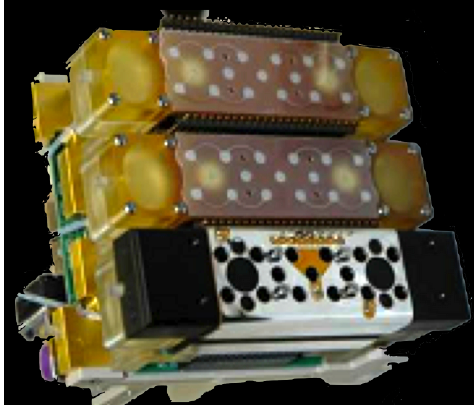
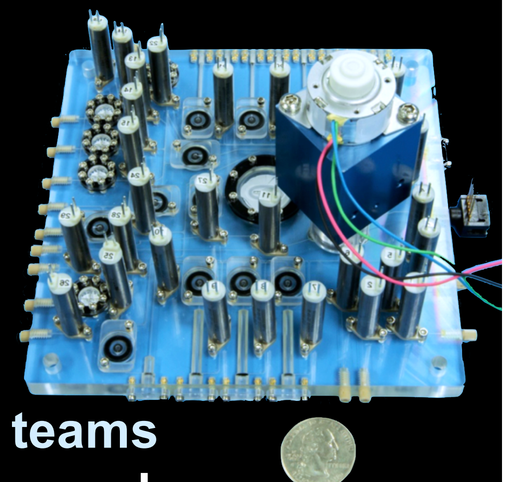
Leveraging Cubesat Payload Technologies to Search for Life on our Solar System's Icy Worlds





Tony Ricco

NASA Ames Research Center

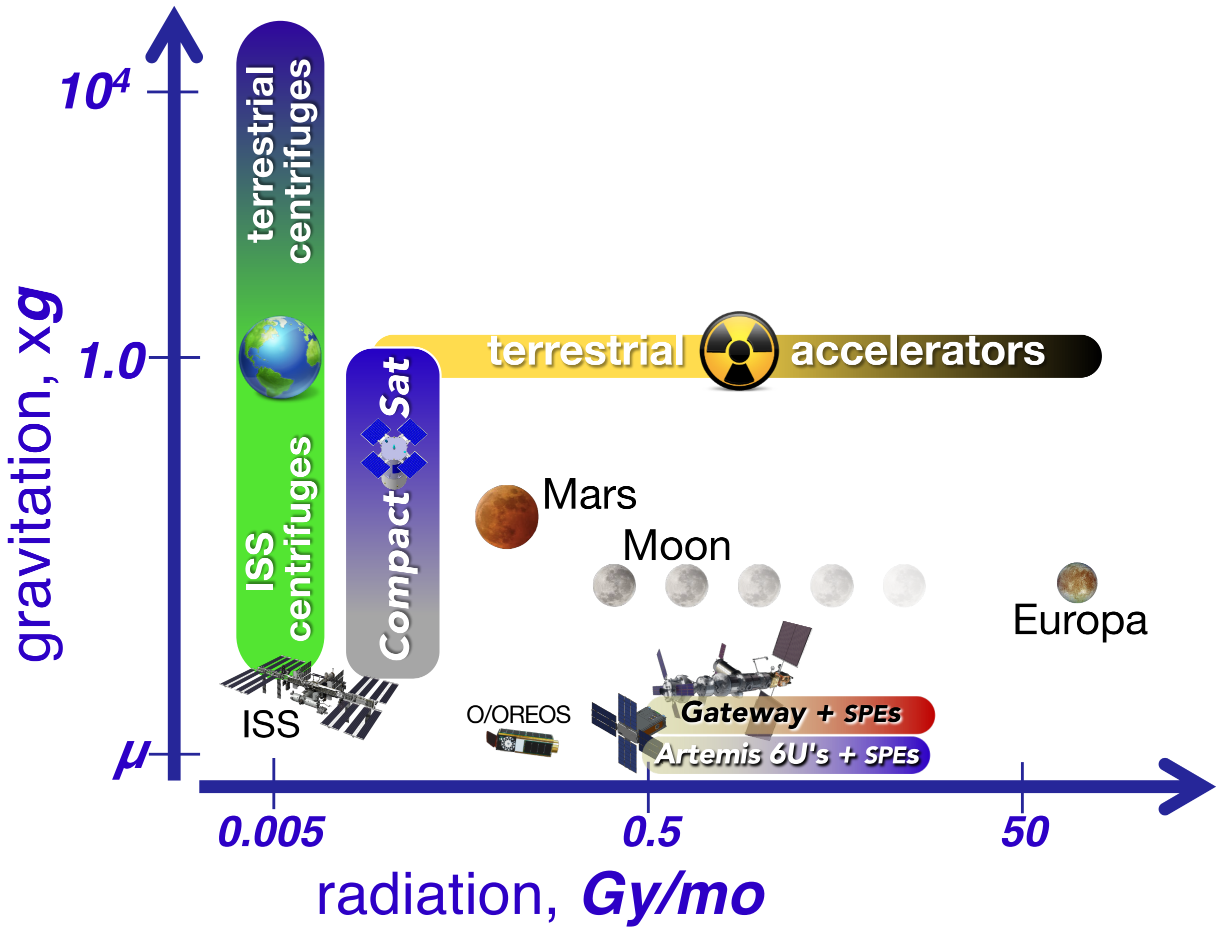
with many thanks to

ARC small payloads and search-for-life tech. dev. teams and the space biologists & astrobiologists everywhere who

Define the science that defines the tech

"BioMapping" the solar system and its gravity-radiation landscape:

- where can terrestrial life exist/thrive?
- where does, or did, non-terrestrial life exist?

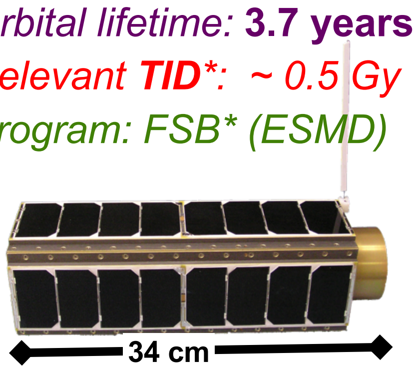


Further considerations: temperature, pressure, chemistry

Integrated Microfluidic Bioanalytical Systems to Grow and Monitor Microbes in Space

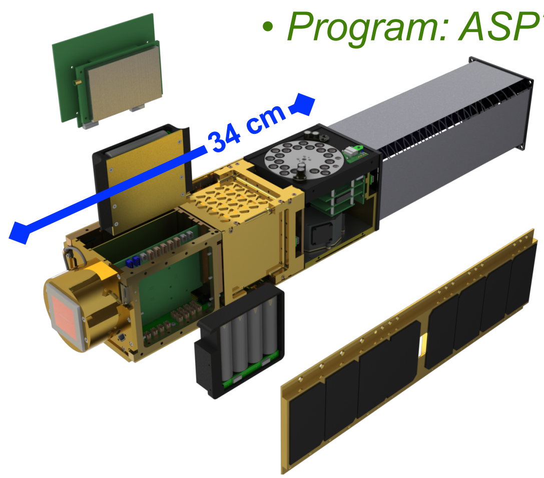
GeneSat (2004-2006-2010)

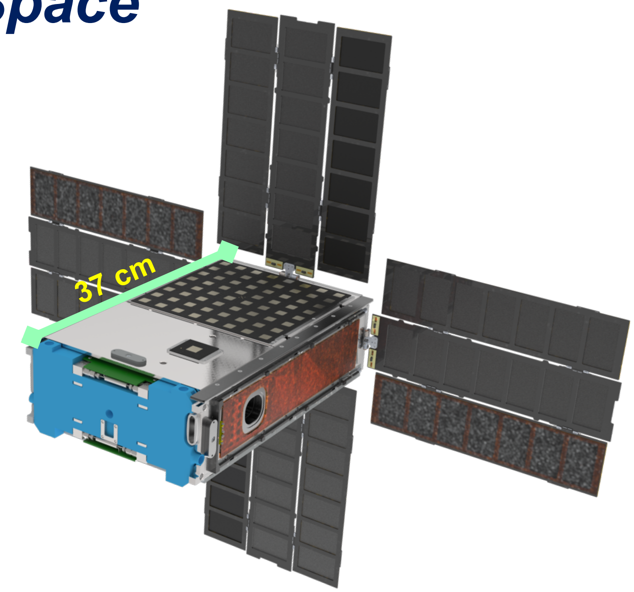
- *Orbit:* **Low Earth Orbit, 440 km**
- *Mission duration:* **1 month**
- *Orbital lifetime:* **3.7 years**
- *Relevant **TID***: ~ 0.5 Gy*
- *Program: FSB* (ESMD)*



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)*



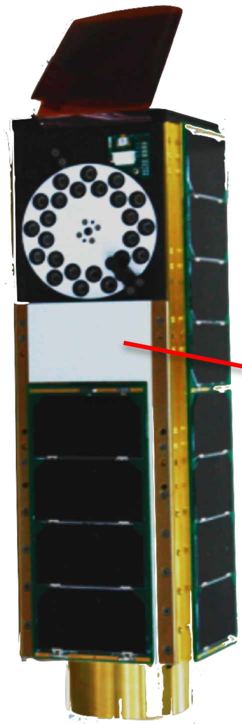


BioSentinel (2013-2021-7500000000)

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

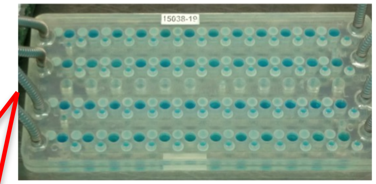
**TID = total ionizing dose*
**FSB = Fundamental Space Biology*   **ASP = Astrobiology Small Payloads*   **AES = Advanced Exploration Systems*

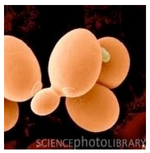
Summary of NASA Ames' Nanosatellite (Astro)Biological Space Missions



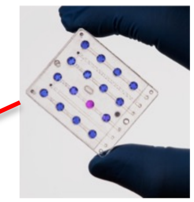
O/OREOS

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

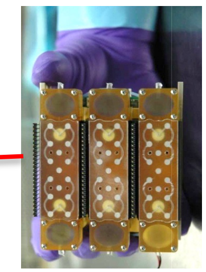


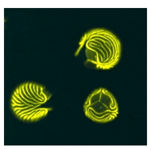


S. Cerevisiae *PharmaSat* (**2009**/3U): ***drug dose response***
BioSentinel (**2021**/6U): ***DNA break/repair***



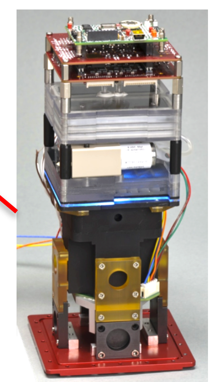
B. Subtilis *O/OREOS** (**2010**/3U): ***survival, metabolism***
*ADRoIT-M*** (6U): ***mutations / lithopanspermia***

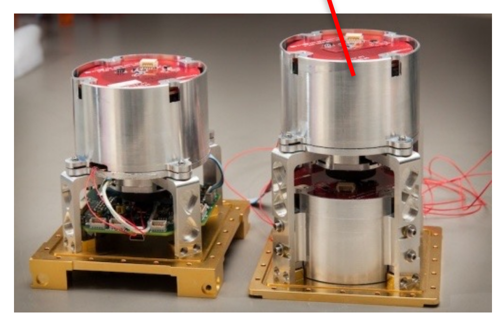




Ceratopteris Richardii *SporeSat-1* (**2014**/3U): **ion channel sensors, μ-centrifuges**
SporeSat-2 (3U): ***plant gravity sensing threshold***

C. Elegans *FLAIR* (3U):
dual-wavelength
fluorescence imager

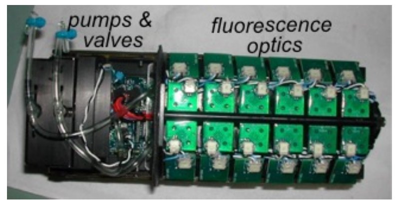


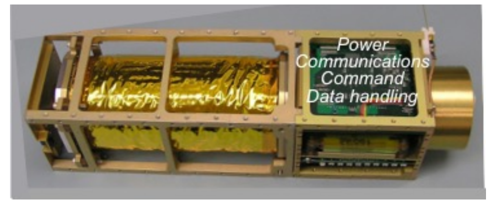


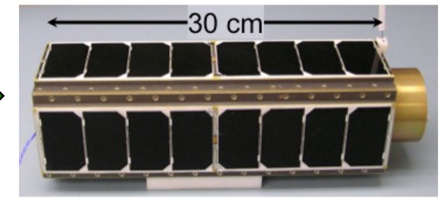
**Organism/Organic Exposure to Orbital Stresses*
***Active DNA Repair on Interplanetary Transport of Microbes*

