EcAMSat Mission

January 11, 2023

Matthew Lera

Background

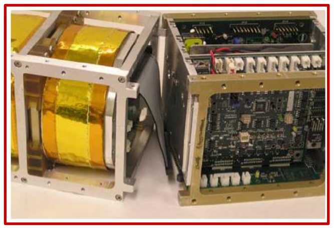
For Astronaut Health During Long Term Spaceflight, Many Questions Remain

- Immediate need to understand antibiotic resistance in microgravity
  - Evidence that bacteria become more virulent and resistant than in normal gravity
  - Astronaut's immune system is compromised in microgravity

- Model Organism: uropathogenic *Escherichia coli* (UPEC)
  - Urinary tract infections have been reported in astronauts
  - WT and ΔrpoS mutant strains
  - rpoS is involved in general stress response and is shown to confer antibiotic resistance to Gentamicin
  - Gentamicin has been used to treat UTI

EcAMSat LINEAGE

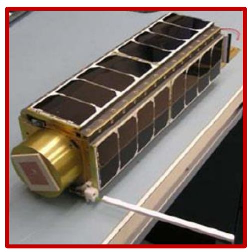
StarDotSat



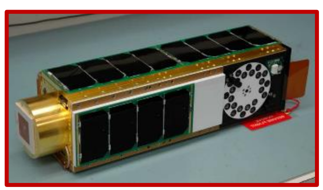
Genebox

GeneSat-1

PharmaSat-1



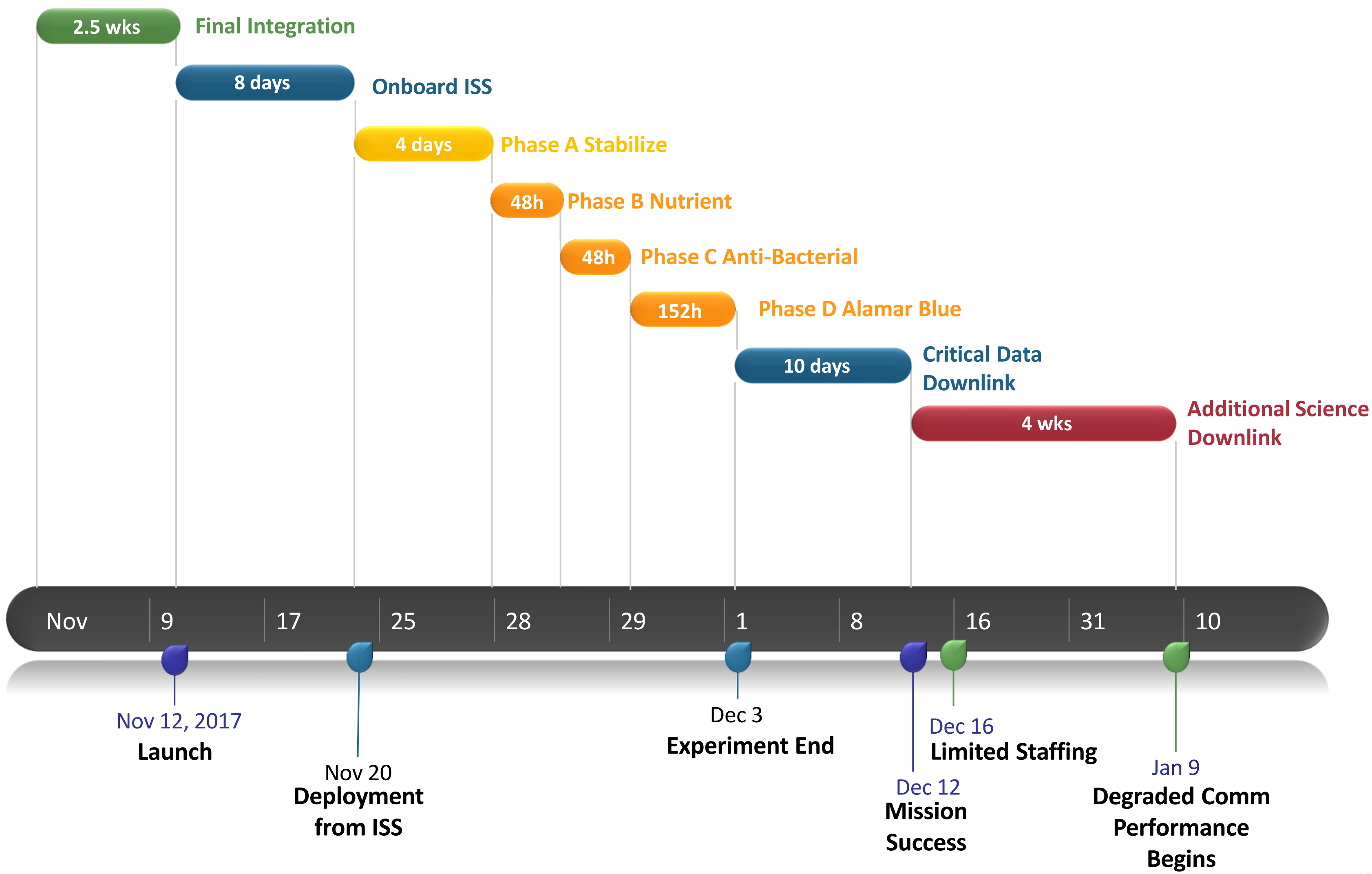
O/Oreos

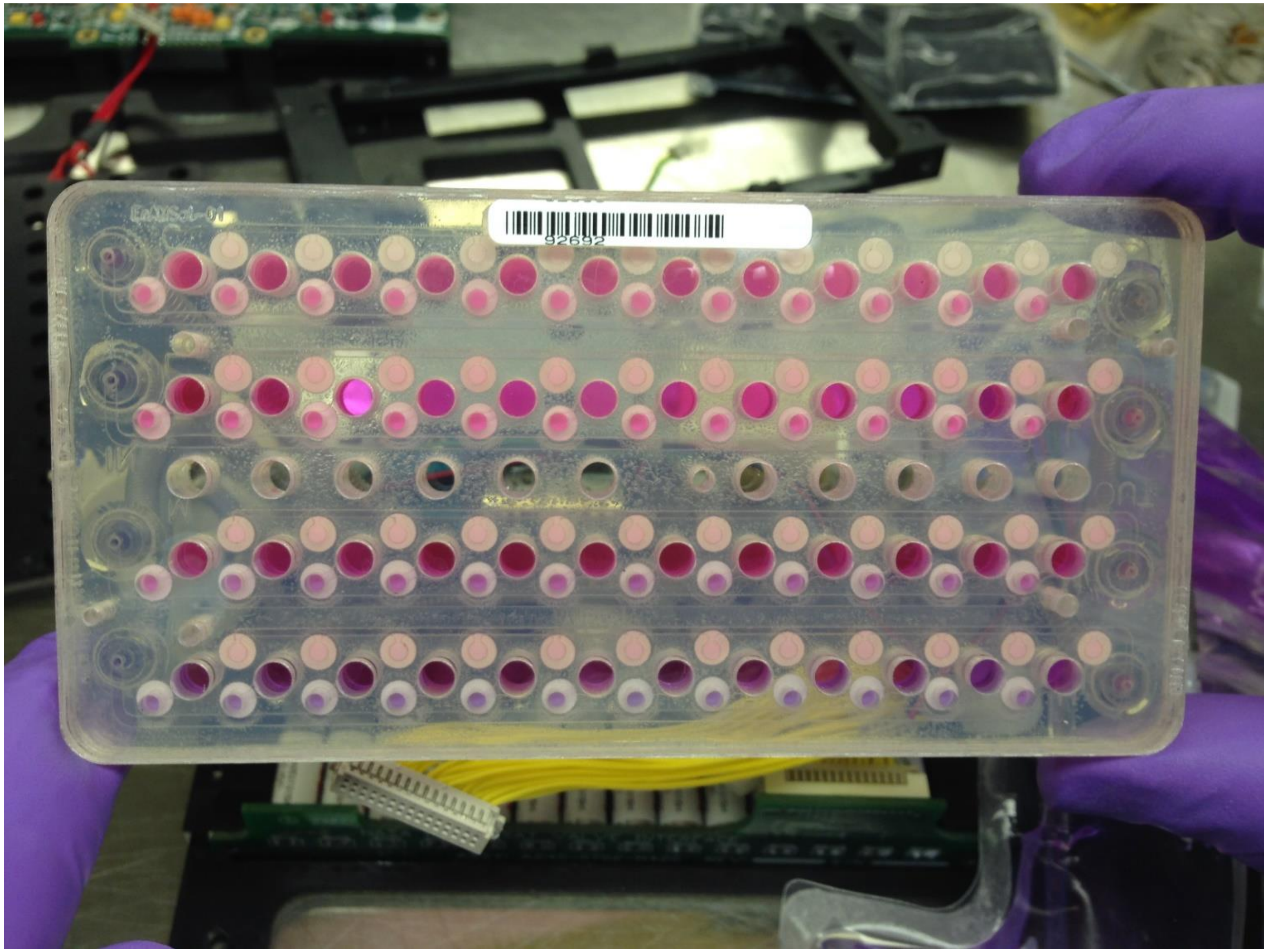


Nanosail-D

EcAMSat

Mission Timeline

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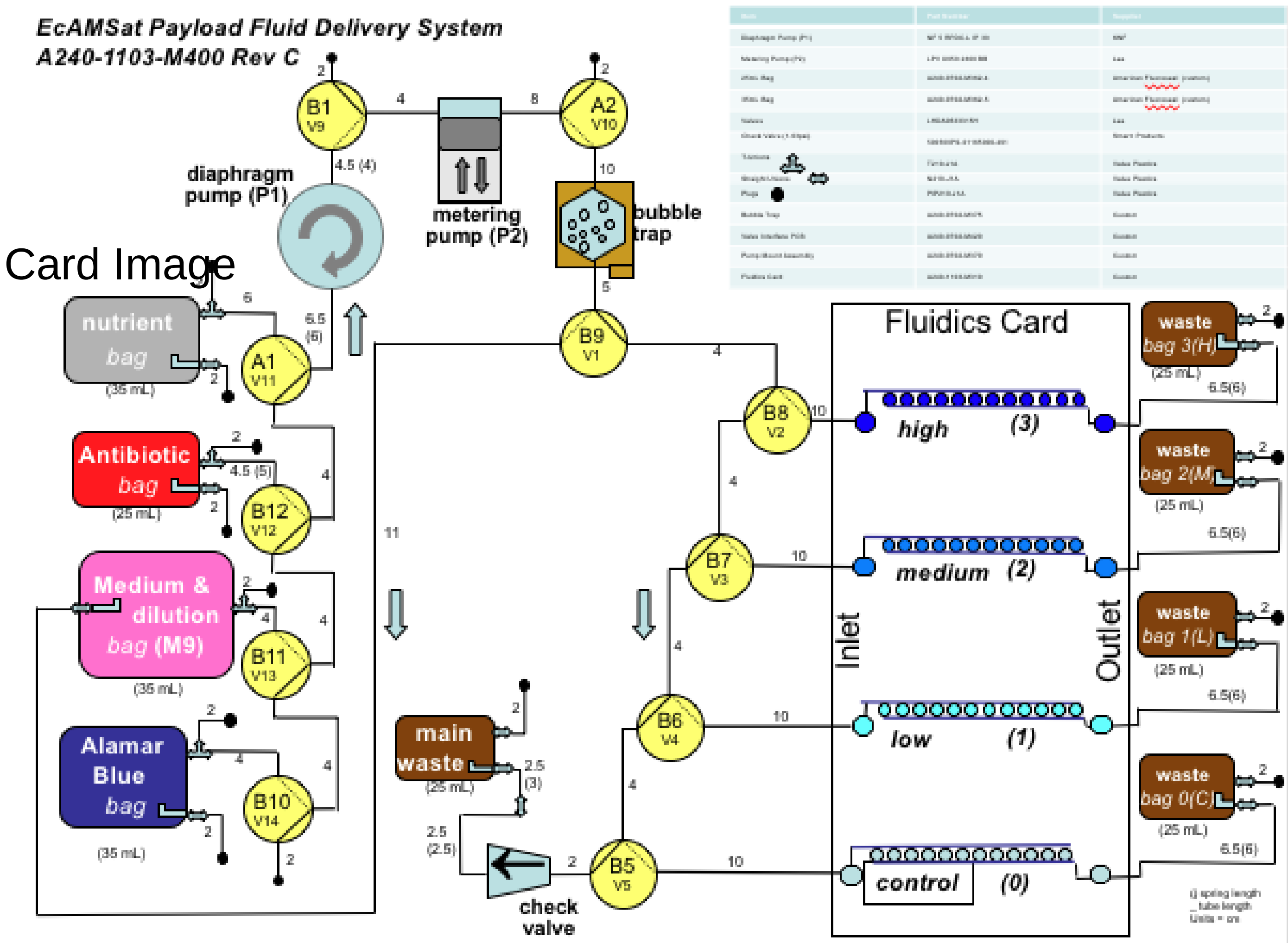
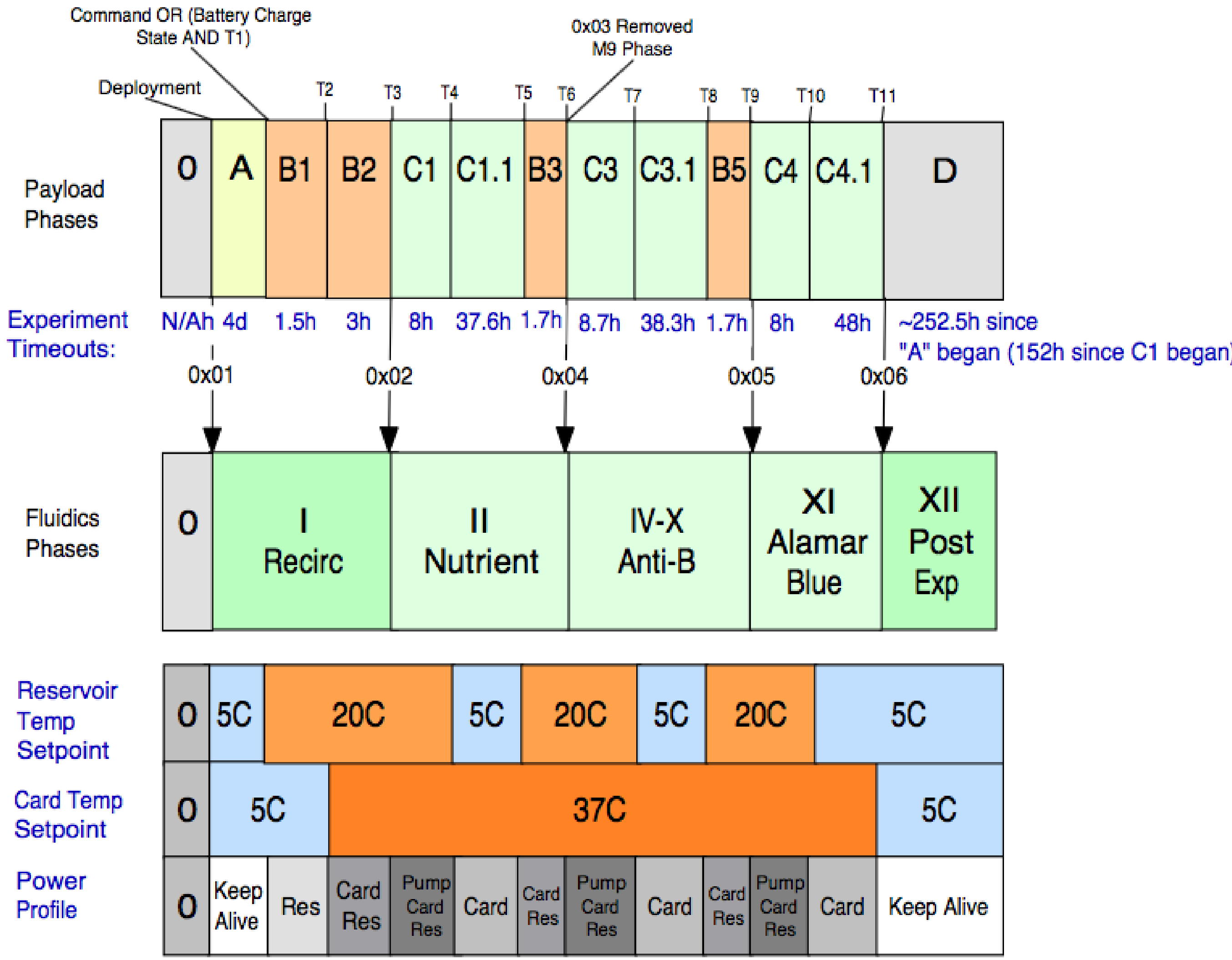
EcAMSat Fluidic Map



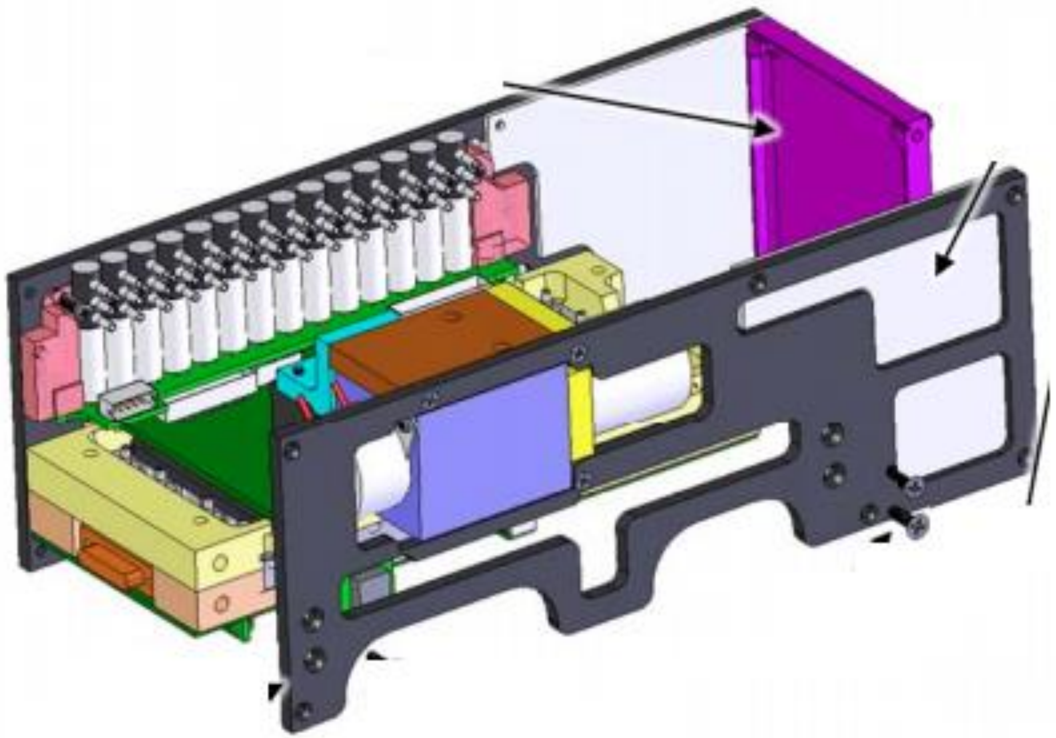
EcAMSat Fluidics Protocol

Full Fluidics Protocol was completed Successfully !!!!

