Third World Academy of Sciences
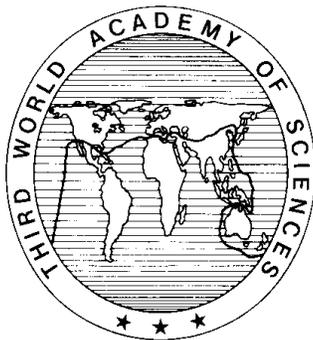
Proceedings

- *TWAS*
  6th General Conference
  9th General Meeting

- *TWNSO*
  5th General Assembly

- *IFS*
  8th Assembly

6-11 September 1997
Rio de Janeiro, Brazil

Direct Methods in Crystallography

Fan, Hai-fu
Institute of Physics, Chinese Academy of Sciences, Beijing 100080, China

An important branch of crystallography is the X-ray diffraction analysis of crystal structures, which aimed at observing the arrangement of atoms in solid state materials and understanding the structure-property relationship. It is well known that X-rays can be diffracted by a crystal. If both the amplitude and the phase of all diffraction beams can be recorded, then a picture of the electron density distribution in the crystal can be obtained by simply Fourier transforming the three-dimensional diffraction pattern. Unfortunately phases were lost in the experiment. Thus, before the crystal structure can be observed, we have to solve the 'phase problem'. Direct methods are that kind of data processing techniques, which can retrieve the lost phases from the corresponding diffraction amplitudes.

In early 60's I was studying the crystal structure of natural organic compounds. I dealt with often crystals containing heavy atoms besides those of carbon, nitrogen and oxygen. Pseudo symmetries were found frequently, which caused much difficulties in solving the phase problem. I thought that direct methods might be useful in solving the problem although they were still in their early days. A modified Sayre equation and the so-called component relations were then proposed in 1965 [1,2].

$$F_{\mathbf{h}} = (\theta_{\mathbf{h},u} / V) \sum_{\mathbf{h'}} F_{\mathbf{h'}} F_{\mathbf{h-h'}} - \sum_p [(\theta_{\mathbf{h},u} / \theta_{\mathbf{h},p}) - 1] F_{\mathbf{h},p} \qquad (1)$$

$$A_{\mathbf{h}} = (\theta_{\mathbf{h},u} / V) \sum_{\mathbf{h'}} (A_{\mathbf{h'}} A_{\mathbf{h-h'}} - B_{\mathbf{h'}} B_{\mathbf{h-h'}}) - \sum_p [(\theta_{\mathbf{h},u} / \theta_{\mathbf{h},p}) - 1] A_{\mathbf{h},p}$$
$$B_{\mathbf{h}} = (2\theta_{\mathbf{h},u} / V) \sum_{\mathbf{h'}} A_{\mathbf{h'}} B_{\mathbf{h-h'}} - \sum_p [(\theta_{\mathbf{h},u} / \theta_{\mathbf{h},p}) - 1] B_{\mathbf{h},p} \qquad (2)$$

The study was soon split into two lines:

i) The problem of pseudo symmetry in crystal-structure analysis has been treated by a variation of Equation (1):

$$F_{wk}(\mathbf{h}) = \frac{2\theta}{V} \sum_{\mathbf{h'}} F_{wk}(\mathbf{h'}) F_{st}(\mathbf{h} - \mathbf{h'}) \qquad (3)$$

The first application was done in 1965 and published ten years later [3]. The result is shown in Figure 1.

ii) The combination of direct methods with single-isomorphous replacement or one-wavelength anomalous scattering data based on equation (2) has been used for resolving the phase ambiguity in crystal-structure analysis of proteins [4]. This is still a hot topic at the present and will be discussed later.

During the 1970's my work was concentrated on the characterization of solid state materials using X-rays. I found often superstructures with the diffraction pattern having very weak satellites besides regular reflections. At that time phases of satellite reflections were assigned only after assuming a superstructure model. A new modified Sayre equation was then derived in 1978 to solve the phase problem without relying on an assumed structure model [5].

$$F_{sate}(\mathbf{h}) = \frac{2\theta}{V} \sum_{\mathbf{h'}} F_{sate}(\mathbf{h'}) F_{main}(\mathbf{h} - \mathbf{h'}) \qquad (4)$$

In the same period I had chance to work in the field of electron microscopy. Electron microscopes can produce for a crystalline sample simultaneously the microscope image and the corresponding diffraction pattern. The resolution of the diffraction pattern is usually much higher than that of the image. In 1972 an idea was proposed that, we can obtain diffraction phases at low resolution by suitable processing of the image. Then by a phase extension using intensities of the diffraction pattern, we can obtain an enhanced image at a resolution far beyond the limit of an electron microscope. A

simulating calculation based on this idea was done thirteen years after [6, 7]. The results are summarized in Figure 2.

A great time came to crystallographers in 1985 that Herb Hauptman and Jerome Karle won the Nobel Price in chemistry owing to their pioneer and outstanding contributions on direct methods. Meanwhile, a question arose: Is this the end of direct methods or what to do next? Our opinion was that while direct methods have been very successful in the field of single-crystal X-ray structure analysis of small molecules, they have little influences outside. Therefore, exploring new applications outside of the traditional field is essential. There are several possible ways to go: i) from single crystal to powder samples; ii) from small molecules to proteins; iii) from ideal to real crystals; iv) from X-ray crystallography to electron microscopy. Our work has been concentrated in the last three topics.

