INVESTIGATION OF ELECTRON BEAM INDUCED MASS LOSS OF EMBEDDING MEDIA IN THE LOW VOLTAGE STEM

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Abstract: This paper deals with usage of a low voltage STEM (*Scanning transmission electron microscope*) for biological purposes. The investigation of electron beam induced mass loss of ultrathin sections of three embedding media is presented. The mass loss of the sample caused by the electron bombardment is not examined in detail in the literature but it seems to be an important fact in investigation of biological samples by low voltage STEM. The samples of different thicknesses were investigated using different microscope settings (acceleration voltage, total dose, probe current, cleaning of the sample surface) and STEM imaging modes (bright-field, dark-field).

Keywords: STEM, mass loss, embedding media, total irradiated dose

1. INTRODUCTION

Nowadays, there is a wide range of machinery and methods for conducting microbiological research. The tendency is to combine the methods or use them in other applications than before, with the aim to obtain better and more reliable results. There is a lack of information about behaviour of electron sensitive materials (e.g. biological samples, embedding media) in the low voltage STEM which warrants the present investigation.

The STEM is useful device combining features of scanning and transmission electron microscopes. The sample in the form of ultrathin section is scanned by the electron probe and the transmitted electrons are detected by the detector under the sample. Aside from in dedicated STEMs [1] this mode can exist as options in both TEM and SEM [2]. The low voltage STEM based on the SEM equipped by a transmission detector (also called STEM-in-SEM) is used for the presented experiments. Nowadays, the low voltage STEM is used more often, and in many cases replaces the typical TEM. However, the TEM is still the unwritten standard in the investigation of samples in the form of ultrathin sections. Here, we report on investigations of embedding media that are typically used for TEM preparation of biological samples.

2. APPLICATION OF THE LOW VOLTAGE STEM

The STEM detector, placed under the specimen, may detect both bright-field and dark-field images (Figure 1). It uses much lower acceleration voltages (30 kV and below) than conventional TEM or dedicated STEM (60-300 kV). Moreover, biological samples suffer from the lack of contrast which is normally enhanced by staining with salts of heavy metals (lead citrate and uranyl acetate). Furthermore, we face the problem of whether the stained structure is real or merely artefact. Thanks to the low voltage STEM (especially dark-field imaging), where the electrons have a lower mean free electron path, the staining is not needed and the obtained images are more reliable. Possibility to achieve better contrast and also better resolution without staining, and low dose imaging makes the STEM suitable for biological applications [3].

Materials such as biological samples, polymers including embedding media are electron beam sensitive. A few types of sample damage by the electron beam are possible. Two the most important are the mass loss and the contamination. Both types of damages depend on the electron

energy used and the electron dose applied to the sample. The mass loss depends on the sample composition and the contamination results from the poor vacuum in the specimen chamber of the SEM, cleanness of the sample surface, etc. It should therefore be possible to measure the mass loss and the data should be reproducible in contrast to the contamination.

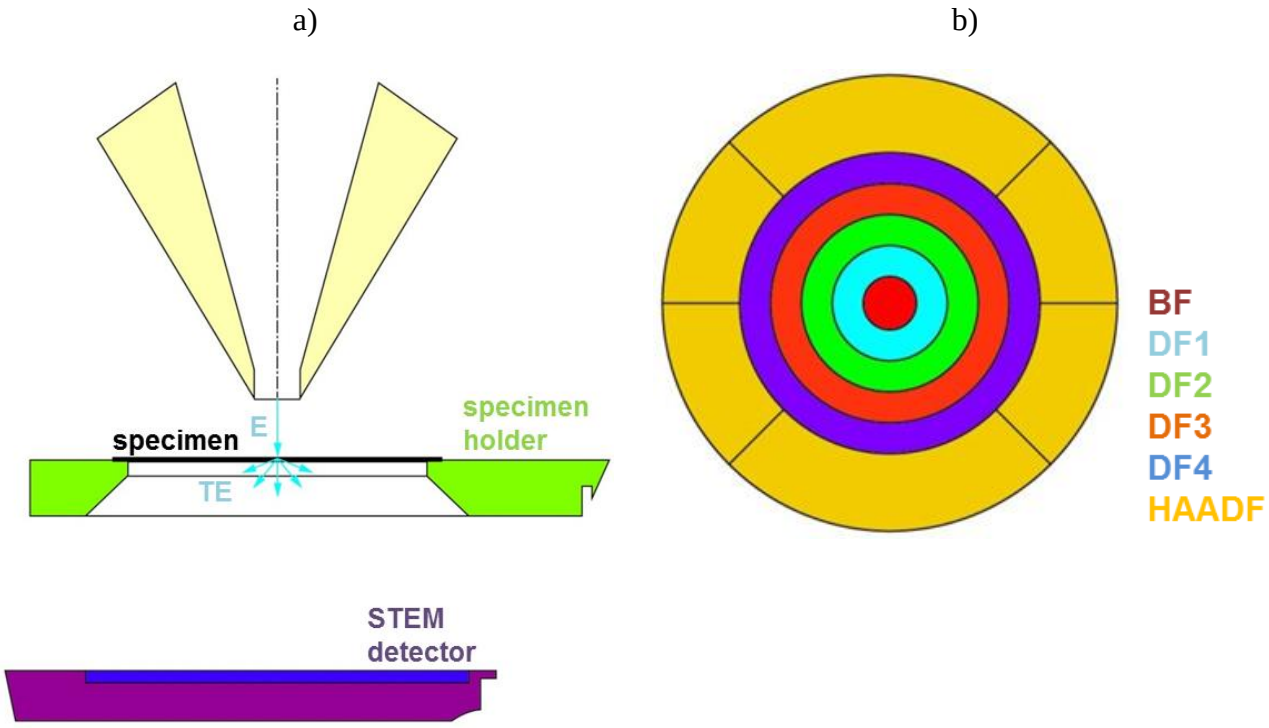

Figure 1: Schematic drawing of the transmission mode in SEM: (a) STEM detector location, (b) solid state STEM detector segments (BF is in the middle of the detector, HAADF on the edge).

