DIRECT METHODS AS A TOOL OF IMAGE PROCESSING IN HIGH RESOLUTION ELECTRON MICROSCOPY

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Introduction

High resolution electron microscopy (HREM) is becoming more and more important in the determination of crystal structures. Apart from that HREM has achieved great progress in recent years, there is another reason: While many crystals important in science and technology are too small and imperfect for carrying out an X-ray single crystal analysis, they are suitable for HREM observation. However, structure analysis by HREM is not as straightforward as that by X-ray single crystal methods, especially when the structure of the crystal is completely unknown. HREM suffers from two disadvantages. First, an electron micrograph (EM) is not a true structure image of the object but rather a convolution of the projected potential distribution with the Fourier transform of the contrast transfer function. Second, the point to point resolution of an EM (about 2Å at present stage) is much lower than that obtainable by diffraction analysis. The above disadvantages can be overcome by some kinds of image processing techniques , namely, the image deconvolution and resolution enhancement.

Direct methods developed in X-ray crystallography belong to a special kind of image processing technique. A three-dimensional X-ray diffraction pattern in which the phase information is lost may be regarded as the Fourier transform of a blurred image of the crystal structure. This blurred image is nothing but the Patterson function. A set of diffraction intensities after processing by a direct method in reciprocal space can be converted to a set of structure factors i.e. the Fourier transform of the structure image. Such a process may be considered as some kind of image deblurring, which clears up the Patterson map to give a true image of the structure.

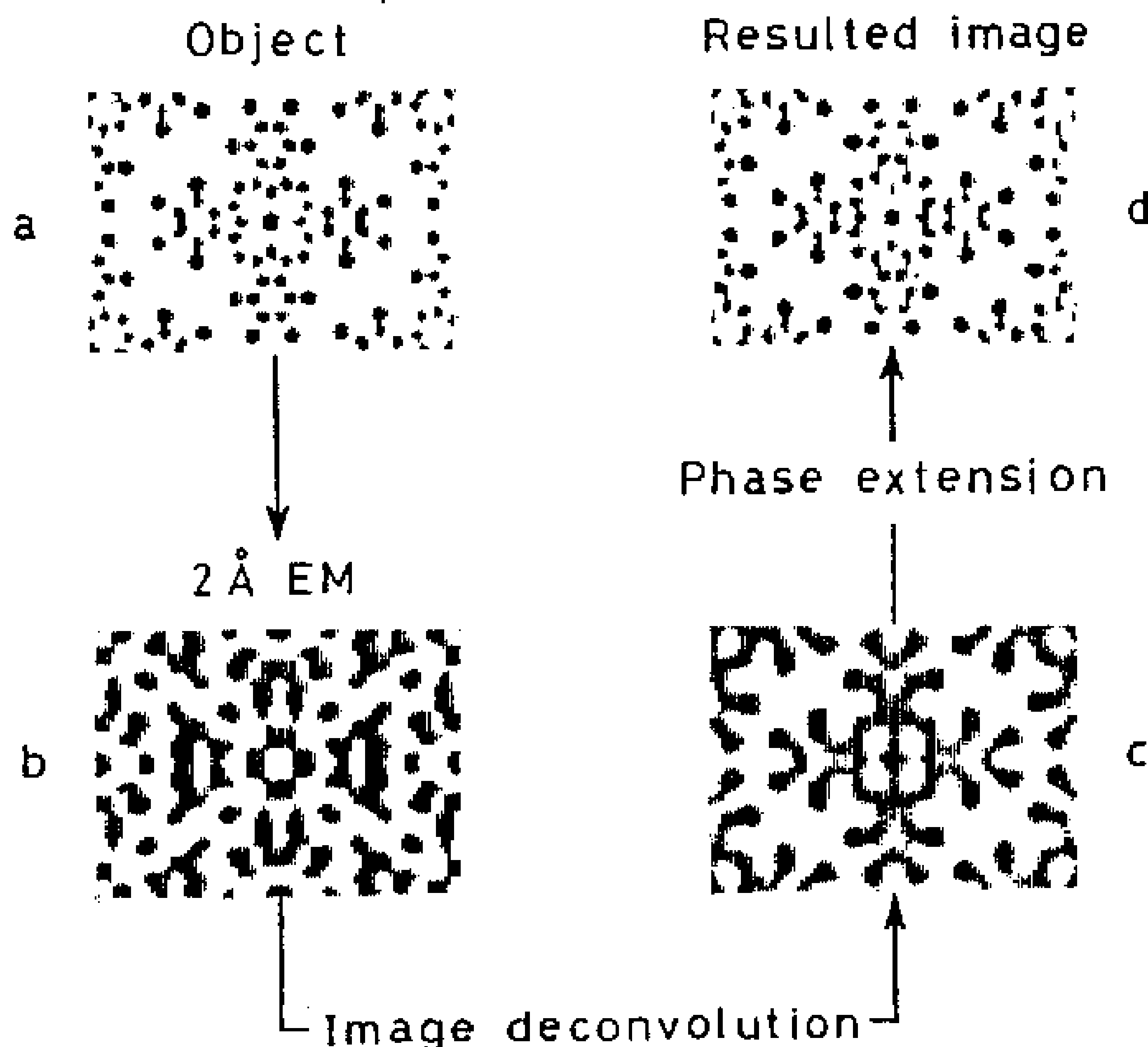
Now we may ask: Whether the direct methods can do something for the image processing in HREM? As a reply, a new technique of image processing in HREM using direct methods has been proposed, which is a new junction of X-ray crystallography and electron microscopy. The procedure is devided into two parts i.e. the image deconvolution and the resolution enhancement . In comparison with the existing techniques, the new technique needs fewer experimental measurements and no preliminary knowledge about the structure. Furthermore, the process is quite simple and easy to implement.

