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Cryo Imaging at 60 keV and 200 keV at a C_s-Corrected ZEISS Libra 200

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TEM-Imaging under cryo conditions is a well established method to investigate beam sensitive samples. Vitrification preserves the native state of biological macromolecules thereby allowing the determination of their morphology and 3-dimensional structure at sub nanometer level. For single molecules and molecular complexes a resolution of 4-10 Å can routinely be reached, depending on symmetry of the sample and the inhomogeneity of the protein preparation. For optimum resolution two critical factors have to be taken into account: The SNR in the acquired images has to be maximized. At the same time the SNR is limited by beam damage of the sample resulting from interactions of the electrons with the specimen and embedding material. To enhance contrast in the images different methods such as zero-loss energy filtering [1], phase-plate imaging [2,3] or C_s-aberration correction [4] have been used successfully. High resolution structural information can exclusively be gained from elastic scattering in the sample. Inelastically scattered electrons blur the image due to their dislocation and the chromatic aberration of the microscope. They are therefore considered as noise and must be removed from the image by zero-loss filtering.
Here we investigate the effect of different electron energies on image contrast and the electron-sample interaction. We collected data of vitrified biological samples imaged at 200 keV (FIG. 1A-C) and 60 keV (FIG. 2A-C) in a C_s-aberration corrected ZEISS Libra 200 FEG-EFTEM. The images were zero-loss filtered and recorded under low dose conditions (typical electron dose of 300 electrons/nm^2). The decreased electron energy leads to an increased image contrast as seen by comparing the images of ribosomes recorded at 200 keV (FIG. 1A) and 60 keV (FIG. 2A). The EELS of protein in ice recorded at 60 keV (Fig. 3) shows that inelastic scattering dominates the imaging process. Even though the majority of electrons is thus removed from the images by zero-loss filtering, it still leads to superior image contrast and better SNR at 60keV electron energy.
The scattering cross section of electrons increases with decreasing electron energy as $1/\beta^2$ for both elastic and inelastic scattering, i.e. at 60 keV the probability of an elastic or inelastic scattering event is about 2,5x higher than at 200 keV. While the inelastic scatter is removed from zero-loss filtered images, the significant increase in elastic signal results in higher structural information at 60kV. At the same time the larger inelastic cross section at 60keV leads to increased beam damage in the sample due to heating and ionization. Finding the ideal set of imaging parameters is therefore crucial for obtaining an optimum ratio between SNR in the images and beam induced sample damage. Experiments related to beam damage at lower electron energies are currently under way [5].

References

[1] R.R. Schröder et al., *J. Struct. Biol.* 105 (1990), 28.
[2] E. Majorovits et al., *Ultramicroscopy* 107 (2007), 213.
[3] K. Murata et al., *Structure* 18 (2010), 903.
[4] H. Stark, Göttingen, *personal communication*.
[5] cf. abstract at this conference R.R. Schröder et al.
[6] the authors thank A. Meinhart (MPI medical research, Heidelberg, Germany) and his group for preparing the ribosomes.

