BioSentinel: Monitoring DNA Damage Repair Beyond Low Earth Orbit on a 6U Nanosatellite

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ABSTRACT

We are designing and developing a "6U" nanosatellite as a secondary payload to fly aboard NASA's Space Launch System (SLS) Exploration Mission (EM) 1, scheduled for launch in late 2017. For the first time in over forty years, direct experimental data from biological studies beyond low Earth orbit (LEO) will be obtained during BioSentinel's 12 to 18-month mission. BioSentinel will measure the damage and repair of DNA in a biological organism and compare that to information from onboard physical radiation sensors. This data will be available for validation of existing models and for extrapolation to humans.

The BioSentinel experiment will use the organism *Saccharomyces cerevisiae* (yeast) to report DNA double-strand-break (DSB) events that result from space radiation. DSB repair exhibits striking conservation of repair proteins from yeast to humans. The flight strain will include engineered genetic defects that prevent growth and division until a radiation-induced DSB activates the yeast's DNA repair mechanisms. The triggered culture growth and metabolic activity directly indicate a DSB and its repair. The yeast will be carried in the dry state in independent microwells with support electronics. The measurement subsystem will sequentially activate and monitor wells, optically tracking cell growth and metabolism. BioSentinel will also include TimePix radiation sensors implemented by JSC's RadWorks group. Dose and Linear Energy Transfer (LET) data will be compared directly to the rate of DSB-and-repair events measured by the *S. cerevisiae* biosentinels.

BioSentinel will mature nanosatellite technologies to include: deep space communications and navigation, autonomous attitude control and momentum management, and micropropulsion systems to provide an adaptable nanosatellite platform for deep space uses.

INTRODUCTION

Ionizing radiation presents a major challenge to long-term human exploration and residence in space. The deep-space radiation spectrum includes highly energetic particles that generate double strand breaks, deleterious DNA lesions that are usually repaired without errors via homologous recombination (HR)[1]. This repair pathway has been conserved in all eukaryotes. While significant progress has been made in identifying and characterizing biological radiation effects using Earth-based facilities, no terrestrial source currently duplicates the unique space radiation environment.

We are currently developing a biosensor-based nanosatellite to fly aboard the SLS EM-1 mission, expected to launch in late 2017. As a secondary payload that will be ejected from the launch vehicle upper stage after capsule separation, BioSentinel will be deployed on a trajectory that results in a lunar flyby that places us into a heliocentric orbit beyond the Earth's magnetic field and radiation belts.

This biosensor will carry a strain of the *S. cerevisiae* organism that contains engineered genetic defects that prevent growth unless a radiation-induced DSB near a reporter gene activates the yeasts' HR repair mechanisms. Culture growth thus directly indicates a

successful DSB-and-repair event. In parallel, HR-defective and wild type strains will be used to provide survival data. Desiccated cells will be carried within independent culture microwells, built into multiple 96-well microfluidic cards. Each microwell set will be activated by media addition at different time points over 18 months. Cell growth will be tracked continuously via optical density. A reserve set of wells will be activated only in the occurrence of a solar particle event (SPE). Biological measurements will be compared to data provided by onboard physical dosimeters and to Earth-based and ISS-based experiments.

The proposed investigation addresses multiple Strategic Knowledge Gaps (SKGs) identified within NASA's Human Exploration and Operations Mission Directorate (HEOMD). The Mars Exploration Program Analysis Group (MEPAG), Small Body Analysis Group (SBAG), and Lunar Exploration Analysis Group (LEAG) have all identified SKGs related to characterizing the ambient space radiation environment as key to maintaining peak human health.

SCIENCE INVESTIGATION

Ionizing radiation presents a major challenge to long-term human exploration and residence in space. Most long-term effects of radiation damage (e.g. cancers) are due to DNA damage. The deep space radiation spectrum includes highly ionizing particles (galactic cosmic rays and high-energy protons) that cause DSBs in DNA[2,3]. Significant progress has been made in identifying and characterizing biological radiation effects using Earth-based proton and heavy-ion radiation facilities; however, no terrestrial sources fully duplicate the full energy spectrum, particle diversity, varying dose rate, and long-term low-dose-rate space radiation environment.

BioSentinel uses a "radiation biosensor" strategy in which the organism *S. cerevisiae* reports the occurrence and repair of DNA DSBs in a single cell. Statistically meaningful rates of this human-relevant damage-and-repair process are measured via the exponential amplification of cells in culture for a period of up to 18 months. BioSentinel also carries a physical radiation spectrometer that will record individual radiation events including estimates of their LET energy and directionality, as well as a total ionizing dosimeter to measure the total dose deposited. Data from the physical spectrometer will be compared directly to the rate of DSB-and-repair events measured by the *S. cerevisiae* biosentinels.

