Beyond *BioSentinel*: Iterative Development of Automated Microfluidics

Mike Padgen

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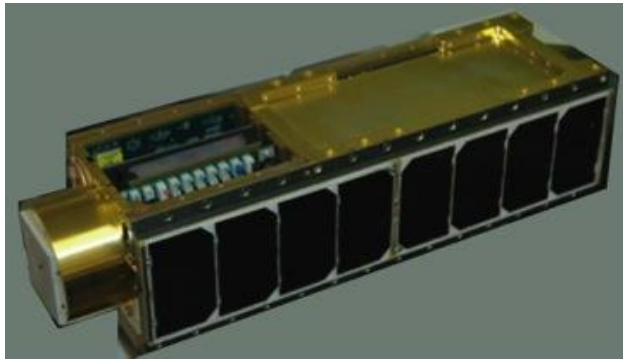
NASA Ames Summer Series

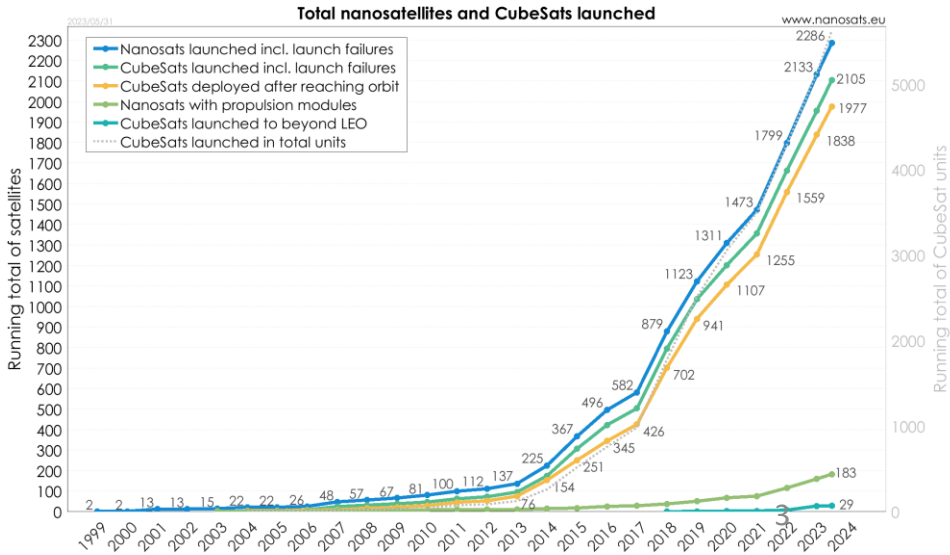
Outline

- A Brief History of Bio-CubeSats
- BioSentinel, LEIA, and Adapting Bio-CubeSat Hardware
- Search-for-Life Sample Processors
- SAMMS and RADREADS: Automated Nucleic Acid Extraction and Analysis
- What's Next

What are CubeSats?

- Small, free-flying satellites containing microprocessor, communications, attitude-control system and a payload
  - Standard form factor “U”, 10 cm cube, <1.33 kg
  - Affordable and accepted platform for space research
- CubeSats developed by middle/high schools, universities, industry and government groups from more than 80 countries
- 2,000+ CubeSats have been launched since 1998
  - >90% since 2013

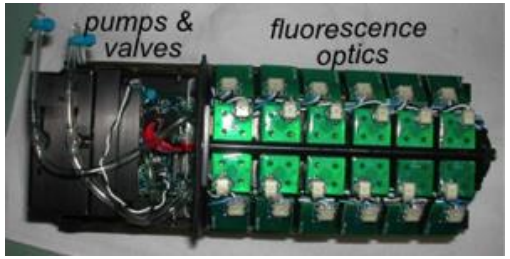


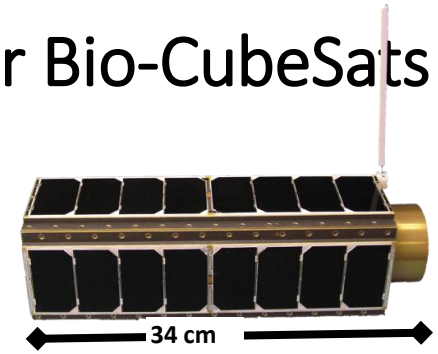


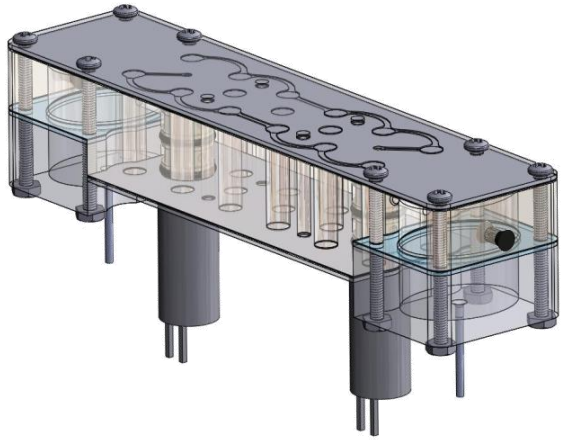
GeneSat-1 images courtesy of NASA Ames
Chart: https://www.nanosats.eu/

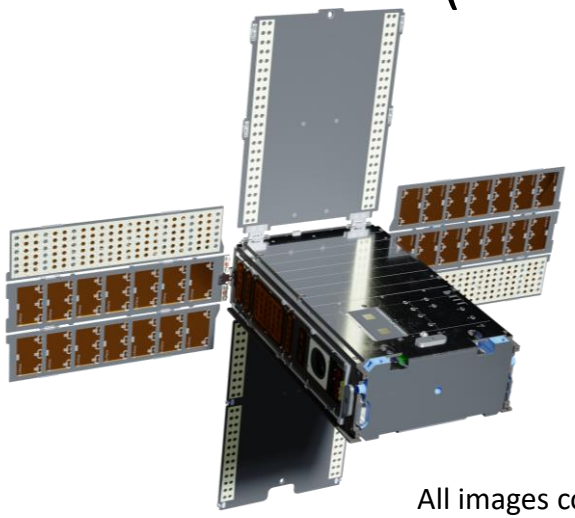
Ames is the Place for Bio-CubeSats

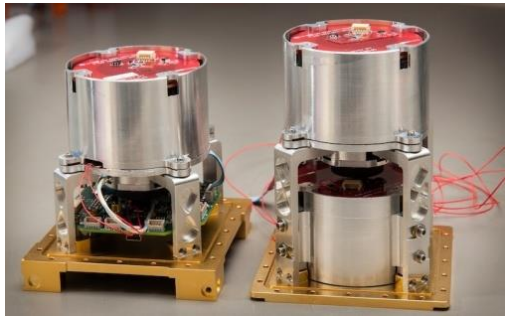
- GeneSat-1 (2006)
- PharmaSat (2009)
- O/OREOS (2010)
- SporeSat (2014)
- EcAMSat (2017)
- BioSentinel (2022)

