Diffraction geometry and integration of diffraction images

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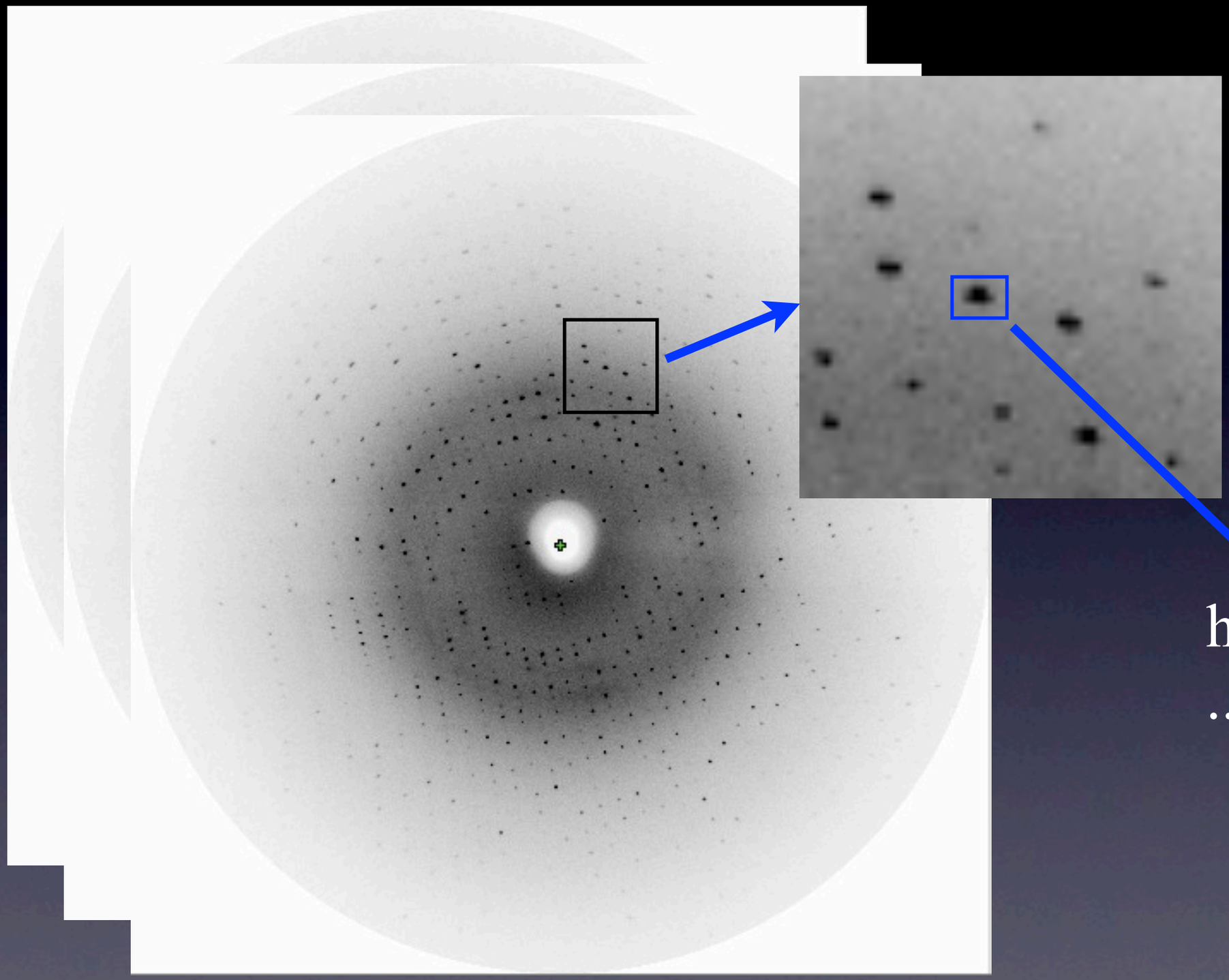
Integration



h k l I σ(I)

...

Image series → Reflection intensity list

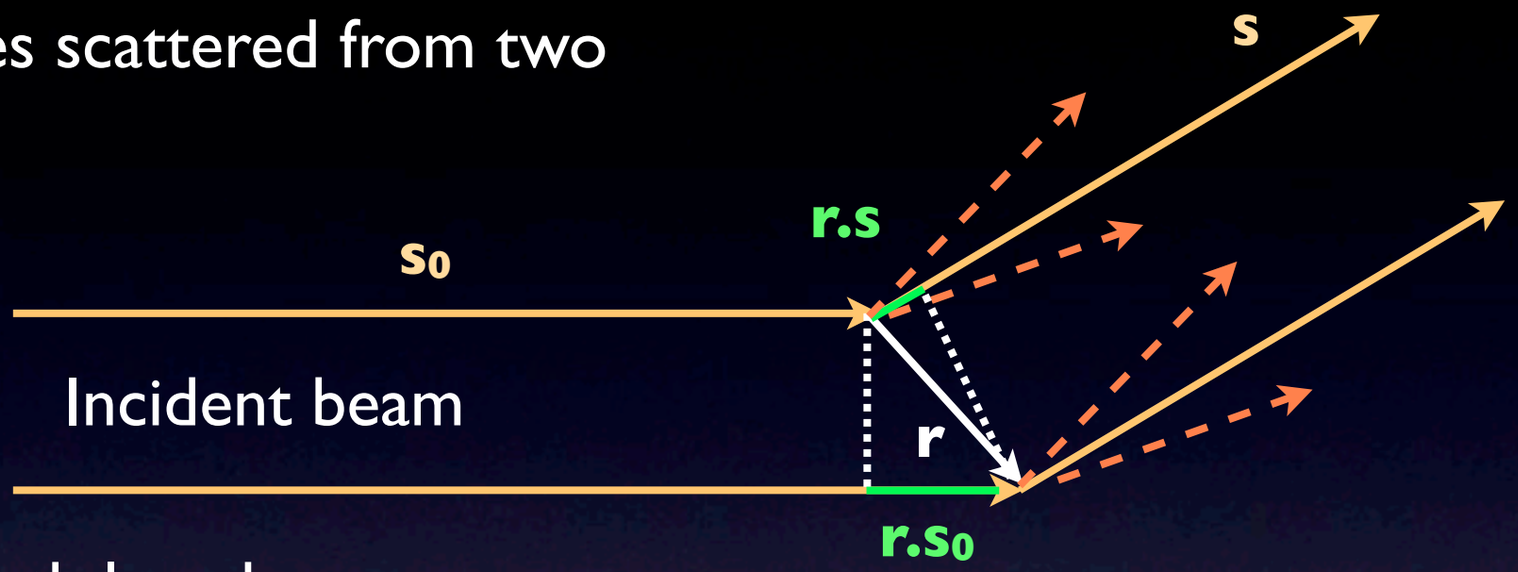
Diffraction Geometry

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

Scattering from two electrons

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

$\delta L = \mathbf{r}.\mathbf{s} - \mathbf{r}.\mathbf{s}_0 = \mathbf{r}.(\mathbf{s} - \mathbf{s}_0)$



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

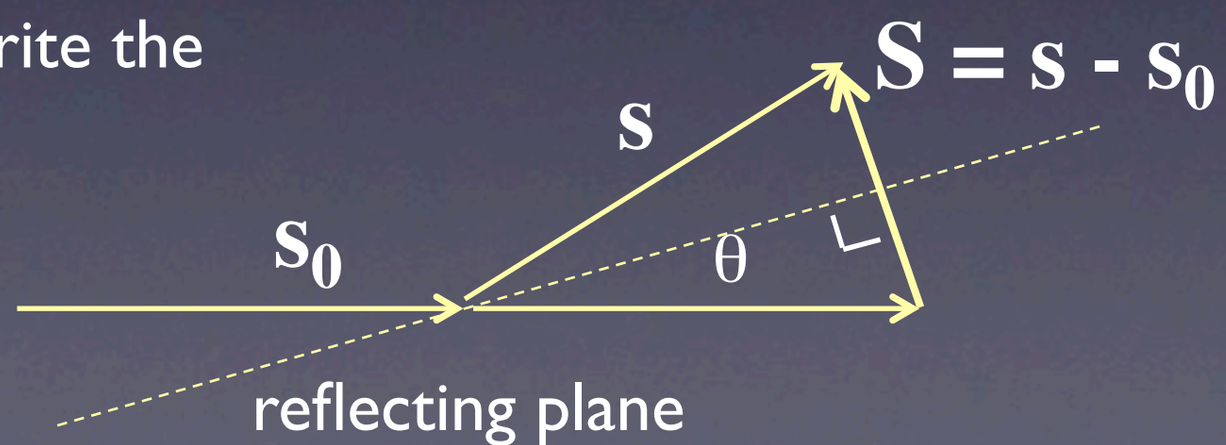
$= 2\pi$ (path difference)$/ \lambda$

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

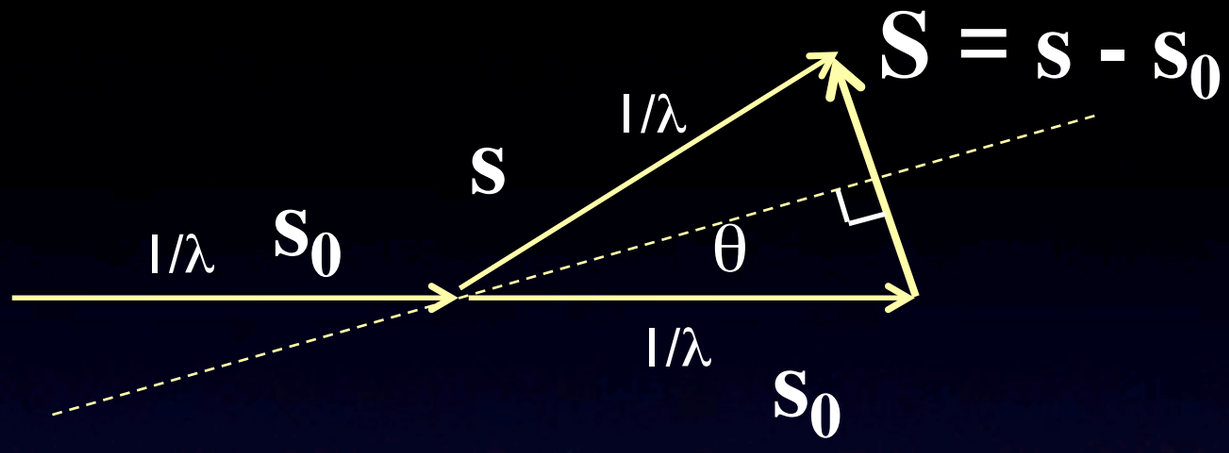
If we make the length of the wave vectors $\mathbf{s}_0$ and $\mathbf{s} = \mathbf{1}/ \lambda$, ie $|\mathbf{s}_0| = |\mathbf{s}| = \mathbf{1}/ \lambda$, then we can write the phase shift $= 2\pi \, \mathbf{r}.\mathbf{S}$ where $\mathbf{S} = \mathbf{s} - \mathbf{s}_0$

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

X-rays scattered in all directions



The Ewald Sphere Construction



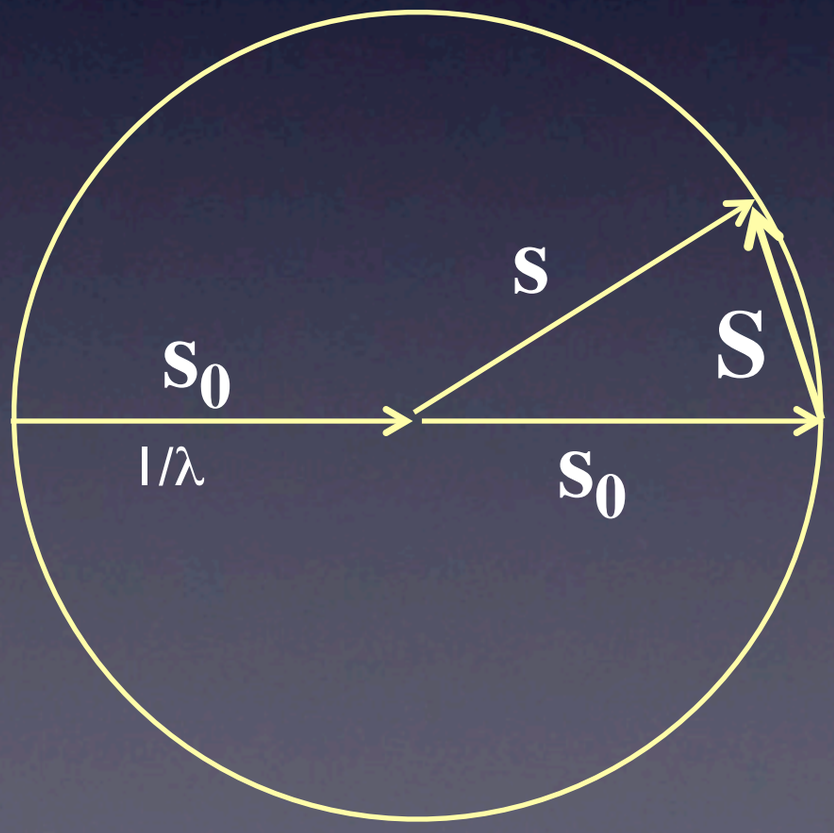
The general condition for diffraction is illustrated by the vector equation

$\mathbf{S} = \mathbf{s} - \mathbf{s}_0$

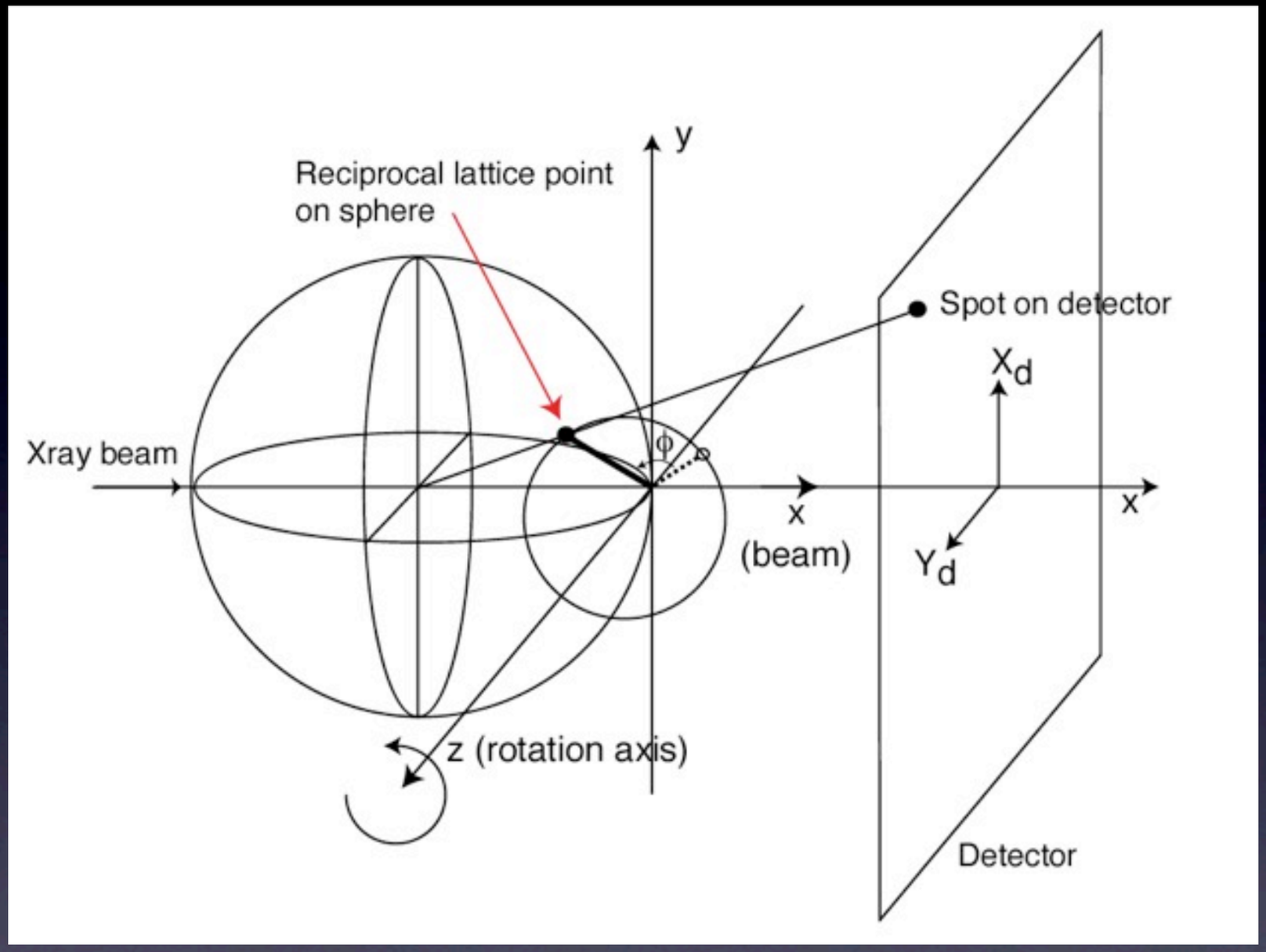
Because $\mathbf{s}_0$ and $\mathbf{s}$ have the same length ($1/\lambda$), we can generalise this diagram by drawing a sphere of radius $|\mathbf{s}_0| = |\mathbf{s}| = 1/\lambda$

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

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

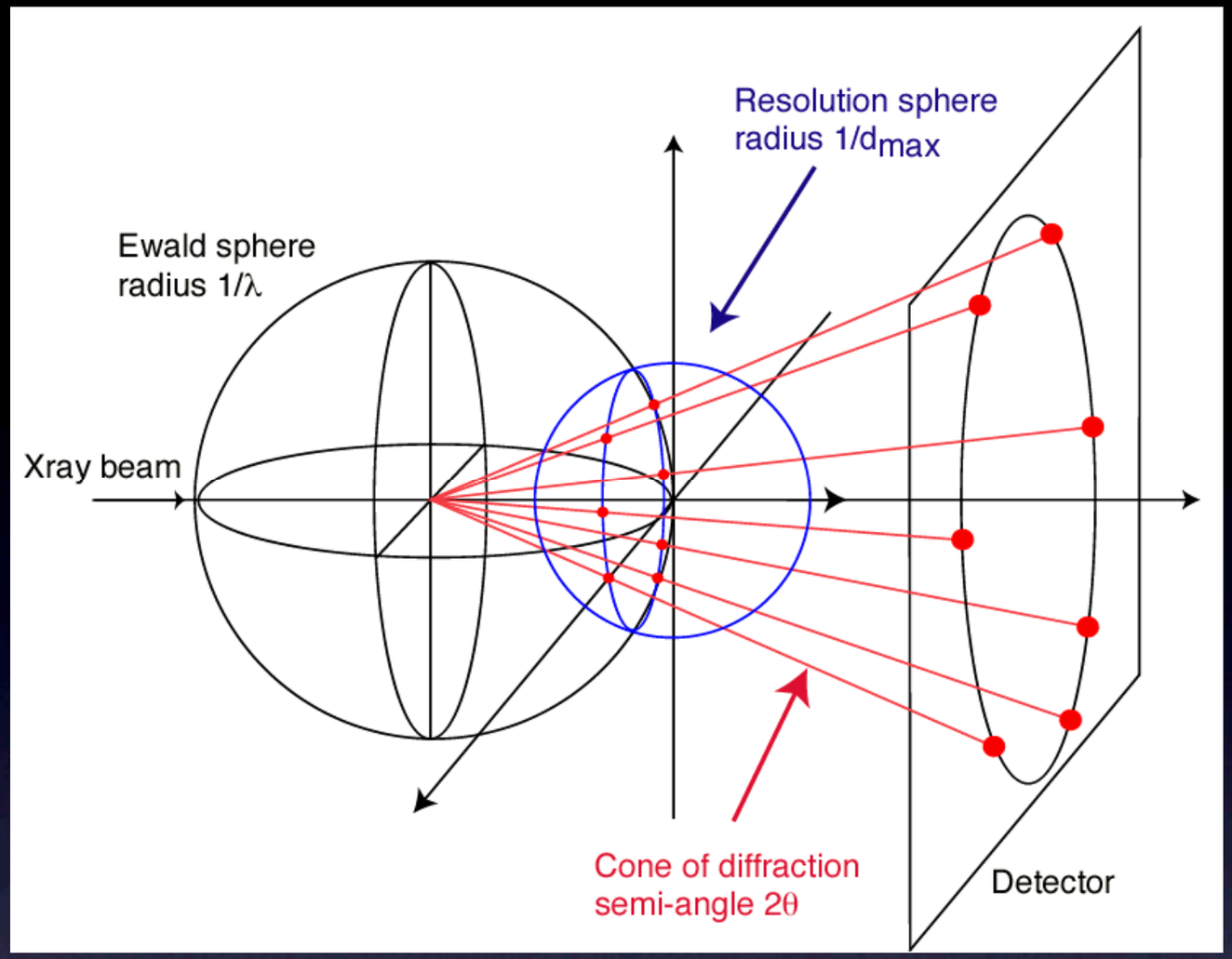


The part of the reciprocal lattice which intersects the sphere is projected on to the detector



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

Detector position



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

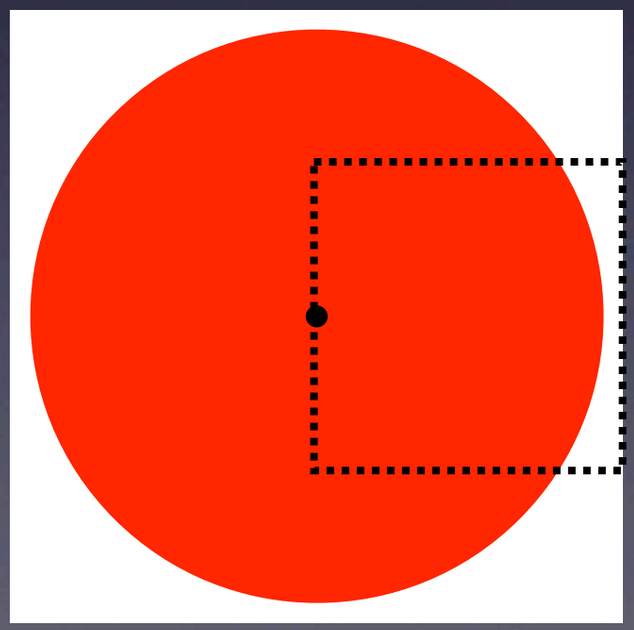
As the crystal rotates, the diffracted beams all lie within a cone of semi-angle $2\theta_{max}$

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

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

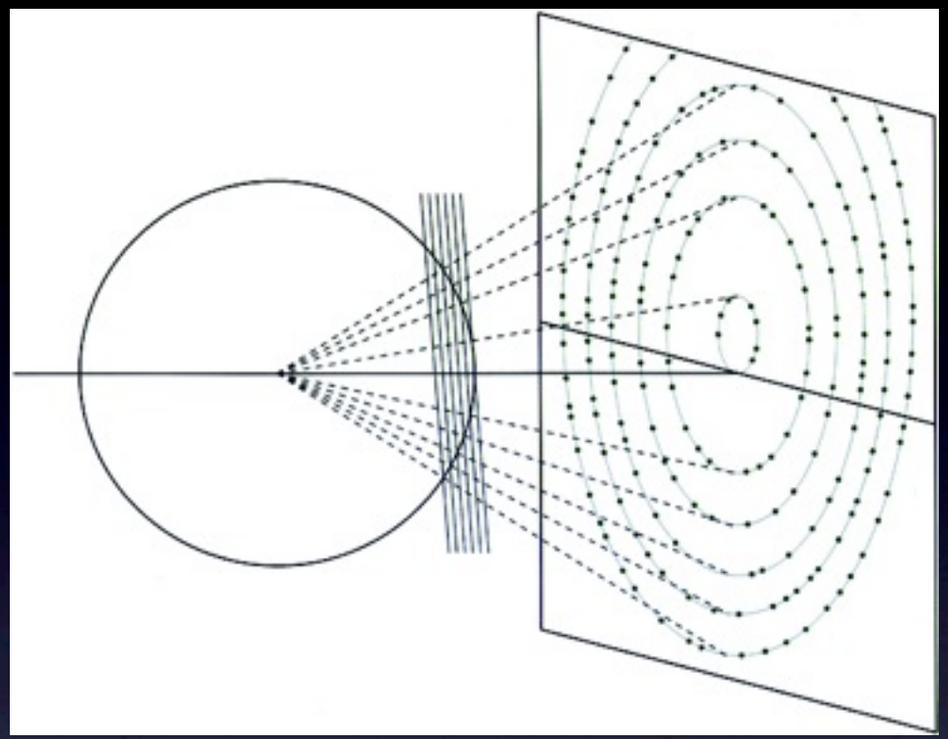


For long axes (close spot separation) it may be necessary to use a long detector distance and an offset detector

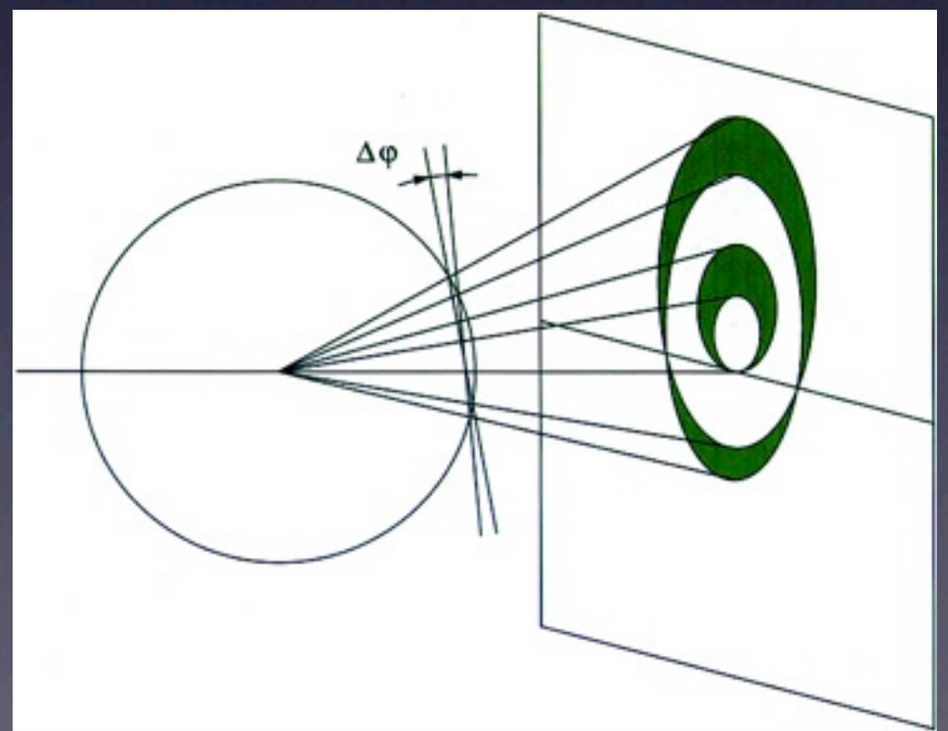
This gives a lower efficiency, and to get complete data requires a complicated strategy

The appearance of diffraction images

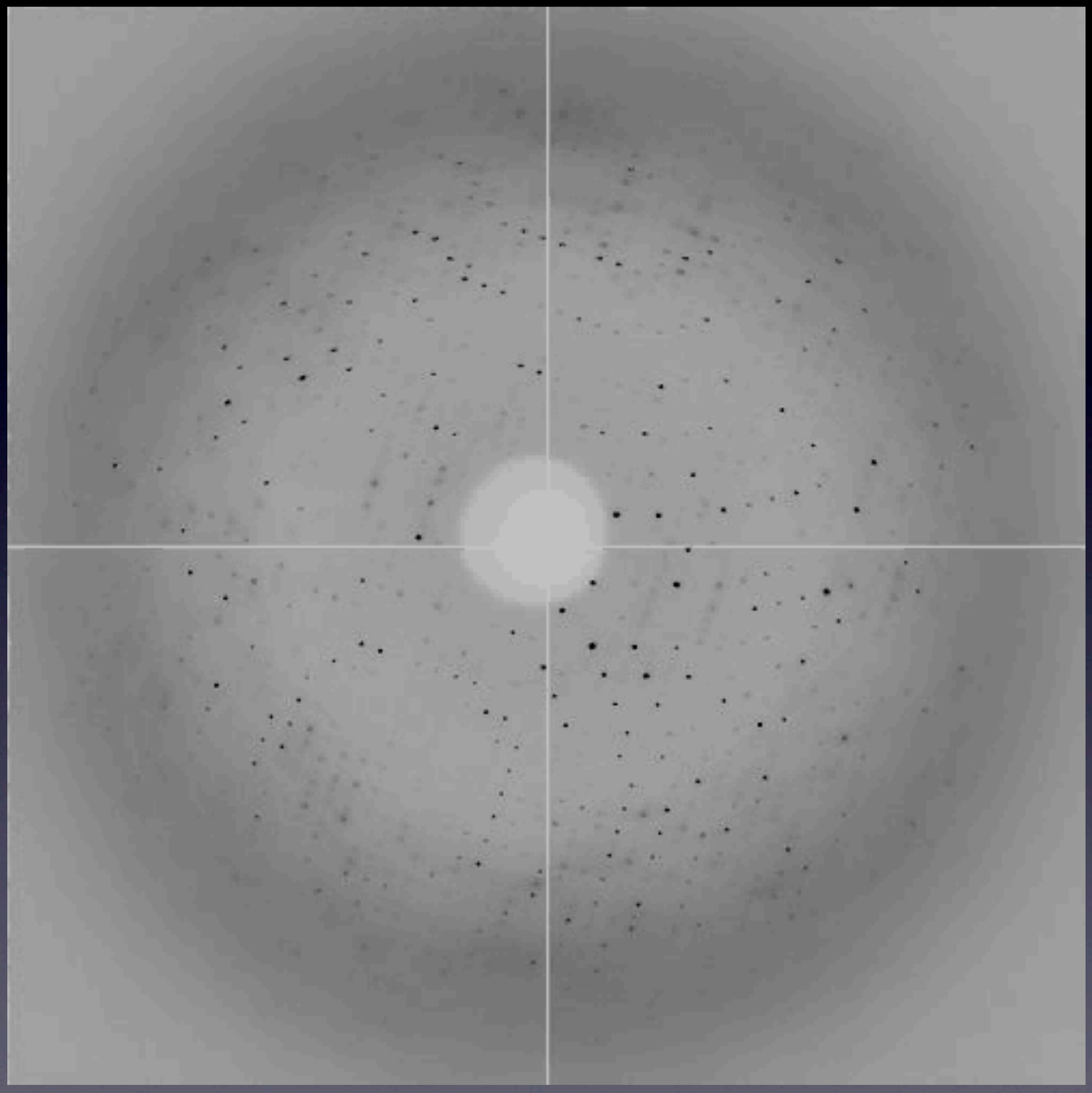
Reciprocal lattice points lie in layers (planes). Each plane intersects the sphere in a circle, and the spots projected on the detector lie in ellipses



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)



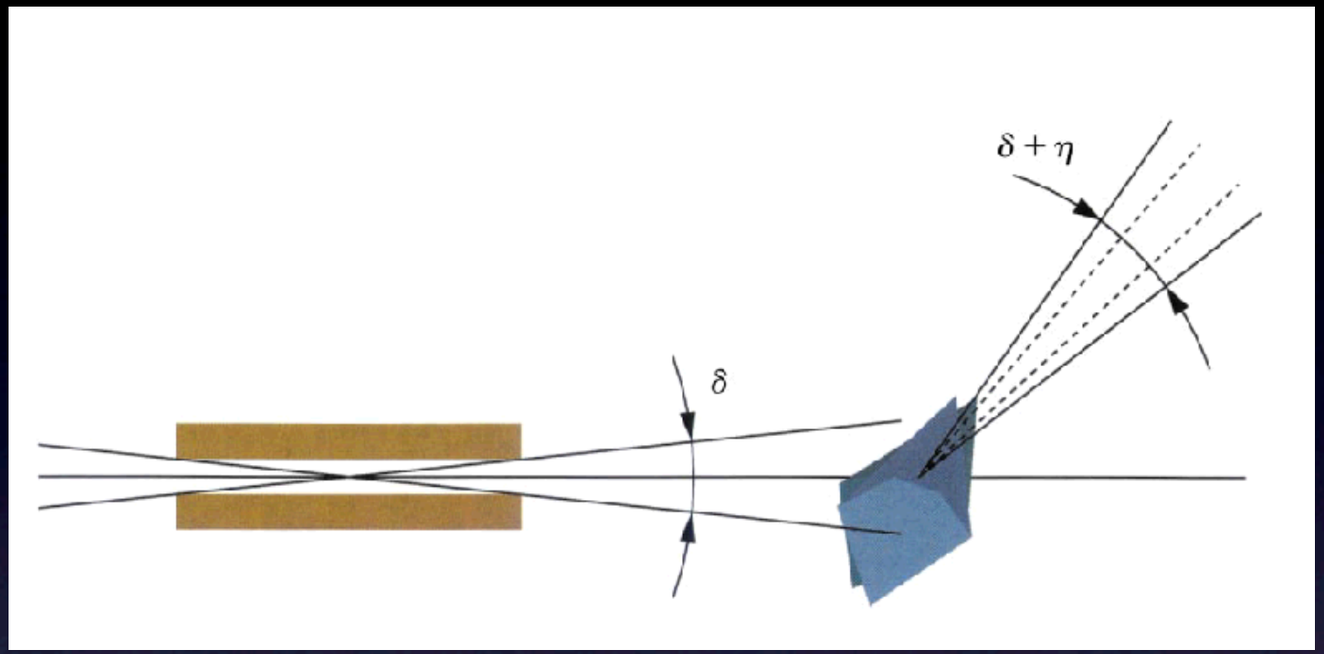
illustrations from Zbyszek Dauter

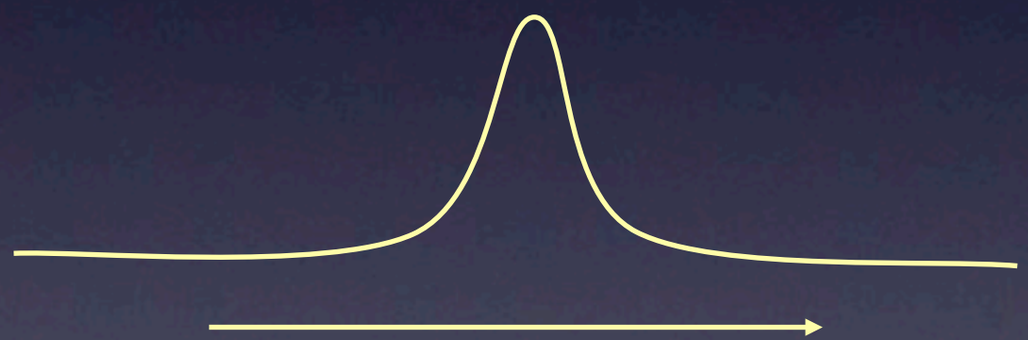


The full diffraction pattern (ie the reciprocal lattice) is 3-dimensional, and we want to measure the whole sphere to the maximum resolution (radius) available.

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.

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



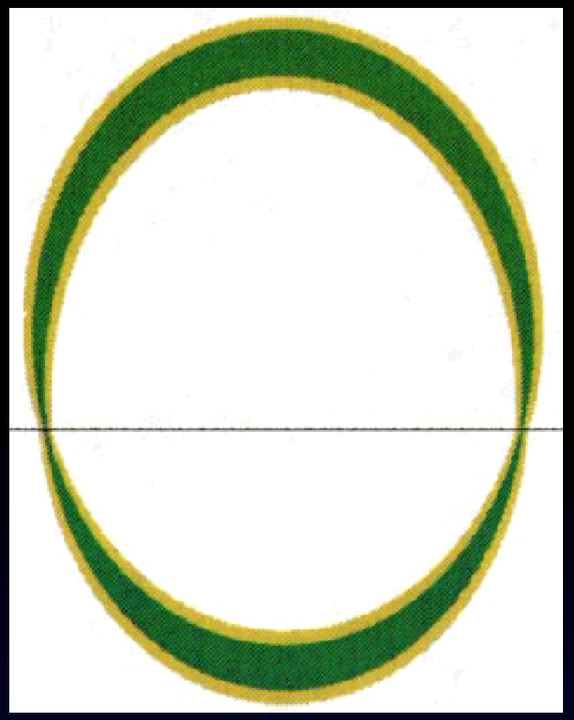


rotation angle ϕ

Reflection width in rotation

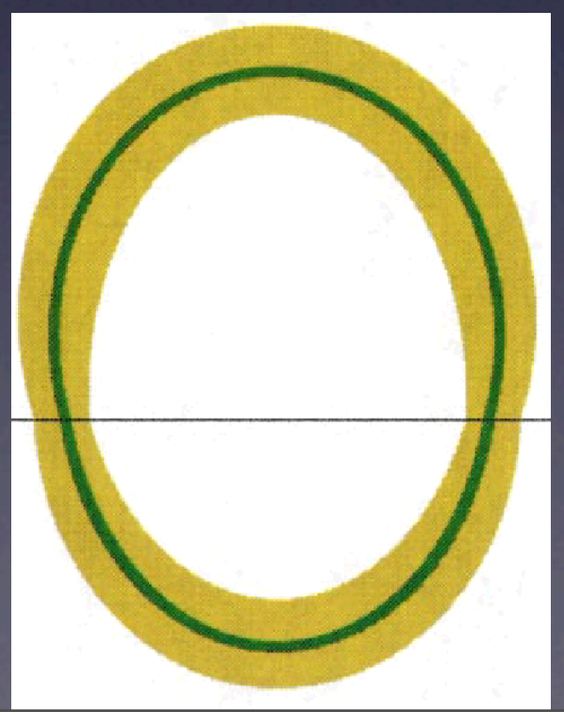
= δ + η + geometric factor

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



High mosaicity causes broadening of the lunes

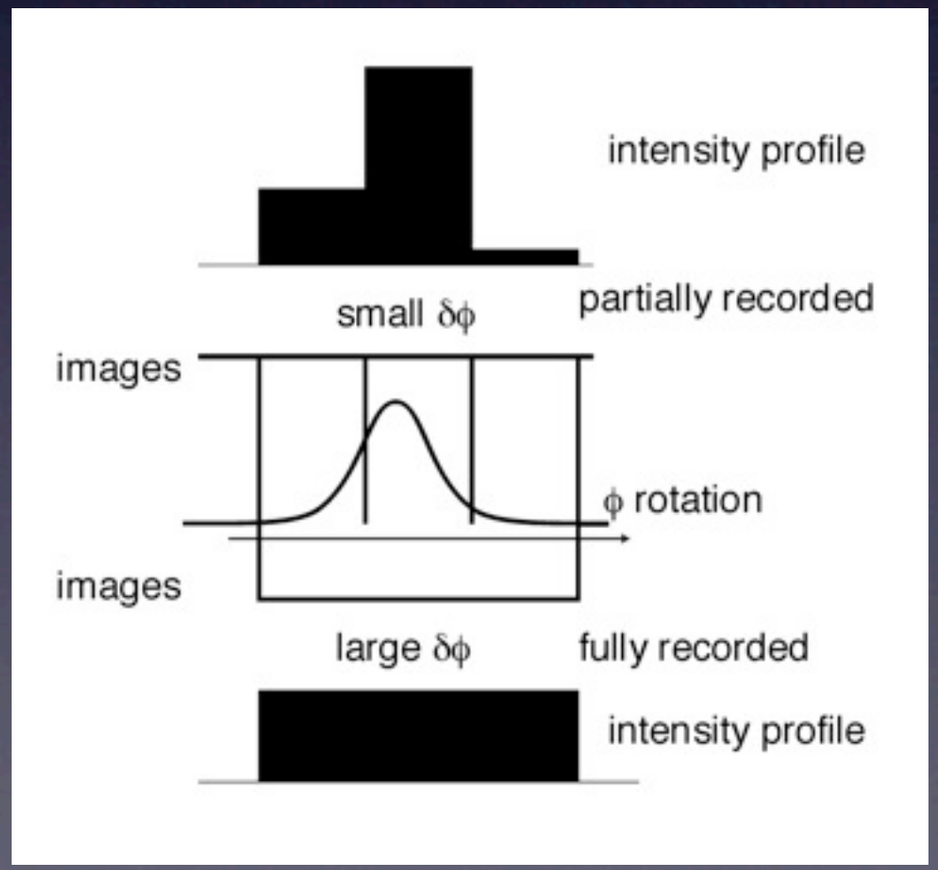
Most obvious along the rotation axis



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.

