

## Imaging and Elemental Mapping of Biological Specimens with the Hitachi HD-2300A Dual-EDS Scanning Transmission Electron Microscope

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Improved specimen preparation techniques, low dose methodologies and related practical developments have significantly advanced “imaging” applications of S/TEM for biological materials, including 3-D tomography and absolute mass determination of macromolecules [1]. However, analytical S/TEM has often been limited to energy-filtered imaging and EELS spectroscopy/mapping for handful suitable elements which offer high cross-section for EELS (e.g., Ca, Fe). On the other hand, chemical analysis and mapping of biological specimens via EDS in S/TEM is limited by specimen stability considerations and poor x-ray collection efficiency (thus the sensitivity) of EDS. With the emerging recognition of the role of metals (e.g., Zn, Cu) in biology and the concomitant need for high analytical sensitivity in EDS, we have designed and developed a dedicated cryo-bio STEM for analytical studies in biology. The new instrument is based on the Hitachi HD-2300A model, equipped with all the traditional high sensitivity electron detectors and significantly reduced radiation damage with a controlled weak probe current (as small as 7 pA), fast scanning, and cryo-compatible operation in low-dose modes. More importantly, the new STEM is equipped with a dual-EDS system with two separate yet integrated EDS detectors (each with ~ 0.38 sr. nominal collection angle) thereby greatly improving the elemental sensitivity and minimum detectability limits. The instrument is naturally compatible with electron energy loss spectroscopy (EELS) and ADF/HAADF imaging for simultaneous imaging and chemical analysis. [2].

Here, we present analytical STEM of the “whole-mount” preparation of model biological specimens such as *Escherichia Coli*, mouse spermatozoa, and red blood cells. Fig. 1 shows secondary electron (SE), BF and ADF STEM images of *E. Coli*. With the SE detector, large cells can be examined by imaging by detecting signals generated only from their surfaces. In Figs. 2 and 3, the Z-contrast images which are collected by annular dark-field detector and x-ray maps of a red blood cell and a mouse sperm cell, respectively, are shown. Their elemental maps confirmed known elemental signatures of these cells such as the compartmentalization of phosphorus in the DNA-rich sperm head. Such multi-modal capabilities of the new dual-EDS STEM promise to open new vistas in analytical microscopy coupled with multi-modal imaging for biological applications. The presentation will cover the relevant aspects of the instrumentation, and illustrative application examples to demonstrate the efficacy of cryo-bio analytical STEM in unraveling important spatio-chemical problems in biology

### References

1. Muller, S.A., Engel, A. (2006). *Chimia*, 60, 749-753.
2. Myers, B.D., *et al.*, *Microscopy and Microanalysis* (2010), submitted.

3. This work was performed in the EPIC facility of NUANCE Center at Northwestern University. Research was supported by the W. M. Keck Foundation Medical Research Award.

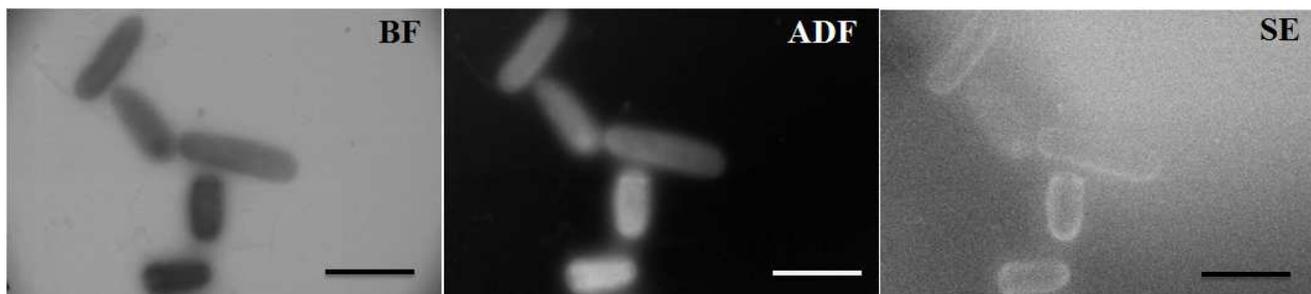


Figure.1 Images of E-coli cell collected by different detectors on HD-2300A. The scale bars are 2 micron.