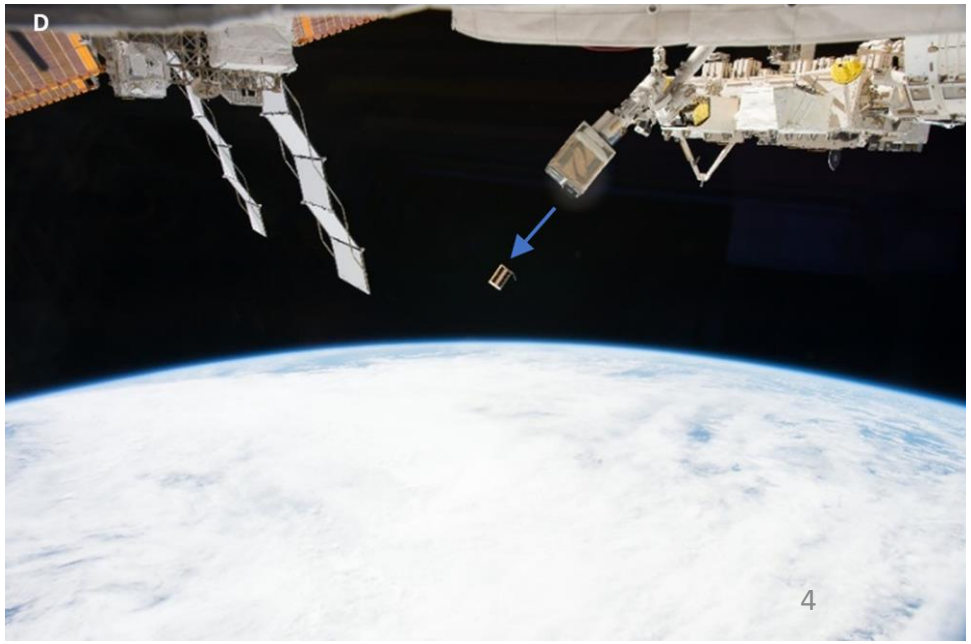








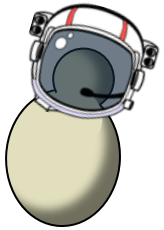




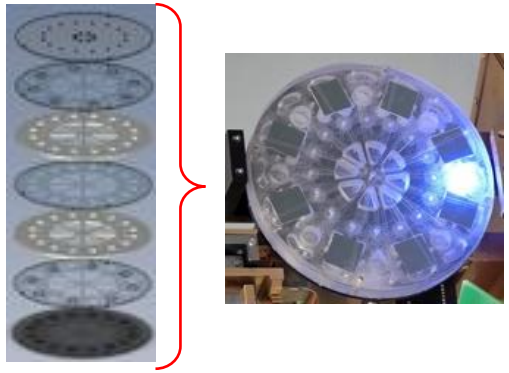
All images courtesy of NASA

Demonstrated Capabilities of Bio-CubeSat Payloads

- *E. coli* culture (*GeneSat-1*, *EcAMSat*)
- Fluorescence measurement of GFP (*GeneSat-1*)
- Optical density of cells (*GeneSat-1, PharmaSat, O/OREOS, EcAMSat, BioSentinel)*
- Sterile assembly (all)



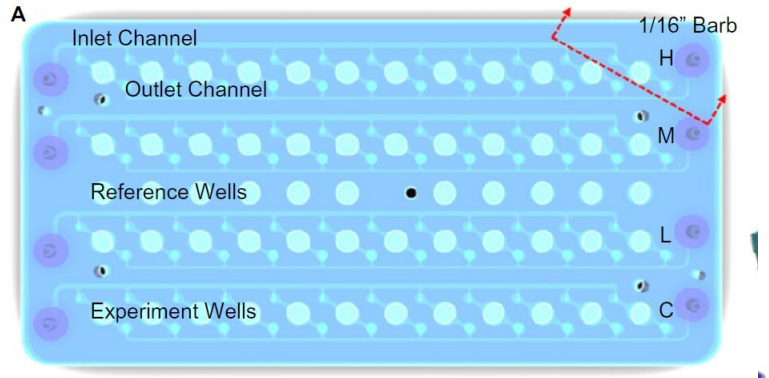
- *S. cerevisiae* culture (*PharmaSat, BioSentinel*)
- Drug dosing and delivery (*PharmaSat, EcAMSat*)
- Metabolic dye measurements (*PharmaSat, O/OREOS, EcAMSat, BioSentinel*)
- Radiation measurement (*O/OREOS, BioSentinel*)
- *B. subtilis* culture (*O/OREOS*)
- Long-term stasis (*O/OREOS, BioSentinel* (*ISS*))
- Centrifuge (*SporeSat*)
- *n* from 10 (*GeneSat*) -> 288 (*BioSentinel*)

