& 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