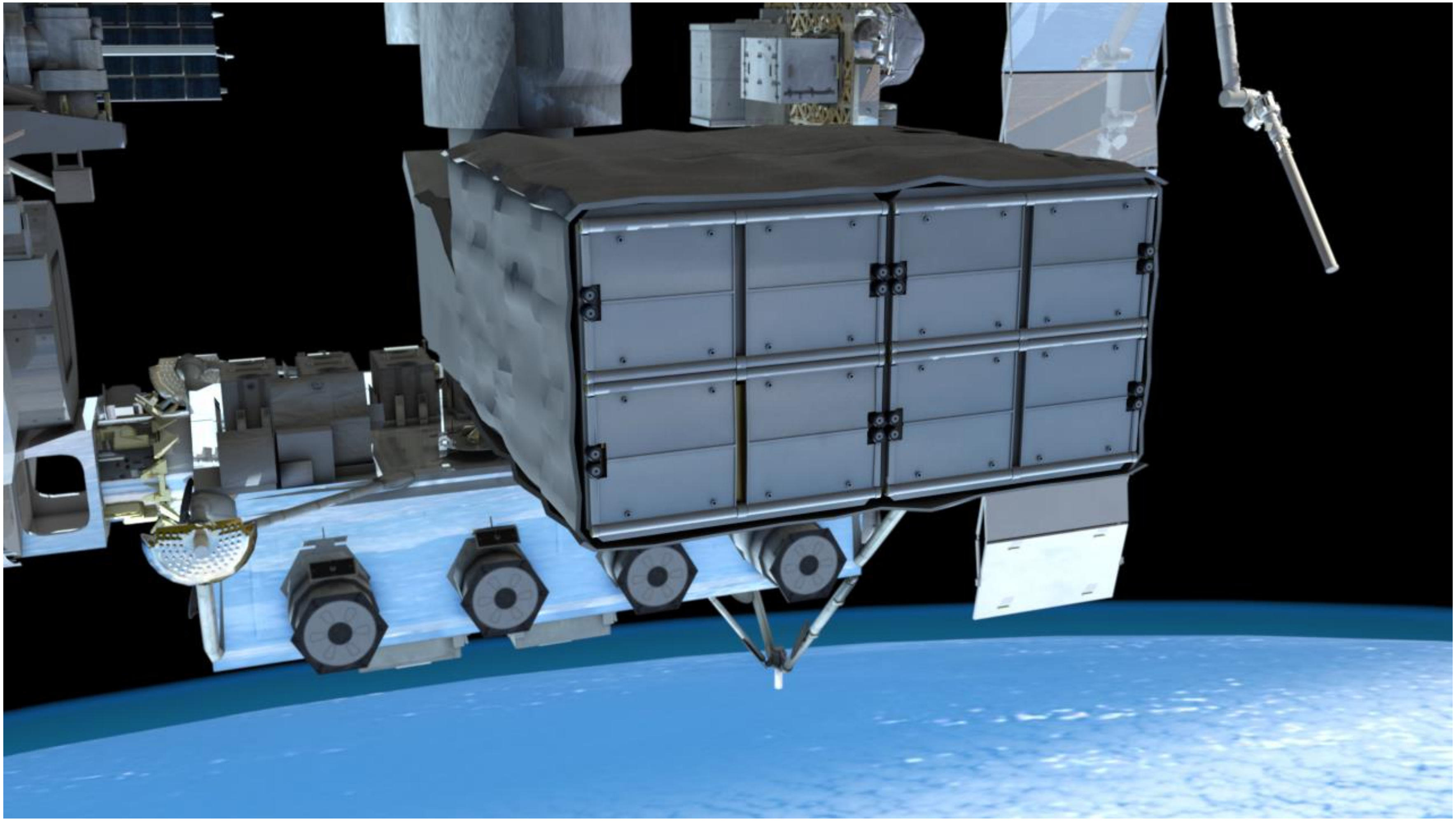


Payload Internals

- Solenoid Valves
- Stepper Pump
- Diaphragm Pump
- Card heaters
- Payload Board
- Detector Board
- Fluidic Card
- Bubble Trap



NASA Ames PAO Animation Example



ISS Video Footage of EcAMSat Deployment

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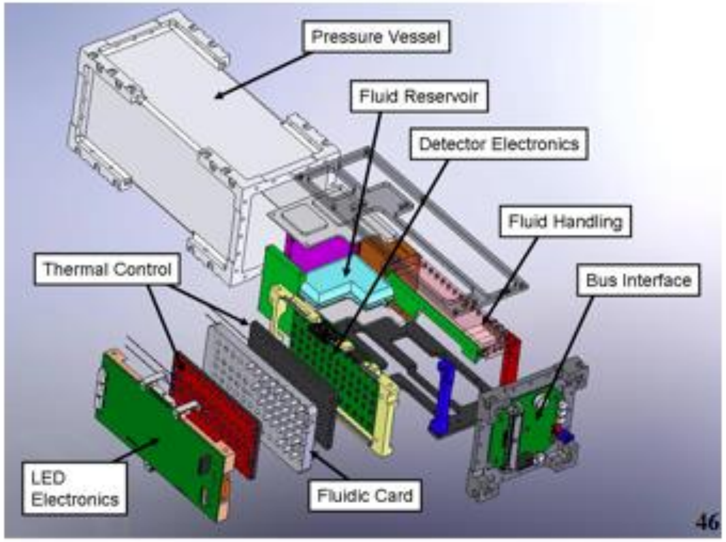
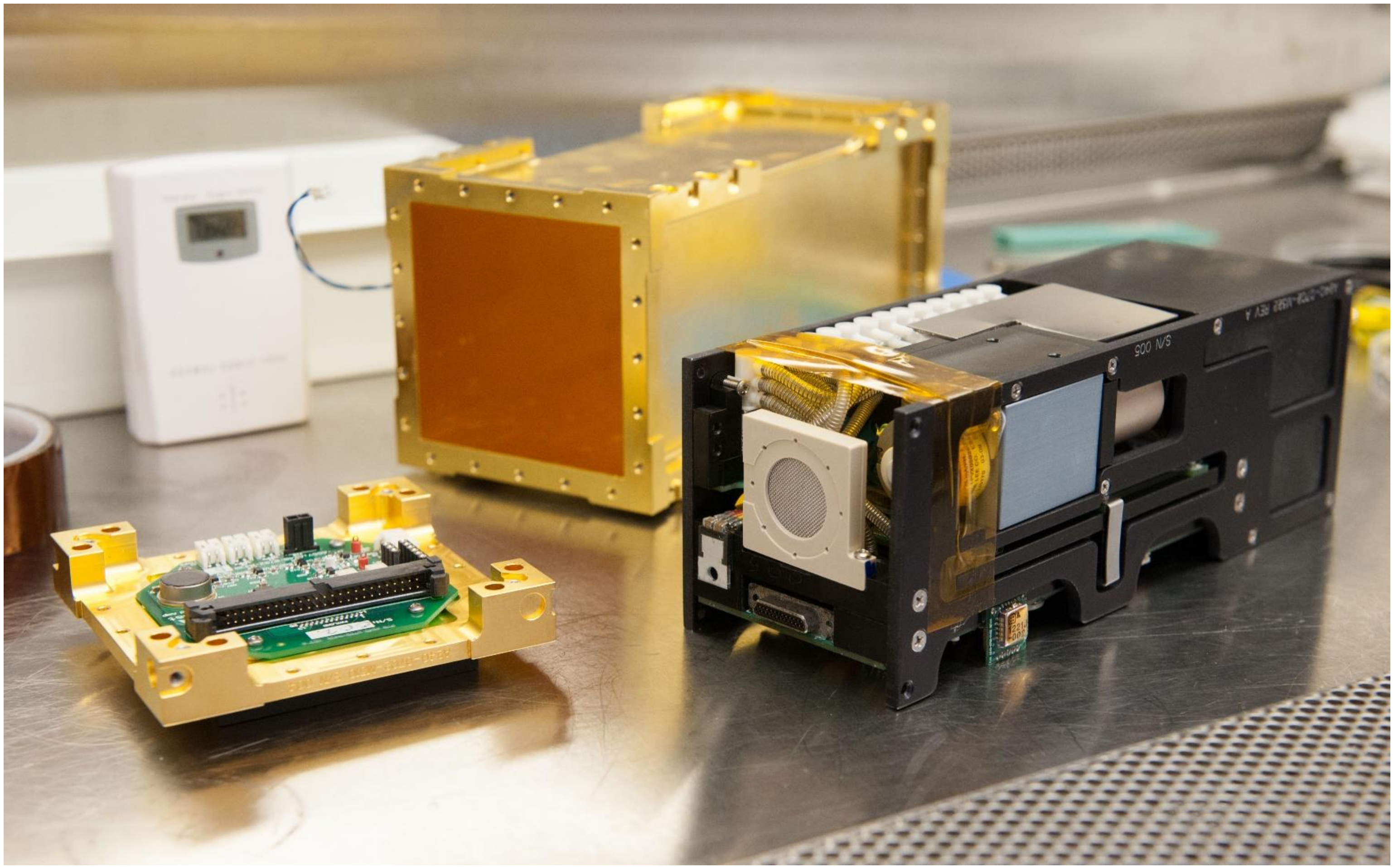
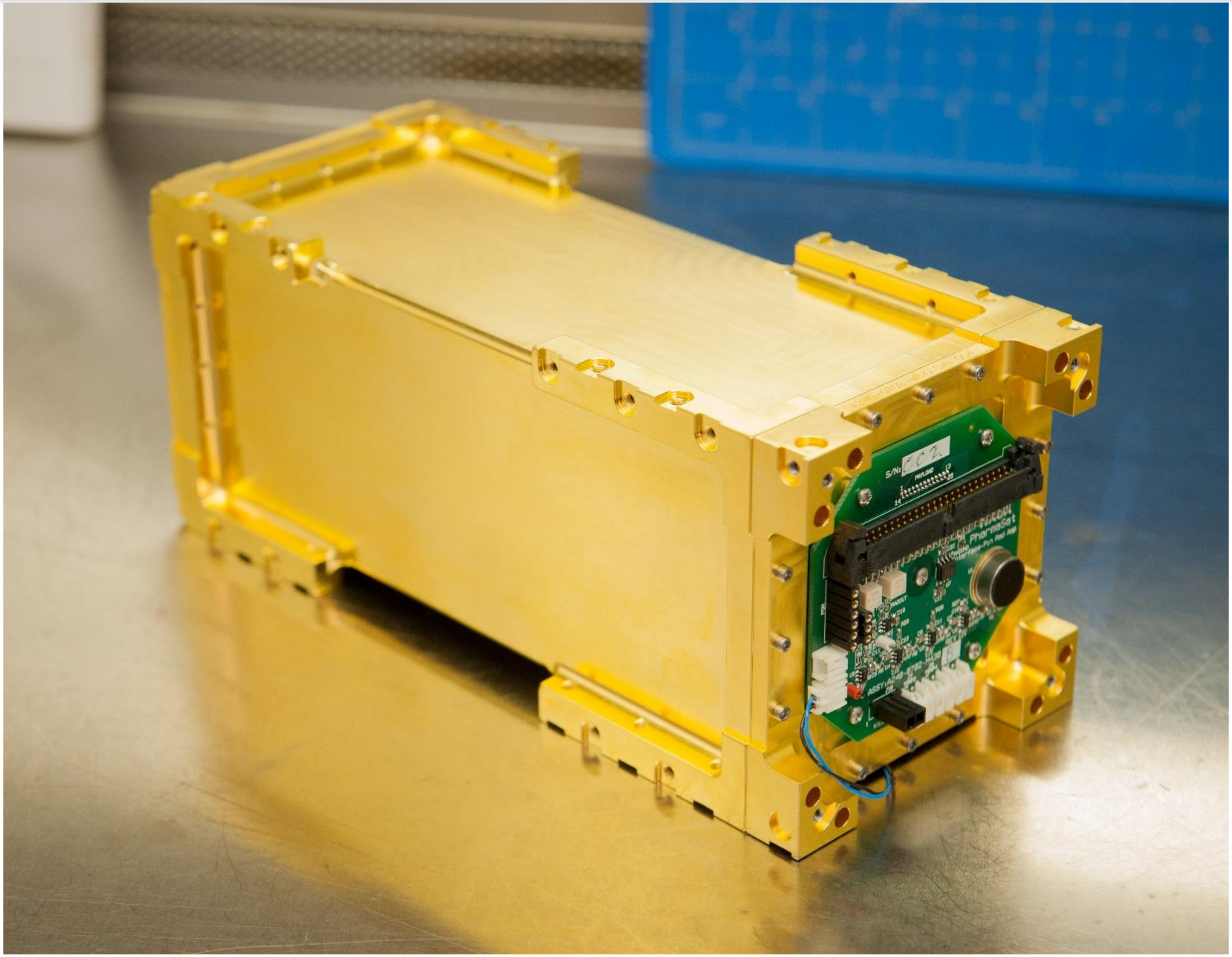

