HASP Student Payload Application for 2015

Payload Title:
SIMBA - StratospherIc Microbe and Bacteria Accumulator

Payload Class: (check one) ■ Small ☐ Large	Institution: University of Colorado, Boulder	Submit Date: 12-19-2014

Project Abstract

SIMBA (Stratospheric Microbe and Bacteria Accumulator) will collect microorganisms from the atmosphere, while simultaneously collecting environmental data about where these microbes are surviving. The purpose of this mission is to discover more about the living conditions of these atmospheric microorganisms, and discover the concentration of these organisms at the altitude HASP will fly. Environmental data will be collected, and Team SIMBA will also culture and attempt to visually identify the microorganisms. Research on these microbes can have a variety of purposes, so any extensive research such as what will be conducted with SIMBA will be beneficial to the scientific community. Similar studies collecting microorganisms from the atmosphere have been conducted on balloons, which is why HASP is an ideal platform for a project such as SIMBA. SIMBA only requires a small payload spot as the weight and size of the accumulator project will remain under the requirements for a small payload.

Team Name: Stratospheric Microbe Accumulator (SIMBA)	Team or Project Website: Under Construction

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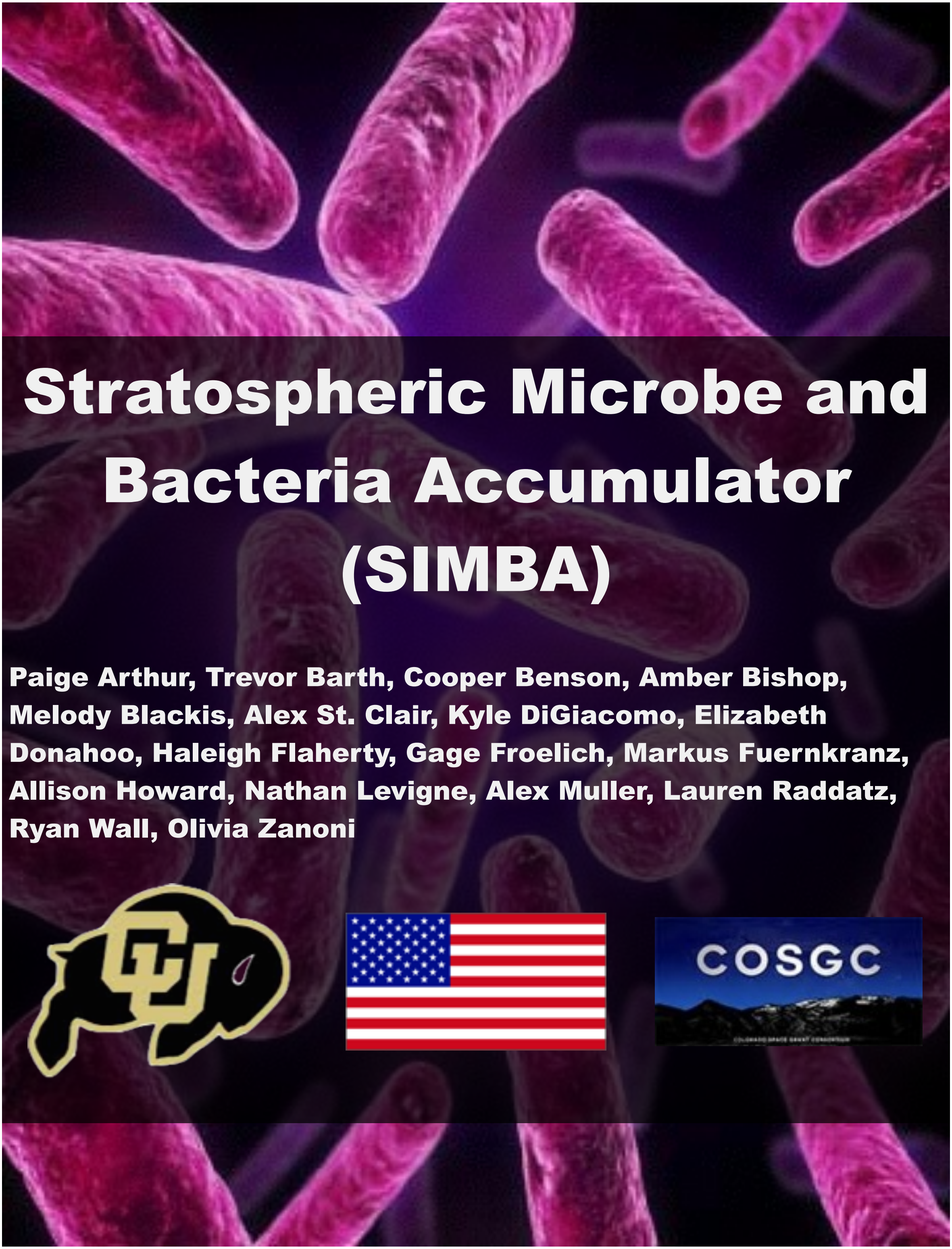

Stratospheric Microbe and Bacteria Accumulator (SIMBA)

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Contents

I. Mission Overview

Team SIMBA shall design and build the Stratospheric Microbe and Bacteria Accumulator (SIMBA) containing a Microbial Capture Unit (MCU) which is capable of capturing microorganisms between 30.0 and 0.2 microns at an altitude of 36 km. In addition to this, SIMBA will utilize humidity, temperature, and pressure sensors to collect environmental data on the living conditions of said microbes collected from the stratosphere.

A. Mission Objectives

- To design, build, and fly a payload that is capable of collecting microorganisms that thrive in the earth's stratosphere.
- To collect microorganisms from the stratosphere using the MCU system.
- To collect environmental data on the living conditions of said microbes using humidity, temperature and pressure sensors.
- Use the fluorescent stain, diamidino phenylindole (DAPI), to determine the concentration of microorganisms at 36 km in the atmosphere.
- Attempt to visually identify the collected microorganisms.

B. Mission Premise

Currently, only a handful of experiments have examined the existence of microorganisms at high altitudes. Out of those studies, even fewer have investigated microbe activity between 25 and 40 kilometers. Since this research is still clearly in its infancy, there is not a sufficiently strong foundation of data to fully explore the applications of the knowledge that these high altitude microorganisms will ultimately provide. As such, the primary goal of SIMBA is to gather as much information about the microorganisms that exist in the stratosphere at approximately 36 km above the Earth's surface. This will ultimately be achieved by collecting the organisms, while simultaneously collecting as much data relevant to the living conditions of these microbes throughout the flight using humidity, temperature, and pressure sensors. By researching the environment that these microbes thrive in, a more thorough understanding of where life can survive can be gained. Through DAPI testing, information on the concentration of microorganisms at 36 km can be found, which will provide insight into where stratospheric bacteria originates from. If there is a low concentration, this is strong evidence that most microbes are lifted to this height via air currents from the earth's surface or lower altitudes. If a high concentration is found, it supports that there are clouds of bacteria that actually inhabit and reproduce at this height. Further, SIMBA will attempt to visually identify the microorganisms in order to find out exactly what microbes exist in these extreme environments.

Although similar experiments have been conducted with a sounding rocket used as the launch vehicle, the vast majority of research done on high altitude microorganisms is carried out using a high altitude balloon. A high altitude balloon platform, such as HASP, is the optimal launch vehicle for this experiment for a few reasons:

1. The payload will be permitted to remain at maximum altitude for an extended period of time, allowing SIMBA to sample a large volume of air during the flight.
2. The payload will be easily retrievable, and will not have to be concerned with the potential loss of data that would come with landing in a body of water.
3. The organisms will not be destroyed by the unavoidable intense heat that comes with the use of a rocket.
4. The use of a high altitude balloon such as HASP is far less expensive than the use of a rocket, thus permitting a greater number of flights.
5. The use of a balloon is far less intrusive to the environment than a rocket or plane, allowing the experiment to record more accurate data.

C. Principle of Operation

SIMBA shall have all of its systems verified prior to launch. HASP integration will occur one month prior to launch. All systems will be powered on a few minutes before launch. The on-board flight sensors (humidity, temperature, and pressure) will record data throughout the rest of the flight. SIMBA shall launch the following day and rise to an estimated altitude of 36,500 meters after approximately one hour. Once maximum altitude is reached (verified by the on-board HASP GPS) the valves will turn permitting air flow into the filter chambers, collecting samples. After fifteen hours the valves will turn once more, stopping the flow of air into the filter chambers. SIMBA shall then power off all systems and begin its descent where a team will recover and extract the digital flight data and filter chambers. The operations diagram is shown in Figure **??** on page ??.