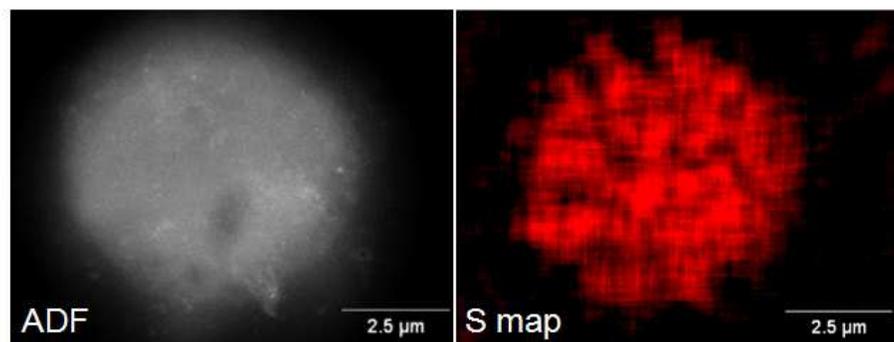


Figure.2 Z-contrast STEM image of red blood cell and x-ray mappings of S.

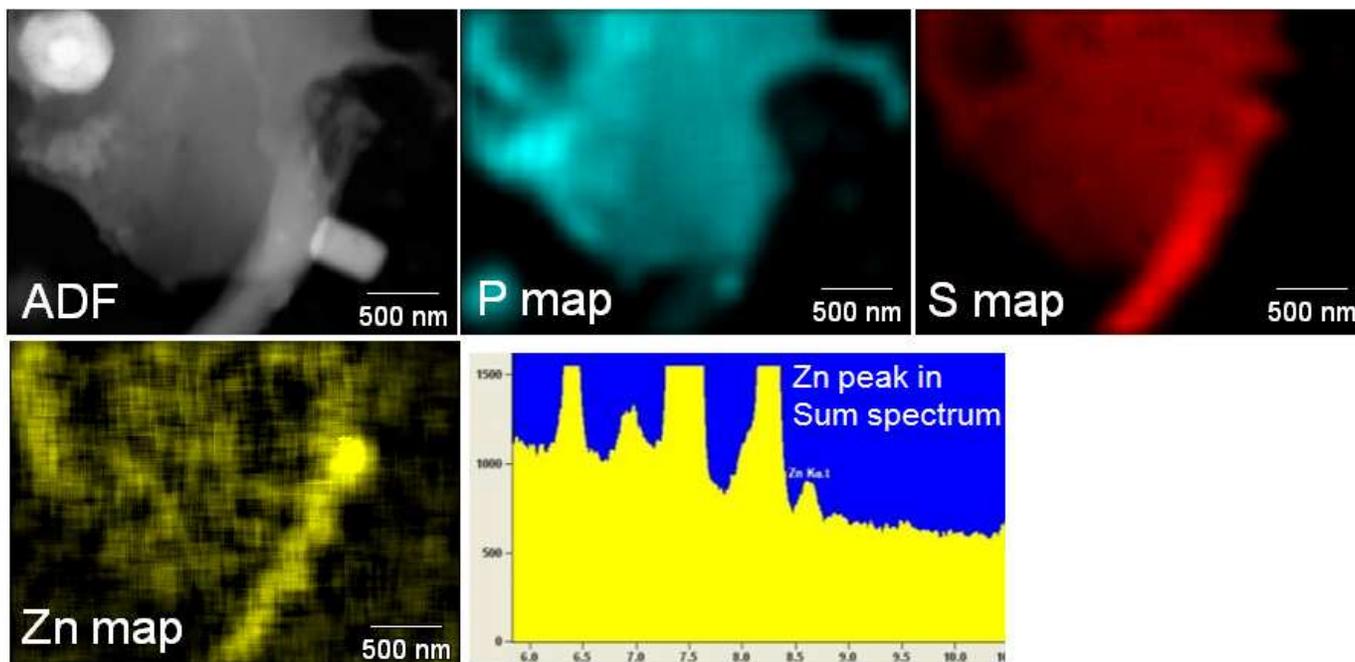


Figure.3 Z-contrast ADF STEM image and x-ray mappings including P, S and Zn maps of a sperm cell. The sum EDS spectra is shown, where Zn peak can be seen.