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

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As a beamline scientist, I oversee the user science programs, in both academia and indsutry that utilize the BL4U-STXM (Scanning Transmission X-ray Microscopy) beamline at the UVSOR research facility. STXM is a form of X-ray absorption-based spectromicroscopy that provides label-free chemical mapping.

STXM is a specialized imaging technique that falls under the category of X-ray absorption-based spectromicroscopy. By measuring how different materials absorb X-rays at specific energies, STXM enables label-free chemical mapping, allowing researchers to visualize the distribution of various chemical components within biological and material samples without the need for dyes or stains. It has a wide range of applications in various fields, including energy materials, environmental and earth sciences, and industrial polymer studies. My current focus is on “beyond organelle mapping,” which requires the advanced spectroscopy to identify the biomacromolecules. Shinohara *et al.* conducted a related study at the BL4U (*cells* 2019). The team presented the quantitative mapping of DNA, RNA, histones, and general proteins in mammalian cells, nuclei, and a chromosomes. This was achieved through the spectral fitting of the reference spectra. To accommodate a broader range of biological samples, including cells and tissues, it is essential to achieve higher chemical sensitivity and enhanced accuracy. “To establish this methodology, two key steps must be taken. First, a basic spectral interpretation of organelles must be conducted. Second, the sample preparation and specimen environment must be optimized. It is imperative to refrain from altering the samples and to preserve 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.” This is my statement from last year. However, due to technical difficulties and the need for a direct comparison between STXM and SEM, I have been working on the biological sample embedded in resin. The sample was ultra-thin sliced using a diamond knife to create a sample 100 nm thick for the STXM measurement. The bottom figures show the STXM results of *Ramazzottius varieornatus*, a tardigrade that is renowned for its anhydrobiotic capabilities, enabling it to survive in harsh, arid environments. The data was collected at the Carbon K-edge absorption for both active (hydrated) and inactive tardigrade samples. The experiment was conducted with the support of ExCELLS. My collaborators prepared the samples and conducted the SEM experiment. The spectra displayed in the Figure 1 correspond to the five significant components that were analyzed using principal component analysis and cluster analysis techniques of the active tardigrade. The following presentation offers a visual representation of the two component maps of C4 and C1 spectra for both active and inactive tardigrades. The distribution of the two components differs between two samples. To further elucidate these results, additional STXM data analysis and the scheduled immunoelectron microscopy experiment will be conducted.

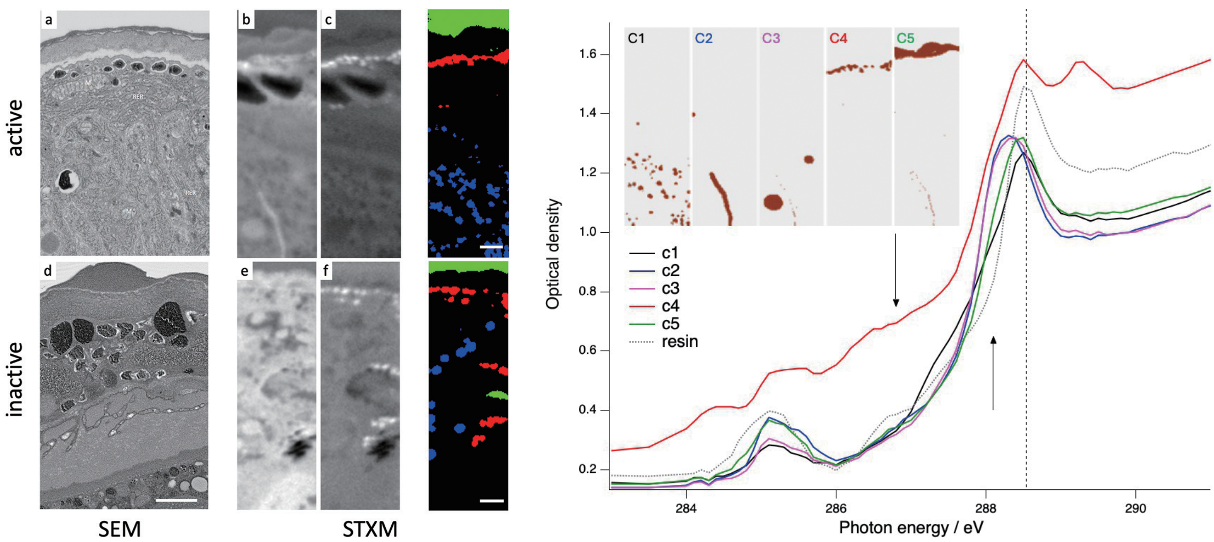

Figure 1. [left] SEM and STXM images of active (top) and inactive (bottom) tardigrade samples. (Scale bar 1 µm) RGB composite component map (red and blue: Two significant components, green: Resin). [right] 5 components map and the corresponding spectra of active tardigrade sample. (Two arrows indicate the photon energies used for the STXM images (b, e: 288.1 eV, c, f: 286.8 eV). The 5 significant components map is shown in C1–C5 images.)