25.2.1.3.2. *Global anisotropic scaling*

With this option, applied after relative Wilson scaling, the unique parameters of a symmetric 3×3 scaling tensor S are determined by two cycles of least-squares minimization of

$$\sum_{hkl} W_{hkl}(F_P - SF_{PH})^2 \qquad (25.2.1.3)$$

with respect to S, where W_{hkl} is a weighting factor,

$$S = S_{11}O_x^2 + S_{22}O_y^2 + S_{33}O_z^2 + 2(S_{12}O_xO_y + S_{13}O_xO_z + S_{23}O_yO_z) \qquad (25.2.1.4)$$

and O_x, O_y, O_z are direction cosines of the reciprocal-lattice vector expressed in an orthogonal system. The derivative data are then placed on the scale of the native by multiplying each derivative amplitude by the appropriate S.

25.2.1.3.3. *Local scaling*

With this option, again applied after relative Wilson scaling, a scale factor for each reflection is also determined by minimizing equation (25.2.1.3) with respect to S, but here S is a scalar and the summation is taken only over neighbouring reflections within a sphere centred on the reflection being scaled. The sphere radius is initially set to include roughly 125 neighbours, and the scale factor is accepted if at least 80 are actually present. If insufficient neighbours are available, then the sphere size is increased incrementally and the process repeated until a preset maximum radius is encountered. If the maximum is reached, the process terminates with the message that the data set is too sparse for local scaling. Scaling is achieved by multiplying each derivative amplitude by the appropriate S.

25.2.1.3.4. *Outlier rejection*

Rejection of outliers is often desirable, as erroneously large isomorphous or anomalous differences can lead to streaks in difference-Patterson maps and complicate identification of heavy-atom or anomalous-scatterer sites. The interactive program *TOPDEL* facilitates identification and rejection of such outliers while selecting reflections for use in difference-Patterson calculations. An input 'scaled' file is read in, and user-supplied resolution and $F/\sigma(F)$ cutoffs are applied. The data are then sorted in descending order of magnitude of ΔF (either isomorphous or anomalous differences) and the largest differences are listed for examination. The user is then prompted to determine which, if any, of the large differences are to be rejected as outliers and to determine what percentage of the remaining largest differences are to be used in the Patterson-map synthesis. The appropriate Fourier-coefficient file is then created.

25.2.1.4. *Fourier-map calculations*

All Fourier maps, including native- and difference-Patterson maps, are computed by the program *FSFOUR*, which runs in batch mode and is a space-group-general variable-radix 3D fast Fourier transform program. Unique reflections are expanded to a hemisphere, and the calculation then proceeds in $P1$. The output map always spans one full unit cell.

25.2.1.4.1. *Submaps*

Selected regions of an electron-density map that are useful for NC symmetry applications can be extracted from the full-cell maps produced by *FSFOUR* with the programs *EXTRMAP* (batch) or *MAPVIEW* (interactive). The 'submap' regions can cover any arbitrary volume and cross multiple cell edges if desired.

25.2.1.4.2. *Orthogonal and skewed maps*

Programs *MAPORTH* and *SKEW* (both run in batch mode) are provided to modify submaps, as modification is sometimes useful or required with NC symmetry applications. *MAPORTH* simply converts the map to correspond to an orthogonal grid, which simplifies refinement of NC symmetry operators. *SKEW* also converts the map to an orthogonal grid, but changes the axis directions such that the new b axis can be arbitrarily oriented. This is useful in NC symmetry applications where one may want to examine maps looking directly down the NC symmetry rotation axis. Both programs compute density values at the new grid points by using a 64-point cubic spline interpolation and can also orthogonalize or skew masks to maintain correspondence with the modified submaps.

25.2.1.4.3. *Graphics maps and skeletonization*

Program *GMAP* (interactive) is used to extract any region from a *FSFOUR* map, possibly crossing multiple cell edges, and convert it to a form directly readable by the external interactive graphics programs *TOM* [SGI version of *FRODO* (Jones, 1978)], *O* (Jones *et al.*, 1991) or *CHAIN* (Sack, 1988). In addition to the output map file, one may also output a corresponding skeleton (Greer, 1974) file (for *TOM*) or skeleton data block (for *O*) to facilitate chain tracing.

25.2.1.4.4. *Peak search*

Program *PSRCH* (batch) lists the largest peaks in a Fourier map and is useful in identifying additional heavy-atom or anomalous-scatterer sites from a map phased by a tentative model. Either positive or negative peaks can be listed, with the latter sometimes useful in MAD phasing applications, depending on the assignment of 'native' and 'derivative' data sets. Only unique peaks are listed, and the peak positions are interpolated from the map.

25.2.1.5. *Structure-factor and phase calculations*

Several methods are used for structure-factor and phasing calculations depending on the nature of the model and how the results will be used. The methods available in the package are described below.

25.2.1.5.1. *By heavy-atom or anomalous-scattering methods*

Phasing by heavy-atom-based methods (isomorphous replacement and/or anomalous scattering) begins when one or more 'scaled' data sets are input to the program *PHASIT* (batch). User-specified rejection criteria are first applied to each data set, and structure factors corresponding to the heavy-atom or anomalous-scatterer substructure are computed from

$$F_{hkl} = Sc \sum_j O_j f_j \exp\{-B_j[\sin^2(\theta)/\lambda^2]\} \exp[2\pi i(hx_j + ky_j + lz_j)], \qquad (25.2.1.5)$$

where O_j is the occupancy, f_j is the (possibly complex) scattering factor, B_j is the isotropic temperature parameter and x_j, y_j, z_j are the fractional coordinates of the jth atom. Sc is a scale factor relating the calculated structure factor (absolute scale) to the scale of the observed data. The summation is taken over all heavy atoms or anomalous scatterers in the unit cell. Alternatively, anisotropic temperature parameters can be used for each atom if desired. A subset of reflections comprising all centric data (plus the largest 25% of the isomorphous or anomalous differences if there are insufficient centric data) is selected and used to estimate Sc by a least-squares fit to the observed differences. Initial estimates of the 'standard error' E (expected lack of closure) are determined from

 references