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Reduced Radiation Damage of Protein Embedded in Vitreous Ice at Low Electron Energies

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The main problem for high-resolution electron microscopy of biological material is sample damage induced by the electron beam. Different mechanisms of damage have been discussed [1,2], which result in mass loss or mass redistribution for organic material embedded in vitreous ice. As possible principal mechanism of damage it is generally assumed that free radicals form in the ice and in the biological material or at the ice/material interface. Such radicals will be highly mobile and will start free-radical chain reactions, which lead to etching of the biological material and the ice layer. Heide and Zeitler pointed out the typical gap-like appearance of damage at the material/ice interface [3].
Recent work within the SALVE project [4] has proven that damage of carbon materials - such as functionalized carbon nanotubes – can be considerably reduced by using electrons at lower electron energy. The reduction of beam damage at 20keV compared to 80keV for fullerene molecules or more complex molecules inside a carbon nanotube has been explained by the reduction of radiation damage effects, likely dominantly attributed to reduction of knock-on damage effects [5].
Here we show first data about the beam damage produced by low energy electrons when used for imaging biological materials embedded in vitreous ice. FIG. 1 shows the EEL spectrum of a typical 50-70nm thick ice layer for 20keV electron energy. As expected inelastically scattered electrons dominate the spectrum implying increased beam damage, as more ionization events are expected. In contrast, imaging shows to be less prone to beam damage. FIG. 2 shows a typical zero-loss filtered image with largely increased contrast. Images of material embedded in free-spanning ice layers can be recorded at a high electron dose. However, imaging on the support carbon film (FIG. 2) induces the usual beam damage already at low dose. Contrary to the expected increase in beam damage by ionization at lower electron energy we find (1) a larger applicable electron dose before visible beam damage occurs, (2) no visible gap formation between biological material and embedding ice, and (3) a reduced mass loss compared to imaging at higher electron energies (80keV and above). At a certain total dose ice crystallization occurs (FIG. 3), starting either spontaneously or at seeds such as the biological material itself. We conclude that the mechanism of beam damage at low electron energy must be different from that at higher energies. The typical damage commonly attributed to free-radical reactions is not observed. Instead crystallization of the ice occurs which can be explained by an increase in the relative importance of electron-phonon interaction.

References

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[2] Y. Talmon et al., *J. Microsc. (Oxford)* 141 (1986), 375.
[3] H.G. Heide and E. Zeitler, *Ultramicroscopy* 16 (1985), 151.
[4] U. Kaiser, et al. *Proc. MC2009*, 3 (2009), 1, doi: 10.3217/978-3-85125-062-6-379
[5] U. Kaiser et al. *Ultramicroscopy* (2011) in press.
[6] Data were obtained at 20keV electron energy at the prototype Zeiss SALVE Libra instrument.

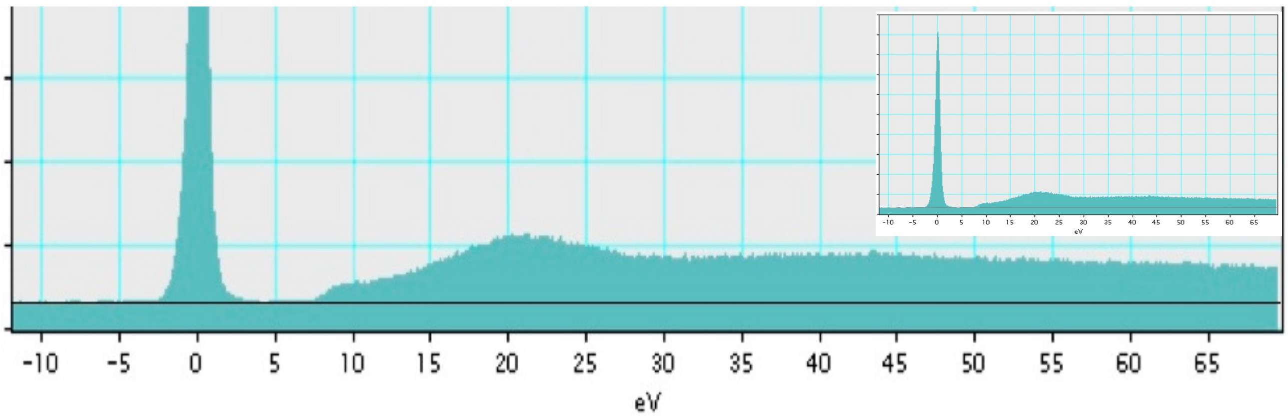

FIG. 1. Electron energy loss spectrum of a 50-70 nm thick ice layer. Note the relatively low zero-loss peak and the majority of inelastically scattered electrons (insert).

