Chemically Sensitive Tomography at 50 nm Spatial Resolution using a Soft X-ray Scanning Transmission X-Ray Microscope

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Previous very successful soft X-ray microscopy tomography experiments [1-3] have been performed at a single photon energy. They have provided high spatial resolution, density based 3D images, but with only limited chemical information. We are developing computed angle-scan tomography in a scanning transmission x-ray microscope (STXM) for chemical visualization of a 3D spatial volume. Our goal is not only to do x-ray tomography at ~50 nm spatial resolution, but also to utilize the capability of STXM to perform energy scanning at high spectral resolution in order to identify and quantitatively map the chemical composition of the sample in a 3D volume.

Transformations in many biological and environmental samples take place on a sub-micrometer scale. There is therefore a growing demand for detailed spatial and chemical analysis at high spatial resolution. The combination of near-edge X-ray absorption fine-structure spectroscopy (NEXAFS) and sub-micron spatial resolution tomography shows great potential when applied to chemical systems, such as multi-phase polymer composites, biomaterial interactions with proteins or metal ions, soil samples, etc [4]. Previously, serial section tomography has been used to provide 3D chemical imaging of toner particles [5]. Figure 1 shows a comparison between a transmission image and tomography slices from a capillary filled with natural river bacterial biofilm. However, the real strength of this new method is to use detailed spectral information to convert image sequences (stacks) into chemical component maps. At each angle, a series of images is acquired at different energies. Different chemical components have different NEXAFS spectra. Thus, singular value decomposition (SVD) based on reference spectra can be used to convert the image sequence into 2D chemical maps. This is illustrated in Figure 2, where the spatial distributions of protein, lipids, water, fused silica and polysaccharide (not shown) were retrieved from one image stack of the tomographic series. Furthermore, when the 2D chemical maps are combined with the angular information, tomography reconstruction yields detailed 3D chemical maps, as illustrated in Figure 3.

We will present our experimental arrangement, sample preparation, and measurement procedures for "chemical tomography". 3D chemical maps of wet biofilms will be shown to illustrate the utility of combining high chemical sensitivity with high spatial resolution.

