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Image Processing in High-Resolution Electron Microscopy using the Direct Method. I. Phase Extension

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Abstract

A procedure to combine the information from an electron micrograph (EM) and the corresponding electron diffraction (ED) pattern is proposed. Here the ED data will be used to obtain a set of amplitudes of the structure factors, while the EM will be used to obtain a set of starting phases assuming the weak-phase-object approximation. A direct method is then used to extend the phase information from a resolution of about 2 to 1 Å. The efficiency of the procedure has been verified by the test calculation on the model structure of copper perchlorophthalocyanine.

Introduction

The idea of combining the information from an EM and the corresponding ED pattern was first proposed by Gerchberg & Saxton (1971, 1972). They showed that the phase problem in both the image plane and the back focal plane can be solved by an iterative procedure. Later, Li (1977) reported the idea of improving the image resolution by the combination of EM and ED. Recently, Ishizuka, Miyazaki & Uyeda (1982) showed that, under the weak-phase-object approximation, improvement in both quality and resolution can be achieved by incorporating the phase correction method (summarized by Gassmann, 1976) into the combination of EM and ED. On the other hand, the application of the direct method, developed in X-ray crystallography, to the image deconvolution in high-resolution electron microscopy was proposed by Li & Fan (1979). This paper describes a procedure that makes use of the direct method in the combination of information from EM and ED. The method was tested under similar conditions assumed by Ishizuka *et al.* (1982).

Method

An ED pattern usually contains observable reflections within a limiting sphere of 1 $Å^{-1}$ radius. This implies that we can obtain from the ED data a structure image of about 1 Å resolution, which is considerably higher than that which can be reached by an EM. In addition, the intensities of the ED pattern from a crystalline specimen are independent of defocus and spherical aberration of the objective lens. Accordingly, under the weak-phase-object approximation,* a set of high-resolution structure amplitudes of good quality can be obtained from an ED pattern. However, the structure analysis by ED alone is subject to the well known difficulty of the 'phase problem'. On the other hand, an EM can provide phase information corresponding to about 2 Å resolution, which can greatly reduce the complexity of the solution of the phase problem. Hence, an improved high-resolution image may be obtained by a phase interpolation and extrapolation procedure using the amplitudes of the structure factors from ED and starting phases from EM.

* The applicability of the weak-phase-object approximation has been demonstrated by Unwin & Henderson (1975) for biological specimens and by Klug (1978/79) for an inorganic compound.

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According to the weak-phase-object approximation, the image intensity is expressed as

$$I(\mathbf{r}) = 1 + 2\sigma\varphi(\mathbf{r}) * \mathcal{F}^{-1}\{A(H)\sin\chi_1(H) \times \exp[-\chi_2(H)]\}, \quad (1)$$

where $\sigma = \pi/\lambda U$, λ is the electron wavelength and U the accelerating voltage. $\varphi(\mathbf{r})$ represents the projection of the potential distribution function. $\mathcal{F}^{-1}$ and * denote the inverse Fourier transform operation and the convolution multiplication, respectively. $A(H)$ is the aperture function, which takes the value of unity inside the aperture and zero elsewhere.

$$\chi_1(H) = \pi\Delta f\lambda H^2 + \tfrac{1}{2}\pi C_s\lambda^3 H^4,$$

where C_s is the spherical aberration coefficient and Δf the defocus amount (negative for underfocus).

$$\chi_2(H) = \tfrac{1}{2}\pi^2\lambda^2 H^4 D^2,$$

where D is the standard deviation of the Gaussian distribution of defocus due to the chromatic aberration (Fejes, 1977). Fourier transforming (1) gives

$$T(\mathbf{H}) = \delta(H) + 2\sigma F(\mathbf{H})\sin\chi_1(H)\exp[-\chi_2(H)]. \quad (2)$$

Except for those reflections for which $\sin\chi_1(H)\exp[-\chi_2(H)] \sim 0$, (2) can be used to derive the amplitudes and phases of structure factors $F(\mathbf{H})$ from an EM within the resolution limit, provided $\chi_1(H)$ and $\chi_2(H)$ are found in advance. The phases so obtained can be used as a starting set for interpolation and extrapolation.

Phase-extension techniques are well developed in X-ray crystallography such as that used for macromolecular structures (summarized by Sayre, 1980) and that in the *MULTAN* program (Main *et al.*, 1980) used for small structures. For the sake of simplicity in implementation, the latter is used in the present work. The amplitudes of structure factors obtained from the ED pattern are input into the *MULTAN* program, while the phases from the EM are used as 'known phases'. A few reflections of unknown phase are added to the starting set for phase permutation. The best solution is selected automatically according to the figures of merit normally used in *MULTAN*. In order to handle the ED and EM data, two minor modifications have been made to the *MULTAN* program.

1. The atomic scattering-factor coefficients for X-rays are replaced by those for electrons (Jiang & Li, 1984).

2. The origin checking in the 'convergence' process is bypassed, since no origin fixation is needed when a set of phases from the EM is available. Moreover, the origin checking procedure in the *MULTAN* program is only suitable for handling three-dimensional diffraction data and is not valid in the two-dimensional case.