3. EXPERIMENTS

For simplicity, we started to measure the mass loss of different types of embedding media for biological sample preparations in transmission electron microscopy. We focussed on three embedding media: Epon, Spurr and LR White. We prepared samples sections of thickness 30, 60, 100 and 200 nm which were cut from blocks of pure embedding media. We then collected bright-field (BF) and dark-field (DF1 and DF2) images (e.g. 25 images in this case) of the same area of the sample with magnification of 100,000x. An overview of the scanned area taken after the last scan at a magnification of 50,000x, shows the increase of the signal due to the mass loss (Figure 2a). The image processing of acquired micrographs was programmed in MATLAB.

In the most destroyed (i.e. the most recently taken) image we defined region of interest and in this area within all images we counted the mean value of the signal in the scanned image vs. the total dose applied to the sample. Figure 2b shows the change of the BF signal to the total dose of electrons for all three embedding media. Results presented in Figure 2 were obtained by SEM Magellan 400L (FEI) at acceleration voltage of 30 kV and probe current of 1.6 pA with 100 nm thin sections of Epon, Spurr and LR White. The mass of the embedding media decreases linearly until the limit dose of the sample. At this point, nonlinearities begin to occur. They are caused by contamination of the sample from resin evaporation and contaminants from the specimen chamber. We used plasma cleaning of the sample surface and the specimen chamber with the aim of supressing the influence of the contamination.

In our experiment, we altered measuring conditions such as acceleration voltage or probe current and compared the results. A calibration procedure was also undertaken which allowed us to measure both the intensity in the image and the mass loss directly.

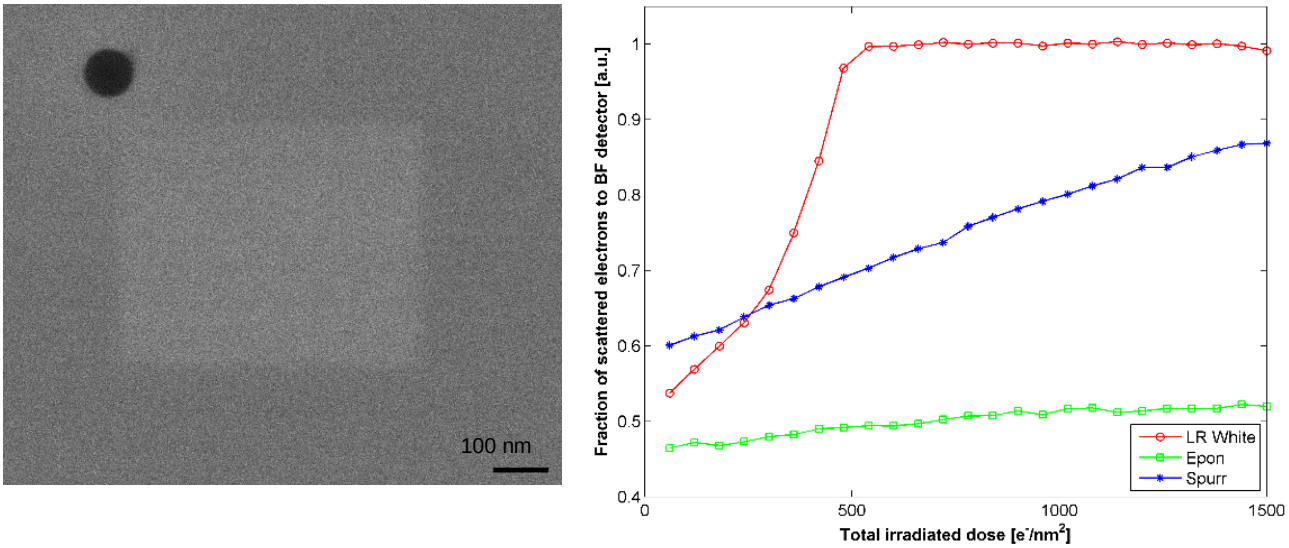

Figure 2: (a) Bright-field micrograph showing an overview of the area scanned many times taken after the last scan at a half magnification of Epon. (b) Mean value of the bright-field signal in the scanned image vs. total irradiated dose for LR White, Epon and Spurr (samples of thickness 100 nm).

4. CONCLUSION

According our measurements, the mass loss of the sample plays important role in investigation of electron beam sensitive samples by low accelerating voltages and attention should be paid to this issue. As is presented in Figure 2, the mass loss at 30 kV can be high even at a dose of 500 el/nm^2, where, for example, the LR white section was completely destroyed. The Epon resin in comparison with the Spurr resin seems to be more stable under an electron beam. The Epon also provides large scattering of the electrons and better contrast than Spurr. The results of plasma cleaning directly in the specimen chamber are that the plasma cleaning supresses the effect of contamination of the sample under the electron beam and the sample tolerates more electrons per square nanometer. Results from investigation of samples using two acceleration voltages (20 kV and 30 kV) show that both the limit dose tolerated by the sample and the mean value of the signal are lower for acceleration voltage of 20 kV. Also when we compare results from DF1 and DF2 of the same sample, the limit dose is approximately same but the mean value of the signal is higher for DF1. When samples of different thickness are compared, the thicker sections have lower variance of the data and even without plasma cleaning the sample tolerates more electrons per square nanometer. To date, we have obtained truly interesting results in this area. However, much research remains to be undertaken.

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