

2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

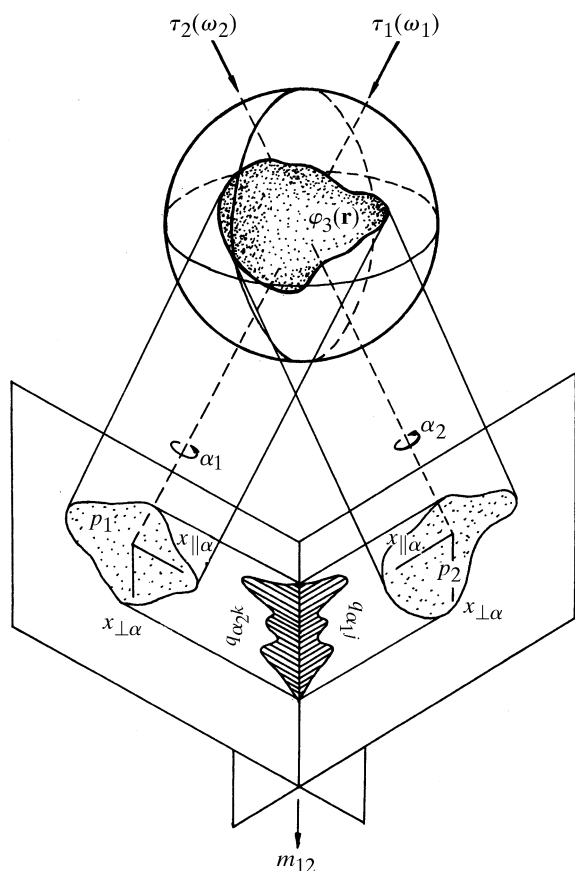


Fig. 2.5.6.6. Relative position of the particle and planes of projection.

accordance with *Lemma 1*, there exists a one-dimensional projection, common for both p_1 and p_2 , which determines angles α_{1j} and α_{2k} along which p_1 and p_2 should be projected for obtaining the identical projection q_{12} (Fig. 2.5.6.5). Comparing $q_{1, \alpha_{1j}}$ and $q_{2, \alpha_{2k}}$ and using the minimizing function

$$D(1,2) = |q_{1, \alpha_{1j}} - q_{2, \alpha_{2k}}|^2 \quad (2.5.6.30)$$

it is possible to find such a common projection q_{12} . (A similar consideration in Fourier space yields Q_{12} .)

The mutual spatial orientations of any three non-coplanar projection vectors τ_1, τ_2, τ_3 can be found from three different two-dimensional projections p_1, p_2 and p_3 by comparing the following pairs of projections: p_1 and p_2 , p_1 and p_3 , and p_2 and

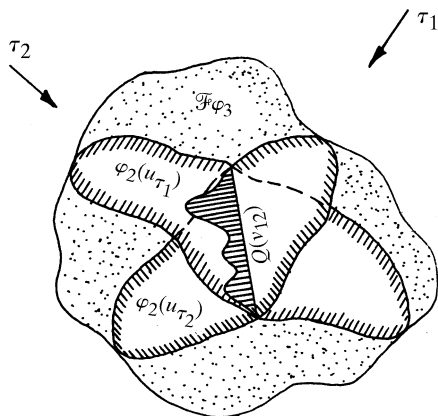


Fig. 2.5.6.7. Section of a three-dimensional Fourier transform of the density of the particles, corresponding to plane projections of this density.

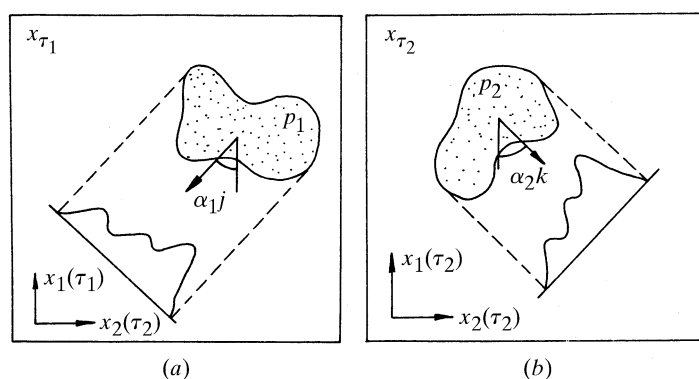


Fig. 2.5.6.8. Plane projections of a three-dimensional body. The systems of coordinates in planes (a) and (b) are chosen independently of one another.

p_3 , and by determining the corresponding q_{12}, q_{13} and q_{23} . The determination of angles ω_1, ω_2 and ω_3 reduces to the construction of a trihedral angle formed by planes h_{12}, h_{13} and h_{23} . Then the projections $p_i(\omega_i)$ with the known ω_i ($i = 1, 2, 3$) can be complemented with other projections ($i = 4, 5, \dots$) and the corresponding values of ω can be determined. Having a sufficient number of projections and knowing the orientations ω_i , it is possible to carry out the 3D reconstruction of the object [see (2.5.6.27); Orlov, 1975; Vainshtein & Goncharov, 1986a; Goncharov *et al.*, 1987].