Yeast Biology

BioSentinel uses the budding yeast *S. cerevisiae* to measure DSBs in response to ambient space radiation. DSBs and their subsequent repair exhibit striking conservation from yeast to humans[4,5]. The biosensor uses yeast because of its similarity to cells in higher organisms. Yeast also has a well-established history of strains engineered to measure DSB repair, flight heritage, and a wealth of available ground and flight reference data. The BioSentinel yeast flight strain contains engineered defects that prevent growth and division until and unless a radiation-induced DSB near the target gene activates the yeast's DNA repair mechanism. Thus, culture growth and metabolic activity directly indicate a DSB and its successful repair. In parallel, a wild yeast strain and a strain engineered to be incapable of repairing DSBs will be used to provide survival data.

In BioSentinel each of the yeast strains is carried in multiple independent culture wells. Sets of wells are activated at different time points over an 18-month mission. Readout sensors will monitor each set of culture wells continuously for 4 weeks before the next set of wells is activated. Cell growth will be measured via optical density and metabolic activity measured using a viability dye.

The anticipated mission orbit will provide biologically significant radiation doses. Far higher doses are expected during a SPE. A reserve set of wells will be retained and can be activated either by ground command in response to an observed solar event or autonomously in response to particle events detected by the dosimeter.

To determine the relative effects of the radiation and gravitational environments on the growth rates of the yeast organism, it will be critical to compare the DSB rate measured by the BioSentinel payload in deep space to the physically measured radiation dose, models of expected DNA damage-and-repair rates, the results of studies in terrestrial facilities, and studies conducted in LEO.

A summary of the different configurations for the BioSentinel sensor package is shown in Table 1. The BioSentinel payload will be flown on a 6U freeflyer beyond the shielding effects of the Earth's magnetic field. An identical payload will also be flown on the International Space Station (ISS) to provide reference growth rates in a microgravity environment which has a benign radiation environment. Ground based payloads will be tested both in a benign laboratory environment and in a radiation beam such as that at Brookhaven National Laboratory.

Table 1: Experimental Setup for BioSentinel Environments

BioSentinel Configuration	Gravity Field	Radiation Environment
EM-1 Freeflyer	μ-g	Deep Space
ISS Instrument	μ-g	LEO, Benign
Ground Control	1-g	Benign
Radiation Ground Control	1-g	Radiation Beam @ BNL

DSBs caused by space radiation and repaired by homologous recombination will restore the function of a selected auxotrophic marker gene, *leu2*. This repair event enables the yeast cells to grow in a selective medium that lacks the amino acid leucine. The yeast thus converts a DSB-and-repair event in the vicinity of the selected locus to a measurable increase in cell number and a detectable colorimetric change due to increased metabolism of actively growing cells. As one control, a wild type yeast strain will be grown in a rich, non-selective medium to estimate if any lethality results from irreparable DNA damage outside of the selected locus[10]. An additional control, in the form of a mutant yeast strain, will carry a deletion of the HR gene *RAD52* and will be unable to repair DSBs by HR. Growth curves from this control will characterize the deleterious effects of DSBs.

Ionizing particle induced DSBs can be repaired effectively in space[7] but the damage and repair rates for actual spaceflight environments have been difficult to measure until now. Diploid yeast cells primarily use HR to repair such DSBs[5], so a well-characterized yeast genetic assay employing HR-mediated repair will quantify the rate of DSBs in the space environment as shown in Figure 1. As shown, a diploid BioSentinel strain contains two non-functional leu2 alleles. If a DSB occurs within the vicinity of one of the alleles, HR-dependent recombination promotes the error-free repair by using the homologous chromosome containing the other *leu2* alleles, promoting gene conversion to a wild type *LEU2* gene.

