Life in Space: Microfluidic Systems Enable the Study of Terrestrial Microbes in Space and the Search for Life on the Solar System's Icy Moons

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with thanks for insights, enlightenment, content:
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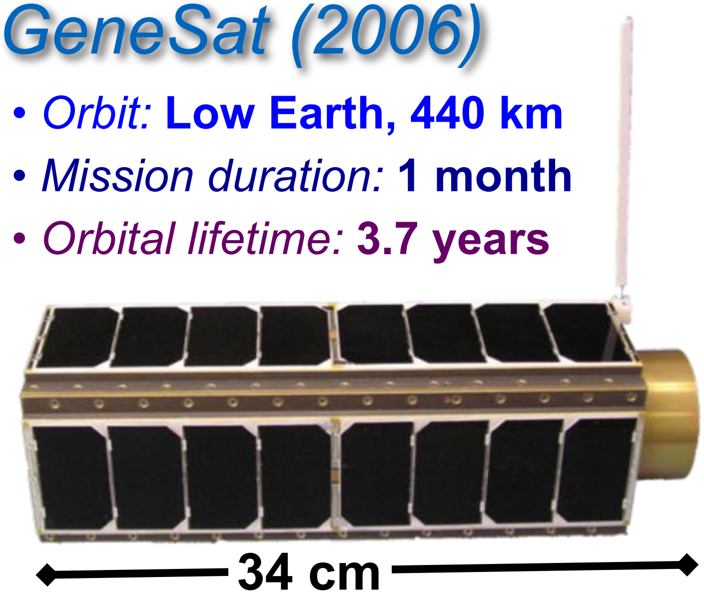
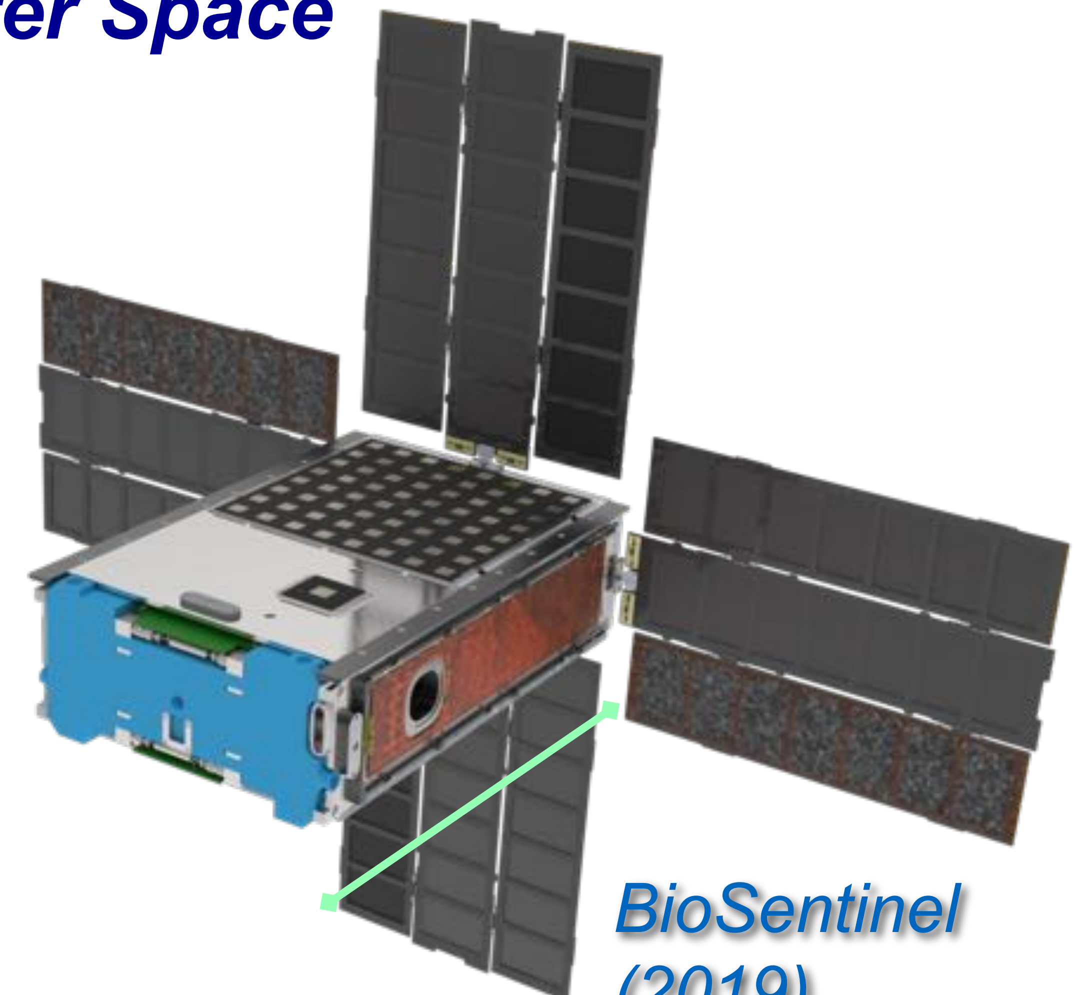
Integrated Microfluidic Bioanalytical Systems: Growing and Monitoring Microbial Cultures in Outer Space

GeneSat (2006)

- *Orbit:* **Low Earth, 440 km**
- *Mission duration:* **1 month**
- *Orbital lifetime:* **3.7 years**





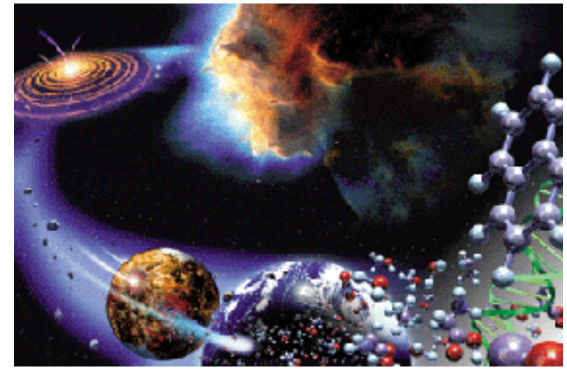
BioSentinel (2019)

- *Orbit:* **Interplanetary** (heliocentric), **0.1 – 100 M km**
- *Mission duration:* **6 – 12 months**
- *Orbital lifetime:* ∞

Astrobiology & Space Biology

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

- ***Why: fundamental understanding of life***
- Understand details & distribution of prebiotic chemistry -- chemical building blocks of life
- Study potential for life to adapt/survive in extraterrestrial environments
- Search for indicators of extant or extinct non-terrestrial life
- Find habitable environments in our solar system & beyond



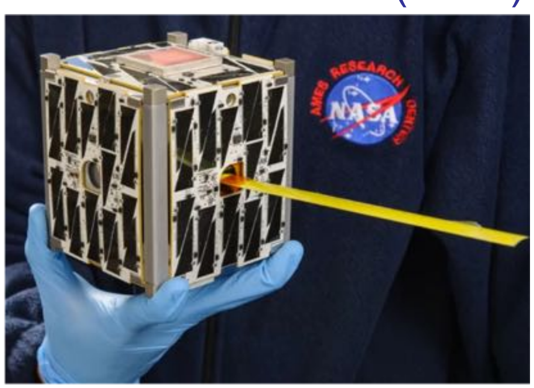
Fundamental Space Biology: effects of the space environment on terrestrial life

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

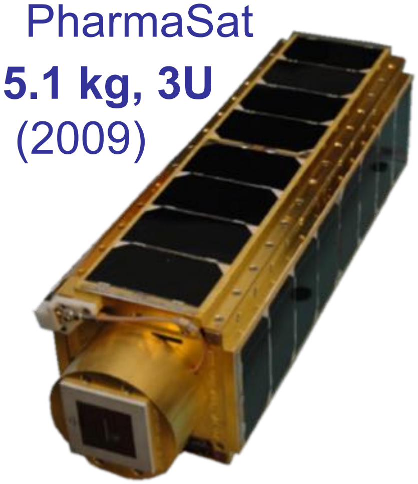
Rationale – Why Small Sats?

- **Small Sats (< 50 kg) are ever more capable:** *Miniature/micro/nano technologies*
  - bioengineered organisms; (micro)fabrication; materials; optics; sensors; actuators; MEMS; fluidics; electronics; communications; instrumentation; data handling & storage
  - Power generation & storage density up; power consumption down
- **Access to space:** *Low-cost* launches as secondary payloads
  - *military, government, commercial*; US, Russia, Europe, India, Japan, Canada …
  - ***Multiple flights possible*** **-** test, learn, iterate
- **Excellent education vehicle:** > 100 universities participating worldwide
- **Autonomous operations:** Less reliance on human crew for operation
- **Technology migration:** ISS; landers/orbiters for moon, Mars, Ocean Worlds

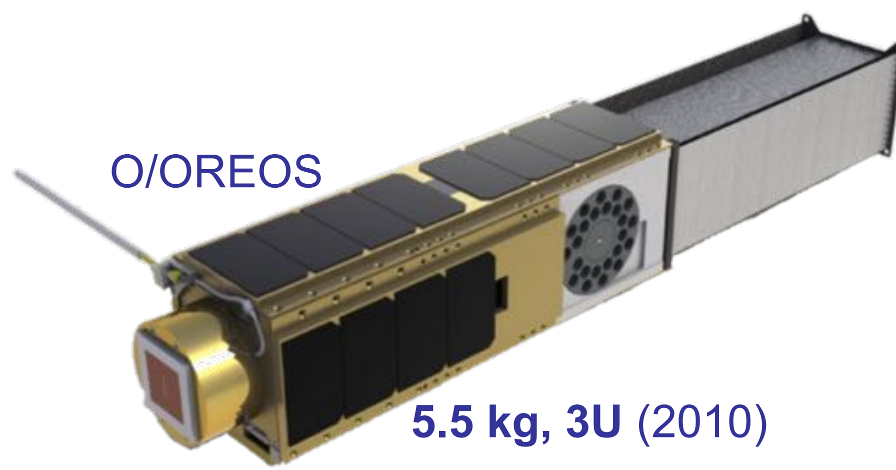
PhoneSat **1U** (2014)



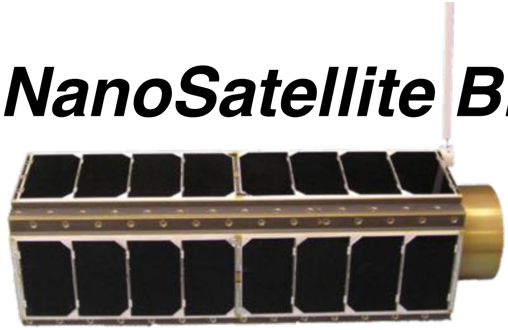
PharmaSat **5.1 kg, 3U** (2009)

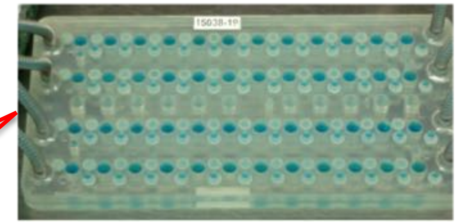


O/OREOS **5.5 kg, 3U** (2010)

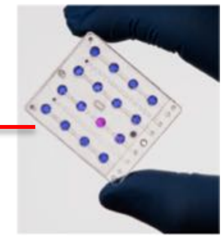


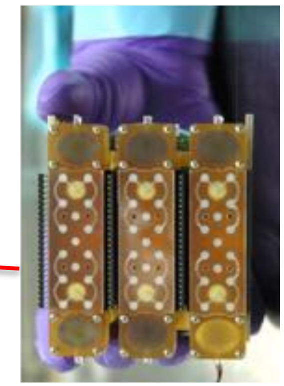
NASA Ames - NanoSatellite Biological Space Missions





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



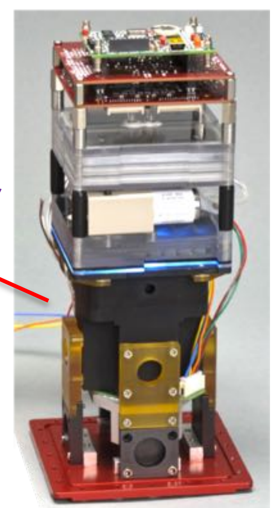


S. Cerevisiae *PharmaSat* (2009/3U): ***drug dose response***
BioSentinel (2019/6U): ***DNA break/repair***

B. Subtilis *O/OREOS** (2010/3U): ***survival, metabolism***
*ADRoIT-M*** (20xx/6U): ***mutations / lithopanspermia***

Ceratopteris Richardii *SporeSat-1* (2014/3U): **ion channel sensors, μ-centrifuges**
SporeSat-2 (20xx/3U): ***plant gravity sensing threshold***

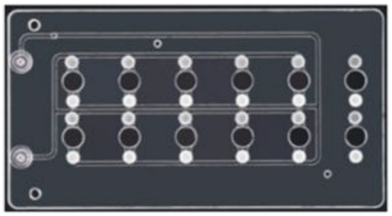
C. Elegans *FLAIR* (20xx/3U):
dual-wavelength
fluorescence imager

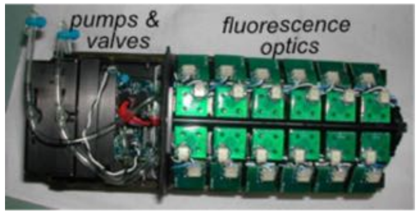


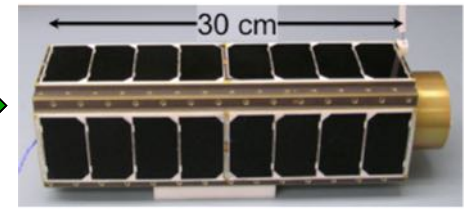
**Organism/Organic Response to Orbital Stress*
***Active DNA Repair on Interplanetary Transport of Microbes*

GeneSat-1: 1st biological nanosatellite in Earth orbit, 1st real-time, *in-situ* gene expression measurement in space

model organism:
E. coli



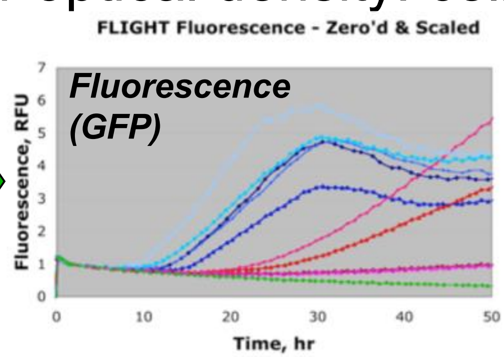




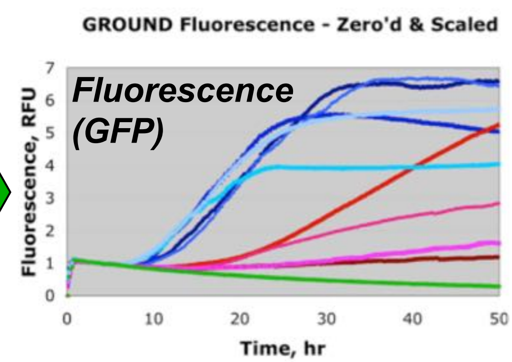
- ~ 0.5 x 2 μm bacteria
- nutrient deprivation in dormant state (6 weeks)
- launch: December 2006 to low Earth orbit (440 km)
- nutrient solution feed upon orbit stabilization, grow *E. coli* in μgravity
- monitor green fluorescent protein: gene expression
- monitor optical density: cell population

16 December 2006

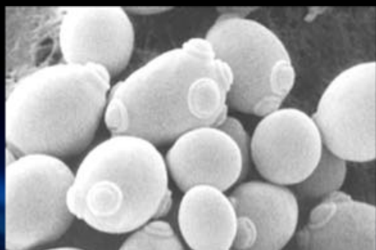
Telemeter data to



Compare to ground data



PharmaSat: *Effect of Microgravity on Yeast Susceptibility to Antifungal Drugs*



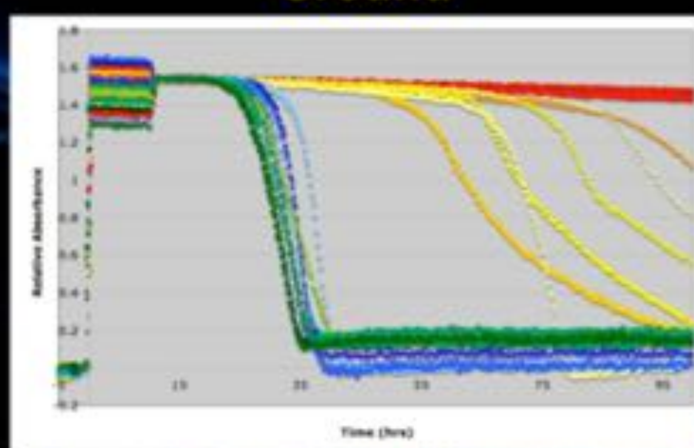
S. cerevisiae

- 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

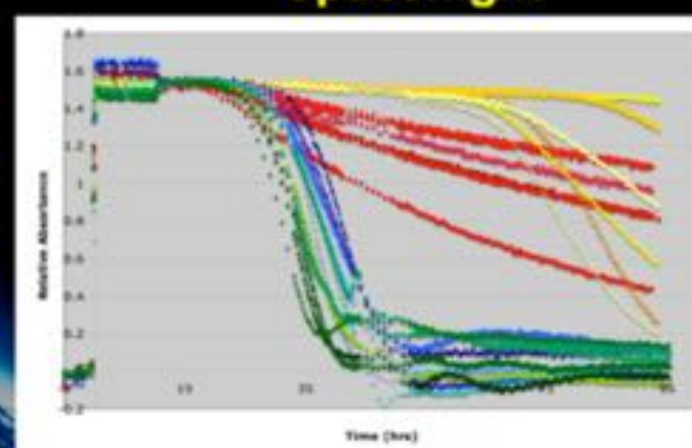
+

19 May 2009

Ground



Spaceflight

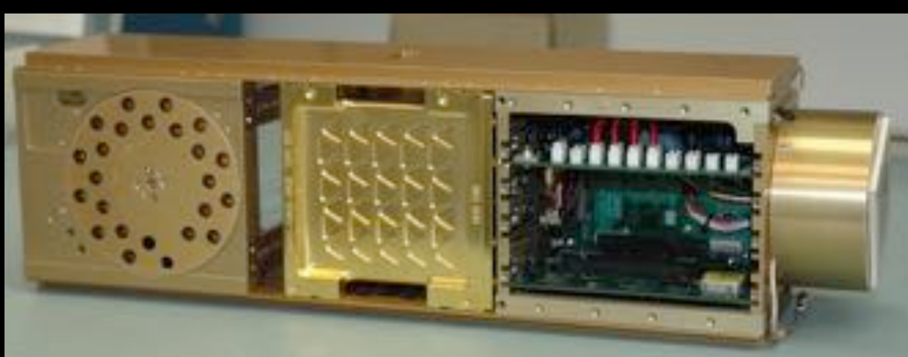


O/OREOS Mission

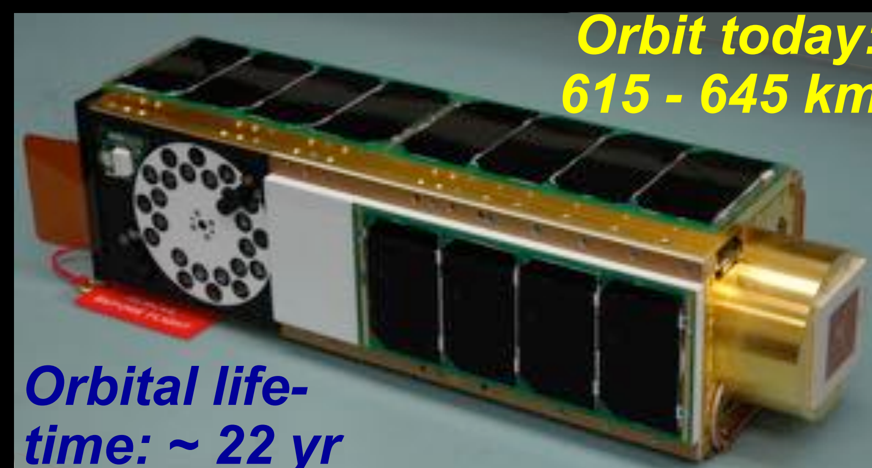
Organism/Organic Response to Orbital Stress

