National Aeronautics and Space Administration

NASA Astrobiology Institute
2016 Annual Science Report
Team Report:

Life Underground,
University of Southern California

Life Underground

Lead Institution:
University of Southern California

Team Overview

Principal Investigator:
Jan Amend

The Life Underground Team is developing and employing field, experimental, analytical, and modeling approaches aimed at detecting and characterizing microbial life in the subsurface—the intraterrestrials—and their host environments. We posit that if life exists, or ever existed, on Mars or other planetary bodies in our Solar System, evidence thereof would most likely be found in the subsurface. Our Team takes advantage of unique opportunities to explore the subsurface ecosystems on Earth through boreholes, mine shafts, sediment cores, marine vents and seeps, and deeply-sourced springs. Access to the subsurface—both continental and marine—and broad characterization of the rocks, minerals, fluids, and microbial inhabitants is central to this study. Our four major research themes are:

- Access to the subsurface
- *In-situ* life detection and characterization
- Guided cultivation of intraterrestrials
- Modeling of ocean sediments

Team Website: https://dornsife.usc.edu/life-underground

2016 Executive Summary

The Life Underground (LU) Team focuses its research efforts on the deep subsurface biosphere on Earth, both continental and marine, with an eye towards potentially habitable environments on other planetary bodies. In 2016, we had major accomplishments in all four of our research themes that led to numerous high impact publications. We also expanded our reach through exciting collaborations, aided graduate students and post-doctoral scholars in advancing their careers, built an interactive computer game, and celebrated a 'genius' in our midst.

Access to the subsurface continued at several unique field sites of astrobiological interest. For example, at the former Homestake gold mine and now Sanford Underground Research Facility (SURF), we established the Deep Mine Microbial Observatory (DeMMO) for long-term geochemical and microbiological monitoring. Numerous student- and postdoc-led projects are now underway, including coordinated microbial diversity, geochemical variability, and lipid biomarker analyses; *in situ* deployment of an electrochemistry cultivation experiment; and a geochemical study of fluid age and CH_4/H_2 sourcing. At the Atlantis Massif core complex in the North Atlantic, members of the LU Team are deeply involved in geomicrobiological studies on serpentinized rocks retrieved on IODP Expedition 357.

As part of our theme on *in situ* life detection and characterization, we developed a spectral imaging pipeline. Rock samples can be analyzed from coarse-scale localization of mineral-associated biomass and bulk mineralogical characterization, to micron-scale UV and Raman analysis and single cell imaging, to even finer scale chemical and isotopic mapping. Part of this analysis suite provides ground-truthing for SHERLOC, a Mars2020 mission flight instrument.

Our guided cultivation of intraterrestrials yielded several novel subsurface isolates, including the first mineral-reducing bacterium from the serpentinizing system at The Cedars in Northern California, two Delftia and Azonexus strains from a deep fractured-rock aquifer in Death Valley, and the first Spirochaete from the deep terrestrial subsurface. Electrochemical enrichment strategies in the field and in the lab were particularly successful in this regard.
In modeling ocean sediments, we combined several global datasets to generate high resolution 3-D descriptions of several