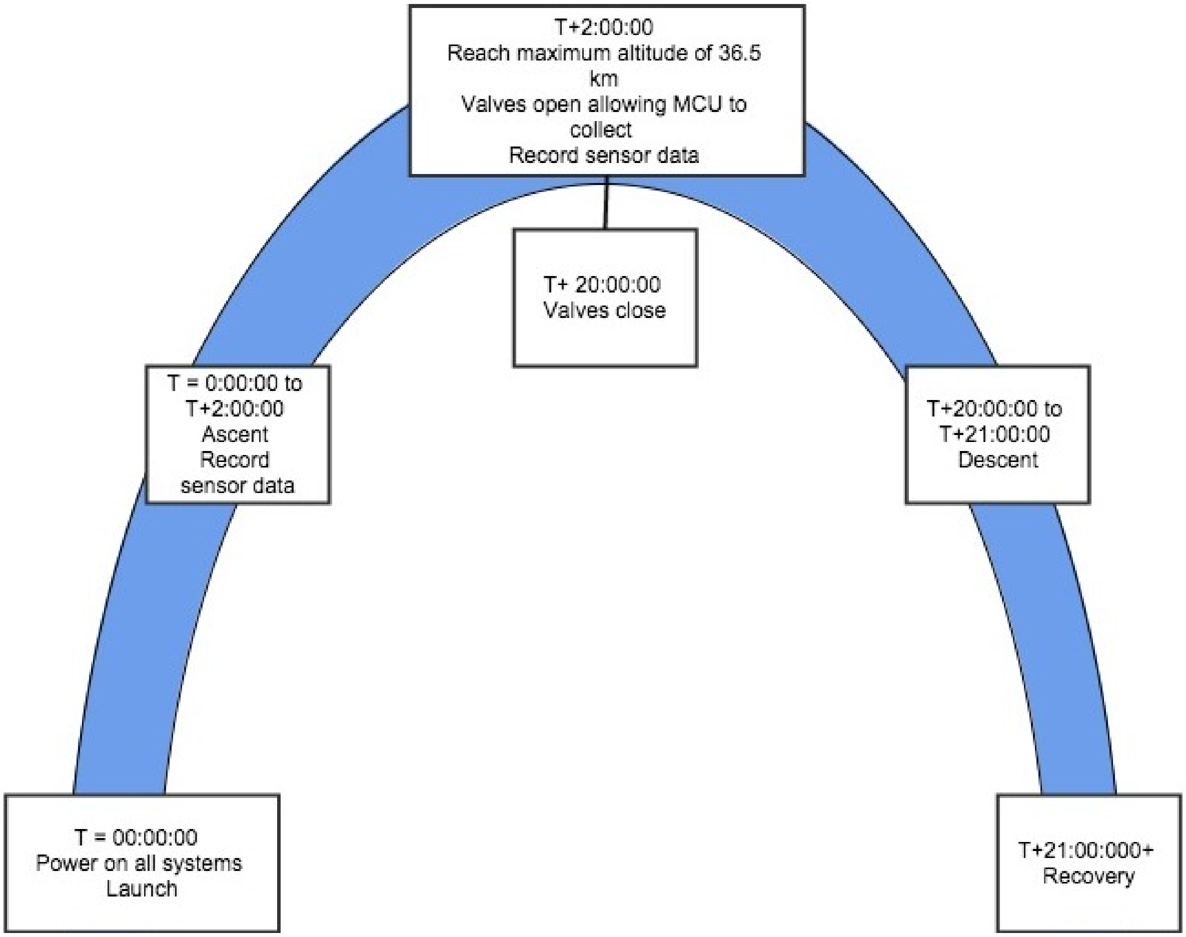

Figure 1: Planned mission operations

II. Requirements

A. Zeroth Level Requirements

#	Requirement	Origin
0	The Stratospheric Microbe and Bacteria Accumulator (SIMBA) containing a Microbial Capture Unit (MCU) capable of capturing stratospheric microorganisms at 36 kilometers and containing them in an isolated environment until they can be cultured and identified.	

B. First Level Requirements

#	Requirement	Origin
0.1	Collect microorganisms from the stratosphere using the MCU system.	0
0.2	Collect environmental data on living environment of microorganisms.	0
0.3	Culture and attempt to visually identify captured microbes.	0
0.4	Use the fluorescent stain, diamidino phenylindole (DAPI), to determine the concentration of microorganisms at 36 km.	0

C. Second Level Requirements

Requirement 0.1: Collect microorganisms from the stratosphere using the MCU system.		
#	Requirement	Origin
0.1.1	MCU shall implement an initial filter to filter out large contaminants.	0.1
0.1.2	MCU shall use vacuum pumps to control airflow.	0.1
0.1.3	MCU shall use solenoid valves to control pumps.	0.1
0.1.4	The payload will be able to receive commands from the ground of size 7 bytes to control the solenoid valves.	0.1
0.1.5	MCU shall implement 0.2 micrometer filters to catch microorganisms.	0.1
0.1.6	MCU shall implement filter chambers to contains and isolate filters.	0.1
0.1.7	MCU shall connect the system using a series of sealed tubes to prevent air leaks.	0.1
0.1.8	Majority of MCU shall be contained in the Clean Zone to prevent contamination.	0.1
0.1.9	Power shall be supplied throughout the entirety of flight at peak height to control solenoid valves and pumps.	0.1
0.1.10	Hardware shall remain uncompromised during the temperature changes of flight.	0.1

Requirement 0.2: Collect environmental data on the living conditions of collected microbes.		
#	Requirement	Origin
0.2.1	SIMBA shall fly a temperature sensor to measure the outside temperature.	0.2
0.2.2	SIMBA shall fly a temperature sensor to measure temperature inside the Clean Zone.	0.2
0.2.3	SIMBA shall fly a humidity sensor to measure the humidity inside the Clean Zone.	0.2
0.2.4	SIMBA shall fly a pressure sensor to measure the pressure inside the Clean Zone.	0.2
0.2.5	Power shall be supplied continuously during flight to these sensors to collect data.	0.2
0.2.6	Sensors shall remain uncompromised during temperature changes throughout the course of the flight.	0.2
0.2.7	The on-board Raspberry Pi shall collect and store sensor data onto a micro-SD card	0.2
0.2.8	The payload will downlink data from the temperature, pressure, humidity sensors along with power draw every ten seconds.	0.2

Requirement 0.3: Culture and attempt to visually identify microorganisms.		
#	Requirement	Origin
0.3.1	MCU shall keep filters with microbes isolated to prevent contamination.	0.3
0.3.2	Clean Zone shall be separate from the electronics housing in order to detach post flight to transport easily to laboratory.	0.3
0.3.3	Structure shall remain uncompromised throughout flight to prevent contamination.	0.3
0.3.4	Team SIMBA shall use the biology laboratory to culture captured microbes using agar.	0.3
0.3.5	Team SIMBA shall use an electron microscope to visually identify captured microbes.	0.3

Requirement 0.4: Use the fluorescent stain, diamidino phenylindole (DAPI), to determine the concentration of microorganisms at 36 km in the atmosphere.		
#	Requirement	Origin
0.4.1	MCU shall keep filters with microbes isolated to prevent contamination.	0.4
0.4.2	Clean Zone shall be separate from the electronics housing in order to detach post flight to transport easily to laboratory.	0.4
0.4.3	Structure shall remain uncompromised throughout flight to prevent contamination.	0.4
0.4.4	Team SIMBA shall use the biology laboratory to incubate captured microbes in phosphate-buffered saline (PBS).	0.4
0.4.5	Team SIMBA shall use an fluorescent microscope to determine concentration of captured microbes.	0.4

D. Third Level Requirements

Requirement 0.1.4: The payload will be able to receive commands from the ground of size 7 bytes to control the solenoid valves.		
#	Requirement	Origin
0.1.4.1	The payload will follow the commands given via uplink to control the operations of the payload.	0.1.4
0.1.4.2	The payload will turn on the air pumps and open the valves upon reaching a certain altitude as indicated by sensor data.	0.1.4

Requirement 0.1.9: Power shall be supplied throughout the entirety of flight at peak height to control solenoid valves and pumps.		
#	Requirement	Origin
0.1.9.1	Power draw will not exceed 30 VDC and 0.5 amps.	0.1.9
0.1.9.2	Payload shall split the provided 30 VDC to control each system.	0.1.9

Requirement 0.1.10: Hardware shall remain uncompromised during the temperature changes of flight.		
#	Requirement	Origin
0.1.10.1	The electrical housing structure shall be insulated to maintain operating temperatures for the components within the structure.	0.1.10
0.1.10.2	All systems will remain within operating temperatures while external temperatures range from −80 °C to 60 °C.	0.1.10

