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Using SEM Micrographs to Examine Hydrothermal Vent Samples

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Honors Senior Capstone Project

Using SEM Micrographs to Examine Hydrothermal Vent Samples

Ceona Koch

Objectives

I started an individual project within Professor Craig Moyer's microbiology lab with the purpose of exploring hydrothermal vent samples using scanning electron microscopy. Moyer has several organisms and core samples cryonically frozen from active sites all over the world, which have not been explored through SEM before. The objectives of the project included looking at three sample types (microbial mats, scale worms, and shrimp) and testing different SEM preparation methods. I focused on two aspects of the samples—their elemental composition and the presence (or absence) of bacteria. The ultimate product was a portfolio of images captured on the scanning electron microscope as well as figures that describe the elemental composition of the samples.

Hydrothermal Vents

Deep-sea vents form in volcanically active places—usually where tectonic plates are spreading. For example, geological hot spots like Hawaii and the Galapagos are very active. Hydrothermal vents release heated, nutrient-rich water into deep-sea habitats that are otherwise inhabitable for most marine organisms. They support very niche biological communities because they create specific chemical and physical conditions through the interactions between magma and seawater. Some of these organisms include chemosynthetic bacteria (the base of the food chain), tube worms, crabs, etc.

Sample Sites & Types

Scale worms are an annelid found across a variety of habitats, including deep-sea habitats. The samples in the Moyer lab were collected from the Lō'ihī Seamount, an active submarine volcano on the southern coast of Hawai'i. Lō'ihī may eventually form the next island on the Hawaii archipelago. The seamount is also where Craig Moyer and two other researchers discovered Zetaproteobacteria, the sixth and most recently described class of Proteobacteria. It is defined by a single species, *Mariprofundus ferroxydans*, which is an iron-oxidizing chemolithoautotroph (which means it gets its energy from chemical compounds). We thought it might be possible to identify *M. ferroxydans* on the scale worms or other samples.

I also looked at a species of shrimp from the Rota vent, a caldera on the Mariana Arc that is approximately 8 km wide and weakly active. The Rota vent is off the coast of Guam in the Philippine Sea.

The last samples were from a core of a microbial mat from Soda Bay near Craig, Alaska. Microbial mats are multi-layered sheets of bacteria and archaea that form where two materials meet (e.g. between magma/seafloor and seawater).

Scanning Electron Microscopy

Scanning electron microscopes produce images by scanning the surface of a sample with an electron beam. The electrons are shot from a 'gun' and focused through a series of condenser

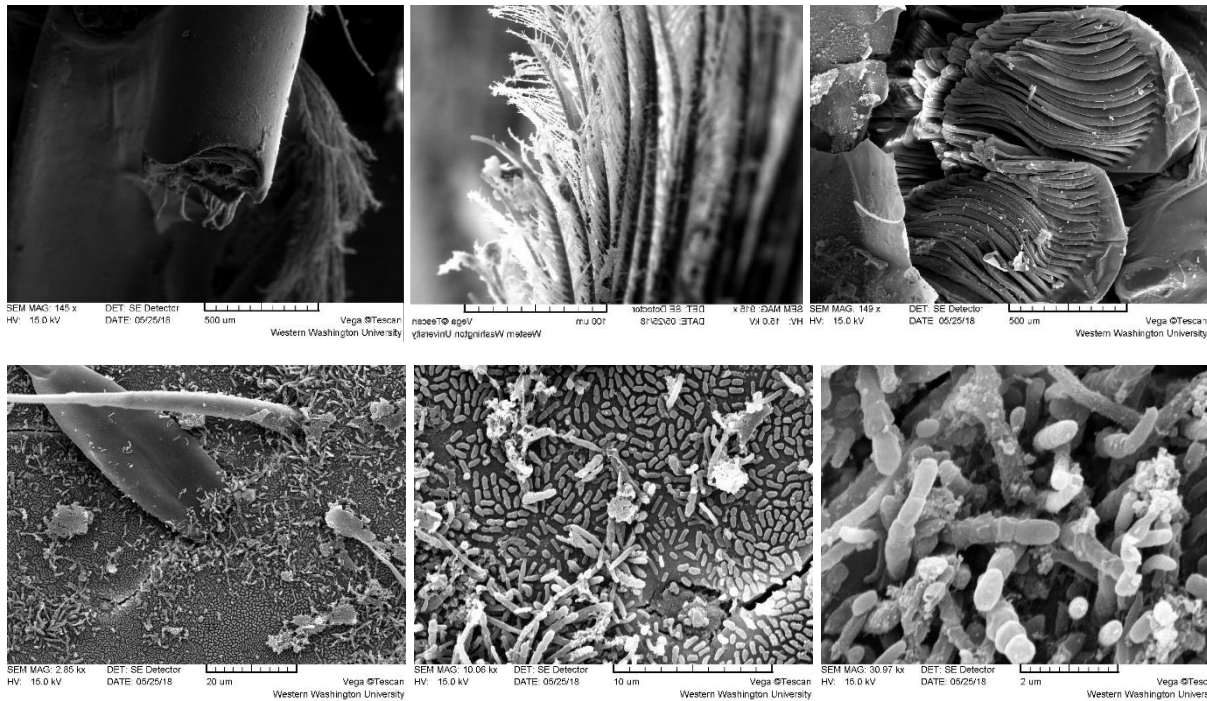
lenses onto the sample. The electrons interact with the atoms of the elements in the sample and the frequency and intensity with which they refract provide information about the topography and composition of the samples.

