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DISTINGUISHING LIVING AND NON-LIVING SURFACE DEPOSITION ON
SERPTENTINE: IMPLICATIONS FOR BIOSIGNATURES AND ASTROBIOLOGY

BY

ALEXANDER SOUSA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE REQUIREMENTS FOR A MASTER OF SCIENCE

IN

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MASTER OF SCIENCE IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
OF

ALEXANDER SOUSA

APPROVED:

Thesis Committee:

Major Professor

Dawn Cardace

Vinka Oyanadel-Craver

Bethany Jenkins

Nasser H. Zawia

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

2019

ABSTRACT

Serpentinizing systems host long-lived reactions in which an ultramafic (or mafic) rock package, rich in reduced species, is progressively metamorphosed via hydration and oxidation into a serpentine-dominated mineral assemblage. On Earth, this process drives redox gradients in related water-rock systems: hydrogen gas is produced, reduced minerals are transformed to oxidized counterparts, and hydrocarbon chains (with potential to fuel chemosynthetic microbes) are released. The life sustaining capacity and abundance of serpentinite on/in silicate planetary bodies make such systems astrobiologically intriguing.

The Mars 2020 rover, equipped with a DUV Raman, is likely to explore serpentinite deposits (exposed selectively at crater rim sites) during its time in Jezero crater.

However, very few studies have explored the efficacy of spectroscopic methods in detecting biosignatures in the challenging context of serpentinites. In this study, Raman and FTIR spectroscopy are used to analyze serpentine minerals after time series incubations in organic solutions with and without an active microbial culture, to determine whether sterile and inoculated (live) organic-rich solutions leave different micro-scale organic signatures. We also tested the impact of dessication on organic films on the mineral surfaces. We find that fluorescence presents significant challenges to detailed analysis. However, changes in spectral character as a function of time and the presence of life are evident. The association of organic material with all samples is rapid, evidenced by quick changes to spectra in the form of fluorescence, aliphatic (simple C-H bonding) peaks from 2800 to 3000 cm^{-1} , amine and amide peaks

above 3000 cm^{-1} detected via Raman spectroscopy, and subtle changes in FTIR spectra. Data interpretation relied on ratioing of peak heights of CH_3 and CH_2 groups ($R_{3/2}$) and principal component analysis (PCA)s were performed but the true strength of these analyses remains in the combination of Raman and FTIR spectroscopic techniques, which produce complimentary data streams and enable the differentiation of all 5 experimental groups (pre-incubation, live incubation, sterile incubation, live desiccated, and sterile desiccated)

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PREFACE

This thesis has been formatted in accordance with the “Manuscript Format” described by the University of Rhode Island’s graduate school and author guidelines for the journal “Astrobiology”. It will be submitted for review promptly after its defense.

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CHAPTER 1

Spectroscopic Investigations of Organo-Mineral and Microbe-Mineral Interactions in Serpentinites.

For submission to Astrobiology

Alexander Sousa^{1*} and Dawn Cardace¹

1. University of Rhode Island, Department of Geosciences, 9 E. Alumni Avenue, Kingston, RI 02881
Telephone: 401-874-9384 Fax: 401-874-2190
Email: cardace@uri.edu
*corresponding author

Abstract

Serpentinizing systems host long-lived reactions in which an ultramafic (or mafic) rock package, rich in reduced species, is progressively metamorphosed via hydration and oxidation into a serpentine-dominated mineral assemblage. On Earth, this process drives redox gradients in related water-rock systems: hydrogen gas is produced, reduced minerals are transformed to oxidized counterparts, and hydrocarbon chains (with potential to fuel chemosynthetic microbes) are released. The life sustaining capacity and abundance of serpentinite on/in silicate planetary bodies make such systems astrobiologically intriguing.

The Mars 2020 rover, equipped with a DUV Raman, is likely to explore serpentinite deposits (exposed selectively at crater rim sites) during its time in Jezero crater.

However, very few studies have explored the efficacy of spectroscopic methods in detecting biosignatures in the challenging context of serpentinites. In this study, Raman and FTIR spectroscopy are used to analyze serpentine minerals after time series incubations in organic-bearing aqueous solutions with and without an active microbial culture, to determine whether sterile and live organic-rich solutions leave different micro-scale organic signatures. We find that fluorescence presents significant challenges to detailed analysis via Raman spectroscopy applied as in this study.

However, changes in spectral character as a function of time and the presence of life are evident. The association of organic material with all samples is rapid, evidenced by fluorescence signals above 3000 cm^{-1} , aliphatic (simple C-H bonding) peaks from $2800\text{ to }3000\text{ cm}^{-1}$ detected via Raman spectroscopy and changing amide III / ester ratios detected via FTIR. We also note differences in CH_2/CH_3 ratios and nitrogenous

to carbonaceous bond signal ratios over time. These findings have important implications for the planetary exploration of serpentinized locales and reveal critical areas for further investigation.

1. Introduction

1.1 Geology and Geochemistry

Serpentinization is the hydration of ultramafic and mafic lithologies to produce serpentine group minerals, molecular hydrogen, and, in the presence of DIC, low-weight hydrocarbons (Oze and Sharma, 2005; Mccollom and Bach, 2008). The following reactions from McCollom (2016) summarize the process of serpentinization and the production of abiotic methane:

1. Olivine $\pm$ orthopyroxene + H₂O $\rightarrow$ serpentine $\pm$ brucite + magnetite + H₂.
2. CO₂ + 4H₂ $\rightarrow$ CH₄ + 4H₂O

Serpentinization is an exothermic, volume-positive, and progressively fracturing process leading to a limited self-catalysis (Russell *et al.*, 2014).

It remains difficult to constrain the absolute degree to which serpentinizing systems pervade Earth. However, as the entire seafloor is underlain by peridotite, serpentinizing systems certainly comprise an important component of our planetary geology.

Because much of this activity takes place kilometers beneath the ocean floor, our knowledge of serpentinizing systems is mainly derived from the study of drill cores, fluids escaping related hydrothermal vent systems (Boetius, 2005), and terrestrial serpentinization typically represented by ophiolite sequences wherein portions of the oceanic plates are emplaced onto land. These ophiolitic rock packages continue to evolve geochemically as a result of disequilibria between component minerals and interactions with surface oxygen and a combination of meteoric or entrapped ground waters. Our samples are from an ophiolitic mélange terrain sited in northern California

known as the Coast Range Ophiolite, where surface exposures and rock cores of variable altered serpentinite are available (Cardace et al., 2013).

Evidence suggests that ultramafic lithology, and in turn serpentinites, are relatively frequent in or on planetary surfaces: Olivine, $(\text{Mg,Fe})_2\text{SiO}_4$, is likely present on many rocky silicate planets, dwarf planets, and even moons. Olivine readily reacts with water (supplied by primary planetary accretion processes or added via comets etc. and heat (supplied by planetary hydrothermalism due to magmatism or impacts may speed serpentization reactions (Müntener, 2010; Etiope *et al.*, 2013; Stüeken *et al.*, 2013). On Mars in particular, observations indicate a strong possibility of subsurface water (Robert, 2001) and serpentinites have been detected from orbit (Ehlmann, Mustard and Murchie, 2010; Greenberger *et al.*, 2015). Moons like Europa and Enceladus are also of high interest in light of their active cryovolcanism and the detection of hydrogen and silica in a geyser plume of Enceladus—leading to multiple suggestions of serpentization at work at the ocean/silicate interface hidden beneath the icy surficial crust (Irwin and Schulze-Makuch, 2001; Vance, 2009; Waite *et al.*, 2017).

1.2 Microbial metabolisms and astrobiology

Single celled organisms are thought to have dominated life on Earth from ~3.7Ga until about 600 mya, about the time when complex life evolved. Given the duration during which Earth's life was essentially microbial, one might expect that extraterrestrial life, if detected, would likely be microbial. However, the mild conditions and abundant chemical resources of Earth are unique. Microbial metabolisms are diverse and appear to exploit unusual biogeochemical niches. Some researchers have even had

success in predicting microbial metabolisms by searching environments for kinetically inhibited chemical disequilibria (Rowe *et al.*, 2017). Hydrogen can fuel extreme life in diverse planetary settings: it provides an important reductant, which may directly power metabolisms (Lin *et al.*, 2005; Brazelton *et al.*, 2013), it is produced by a variety of geochemical and even nuclear reactions (Blair *et al.*, 2007), and it can react with dissolved CO to produce simple hydrocarbons/aromatic compounds via Fischer-Tropsch-Type (FTT) synthesis (Proskurowski *et al.*, 2008). Serpentinized units, and especially those of *mélange* terrains, provide complex chemical landscapes experiencing chemical disequilibria; in the Coast Range Ophiolite (source of serpentinite samples in this study), iron partitions into at least three mineral phases in different amounts and crystallographic sites, representing changing rock-water interactions as the unit evolved through time. The associated bioenergetic dynamics must have driven changing community structures through time also. Indeed, studies have detected life in serpentinization-influenced hydrological flow regimes the world over (Brazelton *et al.*, 2006, 2012, 2013; Tiago and Veríssimo, 2013a; Rempfert *et al.*, 2017; Twing *et al.*, 2017).