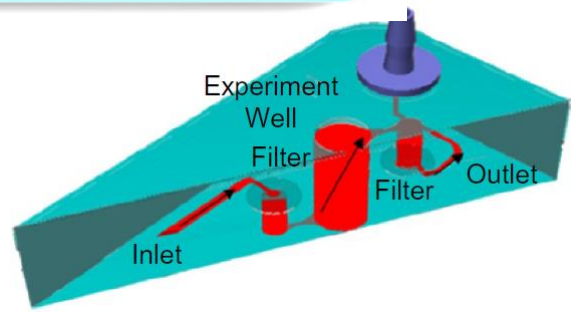


Figures courtesy of NASA Ames

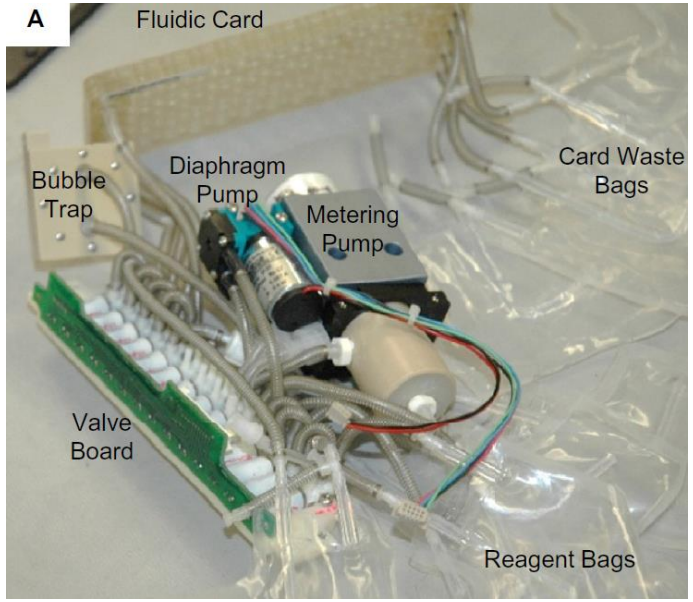
Self-Contained, Automated Bio-CubeSat Fluidic Systems

- Fluidic cards house biology
  - Cells constrained by filters on inlet and exit of microwell (75-100 µL)
- Reagent bags (Growth media, dyes, drugs)
- Pumps and valves to control flow in tubing and channels





SESLO image: Nicholson, et al. Astrobiology 2011



