2.5. ELECTRON DIFFRACTION AND ELECTRON MICROSCOPY IN STRUCTURE DETERMINATION

The inverse transformation has the form

$$\rho(r, \psi, z) = \sum_n \int g_n(r, Z) \exp(in\psi) \exp(2\pi i z Z) \, dZ, \qquad (2.5.6.21)$$

so that g_n and G_n are the mutual Bessel transforms

$$G_n(R, Z) = \int_0^\infty g_n(rZ) J_n(2\pi r R) 2\pi r \, dr \qquad (2.5.6.22)$$

$$g_n(r, Z) = \int_0^\infty G_n(R, Z) J_n(2\pi r R) 2\pi R \, dR. \qquad (2.5.6.23)$$

Owing to helical symmetry, (2.5.6.22), (2.5.6.23) contain only those of the Bessel functions which satisfy the selection rule (Cochran *et al.*, 1952)

$$l = mp + (nq/N), \qquad (2.5.6.24)$$

where N, q and p are the helix symmetry parameters, $m = 0, \pm 1, \pm 2, \ldots$. Each layer l is practically determined by the single function J_n with the lowest n; the contribution of other functions is neglected. Thus, the Fourier transformation of one projection of a helical structure, with an account of symmetry and phases, gives the three-dimensional transform (2.5.6.23). We can introduce into this transform the function of temperature-factor type filtering the 'noise' from large spatial frequencies.

2.5.6.8. *Three-dimensional reconstruction in the general case*

In the general case of 3D reconstruction $\varphi_3(\mathbf{r})$ from projections $\varphi_2(\mathbf{x}_\tau)$ the projection vector $\boldsymbol{\tau}$ occupies arbitrary positions on the projection sphere (Fig. 2.5.6.2). Then, as in (2.5.6.15), we can construct the three-dimensional spatial synthesis. To do this, let us transform the two-dimensional projections $\varphi_{2i}[\mathbf{x}, \boldsymbol{\tau}(\theta, \psi)_i]$ by extending them along $\boldsymbol{\tau}$ as in (2.5.6.13) into three-dimensional projection functions $\varphi_3(\mathbf{r}_{\tau_i})$.

Analogously to (2.5.6.15), such a three-dimensional synthesis is the integral over the hemisphere (Fig. 2.5.6.2)

$$\Sigma_3(\mathbf{r}) = \int_\omega \varphi_3(\mathbf{r}, \boldsymbol{\tau}_i) \, d\omega_\tau = \varphi(\mathbf{r}) * |\mathbf{r}|^{-2}$$
$$\simeq \Sigma \varphi_{3i}[\mathbf{r}_{\tau(\theta, \psi)_i}] \simeq \varphi_3(\mathbf{r}) + B; \qquad (2.5.6.25)$$

this is the convolution of the initial function with $|\mathbf{r}|^{-2}$ (Vainshtein, 1971*b*).

To obtain the exact reconstruction of $\varphi_3(\mathbf{r})$ we find, from each $\varphi_2(\mathbf{x}_\tau)$, the modified projection (Vainshtein & Orlov, 1974; Orlov, 1975)

$$\tilde{\varphi}_2(\mathbf{x}_\tau) = \int \frac{\varphi_2(\mathbf{x}_\tau) - \varphi_2(\mathbf{x}'_\tau)}{|\mathbf{x}_\tau - \mathbf{x}'_\tau|^3} \, ds_{\mathbf{x}'}. \qquad (2.5.6.26)$$

By extending $\varphi_2(\mathbf{x}_\tau)$ along $\boldsymbol{\tau}$ we transform them into $\tilde{\varphi}_3(\mathbf{r}_\tau)$. Now the synthesis over the angles $\omega_\tau = (\theta, \psi, \alpha)_\tau$ gives the three-dimensional function

$$\varphi_3(\mathbf{r}) = \frac{1}{4\pi^3} \int \tilde{\varphi}_3(\mathbf{r}_\tau) \, d\omega_\tau \simeq \sum_i \tilde{\varphi}_{3i}[\mathbf{r}_{\tau(\theta, \psi, \alpha)_i}]. \qquad (2.5.6.27)$$

The approximation for a discrete set of angles is written on the right. In this case we are not bound by the coaxial projection condition which endows the experiment with greater possibilities; the use of object symmetry also profits from this. To carry out the 3D reconstruction (2.5.6.25) or (2.5.6.27) one should know all three Euler's angles ψ, θ, α (Fig. 2.5.6.2).

The projection vectors $\boldsymbol{\tau}_i$ should be distributed more or less uniformly over the sphere (Fig. 2.5.6.2). This can be achieved by using special goniometric devices.

Another possibility is the investigation of particles which, during the specimen preparation, are randomly oriented on the substrate. This, in particular, refers to asymmetric ribosomal particles. In this case the problem of determining these orientations arises.

The method of spatial correlation functions may be applied if a large number of projections with uniformly distributed projection directions is available (Kam, 1980). The space correlation function is the averaged characteristic of projections over all possible directions which is calculated from the initial projections or the corresponding sections of the Fourier transform. It can be used to find the coefficients of the object density function expansion over spherical harmonics, as well as to carry out the 3D reconstruction in spherical coordinates.

Another method (Van Heel, 1984) involves the statistical analysis of image types, subdivision of images into several classes and image averaging inside the classes. Then, if the object is rotated around some axis, the 3D reconstruction is carried out by the iteration method.

If such a specimen is inclined at a certain angle with respect to the beam, then the images of particles in the preferred orientation make a series of projections inclined at an angle β and having a random azimuth. The azimuthal rotation is determined from the image having zero inclination.

