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Molecular Markers Of Different Elemental Compositions Bioengineered For Simultaneous Detection And Localization Of Multiple Biomolecules With Energy And Wavelength Dispersive Spectroscopic Imaging

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Rapid advances in nanobiotechnology call for a technique, which would allow investigators to determine not only biochemical features of the gene expression products (e.g., mass spectroscopy), but also nanomolecular architecture of their interactions. Previously, we have developed spectral labels, which are suitable as molecular labels for use with spectroscopic imaging [1-3]. Substantial progress has been made recently with respect to preparation of nanoparticles consisting of different elements, development of protocols to conjugate them with antibodies and ligands, increased sensitivity of x-ray detectors, stability of the specimen stages, speed of pulse processors, and the design of spectral software. These advances have further enhanced the detectability of molecular markers of different elemental composition by energy and wavelength dispersive spectroscopic imaging (EDS and WDS). The greater ease of detection broadens the applicability of this technology. Nanoparticles made out of Au, Pt, Ag, and Pd were conjugated to antibodies. Conjugates were spread on hydrophilized polystyrene films treated with ECM proteins, supported by carbon grids, and stabilized by sputtering of carbon. Spectroscopy was preformed on the instruments: VG HB501, Philips CM200, LEO 1530, and Cambridge Stereoscan, all equipped with Noran or Oxford EDS and/or WDS systems. Analysis has been performed in three steps: (1) a high resolution image was acquired (Fig. 1); (2) a composite spectrum from the entire area of investigation was collected and energy peak was selected (Fig. 2); and (3) elemental map extracted for each element separately (Fig. 3a and 3b). It was demonstrated that antibody-conjugated nanoparticles made out of different elements can be distinguished from each other with high-resolution of EDS and/or WDS. The spatial resolution attained in the elemental mapping mode was below 5 nm as determined by absence of ZAF contributions in the spectral maps.
Advances in this technology should permit mapping of single labeled biomolecules and open new avenues for studies on biomolecular architectures particularly with respect to co-localization of multiple biomolecules studied by SEM, STEM, as well as TEM. [4]

References.
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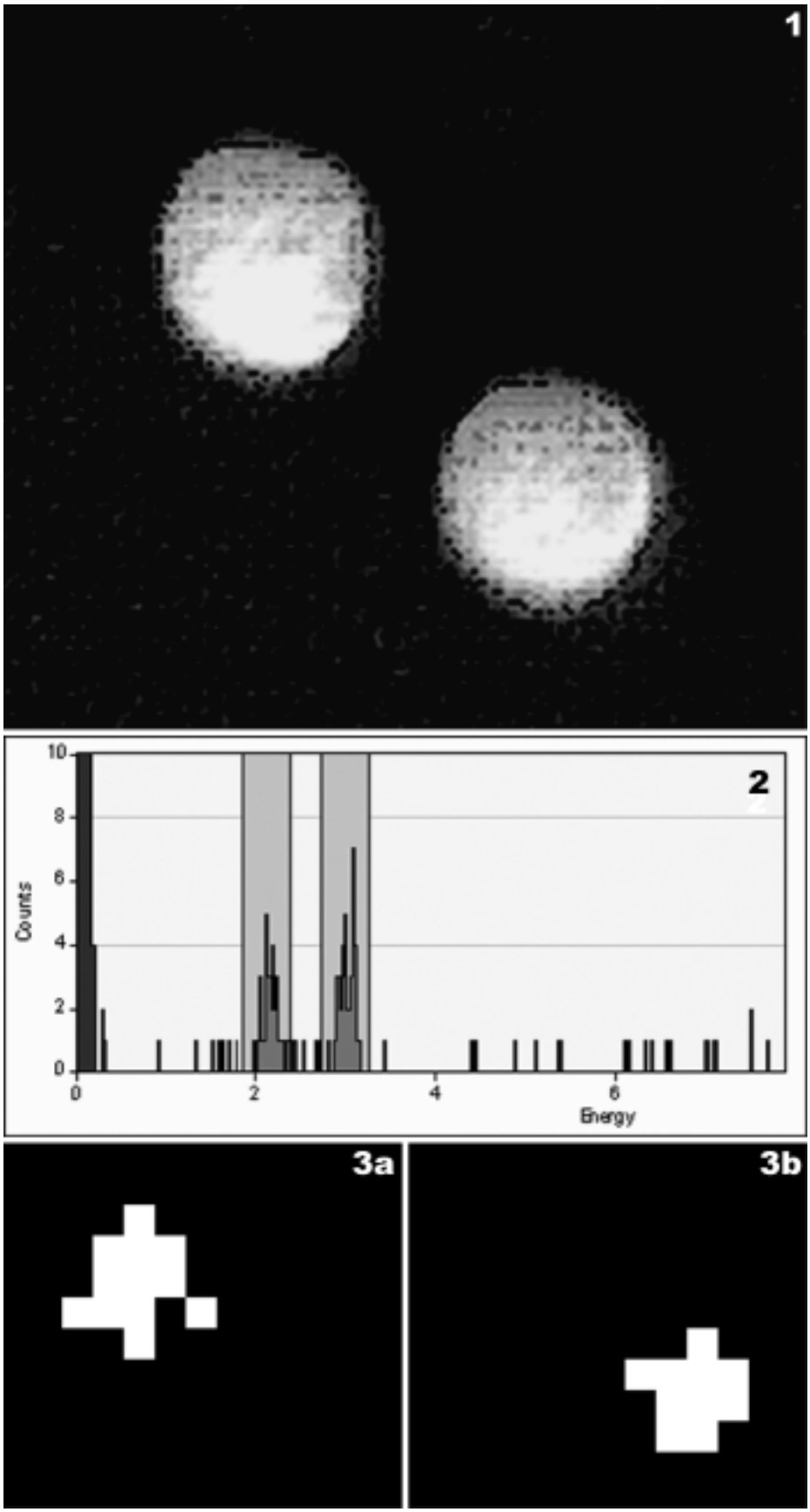
Figure legends.
Fig. 1. High-resolution dark-field image acquired on VG HB 501 illustrating two: Au (lower right) and Ag (upper left) nanoparticles conjugated to antibodies. Horizontal field width 26 nm. Magnification 4.6*10^6 x. **Fig. 2.** Composite spectrum from the entire area of investigation collected with Noran detector under Emispec software allowing selection of the energy peaks: Au – 2.122 keV and Ag – 2.984 keV. Energy in [keV]. Counts in [10^2]. **Fig. 3.** Each elemental map was extracted from the spectral array at 2 nm/pixel, for each element separately as a bitmap in order to facilitate identification of the molecules within the structure pattern presented in Fig.1.



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