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Development and Validation of a Microbe Detecting UAV Payload

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Abstract— **Airborne transport of microorganisms through the atmosphere has widespread implications for many atmospheric processes, ecological processes and human health. The proliferation of infectious disease-causing bacteria and fungi is of particular relevance, as many emerging diseases enter human populations via an atmospheric link to the surrounding environment. *Coccidiodomycosis* (Valley Fever), for instance, is a debilitating fungal disease contracted through the inhalation of *Coccidiodes immitis* and *Coccidiodes posadasii* of central California and elsewhere in the southwestern United States and northwestern Mexico. Recent studies suggest an increase in the incidence of Valley Fever throughout this region, but how the fungus is transported through the atmosphere is not well known. This is due in part to the fact that there is no effective and reliable standardized method for acquiring fungal spores at an elevated altitude, nor to do so rapidly—which would aid in limiting human exposure. This work fills the voids of sensing capability and rapid detection by means of small unmanned aerial systems (sUAS). The use of an sUAS enables low-altitude sampling, in addition to the low-cost development and operation of the payload. The payload consists of two coupled subsystems, which log environment data and extract a bioaerosol sample. The data and sample is analyzed and validated via a variety of molecular biological and microbiological techniques.**

I. INTRODUCTION

The development of sUAS in recent years has lead to valuable research in the field of remote sensing and sampling [1], [2], [3]. Remote sensing by means of unmanned vehicles offers unique high spatial and temporal resolution, formerly unattainable via low-cost or free satellite data. However, parallel to the miniaturization of remote sensing optics, sensors and supporting electronics, various environmental sensors have too been developed for portable field work and intelligent environmental sensor networks (ESN) [4]. While the original intention for the development of these sensor systems was to improve current environmental data collection methods of handheld devices and sensor stations, these sensors are now small enough and light enough to be placed on sUAS platforms.

Sensory electronics help promote the utilization of current field-use environmental sensors in sUAS payloads that can then be post-processed in the lab; however, UAS also offer the possibility of bringing the lab to the field. UAS are obviously well-suited to study atmospheric properties and phenomena that are otherwise difficult to examine. In particular, one component of the atmosphere that is mostly unmonitored and remarkably understudied is the diversity of microorganisms present in the air we breathe. The presence of bacteria, archaea, fungi and viruses in the atmosphere is no surprise, but recent studies have shown just how diverse and potentially important airborne microbes may be [5]. For example, concerns about bioterrorism and airborne transport of human pathogens have promoted application of molecular biological techniques to the rapid detection of pathogens in air DNA samples [6], and exploration of microbial diversity in urban air [7], [8]. Other studies have examined airborne transport of non-human pathogens that cause disease [9], [10]. However, these studies have been largely limited to static, manual sampling platforms located near the land surface.

In 2008, Schmale et al. successfully utilized small UAVs to collect aerobiological samples that are agricultural threats over Virginia Tech's Kentland Farm [11]. One of Schmale's techniques for sample collection was a fixed-wing aircraft outfitted with Petri dishes and servo mechanisms which placed the dishes perpendicular to the aircraft heading upon command. Additional research has been conducted on placing a liquid impinger unit onboard a UAS [12]. While liquid impinging units have a high particulate retention rate, the weight, complexity and fragility of such a payload offsets the usefulness and rapid response of an sUAS. Additionally, both of these designs are best-suited to specific microbiological applications, given the limited biological utility of the samples and their relatively limited number. A UAS capable of sampling comparatively large volumes of air for subsequent analyses using multiple techniques would provide a wide range of applications in atmospheric science, environmental science and the health sciences. This work introduces the design, implementation and validation of such a system.

This work focuses on a fixed-wing unmanned aerial vehicle (UAV), specifically on the payload development and implementation for collecting aeromicrobial samples at altitude. In Section II, an overview of the sUAS utilized in the study is provided, including the payload description, sample collection method, and analog and digital sensors. Section III discusses the methods for validating the sampling payload and data obtained inflight is introduced. Validating results are discussed in Section IV, followed by concluding remarks in Section VI.

