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Electron Beam Induced Mass Loss Dependence on Stained Thin Epon Resin Sections

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Low voltage TEM and STEM (transmission and scanning transmission electron microscope) can be regarded as the method of choice for many structural studies of very thin biological samples like ultrathin sections, viruses etc. [1,2]. Usually, in a conventional TEM (typical acceleration voltage 60 – 300 kV) the image contrast is enhanced by staining using salts of heavy metals (e.g., uranyl acetate, lead citrate) [3]. Low voltage STEM allows to image unstained samples that usually show sufficient contrast, but in some cases the staining can be useful. An important parameter for imaging is a sensitivity of the sample to degradation by electron beam. There have been not many studies describing quantitatively the mass loss of the resin sections; the most comprehensive work was performed on the dedicated STEM for molecular mass measurements [4] done at 80 keV, or even at higher electron energies done by EFTEM [5]. The author in [6] describes that polymers stained with a heavy metal should therefore damage more rapidly through the secondary electron mechanism and higher cross sections for scattering.

However, there is a lack in such measurements at the energies applied in SEM. The mass loss indicates a degree of the radiation damage; therefore, we report here on investigation of the mass loss of embedding medium (Epon resin of middle hardness) in combination of different thickness of the sections with staining. The main technique used for quantitative measurement of the mass loss is based on the mass measurements by dedicated STEM [7, 8], using SEM equipped by a commercial solid-state STEM detector [9, 10]. The imaging was performed by SEM Magellan 400L (FEI) at the acceleration voltage of 30 kV and the lowest possible probe current of 1.6 pA in the bright field (BF) mode using the commercial STEM3 detector (FEI). Electron dose for each scan corresponded to about 60 e^-/nm^2. For our study we used resin sections with thickness 30 and 60 nm. Measurement was performed the second day after their preparation by the ultramicrotome Leica Ultracut UCT. Some slices were stained by uranyl acetate for 30 minutes from the upper side.

Mass loss measurements of the resin slices were performed by imaging the same area at magnification of 100 000 times (field of view 1.024 x 0.884 µm) in a series of many consecutively scanned images (usually 50 first images were recorded). For each experiment, three different places on each sample were imaged for downgrading space dependency of the measurement. Calibration was made using the couple of images recorded for each imaging session, where first of them is absolute dark with beam eclipsed by the grid holder and second one is absolute bright with full beam to the STEM detector. The mass loss was obtained from the normalized BF signals using the Monte Carlo simulation of electron scattering MONCA [11] using the methodology of mass measurements by STEM [12]. All processing was programmed in MATLAB (Mathworks).

As seen on fig. 1 left, the normalized BF signal (showing the fraction of scattered electron to the BF detector) has similar course with nearly linear shape in both thicknesses of the slices without staining; the curves are shifted only by the different thickness. Mass loss (fig. 1 right) shows two groups of thickness independent curves. First one is slice without staining, where at the total irradiation dose of 3000 e/nm^2 still remains 64% of initial mass in the case of 30 nm and 51% for 60 nm slice, respectively.

However, the slices with staining are clearly much higher sensitive to the electron beam (red and blue circles), where curves in BF signal dependency have an exponential shape. Corresponding mass loss shows the same exponential behavior in both thicknesses. They remain only 21% of initial mass for both 30 nm and 60 nm slices at the same dose of 3000 e/nm^2.

We observed higher sensitivity of stained Epon thin sections under incident electron beam. We found change of 43 % mass for 30 nm and 30 % for 60 nm slice. This limits the usable dose for imaging by the low voltage STEM (for 30 kV) because stained sections are more susceptible to burn-out than pure ones.

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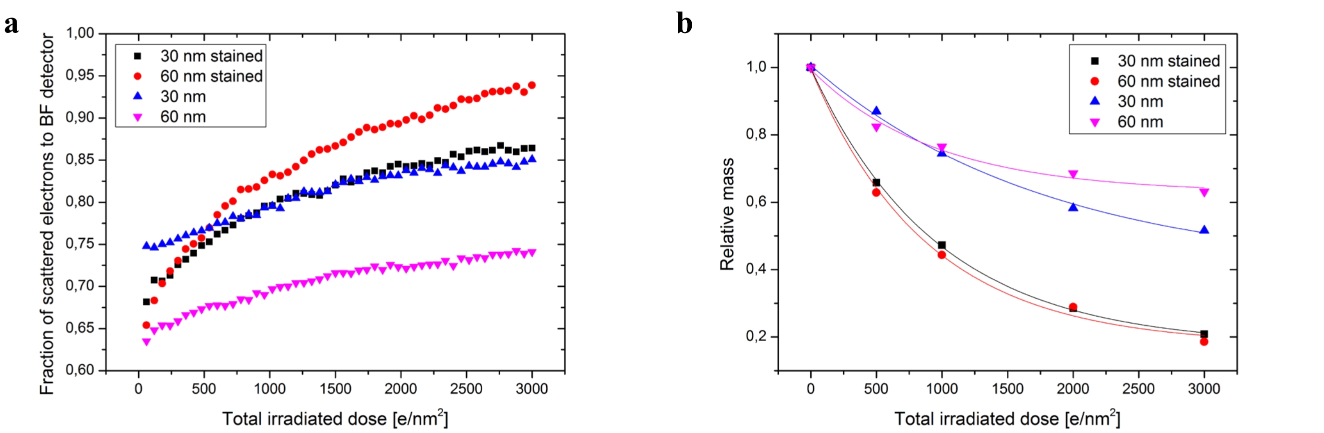

Figure 1. The normalized bright-field (BF) signals (a) and recalculated relative mass (b) dependences on the total irradiation dose on slices of various thicknesses recorded at the second day.

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