### 2.5.6.5. *The method of back-projection*

This method is also called the synthesis of projection functions. Let us consider a two-dimensional case and stretch along $\tau_{\psi_i}$ each one-dimensional projection $L^i$ (Fig. 2.5.6.5) by a certain length $b$; thus, we obtain the projection function

$$L^i(\mathbf{x}) = \frac{1}{b} L^i(x_i) \cdot 1(\tau_i). \tag{2.5.6.13}$$

Let us now superimpose $h$ functions $L^i$

$$\sum_{i=1}^{h} L^i(\mathbf{x}) = \Sigma_2(\mathbf{x}). \tag{2.5.6.14}$$

The continuous sum over the angles of projection synthesis is

$$\Sigma_2(\mathbf{x}) = \int_0^{\pi} L(\psi, \mathbf{x})\, \mathrm{d}\psi = \rho_2(\mathbf{x}) * |\mathbf{x}|^{-1}$$

$$\simeq \sum_{i=1}^{h} L^i = \rho_2(\mathbf{x}) + B(1); \tag{2.5.6.15}$$

this is the convolution of the initial function with a rapidly falling function $|\mathbf{x}|^{-1}$ (Vainshtein, 1971*b*). In (2.5.6.15), the approximation for a discrete set of $h$ projections is also written. Since the function $|\mathbf{x}|^{-1}$ approaches infinity at $x = 0$, the convolution with it will reproduce the initial function $\rho(\mathbf{x})$, but with some background $B$ decreasing around each point according to the law $|\mathbf{x}|^{-1}$. At orthoaxial projection the superposition of cross sections $\varphi_2(\mathbf{x}, z_k)$ arranged in a pile gives the three-dimensional structure $\varphi_3$.

*Radon operator*. Radon (1917; see also Deans, 1983) gave the exact solution of the problem of reconstruction. However, his mathematical work was for a long time unknown to investigators engaged in reconstruction of a structure from images; only in the early 1970s did some authors obtain results analogous to Radon's (Ramachandran & Lakshminarayanan, 1971; Vainshtein & Orlov, 1972, 1974; Gilbert, 1972*a*).

The convolution in (2.5.6.15) may be eliminated using the Radon integral operator, which modifies projections by introducing around each point the negative values which annihilate on superposition the positive background values. The one-dimensional projection modified with the aid of the Radon operator has the form

$$\tilde{L}(x_\psi) = \frac{1}{2\pi^2} \int_0^{\infty} \frac{2L(x_\psi) - L(x_\psi + x'_\psi) - L(x_\psi - x'_\psi)}{x'^2_\psi}\, \mathrm{d}x'_\psi. \tag{2.5.6.16}$$

Now $\varphi_2(\mathbf{x})$ is calculated analogously to (2.5.6.14), not from the initial projections $L$ but from the modified projection $\tilde{L}$:

$$\varphi_2(\mathbf{x}) = \int_0^{\pi} \tilde{L}(\psi, \mathbf{x})\, \mathrm{d}\psi \simeq \sum_{i=1}^{k} \tilde{L}_i(\psi_i, \mathbf{x}). \tag{2.5.6.17}$$

The reconstruction of high-symmetry structures, in particular helical ones, by the direct method is carried out from one projection making use of its equivalence to many projections. The Radon formula in discrete form can be obtained using the double Fourier transformation and convolution (Ramachandran & Lakshminarayanan, 1971).

### 2.5.6.6. *The algebraic and iteration methods*

These methods have been derived for the two-dimensional case; consequently, they can also be applied to three-dimensional reconstruction in the case of orthoaxial projection.

Let us discretize $\varphi_2(\mathbf{x})$ by a net $m^2$ of points $\varphi_{jk}$; then we can construct the system of equations (2.5.6.10).

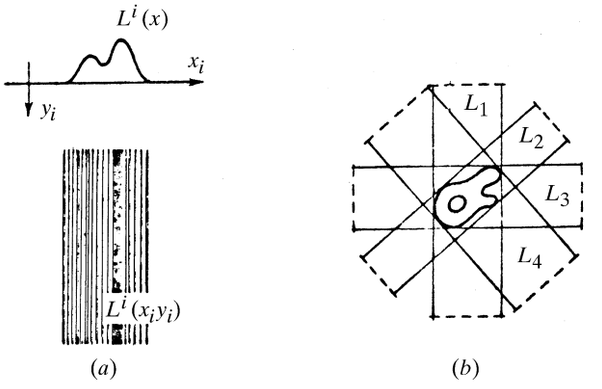


Fig. 2.5.6.5. (*a*) Formation of a projection function; (*b*) superposition of these functions.