From small molecules to proteins

Solving a protein structure by using single isomorphous replacement (SIR) or one wavelength anomalous scattering (OAS) data is important when it is difficult to prepare multiple isomorphous derivatives and to collect multiwavelength anomalous diffraction data. However the phase ambiguity intrinsic in SIR or OAS data obstructs the use of them. The combination of direct methods with the SIR or the OAS technique will solve the problem and simplify the process of analysis. Different groups on the world made significant contributions in this area. The method developed in our group has been tested with experimental diffraction data from known proteins. Recently a group in Leicester, UK used the method to solve an originally unknown protein [8] (see Figure 3).

From ideal to real crystals

In single crystal structure analysis, it is usually assumed that crystals are ideal 3-dimensional periodic objects. However real crystals are never perfect. What we obtained under this assumption is not the real structure but just an averaged structure over a large number of unit cells. Unfortunately knowledge on the averaged structure is often not enough for understanding the properties of many solid state materials. Therefore an important task for methods of solving crystal structures is to extend from ideal periodic crystals to real crystals which contain various kinds of defects. Modulated crystal structures belong to that kind of crystal structures which contain periodic defects, i.e. the atoms in which suffer from certain occupational and/or positional fluctuation. If the period of fluctuation is commensurate with that of the three-dimensional unit cell then a superstructure results, 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. 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. In view of diffraction analysis, it is possible to phase the reflections directly and solve the structure objectively without relying on any assumption about the modulation wave. For this purpose direct methods have been extended from three- to multi-dimensional space [9]. A number of incommensurate modulated crystals including some superconductors have been solved by these methods (Figures 4, 5).

From X-ray crystallography to electron microscopy

For the structure analysis of crystalline materials, electron crystallographic methods are in some cases superior to X-ray methods. Firstly, many crystalline materials important in science and technology, such as high Tc superconductors, are too small in grain size and too imperfect in periodicity for an X-ray single crystal analysis to be carried out, but they are suitable for electron microscopic observation. Secondly, the atomic scattering factors for electrons differ greatly from those for X-rays and it is easier for electron diffraction to observe light atoms in the presence of heavy atoms. Finally the electron microscope is the only instrument that can produce simultaneously for a crystalline sample an electron microscope image and a diffraction pattern corresponding to atomic resolution. In principle either the electron micrograph (EM) or the electron diffraction (ED) pattern could lead to a structure image. However the combination of the two will make the procedure much more efficient and powerful. On the other hand, an electron micrograph needs special processing before it can reveal the true structure image of the sample. A two-stage image processing technique for high resolution electron microscopy using direct methods has been developed [10],

which combines information from electron micrographs and the corresponding electron diffraction pattern. The method has been used in *ab-initio* structure analysis of minute crystalline samples, including bismuth-based high *Tc* superconductors [11] (Figures 6, 7).

