

led to a new class of cyclic receptors and membrane ion carriers.

Mathematics: Servet Martinez, Depto. de Ingeniería Matemática, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile. For his pioneering work in probability theory, particularly conditionally invariant distri-

butions in Markov processes and dynamical systems; and for his studies of deep connections between ultrametricity and potential and the description of cellular automata dynamics.

Physics: Nai-ben Ming, State Key Laboratory of Solid State Microstructures, Nanjing University, China. For the

design and fabrication of periodic and quasiperiodic dielectric superlattices and the realization of second harmonic generation (SHG), multiple wavelength SHG, third harmonic generation, optical stability, polariton excitation and ultrasonic generation with high frequency up to GHz in this kind of synthetic materials.

RESEARCH NEWS

Structure of materials

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The structure of crystalline materials delineate the position of atoms in materials, an information that basic scientists seek to understand and interpret the nature of materials as well as their various physico-chemical properties. Determination of the structure of materials is routinely carried out by well-established techniques of X-ray, electron and neutron diffraction and this is the subject matter of crystallography. The choice of radiation probe depends on the nature of information sought. For example, neutron diffraction is the preferred technique if one were interested in magnetic structures or in determining the position of hydrogens or other light atoms in the presence of heavy atoms.

Several interesting developments have been taking place in the field of crystallography and in this research note attention is drawn to some new results based on recent activities.

Single crystal specimens give rise to three-dimensional diffraction patterns where the overlap amongst different diffraction peaks is minimal; hence single crystal diffraction is a very useful technique for determination of the structure even of complex materials like proteins. However, it may not always be feasible to grow single crystals in sizes large enough to suit the diffraction technique used. One is compelled to use powders in such situations. A large number and variety of materials fall into this category.

When single crystals are not available but crystalline powders are available, one

resorts to studying the diffraction patterns from powders themselves to delineate the crystal structure. A powder diffraction pattern is an average of a large number of (literally thousands of) single crystal patterns – arising from the small crystallites that compose the powder – superposed over one another randomly. The randomness and superposition is so much that every diffraction spot from a single crystal transforms to a spherical distribution for a powder. Consequently the diffraction pattern from a powder is a collection of a larger number of concentric (diffraction) spherical shells; this leads to a simplification also in the sense that one can sample the diffraction pattern of a powder by a one-dimensional traverse along any radius of the diffraction spheres. This is the situation for an 'ideal' powder diffraction pattern. As diffraction peaks from a powder are a result of overlap of individual single crystal diffraction peaks, peak-separation may not easily be possible in the large-angle region. In spite of this handicap, there have been a variety of methods adopted to make use of the powder diffraction patterns and still be able to derive crystal structures.

Analysis of the 'ideal' powder diffraction pattern has been carried out during the past two–three decades using the Rietveld analysis, known after Rietveld who in 1967 proposed the profile refinement technique¹ for refining nuclear and magnetic structures from neutron diffraction data². The technique uses

every point of data and not mainly the peak positions. No attempt is made to resolve overlapping reflections into separate Bragg reflections. Least square fitting procedures of the observed intensities to calculate the overall diffraction profile are adopted. The quantity²,

$$M = \sum_i \omega_i \left[y_i(\text{obs}) - \left(\frac{1}{c} \right) \{ y_i(\text{calc}) \} \right]^2,$$

where $y_i(\text{obs})$ is measured intensity at scattering angle ϕ_i , ω_i its weight factor, c a scale factor and $y_i(\text{calc})$ is sum of contributions from intensities of all Bragg reflections which overlap at point i , is minimized in this procedure. (For further details see refs 1 and 2.) Rietveld showed that the profile refinement procedure leads to more accurate structures compared to conventional integrated intensity refinements. Rietveld's method has undergone considerable improvement over the past three decades and still remains an important tool to analyse powder diffraction data. Manohar³ has reviewed application of Rietveld technique as applied to X-ray diffraction, ab initio determination of an unknown structure and energy dispersive synchrotron structure determination.

