Acta Cryst. (1997). A53, 236–241

Solving the Phase Problem of X-ray Diffraction Using Atomic Resolution X-ray Holograms

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(Received 3 July 1996; accepted 20 November 1996)

Abstract

The recently proposed atomic resolution holographic scheme [Xu (1996). *Appl. Phys. Lett.* **68**, 1901–1903] has been expanded to solve the phase problems of X-ray crystallography. A macromolecular crystal sample is placed on a reference crystal. With the structure of the reference crystal known, the electron density of the specimen can be resolved by the direct mathematical imaging algorithm, without using the phase information. Only Bragg peaks from the macromolecular crystals are needed in the image reconstruction and the requirement to have a high-coherence-length X-ray beam may be reduced.

1. Introduction

X-rays are not unique as probes of molecular structure, but the properties of X-ray scattering combine to make X-ray crystallography the dominant technique for investigating biological structure at atomic resolution. Electrons are scattered much more strongly than X-rays are, but this property leads to multiple-scattering events that complicate the analysis of diffraction from thick samples. Neutrons are non-ionizing and therefore cause none of the radiation damage that afflicts experiments involving X-ray and electron scattering but the weakness of neutron scattering makes it impractical for routine application to great numbers of substances. However, in X-ray diffraction, one is not able to recover the electron density of any structure (including non-crystalline systems) *via* Fourier inversion because X-rays have a frequency of 10^{18} Hz, too fast for any detectors to follow, and only the intensity can be obtained (Bragg, 1913). This constitutes the phase problem, *viz* the phase information is lost for image reconstruction.

2. Solution of the phase problem

Special solutions to the phase problem for crystalline samples have been obtained, such as 'direct' methods pioneered by Karle & Hauptman (1953), so that nearly all small-molecule structures are now solved routinely (Glusker & Trueblood, 1972). Unfortunately, except in rare instances, such methods do not work for macromolecular crystals. Instead, the typical approach becomes experimental, such as using heavy-atom labels (the isomorphous-replacement method) or using multiwavelength anomalous diffraction (Hendrickson, 1995). Often, these approaches are indirect and of trial-and-error types. For non-crystalline structures, solutions to the phase problem have been sought but no general solution has been found (Hukins, 1981). Also, the conventional X-ray hologram cannot be constructed because macroscopically coherent X-ray beams can only be generated with a wavelength of longer than 200 Å (Crasemann, 1994).

To solve the phase problem, a new scheme has been proposed in which the incident X-ray beam is diffracted by a sample–reference-crystal assembly (Xu, 1996). The recorded scattering intensity contains the cross modulation of both electron densities and the unknown electron density can be resolved using a straightforward algorithm without the need for any phase information. Compared with other holographic methods that originated from Gabor (1948), the present method has advantages over soft-X-ray holography (Sayre & Chapman, 1995) because it eliminates the need for a reference beam and thus for a macroscopically coherent source, as well as having advantages over photoelectron holography (Szoke, 1986, 1993) because it does not require all radiating atoms to have identical environments oriented in the same way.

In this paper, the new scheme is expanded from amorphous systems to biological macromolecular crystals and is illustrated using simulated results in 2D. The simulated results show that, contrary to the case of amorphous systems where almost the entire scattering spectrum has to be used, in this case only the Bragg peaks from the macromolecular crystals are used. This makes the scheme extremely simple: one only has to measure the same number of intensities as usual. The difference is that they are now modulated by the presence of the reference crystal.

Fig. 1 shows the experimental set-up similar to those of conventional X-ray scattering, where a monochromatic beam is scattered by a macromole-

cular crystal (S) attached to an inorganic single crystal (R), which also serves as the sample mount. Consider a molecular crystal having a unit cell of 10×10 Å in the (x, y) plane, for which an electron density has been created by a random-number generator, and which has a total of 5 unit cells along the x axis and 10 along the y axis. Attach this to a reference crystal (square lattice of 2×2 Å), which spans from 50 to 100 along the x axis. The combined 2D electron-density function $\rho(x, y)$ is shown in Fig. 2(a). With the assumption of unit amplitude for the incoming beam, the scattered intensity from the sample–reference assembly is given by

