

Gordon Research Conference in Diffraction Methods in Structural Biology

18-23rd July 2010 at Bates College, Lewiston, Maine

Confirmed speakers/discussion leaders :

MG Rossmann, V Ramakrishnan, K Nagai, Z Dauter, S Harris, J Holton, G Evans, V Stojanoff, E Garman, M Mueller, T Schneider, S McSweeney, J Smith, G Winter, C Vonrhein, S Subramanian, H Tsuruta, C Wilmot, P Adam, R Read, G Murshudov, T Terwilliger, M Caffrey, S White, A Perrakis, P Emsley

The meeting will cover all aspects of structure determination from crystallisation to model building and refinement, with additional sessions on complementary methods.

Visit www.grc.org and follow links to Conferences/Current Meetings/Diffraction Methods for more details.

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Structural Biology
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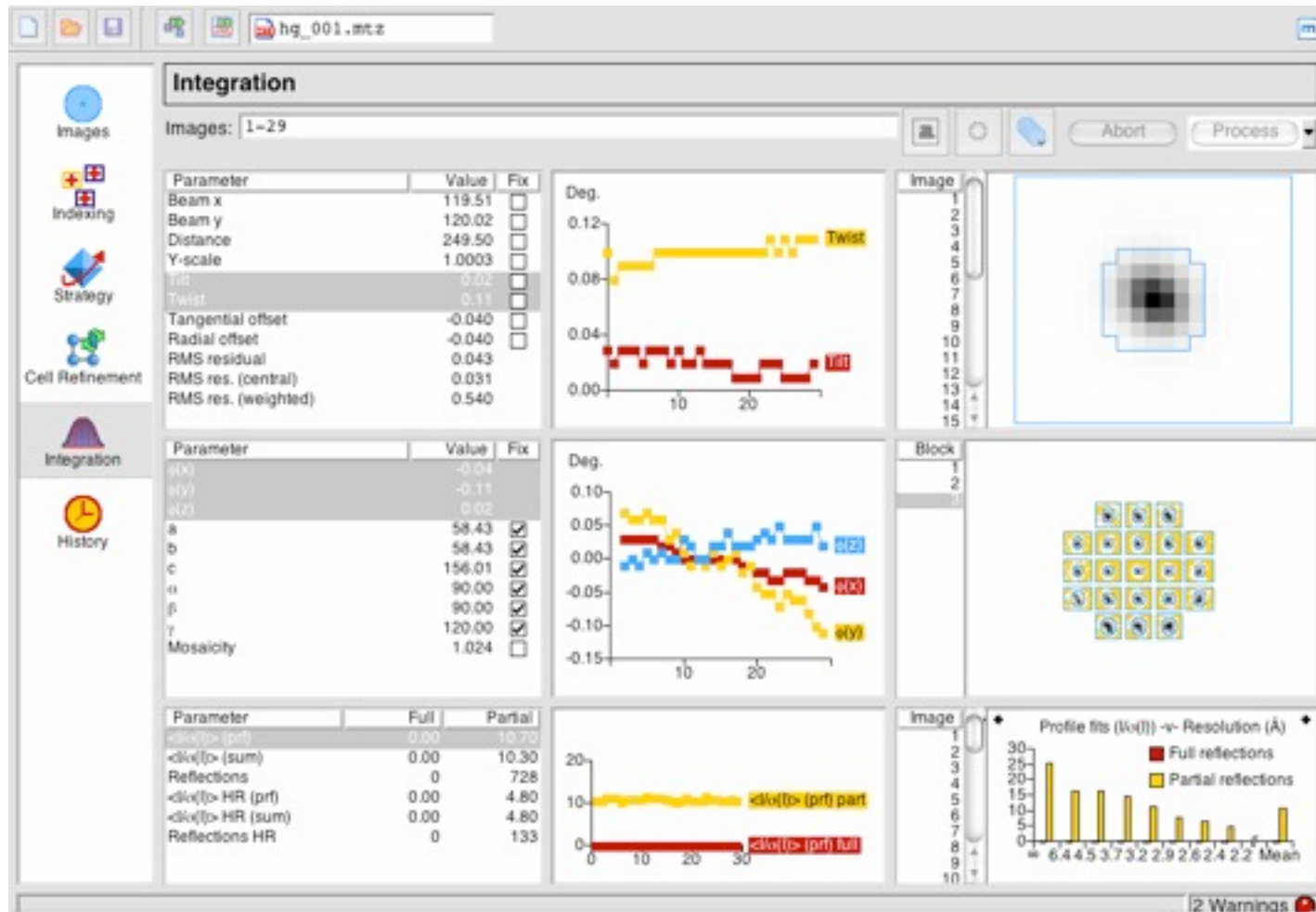
Visit www.grc.org and follow links to Conferences/Current Meetings/
Diffraction Methods for more details.

CCP4 Study Weekend 2010

Spots before your eyes: Diffraction data integration with imosflm

Andrew GW Leslie

MRC LMB, Cambridge



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- Easy to use: Minimal user input and guides the user through the logical steps of data processing

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imosflm is a “front end” graphical user interface to ipmosflm

- Easy to use: Minimal user input and guides the user through the logical steps of data processing
- To provide visual feedback on progress, making it easy to detect when and where problems arise
- To provide all the functionality of ipmosflm for experienced users
- To make data processing fun !

Why use a GUI ?

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)

Why use a GUI ?

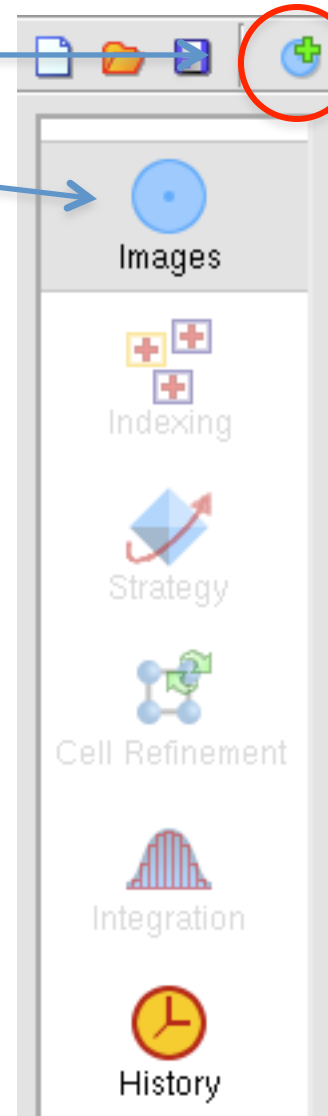
Encourages inspection of the quality of the images (always a good thing) !

- Poor spot shapes
- Anisotropic diffraction
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- Very high mosaicity (increase threshold in indexing)
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- Presence of ice spots or rings (exclude the resolution shells)

However: There is a significant cost in processing time. Can use “batch” submission but the graphing output is (currently) lost.

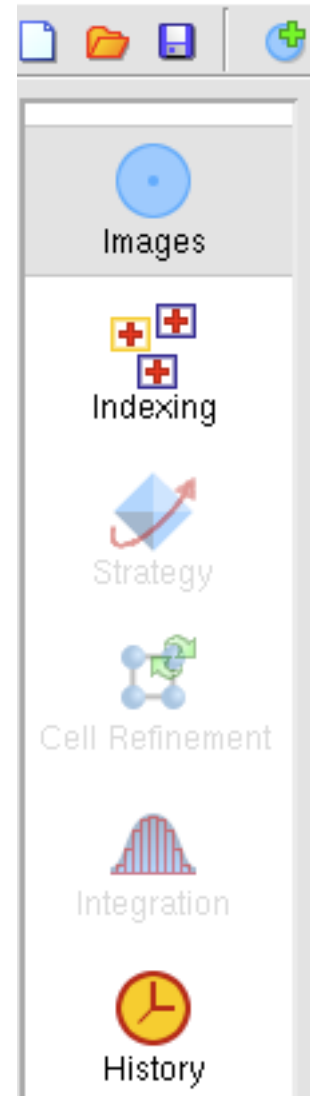
Integration with imosflm

1. Add images
read all images with a common template



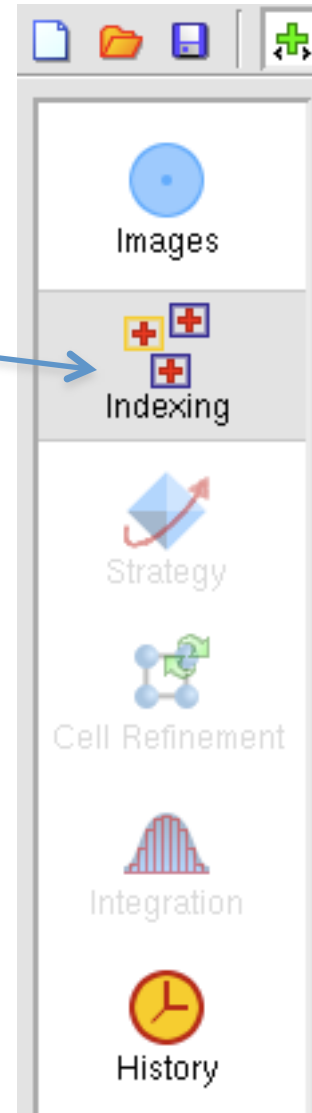
Integration with imosflm

1. Add images
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Integration with imosflm

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2. Find spots and index
find the lattice that fits the spots
check the prediction
estimate the mosaicity



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to get more accurate cell parameters



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5. Integrate the data
usually integrate a few images first

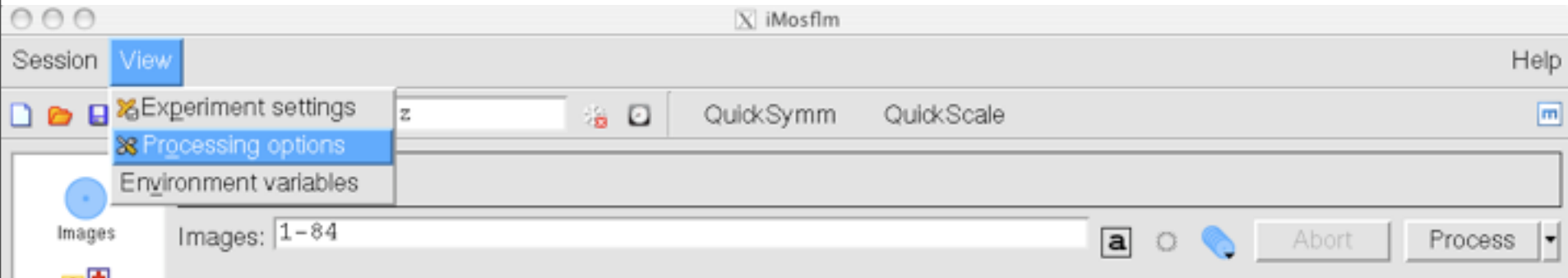


Integration with imosflm

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2. Find spots and index
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to get more accurate cell parameters
5. Integrate the data
usually integrate a few images first
6. Examine mosflm logfile and event history

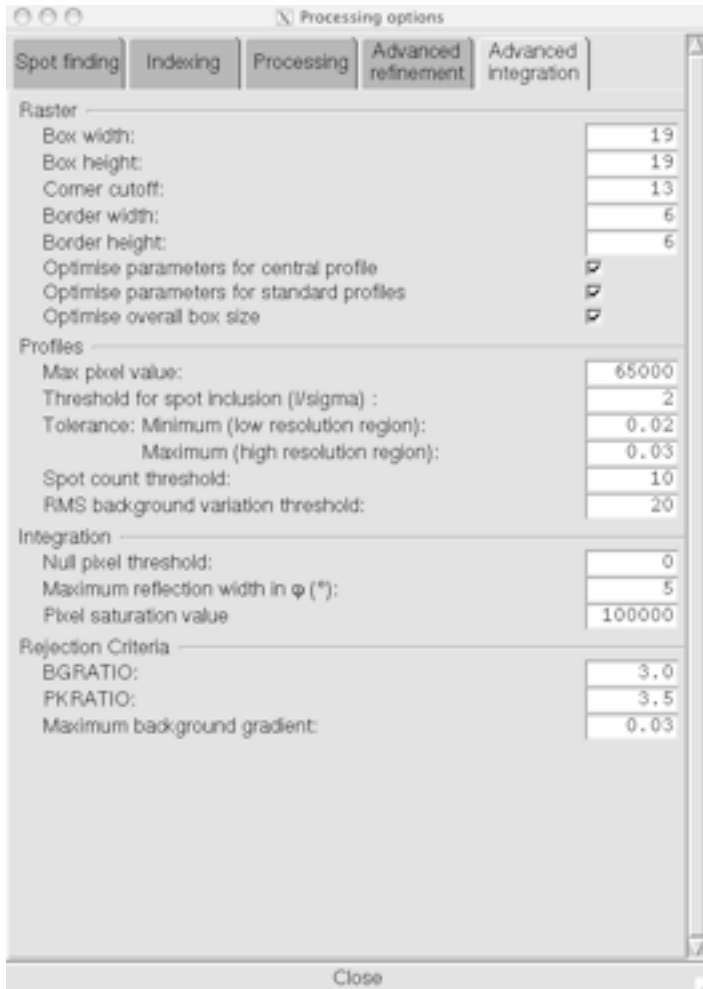


Setting Parameters



Setting Parameters

Processing options



Select the appropriate tab, all the settable parameters are listed.

Setting Parameters

Experiment Settings

Experiment settings

Experiment | Detector

Project:

Crystal:

Dataset:

Title:

Beam position: X (mm):
Y (mm):

Crystal to detector distance (mm):

Beam divergence: X (°):
Y (°):

Wavelength (λ , Å):

Wavelength dispersion (Å):

Beam polarization:

Detector angle (2θ , °):

Reverse direction of spindle rotation

Close

Experiment settings

Experiment | Detector

Refineable detector parameters

Y Scale:

Offsets: radial: tangential:

comega:

Detector tilt: twist:

Non-refineable detector parameters

Detector: model:

Gain:

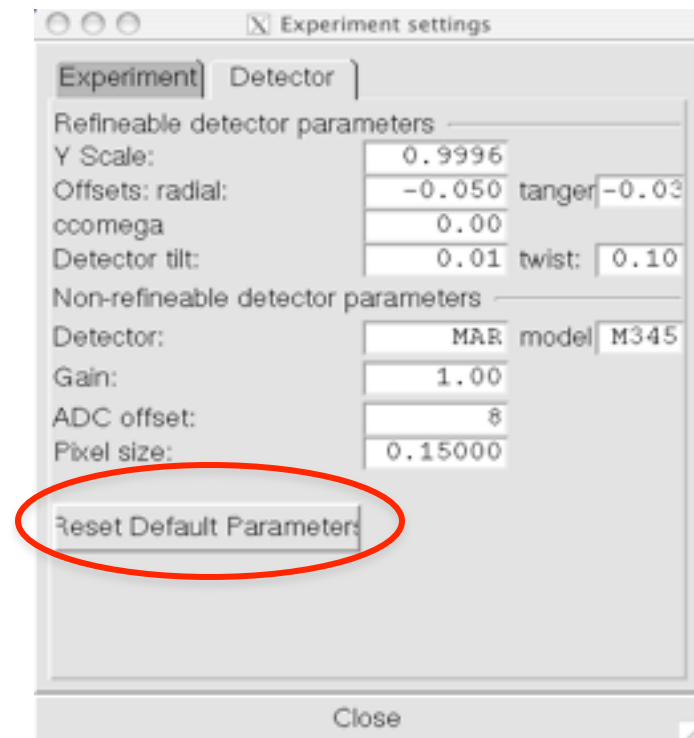
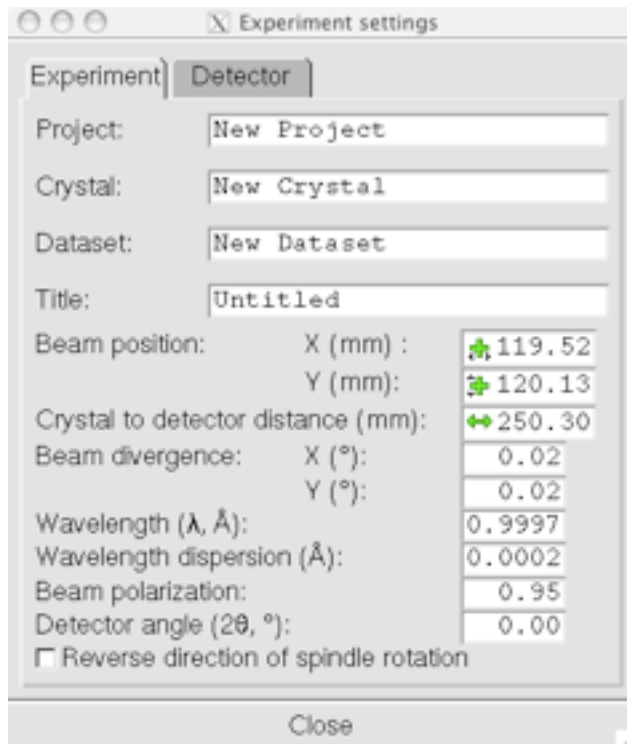
ADC offset:

Pixel size:

Close

Setting Parameters

Experiment Settings



Indexing

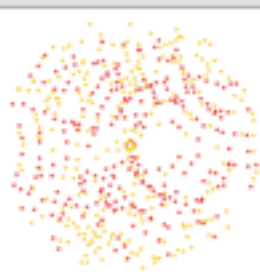
Session View

119.55 120.09 250.00 5.00 10.0 1.05 1.05 0.00 20 25

Autoindexing

Images: 1, 84

| Image | Phi | Auto | Manual | Deleted | > I/sig(I) | Search | Use |
|--------------|-------------|------------|----------|----------|------------|-------------------------------------|-------------------------------------|
| 1 | 0.0 - 1.0 | 294 | 0 | 0 | 148 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| 84 | 83.0 - 84.0 | 222 | 0 | 0 | 105 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| Total | | 516 | 0 | 0 | 253 | | |



Solutions:

| Solution | Lat. | Pen. | a | b | c | α | β | γ | $a(xy)$ | $a(\varphi)$ | δ beam |
|----------|------|------|-------|------|-------|----------|---------|----------|---------|--------------|---------------|
| 1 (ref) | aP | 0 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 120.0 | 0.20 | 0.35 | 0.44 (0.1) |
| 2 (ref) | aP | 0 | 58.5 | 58.6 | 62.1 | 61.9 | 62.0 | 60.0 | 0.20 | 0.35 | 0.44 (0.1) |
| 3 (ref) | mC | 1 | 101.3 | 58.6 | 62.2 | 90.0 | 123.0 | 90.0 | 0.21 | 0.42 | 0.44 (0.1) |
| 4 (ref) | mC | 1 | 101.4 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.39 | 0.45 (0.2) |
| 5 (ref) | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 (0.2) |
| 6 (ref) | hR | 2 | 58.6 | 58.6 | 156.4 | 90.0 | 90.0 | 120.0 | 0.21 | 0.54 | 0.46 (0.3) |
| 7 (ref) | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 (0.2) |
| 8 (reg) | hR | 60 | 60.4 | 60.4 | 144.8 | 90.0 | 90.0 | 120.0 | - | - | - |
| 9 (reg) | mC | 60 | 103.5 | 62.2 | 58.5 | 90.0 | 124.4 | 90.0 | - | - | - |
| 10 (reg) | mC | 60 | 85.3 | 85.4 | 58.5 | 90.0 | 133.2 | 90.0 | - | - | - |
| 11 (reg) | oI | 60 | 58.6 | 62.2 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - |
| 12 (reg) | mC | 61 | 101.4 | 58.6 | 62.1 | 90.0 | 122.9 | 90.0 | - | - | - |
| 13 (reg) | tI | 61 | 60.4 | 60.4 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - |
| 14 (reg) | oI | 61 | 58.5 | 62.2 | 85.4 | 90.0 | 90.0 | 90.0 | - | - | - |
| 15 (reg) | mC | 61 | 101.5 | 58.5 | 62.2 | 90.0 | 122.9 | 90.0 | - | - | - |

Spacegroup: h3

Mosacity: 0.00 Estimate

No Warnings

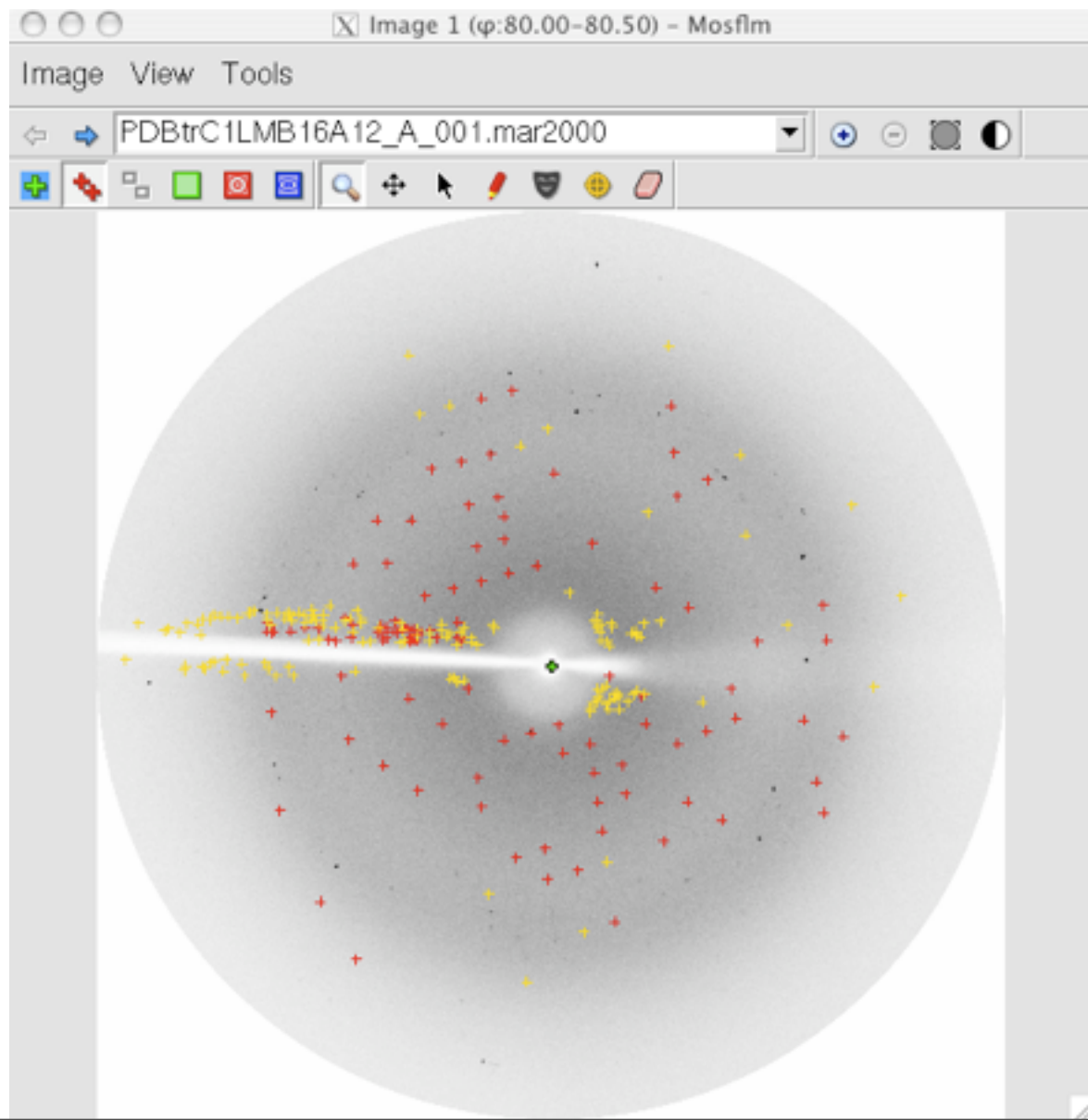
- Selects two images 90 degrees apart (if possible).
- Finds spots on these images
- Selecting “Index” attempts to index using these two images.
- Produces a list of solutions.
- Highlights the “best” solution. (Highest symmetry with a low penalty).