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

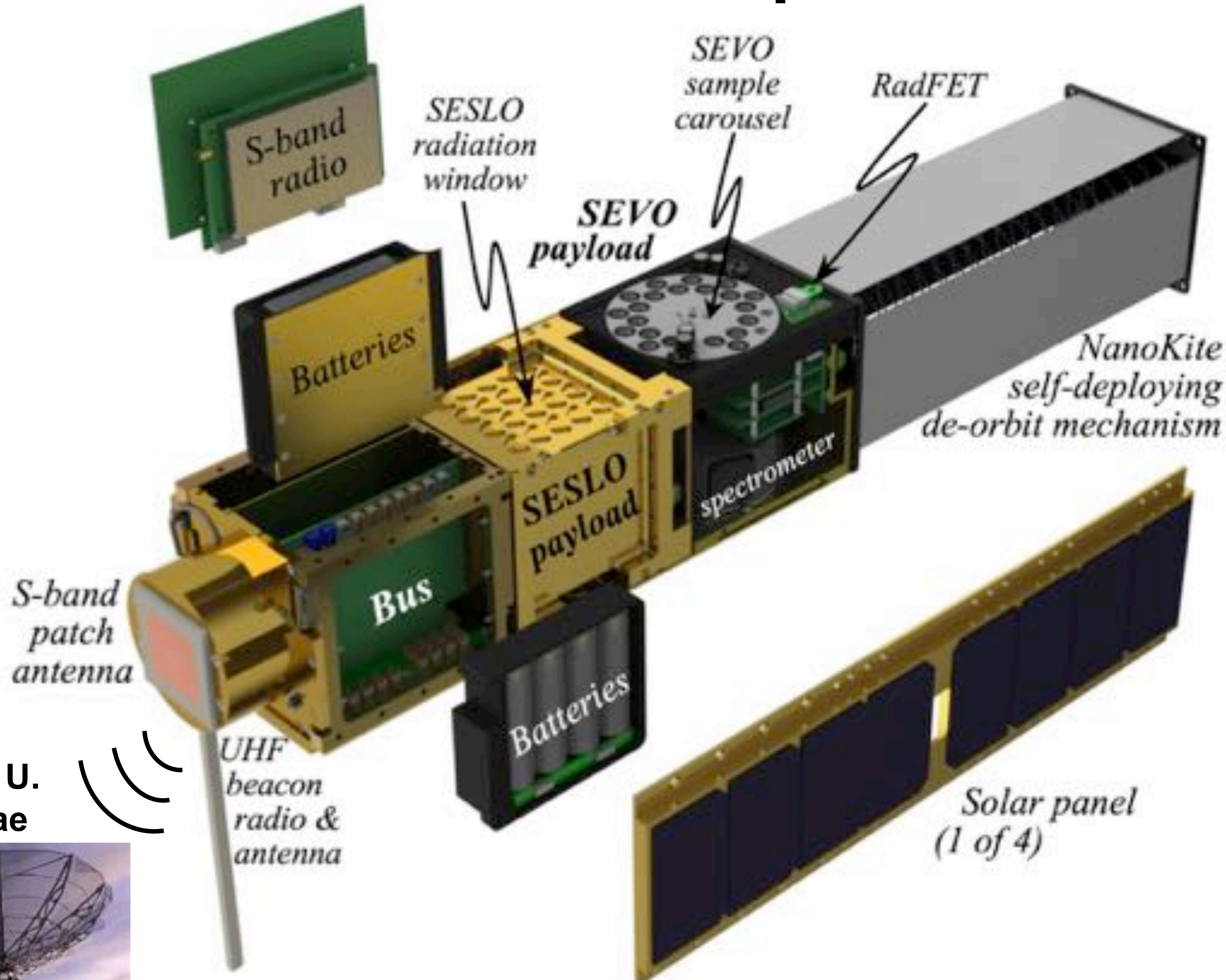
- **Monitor survival, growth, and metabolism of *Bacillus subtilis* using *in-situ* optical density / colorimetry** [SESLO: Space Environment Survival of Living Organisms]
- **Track changes in organic molecules and biomarkers: UV / visible / NIR spectroscopy** [SEVO: Space Environment Viability of Organics]



Flight prototype



O/OREOS Nanosatellite Exploded View



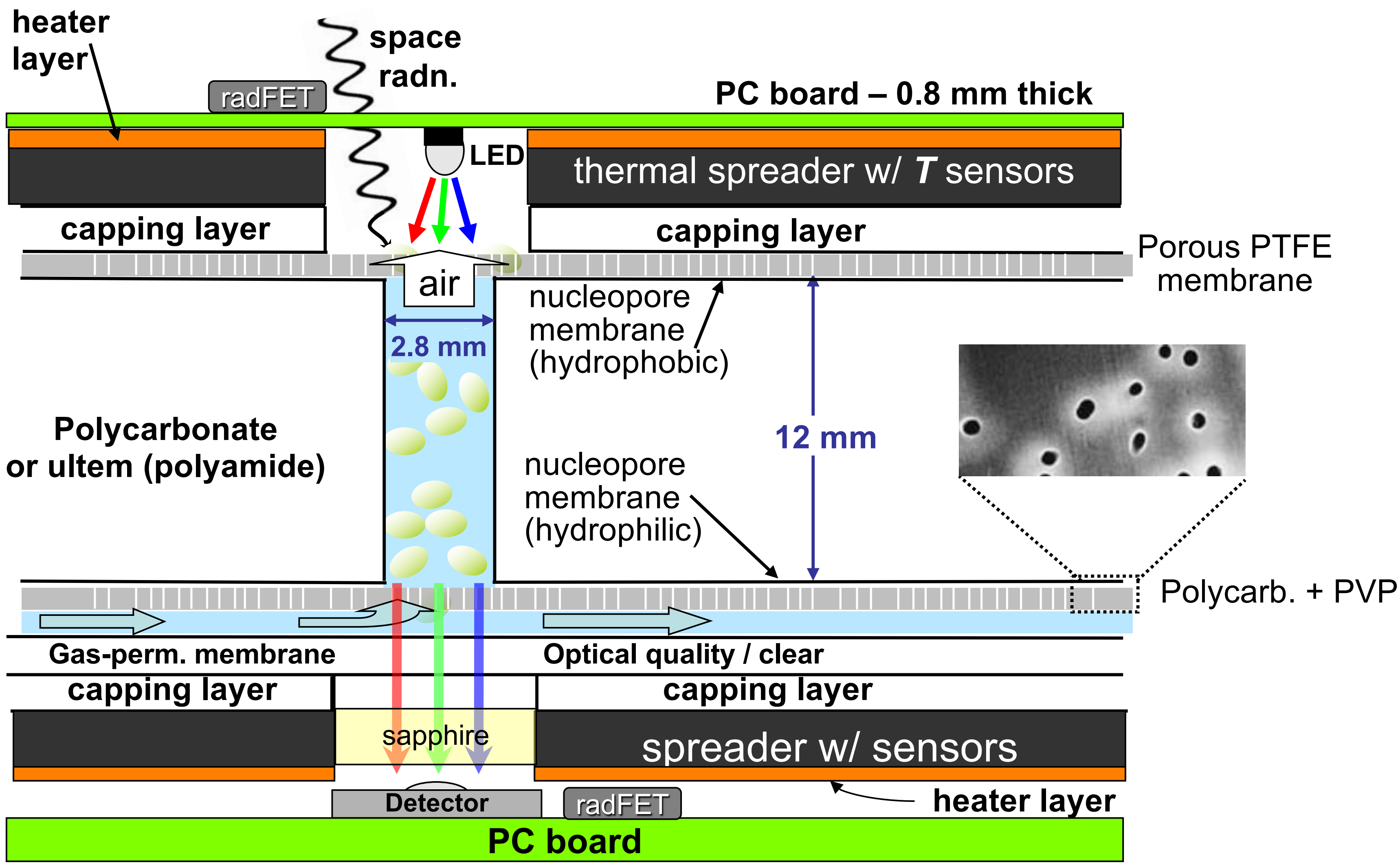
Santa Clara U. 3-m antennae

***SEVO** = *Space environment viability of organics*

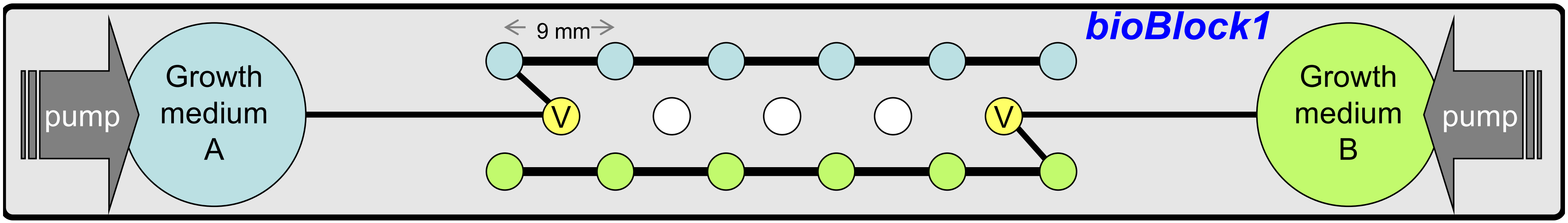
***SESLO** = *Space environment survival of living organisms*

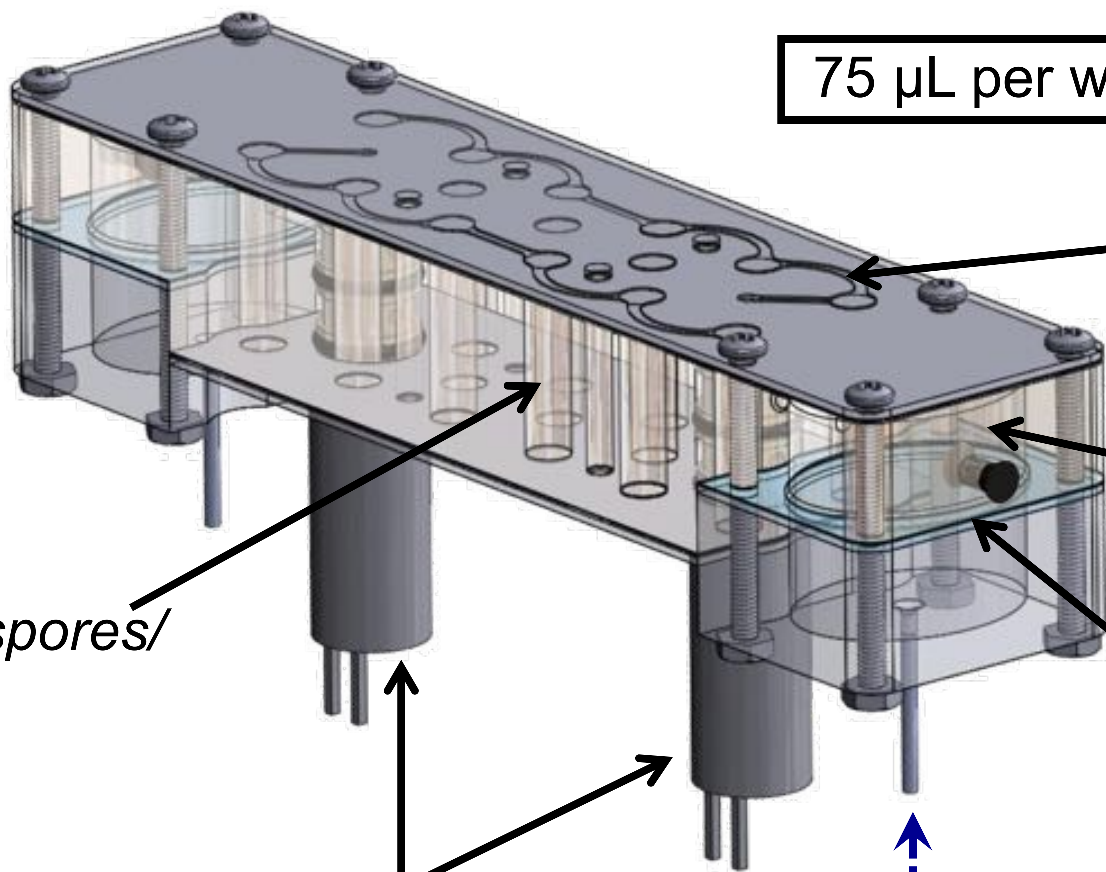
SESLO (bio) Fluidic/Thermal/Optical Architecture

Fluidic / optical / thermal cross-section



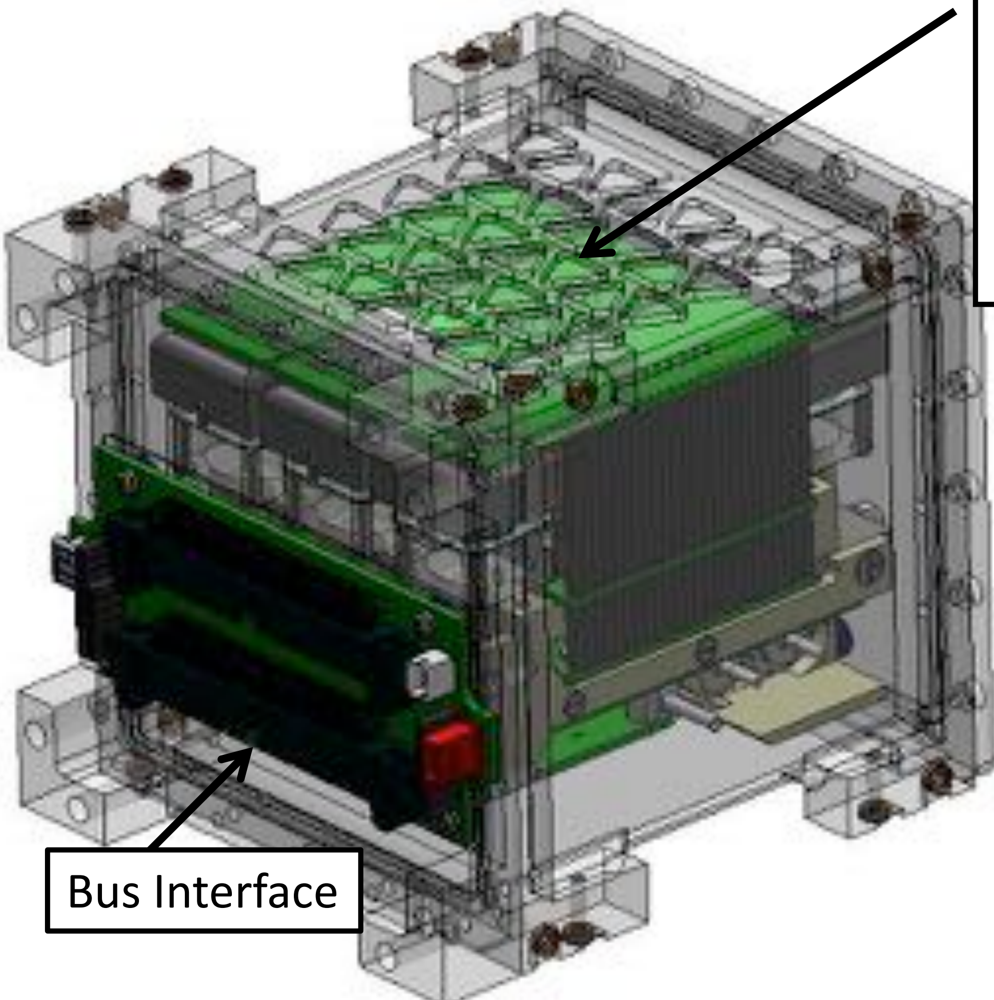
SESLO Integrated Fluidic System: 3 independent bioBlocks





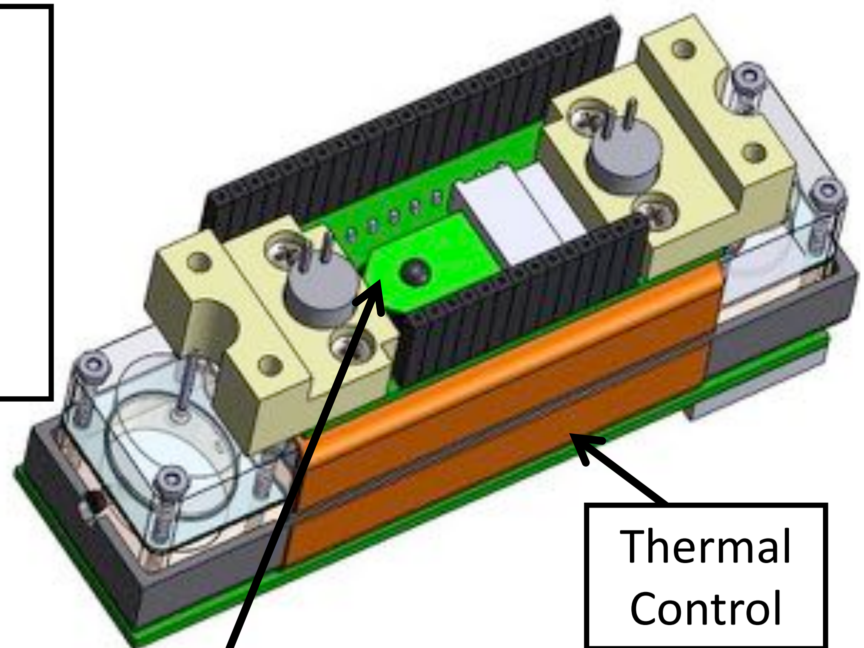
solenoid valves open 2x/day to maintain fluid back-pressure to compensate for evaporation from wells throughout organism growth period

