# Theory of three-dimensional reconstruction. II. The recovery operator

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A direct method of recovering three-dimensional functions from their projections is proposed; it is especially suitable for the recovery of the structure of biomacromolecules from their electron micrographs.

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#### INTRODUCTION

when objects are investigated under transmission radiation, information about the objects is conveyed through their projections. The calculation of the structure of the objects is reduced to the problem of extracting and synthesizing the information locked in their projections. When developing general methods of recovering objects from their projections, we shall illustrate the two aspects of the problem through examples taken from electron microscopy of bimolecules.

In ref. 1 we gave conditions under which the problem of recovering the structure of a given object from a set of its projections could be solved, i.e., that there is an operator  $R\{\tau\}$  which pairs the given projection set  $\rho(x, \tau)$  with the desired function  $\rho(x)$  as follows:

$$\rho(\mathbf{x}) = R\{\tau\} \rho(\mathbf{x}, \ \tau). \tag{1}$$

Let us now formulate the requirements which must be satisfied by the recovery operator.

- 1. The operator  $R\{\tau\}$  must be defined not only on one-dimensional complete sets  $\{\tau\}$  but also on two-dimensional complete sets  $\{\tau\}$ .
- 2. The operator  $R\{\tau\}$  must use all the information locked in each projection which it can process.

At first glance the first conditions can appear redundant if one considers the results of ref. 1, where it was shown that in order to recover the desired function  $\rho(x)$ it sufficed to consider only the projections of the function concentrated along complete curves I on a sphere of directions. In this case R{\tau} could be constructed via results already obtained. For example, for a complete curve the projections of the object corresponding to some semicircle are calculated from Eq. (7) of ref. 1, and then the Radon recovery operator is applied. However, in practice the set of known projections  $\{ au\}$  is discrete, and to obtain a continuous set  $\{\tau\}$  some interpolation procedure is needed. When the original set {7} has certain properties, it can happen that the approximation of  $\{\tau\}$  by a two-dimensional set is more advantageous than the approximation of  $\{\tau\}$  by a one-dimensional set. We give an example of such a situation below for an object with point group 53.

The first requirement implies that the choice of the recovery operator is not unique. In fact, the information locked in the various processable projections for some two-dimensional region on the sphere of directions is strongly redundant. Without loss of information about the

original object, part of the projections can be selected or only selected portions of each projection need be processed. To each such procedure will correspond its own recovery operator. In order to remove the indeterminacy in the choice, practical necessity requires that the second condition be imposed.

## THE RECOVERY OPERATOR IN THE GENERAL CASE

We now show that the recovery operator can be chosen in the form

$$\rho(\mathbf{x}) = -\frac{1}{(2\pi)^2} \Delta \int_{G} dG_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\rho(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'| L([\tau \mathbf{x}'])}, \qquad (2a)$$

$$L([\tau x']) = |x|' \int_{\mathcal{C}} dG_{\tau} \delta(\tau' \tau x'). \tag{2b}$$

In these, G is some complete one- or two-dimensional region;  $\mathrm{dG}_{T}$  is the element of length or area;  $\Delta$  is the Laplace operator;  $\Sigma_{T}$  is the plane passing through the coordinate origin and perpendicular to the vector  $\tau$ ;  $\delta(\tau^{!}\tau x^{!})$  is the one-dimensional delta-function, and  $(\tau^{!}\tau x^{!})$  is the triple scalar product of the vectors  $\tau^{!}$ ,  $\tau$ ,  $x^{!}$ . If G is a two-dimensional region, then L([ $\tau x^{!}$ ]), which depends only on the direction of its argument, is equal to the length of the arc of the great circle lying inside the region G and passing through the ends of the vectors  $\tau$  and  $\mathbf{x}^{!} | \mathbf{x}^{!} |^{-1}$ . If G is a one-dimensional region  $\Gamma$ , then

$$L([\tau x']) = \sum_{k} |m\tau_{k}'|^{-1}, \quad m = \frac{[\tau x']}{|[\tau x']|}, \quad |\tau| = 1, \quad (2c)$$

i.e., L([Tx']) is the sum of reciprocal projections of the unit tangent vector to the curve  $\Gamma$  at all points at which  $\Gamma$  intersects the great circle perpendicular to the vector m, where the projections are along this vector. The integral over the plane  $\Sigma_T$  in (10) has a removable singularity for certain x', when  $\tau$  is a boundary vector.

