Extending X-ray Crystallography to Non-Crystalline Specimens by the Oversampling Method

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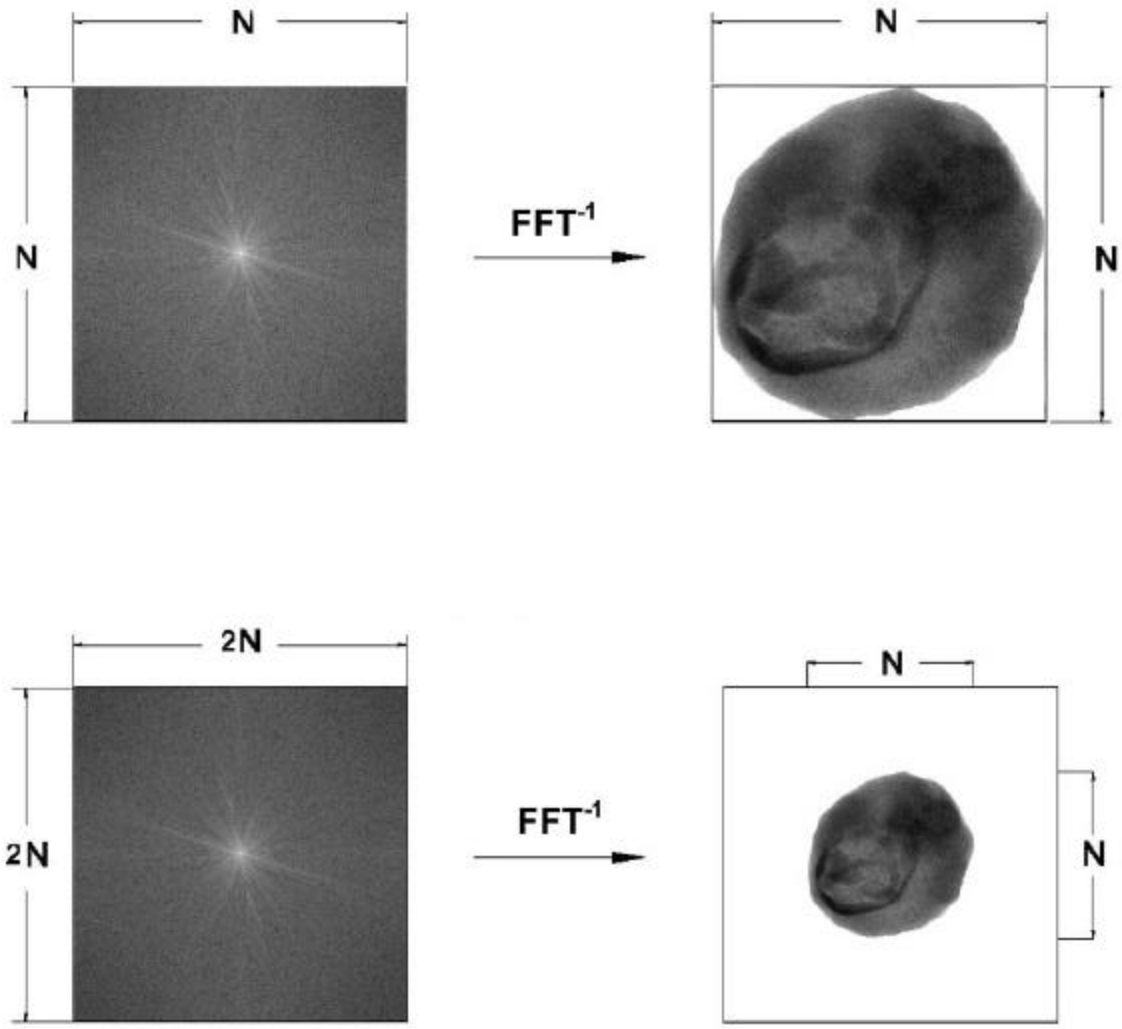
Oversampling Method

- **Sayre, D. (1991) *Note on 'superlarge' structures and their phase problem* in Direct Methods of Solving Crystal Structures, H. Schenk Eds. New York: Plenum, pp 353.**
- **R. H. T. Bates, *Optik(Stuttgart)* 61, 247 (1982).**
- **J. Miao, D. Sayre & H. N. Chapman, *J. Opt. Soc. Am. A* 15, 1662 (1998).**
- **J. Miao *et al*., *Nature (London)* 400, 342 (1999).**
- **J. Miao, J. Kirz & D. Sayre, *Acta Crystallogr.* D 56, 1312 (2000).**
- **J. Miao & D. Sayre, *Acta Crystallogr*. A 56, 596 (2000).**
- **J. Miao, K. O. Hodgson & D. Sayre, *PNAS*, in press (2001).**
- **J. A. Pitney, I. A. Vartaniants & I. K. Robinson, in *Proc. SPIE* 3815, pp. 199-207 (1999).**
- **A. Szöke, (1999) *Chem. Phys. Lett.* 313, 777-788.**