Life in serpentinizing systems faces challenges. Dissolved inorganic carbon can be abiotically mineralized in carbonate minerals, ambient chemistry can be extremely basic, swings in oxidation reduction potential (redox fronts) can stress microbial communities (Schrenk, Brazelton and Lang, 2013). Although these extreme conditions can suppress microbial diversity, serpentinizing systems have shown chemical variability in accordance with terrestrial or oceanic setting, ambient temperatures, water-rock ratios, and aqueous and geochemical regimes: microbial community

compositions demonstrate correlative variability (Schrenk, Brazelton and Lang, 2013). Some microbes are present in many serpentinizing systems. Some systems reveal unique microbial consortia, with novel strains described or swathes of un-identified genetic material (Boetius, 2005; Schrenk, Brazelton and Lang, 2013; Tiago and Veríssimo, 2013b; Meyer-Dombard *et al.*, 2018).

Despite the challenges of existence in these systems, serpentinites may offer refugia for life on otherwise hostile worlds, perhaps through heat generation (to keep temperatures clement and water liquid) and maintenance of elevated temperatures over long timespans, through convection currents and volume expansion (Schulte *et al.*, 2006; McCollom and Seewald, 2013). As evidenced by chemical modeling, benchtop cultivations, and metagenomic studies, microbial life relies on carbon (from methane or other carbon species) and obtains metabolic energy from redox reactions related to the uptake of methane or other carbon species, hydrogen gas, ferrous iron, etc. (Tiago and Veríssimo, 2013b; Rempfert *et al.*, 2017; Rowe *et al.*, 2017; Twing *et al.*, 2017; Meyer-Dombard *et al.*, 2018).

Microbial life utilizes a wide variety of biomolecules to suit its environment and is capable of expressing specific genes and thus shifting metabolisms and production as needed. Some of the most common methods of detecting and analyzing these compounds are inappropriate for planetary exploration: Meta analyses are powerful but often rely on detecting ephemeral compounds, and lipid analysis is a fairly complex multi-step process. Despite the diversity of molecules, all life utilizes nitrogen-based amino acids to generate proteins and enzymes as well as carbohydrate-rich fatty acids and other lipids to create their cell walls. These are the most abundant

compounds, are likely to be the longest lived in hostile environments, and their specific compositional ratio can be indicative of their origin (Marshall *et al.*, 2006a; Igisu *et al.*, 2009; Cao *et al.*, 2011; Newman *et al.*, 2012; Röling *et al.*, 2015). Large biomolecules like complex proteins and lipids are also more likely to interact with inorganic surfaces and become preserved in the rock matrix enabling detecting by a would-be planetary rover (Tipping, 1981; Sansone, Andrews and Okamoto, 1987; Kaiser and Guggenberger, 2000a, 2000b).

1.3 Biosignatures

The search for life on other worlds relies on detecting evidence of life or biosignatures. Biosignatures can take the form of biogenic minerals, fossilized imprints and biogenic debris (Cady *et al.*, 2003; Brack *et al.*, 2006; Marshall *et al.*, 2006b), but can also be inferred from chemical distributions such as fractionated isotopes (Wang *et al.*, 2018) or hydrocarbon distributions (Shultz-Flory distribution) (Lollar *et al.*, 2008; Etiope and Sherwood Lollar, 2013). How biosignatures are created and evolve in serpentinizing systems has only recently been investigated. Ivarsson *et al.* (2017) combined of ESEM, EDS, Raman, and isotopic analysis to confirm the presence of filamentous remains associated with biofilms in serpentinite from the 15°20' N slow spreading ridge (Ménez, Pasini and Brunelli, 2012; Zhu *et al.*, 2014). Certain differentiation between non-living and living biological material remains difficult and largely unexplored (Des Marais *et al.*, 2008). The dynamic geochemistry of actively serpentinizing systems offers multiple pathways through which organic material may be captured and preserved. Although studies of the adsorption of organic materials to other serpentine-related phases

(andradite, magnetite, goethite, hematite) have a strong basis (Ménez, Pasini and Brunelli, 2012; Pasini *et al.*, 2013; Safiur Rahman, Whalen and Gagnon, 2013), the interaction of the serpentine minerals (e.g., chrysotile, antigorite, lizardite) with organic matter is not well understood. The preservation potential of an embedded organic signature in serpentine group minerals is not known.

In general, the difficulty of distinguishing abiotic and biologically derived hydrocarbons and other organic compounds is another obstacle, and subject of ongoing research (Chappelle, Daughtry and McMurtrey, 1932; Sivakumar *et al.*, 2015; Zhou *et al.*, 2015).

Though FTIR and Raman spectroscopy have gross spectral similarities, the techniques are quite different. The Raman laser has a defined frequency (this study, 532 nm). Most of this green laser light is simply reflected or absorbed by the sample with no change in frequency--thus ignored by the Raman detection system. However, a small percentage of incident light interacts with the chemical bonds present within the sample and loses or gain energy upon reflection. This change in the reflected photon's energy is called inelastic scattering or Raman scattering. When many collections of reflected light are performed, a spectrum emerges that is a consequence of the many ways that light may be inelastically scattered upon interaction with the bonding environments present in the sample. In contrast, FTIR spectrometers employ a radiation source that produces light over a broad range of infrared (IR) frequencies. Some of the frequencies interact with chemical bonds present in the sample and excite them causing an array of vibration patterns (vibrational modes). For this reason, FTIR is sometimes referred to as vibrational spectroscopy. Many bonds are capable of

different vibrational modes and bonds that are in a different chemical context behave differently.

Raman spectroscopy, and to a lesser extent Fourier Transform Infrared (FTIR) spectroscopy, are particularly attractive analytical tools in the context of interplanetary exploration and astrobiological research. Given the constraints of space travel, severely limited analytical payloads are required, and complex, multi-step extractions and analyses are virtually impossible. Lightweight spectroscopy tools can distinguish an array of inorganic and organic materials, non-destructively, with comparatively low-power needs, and, in the case of Raman, no sample preparation. Accordingly, a Deep UV (UVC) Raman Spectrometer, in combination with a fluorescence spectrometer, is planned for use aboard the Mars 2020 rover as part of the SHERLOC payload: detection and characterization of organic compounds in diverse Mars materials is anticipated (Beegle *et al.*, 2014).

In this study, we pair complementary Raman and FTIR spectroscopy data to assess detectability of organic compounds relevant to biomarker research in a serpentine-rich matrix. In our experimental design, we subject naturally formed, ophiolite-derived weathering ultramafics dominated by serpentine to sterile and inoculated organic-rich aqueous solutions, and contrast resulting organic films on the serpentine-rich mineral background in dried and desiccated states.

2. Methods

2.1 Summary

Polished wafers of serpentinite were examined before and after incubation in aqueous solutions. Treatments differed with respect to duration and presence of a live microbial culture.

2.2 Sample Preparation

Hand samples of serpentinite were collected from surface outcrops of the Coast Range Ophiolite at the McLaughlin Natural Reserve, Lower Lake CA, USA. Serpentinite samples were broken into smaller pieces via chisel and washed, sonicated for a minimum of 30 seconds, and washed again.

Samples were polished using increasingly finer grit sand paper (methods detailed in Lowenstern and Pitcher, 2013), with an additional, final polishing step using 0.3 micron diamond suspension and lapping film. Samples were sonicated for < 5 seconds to remove debris and loose grains as needed. The final thickness of all wafers was between 100 to 200 microns. After polishing, samples were broken into smaller pieces and mounted to plastic cover slips using commercially available Loctite super glue, rinsed with distilled water, and placed in a desiccator.

2.3. Spectroscopy instrumentation

The nature of Raman spectroscopy is such that non-polar covalent bonds are more readily detected whereas more polar bonds are more easily detected by FTIR. Both Raman and FTIR are referred to as vibrational spectroscopy meaning that in both cases, emitted laser light excites bonds between atoms and the spectrum of light encountered by detectors is altered as a result of these photochemical interactions.

Each material will exhibit this photochemical imprint in a characteristic fashion and to a lesser degree spectrum can be dissected to make educated guesses about the analyzed material.