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

Indexing – Spot Finding

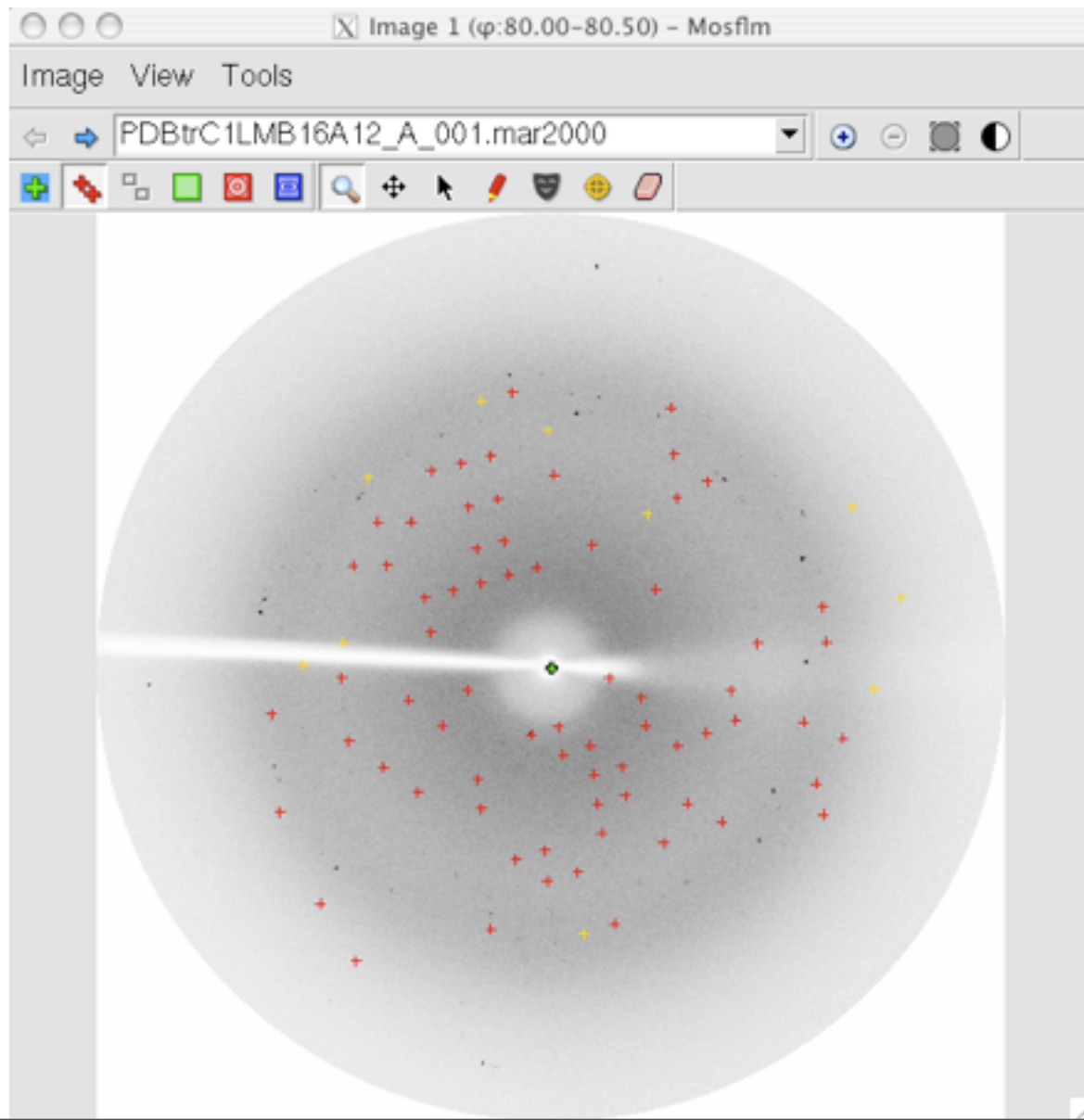
Indexing – Spot Finding

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



Indexing – Spot Finding

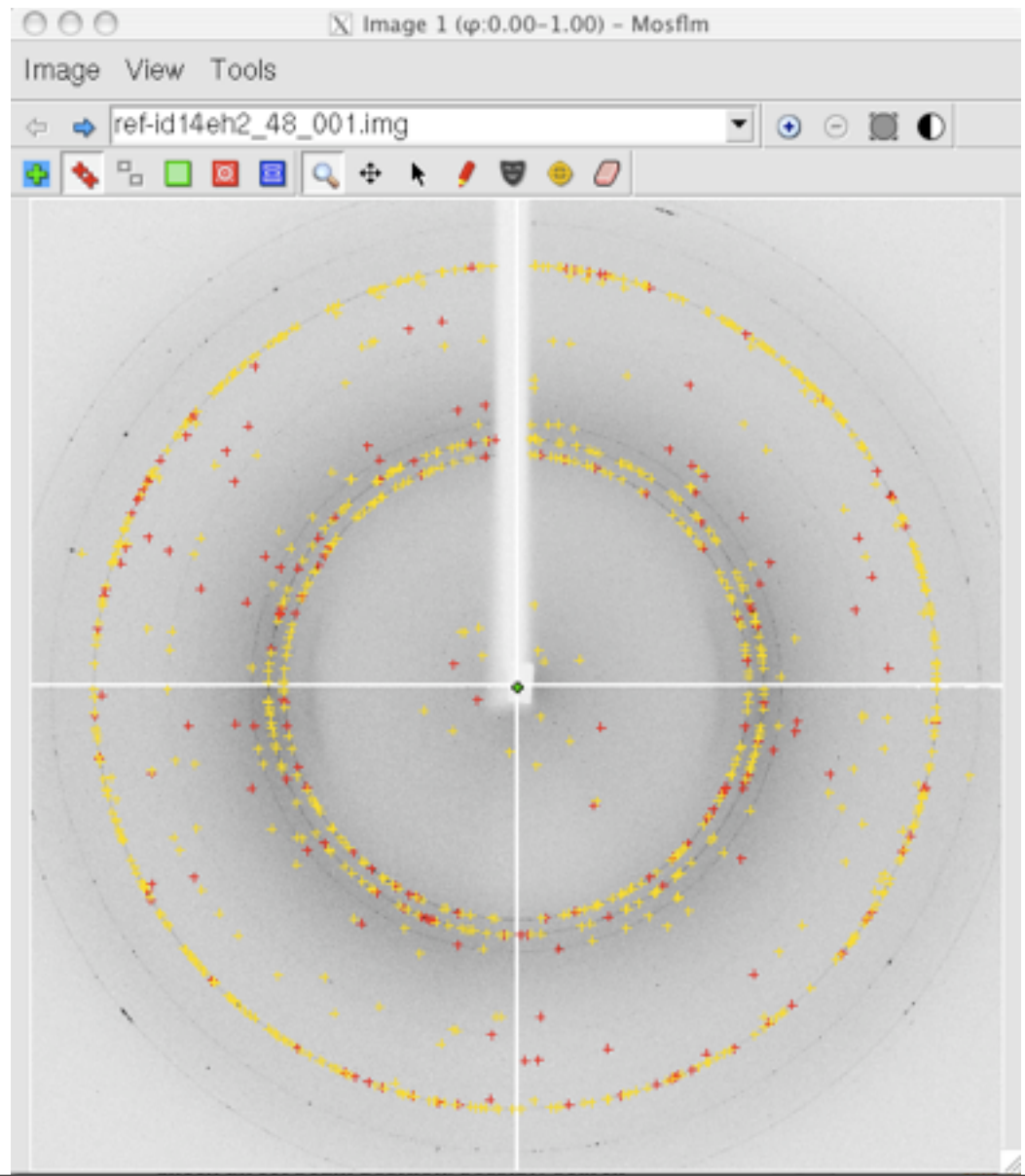
To eliminate “false” spots near shadows, reduce the size of the local background box (100x100)
Eg to 20,20 (pixels)



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To eliminate “false” spots near shadows, reduce the size of the local background box (100x100)
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Ice rings will cause problems

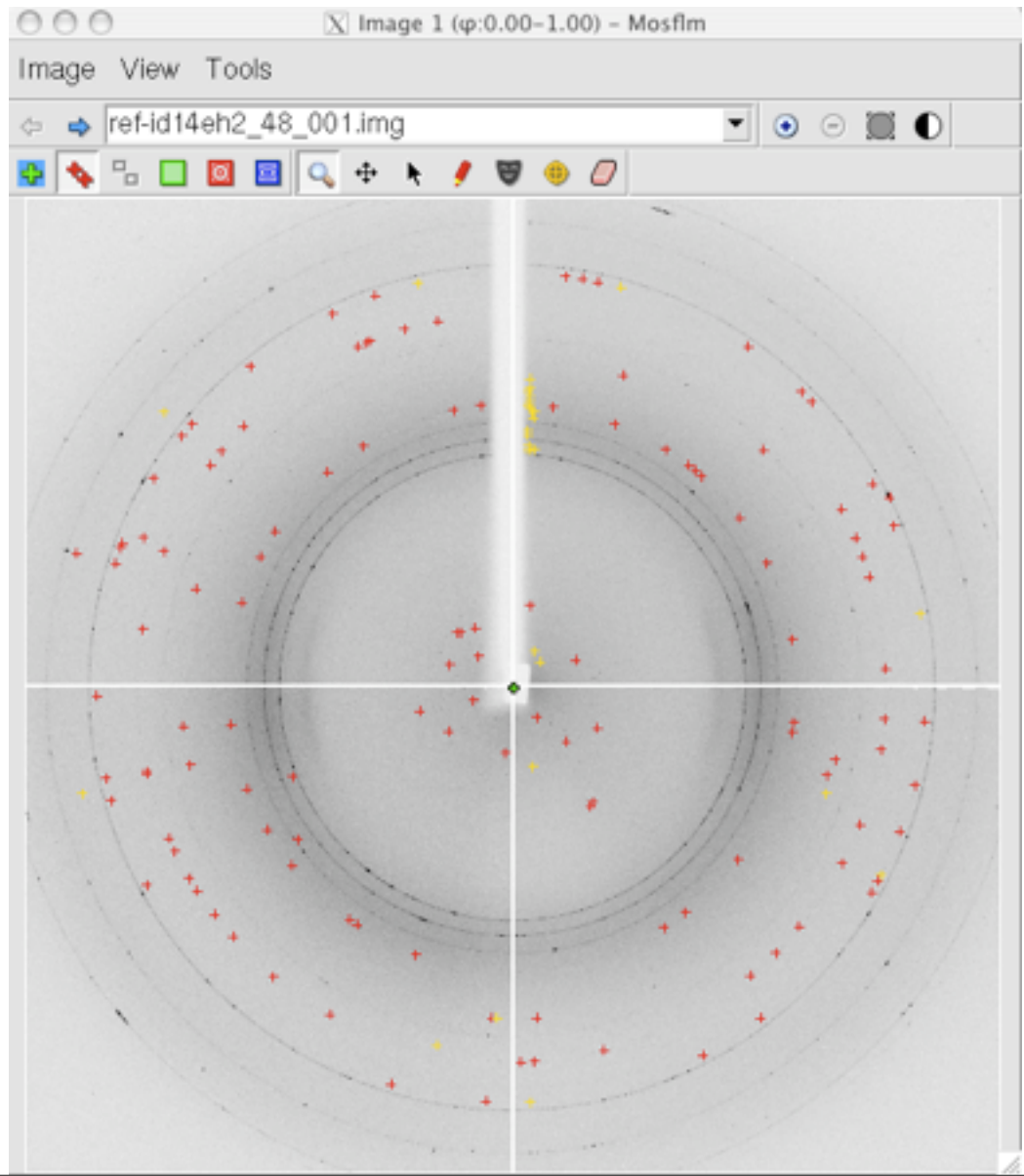


Indexing – Spot Finding

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.



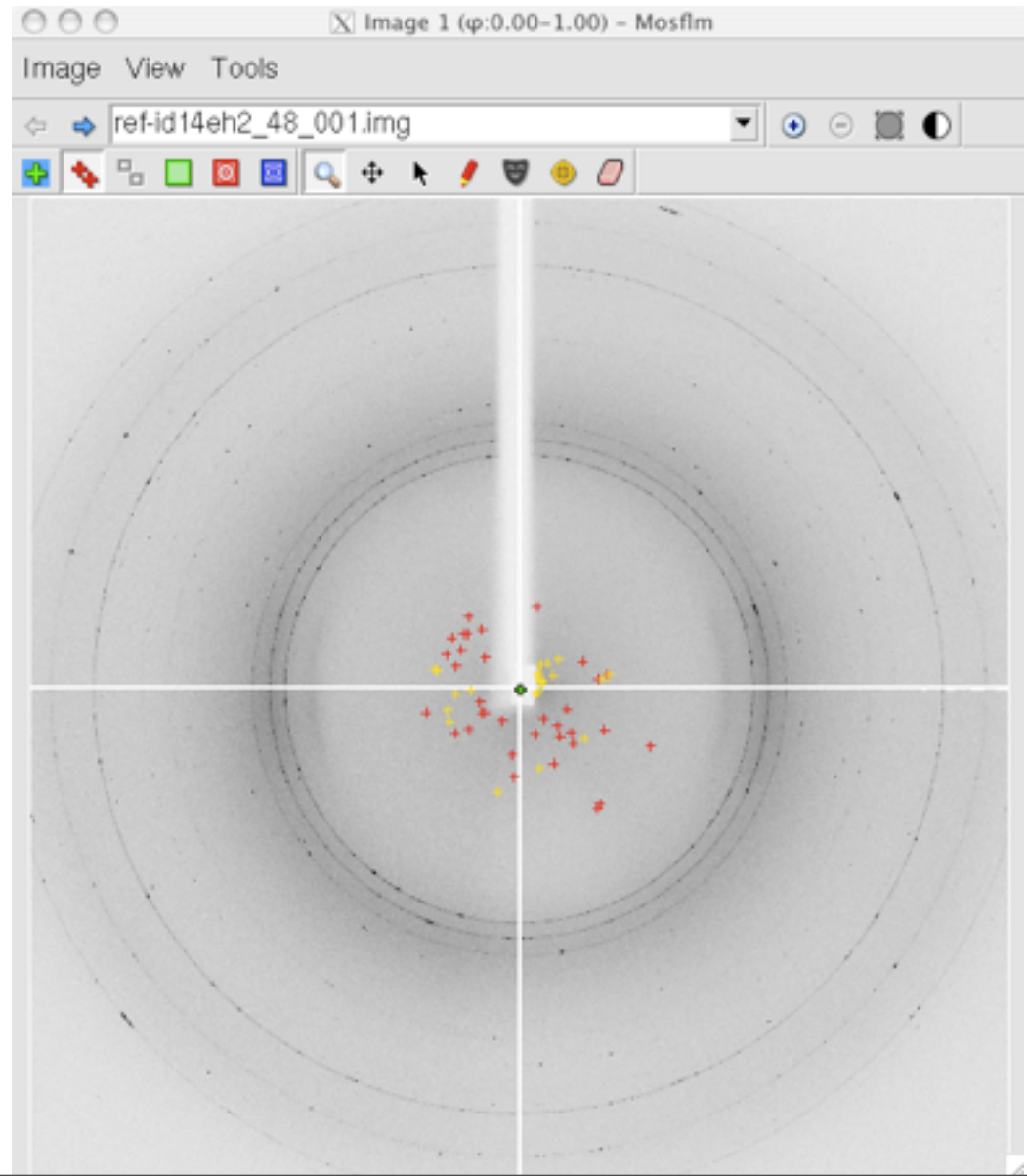
Indexing – Spot Finding

To eliminate “false” spots near shadows, reduce the size of the local background box (100x100)
Eg to 20,20 (pixels)

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



Indexing – Spot Finding

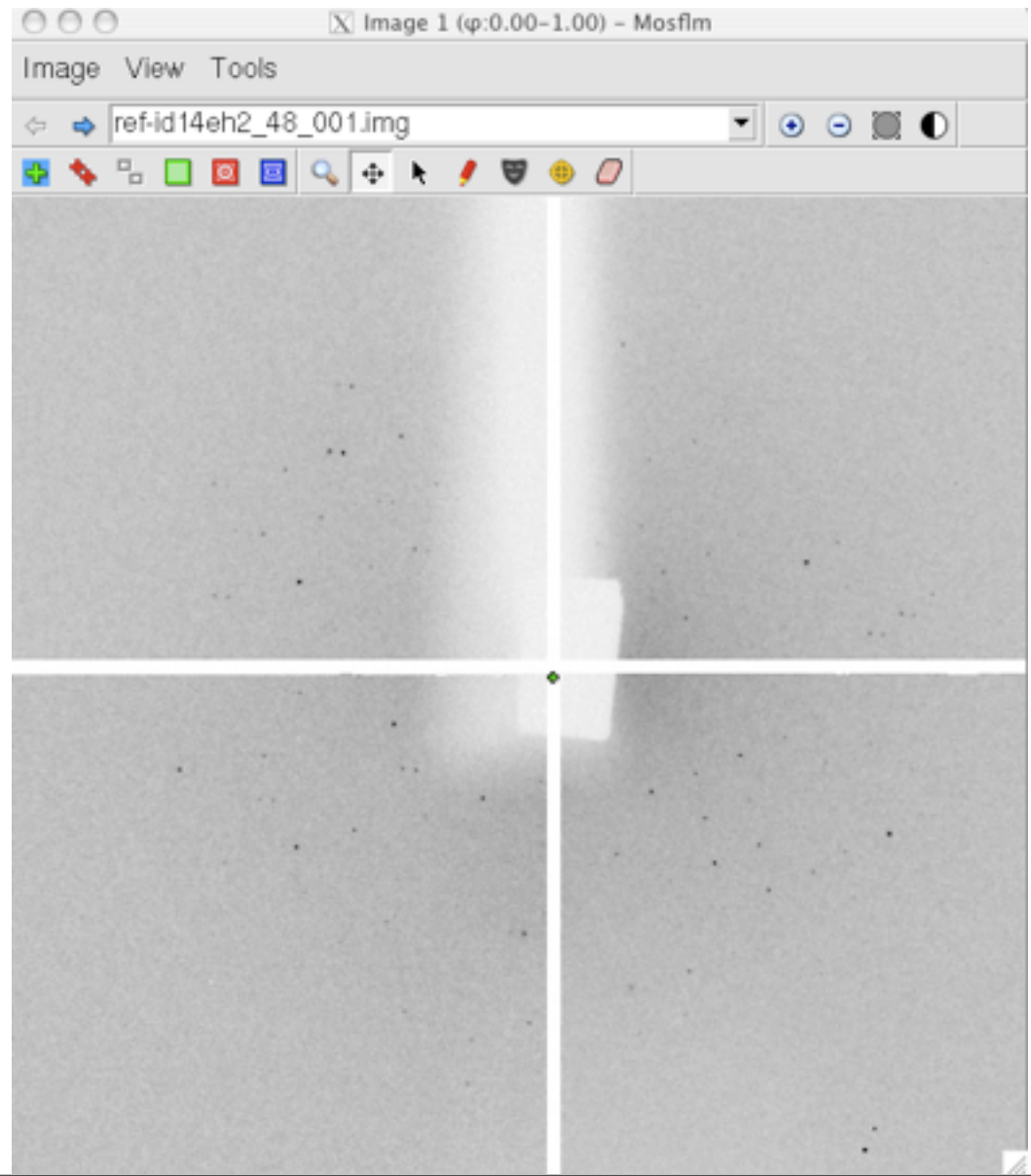
To eliminate “false” spots near shadows, reduce the size of the local background box (100x100)
Eg to 20,20 (pixels)

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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)

