Colorado Space Grant Consortium

# DemoSat
# Design Document

Yeasty Beasties

Written by:
Xavier Cotton, Natalie Smith

Faculty Advisor:
Clara Wente

Front Range Community College (Westminster Campus)

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## 1.0 Mission Overview

This experiment sought to determine how the growth rate and carrying capacity of baker's yeast (*Saccharomyces cerevisiae*) are affected by exposure to gamma radiation in the stratosphere up to an altitude of 100,000ft with external temperatures potentially reaching as low as -80°C.

Yeast is a living biological eukaryotic organism of the kingdom Fungi. Like most organisms, yeast undergoes growth, development, and reproduction when given space, water, and food. For this experiment the yeast was given a solution of water and glucose, and will grow between 10°C and 55°C. Once higher than 55°C, the yeast will begin to denature and below 0°C the yeast will start to crystallize and die. Between 0°C and 10°C yeast is in a dormant state where it is inactive, neither growing or dying. During the payload's flight, it was important that the yeast was not in a dormant state otherwise nothing would happen to it due to exposure to radiation. For a living organism to be affected by radiation, it must be active to show any mutations afterwards. The carrying capacity, which is the maximum population of yeast the environment can contain before there is not enough food or space for the yeast to grow anymore. Theoretically, the yeast will grow exponentially over 10 or so hours (depending on the solutions' starting concentration of yeast) before it starts to level into its carrying capacity where it cannot continue to grow as shown in *Figure 1 (Growth Rate of Yeast)*.

Since it was expected that the environment in which the payload and yeast solutions would be sent to would be as cold as -80°C, glycerol was introduced to help ensure data could still be collected in case the payload got too cold and the yeast solutions crystallized and died. Glycerol lowers the temperature at which the solution would crystallize, but it takes away from the amount of glucose (yeast food) available for the yeast. It was determined that the experiment should include glycerol as an independent variable to determine if it has any affect on the yeast's growth rate during its flight. One group of yeast solutions would have 10% glucose and 90% water. The other group would have 5% glucose, 45% water, and 50% glycerol.

Determining the growth rate of the yeast was the bulk of this experiment and project because it was a time consuming process figuring out how best to grow yeast. To test the concentration of a sample of yeast, one has to set up a spectrophotometer. First the spectrophotometer lamp needs to be heated up. The purpose of the lamp is to shine a light through a cuvette full of solution. The light shines through the cuvette and solution and the light's brightness is measured on the other side. The amount of light that gets through the cuvette full of solution determines how cloudy or concentrated the yeast solution is. After heating up the lamp, one needs to calibrate it with a blank, or in other words a cuvette with just the solution and no yeast in it. Next, the tubes that held that yeast solution would each need to be emptied into a cuvette and tested in the spectrophotometer. Usually the solutions were not perfectly diluted and there was a "cloudiness" that floated around making the solution concentration uneven and making the spectrophotometer read unstable concentrations. When there are three tubes per group, 11 times from 0 hours to 30 hours, and four groups (no glycerol at room temperature, no glycerol at incubating temperature, with glycerol at room temperature, with glycerol at incubating temperature), there are many tubes to test, which is a time consuming process, especially when multiple of these experiments are conducted over the course of weeks because a growth curve was not determined.

Late into the process of experimenting, it was determined that glycerol was actually an agent that inhibits yeast growth, which could have been found out with more preliminary research. Consequently, glycerol ended up not being used for the final experiment and only non-glycerol solutions were made.

## 2.0 Requirements Flow Down

The level 0 requirements are more widely encompassing on what the payload needs to do to be successful and the level 1 requirements give more details on how the level 0 requirements need to be met. See *Figure 2 (Level 0 Requirements)* and *Figure 3 (Level 1 Requirements)*.

## 3.0 Design

The design of the payload had to meet several requirements. It had to safely transport six solutions inside of it without breaking or damaging anything inside of the payload. The second requirement was collecting data on the internal and external environment of the payload to know the conditions that the yeast solutions were under. The final and most challenging requirement was to keep the inside of the payload heated to above 10 degrees Celsius at all times so that the yeast would be in an active state, yet not heat the inside of the payload enough to overheat the yeast.

At the center of the electronics system in Data Handling and Control is an Arduino Uno Rev3. For data collection an Analog Devices TMP36 temperature sensor, a Bosch BME280 atmospheric breakout which monitors temperature, pressure, and humidity; and a Radiation Watch Type 5 Pocket Geiger Radiation Sensor, all from Sparkfun, were supplied power by the Arduino's 3.3v output. Each of these systems operated separately to the Data Handling and Control as seen in *Figure 4 (Block Diagram)*. This data was written to a SparkFun OpenLog SD Card breakout attached to the arduino.

Four Energizer lithium-ion AA batteries were wired in series supplying six volts power the system, with electricity first passing through the power control switch before being junctioned between the Arduino's VIN pin and a Sparkfun Heating Pad which feeds into the drain pin of a Fairchild Semiconductor RFP30N06LE N-channel mosfet transistor, which acts like a valve for the current to the heating pad as the arduino could not supply enough power to generate the required heat. The mosfet's gate pin is wired to the arduino with a junction heading through a 10,000Ω resistor to ground to prevent the heater running randomly.

Four LEDs were used to display the status of the payload's operation, more specifically that 1) the system is powered, 2) the heater is running, 3) the TMP36 is not inactive, freezing, or burning; and 4) the BME280 is operating correctly. The electrical schematic can be found in *Figure 5 (Electrical Schematic)*.

The system software was designed to minimize the amount of power the arduino would draw. To do this the system power modes were used in conjunction with the watchdog timer interrupt to sleep and wake the CPU every 4 seconds. The arduino's external interrupts and corresponding pins were connected to the radiation sensor, with the interrupts briefly waking the CPU to count each signal and noise pulse, and return the CPU to sleep.

When speaking with the 2020-2021 Front Range DemoSat team leader about his team's fungus project and their shortcomings, one of the things brought up was the failure of their heaters. A hypothesis of this failure was that the space the heaters had to heat was so large that it drained the batteries very quickly. This year, the team made sure to make the payload as small as possible, almost too small, which ended up being the biggest limitation in the design. One of the earliest designs shows how very little extra room was allowed for wires. See *Figure 6 (Isometric CAD view)* and *Figure 7 (Front CAD view)* for the CAD drawings.

This design was changed slightly with the original two 9V batteries being swapped for four AA batteries, and allowing for more space for wires. The overall dimensions for the final payload are shown in *Figure 8* (Final Dimensions), but the payload was still very small, making wiring difficult.

There was also a tube holder designed and 3D printed so that it could hold the tubes of solutions during the flight. The tube holder could have been printed with less infill for a smaller weight, but the team was afraid that air pockets might expand in the hold, making it explode. Infill was set at 60% for the print and while heavy, the tube holder did not explode. The team thought it was necessary to have something to hold the tubes during flight as to not allow them to bump into any electronics. The initial 3D print was too small as well, so it was redesigned to be more spacious for the tubes and allow for tape around the caps. See *Figure 9* (Initial Tube Holder).

With the experiment finalized, tube holder printed, payload designed, and electronics largely finished, a mock up payload was made out of cardboard. An early mock up was made based on a CAD design using dimensions of different parts online. It was found out that the dimensions of the Arduino Uno listed on the Spark fun page, did not include the port coming off of the side which made it impossible to fit all the parts into the designed box. After that, all of the parts were physically measured to ensure no error in the design before cuts into the foam board were made. See *Figure 10 (Mock Up Payload)* to see the final cardboard practice payload.

After the payload was ready to be built, rectangles were drawn onto the insulating foam board to be cut out. This led to an issue in cutting the pieces because there was no heating foam cutter on hand. An Exacto knife was used to cut the pieces out which was a long and difficult process. The knife did not cut so easily and at times carried material with it and tore through the board. This made for some crude looking cuts, but the board was still cut well enough to serve its function.

After soldering wires together and finalizing the code, the electronics were integrated into the structure component by component. This was a difficult task to be completed because of how cramped it was and how stiff the wires were. The team wanted to use stiff wires because if the payload got jostled, the wires should not be so loose that they move around and come out of their ports. The stiff wires ended up being a hindrance because they were not so easily moved to where they needed to be, which meant, putting the switch, break out, and LEDs in their cut out divots was also difficult. However, with some tape, wire shortening, wall cramming, and teamwork, the payload was built. See *Figure 11 (Inside of Payload)*, *Figure 12 (Crammed Wires)*, and *Figure 13 (Top View of Electronics)*. See *Figure 14 (Final Payload)* for a picture of the final payload.

