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Visualization of Cells and Cell Organelles Using Coherent X-Ray Diffraction. Yoshinori Nishino[a], Kazuhiro Maeshima[b], Naoko Imamoto[b], Ryuta Hirohata[c], Eiichiro Matsubara[c], Yukio Takahashi[d], Jianwei Miao[e], Tetsuya Ishikawa[a]. [a]*RIKEN SPring-8 Center.* [b]*Cellular Dynamics Laboratory, RIKEN.* [c]*Department of Materials Science and Engineering, Kyoto University.* [d]*Graduate School of Engineering, Osaka University.* [e]*Department of Physics and Astronomy, University of California, Los Angeles.*
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We report our studies on X-ray diffraction microscopy (XDM) for biological samples. XDM requires no lenses, and a sample image is directly reconstructed from the coherent diffraction data. It is ideal X-ray phase-contrast imaging with no contrast degradation due to lenses. XDM is especially useful for observing internal structures of thick objects, such as cells or cell organelles, thanks to high penetrating power of X-rays. The first biological application of XDM was for *Escherichia coli* bacteria cells [1]. The internal distribution of the proteins stained with manganese oxide was two-dimensionally imaged. In the image reconstruction, the Fourier transform of a lower-resolution sample image observed under a full-field soft X-ray transmission microscope was used to make up for missing coherent X-ray diffraction data near-forward direction. The missing data problem was later solved by improvements of microscope instruments to reduce parasitic scattering noise [2,3] and also by improvements of the phase retrieval algorithm, e.g. the iterative normalization algorithm [4]. These improvements have made it possible to reconstruct a sample image only from the coherent diffraction data. Challenges remaining in biological application of XDM have been imaging of unstained samples and three-dimensional imaging. The both have been recently realized by observing an unstained human chromosome in three dimensions [5]. This is the first successful three-dimensional electron density mapping of a cell organelle by using hard X-rays. It is important because XDM is directly connected with X-ray crystallography, which is currently the most powerful method of the atomic structure analysis for proteins. The observed images reveal an axial structure with high electron-density in the chromosome, which other microscopic methods have been unable to visualize under unstained condition. Such mesoscopic-scale structures as cell organelle have been difficult to be observed, although molecular structures of their components like DNA and histone proteins could be studied by X-ray crystallography or electron microscopy. Our result experimentally demonstrates the high imaging ability of coherent X-ray diffraction for unstained biological specimens, which are almost transparent to X-rays, opening a novel and strong means of exploring cellular structures.

[1] Miao J., Hodgson K.O., Ishikawa T., Larabell C.A., LeGros M.A., Nishino, Y., *Proc. Natl. Acad. Sci. USA*, **2003**, 100, 110. [2] Nishino Y. *et al.*, *IPAP Conference Series*, *Proceeding of 8th International Conference on X-Ray Microscopy*, **2006**, 7, 386. [3] Nishino Y. *et al.*, *IPAP Conference Series, Proceeding of 9th International Conference on X-Ray Microscopy*, to be appeared. [4] Nishino Y., Miao J., Ishikawa T., Phys. Rev. B, **2003**, 68, 220101. [5] Nishino Y., Takahashi Y., Imamoto N., Ishikawa T., Maeshima K., *Phys. Rev. Lett.*, **2009**, 102, 018101.

Keywords: diffraction imaging of noncrystalline specimens; X-ray microtomography; chromosome structure

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A Reconstruction Algorithm for Single-Particle Diffraction Imaging Experiments. Duane Ne-Te Loh[a], Veit Elser[a]. [a]*Laboratory of Atomic and Solid State Physics, Cornell University.*
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We introduce the EMC algorithm for reconstructing a particle's 3D diffraction intensity from very many photon shot-noise limited 2D measurements, when the particle orientation in each measurement is unknown. The algorithm combines a maximization step (M) of the intensity's likelihood function, with expansion (E) and compression (C) steps that map the 3D intensity model to a redundant tomographic representation and back again. After a few iterations of the EMC update rule, the reconstructed intensity is given to the difference-map algorithm for reconstruction of the particle contrast. We demonstrate reconstructions with simulated data and investigate the effects of particle complexity, number of measurements, and the number of photons per measurement. The relatively transparent scaling behavior of our algorithm provides a first estimate of the data processing resources required for future single-particle imaging experiments.

[1] Duane Ne-Te Loh, Veit Elser, *Preprint arXiv*, arxiv: 0904.25.81.

Keywords: diffraction imaging of non-crystalline specimens; FEL free electron lasers; phase reconstruction

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Lensless Imaging of Biological Samples with Soft X-Rays. Axel Rosenhahn[a], Ruth Barth[a], Florian Staier[a], Thomas Gorniak[a], Christoph Christoffis[a], Michael Grunze[a]. [a]*Applied Physical Chemistry, University of Heidelberg, Germany.*
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With the newly constructed free electron lasers, powerful x-ray sources which provide only femtosecond pulse length and high peak intensities become available. To fully exploit the great potential these new sources offer, coherent imaging techniques are desired. Digital in-line soft X-ray holography (DIXH) is such a lenseless microscopy technique which we use to investigate biological samples. The experimental setup follows directly the initial idea of Gabor to achieve a magnification of small objects via a holographic projection microscope based on a strongly divergent photon beam.

By creating a large opening angle and the use of short wavelength, a lateral resolution better than 250 nm can be achieved without using any optical elements. To provide a stable and drift free scattering geometry, the new scattering chamber HORST was constructed. This setup can be used for holographic imaging and diffraction microscopy at synchrotron sources and free electron lasers. By tuning the x-ray energy to core resonances, element specific contrast can be obtained. Applications in the field of life sciences and biofouling will be discussed and results obtained at synchrotrons and the free electron laser FLASH will be shown.

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Highly Sensitive Quantitative Biological Imaging by Scanning X-ray Diffraction Microscopy. Klaus Giewekemeyer[a], Sebastian Kalbfleisch[a], André Beerlink[a], Cameron Kewish[b], Pierre Thibault[b], Franz Pfeiffer[c], Tim Salditt[a]. *[a]Institute for X-ray Physics, Georg-August-University, Goettingen, Germany. [b]Paul-Scherrer-Institute, Villigen PSI, Switzerland. [c]Department of Physics (E17), Technical University of Munich, Munich, Germany.*
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Ptychographic diffractive imaging has proved itself a very powerful tool in coherent X-ray imaging as it enables observations with an unlimited field of view and is characterized by fast convergence and a low degree of ambiguity in the reconstruction [1]. Recently it was shown that a pre-knowledge of the complex illuminating wavefield in the sample plane is not necessary any more, but can self-consistently be recovered together with the object transmission function from the same dataset [2]. In this contribution we will report on one of the first applications of this new technique to unstained biological specimens, namely freeze-dried cells of the procaryotic bacterium *Deinococcus radiodurans*, which have been imaged at the cSAXS beamline of the Swiss Light Source using a pinhole (diameter 1.4 microns) as the beam defining optical element at a photon energy of 6.2 keV. We will show how quantitive phase information with very high sensitivity can be extracted from a single dataset taken within 20 minutes at an incident photon flux on the sample of ca. 2e5 photons per second, enabling us to image internal structural features of the cells.

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Keywords: coherent x-ray imaging; phase determination methods; biological structure