$$I(\mathbf{k}) = \sum_{r} \sum_{r'} \rho(\mathbf{r})\rho(\mathbf{r}') \exp[i\mathbf{k}(\mathbf{r} - \mathbf{r}')], \qquad (1)$$

where $\rho(\mathbf{r})$ is the electron density of the assembly at point $\mathbf{r} = (x, y)$ and $\mathbf{k}$ is the scattering vector ($\mathbf{k} = \mathbf{k}_{\text{in}} - \mathbf{k}_{\text{out}}$). The diffracted intensity, I, is shown in Fig. 2(b) as $I^{1/4}$. This is given as the fourth root to shrink the intensity range. The intensity data would, in practice, be recorded by the actual measurement.

According to the proposed scheme (Xu, 1996), the inverse Fourier transform can be performed on $I(k_x, k_y)$ to get the auto-correlation of ρ, also called the Patterson function (P), which is given by (Fig. 2c)

$$F^{-1}_{k \to u,v} I(k_x, k_y) \equiv P(u, v) = \sum_{x,y} \rho(x, y)\rho(x + u, y + v). \qquad (2)$$

Notice, for $u > 50$, the second density term $\rho(x + u, y + v)$ is in the reference-crystal area, whereas the first term, $\rho(x, y)$ (for $x < 50$), is within the unknown sample. Therefore, substitution of the simulated reference density by 0 and 1 makes the whole equation become linear and the unknown density can be recovered by (Xu, 1996)

$$\rho(x_{\max} - u, y_{\max} - v) = P(u, v) + P(u + 2, v + 2) - P(u, v + 2) - P(u + 2, v), \qquad (3)$$

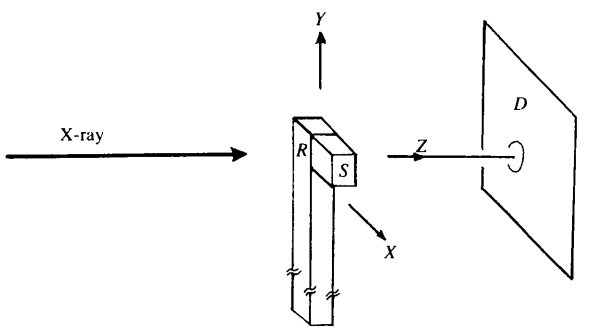


Fig. 1. The experimental set-up showing a specimen (S) attached to a crystal (R) and the 2D X-ray scattering detector (D).

which gives a 2D unit cell with $x = 1$–10, $y = 1$–10, identical to that shown in Fig. 2(a) ($x_{\max} = 50$ and $y_{\max} = 100$).

To demonstrate that one only needs to use the Bragg diffraction from the molecular crystal, a 1D

