International Tables for Crystallography (2010). Vol. B, Figure 2.5.6.5, p. 371.

selecting the order in which pixels enter the correction step (2) in (2.5.6.28). It was observed that if a pixel is selected such that its projection direction is perpendicular to the projection direction of the previous pixel, the convergence is achieved faster (Hamaker & Solmon, 1978; Herman & Meyer, 1993). Interestingly, a random order works almost equally well (Natterer & Wübbeling, 2001).

In single-particle reconstruction, ART has been introduced in the form of 'ART with blobs' (Marabini *et al.*, 1998) and is available in the *Xmipp* package (Sorzano *et al.*, 2004). In this implementation, the reconstruction structure is represented by a linear combination of spherically symmetric, smooth, spatially limited basis functions, such as Kaiser–Bessel window functions (Lewitt, 1990, 1992; Matej & Lewitt, 1996). Introduction of blobs significantly reduces the number of iterations necessary to reach an acceptable solution (Marabini *et al.*, 1998).

The major advantage of iterative reconstruction methods is the ability to take advantage of *a priori* knowledge, *i.e.*, any information about the protein structure that was not initially included in the data processing, and introduce it into the reconstruction process in the form of constraints. Examples of such constraints include similarity to the experimental (measured) data, positivity of the protein mass density (only valid in conjunction with the CTF correction), bounded spatial support *etc*. Formally, the process of enforcing selected constraints is best described in the framework of the projections onto convex sets (POCS) theory (Sezan & Stark, 1982; Youla & Webb, 1982; Sezan, 1992) introduced into EM by Carazo and co-workers (Carazo & Carrascosa, 1986, 1987; Carazo, 1992).

2.5.6.5. *Filtered backprojection*

The method of filtered backprojection (FBP) is based on inversion formulae (2.5.6.11) (in two dimensions) or (2.5.6.14) (in three dimensions). It comprises the following steps: (i) a Fourier transform of each projection is computed; (ii) Fourier transforms of projections are multiplied by filters that account for a particular distribution of projections in Fourier space; (iii) the filtered projections are inversely Fourier transformed; (iv) real-space backprojection of processed projections yields the reconstruction. The method is particularly attractive due to the fact that the reconstruction calculated using simple real-space backprojection can be made efficient if the filter function is easy to compute.

In two dimensions with uniformly distributed projections the weighting function $c(|R|, \Psi)$ in Fourier space is the 'ramp function' $|R|$ [(2.5.6.13)]. In two dimensions with nonuniformly distributed projections, when the analytical form of the distribution of projections is not known, an appropriate approximation to $c(|R|, \Psi)$ has to be found. A good choice is to select weights such that the backprojection integral becomes approximated by a Riemann sum (Penczek *et al.*, 1996),

$$\begin{aligned} c(|R|, \Psi) = |R|\,\mathrm{d}R\,\mathrm{d}\Psi \rightarrow c(R_j, \Psi_i) &= R_j \frac{1}{2\pi}\frac{\Psi_{i+1} - \Psi_{i-1}}{2} \\ &= R_j \frac{\Delta\Psi_i}{4\pi}. \end{aligned} \qquad (2.5.6.29)$$

For a given set of angles the weights $c(R_j, \Psi_i)$ are easily computed (Fig. 2.5.6.5).

In three dimensions, the weighting (2.5.6.29) is applicable in a single-axis tilt data-collection geometry, where the 3D reconstruction can be calculated as a series of independent 2D reconstructions. In the general 3D case, the analogue of weighting (2.5.6.29) cannot be used, as the data are given in the form of 2D projections and it is not immediately apparent what fraction of the 3D Fourier volume is occupied by Fourier pixels in projections. However, the analogue of weighting (2.5.6.29) can be used in the inversion of 3D Radon transforms or in a direction

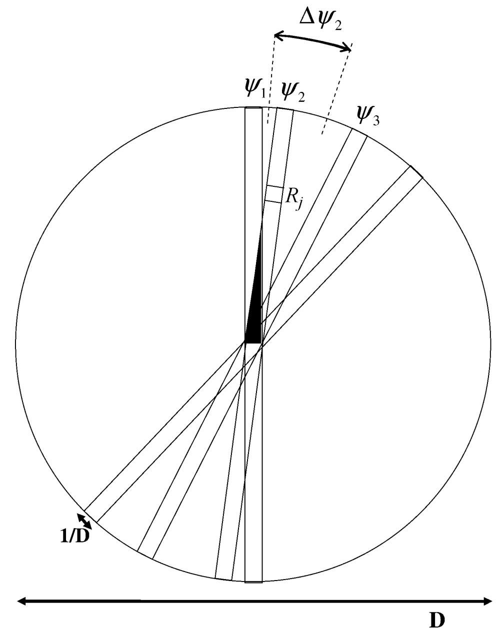

Fig. 2.5.6.5. Nonuniform distribution of projections. The projection weights for the reconstruction algorithms are chosen such that the backprojection integral becomes approximated by a Riemann sum and are equal to the angular length of an arc $\Delta\Psi_i$ (2.5.6.29). In Fourier space, projections of an object with real-space radius D form rectangles with width $1/D$. In the exact filter backprojection reconstruction method, the weights are derived based on the amount the overlap between projections (2.5.6.29).

inversion of 3D ray transforms that is based on an intermediate step of converting 2D projection data to 1D projection data, as described in Section 2.5.6.6.

In order to arrive at a workable solution, the weighting functions applicable to 2D projections are constructed based on an explicitly or implicitly formulated concept of the 'local density' of projections. This concept was introduced by Bracewell (Bracewell & Riddle, 1967), who suggested for a 2D case of nonuniformly distributed projections a heuristic weighting function,

$$c(R_j, \Psi_i) = \frac{R_j}{\sum_l \exp\left[-\mathrm{constant}(|\Psi_i - \Psi_l| \bmod \pi)^2\right]}. \qquad (2.5.6.30)$$

The weighting function (2.5.6.30) can be easily extended to three dimensions; however, it has a major disadvantage that for a uniform distribution of projections it does not approximate well the weighting function (2.5.6.29), which we consider optimal.

Radermacher *et al.* (1986) proposed a derivation of a general weighting function using a deconvolution kernel calculated for a given (nonuniform) distribution of projections and, in modification of (2.5.6.14), a finite length of backprojection (Fig. 2.5.6.3). Such a 'truncated' backprojection is

$$\hat{b}_i(\mathbf{r}) = \varphi_{2_i}(\mathbf{x}_\tau) * l(\mathbf{r}), \quad \boldsymbol{\tau} \perp \mathbf{x} \qquad (2.5.6.31)$$

with projection directions $\boldsymbol{\tau}(\theta, \psi)_i$ and

$$\begin{aligned} l(\mathbf{r}) &= \delta(\mathbf{x}_\tau) t(z_\tau), \\ t(z_\tau) &= \begin{cases} 1 & -(D/2) \le z \le (D/2), \\ 0 & \text{otherwise}, \end{cases} \end{aligned} \qquad (2.5.6.32)$$

where D is the diameter of the object or the length of the backprojection l. By taking the Fourier transform of (2.5.6.31) and using the central section theorem (2.5.6.8), we obtain a 3D Fourier transform of the backprojected projection,

references