Spectral Profiling & Imaging (*SPI*): Extending L.I.F.E. technology for the Remote Exploration of Life in Ice Caves (R.E.L.I.C.) On Earth and Mars

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ABSTRACT

On Earth, the ice of the lakes, glaciers, and caves of the cryosphere (from the ancient Greek word cryos, meaning "cold" or "ice") harbors rich, complex biotic communities. Ice cave habitats have been posited for the Mars cryosphere. Ice in such caves would protect microbes from UV, X-rays, and heavy particle radiation and might be accessible during robotic or astronaut missions. Detection of putative biota-rich ice will require *in situ* detection of biosignatures in cave walls, floor, and ceiling a few centimeters to tens of meters distant from the investigating rover or astronaut. We describe the development of a prototype for a non-destructive, non-contact device that rapidly generates reflectance and fluorescence images and a midline target profile of 960 reflectance and fluorescence spectra. Spectral Profiling and Imaging (***SPI***) requires no irreplaceable consumables and can be sufficiently miniaturized to be used by a single astronaut or a small robotic explorer. The current laboratory instrument is designated ***SPI***^{2} since it generates data sets for two optical phenomena: reflectance and fluorescence. In final form ***SPI***^{4} will be integrated with an autonomous rover and generate data for four optical phenomena: reflectance, fluorescence, Raman scattering, and circular polarization. ***SPI***^{4} will be useful for the Remote Evaluation of Life in Ice Caves (**R.E.L.I.C.**) on planetary bodies whose distance from Earth prohibits real-time mission control.

Keywords: Epifluorescence Imaging, Epifluorescence spectroscopy, Raman Spectroscopy, Mars, Cryosphere, Robotics, Extremophiles, Biomedicine

1. INTRODUCTION

The appearance and evolution of life depends on the existence of ecological niches offering protection from ionizing radiation and access to an efficient energy source. On Earth, a thick atmospheric blanket protects life from heavy particle bombardment or high-energy γ-ray, X-ray, and ultraviolet (UV) photons, but allows visible and infrared sunlight to provide an energy source two orders of magnitude more efficient than the nearest competitive metabolic system. On Mars, in the absence of effective atmospheric blockade, radiation shielding must be provided by ice, rock, or soil to permit survival of microbial life. ESA's ExoMars project has embarked on an ambitious plan to develop drilling technology to search for life beneath the surface of mid-latitude Mars[1]. Maximum drill depth is targeted at 2 meters. Biomass survival simulations for 2-meter depths in a water-ice regolith yield a half-life of less than 10^{6} years[2]. Unless the subsurface of the Mars mid-latitudes is periodically exposed to liquid ground water sufficient to permit cellular metabolic repair of accumulated radiation damage, it is unlikely life will be detected at the 2-meter target depth. Unfortunately, drilling deeper results in prohibitively high mass and fuel budgets for the mission.

Ice caves with equatorial-facing entries or entries facing high albedo ice and snow bathed in sunlight would provide solar energy for photosynthesis, offer protection from desiccation and radiation, and could be accessed by human or robotic explorers without the use of heavy, complex drilling equipment. On Earth the ice of glaciers, lakes, and caves harbor complex, but poorly understood biotic communities[3]. Ice caves are posited for the Mars polar regions[4]. If these ice caves exist in sites accessible to a rover mission, a robotic search for microbial life would

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be quite feasible. Cave exploration would require a rover capable of autonomous navigation and autonomous identification of appropriate samples. Final identification and characterization of putative life forms would require sample return to microbiology laboratories at an astronaut field station or to microbiology and molecular biology laboratories on Earth. Sample selection instruments for such a mission would be required to detect "remote" biosignatures in the ice of cave walls, floor, and ceiling centimeters to tens of meters distant from the rover. Figure 1A and 1B depict the dimensions, the rugged terrain, and the ambient illumination that can be encountered in ice caves by rovers, human explorers, and microbes. Figure 1C depicts the illumination of microbial life using a hand-held 532 nm laser. Instruments for such contamination-sensitive environments must be non-destructive, non-contact devices requiring no irreplaceable consumables.

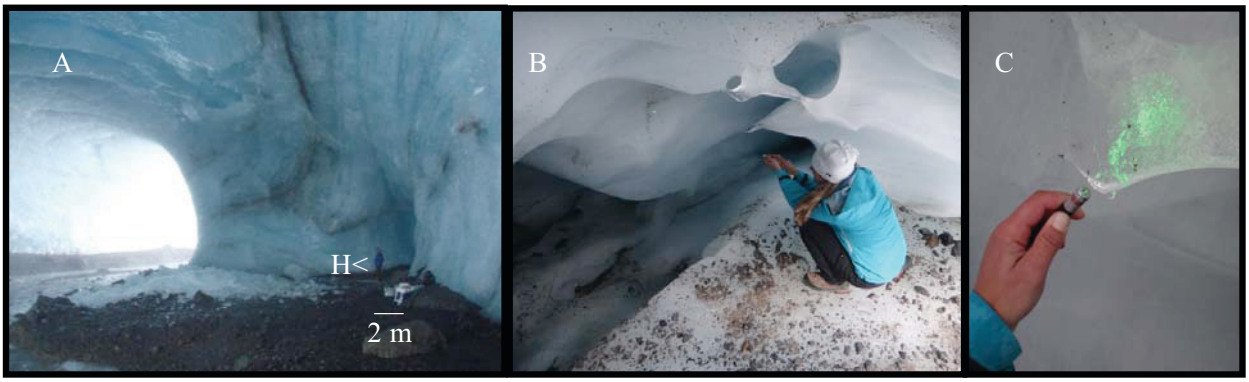

Figure 1. Cave geometry poses significant challenges for either human or robotic exploration. In Iceland's Kverkfjoll ice cave (**A**) instruments will need to navigate a rubble-covered floor and interrogate targets as much as 10 meters overhead (for scale **R.E.L.I.C**. team member Hoover (**H**) stands near far wall). Rotmoosferner ice cave (**B**) in the Austrian Alps poses further challenges for rover navigation including steep terrain and vaulted ceilings. Laser induced fluorescence emission (**L.I.F.E**.) signatures (**C**) are obtained by **a R.E.L.I.C**. team member Sattler.

