Observation of mammalian living cells by a femtosecond single-shot exposure of a soft X-ray free electron laser

Soft X-ray microscopy in the water window (280–530 eV), situated between the carbon *K*-edge (~280 eV) and the oxygen *K*-edge (~530 eV), enables high-spatial-resolution, high-contrast imaging of biological samples in water without the need for labeling, based on contrast mainly originating from carbons in biomolecules. However, because soft X-ray irradiation causes significant radiation damage on living cells, most soft X-ray imaging studies rely on chemically fixed samples. Chemical fixation, while simplifying sample handling, can result in the loss of fine structures and intracellular elements, thus motivating development of a method to observe living cells while minimizing radiation damage.

Recent advances in soft X-ray free electron laser (SXFEL) sources can address this challenge. SXFELs can produce extremely bright femtosecond pulses, so short that the imaging process is finished before severe structural damage can occur on cells. This principle, sometimes called “imaging before destruction,” has already enabled groundbreaking images of living viruses and bacteria [1], but observing larger mammalian cells has been difficult due to their large size. Because mammalian cells are fundamental models in medical and pharmacological research, developing a damage-free, label-free soft X-ray imaging method has remained a critical goal. We developed a soft X-ray transmission microscope with a single-shot femtosecond illumination using an SXFEL for observation of mammalian living cells [2].

To achieve label-free imaging of living mammalian cells with minimal radiation damage, our group constructed a soft X-ray transmission microscope at the soft X-ray beamline of SACLA **BL1** (Fig. 1) [3]. We developed a pair of Wolter mirrors for condenser and objective optics [4]. Wolter mirrors are grazing-incidence reflective optics for soft X-rays. They inherently minimize spherical aberration and commatic aberration, also providing a long working distance and large acceptance area. The long working distance allows the use of thick sample holders filled with culture medium, while the achromaticity of Wolter optics readily accommodates spectrally-resolved imaging across the water window.

In our setup, the third harmonic of the SXFEL beam, typically with a pulse width of around 30 femtoseconds [5], was directed to the sample through Kirkpatrick–Baez (KB) mirrors and a condenser Wolter mirror (CWM). The transmitted beam was then magnified by an objective Wolter mirror (OWM) onto a CCD detector. The spatial resolution is 230 nm at a photon energy of 390 eV and the field of view is $52 \times 52\ \mu m^2$. The microscope is also equipped with visible-light imaging capabilities, allowing sample positioning based on conventional bright-field images. Because of the strong absorption of carbon at these photon energies and the comparatively lower abundances of nitrogen and oxygen, the resulting soft X-ray images primarily reflect the distribution of carbon within the cell.

We observed Chinese hamster ovary (CHO-K1) cells enclosed in a liquid cell holder containing culture medium. The entire holder was sealed and maintained at 37°C to keep the cells alive. The images on the left side of Fig. 2 shows cells captured at a photon energy of 390 eV by a single 30-femtosecond shot by the SXFEL. A variety of cellular structures were visualized in these images. In particular, accumulations of carbon (biomolecules) were observed in regions thought to correspond to nucleoli and the nuclear membrane

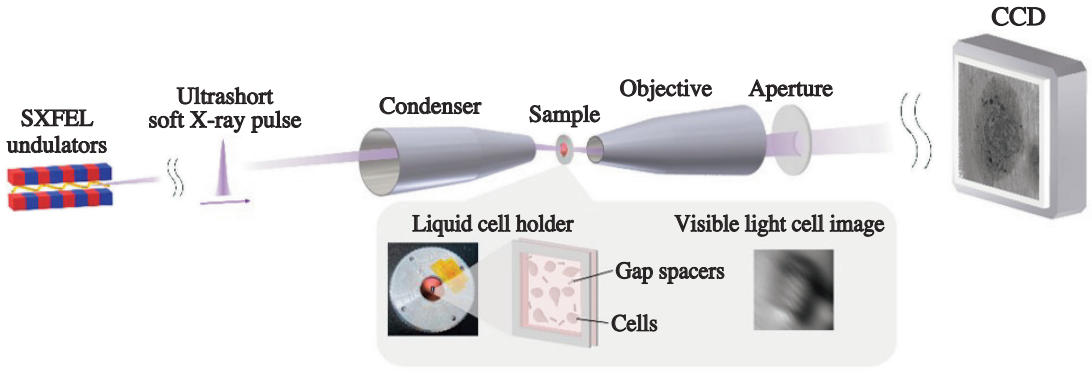

Fig. 1. Schematic illustration of a soft X-ray transmission microscope with SXFEL illumination. [2]

(arrows in Fig. 2). We also detected filamentous structures radiating from the nucleolus to the nuclear membrane. These filamentous structures may constitute an important pathway linking the nucleolus, nuclear membrane, and cytoplasm.

The images on the right side of Fig. 2 are images captured with a 0.5-second multi-shot exposure immediately after the single 30-femtosecond shot. In comparison with the initial single-shot image, we observed some blurriness likely caused by cellular motion or structural changes. Observations were carried out also on paraformaldehyde-fixed cells, but the various structures seen in the living cells were not discernible [2].

In summary, we achieved single-shot label-free imaging of living mammalian cells in culture medium using an SXFEL by overcoming the long-standing issue of X-ray-induced radiation damage on living cells. Numerous cellular structures including previously unknown ones were suggested. As improvements in light source performance allow brighter single-shot images, it will become possible to capture even finer structures with greater clarity. Moreover, soft X-rays can be used not only for carbon imaging but also for analyzing other light elements essential to cellular metabolism such as iron, zinc, phosphorus, and calcium. In the future, this microscope is expected to offer new perspectives in biology by enabling, for example, the visualization of instantaneous changes in chemical states within living cells.

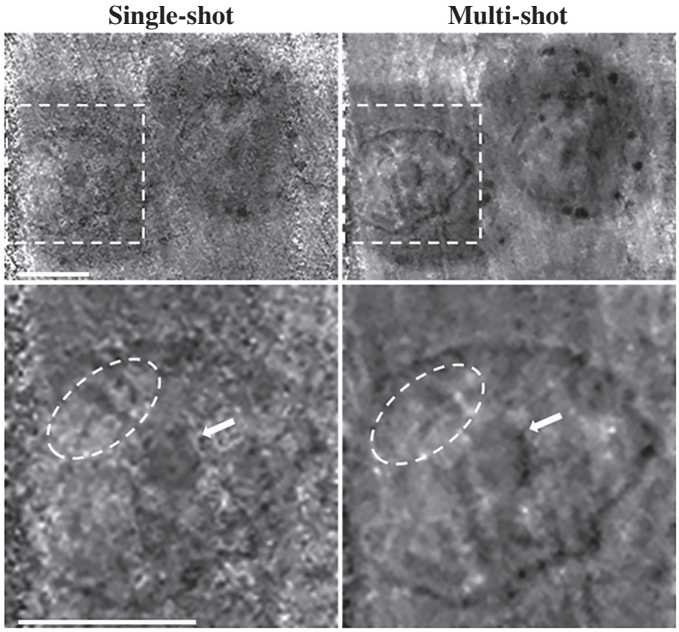

Fig. 2. Soft X-ray images of cells. (Upper left) A soft X-ray image of a living cell captured by a single-shot exposure of an SXFEL pulse. (Upper right) A soft X-ray image of the same cell obtained immediately afterward under 30 shots exposure in 0.5 s. (Lower left and lower right) Magnified views of the cell in the dashed square region. The ovals and arrows (nuclei) indicate the regions at which significant structural changes are observed between the single-shot image and multi-shot image. Scale bars: 5 μm. [2]

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