ORGANIC MATTER IMAGING IN A MARS-ANALOG GLACIAL ENVIRONMENT USING SOLID-PHASE EXCITATION EMISSION MATRIX SPECTROSCOPY. M. C. Willis[1,2], N. Krysiak[1], E. J. Eshelman[1,3], H. J. Smith[1,4], K. Mueller[3] and C. M. Foreman[2,5]. [1]Montana State University, Center for Biofilm Engineering, Bozeman, Montana (madie.willis@gmail.com), [2]Montana State University, Department of Land Resources and Environmental Science, Bozeman, Montana, [3]Impossible Sensing, St. Louis, Missouri, [4]Montana State University, Department of Microbiology and Immunology, Bozeman, Montana, [5]Montana State University, Department of Chemical and Biological Engineering, Bozeman, Montana.

Introduction: Orbital data has revealed that mid-latitude and polar ice on Mars may be layered with varied amounts of clay, dust, or rock [1,2]. On Earth, sediment and other debris in glacial systems have been correlated with increased cell numbers and microbial activity [3]. Debris-rich features such as cryoconite holes are hotspots for glacial microbial communities, acting as refuges for life in otherwise inhospitable environments. Natural organic matter (OM) is a critical carbon source for these organisms and can be produced in situ as a byproduct of metabolic processes or delivered via aeolian deposition from both local and transcontinental terrestrial sources [4].

Excitation emission matrix spectroscopy (EEMS) is a widely used tool for discerning the reactivity of dissolved OM in aqueous samples and can be used to determine whether OM has a microbial origin [5]. Techniques for determining the biogenicity of OM may have applications for future Mars missions with astrobiology science objectives. Here we explored the ability of a novel method, solid-phase excitation emission matrix spectroscopy (SEEMS), to adapt the established EEMS method for the analysis of solid-phase samples without disrupting the natural spatial distribution of OM. This standoff method was used to image OM in lab-synthesized and natural samples, then further tested in a field deployment to an icy Mars analog in Juneau, Alaska.

Methods: *Lab-based method validation:* Organic standards including tryptophan, tyrosine, Suwannee River Natural Organic Matter (SRNOM), Pony Lake Fulvic Acid (PLFA), and others relevant to EEMS and glacial environments were dissolved in aqueous solution in differing concentrations and mixtures. Fluorescence peak components were determined by EEMS collected using a Horiba Fluoromax4 spectrometer (excitation 280nm - 520nm and emission 300nm - 450nm). UV/vis spectra were also collected and used to perform an inner-filter correction for each standard. The standard solutions were then pipetted in 8uL droplets onto a nonfluorescent substrate and imaged using the SEEMS instrument designed by Impossible Sensing in St. Louis, Missouri. The SEEMS instrument excites the sample using 4 LEDs (255nm, 310nm, 365nm, and 510nm) and employs a unique filter array to simultaneously collect emission images from 9 channels. The excitation and emission wavelengths were selected based on the peaks and fluorescence indices for natural OM already established in the EEMS literature [6,7]. Each sample was imaged 50 times, averaged to increase signal, and corrected for background, LED power, and quantum efficiency. A crosstalk correction was then performed to remove ghost pixels from each channel attributed to crosstalk from neighboring bands. The fluorescence data from EEMS and SEEMS were compared for each standard.

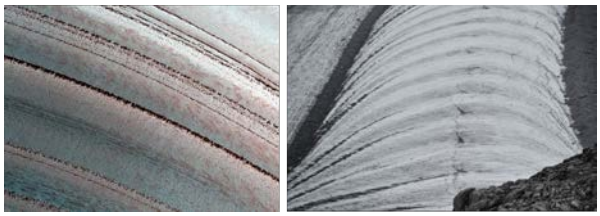

Figure 1. On the left, an image of a 1km wide swath of the northern polar layered deposits on Mars captured by HiRISE (credit: NASA/JPL/UArizona). On the right, ogive banding (~100-meter scale) on the Gilkey glacier (credit: Joel Wilner, the JIRP blog).

Environmental Analog: In July 2022 we conducted an 8-day field campaign to the Gilkey glacier in Juneau, Alaska. The Gilkey glacier is an outlet glacier of the Juneau icefield and was selected for its unique parabolic surface features known as ogives (Fig. 1). These dark and light bands create gradations of sediment on the glacier surface ideal for investigating the impact of varied debris-loading on the habitability of glacier ice. SEEMS images and samples were collected from four environments: cryoconite, supraglacial stream water, and shallow (~1m) ice cores from sediment-rich and sediment-free ice. Samples were returned to MSU for EEMS analysis and imaging with confocal scanning laser microscopy to confirm the presence of OM and microbial cells.

