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# Nano-Particle TEM Sample Preparation Primer

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As nanotechnology continues to drive scientific research, it becomes increasingly difficult to characterize the "nano" structures. Transmission electron microscopy (TEM) has proven to be a significant technique to provide a range of data for most nanomaterials. Point resolutions at 0.24 nm or better with the aberration corrected TEMs can allow for direct imaging of the atomic structure of a sample. [1] It can be a daunting task to determine which TEM grid and or support film to use to obtain the best results for a specific sample type. The old adage of "garbage in, garbage out" is never truer than with electron microscopy sample preparation. Nanomaterial samples seem to magnify this issue at a geometric rate! Often times there is a small amount of the sample with which to work, and when working with "unseen samples" one can sometimes be unsure of adequate sample preparation. To compound these issues is the decision as to which sample substrate should be utilized to provide the best analytical characterization of nanomaterials.

To determine an elementary "go to" protocol for grid and support film selection for TEM examination of nano-particles, copper grids with different support films were evaluated. The support films were lacey carbon (LC), thin holey carbon (HC), holey carbon/Formvar (HCF) and lacey silicon monoxide/Formvar (SiO/F). Carbon black (CB), Cabot Black Pearl 120, and colloidal gold solution (Au), Sigma, were used as the test materials. The CB simulated the lower atomic weight nano-particles while the Au simulated the higher atomic weight nano-particles. The size of the particles are in the 5-100 nm range with the CB at the larger end of the range. Dilution of the CB was at 0.02g/ml and for the Au it was 6.5 x $10^{-5}$ g/ml in 100 ml of $CH_3OH$.

Two methods of dispersing the CB and Au on the TEM grids were evaluated. One method involved the dispersion of the solid in $CH_3OH$ by ultrasound and manually dropping 10 μl of the solution on a TEM grid. The grids were then allowed to air dry. The second method was by using an ASP-1000 Automated Specimen Processor by Microscopy Innovations. TEM grids were placed in the mPrep/g capsules and attached to the reagent lines of the ASP-1000. Initially the suspension liquid was drawn into the loaded capsules and the grids were allowed to dry. After loading, the grids some were rinsed for 5 minutes, with $CH_3OH$, for up to 3 times, Table 1. The grids were allowed to dry in the mPrep capsules. Evaluation of the grids from both sample protocols was completed on a Tecnai F20 at a low and a high magnification, Figure 1. ImageJ as used to determine the percent coverage and percent agglomeration of the grids, Table 1. Other characteristics evaluated were, degree of aggregation and negative support film interference, Table 1.

Single loading with 10 μl and automated loading with no rinsing often resulted in total coverage of the grid. The automated loading with rinsing showed less material on the grid and fewer particles in the agglomerations, Figure 2. Lacey SiO with Formvar film proved to be the best support film, with multiple single particles on the film edges with little or no background artifacts in the TEM images. Automation uses less initial volume and with controlled rinses reduces the amount of agglomerations thus allowing for single particle imaging. As most labs will not have automation capabilities the use of the mPrep capsule fitted with a microliter pipet can have the same affect for sample prep. A basic primer for TEM sample preparation for nano-particles includes a support film with multiple openings, minimal material for loading and multiple rinses for better single particle imaging.

References:

[1] J. C. H. Spence, *et al*, Philos. Mag. 86, (2006), p. 4781.

Table 1 Grids and Characteristics (Number of rinses in brackets preceding value)

| Grid | Loading* | # of Rinses | % Coverage | Agglomeration % (CB) | Film Background |
|---|---|---|---|---|---|
| SiO/F | D | 0 | 80 | Not determined | Multiple over open |
| SiO/F | A | 0, 1, 2, 3 | [1]15 | [1]70, [2]50, [3]20 | Multiple over open |
| HN | D | 0 | 50 | Not determined | Few over open |
| HN | A | 0, 1, 2, 3 | [1]13 | [1]85, [2]50, [3] 25 | Few over open |
| HCF | D | 0 | 100 | Not determined | Few over open |
| HCF | A | 0, 1, 2, 3 | [1]26 | [1]90, [20]50, [1]30 | Few over open |
| LC | D | 0 | 14 | Not determined | Few over open |
| LC | A | 0, 1, 2, 3 | [1]3 | [1]82, [2]60, [3]20 | Few over open |