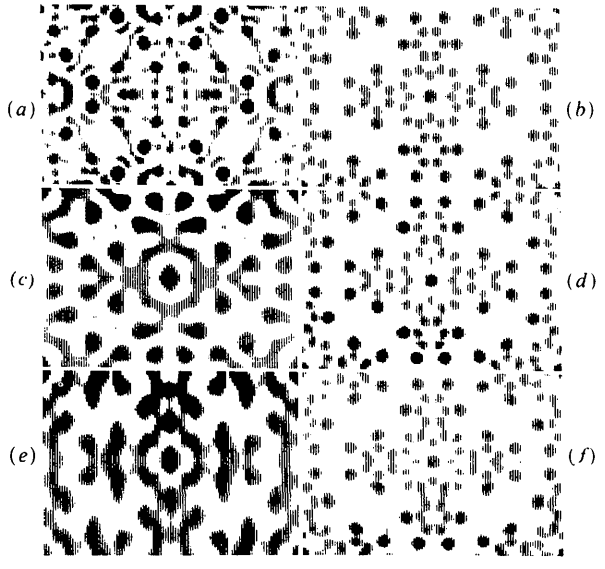

Fig. 1. Projections along the c axis of the unit cell of copper perchlorophthalocyanine, $C_{32}Cl_{16}CuN_8$, $a = 19{\cdot}62$, $b = 26{\cdot}04$, $c = 3{\cdot}76$ Å, $\beta = 116{\cdot}5°$. (*a*) The best E map from *MULTAN*80 using the ED data as input. (*b*) The expected image - potential distribution map calculated using the theoretical $F(\mathbf{H})$'s. (*c*) The starting image at 2·0 Å resolution. (*d*) The resulting image by phase extension from 2·0 to 1·0 Å. (*e*) The starting image at 2·5 Å resolution. (*f*) The resulting image by phase extension from 2·5 to 1·0 Å. All the images from (*c*) to (*f*) were calculated using $F(\mathbf{H})$ as coefficients.

Test data

A model structure of copper perchlorophthalocyanine was used to generate the test data. Structure factors within 1 Å^{-1} (1 Å resolution) were calculated. The amplitudes were used as the data from an ED pattern, while the phases within 0·5 Å^{-1} (2 Å resolution) were used to simulate those obtained from an EM using (2). The imaging condition is assumed to be 500 kV electrons, $\Delta f = -1000$ Å, $C_s = 1$ mm and $D = 150$ Å. Phases of reflections with $|\sin\chi_1(H)\exp[-\chi_2(H)]| \leq 0{\cdot}2$ were regarded as unknown phases.

Test result

1. *Phasing of the ED pattern by the conventional direct method*

It is interesting to see whether a conventional direct method with the ED data alone can lead to a structure image of 1 Å resolution. Using the 189 independent structure-factor amplitudes as input to the *MULTAN* program, 138 phases were assigned. The best E map is shown in Fig. 1(*a*). In comparision with the expected image (Fig. 1*b*), it can be seen that this E map suffers from serious distortion. If nothing is known in advance about the exact structure, further improvement would be rather difficult.

2. *Phase extension from* 2·0 Å *resolution*

Fig. 1(*c*) shows the structure image of 2 Å resolution, which is obtained from the 2 Å EM after the aberrations have been properly corrected. Fourier transforming this image and excluding those reflections satisfying the relation $|\sin\chi_1(H)\times\exp[-\chi_2(H)]|\leq 0{\cdot}2$, we obtain a set of phases for 44 independent reflections within 0·5 $Å^{-1}$. With these starting phases, 116 unknown phases were derived by the *MULTAN* program using a set of normalized structure amplitudes corresponding to 1 Å resolution. The originally unknown phases belong to two categories. One corresponds to those reflections within 0·5 $Å^{-1}$ and satisfying the relation $|\sin\chi_1(H)\exp[-\chi_2(H)]|\leq 0{\cdot}2$, the other corresponds to those reflections between 0·5 and 1 $Å^{-1}$. There are three unknown phases belonging to the first category and all of them had the correct phase (sign) after the *MULTAN* run. On the other hand, there are 113 unknown phases belonging to the second category and 14 of them had their signs wrongly determined. However, most of the wrong signs were associated with a relatively low weight. A weighted Fourier map was then calculated as shown in Fig. 1(*d*). The resulting image is much better than that in either Fig. 1(*a*) or Fig. 1(*c*) and is almost the same as the expected one in Fig. 1(*b*).

3. *Phase extension from* 2·5 Å *resolution*

While the phase extension from 2·0 Å resolution was satisfactory, the phase extension from 2·5 Å resolution was not successful until the normalized structure factors $E(\mathbf{H})$ were replaced by the conventional structure factors $F(\mathbf{H})$ in the *MULTAN* procedure. The starting and resulting images are shown in Fig. 1(*e*) and Fig. 1(*f*), respectively.

Discussion

1. The reason for the *MULTAN* failure in direct phasing of the ED data may be that, in the two-dimensional case, the conventional *MULTAN* procedure is not as powerful as in three-dimensional cases. Besides, in additional to the light atoms, the test structure contains two kinds of heavy atoms, *i.e.* Cu and Cl. This leads to non-negligible deviation of the Sayre equation, which is based on the assumption that the crystal is composed of identical atoms.

2. In a phase-extension process, the phase permutation is not necessary in principle, but it led to a better result in practice. This is due to the inclusion of higher-resolution reflections in the starting set.

3. The effect of truncation will increase with the decrease of starting resolution of a phase-extension process. However, when the whole set of $E(H)$ are replaced by the corresponding set of $F(H)$, the truncation effect can be partially compensated. This explains the unusual result that the phase extension from 2·5 Å failed with a set of $E(H)$ but succeeded with the corresponding set of $F(H)$.

4. With the method described in this paper, only one EM and the corresponding ED data are needed and no *a priori* knowledge of either the complete or the partial structure has to be used. Moreover, the direct-method procedure used here is mainly that commonly used in X-ray crystallography and has not yet been optimized for use with two-dimensional ED data. However, the test results have been shown to be quite satisfactory. Hence, the direct method may become an important tool of image processing in high-resolution electron microscopy.

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