Reprinted from:
"34th Ann. Proc. Electron Miscroscopy Soc. Amer."
Miami Beach, Florida, 1976. G. W. Bailey (ed.).

COMPARISON OF SCANNING TRANSMISSION AND BACKSCATTERED ELECTRON IMAGING IN THE SEM, USING HEAVY METAL STAINED SECTIONS OF BIOLOGICAL TISSUE

Phillip B. DeNee and *Richard G. Frederickson

U.S.Govt/DHEW/ALOSH, Morgantown, W.V., and *Dept. of Anatomy, West Virginia University Medical Center, Morgantown, W.V., 26505

The Transmission Electron Microscope (TEM) has been used extensively for the study of biological tissues in thin section (50-100 nm). For sectioned material greater than 100 nm, the Scanning Transmission Electron Microscope (STEM) and the High Voltage Electron Microscope (HVEM) have become the only alternatives for the study of these tissues at a resolution better than that obtained with the light microscope. Recently, it has been shown(1) that tissue stained with heavy metal can be studied in the Scanning Electron Microscope (SEM) by Backscattered Electron (BSE) imaging to give results similar to those obtained with the TEM. Because BSE imaging is a method complementary to STEM, it seemed worthwhile to compare the two techniques using the same specimens and beam conditions.

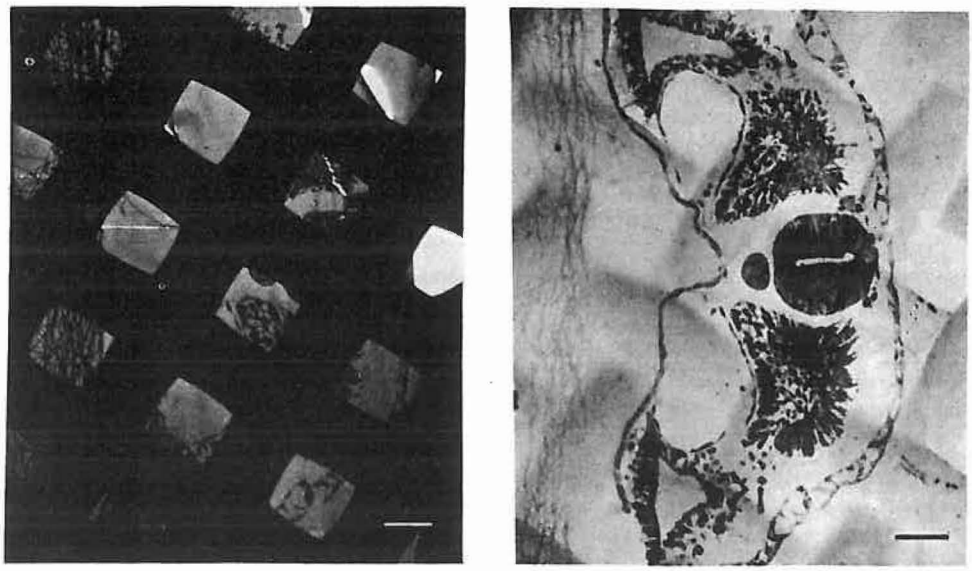
Direct observation of the total specimen is possible with BSE imaging without interference by grid bars. Therefore, an improved perspective of tissue-to-tissue structural relationships can be obtained at a resolution significantly better than that of the light microscope. With the TEM, only a minimal tissue sample can be examined and patterns of tissue organization are difficult to interpret. Morphological studies can be productive with a system of analysis involving a series of microscopic techniques that provide information at different levels of resolution and different levels of cellular and tissue organization. BSE imaging with the SEM provides an extremely significant transition from the poorer resolution of light microscopy to the superior level of detailed visualization of cellular fine structure obtained with the TEM.

For this study we chose embryonic chick tissue already available and previously observed with the 1MeV HVEM. Sections were cut at thicknesses of 60 nm, 100 nm, 200 nm, 400 nm, 600nm, and 800nm, and collected on 300 mesh carbon-coated nylon grids. Each section was stained with uranyl acetate for 60 min., and lead citrate for 20 min. The grids were observed and micrographed using a commercially available SEM, with factory equipped solid state backscatter detector and scintillator/photomultiplier transmission electron detector. Working voltage was 20 Kv and beam current was 0.7 na.