CCD and image plate detectors take a significant time to read out, so for these have to close the shutter & stop the rotation (simultaneously!). Pixel detectors (eg Pilatus) with very fast read-out can be used with continuous rotation with the shutter open. In both cases our sampling of the 3-dimensional reciprocal space is in consecutive slices, typically of between about 0.1° and 1°



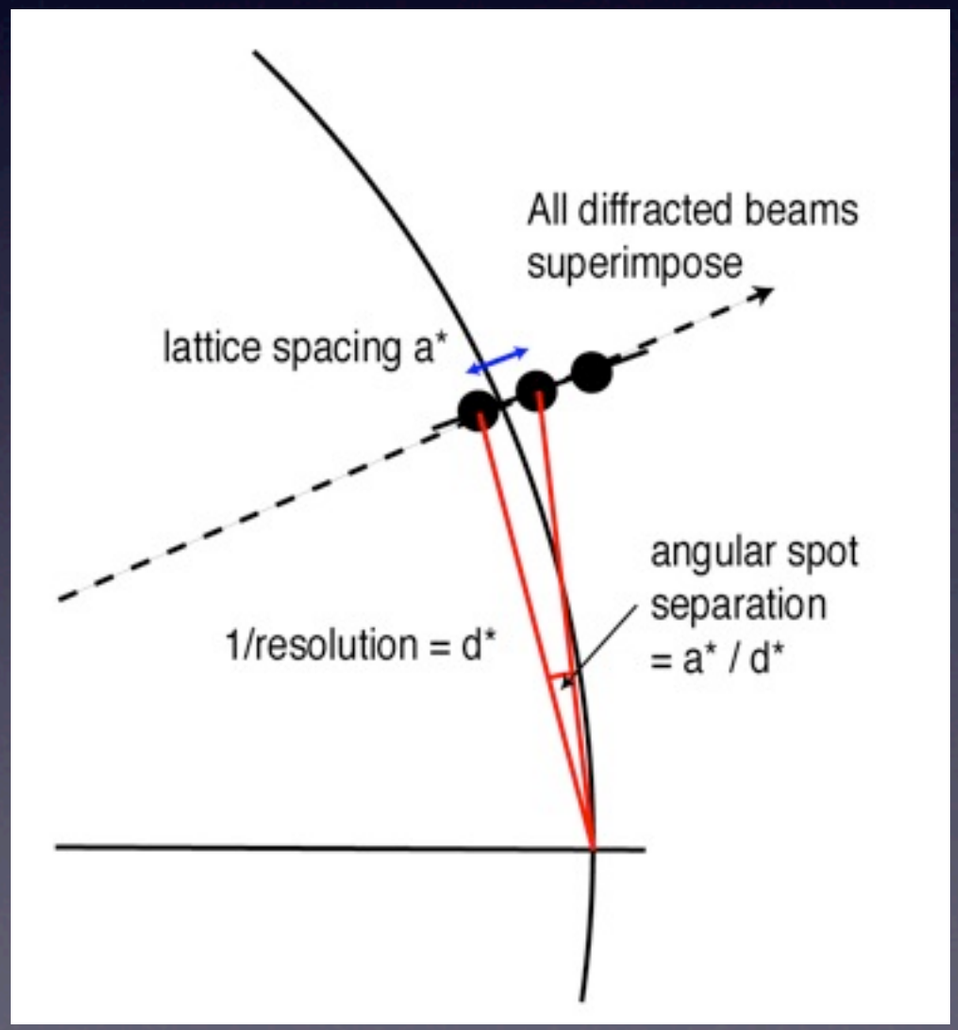
Depending on the slice width and the reflection width a reflection may occur on one image (*full* or *fully recorded*) or on several (*partial* or *partially recorded*)

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

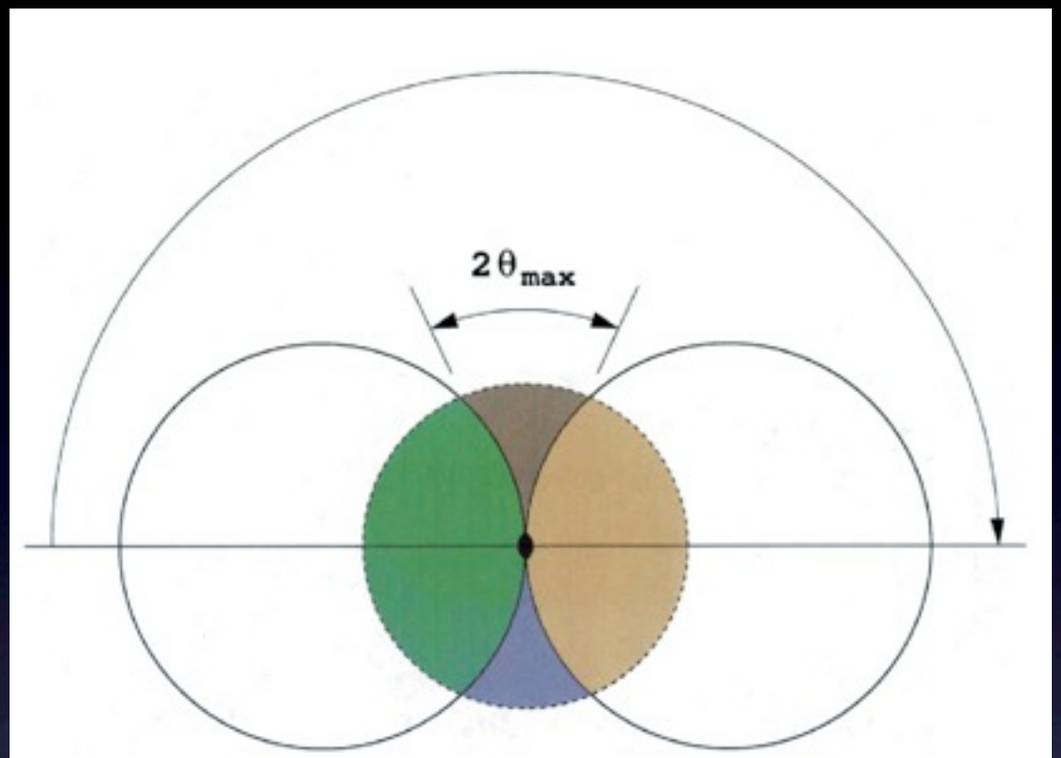
w = reflection width = $\delta + \eta$

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

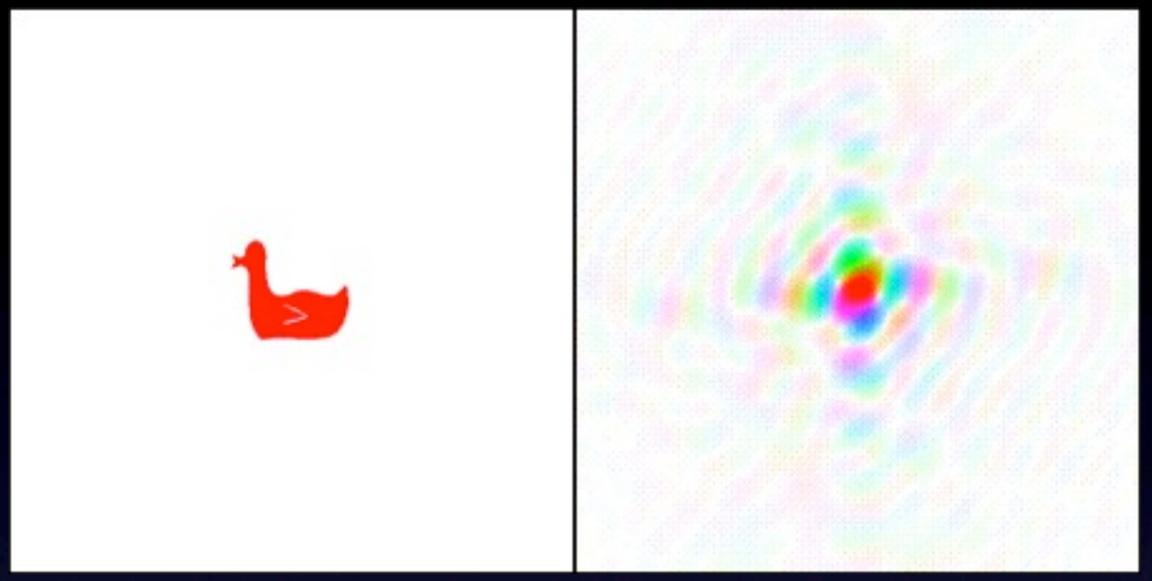


To use the Ewald sphere construction to understand which parts of reciprocal space are measured, it is easier to fix the "resolution sphere" of all reciprocal lattice points within a maximum resolution, and to rotate the Ewald sphere. The region collected is the volume swept out by the leading and trailing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

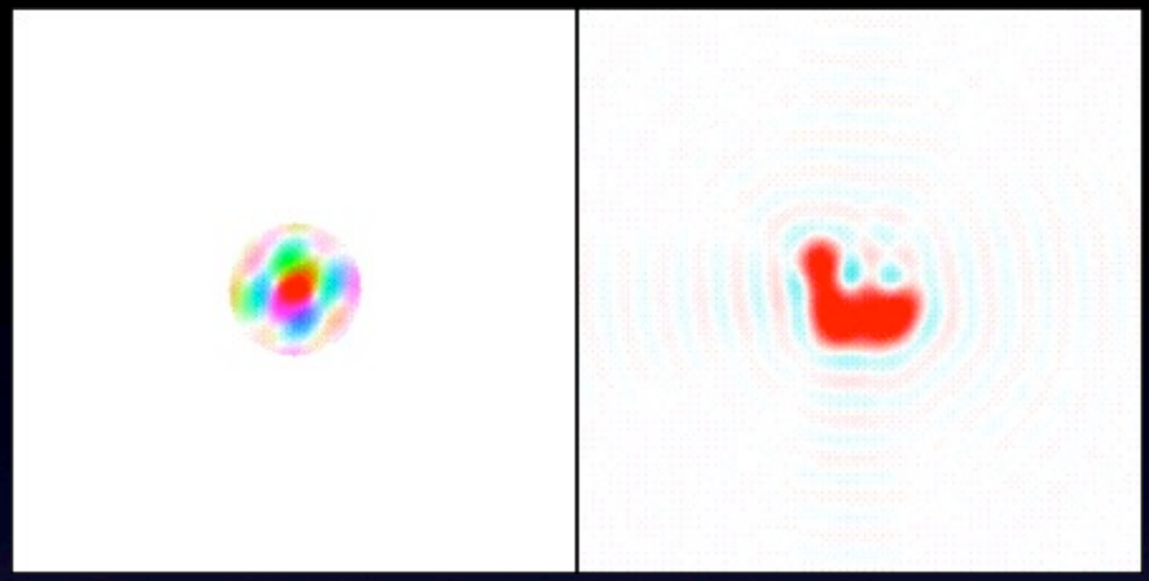
In a rotation of 180° above, the lower boundary of the initial sphere sweeps out the volume coloured green & the upper boundary the light brown part. The dark brown part is measured twice, and the blue part not at all

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)

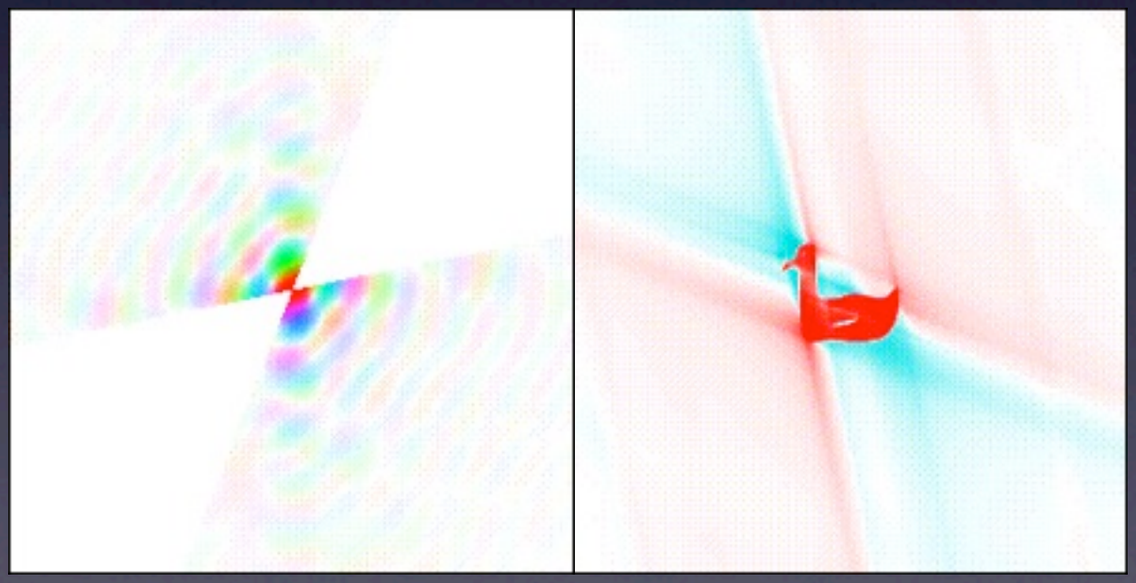
The importance of data completeness



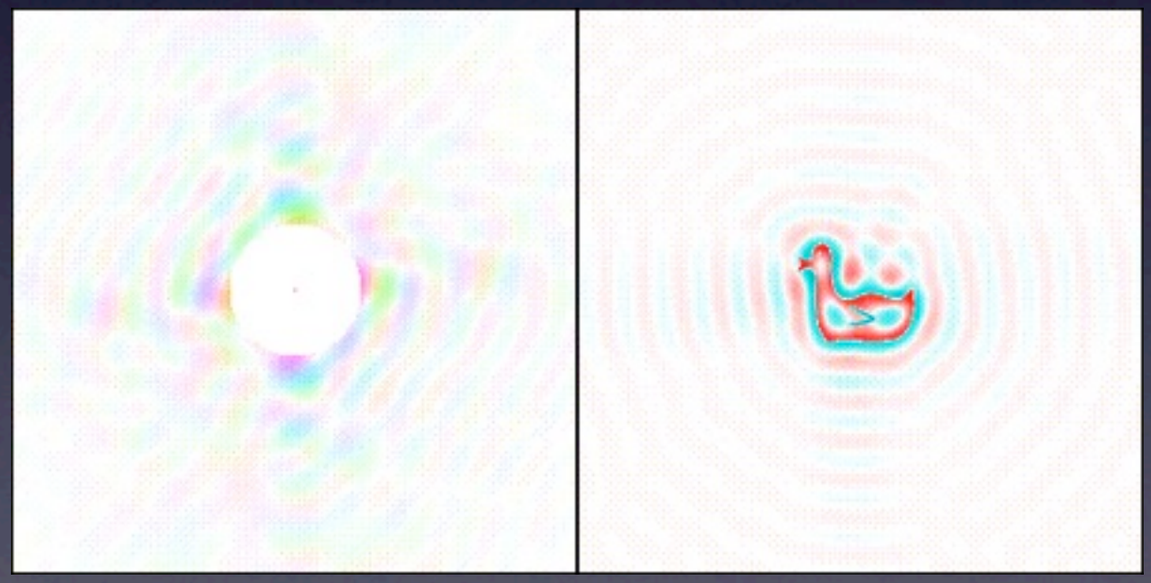
A duck ... ... and its Fourier transform



A low-resolution duck



Incomplete data: missing wedge



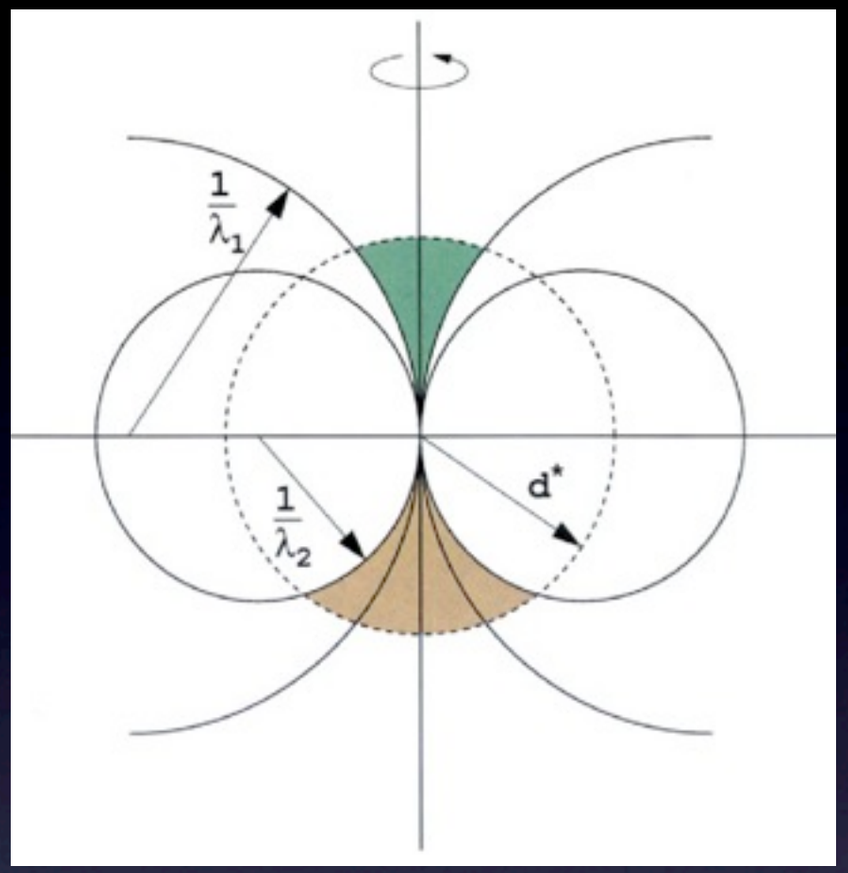
A duck without low-resolution reflections

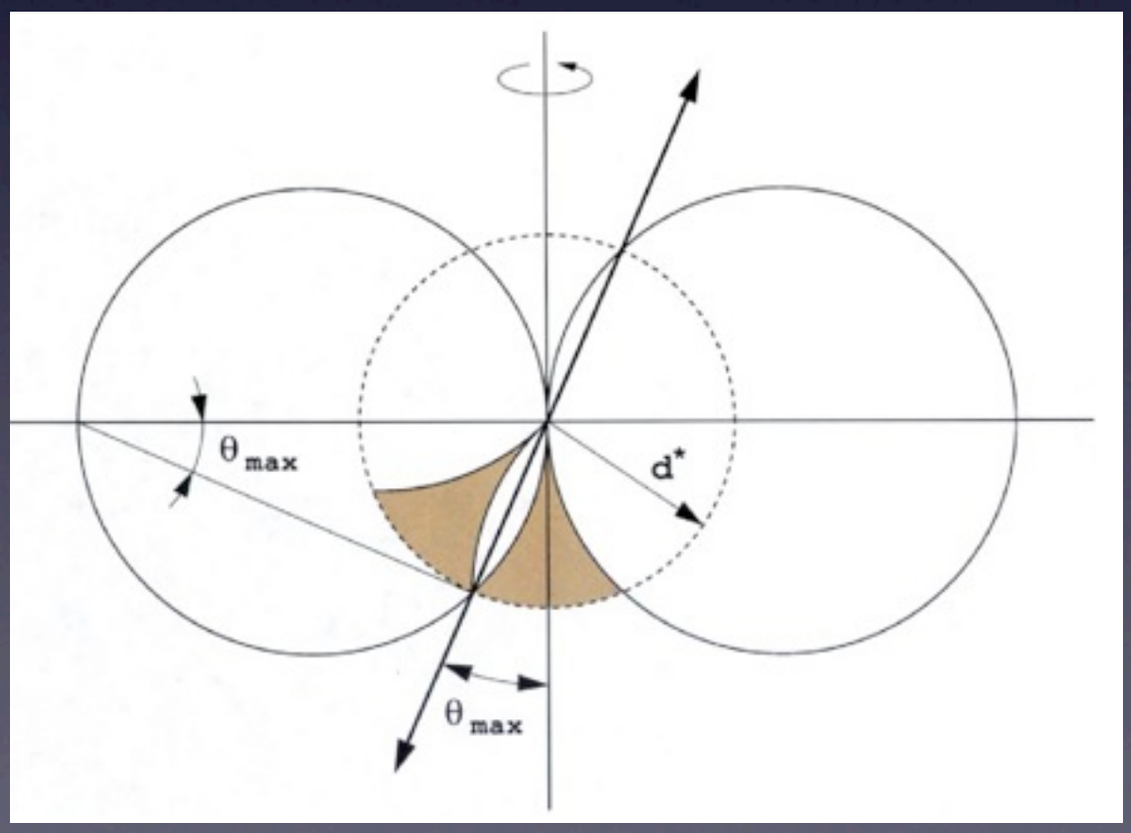
from Kevin Cowtan's "Book of Fourier"

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



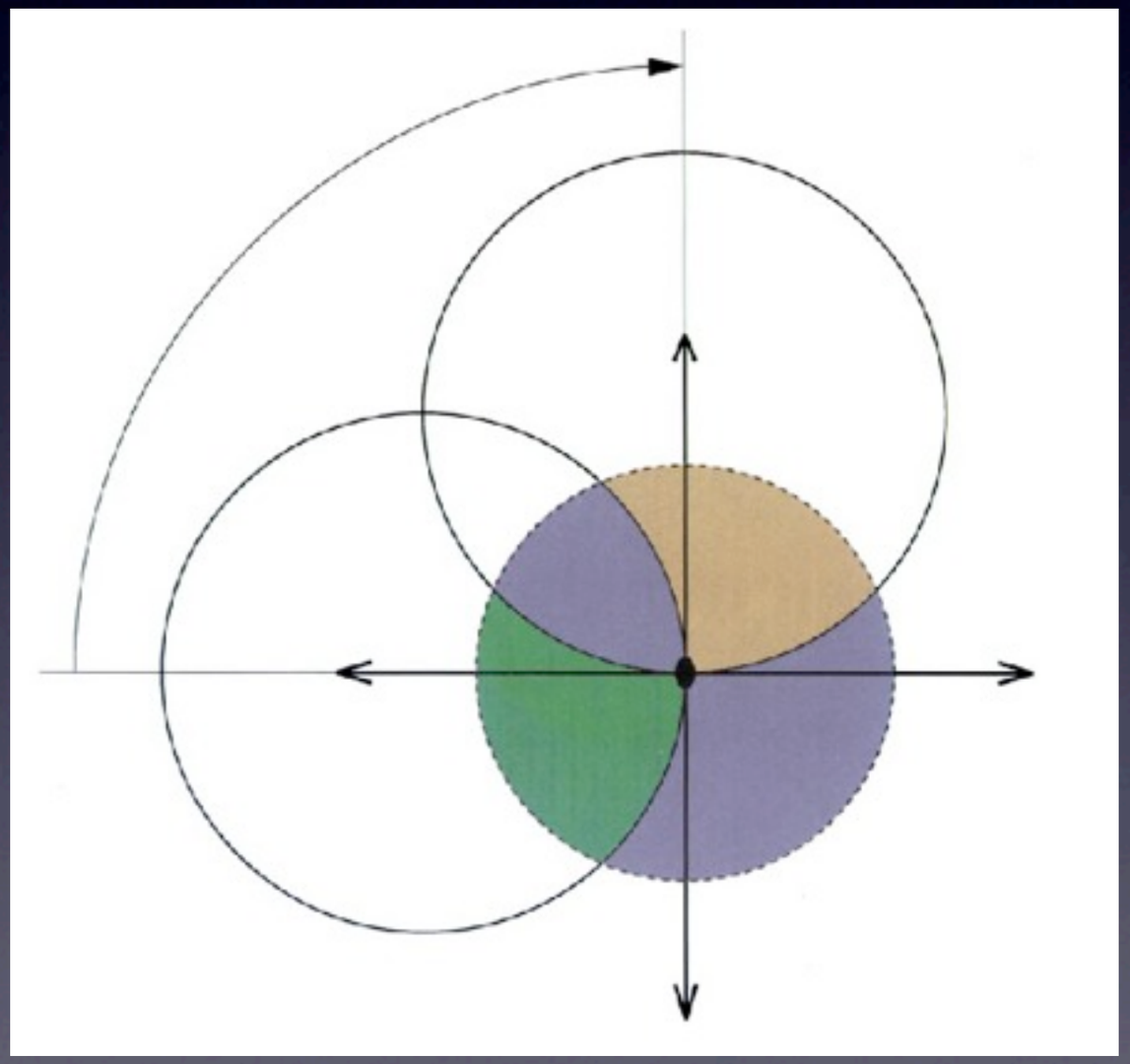


The blind region may be filled in by collecting a second set of data, offsetting the crystal by at least θ_{max} or by symmetry (except in P1)

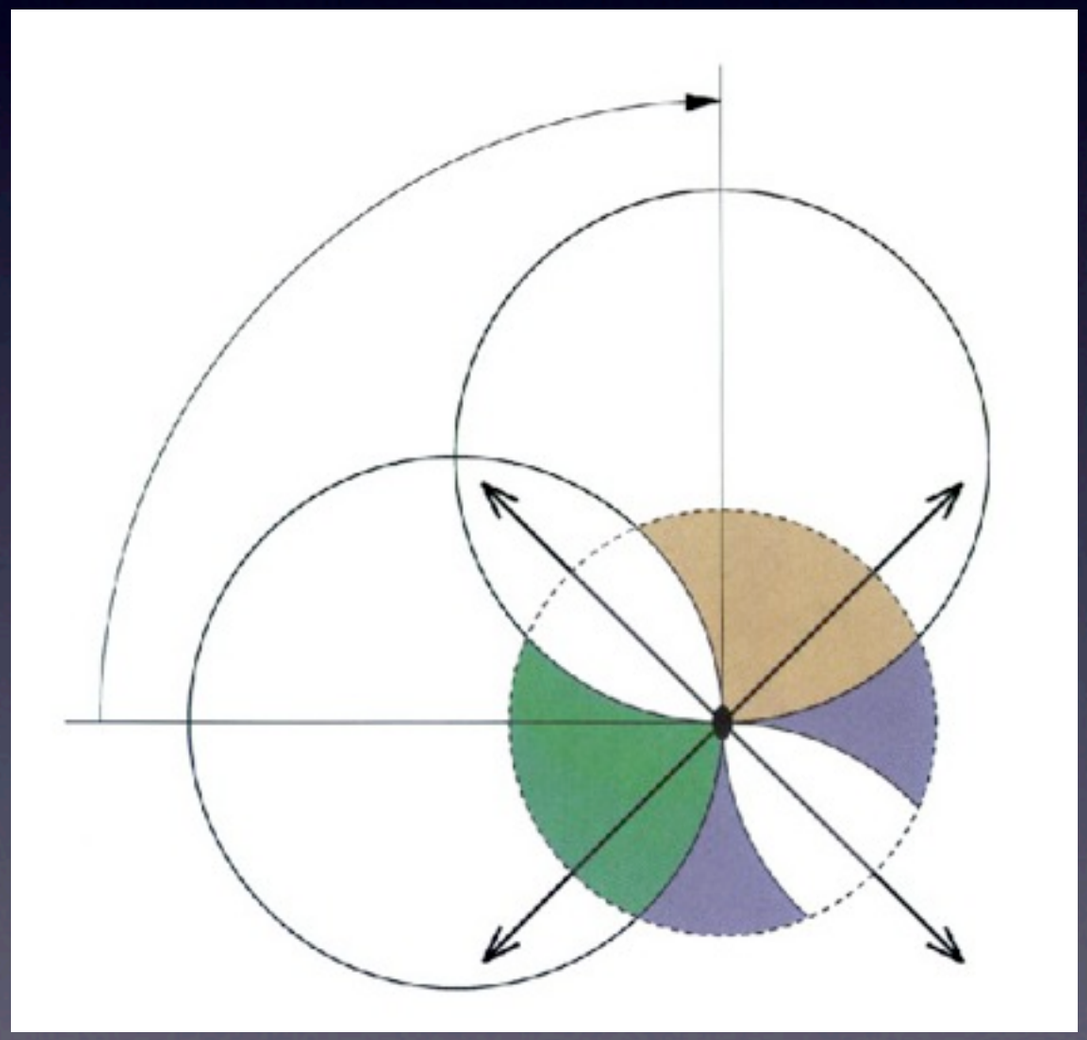
If there is symmetry, offsetting from an axis can remove or reduce the blind region for a single setting

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



	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: MOSFLM DNA (EDNA), BEST

• **Integration**
  • **Index**
  choose lattice
  • **Refine unit cell**
  • **Integrate**
	What is the correct lattice?
	[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)*

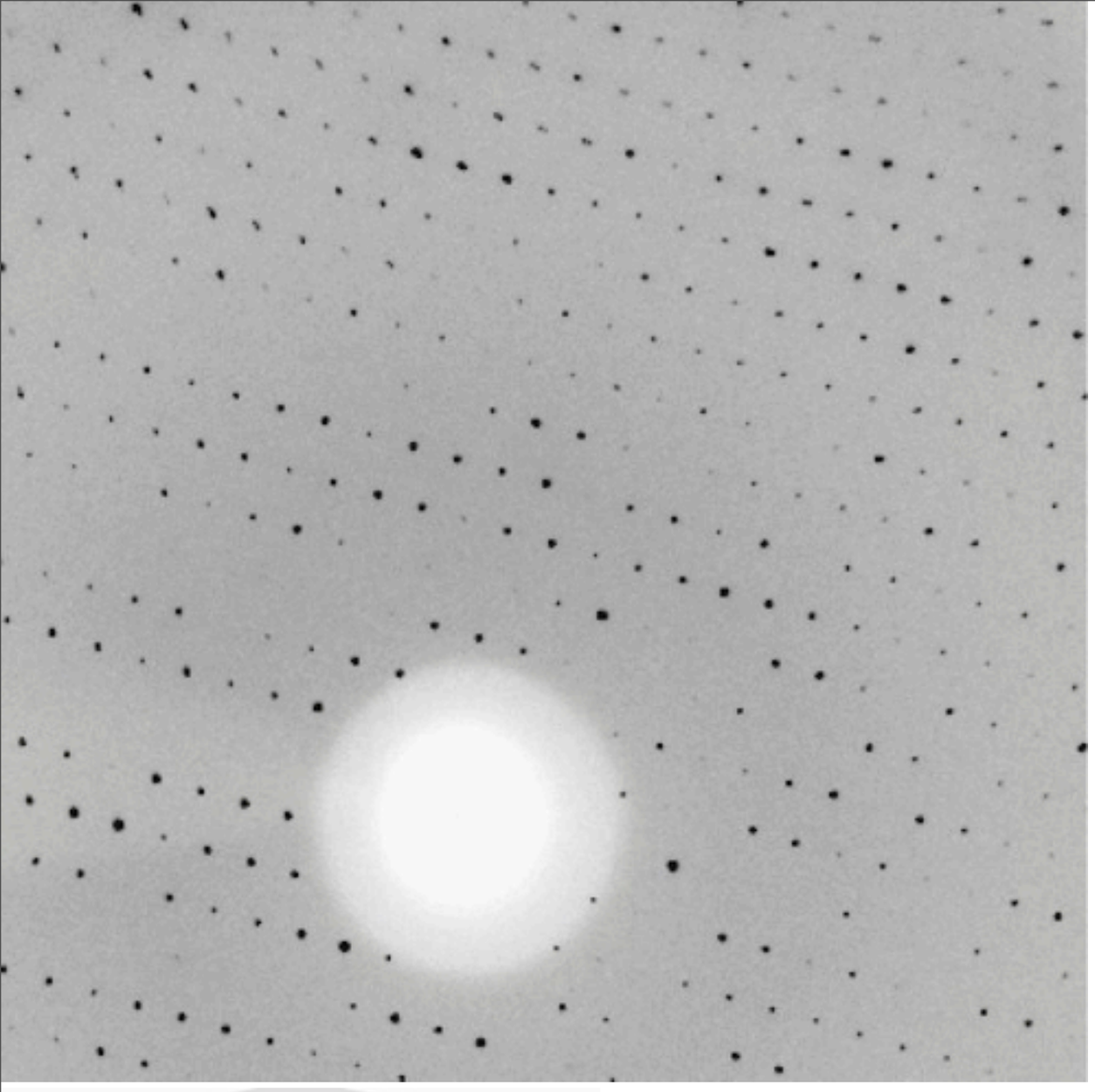
  Optimise cryo procedure, and worry about crystal transfer procedures

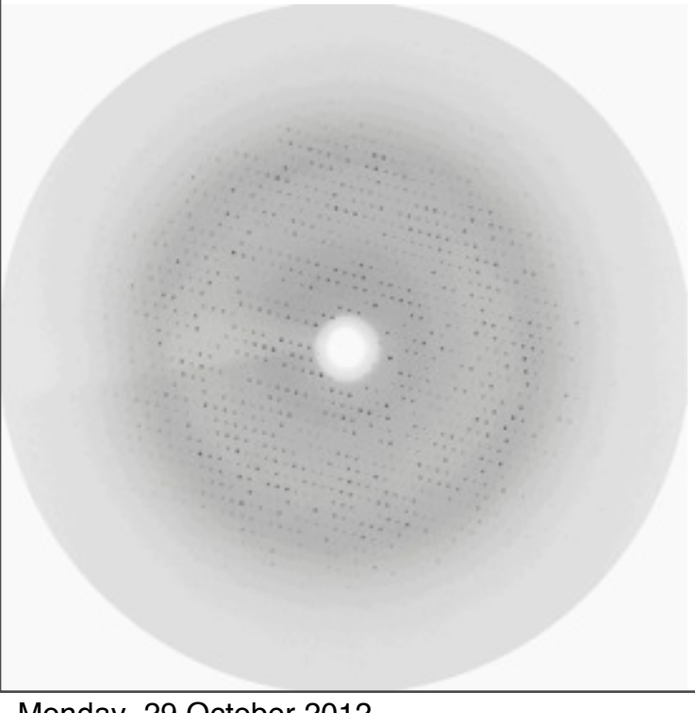
- The best that you have! (the least worst)

