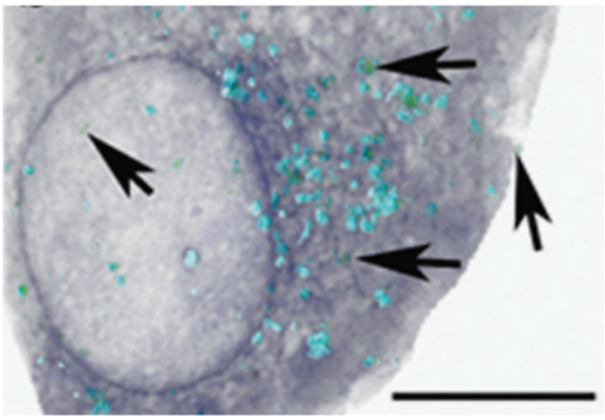
https://doi.org/10.1017/S1551929516001255 Published online by Cambridge University Press

Highlights from Microscopy AND Microanalysis

Biological Applications

Applications in Stimulated Emission Depletion Microscopy: Localization of a Protein Toxin in the 5 Endoplasmic Reticulum Following Retrograde Transport by C Herrera, NJ Mantis and R Cole, *Microsc Microanal* 22(6) (2016) 1113–19

Some plant and bacterial toxins, including cholera and ricin toxin, exploit a specific type of transport (retrograde) to gain entry into host cells. Here we demonstrate the use of new imaging technology, super-resolution stimulated emission depletion (STED) microscopy, combined with live-cell imaging, to visualize ricin toxin within a sub-cellular organelle complex, the endoplasmic reticulum (ER). There is a 52% (0.09 versus 0.19 µm) improvement in resolution obtained by STED, as compared with conventional confocal microscopy (CM). This improved resolution provides a more accurate determination of the amount of ricin that had trafficked to the ER. We present a protocol that offers the possibility of using live-cell imaging and STED microscopy to study the trafficking of biological toxins as the route through retrograde trafficking pathways. This breakthrough opens a new door to study mechanisms such as ricin trafficking. A knowledge of ricin trafficking and how subcellular compartments interact with the toxin is essential in understanding fundamental cellular processes such as retrograde transport.

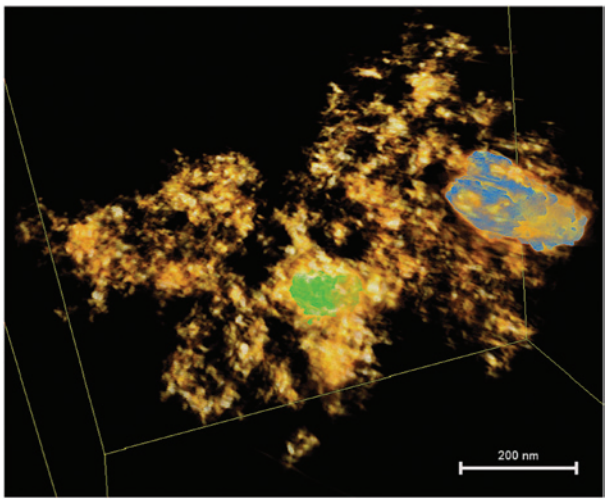


Three-dimensional reconstruction generated from a series collected with STED and CM microscopy. STED imaging here shows ER (blue) and locations where ricin and ER are together (cyan). Arrows correspond to ricin particles that are not localized with the ER. Scale bar = 15 µm.

