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HERON: Demonstrating a Novel Biological Platform for Small Satellite Missions

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ABSTRACT

Long-duration deep space missions pose a significant health risk for both humans and their resident microorganisms. The GeneSat, PharmaSat and O/OREOS missions have previously explored biological questions regarding the effects of spaceflight on *S. cerevisiase*, *B.subtilis*, and *E. coli.* However, there currently exists both a knowledge and an accessibility gap in small satellite biological experiments. These payloads require precise instrumentation and complex platforms that are usually reserved for large research organizations. This makes it difficult for smaller organizations to perform biological research in low Earth orbit (LEO). To address these challenges, the University of Toronto Aerospace Team (UTAT) Space Systems Division is currently developing the HERON CubeSat. HERON houses a payload platform which measures the effects of the LEO environment on the gene expression and drug resistance of *Candida albicans*, a yeast commonly found in the human gut microbiome. Previous research has suggested that *C. albicans* might display increased pathogenicity and drug resistance in response to microgravity, which has important implications for long-duration human spaceflight. The yeast cells are housed in custom acrylic microfluidics chips containing 32 wells with channels for media and drug delivery. A measurement printed circuit board (PCB) contains custom optics capable of measuring minute changes in cell fluorescence. The entire payload stack is then housed in a temperature- and humidity-controlled 2U pressure vessel. Space Systems as a whole is an undergraduate student-led and student-funded design team, dedicated to the development of small satellite missions with a focus on education and undergraduate learning. HERON is scheduled to launch Q1 2022 into a Sun-synchronous orbit via a SpaceX Falcon 9 rocket at an altitude of approximately 550 km. Our platform is open-source and can serve as a low-cost template for future biological CubeSat missions. This paper serves as a technical and scientific description of the platform, along with the lessons learned during the payload design, assembly, and validation processes.

Introduction to the Team

The University of Toronto Aerospace Team (UTAT) Space Systems Division is an interdisciplinary undergraduate student design team that develops and builds spacecraft in the CubeSat size range. UTAT provides an opportunity for students to develop engineering design and leadership skills while making important contributions to the wider scientific and aerospace communities.

UTAT is a fully student-run organization that is funded through a levy on the undergraduate student population. The levy was first established at the University of Toronto in 2017.[1] The team welcomes students from all faculties at the University of Toronto, but primarily caters to undergraduate engineering students. As part of UTAT's commitment to the wider aerospace community and open data, Space Systems aims to make all of its designs available for other student and professional teams to reuse. HERON will be UTAT's first spacecraft to be launched, but is only the start of the world's first fully student-funded satellite program.

The Space Systems team was broken down into nine subsystems for the development of HERON, most of which follow traditional spacecraft subsystems.[2] The subsystems were grouped into three areas of specialization, as follows:

- Mechanical group: Structures, Thermal, and Attitude Determination and Control Systems (ADCS)
- Bus group: Electronics and Power, Communications, and Software
- Payload group: Biology, Microfluidics, and Instrumentation

Each subsystem had an appointed lead who would recruit members as necessary. Members were expected to regularly contribute to a single subsystem, where they would quickly be brought up and develop a specialized skill set. Member turnover was a constant issue, but the HERON team adopted several systems engineering practices to mitigate the issue, which has been covered at length in our other works.[3]

Scientific Background, Hypotheses, and Experimental Aims

The space environment poses several unique challenges to humans and their microbiome including distance, confinement, hostile/closed environments, radiation, and microgravity. These biological challenges have recently received increased attention from the scientific community. In 2020, a group of over 200 investigators from four of the largest space agencies (NASA, JAXA, ESA, and Roscosmos) published a seminal Cell Press collection of 29 papers.[4] In this international collaborative effort, the group established some of the key biological alterations that occur in spaceflight, including mitochondrial dysregulation, DNA damage, oxidative stress, telomere length dynamics, and microbiome shifts.[5,6] Additionally, in the effort to find solutions and mitigate possible microbiome-associated health risks, NASA has utilized the CubeSat platform to study the effects of spaceflight on specific microorganisms. The larger implication of these biological alterations is quickened health deterioration during long-term human space missions. Table 1 in the appendix summarizes the GeneSat,[7] PharmaSat,[8] O/OREOS,[9] SporeSat,[10] Biosentinel,[11] and EcAMSat[12] mission specifications and their major scientific findings to-date.

The role of microbiome shifts/pathogenicity in spaceflight biology is still poorly understood, with multiple groups exploring strategies to tackle this health risk.[13,14,15,16] The microorganism that our team has chosen to study onboard HERON is *Candida albicans*, a yeast member of the human gut microbiota. Previously, Altenburg and colleagues have shown, using simulated microgravity, that *C. albicans* becomes more filamentous in its growth and displays abnormal budding behavior.[17] Confirming and further probing the molecular genetic component of this phenomena, Crabbe and colleagues used a specialized fluid processing apparatus onboard a NASA Space Shuttle to illustrate that *C. albicans* cells experience enhanced aggregation and random budding events.[18] Our team examined their large microarray dataset and chose some promising gene candidates to further probe on HERON's mission, including heat shock protein 90 (HSP90), hyphal wall protein (HWP1), benomyl/methotrexate resistance protein (MDR1), and gpi-anchored aspartic protease (SAP99). Furthermore, it has been extensively documented that humans are likely to experience immuno-compromisation and -suppression, particularly in long-duration, exploration-class spaceflight.[19,20,21,22] Dysregulation of both the innate and adaptive immune system, increased cytokine production, and cell-type specific alterations are just some of the observed changes.[23,24,25] These phenomena, combined with the potentially increased danger from microbiome shifts and infections, contribute to overall increased health risks in human space missions.

We hypothesize that *C. albicans* will display increased genetic pathogenicity by increased expres-

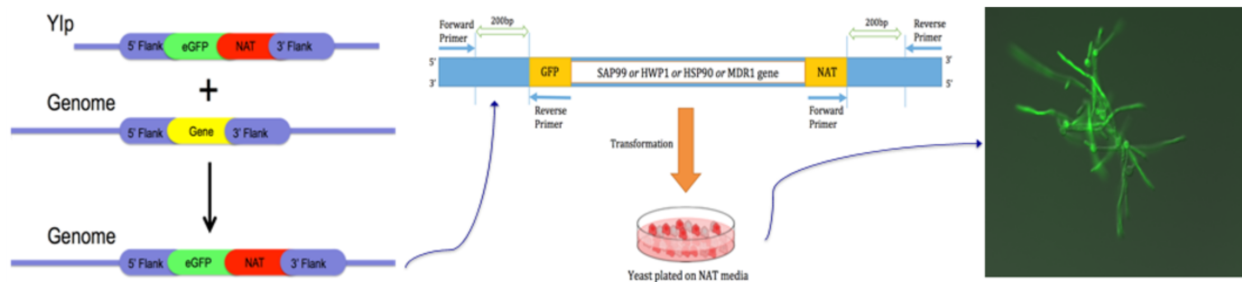

Figure 1: Genetic Engineering of *C. albicans*.

sion of HSP90, HWP1, MDR1 and SAP99 under spaceflight conditions. Experimentally, we have genetically engineered yeast strains to constitutively express green fluorescent protein (GFP) tagged to our genes of interest. These constructs are knocked into our strains' nuclear genomes by site-directed mutagenesis. An illustration of this process is given in Figure 1 for reference. Therefore, our first experiment will compare real-time gene expression patterns to Earth-based controls, using the same instrumentation platform, standardized to on-chip reference controls.