To prove (2), it suffices to show that

$$\delta(\mathbf{x}) = -\frac{1}{(2\pi)^2} \Delta \int_G dG_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\delta(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'| L([\tau \mathbf{x}'])}. \tag{3}$$

In fact, if we replace the left and right sides of (3) by an arbitrary finite function  $\rho(x)$  we get (2). Consider the expression

$$I = -\frac{1}{(2\pi)^2} \Delta \int_G dG_\tau \int_{\Sigma_\tau} dS' \frac{\delta(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'| L([\tau \mathbf{x}'])} =$$

$$= -\frac{\Delta}{(2\pi)^2} \int_{G} dG_{\tau} \frac{|\mathbf{x} - \mathbf{\tau} (\mathbf{x} \mathbf{\tau})|^{-1}}{L([\tau \mathbf{x}])}$$

$$= -\frac{1}{(2\pi)^2} \Delta \frac{1}{|\mathbf{x}|} \int_{G} dG_{\tau} \frac{[1 - (\tau \mathbf{k})^2]^{-1/s}}{L([\tau \mathbf{k}])}, \qquad (4)$$

where  $k = x \cdot |x|^{-1}$ . The integral in (4) does not depend on the vector k. In fact, if we use the representation  $\tau = k(k\tau) + \tau_1$ , then we obtain

$$dG_{\tau} = -d(\tau \mathbf{k}) d\varphi_{\tau_{\perp}},$$

$$\int_{G'} dG_{\tau} \frac{[1 - (\tau \mathbf{k})^{2}]^{-1/2}}{L([\tau \mathbf{k}])} = -\int_{G} \frac{d(\mathbf{k}\tau) d\varphi_{\tau_{\perp}}}{V 1 - (\tau \mathbf{k})^{2}} L([\tau_{\perp} \mathbf{k}])$$

$$= \int_{G} \frac{d\varphi_{\tau_{\perp}} d \arccos(\tau \mathbf{k})}{L([\tau_{\perp} \mathbf{k}])} = \int_{0}^{\pi} d\varphi_{\tau_{\perp}} = \pi.$$
(5)

If we now substitute (5) into (4) and use  $\Delta |x|^{-1} = -4\pi\delta(x)$ , we get  $I = \delta(x)$ , which is what we set out to prove.

We now show that if G is a great semicircle perpendicular to the vector n then (2) becomes Radon's equation. To see this we note that

$$L^{-1}([\tau x']) = \frac{|(nx')|}{|x'|}, \tag{6}$$

$$\Delta \frac{|\mathbf{n}(\mathbf{x}-\mathbf{x}')|}{|\mathbf{x}-\mathbf{\tau}(\mathbf{x}\mathbf{\tau})-\mathbf{x}'|} = -2\delta \mathbf{n}(\mathbf{x}-\mathbf{x}') (\mathbf{n}\mathbf{\tau}\nabla)^2 \ln|\mathbf{x}-\mathbf{\tau}(\mathbf{x}\mathbf{\tau})-\mathbf{x}'|. \quad (7)$$

If we substitute (6) into (2) we use (7), we get Radon's equation:

$$\rho(\mathbf{x}) = \int_{0}^{\pi} d\phi_{\tau} \frac{\Delta}{2\pi^{2}} \int_{-\infty}^{\infty} \rho(\mathbf{x}', \tau) \ln | n\tau(\mathbf{x} - \mathbf{x}') | d\eta, \qquad (8a)$$

where

$$\eta = (\mathbf{n} \tau \mathbf{x}'), \quad \Delta = \left(\frac{\partial}{\partial x_i} - n_i n_k \frac{\partial}{\partial x_k}\right) \left(\frac{\partial}{\partial x_i} - n_i n_m \frac{\partial}{\partial x_m}\right)$$
$$= (\nabla)^2 - (\mathbf{n} \nabla)^2,$$

or

$$\rho(\mathbf{x}) = \frac{1}{2\pi^2} \int_0^{\pi} d\phi_{\tau} \int_{-\infty}^{\infty} \frac{d\eta}{\eta^2} \left[ \rho(\mathbf{x}, \tau) - \rho(\mathbf{x} + [\tau \mathbf{n}] \eta, \tau) \right]. \tag{8b}$$

To recover objects from coaxial projections, a method for the "synthesis of projecting functions" has been proposed in ref. 2, with

$$\Sigma(\mathbf{x}) = \int_{0}^{\pi} d\phi_{\mathbf{x}} \rho(\mathbf{x}, \tau)$$
 (9)

and it gives a fairly good approximation to the function  $\rho$  (x), which is to be recovered. The precise method of recovery defined by (8) can be called modified synthesis of projections.

