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3D Chemical Imaging with STXM Spectro-Tomography

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Three dimensional (3D) chemical imaging using angle scan spectro-tomography in a soft X-ray scanning transmission X-ray microscope (STXM) has been developed and applied to quantitative 3D chemical mapping of fully hydrated biological and polymer systems as well as non-hydrated environmental and biological samples. Soft X-ray tomography using full field X-ray transmission microscopy [1,2] or cryo-STXM [3] has been performed at only one energy which provides density/thickness contrast with limited and potentially ambiguous chemical information. In contrast, spectro-tomography [4-6] acquires tomograms at multiple photon energies to provide true, quantifiable 3D chemical mapping through element- and species-specific, near edge X-ray absorption (NEXAFS) contrast of the components. Fig. 1 shows the in-situ tomography rotator and the sample mounting used, which include pulled glass capillaries, carbon nanopipettes [7], grid strips, and needle frames with wet samples enclose in thin polyimide films. The latter two approaches enable spectro-tomography at energies as low as 150 eV, thus accessing the S 2p edge.

Fig. 2d shows a spectro-tomography derived rendering of the distribution of low density (4% dry weight) acrylate polyelectrolyte ionomer inside 800 nm diameter hollow polystyrene micro-spheres in an aqueous medium in a ~3 micron diameter glass micro-capillary [4]. The uniformity and statistical distribution of filled versus unfilled microspheres was the goal of the study. Specificity to the acrylate filling was achieved from the difference of tomograms recorded at 532.2 eV (O 1s $\rightarrow$ $\pi^*_{C=O}$ peak) and 530 eV (Fig. 2c). A 3D spatial resolution better than 50 nm was documented. The color coding (fig. 2d) reflects the density of acrylate in each voxel which is quantifiable because the differential absorption signal is quantitatively related to the amount of acrylate.

Fig. 3 presents renderings of the protein, all-calcium and intense-Ca signals from a spectro-tomogram recorded at 6 energies at the C 1s and Ca 2p edges. The sample is a single *Synechococcus leopoliensis* PCC 7942 bacterium grown under controlled supersaturation of Ca^{++} and CO_3^{2-} ions in order to study the initial stages of nucleation and the role of aragonite-like $CaCO_3$ which forms as an intermediate in biomineralization of calcite by this species. Detailed analysis of the 3D spatial and compositional correlations of the Ca^{++} and organic carbon signal (Fig 3b,c) in the region of the cell envelope showed that the aragonite layer is rather uniformly distributed in the extra-cellular polymer matrix whereas calcite forms at specific Ca-rich hot spots [6]. [9]

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[9] We thank Michael Schrlau and Haim Bau for providing carbon nanopipettes and Luxel Corporation for polyimide needles. Research supported by NSERC, CFI and the Canada Research Chair program. STXM carried out at ALS BL 5.3.2 (supported by BES-DoE) and CLS 10ID1 (supported by CFI, NSERC, CIHR, NRC and the University of Saskatchewan)

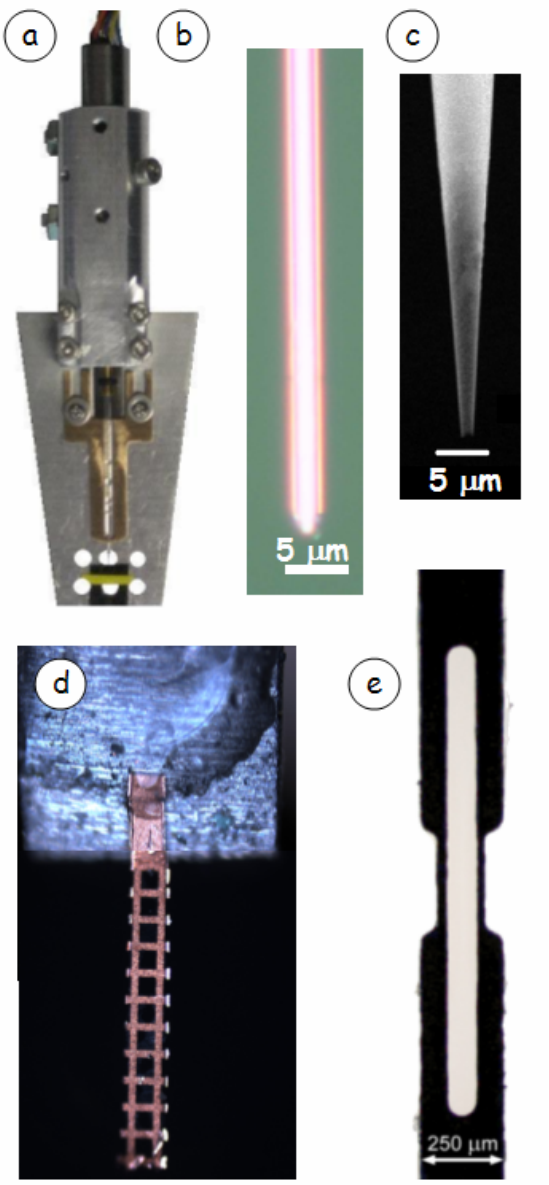

FIG. 1 (a) In-situ rotation system used with (b) pulled glass capillaries, (c) nanopipettes, (d) grid strips, and (e) polyimide needles for STXM spectro-tomography.

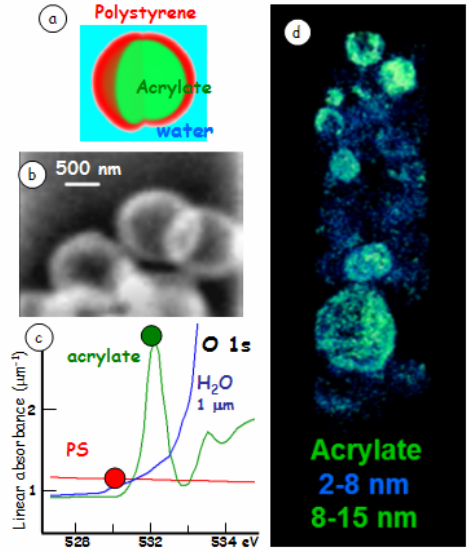

FIG. 2 STXM spectro-tomography of acrylate in PS microspheres. (a) structure, (b) single image, (c) spectroscopy, (d) 3D distributions of acrylate [45]

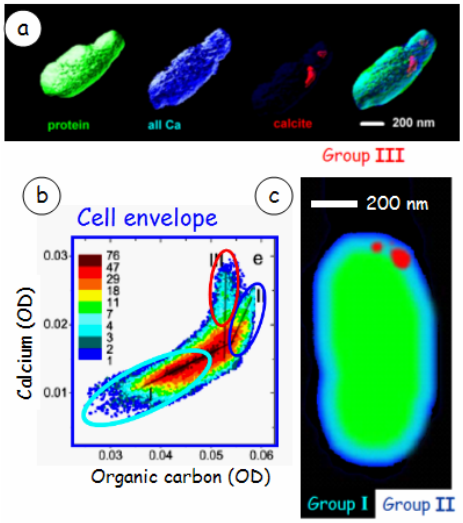

FIG. 3 a) Renderings of protein, all-Ca and hot-spot Ca of a calcifying bacterium. (b) correlation of Ca 2p and C 1s signal in cell envelope showing the aragonite-like Ca is uniformly distributed but calcite forms at hot spots [6]. (d) slice through centre of cell, colorized by the indicated regions in Ca-organic_C space.

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