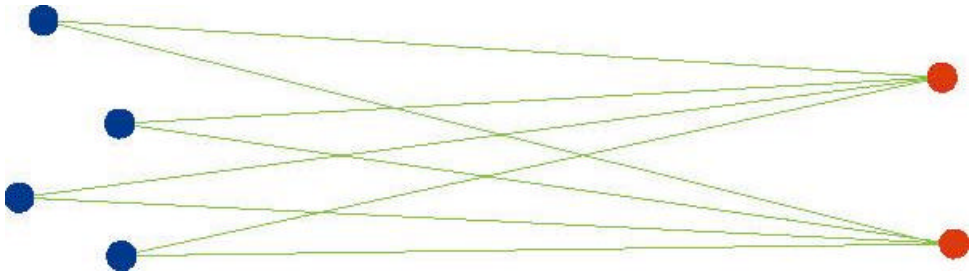
Reciprocal Space **Real Space**



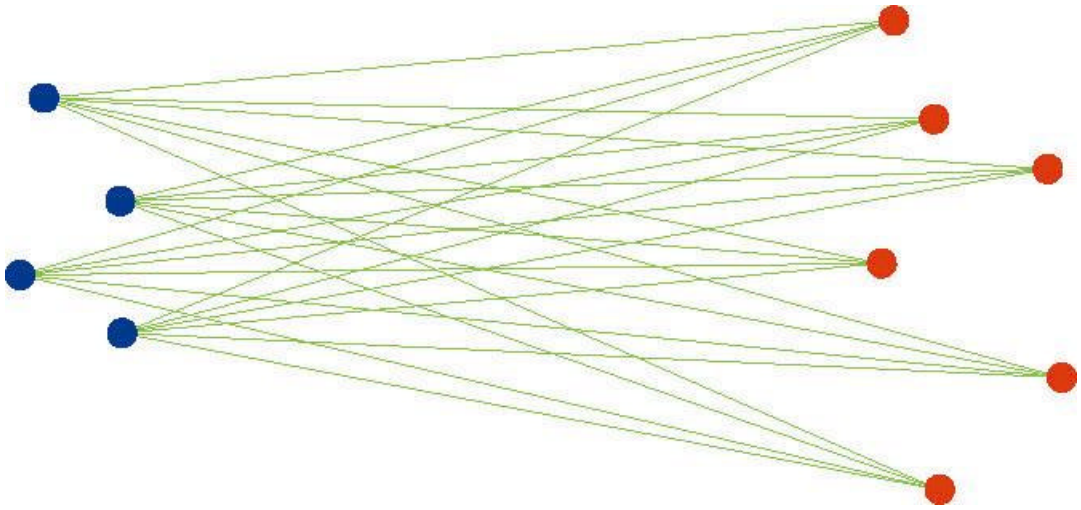
$$s = \frac{electron\ density\ region\ +\ \ no\ density\ region}{electron\ density\ region}$$