*D-Drop, A-Automated

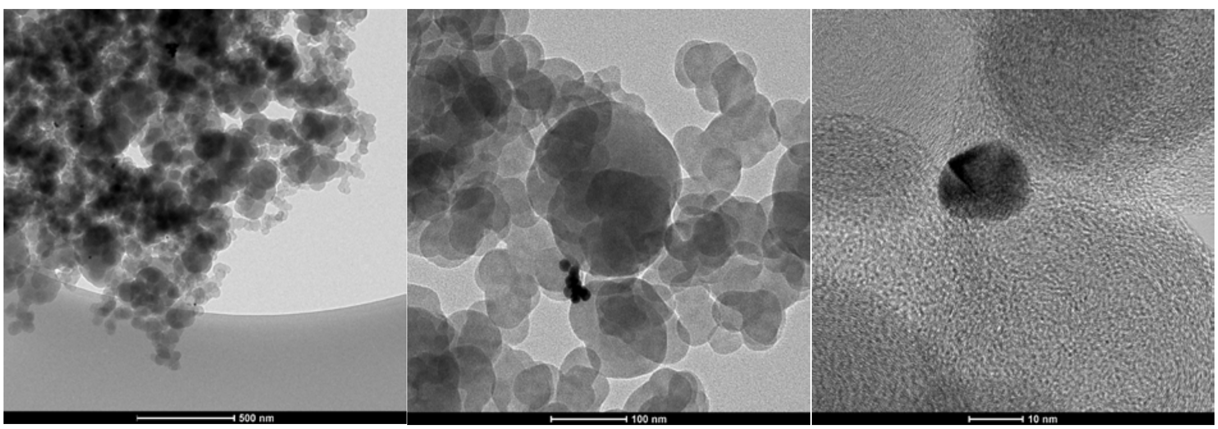

Figure 1 – CB and Au particles on a HCF support film, a. 9,900x, b. 38,000x, c. 285,000x

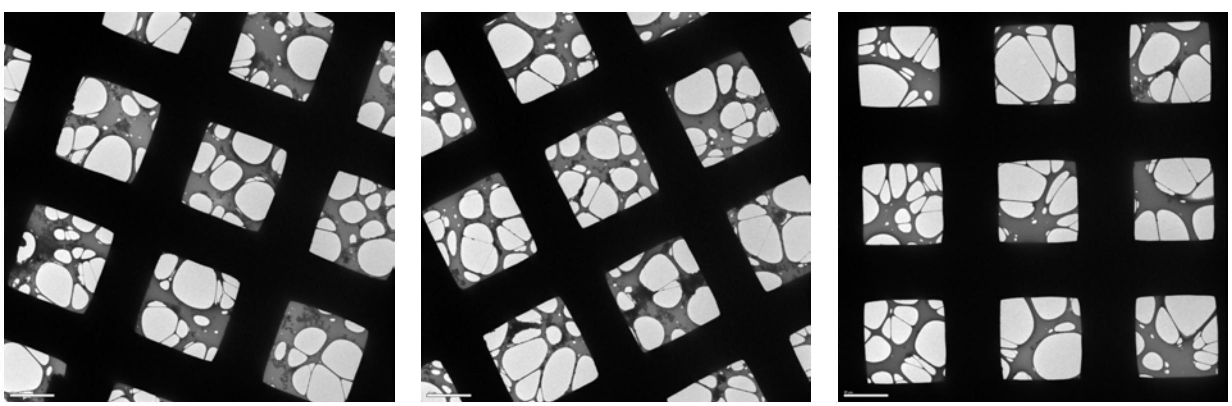

Figure 2 – CB and Au particles on a LC support film at 300x; a, 1 rinse, b. 2 rinses, c. 3 rinses