The efficiency of the technique has been tested by simulating calcu-

lations under different conditions. A typical seriers of results is shown in Fig. 1 from (a) to (d). The object, chlorinated copper phthalocyanine, (a) was photographed (by simulation) to give an electron microscopic image (b), which was strongly blurred by the contrast transfer function. After deconvolution by making use of Sayre's equation, the aberration of (b) was effectively corrected leading to a low resolution structure image (c). Then by combining the information extracted from (c) and the electron diffraction pattern of (a), a phase extension process using a Multan like procedure leads to a high resolution image (d), which is nearly the same as the true structure (a).

Figure 1. Image processing of an EM by direct methods

a. Expected image of chlorinated copper phthlocyanine at 1Å resolution.
b. Calculated EM at 2Å resolution taken under the condition of 500 kV electrons, $\Delta f = -1000$ Å, Cs = 1 mm and D = 150 Å.
c. Structure image at 2Å resolution obtained from b after deconvolution.
d. Structure image at 1Å resolution obtained from c after phase extension.



I. Image deconvolution using Sayre's equation as a criterion

1. Principle of the method

Under the weak-phase-object approximation in which the dynamic diffraction effect is neglected,* the Fourier transform of an EM can be expressed as

$$\underline{T}(\underline{H}) = \delta(H) + 2\sigma \underline{F}(\underline{H}) \sin\chi_1(H) \exp\{-\chi_2(H)\} \,, \tag{1}$$

which can be rearranged to give

$$\underset{H\neq 0}{\underline{F}(\underline{H})} = \underline{T}(\underline{H})/2\sigma \sin\chi_1(H) \exp\{-\chi_2(H)\} \,. \tag{2}$$

Where $\sigma = \pi/\lambda U$. λ is the electron wavelength and U the accelerating voltage. $\underline{H}$ is the reciprocal vector bounded by the resolution limit. $\underline{F}(\underline{H})$ is the structure factor of electron diffraction, which is the Fourier transform of the potential distribution $\varphi(\underline{r})$ of the object. $\sin\chi_1(H)\cdot \exp\{-\chi_2(H)\}$ is the contrast transfer function, in which

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

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

where Δf is the defocus amount, Cs is the spherical aberration coefficient and D is the standard deviation of the Gaussian distribution of defocus due to the chromatic aberration (Fijes,1977). In order to carry out the image deconvolution the valuse of Δf, Cs and D should be found first. Among these three factors, Cs and D can be determined experimentally without much difficulties. Furthermore since H is usually much smaller than unity, the transfer function is not so sensitive to Cs and D as to Δf. Hence the main problem is to find out Δf.

