

## NanoSuit Method for the Observation of Living/Hydrous Organisms in an SEM

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Scanning electron microscopes (SEM) can generate high-resolution images of the nano-sized objects by shooting electrons at their targets and detecting how the electrons react. It is, however, necessary to evacuate the specimen chamber to a high vacuum level ( $10^{-3}$ - $10^{-6}$  Pa) to prevent scattering by molecules in the air. Since living organisms contain approximately 70 to 80% water, it has been thought necessary to pre-treat such biological specimens to achieve minimal tissue damage and maximal preservation of the structure for SEM observations.

We have reported that the polymerization of extra cellular substances on animals by the electron beam of the SEM or by plasma irradiation forms a nano-scale layer—termed the “NanoSuit”—which can act as a barrier and keep organisms alive and hydrous, almost like a miniature space suit, even in a field-emission scanning electron microscopy (FE-SEM) system. We have also examined the response of various plant specimens and shown that, when natural surface substances turn into the NanoSuit, the barrier ability increases. We have further improved the technique to maintain wet tissues freshly excised from intact organisms or cultured cells and found that the fine structure of the specimens protected with the NanoSuit is superior to that of conventionally prepared specimens. Moreover, we have previously examined the suitability of the NanoSuit method for the elemental analyses of biological specimens in FE-SEM. The specimens prepared by the conventional methods displayed additional elemental signals in the EDS data due to the chemicals involved in various pre-treatment processes, whereas the NanoSuit method accurately detected the elemental composition at high-resolutions. Our results demonstrate that the proposed NanoSuit method will be useful for studying hydrous samples using conventional SEM instruments. This concept will improve the depth of our understanding not only in bio-related fields but in a range of other scientific disciplines as well.