Advanced Workshop on Structural Biology:
Using Synchrotron Radiation to Visualise Biological Molecules
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An introduction to diffraction theory

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The Abdus Salam
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for Theoretical Physics
50th Anniversary 1964-2014

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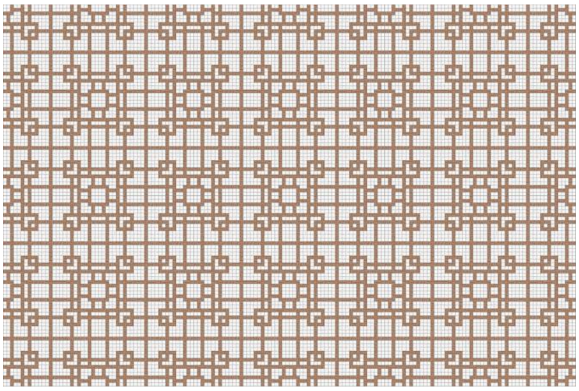
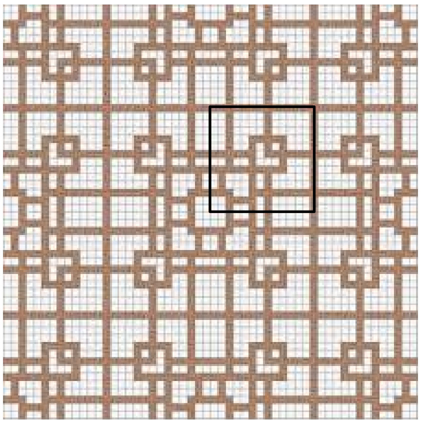
The crystal lattice

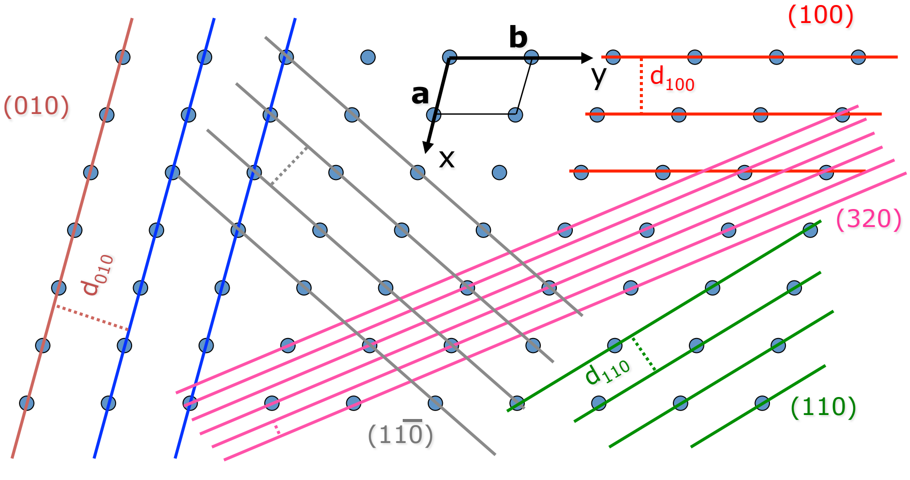


Unit cell



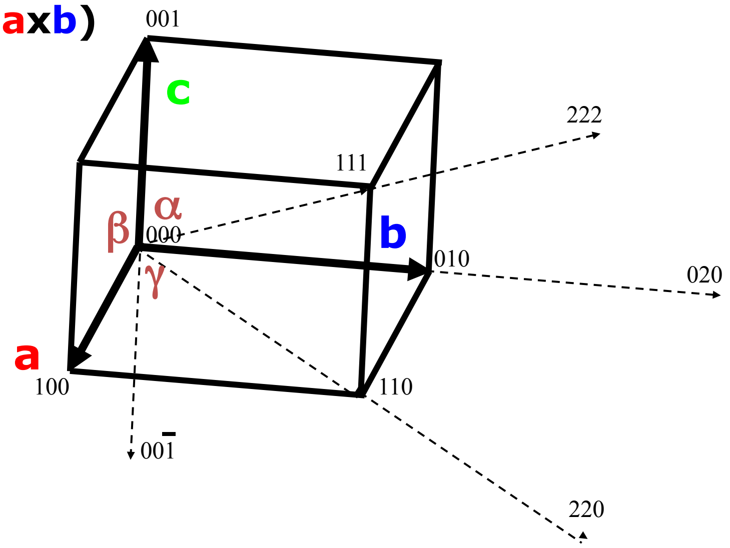
Miller indices (hkl) of lattice planes:

number of divisions the plane-set cuts into **a** (h), **b** (k), and **c** (l)