Mathematically treatment of Oversampling

Real Space **½ ½** **Reciprocal Space**

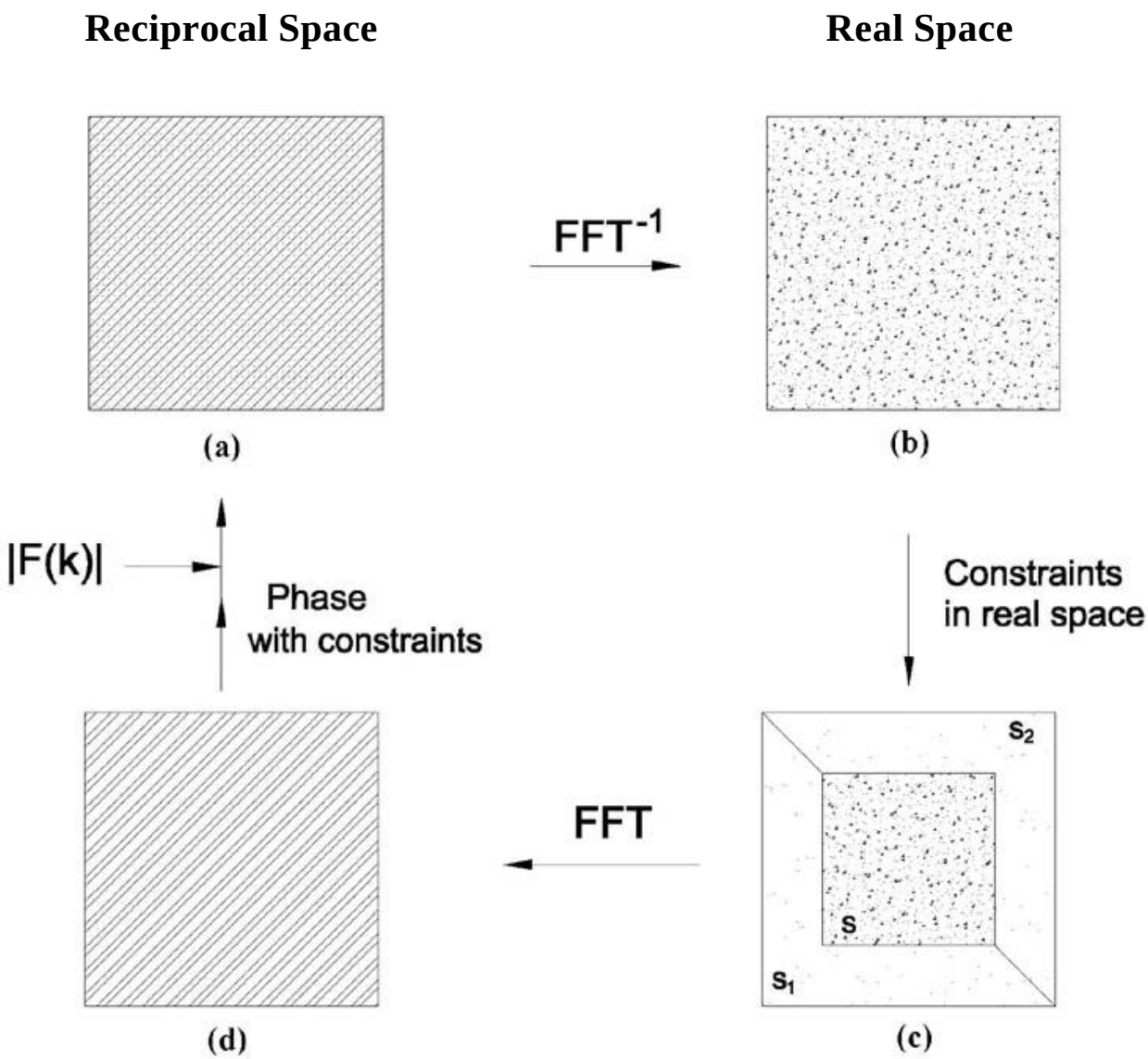


Bragg-peak sampling



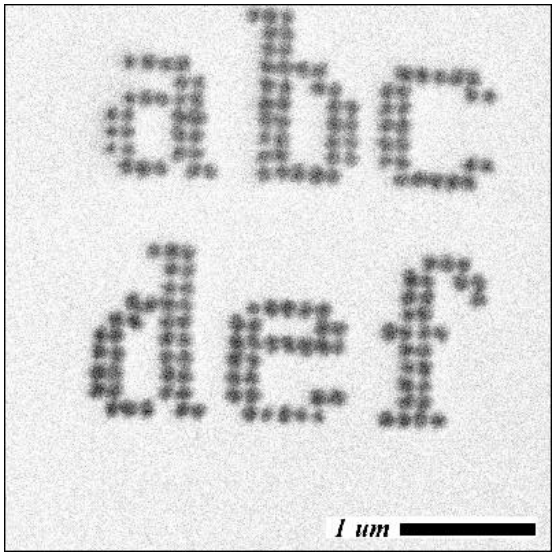
Oversampling

An Iterative Algorithm

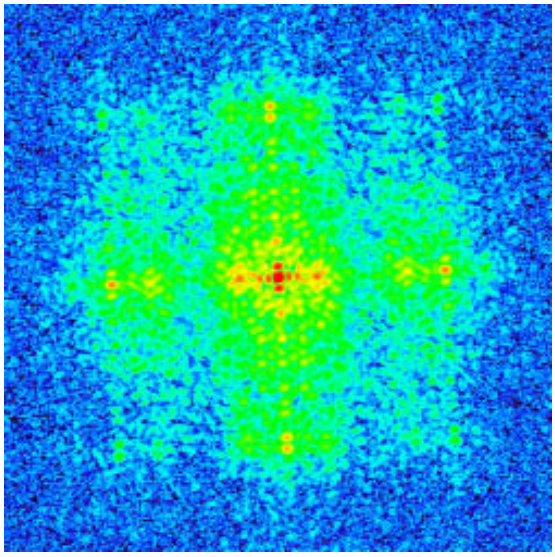


- R. W. Gerchberg & W. O. Saxton, *Optik* 35, 237 (1972).
- J. Fienup, *Appl. Opt.* 21, 2758 (1982).
- J. Miao, J. Kirz & D. Sayre, *Acta Crystallogr.* D 56, 1312 (2000).