Assuming the valuse of Cs and D are known in advance, then by (2) we can calculate a set of $\underline{F}(\underline{H})$ from an EM given a value of Δf. On the other hand a correct set of $\underline{F}(\underline{H})$ should obey the Sayre equation (Sayre, 1952)

$$\underline{F}(\underline{H}) = \frac{\phi}{V} \sum_{H'} \underline{F}(\underline{H}') \underline{F}(\underline{H}-\underline{H}') \tag{3}$$

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* 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.

Hence the true value of Δf can be found by a systematic trial and error method: Assign different values of Δf in a wide range with a small interval, say 10 Å, Calculate a set of $\underline{F}(\underline{H})$ using (2) for each assigned value of Δf, Find out the set of $\underline{F}(\underline{H})$ which satisfies Sayre equation the best. Then the corresponding Δf should be considered as that nearest to the true one. The consistency for a set of $\underline{F}(\underline{H})$ with Sayre equation can be indicated by a figure of merit newly proposed by Debaerdemaeker, Tate & Woolfson (1985), which is defined as

$$S = \frac{\left(\sum_{\underline{H}} \underline{E}(\underline{H})^* \sum_{\underline{H}'} \underline{E}(\underline{H}')\underline{E}(\underline{H}-\underline{H}')\right)^2}{\left(\sum_{\underline{H}} \left|\underline{E}(\underline{H})\right|^2\right)\left(\sum_{\underline{H}} \left|\sum_{\underline{H}'} \underline{E}(\underline{H}')\underline{E}(\underline{H}-\underline{H}')\right|^2\right)}, \tag{4}$$

where $\underline{E}(\underline{H})$ is the normalized structure factor and $\underline{E}(\underline{H})^*$ is the conjugate of $\underline{E}(\underline{H})$. This figure of merit has a value between 0 and 1. The greater the value the better the consistency of $\underline{F}(\underline{H})$ with Sayre equation.

2. Test results

The data used in the test were generated using a model structure of chlorinated copper phthalocyanine:

Chemical formula $C_{32}N_8Cl_{16}Cu$

Plane group of the projection along $\underline{a}$ axis cmm

Unit cell dimensions a=19.62, b=26.04, c=3.76Å, β=116.5°

A series of theoretical EM's with different defocus amount were calculated under the following conditions:

Cs=1mm, D=150Å, $\Delta f = \pm 1000, \pm 800, \pm 600, \pm 400, \pm 200$Å.

The calculated EM's are shown in the first and the third columns of Fig. 2b, while their Δf values are marked in the corresponding positions in Fig. 2a. Each of the ten theoretical EM's was used separately as a starting point of the test calculations, which include the following steps:

1) Calculate a set of $\underline{T}(\underline{H})$ from an EM;

2) Assign different values of Δf in a wide range with an interval of 10 Å. Corresponding to each assigned value of Δf, a set of $\underline{F}(\underline{H})$ is calculated from $\underline{T}(\underline{H})$ using equation (2);

3) Calculate the figure of merit for each set of $\underline{F}(\underline{H})$ using equation (4);

4) Find out the greatest S and then Fourier transform the corresponding set of $\underline{F}(\underline{H})$ to give a structure image.

The results are shown in Table 1 and the second column of Fig. 2a. It shows that, among the ten EM's the deconvolution was very successful in

eight of them, for which the values of Δf were accurately determined and the resulted images were almost the same as the expected one. The remaining two EM's failed in the deconvolution have their Δf values close to that of the optimum under focus (about -400 Å). The failure may be due to two reasons:

1) When the value of Δf is close to that of the optimum under focus, the contrast transfer function will not sensitive to small changes of Δf. This results in a large error in the estimation.

2) The Sayre equation was used without an observed set of $|\underline{F}(\underline{H})|$. Hence the solution may not necessarily be unique.