The Radon equation can be obtained directly from the relation between the two-dimensional synthesis  $\Sigma$  and the desired function, as stated in ref. 2:

$$\Sigma(\mathbf{x}) = \int dS' \frac{\rho(\mathbf{x}')}{|\mathbf{x} - \mathbf{x}'|} = \rho(\widehat{\mathbf{x}}) |\mathbf{x}|^{-1}. \tag{10}$$

This last relation follows immediately from (9) and the equation defining the projection  $\rho(x, \tau)$  from the known function  $\rho(x)$ :

$$\Sigma(\mathbf{x}) = \int_{0}^{\pi} d\phi_{\mathbf{r}} \int_{-\infty}^{\infty} \rho(\mathbf{x} + \tau t) dt = \int_{0}^{2\pi} d\phi_{\mathbf{r}} \int_{0}^{\infty} \rho(\mathbf{x} + \tau t) dt$$

$$= \int_{0}^{2\pi} \int_{0}^{\infty} \frac{\rho(\mathbf{x} + \tau t)}{t} t dt d\phi_{\mathbf{r}} = \int_{0}^{2\pi} dS' \frac{\rho(\mathbf{x} + \mathbf{x}')}{|\mathbf{x}'|} = \rho(\widehat{\mathbf{x}}) |\widehat{\mathbf{x}}|^{-1}. \quad (11)$$

If we regard (10) as an integral equation, we may solve it as follows:

$$\rho(\mathbf{x}) = -\frac{\Delta}{(2\pi)^2} \sum_{\mathbf{x}} \widehat{(\mathbf{x})} |\mathbf{x}|^{-1} = \frac{1}{(2\pi)^2} \int_{\Sigma_{\mathbf{x}}} \frac{\sum_{\mathbf{x}} (\mathbf{x}) - \sum_{\mathbf{x}'} (\mathbf{x}')}{|\mathbf{x} - \mathbf{x}'|^5} dS'.$$
 (12)

Now we substitute (9) into (12):

$$\rho\left(x\right) = -\frac{1}{(2\pi)^{2}} \Delta \int_{0}^{\pi} d\phi_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\rho\left(x',\tau\right)}{\left|x-x'\right|}. \tag{13}$$

If we take into account the fact that the function  $\rho(x,\tau)$  does not depend on the coordinate along the direction of  $\tau$ , we may integrate along this direction and obtain (8). Another derivation of this equation is given in ref. 3.

Now consider the case where the region G is a hemisphere. Then  $L([\tau x']) = \pi$ , and therefore

$$\rho(\mathbf{x}) = -\frac{1}{4\pi^3} \Delta \int_{G} d\Omega_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\rho(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'|}.$$
 (14)

This can be rewritten in the following form:

$$\rho(\mathbf{x}) = \frac{1}{4\pi^3} \int_{\mathcal{C}} d\Omega_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\rho(\mathbf{x}, \tau) - \rho(\mathbf{x}', \tau)}{|\mathbf{x} - \tau(\mathbf{x}\tau) - \mathbf{x}'|^3} . \tag{14a}$$

Equations (14) and (14a) can be derived directly via the operator given in ref. 2; this operator is the spatial synthesis of the projecting function and is defined by the

$$\Sigma(\mathbf{x}) = \int d\Omega_{\tau} \rho(\mathbf{x}, \tau) = \frac{1}{2} \int_{\Sigma} d\Omega_{\tau} \rho(\mathbf{x}, \tau), \quad (15)$$

in which  $d\Omega_T$  is the solid angle element. The integral in the first case is taken over the surface of the hemisphere, and in the second case the integration is extended to the whole sphere G. In this article we have given a relation between the spatial synthesis and the desired function  $\rho(\mathbf{x})$ :

$$\Sigma(\mathbf{x}) = \int \frac{\rho(\mathbf{x}')}{|\mathbf{x} - \mathbf{x}'|^2} dV' = \rho(\widehat{\mathbf{x}}) |\widehat{\mathbf{x}}|^{-2}.$$
(16)

