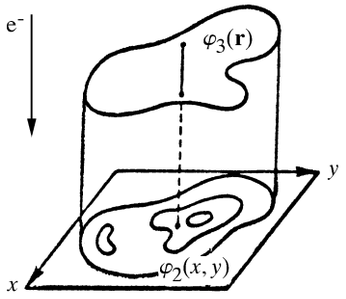

Fig. 2.5.6.1. A three-dimensional object φ_3 and its two-dimensional projection φ_2. The electron beam penetrates the specimen in the direction of the z axis.

The projection direction is defined by a unit vector $\boldsymbol{\tau}(\theta, \psi)$ and it is formed on the plane $\mathbf{x}$ perpendicular to $\boldsymbol{\tau}$. The set of various projections $\varphi_2(\mathbf{x}_{\tau_i}) = \varphi_{2_i}(\mathbf{x}_i)$ may be assigned by a discrete or continuous set of points on a unit sphere $|\tau| = 1$ (Fig. 2.5.6.2).

In Fourier space, the relation between an object and its projection is referred to as the central section theorem: the Fourier transformation of projection φ_2 of a 3D object φ_3 is the central (*i.e.*, passing though the origin of reciprocal space) 2D plane cross section of a 3D transform perpendicular to the projection vector (Bracewell, 1956; DeRosier & Klug, 1968; Crowther, Amos *et al.*, 1970). In Cartesian coordinates, a 3D Fourier transform is

$$\begin{aligned}\mathscr{F}[\varphi_3(\mathbf{r})] &= \Phi_3(u, v, w) \\ &= \iiint \varphi_3(x, y, z) \exp\{2\pi i(ux + vy + wz)\}\, \mathrm{d}x\, \mathrm{d}y\, \mathrm{d}z.\end{aligned} \tag{2.5.6.6}$$

The transform of projection $\varphi_2(x, y)$ along z is

$$\begin{aligned}\mathscr{F}[\varphi_2(x, y)] &= \Phi_3(u, v, 0) \\ &= \iiint \varphi_3(x, y, z) \exp\{2\pi i(ux + vy + 0z)\}\, \mathrm{d}x\, \mathrm{d}y\, \mathrm{d}z \\ &= \iiint \varphi_3(x, y, z)\, \mathrm{d}z \exp\{2\pi i(ux + vy)\}\, \mathrm{d}x\, \mathrm{d}y \\ &= \iint \varphi_2(x, y) \exp\{2\pi i(ux + vy)\}\, \mathrm{d}x\, \mathrm{d}y \\ &= \Phi_2(u, v).\end{aligned} \tag{2.5.6.7}$$

In the general case of projecting along the vector $\boldsymbol{\tau}$, the central section theorem is

$$\mathscr{F}[\varphi_2(\mathbf{x}_\tau)] = \Phi_3(\mathbf{u}_\tau), \quad \mathbf{u}_\tau \perp \boldsymbol{\tau}. \tag{2.5.6.8}$$

From this theorem it follows that the inversion of the 3D ray transform is possible if there is a continuous set of projections φ_τ corresponding to the motion of the vector $\boldsymbol{\tau}(\theta, \psi)$ over any continuous line connecting the opposite points on the unit sphere (Fig. 2.5.6.2) (Orlov's condition: Orlov, 1976). This result is evidenced by the fact that in this case the cross sections $\mathscr{F}[\varphi_2(\mathbf{x}_\tau)]$ that are perpendicular to $\boldsymbol{\tau}$ in Fourier space continuously cover the whole Fourier space, *i.e.*, they yield $\mathscr{F}[\varphi_3(\mathbf{r})]$ and thereby determine $\varphi_3(\mathbf{r}) = \mathscr{F}^{-1}[\Phi_3(\mathbf{u})]$.

In single-particle reconstruction, imaged objects are randomly and nonuniformly oriented on the substrate at different angles (Frank, 2006) and the distribution of their orientations is beyond our control; therefore, the practical impact of Orlov's condition is limited. In fact, it is more useful to determine *a posteriori*, *i.e.*, after the 3D reconstruction of the macromolecule is computed, how well the Fourier space was covered. This can be done by calculating the distribution of the 3D spectral signal-to-noise ratio (Penczek, 2002).