If particles on the substrate have a characteristic shape, they may acquire a preferable orientation with respect to the substrate, their azimuthal orientation α being random (Radermacher *et al.*, 1987).

In the general case, the problem of determining the spatial orientations of randomly distributed identical three-dimensional particles $\varphi_3(\mathbf{r})$ with an unknown structure may be solved by measuring their two-dimensional projections $p(\mathbf{x}_\tau)$ (Fig. 2.5.6.1)

$$p(\mathbf{x}_{\tau_i}) \equiv \varphi_2(\mathbf{x}_{\tau_i}) \simeq \int \varphi_3(\mathbf{r}) \, d\tau_i \quad \mathbf{x} \perp \boldsymbol{\tau}_i; \qquad (2.5.6.1a)$$

if the number i of such projections is not less than three, $i \geq 3$ (Vainshtein & Goncharov, 1986*a,b*; Goncharov *et al.* 1987; Goncharov, 1987). The direction of the vector $\boldsymbol{\tau}_i$ along which the projection $p(\boldsymbol{\tau}_i)$ is obtained is set by the angle $\omega_i(\theta_i, \psi_i)$ (Fig. 2.5.6.2).

The method is based on the analysis of one-dimensional projections q_α of two-dimensional projections $p(\mathbf{x}_{\tau_i})$

$$q(x_{\perp \alpha}) = \int p(\mathbf{x}_{\tau_i}) \, dx_{\|\alpha}, \qquad (2.5.6.28)$$

where α is the angle of the rotation about vector $\boldsymbol{\tau}$ in the p plane.

Lemma 1. Any two projections $p_1(\mathbf{x}_{\tau_i})$ and $p_2(\mathbf{x}_{\tau_2})$ (Fig. 2.5.6.6) have common (identical) one-dimensional projections $q_{12}(x_{12})$:

$$q_{12}(x_{12}) = q_{1, \alpha_1 j}(x_{\perp \alpha_1 j}) = q_{2, \alpha_2 k}(x_{\perp \alpha_2 k}). \qquad (2.5.6.29)$$

Vectors $\boldsymbol{\tau}_1$ and $\boldsymbol{\tau}_2$ (Fig. 2.5.6.3) determine plane h in which they are both lying. Vector $m_{12} = \langle \tau_1 \tau_2 \rangle$ is normal to plane h and parallel to axis x_{12} of the one-dimensional projection q_{12}; both $x_{\perp \alpha_1 j}$ and $x_{\perp \alpha_2 j}$ axes along which the projections q_1 and q_2 are constructed are perpendicular to x_{12}.

The corresponding lemma in the Fourier space states:

Lemma 2. Any two plane transforms, $\Phi_2(\mathbf{u}_{\tau_1}) = \mathscr{F}_2 p_1$ and $\Phi_2(\mathbf{u}_{\tau_2}) = \mathscr{F}_2 p_2$ intersect along the straight line v_{12} (Fig. 2.5.6.7); the one-dimensional transform $Q(v_{12})$ is the transform of $q_{12} : Q(v_{12}) = \mathscr{F}_1 g_{12}$.

Thus in order to determine the orientations $\omega_i(\theta_i, \psi_i, \alpha_i)$ of a three-dimensional particle $\varphi_{3, \omega_i}(\mathbf{r})$ it is necessary either to use projections p_i in real space or else to pass to the Fourier space (2.5.6.5).

Now consider real space. The projections p_i are known and can be measured but angles α_{ij} of their rotation about vector $\boldsymbol{\tau}_i$ (Fig. 2.5.6.8) are unknown and should be determined. Let us choose any two projections p_1 and p_2 and construct a set of one-dimensional projections $q_{1, \alpha_1 j}$ and $q_{2, \alpha_2 k}$ by varying angles α_{1j} and α_{2k}. In

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

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 $(i = 4, 5, \ldots)$ 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-

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

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

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 $\boldsymbol{\tau}_1$, $\boldsymbol{\tau}_2$, $\boldsymbol{\tau}_3$ 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
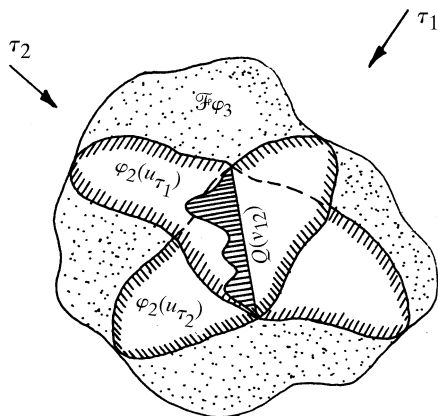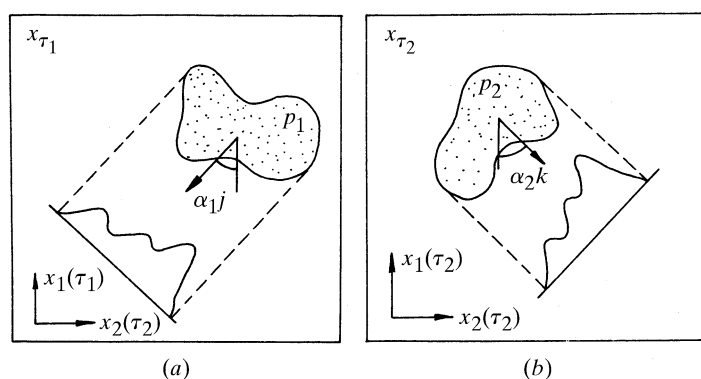

Fig. 2.5.6.7. Section of a three-dimensional Fourier transform of the density of the particles, corresponding to plane projections of this density.