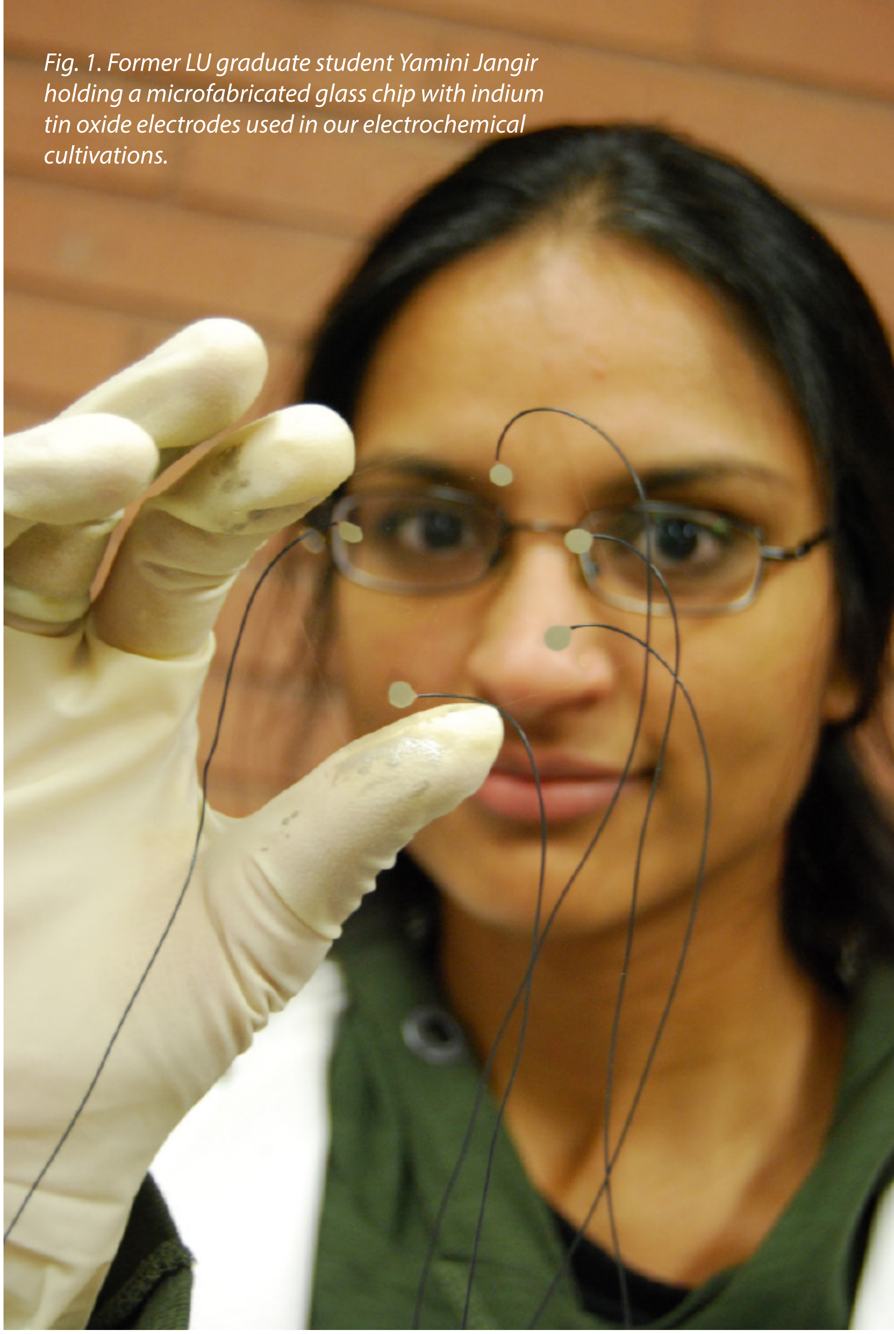

Fig. 1. Former LU graduate student Yamini Jangir holding a microfabricated glass chip with indium tin oxide electrodes used in our electrochemical cultivations.

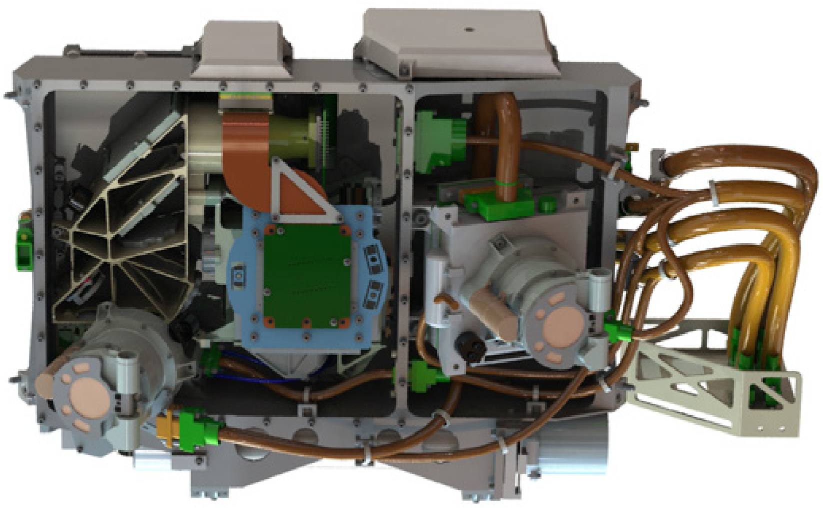

Fig. 2. CAD model of the Mars 2020 mission flight instrument SHERLOC, a deep UV resonance Raman fluorescence spectrometer for detection and characterization of organic compounds and minerals.

