Diffraction Geometry

- Diffraction from a crystal - Laue equations
- Reciprocal lattice
- Ewald construction
- Data collection strategy

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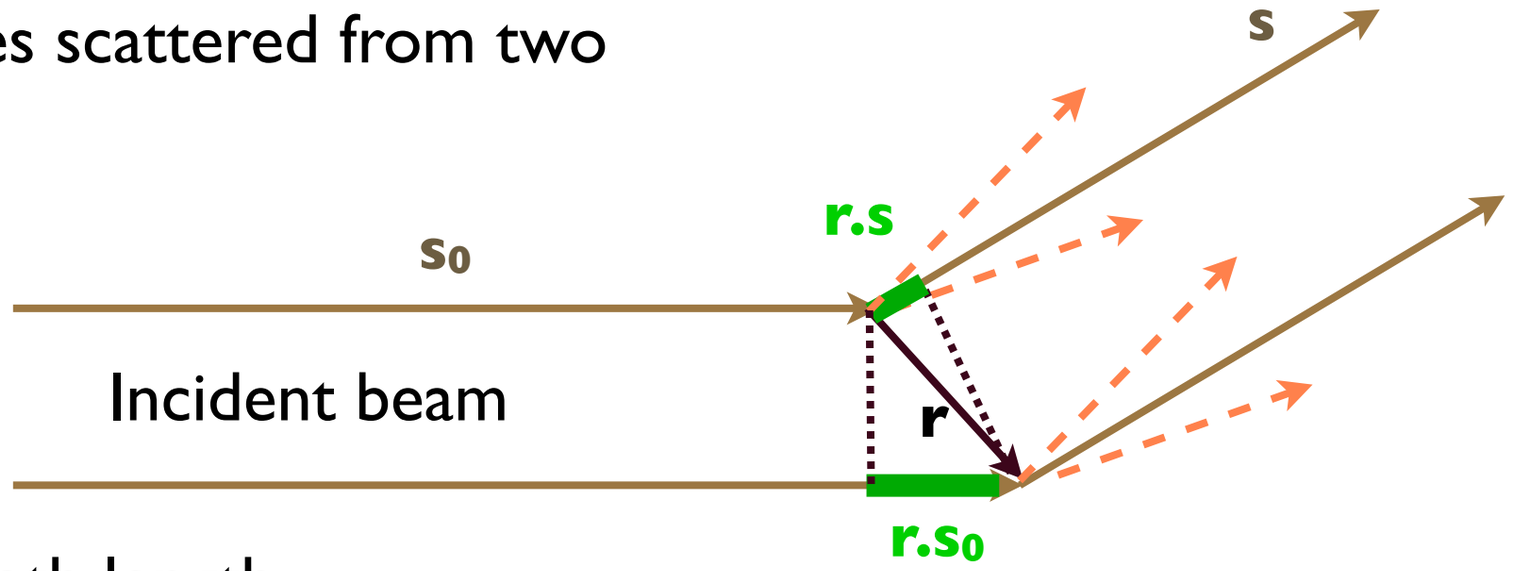
Diffraction conditions & explanation of the Ewald sphere construction

Scattering from two electrons

Path length difference for waves scattered from two points **r** apart in direction **s**

$\delta L = \mathbf{r.s} - \mathbf{r.s_0} = \mathbf{r.(s - s_0)}$



X-rays scattered in all directions

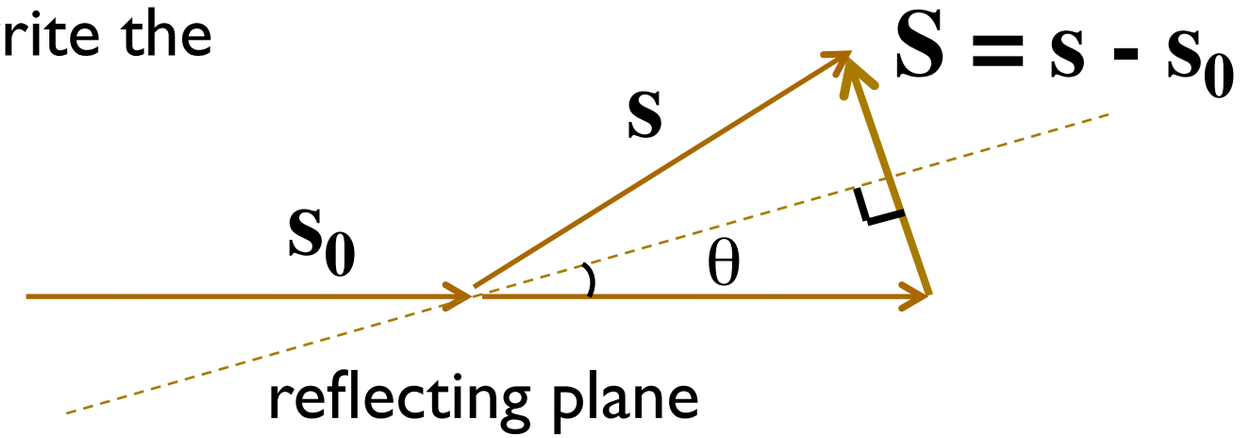
Phase shift corresponding to path length difference δL for a wave with wavelength λ

$= 2\pi$ (path difference)$/ \lambda$

$= 2\pi\ \delta L / \lambda = 2\pi\ \mathbf{r.(s - s_0)}/ \lambda$

If we make the length of the wave vectors $\mathbf{s_0}$ and $\mathbf{s} = \mathbf{1}/ \lambda$, ie $|\mathbf{s_0}| = |\mathbf{s}| = \mathbf{1}/ \lambda$, then we can write the phase shift $= 2\pi\ \mathbf{r.S}$ where $\mathbf{S} = \mathbf{s - s_0}$

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



Diffraction from a one-dimensional crystal

Total wave scattered from n+1 points at $\mathbf{r}_j$ (j = 0, n) is given by

$$\mathbf{F(S)} = \sum_j \exp(i\, 2\pi\, \mathbf{r}_j.\mathbf{S})$$

where $(2\pi\, \mathbf{r}_j.\mathbf{S})$ is the relative phase for the wave from the j'th point

adding up waves represented as $\exp(i\,\varphi) = \cos(\varphi) + i \sin(\varphi)$

A one-dimensional crystal is defined by a series of points at $\mathbf{r}_j = j\,\mathbf{a}$

where **a** is the vector defining the unit cell

then $\mathbf{F(S)} = \sum_j \exp(2\pi i\, \mathbf{r}_j.\mathbf{S})$

$= \sum_j \exp(2\pi i\, j\mathbf{a}.\mathbf{S})$

In general, this is the sum of a series of complex numbers of the same magnitude, but in random directions, so for large n, $|\mathbf{F(S)}| \approx 0$

$$\mathbf{F(S)} = \sum_j \exp(2\pi i\, j\, \mathbf{a} \cdot \mathbf{S}) \quad \text{for } j = 0, n$$

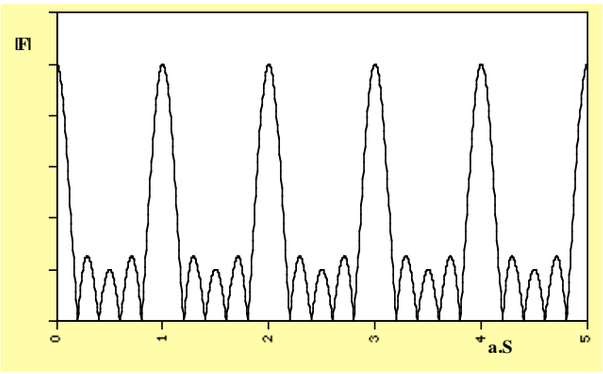
Random

In phase

For large n, $|\mathbf{F(S)}|$ is only non-zero if $\mathbf{a.S}$ is integral, when the terms in the summation add up

$$\mathbf{a.S} = h \qquad (h \text{ is an integer})$$

Note that this is not true for a crystal with few unit cell repeats



n=4

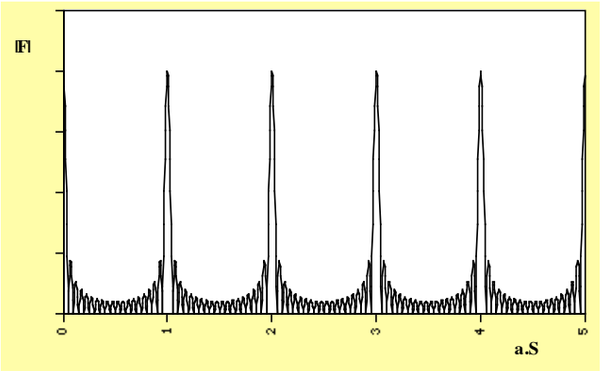
The Reciprocal Lattice

For a given cell repeat **a**, **S** can only take values

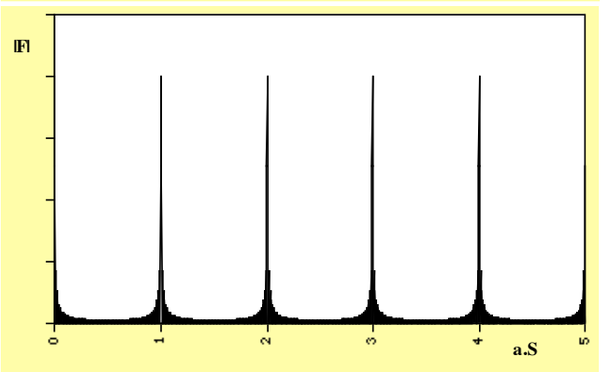
$\mathbf{S} = h\,\mathbf{a^*}$ where $\mathbf{a}.\,\mathbf{a^*} = 1$

a* defines a lattice with spacing **inversely** proportional to the real cell spacing, since

$|\mathbf{a^*}| = 1 / |\mathbf{a}|$



n=19



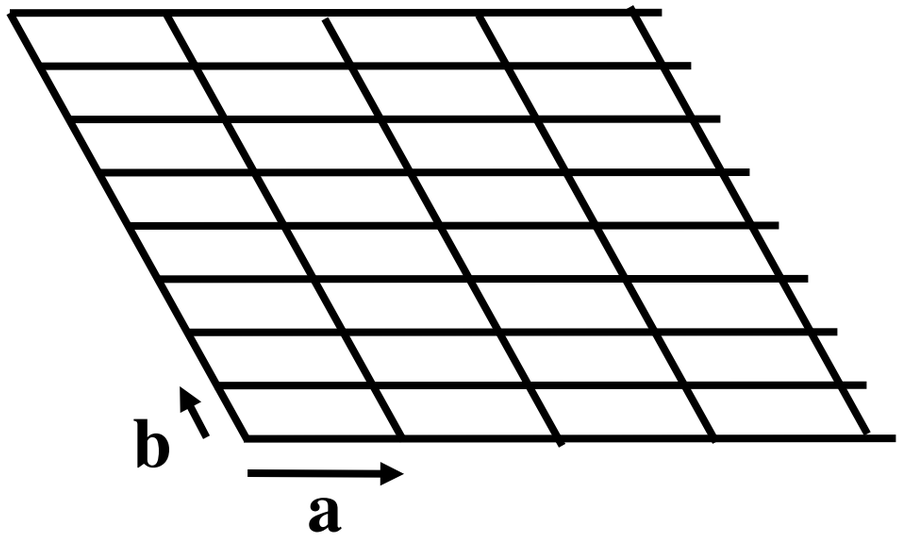
n=99

Three dimensional crystals

A 3-dimensional crystal is defined by three vectors, **a, b, 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

$$\mathbf{F(S)} = \sum_u \sum_v \sum_w \exp(2\pi i\, \mathbf{r_{uvw}} \cdot \mathbf{S})$$

$$= \sum_u \exp(2\pi i\, u\, \mathbf{a} \cdot \mathbf{S}) \sum_v \exp(2\pi i\, v\, \mathbf{b} \cdot \mathbf{S}) \sum_w \exp(2\pi i\, w\, \mathbf{c} \cdot \mathbf{S})$$

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

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

These are the Laue equations

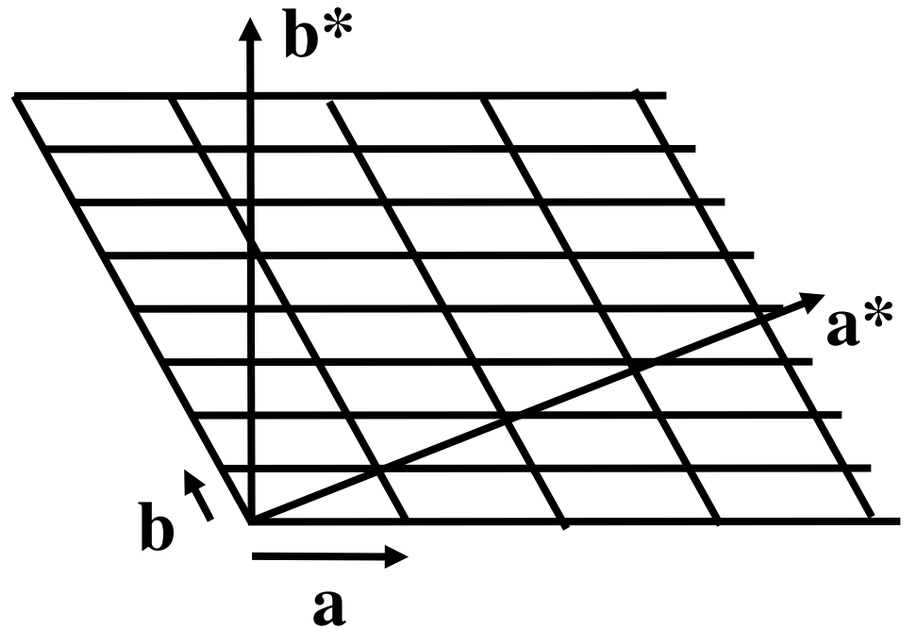
The Laue equations define a **reciprocal lattice** of allowed values of **S**, based on the "reciprocal" vectors **a*, b*, c***

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

a.a* = 1 **a.b*** = 0

b.b* = 1 **b.c*** = 0

c.c* = 1 **c.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)

a* = **(b** × **c**)/V **b*** = **(c** × **a**)/V **c*** = **(a** × **b**)/V V = cell volume

