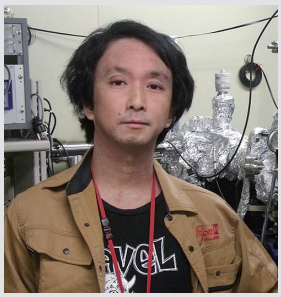
# Application of X-Ray Microscopy

**UVSOR Synchrotron Facility**
**Division of Beam Physics and Diagnostics Research**



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A synchrotron-based scanning transmission X-ray microscope (STXM) is a technique to perform 2-dimensional (2D) X-ray absorption spectroscopy with high spatial resolution. The schematic image is shown in Figure 1. A monochromatic X-ray is focused by an X-ray focusing lens, a Fresnel zone plate, on a sample as a diameter around 30 nm through an order select aperture and the transmitted X-ray is detected. By scanning the sample 2-dimensionally, an X-ray absorption image is obtained. Then, by noticing the near edge X-ray absorption fine structure (NEXAFS) of the specific element, 2D chemical state of the sample can be obtained. Since characteristics of UVSOR is suitable for using extreme ultra-violet and soft X-ray region, the STXM in UVSOR, BL12, is suitable to analyze soft materials and organic materials. The X-ray range from 55 to 770 eV is a unique feature of BL12 and enables to approach lithium K-edge (55 eV~) with spatial resolution at 72 nm.[1] The advantages of STXM, such as high transmittance of X-ray and relatively wide working distance, gain flexibility of the sample and its environment. Therefore, we have been developing special observation/analytical techniques mainly by designing sample cells for STXM.[2] Especially, nowadays, an in-situ/operando analytical technique is attracting more attentions of researchers because that is an important technique to understand intrinsic state of the samples. Recently, heating and cooling of the sample, humidity control system and electrochemistry, 2D orientation of molecules, 3D chemical state mapping, a sample transfer system without exposing to air and microscopic analysis of chemical state of lithium have been developed to explore a new filed of science.[3] These techniques are difficult to perform by using the other microscopic techniques, such as an electron microscope.

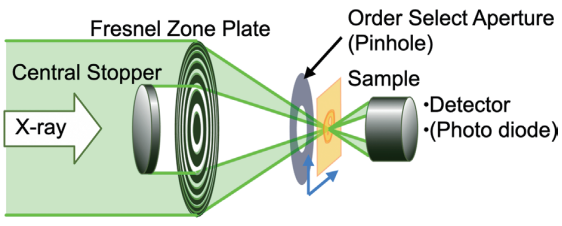


**Figure 1.** Schematic optical system of STXM.

## Selected Publications


- T. Ohigashi and N. Kosugi, "Developments in Sample Environment for a Scanning Transmission X-Ray Microscope at UVSOR-III Synchrotron," *J. Electron Spectrosc. Relat. Phenom.* **266**, 147356 (2023).
- M. Ito *et. al*., "Hayabusa2 Returned Samples: A Unique and Pristine Record of outer Solar System Materials from Asteroid Ryugu," *Nat. Astron.* **6**, 1163–1171 (2022).
- T. Ohigashi, H. Yuzawa and N. Kosugi, "A Low-Pass Filtering Fresnel Zone Plate for Soft X-Ray Microscopic Analysis down to the Lithium K-Edge Region," *Rev. Sci. Instrum.* **91**, 103110 (2020).
- D. Asakura, E. Hosono, M. Kitamura, K. Horiba, E. Magome, H. Setoyama, E. Kobayashi, H. Yuzawa, T. Ohigashi, T. Sakai, R. Kanega, T. Funaki, Y. Sato and A. Ohira, *Chem. –Asian J.* **18**, e202201047 (2023).
- Y.-C. Huang, Y. Li, K. T. Arul, T. Ohigashi, T. T. T. Nga, Y.-R. Lu, C.-L. Chen, J.-L. Chen, S. Shen, W.-F. Pong, C.-L. Dong and W.-C. *Chou, ACS Sustainable Chem. Eng.* **11**, 5390–5399 (2023).

## 1. 3-Dimensional near Edge X-Ray Absorption Fine Structure of an Isolated Cell Nucleus

Computer tomography (CT) is an arithmetic method to reconstruct a 3D structure from serial tilted 2D X-ray absorption images without any destructive process. Reconstructed images of CT with full-rotation (or 180° rotation) data acquisition have quantitative values of X-ray line absorption coefficient. Therefore, by changing the X-ray energies around the absorption edge, 3D nano-NEXAFS can be performed. CT is typically performed by using full-field imaging X-ray microscopy because of shorter acquisition time of 2D X-ray transmission images. On the other hand, in regard to the radiation dose, STXM-CT is one order less than by the full-field imaging CT. This advantage is preferable to analyze 3D nano-NEXAFS of organic and bio samples with complicated structures, such as a cell nucleus. To establish STXM-CT, we have designed a rotating sample cell.[4,5)]

An isolated cell nucleus of a HeLa S3 cell was chemically fixed with glutaraldehyde. After the critical point drying, the cell nucleus was glued on a tip of a tungsten needle (TP-001, Micro Support co., ltd.) by a crystal bond. 50 energy stacks around O K-edge (530 ~ 538 eV) dataset, $f(x,y,\theta,E)$, were obtained with tilting the sample 3.6° each, in total 180° rotation. In the energy stack, X-ray absorption images were acquired by scanning 8×8 μm$^2$ area of the sample with 160 nm pitch. Then, the dwell time was 1 ms per pixel. In total, the whole measurement process took ~12 hours. As pre-reconstruction process, the X-ray absorption images in all the energy stacks were grouped according to the X-ray energy. The 2D cross sectional images were reconstructed from each group and the 3D image was obtained by stacking those images. Finally, a 3D NEXAFS mapping dataset, $F(x,y,z,E)$, was obtained by sorting the reconstructed 3D images by the X-ray energy. For example, a 3D volume projection image is shown in Figure 2(a). A reconstructed cross sectional image in the plane shown in Figure 2(a) by a red dashed line and its local XAS spectra are shown in Figure 2(b) and 2(c), respectively. O K-edge spectra were extracted from structures of cell nucleolus (red and green areas) and cytoskeleton (yellow and blue areas) in Figure 2(b). Figure 2(d) shows RGB color distribution of these chemical components by performing SVD fitting to the 2D NEXFAS by using aXis2000 software. The colors of the plots and of the RGB map are coincident with those of the area in Figure 2(b) except for the yellow plot. In Figure 2(d), the distribution of the green color is not only at the cell nucleolus but also slightly at the cytoskeleton. In the case of the measurement of biological samples, the measurement under cryo condition is necessary to keep samples from radiation damage.

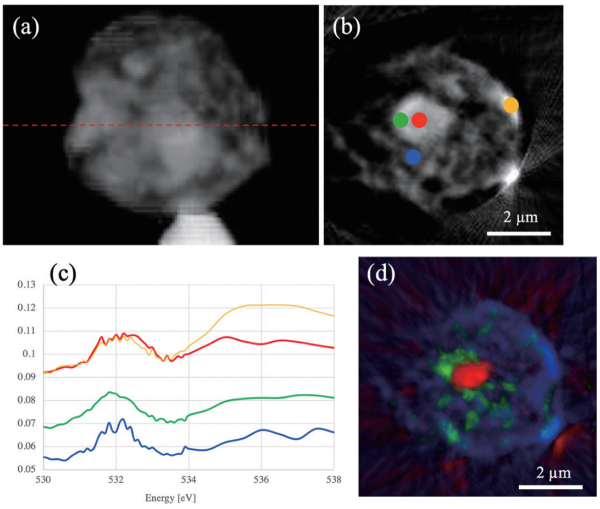


**Figure 2.**[3)] (a) A 3D volume projection image of an isolated cell nucleus of HeLa S3 cell, (b) a reconstructed cross sectional image of the cell nucleus at a red dashed line, (c) X-ray absorption spectra extracted from 2(b) and (d) a RGB color distribution of the spectra. Colors of the plots and the RGB color distribution are coincident with those of areas in (b).

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2) T. Ohigashi, M. Nagasaka, T. Horigome, N. Kosugi, S. M. Rosendahl and A. P. Hitchcock, *AIP Conf. Proc.* **1741**, 050002 (2016).
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4) T. Ohigashi, Y. Inagaki, A. Ito, K. Shinohara and N. Kosugi, *J. Phys.: Conf. Ser.* **849**, 4 (2017).
5) T. Ohigashi, A. Ito, K. Shinohara, S. Tone, Y. Inagaki, H. Yuzawa and N. Kosugi, *Microsc. Microanal.* **24(S2)**, 400–401 (2018).