A SEM Image



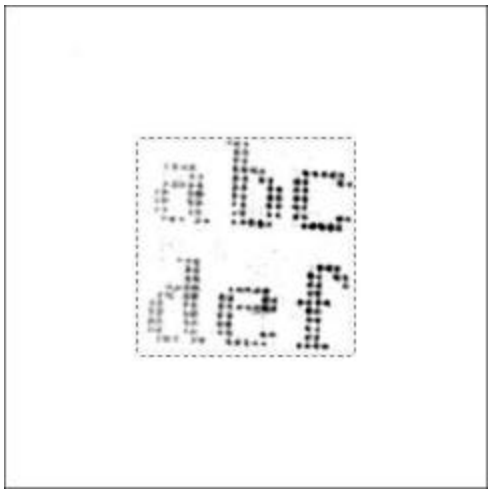
The Diffraction Pattern



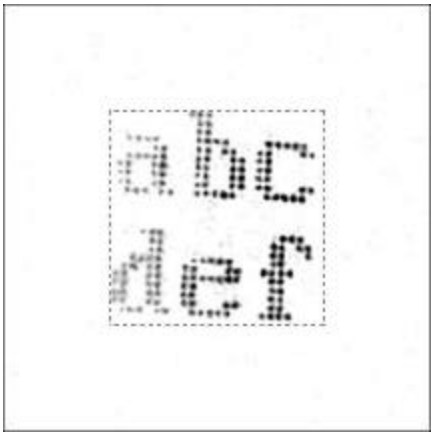
A Reconstructed Image
(after 400 iterations)

Studies of the phase retrieval as a function of s

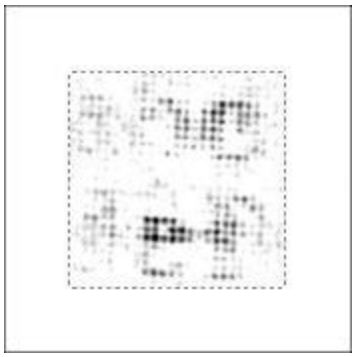
s = 5 (180 x 180 pixels)



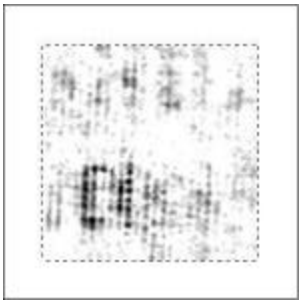
s = 4 (160 x 160 pixels)



s = 2.6 (130 x 130 pixels)

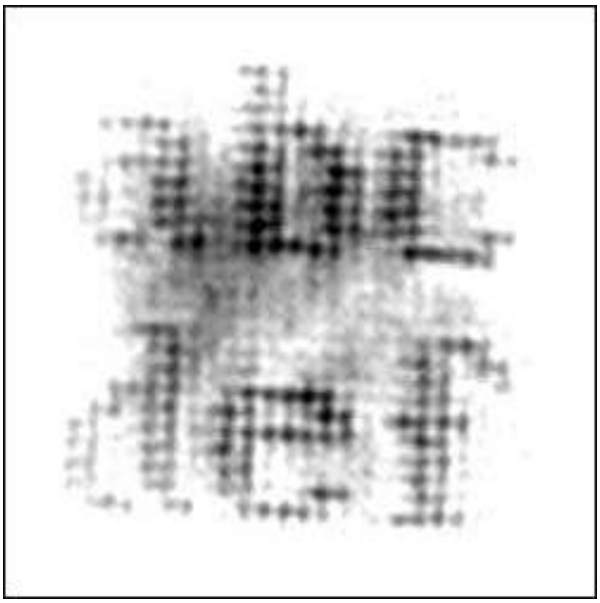


s = 1.9 (110 x 110 pixels)