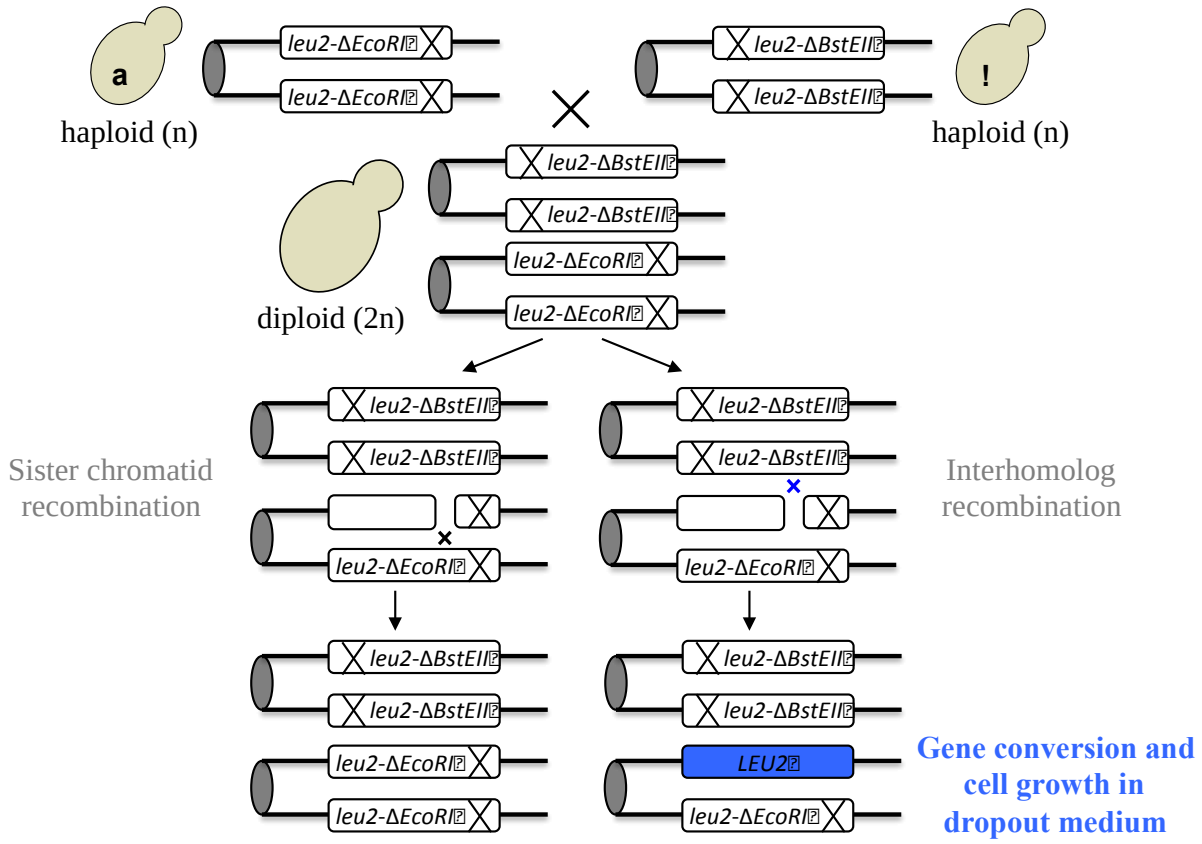

Figure 1: Heteroallelic Recombination Assay

LET Spectroscopy

Significant technological progress has been made in the past few years in miniaturized electronics that opens new possibilities in energized particle detection and imaging. One such family of devices was developed by CERN to meet detection requirements for the Large Hadron Collider[8]. These devices have been incorporated in the Radiation Environment Monitor (REM) and related sensors by the JSC RadWorks group. These devices make possible real-time imaging with high sensitivity, low noise and broad dynamic range. The TimePix generation of sensors allows each imaging pixel to operate in multiple modes. The first mode provides information about the count of detected particles, the second provides information about the energy of particles in each pixel and the last provides information on the time of arrival of particles in each pixel. An example of the data obtained from the sensors is shown in Figure 2.

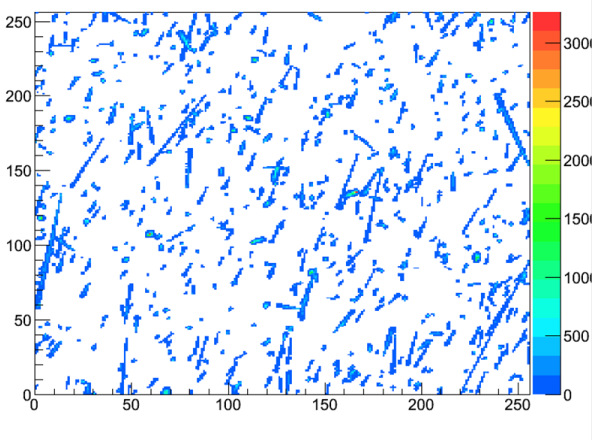

Figure 2: Example data from TimePix based sensors

The data obtained from these sensors can be processed in a variety of ways to complement the yeast biosensors. Particle energy and number can be integrated to calculate the total ionizing dose. Instantaneous particle energy can be used to provide histograms showing the number and intensity of particle impacts helping to calibrate models of contributions from both galactic cosmic rays and solar events. Analysis of the deposited particle tracks can be performed to show the directionality of the incident radiation for some particles. Incident particles of sufficient energy can be analyzed to indicate biosensor wells that may have been impacted by the same particle. Finally, analysis of the dose rate and particle type can be used to autonomously detect the onset of an SPE.

