Direct Methods outside the Traditional Field

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One of the present tendencies on the developments of direct methods is to explore new application fields, i.e. to go outside the traditional field. Which may be defined as: single crystal X-ray analysis on Ordinary Small molecular structures. As is seen here are a number of ways to go:

1. From ***Single crystals*** to ***Powder samples***
2. From ***X-ray crystallography*** to ***Electron microscopy*** ——
Image processing in High Resolution Electron Microscopy
3. From ***Ideal*** to ***Real structures***——
Solving Incommensurate structures
4. From ***Small molecules*** to ***Protein structures***

In the following, the last three topics will be discussed.

I. Direct Methods for Protein Structures

Our discussion will be concentrated on the combination of direct methods with isomorphous replacement and nomalous scattering techniques. Such a combination is important. Because it can reduce the number of heavy-atom derivatives, save time in data collection and simplify the process of structure determination. Early attempts in this respect can be found in 1960's. Great progress has been made in recent years. Some of the works are listed here:

Coulter C.L. (1965). *J. Mol. Biol.* **12**, 292-295.
Fan, H.F. (1965). *Acta Phys. Sin.* **21**, 1114-1118.
Karle, J. (1966). *Acta Cryst.* **21**, 273-276.
Hazell, A.C. (1970). *Nature Lond.* **227**, 269.
Hendrickson, W.A. (1971). *Acta Cryst.* **B27**, 1474-1475.
Sikka, S.K. (1973). *Acta Cryst.* A**29**, 211-212.
Heinerman, J.J.L., Krabbendam, H., Kroon, J. & Spek, A.L. (1978). Acta Cryst. A**34**, 447-450.
Hendrickson, W.A. & Teeter, M.M. (1981). *Nature (London),***290**, 107-113.
Hauptman, H. (1982a). *Acta Cryst.* **A38**, 289-294.
Hauptman, H. (1982b). *Acta Cryst.* A**38**, 632-641.
Giacovazzo, C. (1983). *Acta Cryst.* A**39**, 585-592.
Fan, H.F., Han, F.S. & Qian, J.Z. (1984), *Acta Cryst.* A**40**, 495-498.
Fan, H.F. & Gu, Y.X. (1985). *Acta Cryst.* A**41**, 280-284.
Fortier, S., Moore N.J. & Fraser, M.E. (1985). *Acta Cryst.***A41**, 571-577.
Yao, J.X. & Fan, H.F. (1985). *Acta Cryst.* **A41**, 284-285.

In our group, a direct method has been developed to break the phase ambiguity arising from

single isomorphous replacement or one-wavelength anomalous scattering technique. The main point of the method is that, the phase doublet of each reflection is obtained in usual way, while the direct method is used to make choice between the two possible phases. For this purpose, a probability formula was derived and a procedure to treat the experimental errors was developed. Three sets of experimental one-wavelength anomalous scattering data from small proteins were used to test the method. In all cases, only 1000 largest reflections and 60,000 Σ_2 relationships were used. The results (listed in the following tables) were sorted in descending order of probability and then cumulated into five groups, which include respectively 200, 400, 600, 800 and 1000 reflections.

Insulin OAS data
(Provided by Prof. Guy Dodson & Eleanor Dodson)

Group	%	ER
1	93.0	33
2	94.5	35
3	94.0	37
4	93.5	38
5	92.3	41

RF C OAS data
(Provided by Prof. Nobuo Tanaka)

Group	%	ER
1	95.5	22
2	93.0	25
3	92.2	28
4	88.8	33
5	85.8	36

aPP OAS data
(Provided by Prof. Tom Blundell)

(a) Phased by direct method

Group	%	ER
1	95.0	24
2	90.8	30
3	91.3	29
4	89.0	32
5	86.3	36

(b) Phased by SIR-OAS method

Group	%	ER
1	92.0	16
2	88.8	20
3	87.5	24
4	84.1	30
5	79.4	39

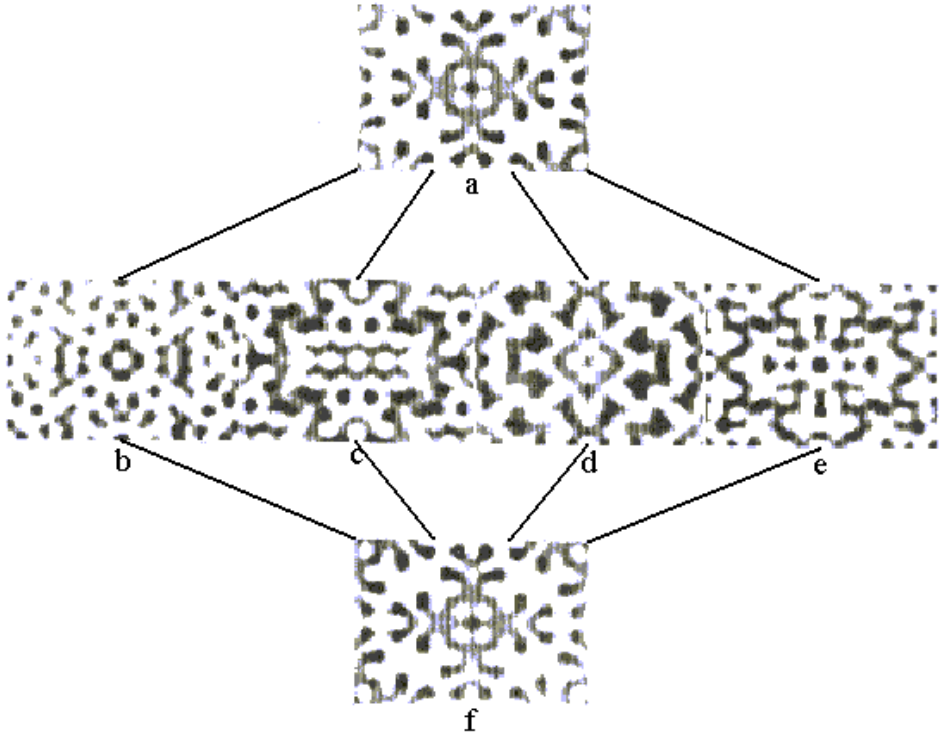
% —— Percentage of reflections with the sign of $\Delta\varphi_H$ correctly determined;
ER —— Averaged phase error in degrees.

As is seen the method is capable of deriving large number of reliable initial phases. Hopefully this method can be used to solve unknown proteins by incorporating suitable phase extension techniques.

II. Direct Methods as A Tool of Image Processing in Electron Microscopy

Apart from X-ray methods, high-resolution electron microscopy is an important tool for structure analysis. Many solid state materials important in science and technology are composed of very small and imperfect crystals. They are not suitable for X-ray analysis, but are suitable for electron microscopic observation. However, high-resolution electron microscopy suffers from two difficulties. First, an electron microscopic image is not a true structure image of the object but just a convolution of the object image with the Fourier transform of the contrast transfer function. Second, the point to point resolution of an electron microscopic image is about 2Å at present, which is not enough to reveal individual atoms in most cases. Hence it is important to have some image processing techniques for image deconvolution and resolution enhancement. On the other hand, direct methods are in fact a special kind of image processing technique. It is reasonable to expect that direct methods can do something in the image processing of high-resolution electron microscopy. New procedures were proposed and tested by simulating calculations. Figure 1 shows an example of image deconvolution.

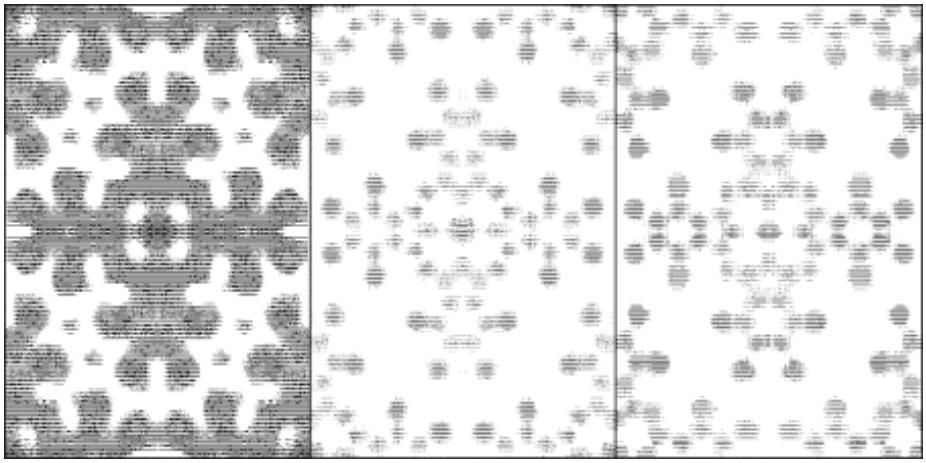
Figure 1. Image deconvolution of theoretical electron-microscope images of copper chlorinated phthalocyanine.



An object like (a) under different photographic conditions can give different electron microscopic images as (b), (c), (d) and (e), none of which resembles the object. In practice some of the photographic conditions such as the defocus value is difficult to measure with enough accuracy. This causes troubles to the image deconvolution. With a newly proposed direct-method processing technique, all these images can be restored to give the picture (f), which is nearly the same of the object (a). Moreover, there is no need to know the defocus value in advance. Figure 2 shows an example of resolution enhancement. There is on the left an artificial electron

microscopic image at 2Å resolution. A structure factor extrapolation based on the Sayre equation was used to extend phases as well as magnitudes of the reflections beyond 2Å and up to 1Å resolution. The resulting image is shown in the middle, which is almost the same as the expected image at 1Å resolution shown at the right. Although the method has just passed through the examination of simulating calculations, they may be practically useful in the near future.

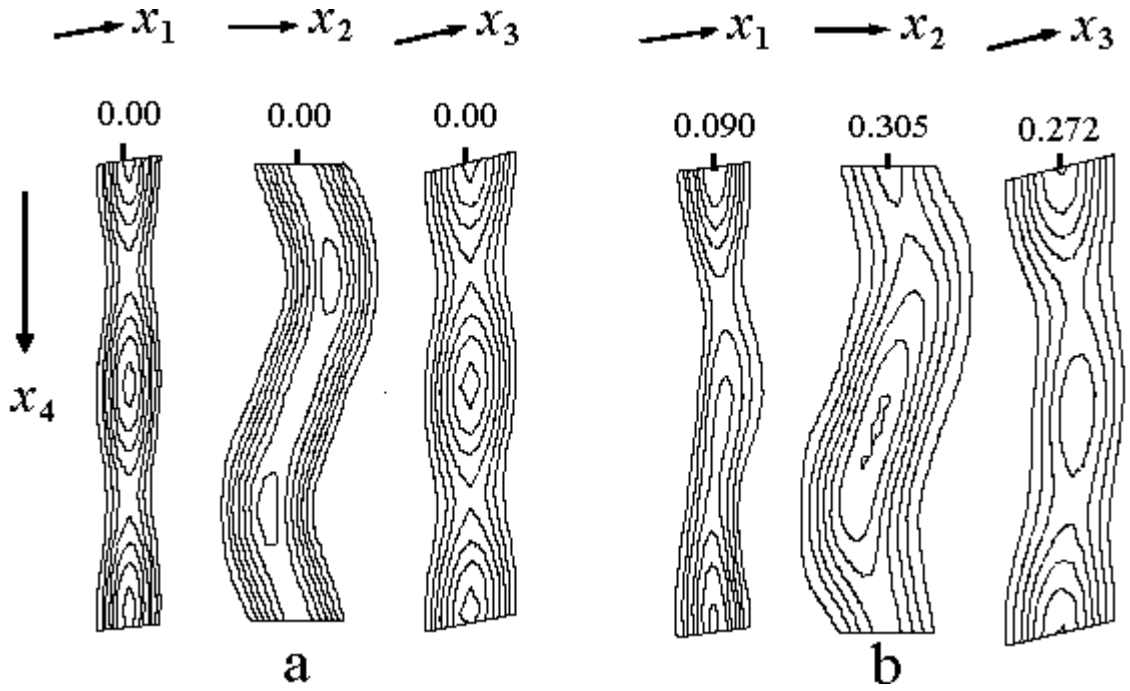
Figure 2. Structure-factor extrapolation of the theoretical electron-microscope image of copper chlorinated phthalocyanine.



III. Direct Methods in Multi-dimensional Space

Modulated structures are that kind of crystal structures, in which the atoms suffer from certain compositional and/or positional fluctuation. If the period of fluctuation is commensurate with that of the 3-dimensional unit cell, then it results in a superstructure, otherwise an incommensurate modulated structure is obtained. Incommensurate modulated phases can be found in many important solid state materials. In many cases, the transition to the incommensurate modulated structure corresponds to a change of certain physical properties. Hence it is important to know the structure of incommensurate modulated phases in order to understand the mechanism of the transition and properties in the modulated state. Incommensurate modulated structures are characterized by their diffraction pattern, in which there are satellite reflections round the main ones. Since the satellites can only be fitted into an n-dimensional (n3) lattice, there is no 3-dimensional reciprocal lattice corresponding to the whole pattern. Up to the present, many incommensurate modulated structures were solved by using some kind of trial-and-error methods. With these methods it is necessary to make assumption on the property of modulation before we can solve the structure. This often causes difficulties and leads easily to errors. A traditional direct method has been extended to multi-dimensional space enabling solution of incommensurate modulated structures by direct phasing main reflections and satellites. The method does not rely on any assumption of the modulation or of the average structure. Text with a known incommensurate modulated structure, γ-sodium carbonate, has been performed.

Figure 3. Sections of the 4-D electron density map of γ-sodium carbonate calculated with the direct-method phasing of experimental X-ray diffraction data.
(a) Sections through the average position of the Na atom;
(b) Sections through the average position of the atom O(1,3).



On the direct-method phased Fourier map (Figure 3), we can see intuitively and objectively 4-dimensional atoms with periodic modulation. Atomic parameters read from the map are consistent with those obtained by the original authors. Recently an important type of condensed matter called quasicrystal has been discovered and extensively investigated. There are some similarities between quasicrystals and incommensurate modulated structures. Direct methods may also find their use in solving quasicrystal structures.

To end up my talk, we can expect that direct methods will be as successful in many new fields as it has been in the traditional field.