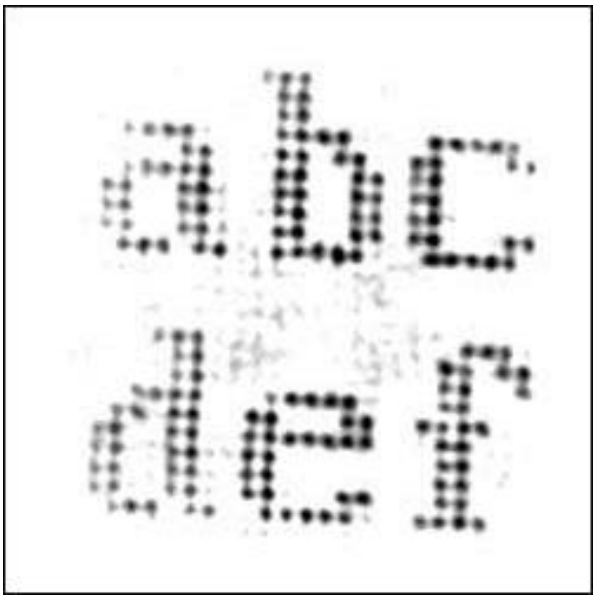


Phase retrieval with some intensity data missing at the center

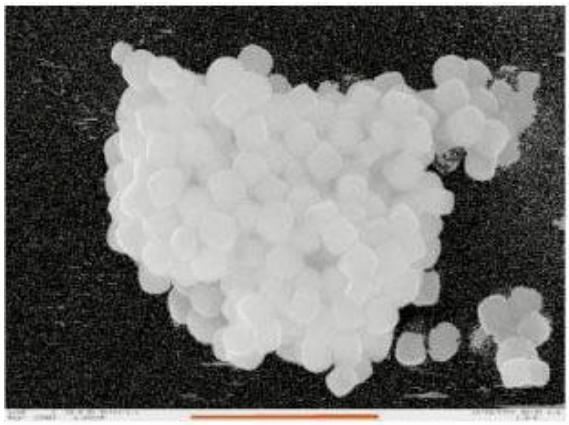
A reconstruction with a 15-pixel-radius circle missing data



A reconstruction with a 6-pixel-radius circle missing data



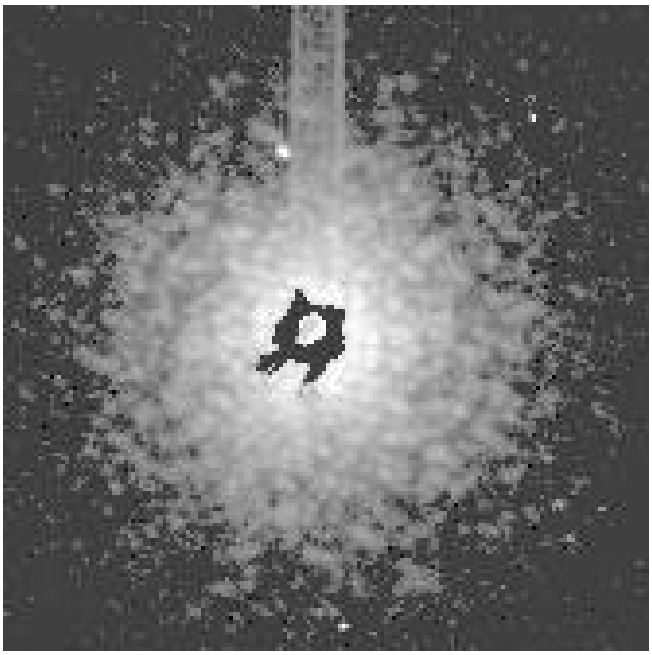
- **Imaging 3D porous nanostructures at nanometer resolution**



6.23 µm

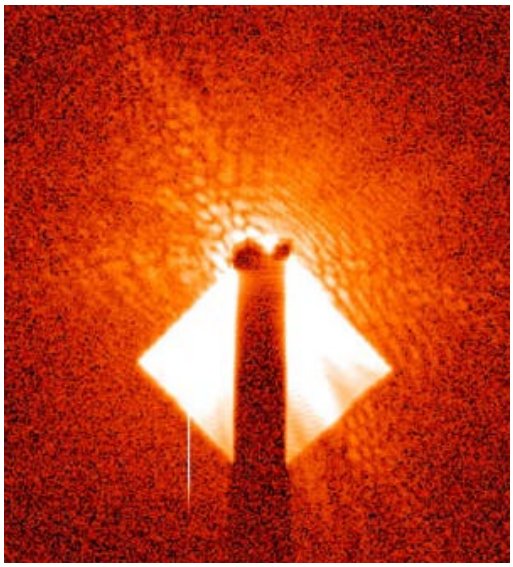
1.45 µm

SEM images of porous MoO_3 materials

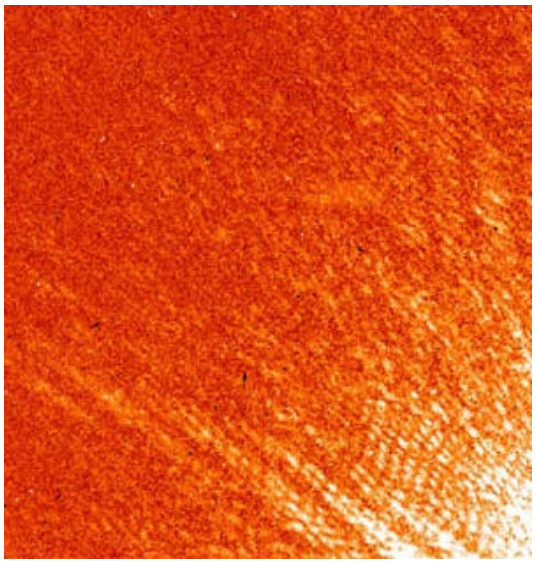


A coherent diffraction pattern from a MCM-48 porous particle (< 1x1x1 mm^3) with l = 1.5Å

