Extending the Methodology of X-ray Crystallography to Non-Crystalline Specimens

Jianwei Miao*, Pambos Charalambous†, Janos Kirz* and David Sayre*

* *Department of Physics & Astronomy, State University of New York, Stony Brook, New York 11794-3800, USA*
† *Kings College, Strand, London WC2R 2LS, UK*

Since the emergence of powerful X-ray sources such as synchrotron radiation, the area of X-ray structure determination gradually evolved into two fields. The relatively small one is X-ray Microscopy which employs X-ray lenses such as zone plates, compound refractive lenses, curved mirrors and glass capillaries to focus X-rays to determine the structures of non-crystalline specimens. The resolution of X-ray Microscopy is limited by the resolution of the X-ray lenses and, for biological specimens, radiation damage. While the radiation damage problem can be alleviated somewhat by cooling the specimens down to liquid nitrogen temperature, the resolution of X-ray lenses is limited by fabrication difficulties. At present, the highest resolution achievable is around 30-50nm[1,2]. The other far more successful field is X-ray crystallography. By employing crystalline specimens, where constructive interference among the large number of identical unit cells generates strong Bragg peaks, X-ray crystallography can achieve atomic resolution without serious radiation damage to the specimens. The major difficulty of X-ray crystallography, however, is that the specimens should be crystalline, while most complex biological specimens, for example, can not be or are too big to be crystallized.

The idea of extending X-ray crystallography to imaging non-crystalline specimens, using a combination of X-ray Microscopy and X-ray Crystallography, was first proposed by Sayre in 1980[3]. However, during almost twenty years, the experimental progress of pursuing the idea was not successful due to two difficulties. First of all, when a specimen is non-crystalline, the diffraction pattern is weak and continuous, which makes the data acquisition challenging[4]. The other is the well-known phase problem. That the oversampling technique could be used to retrieve the phase problem in X-ray diffraction studies of non-crystalline specimens was made by Sayre[5]. By employing this phasing technique, we recently demonstrated for the first time that a soft X-ray diffraction pattern from a micron-size non-crystalline specimen can be recorded and inverted to form a high-resolution image[6,7]. With the experimental setup shown in Fig. 1, we recorded high quality diffraction patterns from non-crystalline specimens. By using a 10 μm pinhole and also placing the specimen only about 30 μm from the corner of the silicon nitride membrane, we obtained a clean diffraction pattern on the detector in three quadrants, free from background due to scattering from the pinhole. The detector was a back-thinned and liquid nitrogen cooled CCD with 512 x

CP521, *Synchrotron Radiation Instrumentation: Eleventh US National Conference,* edited by P. Pianetta, et al.

512 pixels and a 24 um x 24 um pixel size. In front of the CCD were a beam stop to block the intense direct beam and a photodiode to align the specimen. By using this chamber, we studied a few non-crystalline specimens. Fig. 2(a) shows a Scanning

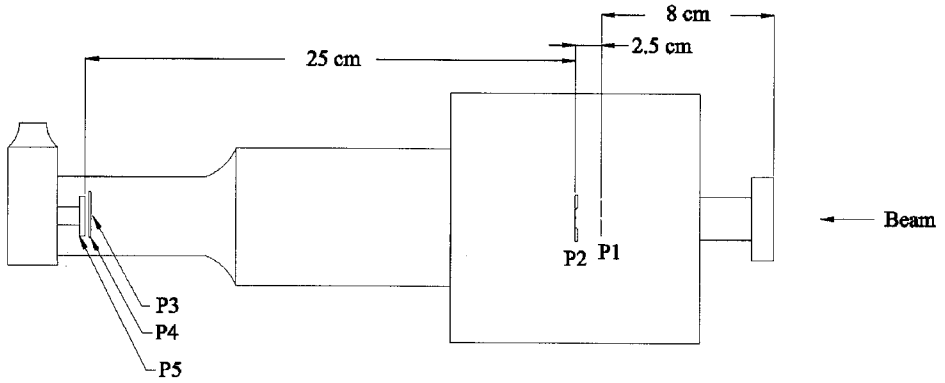
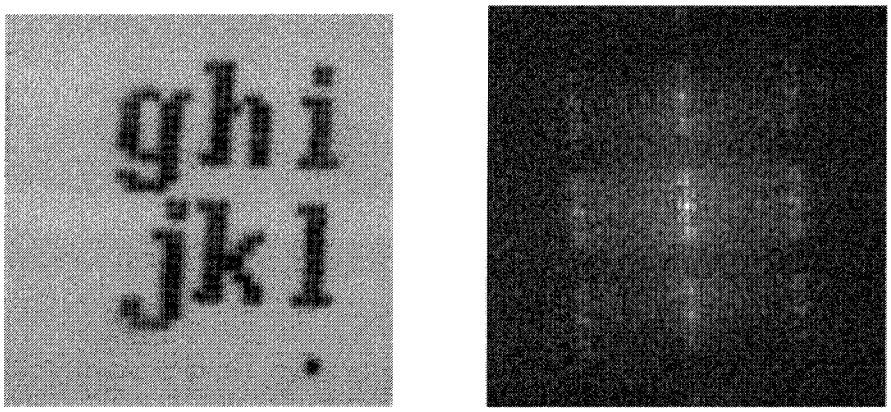


FIGURE 1. Schematic layout of the diffraction chamber. P1: Pinhole, P2: Specimen, P3: Central Stop, P4: Photodiode, and P5: CCD.