# Nano-Particle TEM Sample Preparation Primer

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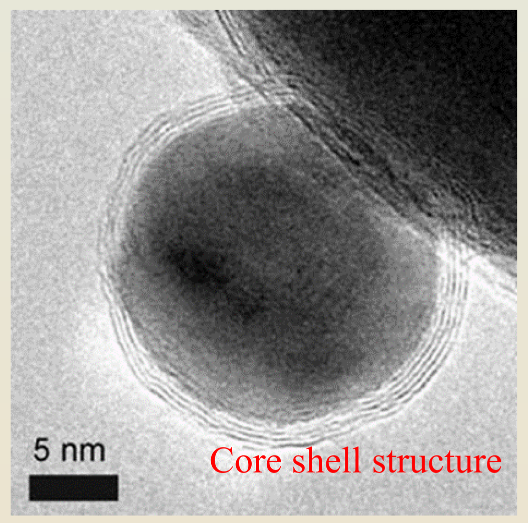
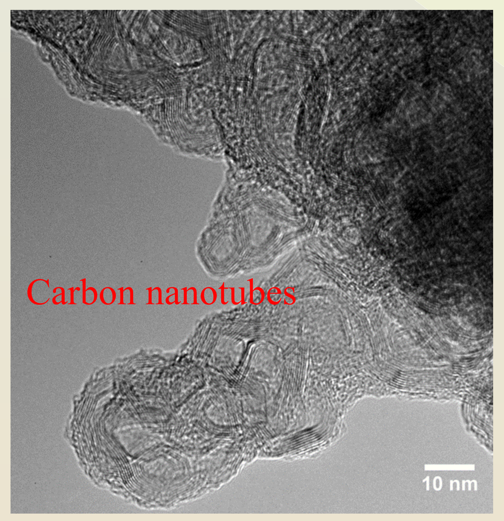
## INTRODUCTION

**As nanotechnology drives scientific research, it becomes increasingly difficult to characterize the latest and greatest "Nano" structures.**




**Transmission Electron Microscopy (TEM) has proven to be the most versatile instrument to deliver an array of data on most nanomaterials**

## OBJECTIVES

- **Best fit TEM grid for nanoparticles**
- **"Go To" Sampling technique**
- **Automated TEM sample prep for material samples**

**Selection of "right" TEM grid or support film has never been an easy task. The only method to determine which grid works best for a particular sample and analysis type is to just "Try It"**

## SAMPLE PREP

**Samples were prepared by suspending carbon black and colloidal gold solution in a methanol matrix.**

**Carbon black was used to represent the lighter nanoparticles such as polymers and carbon nanotubes. Colloidal gold was used to represent the heavy nanoparticle materials such as gold, platinum, iridium, or the transition elements. Methanol was used as it is readily available in most labs.**

**Samples were imaged with a Tecnai F20 TEM at 200 kV.**

- **Cabot Black Pearl 120 Carbon Black (CB), 0.02g/ml, ~100 nm**
- **Sigma Colloidal Gold Solution (Au), 6.5 x $10^{-5}$ g/ml, 5 nm**
- **Reagent grade Methanol, 100 ml**
- **All solutions were ultrasonicated for 15 min. prior to sampling (manual and automated)**

**11 grid types were manually prepared by placing 10 µl of the suspension on the grid and allowing it to dry. These samples were then placed in a TEM storage box after drying.**