Pressure Vessel
Fluid Reservoir
Detector Electronics
Fluid Handling
Thermal Control
Bus Interface
LED Electronics
Fluidic Card
46




PharmaSat

EcAMSat Mission Dashboard

EcAMSat Mission Dashboard

Mission Time
105 Days 16 Hrs 53 Min 10 Sec

Mission Phase
NOV 12, 2017 - Launch
NOV 14, 2017 - ISS Storage
NOV 20, 2017 - Ejection/Initialization
NOV 24, 2017 - Experiment
- Educational Ops
- De-orbit

Mission Status

Experiment / Payload

Science	Complete

Spacecraft Status Summary

S-Band Comm	Operational
Beacon Comm	Operational
Thermal Health	Nominal
Battery Health	Nominal

EcAMSat deployment from the NanoRacks deployer - Courtesy NASA

Spacecraft Status

S-Band Communications	Online
Beacon Communications	Online

The operations team is currently on console

Thank you and congratulations to **JA0CAW** for submitting the first beacon packet data!

Mission Links
NASA EcAMSat Web Site
NASA EcAMSat Fact Sheet
Small Satellite Technology Program
Beacon Decoding Instructions
Beacon Packet Upload Site

Ground Segment Status

Control Nodes

SCU Ops	Primary
CREST Ops	Backup

S-Band Stations - 3m dishes

SCU-A	Operational
SCU-B	Backup
CREST	Backup

UHF Stations

SCU-OSCAR	Operational
SCU-Auto-A	Operational
SCU-Auto-B	Operational
SLU-Auto	Operational
WPI-Auto	Operational
BAY-Auto	Operational

On-orbit mission control for the Nodes mission is being provided by the students, staff and faculty of Santa Clara University's Robotics Systems Laboratory.

Since October 25, 2017, this page has been displayed 90403 times.

Mission Science

Mike Padgen – Fluidics Lead

Macarena Parra – Project Scientist

Matt Lera – Payload Lead

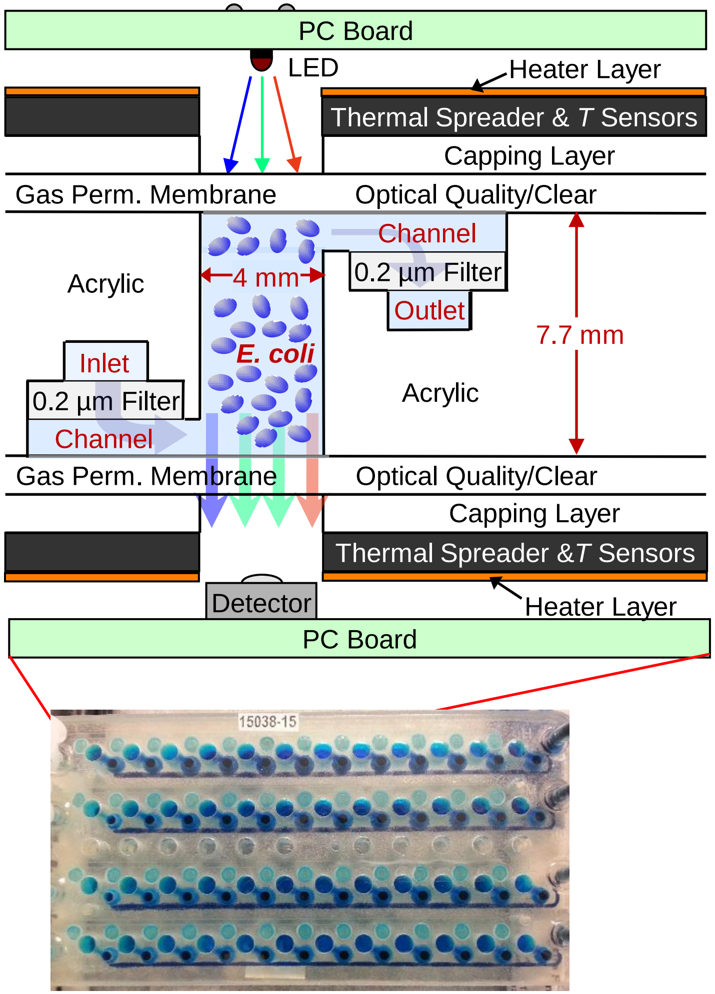
EcAMSat Experiment Background

- Purpose
  - Determine how microgravity alters the antibiotic resistance of uropathogenic *E. coli* (UPEC)
  - Study the role that the critical stress response gene, *rpoS*, plays in antibiotic resistance.

- Experiment
  - Organisms: wild-type UPEC and Δ*rpoS* mutant strain, which does not contain the *rpoS* gene and therefore may not be primed for the anticipated stress response due to microgravity.
  - Antibiotic: Gentamicin
    - Test three concentrations of the antibiotic, plus control.
  - Use Alamar Blue to measure survival after antibiotic treatment.
  - Compare results from flight unit with those from the ground control.

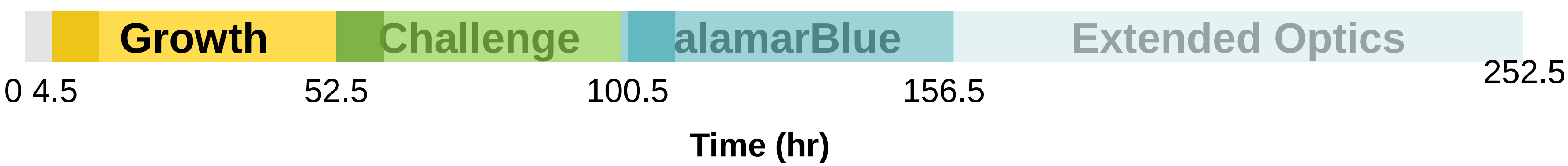
Fluidic Card Stack

- Cells loaded into alternating wells of fluidic card and stored in stasis buffer (M9)
- Thermal spreaders with heaters and embedded temperature sensors on both sides of card
- 3 LED (615, 525, 470 nm) and detector system for each well



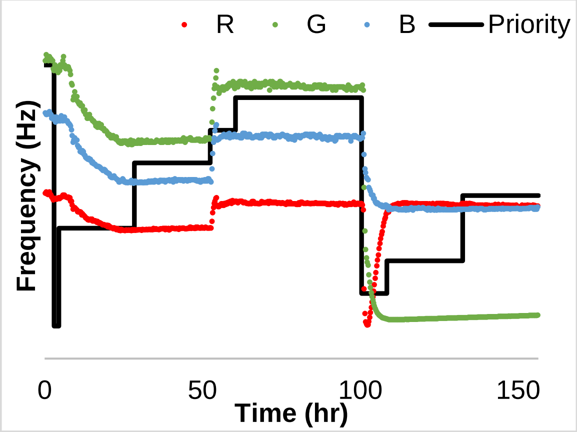
Experiment Protocol

- Heat cards to 37°C (4.5 hr)
- Pump LB to card, allow cells to grow to stationary phase (8 hr pumping, 48 hr total)
- Mix doses of gentamicin in M9, deliver to card and incubate (8 hr pumping, 48 hr total)
- Pump 1X alamarBlue to card, allow reduction (8 hr pumping, 56 hr total)
- Kept card at 37°C after end of nominal experiment, optics data taken for next 96 hr