The micrographs were taken from the 200 nm thick section, which was found to be the optimum thickness for both STEM and BSE imaging. Figs. 1a and 1b illustrate a low power comparison of the same section as viewed with STEM and BSE imaging. In Fig. 1a, only about 25% of the tissue section can be seen in the STEM image because of the grid bars. However, the complete tissue section can be seen in the BSE image (Fig. 1b). The latter allows unrestricted observation of total tissue continuity. Figs. 2a and 2b, show a higher magnification of the tissue illustrating good resolution of cellular detail with both imaging modes. Comparing Figs. 2a and 2b, it can be seen that cellular detail is more distinct in the BSE image (Fig. 2b) while delicate extracellular material (arrow) is more distinct in the STEM image (Fig. 2a). Under the conditions of the experiment it was determined that STEM was marginally adequate for sections of 60 to 100 nm, and optimal at 200 nm. Above 200 nm, resolution deteriorated markedly and was uninformative. Conversely, the BSE image was extremely poor for sections of 60 to 100 nm, but became optimal for thicknesses of 200 nm and greater.

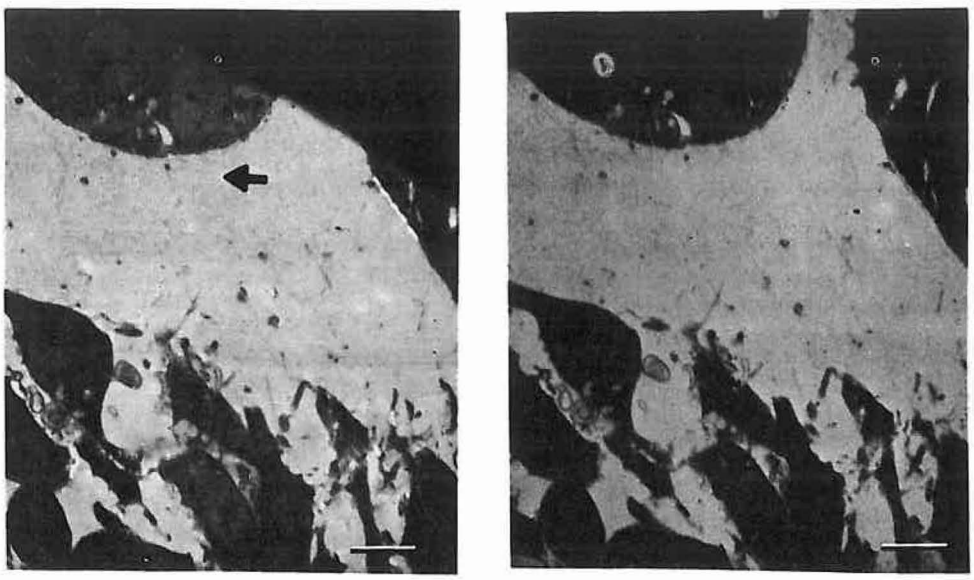
Reprinted from:
"34th Ann. Proc. Electron Microscopy Soc. Amer."
Miami Beach, Florida, 1976. G. W. Bailey (ed.).

1) DeNee, P.B. et al.,"Histochemical stains for the SEM -- qualitative and semi-quantitative aspects of specific silver stains". In: Scan.Elect.Microscopy (O.Johari, ed.) IITRI, Chicago, IL. (1974) p.259.



a b

Fig. 1. Low magnification of 200 nm thick section of chick embryo stained with uranyl acetate and lead citrate. a) STEM image; b) Backscattered electron image with polarity reversed -Note that grid bars do not interfere with the image. Bar = 50 μm.



a b

Fig. 2. Higher magnification of same section shown above. a) STEM image - Note extracellular material (arrow); b) Backscattered electron image with polarity reversed - Note improved cellular details. Bar = 5 um.