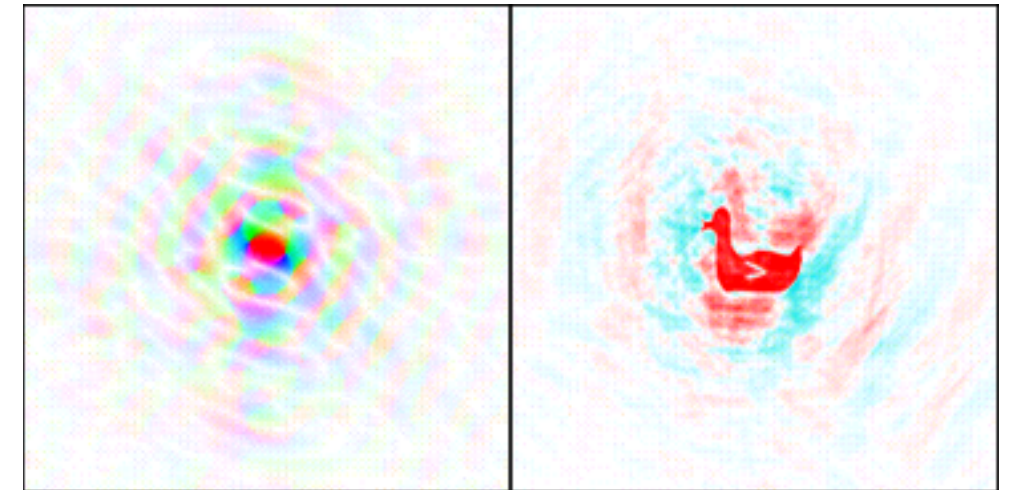
Cat and its transform



*Mix up and
back-transform*



Duck amplitudes, cat phases



Cat amplitudes, duck phases

... but we can't measure phases

Measurement of intensities

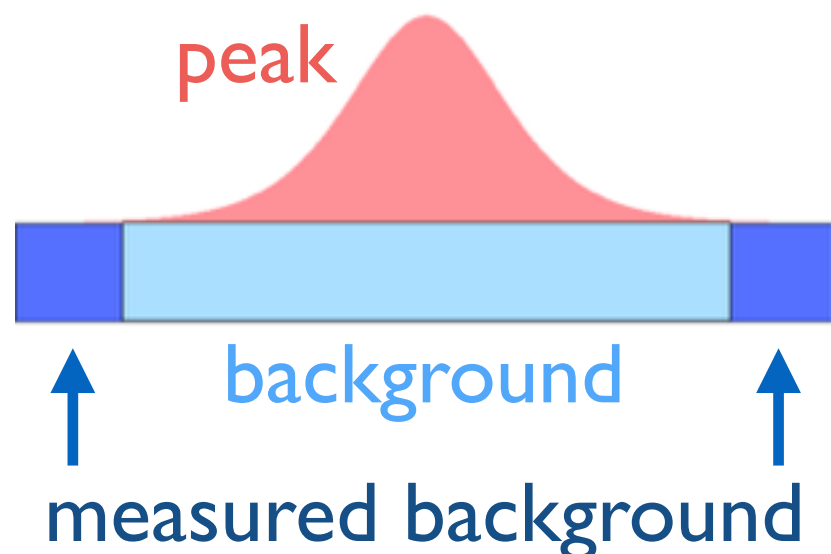
$$\text{Intensity} \propto |F|^2 = F F^*$$

So if we measure intensity we can infer $|F|$, with an arbitrary scale

$$|F| = k \sqrt{I} \quad (\text{in the absence of errors})$$

(we need to allow for errors, particularly for weak intensities)

1D example



To measure peak intensity, we need to estimate the background under the peak (in 2- or 3-D), from neighbouring areas, then subtract background from (peak + background)

Then we have an estimate of intensity I and its error $\sigma(I)$

Intensity of diffraction: the Darwin Equation

$$I = I_0 r_e^2 \boxed{\frac{V_{xtal}}{V_{cell}}} \frac{\lambda^3 L}{\omega V_{cell}} P A |F^2|$$



Sir Charles Galton Darwin (1887-1962)