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II. UAS OVERVIEW

Commercially-off-the-shelf (COTS) available aircraft have proven their worth in the realm of remote sensing research for the past decade or so [13], and allow for smaller research groups to easily multiply their research potential by a large factor. In that same manner, this project leverages these advances in UAV technology in order to develop a novel bioaerosol filtration and collection technique. The UAV utilized is a modified COTS radio controlled (R/C) fixed wing aircraft, aptly named the Scientific Data Drone 1 (SDD1). The avionics onboard SDD1 include six 17g servos, a 45 Amp electronic speed controller (ESC), a 720kV brushless motor, a Spektrum 7 channel receiver and a 3D Robotics Pixhawk autopilot.

The focus of this work is upon the payload and sample collection, thus the Pixhawk autopilot is relied upon to control the attitude, altitude and navigation of the aircraft in its default configuration. What is meant by default configuration is that the PID controllers 'out-of-the-box' are utilized, and basic PID tuning techniques are utilized. The minimum takeoff weight of SDD1 is 1.25 kg, and the maximum takeoff weight is 4 kg. The ground control station (GCS) consists of a Dell Inspirion laptop and the GCS software is Mission Planner. Communication between the GCS and the autopilot is achieved via a 915MHz 3D Robotics telemetry radio system, with an effective range of approximately 1km; however, this range limit will not be reached for the intents and purposes of this project. The maximum flight time attained through experimentation is 1 hour, which introduces substantial constraints on sample collection and will be discussed further in Section II-B.

A. Payload Description

There are two critical constraints when designing any sUAS payload: weight and power. An additional constraint was placed upon the developmental cost of the payload, which is critical for a low-cost, rapidly manufactured and rapidly deployed unit. In this case, the payload is desired to weigh less than 500g, consume less than 20W of power and cost less than $500USD. The payload is controlled through the use of an Arduino Pro Mini board, which utilizes the Atmel ATmega328 8-bit microcontroller. This board was chosen for the sufficient computing power, low weight and low cost. The payload is programmed to sense and log critical environmental factors, control the collection of an air sample and transmit logged data through a live stream to the ground control station.

The payload consists of two subsystems: Sensor Subsystem, referred to as SS1; and Sampling Subsystem, referred to as SS2. SS1 encompasses all the analog/digital (A/D) sensors onboard that are read by the microcontroller, stored onboard via an OpenLog open-source data logger and additionally transmitted wirelessly to the GCS through an XBee Pro Series 1. There is an XBee Pro placed at the GCS and one on the payload, which communicate via IEEE 802.15.4 protocol and allows for serial communication over an ad hoc wireless network. At the GCS, the data stream is visualized on a serial communications port that displays the sensory data in the same manner as it is written to the OpenLog data logger, which is further discussed in Section II-C.

Table I outlines the electronic components utilized in the payload. It can be seen that the payload component weight, cost and power consumption achieves the goals set, along with some margin for each.

B. Sample Collection

Current proven methods in research for extracting a bioaerosol sample by means of a UAV are limited to passive collection, as discussed previously. The payload developed in this work aimed for active sampling control, similar to that used in ground based liquid impinger instruments, but with the light weight and durability of passive collection. Because of this, the "Microbiology Sampling Guide" was used as a reference for determining adequate sample collection volumes necessary for species identification and sample retention [14]. While the guide does not directly provide a range of volumes, for outdoor sampling with an active sampling system one can be interpreted to be from $15-150$L per sample, depending on the unit utilized. Due to weight, size and power consumption, a small 12VDC diaphragm vacuum pump was utilized for sample collection in SS2. The datasheet provided by the manufacturer for this pump indicates a free-flow rate range of $12-15\frac{\text{L}}{\text{min}}$ [15]; however, through the introduction of tubing and filter medium, the average measured flow rate was a mere fraction of that, $0.23\frac{\text{L}}{\text{min}}$. At this flow rate, the range of the volume of air sampled is $13.8-20.7$L, which is on par with the low portion of the range interpreted from [14]. With the pump chosen, the series of events for sampling are described.

