Analysis of localization and chemical status of minor elements in a mammalian cell using soft X-ray contact microscopy

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Abstract. We have developed computer-assisted procedures for mapping minor light elements and their chemical bond information from a set of soft X-ray transmission images of biological specimens. Minor light elements such as Ca, S and P play an important role in the biological function and structure in a cell. Contact X-ray microscopy with an electric zooming tube covering wide wavelength range from the K absorption edge of S to the L absorption edge of P is applied to elemental mapping. The procedure of elemental mapping depends basically on the subtraction of the contribution of major elements such as C, O and N in intracellular absorption spectra to find the presence of the absorption edges of minor elements. The results of simulation experiments were presented. Chemical bond imaging utilizes a XANES spectrum corresponding to a specific chemical bond. Since the intensity is relatively low for minor elements, the ratio between absorption at the peak and at the bottom, which represents the chemical bond distribution, becomes small. We developed the computer program for the enhancement of very small ratio value. This procedure was applied to the S-S and S-C bond imaging at the S-K absorption edge.

1. INTRODUCTION

Minor light elements such as Ca and S have been well recognized to respond to cellular physiological state in their intracellular distribution. The present study deals with the quantitative method for subcellular mapping of such minor elements and the sensitive mapping of chemical bond related to those elements. Soft X-ray microscopy has an advantage in the observation of 1) thin specimen like a cell and 2) the elements having the absorption edges in the soft X-ray region.

A number of studies in the soft X-ray region for mapping the distribution of light element have been focused on the Ca in a biological specimen using highly sharp and intensive XANES peaks in a spectrum at the L absorption edge of Ca [1, 2]. Buckley stressed the high sensitivity of mapping Ca using XANES peaks [3]. However it is rather difficult to obtain quantitative distribution of Ca due to the uncertainty of absorption coefficients at the wavelength of XANES peak. Another approach to obtain element distribution including Ca quantitatively was proposed by Ito et al. [4]. Briefly, from cellular transmittance images, the number of which is larger than that of elements to be considered, elemental content expressed by mass thickness (weight per unit area) is calculated simultaneously using the least square method. For quantitative analysis wavelengths to image should be chosen at which elemental absorption coefficient is listed on the Henke’s table [5]. But to work this method effectively, all the possible elements in a cell should be incorporated into the simultaneous equation.

In the present study, we improved the above method for quantitative elemental mapping particularly for minor elements by subtracting the contribution of major elements from the transmittance in each pixel of the cellular image. For the chemical bond imaging of minor elements, ratio in the height of XANES peaks that correspond to different chemical bonds was enhanced and displayed to detect very small ratio value. These methods were preliminarily applied to human HeLa cell images.
2. MATERIALS AND METHODS

2.1 Procedure of elemental analysis

Elemental content is generally calculated by the subtraction of absorption between at the wavelengths below and above absorption edge. Although absorption jump across the absorption edge is required to be evident in the absorption spectrum of an intracellular area to carry out the subtraction process, the jump at the absorption edges of minor elements is likely to be hidden by the absorption of major elements with much larger content. Therefore to eliminate the contribution of major elements such as C, O and N, the following process was developed.

First, X-ray images taken in the wavelength region covering the absorption edges of constituent elements were prepared. For example, wavelength region from 1.5 nm to 10 nm includes the K edges of major elements C, N, O, and the L-edges of minor elements Ca, P, S, Fe. Then those images are converted to transmittance or absorption images using blank images as expressed below:

\[ A = \ln \frac{1}{T} = \mu_c \rho_c x + \mu_o \rho_o x + \mu_s \rho_s x + \mu_{ca} \rho_{ca} x + \mu_p \rho_p x + \mu_s \rho_s x + \mu_{re} \rho_{re} x + \ldots \ldots \]  

\[ A : \text{Absorption, } T : \text{Transmittance, } \mu_c \text{ etc.}: \text{Mass absorption coefficient of carbon etc., } \rho_c x \text{ etc.: Mass thickness of carbon etc.} \]

At the C, O, and N-K edges, contribution of minor elements can be neglected. Consequently subtraction between both side wavelengths of the absorption edge is expressed as (2) at the C-K edge for example.

\[ A_{\text{corr}} = A_{CL} - A_{CS} = (\mu_{CL} - \mu_{CS}) \rho_c x + (\mu_{OL} - \mu_{OS}) \rho_o x + (\mu_{NL} - \mu_{NS}) \rho_s x \]  

Where suffix CS, OS or NS means wavelength at the shorter side of the C, O or N-K edge, and suffix CL, OL or NL represents wavelength at the longer side of the C, O or N-K edge, respectively. Other two equations are obtained at the O and N-K edges in the similar manner. This simultaneous equation having unknown quantities of \( \rho_c x \), \( \rho_o x \) and \( \rho_s x \) was solved. Subtraction of contribution of C, O and N from equation (1) results in \( A_{\text{corr}} \) as expressed in (3) including only minor elements.

\[ A_{\text{corr}} = \mu_{ca} \rho_{ca} x + \mu_p \rho_p x + \mu_s \rho_s x + \mu_{re} \rho_{re} x + \ldots \ldots \]  

To obtain the mass thickness of a minor element, the ratio image of \( A_{\text{corr}} \) between both side wavelengths of the absorption edge of the minor element should be taken.

2.2 Procedure of chemical bond analysis

Mapping chemical bond was done by subtraction between absorption images at the top and the bottom wavelengths in XANES measured for a standard sample. At the S-K absorption edge glutathiones of reduced form with S-H and S-C bonds (SH compound) and oxidized form with S-C and S-S bonds (SS compound) were adopted. Since both compounds have been reported to exhibit different XANES peaks assigned to S-C and S-S bonds [6], the ratio between the height of S-C peak and S-S peak was calculated and displayed in an enhanced manner when the ratio is very small.
2.3 Contact imaging of human HeLa cells

Contact microscopy with an electronic zooming tube is very effective since it covers wide wavelength range in the soft X-ray region. The detailed layout was shown previously [4]. Human cancer HeLa cells are cultured on an SiN window, fixed with glutaraldehyde and then dried by critical point drying. Monochromatic soft X-rays were obtained at BL-11A and 11B in Photon Factory, Tsukuba, Japan.

3. RESULTS AND DISCUSSION

3.1 Simulation study for elemental analysis in one pixel

Elemental content calculated by the above procedure was compared with assumed values. The assumption of the simulation is as follows: 1) Elemental composition is C, O, N, Ca, P, S and Fe. 2) Wavelengths for imaging are selected from Henke’s table at the both sides of the K-edge of C, O, N and the L-edge of Ca, P, S, Fe. 3) Mass thickness of C is 2.0x10^{-5} g/cm^2, the value in our previous study [4].

Table 1 summarizes the assumed and calculated values for five cases. Case 2 represents the relative contents in human dry body. Case 4 and 5 assumes that the relative content of minor elements is equal but the absolute values are changed. In the case 1 and 3 the number of elements is limited.

<table>
<thead>
<tr>
<th>Element</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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<tbody>
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<td>calc.</td>
<td>given</td>
<td>calc.</td>
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<td>x 10^{-5}</td>
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<td>x 10^{-5}</td>
<td>1.00</td>
<td>x 10^{-5}</td>
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</tr>
<tr>
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<td>x 10^{-6}</td>
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<td>-</td>
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<tr>
<td>P</td>
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<td>x 10^{-7}</td>
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</tr>
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<td>S</td>
<td>-</td>
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<td>-</td>
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<td>x 10^{-9}</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1 (a) shows absorption spectrum of the case 5 in Table 1. No absorption jump was observed for Ca, P and S absorption edges. After the subtraction of C, O and N contribution, distinct absorption jump for these edges appeared. The calculated results in Table 1 show that even if the content of minor elements is equal, error in the content is not the same, but is independent of absolute content from the comparison of the case 4 and 5. The degree of the error may depend on the choice of wavelengths of both sides of absorption edge. While further improvement for reducing errors is in progress, we obtained preliminary image of Ca in a HeLa cell showing Ca accumulation in the nuclear region.
3.2 Distribution of SH and SS compounds in a HeLa cell

Figure 2 shows image of SH compounds having S-C/S-S peak height ratio larger than 1 in HeLa cells. Very small ratio value was visualized by expanding the ratio range from 1.00 to 1.05 to 256 bit gray scale. SH compounds seemed to localize in the cellular region.

The work has been performed at Photon Factory under the application number 2000G328.

References