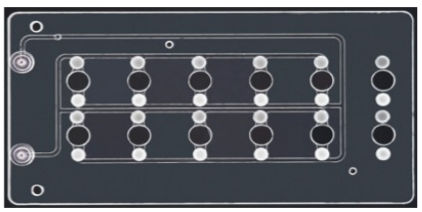
model organism:
0.5 x 2 µm
bacteria
E. coli

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

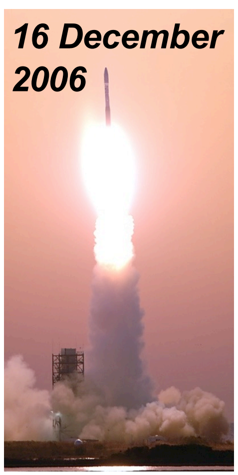




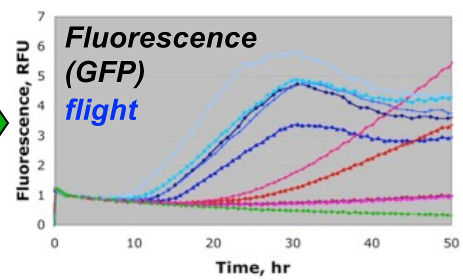




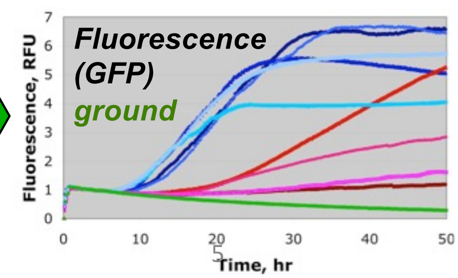
- nutrient deprivation in dormant state (6 weeks)
- launch: December 2006 to low Earth orbit (440 km)
- nutrient solution feed upon orbit stabilization, grow *E. coli* in µgravity
- monitor green fluorescent protein (***GFP***): gene expression
- monitor optical density: cell population



Telemeter data to Earth



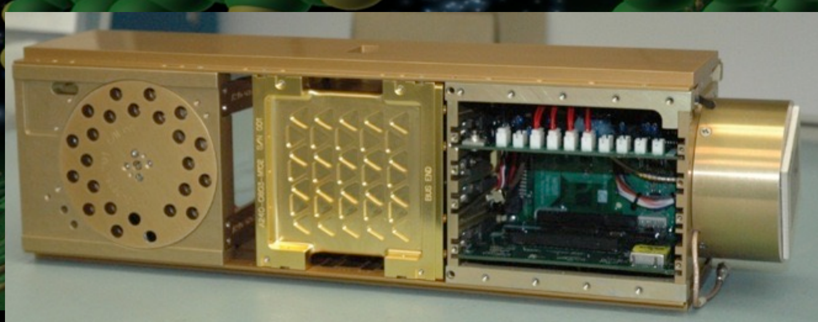
Compare to ground data



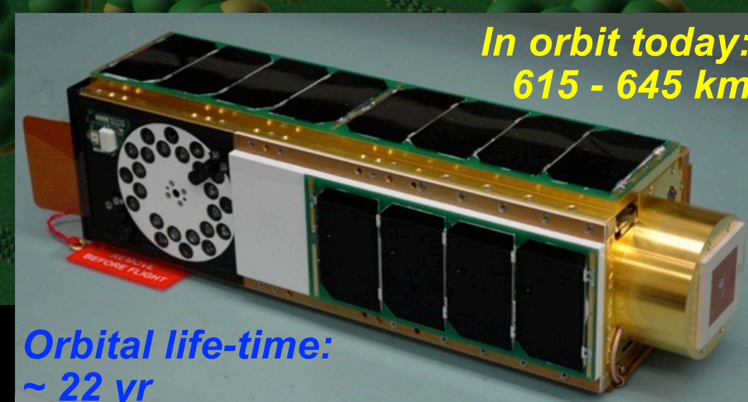
O/OREOS Astrobiology Mission

**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



Flight prototype

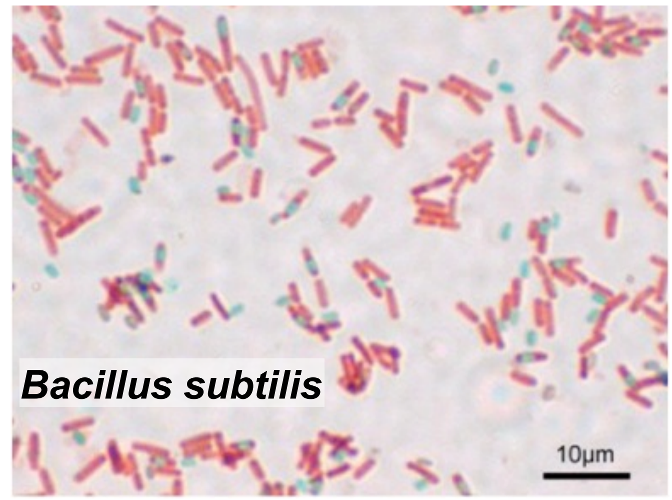


Ehrenfreund *et al*, *Acta Astro 93*, 501 (2014)

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

Organisms, wildtype & mutant, exposed to μgravity & space radiation

- On Earth: dry organisms on microwell walls
- In space: Rehydrate & feed 6 μwells / organism:
  t = 2 wk, 3 mo, 6 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]**

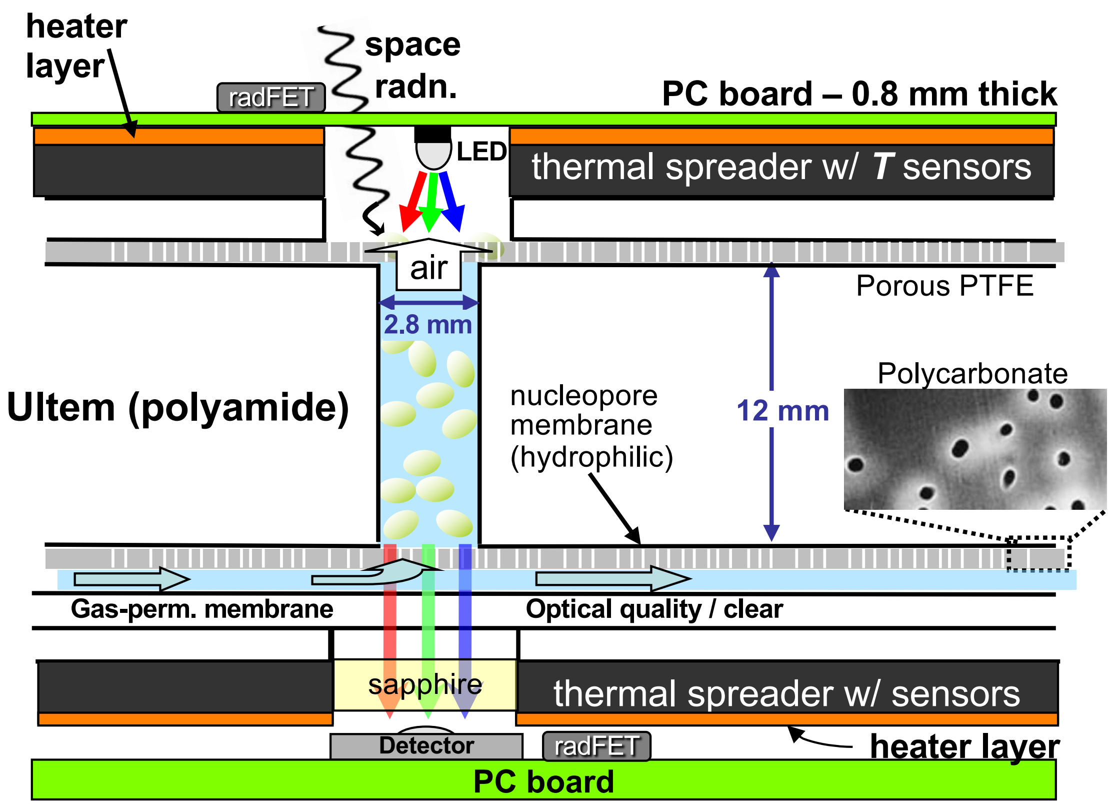


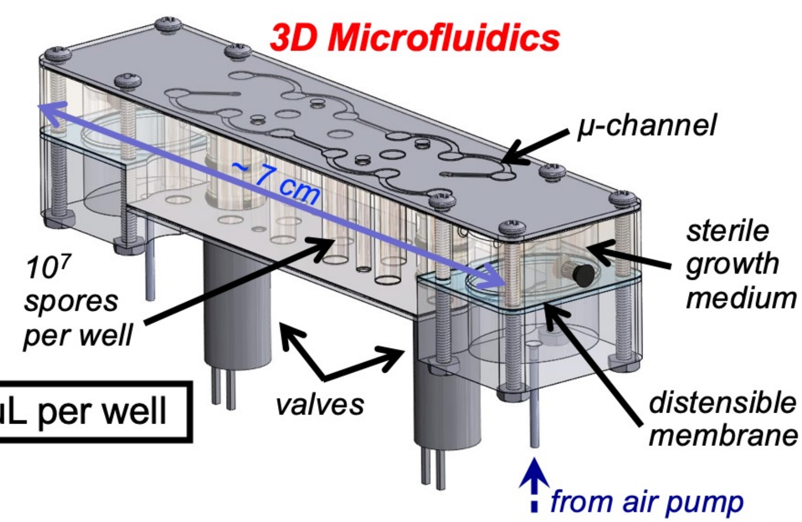
- Sensors: ***T***, ***p***, ***RH***, ***rad*** (integrated dose), **μgrav**
  - » temperature (6 sensors per 12-well bioblock)
  - » pressure, relative humidity (1 sensor each)
  - » radiation total dose @ both ends of wells (2 radFETs)
  - » microgravity levels calc'd. from solar panel currents

Nicholson *et al.*, *Astrobio 11*, 951 (2011)

SESLO payload on O/OREOS

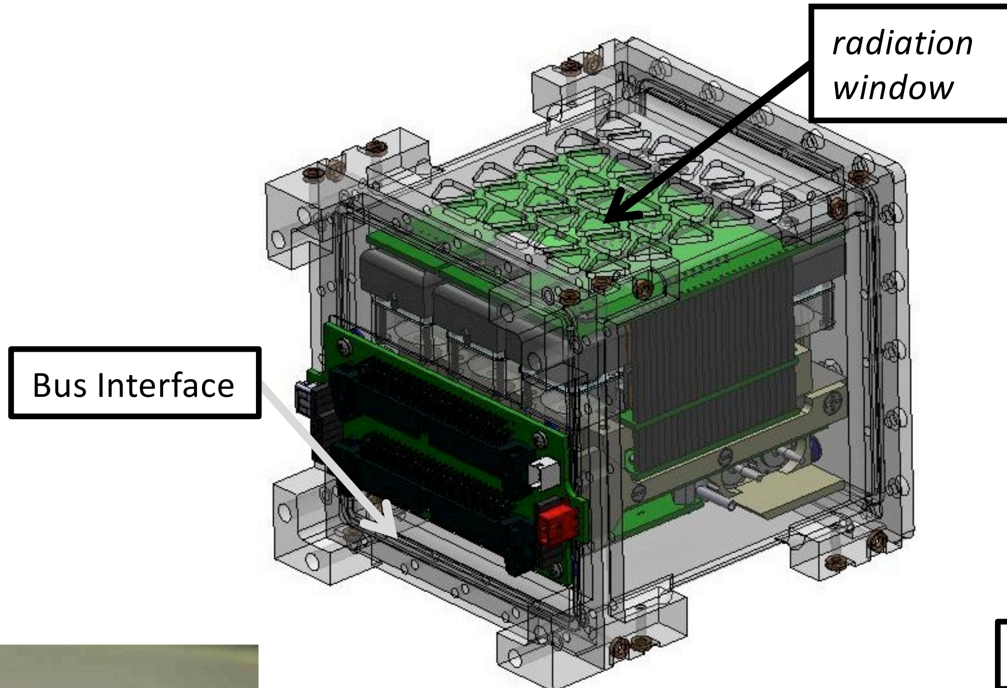
Biological / fluidic / optical / thermal cross-section