Both Raman and FTIR spectroscopy operate by directing a laser toward a sample and analyzing the light reflected or transmitted through the sample. The result is a spectrum, which derives its shape from the intensity of the reflected or transmitted light at a given frequency.

FTIR spectra were obtained using a ThermoFisher Scientific Nicolet IS-50 FTIR with a Continuum IR microscope, operating in reflection mode (University of Rhode Island, Graduate School of Oceanography, Narragansett, RI). Background data were collected before every sample using a reflective gold surface. 125 collections were used for each point, at a resolution of 8 cm^{-1} . Aperture dimensions varied, ranging from $50\text{ }\mu\text{m}^2$ to $30\text{ }\mu\text{m}^2$. Data were collected from 650 to 3000 wavenumbers.

Raman spectra were collected using a Witec alpha300 R Confocal Raman, using a 532 nm beam at either 50X or 100X magnification (University of Rhode Island, Rhode Island Consortium for Nanoscience & Nanotechnology, Kingston, RI). 20 collections were performed at 3 seconds of integration time. Laser power varied from 1mW, 10mW, to 20mW.

2.4. Structured incubation experiments

Incubations were designed to test the effects of two variables on organic loading on mineral surfaces: (1) duration of incubation in organic-rich aqueous solution, and (2) the presence of live cells in solution. Wafers were incubated for different lengths of time (3, 24, and 48 hours). Replicate experiments, inoculated with the biofouling

bacteria *Pseudomonas fluorescens*, were run for identical durations. In both sterile and live trials, the incubation solution consisted of commercially available LB-broth (Difco DF0446-17-3).

Nutrient broth was autoclaved Difco LB broth (Difco DF0446-17-3) at concentrations recommended by the manufacturer (10 g/L). 3 mL of broth were pipetted into 6-well plates (Thermo Scientific PN 130184) for experimental observation. Inoculations were performed by pipetting 10 μ L of vortexed, death phase *P. fluorescens* liquid culture grown in LB broth into the appropriate wells. After sterilized rock samples were added to wells, plates were sealed with electrical tape and placed in an incubation chamber maintained at 25°C and oscillating at 100 rpm. Samples were removed with tweezers at specified time points, dipped in distilled water and placed in a fume hood to dry. After an 8-hour-long drying period, samples were stored in a desiccator until post-incubation spectroscopic scanning was performed.

2.5. Spectral processing

Pre- and post-incubation spectra were processed identically with commercial software. Raman spectra were processed using the cosmic ray removal, Lorentz smoothing, and automatic background subtraction algorithms provided with Witec's proprietary software package, "Control." Deviations from default values were only present in cosmic ray removal (due to the random occurrence and intensity of cosmic rays, a spectrum-specific approach is necessary to remove their effects). FTIR spectra were baseline corrected using built-in automatic baseline subtraction provided by ThermoFisher Omnic software.

2.6 PCA analysis

Raman and FTIR spectral data were normalized by dividing the intensity at each wavenumber by the scan's average intensity. For FTIR, an additional linear baseline correction was performed by linear regression of the spectral intensities.

A principal component analysis of correlations within the intensity data was then performed using JMP statistical software.

3. Results and Discussion

Pre-incubation scans: characterizing the organic backdrop in natural serpentinites

Initial scans of experimental substrate are necessary to characterize background levels of organics in samples of interest. Samples under study are composed primarily of lizardite, magnetite, and very minor talc, with near homogeneity, sample to sample, and peaks correspond well to published data for lizardite (Figure 1 and Figure 12) (Rinaudo and Gastaldi, 2003; Pasini *et al.*, 2013). Both Raman and FTIR resolve organic loading: aliphatic bonding ($\sim 2800\text{--}3100\text{ cm}^{-1}$) is visible in FTIR in some cases but is easily discerned in all Raman spectra. Aliphatic bonds are those between carbon and hydrogen and generally constitute a large fraction of the bonding of any organic molecule, be it biological or abiotically generated. Thus, aliphatic bonding peaks are ubiquitous in biological samples and are even common in inorganic samples unless special precautions are taken to avoid contamination. The presence of these peaks implies the existence of biological material on or within the rock matrix of our samples before incubations, and the lack of other bonds suggests that it is likely a carbon-rich, degraded material. Recall that serpentinization is associated with the abiotic production of hydrocarbons via several putative pathways; this serpentinite was obtained from surface outcrops of the Coast Range Ophiolite, with tens of millions of years of tectonically complex history and through-flowing solutions. Because of this, the observed organic material may contain derivatives of kerogen, humic substances, degraded plant matter, or microbial debris.

Raman spectra demonstrate peaks at wavenumbers associated with OH groups, alcohols, amines and amides, at 3140, 3280, and 3430 cm^{-1} , respectively. The hydroxyl (OH) group is an important constituent of molecular water, sugars, alcohols, and of the serpentinite mineral matrix itself. FTIR data, in contrast, with a lower signal to noise ratio, higher limit of detection, differences in penetration depth, and differences in sensitivities to specific bonds, do not resolve aliphatic peaks as clearly; aliphatic peaks can only be seen in some spectra, when magnified.

End member organics: differentiating the organics in sterile growth media from those in solutions with active cell growth

Raman scans of sterile broth and *Pseudomonas fluorescens* show some aliphatic and nitrogenous bonding around 2900 cm^{-1} and above 3000 cm^{-1} respectively (Figure 2). However, much of the spectra is strongly affected by powerful and broad fluorescence centered around 3300 cm^{-1} . Therefore, spectral processing via baseline correction is necessary in order to standardize data (rendering data internally consistent in this study) and to assess differences qualitatively. Despite fluorescence interference, the changes in relative strength of peaks in the latter portion of the spectra suggest chemical differences between these endmembers. Specifically, the relative heights of peaks and shoulders at 3080, 3100, 3260, and 3440 cm^{-1} , can be used to help differentiate specific amino acids and protein structures (Sinnott, 1983; Dietzek *et al.*, 2010; Smith and Dent, 2019) (Figure 4). These peaks are difficult to characterize but

are a consequence of the abundant peptides in LB broth which includes tryptone as a main ingredient.

FTIR spectra for both sterile broth and microbe-containing solutions overlap in some regions but are not identical (Figure 13). Peaks in complex spectra involve signals at wavenumbers corresponding to myriad chemical and functional groups, such as methyl, methylene, alkyl, carbonyl, amine, and amide, and phosphodiesteres. Overall, the peaks of *P. fluorescens* are more prominent and sharply defined than the broader peaks of the LB broth. This could reflect a simpler bulk chemical composition in the live condition at the time of analysis than with sterile LB broth only (perhaps due to selective microbial metabolism of some compounds, decreasing their presence over time). It may also be that well-formed cellular components (e.g., cell membranes) may resonate more strongly with the Raman technique, yielding a more prominent spectrum. Most importantly, despite many common peaks, spectra for LB broth and *P. fluorescens* are distinct and differentiable by statistical tests.

Results of Incubations

Even for the longest incubation duration condition (48 hours), all coated surfaces of samples consist of comparatively small quantities of biological material on rock substrate, and spectra are dominated by inorganic signals derived from the mineralogical backdrop (SiO₄ and Mg-OH bonds). Since peak prominence is related to abundance, subtle changes due to organic loading are quite difficult to extract. However, there are clear changes in spectral signals, particularly below 2900 and above 2900 cm⁻¹ for FTIR and Raman results, respectively.

Raman spectra show that changes in spectral character occur rapidly at the onset of incubation, although changes below 2900 cm^{-1} are not easily attributed to anticipated chemical changes (Figure 4 and Figure 5). Although low intensity peaks certainly shift, they are too sensitive to baseline-correction induced noise and are not robust enough for deep analysis. Live samples (containing microbial cells in culture) are an exception and remain almost identical to the starting condition. In contrast, changes in peak intensity for other experimental groups are prominent and clear. Desiccated samples in particular show a rapid increase in peak intensities at 3100 , 3290 , and 3440 cm^{-1} (Figure 6 and Figure 7) and reveal previously hidden peaks and shoulders in other spectral positions, such as those at 3880 , 3175 , 3220 cm^{-1} . However, both desiccated samples (whatever the experimental path) are extremely similar in terms of resulting spectra: this indicates that the desiccation process collapses/reduces bond presence and blurs bond detection even as the microscopic scale.

Differences in the amine and amide-rich regions are evident in Raman (Figure 7) and FTIR (Figure 18) spectra. Within Raman spectra, the changing mixes of these nitrogenous bonds, the carbon double bonds of carbonyl functional groups, and the bound hydroxyl groups of sugars and alcohols are evident as changes in individual peak intensity and overall shape from 3000 to 3500 cm^{-1} . This region is also the center of a broad and powerful fluorescence signal, which affects most of the incubated spectra (Figure 2).

