

Imaging live cells by X-ray laser diffraction

Radiation damage is a serious problem in high-resolution biological imaging. It has been impossible to observe living cells at a nanometer resolution by electron microscopy or X-ray microscopy because cells die when exposed to the electrons or X-ray radiation used for the observations. X-ray free-electron lasers (XFELs) can overcome this difficulty by illuminating the sample with ultra-short femtosecond pulses, allowing a snapshot of the intact sample to be acquired before radiation damage [1,2].

To capture ultrafast snapshots of a solution sample at the nanometer resolution using XFELs, we developed a method, which we refer to as pulsed coherent X-ray solution scattering (PCXSS) [3,4]. Figure 1 shows the PCXSS measurement schematically. Water is essential to life. Hence, solution imaging by PCXSS is crucial in biological imaging. In PCXSS, the sample image is reconstructed from the coherent X-ray diffraction (CXD) pattern using lens-less coherent diffractive imaging techniques [5].

PCXSS has a unique feature; the solution sample is placed statically between two thin membranes in a controlled environment. Since XFEL pulses are intense enough to break the membranes in a single shot, we designed a micro-liquid enclosure array (MLEA) chip where many independent micro-liquid enclosures are arranged in a 2D array as shown in Fig. 2(a). The MLEA chips are fabricated by a photolithography process at Hokkaido University using equipment in a clean room operated with support from the Nanotechnology Platform Project of MEXT, Japan.

As the first demonstration of PCXSS measurement, we performed nano-imaging of a living *Microbacterium lacticum* cell. *M. lacticum* is a heat-stable bacterium discovered in milk. It has a typical rod shape, but

is much smaller than ordinary bacteria. Because conventional optical microscopy has an insufficient resolution to observe the internal structures of a sub-micrometer sized cell, little information is available about its cell biology despite its importance in agriculture.

Before performing an XFEL experiment at SACLA, we confirmed that the cells are alive in the MLEA chip. We stained the cells with fluorescent dyes, and observed them by fluorescent microscopy. In Fig. 2(b), live cells are labelled in green and the dead ones are in red. Our experiment shows that 99% of the cells are alive in an MLEA chip one hour after enclosure being placed in the vacuum environment, indicating that MLEA chips are adequate for live cell imaging by PCXSS.

The PCXSS measurement of live *M. lacticum* cells was carried out at beamline BL3 of SACLA. The XFEL beam from the undulator with a photon energy of 5.5 keV was first transported to the optics hutch, and higher harmonics were removed with a pair of flat total reflection mirrors there. The beam was then guided to EH3 and focused onto a $1.5 \mu\text{m} \times 2.0 \mu\text{m}$ spot using a Kirkpatrick-Baez mirror system. At the focus, the XFEL beam illuminates *M. lacticum* cells in a 0.9% (w/v) NaCl solution in an MLEA chip placed in MAXIC (Multiple Application X-ray Imaging Chamber). The CXD patterns of the sample were recorded by MPCCD (Multi-Port Charge-Coupled Device).

Because X-ray scattering from a sub-micrometer sized biological sample is extremely weak, it is important to reduce parasitic background scattering, originating from something other than the sample, for high precision measurements. Consequently, we placed two sets of four-jaw guard slits before the

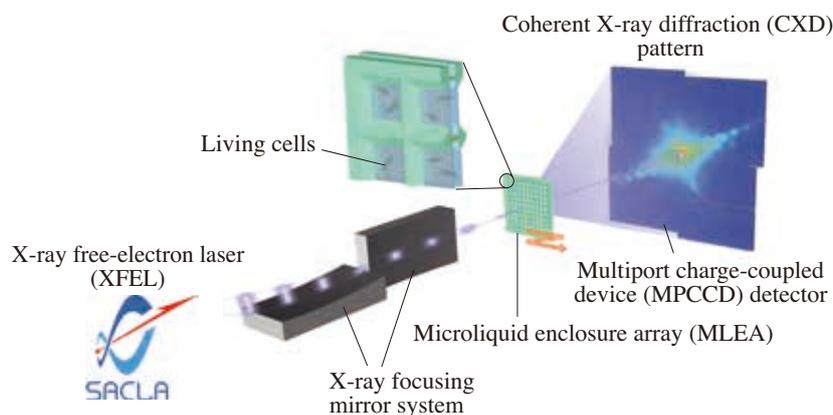


Fig. 1. Schematic of the PCXSS experiment.

