23AG019 BL-5

Visualization of boron distributions in cancer cells dosed with a boron delivery drug

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Keywords: Photoemission electron microscopy, local X-ray absorption spectroscopy, elemental distributions

Boron Neutron Capture Therapy (BNCT) has attracted attention in recent years as a cancer treatment method that aims to destroy only cancer cells without damaging normal cells. It has achieved remarkable results in the treatments of cephalon and cervix cancers. [1]. In this method, cancer cells are destroyed by an alpha-ray and a lithium ion emitted through a nuclear reaction between a boron nuclear and a thermal neutron ray. In order to increase the probability of selectively destroying only cancer cells while causing as little damage to normal cells as possible, it is essential to increase the accumulation of boron compounds on the target cancer cells, which will contribute to maximize the effect of BNCT with thermal neutrons of the lowest possible intensity. Visualization of intracellular boron distribution using microscopic techniques provides important clues for the development of boron compounds that can efficiently accumulate in cancer cells.

Electron probe micro analyzer (EPMA) has been widely used in visualization of microscopic elemental distributions on various material surfaces as one of the most popular techniques, by means of combination of a scanning electron microscope and an X-ray fluorescence analyzer [2]. X-ray fluorescence is produced via relaxation of core-holes excited, in this case, by an electron beam, which is therefore element specific in nature. For light elements such as boron (B), however, Auger transition rates are more than 99.9% in their core-hole relaxation processes, which means that detection of trace light elements by X-ray fluorescence has difficulty. In contrast, X-ray absorption spectroscopy (XAS) with total electron yield (TEY) is suitable to detect trace light elements because TEY is proportional to the amount of secondary electrons generated mainly by Auger electrons [3]. The intensity of photoemission electron microscope (PEEM) is also proportional to the TEY intensity when it is used with X-ray as a light source and without both an energy filter and an energy analyzer [4]. One can obtain a local XAS spectrum by plotting intensities in a certain area on a series of PEEM images as a function of X-ray energies. In addition, an elemental distribution image can be obtained as a difference image of two PEEM images measured at X-ray energies of (1) a pre-edge and (2) a peak position above a certain absorption edge.

In this study, we have performed PEEM and TEY measurements at BL5 in HiSOR for (1) a micro-droplet of solidified L-boronophenylalanine (L-BPA), which is used as a boron delivery drug in clinical BNCTs, on a Si substrate and (2) cancer cells dosed with L-BPA, in order to investigate microscopic chemical states of trace B atoms in them from fine structures in local- and wide-area-XAS spectra near B K-edge and to visualize B distributions on their surfaces. For this kind of measurements with PEEM, we have developed a new auto-measurement system where we can obtain a serial PEEM images with excitation x-ray energies for a certain energy range with a fixed energy step. The experimental station of BL5 is equipped with a PEEM III (Elmitec GmbH) and with a manipulator connected to a digital amperemeter for a TEY measurement. As a light source, the second-order X-rays from the monochromator were used because they have higher intensity and energy resolution around B *K*-edge than the first-order X-ray from the monochromator with the same entrance and exit slit widths. All the measurements were performed at room temperature.

In order to elucidate the XAS signals from L-BPA, first we have performed PEEM and TEY measurements for a micro-droplet of solidified L-BPA on a Si substrate. Figure 1 (a) shows an optical microscope image of

a micro-droplet on a Si substrate. The size of the micro-droplet is about 100 µm. Figure 1 (b) shows a PEEM image of the same micro-droplet measured with Hg lamp showing 2 dimensional local work function distribution for it. The field of view is 150 µm. The blue curve in figure 1 (c) shows plots of averaged intensities per a pixel in blue square in figure 1 (b) as a function of X-ray energy around B K-edge, showing a local XAS spectrum which has a sharp peak structure at 193.6 eV and a small bump at 191.6 eV. These clear structures are not observed in an XAS spectrum measured by TEY (black curve in figure 1 (c)) which provides an averaged information of the sample surface from a wide area illuminated by X-ray (the size of X-ray is about 1 x 5 mm^2 on the sample surface). These results demonstrate the effectiveness of PEEM measurements for detecting signals from small amounts of substances on a sample surface.

