Ultra Uniform Colloidal Particles as Nanoscale Reference Materials

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ABSTRACT

The ability to understand and predict the properties of nanomaterials relies on an accurate measurement of their size, shape, number density, and other physical properties. One challenge associated with many measurement techniques is the lack of available reference materials with narrow specification ranges that can be used for instrument calibration. This paper describes commonly utilized reference materials for transmission electron microscopy and their associated benefits and drawbacks. We also discuss development of an alternative material – polymer-coated colloidal gold nanoparticles with exceptionally narrow size and shape distributions – and their use as calibration standards for electron microscope and particle counting instruments.

Keywords: nanocrystal, reference material, electron microscope, colloid, standard

1 INTRODUCTION

Transmission electron microscopy (TEM) is an important characterization method for measuring the size and cross-sectional shape of nanoparticles. The highest resolution TEM instruments yield near atomic resolution of nanoparticles and provide invaluable information on the structure of small particles. At this scale, it is critical that the dimensions measured are as accurate as possible which requires the generation of a scale conversion factor that translates digital pixels into nanometers. Surprisingly, in the 1 – 100 nm regime, existing TEM calibration standards are not optimal.

TEM micrographs provide information on both the mean diameter of the particles and the size distribution. By dividing the mean diameter by the standard deviation of the diameter, the coefficient of variation (CV) of the particles can be calculated which defines the particle's monodispersity. For anisotropic particles, the aspect ratio can be determined and shape analysis can be used to measure the shape purity of the sample.

The accurate determination of the size of nano-scale colloids and materials using electron microscopy is a complicated topic due to many potential sources of error and uncertainty. There are multiple parameters associated with both imaging and subsequent image processing that require careful attention from the analyst. By carefully selecting each imaging and processing setting, and by selecting an appropriate standard calibration material, the most accurate possible measurements can be enabled. Small absolute variations in measured values can have huge effects in calculated surface area and particle concentration values – cornerstone metrics in many research experiments and an increasing number of products currently on the market.

Here, we demonstrate colloidal nanoparticle synthesis techniques to fabricate highly size- and shape-monodisperse gold nanocrystals in the size range between 20 and 200 nm (Fig. 1). The particles have a high electron density that provides excellent image contrast, and are single crystalline, allowing the atomic lattice to be characterized easily at high magnification. The particles can be coated with a uniform polymer shell and self-assembled to assist with automated sizing of statistically-significant numbers of particles. We describe the potential use of these materials as an alternative to existing electron microscope calibration materials.

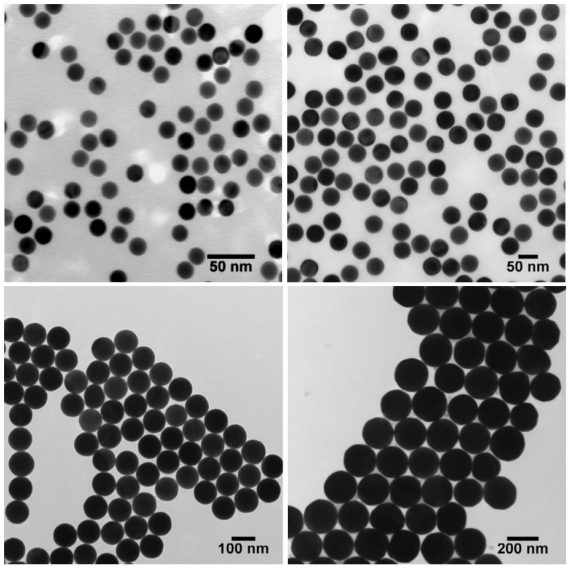

Figure 1: Transmission electron microscope (TEM) images of ultra uniform gold nanoparticles between 20 nm and 200 nm in diameter.

2 RESULTS AND DISCUSSION

Many electron microscopy standards are commonly used for instrument calibration, with different materials commonly utilized for either low, intermediate, or high magnification ranges, but few options exist that can be used across multiple ranges. Below we provide examples of commonly used materials for each size range, and discuss

development of a uniform colloidal particle and associated methods that can be used for instrument calibration.

