Imaging Nanoparticle Arrays

Loading Method for Multiplexing Nanoparticle Characterization

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TEM and SEM measurements are commonly used methods for characterization of nanomaterials. Typically a single sample is applied onto an EM specimen holder or grid. Due to the fact that these grids have to be moved inside a vacuum chamber, the actual sample exchange may take several minutes depending on the EM instrument. Using a new loading technology, utilizing picoliter dispensing to print arrays of up to 100 samples on one EM support, results in a significant increase in sample throughput while decreasing operational costs in EM measurements significantly.

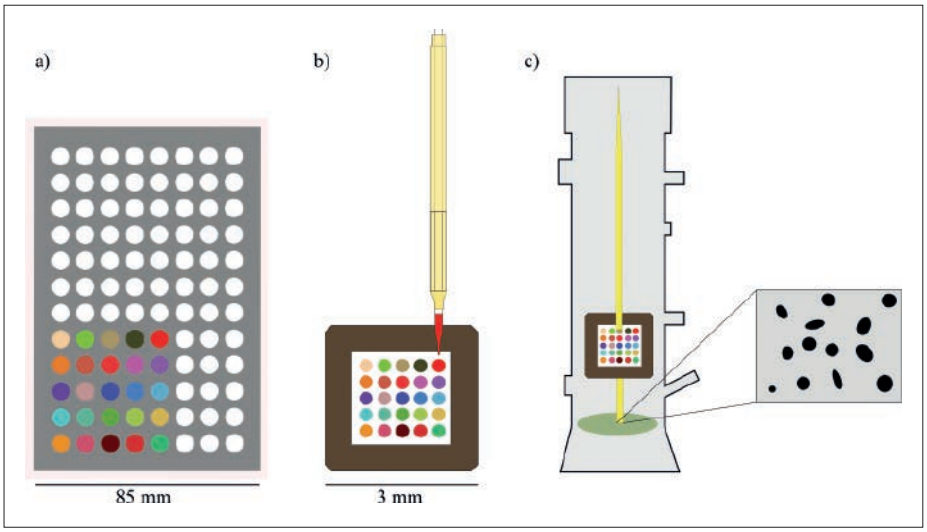

Fig. 1: Illustration of the multiplex procedure for INA-TEM. (a) Sample uptake from 96- or 384-well plate. (b) Piezoelectric dispensing of samples onto the TEM or SEM support. (c) Imaging and characterization of nanoparticle arrays with TEM.

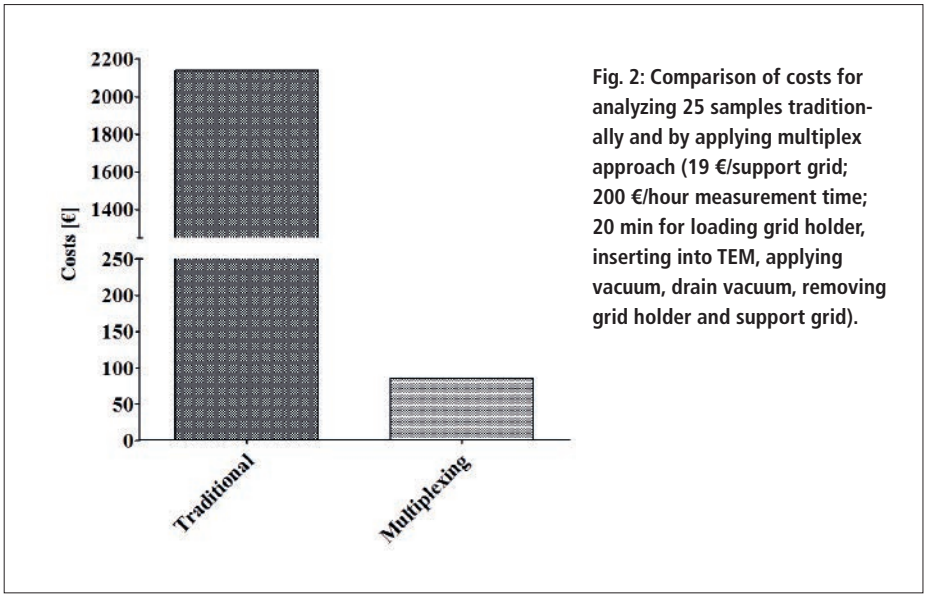

Fig. 2: Comparison of costs for analyzing 25 samples traditionally and by applying multiplex approach (19 €/support grid; 200 €/hour measurement time; 20 min for loading grid holder, inserting into TEM, applying vacuum, drain vacuum, removing grid holder and support grid).

