DETECTING BIOSIGNATURES ON MARS: LESSONS LEARNED FROM MARS ANALOG SITE STUDIES, Jie Wei[1], Alian Wang[1], James L. Lambert[2], David Wettergreen[3], Nathalie A. Cabrol[4], Kimberley Warren-Rhodes[4,] , Fanjing Kong[5], Mianping Zheng[5], [1]Dept of Earth and Planetary Sciences and McDonnell Center for Space Sciences, Washington University in St. Louis, St. Louis, MO, 63130, USA, (jiewei@levee.wustl.edu) [2]Jet Propulsion Laboratory, 4800 Oak Grove Drive, CA, 91109,[3] The Robotics Institute, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh PA 15213,[4] SETI Institute Carl Sagan Center, NASA Ames Research Center, Moffett Field, CA 94035, [5]R& D Center of Saline Lakes and Epithermal Deposits, Chinese Academy of Geological Sciences, Beijing, 100037, China

Introduction: Our understanding of the existence of source elements for life and habitable environmental conditions on Mars has been revolutionarily advanced by the past and current missions to Mars. Now we entered an era to develop missions and robotic investigations for the detection and characterization of the species that might be indicative of martian life, i.e., the bio-signatures or biomarkers.

Studying terrestrial analog sites is a great way to learn lessons on various aspects of robotic planetary missions. Among the selection criteria for a Mars analog site, the most important ones relevant to astrobiology are: (a) having extremely unfavorable conditions to host life; and still, (b) some life-forms were found there. Atacama Desert and a hyper-arid region on Tibet Plateau satisfy these criteria.

We report our field expeditions to these two sites during past six years, using hand-held or rover-deployed instrumentations, along with laboratory analysis of the collected samples for ground-truth. Based on the lessons learned, we developed a strategy for bio-signature detection for future robotic rover explorations on Mars.

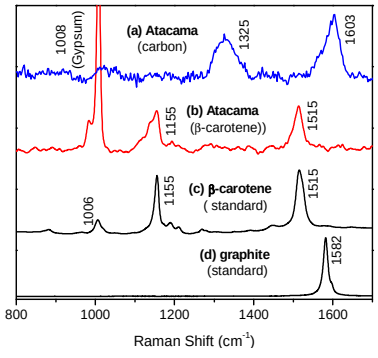
Exploration Technologies: Mars 2020 SDT report (page 42, Fig. 3-15) presents a full evaluation on *estimated detection limits vs. sample processing requirements* among the technologies that are capable for detection and characterization of bio-signatures [1]. During our analog site studies, we deployed four types of *in situ* technologies that require non-sample-processing: laser Raman spectroscopy (LRS), Near IR spectroscopy (WIR), UV-stimulated fluorescence imaging (BUF), and co-registered microscopic imaging (MI).

Atacama Desert and bio-signature detection: The Atacama Desert, which lies in northern Chile between the coast range mountain in the west and the Andes to the east, is one of the driest and oldest desert plateau (2,500 meters above sea level) on Earth. Most area of the desert is hyper-arid with the interior core receives <<5 mm mean annual rainfall [2]. Large amount of sulfates, nitrates, and chlorides signify the final stage of brine evaporation [3]. Despite the cruel environment, bio-signatures were found in the Atacama Desert, including amino acids [4] and photosynthetic micro-organisms [5]. Supported by NASA ASTEP program, Life in the Atacama (LiTA) project has aimed at investigating the regional distribution of life and habitats and also at promoting advanced technology for robotic explorations in future Mars missions [6]. During the LiTA 2012 field campaign, the Mars Microbeam Raman Spectrometer (MMRS), WIR, and BUF were deployed as hand-held or stand-alone. During LiTA 2013 field campaign, MMRS and BUF were installed on Zöe rover, together with a 1-meter drill, and conducted autonomous analyses of surface and subsurface samples during a 50 km traverse. Samples collected during two field campaigns were analyzed using laboratory Raman, IR spectrometers, and BUF for ground truth.

At a playa in the core of the Atacama Desert, MMRS detected *reduced carbons* (spectrum a in Fig. 1) in several samples brought up from subsurface by the 1-meter drill. The relative intensities of G and D bands (at 1325, 1603 cm^{-1}) indicate different degrees of structural ordering. Quantitative analyses using MMRS point counting [7] on playa samples have generated the mineral modes at different depths. Quartz, feldspars, and gypsum were found at all depths, while γ- and β-$CaSO_4$ were found concentrated more at deeper depth. Carbon is found to be a minor phase, while detectable in this playa of sedimentary origin.

In a subsurface salt sample collected from Laguna Azufreras during 2012 LiTA field campaign, typical Raman peaks of carotenoids, one of bio-signature compounds that clearly indicate biological processes, were identified (spectrum b in Fig. 1). The salt sample is mainly composed of gypsum (1008 cm^{-1} peak), while the two relatively weak peaks at 1155 and 1515 cm^{-1} are from C=C stretching and C-C stretching vibrations in β-carotene [8]. Similar Raman spectral patterns were obtained from other spots of the sample, with slightly varied peak positions that indicates the present of different types of carotenoids [8].

