Influence of Electron Noise on Three-dimensional Image Reconstruction

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A three-dimensional reconstruction requires the same integral dose as a conventional two-dimensional micrograph provided that the level of significance and the resolution are identical. The necessary dose D for one of the K projections in a reconstruction series is, therefore, the integral dose divided by K.

Introduction

Three-dimensional image reconstruction from electron micrographs of a tilted object has recently led to first results [1–5]. Obviously of great interest is the electron dose which is needed for a statistically significant image at a given resolution. Already some time ago we have shown [6] that the necessary exposure for imaging is approximately the same for the two- and three-dimensional case. In this paper we complete the more or less qualitative arguments in [6] by quantitative calculations.

We assume a reconstruction method based on the theory of Fourier transformation. Each image shows nearly a parallel projection of the object. In Fourier space each of the projections corresponds to a plane central section normal to the direction of projection. If the number of sections is large enough to fill the Fourier space, the object can be reconstructed by a Fourier transformation.

Experimental conditions do not allow to tilt the object from 0° to 180°. Thus the Fourier space is only partially known (see [3–5]). Further errors are caused by the fact that the image is finite [7], by the transfer function which changes from projection to projection, by some approximations of the imaging theory and by radiation damage. This leads to a deviation between the reconstructed and true object function even if the electron noise is neglected. We want to accentuate that all our results concerning statistics are related to an ideally reconstructed object function. In our calculation we assume, therefore, that a bounded object can be tilted from 0° to 180°, and that the angular increment is so small that no additional clutter will be produced by the reconstruction method. Perhaps the 180°-condition can be realized in the future by the use of whiskers instead of a supporting foil [8].

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Mathematical Background

If the object is tilted about a single rotation axis the mathematical problem reduces to a set of two-dimensional ones. The reconstruction can be done in slices which are normal to the rotation axis (see e. g. [3]). We define the y-axis as rotation axis and the z-axis as optical axis of the imaging instrument. Then the object can be described by a function $\varrho(x, z)$ respectively by its Fourier transform $F(x^*, z^*)$.

$$\varrho(x, z) = \iint F(x^*, z^*)\, e^{2\pi i (x x^* + z z^*)} \mathrm{d}x^*\, \mathrm{d}z^* . \quad (1)$$

Using polar coordinates we write also:

$$\hat{\varrho}(r, \varphi) = \int_{-\pi}^{\pi} \int_{0}^{\infty} \hat{F}(R, \Phi)\, e^{2\pi i r R \cos(\varphi - \Phi)} R\, \mathrm{d}R\, \mathrm{d}\Phi ,$$

where

$$\hat{\varrho}(r, \varphi) = \varrho(r \cos\varphi, r \sin\varphi) , \quad (2)$$

$$\hat{F}(R, \Phi) = F(R \cos\Phi, R \sin\Phi) .$$

Expanding the exponential in (2) in terms of Bessel functions of integer order

$$e^{2\pi i r R \cos(\varphi - \Phi)} = \sum_{n=-\infty}^{\infty} i^n J_n(2\pi R r)\, e^{i n (\varphi - \Phi)}$$

leads together with the integration over Φ to the Fourier coefficients

$$F_n(R) = \frac{1}{2\pi} \int_{-\pi}^{\pi} \hat{F}(R, \Phi)\, e^{-i n \Phi}\, \mathrm{d}\Phi \quad (3)$$

of the function $\hat{F}(R, \Phi)$ which is periodic in Φ,

$$\hat{F}(R, \Phi) = \sum_{n=-\infty}^{\infty} F_n(R)\, e^{i n \Phi} . \quad (4)$$

Thus the object function can be described by

$$\hat{\varrho}(r,\varphi) = 2\pi \sum_{n=-\infty}^{\infty} i^n e^{in\varphi} \int_0^{\infty} R J_n(2\pi R r) F_n(R)\,\mathrm{d}R . \tag{5}$$

We denote by $P(x,\vartheta)$ a projection of the object tilted about the angle ϑ. Between $P(x,\vartheta)$ and the object function $\varrho(x,z)$ exists the relation

$$P(x,\vartheta) = \int_{-\infty}^{\infty} \varrho(x\cos\vartheta + z\sin\vartheta, \; -x\sin\vartheta + z\cos\vartheta)\,\mathrm{d}z \tag{6}$$

which can be expressed by using (1) in Fourier space, too:

$$\int_{-\infty}^{\infty} P(x,\vartheta)\, e^{-2\pi i u x}\,\mathrm{d}x = F(u\cos\vartheta, \; -u\sin\vartheta) . \tag{7}$$

Each point (u,ϑ) from the region $-\infty < u < \infty$, $0 \leq \vartheta < \pi$ corresponds uniquely to a point (R,Φ) in the region $0 \leqq R < \infty$, $-\pi \leq \Phi < \pi$. Therefore the function $F(u\cos\vartheta, \; -u\sin\vartheta)$ determines completely the function $\hat{F}(R,\Phi)$ so that the object function can be calculated using (7), (3) and (5), if all projections $P(x,\vartheta)$ are known.

