

Scanning Confocal Electron Microscopy of Thick Biological Materials

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The pioneering studies of the ultrastructure of thick biological samples were initially performed using the HVEM [1] as at that time this was the only practical way to achieve imaging in very thick samples. Subsequent improvement in ultramicrotomes and specimen preparation permitted use of instruments in the 100kV range, however sometimes at loss of information as sections were typically ≤ 100 nm thick [2]. With the resurgence of interest in tomographic studies [3], work on thicker sections has become of greater interest to the community. Scanning Confocal Electron Microscopy [4] is a new mode which has been shown to be a powerful tool for studying thick (≤ 8 μm) sections of semiconductors. In this work we report on preliminary application of the SCEM to biological materials specifically the ultrastructure of human hair and skin. When dealing with hair medulla, thick sections are essential, as this structure is usually not correctly preserved during ultrathin-microtomy. Moreover, a full understanding of the function of this structure would benefit from the description of its complex 3D organization. In addition, for our research, it was also important to evaluate the possibility to observe the 10 μm thick stratum corneum in its integrality.

Specimens for this work have all been fixed in Müller medium [5], followed by treatment in 2% phosphotungstic acid in ethanol [6] then embedded in Epon from which 1–10 μm sections were prepared, no coatings were applied to the sample for EM observation. SEM, TEM, and SCEM work were conducted respectively on a: Hitachi S4700 FEGSEM (1 kV), Philips CM30 TEM (300 kV), and the ANL SCEM (300 kV), at ANL. All samples for this work have been donated by volunteers while that stratum corneum was isolated from human skin obtained after plastic surgery.

In figure 1, we compare low magnification, TEM and SCEM images of a 5 μm thick section of hair medulla, while in figure 2, we compare 10 μm thick human skin specimen in SEM and SCEM modes. Here we see that the complex 3D nature of the specimen is clearly elucidated by the SCEM mode, yielding information which was not available or is lost using conventional SEM/TEM modes. In all cases we have recorded images at the best resolutions attainable, which for TEM, means operating with small (10 μm) objective apertures and performing a blind through-focal series to obtain a suitable image, since there was insufficient intensity on the TEM screen to perform real-time focusing. EELS spectroscopy of the 5 μm thick hair specimen revealed the complete absence of any elastic peak, having only a broad lorentzian distribution peaked near 600 eV loss, and thus cannot be compensated for through the use of Energy Filtering to provide inelastic filtered imaging. Tilting of the specimen by $\pm 50^\circ$ in the SCEM has also allowed us to observe the three dimensional nature of the samples, and additional work in this area is in progress.

