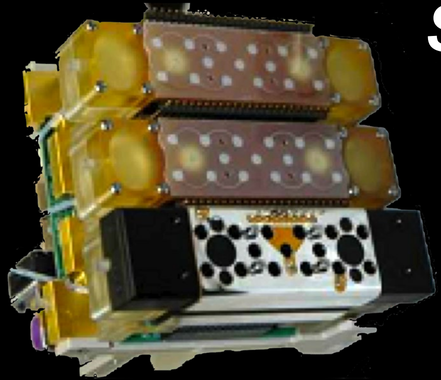
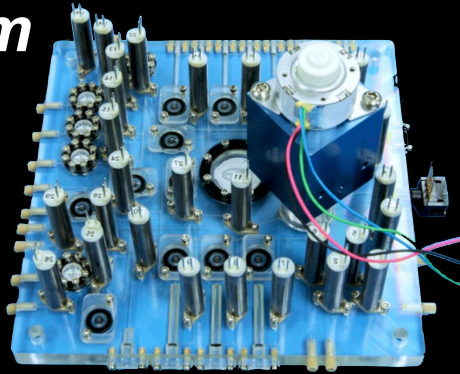
Sensing Systems for Life in Space: Microfluidics to Study Terrestrial Microbes in Space and Search for Life in our Solar System





Tony Ricco

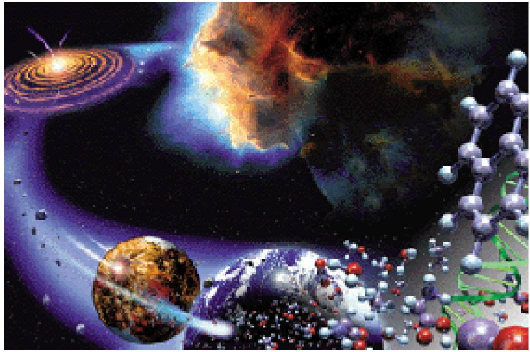
NASA Ames Research Center

with many thanks to

Ames' Small Payloads and Search-for-Life Tech. Dev. teams

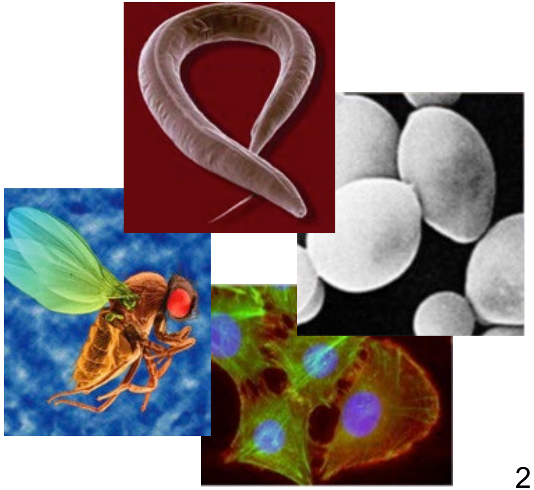
Astrobiology & Space Biology

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

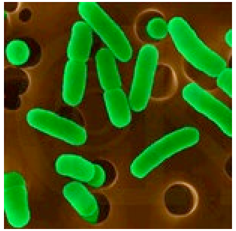
Fundamental Space Biology: space environment effects on terrestrial life

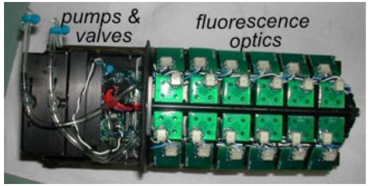


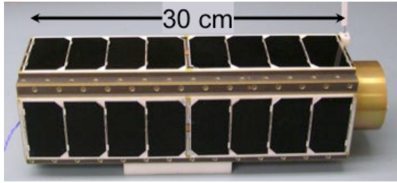
- Reduced gravity effects
  - ***Mammals***: fluid distribution, musculoskeletal loading ⇒ immune stress, decreased bone density, muscle atrophy, slowed wound healing
  - ***Cells***, ***microbes*** in culture: nutrient and waste transport
- Radiation effects: damage from (high-energy) ionizing radiation
  - Greater outside Earth's magnetosphere, ~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***

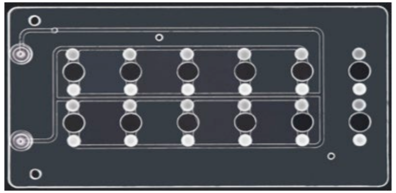
GeneSat-1: 1st biological nanosatellite in LEO, 1st real-time, gene expression measurement in space

model organism:
0.5 x 2 μm
bacteria
E. coli

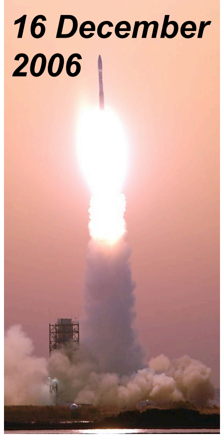




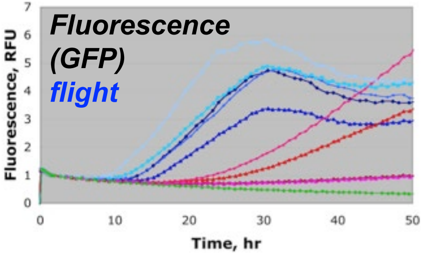




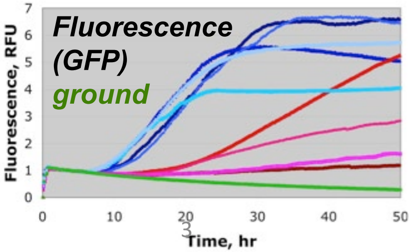
- nutrient deprivation in dormant state (6 weeks)
- launch: December 2006 to low Earth orbit (440 km)
- nutrient solution feed upon orbit stabilization, grow *E. coli* in μgravity
- monitor green fluorescent protein (***GFP***): gene expression
- monitor optical density: cell population