References

1. Fan Hai-fu, *Acta Phys. Sin.* **21** (1965) 1105-1114 (in Chinese); *Chinese Physics* (1965) 1418-1428.
2. Fan Hai-fu, *Acta Phys. Sin.* **21** (1965) 1114-1118 (in Chinese); *Chinese Physics* (1965) 1429-1435.
3. Fan Hai-fu, *Acta Phys. Sin.* **24** (1975) 57-60. (in Chinese)
4. Fan Hai-fu & Gu Yuan-xin, *Acta Cryst.* A**41** (1985) 280-284.
5. Fan Hai-fu, He Luo, Qian Jin-zi & Lui Shi-xiang, *Acta Phys. Sin.* **27** (1978) 554-558. (in Chinese)
6. Fan Hai-fu, Zhong Zi-yang, Zheng Chao-de & Li Fang-hua, *Acta Cryst.* A**41**, 163-165 (1985).
7. Han Fu-sen, Fan Hai-fu & Li Fang-hua, *Acta Cryst.* A**42**, 353-356 (1986).
8. H. F. Fan, Q. Hao, I. Harvey, S. S. Hasnain, Y. D. Liu. Y. X. Gu, C. D. Zheng & H. Ke "Applications of direct methods with single isomorphous replacement or one wavelength anomalous scattering data" (Invited lecture presented by Fan Hai-fu) School on Direct Methods of Solving Macromolecular Structures, Erice, Italy, May 1997.
9. Hao Quan, Lui Yi-wei & Fan Hai-fu, *Acta Cryst.* A**43** (1987) 820-824.
10. Fan Hai-fu & Li Fang-hua, "Direct methods as a tool of image processing in high resolution electron microscopy". (Invited lecture presented by Fan Hai-fu) IUCr Winter School on Direct Methods, Macromolecular Crystallography & Crystallographic Statistics, Madras, India, December, 1985. Published in *<<Direct Methods, Macromolecular Crystallography & Crystallographic Statistics>>* Ed. by H. Schenk, A.J.C. Wilson & S. Pathasarathy, World Scientific, Singapore 1987, pp. 400-409.
11. Fan Hai-fu, Wan Zheng-hua, Li Jian-qi, Fu Zheng-qing, Mo You-de, Li Yang, Sha Bing-dong, Cheng Ting-zhu, Li Fang-hua & Zhao Zhong-xian, "Multi-dimensional electron crystallography of Bi-based superconductors" (Invited lecture presented by Fan Hai-fu) School on Electron Crystallography, Erice, Italy, May 1997.
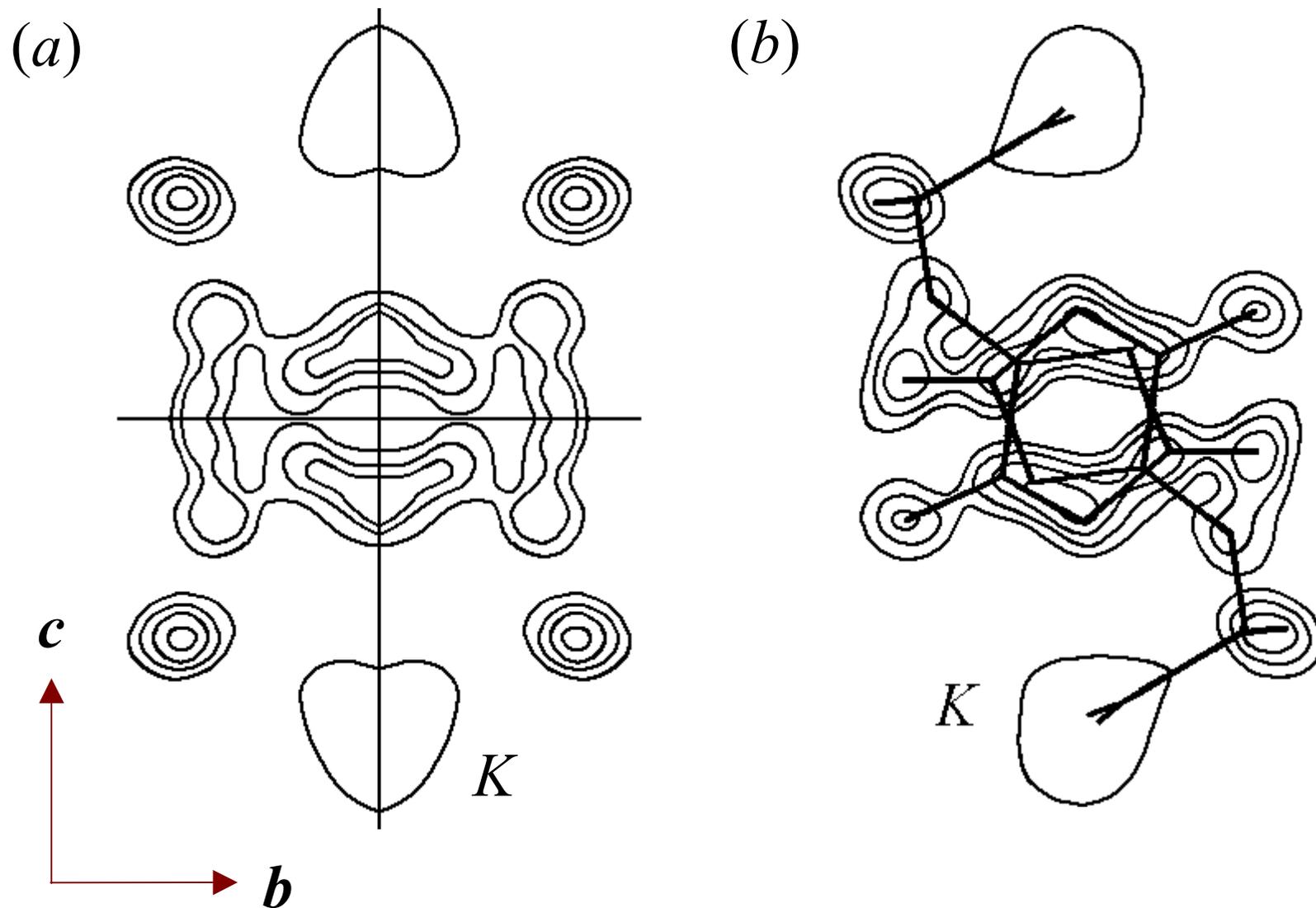
Figure 1. Electron density function projected along the ***a*** axis of the crystallographic unit cell of *SHAS*: (*a*) phased by the known heavy atoms; (*b*) phased by the direct-method. *SHAS* crystallizes in space group P $2_1 2_1 2_1$ with unit cell dimensions a=7.51, b=9.95, c=10.98Å and Z=4. The arrangement of the heavy atoms, potassium, possesses the translational symmetry of ***t***=(***a***+***b***+***c***)/2. Consequently the heavy atoms have no contribution to those reflections with $h+k+l$ odd.

