interactions with the rest of the structure and, following refinement of the structure, by elimination of the peak in the difference map and by a significant improvement in the agreement between the calculated and measured X-ray amplitudes.

4.5.2.6.5. Difference Fourier synthesis

Difference Fourier syntheses are widely used in both protein and small-molecule crystallography to detect structural errors or to complete partial structures (Drenth, 1994). The difficulty in applying difference Fourier techniques in fibre diffraction is that the individual observed amplitudes $|F_o|$ are not available. However, difference syntheses have found wide use in fibre diffraction analysis, one of the earliest applications being to polycrystalline fibres of polynucleotides (*e.g.* Arnott *et al.*, 1967). Calculation of a three-dimensional difference map (for the unit cell) from Bragg fibre diffraction data requires that the observed intensity $I_l(R_{hk}) = I_o$ be apportioned among the contributing intensities $|F_{hkl}|^2 = |F_o|^2$. There are two ways of doing this. The intensities may be divided equally among the contributing $(m/2)$ reflections [*i.e.* $|F_o| = (2I_o/m)^{1/2}$], or they may be divided in the same proportions as those in the model, *i.e.*

$$|F_o| = \left(\frac{I_o}{\sum|F_c|^2}\right)^{1/2} |F_c|. \qquad (4.5.2.63)$$

The advantage of the former is that it is unbiased, and the advantage of the latter is that it may be more accurate but is biased towards the model. Equal division of the intensities is often (but not always) used to minimize model bias. Once the observed amplitudes have been apportioned, an $|F_o| - |F_c|$ map can be calculated as in conventional crystallography, although noise levels will be higher owing to errors in apportioning the amplitudes. As a result of overlapping of the reflections, a synthesis based on coefficients $m|F_o| - (m-1)|F_c|$ gives a more accurate estimate of the true density than does one based on $2|F_o| - |F_c|$, as is described below. Difference syntheses for polycrystalline specimens calculated in this way have been used, for example, to locate cations and water molecules in polynucleotide and polysaccharide structures (*e.g.* Cael *et al.*, 1978), to help position molecules in the unit cell (*e.g.* Chandrasekaran *et al.*, 1994) and to help position side chains, and have also been applied in neutron fibre diffraction studies of polynucleotides (Forsyth *et al.*, 1989).

Sim (1960) has shown that the mean-squared error in difference syntheses can be minimized by weighting the coefficients based on the agreement between the calculated and observed structure amplitudes. Such an analysis has recently been conducted for fibre diffraction, and shows that the optimum difference synthesis is obtained by using coefficients (Millane & Baskaran, 1997; Baskaran & Millane, 1999*a*)

$$\left[w_m \frac{|F_c|(I_o)^{1/2}}{(\sum|F_c|^2)^{1/2}} - |F_c|\right] \exp(i\alpha_c), \qquad (4.5.2.64)$$

where m is the number of degrees of freedom as defined in Section 4.5.2.6.1. If the reflections contributing to I_o are either all centric or all acentric, then the weights are given by

$$w_m = \frac{I_{m/2}(X)}{I_{m/2-1}(X)}, \qquad (4.5.2.65)$$

where $I_m(\cdot)$ denotes the modified Bessel function of the first kind of order m, and X is given by

$$X = \frac{\kappa(I_o)^{1/2}(\sum|F_c|^2)^{1/2}}{\sum_j f_j^2}, \qquad (4.5.2.66)$$

where $\kappa = 1$ for centric reflections and 2 for acentric reflections. The form of the weighting function is more complicated if both centric and acentric reflections contribute, but it can be approximated as w' given by

$$w' = (w_{2N_a} + w_{N_c})/2, \qquad (4.5.2.67)$$

where N_a and N_c are the number of acentric and centric reflections, respectively, contributing. Use of the weighted maps reduces bias towards the model (Baskaran & Millane, 1999*b*).

For continuous diffraction data from noncrystalline specimens, the situation is essentially identical except that one works in cylindrical coordinates. Referring to equations (4.5.2.7) and (4.5.2.10), the desired difference synthesis, $\Delta g(r, \varphi, z)$, is the Fourier–Bessel transform of $G_o - G_c$ where G_o and G_c denote the observed and calculated, respectively, Fourier–Bessel structure factors $G_{nl}(R)$. Since G_o is not known, the synthesis is based on the Fourier–Bessel transform of $(|G_o| - |G_c|)\exp(i\alpha_c)$, where α_c is the phase of G_c. As in the polycrystalline case, the individual $|G_o|$ need to be estimated from the data $I_o^{1/2}$ given by equation (4.5.2.17), and can be based on either equal division of the data, or division in the same proportion as the amplitudes from the model.

Namba & Stubbs (1987*a*) have shown that the peak heights in a difference synthesis are $1/m$ times their true value, as opposed to half their true value in a conventional difference synthesis. The best estimate of the true map is therefore provided by a synthesis based on the coefficients $[m|F_o| - (m-1)|F_c|]\exp(i\alpha_c)$, rather than on $(2|F_o| - |F_c|)\exp(i\alpha_c)$. Test examples showed that the noise in the synthesis can be reduced by using a value for m that is fixed over the diffraction pattern and approximately equal to the average value of m over the pattern (Namba & Stubbs, 1987*a*). Difference Fourier maps for noncrystalline systems have been used in studies of helical viruses to locate heavy atoms, to correct errors in atomic models and to locate water molecules (Mandelkow *et al.*, 1981; Lobert *et al.*, 1987; Namba, Pattanayek & Stubbs, 1989; Wang & Stubbs, 1994).

4.5.2.6.6. Multidimensional isomorphous replacement

At low enough resolution, only one Fourier–Bessel structure factor contributes on each layer line of a fibre diffraction pattern, so that only the phase needs to be determined and the situation is no different to that in protein crystallography. If heavy-atom-derivative specimens can be prepared, the usual method of multiple isomorphous replacement (MIR) (Drenth, 1994) can be applied, which in principle requires only two heavy-atom derivatives. At higher resolution, however, more than one Fourier–Bessel structure factor contributes on each layer line. A generalized form of isomorphous replacement which involves using diffraction data from several heavy-atom derivatives to determine the real and imaginary components of each contributing $G_{nl}(R)$ is referred to as *multidimensional isomorphous replacement* (MDIR) (Namba & Stubbs, 1985). MDIR was first described and used to determine the structure of TMV at 6.7 Å resolution (Stubbs & Diamond, 1975; Holmes *et al.*, 1975), and has since been used to extend the resolution to 2.9 Å (Namba, Pattanayek & Stubbs, 1989). A consequence of cylindrical averaging is that large numbers of heavy-atom derivatives are required: at least two for each Bessel term to be separated. The theory of MDIR is outlined here.

The first step in MDIR is location of the heavy atoms in the derivative structures. The radial coordinate of a heavy atom can be determined by analysis of the intensity distribution in the low-