We report here on preliminary efforts to develop field and laboratory techniques optimized for the Remote Evaluation of Life in Ice Caves (**R.E.L.I.C**.). The effort merges the optical field survey techniques our team has used to find life in ice during Antarctic and Arctic campaigns with microbiology and molecular biology laboratory techniques we have employed to identify microbial species, detect medically important biomolecules, and understand cryosphere carbon budgets. **R.E.L.I.C**. directly engages students in instrument development, field campaigns, laboratory analysis, and development of robotic planetary field survey strategies. In this communication we first describe the use of optical techniques to search for life in the cryosphere. We then review the laboratory procedures most effective for detecting and characterizing microbial extremophile communities. Finally, we report on construction of a unique Spectral Profiling & Imaging (***SPI***) device and review the pattern recognition, communication, and navigation demands for a **R.E.L.I.C**. rover.

2. OPTICAL SIGNATURES IN THE CRYOSPHERE

Reflectance, fluorescence, and Raman techniques have previously been employed by **R.E.L.I.C.** team members to distinguish bacteria from Mars analog soils[5, 6], find microbes in deep subsurface Hawaiian basalts[7], detect microbial life in Antarctic ices[8, 9], and define PAH detection limits for the ExoMars rover enhanced with either 365 nm light emitting diodes[10] or a 375 nm laser diode [6]. Figure 2 depicts the utility of co-registered laser induced fluorescence emission (L.I.F.E.) imaging and Raman spectral data in a Mars mineral analog (Fig. 2A) and a fossilized endolithic community (Fig. 2B) from the McMurdo Dry Valleys. Fig. 2C is a composite of visible light and L.I.F.E. imaging, the latter produce by excitation with 224, 248, or 335 nm lasers (emission following 224 nm excitation is shown). Fig. 2D depicts the resonance Raman (RRS) spectrum following 224 nm excitation. RRS detects signatures in the sandstone for carbonate, nucleic acids, and both bound and unbound water. Longer wavelength 365 nm LEDs and small (5 mW) 532 nm and 640 nm lasers can elicit L.I.F.E. signatures from microbial consortia living within Antarctic lake and glacier ice[6, 8]. L.I.F.E. biosignatures originating in photosynthetic pigments of microbes living in Alaskan river ice can be elicited following excitation with a 532 nm, 5 mW laser diode from a small plane flying at an altitude of approximately 100 meters above the target[11]. While reflectance and L.I.F.E. imaging data for these Antarctic and Alaskan campaigns made it possible to rapidly detect fluorescent

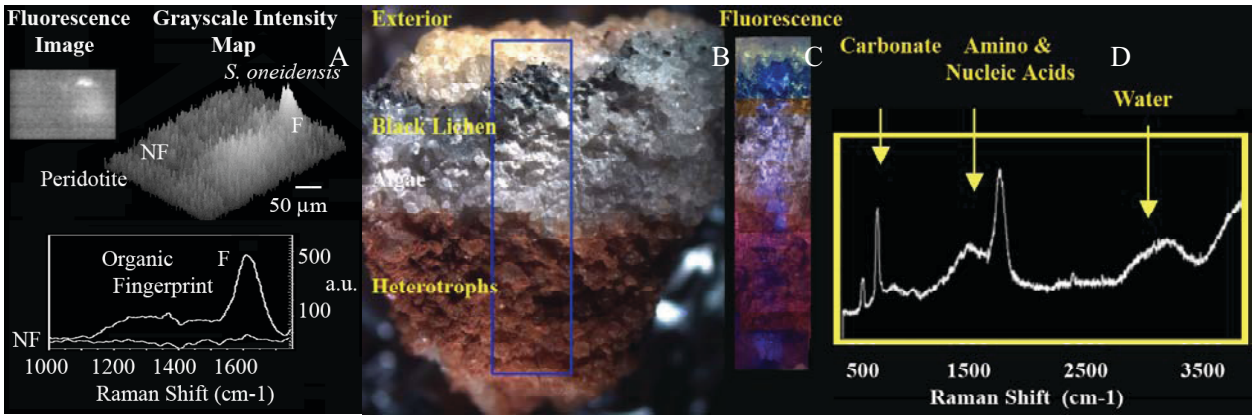

Figure 2. A microorganism, *S. oneidensis*, doped onto Mars analog peridotite (**A**) can be rapidly found by **L.I.F.E.** imaging when excited with 224, 248, or 335 nm lasers. Resonance Raman spectra (**RRS**) for non-fluorescing (**NF**) peridotite differs significantly from the strong organic fingerprint signature seen in the fluorescing (**F**) region. An Antarctic microbial community living within a rock from McMurdo Dry Valleys (B) is detected by **L.I.F.E.** imaging (**C**) when excited at the same wavelengths. **RRS** (**D**) identifies carbonate minerals, amino and nucleic acids, and bound and unbound water. Adapted from Storrie-Lombardi 2005.

material in ice, the lack of spectral chemical signatures co-registered spatially and temporally with the imaging data made it difficult to distinguish false positive mineral signatures from putative microbial signatures. Field data required extensive post-processing after return from the field and close comparison with laboratory microbiological and molecular biology data. In addition, certain photoprotective molecules such as carotenoids may not fluoresce and can effectively quench fluorescence from other pigments and proteins. Carotenoid detection and function can be accomplished even under conditions of extreme desiccation using circular polarization (CP) data derived from reflectance, fluorescence, and/or Raman spectra[12-16].

