APOPTOSIS REVEALED BY EPIFLUORESCENCE CAN BE COMPARED WITH SILICA LOCALIZATION BY SEM BACKSCATTER USING PARAFFIN SECTIONS ON CARBON PLANCHETS

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In investigating pathological changes that occur during development of fibrosis in lungs exposed to silica[1], we found a significant increase in apoptotic cells in rats exposed to silica by inhalation for 80 days. Since silica is not definitively detected at the light level, we sought to use SEM backscatter imaging to correlate the presence of silica with apoptosis identified by fluorescent assays. To accomplish this, paraffin sections of lungs from rats that had been exposed to silica were placed on carbon planchets. Serial sections were placed on glass slides, immediately before and after the section on the planchet. Using a fluorescein apoptosis detection system (Promega G3250), a TdT-mediated dUTP nick-end labeling (TUNEL) assay was performed on both planchets and slides. The planchets and slides were cover slipped with gel/mount (Biomedia) and viewed in an Olympus AZ70 fluorescent photomicroscope with a wide band blue filter cube. Apoptotic nuclei could be seen in similar regions in both the slide and the planchet preparations. After taking photographs, the cover slip was removed from the planchet by soaking in water, and the planchet was dehydrated in alcohol, xylene, and air dried before examination in a JEOL JSM-6400 SEM. Photographs of backscatter images were taken, and elemental identification of silica was made using a PG&T EDS X-ray detector. We attempted to do the reverse procedure, with the silica localization first, then the TUNEL assay. The electron beam modified the tissue area so that broad rectangles of epifluorescence appeared wherever we scanned a region; specific localization of apoptotic nuclei was apparent only outside the scanned regions.

The most tedious part of this procedure was recognizing and orienting the same regions and cells when transferring planchets from light to electron microscopy. To assist, a series of video capture images of the epifluorescence was obtained at 40x, 20x, 10x, and 4x. Using the images as a guide, each of 6-8 apoptotic regions was located on the planchet under a Leica MZ12 gross microscope. Landmarks were identified on a gross photo (Fig. 1), which was used to identify the same higher magnification sites under epifluorescence and SEM (Fig. 3, 4). The apoptotic areas could also be compared to a slide of a serial section stained with hematoxylin and eosin (Fig. 2), using a similar mapping process. In this way we were able to determine that silica was localized in lipoprotein- and macrophage-rich aggregates in the alveoli, and that the apoptotic cells were found in these same regions.

In conclusion: we have found that we can correlate information gained by epifluorescence with SEM backscatter information, using paraffin sections on carbon planchets. This procedure will be very useful as we continue to explore the relationship between silica particles and early indicators of pathology in the development of fibrosis.