Materials Applications

Analytical Multimode Scanning and Transmission Electron Imaging and Tomography of Multiscale Structural Architectures of Sulfur Copolymer-Based Composite Cathodes for Next Generation High-Energy Density Li-S Batteries by VP Oleshko, AA Herzing, CL Soles, JJ Griebel, WJ Chung, AG Simmonds, and J Pyun, *Microsc Microanl* 22(6) (2016) 1198–21

Poly(sulfur-*random*-(1,3-diisopropenylbenzene) copolymers synthesized via inverse vulcanization represent an emerging class of electrochemically active polymers recently used in cathodes for Li-S batteries, capable of realizing enhanced capacity retention (1005 mAh/g at 100 cycles) and lifetimes of over 500 cycles. The composite cathodes are organized in complex hierarchical 3D architectures (see figure), which contain several components and are challenging to understand and characterize using any single technique. Multimode analytical S/(T)EM and EDX/EEL spectroscopies coupled with multivariate statistical analysis and tomography were applied to explore origins of the cathode enhanced capacity retention. We found that replacing the elemental sulfur with organosulfur copolymers improves the compositional homogeneity and compatibility between carbons and sulfur-containing domains down to sub-5 nm length scales resulting in (a) intimate wetting of nanocarbons by the copolymers at interfaces; (b) the creation of 3D percolation networks of conductive pathways involving graphitic-like outer shells of aggregated carbons; (c) concomitant improvements in the stability with preserved meso- and nanoscale porosities required for efficient charge transport.



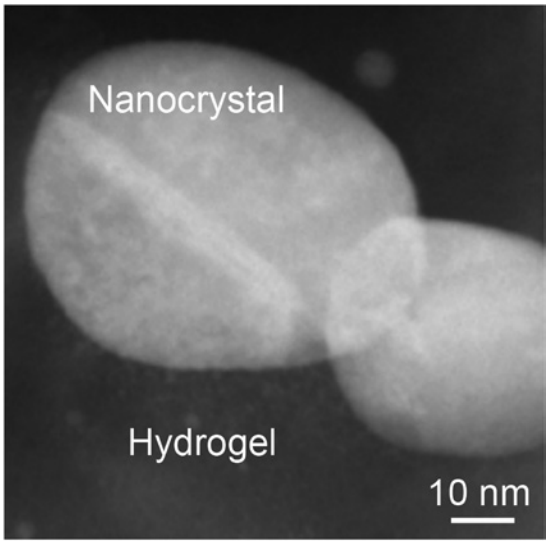
Tilt-angle electron tomography of a poly(*S*-*r*-DIB$_{10\%}$)-based cathode powder using C_s probe-corrected STEM. The reconstructed 3D view of the agglomerated cathode fragment displaying two poly(*S*-*r*-DIB$_{10\%}$) copolymer microparticles colored in blue and green. The copolymer particles are surrounded by aggregated conductive C65 carbons colored in yellow, which form random percolation conductive networks and extended pore structures.

Microscopy AND Microanalysis

Techniques and Instrumentation Development

Site-Specific Preparation of Intact Solid-Liquid Interfaces by Label-Free *In Situ* Localization and Cryo-Focused Ion Beam Lift-Out by MJ Zachman, E Asenath-Smith, LA Estroff, and LF Kourkoutis. *Microsc Microanal* 22(6) (2016) 1338–49

Interfaces between solids and liquids play a key role in a range of processes. For example, in hydrogel-based crystal growth the crystal-hydrogel interface dictates the final crystal morphology and properties, and in batteries the electrode-electrolyte interfaces play a pivotal role in device performance and safety. High-resolution characterization of such complex interfaces with the liquids intact is, however, often lacking. In this paper, we describe a method for preparing liquid/solid interfaces for high-resolution analytical cryo-scanning transmission electron microscopy (cryo-STEM). Access to buried structures inside cryo-immobilized specimens is achieved by cryo-focused ion beam (cryo-FIB) lift-out, which involves site-specific ion milling to produce a cross section of the target interface, extracting the cross section from the larger sample, and thinning it to electron transparency, all near liquid nitrogen temperature to preserve the liquids. To guide the milling process, we introduce a label-free *in situ* method of localizing subsurface structures directly in the cryo-FIB by EDX mapping. After transfer of the sample to the cryo-STEM, the structure, elemental distribution, and local bonding environment of intact liquid/solid interfaces can be determined at the nanometer scale.



Iron oxide nanocrystals in an intact hydrogel imaged by annular dark field cryo-STEM. The sample was prepared by cryo-FIB lift-out and subsequently transferred to the cryo-STEM for analysis.