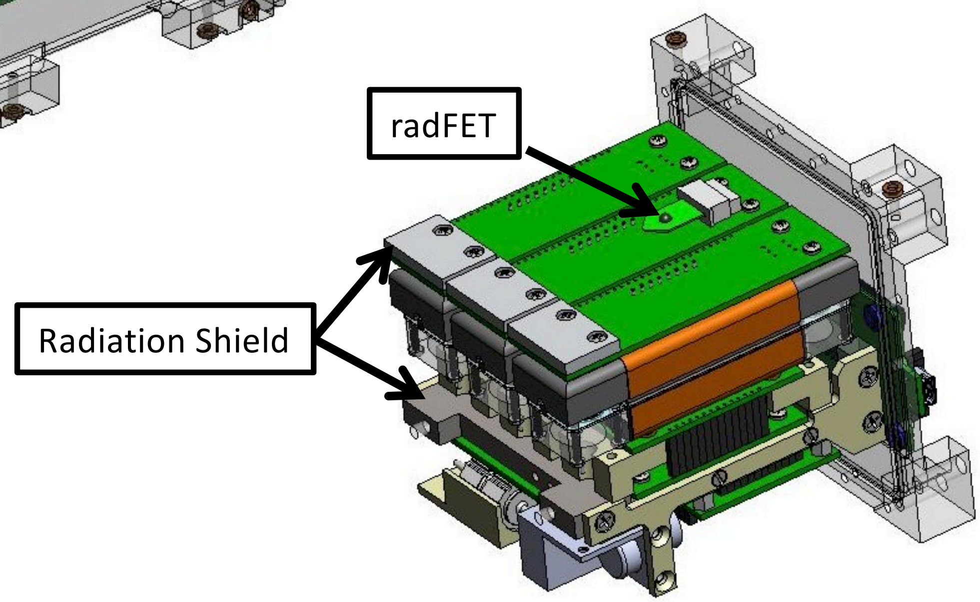




75 μL per well

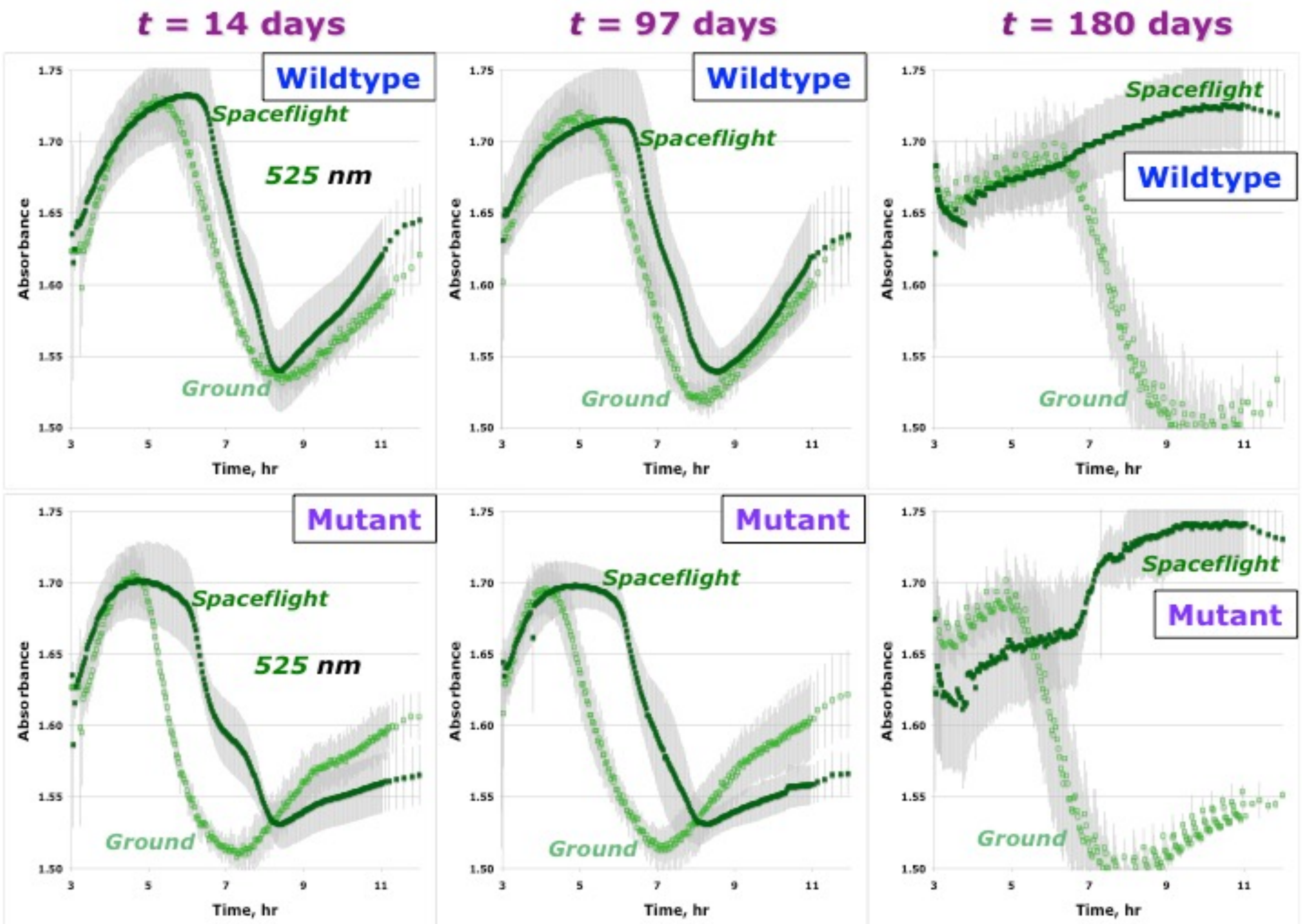
SESLO Integration Summary





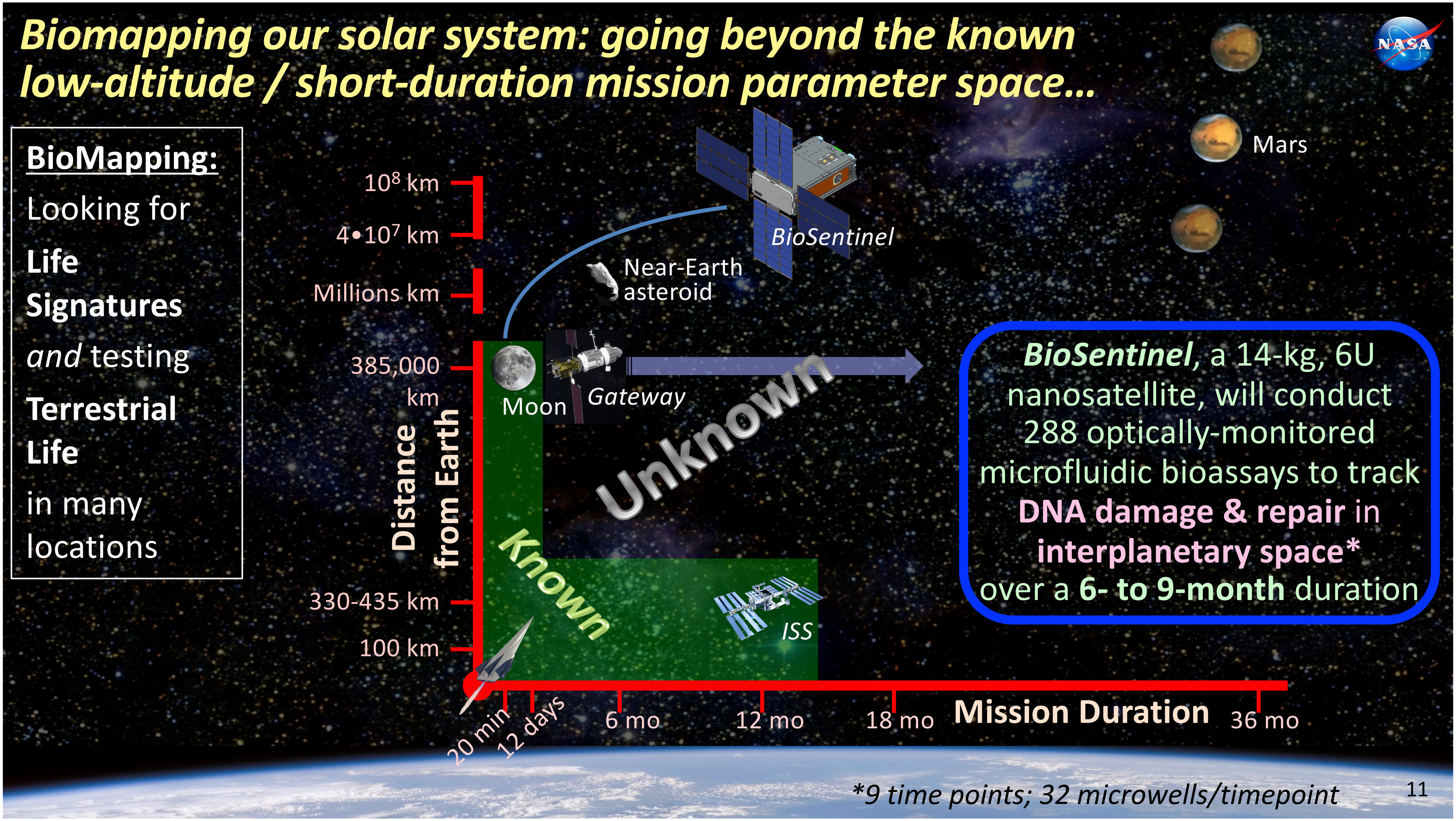
SESLO Spaceflight Results:

Growth of *B. subtilis* in space vs. ground

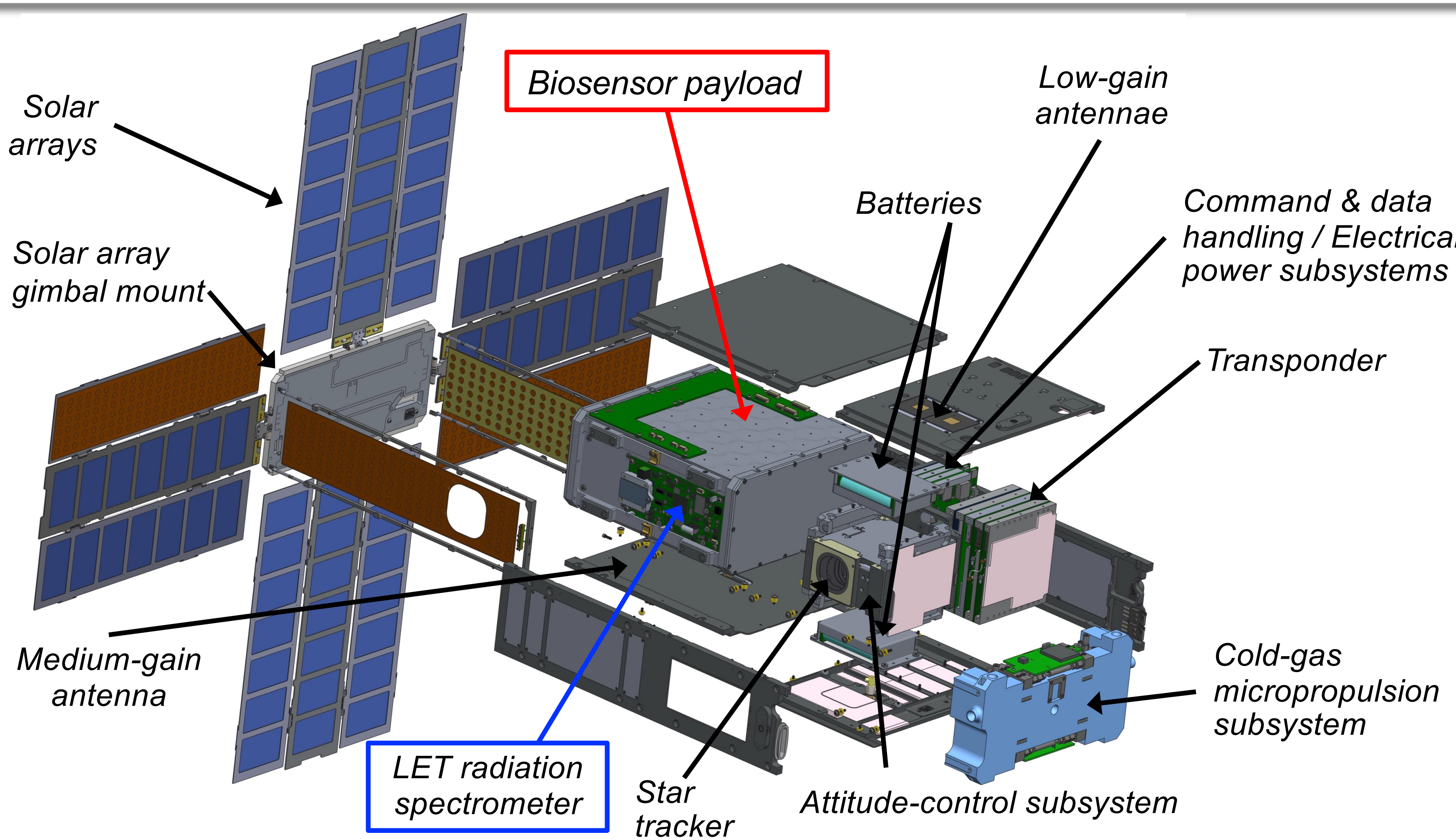


WL Nicholson, AJ Ricco, *et al*, *Astrobiology*, *11*, 951-958 (2011)

