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 $\pm 0.05^\circ$, 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

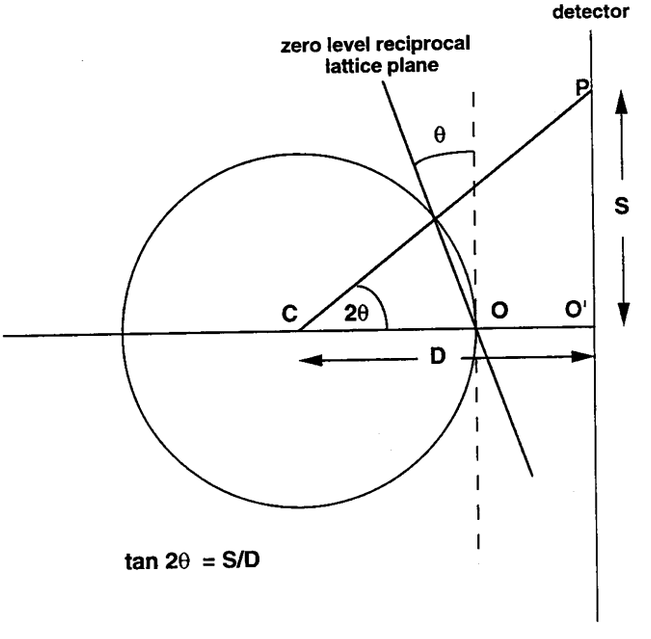

Fig. 3.4.2.1. A zero-layer reciprocal-lattice plane will record on a flat-plate detector placed at a distance D from the crystal C as an ellipsoid of maximum dimension S from the direct-beam position O'.

& Erickson, 1983). In order to minimize the problem of radiation damage, various graphical methods were then devised for determining the precise setting without the need to record 'still' images (Dumas & Ripp, 1986; Moews, Sakamaki & Knox, 1986; Sarma, McKeever, Gallo & Scuderi, 1986). Vriend, Rossmann, Arnold, Luo, Griffith & Moffat (1986) reported a 'post-refinement' technique in which the intensities of partially recorded reflections on oscillation images are compared with their full intensities observed elsewhere on the same or a different image. The degree of partiality is dependent on the crystal orientation so that this provides a very sensitive method of refining the setting parameters (cell parameters, crystal mosaicity and X-ray beam characteristics may also be refined). In a further development, Vriend & Rossmann (1987) described how to determine the orientation from a single oscillation photograph. The method was again devised for crystals that have short lifetimes in the X-ray beam and is based on correlating the unique set of calculated normals to reciprocal-lattice planes with the observed zone axes on the oscillation image.

Currently, auto-indexing procedures based on a single still/oscillation image or preferably several images well separated in reciprocal space are used to determine the precise crystal setting prior to data processing. Kim (1989) has devised an auto-indexing algorithm based on methods previously developed for four-circle diffractometers (*e.g.* Sparks, 1976). The algorithm includes *ab initio* cell-parameter and orientation-matrix determination, followed by reduced-cell calculation and transformation of the reduced cell to one of higher symmetry, where appropriate. Kim's method does require, however, that the diffraction image is large enough to display many lunes. Higashi (1990) has also developed an auto-indexing program for single and or multiple still/oscillation images. The very effective auto-indexing routine of Kabsch (1988*a*, 1993) has been incorporated into the *XDS* program suite (Kabsch, 1988*b*).

Several types of area-detector diffractometer have been developed for fast and accurate measurement of intensity data for macromolecular crystals. Crystal alignment and general strategies for typical devices are described by Xuong, Nielsen, Hamlin & Anderson (1985), Messerschmidt & Pflugrath, (1987), Higashi (1989), and Sato *et al.* (1992).

3.4.2.7. *Diffractometer-setting considerations*

General setting considerations for three- and four-circle diffractometers have been discussed by Busing & Levy (1967). In principle, crystals can be placed on a four-circle diffractometer in any general orientation, although it is often useful to have a setting such that the reciprocal-lattice axis lies parallel to the φ rotation axis. This setting is a prerequisite for effective use of the empirical absorption correction method of North, Phillips & Matthews (1968).