Fig. 3. LU research scientist Brittany Kruger and graduate student Caitlin Casar sampling fluids about a mile underground at SURF in Lead, SD. Credit: Sanford Underground Research Facility

physiochemical properties of marine sediments. These models provide far superior estimates of global sediment volume (~3×10^8 km^3), average sediment thickness (721 m), and accompanying porewater volume (8×10^7 cm^3).

LU members continued fruitful collaborations with other NAI Teams, in particular with the University of Colorado-based Rock-Powered Life group (PI: Alexis Templeton) on serpentinizing systems and the associated biosphere, and with the JPL-hosted Icy Worlds group (PI: Isik Kanik) on the emergence of life and habitability in hydrothermal systems on Earth and other water worlds. New Collaborations with Barbara Sherwood-Lollar at the University of Toronto and TC Onstott at Princeton are expanding our reach into the continental deep subsurface through mines in Canada and South Africa. Led by the Game Innovation Lab at USC, we also finished the production of an interactive, computer-based educational experience (Life Underground—the Game) for middle school science students; teacher feedback evaluations and classroom testing are next. Lastly, the LU Team celebrated our Co-Investigator Victoria Orphan, who received a MacArthur Foundation award for her groundbreaking work on the anaerobic oxidation of methane and the role of archaea in deep-sea sediments.

Fig. 4. The LU Team at the annual meeting held at the USC Wrigley Marine Science Center on Catalina Island, CA.

Project Reports

Access to the Subsurface

Maggie Osburn led the establishment of DeMMO at SURF in South Dakota. DeMMO is a network of six legacy boreholes that tap subsurface fluids at depths ranging from 800 to 4850 feet. The boreholes are fitted with custom made high-density plastic packers that enable the ongoing fluid monitoring for chemistry, microbial communities, and physical attributes. Flow-through cultivation reactors were deployed in July and December 2016 expeditions.

At borehole BLM-1 near Death Valley, graduate Student Sean Mullin is leading *in situ* microbial colonization experiments and the characterization of community composition changes as a function of depth and time. Microbial assemblages were characterized using high throughput sequencing, microscopy, and spectroscopic techniques. In addition, a custom sampling device to allow for at-pressure sample collection was designed and engineered in partnership with McPeak Embedded Designs.

Beth Orcutt was the Co-Chief Scientist on IODP Expedition 357 to the Atlantis Massif in the North Atlantic. The research team is investigating the connection of deep life with serpentinization processes. Following the offshore fieldwork, Orcutt co-led the Onshore Science Party in Bremen, Germany, where the rock cores were split, described, and sampled (Fig. 5). Orcutt's group is focused on examining rates of microbial activity and adapting new single-cell based techniques to identify active microbial groups.