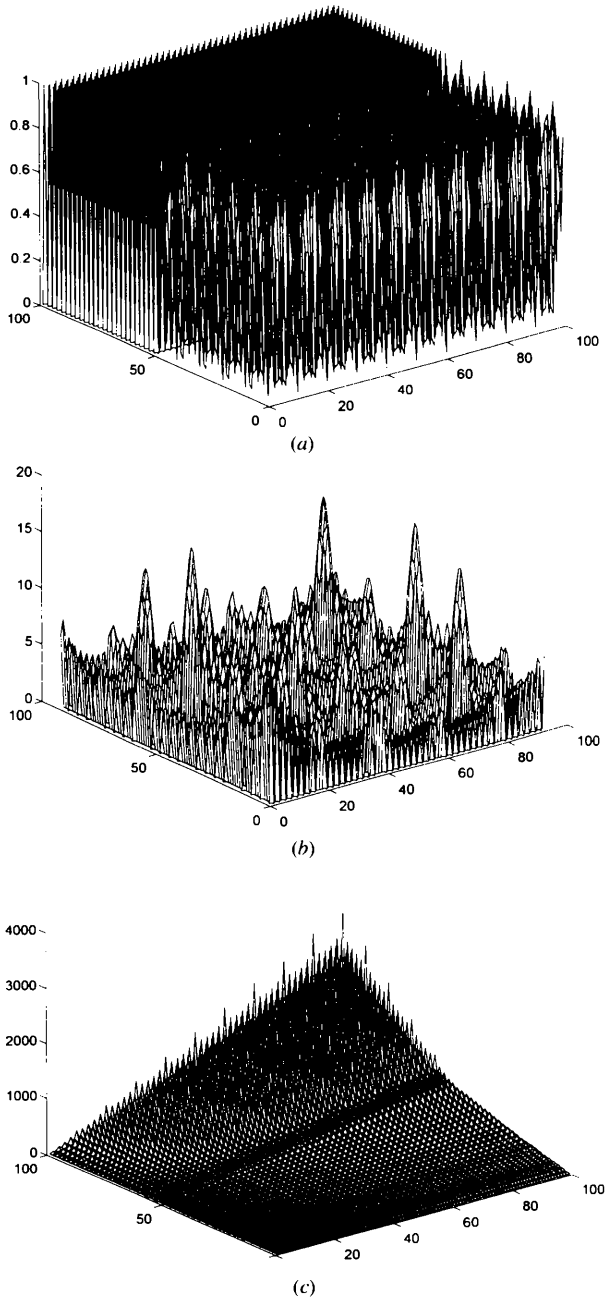


Fig. 2. (a) The 2D electron-density map of the specimen attached to a reference crystal of lattice constants 2×2 valued as 0 and 1. (b) The 2D intensity for the density in (a) ($I^{1/4}$), where both k_x and k_y run from 0 to 100 and are expressed in units of $\Delta k = 2\pi/(200\Delta x)$. Peaks at $(k_x, k_y) = (100, 100)$ and $(0, 0)$ are removed for better viewing. (c) The 2D Patterson function $P(u, v)$ of (a), obtained by 2D Fourier inversion from the intensity (x, y axes inverted).

density $\rho(x, y = 1)$ extracted from the above electron density will be used. It has two different periods (Fig. 3*a*): a molecular crystal of lattice constant 10 and the reference crystal of lattice constant 2. Notice for real specimens that both the number of coordinate points and the number of unit cells are going to be much larger if ångström resolution needs to be achieved (each unit cell has thousands of atoms and the sample will be of micrometre size). Therefore, for illustration, the number of molecular unit cells is now expanded from 5 to 50 and the number of reference-crystal cells from 25 to 250. The 1D scattering intensity of the molecular crystal plus the reference crystal is then given by Fig. 3(*b*), where there are sharp peaks at $k = n \times 200$ $(n = 1, 2, \ldots)$ owing to the Bragg diffraction of the sample and a major peak at $k = 1000$ owing to the reference-crystal lattice of period 2 [note that k is in units of $\Delta k = 2\pi/(2000\Delta x)$].