Next, we hypothesize that *C. albicans* will experience increased drug resistance to fluconazole in spaceflight conditions. Therefore, we will utilize a minimum inhibitory concentration (MIC) assay to determine any shifts in drug resistance during spaceflight versus an Earth-based control, using the same instrumentation platform. Furthermore, we would be able to correlate any shifts in fluconazole drug resistance with shifts in MDR1 (a drug resistance marker) gene expression.

Experimentally, yeast cells are kept in a "stasis" period prior to the start of the mission and are "activated" by the introduction of various mixtures of growth media and drug. The payload electronics record our parameters of interest for a period of 48 hours. UTAT has presented the microbiology experiment, systems engineering challenges, and overall satellite design at five scientific/engineering conferences to-date.[26,27,28,29,3] We received valuable feedback from experts in the field that has facilitated the iterative improvement of HERON over multiple design cycles.

Spacecraft Design

The purpose of this section is to detail the design of HERON. Particular attention is paid to the payload stack and its associated components, which form the primary contribution of this work. Descriptions of the mechanical, thermal, software, and electrical designs are also included. HERON is a 3U CubeSat, a form factor defined by the Cal Poly CubeSat Design Specification (CDS) as a satellite measuring 34 cm × 10 cm × 10 cm and with a maximum 4 kg mass.[30] Figure 2 shows a photo of the completed HERON spacecraft, and Figure 3 shows a cutaway view from computer-aided design (CAD) software illustrating the placement of key satellite components. These components will be described in subsequent sections.

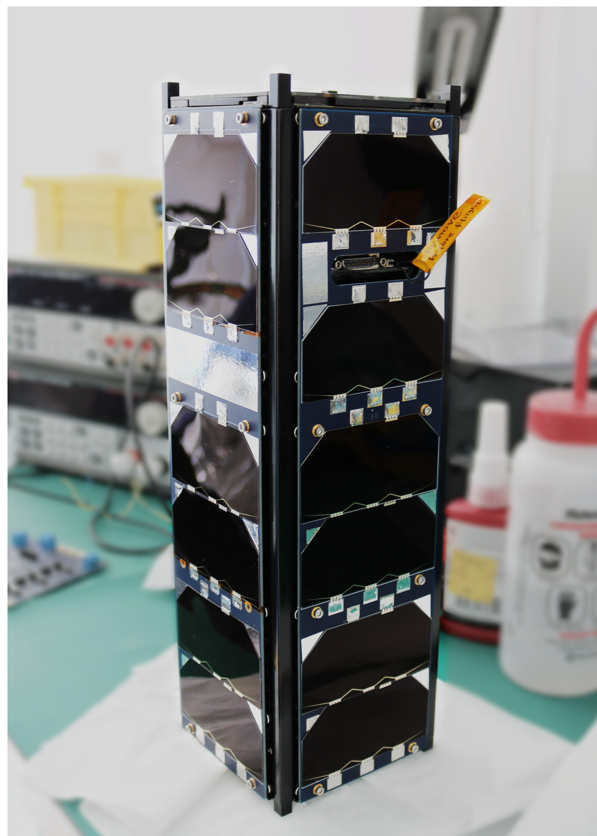

Figure 2: Completed HERON CubeSat

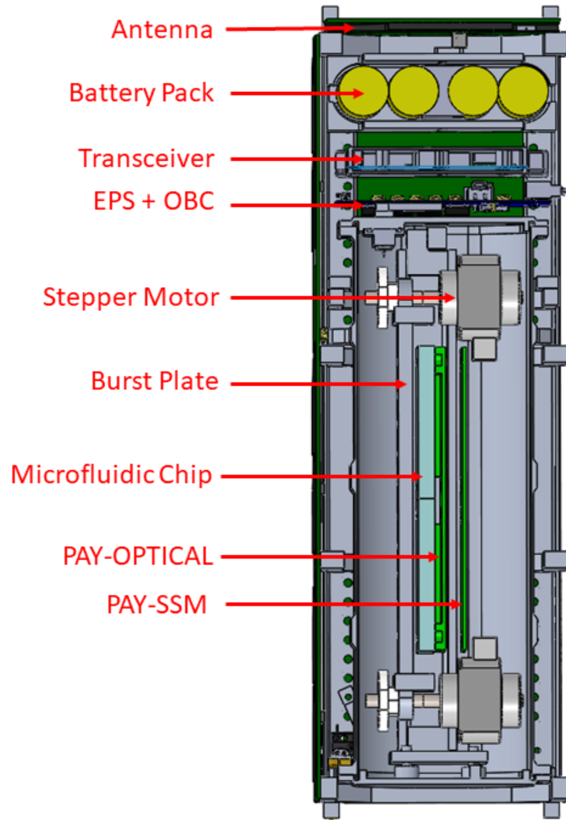

Figure 3: A cutaway rendering of HERON, illustrating key spacecraft electronics and payload components

Payload Stack

Design Overview

This section provides an overview of the payload stack, the primary biological platform around which HERON is built. The payload stack is designed to enable high-sensitivity, high-dynamic-range optical measurements of *C. albicans*, although it could also be applied to similar studies on other organisms. The payload stack is designed to take optical density measurements for population counting and fluorescence intensity measurements which quantify GFP expression. The latter allows us to track our four GFP-tagged genes of interest, each present in a uniquely engineered *C. albicans* strain. Together, these two measurements enable HERON's primary mission.

A diagram of the payload stack is given in Figure 5. The three key components of the payload stack are the microfluidics (MF) chip, optical sensor PCB (PAY-OPT), and optical density PCB (PAY-LED). The microfluidics chip forms the core of the stack and houses 32 oval wells which contain the different strains of *C. albicans*. Each microfluidic well is either used for the Minimum Inhibitory Concentration (MIC) assay or for measuring gene expression, although the optical channel design is identical between the two cases. Not shown in Figure 5 are blister packs which supply the wells with growth media or growth media and fluconazole (an antifungal agent) upon actuation.

