

Triclinic Lysozyme - brief processing notes for iMosflm

Before starting, note the following.

It is assumed that all students will have gone through the online tutorial for iMosflm before even thinking about processing these data! The tutorial is available at

<http://www.mrc-lmb.cam.ac.uk/harry/imosflm/ver105>

(select "documentation" from the left hand menu, and the tutorial specifically for iMosflm, not the "Mosflm User Guide")

The partial dataset that the students have been requested to download is a low-resolution pass from a crystal of triclinic lysozyme. In this case, "low resolution" is probably a higher resolution than most of the students will ever see in their careers as protein crystallographers - using the default settings in iMosflm, data will be integrated to $\sim 1.7\text{\AA}$ (since it integrates to the inscribed circle), but if we integrate to the corners of the detector, there are data to just better than 1.3\AA .

The images in dataset "a" each have an oscillation angle of 3.0° - since the mosaic spread is $\sim 0.7^\circ$, there are insufficient partial reflections to refine the unit cell parameters, so this should not be attempted - Mosflm will fail in an unexpected way.

The route to process these data is therefore -

```
read images
index & estimate mosaicity
integrate
quickscale
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Since there are other (higher resolution) images in the dataset with appropriate oscillation angles that allow for plenty of partials, it is not necessary to worry about the lack of an accurate cell from post-refinement for this partial dataset.

In more detail

Read in the 60 images using the normal iMosflm method.

Go to the indexing pane - unless you have unchecked the options to automatically find spots and automatically index & estimate mosaic spread, the indexing will proceed and you will get one "good" solution, which iMosflm will indicate as its "best guess" choice -

Lat.	Pen.	a	b	c	α	β	γ	$\sigma(x,y)$	$\sigma(\phi)$	$\delta(\text{beam})$
aP	0	27.0	31.4	33.8	104.1	107.8	102.0	1.85	2.16	1.80 (0.2)

Since there is only one solution with a good penalty, the penalty in itself doesn't give very much information. The unit cell dimensions are the right sort of size for triclinic lysozyme, but the $\sigma(x,y)$, $\sigma(\phi)$ and $\delta(\text{beam})$ are all bigger than you might like - values less than one pixel (*i.e.* $< 0.05\text{mm}$) would be good for $\sigma(x,y)$ and $\delta(\text{beam})$.

Check the predictions on the image display - you should see that there is essentially no correspondence between the spots found (and used in indexing) and the predictions. Clearly there is something wrong!

NOTE: There are a few synchrotron beamlines around the world where the scientists in charge have changed the side of the detector that the goniometer is on - this has exactly the same effect as reversing the direction of positive rotation. Mosflm does not automatically detect this from the image headers because there is nothing to stop the beamline scientists "correcting" this or moving the detector to another beamline with the phi rotating in the "normal" direction.

Open "Settings->Experiment Settings->Experiment" and click on the check-box for "Reverse direction of spindle rotation".

Go back to the indexing pane, and notice that the "maximum cell edge" box on the topright-hand side of the display has a figure something like "613" - change this to a sensible value for the crystal - e.g. 100Å. Click the index button to get a solution -

Lat.	Pen.	a	b	c	α	β	γ	$\sigma(x,y)$	$\sigma(\phi)$	$\delta(\text{beam})$
aP	0	27.1	31.3	33.8	88.0	72.0	67.9	0.13	0.72	0.52 (0.1)
aP	21	27.1	31.3	33.8	88.0	108.0	112.1	0.13	0.72	0.52 (0.1)

NOTE: In this case, because the unit cell is rather small, there is no need to change the beam centre to its original value - it is close enough to the right value. This will not normally be true!

In spite of the higher penalty for the second solution, I would choose this since it has two oblique inter-axial cell angles, so is more "conventional" (it is an alternative setting of solution 1, and it is not clear why it should have a higher penalty).

Estimate the mosaic spread again.

Check the predictions on the image display - they should now correspond closely to the found spots.

If you can see the backstop, use the tools to mask it out. I had trouble seeing it, so didn't mask it.

Select the "Integration task". All 60 images should be selected automatically, so just click the "Process button". Integration will take a couple of minutes on a modern computer. The integration should proceed smoothly and there won't be any sharp deviations in any of the plots (except for the RMS residual plots which drop from initial good values to substantially better values, and the $I/\sigma(I)$ plots change between blocks of images).

Check the warnings

You will probably find a few warnings (click on the flag on the bottom right of the GUI for a list). Some of these are "information", some are "warnings"; it may be possible to improve the integration by taking account of these. In this instance, I get 8 in total - 3 "information" and 5 "warning".

Double-click on the text for each warning for detailed information on the problem and how to improve the processing. You may notice that some are contradictory - if this happens, it should usually be possible to just make a mental note, but if you have time you should examine the full-length log file to see if you can determine if the problem happens only with a certain range of images, and displays differently in different parts of the dataset.

Quickscale

Open "Settings->Processing options->Advanced integration", and uncheck the box "Treat anomalous data in Quickscale". For this part of the data we have probably have insufficient information to generate good statistics on the anomalous contribution. After processing all the parts

of the data, we may want to reconsider this.

Select the "Quickscale" task - this launches a Pointless and Scala job - when finished, it will open a web-browser so that you can see the overall statistics. These have been calculated quickly using a small subset of Scala's options, so that you can make sure the images have been processed more-or-less okay. It is probably worthwhile scaling again more carefully to see if you can improve the statistics (dealt with later after integrating all the images).

Checking the results of Quickscale

Check the result from Pointless - does the symmetry agree with what you thought at the start?

Check the plots from Scala - they should vary smoothly through the data processing, values should lie in the "expected" ranges.

Note that Rmerge rises towards the end of the dataset - it may be possible to get better values by indexing originally off three images (1,30,60) so that there is a better fit of calculated reflections, since it was not possible to postrefine the cell.

At this point you may want to process the remaining datasets with iMosflm. If I have time to download the images I'll go through them.