There are two ways to overcome the above difficulty:

1) It is advisable to use an over focus EM rather than an under focus one. As can be seen in Table 1 and Fig. 2a, the deconvolution was successful for all over focus EM's.

2) In addition to the EM, the corresponding electron diffraction pattern is used to provide an observed set of $|\underline{F}(\underline{H})|$. Then for the calculation of the figure of merit S, the phase information can be derived from the EM using equation (2), while the magnitudes of $\underline{E}(\underline{H})$'s can be obtained from the corresponding electron diffraction pattern.

Test results on this modified procedure are shown in Table 2 and Figure 2b .They are much better than those shown in Table 1 and Fig. 2a. For more detail of this image deconvolution technique the reader is referred to Han Fu-son, Fan Hai-fu & Li Fang-hua (1985).

Table 1. Estimation of the defocus amount from a single EM

Δf_{true}	1000	800	600	400	200	-200	-400	-600	-800	-1000
Δf_{est}	1000	800	600	400	210	$-\underline{560}$	$-\underline{520}$	-600	-810	-1000
S	.9704	.9704	.9704	.9704	.9708	.9945	.9742	.9527	.9555	.9527

Table 2. Estimation of the defocus amount from a single EM and the corresponding electron diffraction pattern

Δf_{true}	1000	800	600	400	200	-200	-400	-600	-800	-1000
Δf_{est}	1000	800	600	400	210	-200	-420	-600	-800	-1000
S	.9704	.9704	.9704	.9704	.9708	.9704	.9711	.9704	.9704	.9704

Δf_{true} the true defocus amount of the EM

Δf_{est} the estimated value of defocus

S the figure of merit corresponding to Δf_{est} calculated from equation (4)

