Trace element mapping of a single cell using a hard X-ray nanobeam focused by a Kirkpatrick-Baez mirror system

The elucidation of cellular functions is rapidly progressing owing to techniques that identify and handle genes and proteins. New observation techniques will lead to breakthroughs, as the use of novel microscopes historically paves the way for new discoveries. Here, we describe a new microscope that enables the observation of intracellular elemental distributions with high resolution and high sensitivity [1].

We have developed a scanning X-ray fluorescence microscope (SXFM), which consists of both X-ray fluorescence analysis and X-ray focusing [2]. SXFM is one of the most suitable tools for visualizing elemental distributions in biological samples owing to its capability to obtain multi-element distributions with submicron resolution and sub-femtogram sensitivity and the ready acquisition of images without complex sample preparations such as sectioning and coating. We demonstrated our SXFM performance for biological applications by obtaining intracellular elemental distributions at the single-cell level.

Figure 1 shows the layout of our microscope. Kirkpatrick-Baez (KB) mirrors [3] consisting of two elliptical mirrors aligned at right angles to each other are placed approximately 100 m downstream of the undulator. The working distance is fixed at 100 mm in order to set up various components near samples in the near future. The depth of focus is 50-200 μm depending on the selected beam size, making it unnecessary to consider the thickness of thin samples such as cells. A noticeable feature of our focusing system is that the beam size is controllable within a wide range, from 29×48 nm (diffraction limit) to ~2000×~2000 nm (at 15 keV), which is achieved by adjusting a slit (TC1 slit) installed just downstream of the DCM; that is, we can select the optimum beam size for the scan area (Fig. 2). An energy dispersive detector (SDD) is employed to detect X-ray

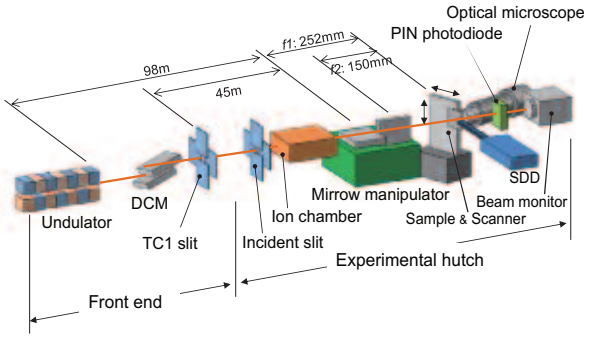

Fig. 1. Layout of the SXFM system.

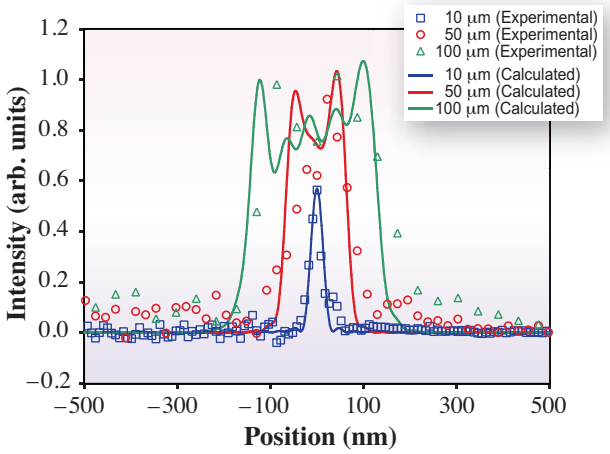

Fig. 2. Profiles of typical X-ray beams used in the study (direction: horizontal focusing, X-ray energy: 15 keV). The lines indicate the profiles measured by a wire scanning method and calculated ones. The values shown in the graph indicate the sizes of the virtual X-ray source formed by the TC1 slit. The narrowest beam has a full width at half maximum of 30 nm with a slit size of 10 μm.

fluorescence. A linear-encoder-based feedback X-Y stage with a positioning resolution of 1 nm is used to obtain a high-resolution raster scan of the sample. The sample and scanner are inclined 60° to the incident X-ray beam to enable the SDD to be set up near the sample. Details of the performance such as the optimum spatial resolution and beam stability have been described in our previous study [2].

X-ray fluorescence spectrum data detected by the SDD are sent to two multichannel analyzers (MCAs) for pulse height analysis and then downloaded to a personal computer. All X-ray fluorescence spectra detected for every pixel are recorded and processed using software to integrate the counts in each region of interest (ROI), as well as to visualize elemental distributions according to the ROI. Recording a large number of raw spectra for every pixel enables background correction, noise reduction, and peak separation to be performed after the observations are completed.