After takeoff, the sUAS is commanded to a set flight altitude of approximately 80 meters above ground level (AGL). At this point, the aircraft is then commanded to circle about a predefined latitude and longitude coordinate with a radius of 200 meters, and subsequently commanded to activate the vacuum pump of the SS2. Once the SS2 is active, aerosol is drawn in from the outside air through a $\frac{1}{4}$" (6.35mm) section of flexible silicone tubing by means of the vacuum pump. The tubing connects to a filter holder, which supports the filter medium and distributes the flow evenly across the filter medium. The filtrate then passes through the flow meter, and finally passes through the vacuum pump and back to the ambient air. The vacuum pump is quite effective for this process, since it induces a substantial pressure gradient between the upstream surface and the downstream surface of the filter medium. The filter is assumed to be a homogenous porous layer and have a property of uniform permeability. A diagram of this process is shown in Figure 1.

Although flight time is limited to approximately 45 minutes by current low-cost battery technology and payload weight, typical sample duration is 1 to 1.5 hours. In this

TABLE I

Component	Weight [g]	Power Consumption [W]	Cost [USD]
Arduino Pro Mini	1	0.1	$9.95
Xbee Radio	0.56	0.71	$37.95
Vacuum Pump	246	10.8	$14.95
OpenLog Data Logger	2	0.02	$24.95
Barometric Pressure and Temperature Sensor	2	0.001	$12.95
Humidity Sensor	0.5	0.11	$16.95
GPS Unit	24	0.11	$89.99
Power Module	0.5	2	$19.95
Flow Sensor	22	0.5	$8.93
Total	**398.56**	**14.541**	**$236.57**

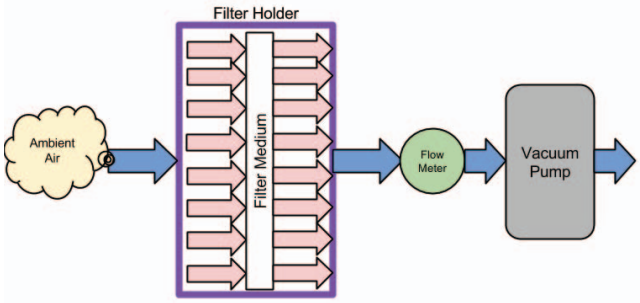

Fig. 1. SS2 Air Sampling unit

scenario, the GCS operator informs the pilot of the low battery level, the payload is then commanded to halt the pumping procedure and the UAV is landed. The discharged battery in the UAV is then replaced with a fully charged battery, the UAV is then launched and the sampling process continues. The total time between pausing and resuming the sample process is approximately five minutes on average.

C. Analog and Digital Sensors

The sensor suite consists of five sensors: barometric pressure sensor, temperature sensor, humidity sensor, power module and flow sensor. These sensors are outlined in Table I, along with the weight, power consumption and cost of each component. The total weight of the sensors is approximately 52g, which is relatively light considering each sensor is packaged with its own PCB breakout. Finally, the power consumption of SS1 is approximately 3.7 Watts. Future iterations of SS1 will consolidate all surface mounted components of the individual sensor breakouts into one cohesive PCB.

Upon powering on the SS1, a new file is written by the OpenLog onto a micro-SD card with a comma delimited header structure written as the first line. The unit iterates through a 'while' loop until a GPS signal is obtained and all I2C communication sensors are active, prior to data logging. All data points are transmitted through the Xbee telemetry unit, as well as logged to the micro-SD card. The values are comma delimited, thus allowing for straight-forward analysis in Microsoft Excel or MATLAB. The XBee telemetry unit enables the realtime visualization of the data stream, allowing the GCS operator to ensure that the payload is properly functioning and to sanity check the values on-the-fly. These values are later downloaded from the micro-SD card and analyzed in MATLAB using a script developed.

