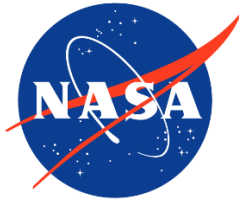# Correlating UV Radiation with Microbial Growth

Team Ultraviolet Voodoo (UVVDU)

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Colorado Space Grant Consortium DemoSat-B Program
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Abstract

The project focus was on measuring ultraviolet (UV) radiation as a function of altitude and, by gathering biological samples over various regions of the atmosphere, attempting to relate UV exposure to microbial growth. This data could shed light on the viability of upper atmosphere microbes, which has an impact on planetary protection protocols developed to prevent contamination of other stellar bodies with life from Earth. Furthermore, UV data through the atmosphere delivered in a low-cost and efficient manner can provide insight for future research. A payload was sent into the upper atmosphere to reach an altitude of 30 km. This payload was designed to survive harsh conditions including: low pressure, low temperature, increased radiation, high rpm, and impact on landing. The design was divided into two main categories: biological and electrical. The biological data focused on collecting microbes that exist in the upper atmosphere and preventing contamination of those samples with ground based organisms. Species such as *Bacillus subtilis*, and *Engyodontium album* were expected to be captured. The electrical components measured UV radiation using photodiodes with optical filters, and a microcontroller to record all the data. The payload survived landing and no major components were damaged.

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1.0 Mission Overview

1.1 Introduction

The sun emits energy in the form of electromagnetic radiation spanning from high energy gamma rays to radio waves. The ultraviolet, visible, and infrared portions of the emission spectrum are required for various biological processes and are responsible for most life on our planet. However, those same wavelengths required for life can also be harmful, especially those in the ultraviolet. The sun emits ultraviolet radiation (UV) in three regions: UV-A (320-400nm), UV-B (280-320nm), and UV-C (200-280nm). UV-A accounts for 90% of the ultraviolet radiation that reaches the surface of the Earth and can cause tissue damage if exposure is prolonged [5]. UV-B is the main cause of sunburn and a principal factor in the growth of skin cancers [5]. UV-C denatures DNA by breaking chemical bonds due to its high energy; however, the upper atmosphere absorbs or reflects it (Fig. 1) [5]. The absorption of UV light as a function of altitude is presented in Figure 1. The entire UV spectrum can be harmful due to the high energy of the light but is also responsible for one positive consequence.
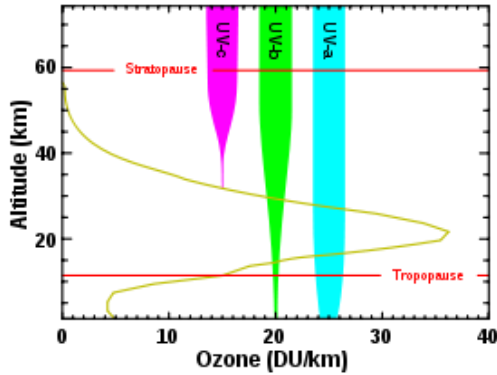

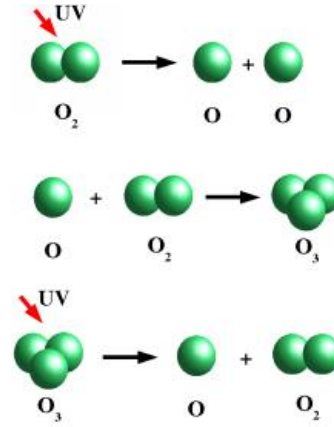Figure 1. *Altitude vs Ozone in relation to different UV regions.* [9]

Figure 2. *Reaction mechanisms for the formation of ozone.* [10]

UV light is responsible for the creation and destruction of the ozone layer, forming a reaction mechanism. Ultraviolet ray's separate diatomic oxygen molecules into two oxygen atoms by breaking the covalent bond via absorption of the high energy ray. These oxygen atoms are very unstable and will form a new bond with dioxygen, forming ozone, as displayed in Figure 2 [10]. Without the separation of the oxygen molecule, ozone cannot exist, but this process requires a lot of energy. The bond enthalpy for the double bond in O_2 is 498 kJ/mol, making the energy required to break one double bond:

$$E = \frac{498\ (kJmol^{-1}) * 1000}{6.02 \text{x} 10^{23} (mol^{-1})} = 8.27 \text{x} 10^{-19} J$$

The maximum wavelength of light required to break the double oxygen bond is:

$$\lambda = \frac{hc}{E} = \frac{\left(6.63 \text{x} 10^{-34} (J\ s) * 3.00 \text{x} 10^8 (ms^{-1})\right)}{8.27 \text{x} 10^{-19} (J)} = 2.41 \text{x} 10^{-7} m = 241\ nm.$$

And therefore, it can be concluded that only the most energetic, UV-C rays, are "absorbed" in the ozone layer.

In addition to ozone, aerosols also play a crucial role in protecting biological life from the harmful effects of radiation. Aerosols, such as sulfates and nitrates, scatter UV radiation in the troposphere, reflecting the rays back into space [4]. Other aerosols, like carbon black, absorb UV creating a heating effect [4]. Usually the UV reduction due to aerosols is minimal because it is highly dependent on jet streams, cloud coverage, gas emission, solar elevation angle, natural disasters (e.g. volcanic eruptions), and varies heavily from region to region. However, in certain areas, aerosol particles can absorb or scatter more than 50 percent of the harmful rays [4].

The amount of radiation that reaches Earth's surface is greatly influenced by ozone, aerosols, and cloud coverage. Each of these factors contribute to UV radiation and microbial growth in the atmosphere. Certain organisms can create a protective layer, called sporulation, to shield from the harmful rays of the sun and other sources of radiation. These microbes adapt to their environment, whether it be the extreme cold, radiation exposure, or low pressure using spores, mini cells, or resistance to survive in such extreme environments [3]. Besides microbes, life on the surface of earth has adapted to UV exposure. Over many years, human cells have developed a special enzyme to combat UV-B damage, and repairs the damaged DNA, making human DNA protected against radiation exposure [5]. However, the repair enzyme is not perfect and does not always repair DNA, which can lead to cancer in some cases [5].

1.2   Mission Goals

The primary mission goal of the project was to collect UV radiation data accurately, precisely, and reliably using photodiodes and optical filters in a payload flown to approximately 30,500 meters on a high-altitude balloon. The information gathered would be used to create a data set for UV intensity throughout the atmosphere. This data could also be correlated with aerosol data collected by AEROSOLUTION. AEROSOLUTION is another team launching a payload from the DemoSat-B program on the same flight. They analyzed carbon black, which is an aerosol that decreases UV exposure on the surface. This presents a unique opportunity to correlate aerosol data with UV data, within the DemoSat program.

The secondary objective of the mission was to expose filters to the atmosphere at 3 different elevations. This can be accomplished with filters that absorb microbes and are sealed for sterility. Microbial activity and radiation exposure can be correlated by analyzing this data post-launch.

