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Quantifying the Dispersion of Nanoparticles by Electron Microscopy

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The concept of *representative* imaging and analysis is a significant challenge in characterisation and is one of vital importance when examining 'real-world' samples, which are often multi-component, complex systems. This is especially evident with nanoparticles, which have increasing applications in strategically important fields, from healthcare to energy generation. When appropriately engineered nanoparticles can be the central and active component of a product, *e.g.* as agents for MRI contrast enhancement or the UV block in suncream.

While it is common to use transmission or scanning electron microscopy (TEM or SEM) to measure primary particle sizes and confirm elemental composition of nanoparticles, there is real potential for more far reaching studies to be performed. Nanoparticles are often dispersed or distributed throughout a matrix, which may be a solid or a liquid, and there is particular interest in determining how the particles and components of a matrix interact to form agglomerates or coatings [1]. A key challenge for examining nanoparticle suspensions by electron microscopy is to remove the liquid or dry the suspensions without re-dispersion of particles or induction of precipitation artefacts [2].

In this work we prepare thin sections of aqueous suspensions of nanoparticles for TEM by plunge-freezing into liquid ethane to ensure the aqueous phase vitrifies with no significant redistribution of suspended material. The frozen hydrated sample can be imaged and analysed by conducting elemental analysis using both energy dispersive X-ray (EDX) and electron energy loss (EEL) spectroscopies provided the applied electron dose does not significantly alter the encapsulating ice [3, 4]. Alternatively, we can add a final sublimation step to remove the aqueous phase to leave the nanoparticles captured in the dispersed state on the remaining carbon film, and permit imaging and analysis at room temperature [2]. We have used this technique to quantify the dispersion of polymer coated quantum dots [2, 5], zinc oxide [6], silica [7] and polystyrene nanoparticles [8] in water and more complex suspensions required for cell culture. This has, for example, enabled us to identify the actual or biological dose of nanoparticles that are presented to a cell surface when exposed to nanoparticle suspensions [8-10].

The systems described above have predominantly been manually analyzed by TEM, in terms of image collection, identification and sizing of agglomerates, all of which limit the sample size and accuracy of the resulting measures. We have used manual segmentation to isolate images of nanoparticle agglomerates on carbon film followed by automated size analysis of the resulting binary images [8] and have recently added automated segmentation procedures including machine learning to increase the analysis rate. Furthermore, to increase the sampling rate, we have incorporated the use of automated data collection by SEM; this is advantageous as multiple signals (e.g. SE, BSE, STEM, EDX) can be collected simultaneously, thus providing several analysis routes for complex nanoparticle samples.

In summary, we demonstrate that through a combination of sample preparation, data collection and analysis approaches there is great potential for representative electron microscopy of real-world complex

multi-component nanoparticle systems, specifically in the quantification of nanoparticle dispersions to provide more accurate distribution profiles which will be useful for product design and performance assessment [11].

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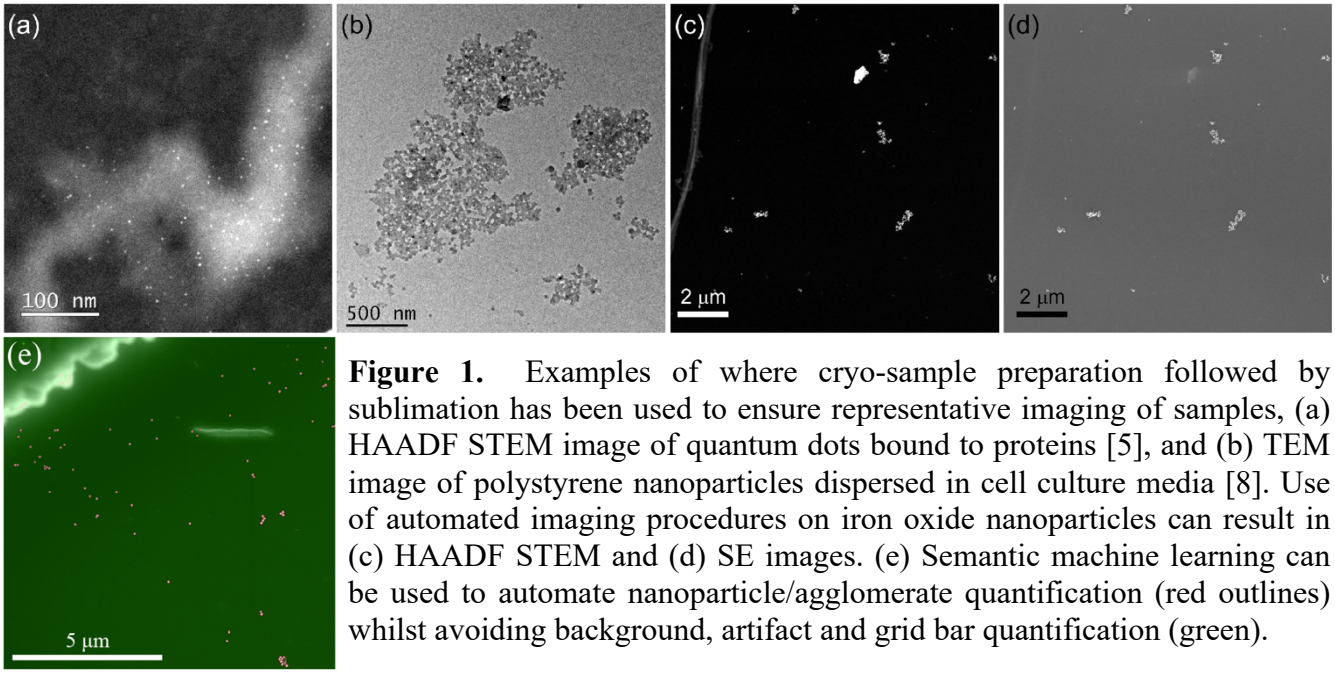

Figure 1. Examples of where cryo-sample preparation followed by sublimation has been used to ensure representative imaging of samples, (a) HAADF STEM image of quantum dots bound to proteins [5], and (b) TEM image of polystyrene nanoparticles dispersed in cell culture media [8]. Use of automated imaging procedures on iron oxide nanoparticles can result in (c) HAADF STEM and (d) SE images. (e) Semantic machine learning can be used to automate nanoparticle/agglomerate quantification (red outlines) whilst avoiding background, artifact and grid bar quantification (green).

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