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This Week's Citation Classic®

Wohlfarth-Bottermann K E. Die Kontrastierung tierischer Zellen und Gewebe im Rahmen ihrer elektronenmikroskopischen Untersuchung an ultradünnen Schnitten (Staining animal cells and tissues for electron microscopical investigation with the aid of ultrathin sections).
Naturwissenschaften 44:287-8, 1957.
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Electron microscopic contrast in ultrathin sections of cells is especially important in revealing faint filamentous structures in the cytoplasm. The introduction of phosphotungstic acid, uranyl acetate, and other heavy metal salts during the dehydration of tissues (thus avoiding the danger of introducing artifacts into an as-yet-incompletely fixed object) broadened the possibilities of an ultrastructural inventory of cells and laid the basis for the subsequent discovery of the cytoskeleton. [The *SCI*® indicates that this paper has been cited in over 370 publications, making it the most-cited article in this journal.]

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Good image contrast in the electron microscope has been a problem since the invention in the 1940s of this powerful tool for studying cell biology and became even more problematic after the introduction of ultrathin sections in the 1950s because the contrast was further reduced by the extreme thinness of the sections. I learned to prepare such sections in the laboratory of F.S. Sjöstrand in 1953 at Stockholm University. Sectioning at that time required preparing suitable knives by sharpening (for hours) already very sharp razor blades. I did this together with H. Fernández-Morán and B.A. Afzelius. Post-war Germany presented many difficulties to researchers, so I was happy to receive, some years later, an appointment at Bonn University, probably due to my skill in preparing supersharp razor blades! Despite the application of this art, however, the problem of contrast remained when viewing the thin products of this stressful procedure in the electron microscope.

I retained the belief of histologists of the preceding century concerning the existence of a general "vital" structure in the cytoplasm of all cells. Thus, it was disappointing to see so little in the respective cell areas, and in some cases to see nothing at all. (Osmium, used at that time for fixation and contrasting, preferentially stains membranes.) Therefore, it was expedient to introduce additional heavy metals to enhance the contrast of structures anticipated in the "cytosol" that might represent the basis of the general "vital" structures postulated in the preceding century. The application of phosphotungstic acid and uranium compounds immediately following fixation by osmium, however, showed deleterious effects on the structural preservation because, at this stage of processing, the tissue is not completely fixed. Thus, contrasting was postponed to the step of dehydration, i.e., the heavy metal compounds were dissolved in ethanol.

The application of this procedure to a promising object (plasmodia of a slime mold) led to the first morphological demonstration of cytoplasmic actomyosin fibrils,[1] also called "stress fibrils," the first of the morphologically viewed group of fibrils constituting the cytoskeleton.[2,3] This discovery may well be one of the reasons for my being awarded the Schleiden-Medal (1977) of the Deutsche Akademie der Naturforscher Leopoldina.

1. **Wohlfarth-Bottermann K E.** Weitreichende, fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. I. Elektronenmikroskopischer Nachweis und Feinstruktur. *Protoplasma* 54:514-39, 1962. (Cited 100 times.)
2. **Komnick H. Stockem W & Wohlfarth-Bottermann K E.** Cell motility: mechanisms in protoplasmic streaming and ameboid movement. *Int. Rev. Cytol.* 34:169-249, 1973. (Cited 165 times.)
3. **Wohlfarth-Bottermann K E.** Biological aspects of motility. (Dove W F. Dee G. Hatano S. Haugli F B & Wohlfarth-Bottermann K E. eds.) *The molecular biology of* Physarum polycephalum. New York: Plenum Press, 1986.