Three basic and critical data points taken are temperature, pressure and humidity. While these values will not be analyzed in depth in the scope of this work, they are essential to much of the in lab post processing and data analysis regarding aerosols. Additionally, a flow sensor is utilized in order to quantify the volume of air sampled during the flight. The flow sensor functions on the utilization of a Hall-effect sensor and impeller; essentially, counting the number of times a magnet located on the impeller changes poles. The microcontroller registers these changes as a binary signal, and thus a frequency can be obtained by dividing the sum of the number of pole changes by a known time interval. This frequency can then be converted to revolutions per minute, and once a proportional correction coefficient is determined, can be converted to flow. The flowrate is then integrated at each time step iteration onboard the SS1 and continually summed to yield the overall volume sampled per sampling cycle.

The A/D sensors onboard SDD1 are critical to obtaining knowledge regarding bioaerosols. Through SS1, a unique view of the region of air between ground level and typical manned aircraft operational altitudes is gained.

III. Experimentation

In order to validate the SS2 sampling ability beyond a reasonable doubt, a proper 'golden-sample' experiment must be performed. In this case, the 'golden-sample' was taken using a single stage liquid impinger sampling unit. A liquid impinger separates and re-suspends particles in a liquid, formerly suspended in aerosols by means of a vacuum and bubbler system. The impinger unit was additionally placed in an ice bath to preserve the sample during collection; however, the filter holder on the SS2 was subject to ambient conditions. The SS2 was placed alongside the impinger unit and both were situated exactly 12 inches off the ground, as shown in Figure 2. The units were set to run for 2 hours, and the samples were immediately refrigerated at the end of the sampling cycle.

Analysis of the samples rendered the result that the bioaerosol sample obtained from the SS2 is favorable when in comparison to the golden-sample. In fact, it was determined that a larger number of particles were collected via

Fig. 2. Liquid Impinger and SS2 test

SS2. While the result of the liquid impinger sample analysis is not presented in this work, the result of the sample taken by the SS2 during this test are shown and discussed in Section IV.

Upon validation, SS1 and SS2 were integrated into SDD1 and a sample set was conducted for seven consecutive days. Due to diffusion and advection phenomena inherently present in environmental systems, a component of characterizing and identifying microbial species suspended in aerosols is recording the altitude at which the particles are collected. As such, a box plot indicating the distribution of the altitudes flown during the sampling process is shown in Figure 3. The desired altitude for flight was 80m AGL, although there was some deviation in the flight path both due to manual R/C control and anomalies in measured pressure (see day 4 maximum outlier). Additionally, the windspeed is recorded during the flight and plotted in Figure 6, which is obtained via the wind triangle and out of the scope of this work [16]. Both of these data points are utilized later on in the post-processing component of microbial species detection and correlation, which will be presented in later work.

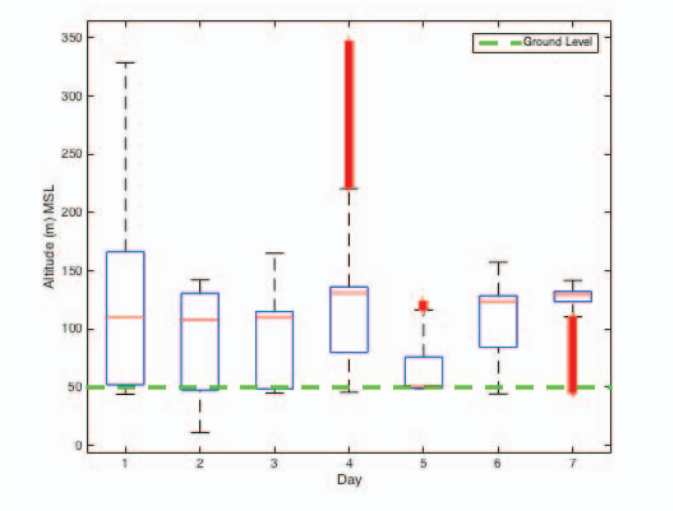

Fig. 3. Box plot of altitudes flown over a seven day period

The flight path flown by the UAS can be seen in Figure 4. In this figure, two flight trajectories can be seen. The first flight trajectory is indicated by the left circle and random movement, while the second flight trajectory is indicated by the right circle. These two trajectories are indicative of the battery swapping process that occurs at approximately three-quarters of the sampling cycle.

