

16. DIRECT METHODS

when all phases are zero. In practice, quartets are rarely used in the minimal function because they increase the CPU time while adding little useful information for large structures.

The cosine function in equation (16.1.4.2) can also be replaced by other functions of the phases giving rise to alternative minimal functions. Examples include an exponential expression that has been found to give superior results for several *P1* structures (Hauptman *et al.*, 1999). In addition, substructure determination using a very simple and computationally efficient modified minimal function,

$$m(\varphi) = 1 - (N_I/N_T) \quad (16.1.4.3)$$

(where I is an arbitrary interval $[-r, r]$, N_I is the number of triplets whose values lie in I and N_T is the total number of triplets), has been reported (Xu & Hauptman, 2004, 2006; Xu *et al.*, 2005) and incorporated into the *BnP* software (see Section 16.1.12.4).

16.1.4.3. Parameter shift

In principle, any minimization technique could be used to minimize $R(\Phi)$ by varying the phases. So far, a seemingly simple algorithm, known as parameter shift (Bhuiya & Stanley, 1963), has proven to be quite powerful and efficient as an optimization method when used within the *Shake-and-Bake* context to reduce the value of the minimal function. For example, a typical phase-refinement stage consists of three iterations or scans through the reflection list, with each phase being shifted a maximum of two times by 90° in either the positive or negative direction during each iteration. The refined value for each phase is selected, in turn, through a process which involves evaluating the minimal function using the original phase and each of its shifted values (Weeks, DeTitta *et al.*, 1994). The phase value that results in the lowest minimal-function value is chosen at each step. Refined phases are used immediately in the subsequent refinement of other phases. It should be noted that the parameter-shift routine is similar to that used in ψ -map refinement (White & Woolfson, 1975) and *XY* (Debaerdemaeker & Woolfson, 1989).

16.1.5. Real-space constraints (*baking*)

For several decades, classical direct methods operated exclusively in reciprocal space, determining phases through statistical relationships between them. Only when this process had converged did the method move into real space by calculating one or more electron-density maps that were examined using stereochemical criteria. In macromolecular crystallography, density modification has always played a central role in phasing. A major advance in direct methods for macromolecules (and large molecules in general) occurred when density-modification methods were incorporated and adapted into the phasing procedure. They are often very simple: peaks which give rise to unrealistic geometries or which are too weak are removed, new structure factors are calculated and hence new phase angles are derived in an iterative process. (They can also be quite sophisticated as in *ACORN2*, which we will discuss in Section 16.1.12.1.) A consequence of this is that the once-clear dividing line between direct methods and other structure-solution techniques has become somewhat blurred.

Peak picking is a simple but powerful way of imposing an atomicity constraint. The potential for real-space phase improvement in the context of small-molecule direct methods was recognized by Karle (1968). He found that even a relatively small, chemically sensible, fragment extracted by manual inter-

pretation of an electron-density map could be expanded into a complete solution by transformation back to reciprocal space and then performing additional iterations of phase refinement with the tangent formula. Automatic real-space electron-density-map interpretation in the *Shake-and-Bake* procedure consists of selecting an appropriate number of the largest peaks in each cycle to be used as an updated trial structure without regard to chemical constraints other than a minimum allowed distance between atoms. If markedly unequal atoms are present, appropriate numbers of peaks (atoms) can be weighted by the proper atomic numbers during transformation back to reciprocal space in a subsequent structure-factor calculation. Thus, *a priori* knowledge concerning the chemical composition of the crystal is utilized, but no knowledge of constitution is required or used during peak selection. It is useful to think of peak picking in this context as simply an extreme form of density modification appropriate when atomic resolution data are available. In theory, under appropriate conditions it should be possible within the dual-space direct-methods framework to replace peak picking by alternative density-modification procedures such as low-density elimination (Shiono & Woolfson, 1992; Refaat & Woolfson, 1993) or solvent flattening (Wang, 1985). The imposition of physical constraints counteracts the tendency of phase refinement to propagate errors or produce overly consistent phase sets. Several variants of peak picking, which are discussed below, have been successfully employed within the framework of *Shake-and-Bake*.