SCIENCE INSTRUMENTATION

The BioSentinel payload system draws heavily on the Ames Research Center (ARC) nanosatellite line including GeneSat-1, PharmaSat and O/OREOS, as well as the JSC ISS REM sensors and related payloads.

The biosensor payload system integrates microorganism growth, optical measurement of viability and growth, thermal control, passive control of relative humidity and pressure, active fluid management, and engineering sensors for temperature, pressure, and relative humidity. The LET spectrometer system integrates the sensing chip with readout electronics, configuration data, and power conversion and conditioning.

BioSensor Instrument

The BioSentinel biosensor instrument uses three multilayer microwell fluidic cards similar to that shown in Figure 3. Each card will contain 96 wells containing around 100 µL of fluid. Final well volume and fluidic card materials will be determined by ground based biocompatibility and functional testing. Membrane filters at each well's entrance retain yeast in the wells during addition of the growth media. Outlet filters enable replacement of the media as well as expulsion of bubbles and spent media.

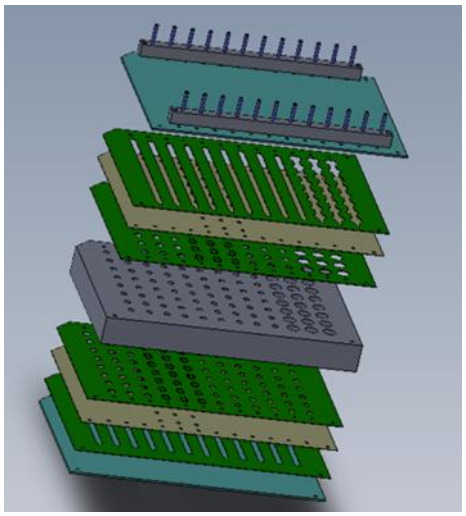

Figure 3: Representative schematic of biosensor fluidic cards

Fluids will be delivered by an independent pump-and-valve system for each fluidic card. Each pumping system will supply nutrient for initial well rehydration[11]. Growth media and any other reagents required by biocompatibility testing will be stored in flexible polymer bags with the polymer material and coating selected to minimize water permeation. The results of initial testing on a card prototype are shown Figure 4. Unmetabolized medium can be seen in dark blue while the metabolized medium has shifted to pink. This resultant color change is used to characterize the growth of the yeast cells.

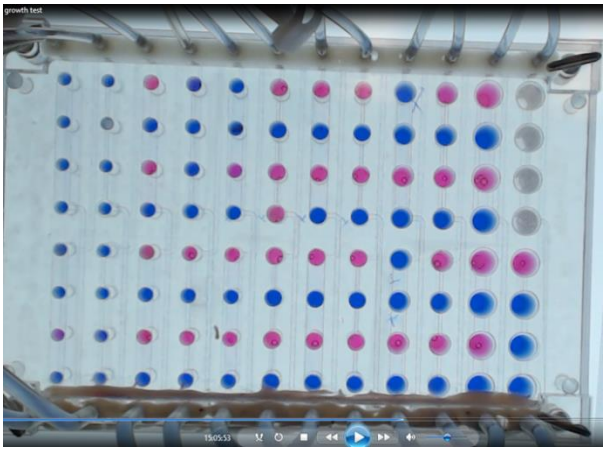

Figure 4: Prototype card showing results of yeast metabolism

The biosensor readout optical systems, based on O/OREOS designs, will provide 3-color LED illumination at the top of each well[12]. Broadband detectors at the bottom will measure the intensity of the light, providing both density and colorimetric measurements.

A thermal control system uses patterned Kapton strip heaters and control sensors to maintain payload temperature at 23°C ± 1°C over the mission lifetime. Engineering sensors will provide the payload internal pressure and relative humidity, medium flow rates, and gravity levels. The payload itself is packaged within a 1 atm containment vessel.

LET Spectrometer Instrument

The LET spectrometer is based heavily on the REM series of instruments flown on the ISS by the JSC RadWorks group, specifically the Battery-operated Independent Radiation Detector (BIRD). This instrument integrates a TimePix sensor with data processing and power conditioning functions.

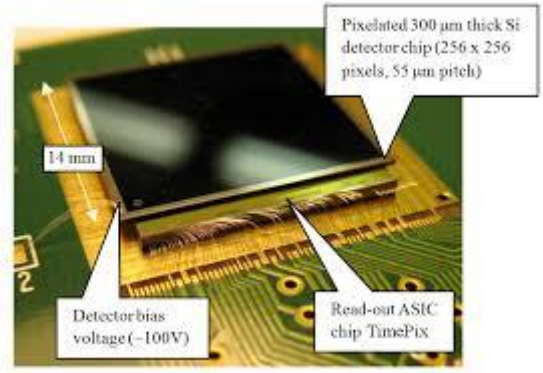

Figure 5: Example of TimePix sensor layout

The TimePix sensors are composed of a semiconductor detector chip, typically silicon, bump-bonded to a readout chip. The sensor contains an area of 256 x 256 square pixels with 55 µm pitch. Each pixel contains a preamplifier, discriminator, 14-bit ADC and shift register. An example TimePix sensor is shown in Figure 5.