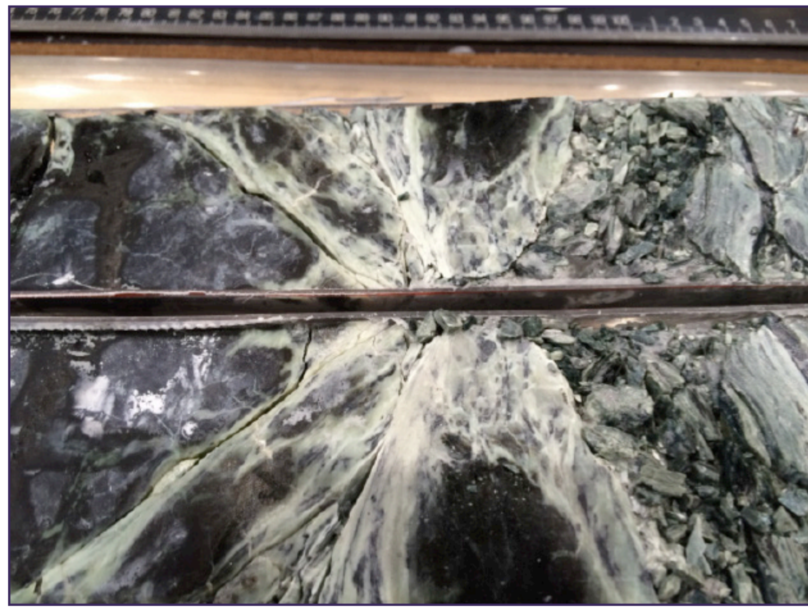

Fig. 5. Fractured and serpentinized rock core collected during IODP Expedition 357 to the Atlantis Massif.

In-situ Life Detection and Characterization

Rohit Bhartia and Victoria Orphan developed a correlated spectral imaging pipeline that fuses mineral, organic, and biologic analyses to bulk analytical methods (genetic diversity/organic inventory) on laboratory standards and at multiple field subsurface environments. This includes *in situ* experiments for assessing mineral-specific microbial colonization and metabolic activity that spans the macro to micro-scale. Field and laboratory incubations of natural and defined minerals in deep subsurface environments including BLM-1, SURF, subseafloor coal and shale beds, and methane seep carbonates have been used for optimization of various components of the pipeline.

The spectral pipeline (Fig. 6, next page) includes coarse-scale localization of mineral-associated biomass (MOSAIC); bulk mineralogical characterization; 10's of μm scale UV and green Raman analysis, and single cell-resolved imaging, chemical, and isotopic mapping using techniques such as SEM-EDX and nanoSIMS. This has led to the incarnation of a biological, organic, and mineral imaging instrument (TORUS), a bench top version of the Mars2020 SHERLOC deep UV Raman/fluorescence instrument. The group also demonstrated fluorescence correlation to biomass, UV Raman analysis of microbial physiological states correlated to variation in nucleic acids and aromatic amino acids content, and mineralogical control of microbial colonization and authigenic biomineralization.
In addition to spectral collection, the LU Team developed a Multi-INstrument-Database (MIND) and a Raman/fluorescence spectral processing pipeline that will also support SHERLOC/Mars 2020 spectral processing and instrument testing efforts.