Unit cell

$V=\mathbf{c}.(\mathbf{a}\mathbf{x}\mathbf{b})$

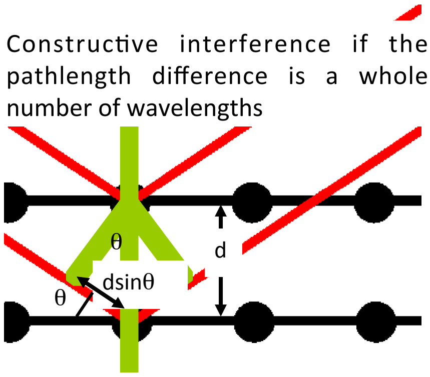


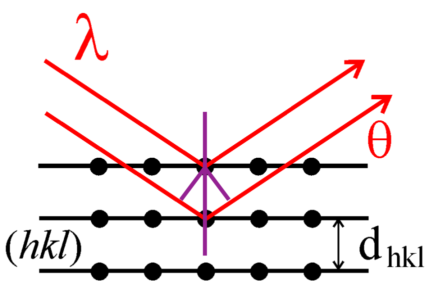
The basis vectors **a**,**b**,**c** define a **lattice**

Bragg's Law

$$\mathbf{(n)\lambda = 2\ d_{hkl}\ sin\theta}$$

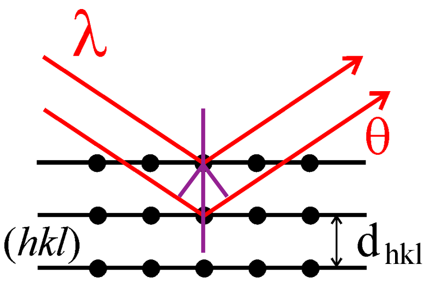
Constructive interference if the pathlength difference is a whole number of wavelengths





Bragg's Law

$$(n)\lambda = 2\, d_{hkl} \sin\theta$$



Consider diffraction as selective reflection :

Only certain angles of reflection *(θ)*
are selected when X-rays of
a given wavelength **(λ)**
are reflected by the lattice planes described by Miller Indices*(hkl)*
with a characteristic interplanar distance (d_{hkl})

The Reciprocal Lattice

The reciprocal lattice is an abstract concept, unlike the crystal (real space) lattice, that is very useful in visualizing diffraction geometry when used together with the Ewald sphere construction.

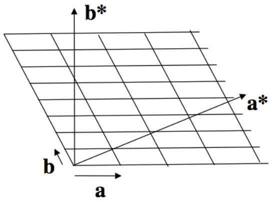
The reciprocal lattice is defined by three vectors **a***, **b***, **c*** (in the same way that the crystal lattice is defined by the three vectors **a**, **b**, **c**) as follows:

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

This definition implies:

$\mathbf{a.a^*} = 1$ $\mathbf{b.b^*} = 1$ $\mathbf{c.c^*} = 1$

A two dimensional example

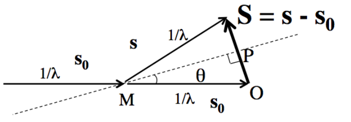


The reciprocal vectors are perpendicular to the *planes* of the real crystal lattice, so **a*** is perpendicular to (100), **b*** to (010) **c*** to (001)

|**a***| is the spacing between the (bc) planes,
|**b***| between the (ca) planes,
|**c***| between the (ab) planes

For the special case of an orthogonal lattice (only),
|**a***| = 1/|**a**|, |**b***| = 1/|**b**|, |**c***| = 1/|**c**|; **a*** ||**a** **b*** ||**b** **c*** ||**c**
We define the scattering vector **S** (or **d***) for a particular reciprocal lattice point as:
S = h **a*** + k **b*** + l **c***

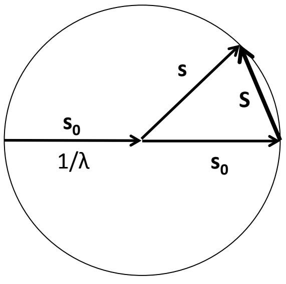
The Ewald Sphere Construction



$\mathbf{s_0}$ is the incident beam vector
s is the diffracted beam vector
S is the scattering vector

