Steere R.L. Electron microscopy of structural detail in frozen biological specimens. *J. Biophys. Biochem. Cytol.* **3**:45-60, 1957.

This paper describes a procedure for the preparation of pre-shadowed replicas which reveal fine ultrastructural details of frozen fractured, etched (by vacuum sublimation) biological specimens. Illustrations reveal the ordered arrays of plant virus particles within crystals, including tobacco mosaic virus crystals within infected cells. [The *SCI*® indicates that this paper was cited 185 times in the period 1961-1977.]

Russell L. Steere
Plant Virology Laboratory
Plant Protection Institute
Beltsville Agricultural Research Center
US Department of Agriculture
Beltsville, MD 20705

January 13,1978

"I believe my pleasure in finding this report in the 'Citation Classics' list results from its appearance as the first successful application of an alternative approach (freeze-etching) to the visualization of biological ultrastructure by electron microscopy. This approach came about as a logical consequence of learned techniques, equipment, and information available to me at the time, and complete freedom to explore the unknown mixed with unsatiated curiosity, eternal optimism, and Steere stubbornness!

"The work was done under the supervision of Robley C. Williams in the virus laboratory at the University of California, Berkeley, where we had just demonstrated that the beautiful crystals found in cells of tobacco mosaic virus (TMV)-infected plants were composed ⅓ of TMV particles and ⅔ of a volatile matrix (probably water). We knew that chemical fixation destroyed these extremely fragile crystals so that thin section electron microscopy could not reveal their internal particle orientation.

"When I discussed the approach described in this paper with Robley in 1952 as a means of visualizing the true crystal structure, he remarked that it would hardly be worth the 3 to 4 years it might take to develop such a procedure just to visualize the ultrastructure of TMV crystals but that, as an alternative procedure for studying cytological ultrastructure, it might be extremely worthwhile. He cautioned me that he couldn't guarantee a promotion and that I might spend 3 to 4 years on such a development, then have to abandon it because of insurmountable obstacles. However, he did promise me that he would guarantee my job for at least 4 years if I wanted to tackle this important work.

"Now, after 20 years and numerous modifications to equipment and procedures, freeze-etching and related techniques have finally come of age. This approach to the study of biological ultrastructure has not only revealed the finite structure of TMV crystals and confirmed many results obtained by thin sectioning and other techniques, but has provided much visual information on membrane and other ultrastructural details unobtainable as yet by other approaches. Recent improvements have pushed the resolution of freeze-etching to the range of 1 nm, and permit the comparison of complementary faces of frozen fractured specimens.

"While doing the research leading to the publication of this paper, I had the rare pleasure of working under exceptional research administrators, whose main concerns were obtaining adequate funding for their programs, selecting qualified scientists, giving them the freedom to explore the unknown, and providing them with adequate protection from the 'publish or perish' philosophy, unnecessary 'busy work,' report writing, meetings, and other distractions."