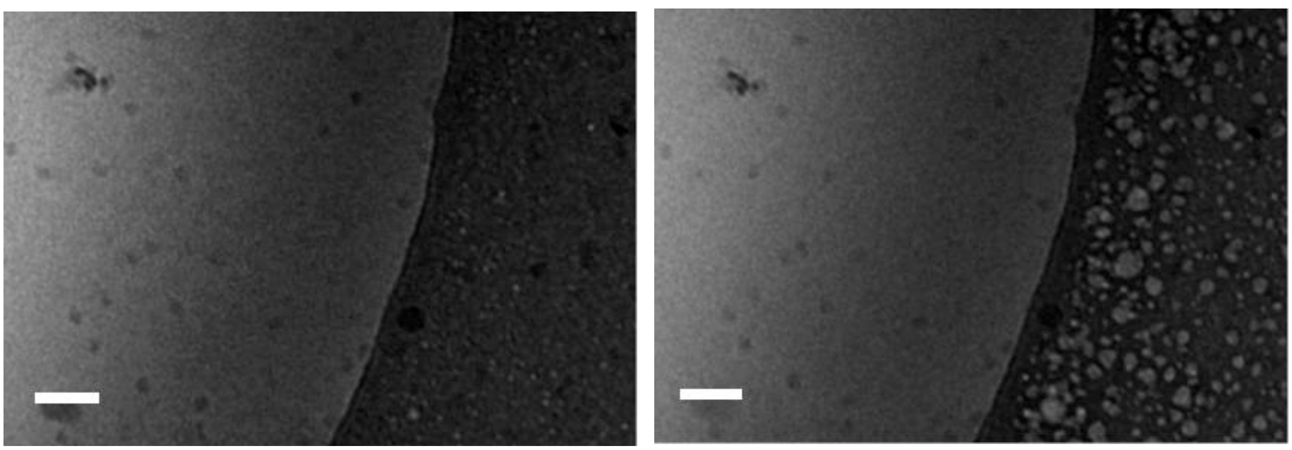

FIG. 2. Zero-loss filtered images of ribosomes embedded in ice. The image was recorded at liquid nitrogen temperature at high defocus in the C_s-corrected low-voltage Zeiss SALVE instrument at an electron energy of 20keV. Electron dose for recording was 240 electrons/nm^2. Scale bar 100 nm. The total dose at the sample was 720 electrons/nm^2 (left) and 2400 electrons/nm^2 (right)

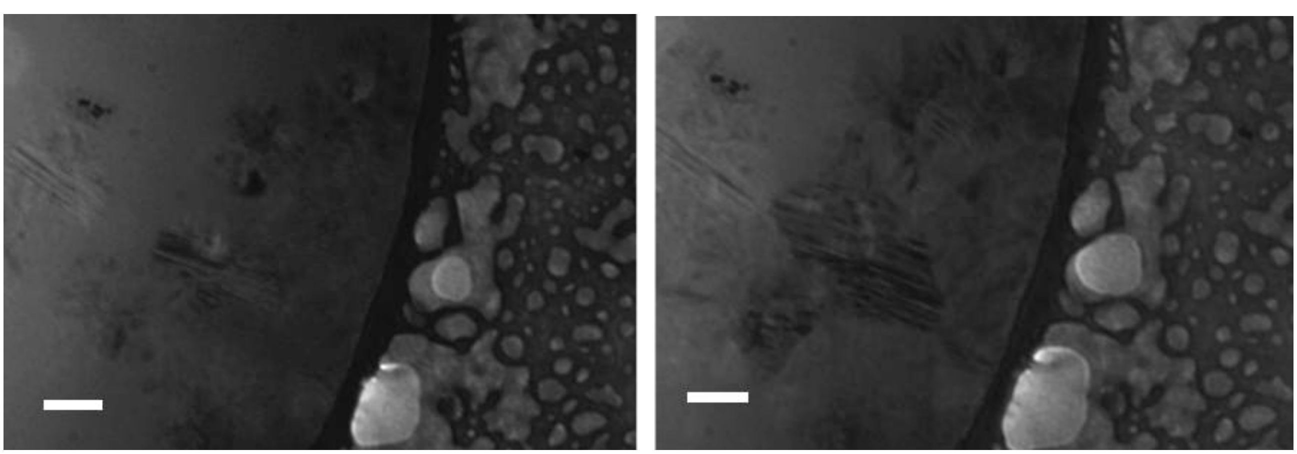

FIG. 3. Specimen areas as in FIG. 2. Electron dose for recording was 1200 electrons/nm^2. Scale bar 100 nm. The total dose at the sample was 12000 electrons/nm^2 (left) and 18000 electrons/nm^2 (right)

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