Microfluidics

Design Overview

The microfluidics system houses all onboard biological materials for the lifetime of the satellite and facilitates the fluid actuation required for the experiment. There are four main design components of the microfluidics system: (1) blister packs, (2) microfluidics chips, (3) the gas-permeable membrane, and (4) the fluid actuation platform.

There are a total of fourteen blister packs, each containing nutrients for biological cell growth to be administered when the satellite is in orbit and the experiment begins. The nutrients consist of a mixture of sterile double-distilled water, glucose (dextrose), yeast extract, and peptone. Different predetermined concentrations of fluconazole are also present in the blister packs for the MIC assay. Each blister pack contains 120 uL of nutrient medium, the amount needed to supply nutrients to two *C. albicans* wells. All blister packs are mounted on the top side of the microfluidics chips at designated locations in the layout, as shown in Figure 4.

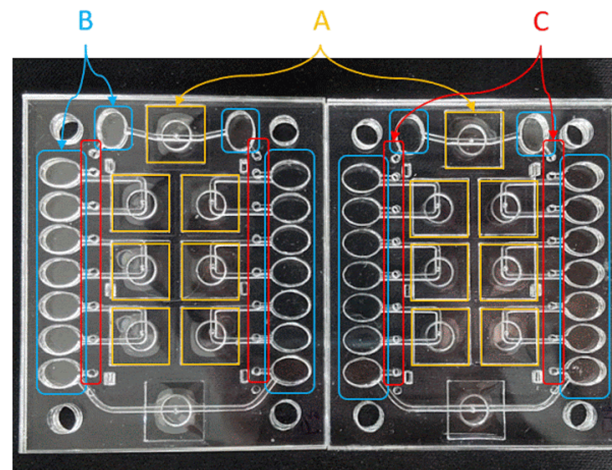

Figure 4: Top view of the microfluidics chips showing the blister pack mounting points (A), the oval wells (B), and the outlet holes (C).

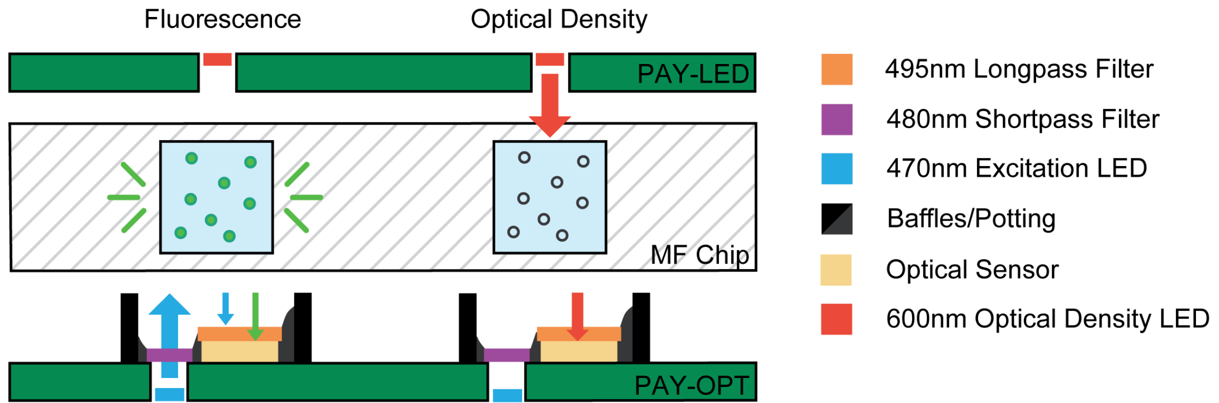

Figure 5: An illustration of the HERON optical stack. The microfluidics chip in the center houses *C. albicans* in several wells. The LED and sensor PCBs to the top and bottom form the optical instrumentation platform for taking optical density and fluorescence intensity measurements. A series of optical filters, baffling, and potting compound can also be seen which provides optical isolation to the platform.

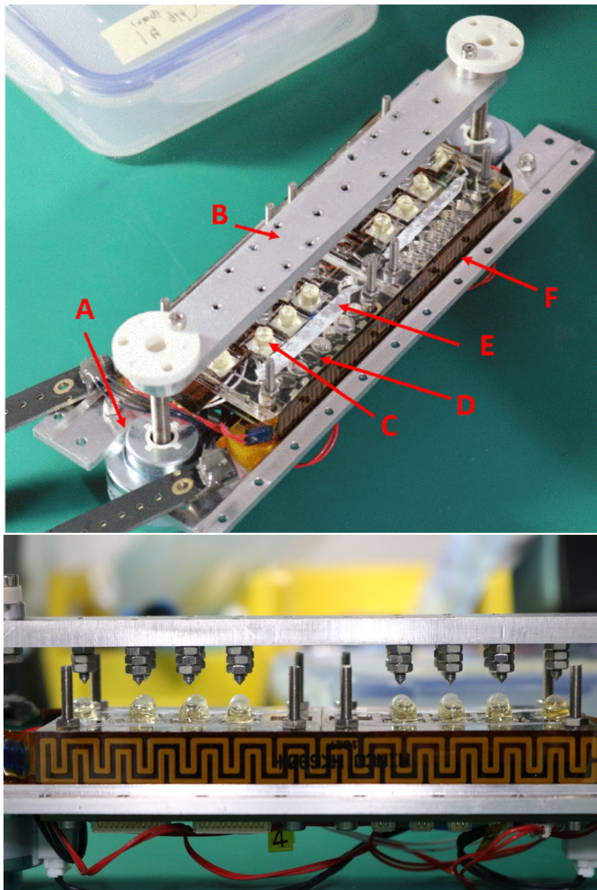

Figure 6: Perspective view (top) and side view (bottom) of the payload stack. A: Stepper Motors. B: Burst Plate. C: Blister Pack. D: Well. E: Gas-permeable membrane. F: Heaters.

There are a total of two microfluidics chips, identical in design. The microfluidics chips house *C. albicans* in solution in oval wells as shown in Figure 4. The microfluidics chips contain a system of fluid channels that connect the blister pack mounting points to the oval wells. Each blister pack mounting point is connected to two oval wells. There are further channels that connect each oval well to a corresponding outlet hole, shown in Figure 4. The outlet holes facilitate passive gas exchange at all times, and are covered with a gas permeable membrane. The material used for the membrane is 0.20 micron pore size PTFE filter paper which allows gas diffusion and blocks water flow. Thus, the membrane contains the well media but allows passive gas exchange for respiration of *C. albicans*. It also allows for air volume displacement when the fluid actuation platform is engaged.

