Correlative Microscopy in Life and Materials Science

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Correlative Light and Low-Voltage Scanning Electron Microscopy

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Conventional correlative microscopy (CLEM) can be used for a relative localization of fluorescent markers in the combination of light and electron microscopy. The fluorescent signal can therefore be used to identify the regions of interest in the light microscope (LM), while electron microscopy (EM) is then used for a detailed analysis of the ultrastructural context of the labelled epitope of interest [1]. This method requires a sample preparation for the electron microscope, where the fluorescent signal is still maintained. And the accuracy of such multi-modal signal identification strongly depends on the resolution and possible alignment of the images from the light and electron microscope.

To overcome the large alignment error in the above described conventional CLEM workflow several approaches were reported for the quasi-molecular, direct or indirect detection of the fluorescent signal in the electron microscope. Jaksch showed that in low voltage scanning electron microscope (LV-SEM) not only material contrast but also compositional contrast becomes visible by detecting the Low Loss Back-Scattered Electrons (LL-BSE) [2]. In collaboration with Heiner Jaksch we tried to verify his experiments for isolated fluorescent markers. The samples used here are purified green fluorescent protein (GFP) dried on Silica wafers or bound to Q-Sepharose. The fluorescent signal was analysed before and after the LV-SEM experiments in a fluorescence LM. LV-SEM images showed a clear material contrast for the silica wafer and signal from contamination on the sample, but the GFP signal was not detected (Figure 1). Images were recorded in a Zeiss Ultra SEM at 0,4-4,0 keV primary electron energy, examining the multidimensional parameter space (high voltage, working distance, dwell time and detector settings) around the condition described by Jaksch. The fluorescent images after the LV-SEM experiments showed, the GFP fluorophor was destroyed by the electron beam (Figure 2). The remaining fluorescent signal did also not colocalize with the LL-BSE signal. A reason could be that the interaction depth of the low voltage electron beam is significantly smaller that the penetration depth of light. More experiments to find imaging conditions, which maintain fluorescence are under way, however, improved imaging parameters have so far not been found.

In another approach photo-oxidation of diaminobenzidine (DAB) is routinely used for the visualization of fluorophores in correlative microscopy [3]. The DAB polymer formed can be stained with osmium tetroxide or other electron-dense compounds [4] for imaging in an electron microscope (Figure 3). Metal ions such as $NiCl_2$, $CoCl_2$ and $CuSO_4$ have been used to modify the colour of the DAB for light microscopy [5].

We will discuss the possibility of using other metal ions for the localization of the DAB-polymer using LV-SEM, and other applications of LV-SEM.

1. As another example of this multi-modality imaging cf I.V. Röder et al., Poster MC 2013.
2. H. Jaksch, LL-BSE electron contrast from Hybridization & Band Gaps, EMC 2012, Manchester UK (Poster)
3. M. Grabenbauer in “Methods in Cell Biology”, ed. T. Müller-Reichert and P. Verkade (Elsevier Inc.) (2013), p. 117-138
4. G.R. Newman, B. Jasani and E.D. Williams, J Histochem Cytochem 1983 p. 1430-1434
5. S.-M. Hsu and E. Soban, J Histochem Cytochem 1982 p. 1079-1082
6. The authors would like to acknowledge many interesting and stimulating discussions with the late Heiner Jaksch. We dedicate this work to him in the interest to contribute to his original ideas and to make them available in future applications.
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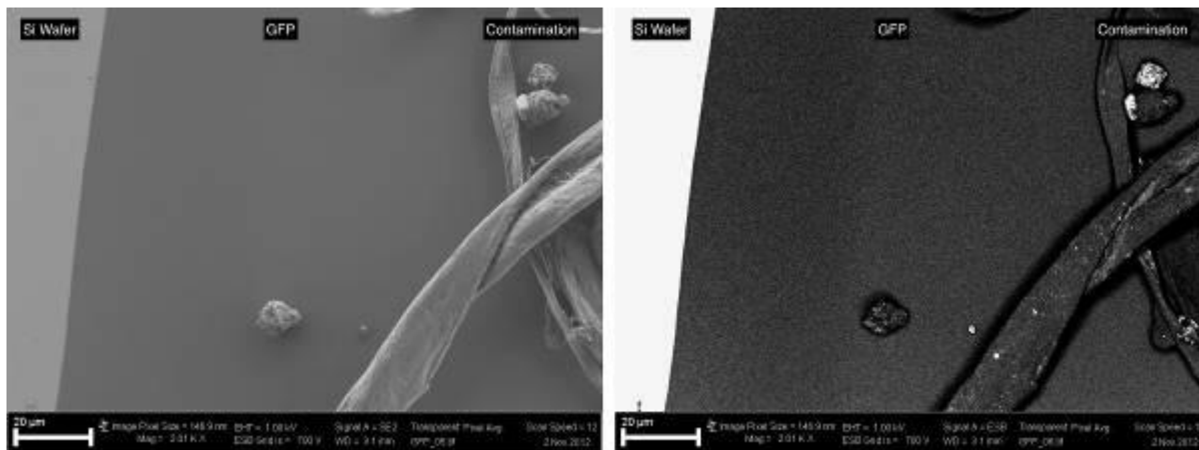