Telemeter data to Earth

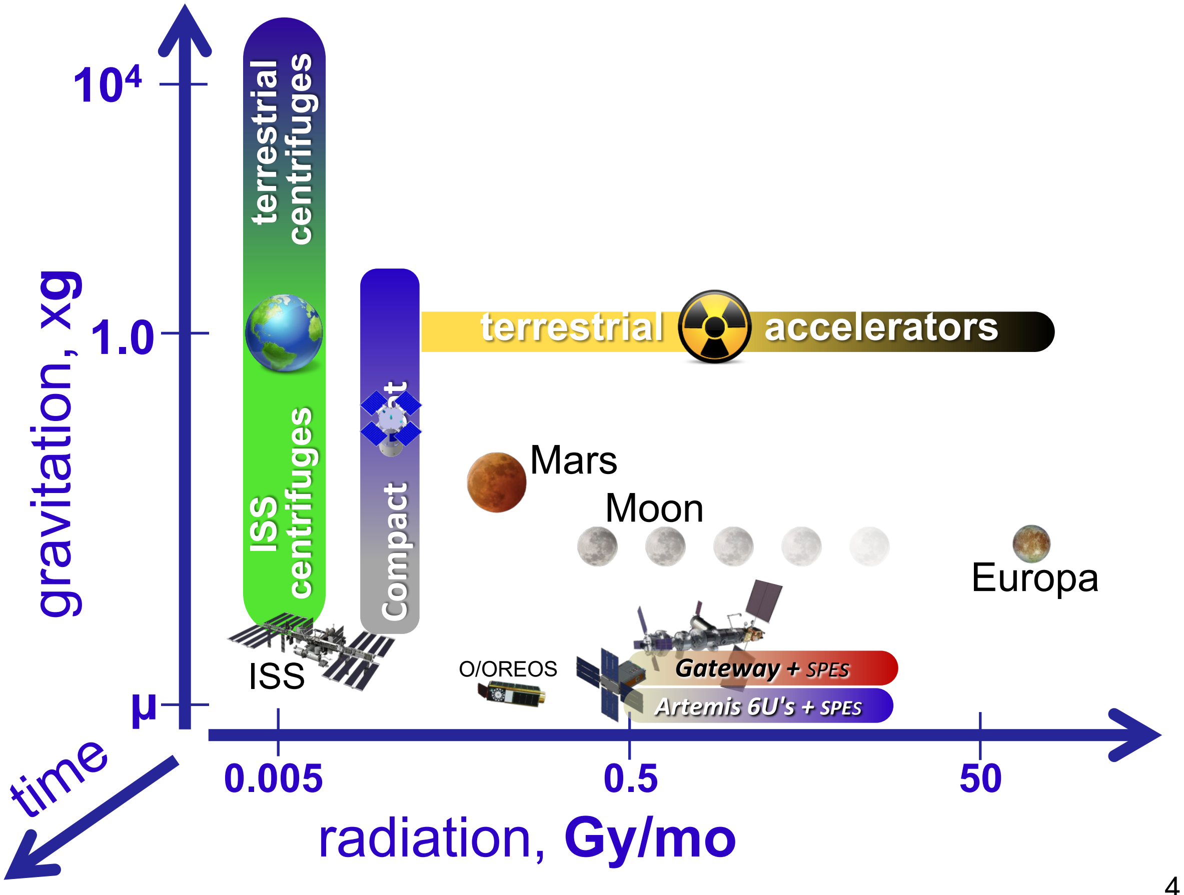


Compare to ground data

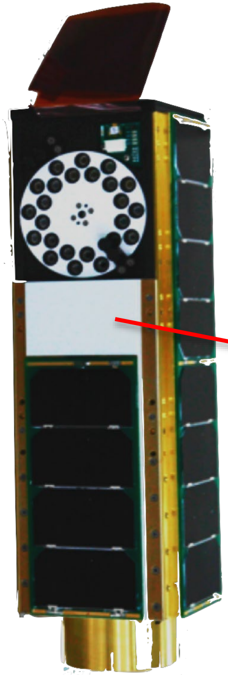


Why Study Biology Autonomously in Space?

To "BioMap" the Gravity-Radiation-Time environment for life

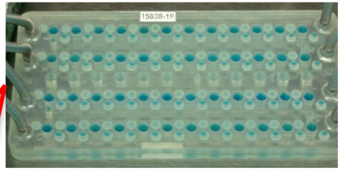


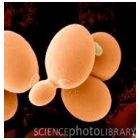
Summary of NASA Ames' Nanosatellite (Astro)Biological Space Missions



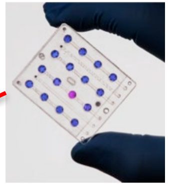
O/OREOS

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

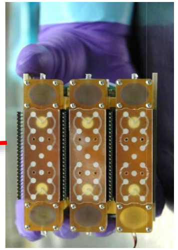


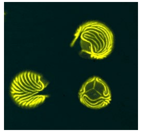


S. Cerevisiae *PharmaSat* (**2009**/3U): ***drug dose response***
BioSentinel (**2022**/6U): ***DNA break/repair***



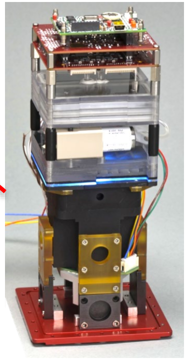
B. Subtilis *O/OREOS** (**2010**/3U): ***survival, metabolism***
*ADRoIT-M*** (6U): ***mutations / lithopanspermia***

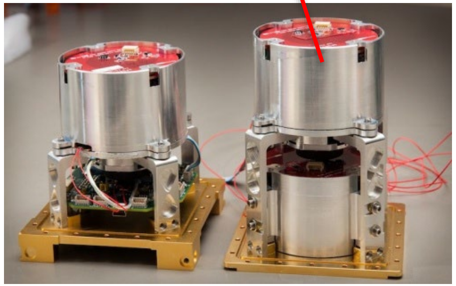




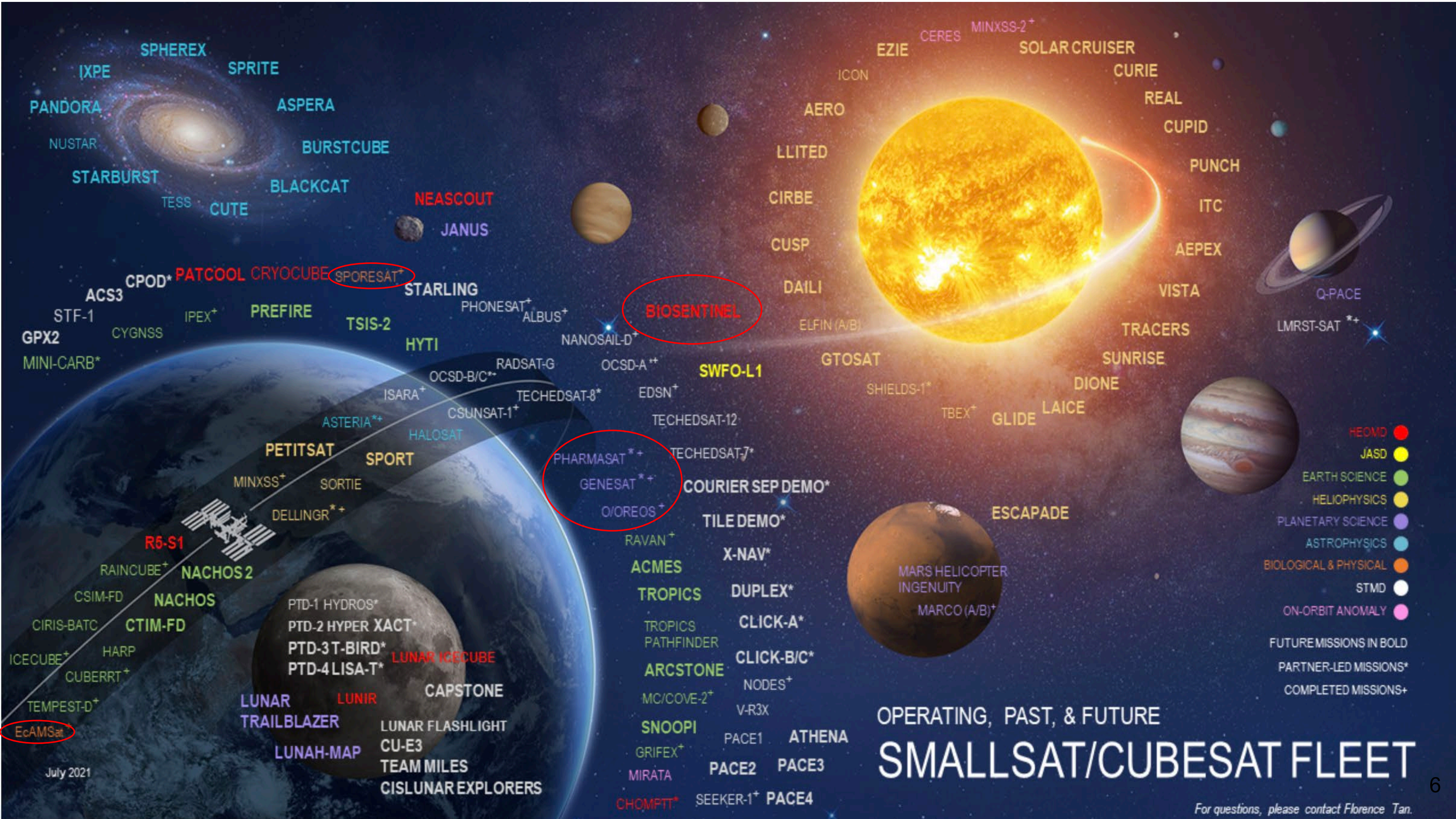
Ceratopteris Richardii *SporeSat-1* (**2014**/3U): **ion channel sensors, μ-centrifuges**
SporeSat-2 (3U): ***plant gravity sensing threshold***