The fluid actuation platform comprises a burst plate connected to two linear stepper motors, as shown in Figure 6. In the pre-experiment position, the burst plate is held at a distance 5–6 mm from the top of the microfluidics chips such that there is no contact between the burst plate and the blister packs. To start the experiment, the stepper motors actuate the burst plate towards the top side of the microfluidics chips. The contact between the burst plate and the blister packs applies a load which causes the bottom film of the blister packs to rupture. This releases the encapsulated media into the channels connected to the oval wells. Continued actuation forces flow from the blister packs and into

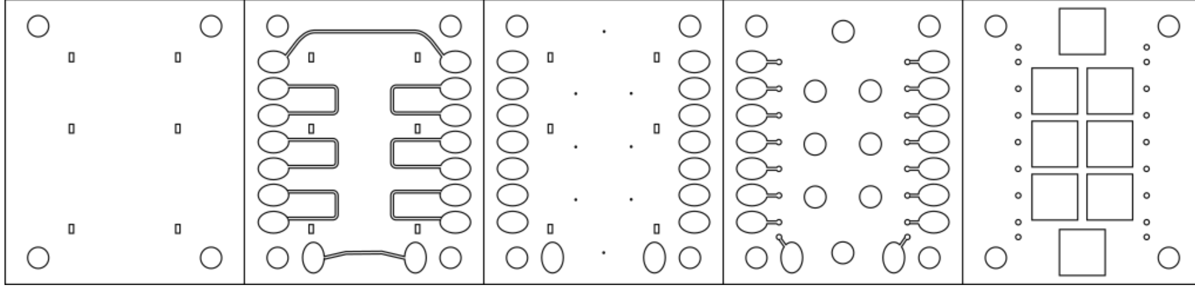

Figure 7: The five layers of the microfluidics chip design from CAD software. The layers are manufactured from laser cut acrylic.

the wells. All design components of the complete microfluidics system are shown in their relative positions in Figure 6.

Component Manufacturing

All components of the microfluidics system are custom manufactured at low cost. The blister packs consist of two sub-components: (1) the thermoplastic domed shell and (2) the aluminum film. To create the thermoplastic domed shell, clear thermoplastic is heated using a heat gun, stretched to a thickness of 0.7 ± 0.3 mm, and formed using a two-part mold. The base of the thermoplastic domed shell and the aluminum film are bonded using ultraviolet-cured (UV-cured) adhesive. The blister packs are bonded to the top side of the microfluidics chips also using UV-cured adhesive.

The microfluidics chips are manufactured using a layered process. Five design layers, as shown in Figure 7, are laser cut onto 1/16" clear acrylic. The acrylic layers are stacked and bonded using a lamination procedure (max temperature: 102°C) carried out by an EVG 520 Hot Embosser. The polytetrafluoroethylene (PTFE) filter paper used for the gas-permeable membrane is bonded to the top side of the microfluidics chips using UV-cured adhesive. The fluid actuation platform is manufactured using standard machining tools and processes.

Optical Instrumentation

Design Overview

The optical instrumentation platform contains all sensors and optical elements needed to take optical density and fluorescence measurements of *C. albicans* in orbit. The platform consists of two PCB assemblies, PAY-LED and PAY-OPT.

PAY-LED is mounted above the microfluidics chips and contains one 600 nm orange light-emitting diode (LED) (P/N: LO P47K-K2M1-24) per well. The LED is mounted such that it shines through the well and into the TLS25911 optical sensor mounted on PAY-OPT. The population counts in each well are inversely proportional to the amount of light making it through the well and into the sensor, as higher population counts will scatter more light away from the optical path.[31]

PAY-OPT is mounted below the microfluidics chips and contains one 470 nm LED (P/N: LB P4SG-S2U1-35-1) per well. The 470 nm LED has a spectral bandwidth of ∼25 nm and was found sufficient to excite the GFP-tagged cells at their excitation wavelength of 475 nm. The optical path was designed such that the excitation illumination would travel up through the wells and away from the TLS25911 optical sensor. Some portion of the 509 nm GFP emission—which is assumed to scatter evenly in all directions—would then travel back towards the sensor. This architecture was inspired by the GeneSat mission[32,7,33] and selected to minimize the amount of excitation light travelling back to the sensor.

Interference from the excitation LED and other light sources was a continuous challenge during design and manufacturing. The final design includes a 480 nm shortpass filter (Omega Optical RPE480SP) to filter the excitation LED. A 495 nm longpass filter (ThorLabs FGL495) was used to further block the excitation and only pass the GFP fluorescence and optical density illumination. Black optical baffles were also used in the final design and are illustrated in Figure 9. The baffles serve two purposes. First, they help eliminate optical pickup from adjacent wells by narrowing the field of view (FOV) of the optical sensor. Second, they serve as a guide which allows the sensor and filters to be potted in

an opaque black potting compound (Loctite Stycast 2850FT BK). Together, the combination of optical filters and potting compound effectively rejects stray interference and ensures isolation of the optical path.

Test results from the optical instrumentation platform were successful. The sensitivity measurements in Figure 8 illustrate the platform's ability to pick up HSP90 strain concentrations down to a dilution factor of a hundredth, which meets the mission requirements. It should be noted that the sensor has a nonlinear response to concentration. Higher well concentrations result in additional optical scattering which shades the cells further back in the well, and reduces the amount of GFP emission making it back to the sensor.

In general, it is difficult to compare absolute optical density and fluorescence measurements between two platforms, even when using commercial equipment.[31] The HERON mission design accounts for this challenge by focusing on the relative differences between the cultures grown in space and the cultures grown in an identical ground copy on Earth.

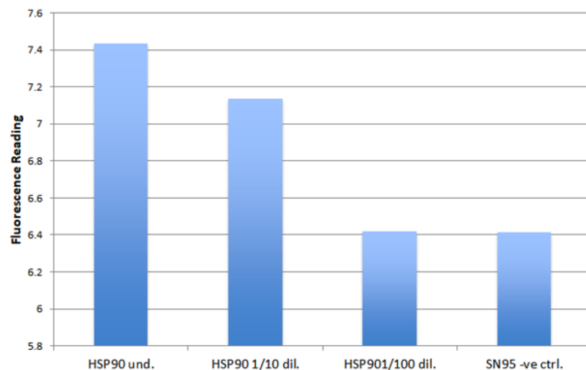

Figure 8: Fluorescence sensitivity testing of the optical instrumentation platform. GFP-CA-HSP90 light detection at different concentrations. The instrumentation platform is able to adequately detect well concentrations down to 1/100 dilution over 100 iterations. Concentrations below 1/100 dilution are indistinguishable from the negative control.

Component Manufacturing

One advantage of this optical platform design is that good tolerances can be achieved by leveraging existing low-cost PCB supply chains. Space Systems opted to have 4-layer boards manufactured with PCBWay using a matte black soldermask to help reduce reflections. The total cost of the entire optical platform came to approximately CA$50 in blank PCBs, CA$200 in electronic components from DigiKey, and CA$400 in optical filters.