Generally, in Raman spectroscopy, fluorescence overwhelms all other data with broad and intense peaks and must be countered/corrected for. This large broad signal, with no chemical specificity, is difficult to correct for, and corrections may result in

specious intensity reads for a given peak. In this study, with the particular baseline curve utilized in the correction strategy, an underestimation of peak intensities is the most likely bias. All spectra presented in figures have been background-corrected, but live samples contained very large fluorescence signals, of which only the most prominent peak tops remain in this region. Although fluorescence is not a concern for FTIR analysis, and FTIR is also capable of detecting and characterizing the same functional groups, the lower resolution of FTIR ($50\text{ }\mu\text{m}^2$ compared to $1\text{ }\mu\text{m}^2$), and lower sensitivity to these bonds makes resolving these peaks from the noise difficult without statistical processing (Figure 14 and Figure 15).

Another easily observed and important spectral feature in Raman spectra are the prominent aliphatic bonding peaks centered around 2900 cm^{-1} (Figure 4, Figure 5, Figure 6, Figure 7, and Figure 8). It is important to note that these peaks are also heavily influenced by the fluorescent interference and correction may lead to underestimation of their intensity as is the case with the nitrogenous bonds. A drastically less prominent set of peaks emerge with FTIR spectral analysis. Few spectra show aliphatic bonding persuasively, and aliphatic bonding is difficult to discern given instrument noise

Within FTIR, spectral changes in both the complex organic region ($1350\text{--}1750\text{ cm}^{-1}$) and aliphatic region ($\sim 2900\text{ cm}^{-1}$) mainly consist of shifting prominence and noise levels (Figure 16, Figure 17, Figure 18, Figure 19). These poorly resolved changes in FTIR spectra are neither consistent nor useful in determining underlying bond character. However, since changes to spectra are rapid, there is little difference between 24- and 48-hour replicates and representative end-member groups can be

studied by averaging the two groups of data. This eliminates some of the variation introduced by the heterogeneous mineral backdrop and non-uniformity of organic film deposition to reveal patterns observable across experimental groups. After data reduction of this type, subtle shifts in peak position can be observed (Figure 18): live (cell-containing) and live desiccated samples display peaks at 1450, 1508, and 1609 cm^{-1} , whereas sterile and sterile desiccated spectra contain these peaks but display more prominently peaks at 1475 and 1580 cm^{-1} . These shifts are likely due to changes in the protein chemistry of the surficial material in sterile samples. Live samples apparently express bonding patterns more similar to the pre-incubation state. Data show that Raman analysis is not much improved by this kind of data reduction: its signals for wavenumber regions of interest are an order of magnitude more prominent than the regions under scrutiny with FTIR and are robust without this treatment. However, these representative group spectra show good agreement with the time series plots from before. (Figure 8)

Relevant geobiological studies allow a deeper understanding of the evolving chemical landscape of the samples in the course of experimental incubations. As demonstrated by Igisu *et al.*, 2012, the ratio of methylene bridges (CH_2) to terminal methylene (CH_3) groups can be used as an indicator of the underlying complexity of molecules present in the sample (Figure 22 and Figure 23). Although Igisu and others applied this technique to FTIR data, we find that interpretations of Raman data differentiate the live sample group much more effectively; it is not as useful for distinguishing other experimental groups. Secondly, the live grouping has a much higher $R_{3/2}$ than any

other group, suggesting simpler molecules dominate the material, unless a correction factor is applied.

Principal Component Analysis (PCA) of Raman spectral data: seeing the forest while in the trees

PCA of complex datasets provides a way of reducing complexity due to many variables (or many discrete wavenumbers of interest) to a few compound variables or “Principal Components”. PCA was performed on Raman and FTIR intensity values separately.

PCA data were visualized in three ways. First, scores for each of samples for four of the principal components (PCs) were plotted against each other to create two-dimensional score plots (Figure 9 and Figure 19). An accompanying table of eigenvalues for FTIR and Raman PCA results shows the degree to which variation in data is explained, for each data stream (Table 3 and Table 4). Second, loadings for these PCs were plotted, and placed above spectra of pre-incubation rock samples and organic end members (Figure 10 and Figure 20). Finally, average PC scores for time intervals were plotted against incubation duration to search for correlations with time (Figure 11 and Figure 21).

The largest changes to the pre-incubation condition appear due to incubation itself (which deposited mixed biological material on the sample surface) and both FTIR and Raman tools were able to distinguish pre-incubation from incubated samples. PCA of Raman data also differentiates desiccated samples from standard samples effectively (Figure 9). Most compellingly, PCA of FTIR data parsed patterns in living samples from other samples, especially in terms of PC1 and PC3 (Figure 19).

With Raman data, PCA-derived clustering of data showed that live samples overlap with all other groups, but especially sterile samples. In fact, in some component comparisons, all samples remain in a linear trend and cluster on the line as if it were a gradation of biological complexity and desiccation; this suggests that the PCs are heavily loaded by signals from similar wavenumbers. In another pairing (PC2 or 1 vs PC3), the statistical results sort samples into two separate linear trends for the undesiccated and desiccated samples.

Raman spectra boast few regions that can be used as diagnostic for live cell signals. the related Loadings plot, with multiple PCs, indicate the importance of bonds from *P. fluorescens* cellular materials and also LB Broth.

In the Raman PCA analysis (Figure 9), PC3 responds strongly to the degree of desiccation: desiccated samples receive higher scores than the other experimental groups. Yet, no single component clearly implicates at the spectral impact of living samples. Upon cross-referencing the component loadings with the endmember spectra, PC3 remains cryptic in terms of loading character. Although PC3 reflects peak presence near where a water band would be ($\sim 3375\text{ cm}^{-1}$) it loads more powerfully from $3050\text{ to }3200\text{ cm}^{-1}$, in the middle of the amide A band for proteins. Leftover proteinaceous material could be transforming during the desiccation process, due to changes in moisture and pH, reducing the spectral signal.

PCA analysis of FTIR data is much more fruitful: samples incubated with a live microbial culture form a statistically separable group, notably in terms of PC2 vs PC3, but in nearly every other relationship as well. Interestingly, this same component

combination also groups live desiccated samples and pre-incubation scans on top of each other. Differing from PCA findings derived from Raman data, there is no apparent distinction of desiccated samples. As previously discussed, the CROMO surface sample used in this study were certainly contaminated with organics derived from atmospheric deposition/groundwater infiltration. Whereas Raman results were very sensitive to chemical changes induced by desiccation, FTIR results were not—and FTIR therefore seems a more appropriate tool for tracking chemical changes associated with the presence of life.

Although samples were normalized to facilitate comparisons, signal intensity of all peaks in live (*i.e.*, containing/exposed to live cell cultures) samples decreases with time.

These results run counter to expectations in two ways. As biological material builds up on the surface, (1) intensities of mineral peaks would be expected to decrease in both samples, and (2) signals due to aliphatic bonding (presumably present in higher concentration in media, apparent as peaks of region 3) would also be expected to increase in both sample sets.

The apparently decreasing signal power in live samples (with cell numbers increasing with time) can be explained through interference by the increasingly powerful fluorescence signal in these samples (Figure 2). Unlike Raman scattering, from which light is emitted at a consistent separation from the incident light for a given bond type, when fluorescent photons are emitted, they do so at a wavelength corresponding to

their resonant structure. Fluorescence thus conveys very different, and much less descriptive, chemical information. Fluorescence is also much more efficient than Raman scattering and produces signals several orders of magnitude more powerful and less defined. In this study, we suggest that fluorescence may be caused by the siderophore pyoverdine: the water-soluble pigment and namesake of *P. fluorescens* (Visca, Imperi and Lamont, 2007). However, typically, concentrations of pyoverdine are drastically reduced (by orders of magnitude) when adequate iron is provided (Meyer and Abdallah, 1978). The strength of the fluorescent signal is therefore surprising given that LB media is not an iron deficient media, and that the fluorescence signal intensity increases rapidly over the course of experiments (Visca, Imperi and Lamont, 2007).

Fluorescence is a common problem in the Raman spectroscopy of biological materials and its mitigation is an ongoing challenge. The major reason for the use of NIR FT-Raman and UV Raman instruments, despite increased complexity in the former and the possibility of sample damage in the latter (Dietzek *et al.*, 2010), does circumvent some of the fluorescence problem. Despite a variety of laser powers, magnification levels and the confocality of our instrument, fluorescence is a prominent spectral interference.