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





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


OPERATING, PAST, & FUTURE
SMALLSAT/CUBESAT FLEET
SPHEREX
IXPE
SPRITE
PANDORA
ASPERA
NUSTAR
BURSTCUBE
STARBURST
BLACKCAT
TESS
CUTE
NEASCOUT
JANUS
ACS3
CPOD*
PATCOOL
CRYOCUBE
SPORESAT+
STARLING
STF-1
IPEX+
PREFIRE
PHONESAT+
ALBUS+
GPX2
CYGNSS
TSIS-2
MINI-CARB*
HYTI
NANOSAIL-D+
BIOSENTINEL
RADSAT-G
OCSD-B/C*+
OCSD-A*+
ISARA+
TECHEDSAT-8*
EDSN+
CSUNSAT-1+
ASTERIA*+
HALOSAT
TECHEDSAT-12
PETITSAT
SPORT
PHARMASAT*+
TECHEDSAT-7*
MINXSS+
SORTIE
GENESAT*+
COURIER SEP DEMO*
DELLINGR*+
O/OREOS+
TILE DEMO*
R5-S1
RAVAN+
X-NAV*
RAINCUBE+
NACHOS 2
ACMES
CSIM-FD
NACHOS
TROPICS
DUPLEX*
PTD-1 HYDROS*
PTD-2 HYPER XACT*
CIRIS-BATC
CTIM-FD
TROPICS PATHFINDER
CLICK-A*
PTD-3 T-BIRD*
LUNAR ICECUBE
ICECUBE+
HARP
PTD-4 LISA-T*
ARCSTONE
CLICK-B/C*
CUBERRT+
NODES+
LUNAR TRAILBLAZER
LUNIR
CAPSTONE
MC/COVE-2+
TEMPEST-D+
V-R3X
SNOOPI
LUNAR FLASHLIGHT
EcAMSat+
LUNAH-MAP
CU-E3
PACE1
ATHENA
GRIFEX+
TEAM MILES
PACE2
PACE3
MIRATA
CISLUNAR EXPLORERS
CHOMPTT*
SEEKER-1+
PACE4
July 2021
CERES
MINXSS-2+
EZIE
SOLAR CRUISER
ICON
CURIE
AERO
REAL
CUPID
LLITED
PUNCH
CIRBE
ITC
CUSP
AEPEX
DAILI
VISTA
Q-PACE
ELFIN (A/B)
TRACERS
LMRST-SAT*+
GTOSAT
SUNRISE
SWFO-L1
SHIELDS-1+
DIONE
TBEX+
GLIDE
LAICE
ESCAPADE
MARS HELICOPTER INGENUITY
MARCO (A/B)+
HEOMD
JASD
EARTH SCIENCE
HELIOPHYSICS
PLANETARY SCIENCE
ASTROPHYSICS
BIOLOGICAL & PHYSICAL
STMD
ON-ORBIT ANOMALY
FUTURE MISSIONS IN BOLD
PARTNER-LED MISSIONS*
COMPLETED MISSIONS+
For questions, please contact Florence Tan.

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

BioMapping:

