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Special Section:

Exploring planetary caves as windows into subsurface geology, habitability, and astrobiology

Key Points:

- Two novel, portable near-infrared (NIR) spectrometers were demonstrated in three geochemically distinct caves
- Acousto-optic tunable filter (AOTF) technology is a viable technique for mineralogical characterization
- AOTF-based NIR spectrometers are an appropriate component of an instrumentation suite that can be used in planetary caves to search for potential biosignatures

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The Development and Demonstration of the Portable Acousto-Optic Spectrometer for Astrobiology in Cave Environments

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Abstract Planetary caves are desirable environments for the search for biosignatures corresponding to extant or extinct extraterrestrial life due to the protection they offer from surface-level solar radiation and ionizing particles. Near-infrared (NIR) reflectance spectroscopy is one of a multitude of techniques that, when taken together, can provide a comprehensive understanding of the geomicrobiology in planetary subsurface regions. To that end, we developed two portable NIR spectrometers that employ acousto-optic tunable filters and demonstrated them in three geochemically distinct cave environments. The instruments were deployed both as stand-alone spectrometers positioned against the targets manually and as a component of an instrument payload mounted on a quadruped robot capable of vertical excursions of several meters. In situ measurements of calcium carbonates, sulfates, metal oxides, and microbial colonies and mats revealed spectral signatures that enable a distinction between the targets of interest and the underlying substrates. The ruggedness and portability of the instruments, and their low size, weight, and power, spectral agility, and active illumination make AOTF-based spectrometers ideally suited for studies of planetary caves.

1. Introduction

The development of in situ instrumentation for the detection of biomarkers in planetary cave environments is critical for the search for evidence of present or past life in our solar system. As refugia from the harsh ionizing radiation environments on the surfaces of airless solar system bodies or those with thin atmospheres, planetary caves represent a tantalizing location in which to conduct searches for extraterrestrial life (Viúdez-Moreiras, 2021). There are myriad challenges associated with investigations of planetary subsurface environments. However, once subsurface access is achieved, the exploration of planetary caves will require robust technologies with low size, weight and power (SWaP) in order to overcome the operational limitations of a small platform.

The detection of extant or extinct life in planetary subsurfaces requires the identification of potential biomarkers, or signatures of physical structure, chemical composition, or metabolic processes associated with living organisms (Uckert et al., 2017). Broadly speaking, a wide range of both passive and active in situ techniques have been developed for and employed in the characterization of planetary surface environments in an effort to identify potential biomarkers. These techniques include (but are not limited to) mass spectrometry (Garcia-Descalzo et al., 2012; Getty et al., 2012), Raman spectroscopy (Tarcea et al., 2007), X-ray diffraction spectroscopy (Blake et al., 2012), imaging microscopy (Núñez et al., 2014), and laser induced breakdown spectroscopy (LIBS) (Pavlov et al., 2012; Schröder et al., 2013). There is general agreement that a multitude of techniques probing a range of spatial and temporal scales is needed to determine the presence of extant or extinct life on another planetary body (Boston et al., 2001; Cady et al., 2003; Conrad & Nealson, 2001; Wynne et al., 2022). Uckert et al. (2017) reviewed the efficacy of several of the aforementioned technologies in a description of a biosignature detection instrument suite for cave environments. In this work, we focus on one technology in particular—near infrared (NIR) reflectance spectroscopy—as such, we describe the evolution of an instrument package optimized for operation within subsurface environments, specifically environments that we expect may most closely resemble the Martian subsurface.

The NIR is a particularly useful spectral region for chemical and mineralogical studies of geologic materials. Vibrational transitions of molecules can be in the form of fundamental modes, in which the atoms in a molecule move in phase and follow a straight path, and some of these motions (e.g., bending and stretching) can be

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symmetric or asymmetric. Additionally, overtone modes occur when the molecules do not follow straight paths as they vibrate (Peters & Englert, 1993). Ultimately the frequencies of these various vibrations correspond to the absorption of photons of those same frequencies, giving rise to the molecular absorptions in NIR spectra of rocks and minerals. Ices, hydrated minerals, oxides, silicates, carbonates, metals, and organics all exhibit distinctive vibrational spectral signatures in the NIR, making it a rich diagnostic spectral region with which to explore the mineralogy and geomicrobiology of planetary subsurfaces.

In previous work (N. J. Chanover et al., 2012; N. Chanover et al., 2013; Tawalbeh et al., 2013), we described our efforts to address the need for a multi-instrument approach to biosignature detection through the development of a NIR point spectrometer, that is, a device that measures the intensity of radiation at a single point on a sample, intended for rapid bulk mineralogical identification of samples. This point spectrometer was built using an acousto-optic tunable filter (AOTF) as the wavelength selecting element (described in more detail in §2), providing a low SWaP approach for quickly obtaining the NIR reflectance spectrum of a sample. The original instrument concept was designed to be paired with a miniature laser desorption time-of-flight mass spectrometer (LDTOF-MS). The goal was to develop an instrument suite that could be used in power and/or mass restricted operational scenarios, such as on a robotic lander or rover, wherein the AOTF point spectrometer would be used to prescreen samples for evidence of volatile or refractory organics prior to the laser desorption step and subsequent mass spectrometer measurement.

As an extension of the AOTF + LDTOF effort, we developed a portable version of the AOTF-based point spectrometer for the characterization of the mineralogy of remote surface and subsurface environments. We demonstrated the capabilities of this technology in several field sites that could serve as terrestrial analogs for extreme environments elsewhere in the solar system. In this paper we summarize the development, field testing, and performance of two instruments: the Portable Acousto-optic Spectrometer for Astrobiology (PASA), and PASA-Lite (PASA-L), which was derived from the PASA concept and designed specifically for a rock-climbing robot developed for traversing cave walls. The workflow for this investigation is shown graphically in Figure 1. In §2 we describe the technology and instrument design, evolution, and operation. Section 3 summarizes the results of the field testing and demonstration of PASA and PASA-L in three geochemically distinct cave environments. We conclude in §4 with a summary of PASA's scientific utility and a future outlook for AOTF-based instrumentation for in situ Martian cave investigations.

2. Instrument Descriptions

The instruments described here were developed with the goal of demonstrating a portable point spectrometer based on AOTF technology for “quick look” in situ detection of organic species at millimeter size scales. As discussed below and in §3, each field campaign revealed new operational or measurement requirements that drove the evolution of the instrument design.

2.1. Technology Overview

The unique aspect of PASA that sets it apart from standard reflectance spectrometers is the use of an AOTF for the wavelength selection element. AOTF devices have been used for multispectral or hyperspectral imaging for several decades (N. J. Chanover et al., 1998, 2003; Glenar et al., 1994, 1997; Smith et al., 1987), but only in more recent years have they been incorporated as elements of spectrometers used for planetary flight projects (Agrawal et al., 2015; Bertaux et al., 2007; Bibring et al., 2017; O. Korablev et al., 2006; O. I. Korablev et al., 2018). AOTFs are recognized as a low power, low mass, radiation tolerant option for space flight, thus they are a suitable technology for a spectrometer intended for operation on a lander or rover platform.

A detailed description of the operating principles of AOTFs is beyond the scope of this paper, but extensive discussions of their operational capabilities are provided in N. J. Chanover et al. (2014), O. I. Korablev et al. (2018) and the references therein. Here we briefly describe the salient features of AOTFs relevant to the PASA development efforts. AOTF systems provide great flexibility, being very compact (a few cm in size), electronically programmable, and requiring low power (~1 W or less of radio frequency (RF) power, depending on the wavelength and aperture size). They can provide selectable spectral samples over a factor of ~2 tuning range by utilizing a birefringent crystal whose optical properties are altered in the presence of an acoustic wave. AOTFs can be made from one of several birefringent materials, including crystalline quartz, magnesium fluoride,

