$\mu(xy)$ which describes phenomenologically the absorption in thin specimens. Then, instead of (2.5.5.5), the wave on the exit surface of a specimen can be written as

$$q(xy) = 1 - i\sigma\varphi(xy) - \mu(xy) \tag{2.5.5.12}$$

and in the back focal plane if $\Phi = \mathscr{F}\varphi$ and $M = \mathscr{F}\mu$

$$Q(uv) = \delta(uv) - i\sigma\Phi(uv) - M(uv). \tag{2.5.5.13}$$

Usually, μ is small, but it can, nevertheless, make a certain contribution to an image. In a sufficiently good linear approximation, it may be assumed that the real part $\cos\chi$ of the phase function (2.5.5.7*a*) affects $M(uv)$, while $\Phi(xy)$, as we know, is under the action of the imaginary part $\sin\chi$.

Thus, instead of (2.5.5.6), one can write

$$Q(\exp i\chi) = \delta(\mathbf{u}) - i\sigma\Phi(\mathbf{u})\sin\chi - M(\mathbf{u})\cos\chi, \tag{2.5.5.14}$$

and as the result, instead of (2.5.5.10),

$$\begin{aligned} I(xy) = 1 &+ 2\sigma\varphi(xy) * \mathscr{F}^{-1}(\sin\chi) * a(U) \\ &- 2\mu(xy) * \mathscr{F}^{-1}(\cos\chi) * a(U). \end{aligned} \tag{2.5.5.15}$$

The functions $\varphi(xy)$ and $\mu(xy)$ can be separated by object imaging using the through-focus series method. In this case, using the Fourier transformation, one passes from the intensity distribution (2.5.5.15) in real space to reciprocal space. Now, at two different defocus values Δf_1 and Δf_2 [(2.5.5.6), (2.5.5.7*a*,*b*)] the values $\Phi(\mathbf{u})$ and $M(\mathbf{u})$ can be found from the two linear equations (2.5.5.14). Using the inverse Fourier transformation, one can pass on again to real space which gives $\varphi(\mathbf{x})$ and $\mu(\mathbf{x})$ (Schiske, 1968). In practice, it is possible to use several through-focus series and to solve a set of equations by the least-squares method.

Another method for processing takes into account the simultaneous presence of noise $N(\mathbf{x})$ and transfer function zeros (Kirkland *et al.*, 1980). In this method the space frequencies corresponding to small values of the transfer function modulus are suppressed, while the regions where such a modulus is large are found to be reinforced.

2.5.5.4. Thick crystals

When the specimen thickness exceeds a certain critical value (~50–100 Å), the kinematic approximation does not hold true and the scattering is dynamic. This means that on the exit surface of a specimen the wave is not defined as yet by the projection of potential $\varphi(xy) = \int\varphi(\mathbf{r})\,\mathrm{d}z$ (2.5.5.3), but one has to take into account the interaction of the incident wave ψ_0 and of all the secondary waves arising in the whole volume of a specimen.

The dynamic scattering calculation can be made by various methods. One is the multislice (or phase-grating) method based on a recurrent application of formulae (2.5.5.3) for n thin layers Δz_i thick, and successive construction of the transmission functions q_i (2.5.5.4), phase functions $Q_i = \mathscr{F}q_i$, and propagation function $p_k = [k/2\pi i\Delta z]\exp[ik(x^2 + y^2)/2\Delta z]$ (Cowley & Moodie, 1957).

Another method – the scattering matrix method – is based on the solution of equations of the dynamic theory (Chapter 5.2). The emerging wave on the exit surface of a crystal is then found to diffract and experience the transfer function action [(2.5.5.6), (2.5.5.7*a*,*b*)].

The dynamic scattering in crystals may be interpreted using Bloch waves:

$$\Psi^j(\mathbf{r}) = \sum_H C_H^j \exp(-2\pi i\mathbf{k}_H^j \cdot \mathbf{r}). \tag{2.5.5.16}$$

It turns out that only a few (bound and valence Bloch waves) have strong excitation amplitudes. Depending on the thickness of a crystal, only one of these waves or their linear combinations (Kambe, 1982) emerges on the exit surface. An electron-microscopic image can be interpreted, at certain thicknesses, as an image of one of these waves [with a correction for the transfer function action (2.5.5.6), (2.5.5.7*a*,*b*)]; in this case, the identical images repeat with increasing thickness, while, at a certain thickness, the contrast reversal can be observed. Only the first Bloch wave which arises at small thickness, and also repeats with increasing thickness, corresponds to the projection of potential $\varphi(xy)$, *i.e.* the atom projection distribution in a thin crystal layer.

An image of other Bloch waves is defined by the function $\varphi(\mathbf{r})$, but their maxima or minima do not coincide, in the general case, with the atomic positions and cannot be interpreted as the projection of potential. It is difficult to reconstruct $\varphi(xy)$ from these images, especially when the crystal is not ideal and contains imperfections. In these cases one resorts to computer modelling of images at different thicknesses and defocus values, and to comparison with an experimentally observed pattern.

The imaging can be performed directly in an electron microscope not by a photo plate, but using fast-response detectors with digitized intensity output online. The computer contains the necessary algorithms for Fourier transformation, image calculation, transfer function computing, averaging, and correction for the observed and calculated data. This makes possible the interpretation of the pattern observed directly in experiment (Herrmann *et al.*, 1980).

2.5.5.5. Image enhancement

The real electron-microscope image is subdivided into two components:

$$J(xy) = I(xy) + N(xy). \tag{2.5.5.17}$$

The main of these, $I(xy)$, is a two-dimensional image of the 'ideal' object obtained in an electron microscope with instrumental functions inherent to it. However, in the process of object imaging and transfer of this information to the detector there are various sources of noise. In an electron microscope, these arise owing to emission-current and accelerating-voltage fluctuations, lens-supplying current (temporal fluctuations), or mechanical instabilities in a device, specimen or detector (spatial shifts). The two-dimensional detector (*e.g.* a photographic plate) has structural inhomogeneities affecting a response to the signal. In addition, the specimen is also unstable; during preparation or imaging it may change owing to chemical or some other transformations in its structure, thermal effects and so on. Biological specimens scatter electrons very weakly and their natural state is moist, while in the electron-microscope column they are under vacuum conditions. The methods of staining (negative or positive), *e.g.* of introducing into specimens substances containing heavy atoms, as well as the freeze-etching method, somewhat distort the structure of a specimen. Another source of structure perturbation is radiation damage, which can be eliminated at small radiation doses or by using the cryogenic technique. The structure of stained specimens is affected by stain graininess. We assume that all the deviations $\Delta I_k(xy)$ of a specimen image from the 'ideal' image $I_k(xy)$ are included in the noise term $N_k(xy)$. The substrate may also be inhomogeneous. All kinds of perturbations cannot be separated and they appear on an electron microscope image as the full noise content $N(xy)$.