1076
doi:10.1017/S1431927619006111
Microsc. Microanal. 25 (Suppl 2), 2019
© Microscopy Society of America 2019

Challenges in Biological X-ray Microanalysis in the AEM

Nestor J. Zaluzec[1], John Seeler[2], Reiner Bleher [3] and Thomas O'Halloran [2,4]

[1.] Photon Sciences Division, Argonne National Laboratory, Illinois, USA.
[2.] Department of Molecular Biosciences, Northwestern University, Evanston, Illinois, USA.
[3.] Department of Materials Science and Engineering Northwestern University, Evanston, Illinois, USA.
[4.] Department of Molecular Biosciences, Department of Chemistry, Chemistry of Life Process Institute, Northwestern University, Evanston, Illinois, USA.

Quantitative high spatial resolution microanalysis of elemental distributions is a challenge in virtually all biological systems with issues ranging from detection sensitivity, radiation damage, through to quantification. This is particularly true when investigating distributions of metals which are found in virtually all cells and organisms. Fluctuations in the concentrations of these species (including Ca, Fe, Cu, Zn,) control a host of functional processes within the cells. Dynamic changes in essential metal ion concentrations play key regulatory roles in physiological events. Furthermore, surges in metal uptake from the extracellular environment can be pathogenic [1,2]. Numerous studies of element distributions have been conducted using synchrotron x-ray fluorescence (S-XRF), due in part to the sensitivity of x-ray excitation for low level concentrations and the ability to study whole cells [3]. Analytical electron microscopy (AEM) applied to organic/inorganic materials is not a new concept and has been applied for many decades using both x-ray and electron loss spectroscopy [4-7], however, advances in detector technology are facilitating new insights for today's research [8]. This evolution has additionally opened up new opportunities to study more challenging systems involving soft-matter and/or biological systems including experiments involving cryo-EM [9].

In order to assess the abilities of the new technologies available for biology system studies, we have used *Xenopus laevis* (African Frog) eggs. This system is often used in biomedical research due to the fact that: they tolerate extensive manipulation; it is easy to inject of a range of nucleic acids into the eggs to prevent protein expression or express mutant proteins; the development of these cells is well understood and allow focused studies and is often used during studies of: ion transport and channel physiology and environmental toxicology. The eggs used in this work were fixed in a solution containing paraformaldehyde, glutaraldehyde, and hydrogen sulfide. Resin-embedded diamond knife ultramicrotome sections (~150 nm thick) were mounted on an aluminum grid coated with a Formvar and a carbon film. Experimental measurements were performed using a ThermoFisher Talos AEM, equipped with the most recent generation of SuperX SDD systems. Specimens were mounted in custom manufactured Beryllium tipped cryo-transfer tomography holder from Fischione Instruments. All observations were made at 200 kV in both TEM and STEM modes at room temperature.

An example of a cross-section of the *Xenopus* egg is shown in Figure 1, in which one can readily see the distribution of vesicles extending from the egg cortex. Temporally resolved hyper spectral imaging (HSI) was utilized to mitigate (but not eliminate) radiation damage effects. Here the probe current employed was 250 pA, at pixel dwell times of 400 usec/pixel for 342 frames resulting in a final DAQ time of ~ 137 msec/pixel. The hyperspectral images shown to the right are taken from the indicated region of interest (ROI) and illustrate the location of metal containing vesicles. Because of the higher spatial resolution achievable in the AEM (nominally 5 nm in this data) in contrast to similar experiments done on an S-XRF beam line at the Advance Photon Source at ANL (~ 200 nm), one can show that the Fe present in this *Xenopus* egg preparation exists as 10 nm nanoparticles in the surrounding matrix

https://doi.org/10.1017/S1431927619006111 Published online by Cambridge University Press

rather than a homogenous distribution. A sum spectrum over the entire ROI field confirms trace levels of Mn and Ni. Key to the success of these measurements was the temporally resolved low dose measurement (compared to typical physical science studies) as well as the higher collection solid angles afforded by the SDD. Follow up experiments to identify the detection limits using traditional XRF composition standards are in progress [10].

