

Integrated Microfluidic Bioanalytical Systems for CubeSats: Growing and Monitoring Microbial Cultures in Outer Space

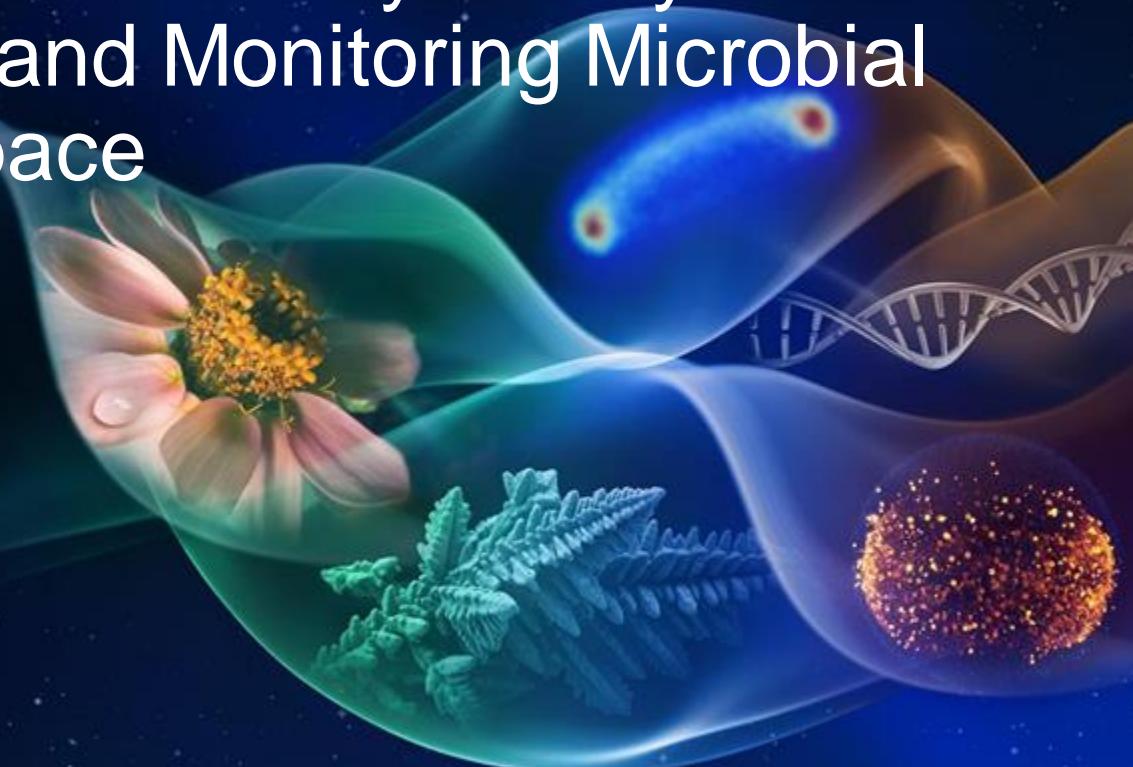
Presentation by:

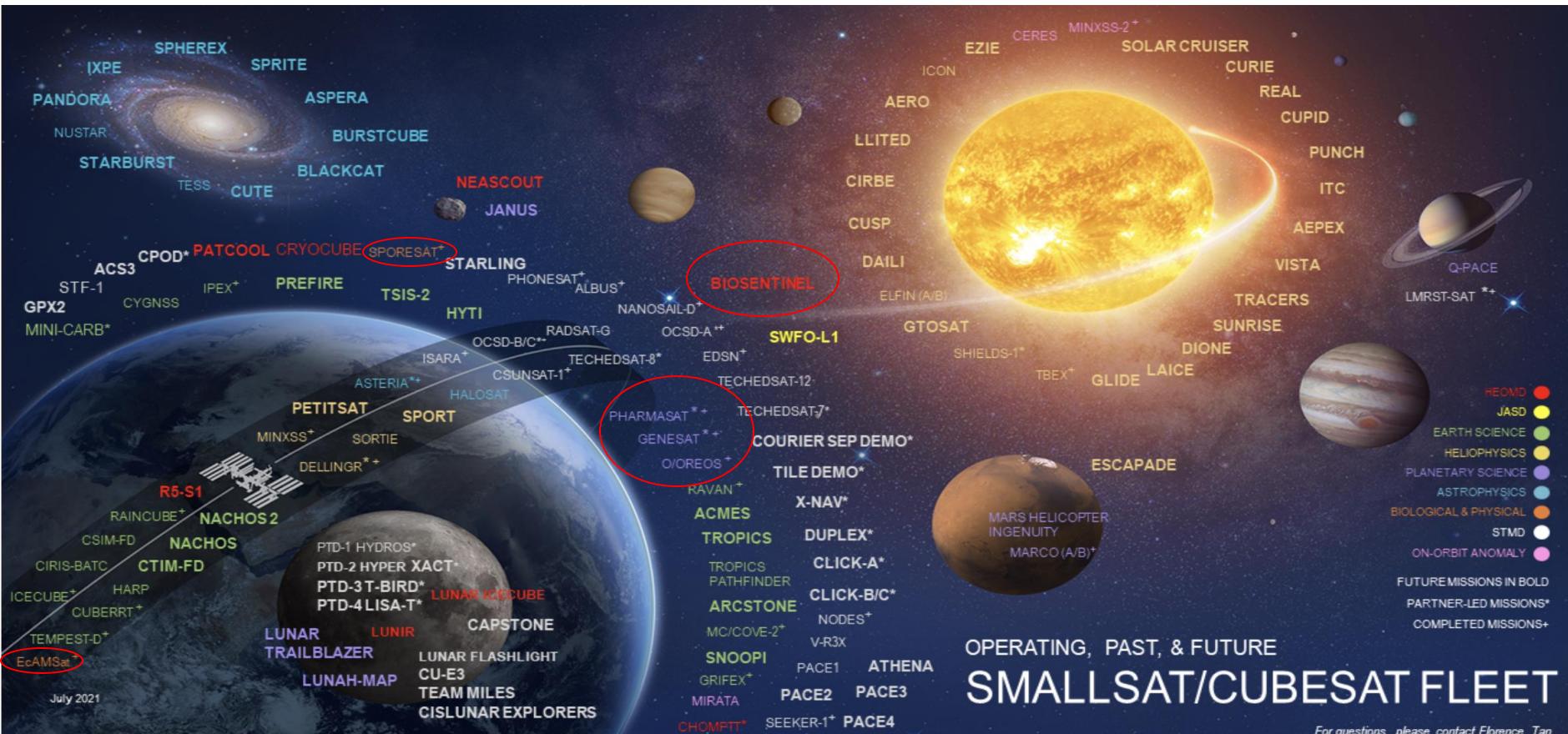
Diana Ly

Macarena Parra

Tony Ricco

NASA Ames Research Center





Ames Biological Small Satellite Payloads & Missions



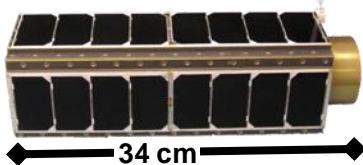
Mission* (format)	Description	Launch Date	Outcome
GeneSat-1 (3U)	<ul style="list-style-type: none"> 2U payload measured expression of GFP in <i>E. coli</i> and tracked microbe population via light scattering 1st NASA nanosatellite mission; 1st biological payload to fly in space in cubesat platform 	2006	Full mission success
PharmaSat (3U)	<ul style="list-style-type: none"> 2U payload measured antifungal drug dose response for <i>S. cerevisiae</i> using colorimetry to measure metabolic activity and population vs. time 1st NASA PI-led nanosatellite mission 	2009	Full mission success
O/OREOS (3U)	<ul style="list-style-type: none"> Two independent 1U astrobiology payloads measured (a) survival of <i>B. subtilis</i> to 6 months and (b) photo-degradation of biomarkers and bio-building blocks for > 1.5 years via UV-visible spectroscopy High-radiation high-inclination orbit; de-orbit mechanism; cells rehydrated in space 	2010	Full mission success, both payloads Spacecraft operable ~ 5 years in orbit
SporeSat-1 SporeSat-2 (3U)	<ul style="list-style-type: none"> 2U payload measures gravitational response of <i>C. richardii</i> fern spores via Ca²⁺ ion channel response Variable g in microgravity ambient using 50-mm μcentrifuges with 32 ion-specific [Ca²⁺] electrode pairs 	SporeSat-1: 2014 SporeSat-2: dhyb	SporeSat-1: Successful spaceflight demo. of μcentrifuges & integral ion-specific electrodes
EcAMSat (6U)	<ul style="list-style-type: none"> 2U payload measured antibiotic resistance in microgravity vs. dose for uropathogenic <i>E. coli</i> 1st biological nanosat deployed from ISS; 1st Ames 6U biological nanosat 	2017	Full mission success
BioSentinel (6U)	<ul style="list-style-type: none"> 4U payload to measure radiation-induced DNA damage in radiation-sensitive <i>S. cerevisiae</i> strain and correlate with physical radiation dosimetry and spectroscopy NASA Ames' 1st deep space nanosat.; 1st biology experiment beyond low Earth orbit since Apollo era 	2021(?)	TBD

Ames Biological Small Satellite Payloads & Missions



GeneSat (2004-2006-2010)

