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*H.N. Chapman, S.P. Hau-Riege, R.A. London,
S. Marchesini, A. Noy, A. Szoke, H. Szoke,
E. Ingerman, J. Hajdu, G. Huldt, M.R. Howells,
H. He, J.C.H. Spence, U. Weierstall*

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Prospects for single-particle imaging at XFELs

H.N. Chapman¹, S.P. Hau-Riege¹, R.A. London¹, S. Marchesini^{1,2}, A. Noy¹, A. Szoke¹, H. Szoke¹, E. Ingerman², J. Hajdu³, G. Huldt³, M.R. Howells⁴, H. He⁴, J.C.H. Spence⁵ and U. Weierstall⁵.

¹Lawrence Livermore National Laboratory, 7000 East Ave, Livermore, CA 94550, USA

²Center for Biophotonics Science and Technology, LLNL, 7000 East Ave, Livermore, CA 94550, USA

³Biomedical Center, Uppsala University, Box 596, SE 751 24, Uppsala, Sweden

⁴Advanced Light Source, Lawrence Berkeley Lab, 1 Cyclotron Rd., Berkeley, CA 94720 USA

⁵Department of Physics and Astronomy, Arizona State University, Tempe AZ 85287-1504 USA

Abstract—X-ray free-electron lasers will produce pulses of x-rays that are 10 orders of magnitude brighter than today's undulator sources at synchrotrons. This may enable atomic resolution imaging of single macromolecules.

I. INTRODUCTION

A new class of x-ray source, called the x-ray free-electron laser (XFEL), is being developed that will produce ultra-short pulsed x-ray beams at 10 orders-of-magnitude higher peak brightness than modern third-generation synchrotron sources. The first XFEL to be built will be the Linac Coherent Light Source (LCLS) [1]. This source will deliver 230-fs duration pulses, with photon energies tunable between 0.8 and 8 keV and $>10^{12}$ photons per pulse. One of the envisioned experiments is atomic resolution imaging of single macromolecules, single virus particles, or nanocrystals [2-4]. Since atomic-resolution lenses for x-rays do not exist, the imaging will be lensless, whereby an image is reconstructed, using phase-retrieval techniques, from the measured continuous diffraction pattern of the sample. The ultra-short pulses of x-rays from the XFEL allow the resolution limit due to radiation damage to be overcome, by delivering the dose in a timescale that is shorter than that for structural change. A complete three-dimensional atomic-resolution structure determination requires many diffraction patterns made at different particle orientations. These will be obtained by injecting identical single particles into the beam and recording one pattern per pulse.

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II. COHERENT X-RAY DIFFRACTION IMAGING

The methods of reconstructing a 3D image from a large number of noisy diffraction patterns of random and unknown orientation are inspired by methods employed in single-particle cryo-electron microscopy [5]. Diffraction patterns are first classified into classes of like-orientation so that they can be averaged to increase the signal relative to noise [6]. The average signal per diffraction pattern at the highest resolution, required for classification, is found to be much less than one photon per pixel, and an incident fluence of 10^8 ph/nm² is sufficient to achieve atomic resolution for particles greater than 15 nm radius [6]. Averaged diffraction patterns must be oriented with respect to each other in 3D Fourier space, which may be achieved by the intersection of common lines. Each diffraction pattern maps onto an Ewald sphere in Fourier space which passes through the zero-frequency origin; two spheres will intersect on an arc, and the intersecting arcs between three spheres of independent orientation will fix the relative orientation of each.

The 3D diffraction transform of a non-periodic particle is continuous. Only the diffraction amplitudes are sampled at discrete points by the pixellated detector and the process of classification. To obtain the image by Fourier inverting the data requires the knowledge of the phases. For objects that have a finite size, or “support,” the Fourier phases can be obtained if the sampling is sufficiently fine—a concept referred to as oversampling [4]. We have applied two classes of algorithms to the phasing of 2D and 3D data. SHRINKWRAP [7] is a modification of iterative transform algorithms [8], in which the known constraints are iteratively applied in real and Fourier space (e.g. the modulus of the diffraction pattern in Fourier space; finite support in real space). In SHRINKWRAP, an initial poor estimate of the support is derived by thresholding the autocorrelation of the image (the Fourier transform