We have to take into consideration that only a finite number K of projections at discrete values ϑ_k ($k = 0, 1, \ldots, K-1$; $0 \leqq \vartheta_k < \pi$) are available. Therefore the function $\hat{F}(R,\Phi)$ is known only on a "star" of $2K$ sampling lines with $\Phi = \Phi_l$ ($l = -K, \ldots, K-1$; $-\pi \leqq \Phi_l < \pi$), where the Φ_l are identical with ϑ_k or $\vartheta_k - \pi$ respectively. If the increment of ϑ is a constant π/K and K is large enough, the relations (3) and (4) are to be replaced by

$$F_n(R) = \frac{1}{2K} \sum_{l=-K}^{K-1} \hat{F}(R,\Phi_l)\, e^{-in\Phi_l} \tag{8}$$

and

$$\hat{F}(R,\Phi) = \sum_{n=-K}^{K-1} F_n(R)\, e^{in\Phi} . \tag{9}$$

Instead of (5) we get the reconstructed object function

$$\tilde{\varrho}(r,\varphi) = 2\pi \sum_{n=-K}^{K-1} i^n e^{in\varphi} \int_0^{R_{\max}} R J_n(2\pi R r) F_n(R)\,\mathrm{d}R . \tag{10}$$

This expression deviates form the true object function $\tilde{\varrho}(r,\varphi)$ for two reasons: a) the finite number of projections, b) the resolution limit $R_{\max}$ which in practice cannot be exceeded. In the limit $K \to \infty$ and $R_{\max} \to \infty$ $\tilde{\varrho}(r,\varphi)$ tends to $\hat{\varrho}(r,\varphi)$.

Influence of the Electron Noise

The shot effect of the imaging electrons causes random fluctuations in the measured projections. The deviation from the theoretically expected value represented by (6) can be described approximately by addition of a stochastic function $n(x,\vartheta)$:

$$P(x,\vartheta_k) = \int_{-\infty}^{\infty} \varrho(x\cos\vartheta_k + z\sin\vartheta_k, \; -x\sin\vartheta_k + z\cos\vartheta_k)\,\mathrm{d}z + n(x,\vartheta_k) . \tag{11}$$

If $P(x,\vartheta)$ is a stochastic function process, $\hat{F}(R,\Phi)$, the coefficients $F_n(R)$ and $\tilde{\varrho}(r,\varphi)$ are stochastic variables too. Using (7), (8) and (10) the moments of them can be calculated from the moments of $n(x,\vartheta_k)$.

The assumption of additive noise can be allowed only if the noise does not depend on the expected signal. Now the number n of electrons which hit a certain area during a given time is distributed according to the Poisson law with a standard deviation nearly $\sqrt{n}$. Nevertheless, additive noise is a good approximation because only phase contrast images of weakly scattering objects are used for reconstruction. Such images show low contrast in comparison with the background caused by the strong primary beam. Therefore the noise determined mainly by the primary beam is independent of the signal and furthermore stationary because of the constant level of the background [9]. Explicitly we assume two conditions to be fulfilled:

1) $n(x,\vartheta_k)$ is stationary with respect to x and ϑ_k.
2) $n(x,\vartheta_k)$ and $n(x',\vartheta_l)$ are statistically independent for $k = l$.

To be stationary with respect to the tilting angle it is necessary to use the same exposure for all projections. The statistical independence of different projections is obvious. From these assumptions follows the existence of an even autocovariance function $\gamma(x)$:

$$\mathrm{Cov}\{P(x,\vartheta_k), P(x',\vartheta_l)\} = \mathrm{Cov}\{n(x,\vartheta_k), n(x',\vartheta_l)\} = \delta_{kl}\,\gamma(x'-x) . \tag{12}$$

Thus the covariance of two points x and x' only depends on the distance $|x-x'|$. The Kronecker symbol δ_{kl} with $\delta_{kl} = 1$ for $k = l$ and $\delta_{kl} = 0$ for $k \neq l$ describes the statistical independence of two different projections.