- Orbit: Low Earth Orbit, 440 km
- Mission duration: 1 month
- Orbital lifetime: 3.7 years
- Relevant TID*: ~ 0.5 Gy
- Program: FSB* (ESMD)

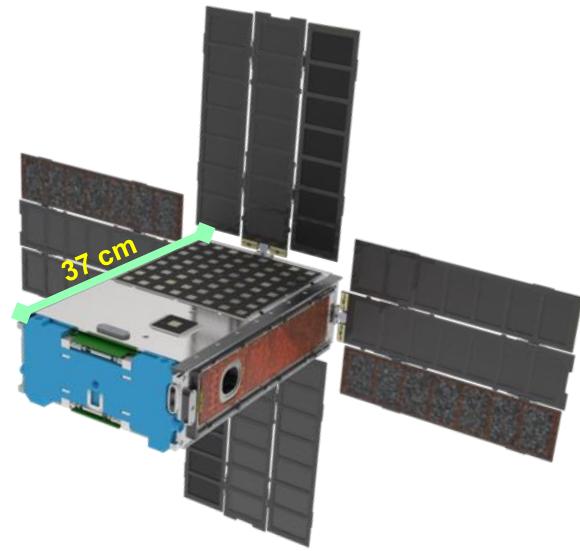
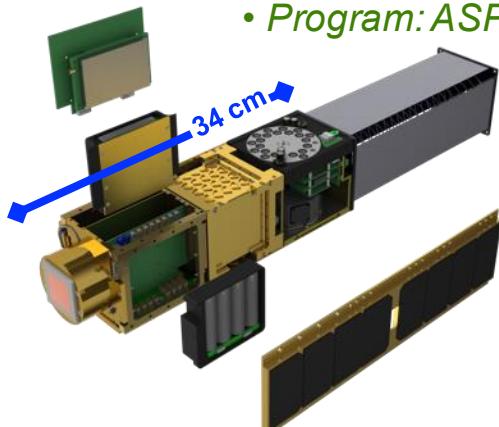


experience

time

O/OREOS (2008-2010-2032)

- Orbit: High-inclination LEO, 650 km
- Mission duration: 6 – 18 months
- Orbital lifetime: ~22 years
- Relevant TID*: 1 – 50 Gy
- Program: ASP* (SMD)



BioSentinel (2013-2021-7500000000)

- Orbit: Interplanetary (heliocentric), 100 k – 60 M km
- Mission duration: 3 – 9 months
- Orbital lifetime: ~ 7.5×10^9 years
- Relevant TID*: ~ 3 Gy
- Program: AES* (HEOMD)

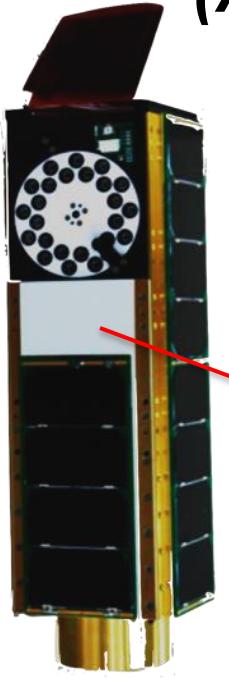
*TID = total ionizing dose

*FSB = Fundamental Space Biology

*ASP = Astrobiology Small Payloads

*AES = Advanced Exploration Systems

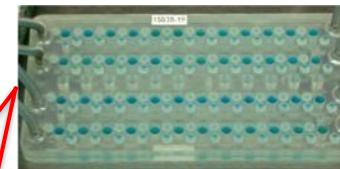
Summary of NASA Ames' Nanosatellite (Astro)Biological Space Missions



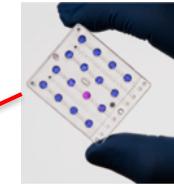
O/OREOS



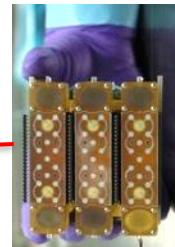
E. coli GeneSat-1 (2006/3U): *gene expression*
EcAMSat (2017/6U): *antibiotic resistance*



S. cerevisiae PharmaSat (2009/3U): *drug dose response*
BioSentinel (2021/6U): *DNA break/repair*



B. subtilis O/OREOS* (2010/3U): *survival, metabolism*



C. richardii SporeSat-1 (2014/3U): *ion channel sensors, μ-centrifuges*
SporeSat-2 (3U): *plant gravity sensing threshold*

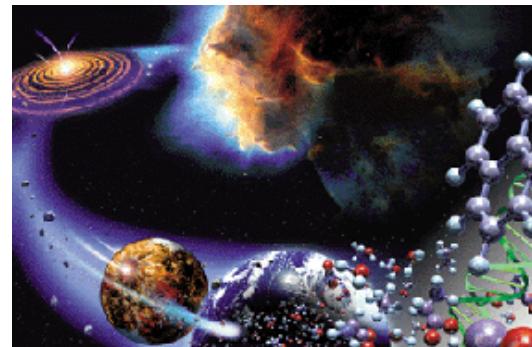


*Organism/Organic Exposure to Orbital Stresses

Astrobiology & Space Biology

Astrobiology: origin, evolution, distribution, & future of life in the universe

- **Why:** fundamental understanding of life
- Prebiotic chemistry – chemical building blocks of life – details, distribution
- Potential for life to adapt/survive in non-terrestrial environments
- Search for indicators of extant or extinct non-terrestrial life
- Find habitable environments in our solar system & beyond



Fundamental Space Biology: space environment effects on terrestrial life

- Reduced gravity effects
 - **Mammals:** fluid distribution, musculoskeletal loading \Rightarrow immune stress, decreased bone density, muscle atrophy, slowed wound healing
 - **Cells, microbes** in culture: nutrient and waste transport
- Radiation effects: damage from (high-energy) ionizing radiation
 - Greater outside Earth's magnetosphere, $\sim 70,000$ km
 - DNA damage: strand breaks, mutations
 - Cell membrane, protein, oxidative damage; cell death
- Bio/chemical effects of extraterrestrial environments: dust etc.
- Synergies of combined effects possible
- **Why:** **human space travel, moon/planetary habitation;**
insights & therapies for human disease, aging, radiation effects



Biology Beyond LEO is integral to NASA's goals

Conditions Beyond Low Earth Orbit cannot accurately be simulated closer to home

- Unique radiation environment
- Combined radiation + partial gravity

BLEO research addresses multiple recommendations of the 2010 Decadal Survey in Biological & Physical Sciences

- P2: robust spaceflight program to understand biological responses to multiple spaceflight stimuli
- CC8, CC9: Use of model organisms to assess radiation risk to humans in space – in a way that cannot be simulated on the ground

Engineering challenges still limit life support/experiment support systems

- Radiation shielding
- Biocompatibility
- Thermal environment
- Data transmission
- Autonomy
- Duration/Late Load