Although an unconventional metric, differences in fluorescence-driven emission between sterile and live samples can be interpreted as an indication of altered biochemical and thus photochemical properties of the scanned material (*i.e.*, the presence of fluorescence here indicates the proliferation of our microbial culture). The extreme fluorescence observed in live samples in this study confirms the need for

Deep UV Raman use on the Mars 2020 rover, which separates fluorescent signals from Raman scattering reliably.

Conclusions

Spectroscopic investigations of planetary surfaces yield important and incompletely tapped data streams. Contextual information combined with the presence or absence of certain bond types allow remote characterization of both organic and inorganic materials. Despite challenges involving fluorescence and signal to noise ratios, Raman and FTIR data streams, when combined and analyzed statistically, allow differentiation between pre-existing carbonaceous material associated with the rock surface, non-living but biologically rich material, an active microbial culture, and these same living and non-living biological material deposited on complex rock surfaces after desiccation.

The ability to distinguish between these different surficial films is strengthened in longer duration experiments and indicates that a well-established microbial colony may be easily distinguished from non-living material when sufficient concentrations (in terms of organic films on mineral surfaces) have been reached.

The Mars 2020 DUV SHERLOC represents an exciting tool with which to probe the Martian surface due to higher resolution of Raman active bonds via elimination of the primary difficulty illuminated in this study: fluorescence (Beegle *et al.*, 2016).

Given previous controversies in the identification of meteor(ite)-bound biosignatures and the likelihood of detecting abiogenic organic material on the Martian surface (especially within serpentinites), the need to probe the intricacies of

de facto biosignatures is critical (Fireman, Rancitelli and Kirsten, 1979; Jull *et al.*, 1998; Achenbach *et al.*, 2015). Timely and trustworthy spectroscopic data acquisition and analysis by which biogenic material can be distinguished from its abiotically produced counterpart stand poised to play a pivotal role in this regard.

Despite the significant challenges of detecting biological material on serpentine-dominated mineral backdrops, by using peak intensity ratios of key biological bonds, FTIR and Raman spectroscopy remain effective tools. FTIR and Raman spectra show promise in distinguishing between living and non-living material, even given these new data for incubations with essentially identical elemental abundances (CNPS ratios). Raman spectra in particular perform well in this regard, yielding $R_{3/2}$ values that display correlations to incubation duration (0-48 hours) and differ substantially in living and non-living material. Durability of organic material in planetary surfaces and near subsurface environments may be a critical factor controlling the applicability of these spectroscopic tools. Future work resolving the limit of detection of these organic compounds in serpentinites and related lithologies is needed.

Tables

Table 1 Raman peak assignments for CROMO serpentine and biological material.

Modified from (1) Rinauldo et al., 2003, (2) Maquelin et al., 2002, and (3) Smith & Dent, 2019a.

Raman Bond Assignments	Typical Range (cm ⁻¹)	Position (cm ⁻¹)	Source
H-O-H group vibrations	200-290	233	1
SiO ₄ bend	290-357	350	1
SiO ₄ symmetric	357-416	388	1
Mg-OH antisymmetric translation*	460-490	374	1
SiO ₄ -AlO ₄ deformation modes	490-519	510	1
OH-Mg-OH antisymmetric translation	590-650	630	1
Si-O-Si	650-750	690	1
OH-Mg-OH outer symmetric translation	750-900	709	1
Si-O	900-1189	1096	1
CH ₂	1420-1500	1450	2
Noise	1200-2500	diffuse	2
CH ₂ str	2750-2850	2860	2
CH ₂ str II	2890-2920	2885	2
CH ₃ str	2825-2975	2930	2 & 3
CH=CH	2980-3080	2980	2 & 3
		3080	
		3100	
Amine and Amide	3155-3475	3260	3
		3440	
OH	2890-3520	3440	3
fluorescence	Diffuse and very broad	3260	3

Table 2. FTIR peaks and bond assignments based on work of (1) Yariv and Heller-Kallai, 1973 (2) Maquelin et al., 2002 and (3) Fuchs, Linares, & Mellini, 1998

FTIR Bond Assignment	Peak position (cm ⁻¹)	Source
Si-O	980	1 & 3
	1090	1 & 3
Assorted Carbohydrates	900-1200	2
Amide III	1216	
C=O	1400	
CH ₂ Deformation	1463	
Amide II	1543	
Amide I	1674	
Ester	1740	
CH ₂ in fatty acids	2850	
CH ₂ alkanes in fatty acids	2930	
CH ₃ in fatty acids	2980	
Amides or Hydroxyls	3300	
Fe-OH	3640	3
Mg-OH	3680	3

Table 3 Eigenvalues associated with principal component analysis of Raman data.

Number	Eigenvalue	Percent	20 40 60 80	Cum Percent	ChiSquare	DF	Prob>ChiSq
1	128.9304	25.035		25.035	308108	132263	<.0001*
2	89.0629	17.294		42.329	296287	132006	<.0001*
3	30.9432	6.008		48.337	285440	131669	<.0001*
4	23.7248	4.607		52.944	279216	131217	<.0001*
5	20.3057	3.943		56.887	273540	130752	<.0001*
6	14.6734	2.849		59.736	268091	130280	<.0001*
7	11.8546	2.302		62.038	263229	129799	<.0001*
8	11.7397	2.280		64.317	258666	129312	<.0001*
9	9.3128	1.808		66.126	254060	128826	<.0001*
10	7.6263	1.481		67.607	249753	128337	<.0001*
11	6.9627	1.352		68.958	245664	127844	<.0001*
12	6.5753	1.277		70.235	241654	127352	<.0001*
13	6.3061	1.224		71.460	237686	126860	<.0001*
14	5.1682	1.004		72.463	233742	126368	<.0001*
15	4.7971	0.931		73.395	229971	125875	<.0001*
16	4.2206	0.820		74.214	226253	125382	<.0001*
17	4.0637	0.789		75.003	222625	124889	<.0001*
18	3.9013	0.758		75.761	219020	124397	<.0001*
19	3.7239	0.723		76.484	215438	123905	<.0001*
20	3.4554	0.671		77.155	211884	123414	<.0001*
21	3.2457	0.630		77.785	208375	122923	<.0001*
22	3.1574	0.613		78.398	204902	122433	<.0001*
23	3.0304	0.588		78.987	201443	121944	<.0001*
24	2.9967	0.582		79.569	198006	121456	<.0001*
25	2.8744	0.558		80.127	194575	120968	<.0001*
26	2.7384	0.532		80.658	191165	120481	<.0001*
27	2.6329	0.511		81.170	187781	119995	<.0001*
28	2.5589	0.497		81.667	184418	119510	<.0001*
29	2.5136	0.488		82.155	181068	119025	<.0001*
30	2.4003	0.466		82.621	177728	118542	<.0001*

Table 4 Eigenvalues for principal component analysis of FTIR data.

Number	Eigenvalue	Percent	20	40	60	80	Cum Percent	ChiSquare	DF	Prob>ChiSq
1	342.0745	49.219					49.219	.	240971	.
2	153.0959	22.028					71.248	.	240959	.
3	68.9562	9.922					81.169	.	240570	.
4	49.4992	7.122					88.291	.	240013	.
5	27.2787	3.925					92.216	.	239419	.
6	16.2405	2.337					94.553	.	238781	.
7	7.3948	1.064					95.617	.	238122	.
8	5.0835	0.731					96.349	.	237446	.
9	4.0982	0.590					96.938	.	236767	.
10	3.0820	0.443					97.382	.	236086	.
11	2.2464	0.323					97.705	.	235405	.
12	1.7101	0.246					97.951	.	234723	.
13	1.2086	0.174					98.125	.	234041	.
14	1.0763	0.155					98.280	.	233359	.
15	0.9725	0.140					98.420	.	232677	.
16	0.8057	0.116					98.536	.	231997	.
17	0.7493	0.108					98.644	.	231317	.
18	0.6629	0.095					98.739	.	230638	.
19	0.6185	0.089					98.828	.	229960	.
20	0.5875	0.085					98.912	.	229283	.
21	0.5168	0.074					98.987	.	228607	.
22	0.4713	0.068					99.055	.	227932	.
23	0.4423	0.064					99.118	.	227258	.
24	0.3984	0.057					99.176	.	226584	.
25	0.3701	0.053					99.229	.	225912	.
26	0.3364	0.048					99.277	.	225241	.
27	0.3040	0.044					99.321	.	224570	.
28	0.2978	0.043					99.364	.	223900	.
29	0.2764	0.040					99.404	.	223232	.
30	0.2546	0.037					99.440	.	222564	.