1.3   Mission Purpose

UV data has applications beyond our project. The radiation data through the atmosphere can be used to fill current gaps in available data sets, help others improve atmospheric models that use UV as an input parameter, and inform future payload designs for the measurement of UV. The irradiance of UV on the ground is well documented, however a gradient through the atmosphere is lacking. There is data for certain altitudes, like 300 km, and 12 km, but a function of altitude versus irradiance for UV exposure is the goal [1], [7]. An additional goal was to gather UV data in an efficient, lost-cost, and sustainable way. The general sensor data that is collected can provide a baseline for future research as well. The payload also contained GPS, accelerometer, temperature, and humidity sensors that were used to monitor the payload.

This project is also exploring microbial growth through the atmosphere. This has potential applications beyond this project since spacecraft travel through the Earth's atmosphere into space. A general concern for spacecraft moving through the atmosphere is what it can transport from outside

the atmosphere [2]. The growing concern about microbes adapting to extreme environments is increased pathogenicity. If traveling spacecraft give microbes the chance to be mutated by UV radiation, then new diseases could arise. Not only on Earth, but exploration to other planets can transport microbes. Whether it be transporting Earth's microbes to other planets, or unknown bacteria from other planets to Earth. Moreover, the adaptations of microbes to extreme environments can apply to new projects about how to improve different processes. Since some microbes that live in the upper atmosphere live on the surface as well, it would be beneficial to compare the differences in their genomes.

1.4 Requirements

The objectives of this mission were to collect radiation data to better characterize how the atmosphere absorbs or reflects harmful rays. This introduces a level breakdown, with level 0 requirements being related to the primary mission goal. Level 0 is the first mission requirement this project focused on, and so forth until level 2. To accomplish this, the sensors needed to be accurately calibrated and placed in a location suitable for measurements. The UV sensors must face the sun, since they are photodiodes and only receive signals based on light. The recovery of the payload was crucial, as the data was located on the SD card inside the payload. Additionally, the payload is sustainable only if recovery is successful.

Level 1 requirements for the microbial sampling device was the air sampling device. This includes the pump, filters, servo, and other minute mechanical aspects. The system consisted of a filter, pump, servo, tubing, and a manifold block. All the components needed to work in parallel for the biological data to be collected.

The level 2 requirement is contamination. This is an important objective because if the samples are contaminated, that means they do not accurately represent what is growing in the upper atmosphere.

Table 1. *Requirements diagram.*

Level 0	- UV-A, UV-B, UV-C sensors calibrated - Collect accurate and reliable data - Record for the entire flight - Recovery after flight - Less than 1000 grams - Less than $1000
Level 1	- Collect air samples at three different heights - Pump generates volumetric flow for the entire flight - Servo ensures air is directed to right filter
Level 2	No contamination

2.0 Materials and Methods

2.1 General Payload Design

The payloads function was divided into two major components: biological filtration and all other sensor data acquisition. The primary reasoning for the division was for space and weight distribution of the system. The filtration system components were nearly the same weight as the electrical components and battery, creating a center of mass near the flight tube. When optimizing the space, it became evident that dividing the two systems would be best. Shown in Figure 3, one side of the payload has the integrated electronics, and Figure 4 shows the biological sampling device on the opposite end.

Figure 3. *Electronics layout on payload.*

Figure 4. *Biological layout on payload.*

The filtration subsystem consisted of a central manifold block (the container for holding and sealing the sterile filters), a pump, a 4-way valve, temperature sensor, and heater. The system was designed for the pump to run continuously throughout the flight, with the air flow over each filter being controlled with the 4-way valve. Additionally, because the pump was located after filters, there would not be a need to sterilize the pump and the risk of contamination could be further reduced.

The sensor subsystem was controlled with a microcontroller and was designed to operate without user input for the duration of the flight. An Arduino Mega was selected to support the considerable number of I/O's (16) required, with enough pins to run the needed sensors, heaters, and mechanical systems. The primary sensors were the three photodiodes, one for UV-A, UV-B and UV-C. The other sensors included an accelerometer, magnetometer, gyroscope, GPS module, and two temperature and humidity sensors.

The payload's layout is centered around lightweight and durable material and was designed to be sustainable and affordable. Recycled 1½" Styrofoam core was used for the structure material to insulate and provide cushion for landing. Aluminum tape on the outside of the payload proved to be the sturdiest for flight, surviving the drop test, stair test, and whip test with minimal surface damage. The interior of the payload measured 12" x 6" x 5" with an exterior dimension of 15" x 9" x 8". Each of the panels that made up the box were cut with a 45-degree taper to ensure simultaneous contact along all surfaces as shown in Figure 5. The 45-degree cut also ensured that some of the force on heavy impacts would be evenly distributed throughout the joint. The final dimensions allowed for both the manifold assembly and the Arduino assembly to sit flat on the bottom of the payload with extra space for assembly purposes.
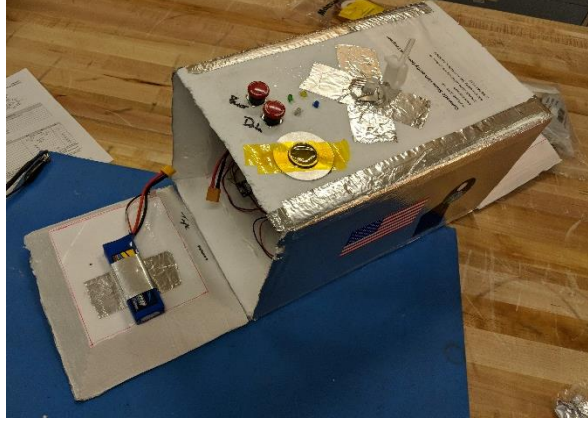
Figure 5. *Final exterior of payload.*

2.2 Subsystems

2.2.1 Sensor

The electrical system was comprised of multiple sensors and circuits with an Arduino Mega acting as the main computing and recording device. All components were powered using a 2-cell lithium polymer battery connected to a 5V switching voltage regulator. The UV sensors used were SGLux SG01S–A18, SG01S-B18, and SG01S-C18 for UV-A, UV-B, and UV-C respectively. A perfboard for the circuit shown in Figure 7 was created, which included an operational-amplifier and resistors to set the gain for each sensor.
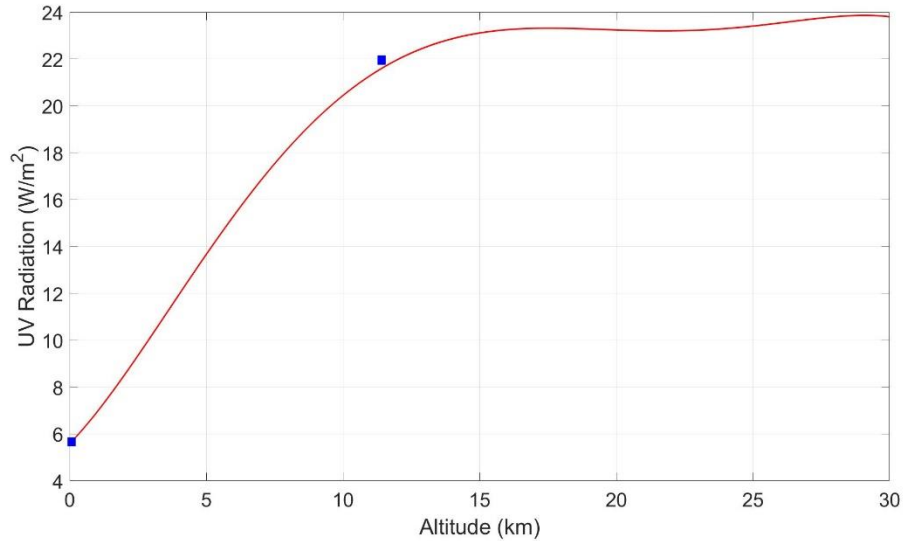