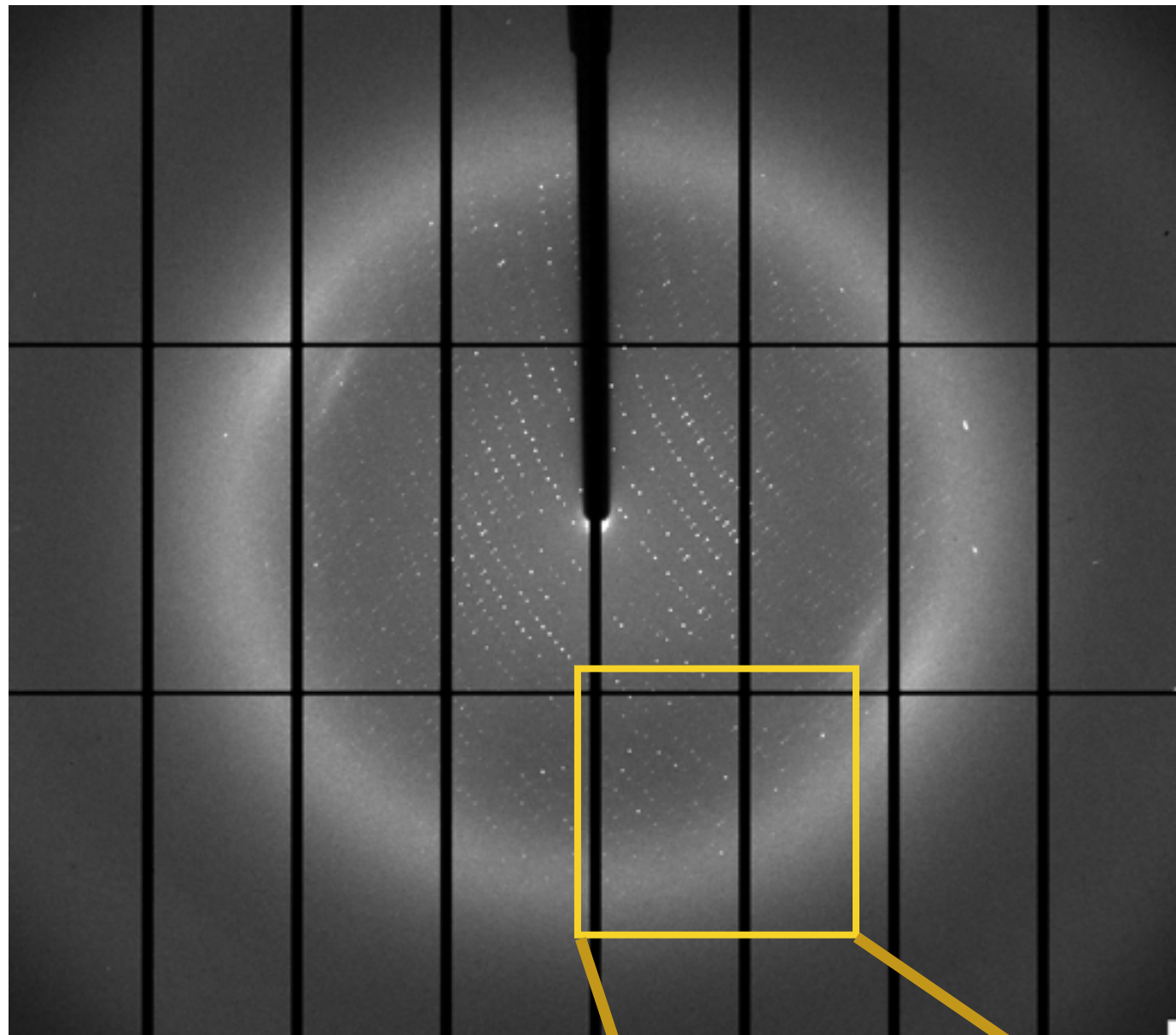
- I_0 incident beam intensity
- r_e radius of electron
- V_{xtal} volume of crystal
- V_{cell} volume of unit cell
- λ wavelength
- ω rotation rate
- L Lorentz factor – relative speed along sphere normal
- P polarisation
- A transmission – 1/absorption in crystal
- $|F|$ structure factor amplitude

