

THE DETECTION OF BIOSIGNATURES BY LASER RAMAN SPECTROSCOPY FOR MARS EXPLORATION Jie Wei<sup>1</sup>, Alian Wang<sup>1</sup>, Yanli Lu<sup>1</sup>, Kathryn Connor<sup>1</sup>, Alex Bradley<sup>1</sup>, Craig Marshall<sup>2</sup> and Andrew Steele<sup>3</sup>, <sup>1</sup>Dept of Earth and Planetary Sciences and McDonnell Center for Space Sciences, Washington University in St. Louis, St. Louis, MO, 63130, USA, ([jjewei@levee.wustl.edu](mailto:jjewei@levee.wustl.edu)) <sup>2</sup>Dept. of Geology, University of Kansas, 1475 Jayhawk Blvd., Lawrence, KS 66045, <sup>3</sup>Geophysical Laboratory, Carnegie Institution of Washington, 5251 Broad Branch Rd. NW, Washington DC, 20015.

**Seeking sign of life:** The first few billion years of Mars' geologic history records surface environments considerably different than the surface today, prompting a succession of coordinated surface and orbiter missions over the past two decades aimed ultimately at determining if Mars ever had an early biosphere. Past missions have sought environments where water was abundant and possibly long-lived. Curiosity rover has shown that Gale crater once possessed a habitable fluvio-lacustrine environment at Yellowknife Bay, Gale Crater.[1] Though Curiosity suggested that indigenous martian or meteoritic organic C sources may be preserved[2], no organic matter that might have come from martian microbes has been directly detected by the Mars exploration missions. NASA's 2020 rover mission to Mars calls the detection of biomarkers reflecting bio-history on Mars as one of its primary goals.

Biomarkers are organic compounds with structures and chemistry that are diagnostic for biological processes, particularly those that are stable under harsh environmental conditions in long period of time. Ancient terrestrial biomarkers that have survived extensive diagenesis and catagenesis are suggestive of the compound classes that might be most recalcitrant. If biomarkers were preserved at surface and upper-subsurface on Mars, the concentration would be extremely low, although the local enrichment at fine-scale cannot be excluded. In order to look for needles in hay stacks, analytical tools for molecular phase identification at fine scale is essential, for which a microbeam laser Raman spectrometer (LRS) with scan capability is the right technology.

**Microbeam laser Raman spectrometer:** LRS provides chemical and structural information of a molecule (organic or inorganic), with much sharper spectral peaks than mid-IR spectroscopy. Raman signal strength is proportional to the covalency of major chemical bonds in molecules, thus is extremely sensitive to carbon-carbon bonds (100% covalency but forbidden by IR selection rule) in kerogen and carbon-(H, N, O) bonds in organic matter (>90% covalency). For these reasons, LRS has been a go-to tool, in the past 30 years, to study carbonaceous materials in extra-terrestrial samples (meteorites, stardust, martian meteorites, IDPs). Furthermore, photosynthetic microorganisms was identified using LRS in the interior of halite crusts in the Atacama Desert [3]. Carotenoids

were identified in ancient (1.44 Ma) halite brine inclusions from borehole cores [4].

A LRS system developed for flight, the Microbeam Raman Spectrometer (MMRS), was installed on Zoë rover and conducted autonomous subsurface soil analysis at the Atacama desert during a field campaign in 2013 [10]. A new version of MMRS, Compact Integrated Raman Spectrometer (CIRS), is ready to fly.

This abstract reports our preliminary tests, using a laboratory LRS with equivalent performance as MMRS, to determine the feasibility of detecting potential biosignatures at low concentrations.