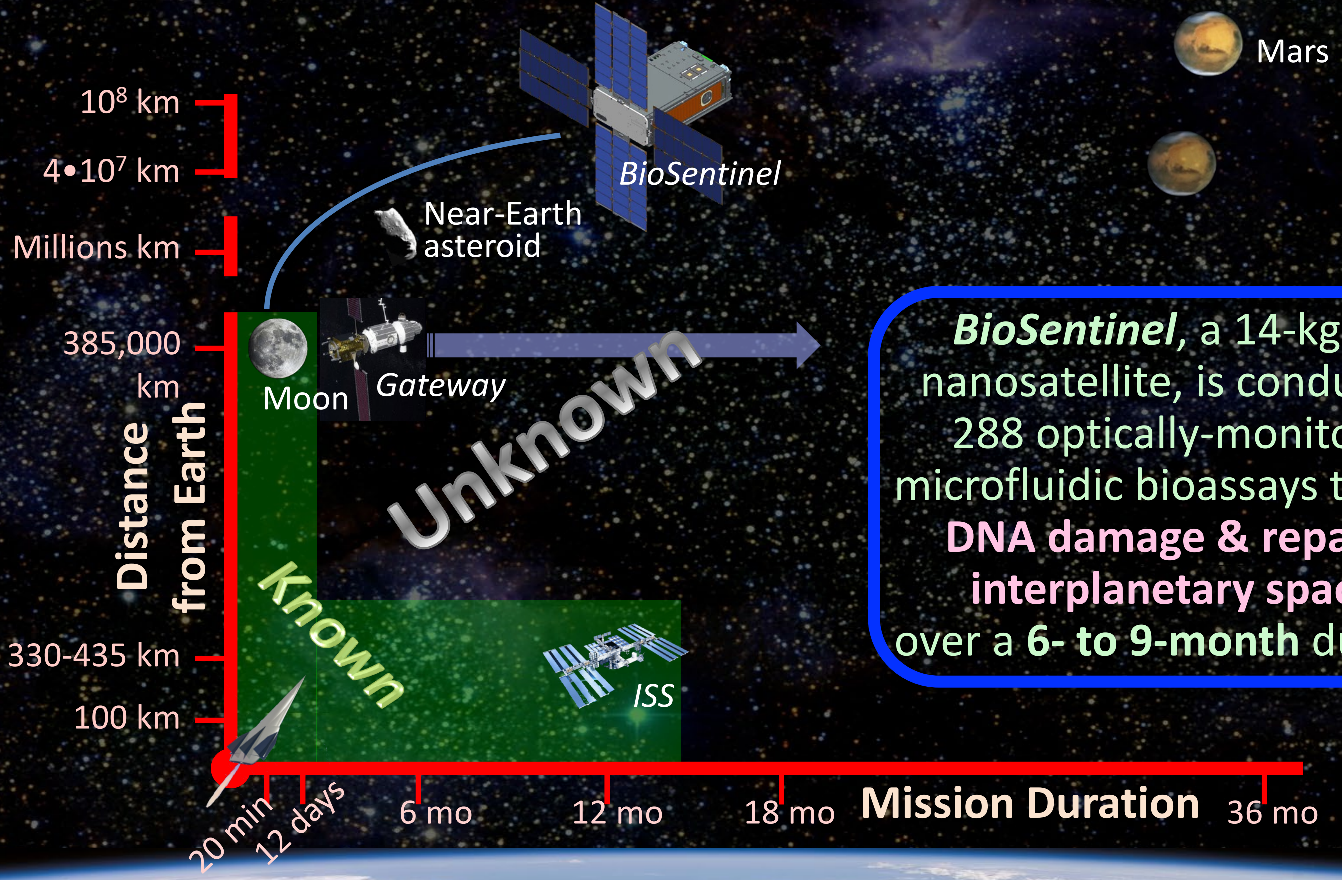
Looking for

Life Signatures

and testing

Terrestrial Life

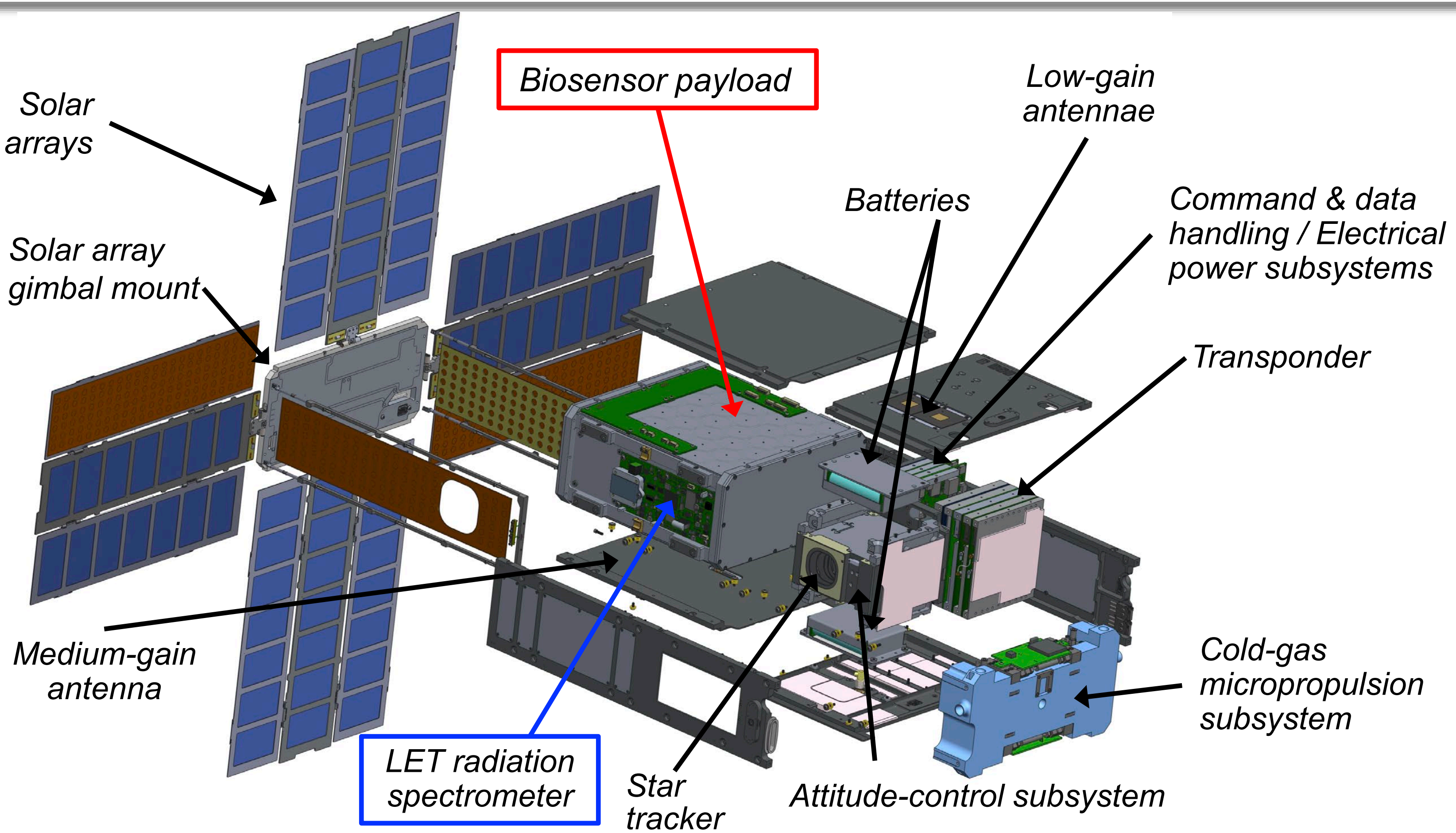
in many locations



BioSentinel, a 14-kg, 6U nanosatellite, is conducting 288 optically-monitored microfluidic bioassays to track **DNA damage & repair** in **interplanetary space*** over a **6- to 9-month** duration

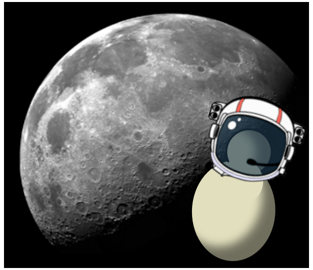
**9 time points; 32 microwells/timepoint*

BioSentinel Subsystem Overview



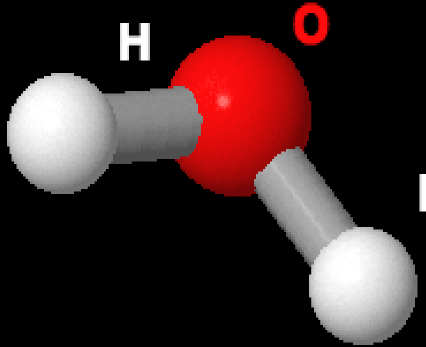
BioSentinel Science Mission: *"Canary in a Coal Mine"*

