Re-Viewing of Stereoscopic imaging: a real alternative to EM tomography techniques?

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Many relatively new techniques as TEM tomography and FIB-SEM serial sectioning are applied to get three dimensional (3D) information of a sample. In this work we will demonstrate that sometimes stereoscopy, a comparatively simple technique, could provide a very good overview of the three dimensional arrangement of the features in a specimen. Whenever e.g. the connectivity of fine networks is of interest (in nanotube or nanofiber networks), the only way to check the connectivity is to collect 3D information.

Stereoscopy is a very fast and simple method, to collect 3D information of a sample with a transmission electron microscope (TEM) as well as with a scanning electron microscope (SEM). It only requires two images (stereo pair) from the sample, which has to be tilted by some degrees after collecting the first of the two images. Even if the sample is sometimes not stable and moves around under the electron beam, usually due to charging effects, there is a good chance to collect a stereo pair because the sample must stay on its position only for a short exposure time (10 to 20 seconds) and the stability may be further reduced by low dose techniques. For other methods like TEM tomography or serial sectioning which deliver also 3D information an elaborated sample preparation - fine networks must be embedded to be stable - is necessary. Then a time-consuming data collection of multiple projected images followed by a complex data analysis is needed to obtain a complete 3D data set of the sample. By contrast, to produce a stereoscopic image out of the two tilted images is very easily done by hand or can be performed by one of the numerous stereoscopy software packages.

Many TEM images give the impression that the sample is completely flat, while in fact it is not. The silver nanoparticles shown in figure 1 are located on a three dimensional network built by an amorphous silica substrate. For the latex nanoparticles shown in figure 2 it is impossible to make an interpretation on the arrangement in the three dimensional space, but it is be clearly recognizable in the stereoscopic image, which particles are on top and which are below the others. Also in the SEM image figure 3 the stereoscopic image gives a perfect impression of 3D arrangement of the membrane ruffles induced by the bacteria.

The TEM images shown below were taken with a Philips CM30 LaB6 at 300kV and the SEM images are collected with a XL-30 FEG SEM FEI. To mount the stereoscopic images the program StereoPhoto Maker [1] was used.

Stereoscopic imaging in EM is a very helpful technique to explore the third dimension (z-direction) of filigran nanowire networks or other complex samples. It helps e.g. to decide if an image feature is above or below the other one as it is demonstrated in the figures 1 and figure 2 and the connection as well as the arrangement of particles or tubes could easily be checked.

1. http://www.stereomaker.net/eng/stphmkr/

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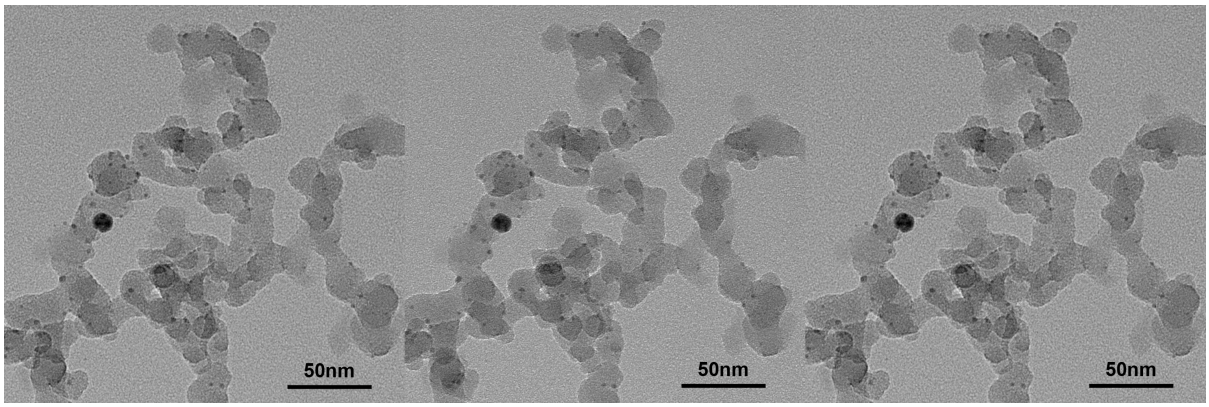

Figure 1. Stereoscopic TEM-image of silver nanoparticles (small black dots) on amorphous silica. Left and middle image are a stereo pair for parallel view, the middle and the right image build a stereo pair for crossed eye viewing.