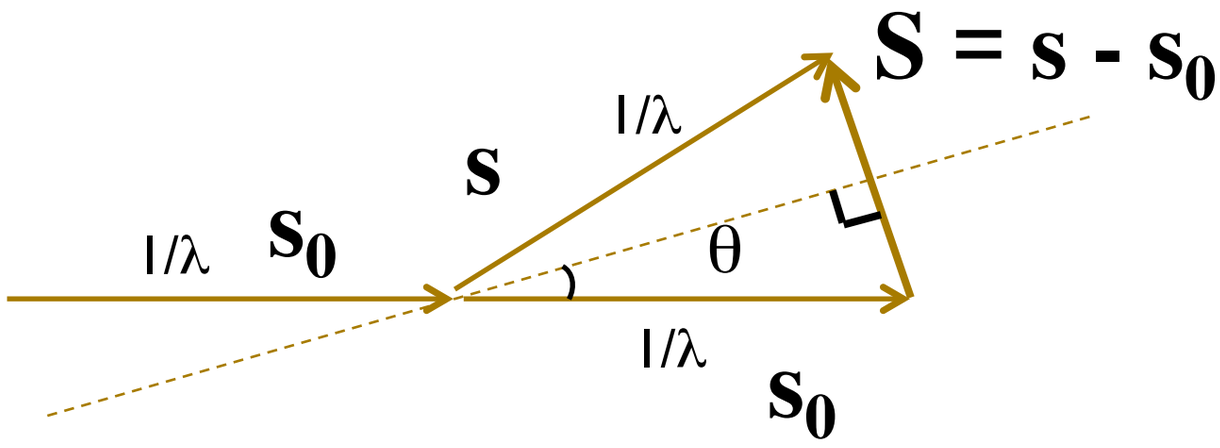
|**a***| is the spacing between the (bc) planes, |**b***| between the (ca) planes,

|**c***| between the (ab) planes

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

|**a***| = 1/|**a**|, |**b***| = 1/|**b**|, |**c***| = 1/|**c**|

The Ewald Sphere Construction



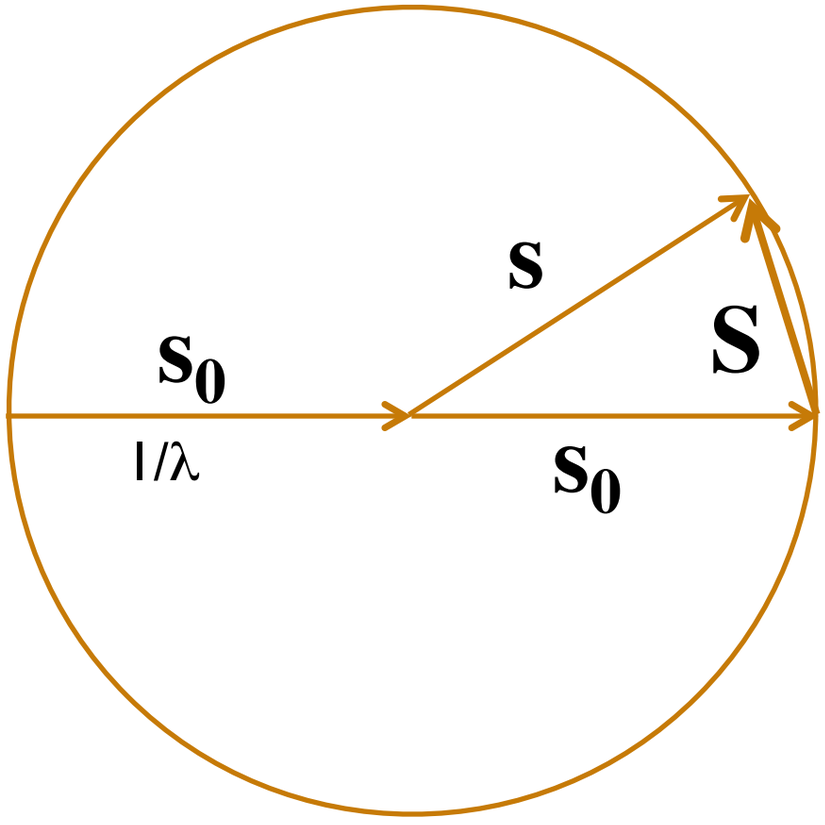
The general condition for diffraction is illustrated by the vector equation

$\mathbf{S} = \mathbf{s} - \mathbf{s_0}$

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

$\mathbf{S}$ is the diffraction vector in *reciprocal space*

For a crystal, $\mathbf{S}$ may only take certain values,
$\mathbf{S} = h\,\mathbf{a^*} + k\,\mathbf{b^*} + l\,\mathbf{c^*}$



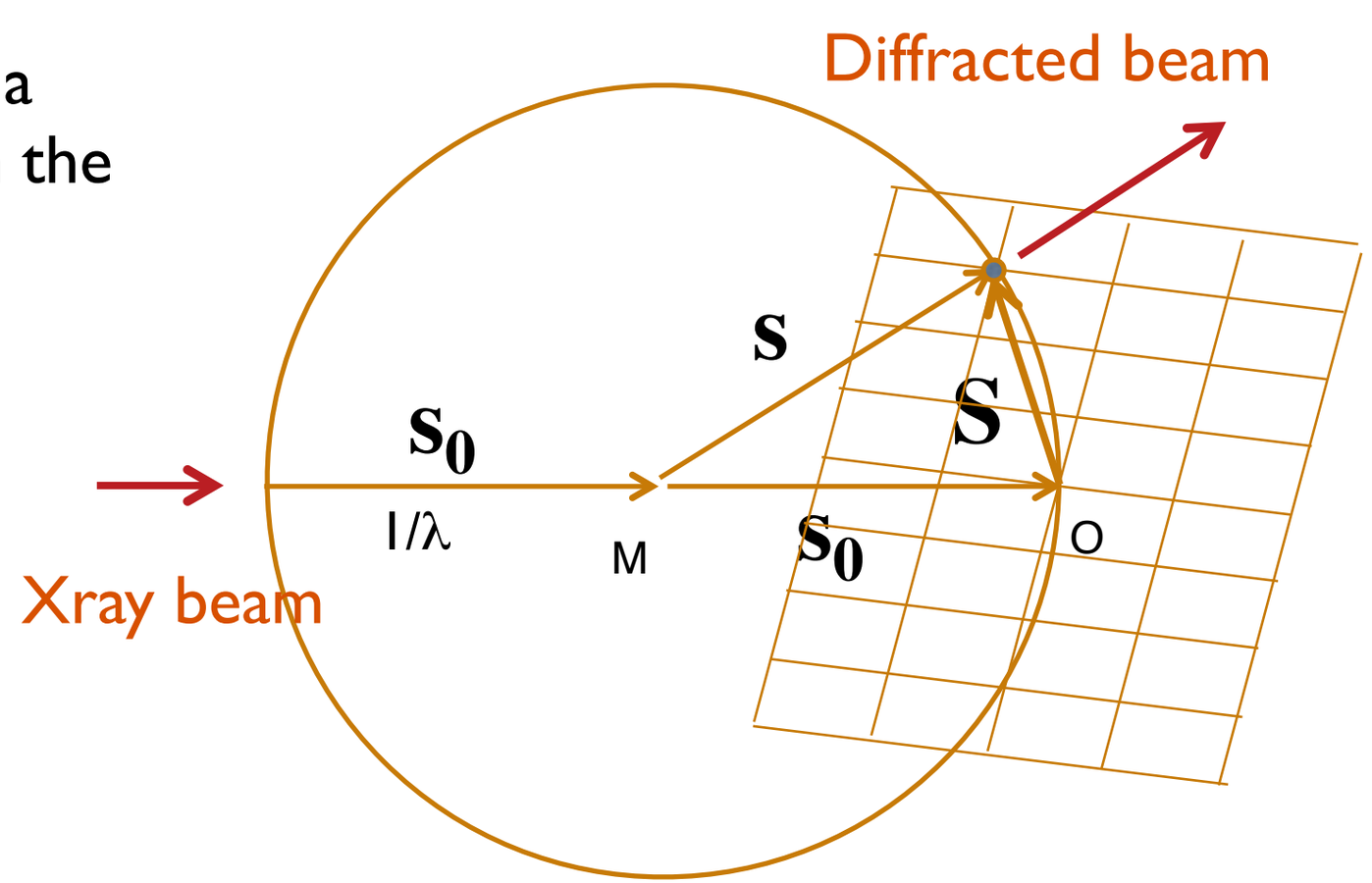
The Ewald sphere is only a construction but is very useful to understand the *geometry* of diffraction. Confusingly, it has two origins:-

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 beam **s**

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

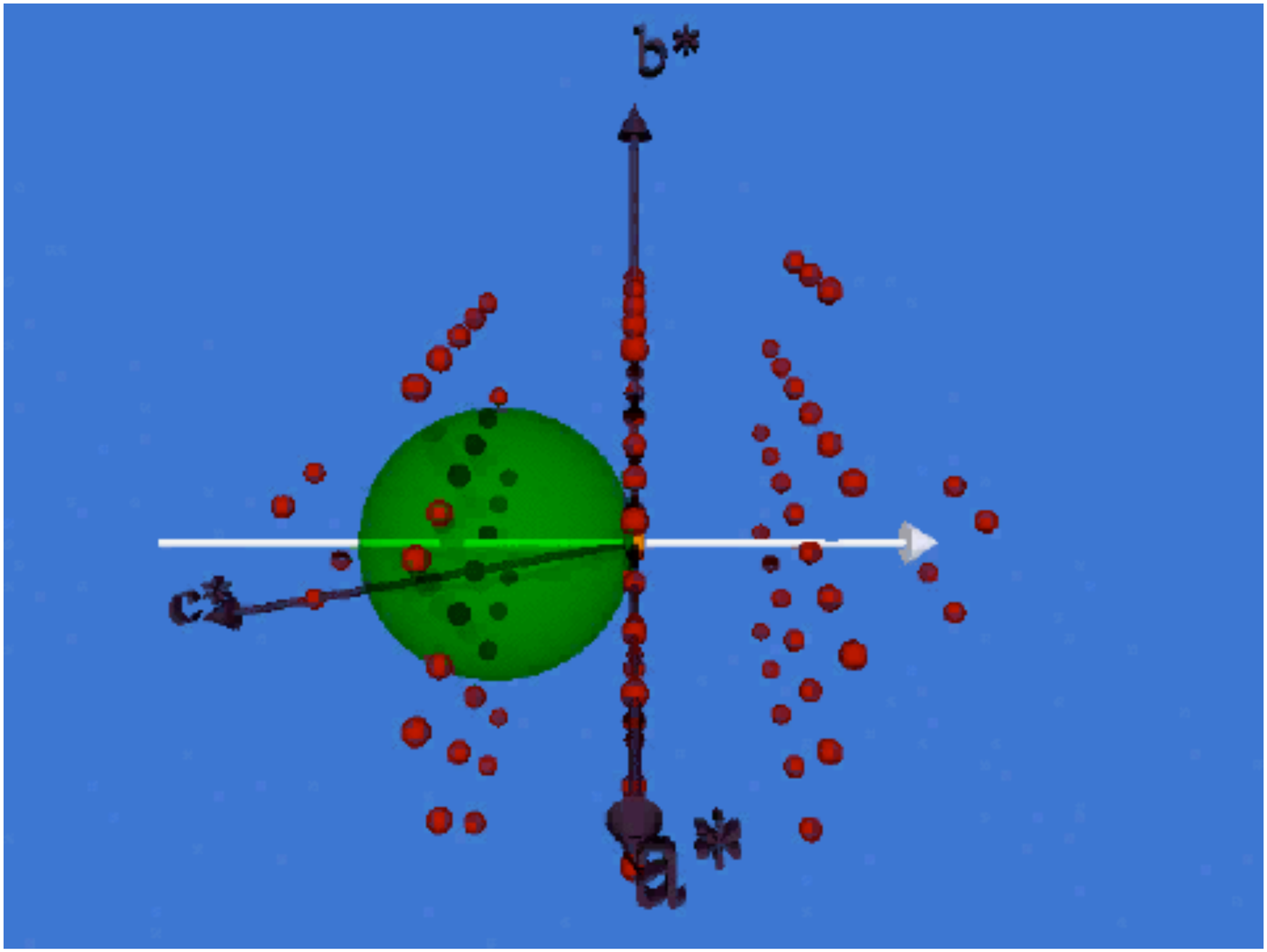
As the crystal rotates, the reciprocal lattices rotates in exactly the same way