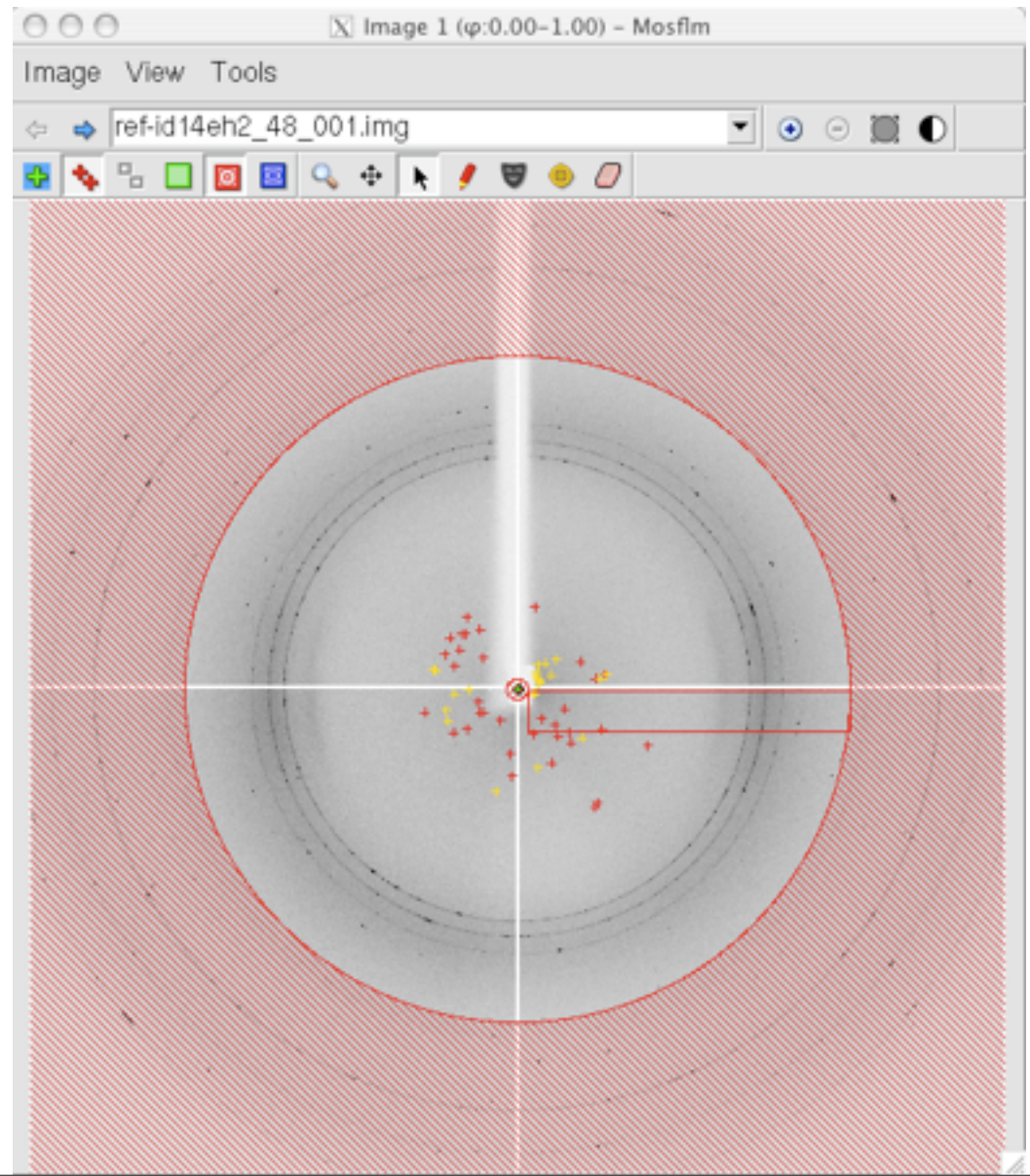
Ice rings will cause problems

So mosflm rejects all spots at the resolutions corresponding to ice.
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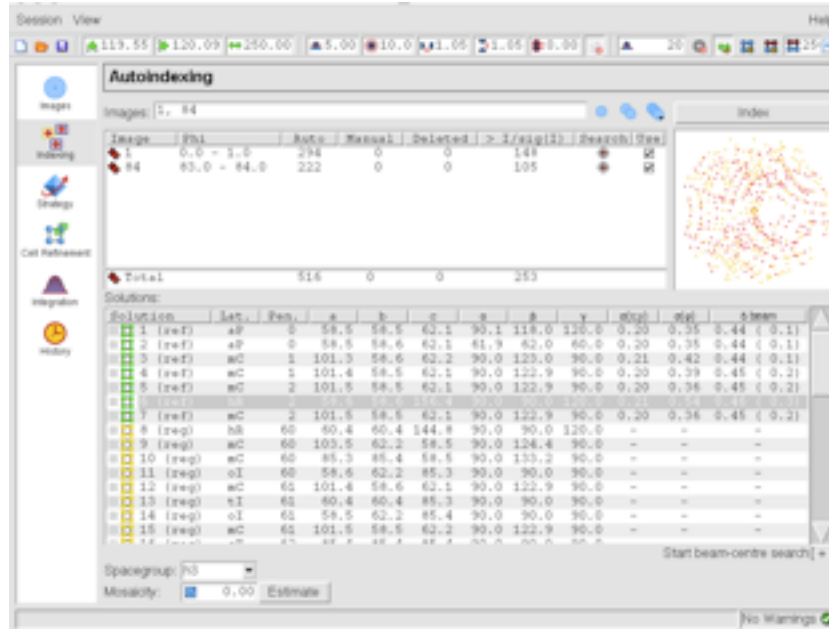
Because the diffraction is very weak, mosflm rejects all spots beyond 4.5Å

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 $\sigma(xy)$ small ? (typically 0.1-0.3mm, but can be up to 1mm if spots are split)

Sometimes it will not give a solution....

What can be done if indexing fails ?

What can be done if indexing fails ?

The screenshot shows the Mosflm software interface. The main window is titled "Mosflm" and has a "Session View" header. The "Autoindexing" panel is active, showing a table of indexed images and a scatter plot of reflections. A dialog box with a warning icon is overlaid on the interface, displaying the following text:

The indexing process has failed. It might be worthwhile trying again with:

1. A large or smaller longest cell edge
2. Using more or fewer reflections (200 - 1000 is best)
3. Using more and/or different images
4. Checking your direct beam position carefully

The dialog box has a "Dismiss" button at the bottom.

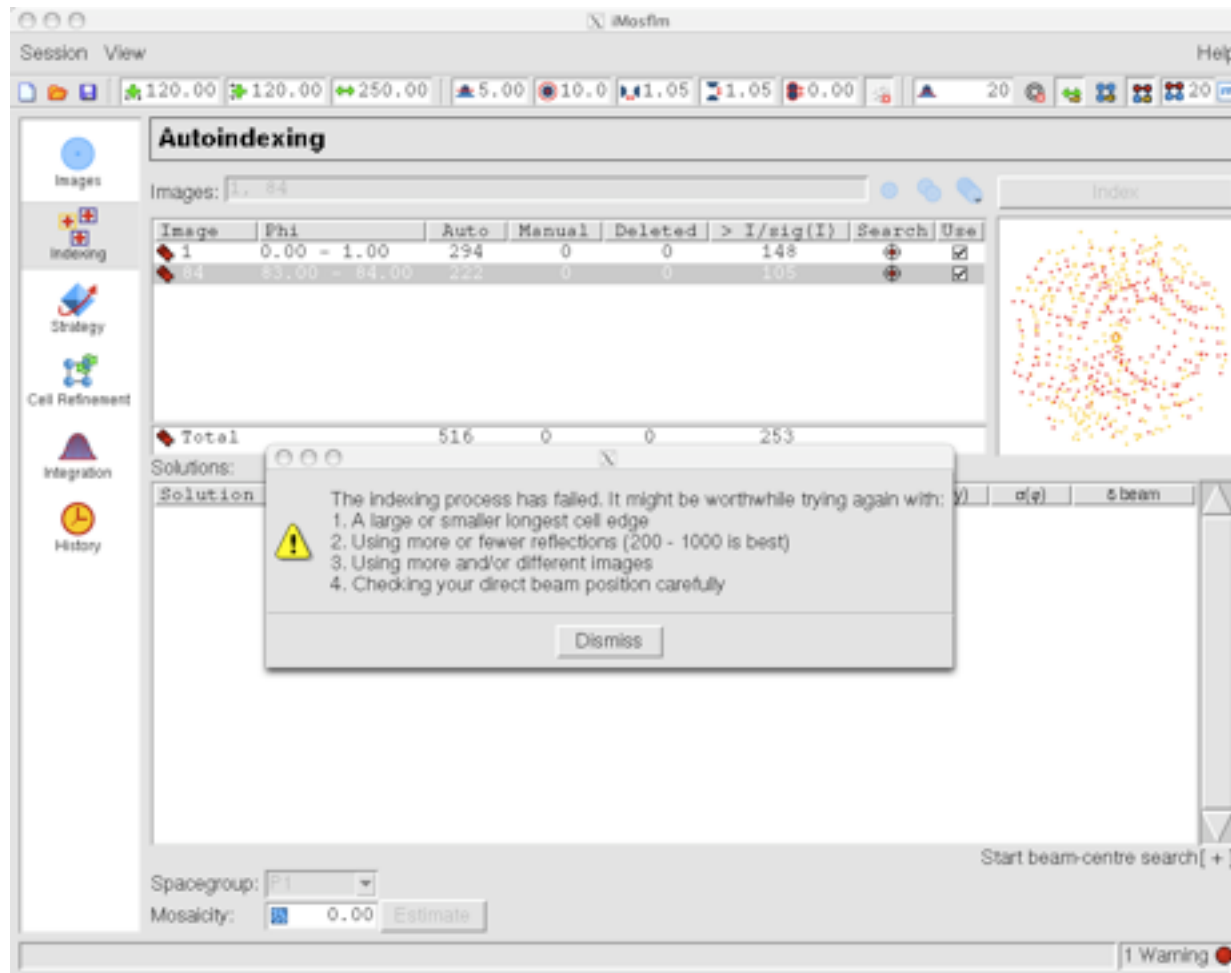
The "Autoindexing" panel includes a table with the following data:

| Image | Phi | Auto | Manual | Deleted | > I/sig(I) | Search | Use |
|-------|---------------|------|--------|---------|------------|-------------------------------------|-------------------------------------|
| 1 | 0.00 - 1.00 | 294 | 0 | 0 | 148 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| 84 | 83.00 - 84.00 | 222 | 0 | 0 | 105 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| Total | | 516 | 0 | 0 | 253 | | |

The "Index" panel shows a scatter plot of reflections. The "Solutions" panel is empty. The "Spacegroup" is set to P1 and the "Mosaicity" is 0.00. A status bar at the bottom right indicates "1 Warning".

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)



The screenshot shows the iMosflm software interface. The main window is titled "iMosflm" and has a "Session View" header. The "Autoindexing" panel is active, showing a table of indexed images and a "Total" row. A dialog box with a yellow warning icon is overlaid on the interface, displaying the following text:

The indexing process has failed. It might be worthwhile trying again with:

1. A large or smaller longest cell edge
2. Using more or fewer reflections (200 - 1000 is best)
3. Using more and/or different images
4. Checking your direct beam position carefully

The dialog box has a "Dismiss" button at the bottom. The background interface shows a table with the following data:

| Image | Phi | Auto | Manual | Deleted | > I/sig(I) | Search | Use |
|-------|---------------|------|--------|---------|------------|-------------------------------------|-------------------------------------|
| 1 | 0.00 - 1.00 | 294 | 0 | 0 | 148 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| 84 | 83.00 - 84.00 | 222 | 0 | 0 | 105 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| Total | | 516 | 0 | 0 | 253 | | |

The "Index" panel on the right shows a circular pattern of reflections. The "Solutions" panel is empty. The "Spacegroup" is set to "P1" and the "Mosaicity" is set to "0.00". A "Warning" icon is visible in the bottom right corner of the software window.

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)

The screenshot shows the 'Autoindexing' window of a software application. The top bar displays various parameters: 119.55, 120.09, 250.00, 5.00, 10.0, 1.05, 1.05, 0.00, 20, and 25. The 'Images' field contains '1, 84'. The 'Index' field is empty. The main table lists indexed images with their respective parameters.

| Image | Phi | Auto | Manual | Deleted | > I/sig(I) | Search | Use |
|--------------|-------------|------|--------|---------|------------|-------------------------------------|-------------------------------------|
| 1 | 0.0 - 1.0 | 294 | 0 | 0 | 148 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| 84 | 83.0 - 84.0 | 222 | 0 | 0 | 105 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| Total | | 516 | 0 | 0 | 253 | | |

Below the table, there are sections for 'Solutions' and a detailed parameter table. The 'Solutions' section lists four solutions (4, 5, 6, 7) with their respective parameters. The detailed table below provides specific values for each parameter.

| Solution | Lat. | Pen. | a | b | c | α | β | γ | $\sigma(x,y)$ | $\sigma(\varphi)$ | δ_{beam} |
|----------|------|------|-------|------|-------|----------|---------|----------|---------------|-------------------|-----------------|
| 4 (ref) | mC | 1 | 101.4 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.39 | 0.45 (0.2) |
| 5 (ref) | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 (0.2) |
| 6 (ref) | hR | 2 | 58.6 | 58.6 | 156.4 | 90.0 | 90.0 | 120.0 | 0.21 | 0.54 | 0.46 (0.3) |
| 7 (ref) | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 (0.2) |

Below the solutions table, there is a 'Start Beam-centre search' button and a detailed table of beam parameters.

| Beam x | Beam y | Beam x ref | Beam y ref | a | b | c | α | β | γ | $\sigma(x,y)$ | $\sigma(\varphi)$ | δ_{beam} |
|--------|--------|------------|------------|------|------|------|----------|---------|----------|---------------|-------------------|-----------------|
| 118.5 | 119.1 | 119.57 | 120.09 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 119.9 | 0.22 | 0.34 | 1.40 |
| 118.5 | 119.6 | 119.57 | 120.09 | 58.5 | 58.6 | 62.1 | 90.1 | 118.0 | 120.0 | 0.19 | 0.54 | 1.10 |
| 118.5 | 120.1 | 119.57 | 120.09 | 58.5 | 58.6 | 62.1 | 90.1 | 118.0 | 120.0 | 0.19 | 0.54 | 1.00 |
| 118.5 | 120.6 | 119.57 | 120.09 | 58.5 | 58.6 | 62.1 | 90.1 | 118.0 | 120.0 | 0.19 | 0.54 | 1.10 |
| 118.5 | 121.1 | 119.56 | 120.09 | 58.6 | 58.5 | 62.1 | 118.0 | 90.1 | 120.0 | 0.21 | 0.54 | 1.40 |
| 119.0 | 119.1 | 119.57 | 120.08 | 58.5 | 58.5 | 62.1 | 118.0 | 90.1 | 119.9 | 0.21 | 0.35 | 1.10 |
| 119.0 | 119.6 | 119.57 | 120.09 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 120.0 | 0.20 | 0.35 | 0.72 |
| 119.0 | 120.1 | 119.57 | 120.09 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 120.0 | 0.20 | 0.35 | 0.52 |
| 119.0 | 120.6 | 119.57 | 120.08 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 120.0 | 0.20 | 0.35 | 0.72 |
| 119.0 | 121.1 | 119.57 | 120.08 | 58.5 | 58.5 | 62.1 | 118.0 | 90.1 | 119.9 | 0.21 | 0.35 | 1.10 |
| 119.5 | 119.1 | 119.57 | 120.08 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 119.9 | 0.21 | 0.35 | 0.99 |
| 119.5 | 119.6 | 119.57 | 120.09 | 58.5 | 58.5 | 62.1 | 90.1 | 118.0 | 120.0 | 0.20 | 0.35 | 0.50 |

The 'Spacegroup' is set to 'h3' and 'Mosaicity' is 0.00. The 'Estimate' button is visible. The bottom right corner shows 'No Warnings' with a green checkmark.

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 | $\sigma(xy)$ | $\sigma(z)$ | 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) | aP | 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) | aP | 1 | 81.3 | 82.3 | 81.8 | 90.0 | 90.0 | 90.0 | 0.18 | 0.44 | 0.00 (0.0) |
| 5 (ref) | aP | 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) | wC | 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) | wC | 3 | 116.2 | 115.8 | 81.4 | 90.0 | 90.2 | 90.0 | 0.18 | 0.48 | 0.00 (0.0) |
| 11 (ref) | wC | 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) | wC | 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) | wC | 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 $\sigma(xy)$ value of 0.34mm while for the (correct) orthorhombic 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 ($\sigma(xy)$), 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

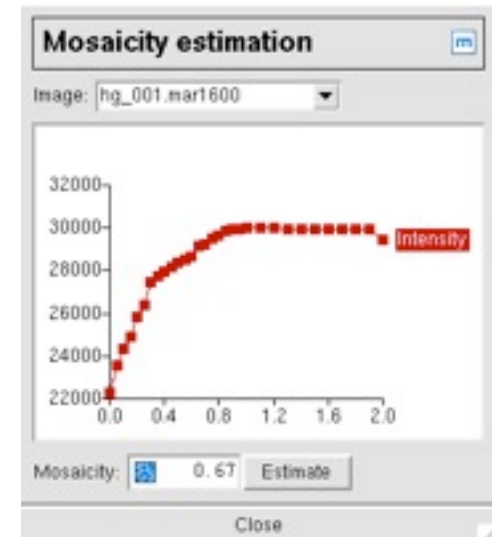
| | | | | | | | | | | | | | |
|----|-------|----|----|-------|------|-------|------|-------|-------|------|------|------|--------|
| 2 | {ref} | aP | 0 | 58.5 | 58.6 | 62.1 | 61.9 | 62.0 | 60.0 | 0.20 | 0.35 | 0.44 | (0.1) |
| 3 | {ref} | mC | 1 | 101.3 | 58.6 | 62.2 | 90.0 | 123.0 | 90.0 | 0.21 | 0.42 | 0.44 | (0.1) |
| 4 | {ref} | mC | 1 | 101.4 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.39 | 0.45 | (0.2) |
| 5 | {ref} | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 6 | {ref} | hR | 2 | 58.6 | 58.6 | 156.4 | 90.0 | 90.0 | 120.0 | 0.21 | 0.54 | 0.46 | (0.3) |
| 7 | {ref} | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 8 | {reg} | hR | 60 | 60.4 | 60.4 | 144.8 | 90.0 | 90.0 | 120.0 | - | - | - | - |
| 9 | {reg} | mC | 60 | 103.5 | 62.2 | 58.5 | 90.0 | 124.4 | 90.0 | - | - | - | - |
| 10 | {reg} | mC | 60 | 85.3 | 85.4 | 58.5 | 90.0 | 133.2 | 90.0 | - | - | - | - |
| 11 | {reg} | oI | 60 | 58.6 | 62.2 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 12 | {reg} | mC | 61 | 101.4 | 58.6 | 62.1 | 90.0 | 122.9 | 90.0 | - | - | - | - |
| 13 | {reg} | tI | 61 | 60.4 | 60.4 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 14 | {reg} | oI | 61 | 58.5 | 62.2 | 85.4 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 15 | {reg} | mC | 61 | 101.5 | 58.5 | 62.2 | 90.0 | 122.9 | 90.0 | - | - | - | - |

Spacegroup: h3
Mosaicity: 0.00 Estimate

Start beam-centre search [+]

No Warnings

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

History

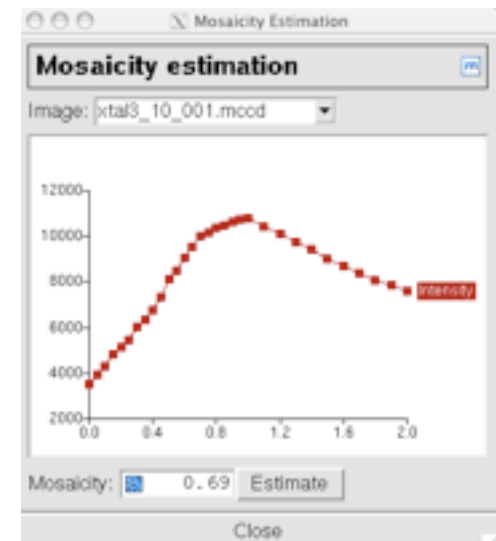
| | | | | | | | | | | | | |
|----------|----|----|-------|------|-------|------|-------|-------|------|------|------|--------|
| 2 (ref) | aP | 0 | 58.5 | 58.6 | 62.1 | 61.9 | 62.0 | 60.0 | 0.20 | 0.35 | 0.44 | (0.1) |
| 3 (ref) | mC | 1 | 101.3 | 58.6 | 62.2 | 90.0 | 123.0 | 90.0 | 0.21 | 0.42 | 0.44 | (0.1) |
| 4 (ref) | mC | 1 | 101.4 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.39 | 0.45 | (0.2) |
| 5 (ref) | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 6 (ref) | hR | 2 | 58.6 | 58.6 | 156.4 | 90.0 | 90.0 | 120.0 | 0.21 | 0.54 | 0.46 | (0.3) |
| 7 (ref) | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 8 (reg) | hR | 60 | 60.4 | 60.4 | 144.8 | 90.0 | 90.0 | 120.0 | - | - | - | - |
| 9 (reg) | mC | 60 | 103.5 | 62.2 | 58.5 | 90.0 | 124.4 | 90.0 | - | - | - | - |
| 10 (reg) | mC | 60 | 85.3 | 85.4 | 58.5 | 90.0 | 133.2 | 90.0 | - | - | - | - |
| 11 (reg) | oI | 60 | 58.6 | 62.2 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 12 (reg) | mC | 61 | 101.4 | 58.6 | 62.1 | 90.0 | 122.9 | 90.0 | - | - | - | - |
| 13 (reg) | tI | 61 | 60.4 | 60.4 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 14 (reg) | oI | 61 | 58.5 | 62.2 | 85.4 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 15 (reg) | mC | 61 | 101.5 | 58.5 | 62.2 | 90.0 | 122.9 | 90.0 | - | - | - | - |

Spacegroup: h3
Mosaicity: 0.00 Estimate

Start beam-centre search[+]