Figure 2 (a) shows an optical microscope image of a colony of cancer cells dosed with L-BPA on a silicon substrate. Figure 2 (b) shows a PEEM image of the same colony measured with X-ray of 195 eV. The field of view is 150 µm. The red curve in figure 2 (c) shows plots of averaged intensities per a pixel in red square in figure 2 (b) as a function of X-ray energy around B K-edge. For the blue curve in figure 2 (c), 30 PEEM images are summed for each X-ray energy, providing better S/N ratio of the blue curve than the one of the red curve. The small structures in this local-XAS spectrum for the cancer cell does not resemble the ones in figure 1 (c) for micro-droplet of L-BPA. This suggests that the L-BPA may be decomposed in the cancer cell. Further measurements to obtain more better S/N ratio in a local-XAS spectrum for the cancer cells are in progress.

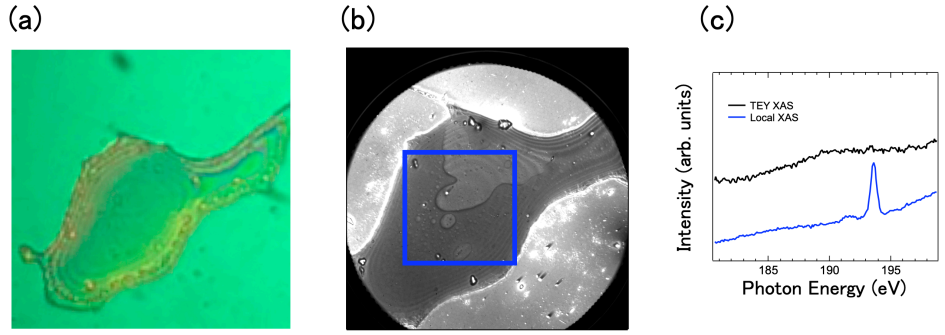

FIGURE 1. (a) An optical microscope image of a micro-droplet on a Si substrate. The size of the micro-droplet is about 100 µm. (b) A PEEM image of the same micro-droplet measured with Hg lamp. The field of view is 150 µm. The blue curve in (c) shows plots of averaged intensities per a pixel in blue square in figure 1 (b) as a function of X-ray energy around B K-edge. The black curve shows an XAS spectrum measured by TEY.

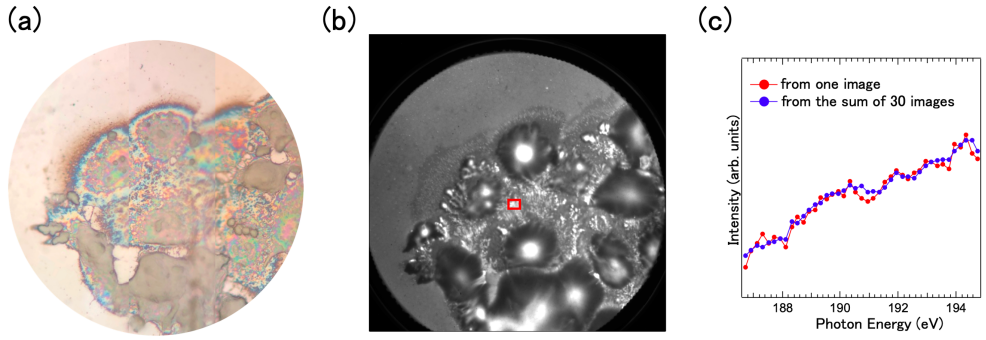

FIGURE 2. (a) An optical microscope image of a colony of cancer cells dosed with L-BPA on a silicon substrate. (b) A PEEM image of the same colony measured with X-ray of 195 eV. The field of view is 150 µm. The red curve in (c) shows plots of averaged intensities per a pixel in red square in figure 2 (b) as a function of X-ray energy around B K-edge. For the blue curve in figure 2 (c), 30 PEEM images are summed for each X-ray energy.

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