Requirement 0.2.5: Power shall be supplied continuously during flight to these sensors to collect data.		
#	Requirement	Origin
0.2.5.1	Power draw will not exceed 30 VDC and 0.5A.	0.2.5
0.2.5.2	Payload will split the provided 30 VDC to control each system.	0.2.5

Requirement 0.2.6: Sensors shall remain uncompromised during temperature changes throughout the course of the flight.		
#	Requirement	Origin
0.2.6.1	The electrical housing structure shall be insulated to maintain operating temperatures for the components within the structure.	0.2.6
0.2.6.2	All systems will remain within operating temperatures while external temperatures range from −80 °C to 60 °C.	0.2.6

Requirement 0.2.8: The payload will downlink data from the temperature, pressure, humidity sensors along with power draw every ten seconds.		
#	Requirement	Origin
0.2.8.1	The payload will be able to communicate with the HASP platform to downlink a package of data sized no more than 100 bits per second	0.2.8

Requirement 0.3.3 Structure shall remain uncompromised throughout flight to prevent contamination.		
#	Requirement	Origin
0.3.3.1	Clean Zone structure shall stay stable through the temperature change and forces up to 10g.	0.3.3
0.3.3.2	Electrical housing structure shall stay stable through the temperature change and forces up to 10g.	0.3.3

Requirement 0.4.3 Structure shall remain uncompromised throughout flight to prevent contamination.		
#	Requirement	Origin
0.3.3.1	Clean Zone structure shall stay stable through the temperature change and forces up to 10g.	0.3.3
0.3.3.2	Electrical housing structure shall stay stable through the temperature change and forces up to 10g.	0.3.3

III. Design Overview

SIMBA is made of two connected structural components; the Clean Zone that houses the air sampling equipment and the electronics housing attached underneath. The clean zone is uninsulated and contains structural support cylinders, internal polycarbonate filters, external barrier filters, two filter chambers containing 0.2 µm filters, two metal shut-off solenoid valves, and temperature sensors for each valve. The bottom component holds the Electronic Power System (EPS), a Command and Data Handling system (CDH), and two Boxer 10K pumps. It is insulated to ensure that the devices are maintained in a suitable temperature range. A temperature sensor shall be placed on each pump and the Raspberry Pi B+ microprocessor to indicate their temperatures while operating. Externally, three sensors are placed on SIMBA to record the atmospheric humidity, temperature, and pressure. With these sensors, environmental data shall be collected on the living conditions of the accumulated microbes.

A. Structural Design

The SIMBA design will consist of two major substructures: The Clean Zone and the electronics housing, shown in Figures **??** on page ?? and **??** on page ??. Both structures will be made of machined Aluminum, with the Clean Zone also having a portion of one of the walls made out of Tyvek. This will allow the Clean Zone to depressurize as SIMBA increases in altitude, without allowing any contaminants into the structure. The Clean Zone will be 14 cm x 14 cm x 15 cm and will contain two solenoid valves, as well as two filter chambers that will contain 0.2 µm filters. The entrances and exits of the Clean Zone will be one-way valves to prevent contamination. Above the Clean Zone will be two cylindrical structures with a height of 3 cm and a radius of 0.625 cm, which will support larger external filters to prevent any large contaminants from entering the system. The Clean Zone structure will then be bolted to the 15 cm x 15 cm x 12 cm electronics housing. The electronics housing will contain the CDH system, EPS, as well as the pumps. This structure will be internally insulated to ensure that each of the components work to the desired specifications. To allow the necessary volumetric flow, 0.625 cm (0.25 in) tubes will be used throughout the system to connect the pumps, filters, and valves to the external environment. Appropriate simulations will be run to ensure that expansion as well as impact forces will not affect the structural integrity of the system.

1. Structural Integration

The baseplate of the electronic housing will be connected to the four side plates, as well as the HASP mounting plate by eight blots. See Figure 4 on page 12 for the planned adjustments to the provided baseplate. Next the Clean Zone will be secured on top of the electronics housing. Both the EDAC connectors and the DB9 DTE serial connectors will be placed in the established positions ready to be interfaced on the bottom with the corresponding plug.

B. Science Design

1. Filter System

Above the Clean Zone, two cylindrical structures are supported. Through these, stratospheric air will be drawn into the MCU. Polycarbonate filters with a pore size of 20 to 100 µm will be used externally on the cylindrical structures, to ensure that large contaminants do not enter the system. Inside of each of these will be a column wrapped in 0.2 µm polycarbonate filters, which allows the air samples to flow through the rest of the MCU. A Polypropylene 47 mm filter holder will be utilized with the inner filter. Two filter holders, containing 0.2 µm filters are attached to the filter holder via tubing, and are where the microbes will collect. This is illustrate in Figure 5 on page 13.

2. Pumps

In order to collect a sufficient amount of microorganisms SIMBA will require a method of processing vast quantities of air through the on-board polycarbonate filters. To accomplish this two double-headed pumps will be installed within the base of SIMBA and will have a tube connecting it to the filter chambers within the clean room. This particular pump is capable of processing 6L/min of air which allows SIMBA to process nearly 1,800L of air throughout the planned 5 hours of operation. A Raspberry Pi B+ will control the pumps

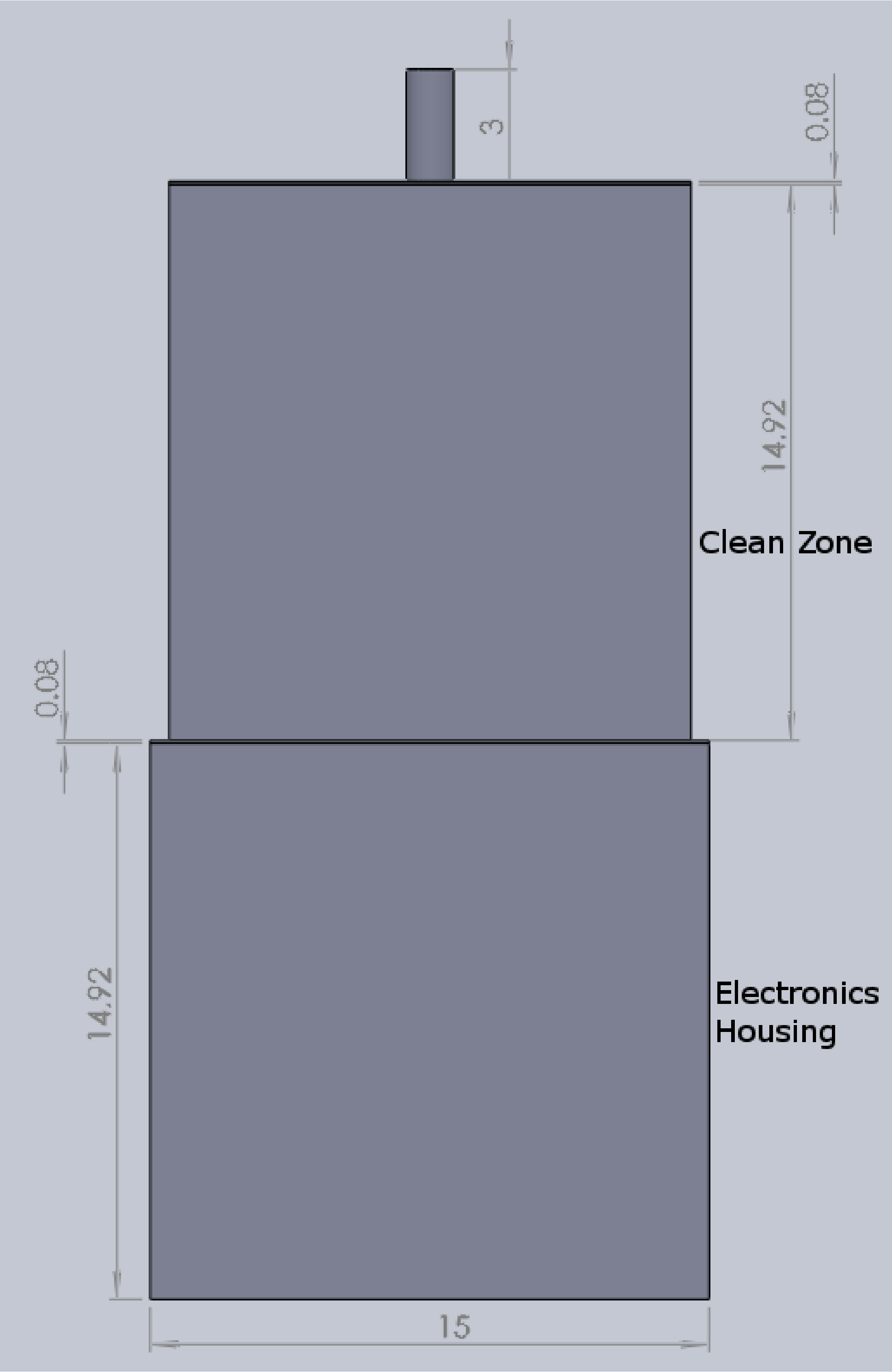

Figure 2: Side View of SIMBA with both sections labeled (all units are in cm)