The general condition for diffraction is illustrated by the vector equation
$\mathbf{S} = \mathbf{s} - \mathbf{s_0}$

In the triangle MOP, sin(θ) = OP/OM = λd*/2 = λ/2d



Because $\mathbf{s_0}$ and **s** have the same length (1/λ),
we can generalise this diagram by drawing a sphere of radius

$|\mathbf{s_0}| = |\mathbf{s}| = 1/\lambda$

S is the diffraction vector in *reciprocal space*

For a crystal, **S** may only take certain values, **S** = h **a*** + k **b*** + l **c***

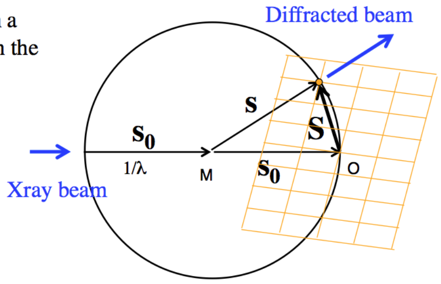
The Ewald sphere is only a construction but is very useful to understand the *geometry* of diffraction. Confusingly, it has two origins:

M is the centre of the sphere, and may be considered as the position of the crystal, since this is the source of the secondary (diffracted) beam **s;**

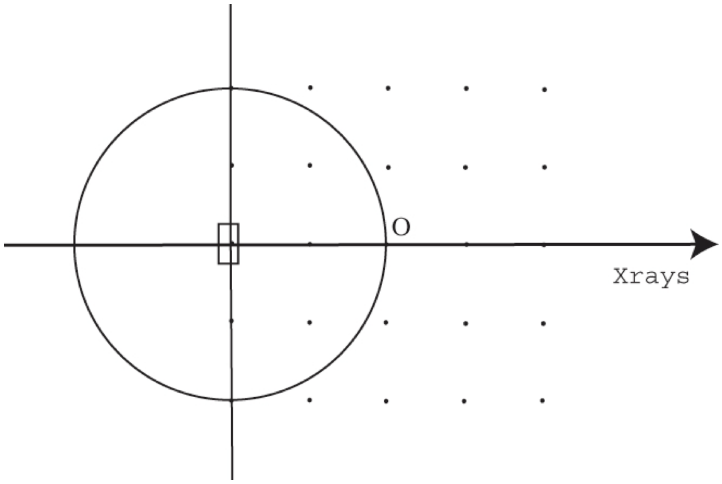
O is the origin of reciprocal space, the origin of the diffraction vector S, and the centre of the reciprocal lattice

As the crystal rotates, the reciprocal lattices rotates in exactly the same way

Diffraction only occurs when a reciprocal lattice point lies on the sphere

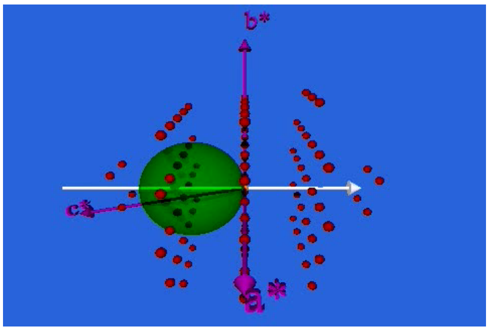


A two dimensional example



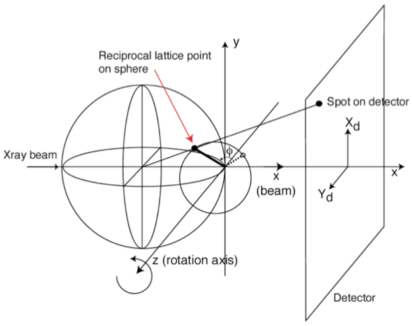
In three dimensions

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



http://escher.epfl.ch/x-ray/diff.mpeg

What happens on the detector



As the crystal rotates, each lattice point in turn passes through the sphere, and a spot is recorded on the detector.
We can use the Ewald construction to understand

- what diffraction images look like
- how to collect a complete dataset without missing bits

Ewald sphere

Ewald construction helps to understand

- what diffraction images look like
- how to collect a complete dataset without missing bits

We can imagine the reciprocal lattice sitting on the crystal on the camera, and rotating as the crystal rotates

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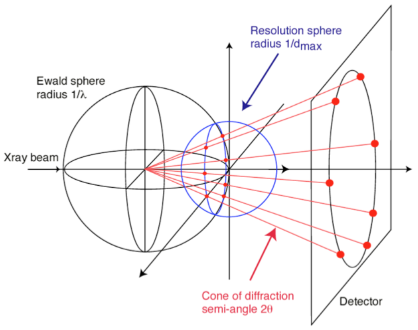


