(2) Parameters that can be determined from internal self-consistency of the data, but for which least squares is not implemented. For example, error-estimate parameters are in this category.

(3) Parameters that have to be established in a separate experiment, *e.g.* pixel sensitivity from flood-field exposure.

(4) Parameters that are obtained from hardware description.

The least-squares method is based on minimization of a function that is a sum of contributors of the following type:

$$(\text{pred} - \text{obs})^2/\sigma^2 = \chi^2, \qquad (11.4.5.1)$$

where pred is a prediction based on some parameterized model, obs is the value of this prediction's measurement and σ^2 is an estimate of the measurement and the prediction uncertainty. *DENZO* has the following least-squares refinements:

(1) refinement of unit-cell vectors in autoindexing;

(2) refinement of background and background slope; and

(3) refinement of crystal orientation, unit cell, mosaicity, beam focus and position, detector orientation and position, and geometrical distortions that are parameterized differently for different detectors.

SCALEPACK can refine the following parameters by least-squares methods:

(1) unit cell, crystal orientation and mosaicity, including changes of these parameters during an experiment;

(2) goniostat internal alignment angles;

(3) crystal absorption, using spherical harmonics (Katayama, 1986; Blessing, 1995) expansion of the absorption surface;

(4) uniformity of exposure, including shutter timing error;

(5) correction to the Lorentz factor resulting from a misalignment of the spindle axis;

(6) reproducible wobble of the rotation axis resulting from a misalignment of gears in a spindle assembly;

(7) non-uniform smooth detector response, for example, resulting from decay of the image-plate signal during scanning; and

(8) other factors contributing to scaling resulting from a slow fluctuation of beam intensity, change in exposed volume, overall crystal decay and resolution-dependent crystal decay.

11.4.5.4. *Correlation between parameters*

Occasionally, the refinement can be unstable due to high correlation between some parameters. High correlation results in the errors in one parameter compensating for the errors in other parameters. In the case where compensation is 100%, the parameter would be undefined, but the error compensation by other parameters would make the predicted pattern correct. In such cases, eigenvalue filtering [related to singular value decomposition, described by Press *et al.* (1989) in *Numerical Recipes*] is employed to remove the most correlated components from the refinement to make it more stable. Eigenvalue filtering works reliably when starting parameters are close to the correct values, but may fail to correct large errors in the input parameters if the correlation is close to, but not exactly, 100%. Once the whole data set is integrated, global refinement [also called post refinement: Rossmann *et al.* (1979); Winkler *et al.* (1979); Evans (1987); Greenhough (1987); Evans (1993); Kabsch (1993)] can refine crystal parameters (unit cell and orientation) more precisely and without correlation with detector parameters. The unit cell used in structure-determination calculations should come from the global refinement (in *SCALEPACK*) and not from *DENZO* refinement.

11.4.5.5. *Single- and multiframe refinement*

The crystal and detector orientation parameters can be refined for each group of images or for each processed image separately. Refinement performed separately for each image allows for robust data processing, even when the crystal slips considerably during data collection.

11.4.5.6. *Active area*

Not every pixel represents a valid measurement. Specification of the active detector area in *DENZO* is derived from the format and the definition of the detector size. Detector calibration with flood-field exposure will calculate the sensitivity for each pixel and will also determine which pixels should be ignored. The input command can additionally label some areas of the detector to be ignored, most frequently the shadow caused by the beam stop and its support. To define the shape of the area shadowed by the beam stop, the useful commands are *ignore circle* and *ignore quadrilateral.* There are also commands to ignore triangular shapes, margins of the detector and a particular line or pixel.