## 4.0 Management

The team consisted of Xavier Cotton and Natalie Smith with support from the faculty advisors Clara Wente and Jen Cappa.

| **Name** | **Responsibilities** |
|---|---|
| Xavier Cotton | CAD Designs, Yeast Analysis, Structural Building |
| Natalie Smith | Schematics, Electronics, Code |

## 5.0 Budget

A pack of Energizer lithium-ion AA Batteries were purchased from King Soopers. The geiger counter was purchased from Sparkfun and is called, “Pocket Geiger Radiation Sensor - Type 5”. The “If Found” sticker was printed for free on campus and two American flag stickers were purchased from Amazon; they are called, “American Flag Hard Hat Stickers - 1.8" x 3" American Flag Decals (2-Pack)”. 500 5 mL Eppendorf Tubes were purchased from “avantor” supplied by “vwr” and they are called, “VWR® Screw-Cap Centrifuge Tube, 5 ml”. The battery holder was already owned by teammate

Natalie who got it on Amazon in the past. See *Figure 15 (Weight and Cost Rundown)* for a complete list of everything in the payload, their costs and their weights.

## 6.0 **Test Plan and Results**

To test the payload and all of the subsystems, regular code and structural tests were conducted. After the initial whip test, the system turned off so the payload was opened up and it was found that the batteries in the pack slipped out of their enclosure. The batteries were simply wrapped all around the battery pack and tests resumed. The payload and its contents survived the next whip tests, drop tests, and stair pitch tests with only some dents on the corners and edges. See *Figure 16 (Whip Test)*, *Figure 17 (Stair Pitch Test)*, and *Figure 18 (Drop Test)*.

Afterwards, a cold test was conducted with mostly success. The payload (without the yeast solutions) was placed in a -80°C freezer for three hours to mimic the worst temperature conditions possible during the flight. The payload worked as planned, fluctuating the inner temperature between 40 and 10°C for ten minutes and gradually lowered to being between 30 and -3°C until the arduino triggered a brown-out reset (BOR) due to insufficient power. The payload continued to collect data and supplied current to the heater while resetting repeatedly for an additional 30 minutes. However, the batteries tested were alkaline batteries, which behave significantly worse than lithium ion batteries in cold conditions, and the payload was put under the absolute coldest conditions from the get go. The team confidently moved forward knowing there would be batteries better suited for the task and a warmer environment. See *Figure 19 (Cold Test-Temperature)* for the inside temperature of a cooler with the heater and alkaline batteries.

The geiger counter was also tested during the cold test and the data retrieved was promising. The geiger counter measures radiation events, the striking of radioactive particles against a material wrapped around the hardware, in counts per minute with a count per minute fluctuating between 14 and 0, about 0.1 μSv/hr (micro Sieverts per hour) being standard for a room on Earth with minimal radiation exposure. See *Figure 20 (Geiger Counter Cold Test)* for a graph of the collected data. Other tests were also conducted with a piece of uranium glass placed near the testing rig, in which the count per minute exceeded the maximum 14 up to 22 counts per minute.

## **7.0 Expected Results**

The first basic freezer test utilized a heating pad operating in conjunction with a temperature sensor inside a styrofoam cooler in a consumer freezer set to -2°C; the test was successful with a total running time of two hours resulting in an even cycling of the heater, which inspired confidence in the chosen hardware configuration.

The first cold test in a freezer set to -80°C, however, had an issue where the temperature sensor had the wires connecting to power and ground swapped, resulting in an average temperature reading of 240°C. The heater thus partially melted the breadboard underneath. No other damage occurred and the sensor continued to function correctly despite the error, although the test was determined a failure as the heating pad never drew current. This error was never replicated.

Two sets of tests were too difficult to perform with thorough data collection due to the restriction of available altitude. The atmospheric pressure corresponding to increase in altitude was calculated with the formula in *Figure 21 (Predicted Atmospheric Pressure)*, and the amount of ionizing radiation with increasing altitude was determined from an external source graphed in *Figure 22 (Predicted Radiation vs Altitude)*.

Theoretically, the yeast was supposed to grow at an exponential rate and then level off into carrying capacity as shown in *Figure 1 (Growth Rate of Yeast)*, but all of the tests done on the yeast solutions with the spectrophotometer yielded a level curve as shown in *Figure 23 (Yeast Test Results)*. A flask of yeast solution was made with .3g of yeast and 100mL of 10% glucose solution. This solution was then put in tubes with caps on and incubated for varying amounts of time up to 30 hours. Some of the yeast solutions exploded and others popped open when they were about to be tested. This clearly shows that there was growth because of the excess $CO_2$. This graph however, makes it appear like the yeast had little to no growth. The spectrophotometer was calibrated every time so maybe better data would have been collected if the starting concentration was less, giving more room for the yeast to grow, a superior environment was given for the yeast solutions to grow, and/or many more solutions were made over a greater period of time. That being said, after the flight, and assuming gamma radiation improves growth rate, the yeast solutions were predicted to have a higher concentration than the control yeast solutions coming out of the payload and then have a level growth curve after being incubated more.

## 8.0 Launch and Recovery

On the day of launch, the team met at Deer Trail Elementary School in the morning to prepare the payload for flight. Natalie brought the payload with her and Ms. Wente brought the prepared, cooled yeast solutions from the campus in a cooler with ice. The team put the yeast solutions in the payload and then it was taped and weighed for a final weight of 524g. Later, it was put on the flight string and as the payload handler, Natalie lifted the payload off the ground to flight. After a flight time of one and a half hours, the payload and balloon landed in a dirt field on private land, east of the launch site. The payload appeared unharmed and was safely retrieved. However, the payload did sit in the warm field for around an hour until the landowner could come and let the team go through their land to retrieve the payload, allowing time for the yeast solutions in the payload to warm and grow. When the team retrieved it, the payload was structurally sound, but had lost power. The yeast solutions and SD card were removed from the payload for analysis of the collected data, and the yeast solutions were placed into a cooler and refrigerated at the campus until the growth rate could be analyzed. See *Figure 24 (Payload Retrieval)* for a picture of the payload at the landing site.

## 9.0 Results, Analysis, and Conclusions

Despite thorough testing and planning, the results of the launch and experiment were largely a failure. The payload was active for 9.5 minutes before the payload and balloon lifted off and continued for 23 minutes after the balloon took flight. After 23 minutes had passed the heaters and all of the sensors lost power. The batteries did not die however, because the payload was able to be turned back on after it was retrieved, capable of continuing operation. The theory for this electronics failure was a loose connection that was jostled during flight and after it landed, but it remains unknown.

Continuing the theme of equipment issues, and despite having sealed screw cap tubes with electric tape around the cap, after the payload and the yeast solutions were retrieved, the yeast solutions had still leaked from the tubes. There was also the presence of dried glucose at the top of the tubes, invalidating any results that could have been measured from the yeast solutions.

The data retrieved from the payload indicated a very unstable internal temperature, with the heater constantly cycling every two cycles of the watchdog timer to keep the inside of the payload warm enough for operation as seen in *Figure 25 (Flight Internal and External Temperature Results)*. The geiger counter data does show a steady increase in the average number of counts per minute in alignment with the predicted data. Of particular note is the spike of radiation event counts during launch where the amount exceeded 40 counts per minute as seen in *Figure 26 (Flight Geiger Counter Results)*, which corresponds to the beginning of decrease in atmospheric pressure as seen in *Figure 27 (Flight Atmospheric Pressure)*, which indicated the beginning of the flight.

## 10.0 Ready for Flight

A few modifications are required in the event of further flights. One to three additional temperature sensors are placed throughout the payload in order to determine which components are actually receiving heat rather than just one placed between the geiger counter and the arduino. The software needs to be updated to utilize these sensors as well as modifications to the portion handling power management, as CPU components were not deactivated and reactivated optimally. Design flaws in the external shell required better sealing around the face adjacent to the external electronics. All components with exception of the batteries will last until an additional flight. Of course, yeast solutions will need to be remade since the tubes holding them leaked and the glucose dried.

## 11.0 Conclusions and Lessons Learned

The team learned a lot about the growth cycle of yeast and environmental factors that impact yeast's growth. We also got to learn about and use some interesting new technology including the pocket geiger counter and spectrophotometers, as well as more depth to current technology such as the interfacing of AVR C power management code, which is the architecture the Arduino's CPU operates under, with arduino code.

The biggest change that the team would make if we got to do this again next year would be to pick a less ambitious research topic. With only two people, a biology topic introduced a lot of challenges on the team that demanded a lot for only two people. Even with assistance from mentors, the workload of this project during school was a lot, so if there was a next time, we would only choose a lighter research topic or try to get more people to join the team.

## 12.0 Message to Next Year

Glycerol was suggested as an agent to bring the freezing point of yeast down and allow for it to grow under colder conditions. It took many tests to determine that this was not a good idea and it was very difficult to get yeast to exhibit growth in glycerol.

Also, the team wishes more time and help was put into making and testing the electronics and code. The code worked on Earth, under ideal conditions, but more testing could have shown that it would have failed while in flight. Another consideration when required to determine the internal temperature is to utilize several internal temperature sensors in several locations in the payload, rather than just one. An important suggestion is to avoid using the Sparkfun heating pads for heat generation in cramped structures.