Figure 6. *UV data fit. Blue dots are known points, red line is 5th order polynomial fit. 300km point not shown.*

The op-amp was required to boost the output signal to a measurable value, as the sensors have an output current in the nano-amp range. These resistor values were 30 Mohm for UV-A, 30 Mohm for UV-B, and 60 Mohm for UV-C, which were determined by testing various resistor combinations and comparing the output from the UV-A sensor to calculate theoretical values. Figure 6 shows the 5th order polynomial data fit created from surface, 12 km, and 300 km data (300km point not shown on figure) [12], [7], [1]. These theoretical values were created by taking the fit data and using the sensor

responsivity and sensor size given in the datasheet to generate expected output current values from each sensor. The op-amp datasheet provides an equation to determine the output voltage with a known input current and gain resistor. Testing showed that this method was accurate to ~15%. Only the UV-A could be tested, as the amount of UV-C that reaches the ground is negligible and, if the gain is set high enough to read UV-B on the ground, the sensor will saturate the op-amp before the burst altitude during flight. Additionally, a UV glass diffuser (Thorlabs, DGUV10-120) was used to smooth the data and provide equal light to each sensor, done by taking the concentrated sun rays and spreading them equally across all three sensors. This board, and all other sensors, were connected to a custom perfboard shield that fit on top of the Arduino, shown in Figure 8.
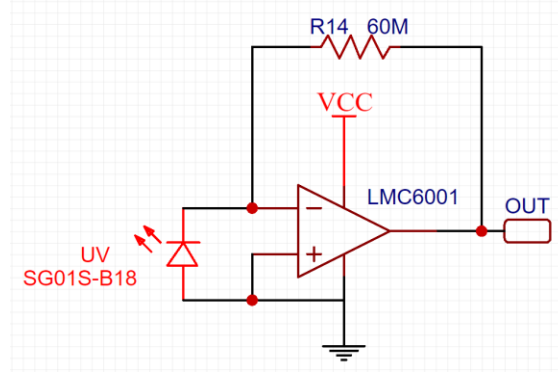

Figure 7. *Circuit for Op-Amp.*

An accelerometer, magnetometer, and gyroscope module (MPU-9250) was used to determine the orientation of the payload throughout the flight and was connected through a serial peripheral interface. The heater and pump were connected to individual N-channel metal-oxide-semiconductor field-effect-transistors, which allowed them to pull as much power as required while still being controlled via the Arduino. The DFRobot DSS0M15S 270-degree servo was used to control the valve position via pulse-width-modulation, and the two digital temperature/humidity sensors were DHT22s. A NEO-6M GPS module that used serial communication monitored altitude and was selected for its ability to function up to 36,500 m. Finally, the SparkFun OpenLog was used to write data to the Micro-SD card over serial communication.
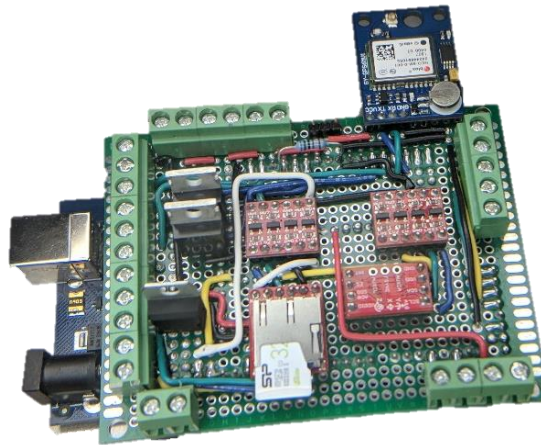

Figure 8. *Perfboard shield on Arduino.*

On the exterior of the payload are two push buttons and four light emitting diodes, shown in Figure 9. One button was connected in line with the battery, acting as the main power switch, and the

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other was connected to the Arduino and served as a data record switch. The four LEDs each had a status associated with them: yellow for power, blue for data recording, green for GPS lock, and white for accelerometer calibration. These allowed for the payload to be taped closed, but still have confirmation that the Arduino was operating as required.

Figure 9: *Button layout on top of payload.*

The code logic was simple, and continuously ran in a loop that checked multiple conditions to determine the next step. These included checking the altitude to determine if the pump needed to be on or if the servo needed to move to its next position and checking the interior temperature sensor to see if the heater needed to be on. Each loop also wrote data to the SD card if the record switch was engaged.

2.2.2 Biological

The biological subsystems primary concerns were that of sterility and mass flow per unit area. Sterility is the most important. If sterile conditions cannot be guaranteed, the microbes that were captured may not be from the upper atmosphere. Although control samples are used, it may be possible to capture the same microbes in the upper atmosphere as those found at the surface. By maintaining sterility, a greater correlation can be made with what grows from the filtration system and with what may be found in the upper atmosphere. The next concern was ensuring that the filters would have the necessary mass flow rate to guarantee an incidence of microbes. As altitude increases the microbial density decreases. Compounded with this is the decreasing atmospheric density. The design requires that both flow rate and filter surface area is maximized. Other important design complications included ensuring the materials seal on surfaces, finding a lightweight material for mounting, and the 4-way valve.

As previously mentioned, the filter system has three main components: the manifold block (the device that holds and seals the four filters), the 4-way valve, and the pump. Air from the atmosphere enters the system through an inlet tube connected to the 4-way valve. Depending on the current altitude, the valve will direct the air to one of three filters. A pump is attached at the rear of the system generating mass flow across each filter. This design was developed for a compact and minimalist layout. The pump is a 5-volt Parker CTS Micro 2.5 L/min diaphragm pump designed for medical equipment, which is lightweight, compact, and easy to use. The valve used a 270-degree high torque servo to actuate the inner valve mechanism. The manifold block was cleaned with 99% isopropanol before flight. There was also a control filter placed in the manifold block, but not exposed to the environment as a negative control. The process flow diagram, shown in Figure 10, details the process from start to finish.
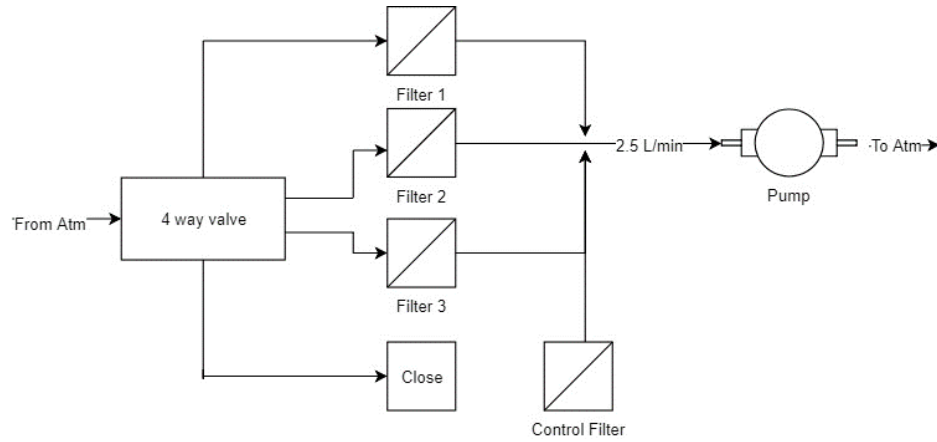
Figure 10. *Process flow diagram of biological components.*

Due to the unique design and custom parts, advanced manufacturing was required. Most of the parts in the filter assembly were made from polyethylene. Low density polyethylene (LDPE) was used for the main body of the valve. However, due to increased manufacturing difficulties, high density polyethylene was used for the manifold block. This decision largely did not affect the final product as HDPE is only marginally heavier than LDPE but had significantly better machining quality.