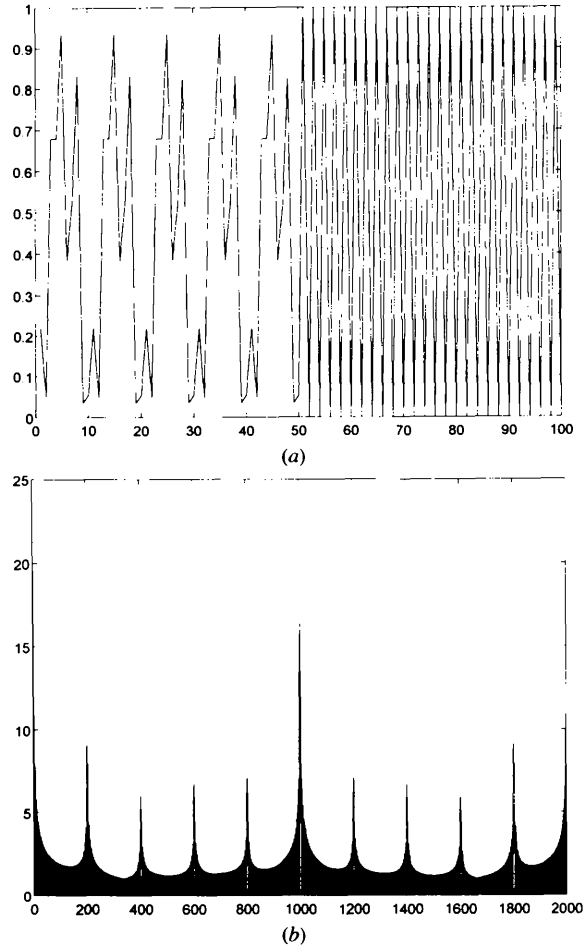


Fig. 3. (*a*) The 1D electron-density map along the x axis ($y = 1$ of Fig. 2*a*). (*b*) The 1D intensity ($I^{1/4}$) for 50 sample unit cells plus 250 reference-crystal unit cells, where k (= 0–2000) is in units of $\Delta k = 2\pi/(2000\Delta x)$.

Unlike the amorphous cases, where the entire diffraction spectrum is needed for the image reconstruction (Xu, 1996), here one can neglect the scattering located between the Bragg peaks (Fig. 4*a*). In fact, in this example, one will get the Patterson function, from which all 50 molecular unit cells can be obtained. Since they are assumed to be identical, the best result will be given by the arithmetic average of these 50 unit cells [Fig. 4(*b*) shows the result of such an average, which is repeated five times for illustration]. The small distortion in the final result was attributed to the fact that there are only 50 unit cells and the Bragg peaks are not perfectly sharp.

In order to avoid the strong Bragg diffraction of the reference crystal eclipsing the scattering from the molecular crystal and to avoid the measurement of intensity with k values near zero, the above 1D intensity was further modified to remove the large values at k near 0 and $k = 1000$ (when the reference crystal and sample are incommensurate, the reference Bragg peaks can be removed without having to remove any sample

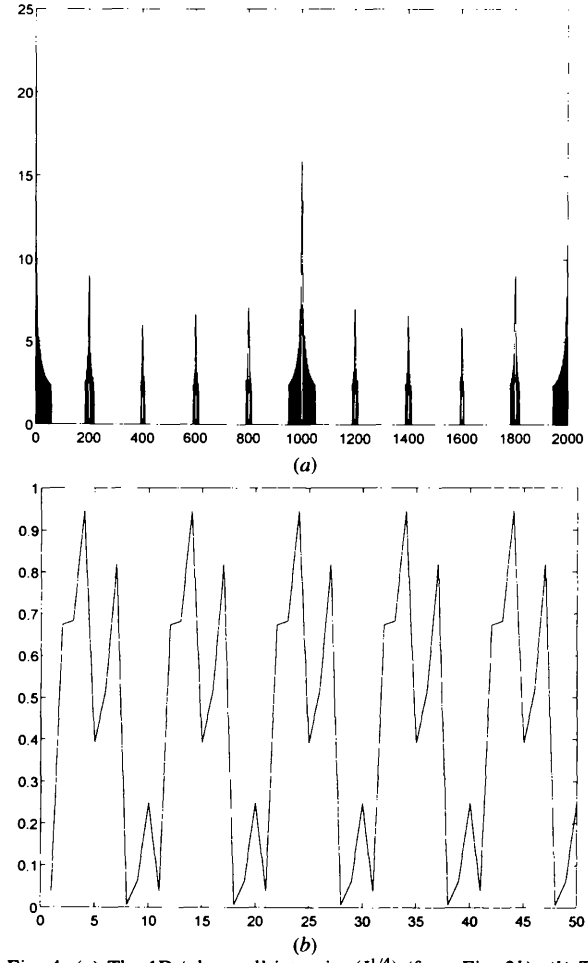
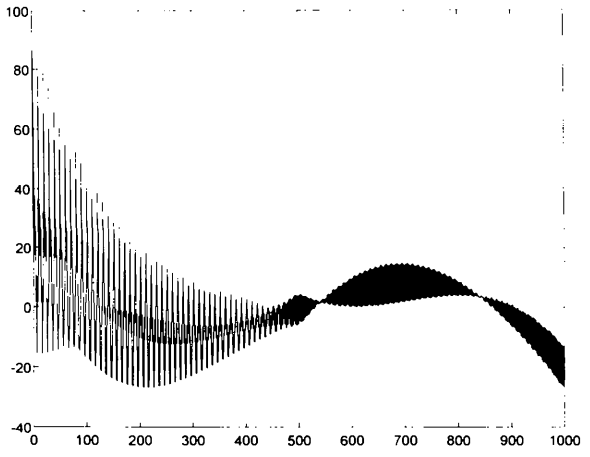
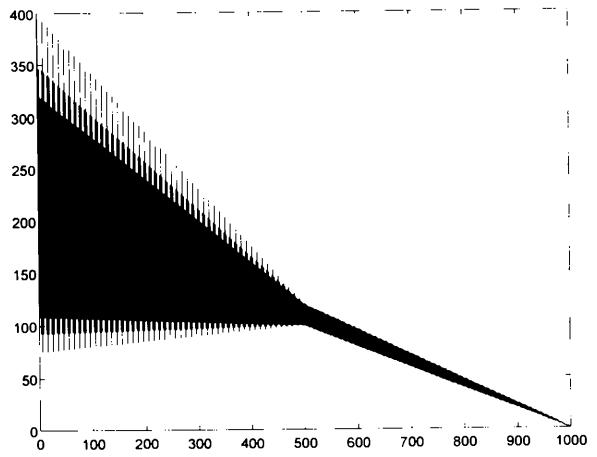