References

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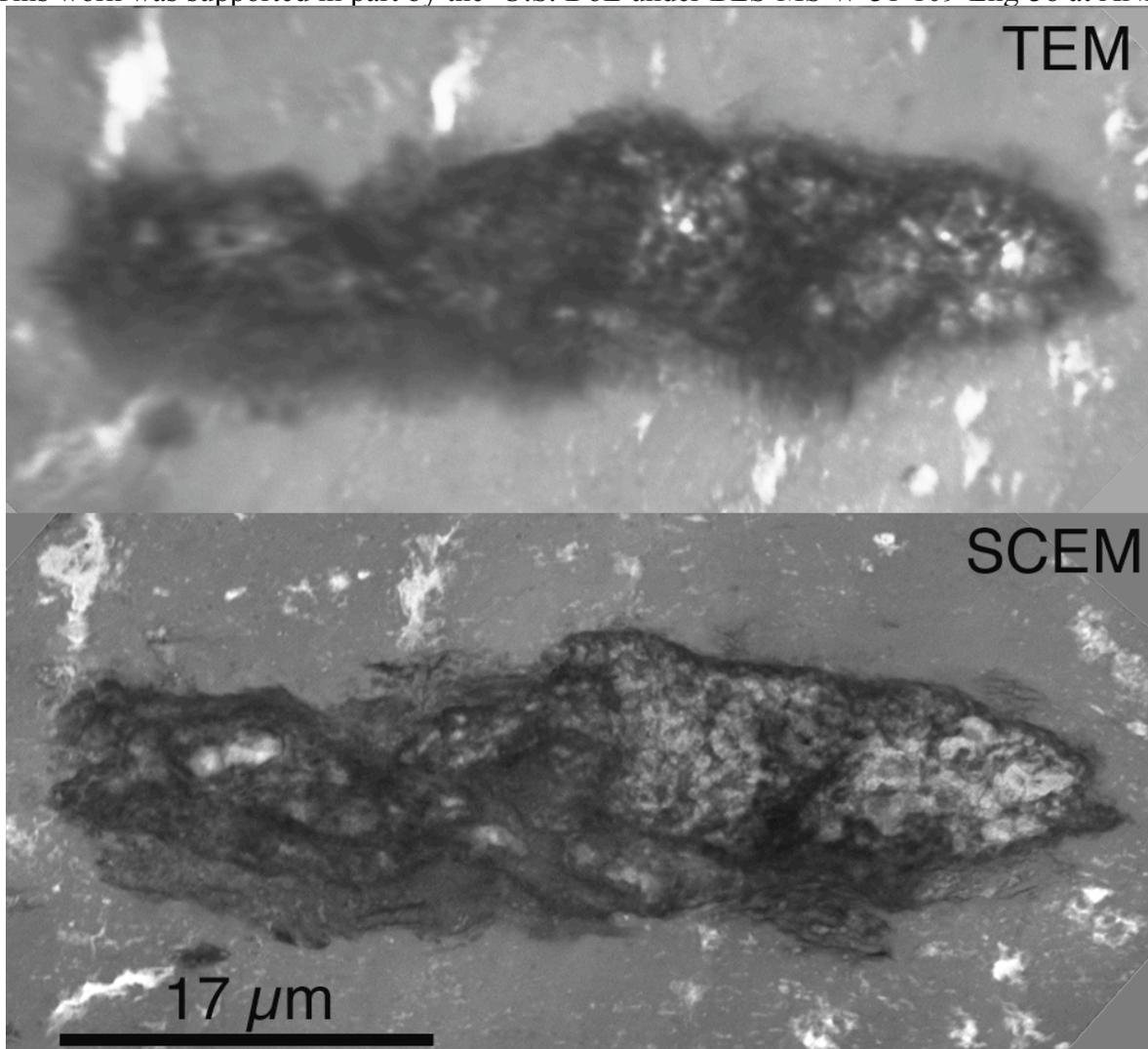


FIG. 1. Comparison of TEM and SCEM images from a 5 μm thick section of hair medulla

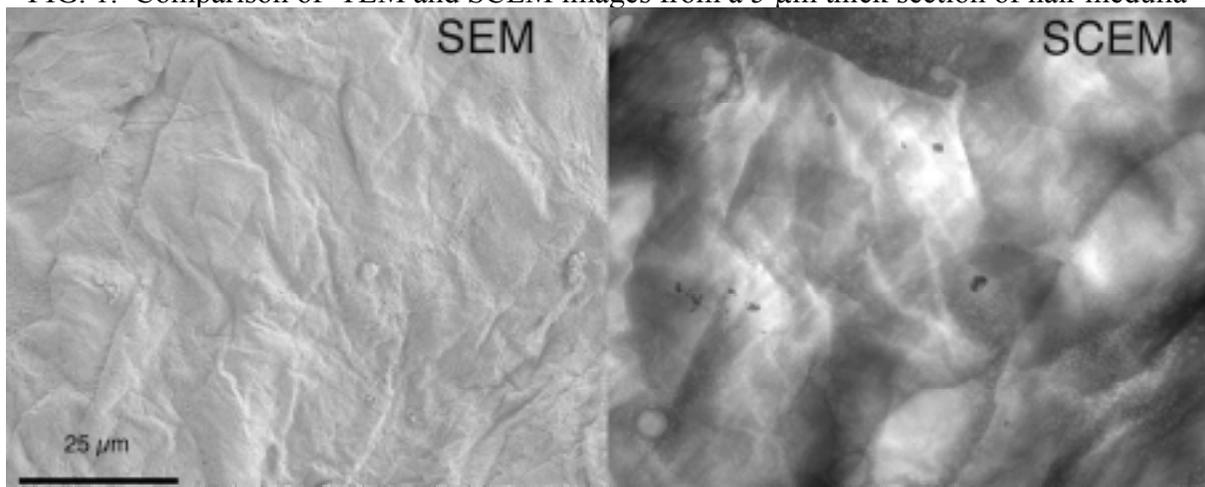


FIG. 2. Comparison of SEM and SCEM images of a 10 μm thick human skin specimen.