No Warnings

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

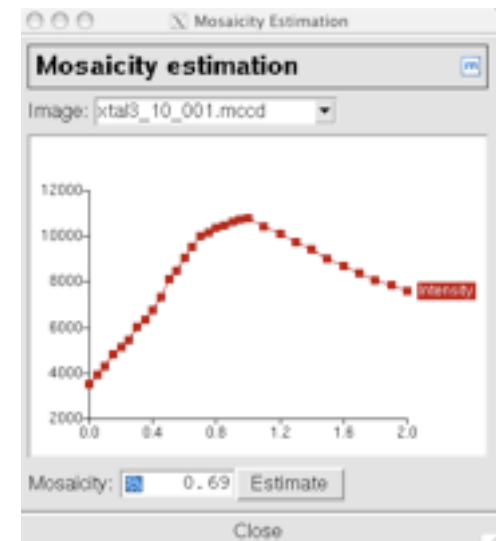
| | | | | | | | | | | | | | |
|----|-------|----|----|-------|------|-------|------|-------|-------|------|------|------|--------|
| 2 | {ref} | aP | 0 | 58.5 | 58.6 | 62.1 | 61.9 | 62.0 | 60.0 | 0.20 | 0.35 | 0.44 | (0.1) |
| 3 | {ref} | mC | 1 | 101.3 | 58.6 | 62.2 | 90.0 | 123.0 | 90.0 | 0.21 | 0.42 | 0.44 | (0.1) |
| 4 | {ref} | mC | 1 | 101.4 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.39 | 0.45 | (0.2) |
| 5 | {ref} | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 6 | {ref} | hR | 2 | 58.6 | 58.6 | 156.4 | 90.0 | 90.0 | 120.0 | 0.21 | 0.54 | 0.46 | (0.3) |
| 7 | {ref} | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 8 | {reg} | hR | 60 | 60.4 | 60.4 | 144.8 | 90.0 | 90.0 | 120.0 | - | - | - | - |
| 9 | {reg} | mC | 60 | 103.5 | 62.2 | 58.5 | 90.0 | 124.4 | 90.0 | - | - | - | - |
| 10 | {reg} | mC | 60 | 85.3 | 85.4 | 58.5 | 90.0 | 133.2 | 90.0 | - | - | - | - |
| 11 | {reg} | oI | 60 | 58.6 | 62.2 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 12 | {reg} | mC | 61 | 101.4 | 58.6 | 62.1 | 90.0 | 122.9 | 90.0 | - | - | - | - |
| 13 | {reg} | tI | 61 | 60.4 | 60.4 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 14 | {reg} | oI | 61 | 58.5 | 62.2 | 85.4 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 15 | {reg} | mC | 61 | 101.5 | 58.5 | 62.2 | 90.0 | 122.9 | 90.0 | - | - | - | - |

Spacegroup: h3
Mosaicity: 0.00 Estimate

Start beam-centre search[+]

No Warnings

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



Indexing – Mosaicity estimation

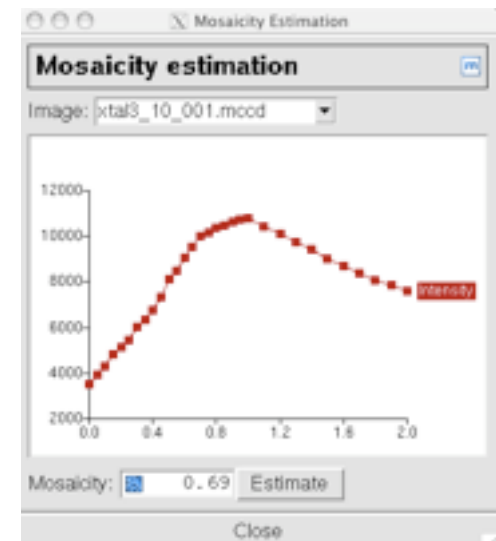
| | | | | | | | | | | | | | |
|----|-------|----|----|-------|------|-------|------|-------|-------|------|------|------|--------|
| 2 | {ref} | aP | 0 | 58.5 | 58.6 | 62.1 | 61.9 | 62.0 | 60.0 | 0.20 | 0.35 | 0.44 | (0.1) |
| 3 | {ref} | mC | 1 | 101.3 | 58.6 | 62.2 | 90.0 | 123.0 | 90.0 | 0.21 | 0.42 | 0.44 | (0.1) |
| 4 | {ref} | mC | 1 | 101.4 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.39 | 0.45 | (0.2) |
| 5 | {ref} | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 6 | {ref} | hR | 2 | 58.6 | 58.6 | 156.4 | 90.0 | 90.0 | 120.0 | 0.21 | 0.54 | 0.46 | (0.3) |
| 7 | {ref} | mC | 2 | 101.5 | 58.5 | 62.1 | 90.0 | 122.9 | 90.0 | 0.20 | 0.36 | 0.45 | (0.2) |
| 8 | {reg} | hR | 60 | 60.4 | 60.4 | 144.8 | 90.0 | 90.0 | 120.0 | - | - | - | - |
| 9 | {reg} | mC | 60 | 103.5 | 62.2 | 58.5 | 90.0 | 124.4 | 90.0 | - | - | - | - |
| 10 | {reg} | mC | 60 | 85.3 | 85.4 | 58.5 | 90.0 | 133.2 | 90.0 | - | - | - | - |
| 11 | {reg} | oI | 60 | 58.6 | 62.2 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 12 | {reg} | mC | 61 | 101.4 | 58.6 | 62.1 | 90.0 | 122.9 | 90.0 | - | - | - | - |
| 13 | {reg} | tI | 61 | 60.4 | 60.4 | 85.3 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 14 | {reg} | oI | 61 | 58.5 | 62.2 | 85.4 | 90.0 | 90.0 | 90.0 | - | - | - | - |
| 15 | {reg} | mC | 61 | 101.5 | 58.5 | 62.2 | 90.0 | 122.9 | 90.0 | - | - | - | - |

Spacegroup: h3
Mosaicity: 0.00 Estimate

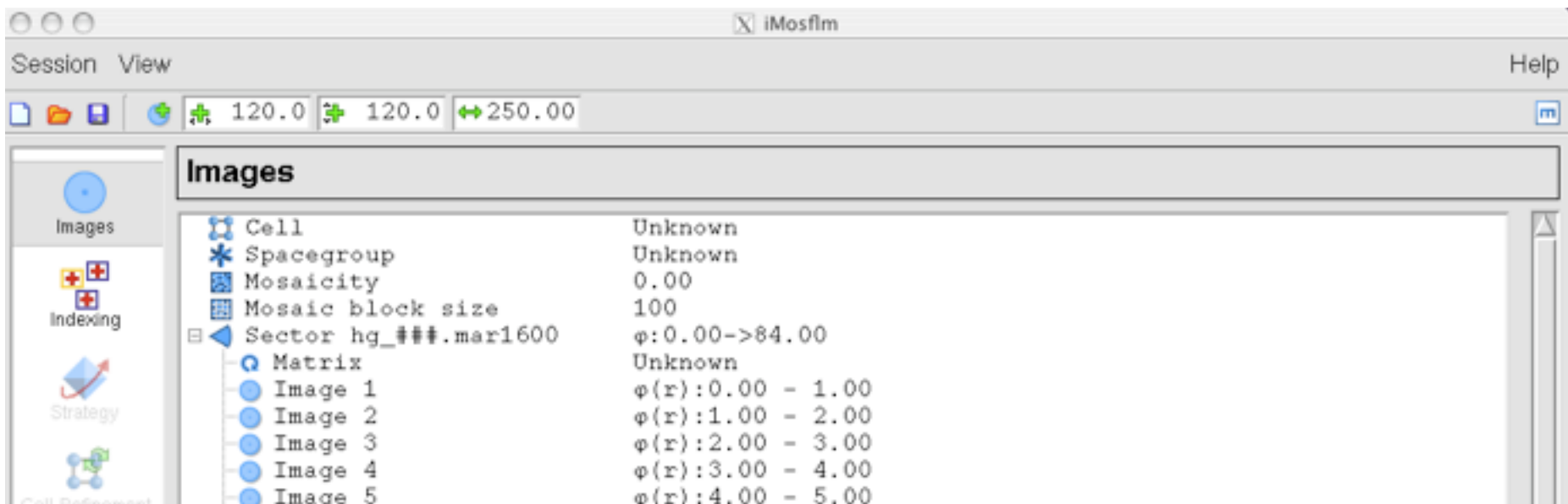
Start beam-centre search [+]

No Warnings

Warning: Due to a bug in the current version, if you go back into the indexing pane after refining the cell or integrating and repeat the mosaicity “Estimate”, imosflm will crash !

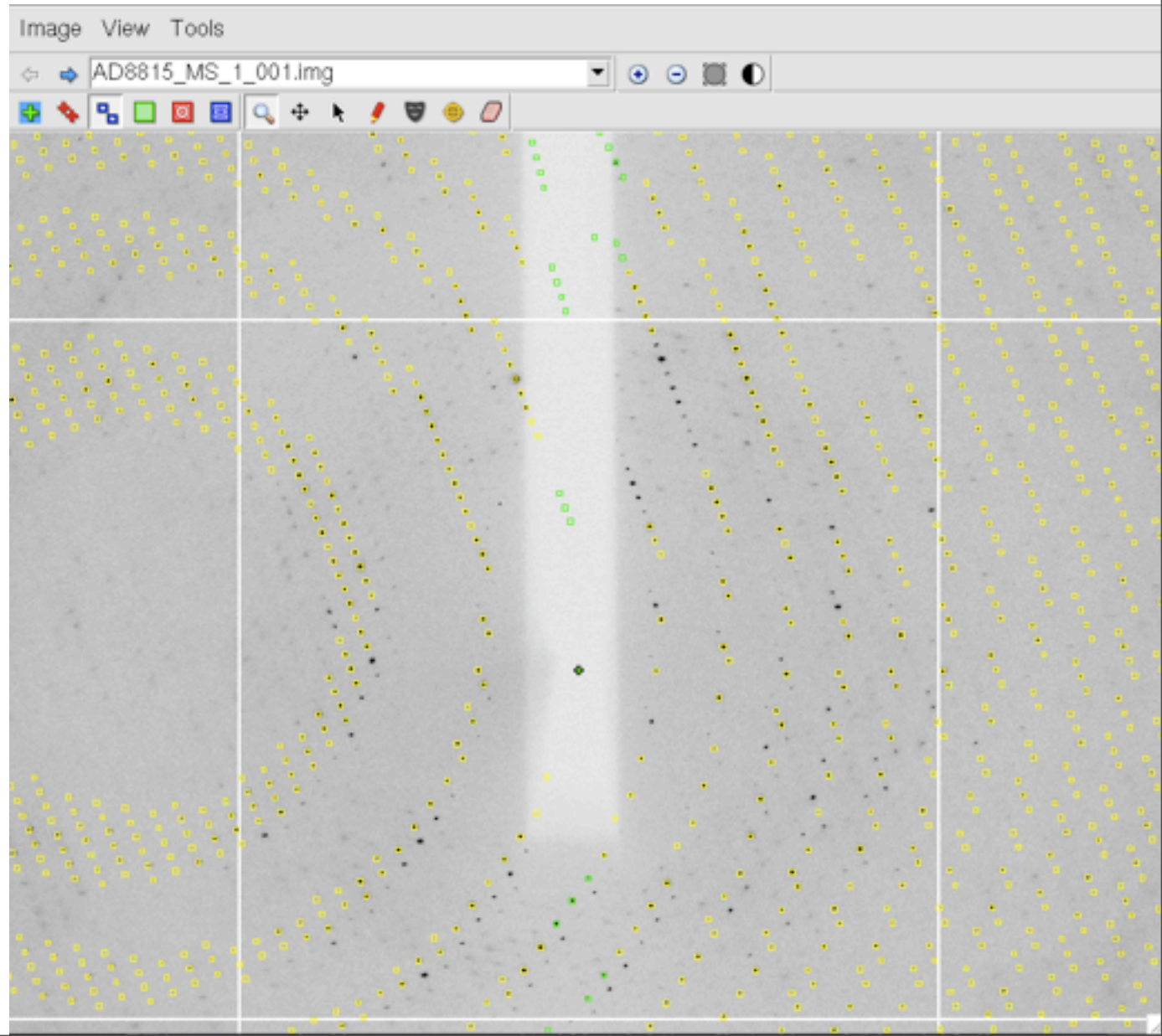


Mosaicity – the effect of the mosaic block size



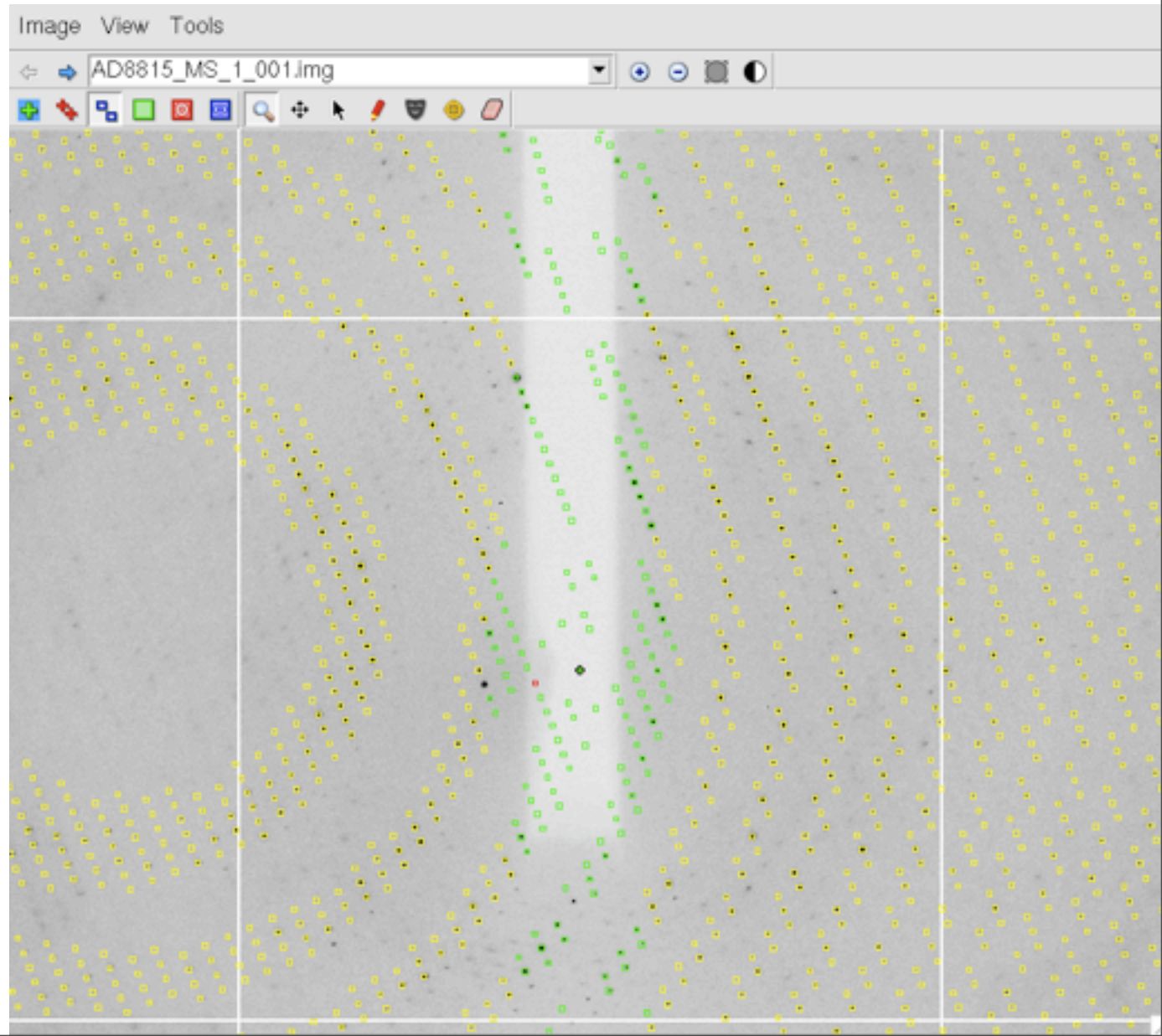
Decreasing the mosaic block size has the effect of increasing the apparent mosaic spread at low resolution (Nave, C. (1998) Acta Cryst D54, 848-853)

Mosaicity – the effect of the mosaic block size



Blocksize 100 μ

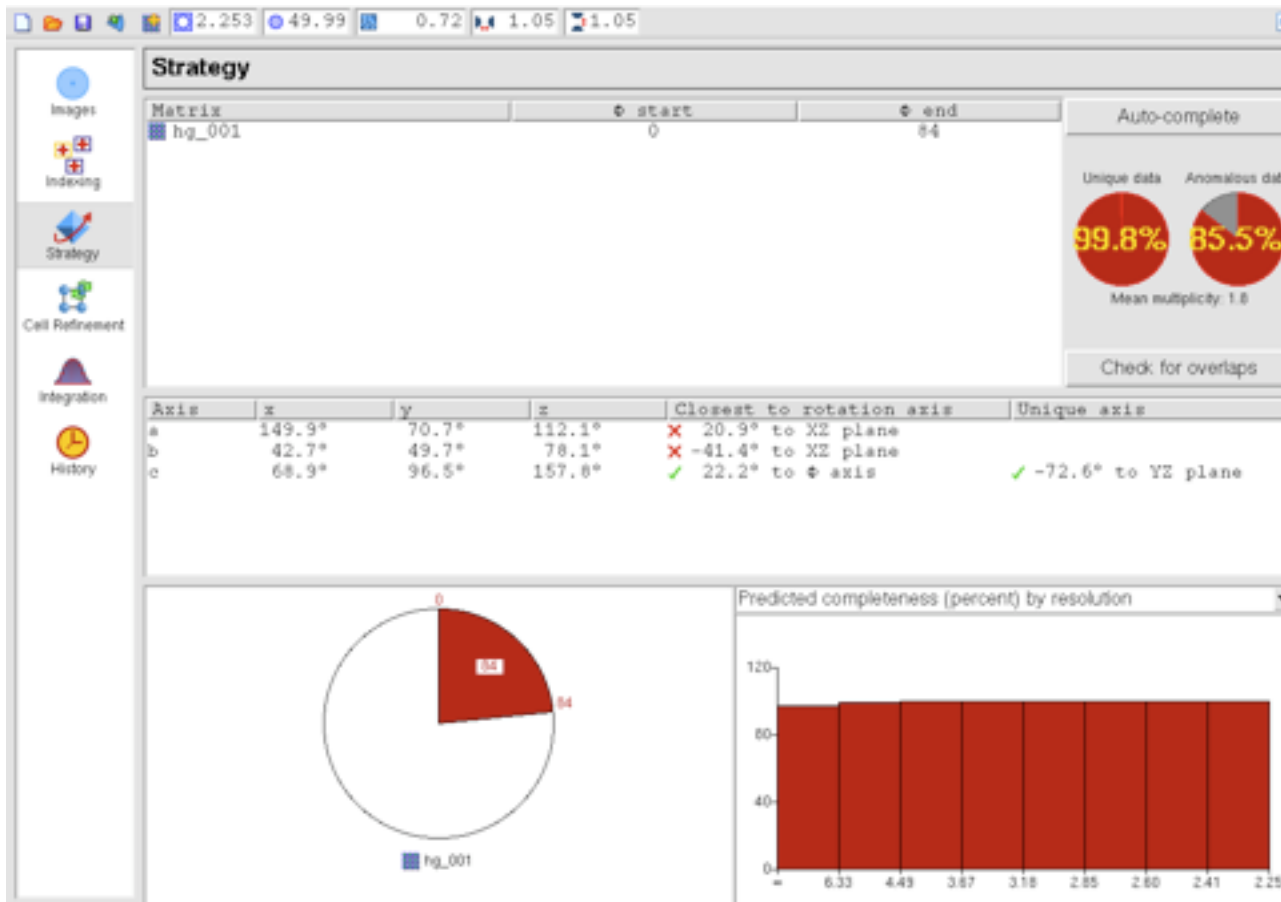
Mosaicity – the effect of the mosaic block size



Blocksize 0.2μ

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

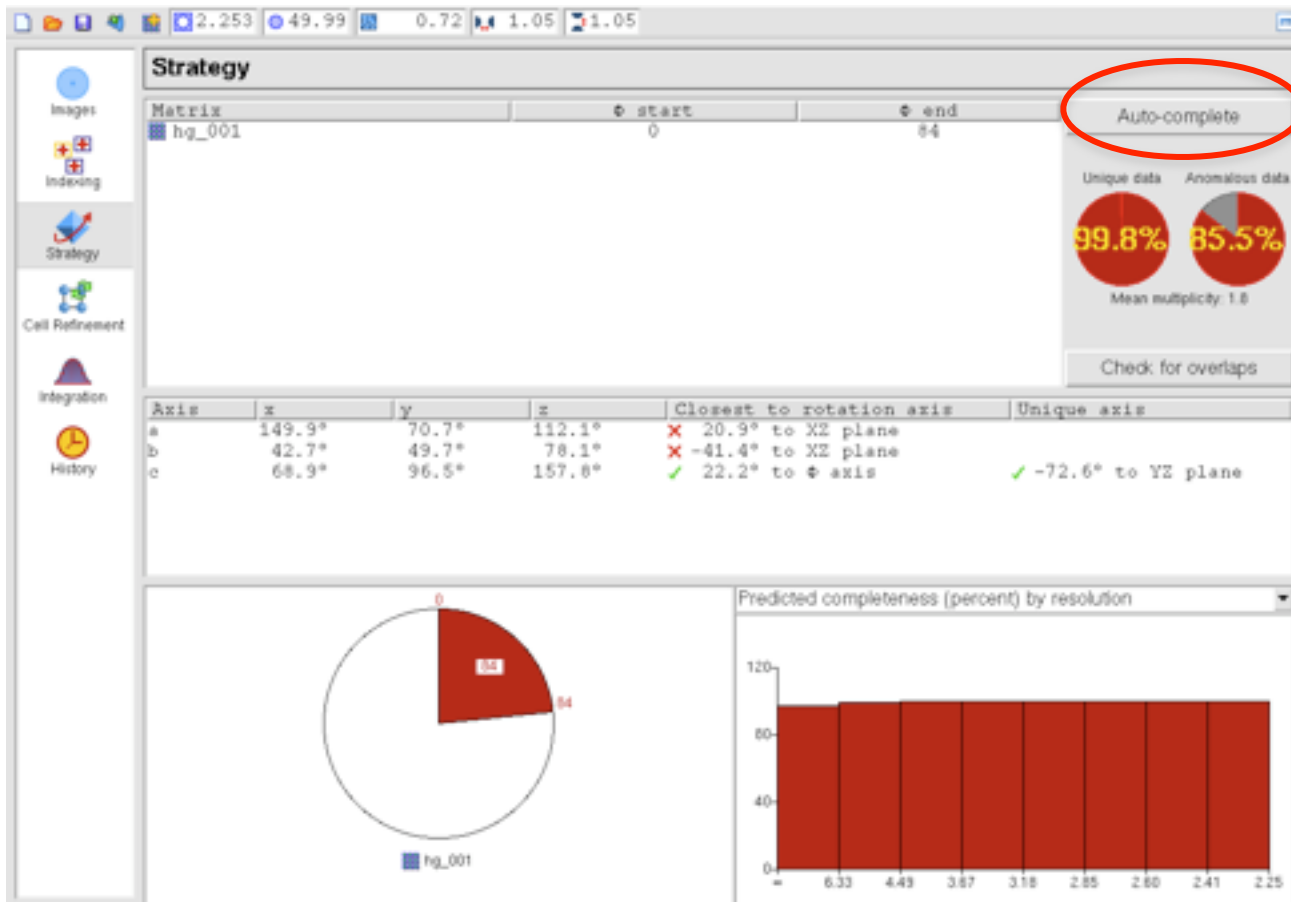
Strategy



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.