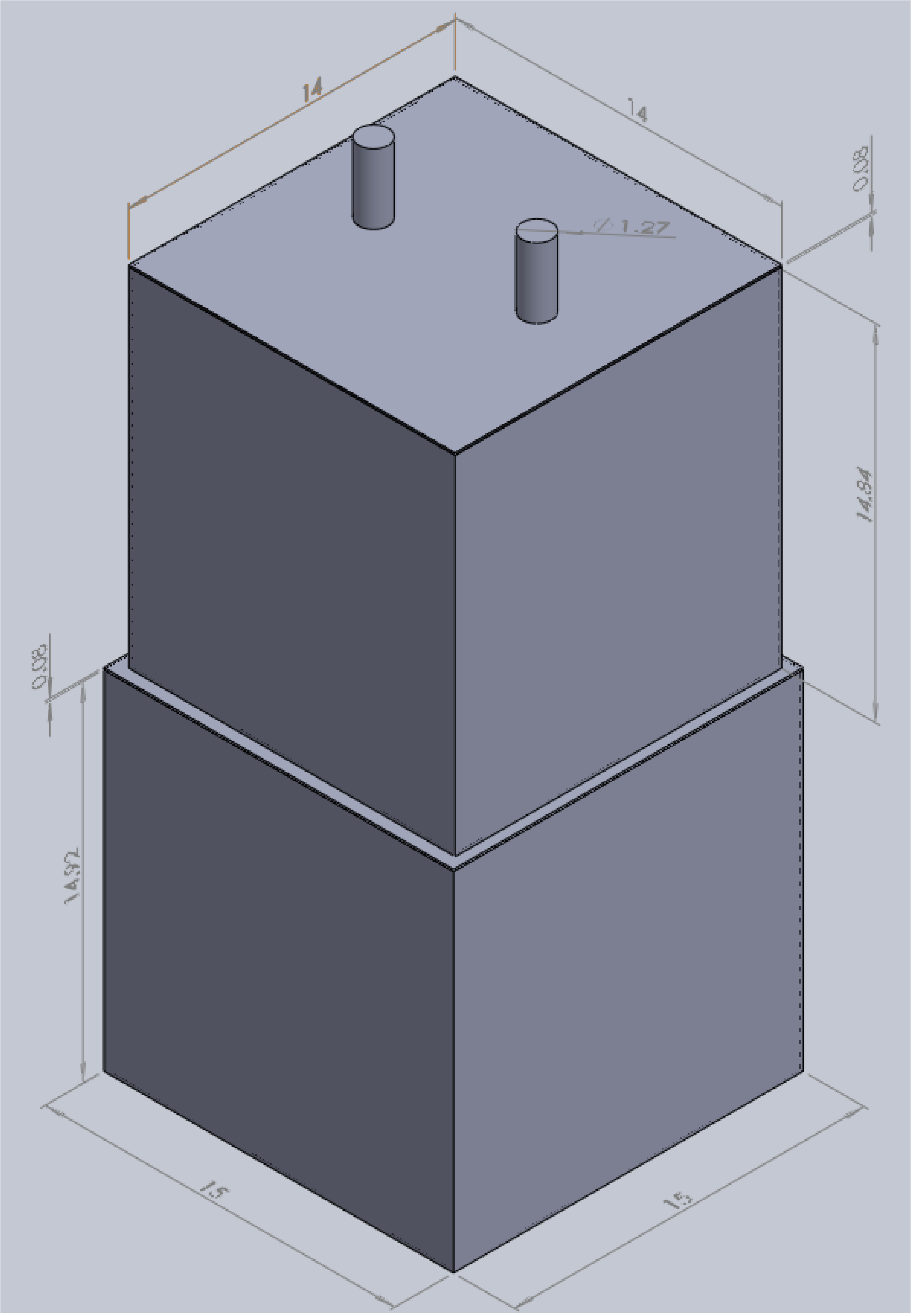

Figure 3: Isometric view of SIMBA (all units are in cm)