Fig. 4. (*a*) The 1D 'chopped' intensity ($I^{1/4}$) (from Fig. 3*b*). (*b*) The 1D density obtained from (*a*) using algorithmic average.



(*a*)



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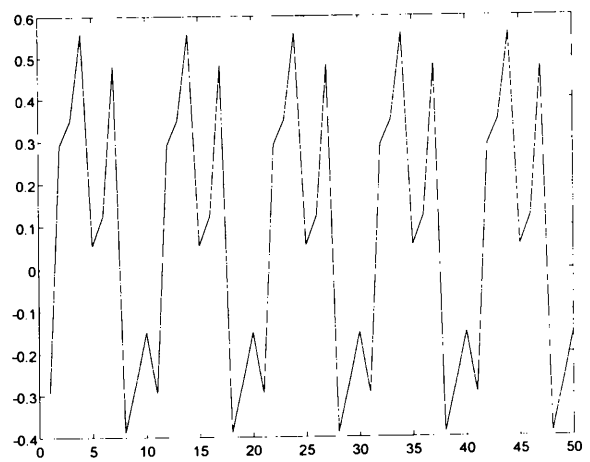
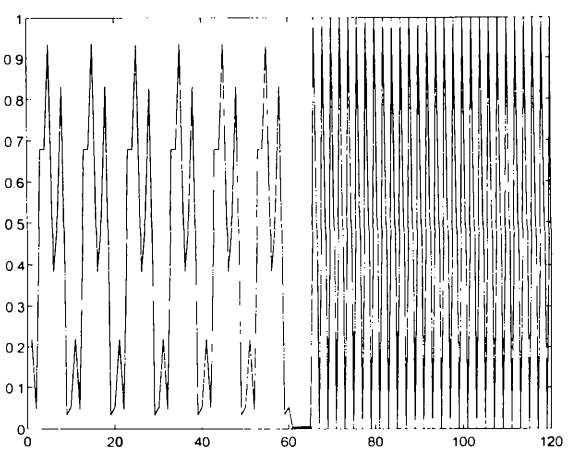


(*c*)

Fig. 5. (*a*) The 1D Patterson function of 'chopped' intensity (Fig. 4*a*) with peaks of $k = 1000$ and $k = 0$ removed. (*b*) The 1D Patterson function of 'chopped' intensity (Fig. 4*a*) before removing the peaks at $k = 1000$ and $k = 0$. (*c*) The 1D density obtained from (*a*) using the algorithmic average.