Figures

Figure 1. Raman spectra of serpentine in CROMO surface sample. Raw (top) and processed (bottom) spectra are annotated to indicate mineral peaks (violet bar), aliphatic and amine/amide peaks (green bar). Data were collected at 50x magnification with 10mW of laser power (spot size $<1 \mu\text{m}^2$) on a Witec alpha300 R confocal Raman. Spectra were baseline corrected, smoothed, , cleaned of cosmic rays.

*Figure 2 Raw (top) and processed (bottom) average Raman spectra of *P. fluorescens* and LB Broth (N=5). Powerful fluorescent signals dominate the spectra, but after baseline correction, the Si-O peak at 150 cm^{-1} , and peaks above 3000 cm^{-1} can be used to distinguish between the organic constituents. Noise (500 to 2500 cm^{-1}) makes a more nuanced analysis of biological loading impossible.*

Figure 3 Closeup of higher wavenumbers in average Raman spectra of culture and media (n=10). Peaks at 2885 and 2980 correspond to CH_2 . 2930, CH_3 . 3080, $\text{CH}=\text{CH}$. >3100, amines and amides. It is difficult to link peaks to specific molecular structures above 3100 but they do suggest proteins (see Table 1 Raman peak assignments for CROMO serpentine and biological material. Modified from (1) Rinauldo et al., 2003, (2) Maquelin et al., 2002, and (3) Smith & Dent, 2019a.)

Time Series

Figure 4 Spectral averages ($n > 5$) for regions of interest on serpentine after 0, 3, 12, 24, 48 hours' exposure to organic-bearing solutions. Small peaks between prominent mineral peaks may be noise caused by background correction. These peaks are much less prominent in live samples, almost identical to the pre-incubation condition.

Figure 5 Spectral averages created from several point collections ($n > 5$ ea.). Small changes are evident but difficult to explain chemically. Small peaks between prominent mineral peaks may be noise caused by background correction

Figure 6 Raman spectra from 2800 to 3500 cm^{-1} . Aliphatic, protein, and hydroxide bonding peaks for live and sterile incubations of serpentine are prominent. Spectral averages ($n > 5$) change with incubation time and involve large increases in peaks centered at 3100, 3220, 3290, and 3440 cm^{-1} , a series of peaks which all fall within amine and amide ranges. Peaks in the aliphatic range of 2900 cm^{-1} change only slightly.

Figure 7 Raman spectra aliphatic, protein, and hydroxide bonding peaks for live and sterile incubations of serpentine. Spectral averages ($n > 5$) show that after desiccation, samples become spectrally similar. Peaks in the 2900s are all aliphatic, whereas large peaks centered at 3100, 3220, 2990, and 3440 fall within amine and amide ranges. N.B. spectra are offset by 0.2 a.u.

Experimental groupings

Figure 8. 48-hour scans were averaged to create representative spectra for each experimental group ($n > 7$). Changes in spectral character in mineral peaks (left) are subtle and hard to distinguish from noise. However, differences amongst the organic bonds (left) are stark, in terms of peak intensities and peak prominence. NB. spectra have been normalized to maximum heights.

PCA Analysis

Figure 9 Score plots of loadings generated via PCA of 240 Raman spectra effectively distinguish between unincubated and incubated material and undesiccated from desiccated samples. PCA analysis also shows a limited ability to isolate live samples from sterile and to indicate the presence of an active microbial culture, and desiccation.

Figure 10. This region of the Raman spectra ($2800\text{-}3600\text{ cm}^{-1}$) is where the two organic treatments can be differentiated by eye and also by PCA. PC1 appears to be

highly loaded by variables for which peaks which sterile broth has its highest intensity. The same applies to PC2 and the peaks of *P. fluorescens*.

Figure 11 Average score for a given time interval was calculated and plotted against its associated incubation duration. For each time interval, the minimum number of scores averaged was 3.

Figure 12 Averaged FTIR spectrum of serpentine in CROMO surface sample (n>20).

Mineral peaks at 695, 1054, 1131, 1267, 1771, and 3700 are primarily due to interactions between Si, O, Mg, and OH. Organic peaks are not visible.

Figure 13 FTIR analysis is well suited to producing spectra for LB broth and *P. fluorescens*. Averaged spectra ($n=10$) retain peaks related to amide III at 1260, C=O and C-H deformation at 1400 and 1460, amide II at 1530, amide I at 1660, CH₂ and CH₃ bonding at 2850, 2930, 2960, and N-H stretching at 3300.

Figure 14. Representative FTIR spectra for all incubation durations ($n > 3$). This region of the IR spectrum contains peaks that correspond to the carbonyl bonds of aldehydes and ketones, various amine and amides and larger protein structures. Differences between time steps are not easily observed. Spectra have been offset to facilitate comparisons.

Figure 15 Representative FTIR spectra for incubations for the specified time point ($n>3$). This region of the IR spectrum contains peaks which correspond to the carbonyl bonds of aldehydes and ketones, various amine and amides and larger protein structures. Differences between time steps are not easily observed. Spectra have been offset to facilitate comparison.

Figure 16 Representative FTIR spectra for incubations for the specified time points ($n > 3$). This region of the IR spectrum contains peaks that correspond to aliphatic CH_2 and CH_3 bonding. Changes in peak heights, position, or prominence are not easily observed through time. Spectra have been offset to facilitate comparisons.

Figure 17. Representative FTIR spectra for incubations for the specified time point ($n>3$). This region of the IR spectrum contains peaks that correspond to aliphatic CH_2 and CH_3 bonding. Changes in peak heights, position, or prominence are not easily observed through time. Spectra have been offset to facilitate comparisons

Experimental groupings

Figure 18 Representative FTIR spectra for experimental groups were computed from all 24- and 48-hour sample scans for the specified group (n>6). Desiccation intensifies differences between sterile and live experimental groups.

PCA Analysis

Figure 19 Principal component analysis of 80 FTIR spectra. Color coding connotes live/sterile condition and air dried/dessicated condition. PC3 separates live from sterile in this study.

Figure 20. Colored bars correspond to important peaks and are color coded according to the organic endmember to which they correspond below. PCA loadings indicate that some peaks in the spectra of LB broth and *P. fluorescens* load strongly on particular PCs..

Figure 21 Average score for a given time interval was plotted as a function of incubation duration. For each time interval, the minimum number of scores averaged 3.

Figure 22. Peak height ratios of the heights of the CH_3 peak located at $\sim 2450\text{ cm}^{-1}$ in FTIR, divided by that of the CH_2 peak at $\sim 2945\text{ cm}^{-1}$. Corresponding peaks for Raman data treatment place the CH_3 peak at $\sim 2980\text{ cm}^{-1}$ and the CH_2 peak at $\sim 2900\text{ cm}^{-1}$. Decreasing CH_3/CH_2 ratio can indicate an increase in the average length of carbon chains such as in the creation of phospholipids and the presence of cyclic compounds such as those located in polysaccharides (Igisu, 2006; Igisu et al., 2012). These chemical differences would degrade over time since many complex biological molecules are poorly preserved.

Figure 23 Consistent with Figure 12, live samples can be distinguished with both spectroscopic tools. However, Raman is a more sensitive to aliphatic bonds and thus provides better data for calculating $R_{3/2}$.

Spectrophotometer

Figure 24. Optical density at 600 nm demonstrates that contamination of sterile samples remained below the limit of detection, with a near-zero density value, throughout the experiment.

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APPENDIX A

Experimental Forays:

X-ray photoelectron spectroscopy assessment of organic solvent cleaning protocols.

Introduction:

An experiment to assess the effect of organic solvent washing, the applicability of X-ray photoelectron spectroscopy, and sputter cleaning to biosignature detection was undertaken.

Three major questions were asked

- 1) Can XPS technology be leveraged for biosignature research?
- 2) Is there a measurable difference between olivine and serpentinite after washes with organic solvents?
- 3) How does sputter cleaning effect the carbon loading on samples?

X-ray Photoelectron spectroscopy operates by exciting electrons within incident orbitals: X-ray photons are directed toward the sample and the emission of excited photoelectrons is quantified. In this way a sample's surface can be scanned and detected photoelectrons can be linked to specific bond types between specific atoms.

