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Development of a Correlation of Soft X-Ray Tomography with Fluorescence Microscopy at Taiwan Photon Source

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We present the construction of a full-field transmission soft X-ray tomography (SXT) beamline at Taiwan Photon Source (TPS). The beamline, designed with a soft X-ray energy range of 200~3,000 eV, is used to image frozen-hydrated biological specimens. Owing to the organic compositions of such specimens, they have a high natural absorption contrast with the water in the energy range of water window which is the energy between K-edge absorption of carbon (284 eV) and oxygen (543 eV). The penetration depth of soft X-ray energy of water window in bio-specimen is about 10 μm. Indicating that acquiring of 3D image from nearly native cells is possible without the need for staining and sectioning [1]. SXT has been revealed to visualize the internal structures of whole cells [2]. To meet the design demands of a condenser in X-ray microscopy, the optics of varied-lines-spacing plane-grating monochromator (VLS PGM) are applied to provide a secondary source with a fixed focus during the energy scanning [3]. To obtain highly and homogeneously efficient reflectivity at different energies, the sides of the mirrors are coated with the materials of gold and rhodium, including a horizontal focus mirror (HFM), a vertical focus mirror (VFM) and a vertical refocus mirror (VRFM). The gold-coated mirrors are applied at low energy part, 200~1,200 eV, and the rhodium-coated mirrors are utilized at high energy part, 1,200 ~3,000 eV [4]. Photons with different energies are focused at $slit_2$ where is the secondary source for the condenser in SXT microscopy. Figure 1(A) shows the optical layout of the beamline and the microscope.

The SXT microscopy is used with a high demagnification capillary condenser and an objective Fresnel zoneplate with a spatial resolution of 15~30 nm for 2D imaging and 50 nm for 3D tomography [5]. However, since most of the organelles have similar contrasts and structures, the location of region of interest (ROI) in cellular structure cannot be easily determined by only an SXT image. Accordingly, the use of correlation of fluorescence microscopy with SXT helps to obtain 3D images of the ROI [6]. Therefore, this work develops on-line correlated fluorescence structured illumination microscopy (SIM) with SXT at TPS, to provide functional and structural information about the ROI of a biological specimen. The fluorescence detector is oriented 70° away from the SXT beam direction, as depicted in the photograph in Fig. 1(B). The construction of the beamline and the microscope is complete. The system is now commissioned. Figure 2(A) displays the endstation, including the direction of the soft x-ray input and CCD, the orientation of optical components in fluorescence microscope, and the locations of the liquid nitrogen inlet and the cryo-sample load lock system. To determine the direction of the beam, a soft X-ray-sensitive phosphor screen is located downstream of the zoneplate as a monitor. Figures 2(B-D) present the photons on the phosphor screen under various conditions as preliminary results during the optical alignment of the capillary condenser in the endstation. Figure 2(B) displays a bright spot on the screen, indicating a direct beam of photons with an energy of 520 eV on the screen unfocused by the capillary condenser. Figure 2(C) shows two small symmetric arc shapes on the screen, indicating the photons with an energy of 520 eV that are focused by the capillary condenser during the opening of rectangle shape of X-ray beam position monitor (XBPM) to 12 x 2 mm^2 (H x V). The location of XBPM is upstream of the

HFM. Once the XBPM opens to 20 x 20 mm^2 (H x V), a ring shape is observed on the phosphor screen, demonstrating that all photons are focused by the capillary condenser. The central spot on Fig. 2(C) and 2(D) indicates the direct beam of photons with an energy of 520 eV unblocked by the stopper. The stopper is located between $slit_2$ and the capillary condenser. We expect to obtain the first image of a sample at room temperature very soon.

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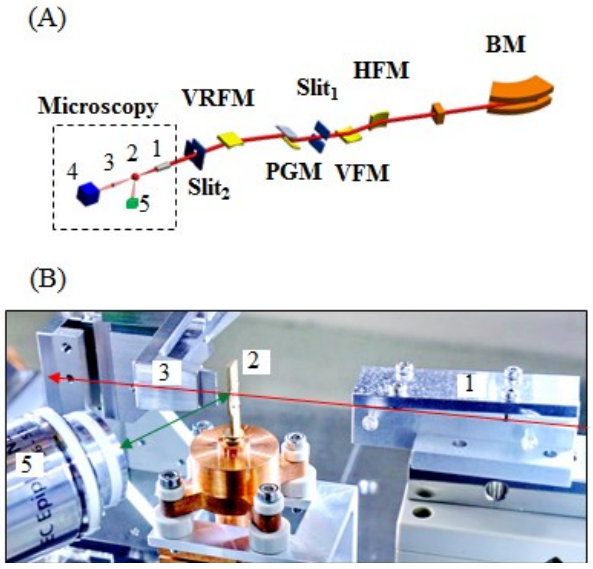

Figure. 1. (A) Layout of beamline for SXT. (B) Photography of SXT with fluorescence microscope in vacuum chamber. 1-Capillary condenser; 2-Sample; 3-Zoneplate; 4-CCD; 5-Fluorescence detector.

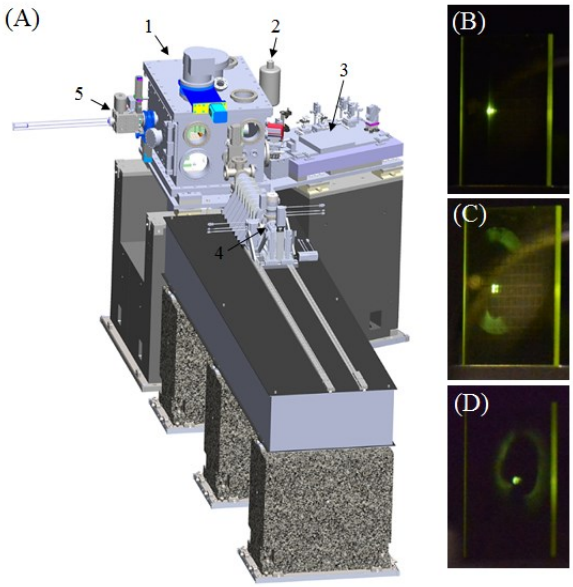

Figure. 2. (A) Drawing of endstation. 1-Soft X-ray in; 2-Liquid N_2 inlet; 3-Optical components of fluorescence microscope; 4-CCD for SXT; 5-Cryo-sample load-lock system. (B) Direct beam of 520 eV photons on screen, not focused by capillary condenser. (C) Photons with energy of 520 eV are focused by capillary condenser with XBPM opening of 12 x 2 mm^2 (H x V). (D) As (C), but XBPM opening is 20 x 20 mm^2 (H x V).