Cryogenic analytical electron microscopy for native state imaging of nanomaterials

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A continuous problem in characterisation surrounds the concept of 'representative' imaging. While electron microscopy is ideally suited to the nanoscale imaging and analysis of materials, with the spatial resolution and availability of advanced spectroscopic techniques affording detailed structural and compositional investigations, limitations are encountered in terms of the samples that can be examined as the vacuum requirements exclude many *in situ* studies and sample preparation can often lead to artifacts. This is especially important when examining 'real-world' samples, which are often multi-component, complex systems.

To examine such systems, our group at the University of Leeds has implemented cryogenic sample preparation and transfer for two of our physical sciences-based electron microscopes, a Titan Themis[3] 300 TEM and a Helios G4 CX FIB-SEM. Both microscopes are optimised for analytical work, with a SuperX 4 EDX detector arrangement and Gatan Quantum spectrometer on the Titan, and an Oxford Instruments 150 mm^2 EDX detector on the Helios. While both are often used for conventional imaging and analysis, we can also utilise a FEI Vitrobot for sample preparation, a Gatan 914 TEM cryo-holder and Quorum SEM cryo-transfer unit, to examine frozen samples.

This setup has permitted the native state or *in situ* analysis of a range of nanomaterials, with applications in areas such as medicine, personal products and environmental science. This presentation will cover our move from using cryogenic approaches for representative sample preparation[1,2,3] through to cryo-(S)TEM to overcome sample preparation artefacts[4] and recent cryo-analytical (S)TEM and cryo-FIB-SEM results, including the application of monochromated EELS to frozen, hydrated samples and 3D volume analysis of frozen dispersed nanoparticles.

The advantage to this approach will be detailed through examples of nanoparticle-stabilised emulsion droplets and nanoparticles dispersed in complex suspensions for toxicological testing, both of which suffer from significant sample preparation derived artifacts when examined by conventional electron microscopy. Furthermore, the limitations and challenges will be discussed, with the importance of further correlation to other microscopy techniques (e.g. liquid cell TEM or atmospheric SEM) and bulk measures detailed.

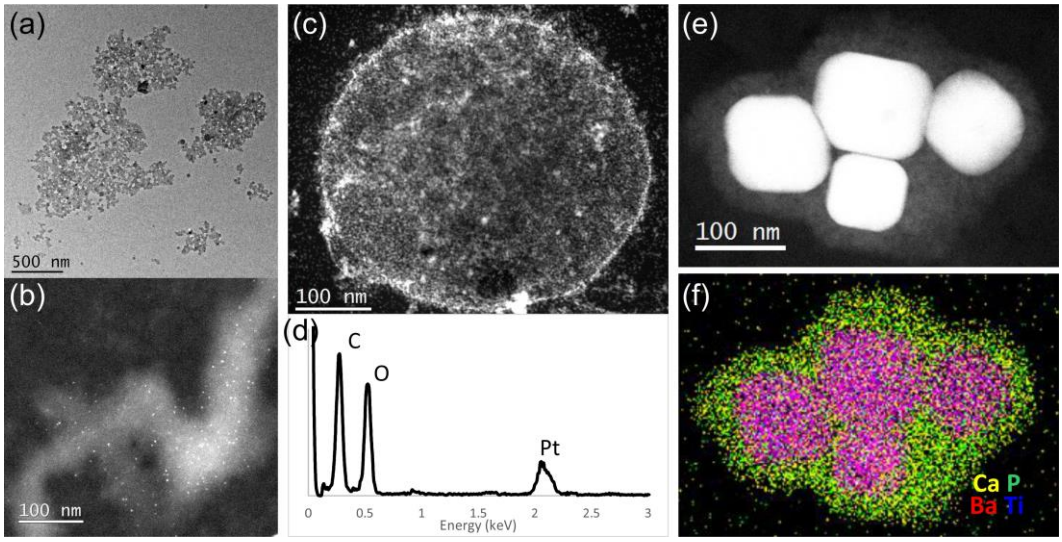

Figure 1. (a) and (b) Examples of where cryo-sample preparation has been used to ensure representative imaging of samples, (a) TEM image of polystyrene nanoparticles dispersed in cell culture media,[1] and (b) HAADF STEM image of quantum dots bound to proteins.[2] (c) Cryo-HAADF STEM image of a platinum-nanoparticle stablised emulsion droplet, with corresponding EDX spectrum (d). (e) cryo-HAADF STEM image and (f) EDX map of barium titanate nanoparticles dispersed in cell culture media.

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