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Compositional Imaging of Cells and Bionanoparticles by EFTEM

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Elemental mapping based on electron energy loss spectroscopy (EELS) provides cell biologists with information that complements the ultrastructure obtained by conventional transmission electron microscopy (TEM) [1]. Despite the lower collection efficiency of energy filtered TEM (EFTEM) relative to spectrum-imaging in the scanning TEM (STEM) due to the EFTEM's sequential acquisition of the EELS information one energy band at a time, much higher total currents (~100 nA) are available with wide-beam illumination [2-5]. This means that in EFTEM large numbers of pixels (~10^6) can be read out in a few seconds [6-9]. In particular, EFTEM mapping when combined with electron tomography facilitates quantitative electron spectroscopic tomography (QuEST), which provides three-dimensional elemental distributions from a tilt series of two-dimensional projections [10-12]. It is then possible to interrogate specific voxels in the 3D elemental maps to determine numbers of atoms in structures of interest.

In the first example, we have used a correlative microscopy approach based on light microscopy, EFTEM imaging, and STEM to localize specific protein complexes. We have explored the ultrastructure of the well-studied *Drosophila melanogaster gypsy* chromatin insulator body by immunolabeling a key insulator protein CP190 using a fluoronanogold conjugated antibody probe. We have used fluorescent imaging to identify nuclei that contain the insulator bodies, which are rare structures within the thin sections. A comparison of low-magnification electron micrograph of a whole cell with the corresponding fluorescent image reveals the approximate location of the structure of interest. The fluorescence signal observed by light microscope guarantees the presence of the conjugated nanogold, which can be visualized using STEM, and used to locate precisely the labeled CP190 proteins. The EFTEM and QuEST techniques are then performed to image the nitrogen and phosphorus and thus to map distributions of protein and nucleic acid within the cell nucleus. From our quantitative analysis of these two elemental distributions, it is evident that the insulator body contains an abundance of protein but a small quantity of nucleic acid. Even though dense chromatin surrounds the insulator body, it is difficult to determine whether the low levels of phosphorus within the insulator body structures correspond to DNA or RNA; this requires further investigation.

In the second example we use EFTEM imaging together with electron tomography to characterize different types of nanoparticles, which have potential use in diagnostic medical imaging. For some types of nanoparticles it was more efficient to generate 2D EFTEM maps for one projection only, and then combine those with structural information from conventional TEM tomography. For other nanoparticles it was necessary first to perform EFTEM spectrum-imaging and then STEM tomography. These hybrid nanoparticles are composed of a core, which can include the therapeutic drug, and outer layer that can be functionalized with a targeting peptide and/or an imaging probe. The chemical compositions of all these components and their 3D organization can be critical to how these nanoparticles behave in the biological microenvironment, i.e., *in vivo.*

One such nanoparticle results from self-assembly of three components, a mixture of feraheme, protamine and heparin, which form a complex that has been proposed for labeling and tracking infused

or implanted stem cells using magnetic resonance imaging [13]. From our STEM tomographic data we have found 6-nm feraheme particles attached to the surface of a protamine-heparin soft core. The EFTEM elemental maps obtained from spectrum-images showed iron on the outer surface of the particle with nitrogen and sulfur-rich regions in the inner part. These correspond, respectively, to feraheme, protamine, and heparin-rich regions in the nanoparticles. Overlaying these elemental maps revealed that heparin is confined to the core of the nanoparticles, whereas the surface is enriched in protamine and feraheme.

Another example of particle that we characterized is a flower-shaped synthesized Au-Fe_3O_4 optical nanosensor that has been proposed for imaging protease expressions *in vivo* [14]. From our EFTEM elemental maps, we have concluded that these nanoparticles are composed of gold cores and their flower-like petals are the iron oxide phase. We have been able to analyze several other types of nanoparticles that can be used either as imaging probes or drug delivery systems, or both

In summary acquisition of elemental maps in the analytical electron microscopy operated in the EFTEM mode gives useful complementary information about subcellular distributions of biomolecules (e.g., proteins and DNA) that cannot be determined from ultrastructure alone. Furthermore, the technique also provides key information about the architecture of bionanoparticles, which have been proposed for potential clinical applications. However, the task of mapping most types of nanoparticles inside thin sections of cells based on EFTEM and EELS remains a challenge, which has still to be addressed [15].

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