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Imaging and Energy-Loss Characteristics for *in vitro* TEM Analysis of Nanoparticles

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With the vast potential applications of nanomaterials, there is rising concern over the still undetermined toxicity and fate of engineered nanoparticles upon their release into our bodies and our surroundings. Toxicology studies in the literature, for the most part, consist of highly conflicting, incomparable results due to many uncertainties in the critical material properties. There is an urgent need for a technique that combines the ability to characterize functionalized nanostructures in their *relevant* aqueous (*in vitro)* environment with the high spatial resolution necessary to resolve *individual* nanostructures. The development of such a technique would provide unique and pertinent insights into the behavior of functionalized nanostructures, and would open the door to dynamic studies that could not otherwise be addressed.

This research seeks to establish methods for the characterization of nanoparticles in solution by conventional transmission electron microscopy (TEM) using a customized liquid flow cell specimen holder [1]. This paper presents an overview of the approach and some preliminary results toward characterizing the bright-field imaging of Au nanoparticles through a fluid layer of several micrometers thickness. Data were acquired from a liquid cell with a buffer solution contained between two ultra-thin SiN windows using a using a 300 kV FEG TEM equipped with an imaging energy filter. A specialized holder interfaces with the specimen cell to provide fluid circulation. In order to characterize the flow cell thickness (t) and intrinsic properties, 10-nm-diameter Au nanoparticles (NIST reference material 8011) were deposited onto the top and bottom exterior membrane surfaces. The thickness of the fluid layer was determined by parallax shift (p) over a tilt angle of $\pm\gamma$, where $t = p/2\sin\gamma$ [2]. A parallax shift of 187 nm over ±1° tilt, implying a fluid thickness of ≈ 5.3 μm, is shown in Fig. 1. Interestingly, it was found that local displacement of the fluid occurred upon irradiation above a threshold dose, thus allowing the imaging characteristics and inelastic scattering response to be collected and compared from the same flow cell through liquid water and water vapor (essentially corresponding to the filled and unfilled cell), as shown in Fig. 2. While the energy-loss profile for the unfilled cell is typical of that for electron transparent specimens with thickness a fraction of an inelastic mean free path, the zero-loss intensity in the liquid-filled cell is negligible due to multiple inelastic scattering through the water, and the intensity maximum is ≈ 540 eV. Furthermore, while high resolution and good signal-to-noise ratio can be obtained from the Au nanoparticles on the unfilled cell, with the introduction of a fluid layer, it becomes advantageous to acquire filtered images integrated over the broad energy-loss range [3].

References

[1] N. de Jonge et al., *PNAS* 106 (2009) 2159.
[2] L. Reimer and H. Kohl, *Transmission Electron Microscopy,* Springer, New York, 2008.
[3] We are grateful to Protochips Inc. and for support from Vanderbilt University Medical Center (NJ) and the National Research Council Postdoctoral Research Associateship Program (KLK).

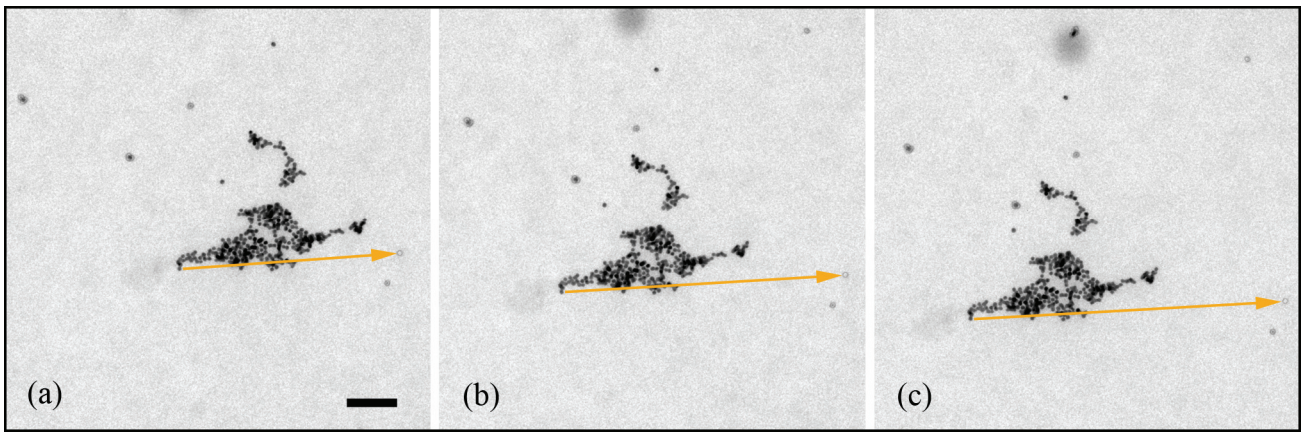

FIG 1. Parallax shift of 10 nm Au nanoparticles through tilts (a) -1° to (b) 0° to (c) +1°, used to determine the flow cell thickness *t*. Scale bar is 100 nm.