This relation is proved in the same way as (10):

$$\Sigma(\mathbf{x}) = \frac{1}{2} \int_{\mathbf{x}} d\Omega_{\tau} \int_{-\infty}^{\infty} \rho(\mathbf{x} + \tau t) dt = \int_{\mathbf{x}} d\Omega_{\tau} \int_{\mathbf{0}}^{\infty} \rho(\mathbf{x} + \tau t) dt$$
$$= \int_{\mathbf{x}} \int_{\mathbf{t}}^{\infty} \frac{\rho(\mathbf{x} + \tau t)}{t^{2}} t^{2} dt d\Omega_{\tau} = \int_{\mathbf{x}} dV' \frac{\rho(\mathbf{x} + \mathbf{x}')}{|\mathbf{x}'|^{2}}. \tag{17}$$

If we consider (16) as an integral equation defining the function  $\rho(x)$ , we may then write its solution as

$$\rho(\mathbf{x}) = -\frac{1}{(2\pi)^2} \Delta \Sigma(\widehat{\mathbf{x}}) |\widehat{\mathbf{x}}|^{-2}.$$
 (18)

If we now substitute (15) into (18) and then integrate with respect to the coordinate along the direction  $\tau$  we get (14).

### RECOVERY OPERATOR FOR SYMMETRIC OBJECTS

If the object has point symmetry group g, then (2) can be so modified that the invariance of the function to be recovered under the transformation operators  $F_1, F_2, \ldots, F_D$ of the given group g of order p can be directly inferred from the recovery procedure itself. Just as in ref. 1 we introduce the group g, which differs from the group g only by the addition of a center of inversion (of course, if the voriginal group g has a center of inversion, then  $\bar{g} = g$ ), and we partition the sphere of directions S into independent regions Go(k):

$$S = \sum_{k=1}^{\overline{p}} \overline{F}_{k} G_{0}^{(k)} = \sum_{k=1}^{\overline{p}} G_{0}^{(k)}, \qquad (19)$$

where  $\overline{\mathbf{F}}_{\mathbf{k}}$  are the transformation operators of the group g, and p is the order of this group. Let us now suppose that the projections of the symmetric object are known, where the projections correspond to some complete region Go\* CGo. In ref. 1 we indicated the properties which the regions Go\* must have for all groups.

From regions of type Go\* form the region

$$G = \sum_{k=1}^{p} \bar{F}_k G_0$$
 (20)

and introduce

$$L^{\bullet}(\mathbf{x}) = |\mathbf{x}| \int_{G_{\mathbf{x}}} dG_{\mathbf{x}} \delta(\tau \mathbf{x}), \tag{21a}$$

$$L(\mathbf{x}) = \sum_{k=1}^{\bar{p}} L^{\bullet}(\bar{F}_k \mathbf{x}) = \frac{\bar{p}}{p} \sum_{k=1}^{\bar{p}} L^{\bullet}(F_k \mathbf{x}). \tag{21b}$$

Then

$$\rho(\mathbf{x}) = \sum_{k=1}^{p} \rho^{*}(F_{k}\mathbf{x}), \qquad (22a)$$

$$\rho^{*}(\mathbf{x}) = -\frac{1}{(2\pi)^{2}} \frac{\bar{p}}{p} \Delta \int_{G_{0}}^{1} dG_{\tau} \int_{\Sigma_{\tau}}^{1} dS' \frac{\rho(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'| L([\tau \mathbf{x}'])}$$

$$= -\frac{1}{(2\pi)^{2}} \Delta \int_{G_{\tau}}^{1} dG_{\tau} \int_{\Sigma_{\tau}}^{1} dS' \frac{\rho(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'| \sum_{r}^{p} L^{*}(F_{k}[\tau \mathbf{x}'])}. \qquad (22b)$$

To prove (22) one need use only (2) for the region (20) and the relations

$$\rho(\mathbf{x}, F\tau) = \rho(F^{-1}\mathbf{x}, \tau), \tag{23}$$

$$L(F[\tau x']) = L([\tau x']). \tag{24}$$

If Go\* coincides with the region Go, then (22) are simplified since  $L([\tau x]) = 2\pi$ .