EcAMSat images: Padgen, et al. Acta Astronautica 2021

BioSentinel Mission

- 14-kg, 6U nanosatellite
- BioSensor payload
  - Measure effects of deep space radiation on yeast growth and metabolism
- LET radiation detector (JSC)
- Secondary payload on Artemis 1
  - Launch: Nov 16, 2022
  - Lunar Flyby: Nov 20
  - First biology experiment started: Dec 5
- BioSensor and LET payload sent to ISS in 2022

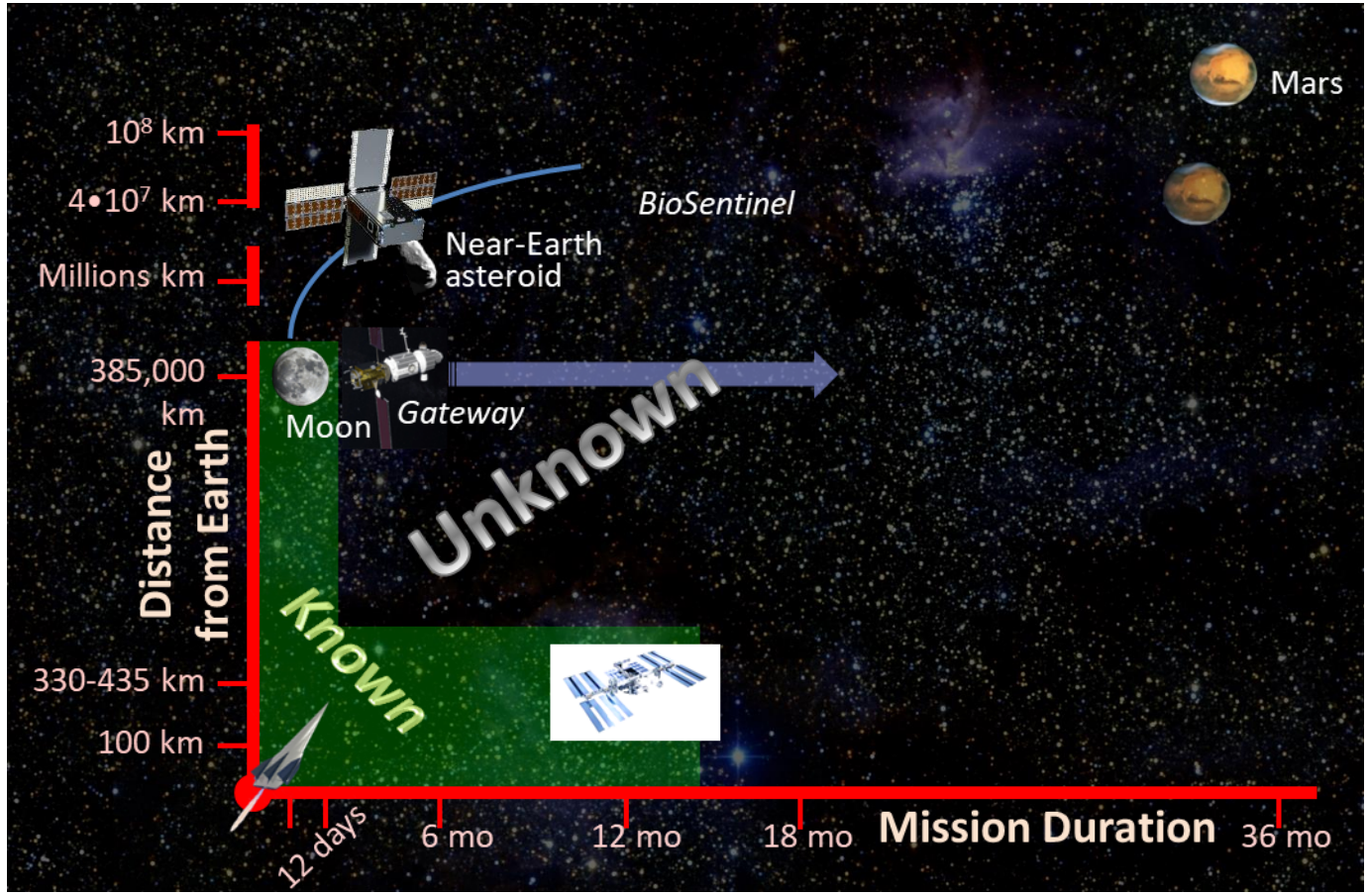
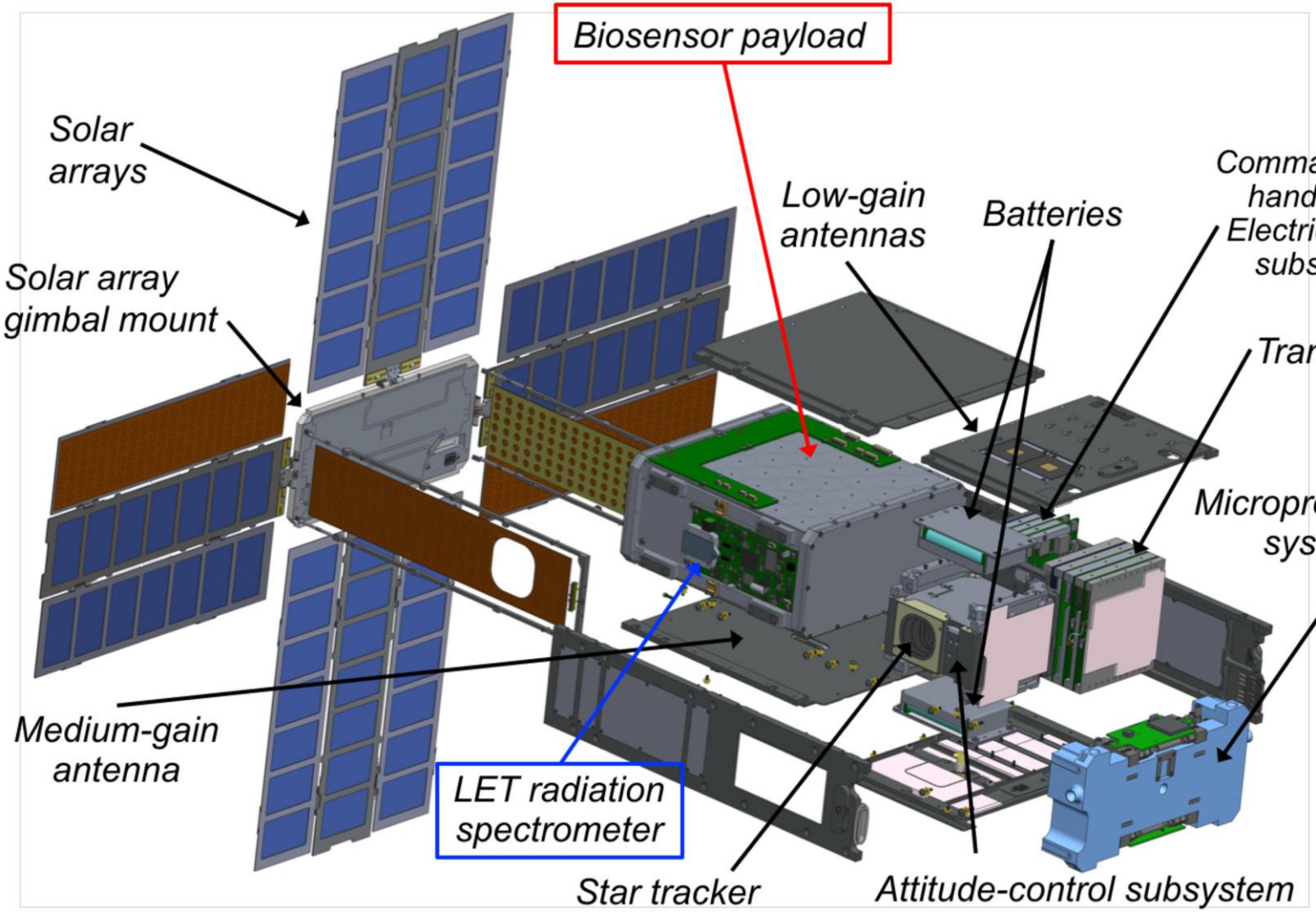