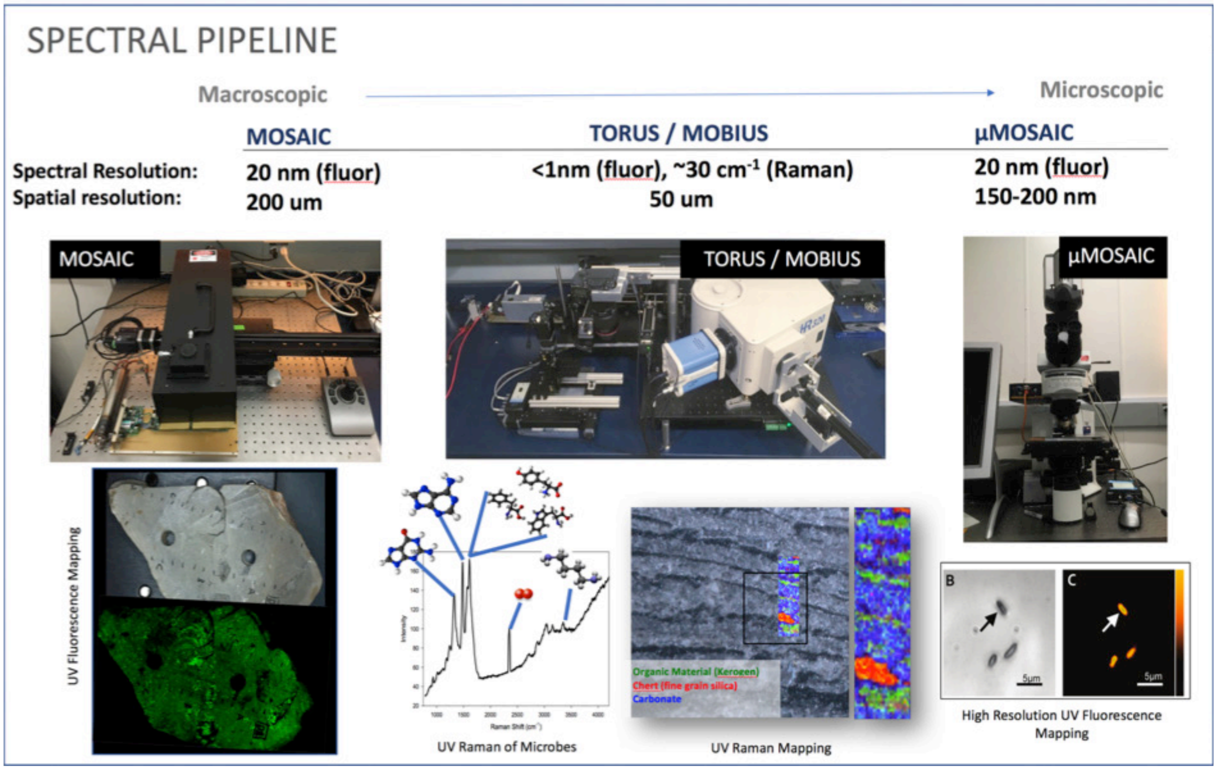

Fig. 6. A pipeline for spectral analysis of samples from microscopic to macroscopic scales, with a focus on handling increasing datasets and bringing new instruments on line (e.g., TORUS).

Guided Cultivation of Intraterrestrials

Postdoctoral fellow Annette Rowe has isolated and characterized the first mineral-reducing microbe from a serpentinizing system (The Cedars, Northern California). An electrochemical enrichment strategy (Fig. 7) provided evidence of active extracellular electron transfer *in situ* within this astrobiologically important environment. The isolated microbe is capable of reducing magnetite—a common end product of serpentinization. Furthermore, pre and post mineral mapping (deep UV fluorescence, Raman, 16S rRNA gene characterization) is providing insight into the selective attachment of microbes to various minerals, and in some cases the altered mineralogy corresponding with microbial attachment. Graduate student Yamini Jangir also reported electrochemical enrichment and isolation of Delftia and Azonexus strains from a deep fractured-rock aquifer in Death Valley.

Graduate students Yamini Jangir and Lily Momper isolated and characterized novel organisms from SURF. Jangir applied electrochemical techniques *in situ* at the 4850 ft level of the fomer gold mine, using four electrodes mimicking different metabolisms. The active community (16S rRNA) on these electrodes largely captured the community present in the borehole water. We subsequently isolated electrode-respiring strains from the genera *Bacillus, Anaerospora, Cupriavidus, Azonexus,* and *Comamonas*. Momper isolated and characterized a novel genus (*Spirosphaera subterraneum gen. nov, sp. nov*) from SURF fluids; this is the first isolate within the phylum Spirochaetes from the deep terrestrial subsurface. It demonstrates pleomorphy under high vs. low nutrient stress regimes, likely an adaptation to the nutrient-poor, energy-limited deep subsurface.

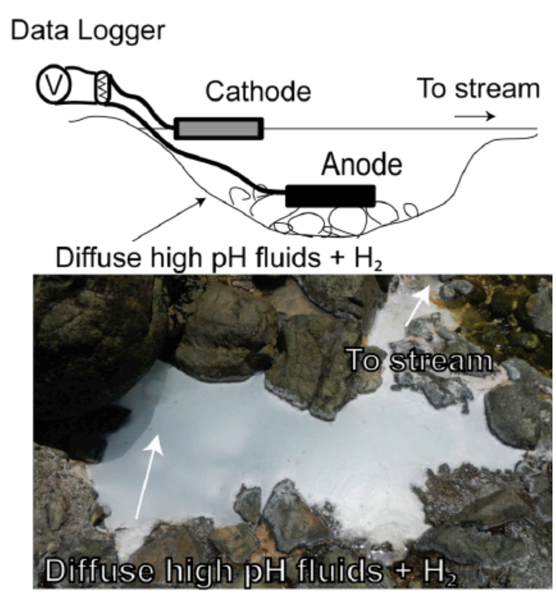

Fig. 7. A schematic (top) and site image (bottom) of the in situ electrochemical enrichment at The Cedars serpentinizing system in Northern California.

