

KOTOBUKI-1 apparatus for cryogenic coherent X-ray diffraction imaging

Coherent X-ray diffraction imaging (CXDI) is a technique to visualize three-dimensional structures of non-crystalline particles with micrometer to sub-micrometer dimensions. In CXDI experiment, an isolated particle is irradiated by X-rays with a high transverse coherence, and then the Fraunhofer diffraction pattern is recorded so that the number of pixels in the detector is more than the twice that in the electron density map of the particle at a desired resolution (oversampling). The electron density map is, in principle, reconstructed by applying the phase-retrieval algorithm to the oversampled diffraction pattern. The weak electromagnetic interaction of hard X-rays with atoms allows the long penetration depth to visualize thick specimens, which are too opaque for electron microscopy without multiple scattering.

One application of CXDI is the structure analyses of cellular components, such as organelles and whole cells, which have never been crystallized. Biological specimens should be kept in a fully hydrated state to visualize their functional structures. Cryo-cooling of specimens used in electron microscopy and protein crystallography avoid degradation in a vacuum environment, which is necessary to reduce background scattering, and radiation damage in synchrotron experiments. Diffraction data collection at liquid nitrogen temperatures dramatically reduces radiation damage of specimens. In addition, flash-cooling allows the functional structures of specimens to be observed even under a vacuum [1,2].

We developed a diffraction apparatus named KOTOBUKI-1 (Fig. 1) for cryogenic CXDI experiments of frozen-hydrated non-crystalline particles at SPring-8 and SACLA [1]. The apparatus is composed of three major components: a vacuum chamber equipped with a cryogenic pot connected to a goniometer, a loading device to transfer frozen-hydrated specimens to the pot, and an alignment table to mount the chamber and the loading device. The cryogenic pot can be operated at 66 K through the evaporation cooling effect of liquid nitrogen, suppressing the positional fluctuations of specimens to less than 0.4 μm . A set of loading devices enables the specimen holders stored in liquid nitrogen to be mounted onto the specimen stage in the vacuum chamber.

Recently, we have been using the apparatus together with two multi-port CCD detectors for single-shot XFEL-CXDI experiments of non-crystalline particles with the dimensions of 100 – 1000 nm at beamline BL3 of SACLA. We usually use X-rays with

the photon energy of 5.5-keV and the intensity of 10^{10} – 10^{11} photons/ μm^2 /pulse. Because an extremely intense X-ray pulse destroys particles at the atomic level, the specimen disk is raster-scanned by the goniometer to supply fresh particles to the irradiation area. Collected diffraction patterns are processed using the program suite SITENNO [3,4]. Immediately after completing each data collection run, SITENNO automatically selects diffraction patterns with a significant level of intensity and conducts phase retrieval calculations [5-8].

As illustrated in Fig. 2, the diffraction pattern from cube-shaped particles with dimensions of 500 nm is approximated with the Fraunhofer diffraction pattern expected from a square-shaped aperture. This finding indicates that diffraction from the particle occurs before

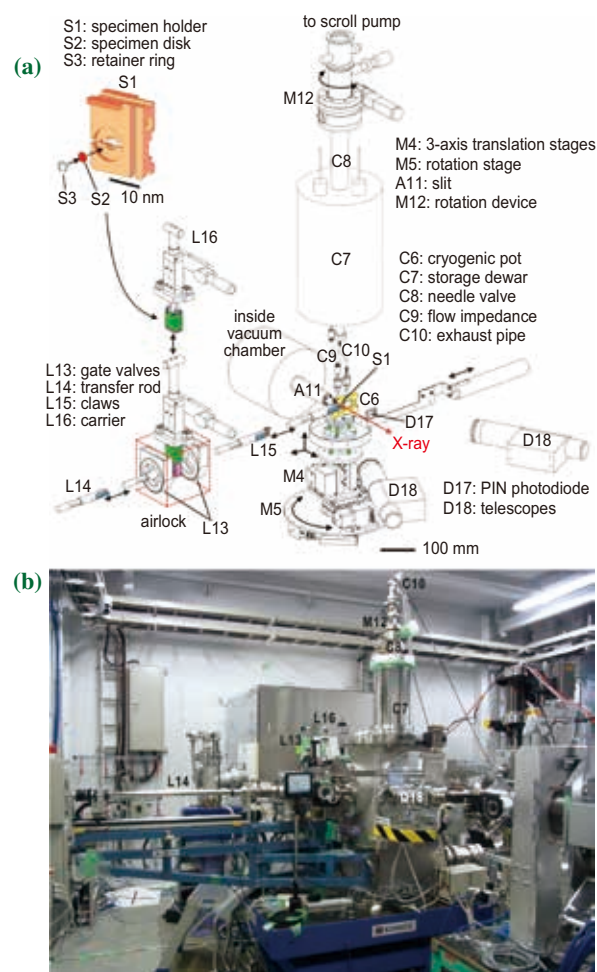


Fig. 1. (a) Illustration showing the arrangement of key components and (b) photograph of the KOTOBUKI-1 apparatus viewed downstream.

