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Life, Earth and Planetary Sciences

BL2A

Mo L_{III}-Edge XANES Study of Active Mo Species on H-GaAlMFI Catalysts for Methane Dehydroaromatization

H. Aritani[1], T. Sugawara[1], N. Naijo[1], S. Mogi[1], Y. Takayama[1] and A. Nakahira[2]
[1]*Department of Life Science & Green Chemistry, Saitama Institute of Technology, Fukaya 369-0293 Japan*
[2]*Graduate School of Engineering, Osaka Prefecture University, Sakai 599-8531 Japan*

MoO_3-modified H-MFI (Mo/H-MFI) is a typical catalyst for methane dehydroaromatization, which is an important reaction for direct GTL (Gas to Liquid) processes. Many workers revealed that MoO_3-modified H-MFI zeolite (SiO_2/Al_2O_3=28-40) shows catalytically high activity for MTB (Methane to Benzene) reaction, which is so-called methane dehydroaromatization. The unique activity is based on strong acidity and sieving effects onto MFI zeolite support. During the reaction, MoO_x species must be reduced and carbonized to form carbide-like species (MoC_xO_y). But catalytic deactivation is brought about at the same tome by carbon contamination on MFI. Because the deactivation strongly depend on the strong acidity on H-MFI, an improvement of surface property onto H-MFI is called for. In our recent study, Ga-containing H-MFI (GaAl-MFI) supports have been synthesized hydrothermally,[1] and Mo-modified GaAl-MFI catalysts have been applied to employ the methane dehydroaromatization. By partially substitution of Ga ions onto H-MFI framework (Ga/Al=50-100), the strong acidity is slightly suppressed. The effect of Ga ion onto H-MFI, highly active and durable catalysts can be expected. In the present report, Mo L_{III}-edge XANES study is applied to characterize the Ga incorporation or surface modification onto Mo/H-MFI to evaluate the active Mo species after the MTB reaction.

Catalysts were prepared by impregnation of H-GaAlMFI support with $MoO_2(acac)_2$–$CHCl_3$ solution, and followed by drying overnight and calcination at 773 K for 3 h. The amount of MoO_3-loading is 5.0 wt% in this study. H-GaAlMFI supports were synthesized hydrothermally at 413 K for a week, and followed by ion-exchanging with NH_4Cl and calcination at 873 K. The catalytic activity of methane dehydroaromatization was evaluated by means of fixed bed flow reaction, as described in a separate paper [2]. The Ga-incorporated catalyst samples are denoted as Mo/GaMFI*n*, in which *n*=Si/Al_2 atomic ratios. The Ga-modified ones (in extraframework) are denorted as Mo/Ga/MFI*n*. Mo L_{III}-edge XANES spectra were measured in BL2A of UVSOR-IMS in a total-electron yield mode using InSb double-crystal monochromator. Photon energy was calibrated by using Mo metal-foil at Mo L_{III}-edge, and normalized XANES spectra and their second derivatives are presented. REX-2000 (Rigaku) software was used by normalization of each XANES spectrum.

Figure 1 shows the Ga-incorporated or modified Mo/MFI28 (upper). By Ga modification, edge energy of XANES becomes lower, suggesting the promotion of Mo reduction to form Mo_2C species. In case of Mo/MFI56 catalysts (Fig. 1, lower), similar feature of the results can be shown. But Mo/GaMFI in Mo/Ga=50-100 catalysts only enhance the catalytic MTB activity by Ga incorpotration. It is likely the modified Mo species are only present on MFI extraframework, and thus, Ga incorporation brings about the both inhibition of acidity and reduction of Mo species. Ga modification gives similar effect only for Mo species on MFI, but the acid site is poisoned. Thus the catalytic activity becomes lower.

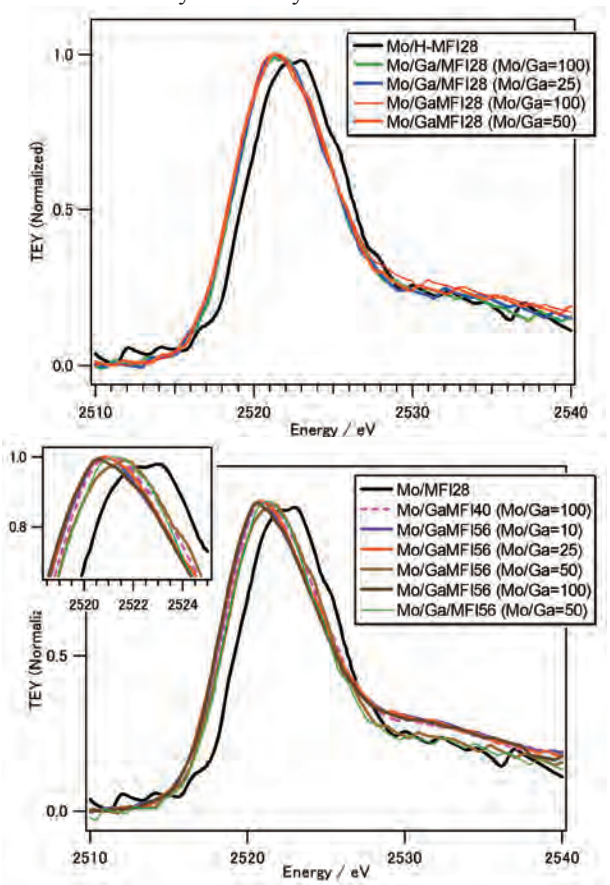

Fig. 1. Mo L_{III}-edge XANES of Ga- incorporated or modified Mo/MFI28 (upper) and Mo/MFI56 (lower) catalysts after MTB reaction at 1023 K.

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BL4U

Uptake of Dexamethasone into Human Skin Investigated by Soft X-Ray Spectromicroscopy

R. Flesch[1], T. Ohigashi[2], S. Küchler[3], K. Yamamoto[1], S. Ahlberg[4], F. Rancan[4], A. Vogt[4], U. Blume-Peytavi[4], P. Schrade[5], S. Bachmann[5], M. Schäfer-Korting[3], N. Kosugi[2] and E. Rühl[1]

[1]*Physical Chemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany*
[2]*UVSOR Facility, Institute for Molecular Science, Okazaki 444-8585, Japan*
[3]*Institut für Pharmazie, Freie Universität Berlin, 14195 Berlin, Germany*
[4]*Charité Universitätsmedizin, 10117 Berlin, Germany*
[5]*Abteilung für Elektronenmikroskopie at CVK, 13353 Berlin, Germany*

The uptake of drugs, such as dexamethasone, topically applied onto human skin is investigated by soft X-ray spectromicroscopy. Dexamethasone is a widely used for the treatment of inflammatory skin diseases such as atopic dermatitis. It is aimed to study the depth profile of dexamethasone, so that specific information on the uptake process is derived. Dexamethasone was dissolved in ethanol and this 0.5% solution was applied onto the skin sample for 4 h. Subsequently, the sample was fixed and sliced into 300 nm thick sections.

The experiments were performed at the BL4U beamline at UVSOR III using a scanning X-ray microscope (STXM) [1]. Chemical selectivity is obtained from excitation at the O 1s-edge (525-560 eV). Figure 1 shows a comparison of the O 1s-absoption of fixed human skin and dexamethasone. Both spectra are similar in shape, showing an intense O 1s→ π* resonance dominating the pre-edge regime. This resonance occurs at slightly lower energy in dexamethasone (E=530.5 eV) than in skin (E=532.2 eV), providing chemical selectivity for probing the drug uptake into skin.

Figure 2 shows a comparison of a skin sample exposed to dexamethasone probed by optical microscopy and soft X-ray microscopy. Figure 2(a) clearly shows the layered structure of the stratum corneum, the outermost skin layer, probed by optical microscopy. It is followed by the viable epidermis and the dermis. Figure 2(b) shows for the same section of the skin sample the spatial distribution of absorption, which is obtained from a difference image in X-ray absorption measured at 528 eV (pre-edge regime) and on the O 1s→π*-transition (530.5 eV) of dexamethasone (cf. Fig. 1) providing chemical selectivity. The spatially resolved results indicate that highest absorption contrast is found in the stratum corneum, as indicated by red color. In contrast, lower concentration is observed in the viable epidermis and no change in absorption contrast occurs in the dermis. It is also evident that the cells nuclei in the viable epidermis (circular structures in Fig. 2(a)) do not show any evidence for drug uptake.

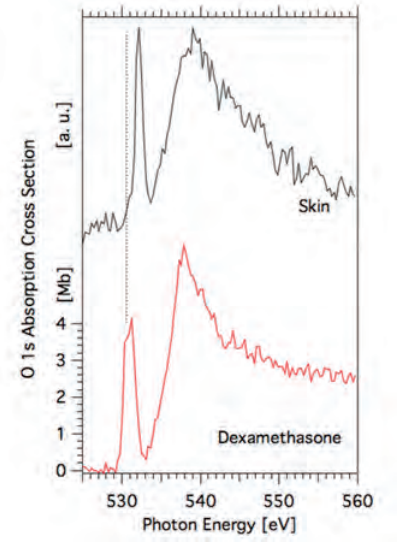

Fig. 1. O 1s excitation of fixed human skin (black curve) and dexamethasone (red curve).

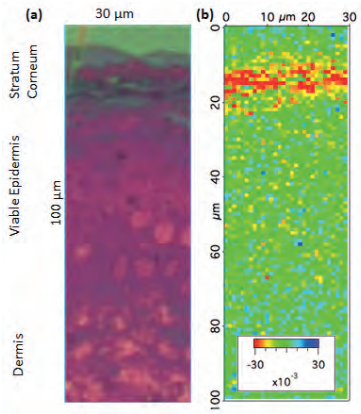

Fig. 2. (a) optical microscopy image of human skin; (b) spatial distribution of dexamethasone in the same skin section, as shown in (a). See text for further details.

[1] T. Ohigashi, H. Arai, T. Araki, N. Kondo, E. Shigemasa, A. Ito, N. Kosugi and M. Katoh, J. Phys. Conf. Ser. **463** (2013) 012006.

BL4U

Nanoscale Analysis of Microbe-Mineral Interface in Bioleaching Process by STXM Technique

S. Mitsunobu[1], S. Ming[1], H. Makita[2] and T. Ohigashi[3]
[1]*Institute for Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*
[2]*Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka 237-0061, Japan*
[3]*UVSOR Facility, Institute for Molecular Science, Okazaki 444-8585, Japan*

Some kind of microbe can dissolve and leach "solid mineral" to obtain life energy from the solid phase. This biotic leaching rate of minerals is much fast compared with its abiotic leaching rate [1]. Accordingly, the bacterial mineral leaching is recently used as a save-cost and save-energy technique for extracting metals (eg., Cu, Ni, Zn) from sulfide ores such as pyrite [2]. Previous papers imply that the microbes would produce some powerful extracellular substances to promote dissolution of the minerals effectively. On the other hand, the leaching of sulfide minerals leads to the formation of acid mine drainage. It is also important subject to understand the bioleaching process from a viewpoint of environmental sciences. However, little is known about the interfacial process at mineral-bacteria interface leading to the degradation of metal sulfides, because the interface is microscopic region and it is difficult to analyze it directly.

Here, we have tried to analyze the extracellular substances in bacteria-mineral interface in pyrite (FeS_2) bioleaching with a high spatial resolution by scanning transmission X-ray microscopy (STXM) installed at UVSOR BL4U, where is the first STXM beamline in Japanese synchrotron facilities. In this study, powdered natural pyrite (Navajún mine, Spain) and *Acidithiobacillus ferrooxidans* (JCM 7812 and ATCC 23270) were used for the sulfide mineral and leaching bacteria, respectively. The acidophilic, iron(II)-oxidizing bacteria *A. ferrooxidans* is one of the most important mesophiles for the extraction of metals from sulfidic ores by the bioleaching [1-2]. Samples for the STXM analysis were collected after 30 days incubation. The sample was dropped on Si_3N_4 membrane and air-dried at RT.

Figure 1 shows STXM images of *A. ferrooxidans* cells accreted on the pyrite particle for oxygen (O) and nitrogen (N). Characterization of extracellular matter in the bacteria-mineral interface was performed by near edge X-ray absorption fine structure (NEXAFS) at the O K-edge (Fig. 2). Features around the absorption edge and post-edge of lipid, DNA, sugar, and protein clearly varied. Spectral features of *A. ferrooxidans* cell are mostly similar to those of alginate and albumin representative for sugar and protein, respectively. Thus, O NEXAFS results indicate that *A. ferrooxidans* forms the extracellular substances on the pyrite surface containing the sugar such as polysaccharide addition to the protein, which was also supported by N NEXAFS and lectin-staining results (data not shown). The biogenic polysaccharides often form a strong complex with Fe ion under wide pH region [3-4]. Thus, our findings imply that *A. ferrooxidans* could produce the polysaccharides to accelerate the dissolution of pyrite and/or mediate contact between the cell and pyrite surface.

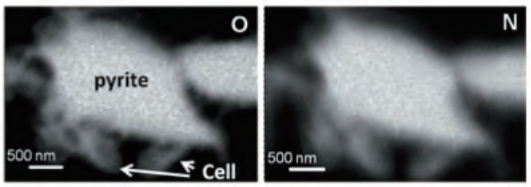

Fig. 1. STXM-derived elemental maps for oxygen (550 eV) and nitrogen (410 eV) of cells and surrounding pyrite particles.

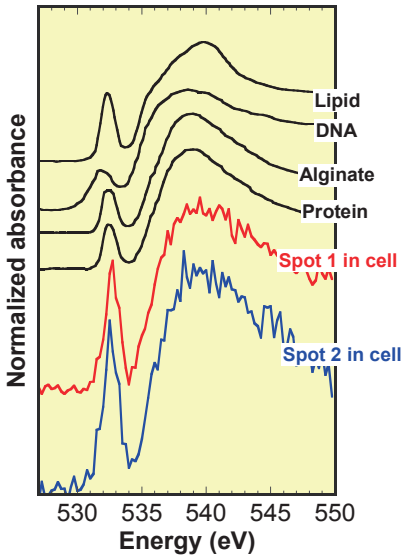

Fig. 2. STXM-based oxygen 1s NEXAFS spectra of standards and samples. Standards contain bovine serum albumin (protein), alginate (acidic polysaccharide), *E. coli* DNA and 1,2-dipalmitoyl-sn-glycero-3-phospho ethanolamine (lipid).

[1] H. Tributsch, Hydrometallurgy **59** (2001) 177.
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[3] C. Chan *et al.,* Geochim. Cosmochim. Acta **73** (2009) 3807.
[4] S. Mitsunobu *et al.,* Environ. Sci. Technol. **46** (2012) 3304.

BL4U

Observation of DNA and Protein Distributions in Mammalian Cell Nuclei Using STXM

T. Ohigashi[1,2], A. Ito[1,3], K. Shinohara[3,4], S. Tone[5], M. Kado[4], Y. Inagaki[1], Y. F. Wang[1] and N. Kosugi[1,2]

[1]*UVSOR Facility, Institute for Molecular Science, Okazaki 444-8585, Japan*
[2]*The Graduate University for Advanced Studies (SOKENDAI), Okazaki 444-8585, Japan*
[3]*Tokai University, Hiratsuka 259-1292, Japan*
[4]*Japan Atomic Energy Agency, Kizugawa 619-0215, Japan*
[5]*Kawasaki Medical School, Kurashiki 701-0192, Japan*

To observe the structure of biological samples, an electron microscope and a fluorescence microscope are extensively used. However, the former requires specimen in vacuum and the latter has relatively lower spatial resolution. Moreover, the electron microscope usually requires several preparation processes for the samples, such as fixing, slicing and staining. On the other hand, a soft X-ray microscopy is applicable to relatively thick specimen even under hydrated condition at high resolution, and is expected to be complementary to these two types of microscopes. A scanning transmission X-ray microscope (STXM) must be a powerful tool for this purpose [1]. The STXM has high spatial resolution, high transmittance and lower radiation damage than the electron beam. Especially, chemical analysis combined with near edge X-ray absorption fine structure (NEXAFS) enables us to obtain 2-dimensional chemical information of the sample [2]. In this study, nuclei of cultured human cells were observed with the STXM installed on UVSOR BL4U to image the distributions of DNA and protein separately.

NEXAFS spectra of the DNA and histone, a nuclear protein, were measured by the STXM as reference data. Their suspensions were dropped onto 100 nm-thick silicon nitride membranes and were dried in the air. Their NEXAFS spectra around nitrogen 1s are shown in Fig. 1. In these spectra, a remarkable feature to discriminate the DNA from the protein is seen on a peak at 400.8 eV as nitrogen 1s→π* resonance arising from C=N double bonds in the DNA.

Human A549 cells derived from lung cancer were cultured directly on the silicon nitride membrane, fixed with glutaraldehyde, and dried in the air. The sample was placed in the STXM chamber, which was then evacuated and was filled by helium to 30 mbar. The 51 X-ray transmission images (an energy stack) were acquired with changing the X-ray energies from 399 to 404 eV. The dwell time and the scanning pitch of the specimen were 5 ms and 0.2 μm step, respectively. The reference spectra of the DNA and the histone in Fig. 1 were fitted to the energy stack by using aXis2000 software [3] and their distributions are shown in Fig. 2. Figures 2 (a) and (b) show distributions of the DNA and the histone (protein), respectively. Figure 2 (c) shows the distribution of constant profile with no spectral feature, suggesting that in the nucleolus molecules other than the DNA and/or the histone (protein) are densely accumulated. The results show that the DNA was distributed over the nucleus, while the histone was poorly distributed in the nucleolus. Considering that RNA is rich in the nucleolus, the RNA may be present with less protein in the nucleolus.

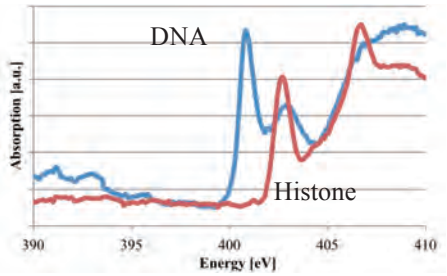

Fig. 1. X-ray absorption spectra of the DNA and the histone (protein) around nitrogen 1s resonance.

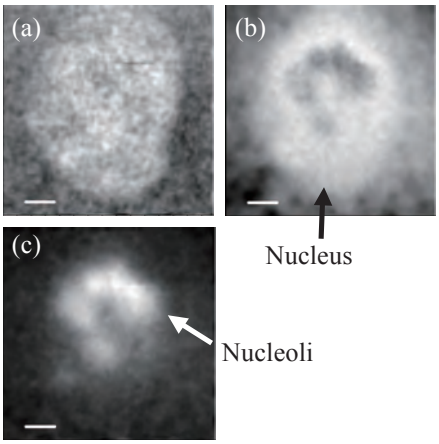

Fig. 2. Distributions of the DNA (a), the histone (protein) (b) and constant (c) in the cell. Bright color shows high density. Scale bars are 2 μm.

[1] J. Kirz *et al*., Nucl. Instr. and Meth. B **87** (1994) 92-27.
[2] T. Ohigashi *et al*., J. Phys. Conf. Ser. **463** (2013) 012006.
[3] http://unicorn.mcmaster.ca/aXis2000.html

BL4U

Nitrogen- and Oxygen-XANES Spectral Analysis of Mineral Olivine and Organic Material from Meteorite: Preliminary Measurements for Implementation Planning of a Synchrotron Cosmochemistry Research Base at UVSOR BL4U

H. Yabuta
Department of Earth and Space Science, Osaka University, Osaka 560-0043, Japan

The PI has eight years of experiences of a scanning transmission x-ray microscope (STXM) operations and analyses in cosmo- and geochemistry [e.g., 1-3]. Making great use of her experiences, she is planning to construct a synchrotron cosmochemistry research base at BL4U, UVSOR, under cooperation with a beamline manager. Because of the first use of the STXM (Bruker) built at BL4U, UVSOR, this year, the initial purpose of this study was to acquire the operation of the STXM and to confirm the reproducibility of the STXM measurements of organic materials from meteorites. However, unfortunately, it was impossible to measure Carbon K-edge XANES spectra, due to a bad condition of the STXM stage motions and contamination of the mirror. This report summarizes the preliminary result of Nitrogen and Oxygen K-edge XANES.

As samples, mineral olivine (magnesium iron silicate) and acid-insoluble organic solids (IOM) isolated from carbonaceous meteorite Asuka 881458 were used. Thin sections (100 nm thickness) of the individual samples were prepared by a focused ion beam (FIB) - scanning electron microscope (SEM). FIB sections of olivine Nos. 1 and 2 were prepared before and after that of IOM was prepared, respectively. Nitrogen- and Oxygen-X-ray absorption near edge structure (XANES) spectra of the FIB sections were acquired using a scanning transmission x-ray microscope (STXM) at the BL 4U, UVSOR.

Nitrogen- and Oxygen-XANES spectra of olivine (Nos. 1 and 2) and IOM are shown in Fig. 1. O-XANES spectrum of meteorite Asuka (A) 881458 IOM exhibits a peak of organic carbonyl group (C=O) at 532 eV. This peak was not identified from the O-XANES spectra of olivine samples 1 and 2, demonstrating that the previous sample did not contaminate the latter sample during the FIB procedures. Likewise, N-XANES spectrum of meteorite Asuka (A) 881458 IOM exhibits absorption peaks of organic imine (C=N), nitrile (C≡N), and amide (NHx(C=O)C) groups at 399, 400, 402 eV, respectively, while those of olivine samples 1 and 2 do not.

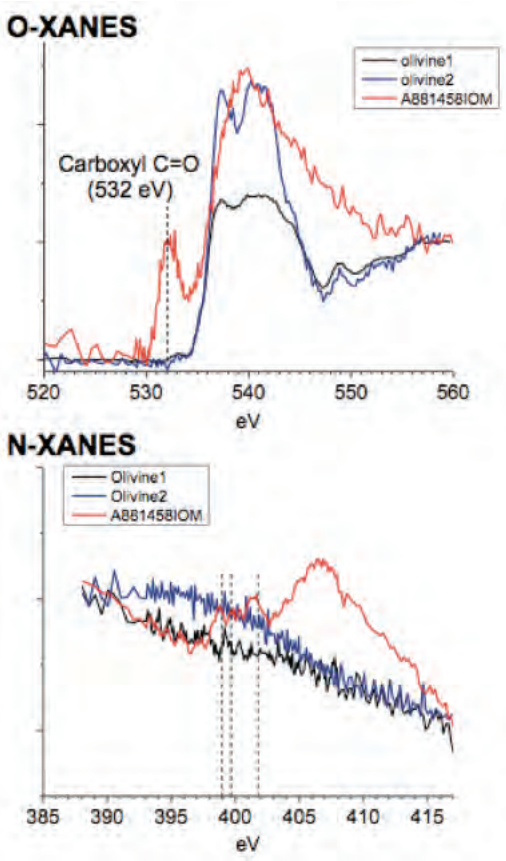

Fig. 1. N- and O-XANES spectra of mineral olivine samples Nos. 1 (black) and 2 (blue) and acid-insoluble organic solids isolated from carbonaceous meteorite Asuka (A) 881458 (red).

[1] S. A. Sandford *et al.,* Science **314** (2006) 1720-1724.
[2] G. D. Cody *et al.,* Meteoritics & Planetary Science **43** (2008) 353 – 365.
[3] H. Yabuta *et al.,* The 44th Lunar and Planetary Science Conference. Abstract (2013) #2335.

BL4U

Organelle-Spectra of a Leydig Cell in Cultural Fluid

T. Ejima[1], R. Hirose[1], M. Ishino[2], M. Kado[2], M. Aoyama[3], K. Yasuda[3] and T. Tamotsu[3]
[1]*IMRAM, Tohoku University, Sendai 980-8577, Japan*
[2]*Japan Atomic Energy Agency, Kizugawa 619-0215, Japan*
[3]*Dept. of Biological Sciences, Fac. of Science, Nara Women's University, Nara 630-8263, Japan*

To investigate suitable wavelength for observing organelles in a bio-cell with fluid in Water-window wavelength region, contrast of each organelle in a bio-cell to fluid or the other organelles will be determined by absorption spectra of each component. For this purpose, soft X-ray absorption images of Leydig cells in cultural fluid were observed controlling the thickness of the fluid. Spectra of both organelles and fluid will be obtained from the images that irradiate wavelength was changed.

At first, Leydig cells of mouse testes cultured and fixed on a SiN membrane were preserved in the cultural fluid during the X-ray observation. Thickness of the cultural fluid was controlled by a SU8 film deposited around the membrane. The membrane with the bio-cells was placed in a holder and the holder was set to the stage under He atmosphere to avoid leak of the fluid during the observation. The observation was made at 2.9～3.1nm (395～430eV) wavelength region under the conditions that the step width was 300nm, exposure time, 5 msec/pixel, and wavelength resolution λ/Δλ, 5000.

Organelle structures in a cell and spectra corresponding to the structures were obtained applying PCA and Cluster analyses to the observation results [1]. Figure 1 (a) shows an eigen-image that shows maximum value of the contribution ratio obtained from the PCA analysis. The eigen-image represents that several large organelles exist in the star-shaped bio-cell and many dot-structures are around the large organelles.

According to Cluster analysis, these organelles are categorized by the degree of similarity as the map represented in Fig. 1 (b). In the map, blue area represents the culture fluid and the other areas, a bio-cell and organelles in the cell.

Figure 1 (c) shows that the absorption spectrum that corresponds to the yellow area of Fig. 1(b). In the spectrum, there is a sharp peak at 400eV and a broad peak with a shoulder structure at 405 eV. In addition, small structures exist at 398 eV, 402 eV, and 404 eV. The sharp peak at 400 eV and the broad one at 405 eV should be originated from the π* and σ* structures, respectively, of C-N bonds in the organelles [2]. Small and shoulder structures will be satellite structures of C-N bonds and the details of the origin need further investigation.

Small organelles such as dot structures in Fig. 1(a) are recognized in the eigen-images, but the spectra of the small orgranelles are not clearly separated from the other organelles. The experimental conditions and image-analysis techniques will be improved.

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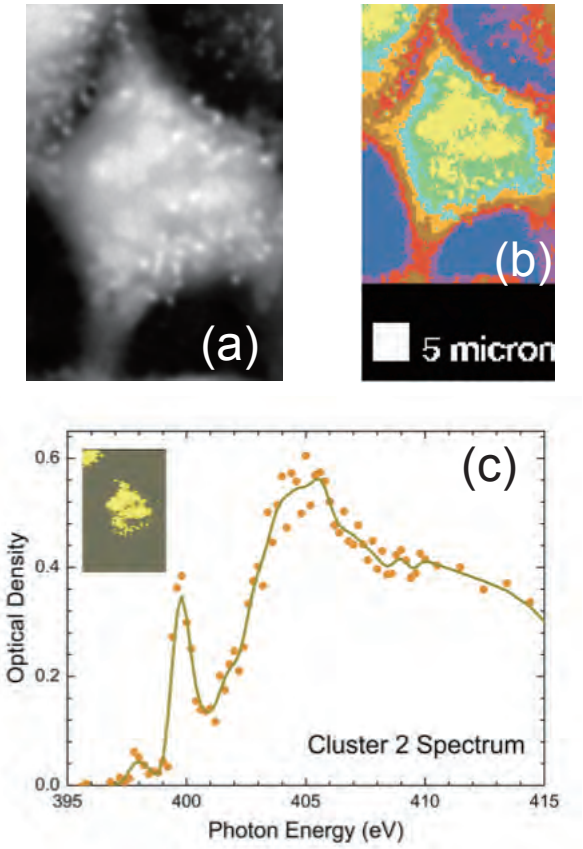

Fig. 1. (a) Optical density image of a Leydig cell. (b) Clusters obtained from cluster analysis on the basis of the results of PCA analysis. (c) Optical density spectrum of the yellow area represented in (b) (inset).

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[2] Y. Zubavichus, A. Shaporenko, V. Korolkov, M. Grunze and M. Zharnikov, J. Phys. Chem. B **112** (2008) 13711-13716.