In spite of the interest in powder diffraction techniques using Rietveld analysis, several approaches have been tried to overcome the limitations of powder data based on single crystal methods⁴ or by maximum entropy technique⁵. A plausible method of solving structures using full powder pattern

analysis is based on Monte Carlo schemes also, to locate global minimum of the figure of merit instead of local minima. Here one resorts to generating trial atomic positions in the unit cell which are ultimately tested for agreement with the measured diffraction profile. The Monte Carlo method has been combined with simulated annealing technique also⁶. These approaches have had better success than the previous ones.

Direct methods are expected to solve, in practice, the phase problem for small molecules in the case of single crystal diffraction. In the case of powders even for a crystal structure with a few atoms/asymmetric unit cell, the problem is challenging⁷.

It is against this background that we shall now present some of the recent developments:

Single crystal-like diffraction data from powders

As was stated earlier, powder diffraction data tend to miss (or lose) the quality of single crystal data. Since one does not always deal with 'ideal' powder specimens, attempts have been made to obtain different powder diffraction patterns from the same sample. Such patterns are used simultaneously to extract a single set of diffraction intensity information. Two such approaches are based on: (a) measurement of powder diffraction patterns for the same sample at different temperatures – If there are no phase changes occurring in the temperature range but if anisotropic differential thermal expansion occurs as a function of temperature, then relative positions of reflections change so much that lines that are superposed at one temperature will separate at some other temperature; and (b) looking at a sample that is not an ideal powder but a partially textured sample.

Thomas Wessels *et al.*⁸ have reported solution of the structure of as-synthesized form of zeolite UTD-1, which contains as many as 69 non-hydrogenous atoms in the asymmetric unit cell by full quantitative texture analysis of a single sample. (In addition to structure of zeolite UTD-1, these authors have solved⁸ the structure of two other complex systems, namely Zeolite ZSM-5 and a molecular sieve Mu-9.) Whereas an ideal powder sample provides less information compared to its single crystal counterpart, a

textured sample may be considered as providing more information than the ideal powder but less than that from a single crystal. That is to say that if diffraction from some (*hkl*) plane corresponds to an ideal diffraction peak at a specific orientation of the sample in the case of a single crystal and that from its powder a spherical intensity distribution irrespective of orientation of the sample, the diffraction from a textured sample, oriented preferentially in directions similar to those of the single crystal, gives rise to broadly smeared diffraction peaks. Texture diffraction analysis techniques lead one to construct 'pole figures'. Wessels *et al.*⁸ carried out measurements of pole-figure data for a few selected reflections (seven, to be specific) from a textured sample of UTD-1, using a modified powder diffractometer at the European Synchrotron Radiation facility at Grenoble which provided them the orientation of the crystallites in the sample. Then they measured complete diffraction patterns $y(2\theta, \text{orientations})$ at several (five, to be specific) sample orientations. The diffracted intensity of a textured sample at any scattering angle and orientation is related to the pole-density distribution function $P_{hkl}(\text{orientation})$ by the relation,

$$y(2\theta, \text{orientations}) = \sum_{hkl} I_{hkl} P_{hkl}(\text{orientation}) G(2\theta - 2\theta_{hkl}),$$

where I_{hkl} is the true intensity of the (*hkl*) reflection, with $G(2\theta - 2\theta_{hkl})$ being the peak profile function. Since $P_{hkl}(\text{orientation})$ is known, one can derive I_{hkl} corresponding to that of the single crystal for even overlapping reflections. From such derived intensities, Wessels *et al.*⁸ were able to derive the correct space group and detailed crystal structure of the zeolite UTD-1 by a combination of direct methods as well as subsequent Rietveld refinement; the system possesses non centrosymmetric space group *Pc* with 117 atoms in the asymmetric unit cell.

This study has demonstrated the usefulness of texture analysis technique for deriving crystal structure of samples that can be obtained with preferred orientation.