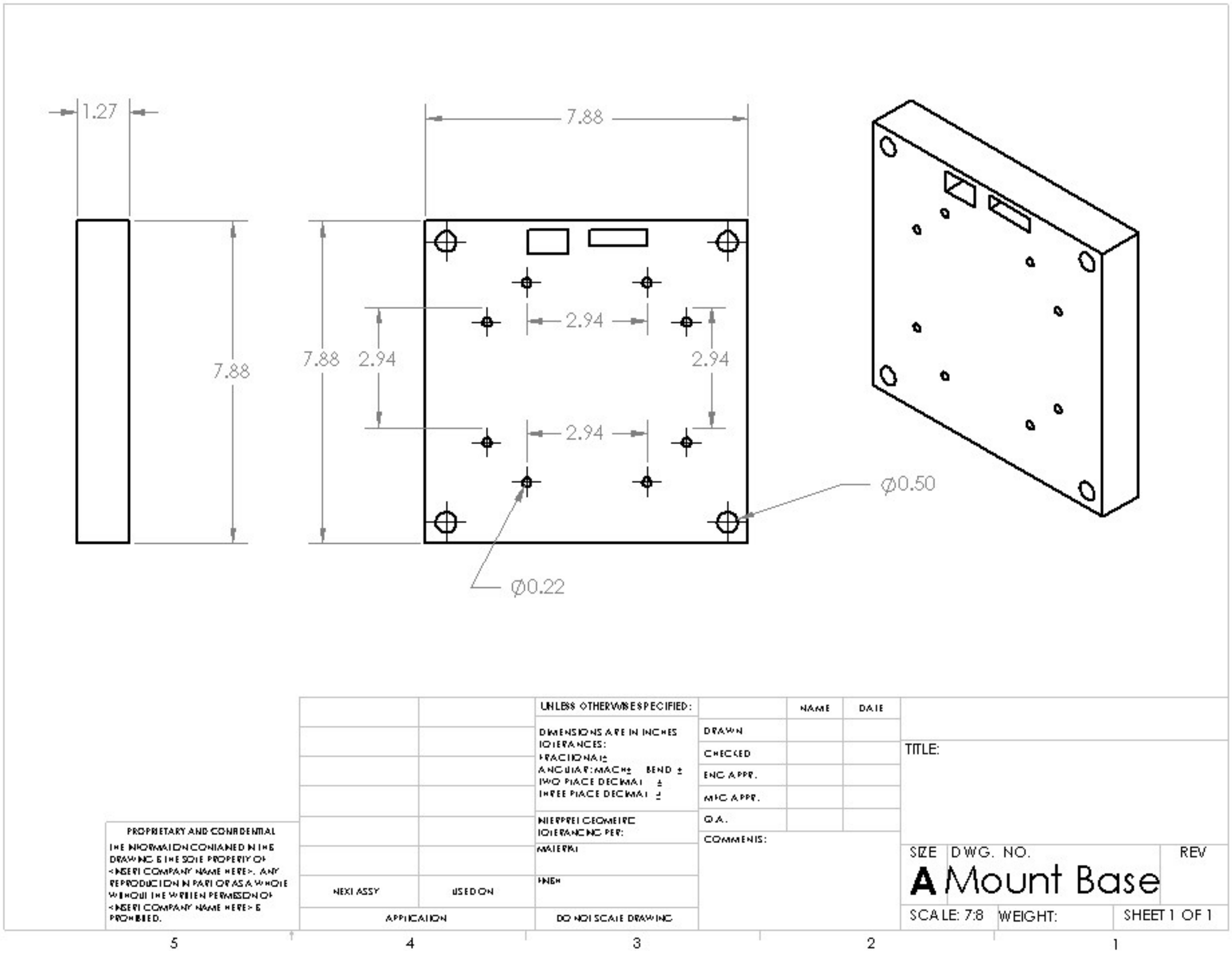

Figure 4: Mounting Plate Specification

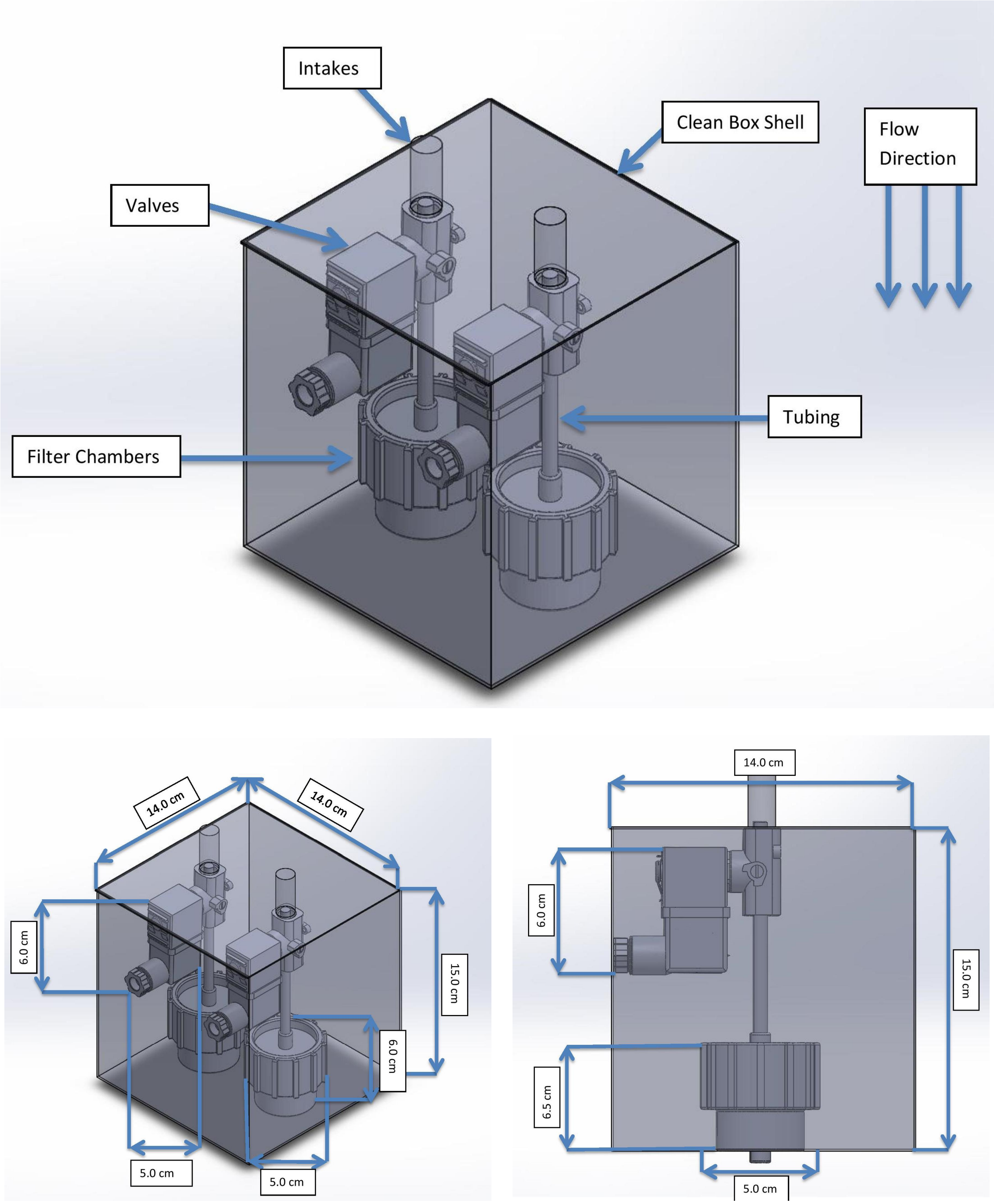

Figure 5: Clean Zone

by commanding them to turn on when the on-board pressure sensor gets a pressure reading that matches 36 km. A buck converter will supply the pumps with 12V of power in order to operate.

3. Valves

For this mission to succeed air samples must only be taken from an altitude of 36 km. To ensure that no air enters prior to or after the intended collection time SIMBA will utilize two 0.55 Watt 8314 series solenoid valves. These motorized valves allow SIMBA to remotely permit the flow of air into the filter chambers when the correct altitude is reached. These valves are capable of operating in temperatures as low as −40 °C which is important as they will be housed in the uninsulated Clean Zone.

C. Thermal

In order to prevent components from overheating or being below their operating temperatures, the electronics housing in SIMBA shall be thermally insulated around the inner perimeter.

The components of the electronics housing are the Raspberry Pi B+, two buck converters, and the two pumps. The Pi operates in a −25 °C to 80 °C temperature range. The buck converters operate in a −40 °C to 150 °C temperature range. The pumps operate in a 0 °C to 50 °C range, making them the most sensitive to temperature. As these components are integral to the optimal operation of SIMBA, an ambient temperature between 0 °C and 50 °C must be maintained. To keep these devices in this range, a multi-layer insulation (MLI) shall thermally insulate this region.

1. Sensors

a. Humidity

A humidity sensor that draws 200 µA and operates in a 0-100%RH range shall be placed on the exterior of the payload.

b. Temperature

Six temperature sensors that draw less than 0.5 µA each and operate in a −40 °C to 125 °C range shall be used with SIMBA. The devices output voltage at a scale factor of 10 mV/°C , allowing the CDH to record data of the ambient temperature and of SIMBA's components. Internal sensors are necessary as many of the devices contained in the structure are electronic and can only operate in a certain temperature range, and also run the risk of overheating. Internal sensors shall be placed on the valves in the Clean Zone, and also on the pumps and Raspberry Pi B+ in the insulated electronics housing. An external one is utilized to gather data about the living conditions of the collected microbes.

c. Pressure

A digital barometer that draws 3 µA (in advanced mode) and operates in a -4.0 hPa to +2.0 hPa range shall be used. The purpose of the pressure sensor is to measure the pressure as the payload ascends.

D. Command and Data Handling

To control the experimental setup and communicate with HASP, the payload's primary microprocessor shall be a Raspberry Pi B+ carrying the Raspbian operating system. To read from the sensors and control the experimental mechanics, the Pi shall utilize I2C protocol with on-board hardware. This allows the system to use fewer general purpose input-output (GPIO) pins than would be used if I2C were not in play. While this will require specific implementation in Raspbian and the hardware itself, this is a superior option, as not using I2C would possibly require an addition to the Pi's GPIO pins and would be far less clean. Additionally, the Pi will utilize Python code to control the overhead for its operations. Python gives direct hardware access while simultaneously offering high-level computation, and it is a very versatile language.

All data recorded from the sensors shall be recorded onto the micro-SD card for analysis post-flight, and the Pi shall communicate to ground via the Pi's USB connections through a USB-to-RS232 converter. The communication between the Pi and HASP shall consist primarily of uplink-downlink. Additionally, to aid in the implementation of the experiment, the Pi shall utilize the HASP GPS and temperature data, which shall be optimally provided every ten seconds. Sensor data from the on-board temperature, humidity, pressure sensors and the current draw of the entire system shall also be downlinked to ground. The payload will be able to receive four types of commands from ground: Manual Stop, Manual Start, Automatic, and Ping. These commands will serve as a fail-safe in the case of current overdraw or components overheating, indicated by the internal temperature data and the current overdraw of the individual components. In the case of either of these nearing the operational limit, the Manual Stop command will be for the component in question to allow for a reset period. After this period, the Manual Start command will be sent to the component to restart its functionality. These commands will provide the ability to switch between the two pumps as needed. The Automatic command will return all components to their pre-programmed functions. The Ping command will prompt the Pi to return a code within the downlink, which indicates that the payload is listening and is responsive. This command will be sent every ten minutes.

Command Type	Command Functionality
Manual Stop	Sent to individual component to halt function
Manual Start	Sent to individual component to return functionality
Automatic	Returns all components to their initial status
Ping	Indicates for the Pi to send a return code indicating functionality and responsiveness

The total size of the downlink shall not exceed 100 bits per second, and downlink will occur every ten seconds. Included in the downlink will be a time stamp, the sensor data, and current draw.

The Pi was chosen for several reasons, the first and foremost of which is the ease it would provide in communicating to the primary platform, as well as its versatility and flexibility. The Pi is an easily implementable and powerful system that would allow for simple system communication and control.

E. Electric Power System

SIMBA shall receive 30 V DC from the HASP platform. One buck converter will be used to initially reduce the voltage to 5 V for the Raspberry Pi B+ microprocessor and environmental sensors (temperature, humidity, pressure and acceleration). A second buck converter will be used to initially reduce the voltage to 12 V for the pumps and valves. The Raspberry Pi will operate one pump and valve system at a time once maximum height is reached It is important to note that only one pump will be running at a time, so the total available power of 15 W will not be passed. The second pump and valve system is simply a backup in case the first system fails. Figure 6 on the next page is a Functional Block Diagram depicting the flow of Power and Data.