Sample Preparation

Traditionally, biological samples are preserved in a liquid solution and have to be dried completely before going into the SEM so that they don't shrivel under the heat and pressure from the electron beam. Both the shrimp and the worms were preserved in glutaraldehyde, a fairly toxic preservative. First, I took the samples through a series of ethanol washes of increasing concentration. This replaced all of the glutaraldehyde with ethanol, which is more miscible with liquid CO₂—the next step in the process. I closed the samples in the critical point dryer, which fills a small chamber with liquid CO₂ and raises the temperature and pressure until the CO₂ reaches a supercritical state (neither solid, liquid, nor gas) and is vented off, resulting in a completely dry sample. After mounting the samples on a small metal post, they are coated with gold-palladium, a conductive surface that protects the samples from heat. It also keeps them from charging, which is when electrons from the beam start to build up on the surface of the sample and result in bright white spots on the images.

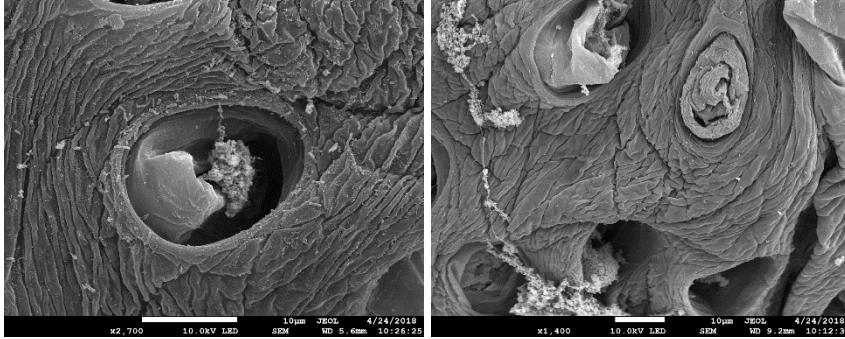
I looked at these samples with both microscopes that WWU has, the Tescan Tungsten and the Jeol Field Emission. The Jeol is the newer, more expensive microscope, which produces clearer images with less electrostatic distortion and is more user-friendly, though there are more steps to take to capture a well-composed image.