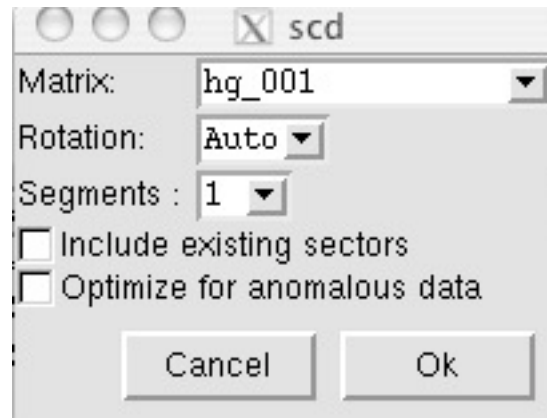
Strategy



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

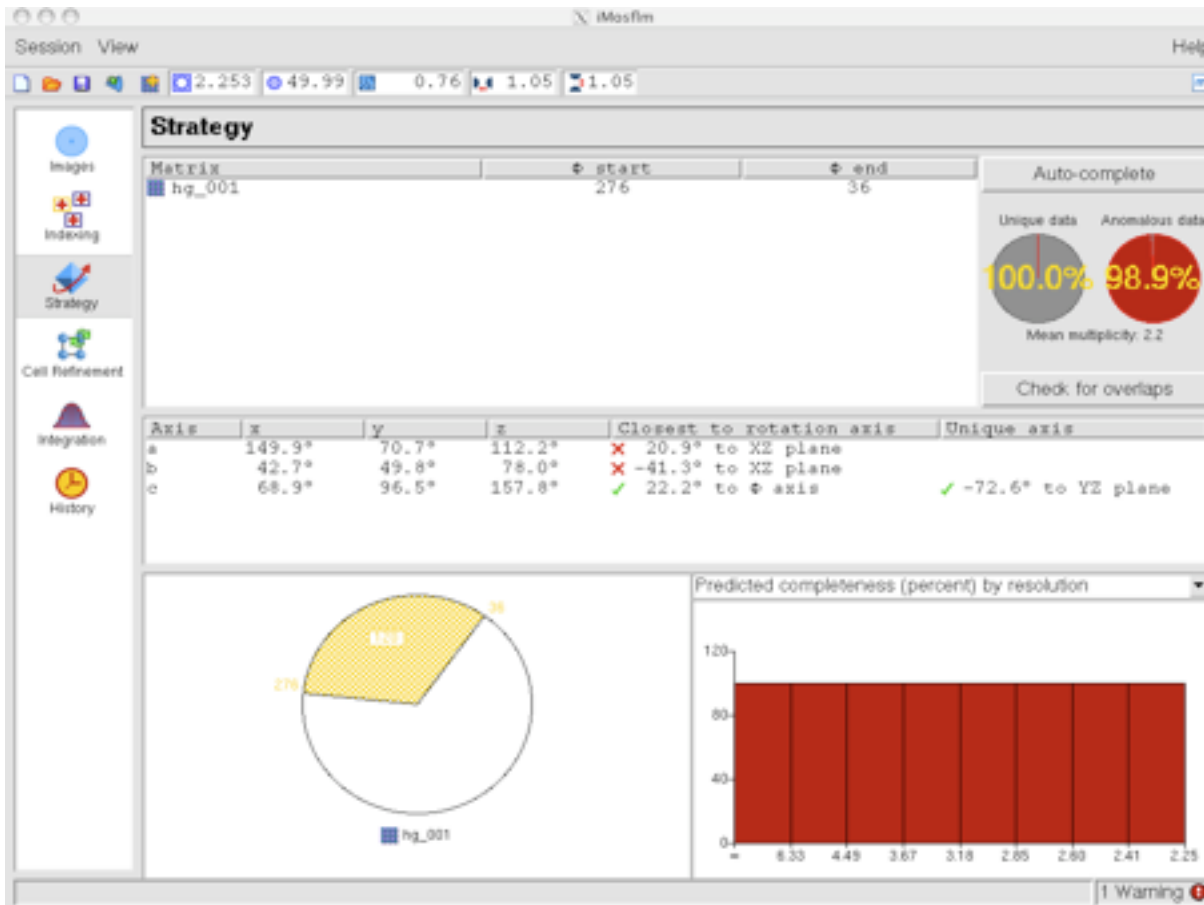


Use defaults for a normal strategy calculation

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.

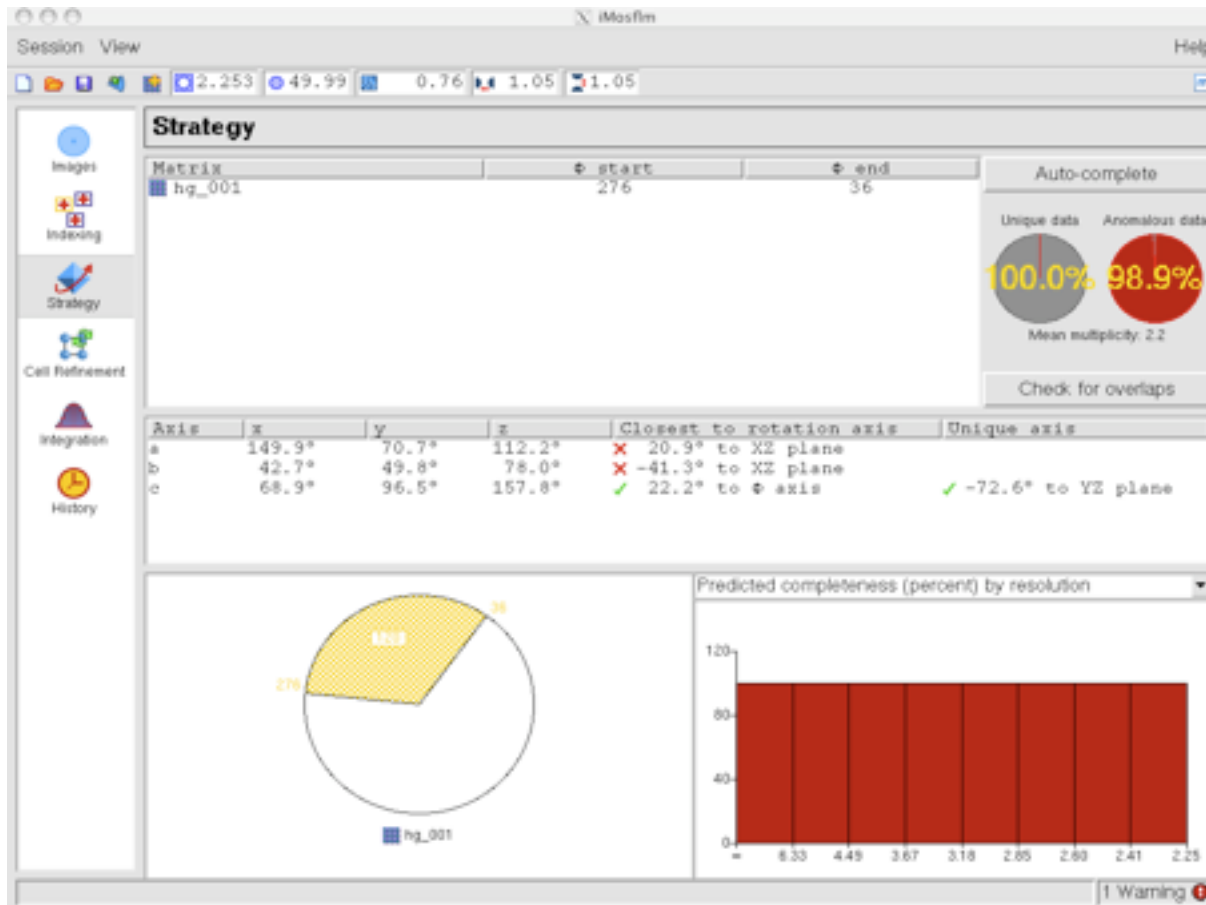
If collecting anomalous data, remember to tick the box !

Strategy



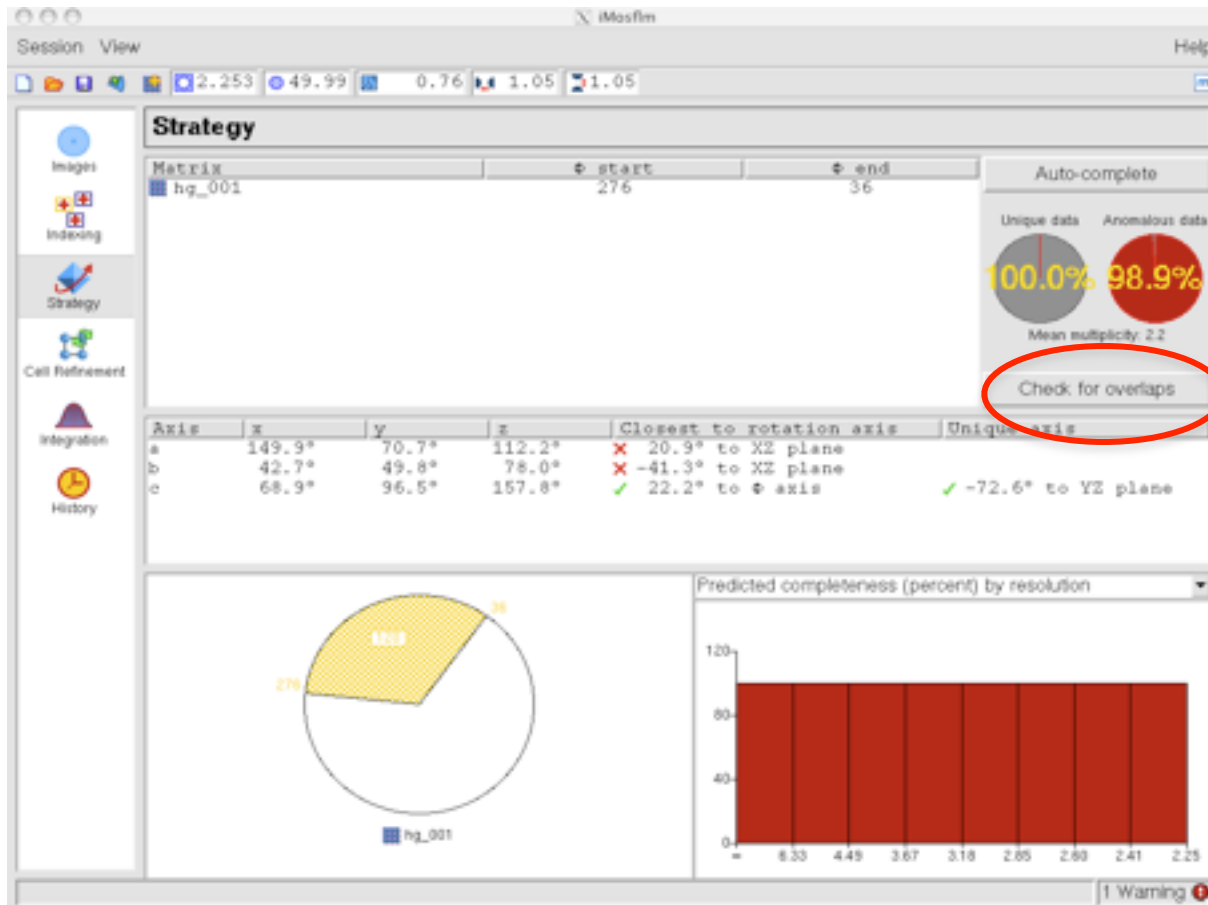
The start end end point of the suggested strategy can be dragged with the mouse to test other rotation ranges.

Strategy



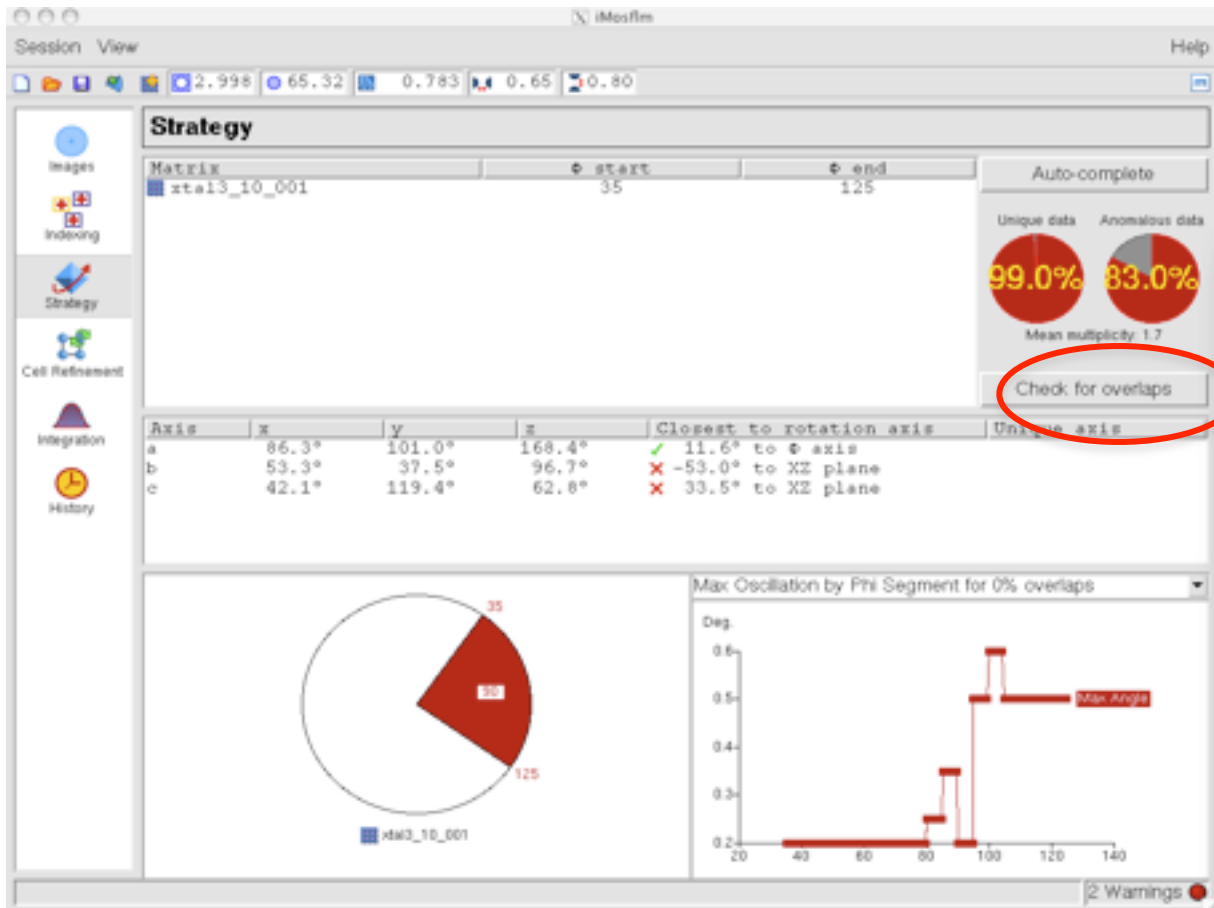
Select “Check for overlaps” to determine maximum oscillation angle without overlaps

Strategy



Select “Check for overlaps” to determine maximum oscillation angle without overlaps

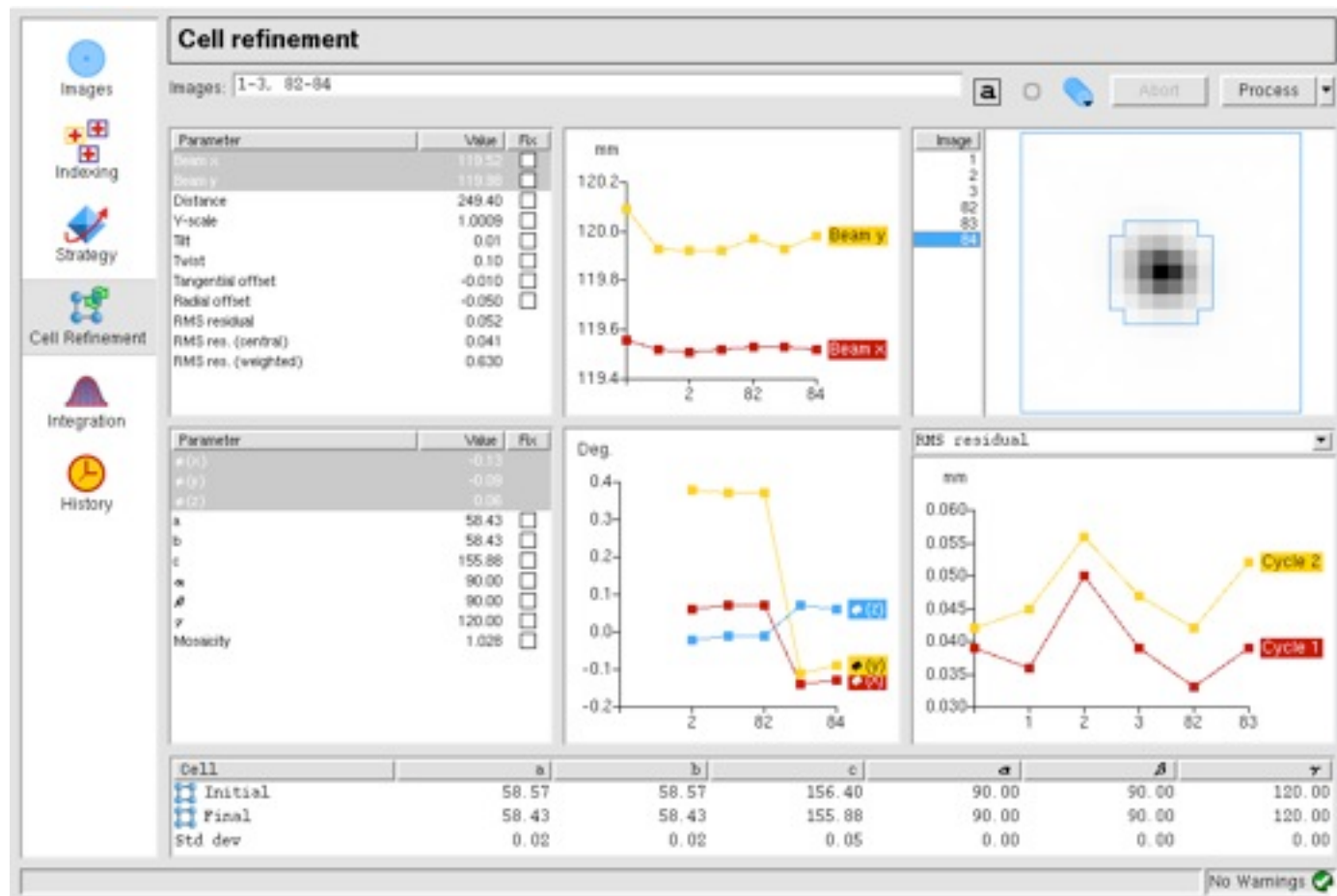
Strategy



Select “Check for overlaps” to determine maximum oscillation angle without overlaps

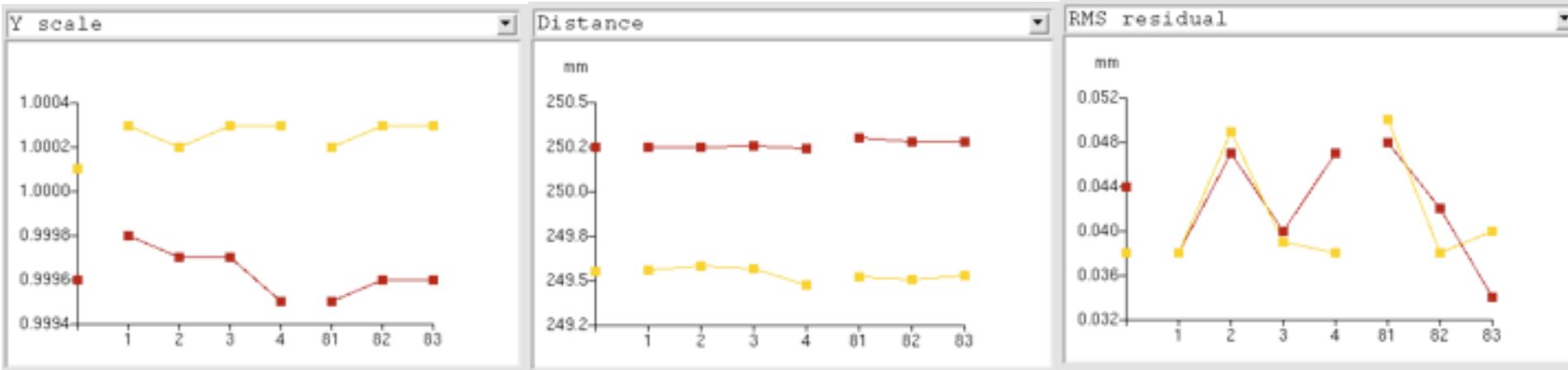
Cell refinement

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 0.1\AA .