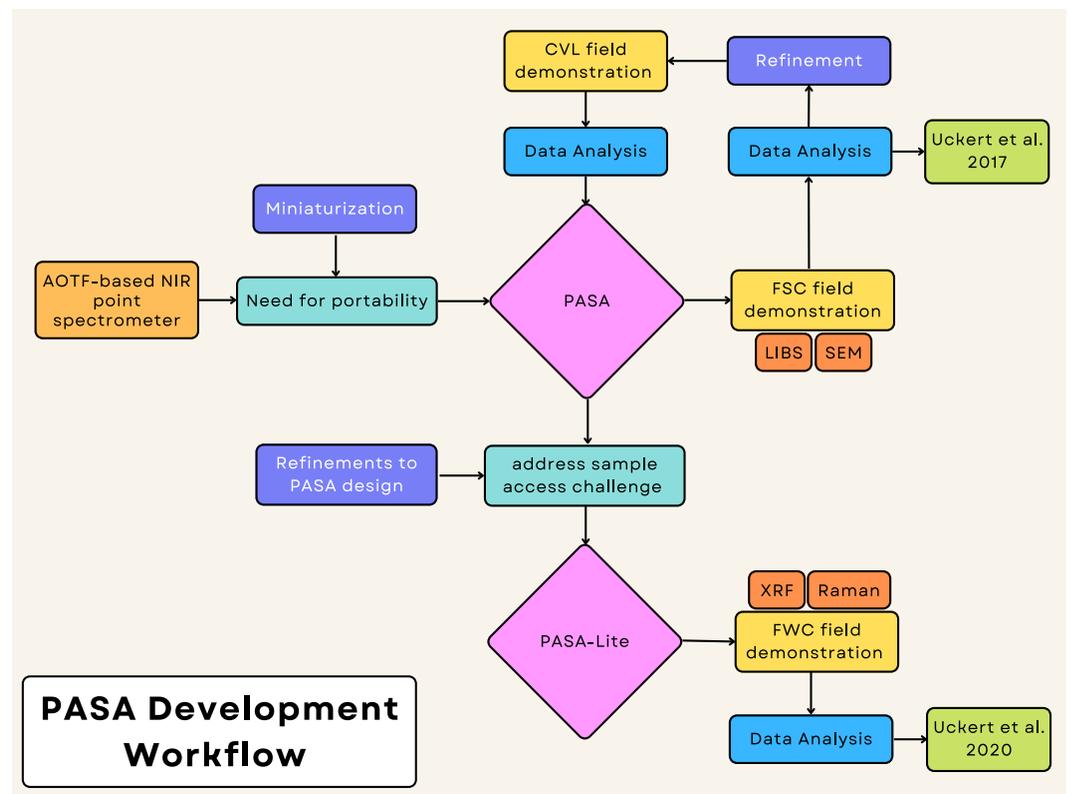


Figure 1. Graphical depiction of the workflow of the portable acousto-optic spectrometer for astrobiology and PASA-Lite development, field testing, data analysis, and dissemination of results.

sapphire, thallium-arsenic-selenium, and tellurium dioxide (TeO_2). Tellurium dioxide has the widest operating wavelength range of ~ 0.4 to nearly $5 \mu\text{m}$ (Smith et al., 1987), and consequently it is the most commonly used for AOTF devices.

An AOTF operates through the principle of optical diffraction by a traveling acoustic wave passing through a birefringent crystal. The basic geometry of an AOTF device and its orientation relative to an instrument optical axis is shown in Figure 2. In the plane of diffraction, a traveling acoustic wave (k_a) undergoes vector addition with randomly polarized incident light (k_i) if both are launched at the proper directions into the crystal. This acoustic wave is set up within the medium by applying an RF signal via a piezoelectric transducer, typically made of lithium niobate, bonded to the crystal. The optical and acoustic signals produce orthogonally polarized diffracted beams ($k_d = k_i \pm k_a$) that are deflected nearly symmetrically with respect to the input optical axis. Hence, with broadband light as an input, orthogonally polarized, spectrally narrow beams are diffracted within the crystal and emerge at a small angle relative to the exit face of the crystal. The AOTF material, TeO_2 , is inherently radiation hard (Prasad et al., 2007; Trivedi et al., 2006). Furthermore, these devices have no moving parts, making them an attractive option for landed surface platforms. Newly available light sources allow AOTFs to be used for in situ applications without ambient light, hence they are a superior choice for instrument development for a landed mission with a focus on the subsurface.

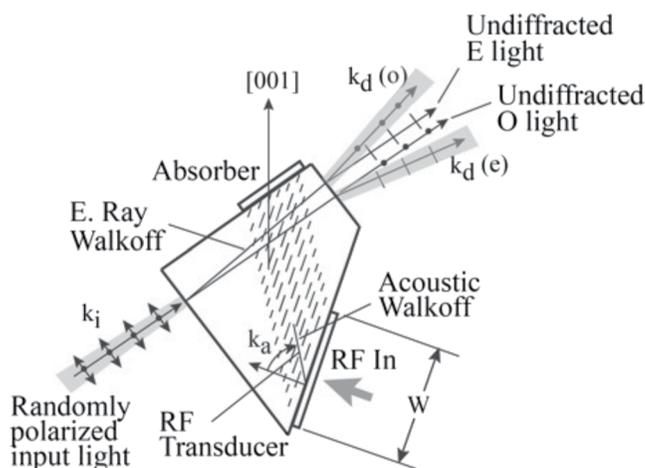


Figure 2. Geometry of an acousto-optic tunable filter crystal. Orthogonally polarized, spectrally narrow output beams are indicated by (o) and (e).

2.2. Instrument Design, Packaging, and Operation

The optical design of PASA is based on that of its predecessor, which we paired with an LDTOF-MS at NASA Goddard Space Flight Center as part of

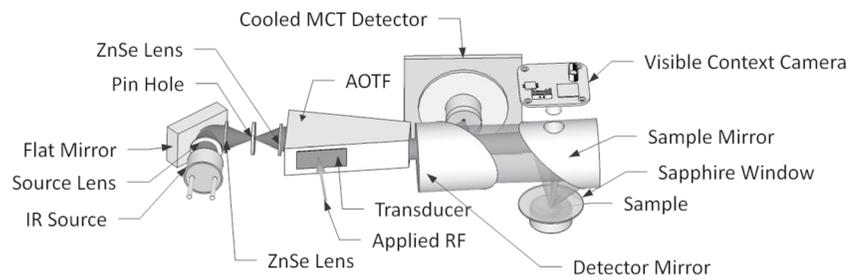


Figure 3. Schematic showing the optical layout of the source, acousto-optic tunable filter, sample, and detector (Uckert et al., 2020).

an instrument suite (N. Chanover et al., 2013; Getty et al., 2012; Tawalbeh et al., 2013). In brief, it consists of a detector element, a light source, the AOTF, focusing optics, and an electronics module. Light from a broadband infrared (IR) source, a Helioworks lamp, is passed through the AOTF crystal, which produces the narrowband output with a center wavelength that is a function of a sweeping RF signal that is driven into the crystal (Figure 3). One narrowband output beam is selected and reflected off a sample mirror, which focuses the light on the sample. The back-reflected light is collected by the sample mirror and sent to a detector mirror that focuses the light onto an HgCdTe detector. The recorded signal amplitude provides a measurement of the sample reflectance as a function of wavelength, yielding an IR spectrum spanning 1.6–3.6 μm with a spectral resolution ($R = \lambda/\Delta\lambda$) of ~ 267 –383 from the long wavelength end of our spectral range to the short wavelength end. Figure 4 illustrates the bandpass shape measured for two wavelengths within the spectral range of PASA. Non-collinear AOTFs, that is, those in which the acoustic and optical waves are physically separated, ideally have a bandpass shape that is represented by a sinc^2 function (Georgiev et al., 2002). The bandpasses for PASA deviate from this ideal functional form as a result of minor spectral leakage (Tawalbeh et al., 2013).

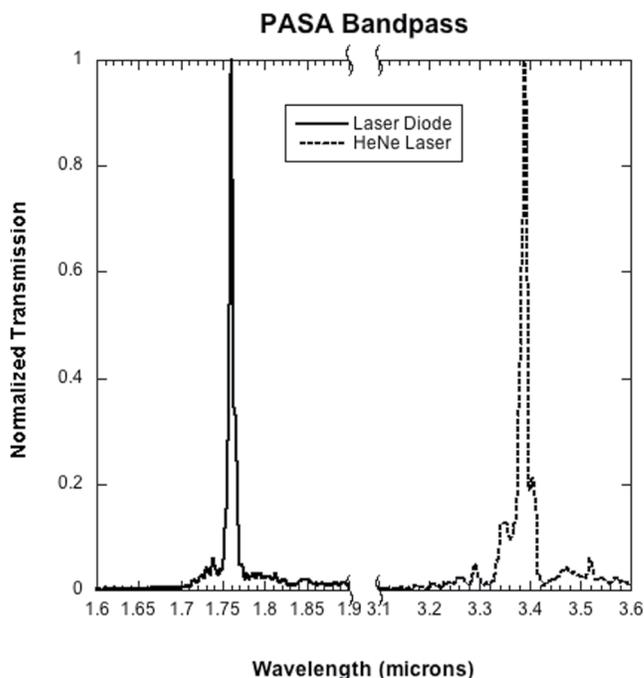


Figure 4. Bandpass functions for portable acousto-optic spectrometer for astrobiology (PASA). A laser diode beam at 1.76 μm and a HeNe laser at 3.39 μm were used to map the bandpass shape as well as determine the spectral resolution of PASA at these wavelengths. The measured full width at half maximum (FWHM) of the laser diode beam is 0.0046 μm , corresponding to a resolution $R = \lambda/\Delta\lambda = 382.6$. For the HeNe laser, the measured FWHM is 0.0127 μm and $R = 266.9$.