Figure courtesy of Tony Ricco

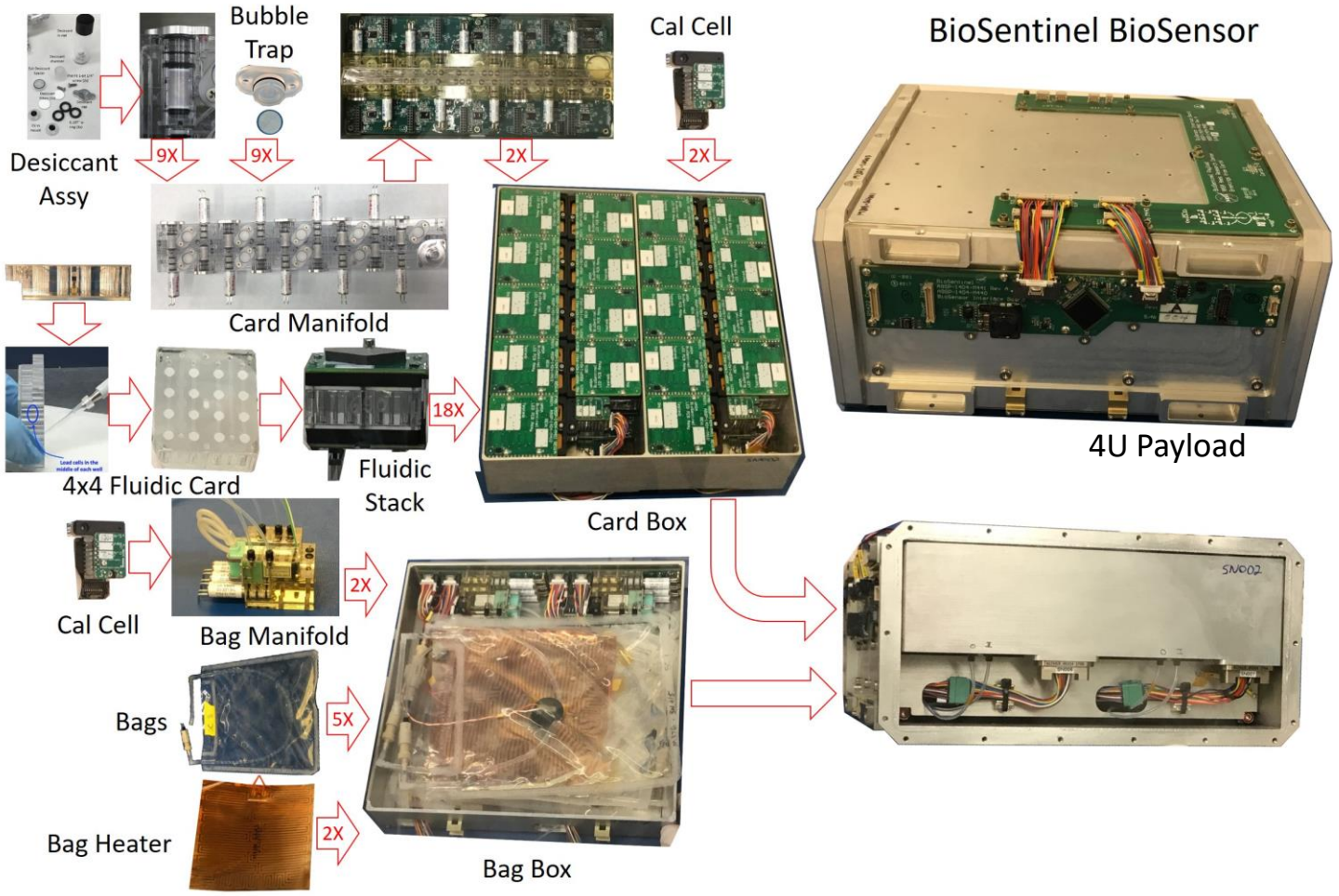
BioSentinel Spacecraft Configuration



Ricco, et al. IEEE 2020

BioSentinel BioSensor

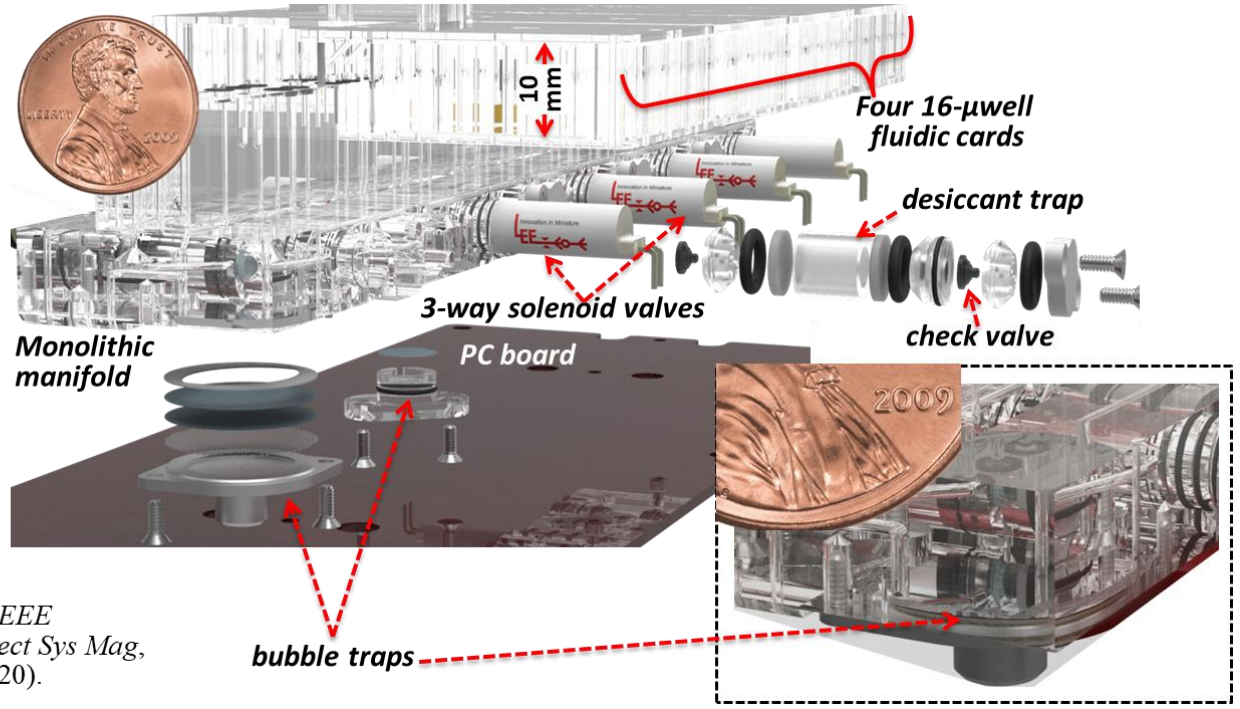
- 2 card manifolds
  - 9 cards, 16 wells/card
  - Check valves, desiccant, bubble traps
- Bag manifold in separate box
  - aB/SC stored separately, mixed by toggling valve
- Lessons learned
  - Assembly/access
  - Stasis
  - Humidity



Images courtesy of NASA Ames

BioSentinel's Thermally Fused Monolithic Manifolds

- Multiple layers of machined polycarbonate (PC) thermally fused together
  - Higher density of functionality
  - More robust
  - Fewer fluidic connections
  - Less dead volume
    - Silicone tubing 20 µL/cm
    - PC 10 µL/cm
    - COP 0.31 µL/cm

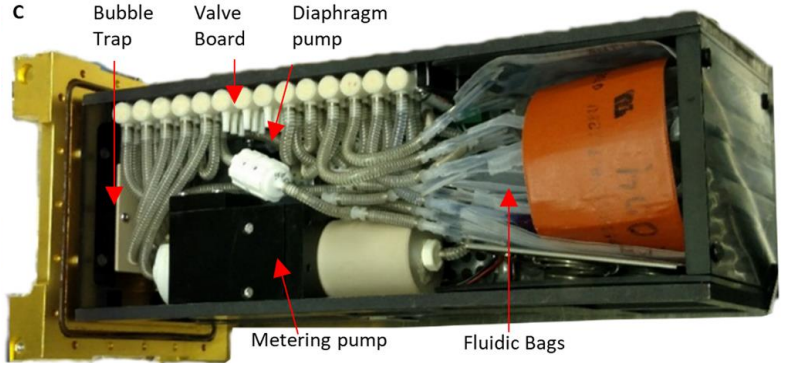


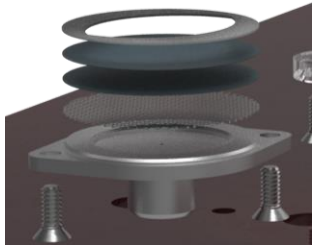
Ricco et al., *IEEE Aerospace Elect Sys Mag*, *35*, 18-24 (2020).