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

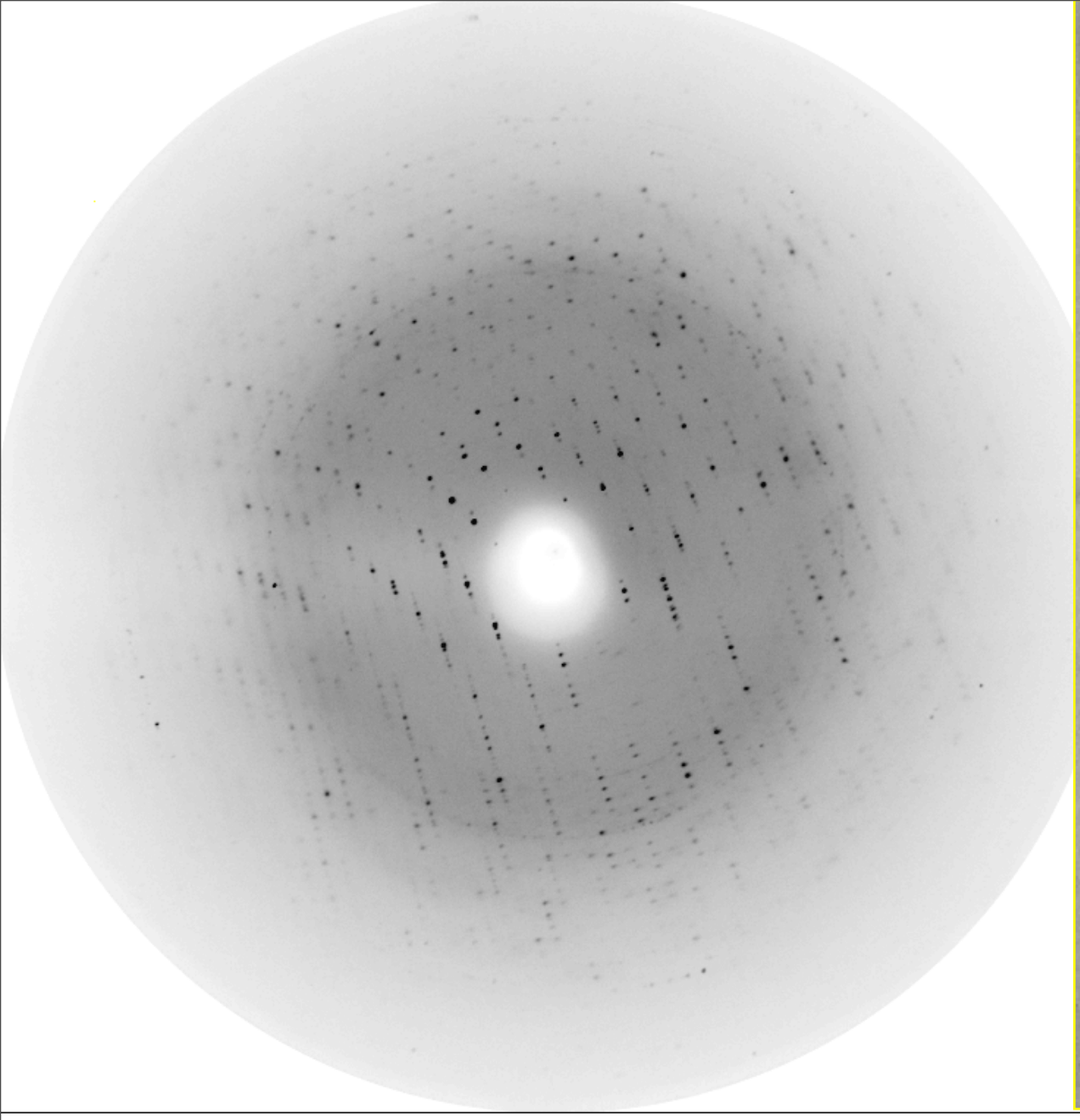


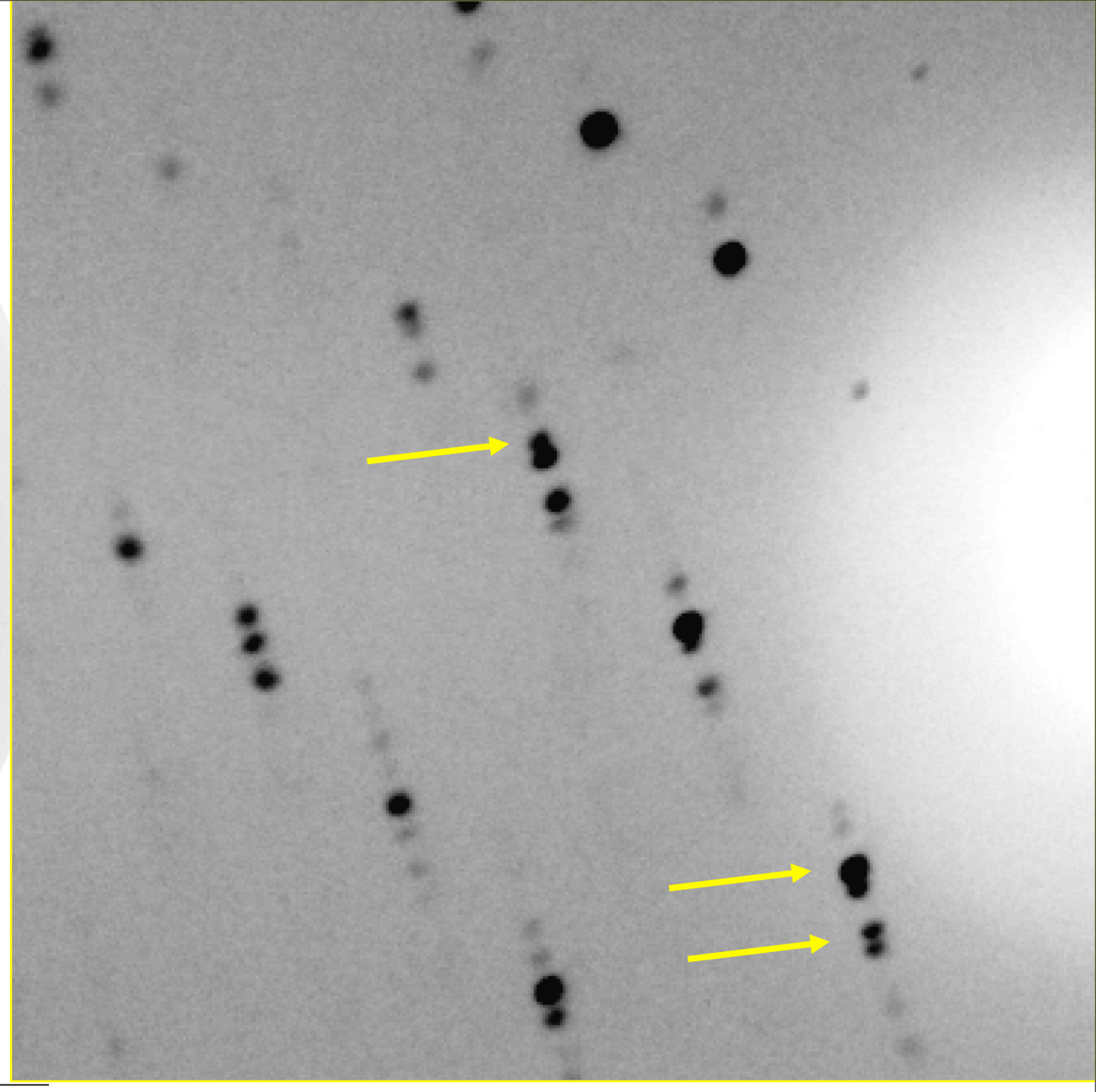


Phi = 0°

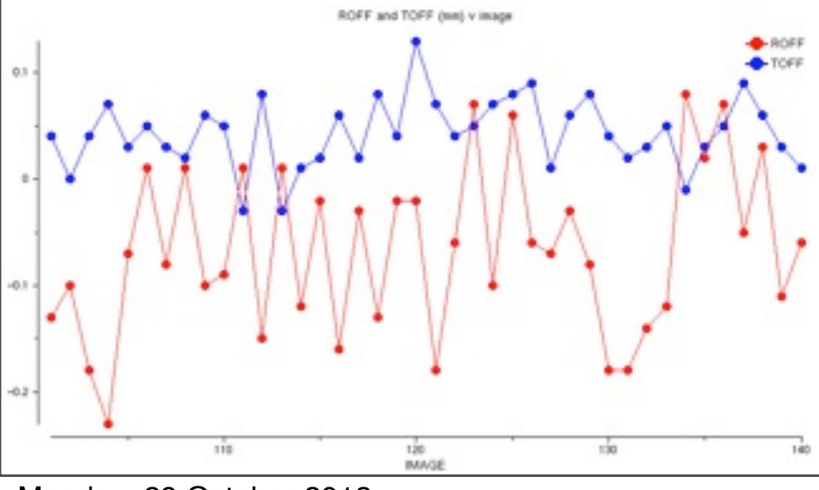
Phi = 90°

Always check diffraction in two orthogonal images !

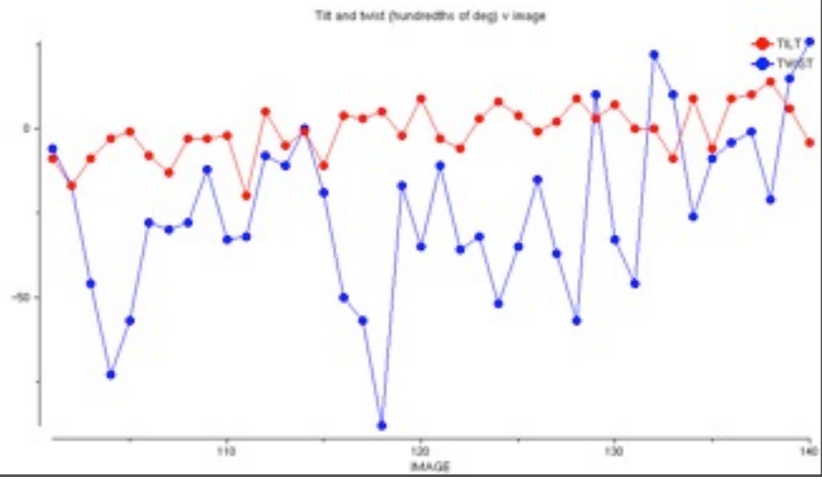




Additional spots present, not resolved.



Results in instability in refinement of detector parameters.



Data collection strategy

Compromise between statistics (enough photons/reflection, and multiplicity) and radiation damage. **On synchrotrons, 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: **1° is usually wrong**!

  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 redundancy 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
  - 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.

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

Exposure time/image

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

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)

Not a problem for shutterless continuous collection with fast read-out detector

Integration of diffraction images

Integration

Images → hkl I σ(I)

Two distinct methods:

- 2-D: integrate spots on each image, add together partially recorded observations in the scaling program. MOSFLM, DENZO, HKL2000, etc
- 3-D: integrate 3-dimensional box around each spot, from a series of images. XDS, D*TREK, SAINT etc

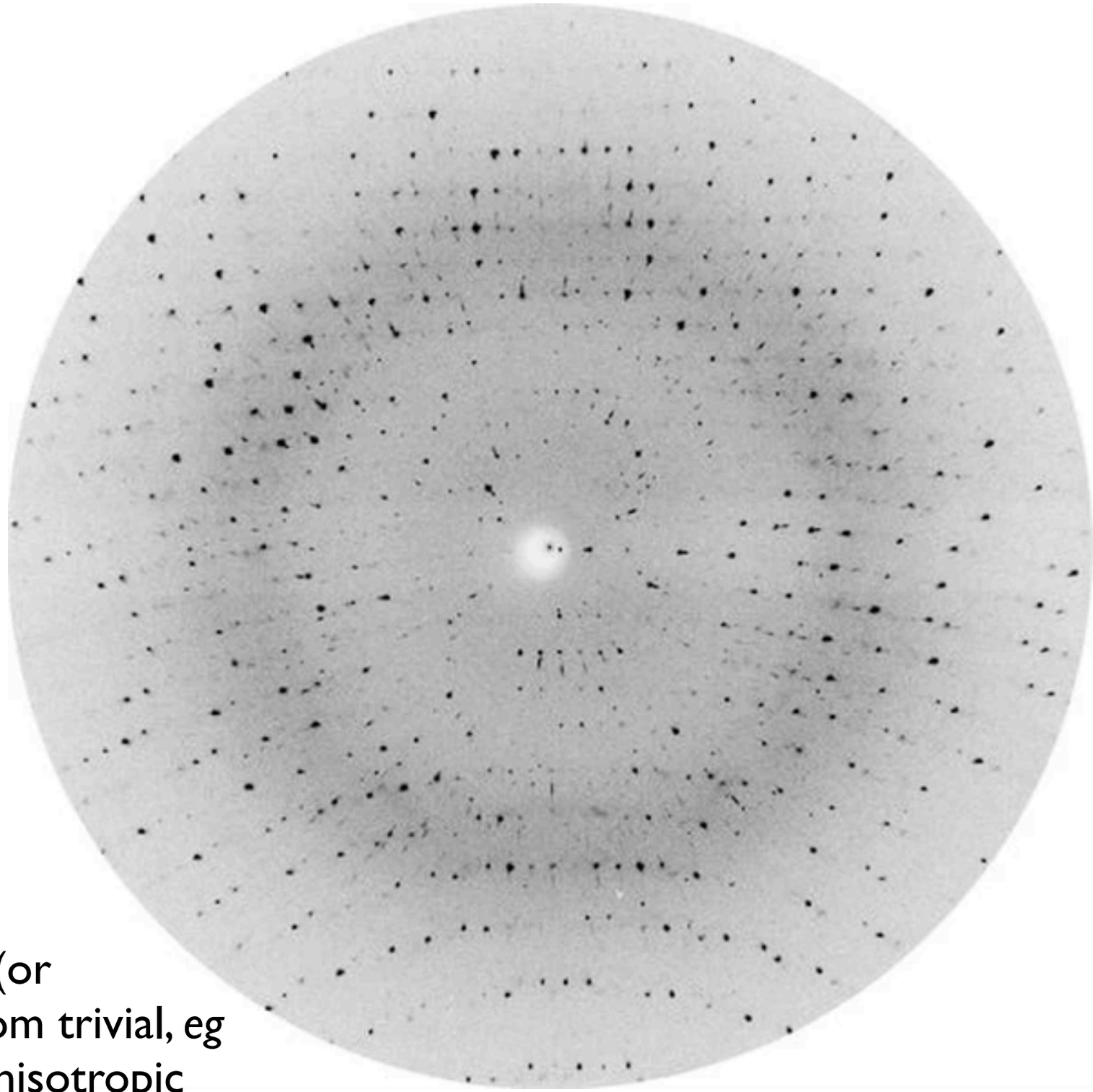
For today: MOSFLM

Starting Point: A series of diffraction images, each recorded on a 2D area detector while rotating the crystal through a small angle (typically 0.2-1.0° per image) about a fixed axis (the Rotation/Oscillation Method).

Outcome: A dataset consisting of the indices (h,k,l) of all reflections recorded on the images with an estimate of their intensities and the standard uncertainties of the intensities: h, k, l, I(hkl), σ(I)

integration slides from Andrew Leslie

Note that a series of images samples the full 3-dimensional reciprocal space, Bragg diffraction and any other phenomena, all scattering from crystal and its environment.



In practice, defects in the crystals (or detectors) make integration far from trivial, eg weak diffraction, crystal splitting, anisotropic diffraction, diffuse scattering, ice rings/spots, high mosaicity, unresolved spots, overloaded spots, zingers/cosmic rays, etc, etc.

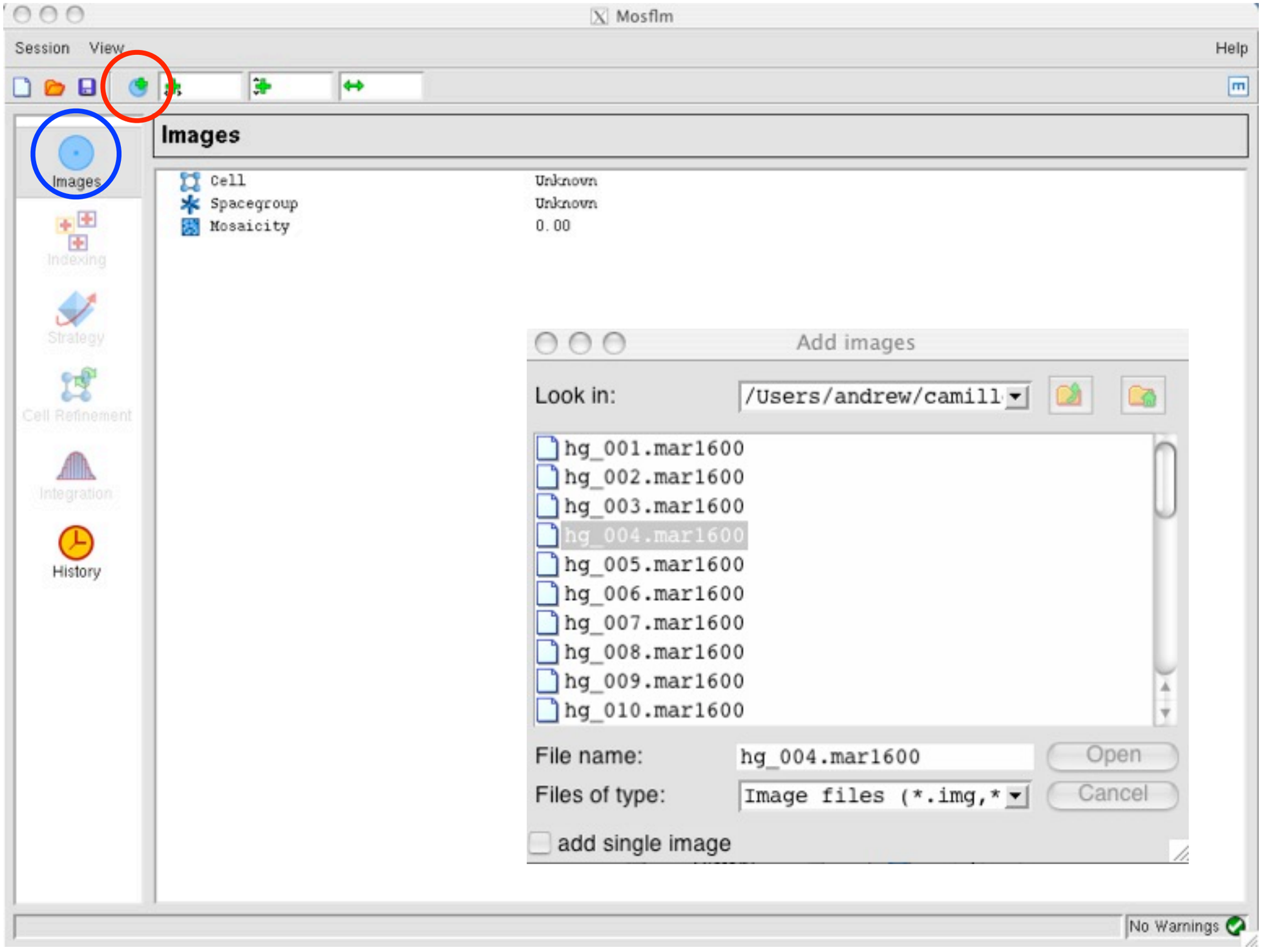
Integration

We want to calculate the intensity of each spot: then working backwards –

- The simplest method is draw a box around each spot, add up all the numbers inside, & subtract the background (or better, fit profile)
- To do this, we must know **where** the spot is: this needs
  - the unit cell of the crystal
  - the orientation of the crystal relative to the camera
  - the exact position of the detector
- To find the unit cell and crystal orientation, we must index the diffraction pattern
  - this can be done by finding spots on one or more images

Tools: the iMosflm interface

Images window: select & load images (all images with a common template)



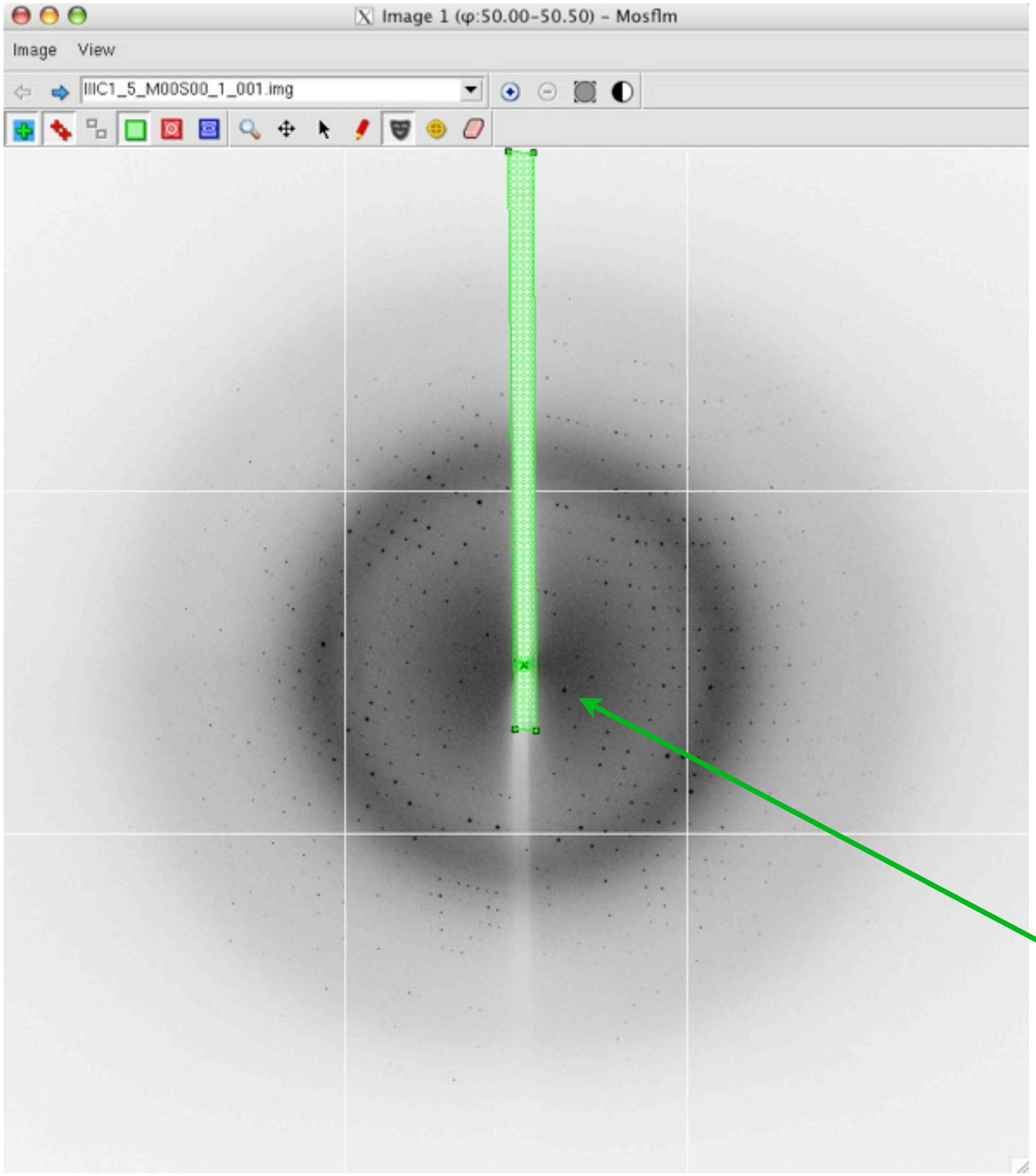


Image Display

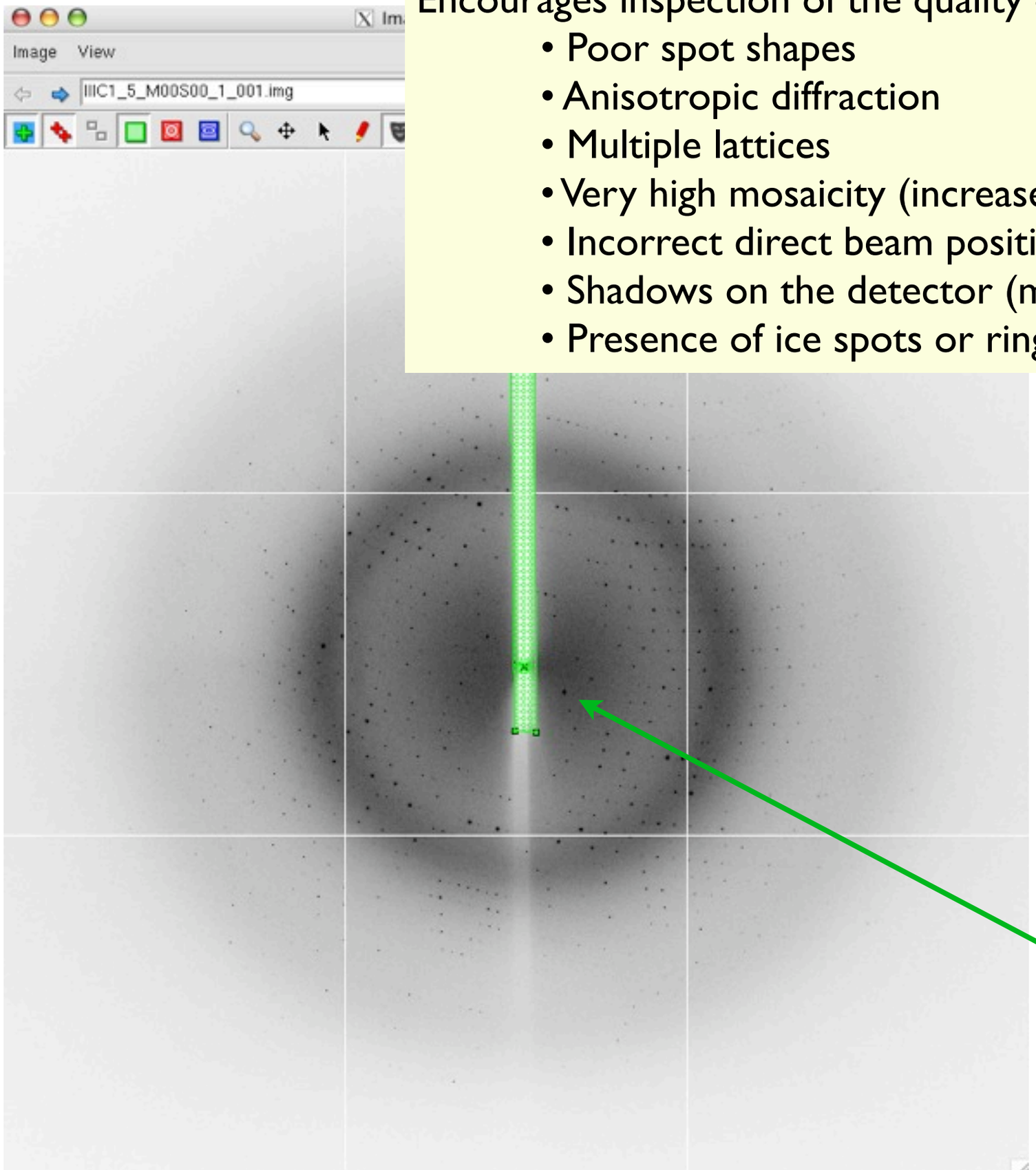
Simple control over:

- Found spots
- Predicted pattern
- Direct beam position
- Resolution limits
- Masking function
- Panning and Zooming

Note manually drawn mask for beam-stop shadow

Encourages inspection of the quality of the images (always a good thing) !

- Poor spot shapes
- Anisotropic diffraction
- Multiple lattices
- Very high mosaicity (increase threshold in indexing)
- Incorrect direct beam position (move it, try direct beam search)
- Shadows on the detector (mask them)
- Presence of ice spots or rings (exclude the resolution shells)



Note manually drawn mask for beam-stop shadow

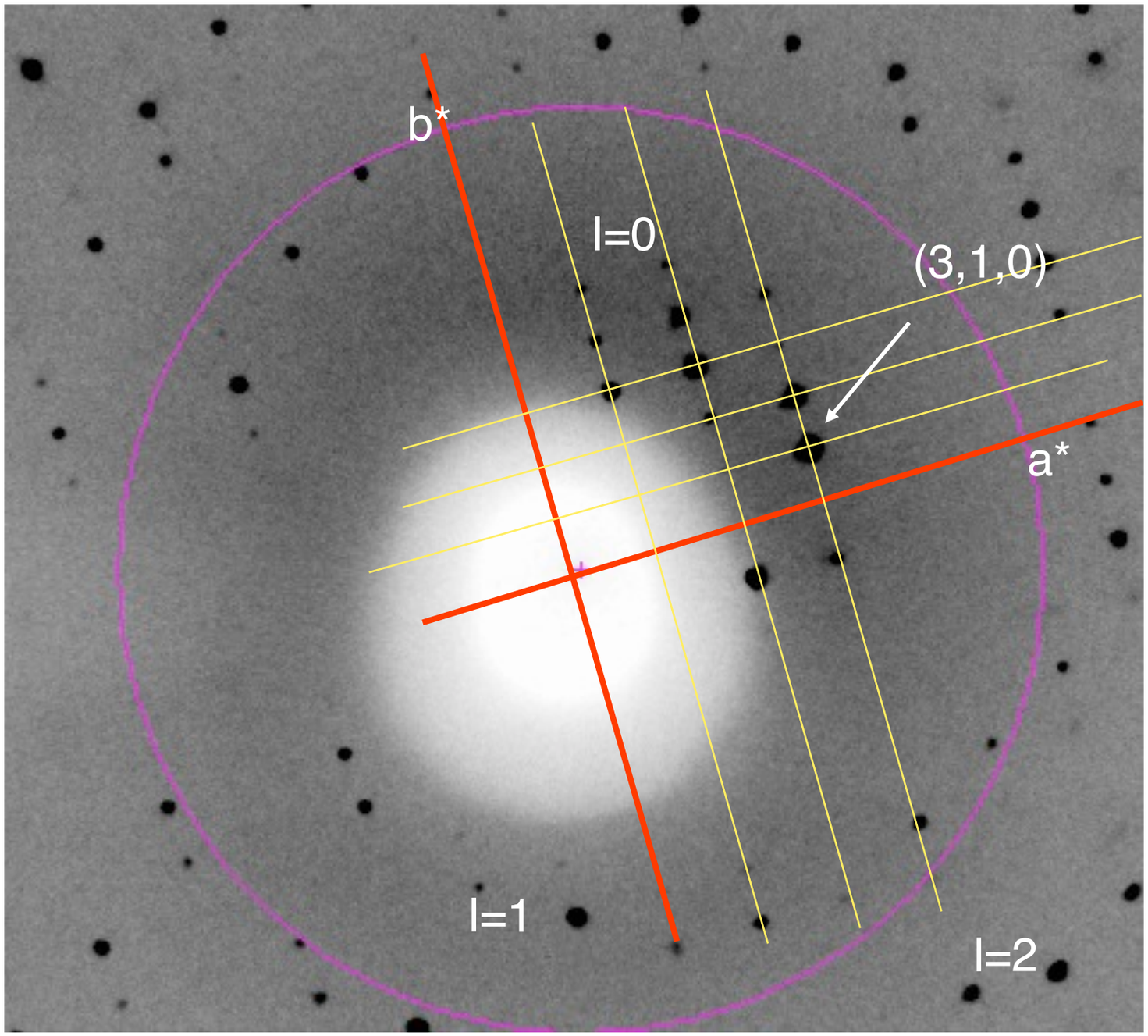
Integration procedure in iMosflm

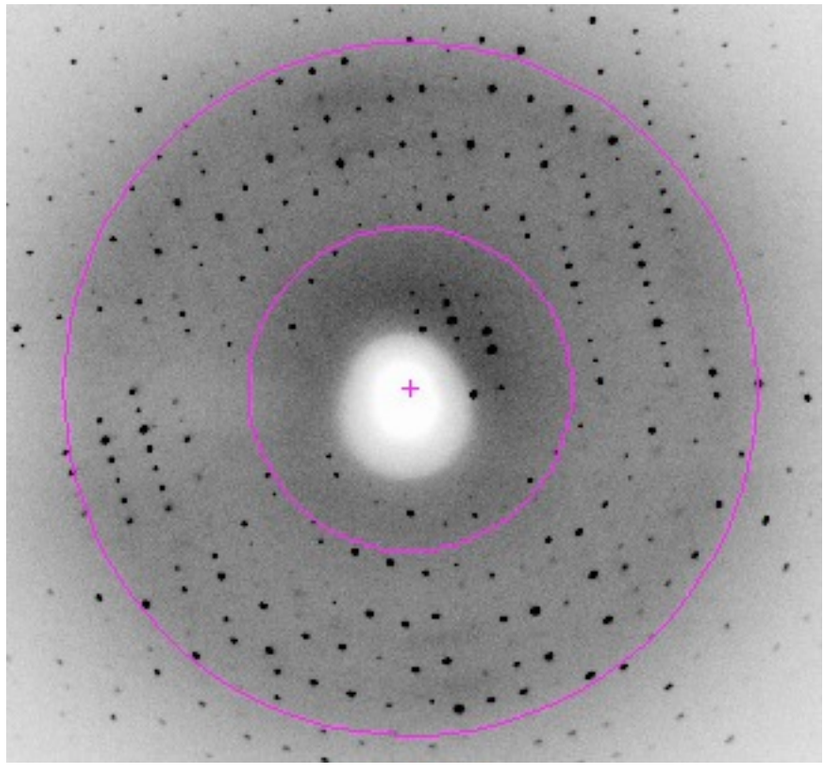
1. **Find spots**
   what is a spot? should have uniform shape, not streak
2. **Index**
   find *lattice* which fits spots
3. **Estimate mosaicity**
   improve estimate later
4. **Check prediction, on images remote in φ (90° away)**
   is the indexing correct?
5. **Refine cell**
   use two wedges at 90°, or more in low symmetry
6. **Mask backstop shadow**
   not (yet) done automatically by program
7. **Integrate one (or few) image**
   to check resolution etc
8. **Integrate all images**
   may be run in background for speed

Strategy option, for use before data collection

Indexing

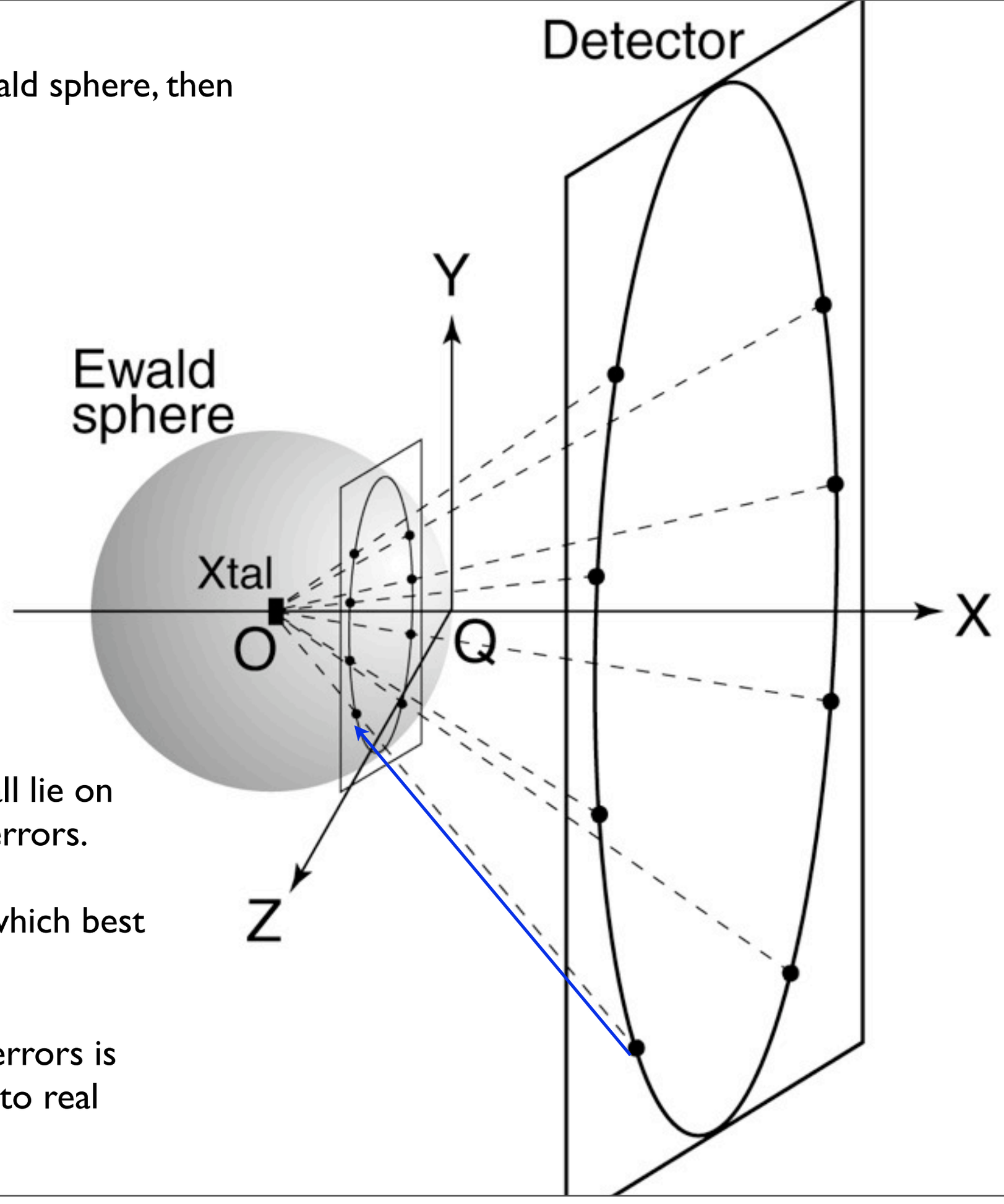
If we know the main beam position on the image, we can count spots from the centre





To do it properly, we need to put the spots into 3 dimensions, knowing the rotation of the crystal for this image

Back-project each spot on to Ewald sphere, then rotate back into zero-φ frame

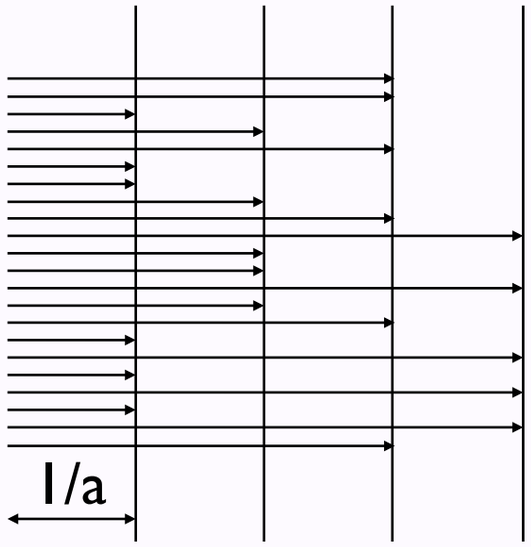


This gives a list of vectors which all lie on the reciprocal lattice, with some errors.

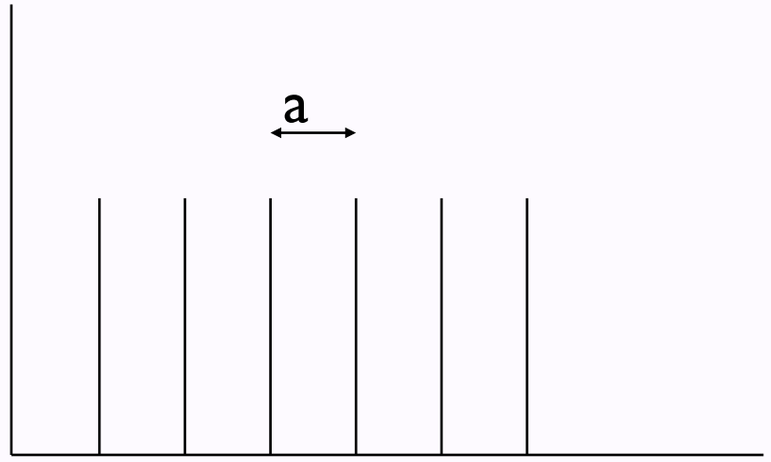
We then want to find a **lattice** which best fits these vectors.