2.1 Existing Calibration Materials

Transmission electron microscopes can typically provide images of large objects with dimensions of hundreds of nanometers up to micrometer sizes at low magnification, materials in the 10 to 100 nm range at intermediate magnification, and particles less than 10 nm and atomic lattice imaging at high magnification, depending on instrument resolution.

At low magnification, replicas of optical diffraction gratings are often used for instrument calibration, and a typical calibration grid is shown in Fig. 2. The line spacing of the original grating can be determine from optical measurements, providing a known distance for determining a scale factor at a given magnification. Such grating replicas are most useful at low magnification, where a large number of line spacings can be measured to decrease measurement error. At higher magnification, potential sources of error reduce the usefulness of the gratings, since the line edges are relatively ill defined, and fewer lines are visible. Some standards include the co-deposition of latex spheres of a known size, the grey translucent spheres in Fig. 2, for example, though the potential exists that the spheres can be damaged during exposure to the electron beam.

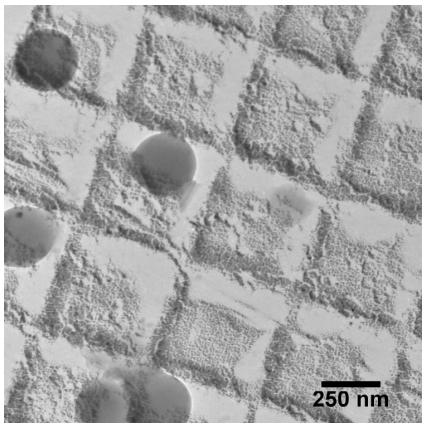

Figure 2: TEM image a grating replica with deposited latex spheres for low magnification calibration.

At intermediate magnification ranges, some nanosized colloidal materials are available, including NIST Reference Materials. Shown in Fig. 3 is a TEM image of citrate-stabilized 30 nm gold nanoparticles (NIST RM8012 [1]). The clear benefit of such a material is the extensive characterization data provided by an internationally-recognized standards organization. For general calibration use, however, such samples present users with some challenges. Perhaps most significantly is the substantial number of shape impurities in the sample; Fig. 3 shows the presence of many rod- and faceted prism- shaped particles, where the particle size presented into the two-dimensional projection view is influenced by orientation on the substrates. The small-molecule citrate coating also leads to closely-packed assemblies, which inhibits the use of automated particle identification and sizing routines that depend on larger interparticle spacing. Finally, the particles exhibit a large amount of internal contrast variation, indicating that they are polycrystalline and contain large numbers of atomic defects, which complicates lattice imaging and analysis for high-resolution calibration.

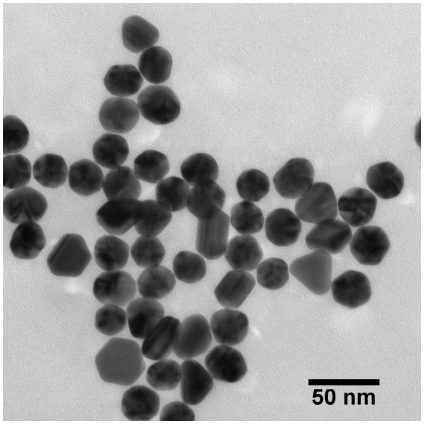

Figure 3: TEM image of a NIST gold nanoparticle reference material (RM8012) used for intermediate magnification calibration.

At high magnification suitable for lattice-scale imaging, many options exist using thin metal or semiconductor films, in which the atomic spacing is well known from X-ray diffraction measurements and the atomic structure of the films can be assumed to be unchanged from the bulk material. For lower-resolution instruments at high magnification, samples with nanometer-scale lattice spacings can be used, including catalase crystals [2] (e.g., Fig. 4).

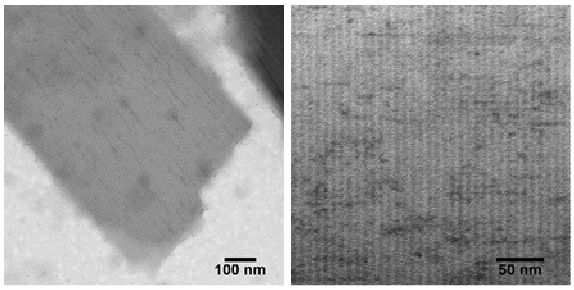

Figure 4: TEM image of a beef catalase crystal used for high magnification calibration at low magnification (left) to show the crystal morphology and at higher magnification (right) to illustrate the internal lattice structure of the crystal.