The manifold block was designed with a compact two-by-two filter layout. This design made it possible to fit the three necessary filters for testing and a single filter as a control. The setup maximized the filter surface area while minimizing the material needed to maintain stiffness and reducing weight. Originally the intention was to 3D print the manifold block out of PETG; however, sealing became an issue. It was then required to simplify the design and 3-axis CNC machine the part out of HDPE.
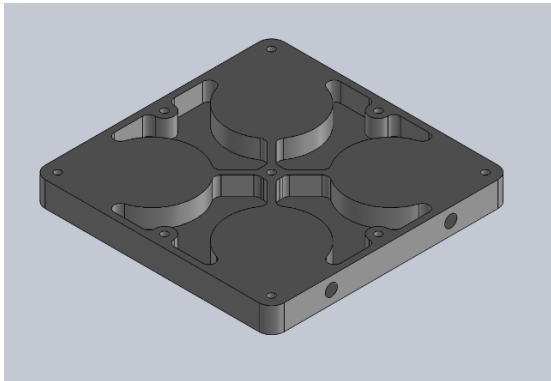

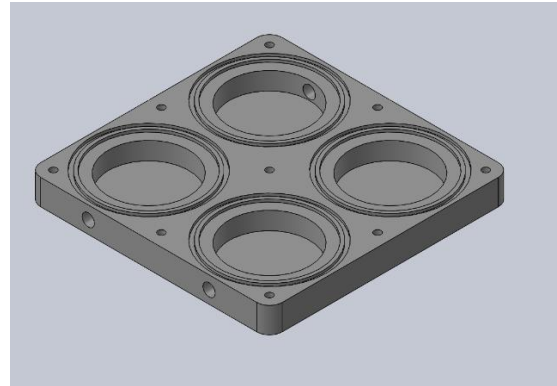Figure 11. *Bottom of lower manifold block assembly.*

Figure 12. *Top of lower manifold block assembly.*

Some of the benefits of machining are that the tolerances may be held tighter, meaning a better surface finish. Also, directly tapping into the material is no longer a concern as 3D printed part tend to fail under those types of stresses and therefore further simplifies the design. Each of the filters bays had an O-ring sealing itself from the rest of the system and a stainless-steel mesh to support the filter and prevent the filter from tearing while experiencing high mass flow rates.

Figure 13. *O-ring and stainless steel mesh.*

Of the systems designed, the valve assembly was the most complicated. The assembly consists of a valve body, inner valve, servo mount, and the high torque servo. The basic design of the valve is that air enters through the front of the mechanism and was either blocked from passing (closed position) or directed through one of three ports. When the inner valve is pointed straight down the flow rate was reduced to zero. This made it possible to maintain sterility during launch and recovery.
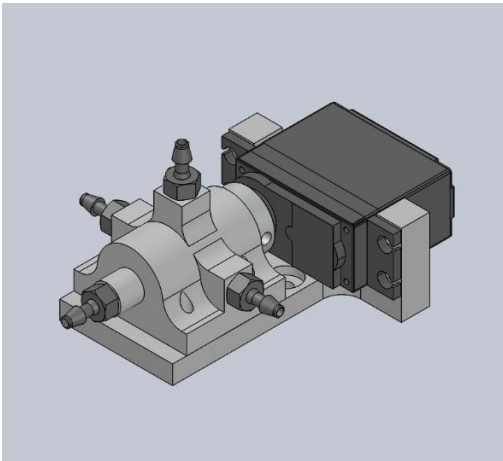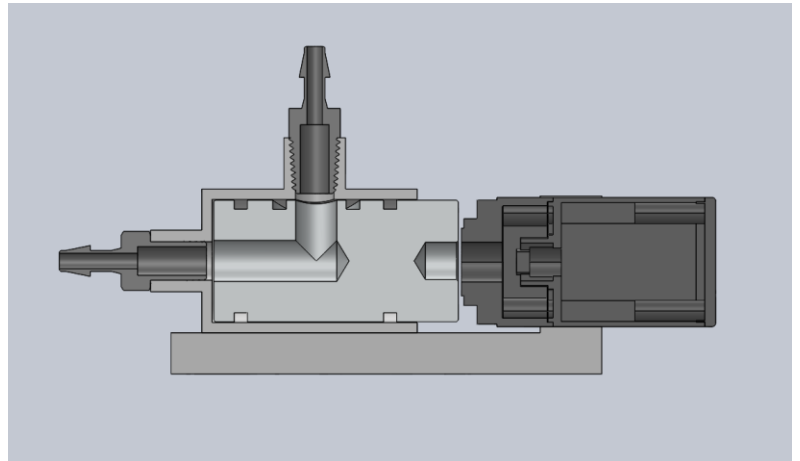

Figure 14. *Complete 4-way valve assembly.*

Figure 15. *Cross-section of the valve assembly.*

The inner valve required three O-rings to maintain the needed sealing. The first two were designed to seal all the ports from any outside atmosphere using 3/4-inch O-rings at either end of the valve. The third was the face O-ring that sealed an individual port from the rest of the system. This also acted as a seal for the entire system when the O-ring was not angled toward a port. Of the parts in the valve assembly, only the inner valve was made of aluminum and the rest was made from LDPE. The decision to manufacture the inner valve out of aluminum was made after a failed attempt to machine the face groove out of LDPE. Since surface finish is a concern when attempting to create a seal, aluminum was selected for its low cost and ease of machining.

Figure 16. *Details of the inner valve.*

The filter is made from a mixed cellulose ester (MCE) membrane with 0.45-micron pores. The filter pore size is ideal for bacteria and yeast samples, and the membrane has been used before in previous years for collecting samples with relative success [8]. This filtering device will capture microbes from the air, such as bacterial species *Bacillus subtilis, Staphylococcus pasteuri,* and yeast culture *Engyodontium album* [3], [6], [13]. These are all well documented bacteria, and nonpathogenic to humans. To correlate microbial activity with radiation exposure, the captured microbes from the upper atmosphere can be analyzed on the ground. Since these microbes already survive on the ground, it would be interesting to study their evolutionary aspects to adapt to new environments.

The sterilization techniques were adapted from aseptic techniques used in microbiology labs. Since the air is full of contaminants, the testing was performed near an alcohol burner filled with denatured ethyl alcohol. The function of the open flame is to create an updraft, thereby cleaning the air near the flame of possible microbes. The sterilization techniques enumerated by common microbiology procedures were implemented [11]. The sterility was tested beforehand and during testing with a negative control agar plate that was placed within the sterile field to show no growth from the tabletop, air, or dust. Any inanimate object was sanitized using 99% isopropyl alcohol or heat, and the filter sampling device was sealed pre-flight using clamps.