One of the reasons the yeast solutions' concentrations were unreasonable was because they were trapped in a closed tube. As the yeast grows, $CO_2$ is released, making the air inside the tube expand. Since the tube was closed, there was no room for the air to expand, making some tubes explode and constricting other solutions' growth rate. A future experiment would need to completely redesign how the yeast solutions are allowed to grow so that they have less restricted space.

Future teams should also pick a topic sooner than the team did this year. A final topic was not picked until a couple weeks into the second semester, making everything rushed to an extent. Future teams should pick a project and make a research question before the end of the first semester. Even if it is not perfect, run with it because time is limited.

Finally, make sure the team has the people for the job. If the team is building new code that uses different batteries and new technology, it would be a good idea to have a couple advanced coders or a computer science mentor. If the team is going to use bacteria or fish eggs, ensure they have access to one or two people with biology experience.

Learning new things is fun, but it can quickly become overwhelming without proper guidance.

Appendix

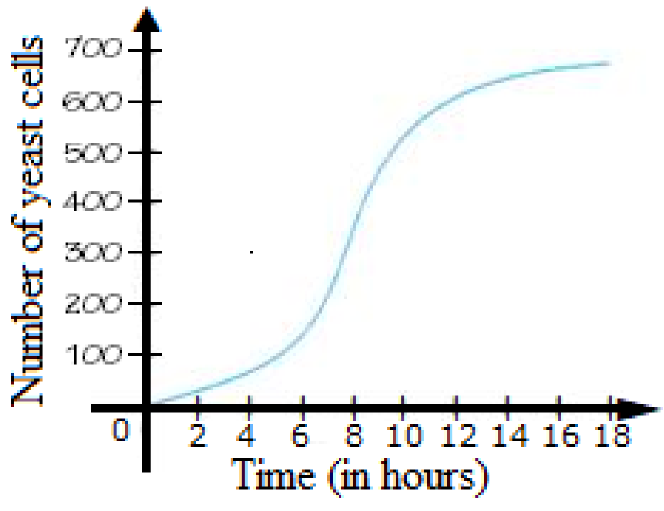


Figure 1 (Growth Rate of Yeast) (Source: study.com)

| Level 0 Requirements | Description |
|---|---|
| 0.0 | The payload needs to meet the launch requirements by launch day |
| 0.1 | The yeast solutions shall be warm enough to not crystalize |
| 0.2 | The payload shall be still working and retrieved after flight |

Figure 2 (Level 0 Requirements)

| Level 1 Requirements | Description | Origin |
|---|---|---|
| 1.0 | The payload shall be under 750 g | 0.0 |
| 1.1 | The payload shall be survive rigorous structural tests | 0.0 |
| 1.2 | The heater and batteries shall keep the yeast solutions above 10°C for 3 hours | 0.1 |
| 1.3 | The payload's radiation and temperature data shall be collected and analyzed | 0.2 |

| | | |
|---|---|---|
| 1.4 | The payload's final code shall be optimized to not drain the battery completely and collect all data | 0.2 |

Figure 3 (Level 1 Requirements)

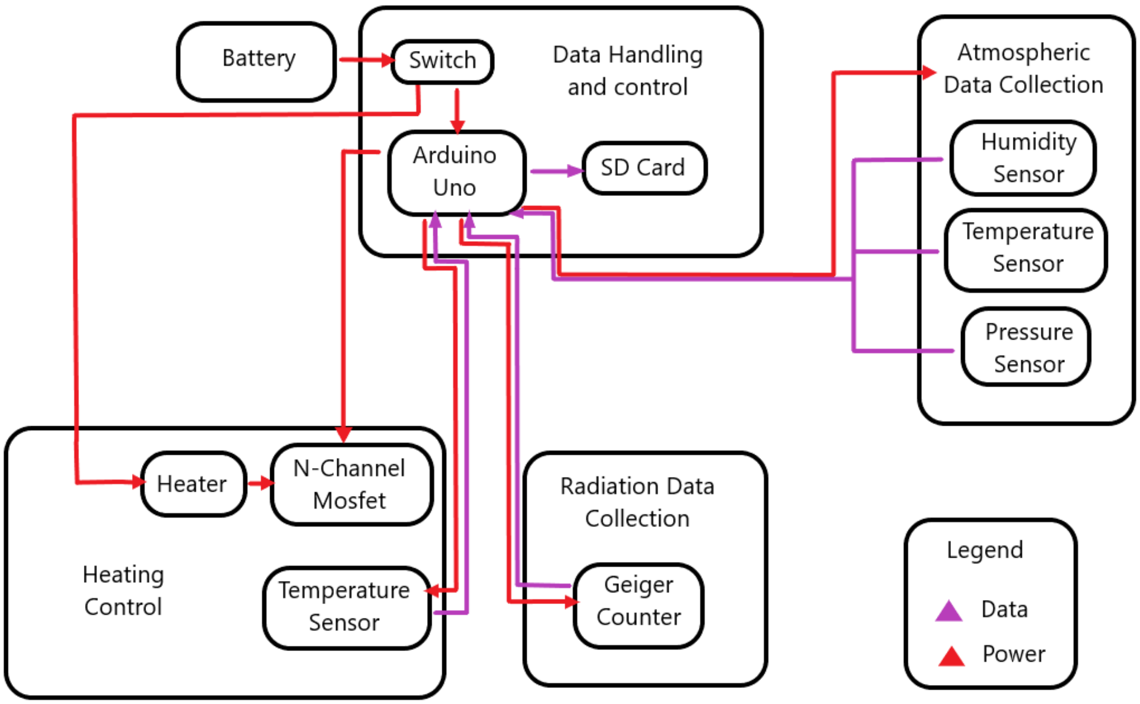


Figure 4 (Block Diagram)

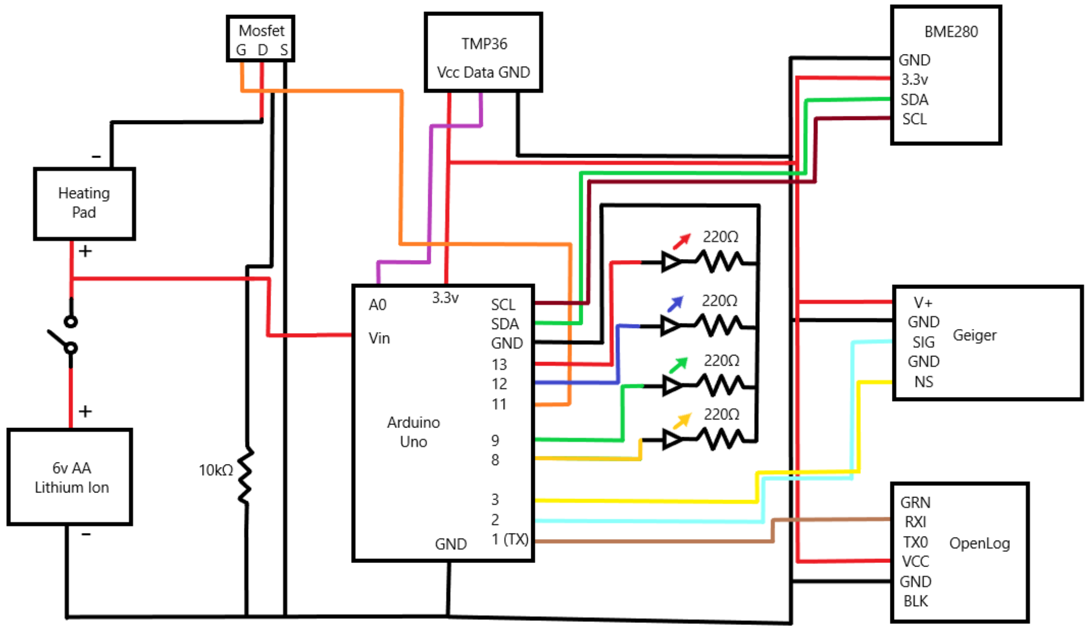


Figure 5 (Electrical Schematic)