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

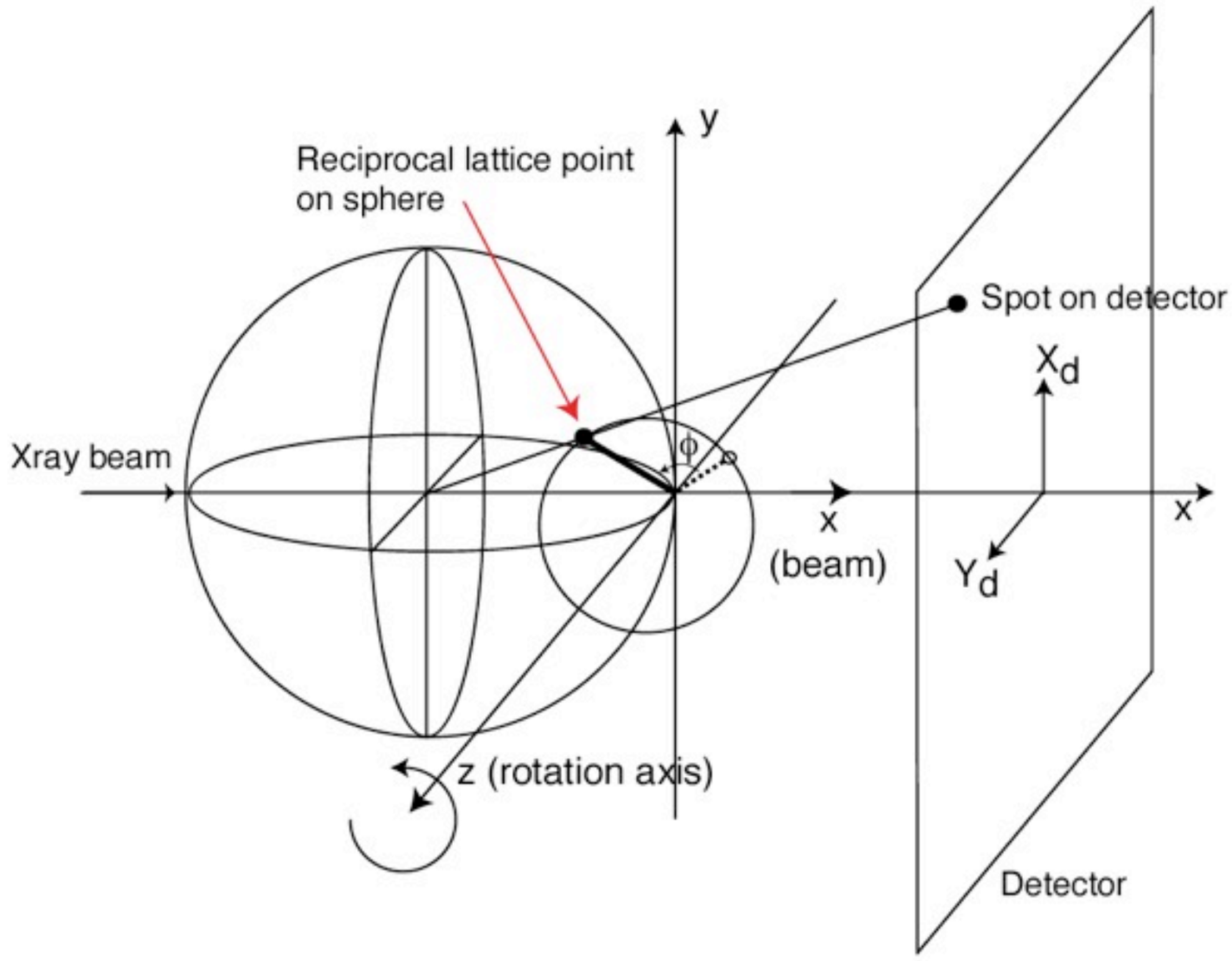


As the crystal rotates, so does the the reciprocal lattice

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



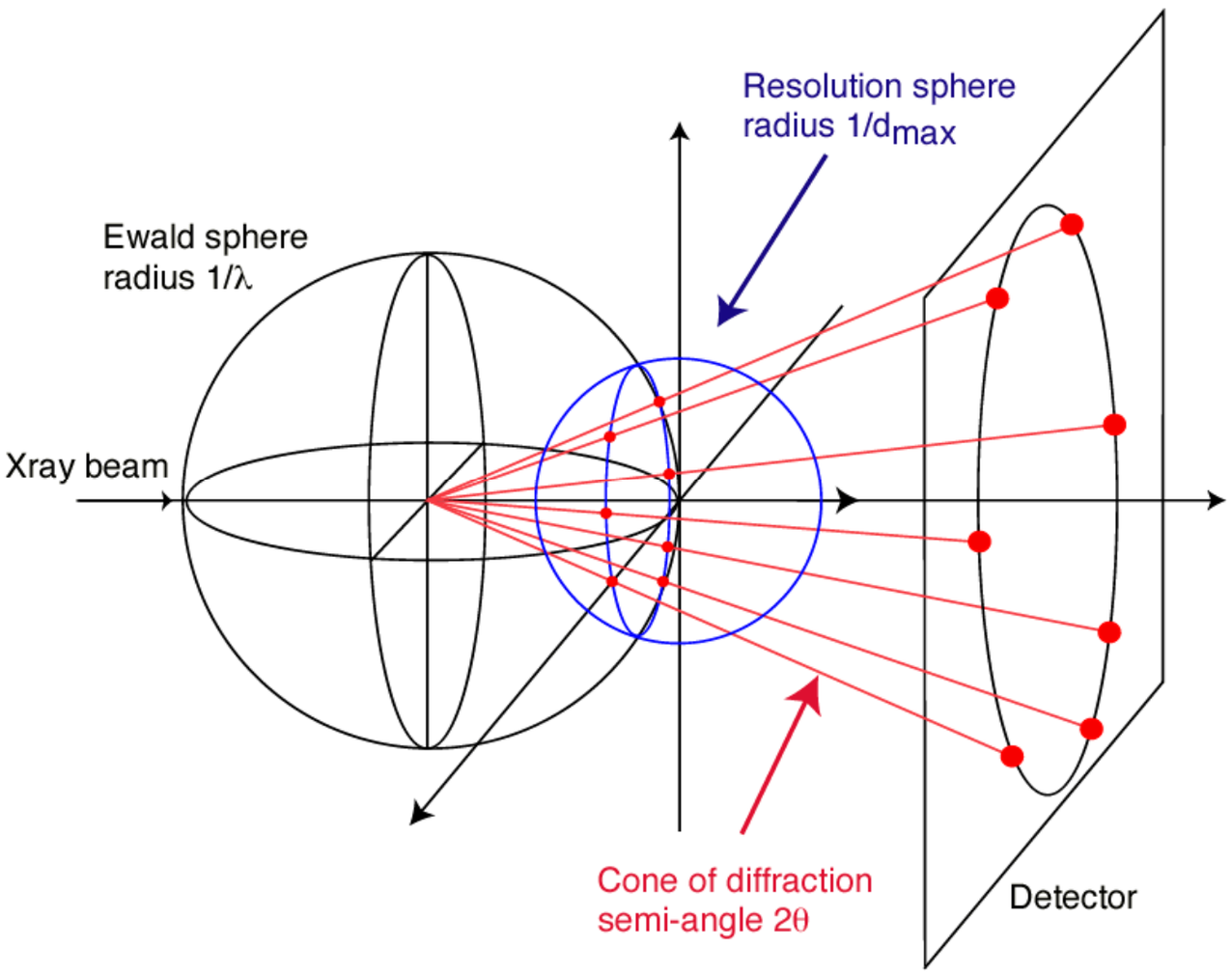
As the crystal rotates, each lattice point in turn passes through the sphere, and a spot is recorded on the detector

We can use the Ewald construction 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

Detector position



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

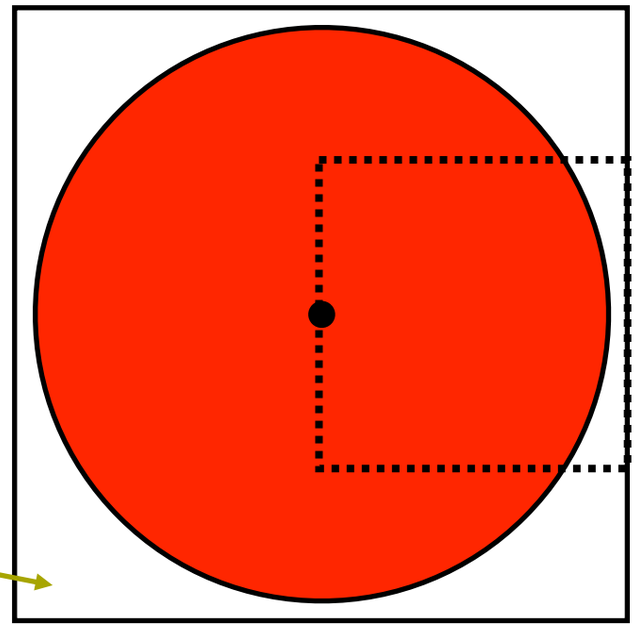
As the crystal rotates, the diffracted beams all lie within a cone of semi-angle $2\theta_{max}$

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

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

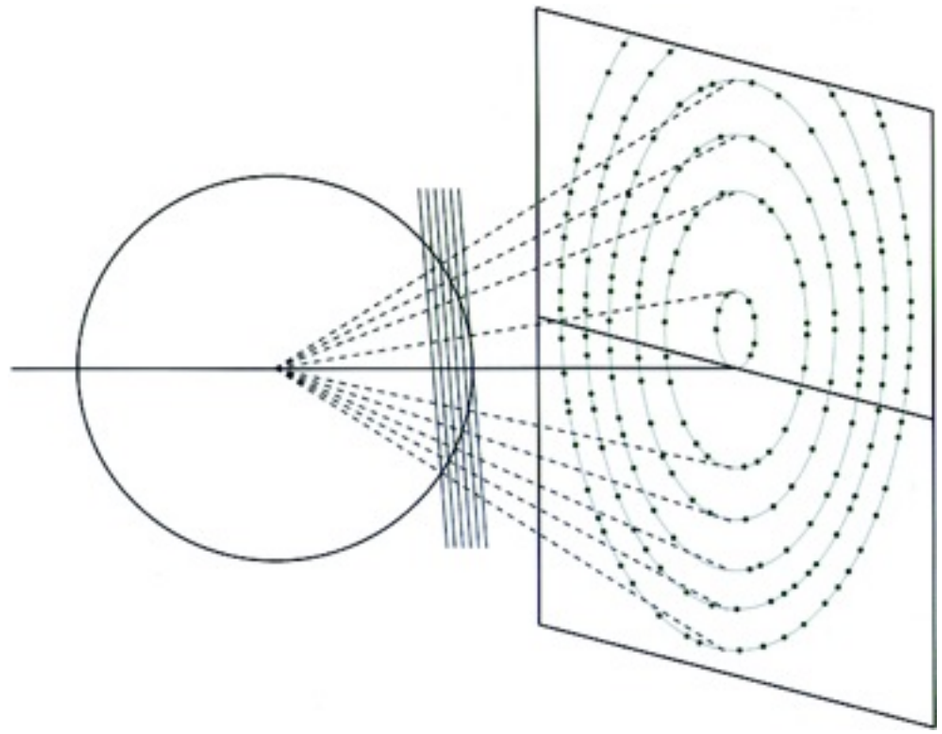


For long axes (close spot separation) it may be necessary to use a long detector distance and an offset detector

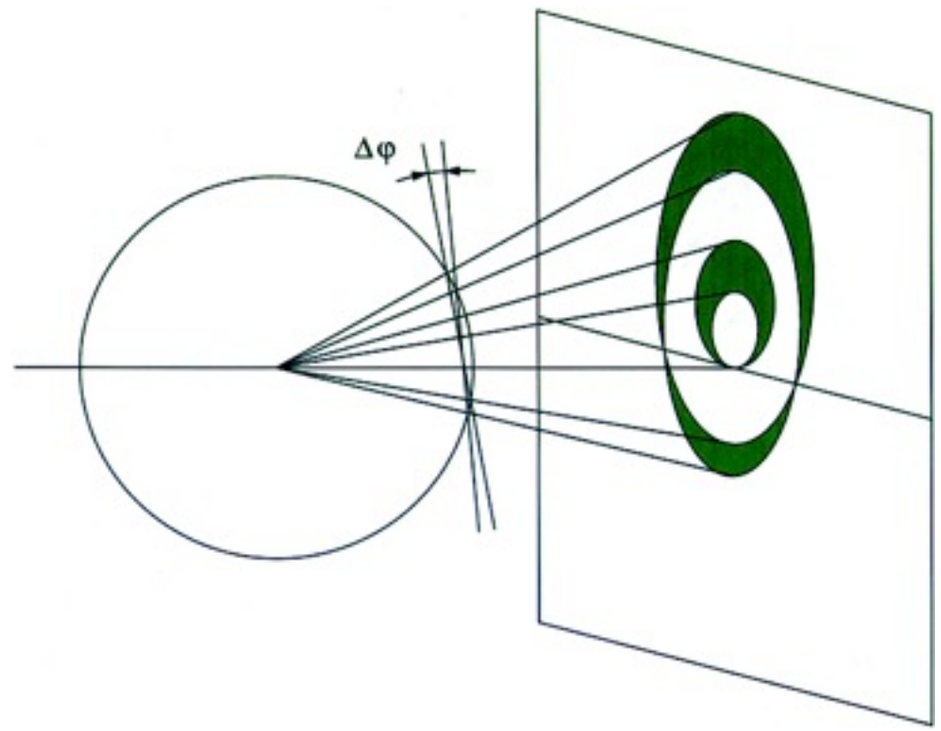
This gives a lower efficiency, and to get complete data requires a complicated strategy

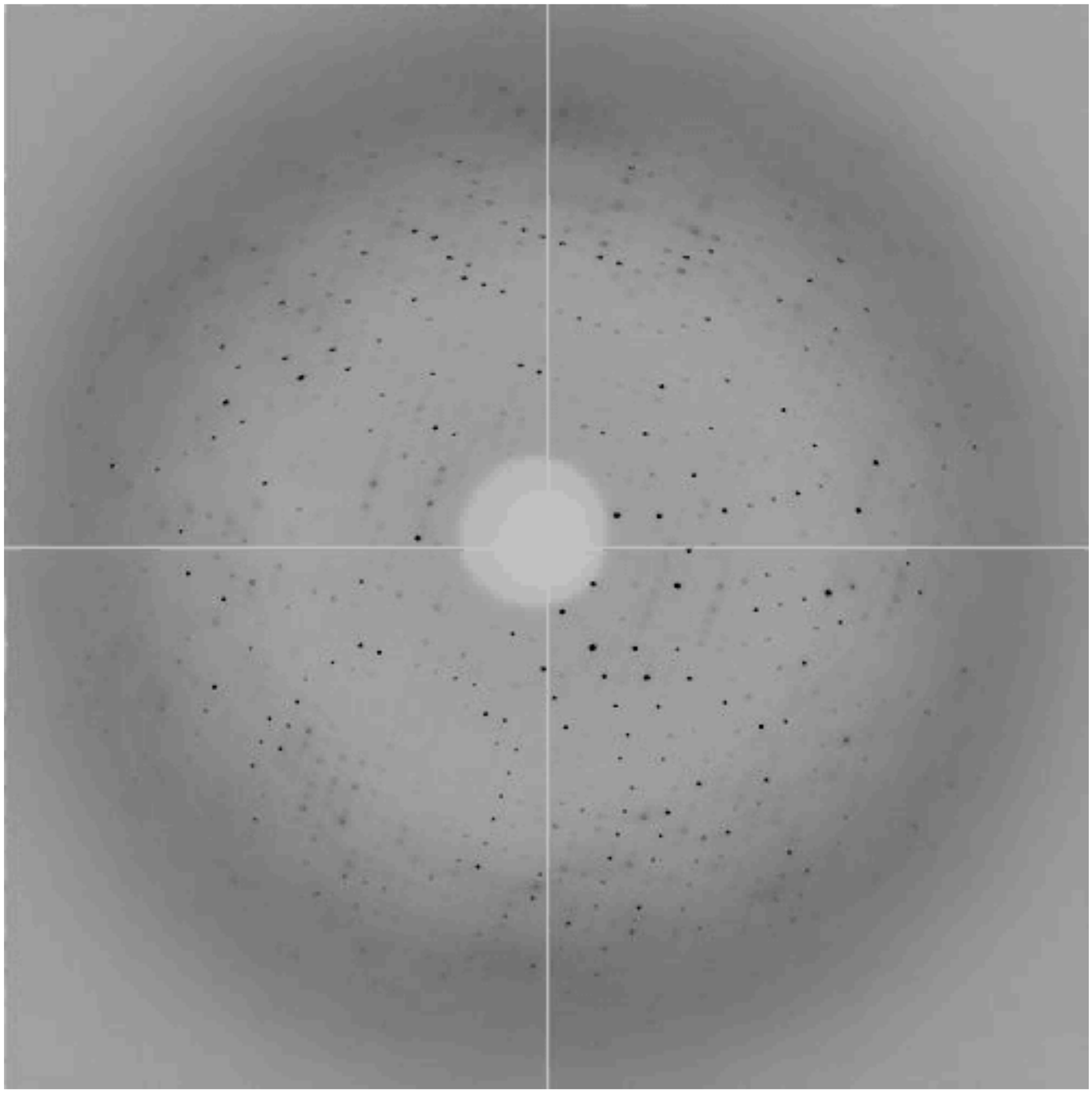
The appearance of diffraction images

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)





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.

