Introduction: Understanding the effects of microorganisms on mineral alteration requires the ability to recognize the effects of bacteria-promoted dissolution on mineral surfaces. We have utilized atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) to investigate surfaces under bacteria colonies. In order to observe the effects of long-term growth of microbes on mineral surfaces, however, microbes must be removed from the surfaces without chemically or physically changing the surface. In previous work we removed bacteria from surfaces of hornblende glass and crystal with lysozyme, an enzyme that lyses bacterial cell walls. We thought lysozyme was adequate in removing bacteria based upon SEM analyses of the cleaned mineral surfaces [1]. However, when such surfaces were analyzed at nanoscale in this study by AFM, we observed residual biomatter. The hornblende glass was synthesized in order to prepare a chemically homogeneous and smooth surface similar to the iron-silicate mineral hornblende. Such smooth surfaces aid us in recognizing features attributable to bacteria.

Approach: In this study, monocultures of the soil bacterium Arthrobacter sp. were incubated for 13 and 77 days in an iron-deficient medium in the presence of hornblende glass planchets. Arthrobacter sp. is known to produce siderophores that enhance removal of Fe from hornblende [1]. No attempt was made to replenish glucose in these experiments. Removal of bacteria with CO₂ snow-cleaning (Applied Surface Technologies), and four detergents (sodium tetraborate, sodium pyrophosphate, sodium dodecyl sulfate (SDS), and Triton X-100) was tested.

Results: AFM observations revealed cellular residue on glass surfaces that underwent CO₂ snow-cleaning after 13 days of growth. In contrast, treatment with each of four detergents followed by acetone completely removed Arthrobacter sp. even after 77 days of growth. Furthermore, these treatments did not alter the topography of the surfaces as observed under AFM. To evaluate chemical alteration by the treatments, XPS was used to measure the chemistry of the upper ~25 Å of the glass surfaces. Preliminary evaluations by XPS suggest greater variation in surface chemistry of samples cleaned with sodium tetraborate and sodium pyrophosphate than with SDS and Triton X-100. However, an organic layer was detected on all surfaces, such that C comprised as much as 59.4 atomic% of the 25 Å analyzed. One sample was re-analyzed by XPS after most of the carbon was removed by ultraviolet ozone cleaning (UVOC). Small but significant differences in elemental abundances were observed before and after the UVOC treatment. Thus, the remaining samples will be cleaned by UVOC and re-analyzed. Three types of polished glass planchets were imaged under AFM after detergent cleaning: planchets that were untreated (blanks), planchets that were incubated for 77 days in medium (controls), and planchets that were incubated for 77 days in medium inoculated with bacteria (samples). AFM analyses revealed etch pits similar in size and distribution to small colonies of Arthrobacter sp. on the samples. Although occasional large pits (6 to 20 µm across and 200 to 400 nm deep) were observed on all surfaces (blanks, controls, and samples), only samples incubated for 77 days with Arthrobacter sp. contained frequent small pits (diameter = 300 to 4000 nm, depth = 30 to 170 nm). These smaller pits were irregularly shaped and concentrated along polishing scratches; they were not observed on samples that were incubated for 13 days, abiotic controls incubated for 13 or 77 days, or on blanks.

To determine the mechanisms of pitting and bacterial attachment and to develop a set of indicators for distinguishing bacteria-produced etch pits from those formed by abiotic processes, hornblende glass planchets were incubated with Arthrobacter sp. for 46 days with parallel abiotic experiments using desferrioxamine mesylate (DFAM, a commercially available siderophore) or oxalic acid instead of bacteria. In these experiments, glucose was replenished periodically. Weekly sampling and analysis of all sample solutions (with and without bacteria) revealed more release of Fe, Al, and Si in the DFAM experiments than the others, and negligible changes in pH. Preliminary AFM analyses confirm the presence of bacteria-related etch pits on sample surfaces, with comparable abundance and distribution as observed previously.

Discussion: We expect that further analysis of surfaces exposed to oxalic acid and DFAM will demonstrate that chelation of iron by siderophores rather than dissolution by organic acids is responsible for the etch pits. Additionally, the distribution of pits and strong bacterial attachment to the surfaces suggest that pitting is related to colonization; for example, the pits may be due to high concentrations of siderophores contained in glycocalyx. If these observations are confirmed, it may be the first documented case in which pitting is observed due to siderophore production.