Three-Dimensional Visualization of a Human Chromosome Using Coherent X-Ray Diffraction

When we want to know the structure of a protein or its complex, we have to make its crystals in advance. If we are lucky, we will obtain the crystals and then X-ray crystallography will yield the structure of the protein or its complex with about 1 Å resolution. However, when we want to know the detailed structure of much bigger objects such as cellular organelles, for example, chromosomes or nuclei, what should we do? Such objects with varying sizes and shapes do not form crystals for X-ray crystallography. Moreover, they are too thick for electron microscopy. Coherent X-ray diffraction microscopy (XDM) is the method to achieve it [1].

When a noncrystalline object is illuminated by coherent, monochromatic X-rays having a well-defined phase, a continuous diffraction pattern called speckles occurs (Fig. 1(b)). In the case of light microscopy, this kind of diffraction from an object is converted into a real-space image by lenses. Unfortunately, we do not have good optical lenses for X-rays. Recently, it became possible to convert the diffraction into a real-space image by computational calculation, instead of lenses [2]. If the diffraction speckle pattern is recorded finely enough to satisfy the "oversampling condition," which is related to the Shannon sampling theorem [3], the structure can be reconstructed by an "iterative phase retrieval method," a process by which computers find a structure to fit the diffraction pattern [2]. That is, XDM is an emerging technique for "lensless" high-resolution X-ray microscopy.

We observed a human chromosome using XDM [1]. Chromosomes are essential organelles for the transmission of replicated genomic DNA into two daughter cells during cell division [4]. Although more than 100 years have passed since chromosomes were first observed, how a long string of genomic DNA is packaged into compact chromosomes remains unclear.

A schematic of XDM measurement of a human chromosome at beamline **BL29XUL** is shown in Fig. 1(a). X-rays at an energy of 5 keV passed through a 20-μm-diameter pinhole to illuminate the target chromosome only, which was stuck to a thin silicon nitride membrane. The coherent diffraction (speckles) from the chromosome was recorded with an X-ray direct-detection charge-coupled device (CCD) 1.32 m downstream from the sample. A beamstop before the CCD blocked the unscattered direct X-ray beam.

From the coherent diffraction pattern (Fig. 1(b)), a human chromosome image was reconstructed. A two-dimensional (2D) reconstruction of the human chromosome is shown in grayscale (Fig. 2(a)) and in color (Fig. 2(b)). Note that the intensities are proportional to the projection of the electron density. The spatial resolution of the 2D reconstruction is 38 nm. The most striking feature in the reconstruction is the high-intensity region near the chromosome axes with a width of ~200 nm (Fig. 2(b)). The axial structure has so far been detected only using immunoelectron microscopy or fluorescence microscopy on labeling condensin and topoisomerase IIα, which are essential proteins for the chromosome assembly process [4].

