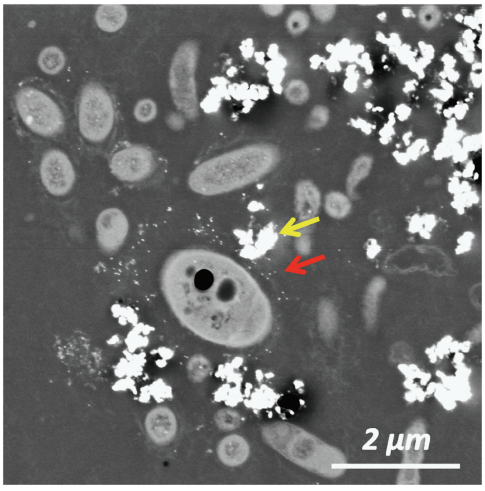
Highlights from Microscopy AND Microanalysis

Techniques and Biological Applications

Cryo-Scanning Electron Microscopy (SEM) and Scanning Transmission Electron Microscopy (STEM)-in-SEM for Bio- and Organo-Mineral Interface Characterization in the Environment by G Wille, J Hellal, P Ollivier, A Richard, A Burel, L Jolly, M Crampon, and C Michel, *Microsc Microanl* 23(6) (2017) 1159–72.

Understanding biofilm interactions with nanoparticles can benefit from the array of microscopy tools. Various approaches in biofilm preparation, labeling (cells via DAPI and extracellular polymeric substances (EPS) via lectins coupled to fluorescent dye or gold nanoparticles), and observations by fluorescent or electron microscopy were applied to study biofilm interactions with zero-valent iron nanoparticles (nZVI). Fluorescence microscopy revealed nZVI embedded in the biofilm structure as aggregates. Cryo-SEM observations showed nZVI aggregates close to bacteria. STEM-in-SEM showed that nZVI aggregates could enter the biofilm to a depth of 7–11 µm. Bacteria were surrounded by a ring of EPS preventing direct nZVI/membrane interactions. EDS revealed a co-localization of nZVI with lectin-gold labeling suggesting a potential role of EPS in nZVI embedding. The combination of various microscopy approaches enabled access to the inside and outside of the biofilm at different scales of magnitude, allowing visualizations of the interactions between biofilm and nZVI. This approach allows nZVI characterization of size and aggregation inside the biofilm.

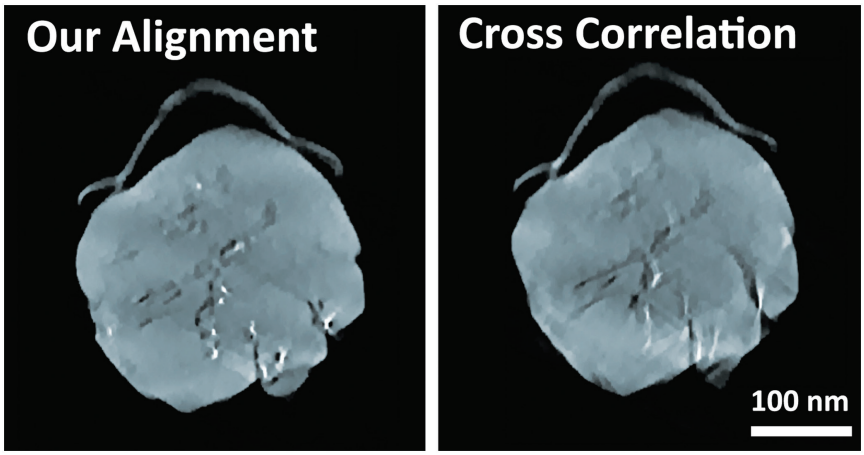


Distribution of nZVI (yellow arrow) and EPS lectin-gold labeling (red arrow) at the periphery of a biofilm (STEM-in-SEM). Labeling was performed on the fresh biofilm (before fixation) using lectins coupled to gold particles. nZVI were observed as aggregates embedded in the biofilm structure. No nZVI was observed in direct contact with bacteria, suggesting a protective role of EPS.