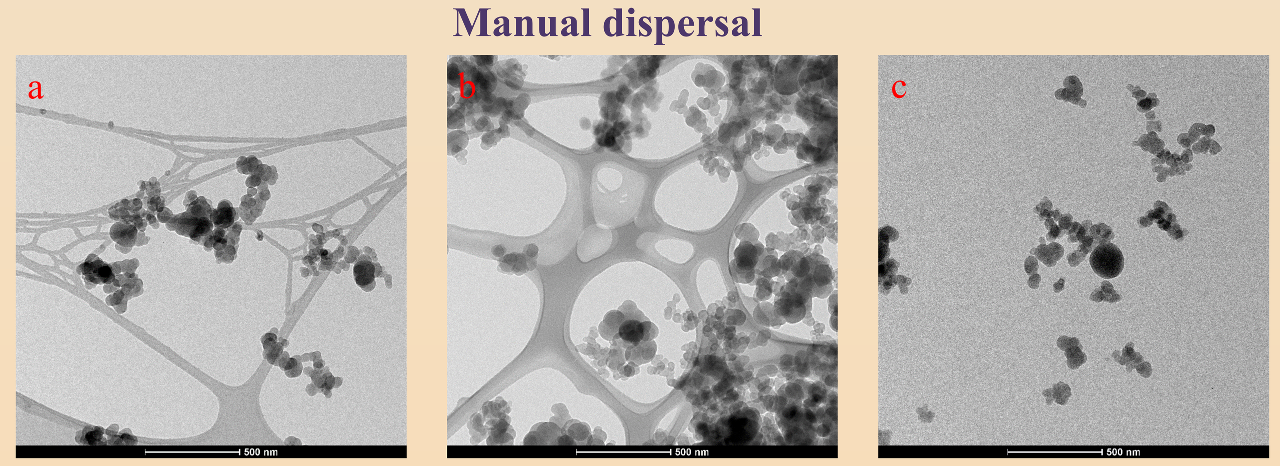
**Of those 11, four types were selected to be evaluated with the mPrep robot system of Microscopy Innovations.**

**Using an ASP-1000 Automated Specimen Processor by Microscopy Innovations. TEM grids were placed in the mPrep/g capsules and attached to the reagent lines of the ASP-1000. Initially the suspension liquid was drawn into the loaded capsules and the grids were allowed to dry. After loading, some grids were rinsed for 5 minutes, with $CH_3OH$, for up to 3 times. The grids were allowed to dry in the mPrep capsules.**

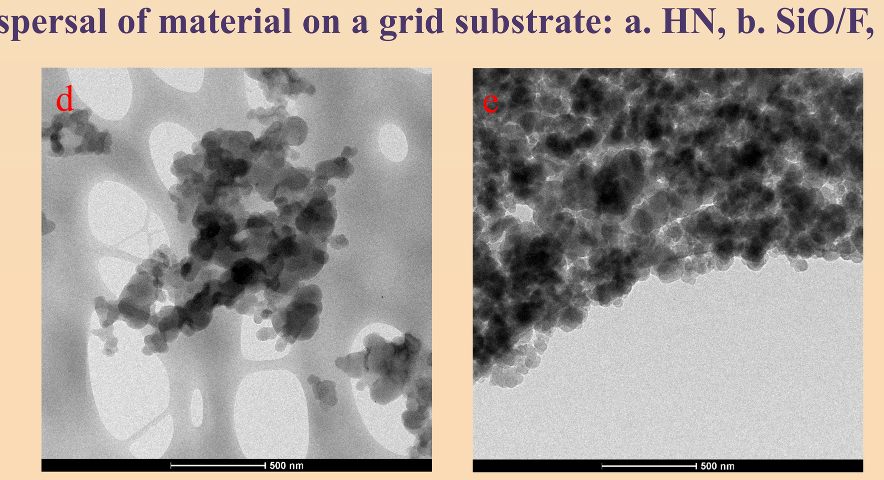
| Grid | Manual | Automated |
|---|---|---|
| Thick holey carbon 400 Mesh (THC) | X | |
| Quantafoil R1 2/1.3 400 Mesh (Q) | X | |
| Lacey carbon 400 Mesh (LC) | X | X |
| Thin holey carbon 400 mesh (HN) | X | X |
| Holey carbon/Formvar film 400 mesh (HCF) | X | X |
| C-Flat Holey carbon film 400 mesh (CFH) | X | |
| Lacey Silicon Monoxide/Formvar 300 mesh (SiO/F) | X | X |
| Thick holey carbon/Formvar 200 mesh (THCF) | X | |
| Lacey carbon/Formvar 300 mesh (LCF) | X | |
| Holey carbon/Ultra thin carbon 400 mesh (HCUT) | X | |
| Ultra thin carbon (~ 3 nm) 400 mesh (UTC) | X | |