16.1.5.1. Simple peak picking

In its simplest form, peak picking consists of simply selecting the top N_u *E*-map peaks where N_u is the number of unique non-H atoms in the asymmetric unit. This is adequate for true small-molecule structures. It has also been shown to work well for heavy-atom or anomalously scattering substructures where N_u is taken to be the number of expected substructure atoms (Smith *et al.*, 1998; Turner *et al.*, 1998). For larger structures ($N_u > 100$), it is likely to be better to select about $0.8N_u$ peaks, thereby taking into account the probable presence of some atoms that, owing to high thermal motion or disorder, will not be visible during the early stages of a structure determination. Furthermore, a study by Weeks & Miller (1999b) has shown that structures in the 250–1000-atom range which contain a half dozen or more moderately heavy atoms (*i.e.*, S, Cl, Fe) are more easily solved if only $0.4N_u$ peaks are selected. The only chemical information used at this stage is a minimum inter-peak distance, generally taken to be 1.0 Å. For substructure applications, a larger minimum distance (*e.g.* 3 Å) is more appropriate, provided that care is taken with disulfide bridges (Section 16.1.11).

16.1.5.2. Iterative peaklist optimization

An alternative approach to peak picking is to select approximately N_u peaks as potential atoms and then eliminate some of them, one by one, while maximizing a suitable figure of merit such as

$$P = \sum_{\mathbf{H}} |E_c^2| (|E_0^2| - 1). \quad (16.1.5.1)$$

The top N_u peaks are used as potential atoms to compute $|E_c|$. The atom that leaves the highest value of P is then eliminated. Typically, this procedure, which has been termed *iterative peaklist optimization* (Sheldrick & Gould, 1995), is repeated until only $2N_u/3$ atoms remain. Use of equation (16.1.5.1) may be regarded as a reciprocal-space method of maximizing the fit to the origin-removed sharpened Patterson function, and it has been used for

this purpose in molecular replacement (Beurskens, 1981). Subject to various approximations, maximum-likelihood considerations also indicate that it is an appropriate function to maximize (Bricogne, 1998). Iterative peaklist optimization provides a higher percentage of solutions than simple peak picking, but it suffers from the disadvantage of requiring much more CPU time and so is less effective than the random-omit method described in the next section.

16.1.5.3. Random omit maps

A third peak-picking strategy involves selecting approximately $1.3N_u$ of the top peaks and eliminating some, but, in this case, the deleted peaks are chosen at random. Typically, one-third of the potential atoms are removed, and the remaining atoms are used to compute E_c . By analogy to the common practice in macromolecular crystallography of omitting part of a structure from a Fourier calculation in the hope of finding an improved position for the deleted fragment, this version of peak picking is described as *random omit*. This procedure helps to prevent the dual-space recycling from getting stuck in a local minimum and is thus an efficient search algorithm.

16.1.6. Fourier refinement

E-map recycling, but without phase refinement (Sheldrick, 1982, 1990; Kinneging & de Graaff, 1984), has been frequently used in conventional direct-methods programs to improve the completeness of the solutions after phase refinement. It is important to apply Fourier refinement to *Shake-and-Bake* solutions also because such processing significantly increases the number of resolved atoms, thereby making the job of map interpretation much easier. Since phase refinement *via* either the tangent formula or the minimal function requires relatively accurate invariants that can only be generated using the larger *E* magnitudes, a limited number of reflections are phased during the actual dual-space cycles. Working with a limited amount of data has the added advantage that less CPU time is required. However, if the current trial structure is the ‘best’ so far based on a figure of merit (either the minimal function or a real-space criterion), then it makes sense to subject this structure to Fourier refinement using additional data, thereby reducing series-termination errors. The correlation coefficient

$$\begin{aligned} \text{CC} = & \left[\left(\sum wE_o^2 E_c^2 \sum w \right) - \left(\sum wE_o^2 \sum wE_c^2 \right) \right] \\ & \times \left\{ \left[\left(\sum wE_o^4 \sum w \right) - \left(\sum wE_o^2 \right)^2 \right] \right. \\ & \left. \times \left[\left(\sum wE_c^4 \sum w \right) - \left(\sum wE_c^2 \right)^2 \right] \right\}^{-1/2} \end{aligned} \quad (16.1.6.1)$$