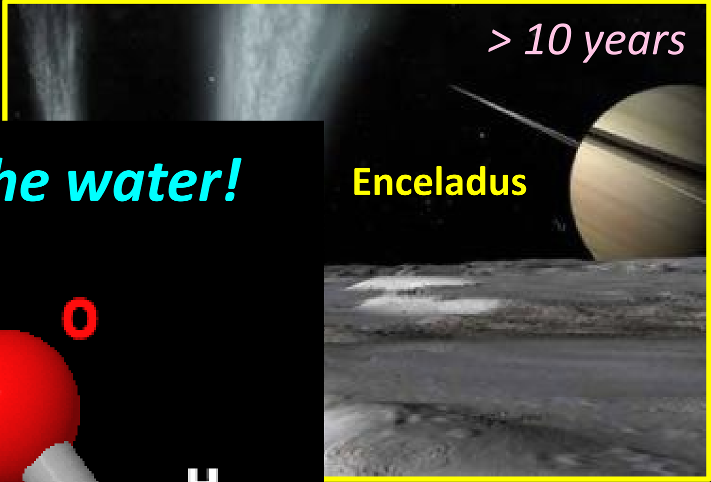
- ***Quantify DNA damage from space radiation environment***
  - Deep space environment cannot be reproduced on Earth: *omnidirectional, continuous, low flux, variety of particle types*
  - Health risk for humans beyond LEO, esp. particle events (SPEs)
- **Yeast assay: microfluidic arrays monitor DNA damage**
  - Two strains of *S. cerevisae*: 1 control (wild-type), 1 mutant (*rad51*Δ)
    - *mutant sensitive to DNA damage: double-strand breaks not repaired well*
  - Wet and activate multiple banks of yeast in µwells over mission duration
- **Correlate biological response with physical radiation measurements**
  - ***Linear Energy Transfer*** (LET) spectrometer bins & counts particle events by LET
  - Total Ionizing Dose (TID): LET systems calculates integrated deposited energy
- ***BioSentinel* now operating in interplanetary space**
  - Launched with Artemis-1, 16 November 2022
  - Companion BioSentinel payload experiment on ISS in LEO complete
  - Lunar BioSentinel experiment (LEIA) in development

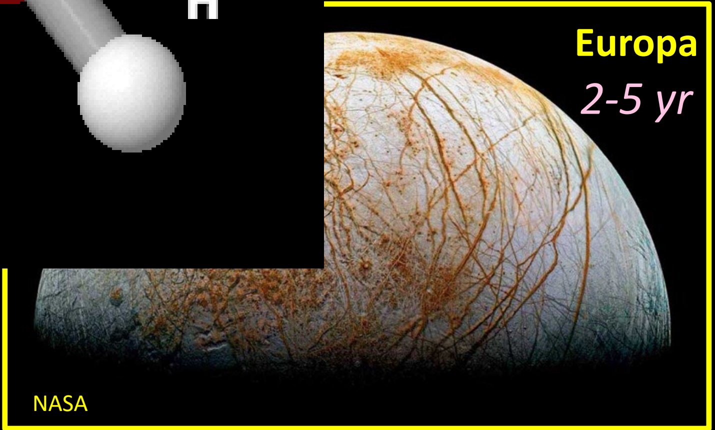


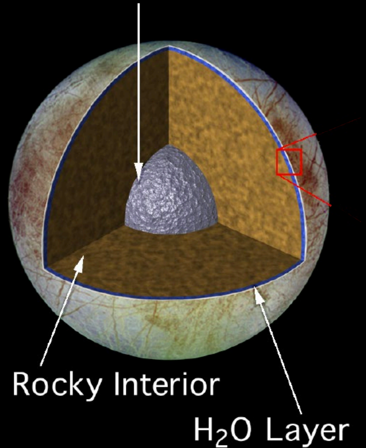
Life-Search Exploration Targets

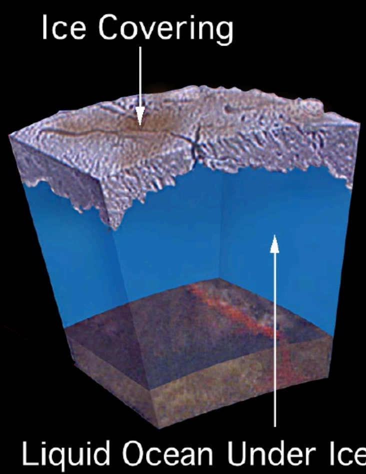
Follow the water!

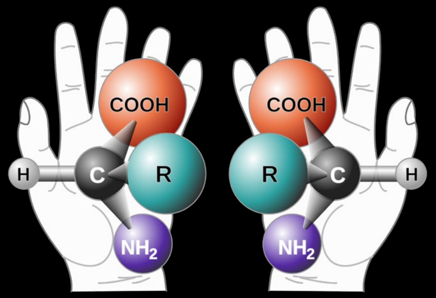






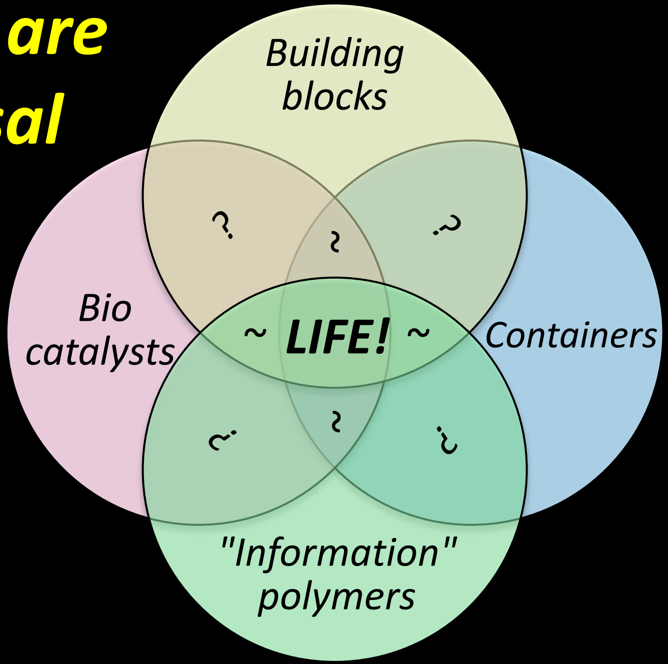






Life Detection Strategy: *some aspects of life are likely to be universal*

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



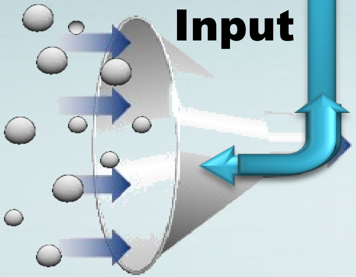
Together, these indicators could provide conclusive evidence of life…

but such an exceptional claim will require exceptional proof →

Use a suite of complementary instruments

Fluidically Enabled Instrument Suite

Sample Input



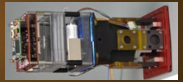
This powerful approach is not limited to searching for life

Fluidics Processor

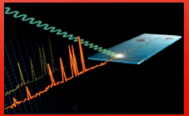
- Deliver extraction solution
- Retrieve sample with particles
- Separate particles • Add dyes
- Degas / de-bubble
- Adjust ionic strength
- Remove interfering ions
- Adjust pH or solvent polarity
- Adjust solvent polarity
- Concentrate samples
- Store & reconstitute reagents
- Provide calibration standards
- Provide controls / blanks
- Deliver particle-free aliquots

