Quantitative High-Resolution TEM/STEM and Diffraction

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Factors influencing high quality low voltage TEM imaging of biological routinely stained specimen

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Low-voltage TEM imaging requires sufficient electron transmittance. One advantage of low-voltage TEM is the increased electron scattering resulting in higher contrast. Principally this implies that negative stain contrast agents may not be needed which would be beneficial since they inherently reduce the electron transmittance. However, negative stains are not only used to increase the contrast when imaging biological specimens. They also help in fixation and protect the specimen from dehydration. Hence, a requirement to acquire high quality images of biological specimen using a low-voltage TEM is fixation/staining while maintaining enough electron transmittance. This identifies and a few factors that can be investigated in order to acquire images of biological specimens using low-voltage TEM: supporting film, choice (molecular weight) of stain and thickness of stain layer. Here we have focused on the first factor, the supporting film. More specifically, we have focused on the thickness of amorphous carbon films since such supports tend to have negligible influence of the specimen, and the negative stains generally distribute evenly (which to our experience is not the case when using very thin graphene grids).

We here present a small study where the influence of the thickness of the carbon supporting film on electron transmittance has been investigated. The electron transmittance for two thicknesses of carbon films (EMS grids >30 nm and KC S6 grids <10 nm) has been measured at a range of energies, from 5 keV to 100 keV in an LVEM 5 (5keV), and a Tecnai Spirit BioTwin (20 to 100keV). The transmittance was derived as the ratio of the mean intensity values of images acquired with and without the grids inserted into the electron beam. How the transmittance for the two carbon film thicknesses varies for different electron voltages is shown in Figure 1.

To illustrate how the final low-voltage TEM image quality is influenced by the carbon film thickness, thick and thin carbon film grids containing BK virus-like particles stained with 2% UAc were prepared and imaged at 20 keV in a Tecnai Spirit BioTwin microscope at magnifications ranging from 6000 to 87000. Example images of the EMS and KS C6 grids are shown in Figure 2 together with their corresponding histograms.

In conclusion: reducing the carbon layer thickness from ~30 nm to <10 nm results in a significant increase in electron transmittance showing the following benefits:

- Improved image quality
- Reduced risk for dehydration effects
- Rapid and reproducible preparation procedures
- Conventional nsTEM procedures can be utilized
- Reduced chromatic aberration originating from the non-elastic scattering of the incident beam in the sample

1. This work was carried out jointly by the Centre for Image Analysis at Uppsala University and Swedish University of Agricultural Sciences, Vironova AB, and Delong Instruments, as part of the Eurostars miniTEM project, in which a low-voltage TEM with built in automated acquisition and analysis functionally is being developed.

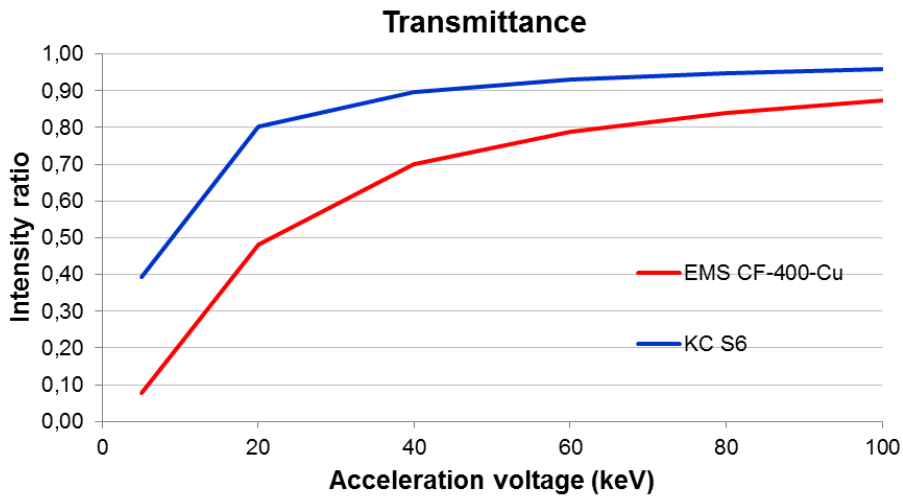

Figure 1. Electron transmittance of KC S6 grids (blue) and EMS CF-400-Cu grids (red) at energies from 5 to 100 keV. The values are derived as the ratio of the mean intensity values of images acquired with the grid inserted in the electron beam and images acquired with no grid inserted.

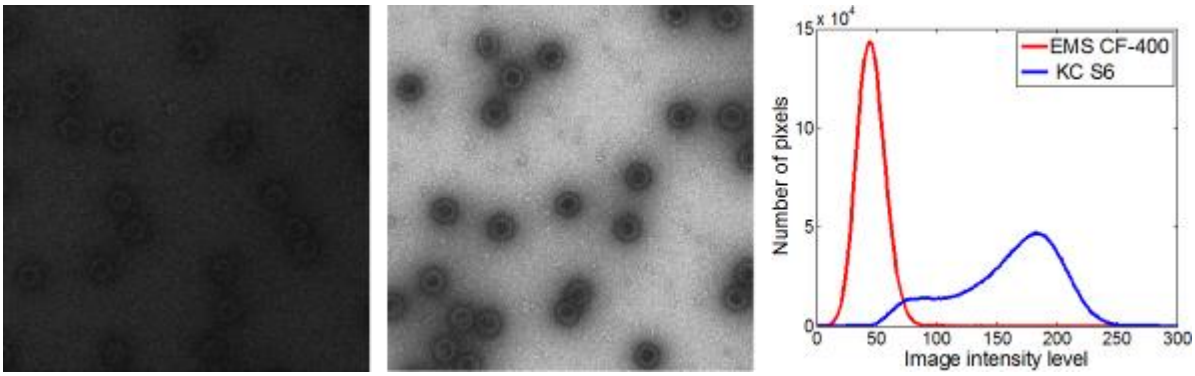

Figure 2. Example images of rBK viruses acquired at 20keV and 60 000 magnification on an EMS CF-400 grid (left),and KC S6 grid (middle). The corresponding histograms (right) are shown in red for EMS CF 400 and blue for KC S6. The images were acquired as 16bit images whereof the intensity range [0 300] is displayed here.