Cell refinement

Has it worked ?



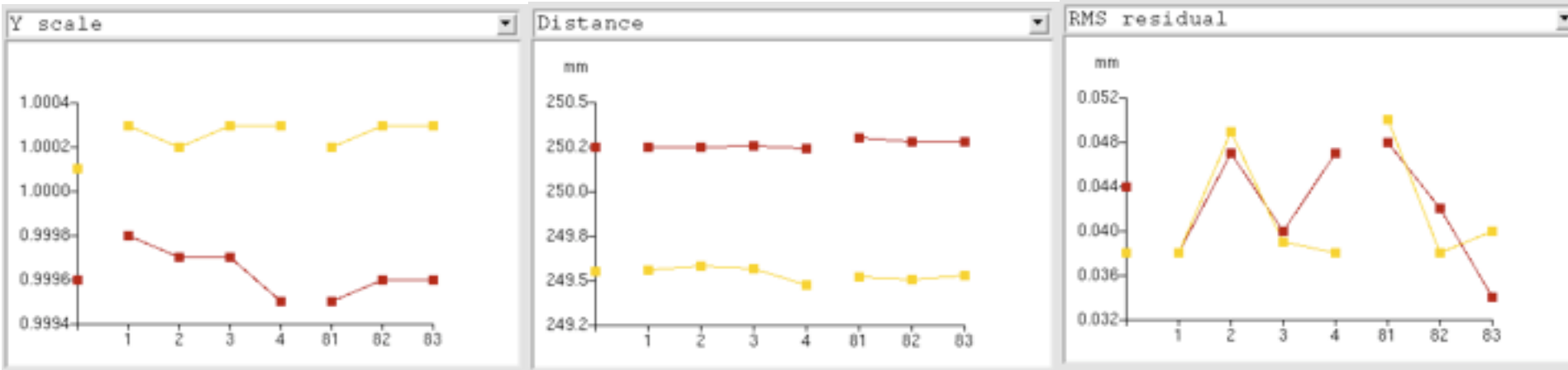
At resolutions less than $\sim 3.3\text{\AA}$, the cell refinement may not work well.

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.

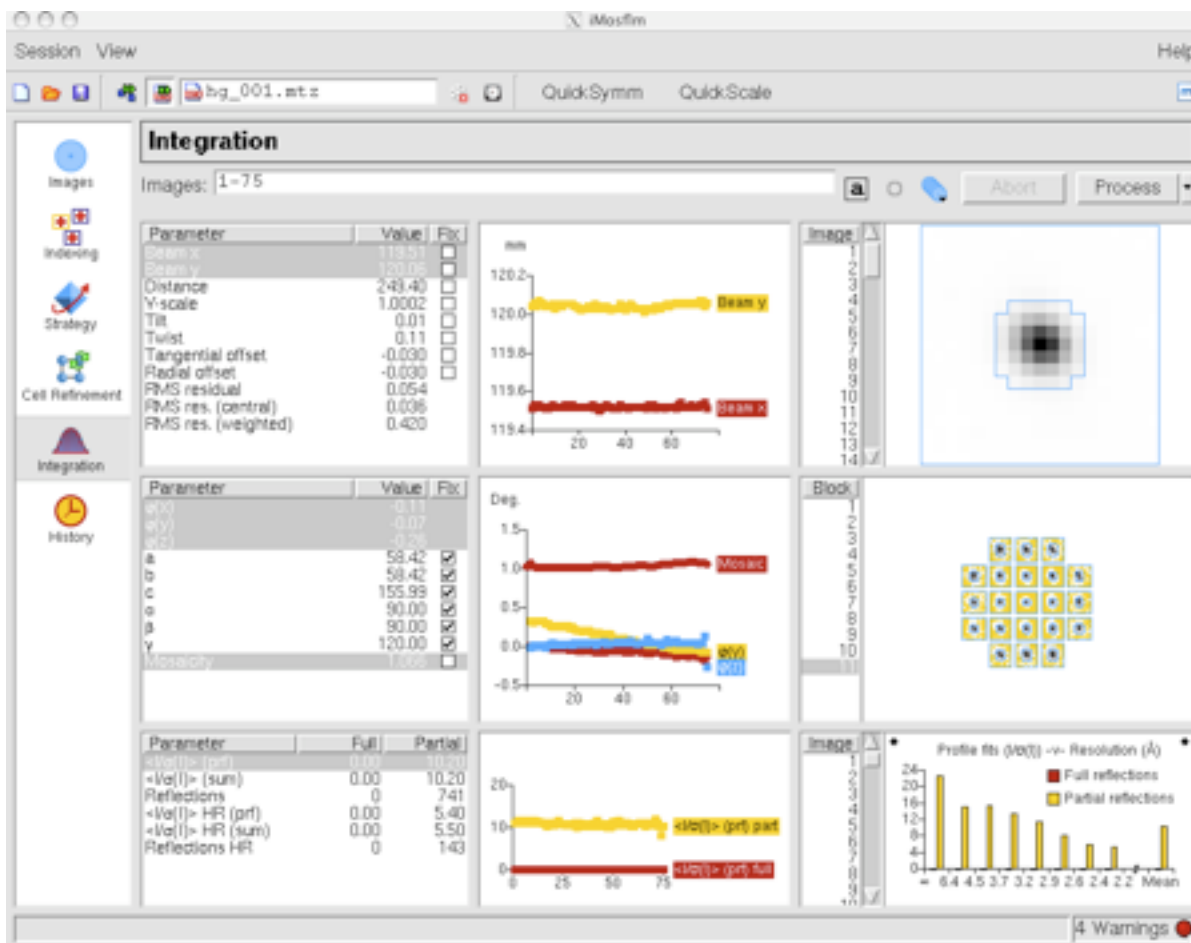
Cell refinement

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

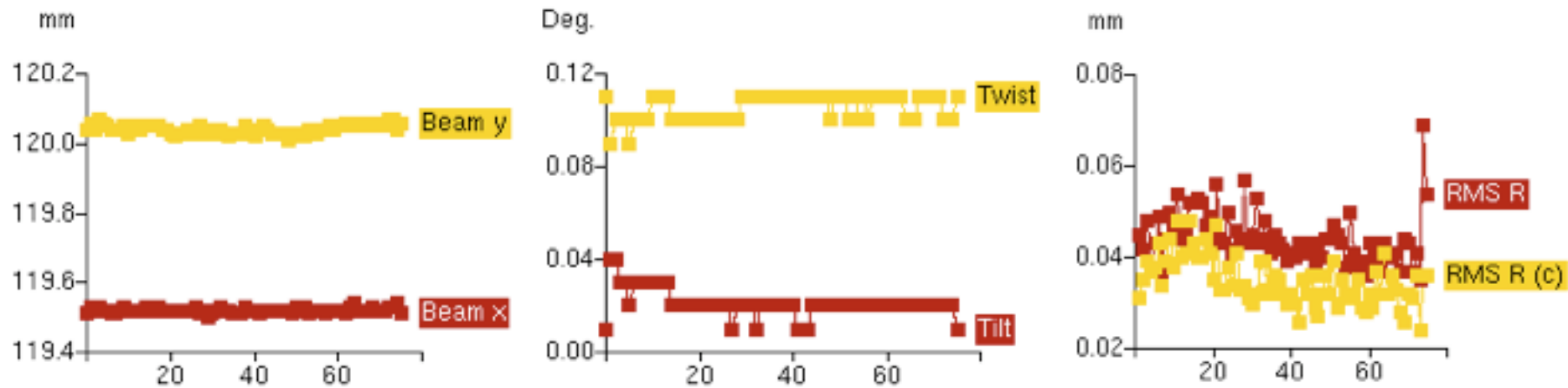


During integration, the refined detector and crystal parameters are plotted as a function of image number.

Also displayed are the average spot profile in the centre of the detector and the standard profiles and an indication of data quality ($I/\sigma(I)$) as a function of resolution)

Integration

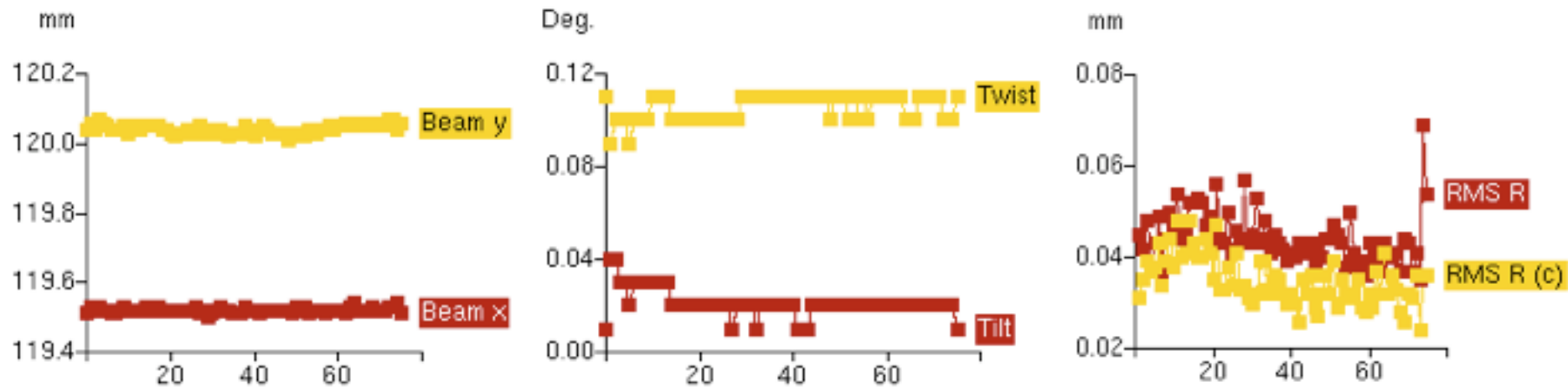
Integration - Stability of refined detector parameters



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



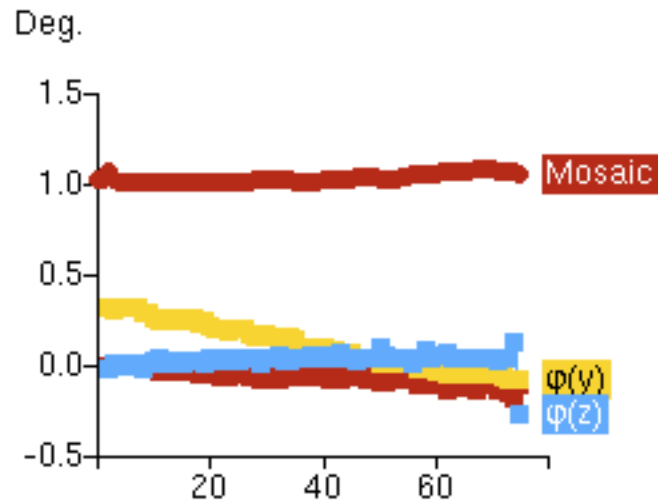
Detector parameters should remain stable during integration

- 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 !

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

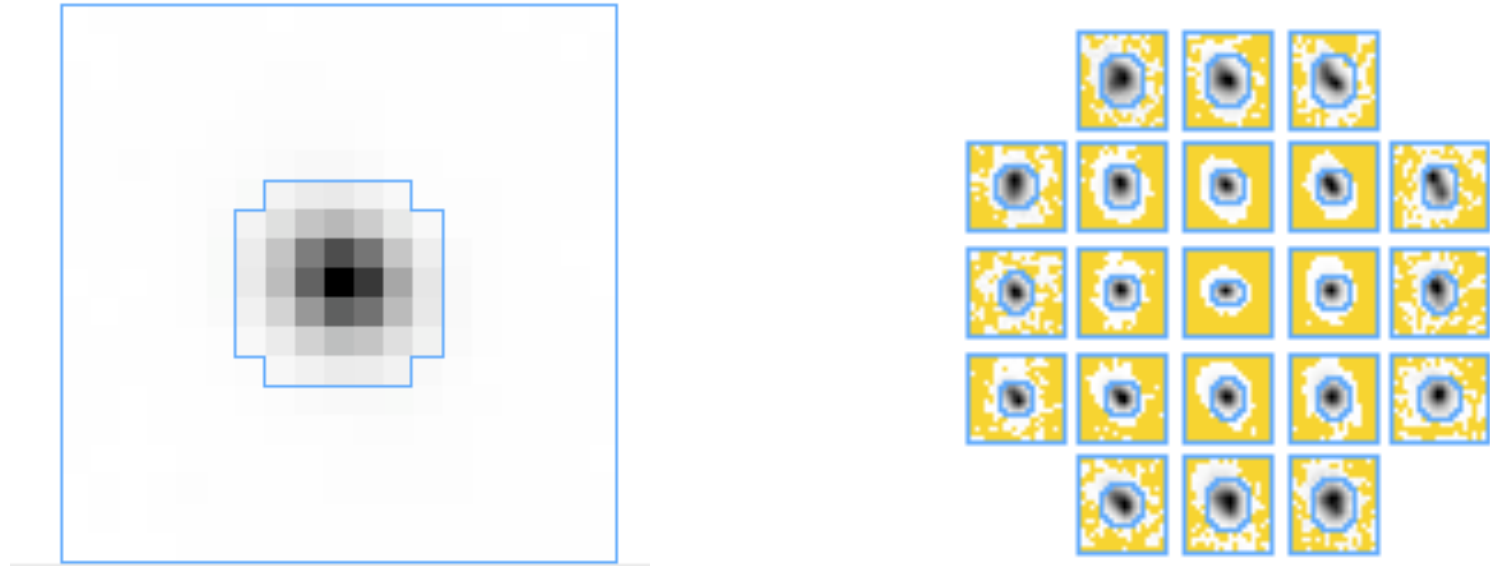
Integration – Stability of refined crystal parameters



Refined missets $\phi(x)$, $\phi(y)$, $\phi(z)$ should vary less than $0.1 \times$ 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.

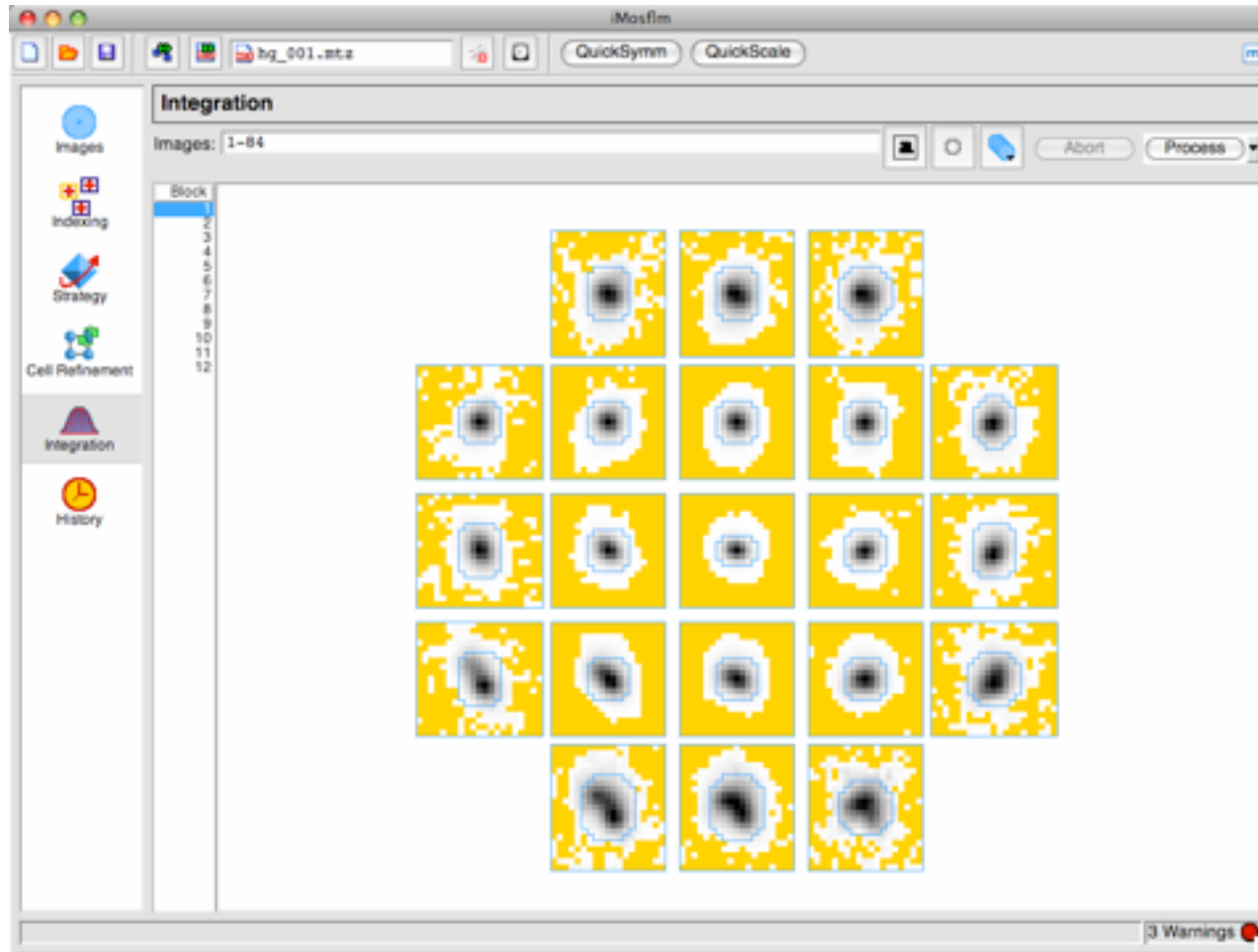
Integration – Spot profiles



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.

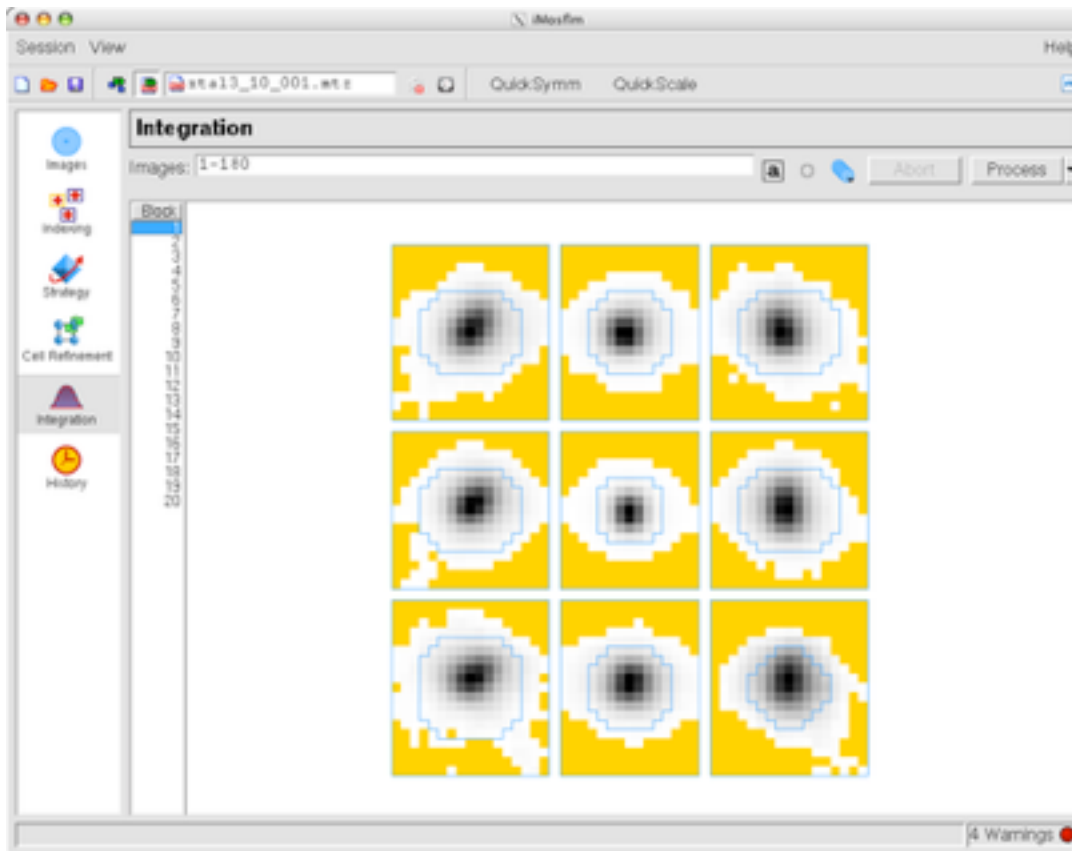
Integration – Spot profiles



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

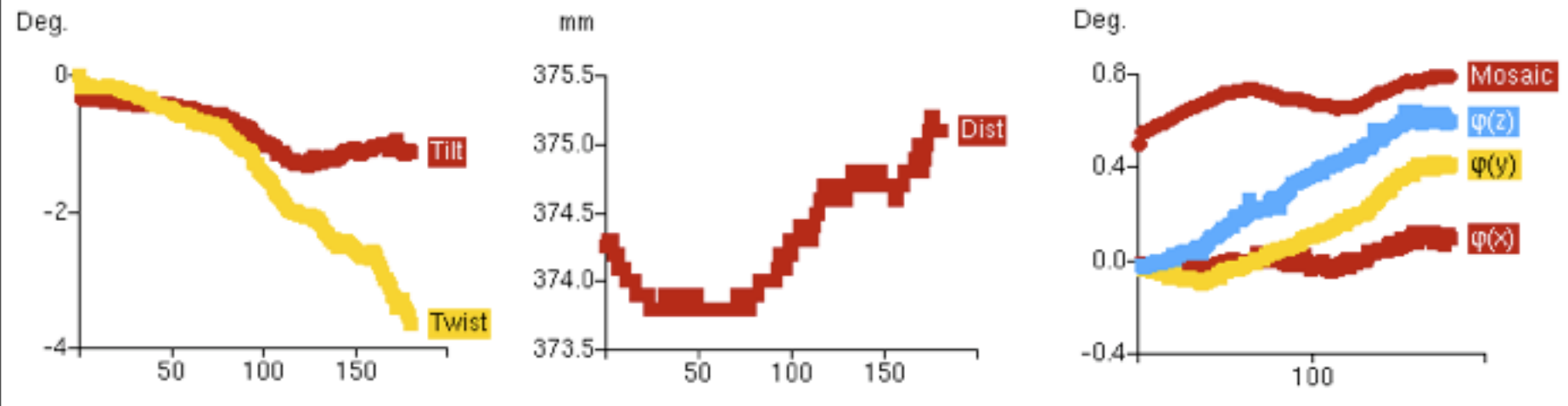
Integration – what should NOT happen

3Å weakly diffracting data, monoclinic cell, *indexed from the first image only*



Integration – what should NOT happen

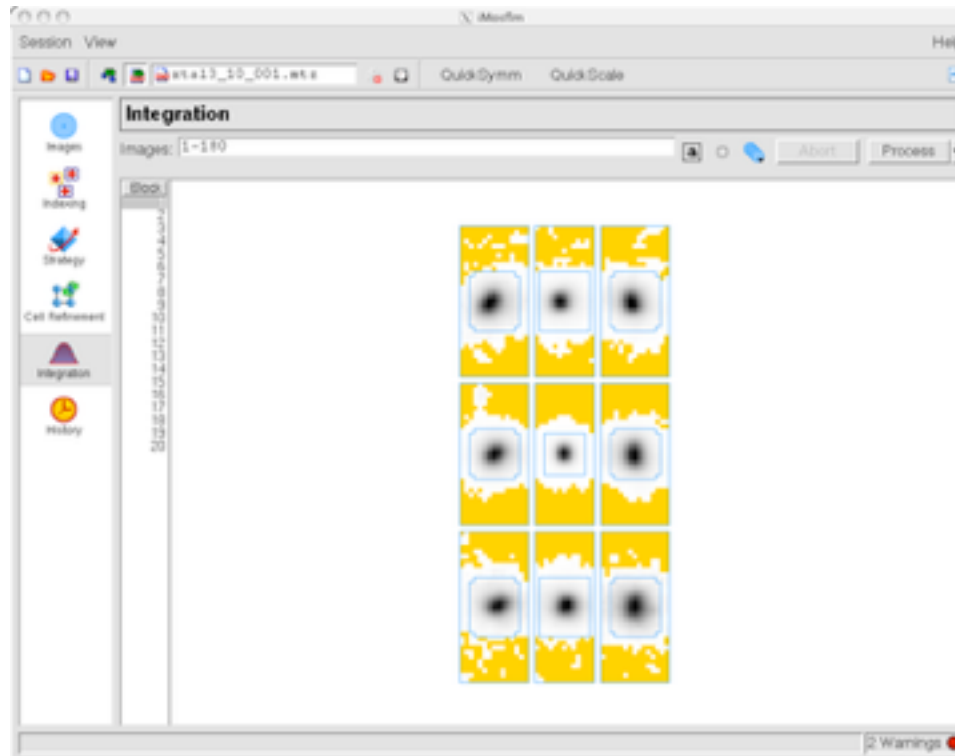
3Å weakly diffracting data, monoclinic cell, *indexed from the first image only*