Figure 2a. Results on the test of image deconvolution using a single EM

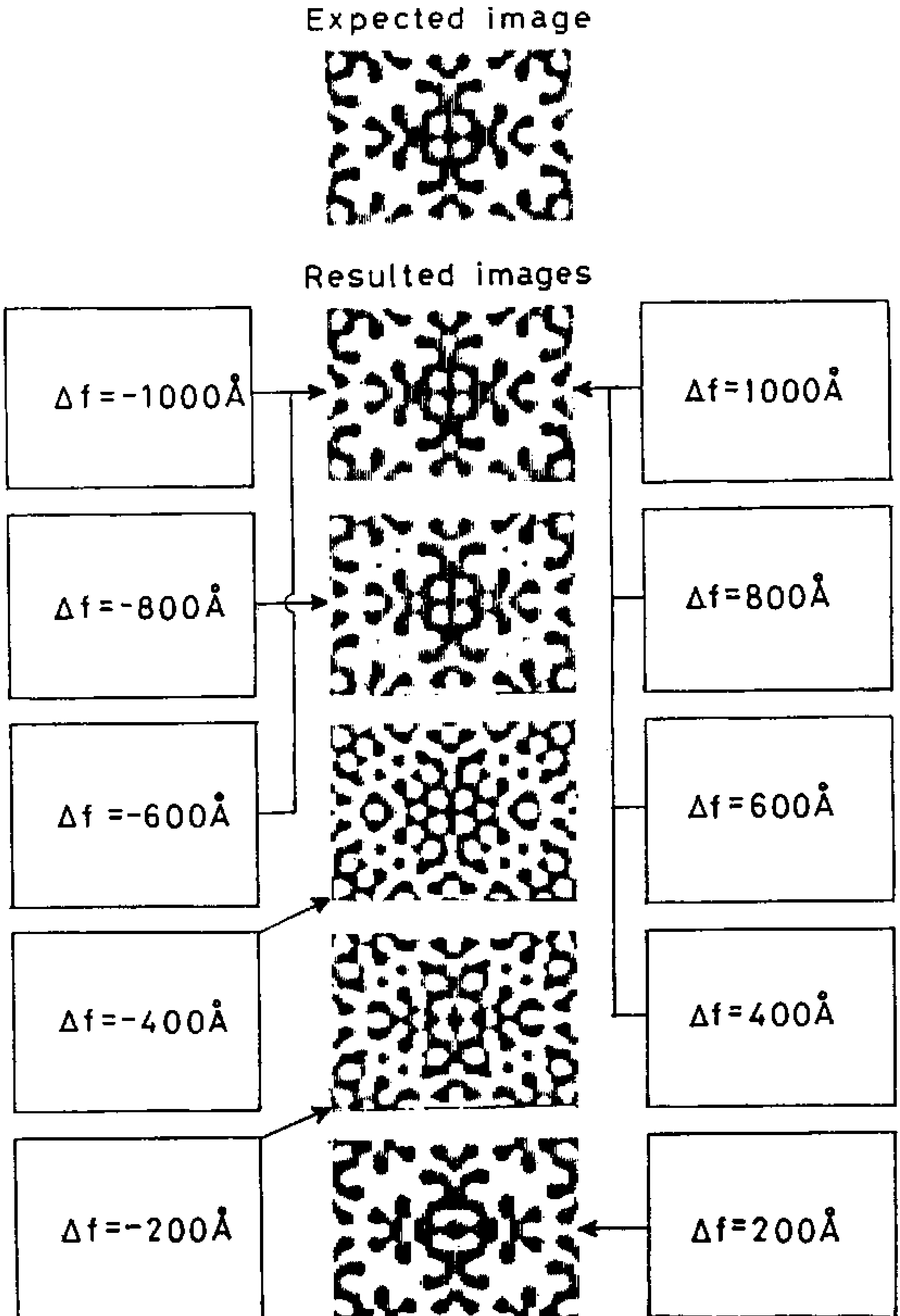
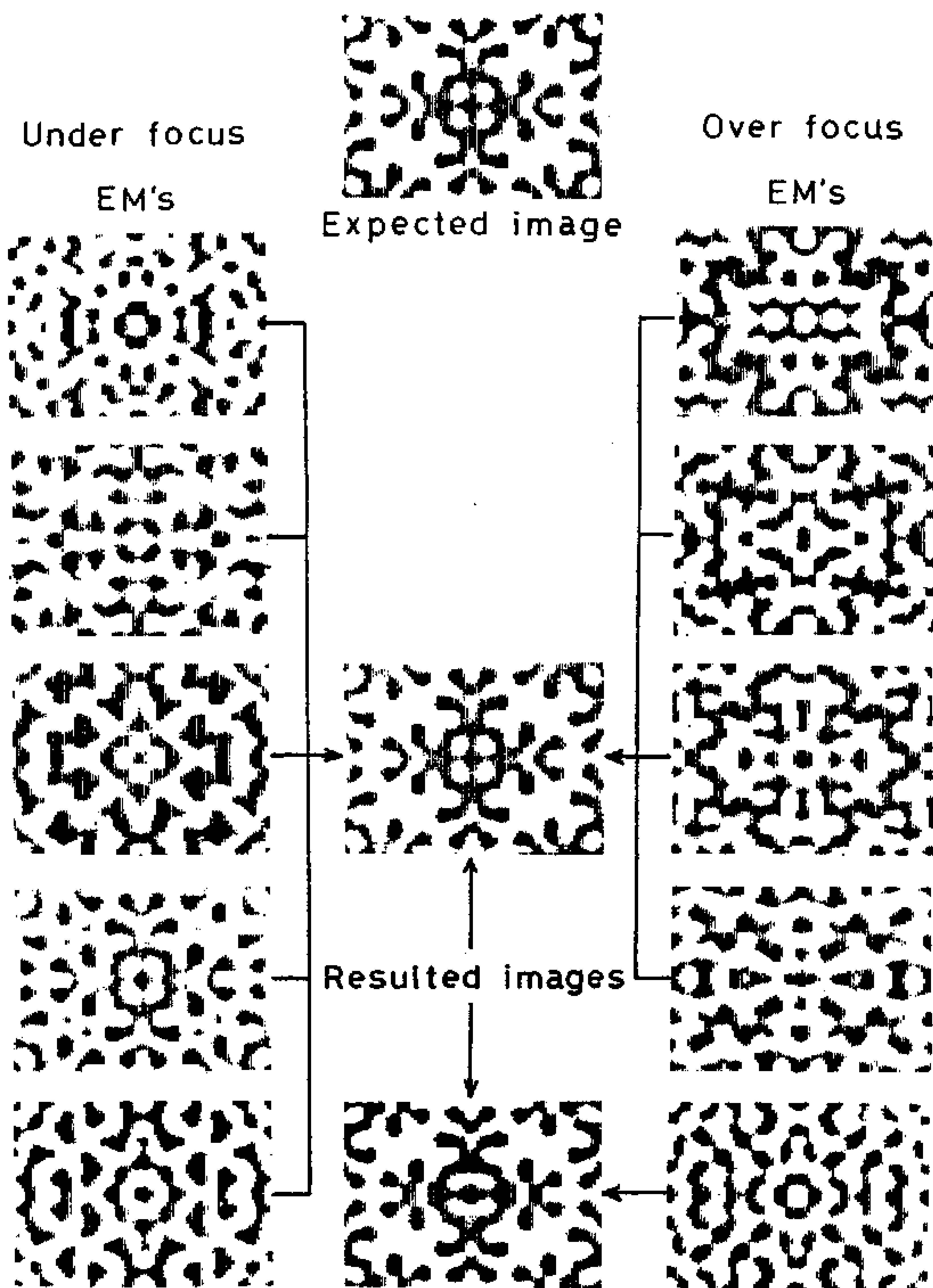