Object

Resulting image

2Å EM

Resolution Enhancement

Image deconvolution
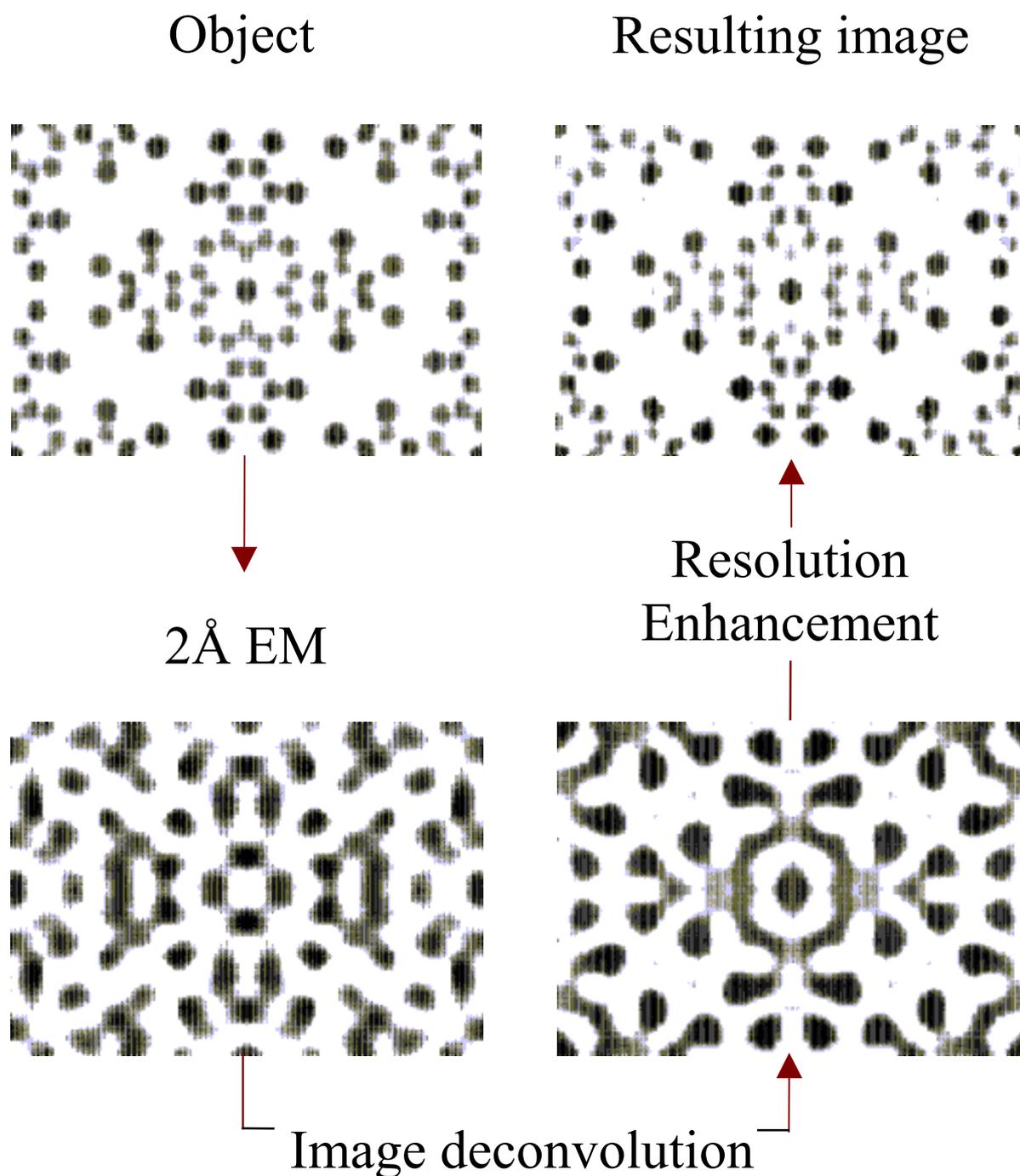
Figure 2. Simulating calculations of the two-stage image processing in high resolution electron microscopy. *Object*: [001] projection at 1Å resolution of the sample, crystalline chlorinated copper phthalocyanine (chemical formula: $C_{36}N_8Cl_{16}Cu$; space group C2/c; unit-cell dimensions: a=19.62, b=26.04, c=3.76Å, β=116.5^o). *EM*: electron microscope image of chlorinated copper phthalocyanine at 2Å resolution taken with the incident electron beam parallel to the *c* axis with photographic conditions of 500 kV electrons, Δf=−1000Å, Cs=1mm and D=150Å. *Image