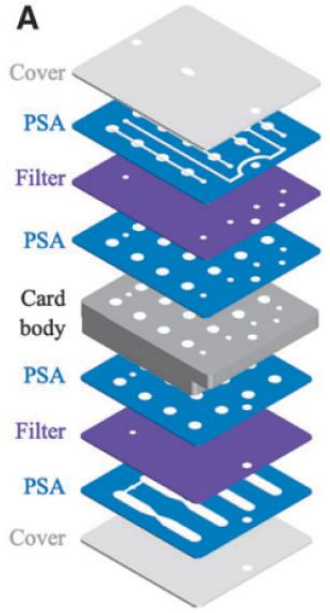
COP: cyclo olefin polymer, trade name Zeonor

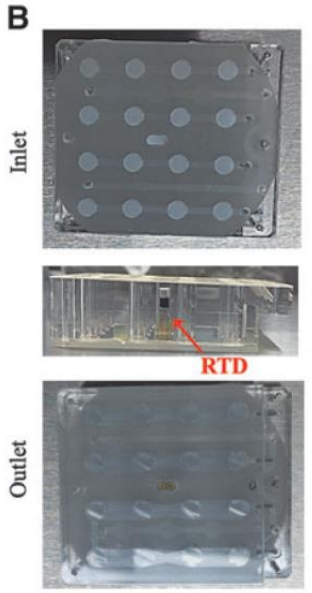
Adapting Bio-CubeSat Hardware

- EcAMSat used leftover PharmaSat hardware
  - Fluidic card filter change
  - PowerCell payload used a similar fluidic card
- BioSentinel adapted design free-standing bubble trap in the PharmaSat/EcAMSat payload, mounted to manifold
- BioSentinel card adopted assembly processes from EcAMSat
- Reuse COTS pumps and valves with flight heritage





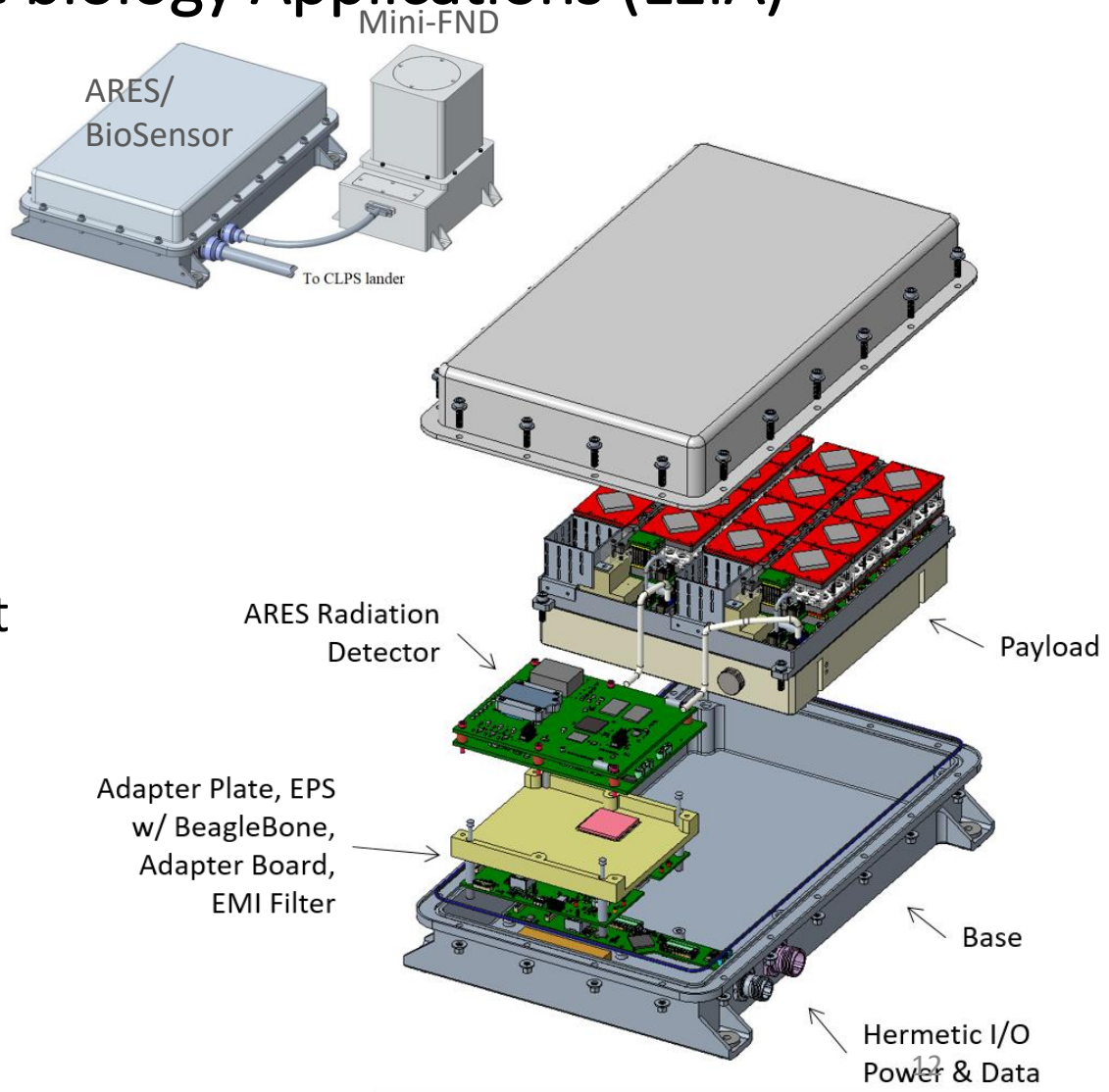




EcAMSat images: Padgen, et al. LSSR 2020
BioSentinel fluidic card: Padgen, et al. Astrobiology 2021
BioSentinel bubble trap: Ricco, et al. IEEE 2019

Lunar Explorer Instrument for space biology Applications (LEIA)

