



POSTER #13

**CATHODOLUMINESCENCE IMAGING AND MAPPING OF
BIOLOGICAL SAMPLES IN CRYO-SEM**

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Many substances ordinarily imaged in scanning electron microscopy (SEM) are characterized by cathodoluminescence (CL) behaviour. In general it can be biological samples, some chemical and mineral compounds. It brings complication in localization of places of the interest where CL labels are situated, because signals coming from the labels are overlaid with background (in polychromatic CL mode all CL emitting structures are visible). This problem can be solved by using the monochromatic CL mode and by the known wavelength in maximum of peak (or shape of CL spectrum); or more general by combination of both methods, the cryo-SEM and the CL, where data from topographical image are complemented by CL activity image. Measurement of the characteristic and specific CL spectrums of the CL labels can be useful but the shape of their spectrum is unfortunately overlaid by another compounds from the frozen sample, probably of biological origin or buffer.

In our experiment THP1 cells were cultivated with nanodiamonds for 24 hours and frozen by high pressure freezing (HPF EM ICE, Leica Microsystems). The samples were fractured in the ACE600 device (Leica microsystems), and were transferred in the high vacuum and under low temperature to the SEM Magellan 400 (FEI) using the shuttle VCT100 (Leica microsystem). The SEM is equipped with a cryo-stage (Leica microsystems) and a detection system MonoCL4 (Gatan), which were used for the CL analysis. Samples were imaged with various acceleration voltages (1, 5, 10, 15, 30 kV) and beam currents (50 pA, 0.1 nA, 0.4 nA) in polychromatic CL mode. At the end of cryo-experiment the samples were transferred back to the ACE600 device and completely freeze-dried for further investigation at room temperature.

The CL panchromatic image shows active places with the best contrast at acceleration voltage of 5 kV. In comparison with images taken at 1 kV there are clearly visible areas of individual cells with bright spots where CL active material is localized (fig 1). Higher acceleration voltage gives more signal from deeper parts of the sample and therefore contrast of bright spots is lower. Very important reason for using cryo method is absence of crystals which are formed during drying, apparently from culture solution or wash buffer. Those can be misunderstood in CL image as particles of interest, because they are also clearly visible (fig 2). The biggest technical issue for imaging of nanometer-scaled labels is necessity of using long working distance (CL detector mirror is pushed between SEM pole piece and the sample) and impossibility to use high magnification mode. But in the comparison with imaging using secondary electrons the CL signal initiation is depend on higher probe current. Therefore, the disadvantages of this method are beam damage and surface charging, both caused mainly by high beam currents.

Nevertheless, the cryo-CL-SEM in polychromatic mode is powerful method which may differentiate individual cells in frozen sample and localize tagged particles inside cells. Subsequently, identified positions of points of interest (labels, cells and structures) can be used for closer investigation of these fine structures by another imaging techniques.



Acknowledgement:

This study was supported by the Czech Science Foundation GA17-15451S and GBP503/12/G147, the Ministry of Education, Youth and Sports (LO1212 and CZ.02.1.01/0.0/0.0/15_003/0000495). We would like to thank A. Nowak (Leica Microsystems) for providing the HPF EM ICE.

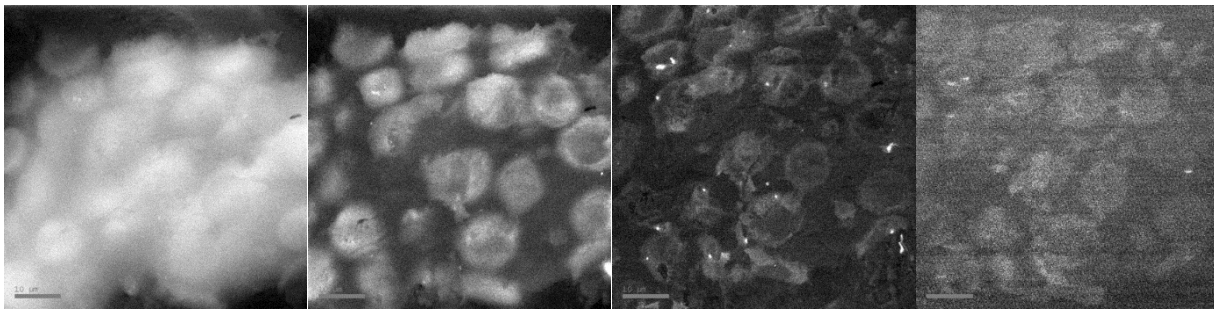


Figure 1: Cryo-CL-SEM images of hydrated frozen sample with CL active spots at acceleration voltages 30, 15, 5 and 1kV. Bar 10 μm .

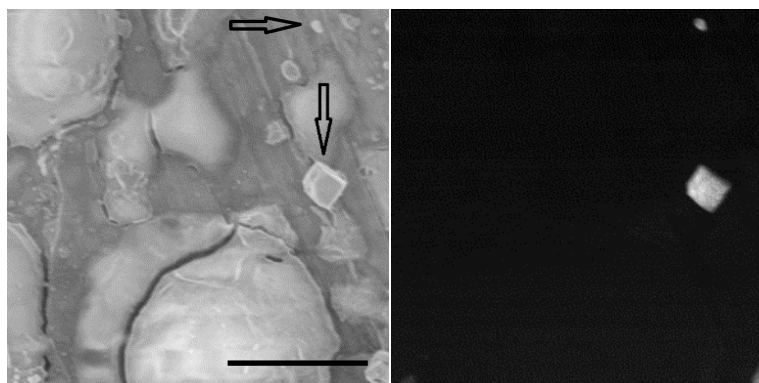


Figure 2: Crystal formed during drying from culture solution or wash buffer in SE and CL image. Bar 5 μm .