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

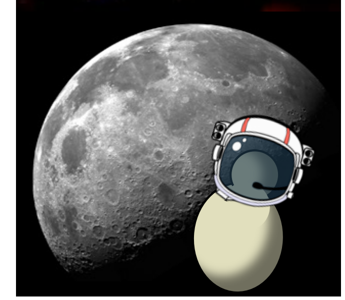

Biomapping our solar system: going beyond the known
low-altitude / short-duration mission parameter space…
BioMapping:
Looking for
Life
Signatures
and testing
Terrestrial
Life
in many
locations
10^8 km
4•10^7 km
Millions km
385,000 km
330-435 km
100 km
Distance from Earth
Mars
BioSentinel
Near-Earth asteroid
Moon
Gateway
Unknown
Known
ISS
20 min
12 days
6 mo
12 mo
18 mo
Mission Duration
36 mo
BioSentinel, a 14-kg, 6U nanosatellite, will conduct 288 optically-monitored microfluidic bioassays to track DNA damage & repair in interplanetary space* over a 6- to 9-month duration
*9 time points; 32 microwells/timepoint
11

BioSentinel Overview: Taking Biofluidic Systems to Deep Space



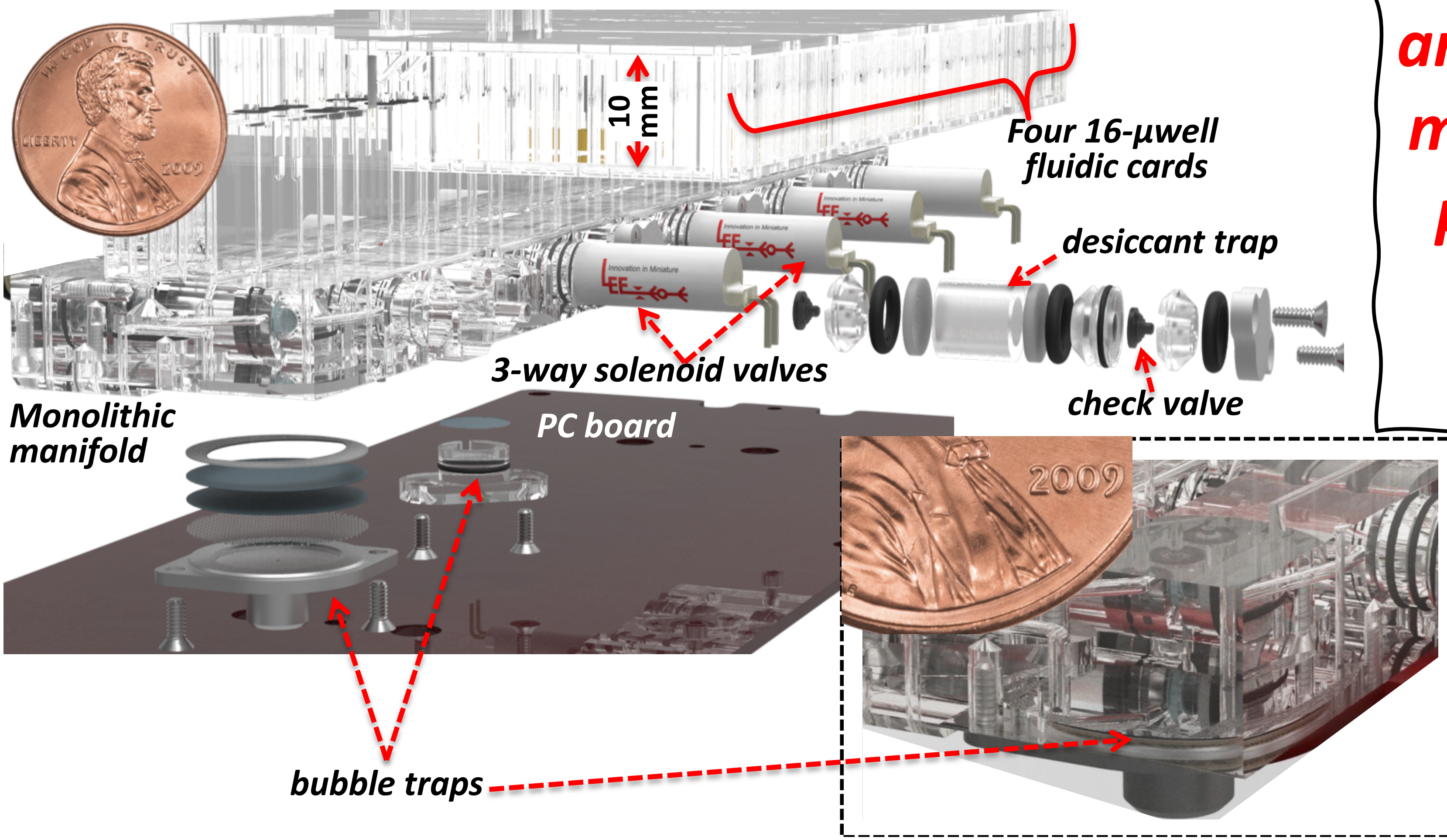
BioSentinel Science Mission: "*Canary in a Coal Mine*"

- ***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. cerevisae*: 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 µwells 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



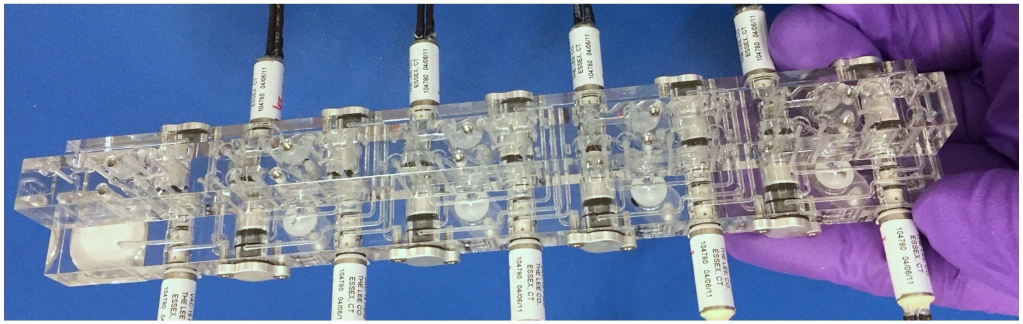
Predecessor to Search-for-Life Fluidic Technologies:

BioSentinel's Fluidic Processor, aka manifold



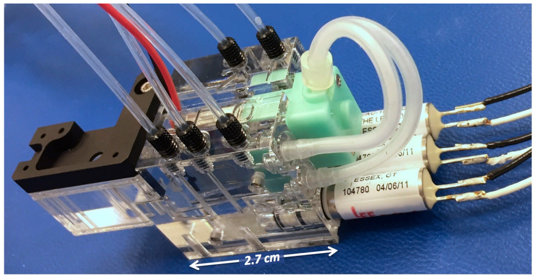
The fluidic analogy of multilevel printed-circuit boards

BioSentinel — *Biofluidic Subsystem*



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

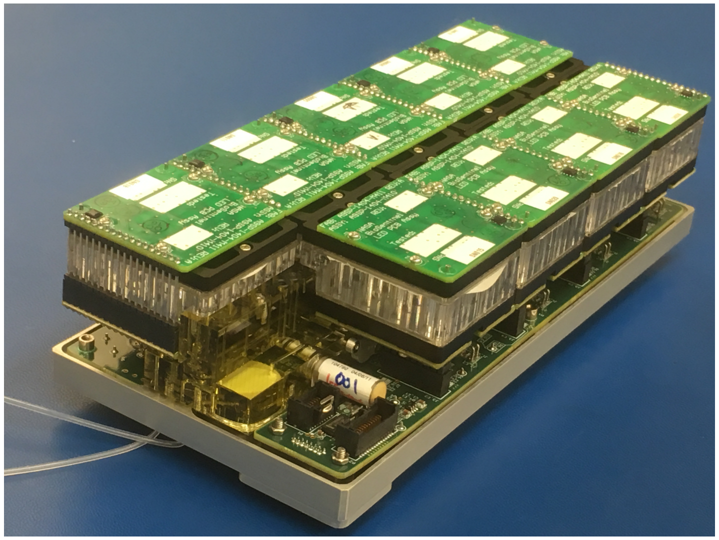
Manifold-integrated components:
- ***active & check valves***
- ***bubble traps***
- ***desiccant traps***
- ***optical calibration cells***



Reagent-and-pump manifold [1 of 2]

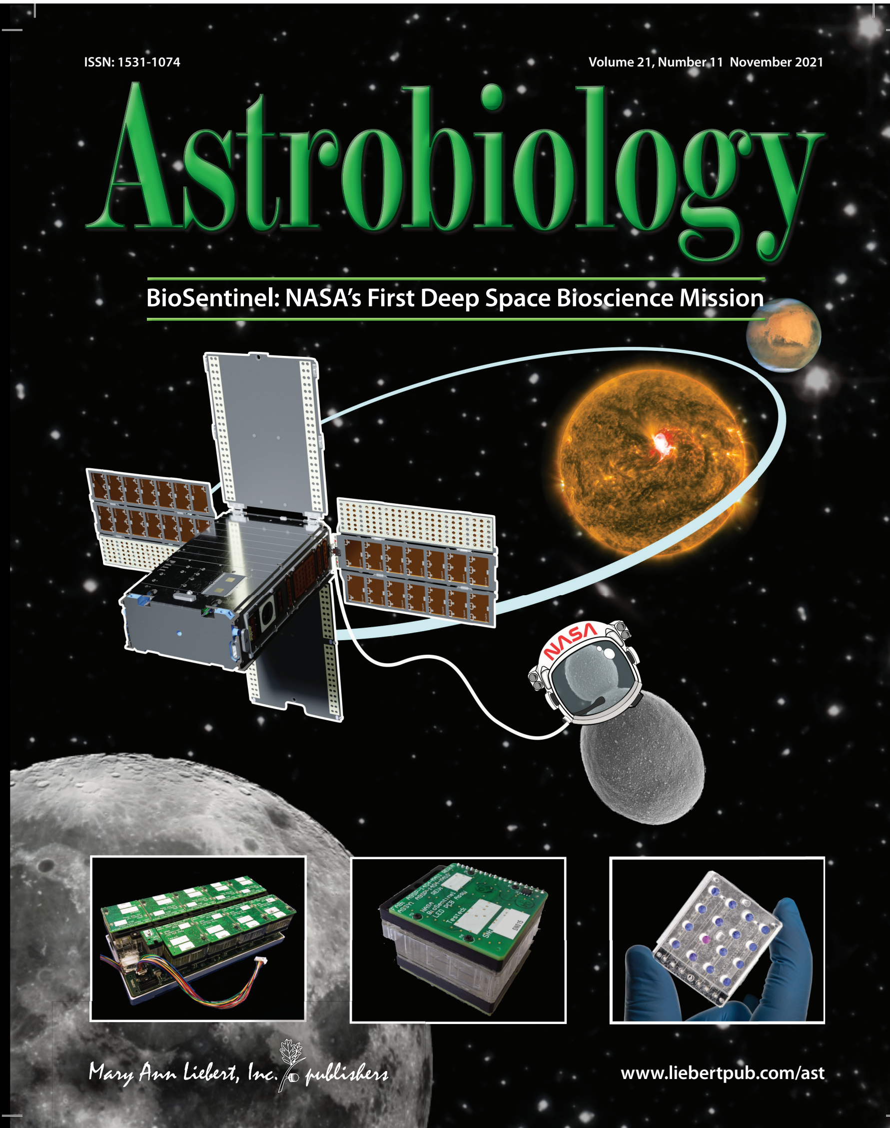
Tally of components:
- ***2 pumps, 2 main bubble traps***
- ***24 active valves, 38 check valves***
- ***16 fluidic cards with 16 small bubble traps, 16 desiccant traps, 288 wells total***

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



MR Padgen *et al.*,
Astrobiology, *21(5)*,
11 pp (2021).