SESLO Design Summary



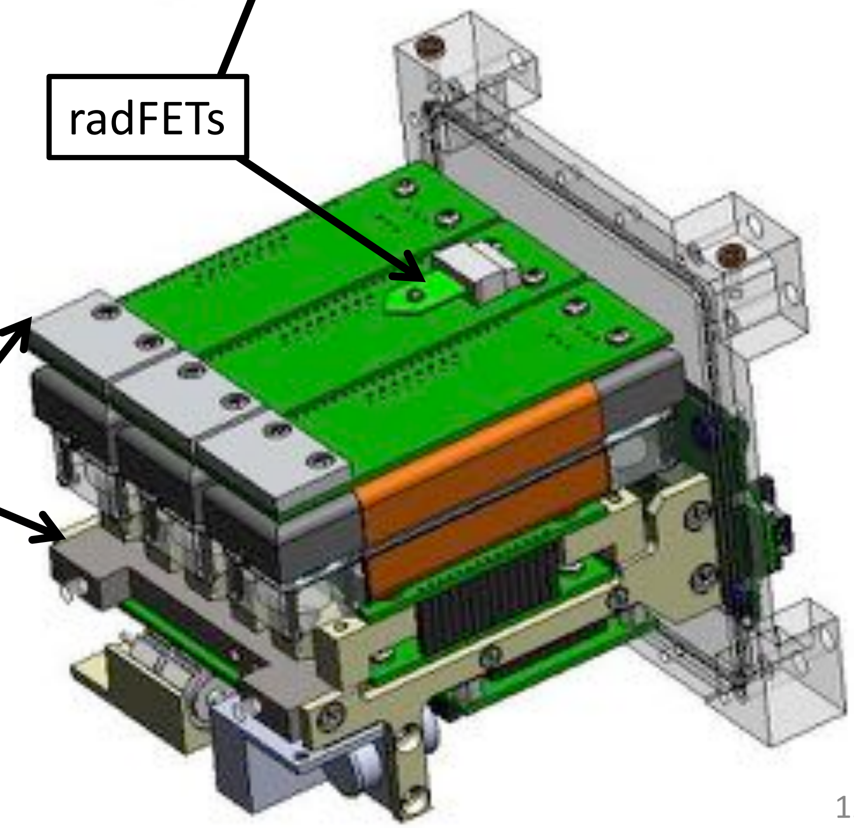
Thinned region of pressure vessel: *radiation window*

Bus Interface

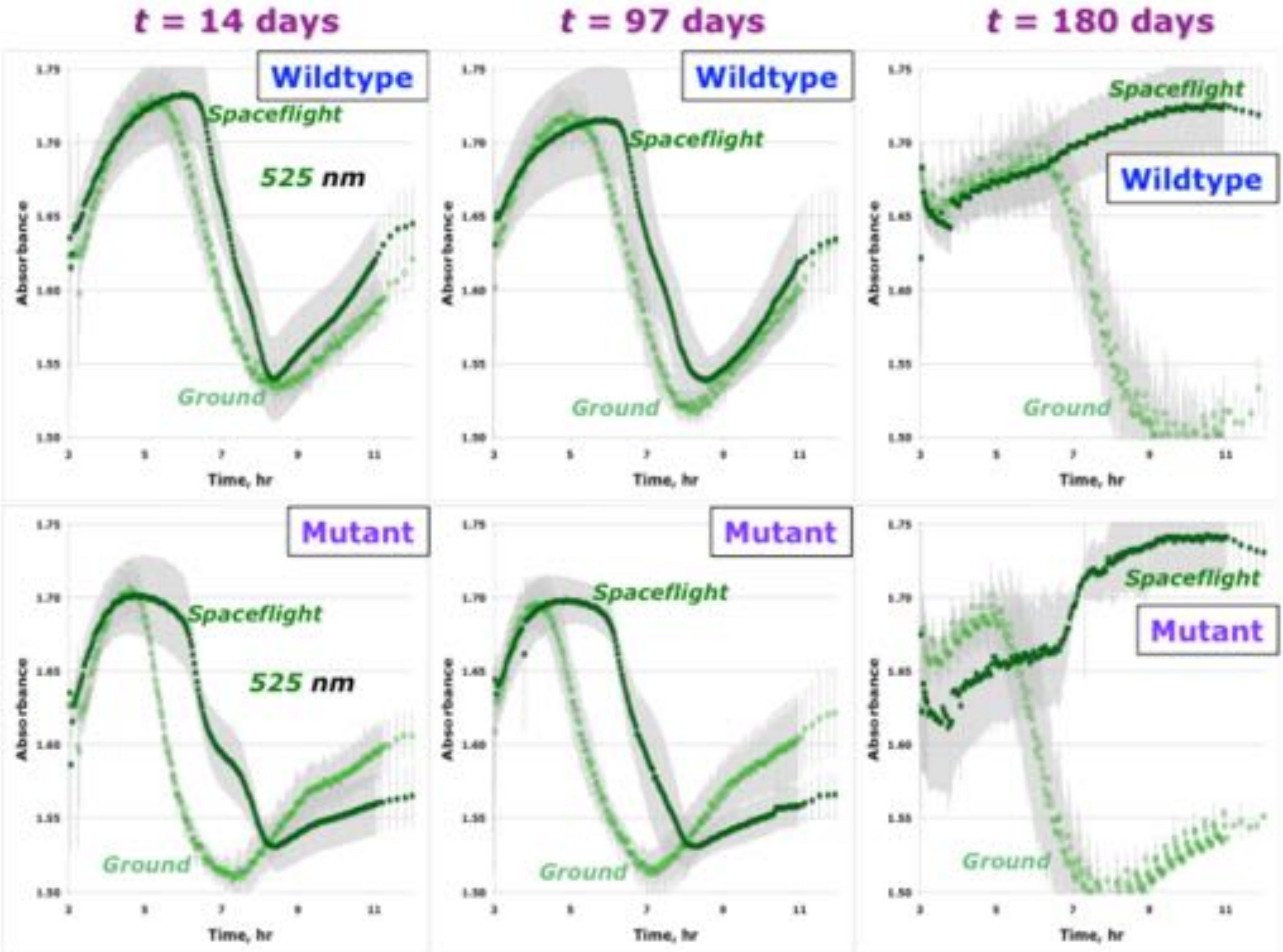


Thermal Control

radFETs



Radiation Shield

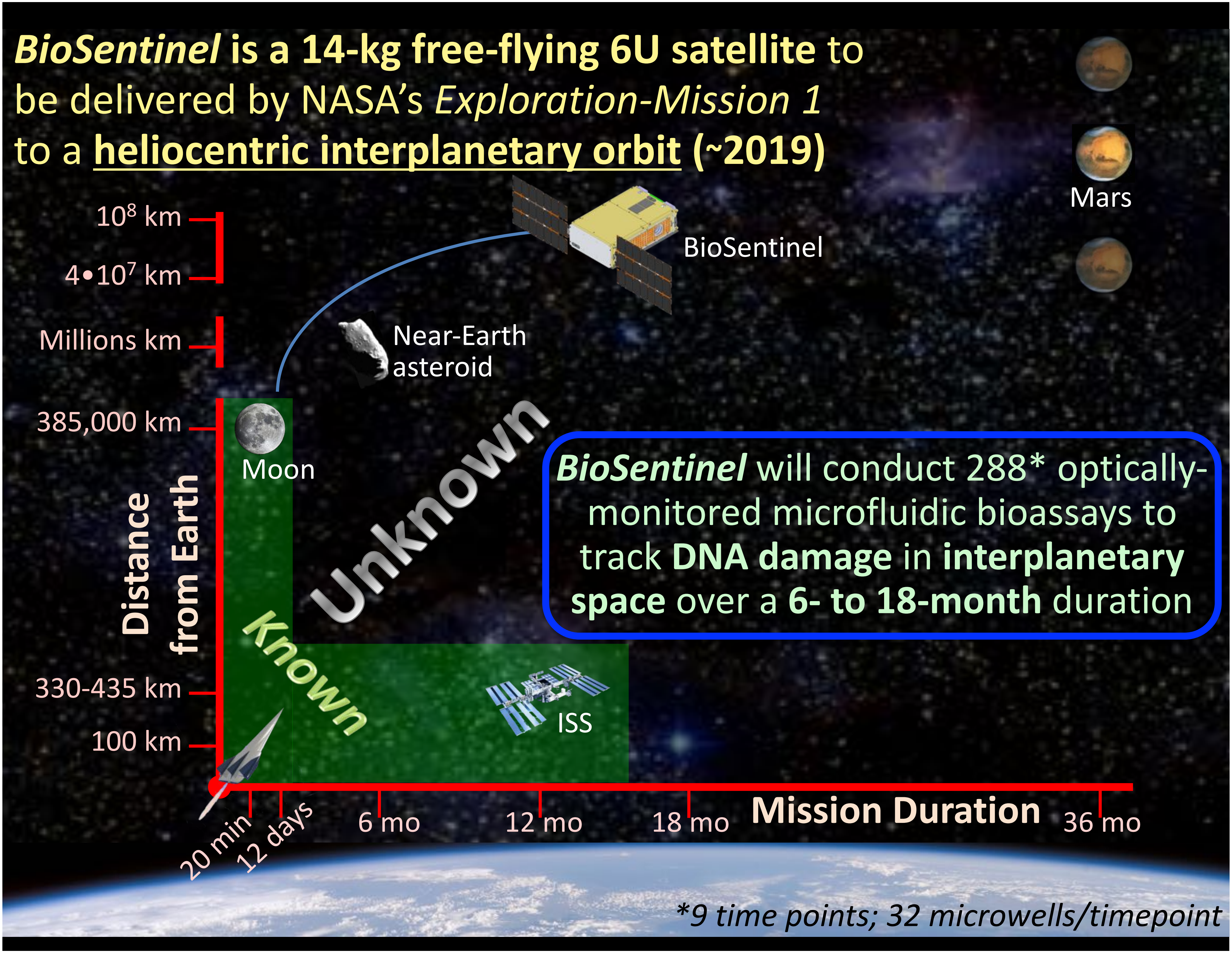


t = 14 days
t = 97 days
t = 180 days
Wildtype
Spaceflight
525 nm
Ground
Absorbance
Time, hr
Wildtype
Spaceflight
Ground
Absorbance
Time, hr
Spaceflight
Wildtype
Ground
Absorbance
Time, hr
Mutant
Spaceflight
525 nm
Ground
Absorbance
Time, hr
Mutant
Spaceflight
Ground
Absorbance
Time, hr
Spaceflight
Mutant
Ground
Absorbance
Time, hr

BioSentinel Mission: Biological Effects of Deep-Space Radiation

1st Biology Experiment beyond Low Earth Orbit since Apollo (1972)

- **Limits of life in space, as studied to date:**
  - 12 days on a lunar round trip (furthest distance)
  - ~ 1.5 years in low Earth orbit (longest duration)
- **If humans are to go beyond LEO for longer times:**
  - model organisms can help us understand / mitigate biological risks
    - **direct measure of factors that impact human health or performance**
    - **impact on biota that accompany humans**
    - **impact on organisms for processing waste or producing food**
- **Interplanetary space: biological access enables new astrobiological studies** in deep space's complex radiation field
    - **microbial evolution, development, survival**
    - demonstration of technologies relevant to **life detection** far from Earth

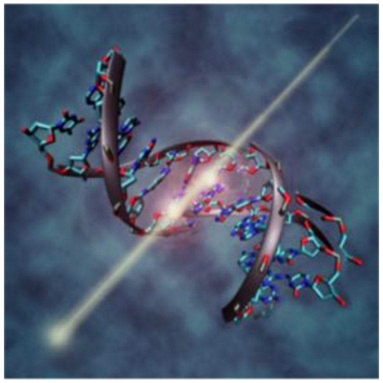


BioSentinel is a 14-kg free-flying 6U satellite to be delivered by NASA's *Exploration-Mission 1* to a heliocentric interplanetary orbit (~2019)
Mars
BioSentinel
Near-Earth asteroid
10^8 km
$4 \cdot 10^7$ km
Millions km
385,000 km
330-435 km
100 km
Distance from Earth
Moon
Unknown
Known
ISS
BioSentinel will conduct 288* optically-monitored microfluidic bioassays to track DNA damage in interplanetary space over a 6- to 18-month duration
20 min
12 days
6 mo
12 mo
18 mo
Mission Duration
36 mo
*9 time points; 32 microwells/timepoint

Why Study (Astro)Biology in Deep Space?

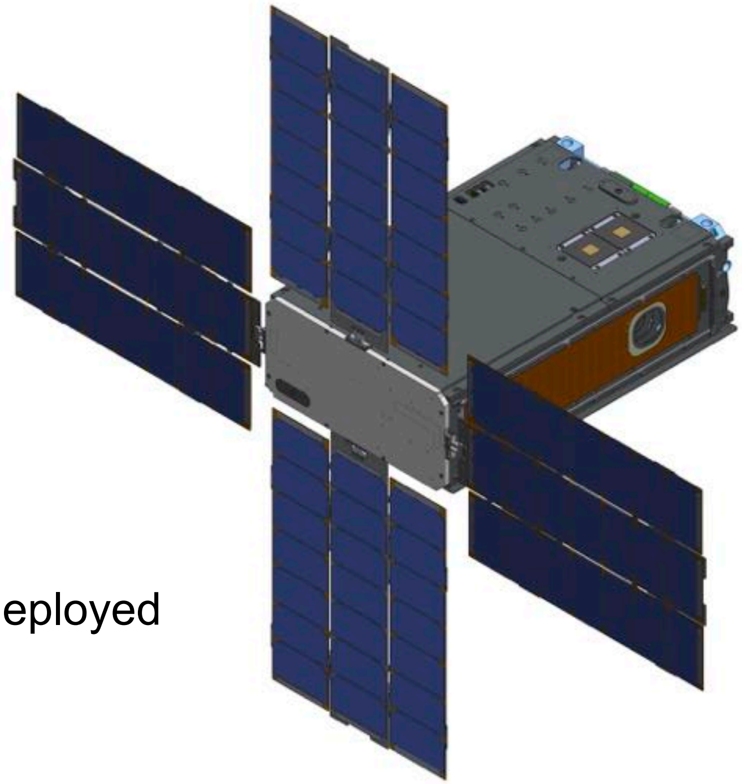
Low Earth Orbit provides perfectly adequate μ-gravity

***Answer*: Radiation**

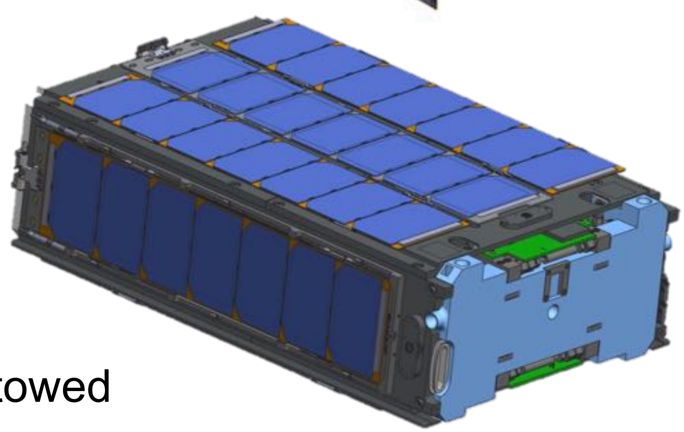


- Space beyond Earth's magnetosphere hosts a complex mixture of particle types
  - each particle type has its own energy spectrum
  - also: electromagnetic radiation extending into vacuum UV
- For some biological processes, **effects** of chronic low dosage of multiple particle types & energies ≠ acute dose of 1 or 2 particle types, 1 energy
  - Biology can self-repair. Solid-state materials, devices (generally) do not.
  - Repair (and mutation) can profoundly impact long-term radiation effects in biological organisms that are not simulated by non-living materials.
  - Cells communicate. Damage of a few cells can indirectly affect many others.
  - Cell lethality is typically not the main concern – the problem is those that survive a "hit".
- High-radiation environments available in "special" cases of LEO
  - polar orbits, dense regions of Van Allen belts, So. Atlantic Anomaly
  - BUT these are not the same as deep space: GCR is shielded/modified by magnetosphere and SPEs are highly attenuated