1. Power Budget

Table 1 depicts the estimated power usage of SIMBA by component. All of the environmental sensors will be powered by the Raspberry Pi B+. Note that the values given below are maximum estimates for each of the components to ensure that the power use does not surpass 15 W. It is also worth noting that there is no point at which all of the components will be running at once. Thus, SIMBA will not use more than 12.5 W of the available 15 W.

Component	Voltage (V)	Current Draw (A)	Power Draw (W)
Raspberry Pi B+	5	1.4	7
Pump	12	0.39	4.68
Valve	12	0.046	0.55
Maximum Total Power Draw: 12.23 W of the 15 W Allowed			

Table 1: Power Budget

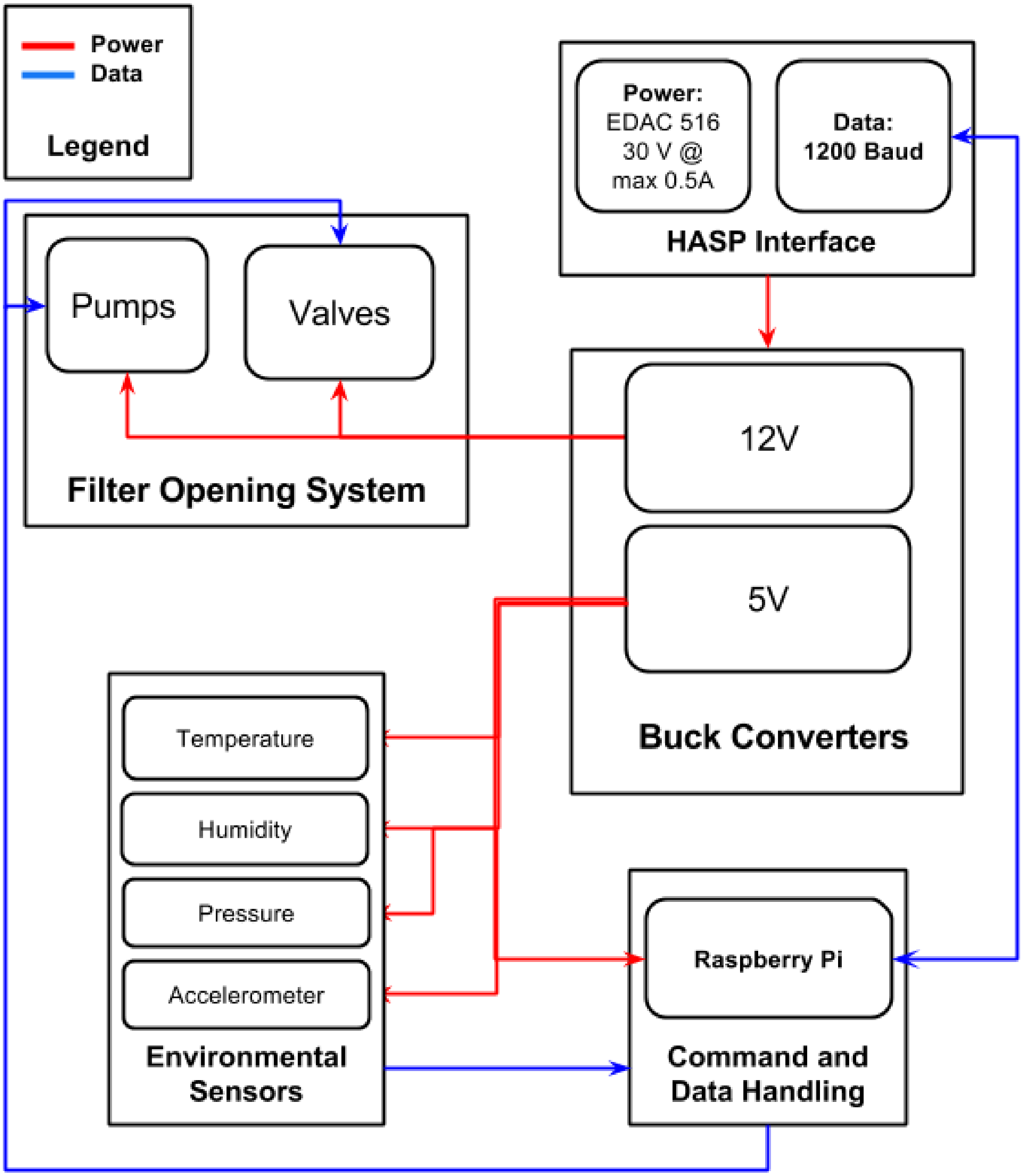

Figure 6: EPS Functional Block Diagram

IV. Experimental Procedure

A. Sterilization

For the results of this mission to be accurate the polycarbonate filters must be clean of any contaminants. To address this SIMBA has implemented a chamber, known as the Clean Zone, that houses all of the air sampling equipment (valves, flow tubes, structural support cylinder, filter chambers, polycarbonate filters membrane filters, and electrical wiring). The Clean Zone will have strict sterilization procedures that utilize an autoclave machine and isopropyl alcohol. An autoclave machine is a device that sterilizes objects by exposing them to high pressure and temperature steam for 10 to 20 minutes. The intense heat and pressure causes the enzymes and structural proteins of microorganisms to irreversibly denature, thus killing them. This method is very effective as it destroys nearly 100 percent of all organisms and their endospores, this reputation has made it the industry standard for sterilization of medical equipment. Although autoclaving metal and certain plastics is safe, some of the equipment and materials aboard SIMBA would be destroyed in the autoclaving process e.g., pump tubes, solenoid valves, and filter chambers. To sterilize these parts they will be hand cleaned with 91 percent isopropyl alcohol. Isopropyl alcohol kills microorganisms by denaturing proteins and dissolving the lipid membrane. Now that the two methods for sterilization have been defined, the specific sterilization procedures are listed below. There are three different procedures for construction, pre-launch, and recovery. Construction sterilization procedure is to be followed during any mechanical/electrical work or testing done on the Clean Zone prior to pre-launch. Pre-launch sterilization procedure is to be followed during the assembly of the final Clean Zone that is to be integrated onto the HASP. Recovery Sterilization Procedure is to be followed once the flight has concluded and the polycarbonate membrane filters are ready for extraction and analysis. Procedures including the isopropyl alcohol will be carried out in the clean room in the Colorado Space Grant facilities at CU Boulder. The autoclaving procedures will occur in the Copley Lab, also located at CU Boulder.

1. Construction Sterilization Procedure

1. The Clean Zone is to be placed in an autoclave bag (aka sterile bag) at all times when not in use.
2. Prior to handling the Clean Zone, or any of its parts, all personnel are required to wear new latex gloves that have been cleaned with 91 percent isopropyl alcohol.
3. Any equipment or tools that are to be used on the Clean Zone must be wiped down with 91 percent isopropyl alcohol prior to use.

2. Pre-launch Sterilization Procedure

1. Prior to handling the Clean Zone or any of its parts all personnel are required to wear new latex gloves that have been cleaned with 91 percent isopropyl alcohol.
2. Any equipment or tools that are to be used on the Clean Zone must be wiped down with 91 percent isopropyl alcohol prior to use.
3. The Clean Zone's aluminum structural support cylinder, filter holders, and aluminum shell are to be placed into the autoclave machine for 15 minutes at 126-129C and 2.5 atm.
4. The Clean Zone's pump tubes, solenoid valves, and wiring are to be hand cleaned with 91 percent isopropyl.
5. Polycarbonate filters are to be handled with tweezers sterilized with dry heat at all times.
6. Finally assembly of the Clean Zone is to be done in a sterilized hood, where sterilization samples will be taken.*
7. After the Clean Zone has been installed onto SIMBA the entire device must be placed in an autoclave bag for transportation.

3. Recovery Sterilization Procedure

1. Prior to handling the Clean Zone or any of its parts all personnel are required to wear new latex gloves that have been cleaned with 91 percent isopropyl alcohol.
2. Any equipment or tools that are to be used on the Clean Zone must be wiped down with 91 percent isopropyl alcohol prior to use.
3. Polycarbonate filters are to be handled with tweezers sterilized with dry heat at all times.