Figure 2b. Results on the test of image deconvolution using a single EM and the corresponding ED pattern



II. Resolution enhancement by a Multan like procedure

1. Principle of the method

An electron diffraction (ED) pattern usually contains information up to 1Å 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 after suitable deconvolution can provide phase information corresponding to about 2 Å resolution. This 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 Multan-80 program has been modified to satisfy the requirements of this method. The practical process is as follows:

1) The amplitudes of structure factors obtained from the ED pattern are input into the program as a set of diffraction data.

2) The phases from the EM are used as 'known phases' in the program.

3) A few reflections of unknown phase are added to the starting set for phase permutation.

4) The best solution is selected automatically according to the figures of merit normally used in Multan.

2. Test results

The same model structure described in section I was used. A set of structure factors at 1Å resolution were calculated. The amplitudes were used to simulate the data from an ED pattern, while the phases within certain resolution limit lower than 1Å were used to simulate those obtained from an EM after deconvolution. The imaging condition is assumed to be 500 kV electrons, $\Delta f=-1000$ Å, Cs=1 mm and D=150 Å. Phases of reflections with $\left| \sin \chi_1(H) \exp\{-\chi_2(H)\} \right| \leqslant 0.2$ were regarded as unknown phases. The test calculation included the following three parts:

1) The Multan-80 program was used to solve the structure with ED data alone. The resulted best E-map is shown in Fig. 3a. In comparison with the expected image (Fig. 3b), 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) The 2Å resolution EM after deconvolution (Fig. 3c) was used as the starting image.Phase extension to 1Å resolution resulted in the image shown in Fig. 3d, which is almost the same as the expected one (Fig. 3b).

3) The 2.5Å EM after deconvolution (Fig. 3e) was used as the starting image. Phase extension to 1Å resolution resulted in the image shown in Fig 3f. It is also quite satisfactory.

According to the above results we concluded that, while Multan-80 could not solve the structure with ED data alone, the phase extension, which leads to the resolution enhancement of the EM, was very successful by a Multan like procedure. For more detail of this resolution enhancement technique, the reader is referred to Fan Hai-fu, Zhong Zi-yang, Zheng Chao-de & Li Fang-hua (1985).