While reflectance and fluorescence imaging can quickly (milliseconds) identify targets of possible interest and place them in proper spatial context, these imaging signatures are not sufficiently unique to be considered "proof of life". Hyperspectral reflectance, fluorescence, or Raman imaging is technically possible, but comes with gigabyte to terabyte image processing, storage, and transmission costs that are excessive for a real-time field pattern recognition system. Such demands from hyperspectral imaging data pose an overwhelming challenge for the Deep Space Network. Chemical information derived from co-registered fluorescence, Raman and CP spectral profiles extracted from a midline transect through a context reflectance or fluorescence image could identify both the molecular components of living organisms and also detect metabolic signs of extant life while requiring minimal storage and processing overhead compared to the original RGB context image. The next section describes the first implementation of a unique imaging and spectral profiling instrument, a prototype of a system under construction that will acquire co-registered reflectance, fluorescence, Raman, and CP data.

3. SPECTRAL PROFILING AND IMAGING (*SPI*): Optimizing Information While Minimizing Data Loads

Rapidly surveying extreme environments and transmitting maximum information back to mission control while using minimal bandwidth is the core challenge for robotic exploration of our solar system. For life detection tasks the probe should simultaneously acquire both spatial and chemical information from both the putative concentration of biological material and also from the surrounding habitat. In the case of organisms encapsulated in cryoconites the device should capture in a single image and spectra the well-defined mixture of microorganisms and minerals as well as the surrounding ice. In the case of endolithic communities the system should be able to capture multiple layers and detect shifts in either the metabolic activity and/or the dominant species in each level of the layered community across a day/night cycle. During the past two months, we have constructed an optical bench prototype of an instrument that meets these requirements. The basic optical track appears in Figure 3. The device currently consists of two primary components: a physical optical track that manipulates incoming light and software that records and analyzes images from two CCDs. In brief, a sample is illuminated by ambient light, 5800K temperature white LEDs, 365nm ultraviolet LEDs, or via lasers of multiple wavelengths. The optical track then simultaneously forms two images onto two 1.3 megapixel camera CCDs. One image is a color image of the sample

(either reflectance or fluorescence), while the other grayscale image is of a grating-diffused light spectrum corresponding to a thin vertical column transecting the midline of the sample image. The software interface interacts with the two cameras to capture and process these two images. The final result of this data processing is an RGB color image and 960 spectral plots each representing chemical data from a 1-10 pixel wide band vertically traversing the sample at the image midline.

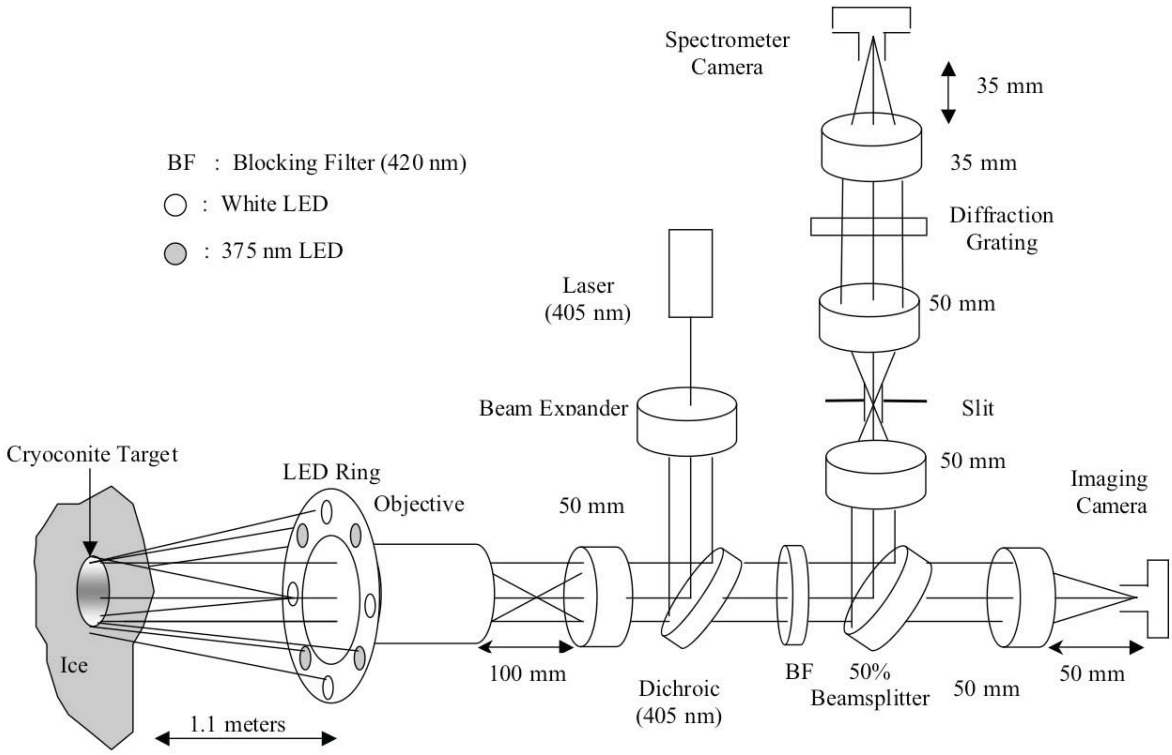

Figure 3. Optical layout for prototype Spectral Imaging & Profiling (***SPI***^{2}) system capable of collecting reflectance and fluorescence information.

