Abstract

A procedure for refining a crystal orientation matrix for the flat-cone diffractometer is discussed. The positions of the centers of gravity of reflections obtained during routine data collection are transformed in such a way that they can be used as input to the least-squares procedures of Busing & Levy [Acta Cryst. (1967), 22, 457-464] or Shoemaker & Bassi [Acta Cryst. (1970), A26, 97-101]. The orientation matrix can be refined on the basis of the positions of all observed reflections, and not only of a selected sample, thus increasing its reliability. The procedure is particularly suited for protein crystallographic studies, as it makes it possible to compensate for crystal movements encountered during data collection.

Introduction

A diffractometer utilizing a linear position-sensitive detector has been described by Prince, Wlodawer & Santoro (1978). A neutron version of this instrument (called a 'flat-cone diffractometer' because of its mode of operation) has been in use at the National Bureau of Standards reactor facility since 1977 and has provided the data used in the refinement of ribonuclease-A (Wlodawer, 1980; Wlodawer & Sjölin, 1981a).

The original description of the instrument (Prince, Wlodawer & Santoro, 1978) contained the definition of the orientation matrix and a procedure to evaluate it. This definition closely followed the formalism adopted for three- and four-circle diffractometers by Busing & Levy (1967). Subsequently, expressions for the calculation of a semi-empirical absorption correction for this instrument (as well as for diffractometers utilizing other Weissenberg geometries) were given by Santoro & Wlodawer (1980). The remaining problems found troublesome during the data collection for ribonuclease were caused by the poor signal-to-noise ratio for the reflections collected from a macromolecular crystal and by the shifts of reflection centers from the predicted positions caused by an inaccurate knowledge of the orientation matrix.

The first problem was solved by developing an algorithm based on analyzing the variances of the data collected for each reflection, a technique which has been called the 'dynamic mask procedure' (Sjölin & Wlodawer, 1981; Wlodawer & Sjölin, 1981b). With this procedure, each reflection is carefully examined in its two-dimensional box which is defined in one direction by a number of pixels along the linear detector and in the other direction by a number of steps, $\Delta \psi$, of the angle $\psi$ describing the rotation about the zone axis selected as the rotation axis of the flat-cone geometry. Thus, in addition to the integrated intensity, the position of each reflection can also be accurately determined. This information can in turn be used to improve the orientation matrix of the crystal, as will be shown below.

Mathematical analysis

Let us assume that the position of each reflection has been accurately determined by finding its center of gravity in the $(d, \psi)$ space, where $d$ represents the position of a point along the counter and $\psi$ the rotation angle. This can be used to obtain better estimates of the orientation matrix and the lattice parameters of the crystal with a method similar to that proposed by Busing & Levy (1967) or with the procedure given by Shoemaker & Bassi (1970) and Shoemaker (1970). If we adopt the first method, we may assume that the Bragg condition is satisfied for the center of gravity of a reflection, and we may take as an observation the value $\psi_d^*$, corresponding to this point.

*Here and in what follows we will adhere to the procedure of Busing & Levy (1967) of indicating with the subscript $d$ the instrumental dial readings and their matrices, with the subscript $c$ the calculated angles and their matrices, and without subscripts the quantities evaluated from the trial parameters. All other symbols, as well as the reference systems used here, are defined in Busing & Levy (1967) and/or Prince et al. (1978).
The only requirement here is that the scattering vector \( \mathbf{u} \) make an angle of \( 90^\circ + \theta \) with the primary beam direction, i.e.

\[
\mathbf{u}_{12} = -\sin \theta,
\]

where

\[
\mathbf{u}_i = \mathbf{M}_d \Psi \mathbf{u}_o.
\]

From (1) and (2), we obtain

\[
(u_{o3} \cos \mu_1) \sin \psi_1 + (u_{o2} \cos \mu_1) \cos \psi_1 = (u_{o1} \sin \mu_1 - \sin \theta)
\]

and the calculated value \( \psi_1 \) corresponding to the observation \( \psi_1 \) can be obtained since

\[
\mathbf{u}_o = \mathbf{U} \mathbf{B} \mathbf{h}_o/q.
\]

From the above considerations it is clear that the observations most easily obtainable with a linear detector operated in flat-cone geometry are analogous to the type 5 observations analyzed by Busing & Levy (1967).