The dataset should also be complete in dynamic range, including weak & strong spots, ie avoiding too many *overloads*, since the structure of the asymmetric unit is inferred from the measured intensities.

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

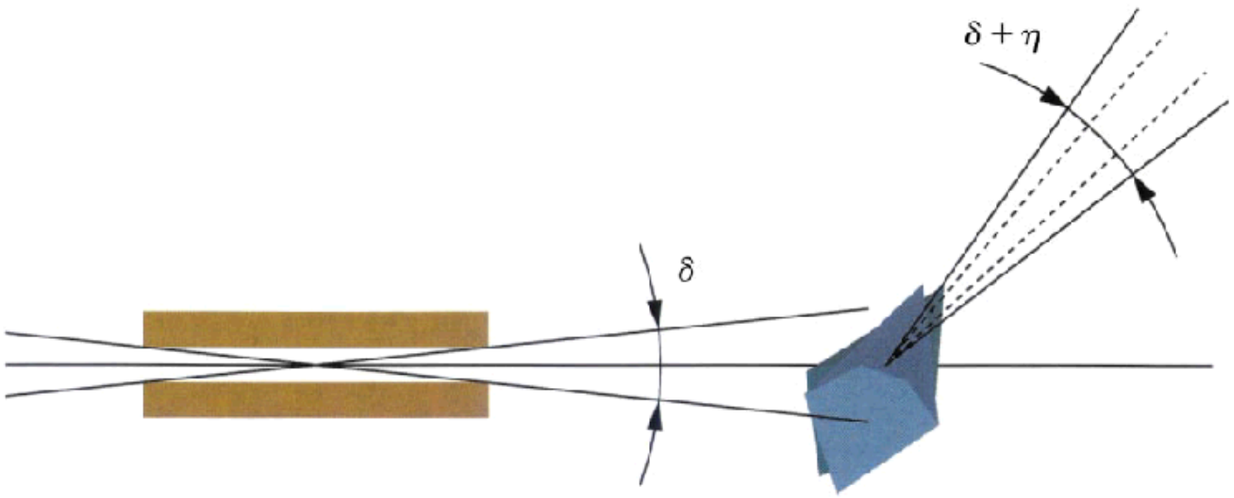
- the incident beam has a finite width and is not exactly parallel ***(beam divergence)***
- the beam is not entirely monochromatic ***(dispersion)***

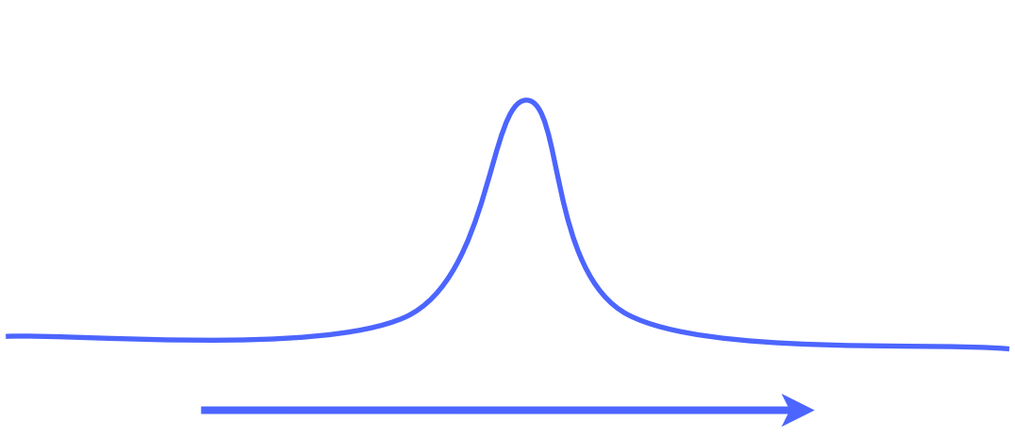
The crystal

- 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

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



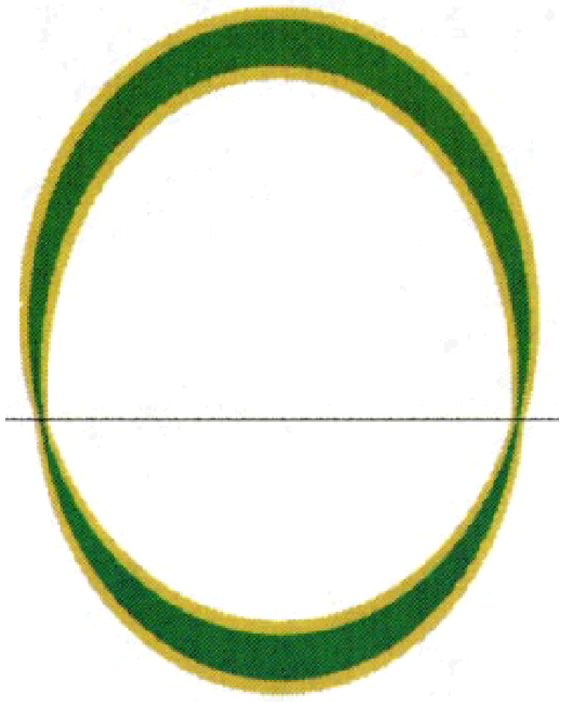


rotation angle ϕ

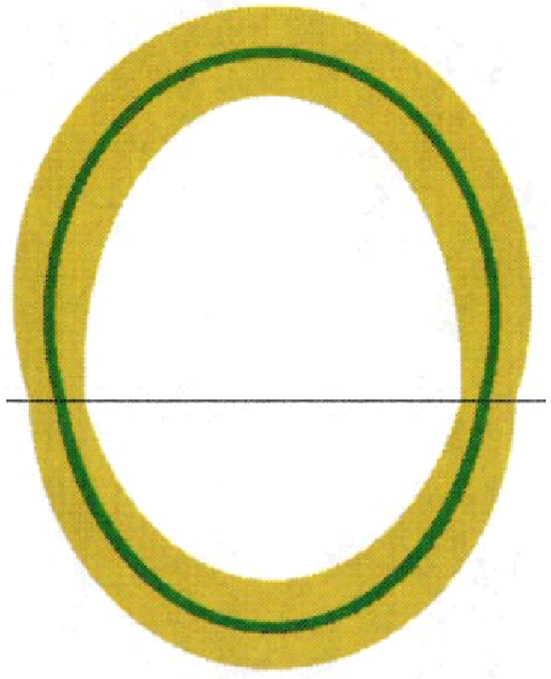
Reflection width in rotation

= δ + η + geometric factor

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



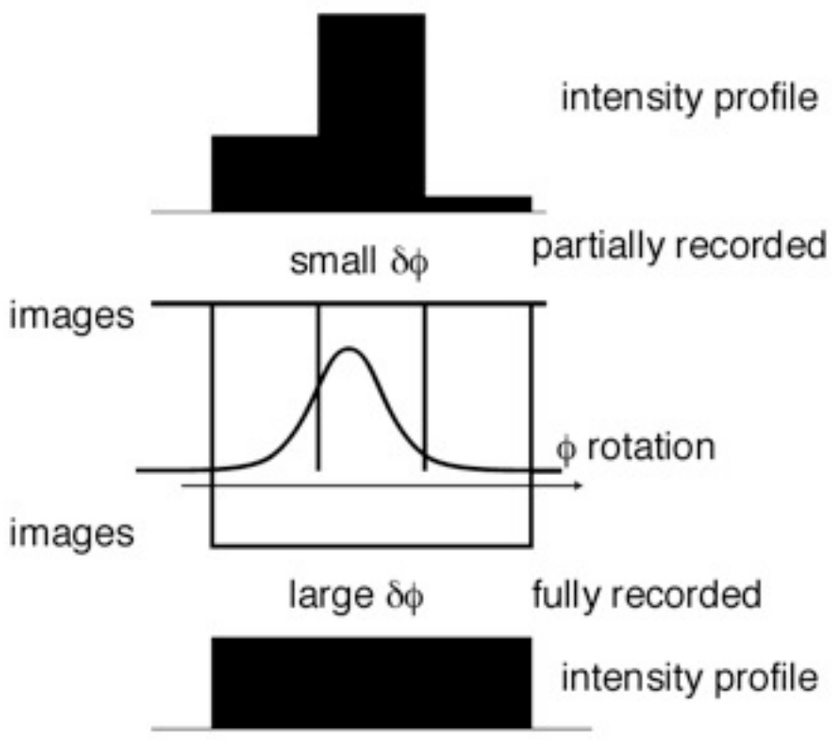
High mosaicity causes broadening of the lunes Most obvious along the rotation axis



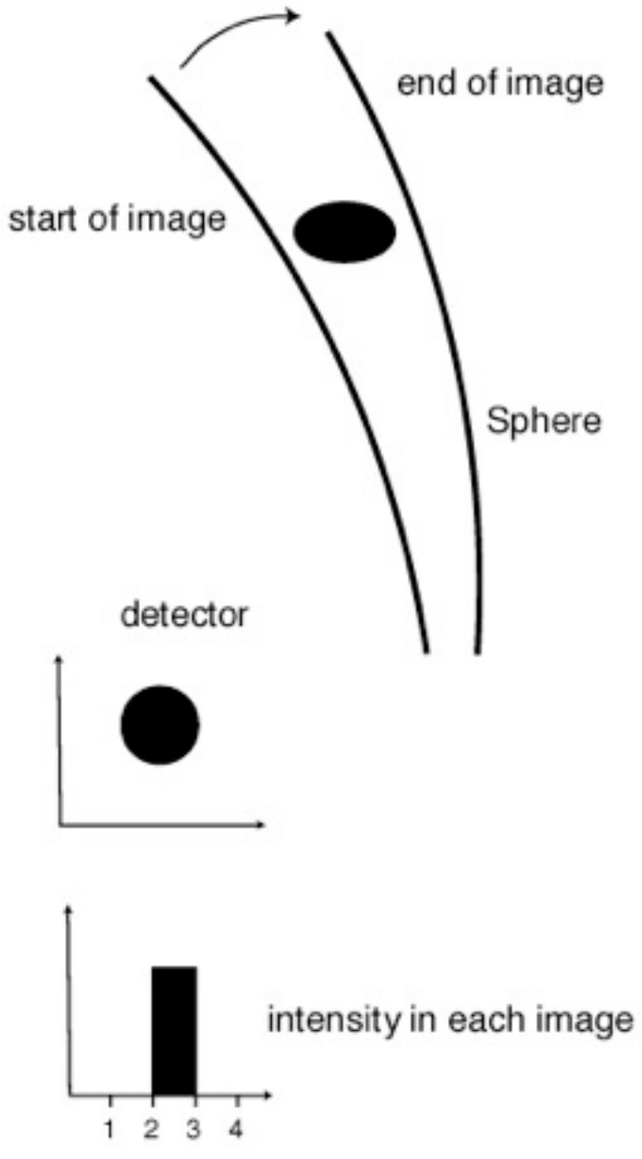
Images: fully recorded and partially recorded reflections

We want to determine the intensity of a reflection, integrated over its extent in reciprocal space by rotating the crystal so that the extended reciprocal lattice point passes through the sphere.

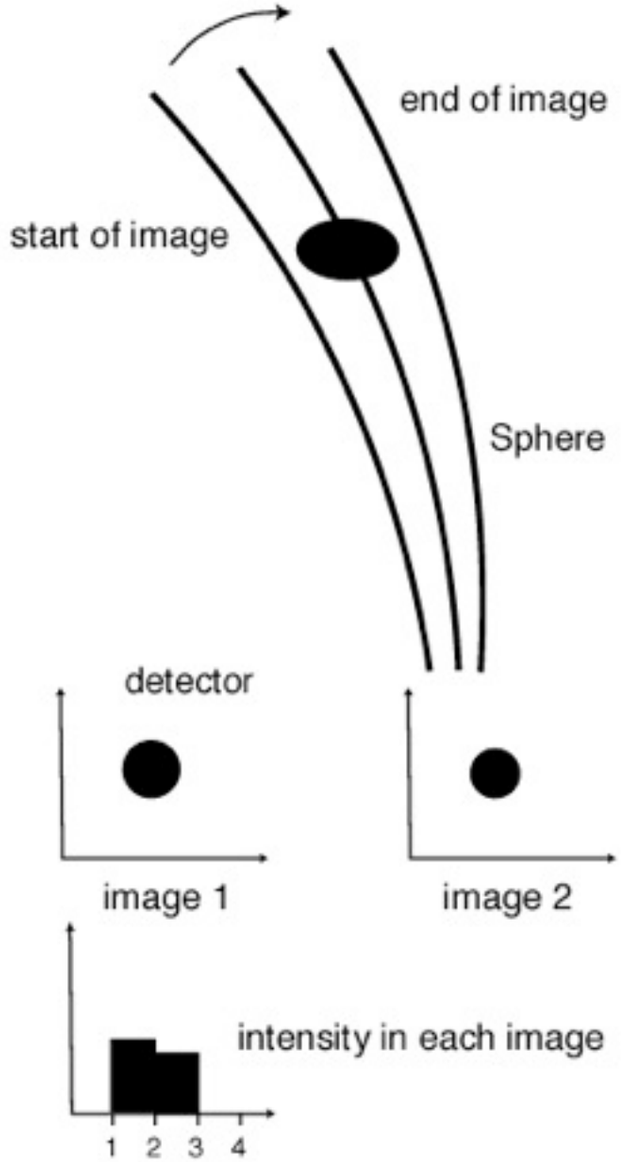
Our sampling of the 3-dimensional reciprocal space is in consecutive slices, typically of between about 0.1° and 1°. Some detectors (image plates, CCDs) take a significant time to read out, so then we have to close the shutter & stop the rotation (simultaneously!).