Hard X-ray holography for imaging 3D crystal structure

A new technique referred to as X-ray holography has been tried out for over-

coming the problems of non-availability of single crystals and the phase problem⁹. It is based on holography invented by Denis Gabor some 50 years ago¹⁰. In the technique of holography (complete writing), optical information about an object or a scene is recorded in a complete manner in an emulsion plate and subsequently the object/scene is made visible or reproduced realistically in all its true spatial 3D aspects with its shadows and varying intensities when illuminated suitably on the image record. The photo-emulsion plate is referred to as a 'hologram'. When the plate itself is examined, there is nothing visible to the naked eye that has any resemblance to the object/scene. In fact the plate contains information about the scene coded in the form of interference patterns. It is only when the hologram is illuminated suitably that the coded information can be decoded and the scene made visible. A hologram is prepared like the negative of an ordinary photograph. A source of light illuminates the scene and activates the emulsion plate after scattering from the scene. But unlike in ordinary photography, a diverging laser beam that provides coherent light is used in preparing the hologram. While the laser beam is illuminating the object, it illuminates a flat mirror also simultaneously which is mounted such that the reflected beam from the mirror is also incident on the emulsion plate. Hence the plate records an interference pattern due to the object beam (from the object/scene) as well as a reference beam (from the mirror); the phase and amplitude information of scattering from the object is thus stored in the emulsion plate. As in the case of the ordinary photography, the plate is processed and one has the negative of the interference pattern. This is the hologram, created by a lens-less situation on a very high resolution emulsion plate. Holography using lasers has been able to store 3D spatial information on a 2D surface without optical elements; the information is retrievable.

Recently, high energy X-rays or gamma rays have been used in holograms that allow one to access atomic resolution in the images. Limits to resolution have been due to limits to miniaturization – preparing sources and detectors of sizes less than a few hundred angstroms. Researchers have used what are referred to as 'inside source' and 'inside detector' concepts using high energy electrons to form holograms. Holography with soft X-

rays is a straightforward extension, in principle, of the optical holographic principle to shorter wavelengths. This became possible with the development of undulator X-ray sources on synchrotron storage rings, as these provide high intensity X-rays with very large coherence lengths¹¹. The holographic principle comes into vogue because of interference between the X-ray beam that reaches the detector directly and which gets scattered off atoms in the material. Tegze *et al.*¹² have used the X-ray holographic technique to unravel the structure of CoO using combination of normal and inverse, synchrotron and lab experiments but without a priori knowledge of the structure.

The experiment relates to measurement of normal and inverse holograms at several X-ray energies in the range of 5 to 20 keV, on a single crystal CoO sample of mosaic spread of about 0.3°. The measured CoK_α fluorescent intensity was corrected for θ -dependent absorption of emitted fluorescence, modulation of fluorescence and filtering line patterns due to X-ray standing waves, etc. The atomic images were reconstructed using Helmholtz–Kirchhoff formula¹¹, after extending the data into 4π solid angle, summing holograms taken at different energies (see ref. 12 for details) and combining holograms taken in the normal and inverse modes using both synchrotron and lab data. The resulting 3D image is shown in Figure 1.

One can see that this structure is reproducing the well-known structure of this material obtained by conventional procedures but without any a priori knowledge of the structure in processing the holographic data.

Mapping of electronic orbitals in crystals

Synchrotron-based X-ray diffraction techniques have been used for mapping electron density distribution in molecular crystals routinely¹³; however in ionic crystals extinction effects in X-ray diffraction have prevented similar approaches. Zuo *et al.*¹⁴ have used a recently developed technique called convergent beam electron diffraction¹⁵ over nanometer dimension perfect crystal regions of a natural Cu_2O (cuprite) crystal for experimental mapping of charge density distribution in Cu_2O . The low-order crystal structure factors measured were

combined with structure factors from X-ray diffraction results for high-order reflections. The crystal charge density was derived by a multipole refinement of this data. The charge distribution found around Cu is remarkably similar to the textbook d_{z^2} orbitals. Non-spherical distortions of Cu and O were also the outcome of detailed analysis of the data. A three-dimensional plot (Figure 2) showing the difference between the static crystal charge as obtained by the multipole fitting to the data and charge density