Functionally, PASA operated in the same manner as the AOTF-based point spectrometer described in Tawalbeh et al. (2013). However, we made several modifications to the original instrument in the construction of a more rugged, portable version:

1. AOTF crystal. We used a noncollinear AOTF device manufactured by NEOS as the dispersive element in PASA. The specifications of this crystal (Table 1) are similar to that used in the point spectrometer paired with the Goddard LDTOF-MS.
2. Sapphire window. A sapphire window was placed at the bottom of the steel box used to house the AOTF fixture. This served to protect the optics from environmental contamination or damage (e.g., from moisture, dust, or mud) while acquiring measurements of field samples. The installation of the sapphire window resulted in a reduction of overall optical signal efficiency of 25% due to Fresnel reflections at the surfaces of the uncoated window, but this was deemed a necessary tradeoff to reduce the risk of damage to the optics. On the other hand, the window had the positive effect of extending the illumination focal spot slightly further from the box surface, about 1.5 mm, which provided an additional 1.7 mm of alignment relief when working with rough surfaces.
3. Context camera. Unlike the AOTF spectrometer that we placed in the LDTOF-MS vacuum chamber, in the PASA optical design we included a small context camera to enable the acquisition of near-simultaneous visible images of the samples. This required no change in the optical design, as there was already a hole in the parabolic mirror that was originally intended for the LDTOF ion inlet (shown in Figure 3). The camera did not need to be vacuum rated as it was not going to be placed in the LDTOF vacuum chamber, therefore an off-the-shelf camera was adequate for our purposes. We used a color complementary metal-oxide-semiconductor

Table 1
Properties of Acousto-Optic Tunable Filter Crystals Employed in Portable Acousto-Optic Spectrometer for Astrobiology and PASA-Lite

Property	Value
Crystal material	TeO ₂
Wavelength range	1.6–3.6 μm
Spectral resolution ($\lambda/\Delta\lambda$)	~250–400

camera with 744×480 pixels from The Imaging Source (<https://www.theimagingsource.com>; model number DFM 22BUC03-ML) and connected it via a USB 2.0 connection to our data acquisition system.

4. Data collection software. Data acquisition for both the point spectrometer and the context camera was performed using custom-built LabView control software and an associated graphical user interface. The LabView software controls the acquisition and display of the context camera images, triggers the RF synthesizer, performs a sweep of our spectral range by incrementing the RF, and triggers the data acquisition at each frequency. The data acquisition software was generally operated in a “scanning” mode, whereby spectral measurements were obtained by sweeping across the entire wavelength range of operation. Acquisition of single sweep took approximately 0.1 s, and we typically acquired 256 or 512 spectra of a sample (which takes roughly 30 or 60 s, respectively) and averaged them together to increase our signal-to-noise ratio. An averaged spectrum can be viewed immediately after acquisition so that the data quality and signal-to-noise can be rapidly assessed.
5. Miniaturization. Because the optical design remained virtually unchanged between PASA and its predecessor, most of the miniaturization was achieved in area of the instrument controls. For example, we eliminated the RF power meter that continuously monitored the power reaching the AOTF crystal. We also implemented switching power supplies that require one battery to run multiple voltages. For the instrument in the LDTOF vacuum chamber this would have resulted in too much electronic noise, but since PASA was generally operated roughly a meter away from the accompanying electronics module, electronic isolation of all power supplies was not as critical. We used a differential input at the A-to-D converter as a means of further reducing electronic noise in the system.
6. Packaging. The optics, the AOTF, and the lamp, detector, and context camera were housed in an optics module, which can be transported in a 34 cm \times 30 cm \times 15 cm sealed equipment case. The electronics module contained the frequency synthesizer, the thermoelectric cooler controller, the RF circuitry, various analog-to-digital converters, and the lamp circuit. These were all housed in a second equipment case of the same size. The electronics control and data acquisition software were run on a ruggedized laptop computer, which was stored in a third, smaller case. The entire portable instrument assembly is shown in Figure 5.
7. Power. Because PASA was intended to be operated in field locations without AC power, it was powered through a portable battery pack containing nickel-metal hydride rechargeable batteries, which can be worn or carried. These were rated to a total capacity of 10 Amp-hours. Each battery pack allows for approximately 4 hr of continuous operation (given our total current requirements of ~2.5 Amp). This represented a lower limit since power can be conserved by shutting off some of the electronics modules when repositioning the instrument or examining the data files immediately after acquisition. Hence, 8 hr of intermittent use was possible with a single battery pack.

The portable version of our AOTF-based point spectrometer serves as a logical next step in technology readiness level (TRL) progression of this technology. With the integration of our brassboard AOTF point spectrometer with the LDTOF-MS we achieved TRL 4 (component and/or breadboard validation in laboratory environment), whereas these field demonstrations with PASA bring this technology to TRL 5–6 (system/subsystem model or prototype demonstration in relevant environment). This was a necessary progression in the development of AOTF-based instrumentation as possible candidates for future flight opportunities.

2.3. Data Acquisition and Analysis Strategy

In order to account for variations in instrument response as a function of wavelength, we acquired spectra of a Lambertian scattering surface, a small infragold target, immediately before or after any science data acquisition. Our infragold reference target was a round disk of aluminum, approximately 4 cm in size, that was sintered to generate a rough Lambertian surface and then gold plated. The reflectance standard was housed in an aluminum mount with rare-earth magnets in the corners, which enabled us to quickly attach and remove the puck from the steel box containing the aperture to the instrument. A typical spectrum and context image of the infragold target are shown in Figure 6.

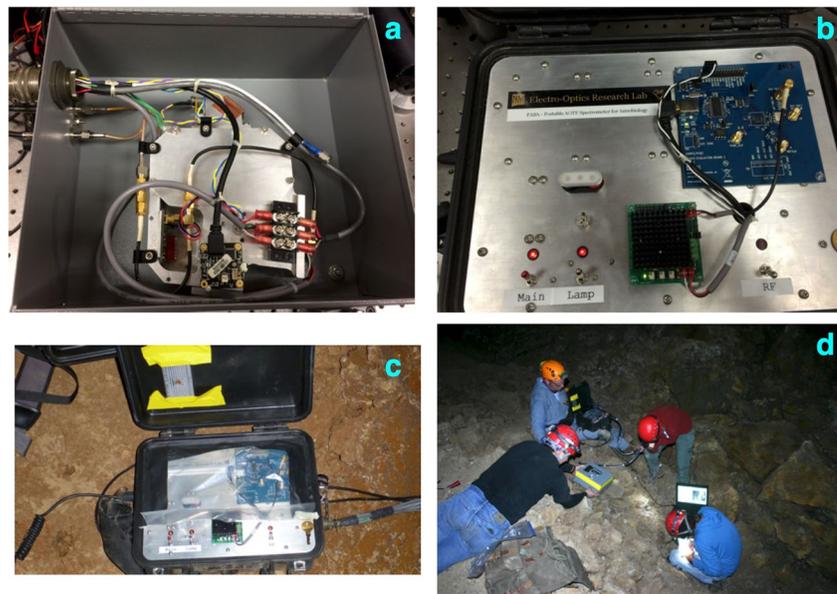


Figure 5. (a) Top-down view of the aluminum optical head that contains the acousto-optic tunable filter crystal and focusing optics. The optical head is affixed inside the stainless steel housing box with a sapphire window over the optical aperture. (b) Top-down view of the electronics box, which contains controllers for the main power, detector thermoelectric cooler, the RF synthesizer, and the lamp. (c) View of the electronics box as deployed in Fort Stanton Cave. The switches and boards are covered with a plastic sheet to protect them from dust and moisture, and the rugged case is equipped with desiccant (taped to the lid) to control the moisture level near the electronics. Photo courtesy of R. Hull. (d) The portable acousto-optic spectrometer for astrobiology instrument in operation. Four team members are managing the instrument control laptop (lower right), cables (upper right), electronics module (upper left), and the spectrometer module (lower left). The battery pack is being worn around the waist of one of the team members (upper left). Photo courtesy of W. Walker.

A standard data acquisition sequence entails an infragold measurement followed by the target measurements. All sample measurements presented in this manuscript were of natural surfaces; no additional sample preparation was performed in the field or in the laboratory. Targets were positioned in front of the instrument aperture using the context camera, and then the instrument was manually held in position while the data were acquired (Figure 7). Visual inspection of the data provides an indication of the signal-to-noise ratio, and the instrument was subsequently moved to a different location or repositioned for additional measurements. In post-processing, the user had the ability to reject the spectral scans that deviated substantially from the average, for example, due to a slight movement of the instrument housing during the data acquisition. Figure 8 shows an example of the raw output from PASA measurements acquired in the laboratory. In this case, PASA was configured to collect 256 spectra of a bentonite mineral standard over ~ 30 s. The median value within each channel was calculated and adopted as the “final” spectrum of the sample to limit contributions from slight positional movements and noise spikes.

After our second PASA field campaign, when the instrument was deployed to Cueva de Villa Luz (CVL) (§3.2), the PASA data acquisition software was updated to incorporate reference spectra from the USGS Spectral Library (Clark et al., 2007), which included common minerals measured in these environments (e.g., carbonates, sulfates, phyllosilicates). This spectral comparison and visualization tool was written in Python and enabled interpretation of PASA data in the field through the comparison of field data with standard mineralogical end members (Uckert, 2016b).

