Mapping of intracellular light elements including Ca, P and S in mammalian cells by soft X-ray contact microscopy

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Soft X-ray microscopy is a powerful tool for high-resolution mapping of light elements in thin specimens such as a biological cell with a few-micron thickness. We have explored the method to obtain intracellular distribution of light elements particularly of minor light elements such as Ca, P, and S. Since abundant major light elements, C, O and N are constituent elements of most biomolecules, the distributions of those elements are similar to a cell image formed by X-ray absorption. On the other hand minor light elements are contained in specific biomolecules; for example, nucleic acids and phospholipids contain phosphorus, while proteins do not. In addition in some cases the distribution of those elements is known to reflect physiological response.

For the elemental mapping we have used contact microscopy with an electronic zooming tube that has sensitivity over the wide wavelength range covering the absorption K or L edges of constituent elements in a cell. From the absorption spectrum of one pixel in a cell image we have developed a computer program to obtain the quantities of minor elements whose absorption edges are hidden by large absorption of major light elements. In our previous algorithm, elemental content of C, O and N that is calculated from the absorption jump across the K absorption edge is subtracted from the absorption spectrum, resulting in the appearance of the absorption jump at the absorption edges of minor elements. The elemental content of these elements is obtained by the same procedure as major elements [1].

In the present study, we have improved the above method by introducing iteration procedure until absorption jumps at all the detected absorption edges become zero. In every iteration process we can find new elements in a specimen by the recognition of absorption jump at the absorption edge and improve the accuracy of elemental content. The content of all the elements whose absorption edges become evident is calculated by solving a simultaneous equation in which absorbance is expressed as a linear combination of mass thicknesses of constituent elements. The algorithm was checked for the images of dried KNO_3 and 2'-deoxyguanosine ($C_{10}H_{13}N_5O_4$) with known elemental composition. With these standard specimens we had a good agreement with the expected values by choosing appropriate wavelengths for imaging at the both sides of the absorption edges of these elements. Then the program was applied to the image of dried human HeLa cells. Distribution of phosphorus was more preferential in the nuclear region than those of C, O or N, while sulfur seems to distribute uniformly in a cell. Interestingly calcium was mainly accumulated in the nuclear region. A simplified method based on the similar iteration algorithm is now being developed.

[1] A. Ito et al., J. Phys. IV France, **104**, 297-300 (2003).