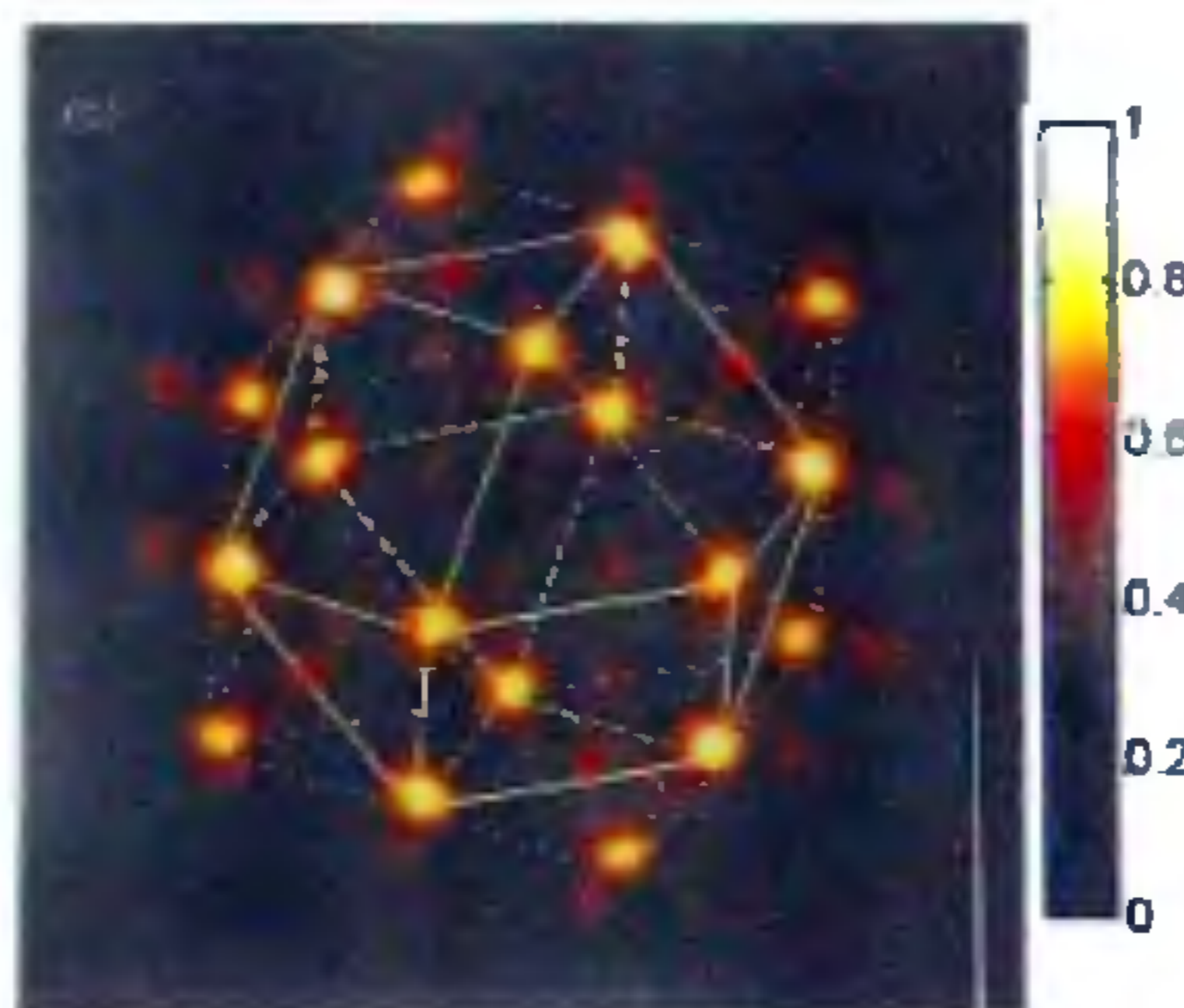


Figure 1. Three-dimensional image of CoO reconstructed from X-ray holograms showing the first (solid and dashed lines) and second (dotted lines) Co neighbours of a central Co atom; the contribution to this image from the central Co atom is removed by the reconstruction algorithm (from ref. 12).