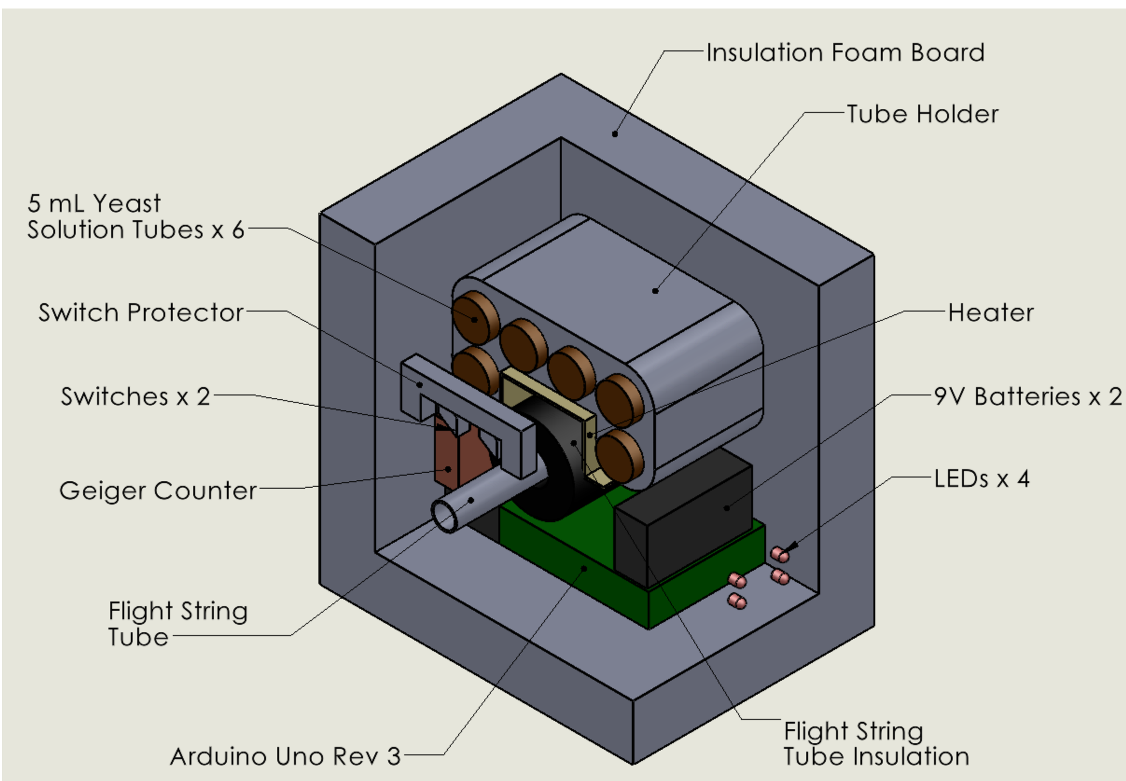


Figure 6 (Isometric CAD view)

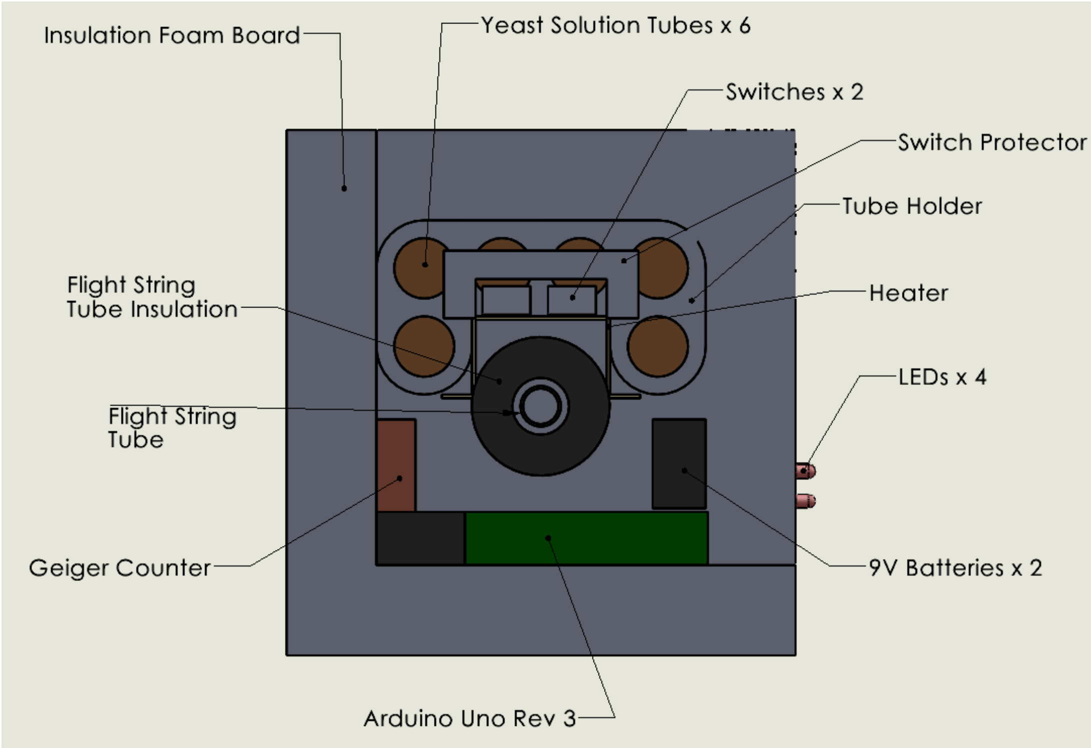


Figure 7 (Front CAD view)

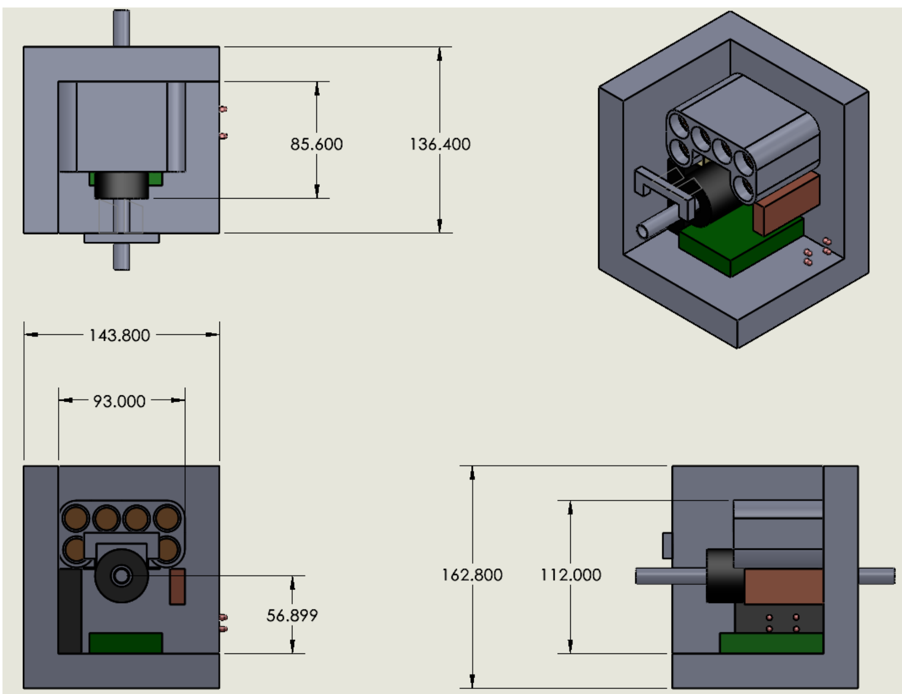


Figure 8 (Final Dimensions)

Figure 9 (Initial Tube Holder)

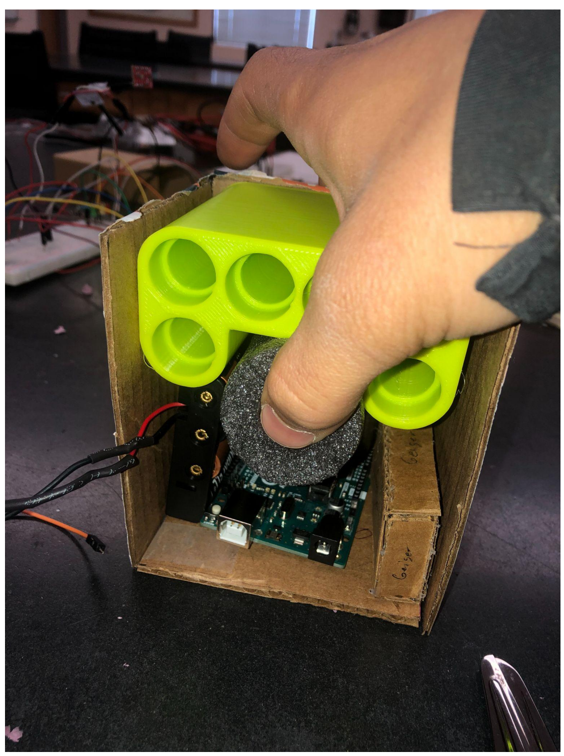

Figure 10 (Mock Up Payload)