(Fujinaga & Read, 1987), where weights $w = 1/[0.04 + \sigma^2(E_o)]$, has been found to be an especially effective figure of merit when used with all the data and is, therefore, suited for identifying the most promising trial structure at the end of Fourier refinement. Either simple peak picking or iterative peaklist optimization can be employed during the Fourier-refinement cycles in conjunction with weighted *E* maps (Sim, 1959). The final model can be further improved by isotropic displacement parameter (B_{iso}) refinement for the individual atoms (Usón *et al.*, 1999) followed by calculation of the Sim (1959) or sigma-A (Read, 1986) weighted map. This is particularly useful when the requirement of atomic resolution is barely fulfilled, and it makes it easier to interpret the resulting maps by classical macromolecular methods.

16.1.7. Resolution enhancement: the ‘free lunch’ algorithm

Direct methods take a set of phases, refine them and also determine new ones. There is no reason, however, why they cannot be used to predict new amplitudes as well. If density modification of a real-space map is performed, then any process of real-space density modification will, following a Fourier transformation, give structure-factor amplitudes for reflections that were not used to generate it, and these can be outside the resolution limit. If direct methods require atomic resolution data, can we use these techniques to extrapolate structure factors (*i.e.* predict not only phases, but also amplitudes for missing data) and extend data resolution? The idea is not new, but it has been quite extensively studied in recent years. Sheldrick has termed such algorithms ‘free lunch’, with reference to the saying: ‘There is no such thing as a free lunch’! In one example (Usón *et al.*, 2007), weak SIRAS starting phase information followed by density modification led to an $|F_o|$ weighted mean phase error (MPE) of 54° at 1.98 Å resolution, but when the density modification was performed with amplitude extrapolation to 1.0 Å, the MPE fell to 17° . Caliandro *et al.* (2005a,b) used Patterson or direct methods to obtain trial phases that are submitted to various density-modification methods. Following this, extrapolated phases were generated. This too transformed uninterpretable maps into a solution amenable to automatic tracing. Palatinus *et al.* (2007) used maximum entropy (ME) methods for amplitude extrapolation. In some ways these should be ideal for this purpose, and it is worth noting that ME maps have, *de facto*, optimal resolution enhancement built in, although they can be difficult to generate for large structures.

Why does this work, and why is it sometimes so spectacular? The answer probably lies with the fact that maps are much more sensitive to phases than amplitudes and, if the model bias of predicting new amplitudes is not too great, then using a nonzero value is better than zero, which is the default. Fourier-truncation errors may also be reduced, resulting in less spurious map detail.

16.1.8. Utilizing Pattersons for better starts

When slightly heavier atoms such as sulfur are present, it is possible to start recycling procedures from a set of atomic positions that are consistent with the Patterson function. For large structures, the vectors between such atoms will correspond to Patterson densities around or even below the noise level, so classical methods of locating the positions of these atoms unambiguously from the Patterson are unlikely to succeed. Nevertheless, the Patterson function can still be used to filter sets of starting atoms. This filter is currently implemented as follows in *SHELXD*. First, a sharpened Patterson function (Sheldrick *et al.*, 1993) is calculated, and the top 200 (for example) non-Harker peaks further than a given minimum distance from the origin are selected, in turn, as two-atom translation-search fragments, one such fragment being employed per solution attempt. For each of a large number of random translations, all unique Patterson vectors involving the two atoms and their symmetry equivalents are found and sorted in order of increasing Patterson density. The sum of the smallest third of these values is used as a figure of merit (PMF). Tests showed that although the globally highest PMF for a given two-atom search fragment may not correspond to correct atomic positions, nevertheless, by limiting the number of trials, some correct solutions may still be found. The two-atom vectors are chosen by biased random sampling that favours the vectors corresponding to higher Patterson values. The two atoms