The best way to average out the errors is to use a **Fourier transform** into real space

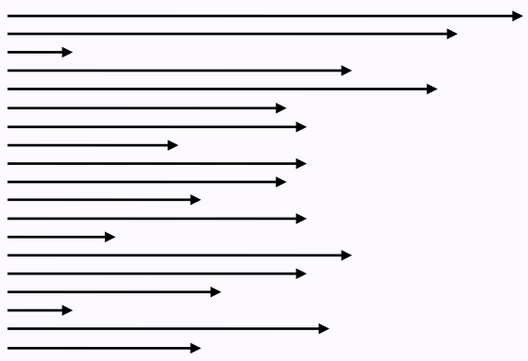
Consider every possible direction in turn as a possible **real-space** axis, ie perpendicular to a reciprocal lattice plane. Project all observed vectors on to this axis



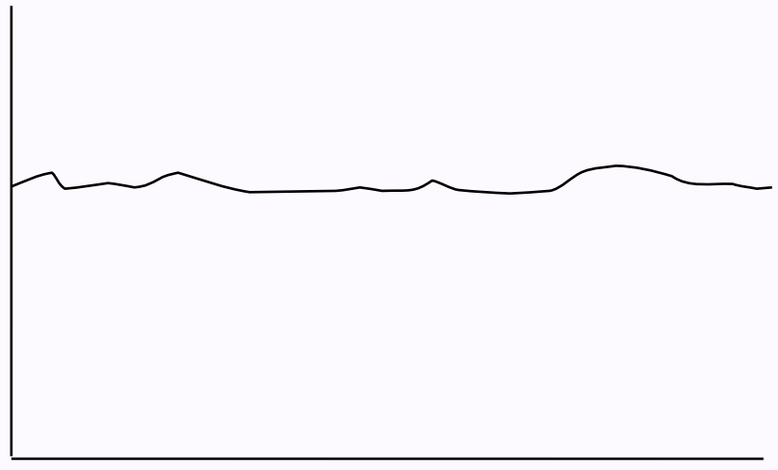
Fourier transform



Lattice plane normal to lattice plane: vectors cluster at lengths which are multiples of the lattice spacing. Fourier transform shows sharp peaks

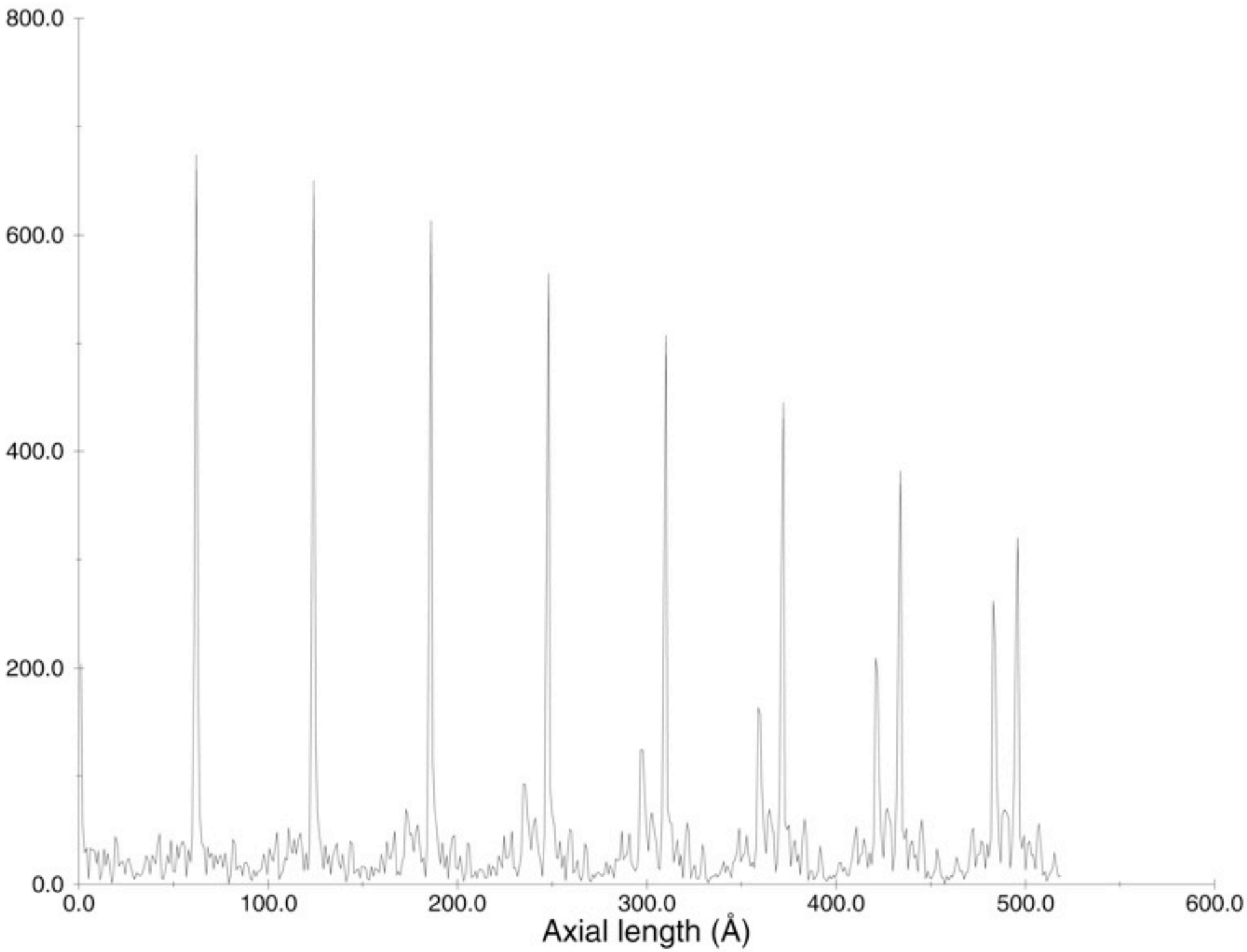


Fourier transform



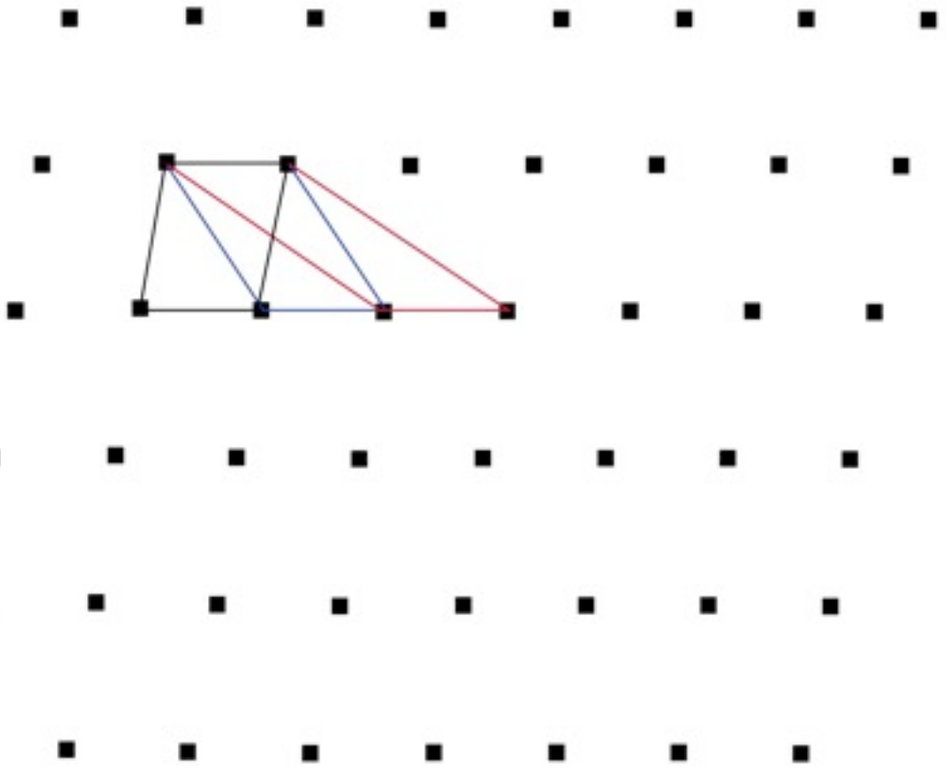
Non-lattice direction, random length. No peaks in Fourier transform

1D Fourier transform of projected scattering vectors



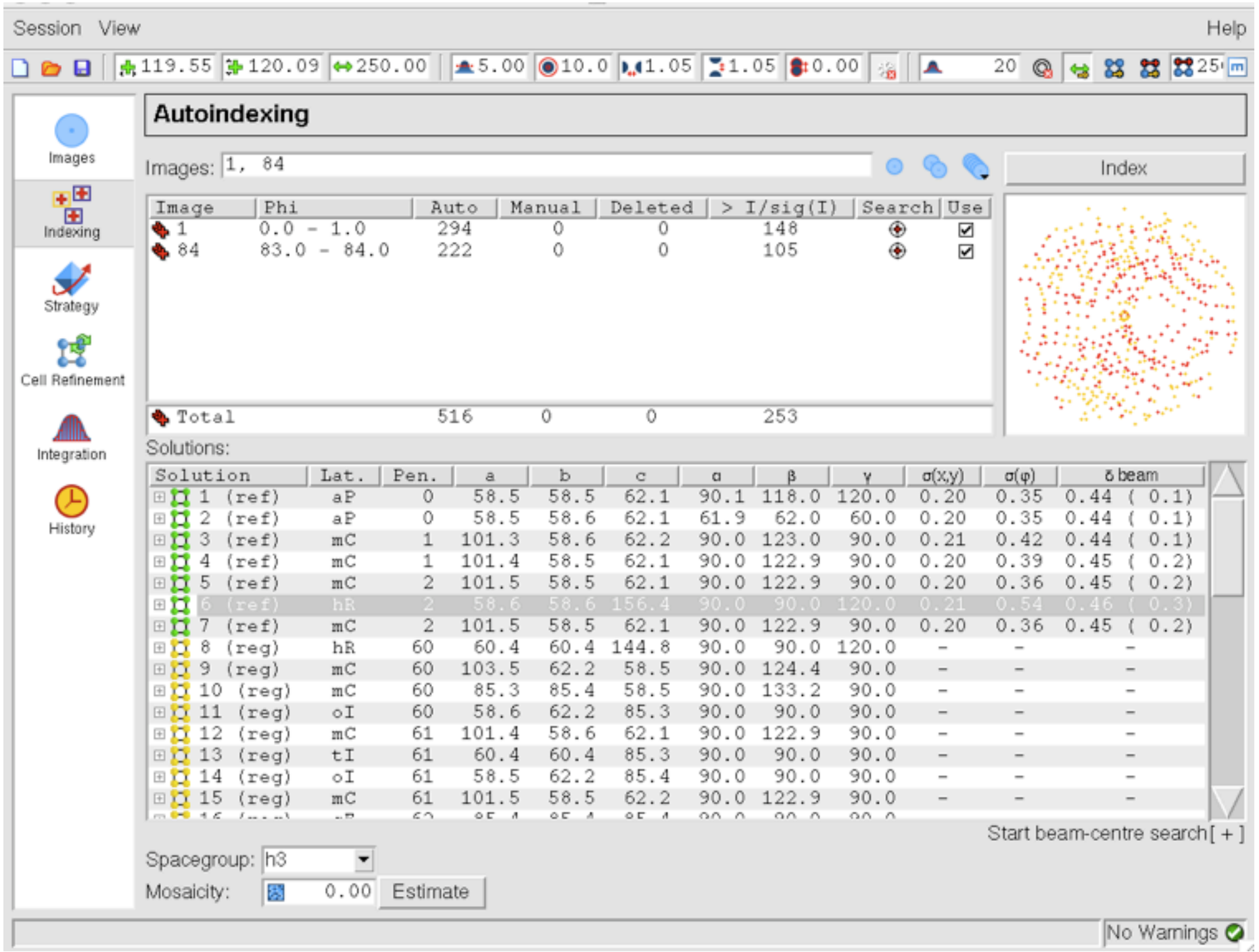
Pick three non-coplanar directions which have the largest peaks in the Fourier transforms to define a lattice.

This is not necessarily the simplest lattice (the "reduced cell")



In the 2D example shown, the black cell corresponds to the reduced cell, while the red or blue cells may have been found in the autoindexing.

Indexing

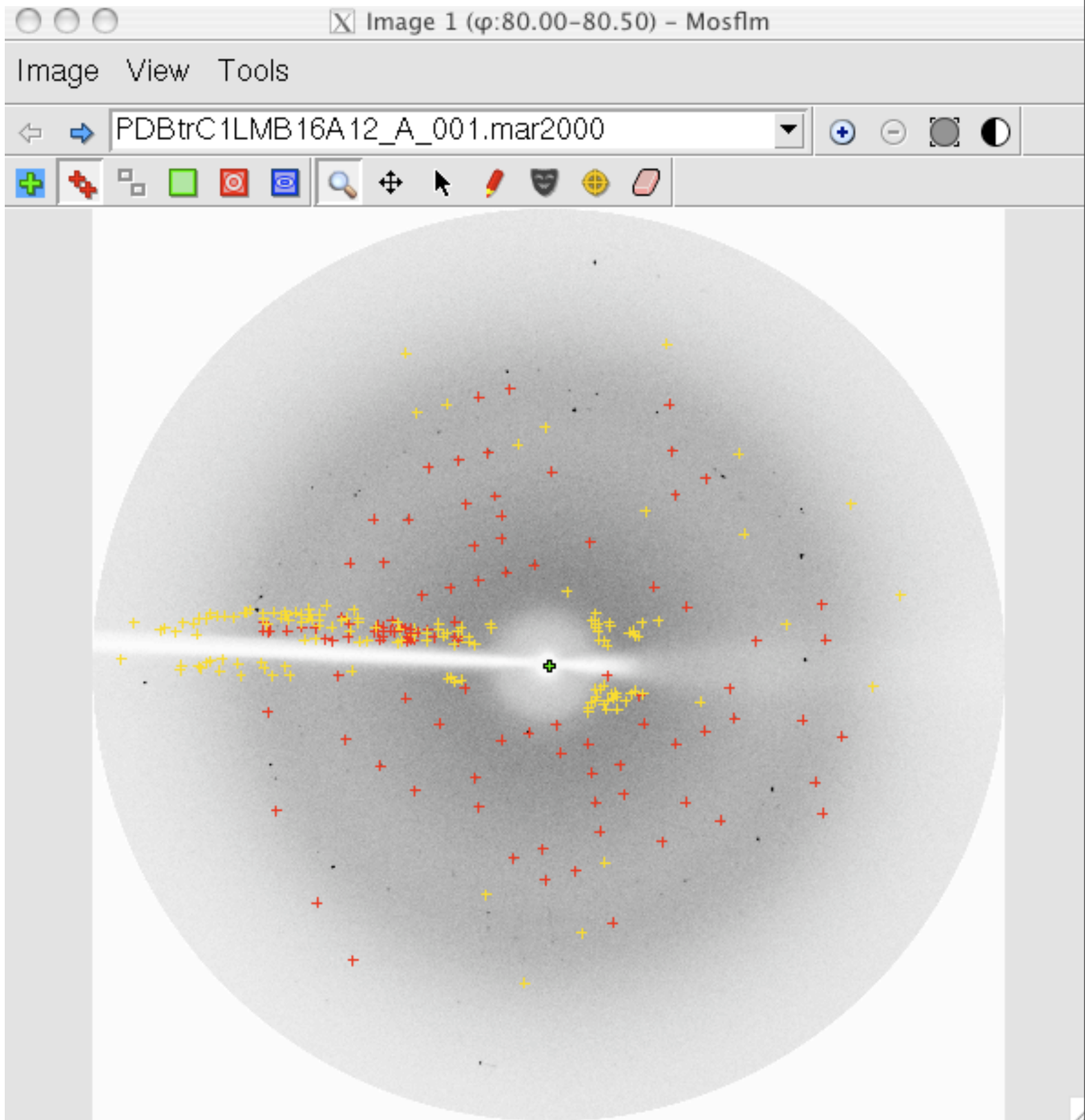


- Selects two images 90 degrees apart (if possible).
- Finds spots on these images
- Selecting "Index" attempts to index using these two images.
- Produces a list of solutions.
- Highlights the "best" solution. (Highest symmetry with a low penalty).

We only have information about the lattice shape at this stage, so assignment of symmetry is an assumption.

Indexing – Spot Finding

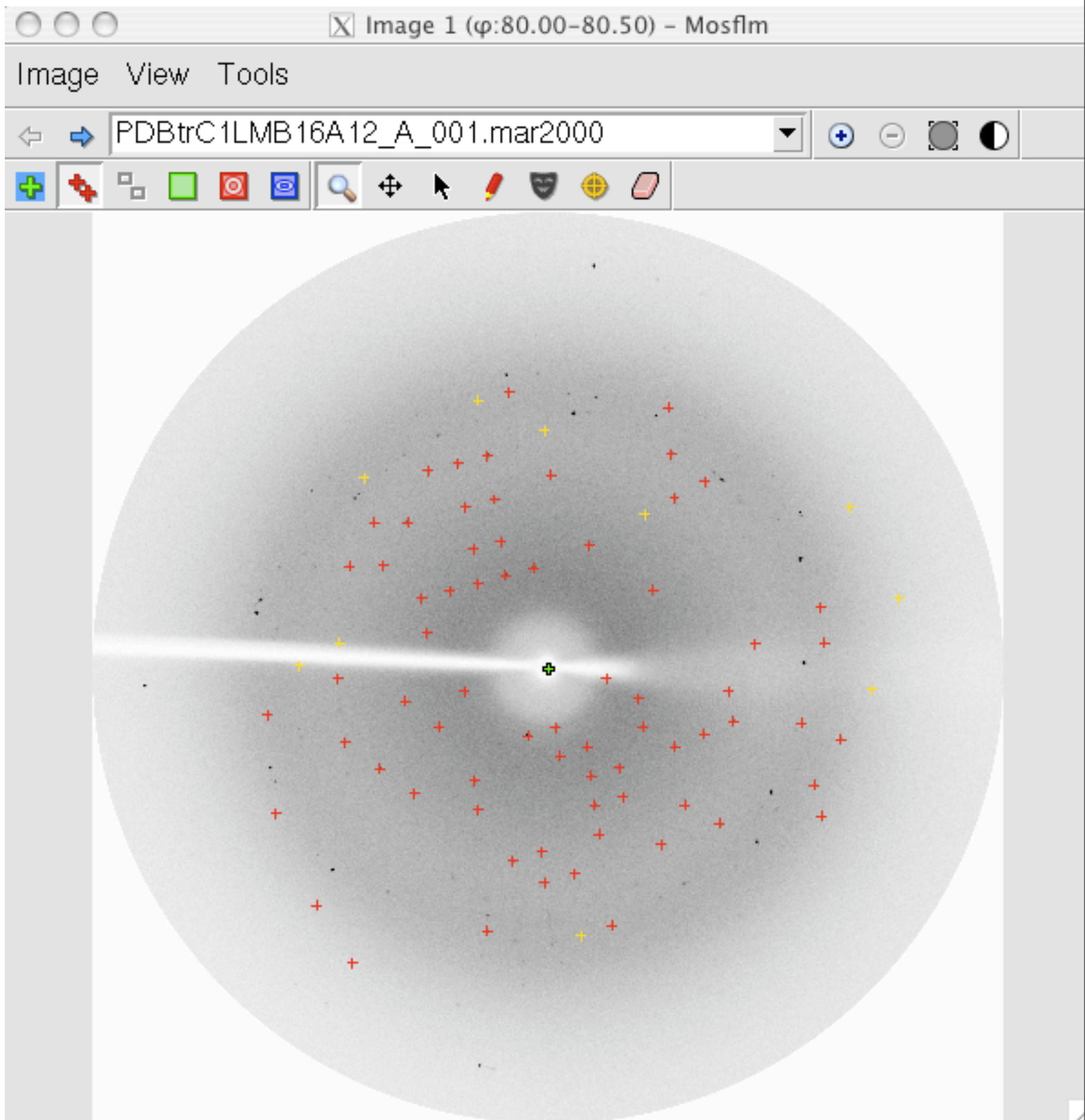
To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)



Indexing – Spot Finding

To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)

eg to 20,20 (pixels)

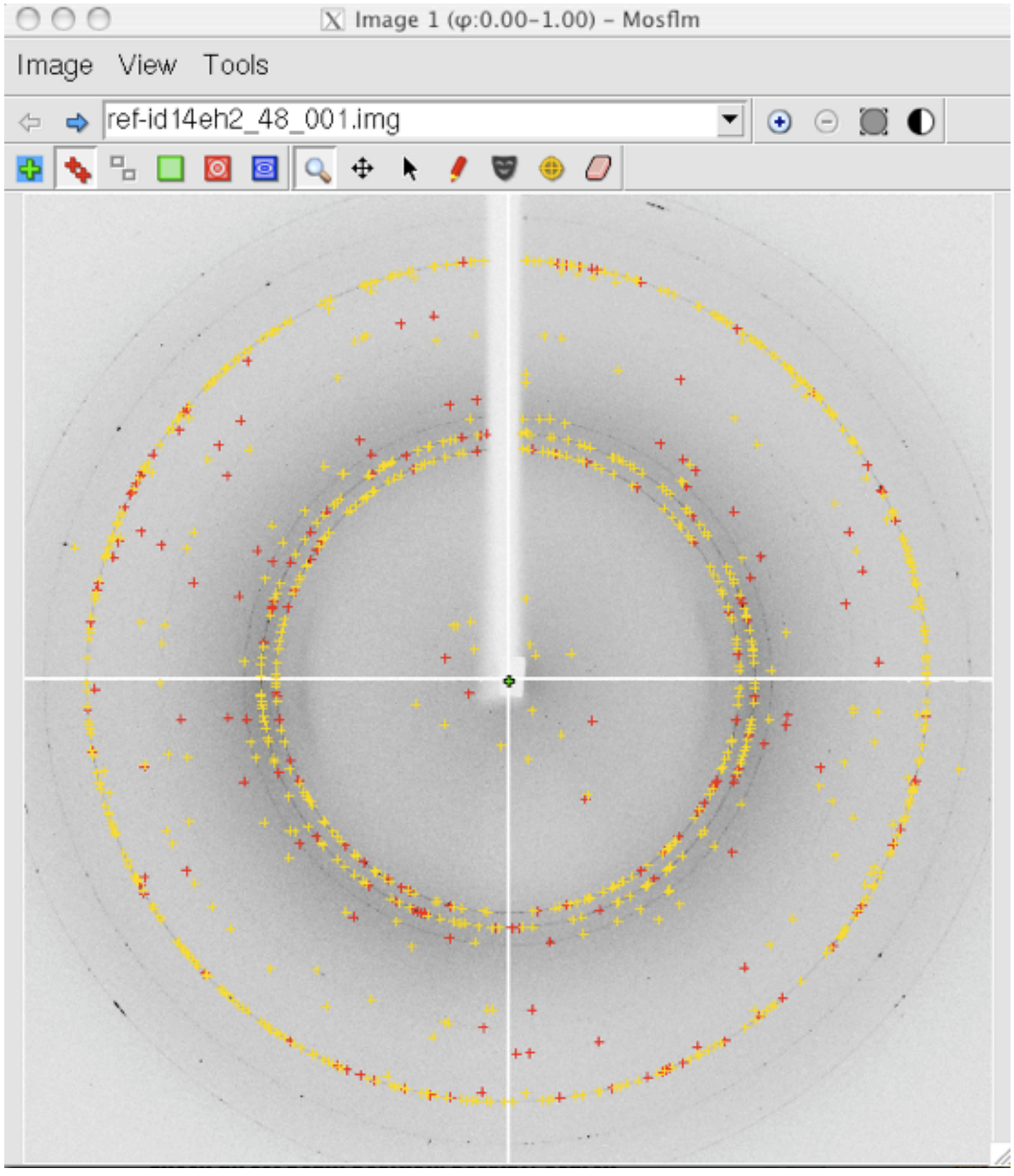


Indexing – Spot Finding

To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)

eg to 20,20 (pixels)

Ice rings will cause problems



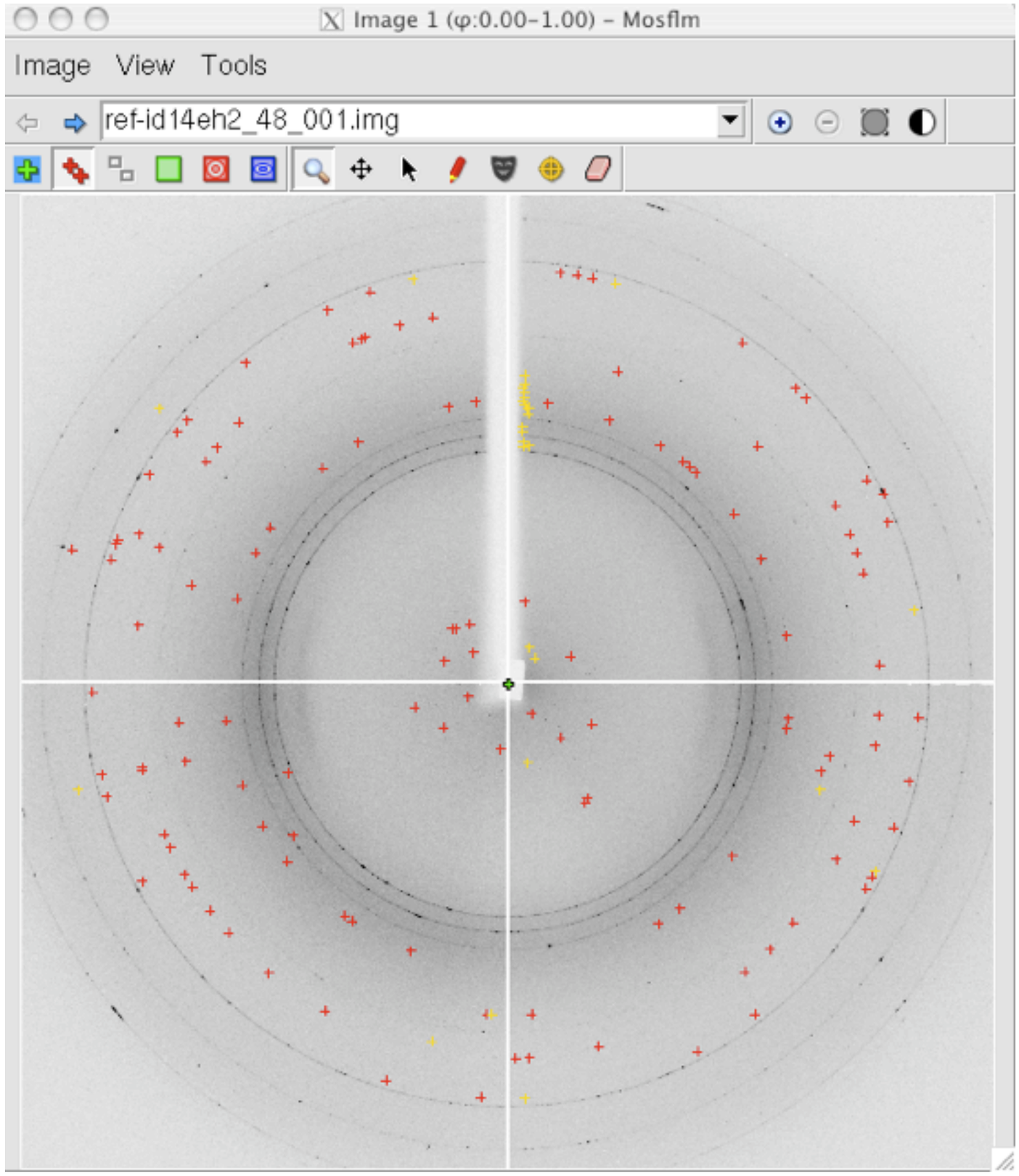
Indexing – Spot Finding

To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)

eg to 20,20 (pixels)

Ice rings will cause problems

So mosflm rejects all spots at the resolutions corresponding to ice. Additional rings still give problems in this case.



Indexing – Spot Finding

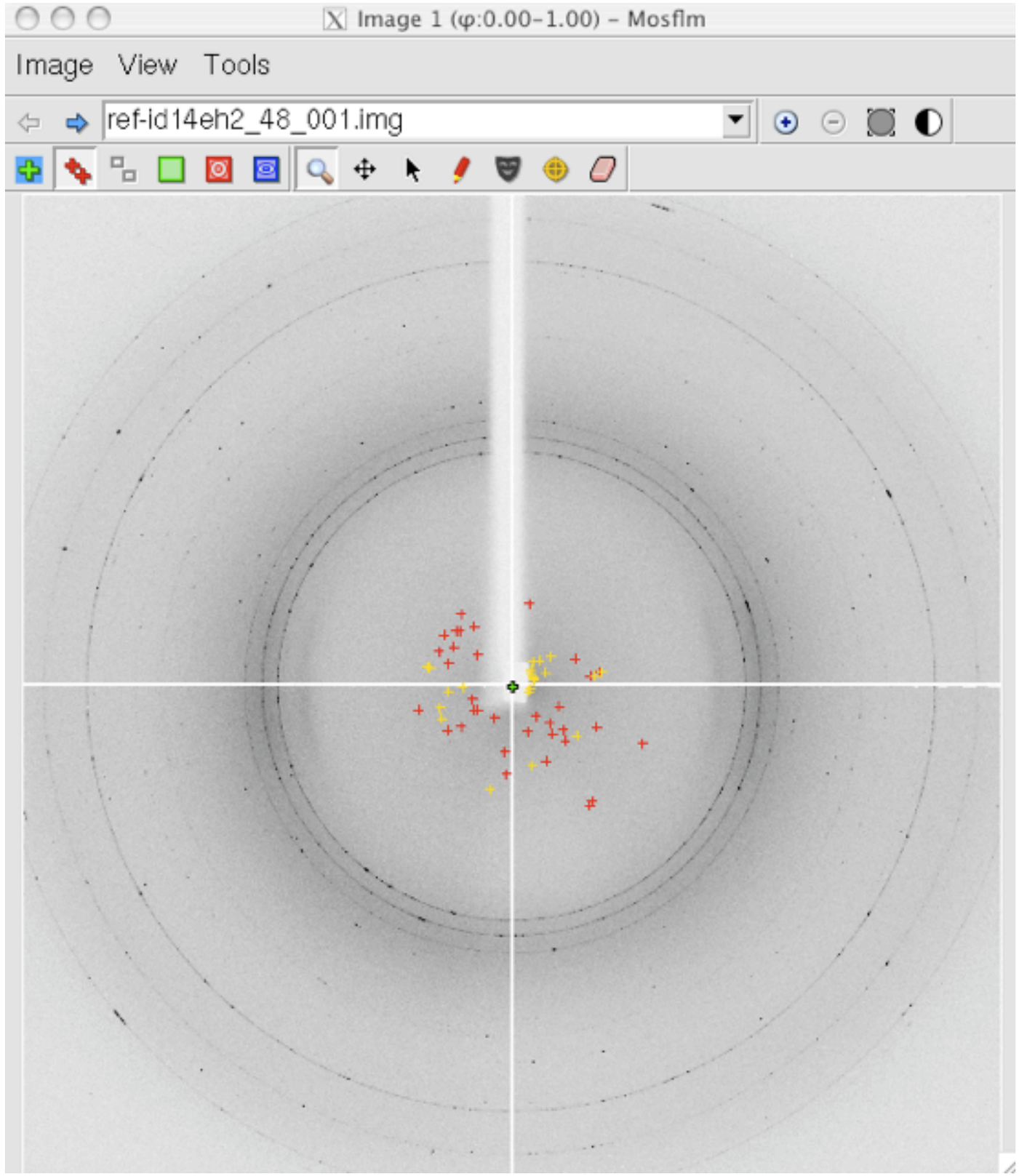
To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)

eg to 20,20 (pixels)

Ice rings will cause problems

So mosflm rejects all spots at the resolutions corresponding to ice. Additional rings still give problems in this case.

Because the diffraction is very weak in this case, mosflm rejects all spots beyond 4.5Å



Indexing – Spot Finding

To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)

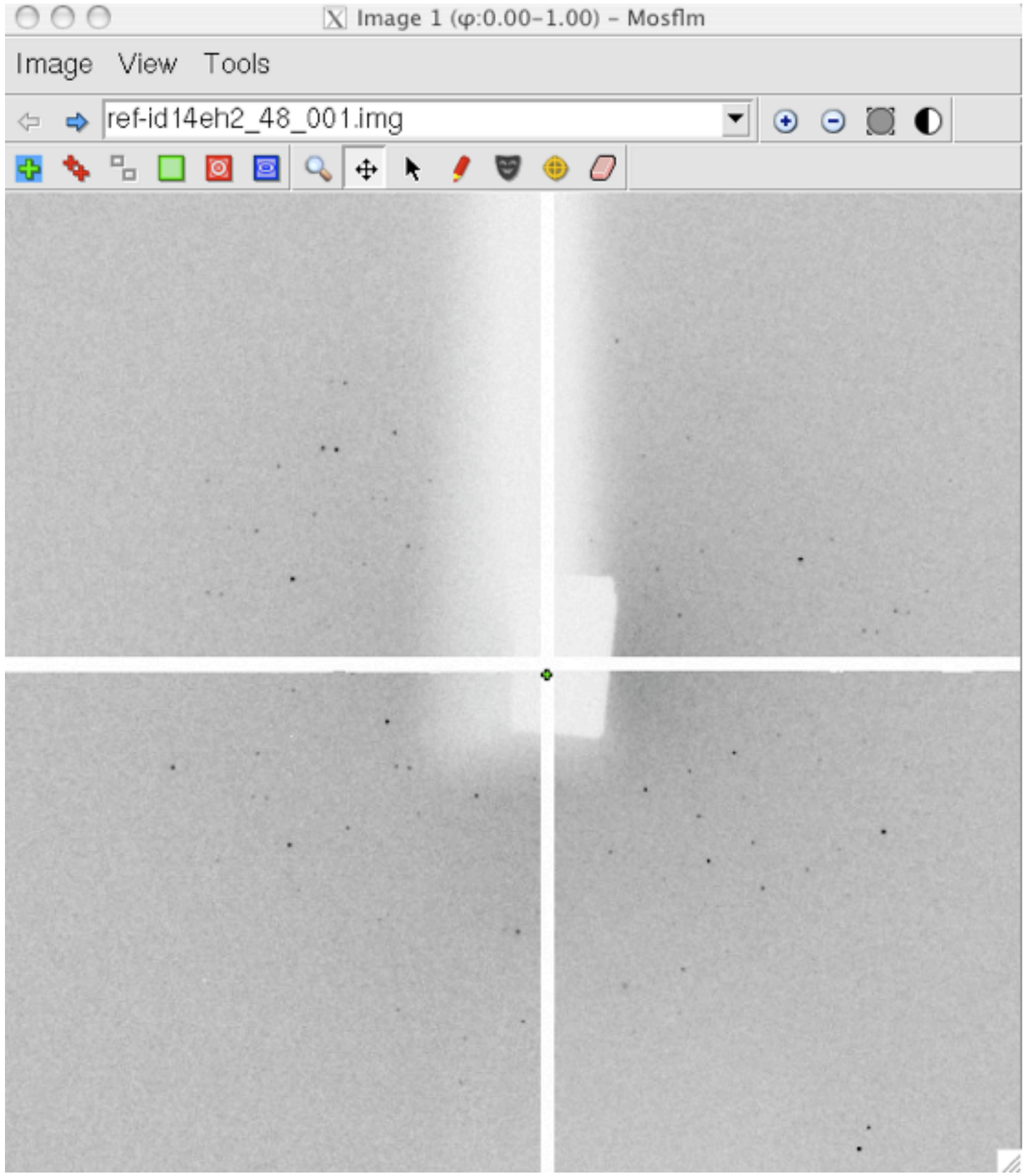
eg to 20,20 (pixels)

Ice rings will cause problems

So mosflm rejects all spots at the resolutions corresponding to ice. Additional rings still give problems in this case.

Because the diffraction is very weak in this case, mosflm rejects all spots beyond 4.5Å

In addition, mosflm lowers the threshold for finding spots and the minimum spots size (in pixels)



Indexing – Spot Finding

To eliminate "false" spots near shadows, reduce the size of the local background box (100x100)

eg to 20,20 (pixels)

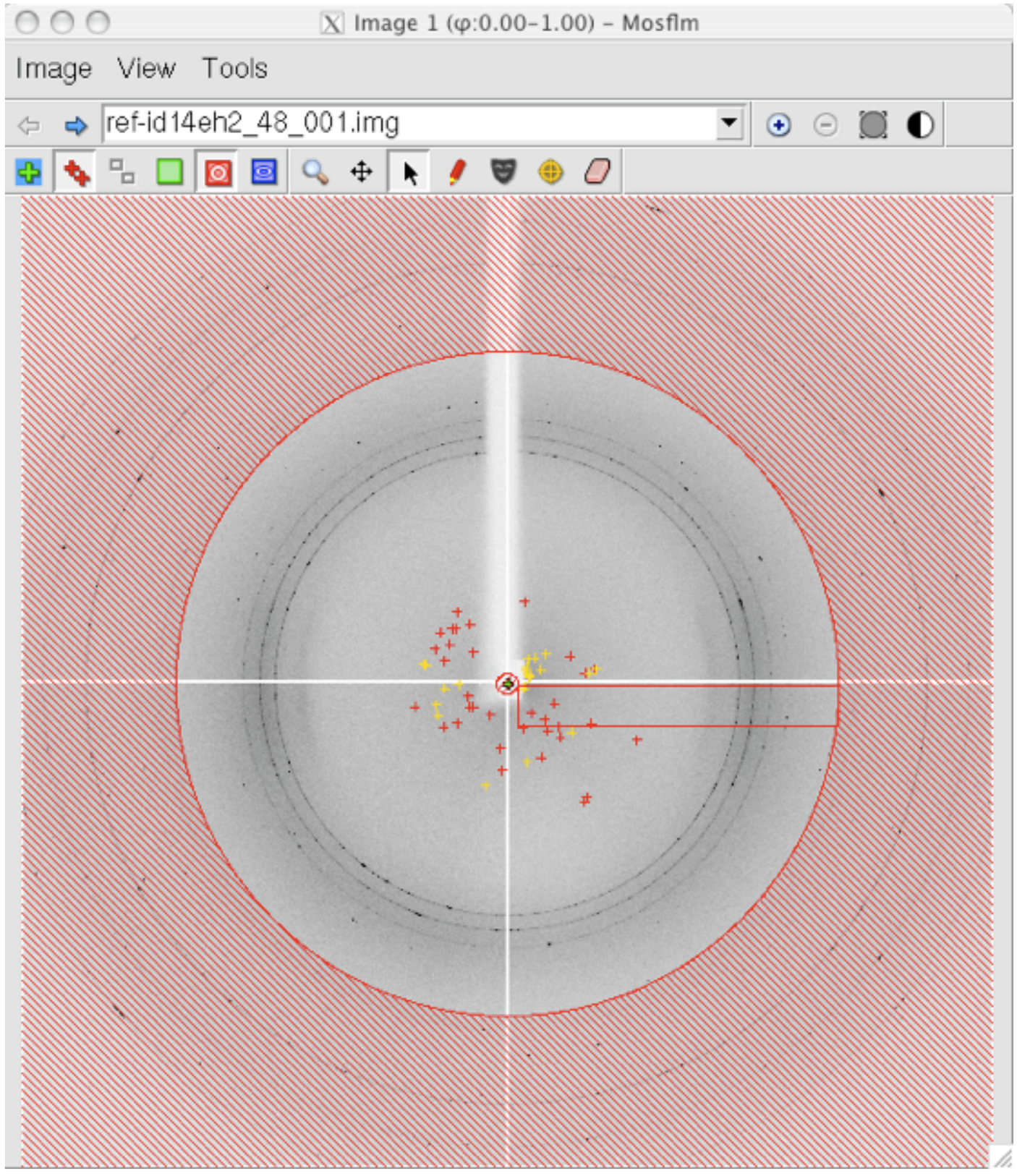
Ice rings will cause problems

So mosflm rejects all spots at the resolutions corresponding to ice. Additional rings still give problems in this case.