Tescan Images

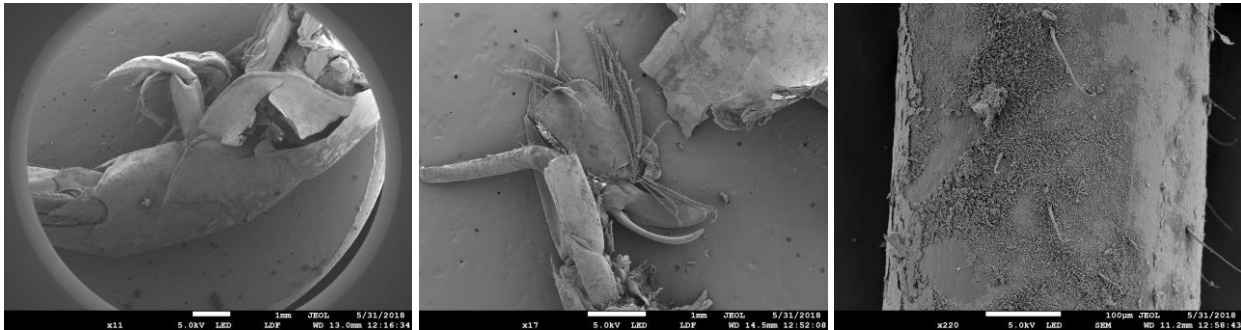


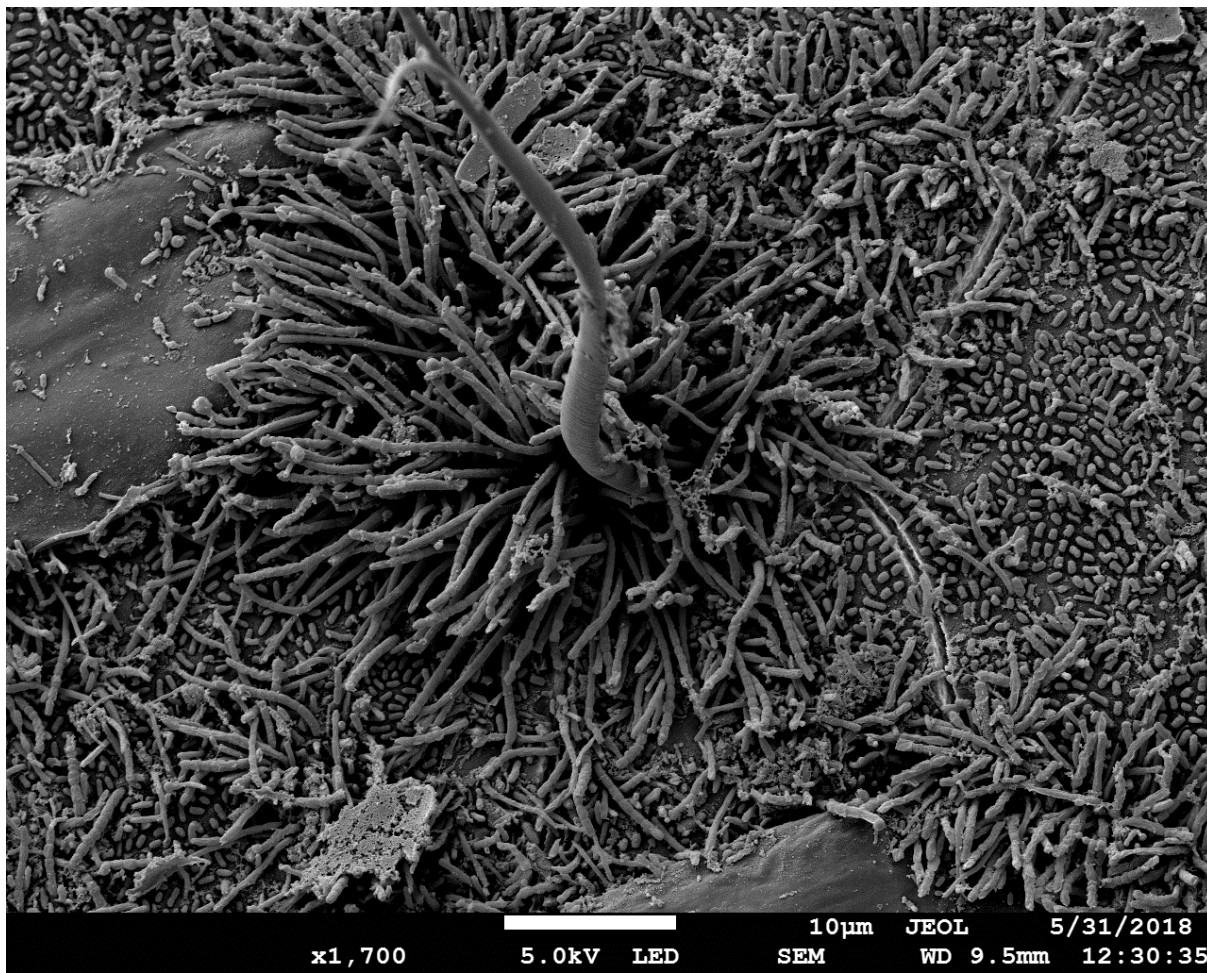
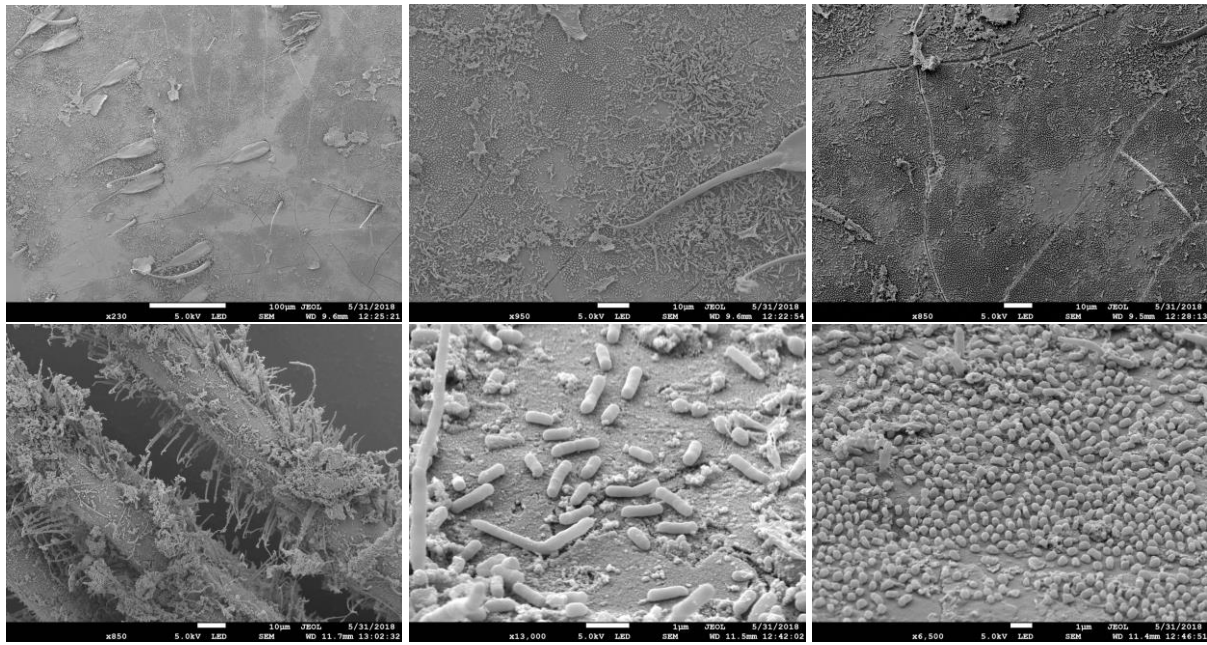
The top three images are distinctive parts of the shrimp samples—it's mouthparts, tail, and gills. The bottom three images show bacteria on the carapace of the shrimp in order of increasing magnification. The bacteria were most dense on the carapace but were also found on appendages and on the feather-like tail structures.