Modeling of Ocean Sediments

Doug LaRowe led a study that combined global datasets describing bathymetry, heat conduction, bottom-water temperatures, and sediment thickness to quantify the three-dimensional description of the physiochemical properties of marine sediments (Fig. 4). They showed that >75% of marine sediments are at <80 °C, temperatures where microorganisms can play central roles in biogeochemical processes. Temperature is a master variable that influences the thermodynamic tendency of reactions to happen, the kinetics of these reactions, the diffusion of chemical species, and the physical properties of water that dictate the direction and speed of fluid flow. The modeling study further determined that globally there are ~3×10^8 km^3 of ocean sediment, with an average thickness of 721 m. These sediments are saturated with 8×10^7 km^3 of porewater, making this the second largest reservoir of water at or near the surface of the Earth.

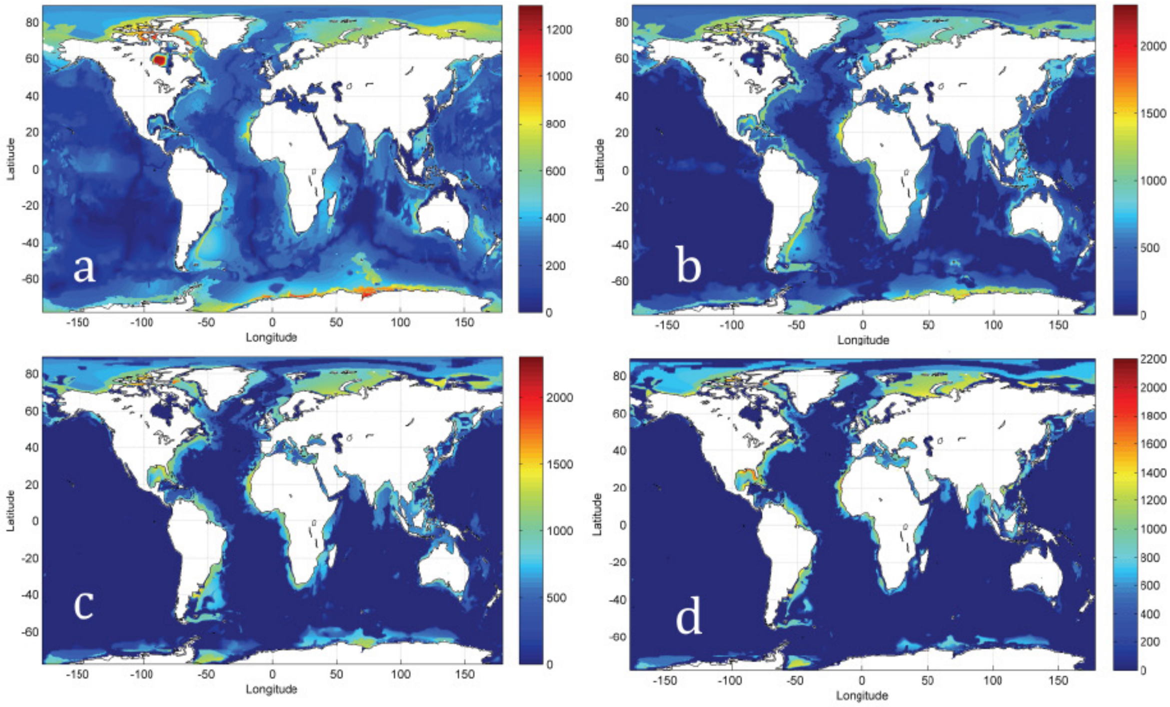

Fig. 8. Global distribution of temperature in marine sediments. In each panel ((a) 0-20 °C, (b) 20-40 °C, (c) 40-60 °C, (d) 60-80 °C), the color scale represents the thickness (in m) of sediment in the corresponding temperature range.

Team Members

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Yuri Gorby
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Brandi Reese
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Everett Salas
Cecilia Sanders
Haley Sapers
Pratixa Savalia
Barbara Sherwood-Lollar
Daan Speth
Elizabeth Trembath-Reichert
Greg Wanger
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Holly Willis
Hank Yu

Life Underground: 2016 Publications

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