The associated processor board performs low level voltage conversion, configures the TimePix sensor modes, extracts data from the TimePix sensor and stores the radiation frame data. The power supply converts unregulated bus power to the voltages required for processing.

MISSION DESIGN

BioSentinel is being developed to fly as a 6U nanosatellite that will be deployed from the SLS EM-1 flight, currently scheduled for launch in late 2017. The SLS vehicle has the capacity to host up to 12 6U CubeSats as secondary payloads per flight. The nanosatellites are mounted between the Interim Cryogenic Propulsion Stage (ICPS) and the Orion crew vehicle as shown in Figure 6[9].

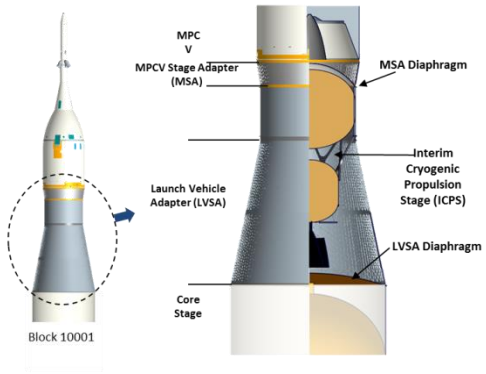

Figure 6: Nanosatellite Accommodation on SLS

The SLS vehicle is designed to deliver astronauts to locations such as Mars, the Moon, asteroids, and the Earth's Lagrangian points. The EM-1 mission is specifically designed to deliver the Orion capsule on a circumlunar trajectory. After the Orion capsule separates, a disposal maneuver is performed by the upper stage. At this point, the secondary nanosatellites are planned to be deployed over a period of several hours. As shown in Figure 7, BioSentinel will follow a trajectory from this point resulting in a ~700 km lunar flyby. The lunar flyby will cause BioSentinel to enter a heliocentric orbit with 0.93 x 0.98 Astronomical Unit (AU) dimensions.

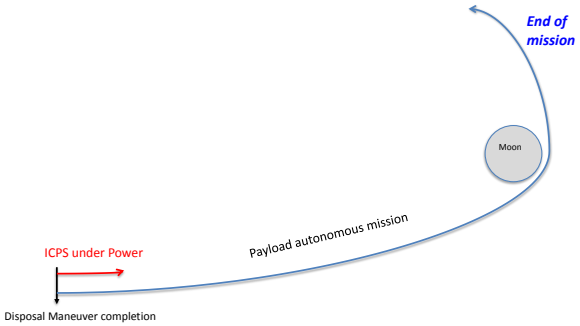

Figure 7: ICPS Trajectory post Orion Disposal

The resultant heliocentric orbit is shown in Figure 8. In this figure, the Sun is centered with the Earth's orbit shown in blue and BioSentinel's orbit shown in red.

Due to the BioSentinel orbit being interior to that of the Earth's, it will move away from the Earth as its orbital period is slightly smaller. This relative motion results in the BioSentinel-to-Earth ranges shown in Figure 9. At the 12-month nominal mission duration, BioSentinel will be 0.43 AU from the Earth and 0.73 AU at its 18-month extended mission duration.