Jeol Images



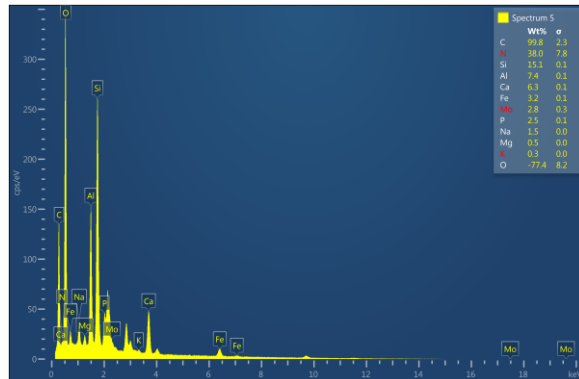
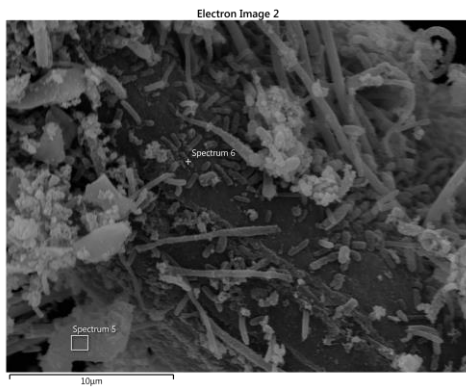
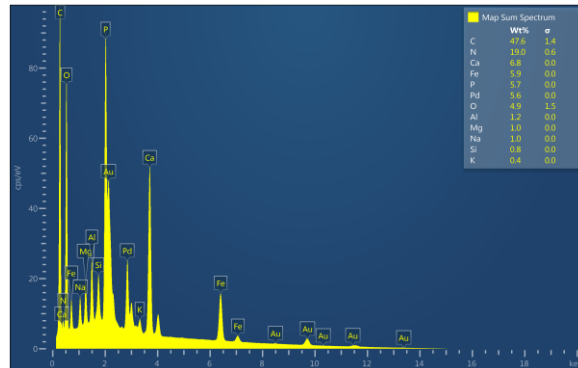
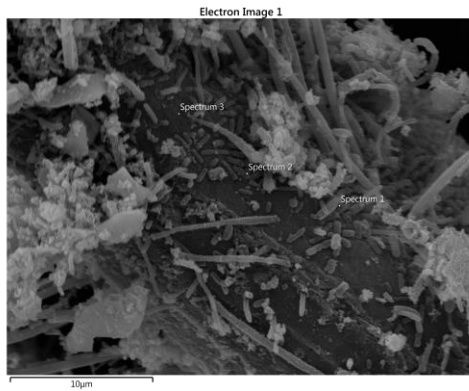
Other undergraduate students spent more time looking at the scale worms than I did, but never encountered identifiable bacteria. They still have a complex surface morphology that was interesting to look at with SEM.