Instrument Suite Candidates

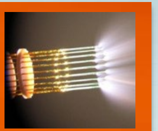
Fluorescence microscopy



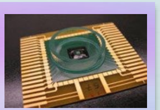
Microchip capillary electrophoresis with laser-induced fluorescence



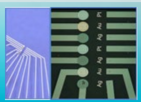
Mass spectrometry w/ electrospray, GC, or (MA)LDI “front end”



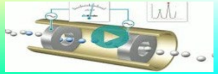
Electrochemical biosensors



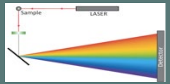
Ion-selective electrodes [Habitability, energy]



Ion chromatography [Habitability]



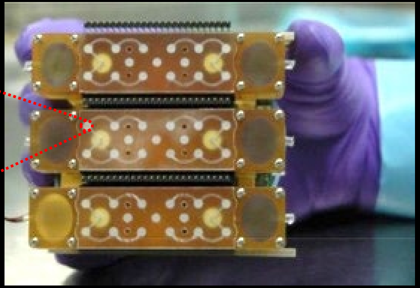
Vibrational spectroscopy

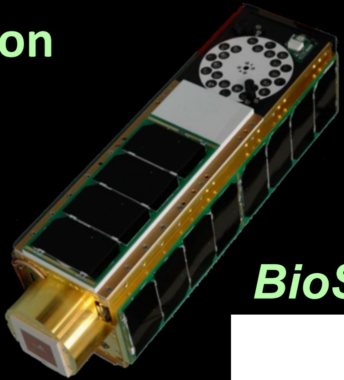


Ames Research Center

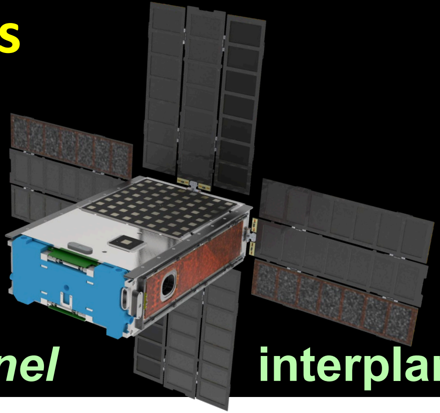
Astro/Biological Space Missions: Enabling Technologies from ARC Cubesat Payloads

O/OREOS – multiyear mission





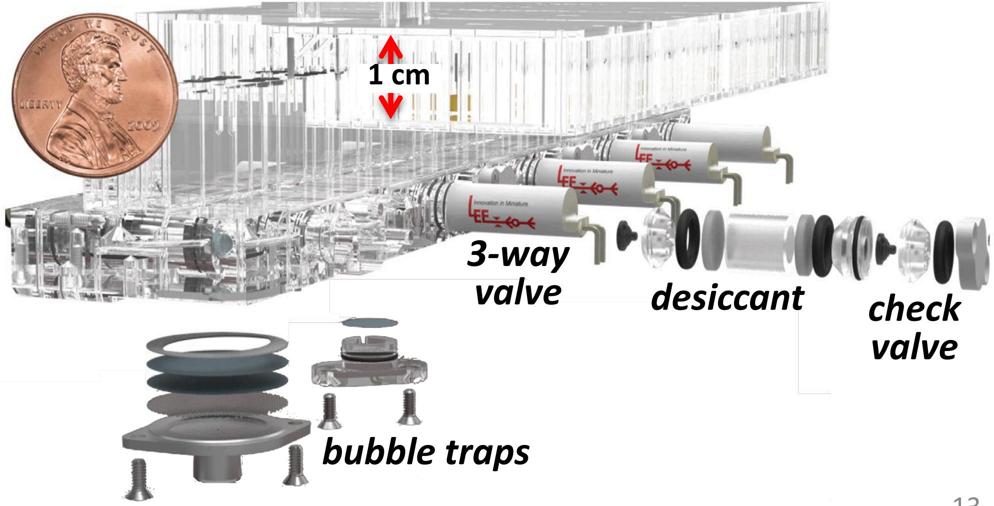
BioSentinel interplanetary mission



<u>Relevant payload technologies</u>

- Handling biological specimens in space
- Perfect sterility (flight-proven)
- Ultra-low organic contamination
- Biocompatible materials
- Manipulate µL volumes
- Fly dry; wet-out fluidics in reduced gravity
- Functional in high-radiation environment

Monolithic multilevel fused manifolds

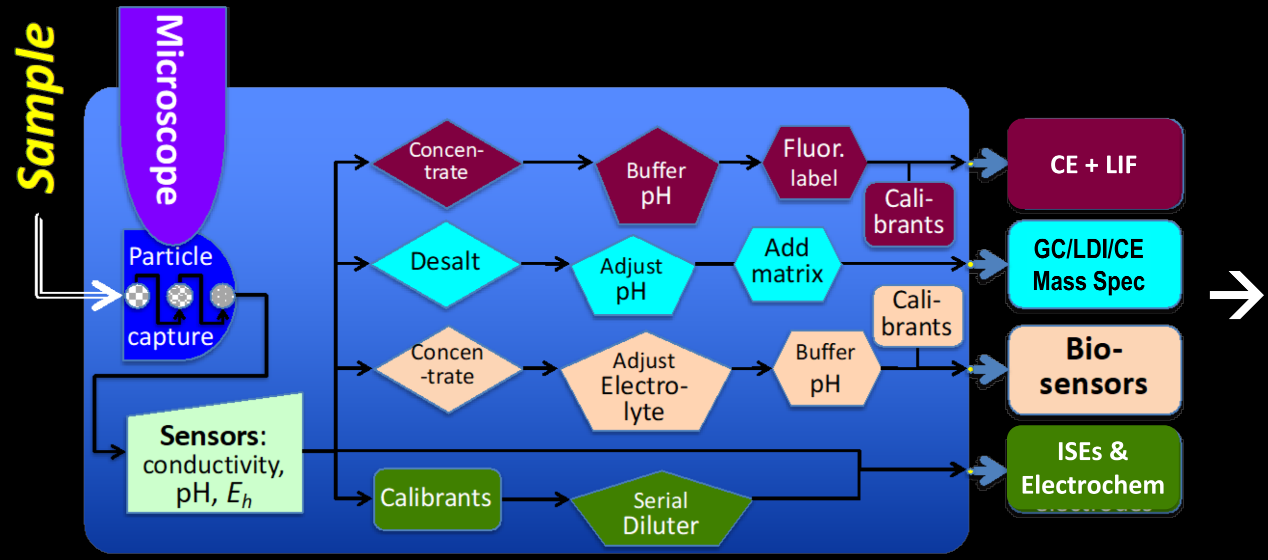


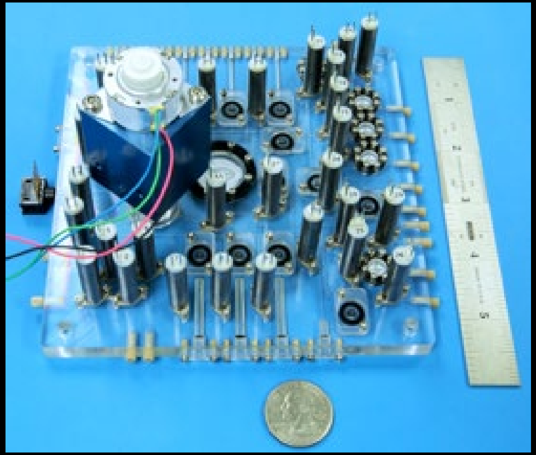
SPLIce: **Sample Processor for Life on Icy Worlds**