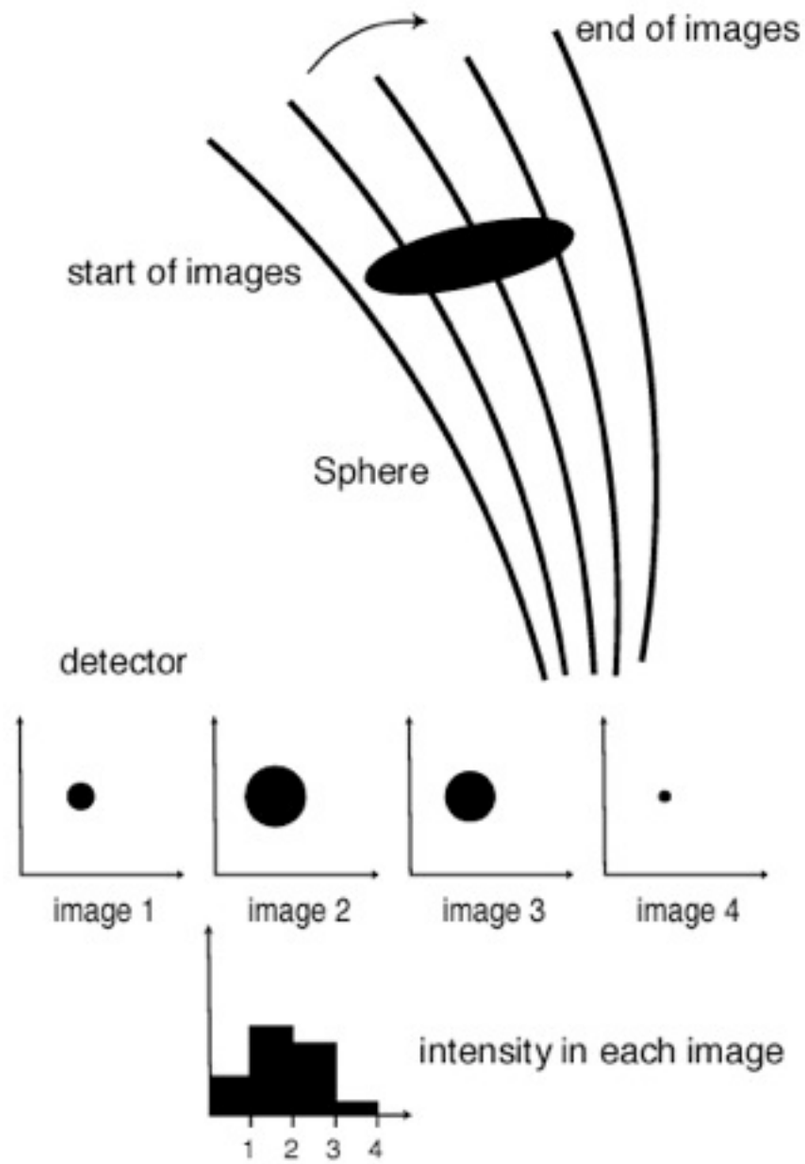
Depending on the slice width and the reflection width a reflection may occur on one image (*full* or *fully recorded*) or on several (*partial* or *partially recorded*)



A fully-recorded spot is entirely recorded on one image



Partials are recorded on two or more images



"Fine-sliced" data has spots sampled in 3-dimensions

Perhaps best processed with a 3D program (eg d*trek, XDS)

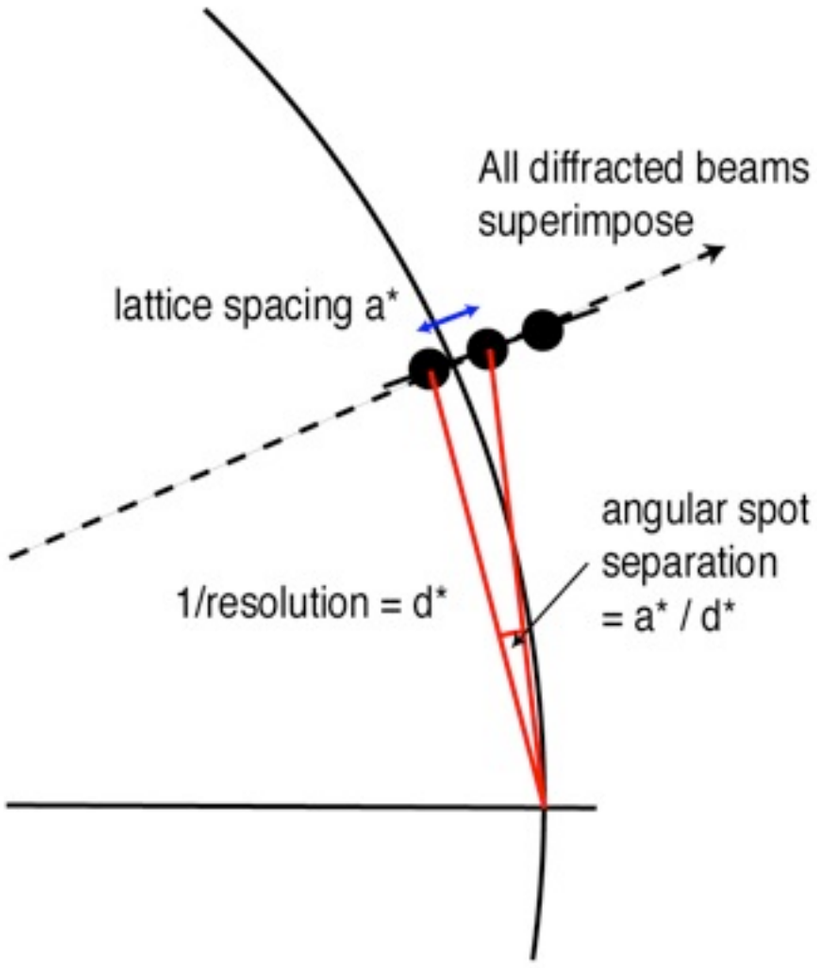
based on figure by Elspeth Garman

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



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

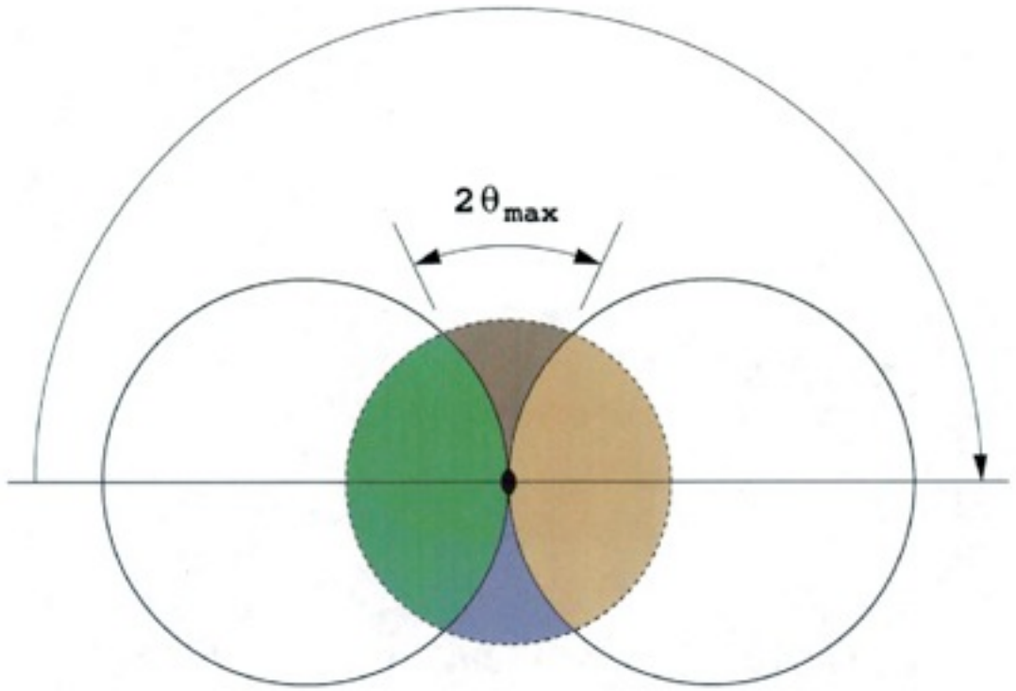
w = reflection width = $\delta + \eta$

eg 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

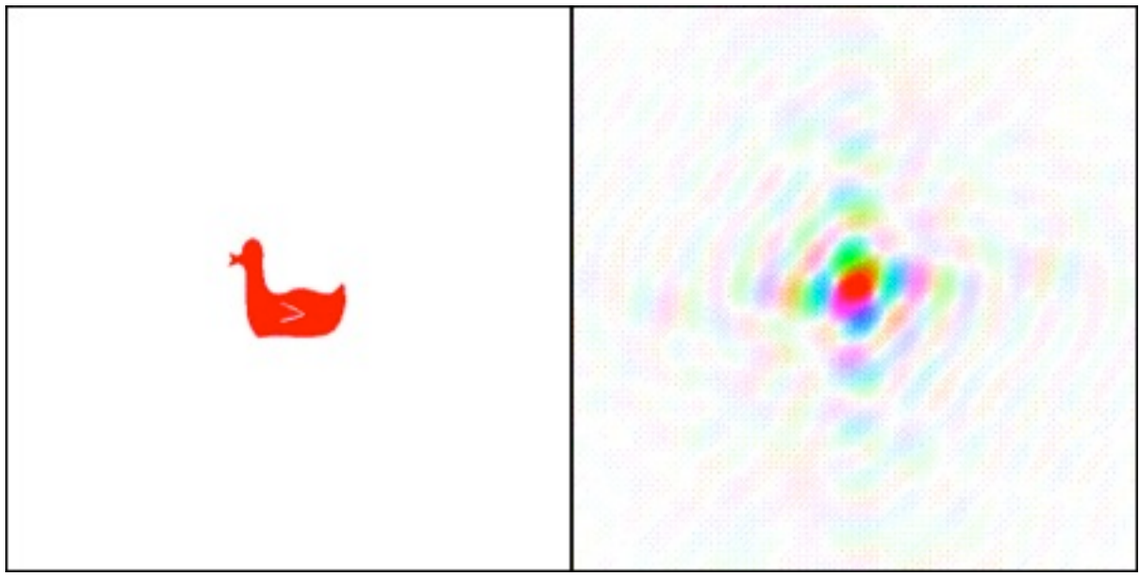


To use the Ewald sphere construction to understand which parts of reciprocal space are measured, it is easier to fix the "resolution sphere" of all reciprocal lattice points within a maximum resolution, and to rotate the Ewald sphere. The region collected is the volume swept out by the leading and trailing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

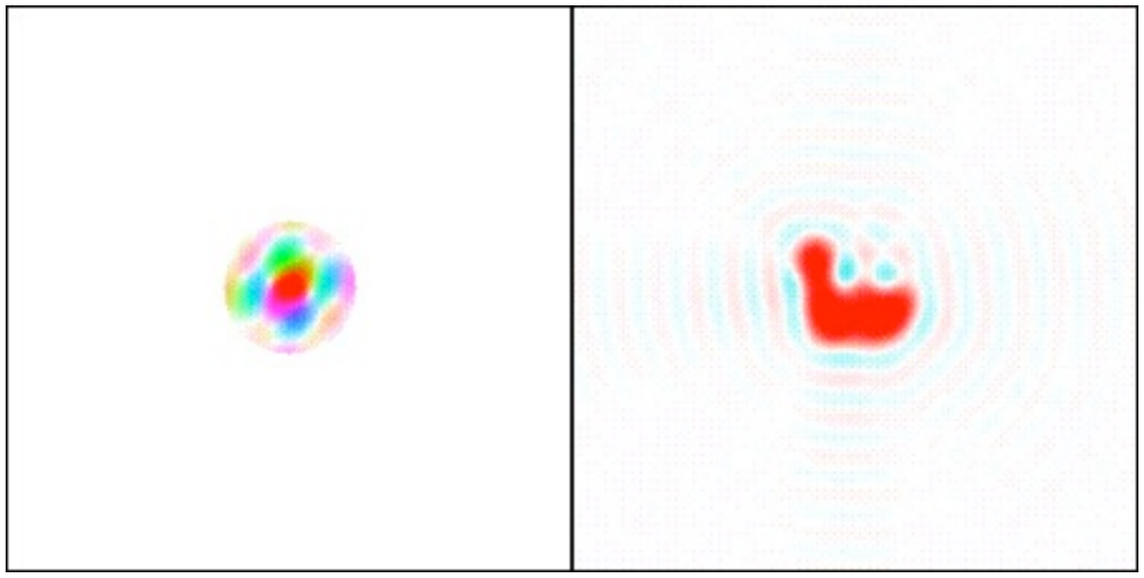
In a rotation of 180° above, the lower boundary of the initial sphere sweeps out the volume coloured green & the upper boundary the light brown part. The dark brown part is measured twice, and the blue part not at all