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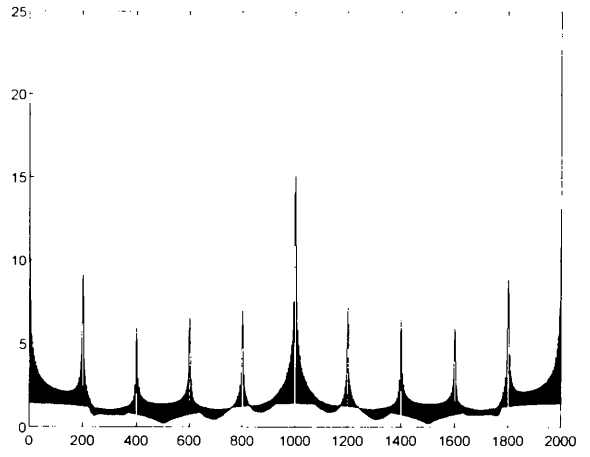

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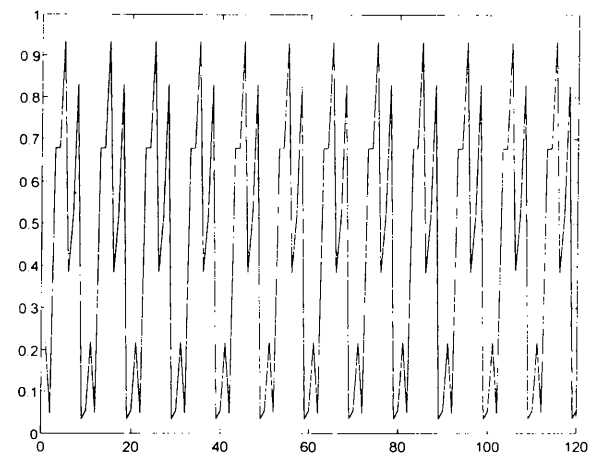
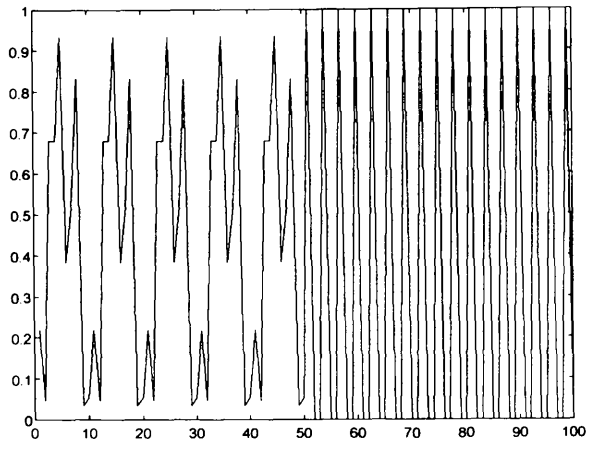
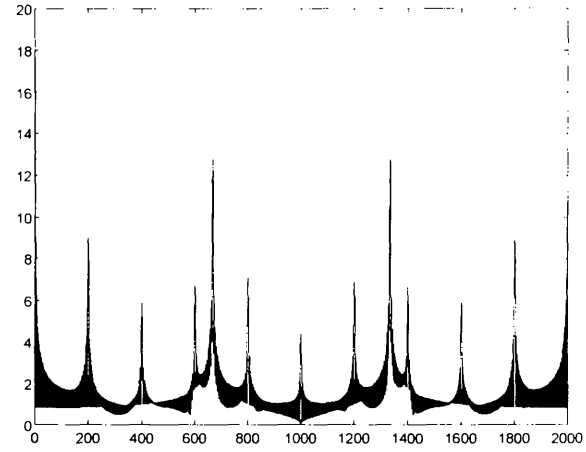


(*c*)

Fig. 6. (*a*) Part of the 1D electron-density map, where there is a 'gap' at the junction between the sample (50 unit cells) and the reference (248 unit cells). (*b*) The 1D intensity ($I^{1/4}$) for 50 sample unit cells plus 248 reference unit cells, with k in units of $\Delta k = 2\pi/(2000\Delta x)$. (*c*) The 1D density obtained recovered from (*b*).