When $h$ projections are available the condition of unambiguous solution of system (2.5.6.10) is: $h \geq m$. At $m \simeq (3\text{–}5)h$ we can, in practice, obtain sufficiently good results (Vainshtein, 1978).

Methods of reconstruction by iteration have also been derived that cause some initial distribution to approach one $\varphi_2(\mathbf{x})$ satisfying the condition that its projection will resemble the set $L^i$. Let us assign on a discrete net $\varphi_{jk}$ as a zero-order approximation the uniform distribution of mean values (2.5.6.7)

$$\varphi^0_{jk} = \langle \varphi \rangle = \Omega / m^2. \tag{2.5.6.18}$$

The projection of the $q$th approximation $\varphi^q_{jk}$ at the angle $\varphi_i$ (used to account for discreteness) is $L^{iq}_n$.

The next approximation $\varphi^{q+1}$ for each point $jk$ is given in the method of 'summation' by the formula

$$\varphi^{q+1}_{jk} = \max[\varphi^q_{jk} + (L^i_n - L^{i,q}_n)/N^i_{L_n};\, 0], \tag{2.5.6.19}$$

where $N_{L^i}$ is the number of points in a strip of the projection $L^i_n$. One cycle of iterations involves running $\varphi^q_{jk}$ around all of the angles $\psi_j$ (Gordon *et al.*, 1970).

When carrying out iterations, we may take into account the contribution not only of the given projection, but also of all others. In this method the process of convergence improves. Some other iteration methods have been elaborated (Gordon & Herman, 1971; Gilbert, 1972*b*; Crowther & Klug, 1974; Gordon, 1974).

### 2.5.6.7. *Reconstruction using Fourier transformation*

This method is based on the Fourier projection theorem [(2.5.6.3)–(2.5.6.5)]. The reconstruction is carried out according to scheme (2.5.6.6) (DeRosier & Klug, 1968; Crowther, DeRosier & Klug, 1970; Crowther, Amos *et al.* 1970; DeRosier & Moore, 1970; Orlov, 1975). The three-dimensional Fourier transform $\mathscr{F}_3(\mathbf{u})$ is found from a set of two-dimensional cross sections $\mathscr{F}_2(\mathbf{u})$ on the basis of the Whittaker–Shannon interpolation. If the object has helical symmetry (which often occurs in electron microscopy of biological objects, *e.g.* on investigating bacteriophage tails, muscle proteins) cylindrical coordinates are used. Diffraction from such structures with $c$ periodicity and scattering density $\varphi(r, \psi, z)$ is defined by the Fourier–Bessel transform:

$$\Phi(R, \Psi, Z) = \sum_{n=-\infty}^{+\infty} \exp\left[in\left(\Psi + \frac{\pi}{2}\right)\right] \int_0^{\infty} \int_0^{2\pi} \int_0^{l} \varphi(r, \psi, z)$$

$$\times J_n(2\pi r R) \exp[-i(n\psi + 2\pi z Z)] r\, \mathrm{d}r\, \mathrm{d}\psi\, \mathrm{d}z$$

$$= \sum_n G_n(R, Z) \exp\left[in\left(\Psi + \frac{\pi}{2}\right)\right]. \tag{2.5.6.20}$$

The inverse transformation has the form

$$\rho(r,\psi,z)=\sum_n \int g_n(r,Z)\exp(in\psi)\exp(2\pi izZ)\ \mathrm{d}Z, \qquad (2.5.6.21)$$

so that $g_n$ and $G_n$ are the mutual Bessel transforms

$$G_n(R,Z)=\int_0^\infty g_n(rZ)J_n(2\pi rR)2\pi r\ \mathrm{d}r \qquad (2.5.6.22)$$

$$g_n(r,Z)=\int_0^\infty G_n(R,Z)J_n(2\pi rR)2\pi R\ \mathrm{d}R. \qquad (2.5.6.23)$$

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, $m=0,\pm1,\pm2,\ldots$. 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)

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

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 $\varphi_2(\mathbf{x}_{\boldsymbol{\tau}})$, the modified projection (Vainshtein & Orlov, 1974; Orlov, 1975)

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

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

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

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 $\psi$, $\theta$, $\alpha$ (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 $\beta$ 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 $\alpha$ 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)

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

if the number $i$ of such projections is not less than three, $i\geq3$ (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 $q_\alpha$ of two-dimensional projections $p(\mathbf{x}_{\tau_i})$

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

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

*Lemma* 1. Any two projections $p_1(\mathbf{x}_{\tau_1})$ 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 $\alpha_{ij}$ 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 $\alpha_{1j}$ and $\alpha_{2k}$. In



references