**LRS Detection of Carbon:** In living organisms, organic material typically occurs with specific stereochemistry and heteroatom (O, N, S, P) functionalities. Over geological time these functionalities are lost and begin to form a complex cross-linked aromatic-carbon dominated moiety called kerogen. Thus the first step to characterize potential bio-signatures on Mars is the detect and characterize kerogen. Figure 1 shows Raman spectra from two meteorites and two terrestrial chert samples. Two Raman peaks at 1581 and 1355  $\text{cm}^{-1}$  are characteristic G band ("graphitic") and D band ("disorder") of carbonaceous materials [6]. The shape and relative intensity of the two bands reflect the degree of their structural disorder and thus the temperatures that they have experienced.

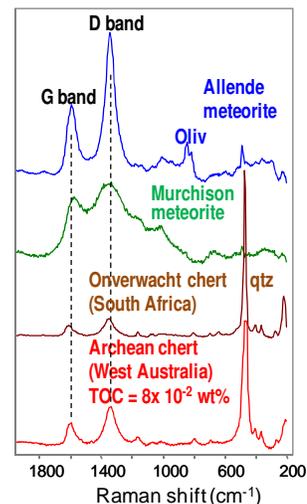


Figure 1. Typical LRS spectra of carbonaceous materials

**LRS Detection of Organic Matter (OM):** Other functional groups in complex organic matter (OM) have distinct fingerprint Raman spectral peaks. In Fig. 2, Raman spectra of some biomarkers, which belong to lipids, amino acids, fatty acids and alkanes groups, are presented. An advantage of LRS is that the C-C (single, double and triple bonds) groups and C-N groups have stretching vibrations resulting in strong peaks, while in IR spectra these peaks are, generally, weak or inactive. Aromatic groups may also be easily examined by LRS. In Fig. 2, symmetric and anti-symmetric C-H stretch-

ing vibrations in CH<sub>2</sub> and CH<sub>3</sub> groups dominates the region from 2800 to 3000 cm<sup>-1</sup>. Weak N-H stretching bands show at shifts bigger than 3100 cm<sup>-1</sup>. No O-H stretching vibration peaks are presented.

Carotenoids are p-electron-conjugated chain molecules. The presence of both intact and diagenetically altered carotenoids in sediments dating back as far as the Miocene has been indicated GC/MS analysis. The peaks in Raman spectrum of β-carotene at 1515, 1155 and 1006 cm<sup>-1</sup> are due to C=C stretching, C-C stretching and CH<sub>2</sub> scissoring modes, respectively [6].

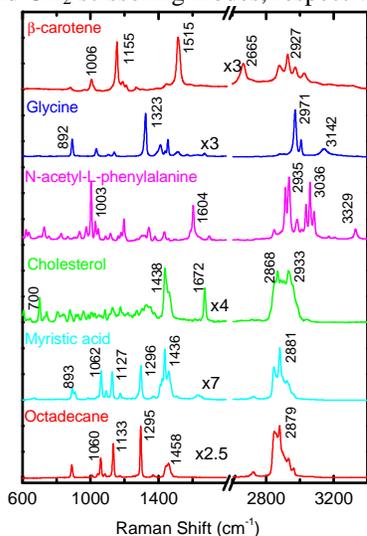


Figure 2. Raman spectra of some potential biomarkers

Glycine is the simplest amino acid. The NH stretching vibration produces the peak with the highest shift of 3142 cm<sup>-1</sup>. The CH<sub>2</sub> stretching vibration produces the strongest peak at 2971 cm<sup>-1</sup>. Of the peaks below 1670 cm<sup>-1</sup>, NH<sub>2</sub> twisting and C-C stretching modes are responsible for the two prominent peaks at 1323 and 892 cm<sup>-1</sup> [7]. The 892 cm<sup>-1</sup> band can be ascribed to the ν(CNC) symmetric stretch of the amino group [8].

Another amino acid that acts as a biomarker is N-acetyl-L-phenylalanine, which has been detected in meteorites. Like glycine. The N-H stretching produces the peak at the highest Raman shift of 3329 cm<sup>-1</sup>. As L-phenylalanine, a benzene structure is contained, which produces intense bands. The peak at 1003 cm<sup>-1</sup> can be attributed to the trigonal ring breathing, and the 1604 cm<sup>-1</sup> band the ring stretching [8]. The peaks between 3000 to 3100 cm<sup>-1</sup> are characteristic of aromatic C-H stretching modes, which is higher than C-H stretch in CH<sub>2</sub> and CH<sub>3</sub> groups.

