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Correlation of Soft X-ray Tomography with Fluorescence Microscopy in Biological Study

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Soft X-ray tomography (SXT) is a newly developed technique for 3D whole cell image. Imaging the biological specimen with SXT is based on the organic composition of subcellular constituents. The soft X-ray with energy of water window, which is between the K-edge absorption of carbon (284 eV) and oxygen (543 eV), can produce high different absorption coefficient between biological samples and water to make a natural contrast of the image. The penetration depth of the water window in biological samples can be about 10 μm, indicating it is able to acquire a 3D image from nearly native thick cells without the need of staining and sectioning of the specimen [1,2]. SXT has been demonstrated to visualize the internal structure of whole cells [3]. With the demand of a biological community, SXT beamline and endstation are under construction at Taiwan Photon Source (TPS) in National Synchrotron Radiation Research Center (NSRRC). The beamline covered the energy range of 0.26 ~ 2.6 keV is dedicated to image frozen-hydrated biological samples.

The SXT microscope is designed with a high demagnification condenser and an objective Fresnel zoneplate with spatial resolution of 15~30 nm for 2D imaging and 50 nm for 3D tomography, which can fill the gap between the fluorescence microscopy and electron microscopy in biological studies. However, the location of functional proteins cannot be identified directly from SXT images. Therefore, it is important to correlate a fluorescence microscope with SXT for the complements to have 3D images in the region of interest (ROI) [4]. In SXT endstation, an on-line fluorescence structure illumination microscope (SIM) is adopted to be correlated with SXT to provide biological samples with functional and structural information in the ROI, as shown in figure 1. The endstation is planned to be in commissioning in the middle of 2017.

Besides a construction, a preliminary experiment of visualization mast cell degranulation by the correlation of X-ray tomography with fluorescence microscopy was performed in U41-SGM beamline at BESSY II, Germany [5]. To examine the location of the granule, it is important to prelabel the cells with a fluorescent dye that highlights granules and mitochondria. Frozen cells were prepared by cryo-fixation with plunge freezing in liquid ethane. The prepared frozen samples were firstly imaged by a cryo-screening system with brightfield and fluorescence microscopy to select the samples with suitable ice condition, and also to determine the location of ROI from a merged fluorescence image, before the soft-X-ray imaging. Once the determination, tilt images of selected cell in ROI were collected by rotating the sample with the increment of 1 degree. We applied a free software of IMOD to complete the image reconstruction, as shown in figure 2.

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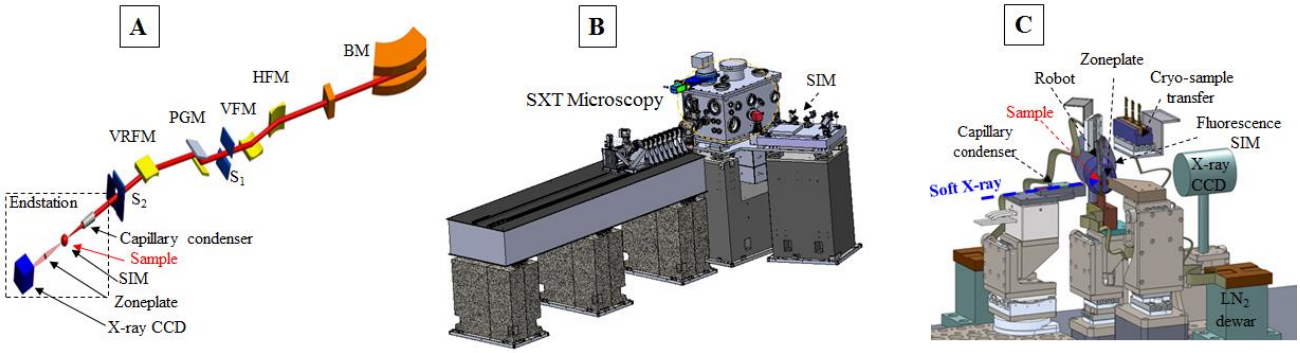

Figure 1. Conceptual design of soft X-ray tomography beamline and endstation. (A) Optical layout of SXT beamline and microscopy. (B) Drawing of the endstation, including the SXT microscopy and structural illumination fluorescence microscopy (SIM). (C) Conceptual design of the endstation inside the vacuum chamber.

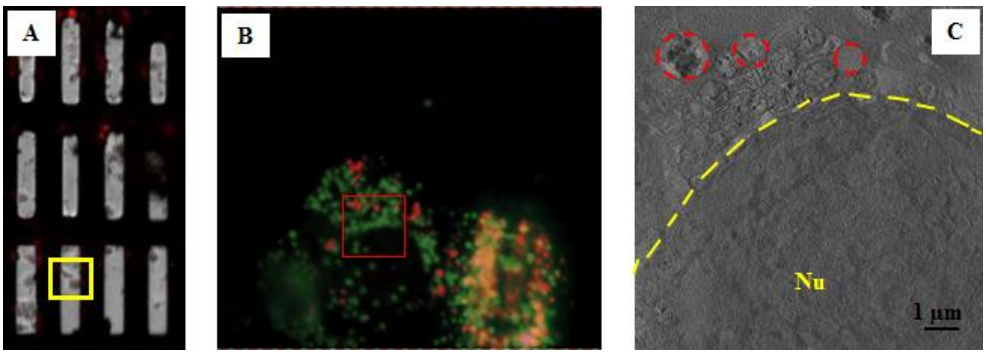

Figure 2. Correlation of soft X-ray tomography with fluorescence microscopy on prelabeled granules. (A) Merged image of cryo-cells from brightfield and red-fluorescence images. That image was captured with a 10x objective lens on the cryo-fluorescence screening system; (**B**) Merged fluorescent image of cryo-cells. That image was captured with a 100x objective shown in (A). Mitochondria (green) and granules (red) are visible. (C) A reconstructed image from SXT was coordinated with the fluorescent dye stained granules (circle labeled) shown in (B). Nu: nucleus.

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