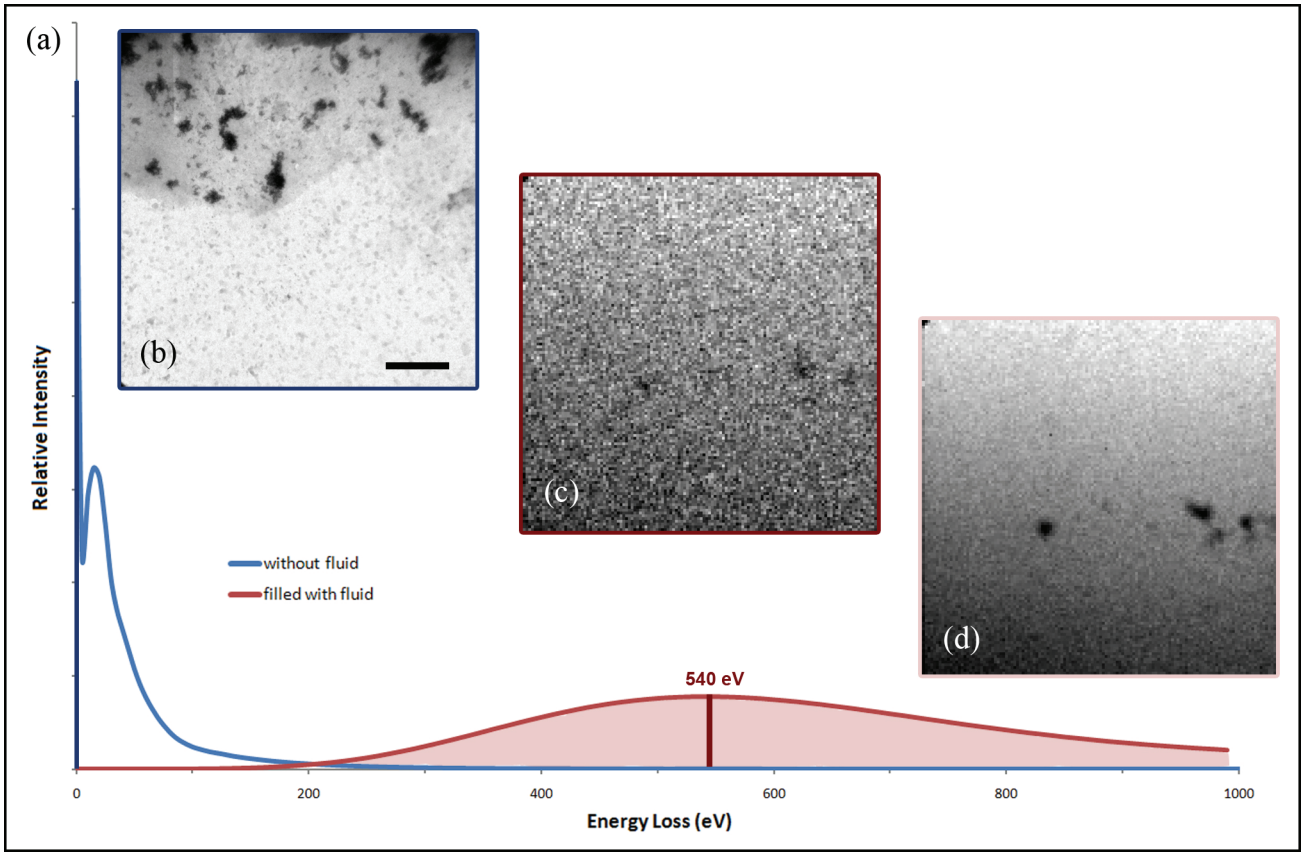

FIG 2. Energy-loss profiles and corresponding images extracted from EFTEM spectral images (SIs) acquired from a flow cell with and without fluid. 128x128 pixel SIs were acquired with 1 s per frame, a 20 eV slit and 10 eV step size. All images are at the same magnification; the scale bar is 500 nm. (a) The presence of the fluid dramatically changes the energy-loss profile. The intensity axis is scaled so that the two profiles have the same integrated intensity. (b) Zero-loss image acquired from the unfilled cell shows aggregates of Au nanoparticles on the flow cell membrane. (c) An individual filtered image slice at 540 eV through ≈ 5.3 μm of fluid exhibits low signal-to-noise ratio, so that particles on the membrane are barely distinguishable. (d) Particle aggregates are clearly visible through the fluid-filled cell with the SI integrated from 0 to 1000 eV.

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