Deconvolution*: assuming that the defocus value Δf of the *EM* is unknown, a direct method was used to find out the value and then the *EM* was deconvoluted. *Resolution enhancement*: a direct-method phase extension was performed with starting phases from the deconvoluted image and magnitudes from the corresponding electron diffraction pattern (not shown in the figure).
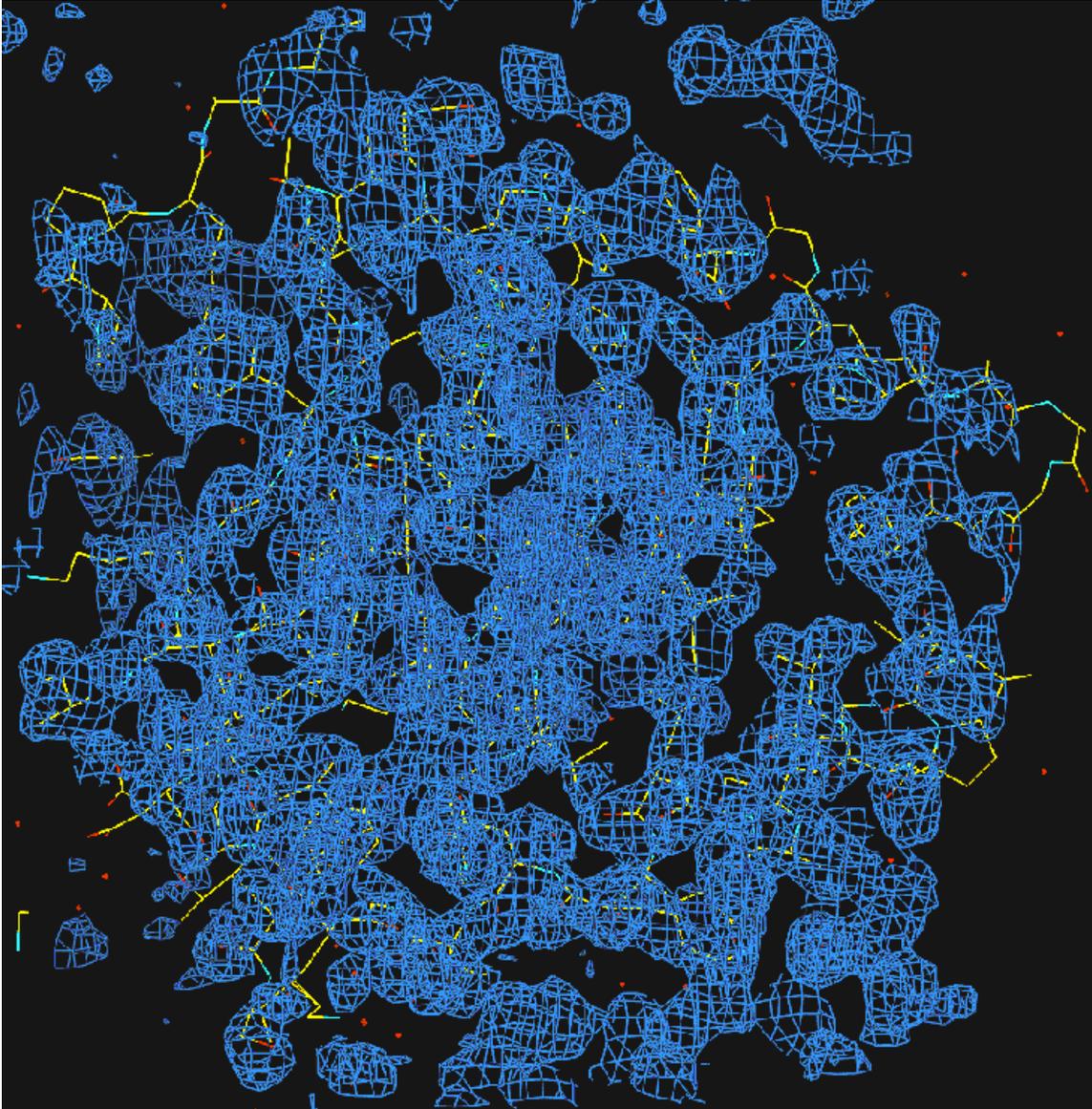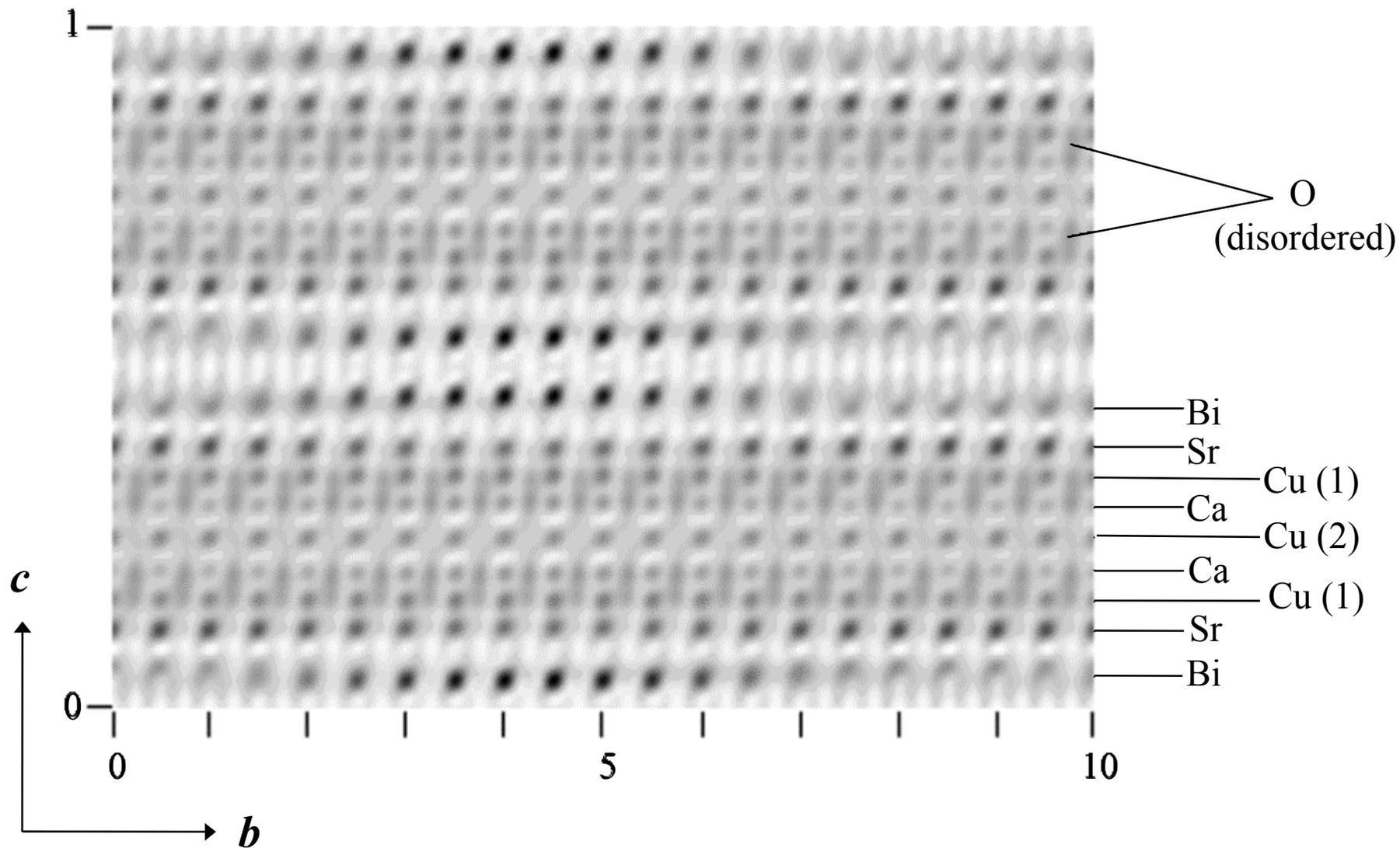
Figure 3. Electron density map of the protein Rusticyanin from the one-wavelength anomalous scattering data. Diffraction phases were obtained by using the direct method followed by the density modification (with the program 'dm' in CCP4* suit).