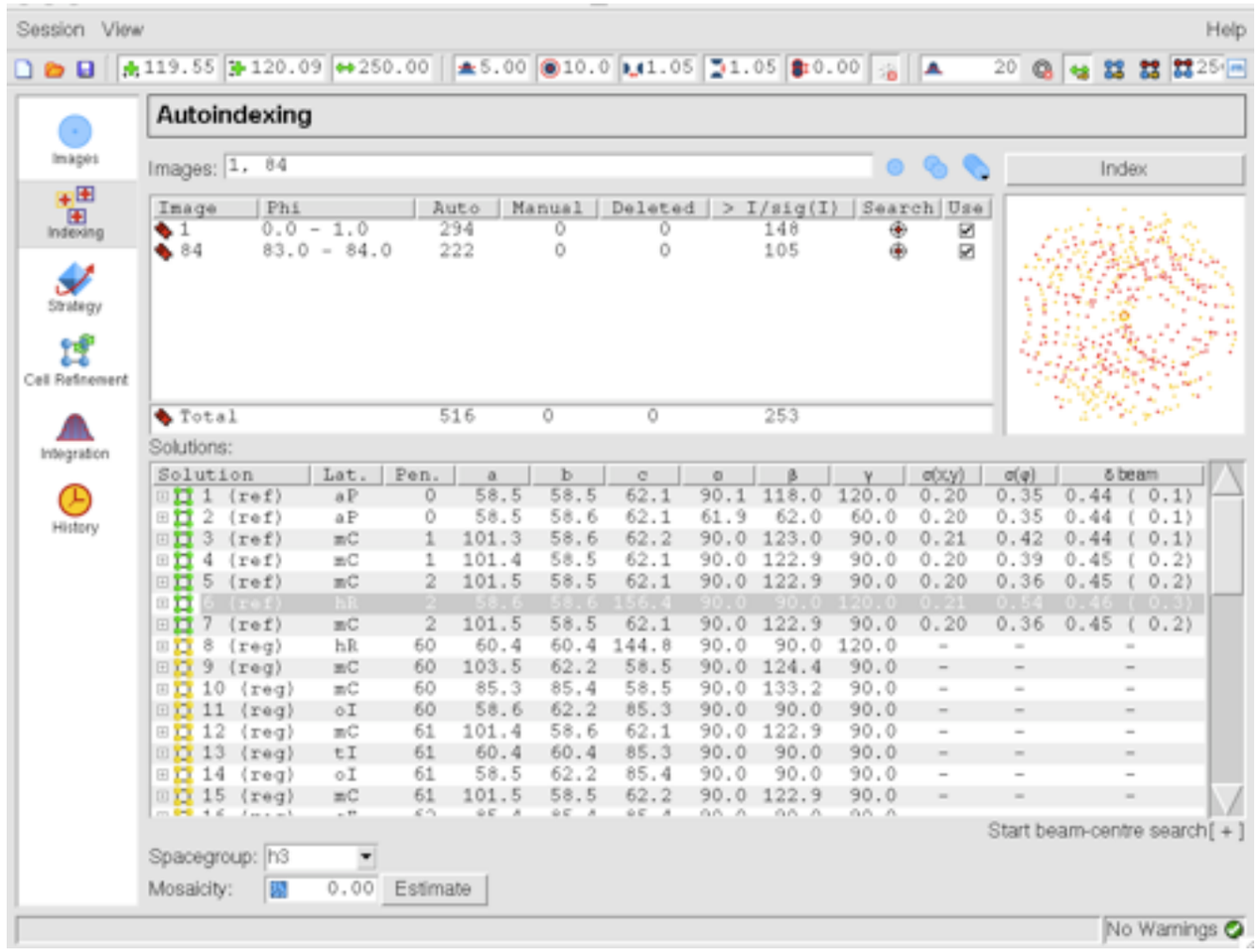
Because the diffraction is very weak in this case, mosflm rejects all spots beyond 4.5Å

In addition, mosflm lowers the threshold for finding spots and the minimum spots size (in pixels)

The spot finding radii can be set, and regions masked out for difficult cases



Indexing



How to tell if the solution is correct ...

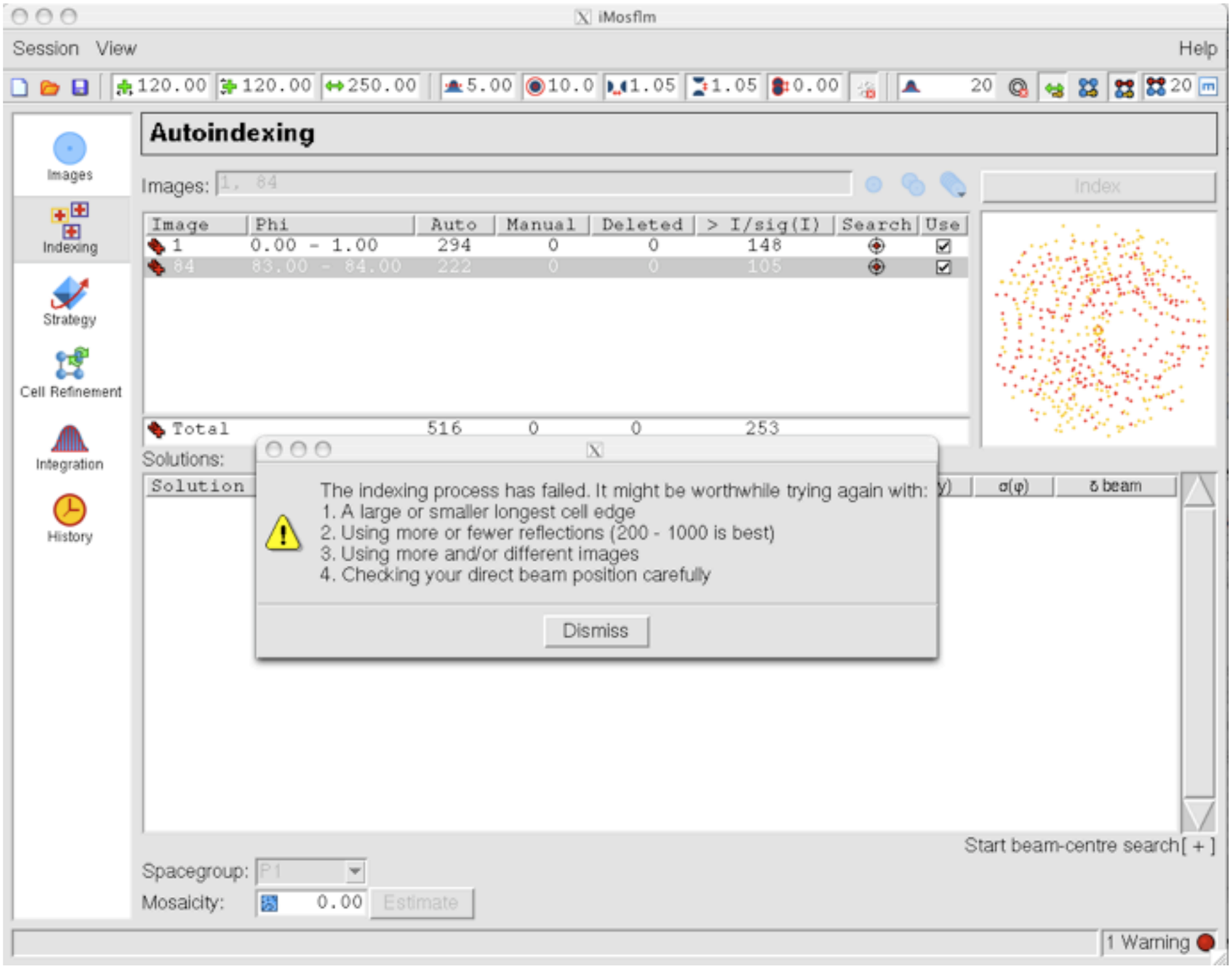
Look at the image !

- Do the predicted spot positions agree with the real spot positions ?
- Is the overall appearance of the lunes correct ?
- Is the positional error σ(xy) small ? (typically 0.1-0.3mm, but can be up to 1mm if spots are split)

Note that the list of solutions given by Mosflm are in fact all the ***same*** solution, with different lattice symmetries imposed, so that if the triclinic solution (number 1) is wrong, then all the others are too

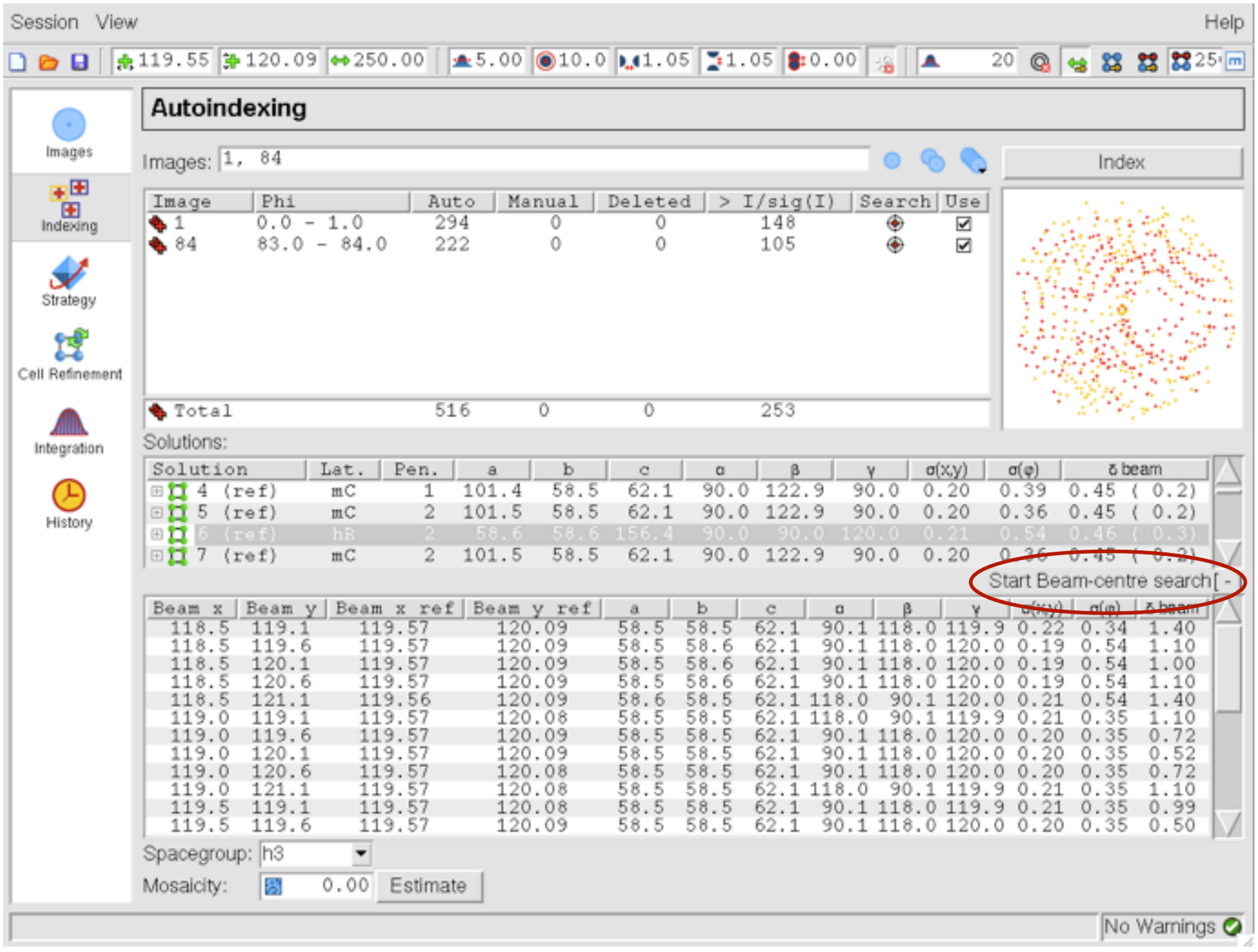
What can be done if indexing fails ?

What can be done if indexing fails ?



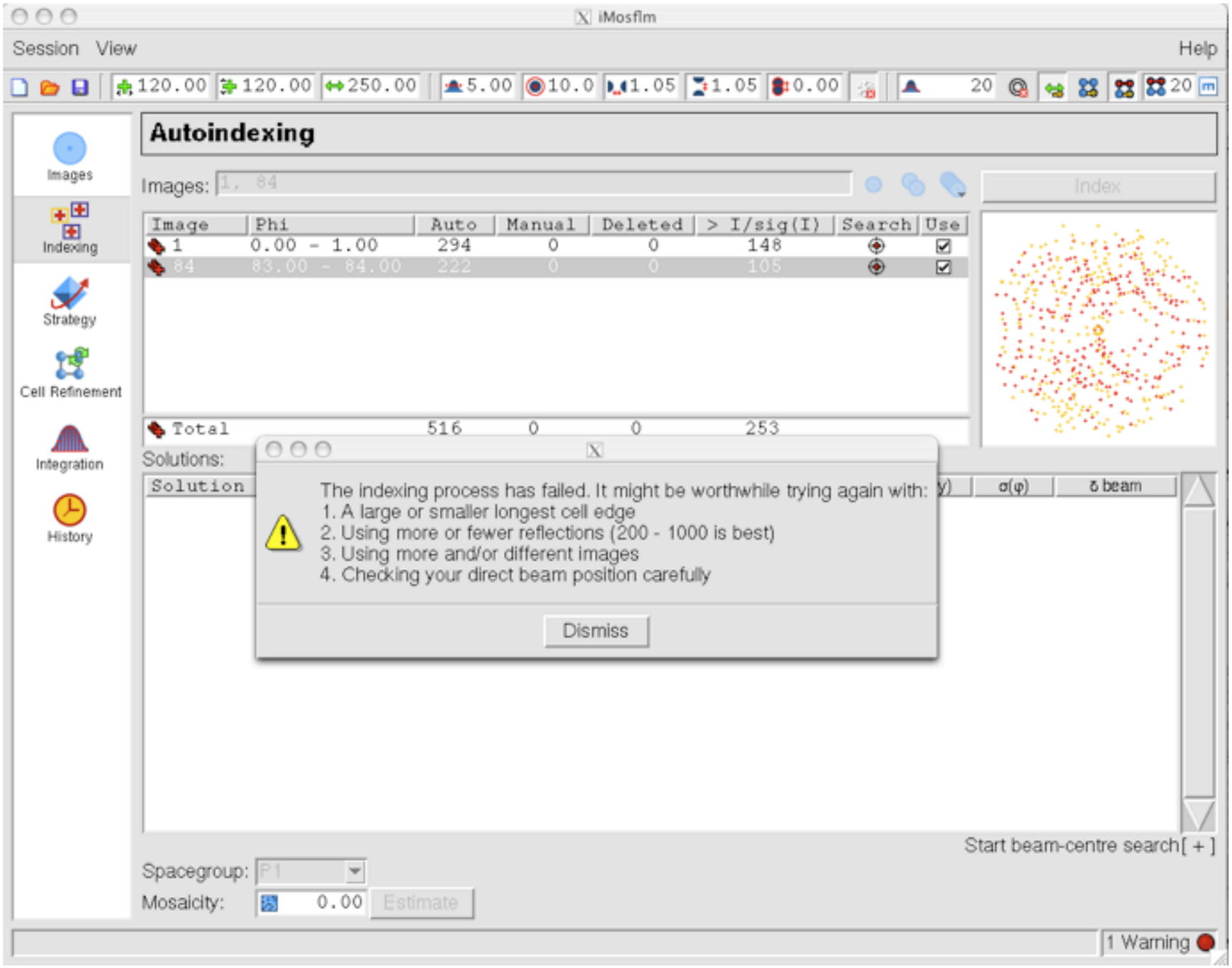
What can be done if indexing fails ?

Check the direct beam (shown as a green cross on the image), if uncertain try a direct beam search (works best with 2 images)



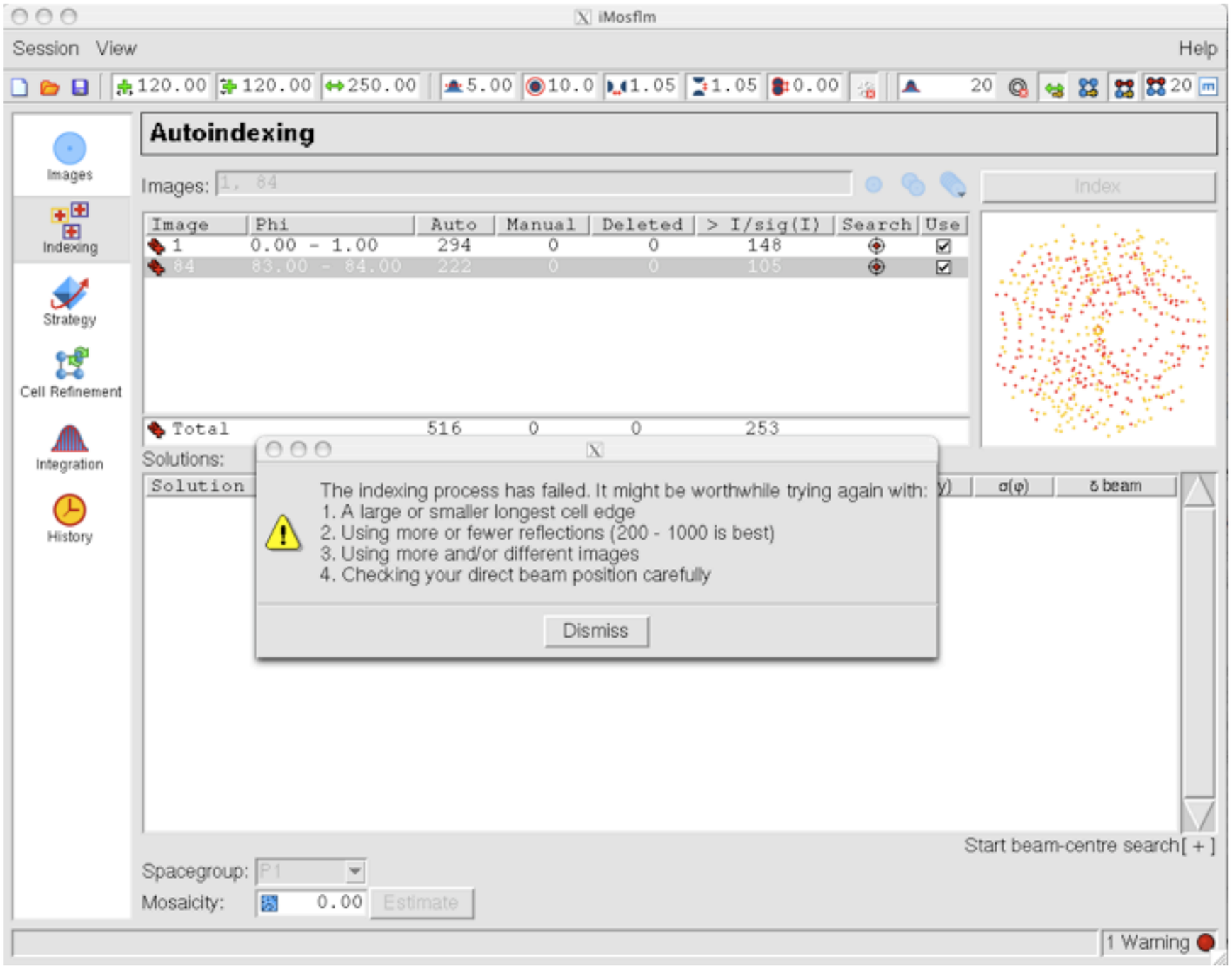
Beam search

What can be done if indexing fails ?



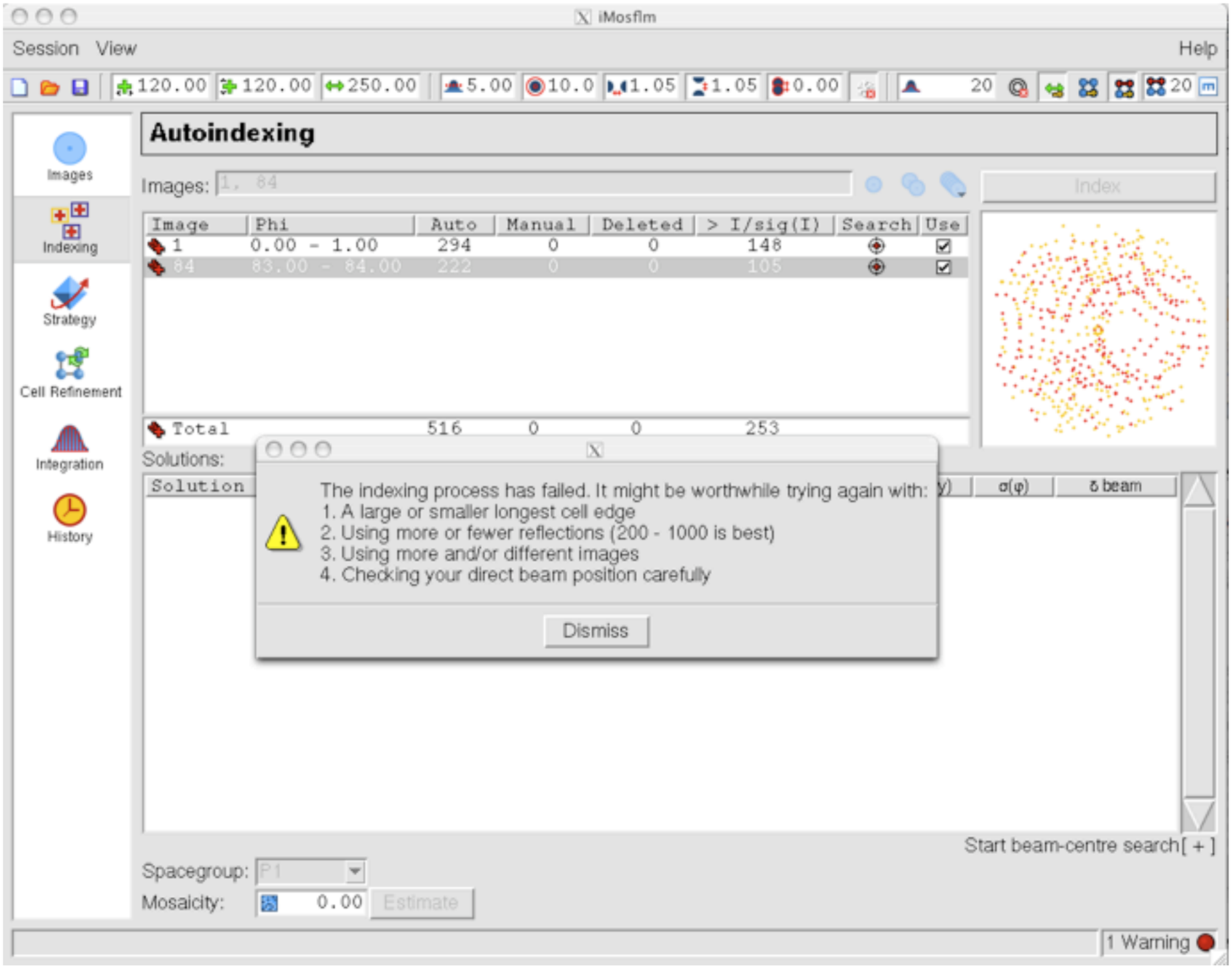
What can be done if indexing fails ?

Try only using one image, especially if disorder/multiple lattices are only present in one of the images or if the crystal has "died" by the last image.



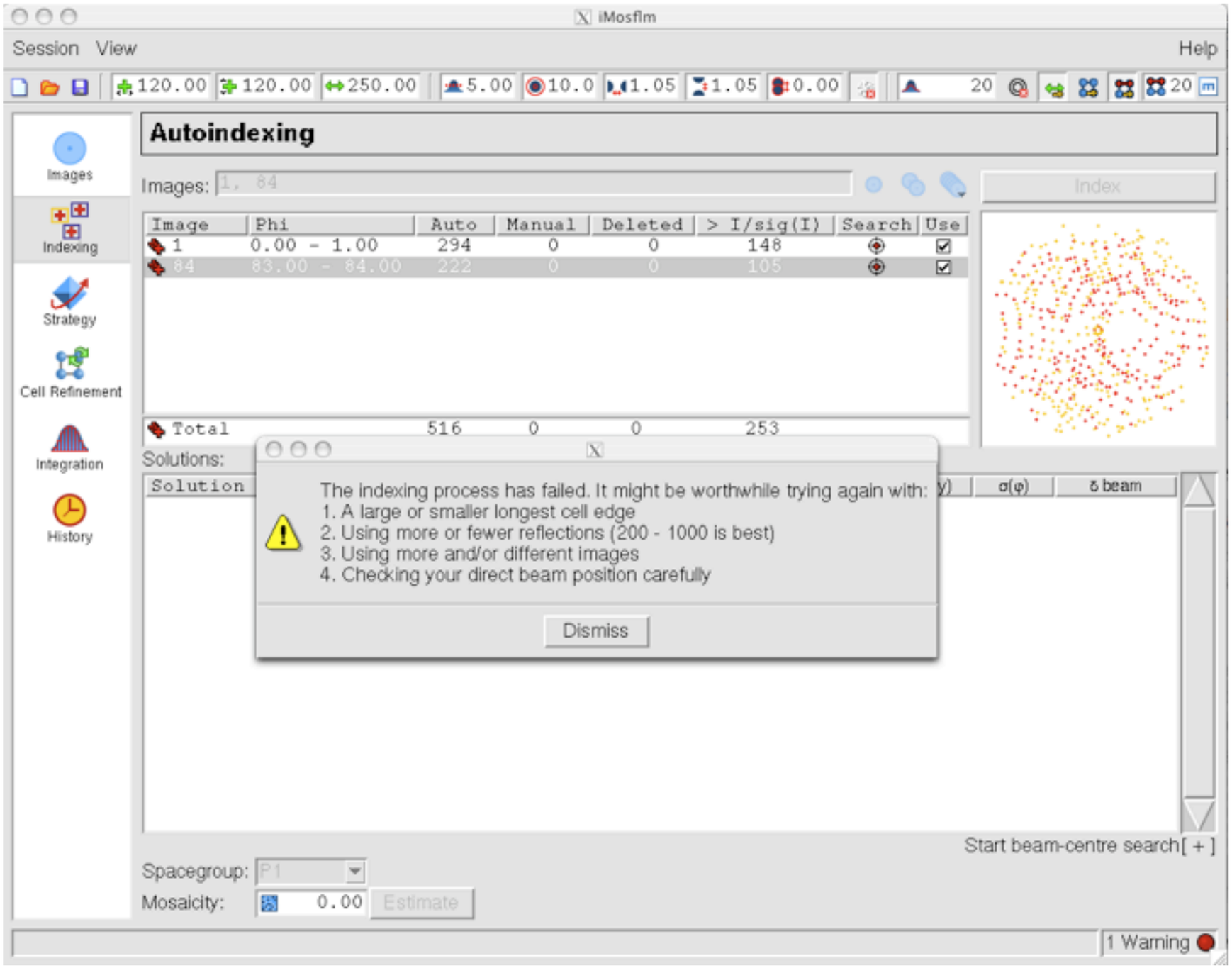
What can be done if indexing fails ?

If very mosaic, or there are multiple lattices, increase the threshold for spots to be used up to ~100 (this is set automatically to 5, 10 or 20 by mosflm depending on strength of the image).



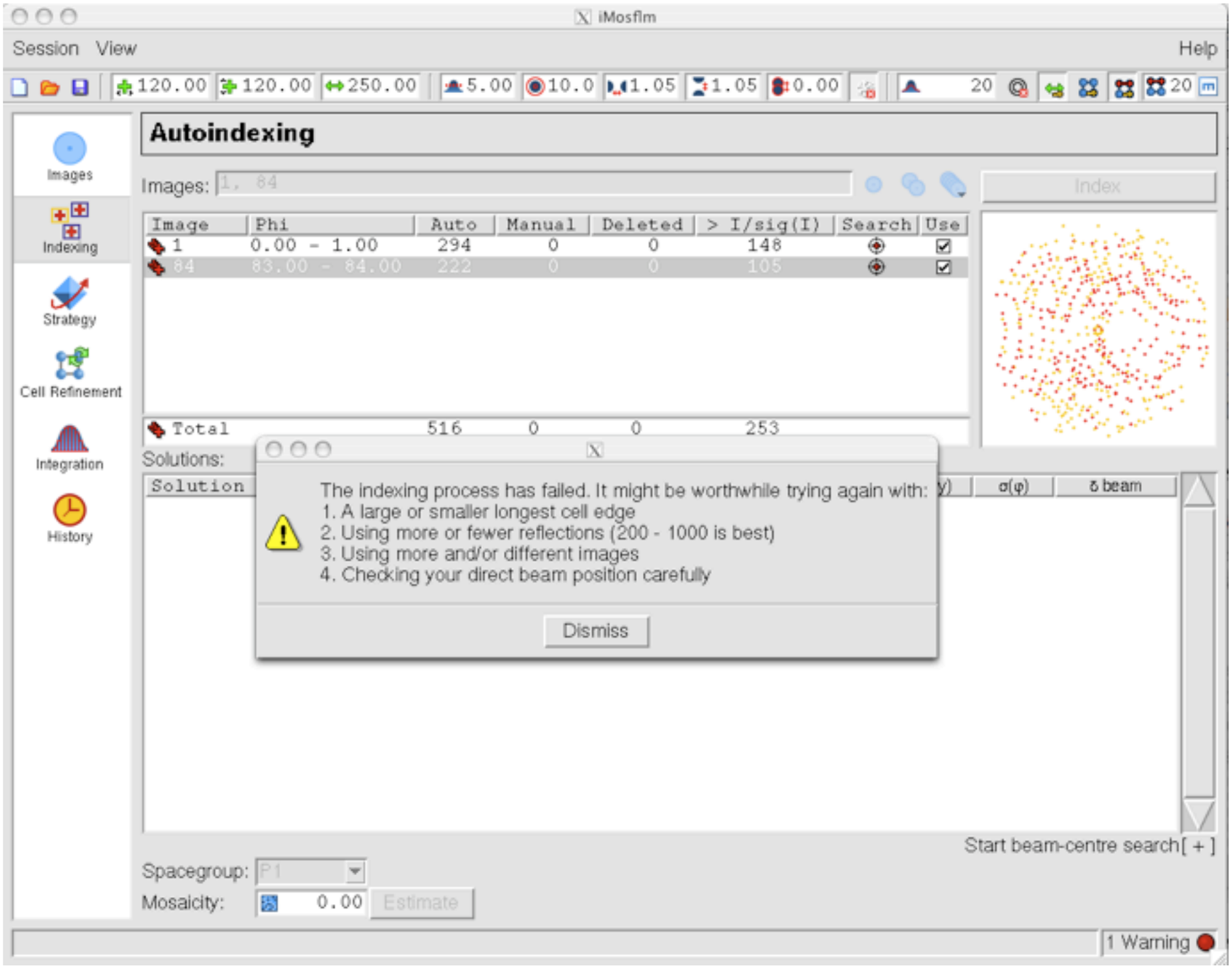
What can be done if indexing fails ?

Even if not mosaic, try different thresholds, in range 5-100.



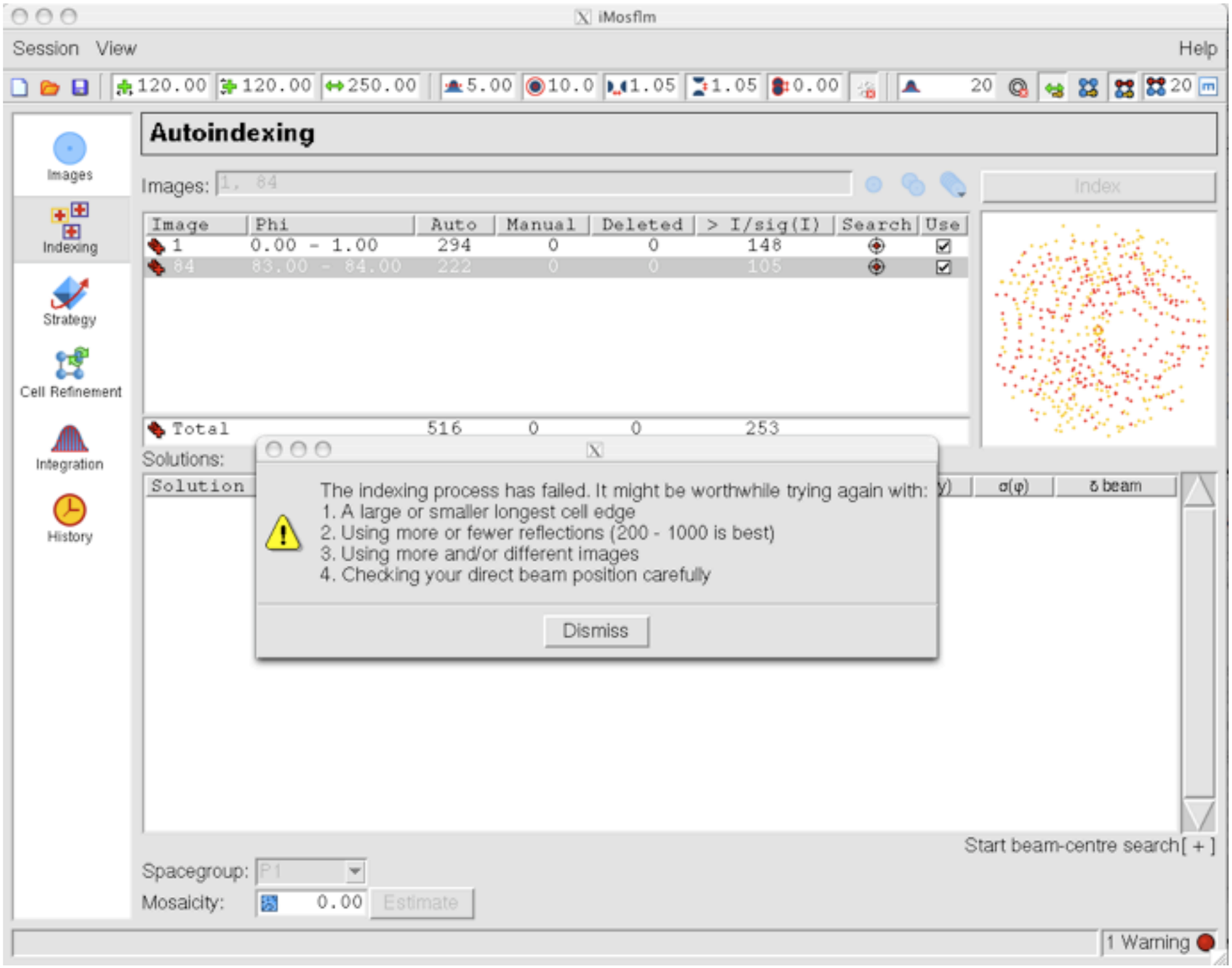
What can be done if indexing fails ?

Decrease the maximum cell length (worked out based on the spot size, so for small spots may be very large, eg 700Å).



