

## Preparation of STEM samples Coating of Cu grids with Nanoparticles from solution

1. If possible, only touch the Cu-Grid at the edge, outside the grid, with very fine inverted tweezers. If necessary, work under a magnifying glass. Pay attention to the correct side of the grid (the glossier side).

Inside the grid is the carbon layer in between and it is very sensitive and can break. The Cu grid should not be corrugated after the preparation. If the carbon film breaks before coating with the sample, there are usually particles on both sides of the grid, which is undesirable.

- 2. Establishing the best nanoparticle concentration in a suspension (e.g. in ethanol) is an important aspect. The solution of the suspension should not react with the nanoparticles or dissolve the particles themselves. If the concentration is too high, particle agglomerates can form during drying and these can lie on top of one another, so that the sample cannot be used for analysis. Experience shows that the appropriate particle concentration is about 10-100 times higher than for DSL. However, it depends on the nanoparticle size, the scattering properties and the electron density of the material.
- 3. A drop of this prepared suspension is applied to the TEM-Cu grid and this is allowed to dry. The particles can rearrange themselves during the drying process. A monolayer would be desirable.
- 4. You should also take into account the drying process, especially if you are working with organic molecules, that you leave your suspension on the TEM grid overnight until it is dry. Proteins and other components from liquids may crystallize on the grid. When working with concentrated liquids and nanoparticles that are not very electron dense (such as silicon dioxide), the dried molecules can make it difficult to distinguish the nanoparticles on the lattice (artifacts).
- 5. If you intend to analyze the aggregation / agglomeration of nanoparticles in your media, you should consider whether the agglomeration is real or whether it is an artifact of TEM sample preparation. Suspensions with low concentrations partially solve this problem.

Ultimately, the optimization of the concentration is an empirical task that is improved and adapted from preparation to preparation.

At the edge of the Cu grid, there should not be too many particles if possible, otherwise the STEM holder will be contaminated and the particles may fall down onto the detector.