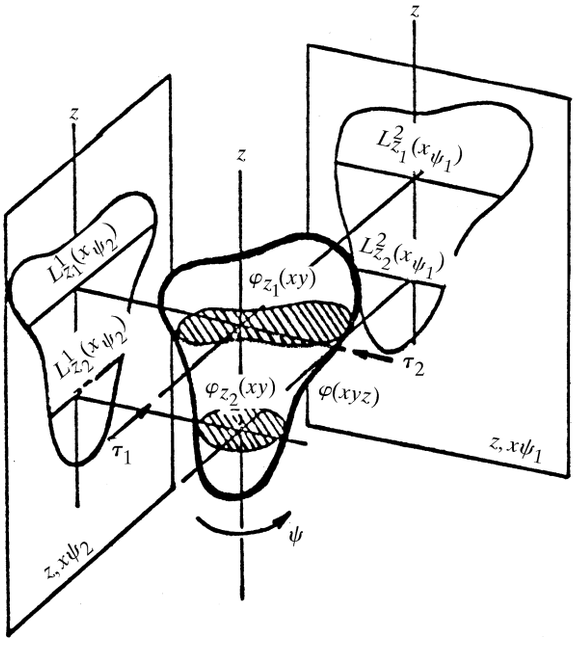

Fig. 2.5.6.3. Orthoaxial projection.

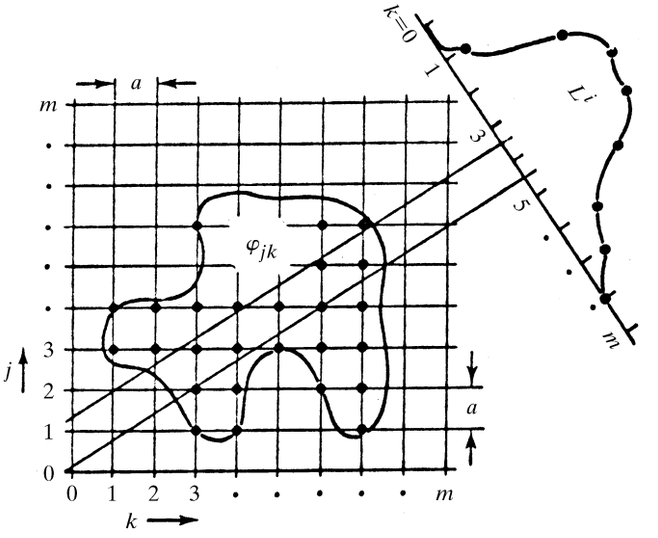

Fig. 2.5.6.4. Discretization and oblique projection.

In this case, the reconstruction is carried out separately for each level z_l:

$$\text{set } \varphi_{1,\,z_l}(x_\psi) \equiv \text{ set } L^i_{z_l} \rightarrow \varphi_{2i}(xyz_l) \qquad (2.5.6.9)$$

and the three-dimensional structure is obtained by superposition of layers $\varphi_{2z_l}(xy)\Delta z$ (Vainshtein *et al.*, 1968; Vainshtein, 1978).

2.5.6.3. *Discretization*

In direct methods of reconstruction as well as in Fourier methods the space is represented as a discrete set of points $\varphi(\mathbf{x}_{jk})$ on a two-dimensional net or $\varphi(\mathbf{r}_{jkl})$ on a three-dimensional lattice. It is sometimes expedient to use cylindrical or spherical coordinates. In two-dimensional reconstruction the one-dimensional projections are represented as a set of discrete values L^i, at a certain spacing in x_ψ. The reconstruction (2.5.6.9) is carried out over the discrete net with m^2 nodes φ_{jk}. The net side A should exceed the diameter of an object D, $A > D$; the spacing $a = A/m$. Then (2.5.6.8) transforms into the sum

$$L^i = \sum_k \varphi_{jk}. \qquad (2.5.6.10)$$

For oblique projections the above sum is taken over all the points within the strips of width a along the axis $\boldsymbol{\tau}_{\psi_i}$ (Fig. 2.5.6.4).

The resolution δ of the reconstructed function depends on the number h of the available projections. At approximately uniform angular distribution of projections, and diameter equal to D, the resolution at reconstruction is estimated as

$$\delta \simeq 2D/h. \qquad (2.5.6.11)$$

The reconstruction resolution δ should be equal to or somewhat better than the instrumental resolution d of electron micrographs ($\delta < d$), the real resolution of the reconstructed structure being d. If the number of projections h is not sufficient, *i.e.* $\delta > d$, then the resolution of the reconstructed structure is δ (Crowther, DeRosier & Klug, 1970; Vainshtein, 1978).

In electron microscopy the typical instrumental resolution d of biological macromolecules for stained specimens is about 20 Å; at the object with diameter $D \simeq 200$ Å the sufficient number h of projections is about 20. If the projections are not uniformly distributed in projection angles, the resolution decreases towards $\mathbf{x} \perp \boldsymbol{\tau}$ for such $\boldsymbol{\tau}$ in which the number of projections is small.

Properties of projections of symmetric objects. If the object has an N-fold axis of rotation, its projection has the same symmetry. At orthoaxial projection perpendicular to the N-fold axis the projections which differ in angle at $j(2\pi/N)$ are identical:

$$\varphi_2(\mathbf{x}_\psi) = \varphi_2[\mathbf{x}_{\psi + j(2\pi/N)}] \quad (j = 1, 2, \ldots, N). \qquad (2.5.6.12)$$

This means that one of its projections is equivalent to N projections. If we have h independent projections of such a structure, the real number of projections is hN (Vainshtein, 1978). For a structure with cylindrical symmetry ($N = \infty$) one of its projections fully determines the three-dimensional structure.

Many biological objects possess helical symmetry – they transform into themselves by the screw displacement operation $s_{p/q}$, where p is the number of packing units in the helical structure per q turns of the continuous helix. In addition, the helical structures may also have the axis of symmetry N defining the pitch of the helix. In this case, a single projection is equivalent to $h = pN$ projections (Cochran *et al.*, 1952).

Individual protein molecules are described by point groups of symmetry of type N or $N/2$. Spherical viruses have icosahedral symmetry 532 with two-, three- and fivefold axes of symmetry. The relationship between vectors $\boldsymbol{\tau}$ of projections is determined by the transformation matrix of the corresponding point group (Crowther, Amos *et al.*, 1970).

2.5.6.4. *Methods of direct reconstruction*

Modelling. If several projections are available, and, especially, if the object is symmetric, one can, on the basis of spatial imagination, recreate approximately the three-dimensional model of the object under investigation. Then one can compare the projections of such a model with the observed projections, trying to draw them as near as possible. In early works on electron microscopy of biomolecules the tentative models of spatial structure were constructed in just this way; these models provide, in the case of the quaternary structure of protein molecules or the structure of viruses, schemes for the arrangement of protein subunits. Useful subsidiary information in this case can be obtained by the method of optical diffraction and filtration.

references