AJ Ricco *et al.*,
IEEE Aerospace Elect Sys Mag, *35*,
18-24 (2020)



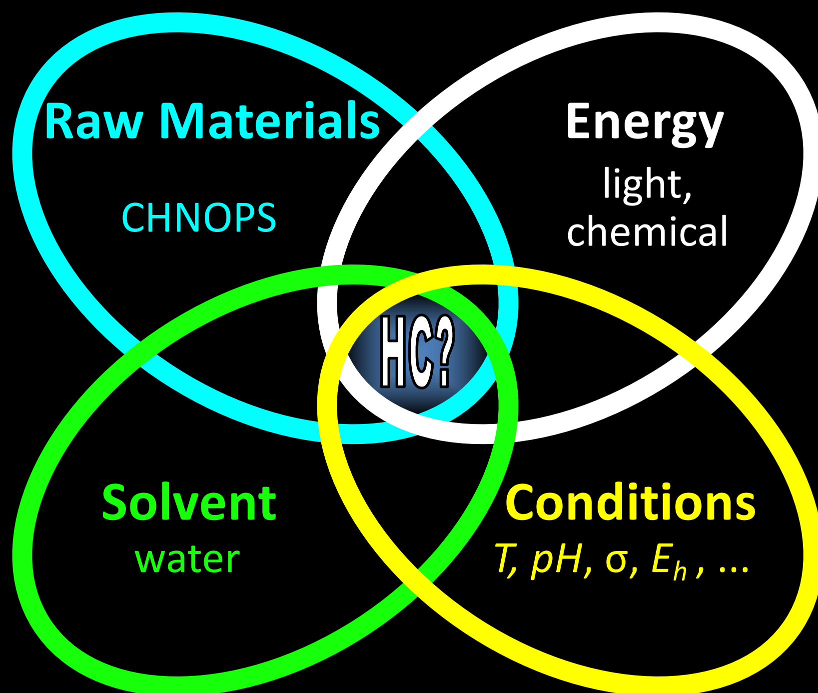
Ames Biological Small Satellite Payloads & Missions

Mission* (format)	Description	Launch Date	Outcome
GeneSat-1 (3U)	• 2U payload measured expression of GFP in *E. coli* 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)	• 2U payload measured antifungal drug dose response for *S. cerevisiae* using colorimetry to measure metabolic activity and population vs. time • 1st NASA PI-led nanosatellite mission	2009	Full mission success
O/OREOS (3U)	• Two independent 1U astrobiology payloads measured (a) survival of *B. subtilis* 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
GraviSat (3U)	• 2U payload integrating microcentrifuge to support microalgae (*C. vulgaris, D. bardawil*) in multiple wells with pulse-amplitude-modulated fluorescence & carbonate ion measurements	N/A	Payload qual'd. to TRL 5
MisST (3U)	• 2U payload integrating a multi-strain *C. elegans* fluidic habitat with a 2-color fluorescence microscope	N/A	Payload qual'd. to TRL 6
SporeSat-1 SporeSat-2 (3U)	• 2U payload measures gravitational response of *C. richardii* fern spores via Ca^{2+} ion channel response • variable **g** in microgravity ambient using 50-mm µcentrifuges with 32 ion-selective [Ca^{2+}] electrode pairs	SporeSat-1: 2014 SporeSat-2: tbd	SporeSat-1: Successful spaceflight demo. of µcentrifuges & integral ion-selective electrodes
EcAMSat (6U)	• 2U payload measured antibiotic resistance in microgravity vs. dose for uropathogenic *E. coli* • 1st biological nanosat deployed from ISS; 1st Ames 6U biological nanosat	2017	Full mission success
Eu:CROPIS / Powercells ("minisat")	• Secondary payload, aboard DLR (German Aerospace Center) rotating 1-meter-diameter, 250-kg artificial-gravity satellite, to demonstrate synthetic biology-relevant processes	2018	Success Further details TBD
BioSentinel (6U)	• 4U payload to measure radiation-induced DNA damage in radiation-sensitive S. cerevisiae 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

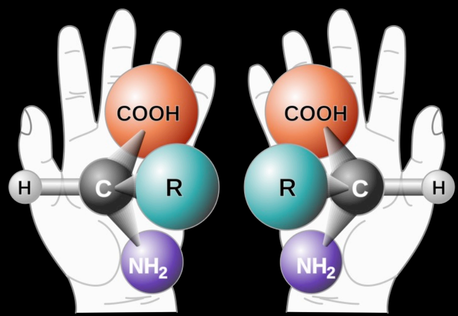
The Search for Life & Habitability

Environmental Requirements to Sustain Life

HC = HABITABLE CONDITIONS



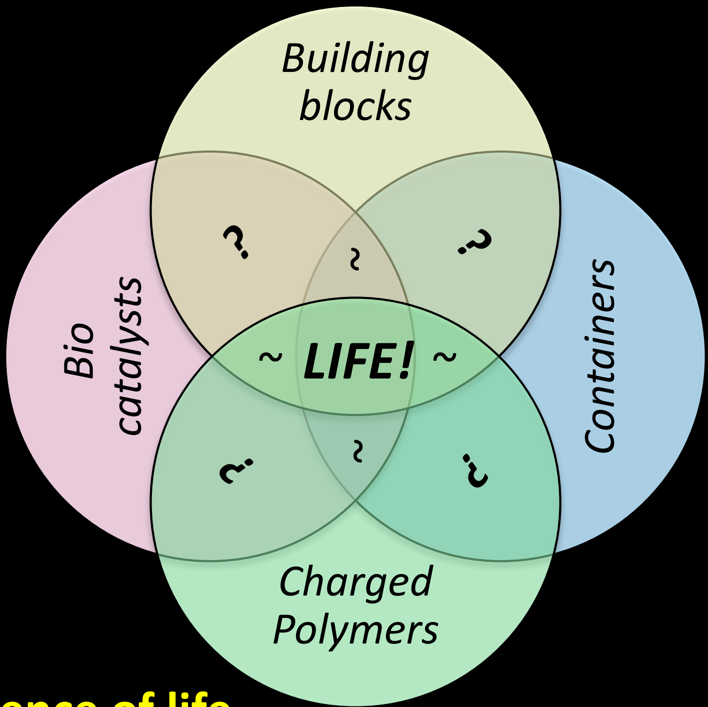
Present **habitability easier to assess than Past…**



Searching for Life: What to Detect?

some aspects of life are likely to be universal…

- Versatile chemical building blocks
- Complex multimeric biomolecules
- Containment structures
- Function-specific molecules



Arguably, all are required for life

- Combined, these indicators could provide conclusive evidence of life
- *Technologies needed to enable the search in an icy-moon environment?*

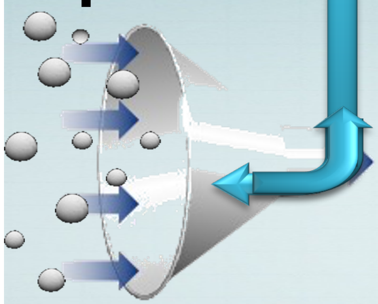
Targets, Rationale, and Instruments for Life Search

Ames Research Center

Measurement Target	Observed Parameter	Life Detection Rationale *examples*	Analytical Approach
Molecular building blocks	Chirality	**Enantiomeric excess** enables biochemistry *amino acids, saccharides*	*Capillary Electrophoresis* *Mass Spec*
Functional molecules	Catalysis	**Biochemical processes; electron transfer** *kinases; quinones*	*Electrochemical BioSensors* *Mass Spec*
Biogenic organic polymers	'Structural' polymers	**Containers, energy, biochemistry** *lipids*	*Mass spec* *Capillary Electrophoresis*
	'Information' polymers	**Information storage and transfer** *poly nucleic acids*	*Sequencing* *Mass Spec*
Containers	Morphology	**Containers, structures, barriers** *cells, membranes*	*Fluorescence Microscopy with staining*

Fluidics Processor

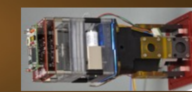
Sample Input



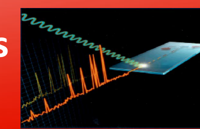
1. Deliver extraction solution
2. Retrieve sample with particles
3. Separate particles 4. Add dyes
5. Degas / de-bubble
6. Adjust ionic strength
7. Remove interfering ions
8. Adjust pH or solvent polarity
9. Adjust solvent polarity
10. Concentrate samples
11. Reconstitute standards/reagents
12. Provide calibration standards
13. Provide controls / blanks
14. Deliver particle-free aliquots

Instrument Suite Candidates

- Fluorescence microscopy

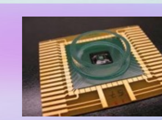


- Microchip capillary electrophoresis with laser-induced fluorescence

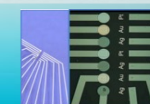


- Mass spectrometry w/ electrospray, GC, or (MA)LDI "front end"

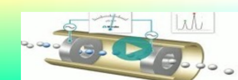
- Electrochemical biosensors



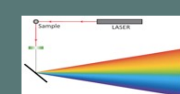
- Ion-selective electrodes [Habitability, energy]



- Ion chromatography [Habitability]