2.4. PASA-Lite

The deployment and demonstration of PASA in several terrestrial caves, described further in §3, was the first step in demonstrating that an AOTF-based point spectrometer could be successfully operated in the challenging power- and illumination-restricted subsurface environments. However, these campaigns led us to identify a third challenge—sample access—that could not be overcome with PASA. Data acquisition with PASA required manually positioning the entrance aperture of the instrument over the sample region of interest (Figure 7); thus, it was limited to those

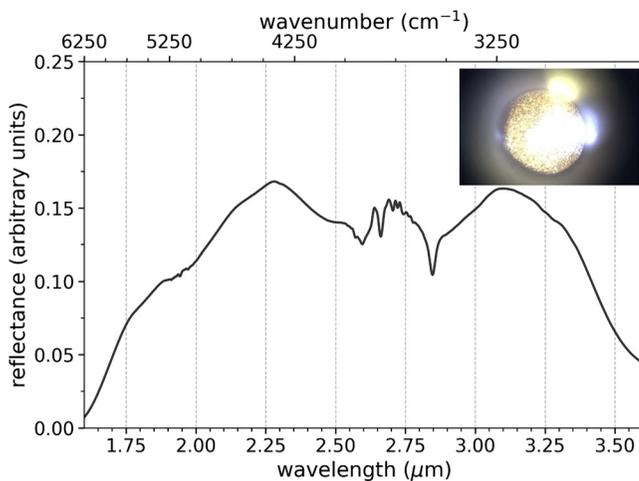


Figure 6. Infragold spectrum acquired with PASA-Lite (\$2.4) and corresponding context camera image (inset).



Figure 7. Data acquisition using portable acousto-optic spectrometer for astrobiology in Fort Stanton Cave on 31 October 2013. The acousto-optic tunable filter fixture is being held against a surface of interest while the data acquisition sequence is initiated on the laptop. The electronics module is tethered between the optics module and the laptop.

regions that we could reach by hand. Even if PASA were integrated onto a wheeled platform that uses a robotic arm to position the instrument, that still would not permit exploration of the rich geologic diversity of the stratigraphic columns associated with cave entrances. Furthermore, the short focal length of PASA and the rigid nature of its stainless steel housing made it challenging if not impossible to acquire spectra of samples on very rough terrain.

To address this challenge we developed a lightweight version of PASA, PASA-L, to integrate with the Limbed Excursion Mechanical Utility Robot (LEMUR) 3, a quadruped prototype robot with seven degrees of freedom for each limb (Parness et al., 2017). This robot uses hundreds of microspine grippers (Parness et al., 2013) at the end of each limb to climb cliff faces. Through a judiciously selected payload, LEMUR 3 could be used to evaluate the walls of subsurface environments for the presence of volatiles, minerals, and biomarkers (Uckert et al., 2020).

The optical design of PASA-L is nearly identical to that of PASA. We made several modifications to the instrument and ancillary components when building PASA-L that enabled its implementation on LEMUR 3:

1. Reduction in mass. We significantly reduced the mass of PASA-L compared to that of PASA by replacing the aluminum fixture used to house the AOTF, other optical elements, and the detector with a lightweight, 3D-printed plastic fixture.
2. Longer focal length. PASA-L was mounted to the bottom of the LEMUR 3 body, and proximity to measurement targets was achieved by the lowering (“squatting”) of LEMUR 3 to bring the body as close to the target as possible. The standoff distance of the LEMUR 3 body still required us to increase our focal distance from 25 mm (PASA) to 50 mm (PASA-L). The PASA-L focus mechanism was motorized and controlled by the instrument operator, who determined the best focus position by performing a visual optimization of the signal-to-noise of the target spectra using the data acquisition software. This new focus mechanism provided a greater standoff distance from the wall to collect measurements, which improved our ability to access samples in rough terrain.
3. Higher power lamp. The aforementioned longer focal distance caused a reduction in intensity of the illumination from our light source, the Helioworks lamp. We compensated for this effect by employing a higher power lamp (10 vs. 2.4 W).
4. Context imager location: The context imager was moved outside of the spectrometer housing. Although the image and spectra were no longer co-located, the off-axis parabolic mirror no longer required a borehole, increasing light throughput, and the larger field of view of the image provided better geologic context of the sampling area with which to interpret the spectra.

A comparison of the PASA and PASA-L specifications is provided in Table 2.

The addition of PASA-L to LEMUR 3 enabled measurements of locations in the vertical direction that would have been impossible to access with PASA. A detailed discussion of the field campaigns with LEMUR and its instrument suite, which included PASA-L, can be found in Uckert et al. (2020).

3. Results

The PASA and PASA-L instruments were deployed to three geochemically distinct cave environments in an effort demonstrate the AOTF-based

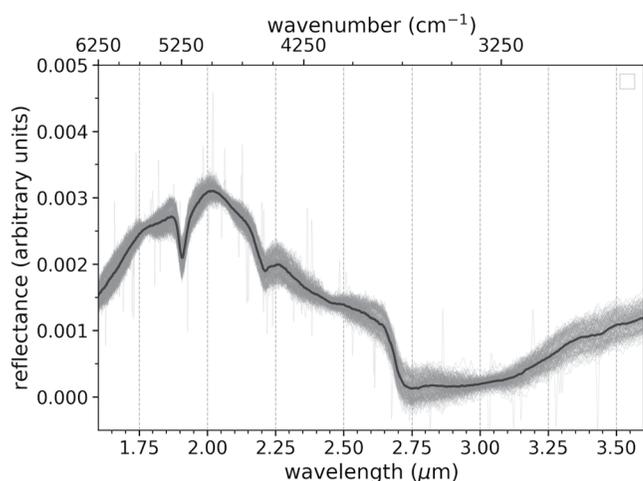


Figure 8. An unprocessed spectrum of a bentonite mineral standard acquired in the laboratory, showing all 256 spectra stacked (thin gray traces) and the median spectrum (darker black trace). These spectra have not been calibrated against an Infragold standard.

NIR point spectrometer technology for advancing our understanding of the Martian subsurface environment. Results from each of the three field campaigns are presented in the following subsections, which are presented in the chronological order of the instrument development efforts.

3.1. Fort Stanton Cave

The first field measurements made with PASA were in Fort Stanton Cave, a limestone cavern in southeastern New Mexico. This is a relatively young cave, having formed several million years ago through the process of carbonic acid dissolution. Its designation as a National Conservation Area by the Bureau of Land Management restricts access by recreational cavers, thereby offering an ideal site for investigations of cave dwelling microbial colonies.

The Fort Stanton Cave field campaigns with PASA were conducted on 30–31 October 2013 and 3 May 2014. Prior to the field campaigns we retrieved samples from Fort Stanton Cave on 1–2 May 2013, for in-lab demonstration measurements using PASA. For laboratory measurements, samples were selected from inconspicuous locations within the cave from a variety of locations to ensure that the collection would be geologically diverse. Samples were packaged in foil and stored in sterile bags and containers to limit contamination in transit. The locations of the PASA sampling measurements are shown in Figure 9.

We used PASA to acquire NIR spectra of a range of speleothems and formations of varied morphologies in an effort to demonstrate the instrument on surfaces exhibiting a range of roughness and reflectance and to investigate the presence of biosignatures. The measured samples included calcium carbonates, sulfates, and metal oxides (Figure 10). To complement the PASA measurements, we also studied the samples returned to the laboratory with scanning electron microscope (SEM) imagery and a LIBS instrument. As detailed in Uckert et al. (2017), the biogenicity of a calcite sample was determined through an examination of morphological indicators of biomineralization as seen in SEM imagery. We used a commercial Hitachi S-3400N Type II SEM to examine mm-sized regions of the samples retrieved from Fort Stanton Cave to identify the presence of morphological structures consistent with features associated with microbial activity. The PASA spectra alone were not sufficient to definitively distinguish between minerals precipitated abiologically and those precipitated through biomineralization. Biologic signatures identified in some of the SEM images include filaments and spheroid structures, although it is important to recognize that similarities to microbial structures is not sufficient evidence to definitively claim life detection. Nonetheless, the SEM images provided guidance on which regions of each sample showed promise for further analysis. We also used a commercial Ocean Optics LIBS 2500+ instrument, which operated between 0.2 and 0.98 μm to determine the variation in elemental composition within the retrieved samples.

After samples from Fort Stanton Cave were sorted into biologic and abiotic groups using the SEM data, in order to quantitatively assess the ability of PASA and a LIBS instrument to differentiate between biomineralized and inorganically precipitated calcite specimens, the PASA spectra and concomitant LIBS data were analyzed with two statistical methods. Using a Principal Component Analysis, we found that specific features in the PASA spectra of both the biologic and abiotic samples were responsible for 9% of the variance in principal component 2, indicating that the strength of these features due to carbonates, CH, OH and water can be indicative of potential biologic activity (Uckert et al., 2017). Using a Partial Least Squares Regression analysis, we trained a model on a subset of samples that were ascribed biologic or abiotic origin a priori using the SEM imagery and then modeled the remaining samples to determine their biogenicity. The model correctly classified ~90% of the calcite samples (McMillan et al., 2014), demonstrating that IR spectroscopic data coupled with multivariate analysis can be a powerful technique for assisting with the identification of biomineralized calcites. These results are consistent with previous findings concerning the utility of

Table 2
Specifications of Portable Acousto-Optic Spectrometer for Astrobiology and PASA-Lite

Property	PASA value	PASA-Lite value
Mass (g)	2,000	990
Power (W)	Standby: 7.8	Standby: 10.4
	Operational: 31	Operational: 37.4
Physical dimensions (cm)	11.4 × 12.7 × 4.1	11.4 × 17.5 × 4.1
Focal spot diameter (μm)	≤2,000	≤1,000
Focus adjustment	Fixed	±2 cm
Data acquisition time (sec) ^a	0.1–30	0.1–30

^aThe range of data acquisition times is dictated by the number of individual spectra (each of which has a minimum acquisition time of 0.1 s) that are coadded together.