(*a*)



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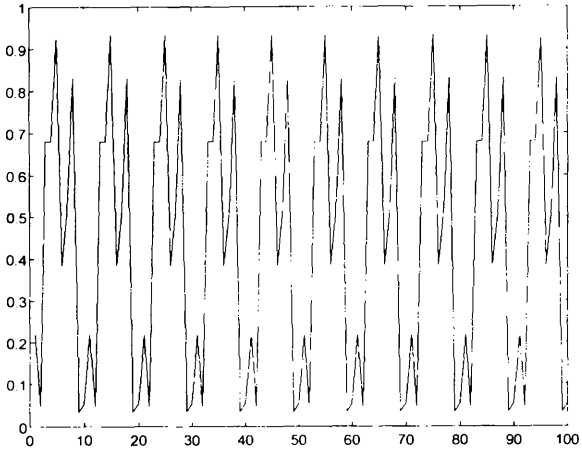

(*c*)

Fig. 7. (*a*) Part of the 1D electron-density map, where there are 50 sample unit cells of period 10 and 166 reference unit cells of period 3. (*b*) The 1D intensity ($I^{1/4}$) of (*a*) with two Bragg peaks located near $k = 660$ and $k = 1330$ from the reference crystal [k is in units of $\Delta k = 2\pi/(2000\Delta x)$]. (*c*) The 1D density recovered from (*b*).

Bragg peaks). The resulting Patterson function is shown in Fig. 5(*a*), where some negative values and low-frequency oscillations appear, which is compared with the Patterson function before removal of the reference-crystal reflections (Fig. 5*b*). This is because the intensities at k near 0 represented the constant and low-frequency oscillation components. Nevertheless, following the same procedure (plus the arithmetic average), one can still obtain a reasonably good electron density (Fig. 5*c*).

Moreover, because of the fact that only Bragg peaks from the sample (molecular crystals) are used, one is able to sacrifice more intensity on a synchrotron to gain longer coherence lengths (using finer pinholes *etc.*) to cover the entire sample assembly. Moreover, when the coherence lengths are smaller than the assembly, this scheme may still be used. In that case, the intensity signal contributed by the sample crystal alone will have to be removed from the data (whereas the intensity attributed to the reference crystal alone, the major Bragg peaks, will be removed anyway). The ratio of this intensity from the sample alone *versus* the intensity of both sample and reference crystal will have to be estimated from the sample size and coherent lengths. Also, since the only difference here is the addition of the reference crystal, no extra radiation damage should occur.

Finally, to test the stability and robustness of the scheme, as well as to show that the two lattice periods (sample and reference) do not need to have integer ratio, a number of numerical experiments are provided. In the first case, the reference crystal is shifted away from the sample, or chopped off by a few coordinate points at the juncture (Fig. 6*a*), so that there is a gap between the two. The resulting intensities and the recovered densities show very little change from the ideal case (Figs. 6*b*, *c*). This is because the proposed algorithm is expressed by the linear equation (2), which not only provides a unique solution but also has good stability. The important consequence of the test is that there is no need for the sample to be firmly attached to the reference crystal, nor a necessity to know the exact size of the reference crystal. In the second case, the periodicity of the reference crystal is changed from 2 to 3 (Fig. 7*a*) and the resulting intensity shows two Bragg peaks of the reference crystal at incommensurate positions to those of the sample (Fig. 7*b*). From the same algorithm [equation (3) except 2 has to be replaced by 3], an accurate sample density is recovered (Fig. 7*c*). In the last case, a combined test is performed, where not only a gap is added between the sample and the reference (which has a period of 3), but also a number of defects – vacancies and interstitials are incorporated into the references (Fig. 8*a*). The intensity is shown in Fig. 8(*b*) where some noise is also visible. As a result, there are a small number of bad unit cells in the recovered sample density (Fig. 8*c*). However, after