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

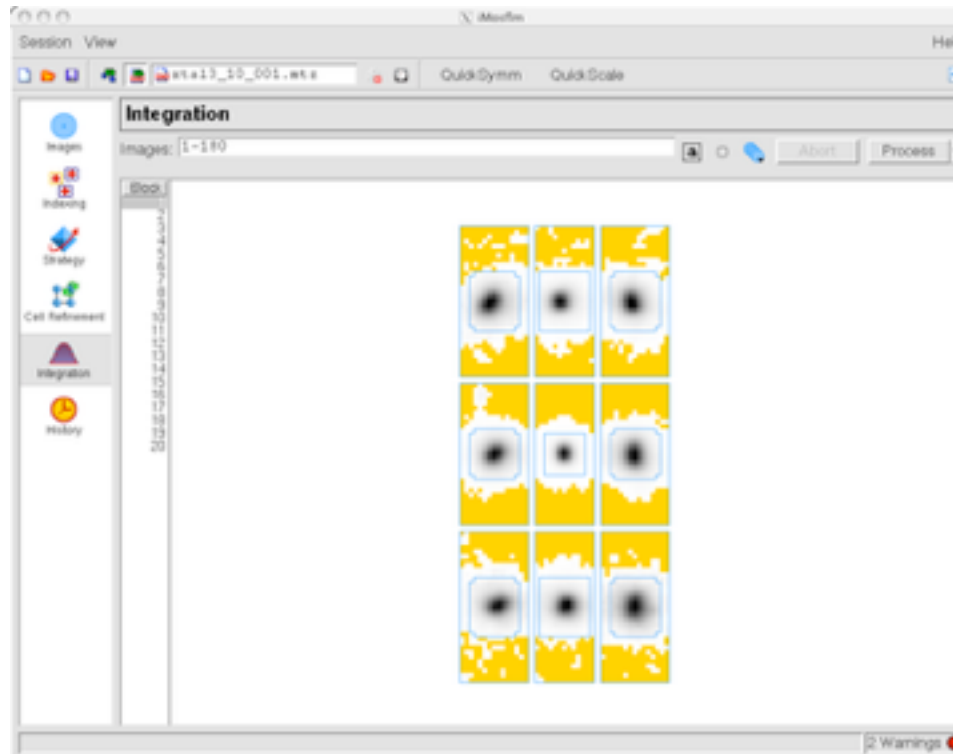
Integration – what should NOT happen

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



Integration – what should NOT happen

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



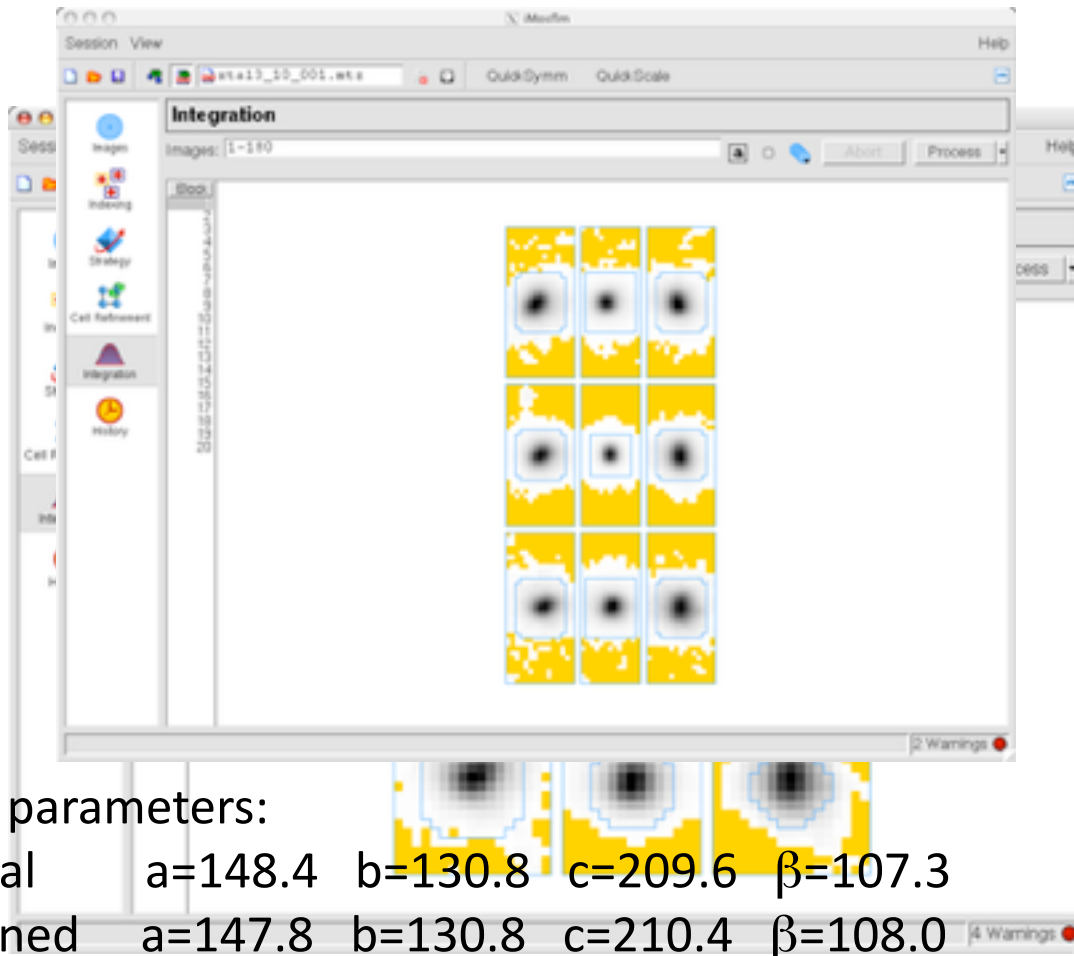
Cell parameters:

Initial a=148.4 b=130.8 c=209.6 β =107.3

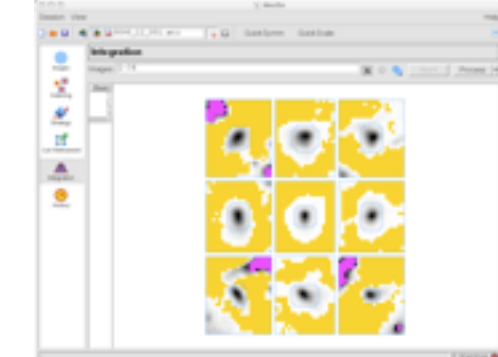
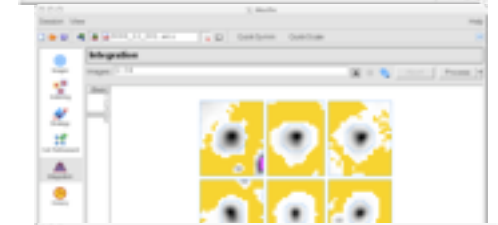
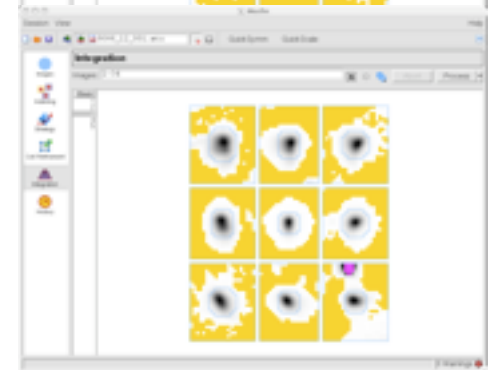
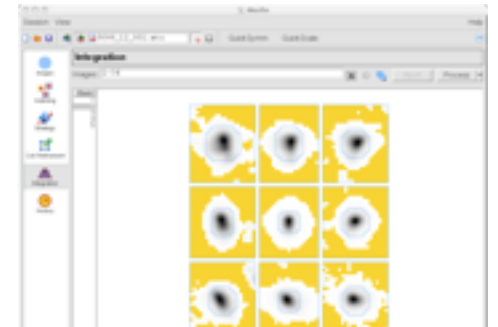
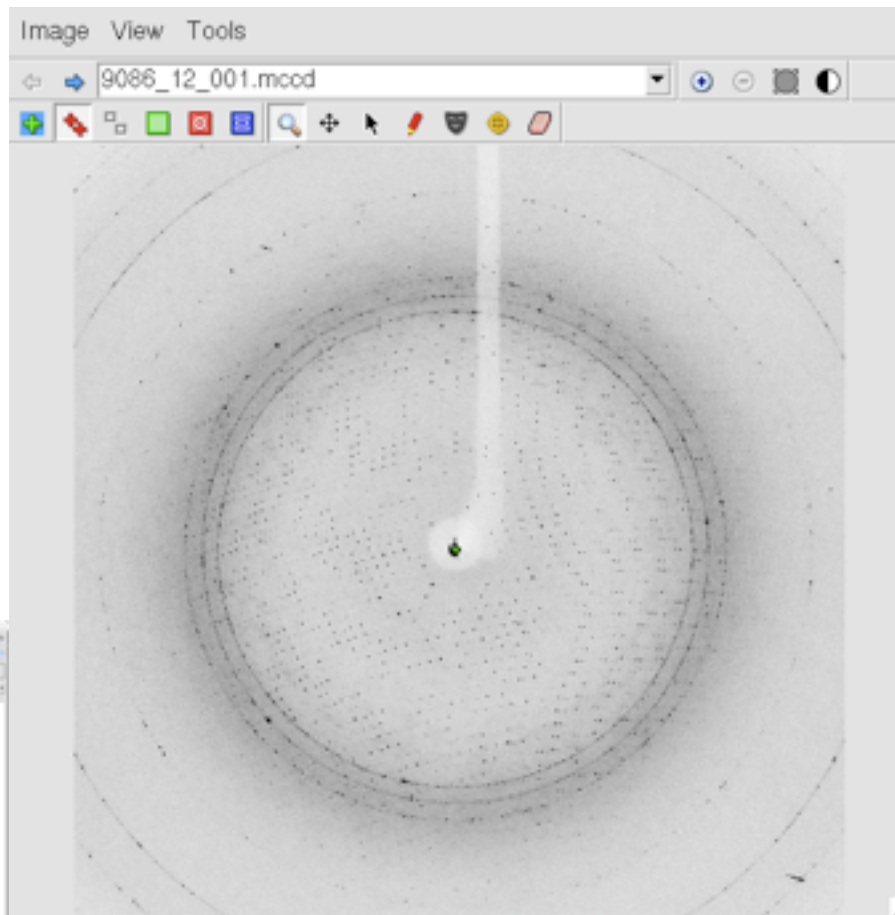
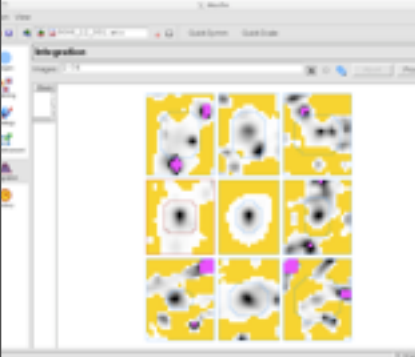
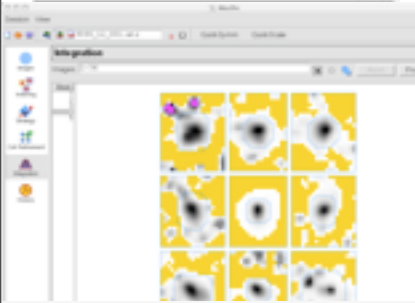
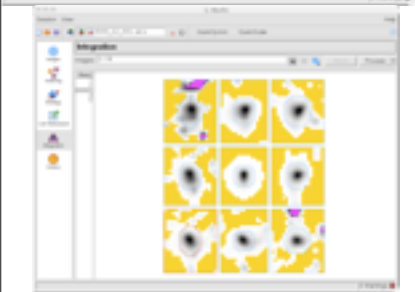
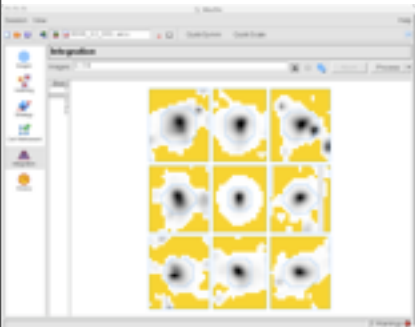
Refined a=147.8 b=130.8 c=210.4 β =108.0

Integration – what should NOT happen

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

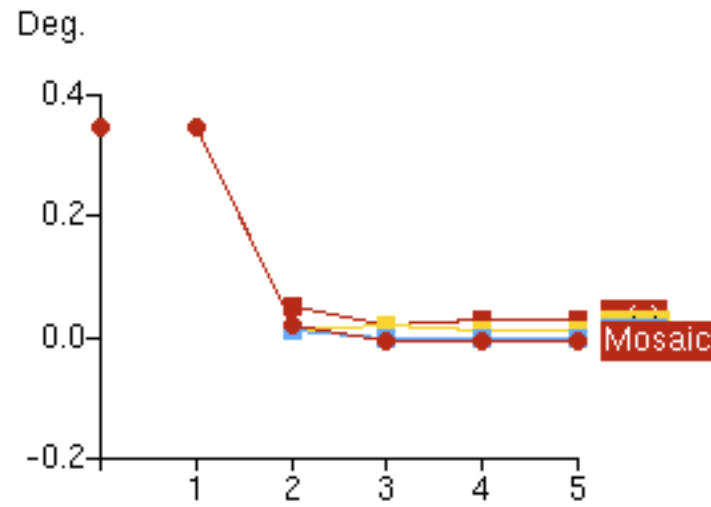


Icy Issues

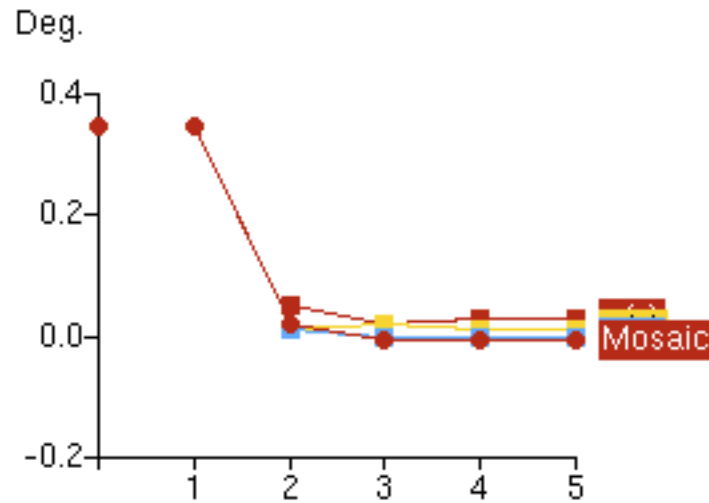


Excluding spots in the resolution shells corresponding to the rings helps improve the profiles, but leads to lower completeness (eg 98->89% at 2.6 Å)

More on what should not happen ...

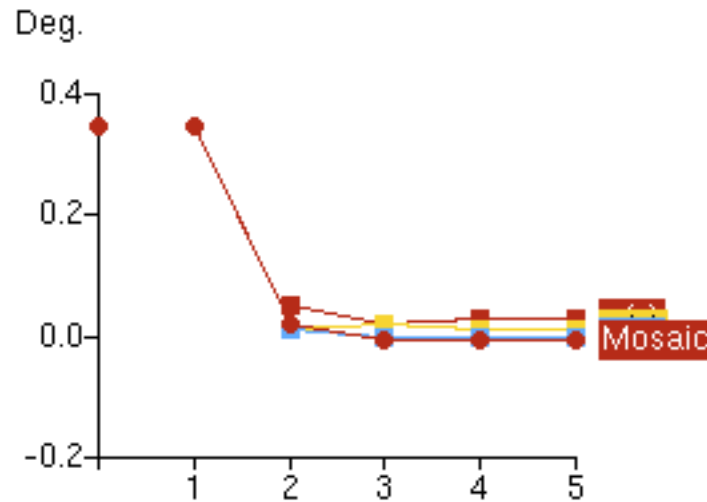


More on what should not happen ...



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

More on what should not happen



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

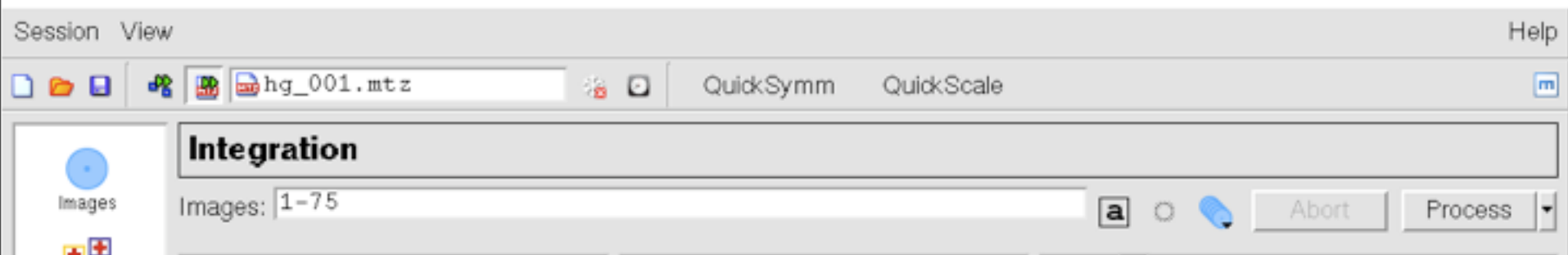
Possible causes:

- Inaccurate cell parameters
- Inaccurate crystal orientation

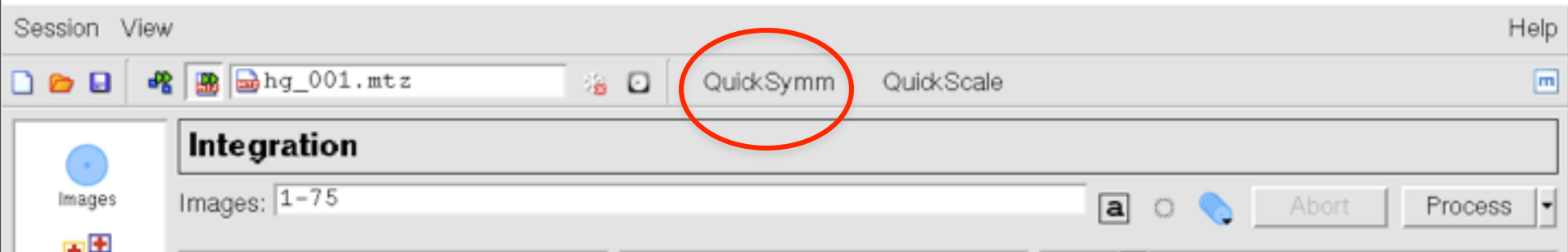
Possible solutions:

- 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 Scala

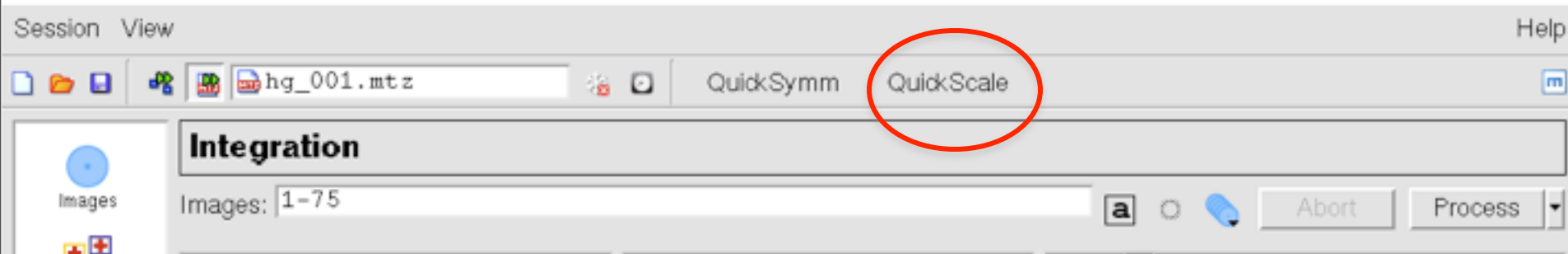


After integration Pointless and Scala



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

After integration Pointless and Scala



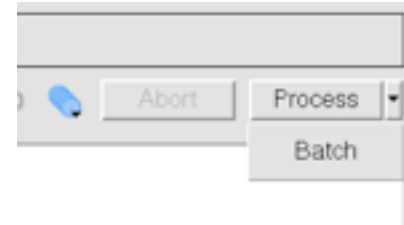
Selecting “Quicksymm” will run POINTLESS to determine the true symmetry. The results will be displayed in a browser window.