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

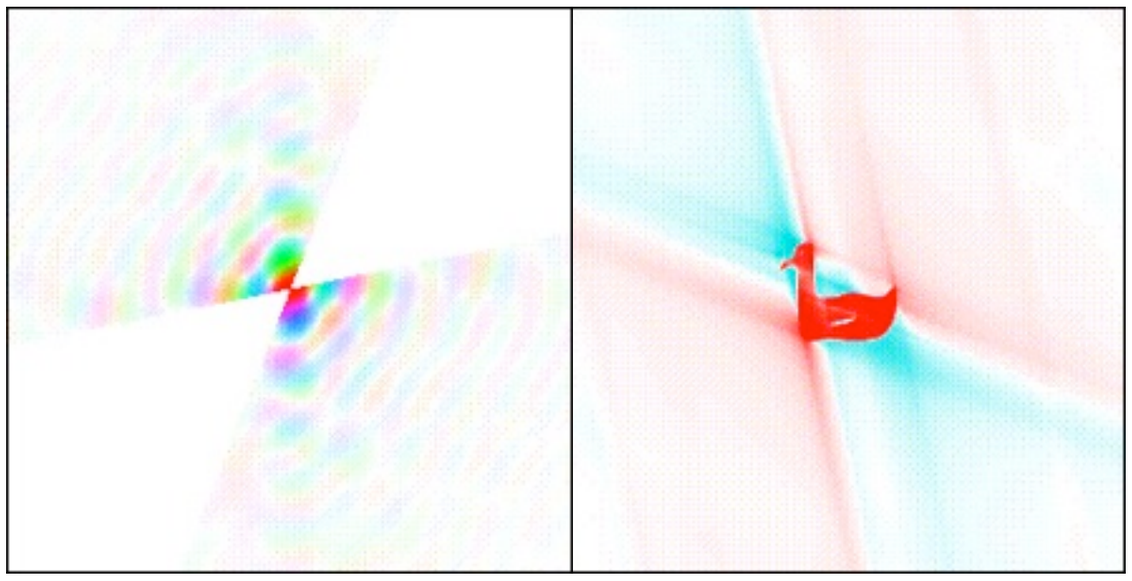
The importance of data completeness



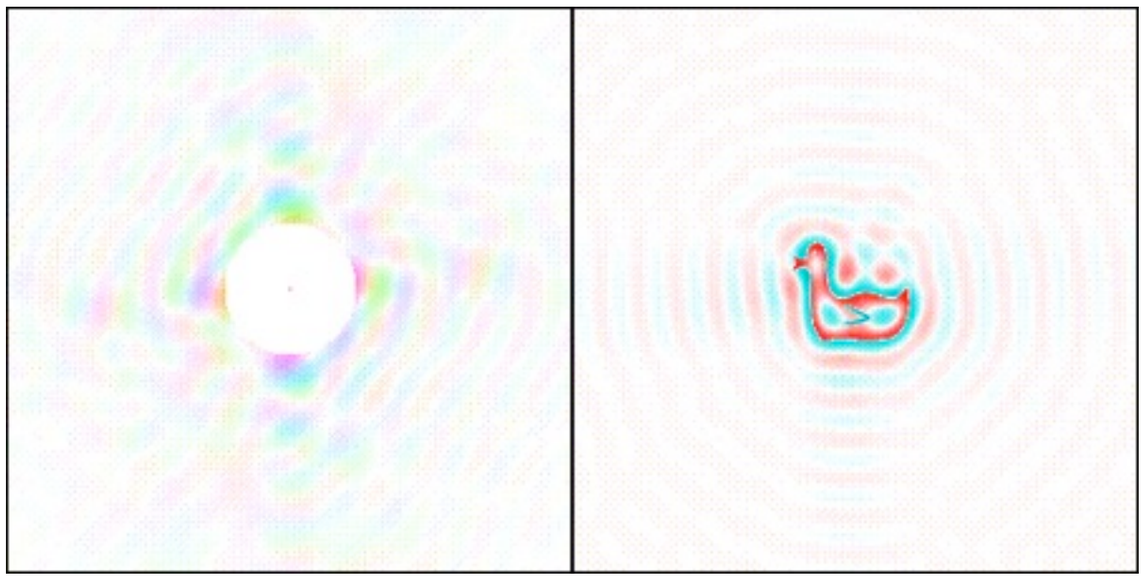
A duck ... ... and its Fourier transform



A low-resolution duck



Incomplete data: missing wedge



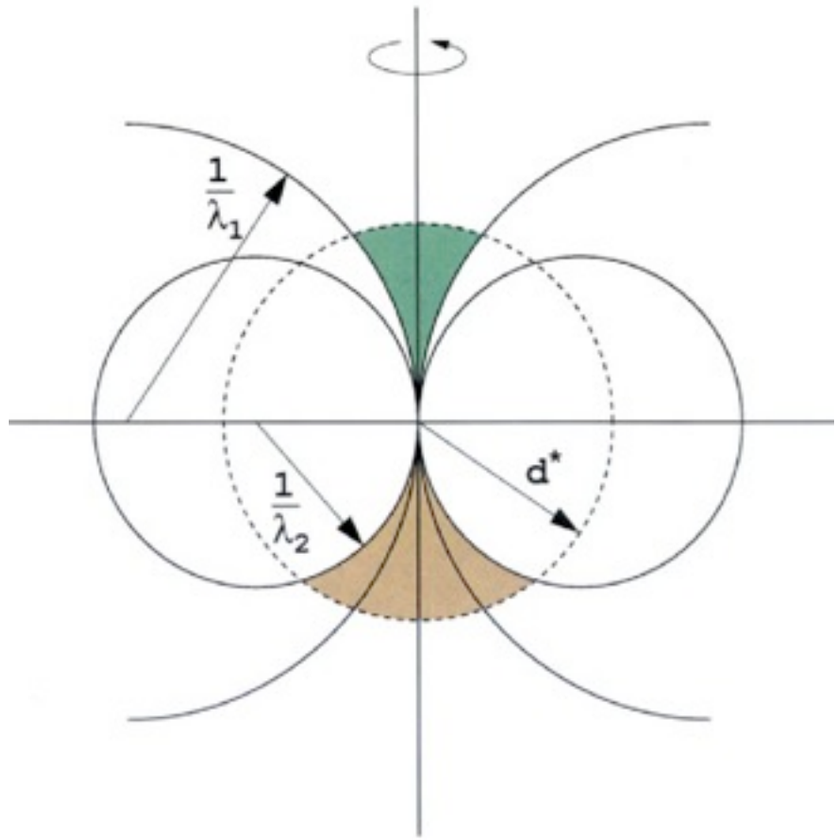
A duck without low-resolution reflections

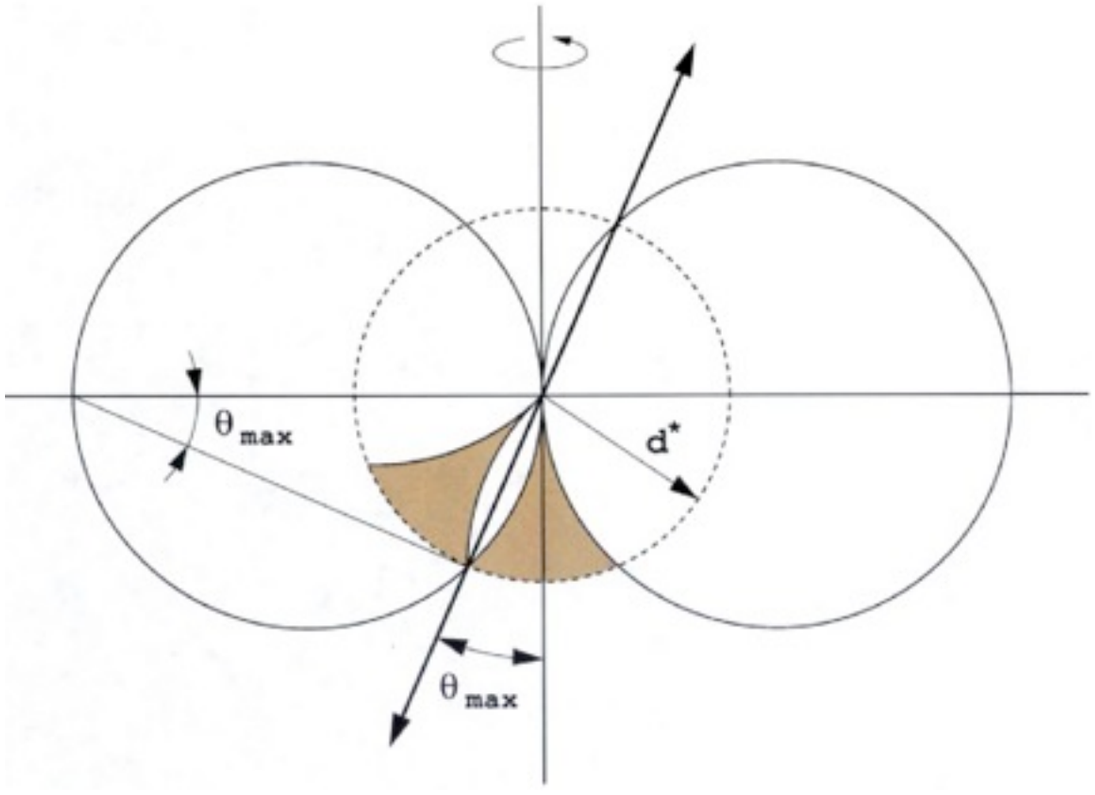
from Kevin Cowtan's "Book of Fourier"

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



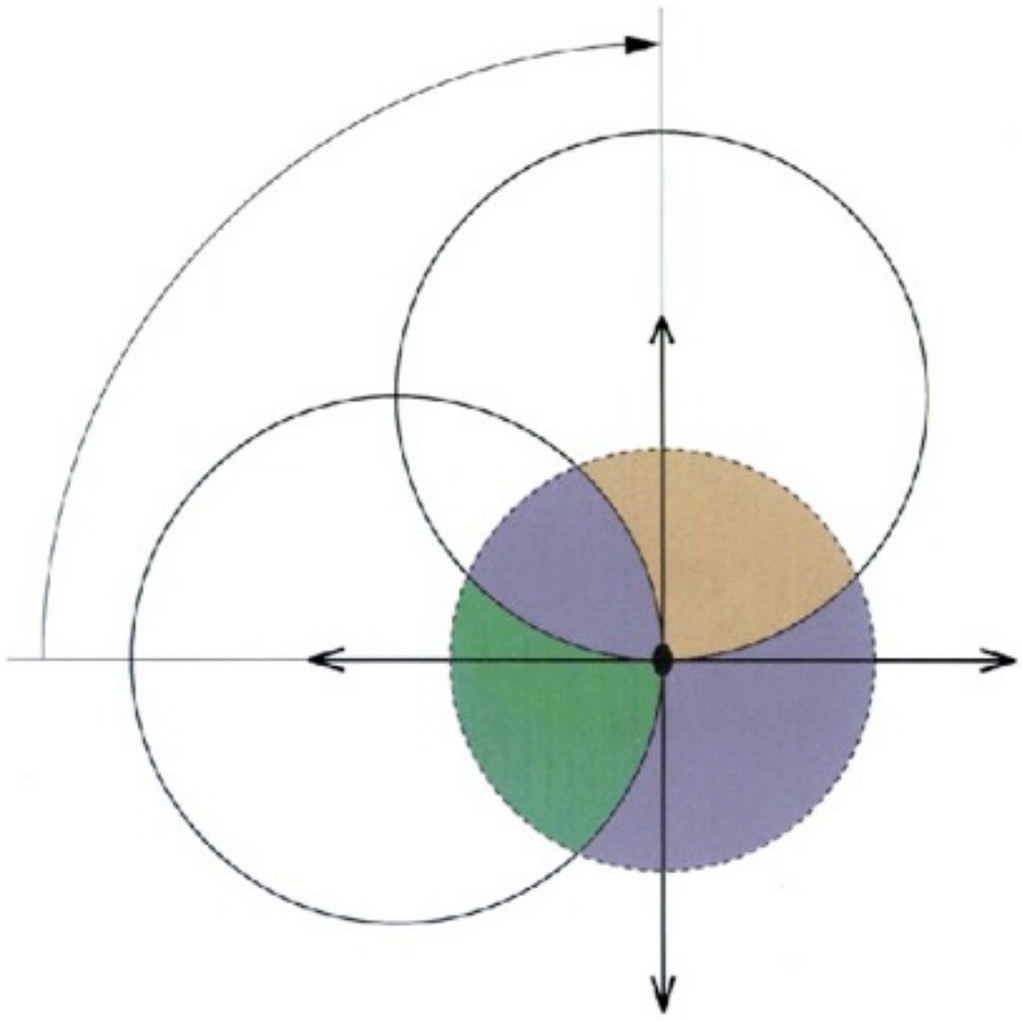


The blind region may be filled in by collecting a second set of data, offsetting the crystal by at least θ_{max} or by symmetry (except in P1)

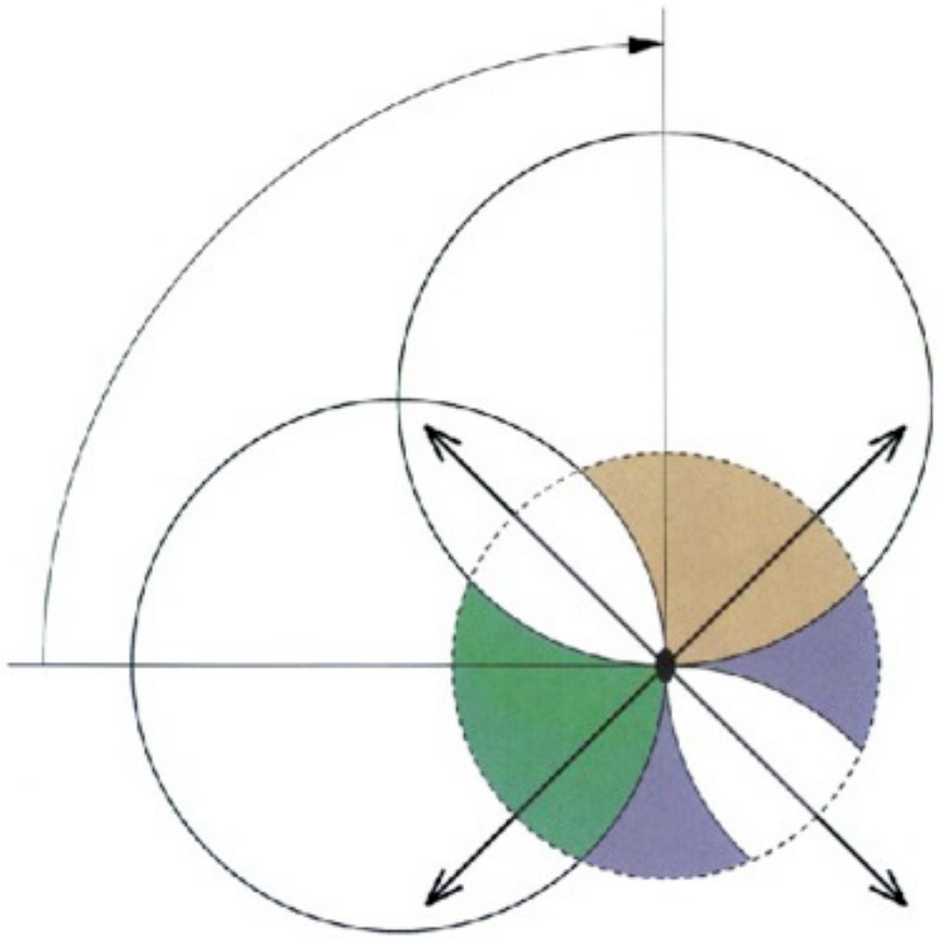
If there is symmetry, offsetting from an axis can remove or reduce the blind region for a single setting