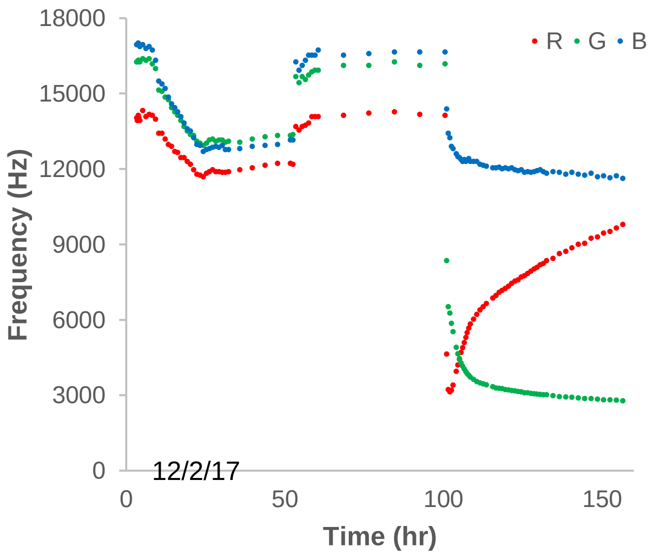


Data Downlink

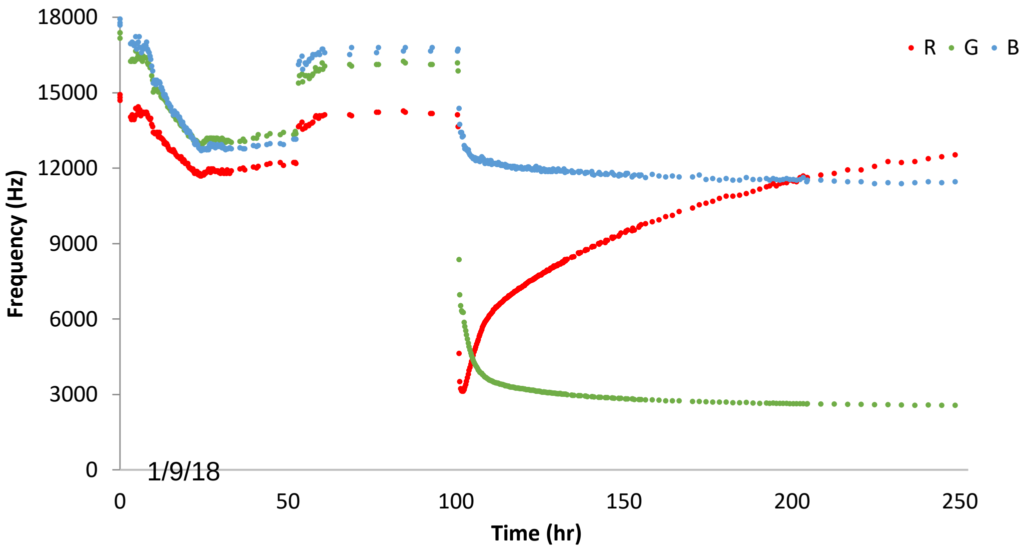
- Prioritize baseline and pumping phases
- Then AB reduction and growth
- Received all minimum required data
  - Received all pages* in critical phases
  - 824 payload pages received



Minimum Data Set



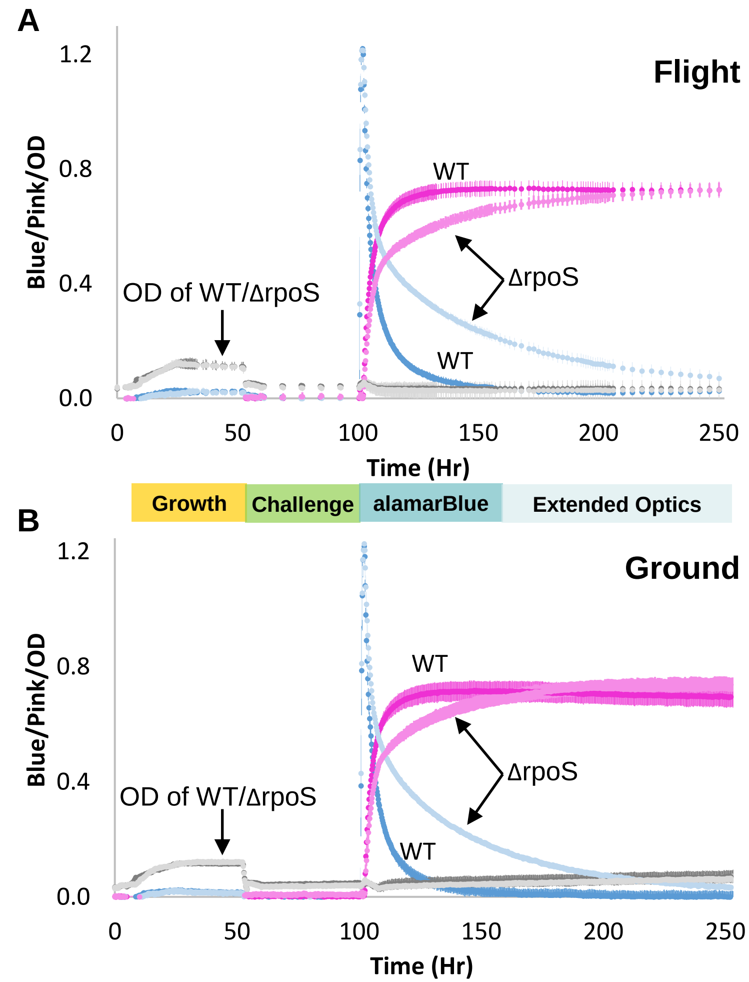
Received Data Set



*Each pair of pages includes data for all 60 wells during one 15 min period

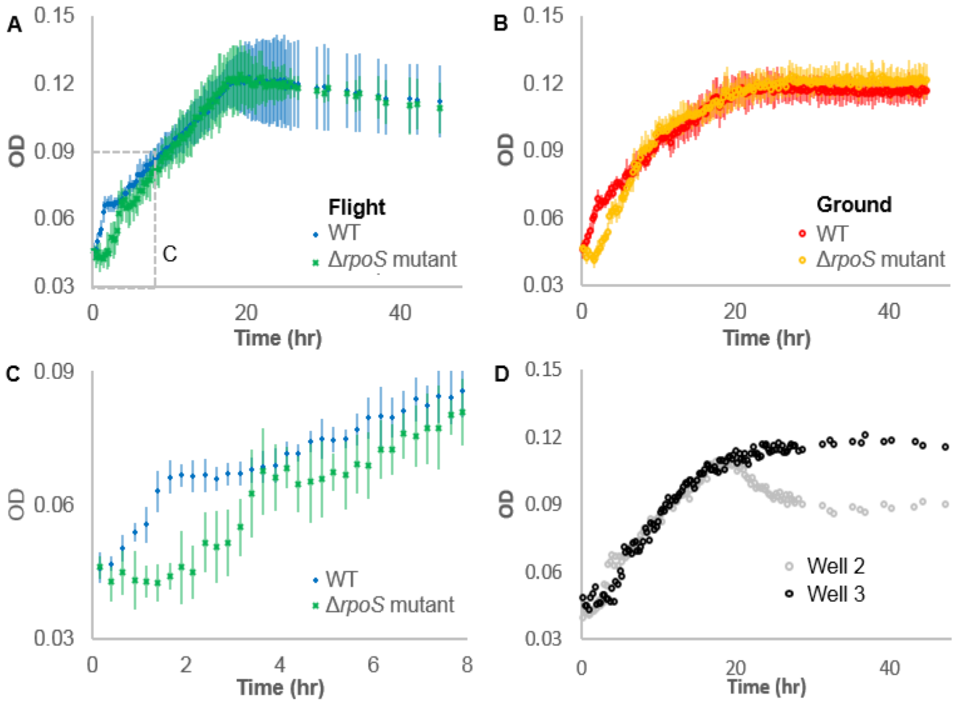
Flight vs Asynchronous Ground Control

- Asynchronous ground control autonomously "deployed" 48 hours after flight
  - Autonomously started experiment 4 days later
- Flight and ground performed similarly
  - The relative concentrations of the oxidized (Blue) and reduced (Pink) forms of AlamarBlue® are shown for Flight (top) and Ground (bottom).
  - The cell OD throughout the experiment is also shown (gray)
  - Control bank shown suggests no large differences in timing between flight and ground and no difference in baseline stress between card load and experiment initiation



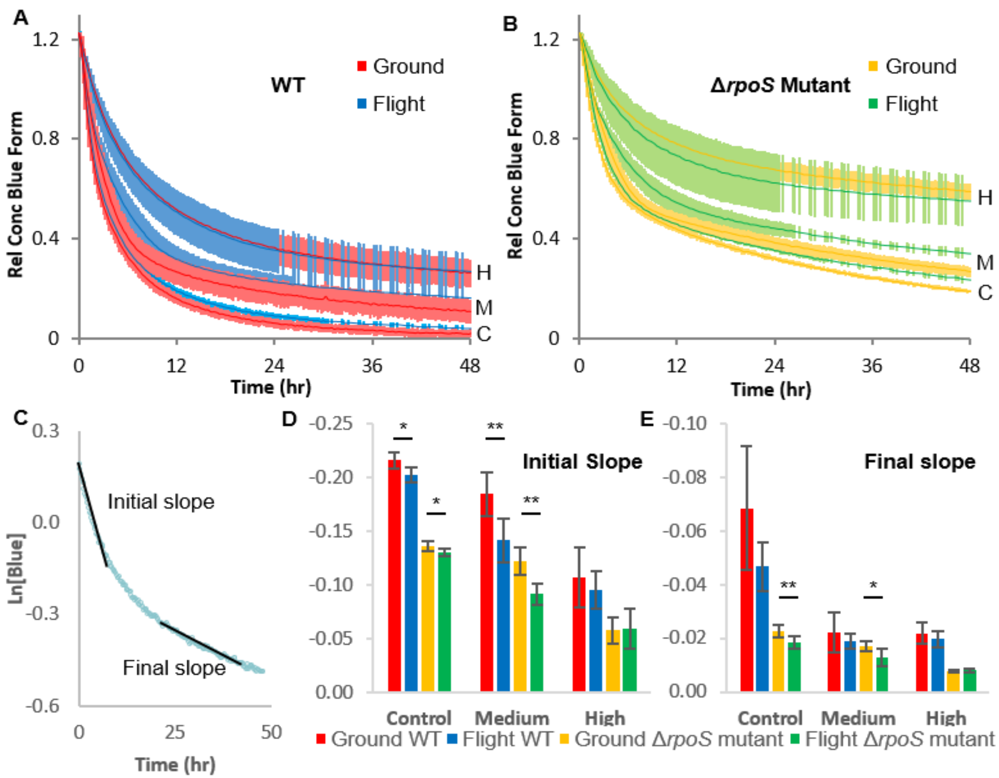
Full experiment control bank A) flight and B) ground (error bars +/- SD)

OD during growth phase



- Growth of single wells of WT and mutant in flight and ground
- Some wells in flight experienced a dip in OD while approaching stationary phase. Such wells were random and did not correlate to differences in AlamarBlue® reduction later in the experiment.

AlamarBlue® reduction after exposure to varied Gm concentrations



- AlamarBlue® reduction shown for wildtype and mutant strains at no (control), medium, and high concentrations of gentamicin in flight and ground
- Averages of all banks showed a combined effect of microgravity and gentimicin on reducing AlamarBlue® reduction in the medium dose exposure
- No significant difference seen in high dose banks

Conclusions

- EcAMSat mission achieved full success, completing all required objectives.
- While the various stresses imposed on the cells by the payload hardware, by the antibiotic, and due to microgravity could not be completely separated, it appeared that the effect of microgravity was greatest for both strains in the medium Gm dose case.
- The largest difference between the two strains was at the high dose.
- The results demonstrate that the *rpoS* gene and its downstream products are important therapeutic targets for treating bacterial infections on Earth and in space.