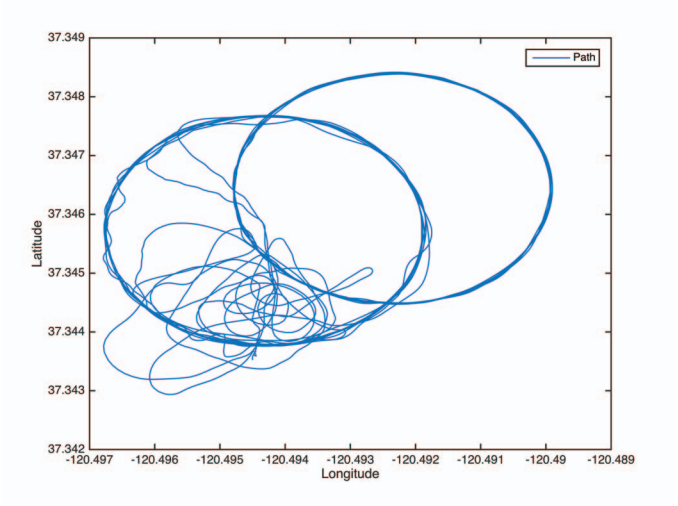

Fig. 4. Flight path of SDD1. Two intersecting circles indicate the flight paths of a sampling operation. The left circle is the first portion of the sampling cycle, and the right circle is the final portion.

Figure 5 shows temperature and altitude as a function of flight time. As flight altitude increases, an initial drop in temperature is seen over the first five to ten minutes of flight time. While altitude remains relatively constant, the temperature then begins to increase gradually. This fluctuation in temperature at a constant altitude and at altitude transitions can aid in the understanding of the dynamics behind bioaerosol transport and microbial life spans while in the state of bioaerosol.

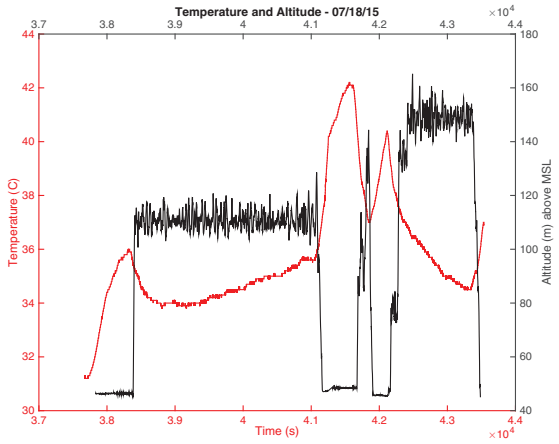

Fig. 5. The left axis is temperature and the right axis is altitude ASL of a sampling flight.

IV. Validation

Filtered aerosol material collected via the UAS-based sampling system was analyzed via multiple molecular biological and microbiological techniques, demonstrating the efficacy of the system. Successful recovery of airborne cells was verified using flow cytometry, which can subsequently be refined to detect and enumerate specific microbial groups.

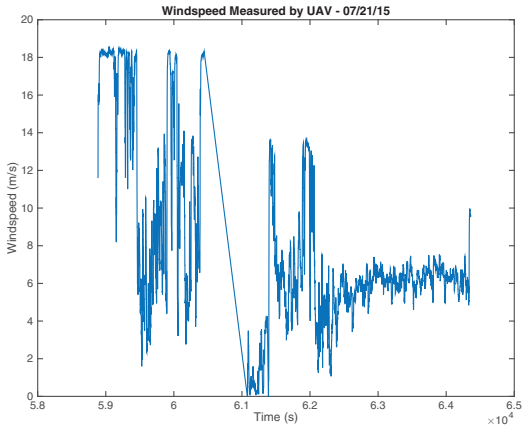

Fig. 6. Typical windspeed graph during sampling operation.