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



How to collect good data

Crystal

↓ Data collection

Diffraction geometry
Strategy

Images

↓ Integration

Indexing

h k l I σ(I)

↓ Scaling & merging (data reduction)

Space group determination
Quality assessment

h k l F σ(F)

Decisions

- **Select crystal**

  Collect a few images to judge quality

  Is this your best crystal? Mosaicity, resolution, size, ice

- **Decide strategy and collect all images**

  Total rotation, rotation/image, exposure time, position of detector. Programs: BEST, Mosflm, etc

- **Integration**
  - **Index**

    choose lattice

    What is the correct lattice?
  - **Refine unit cell**
  - **Integrate**

    [Integration parameters: box size, overlap check]

- **Choose Laue group (point group)**

  What Laue group, space group?

- **Scale & merge**

  How good is the dataset? Any bad bits?

- **Convert I to F**

  Is the crystal twinned?

What is a good crystal?

- Single only one lattice

  check by indexing pattern and looking for unpredicted spots

- Diffracts to high angle
- Low mosaicity
- Large *the diffracted intensity is proportional to the number of unit cells in the beam: not much gain for a crystal much larger than beam (100–200μm). Smaller crystals may freeze better (lower mosaicity)*
- Good freeze *no ice, minimum amount of liquid (low background)*

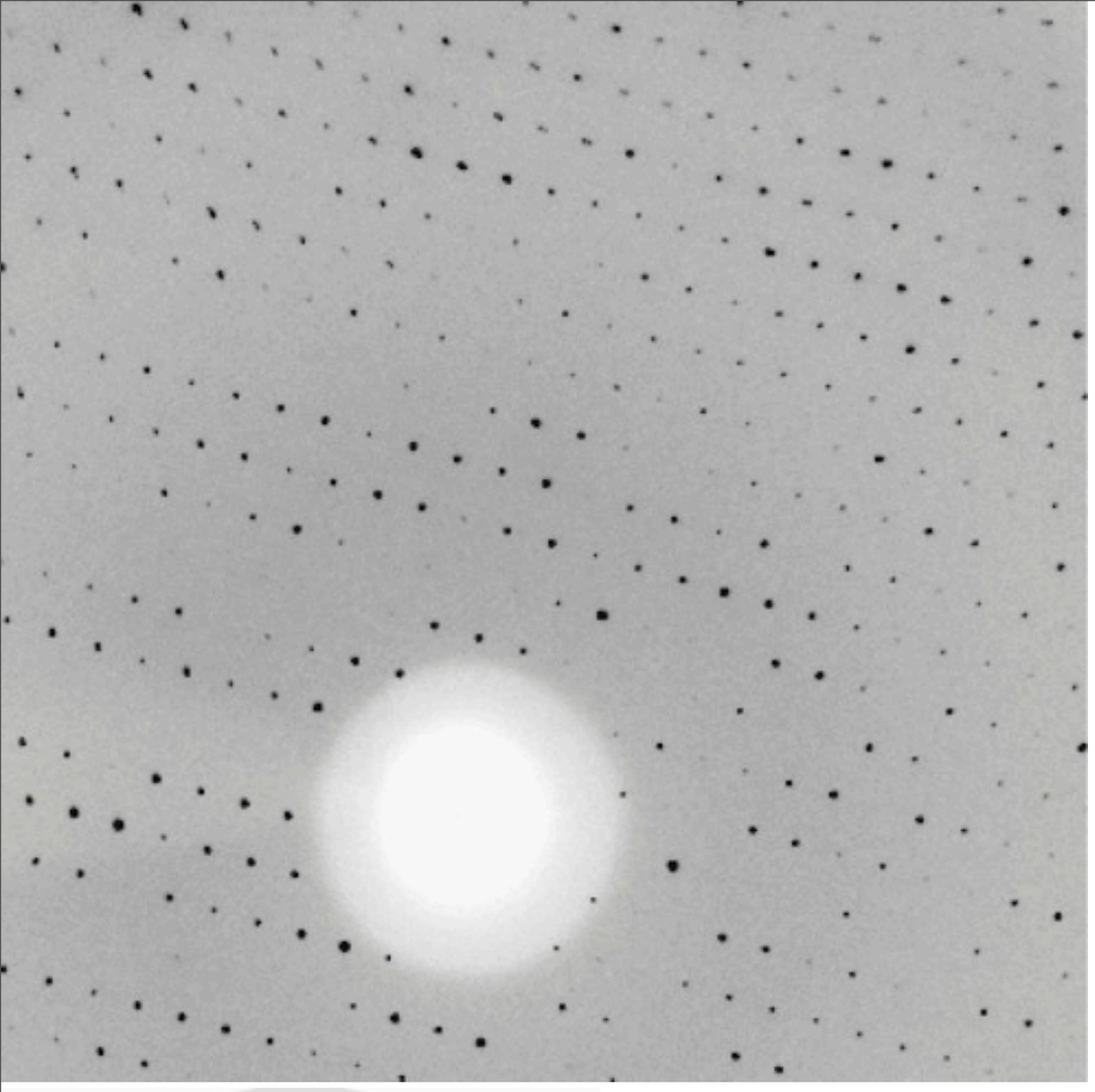
  Optimise cryo procedure, and worry about crystal transfer procedures

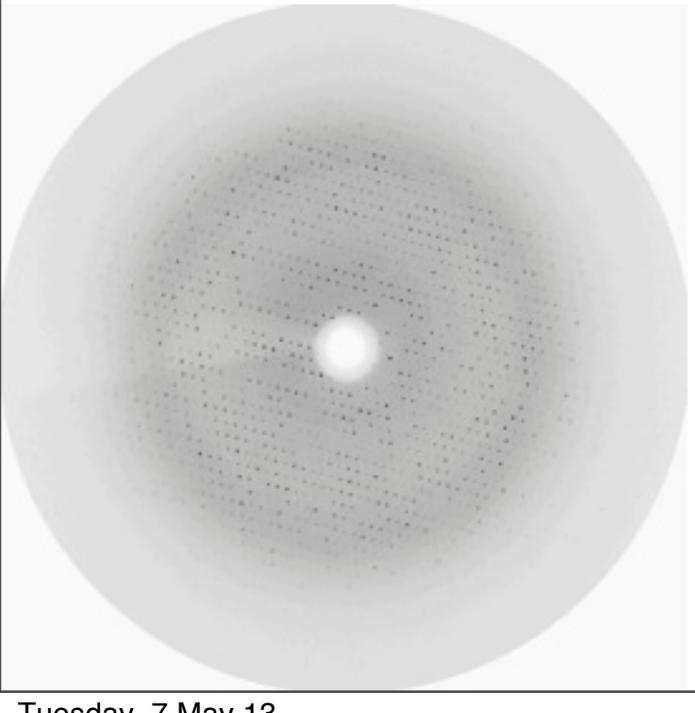
- The best that you have! (the least worst)

  Beware of pathological cases (twinning etc)

The quality of the crystal determines the quality of the dataset

You can get bad data from a good crystal,
but you can't get good data from a bad crystal