The cholesterol Raman spectrum has characteristic peaks at 1672 and 1438 cm<sup>-1</sup> due to C=C stretching and CH<sub>2</sub> and CH<sub>3</sub> bending vibrations, respectively. The steroid rings stretching modes produce the peak at 700 cm<sup>-1</sup> and the weak bands around it [8].

Myristic acid is an unbranched saturated fatty acid. The bands between 1050 and 1130 cm<sup>-1</sup>, the band at

1296 cm<sup>-1</sup>, and the band at 1436 cm<sup>-1</sup> are due to C-C stretching vibration, CH<sub>2</sub> twisting vibration and CH<sub>2</sub> or CH<sub>3</sub> deformations, respectively.[8] The bands at 893 cm<sup>-1</sup> is due to in-plane CH<sub>2</sub> bending (rocking) [8].

Alkane-containing molecules are used in biological membranes. As a saturated alkane, octadecane is very stable. The Raman peaks at 1060 and 1133 cm<sup>-1</sup> are due to C-C stretching vibration; the peak at 1295 cm<sup>-1</sup> is CH<sub>2</sub> out-of-plane bending (twisting); and the band at 1458 cm<sup>-1</sup> is due to CH<sub>2</sub> and CH<sub>3</sub> bending or scissoring motions.[9] The peak at 890 cm<sup>-1</sup> can be assigned to CH<sub>2</sub> rocking vibration.

**LRS detection sensitivity of carbon and biomarkers:** No systematic data have been published on the absolute Raman cross sections of solids. In order to determine the relative LRS signal strength of potential bio-signatures, we made mixtures of these materials with standard minerals.

The Archean chert has a total organic carbon (TOC) of 8x10<sup>-2</sup> wt%. We further reduced carbon concentration by mixing Archean chert grain (<88 μm size) with quartz grains and conducted LRS line scan without auto-focusing. Carbon peaks were detected in 7 of 100 LRS spectra for a 1% mixture (TOC ~8x10<sup>-4</sup> wt%). These mixing experiment revealed a relatively large Raman cross section of carbon, compared with quartz.

Table 1. number of LRS spectra among 100 showing detectable biomarkers' peaks

	1 mole%	0.1 mole%	0.01 mole%	0.001 mole%
N-Acetyl-L-phenylalanine	24	2-3	0	
β-Carotene	100	100	97	10
Cholesterol	15	2-3	1	0
Octadecane	39	2-3	0	

Furthermore, we desolved four potential biomarker compounds in organic solvent, mixed the solutions with powdered gypsum at various concentrations, and run LRS 100 spot line scan on each. The number of LRS spectra with detectable characteristic peaks of biomarkers are shown in table 1.

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**References:** [1] Grotzinger et al. (2013), *Science*, 10.1126/science.1242777. [2] Ming et al. (2013), *Science*, 10.1126/science.1245267. [3] VITEK et al. (2010), *Phil. Trans. R. Soc. A*, 368, 3205–3221. [4] Winters et al. (2013), *Astrobiology*, 13,1065–1080. [5] Livneh et al. (2002), *Phys. Rev. B*, 66, 195110. [6] Marshall C. and Marshall A (2010), *Phil. Trans. R. Soc. A*, 368, 3137–3144. [7] Zhang et al. (2006), *Acta Physica Polonica A*, 109, 399–404. [8] Gelder et al. (2007), *J. Raman Spectrosc.*, 38, 1133–1147. [9] Orendorff et al. (2002), *J. Phys. Chem.*, 106, 6991–6998. [10] Wei et al. (2014), *this GeoRaman Conference*.