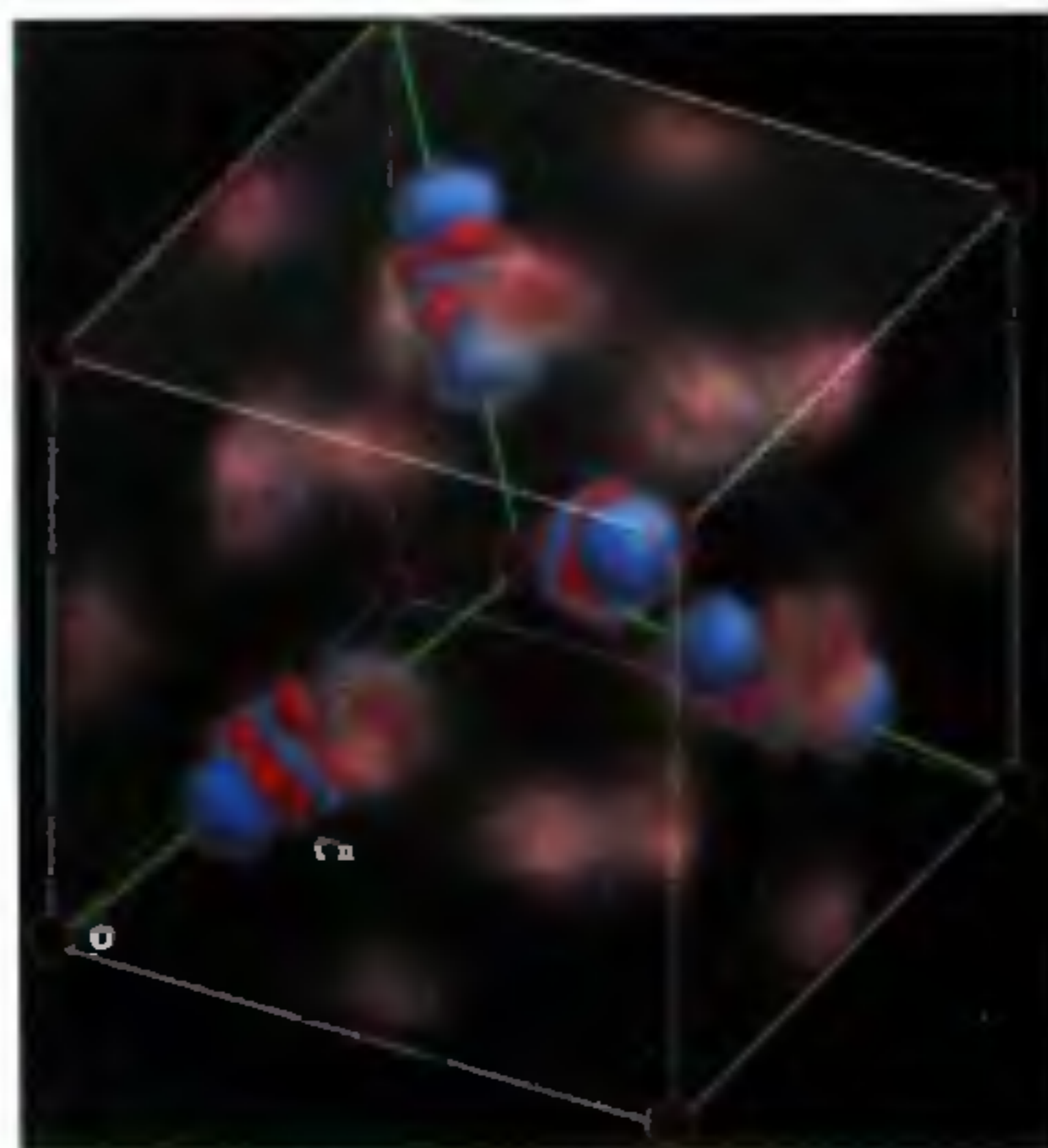


Figure 2. Three-dimensional image of bond-electron density in copper oxide. Difference between the experimental charge density and spherical O^{2-} and Cu^+ ions is shown (blue, d_{z^2} charge holes; red, surplus). The map was obtained by combining experimental extinction-free low-order electron structure factors (CBED) with high-order ones from X-ray scattering using a multipole model (Courtesy Dr J. M. Zuo).

of spherical O^{2-} and Cu^+ ions, reveals clearly a covalent bond between Cu atoms as expected in theoretical studies.