The team opted to assemble the PCBs in-house, utilizing a DIY reflow oven to solder the boards. Optical filters were cut by hand from the stock material using a glass cutter, and assembled into place using the baffles and potting compound. Particular attention was paid to ensuring that no potting compound interfered with the optical path of the sensor. The photograph in Figure 9 shows some potting compound on top of the filters, but this was polished off after fully curing.

Several improvements could be made to the platform, particularly regarding manufacturing. Hand-cutting the optical filters was a cost-saving measure, but resulted in large manufacturing tolerances. Once the tolerances are brought down, design of a housing which mounts on PAY-OPT and provides a better alignment of the optical filters would be a significant improvement. Such a part could be easily 3D printed, as the payload bay is maintained at 1 atm in orbit and outgassing is therefore not an issue. As it is, mounting and potting of the misshapen glass pieces was a time-consuming and error-prone process which required careful attention to detail.

Payload Subsystem Main Board

The payload subsystem main (PAY-SSM) PCB is responsible for thermal control of the microfluidics chips and motor actuation to start the experiment. PAY-SSM also collects housekeeping data from pressure, humidity, and temperature sensors inside the payload and sends it to the Onboard Computer (OBC) for later transmission to the ground.

PAY-SSM constantly monitors the microfluidics chip temperatures to keep the cell cultures around a desired set point. Outside the experiment, *C. albicans* is kept in stasis around 14°C. The temperature is then brought up to 37°C to start the experiment. The orbit selection process determined that a minimum of 2.5 W of heating is required to maintain the payload at this setpoint. This is accomplished through the use of five flexible polyimide heaters running off a 6 V rail, and twelve thermistors embedded in the microfluidics chips. Figure 6 shows the placement of the heaters around the chips, and Figure 7 shows the six small thermistor slots on the first, second, and third layers of the chip.

The control algorithm PAY-SSM uses is a simple bang-bang controller with a weighted thermistor grouping as the input to each heater controller. Different grouping schemes were tested to find the one that yielded the best temperature uniformity across

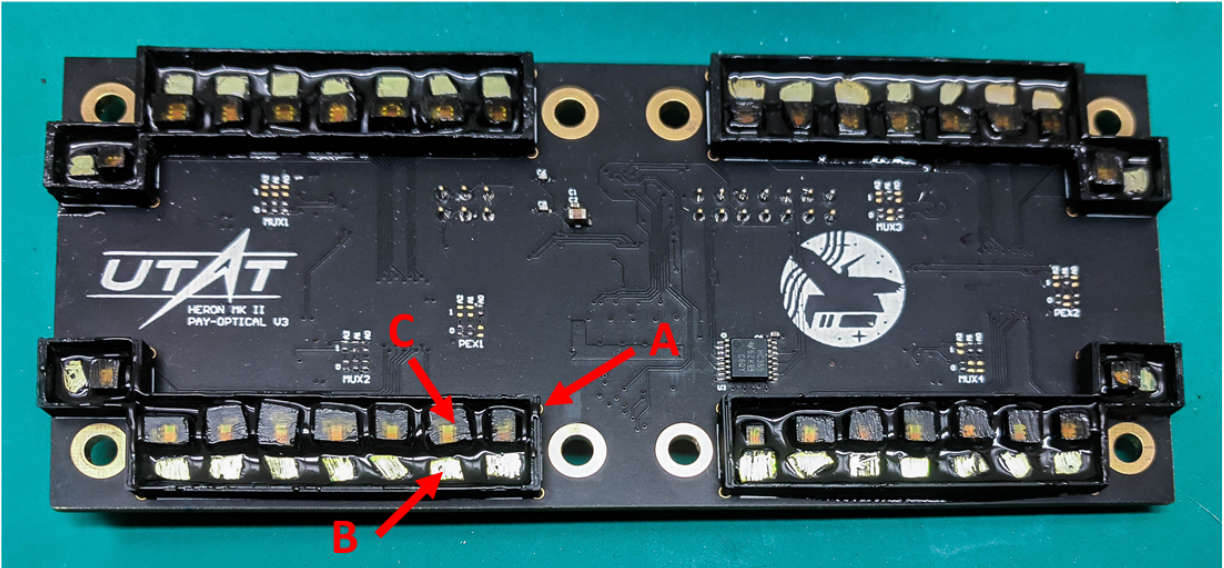

Figure 9: Top view of PAY-OPT showing the baffles (A) with potting compound, 480 nm shortpass filter (B), and 495 nm longpass filter (C). The TLS25911 optical sensor is mounted underneath the 495 nm filter, and the 470 nm excitation LED is reverse-mounted underneath the 480 nm filter.

the microfluidics chips. The control loop is run once for each heater every minute, as this was found to be sufficient given the system's slow response time requirement.

PAY-SSM is also responsible for actuating the two stepper motors which rupture the blister packs to begin the experiment. The motors are run off a dedicated 10 V regulator and chopper drive to maximize torque. The actuation plate is moved down one step at a time until contact is made with two limit switches. The switches are placed such that the blister packs are fully deployed at the end of travel.

Many designs were explored when coming up with the payload actuation mechanism. One intuitive and early concept was to use small pumps to release fluid into the wells from a larger reservoir. However, the MIC assay in particular would have required a complex fluid manifold design to accurately mix the correct concentrations of fluconazole into each well. The advantage of the blister pack design is that the fluid for every two wells can be customized, and stepper motors were found to be the simplest means of applying even pressure.

Other designs for actuation feedback were also explored, including optical or resistive-based linear slider mechanisms. However, given that the payload only needs to be actuated once, it was found that aligning the burst plate with the wall of the payload bay and actuating until the limit switches were hit was the simplest and most reliable method. This technique is used in some 3D printers, for example, to align the Z-axis.

Mechanical and Thermal Design

Several mechanical considerations went into the payload design. The entire payload is housed in a pressure vessel which maintains an internal pressure of 1 atm and humidity near 100%. A single axial seal with a viton O-ring is used on the top and bottom pressure vessel caps and is kept in compression through sixteen flange screws. Testing determined that this pressure vessel has a leak rate of 51.0 Pa per day in vacuum and with 1 atm internal pressure. This rate of pressure loss ensures the vessel will maintain sufficient pressure to carry out the experiment for several months while in orbit.

The thermal subsystem is concerned with temperature regulation of certain spacecraft components, namely the payload. This has implications on many aspects of HERON. It has driven the mission orbit selection in addition to significant mechanical design.