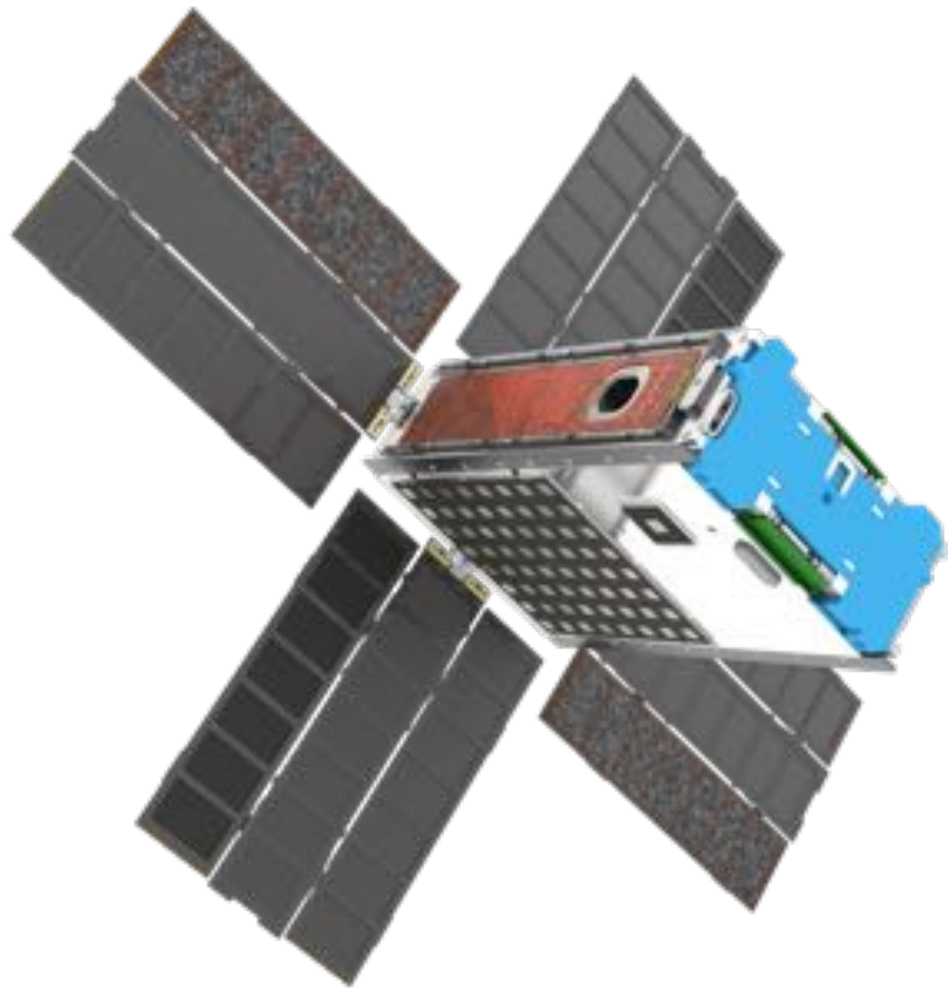
BioSentinel: Deployed & Stowed



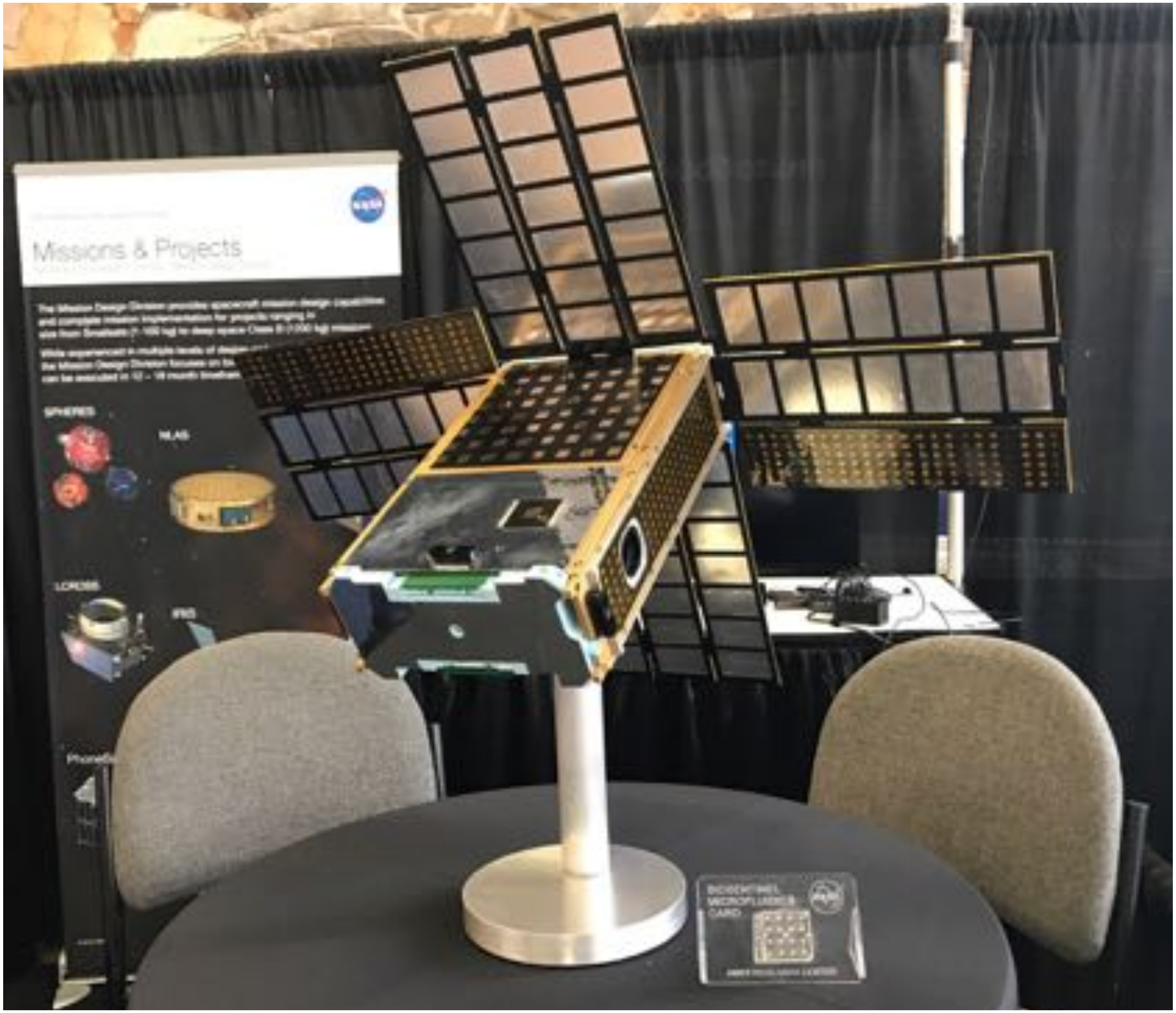
Deployed



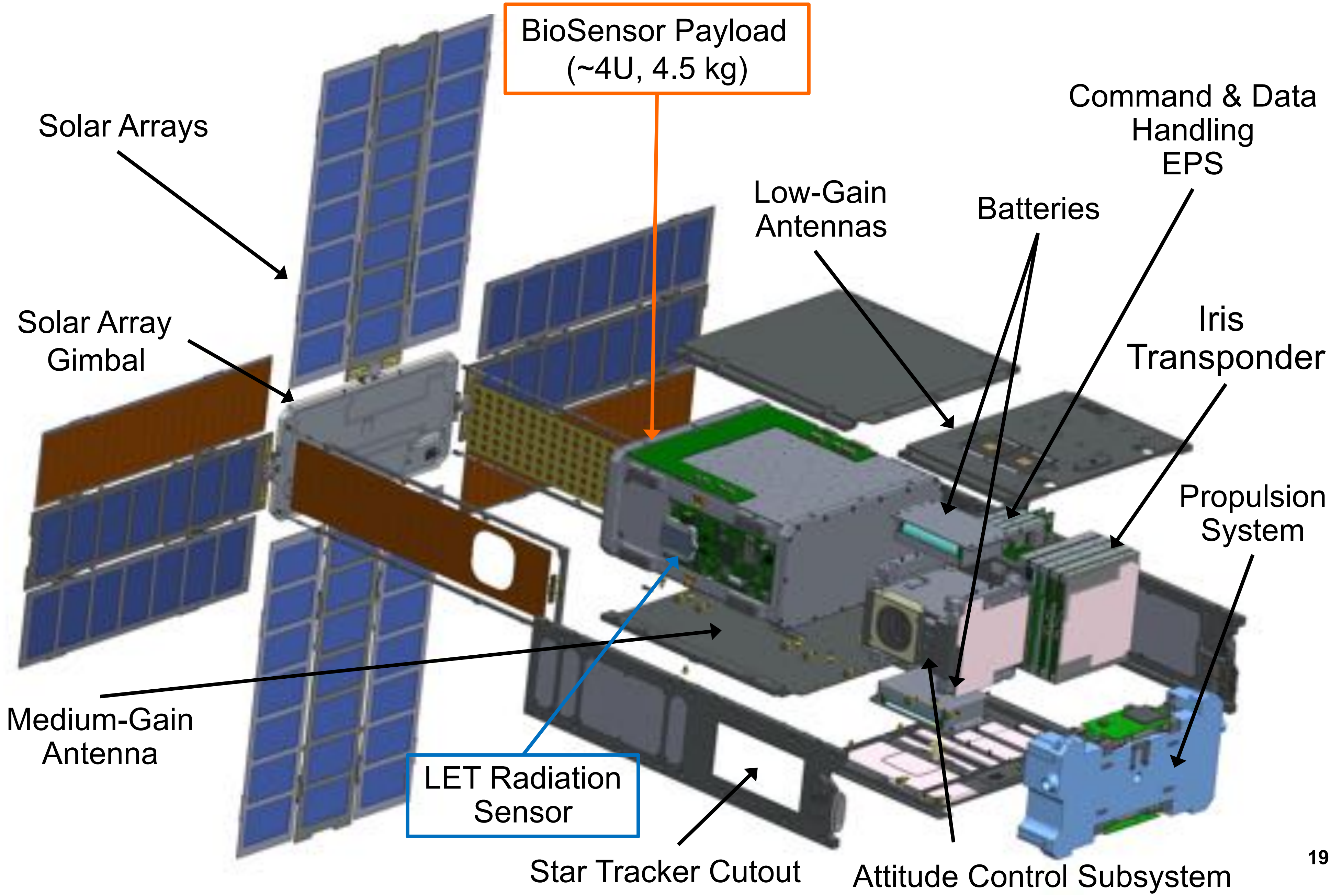
Stowed



BioSentinel: Deployed & Stowed



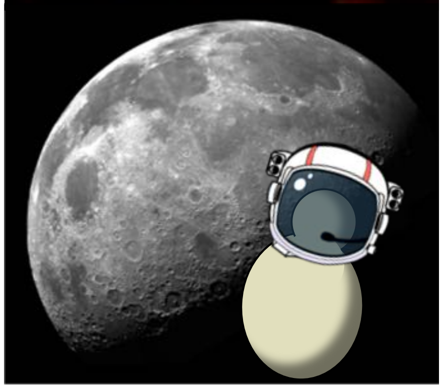
BioSentinel Subsystem Overview



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

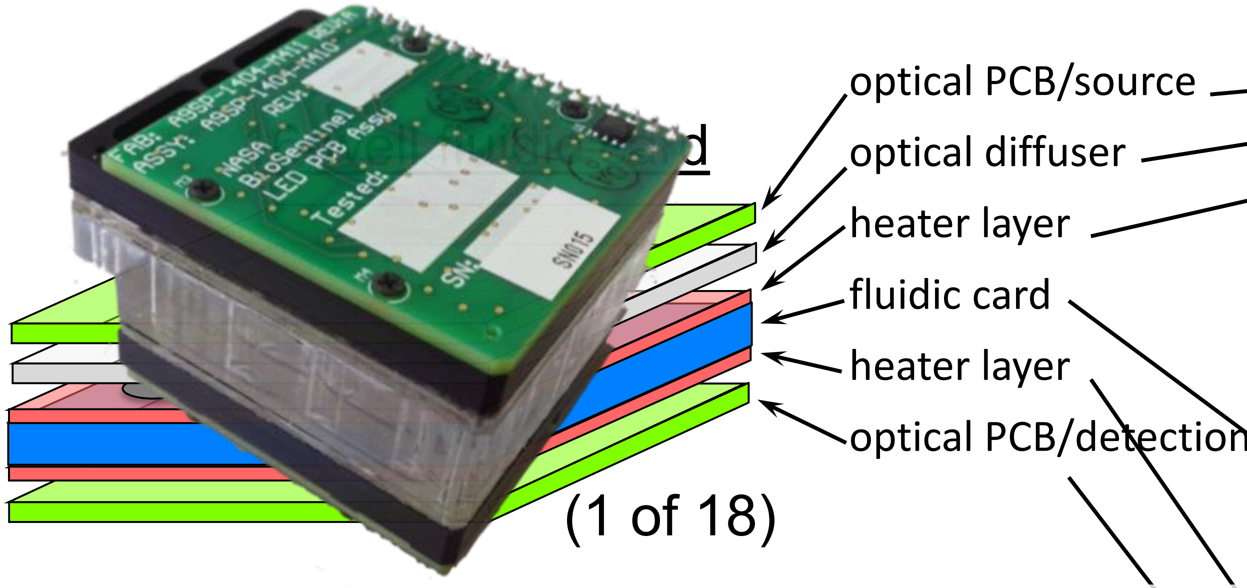
- ***Quantify DNA damage from space radiation environment***
  - Deep space environment cannot be reproduced on Earth: *omnidirectiona continuous, low flux, variety of particle types*
  - Health risk for humans spending long durations beyond LEO
  - Radiation flux can spike 1000x during a solar particle event (SPE)

- **Yeast assay: microfluidic arrays monitor DNA damage**
  - Two strains of *S. cerevisae*: 1 control (wild-type), 1 engineered
    - *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 Δrad51 mutant
  - Reserve wells for solar particle event: autonomous activation

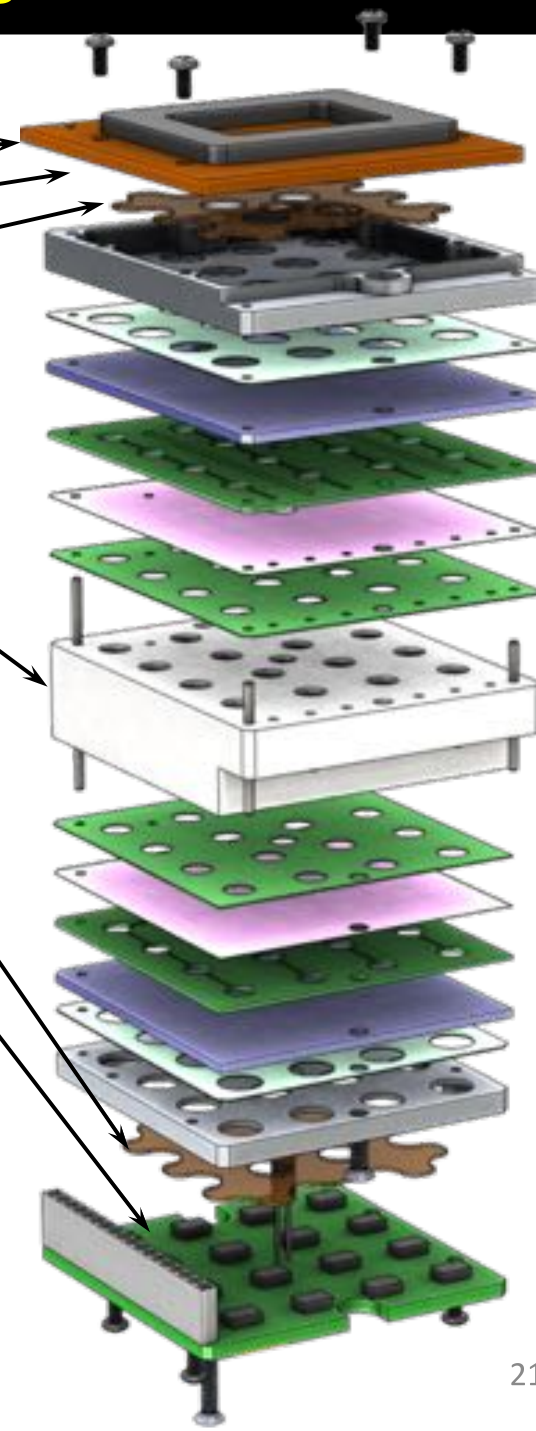


- **Correlate biological response with physical radiation measurements**
  - ***Linear Energy Transfer*** (LET) spectrometer bins and counts particle events by their LET
  - Total Ionizing Dose (TID): calculation of integrated deposited energy by LET system

BioSentinel — *BioSensor Payload Configuration*

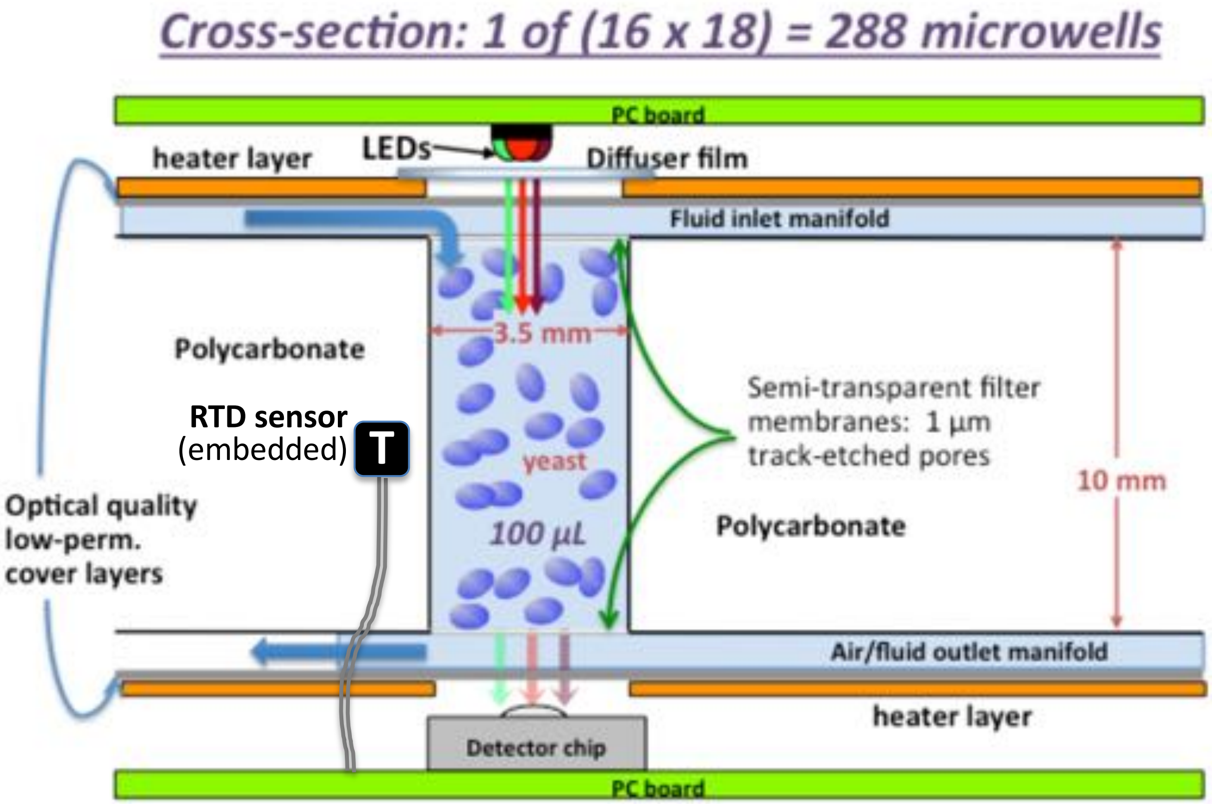


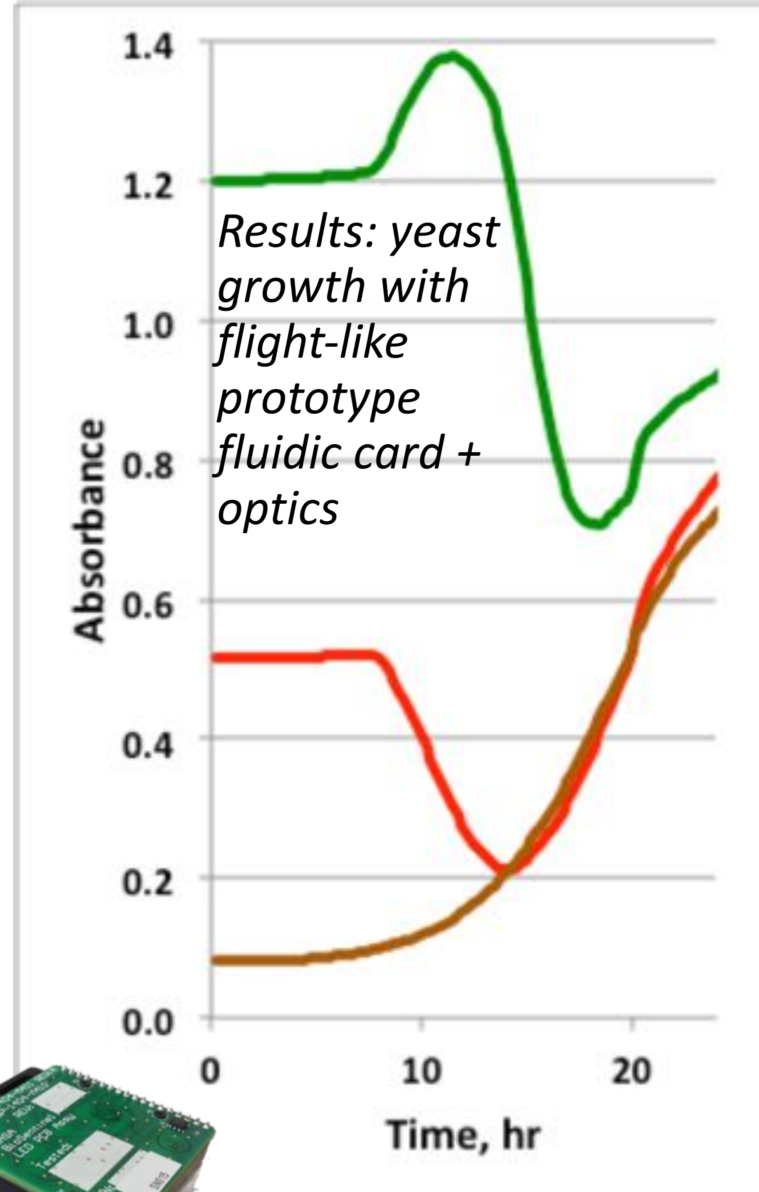
- **Optical absorbance measurement per well**
  - ➢ Dedicated 3-color optical system at each well
  - ➢ Measure dye absorbance & optical density (cell population) with stray light correction
  - ➢ Ground pre-calibration + in-flight "active" cal.
- **Pressure & humidity sensors** in P/L volume
- **Dedicated thermal control system per card**
  - ➢ 16 – 23°C; 1 °C uniformity, accuracy, stability
  - ➢ 1 RTD sensor per card: closed-loop control