Detector position

For a maximum resolution of d_{max}, all diffraction vectors **S** must lie within a resolution sphere of radius $1/d_{max}$

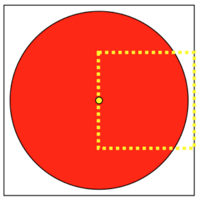
As the crystal rotates, the diffracted beams all lie within a cone of semi-angle $2\theta_{max}$

$\lambda/d_{max} = 2 \sin \theta_{max}$

A detector centered on the beam collects the whole cone

This gives optimum efficiency and simple strategy

The corners of a square detector collect incomplete data

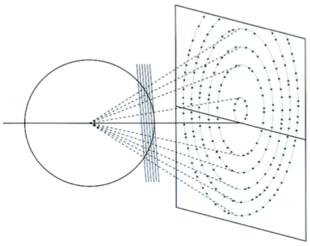


For long axes (close spot separation) it may be necessary to use a long detector distance and an offset detector

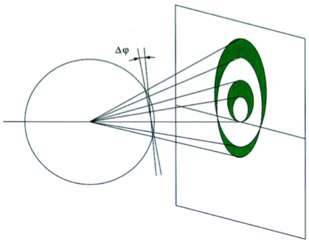
This gives a lower efficiency, and to get complete data requires a complicated strategy

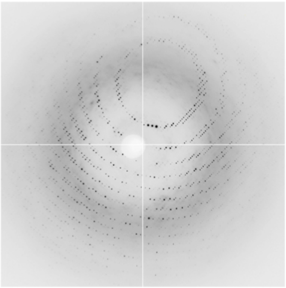
The appearance of diffraction images

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

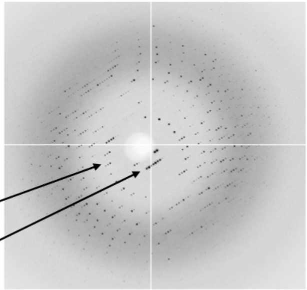


If the crystal is rotated through a small angle,each circle is broadened into a ***lune***. All the spots in a lune belong to one plane of the reciprocal lattice (not necessarily a principal plane)



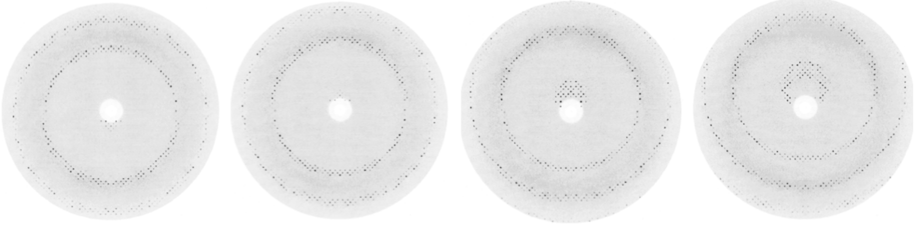


Examples of lunes in rotation images

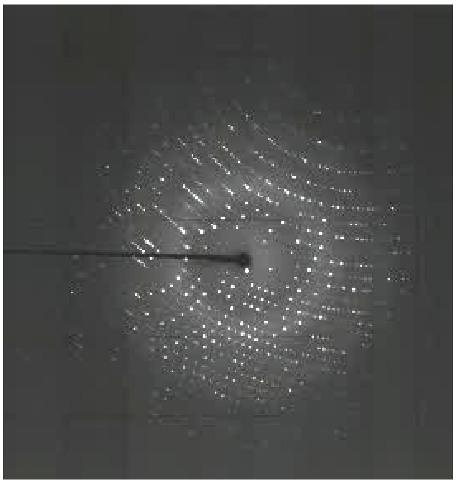


Lunes corresponding to different sets of planes

A series of successive rotation images showing the lunes moving up the image



A diffraction movie of lysozyme diffraction



These images were recorded by a CMOS flat panel detector C7942 by Hamamatsu Photonics. The crystal was cooled by liquid nitrogen and rotated by 0.5 deg/frame. The data were recorded at BL38B1 in SPring-8. (http://yagi.spring8.or.jp/lysozyme.html)

The size of spots in reciprocal space and on the detector

Real observed diffraction is complicated by the imperfections of real crystals and X-ray beams