Figure 4 demonstrates some of the data output of the system. The target is an artificial cryoconite ice core 9 cm in diameter created from Mono Lake cyanobacteria-dominated tufas. The lake is a highly alkaline soda lake that is used frequently by the astrobiology community to investigate microbial biodiversity in glacial lake systems and to test robotic field survey instruments in a harsh, alkaline, cryosphere environment[9, 12, 17-19]. Mono Lake microorganisms resident in the tufas experience annual lake surface freeze-thaw cycles. A calcium carbonate tufa and the resident microbial assemblage were frozen overnight in pH 11.5 lake water in the shape of a standard ice core. The artificial core was illuminated first with two white LEDs (Fig. 4A) and then a 5 mW 405 nm laser (Fig. 4B) from a distance of 1.1 meters for 0.5 seconds. The sparkling green-white upper half of the reflectance image (F.4A) is a frozen mixture of water, salts, organisms and metabolic products. The bright white region at 7 o'clock is an exposed piece of the tufa. The dark mass between 5 and 6 o'clock is a small biofilm. In the **L.I.F.E**. image (Fig. 5B), everything visible is fluorescence following excitation at 405 nm (λ_{Ex}=405nm) with emission wavelengths longer than the 420 nm blocking filter (BF) cutoff (λ_{Em} >420nm). The diffuse red-brown fluorescence in the upper half arises from the organisms and their metabolites, particularly photosynthetic pigments suspended in ice. The tufa produces a broad (white) fluorescence). Biofilms on the tufa appear as bright red localized L.I.F.E signatures. Spectral profiles (960 reflectance and 960 fluorescence spectra) were obtained from a midline transect of the target. Four reflectance spectra are depicted in Fig. 5C and co-registered fluorescence spectra appear in Figs. 5D-G. Each plot represents the mean of 10 contiguous spectra. Spectra are coded according to type and number of pixels from top of image. For example, Rp250 and Fp250 would be the Reflectance and Fluorescence spectra acquired from row 250 on the spectra CCD that is co-registered with midline data from row 250 on the RGB image. "p" refers to the chemical profile of the target generated by the full set of 960 spectra. Qualitative differences are apparent in the reflectance and fluorescence spectra for the ice mixture (Rp|Fp250 and Rp|Fp400) compared to the tufa (Rp|Fp550)