**To verify that no contaminants are present the Clean Zone will be swabbed at random locations prior to transportation. These swabs will then be cultured, if no colonies grow then sterilization will be confirmed.*

B. Sampling and Microfiltration

The objective of the Microbial Capture Unit (MCU) is to capture stratospheric microorganisms and contain them in the Clean Zone to avoid contamination. Once SIMBA reaches its maximum height of approximately 36 kilometers, the MCU will commence operations. The two solenoid valves within the MCU will turn and the external air from the stratosphere will flow into the system as controlled by the two Boxer 10K pumps. Air will flow first through the two larger cylindrical filters above the Clean Zone to filter out larger contaminants. Then the air will be carried through the tubing system to the two filter chambers within the Clean Zone, each containing 0.2 micrometer filters, to capture the stratospheric microorganisms. The air will lastly flow through the rest of the tubing system and out through the Boxer pumps. This process will continuously transpire during the time at maximum height, or approximately five hours before the valves turn again, ceasing operations. Once the valves conclude operations, the filter chambers will be independent of their environment within the Clean Zone, preventing contamination of the samples. The samples will remain isolated until after landing, when the team has separated them from the MCU without contamination and transported them to the laboratory to be cultured and identified.

C. Culturing and Identification

This mission requires a significant amount of post flight procedures to culture the captured microorganisms. Specifically, the goal is to capture four members of the Bacillus genus: Bacillus aerius, Bacillus aerophilus, Bacillus altitudinis, and Bacillus stratosphericus. Once the payload is retrieved, the MCE filters will be removed and placed in sterile petri dishes to be taken to the Pace Laboratory at the University of Colorado at Boulder for culturing. Due to the nature of theses microbes to withstand harsh conditions, they do not need to be cultured under the same conditions at which they were collected. Below is a chart of ideal culturing conditions for each type of genus. This information is from a previous study done by Shivaji et al.[4] and recommendations from Dr. Norman Pace.

Genus	Temperature	pH Level	Percent NaCl
Bacillus aerius	8 °C to 37 °C	6 to 10	11.6 percent
Bacillus aerophilus	8 °C to 37 °C	6 to 10	11.6 percent
Bacillus altitudinis	8 °C to 45 °C	5 to 8	
Bacillus stratosphericus	8 °C to 37 °C	6 to 10	17.4 percent

From these specifications, the team has determined to culture the filters under the following conditions to allow for all four types of genus to grow. Using a nutrient agar, the samples will be cultured between 8 °C to 37 °C, with a pH level between 5 to 8, and a 2% salt content in the agar. The samples will be allowed to culture for a week with monitored progress every two days. The study done by the Shivaji et al. also provides information on how the genus colonies can be identified visually.

Genus	Colony Diameter	Color	Other Visual Identifiers
Bacillus aerius	3 to 5 mm	white	irregular, raised
Bacillus aerophilus	5 to 8 mm	white	irregular, raised
Bacillus altitudinis	2 to 3 mm	white	regular margin, convex
Bacillus stratosphericus	3 to 5 mm	white	17.4 percent

As seen above, Bacillus aerius and Bacillus stratosphericus have the same visual identifiers. If the colonies grown match their descriptors, the samples will be placed in a new agar solution with a higher salt concentration of above 11.6%. If the sample continues to thrive, it can then be identified as Bacillus stratosphericus or if the sample dies, it can be identified as Bacillus aerius.

D. Diamidino Phenylindole (DAPI)

Diamidino Phenylindole (DAPI) is a fluorescence stain that, when poured onto a biological sample, will bind to DNA strains on the sample. Once viewed under a fluorescent microscope, the DAPI stain will illuminate any cells on the sample. With this, the filter can be divided into sections whose area and microbe concentration can be calculated. Using the concentration and comparing it to the amount of air pumped through the filters, an estimation on the atmospheric concentration of microorganisms can calculated. The DAPI testing will also provide data on the variety of life living at 36 km since the images from the fluorescent microscope will show basic cell shapes. Ideally Team SIMBA would aim to identify the specific kinds of microbes found, but through the personnel and resources available, this is not a feasible mission for the team. Identification would require polymerase chain reaction (PCR) testing. Some of the problems that make PCR an unlikely option for the SIMBA mission include the cost and time required for it, but mainly the level of biological expertise needed for such a complex procedure.

1. Procedure for DAPI testing:

1. Polycarbonate filters must be retrieved from the Clean Zone post landing.
2. Filters will be cut in quarters, this ensures that if a mistake is made and the sample is damaged, not all of the data will be lost.
3. Place the filter piece on a sterilized petri dish.
4. Dilute DAPI stain to 300 nM 300 phosphate-buffered saline (PBS).
5. Add 300 µL of the diluted mixture to the filter piece, making sure that the whole sample is covered.
6. Let the samples incubate for 1-5 mins.
7. After incubation period, rinse using PBS then drain any excess off the sample.
8. View under an fluorescent microscope.

V. Management

SIMBA consists of 16 members. Each member shall be on a subsystem based on interest and/or experience with the subsystem. SIMBA shall work with faculty advisers and members of the HELIOS IV team to ensure the successful design and construction of the payload. The HELIOS IV advisers shall work with the SIMBA team in the subsystems of their expertise in order to give their knowledge and experienced gained from the 2014 HASP mission and from the current work for the 2015 HASP mission. The members of SIMBA and leads are subject to change. The current members affiliation is based on proposal writing team, while the final team selection will occur if SIMBA is awarded a seat.

A. Team Organization

Name	Affiliation	Email	Phone Number
Chris Koehler	Project Adviser	Koehler@colorado.edu	303-492-3141
Chris Koehler	Faculty Adviser	Koehler@colorado.edu	303-492-3141
Paige Arthur	Project Manager	paar5780@colorado.edu	303-957-7360
Alex St. Clair	Systems Engineer	david.stclair@colorado.edu	303-898-3902
Ryan Wall	Structures Lead	ryan.wall@colorado.edu	858-353-4609
Kyle DiGiamcomo	CDH Lead	kydi3385@colorado.edu	303-956-5660
Allison Howard	EPS Lead	allison.howard@colorado.edu	303-947-5935
Haleigh Flaherty	Science Lead	haleigh.flaherty@colorado.edu	719-660-8177

Table 2: Team Organization

B. Scheduling

In addition to these scheduled meetings and conferences below, SIMBA shall meet a minimum of once a week to accomplish project tasks and meet with SIMBA's project adviser, Chris Koehler. During these meetings, team members shall discuss the current state of the project, and each system lead shall submit progress reports to the Systems Engineer and Project Manager. Each sub-section shall meet at their discretion and work on their system tasks. Work hours will be held at times that are convenient for members of different sub-teams to work together. Reviews will be held with both SIMBA's faculty and science advisers plus current and past COSGC students. A preliminary schedule is presented in figure **??** on page ??. A more detailed schedule is currently in development and incorporates lessons learned from previous HASP missions, in particular HELIOS III.

Milestone	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Notes
Proposal Team Formed	X														
Submit Proposal		X													
Payloads Selected			X												
Submit Selection Response			X												Address comments in proposal
Preliminary Design Review				X											Present to Space Grant
Critical Design Review					X										Present to Space Grant
Order Parts					X										
Preliminary PSIP Due						X									
Finalize Science								X							Sterilization tested
Finalize EPS								X							Board tested wth all systems
Finalize CDH								X							Code complete
Finalize Structure								X							Structure assembled
Final PSIP Due								X							
FLOP Due									X						
Systems Test									X						Entire payload tested together
Integration at CSBF										X					
HASP Flight Preparation										X					
Target Flight Ready										X					
Target Launch Date											X				
Flight Operations											X				
Recovery											X				
Finalize Data Analysis												X			
Final Science Report														X	