Microfluidic technology development for Enceladus & Europa life search

Multifunctional sample processing hub for science payload integration

Functional highlights: > 95% sample recovery; long-term on-board reagent storage / radiation stability / reconstitution; bubble trapping and bubble generation; 128x sample concentration; autonomous sequenced processing; integrated sensors; metered delivery; particle trapping; dye addition

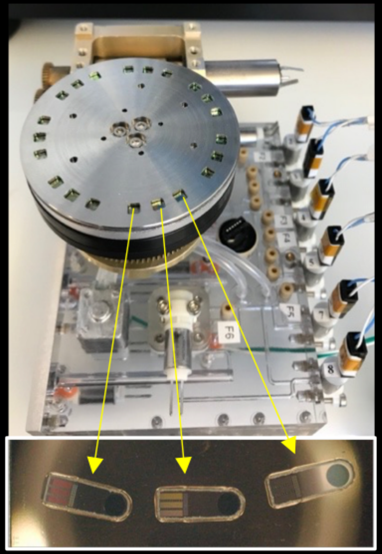




Microfluidic multilayer "circuit board"

Luminescence Imager for Exobiology (LIfE)

Integrated Fluidic-Sample Processor and Microscope



- 3-stage inline filtering:
  - Silicon nitride particle-capture filters w/ etched-on calibration targets
- FOV-optimized geometry

Monolithic Multilevel Manifold

- pH, conductivity, pressure sensors
- Fluorescent stains: porous polymer stabilization, storage and controlled delivery

Rotary Imaging Stage w/ Fluidic Actuator

- Integrates multiple key fluidic functions and image location with a single rotary valve

Bright Field & Epifluorescence Microscope

- Lateral resolution: < 0.5 µm
- Depth of field: 2 µm
- Z motion: 400 µm ± 1 nm
- LED excitation: 275, 375, 470, 525 nm
- Emission filters: 334, 470, 529, 579 nm

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

Fluidically Integrated Electrochemical Habitability Assessment for Icy Worlds

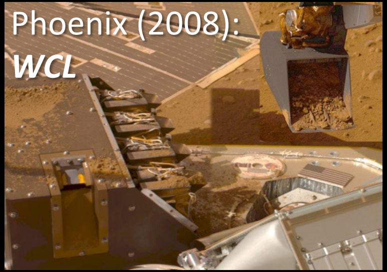
Heritage: Phoenix Wet Chemistry Lab (WCL) + COLDTech + ICEE-2

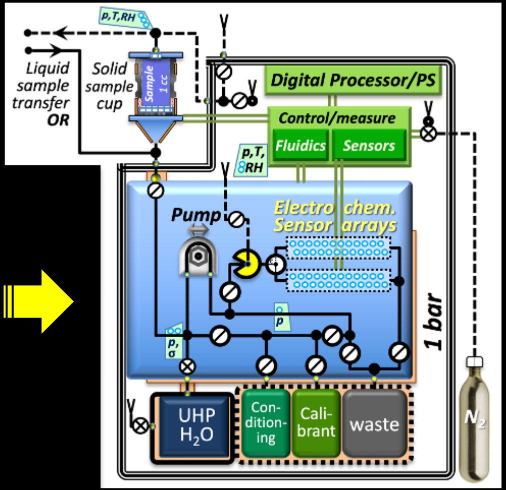
Lead: **ARC** Partners: **JPL, Tufts, MIT, U. of Alberta, Honeybee Robotics**

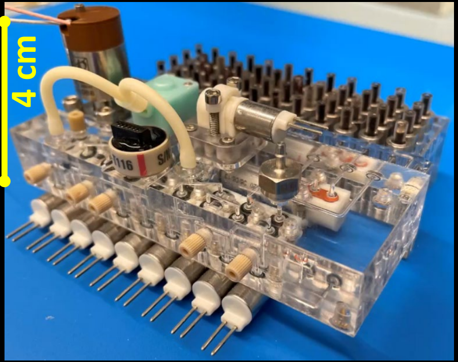
Fluidic Functions:

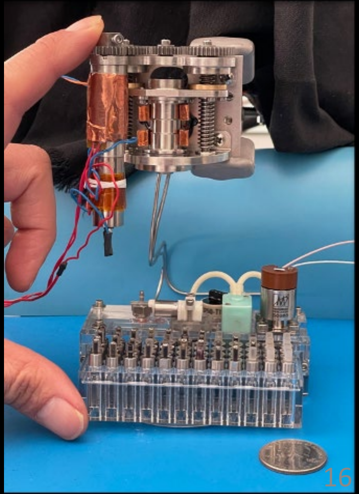
- receive (and melt) icy / regolith samples
- prepare/deliver conditioning/calibrant solutions
- control temperature & pressure
- execute & store measurements

Measurements	pH, ionic conductivity, E_h
Gases	O_2, SO_2
Cations	Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+
Anions	Cl^-, I^-, NO_3^-, PO_4^{3-}, CO_3^{2-}, ClO_4^-, ClO_3^-, SO_4^{2-}, SO_3^{2-}, S^{2-}
ROS	O_2^-, H_2O_2

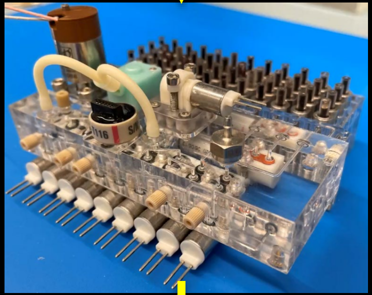


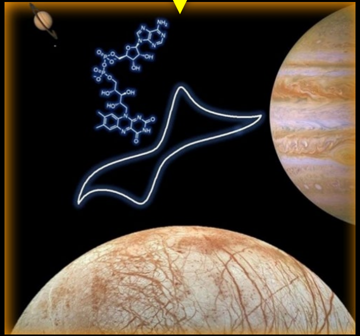




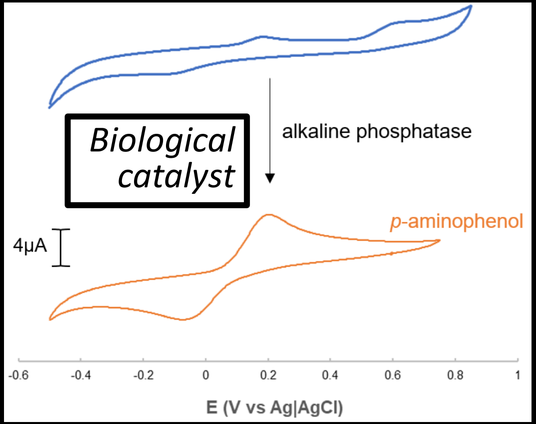


Electrochemical Detection of Catalysts and Electron Transfer as Signatures of Life

