360° TEM/STEM Nanoanalysis: Functionalized Holders for 3D Electron Microscopy

360° TEM/STEM Nanoanalysis: Functionalized Holders for 3D Electron Microscopy. A new protocol for functionalizing sample holders has been developed for 360° TEM/STEM observation of nanoparticles and nanostructures. The three step process includes FIB milling to customize sample stub geometry, thin film deposition for substrate selection and subsequent chemical functionalization for nanoparticle adhesion. This protocol was used to determine the morphology and local material properties of individual Au/SiO2 core-shell nanoparticles used in a DNA detection assay.

Nanoscience Imaging & Spectroscopy

Tools for characterizing nanoparticles with complex three-dimensional (3D) geometrics are required to further nanoscience research and enable the continuing development of viable nanotechnologies. In this manuscript we report a protocol which combines focused ion beam milling (FIB), thin film deposition and solution chemistry to customize a rotation holder [1] for 3D structural and chemical analysis of nanoparticles with transmission, and scanning transmission electron microscopy (TEM and STEM). By using this protocol the geometry, substrate material and chemistry of the STEM or TEM rotation holder can be optimized for the nanoparticle system of interest. To illustrate this protocol, 3D STEM imaging rotation holders were modified for analysis of core-shell nanoparticles used in a DNA detection assay. The details of the Au/SiO2 core-shell nanoparticle synthesis and resulting increased DNA detection efficiency are reported elsewhere [2]. Electron microscopy techniques are widely used to characterize morphology and electronic properties of materials at the near-atomic scale but are not well suited for visualizing complex structures in 3D [3, 4]. A nanoparticle must be either tilted or rotated with respect to the electron beam to enable multiazimuthal observation and 3D analysis. This requires a "nanoparticle holder" which can be either rotated or tilted in a TEM or STEM. A 3D rotation holder, with sample location indicated by the arrow in Figure 1A, allows analysis from a full 360 degrees as opposed to traditional tomographic tilt-series based analysis which suffers from the missing wedge [5].
Unfortunately the scanning electron microscopy (SEM) and focused ion beam (FIB) based nano-manipulation techniques traditionally used for attaching samples to such rotation holders are not well suited for mounting individual nanoparticles [6-8]. Due to the small particle size and the practical difficulties of attaching and depositing individual particles with nanomanipulators a new technique is needed. To enable 3D STEM or TEM analysis of nanoparticles a three step process was developed to functionalize the brass needle stubs used in the rotation holder. In the first step a FIB is used to customize the geometry of a brass needle stub. Then a ion beam sputter-deposition system (Gatan 682 PECS) is used to coat the surface of the needle stub with a substrate material ‘matched’ to the nanoparticle of interest. In the final step, the surface chemistry of the stub or nanoparticle is optimized to control the adhesion between these two surfaces. To demonstrate this protocol, functionalized rotation holders were created to enable 3D STEM imaging and analysis of core-shell nanoparticles used in a DNA detection assay [2]. The functionalized holders were used to rotate individual Au/SiO2 nanoparticles a full 360° with respect to the electron beam of the STEM as illustrated in figure 1B. High angular annular darkfield (HAADF or Z-contrast (ZC)), bright-field (BF) and secondary electron (SE) STEM signals were recorded at regular angular intervals to study the 3D morphology and composition of the nanoparticle. Electron energy loss spectroscopy (EELS) spectrum images [9] were recorded at several angular intervals to map optoelectronic and material properties of the core-shell nanoparticle.