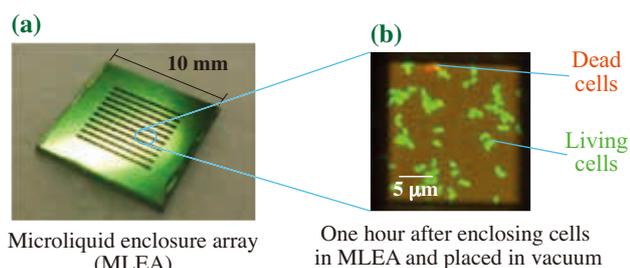


Fig. 2. (a) MLEA chip. (b) Result of fluorescence microscopy study confirming that cells can be kept alive in MLEA chips.

sample in MAXIC. Moreover, we confirmed that the silicon window frame of an MLEA chip with an aperture size of $\sim 20 \mu\text{m}$ acts as an additional guard slit in the sample plane, and plays an essential role in reducing parasitic scattering.

Figure 3(a) shows a single-shot CXD pattern of a live *M. lacticum* cell. An interference fringe extending in one direction is clearly observed. The fringe interval indicates that the sample has a width of 194 nm. Figure 3(b) shows the reconstructed sample image. In the image reconstruction, we used the relaxed averaged alternating reflections (RAAR), the noise-

tolerant hybrid input-output (HIO), and shrink-wrap algorithms. The full-period resolution of the image, which was estimated using the phase-retrieval transfer function, is 37 nm.

The reconstructed cell image is rod-shaped with a width and length of $\sim 194 \text{ nm}$ and $\sim 570 \text{ nm}$, respectively. The lower part of the cell image contains a dumbbell-shaped high image-intensity region, indicative of a nucleoid, a DNA-rich structure in prokaryotic cells. In fact, the image intensity difference between the upper and lower regions of the cell can be roughly explained by assuming that they are mostly composed of protein and nucleic acids, respectively.

Our research demonstrates that the PCXSS method using XFEL has great potential for observing biological samples close to their natural state at a nanoscale resolution without staining with heavy metals or thin sectioning. As the achieved resolution greatly exceeds conventional optical microscopy, PCXSS should help reveal important cellular events, such as genome replication and subsequent cell division. By further improving the resolution, nanostructures of natural-state biological molecules will be also imaged. In addition, PCXSS can be usefully applied to imaging nanostructures of materials functioning in solution.

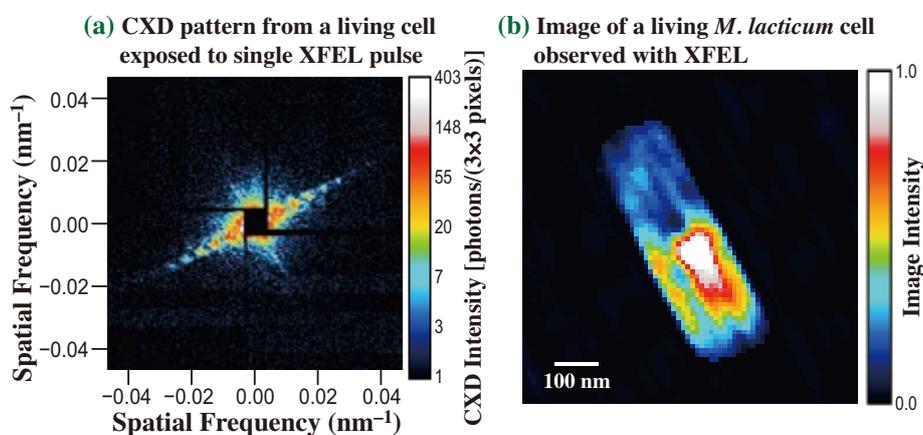


Fig. 3. (a) Single-shot CXD pattern of a living *M. lacticum* cell. (b) Reconstructed image obtained by applying iterative phase retrieval algorithms to the CXD pattern.

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