- **Imaging large biological structures (whole cells, molecular clusters, *etc*.) at high resolution**



(a)



(b)

Coherent diffraction patterns from an air-dried yeast cell (~ 3 mm in diameter) with l = 1.3 nm. a, After 10 minute exposure b, Part of the speckle pattern with resolution up to 18 nm after 60-minute exposure.

D. Sayre, in *Imaging Processes and Coherence in Physics* (eds. M. Schlenker et al.) 229-235 (Springer, Berlin, 1980).

On the Possibility of Imaging Single Biomolecules

- **Short pulse X-FELs and the radiation damage problem**
- **Recording the diffraction patterns from a simulated series of X-FEL exposures of rubisco molecules**
- **Phasing the 3D diffraction pattern of the molecule**

• A simulated X-FEL

Wavelength: 1.5 Å

Pulse flux: 2 x 10^{12} photons

Focal spot: 0.1 mm (~ 2.55 x 10^{6} photons/ $Å^2$)

Pulse duration: 10 fs

Pulse repetition rate: 120 Hz

• Radiation damage

Solem & Baldwin, *Science* 218, 229-235 (1982).

⇒ **With picosecond pulse duration X-rays, biological specimens remain morphological unchanged to an accuracy of a few nm.**

Neutze et al., *Nature* 400, 752-757 (2000).

⇒ **With an X-FEL of pulse leng. < 50 fs and 3 x 10^{12} photons focused down to a spot of ~ 0.1 mm, a 2D diffraction pattern could be recorded from a biomolecule before the radiation damage manifests itself.**

• Sample handling

Using spray techniques, *i.e.* mass spectrometers to insert molecules one-by-one in random orientation into the X-ray beam.

M. A. Tito *et al*., *J. Am. Chem. Soc*. 122, 3550 (2000).

• Orientation determination

Use the methods developed in cryo-EM to determine the molecule orientation based on many 2D diffraction patterns.

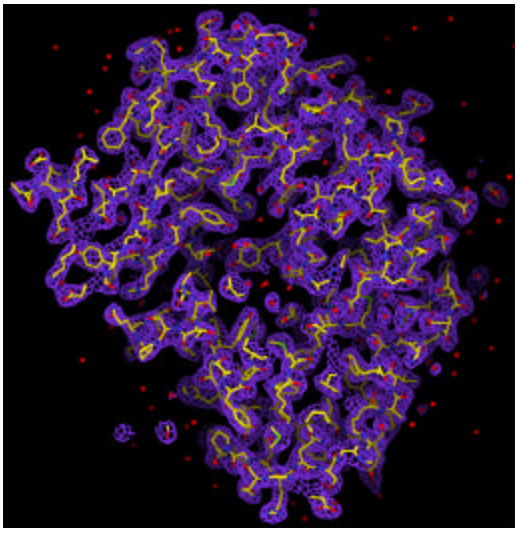
Crowther, *Phil. Trans. Roy. Soc. Lond*. B. 261, 221 (1971).

J. Frank, in *Three-Dimensional Electron Microscopy of Macromolecular Assemblies*, Academic Press (1996).

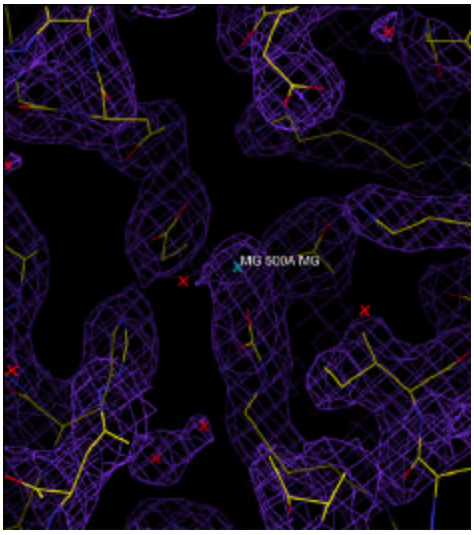
Use laser fields to physically align each molecule.