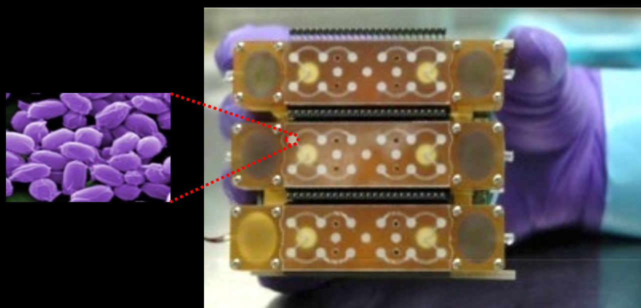


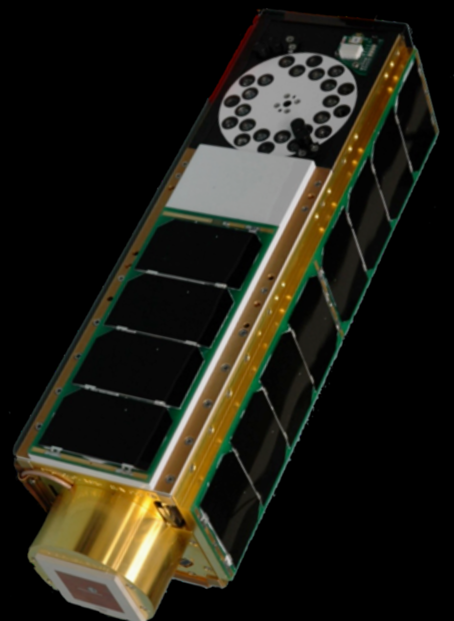
- Raman spectroscopy

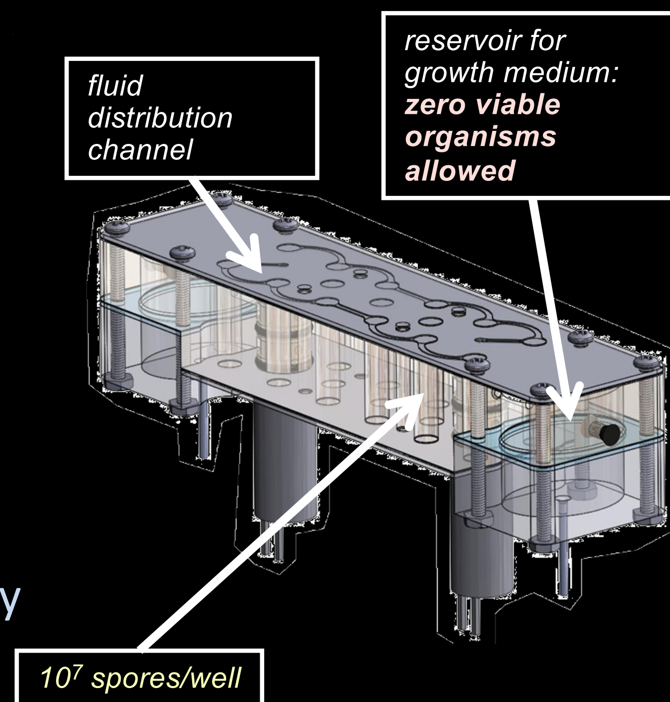


Past Biological Space Missions provide Enabling Technologies

O/OREOS







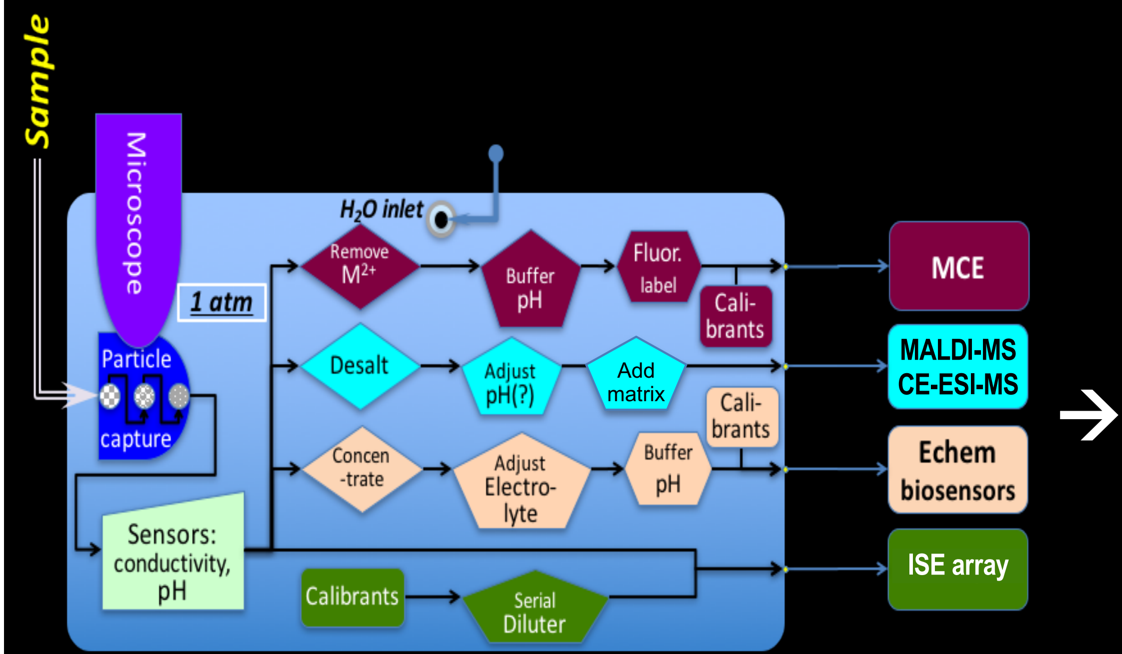
Relevant payload technologies

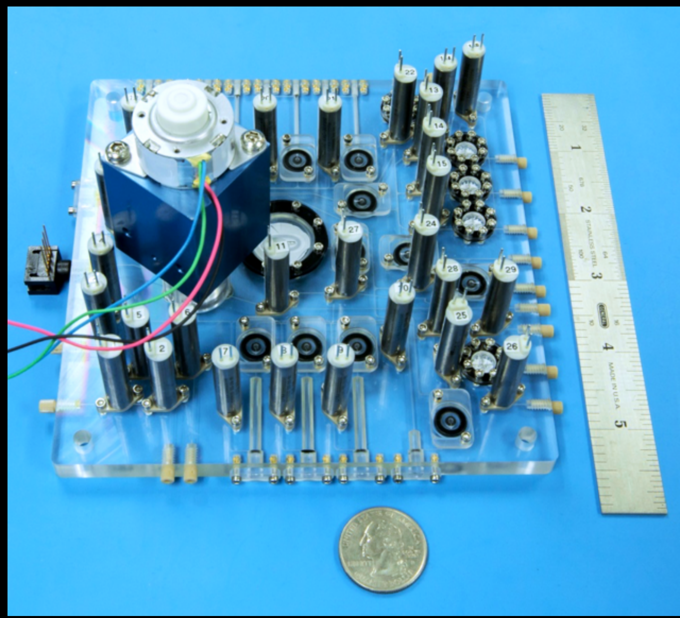
- Handling biological specimens in space
- Perfect sterility (flight-proven)
- Ultra-low organic contamination
- Biocompatible, low-leach-and-offgas materials
- Fly dry, then wet-out fluidics “much later” in reduced gravity
- Manipulate nL – µL volumes
- Functional in high-radiation environment

Integrated Life Detection Payloads

Sample Processor for Life on Icy Worlds (*SPLIce*):

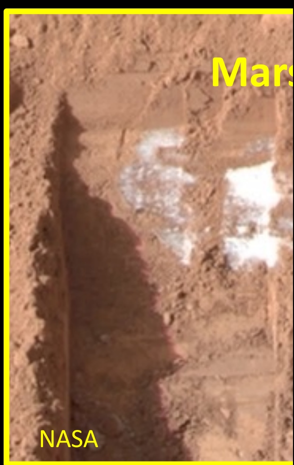
- ***Technology development for Enceladus & Europa life search***
- Partners: APL (Johns Hopkins U.), NASA/JPL, NASA/Goddard, Tufts University



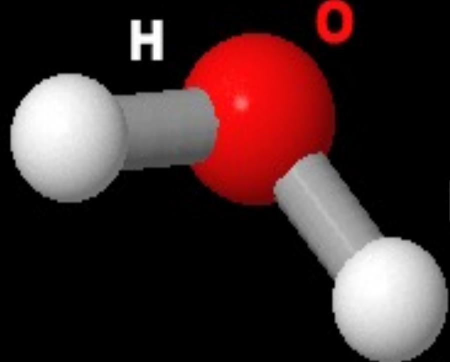


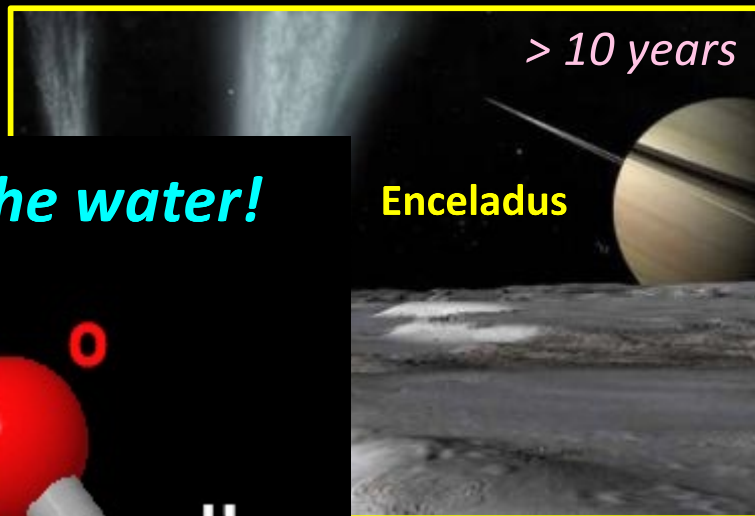
SPLIce *Monolithic Manifold*

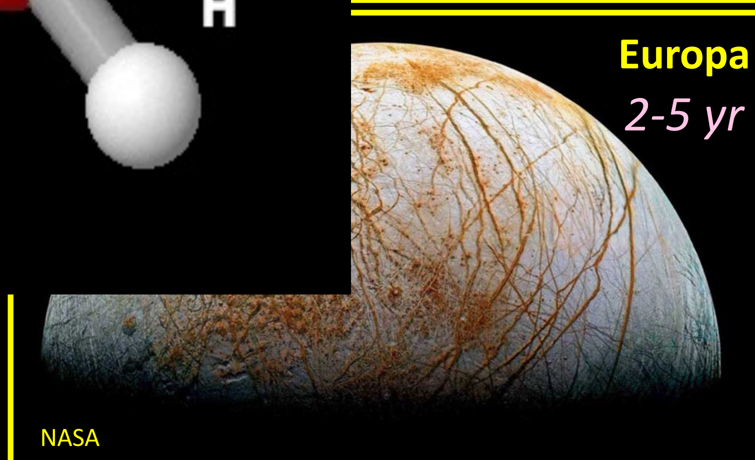
Life-Search Exploration Targets

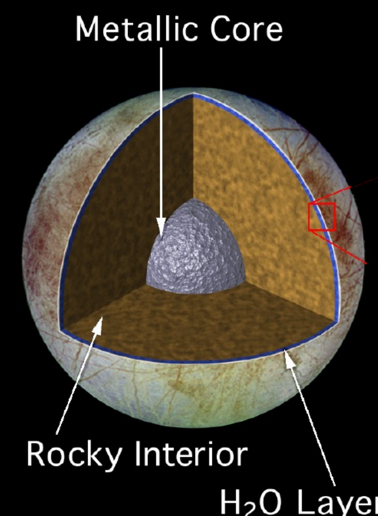


Follow the water!

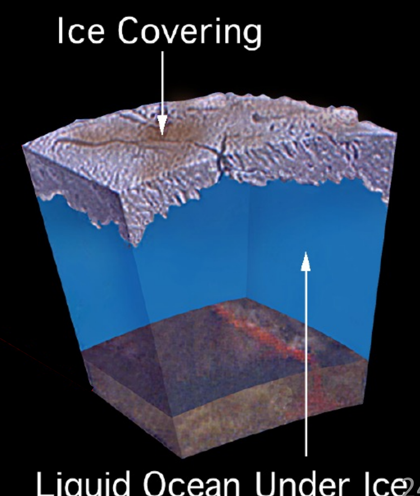








Europa

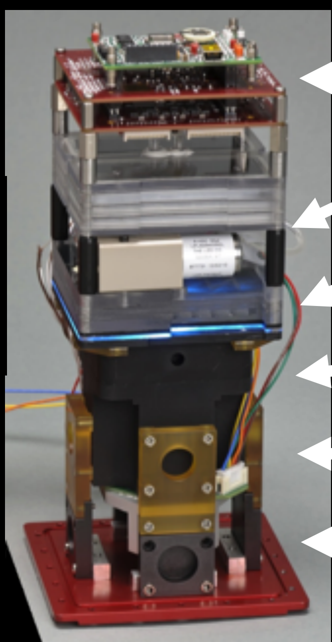


ELM: Europa Luminescence Microscope

Based on ARC's nanosatellite 2U **fluorescence & fluidics** imager for *C. elegans*