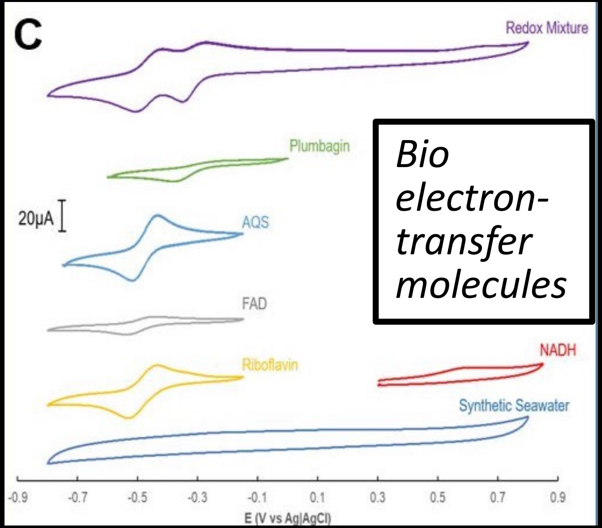


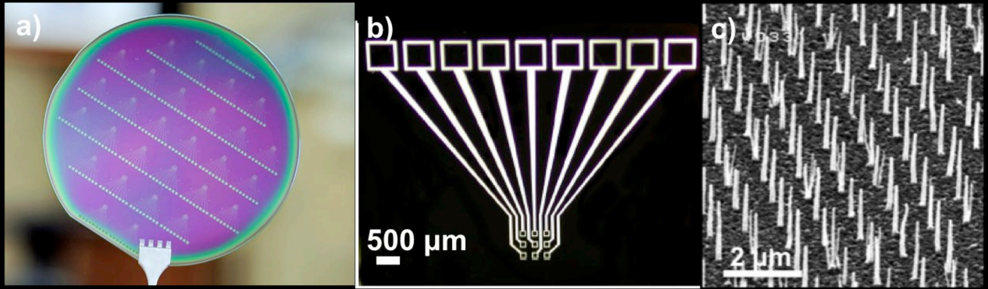


- Example targets: phosphatases, proteases, kinases; bio. electron-transfer molecules
- Habitability, energetics, e⁻ transfer w/ 1 instrument



Thomson, Quinn, Ricco, Koehne, *ChemElectroChem* **2020**, 7, 1-11.



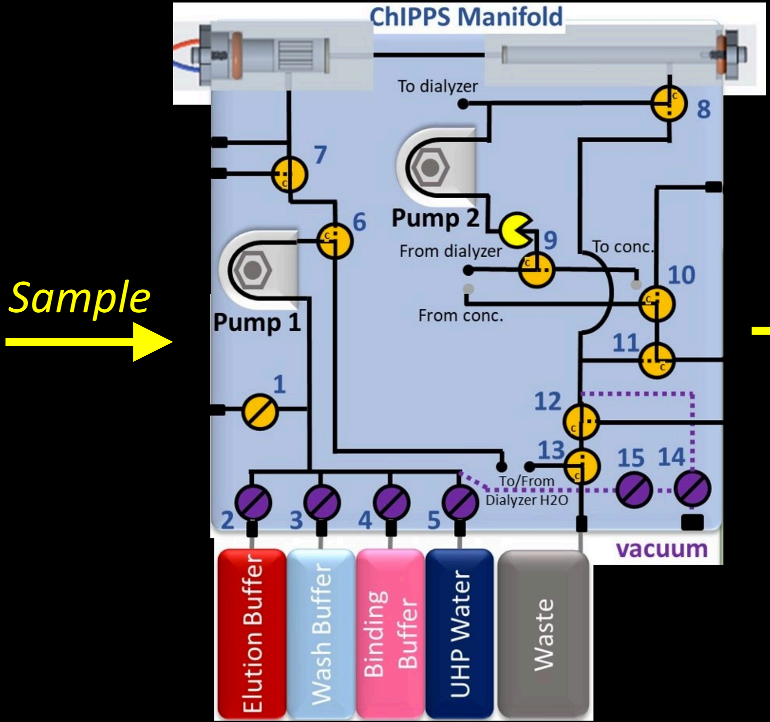


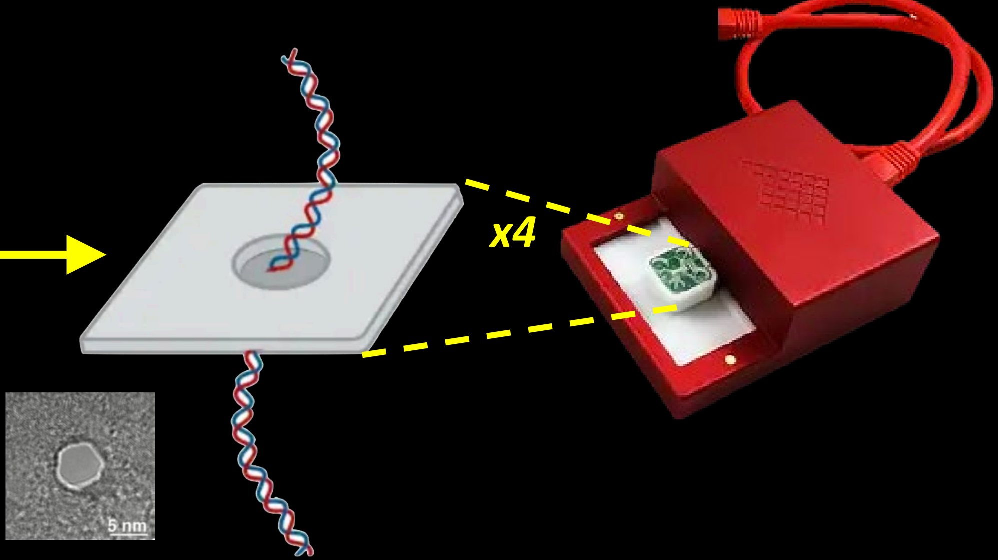
Nanoelectrode array sensing electrodes provide higher signal to noise

Solid-State Nanopore Life-Detection Technology

- Robust silicon nitride nanopore membranes for spaceflight missions
- Detection of multiple types of charged polymers (polyelectrolytes), particles
- Fluidic processor front-end to prepare samples (extract/isolate; de-salt, concentrate)

ARC; Goeppert Inc.; Elements SRL





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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


EcAMSat above Earth

Conclusions


ELSAH below Enceladus

- **Synergies & technology overlaps between space biology and astrobiology should be *identified* and *exploited***
  - Microfluidics development at ARC has ~ followed this strategy
  - Paradigm: funding → science → technology (& mission)
- **HERITAGE of astro- & fundamental biology experiments in low-Earth orbit is a major enabler for interplanetary biological missions**
  - 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
  - High-heritage sensors, fluidic components & approach; high-efficiency / uniformity thermal control, managing pressure and condensation …