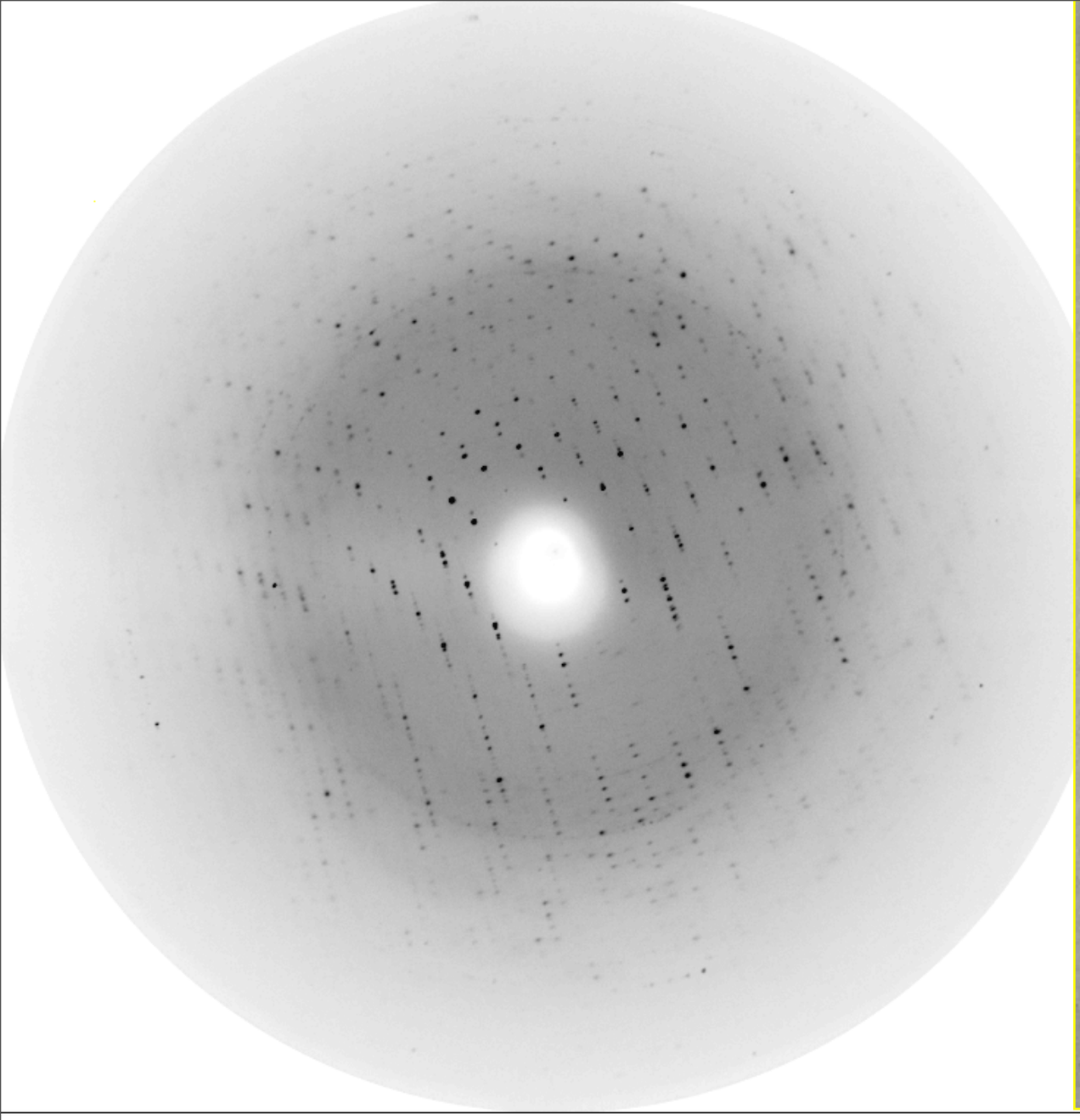


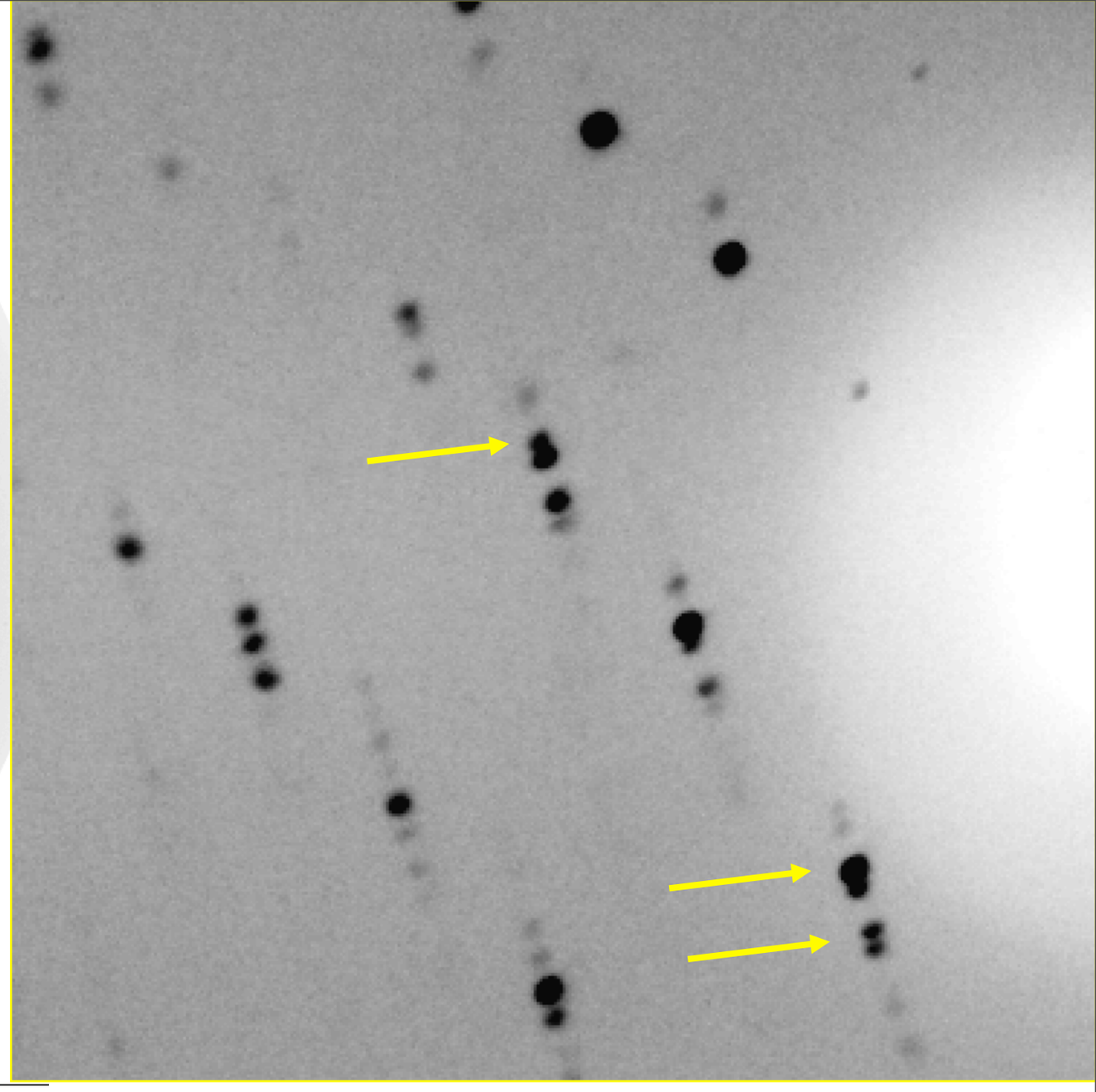


Phi = 0°

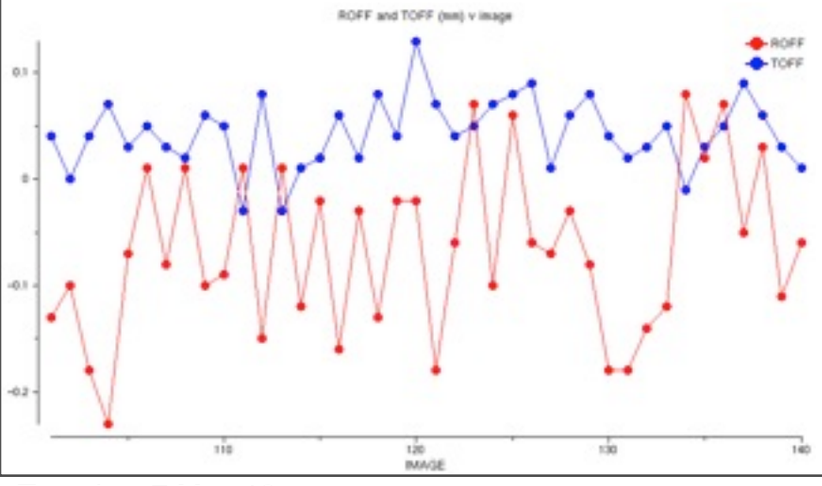
Phi = 90°

Always check diffraction in two orthogonal images !

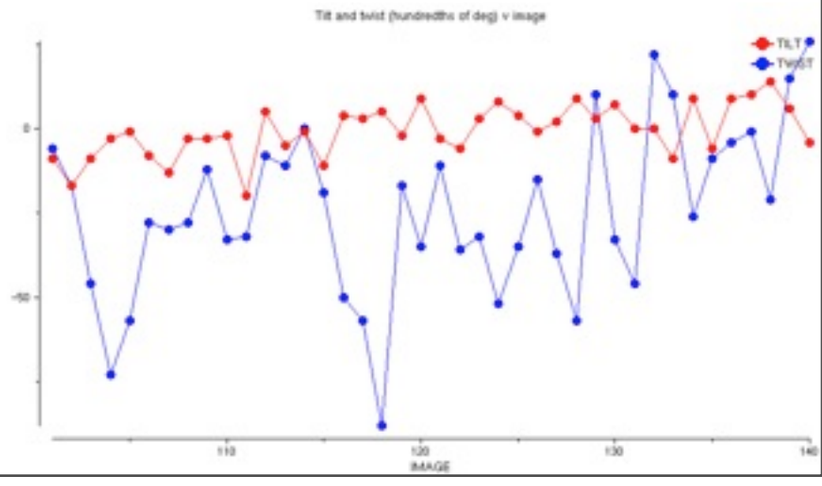


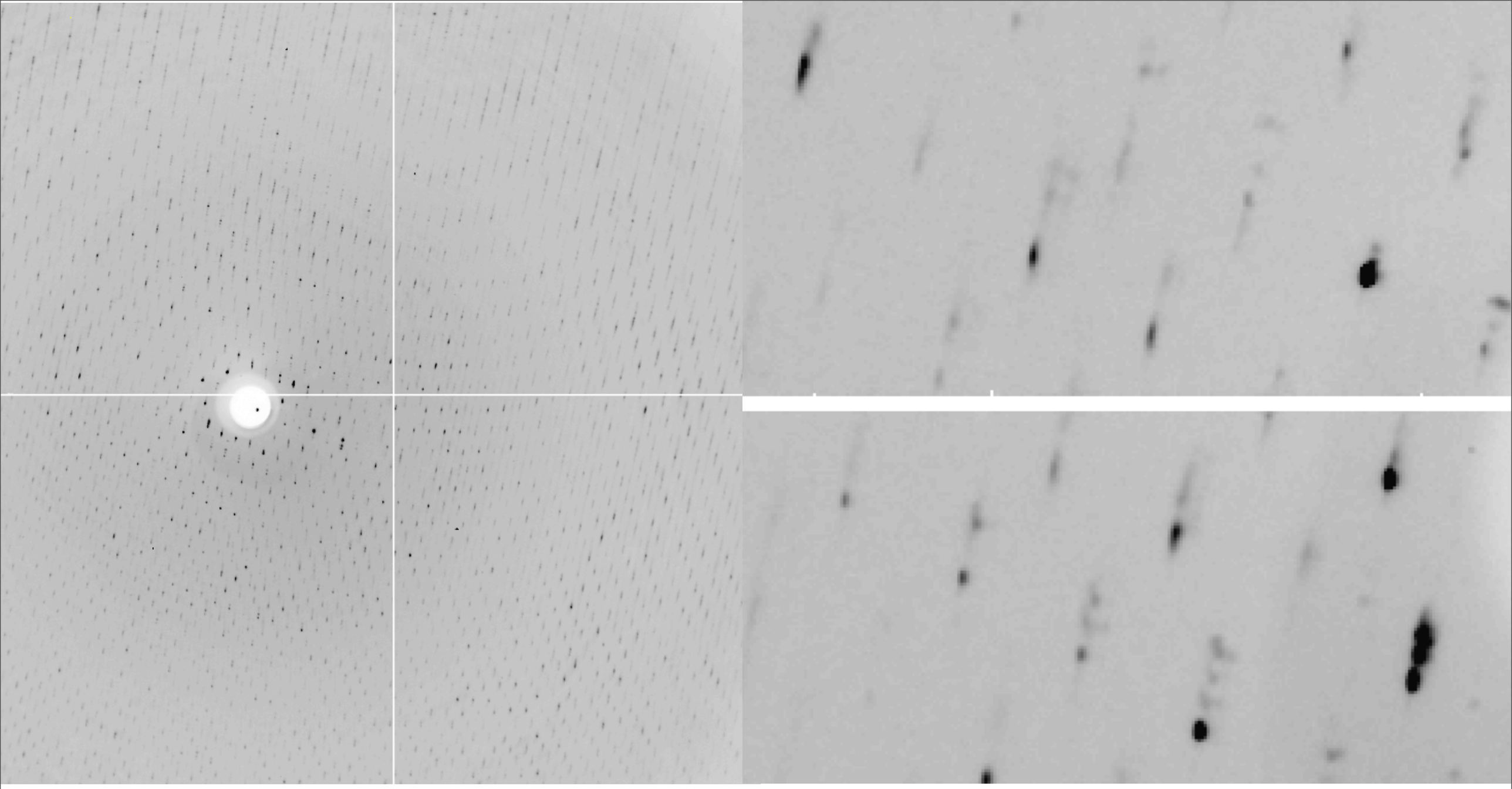


Additional spots present, not resolved.



Results in instability in refinement of detector parameters.





Spots not resolved, very poor spot shape and streaking

Data collection strategy

Compromise between statistics (enough photons/reflection, and multiplicity) and radiation damage. **Radiation damage is the big problem.**

Radiation damage controls the total time available for crystal exposure.

Data collection strategy

Compromise between statistics (enough photons/reflection, and multiplicity) and radiation damage. **Radiation damage is the big problem.**

Radiation damage controls the total time available for crystal exposure (~1 to 3 minutes on a synchrotron). Radiation damage is worst at an absorption edge.

- Total rotation range

  Ideally 180° (or 360° in P1 to get full anomalous data)

  Use programs (eg Mosflm) to give you the smallest required range (eg 90° for orthorhombic, or 2 x 30°) and the start point.

- Rotation/image: **(almost) never 1°**!

  good values are often in range 0.1° - 0.5°, minimize overlap and background

- Time/image *depends on total time available*
- Detector position: *further away to reduce background and improve spot resolution*

Two Cases:

Anomalous scattering, MAD

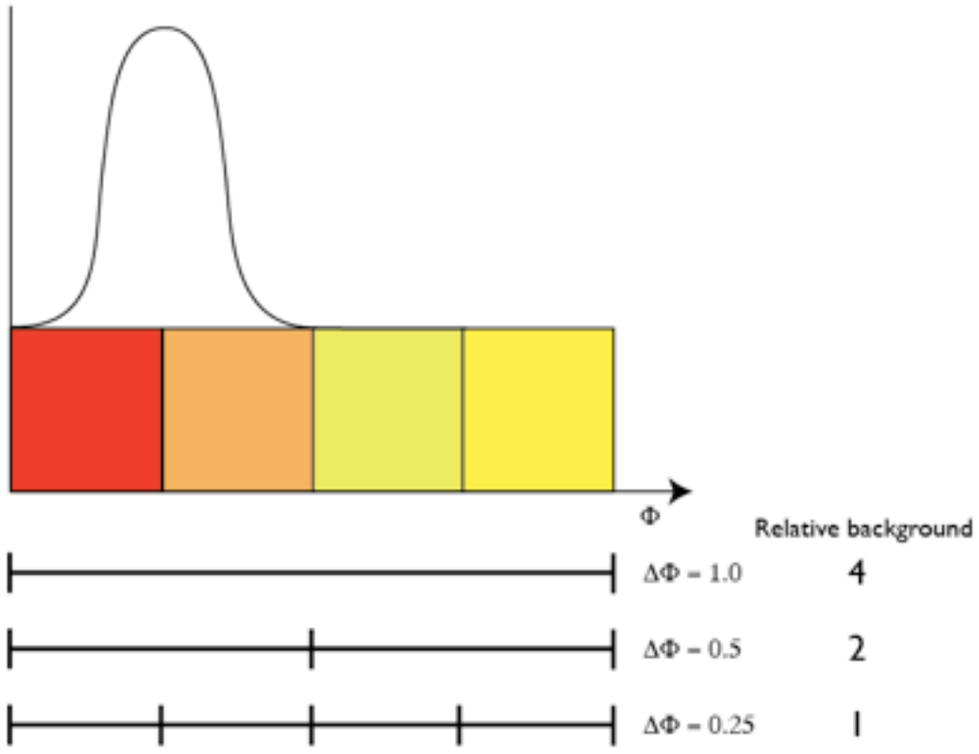
- High multiplicity is better than long exposures (eliminates outliers)
- Split time between all wavelengths, be cautious about radiation damage, reduce time & thus resolution if necessary
- Collect Bijvoet pairs close(ish) together in time: align along dyad or collect inverse-beam images
- Maybe recollect first part of data at end to assess radiation damage

Data for refinement

- Maximise resolution: longer exposure time (but still beware of radiation damage)
- High multiplicity less important, but still useful
- Use two (or more) passes with different exposure times (ratio ~10) if necessary to extend range of intensities (high & low resolution)
- Short wavelength (<1Å) to minimise absorption
- Collect symmetry mates at different times and in different geometries, to get best average (even with higher R_{merge}!). Rotate about different axes.

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 $I/\sigma(I)$.

Fine phi slicing *should* give better data but:

1) Assumes no errors in shutter synchronisation
   this is demanding for very short (<0.5 sec) exposures, but OK with fast detectors (Pilatus etc) using shutterless collection
2) There will be detector readout noise in each image - not a problem with Pilatus
3) There is not much advantage in slice widths less than ~ 1/5 reflection width

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

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 180°/n for 2- & 4-folds, 360°/n for 3- & 6-folds (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

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 (eg MOSFLM) to determine range and start point
- Collect 180° or 360° and start anywhere, but still better to start at the "right" point

Exposure time/image

- Compromise between high resolution (good) and radiation damage (bad)
- Process & assess first data collection, then revise strategy
- Use estimates from BEST etc

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 errors in synchronisation of shutter opening and rotation (this potentially adds an error for each image)

Other considerations not discussed here

- Practical aspects of mounting and centering crystals
  - ⋆ grid searches for finding small crystal or centering

- What to do if radiation damage is limiting?
  - ⋆ use multiple positions on a large crystal (not too close together)
  - ⋆ collect from multiple (many) *isomorphous* crystals, select "best" ones

Data collection is your last experimental step: don't mess it up

Some references:

Diffraction geometry, Ewald sphere

Text books, eg J.Drenth

Data collection strategy

Radiation damage in macromolecular crystallography: what is it and why should we care?, E.Garman, Acta Cryst. D66, 339-351(2010).

Data Collection Strategies, Dauter, Z. Acta Cryst. (1999). D55, 1703-1717.

Optimal Fine phi-slicing for Single-Photon-Counting Pixel Detectors
Marcus Mueller, Meitian Wang, and Clemens Schulze-Briese, Acta Cryst. (2012) D68, 42-56

The finer things in X-ray diffraction data collection
J. Pflugrath, Acta Cryst D55, 1718-1725, 1999