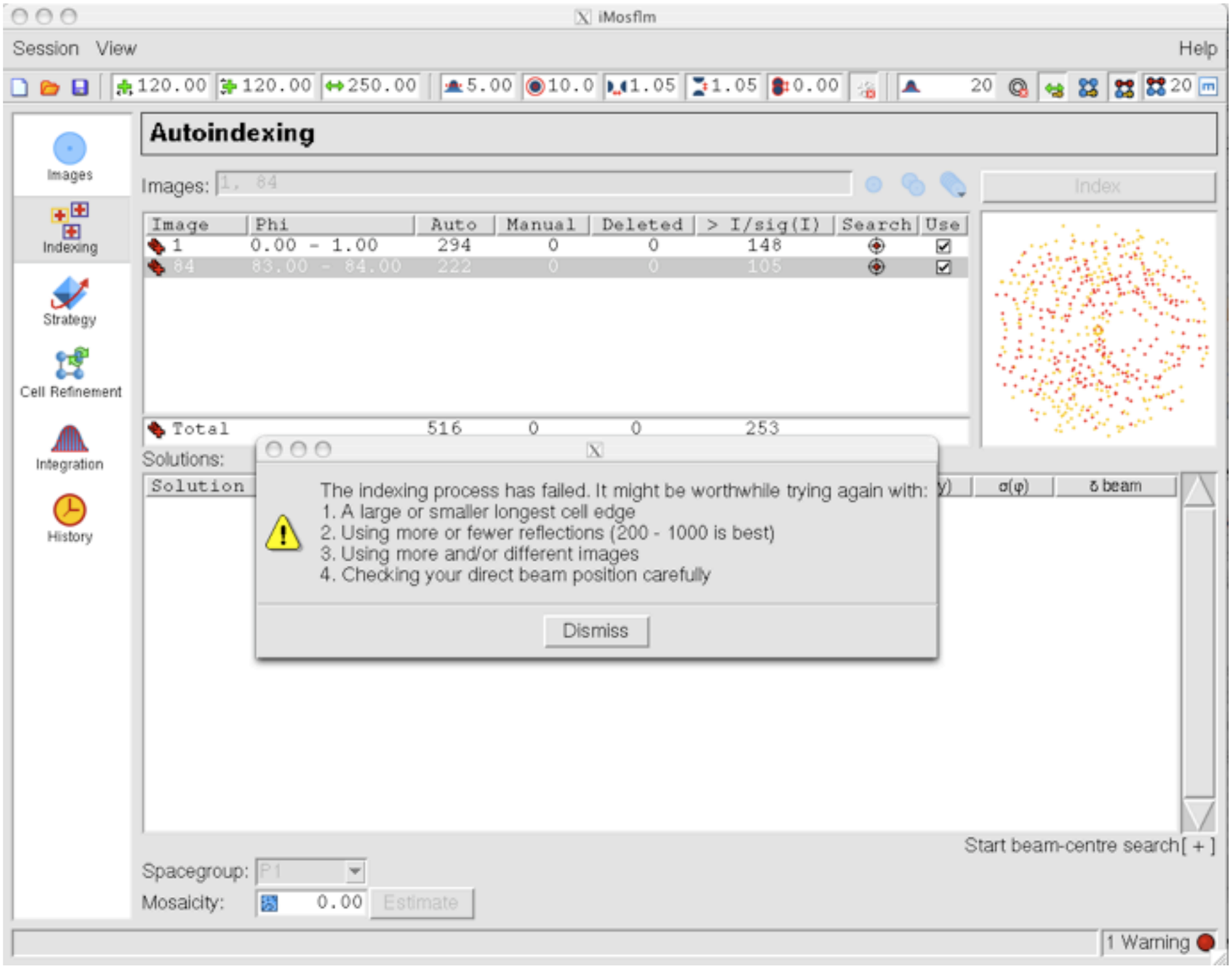
What can be done if indexing fails ?

Try using more images (if only a few spots on each image). Indexing usually works best with a few hundred reflections.



What can be done if indexing fails ?

Many of the parameters that influence the spotfinding and indexing can be changed in the top line of the gui.



Indexing – choosing the “best” solution

Solution	Lat.	Pen.	a	b	c	α	β	γ	σ(x,y)	σ(φ)	δ beam
1 (ref)	aP	0	81.3	81.9	82.3	90.0	90.1	90.0	0.17	0.43	0.01 (0.0)
2 (ref)	mP	0	81.3	81.9	82.3	90.0	90.1	90.0	0.16	0.43	0.00 (0.0)
3 (ref)	aP	0	81.3	81.9	82.3	90.0	89.9	90.0	0.17	0.43	0.01 (0.0)
4 (ref)	mP	1	81.3	82.3	81.8	90.0	90.0	90.0	0.18	0.44	0.00 (0.0)
5 (ref)	mP	1	81.8	81.3	82.3	90.0	90.0	90.0	0.18	0.44	0.00 (0.0)
6 (ref)	oP	1	81.3	81.8	82.3	90.0	90.0	90.0	0.18	0.44	0.00 (0.0)
7 (ref)	mC	3	116.2	115.8	81.4	90.0	90.2	90.0	0.18	0.48	0.00 (0.0)
8 (ref)	tP	3	82.1	82.1	81.3	90.0	90.0	90.0	0.22	0.45	0.00 (0.0)
9 (ref)	oC	3	116.0	116.1	81.3	90.0	90.0	90.0	0.21	0.46	0.00 (0.0)
10 (ref)	mC	3	116.2	115.8	81.4	90.0	90.2	90.0	0.18	0.48	0.00 (0.0)
11 (ref)	mC	4	115.5	115.3	82.2	90.0	90.1	90.0	0.24	0.43	0.01 (0.0)
12 (ref)	oC	4	115.5	115.3	82.3	90.0	90.0	90.0	0.24	0.42	0.01 (0.0)
13 (ref)	tP	4	81.6	81.6	82.3	90.0	90.0	90.0	0.24	0.42	0.01 (0.0)
14 (ref)	mC	4	115.5	115.3	82.2	90.0	90.1	90.0	0.24	0.43	0.01 (0.0)
15 (ref)	hR	6	115.5	115.5	142.4	90.0	90.0	120.0	0.28	0.49	0.01 (0.0)
16 (ref)	hR	6	115.7	115.7	141.7	90.0	90.0	120.0	0.35	0.44	0.01 (0.0)
17 (ref)	cP	7	81.8	81.8	81.8	90.0	90.0	90.0	0.34	0.44	0.01 (0.0)
18 (reg)	mC	248	81.3	183.3	81.9	90.0	90.0	90.0	-	-	-
19 (reg)	oC	248	81.3	183.3	81.9	90.0	90.0	90.0	-	-	-

Spacegroup: P23

Mosaicity: 0.92 Estimate

Normally the solution with the highest symmetry from the group of solutions with low penalties is correct. However, beware of pseudosymmetry !

Note here that the chosen solution (cubic) has a σ(xy) value of 0.34mm while for the (correct) orthorhomic solution this value is 0.18mm

The true symmetry can only be determined by integrating some of the images

If known, the true space group can be selected from the drop down menu

Indexing – Other tips

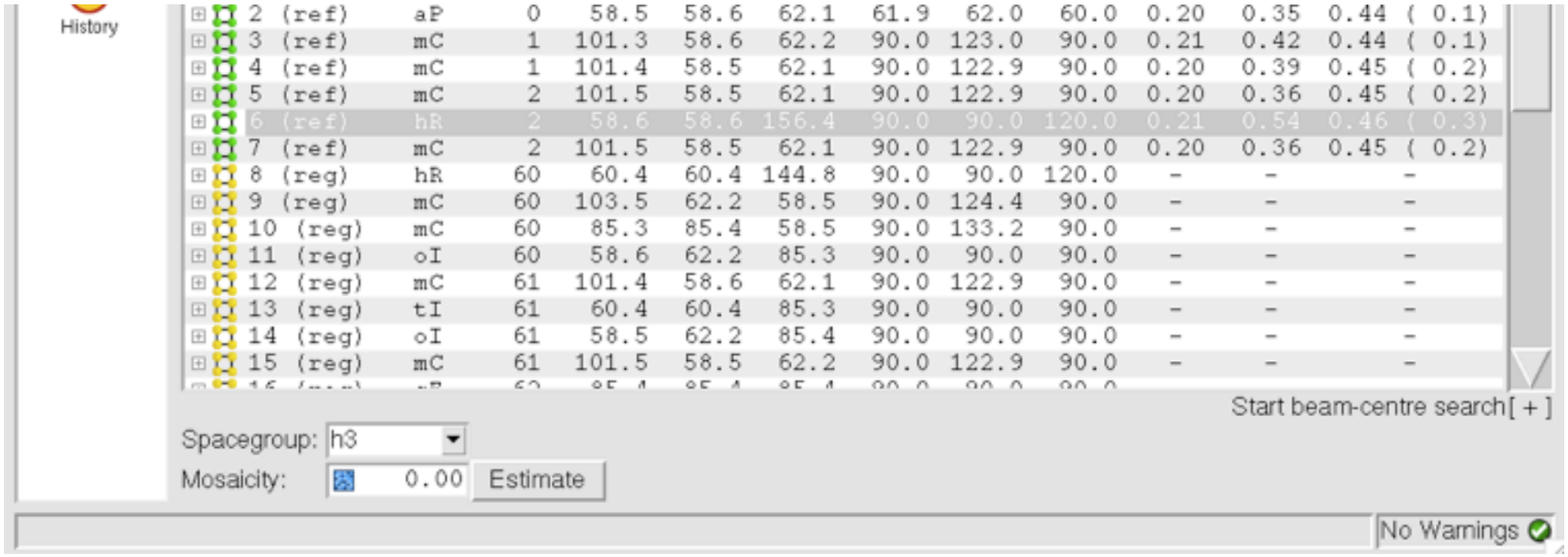
• If even the triclinic solution (solution 1) has a high positional error (σ (xy)), sometimes selecting this triclinic solution and then repeating the indexing will help find the correct solution. This is because the direct beam coordinates are refined as part of the indexing.

Incorrect direct beam coordinates are the most common cause of indexing failure.

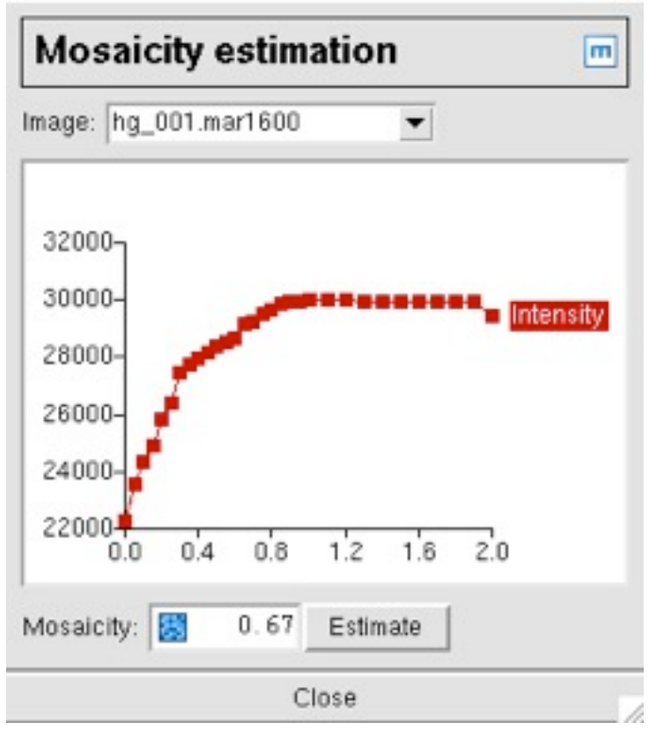
• Beware ! Indexing from a single image can give incorrect results for low symmetry space groups. The prediction will fit for the image used in the indexing, but not for images at very different phi values.

• Beware of pseudo centering or lattice repeats which result in a class of reflections being systematically weaker than the rest (eg a pseudo lattice translation of a/2 will mean that all *h* odd reflections are weak). If only the strongest reflections are used in indexing, the resulting cell will be too small.

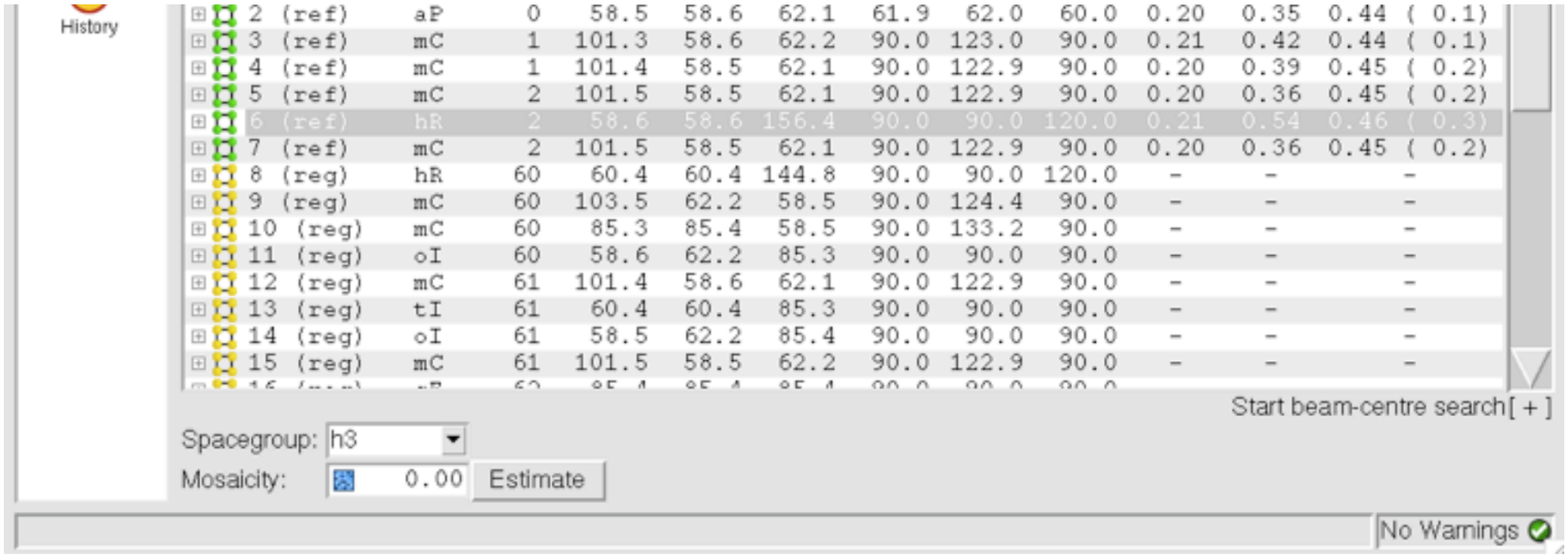
Indexing – Mosaicity estimation



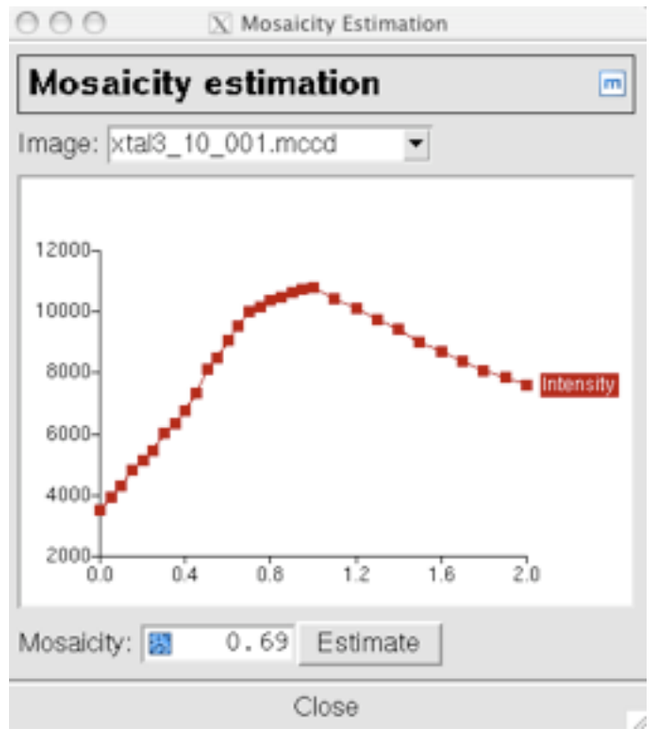
The image is integrated many times with increasing values of the mosaic spread, and the total intensity of all predicted spots is plotted against mosaicity. A point corresponding to the shoulder of this curve is taken as the best estimate.



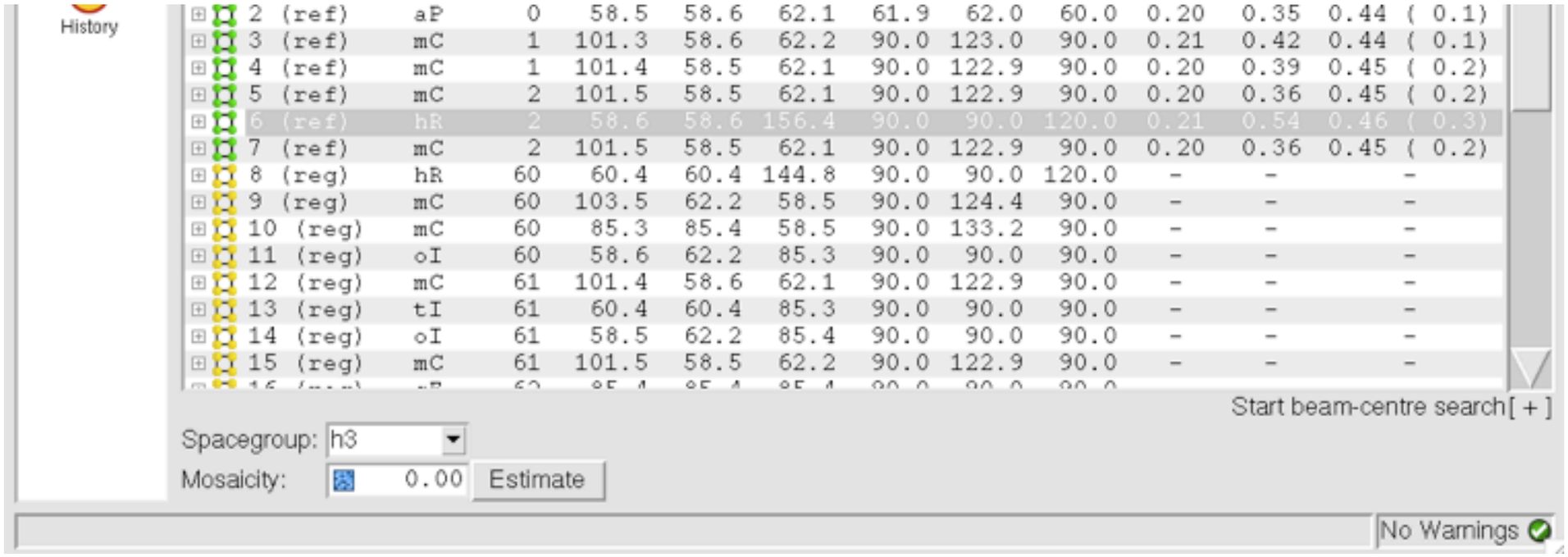
Indexing – Mosaicity estimation



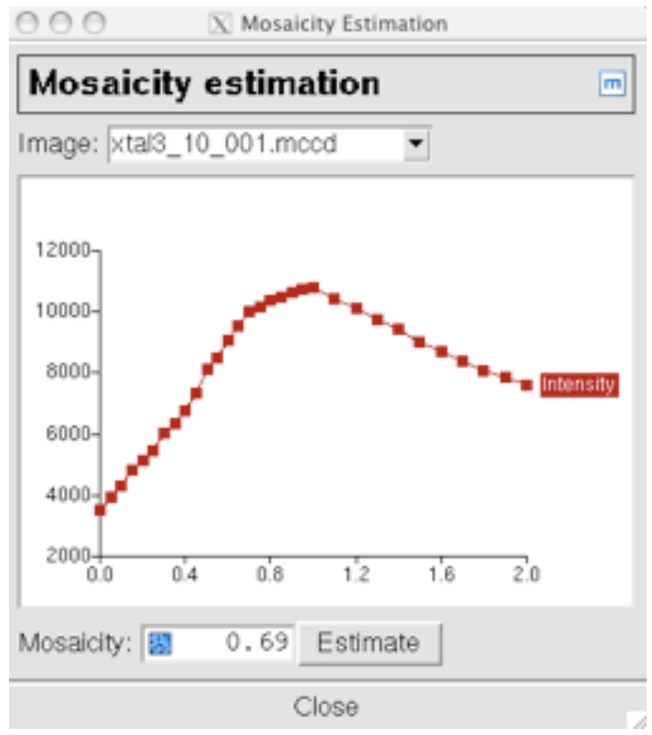
If the unit cell is large, the total intensity will drop at high values of mosaic spread, because overlapping reflections are not included.



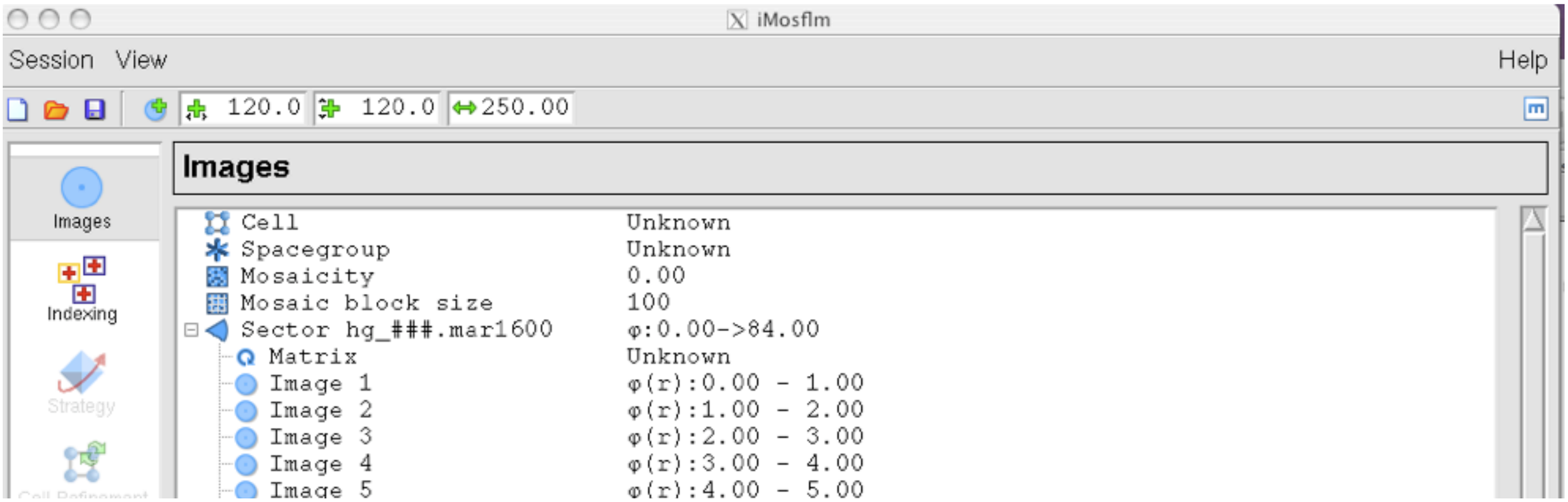
Indexing – Mosaicity estimation



If the initial orientation is incorrect, the mosaicity will be over-estimated. This will be corrected during integration.



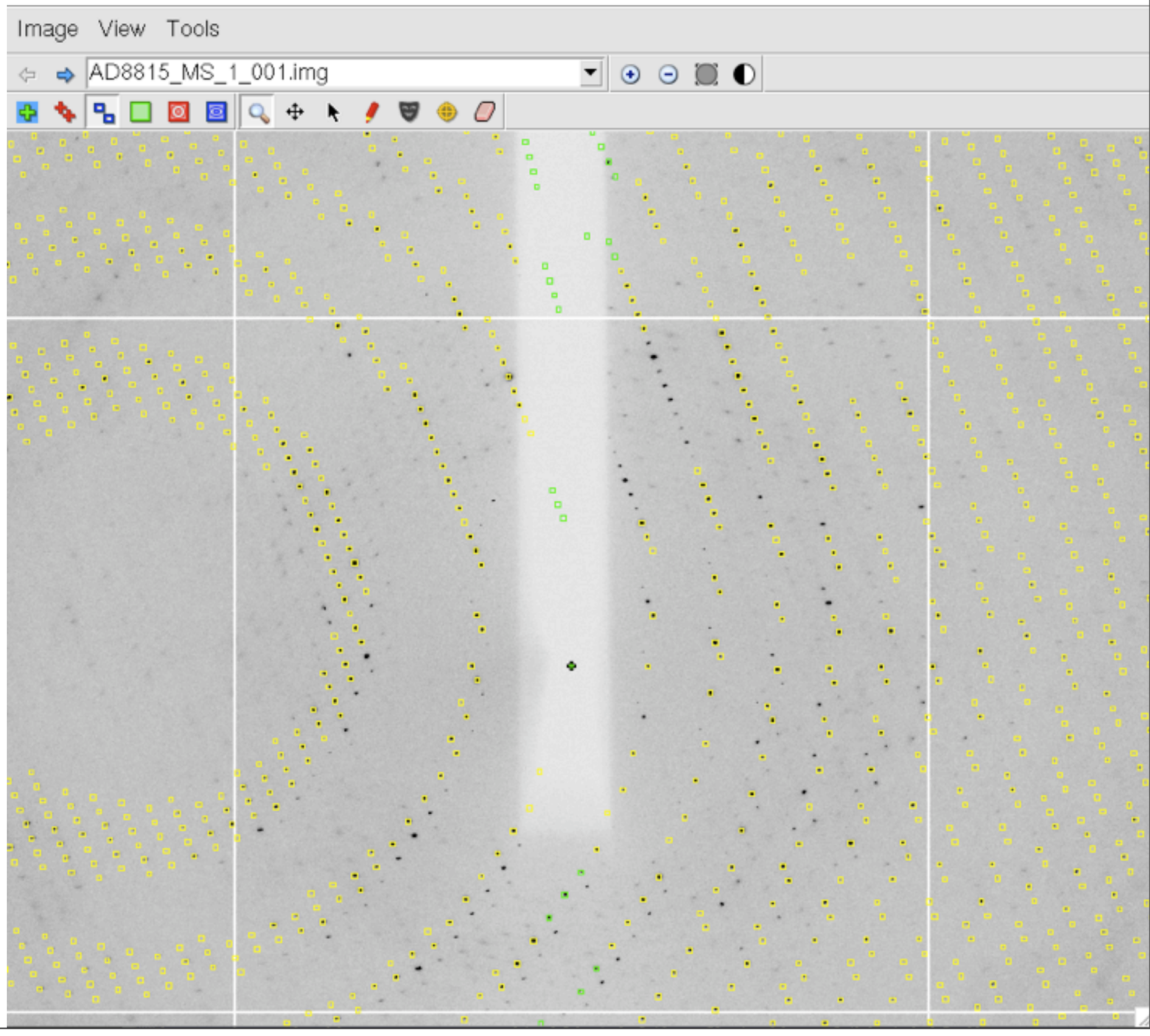
Mosaicity – the effect of the mosaic block size



Decreasing the mosaic block size has the effect of increasing the apparent mosaic spread at low resolution (Nave, C. (1998) Acta Cryst D54, 848-853)

Mosaicity – the effect of the mosaic block size

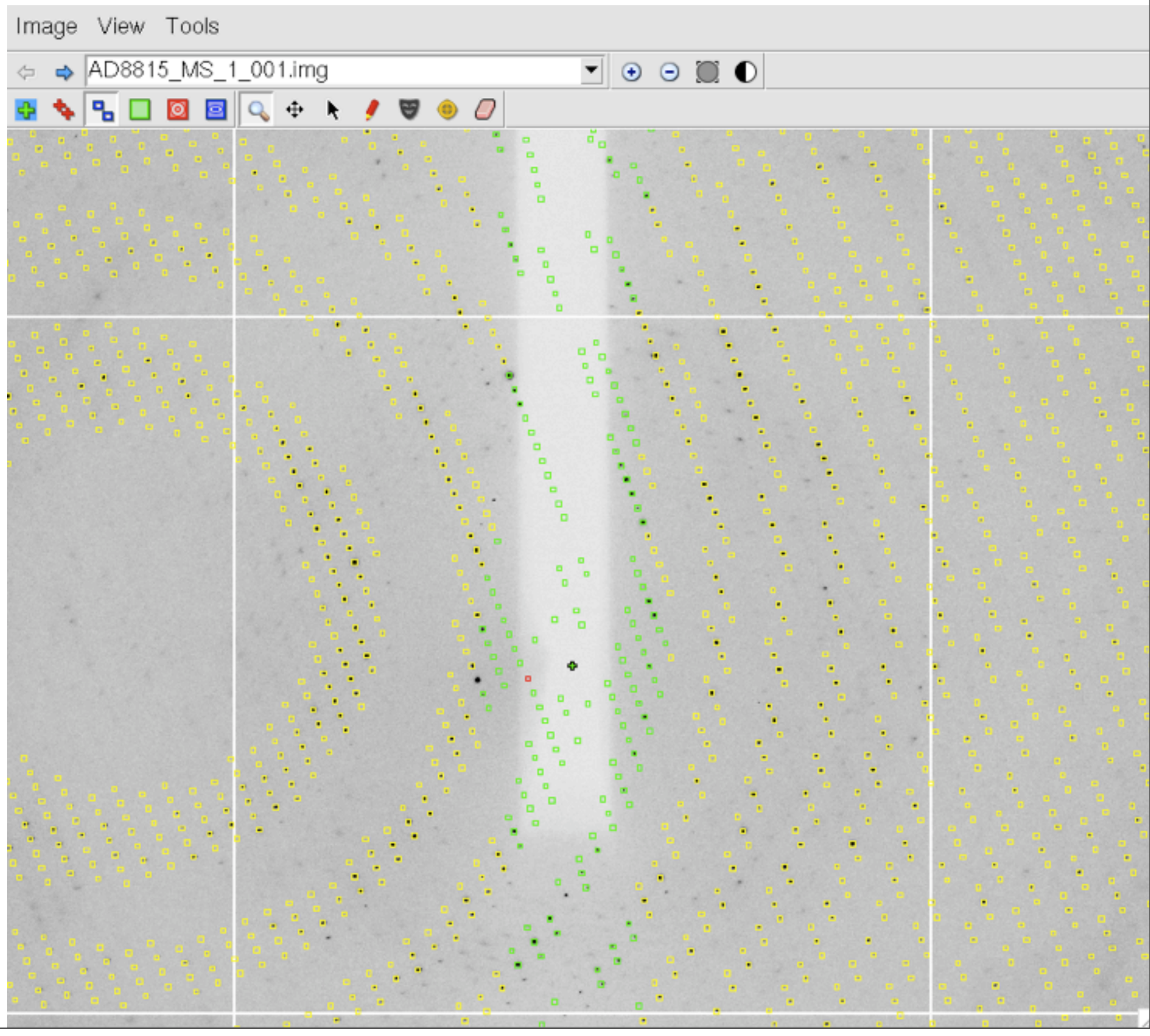
Blocksize 100μ



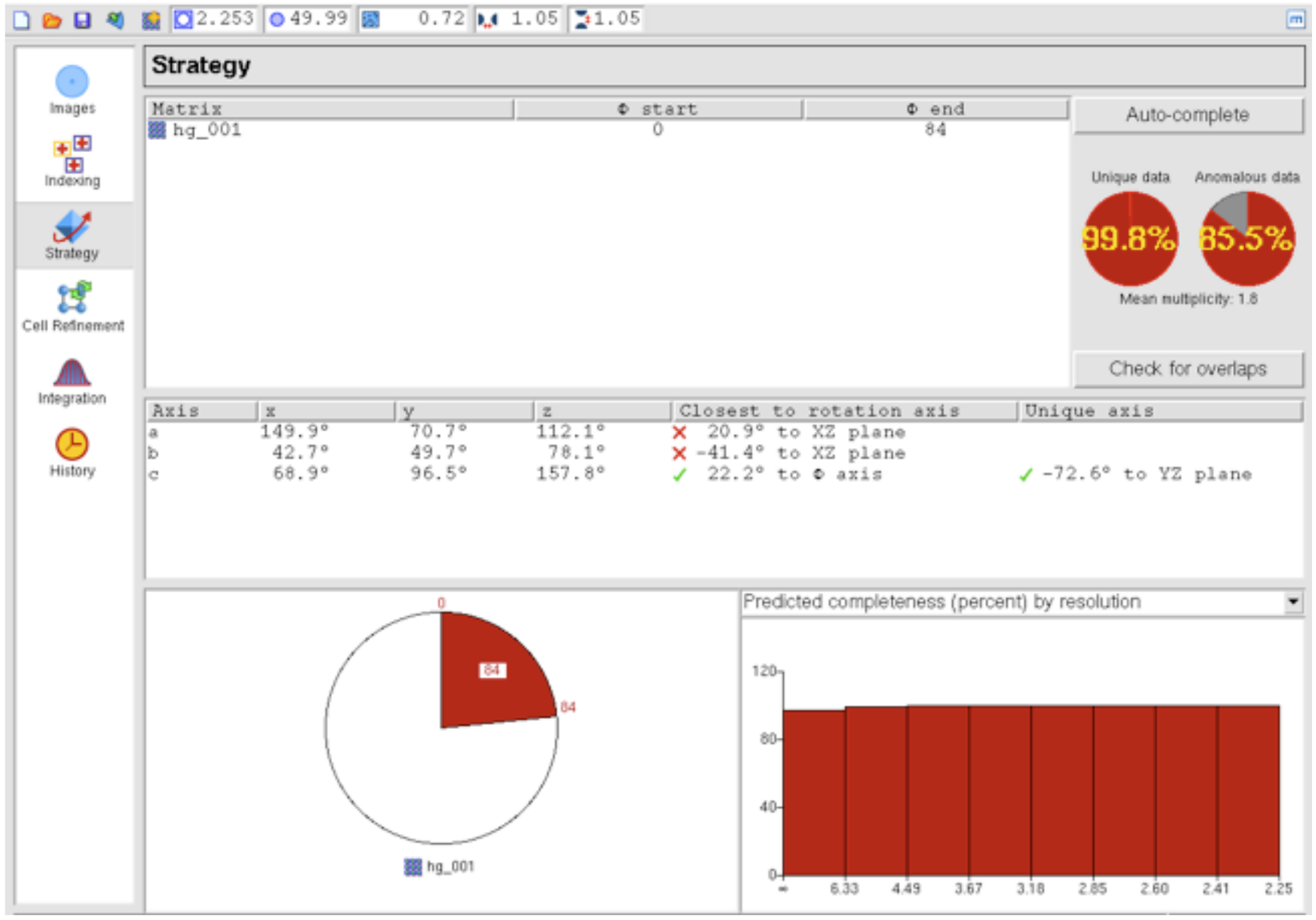
Mosaicity – the effect of the mosaic block size

Blocksize 0.2μ

It may be necessary to increase the maximum reflection width to avoid losing reflections (green boxes)



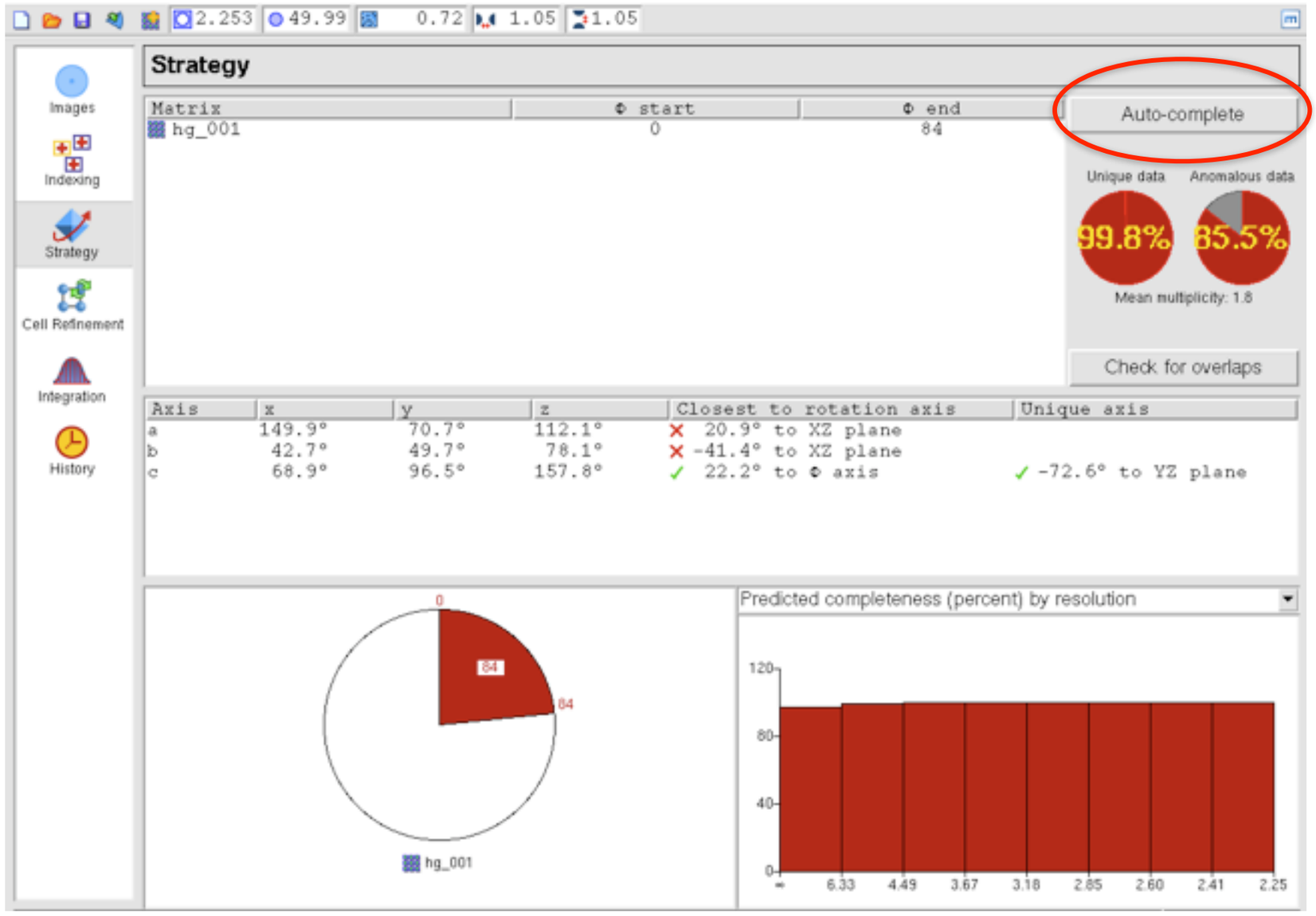
Strategy



Initial strategy results assume that all data between phi start and phi end have been collected !

To calculate a strategy, select the “Auto-complete” button.

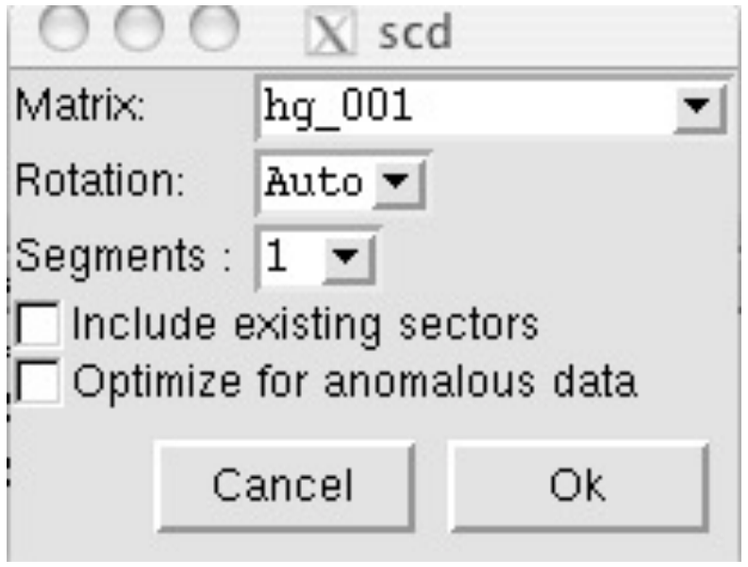
Strategy



Initial strategy results assume that all data between phi start and phi end have been collected !