DNA extracted from these cells can be used in a variety of downstream applications, including polymerase chain reaction (PCR) amplification of specific genes from specific organisms—such as *Coccidiodes sp.* fungi—or analysis of entire microbial communities present in air samples.

We initially used flow cytometry to verify the presence of soil particles on filters to estimate the total number of particles, and to ultimately determine whether some of these particles represent living cells. Flow cytometry is used to quantify and sort particles based on their optical properties; particles are suspended in liquid, introduced into the instrument in a single stream, and interrogated via lasers of different wavelengths with the instrument collecting data on absorption, scattering (in multiple directions), and emission for the different wavelengths. Particles analyzed via flow cytometry are most commonly cells. For initial tests, aerosolized soil particles that were collected onto 0.2 micron Durapore filters (Millipore) by the SS2 filter system were then re-suspended from the filter via vortexing in sterile water and analyzed via flow cytometry.

We first determined whether particle counts for filters exposed to soil were substantially higher than background counts, and found that they were typically $2-3$ orders of magnitude larger. This indicates that the filter system captures sufficient aerosol material. Many of these particles reflect non-living mineral or organic compounds present in air; identification of actual cells was achieved by using a dye that binds to DNA and exhibits specific optical properties when bound to DNA. 4',6-diamidino-2-phenylindole, or DAPI, is excited by 350nm light and emits at 470nm when bound to DNA. Bacterial cultures (*E. coli*) were used as a positive control during analysis. Based on analysis of several test samples, we found that samples treated with DAPI (Figure 7B and 7E) had significantly different emission properties than when left untreated (Figure 7A and 7D). Based on comparisons with the optical properties of the positive control, $29.74-44.23\%$ of the aerosol particles present in samples collected by the SS2 filter system contained DNA. These numbers are in line with estimates of the bioaerosol fraction present in air samples [17]. To our knowledge, this is one of the first applications of flow cytometry to aerosol particles other than Bowers et al. [18], who quantified airborne microbes in samples collected at a single location using flow cytometry.

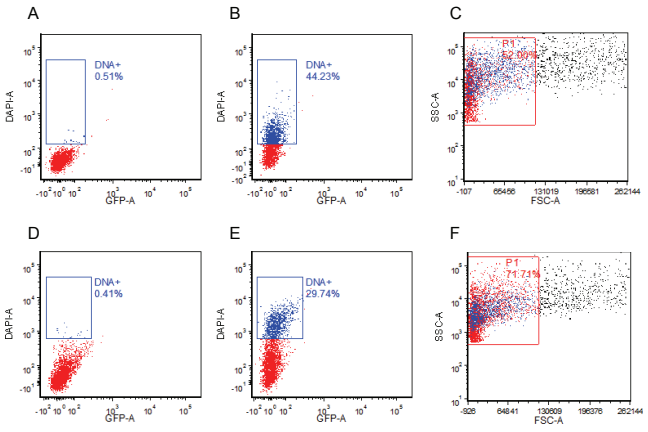

Fig. 7. Flow cytometry results from test samples. Top panels (A-C) and bottom panels (D-F) represent two individual test samples. (A and D) Background DAPI emission versus green fluorescent protein (GFP) emission for samples without DAPI added. (B and E) DAPI emission versus GFP emission for samples following the addition of DAPI. (C and F) Side scattering (SSC) versus forward scattering (FSC) for samples following the addition of DAPI; FSC represents an index of particle size.

Given the presence of significant cell numbers in aerosol samples collected using the UAS filter system, it is possible to extract sufficient quantities of DNA from these samples for subsequent molecular biological analysis. Earlier work using UAS to collect microbial samples have all focused on culturing microorganisms [11]; however, it is well-accepted that the vast majority of microorganisms (ca. 99%) are resistant to cultivation in a laboratory setting. For this majority, molecular techniques offer the possibility of analyzing and quantifying microbial DNA, rapidly and accurately, without cultivation. For these reasons, PCR-based tests are emerging as ideal for detection and monitoring of a variety of airborne infectious diseases [6], [19], [20]. Once DNA is recovered, it can also be analyzed at a variety of levels of resolution that range from detecting specific pathogens such as *Coccidiodes sp.* fungi, to examining a broad diversity of microbial groups.

