Correlative cryo-qSTEM-CL imaging in a SEM

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Nowadays, a correlative microscopy of several signals is one of the main topics in the electron microscopy field. In some studies, a localization, distribution and density of light emitting labels or particles in a sample is important. Unfortunately, not all light emitting structures are possible to be imaged at room temperature. Very fine particles like quantum dots with diameter in terms of nm are recognizable only at very low temperatures and with appropriate anticontamination shielding, because a contamination layer of hydrocarbon deposits can disturb whole measuring at the first electron beam scan. Qualitative imaging can be changed to quantitative by using Monte Carlo (MC) simulations of samples, with known composition and density, which can bring a new dimension to thin samples imaging by STEM (Scanning Transmission Electron Microscopy). Recalculation of calibrated images via MC gives the information about the local thickness of the sample and total mass of the imaged particle with high precision (approach published in [1] was used). This information can be compared with local cathodoluminescence (CL) activity or quantitative imaging in backscattered electrons.

This instrumental study was performed on a sample of high luminescence nanodiamonds placed on thin carbon covered lacely TEM grid. Bright field (BF) STEM image captured in SEM Magellan 400L (FEI) at acceleration voltage of 30 kV and probe current of 3.1 pA is shown in Figure 1a (mean size of nanodiamonds is around 100 nm). Corresponding thickness-map (1b) is made by comparison of BF image with MC simulation calculated in CASINO program [2]. There are visible places of the highest thickness (yellow) at the level of 100 nm. The arrows indicate two nanodiamond pieces that are overlapped. Denoised CL image captured at same microscope settings is shown in Fig. 1c, with recognisable borders of those particles. Our results show that the CL activity of the sample is given by both local thickness and "point to edge" distance. In practice it means that the area of the highest CL intensity is not the same as the place with the highest sample thickness. These results are useful in investigation of CL active nanoparticles like nanodiamods and quantum dots, because particles with the same total mass can vary in CL activity. With such knowledge, its preparation process can be modified for higher CL yield. This may be of interest, e.g., for more precise analysis of labelling in life-science applications.

[1] Krzyzanek V. et al: Journal of Structural Biology 165 (2009), 78-87.

[2] Demers H. et al: Microscopy and Microanalysis 17(S2) (2011), 612-613.

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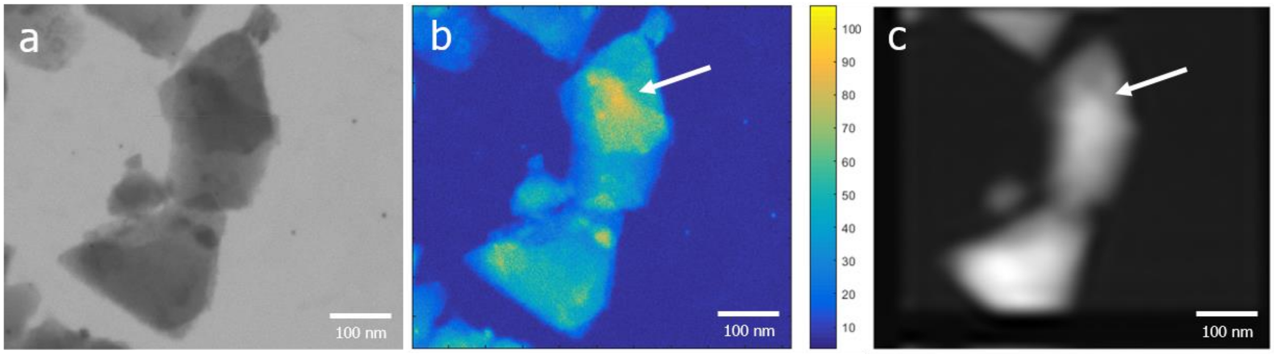

Figure 1: Bright field STEM image of nanodiamonds on thin carbon layer (a). Corresponding thickness-map with the scalebar in nm (b). Cathodoluminescence activity of the same area after image coregistration (c)