Instead of the autocovariance function it is possible to use its Fourier transform, the power spectrum $\Gamma(u)$:

$$\Gamma(u) = \int_{-\infty}^{\infty} \gamma(x)\, e^{-2\pi i u x}\,\mathrm{d}x . \tag{13}$$

$\Gamma(u)$ shows how the noise of the projection is distributed over space frequency because

$$\text{Var}\{P(x,\vartheta_k)\} = \gamma(0) = \int_{-\infty}^{\infty} \Gamma(u)\,\mathrm{d}u\,. \quad (14)$$

In the appendix we calculate the covariance of two points of the reconstructed object function. Following Eq. (A 4) we get for $r = r'$ and $\varphi = \varphi'$:

$$\text{Var}\{\tilde{\varrho}(r,\varphi)\} = \frac{2\pi^2}{K} \int_0^{R_{\max}} R^2\,\Gamma(R)\,\mathrm{d}R\,. \quad (15)$$

This expression is independent of r and φ just as the variance of a projection represented by (14) is independent of x and ϑ_k. The power spectrum $\Gamma(R)$, however, is weighted here with a factor $(2\pi R)^2/2K$, so that higher space frequencies contribute much more to the noise than lower ones. This follows from the fact, that on each circle in Fourier space with radius R always $2K$ sampling points are known. Thus the density of sampling points and of information decreases with increasing R [10].

Consideration of the Electron Dose

The density of an electron micrograph can be described by the values I_i $(i = 1, 2, \ldots, N)$ of N image elements, which are the results of a densitometer measurement. For reconstruction, however, we use the normalized values

$$P_i = I_i/I_0 - 1 \quad \text{with} \quad I_0 = \frac{1}{N}\sum_{i=1}^{N} I_i\,. \quad (16)$$

The P_i are the discrete analog to the projection function $P(x)$ in equation (11). (Here we have omitted the unimportant argument ϑ_k.) This scaling makes the projection function identical with the contrast defined by Scherzer [11]. Its advantage is that the expectation values of the P_i and also of $\tilde{\varrho}(r,\varphi)$ are independent of the electron dose.

If the photographic plate is used in its linear range then the measured I_i are proportional to the number ν_i of electrons which have hit the i-th image element during the exposure time of one projection

$$P_i = \frac{\nu_i}{\nu_0} - 1 \quad \text{with} \quad \nu_0 = \frac{1}{N}\sum_{i=1}^{N} \nu_i\,. \quad (17)$$

The ν_i are distributed according to the Poisson law. Their most important statistical properties are the equality of expectation value and variance and the independence of different image elements,

$$\text{Var}\{\nu_i\} = M\{\nu_i\} = \lambda_i\,,$$

$$\text{Cov}\{\nu_i, \nu_j\} = \delta_{ij}\,\lambda_i\,,$$

where λ_i denotes the expectation value of ν_i. Using only these second order moments of the ν_i and starting from (17) we can calculate approximately

$$\text{Cov}\{P_i, P_j\} = \frac{1}{\lambda_0}\left(\frac{\lambda_i}{\lambda_0}\,\delta_{ij} - \frac{1}{N}\,\frac{\lambda_i\,\lambda_j}{\lambda_0^2}\right),$$

with

$$\lambda_0 = \frac{1}{N}\sum_{i=1}^{N} \lambda_i\,.$$

The assumption of stationarity made above means that nearly $\lambda_i = \lambda_j = \lambda_0$ so that for large N the equation

$$\text{Cov}\{P_i, P_j\} = (1/\lambda_0)\,\delta_{ij} \quad (18)$$

is valid. If we replace this relation by its continuous analogy and use (13) we get

$$\Gamma(u) = 1/D\,, \quad (19)$$

a constant power spectrum representing a white noise process. D is the number of electrons which in the average occupy the unit length within the exposure time of a projection, i. e. the dose per unit length. λ_0 follows from D by multiplication with the length of one image element.