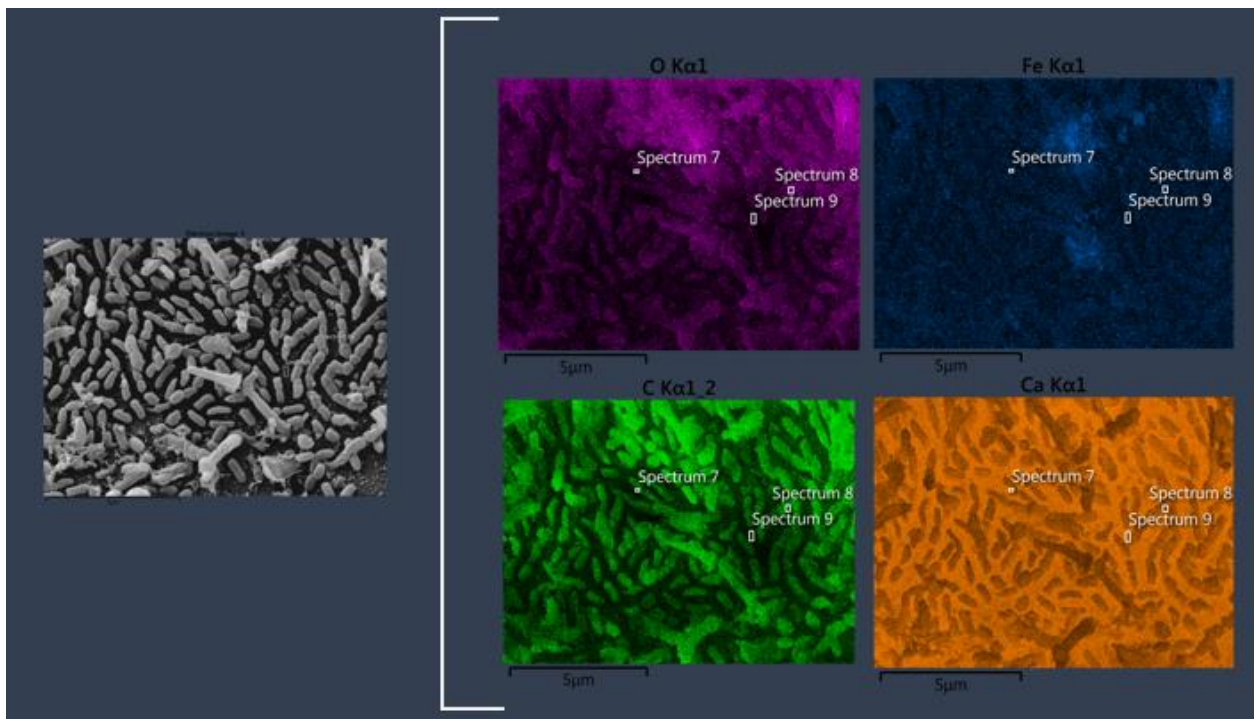
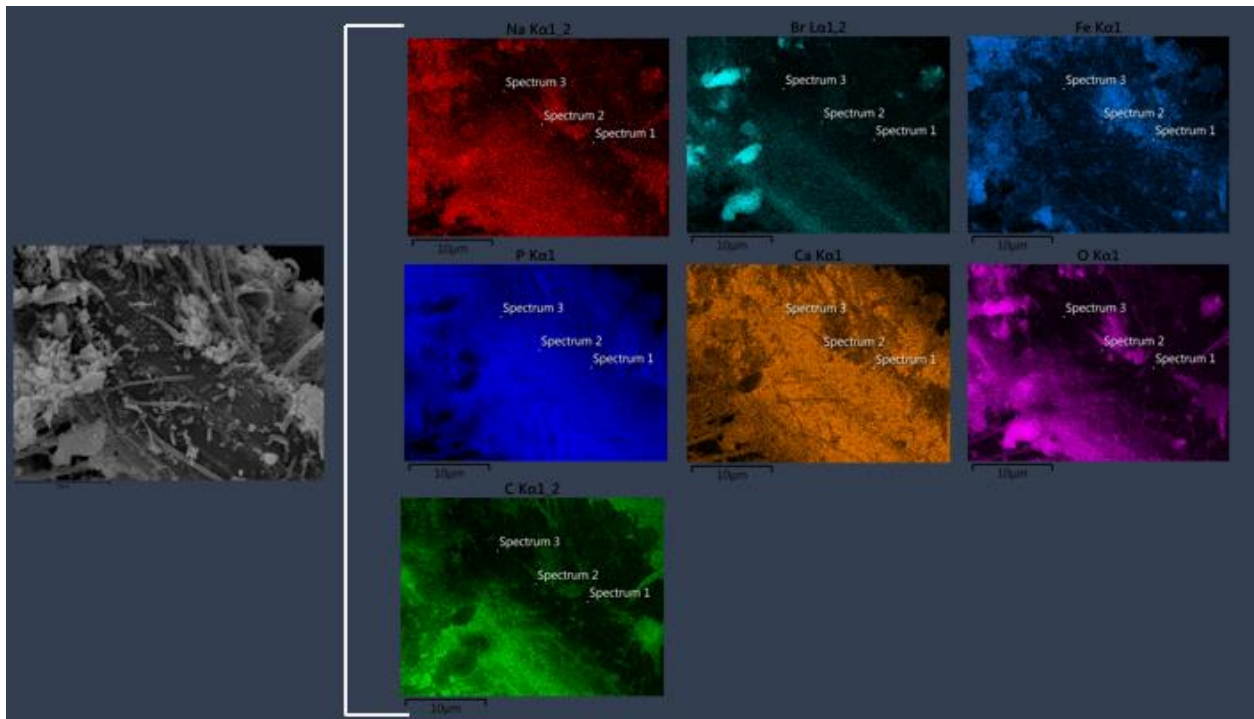




Element Composition

I also used energy dispersive X-ray spectroscopy (EDS) to look at the elemental composition of the shrimp samples. The EDS machine is attached to the SEM and focuses an x-ray beam on the surface of the sample. Similar to the electron beam, the number and energy of the x-rays emitted are measured by a spectrometer and the energy differences indicate which elements are present. It then generates graphs and maps on the computer.