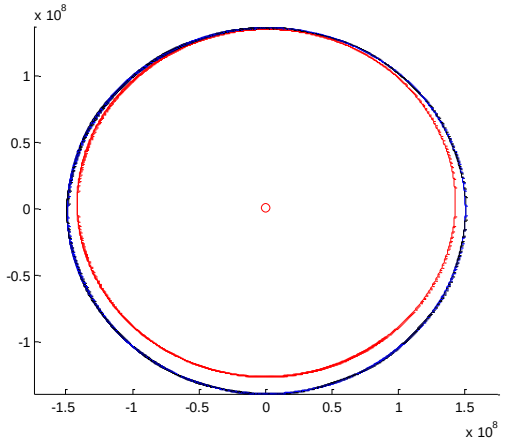

Figure 8: BioSentinel orbit compared to Earth orbit

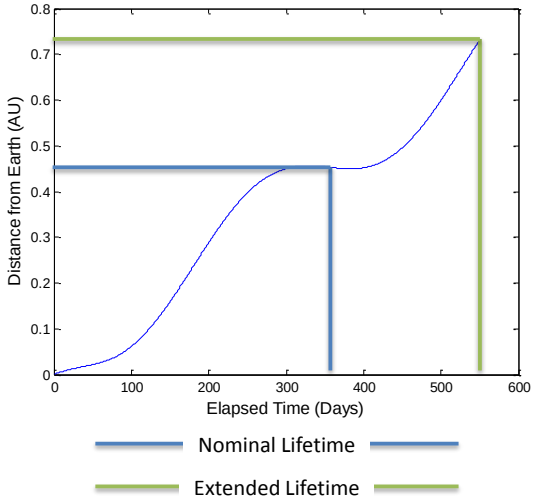

Figure 9: BioSentinel to Earth range

There is the possibility that a small propulsive maneuver performed after separation could result in capture of BioSentinel within the Earth-Moon system. This maneuver would have the benefit of minimizing communications range over the mission. However, this would require mass and volume to be diverted from the payload to support a propulsion system. Analysis of this tradeoff will be performed as the mission is further developed. The BioSentinel science goals require only that BioSentinel be in an orbit outside the benefits of the Earth's magnetic fields. Thus, no further maneuvers are required from the baseline EM-1 mission to place BioSentinel into a viable mission orbit[10].

SPACECRAFT

The BioSentinel spacecraft is being implemented using lessons learned from both the ARC Nanosatellite line as well as from the Lunar Atmosphere and Dust Environment Explorer (LADEE) mission. A drawing showing the packaging of the BioSentinel spacecraft is shown in Figure 10. The spacecraft has been designed to allocate 4U of volume to the payload while containing bus functionality in the remaining 2U volume.

The BioSentinel spacecraft is implemented as a 3-axis controlled system with a micropropulsion system for momentum management. A coherent X-band transponder provides command, data, and navigation capabilities. Deployed solar arrays are used to provide the power required for processing and communications. The structural subsystem leverages the biosensor containment system to minimize mass as well as distribute heat. Thermal control is implemented with a cold-biased system with heaters, sensors, and passive coatings.

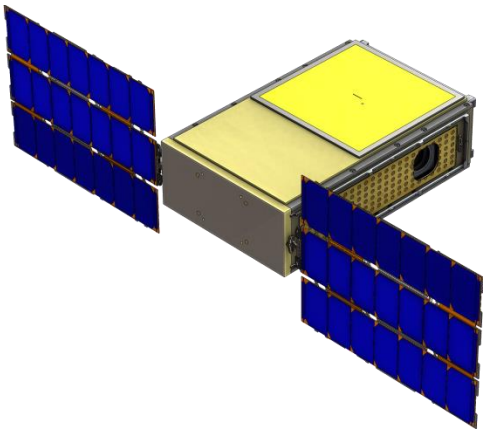

Figure 10: BioSentinel Spacecraft CAD Layout

Guidance, Navigation, and Control (GNC)

The BioSentinel GNC system is required to detumble the spacecraft after deployment, orient solar arrays toward the sun, point communications antennae to the Earth, support a robust safe mode, and perform these functions autonomously. Typical CubeSat control solutions are not applicable as no magnetic field or GPS signals are available most of the mission.

The current BioSentinel GNC system relies on reaction wheels for primary control, a star tracker for nominal determination, an IMU for deployment and safe mode, and a propulsion system for momentum management and detumbling. An Extended Kalman Filter based on LADEE and EDSN software is used for knowledge estimation as it easily meets the 5° pointing accuracy requirement for solar array and antenna orientation.

The BioSentinel GNC team has built a small testbed with a variety of sensors and actuators to do early prototyping of control algorithms. An example of detumble analysis performed is shown in Figure 11.

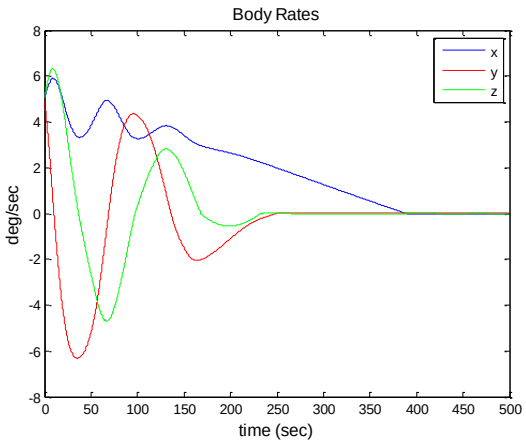

Figure 11: Detumble Analysis

Propulsion

As stated previously, the EM-1 disposal trajectory is compatible with BioSentinel mission requirements and no additional propulsive maneuvers are required to enter a mission orbit. While the dispenser to be used by SLS has not been finalized, the BioSentinel GNC team performed analysis using typical tipoff rates for CubeSat dispensers and showed that available reaction wheels would saturate performing detumble. A propulsion system is then required and also used for momentum management. Further analysis on trajectory management using both impulsive and low-thrust electric propulsion systems is underway.