2U → 3U

~ 16 cm



Electronics

Fluidics Manifold

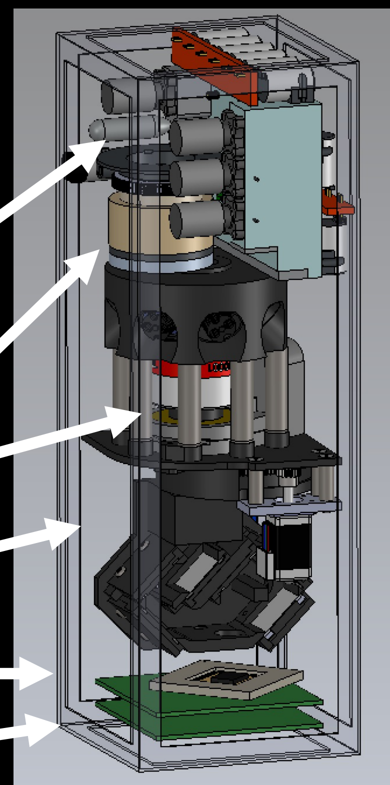
Fluidic Sample Stage

LED Excitation

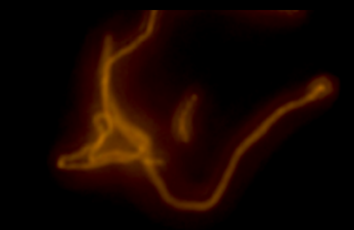
Optics Assembly

Camera Board

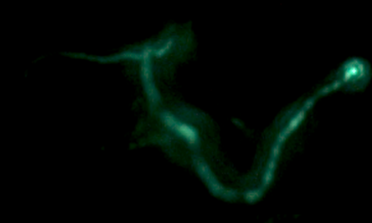
Electronics



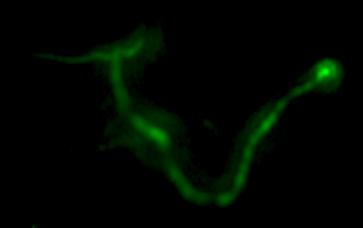
P. boydii ~ 1 µm Wide



Cell Mask – Membrane Lipids



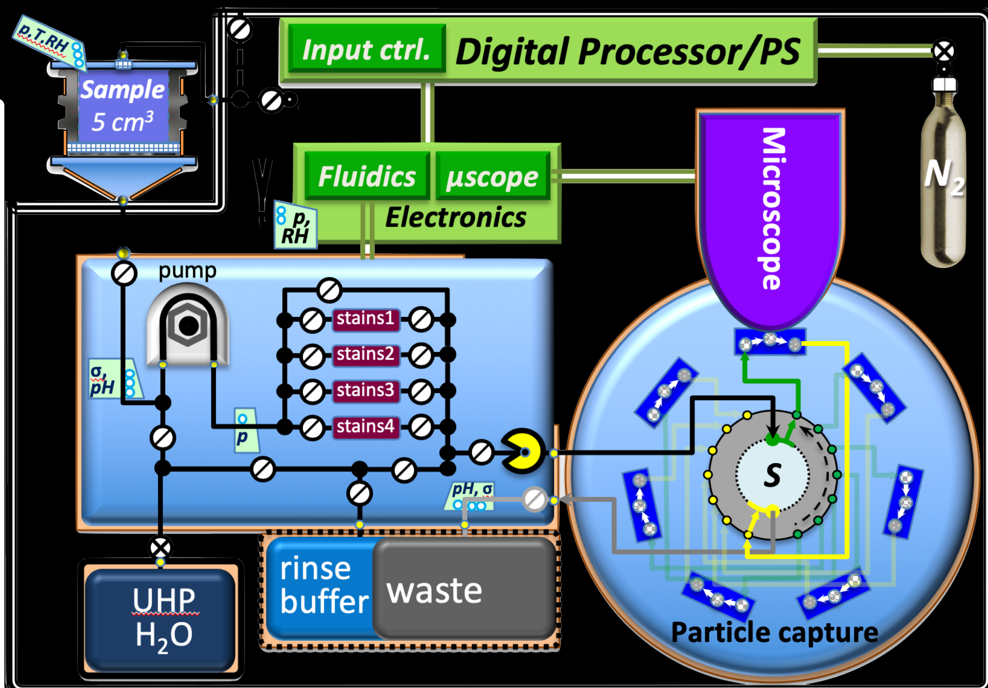
Alexa Fluor 405 - Membrane Proteins



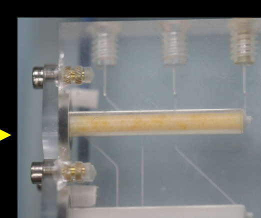
SYTO9 – Nucleic Acids

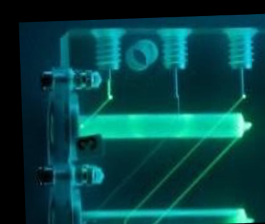
Europa Luminescence Microscope – Block Diagram & Fluidics Breadboard

MisST Imager: 2U Nanosat payload



Fluorescent stains: Porous-polymer supports for anhydrous storage, aqueous rehydration

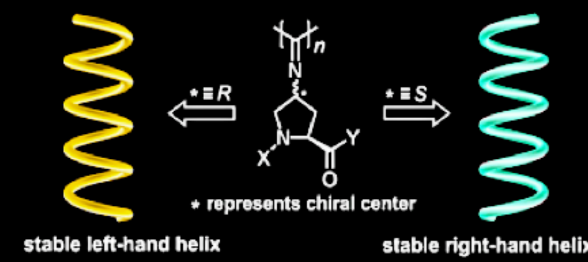




Microchip Capillary Electrophoresis (JPL, ARC)

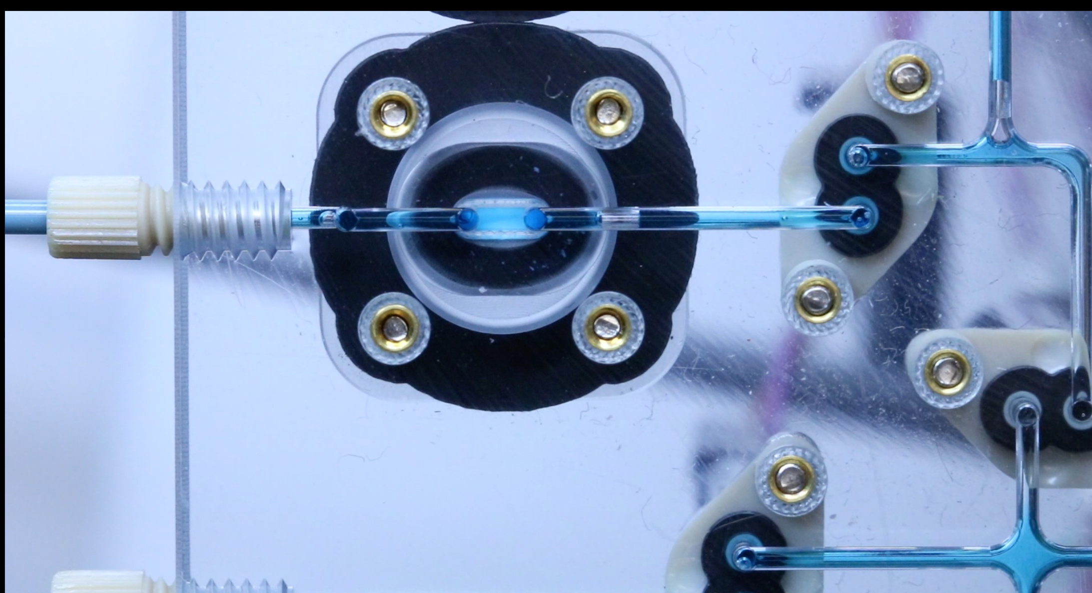
Chiral separations, e.g. amino acids (JPL: field demos.)

- Ultrasensitive laser-induced-fluorescence detection

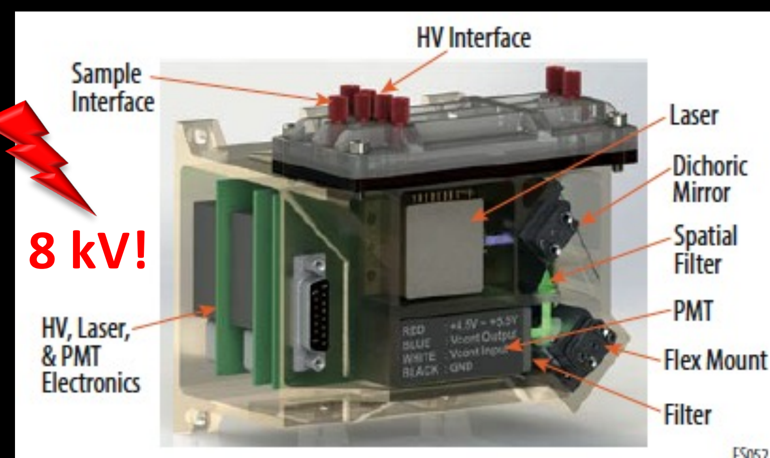


ARC sample-processing “front end” (COLDTech/ICEE2)

- Combination bubble trap / air-gap generator



8 kV!



MICA: **Microfluidic Icy-world Chemical Analyzer**

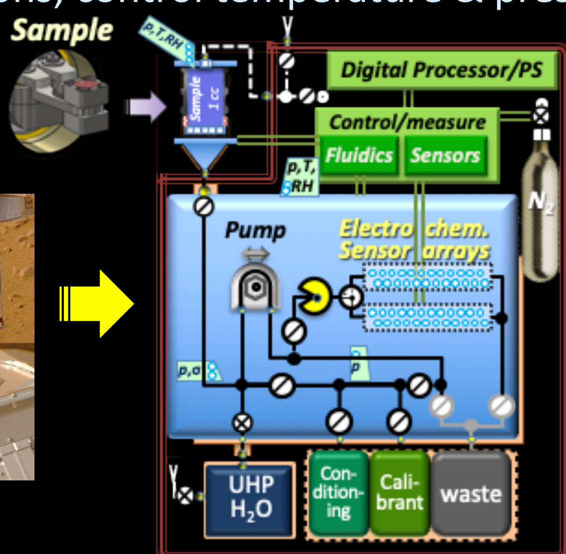
Fluidically Integrated Habitability Assessment for Icy Worlds

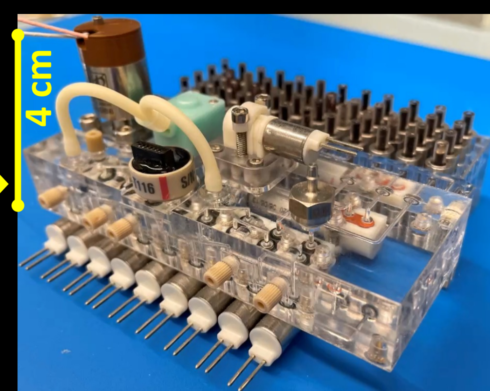
Heritage: Phoenix Wet Chemistry Lab (WCL) + COLDTech, ICEE-2 projects

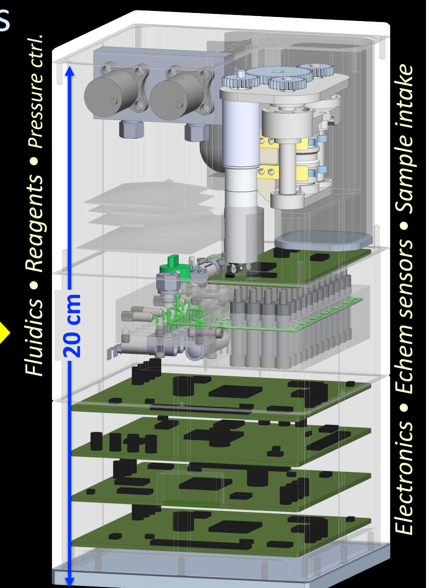
Lead**: ARC** Partners**: JPL, Tufts, MIT, U. of Alberta, Honeybee Robotics**

Key Measurements: pH, conductivity, ions, gases, ROS: Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, CO_3^{2-}, NH_4^+, NO_3^-, ClO_4^-, ClO_3^- PO_4^{3-}, SO_4^{2-}, SO_3^{2-} ; O_2, SO_2, O_2^- , H_2O_2; solution energetics: discrete & average of redox-active species (E_h)

Fluidic Functions : Receive & melt icy samples; prepare/deliver conditioning, blank, & calibrant solutions at multiple concentrations; control temperature & pressure; execute & store measurements

