Correlative Microscopy in Life and Materials Science

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Detection of specific lanthanum signal in a metal-organic compound as approach for correlative high-resolution microscopy

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Correlating information from light and electron microscopes from identical specimen areas helps in understanding function in relation to cellular structures at high resolution [1]. Such an approach requires super-imposing regions of interest from two different imaging modalities. This is still a challenging endeavour since fluorescence emission from light microscopy creates an entirely different visualization of the object than electron microscopy. For the latter bright-field transmission electron microscopy (TEM) is typically applied. In the past, analytical TEM has been shown to be useful to map spectroscopic signals. This is also feasible with bio-organic materials, which can be associated to specific energy-loss spectra [2]. Direct detection of a functional signal at TEM level would allow for direct embedding of targeted molecules into the surrounding ultrastructure. This was shown for fluorescent nanocrystals, which were identified by optical absorption signals from inelastic scattering using low energy-loss electron spectroscopic imaging (LoESI) [3].

Additionally, by spectral visualization of core-losses analytical TEM offers mapping of element specific ionization signals [4]. Thus, introducing non-natural metals near target molecules and spectroscopic imaging at the respective ionization energies should allow for another possibility to directly combine functional and ultrastructural imaging in TEM. This can be done by adding metals to an organic, polymeric precipitation in the vicinity of fluorescent markers. Large precipitates were already found to bind to nickel or cobalt metal ions as visualized by light microscopy [5]. Here, we show that a specific lanthanum signal at organic precipitations can be mapped by analytical TEM. Precipitates are found at membranes of resin embedded HeLa cells (Fig. 1A). As shown in Fig. 1B, high-loss electron energy-loss spectroscopy (EELS) reveals $M_{4,5}$ edges of lanthanum at 832 and 849 eV in such areas. Since LoESI requires much less electron dose for collection of significant signal we also performed a multispectral analysis of a set of ESI images from 4 to 80 eV (in steps of 1 eV). Segmentation of membrane associated metal-organic precipitates is depicted in Fig. 1C. Extracted spectra from segmented and background regions, can be used to compute a difference spectrum (Fig. 1D). It reveals specific features, e.g. around 30 and 60 eV, which can again be attributed to specific lanthanum edges.

The technique illustrated here can be used to identify different metals with distinguishable features in the analytical electron microscope. Targeting multiple metal-organic compounds specifically to various structures or molecules of interest might allow labelling and detection of multiple cellular targets with simultaneous correlation to their local structural environment.

1. M. Grabenbauer, W.J.C. Geerts, J. Fernadez-Rodriguez, A.T. Nilsson, Nature Methods 2 (2005), p. 857.
2. M.A. Aronova and R.D. Leapman, MRS Bulletin 37 (2011), p. 53.
3. M. Pfannmöller, I. Röder, G. Benner, I. Angert, I. Wacker and R.R. Schröder, Microscopy and Microanalysis 17 (2011), p. 818.
4. R.F. Egerton, Springer, New York (2011).
5. S.M. Hsu and E. Soban, Journal of Histochemistry & Cytochemistry 30 (1982), p. 1079.
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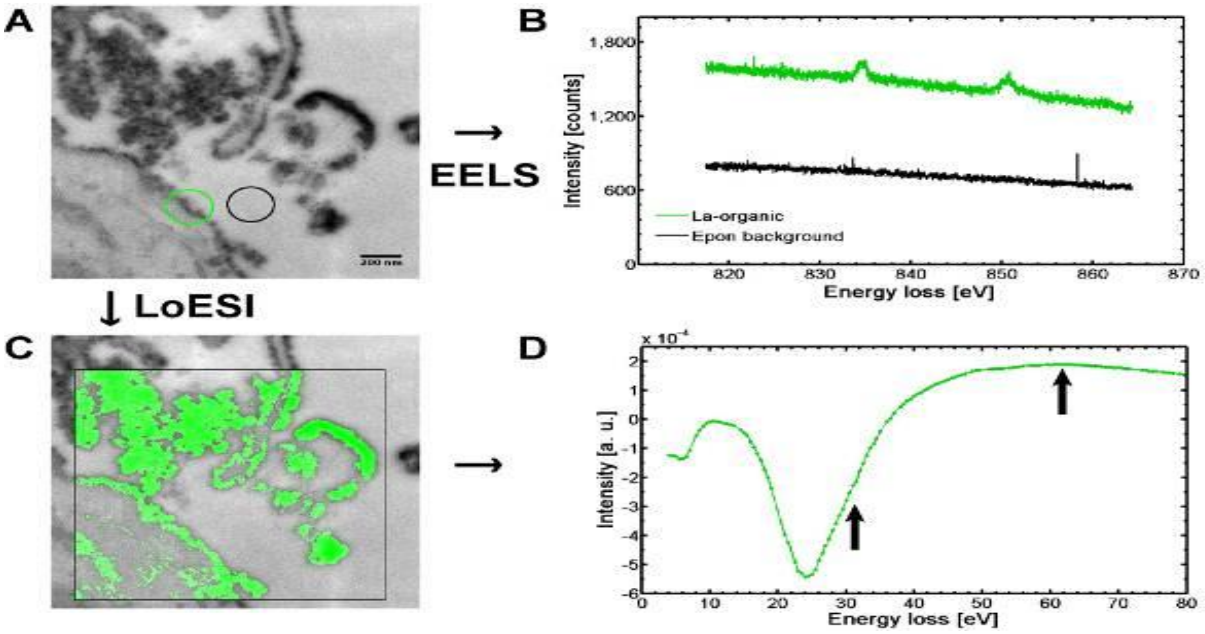

Figure 1. Segmentation of lanthanum-organic precipitates by EELS and ESI. A: Transmission electron micrograph of resin embedded HeLa cells with precipitates at membranes. B: EELS spectra from selected areas (circles in A) with precipitate and with resin background only, showing $M_{4,5}$ edges at 832 and 849 eV energy-loss. C: Multispectral segmentation using a low energy-loss ESI series with inelastic images from 4 to 80 eV using steps of 1 eV. Membrane regions with precipitates are well separated from resin background and intracellular regions. D: Normalized spectrum of segmented pixels in C (difference between averaged ESI spectrum of segmented pixels and background pixels). Features around 30 and 60 eV (arrows) indicate specific lanthanum signals from the $O_{2,3}$ edges.