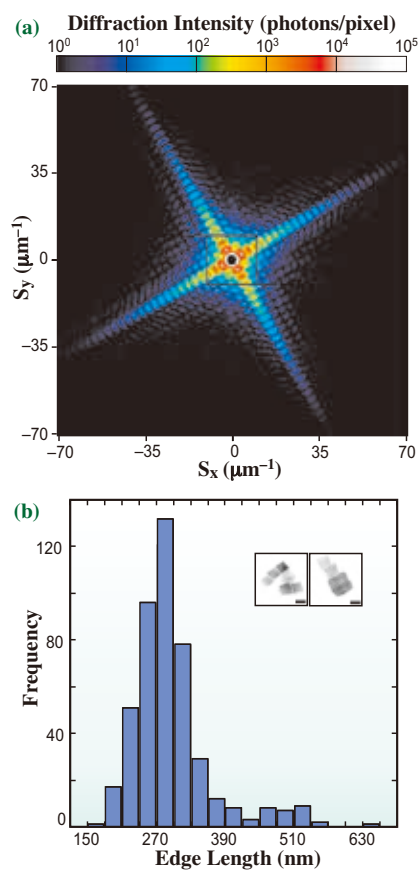


Fig. 2. (a) Diffraction pattern from a cube-shaped copper-oxide particle and (b) the size distribution determined from the electron density maps of 1276 particles.

the destruction. The XFEL-CXDI experiment allows us to collect a huge number of diffraction patterns within a short time. As an application, we tried to determine the size distribution of synthesized nano-metal particles. Even from aggregates of several particles, the phase-retrieval software robustly reconstructs the projection electron density maps as displayed in the upper half of Fig. 3 [3,7]. The distribution is consistent with that from scanning electron microscopy prior to the XFEL-CXDI experiment [9,10].

In addition to applications for material sciences and industry, XFEL-CXDI allows the structure of organelles in biological cells to be visualized. Organelles are dispersed on thin membranes with a small amount of buffer solutions to reduce background scattering and to ensure the contrast in the projected electron density maps. When the number density is $10/10 \times 10 \mu\text{m}^2$, the hit rate by XFEL pulses exceeds 50%. Recently we successfully visualized the electron density distribution in chloroplasts and nuclei of eukaryote cells [11]. XFEL-CXDI would become a suitable tool to visualize intact and whole organelles in addition to sophisticated imaging techniques such as

fluorescence microscopy, electron microscopy, X-ray microscopy, etc. The complementary use of XFEL-CXDI together with these techniques is expected to bridge the resolution gap between cell biology and structural biology.

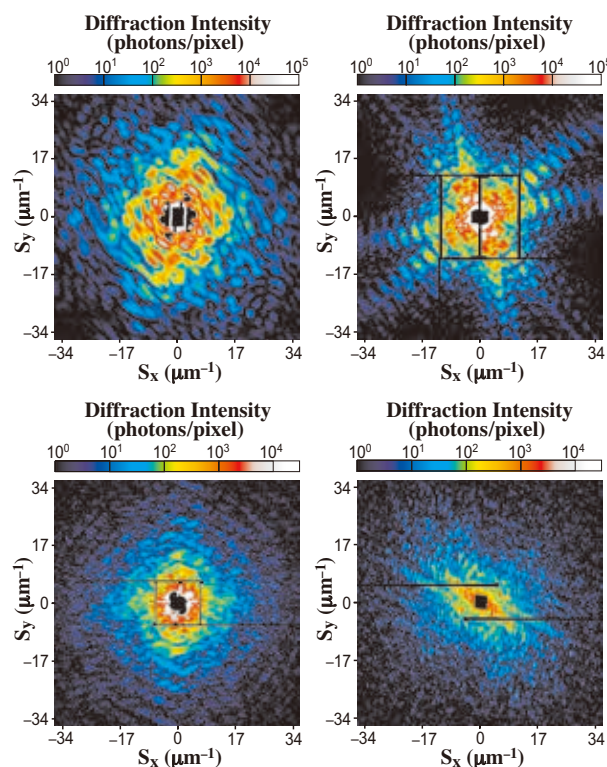


Fig. 3. Representative diffraction patterns observed in XFEL-CXDI experiments for aggregates of synthesized non-metal particles (upper panel) and for organelles of eukaryote cells.

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