NIH/3T3 cells, which were plated on a polymer film of 4 μm thickness, were observed at the second experimental hutch (EH2) at beamline **BL29XUL**. An X-ray energy of 15 keV was selected for detecting of elements from Na (Z=11) to Pb (Z=82). In this experiment, the temperature of the whole SXFM system was maintained within ±0.1°C to ensure the stability of beam position and size. Elemental distribution maps obtained by SXFM at the single-cell

level are shown in Fig. 3. The Au distribution image and the fluorescence image obtained using visible light correspond to the mitochondrial distribution, as a result of the use of antibodies with fluorescence dye and colloidal gold. Compared with other elements, Zn and Cu are particularly colocalized with the Au distribution, except at the nucleus, while P, Ca, and Fe are highly concentrated at the nucleus. Zn and Cu seem to be mainly included in the mitochondria and nucleus.

To acquire higher-resolution images of the mitochondria, the beam was sharpened by closing the TC1 slit. Figure 4 shows high-resolution elemental distribution maps. As can be seen from the distribution maps, the signal intensities of Zn were partially colocalized with those of Au (indicated by arrow). The signal intensities of Ca, P, and Zn, which appear granular, likely correspond to other organelles that could not be identified in the experiments.

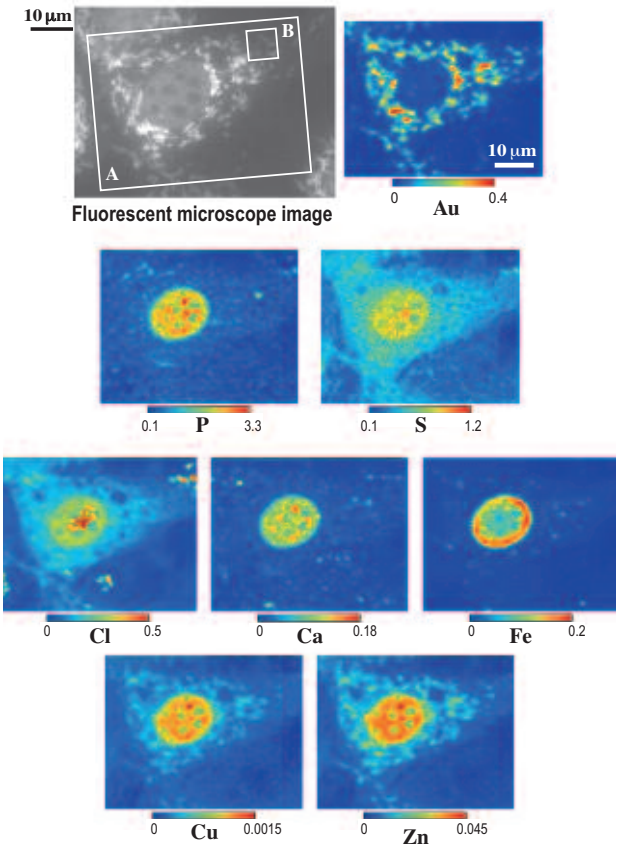

Fig. 3. Fluorescent microscope image obtained using a visible light microscope and elemental distribution maps at a single cell level in area A. The fluorescent microscope image and the Au distribution map show the existence of ATP synthase β localized in mitochondria. Color bars indicate the elemental contents (fg) of an irradiation area.

Recently, we have improved the SXFM to enable observation at cryogenic temperatures [4] of specimens with user-friendliness. The improvements allow us to accelerate the study of the biological and medical applications of SXFM. We believe that the accurate visualization of intracellular elemental distributions will help to clarify cellular functions and to overcome diseases. SXFMs are thus likely to be an important tool for such investigations in the near future.

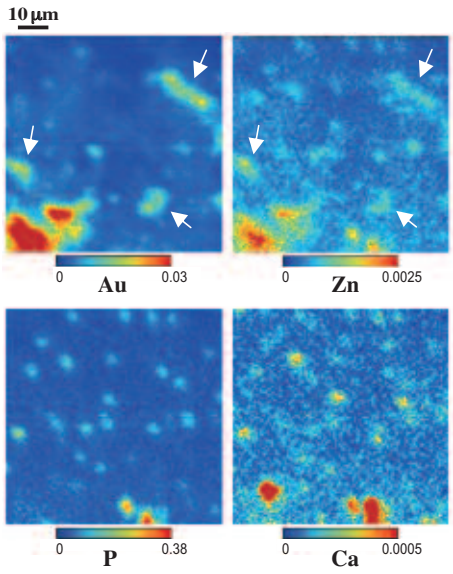

Fig. 4. High-resolution elemental distribution maps in area B in Fig. 3. The elliptical object indicated by arrows in the Au distribution map shows a single mitochondrion and the dense region at the lower left represents piles of mitochondria. Color bars indicate the elemental contents (fg) of an irradiation area.

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