TRANSMISSION ELECTRON MICROSCOPY ANALYSIS OF NANOPARTICLES

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Note to the Reader: *We at nanoComposix have published this document for public use in order to educate and encourage best practices within the nanomaterials community. The content is based on our experience with the topics addressed herein, and is accurate to the best of our knowledge. We eagerly welcome any feedback the reader may have so that we can improve the content in future versions.*

Please contact us at info@nanocomposix.com or 858-565-4227 with any questions or suggestions.
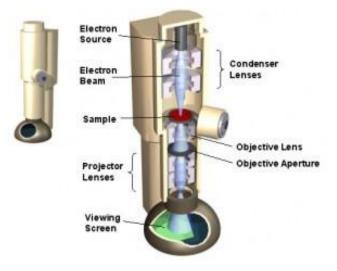
Introduction

Transmission Electron Microscopy (TEM) is a vital characterization tool for directly imaging nanomaterials to obtain quantitative measures of particle and/or grain size, size distribution, and morphology.

TEM images the transmission of a focused beam of electrons through a sample, forming an image in an analogous way to a light microscope (Figure 1). However, because electrons are used rather than light to illuminate the sample, TEM imaging has significantly higher resolution (by a factor of about 1000!) than light-based imaging techniques. Amplitude and phase variations in the transmitted beam provide imaging contrast that is a function of the sample thickness (the amount of material that the electron beam must pass through) and the sample material (heavier atoms scatter more electrons and therefore have a smaller electron mean free path than lighter atoms).

Figure 1. Schematic of a transmission electron microscope.

Successful imaging of nanoparticles using TEM depends on the contrast of the sample relative to the background. Samples are prepared for imaging by drying nanoparticles on a copper grid that is coated with a thin layer of carbon. Materials with electron densities that are significantly higher than amorphous carbon are easily imaged (Figure 2). These materials include most metals (e.g., silver, gold, copper, aluminum), most oxides (e.g., silica, aluminum oxide, titanium oxide), and other particles such as polymer nanoparticles, carbon nanotubes, quantum dots, and magnetic nanoparticles.

At nanoComposix, we use a JEOL 1010 transmission electron microscope operating at an accelerating voltage of 100 keV and an AMT XR41-B 4-megapixel (2048 x 2048) bottom mount CCD camera. The camera's finite-conjugate optical coupler provides high resolution and flat focus with less than 0.1% distortion for magnifications as high as 150,000x. Images from
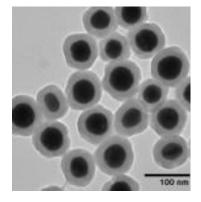

Figure 2. TEM image of silica-coated gold nanoparticles. The gold cores look distinctly darker than the silica shells due to the much higher electron density.

the camera are saved as high-resolution TIFF files, and image processing software packages are used to quantify particle/grain size, size distribution and morphology.

What to Expect

We deposit samples onto TEM grids coated with a thin carbon or polymeric support film; since the film is amorphous, thin (~20 nm) and has a relatively low electron density, it provides a uniform substrate for imaging samples. A small drop of solution is placed onto a grid and allowed to evaporate, typically under vacuum. A research associate will image the sample, taking digital pictures of several locations on the grid to obtain a representative set of images.

When submitting a sample, please specify what type of data you are most interested in obtaining:

> ***Statistics on size and morphology*** – Lower magnification images (typically 2,000-25,000x magnification) are obtained, typically capturing between 50-500 particles per image, providing a sufficient number of particles that can be measured to generate size and shape statistics on the sample.

> ***Sample survey*** – Images are captured at a range of magnifications (typically 2,000-150,000kx magnification, as appropriate for a given sample) to provide data for generating size and shape statistics on the sample, as well as higher magnification close-up images of the particle surface.

> ***High magnification*** – Images of single particles or small numbers of particles are obtained at high magnification (80,000x-200,000x) to provide a close-up image of particle morphology and surface, thickness of surface coating, etc.

We will provide you with a set of high-resolution digital TIFF images for each sample. We also offer a service to measure particle dimensions to calculate average particle size, size distribution and aspect ratio. If you opt for this service we provide a summary of the measurements as well as the raw data. If you prefer to make particle measurements yourself there are a number of software packages with options for image processing and measuring. One common package is ImageJ, a free Java-based software package provided by the National Institutes of Health (NIH).

Sample Preparation Advice

TEM is an important and often-used tool for us at nanoComposix. While interpretation of the resulting images is typically straightforward, sample preparation is extremely important in order to obtain the highest-quality images possible. In addition to imaging samples as-received, our research associates can assist with cleaning or concentrating samples prior to imaging, and measuring the size distribution of samples.

In order to obtain high-quality images, with the greatest amount of contrast between the particles and the surroundings, we suggest:

1) **Thoroughly clean samples**: TEM imaging relies upon differences in electron density between the sample and the surrounding substrate/matrix – the presence of involatile organic molecules that are not removed under vacuum during sample preparation, or the presence of inorganic species (salts, dissolved silica, etc.) that are concentrated while drying a sample onto a TEM grid, decrease the contrast between the sample and the surroundings and reduce the image quality.

2) **Concentrate samples**: If your sample is very dilute, we suggest centrifuging the sample to concentrate it, or allowing some solvent to evaporate. Typically, if the particle dispersion has a visible color then the sample is sufficiently concentrated. We are still able to obtain images from dilute samples, but the number of particles per image will likely be low.

