DETECTING CORRELATIVE LIGHT AND ELECTRON MICROSCOPIC LABELS BY ELECTRON SPECTROSCOPIC IMAGING

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To bridge the gap in resolution and to localize molecules of interest in high-resolution light and electron microscopy a number of correlative approaches have been proposed. One possibility is the use of correlative markers, e.g. fluorescent Quantum Dots (Qdots), which in TEM are imaged and distinguished by their contrast and shape. In 2007 Mhawi et al. demonstrated a more direct possibility, the visualization of electron low energy loss, which represents the TEM equivalent of fluorescence excitation. Such spectral information in the low energy-loss range was used to identify doxorubicin introduced into cells to label nuclei.

Today, novel microscopes with monochromated beam and highly corrected energy filters allow electron spectroscopic imaging with much higher sensitivity. A large continuous energy-loss range can be probed by a series of images, e.g., from 1 to 30 eV energy loss. In combination with multivariate statistical analysis of such multispectral data sets, we aim at identifying single fluorescent particles as correlative markers in the TEM by means of their spectroscopic signal. To this point we examined marker labelled ultra-thin sections of resin embedded, high-pressure frozen cells and muscle tissue. Antibody-conjugated CdTe/ZnS Qdots were used to label sarcoplasmic reticulum calcium ATPase. It was possible to discriminate these Qdots, as well as DNA (heterochromatin) from the surrounding environment by means of their spectral, low energy-loss features. In addition, we investigated resin embedded *Trypanosoma*, which were labelled with two different sets of antibody-conjugated Qdots against aldolase and tubulin. We show that, similar to the case of separation of DNA and one set of Qdots, both sets of Qdots can be distinguished from the background and each other. This shows that the spectral differences of the low energy-loss absorption for different fluorescent nanoparticles may be specifically used for multicolour marker detection in TEM.

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