The tight thermal constraints imposed by the payload drive the selection of a Sun-synchronous orbit (SSO) with a local time at descending node (LTDN) between 9:30 and 11:30 and an altitude be-

tween 500 and 600 km. This corresponds to an orbital inclination of 97.4 to 97.8 degrees. The stable thermal and power profile of SSO is preferable to an International Space Station (ISS) orbit where large fluctuations in the eclipse fraction lead to extreme cold and hot epochs. The satellite orbit is selected to cold-bias the thermal payload, such that the steady-state temperature of the satellite does not exceed the maximum allowable payload temperature in a hot-case analysis. This removes the need for active cooling, but does require active heating. Thermal and power simulations show that the satellite will operate with a small negative power margin during the 48-hour experiment under the worst cold-case conditions. However, the battery capacity has been selected to ensure that the satellite has sufficient energy even in this worst case.

Onboard thermal control uses a combination of three methods: insulation blankets, surface coatings, and heat path control. Insulation blankets were wrapped around both the payload pressure vessel and the battery pack. Instead of conventional multi-layer insulation (MLI), Space Systems developed a composite aerogel blanket consisting of a layer of aerogel insulation encased in kapton sheets. This insulation method was chosen to take advantage of the low thermal conductivity of aerogel material, as these blankets are in direct contact with the payload vessel or battery. It was deemed to be easier to manufacture than conventional MLI for similar effectiveness, as it did not require careful spacing and stitching of multiple kapton layers. Moreover, conventional MLI has been shown to perform poorly on small surface areas characteristic of CubeSats.[34]

There are disadvantages to this form of insulation. Even thin aerogel leads to a bulky insulation blanket, which occupies significant volume. Furthermore, aerogel generates fine dust when handled. This dust poses a hazard for personnel and prevents adhesive tapes from adhering. This dust is also an outgassing concern, as air evacuating the insulation during ascent could carry out fine particles. This was addressed by sealing the aerogel with kapton and covering vents with a NIOSH N95 filter to capture particles. Vacuum chamber testing of the insulation verified this design.

Surface coatings were employed in many areas of HERON. To reduce radiative heat transfer, low-emittance tape was used to extensively cover the interior and exterior of the payload pressure vessel, the inward facing surfaces of the primary structure, and the battery support structure. Tapes with other absorptance and emittance values were used on the exterior of the spacecraft to optimize heat absorption and loss during an orbital period.

Consideration was given to structural joints that would permit conductive heat transfer between the payload pressure vessel and the spacecraft structure. The payload vessel has integrated mounting points for bolting to the primary spacecraft structure. To minimize conductive heat loss through these joints, polyether ether ketone (PEEK) plastic washers were used to eliminate all metal-to-metal contacts. PEEK plastic was chosen because it is readily available, rigid, and has outgassing properties suitable for spaceflight. Several layers of these washers were placed between the bolt head or nut and the structure.

Electronics Stack

The electronics or "bus" stack consists of the Electrical Power System (EPS) PCB, Onboard Computer (OBC) PCB, and RF transceiver. The bus stack is visible in Figure 3 above the payload bay. The transceiver is a commercial off-the-shelf (COTS) EnduroSat Ultra-High Frequency (UHF) Transceiver Type II module, mounted on top of EPS using a PC-104 connector. EPS also connects to the satellite antenna, a COTS EnduroSat UHF Antenna II quad turnstile antenna module.

The EPS PCB interfaces with four solar panels located on the long faces of the satellite, and a 1S4P battery pack of 2170 Li-ion cells. Each solar panel uses a 2S3P configuration of AzurSpace 3G30A solar cells. EPS regulates the battery voltage to produce 3.3 V and 5 V outputs which are distributed across the satellite, with the raw battery voltage being sent to PAY-SSM for downstream regulation. Figure 10 illustrates the power distribution architecture.

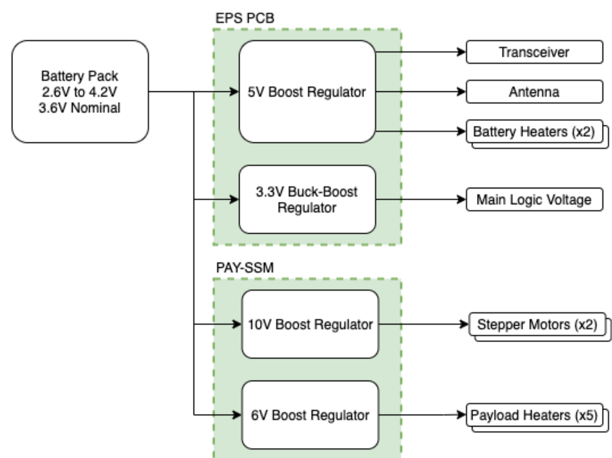

Figure 10: HERON Power Distribution from Battery Voltage to End-point Loads

The OBC microcontroller unit (MCU) communicates via the universal asynchronous receiver-transmitter (UART) protocol with the transceiver for on-orbit communication. It uses an inter-integrated circuit (I2C) interface to deploy the antenna 30 minutes after launch. The OBC contains 6 MB of external flash memory for aggregating and storing all satellite data to be later downlinked to the ground station. The board also contains a real-time clock (RTC) module for precise timestamping of the collected data.

A 25-position D-Sub Micro-D umbilical cable is used to interface with the satellite when fully assembled. A development and testing "Systems" (SYS) PCB connects to the umbilical for UART access and the ability to reprogram the OBC, EPS, and PAY MCUs.

Software

The software architecture defines OBC, EPS, and PAY as the three primary subsystems of the satellite, each with their own ATmega64M1 AVR MCU connected to a common controller area network (CAN) bus. All MCUs use a superloop rather than a real-time operating system (RTOS) for simplicity and ease of software development, which was important to quickly bring up new team members.

Given the nature of the biology experiment, the satellite needs to autonomously collect and store measurement data every 30 minutes during the 48-hour experiment. The period of data collection can be adjusted from the ground station. OBC coordinates data collection and requests all necessary data fields (measurements) from other subsystems. The OBC writes all data fields to flash memory with a timestamp from the RTC module.

We define several "block types", where each block type specifies a group of data fields to collect. Optical measurements have a separate block type from housekeeping measurements, which track the general state of the satellite, e.g. voltage, current, temperature, humidity, pressure, and reset events.

The ground station can request saved data whenever it is convenient. During a pass, the ground station can request to downlink data of a certain block type and "block number", which is a sequential index indicating the order of block collection. The ground station can poll OBC for the most up-to-date block number to determine which blocks to downlink. If a packet is lost in transmission, the ground station can request the same block again.

For commands that change satellite operation, e.g. heater setpoints or data collection periods, the OBC executes simple individual commands while the ground station must coordinate sending multiple commands. This architecture places most of the computational complexity on the ground station, which can use a modern single-board computer (SBC) with plenty of processing power, reducing possible satellite failure modes. We also anticipated that our on-orbit operational plans would change between the satellite development and satellite operation phases of the mission. If complex commands were implemented on OBC, it would be difficult to customize them later since HERON lacks on-orbit reprogramming capabilities.