11.4.5.7. *Flood field*

The basic method for calibration of the spatial dependence of detector sensitivity is to measure the response to a flood-field exposure. The amount of relative exposure per pixel needs to be known. *DENZO* allows for either a uniform or an isotropic source. If the source is at the crystal position, *DENZO* refinement (with a separate crystal exposure) can be used to define the geometry of the source relative to the detector. To calculate the flood-field response, an earlier determination of the detector distortion is required. The flood-field response is converted to a sensitivity function. Large deviations from the local average are used to define inactive pixels. The edge of the active area needs special treatment, depending on the method of phosphorus deposition.

11.4.5.8. *Absolute configuration*

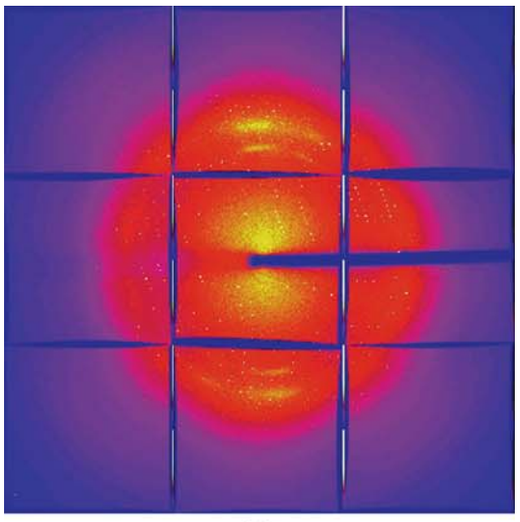
Absolute configuration is defined relative to the data-coordinate system and is only affected by the sign of the parameter *y scale.* A mirror transformation of the data does not affect the self-consistency of the data. Thus, the correctness of the absolute configuration cannot be verified by data-reduction programs.

11.4.5.9. *Correcting diffraction images*

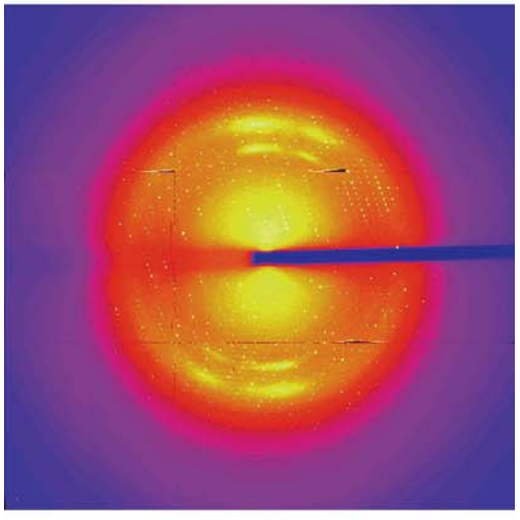
HKL can also generate data corrected for the above factors and/or for geometrical conversion and distortion in uncompressed, lossless compressed and lossy (non-reversible to the last digit) compressed modes in linear or 16 bit floating-point encoded format. Fig. 11.4.5.1 shows data from the APS-1 detector in (*a*) uncorrected mode, (*b*) transformed to an ideal rectangular detector and (*c*) transformed to a spherical detector.

11.4.5.10. *Detector goniostat*

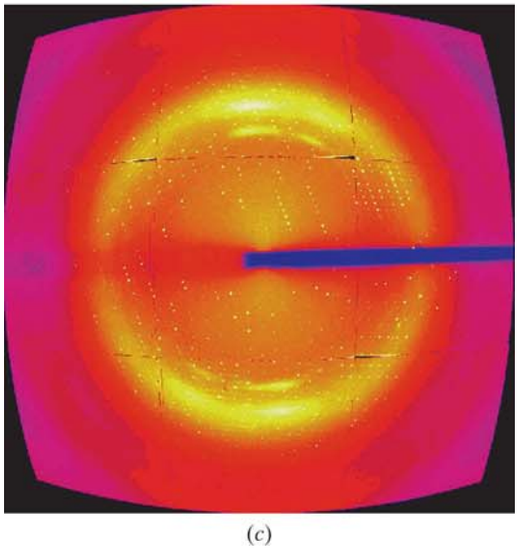
The detector goniostat in *DENZO* can have only one rotation axis – 2θ. In the complex transformations described in equation (11.4.2.8), the geometrical scale is affected by pixel-to-millimetre conversion and distortion. For different instruments, the scale is defined differently. For detectors without distortion, the scale is defined by the value of the pixel size in the 'slow' direction. For detectors with distortion characterized by polynomials (*e.g.* CCD detectors), the scale is also defined by the way the distortion was determined. In such a case, the source of scale is the separation between holes in the reference grid mask or, alternatively, the goniostat translation. As the distance of the detector active surface from the crystal cannot be measured precisely, the difference between the two distances is the ultimate source of the scale reference. The angle between the detector distance translation and



