Soft X-Ray Spectro-Microscopy and Scattering for Life Science—beyond Organelle Mapping

UVSOR Synchrotron Facility
Division of Advanced Photochemistry

ARAKI, Tohru
Senior Researcher

Beside running the user programs using the BL4U at the UVSOR, which is a STXM (Scanning Transmission X-ray Microscopy) beamline. STXM is one of the x-ray based spectro-microscopy techniques providing us a label-free chemical mapping for a wide variety of sciences like energy materials, environmental and earth science, and many in the industrial science as well. I want to push this technique applying for a life science, especially with soft x-rays, which has been studied by mainly hard x-ray regimes such as protein crystallography and small angle scattering at the synchrotron radiation facilities so far.

One of the most effective soft X-ray microscopes, the so-called soft X-ray tomography (SXT), can offer unparalleled 3D organelle mapping of entire biological cells larger than 10 micrometres. The SXT uses a fixed photon energy, just before the O K-edge absorption at 520 eV, to observe the hydrated sample using the "water window" energy region. This allows us to see the absorption contrast of the biological sample, which comes from the carbon and nitrogen content inside them. The distinctive LAC (linear absorption coefficient) at 520 eV of each organelle enables the delineation of these structures. I intend to extend this analysis by employing the full potential of the SXAS (soft X-ray absorption spectroscopy) technique, which allows for the utilisation of a range of photon energies. The utilisation of SXAS-based spectro-microscopy and spectroscopy-scattering enables the differentiation of analogous organelles and oxidation states of metal/ion within cellular structures. In order to establish this methodology, two key steps must be taken: Firstly, a basic spectral interpretation of organelles must be conducted, and secondly, the sample preparation and specimen environment must be optimised. It is of particular importance to refrain from any modifications of the samples and to maintain the native states of the cells, including the loss of metals or ions, throughout both the sample preparation and the data collection process, in order to prevent radiation damage.

In the current year, I conducted a collaborative research project on *Ramazzottius varieornatus*, a tardigrade that is renowned for its anhydrobiotic capabilities, enabling it to survive in harsh, arid environments. My colleague prepared the thin section samples embedded in resin for a comparative morphometric analysis of their microscopic anatomy using both scanning electron microscopy (SEM) and STXM. Figure 1 (a) illustrates a representative optical microscope image. In order to enhance contrast, a staining agent must be applied to the sample prior to conducting a SEM analysis. However, it offers a higher spatial resolution than other techniques. In comparison, STXM is a label-free method that provides a lower spatial resolution, yet still achieves a reasonable resolution of tens of nanometres. Figure 1 (b) depicts a STXM transmission raw image collected at the protein characteristic photon energy. Figure 1 (c) presents a RGB composite map, comprising the three STXM images acquired at distinct photon energies. The images provide insight into the structural and compositional characteristics of different organelles and biological molecules.

These results prove the STXM's capabilities. However, there is still work to be done to fully understand the images and extract all the information they contain. One possible approach is the cryogenically vitreous ice-covered sample prepared by the plunge-freeze method. This is currently regarded as the gold standard for such sample preparation in the fields of Cryo-EM and Cryo-SXT.

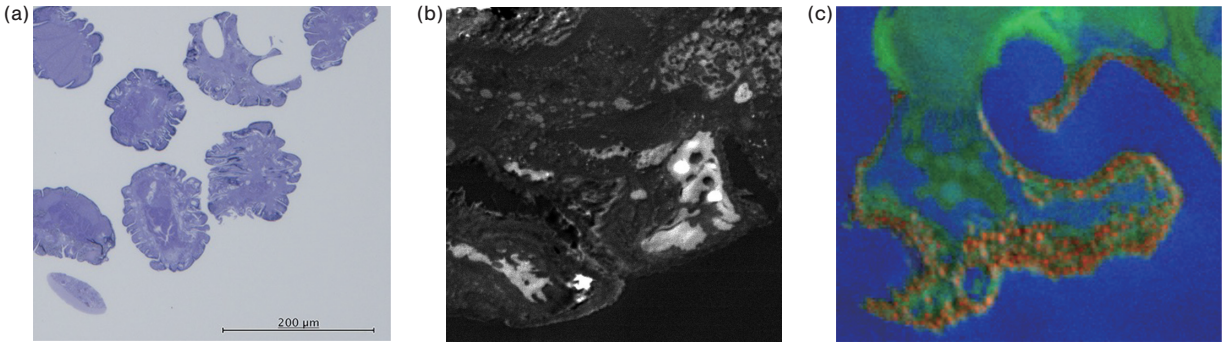

Figure 1. (a) The optical microscope images of the stained tardigrade thin section tissue samples. (b) The STXM transmission image of the tardigrade thin section tissue sample. (30 × 30 μm). (c) The RGB composite map of the tardigrade samples obtained by the STXM images at the three different photon energies. (16 × 14 μm).