



Supplemental Movie 1. Morphological changes of frozen-hydrated biofilm during gradual temperature increase captured by cryoSEM. Highly hydrated EPS and cells on the biofilms surface (-160 °C) get more pronounced as the temperature increases (-140 °C). EPS started contracting due to the water loss at sublimation, and cracks started to appear (-120 °C). Notice the stretched areas getting pulled apart, creating elongated features (-100 °C). The sudden overall ESP collapse occurred at around -90 °C, with more crevasses developing (-40 °C, lower image part). Finally, at -20°C, the material shrinkage due to the water loss caused massive cracks, and the imaged area was lost. Bright vertical area was caused by the electron charging on newly exposed underlying material. Time sequence of the gradual warm-up from -180 °C to -40 °C was 140 minutes.