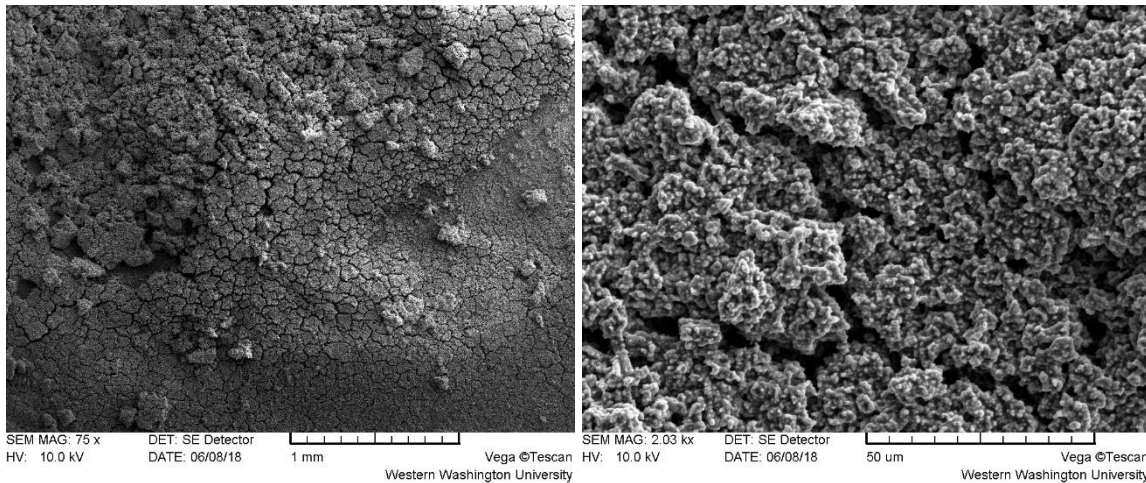


New SEM Method

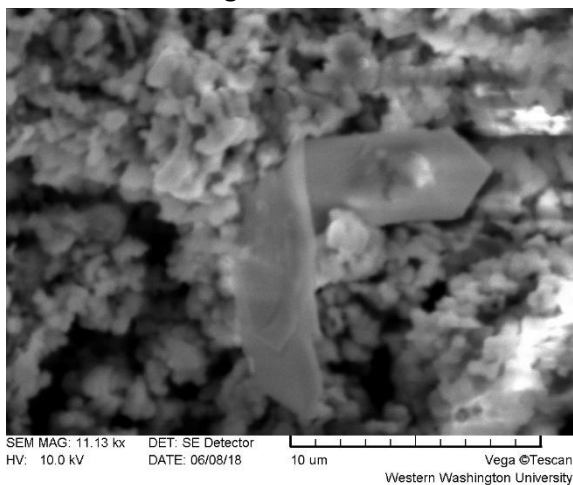
I was working with Dan Carnevale from WWU SciTech on both microscopes and he found a 2016 paper that had tested a new sample preparation method on bacteria and viruses. We decided to

try this method with the microbial mat samples as it was likely the most similar to the samples used by the authors of the paper. Using this method, instead of coating the sample with gold-palladium, we first coated small rounds of filter paper. These fit into syringes through which we filtered the liquid part of the microbial mat samples. We cut the filter paper (where the sediment from the liquid settled) to fit the stub and posted the sample. Immediately, we added a few drops of an ionic liquid (1-Butyl-3-methylimidazolium), blotted it off and then inserted the sample into the SEM. The ionic liquid has a very high vapor pressure, so it doesn't volatilize, and it also acts as a substitute for the Au/Pd coating because it is a conductive surface. This method is much faster—less than an hour compared to up to 24.

Ionic liquid Images (Tescan)



In these two images you can't distinguish any clearly visible bacteria. Though the method worked well and the images are good (just slightly blurrier than the traditional method), this sample was not filtered enough and all we could see was sediment/minerals.



We used EDS to identify this crystal as barite. Barite is interesting and somewhat uncommon because it requires the direct mixing of barium-rich hydrothermal fluid with sulfate-rich

seawater. It has a low solubility, which allows barite crystals to preserve geochemical footprints. This essentially provides information about the conditions of formation.

Moving Forward

As other students in Moyer's lab continue their research and as Dan works to improve the ionic liquid methods, it will take time to figure out how the microbial mat samples should be processed prior to any sort of SEM preparation. It will likely need to be filtered several times with varying pore-sizes so that the bacteria are isolated from the sample.

Further research will also be necessary to identify what species of bacteria we may have found, if possible.

Acknowledgements

I am thankful for the opportunity to join Craig Moyer's lab if even for a short time, and I am so appreciative of all the time Dan Carnevale put into working with me—countless hours training on the microscopes, using them to capture images, and testing new methods. Charles Wandler (another SciTech staff member) gave me my first to SEM trainings and gave me the basic skills. Also, I want to thank the honors program for the opportunity to embark on this little passion project and be able to present some interesting results.