Lessons Learned & Best Practices

- VOCs likely masked the science results
  - Activated carbon likely becomes saturated
  - Isolating the Biology from air exchange a potential solution
- There is no such thing as a small change. Changing organisms from previous mission resulted in significant hardware modifications that impacted cost and schedule.
- There is a lot you can tune in software
- Biocompatibility, biocompatibility, biocompatibility
- Distribute your samples
- Prioritize your data downlink

Thriving in Deep Space (TIDES)

Challenge:

- Need robust prediction of biological responses to long-duration exposure to deep space to enable crew, plants, and microbiome to thrive in deep space exploration
- Combined effects of stressors (e.g., radiation, reduced gravity, elevated CO_2, circadian disruption) are unknown

Impact:

- Use of well-characterized biological models, from the simplest to more complex organisms, has been utilized to understand Earth-based biological processes and has led to several Nobel prizes. BPS needs to use a similar approach to understand the effects of the novel environment of deep space on crew, plants, and microbiome.
- Will provide mitigation methods for risks to crew health and performance; design guidance for plants and microbiome; biomarkers to monitor the health of crew, plants, and microbiome
- Will provide insight into development, aging, and disease mechanisms of interest to NIH, NSF, and biotechnology industry

Approach:

- Conduct a series of Earth-based, Low Earth orbit, and **BLEO investigations**
- Omics and physiological data from cells, organs/tissues, and whole organisms will be compared within populations and across organisms to develop models that predict biological responses to long-duration exposure to deep space
- Technology development for dosimetry, in flight radiation source and artificial gravity

Beyond LEO Instrumentation & Science Series (BLISS)

BLISS
Matthew Lera

- **LEIA**
  Dave Pletcher
- **Science Working Group**
  Jessica Lee/Matthew Lera
- **Mini-ECLSS (MSFC/ARC)**
  Tara Samuels/Matthew Lera
- **Artemis II**
  Kevin Martin
- **BLISS-2**
  (On hold)

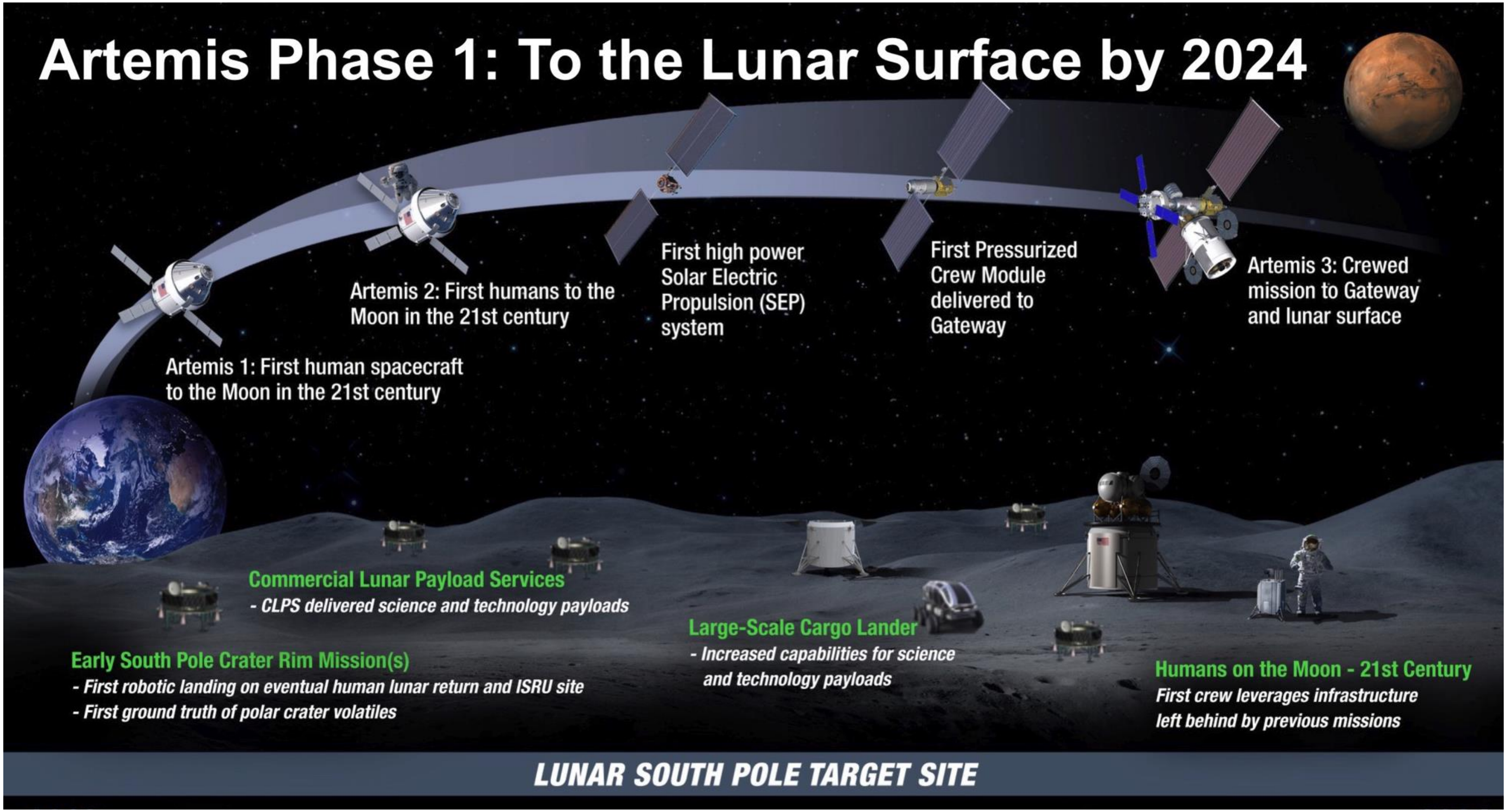
LEIA: Yeast experiment utilizing BioSentinel heritage payload and a new neutron detector (Mini-FND) on a CLPS lander to study impacts of lunar environment on biology. (Page 2)

BLISS-SWG: Group of scientists, skewed external to NASA, who offer opinion on what research the community thinks is important to conduct BLEO. Meet monthly on 3rd Thursday. (Page 6)

Mini-ECLS: Joint effort with MSFC to develop early concepts for small environmental control and life support systems for hosting biological payloads. (No updates)

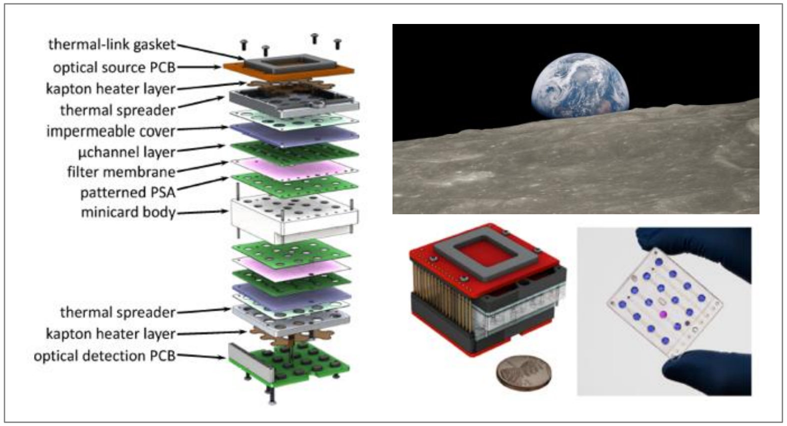
Artemis II: Passive fruit fly and nematode experiment in the Orion capsule on Artemis II based on previous Fruit Fly Lab ISS missions. (Page 5)

Pre-phase A to begin FY24 (No updates)


Artemis Phase 1: To the Lunar Surface by 2024
Artemis 1: First human spacecraft to the Moon in the 21st century
Artemis 2: First humans to the Moon in the 21st century
First high power Solar Electric Propulsion (SEP) system
First Pressurized Crew Module delivered to Gateway
Artemis 3: Crewed mission to Gateway and lunar surface
Commercial Lunar Payload Services
- CLPS delivered science and technology payloads
Early South Pole Crater Rim Mission(s)
- First robotic landing on eventual human lunar return and ISRU site
- First ground truth of polar crater volatiles
Large-Scale Cargo Lander
- Increased capabilities for science and technology payloads
Humans on the Moon - 21st Century
First crew leverages infrastructure left behind by previous missions
LUNAR SOUTH POLE TARGET SITE

Lunar Explorer Instrument for space biology Applications (LEIA)

- Mission Directorate: Science Mission Directorate (SMD)
  - **Theme**: Biological and Physical Sciences (BPS)
  - **Program**: Space Biology
- Key Personnel
  - **Project Manager**: Brandon Maryatt (ARC)
  - **Project Scientist**: Jessica Lee (ARC)
  - **PI:** Mark Settles (ARC)
  - **PI:** Sergio Santa Maria (ARC/UNM Cosmiac)
  - **Co-I:** Don Hassler (SwRI)
  - **Systems Engineer:** Leandro James (ARC)

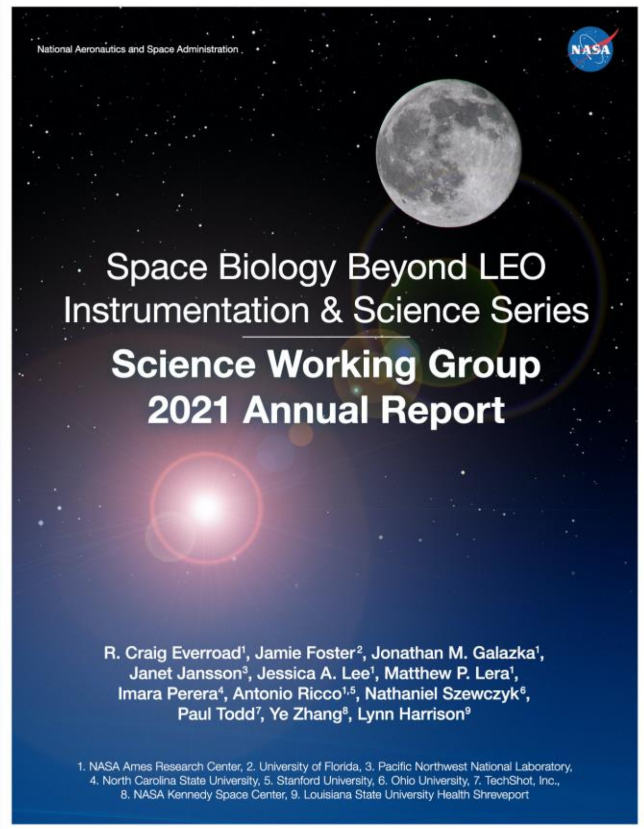


Diagrams of exploded (left) and assembled (center bottom) microfluidic minicard from BioSensor payload, with thermal control and optical measurement components. From Ricco et al. (2019) DOI:10.1109/MAES.2019.2953760