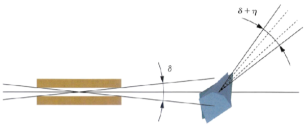
The X-ray beam
- the incident beam has a finite width and is not exactly parallel ***(beam divergence)***
- the beam is not entirely monochromatic ***(dispersion)***

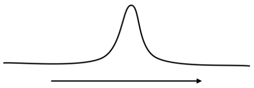
The crystal
- the crystal has a finite size
- the crystal is not perfect, but may be considered a mosaic of blocks in slightly different orientations ***(mosaicity)***

The effect of these factors can be considered as a broadening of the reciprocal lattice points, giving them a non-zero size and therefore a finite reflecting range

The size of spots in reciprocal space and on the detector

Beam divergence δ and mosaicity η
add up to increase the angular width
of the diffracted beam

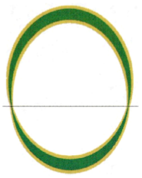




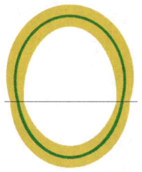
rotation angle ϕ

Reflection width in rotation
= δ + η + geometric factor

(geometric factor depends on angle between the rotation axis & **S**)

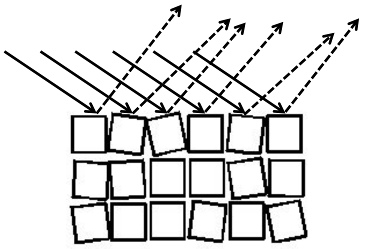


High mosaicity causes
broadening of the lunes
Most obvious along the
rotation axis



Crystal Mosaicity (η)

angular measure of the degree of long-range order of the unit cells within a crystal. Lower mosaicity indicates better ordered crystals and hence better diffraction



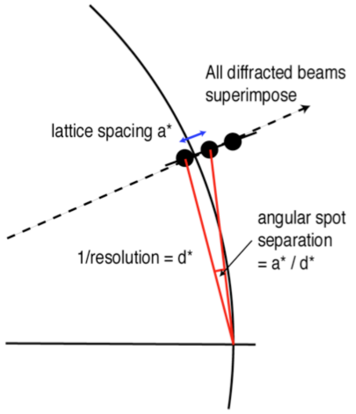
Highly exaggerated representation
of crystal mosaicity

Typical values for a protein crystal 0.05° - 0.7 degree

Overlaps and rotation range

- Current integration programs assume that spots are resolved, both on the detector and on rotation ϕ. This means that the intensity goes down to background all round the spot
- Resolution is a problem for large unit cells, high mosaicity and high resolution
- Overlap between spots on the detector is easy to see, but to understand
- Overlap on ϕ we need to look in reciprocal space

Overlaps and rotation range



When a closely-spaced row of spots (eg along a*) is moving perpendicularly into the sphere, their diffracted beams almost coincide.
The spots are on top of each other on the detector, and are only separated on ϕ

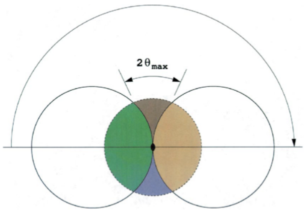
Maximum slice width = (a*/d*) - w = d/a -w
w = reflection width = δ + η

Example:

cell = 200Å,
resolution = 2Å,
width = 0.3°
Maximum Slice = 0.27°

If possible, orient a long axis along the rotation axis to minimise overlap problems

Completeness: total rotation range and the blind region



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

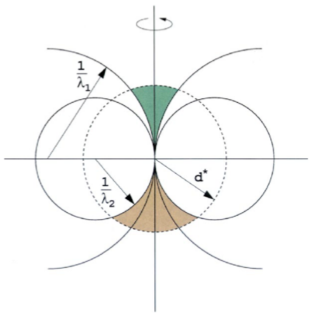
In a rotation of 180° above, the lower boundary of the initial sphere sweeps out the volume coloured green & the upper boundary the light brown part. The dark brown part is measured twice, and the blue part not at all

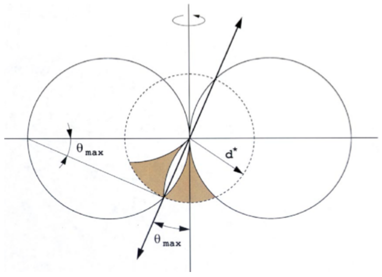
Because of Friedel's law, this dataset is complete (apart from the *blind region*), but if complete anomalous differences are required, then 180° + 2θmax is required (unless there is symmetry)