(*a*)



(*b*)



(*c*)

Fig. 11.4.5.1. The transformations in *DENZO* applied to APS-1 detector data. (*a*) Raw data are affected by geometrical distortion introduced by nine fibre-optic tapers; (*b*) the same image converted to planar Cartesian space; (*c*) the same data converted to a virtual spherical detector.

the X-ray beam completes the definition of the detector goniostat in *HKL*.

11.4.5.11. *Crystal goniostat*

The physical goniostat is defined by six angles. Two angles define the direction of the main axis (ω) in the *DENZO* coordinate system. The third angle defines the zero position of the ω axis. The fourth is the angle between ω and the second axis (κ or χ). The fifth defines the zero position of the second axis. The sixth is the angle between the second and the third axes. This type of goniostat definition allows for the specification of any three-axis goniostat (*EEC Cooperative Workshop on Position-Sensitive Detector Software*, 1986). Each type of goniostat is represented by six angles. Misalignment of the goniostat is represented as an adjustment to these angles, which can be refined by the *HKL* system.

11.4.5.12. *Crystal orthogonalization convention*

Crystal orientation specified by the three angles needs a definition of a zero point. Any crystal axis, or its equivalent reciprocal-space zone perpendicular to it, can be used as a reference. The definition of zero point aligns the crystal axis with the beam direction and one of the reciprocal axes with the x direction. The user can specify both axes.

11.4.5.13. *Refinement and calibration*

Both the refinement and calibration procedures determine the properties of the instrument. The principal difference between refinement and calibration is that calibration is performed with data obtained outside the current diffraction experiment, and refinement uses data obtained during the current diffraction experiment. *DENZO* performs both refinement and calibration, and in some cases the difference between calibration and refinement is a question of semantics, as the refined data from one experiment can be used as a reference for another experiment, or even as a reference for a subsequent refinement cycle or for another part of the same experiment.

11.4.6. Prediction of the diffraction pattern

The autoindexing procedure assigns Miller indices only to strong spots, ones that can be found through a peak search. The target of the experiment is to estimate structure factors for all reflections captured by the detector. Therefore, positions of all spots need to be predicted by applying the following equations to all possible triplets **h**. Using

$$\mathbf{S} = [A]^{-1}\mathbf{h}, \qquad (11.4.6.1)$$

we have to find the matrix $[A]$ that generates the vector **S**, which satisfies the diffraction condition [equation (11.4.2.1)], knowing that the matrix $[A]$ is a function of the crystal orientation [equation (11.4.2.6)]. The rotation of the crystal during the experiment creates a straightforward algebraic problem that results in a complex equation defining the angle at which the reflection occurs. This angle also defines the image at which the reflection appears. Knowing this angle, the vector **S** can be calculated, and, from equation (11.4.2.5), the direction of the vector **X** can be found:

$$\mathbf{X}/|\mathbf{X}| = \lambda(\mathbf{S} - \mathbf{S}_0). \qquad (11.4.6.2)$$

Calculation of the length of vector **X** requires knowledge of the detector orientation, which, for flat detectors, is described here by vector **G**, perpendicular to the detector and with length equal to the crystal-to-detector distance: