11.4.7. Detector diagnostics

The *HKL* package has a number of tools that can detect possible detector or experimental setup problems (Minor & Otwinowski, 1997). Visual inspection of the image may provide only a very rough estimate of data quality. A check of the analogue-to-digital converter can provide rough diagnostics of detector electronics. Examination of the background can provide information about detector noise, especially when uncorrected images can be examined in the areas exposed to X-rays and areas where pure read-out noise can be observed. *DENZO* provides several diagnostic tools during the integration stage, as the crystallographer may observe crystal slippage, a change of unit-cell parameters or a change of the values of positional and angular χ^2 during the refinement. Even more tools are provided at the data-scaling stage. By observing scale factors, poor crystal alignment can be detected. Other tools may help diagnose X-ray shutter malfunction, spindle axis alignment and internal detector alignment problems. The final inspection of outliers may again provide valuable information about detector quality. The clustering of outliers in one area of the detector may indicate a damaged surface; if most outliers are partials, it may indicate a problem with spindle backlash or shutter control. The zoom mode may be used to display the area around the outliers to identify the source of a problem: for example, the existence of a satellite crystal or single pixel spikes due to electronic failure. Sometimes, even for very strong data, a histogram of the pixel intensities may stop below the maximum valid pixel value, indicating saturation of the data-acquisition hardware or software.

11.4.8. Multiplicative corrections (scaling)

Proper error estimation requires the use of Bayesian reasoning and a multi-component error model (Schwarzenbach *et al.*, 1989; Evans, 1993). In *SCALEPACK*, the estimated error of the measurement is enlarged by a fraction of the expected, rather than the observed, intensity. This algorithm reduces the bias towards reflections with an integrated intensity below the average.

The scaling model allows for a large number of diverse components to contribute to the multiplicative correction factor s for each observation,

$$s = \exp\left[\sum_i p_i f_i(hkl)\right], \qquad (11.4.8.1)$$

where p_i are *a priori* unknown coefficients of the scaling components and f_i represent different functional dependence of the scale factor for each observation. The simplest scaling model has a separate scale factor for each group (batch) of data, for example, one scale factor per image. In such a case,

$$f_i = \delta_{ij}, \qquad (11.4.8.2)$$

where j is the batch index for a particular reflection. For resolution-dependent decay, represented by one temperature factor per batch of data,

$$f_{i+n} = [(\mathbf{S}\cdot\mathbf{S})/2]\delta_{ij}, \qquad (11.4.8.3)$$

where n is the number of batches needed to make p_i represent the logarithm of the overall ith batch scale factor and p_{i+n} represents the temperature factor of batch i. $\mathbf{S}$ is the scattering vector for each reflection.

Coefficients of crystal absorption are much more complex. Scaling coefficients are associated with spherical harmonics (Katayama, 1986; Blessing, 1995) as a function of the direction of vector $\mathbf{S}$, expressed in the coordinate system of the rotating crystal. Each spherical harmonic index lm has two (or, in the case of $m = 0$, one) p_g coefficients. One of these spherical harmonic functions is given by

$$f_g = \left[\frac{(2l+1)}{4\pi}\frac{(l-m)!}{(l+m)!}\right]^{1/2} P_{lm}(\cos\Psi)\cos(m\Phi), \qquad (11.4.8.4)$$

where Ψ and Φ are polar-coordinate angles of vector $\mathbf{S}$ in the crystal coordinate system, and P_{lm} is a Legendre polynomial (Press *et al.*, 1989). The other spherical harmonic of index lm has a sine instead of a cosine as the last term.

The multiplicative factor is applied to each observation and its σ to obtain the corrected intensity $I_{\rm corr}$ and associated σ. The averaged intensity over all symmetry-related reflections $\langle I_{\rm corr}\rangle$ is obtained by solving the two following equations:

$$W = 1\Big/[(\sigma E_1)^2 + (\langle I_{\rm corr}\rangle E_2)^2], \qquad (11.4.8.5)$$

where E_1 and E_2 are the user-specified *error scale factor* and *estimated error*, respectively, and

$$\langle I_{\rm corr}\rangle = \sum I_{\rm corr} W \Big/ \sum W. \qquad (11.4.8.6)$$

Thus,

$$\sigma(I) = I\Big/[\textstyle\sum W]^{1/2}. \qquad (11.4.8.7)$$

During parameter refinement, the scale (and B, if requested) for all scaled batches are refined against the difference between the $\langle I_{\rm corr}\rangle$'s and $I_{\rm corr}$'s for individual measurements, summed over all reflections (Fox & Holmes, 1966; Arndt & Wonacott, 1977; Stuart & Walker, 1979; Leslie & Tsukihara, 1980; Rossmann & Erickson, 1983; Walker & Stuart, 1983; Rossmann, 1984; Schutt & Evans, 1985; Stuart, 1987; Takusagawa, 1987; Tanaka *et al.*, 1990). $\langle I_{\rm corr}\rangle$'s are recalculated in each cycle of refinement. There is full flexibility in the treatment of anomalous pairs. They can be assumed to be equivalent (or not) and they may be merged (or not). This approach allows the crystallographer to choose the best scaling and merging strategy.

11.4.8.1. *Polarization*

A polarization correction may be applied during *DENZO* calculations. Sometimes the exact value of polarization is not known. This error may be corrected during the scaling procedure. This feature can be used to refine the polarization at synchrotron beamlines. Very high resolution data should be used for this purpose.

11.4.9. Global refinement or post refinement

The process of refining crystal parameters using the combined reflection intensity measurements is known as global refinement or post refinement (Rossmann, 1979; Evans, 1993). The implementation of this method in *SCALEPACK* allows for separate refinement of the orientation of each image, but with the same unit-cell value for the whole data set. In each batch of data (a batch is typically one image), different unit-cell parameters may be poorly determined. However, in a typical data set, there are enough orientations to determine all unit-cell lengths and angles precisely. Global refinement is also more precise than the processing of a single image in the determination of crystal mosaicity and the orientation of each image.

11.4.10. Graphical command centre

The goal of the command centre is to coordinate all phases of the experiment and to facilitate interactive experiments in which data

references