## RESULTS

### Manual dispersal

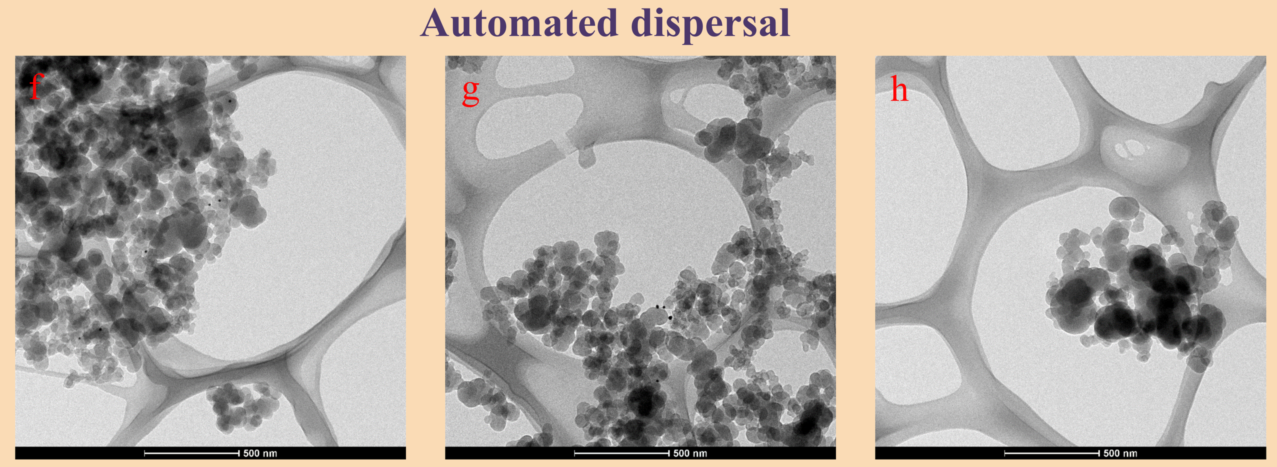



**Good dispersal of material on a grid substrate: a. HN, b. SiO/F, c. THCF**

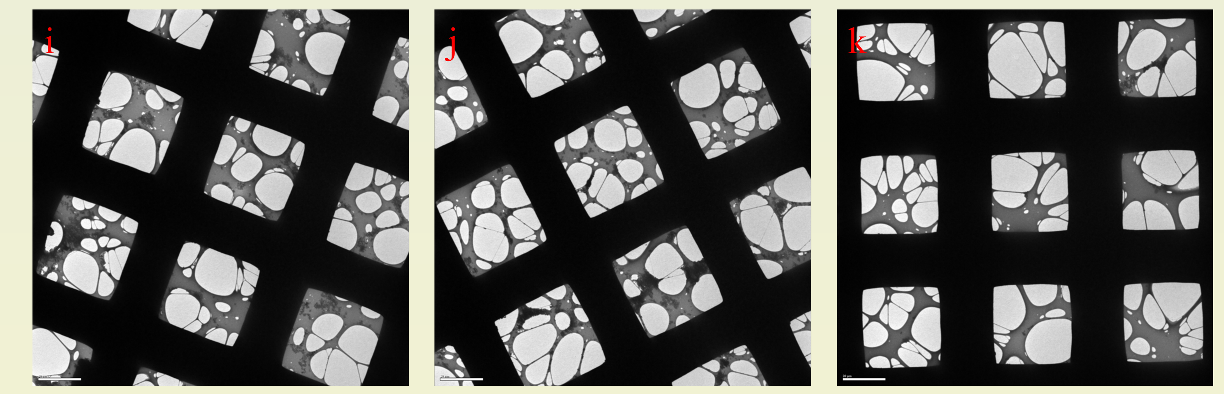


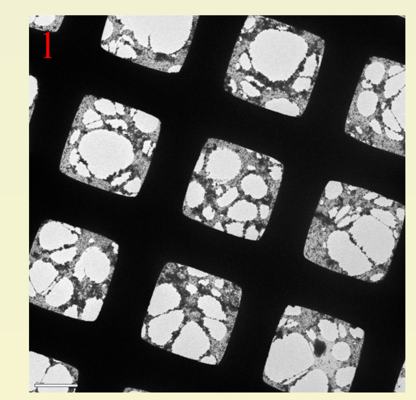

**Poor dispersal of material on a grid substrate: d. LCF, e. CFH**

### Automated dispersal

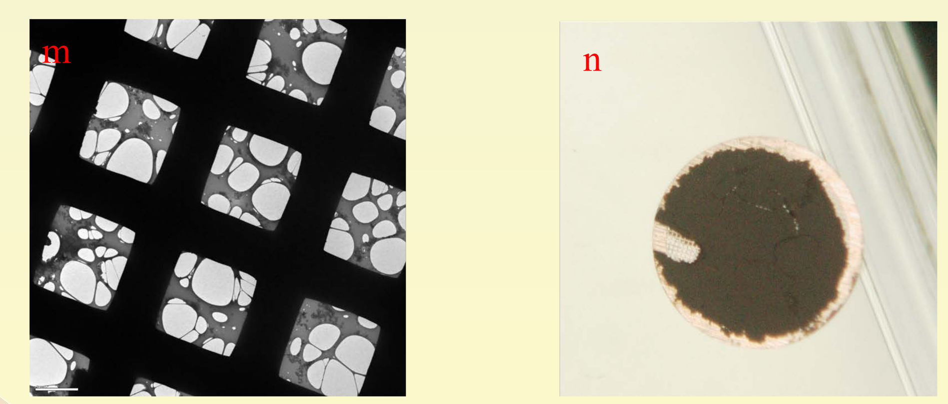