Results: EEMS matrices and fluorescence index values of the organic solutions matched similar data found in the literature for these standards. As expected, EEMS of the amino acids tryptophan and tyrosine

indicated protein-like fluorescence and had high biological (>1) and fluorescence index (~1.7) values. When identified in natural samples these values are indicative of OM with a recent, microbial origin (ex: the extracellular leachate of proteins from bacteria and algae). Conversely, the fluorescence components in the SRNOM and PLFA standards were indicative of humic-like fluorescence often associated with larger molecular weight organic compounds such as degraded plant matter and polycyclic aromatic hydrocarbons (PAHs).

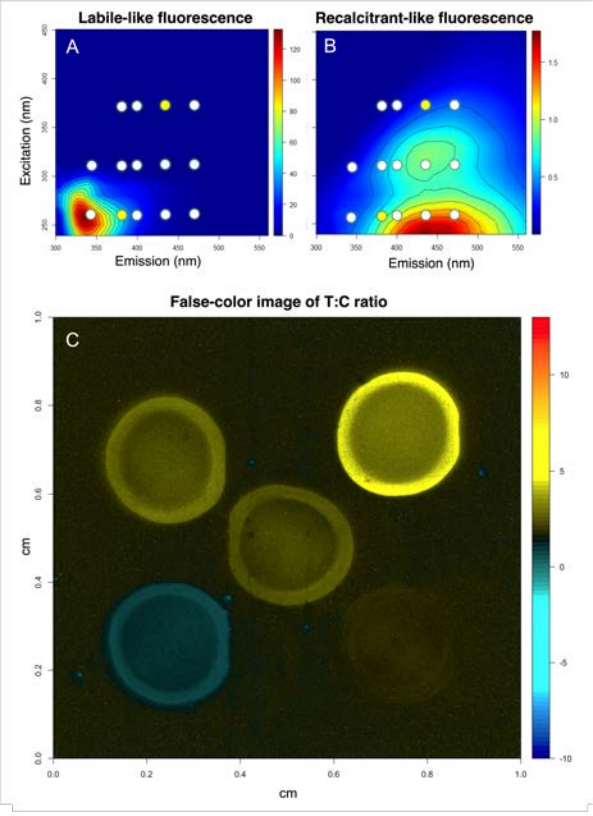

Figure 2. Representive EEMs of tryptophan **(A)** and SRNOM **(B)**. Circles indicate coordinates of ex/em wavelengths imaged by SEEMS. **(C)** SEEMS image showing droplets containing 3:1 tryptophan:SRNOM, 100% tryptophan, 1:1 tryptophan:SRNOM, 100% SRNOM, and 1:3 SRNOM:tryptophan.

SEEMS images of droplets with equal carbon concentrations, but differing ratios of protein-like vs humic-like compounds, showed variation in the fluorescence components within the same image (Fig. 2). This was successful for peak intensity ratio T:C, for which a higher value indicates a more labile-like vs recalcitrant-like substance. However, due to low power from the 310nm LED and attenuated signal from emission wavelengths below 380nm, we were unable to image differences in the humification, fluorescence, and biological indices. SEEMS images collected during the field deployment also showed variation in OM quality across the surface of glacial samples. An example is shown in figure 3.

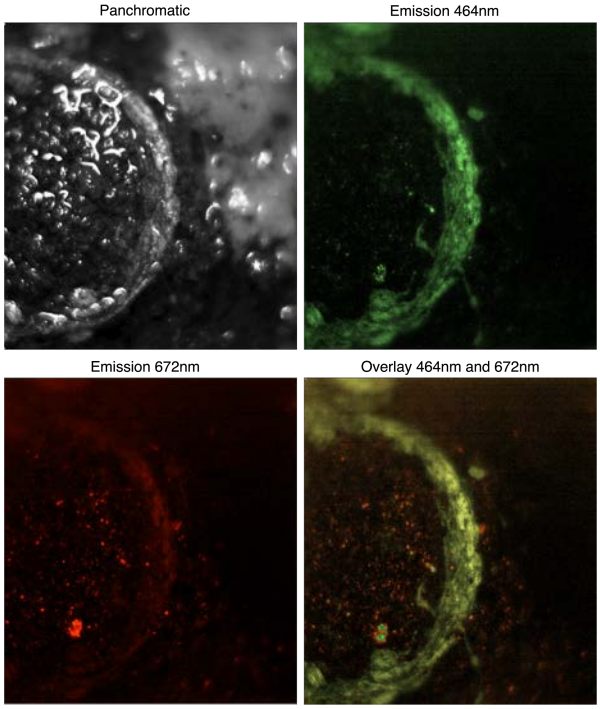

Figure 3. Black and white visible image of biofilms on cryoconite surface and corresponding fluorescence channels collected with 365nm excitation.

Conclusions: SEEMS can differentiate and image the spatial distribution of OM quality across a sample surface. However, further development of the technique and instrumentation is needed to extend the capability further into the UV to gain more information about the protein-like fraction of OM, and to be able to correlate SEEMS data to the EEMS fluorescence indices used to determine OM reactivity and origin.

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