J. J. Larsen, K. N. Hald, Bjerre, H. Stapelfeldt & T. Seideman, *Phys. Rev. Lett*. 85, 2470-2473 (2000).

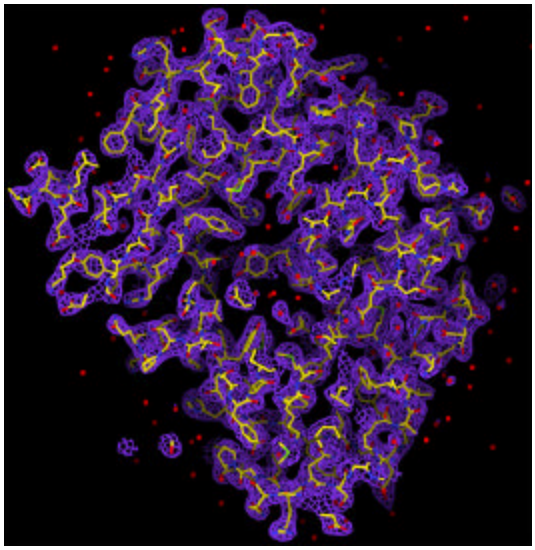
The 3D electron density map of the rubisco molecule



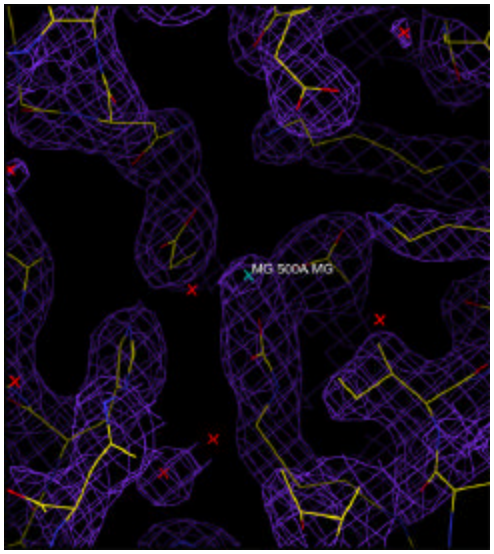
The active site of the molecule



The reconstructed 3D electron density map

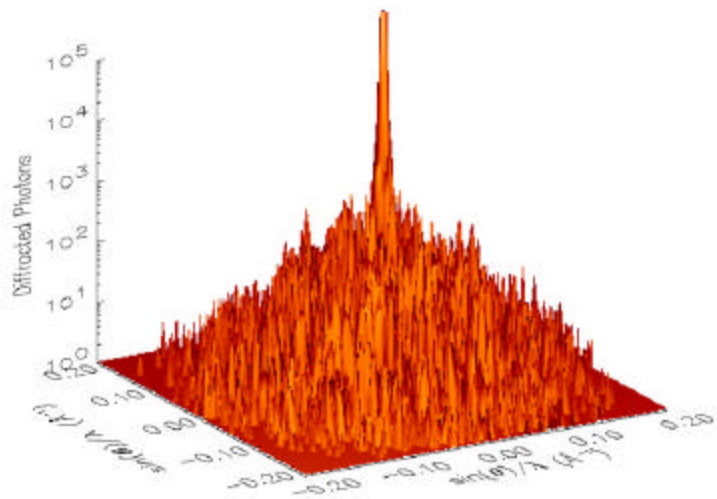


The reconstructed active site

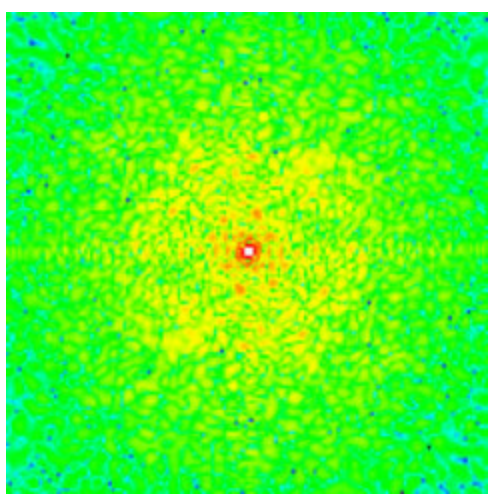


Poisson noise:

$$R_I = \frac{\sum_{k_x,k_y,k_z} \left| I_{calculated}(k_x,k_y,k_z) - I_{noisy}(k_x,k_y,k_z) \right|}{\sum_{k_x,k_y,k_z} \left| I_{calculated}(k_x,k_y,k_z) \right|}$$

