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On the Benefit of Aberration Correction in Cryo Electron Microscopy

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Aberration correction [1] is very successful in materials science since about 20 years and enables reliable materials characterization with true atomic resolution. In cryo electron microscopy (cryo EM) the resolution of the TEM hasn't been the limiting factor for a long time. Recently, however, cryo EM has reached the single Ångstrom level [2] where the aberrations of the TEM start to play a significant role in the process of image formation.

Although the images in cryo-EM are recorded with a relatively large defocus which is taken into account during a-posteriori CTF-correction, the coma-free alignment, i.e. adjustment of the beam tilt, is extremely important and imposes very strict requirements to the accuracy and the stability of the illumination alignment of the TEM [3]. As an example, to maintain a reasonably small axial coma, e.g. B2 < 100 nm, the beam tilt has to be correctly adjusted to the level of only 30 µrad for a cryo lens at 300 kV. Otherwise, the spherical aberration of the objective lens causes a severe amount of beam-tilt-induced axial coma. Moreover, this beam alignment has to be stable for a very long time in order to meet the requirements for the automated data acquisition. The situation becomes much more relaxed in a Cs-corrected TEM, where even a beam tilt of several mrad is acceptable without noticeably introducing axial coma.

For improved data acquisition rates it is common practice to use combined beam-image-shifts during image recording rather than shifting the specimen. This virtual specimen shift, however, is affected by the unavoidable off-axial coma of the objective lens (Fig. 1) which compares to an azimuthal beam tilt orthogonal to the direction of image shift [3]. It has been shown that a precise synchronous adjustment of the beam tilt during beam-image-shift allows to compensate for the effect of the off-axial coma [4]. In a Cs-corrected TEM, however, the corrector has to be used to actively compensate the shift-induced coma below the object during image shift. The beam tilt above the specimen does not need to change and remains flexible, e.g. for correction of dispersion by beam tilt in the presence of chromatic aberration Cc. An aberration-free beam-image-shift of up to 10 µm is feasible, which is extremely useful for an efficient work-flow in data recording for single particle reconstructions.

Large cameras with a high number of pixels allow to record much more particles per image hence are important to increase throughput. The large field of view which is captured at once, however, is affected by the off-axial coma in that the coma increases linearly from the center of the image towards the outer areas. While this effect is negligible for 2k cameras, it can have a distinct impact on 4k detectors with a similar pixel size. Fortunately, this limitation can be overcome by the off-axial coma corrector (BCOR) which enables aplanatic imaging, i.e. freedom from spherical aberration and off-axial coma [5].

A considerable fraction of electrons that interacted with a cryo specimen has lost some energy in the object (inelastic scattering). Due to the chromatic aberration of the objective lens these electrons are focused differently and consequently do not contribute to image contrast but instead just form a blurred background intensity. A Cc/Cs-corrector (CCOR) images all electrons independent of their energy to the same image plane [6] (compare Fig. 2). It is expected that in this case the inelastically scattered electrons contribute to the image contrast as well. The more inelastic interaction happens in the specimen the larger this advantage of chromatic aberration correction could be. Therefore, we have good reason to believe that a Cc/Cs-corrector is especially beneficial for cryo tomography, where the projected sample thickness can be quite large for the high specimen tilt angles. In addition, the CCOR simultaneously corrects for the spherical aberration and, like the BCOR, for the off-axial coma (achroplanatic imaging). [$]

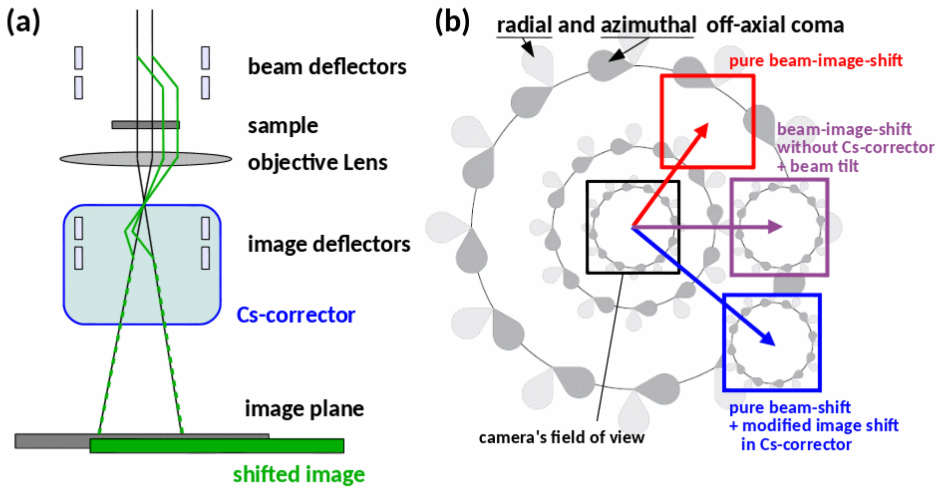

Figure 1. (a) Synchronous use of beam and image deflectors is commonly used for virtual object shifts. However, this shift is strongly affected by off-axial coma (b) as illustrated in red. In an uncorrected TEM, an additional beam tilt can be used to counterbalance the off-axial coma effect (magenta). In a Cs-corrected TEM, the corrector elements are coupled such that the off-axial coma contribution is compensated for (blue), the beam tilt remains flexible. For large cameras the off-axial coma even changes the image quality within a single camera frame. To remove this effect as well, a corrector for off-axial coma (e.g. BCOR) has to be used.

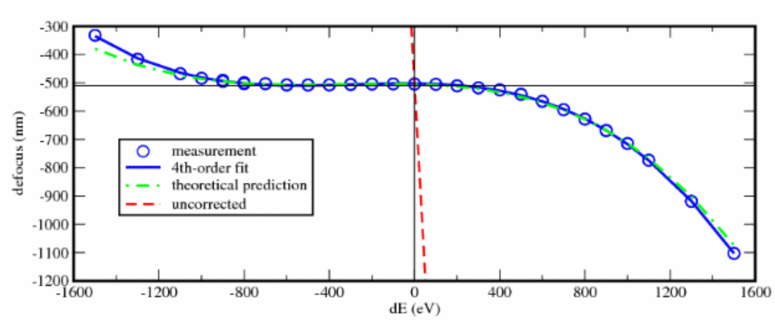

Figure 2. Change of focus vs. energy change (dE): In a Cc-corrected TEM electrons with a difference in energy of many hundreds of eV are focused to an identical image plane (blue curve) [5]. In a Cc-uncorrected TEM the defocus changes dramatically with change of energy (red dashed curve), consequently the resulting image contrast is strongly reduced.

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