TEM images of a bovine catalase crystal are shown in Fig. 4, and the higher magnification image reveals a crystalline lattice structure with relatively large lattice spacings, between 6 and 9 nm. Such large spacings are readily observable even on instruments without atomic-scale imaging capability, allowing calibration at high

magnifications, though the measured spacing is not absolute in that it depends on the methods used to prepare and fix the crystals prior to imaging, which can result in potential sample-to-sample or site-to-site variation.

2.2 Ultra Uniform Gold Nanospheres as Calibration Materials

Using colloidal synthesis methods, gold nanosphere samples with high size- and shape-uniformity were fabricated (Fig. 1). The gold nanospheres have high contrast in transmission imaging, exhibit minimal surface faceting and have circularity values and aspect ratios close to unity, and are typically single crystals with few lattice defects. Following synthesis, the nanospheres are water soluble due to stabilization with highly charged, small molecule surfactants, which produces assemblies of particles with only very small particle separation upon drying. To increase the edge-to-edge separation distance, with the goal of utilizing automated sizing routines, the original surface molecules were exchanged for a high molecular weight polystyrene chain, that covalently binds to the gold surface through a terminal thiol. The resulting particles can be dispersed in organic solvents, and when deposited onto TEM grids produce extended close-packed monolayer films with large edge-to-edge spacing (Fig. 5).

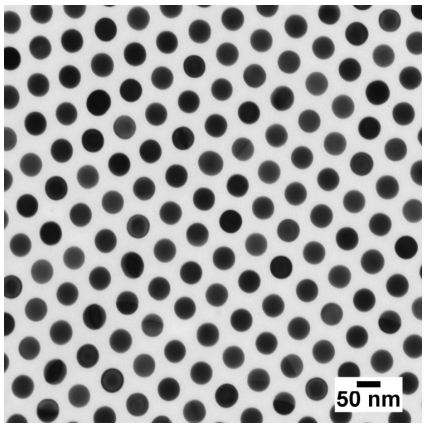

Figure 5: TEM image of polymer-coated ultra uniform gold nanoparticles.

Imaging the ultra uniform nanospheres at high resolution enables the crystal structure of the particles to be observed and measured. A high-resolution image of a gold particle is shown in Fig. 6 (left), and the calculated fast Fourier transform (FFT) of the particle is shown in the right panel. Because the particle is a single-crystal, the FFT shows a single set of diffraction spots that can be readily indexed to the *fcc* crystal structure of gold, imaged along the [011] zone axis for this particle. Assignment of the diffractions spots to the indicated lattice spacings, and comparison of the measured distance for each spacing with the known lattice distances in bulk gold, allows a calibration scale to be readily obtained. Because of the relatively large particle size, any surface reconstruction is expected to contribute negligibly to the measured lattice spacing, allowing the use of bulk lattice constants.

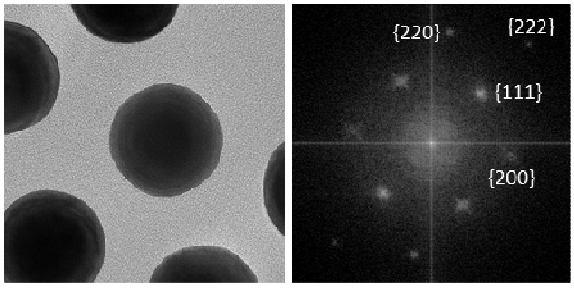

Figure 6: High-resolution TEM image (left) of ultra uniform gold nanoparticles. The calculated FFT (right) of the central particle shows diffraction spots from the gold lattice along the [011] zone axis.

By carrying out calibration using the atomic gold lattice at high magnification, the size of a selected particle can be measured with a high degree of precision. Carrying out additional imaging of the same particle at lower magnification allows a calibration scale to be established using the known particle size.