Techniques and Material Applications

Improved Three-Dimensional (3D) Resolution of Electron Tomograms Using Robust Mathematical Data-Processing Techniques by T Sanders and I Arslan, *Microsc Microanl* 23(6) (2017) 1121–29.

Electron tomography is an essential tool for 3D characterization of nanomaterials. In recent years advances have been made in specimen preparation and mounting, acquisition geometries, and reconstruction algorithms. However, one important component of the data-processing has received less attention: the 2D tilt-series alignment. All the images need to remain coherently aligned over the full range of angles. An inaccurate alignment may be difficult to identify yet can significantly limit the final 3D resolution. This work presents an improved center-of-mass alignment model that overcomes discrepancies from unwanted objects that enter the imaging area throughout the tilt series. In particular, an approach is developed to overcome changes in the total mass upon rotation of the imaging area. Our method is applied to accurately recover small Pt nanoparticles embedded in a zeolite that might otherwise go undetected in both the 2D microscopy images and the 3D reconstruction. This example illustrates the importance of an accurate tilt-series alignment and can increase resolution and clarity for tomograms in both physical and biological sciences.



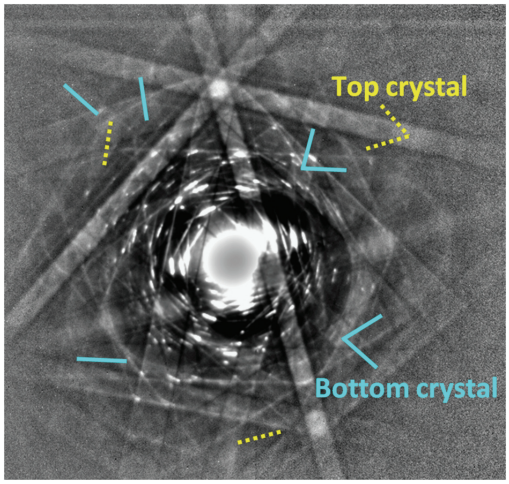
A comparison of one slice through a 3D reconstructed volume of Pt nanoparticles embedded in a zeolite. The same reconstruction algorithm was applied to identical input images, yet with a different intermediate tilt series alignment step. Our alignment shows Pt particles that are distinct and clearly visible. The standard cross correlation alignment shows particles that are blurry or not present.

Microscopy AND Microanalysis

Techniques and Material Applications

Depth Resolution Dependence on Sample Thickness and Incident Energy in On-Axis Transmission Kikuchi Diffraction in Scanning Electron Microscopy (SEM) by E Brodu and E Bouzy, *Microsc Microanal* 23(6) (2017) 1096–1106.

Transmission Kikuchi diffraction (TKD) is a powerful emerging technique allowing nanoscale orientation mapping of electron-transparent samples in SEM. The spatial lateral resolution of this technique is below 10 nm, enabling the study of finer microstructures than by EBSD. The "on-axis" TKD configuration was then developed at LEM3 with the objective of reducing drastically the acquisition time. This study investigates the depth resolution of the "on-axis" configuration, defined as the thickness of the emitting layer at the bottom of samples. Thanks to a silicon sample specifically designed and produced, the depth resolution (or selectivity) of the Kikuchi diffraction is measured as a function of sample thickness and incident beam energy: it ranges on silicon from 30–65 nm in the range 10–30 keV with a close-to-linear dependence with energy and no dependence with sample thickness. This depth resolution, making only the bottom of samples visible by TKD, results from the absorption of the Kikuchi diffraction emitted by the top layers by the bottom layers. The mean absorption coefficient is then proposed to model this selectivity.



The depth resolution, or selectivity, of the Kikuchi diffraction by "on-axis" TKD in SEM was measured via the analysis of the diffraction patterns produced on line scans, which are crossing a silicon twin boundary.