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Intracellular Elemental Mapping using Simultaneous EELS and EDS: A Combined Approach to Quantifying Na, K and Ca

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The Scanning Transmission Electron Microscope (STEM) is routinely used in the physical sciences to characterize materials at high spatial resolutions. Advances in quantitative imaging techniques such as high-angle annular dark field (HAADF), combined with careful simulation, now permit atom counting sensitivities at the atomic level [1]. In parallel, developments in analytical STEM grant access to chemical information about a sample. In particular, electron energy loss spectroscopy (EELS) and energy dispersive x-ray spectroscopy (EDS) reveal insights into the composition of a given sample, and can themselves be quantified in terms of atom counts [2].

Sodium, potassium and calcium are all key elements in maintaining cellular function within nerve tissue, and small changes in levels of any can have significant physiological impact [e.g. 3]. However, most approaches for measuring these elements both in- and ex-vivo face technical limitations; many conventional techniques lack either the spatial resolution or the sensitivity required to accurately track small changes in these key cellular elements. Recent developments in genetically-encoded fluorescent Ca^{2+} markers are a promising avenue for dynamic calcium imaging, but targeting of specific organelles of interest remains challenging [4].

While a number of studies have used either EDS or EELS alone to examine questions posed by life sciences [5, 6], few have attempted to fully utilize the analytical capabilities of the STEM. This is a result of challenges related to electron beam-induced damage, sample preparation of tissue sections, and that there has been relatively poor sharing of skill-sets of scientists in the "soft and squishy" and the "hard and dry" scientific worlds [7].

We present our recent work examining the viability of a combined, simultaneously acquired, STEM-EDS-EELS approach for investigation of Ca, K and Na levels in sub-cellular structures. We show methods of approaching and overcoming challenges associated with acquiring these measurements, including the large carbon background underneath the Ca $L_{2,3}$ edge in EELS, and the substantial overlap of the calcium K_α and (significantly more abundant) potassium K_β peaks in EDS [Figure 1].

We discuss advantages and drawbacks of existing quantification techniques for both spectroscopies and demonstrate a new approach using partial cross-sections for absolute measurement. We have, at the sub cellular level, investigated quantification of K, Na and Ca in units of atoms/μg dry weight. We also discuss important considerations when preparing a sample for this analysis, such as minimizing sample damage and the diffusion of mobile elements [8].

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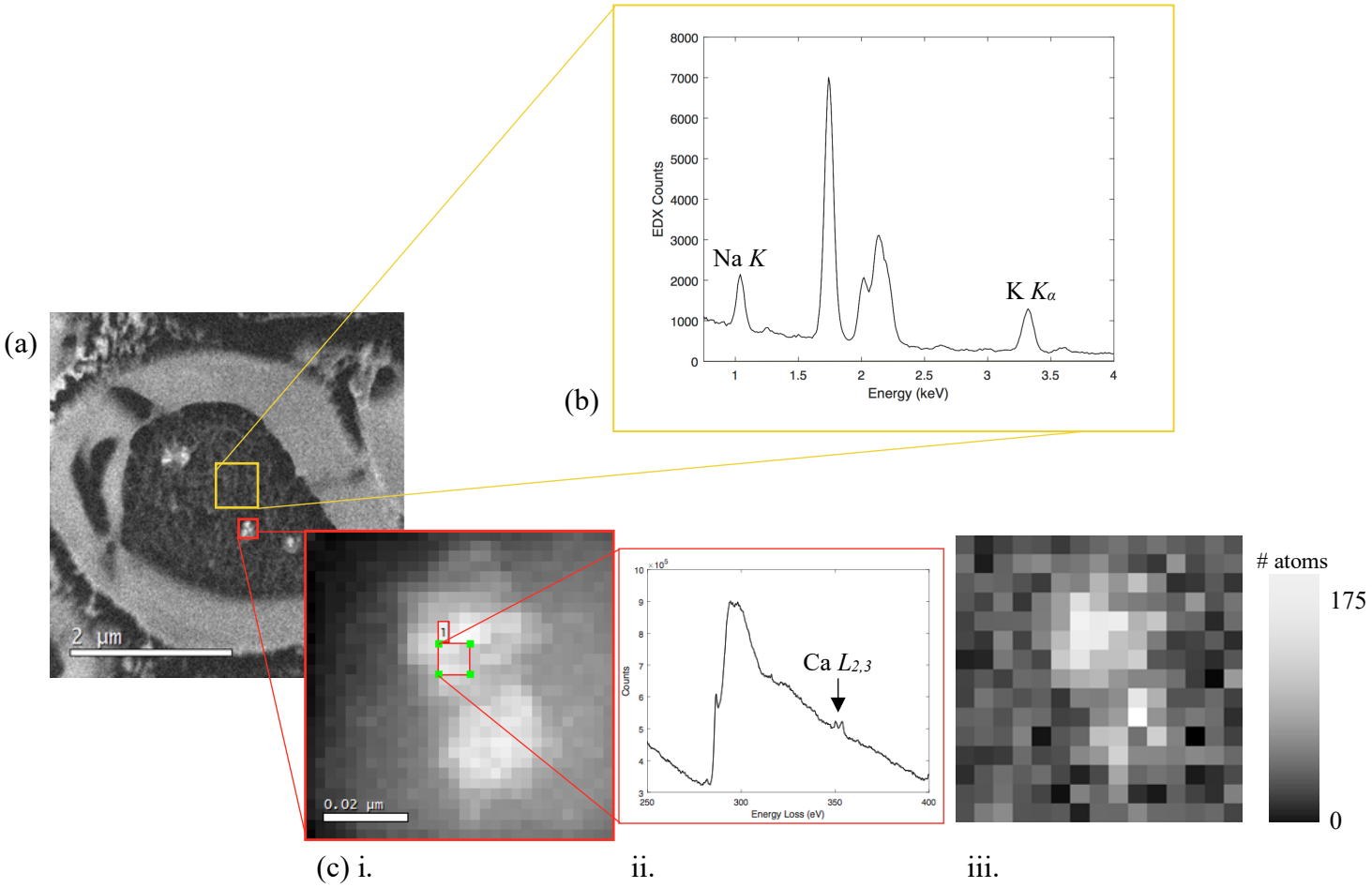

Figure 1. (a) HAADF image of typical cryosectioned myelinated peripheral nerve cell. (b) EDS obtained from within the lumen of cell shows clear Na and K peaks, which are readily quantified. (c) Ca clusters within a myelinated axon imaged using HAADF (i) can be quantified using EELS (ii). In the resulting chemical map, atom counts range between 0 and 175 atoms per 25nm^2 pixel.

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