Therefore, in this case recovery is achieved as follows:

$$\rho(\mathbf{x}) = \sum_{k=1}^{p} \rho^{*}(F_{k}\mathbf{x}), \tag{25a}$$

$$\begin{split} \rho^{\star}(\mathbf{x}) &= -\frac{1}{8\pi^{3}} \frac{\bar{p}}{p} \Delta \int_{G_{\bullet}} dG_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\rho(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'|} \\ &= \frac{1}{8\pi^{3}} \frac{\bar{p}}{p} \int_{G_{\bullet}} dG_{\tau} \int_{\Sigma_{\tau}} dS' \frac{\rho(\mathbf{x}, \tau) - \rho(\mathbf{x} - \mathbf{x}', \tau)}{|\mathbf{x}'|^{3}} \,. \end{split}$$
(25b)

### CALCULATION OF STRUCTURE FUNCTIONS OF BIOLOGICAL OBJECTS

Let us now discuss the application of the theory developed above to actual calculations of the structures of biological macromolecules. To do this we must make the following observations.

1. Electron microscopy of biomacromolecules studies objects via "models" of them, models which are obtained from contrasting specimens, whose dispersions are characterized by the length  $\lambda = 15-30$  Å. The real distribution of the contrast density  $\rho^{ex}(x)$  can be expressed in the form:

$$\rho^{ex}(\mathbf{x}) = \rho(\mathbf{x}) + \varphi(\mathbf{x}), \tag{26}$$

where  $\rho(x)$  is functionally related to the density of the material of the object itself, and  $\varphi(x)$  is a function describing the noises present even when  $\rho(\mathbf{x})$  is homogeneous. The characteristic length for the noise variation is of the order of the length dimension of the macromolecule, D. Photometry of micrographs of the object therefore leads to the function:

$$\psi_{\tau}(\mathbf{x}) = \rho(\mathbf{x}, \ \tau) + \bar{\varphi}(\mathbf{x}), \tag{27}$$

where  $\hat{\phi}(\mathbf{x})$  is the microphoto noise function. The theory developed in this article ignores this. Therefore, the presence of noise must be allowed for when the recovery algorithm is constructed.

- 2. Only a finite number of microphotographs of the object are ever at the disposal of the experimenter. Therefore when the recovery algorithm is constructed, we must consider how many projections we will need in order to recover the function ρ(x) to the specified accuracy.
- 3. The determination of the orientation of the projection of general position from the micrographs is a problem in itself; it has not yet been solved.

The first difficulty can be overcome within the theory we have developed. To see this we note that (2), (8), (14), and (22) contain the Laplace operator. If we take into account the fact that  $D \gg \lambda$  is usually the case for electron microscopy of biomacromolecules and therefore  $\widetilde{\varphi}(\mathbf{x})$  is a slowly varying function when compared with  $\rho$  (x,  $\tau$ ), then we have that

$$\Delta \psi_{\tau}(\mathbf{x}) = \Delta \rho(\mathbf{x}, \tau) \left[ 1 + O(\lambda^2/D^2) \right]. \tag{28}$$

Thus with an accuracy of 1% the Laplacian of the function given by the photometer coincides with the Laplacian of the projection. Recovery is defined by the procedure

$$\rho(\mathbf{x}) = \int_{\mathcal{C}} dG_{\tau} \Phi(\mathbf{x}; \tau), \tag{29a}$$

$$\Phi(\mathbf{x}; \tau) = -\frac{1}{(2\pi)^2} \int_{\Sigma_{\tau}} dS' \, \frac{\Delta \psi_{\tau}(\mathbf{x} + \mathbf{x}')}{|\mathbf{x}'| L([\tau \mathbf{x}'])} \,. \tag{29b}$$

The same result follows if the calculations are derived from (8), (14), and (22). We note also that the proposed direct method of recovery requires fewer calculations as compared with the binary Fourier transform method of ref. 4, and this means that the noise can be more effectively tuned out.2) The dimensions of the calculation region almost coincide with the dimensions of the object itself. Let us now calculate the number of projections of the object we need in order to determine the function  $\rho(x)$  to the specified accuracy. In order not to make the derivation overlong, let us consider the coaxial projection case. Let us write (13) as

$$\rho \left( \mathbf{x}\right) =\Delta \int\limits_{0}^{\pi }d\phi _{\tau }\Phi \left( \mathbf{x};\tau \right) ,$$

where we use the notation

$$\Phi(\mathbf{x};\tau) = -\frac{1}{(2\pi)^2} \int_{\Sigma_{\tau}} dS' \frac{\rho(\mathbf{x}',\tau)}{|\mathbf{x}-\mathbf{x}'|}.$$
 (30)