DNA was extracted from UAS-collected aerosol material using standard DNA extraction approaches developed for environmental samples (MoBio PowerSoil DNA extraction kit). Given the relatively low concentrations of cells in the atmosphere, overall DNA yields were low ($0.1-3$ng of DNA) but sufficient for sensitive molecular analyses. More lengthy sampling and improvements to the pump flowrate could further increase yields. Ongoing work is aimed at quantifying DNA from specific microbial groups of interest, including *Coccidiodes sp.* fungi.

V. Future Work

The ultimate goal of this project is to rapidly identify and locate harmful bioaerosols in the air surrounding communities. To accomplish this, rigorous source seeking algorithms alongside real-time identification technology must be developed leveraging theoretical works found in literature [21] [22]. While brute-force methods such as lawn-mower patterns or circling patterns are relatively effective, they are inefficient, costly and time consuming, thus negating the benefits of utilizing sUAS. Additionally, there needs to be further analysis performed on the perturbations introduced by the UAV on the volume of air being sampled, thus dispersing the concentration of the microbes. Therefore, future theoretical work and emphasis is placed on optimal path planning, source seeking and multi-agent cooperation.

In addition to theoretical work, application based developments are underway. Complete autopilot integration for data stream via the sUAS telemetry link and live data view in Mission Planner is under development. Research on a field test kit, a future iteration of the current sampling unit, in which the results of key microbes of interest can be identified at the source in real-time. A designed PCB of the field test kit is shown in Figure 8. This field kit could grant up to date knowledge of air quality to public health officials and aide in the rapid response of nearby communities, especially in case of a significant presence of Valley Fever.

Specific to Valley Fever research, prior work utilizing infrared imagery can be leveraged, such as [23]. In this work, a correlation between Normalized Differential Vegetation Index (NDVI) time series using satellite imagery and disease incidences is made. Additionally, there is much research establishing the benefits of aerial imagery collected by UAVs in comparison to the aerial imagery collected by satellites.

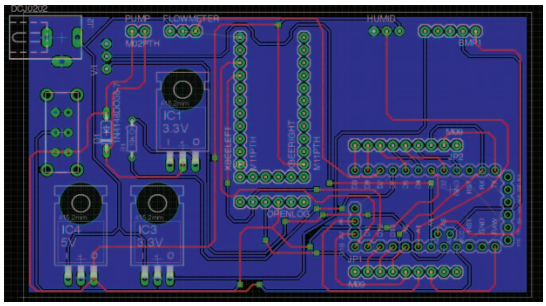

Fig. 8. PCB Schematic for SS1 v2

VI. Conclusion

Over the past few years, research into the aeromicrobiome has demonstrated that living cells are relatively common component of the atmosphere, where they have surprising impacts. For example, [24] showed that biological aerosols may affect the formation of precipitation in the western United States, and that some of these cells can be transported across entire ocean basins. Other work has hinted at cross-ocean transport of disease-causing organisms [25]. However, most studies examining microbes in the atmosphere are based on samples collected at a limited number of locations near ground level, and we consequently lack a four dimensional view of the constantly-changing atmosphere. We demonstrate that this void can be addressed in the lower atmosphere via UAS-based microbiological sampling. We focused on the development of a system that can be readily adapted to a variety of research and health applications—from basic ecological questions to the detection of specific pathogens in atmosphere. This has the added benefits of being extremely rapid, with the potential for end-to-end automation. In the case of Valley Fever, rapid detection could aid in public awareness and rapid response of public health officials to notify nearby communities to stay indoors or wear protection from inhalation of the harmful fungi. The flexibility of our system—a filter than can be analyzed in parallel via multiple techniques—allows this approach to be readily adapted to other airborne infectious diseases. The relatively low cost of our system also allows synchronous deployment in a variety of locations, increasing spatial and temporal sampling resolution.

Acknowledgment

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