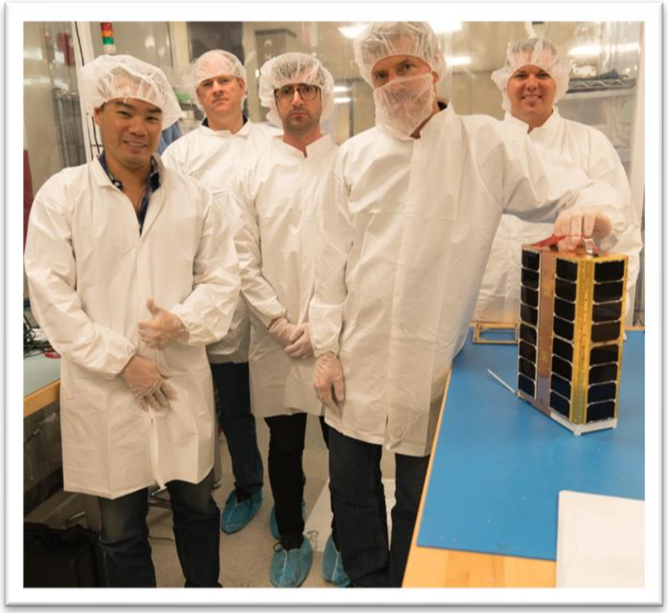
- Summary of Project
  - Autonomously culture a model organism on the lunar surface over the single-lunar day duration of a Commercial Lunar Payload Services (CLPS) mission, and measure the radiation environment and any biological radiation effect that may occur

Science Working Group

- **BLISS Science Working Group**
  - Consists of members of research community representing subject matter experts in fundamental Space Biology focusing on diverse model organisms
  - Skewed heavily external from research institutions, with support from NASA internal SMEs mostly in facilitator roles
  - Allows us to hear directly from research community on what they think is important research that needs to be conducted BLEO
- **2021 Annual Report released last fall**
  - Represents opinion of science community on what research can and should be conducted in BLEO environment in next 5 years
  - Adding members to expand 2022 SWG to include experts on higher organisms and AI/ML
- **Lunar Biology Technology (LBTech) Workshop**
  - April 20-21, 2022
  - Facilitated discussion between Science community, Payload Developers, and Platform Providers to strategize on BLEO research and infrastructure
  - We will compile breakout group discussions in an LBTech report for the community



Thank You



Michael Padgen , Matthew Lera, Macarena Parra, Tony Ricco, Matt Chin, Tori Chinn, Aaron Cohen, Charlie Friedericks, Mike Henschke, Tim Snyder, Stevan Spremo, Jing-Hung Wang, A C Matin

> Life Sci Space Res (Amst). 2020 Feb;24:18-24. doi: 10.1016/j.lssr.2019.10.007. Epub 2019 Oct 31.

EcAMSat spaceflight measurements of the role of σ^S in antibiotic resistance of stationary phase Escherichia coli in microgravity

Michael R Padgen [1], Matthew P Lera [2], Macarena P Parra [2], Antonio J Ricco [2], Matthew Chin [2], Tori N Chinn [2], Aaron Cohen [2], Charlie R Friedericks [2], Michael B Henschke [2], Timothy V Snyder [2], Stevan M Spremo [2], Jing-Hung Wang [3], A C Matin [4]

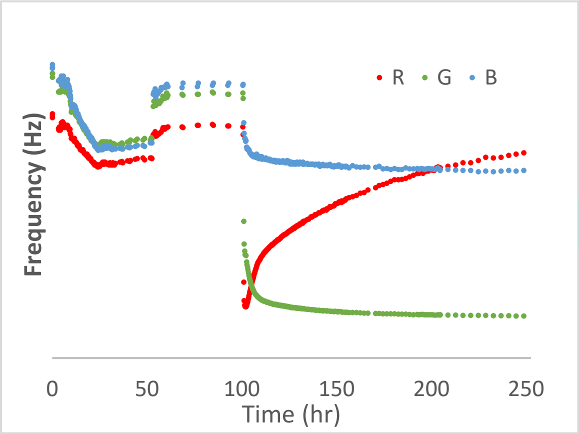
Affiliations + expand

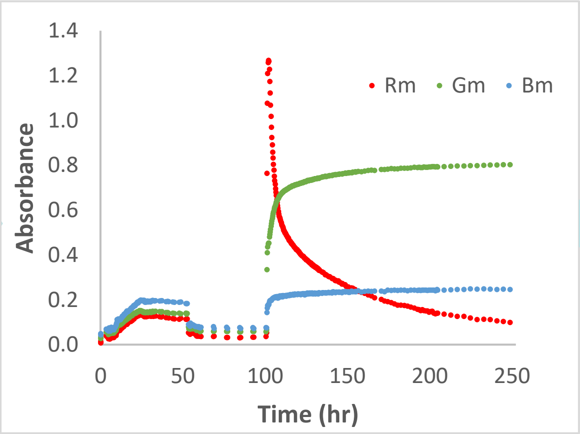
PMID: 31987476 DOI: 10.1016/j.lssr.2019.10.007

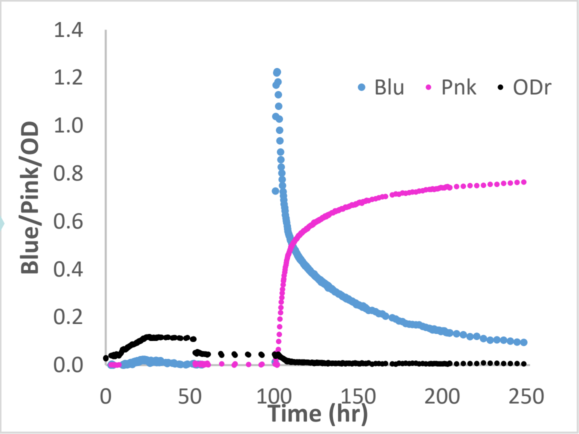
Back-up

Data Calibration

- Two critical regions for data calibration
  - Baseline (last hour of heating, only abs from cell OD)
  - Max AB (at end of AB pumping phase)
- Known absorbance at these points used to determine stray light corrections and calculate absorbance at all time points
- Measured absorbance of oxidized and reduced AB at different wavelengths used to separate Blue (ox) and Pink (red) forms of AB and cell OD

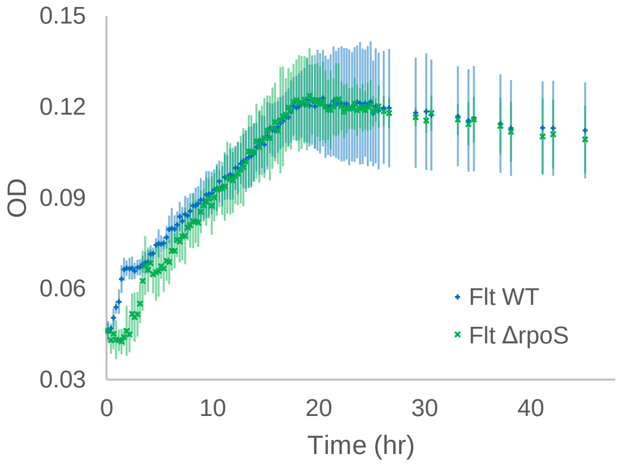


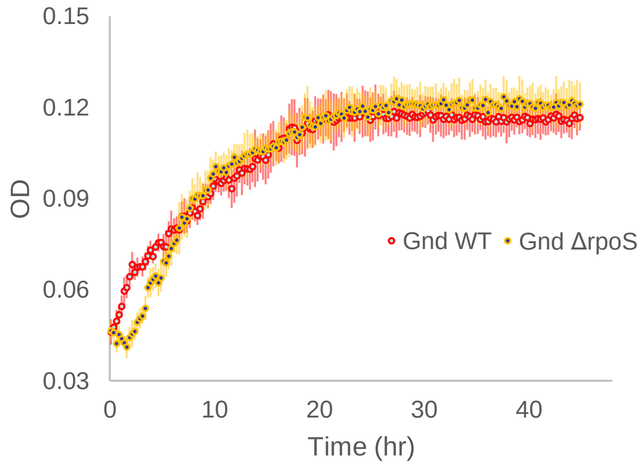




Flight vs Ground – Growth Phase

- Similar growth seen in flight and ground.
  - Max OD at lag phase similar between flight and ground.
- Average OD after Max OD reached declined in flight samples.
  - No such decline seen in ground samples or in any preliminary science testing