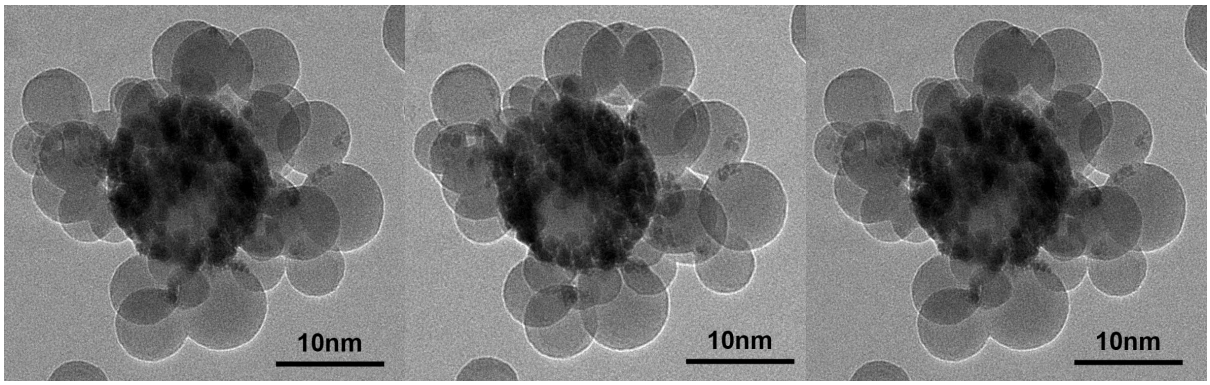

Figure 2. Stereoscopic TEM-image of latex nanoparticles. Left and middle image form a stereo pair for parallel view, the middle and the right image build a stereo pair for crossed eye viewing.

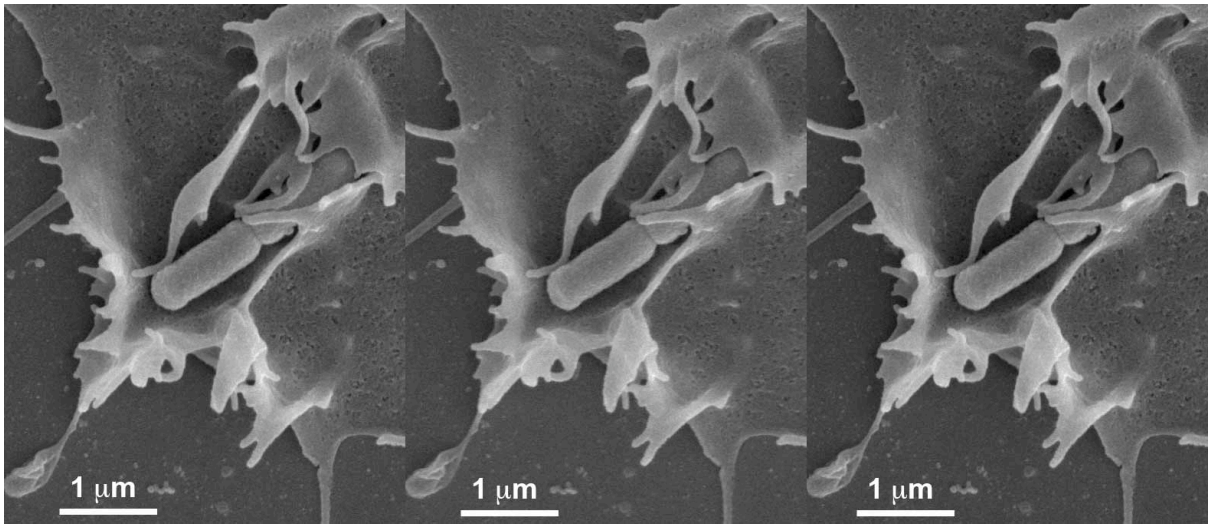

Figure 3. Stereoscopic SEM-image of a bacterium on a hela-cell. Left and middle image are a stereo pair for parallel view, the middle and the right image build a stereo pair for crossed eye viewing.

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