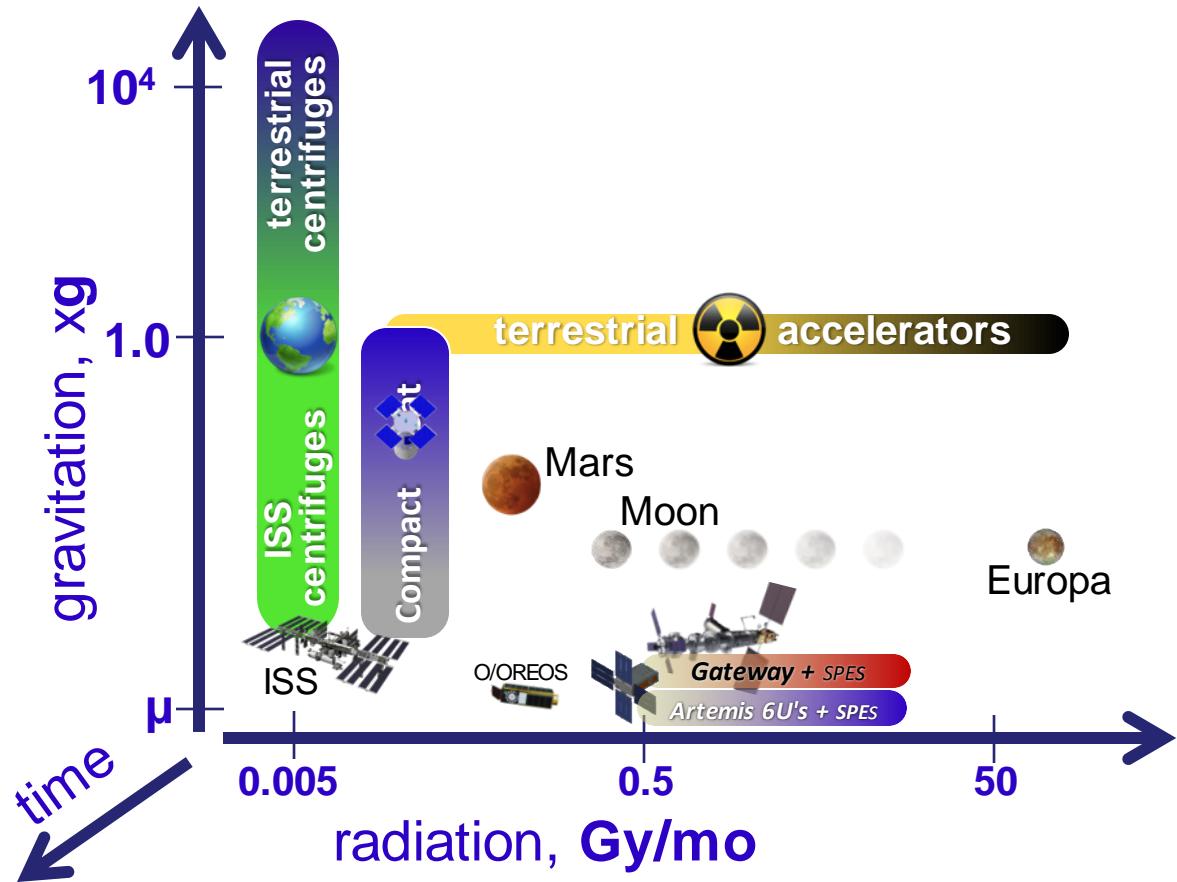


Theme	Strategic Goal	Strategic Objective
DISCOVER	EXPAND HUMAN KNOWLEDGE THROUGH NEW SCIENTIFIC DISCOVERIES.	1.1: Understand the Sun, Earth, Solar System, and Universe. 1.2: Understand Responses of Physical and Biological Systems to Spaceflight.
EXPLORE	EXTEND HUMAN PRESENCE DEEPER INTO SPACE AND TO THE MOON FOR SUSTAINABLE LONG-TERM EXPLORATION AND UTILIZATION.	2.1: Lay the Foundation for America to Maintain a Constant Human Presence in Low Earth Orbit Enabled by a Commercial Market. 2.2: Conduct Exploration in Deep Space, Including to the Surface of the Moon.

In NASA's Strategic Plan 2018, the Discover and Explore themes prompt Astrobiology and Space Biology BLEO research

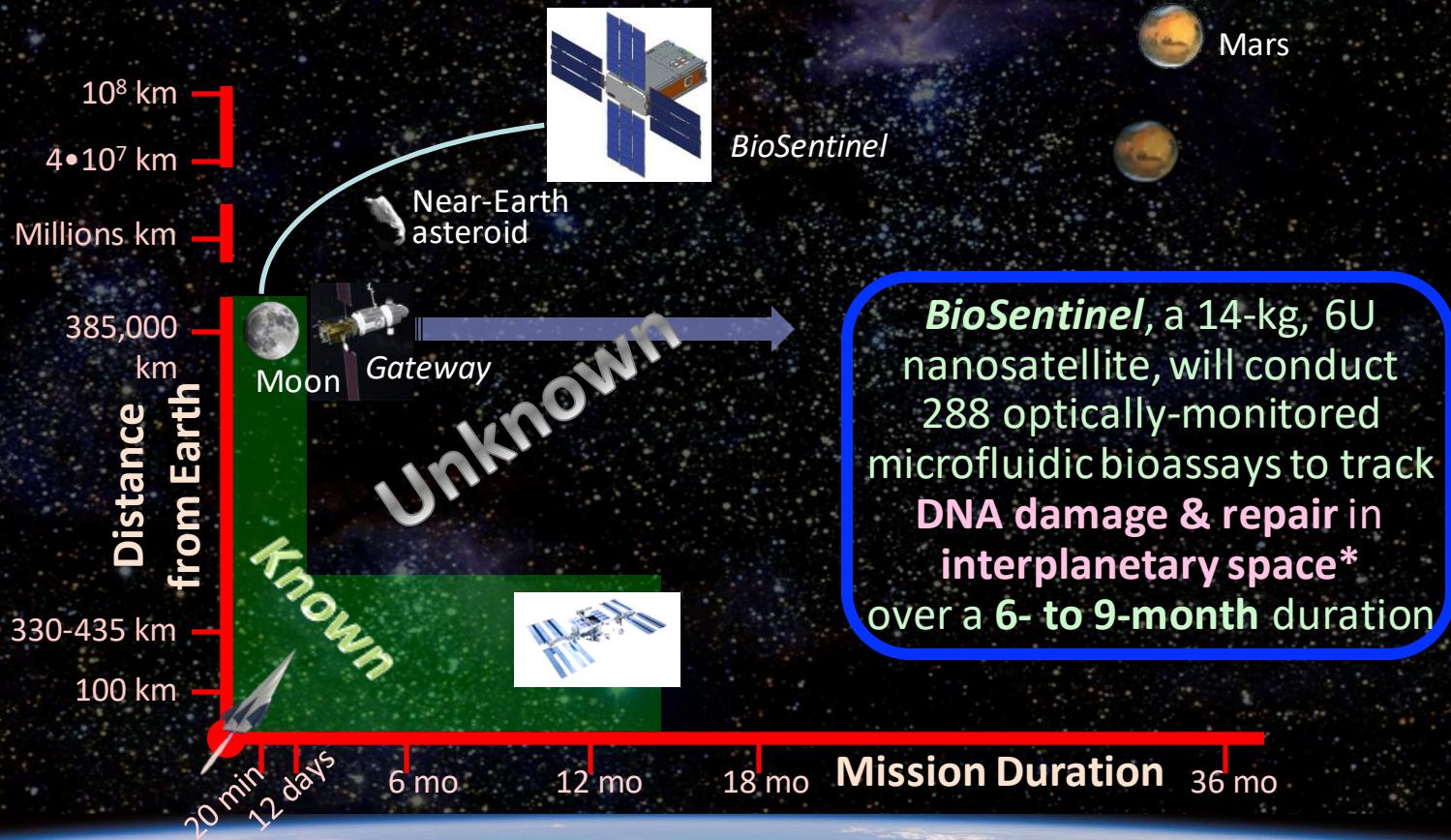
BioMapping the gravity-radiation-time "landscape"

New options for orbits / destinations, rotation rates, and mission duration are expanding parametric coverage



"Biomapping" our solar system: going beyond the known low-altitude / short-duration mission parameter space...

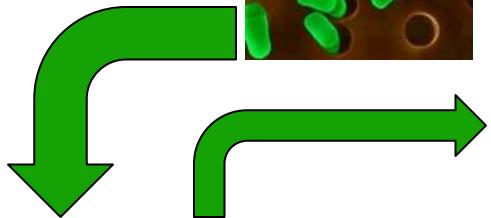
BioMapping:
Looking for
Life
Signatures
and testing
Terrestrial
Life
in many
locations



model organism:
0.5 x 2 μm
bacteria
E. coli



GeneSat-1: 1st biological nanosatellite in LEO, 1st real-time, gene expression measurement in space

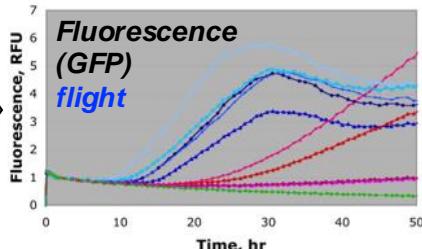


- nutrient deprivation in dormant state (6 weeks)
- launch: December 16, 2006 to low Earth orbit (440 km)
- nutrient solution feed upon orbit stabilization, grow *E. coli* in μg gravity
- monitor green fluorescent protein (**GFP**): gene expression
- monitor optical density: cell population

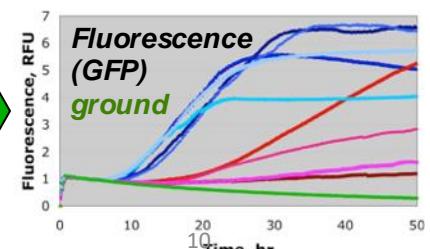
16 December
2006



Telemeter data
to Earth



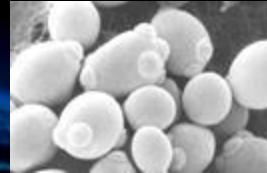
Compare to
ground data





PharmaSat: Effect of Microgravity on Yeast Susceptibility to Antifungal Drugs

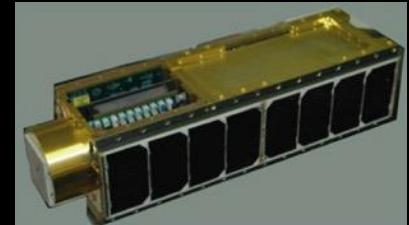
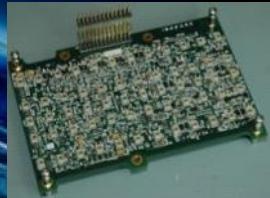
- Grow yeast in multiwell fluidics card in μ -gravity
 - Measure inhibition of growth by antifungal
 - Optical absorbance (turbidity: cell density)
 - Metabolism indicator dye: Alamar Blue
 - Control + 3 concentrations of antifungal