Reference

1. Porter, D.W., *et al.*, (1999) The Toxicologist 48(1-S): 132.

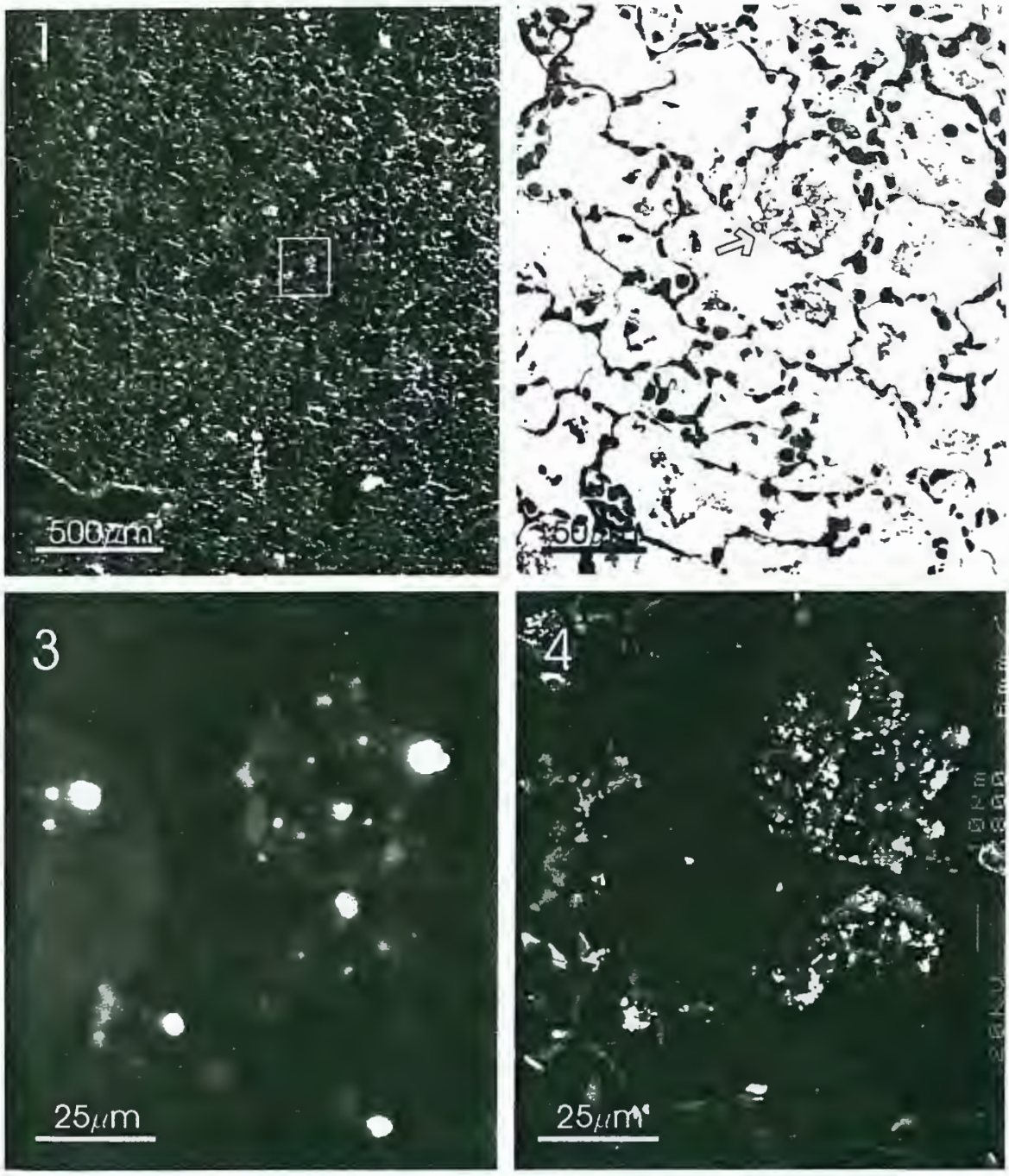

Fig. 1. Gross photo of paraffin section on planchet. Rectangular region corresponds to the area shown in Figures 3 and 4.

Fig. 2. Adjacent section on glass slide, stained with hematoxylin and eosin, showing same general area as figures 3 and 4. Arrow: lipoprotein-rich material within alveolus.

Fig. 3. Region of fluorescence in paraffin section on planchet after TUNEL assay. Large bright areas correspond to apoptotic nuclei.

Fig. 4. Backscatter image of same region seen in figure 3, showing localization of silica particles (bright clusters).

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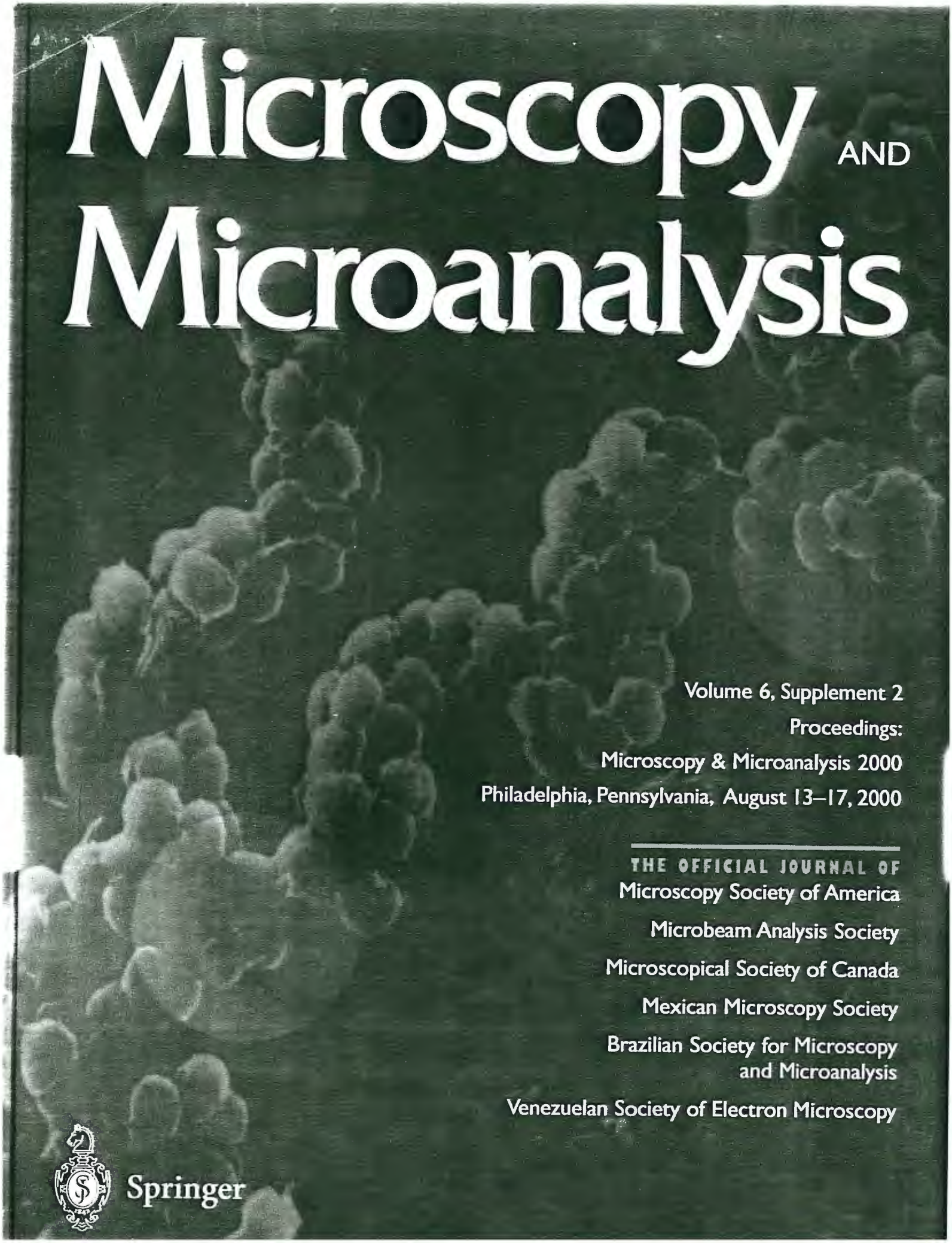

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