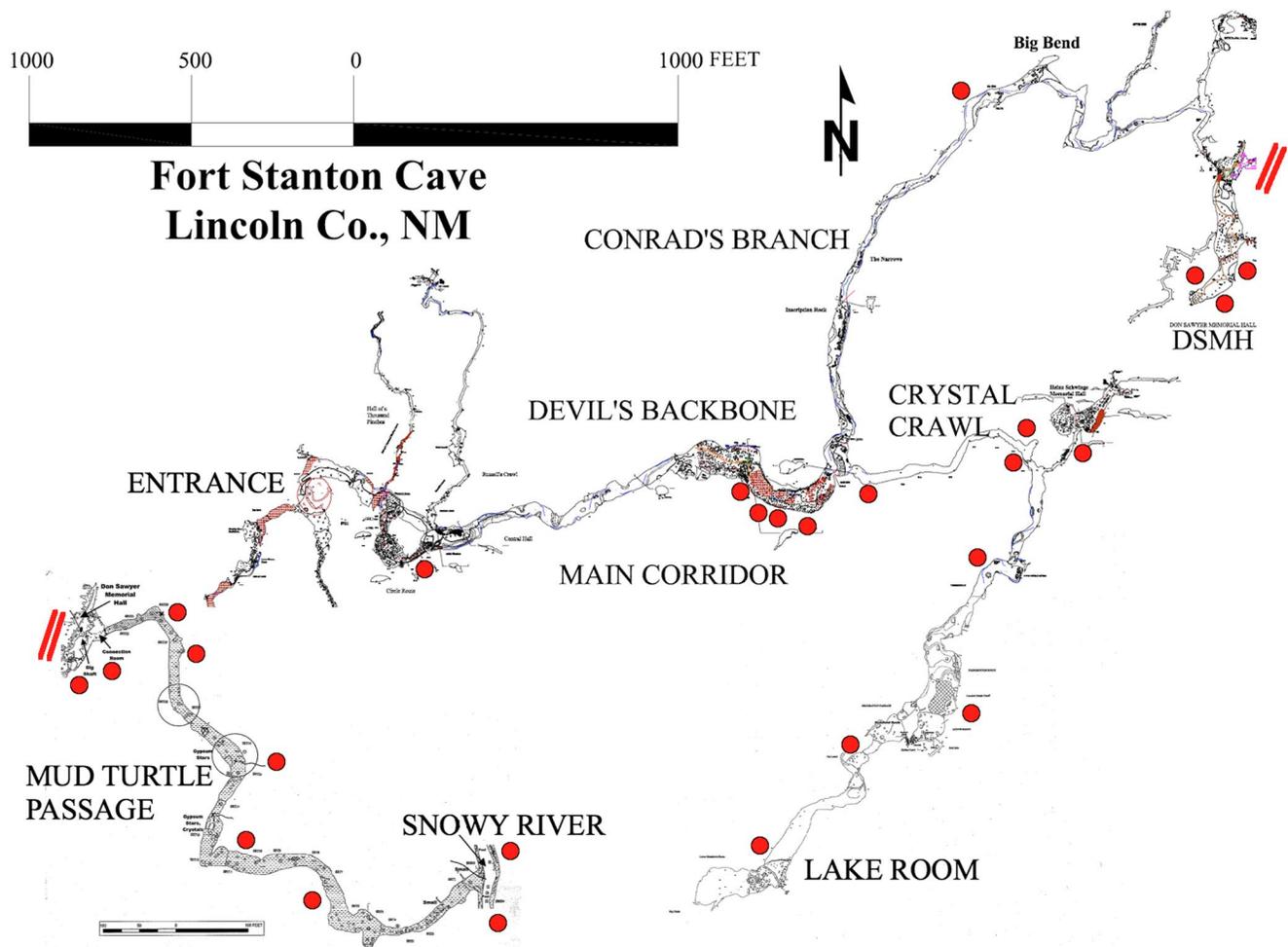


Figure 9. A map of the first several kilometers of passage of Fort Stanton Cave, with the approximate portable acousto-optic spectrometer for astrobiology sampling locations annotated by red circles. Map courtesy of the Fort Stanton Cave Study Project.

NIR spectroscopy to determine sample biogenicity (Berg et al., 2014; Orofino et al., 2007; Regev et al., 2010). The spectral and operational agility of PASA demonstrated the utility of a hand-held NIR spectrometer for exploring planetary subsurface environments, particularly when used as part of an instrument suite comprised of complementary techniques for identifying biosignatures.

3.2. Cueva de Villa Luz

The second field environment in which PASA was deployed for subsurface characterization is CVL, in Tabasco, Mexico. CVL is a young hypogenic cave, approximately 2 km in length, in which H₂S-rich water reacts with oxygen and water to dissolve limestone and create gypsum, resulting in active speleogenesis. As in the work described in Uckert et al. (2017), for the CVL expedition PASA was included as one component of a larger, multi-instrument campaign designed to study the bizarre lifeforms in this sulfuric acid cave as a model for extra-terrestrial life detection missions. CVL is replete with various exotic microbial colonies, including white mucous-like straws and loops suspended from the ceilings and walls (dubbed “snottites”) and splotchy biovermiculation colonies (Hose & Pizarowicz, 1999). These chemoautotrophic microbes thrive in the ultra-low pH environment of CVL, thus most of our PASA measurements were made in situ. Because we were unable to retrieve a sufficient number of samples from CVL to conduct complementary measurements in the lab with SEM and/or LIBS, or to do statistical analyses like those described in Uckert et al. (2017), our results from CVL are strictly qualitative.

We deployed PASA in CVL on 17–20 December 2013 in a series of ~2-hr forays into the cave. The toxic levels of atmospheric H₂S and CO necessitated the use of protective gas masks for everyone involved in the field

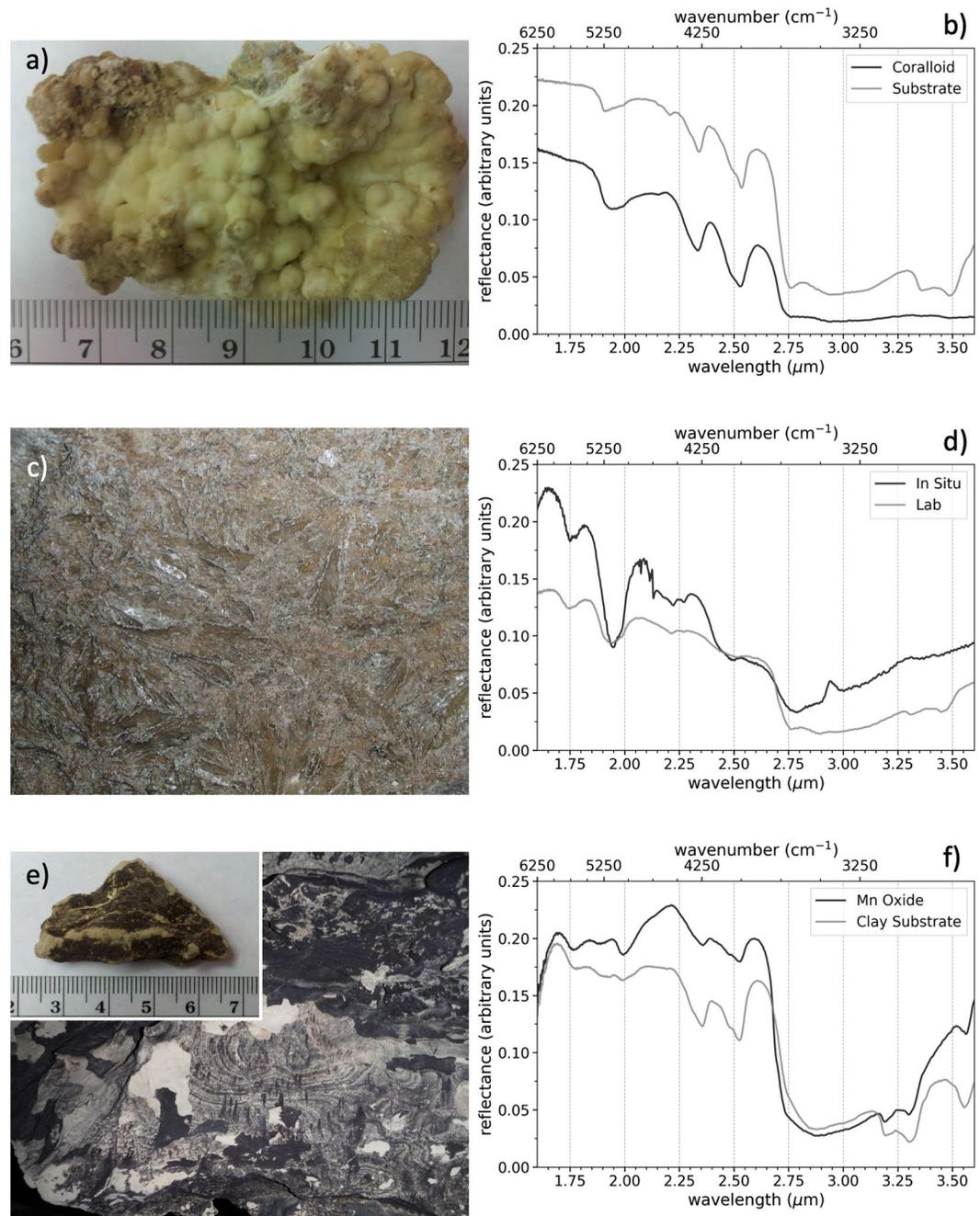


Figure 10. (a) A photograph of a coralloid sample retrieved from Fort Stanton Cave with a portable acousto-optic spectrometer for astrobiology (PASA) infrared (IR) spectrum of this sample (b), measured in the laboratory, showing the IR reflectance of the coralloid deposit and the underlying substrate. (c) A gypsum star in Fort Stanton Cave measured by PASA (d) both in situ and in the laboratory, showing changes in hydration states. (e) A photograph of a Mn oxide laminated deposit in Fort Stanton Cave. Inset is a returned sample from Fort Stanton Cave. (f) A PASA IR spectrum, acquired in the laboratory, of the Mn oxide and the underlying clay substrate.

