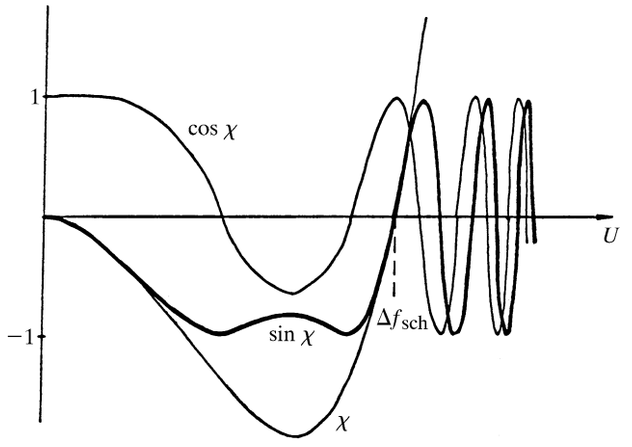

Fig. 2.5.5.1. The χ function and two components of the Scherzer phase function $\sin \chi(U)$ and $\cos \chi(U)$.

$q(\mathbf{x}) = \exp[-i\sigma\varphi(\mathbf{x})]$ (2.5.2.42), and for weak phase objects the approximation $[\sigma\varphi \ll 1]$

$$q(\mathbf{x}) = 1 - i\sigma\varphi(\mathbf{x}) \tag{2.5.5.5}$$

is valid.

In the back focal plane of the objective lens the wave has the form

$$Q(uv) \cdot T(U) \tag{2.5.5.6}$$

$$T = A(U)\exp(i\chi U) \tag{2.5.5.7a}$$

$$\chi(U) = \pi\Delta f\lambda U^2 + \frac{\pi}{2}C_s\lambda^3 U^4, \tag{2.5.5.7b}$$

where $U = (u^2 + v^2)^{1/2}$; $\exp[i\chi(U)]$ is the Scherzer phase function (Scherzer, 1949) of an objective lens (Fig. 2.5.5.1), $A(U)$ is the aperture function, C_s the spherical aberration coefficient, and Δf the defocus value [(2.5.2.32)–(2.5.2.35)].

The bright-field image intensity (in object coordinates) is

$$I(xy) = |\psi_1(xy) * t(xy)|^2, \tag{2.5.5.8}$$

where $t = \mathscr{F}^{-1}[T]$. The phase function (2.5.5.7) depends on defocus, and for a weak phase object (Cowley, 1981)

$$I(xy) = 1 + 2\sigma\varphi(xy) * s(xy), \tag{2.5.5.9}$$

where $s = \mathscr{F}^{-1}[A(U)\sin\chi]$, which includes only an imaginary part of function (2.5.5.6). While selecting defocus in such a way that under the Scherzer defocus conditions [(2.5.2.44), (2.5.2.45)] $|\sin\chi| \simeq 1$, one could obtain

$$I(xy) = 1 + 2\sigma\varphi(xy) * a(xy). \tag{2.5.5.10}$$

In this very simple case the image reflects directly the structure of the object – the two-dimensional distribution of the projection of the potential convoluted with the spread function $a = \mathscr{F}^{-1}A$. In this case, no image restoration is necessary. Contrast reversal may be achieved by a change of defocus.

At high resolution, this method enables one to obtain an image of projections of the atomic structure of crystals and defects in the atomic arrangement – vacancies, replacements by foreign atoms, amorphous structures and so on; at resolution worse than atomic one obtains images of dislocations as continuous lines, inserted phases, inclusions *etc.* (Cowley, 1981). It is also possible to obtain images of thin biological crystals, individual molecules, biological macromolecules and their associations.

Image restoration. In the case just considered (2.5.5.10), the projection of potential $\varphi(xy)$, convoluted with the spread function, can be directly observed. In the general case (2.5.5.9), when the aperture becomes larger, the contribution to image formation is made by large values of spatial frequencies U, in which the function $\sin\chi$ oscillates, changing its sign. Naturally, this distorts the image just in the region of appropriate high resolution. However, if one knows the form of the function $\sin\chi$ (2.5.5.7), the true function $\varphi(xy)$ can be restored.

This could be carried out experimentally if one were to place in the back focal plane of an objective lens a zone plate transmitting only one-sign regions of $\sin\chi$ (Hoppe, 1971). In this case, the information on $\varphi(xy)$ is partly lost, but not distorted. To perform such a filtration in an electron microscope is a rather complicated task.

Another method is used (Erickson & Klug, 1971). It consists of a Fourier transformation $\mathscr{F}^{-1}$ of the measured intensity distribution TQ (2.5.5.6) and division of this transform, according to (2.5.5.7*a*,*b*), by the phase function $\sin\chi$. This gives

$$\frac{TQ}{\sin\chi} = Q(uv)A(U). \tag{2.5.5.11a}$$

Then, the new Fourier transformation $\mathscr{F}QA$ yields (in the weak-phase-object approximation) the true distribution

$$\varphi(xy) * a(xy). \tag{2.5.5.11b}$$

The function $\sin\chi$ depending on defocus Δf should be known to perform this procedure. The transfer function can also be found from an electron micrograph (Thon, 1966). It manifests itself in a circular image intensity modulation of an amorphous substrate or, if the specimen is crystalline, in the 'noise' component of the image. The analogue method (optical Fourier transformation for obtaining the image $\sin\chi$) can be used (optical diffraction, see below); digitization and Fourier transformation can also be applied (Hoppe *et al.*, 1973).

The thin crystalline specimen implies that in the back focal objective lens plane the discrete kinematic amplitudes Φ_{hk} are arranged and, by the above method, they are corrected and released from phase distortions introduced by the function $\sin\chi$ (see below) (Unwin & Henderson, 1975).

For the three-dimensional reconstruction (see Section 2.5.6) it is necessary to have the projections of potential of the specimen tilted at different angles α to the beam direction (normal beam incidence corresponds to $\alpha = 0$). In this case, the defocus Δf changes linearly with increase of the distance l of specimen points from the rotation axis $\Delta f_\alpha = \Delta f_0(1 + l\sin\alpha)$. Following the above procedure for passing on to reciprocal space and correction of $\sin\chi$, one can find $\varphi_\alpha(xy)$ (Henderson & Unwin, 1975).

2.5.5.3. An account of absorption

Elastic interaction of an incident wave with a weak phase object is defined on its exit surface by the distribution of potential projection $\varphi(xy)$; however, in the general case, the electron scattering amplitude is a complex one (Glauber & Schomaker, 1953). In such a way, the image itself has the phase and amplitude contrast. This may be taken into account if one considers not only the potential projection $\varphi(xy)$, but also the 'imaginary potential'