2.5.7. Direct phase determination in electron crystallography (D. L. DORSET)

2.5.7.1. Problems with 'traditional' phasing techniques

The concept of using experimental electron-diffraction intensities for quantitative crystal structure analyses has already been presented in Section 2.5.4. Another aspect of quantitative structure analysis, employing high-resolution images, has been presented in Sections 2.5.5 and 2.5.6. That is to say, electron micrographs can be regarded as an independent source of crystallographic phases.

Before direct methods (Chapter 2.2) were developed as the standard technique for structure determination in small-molecule X-ray crystallography, there were two principal approaches to solving the crystallographic phase problem. First, 'trial and error' was used, finding some means to construct a reasonable model for the crystal structure *a priori*, e.g. by matching symmetry properties shared by the point group of the molecule or atomic cluster and the unit-cell space group. Secondly, the autocorrelation function of the crystal, known as the Patterson function (Chapter 2.3), was calculated (by the direct Fourier transform of the available intensity data) to locate salient interatomic vectors within the unit cell.

The same techniques had been used for electron-diffraction structure analysis (nowadays known as *electron crystallography*). In fact, advocacy of the first method persists. Because of the perturbations of diffracted intensities by multiple-beam dynamical scattering (Chapter 5.2), it has often been suggested that trial and error be used to construct the scattering model for the unit crystal in order to test its convergence to observed data after simulation of the scattering events through the crystal. This indirect approach assumes that no information about the crystal structure can be obtained directly from observed intensity data. Under more favourable scattering conditions nearer to the kinematical approximation, *i.e.* for experimental data from thin crystals made up of light atoms, trial and error modelling, simultaneously minimizing an atom–atom nonbonded potential function with the crystal-

2.5. ELECTRON DIFFRACTION AND ELECTRON MICROSCOPY IN STRUCTURE DETERMINATION

lographic residual, has enjoyed widespread use in electron crystallography, especially for the determination of linear polymer structures (Brisse, 1989; Pérez & Chanzy, 1989).

Interpretation of Patterson maps has also been important for structure analysis in electron crystallography. Applications have been discussed by Vainshtein (1964), Zvyagin (1967) and Dorset (1994a). In face of the dynamical scattering effects for electron scattering from heavy-atom crystals realized later (*e.g.* Cowley & Moodie, 1959), attempts had also been made to modify this autocorrelation function by using a power series in $|F_{\mathbf{h}}|$ to sharpen the peaks (Cowley, 1956). (Here $F_{\mathbf{h}} \equiv \Phi_{\mathbf{h}}$, replacing the notation for the kinematical electron-diffraction structure factor employed in Section 2.5.4.) More recently, Vincent and co-workers have selected first-order-Laue-zone data from inorganics to minimize the effect of dynamical scattering on the interpretability of their Patterson maps (Vincent & Exelby, 1991, 1993; Vincent & Midgley, 1994). Vainshtein & Klechkovskaya (1993) have also reported use of the Patterson function to solve the crystal structure of a lead soap from texture electron-diffraction intensity data.

It is apparent that trial-and-error techniques are most appropriate for *ab initio* structure analysis when the underlying crystal structures are reasonably easy to model. The requisite positioning of molecular (or atomic) groups within the unit cell may be facilitated by finding atoms that fit a special symmetry position [see *IT A* (1995)]. Alternatively, it is helpful to know the molecular orientation within the unit cell (*e.g.* provided by the Patterson function) to allow the model to be positioned for a conformational or translational search. [Examples would include the polymer-structure analyses cited above, as well as the layer-packing analysis of some phospholipids (Dorset, 1987).] While attempts at *ab initio* modelling of three-dimensional crystal structures, by searching an n -dimensional parameter space and seeking a global internal energy minimum, has remained an active research area, most success so far seems to have been realized with the prediction of two-dimensional layers (Scaringe, 1992). In general, for complicated unit cells, determination of a structure by trial and error is very difficult unless adequate constraints can be placed on the search.

