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 *P*1 structures (Hauptman *et al.*, 1999). In addition, substructure determination using a very simple and computationally efficient modified minimal function,

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

(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 *XMY* (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 interpretation 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 (1999*b*) 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

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

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