Let us divide the interval  $[0,\pi]$  into N parts and use the trapezium and Simpson's rules to estimate the integral. In the first case we have

$$\rho(x) = \Delta \left\{ \frac{\pi}{N} \left[ \Phi_0 + \Phi_1 + \dots + \Phi_{N-1} \right] \left( 1 - \frac{\pi^2}{12N^2} \frac{1}{\Phi^*} \frac{\partial^2}{\partial \phi^2} \Phi^* \right) \right\}, (31)$$

where

$$\begin{split} \Phi_{\lambda} = &\Phi\left(\mathbf{x}; \ \tau_{\lambda}\right), \ \tau_{\lambda} = &\tau\left(\phi_{\lambda}\right) = \tau\left(\frac{\pi}{N} \, k\right), \quad \Phi^{\star} = &\Phi\left(\mathbf{x}; \ \tau^{\star}\right), \\ &\tau^{\star} = &\tau\left(\phi^{\star}\right), \ 0 < \phi^{\star} < \pi. \end{split}$$

In the second case we have

$$\rho(\mathbf{x}) = \Delta \left\{ \frac{2\pi}{3N} \left[ \Phi_0 + \Phi_2 + \dots + \Phi_{N-2} + 2(\Phi_1 + \Phi_2 + \dots + \Phi_{N-1}) \right] \right.$$

$$\times \left( 1 - \frac{\pi^4}{180N^4} \frac{1}{\Phi^4} \frac{\partial^4}{\partial \Phi^4} \Phi^4 \right) \right\}. \tag{32}$$

In this we have assumed that N is even. We now show that  $(1/\Phi)$   $(\partial^2/\partial \varphi^2)\Phi \approx (D^2/\lambda^2)$ . If we use (30) we get

$$\frac{\partial^{2}}{\partial \varphi^{2}} \Phi(\mathbf{x}, \mathbf{\tau}) = -\frac{1}{(2\pi)^{2}} \int dS' \frac{1}{|\mathbf{x} - \mathbf{x}'|} \frac{\partial^{2}}{\partial \varphi^{2}} \rho(\mathbf{x}, \mathbf{\tau})$$

$$= \frac{1}{(2\pi)^{2}} \int \frac{dS'}{|\mathbf{x} - \mathbf{x}'|} \left[ \rho(\mathbf{x}, \mathbf{\tau}) - \Delta \int_{-\infty}^{\infty} t^{2} \rho(\mathbf{x} + \mathbf{\tau}t) dt \right]. \tag{33}$$

Since the dispersion of the contrast density is characterized by the length  $\lambda$ , we have  $\Delta \rho(\mathbf{x}) \approx \rho^{(\mathbf{x})}/\lambda^2$ . Therefore

$$\Delta \int t^2 \rho(\mathbf{x} + \mathbf{\tau}t) dt \approx \frac{1}{\lambda^2} \int t^2 \rho(\mathbf{x} + \mathbf{\tau}t) dt \geqslant \frac{D^2}{\lambda^2} \rho(\mathbf{x}, \mathbf{\tau}). \tag{34}$$

Substituting (34) into (33), we get, if we take  $\lambda \ll D$ ,

$$\frac{\partial^2}{\partial \omega^2} \Phi(\mathbf{x}; \tau) \approx \frac{D^2}{\lambda^2} \Phi(\mathbf{x}; \tau). \tag{35}$$

If we now substitute (35) into (31) and (32), we get

$$\rho(\mathbf{x}) = \frac{\pi}{N} \left( 1 - \frac{\pi^2 D^2}{12N^2 \lambda^2} \right) \Delta \left[ \Phi_0 + \Phi_1 + \dots + \Phi_{N-1} \right]$$
 (36)

and

$$\rho(\mathbf{x}) = \frac{2\pi}{3N} \left[ 1 - \frac{1}{180} \left( \frac{\pi D}{N\lambda} \right)^4 \right] \Delta \left[ \Phi_0 + \dots + \Phi_{N-2} + 2\Phi_1 + \dots + 2\Phi_{N-1} \right].$$
(27)

In order to achieve an accuracy of p%, we must have

$$N_{\rm T} \approx \frac{D}{\lambda} \frac{9}{\sqrt{p}} \tag{38}$$

projections if the trapezium rule is used, and

$$N_{\rm S} \approx \frac{3}{p^{\prime\prime}} \frac{D}{\lambda}$$
 (39)

projections if Simpson's rule is used. For a specified calculation accuracy Simpson's rule requires fewer projections. For example,  $\delta = N_T/N_S = 2.8$  when p = 1%, and  $\delta = 1.7$  when p = 10%.