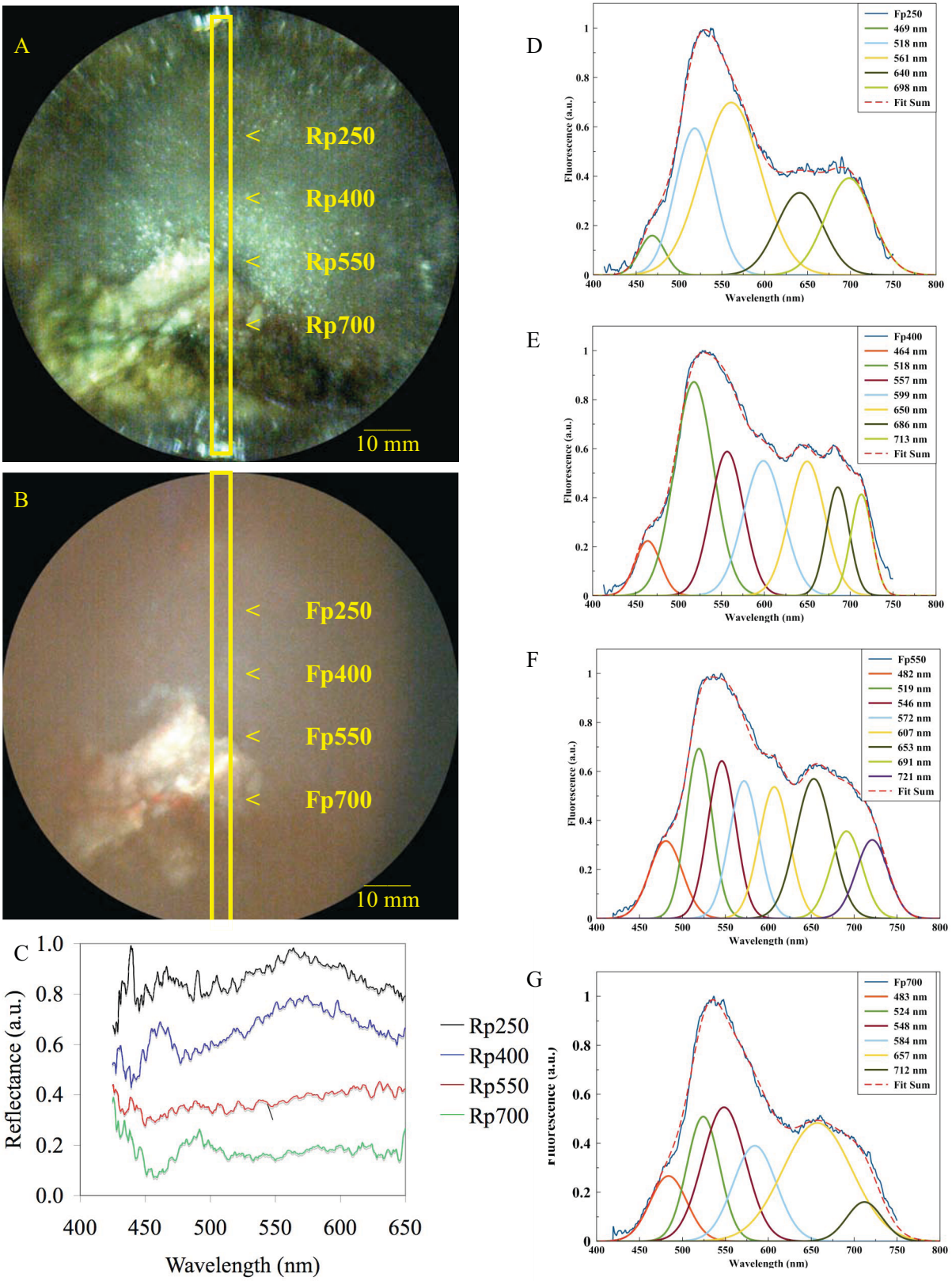

Figure 4. Mono Lake cryoconites at a distance of 1.1 meters with white LED and 405 nm laser illumination producing (**A**) RGB image, (**B**) fluorescence image, (**C**) reflectance spectra profiles (**Rpn**), and (**D, E, F, & G**) fluorescence spectral profiles (**Fpn**) with Gaussian fitting. (4 of 960 spectra shown).

and thick biofilm (|RpFp700) regions. To establish statistically reliable quantitative differences Gaussian modeling

and deconvolution of the spectra is accomplished. Comparison of maxima and integrated power for the Gaussian set required to produce a target spectrum is the first step in identifying the molecular sources of the **L.I.F.E**. signature. Gaussian curves and the synthetic spectra they produce appear in Figs. 5D-G. Spectral differences arise from variations in dominant species and/or metabolic state. Since the instrument provides co-registered reflectance and fluorescence images and midline spectral profiles, it rapidly identifies "regions of interest" and can detect localized spectral shifts across time characteristic of metabolic activity in a portion of living microbial community. More precise chemical identification of pigments and proteins requires Raman and CP data, as well as multiple laser sources. Work planned for the fall school term at HMC includes installation of 532 and 640 nm lasers. This version of the **S**pectral **P**rofiling and **I**maging device is designated ***SPI*^{2}** since the prototype generates two information data types: reflectance and fluorescence. The final configuration, ***SPI*^{4}**, will generate reflectance, fluorescence, Raman, and CP data using 375, 405 455, 532, and 640 nm lasers.

4. LABORATORY MICROBIOLOGICAL & MOLECULAR BIOLOGICAL CHARACTERIZATION OF CRYOSPHERE HABITATS

Microbiological laboratory studies by **R.E.L.I.C.** team members have led to discovery of new genera and species of bacteria from the polar regions of Earth[20, 21], microbial communities in cloud ices[22], environmental pathogenic bacteria[23], and biomolecules important for cryopreservation[24] and bioremediation[25]. These microbial characterization techniques include pyrosequencing (biodiversity), field emission scanning electron microscopy (FESEM) with EDS (elemental abundance determination), GC-MS (organics identification), flow cytometry, aerobic and anaerobic cultivation techniques; and epifluorescence, phase contrast and high magnification dark-field video photomicroscopy for analyses of cell morphology and motility.

Identifying a Living Entity & New Species Discovery - Discovery of new microbial species begins with description of morphology and verification that the microbe is alive. The observation of non-Brownian, non-linear self-propelled locomotion (motility) provides clear and convincing evidence of extant life[26]. It was the motility of "little animals" that allowed the father of microscopy, Antoni van Leeuwenhoek, to discover bacteria three centuries ago and recognize them as living organisms[27, 28]. Motile prokaryotes move in aqueous environments by swimming or by gliding along surfaces. The swimming, twitching, rotating or gliding motility of bacteria is produced by a variety of structures including flagella, axial filaments, pilli, fimbriae, slime and possibly other processes that are not as yet fully understood[29, 30]. Differentiation of motility from the movements of abiotic particulates or dead bacterial cells (generated by capillary action, convection currents, or Brownian motion) can be accomplished in minutes by a microscopist in a field laboratory and is amenable to robotic image analysis techniques. Observations of rapidly swimming bacteria from the interior of a field-melted ice core extracted from a golden brown zone of a frozen Pleistocene thermokarst pond in the CRREL Permafrost Tunnel in Fox, Alaska allowed **R.E.L.I.C.** team member Hoover to detect cryopreserved living bacteria in this ancient ice. These motile, rod-shaped, Gram-positive, chemorganoheterotrophic, psychrotolerant bacteria are now formally recognized as *Carnobacterium pleistocenium* [21]. This methodology of ultra-high magnification, dark-field video microscopy has been used to evaluate hundreds of ice samples during field expeditions to Alaska, Antarctica, Patagonia, Siberia, Iceland, and Austria. The technique and will be employed for preliminary field laboratory assessment of the viability of cryosphere samples identified by the **R.E.L.I.C.** field instrument.

While observation of bacterial motility unambiguously demonstrates that organisms are alive, the absence of motility does not establish whether the putative bacterium is dead or is an inorganic particle. Microorganisms, particularly psychrophiles, may be non-motile, inactive, or adhered to a mineral substrate. In fact, for psychrophiles growth and production of metabolic products can be extremely slow (months to years). As a result, other tests for life are employed in the laboratory including epifluorescence microscopy, scanning electron microscopy, and culture-dependent methods. Of great utility is the combination of Field Emission Scanning Electron Microscopy (FESEM) images with Energy Dispersive X-Ray Spectroscopy (EDS) to characterize the elemental abundances of discrete targets and the soil or rock habitat. Extant microbial life can be distinguished from fossil relics (and both living and fossilized microbes from minerals) and from the surrounding mineral matrix by comparing carbon, nitrogen, oxygen, phosphorous, and trace metal abundances[31-33].