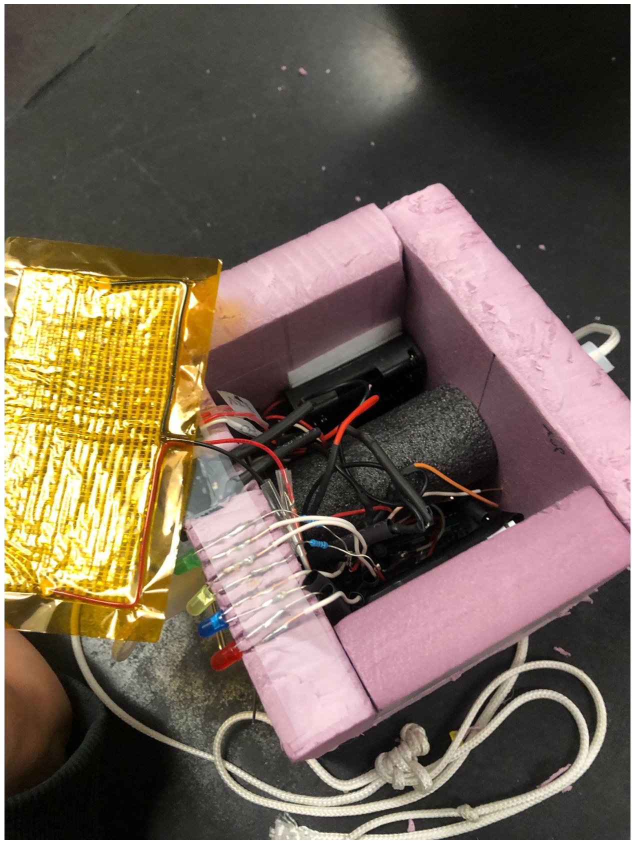

Figure 11 (Inside of Payload)

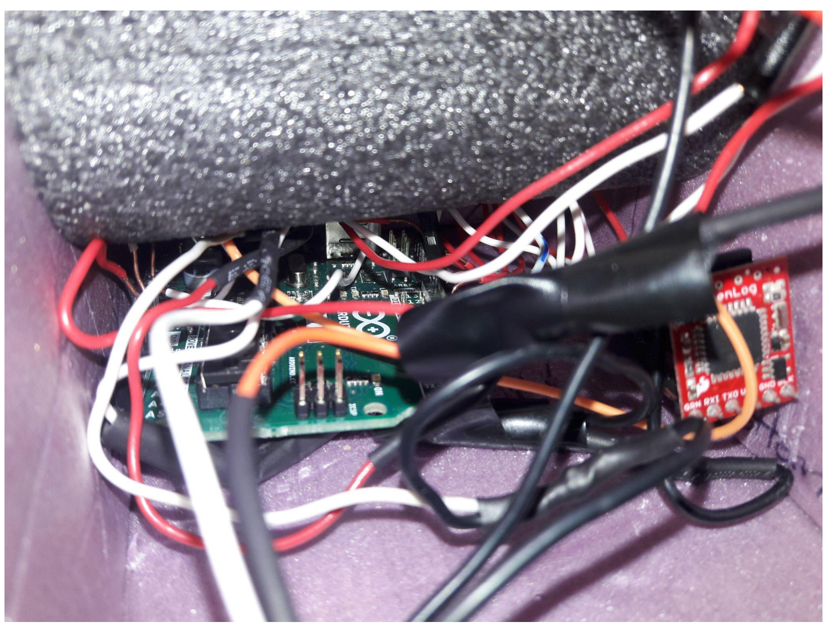

Figure 12 (Crammed Wires)

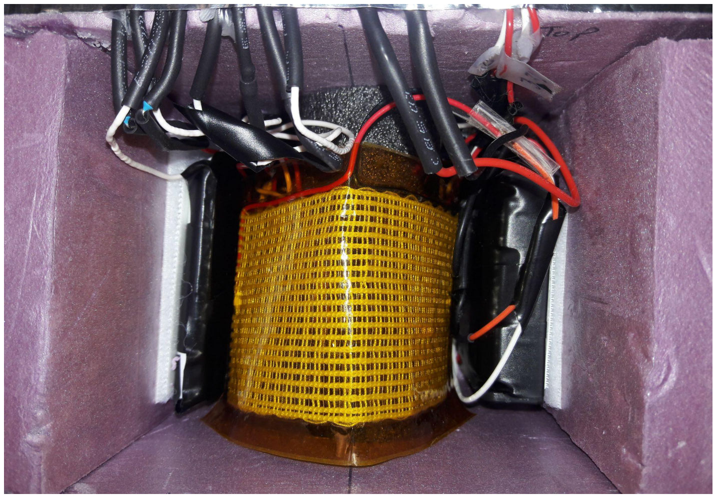

Figure 13 (Top View of Electronics)

Figure 14 (Final Payload)

| Part | Quantity | Cost ($) | Weight (g) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Insulated Foam Core/Tape | | Free | 156.3 |
| Flight Tube | 17 cm | Free | 8.3 |
| Flight Tube Insulation | 8.6 cm | Free | 1.3 |
| Heating Pad | 1 | Free | 2.9 |
| Power Switch | 1 | Free | 4.3 |
| OpenLog SD Card Breakout | 1 | Free | 2.1 |
| Bosch BME280 Atmospheric Breakout | 1 | Free | 1 |
| Analog Devices TMP-36 Temperature Sensor | 1 | Free | .2 |
| Resistor | 5 | Free | (.2 * 5) = 1 |
| Wires | | Free | 3.1 |
| LEDs | 4 | Free | (.3 * 4) = 1.2 |
| AA Batteries | 4 | 10.99 | (15 * 4) = 60 |
| Washers | 2 | Free | (16.5 * 2) = 33 |
| Paper Clips | 2 | Free | (.95 * 2) = 1.9 |
| MicroSD Card | 1 | Free | .3 |
| Stickers | 2 | 4.99 | (.9 + 1.8) = 2.7 |
| Geiger Counter | 1 | 69.95 | 7 |
| Arduino Uno Rev 3 | 1 | Free | 25 |
| 5 mL Eppendorf Tubes & Solutions | 6 | 96.74 | (10.7 * 6) = 64.2 |
| Mosfet Transistor | 1 | Free | 2.5 |
| AA Battery Holder | 1 | Free | 16.4 |

| | | | |
|---|---|---|---|
| Velcro | | Free | 7.4 |
| Tube Holder | 1 | Free | 121.9 |
| | | **Total** | **Total** |
| | | $182.67 | 524 g |

Figure 15 (Weight and Cost Rundown)


Figure 16 (Whip Test)

Figure 17 (Stair Pitch Test)


Figure 18 (Drop Test)

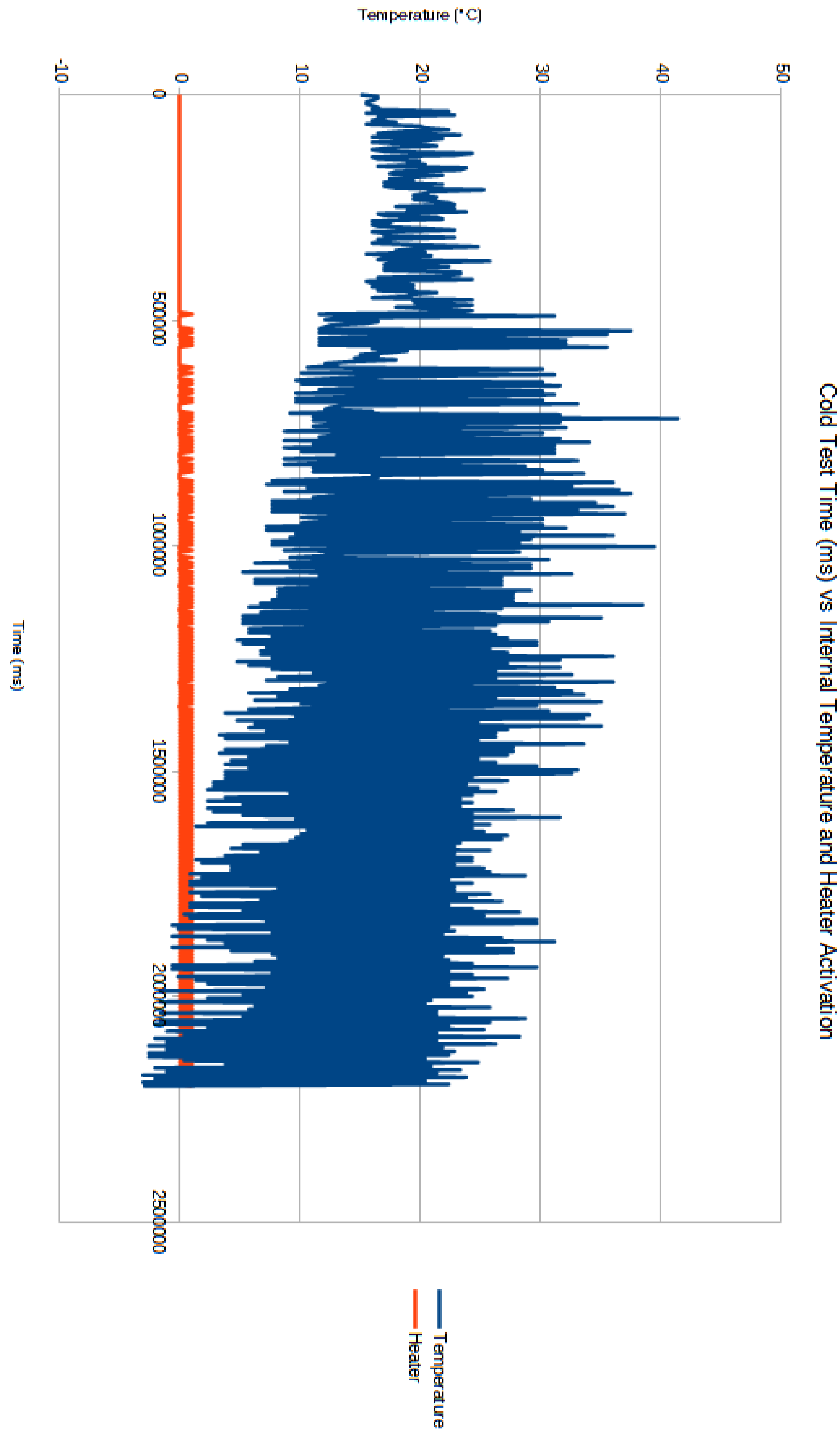


Figure 19 (Cold Test-Temperature)