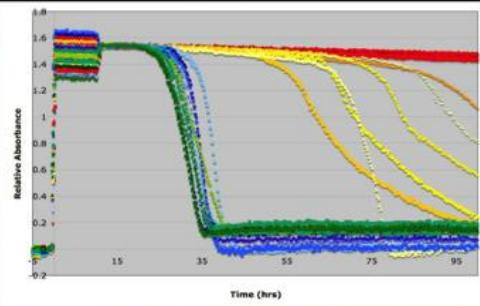
S. cerevisiae



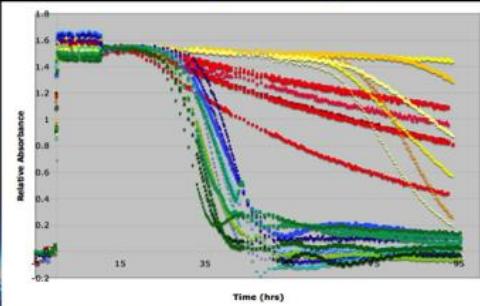
19 May 2009



Ground



Spaceflight





O/OREOS Astrobiology Mission



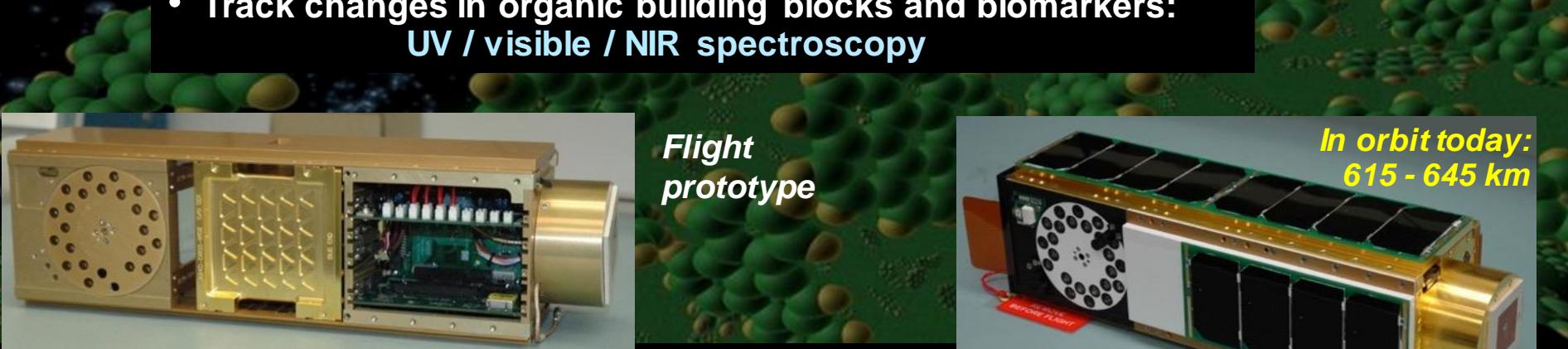
Kodiak,
Alaska
Nov
19,
2010

Effects of space exposure on
biological organisms (6 mos.)
& organic molecules (18 mos.)

- Monitor survival, growth, and metabolism of *Bacillus subtilis*: *in-situ* optical density / colorimetry
- Track changes in organic building blocks and biomarkers: UV / visible / NIR spectroscopy



Minotaur IV



Flight
prototype

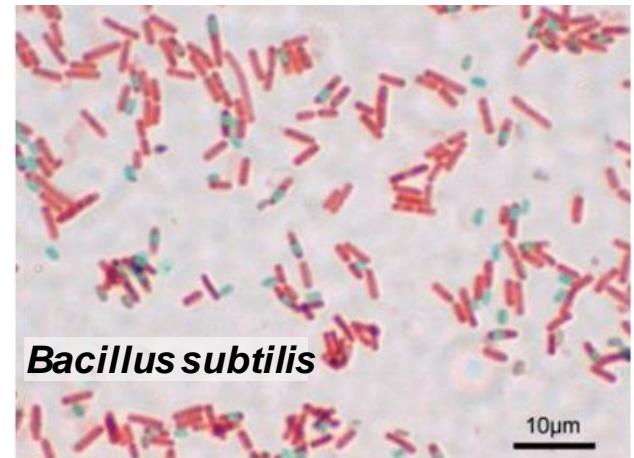
In orbit today:
615 - 645 km

Orbital life-time:
~ 22 yr

Payload 1: Space Environment Survivability of Live Organisms (SESLO)

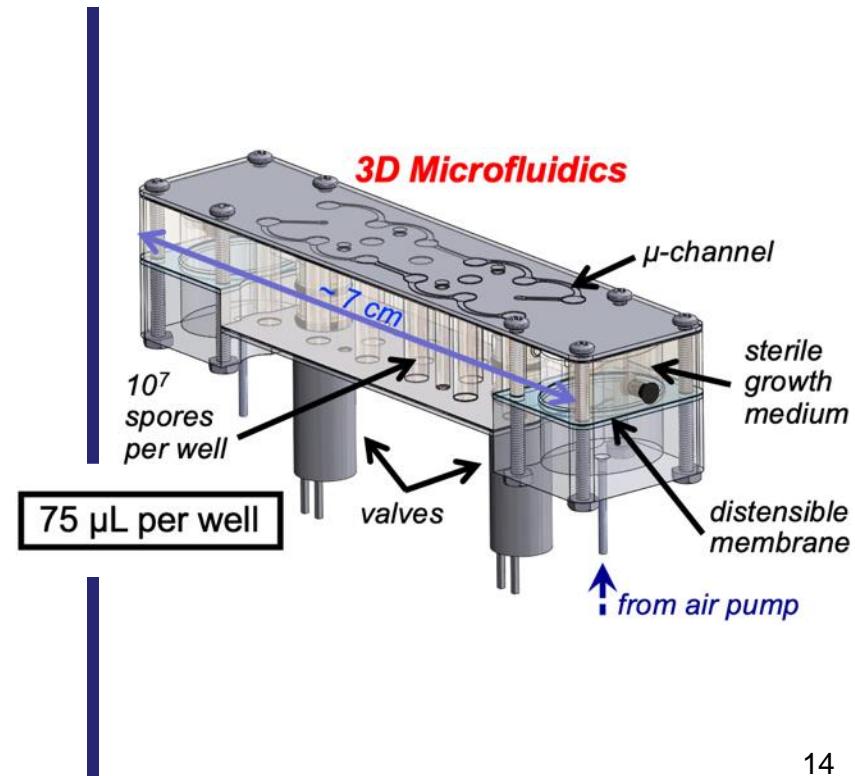
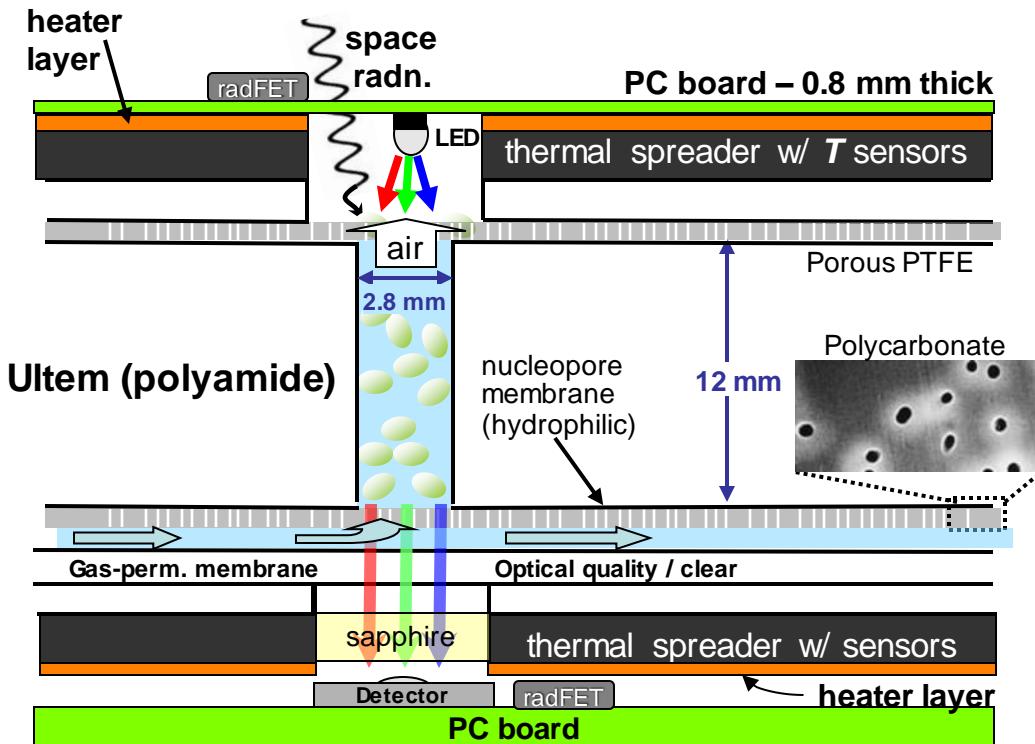
Organisms, wildtype & mutant, exposed to μ gravity & space radiation

- On Earth: dry organisms on microwell walls
- In space: Rehydrate & feed 6 μ wells / organism:
 $t = 2 \text{ wk}, 3 \text{ mo}, 6 \text{ mo}$ (**requires perfect sterility**)
- Grow @ 37°C for ~3 days
- Measure **RGB** absorbance@ **615, 525, 470 nm**
 - track culture population via optical density
 - track metabolic activity via [Alamar Blue]
- Sensors: **T, p, RH, rad** (integrated dose), **μ grav**
 - » temperature (6 sensors per 12-well bioblock)
 - » pressure, relative humidity (1 sensor each)
 - » radiation total dose @ both ends of wells (2 radFETs)
 - » microgravity levels calc'd. from solar panel currents