Table 3: Planned SIMBA schedule

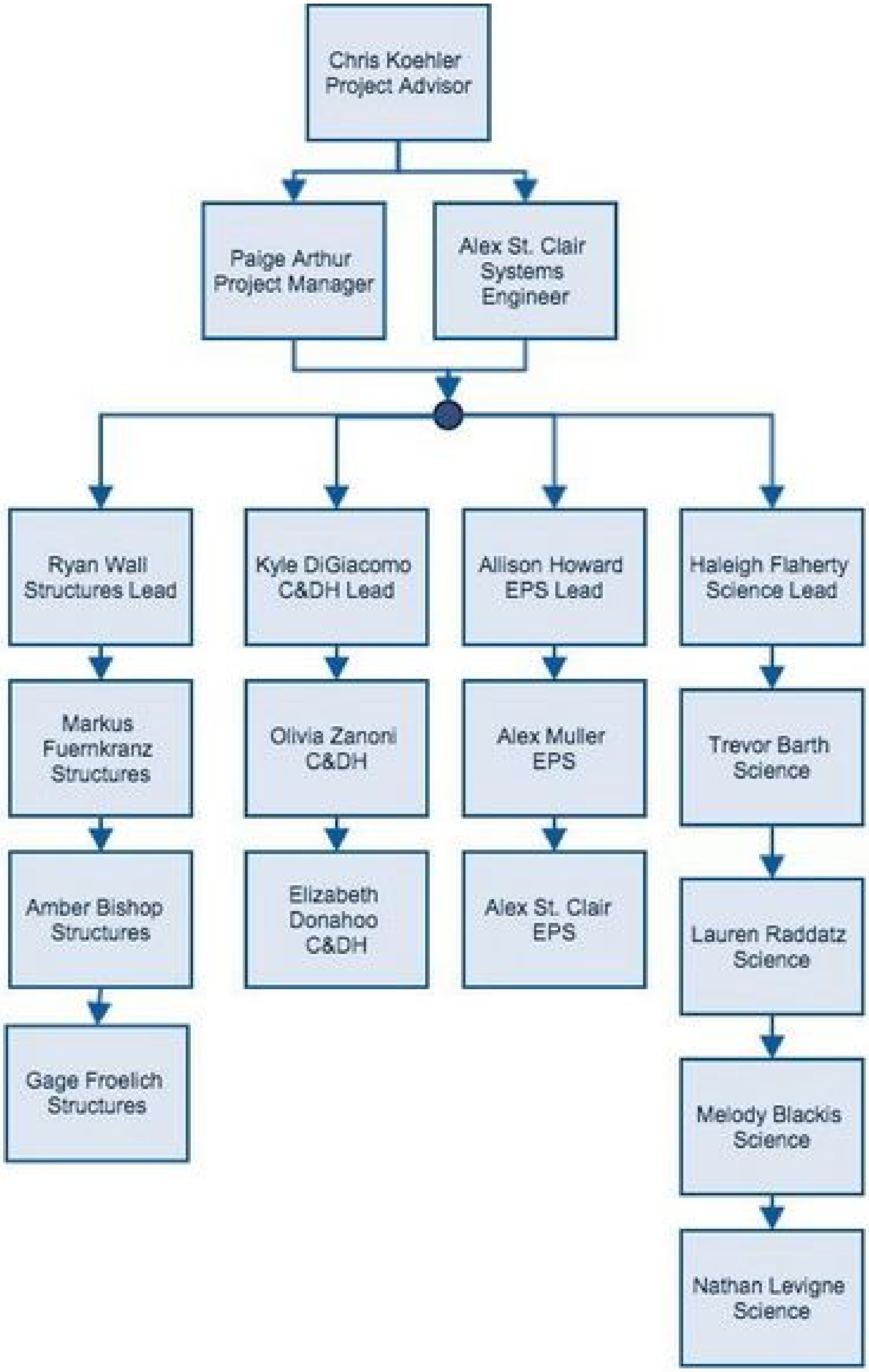

Figure 7: Team Organization Chart

VI. Weight Budget

Component	Number	Weight (kg)	Uncertainty (kg)
Raspberry Pi B+	1	0.05	0.005
Boxer 10K Pump	2	0.32	0.010
Environmental Sensors	4	0.01	0.005
Button Sampler	2	0.09	0.008
Filters	2	0.00	0.001
Wiring	-	0.01	0.005
Valves	2	0.30	0.100
Tubing	-	0.01	0.010
Buck Converters	2	0.09	0.008
Structure	-	0.55	0.050
Total		1.43	0.202

Table 4: Weight Budget

This weight budget was created by measuring or calculating the mass of components. Calculations were done using SolidWorks and the structural designs. A margin of error is included in the uncertainty.

VII. Integration and Launch

Upon integration of SIMBA into the HASP platform, a total of six people including all System Leads, the Systems Engineer, and the Project Manager shall ensure proper integration procedure is followed. A comprehensive checklist shall be used to confirm a successful integration of the SIMBA payload. The Systems Engineer shall test all communication processes and equipment throughout integration to assure proper function. SIMBA plans to keep the payload attached to HASP mounting plate after integration. Below is a detailed integration plan SIMBA will follow at NASA's Columbia Scientific Balloon Facility in Palestine, TX. The SIMBA payload has no orientation requirements.

1. The payload does not exceed the mass constraint of 3 kg, the footprint constraint of 15 cm x 15 cm, or the height constraint of 30 cm.
    (a) The payload will have a 15 cm x 15 cm footprint, be 30 cm tall, and have a mass of 1.373 kg plus the mass of the structure, which will be less than 1.627 kg.
2. The payload base fits within the 5.875 in x 5.875 in allowed area.
3. Ensure that the SIMBA Payload is physically attached to the HASP Platform.
4. Ensure that the PVC plate is secured to the platform.
5. Ensure that the SIMBA Payload is secured to the PVC plate.
6. Ensure proper connection of electrical wiring and digital communication lines to all systems.
7. Ensure correct HASP wiring hookups
    (a) Check and confirm serial hookup.
    (b) Check and confirm EDAC hookup.
8. Structures Attachment
    (a) Ensure all structural components are attached to the HASP Platform.
9. EPS Hookup
    (a) Ensure EPS is connected to HASP power line through EDAC 516 interface
    (b) Ensure all wiring is properly secured
10. CDH Hookup
    (a) Ensure flight computer connected to EPS power board
    (b) Ensure environmental sensors are connected to flight computer
    (c) Ensure all serial communication lines connected to DB9 connector
11. Science Hookup
    (a) Ensure that all pipes, valves, and pumps are connected correctly
    (b) Ensure that the system is sealed
12. Verify functionality of individual subsystems:
    (a) EPS
        i. Ensure power reception from EDAC 516 interface
        ii. Ensure power distribution to all systems
        iii. Verity that the power draw does not exceed 30V at 0.5A DC
    (b) CDH
        i. Ensure functionality of serial communication through DB9 connector

        ii. Confirm that the serial communication is at 1200 baud
        iii. Ensure that CDH is communicating with all other subsystems
        iv. Check the functionality of EDAC 516 command channels (K,L and M,R)
        v. Send command bit to each individual system
        vi. Ensure CDH is receiving signals from all systems and environmental sensors
    (c) Science
        i. Verify valve and pump functionality

13. Verify entire payload functionality
    (a) Command CDH to power on/off payload
    (b) Command CDH to give Health and Status to ground station
14. Ensure communication between EPS, CDH, and Science
    (a) Command CDH to power on/off Science system to test flow functionality
15. Decontaminate Science Clean Zone
16. Final Mission Simulation
    (a) Activate payload using serial command from ground station

A. Launch Procedures

Table 5 shows an approximation of flight times, altitudes, and events planned for the HASP flight at Ft. Sumner, NM. The leftmost column contains estimated times in the hour-minute-second format. In the rows associated with each time are estimated altitudes, flight events, and system directives. The Project Manager is responsible for ensuring that all of the flight procedures are properly followed and completed. The team leads of each subsystem shall be responsible for ensuring that their respective subsystems successfully undergo integration into the HASP platform. During the flight the CDH lead shall be responsible for sending all commands to the SIMBA payload on the HASP platform.

Est. Time	Est. Altitude (m)	Flight Event	System Event	Event Sub-Tasks
T 1 day	0	N/A	Pre-Launch Check	Verify All Systems
T 1 day	0	HASP Integration	HASP Integration	
T 01:00:00	0	N/A	Flight Line Setup	
T = 00:00:00	0	Launch	N/A	
T + 02:00:00	36,576	Max Altitude	All Systems On	
T + 02:30:00	36,576	Float	Begin Sample Collection	Open Valves, Start Pumps
T + 06:00:00+	36,576	Float	End Sample Collection	Close Valves, Power off Pumps
T + 06:00:00+	36,576	Float	Disable Systems, Power Down	
T + 06:30:00+	36,576	Begin Descent	N/A	
T + 08:00:00+	0	Landing	N/A	

Table 5: Launch Procedures

VIII. Conclusion

The goal of SIMBA is to collect stratospheric microorganisms to determine their concentration in the stratosphere and analyze the environment in which they survive. SIMBA shall demonstrate the feasibility of capturing stratospheric microorganisms at high altitudes, and furthermore, shall show that it is possible to identify the characteristics of their environment. To demonstrate this, SIMBA shall fly two Boxer 10K pumps controlled with two solenoid valves in order to pull air from the stratosphere through filters of varying pore size, capturing microorganisms and holding them in an isolated chamber to prevent contamination. SIMBA will keep these microorganisms isolated in the Clean Zone until Team SIMBA can transport them to a lab for DAPI testing. Should the SIMBA mission prove successful, it shall show that high altitude balloons can offer the ability to gather more information regarding where life can survive.

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