Communications

The BioSentinel communications system is required to receive commands, transmit telemetry to the ground and support navigation and ranging functions. Based on spectrum management recommendations, BioSentinel has baselined the use of a coherent X-band transponder. Two patch antennae, one omnidirectional and one medium gain antenna (MGA), are specified as well. Placing the antennae on opposite faces of the spacecraft provides communications coverage over a majority of the geometric sphere around BioSentinel. An MGA with ~15 dB gain is assumed as well.

Using this MGA with available CubeSat transponders, BioSentinel can close data links to 34m Deep Space Network (DSN) assets at data rates ranging from 62.5 – 6000 bps over its lifetime. Investigation into alternative assets such as those provided by the Universal Space Network (USN), Stanford Research Institute (SRI), or Morehead State University is in progress. BioSentinel currently estimates the use of DSN assets for 4 hours per pass, twice per week to retrieve mission science data.

Structures

The BioSentinel structural system design is based on lessons from NASA ARC's SporeSat and EcAMSat. Aluminum structural panels are joined to the biosensor payload canister and to frames holding the electronic components within the system. The interfaces are tailored using materials such as Delrin, Ultem or indium to meet thermal system requirements. While the official dispenser has not been selected, BioSentinel has been designed to be compatible with the NanoSat Launch Adapter System (NLAS) developed by NASA Ames Research Center or the Canister Satellite Dispenser (CSD) system from Planetary Systems. Other 6U options will be evaluated if selected for use on SLS.

Thermal Control System (TCS)

The BioSentinel TCS design approach is to cold bias the payload and to use passive coatings or interface materials and heaters for trim control. Metallized Teflon or fluorinated ethylene propylene (FEP) tapes or paints are used for surface optical property control. Insulating or conductive gaskets are used for interface thermal control. The BioSentinel payload is designed to stay at 23°C ± 1°C and the BioSentinel bus is designed for temperatures from 0°C to 70°C. Individual excursions will be analyzed against the component properties.

Figure 12 shows preliminary thermal model results with maximum external temperatures of 41°C and minimum external temperatures of 6°C. The absence of a central eclipsing body in the BioSentinel trajectory allows temperatures to approach steady state conditions as fluctuations are driven primarily by component duty cycles rather than eclipse, IR and albedo from a central body.

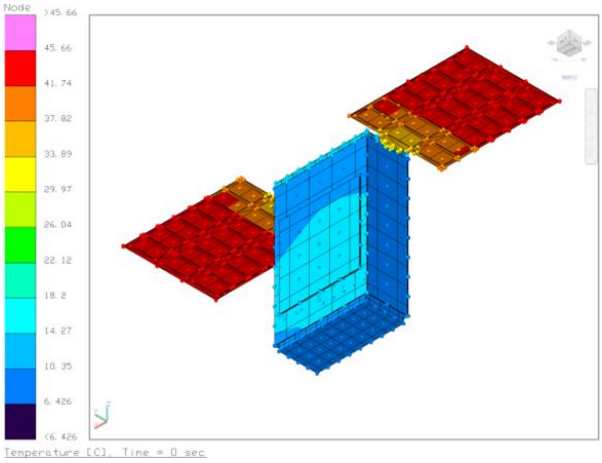

Figure 12: Preliminary Thermal Model Results

Command and Data Handling (C&DH)

The BioSentinel C&DH system is designed to provide reliable, cost-effective control of spacecraft functions. The system has been designed to leverage LADEE flight software heritage. The C&DH team evaluated processing solutions to find options that provided ~100 Million Instructions Per Second (MIPS) with floating point capacity that were compatible with the VxWorks, RTEMS and Linux operating systems. Solutions based on ARM, Zynq, Freescale, and LEON3 based processors, among others, were evaluated. Boards representative of the solutions were procured and the flight software team is beginning the process of porting flight software to the solutions. Early prototyping of the hardware is used to inform procurement decisions.

Software

BioSentinel flight software (FSW) is architected to be based on LADEE flight software to the greatest extent possible. LADEE flight software was executed on the VxWorks operating system, built using the Core Flight Services / Core Flight Executive (CFS/CFE), and developed using Matlab/Simulink model based software that was autocoded to C.