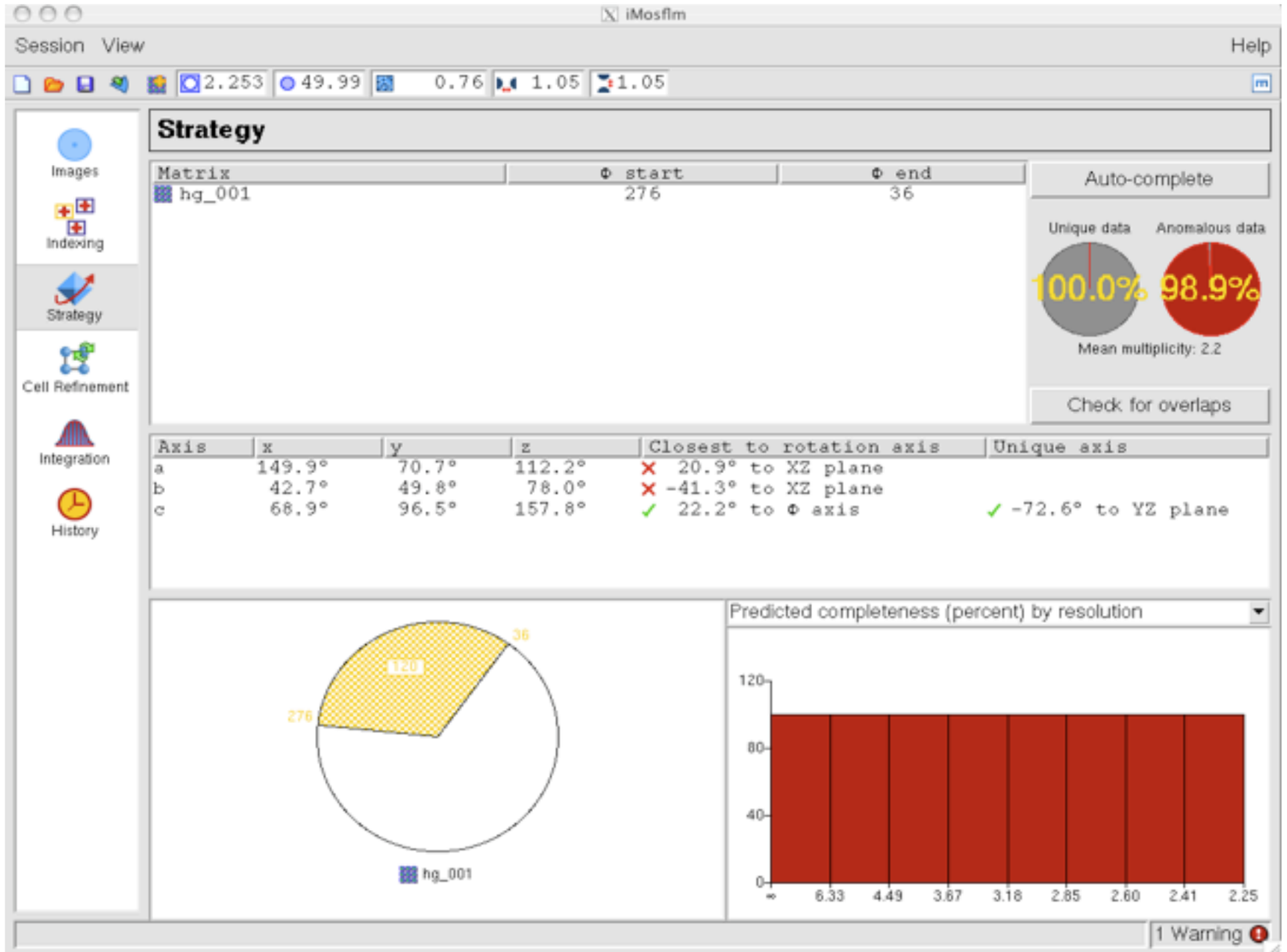
To calculate a strategy, select the “Auto-complete” button.

Strategy



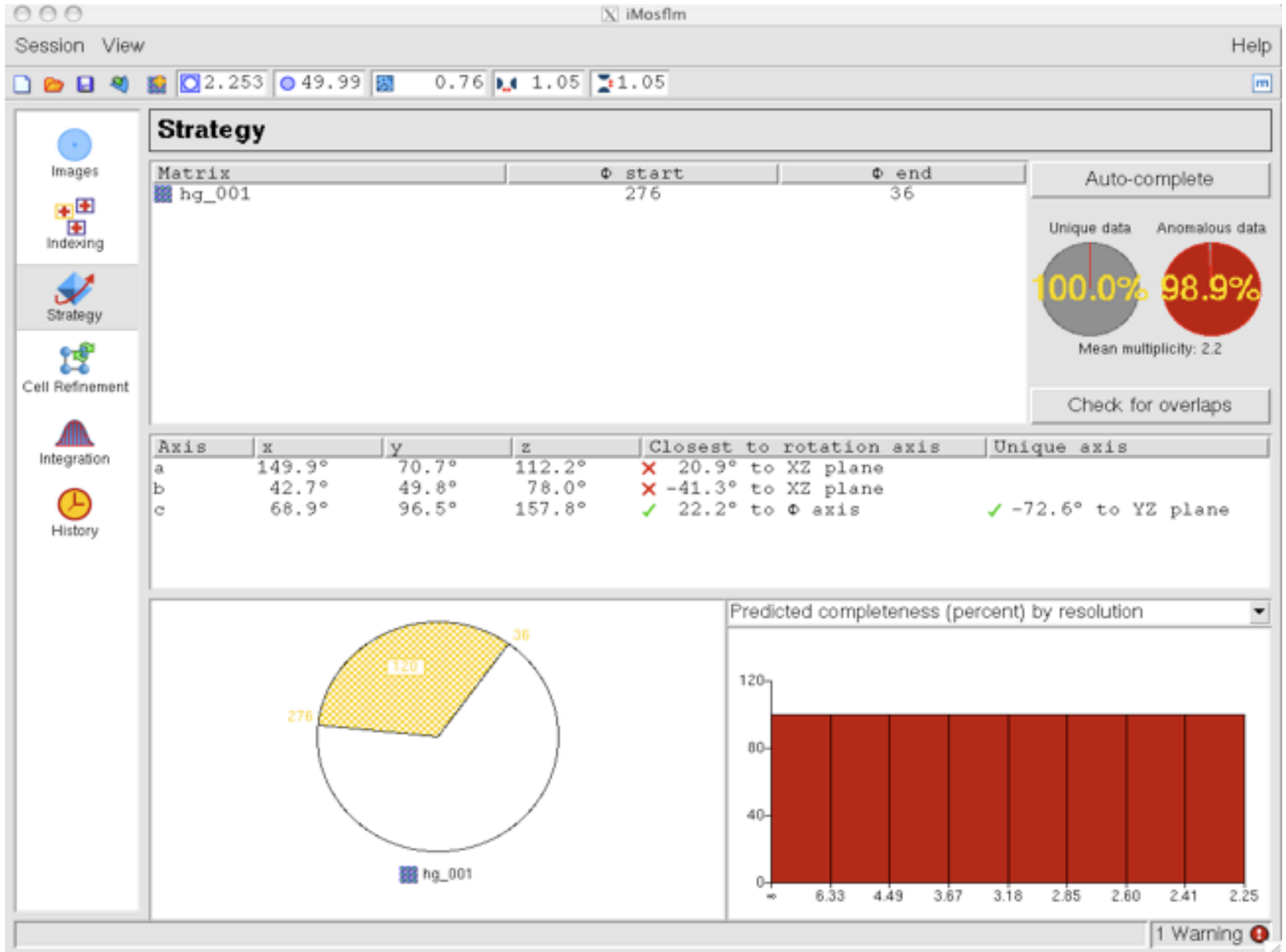
Use defaults for a normal strategy calculation
High completeness (>94%) can be achieved with a smaller total rotation by collecting the data in segments. Eg 2 segments of 30° each for orthorhombic. Select the desired total rotation and the number of segments from pull down menus.
If collecting anomalous data, remember to tick the box !

Strategy



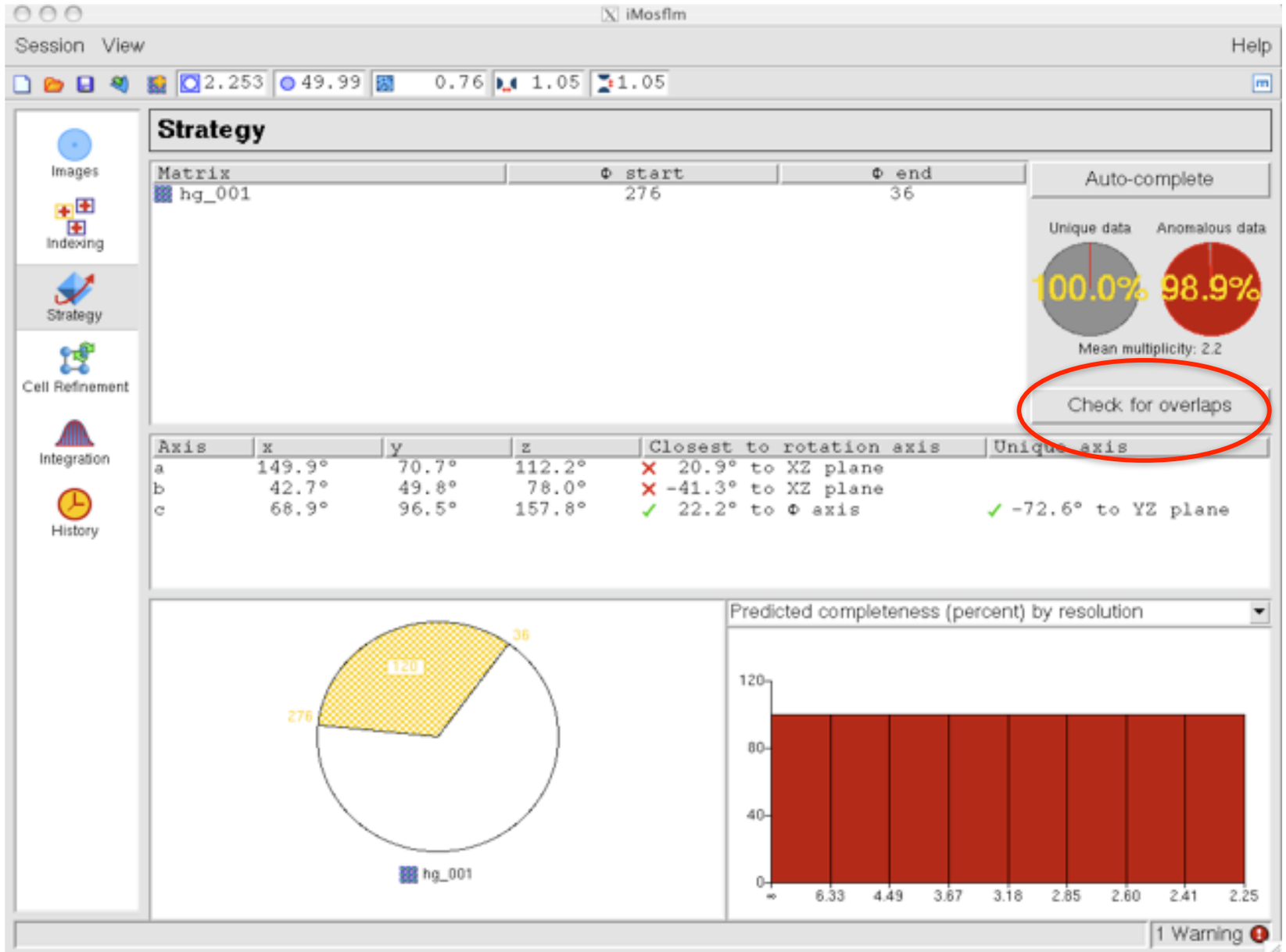
The start end end point of the suggested strategy can be dragged with the mouse to test other rotation ranges.

Strategy



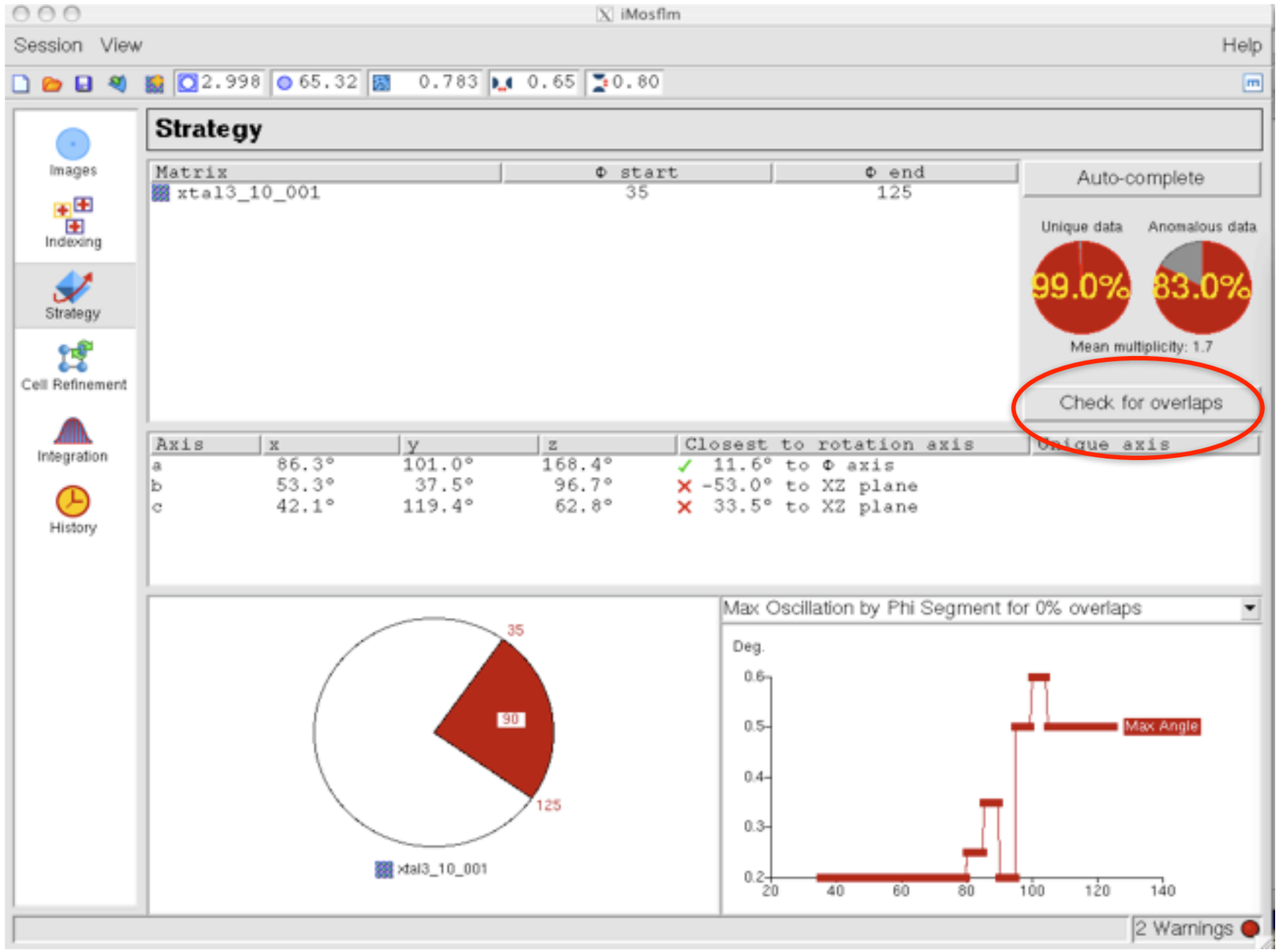
Select "Check for overlaps" to determine maximum oscillation angle without overlaps

Strategy



Select "Check for overlaps" to determine maximum oscillation angle without overlaps

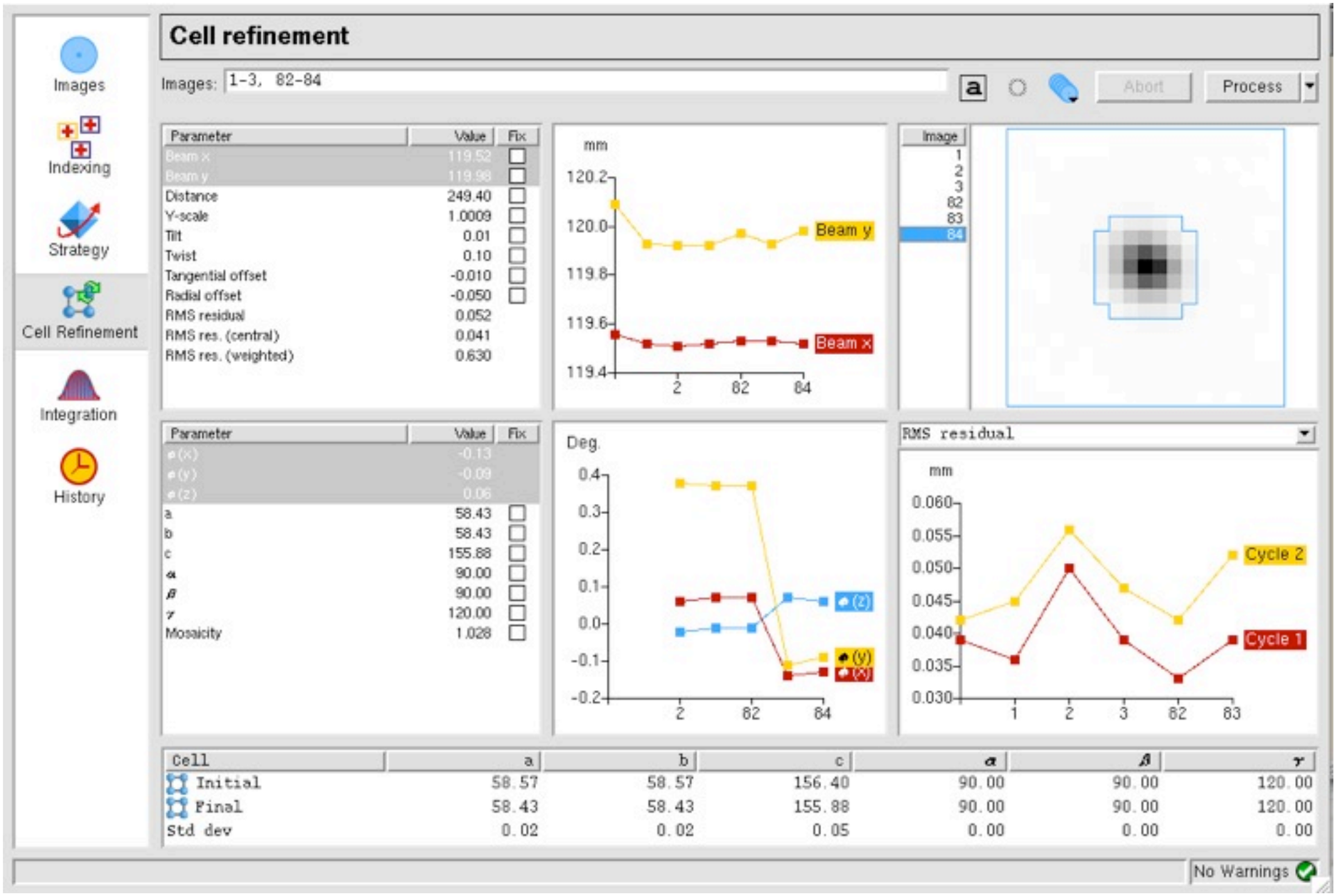
Strategy



Select "Check for overlaps" to determine maximum oscillation angle without overlaps

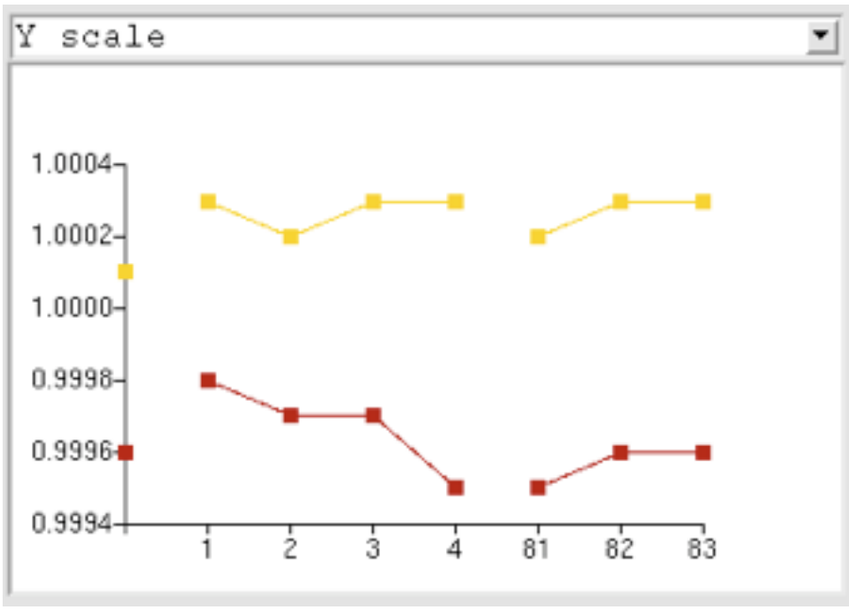
Cell refinement

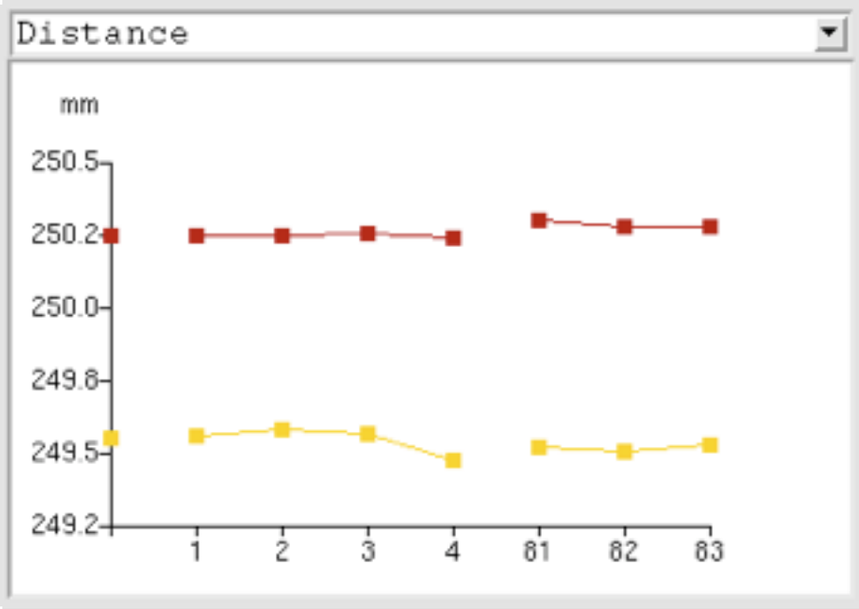
For cell refinement, two small wedges of data separated by about 90° in phi are integrated. The way in which the total spot intensity of partially recorded reflections is distributed across adjacent images is used to refine crystal cell parameters, orientation and mosaicity. Final cell sds should ideally be less than 0.1Å.

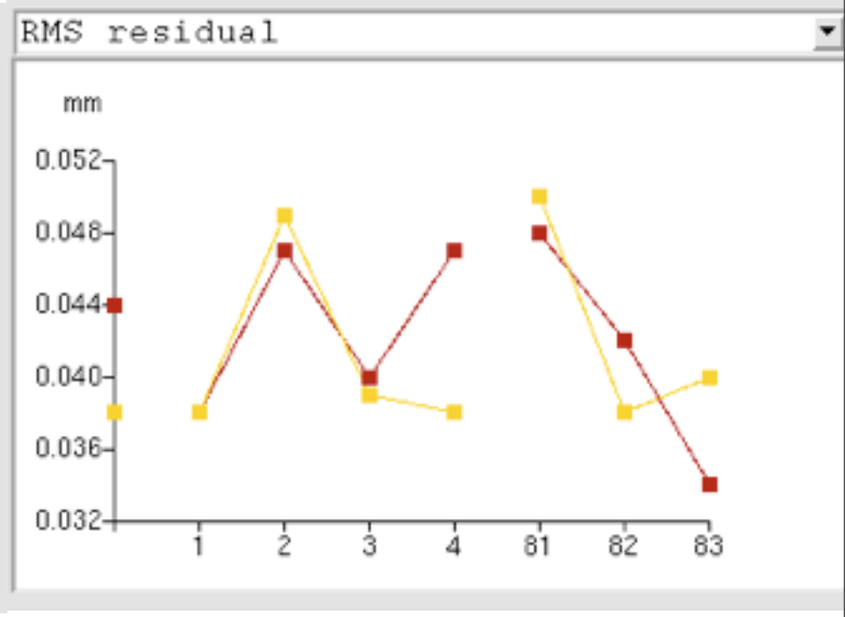


Cell refinement

Has it worked ?







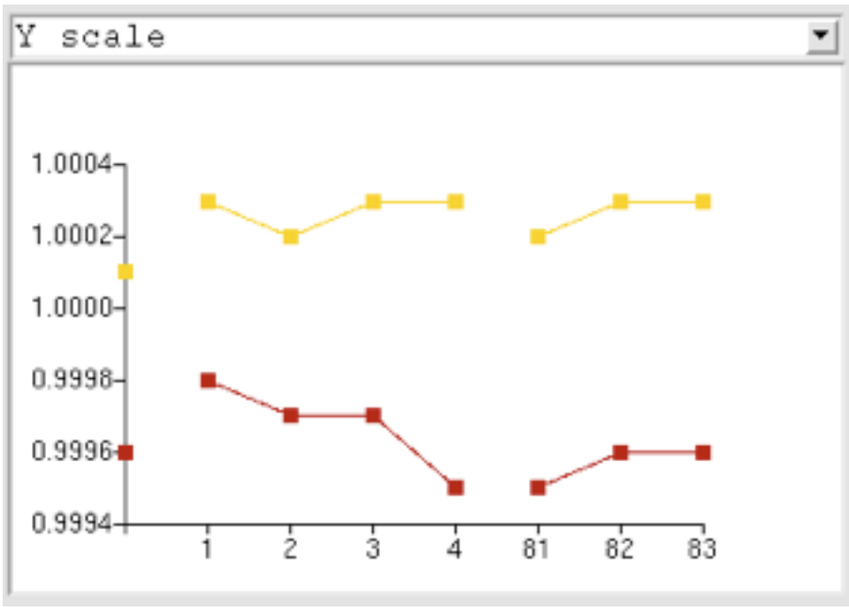
At resolutions less than ~3.3Å, the cell refinement may not work well.

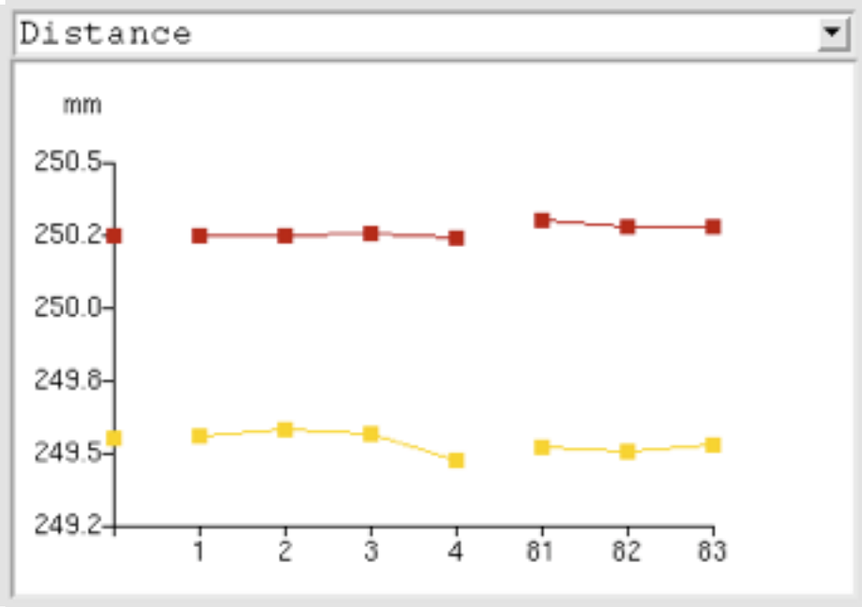
Indicators that it has worked are:

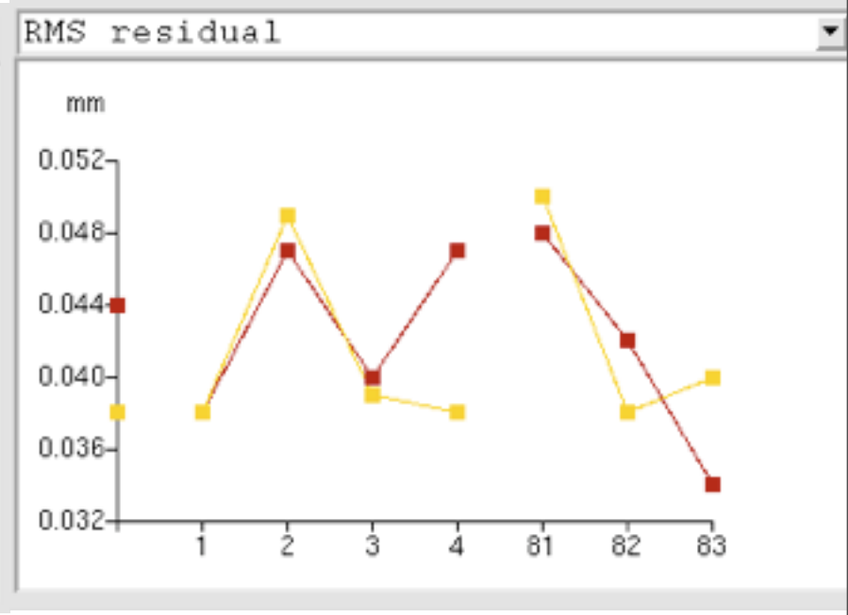
- Yscale is close to 1.0 for all images
- The detector distance is the same for all images
- The rms residual is smaller at the end of refinement.

Cell refinement

Has it worked ?

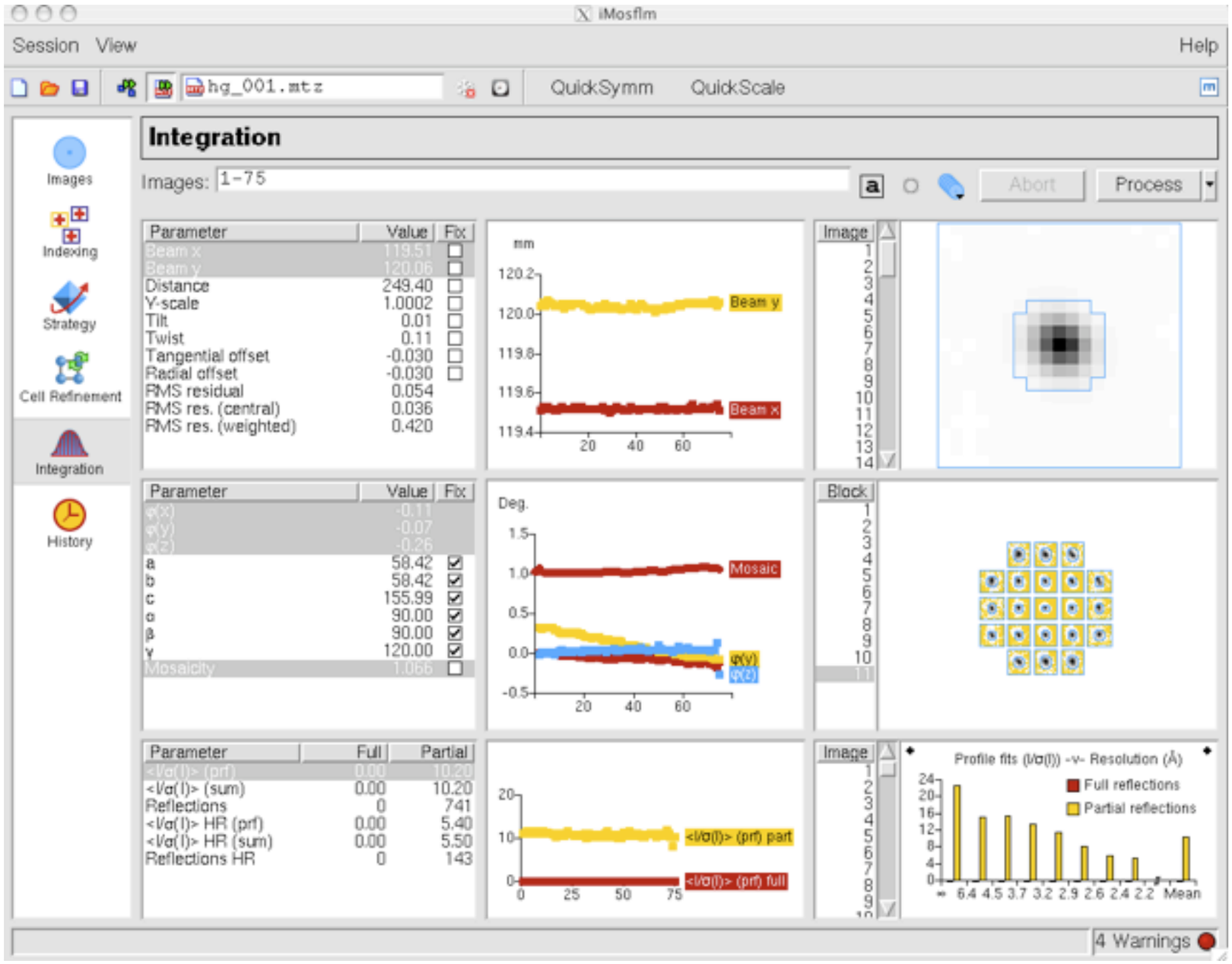






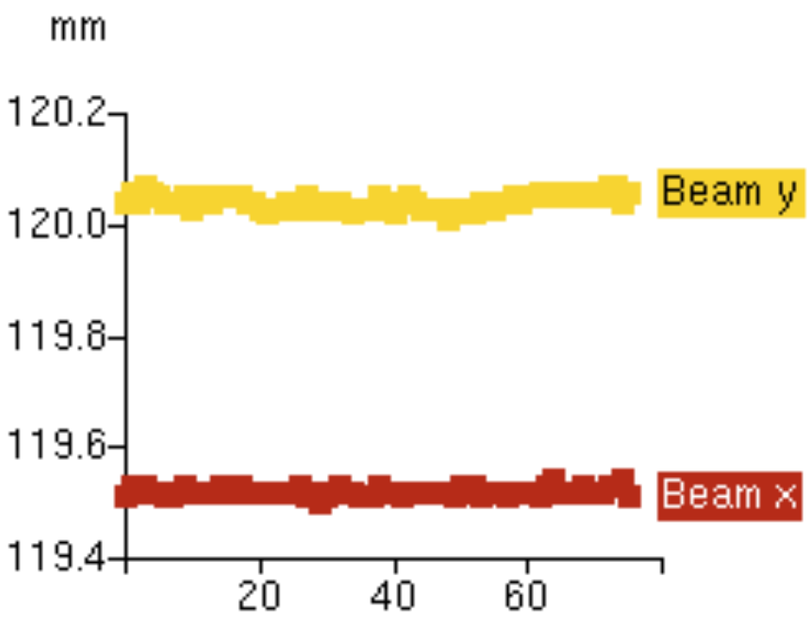
If cell refinement does not work well, simply use the cell derived from autoindexing on two images (or more for low symmetries).