* Collaborative Computational Project, Number 4 (1994). *Acta Cryst*. **D50**, 760-763.

Figure 4. Incommensurate modulation in the Pb-doped Bi-2223 superconductor solved by multidimensional direct-methods revealing the projected potential distribution along the *a* axis. Ten unit cells are plotted along the *b* axis, showing the details of the modulation.
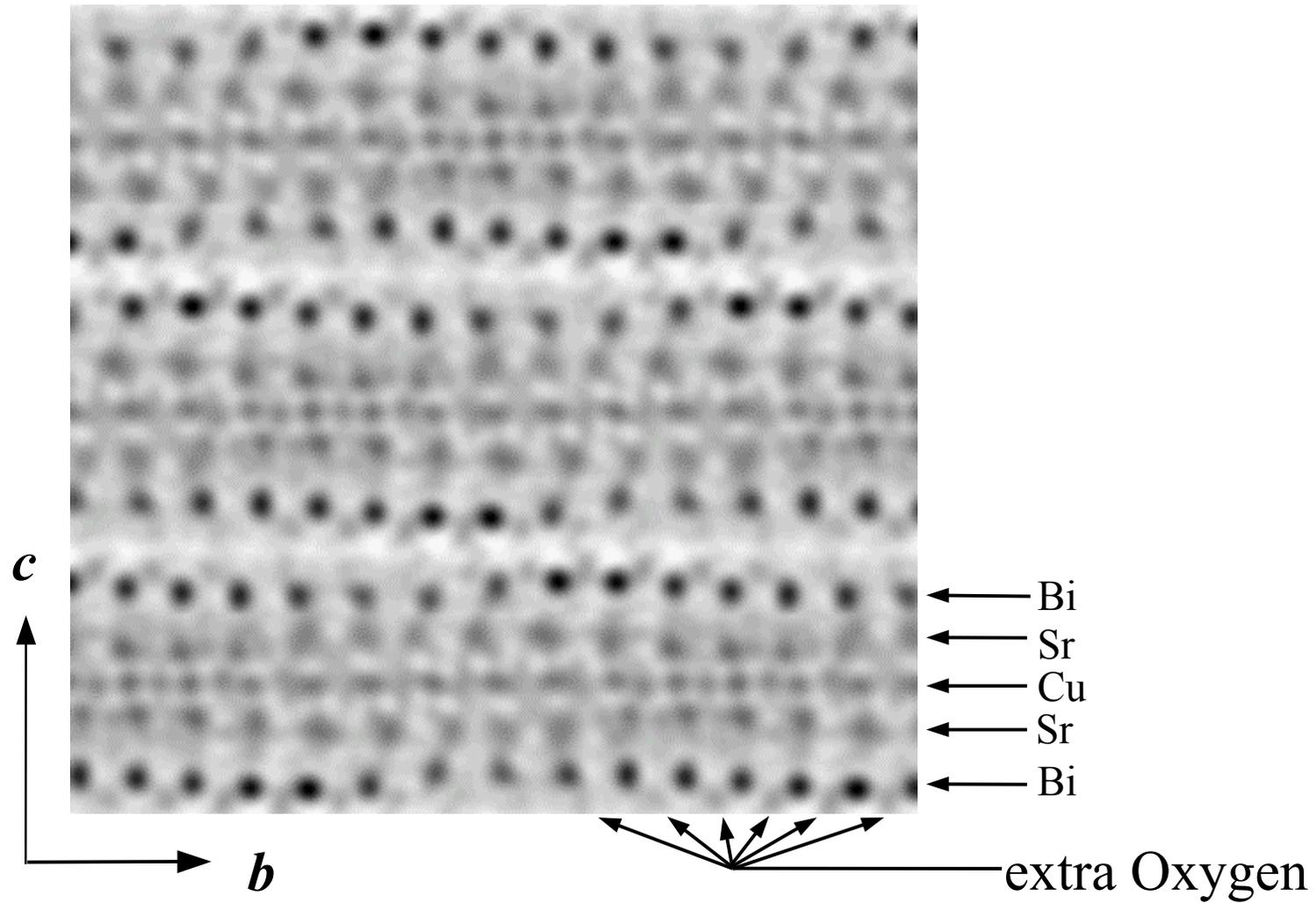
Figure 5. Multidimensional direct-methods study of the Bi-2201 superconductor revealing the incommensurate modulation and extra oxygen atoms on the [100] projection of the potential distribution function.
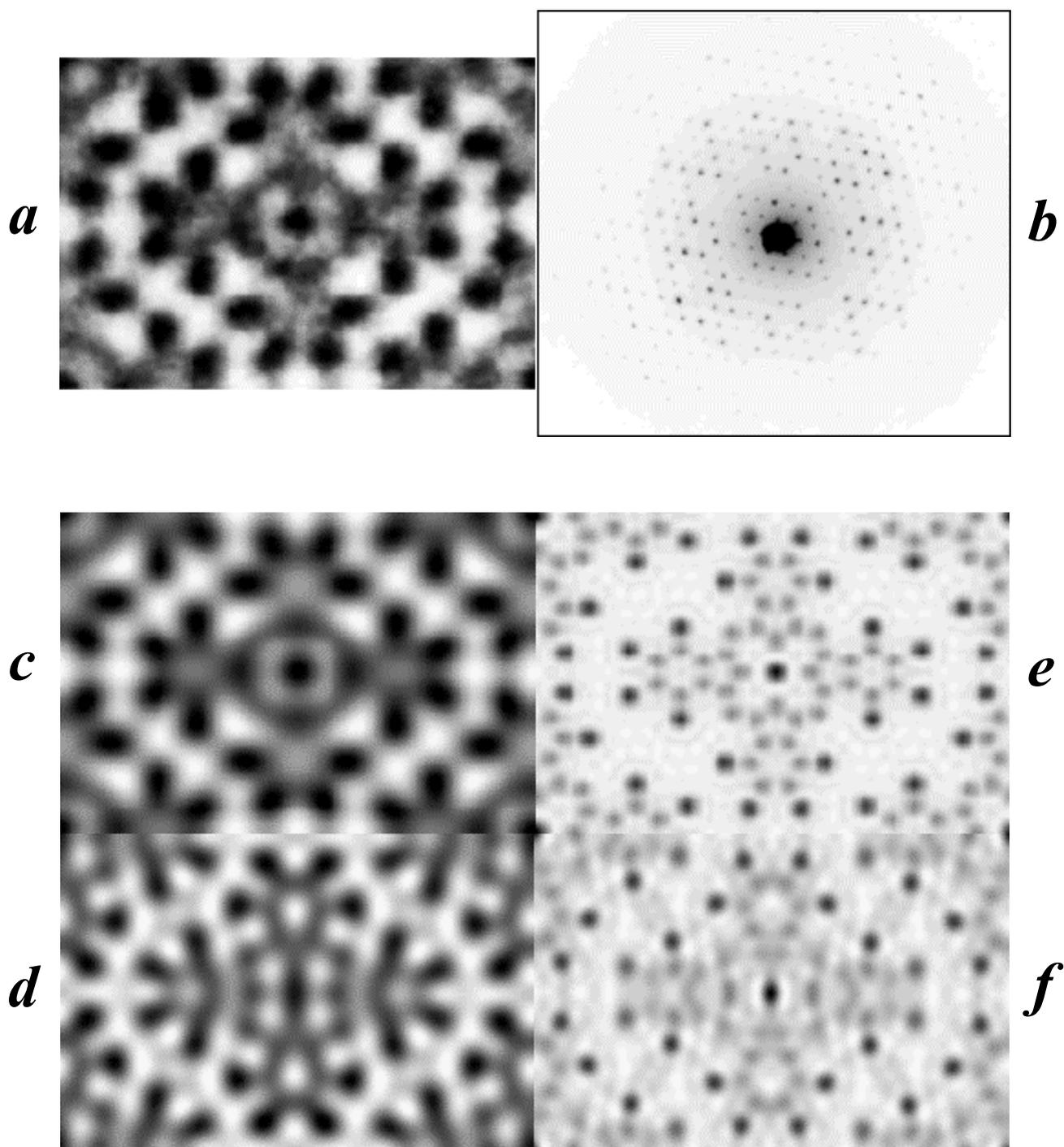
Figure 6. Image processing in high resolution electron microscopy using direct methods: (*a*) experimental electron micrograph (EM) of chlorinated copper phthalocyanine at 2Å resolution taken with the incident electron