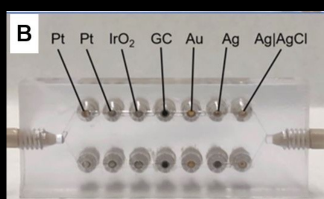


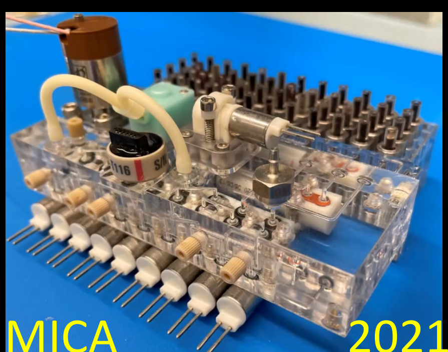




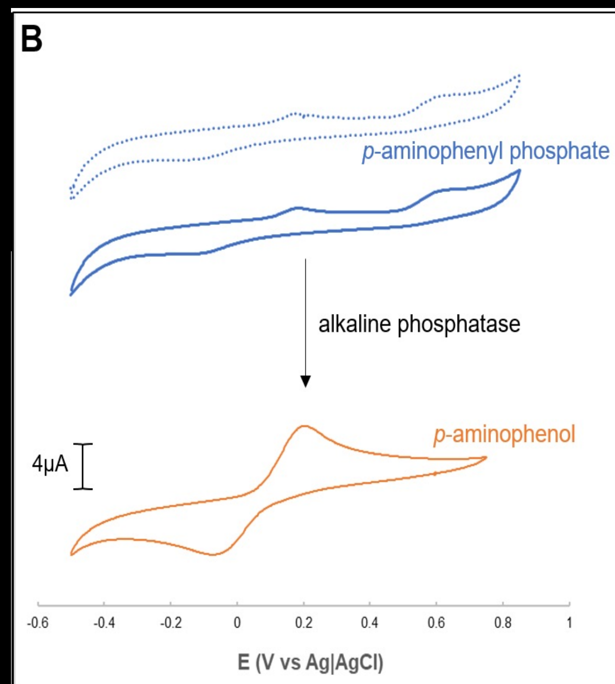
Electrochemical Detection of Catalysts as Signatures of Life

- Leverages Phoenix Wet Chemistry Lab technology
- Example targets: kinases, phosphatases, proteases
- Same instrument characterizes habitability & energetics





Ames, JPL, Tufts U., MIT

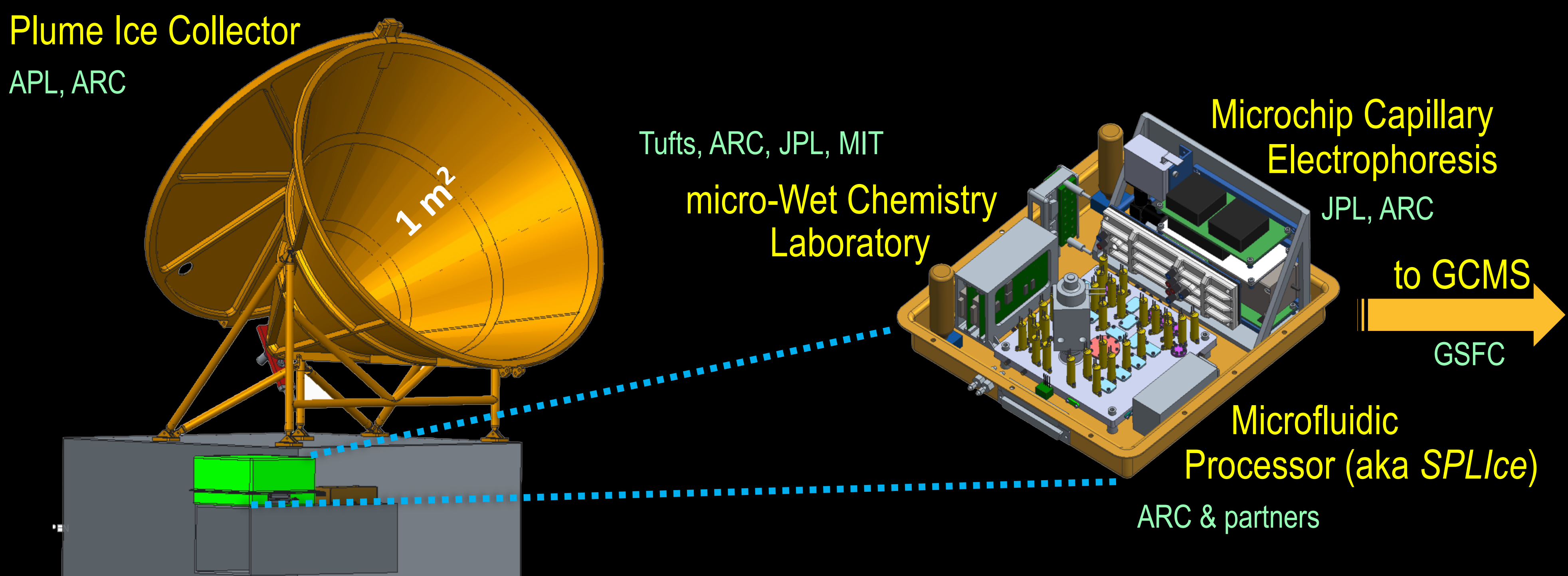


SD Thomson *et al.*, *ChemElectroChem* **2020**

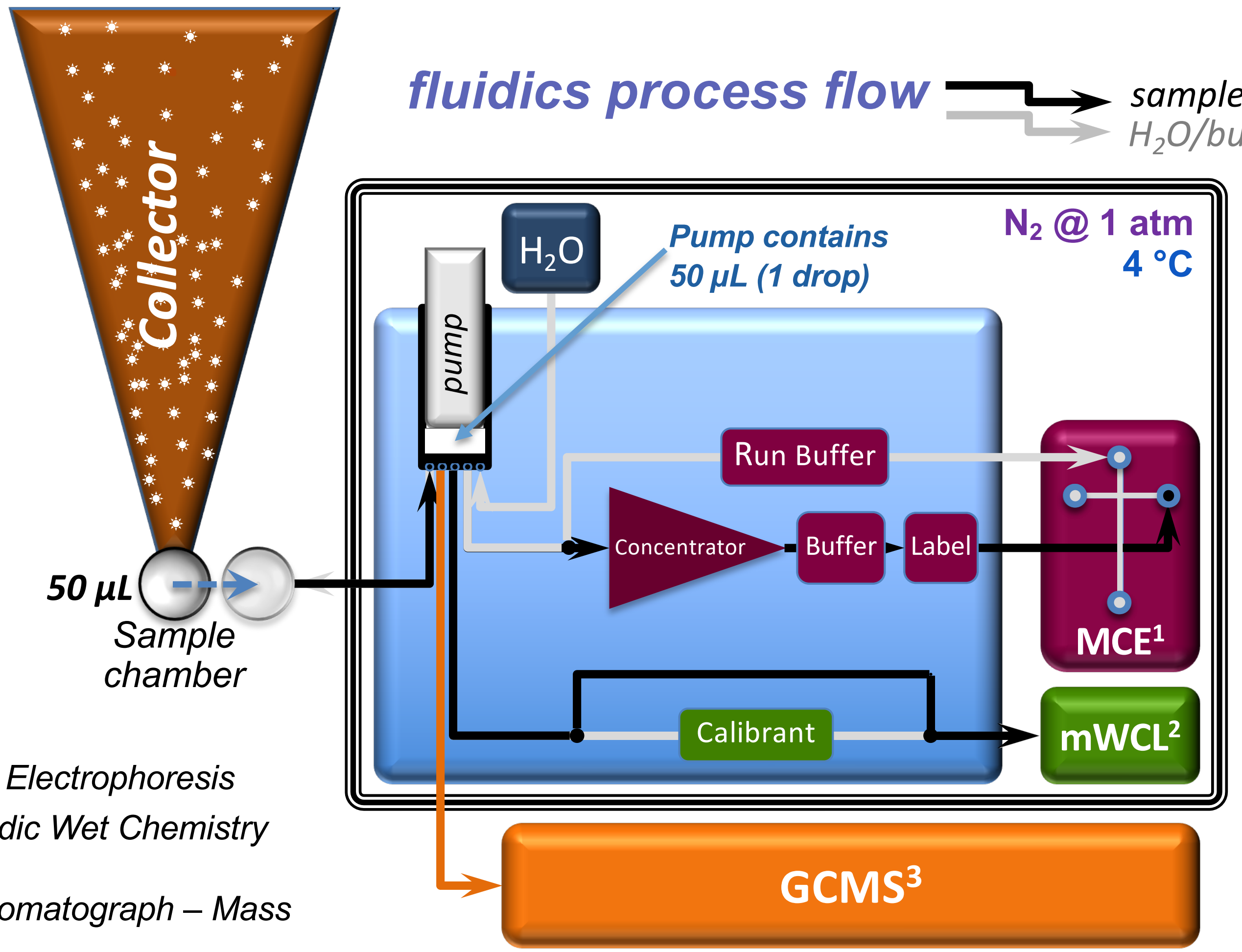
ELSAH: Enceladus Life Signatures and Habitability

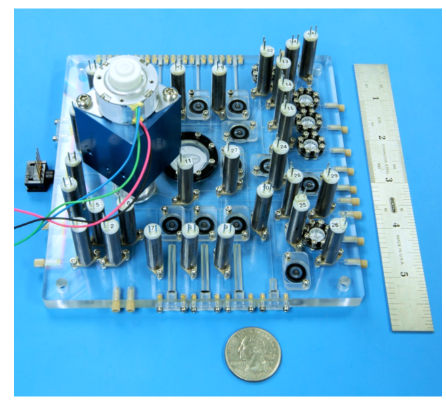
New Frontiers 4

μCAFE: micro Chemical Analyzer of Fluids for Exobiology



Sample Processor for Life on Icy worlds: "SPLIce" as the core of a Life-Search System for Enceladus

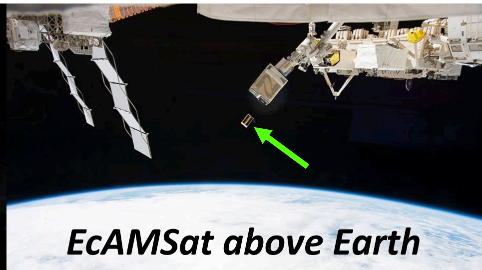




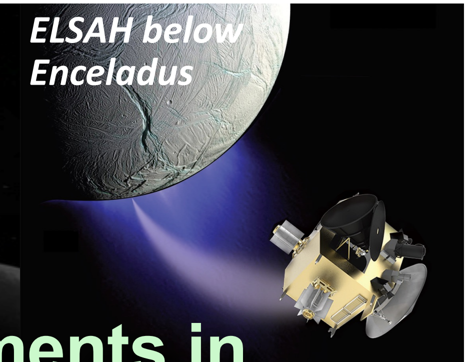
[1]MCE = MicroChip Electrophoresis

[2]mWCL = Microfluidic Wet Chemistry Laboratory

[3]GCMS = Gas Chromatograph – Mass Spectrometer


EcAMSat above Earth

Conclusions


ELSAH below Enceladus

- **HERITAGE of astro- & space-biology experiments in low-Earth orbit is a major enabler for interplanetary astro/biological missions, including the search for life on icy moons**
  - Full autonomy, flying dry, filling fluidic systems in μ-gravity, managing bubbles, …
  - Long-term materials biocompatibility, perfect sterility, contamination control
  - Radiation-tolerant design: *O/OREOS* functional to 5 years (15x ISS radn. levels); *BioSentinel* design for 2 yr in deep space, fluidics radn. sterilized at 3.5 Mrad (35 kGy)
  - High-heritage sensors, fluidic components & approach; high-efficiency / uniformity thermal control, managing pressure and condensation …

Thanks!

O/OREOS: Pascale Ehrenfreund, John Hines, Dave Squires, Chris Kitts, Charlie Friedericks, Dave Landis, Elwood Agasid, Matthew Piccini, Chris Beasley, Nathan Bramall, Gio Minelli, Greg Defouw, Laura Bica, Katie Bryson, Roland Burton, Julie Chittenden, Amanda Cook, Millan Diaz-Aguado, Shak Ghassemieh, Mike Henschke, Ed Luzzi, Diana Ly, Nghia Mai, Rocco Mancinelli, Andy Mattioda, Mike McIntyre, Mike Neumann, Wayne Nicholson, Macarena Parra, Richard Quinn, Mike Rasay, Bob Ricks, Orlando Santos, Aaron Schooley, Eric Stackpole, Linda Timucin, Bruce Yost, Anthony Young

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NASA/Ames

$ GeneSat: NASA Fundamental Space Biology, ESMD (now ~ SLPSRA/HEOMD)
$ O/OREOS: NASA Astrobiology Small Payloads Program, SMD
$ BioSentinel: NASA Advanced Exploration Systems, HEOMD
$ Search for Life Tech.: NASA COLDTech and ICEE2 Programs, SMD