This result allows us to prove an important statement. If the noise of the projection has the same band limit as $\tilde{\varrho}(r,\varphi)$ we get from Eq. (14)

$$\text{Var}\{P(x)\} = (2/D)\,R_{\max}\,. \quad (20)$$

According to (15) the variance of the reconstructed object function is

$$\text{Var}\{\tilde{\varrho}(r,\varphi)\} = (2\pi^2/3\,K\,D)\,R_{\max}^3\,. \quad (21)$$

Halving the dose D does not change the functions $P(x)$ and $\tilde{\varrho}(r,\varphi)$. However the standard deviations (i. e. the square roots of the variance) of both functions increase by a factor of $\sqrt{2}$. If at the same time the number K of projections is doubled the term $K\,D$ and the standard deviation of the reconstructed object function remain constant. Denoting $2\,a$ for the extension of the object, the term $2\,a\,K\,D$ corresponds to the integral electron dose. Thus it is possible to reconstruct a significant object from non-significant projections, if K is large. In practice there is only the additional condition that the determination of the tilt axis by correlation of successive projections must be significant (see [4]).

An Example

We demonstrate by an example that imaging of a detail of an object is possible with the same dose in the two- and in the three-dimensional case. Let us regard a schematic object consisting of a transparent and uniform plate with thickness t and density C_2. This plate contains a sphere of radius r_0 and density C_1 centred at the point (x_0, z_0) in one of the sections perpendicular to the y-axis. This model can be understood as a heavy atom cluster embedded in a light atom foil (at a resolution where the structure of the foil can be neglected) or – in the case $C_1 < C_2$ – as a hole surrounded smoothly by atoms. We can detect the sphere in a conventional micrograph as well as by three-dimensional image reconstruction. However, only the latter method provides information concerning the z-coordinate. As signal we use the measured projection function $P(x)$ at the tilting angle $\vartheta = 0$ in the two-dimensional case and the reconstructed object function $\hat{\varrho}(x, z)$ in the thre-dimensional case. Both functions describe the sphere according to the scaling (16) by a deviation from the zero level. The detection of the sphere is significant if $P(x)$ or $\tilde{\varrho}(x, z)$ are large enough at the point x_0 or (x_0, z_0) compared to the standard deviation (20) or (21).

We assume K to be large enough so that the expectation value of the reconstructed object function $\tilde{\varrho}(x, z)$ may be replaced by the true object function, namely

$$M\{\tilde{\varrho}(x_0, z_0)\} = C_1 .$$

The expectation of $P(x)$ at the point x_0 is $2 r_0 C_1 + (t - 2 r_0) C_2$ or approximately

$$M\{P(x_0)\} = 2 r_0 C_1$$

according to the scaling (16). Together with (20) and (21) we get for the signal to noise ratio in the case of the reconstructed object function

$$\frac{M\{\varrho(x_0, z_0)\}}{\sqrt{\mathrm{Var}\{\tilde{\varrho}(x_0, z_0)\}}} = C_1 \sqrt{\frac{D}{2 R_{\max}}} \frac{\sqrt{3 K}}{\pi R_{\max}}$$

and in the case of the projection

$$\frac{M\{P(x_0)\}}{\sqrt{\mathrm{Var}\{P(x_0)\}}} = C_1 \sqrt{\frac{D}{2 R_{\max}}}\, 2 r_0 .$$

If the diameter $2 r_0$ of the sphere is equal to the nominal resolution $0.6/R_{\max}$ the signal to noise ratio of the reconstructed object function exceeds that of the projection by a factor of $\sqrt{3 K}/0.6 \pi \approx \sqrt{K}$. In other words: To detect the sphere by a single projection with the same significance the dose of this projection must be equal to integral dose $K D$ necessary for three-dimensional reconstruction.

If in our model a second sphere were situated above the first the signal to noise ratio of the projection at the point x_0 would be doubled. It is possible to decide between one or two spheres by projection if one uses two density levels. One can show that the significance of decision between the first and the second level is the same as between the first and the noise level. However, this method works only if one can be sure that there are equal spheres with density C_1. Decision between a sphere with density $2 C_1$ and two spheres with density C_1 is only possible by the use of three-dimensional image reconstruction which, furthermore, provides the z-coordinates of the spheres.

At atomic resolution we can represent all objects in good approximation by spheres of different densities ("atoms"). Generalizing the example it is obvious that three-dimensional image reconstruction provides more information about the object compared to a single projection with the same integral dose and resolution.

Conclusions

The result derived above is of general interest in the treatment of information. Let us regard an ensemble of N statistical events (e. g. N scattered electrons). Each event is characterized by several parameters (e. g. their positions in the image plane). A further discrimination of the N events with respect to an additional parameter (in our case orientation of the specimen) leads to new information (in our case differentiation of the structure along the third coordinate). The argument can be generalized – for example another additional significant parameter in electron microscopy of radiation sensitive specimens is the time t of the scattering event. These ideas lead immediately to the concept of trace structure analysis [12]. Another type of information gain – not discussed in this paper – is possible, if redundancies can be utilized. A striking example is the retrieval of phase information in X-ray crystallography based on the positive and point like ("atomic") structure of the electron density (see also the use of a different type of redundancies in [12]).

