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Three Dimensional Imaging of Biological Samples and Nano-materials Using Soft X-ray Microscopy

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Abstract:
The soft X-ray Microscopy beamline BL07W at National Synchrotron Radiation Laboratory is devoted to cryo nano-tomography for biological applications in the water window (284 - 530 eV) and for imaging of nanomaterials from 200 to 2500 eV. An ellipsoidal capillary used as condense to focus monochromatic light onto the sample. Two Ni zone plate (ZP) lenses made by Zeiss with 40 nm and 25 nm outer most zone widths, respectively, are available, giving spatial resolution in 2D of down to 40 nm and 30 nm, respectively. Hydrated biological specimens had been imaged in the water window photon energy range without chemical fixation, dehydration, chemical staining and physical sectioning. In addition, other applications such as nanomaterials imaging had been demonstrated.

Keywords:
Soft X-ray Microscopy, water window, cryo nano-tomography, nanomaterials imaging

Introduction:
Soft X-ray microscopy can provide a complementary spatial resolution between optical and electron microscopy, and to make full use of the high natural absorption contrast between organic materials and water in the photon energies which is between the K-absorption edge of oxygen ($E \approx 530$ eV or $\lambda = 2.34$ nm) and carbon ($E \approx 280$ eV or $\lambda = 4.43$ nm). The photon energies so called "water window" are especially suitable for imaging samples in aqueous media. Thus, hydrated biological specimens can be imaged in the water window photon energy range with no chemical fixation, dehydration, chemical staining and physical sectioning. Soft X-ray Nano-Computer Tomography can provide three-dimensional tomographic volumes to visualize subcellular structures with high resolution, up to 30 nm. Cryo- Soft X-ray microscopy had been used to visualize the internal architecture of fully hydrated cells [1-3] at high spatial resolution. In this paper, we will introduce the imaging results of biological samples and Nano-materials based on soft X-ray microscopy at National Synchrotron Radiation Laboratory (NSRL), Hefei, China.

Method:
The nanotomography experiments were performed at NSRL. A transmission soft X-ray microscope was installed in this beamline. The X-ray beam is focused on the sample by an elliptical capillary condenser. Then, A high resolution zone plate objective with outermost zone width of drN = 40 nm providing a spatial resolution of down to 40 nm was used. A tilt series images were collected from -60^{o} + 60^{o} at 1^{o} degree intervals at 520 eV X-ray energy. Each projection was collected with an exposure time of 1 seconds. Alignment and reconstruction of the tilt series were carried out with filtered back-projection algorithm from Carl Zeiss X-ray Microscopy Inc. Typically, 100-mesh TEM grids were used to carry the samples. Grids with biological samples were mounted in the homemade plunge freezer, and rapidly put dropped into the liquid nitrogen in the movable cryo-preserving container for cooling down to liquid nitrogen temperature. The rapid plunge procedure was to avoid contamination caused by ice crystallization so as to protect biological samples from structural damage. The grids with biological samples were transferred

into the soft X-ray imaging vacuum cryogenic chamber by the sample transfer chamber to ensure the next step of imaging.

Result:
A hydrated yeast had been imaged by soft X-ray microscope at 109 K (Figure 1) [2]. The 3D structure of the yeast was reconstructed from 121 projections (-60 to 60 degree). From the 2D slice, the subcellular organelles, such as vacuole, cell nucleus and mitochondria, can be identified. This information cannot be obtained only from 2D projection. Ultrafine PDA-rGO fibers were imaged by soft X-ray microscope. As shown in Figure 2, linear PDA-rGO fibers consist of large wrinkled lamellar building blocks on the macro level, which exhibits highly axial orientation and close packing in the axial direction. The results indicated that GO@PDA was assembled into flexible GBFs with uniform linear morphology and highly aligned, close-packed lamellar microstructures [4].

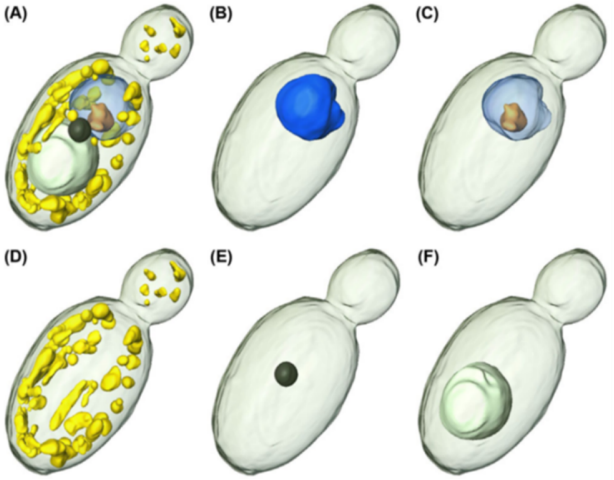

Figure. 1. Rendering images of yeast. (A) All segmentations of the reconstruction. (B-F) Five types of segmented regions. The corresponding organelle identification of the segmented regions are in the order of nucleus (B), nucleolus (C) inside the nucleus (B), mitochondria(D), lipid body (E) and vacuole (F) [3].

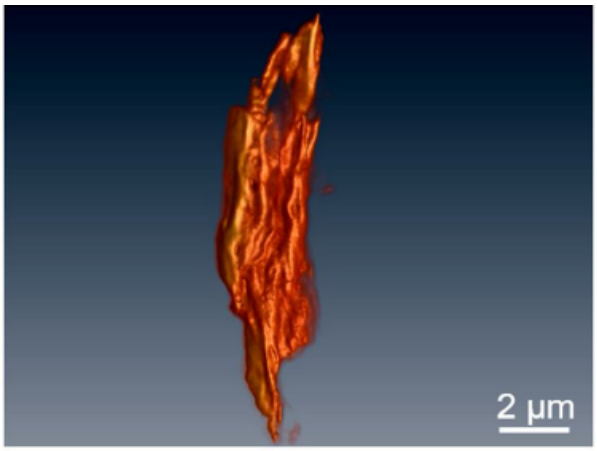

Figure. 2. Soft X-ray image of a fragment from RGO@PDA fibers with a spatial subcellular structures [4].

Conclusion:
We had demonstrated cryo soft X-ray tomography of frozen hydrated yeasts in the water window energy range with spatial resolution down to 40 nm and the studies of nano-materials. The results shown that soft X-ray nanotomography can provide an alternative tool for people to study hydrated biological specimens and nano-materials.

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