BioSentinel would like to provide a C&DH and FSW that can use either a low-cost OS for risk-tolerant, cost-constrained customers or a higher reliability OS for more risk-averse stakeholders. CFS/CFE has been successfully ported to VxWorks, RTEMS, and RT Linux providing a range of possible solutions. Using the development hardware procured, the FSW team is working to port CFS to RTEMS and Linux on the processing solutions available to meet this goal.

Electrical Power System (EPS)

BioSentinel's EPS design is driven by three primary requirements. It must provide enough electrical power to handle all electrical services, it must provide systems compatible with launch safety, and it should use hardware compatible with the selected C&DH system. The primary load on the BioSentinel system is the X-band transponder. Current solutions require 14 – 20W of DC input power and communications passes of 4 hours or more to retrieve data preclude the traditional power cycling approach. Deployable arrays providing ~ 35W are being evaluated for use. The lack of a central body alleviates the battery from deep discharges. Lithium-ion 18650 cells are baselined due to flight heritage. Additional study will take place as the safety review process progresses. Finally, processing solutions with associated EPS products are being evaluated for compatibility. Adapting heritage ARC nanosat EPS designs for processors that do not have associated EPS solutions is also in progress.

TESTING

BioSentinel is being developed using multiple testbeds as early as possible in the development process. While in Phase A, testbeds exist for GNC, C&DH, and communications hardware. Payload testbeds are under development as well.

Figure 13: LADEE / BioSentinel RF Testbed

The LADEE RF testbed is shown in Figure 13. This testbed was built to interface with multiple antennae and test ports on a transponder and a modem test set. It allowed commands from a local operator or remote mission operations center to be sent to a transponder over Ethernet connections and be switched through multiple paths. It also includes multiple sensors designed to measure downlink power, spectrum performance and noise as well as to inject interfering carrier and other noise sources. BioSentinel envisions using this system for transponder and spacecraft checkout.

The BioSentinel GNC team has built a separate testbed with representative processing and power system hardware, a 3 degree of freedom (3-DOF) air bearing, Helmholtz coil, and star field emulator. The testbed is being used to develop and benchmark control algorithms, evaluate components, and test safe mode and fault response.

The BioSentinel C&DH and FSW teams have also started construction of testbeds for software development and test. LADEE benefitted from the availability of multiple processor- and hardware-in-the-loop testbeds. BioSentinel plans on procuring multiple processor-and-hardware sets to be used for software development and interface verification with bus and payload components.

Finally the BioSentinel payload development team is developing multiple testbeds as well. One major challenge in developing biological payloads is ensuring that the components used are compatible with micro-organism biology. Multiple polymers and adhesives are used for fabricating and sealing the microwell cards. The BioSentinel payload team has developed miniature fluidic card designs, each containing 32 wells. The smaller cards can be rapidly procured and manufactured in multiple combinations allowing scientists to test the yeast with a variety of materials, growth media, storage conditions and storage temperatures. One of the minicards is shown in Figure 14.

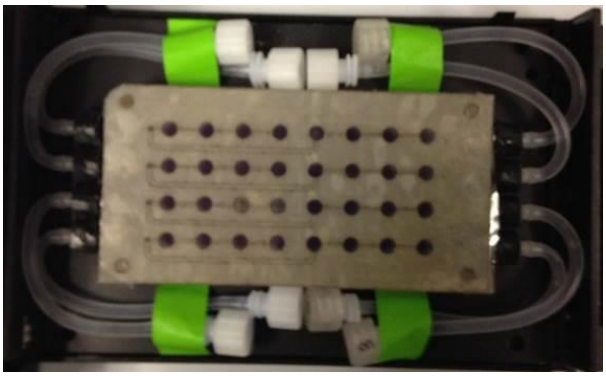

Figure 14: Biosensor payload minicard testbed

CONCLUSION

The BioSentinel mission will perform the 1st NASA biology study beyond LEO in the last 4 decades. By enabling the comparison of DSB-and-repair data across multiple radiation and gravitation environments, BioSentinel will resolve questions about the relative contributions and impacts of those environments. The BioSentinel design will result in a payload design that can fly on a freeflying spacecraft to desired environments or be a hosted payload on a variety of platforms.

Ionizing radiation presents a major challenge to long-term human exploration of space as most of the effects of radiation damager are due to DNA damage. BioSentinel will develop a “radiation biosensor” in combination with an LET spectrometer and TID dosimeter. By combining these data types, BioSentinel will meet multiple SKGs across multiple teams with

NASA's Human Exploration and Operations Mission Directorate. These SKGs deal with characterizing the ambient space radiation environment as being key to maintaining peak human health.

In addition, the BioSentinel spacecraft design will validate nanosatellite technologies that can be used for deep space navigation, attitude control, and propulsive maneuvering. By providing a low-cost implementation of such capabilities it will enable low-cost, high capability missions in the future.

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