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Synchrotron-based X-ray Fluorescence Microscopy as a Complementary Tool to Light Microscopy/Electron Microscopy for Multi-scale and Multi-modality Analysis

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Correlative microscopy allows us to identify sites-of-interest across different length scales and perform both structural and chemical analysis by combining the use of light, electrons and x-ray photons. It becomes an essential method to address questions for both biological and materials sciences. We have presented here example studies as well as the challenges of using synchrotron-based x-ray fluorescence microscopy (SXFM) as a complementary tool to either light microscopy (LM) or transmission electron microscopy (TEM) for trace element analysis.

Trace elements are essential for the existence of life. It is estimated that one-third of all known proteins contain metal cofactors, and the majority of these function as essential metalloenzymes. For example, Mn has vital roles in enzyme reactions, however excessive Mn can induce neurological diseases [1]. In a recent study using rat primary midbrain neurons, we have found that αSynulein (αSyn) alters the level of intracellular Mn, which may contribute to chronic neurodegeneration [2]. By using SXRF in conjunction with the GFP fluorescence, we were able to measure the intercellular elements in GFP-positive cells, i.e. cells that were overexpressing αSyn (Figure 1). In order to minimize structural/elemental alternation and reduce radiation damage under x-ray exposure, the samples were cryopreserved. The measurements were carried out in cryogenic conditions at both a light microscope and the Bionanoprobe, i.e. an SXFM instrument dedicated to cryogenic studies at sub-100 nm resolution at the Advanced Photon Source [3]. In a different study, Mn was used to enhance magnetic resonance imaging of brain to trace neuronal connections [4]. In order to elucidate the mechanism of Mn uptake, rat hippocampal slices of 80 nm were imaged using a TEM first and then measured at the BNP with a photon energy above Mn k-edge. The intercellular Mn distribution was then correlated with the TEM ultrastructure [5]. SXFM delivers unprecedented elemental sensitivity, which well complements the higher spatial resolution offered by TEM.

Trace elements also play important roles in materials science. For example, addition of elements in trace amount to Al alloys, i.e. microalloying, is an effective way to improve their mechanical and anticorrosive properties [6]. Our present work has been focusing on a novel microalloyed 5xxx Al alloy with a nominal composition of Al-6Mg-0.9Mn-0.07Zr-0.2Er (wt.%). The microstructure and elemental distributions have been characterized using both SXFM beamlines and an analytical TEM. While the TEM was able to detect Er- and Mn-enriched precipitates of sub-10 nm in size, SXFM was used to survey relatively large and thick regions (Figure 2). Further quantitative analysis and correlation will be carried out.

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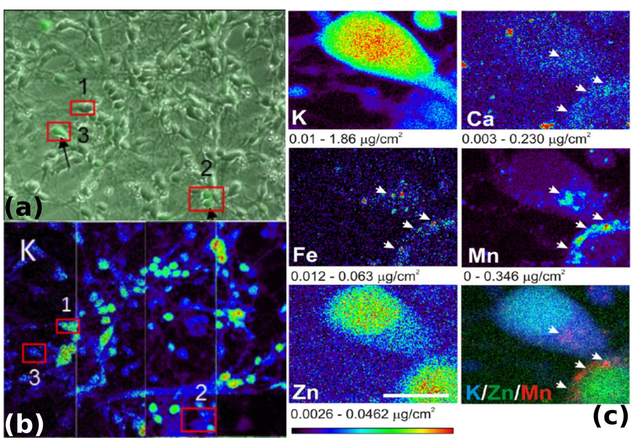

Figure. 1. Correlative LM and SXFM imaging of a rat midbrain neuron culture sample: (a) overlay of optical phase contrast and GFP fluorescence images of the culture sample with the arrows indicating a few GFP-positive cells; (b) x-ray fluorescence image showing K distribution of the same region; and (c) x-ray fluorescence images of cell#3 showing subcellular distribution of multiple elements with arrows indicating Mn-enriched cellular compartments [2]. Scale bar: 10 µm.

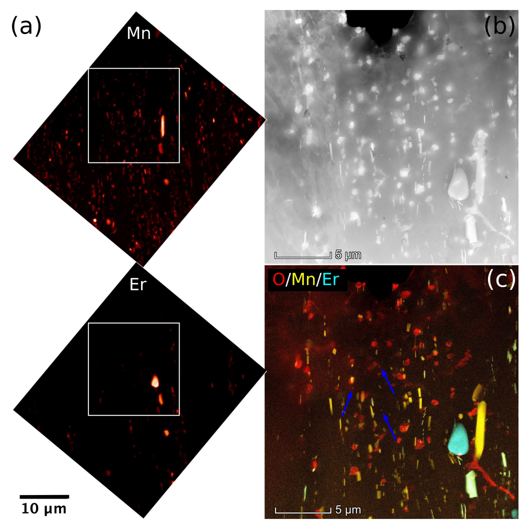

Figure. 2. Correlative SXFM and analytical TEM study of a microalloyed Al alloy sample: (a) x-ray fluorescence images showing the distributions of Mn and Er over a 37 µm x 42 µm region; (b) high angle annular dark field image acquired using an FEI Talos 200kV S/TEM; and (c) EDS image showing the overlay of O, Mn, and Er, with blue arrows indicating nanoscale precipitates.

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