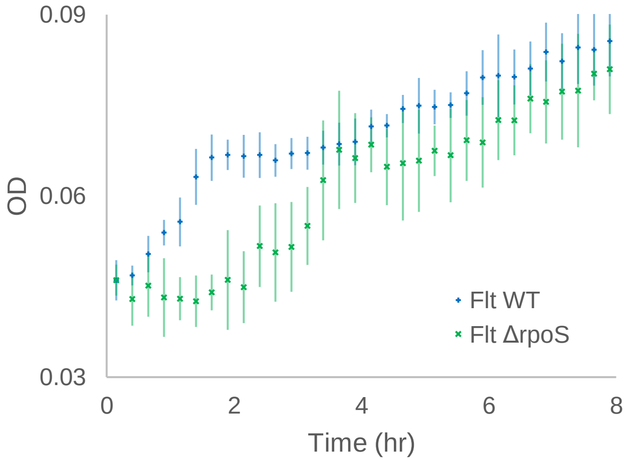


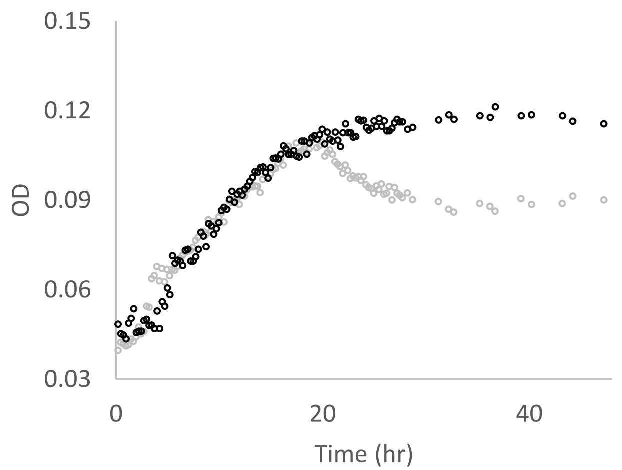


Averages of one bank during growth phase from flight (top) and ground (bottom) (error bars +/- SD)

Flight vs Ground – Growth Phase

- The lag in growth of ΔrpoS compared to the WT was seen in flight as expected (top)
- Roughly half the wells of the flight experiment displayed a decline in OD after the Max OD was achieved (bottom)

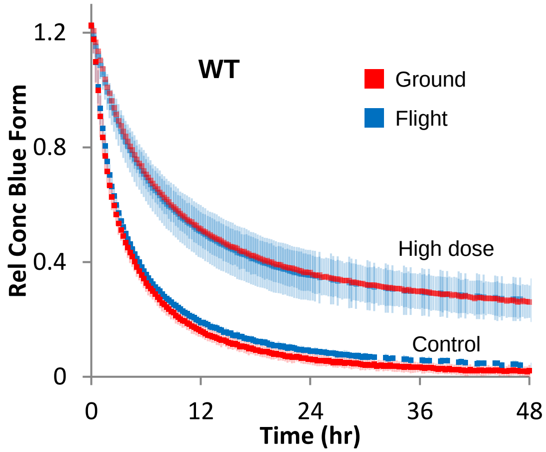


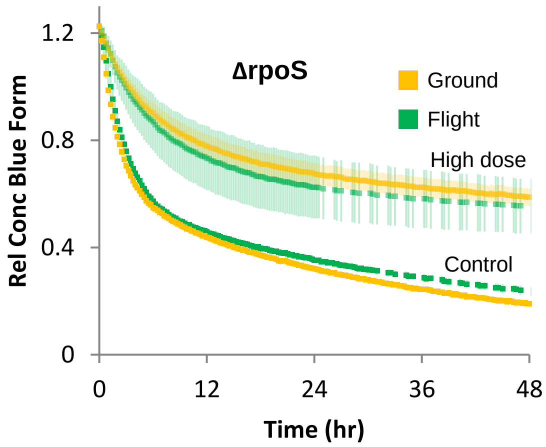


Zoom of previous figure showing typical lag in growth of ΔrpoS vs WT (top) and aforementioned dip in OD (bottom)

Flight vs Ground – Alamar Blue

- WT cells from the high antibiotic dose bank showed slower reduction than those from control bank as expected (top).
- ΔrpoS cells in the high antibiotic dose bank also reduced alamar blue more slowly than in the control bank (bottom).



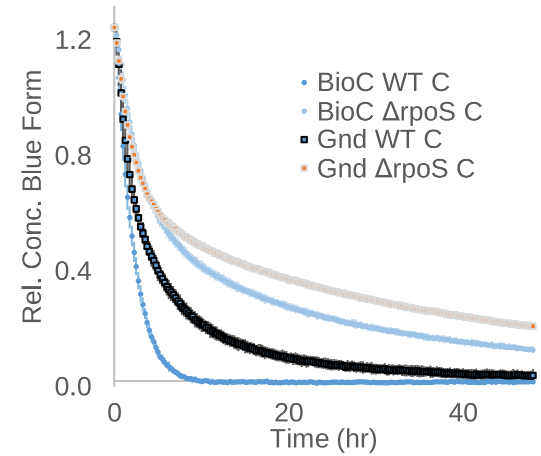


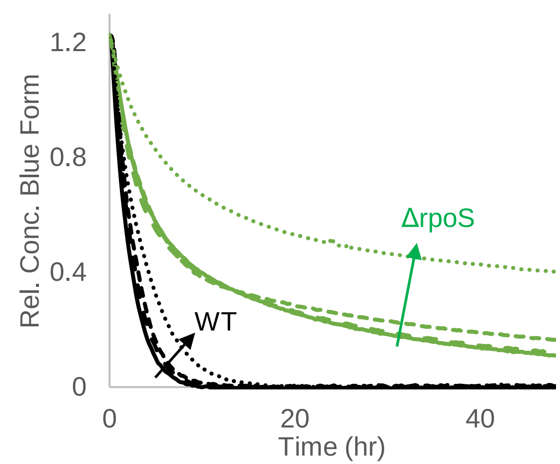
Averages of high dose and control banks of flight and ground in WT (top) and ΔrpoS (bottom) (error bars +/- SD)

Ground vs. Biocomp Control – Alamar Blue

- A second Biocompatibility Control was run to explore effects of system itself on alamar blue reduction
  - Ground control spent stasis time in can
  - Bicompatibility Control spent less that 1 day in can prior to experiment initiation.
- WT and ΔrpoS both reduced alamar blue more rapidly in the biocompatibility control than in ground control
- Only the highest concentration of antibiotic seemed to affect the alamar blue reduction in either strain in the biocomp control.

Run	Cell Load	Assy	LB Feed	Stasis	Can Time
Flight	9/27/17	10/3/17	11/24/17	58 days	54 days
Ground 2	9/27/17	10/11/17	11/26/17	60 days	46 days
Ground 3	9/27/17	12/12/17	12/13/17	77 days	<1 day

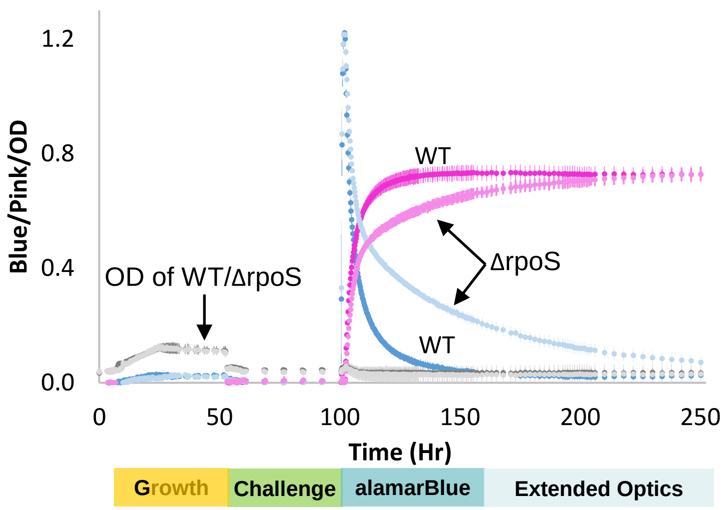


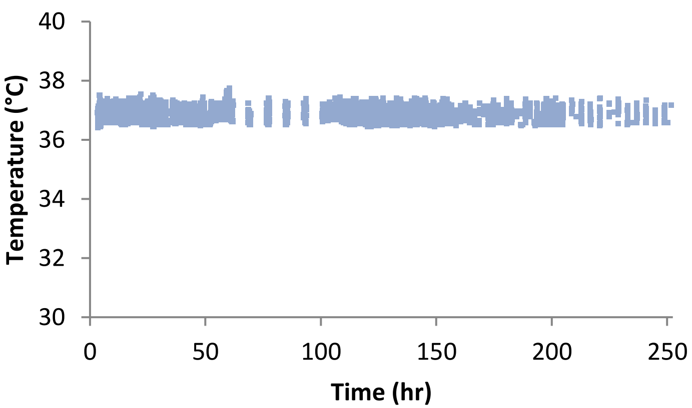


Reduction of alamar blue in biocomp vs ground control (top). Reduction of alamar blue of WT and ΔrpoS of high, medium, low, and no antibiotic.

Flight Payload Performance

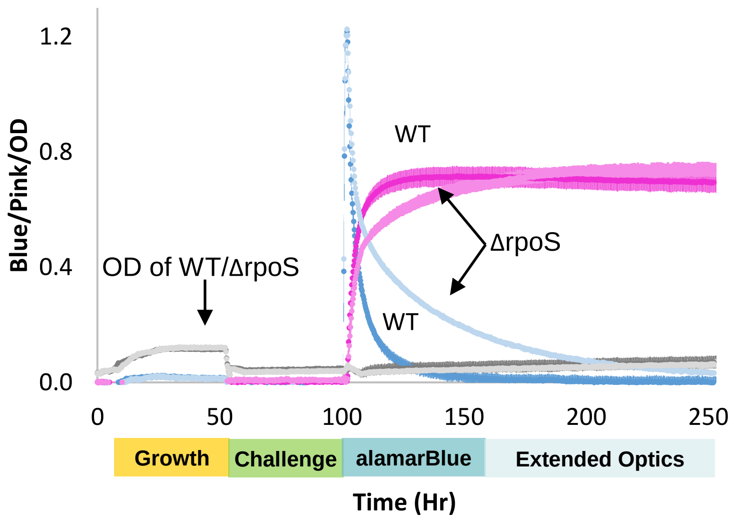
- Experiment started autonomously
- Fluidic Card
  - Performed nominally
- Fluidic System
  - All pumping was nominal
- Optics
  - 1 Blue LED lost (n=5 Δrpos C)
    - Historically better performance than PharmaSat, O/OREOS
  - Recorded extra 96 hr data
- Thermal
  - Card temp remained 37+/-0.6°C for nominal experiment plus 96 hr





Ground Control #2 Performance

- Experiment started autonomously 2 days after flight to mimic flight starting condition
- Fluidic Card
  - Internal leak, known failure path
  - No hardware damage/minimal loss of science



- Fluidic System
  - All pumping was nominal
- Optics
  - 5 Blue LEDs lost (3 experiment wells, 2 reference wells)
  - Recorded extra 96 hr data
- Thermal
  - Temp remained 37 +/- 0.5°C for nominal experiment plus 96 hr
- No contamination found