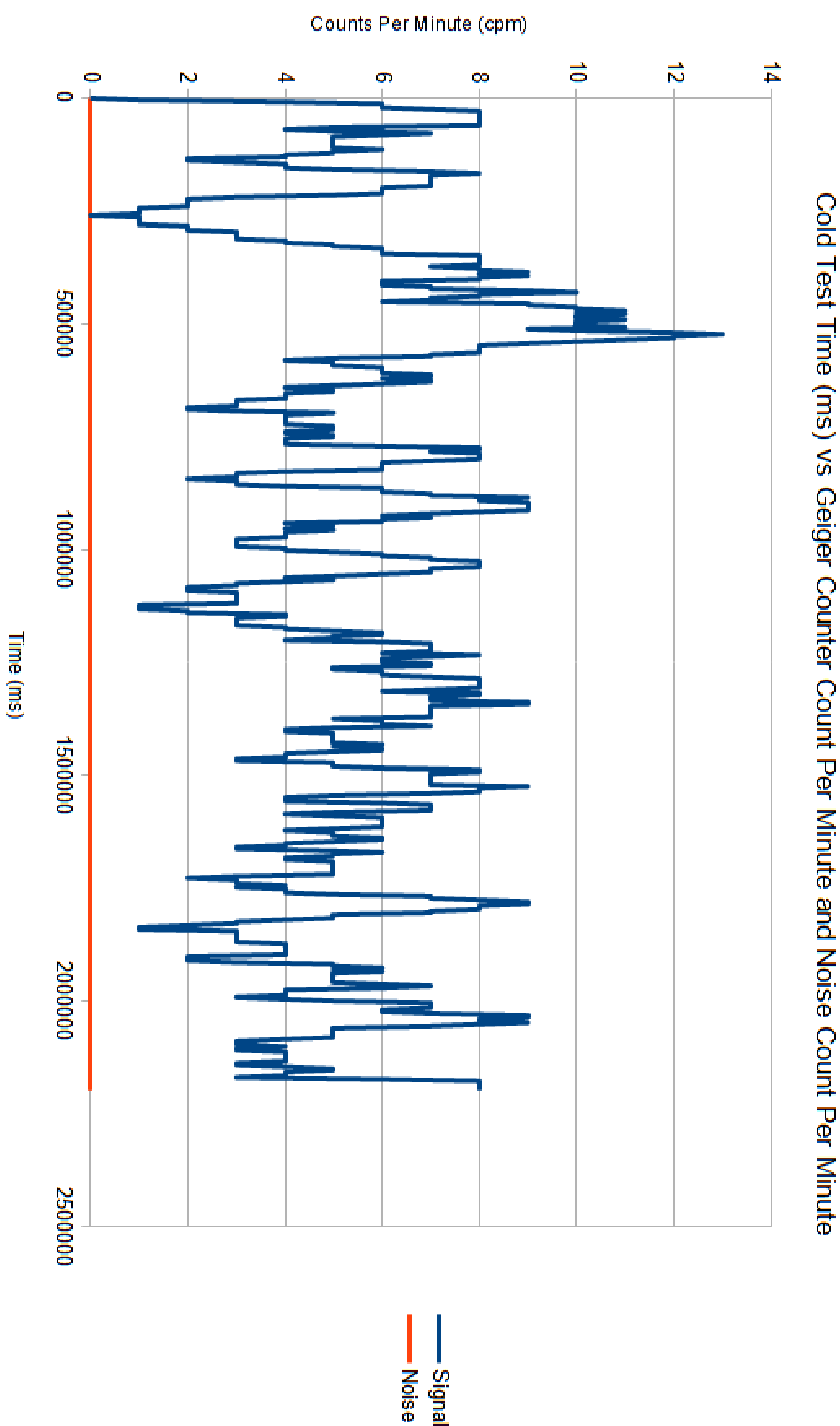


Figure 20 (Geiger Counter Cold Test)

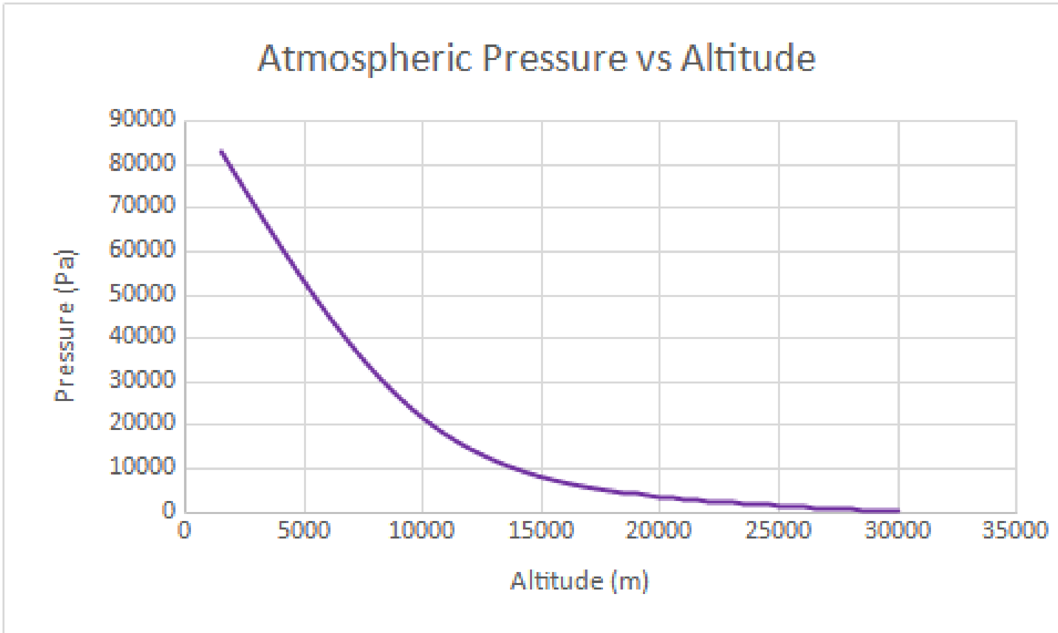


Figure 21 (Predicted Atmospheric Pressure)

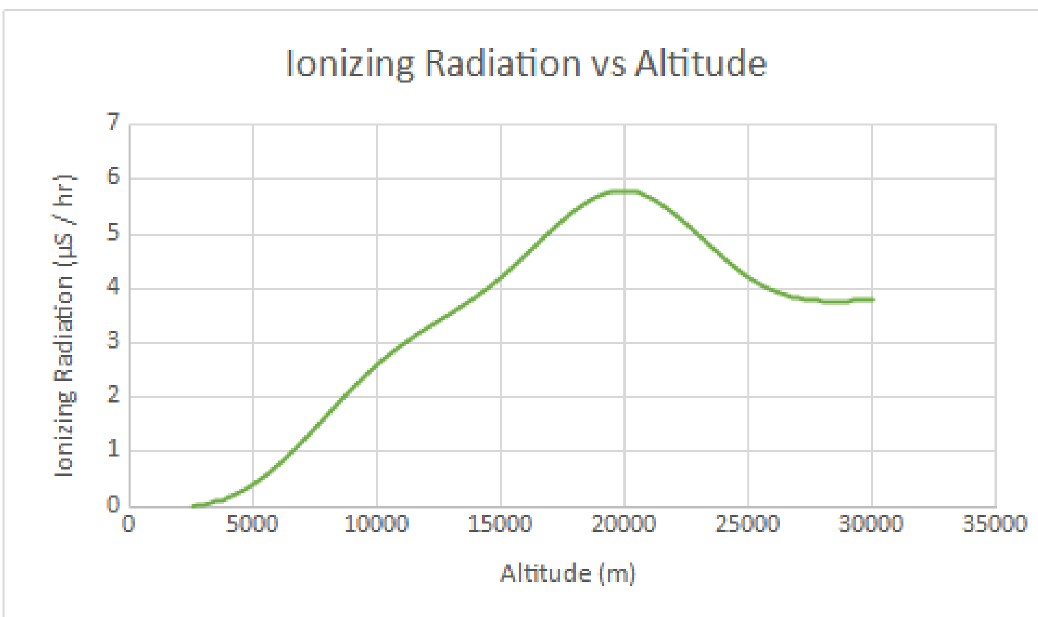


Figure 22 (Predicted Radiation vs Altitude) (Source: Space Weather Archive)