Selecting “Quickscale” will run POINTLESS, followed by running 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.

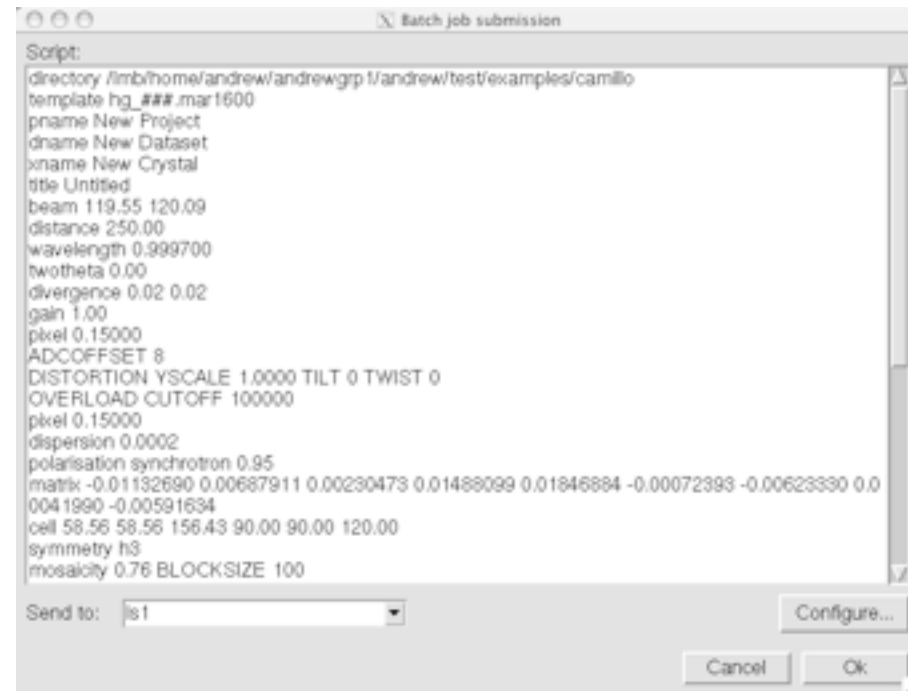
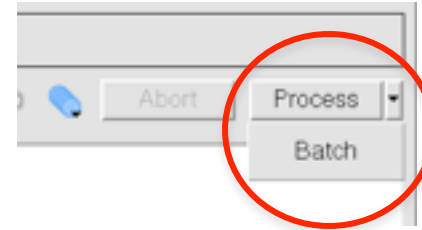
Go faster ... background job submission from the GUI

Selecting “Batch” from the Process pull down menu allows submission of an integration run in background.



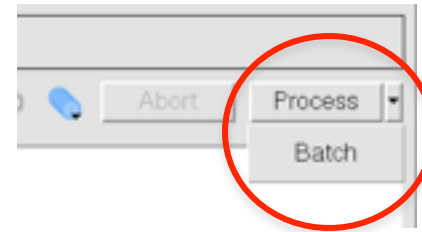
Go faster ... background job submission from the GUI

Selecting “Batch” from the Process pull down menu allows submission of an integration run in background.



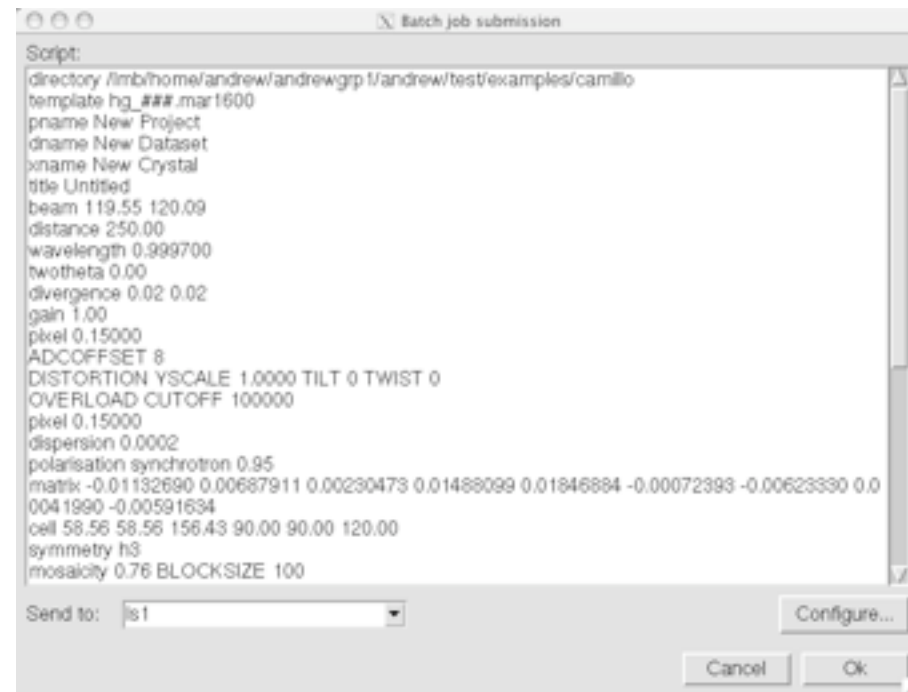
Go faster ... background job submission from the GUI

Selecting “Batch” from the Process pull down menu allows submission of an integration run in background.

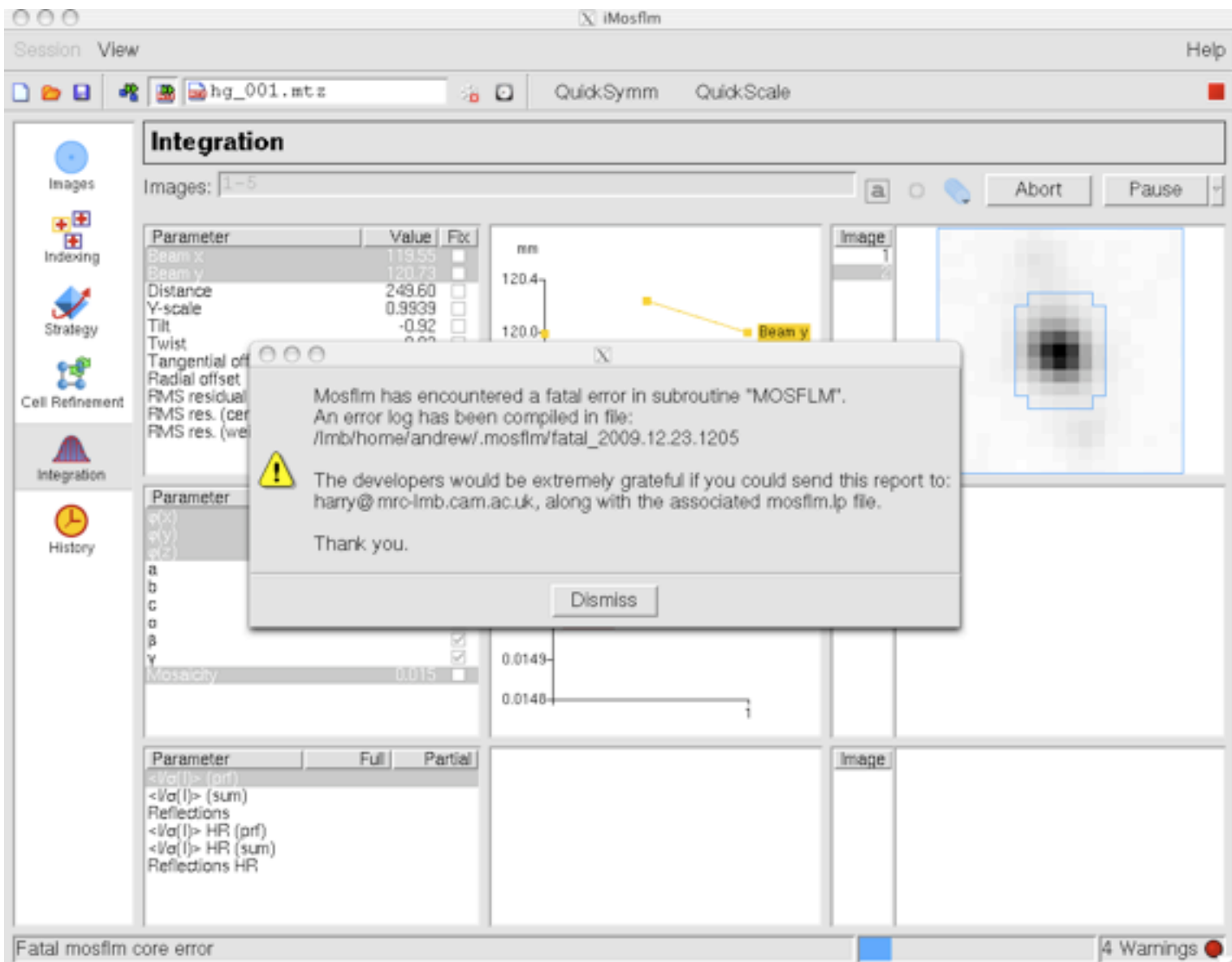


This will be quicker, but the imosflm graphical output is lost (although very similar graphs are produced by running loggraph on the mosflm summary file).

This also provides a way of adding keywords for features not yet available in imosflm, or the commands can be cut and pasted into a “standard” command script.



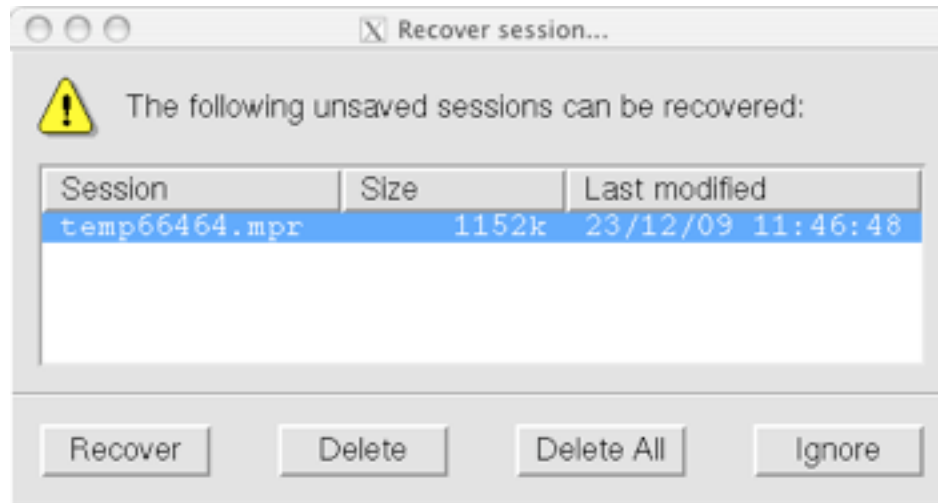
It's crashed again !!!



It's crashed again !!!

Solutions:

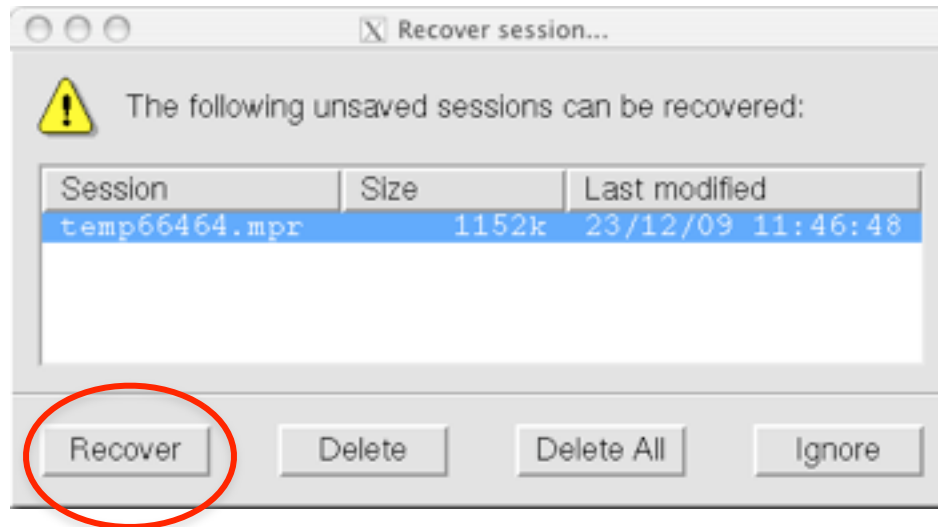
Kill the imosflm process and restart it, usually the option to recover the session will be available.



It's crashed again !!!

Solutions:

Kill the imosflm process and restart it, usually the option to recover the session will be available.



It's crashed again !!!

Solutions:

Kill the imosflm process and restart it, usually the option to recover the session will be available.

With the latest (1.0.4) version, it will revert to the Images pane, allowing parameter changes and another attempt to carry out the same task.

Acknowledgements



Harry Powell



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Phil Evans and many users for feedback

CCP4 and BBSRC for support

Acknowledgements



Harry Powell



Geoff Batty



Luke Kontogiannis



Phil Evans and many users for feedback

Please give us feedback !

CCP4 and BBSRC for support

Other tips

Specifying the “Minimum spot separation” (to be the actual spot size) can help spotfinding in very difficult cases.

Turning off “Local background determination” can sometimes help in very weak images (although it usually makes things worse).

Reducing “Spot rms variation” can help if spots are large and diffuse and obvious spots are not being detected.

More on dealing with multiple sectors ... choice of segments for cell refinement.

Strategy

If only two reference images have been collected, the Strategy pane can be used to design a data collection strategy.

The Strategy pane displays the following information:

Matrix: hg_001

start: 0

end: 64

Auto-complete: [button]

Unique data: 99.8%

Anomalous data: 85.5%

Mean multiplicity: 1.0

Check for overlaps: [button]

| Axis | x | y | z | Closest to rotation axis | Unique axis |
|------|--------|-------|--------|--------------------------|----------------------|
| a | 149.9° | 70.7° | 112.1° | ✗ 20.9° to XZ plane | |
| b | 42.7° | 49.7° | 78.1° | ✗ -41.4° to XZ plane | |
| c | 68.9° | 96.5° | 157.8° | ✓ 22.2° to ϕ axis | ✓ -72.6° to YZ plane |

Donut Chart: Shows the distribution of data. The red segment represents 64 unique data points, and the white segment represents 0 anomalous data points.

Predicted completeness (percent) by resolution:

| Resolution (Å) | Completeness (%) |
|----------------|------------------|
| 6.33 | ~90 |
| 4.43 | ~95 |
| 3.67 | ~95 |
| 3.10 | ~95 |
| 2.85 | ~95 |
| 2.60 | ~95 |
| 2.41 | ~95 |
| 2.25 | ~95 |

Integration procedure in iMosflm

1. Read Images

read all images with a common template

2. Find spots and Index

find *lattice* which fits spots

3. Check prediction

is the indexing correct?

4. Estimate mosaicity

improve estimate later

5. Refine cell

use two wedges at 90°, or more in low symmetry

6. Mask backstop shadow

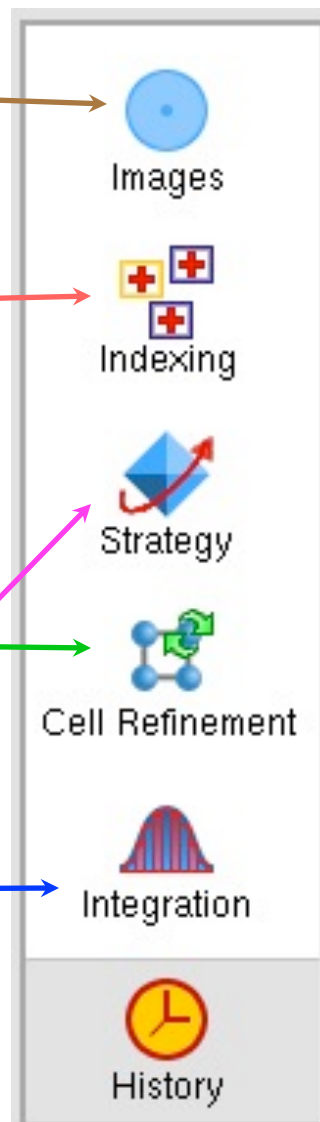
not (yet) done automatically by program

7. Integrate one (or few) image

to check resolution etc

8. Integrate all images

optionally run in background for speed



Strategy option, for use before data collection

GUIs, new and old

Integration

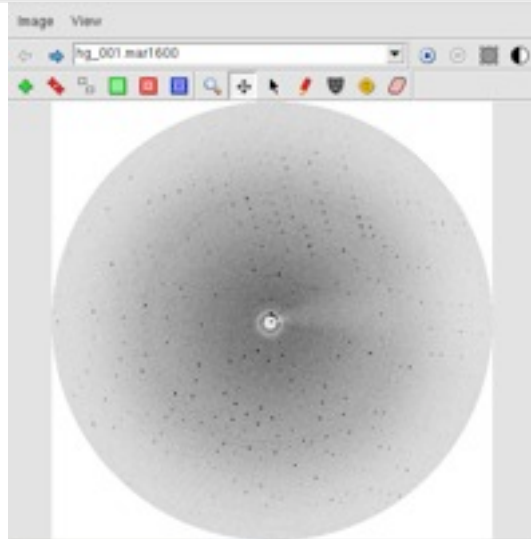
Images: 1-29

| Parameter | Value | Fix |
|---------------------|--------|--------------------------|
| Beam x | 178.61 | <input type="checkbox"/> |
| Beam y | 120.02 | <input type="checkbox"/> |
| Distance | 249.50 | <input type="checkbox"/> |
| Y-scale | 1.0000 | <input type="checkbox"/> |
| tau | 0.00 | <input type="checkbox"/> |
| Tangential offset | -0.040 | <input type="checkbox"/> |
| Radial offset | -0.040 | <input type="checkbox"/> |
| RMS residual | 0.043 | <input type="checkbox"/> |
| RMS res. (central) | 0.031 | <input type="checkbox"/> |
| RMS res. (weighted) | 0.540 | <input type="checkbox"/> |

| Parameter | Value | Fix |
|-----------|--------|--------------------------|
| a | 58.43 | <input type="checkbox"/> |
| b | 58.43 | <input type="checkbox"/> |
| c | 156.01 | <input type="checkbox"/> |
| alpha | 90.00 | <input type="checkbox"/> |
| beta | 90.00 | <input type="checkbox"/> |
| gamma | 120.00 | <input type="checkbox"/> |
| Mosaicity | 1.034 | <input type="checkbox"/> |

| Parameter | Full | Partial |
|-----------------------------------------|------|---------|
| $\langle d \rangle$ (sum) | 0.50 | 10.30 |
| /Reflections | 0 | 728 |
| $\langle d \rangle$ HR (sum) | 0.00 | 4.80 |
| $\langle d \rangle$ HR (sum) | 0.00 | 4.80 |
| /Reflections HR | 0 | 133 |

Plots: Deg vs. Resolution (Å), Profile fit (sum) vs. Resolution (Å)



MOSFLM version 7.0.5 Image Display

hg_001.mar1600

Min 1 Max 473 auto=410. auto=079

Overlay on Contrast

Colour Click on white Mag =4

Please use imosflm GUI instead .. waiting for input

Main menu

- Feed image
- Find spots
- Edit spots
- Clear spots
- Select images
- Autoindex
- Estimate mosaicity
- Predict
- Clear prediction
- Adjust
- Refine cell
- Integrate
- Strategy
- Keyword input
- Find hkl
- Pick
- Measure cell
- Circles
- Beam / mask areas
- Save/Exit

Processing parameters

- alpha 0.00
- beta 0.00
- gamma 0.00
- tau 0.00
- tau2 0.00
- tau3 0.00
- Mosaic 0.000
- Dist 0.000
- Leadin 1.000
- Distance 250.00
- Beam X 120.00
- Beam Y 120.00
- COORDIN 0.000
- DIFF 0.00
- DIFF 0.00
- TRIAL 1.0000
- Pick area X 11
- Dot threshold 11
- Dot threshold 20
- Vector scale 1
- Div theta 0.00
- Resolution 0.00
- "SPY Stable" 0.00
- Threshold 5.00
- Gain 1.91
- Gain 100.00
- Z offset 0.00
- Z offset 0.00
- Min X size 0.50
- Min X size 10.00
- Min Y size 0.50
- Min Y size 10.00
- Min no of pix 4
- Z splitting 0.00
- Z splitting 0.00
- "AUTOINDEXING"
- Min I/sig(I) 0.00

Output

- Frame 1601
- Min X 240.0 0.0
- Resolution 0.00
- Radius 0.0 0.0
- F PA 0.00 width 0.00
- Intensity 0
- Sign 0
- Spacing A 0.000
- spacing 0.0
- Scan 0.0
- Wavelength 0
- Comfactor 0
- Circle resolution A 0.0 0.0 0.0
- Phi 0.00 0.00 1.00