Figure 3. Results on the test of resolution enhancement

a. Multan solution of the ED data

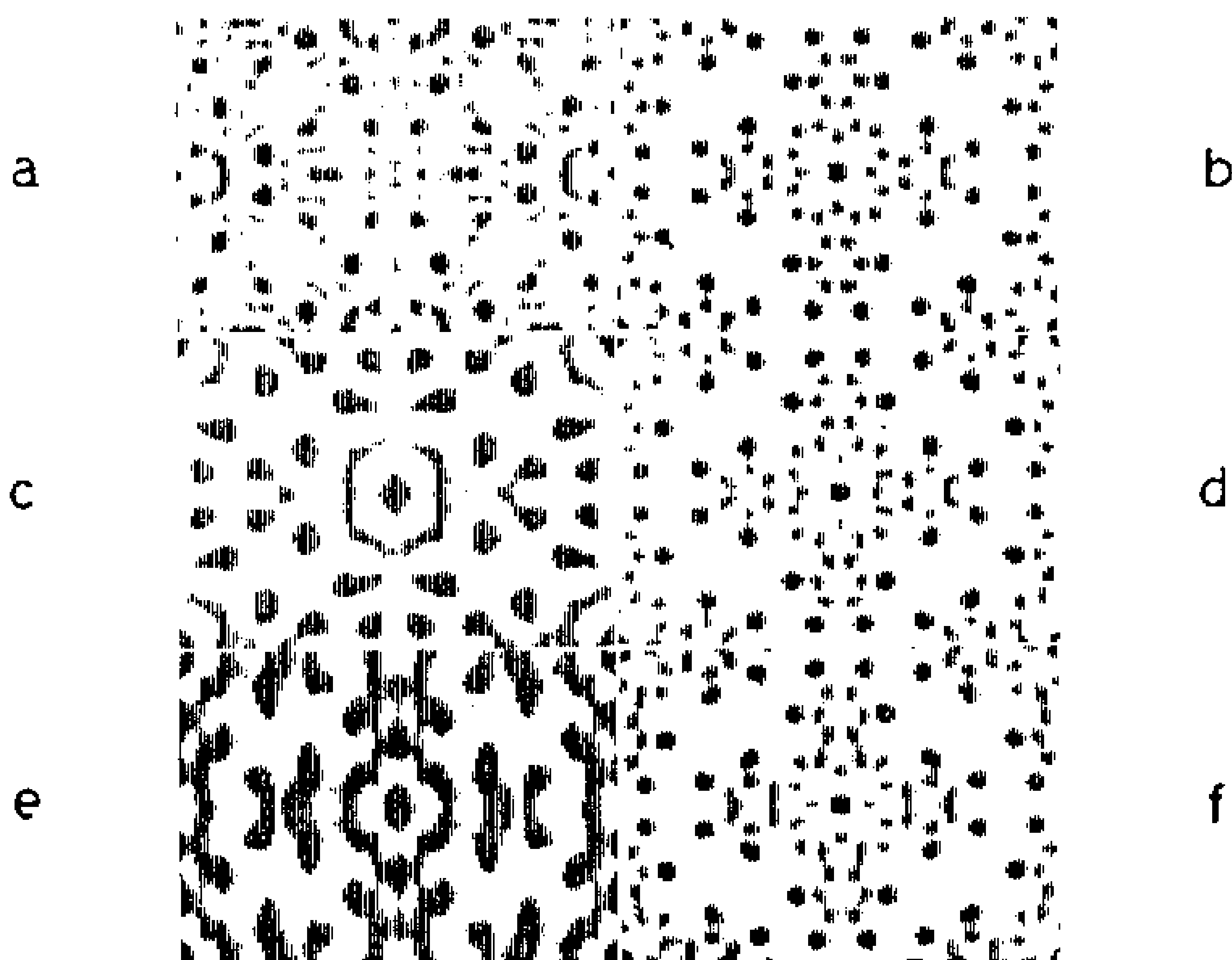
b. Theoretical structure image at 1Å resolution

c. Calculated EM at 2Å resolution after deconvolution

d. Result of phase extension from 2 to 1Å

e. Calculated EM at 2.5Å after deconvolution

f. Result of phase extension from 2.5 to 1Å



Conclusion remarks

The method described in this paper has been proved to be successful for processing theoretical images. Next step of the study would be that of applying the method to handle experimental EM's. Another important work remains to be done is to extend the method to satisfy the case in which dynamic diffraction effect could not be neglected.

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