**Rinse on dispersal for SiO/F: f. 1 rinse, g. 2 rinses, h. 3 rinses**

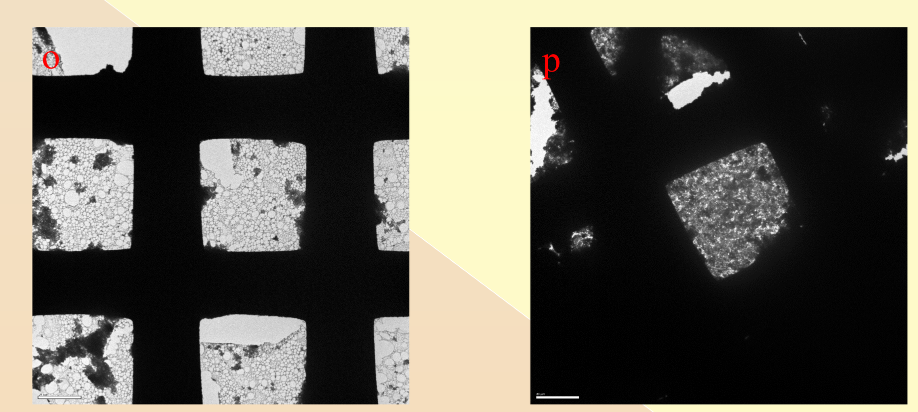



**LC @300x, a. 1 rinse, b. 2 rinses, c. 3 rinses, d. 10 µl dropwise addition. Approximately 3% coverage of CB for one rinse vs 14% coverage of CB with drop addition.**