Detection and Characterization of Microbial Food Webs - Ice caves can be either relatively short-lived glacial caves, or rock caves containing long lasting ice. The latter are more stable in shape, duration, and luminosity. Caves with permanent ice bodies are well known as refuges for microbial communities which need to adapt to long-duration conditions such as permanent cold temperatures near freezing point, low light levels, low organic content of nutrients, and relatively few primary producers (photosynthetic microbial species). Both types of caves can exhibit

low light levels and protection from radiation. At present relatively little is known about the microbial diversity and interlocking food web dependencies of either rock or glacier ice cave ecosystems. Investigation of ice caves in the Austrian Alps and Antarctica has shown that cave floor microbial species are similar to the populations found in the water and air flowing into the cave. Moreover, there is a gradient of autotrophic organisms from the cave entrance into the inner, darker portions of the cave. Studies from Grubstein ice cave in the Dachstein Alps (Austria) showed very low similarity of the microbes found in sand in the interior compared with ice cores. There is no evidence that multicellular organisms such as tardigrades are present. If that observation is confirmed, the living communities in ice caves are microbially dominated meaning that the food webs are truncated and consist of viruses, bacteria (auto- and heterotroph) and algae (mainly snow algae at the cave entrance). These organisms and their food webs have not been described.

Discovery of Biomolecules for Molecular Medicine - The adaptive capabilities of bacteria in Earth's cryosphere are often marked by their ability to synthesize secondary metabolites. These structurally and functionally diverse compounds provide bacteria competitive advantage over other microflora or protect them from subzero temperatures, desiccation and radiation damages. Metabolite diversity in microbial populations is enhanced by horizontal gene transfer allowing the rapid development and spread of bioactive compounds between disparate species [34], a process particularly prevalent in isolated extremophiles ecosystems[18, 19]. Some of these natural products or bioactive compounds have resulted in the discovery of novel antimicrobial agents against a growing number of multidrug resistant human pathogens [35, 36] and anticancer agents against a wide range of cancer cells[37, 38]. Studies have shown that cold-adapted microorganisms could be valuable source of bioactive compounds[39]. In a previous study, 22 out of 580 bacterial strains from terrestrial and marine locations across the Terra Nova Bay of Antarctica, exhibited antimicrobial activity against *Escherichia coli*, *Proteus mirabilis*, *Micrococcus luteus*, and *Bacillus subtilis* [40].

Cryosphere cave ecosystems have been shown to be a rich source of cold-adapted organisms producing a variety of bioactive antimicrobial compounds. Cave temperature stability near 0 ^{o}C and the absence of freeze–thaw cycles selects for cold-adapted bacteria (Gounot, 1999). A variety of microbial species isolated in an extensive Kentucky cave system for ~2 My years exhibit significant resistance to a multiple antibiotics[41]. Actinomycetes found in Thailand cave soils exhibit antifungal activity[42] and Actinomycetes from karstic caves in Turkey exhibit antimicrobial activity to multiple bacterial, filamentous fungi, and algae species[43]. An antimicrobial metabolite extracted from *Streptomyces* sp. 1492 exhibited bactericidal activity against antibiotic resistant clinical bacteria strains including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterobacter faecium* (VRE), and *Acinetobacter baumanii*. The cave microbe *Streptomyces tendae* produces cervimycin, a polyketide glycoside exhibiting bactericidal activity against VRE pathogens[44].

Recently, **R.E.L.I.C.** team member Bej and co-workers identified and characterized a purple violet pigment (PVP) from an Antarctic icy lake bacterium, *Janthinobacterium* Ant5-2 (U.S. patent application #PCT/US10/24823, August 19, 2009), that exhibited strong antimicrobial activity against XDR and MDR strains of *Mycobacterium tuberculosis*[35] and MDR/MRSA strains of *Staphylococcus aureus*.[45] In addition, the PVP also exhibited effective anticancer activity on skin fibrosarcoma and melanoma cancer cell lines *in vitro* without any adverse effects on the non-cancerous normal keratinocytes and skin fibroblast cells.[37] *We hypothesize that the perennial ice layers in ice caves provide ecologically extreme conditions for microorganisms to synthesize secondary metabolites that could have direct applications in biomedicine*. Our objective over the next five years will be to isolate pigmented bacteria from cave ice samples and test the antimicrobial activities of their crude extract against human pathogens by high-throughput (HTS) screening method. We will employ 96-well format cell viability colorimetric resazurin assay for initial screening. We have successfully used this method in the lab for bacterial pigments.[35] MTT assay will be employed to screen for initial screening to detect anticancer activity of the bacterial extract. The crude extracts exhibiting inhibitory activities will be subjected to further purification using standard protocol (HPLC) and the potent pigments or biomolecules will be identified by chemical structure analysis using mass spectrometry and NMR.[35, 37, 46]

4. AUTONOMOUS ROBOTIC SAMPLE SELECTION IN REMOTE ICE CAVES

While cryosphere cave structure is unknown for putative sites on Mars, the ice moons of the outer planets, or as yet unexplored terrestrial exoplanets, the caves of Earth's own cryosphere provide a rigorous set of field sites to develop **R.E.L.I.C.** technology. Field sites currently under investigation by human members of the R.E.L.I.C. team include both rock and glacier caves in Iceland and the Austrian Alps. The Austrian Alps provide some of the largest ice caves in the world such as Dachstein Eisriesenwelt, an excellent representative of ice-covered rock caves.