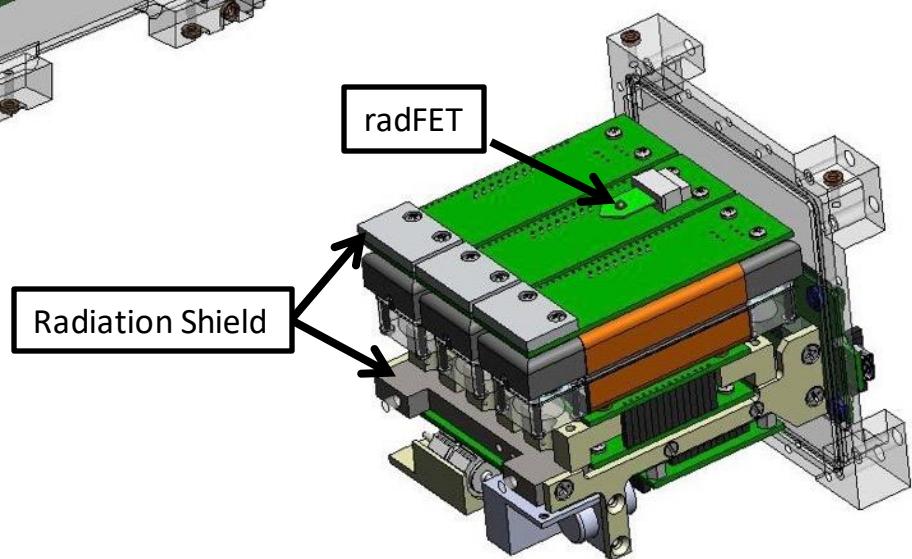
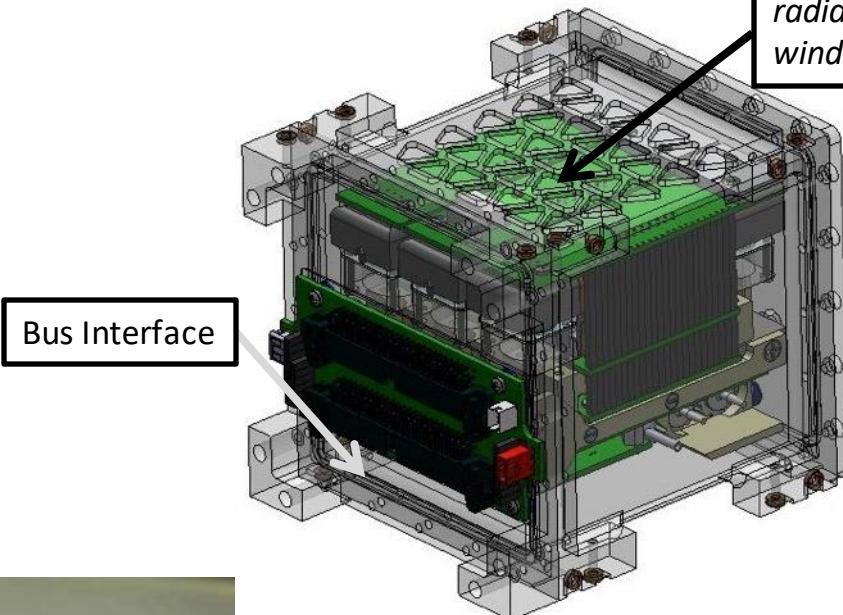


SESL payload on O/OREOS

Biological / fluidic / optical / thermal cross-section



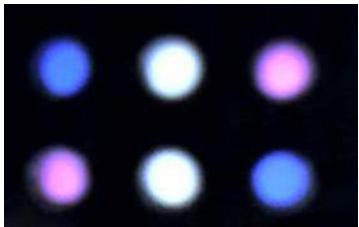
SESL Integration Summary



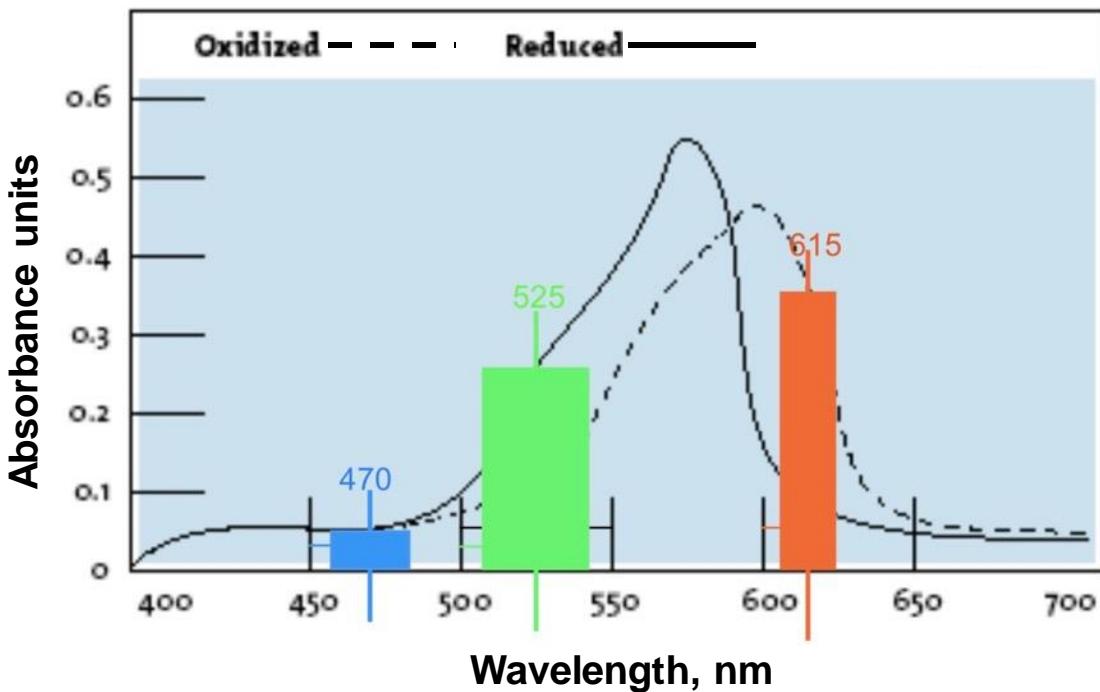
AlamarBlue:

“viability dye”
changes color &
becomes
fluorescent in
response to
metabolic activity of
almost any cell type

— some cells under
some conditions
subsequently convert
pink form to colorless



Alamar Blue spectra in both forms with LED wavelengths & bandwidths

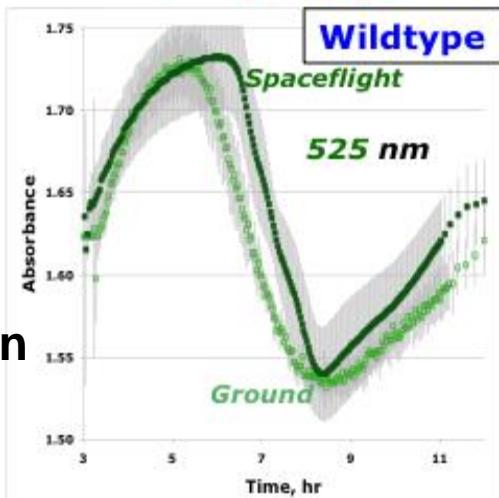


SESLO
Spaceflight
Results:
Growth of
B. subtilis in
space vs.
ground

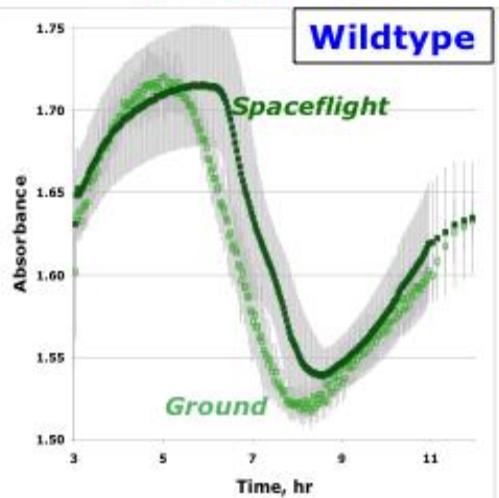
WL Nicholson,
AJ Ricco, et al,
Astrobiology, 11,
951-958 (2011)

WL Nicholson
and AJ Ricco,
Life, 10, 1-14
(2020)

