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Chemical Imaging by Soft X-ray Scanning Transmission X-ray Microscopy

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The X-ray absorption signal in synchrotron based soft X-ray scanning transmission X-ray microscopy (STXM) is providing quantitative maps of chemical species in many samples, under a wide range of environments (wet, variable temperature, pH, stress, electrochemical control, etc). State-of-the art zone plates provide a spatial resolution of 15 nm [1], while 35 nm resolution is achieved routinely in STXMs at the Advanced Light Source (ALS) and at the National Synchrotron Light Source (NSLS) [2]. With appropriate software [3], sequences of images recorded over a range of photon energies spanning one or more core excitation edges can be inverted by multivariate statistical analysis methods [4] or by pixel-by-pixel spectral fitting to generate quantitative chemical component maps. **Figure 1** shows an example from a study of metal and Ca ions relative to the biochemistry of a natural river biofilm exposed to 10 ppm $NiCl_2$ for 24 hours. In addition to mapping the majority macromolecules of the system from their C 1s, N 1s and O 1s response, these metal 2p edge signals provide oxidation state sensitivity for multiple metal species [5]. Organic antimicrobial compounds and their effect on environmental and cultured biofilms are also under investigation [6]. Such studies contribute to understanding the organization of biofilms and their capacity to sequester organic and inorganic compounds from the surrounding environment.

In addition to mapping chemistry, the polarization properties of synchrotron light are being exploited to measure orientation properties of samples at high spatial resolution with STXM. The linear dichroic signal is being used to map β-sheet crystallite distributions in *B. mori* cocoon silk [7] and dragline spider silk. **Figure 2** presents an optical density image at 288.2 eV (protein π^*_{amide} band) of one orientation of a longitudinal section of dragline silk from *N. clavipes* mechanically pulled from an immobilized spider at a controlled speed of 0.5 cm/sec. Taking differences of such images with the fiber oriented perpendicular and parallel to the E-vector allows visualization and quantization of the distribution of alignment of the β-sheet crystallites which are the origin of the dichroic signal. We hope such studies will help to understand the origins of the remarkable mechanical properties of silk, and the flexibility with which spiders can produce silk materials with quite variable properties.

References

[1] W. Chao, et al, Nature 435 (2005) 1210
[2] A.L.D. Kilcoyne et al, J. Synchrotron Rad. 10 (2003) 125.
[3] aXis2000, available free for non-commercial use at http://unicorn.mcmaster.ca/aXis2000
[4] M. Lerotic, et al. J. Electron Spectrosc. Rel. Phen. 114 (2005) 1137.
[5] J.J. Dynes et al. Enviro. Sci. Tech. (2006) in press. (DOI 10.1021/es0513638)
[6] J.J. Dynes et al. Science of the Total Environment (2006) submitted
[7] D. Hernández Cruz, et al. Biomacromolecules (2006) in press
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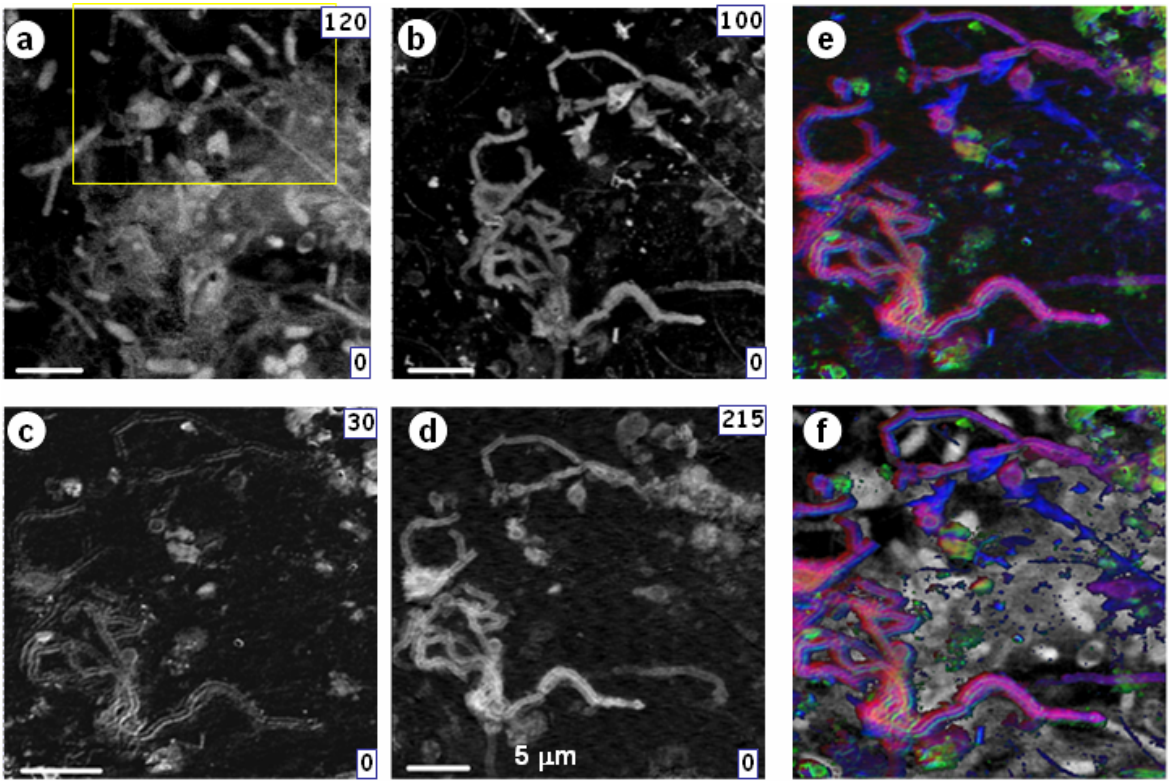

Fig. 1. (a) Map of biological components ($OD_{288.2} - OD_{282}$) of a river biofilm exposed to 10 ppm Ni^{2+}. Difference maps (on – off resonance) of (b) Ca^{2+} , (c) $Fe^{(III)}$, (d) Ni^{2+}. (e) Composite of Ni (red), Fe (green), Ca (blue) with rescaling. (f) Composite of metal maps superimposed on bio map. (g) Bio-macromolecule maps of the region in yellow box in (a). Protein (red), Polysaccharide (green), Lipid (blue). The upper and lower numbers are grey scale limits indicating thickness in nm.

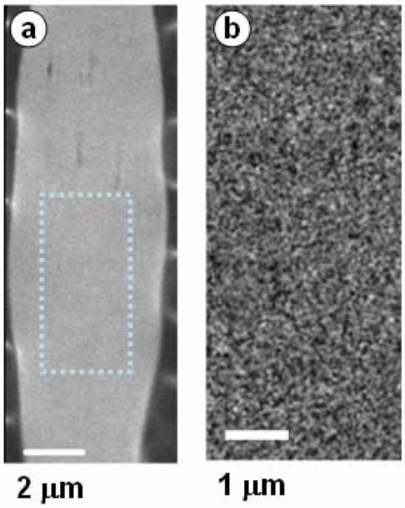

Fig. 2 (a) Optical density image at 288.2 eV (protein $\pi^*_{C=O}$ band) of a longitudinal section of *N. clavipes* dragline silk generated at 0.5 cm/s. (b) Dichroic signal extracted by taking difference of images from the fiber oriented perpendicular and parallel to the E-vector of the light.