National Aeronautics and Space Administration

FY23 Topic Areas Research and Technology Development (TRTD)

Microbial Pigments and Their Degradation Products as Biosignatures

Principal Investigator: Michael Malaska (322)**; Co-Investigators:** Sunanda Sharma (322), Edith Fayolle (322), Tuan Vu (322); **External Co-Investigator**: Carina Lee (Texas State, Jacobs, NASA Johnson)

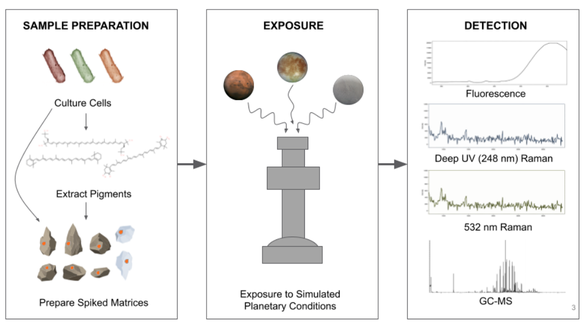
Strategic Focus Area: Preparing for returned sample investigations

Objectives: Our objective is to investigate the detectability of pigments, a class of molecules that have diagnostic structural features considered to have high biosignature potential for detecting microbial life on other planets, with different spectroscopy methods to inform future instrument selection for astrobiology-focused missions. Specifically, we aim to:

1) Evaluate the likelihood that unique spectral properties of carotenoid pigments, or their refractory byproducts, would be preserved and detectable on a remote planetary surface by exposing the pigments to simulated conditions for Mars, Europa, and Enceladus.
2) Demonstrate that current mission instruments (Fluorescence, deep UV/visible Raman spectrometers, GC-MS) could be able to detect these compounds to identify high-priority samples for possible return.
3) Determine limits of detection and ability to identify the biogenic carotenoid marker and its characteristic breakdown products, to provide a potentially more feasible approach for biosignature detection.

Approach and Results: The aim for FY23 was to establish setups, create and validate protocols, and acquire baseline measurements.

1. *Culturing model organisms:* We established stable cultures of two extremophiles relevant to Mars and Ocean Worlds: *Chroococcidiopsis cubana,* a salt-, desiccation-, and ionizing radiation-tolerant cyanobacterium that produces chlorophylls and carotenoids; and *Halobacterium salinarum*, a halophilic archaeon that produces carotenoids and is tolerant to UV, ionizing radiation, and desiccation.
2. *Pigment characterization:* We performed initial comparisons with three carotenoids using 248 (SHERLOC analog), 532, and 633 nm excitation Raman spectroscopy and DUV fluorescence. Our results were surprising - all the carotenoids had different peaks in DUV vs. visible Raman. This is underreported in literature, a majority of which uses visible Raman and includes UV-Vis absorption data above >300nm. We hypothesized that there may be a different resonance enhancement effect possibly related to the end groups. We ruled out UV damage, contamination, solvent effects, instrument miscalibration, etc. We presented results at conferences [B, C] and received suggestions to consider two other references (add citations) and use model compounds to test our hypothesis. We used UV-Vis spectrophotometry to confirm that the pigments and model compounds have a <300nm absorption peak responsible for the different enhancements in DUV vs. visible Raman.
3. *Preparation of matrices:* We designed and sent our tabs (that will hold the samples in the chambers) for fabrication; they have been received. In our initial proposal, we narrowed our choices to gypsum, calcite, olivine, halite, and montmorillonite for matrix materials. However, after initial testing, we realized that we could not make a thin enough mineral layer that is easily attached to the surface of the sample tabs. After a thorough literature search, we switched our selections to sodium sulfate, sodium (bi)carbonate, and halite, all of which are soluble and can easily be spiked with organics.
4. *Set up of environmental chambers:* We sourced the needed parts to complete these setups. For Mars, we are using the environmental chamber with a CO_2-rich atmosphere and UV radiation simulating the solar spectrum via an Ar mini-arc lamp, under 5 torr pressure at 200K. For Enceladus, we will use UV flux simulating the solar spectrum under vacuum at 60K. For Europa, we will use high-energy electrons under vacuum at 100K.
5. *Exposure of spiked mineral and ice samples to planetary surface conditions:* The exposure of samples is planned for FY24.
6. *Detection with Raman, fluorescence spectroscopy, and GC-MS:* This is planned for FY24, after sample exposure.



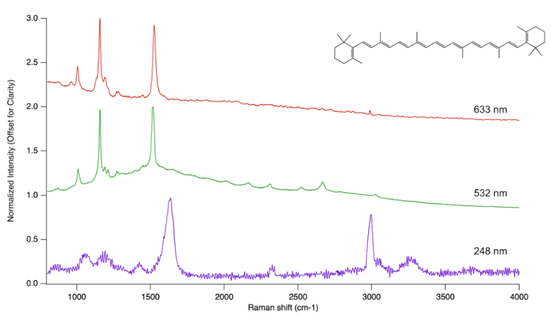


Figure 2. Beta-carotene in dichloromethane evaluated by 633nm, 532 nm, and 248nm Raman spectroscopy. All data are normalized and offset for clarity.