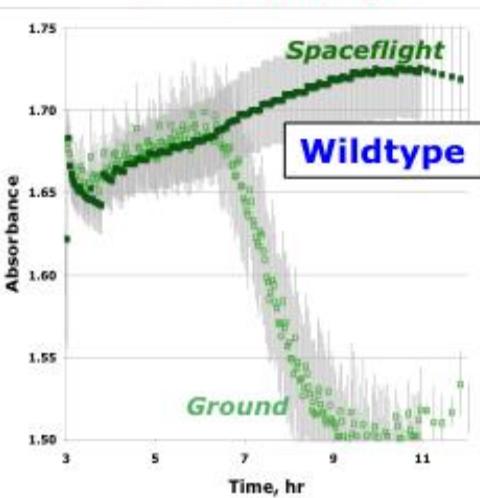
t = 14 days



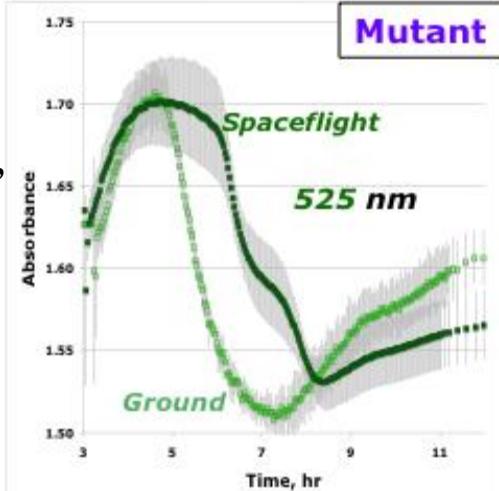
t = 97 days



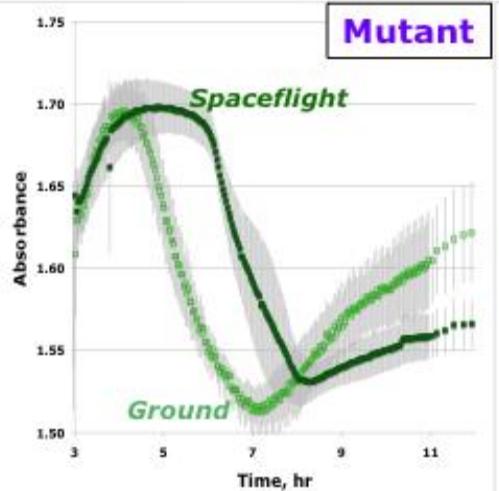
t = 180 days



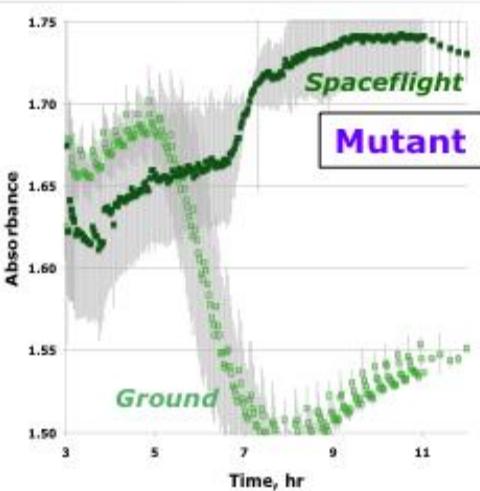
Mutant



Mutant

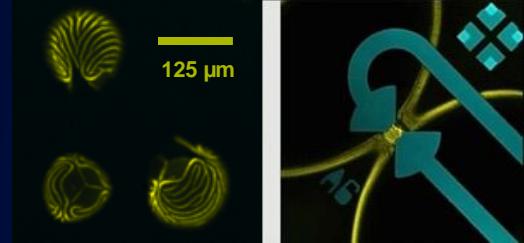


Mutant



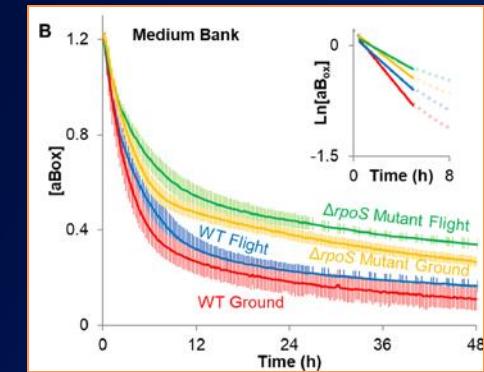
SporeSat

- PI team: J. Rickus, A. Salim, Purdue University; S. Roux, University of Texas, Austin
- Objective: Investigate gravity dependence of fern-spore single-cell calcium ion fluxes during germination
- System: *Ceratopteris richardii*
- Variables: Gravitation: micro- \mathbf{g} to 2x \mathbf{g}
- Measurement: Differential calcium ion concentration between gravitational “top” and “bottom” of each spore
- 3 U, 5.5 kg (SporeSat-1)
- 18 Ap 2014 Launch, SpaceX Dragon Cargo Capsule, CRS3
- 18 Ap 2014, deployed at 400 km altitude prior to ISS arrival
- ~100 kB payload data
- Findings:
 - Long pre-launch duration (> 100 days) + illumination source failure prevented spore germination
 - Ion-selective electrode pairs, mini-centrifuges, thermal-control functioned nominally: provided baseline response
 - Ground experiments demonstrated variable-gravity response to net acceleration (rotation plus gravitation)
 - SporeSat-2 developed to address SS-1 hardware issues



E. coli AntiMicrobial Satellite (EcAMSaT)

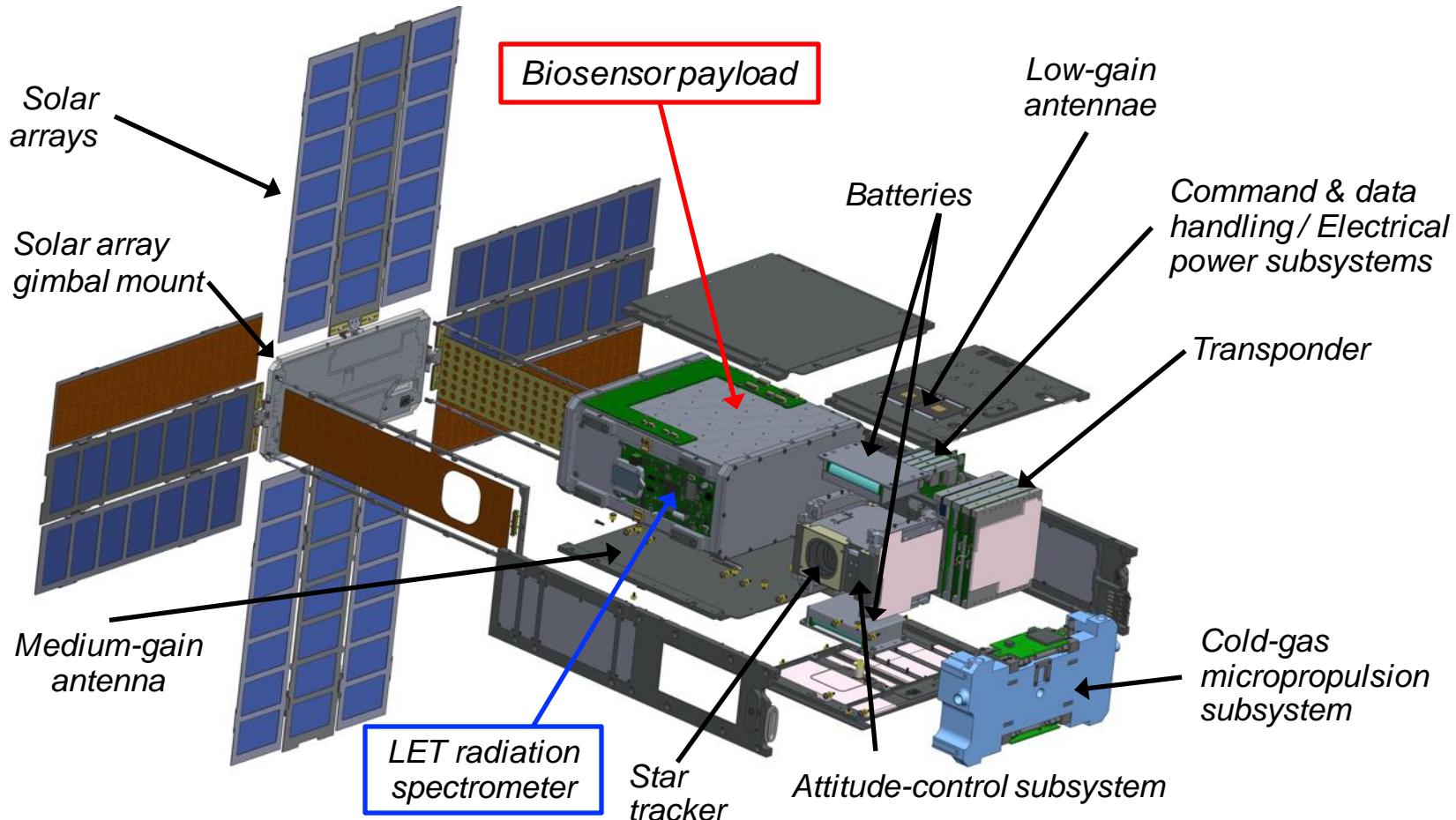
- PI: AC Matin, Stanford University
- Objective: Determine how microgravity alters the antibiotic resistance of *E. coli* and the role of the gene *rpoS* in antibiotic resistance
- System: *E. coli* wild-type and $\Delta rpoS$
- Variables: zero, low, medium, high dose of gentamicin
- Measurement: Alamar Blue absorbance
- 6 U, 11 kg
- 12 Nov 2017 Launch
- 20 Nov 2017 ISS Deployment (NanoRacks)
- ~100 kB payload data
- Findings:
 - Microgravity reduced resistance in both strains
 - Multi-week storage made $\Delta rpoS$ less metabolically active
 - Loss of *rpoS* makes *E. coli* more susceptible to stressors