(a) **(b)**

FIGURE 2 (a) A STXM image of the specimen. **(b)** A diffraction pattern of the specimen (using a logarithmic scale).

Transmission X-ray Microscope (STXM)[8] image of one specimen. It was a collection of gold dots, each 100 nm in diameter and 80 nm thick, to form a set of six letters. Fig. 2(b) shows the experimental diffraction pattern of the specimen with λ = 1.7 nm, in which the fourth-quadrant data were obtained by using central symmetry. Since the central area of the X-ray diffraction pattern was blocked by the beam stop, we replaced the central area--a 19-pixel radius circle--by a patch consisting of the low-resolution part of the squared magnitude of the Fourier transform of Fig. 2(a). To invert the diffraction pattern to an image, one has to solve the phase problem. During the last few years, we gradually carried out the oversampling technique by developing a theory and

an iterative phasing algorithm[9,10]. By employing the iterative algorithm with constraints in both the real and reciprocal space, we are able to recover the phase information directly from the diffraction patterns. Fig. 3 shows an image reconstructed from Fig. 2 with 400 iterations. The resolution of Fig. 4 is ~ 60 nm which was set by the angular extent of the CCD detector. We believe that the successful recording and reconstruction of the non-crystalline specimens opens a door for high-resolution three

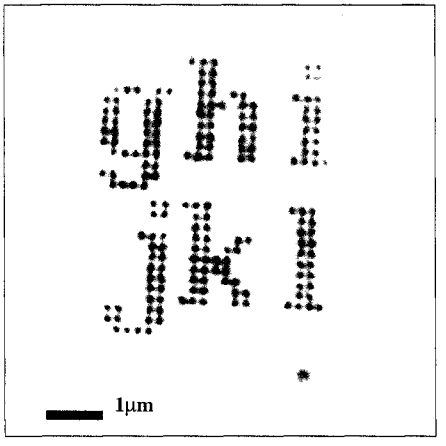


FIGURE 3. A high quality reconstruction from the diffraction pattern (after 400 iterations).

dimensional structure determination in both material science and structural biology. In the long run, with the more powerful, coherent and short-pulsed X-ray sources such as free electron lasers, we may have a chance to circumvent the radiation damage problem by recording the diffraciton patterns in a single pulse, and thus may be able to image biological specimens such as single molecules at very high resolution.

ACKNOWLEDGMENTS

The decision to try oversampling as a phasing technique was arrived at in a conversation in the late 1980s with G. Bricogne. W. Yun and H. N. Chapman also participated in early parts of this experiment. These contributions are gratefully acknowledged. We are grateful to C. Jacobsen for his help and advice especially with the numerical reconstruction. We thank him and M. Howells for use of the apparatus in which the exposures were made, and S. Wirick for her assistance in the data acquisition. P. Charalambous thanks the Leverhulme Trust Great Britain for supporting the nanofabrication program at King's College, London. This work was carried out at the National Synchrotron Light Source, which is supported by the Department of Energy. Our work is supported in part by grant # DE-FG02-89ER60858 from the Department of Energy.

REFERENCES

1. Jacobsen, C., Kirz, J., and Williams, S., *Ultramicroscopy* **47**, 55-79 (1992).
2. Meyer-Ilse, W., Warwick, T. and Attwood, D. (eds), *X-ray Microscopy,* Berkeley, California, 1999, to be published.
3. Sayre, D., "Prospects for long-wavelength x-ray microscopy and diffraction," in *Imaging Processes and Coherence in Physics*, edited by Schlenker, M. et al., Springer, Berlin, 1980, pp. 229-235.
4. Sayre, D., Chapman, H. N., and Miao, J., *Acta Cryst.* A **54**, 233-239 (1998).
5. Sayre, D. "Direct Methods of Solving Crystal Structures", in *NATO ASI Series B (Physics),* Vol. **274**, edited by Schenk, H., New York: Plenum, 1991, pp. 353-356.
6. Miao, J., Charalambous, C., Kirz, J., and Sayre, D., *Nature* 400, 342-344 (1999).
7. Miao, J., Charalambous, C., Kirz, J., and Sayre, D., "Extending the methodology of X-ray Crystallography to allow X-ray Microscopy without X-ray optics," in *X-ray Microscopy,* edited by Meyer-Ilse, W., Warwick, T. and Attwood, D., Berkeley, California, 1999, to be published.
8. Kirz, J., Jacobsen, C., and Howells, M., *Q. Rev. Biophys.* **28**, 33-130 (1995).
9. Miao, J., Sayre, D., and Chapman, H. N., *J. Opt. Soc. Am.* A **15**, 1662-1669 (1998).
10. Miao, J., and Sayre, D., "Solving the phase problem in perfect or imperfect small crystals and randomly oriented specimens by using the oversampling technique," in preparation.