For each command, the ground station sends one packet (a request) and the OBC sends two packets in return, an acknowledgement (ACK) and a response. OBC sends an ACK back to the ground station immediately after receiving a request, which is valuable for commands that take a long time to execute, such as motor actuation. If the ground station does not receive the ACK, it resends the request until it receives an ACK. A sequential command counter is included in all packets. The ground station can harmlessly retransmit the same request multiple times until an ACK is received, and OBC will only execute the command once. After a command has finished executing, OBC sends a response packet with the final result.

The OBC saves a command log to external flash memory, keeping track of all commands executed by the satellite and flagging those that were executed autonomously. The ground station may simply read from the command log to see if a command was executed.

This communication scheme is well-suited to the HERON mission. High data throughput is not a concern, and this scheme keeps the spacecraft architecture simple and robust to errors.

Open-Source Design Resources

Part of UTAT's core mission is to make aerospace more accessible to students and to contribute to the broader aerospace community. To foster a spirit of collaboration and open-source design, UTAT has made all major design materials available on utat.ca/heron. This includes GitHub repositories for both flight and testing code, GrabCAD files for the full satellite structure, and various design artefacts including test specifications, command architecture, and analyses. More photos of the satellite and various components can also be found on our website.

Challenges Encountered and Lessons Learned

The HERON mission was not without its challenges. The Space Systems team faced several technical and organizational challenges during development, many of which were driven by the limitations of being an undergraduate student team. This section outlines our design process, several of the associated challenges, and some lessons learned that future missions may be able to take advantage of.

To close out the HERON spacecraft for launch, a set sequence of tasks had to be carried out to account for the needs of both payload biology and launch provider requirements. This timeline is illustrated in Figure 11. The payload biology could be integrated into the spacecraft no earlier than L-5 months (five months prior to launch). This requirement was driven by stasis testing of the payload, which demonstrated that the cells remain viable for at least seven and a half months. A plot showing the viability up to four and a half months in different media is given in Figure 12, and drove our selection of ddH20 as the suspension media. Two and a half months of margin was then added to the final requirement to account for the launch date being a no-earlier-than (NET) date at the start of a two-month launch window. Note that no data was collected beyond seven and a half months, although literature suggests that the cells could stay alive for longer.[35,36]

In addition to the biological requirement, the launch provider required that the spacecraft pass proto-qualification vibration testing prior to launch and that the spacecraft be handed off at L-60 days. The fact that the HERON spacecraft is assembled around the biological payload meant that the vibration and handoff pipelines were blocked until *C. albicans* could be integrated. Altogether, this created the need to ready the biology and be able to complete assembly and vibration testing in three months. Successfully meeting this objective required balancing the competing demands from the launch provider, the sponsored vibration testing, the biological lab space, and the cleanroom assembly space against the challenges of the ongoing COVID-19 pandemic and the resulting fluctuating closure of the University of Toronto.

This complicated pipeline demonstrates the need for a mindset focused on integration early in the design process. Many dry runs of spacecraft assembly were conducted to build up a strong base of documentation to support a correct final assembly. Test plans and smaller system-level tests were completed to build up from component-level to system-level functionality. Some satellite-level tests were conducted prior to the biology integration as well, namely the thermal bakeout and thermal vacuum chamber testing. A lesson learned is to plan out and mock up wire harnesses and connectors in the integrated spacecraft as thoroughly as possible. Late-stage design changes were required in the PCBs responsible for electrical power and onboard computing to accommodate an interfering payload wire harness.

On-orbit communications and mission operations were also areas that should have been focused on earlier in the project. Unexpected regulatory concerns and late integration of the ground station into the overall satellite design plan made the final integration pipeline more uncertain. In the future, a clear operations plan should be established earlier in the spacecraft design process. This should explicitly prioritize regulatory requirements for on-orbit communications to avoid launch delays.

Regulatory concerns are not limited to communications. Hazardous materials such as the biological payload of HERON have also created their own set of challenges. The *C. albicans* yeast used in the payload has a biohazard safety level of two (BSL-2). Anyone transporting or handling this payload is required to receive specialized biosafety training. Both the biologicals (UN3373 Category B) and the lithium-ion batteries (UN3481) are UN-recognized dangerous goods that are subject to Canadian Transportation of Dangerous Goods Regulations, which stipulate adequate means of containment, marking, training, and accident reporting measures for any movement of dangerous goods within Canada and across the US-Canada land border. The delivery of the satellite across the US-Canada border was further complicated by travel restrictions implemented in response to the COVID-19 pandemic.

Overall, the selection of a biological payload imposed tough requirements on the team from an integration, manufacturing, and regulatory standpoint. Ultimately, the Space Systems team decided to integrate HERON roughly a year in advance of the launch. This was driven by the launch being pushed back and a loss of expertise with several key members graduating from the team. The stasis results from literature[35,36] and our own test results support this decision, although it introduces additional uncertainty and risk. To help reduce uncertainty, the ground copy is being stored under the same conditions as the flight model for later comparison. Our recommendation is that stasis testing and proper storage procedures are essential for future biological missions. The ability to keep a biological payload in

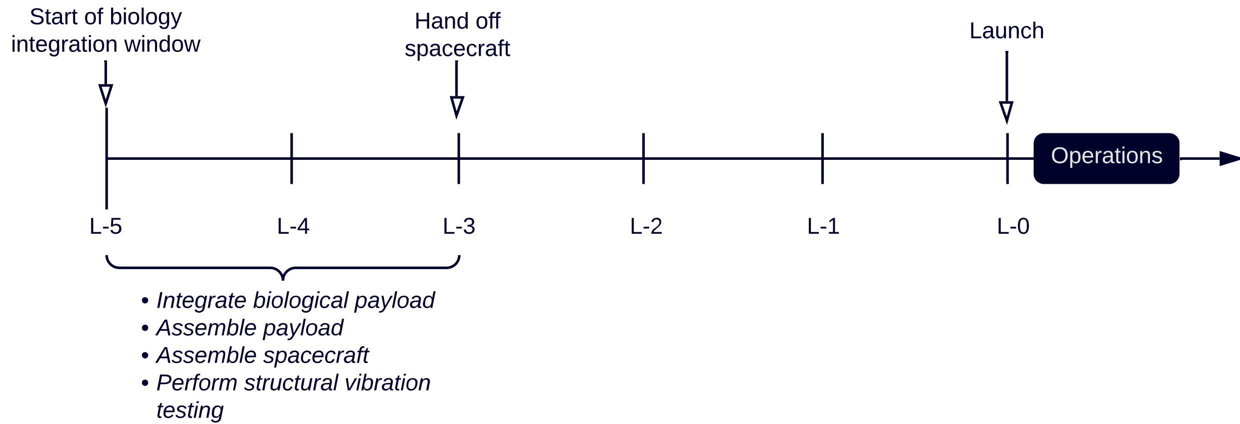

Figure 11: Timeline leading up to launch. The L-minus numbers below the timeline correspond to the number of months before launch.

