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). Misalignment of the goniostat is represented as an adjustment to these angles, which can be refined by the *HKL* system.

11.4.5.11. Crystal orthogonalization convention

Crystal orientation specified by the three angles needs a definition of a zero point. Any crystal axis, or the 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 in the *DENZO* coordinate system. The user can specify which crystal axes will be aligned at zero crystal orientation angles by specifying the so-called reference zone.

11.4.5.12. 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 not obtained during the current diffraction experiment, and refinement uses data obtained during the current diffraction experiment. *DENZO* performs both refinement and calibration. 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 procedure. The target of the experiment is to estimate structure-factor amplitudes 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

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

a matrix [**A**] must be found that generates the vector **S** that satisfies the diffraction condition [equation (11.4.2.1)], knowing that the matrix [**A**] is a function of the crystal orientation at the goniostat angles that generated the reflection with indices **h** [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 consequently defines the image or images on 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:

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

Calculation of the length of vector **X** requires a knowledge of 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:

$$\mathbf{Y} = [\mathbf{R}_{2\theta}]^{-1}\lambda(\mathbf{S} - \mathbf{S}_0), \tag{11.4.6.3}$$

$$\mathbf{H} = [\mathbf{R}_z]^{-1}[\mathbf{R}_y]^{-1}[\mathbf{R}_x]^{-1}\mathbf{G}, \tag{11.4.6.4}$$

$$\mathbf{X} = [\mathbf{R}_{2\theta}]\frac{\mathbf{H}\cdot\mathbf{H}}{\mathbf{Y}\cdot\mathbf{H}}\mathbf{Y}. \tag{11.4.6.5}$$

Then, by inverting equation (11.4.2.8), the position in pixels $\{p, q\}$ of the reflection can be calculated:

$$\{p, q\} = [\mathbf{D}]^{-1}[\mathbf{K}]^{-1}([\mathbf{L}]^{-1}([\mathbf{R}_{2\theta}]^{-1}([\mathbf{R}_z]^{-1}[\mathbf{R}_y]^{-1}[\mathbf{R}_x]^{-1}(\mathbf{X} - \mathbf{T}_D) + \mathbf{T}_D) - \mathbf{T}_D) + \mathbf{B}). \tag{11.4.6.6}$$

11.4.6.1. Refinement of crystal and detector parameters

The precision of the integration step depends on precise knowledge of the peak positions. The autoindexing step provides only an approximate orientation of the crystal, and the result of that step is imprecise if the initial values of the detector parameters are poorly known. A nonlinear least-squares refinement process is used to improve the prediction (EEC Cooperative Workshop on Position-Sensitive Detector Software, 1986). Depending on the particulars of the experiment, the same parameters (*e.g.* crystal-to-detector distance) may either be known more precisely *a priori*, or are better estimated from the diffraction data. *DENZO* allows either fixing or refining of each of the parameters separately. This flexibility is important when characterizing a detector, but when detector parameters are already known, the *fit all* option and detector-specific default values are quite reliable.

DENZO can refine the six parameters describing the position and orientation of the detector in space. It can also refine internal parameters of the detector including:

(1) curvature radius and rotation of the active surface – *film rotation*, for cylindrical detectors;
(2) x to y scale and x to y skew;
(3) radial/angular distortion for spiral scanners; and
(4) polynomial distortion, separate for each CCD module of multi-CCD detectors (Naday *et al.*, 1998).

Detector- and crystal-parameter refinement in *DENZO* is achieved by minimizing the sum of three functions [equations (11.4.6.7), (11.4.6.8) and (11.4.6.11)] of the type in equation (11.4.5.1). The contribution resulting from the measurement of position $\{p, q\}$ of the reflection is

$$\chi_p^2 = \sum_{hkl}(p_{\rm pred} - p_{\rm cent})^2/\sigma_p^2, \tag{11.4.6.7}$$

$$\chi_q^2 = \sum_{hkl}(q_{\rm pred} - q_{\rm cent})^2/\sigma_q^2. \tag{11.4.6.8}$$

where $p_{\rm pred}, q_{\rm pred}$ is the predicted position of the reflection in pixel coordinates, $p_{\rm cent}, q_{\rm cent}$ is the centroid position of the observed reflection in pixel coordinates and σ_p, σ_q are combined estimates of uncertainties of the observed and predicted positions.

11.4.6.2. Bragg's law for non-ideal conditions: mosaicity

The Bragg condition [equation (11.4.2.1)] assumes diffraction from ideal crystals and a parallel X-ray beam. In reality, crystals are mosaic and the beam has some angular spread. The value of the *mosaicity* keyword describes the range of orientations of the crystal lattice within a sample. As the impacts of mosaicity and the beam's angular spread on the angular width of reflections are