$$\langle |F^2| \rangle \approx f_{atom} \sqrt{n_{atoms}} \propto V_{cell}$$

so $I \propto V_{xtal} / V_{cell}$ = number of unit cells in beam

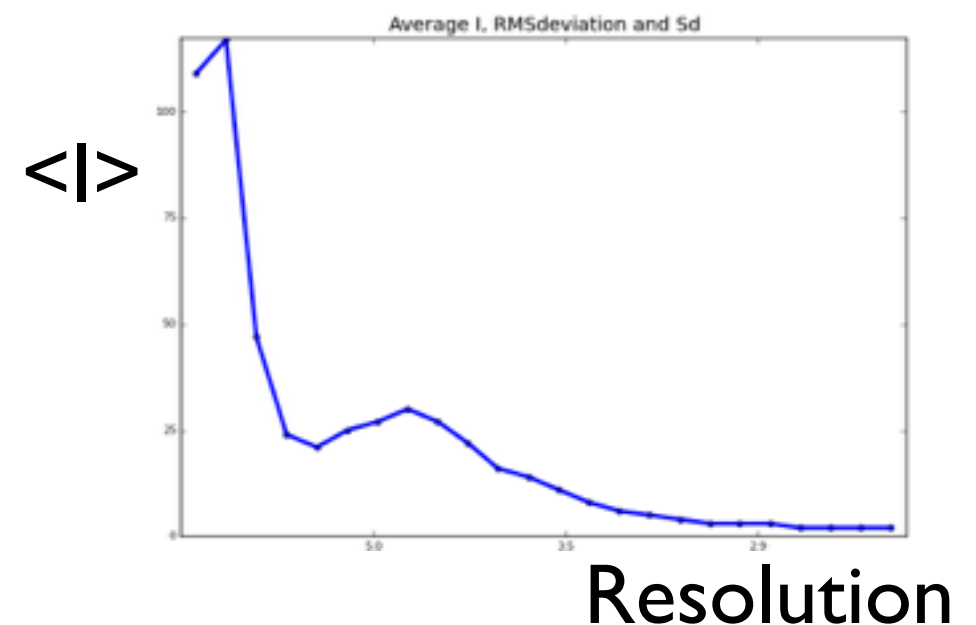
Holton JM, Frankel KA. The minimum crystal size needed for a complete diffraction data set. Acta Crystallogr D (2010) 66, 393–408.

Real diffraction patterns show other effects which we need to consider

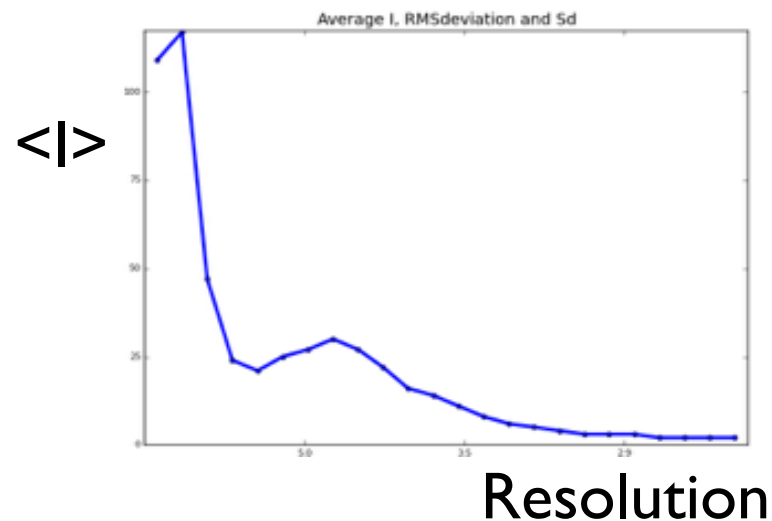


Summed images
 $10 \times 0.15^\circ = 1.5^\circ$

- background due to scattering by water etc
- sometimes ice spots or rings
- scattering from loop etc
- beamstop shadows
- detector tile boundaries
- intensity falls off at higher angles (higher resolution)



Intensity and $|F|$ fall off at high resolution due to dynamic and static disorder



*You can measure useful intensities a little beyond where you can see spots
(see later lectures)*

$$F(\mathbf{h}) = \sum_j f_j(\mathbf{h}) T_j(\mathbf{h}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j)$$

Modify the structure factor equation to include a disorder term (temperature factor) $T_j(\mathbf{h})$ to model the “disorder” of each atom

$$T_j(\mathbf{h}) = \exp(-B_j \sin^2 \theta / \lambda^2) = \exp(-B_j |S|^2 / 4) = \exp(-B_j / 4d^2) \quad \text{isotropic}$$

or $T_j(\mathbf{h}) = \exp(-\mathbf{h}^T \boldsymbol{\beta}_j \mathbf{h})$ *anisotropic*

The electron density image from crystallography is

- ♦ a space average over all unit cells
- ♦ a time average over the data collection times

Phasing

How to get phases? We have observed amplitudes (intensities)

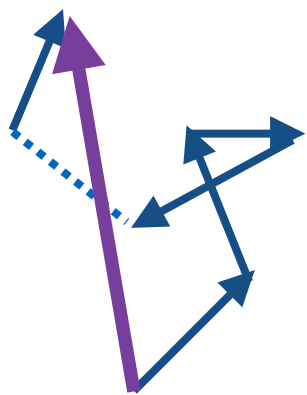
- Molecular replacement – we have a related model, try all possible positions and orientations to find one that fits the observed data
- Experimental phasing – add a small(ish) number of heavy atoms or anomalous scatterers, observe the different F_s (I_s) with and without, or Friedel pairs; locate substructure from difference; use the known positions as phase references (*determine phase relative to substructure phase*)
- Direct methods – very high resolution, resolved atoms $< \sim 1.2 \text{ \AA}$ resolution. Algebraic phase relationships as used for small molecules

General and restricted phases

$$F(\mathbf{h}) = \sum_k \exp(2\pi i \mathbf{h} \cdot \mathbf{t}_k) \sum_j f_j T_j(\mathbf{h}) \exp(2\pi i \mathbf{h} \cdot (\mathbf{R}_k \mathbf{x}_j))$$

$$= \sum_k \exp(2\pi i \mathbf{h} \cdot \mathbf{t}_k) F_k(\mathbf{h})$$

Structure expression summed over crystallographic symmetry operators $\mathbf{R}_k \mathbf{x}_j + \mathbf{t}_k$



Summation of contributions from each atom in cell

Example: space group P2, operators x, y, z and $-x, y, -z$

no translations, $\mathbf{t}_k = \mathbf{0}$, so

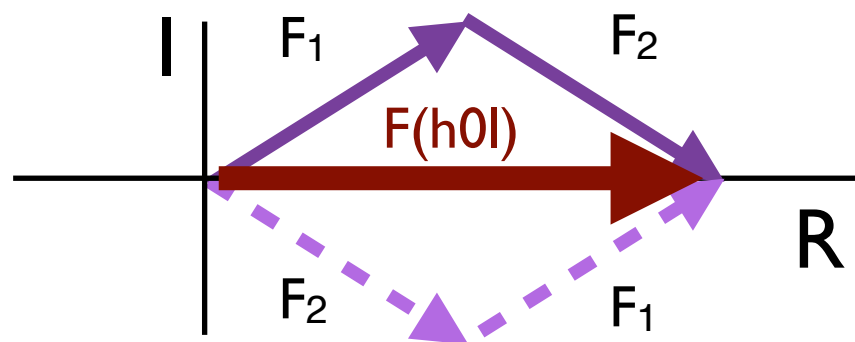
$$F(\mathbf{h}) = F_1 + F_2 = \sum_j f_j \exp(2\pi i (h x_j + k y_j + l z_j))$$

$$+ \sum_j f_j \exp(2\pi i (-h x_j + k y_j - l z_j))$$

for the special case of $k=0$,

$$F(h0l) = \sum_j f_j \exp(2\pi i \mathbf{h} \cdot \mathbf{x}) + \sum_j f_j \exp(2\pi i -\mathbf{h} \cdot \mathbf{x})$$

ie $F_2 = F_1^*$ and the phase of $F(h0l) = 0$ or π (180°)



Centric reflection. Restricted phase, only two possibilities, so more accurate

Centric zones are zero levels perpendicular to an even-fold axis

Good luck!