To prepare a sample, we ask that you send at least ~500 μL of solution, or ~10 mg of dried material along with information on what solvents can be used to resuspend your sample. If you are providing samples that you wish for us to concentrate or clean before imaging, we ask that you send at least 5 mL of solution.

Before shipping, transfer the solution into a small volume container that will not leak. Tightly screw the cap on and seal with parafilm or electrical tape to prevent leaking; clearly label each sample with a unique ID. Enclose all samples in a secondary container and add padding as needed to prevent breakage of your container.

Sample Analysis

SIZE AND MORPHOLOGY CHARACTERIZATION

The measurement of individual unagglomerated spherical particles is straightforward. However, when nanoparticles are bound together or have an irregular shape, accurate size statistics can be more complicated to obtain. For particles that are bound together, the necking between particles is examined to determine if the particles were potentially individual in solution or whether the particles are sintered together. If potentially individual, the diameter of each particle is used as the measurement for the size statistics. If sintered or irregularly shaped, the particle diameter is measured at a fixed angle for all particles in the image. Since the orientation of the particles is typically random, the resulting statistics are representative of the average and standard deviation of the sample (Figure 3). An alternative method of analyzing sintered or non-spherical particles is to use image analysis software to determine the cross-sectional area of the particles and convert the area to an equivalent spherical diameter.
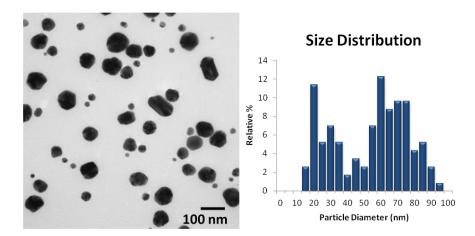

Figure 3. For particles that are size and shape polydisperse, a size distribution can be obtained by measuring each particle diameter at a fixed angle. The random orientation of particles allows for a statistical measure of the size distribution to be generated.

POSSIBLE ISSUES DURING IMAGE ANALYSIS

While TEM is a powerful tool for directly imaging nanomaterials, there are some potential complications that may arise during imaging. Our service team can assist you with sample preparation and offer additional guidance for obtaining the best images of your material. Some possible issues with imaging that can arise include:

Particle Agglomeration: A number of factors influence how a given sample deposits onto a TEM grid, including the solvent evaporation dynamics and the wetting behavior of the solvent on the TEM grid, if the particles are electrostatically or sterically stabilized, and the diffusion of the particles through the solvent during the drying process, and sample concentration. Hence, it is not necessarily the case that the presence of aggregates viewed by TEM is indicative of aggregates in the starting solution.

> *Solution:* At nanoComposix, TEM is used primarily to determine particle size and shape while other measurement techniques such as dynamic light scattering (DLS) and centrifugal particle sizing (CPS) are used to determine the agglomeration state of nanoparticles in solution. Comparing the average particle size obtained using both methods gives useful guidance on the presence or absence of aggregates in solution. In order to reduce agglomeration on the grid to improve imaging, we may be able to use specially functionalized grids and sample deposition techniques; an analytical specialist can assist in selecting alternate preparation methods.

Impurities in the Sample: TEM imaging relies upon differences in electron density between the sample and the surrounding substrate/matrix – the presence of involatile organic molecules that are not removed under vacuum during sample preparation, or the presence of inorganic species (salts, dissolved silica, etc.) that are concentrated while drying a sample onto a TEM grid, decrease the contrast between the sample and the surroundings and reduce the image quality.

> *Solution:* Image quality can typically be improved by removing such impurities before sample preparation. For samples of water-soluble nanoparticles with dissolved impurities, this can be accomplished by centrifuging samples at high speed to form a concentrated "pellet" at the bottom of the centrifuge tube, and carefully removing the impurity-containing supernatant (see Figure 4). Our analytical specialists can assist with sample cleaning prior to TEM imaging.

Figure 4. The colloids in a dilute solution (left) is concentrated into a small volume via centrifugation (right). Other strategies, including filtration, can be used to concentrate samples and remove impurities.

Few Particles in Images: Two primary factors influence how a sample is distributed on a TEM grid following preparation, dynamics associated with the drying process and solution concentration. The former process typically leads to a non-uniform distribution of particles on the TEM grid, with some areas of the TEM grid containing few particles and some areas of the grid containing a denser sample. Our imaging experts scan large areas of the grid in order to locate the most densely populated areas of the grid for imaging. For starting solutions that are very dilute, however, even relatively concentrated areas on the grid may only contain a few particles within a given area.

> *Solution:* Often, dilute dispersions can be concentrated through centrifugation (Figure 4) or partial solvent evaporation, and our research associates can assist with this type of sample preparation if it is not convenient to send us concentrated samples. For some samples, repeated cycles of sample deposition and drying on a TEM grid can increase the density of sample on the grid, however this may also lead to a loss of image quality due to an increase in the amount of organic or inorganic impurities that are also deposited from the sample.

Improving Data Quality

After examining your TEM data, a nanoComposix analytical specialist may make recommendation on further processing that could be done to improve the quality of your data. Additional samples can be sent in for analysis or sample optimization of the already provided sample can be conducted at nanoComposix.