Earth Orbit & Beyond: Radiation Environments

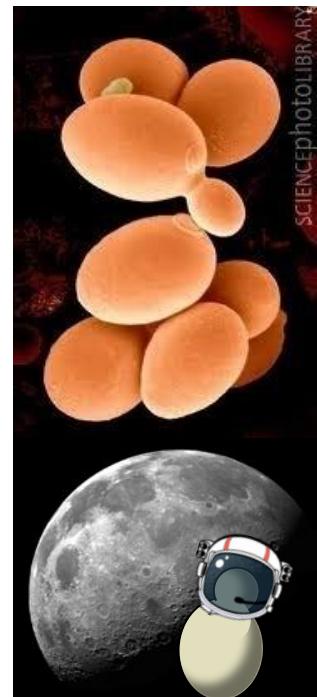
Orbit	Altitude (from sea level, km)	Orbital Inclination ^a	Orbital Period around Earth	Predominant Particle Radiation Sources	Shielding-Dependent Monthly Radiation Dose ^b (Gy)	
					1 mm ^c	5 mm ^c
Low Earth Orbit (LEO)	300 – 2000	0 – 55°	90 – 127 min	electrons, protons	0.0061 – 660	0.0041 – 36
ISS in LEO	330 – 435	51.6°	91 – 93 min	electrons, protons	5 – 30	0.34 – 0.020
High-inclination LEO^d	400 – 2000	65° – 115°	92 – 127 min	electrons, protons, GCRs, SEPs ^h	40 – 1500	0.69 – 140
Sun Synchronous LEO, including (near-) polar	400 – 1000 (typical)	~98° & others	92 – 105 min	electrons, protons, GCRs, SEPs	40 – 180	0.86 – 10
Medium Earth Orbit (MEO)	2000 – 35 750	Various	2 – 23.9 hr	electrons, protons (Van Allen Belts)	40 – 9700	0.69 – 190
Geosynchronous Equatorial Orbit (GEO)	35 786	0°	23.93 hr	electrons (Outer Van Allen Belt)	3300	32
Highly Elliptical Orbit (HEO)^e	perigee < 1000 apogee > 35 800	Various	10.6 – 26 hr	electrons, protons (Van Allen Belt(s))	4.7 – 11000	1.3 – 190
Lunar libration points^f	L1: 326 400 L2: 444 400	5°	27 – 29 d	GCRs, SEPs	11 – 140	0.55 – 21
Lunar orbit^j	perigee: 363 104 apogee: 405 700	5°	27 d	GCRs, SEPs, neutrons	7.7 – 96	0.38 – 15
Interplanetary space^g	> ~ 100 000		N/A	GCRs, SEPs	11 – 140	0.55 – 21

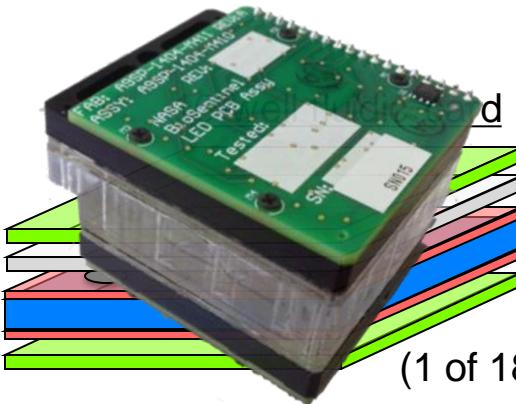
BioSentinel Subsystem Overview



BioSentinel Science Mission: “*Canary in a Coal Mine*”

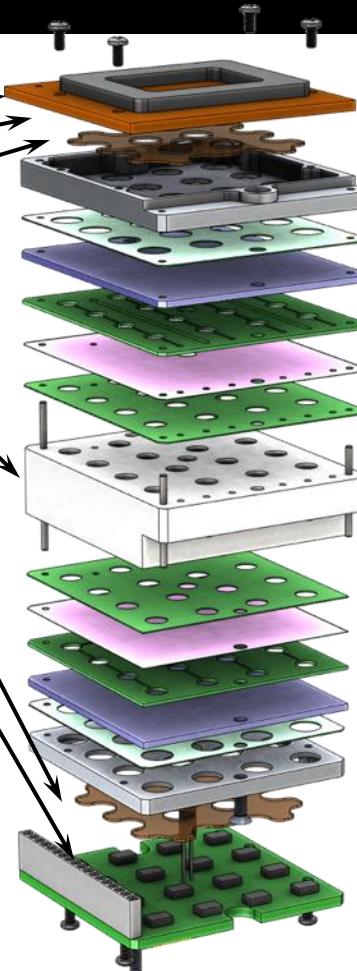
- **Quantify DNA damage from space radiation environment**
 - Deep space environment cannot be reproduced on Earth: *omnidirectional, continuous, low flux, variety of particle types*
 - Health risk for humans spending long durations beyond LEO
 - Radiation flux can spike 1000x during a solar particle event (SPE)
- **Yeast assay: microfluidic arrays monitor DNA damage**
 - Two strains of *S. cerevisiae*: 1 control (wild-type), 1 mutant
 - *engineered strain is sensitive to DNA damage, esp. double-strand breaks (DSBs)*
 - Wet and activate multiple banks of yeast in μ wells over mission duration
 - DNA damage impairs cell growth & division, esp. for *rad51 Δ* mutant
- **Correlate biological response with physical radiation measurements**
 - **Linear Energy Transfer** (LET) spectrometer bins and counts particle events by their LET
 - Total Ionizing Dose (TID): calculation of integrated deposited energy by LET system





(1 of 18)

optical PCB/source
optical diffuser
heater layer
fluidic card
heater layer
optical PCB/detection



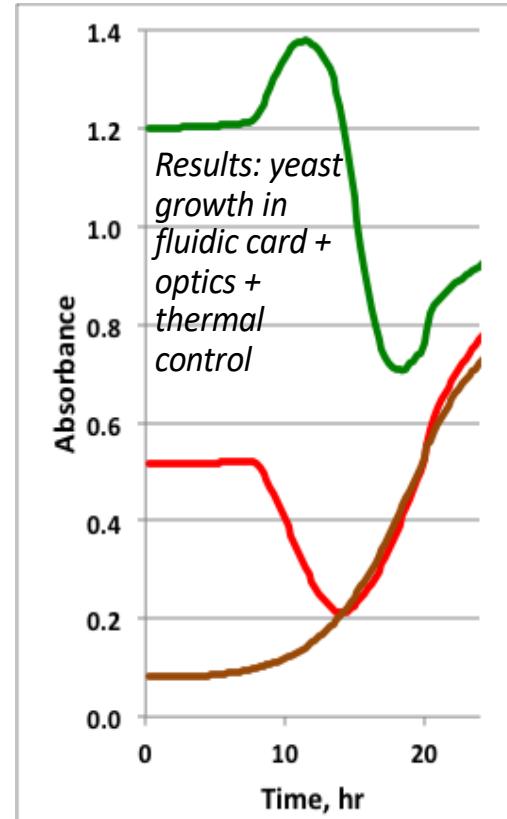
- **Optical absorbance measurement per well**

- Dedicated 3-color optical system at each well
- Measure dye absorbance & optical density (cell population) with stray light correction
- Ground pre-calibration + in-flight “active” cal.

- **Pressure & humidity sensors** in P/L volume

- **Dedicated thermal control system per card**

- 16 – 23°C; 1 °C uniformity, accuracy, stability
- 1 RTD sensor per card: closed-loop control

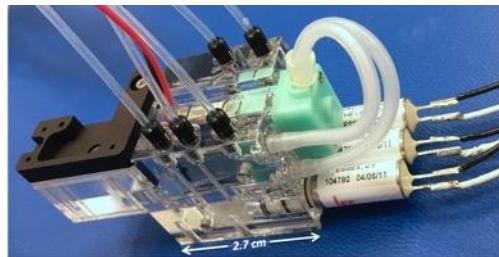




Biofluidic Subsystem



9-fluidic-card manifold (144 wells) [1 of 2]



Reagent-and-pump manifold [1 of 2]

Tally of components:

- 2 pumps, 2 main bubble traps
- 24 active valves, 38 check valves
- 16 fluidic cards with 16 small bubble traps, 16 desiccant traps, 288 wells total

9 fluidic cards integrated with measurement optics, thermal control, and fluidic manifold
[1 of 2]



Manifold-integrated components:

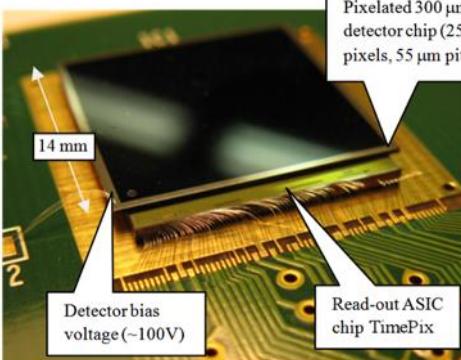
- active & check valves
- bubble traps
- desiccant traps
- optical calibration cells