Very few investigations have been completed on the cave's microbial communities, but efforts are underway to date ice cores from the cave at the University of Heidelberg. For a glacial cave we have chosen the Eispalast at Hintertux Glacier, Austria (2.900m a.s.l.) for field tests. This cave is 15m under the glacier itself and provides many chambers and channels to test the rover under controlled conditions. A collaborative investigation by the Hoover and Sattler labs has produced preliminary culture and biogeochemical data on this cave system. In the past months, a subglacial lake has been detected in this cave which carries millions of liters of water. A combination of ice and rock caves can be found at Hundalmhöhle, near Innsbruck, Austria. This cave offers multiple levels to test rover mobility. The upper is an ice camber, the lower one a rock chamber containing moonmilk.

Iceland's Kverkfjöll ice caves are formed by volcanic springs beneath the Vatnajökull ice cap, the largest glacier mass in Europe. The cap spans volcanic fissures of the Mid-Atlantic Ridge Plates, and the ice caves encountered in the Kverkfjöll snout provide Mars analog environments for extremophiles. Ice microbiota from the glacier are continually released in hot air rising to the cave roof and then fall directly into the heated river that emerges from the massive glacial snout. Samples collected from the ice and the hot stream inside the mouth of the cave by Hoover in 2009 are rich in filamentous cyanobacteria and other microbial extremophiles. The Lower Kverkfjöll Ice Cave is located at the spring of the Jökulsá á Fjöllum, the largest river in central Iceland. The cave is near the Sigurðarskali hut, and is readily accessible by 4-wheel drive vehicle. The hut has heat and electrical power, and can provide both living accommodations and laboratory research space for the field campaigns. The Upper Kverkfjöll Ice Cave, located near the Chocolate Hill nunatak in an active geothermal area with hot sulfur springs and rhyolite silts, is also accessible. This convergence of terrestrial ice caves with volcanic rocks between pillow hyaloclastite ridges makes Kverkfjöll glacier region an excellent analog for ice caves that might be encountered on Mars. Reasons to investigate these caves include: (1) each cave site has been previously examined by one or more **R.E.L.I.C.** investigators; (2) the caves are easily accessible, an advantage for time, effort, budget, and safety (of particular concern since **RE.L.I.C.** relies so heavily on student participation); (3) their ease of access to one of us (BS) makes it possible to do repeated seasonal sampling; (4) each cave is accessible to a rover; (5) all the caves present variance in illumination (light and dark regions) sufficient to contain regions dominated by photosynthetic species, as well zones dominated primarily by heterotrophes.

The field system to explore these caves will consist of an optical life detection instrument and an autonomous rover for field deployment. These two devices will be constructed for the **RE.L.I.C.** project over the next five years by undergraduate students in Senior Engineering Clinics and Summer Research Projects at Harvey Mudd College in Claremont California. The robotic system will be able to navigate a cave and select ice samples using real-time on-board data analysis without human intervention. The optical system will obtain spatially co-registered reflectance and fluorescence images, and then generate four spectral profiles (reflectance, fluorescence, Raman, and circular polarization) of targets in the ice walls and ceilings of cryosphere caves at distances of 0.1 to 20 meters. Broadband sample illumination will be provided either by ambient light or white LEDs. High intensity epi-illumination will be provided by 375 nm, 405 nm 455 nm, 532 nm, and 640 nm laser diodes to probe discrete sets of biomolecules. During image acquisition the Spectral Profiling and Imaging system, ***SPI***^{4}, will extract ~1000 reflectance, fluorescence, Raman, and circular polarization (CP) spectra from a midline transect of the imaged target. Each spectrum will represent from 1-10 target pixels with final pixel binning dependent on S/N requirements. The images provide ecosystem context for the chemical information contained in the spectral profiles. As the ***SPI***^{4} data set increases, machine algorithm image feature extraction will be used to identify spectral biosignatures. These data will be used to train a stochastic artificial neural network (ANN) to generate Bayesian probabilities of correct target classification.[47-50] System software will be developed to allow intelligent sample detection and selection by the autonomous rover described in the next section using ANNs trained on **R.E.L.I.C.** field data. All electronics, optical elements, and power supplies will be encased in a waterproof, rugged field pack with thermal regulation to match laser operational requirements.

To accomplish *in-situ* sample identification within remote ice caves, ***SPI***^{4} will be mounted on an autonomous rover. The rover will be equipped with the following capabilities:

1) Autonomous Navigation – The rover must navigate ice caves without user guidance. This includes localizing the rover without the use of GPS.
2) Mapping – The rover must map the cave's geometry and landmark sampling locations.
3) Optimal Planning – The rover must construct and follow paths that maximize information gain.
4) Minimum Range - To allow sampling at various habitat light levels, the rover must be able to travel 100-200 meters into the cave.
5) Versatile Locomotion – The rover must be able to climb up and down inclines of up to 35 degrees and navigate various terrains (sand, snow, ice, and rocks up to 0.25 meters high.
6) Robust to Cold Weather – The rover must operate for extended periods at temperatures of -10^{o} Celcius.

7) Minimum Payload – The rover must be capable of carrying the optical imaging system
8) Reliable Communication – The rover must be able to communicate with at least one sentinel node located outside the cave.

