Integrated Nano-Scale Imaging for Investigating Applications and Implications of Nanomaterials in the Living Cell

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Pacific Northwest NATIONAL LABORATORY
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Project 1.2; October 2010 – September 2013

Purpose

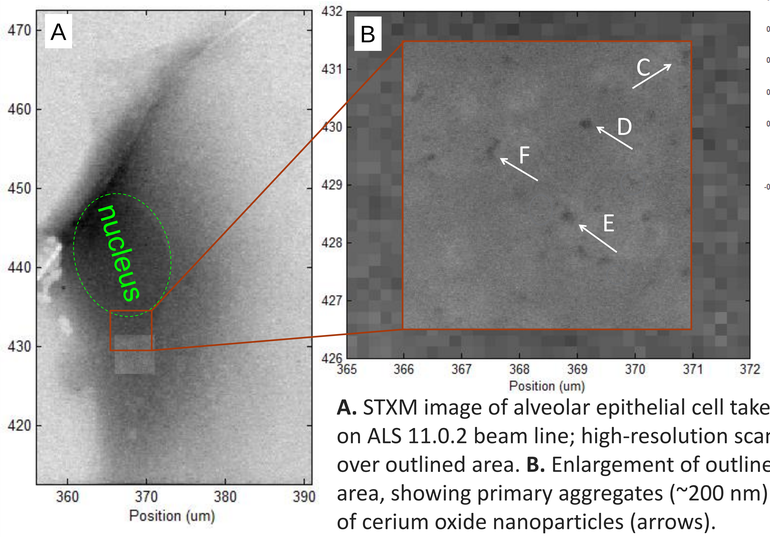
Investigate mechanisms underlying cellular interactions and fate of individual nanoparticles in the intact hydrated cell with nanometer resolution using PNNL-developed integrated super-resolution fluorescence (SRF) and X-ray imaging techniques

Key Successes

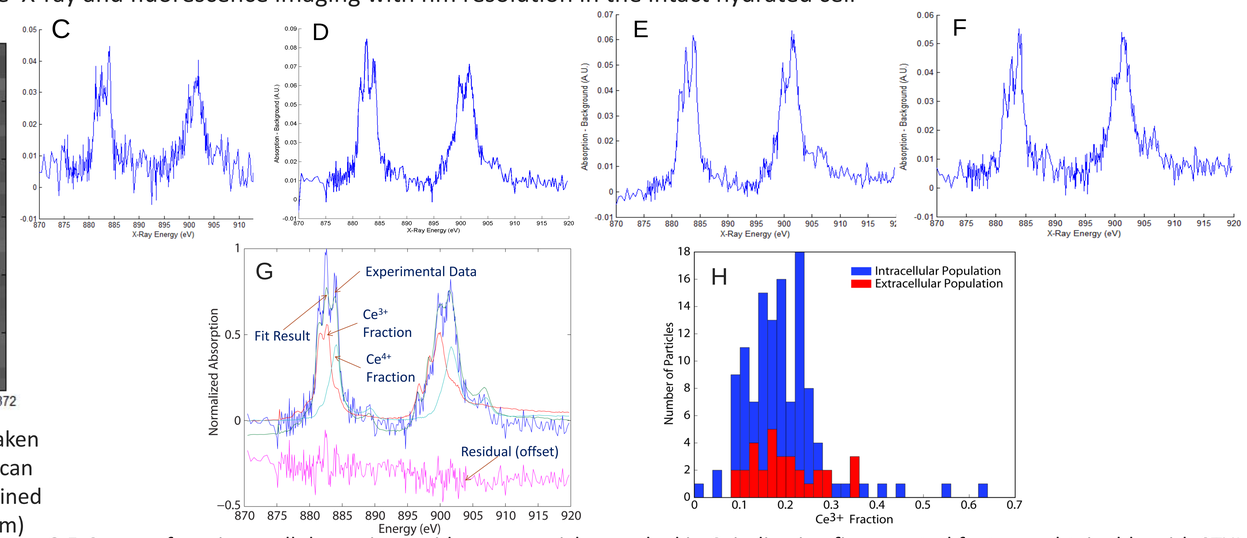
- Established correlative SRF imaging and scanning transmission X-ray microscopy (STXM) in intact hydrated cells at unprecedented resolution
- Gained new understanding of molecular interactions that occur between cerium oxide nanoparticles and the intracellular environment by quantifying the oxidation state of untagged pristine particles inside organelles using integrated spatial and chemical information with nanometer resolution

Research Accomplishments

Understand cellular interactions of nanoparticles using correlative X-ray and fluorescence imaging with nm resolution in the intact hydrated cell

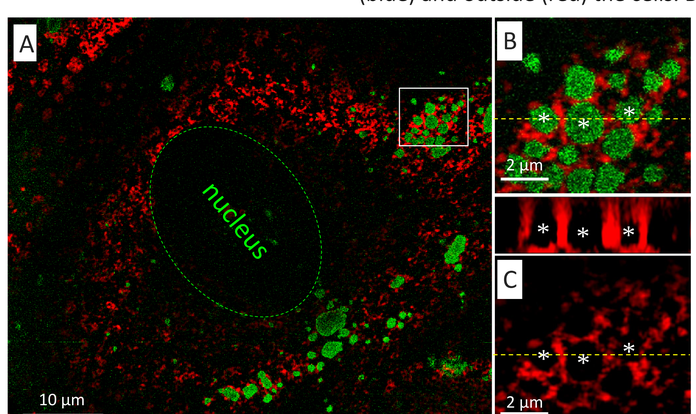


A. STXM image of alveolar epithelial cell taken on ALS 11.0.2 beam line; high-resolution scan over outlined area. **B.** Enlargement of outlined area, showing primary aggregates (~200 nm) of cerium oxide nanoparticles (arrows).



C-F. Spectra from intracellular cerium oxide nanoparticles marked in C, indicating fine spectral features obtainable with STXM. **G.** Single value decomposition (SVD) analysis of Spectrum C based on pure Ce^{3+} and Ce^{4+} reference spectra, showing the nanoparticle consists of 55% Ce^{3+} and 45% Ce^{4+}. **H.** SVD analysis of 150 nanoparticle spectra for nanoparticle populations: within (blue) and outside (red) the cells. Both populations center at 19% Ce^{3+} fraction.

A. Correlative STXM and structured illumination fluorescence microscopy (SIM) image of intact hydrated alveolar epithelial cell at 50- and 120-nm resolution, respectively, showing ceria nanoparticles (green) and lysosomes (red). **B.** Enlargement of rectangle in A. **C.** Presence of ceria particles within lysosomes determined by 3D analysis of SIM image. Upper panel: side view along dashed line, confirming tight fit of red lysosomal marker around the particles in 3D. ~50% of the particles were encased within lysosomes.



- Successful National Science Foundation (NSF) proposal for a multi-institutional Center for Chemical Innovation (CCI)
- Four successful proposals for highly competitive ALS beam lines
- Two invited presentations
- Four publications (2 accepted, 1 under revision, 1 in preparation)

Next Steps

- The multi-institutional NSF Center will compete for Phase 2 in 2014
- Proposal to National Institute for Environmental Health Sciences is pending review
- Submit technology maturation LDRD proposal

PNNL-SA-96601