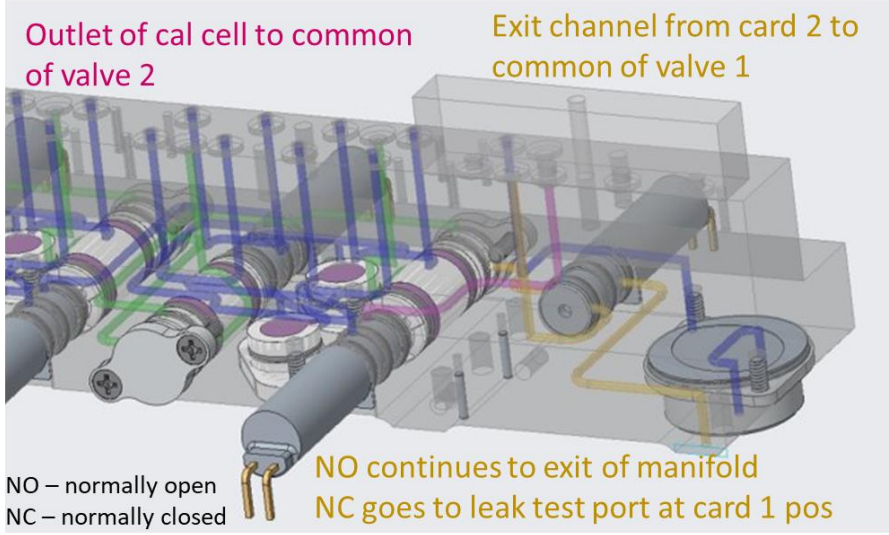
- Commercial Lunar Payload Services (CLPS) CP-22 mission
- Modify BioSensor, address lessons learned
  - Assembly/access, stasis, humidity
- Biology goals:
  - Cellular sensitivity to the Lunar environment
  - Production of antioxidant carotenoids for crew dietary supplementation.
  - Engineered genes from tardigrades and yeast for enhanced tolerance to the lunar surface.
- ARES radiation detector (JSC) and Mini Fast Neutron Detector (SwRI)
  - Biologically relevant radiation at the south pole landing site.

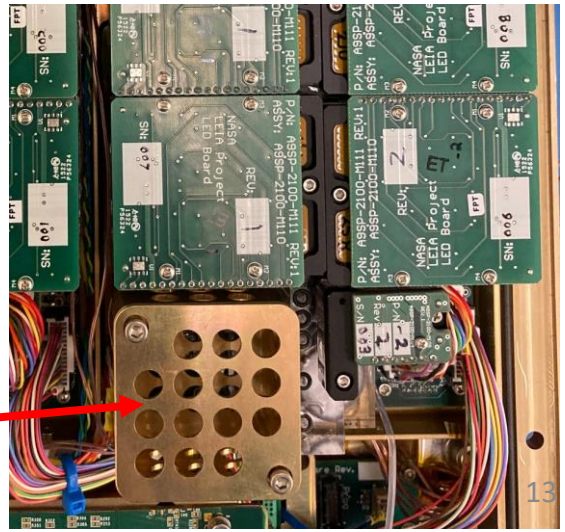


Figures courtesy of NASA Ames

LEIA Modifications to the BioSensor

- Base and lid enclosure
  - Provides access to cards to perform late load
- Card manifold (where yeast is stored)
  - Removed one card on each bank
  - Created dry leak test port
  - Space used for desiccant
- Bag manifold – humidity seal
  - Electronics separated from bags and humidity during stasis
- Late load: Biology installed after acceptance testing, limit stasis





Figures courtesy of NASA Ames

BioSentinel and LEIA Status

- BioSentinel Free-Flyer still collecting radiation data ~22 million km from Earth
  - BioSensor testing complete March 2023
- BioSentinel ISS unit ran ~6 months, returned August 2022
- LEIA prototype completed thermal testing
  - Prepping for CDR
  - CLPS vendor TBD
  - Refurbishing prototype for Science Verification Test
  - Assembling flight-like Qualification unit for further testing

Search for Life Sample Processors

- Several projects focused on developing sample preparation technologies for life detection missions to icy worlds
  - Monolithic manifolds in PC or COP, leveraging flight proven hardware
  - Interfacing to sample collectors (<50 μL) and downstream analysis
- What to look for?
  - Versatile chemical building blocks
  - Complex multimeric biomolecules
  - Containment structures
  - Function-specific molecules