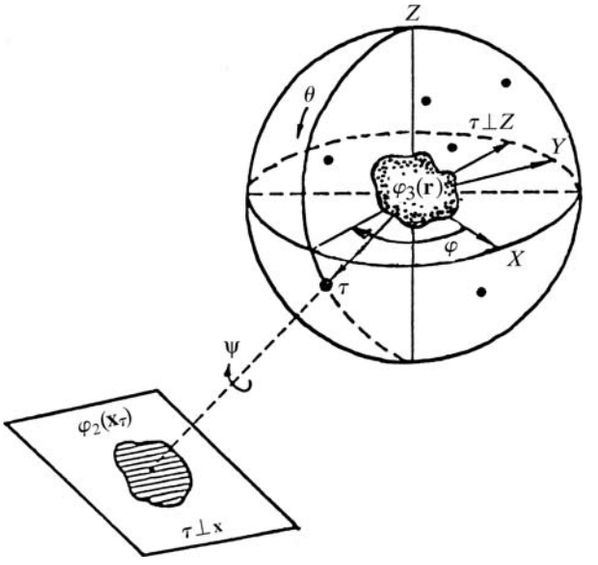

Fig. 2.5.6.2. The projection sphere and projection $\varphi_2(\mathbf{x}_\tau)$ of $\varphi_3(\mathbf{r})$ along $\boldsymbol{\tau}$ onto the plane $\boldsymbol{\tau} \perp \mathbf{x}$. The case $\boldsymbol{\tau} \perp Z$ represents orthoaxial projection. Points indicate an arbitrary distribution of projection directions $\boldsymbol{\tau}$.

2.5.6.2. 3D reconstruction in the general case

In the general case of the 3D reconstruction of $\varphi_3(\mathbf{r})$ from projections $\varphi_2(\mathbf{x}_\tau)$, the projection vector $\boldsymbol{\tau}$ occupies arbitrary positions on the projection sphere (Fig. 2.5.6.2). First, let us consider the case of a 2D function $\rho_2(\mathbf{x})$ and its ray transform $\varphi_1(x, \psi)$. We introduce an operation of backprojection b, which is stretching along $\boldsymbol{\tau}_\psi$ each 1D projection $\varphi_1(x_\psi)$ (Fig. 2.5.6.3). When the result is integrated over the full angular range of projections $\varphi_1(x, \psi)$, we obtain the projection synthesis defined as

$$b(x, y) = \int_0^\pi \varphi_1(x \cos\psi + y \sin\psi, \psi)\, \mathrm{d}\psi. \tag{2.5.6.9}$$

However, the backprojection operator is not the inverse of a 2D ray transform, as the resulting image b is blurred by the point-spread function $(x^2 + y^2)^{-1/2}$ (Vainshtein, 1971):

$$b(x, y) = \rho(x, y) * (x^2 + y^2)^{-1/2}. \tag{2.5.6.10}$$

By noting that the Fourier transform of $(x^2 + y^2)^{-1/2}$ is $(u^2 + v^2)^{-1/2}$ and by using the convolution theorem $\mathscr{F}[f * g] = \mathscr{F}[f]\mathscr{F}[g]$, we obtain the 'backprojection-filtering' inversion formula:

$$\begin{aligned}\rho(x, y) &= b(x, y) * (x^2 + y^2)^{1/2} = \mathscr{F}^{-1}\big[|\mathbf{u}|\mathscr{F}[b]\big] \\ &= \text{Filtration}_{|\mathbf{u}|}[\text{Backprojection}(\varphi_1)].\end{aligned} \tag{2.5.6.11}$$

The more commonly used 'filtered-backprojection' inversion is based on the 2D version of the central section theorem (2.5.6.8):

$$\mathscr{F}[\varphi_1(x_\psi)] = \Upsilon_2(\mathbf{u}_\psi) = \Upsilon_2(R, \Psi), \tag{2.5.6.12}$$

where $\mathscr{F}[\rho_2] = \Upsilon_2$. With this in mind, $\rho_2(\mathbf{x})$ can be related to its ray transform by evaluating the Fourier transform of ρ_2 in polar coordinates: