Development of Soft-X-ray Tomography for Biomedical Research

The soft-X-ray tomography (SXT) beamline is the first beamline in the second phase of construction of Taiwan Photon Source (TPS). This beamline, covering the energy range of 200–3,000 eV, is dedicated to a transmission full-field microscope to image 3D frozen-hydrated whole cells and tissue. Based on the organic composition of subcellular constituents, the energy in the water window, which is between the K-edge absorptions of carbon (284 eV) and oxygen (543 eV), can derive a high-absorption natural contrast of a biological sample from the water environment without staining. The depth of penetration of a biological specimen in the energy range of the water window is about 10 μm, which indicates that a native 3D cell can be imaged directly without sectioning.[1,2] To increase the probing depth, we can increase the X-ray energy to 3,000 eV. Another window of energy range 2,000–3,000 eV is consequently designed for the phase contrast of a biological sample. High energies can expand the depth of the focus and allow imaging of the tissue sample of thickness up to 50 μm.

Located at TPS port 24, the SXT beamline adopted a horizontal acceptance 1.2 mrad from the bending-magnet source. The photon beam from this source is collected with a pair of Kirkpatrick-Baez (KB) mirrors – a horizontal focusing mirror (HFM) and a vertical focusing mirror (VFM), to focus the beam horizontally on the position of the exit slit and vertically on the position of the entrance slit. To meet the demand for an endstation of full-field transmission soft-X-ray tomography, the optics of a plane-grating monochromator with varied line spacing (VLS PGM) has been adopted to provide a virtual source with a fixed position for a condenser in a X-ray microscope.[3] Three gratings are planned to cover the entire energy range, 200–3,000 eV. The last mirror, a vertical refocusing mirror (VRFM), refocuses the photons from a virtual image of the VLS PGM onto the position of the exit slit. **Figure 1** displays a basic concept of the beamline and microscope. **Figure 2** shows a photograph of the HFM and VFM that are located upstream of the beamline at 26 m and 28 m from the source, respectively. The photon flux at 520 eV is about 2.82 x 10^{11} photons s^{-1}. The microscope is designed with a combination of a capillary condenser and an objective Fresnel zone plate as the object lens.[2] The light from the virtual source at the exit-slit position is collected and focused with a capillary condenser (CC) on the sample position. The light transmitted from the sample is refocused with a zone plate (ZP) onto the position of a charge-coupled device (CCD). This microscope is designed to include a low-energy region, 200–1,200 eV, and a high-energy region, 1,200–3,000 eV. The microscope in the low-energy region can observe a 3D structure of nearly native cells; the magnification of an image is 1400 for energy in the water window. The microscope in the high-energy region can observe the 3D tissue structure; the magnification of image is 500 for energy 3,000 eV. Two objective zone plates with widths 25 and 40 nm of the outermost zone are provided. A spatial resolution 15–30 nm is expected to be achieved for 2D imaging and 50 nm routinely for 3D tomography. SXT can fill the gap between a fluorescence microscope and an

