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SEM Visualization of Biological Samples using Hitachi Ionic Liquid HILEM® IL 1000: a Comparative Study.

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SEM observation of biological specimens requires a series of processing steps to render the specimen conductive for electron beam scanning, and resilient to vacuum conditions in the SEM specimen chamber. This generally implies fixation, gradual dehydration, drying with solvent or at critical point with liquid CO_2, followed by mounting and metal (Au/Pd/Pt) sputter-coating of the sample. In recent years Variable Pressure-SEM enabled imaging of hydrated specimens under extended pressures (50-300Pa), but with resolution limitations due to decreased SNR (signal to noise ratio) caused by gas and moisture, and availability of mainly backscattered electron detection (BSD) under these conditions. It has been discovered that ionic liquids can be observed by SEM without accumulation of electron charges, indicating that the liquid behaves as an electronically conducting material. Ionic liquids (which can be described as a salt that exists in the liquid state at room temperature) can therefore potentially be used to supply electronic conductivity to samples in place of carbon or metal coating, while insulating them [1]. It is suggested that biological samples coated with an ionic liquid do not require fixation, dehydration or metal coating because ionic liquids do not evaporate under vacuum conditions and are electrically conductive [2,3]. These recent studies showed excellent resolution and structural preservation in biofilms using ionic liquids and secondary electron imaging, both with and without aldehyde fixation.

Hitachi High-Technologies recently introduced their HILEM® IL 1000 ionic liquid for visualization of biological samples under high vacuum and using SE imaging without conventional fixation, drying or sputter-coating. We evaluated this novel technology to find the range of conditions that will be most appropriate for imaging of various biological samples in a minimalistic way. Among the parameters evaluated were: (a) vacuum conditions (b) accelerating voltage (c) detectors (d) aldehyde fixation (e) mounting substrate, and (f) concentration of ionic liquid.

We examined a variety of specimens, varying from multicellular plant tissue to microbial biofilms and single cells (Fig.1). Conductivity is provided through a range of accelerating voltages and at various concentrations of ionic liquid, with 10% IL and 5-10kV being optimal for SEM, 2-3kV for FESEM and 15kV for VP-SEM (Hitachi S3400N). Fixation in glutaraldehyde may help to preserve cellular structure in microbes and single cells, while residual liquid may limit observations of fine features and edges. Removal of most liquid through centrifugation, absorbent paper or vacuum filtering through microporous filters, followed by an hour of incubation in a desiccator, efficiently dries samples before imaging under high vacuum. Substrate conductivity as well as surface topography may limit imaging under higher accelerating voltage. Under high magnification residual liquid may show areas of beam interference as small bubbles or linear markings. Future research may help to alleviate some of these problems. Ionic Liquid applications provide a valuable new tool for educational purposes, and conditions where conventional drying and sputter-coating are not available or allowed by experimental requirements.

References:

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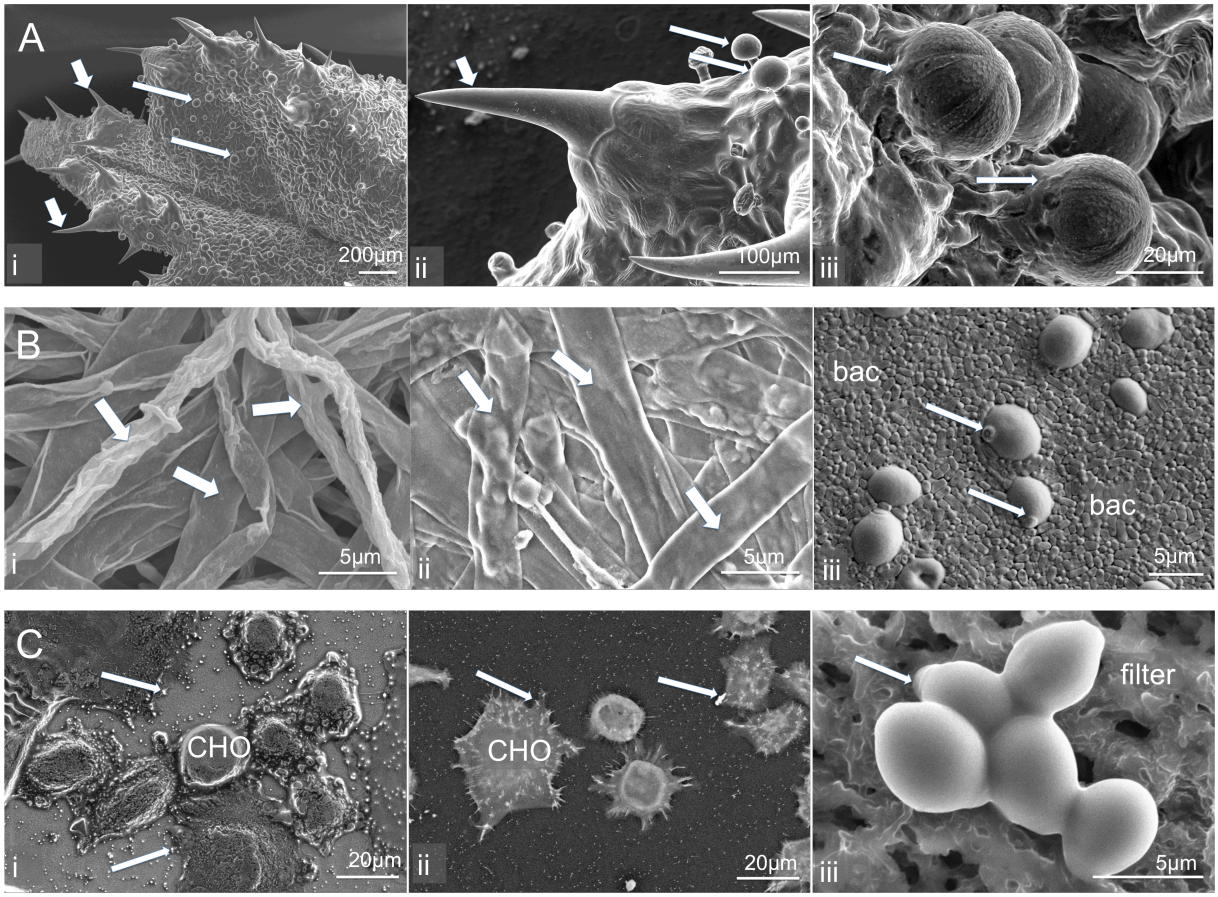

Figure 1: Specimens treated with 10% HILEM® IL 1000 ionic liquid, visualized with Hitachi S3400N at 5-10kV using Secondary Electron (SE) detection except for C(ii) where VP-SEM with BSD was used and B(iii) imaged with FESEM using SE detection at 3kV.
A: Plant material: (i-ii) *Pelargonium* leaf with glandular hairs (Long arrows) and spiny trichomes (short arrows), (iii) Pollen grains with evidence of residual ionic liquid (arrows),
B: Microbial biofilms (i-ii): *Aspergillus fumigatus* with hyphae (arrows) forming an interwoven network (i) conventional processing with CPD and sputter coating, (ii) treated with 10% IL, (iii) mixed culture yeast (*Saccharomyces cerevisiae)* and bacteria (bac) on agar. Budding yeast indicated with arrows.
C: Single cell applications: (i-ii) Chinese Hamster Ovary (CHO) cells on nanostraw (arrows) substrate (i) treated with IL, (ii) aldehyde-fixed and hydrated, using VP-SEM at 50Pa with cool-stage at -20C; (iii) *Saccharomyces cerevisiae* (arrows) on microporous filter.

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