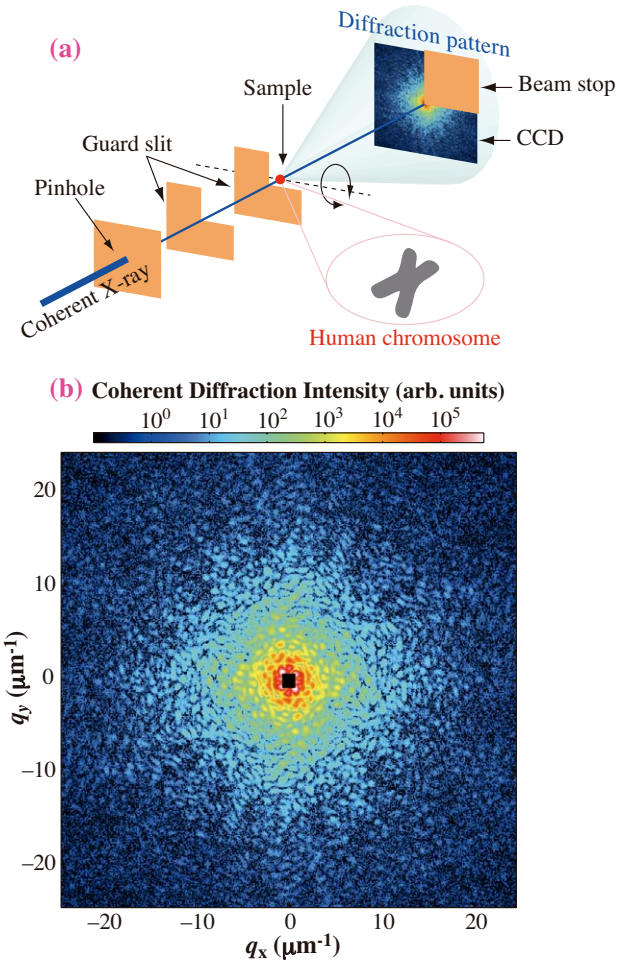

Fig. 1. **(a)** Schematic of XDM measurement of an unstained human chromosome at BL29XUL. **(b)** Coherent diffraction (speckles) from the chromosome.

For three-dimensional (3D) reconstruction, we obtained data at different incident angles ranging from –70° to +70°(38 diffraction data sets). A reconstructed 3D electron density map of the chromosome is shown in Fig. 2(c). The reconstructed chromosome image also has the highest electron density around the centromere. This can be explained by the fact that the centromere region is more condensed than other chromosomal regions. We estimate the spatial resolution of the 3D reconstruction to be 120 nm. This is the first 3D electron density mapping of an unstained cellular organelle using XDM.

Since the spatial resolution of microscopy is determined by the wavelength, hard X-rays can achieve atomic resolution in principle. With third-generation synchrotron radiation X-rays, the spatial resolution is often limited by the radiation damage or the X-ray intensity. Both limitations may be removed or lowered dramatically with the X-ray free electron lasers (XFELs) to produce high peak-brilliance coherent hard X-rays, which is currently being developed [6]. X-ray diffraction resolved the structure of DNA almost 50 years ago [5] and it resolved that of the nucleosome, where DNA is wrapped around histone proteins, 10 years ago [4]. XFELs will enable a dramatic improvement in the spatial resolution of XDM and would accelerate research for the atomic structure analysis of chromosomes and other cellular organelles, including macromolecular complexes [6].

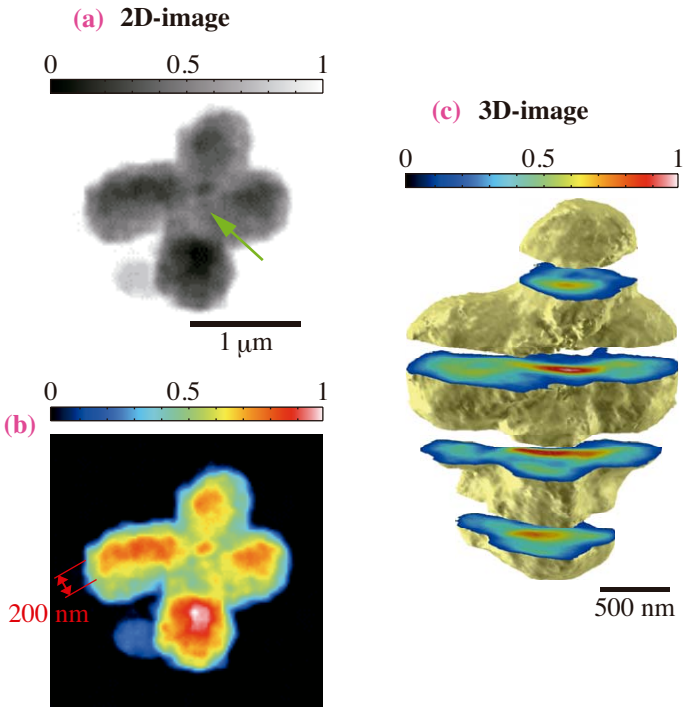

Fig. 2. A two-dimensional reconstruction of the human chromosome is shown in grayscale **(a)** and in color **(b)**. Note that the intensities are proportional to the projection of the electron density. The centromere region is indicated by an arrow in **(b)**. **(c)** Reconstructed 3D electron density map of the chromosome. Cross-sectional images of the chromosome at 409 nm intervals are shown.

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