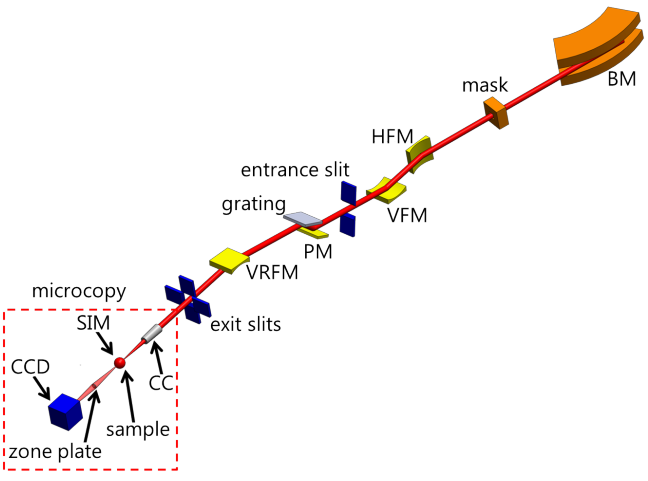

Fig. 1: Layout of the soft-X-ray tomography beamline and end station

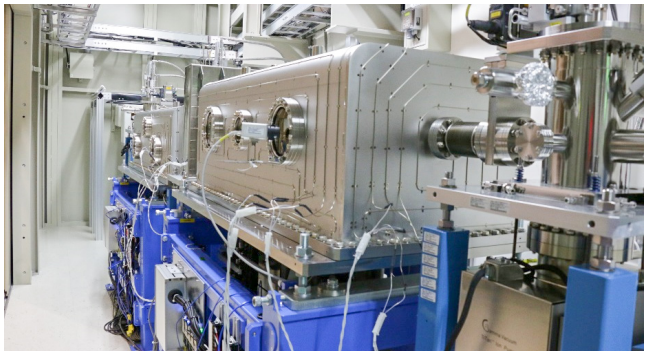

Fig. 2: HFM and VFM chambers

electron microscope in biological investigations. As the location of functional proteins in a cell cannot be identified directly from SXT images, it is important to have a fluorescence microscope to complement SXT to image a cell in a region of interest.[4-6] Herein, we adopted a high-resolution fluorescence structured- illumination microscope (SIM) that is correlated online with the SXT to derive from a biological specimen the desired structural and functional information. The light path of the fluorescence SIM is 70° off the beam of the SXT. **Figure 3** shows a photograph of a correlation of SXT and fluorescence SIM; that correlative system is inside the vacuum chamber. To prevent radiation damage, a sample and its environment should be kept under cryogenic conditions, for which reason samples must be prepared by quick freezing using either a plunge freezer or a high-pressure freezer to avoid the formation of ice crystals. Some biomedical subjects can be implemented, including an investigation of molecular events within cells, changes in cellular architecture, interaction or communication between cells, interaction between host and microorganisms, and structural changes of tissue. The construction of the beamline and end station in energy range 200–1,200 eV is complete; the commissioning began from the end of 2017. (Reported by Lee-Jene Lai)

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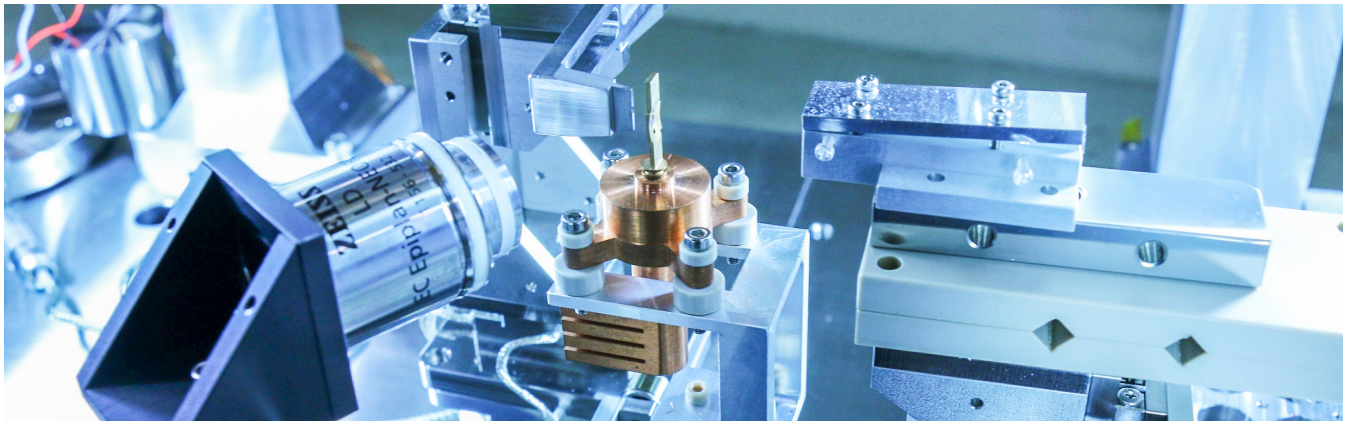

Fig. 3: Fluorescence SIM correlation with SXT inside the SXT chamber

The Installation of the Instrument for Bragg Coherent Diffraction Imaging

A real-space image is more interesting than a one-dimensional reduction scattering profile with a model fitting curve. However, the resolution of the image is subject to the optics for the traditional X-ray microscopies. The lensless imaging technique, coherent X-ray diffraction imaging (CXDI), can overcome the resolution limit affected by the optics, but its development is limited by the coherent X-ray beam quality. The implementation of coherent X-ray scattering techniques has been initiated since high brightness synchrotron sources started producing highly coherent X-ray beams. The Coherent X-ray Scattering (CXS) Beamline, **TPS 25A**, is one of the dedicated beamlines designed for the coherent X-ray scattering experiments and it has been opened to users. In traditional microscopies, the optics is used to obtain the images.