On the other hand, the input required by the procedure of Shoemaker & Bassi (1970) and Shoemaker (1970) requires precise values of the setting angles of reflections analyzed with the geometry of a four-circle diffractometer. From these the coordinates \( \mathbf{h}_o \) of the corresponding reciprocal-lattice points can be evaluated, and the observational equations are

\[
\mathbf{V}^{-1} \mathbf{h}_o = \mathbf{V}^{-1} \mathbf{V} \mathbf{h} = \mathbf{h}',
\]

where all the symbols have the same meaning as those defined by Shoemaker & Bassi (1970). This procedure can be extended to flat-cone geometry in the following way. From the settings \( \phi, \chi, \omega, \mu, \) and \( \gamma \), which center a reflection on the linear detector, we may calculate the vector \( \mathbf{h}_o \) in the Cartesian system attached to the \( \Phi \) circle with the equations

\[
\mathbf{h}_o = \mathbf{P}^{-1} \mathbf{h}_i,
\]

where

\[
\mathbf{P} = \mathbf{M} \Omega \chi \Phi
\]

and

\[
\begin{align*}
\gamma_1 &= \cos \gamma \sin \mu \\
\gamma_2 &= \cos \gamma \cos \mu - 1 \\
\gamma_3 &= \sin \gamma.
\end{align*}
\]

The coordinates \( \mathbf{h}_o \) (equation 6) are sufficient as input to Shoemaker’s procedure, but their use requires some modification. If one desires to use the unmodified procedure, the required input consists of the setting angles of reflections in four-circle geometry which are given by

\[
\begin{align*}
\varphi_0 &= \tan^{-1} (h_{2o} h_{1o}) \\
\chi_0 &= \tan^{-1} \left[ (h_{3o}/h_{1o}^2 + h_{2o}^2)^{1/2} \right] \\
2\theta_0 &= \cos^{-1} (\cos \gamma \cos \mu) \\
\omega_0 &= \frac{\pi}{2}.
\end{align*}
\]

**Implementation**

We have chosen, in practice, a method based on the procedure of Shoemaker & Bassi (1970) because of the availability of necessary computer programs. We have modified a Fortran program written by C. Hubbard (personal communication).

An initial orientation matrix for a crystal to be aligned was obtained in the usual way — by centering about a dozen reflections in equatorial geometry and using the least-squares procedure of Shoemaker & Bassi (1970). This initial orientation matrix is subject to errors because of the weakness of reflections from a protein crystal, difficulty in establishing exactly the horizontal plane of the instrument, and possible crystal movement.

Subsequently, reflection data are calculated level by level, and each level is normally processed before the next one is started. For ribonuclease, between 1000 and 1500 reflections are accessible in each level to 2.0 Å resolution, and the positions of all reflections with intensities \( I > 10 \sigma(I) \) are used as input to the procedure (equations 5–9). While the position of each individual reflection is not established with an accuracy as high as that possible during alignment centering, the number of reflections available for refinement is very large. For a typical level, data from as many as 300 reflections are used to recalculate the orientation matrix. Most importantly, obtaining these data did not require any additional measurement time, and thus did not prolong the data collection. To avoid problems in defining the parameters related to the rotation axis of the crystal, only reflections belonging to upper levels are used in the procedure.

The procedure was tested on a crystal which was deliberately misset and was shown to perform successfully. Even when the maximum misalignment for individual reflections was as large as \( 0.5^\circ \) in \( \psi \), the recalculated matrix properly centered all reflections. The limits of convergence were reached when the reflections were becoming only partially recorded in their boxes, since under these conditions the apparent centers of gravity would no longer correspond to the true position of each reflection. Since incompletely recorded reflections can be recognized as such and properly marked, it is possible to extrapolate the positions of their centers of gravity and to use them in the recalculation of the orientation matrix. Under these conditions the intensity data are not useful anyway, and the level has to be recollected. We have found that, as long as the centers of gravity of the reflections did not fall outside their predicted boxes, the procedure was powerful enough to ensure that during a subsequent scan they would be completely enclosed within their boxes, even though they would not necessarily be very well centered.
In our implementation this procedure is employed 'off-line', with the orientation matrix recalculated after each level has been collected. This can compensate for possible crystal movement. An alternative implementation is to perform the calculations 'on-line' during data collection, with a list of reflections that is constantly updated as more are processed. This latter implementation is particularly well-suited to processing the data from an area detector. In either case we are utilizing a major advantage of position-sensitive detectors over standard counters, namely the ability to follow the movement of reflections in space to prevent the introduction of systematic errors due to crystal misalignment.

References