Post-launch processing included analyzing the growth of microbes using selective media. The bacteria were grown on Wallerstein Differential (WLD) media, the yeast was grown on yeast malt agar (YMA) media, and there was a control for each filter. At each altitude sampled the filters are cut in half, one for each media. The positive control was growth of expected microbes on the media pre-launch, and the negative control was the sterilization, growing nothing in the sterile field for extraction and placement of filters. After incubation at 30 degrees Celsius for at least 72 hours, growth was analyzed under a bright-field microscope at 40x and 100x to compare morphologies and colony growth (Fig. 17).
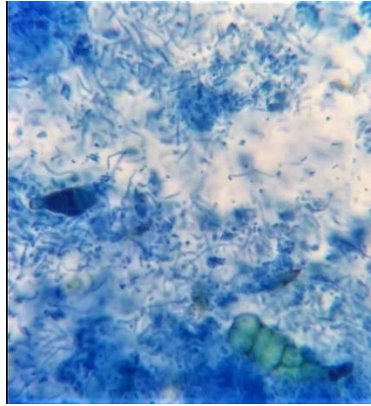
Figure 17. *Methylene blue stain including bacillus and cocci bacteria viewed at 40x.*

2.3 Management

Tasks were assigned to each person within the team based on strengths. Matt Kronwall handled mechanical aspects, modeling, and machining parts due to his experience with machining in the past. Hayden Deatherage worked on electronics and coding throughout the project due to his experience with robotics and Arduino. Rachel Jackson handled the biological aspects and experimental methods pertaining to microbes because of her experience in a microbiology lab and chemistry lab. Overall the team worked effectively and efficiently following a schedule that accounted for some setbacks.

2.4 Budget

There was a mass and monetary constraint for this project, which dictated a lightweight, sustainable, and affordable payload design. The payload was successfully both under budget, and under the mass allowance. The total mass was 980 grams and the budget spent was $797.28, meeting both requirements shown in Table 2.

Table 2. *Cost breakdown based on subsystem.*

Subsystem	Cost
Biological	$232.19
Electrical	$479.47
Mechanical	$85.62
Total	**$797.28**

3.0 Test Plan and Results

There was vigorous testing throughout the project with a focus on structural integrity, environmental tests, and functionality of parts.

3.1 Structural Tests

Whip Test Requirements: This simple test simulates post burst where maximum force is applied to the payload. The payload must operate under these conditions.

    Test Procedure:
        i.   Fill payload with 1 kg of distributed weight
        ii.   Attach a string through the center of mass of the payload, and through flight tube
        iii.   Spin the payload around as fast as possible to simulate 15 g's of force
        iv.   Simulate sudden changes in force and direction
        v.   Analyze the condition of payload and flight string tube

Test Results: The payload suffered no damage from the whip test. The flight string was not held in place by the paperclip. For the final design, sturdier wire and a more intricate wrap-around technique was used to insure the integrity of the flight tube.

Figure 18. *String up used for whip test.*

Drop Test Requirements: The payload must withstand a drop that will simulate worst-case landing conditions. The payload must be able to survive a 15-20-foot drop onto a solid surface.

    Test Procedure:
        i.   Fill payload with 1000 g of mass
        ii.   Drop payload from 15, and 20 feet
        iii.   Analyze structural damage

Test Results: Three identical payloads were created using aluminum, duct tape, and packaging tape. The duct tape suffered minimal damage, but some tape came unstuck. The payload survived from 20 feet and 60 feet but was the heaviest. The packaging tape survived the fall from 20 feet and 60 feet. However, the tape did not hold the structure in place and failed to adhere well. The aluminum tape's integrity held and did not break from 20 feet or 60 feet. Additionally, wooden dowels inserted as reinforcements were tested. The strongest design, and recommendation for flight, is three pieces of aluminum tape lengthwise and one long piece widthwise.

Stair Test Requirements: The payload must withstand being thrown down a staircase made of concrete. This will simulate harsh landing conditions due to high winds re-inflating the parachute.

    Testing Procedure:
        i.   Fill payload with 1000 g of mass
        ii.   Drop and throw payload down a flight of concrete stairs
        iii.   Analyze structural damage

<u>Test Results:</u> The payload took on minimal damage to corners, with the aluminum tape crumbling to absorb the force. The washer on the flight tube fell off, due to failure of the paperclip. For the final design, hot glue is recommended.

Figure 19. *Damage inflicted by stair test.*

3.2 Functional Tests

<u>Cooler Test Requirements:</u> Extreme environments can negatively impact the inside and outside of the payload. The payload must withstand low temperature.

> Test Procedure:
> i. Assemble fully operational payload as it would be on launch day
> ii. Distribute dry ice evenly into a large cooler
> iii. Place fully operational payload on top of the dry ice, Figure 20
> iv. Turn payload on and write data to SD card
> v. Make sure that the temperature of the cooler and the internal temperature are recorded
> vi. Close the lid and return in three hours
> vii. Analyze functionality of electronics, mechanical aspects, and biological aspects.

<u>Test Results:</u> The inside temperature remained within the desired range, varying between 10 and 12 degrees Celsius, which is exactly as designed (Figure 21). The exterior temperature was -40 degrees Celsius, which simulated the ambient temperature expected during flight. The Arduino recorded data for the entire test, and no components were damaged. The flight was also simulated using time delays for the biological system, and the servo cycled through all 3 positions and closed at the correct time, indicating a successful test. The battery's voltage dropped from 8.4V to 7.6V also confirming the battery's capacity was suitable for launch.
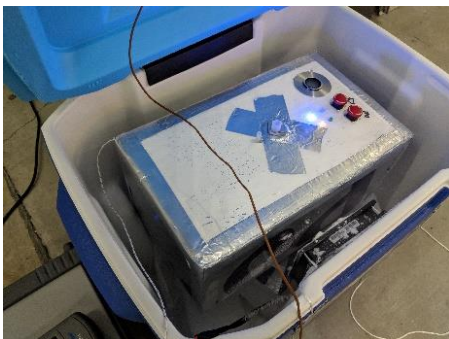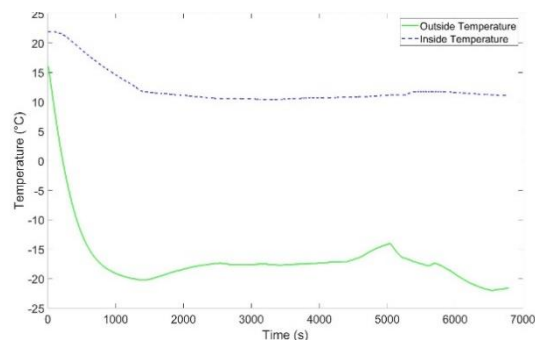

Figure 20. *Payload in cooler.*

Figure 21. *Temperature vs. Time.*

<u>Sensor and Memory Test Requirements:</u> All sensors must accurately, precisely, and reliably record data in all extreme environments.