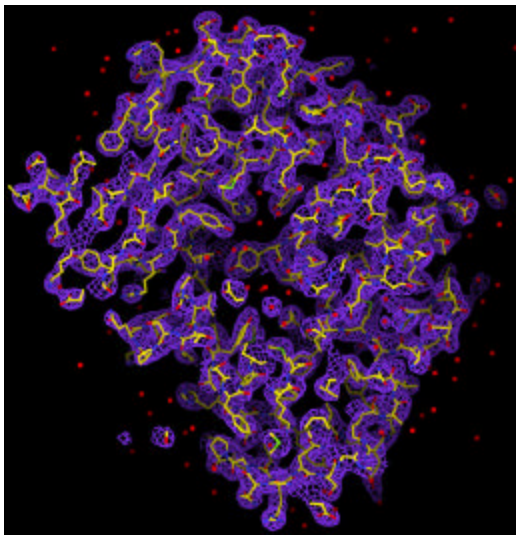


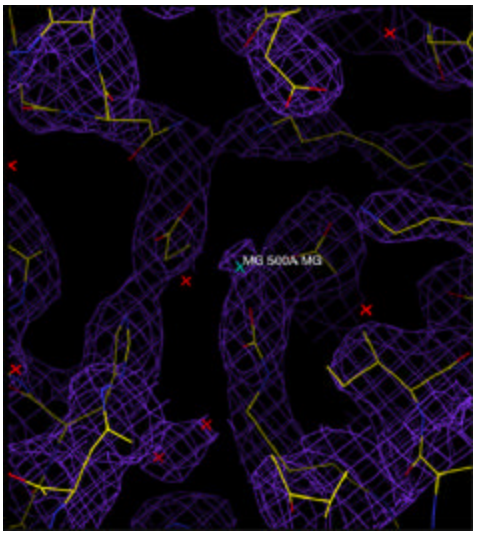
(a) Obtained from 10^6 identical rubisco molecules with R_I = 9.8% and 3x3x3 central pixels removed



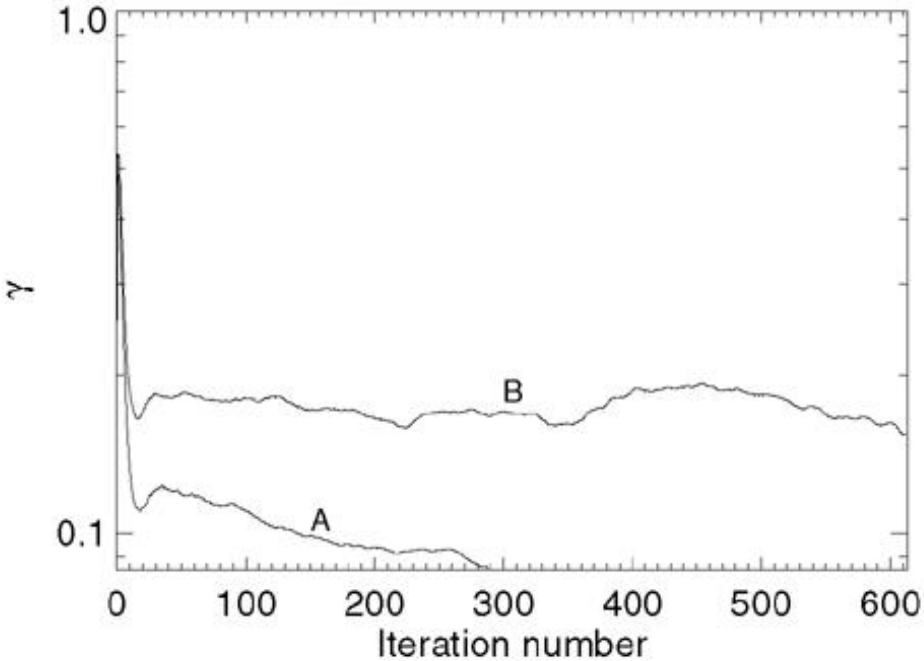
(b) Top view of (a)

Reconstruction from the 3D diffraction pattern of 3 x 10^5 identical molecules with R_I = 16.6% and 3 x 3 x 3 central pixels removed.





The convergence of the reconstructions



Summary

- **When a diffraction pattern is sampled at a spacing finer than the Bragg-peak frequency ($s > 2$), the phases are in principle uniquely embedded inside the diffraction pattern and can be directly retrieved by using an iterative algorithm.**

- **Experimentally demonstrated the phases can be directly retrieved from a coherent diffraction pattern of a micron-sized non-crystalline specimen.**

- **With 3rd and 4th generation coherent X-ray sources, this methodology could provide a major new opportunity for the high-resolution three-dimensional structure determination of materials science nanostructures, large biological structures, and even single biomolecules.**