25.2.1.9.2. *σ_A weights*

As an alternative to the procedure above, in the *BNDRY* program the weights, W, used when constructing the unimodal probability distributions in equations (25.2.1.30) can be computed according to

$$W = \frac{2\sigma_A E_{\rm tot} E_{\rm par}}{1 - \sigma_A}, \qquad (25.2.1.31)$$

where $E_{\rm tot}$ and $E_{\rm par}$ are normalized structure-factor amplitudes for the observed and calculated structure factors, respectively, and σ_A is determined by the procedure described by Read (1986). For acentric reflections, equation (25.2.1.31) is used whereas for centric reflections, W is one half the value given by equation (25.2.1.31).

25.2.1.9.3. *Damping contributions*

Normally, the distributions constructed for the calculated phases are combined with those for the anchor set with full weight in equation (25.2.1.10). However, in *BNDRY*, one can supply a damping factor in the range 0–1 to down-weight the contributions of the anchor set. The damping factor simply multiplies the distribution coefficients such that a factor of 1 (default) indicates no damping, and values less than one place more emphasis on the map-inverted or partial structure phases. If set to zero, the calculated phases are accepted as they are, since there is effectively no phase combination with the anchor set.

25.2.1.9.4. *Phase extension*

If phase extension is requested during the phase combination step, an additional file (prepared by the interactive program *MISSNG*) is also supplied to the *BNDRY* program. This file contains unique reflections absent from the anchor set but for which observed amplitudes (and possibly phase probability distribution coefficients) are available. Phase combination then proceeds exactly as above, except that for any extended reflections lacking phase probability information, the calculated phases are accepted as they are. Phase extension is required when phasing purely by SAS methods as it is the only way to phase centric reflections. As a final option, phase and amplitude extension is possible, in which case both the calculated amplitude and phase are accepted as they are for reflections having only indices provided on the extension file. This is sometimes desirable to include low-resolution reflections that may have been obscured by the beam stop.

25.2.1.10. *Noncrystallographic symmetry calculations*

Several programs are provided to carry out noncrystallographic symmetry averaging within submaps and are briefly described below.

25.2.1.10.1. *Operator representation and definitions*

NC symmetry operators are specified in terms of the parameters φ, ψ, χ, O_x, O_y, O_z and t, which refer to a Cartesian coordinate system in Å, obtained by orthogonalization of the unit cell as in the Protein Data Bank (Bernstein *et al.*, 1977). The angles φ and ψ determine the direction of the NC rotation axis, while χ determines the amount of rotation about it. O_x, O_y and O_z are coordinates of a point through which the rotation axis passes, and t is a post-rotation translation parallel to the rotation axis. The relationships between the angles, orthogonal reference axes X, Y, Z and the unit cell are given in Fig. 25.2.1.2. Coordinates for a pair of points related by NC symmetry are then expressed in the orthogonal system by

$$P_2 = R_{\varphi,\psi,\chi}(P_1 - O) + O + tD_{\varphi,\psi}, \qquad (25.2.1.32)$$

where P_1 and P_2 are three-element column vectors containing coordinates for the related points, $R_{\varphi,\psi,\chi}$ is a 3×3 rotation matrix derived from the angles, O is a three-element column vector containing coordinates for a point through which the rotation axis passes, t is the post-rotation translation scalar in Å and $D_{\varphi,\psi}$ is a three-element column vector containing direction cosines of the rotation axis. This type of parameterization simplifies transfer of information from self-rotation functions, which are usually calculated in spherical polar angles anyway, and also makes obvious pseudo-space-group symmetry type operations such as pseudo-screw axes. For convenience, a program *O_TO_SP* is provided to convert from a 3×3 rotation matrix and 1×3 column vector representation of the NC symmetry operation, as used in some programs, to the parameters described here.

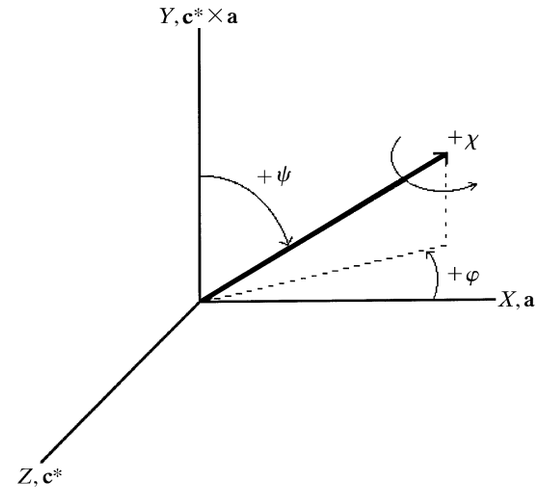

Fig. 25.2.1.2. Relationships between noncrystallographic symmetry rotation axis direction, orthogonal reference system axes X, Y, Z and crystallographic axes. The X axis is aligned with the crystal **a**. The Y axis is parallel to $\mathbf{a} \times \mathbf{c}^*$. The Z axis is parallel to $\mathbf{X} \times \mathbf{Y}$, *i.e.* $\mathbf{c}^*$. ψ is the angle between the NC rotation and $+Y$ axes. φ is the angle between the projection of the NC rotation axis in the XZ plane and the $+X$ axis, with $+\varphi$ counterclockwise when viewed from $+Y$ toward the origin. χ is the amount of rotation about the directed axis, with $+\chi$ clockwise when viewed from the axis toward the origin.

25.2.1.10.2. *Operator refinement*

Refinement of the NC symmetry operator parameters is achieved by least-squares minimization of the squared difference in electron density for all NC-symmetry-related points. Thus, one minimizes

$$\sum\{\rho(r) - \rho[R_{\varphi,\psi,\chi}(r - O) + O + tD_{\varphi,\psi}]\}^2 \qquad (25.2.1.33)$$

with respect to the operator parameters, where the sum is taken over all points within the appropriate averaging envelope(s). One starts refinement with low-resolution data (~6 Å) on a coarse (~2 Å) grid and monitors progress by following the correlation coefficient between the related electron-density values. Once convergence is obtained, the calculation is resumed with higher-resolution data on a finer grid. Typically, a correlation coefficient of around 0.4 or higher (for a 3 Å MIR map, 1 Å grid) indicates that the operator has been correctly located. The operator refinement is confined to submaps and is facilitated by use of an orthogonal grid. A submap containing the molecules to be averaged is obtained from the programs *MAPVIEW* or *EXTRMAP* and can be converted to an orthogonal grid, if needed, by the program *MAPORTH*, as described earlier.

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references