**Functionalized Holder Preparation**

The sample stub used for 360° rotation in a STEM/TEM is composed of brass and measures 3 mm in length along the rotation axis, ~300 um in diameter and is tapered to a point at the tip with a 30 um diameter flat (fig. 2A). The flat at the apex is well suited for attaching large micronsized samples with FIB or SEM based nano-manipulation techniques. However these techniques are not well suited for
nanoparticles and the roughness of the 30 um flat can easily obscure the nanoparticles from the electron beam at certain angles of rotation. To improve the sample stub geometry, a FIB was used to customize the shape of the holder apex. For holding the 100 nm core-shell nanoparticles used in the DNA assay, the 30 um flat was FIB milled into a 5 x 5 array of pillars. This array of pillars minimizes potential shadowing from the substrate during rotation and increases the number of potential sites well suited for TEM/STEM analysis of individual nanoparticles. The apex of the rotation holder before and after FIB modification is shown in figures 2A and B. A variety of custom holder geometries can created using FIB modification and application specific solutions are only limited by creativity. The nanoparticle system of interest dictates which substrate materials are best suited for controlling of the adhesion and dispersion of the nanoparticles. Fortunately there are a variety of thin film deposition techniques and materials which can be used to coat the FIB modified brass sample stub with a well 'matched' substrate material. For the silica core-shell particles, a 10-20 nm thick silica film was deposited on the stub using a Gatan 682 PECS ion beam sputter coater. By coating the stub with silica, solution chemistry could be varied in a predictable way to control the adhesion and dispersion of the core-shell particles used in the DNA assay. The nanoparticles were deposited onto the coated and FIB modified sample holder by placing a 20 uL droplet of the nanoparticles in solution onto the apex of the customized sample holder. Several nanoparticle solution chemistries, from Ref. [2], were tested to determine which solution resulted in the best adhesion, dispersion and produced minimal beam induced carbon deposition. For some solutions, several layers of nanoparticles adhered to apex, or analysis region of the pillars as shown in figures 3A and B. For other solutions, the nanoparticle-substrate adhesion was reduced, and individual nanoparticles were found near the apex of several pillars as shown in Figure 4A. The effect of solution chemistry on the surface charge of the nanoparticle and substrate silica is thought to explain these differences in adhesion and dispersion, though this has not yet been independently verified. This example of functionalizing sample holders for a particular nanoparticle system can be adapted for many different types of nanoscience applications by customizing the holder geometry, the composition of the thin film coating and/or by modifying the surface chemistry of the sample stub for the system of interest.

360° Rotation STEM Imaging

The ability to rotate individual nanoparticles 360° with respect to the incident electron beam greatly simplifies nanoscale structural characterization. The micrographs of figure 4A-D show a single silica core-shell nanoparticle which adhered near the apex of a functionalized 3D holder. The size, shape, surface features and chemical composition of this core-sell nanoparticle was mapped in 3D
by acquiring STEM ZC, BF, and SE images while rotating this nanoparticle a full 360°. Z-contrast images from another core-shell nanoparticle, containing two Au cores, acquired at 20° intervals are shown in figure 5. The dual core nanoparticle shown in figure 5A could have been mistaken for a single core nanoparticle (fig. 5F) if rotation was not possible. Using this same approach, images of yet another nanoparticle were acquired in 5° intervals and then used to create animations and tomographic reconstruction (to be published). EELS spectrum images were also acquired at several angular increments to determine the optoelectronic properties of the sol-gel silica shell. The EELS data showed the silica to have a band gap of 8.9 eV and a chemical composition ratio of 1:2 for silicon to oxygen (to be published), similar values to those reported in the literature for evaporated microelectronics grade silica [9, 10].

Summary

A new electron microscopy protocol has been developed for functionalizing sample holders to enable 360° TEM/STEM nanoanalysis of individual nanoparticles. This protocol can be easily adapted for a wide variety of nanoparticle systems, and was demonstrated by preparing and then using functionalized holders for the analysis of core-shell nanoparticles used in a DNA detection assay. The functionalized holders were used for 360° STEM/EELS imaging and analysis of individual nanoparticles cantilevered over vacuum. Local material properties responsible for the increased DNA detection efficiency of these core-shell nanoparticles were mapped in 3D by combining information taken at regular angular intervals. By controlling the sample holder’s geometry, coating and surface chemistry this three-step protocol can be adapted to enable the 360° TEM/STEM analysis of many nanoparticle systems.

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References:


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