measurements, limiting our time below the surface. The measurement objectives for PASA specifically were to characterize the NIR spectral signatures of the snottites and other microbial colonies and biologically mediated mineral deposits, while the operational goals were to acquire sample measurements throughout the cave, thereby exercising the PASA components in a wet and toxic subsurface environment. The locations of the CVL sampling measurements are shown in Figure 11.

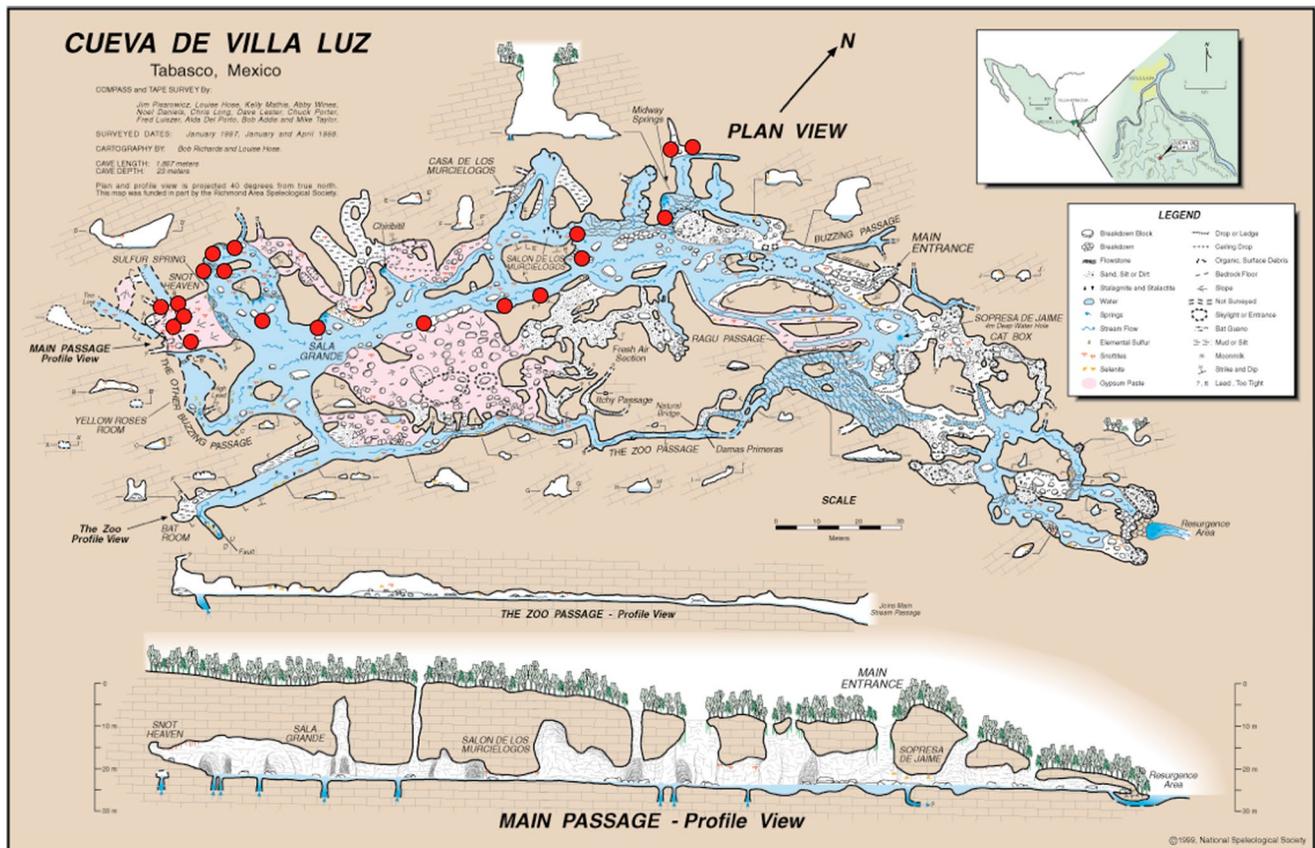


Figure 11. A map of Cueva de Villa Luz with the portable acousto-optic spectrometer for astrobiology sampling locations annotated by red circles. Map adapted from Hose and Pizarowicz (1999).

Figure 12 shows photographs, NIR reflectance spectra acquired with PASA, and reference images acquired with the PASA context camera for two samples: a snottite and a sulfur coated gypsum rock that was measured both in situ and in the laboratory. The snottites contain microbes that oxidize sulfuric acid (Hose et al., 2000), but as shown in panel (b) of Figure 12, at the spectral resolution of PASA the NIR spectrum of snottites is dominated by water features. The presence of sulfate mineral classes in CVL, particularly gypsum, is not surprising given that the oxidation of hydrogen sulfide (H_2S) to sulfuric acid (H_2SO_4) produces gypsum (Onac & Forti, 2011), and this process is accelerated in low pH environments (Forti et al., 2002). In the comparison of the NIR spectra of the sulfur-dusted sample from CVL and the synthetic mixture of sulfur and gypsum (Figure 12d) we see stronger features corresponding to bound water (at 1.70, 2.50, and 2.65–3.1 μm) in the field sample. Furthermore, the weak features near 2.2 and 2.3 μm corresponding to metal hydroxides—likely AlO, MgO, or FeO—are absent from the lab sample. Finally, the C-H absorption feature near 3.3 μm is only seen in the spectrum of the CVL sample, suggesting the possible presence of hydrocarbons that could be measured in situ in CVL.

3.3. Four Windows Cave

The development of PASA-L was motivated by our desire to use an AOTF-based NIR point spectrometer to explore planetary subsurfaces from a platform with vertical mobility. This led to the addition of PASA-L to the LEMUR 3 robot instrument payload, and the third cave environment explored with the PASA technology was Four Windows Cave, located in El Malpais National Monument. This lava tube cave is one of 15 major lava tubes formed in central New Mexico approximately 11,000 years ago during the Bandera lava flow (KellerLynn, 2012).

In an effort to demonstrate the efficacy of PASA-L in this lava tube environment not unlike what might be expected on other planetary bodies (Hong et al., 2014; Kaku et al., 2017), we deployed PASA to Four Windows Cave on 8 May 2015, and we used both PASA and PASA-L (integrated with LEMUR 3 and as a stand-alone instrument) in Four Windows Cave on 10–22 September 2015. We acquired NIR spectra from three regions of