References:

[1] E Velasco et al., Sci Rep. **8(1)** (2018), p. 6535. doi: 10.1038/s41598-018-24964-1.
[2] AJ Morgan et al., in "Electron Probe Microanalysis" (Springer, Berlin, Heidelberg). https://doi.org/10.1007/978-3-642-74477-8_5
[3] T Paunesku et al., J. Cellular Biochem **99** (2006), p. 1489.
[4] TA Hall, Physical Techniques in Biological Research, IA, 2nd Academic Press, (New York).
[5] P Echlin, Microsc. Microanal. **4** (1999), p. 577.
[6] RD Leapman et al., J. Microsc. **133** (1984), p. 239.
[7] AP Somlyo and H Shuman, Ultramicroscopy **8** (1982), p. 219.
[8] NJ Zaluzec, Microsc. Microanal. **20** (2014), p. 1318.
[9] M Ovsyanko, et al., Microsc. Microanal. **24(1)** (2018), p. 776. doi:10.1017/S1431927618004373
[10] This research was funded in part by Laboratory Directed Research and Development (LDRD) funding No. 2017-153-N0 at Argonne National Laboratory by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences , it employed resources in the Center for Nanoscale Materials, an Office of Science user facility, This research also made use of the BioCryo facility of Northwestern University's NU*ANCE* Center, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205); the MRSEC program (NSF DMR-1720139) at the Materials Research Center; the International Institute for Nanotechnology (IIN); and the State of Illinois, through the IIN.as well as through the grants NIH R01 GM115848 and NIH T32 GM008061.

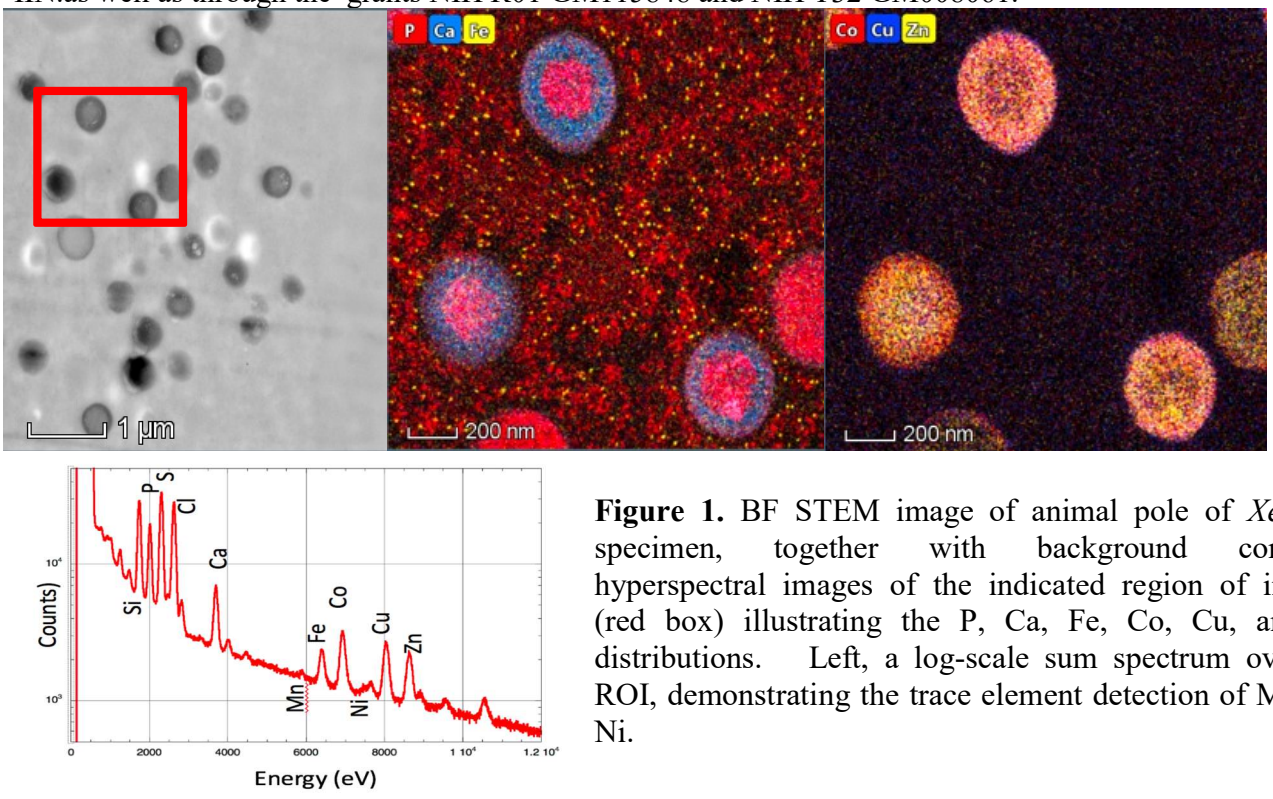

Figure 1. BF STEM image of animal pole of *Xenopus* specimen, together with background corrected hyperspectral images of the indicated region of interest (red box) illustrating the P, Ca, Fe, Co, Cu, and Zn distributions. Left, a log-scale sum spectrum over the ROI, demonstrating the trace element detection of Mn and Ni.

https://doi.org/10.1017/S1431927619006111 Published online by Cambridge University Press