Investigation of the Electron Beam Dose Tolerance of GFP in Liquid

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The investigation of biological specimens with electron microscopy (EM) is often hampered by radiation damage of samples, in particular because typical biological specimens are electron beam radiation sensitive. Traditionally, sample preparation for EM includes drying or freezing of the specimens but the observation of biological samples and processes in their native, liquid environment is of major interest in EM research for which liquid phase electron microscopy is a new option [1]. To accomplish this, it is necessary to know the dose tolerance of the specimens such that specimens can be kept intact during microscopy.

Here, the electron dose tolerance of biological specimens in liquid was investigated. As a model, the green fluorescent protein (GFP) was used. For sample preparation, GFP was bound to SiN microchips via biotin-streptavidin binding. The fluorescence intensity loss of GFP was investigated upon electron beam radiation in transmission electron microscopy (TEM) and scanning electron microscopy (SEM) with varying electron flux and electron dose. The loss of fluorescence intensity was associated with the loss of protein function and the degradation of the protein [2].

The fluorescence intensity was analyzed over the electron dose from 0.001 – 10 $e^-/Å^2$. The electron flux varied from 0.001 – 10 $e^-/Å^2s$ to analyze the impact of electrons passing the sample per unit area per unit time. It was found that in SEM, the fluorescence intensity decreased and was comparable to background level at an accumulated electron dose of 1$e^-/Å^2$. At this dose, GFP lost its fluorescence function and was considered as no longer intact. Varying the electron flux did not have a large impact on the electron dose threshold of GFP.

[1] Wu, H., et al. Advanced Materials 32.25 (2020): 2001582

[2] Mazzola, P., et al. Biotechnology Progress 22.6 (2006): 1702-1707