PROCESSING DIFFRACTION DATA WITH MOSFLM

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Abstract: Processing diffraction data falls naturally into three distinct steps: First, determining an initial estimate of the unit cell and orientation of the crystal; second, obtaining refined values for these parameters; and third, integrating the diffraction images. The basic principles underlying autoindexing, parameter refinement, and spot integration by summation integration and profile fitting are described.

Keywords: data processing; profile fitting; autoindexing; postrefinement.

1. Introduction

This chapter will describe in outline the procedure for integrating monochromatic diffraction data from macromolecules. It is assumed that the diffraction images have been collected using the rotation method. Although the procedures will be described with reference to the MOSFLM program, the basic principles involved are common to most, if not all, data integration programs currently in use. More detailed accounts of many aspects of data processing are covered in the proceedings of a recent CCP4 Study Weekend [1].

2. Collecting the images

While the focus of this chapter is on data integration rather than data collection, it is worth emphasizing that successful data integration depends on the choice of appropriate experimental parameters during data collection. It is therefore crucial that the diffraction experiment is correctly designed and executed. A list of the most important issues that need to be considered is given below.

- Is the crystal single? Is the diffraction highly anisotropic? Two diffraction images 90° apart in phi should be examined carefully for evidence of split...
spots or the presence of a second lattice. A single image can easily be misleading in this respect.

- Can the image be successfully indexed? Failure of the indexing could indicate the presence of a second lattice. Does the derived cell and orientation account for all the spots on the image (with an appropriate mosaic spread)? Are there lines of weak spots between those predicted (indicative of a pseudocell)? Are there additional spots due to the presence of a satellite crystal?

- Does the crystal really diffract to the edge of the detector? If not, either increase the exposure time or move the detector further away to improve the data quality (signal to noise).

- Is the collimation adequate to resolve adjacent reciprocal lattice spots for the longest cell spacing? If not, move the detector further back or try reducing the incident beam size or, in some circumstances, the beam divergence.

- Is the dynamic range of the detector sufficient to avoid overloaded reflections at low resolution? If not, a rapid pass may be necessary to measure these strong reflections. Ideally, collect this rapid pass first.

- What is the optimum rotation angle per image? Too large a value will result in spatial overlap of spots in adjacent lunes. Too small a value will give a poor duty cycle, as the exposure time becomes comparable with the detector readout time. Very short exposure times (less than ~0.5 s) on modern synchrotron sources can lead to problems with shutter synchronization.

- Ideally, aim for high data multiplicity as this will improve the overall quality of the data by reducing random errors and facilitating outlier identification. If this is not possible, aim for high completeness, possibly by collecting several segments of data rather than a single large rotation. Be conservative in the choice of exposure time, so that the data set is complete before the onset of serious radiation damage.

- Always integrate at least some (and preferably all) the diffraction images during data collection, to check for unforeseen problems and to get a quantitative estimate of data quality. Soon it should be possible to do this automatically.

3. Determining the crystal cell parameters and orientation

The autoindexing algorithms currently in use are extremely powerful and in general it will be possible to determine the unit cell dimensions and crystal orientation from a single diffraction image, providing that the direct