<div style="padding-left: 2em">
<u>Test Procedure:</u>
</div>

i. Test each sensor in its respective environment
   1. For UV sensors, test inside and outside
   2. For accelerometer rotate along yaw, pitch, and xyz axes
   3. For temperature sensors test in ice water bath and hot water bath
   4. For GPS test outside at differing altitudes
ii. Compare data in different, and varying scenarios
iii. Write all data to SD card

<u style="color:red">Test Results:</u> All sensors are correctly calibrated and are working functionally in the code. All the sensors are writing to the SD card and they output correct data for the given situation. The circuit was then moved to a soldered perfboard setup, which did not work initially. After extensive testing, it was determined that the flux of the solder created a large (>60Mohm) resistance throughout the board, which was enough to cause failure. This was hard to track down, as the resistance was too large to be measured by a multimeter, but small enough to make the board malfunction. Removing the flux from the board with isopropyl alcohol in an ultrasonic cleaning machine fixed the issue.

Figure 22. *Electronic testing on breadboard, UV sensor array.*

3.3 Biological Testing

<u>Collection Test Requirements:</u> Collect microbes in upper atmosphere and analyze on the ground. The samples must remain sterile before the flight, during, and after the flight via a sealed chamber.

<div style="padding-left: 2em">
<u>Test Procedures:</u>
</div>

i. Extract sterile sample from payload
ii. Cut up filter in half
iii. Place on 2 different agar plates (YMA for yeast, and WLA for bacteria)
iv. Place agar plates in incubation chamber at constant 30 degrees C and grow for 3-4 days checking for growth every 24 hours.
v. Prepare samples for viewing under microscope using correct staining procedures
vi. Analyze samples under microscope and identify different bacteria, archaea, fungi, and protists. This will be done at 40x, 100x, and 400x under a fluorescent stain and gram stain using bright field microscope, and phase contrast.

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<u>Test Results:</u> The biological samples were plated upon returning from launch and everything was worked inside the sterile field. The growth of the samples did not appear. The control groups pre-launched were analyzed with gram stain. There was considerable mold contamination within the control groups, this is caused by the alcohol burner not creating an updraft (Figure 23). Post-launch data includes no growth from the samples due to the servo not cycling, and not collecting enough mass from the upper atmosphere.

Figure 23. *Mold cells from control.*

<u>Sterilization Test Requirements:</u> Inside the chamber all biological material, including filters, valves, electronics, and tubing, must remain sterile. If sterility is broken, the test of biological material in upper atmosphere has failed.

> <u>Test Procedures:</u>
> i. Clean all equipment with heat, if it cannot withstand heat, use isopropanol
> ii. Clean before and after launch to ensure sterile conditions
> iii. Turn on alcohol burner and work inside the sterile field
> iv. When placing filters in device, make sure you do not remove plastic guard until the rest is fully assembled.
> v. When extracting filters, to ensure sterility be next to flame – use negative control group
> vi. Transfer filters to agar plates
>   1. Note: The agar plates will be controlled - no growth for at least 3 days prior to launch to ensure sterility of burner
> vii. Grow on plates at 25-30 degrees C for 3-4 days and check for growth every 24 hours
> viii. When done growing view under free microscope then proceed to staining procedures

<u>Test Results:</u> The sterilization process was difficult and arduous; however, our control was contaminated with mold. This confirms that our sterile technique was flawed. The alcohol burner's effectiveness is less than ideal. The possible sources of contamination include: moving too fast through the sterile field, sterilizing with isopropyl alcohol, and/or moving out and back into the sterile field. These mistakes are hard to catch, but in the end the only source of contamination was mold. Due to the complex design of the manifold block there was a multitude of components that needed to be cleaned. The grease in the joints were broken down by the alcohol, causing issues with the manifold block assembly. Moreover, the contamination could have arose from using multiple components instead of one fluid design.

3.4 Expected Results

It is expected that the data will correlate to previous research. This includes UV sensor data, previous DemoSat projects data, as well as microbes that have been captured in the upper atmosphere. This experimental goal is to validate existing data and record the accuracy of different sensors on the flight through the upper atmosphere.

The UV data, presented in Figure 6, is a 5th order polynomial fit based on data at three different altitudes: ground, 12 km, and 300 km. Due to the lack of UV-A, UV-B, and UV-C data through the atmosphere, the gradient had to be assumed (Fig. 20).

The magnetometer was used to correlate the UV sensor position with respect to the sun through processing the yaw, pitch, and roll data.

The microbial growth was analyzed post-launch via growth on selective media for bacteria and fungi. The filters were grown on YMA, and WLD to analyze the bacterial colonies and fungal colonies. To identify differing morphologies and structures, a neutral gram stain can be used. During flight, the pump ran at 1.2 L/min, as the filters restricted airflow. 12.48 L of air was moved during the 10-15 km region, 18.14 L through the 15-20 km region, and 27.84 L during the 20-27 km region. Based on previous research collected in similar cases, 10-15 km should capture 0.0098 colony forming units (CFU), 15-20 km should capture 0.00808 CFU, and 20-27 km should capture 0.01273 CFU [3], [13], [14]. While the chances are small, it is desired to capture at least one.

4.0 Launch Results

The payload took minimal damage upon landing and recovery of the payload was successful. The battery lasted the entire flight and recovery time, decreasing from 8.4 V to 7.6 V. The sensors all successfully wrote data to the SD card for the duration of the flight, and all sensors functioned as expected. However, the UV-B and UV-C sensors did not collect reliable data, and the UV-A data was not as expected. Additionally, the payload is ready for another launch, meeting the sustainability requirement. Figure 24 shows a successful launch, with all payloads attached with this project on the bottom. Figure 25 depicts how the payload landed, with the UV sensors in a shadow and minimal surface damage. Figure 26 shows how the payload landed, not destroying any major components, and with the structural integrity suitable for another launch.

