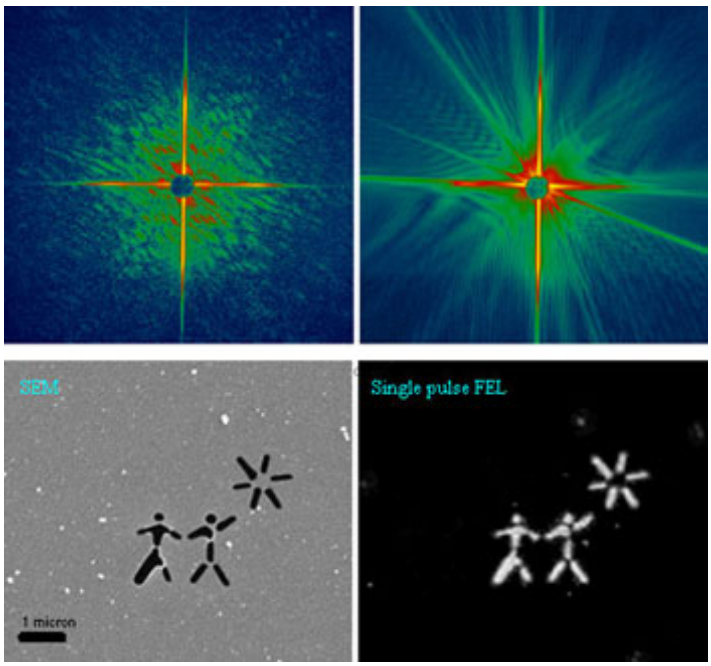
Femtosecond Diffractive Imaging with a Soft-X-ray Free-Electron Laser

We have demonstrated flash diffractive imaging of nanostructures using pulses from the first soft-X-ray free-electron laser in the world, FLASH. This is the first step in the development of single-molecule diffractive imaging (Neutze *et al.*, Nature, **406**, pp. 752-757, 2000), where atomic-resolution images of macromolecules will be obtained, without the need for crystallization of the molecules.

As reported in the December issue of Nature Physics, we used an intense, ultrafast pulse from the FLASH soft-X-ray free-electron laser to record a coherent X-ray diffraction pattern from a nanoscale object. We inverted this pattern to form a high-resolution image of the object, without the need for any lenses, using the Shrinkwrap algorithm (Marchesini *et al.*, Phys Rev B, **68**, 140101(R) 2003). The flash image resolves 50 nm features, was recorded in 25 fs, and is the fastest image ever recorded with sub-optical resolution. The 25 fs pulse contained about 10^{12} photons and was focused down onto the sample to give an intensity of 4×10^{13} W/cm^2, at a wavelength of 32 nm. The coherent diffraction pattern was obtained before the sample exploded at a temperature of 60,000 K. No evidence of sample damage could be seen in the reconstructed image.



Top left: A diffraction pattern recorded with a single FLASH FEL pulse from a test object placed in the focused beam. **Top right**: The diffraction pattern recorded with the second pulse, showing diffraction from the hole in the sample created by the first pulse. The sample was a pattern milled from a 20-nm thick silicon nitride membrane, shown **Bottom left**. **Bottom right**: The image reconstructed from the single-shot diffraction pattern using our "Shrinkwrap" phase retrieval algorithm. The algorithm only used the measured diffraction intensities and the knowledge that the diffraction pattern was oversampled. We did not use the SEM image in the reconstruction.

We measured the diffraction pattern with a sensitivity of single photon detection, even though the sample radiates after explosion and the intense pulse vaporizes anything else in its path. This was achieved with a novel diffraction camera consisting of a graded multilayer mirror which varies in period by a factor of two across its face. The direct beam passed through a hole in the mirror, while the coherent scattering from the sample was reflected by the mirror onto a CCD. The mirror efficiently filtered out radiation of other wavelengths and radiation travelling in the wrong direction (i.e. not emanating from the sample).

Today, the bottleneck in the atomic resolution imaging of large macromolecules and macromolecular complexes is a fundamental need for crystals. This limits the scope of detailed structural analysis to molecules and assemblies which can be crystallized. Many biologically important target complexes are difficult or impossible to crystallize. There is therefore a great need to develop new structural determination methods.

It was suggested by Neutze *et al*. (Nature, **406**, 752-757, 2000) that ultrafast pulses from a free-electron laser could be intense enough to give a measurable diffraction pattern from a single uncrystallized macromolecule. The incident X-ray dose would exceed the usual tolerance of biological materials to keep their structural integrity, by five orders of magnitude. In fact, the interaction with the pulse would be so violent that the molecule would completely vaporize. However, due to the inertia of the atoms, this damage process would not start until after the pulse had carried away the information of the undamaged molecule.

To build up a three-dimensional image requires many coherent diffraction patterns from many, identical, macromolecules. There are many challenges yet to be addressed to carry out single-molecule imaging, such as delivering the samples, orienting the diffraction data (or the molecules) and combining the data. However, this current work gives great hope to the method, as it was demonstrated that it is possible to record a diffraction pattern, with single photon sensitivity, even when the illuminating pulse destroys everything in its path, and turns the sample into a radiating plasma. The image was reconstructed at the highest resolution consistent with the illumination and collection aperture of the detector.

The experiments were carried out by a collaborative team from Lawrence Livermore National Laboratory, the NSF Center for Biophotonics Science and Technology, Stanford Synchrotron Radiation Laboratory, Uppsala University, the Deutsches Elektronen-Synchroton (DESY), and Technische Universität Berlin. The FLASH FEL began operations at DESY in August, 2005.

Primary Citation

H. N. Chapman, A. Barty, M. J. Bogan, S. Boutel, M. Frank, S. P. Hau-Reige, S. Marchesini, B. W. Woods, S. Bajt, W. Henry. Benner, R. A. London, E. Plönjes, M. Kuhlmann, R. Treusch, S. Düsterer, T. Tschentscher, J. R. Schneider, E. Spiller, T. Möller, C. Bostedt, M. Hoener, D. A. Shapiro, K. O. Hodgson, D. van der Spoel, F. Burmeister, M. Bergh, C. Caleman, G. Huldt, M. Seibert, F. R. N. C. Maia, R. W. Lee, A. Szöke, N. Timneanu and J. Hajdu, "Femtosecond Diffractive Imaging with a Soft-X-ray Free-Electron Laser", *Nat. Phys.* Published online: 12 November 2006 | doi:10.1038/nphys461