beam parallel to the *c* axis, only one unit cell is shown; (*b*) the corresponding experimental electron diffraction (ED) pattern at 1Å resolution; (*c*) the EM after symmetry averaging; (*d*) image resulting from the direct-method deconvolution of (*c*); (*e*) image at 1Å resolution resulting from the direct-method phase-extension with information from (*d*) and (*b*); (*f*) the theoretical image at 1Å resolution calculated from the structure model. EM and ED patterns were kindly provided by professor N. Uyeda.
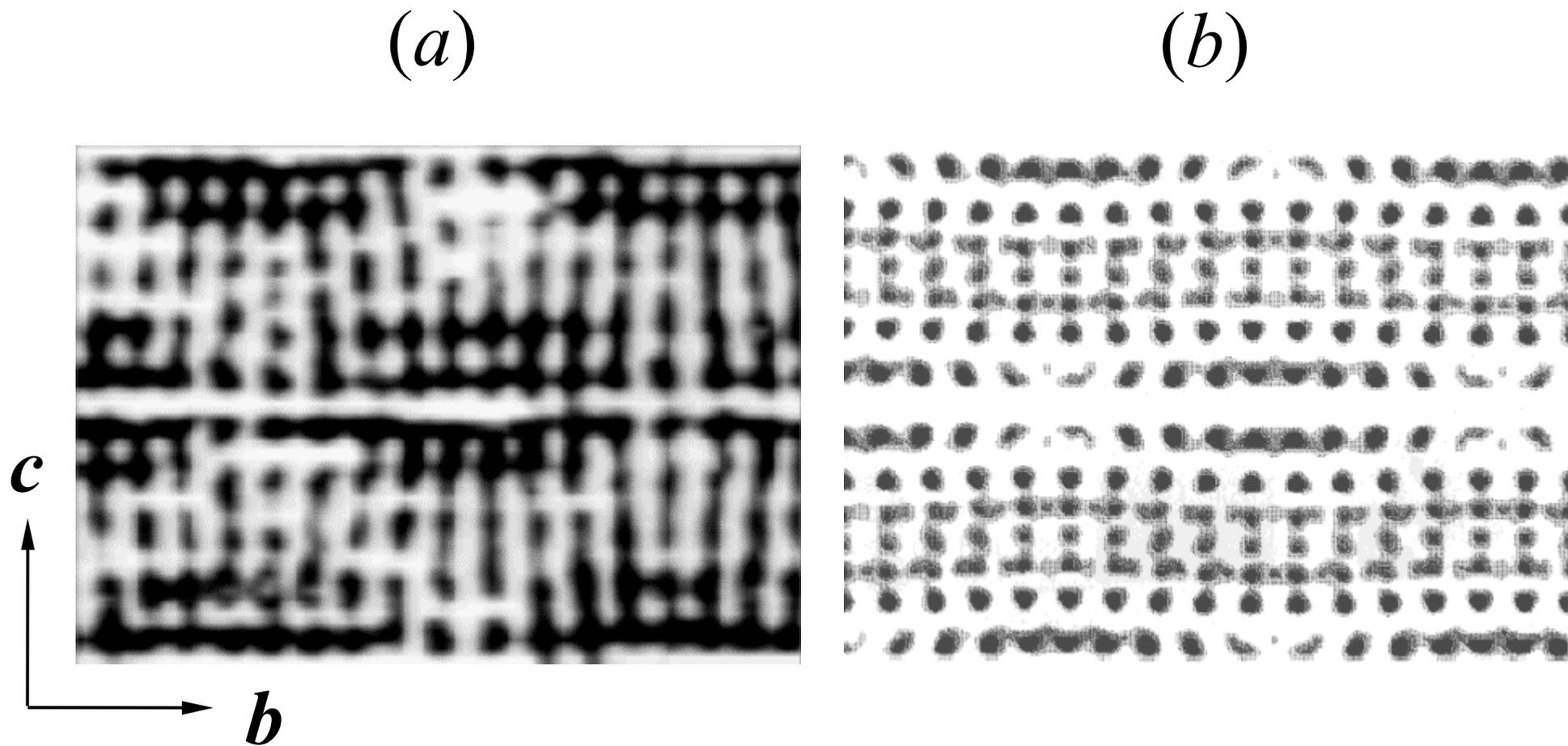
Figure 7. The incommensurate modulation in the Bi-2212 superconductor revealed by multidimensional direct methods combining the information from a single electron micrograph (EM) and the corresponding electron diffraction (ED) pattern. (a) experimental EM taken with the incident electron beam parallel to the *a* axis (an area of 8*b*×2*c* are shown); (b) the potential distribution function projected along the *a* axis obtained by multidimensional direct-method phase extension with starting phases from the EM and magnitudes from the corresponding ED pattern. The experimental electron microscope image was kindly provided by Dr. S. Horiuchi

Direct Methods

Sayre's equation
(D. Sayre 1952)

Modified Sayre equations
1963

Direct Methods Dealing with Pseudo Symmetries
1965-1985

Breaking Translational
Phase Ambiguities
1965 - 1978

Breaking Enantiomor-
phous Phase Ambiguities
1965 - 1978

Solving Superstructures
1978 - 1982

Direct Methods
in Protein Crystallography
(1965), 1983 -

Direct Methods
Solving Structures with
Periodical defects
1987 -

Solving
Composite Structures
1992 -

**Image Processing in
High Resolution
Electron Microscopy
1972-**