Figure 1. Green fluorescent protein on Silica wafer in LV-SEM, detection of secondary electrons (left) and low loss back-scattered electrons (right). Scale bar 20 µm

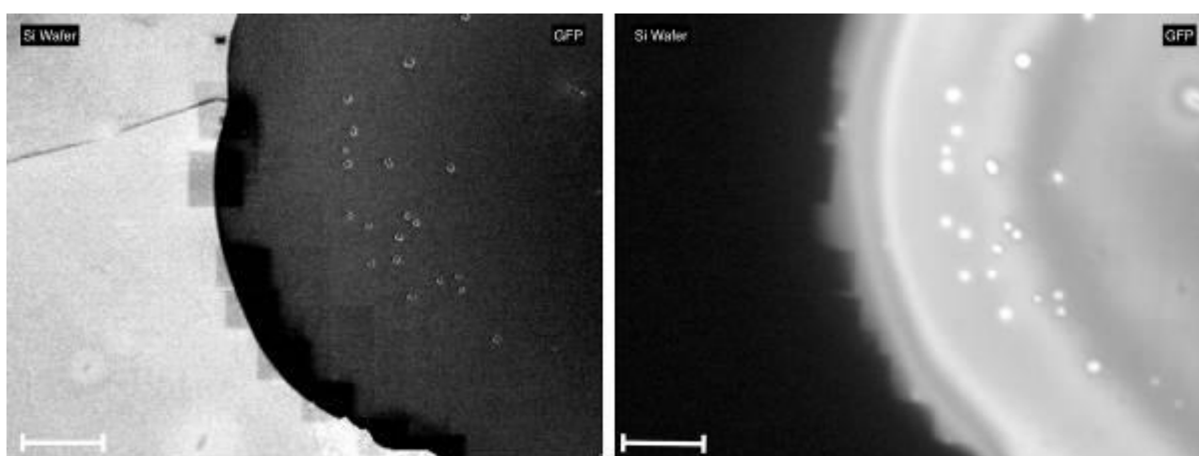

Figure 2. Green fluorescent protein on silica wafer after LV-SEM imaging. LV-SEM image, using the Inlens-SE detector showing the scanned – and therefore bleached – areas (left) and fluorescent LM (right). Scale bar 50 µm

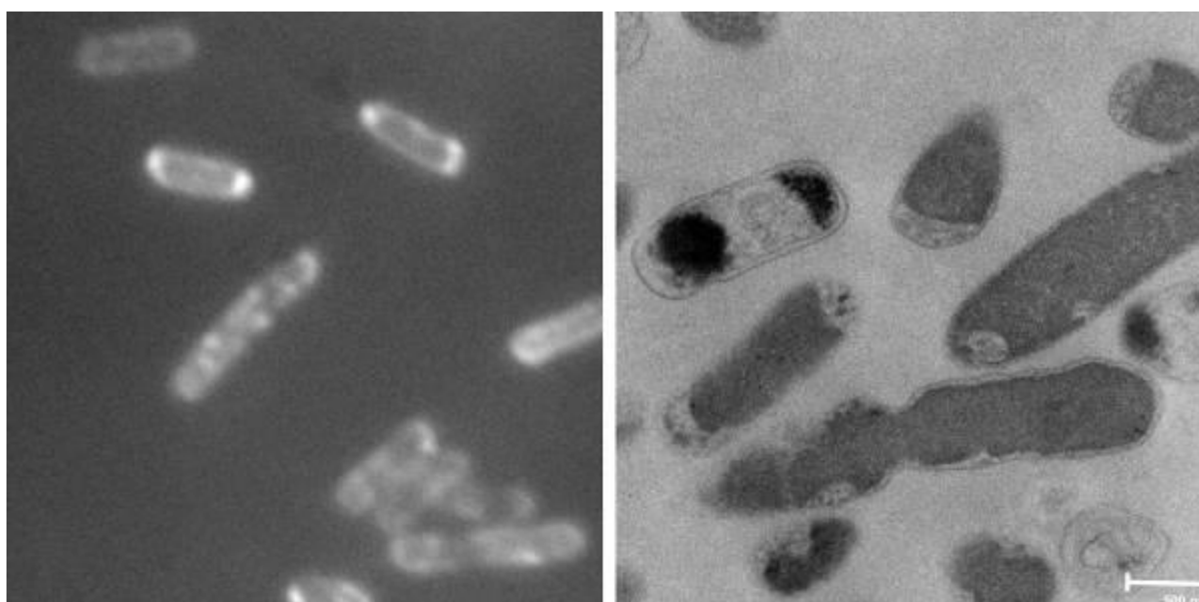

Figure 3. Fluorescence LM of GFP fused to the E. coli glutamate transporter expressed in E.coli (left). Photo-oxidation of DAB through GFP (right). Scale bar 500 nm.