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



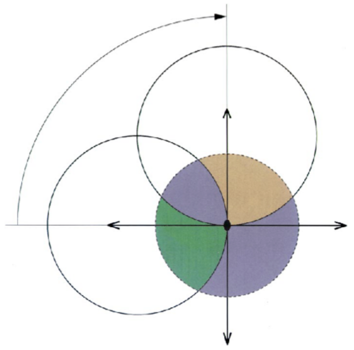


The blind region may be filled in by collecting a second set of data, offsetting the crystal by at least θ_{max} or by symmetry (except in P1)

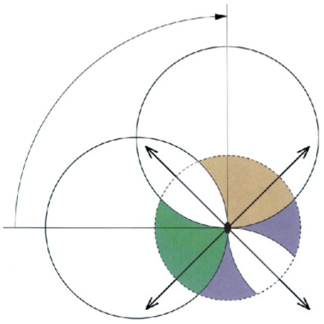
If there is symmetry, offsetting from an axis can remove or reduce the blind region for a single setting

Symmetry and 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



Summary of strategy choices

Detector position

- Place detector far enough away to resolve spots (or reduce beam size)
- Use the whole detector area (don't have blank region around edge)
- Don't use an offset detector unless desperate for spot resolution. If you have to offset the detector, be ***very careful*** in strategy planning

Total rotation range

- If possible, collect 180° (360° in P1 with anomalous). High redundancy is *Good*, provided that radiation damage is not serious
- When rotating around (or close to) a symmetry axis of order n, the minimum rotation is about 360°/n (more complicated in dihedral or cubic symmetry)
- With an offset detector, a larger rotation range is needed, as only one surface of the Ewald sphere is active rather than two

Summary of strategy choices

Crystal orientation and rotation start point
- To remove the blind region, avoid rotating exactly around a symmetry axis
- To optimise anomalous differences with respect to absorption, rotate exactly around a symmetry axis (even-fold only)
- Use a strategy program to determine range and start point
- Collect 180° or 360° and start anywhere

Image rotation range (slicing)
- Use a strategy program to determine optimum width
- Set width < (maximum resolution)/(longest axis not along spindle) - spotwidth
- Process data & check for overlaps
- Fine-slicing is more sensitive to readout noise and errors in synchronisation of shutter opening and rotation (this potentially adds an error for each image) but this is not an issue with Pilatus detectors.

2D vs 3D Data Integration and Coarse vs Fine phi slicing

In 2D integration, the intensity of partially recorded reflections is evaluated separately on each image and only summed at the data scaling stage.

In 3D integration, the different parts of a partially recorded reflection on different images are assembled by the integration program to give a 3D profile (shoebox) of the reflection which is then integrated to yield a "fully recorded" intensity.

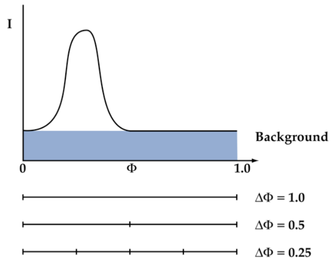
Coarse phi slicing uses a rotation angle per image that is greater than the mosaic spread (plus beam divergence), so there will be some fully recorded reflections.

Fine phi slicing uses a rotation angle per image that is significantly less than the crystal mosaic spread (eg less than half), so that **all** reflections are partially recorded in that they are spread over several images.

Note that fine sliced data can be processed using either 2D or 3D integration methods, because with current software there is no difficulty scaling data that has no fully recorded reflections (this was not always the case).
(See J. Pflugrath, Acta Cryst **D55**, 1718-1725, 1999 for a discussion of fine phi slicing).

Advantages of fine phi slicing

Using an oscillation angle smaller than the reflection width (in phi) will improve signal to noise for weak reflections by minimising the background:



$$I_{spot} = I_{tot} - I_{bck}$$

$$var(I_{spot}) = var(I_{tot}) + var(I_{bck})$$

$$= I_{tot} + I_{bck}$$

$$I/\sigma(I) = (I_{tot} - I_{bck})/(I_{tot} + I_{bck})^{1/2}$$

For weak reflections, the background will dominate I /σ(I).

Fine phi slicing *should* give better data but:

1) Assumes no errors in shutter synchronisation (demanding for very short (<0.5 sec) exposures).
2) There will be detector readout noise in each image (except Pilatus).

(see J. Pflugrath, Acta Cryst **D55**, 1718-1725, 1999).