Although Patterson techniques have been very useful in electron crystallography, there are also inherent difficulties in their use, particularly for locating heavy atoms. As will be appreciated from comparison of scattering-factor tables for X-rays [*IT C* (1999) Chapter 6.1] with those for electrons, [*IT C* (1999) Chapter 4.3] the relative values of the electron form factors are more compressed with respect to atomic number than are those for X-ray scattering. As discussed in Chapter 2.3, it is desirable that the ratio of summed scattering factor terms, $r = \sum_{\text{heavy}} Z^2 / \sum_{\text{light}} Z^2$, where Z is the scattering factor value at $\sin \theta / \lambda = 0$, be near unity. A practical comparison would be the value of r for copper (DL-alaninate) solved from electron-diffraction data by Vainshtein *et al.* (1971). For electron diffraction, $r = 0.47$ compared to the value 2.36 for X-ray diffraction. Orientation of salient structural features, such as chains and rings, would be equally useful for light-atom moieties in electron or X-ray crystallography with Patterson techniques. As structures become more complicated, interpretation of Patterson maps becomes more and more difficult unless an automated search can be carried out against a known structural fragment (Chapter 2.3).

2.5.7.2. Direct phase determination from electron micrographs

The 'direct method' most familiar to the electron microscopist is the high-resolution electron micrograph of a crystalline lattice. Retrieval of an average structure from such a micrograph assumes that the experimental image conforms adequately to the 'weak phase object' approximation, as discussed in Section 2.5.5. If this is

so, the use of image-averaging techniques, *e.g.* Fourier filtration or correlational alignment, will allow the unit-cell contents to be visualized after the electron-microscope phase contrast transfer function is deconvoluted from the average image, also discussed in Section 2.5.5. Image analyses can also be extended to three dimensions, as discussed in Section 2.5.6, basically by employing tomographic reconstruction techniques to combine information from the several tilt projections taken from the crystalline object. The potential distribution of the unit cell to the resolution of the imaging experiment can then be used, *via* the Fourier transform, to obtain crystallographic phases for the electron-diffraction amplitudes recorded at the same resolution. This method for phase determination has been the mainstay of protein electron crystallography.

Once a set of phases is obtained from the Fourier transform of the deconvoluted image, they must, however, be referred to an allowed crystallographic origin. For many crystallographic space groups, this choice of origin may coincide with the location of a major symmetry element in the unit cell [see *IT A* (1995)]. Hence, since the Fourier transform of translation is a phase term, if an image shift $[\delta(\mathbf{r} + \mathbf{r}_0)]$ is required to translate the origin of the repeating mass unit $\varphi(\mathbf{r})$ from the arbitrary position in the image to a specific site allowed by the space group,

$$g(\mathbf{r}) = \varphi(\mathbf{r}) \otimes \delta(\mathbf{r} + \mathbf{r}_0) = \varphi(\mathbf{r} + \mathbf{r}_0),$$

where the operation ' \otimes ' denotes convolution. The Fourier transform of this shifted density function will be

$$G(\mathbf{s}) = F(\mathbf{s}) \exp(2\pi i \mathbf{s} \cdot \mathbf{r}_0) = |F(\mathbf{s})| \exp[i(\phi_s + 2\pi i \mathbf{s} \cdot \mathbf{r}_0)].$$

In addition to the crystallographic phases ϕ_s , it will, therefore, be necessary to find the additional phase-shift term $2\pi i \mathbf{s} \cdot \mathbf{r}_0$ that will access an allowed unit-cell origin. Such origin searches are carried out automatically by some commercial image-averaging computer-software packages.

In addition to applications to thin protein crystals (*e.g.* Henderson *et al.*, 1990; Jap *et al.*, 1991; Kühlbrandt *et al.*, 1994), there are numerous examples of molecular crystals that have been imaged to a resolution of 3–4 Å, many of which have been discussed by Fryer (1993). For π -delocalized compounds, which are the most stable in the electron beam against radiation damage, the best results (2 Å resolution) have been obtained at 500 kV from copper perchlorophthalocyanine epitaxially crystallized onto KCl. As shown by Uyeda *et al.* (1978–1979), the averaged images clearly depict the positions of the heavy Cu and Cl atoms, while the positions of the light atoms in the organic residue are not resolved. (The utility of image-derived phases as a basis set for phase extension will be discussed below.) A number of aromatic polymer crystals have also been imaged to about 3 Å resolution, as reviewed recently (Tsuji, 1989; Dorset, 1994b).

Aliphatic molecular crystals are much more difficult to study because of their increased radiation sensitivity. Nevertheless, monolamellar crystals of the paraffin n -C₄₄H₉₀ have been imaged to 2.5 Å resolution with a liquid-helium cryomicroscope (Zemlin *et al.*, 1985). Similar images have been obtained at room temperature from polyethylene (Revol & Manley, 1986) and also a number of other aliphatic polymer crystals (Revol, 1991).

As noted by J. M. Cowley in Section 2.5.1, dynamical scattering can pose a significant barrier to the direct interpretation of high-resolution images from many inorganic materials. Nevertheless, with adequate control of experimental conditions (limiting crystal thickness, use of high-voltage electrons) some progress has been made. Pan & Crozier (1993) have described 2.0 Å images from zeolites in terms of the phase-grating approximation. A three-dimensional structural study has been carried out on an aluminosilicate by Wenk *et al.* (1992) with thin samples that