It is believed that this is the first time that one is able to observe the bonding orbital of metal ions.

Direct imaging of non-crystalline entities using soft X-rays

As the wavelength of electromagnetic radiation decreases from that of visible light at one end to hard X-rays at the other end, visibility of finer entities becomes increasingly possible; hence the X-ray holograms have been able to reveal atomic sizes as discussed here. Compared to electrons X-rays penetrate matter more, allowing to probe 'thick' materials. Recently soft X-ray diffraction as opposed to hard X-ray diffraction has been used to image non-crystalline materials albeit with poor resolution of several nanometers. The sizes of entities being imaged are nearly two orders larger in magnitude than atomic sizes.

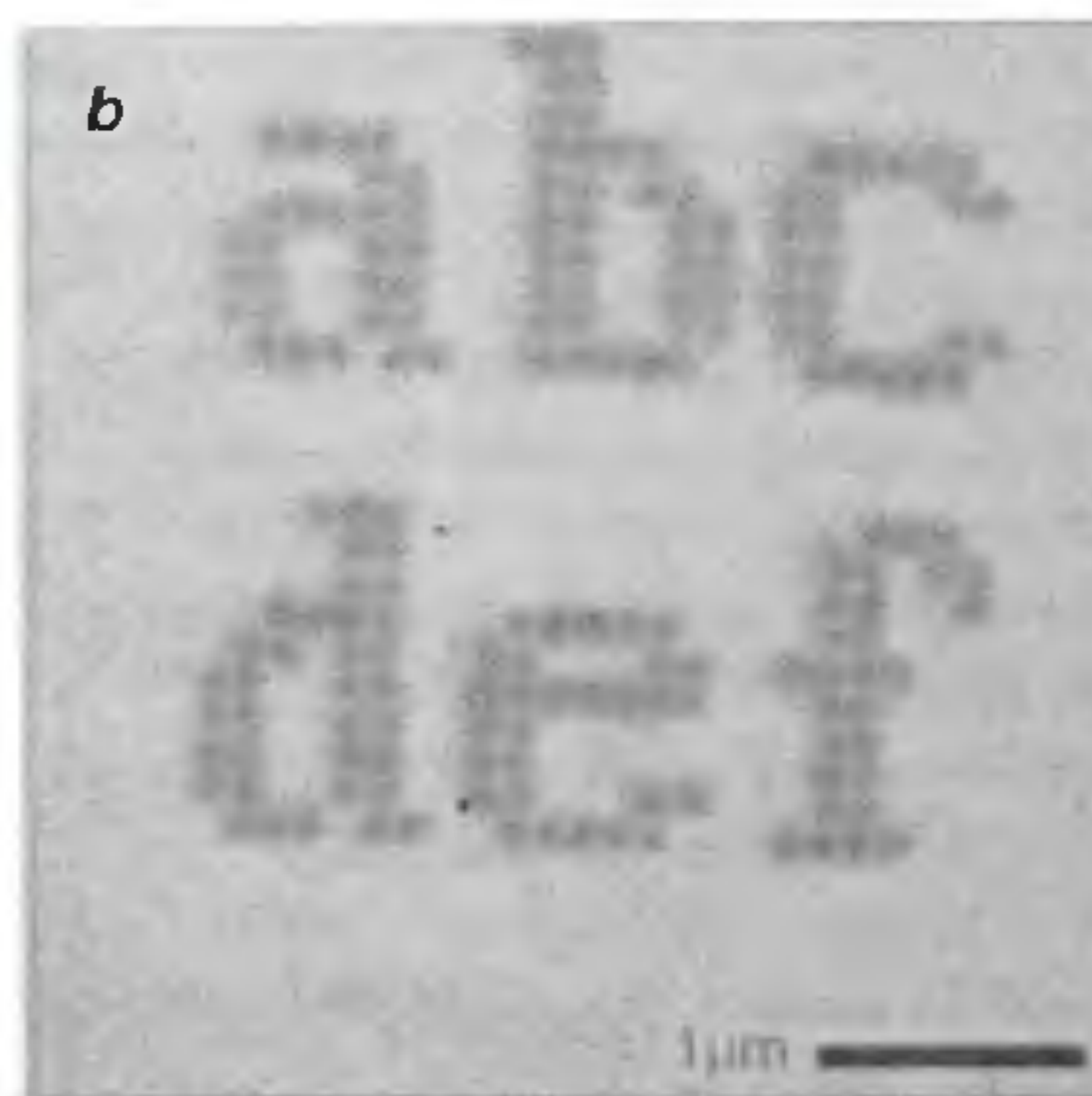
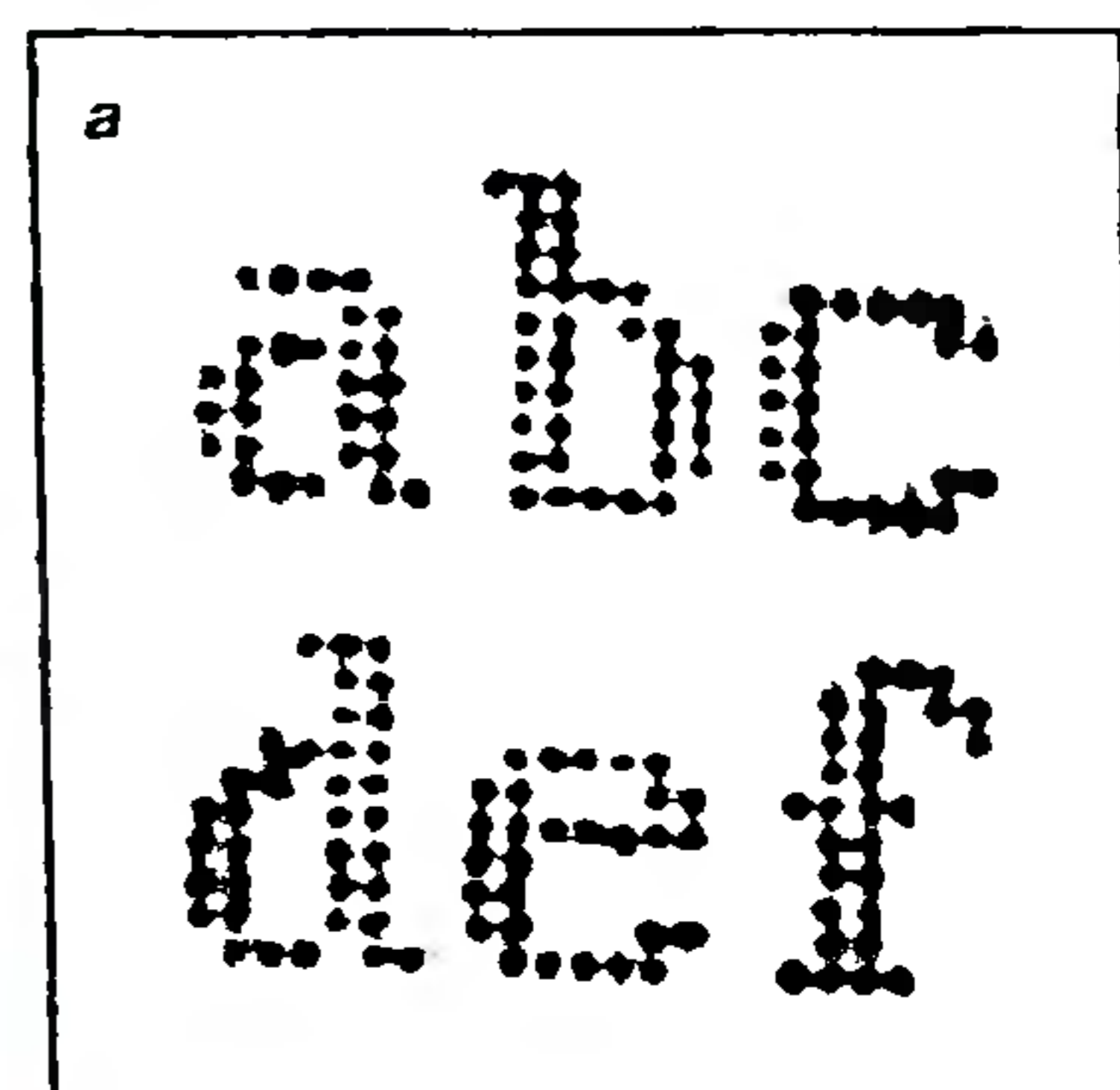