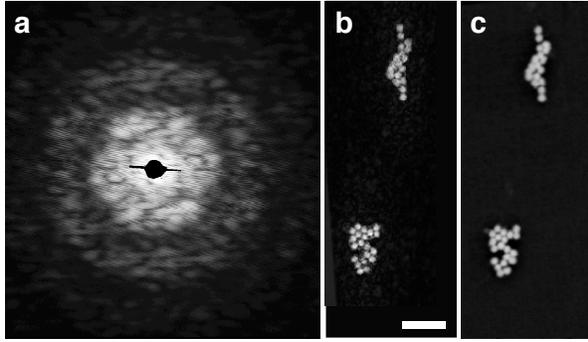


Fig. 1. (a) Coherent diffraction pattern recorded with a wavelength of 2.1 nm. (b) Image reconstructed from (a) using Shrinkwrap. The unrecorded intensities behind the central beamstop of (a) were unconstrained in the reconstruction. (c) SEM image of the object, which consists of 50-nm diameter gold spheres on a silicon nitride membrane. The scalebar in (b) is 300 nm. See [7] for more details.

of the diffraction intensities). From an initial trial of random phases we run about 20 iterations of a transform algorithm using this initial support constraint. The subsequent image is blurred and thresholded and used to improve the estimate of the support, and the process repeated. After about 100 cycles the support has usually shrunk around the outline of the object, giving a very good estimate of the image. Thus SHINKWRAP reconstructs the image *ab initio* without supplying any *a priori* knowledge. An example of a reconstructed image is shown in Fig. 1.

Another program that we have used to successfully phase experimental data is SPE DEN [9]. This uses a constrained conjugate gradient solver to find the amplitudes of 3D gaussian blobs whose calculated diffraction intensities match the measurements while also minimizing cost functions based on constraints (including a low-resolution target, 2D projections or known phases). The algorithm finds the optimal image that fits all the constraints and, since it only ever performs calculations from real to Fourier space, it never needs to interpolate data onto a regular grid. However, as a local optimizer, it does not have as large a volume of convergence as the iterative transform algorithms and we expect to use it in XFEL imaging as a way to refine images produced by SHRINKWRAP and avoid artifacts due to missing data.

III. DYNAMICS OF THE DAMAGE PROCESS

Studies of the dynamics of small particles irradiated by intense XFEL pulses were first performed by Neutze *et al.* [2] using molecular dynamics calculations. In these calculations, atoms are ionized by the photoelectric effect, and the photoelectrons are assumed to go to infinity, leaving the particle charged. As the charge builds up the particle explodes due to Coulomb repulsion. We have performed calculations using a hydrodynamic model [10] that includes the trapping of

photoelectrons, which the Neutze work omitted. We find that the Auger electrons start becoming trapped after about <1 to 2 fs. Also the photoelectrons become trapped after about 10 fs if the particle is large (~ 100 Å diameter). Since trapped electrons lead to further unbound electrons through collisional ionization, these cascades quickly dominate the damage process. It is also found that the trapped electrons quickly relax in energy and position to form a cloud around the positive ions, leaving a neutral core and a positively charged outer shell (similar to Debye shielding). The ion motion therefore peels off from the outer shell. In the inner core there is hardly any ion motion but the high electron temperature leads to a great amount of ionization and blurring of the electron density. It is this latter effect that requires pulse lengths of 10 fs or less to overcome damage with pulse fluences greater than 10^4 ph/nm².

IV. PROSPECTS FOR ATOMIC RESOLUTION IMAGING

There are many challenges that need to be solved to perform single-particle diffraction imaging at XFELs. Samples must be purified and injected into the beam, one particle at a time. Techniques from electrospray mass spectrometry and laser cooling may be employed. Calculations from our model show that the initial 230 fs pulses of LCLS could be used to achieve atomic resolution if the pulse fluence was decreased by 10 to 100 times. For a single particle this does not give enough diffracted signal to classify the pattern. However, if the orientation of the particle could be fixed (e.g. by a polarized laser) then patterns can be averaged. Alternatively, the particles could be engineered to give stronger diffraction signals. Nanocrystals of only $3 \times 3 \times 3$ unit cells will diffract quasi-Bragg peaks that are almost 1000 times stronger than the intensities of a single unit cell, enabling classification (see [4]). Longer pulse durations at high fluence may be acceptable if it were possible to reconstruct atomic positions from partially ionized atoms. As yet, none of the damage models have been validated, since no source yet is available. The real potential for XFEL single-particle imaging will not be known until these new sources become operational.

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