BioSentinel

Bio-fluidic-opto-thermal Cross Section



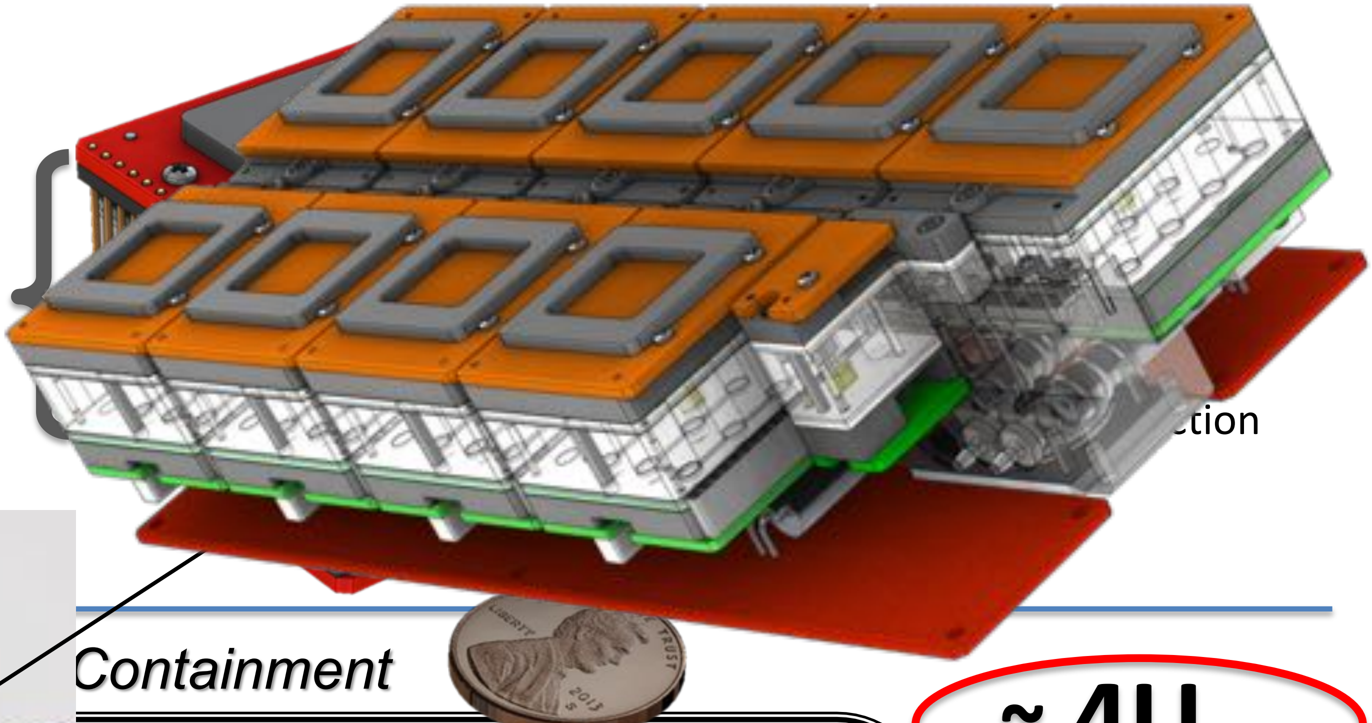


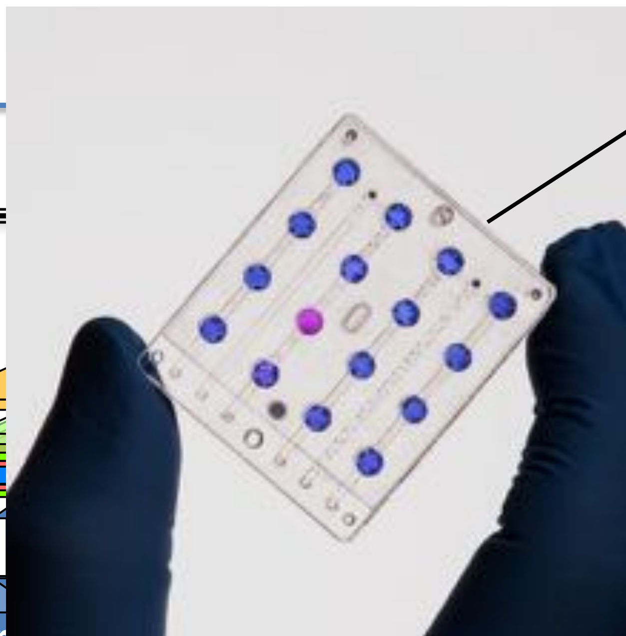
- Yeast dried onto µwell walls prior to integration & launch
- Pairs of 16-µwell cards wetted periodically
- 3 LEDs + detector, per well, track growth *via* optical density and cell metabolic activity *via* dye color changes.
- LEDs: 570, 630, 850 nm

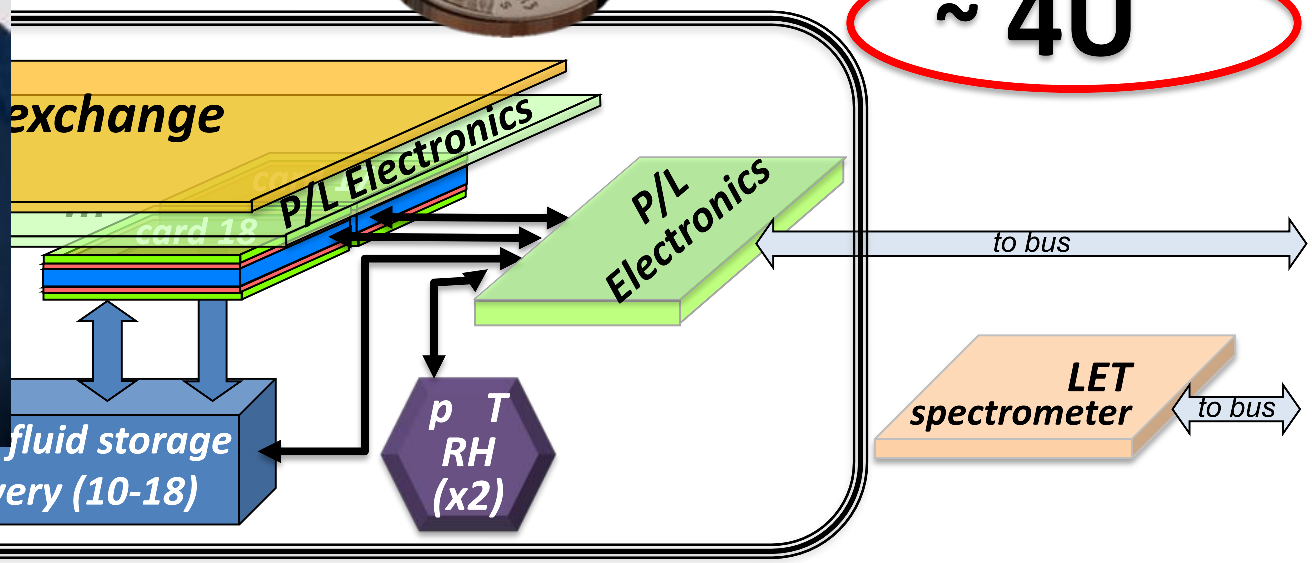
BioSentinel

Bio-fluidic-opto-thermal Configuration

16-well card
= 1 “set”
(18 sets total)







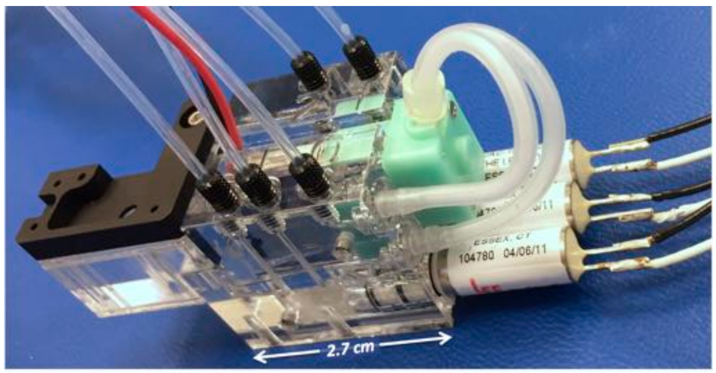
~ 4U

Biofluidic Subsystem

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

Manifold-integrated components:

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



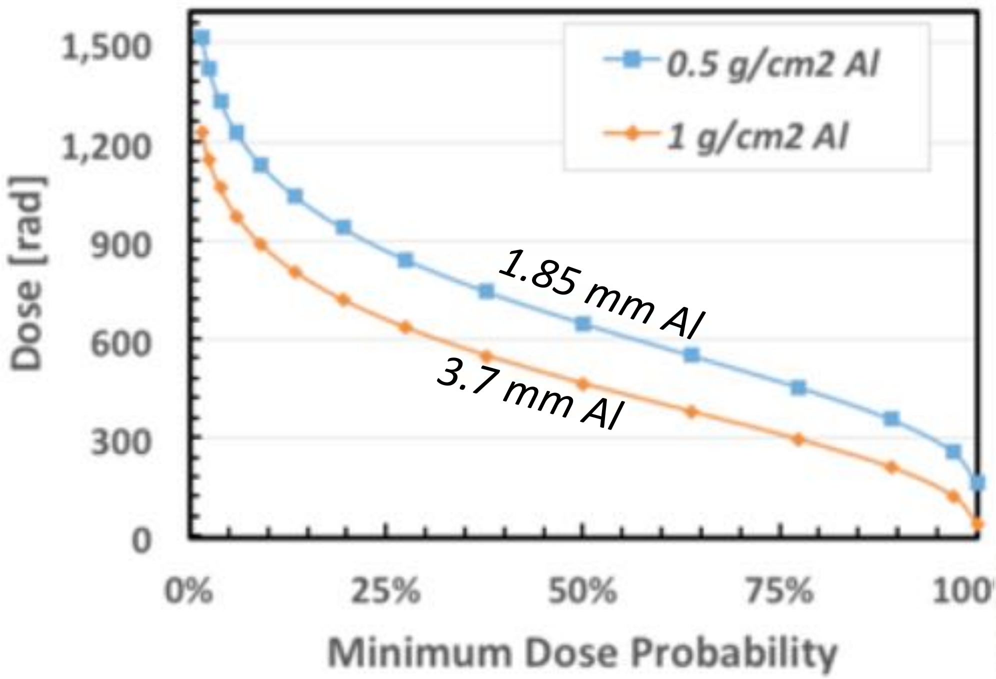
Reagent-and-pump manifold [1 of 2]

Tally of components:

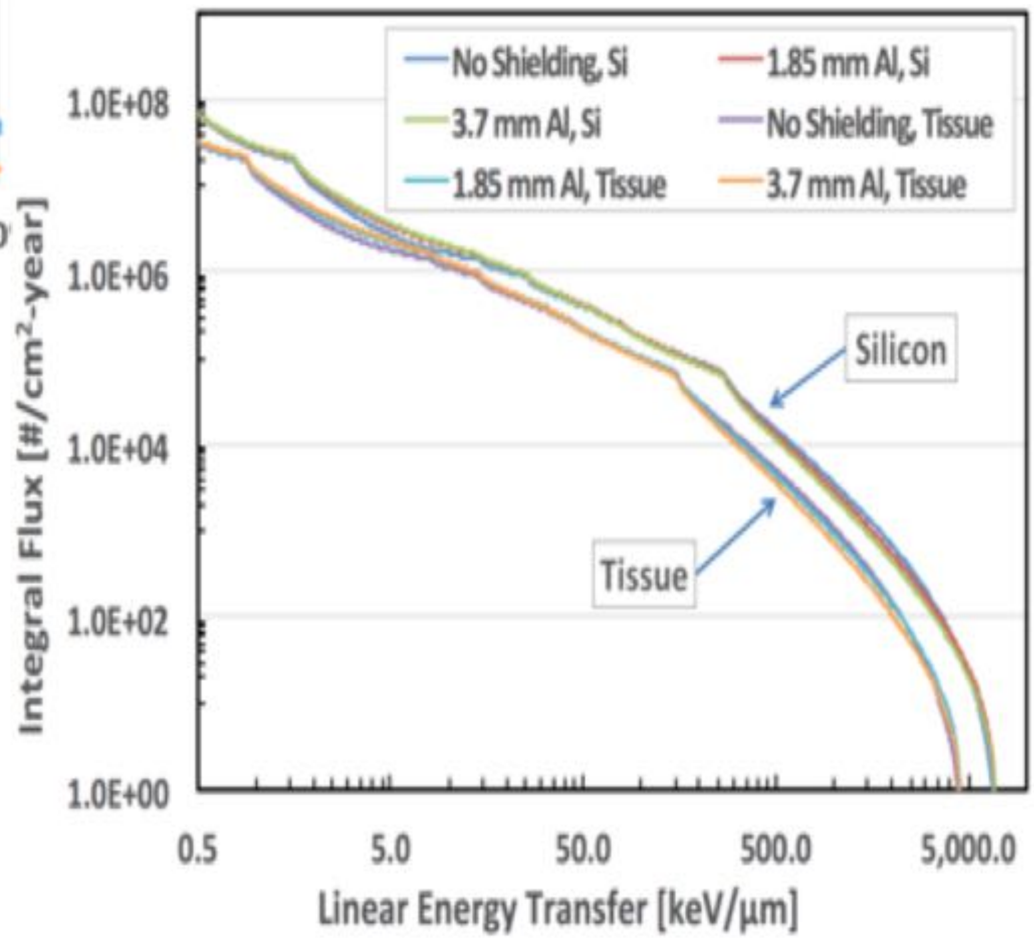
- ***2 pumps, 2 main bubble traps***
- ***24 active valves, 38 check valves***
- ***16 fluidic cards with 16 small bubble traps, 16 desiccant traps, 288 wells total***

BioSentinel

Interplanetary Radiation Environment



Total Ionizing Dose (Si) in 1 year: Ambient Flux + possible SPE(s)



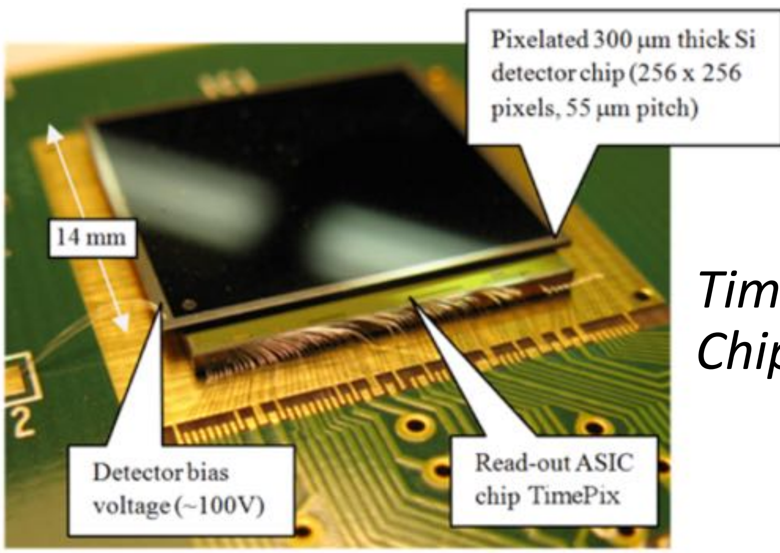
Flux (1 year) vs. linear energy transfer (LET) of particles for varying shielding thickness

BioSentinel

Radiation Sensing

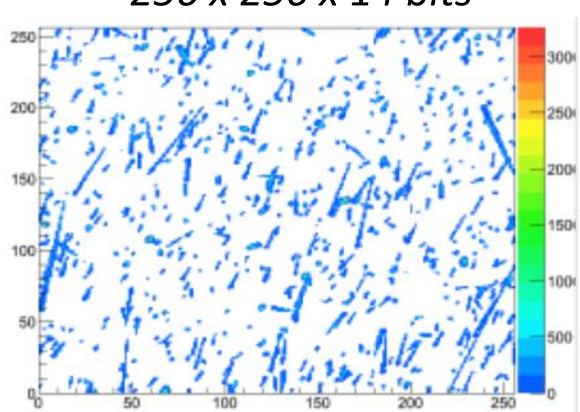
- LET "spectrometer": TimePix solid-state device
  - measures **linear energy transfer** spectra
  - time-over-threshold (TOT) mode. Wilkinson-type ADC
    - *direct energy measurement per pixel*
  - LET 0.2 – 300 keV/µm into 256 bins, each 3% width; store hourly bin totals
  - Download "local space weather" periodic snapshots
  - Also reports **TID** (total ionizing dose)

