3.4. MOUNTING AND SETTING OF SPECIMENS FOR X-RAY CRYSTALLOGRAPHIC STUDIES

3.4.2.5. *Setting and orientation with stationary-crystal methods*

3.4.2.5.1. *Laue images – white radiation*

The azimuthal and back-reflection Laue methods for setting crystals with relatively small unit cells have been described by Jeffery (1971). The former is capable of achieving an accuracy of setting of $\pm 0.05°$, whereas the latter is important in metallurgy, where the Laue method is often the only possibility because of the large size of the specimens. Schiller (1985) has emphasized the importance of the back-reflection Laue technique for setting specimens with a precision of $0.1°$ needed in semiconductor surface preparation.

In recent years, there has been a resurgence of the Laue technique, in conjunction with synchrotron radiation, to record intensity data from biological macromolecules in very short time scales. The overall experimental strategies involved are described by Helliwell *et al*. (1989) and Clifton, Elder & Hajdu (1991). Crystals are not usually set in a precise orientation for these types of experiment prior to data acquisition because of radiation damage. The post-determination of the precise crystal orientation with respect to the instrument axes from the recorded Laue pattern therefore forms an essential part of the data processing. Most methods are based on the indexing procedure of Riquet & Bonnet (1979), and an interactive computer program for the interpretation and simulation of Laue patterns has been written by Laugier & Filhol (1983). An orientation-matrix approach has been reported by Jacobson (1986), and the work of Helliwell *et al*. (1989) has led to a comprehensive set of Laue processing programs. In addition to enabling trial-and-error visual matching of images, this program suite includes an auto-indexing procedure based on a known unit cell, and refinement of the orientational parameters. More recently, Carr, Cruickshank & Harding (1992) have developed a method whereby a gnomonic projection of the Laue diffraction pattern can be used to determine the cell dimensions and orientation of a crystal. The axial ratios and interaxial angles can be determined precisely, but the absolute scaling of the cell is dependent on the accuracy with which the minimum wavelength used in the experiment is known.

3.4.2.5.2. *'Still' images – monochromatic radiation*

More recently, the azimuthal method has proved of great value in the rapid alignment of crystals with large unit cells prior to data collection on devices using rotation geometry. After optical alignment, a 'still' photograph taken with monochromatic radiation (or a very small angle rotation photograph, typically $0.05–0.20°$), is used to locate a zero-layer reciprocal-lattice plane (Fig. 3.4.2.1). Such a plane will record on a flat detector placed at a distance D mm from the crystal, C, as an ellipsoidal trace of maximum dimension S mm from the direct-beam position, O'. In order to make the plane perpendicular to the X-ray beam (*i.e.* the real axis parallel to the X-ray beam), it must be rotated through an angle θ such that $\tan 2\theta = S/D$.

If the vector $O'P$ makes an angle α with the rotation axis, the angle θ can be resolved into a vertical component, $\theta \sin \alpha$, corresponding to a rotation of the spindle axis, and a horizontal component, $\theta \cos \alpha$, corresponding to a rotation of the goniometer arc whose axis is perpendicular to the X-ray beam (assuming a perpendicular and parallel setting of the goniometer head). Rotation of the reciprocal-lattice plane within its own plane can then be achieved with the goniometer arc whose axis is parallel to the beam. This technique is also applicable to preliminary setting on a precession camera.

However, with very radiation sensitive crystals, it is inadvisable to waste time accurately setting the crystal prior to data collection, since the crystal is subject to continuous radiation damage from the beginning of the first exposure (Rossmann & Erickson, 1983). In this case, two 'still' images are collected, preferably separated by a $90°$ rotation, after data collection but before the crystal is irretrievably damaged. In principle, the orientation can be determined from a single still, but the precise crystal orientation is better determined by identifying and measuring the orientations of two real axes relative to the camera axes, from the sets of ellipses on two stills. The orientation of the reciprocal axis, perpendicular to these two real axes, can then be calculated, and, provided that the unit-cell dimensions are known, the orientation of the third real axis readily determined. Given the directions of the three real axes, the direction cosines of the reciprocal axes can be computed and a matrix determined that specifies the crystal orientation with respect to the camera axes. This method obviates the need to index the 'partial' reflections on still images (Jones, Bartels & Schwager, 1977).

3.4.2.6. *Setting and orientation for crystals with large unit cells using oscillation geometry*

The use of the screenless rotation technique is now routine as a method for large-molecule data collection (Arndt & Wonacott, 1977; Usha *et al*., 1984). In general, the setting of the crystal for data-collection purposes does not need to be precise, although efficient data collection may dictate that a particular direct axis is set along the rotation axis (Munshi & Murthy, 1986), and subsequent data processing may be simpler. An accurate knowledge of the crystal orientation relative to the axial system of the camera is, however, absolutely essential for the final data processing.

Historically, determination of the crystal setting was normally undertaken using 'still' photographs (see Subsection 3.4.2.5) and the final orientation then determined from two such photographs taken orthogonally (Jones, Bartels & Schwager, 1977; Rossmann
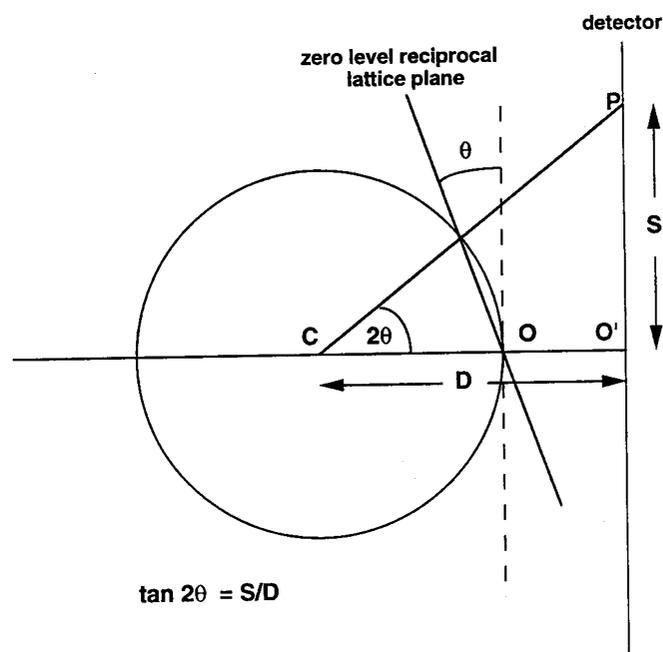

Fig. 3.4.2.1. A zero-layer reciprocal-lattice plane will record on a flat-plate detector placed at a distance D from the crystal C as an ellipsoid of maximum dimension S from the direct-beam position O'.

references