Figure 24. *Balloon launch with payloads.*

Figure 25. *Payload top upon landing, clamps attached when found*

Figure 26. *Payload side upon landing.*

4.1   Sensor Data

The sensors on the payload recorded data for the entire flight and during recovery as well. The temperature sensors on the inside and outside are graphed in Figure 28 showing both temperature sensors as a function of time. The payload's altitude as a function of time was recorded as well in Figure 29. Furthermore, the humidity was recorded throughout the entire flight, shown in Figure 30.
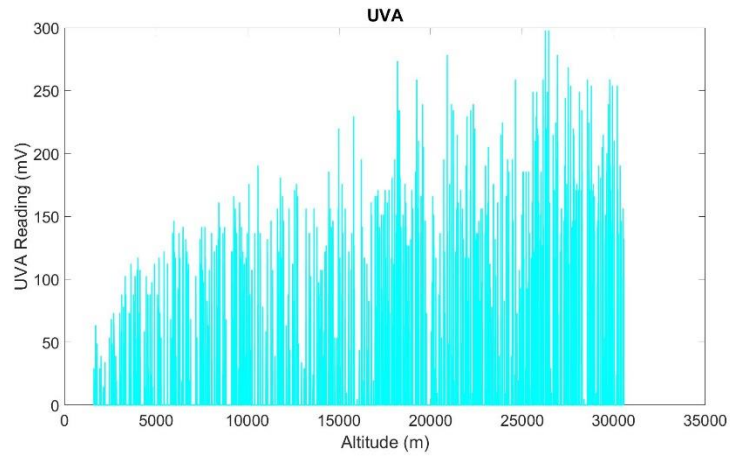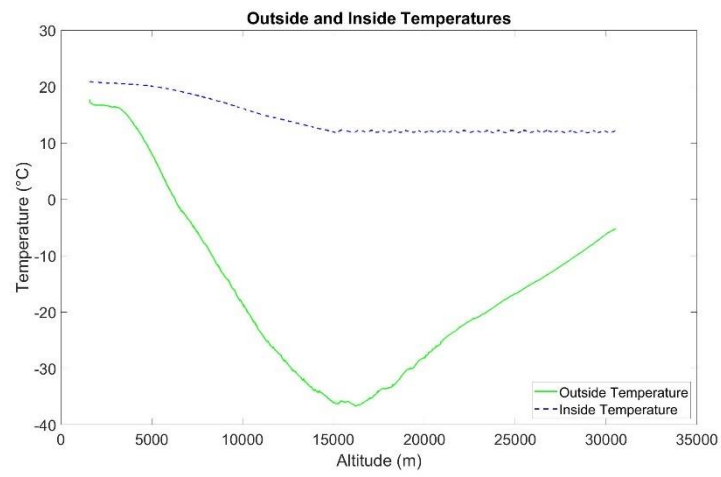
Figure 27. *UV-A Reading vs. Altitude.*
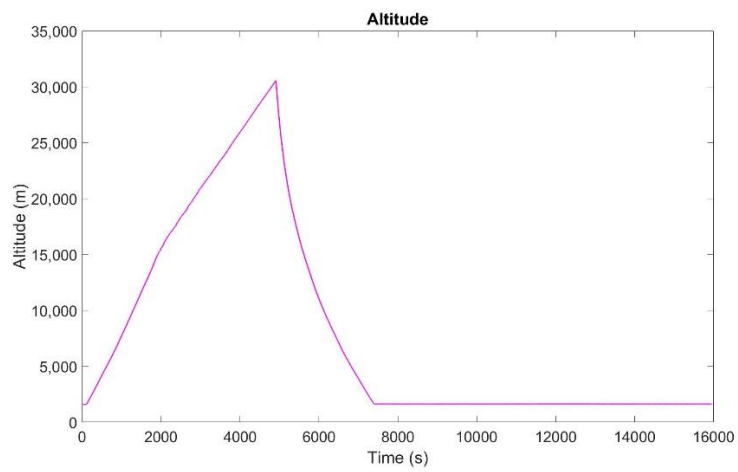

Figure 28. *Temperate vs. Altitude.*
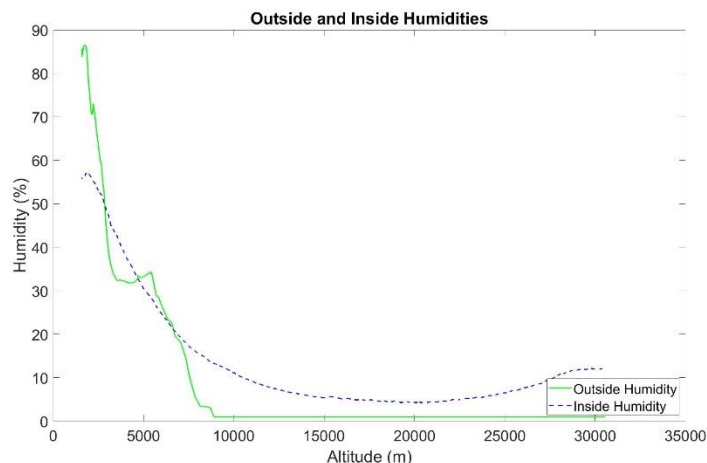

Figure 29. *Altitude vs. Time.*

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Figure 30. *Humidity vs. Altitude.*

4.2 Biological Data

The filters were placed post-launch in the sterilization room by the alcohol burner. There was a negative control in the sterile field to check for contamination and a positive control outside the sterile field for possible contaminants as well. The growth in the petri dishes were checked every 24 hours, and after 3 days some mold started growing on the positive control (Fig. 33). After 6 days the samples from the upper atmosphere (Fig. 31 and Fig. 32) did not grow anything except contamination in one plate (Fig. 33). With this, the colonies are believed to not have been sampled due to the servo not cycling between positions. There was no growth on the dishes after 6 days, with the conclusion that the experiment failed.
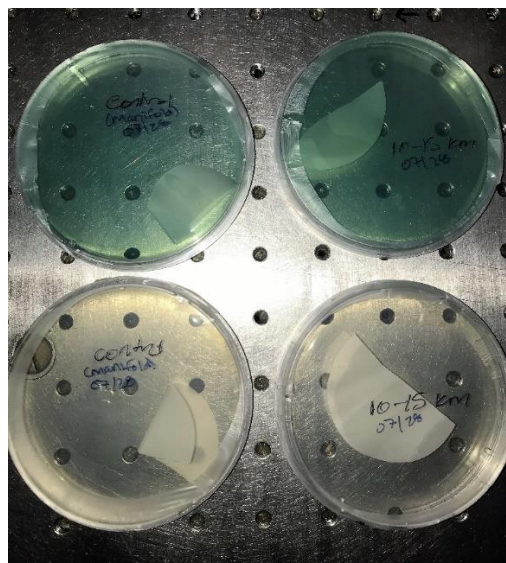

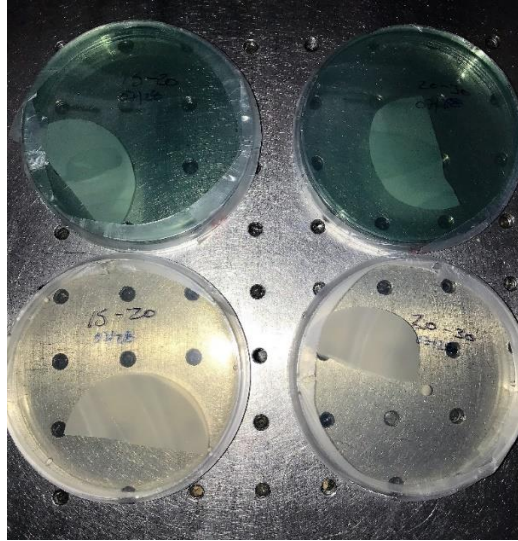Figure 31. *Control and 10-15 km samples with no growth after 6 days.*

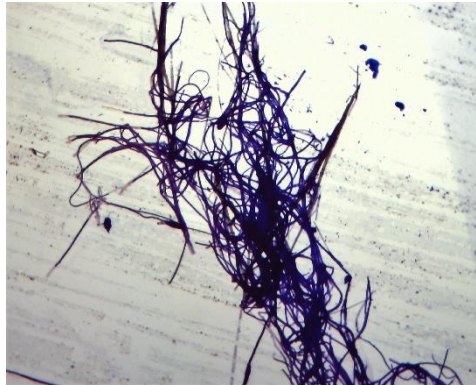Figure 32. *15-20 km and 20-30 km sample with no growth after 6 days. One plate with mold contamination.*

Figure 33. *Mold contamination on 20-30 km plate.*

5.0 Analysis

The payload survived the launch, with the balloon bursting at 30,500 meters. This was expected from the structural testing completed pre-launch. The only damage to the payload was a direct impact on a corner, but the aluminum tape and Styrofoam absorbed the impact with no damage to the interior of the payload.