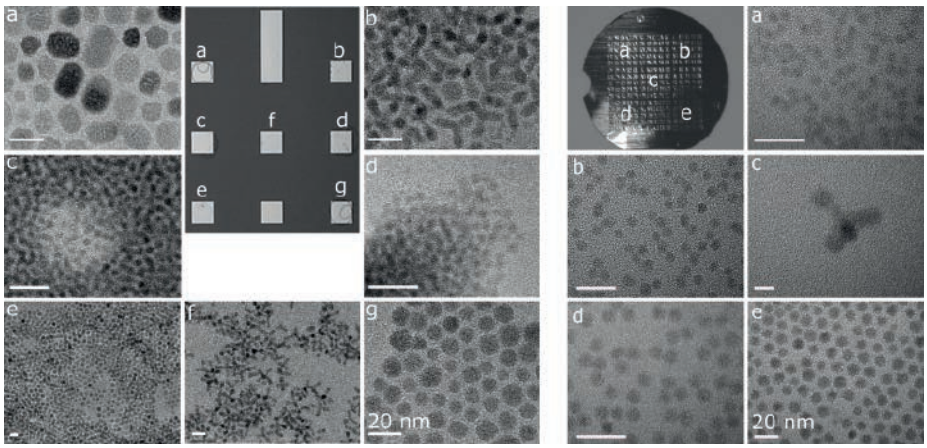

Fig. 3: TEM images of different printed toluene-based samples on a support. (Left) silicon nitride support with (a) $NaYF_4$ <100 nm; (b) CdTe 3x10 nm; (c) CdSe 4 nm, diluted; (d) CdSe 3x10 nm; (e) CdSe 4 nm undiluted; (f) $NaYF_4$ 9 nm; (g) $NaYF_4$ < 100 nm. (Right) Copper grid with carbon film loaded with (a) CdSe 4 nm; (b) CdSe 6 nm; (c) CdTe 3x10 nm; (d) $NaYF_4$ 9 nm; (e) $NaYF_4$ 6 nm.

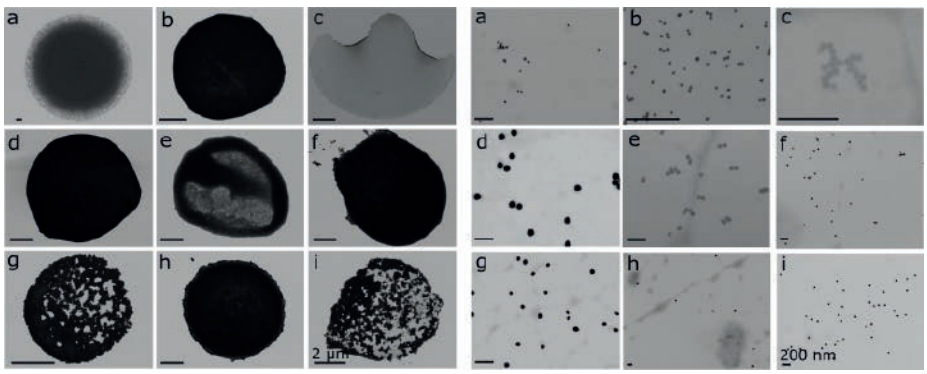

Fig. 4: SEM images of printed nanoparticle samples on a single EM support. (Left) Unmodified silicon nitride support, causing particle agglomerations during drying. (Right) Surface modified silicon nitride support with homogeneous particle distribution. (a) Silverplates; (b) Citrate-stabilized gold 15 nm (c) Silica 20 nm; (d) Platinum 70 nm; (e) Silica 50 nm; (f) Citrate-stabilized gold 40 nm; (g) bPEI-stabilized gold 50 nm; (h) Silver 80 nm; (i) PEG-stabilized gold 40 nm.

Introduction

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) measurements are standard methods for characterization of nanoparticles. Typically sample preparation for imaging occurs by manually preparing a single nanoparticle sample on an electron microscope (EM) grid utilizing standard laboratory µL pipets. In addition, many delicate handling steps have to be done using tweezers to move grids out of their packaging or to place it on the lab bench to load the grid with a sample. In these approaches, one sample is placed on one EM support. If more than one sample has to be investigated using EM, several grids have to be prepared and subsequently inserted into the instrument one after the other. For a sample/grid exchange the TEM has to undergo a complete cycle of losing and reestablishing vacuum conditions, which easily can take up to 20 minutes. Having the capability to insert multiple samples at once inside the TEM and acquiring all images without breaking vacuum for each one results in a significant sample throughput per EM instrument, that can be analyzed per working day. Arraying multiple samples onto an EM for this "Imaging Nanoparticle Arrays using TEM" (INA-TEM) technology can be achieved with a picoliter-dispensing system to print small amounts of multiple different nanoparticle suspensions onto a single support (fig. 1). Using a dedicated TEM sample preparation set-up, grids can be left in their packaging to load them with up to 100 different samples. A homogeneous distribution of the nanoparticle samples within the individual spots of the printed arrays can be adjusted by drop volume, environmental conditions (temperature/humidity inside the TEM sample preparation set-up) and grid surface.