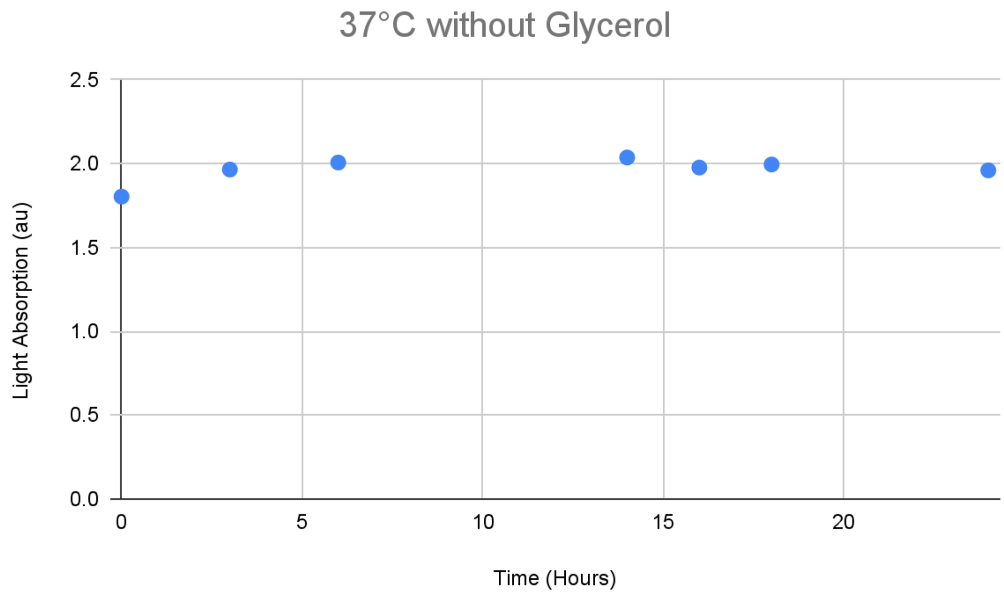


Figure 23 (Yeast Test Results)

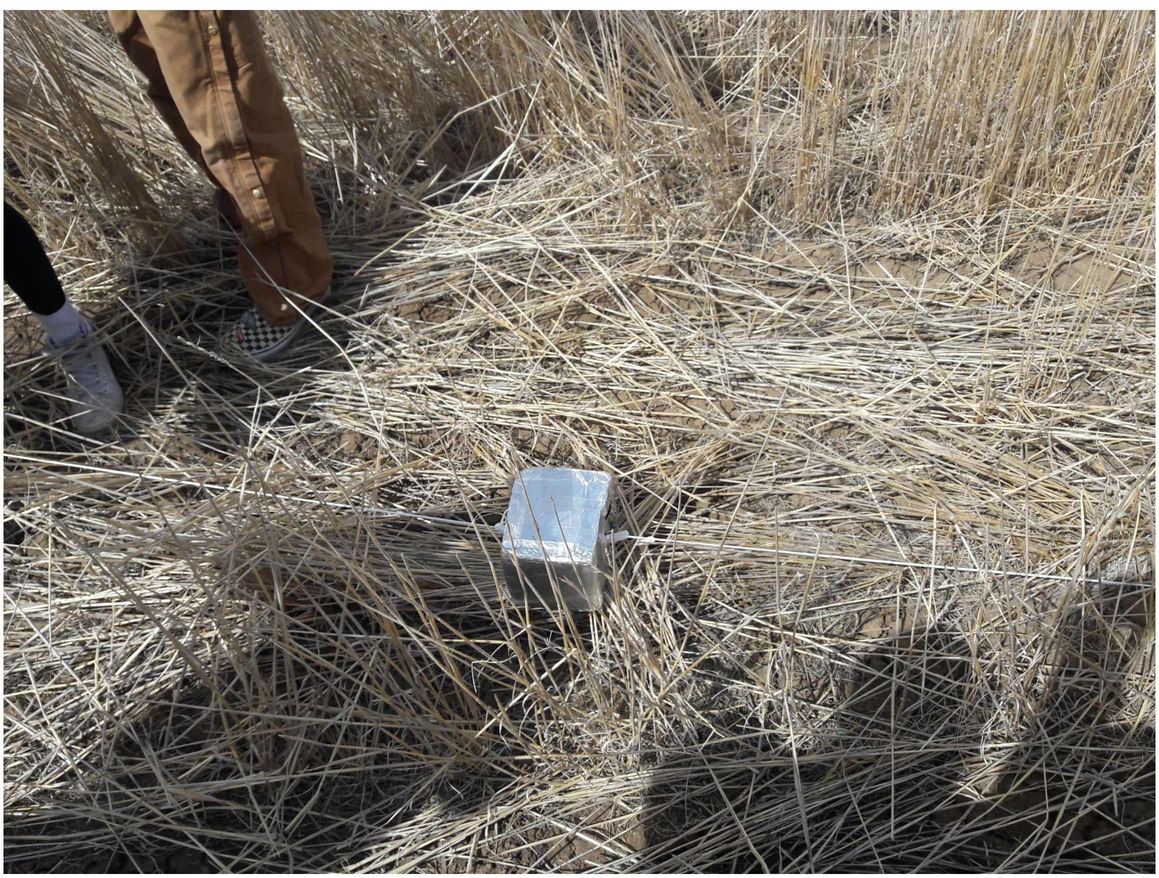

Figure 24 (Payload Retrieval)

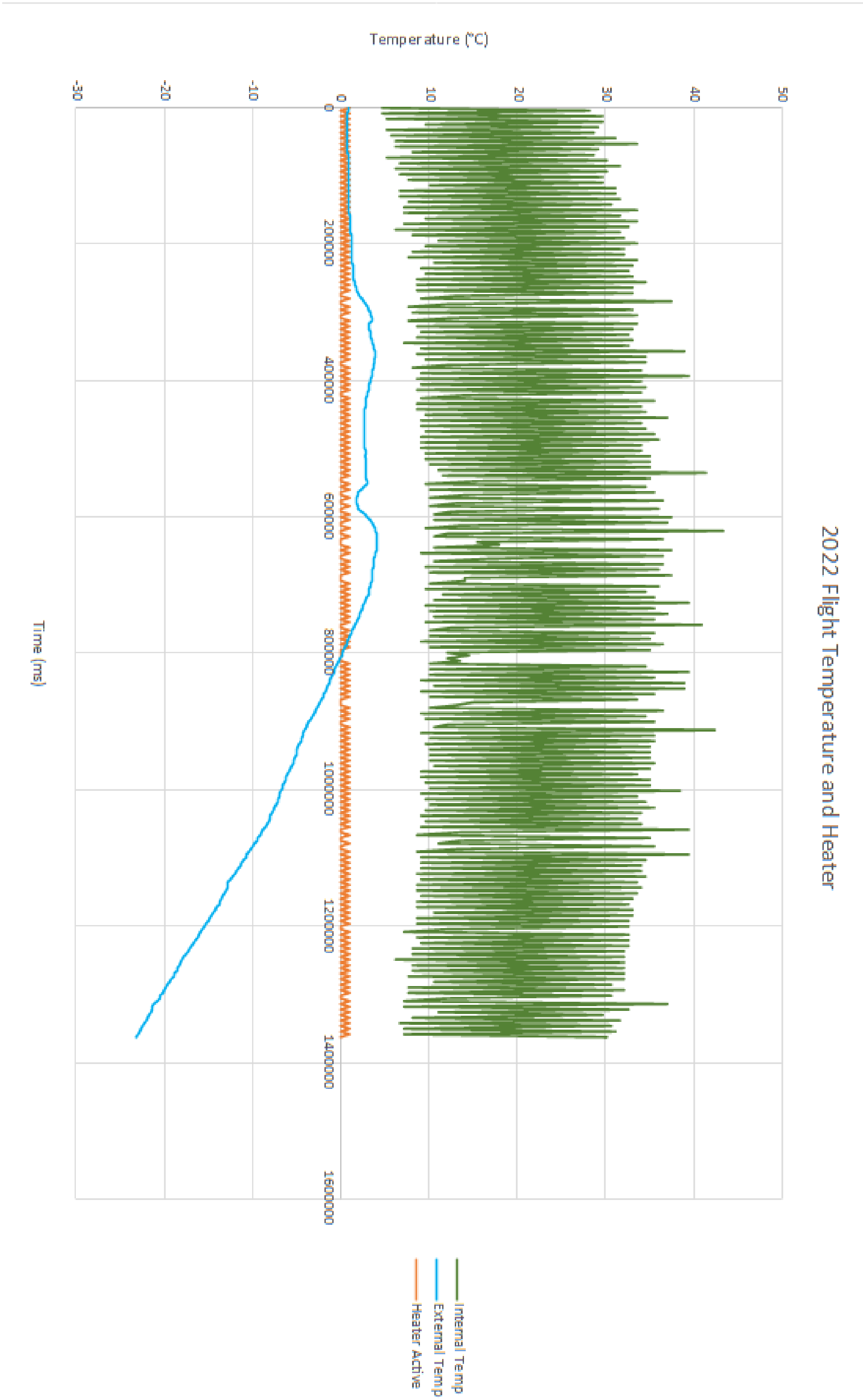


Figure 25 (Flight Internal and External Temperature Results)