Our exploration of this technique primarily focused on carbon as a marker of contamination. Despite the relative abundance of inorganic carbon in serpentinites. XPS is an extremely surficial technique resolving thin films of tens of angstroms thus measuring the chemistry of only the top few atoms. This makes it ideally suited to measuring contamination. However, the large resolution ($\sim 300\mu\text{m}$) (Moulder, 1992) reduces its utility in rock types like serpentinite which have extremely heterogenous surfaces and for which a more nuanced understanding of carbon loading may be

necessary— organic material has shown very different sorptive proclivity for different minerals and particularly different valence states of iron (Tipping, 1981; Filius, Hiemstra and Riemsdijk, 1997)

Methods:

Samples of olivine and serpentinite were collected from Ward's Scientific and the Coast Range Ophiolite respectively and formed into thin wafers of rock (<100 μm thick) in accordance with methodology detailed in Lowenstern and Pitcher, 2013. All samples were then rinsed in ultrapure water and half of the samples were washed with for 5 minutes each in benzene, dichloromethane, and hexane successively in a simplified variation of the methodology utilized in Hays 2010. Samples were transported by being sandwiched between ultrapure water-washed glass slides wrapped in commercial grade aluminum foil. When needed, samples were only manipulated with steel tweezers.

Samples were then scanned with a Perkin-Elmer 550 Multitechnique Surface Analyzer (URI-ENG) “as-received” and after successive rounds of Ar⁺ sputter cleaning.

Results/Discussion:

Since this was an exploratory study with time constraints, data collection was not perfectly systematic, and n is confined to 1 sample of each rock type (serpentinite and olivine). It should be noted that a comparison of how carbon loading changes with respect to cleaning and sputter cleaning is probably best visualized by figures 3 and 4 but these rely on extrapolations from extremely limited data. The area scanned by the

XPS instrument used covers around $\sim 300\mu\text{m}$ and is thus a more reliable indicator of the surface chemistry. As a consequence, despite interesting results all data should likely be treated as insignificant.

Comparing between rock types, it seems that olivine may carry a slightly larger load of carbon and thus organic material; (olivine peak counts >275 , serpentinite counts <250) despite the comparative abundance of oxidized iron phases known for their sorption of organic materials in serpentinite (goethite, magnetite, etc.). Due to rigorous polishing procedures it seems unlikely that this is a consequence of previous handling regimes and either indicates a wide range (high standard deviation) of carbon content in our samples or a true trend of higher carbon overall in olivine samples.

We also see an unexpected trend within rock types wherein serpentinite samples subject to washes in organic solvents had only small decreases in carbon loading relative to unwashed replicates and washed olivine samples actually generated higher counts. This work was published in part at Goldschmidt 2017. The poster can be found in APPENDIX B

Data:*Table 5. Table of **Serpentinite** XPS counts at varying binding energies (eV)*

	Dirty	Dirty	Clean	Clean
sputter cleaning (s)	6	12	0	6
300	15.4153	-59.0032	8.4629	16.6483
299.2	8.33	-46.1205	41.366	-15.9719
298.4	-29.2244	-66.1634	-10.592	-3.1641
297.6	28.2634	-6.1888	-12.5533	-1.9842
296.8	-9.1916	3.9018	-5.8091	9.7322
296	-19.0718	2.2871	18.3624	-7.748
295.2	-11.8112	12.4164	-33.6316	-3.1114
294.4	20.6197	14.3868	-13.857	-31.2309
293.6	33.3778	36.434	8.1242	-8.0371
292.8	58.1057	44.6052	-4.6969	8.2829
292	49.8892	-1.5243	45.6008	4.8037
291.2	70.7266	23.6439	41.5441	-2.2193
290.4	139.2945	-15.8377	100.6192	39.8722
289.6	161.9233	-4.0018	205.5035	52.973
288.8	96.0322	5.6246	245.2293	101.2744
288	52.2681	-5.9113	202.281	104.2348

287.2	25.4601	29.606	128.7867	46.3262
286.4	-2.8198	-52.5484	46.4724	31.3299
285.6	8.4307	-16.7	-14.1943	-7.1913
284.8	38.2585	13.7987	1.2488	0.8466
284	14.004	8.1154	16.7148	0.737
283.2	-27.583	-15.4352	10.4318	-29.8189
282.4	24.5281	30.3344	-17.6328	9.6539
281.6	13.4173	12.9845	2.1582	-20.8545
280.8	-3.049	11.8557	48.1047	2.2759
280	0.2358	-10.6095	15.7414	-14.2474

Table 6. Table of *Olivine* XPS counts at varying binding energies (eV)

Binding Energy (eV)	Dirty	Dirty	Clean	Clean	Clean
Sputter clean (s)	0	12	0	6	12
300	-16.6124	-58.0952	12.83	15.3044	9.6918
299.2	-21.0215	3.0952	47.384	29.3463	21.8445
298.4	-12.5802	18.5714	-21.814	-4.4869	10.7113
297.6	19.376	25.4762	-8.8368	5.9195	-20.2702
296.8	24.6179	-13.3333	-2.9885	3.5151	24.4436
296	-43.9939	-12.1429	11.8254	-9.4347	-4.1734
295.2	-7.6555	-2.381	37.4184	28.9925	9.1031
294.4	4.3539	80.2381	23.6886	-10.7216	-5.5664
293.6	-16.3842	4.2857	67.213	19.499	33.3514
292.8	40.0902	12.619	57.1381	34.6837	51.384
292	54.8138	23.8095	184.8921	32.4065	54.3063
291.2	52.0556	-2.1429	187.6508	35.9566	42.0793
290.4	124.3139	43.3333	279.9315	121.3838	63.6272
289.6	140.8777	23.0952	265.0276	65.7854	17.6134
288.8	145.8335	8.5714	212.4848	44.9436	2.9558
288	122.6632	-14.5238	93.5601	47.3644	73.362
287.2	45.327	-4.7619	51.3099	10.0295	-19.2625

286.4	6.0142	10.7143	1.7871	56.6764	26.6021
285.6	-7.8468	-32.3809	4.0547	11.1952	-5.5242
284.8	15.6909	-8.3333	-7.9959	-19.3504	3.0681
284	-2.8012	-41.4286	8.5667	-2.1278	14.013
283.2	-5.0442	-31.6667	3.6868	-9.3707	19.1559
282.4	5.5832	-10.4762	4.164	14.9155	7.9898
281.6	-21.2378	-6.4286	-9.6816	-12.1216	-24.1906
280.8	7.9942	-25.2381	-14.6125	13.3074	4.1662
280	-7.0992	15.9524	-10.6055	-10.2413	-21.0314

Figures

Figure 25. Samples which have received no special treatment are referred to here as “Dirty”. Cleaned samples have been washed in organic solvents. Although direct comparisons are not possible. Sputter cleaning (SC) reduces carbon bonding intensity in both replicates. Refer to Table 5 for sputter cleaning durations.

Figure 26. Samples which have received no special treatment are referred to here as “Dirty”. Cleaned samples have been washed in organic solvents. Sputter cleaning reduces carbon bonding. Refer to Table 6 for data.

Figure 27. peak maximums for 280-300 eV. Extrapolations show little difference between clean and dirty replicates.

Figure 28. sputter cleaning reduces carbon loading on olivine samples but seems more effective in Clean olivine samples where more carbon is initially detected.

CROMO In-situ flow-through, microbial colonization capsules (MCCs).

Abstract:

Samples of CROMO Serpentine, White Seep Travertine, Dolomite, corundum, Diopside, Twin Sisters Olivine, Ishpameg Magnetite, Yellow Dog Peridotite, and Augite, were polished using successively higher grit sandpaper until a final grit of .3 micron diamond paste was reached. Samples were placed in chambers made from individual PVC pipe sections screwed together with sections of nylon window screen between them. Each pipe section was also drilled with upwards of 12 holes to allow water to pass through them. Capsules were placed in CSW 1,1, CSW 1,4, QV 1,1, QV 1,2 December 15th and 16th of 2016. As of the time of this writing they have been deployed for 3 years.

Figures

Figure 1. Schematic representation of a deployed incubation chamber.

Figure 2. Photograph of deployed incubation chamber containing various rock and mineral samples. System is fully customizable and can be re-designed with different mineral baits. Four Microbial Colonization Capsule systems (MCC) (see above) were constructed of PVC pipe, and nylon mesh and deployed into monitoring wells at the CROMO in December 2016.

Table1. Capsules and mineral samples contained within. This table has been rearranged from easy comparison between chambers.

Capsules and Chambers (rearranged for easy comparison)			
Capsule 1 (Orange)	Capsule 2 (Green)	Capsule3 (Blue)	Capsule 4 (Pink)
Srp	Srp	Srp	Srp
Tra	Tra	Tra	Tra
Di	Di	Di	Di
Ol	Ol	Ol	Ol
Mag	Mag	Mag	Mag
Per	Per	Per	Per
Aug	Cor	Cor	Srp
Dol	Dol	Dol	Dol

Table 2. Capsules and mineral samples contained within. This table corresponds to the actual arrangement of rocks and minerals

Table 2			
Capsules and Chambers (Spatial Arrangement)			
Capsule 1 (Orange)	Capsule 2 (Green)	Capsule 3 (Blue)	Capsule 4 (Pink)
Srp	Srp	Srp	Srp
Aug	Cor	Cor	Tra
Tra	Tra	Ol	Di
Di	Di	Di	Mag
Mag	Mag	Mag	Ol
Ol	Ol	Tra	Per
Per	Per	Per	Dol
Dol	Dol	Dol	Srp

Table 3. Wells and associated dates of deployment.