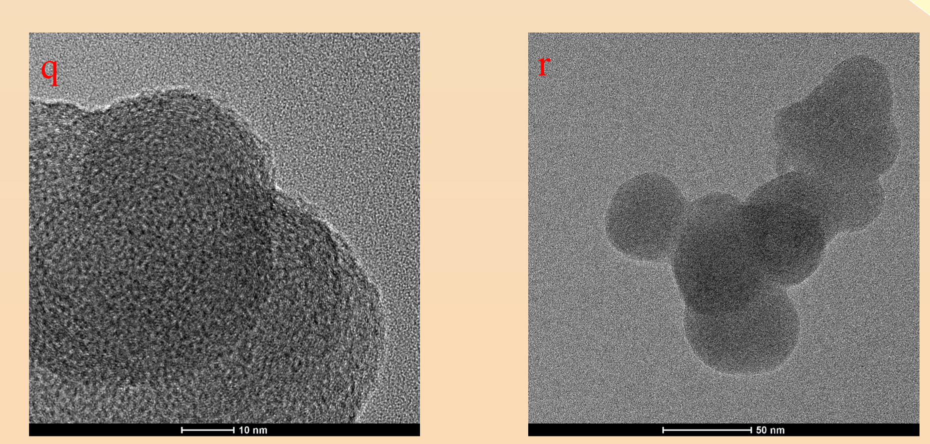



**HFC: m. one rinse, n. dropwise addition to grid, total coverage of grid**

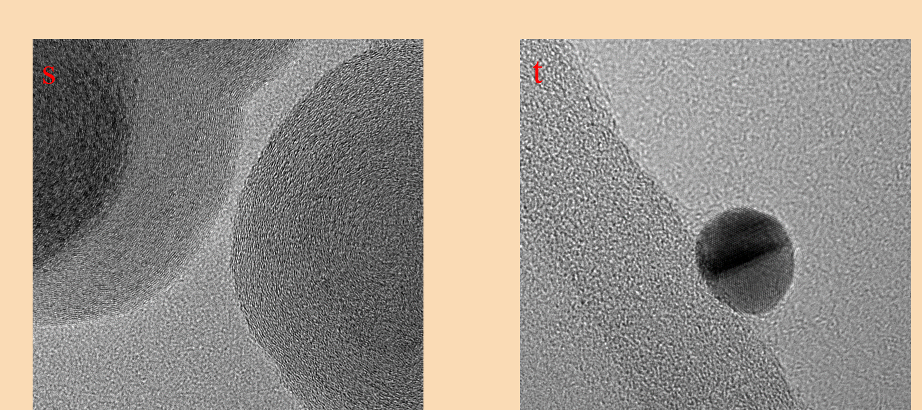



**SiO/F: o. one rinse, p. dropwise addition grid**

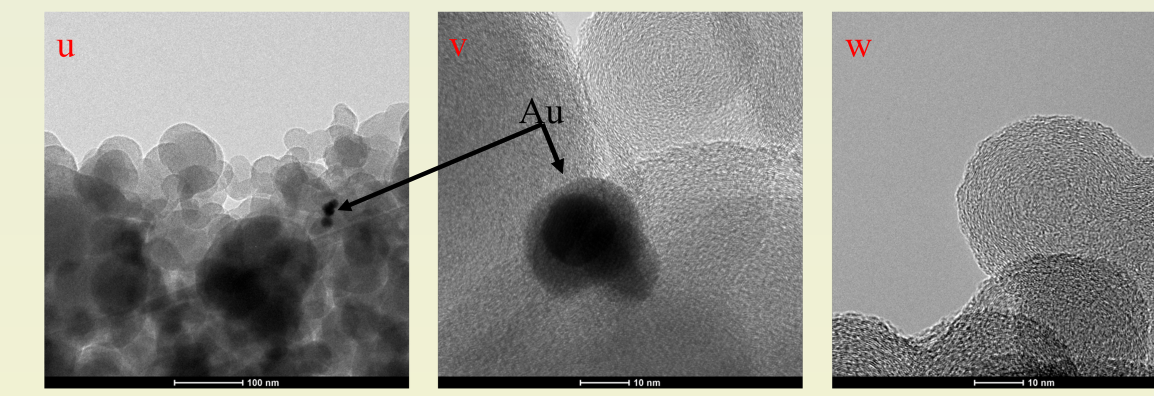
### Contrast



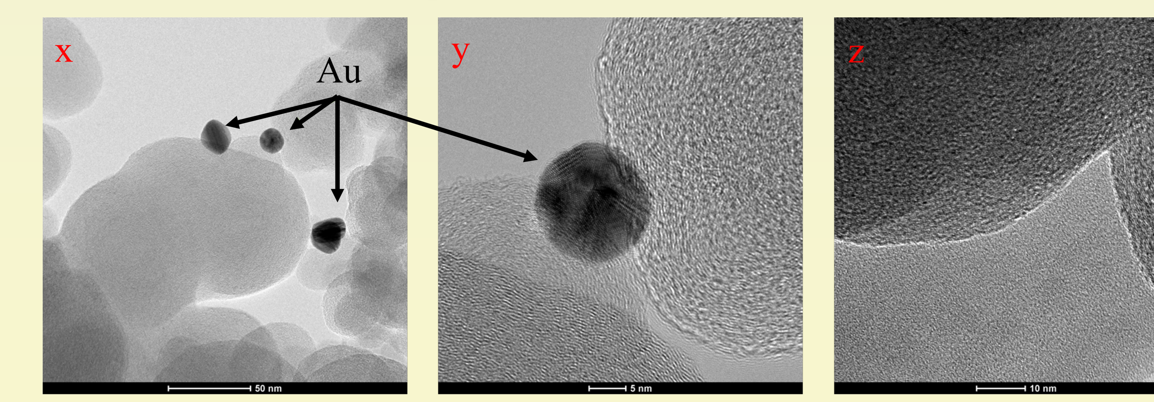

**Poor contrast over the substrate: q. LCF, r. THCF**



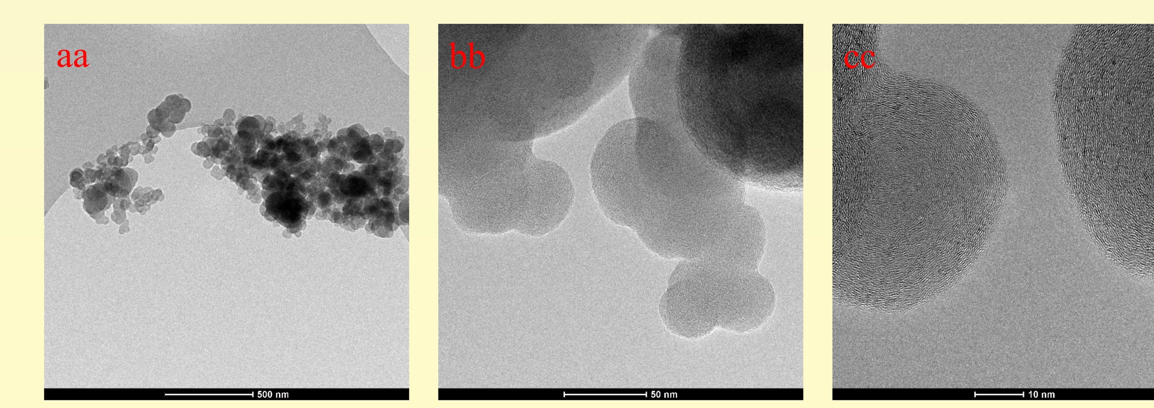

**Good contrast over the substrate, UTC: s. CB, t. Au**




**HCF grid had multiple particles over the open areas. When the material is on the carbon film it becomes difficult to adjust the contrast of the structure due to interference from the film. Particles over the open areas showed a layering of particles, making it difficult to differentiate individual particles.**




**SiO/F grid displayed the least agglomeration of the materials with a large concentration of the material over the openings in the film. Although there was some agglomeration it was easier to do single particle analysis. The structure of the Au particle is seen clearly as well as the turbostratic nature of the CB.**




**Q grid displayed larger areas for materials to adhere and extend over an opening. There is still agglomeration of particles but there were areas of low particle density allowing for individual particle analysis.**

## CONCLUSION

**Single loading with 10 µl and automated loading with no rinsing often resulted in total coverage of the grid. The automated loading with rinsing showed less dense material with fewer particles in the agglomerations on most grids. SiO/F film proved to be the best support film, with good dispersion, multiple single particles on the film edges with little or no background artifacts in the TEM images. Although this grid provided the best support it was less robust under the beam than other support films. The Q grids also showed multiple particles on the hole edges, but with larger agglomerations, and proved to be more robust under the beam.**

**Automation uses less initial volume and with controlled rinses reduces the amount of agglomerations thus allowing for single particle imaging. As most labs will not have automation capabilities the use of the mPrep capsule fitted with a microliter pipet can have the same affect for sample prep. A basic primer for TEM sample preparation for nano-particles includes a support film with multiple openings, areas for good contrast during imaging, minimal material for loading, and multiple rinses for better single particle imaging.**

## REFERENCES

J. C. H. Spence, et al, Philos. Mag. 86, (2006), p. 4781.