Introduction: The NASA Astrobiology Roadmap identifies searching for biosignatures in the subsurface of Solar System bodies as a future exploration objective [1]. Subsurface lava tubes can provide more stable conditions than surface environments, reduced exposure to radiation, and protection from small meteorite impacts [2-4]. Terrestrial lava tubes contain biological microbial mats as well as secondary mineral deposits that host microbial communities [4-9]. By preserving associated organic material through time, secondary minerals provide constraints on the chemical/physical conditions of the fluids involved in their formation and would be a valuable science target on Mars. Lava tubes are identified and mapped on Mars [10-12] and could also provide protection for future exploration missions [13]. Human observation and exploration of lava tubes must be conducted carefully to avoid contamination of potential records of past or present life and avoid negative impacts on crew health.

Objectives: We have begun a study of two lava tube segments on the north flank of Mauna Loa, Hawai‘i as a testbed for determining 1) how best to identify and map subsurface void spaces from the surface while 2) assessing if the subsurface environment hosts native biology. Terrestrial lava tubes are studied as analogs for Mars with respect to microbiology, mineralogy, and as targets of exploration from the surface [14]. However, no study has yet been conducted to explore the balance between lava tubes as an exploration and science target during human operations. Here we report on our first steps in conducting such a study.

Field site: Our research is a collaboration between the Hawai‘i Space Exploration Analog and Simulation (HI-SEAS) team and the Solar System Exploration Research Virtual Institute’s (SSERVI) Remote, In Situ and Synchrotron Studies for Science and Exploration (RIS5E) team. The HI-SEAS station is located on the north flank of Mauna Loa at ~2400 m elevation. This site provides unique, Mars-relevant environmental conditions ideal for this joint NASA Human Exploration Operations Mission Directorate and Science Mission Directorate project.

The studied lava flows are geologically young (~200 years old). Aqueous alteration processes generally happen more quickly on Earth than Mars. As a result, the much older martian basalts’ “alteration state” can be best represented by young terrestrial basalts. The young age and altitude combine for sparsely vegetated terrain, reducing the chance of plant derived organics in cave fluids. The geographic location leads to intermittent exposure to acidic aerosols from degassing at the Kilauea summit lava lake (2008-present) and adjacent Mauna Loa lava flows, emplaced <1 km away (1880-1940). Interactions with volcanic acidic aerosols are a commonly hypothesized mechanism for widespread acidic alteration during parts of martian history [15, 16]. Furthermore, the study site is at a sufficiently high elevation to avoid direct input of oceanic aerosols. Secondary salt cations and anions are thus likely to derive from the basalt or volcanic aerosols.

Methods: The lava tubes were first explored from the surface using Ground Penetrating Radar (GPR) and Magnetic (Mag) surveys. GPR surveys were performed using a GSSI SIR 3000 system with a 270 MHz antenna capable of penetrating to depths of 0-6 meters. Due to the field site’s rough terrain, readings were taken in sounding mode at discrete stations spaced on 5 and 2 m grids. Mag surveys were performed with total field GEM GSM-19 Overhauser and Geometrics G-859 cesium vapor walking magnetometers. Readings were recorded at a 1 Hz sampling rate, along a repeated grid survey with a spacing of 10-20 meters. Mag readings display drops in remnant background magnetic fields associated with subsurface cavities and tubes and enable mapping of the subsurface lava tube locations.

Next, we conducted scans of the tube interiors via Light Detection and Ranging (LiDAR) with a Riegl VZ400. Each scan utilized 0.04° (vertical and horizontal) angular spacing, achieving 7 cm spacing between points 100 m from the scanner. Digital photographs facilitated colorization of the LiDAR point cloud for context. For scans outside the lava tube, a Trimble R8 Global Navigation Satellite System (GNSS) Global Positioning System (GPS) receiver provided a geographic reference frame for the data. We produced a detailed point cloud of the interior for comparison with geophysics surveys and onto which we can map changes in interior mineralogy, chemistry and biology.