CSW 1,1	12/16/2016
CSW 1,4	12/15/2016
QV 1,1	12/16/2016
QV 1,2	12/16/2016

*Capsules placed just above the data loggers

Table 4. Mineral Sample histories/origins:

Srp	Serpentintie	CROMO	Hand sample gathered from surface
Tra	Travertine	White Seep CROMO	Hand sample gathered from surface
Di	Diopside	Bird CK, Herschel, ON, Canada	Mineral standard : Ward's scientific
Ol	Olivine	Twin sisters, mountain's in WA	Mineral standard : Ward's scientific
Mag	Magnetite	Standard Sharon MA	Mineral standard : Ward's scientific
Per	Peridotite	Yellow Dog, MI - std	Mineral standard : Ward's scientific
Aug	Augite	Not specified by packaging	Mineral standard : Ward's scientific
Dol	Dolomite	Sussex County NJ (– std)	Mineral standard : Ward's scientific
Cor	Corundum	Not specified by package. (massive)	Mineral standard: Ward's scientific

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Analysis of Organic Films on Serpentine Wafers Via Micro Fourier Transform Infrared (μ FTIR) Spectroscopy and X-Ray Photoelectron Spectroscopy (XPS) Analyses

Alexander Sousa* and Dawn Cardace: University of Rhode Island, Kingston RI *alexander_sousa@uri.edu

Introduction

Serpentinization is the metamorphic alteration of ultramafic rock¹. This process occurs in diverse planetary settings^{1,2} including Mars^{3,4} and ocean worlds^{5,6}, may support deep life on earth^{7,8}, and is thought to be an abiotic source of low-weight hydrocarbons. Combined these factors make Serpentinization an attractive astrobiological target. However, the abiotic production of hydrocarbons complicates the de facto identification of biosignatures. Here we use spectroscopic techniques⁹⁻¹⁰ to investigate and characterize both organic material which may be preserved within serpentine samples as well as their micro-scale mineralogical context.

Geologic Context

Serpentine samples from the Coast Range Ophiolite Microbial Observatory (CROMO) depicted in Fig 2; a series of monitoring wells drilled into an actively serpentinizing ophiolite in Lower Lake, CA¹⁰. The serpentinites contain the standard assemblage of minerals some of which reflect changing redox conditions (Fig 2).

Fig 1. Northern CA, serpentine deposits in blue. Map originally created for Cardace et al., 2013 generated from USGS data.

Fig 2. Eh-pH diagram (made using Geochemists Workbench ver. 10) shows stability fields of hematite, magnetite, Fe³⁺ and Fe²⁺. Subtle shifts in Eh or pH cross hematite-magnetite boundary.

Methods

Sample Preparation. For External Reflection FTIR (R-FTIR) and X-Ray Photoelectron Spectroscopy (XPS) samples were doubly polished¹⁴ down to ¼ micron grit size¹¹. In some cases samples were washed for 5 min. each in benzene, dichloromethane, and hexane¹². For Total Attenuated Reflectance (ATR) FTIR samples were crushed using a percussion mortar and passed through a 100 micron sieve. Limit of detection experiments were performed using ion chromatography standard SPEX CertiPrep by allowing 200microliters of liquid to dry to 50mg of sample placed in a 96 well plate. *Pseudomonas fluorescens* liquid cultures were produced from FisherSci Cult-loops and were grown using manufacturer's recommendation (LB Broth at 25C for 24 hrs). After 24 hrs 150mL of media was centrifuged at 10,000G for 15 minutes, the supernatant was removed and the pellet was left in a desiccator for 48 hours. This dry material was resuspended in 2mL of water and vortexed creating a 100mg/mL solution.

Micro-FTIR (μ FTIR). FTIR spectroscopy allows resolution of relevant bond energies. IR light is transmitted through the prepared wafer thus data integrate the entire path length; we used a Nicolet iS50 FTIR Spectrometer with Continuum microscope (URI-GSO, Kelley lab).

X-ray Diffraction (XRD). Diffraction patterns serve as mineral fingerprints; data were collected with Co tube and an Olympus Terra XRD (URI-GEO, Cardace lab). Weight percentages below ~3% are not resolvable. XRD served to groundtruth rock and mineral standards

X-ray photoelectron spectroscopy (XPS). On surfaces, XPS analyzes only the first tens of angstroms⁷, resolving thin films. We used a Perkin-Elmer 550 Multitechnique Surface Analyzer (URI-ENG).

Fig 3. Schematic representation and photograph of a desiccated incubation chamber containing various rock and mineral samples. System is fully customizable, and can be re-designed with different mineral baits. Four Microbial Colonization Chambers systems (MCC) (one shown) were constructed of PVC pipe, and nylon mesh and deployed into monitoring wells at the CROMO in December 2006.

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Results

Organic bonds are resolvable in hand samples from CROMO (Figure4). The R-FTIR spectra (Figure 5) is visibly similar. Sensitivity of ATR FTIR is drastically affected by surrounding minerals. Identical concentrations of organic material generate far less pronounced peaks and are swallowed by background noise at lower concentrations in serpentinites (compare figures 6 and 7) as compared to olivine. In ATR mode, organic material in an olivine matrix fails to reliably display organic peaks around 100ppm but is this limit is likely much lower utilizing Reflection and Transmission mode. XPS analysis indicates that Serpentinites should at contain more organic matter on their surfaces¹³ and that Organic solvents can effectively reduce the degree of material of their surfaces.

Figure 4. Transmission-FTIR SPECTRA of a CROMO sample display organic signals (green box). Inset: A transmitted light image of the wafer displays varied mineralogy including: Lizardite, Magnetite, Goethite, Talc and others.

Figure 5. Organic signals detected on glass wafer with concentrated *Pseudomonas fluorescens*

Figure 6. panel 1 (top) displays a pure olivine spectra. Panel 2 (middle) The organic peaks of acetate are visible near 1400, 1550, and 3400 wavenumbers. Panel 3 (bottom) Organic peaks of formate are visible at 1350,1600, and 3400.

Figure 7. panel 1 (top) displays a pure serpentine spectra. Panel 2 (middle) The organic peaks of acetate are visible near 1400, 1550, and 3400 wavenumbers. Panel 3 (bottom) Organic peaks of formate are visible at 1350,1600, and 3400.

Figure 8. Serpentine shows a comparatively weaker organic absorbance relative to mineralogical absorbance whereas on olivine the mineralogical spectra is dwarfed by the organic peaks.

Fig 9. Organic peaks of formate are resolvable to approximately 100ppm using ATR-FTIR

Conclusions

ATR-FTIR analysis (Fig. 6, 7, and 8) demonstrates the importance of mineralogical context in the detection of organic Signatures. XPS analysis (Fig. 11, 12 and 13) reinforces this disparity by demonstrating significantly higher intensities of organic peaks in olivine relative to serpentine. ATR data (Fig. 9) and the suppressive effect of serpentine on organic material suggests that the carbonaceous matter detected in field samples is at relatively high concentrations, or that a changing mineral backdrop may be amplifying the signal. Analysis of Goethite Spectra (not pictured) yielded massive peaks in the same area as amorphous organic material most likely indicating that our Goethite sample (URI Geoscience Collection) was compromised. This may constitute a promising target for organic solvent washes. Goethite grains in CROMO samples may be sorbing organic material during the aqueous process that constitutes serpentinization. The Coast Range has a particular tumultuous history likely traversing many Eh, pH, and Redox paths (Fig 2). Beyond organo-mineral interactions, differences in photoelectric properties, density, or simple optics (translucence) maybe the primary driver of spectroscopic. This work makes clear the need for combination organo-mineral standards to ground in-situ observations and properly prepare for analysis of MCCs and serve as a proxy for environmentally derived positive controls.

Figure 10. The C1s binding electron is detected around 290 eV. Their presence in this sample indicated a level of background loading.

Figure 11. Here we show the efficacy of Ar Ion sputter cleaning in removing carbonaceous material on olivines and serpentinites. (Olivine offset by 200 units)

Figure 12. Samples washed with organic solvents show a substantial decrease in carbonaceous material

Citation

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