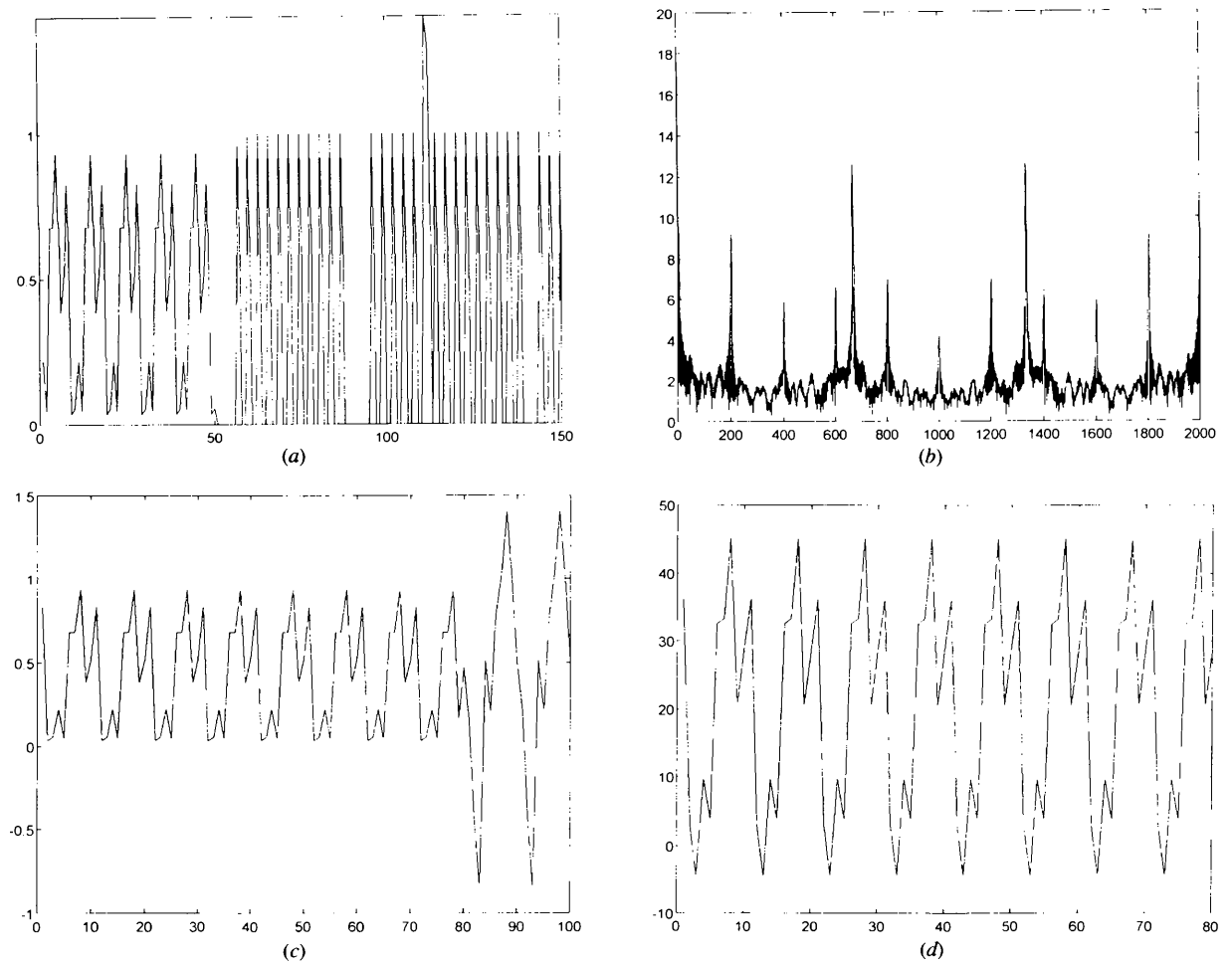

Fig. 8 (*a*) Part of the 1D electron-density map, showing a 'gap', a couple of 'vacancies' and an 'interstitial' on top of the 166 reference unit cells of period 3. (*b*) The 1D intensity ($I^{1/4}$) of (*a*) with some 'noise' [k is in units of $\Delta k = 2\pi/(2000\Delta x)$]. (*c*) Part of the 1D density recovered from (*b*), containing a few 'bad' unit cells. (*d*) Result of (*c*) after the arithmetic average.

using the arithmetic average described above, a good result is obtained again (Fig. 8*d*).

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