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This Week's Citation Classic

Branton D. Fracture faces of frozen membranes.
Proc. Nat. Acad. Sci. US **55:** 1048-56. 1966.

Freeze-etching, a new method of preparing specimens for electron microscopic examination, splits membranes in the frozen specimen so as to expose inner membrane faces. Examination of these suggests that the organization of biological membranes is an extended bilayer interrupted by globular subunits. [The *SCI*® indicates that this paper was cited 468 times in the period 1966-1977.]

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"This paper was the product of my first effort as an independent scientist As a post-doctoral student I had worked with Kurt Mühlethaler and Hans Moor in Zurich, Switzerland. While I was in Switzerland, Hans had been developing freeze-etch methods that made it possible to prepare biological specimens for electron microscopy without subjecting them to the usual chemical fixatives and embedments required in other preparatory techniques Fascinated by the prospect of studying biological ultrastructure in as life-like a state as possible, my colleague, Roderick Park, and I purchased the first commercially available freeze-etch unit for use in our research.

"This sturdy first unit, coyly identified as serial number 101 by its manufacturer, and lovingly called 'Number One' by its users, launched Park and me into a series of studies whose results were far more exciting than any we had anticipated. (The *SCI*® indicates that the results obtained in 32 studies with Number One were cited 2,104 times in the period 1961-1975.Unfortunately, I cannot judge whether this makes Number One a Classic Instrument ISI® has not yet published an Equipment Citation Index.)

"From the start, it was apparent that freeze-etching frequently exposed vast expanses of a cell's membranes to inspection in the electron microscope.Hans Moor interpreted these expanses as the surfaces of the various membrane systems found in all cells, and this interpretation guided all of the investigators, including myself, who were then using freezeetch techniques. But gradually I became aware that this interpretation was based on a set of contradictory assumptions which could not be reconciled with the known surface properties of biological membranes This awareness put me on the alert for data that would suggest alternate interpretations of freeze-etching

"Late in 1963, while reexamining some old electron micrographs on onion root-tip cells, I discovered what had been staring me in the face all along. I saw that the fracture process used in freeze-etching was splitting biological membranes, which are only about 85Å thick, into two even thinner halves! In other words, what all of us had been interpreting as the surfaces of membranes were in fact fracture faces within the membrane itself.I was so astonished that I could not at first believe what was plain to see.

"Excitedly, I sent Hans Moor a letter and diagram telling him of my discovery.To my dismay he responded that he did not agree with my interpretation. I soon found that Hans was not the only skeptic. During the next several years, I found myself advancing arguments that few cell biologists accepted and which several leading journals rejected.Everyone continued to believe the interpretations advanced by Moor and his colleagues until, in 1968, the evidence amassed by Rod Park, myself, and our students led even the Swiss group to acknowledge that freeze-etching could split biological membranes.

"It is particularly gratifying to learn that an article which at first elicited only controversy has now been identified as a citation classic. I believe there are two reasons for its citation record. First, freeze-etching has become a routine method for preparing biological samples for electron microscopy. Although my article is not a 'methods' paper, it does define the utility of freeze-etching by indicating what other investigators can expect to see and not see when using this technique to investigate their own preparations.Second, my interpretation of freeze-etch results helped to distinguish between various models for the molecular organization of biological membranes While showing that the membrane continuum was composed of a bilayer, it explicitly denied the notion of a biological membrane that was spatially uniform.Thus, the correct interpretation of freeze-etch results advanced in this paper provided important evidence for our current notions of how biological membranes are organized. "