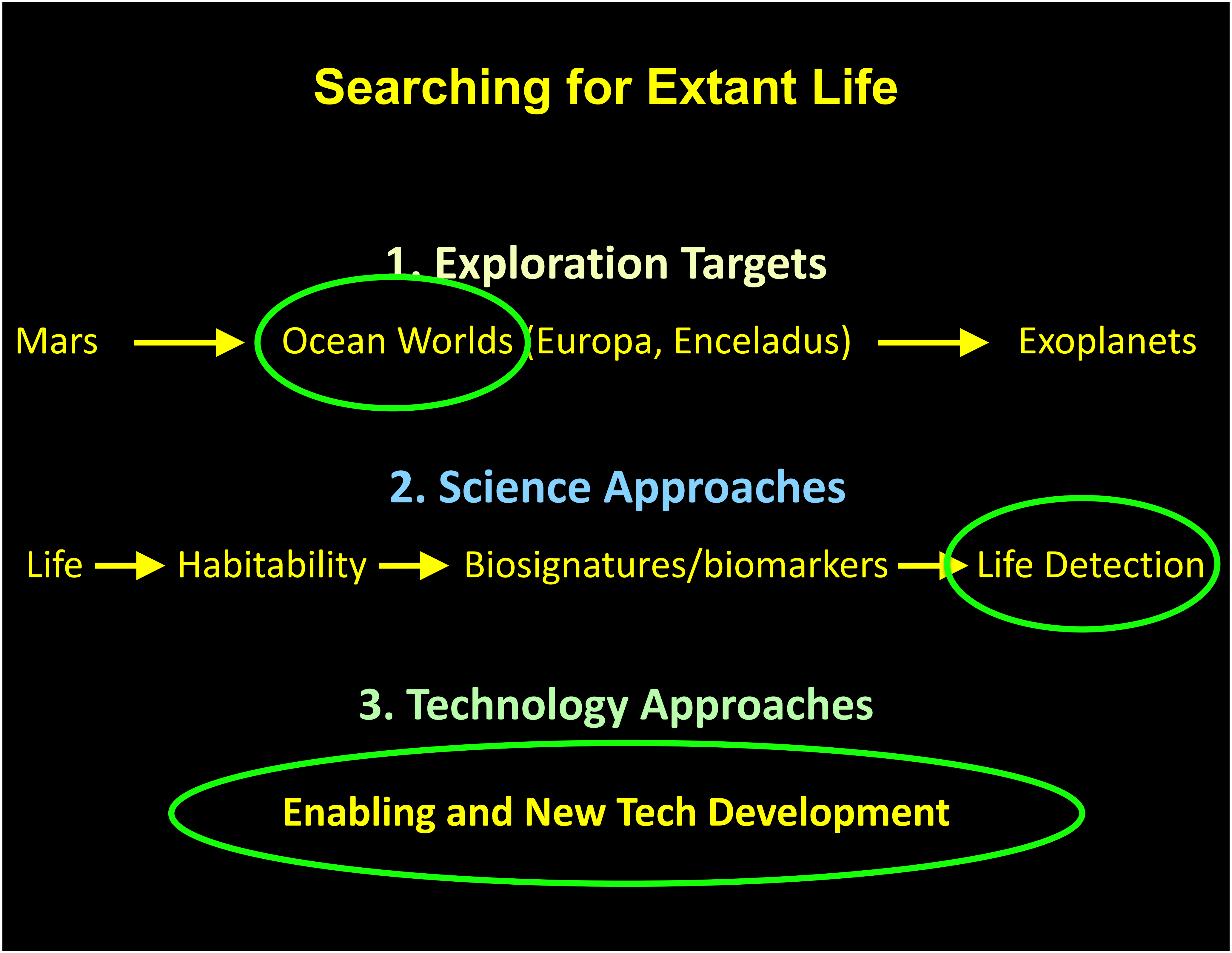
- SPE Trigger: TID rate increase causes wet-out of a pair of fluidic cards
  - Ground command as backup



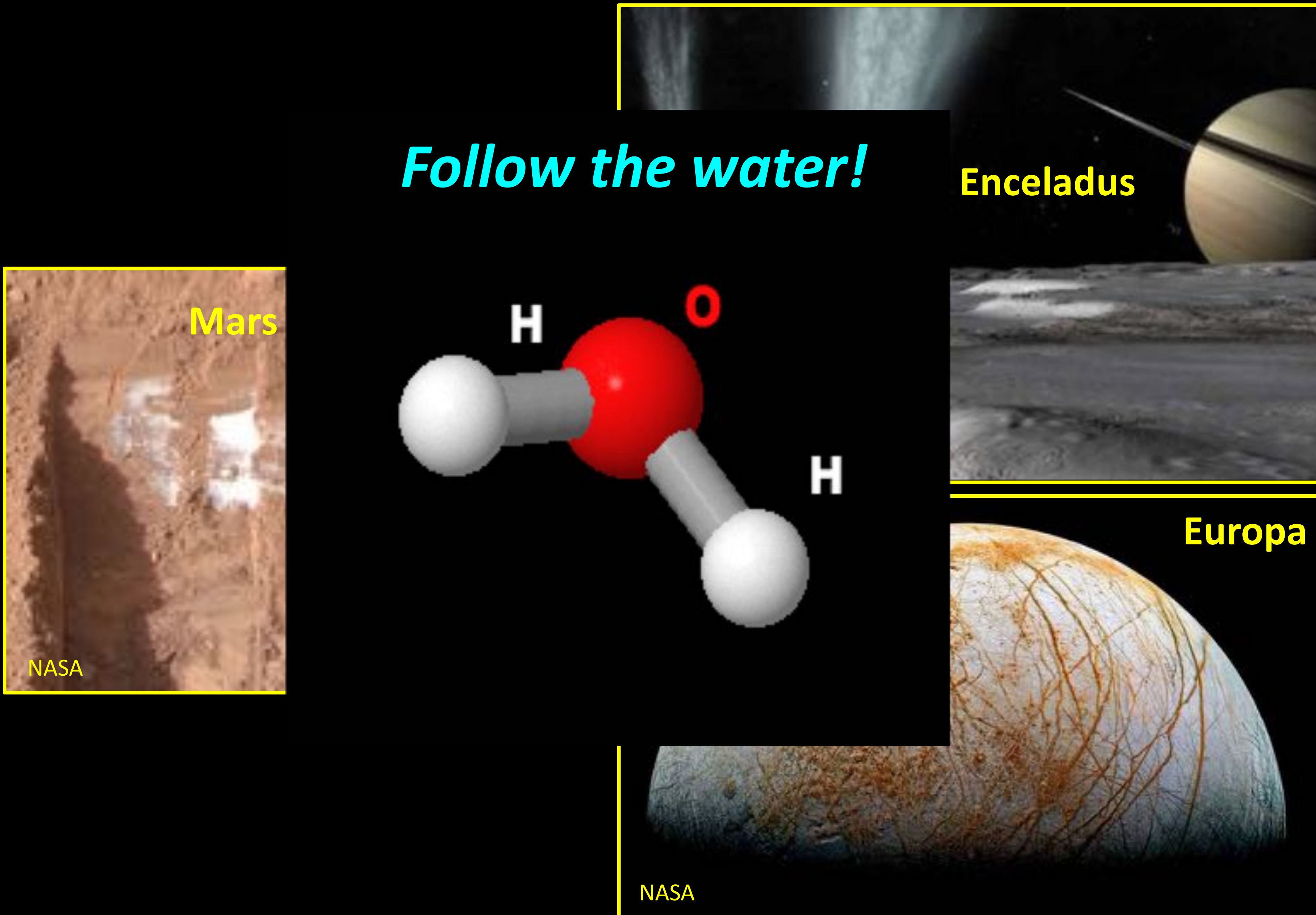
TimePix Chip

Typical TimePix frame:
256 x 256 x 14 bits




Searching for Extant Life
1. Exploration Targets
Mars
Ocean Worlds (Europa, Enceladus)
Exoplanets
2. Science Approaches
Life
Habitability
Biosignatures/biomarkers
Life Detection
3. Technology Approaches
Enabling and New Tech Development

Exploration Targets



Exploration Methodologies

- Contemporary Tools for (Mars) Exploration
  - ✓ Rocks, Dirt, Atmospheres
  - X Endogenous Water/Ice

- Flight Predecessors Limited
  - ➢ Viking "Biology" Experiments
  - ➢ Focus turned to habitability
  - ➢ Mars Phoenix Wet Chemistry Laboratory

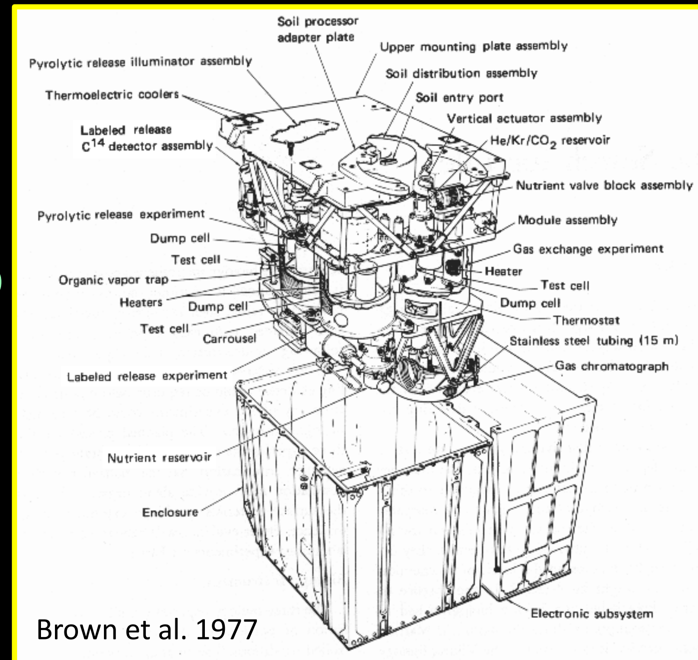
- To seek life: New Class of "Life Search" Instruments needed

- *Automated* (Micro)fluidic Systems with Sensors to enable Full Autonomy

- New methods for contamination control

- Leverage Biotech, Biomed, Process control

Viking

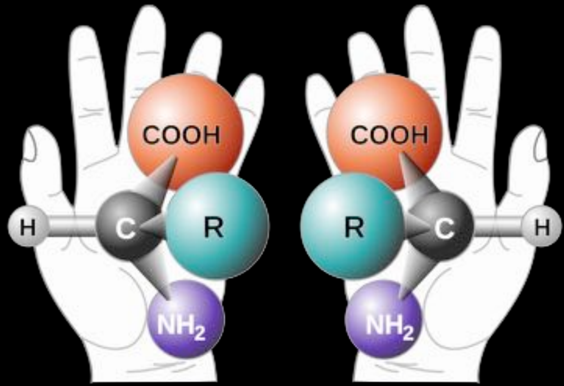


Brown et al. 1977

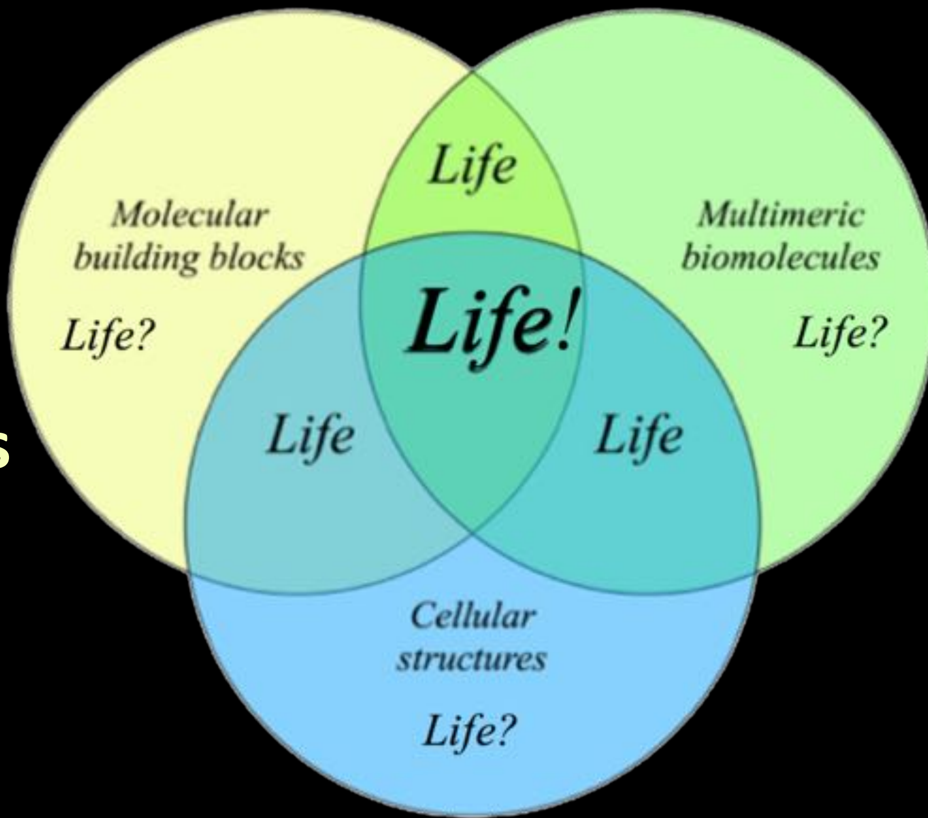
Phoenix

Life Detection Approaches

aspects of life likely to be universal



- "Simple" chemical building blocks
- Complex biomolecules
- Cellular structures



Arguably, all are required for life to exist in an ocean world

- Combined, these indicators could provide conclusive evidence of life
- *What technologies can enable the search in an icy-moon environment?*

(Partial) Traceability Matrix

Measurement Target	Observed Parameter	Life Detection Rationale	Analytical Approach
Molecular building blocks	Chirality	**Enantiomeric excess**: distinct feature, arguably necessary for biochemistry, e.g. *amino acids, saccharides*	*Capillary Electrophoresis* *Mass Spec*
Functional molecules	Catalysis	**Enzymatic change; facilitated electron transfer**: search by function, not specific molecule	*Electrochemical BioSensors* *Mass Spec*
Biogenic organic polymers	'Simple' polymers to build & contain	**Amphiphilic polymers:** construction materials for cellular life's structures & containments in aqueous environments, e.g. *lipids*, particularly *fatty acids*	*Mass spec* *Capillary Electrophoresis*
	'Complex' polymers to store & transfer information	**High molecular weight polymers** made of subunits with (1) diversity to store information and (2) means to interact or dissociate to transfer information, e.g. *poly nucleic acids*	*Sequencing* *Mass Spec*
Containment structures	Whole cells or membrane fragments	**Containers and barriers:** Key to even the simplest forms of terrestrial life, e.g. *containment and separation (membrane-like) structures*	*Fluorescence Microscopy with staining/labeling*

Analytical Measurement Technologies (Instruments): *Critical Performance Parameters and Selection Basis*

- Measurable analytes (amino acids, lipids, ions, …)
- Limit of detection (LOD) [≠ sensitivity]
- Dynamic range
- Physical characteristics: size, mass, power, data, thermal
- Heritage / maturity
- Complementarity/orthogonality to the rest of the suite

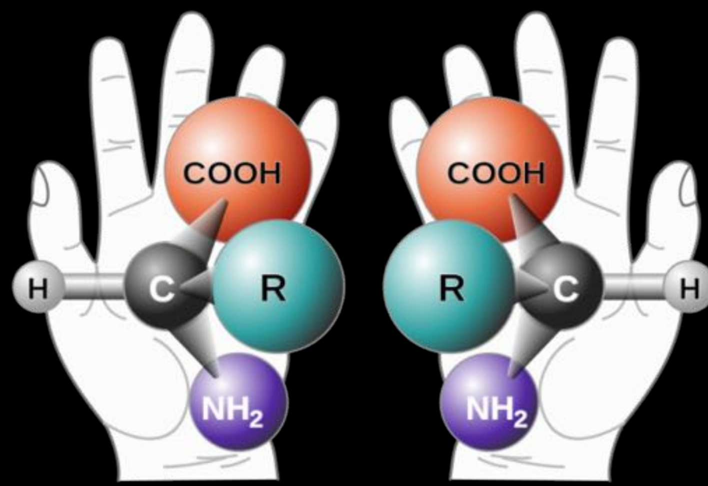
Microchip Capillary Electrophoresis (MCE)

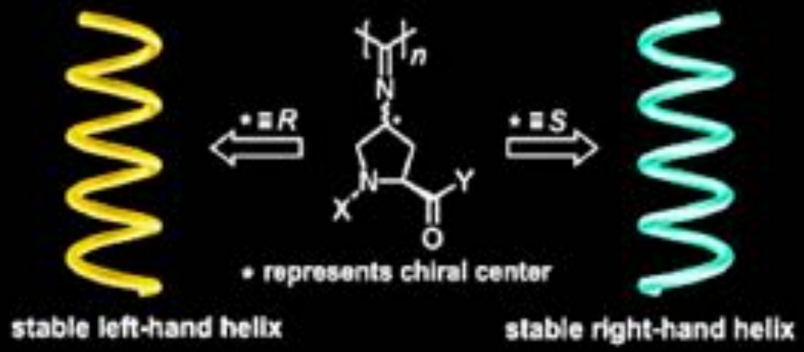
Chiral Separations (Amino Acid)

ARC Cubestat Microfluidic Sample Handling and Processing Heritage

Laser-Induced Fluorescence Detection

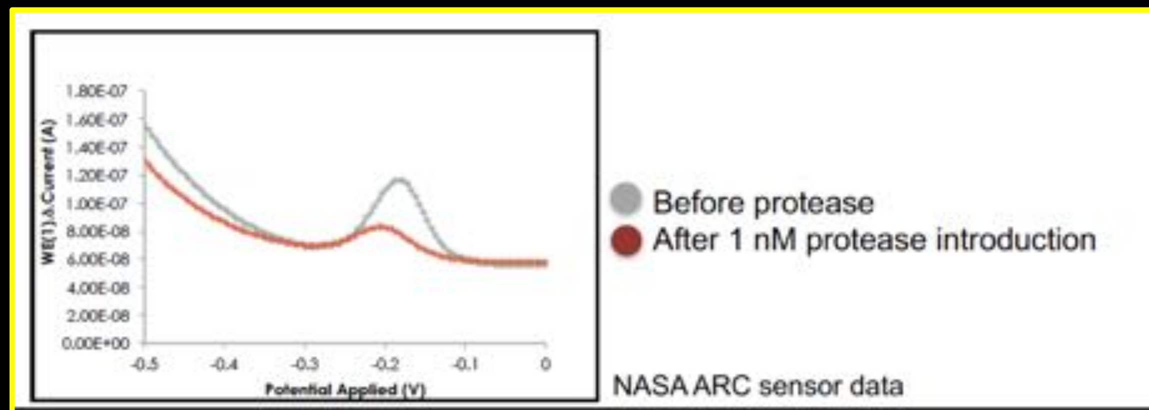
NASA JPL and SBIR Partnership

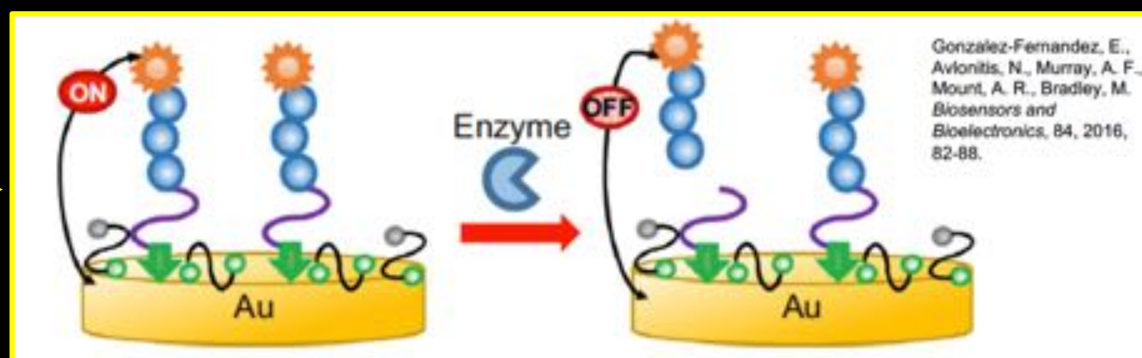




Electrochemical Detection of Biological Catalysts as Signatures of Life

ARC Center Innovation Fund
Electrochemical Extant Life Detection
Phoenix Wet Chemistry Laboratory Lineage





Solid-State Nanopore Life Detection Technology

Concepts for Ocean worlds Life Detection Technology (COLDTech)

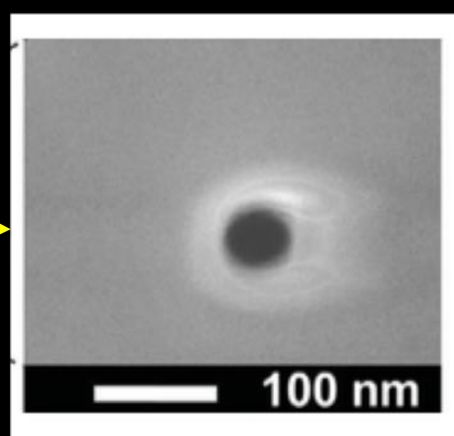
Detection of multiple types of biopolymers