Subsequently, mineralogical characterization of cave materials (floor deposits, rock coatings/rinds, etc.)
began with X-Ray Diffraction (XRD) and thermal analysis (thermogravimetry, differential scanning calorimetry, evolved gas analysis mass spectrometry (TG/DSC/EGA MS)) of samples representing the tube interior rocks and secondary mineral coatings. XRD sample patterns were acquired with a Bruker D8 Discover diffractometer from 2-90° 2θ at least 0.02°/step and at least 0.2 sec/step. Mineral compositions and abundances were determined with Rietveld refinement and whole pattern fitting. For thermal analysis, a Setaram LabSys TG/DSC instrument coupled to a Pfeiffer OmniStar mass spectrometer (MS) was used to heat powders to ~1400°C at 35°C/min in a He carrier gas. The evolved volatiles help constrain sample minerals, including minerals present below the XRD detection limit or difficult to characterize with XRD (i.e., non-crystalline materials). Additionally, we used an Olympus Innov-X DELTA Premium Handheld X-ray Fluorescence Analyzer to assess chemistry. The XRF is equipped with a large-area silicon drift detector (with a resolution of approximately 185 eV), and a 4W Rh anode x-ray tube that provides the excitation source. The specific x-ray tube geometry and variable excitation source configurations allow for semi-quantitative analysis of a large range of the periodic table. This instrument provides near real-time feedback, a capability that humans on Mars may have to assess the potential for materials that might harbor life.

Lava tube samples were steriley collected and preserved in Zymo DNA/RNA Shield at the same locations sampled for mineralogical analysis. Next generation sequencing of this initial set of samples, including many secondary mineral deposits, has been completed in collaboration with Georgetown’s Shared Genomic and Epigenomic Sequencing Center, and metagenomic analysis is underway. This sequencing will enable evaluation of what types of microorganisms are present as well as their metabolic pathways. Results will help illuminate the role of microbial mediation in the formation of these rare cave deposits, with implications for astrobiology and life detection on Mars, as well as shed light on the minimum environmental conditions for sustaining life in young basaltic terrains.

**Results:** Lava tubes were accessed via skylights to map the paths of lava tube segments. Geophysics data supports interpretations of subsurface void space and could be acquired by one crew member with a suit-mounted Mag instrument. LiDAR data has confirmed the exact location and internal structure of the tube, as well as identified physical hazards. Our results indicate that geophysics work from the surface confirms the presence of the void space, but additional subsurface analyses are required to map tube dimensions.

Compositional XRF analyses indicate in real-time that secondary materials were Na Sulfates, interpretations confirmed by longer integration time XRD analyses and TG/DSC and MS analyses that also identified calcite, gypsum, and monohydrocalcite, a metastable phase that forms from a Mg-bearing amorphous Ca carbonate precursor. Preliminary research indicates that these materials are dominated by Proteobacteria and Actinobacteria and deeper sequencing is underway to elucidate metabolic pathways.

Results obtained thus far are focused on evaluating exploration operations techniques for crew to interact with lava tubes on other Solar System objects. The diverse biology observed in Earth’s lava tubes may be observed in some form in Mars’ lava tubes. The HI-SEAS field station enables an assessment of how best to combine science goals with human exploration goals that might consider lava tubes as a resource. We seek to refine approaches to characterize lava tube dimensions and physical hazards from the surface and search for materials that might indicate presence of biology prior to humans entering the lava tube. This work will continue at the HI-SEAS site as well as in the Lava Beds National Monument during our recently funded Lava Tube Explorer project funded through NASA’s PSTAR program.

**Acknowledgements:** This project is funded by the HI-SEAS team via NASA’s Human Research Program and SSERVI to RIS’E. All research was conducted under a data collection permit from the Office of Conservation and Coastal Lands at the Department of Land and Natural Resources and a Conservation Distributed Use Permit (HA-3665).