Figure 1. Atacama Desert: carbon and β-carotene detected by LRS



DLT on the Tibet Plateau and bio-signature detection: The Tibet Plateau in southwestern part of China is the world's highest (average elevation ~ 4500 meters) and largest plateau. The blockage of the humid air from the Indian Ocean by the highest mountain chain, the Himalaya (average elevation ~ 6100 meter), has generated a hyper-arid region in the northeast corner of Tibet Plateau, the Qaidam Basin, with the aridity index (AI) near 0.01. This extremely low relative humidity, together with the high altitude induced low atmospheric pressure, high UV radiation, and large temperature swing, have dictated an extremely harsh environment for hosting terrestrial life. Our study site, Dalangtan (DLT) saline playa, preserved the oldest salt deposits (Eocene-Oligocene) in the region, where the salt mineralogy represents a final stage of evaporation of K, Na, Ca, Mg, Fe, C, B, S, and Cl-bearing brines. This super saline environment further presents an extremely harsh condition for life. Yet, life of different forms was found in the subsurface regolith at DLT.

Based on a collaborating agreement between WUSTL-EPSC and CAGS-RDCSLED, field expeditions to DLT were conducted by a combined team in 2008, then by a CAGS team in the following years. WIR was deployed directly on the soil or the rock samples in the field, which revealed information on hydrous salts. Laser Raman spectroscopic measurements were made on all collected samples in laboratory, which revealed comprehensive mineralogy to fine-scale. Furthermore, LRS detected *reduced carbon* (Fig. 2a) in several samples collected from subsurface. To confirm the bio-origin of the detected carbon, culture development under aerobic and anaerobic conditions were made. *Two types of bacteria*, a cocoid-shaped and a rod-shaped of µm sizes, were successfully produced. Their 16 SrRNA gene sequences suggest that they belong to the halophile class. Furthermore, direct fluorescent microscopic imaging shows variety of UV-stimulated fluorescent centers at microscopic scale (Fig. 2b). Direct observation using Scanning Electron Microscope has discovered rod-shaped objects of µm sizes (Fig. 2c) over the salt surface, very similar to the rod-shaped halophile produced by culture.

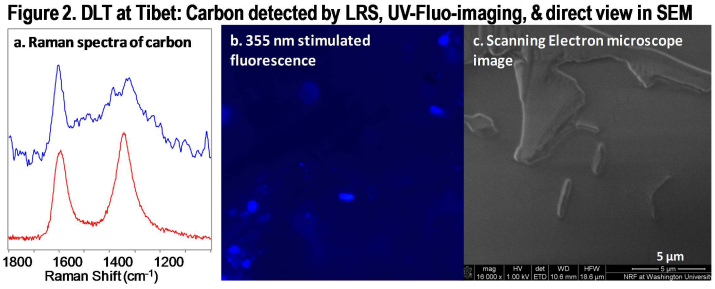
Detecting bio-signature in future missions: Through years' investigations in laboratories on extra-terrestrial samples, it became more and more clear that the definitive answer to *life on Mars* will have to be made based on comprehensive evidences provided by sophisticated laboratory instrumental analyses on the returned samples. For next landing mission to Mars, the strategy should be to identify the potential bio-signatures (PBS) of six categories: organic, isotopic, mineralogical, chemical, macro- and micro-textural in the samples that have well established geological context and evidences for PBS preservation potential.

Experiences through above analog site studies tell us that the search for PBS on Mars has to be done in steps. The ultimate first step should be *in situ* search for *reduced carbon* in routine examinations of martian samples. A sample containing reduced carbon would stimulate the follow-up cross-examination by all payloads. In a mission without extensive sample processing, the detection of reduced carbon can only be achieved by LRS (forbidden for IR spectroscopy by selection rules). Furthermore, LRS has to be made *in situ*, using tightly focused laser beam and checking > 100 spots in a sample, to increase the detection probability.

The follow-up LRS (mid-IR as well) study would be *the characterization of C-H bonds and other chemical bonds made of C, H, O, S, N, Cl in organic maters.* However, the study's feasibility is highly depending on the concentration, thus the tighter sampling spot, the higher detection probability.

UV-stimulated natural fluorescence emission from bio-originated species can be a complimentary evidence for PBS. An UV-Fluo-imager is less information-specific than molecular spectrometers, but will provide spatial distribution that could help the building-up of spatial correlation with micro fabric and molecular IDs, if it will be co-registered with an optical microscopic imager and a line-scan LRS. An instrument suit with all three functions, the Compact Integrated Raman Spectrometer (CIRS), will be able to address three of the six categories of PBS, organic, mineralogical, micro textural. CIRS was proposed to the Mars 2020 mission.

Figure 2. DLT at Tibet: Carbon detected by LRS, UV-Fluo-imaging, & direct view in SEM



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