References

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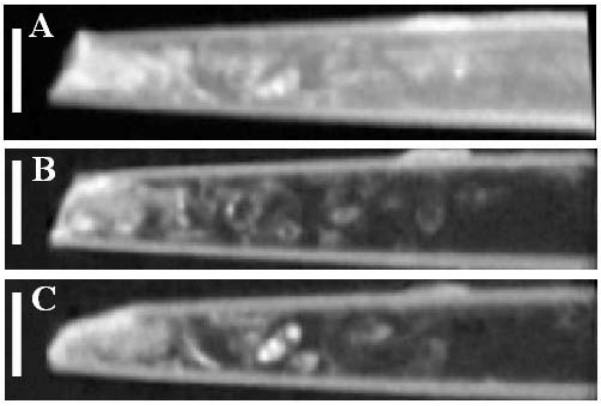
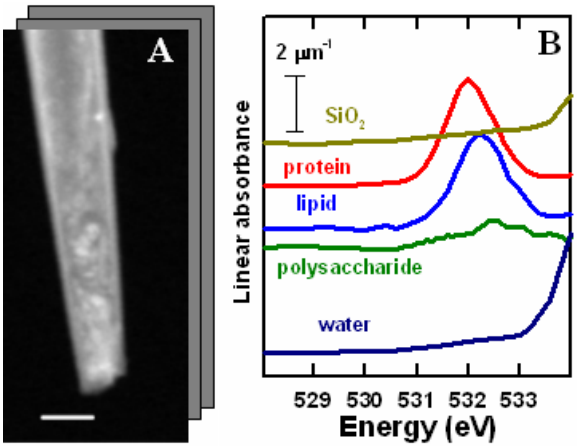
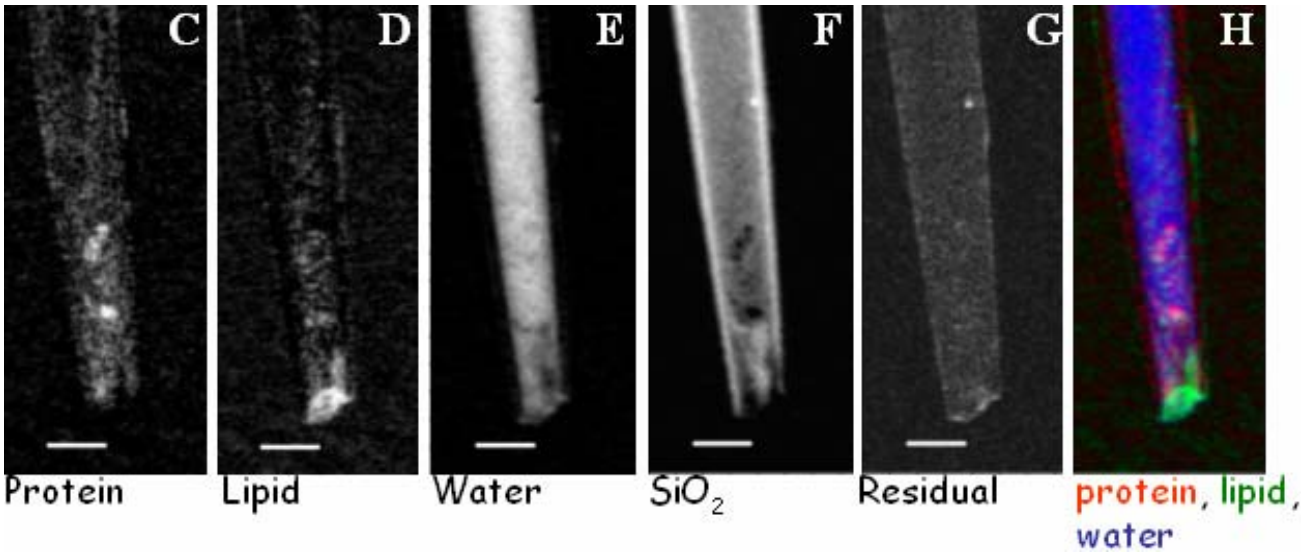


Fig. 1. Comparison between a x-ray transmission image (A) and tomography slices (B-C) of a capillary filled with bacterial biofilm. (A) is an absorption transmission image made by a Scanning Transmission X-Ray Microscope at 532.2 eV photon energy and it is show in an optical density scale. (B-C) are slices from a tomography reconstruction based on 36 STXM images from 0 to 180 degrees. Scale bar = 2 μm.

Fig. 2. The spectral image stack (A) can be converted into chemical component maps (C-H). At each angle, a stack of images (A) is acquired for a range of energies (e.g., 528-534 eV). Since the fine-structure near an absorption edge is very different for different chemical components, singular value decomposition (SVD) procedure can utilize reference spectra (C) and convert the image stack (A) into a 2D chemical maps of, e.g., protein, lipids, water, fused silica (C-F). Scale bar = 2 μm.





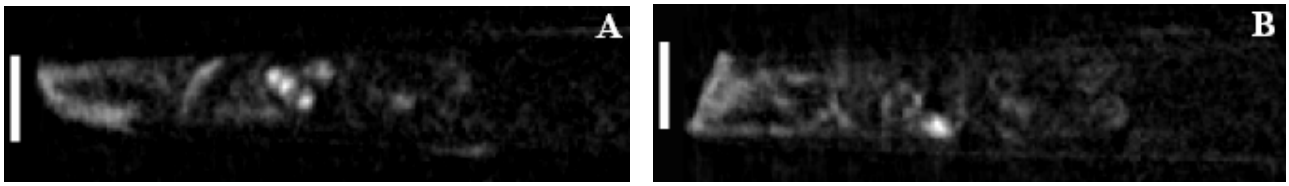


Fig. 3. 2D slices (A-B) from a 3D protein map generated by tomography reconstruction of a 0 to 180 degrees rotation and 528 to 534 eV photon energy image stack. Scale bar = 2 μm.