BioSentinel

Monitoring Radiation: Single-PCB Linear Energy Transfer Spectrometer

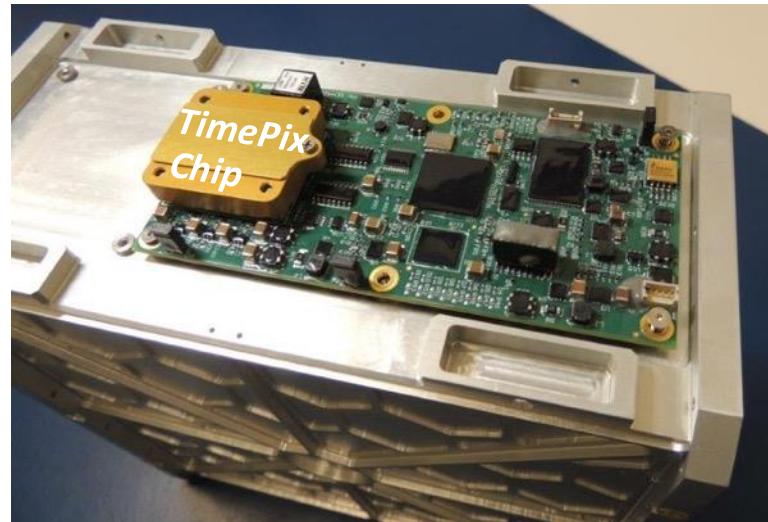
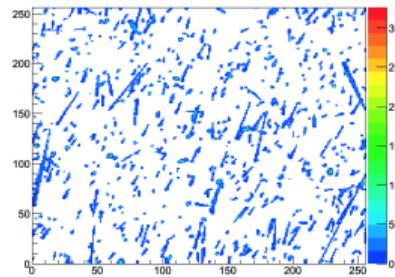
- LET “spectrometer”: TimePix solid-state device
 - measures **linear energy transfer** spectra
 - time-over-threshold (TOT) mode. Wilkinson-type ADC
 - ❖ *direct energy measurement per pixel*
 - LET 0.2 – 300 keV/ μm into 256 bins, each 3% width; store hourly bin totals
 - Download “local space weather” periodic snapshots
 - Also reports **TID** (total ionizing dose)
- SPE Trigger: TID rate increase causes wet-out of a pair of fluidic cards



TimePix
Chip

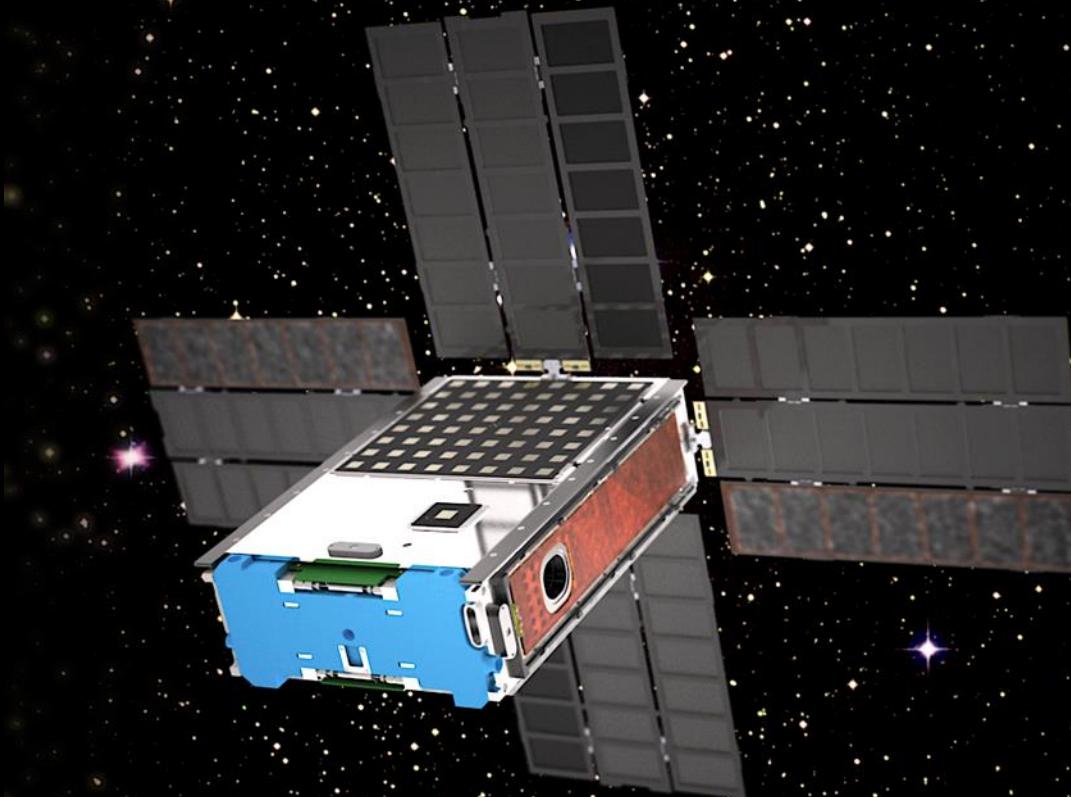


Pixelated 300 μm thick Si
detector chip (256 x 256
pixels, 55 μm pitch)



Single-board LET spectrometer mounted
on *BioSentinel* biosensor payload
enclosure

Typical TimePix frame:
256 x 256 x 14 bits

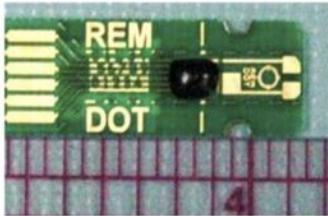


AJ Ricco, SR Santa Maria, RP Hanel, S Bhattacharya, *The Radworks Group*, and the *BioSentinel Team*, *IEEE Aerospace Elect Sys Mag*, 35, 18-24 (2020).

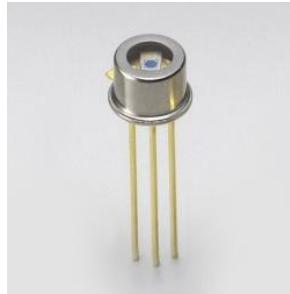
Radiation Dosimeters

Critical adjunct to biology experiments

- Correlate radiation dose, dose rate, or time-varying spectrum with biological effects
- Radiation details can vary locally: embed sensors with bio payloads even if host platform includes integral radiation measurements
- Tissue-equivalent sensors (proportional counter or plastic scintillator) measure biologically-relevant exposure
- Science objectives drive dosimeter selection – more than one dosimeter technology may be needed



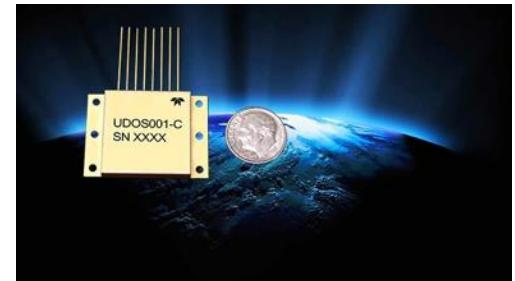
RadFET: Total Ionizing Dose (TID), records TID with power off or on
8/18/2021



PIN diode: Real-time dose rate, low cost for multi-location applications



Plastic scintillator: radiation dose response similar to tissue, including neutron and proton responses



Total Ionizing Dose (TID) – Teledyne μDOS



Single-Board Linear Energy Transfer (LET) Radiation Spectrometer



Lessons Learned

- No such thing as a ‘simple’ modification
- Be sure to test biocompatibility of all parts that come in contact with the science, including the treatment of the materials before integration with the biology (e.g., autoclave, EtO, etc.)
- Be sure to offgas all materials in the cubesats since the long turnover period may be detrimental to the science before activation
- Negotiate a late turnover to minimize the offgassing effects
- Perform end-to-end tests, including long-duration stasis period
- Murphy’s law of bubbles in microgravity
- Science and engineering teams need to work side-by-side for biological experiments



Lunar Explorer Instrument for space biology Applications (LEIA)

- Anticipated landing site for potential LEIA mission is lunar south pole (84-90 deg S)
- BioSensor payload must be adapted for operation during lunar daytime environment (south pole) and interface with Commercial Lunar Payload Services (CLPS) vehicle
 - Lunar south pole environment is cold and LEIA may be in shade of CLPS lander; LEIA BioSensor may require additional insulation and increased heater power
 - LEIA enclosure must shield electronics but not biology from radiation
 - LEIA must protect against interference from adjacent payloads (e.g. EMI, thermal)
 - BioSentinel mission (6-12 months) substantially longer than LEIA mission (<14 days). LEIA BioSensor fluidics “cards” will all need to run in parallel, affecting thermal profile and power draw.
- Short LEIA mission timeframe drives selection of biology that is more sensitive to radiation, but may be less robust to long-term stasis. LEIA’s BioSensor payload will incorporate a “late load” capability to substantially reduce time the biology is in stasis.
- LEIA to include charged particle radiation detector to correlate radiation data with biological data. A high energy neutron detector is also under consideration.

Future Plans for BLEO Science

- Annual Space Biology call for BLEO science
 - CLPS
 - Gateway
 - SmallSat/CubeSats
- Need both scientists and engineers to be successful!



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LEIA: Jennifer Lee, Matthew Lera, Brandon Maryatt

NASA/Ames

\$ **GeneSat/PharmaSat: NASA Fundamental Space Biology, ESMD (now BPS/SMD)**

\$ **O/OREOS: NASA Astrobiology Small Payloads Program, SMD**

\$ **BioSentinel: NASA Advanced Exploration Systems, HEOMD**

The background of the slide features a dark blue gradient with a subtle starry texture. Overlaid on this are several glowing, translucent spheres of various sizes and colors. A prominent yellow sphere contains a detailed image of a pink flower with yellow stamens. To its right is a large blue sphere containing a glowing red dot, with a silver DNA double helix wrapped around its lower edge. Below the blue sphere is a smaller green sphere containing a green fern frond. In the bottom right corner, there is a small orange sphere filled with numerous small, glowing orange particles.

Backup Slides

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