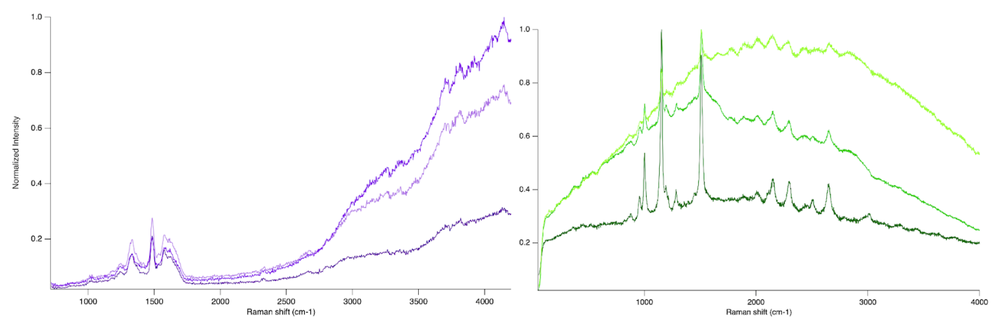


Figure 5. Comparison of different preparations of pigmented H. salinarum cells under deep UV (left) versus 532 nm (right) Raman. In each graph, the top spectrum is a drop taken from the liquid culture with media, the middle is pelleted and washed cells from liquid culture, and the bottom is a solid culture grown on agar.

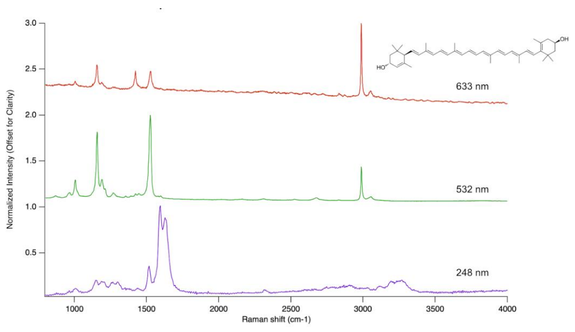


Figure 3. Lutein (solid) evaluated by 633nm, 532 nm, and 248nm Raman spectroscopy. All data are normalized and offset for clarity.

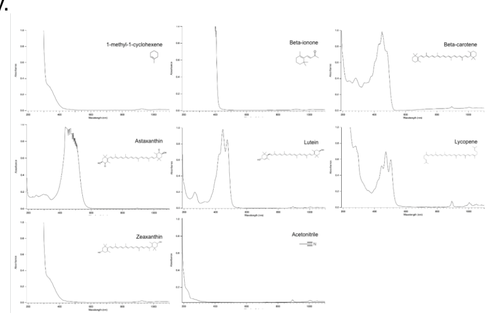


Figure 4. UV-Vis absorption of all pigments and model compounds, as well as solvent (acetonitrile).

Significance/Benefits to JPL and NASA: Our major finding is that carotenoids yield distinct Raman spectra under DUV vs. visible excitation due to different pre-resonant enhancement. We address a comparative dearth in the literature on DUV Raman assignments and mechanistic understanding of organics. Our findings could be especially applicable to diagenetically altered molecules that can retain end groups or are aromatized, underscoring the premise that using two different wavelengths enables observation of molecules that could be associated with both extant and extinct life. In FY23, we addressed our detectability-focused objectives and paired absorption and Raman spectroscopy to understand the mechanism underlying the spectral differences. We are now well-placed to perform the environmental exposures and analysis in FY24. As JPL is a leader in Raman and fluorescence spectroscopy for astrobiology/planetary exploration, it is critical that we advance scientific understanding and mission-centric application in this area. Raman spectroscopy and DUV fluorescence have been explicitly mentioned by the Mars Sample Return Planning Group 2 and for proposed missions. Our data augment the existing JPL-led spectral libraries relevant for future instrument development and our study of damage, alteration, and appropriate parameters for organic analysis is highly relevant for sample return science.

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Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California

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Publications:

[A] Sunanda Sharma, Tuan Vu, Edith Fayolle, Carina H. Lee, Michael Malaska, Jessica Weber. “Deep Ultraviolet Raman Spectroscopy of Carotenoid Pigments: Implications for Biosignature Detection,” *In preparation*, 2023.
[B] Sunanda Sharma, Carina Lee, Michael Malaska, Edith Fayolle, Tuan Vu, Rohit Bhartia, “Detecting Pigments As Potential Biosignatures With Deep UV Raman Spectroscopy,” *Lunar and Planetary Science Conference*, #2666. Houston, TX 2023.
[C] Sunanda Sharma, Carina Lee, Michael Malaska, Rohit Bhartia, “Detecting Pigments with Deep UV Raman and Fluorescence Spectroscopy,” *Gordon Research Conference Geobiology*. Ventura, CA 2022.

PI/Task Mgr. Contact Information:

8183547652 / Michael.j.malaska@jpl.nasa.gov