In instances where a microscope does not have sufficient resolution to perform atomic-scale imaging, the sizes of large numbers of particles can be measured at lower magnification and compared with previously determined values. The accuracy of such measurements depends in part on the quality of images obtained, and consistency of the measurement method that generates particle sizes for comparison. Image quality requires both high contrast between the particles and the carbon substrate; since the elemental content of both materials is fixed, contrast then is determined by the electron beam alignment and intensity at a given magnification. Ensuring that the image is not stigmated is useful, as is that the image is properly focused. At true focus there should be no bright or dark halos surrounding the particles (Fresnel fringes). The central image in Fig. 7 below is in focus, while the images to the left and right exhibit bright and dark halos surrounding the particles, due to being under- and over-focused, respectively.

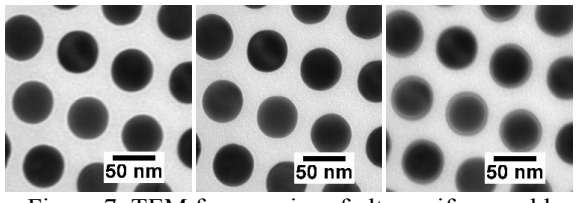

Figure 7: TEM focus series of ultra uniform gold nanospheres.

The high-contrast of the particles, large particle-to-particle separation, and monolayer films of unagglomerated particles allow for automated image analysis and sizing of the particles, using tools such as ImageJ [3]. Here, there is still some user input required for the image analysis to

obtain consistent results between different users. The autosizing feature in ImageJ requires conversion of a grey-scale TEM image to a binarized black and white version that allows edge detection and other calculations to be easily performed. While thresholding algorithms can be used to convert images to black and white, they may not work uniformly across all instruments and some user input may be necessary to ensure that the conversion is performed properly. As one example (Fig. 8), a default algorithm setting correctly selects most of a nanoparticle, but underestimates the particle size at the particle edges where contrast is lower. Manual adjustment of the thresholding parameters may be necessary in some cases to completely capture particles prior to measurement.

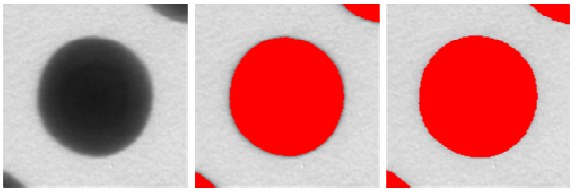

Figure 8: A magnified TEM image of a single particle (left). Applying the default threshold settings (center) in ImageJ produces a red selection with a dark ring, the latter corresponding to lower-contrast edge pixels. Adjusting the threshold values to increase the range of grey values selected (right) accurately selects the edge pixels for sizing.

Carrying out imaging in this way allows hundreds or thousands of particles to be rapidly measured. From n = 500 particles, for example, we obtain size and shape metrics for the gold nanosphere sample shown in Fig. 5., summarized below in Table 1. Here, the Area Equivalent Diameter (AED) is defined as the diameter of a circle having the same area as the projected image of the particle (A), and is calculated as $AED = \sqrt{(4A/\pi)}$.

Particle Characteristic	Measurement (Avg. ± St. Dev.)
Area Equivalent Diameter (AED)	52.0 ± 2.0 nm
Average Feret Diameter	52.5 ± 2.0 nm
Feret (maximum)	53.8 ± 1.7 nm
Feret (minimum, minFeret)	51.1 ± 1.1 nm
Projected Area	2127 ± 103 nm^2
Circularity	0.906 ± 0.006
Aspect Ratio	1.04 ± 0.02

Table 1: Size and shape metrics from automated particle measurements.

3 CONCLUSIONS

A colloidal gold nanoparticle material with high size and shape uniformity was produced using colloidal methods, and functionalized with an electron-transparent polymer coating that produced self-assembled monolayers of well-spaced particles for characterization. In addition to the uniformity of the material, the high image contrast, large particle spacing, controllable size, and single-crystal structure provide several advantages compared with existing materials used for transmission electron microscope calibration. The size of the particles and the well-defined lattice structure of gold make them useful for instrument calibration across a wide range of magnifications, compared with many standards that can only be used for calibration over more narrow magnification windows. Further development of such standard materials will improve the accuracy of nanomaterial size measurements and associated analytical techniques.

REFERENCES

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