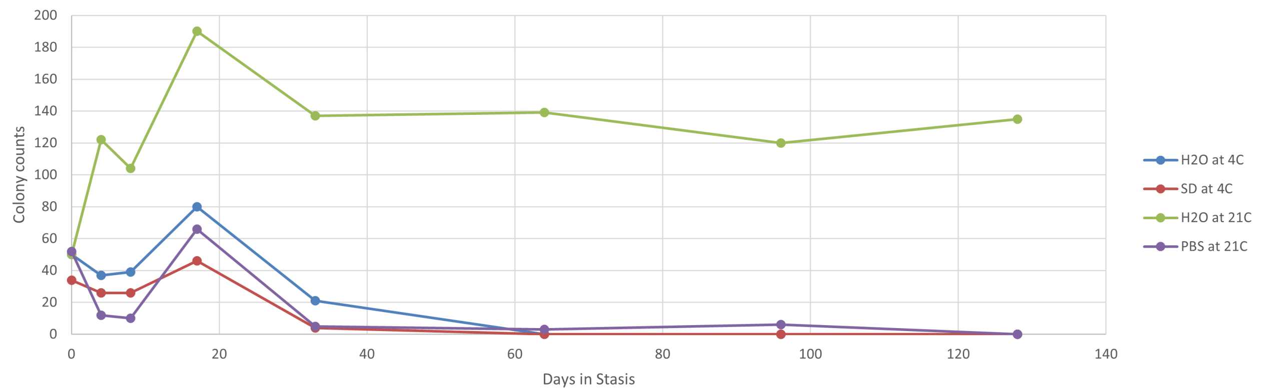

Figure 12: Stasis testing and viability of *C. albicans*. Stasis cell colony counts, over 130 days. Testing in various fluids illustrate that a sterile water suspension keeps the cells viable for the longest period of time. A solution of SN95 cells, a strain of *C. albicans*, suspended in double distilled water (ddH2O) was kept in a microfluidics chip within a high humidity chamber.

storage helps buffer against the risk introduced by launch delays or global pandemics, and helps mitigate the loss of expertise resulting from turnover in student teams.

Conclusion

Summary of Contributions

This paper has presented a detailed overview of the design and development of HERON. An overview of current research into the effects of microgravity on microorganisms was presented, along with HERON's core objective to study the effects of the LEO environment on *C. albicans*. A brief introduction to the team was presented, with much of the paper spent on a detailed overview of the satellite design. Particular emphasis was placed on the microfluidics, optical instrumentation, and payload actuation platforms. Several possible improvements to the current design were identified, which can lay the groundwork for future missions. Overall, the designs were found to meet the mission objectives while keeping HERON relatively low-cost. The entire budget for development, manufacturing, and testing (not including launch) came to roughly 50,000 CAD. Considerations regarding thermal, electrical, and software design were also discussed, followed by a short introduction to the design resources available on our website at utat.ca/heron.

Future Work

UTAT hopes that this work can help kickstart future teams intending to perform similar biological experiments on-orbit. In particular, future work should focus on making the microfluidics and optical platform manufacturing processes more reliable. Future work should also look to build upon other results from the seminal collection of Cell Press papers, as biological research in space is still a relatively new and open field for contributions. Two important lessons from development are to test like you fly and to test early. Several problems were only encountered during the final stages of assembly, which would have been caught months in advance had dry runs been scoped better and performed on flight-representative hardware. The human brain is surprisingly bad at predicting all the ways that small spacecraft can go wrong, and testing flight-like components early is the best remediation.

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This mission would not have been possible without the contributions of hundreds of Space Systems members, going back to the conception of HERON in 2014. A full list of contributors can be found on utat.ca/heron. We would also like to thank Prof. Jason Harlow and the Department of Physics at U of T for our engineering lab space; the William Navarre and Leah Cowen Laboratories, the Department of Molecular Genetics at U of T, for our biological lab space; the U of T Centre for Microfluidic Systems and Dan Voicu for access to microfluidics manufacturing tools; the Wheeler Microfluidics Laboratory for the microfluidics prototyping support; Prof. Christopher Damaren for his supervision; Doug Sinclair and Sinclair Interplanetary for their mentorship; Larry Reeves and the Canadian Satellite Design Challenge for their feedback and testing resources; MDA for their generous donation of environmental testing services; Kepler Communications for their technical mentorship and access to facilities; our launch provider, Spaceflight Industries, for their continuous understanding and support; as well as the University of Toronto undergraduate student body for their support via the levy, making our dreams possible. In loving memory of Alejandro Duque, a passionate and dedicated leader in the initial conceptions of the biological payload.

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Table 1: Comparison of Biological CubeSat Missions

Satellite	Launch Year	Organization	Organism	Mission Lifetime	Size and Mass	Number of Wells	Bay Volume [L]	Well Volume [μL]	Thermal Stability [°C]
GeneSat[32,37]	2006	NASA Ames	*E. coli*	2 weeks	3U+, 4.4kg	12	0.9	110	35 ± 0.5
PharmaSat[37,8]	2009	NASA Ames	*S. cerevisiae*	2 weeks	3U+, 5kg	48	1.2	100	27 ± 0.3
O/OREOS[9,37]	2010	NASA Ames	*B. subtilis* *H. chaoviatorus*	97 days	3U+, 5.5kg	36	-	75	>0
SporeSat[10,37]	2014	NASA Ames	*C. richardii*	2 months	3U+, 5.5kg	-	-	-	-
BioSentinel[11]	2017	NASA Ames	*S. cerevisiae*	12-18 months	6U, <12kg	288	~4.3	100	23 ± 1
EcAMSat[38]	2017	NASA Ames	*E. coli*	About 1 week	3U+, 5kg	48	1.2	100	37 ± 1

Satellite	Main Goal	Results
GeneSat[32,37]	Characterize bacterial growth and metabolism using E. coli, and validate technologies for low-cost spaceborne biological resources.	Successful technological demonstration mission for spaceborne studies, shows good proof of concept of instrumentation.[7]
PharmaSat[37,8]	Study the effects of microgravity on yeast growth, metabolism, and antifungal efficacy.	Positive evidence of yeast growth and working instrumentation[39]
O/OREOS[9,37]	Study the effects of microgravity and radiation on bacterial spores.	Microgravity cells grow more slowly than those in gravity, and grow faster when sensitive to radiation.[40]
SporeSat[10,37]	Study the mechanisms of plant cell gravity sensing.	Awaiting results.
BioSentinel[11]	Investigate the damage and repair of biological DNA in microgravity.	Awaiting results.
EcAMSat[38]	Investigate spaceflight effects on bacterial antibiotic resistance and its genetic basis.	Awaiting results.