Major Partner: UCSC

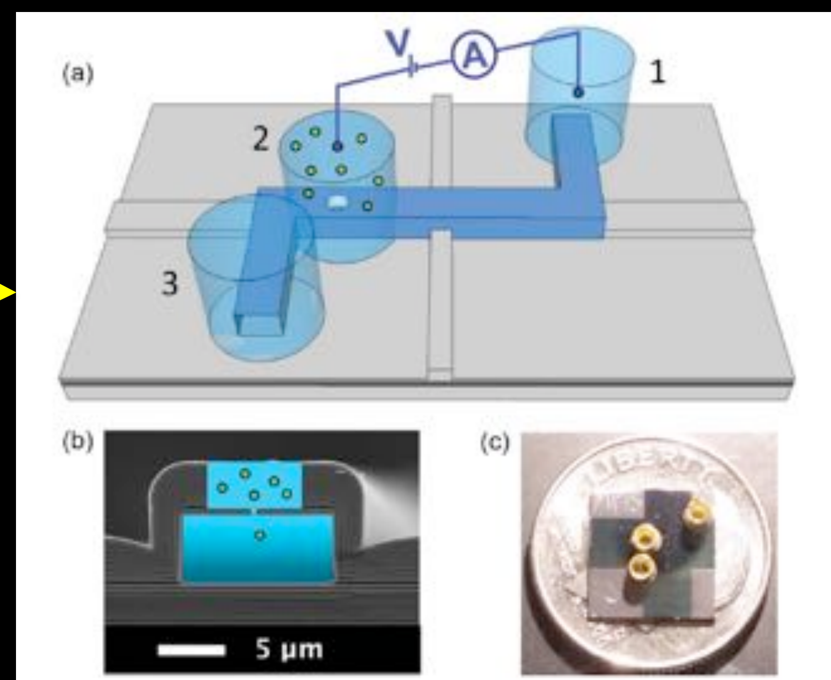
Oxford MinION Inspired

Robust silicon nitride nanopore membranes for flight missions

Oxford Nanopore
Biological nanopore membrane



Rudenko et al. 2011



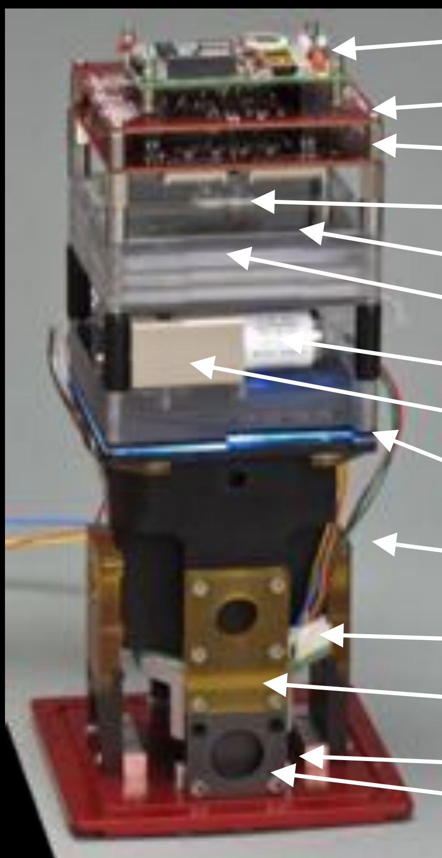
LIfE: Luminescence Imager for Exploration

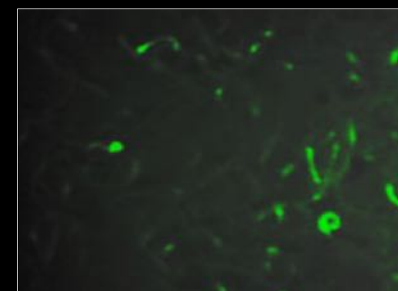
Fluorescence Microscope for Ocean World Life Detection

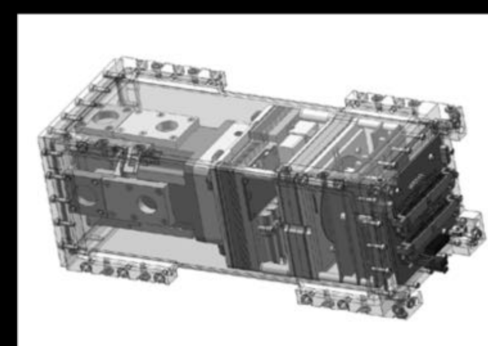
COLDTech Development: Automated Analytical Fluidic-Platform

FLAIR: Fluorescence Analysis for In-situ Research on Nanosatellites

2U dual-wavelength **fluorescence + fluidics** imager payload

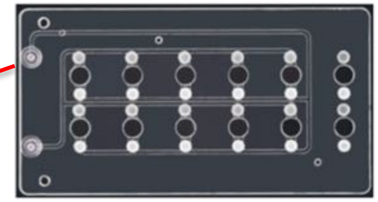


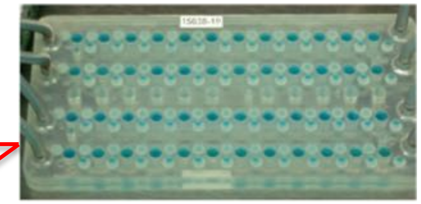




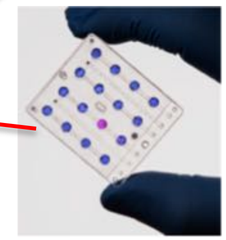
Building on what we Know How to Do:
Ames Pioneering **CubeSat* Biological Space Missions**

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

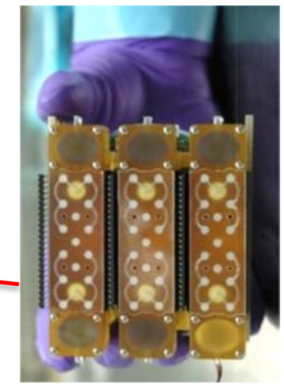


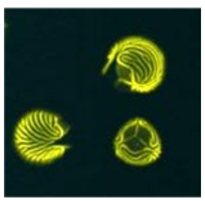


S. Cerevisiae PharmaSat (2009): ***drug dose response***
BioSentinel (2020): ***DNA damage***



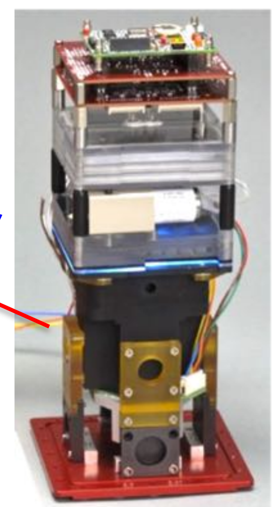
B. Subtilis O/OREOS** (2010): ***survival, metabolism***
ADRoIT-M*** (20xx): ***mutations / lithopanspermia***





Ceratopteris Richardii SporeSat-1 (2014): **ion channel sensors, μ-centrifuges**
SporeSat-2 (20xx): ***plant gravity sensing threshold***

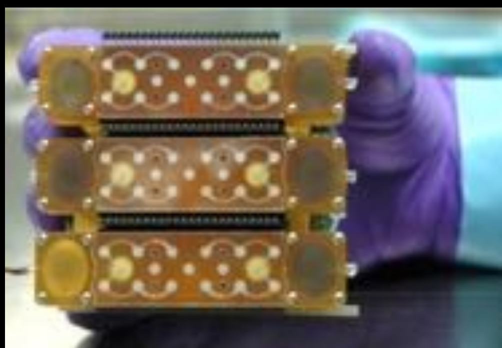
C. Elegans FLAIR (20xx):
dual-wavelength
fluorescence imager

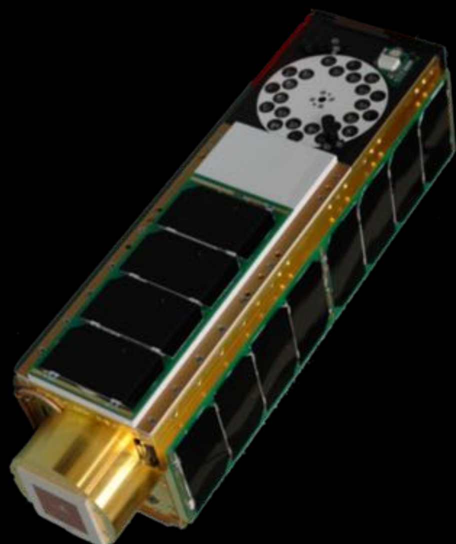


**All are either 3U or 6U form factor*
***Organism/Organic Response to Orbital Stress*
****Active DNA Repair on Interplanetary Transport of Microbes*

Astro/biological Space Missions as a Source of Enabling Technologies*

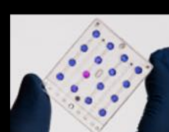
Organism/Organic Response to Orbital Stress (3U/2010)



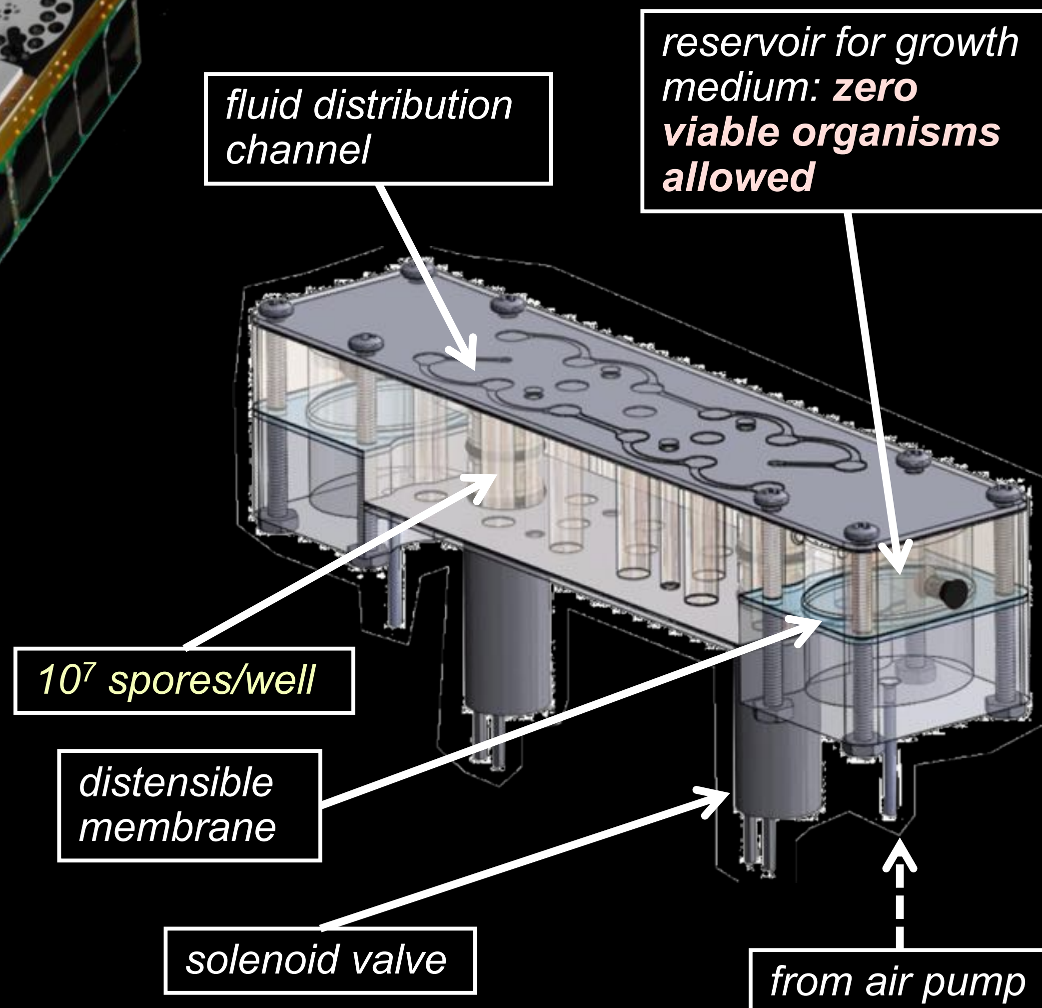


<u>Astrobiology Payload</u>

- 1U Payload1: *B. Subtilis*
  6 months: Survival, Metabolism
- Perfect Sterility
  11 months

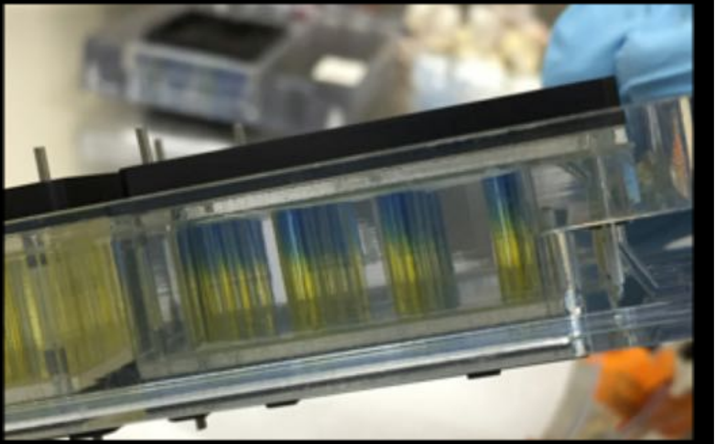


- Hydrophobic Membrane for Air Expulsion
- High-radiation LEO (72°, 650 km)
- Functional for 5 years



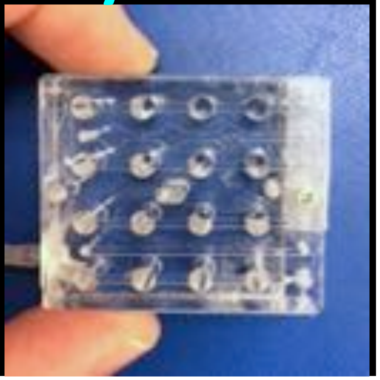
****ARC is the leading center for implementation of automated fluidics systems in space***

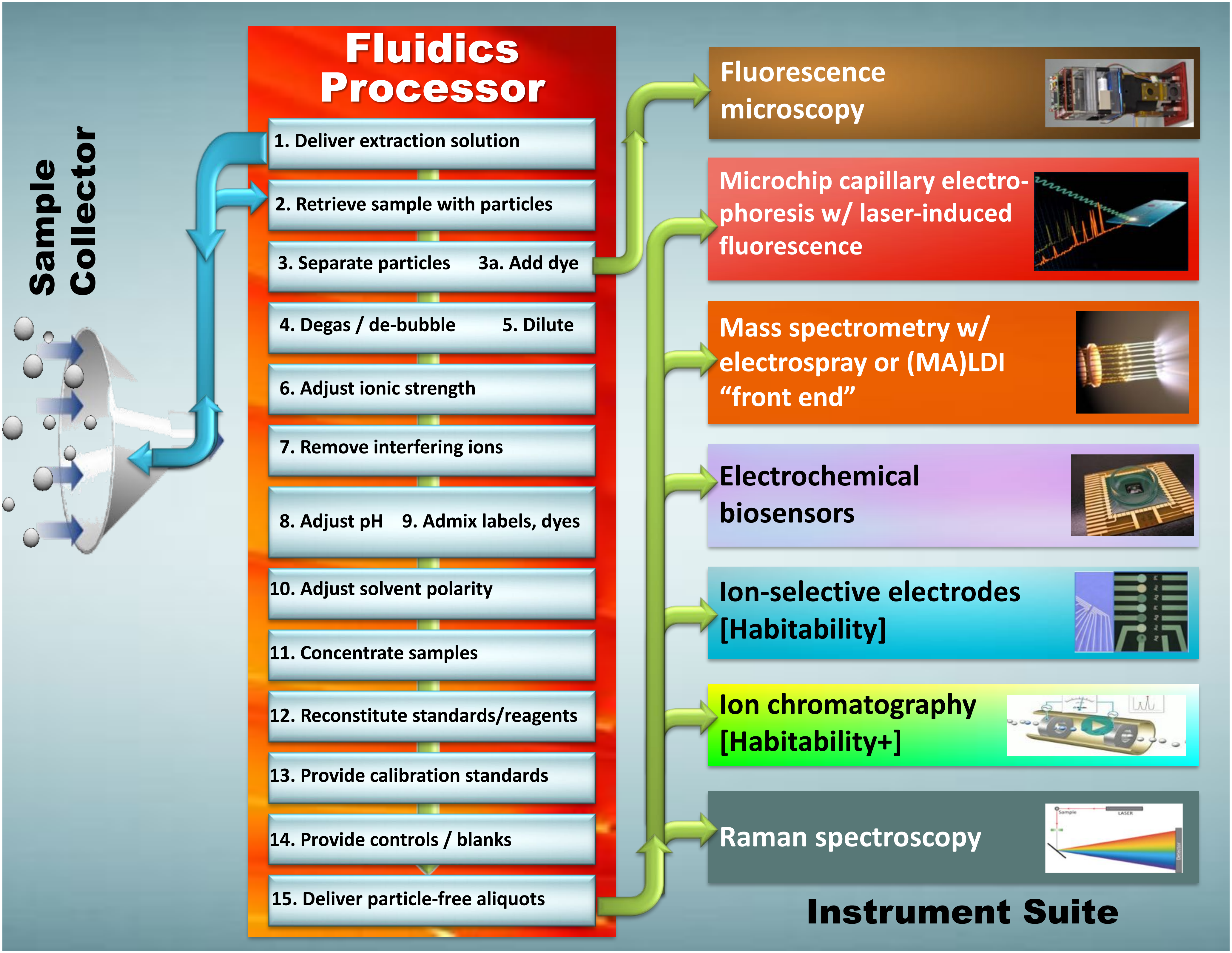
Enabling Technologies: Key Functionalities of Ames Bio-Cubesats



Sample Processor for Life on Icy Worlds (SPLIce)

- sensitive bioanalysis
- **requirement for perfect sterility**
- ultra-low mass/volume/power budgets
- localized precision thermal control (± 1 °C typ)
- **ultra-low organic surface & volatile contamination, biocompatible materials**
- materials selection: non-reactive interfaces between polymers, metals, ceramics
- precision electrical/optical measurements in an environment w/ fluids nearby
- extended stasis for fluid & reagent systems (up to 2 years for *BioSentinel*)
- managing gas/fluid interfaces, elimination of bubbles, expulsion of air (N_2)
- **handling μL fluid volumes; flying dry, then wetting out a fluidic system**
- maintaining 1 atm in space environment with ultralow leakage
- managing sample pH
- managing a humid, potential condensing environment
- accounting for radiation effects on polymers (tested to 4 Mrad)



