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

Alexandra A. Sheader1*, Gema Vizcay-Barrena2, Roland A. Fleck2, Sarah J. L. Flatters3 and Peter D. Nellist1

1 Department of Materials, University of Oxford, UK.
2 Centre For Ultrastructural Imaging, King’s College London, UK.
3 Wolfson Centre for Age-Related Diseases, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, UK.
*Corresponding author: alex.sheader@materials.ox.ac.uk

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 Ca2+ 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 L2,3 edge in EELS, and the substantial overlap of the calcium Kα and (significantly more abundant) potassium Kα 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].
References:
[8] The authors acknowledge use of the South of England Analytical Electron Microscope (EP/K040375/1), within the David Cockayne Centre for Electron Microscopy, Department of Materials, University of Oxford, alongside financial support provided by the Henry Royce Institute (Grant ref EP/R010145/1). This research was supported by DTP studentship awarded by the University of Oxford Department of Materials (AAS), and a Physiological Society Research Grant 2015 awarded to SJLF.

**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 25nm² pixel.