Equations (38) and (39) are obtained when the distribution of the corresponding projections of points on the semicircle  $\varphi_1, \varphi_2, ..., \varphi_N$  can be assumed uniform. When this cannot be assumed, (38) and (39) must be supplemented so that allowance is made for the nature of the distribution of the corresponding projections of points on the sphere S, and this can be described by the quantity

$$\gamma = \frac{N^2}{\pi^2} \overline{(\phi_k - \phi_{k-1})^2} - 1,$$

in which the bar denotes that an average is taken. The estimate shows that to achieve an accuracy of p% with specified  $\gamma$ , one must take

$$N_{\rm T} \approx \frac{D}{\lambda} \cdot 9\sqrt{(1+3\gamma)/p},$$
 (38a)

$$N_{\rm S} \approx \frac{D}{\lambda} \cdot 3\sqrt[4]{(1+10\gamma)/p}$$
. (39a)

For the general case, the estimates found for the required number of projections still hold. It can be assumed with a good degree of safety that to achieve an accuracy of  $5\lambda$ ,  $N\approx 3D/\lambda$  projections are required.

Thus we see that the natural difficulty is that of determining the orientation of projections of general position. However, for highly symmetric objects this obstacle can be overcome since it is easy to pick out projections taken along the axes of symmetry among microphotographs of such objects.

Let us now consider an example; we consider an object having icosahedral symmetry group 53 (p = 60). On the sphere of directions, the total number of projections along the five-, three-, and two-fold axes is  $5 \cdot 3 + 5 \cdot 2 + 3 \cdot 2 = 31$ . To calculate  $\rho(x)$ , Eq. (23) is the suitable one, with the region  $G_0$  the spherical triangle with vertices at the exit points on the sphere of directions of the five-, three-, and two-fold axes;  $F_k$  are the transformation operators of the group 53. We write the general form of the rotation operator of angle  $2\pi m/n$  around an n-fold axis of direction k:

$$\mathbf{F}_{\mathbf{k}_{m}} = \mathbf{I}\cos\frac{2\pi}{n}\,m + \mathbf{k} \times \mathbf{I}\sin\frac{2\pi}{n}\,m + \mathbf{k} \cdot \mathbf{k}\left(1 - \cos\frac{2\pi}{n}\,m\right). \tag{40}$$

If we join the middle of the base of the spherical triangle with the midpoints of the other two sides, the region is divided into three parts, two of which (containing the axes 5 and 3) will be congruent, while the third (containing the axis 2) will have an area equal to the sum of the other two. As a result, we have

$$\rho^{*}(\mathbf{x}) = \frac{1}{960\pi^{2}} [T(\mathbf{x}; \tau_{5}) + T(\mathbf{x}; \tau_{5}) + 2T(\mathbf{x}; \tau_{2})], \tag{41a}$$

$$T(\mathbf{x};\tau) = -\Delta \int_{\Sigma_{-}} dS' \frac{\rho(\mathbf{x}+\mathbf{x}',\tau)}{|\mathbf{x}'|}, \qquad (41b)$$

where  $\tau_5$ ,  $\tau_3$ , and  $\tau_2$  are the directions of the five-, three-, and two-fold axes, whose exit points are the vertices of the triangle  $G_0$ .

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2) In the binary Fourier transform method mentioned noise can be eliminated by increasing the calculation region in k space.

<sup>1</sup>S. S. Orlov, Kristallografiya, <u>20</u>, 511 (1975) [Sov. Phys. - Crystallogr., <u>20</u>, 312 (1975)].

<sup>2</sup>B. K. Vainshtein, Dokl. Akad. Nauk SSSR, <u>196</u>, 1072 (1971) [Sov. Phys. – Dokl., <u>16</u>, 66 (1971)].

<sup>3</sup>B. K. Vainshtein and S. S. Orlov, Kristallografiya, <u>17</u>, 253 (1972) [Sov. Phys. – Crystallogr., <u>17</u>, 213 (1972)].

<sup>4</sup>D. J. De Rosier and A. Klug, 217, 130 (1968).

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 $<sup>1)</sup>_A$  more advantageous procedure for recovering  $\rho(x)$  would be one such that when both the resolving power of the method of obtaining the projections and the number of projections are specified it gives the smallest error.