Appendix: Calculation of the covariance of two points of the reconstructed object function.

Using (7), (12) and (13) the covariance of two points in the Fourier space can be described by

$$\mathrm{Cov}\{F(u\cos\vartheta_k, -u\sin\vartheta_k), F^*(u'\cos\vartheta_l, -u'\sin\vartheta_l)\} = \delta_{kl}\,\Gamma(u)\,\delta(u'-u)\,. \tag{A 1}$$

Now $F(u\cos\vartheta, -u\sin\vartheta)$ is identical with $\hat{F}(R, \Phi)$. Explicitly we state the relation

$$\hat{F}(R, \Phi_k) = \begin{cases} F(R\cos\vartheta_k, -R\sin\vartheta_k) & \text{for } k = 0, 1, \ldots, K-1\,, \\ F(-R\cos\vartheta_{k+K}, R\sin\vartheta_{k+K}) & \text{for } k = -K, -K+1, \ldots, -1\,. \end{cases}$$

Considering furthermore the symmetry of $\Gamma(u)$ and $\delta(u)$ were replace (A 1) by

$$\mathrm{Cov}\{\hat{F}(R, \Phi_k), \hat{F}^*(R', \Phi_l)\} = \Gamma(R)\,[\delta_{kl}\,\delta(R-R') + \delta_{k+K,l}\,\delta(R+R')]\,. \tag{A 2}$$

We omit the δ-function with the argument $R+R'$ because R and R' are non-negative. Combining (8) and (A 2) gives the covariances of two Fourier coefficients $F_n(R)$ and $F_m(R')$:

$$\mathrm{Cov}\{F_n(R), F_m^*(R')\} = \frac{1}{4K^2}\,\Gamma(R)\,\delta(R-R')\sum_{k=-K}^{K-1} e^{-i(n-m)\Phi_k}\,.$$

Obviously the summation over k is equal to $2K$ in the case $m=n$. For $m \neq n$ the summation is zero which can be proved using the constant increment of Φ and the addition theorems of trigonometric functions. So we get

$$\mathrm{Cov}\{F_n(R), F_m^*(R')\} = (1/2K)\,\delta_{mn}\,\Gamma(R)\,\delta(R-R')\,. \tag{A 3}$$

Now the covariance of the reconstructed object function $\tilde{\varrho}(r, \varphi)$ at two points (r, φ) and (r', φ') can be expressed according to (10) by a summation over all covariances of the Fourier coefficients. Substituting (A 3) leads to

$$\mathrm{Cov}\{\tilde{\varrho}(r, \varphi), \tilde{\varrho}^*(r', \varphi')\} = \frac{2\pi^2}{K}\sum_{n=-K}^{K-1} e^{in(\varphi-\varphi')}\int_0^{R_{\max}} R^2\,J_n(2\pi r R)\,J_n(2\pi r' R)\,\Gamma(R)\,\mathrm{d}R\,.$$

We can simplify this experession using the relation

$$J_0\left(2\pi R\sqrt{r^2+r'^2-2rr'\cos(\varphi-\varphi')}\right) = \sum_{n=-\infty}^{\infty} J_n(2\pi R r)\,J_n(2\pi R r')\,e^{in(\varphi-\varphi')}\,,$$

which may be approximated by a limited summation from $-K$ to $K-1$.

$$\mathrm{Cov}\{\tilde{\varrho}(r, \varphi), \tilde{\varrho}^*(r', \varphi')\} = \frac{2\pi^2}{K}\int_0^{R_{\max}} R^2\,J_0\left(2\pi R\sqrt{r^2+r'^2-2rr'\cos(\varphi-\varphi')}\right)\Gamma(R)\,\mathrm{d}R\,. \tag{A 4}$$

It is interesting to note the condition for the validity of this approximation. As $J_n(x) \approx 0$ for $0 \leq x < |n|-2$ the limited summation is allowed if $2\pi R r < K-2 \approx K$. If the object lies within a circle of radius a the largest possible value of $2\pi R r$ is $2\pi R_{\max} a$. So the condition $R_{\max} < K/2\pi a$ [7] assures that the statistical properties of $\tilde{\varrho}(r, \varphi)$ are stationary and as shown in [13] that the resolution is uniform.

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