Automated Multiplexing Procedure

For high precision loading of multiple nanoparticle dispersions on TEM and SEM supports a drop on demand spotter was used (sciTEM, Scienion, Berlin, Germany). This instrument allows printing of single drops of volumes between 50 - 800 pL onto a 1 mm x 1 mm grid window. The resulting spot diameters are typically between 50-500 µm. While similar technologies have been used to investigate proteins using TEM, sciFlexarrayer S3 [1], the sciTEM instrument provides automated grid recognition and QC imaging of the printed samples on one or multiple grids. With the tech-

nology developed, the costs for TEM supports and time for loading and unloading a TEM can be reduced significantly. For example when a 5 x 5 sample pattern is printed, 25 samples can be placed for imaging on a single grid, thereby saving the costs for 24 other grids that traditionally would have been used. At the same time multiple hours of EM instrument operation can be saved by avoiding to perform 24 sample exchanges / venting / vacuuming cycles (fig. 2).

Toluene- and Water-based Nanoparticle Dispersions

Samples of nanoparticles are commonly synthesized and prepared in organic solvents or aqueous solutions [2]. Due to their different rheological behavior, these solvents require different handling when applying non contact piezoelectric deposition technology. Dispensers with tailor-made surface coatings have been developed for printing of toluene-based as well as water-based nanoparticle suspensions.

High quality SEM/TEM images require a homogeneous distribution of nanoparticles on the TEM supports. This is mainly determined by the nature of the nanoparticles, the solvents used and the wettability of the support films. Figure 3 shows the TEM images of seven different toluene-based nanoparticle samples printed onto a single silicon nitride support (SimPore, West Henrietta, NY, USA) and in addition five toluene-based samples printed onto a single copper grid with a carbon film (TedPella, Redding, CA, USA).

Printing of 190 picoliter droplets of nanoparticle suspensions in toluene on silicon nitride supports or copper grids with carbon film typically results in an uniform distribution of the nanoparticles within the spots after evaporation of the solvent due to the relatively low surface tension and high vapor pressure of toluene. This eases the imaging of multiple nanoparticle samples on a single grid during one measurement cycle and allows imaging of the sample in different regions of the spots.

In contrast when using aqueous nanoparticle suspensions strong agglomeration effects of the nanoparticles within the spots can occur after the drying process as result of high surface tension, evaporation flux and Marangoni flow. Methods of surface functionalization of the support films have been developed to overcome these effects. Hydrophilicity of the film surfaces can be increased or ionic groups can be introduced by application of these methods without increasing the background signal in SEM/TEM measurements. For comparison the SEM images of nine different nanoparticle samples (NanoComposix, San Diego, CA, USA) loaded on an unmodified and surface modified EM support are shown in figure 4. The nanoparticles distribution within the spots of the printed arrays could be significantly optimized and agglomeration effects minimized when using the surface modified supports compared to uncoated grids.

Finding and imaging the single spots on the respective grids during the TEM measurement can result in some challenges when using supports with no indexing. (Due to the small field of view in a TEM instrument, some time has to be spend to identify the spotted regions on a grid.) The procedure becomes more straightforward when indexed grids as those shown in figure 3 were used. Alternatively, the spotted positions on a single grid are available from the printer after loading with the nanoparticle samples, and these might be imported into TEM instruments for automated measurements in future applications.

Conclusion

Applying a picoliter liquid dispensing technology for sample preparation on EM grids allows multiplexing of nanoparticle characterization using TEM, introduced as INA-TEM. This new technology can be applied for water- and toluene-based nanoparticle solutions. It reduces significantly the effort of manual and tedious handling of flimsy and fragile grids. In addition to precise dispensing of picoliter samples onto silicon nitride grids, surface coatings of the support films have been developed to obtain homogeneous distribution nanoparticles within the spots. This new multiplex methodology for sample preparation on EM grids increases sample throughput of an EM instrument in combination with reduced costs for the consumable needed.

Acknowledgements

We thank Dr. Emory Chan, Lawrence Berkeley National Laboratory, Berkeley, USA, Dr. Werner Oesterle and Dr. Erik Ortel, Federal Institute for Material Research and Testing, Berlin, Germany, for support and TEM / SEM measurements.

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