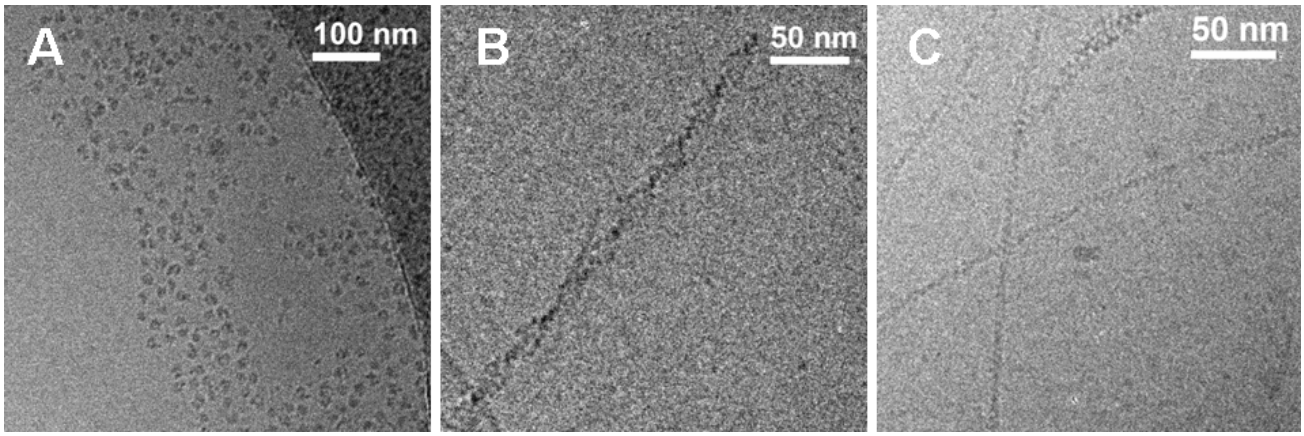

FIG. 1. Zero-loss filtered C_s-aberration corrected images of biological macromolecules in vitreous ice recorded at **200 keV** at liquid nitrogen temperature. The total electron dose per image was about 300 e^-/nm^2. A) ribosomes, B) myosin-S1-decorated actin filament, C) actin filaments.

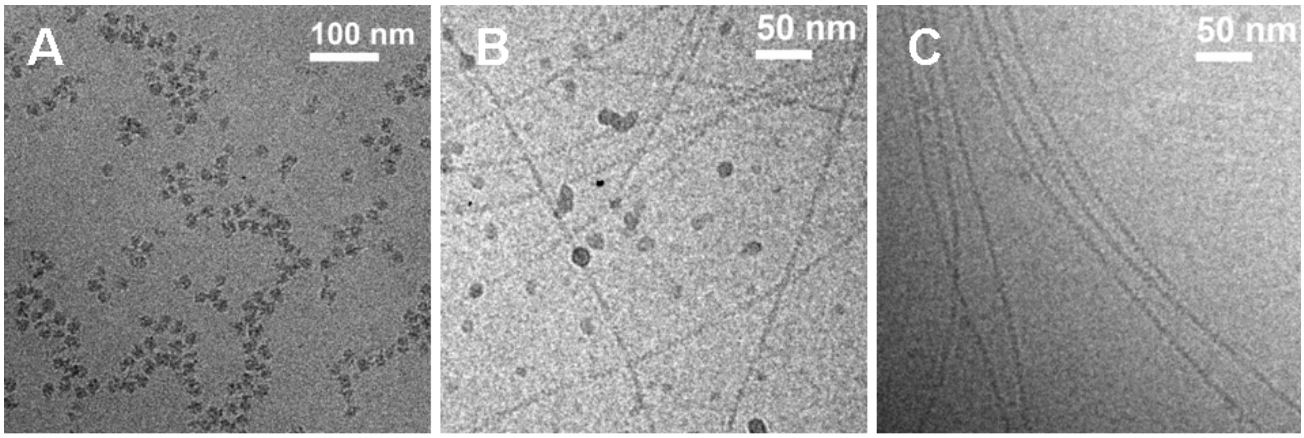

FIG. 2. Zero-loss filtered C_s aberration corrected images of biological macromolecules in vitreous ice recorded at **60 keV** at liquid nitrogen temperature. The total electron dose per image was about 300 e^-/nm^2. A) ribosomes, B) and C) actin filaments.

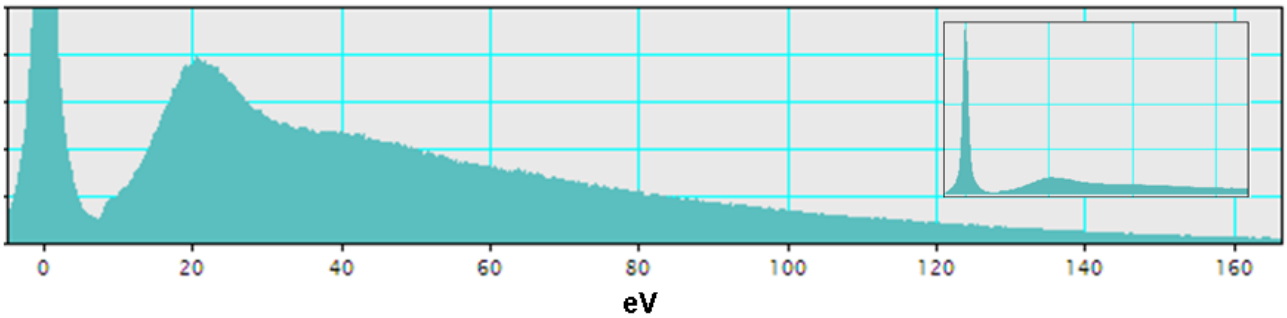

FIG. 3. Electron energy loss spectrum of an approximately 80 nm thick ice layer at 60 keV primary electron energy. Note the multiple plasmon scattering in the energy loss part of the spectrum. Insert: same spectrum showing the complete zero-loss peak.

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