The field of autonomous robotics has progressed considerably over the last decade. Robots now automate warehouse distribution (Kiva Systems, http://KivaSystems.com), sample the depths of the ocean,[51] explore Mars,[52] and drive on our roads.[53] To accomplish these tasks autonomously, robots must navigate on their own. Autonomous navigation has been well studied and typically consists of a feedback control system that uses perception of the environment, localization and mapping, planning, and motion control [54] – all of which are required for **R.E.L.I.C.**

Significant heritage exists for **R.E.L.I.C.** rover construction. A robot "groundhog" has been deployed to autonomously navigate and map underground mines[55] and an autonomous sampling strategy for Mars missions has been demonstrated,[56] including autonomous identification of sample locations and navigation toward such locations. Mapping and navigation of an underwater cave has also been conducted [57]. Much of this prior work has relied on a Simultaneous Localization and Mapping (SLAM) system that uses a variety of sensors and appropriate algorithms to build a local map while at the same time determining the position of the robot within that map. Most SLAM approaches are based on Kalman Filtering or Particle Filtering.[58] **R.E.L.I.C.** requires two new problems be addressed. First, a motion planner is required that constructs paths within a 3D environment that are optimal with respect to information gain from the optical imaging system. Second, an appropriate localization system must be developed that allows for safe entry and exit of the rover within the cave. **R.E.L.I.C.** Co-I Clark has addressed similar problems including multi-robot motion planning within tunnel environments using modified A* tree-searches[59] and Probabilistic Road Maps[60]. The work has led to the successful mapping of ancient Maltese underwater tunnels,[61] localization in urban environments where no GPS is available,[62] and detecting the likelihood of Arctic ice sheets above an autonomous underwater vehicle.

For **R.E.L.I.C.** an off-the-shelf mobile robot basic platform will be modified. For example, Husky A200 (Fig. 5A, ClearPath Robotics) is a rugged, versatile, and easily programmable platform packaged with ROS, a standard robot operating system with a variety of sensor drivers. The 990x670x390mm 50kg platform has a differential drive system, a max speed of 1.0 m/s, wheel encoders, and an operating temperature of -10 to +30 degrees C. The platform will be equipped with a laptop processor, an IMU, 3-axis compass, omni-directional video camera, GPS, and two Hokuyo laser scanners mounted on a tilt servo for 3D scanning. Possible final configuration with the **R.E.L.I.C.** ***SPI***^{4} laser system appears in Fig. 5B. Hardware development will consist of design/construction of the 3D laser scanner system, a landmark based localization system, and sensor integration. There will be considerable software development to enable autonomous navigation and sampling. The control flow from sensors to actuators is depicted in Fig. 5C, where key software modules are shaded gray. The continuous feedback loop will first take all *Navigation Sensor* measurements and fuse them within a Localization and Mapping algorithm (e.g. Particle Filter based SLAM) to calculate rover position and map estimates. The *Sample Location Planner* will use these estimates along with optical imaging measurements to calculate the next desired location for sampling. The *Path Planner* will determine a collision-free path to the desired sampling location. A velocity *Motion Controller* that will be used to track the path by setting appropriate wheel speeds.

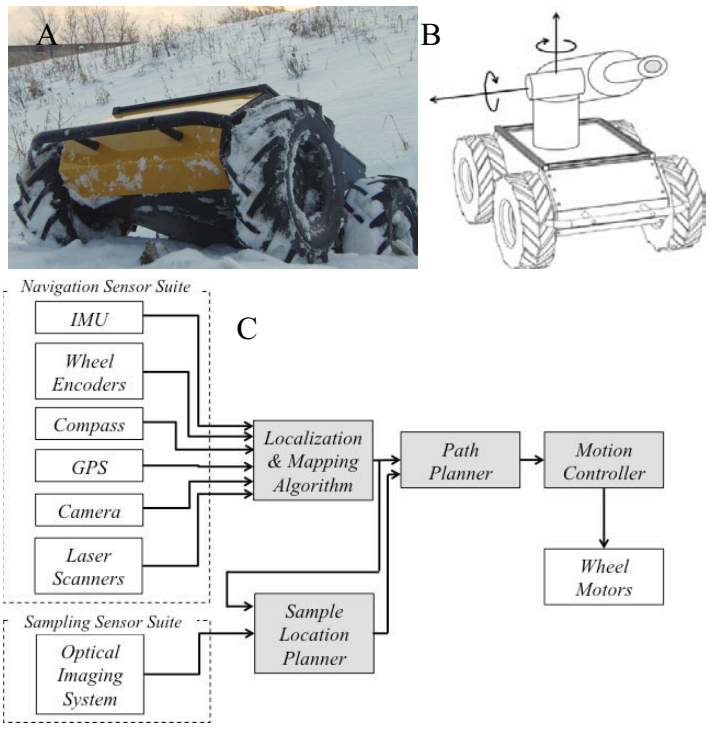

Figure 5. The Husky A200 shown manuvering in snow (**A**) can be configured with the **R.E.L.I.C.** ***SPI***^{4} laser system mounted on a pan-tilt swivel (**B**). Tne hardware and software flow chart for autonomous navigation and sample selection appears in **C**.

5. SUMMARY

Earth, Mars, the icy moons of the gas giants, and perhaps even our own moon possess a cryosphere. These zones offer water, sunlight, biogenic elements, and protection from lethal levels of radiation, critical attributes necessary for the appearance, persistence, and evolution of life. On Earth, microbial extremophiles are able to live within ice itself and perform metabolic tasks even at sub-freezing temperatures. Indeed, microbial life has developed effective strategies to live within glaciers and ice caves of the cryosphere and make use of the ice itself for protection from desiccation, radiation, and transient changes in planetary conditions. In this communication we have outlined a strategy to (1) integrate a novel, non-destructive, non-contact imaging spectrometer with laboratory microbiology and molecular biology techniques to find and characterize microbial life in ice caves on Earth, (2) develop a rover capable of autonomous navigation of cave systems and autonomous sample selection/acquisition, and (3) use the lessons learned from exploring ice caves in the Austrian Alps and Iceland to develop a comprehensive field and laboratory strategy for the non-destructive, non-contact Remote Evaluation of Life in Ice (**R.E.L.I.C.**) on Mars, the ice moons, and exoplanets, and (4) contribute to the development of the next generation of space science engineers by centering the design, fabrication, and testing of the field optical and robotic systems on college students participating in the Senior Physics and Engineering Clinics of Harvey Mudd College.

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