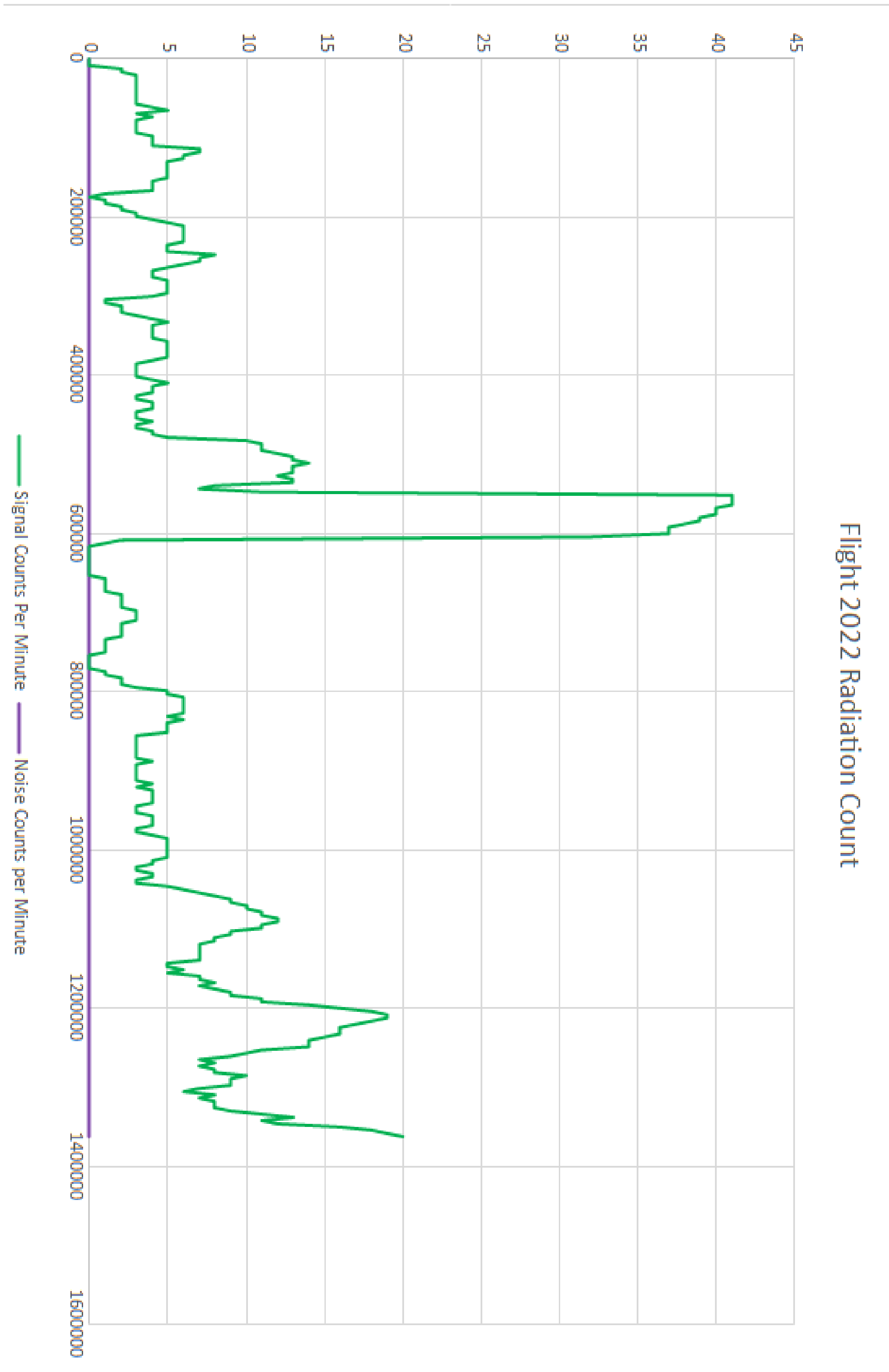


Figure 26 (Flight Geiger Counter Results)

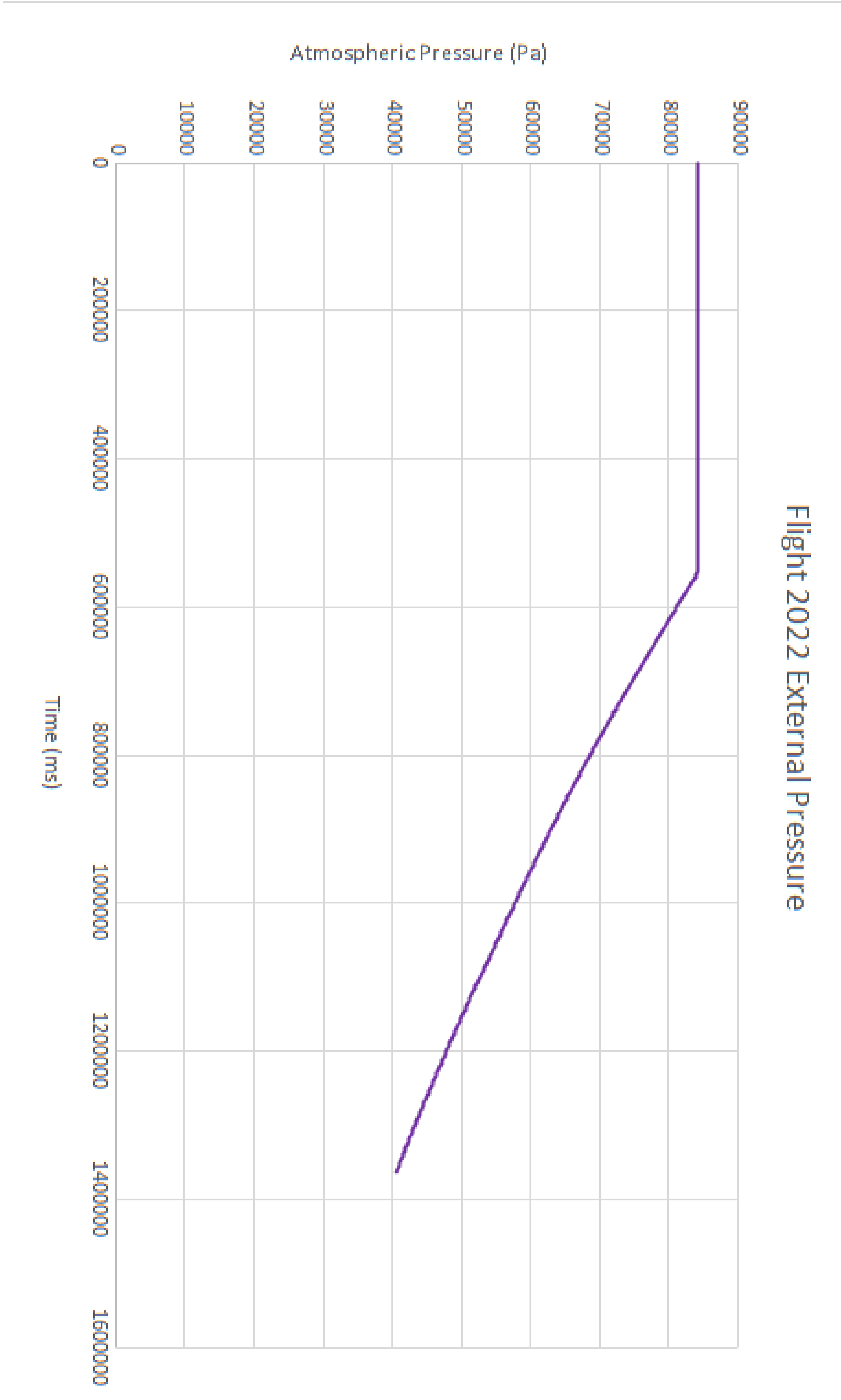


Figure 27 (Flight Atmospheric Pressure)