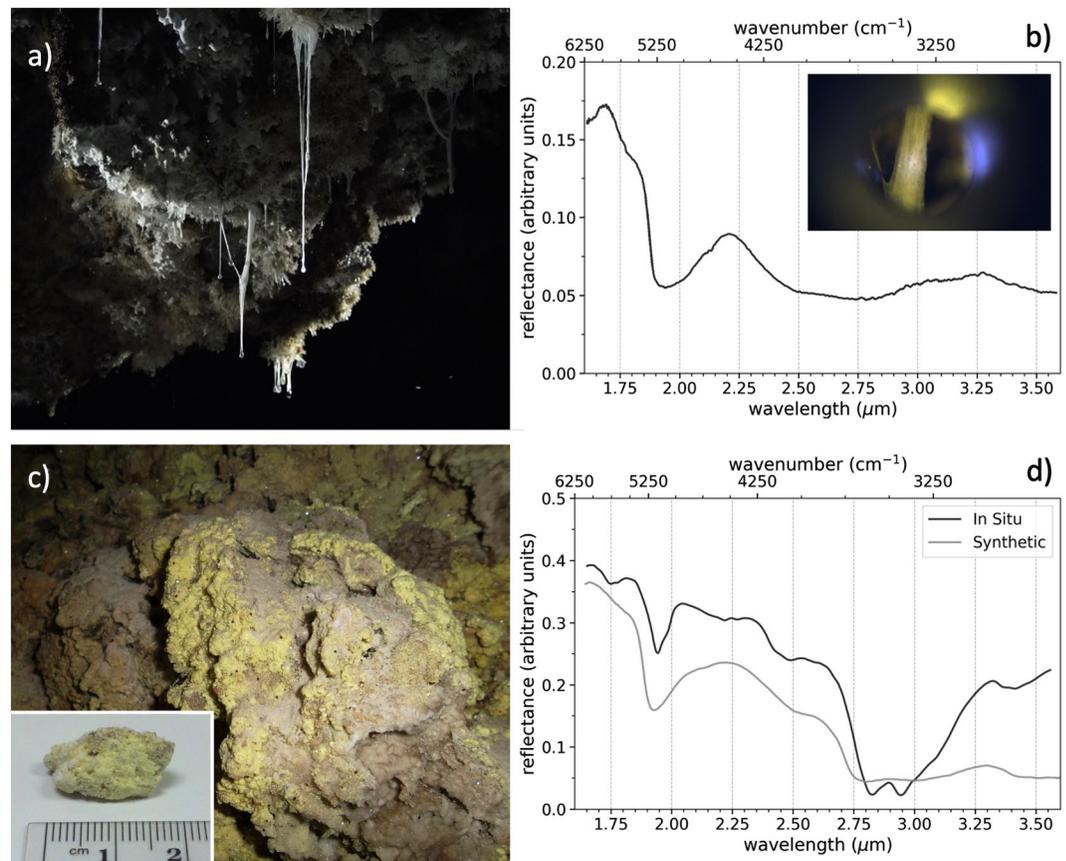


Figure 12. (a) A photograph of a snottite suspended from the ceiling of Cueva de Villa Luz (courtesy of Val Hildreth-Werker). (b) A portable acousto-optic spectrometer for astrobiology (PASA) infrared (IR) spectrum of a snottite, with an inset showing the PASA context image of the sample. (c) A sulfur-dusted gypsum rock measured in situ and in the lab (inset). The yellow regions on the sample correspond to sulfur dust, while the whitish tan substrate on which the sulfur is deposited is predominantly gypsum. (d) A PASA IR spectrum of the sulfur-dusted gypsum sample measured in situ and a synthetic mixture of powdered gypsum and sulfur.

the cave with distinct illumination conditions: the entry, which is subject to direct as well as scattered sunlight, the “twilight zone,” which is only faintly illuminated by scattered light, and the interior of the cave, which is in complete darkness. Figure 13 illustrates the sampling locations of PASA-L in Four Windows Cave, both mounted to LEMUR 3 and positioned by hand against the cave walls and regions of interest.

Because our instrument demonstration efforts were part of a broader effort to refine the strategies to detect organic minerals and differentiate geological patterns of biotic versus abiotic origin, a critical gap in current capabilities, we focused our observations on biosignatures throughout Four Windows Cave (Figure 14). In this operational scenario there was no opportunity to retrieve a statistically significant number of samples from the site and analyze them in the laboratory using SEM or LIBS instruments, thus the results from Four Windows Cave are qualitative and are intended to demonstrate the ability of PASA-L to function as part of the LEMUR payload. The entrance region was home to several microbial colonies of cyanobacteria, that is, bacteria capable of photosynthesis. As seen in Figure 14b, the microbial mat was more reflective than the underlying substrate at all wavelengths, and in both the microbial mat and the underlying basalt we see signatures of H₂O (symmetric bend and asymmetric stretch combined band at 1.95 μm), OH (stretching and bending modes near 2.4 μm), and the broad H₂O and OH⁻ vibrational mode from 2.6 to 3.0 μm (Pieters & Englert, 1993).

The cave walls in both the twilight zone and the dark zone of Four Windows Cave were replete with colorful colonies of actinobacteria. Figure 14d illustrates that the actinobacteria in the twilight zone exhibited stronger OH⁻ and bound H₂O absorption (1.9 and 2.4 μm). These spectra were acquired when PASA-L was not mounted to LEMUR 3; instead, the instrument was positioned by hand directly against the cave walls.

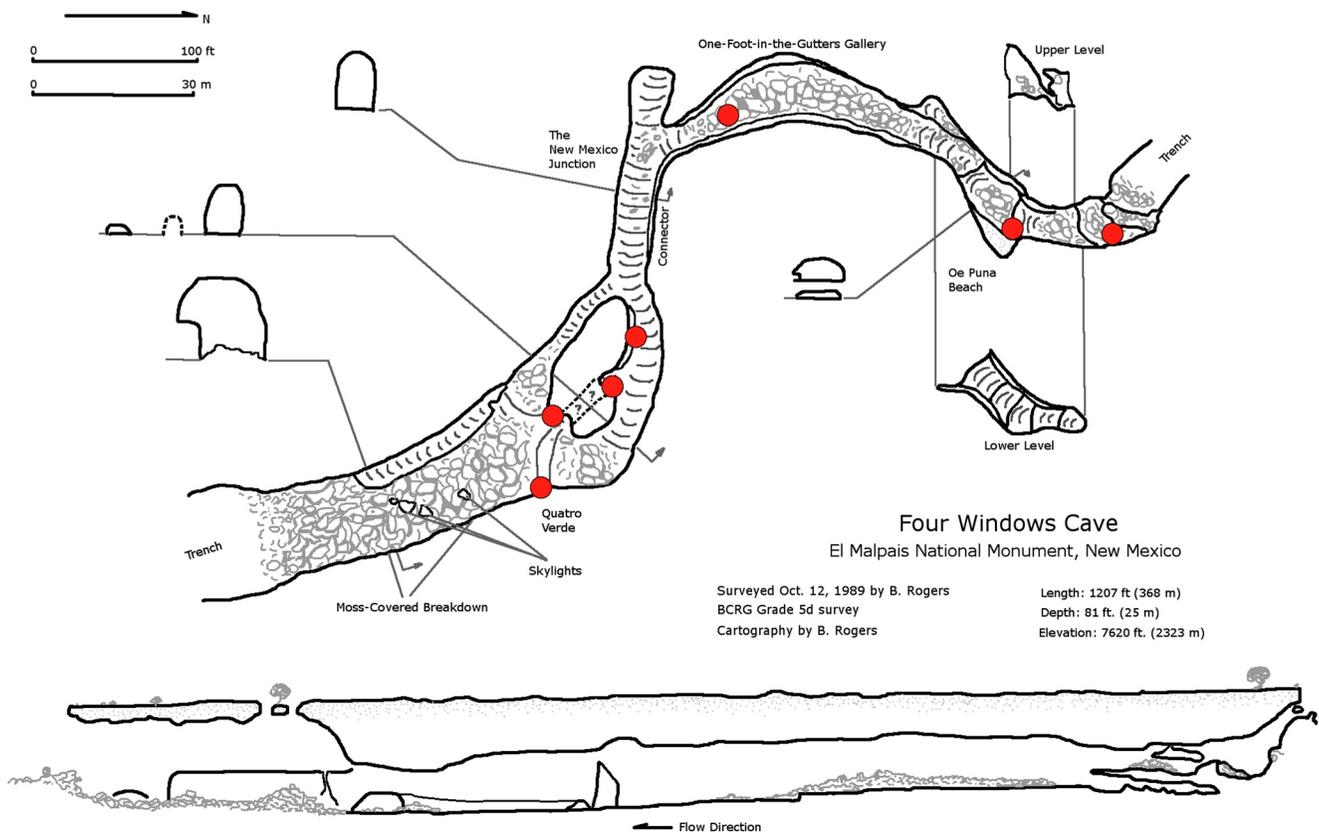


Figure 13. A map of Four Windows Cave in the El Malpais National Monument, with the portable acousto-optic spectrometer for astrobiology sampling locations annotated by red circles. This map is replicated from Uckert et al. (2020) and was adapted from the original survey by B. Rogers (courtesy of El Malpais National Monument).

Finally, Four Windows Cave contains several examples of biovermiculations, which are splotchy bacterial colonies that are relatively thin (a few mm thick) but with anomalously low pH values (~ 3) compared to their surroundings. It is unclear how such a steep pH gradient could be maintained without an impermeable barrier, thus they represent an intriguing example of exotic microbial colonies that could be studied with a portable instrument suite. Given that NIR reflectance spectroscopy is a widely used technique for mineralogical classification, we used PASA-L to determine whether it is possible to detect spectroscopic signatures of microbial alteration of underlying mineralogy, and whether the biovermiculations themselves exhibit unique spectra that contain clues about their origin. Figure 14f illustrates the NIR spectrum of a biovermiculation as well as the underlying basalt. The PASA-L spectra confirm that the basalt is less reflective at all wavelengths, that both the biovermiculation and the underlying substrate share the strong bound water absorption feature at $3 \mu\text{m}$, and that several features corresponding to OH and CH symmetric bend (ν_2) and asymmetric stretch (ν_3) at 1.90 and $2.20 \mu\text{m}$ are stronger in the biovermiculation spectra. A more extensive analysis of the biosignatures encapsulated in the biofilms and their underlying substrates would require the use of a portable instrument suite with a multitude of techniques, with PASA serving as one component of the payload.