In the case where the crystal orientation is not precisely determined, setting is normally achieved using automatic procedures that involve finding a set of general reflections and generating a **UB** matrix from their angular positions. Generation of the **UB** matrix can be achieved by finding the shortest non-coplanar reciprocal-lattice vectors and assigning these as the reciprocal-cell axes (Hornstra & Vossers, 1974). The resulting unit cell is always primitive, and additional manipulations are required to determine the conventional cell and type of Bravais lattice. This reciprocal-space method is adopted by the Nonius CAD4 diffractometer software (CAD4 Manual, 1989). Alternatively, the 'auto-indexing' method originated by Sparks (1976, 1982) and Jacobson (1976) can be used whereby direct-lattice vectors are generated, again through an initial cell. Clegg (1984) has described an enhancement of the direct-lattice vector method so that the initial cell is used to produce direct-lattice vectors systematically. In order to confirm that a generated vector is a true direct vector, the condition is applied that the scalar multiplication of a true direct vector and any true reciprocal vector (*i.e.* the observed reflection vectors) results in an integer. If a great majority of the products of a putative direct vector and each of the measured observed reflection vectors are integers, the direct vector is accepted. The final cell can be obtained from the set of accepted direct vectors. Subsequently, Duisenberg (1992) developed a method of auto-indexing that is particularly applicable to difficult cases such as twin lattices, incommensurate structures, fragmented crystals, long axes, and even unreliable data. Finding the reciprocal lattice from a distribution of reciprocal-lattice points (*i.e.* observed reflections) is reduced to finding elementary periods in one-dimensional rows, obtained by projecting all observed points onto the normal to the plane formed by any three of these points. Row periodicity and offending reflections are readily recognized. Each row, by its direction and reciprocal spacing, defines one direct-axis vector, based upon all co-operating observations. A primitive cell can be obtained from the direct vectors and refined against the fitting reflections, resulting in one main lattice, or a main lattice and a set of alien reflections (see also Subsection 3.4.2.6).

3.4.2.8. *Crystal setting and data-collection efficiency*

Although it has become modern practice to determine the orientation of crystals after data collection using auto-indexing procedures, rather than to carry out accurate alignment prior to data collection, such a procedure, as indicated earlier in this section, can lead to inefficient data collection. In the case of anomalous-dispersion measurements, and particularly multiple-wavelength anomalous diffraction (MWAD) for phase determination (*e.g.* Kahn *et al.*, 1985), it is often very important to orientate the crystal so that Bijvoet pairs of reflections are recorded simultaneously. The use of synchrotron radiation, where access is usually very limited and crystals are highly radiation sensitive, often leads to insufficient care being taken in the data-collection procedure. An efficient data-collection strategy should aim to measure a set of data as complete as possible (preferably $> 90\%$) in the shortest possible time. Contiguous regions of reciprocal space, such as the 'cusp' region for oscillation geometry, and low-resolution shells should *not* be omitted. In addition, a reasonable number of reflections should be measured more than once to check for internal consistency in the data set. For biological macromolecules, in particular, the temptation to collect data beyond the practical resolution limit should be avoided. Two useful indicators from the outer resolution shell are (*a*) the proportion of significant data should not fall below 70%, and (*b*) the internal consistency index for data measured more than once should not rise above 20%. In general, rotation of crystals along the highest rotation symmetry axis (*i.e.* the fourfold axis for tetragonal systems) will require the least amount of data to be collected, and it is advisable to mount crystals so that this rotation axis is parallel to the fibre or capillary axis, provided that this is sensible in terms of the crystal morphology.

Munshi & Murthy (1986) have discussed strategies of data collection using the screenless oscillation method based on the Laue group and the nature of the crystal axis parallel to the rotation axis. More general strategies for area-detector systems have been reported by Xuong, Nielsen, Hamlin & Anderson (1985) and Zhang & Matthews (1993).

references