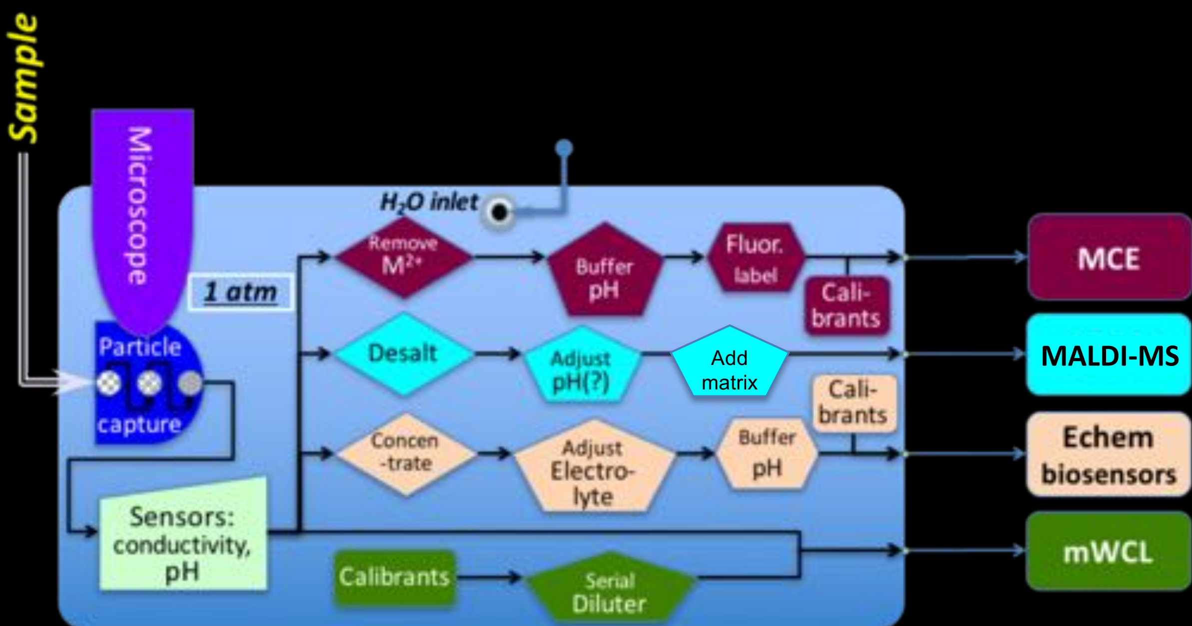

Sample Collector
Fluidics Processor
1. Deliver extraction solution
2. Retrieve sample with particles
3. Separate particles
3a. Add dye
4. Degas / de-bubble
5. Dilute
6. Adjust ionic strength
7. Remove interfering ions
8. Adjust pH
9. Admix labels, dyes
10. Adjust solvent polarity
11. Concentrate samples
12. Reconstitute standards/reagents
13. Provide calibration standards
14. Provide controls / blanks
15. Deliver particle-free aliquots
Fluorescence microscopy
Microchip capillary electro-phoresis w/ laser-induced fluorescence
Mass spectrometry w/ electrospray or (MA)LDI "front end"
Electrochemical biosensors
Ion-selective electrodes [Habitability]
Ion chromatography [Habitability+]
Raman spectroscopy
Instrument Suite

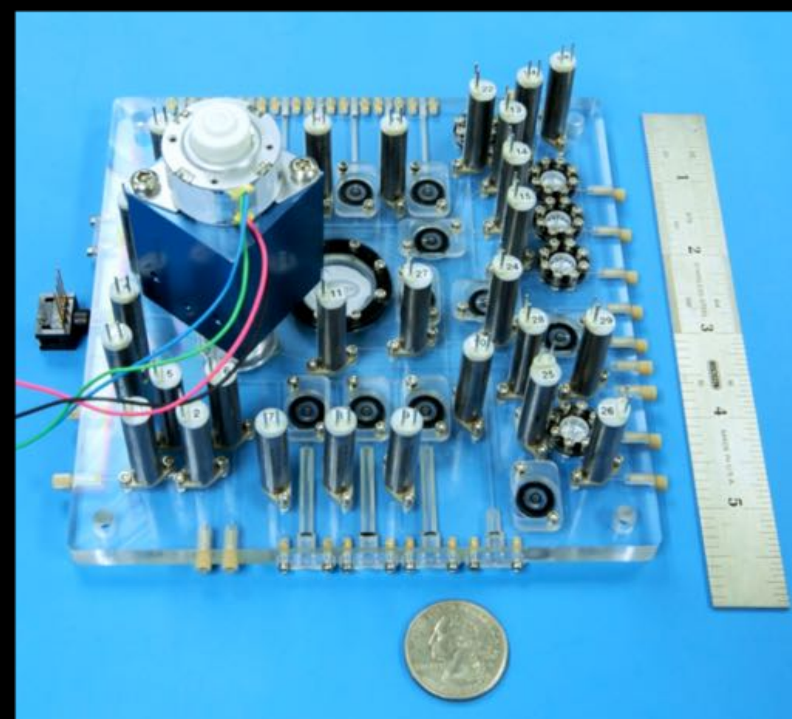
Integrated Life Detection Payloads

Sample Processor for Life on Icy Worlds (SPLIce): COLDTech (SMD)

- *Tech. dev. tailored to Enceladus & Europa targets*

Partners: APL, JPL, GSFC, Tufts

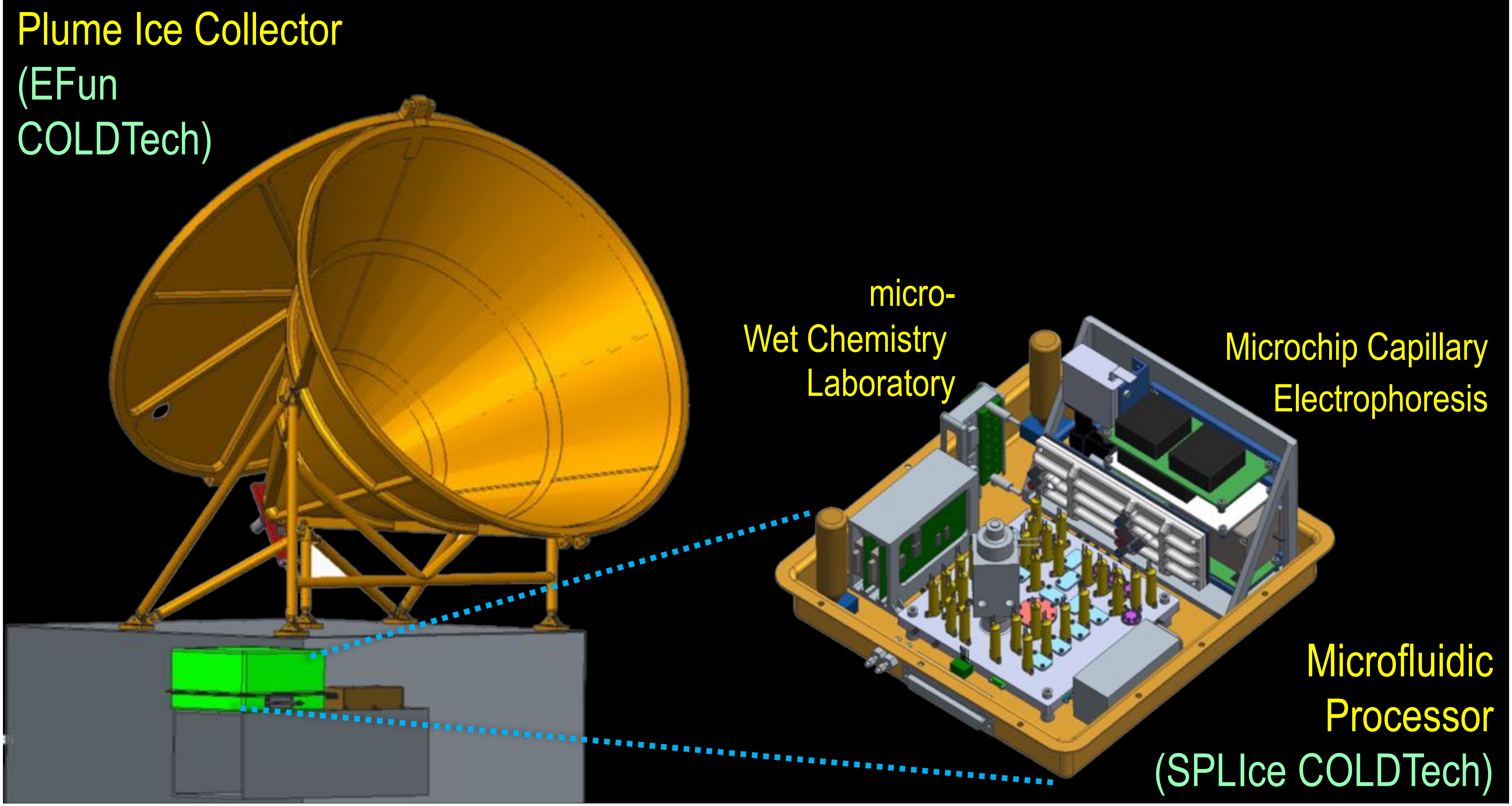




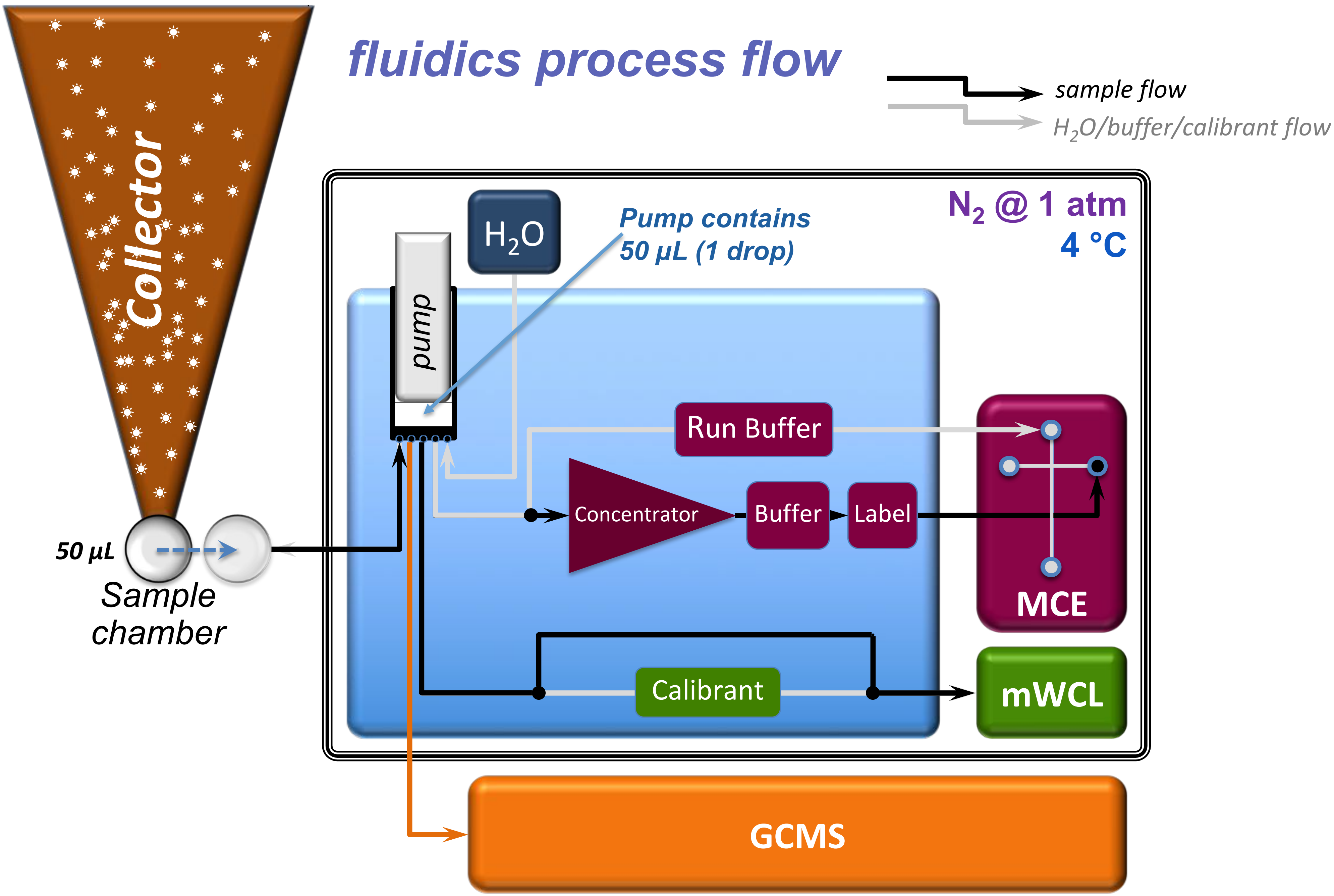
SPLIce Engineering Team

Integrated End-to-End Life Detection System Concept

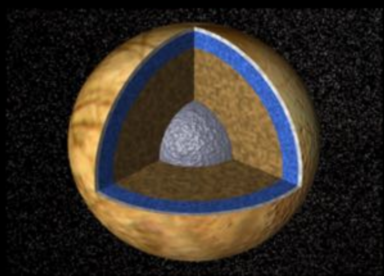
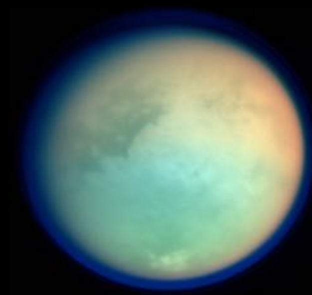
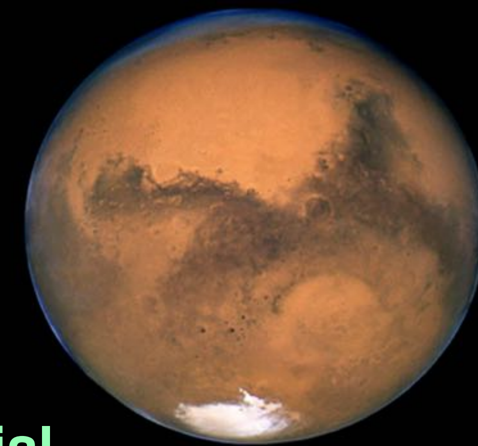
Major Partners: APL, GSFC, & JPL



EFun + SPLIce = µCAFE:
microChemical Analyzer of Fluids for Exobiology



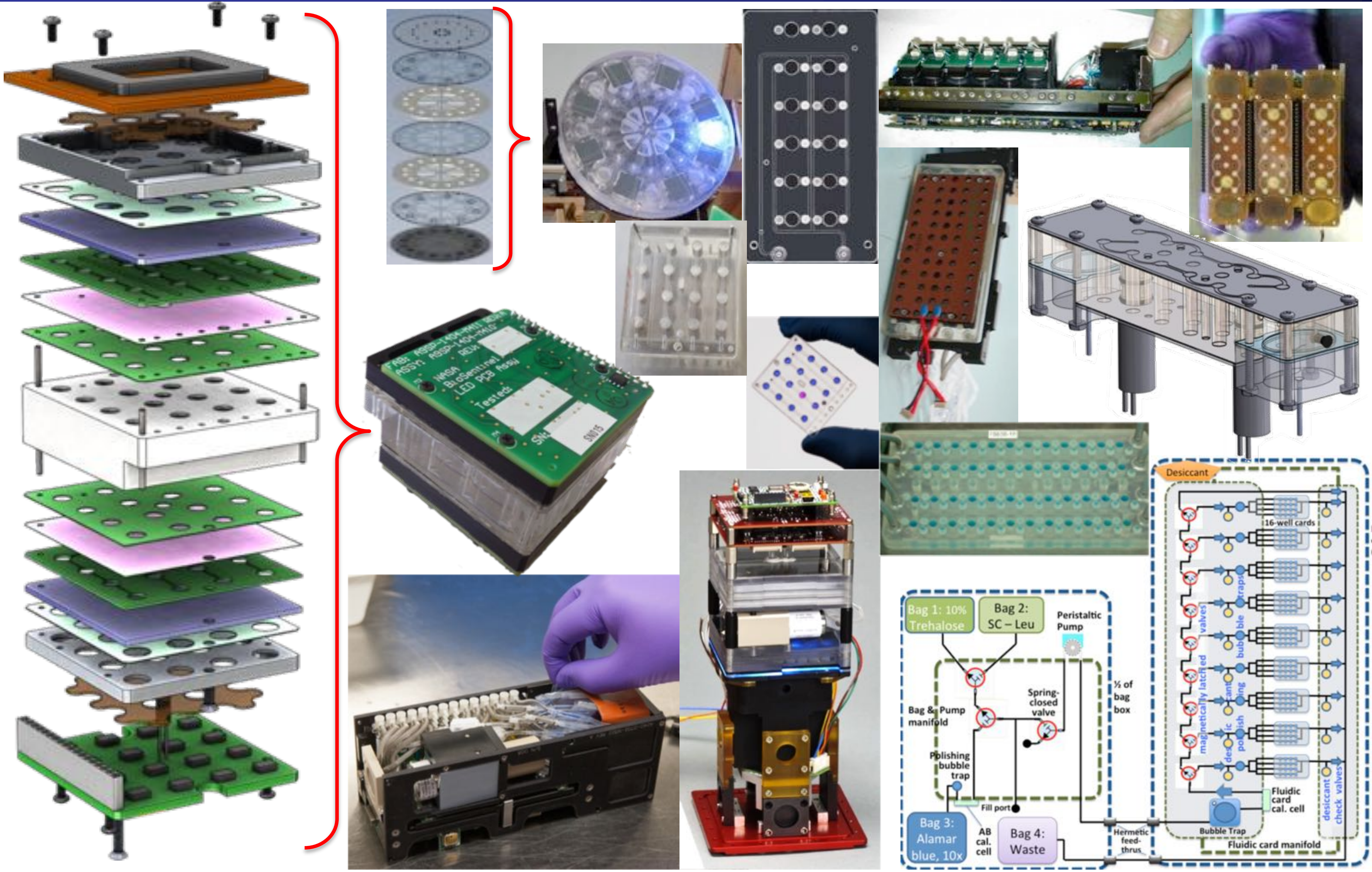
Conclusions

PLAY TO YOUR STRENGTHS!

- Deep Knowledge of the scientific challenge is crucial
  - excellent astrobiologists necessary to create a winning astrobiology mission!
- Technological solutions can/should be adapted from everywhere
  - don't drive screws with a hammer –
  - but if your screwdriver has a massive handle, it may be a great nail driver
- Experience & Heritage can give you a Massive Advantage
  - powerful to have already done approximately what you need to do: *spaceflight missions are too challenging to start from scratch*
- Science and Engineering must work hand in hand
  - no chucking things over the fence!
- Creativity is most powerful as a means to adapt, rather than an excuse to ignore

Questions?


Desiccant
16-well cards
magnetically latched valves
desiccant bubble traps
polishing
desiccant check valves
Fluidic card cal. cell
Bubble Trap
Fluidic card manifold
Bag 1: 10% Trehalose
Bag 2: SC – Leu
Peristaltic Pump
Spring-closed valve
Bag & Pump manifold
½ of bag box
Polishing bubble trap
Fill port
Bag 3: Alamar blue, 10x
AB cal. cell
Bag 4: Waste
Hermetic feed-thrus