4. Conclusions

Through the development of two NIR point spectrometers that use AOTFs as the wavelength selecting element, we demonstrated the efficacy of this technology for studies of subsurface environments. The terrestrial application of these instruments in three geochemically diverse caves supports their potential use on other planetary bodies. In each case, PASA or PASA-L was one component of an instrument suite, either used as a standalone instrument but in tandem with other technologies or integrated onto a single robotic platform in the case of PASA-L and LEMUR 3. As previously demonstrated in Uckert et al. (2017, 2020), and reaffirmed by a broad community of planetary cave exploration experts (Wynne et al., 2022), the power of an instrument suite cannot be understated.

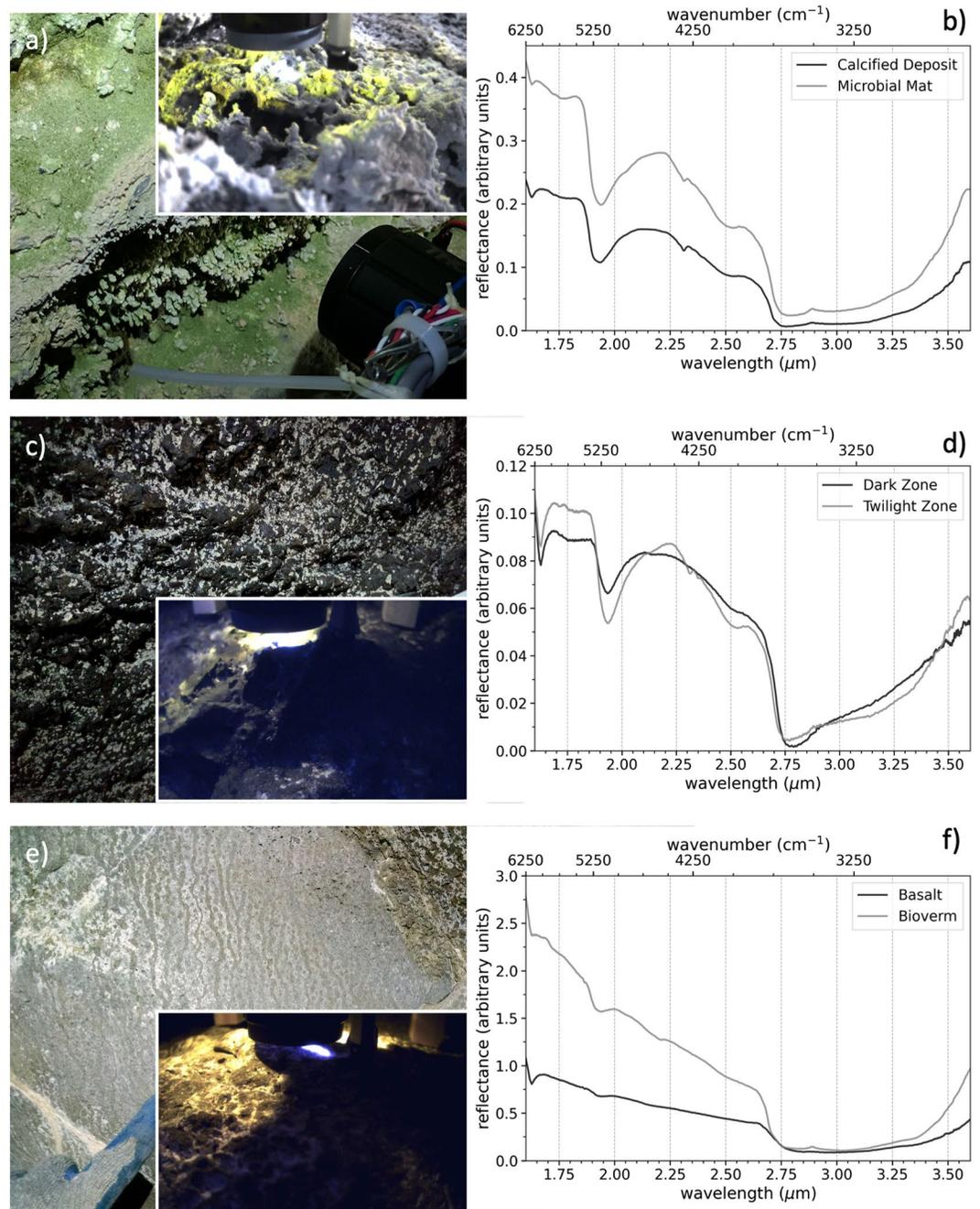


Figure 14. (a) A photograph of the cyanobacteria-rich wall near the cave entrance, with the portable acousto-optic spectrometer for astrobiology (PASA) objective in the foreground. The inset shows the image of the sample collected by the PASA-Lite (PASA-L) camera. (b) A PASA infrared (IR) spectrum of the cyanobacteria microbial mat and the underlying calcified basalt. (c) A photograph of the actinobacteria-rich basalt wall collected in the Twilight Zone. The inset shows the image of the sample collected by the PASA-L camera. (d) A PASA IR spectrum of an actinobacteria-rich wall measured in the Twilight Zone and Dark Zone. (e) A photograph of a biovermiculation pattern identified, with an inset showing the region of the sample collected by PASA-L. (f) A PASA IR spectrum of the biovermiculation pattern and the underlying basalt substrate.

NIR spectroscopy is a tool that can distinguish broad functional groups in mineralogical studies, which is an important contribution to our understanding of planetary subsurfaces.

AOTF-based instruments offer several advantages over traditional NIR spectrometers. The low SWaP afforded by our optical design makes PASA and PASA-L ideally suited for exploration of remote planetary environments.

The inherent ruggedness of these solid state devices also makes them ideally suited for field measurements where access to the environments of interest can be challenging and the environmental conditions themselves are inhospitable. Finally, the spectral agility of AOTFs provides flexibility in terms of the ability to focus on specific spectral regions of interest or acquiring spectral scans of the entire wavelength range of the instrument. Furthermore, the rapid integration times for both data acquisition modalities of PASA and PASA-L are an improvement over more traditional dispersive spectrometers.

Operationally, we learned several important lessons concerning the use of AOTF-based spectrometers in cave environments. First and foremost, working in the subsurface requires judicious selection of an appropriate illumination source. As we observed in Four Windows Cave, the low reflectivity of dark basaltic lava required the use of a higher power NIR lamp. This same issue would need to be overcome when exploring caves on Mars. If integrated into a robotic platform that housed multiple instruments, the desire to enhance the signal-to-noise ratio of our spectra would need to be balanced by the power availability of the rover and the power requirements of the remaining instruments that comprise the payload.

Second, in CVL we found that some of our measurements of morphological features with high water content (e.g., snottites) were more challenging due to the fact that multiple reflections of the light source inside the water droplets caused an increase in scattered radiation overlying the spectral data of interest. While we would not expect to encounter liquid water in the Martian subsurface, even the presence of frozen ice mixtures could pose similar challenges. This feature is inherent to the AOTF instrument optical design, which was optimized for diffuse reflection. The enhanced scattered light caused by the specular reflections manifested in the resultant spectra as an increased bias level or even saturation of the detector. To mitigate this in CVL, the instrument was repositioned in front of the sample by hand to reduce the scattered light that caused the internal reflections. Short-term variations in the spectra due to slight movement of the window were reduced by taking numerous spectra (typically 256) to generate a final spectrum from the median of the ensemble. This would be more challenging to execute in an autonomous operational scenario, and thus should be an important consideration for future iterations of the instrument design.

In addition to these lessons learned, we demonstrated that AOTF-based point spectrometers can be used in a wide variety of subsurface environments. A single instrument that is versatile enough to operate successfully in a wide range of cave types and contribute to the scientific goals of mineralogical and astrobiological characterization would be a powerful addition to an instrument suite designed to study planetary caves.

Development of PASA and the subsequent improvements that led to PASA-L were influenced from lessons learned from field expeditions in terrestrial caves. The scale and chemical composition of these caves are more relevant to an expected Mars subsurface environment than other planetary caves. While we report the TRL of PASA as 5–6 (system/subsystem model or prototype demonstration in relevant environment), we note that this TRL is based on expected Mars subsurface environments, which are likely to be the first targets for subsurface astrobiology missions.

In order to advance this technology further to make it a viable option for future planetary flight projects, AOTF-based NIR spectrometers would need to undergo additional demonstrations in a broader range of cave environments analogous to those we might expect on other solar system bodies, such as those described in Boston (2004). They would also need to be subjected to standard environmental tests such as thermal cycling and vibration testing to ensure that such an instrument could survive the rigors of launch, deep space travel, and entry, descent and landing (Titus et al., 2021). Finally, further miniaturization could be explored, for example, by making the optical design more compact, although given the compactness and low SWaP of PASA-L this is likely to only provide incremental improvement. We demonstrated that AOTF-based spectrometers are a powerful tool that can be used to explore planetary subsurface environments, and with ongoing improvements and modifications they are a logical choice for the payloads of future planetary cave explorers (specifically for Mars).

Data Availability Statement

The software developed for the analysis of PASA data can be accessed via https://github.com/kyleuckert/PASA_view (Uckert, 2016b). The data supporting the conclusions of this study, which focus on a technology and operational strategies for the acquisition of NIR spectra in planetary caves, can be accessed via <http://doi.org/10.5281/zenodo.7036224> (Uckert, 2016a).

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