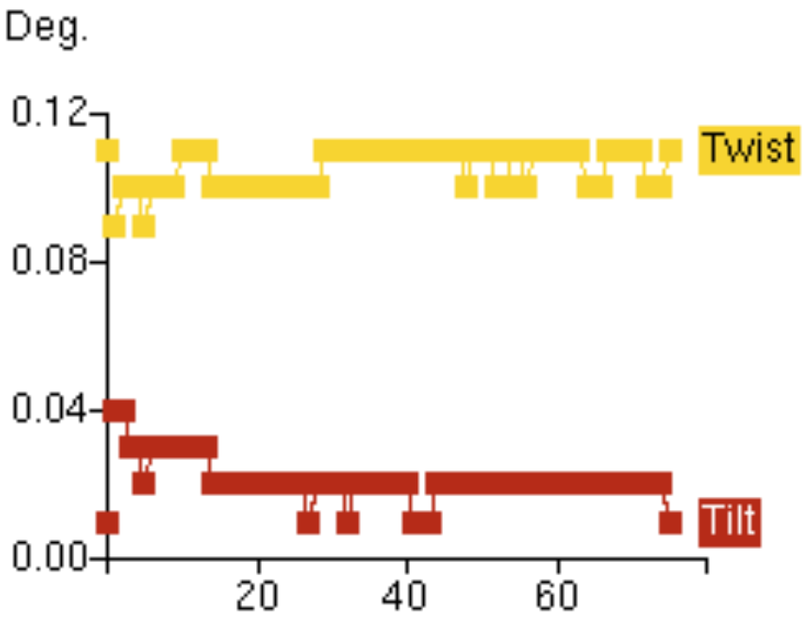
Integration

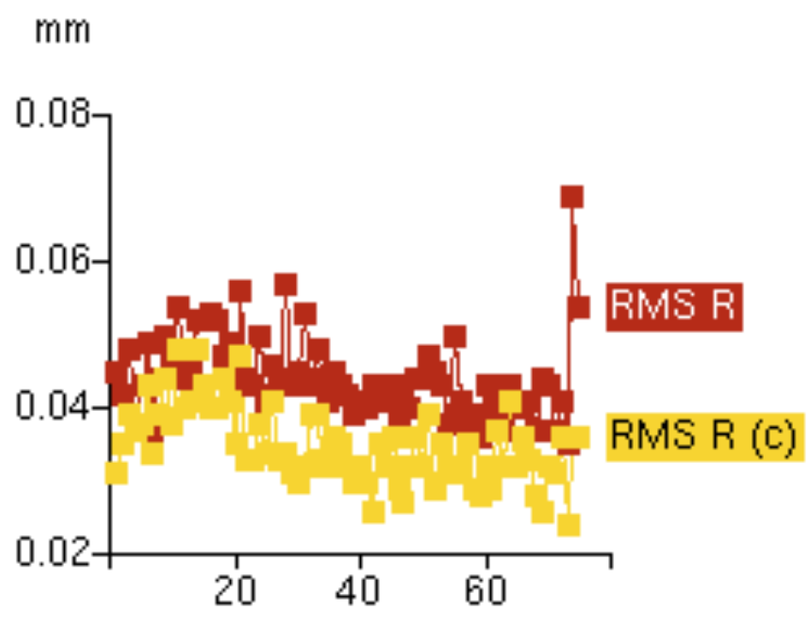


During integration, the refined detector and crystal parameters are plotted as a function of image number.
Also displayed are the average spot profile in the centre of the detector and the standard profiles and an indication of data quality (I/σ(I)) as a function of resolution)

Integration - Stability of refined detector parameters





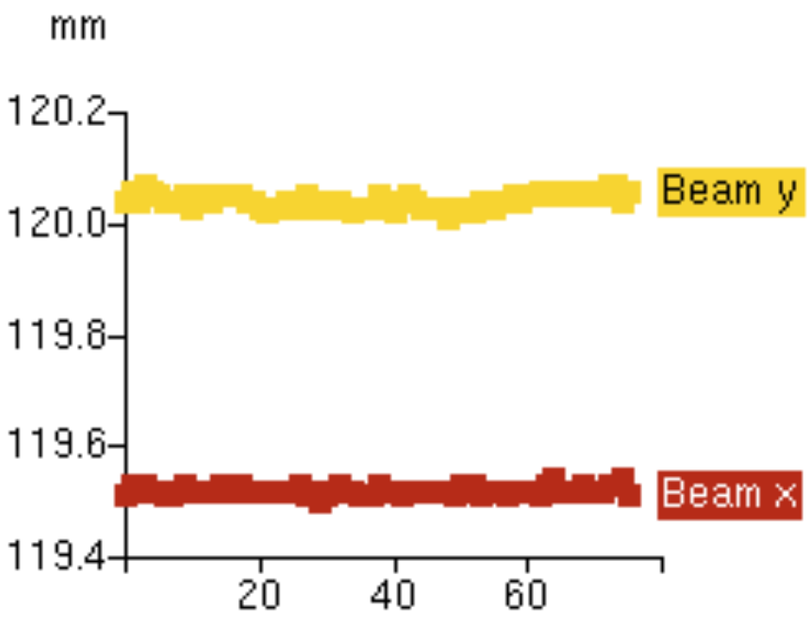


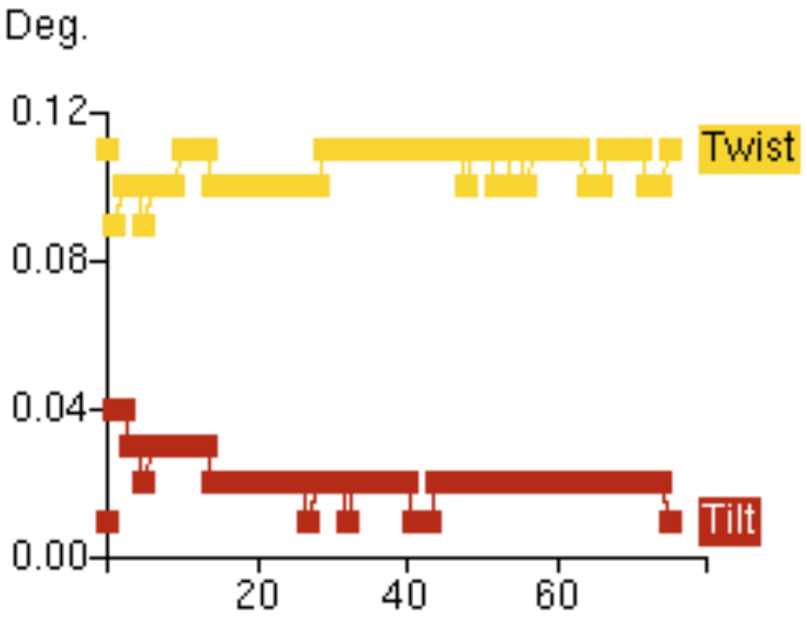
Detector parameters should remain stable during integration

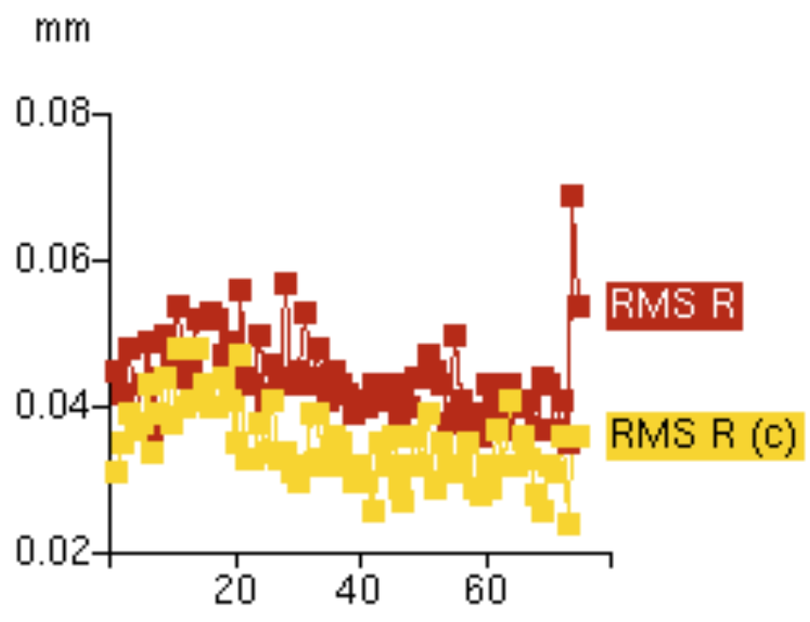
- Direct beam coordinates should not vary by more than 0.1-0.2mm
- Detector tilt and twist should not vary more than 0.2-0.3 degrees
- Rms error in spot positions should be more or less constant (unless spot size/quality or strength of diffraction is changing significantly).

Parameters can be fixed if necessary, but only as a last resort !

Integration - Stability of refined detector parameters







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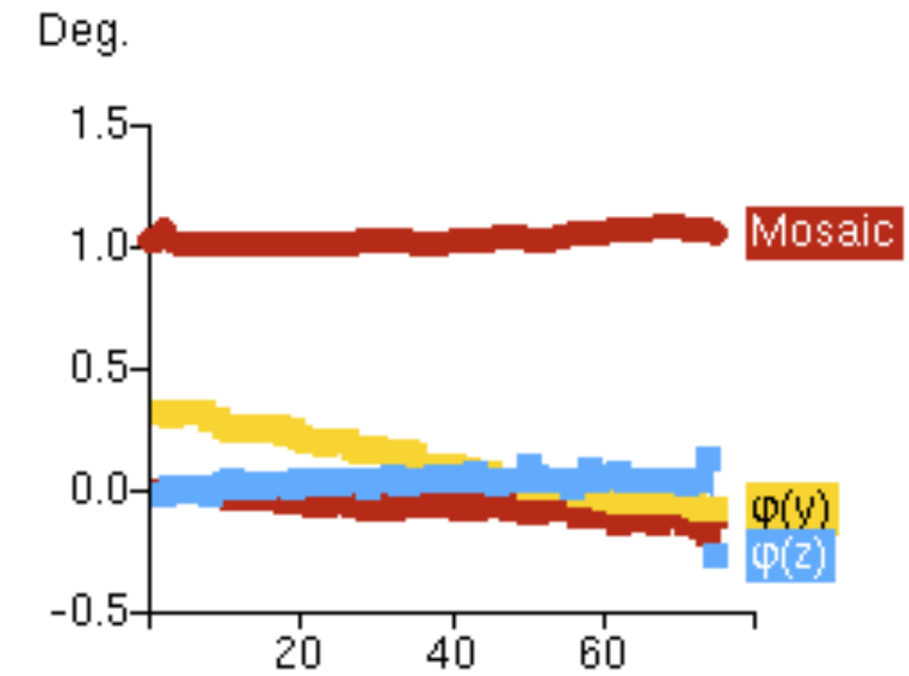
- Detector tilt and twist should not vary more than 0.2-0.3 degrees
- Rms error in spot positions should be more or less constant (unless spot size/quality or strength of diffraction is changing significantly).

Parameters can be fixed if necessary, but only as a last resort !

Beware: Beam Y coordinate will appear to change if Yscale parameter changes (compensates for error in cell parameters).

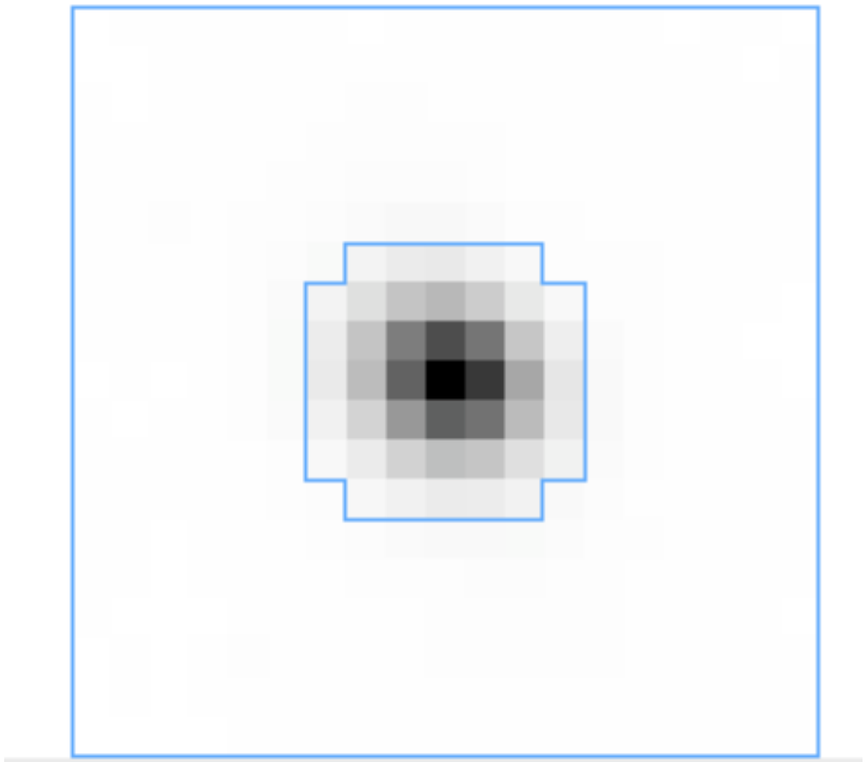
Integration – Stability of refined crystal parameters

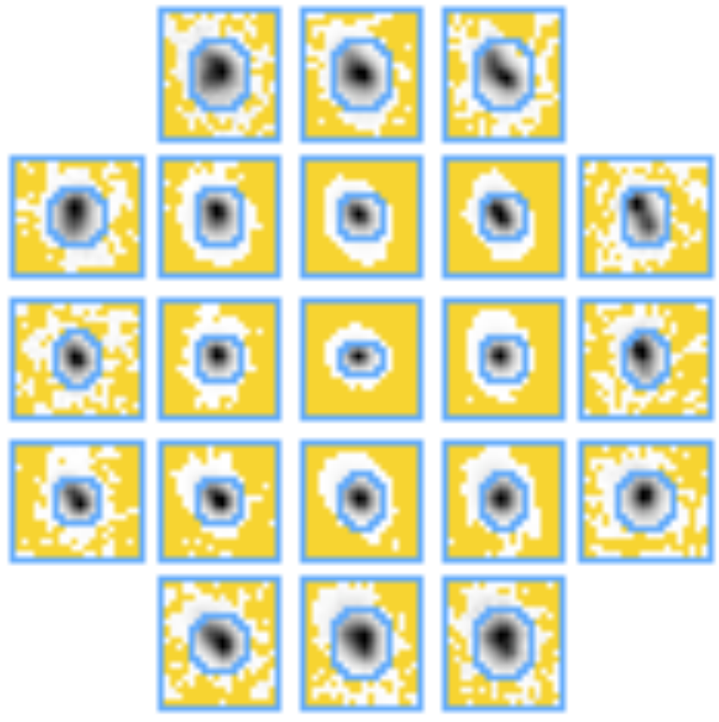


Refined "missets" φ(x), φ(y), φ(z) should vary less than 0.1 × mosaic spread from one image to the next. A smooth variation is probably compensating for a non-orthogonal beam and rotation axis.

Refined mosaic spread should vary smoothly.

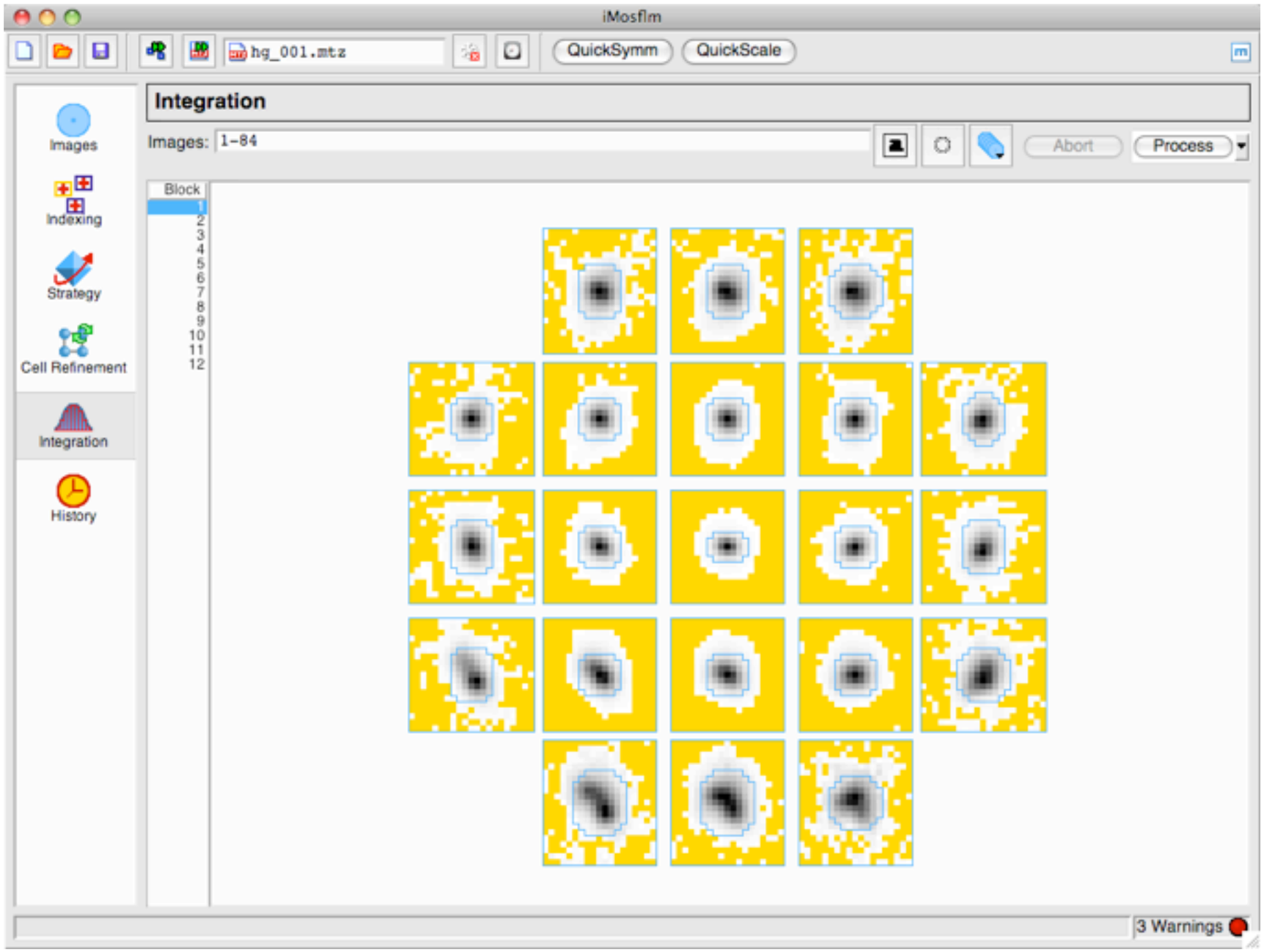
Integration – Spot profiles





The average spot profile in the central region of the detector is displayed for every image, and the standard profiles are displayed for every block of images.
The spot should be positioned centrally within the peak region of the box (inner blue boundary). The Profile Tolerance parameters can be used to make the peak region smaller or larger for special cases.

Integration – Spot profiles

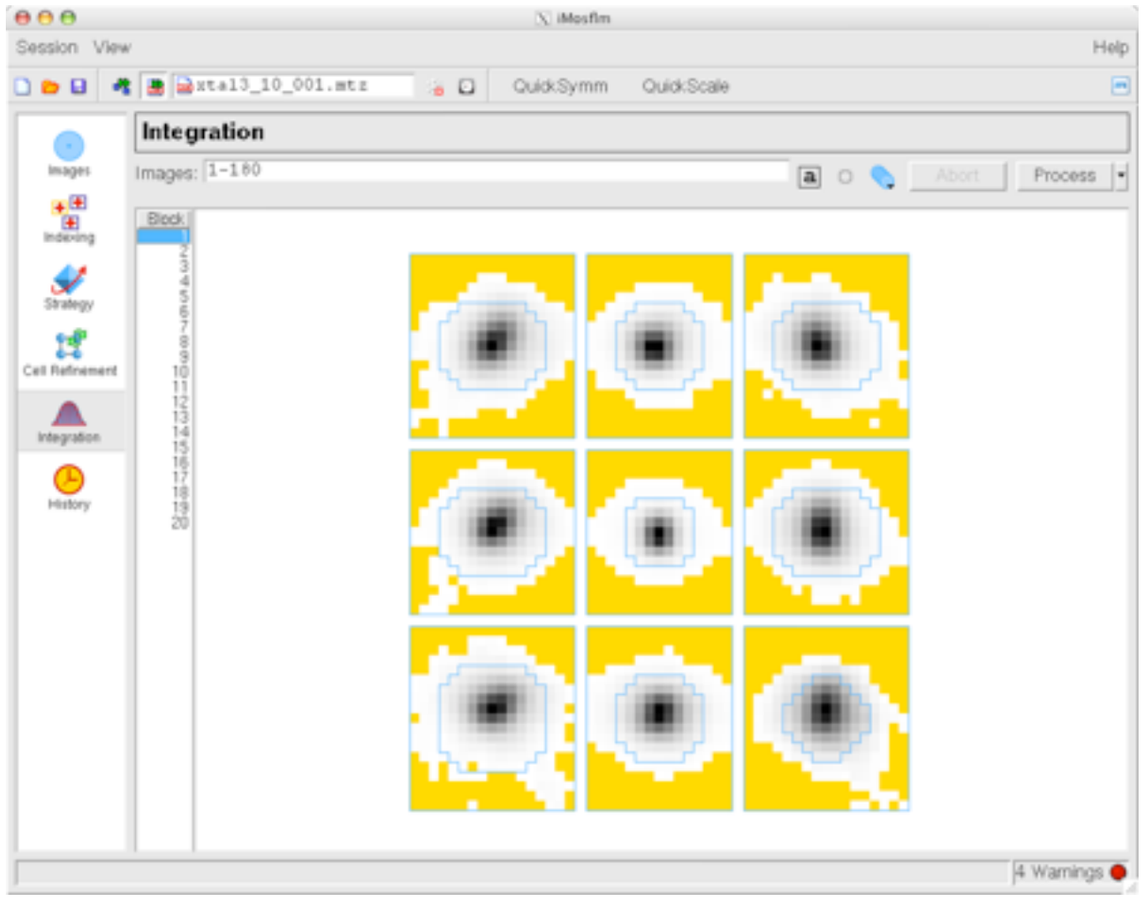


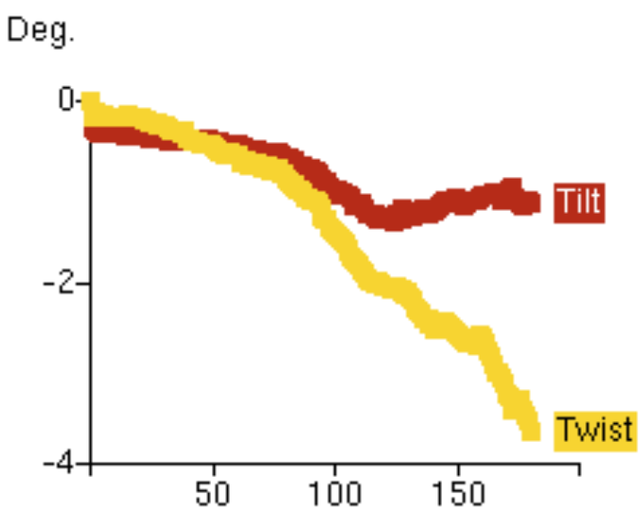
The standard profiles should be well defined for all blocks of data in the dataset

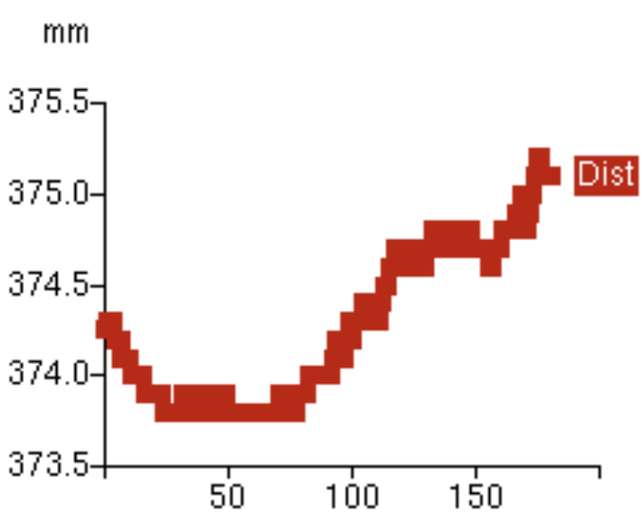
Integration – what should NOT happen

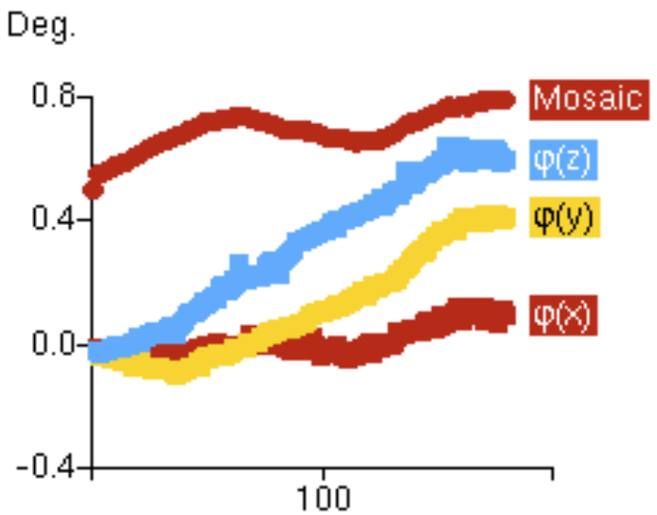
3Å weakly diffracting data,
monoclinic cell

indexed from the first image only





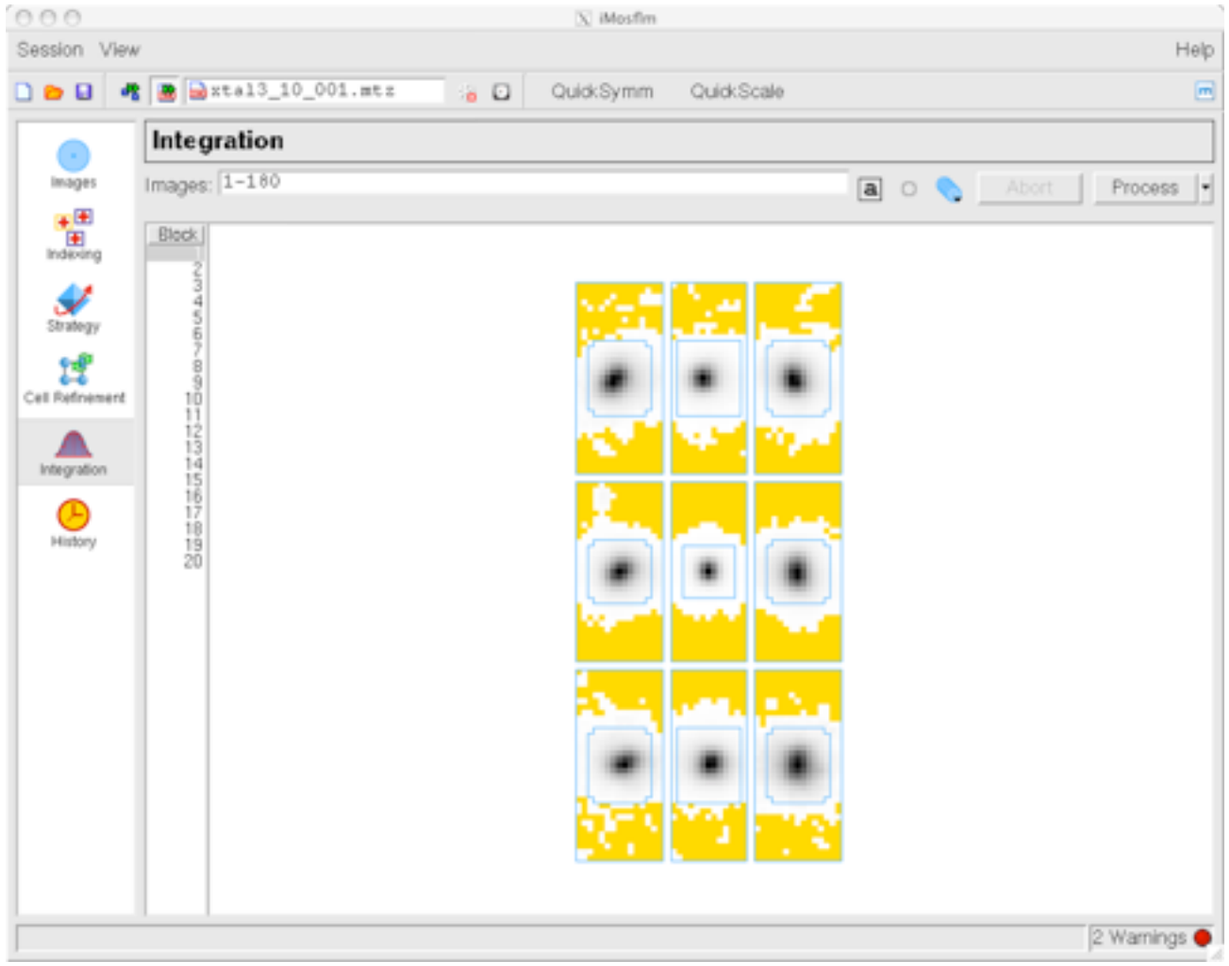




Excessive variation in detector Tilt/Twist and distance also indicate a problem

Integration – what should NOT happen

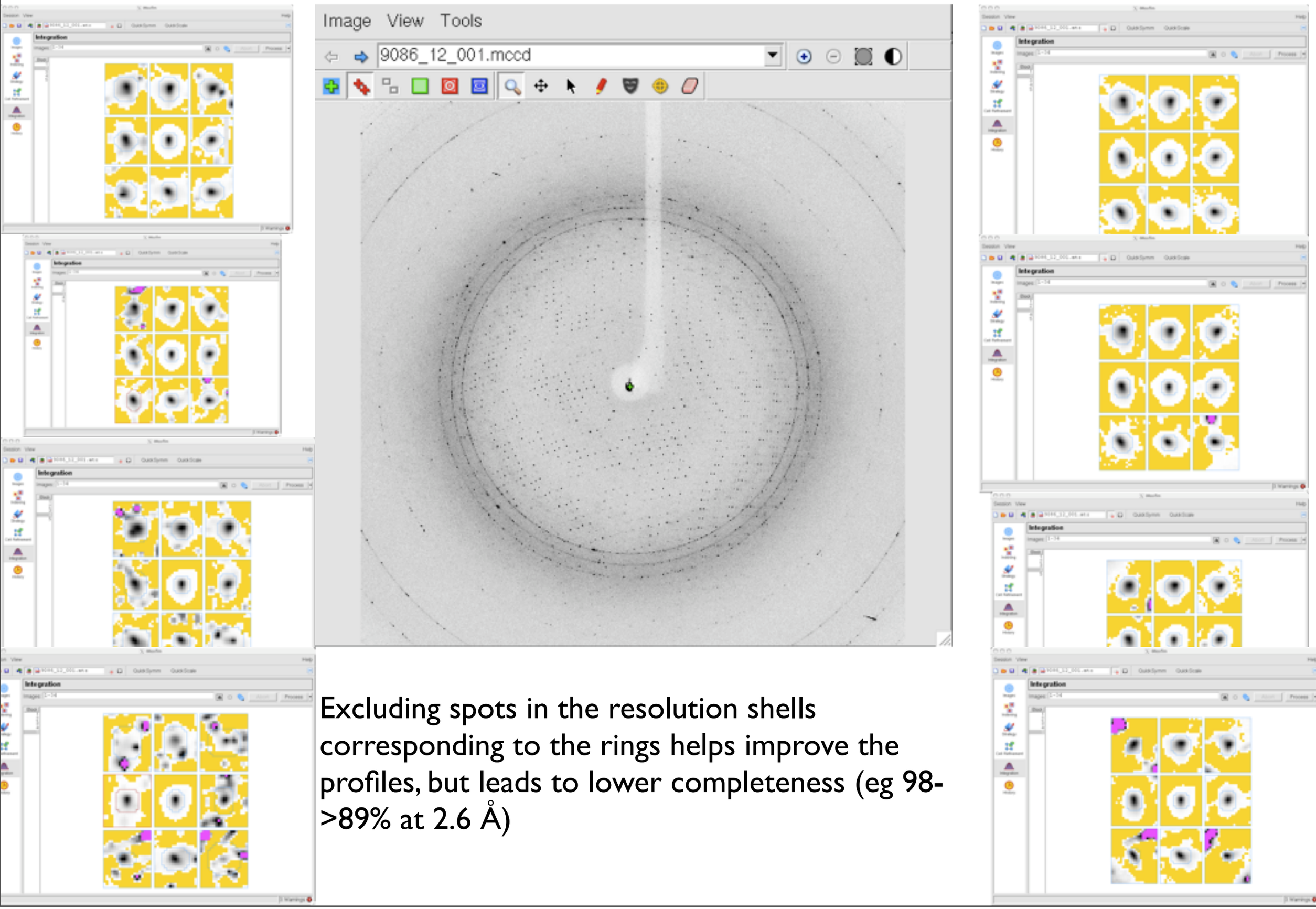
The situation improves significantly if the cell is refined (using 2 segments of data)



Cell parameters:

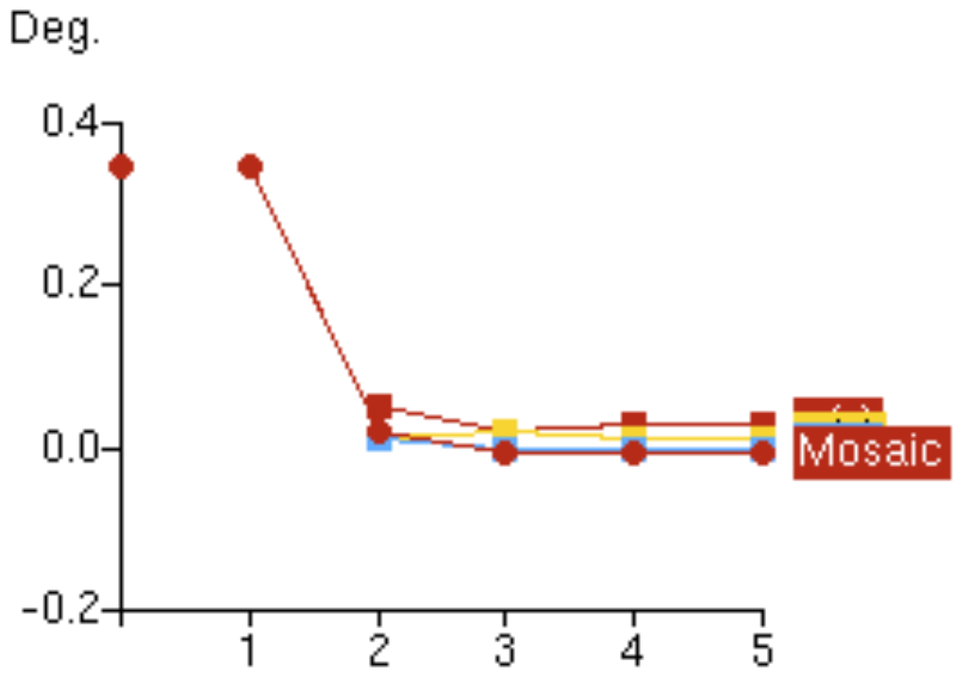
Initial	a=148.4	b=130.8	c=209.6	β=107.3
Refined	a=147.8	b=130.8	c=210.4	β=108.0

Icy Issues



Excluding spots in the resolution shells corresponding to the rings helps improve the profiles, but leads to lower completeness (eg 98->89% at 2.6 Å)

More on what should not happen ...



If the mosaicity refines to a value close to zero, all subsequent processing will be junk !

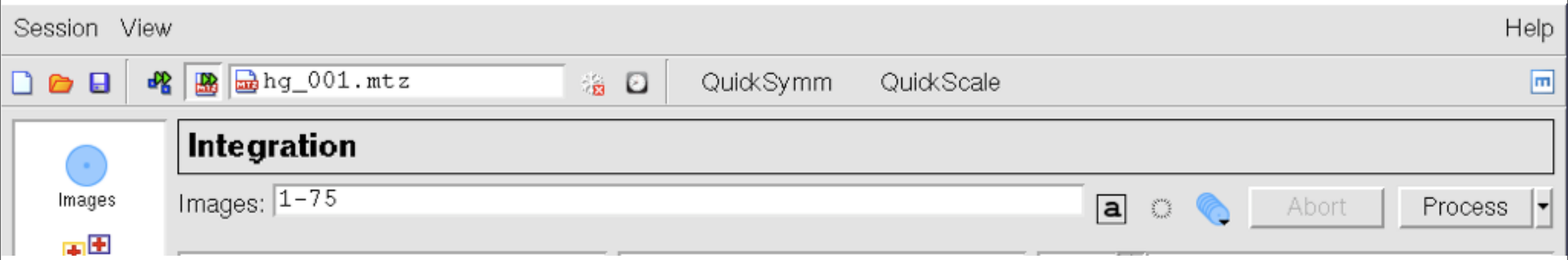
Possible causes:

- Inaccurate cell parameters
- Inaccurate crystal orientation

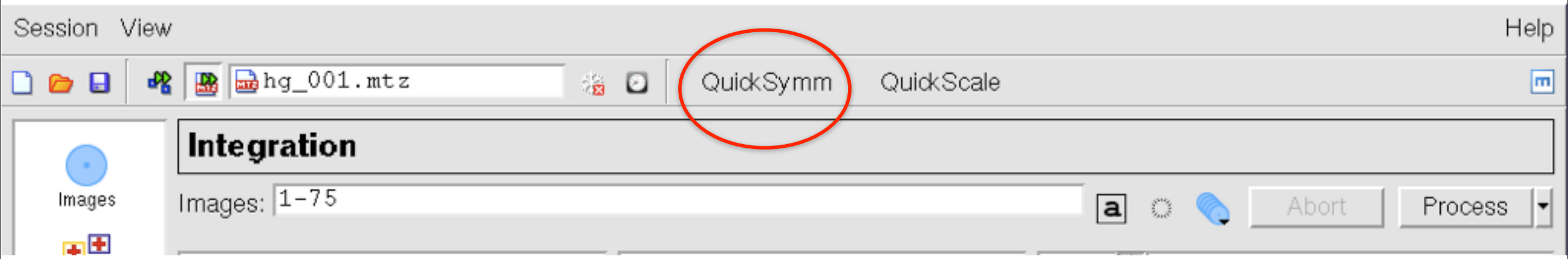
Possible solutions:

- Try to get an accurate cell (Cell refinement)
- Reset the mosaicity to a sensible value and repeat integration of a few images (5-10), so that orientation is updated.
- **Fix** the mosaic spread at a suitable value (look at the predicted spots).

After integration ... Pointless and Aimless/Scala

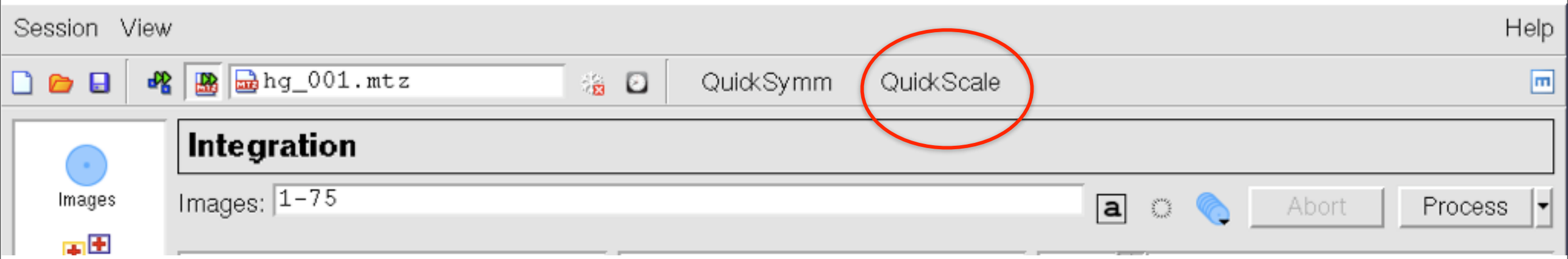


After integration ... Pointless and Aimless/Scala



Selecting "Quicksymm" will run POINTLESS to determine the true symmetry. The results will be displayed in a browser window.

After integration ... Pointless and Aimless/Scala



Selecting "Quicksymm" will run POINTLESS to determine the true symmetry. The results will be displayed in a browser window.

Selecting "Quickscale" will run POINTLESS, followed by running AIMLESS or SCALA with the space group symmetry assigned by POINTLESS. Results are displayed in a browser window.

At present, both programs can only be run with the default options.

Acknowledgements

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Luke Kontogiannis*

** past team members*

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