Figure 3. *a*, Reconstructed image of a non-crystalline specimen; *b*, SEM image of the specimen (from ref. 16).

Miao *et al.*¹⁶ have carried out soft X-ray (wavelength ≈ 1.7 nm) diffraction using an undulator beam line at NSLS. The specimen examined was a collection of gold dots (≈ 100 nm³ each) arranged in the form of English alphabets. The diffraction was registered using a 512×512 CCD pixels detector. Data analysis is based on reconstruction using oversampling technique proposed by Bates¹⁷ earlier. Figure 3a shows the reconstructed image of the specimen shown in Figure 3b, demonstrating successfully that soft X-ray diffraction is indeed useful in such imaging requirements.

It is believed that one can extend this technique to image large cell structures in biological systems and other materials.

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Three dimensional structures at the heart of the central dogma of molecular biology: An end of millennium gift-pack from crystallographers

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The fundamentals of gene expression were established by the mid-1960s, primarily from the enunciation of the central dogma of molecular biology, i.e. DNA \rightarrow RNA \rightarrow protein and the pioneering work of Francois Jacob and Jacques Monod on the regulation of this circuit. Thus, several research groups all over the world spent over 30 years to understand every step associated with the central dogma. A few organisms were the hot favourites like the bacterium *Escherichia coli* and the common yeast *Saccharomyces cerevisiae*. Around the mid-1980s, all the broad outlines of the steps leading to protein synthesis from DNA, were unveiled. However, there remained major gaps in our understanding of the molecular details of the processes of transcription and translation.

Whenever there is a technological hang-up scientists spend money on a different route or a different technology. We knew about the DNA replication cycle and the role of DNA polymerase, the complexity of eukaryotic genome like

chromatin was well established, the mechanisms of RNA synthesis and protein synthesis on mRNA templates was clearly understood. However, the structure of chromatin was not known and therefore we were surprised by the rapidity with which transcription processes can continue over a mass of DNA so tightly bound with the histone octamer. Similarly, the structure of RNA polymerase which actively transcribes the DNA chain or the structure of the ribosome eluded us and as a result the beauty of the cell machinery remained obscure. From our knowledge and experience, it was clear that single crystal X-ray diffraction analysis at atomic dimensions, is a necessity for a clear understanding of these basic biochemical events. There were however, formidable obstacles to be overcome in the crystallization of the immensely complex entities involved in protein synthesis, based on direction encoded in the DNA template. Alternate approaches like NMR measurements, molecular modelling, analysis of the structure in parts,

photochemical cross linking, fluorescence energy transfer, etc. yielded a large volume of data on the structures of the nucleosome, RNA polymerase and ribosome; but these were almost like listening to cricket commentaries on the radio when you are deprived of a ticket at the venue (in pre-television days)! It is needless to say we have been all waiting for X-ray structures of these macromolecules and news (gossip?) arrived at regular intervals that things were happening!

But, what was the problem? The molecular complexity of the nucleosome, RNA polymerase and ribosomes is the reason for their obscurity! They have too many components within them; the nucleosome has a histone octamer bound to short DNA, RNA polymerase from bacteria have 5–6 subunits whereas the yeast enzyme has 10–12 subunits, and the ribosome from bacteria has 54 proteins with 3 RNA molecules. In addition, there is no apparent symmetry in these molecular assemblies, unlike viruses. Furthermore, the choice of the bacterium *E. coli*