The temperature sensors recorded data throughout the entire flight, with the inside temperature remaining above 10 degrees Celsius, which is exactly as coded. Furthermore, the Styrofoam core provided enough insulation, so the heaters only needed to turn on for part of the flight, shown in Figure 28 at altitude 15000 – 30000 m. The temperature of the inside remained around 10 degrees Celsius, whereas the outside temperature dropped down to -40 degrees Celsius. This proves that the code worked, and that none of the components were damaged during flight due to extreme temperatures. The humidity of the payload decreased sharply after launch, and it was slightly higher on the inside of the payload due to the heater cycling.

The accelerometer recorded data related to yaw, pitch, and roll. The payload rotated almost continuously at 6 rotations per minute (rpm). Based on the pitch and roll, the payload experienced

some spin and shaking, as expected. Upon recovery nothing was moved around, but the servo pin was disconnected. This is assumed to be a result of the launch. The recorded data showed that the code told the servo to cycle properly, but it is unknown if it did.

The UV index for the day started at 1 at 6:33 am for takeoff and increased steadily to 3 at 8 am for landing. At the landing site, shown in Figure 25, the UV sensors were in a shadow and did not record any UV data. Data was collected throughout the entire flight, but the solar elevation angle was lower than expected. At the time of launch the angle was 16 degrees, but an angle of at least 30 degrees was needed for the data to provide the calculated, theoretical values. UV-A data was collected and is shown in Figure 27. UV-B and UV-C data was nominally zero, attributable to the low light values. Post launch, another test was run, and the UV sensors performed flawlessly. This proves that the sensors work, but they were not positioned well during the launch for the given conditions. They are still functional and ready for another launch. Due to the UV photodiodes not collecting reliable data, the microbial growth analysis was flawed, however there were other issues as well.

The sterilization technique was imperfect. The design focused on functionality over simplicity. Contamination was minimized by using one pump and one valve for all the filters. However, anytime a component shares functionality contamination can occur. This is most apparent in the valve. As air flowed through the valve there was a chance that a microbe may become attached to the wall of the inlet tube. If that is the case, the microbe now has a chance to contaminate any of the filters. The grease that lines the valve can also act as a contaminating agent. As bacteria gets trapped in the grease it can then spread across the surface of the valve as the inner valve mechanism rotates.

Another flaw to take into consideration is that the two remaining ports are connected to each other when the valve is indexed to a port. This is from a small clearance gap between the valve body and the rotating inner valve, which is only 0.005". However, because all the filters use the same pump, a small bypass leakage may be present. The decision to use this system was made because the chance of bypass is low, and if there was any, the flow rate would be negligible.

The biological data was a failed experiment. There was no growth on any of the dishes except for one plate with one mold spore. This mold spore is originating from moving too fast within the sterile field. Since mold exists in the air, it can contaminate anything. The plates that were used did not have mold inhibitors, and therefore the mold was able to grow. Furthermore, since the dishes, except one, were sterile it can be concluded that the sterile technique works but needs some modifications. However, due to one dish being contaminated the flaw in the sterile technique arises from disturbing the sterile field. This is caused by moving too fast, too much air flow within the sterile field, or bad ventilation. In the scope of DemoSat, biological air sampling experiments are very hard to accomplish. The amount of air required to have a chance of growing anything is immense, and a much larger pump than can fit within a 1 kg payload is required. Past experiments that had growth were most likely due to contamination.

6.0 Conclusion

The mission was a mixed success. Much of the data collected was incomplete. This was due to a few errors made during the design process. As a whole; however, the payload functioned as expected. The Arduino collected data from all the sensors throughout the flight and the temperature

of the payload maintained the desired 10 degrees Celsius. And the payload sustained minimal damage in flight with the potential for a second flight.

Although the UV radiation data collected was not complete, the data that was collected did reinforce predictions made in the beginning of the project. Of the UV data collected only the UV-A sensor collected data. The data itself was lower in irradiance than expected. This is most likely due to the low zenith angle and greater Rayleigh scattering seen in the morning. That being said the UV-A data does follow the same trend as the predicted data set, Figure 6. Some of the problems experienced could have been solved by changing the location of the UV sensor array or launching later in the day.

The success of the biological testing component is a bit harder to discern. Upon the retrieval of the payload, the interior was inspected for damage. During this inspection it was found that the 3-pin connecter for the servo motor, which controls the 4-way valve, was disconnected. Where or when the servo was disconnected is impossible to tell. After 9 days none of the samples had any significant growth. There was some growth on the 20-30 km samples; However, the mold that was growing was also found inside the lab where the samples were processed and therefore is not scientifically significant and can be assumed to be contamination. The fact that nothing significant grew leads to the assumption that the servo may not have been collected at all and did not run properly. However, when correlating the time that the payload spent at each altitude and the volumetric flow rate of the pump, it was found that there was a 1.5% chance to encounter a colony forming unit in the stratosphere. This then means that the servo may have ran properly but not encountered any microbes. The solution for future experiments would be to ensure that the servo is properly secured and increase the flow rate of the pump.

Overall, the payload met many of the desired goal for the project. Most important of which were maintaining the proper weight and budget requirements. The payload passed all preliminary testing and only suffered superficial damage during the flight. With minimal adjustments the experiment may be able to be launched again with greater success.

7.0 Revisions for Next Launch

The takeaway from this project is to test all equipment in varying environments, even if it seems extreme. The varying aspects of launch day are hard to account for. This project also recommends that a sharp vision from the start of the project is required. If a clear, concise project starts with a focused idea then the road through design will be more straightforward. Do not stretch the project to complete multiple different ideas. In hindsight, this project should have either focused on UV data or biological data, not both. Additionally, mistakes always lead to learning opportunities.

To get proper data from the UV sensors, a few things could be changed. The easiest solution would be to launch later in the day when the solar elevation is above 40 degrees but less than 70 degrees. This range allows for the sun to properly reach the sensors, while not having shadows from payloads or the balloon above. Another method would be to launch at the same time of day but put the sensors on the side of the payload, facing the horizon. The payload will be spinning, and not always facing the sun, but the data could be correlated to the data from a magnetometer to give accurate data. In either situation, good data should result, assuming all sensors are operating correctly.

Many improvements to the filtration system can be made. Although testing did not indicate any immediate problems, corrections need to be made. The valve was the best solution when considering its weight, but more importantly its power consumption. In this case, it would have been more beneficial to have three separate inlet tubes for each filter rather than just the one. The use of small medical grade solenoids acting as a shut off valve would replace the function of the 4-way valve. By doing so, cleaning the system would be greatly simplified and only have a marginal effect on the weight. However, further power consumption testing would be needed to guarantee mission success.

The biological data could be improved as well. Having a clean chamber, instead of just an alcohol burner would be ideal. A second alcohol burner could improve the sterility of the sterile field as well, creating a larger updraft. The sterile conditions were met but could be improved even further. The source of contamination arose from disturbing the sterile field. In the future, the recommended approach is to move slower and have less air flowing through the room around you. This can be accomplished by not opening any doors to the room, having less people move in the room, and less movement within the sterile field. The main source of contamination in any sterile environment is the air, and if you can easily eliminate any unnecessary transportation of microbes through the air that would improve the odds for sterility.

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