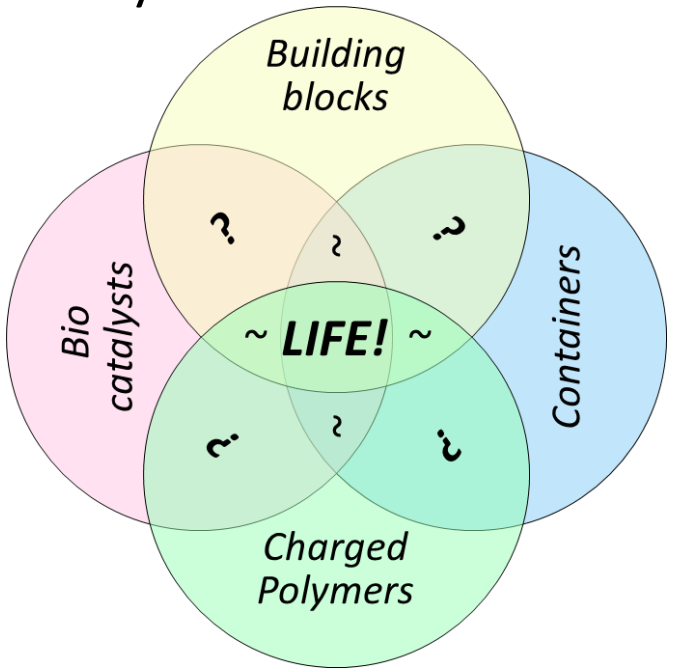
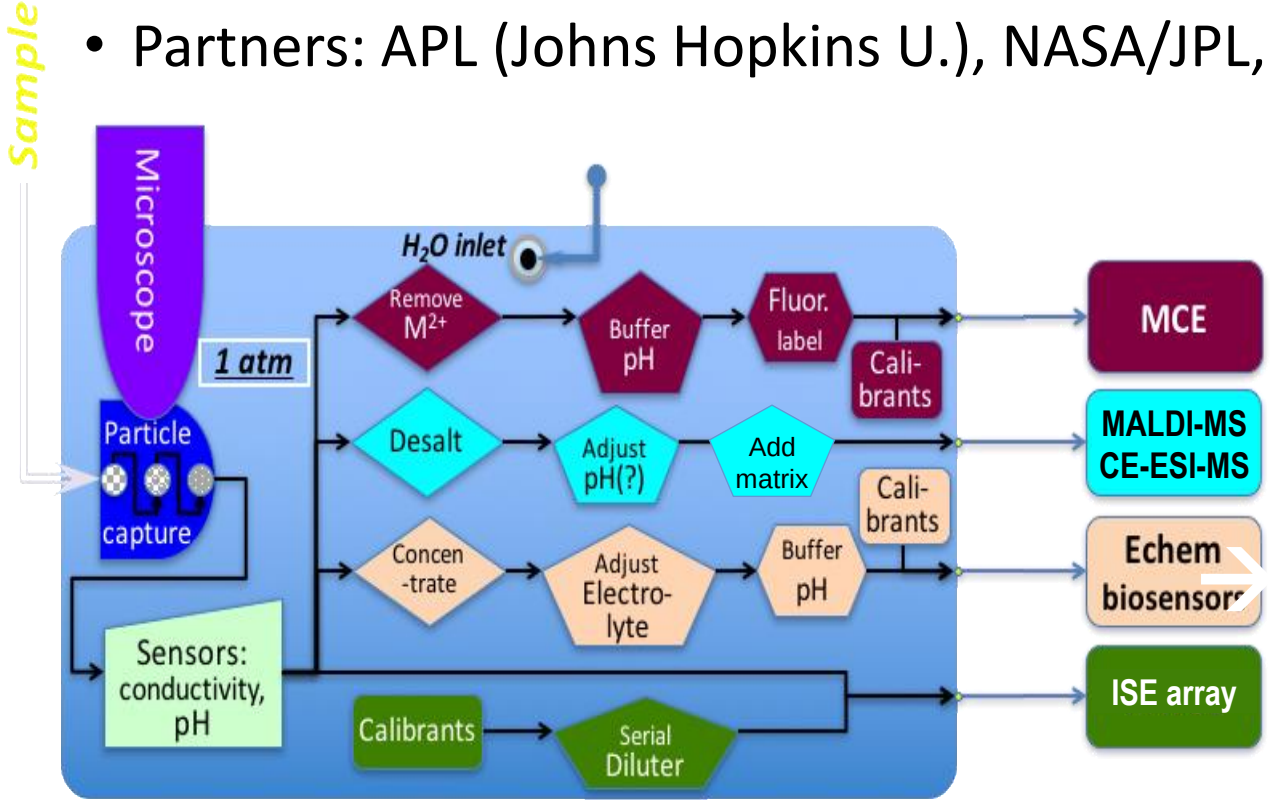
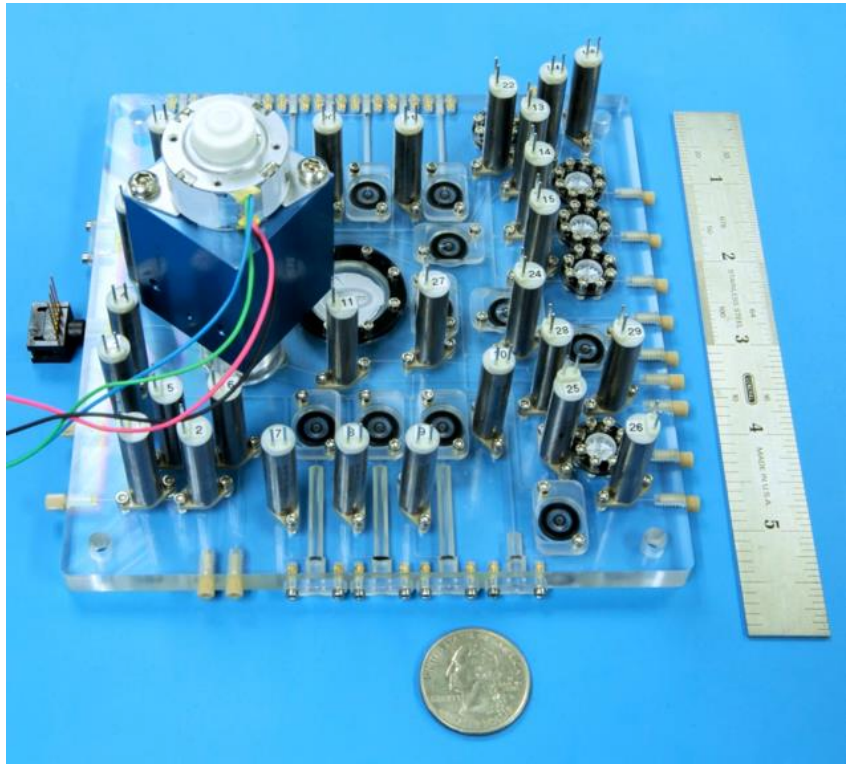


Figure courtesy of Tony Ricco

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

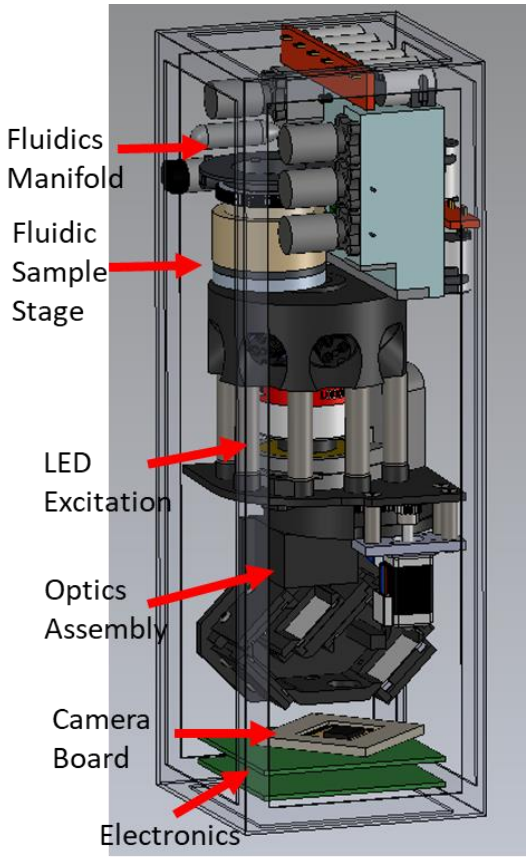


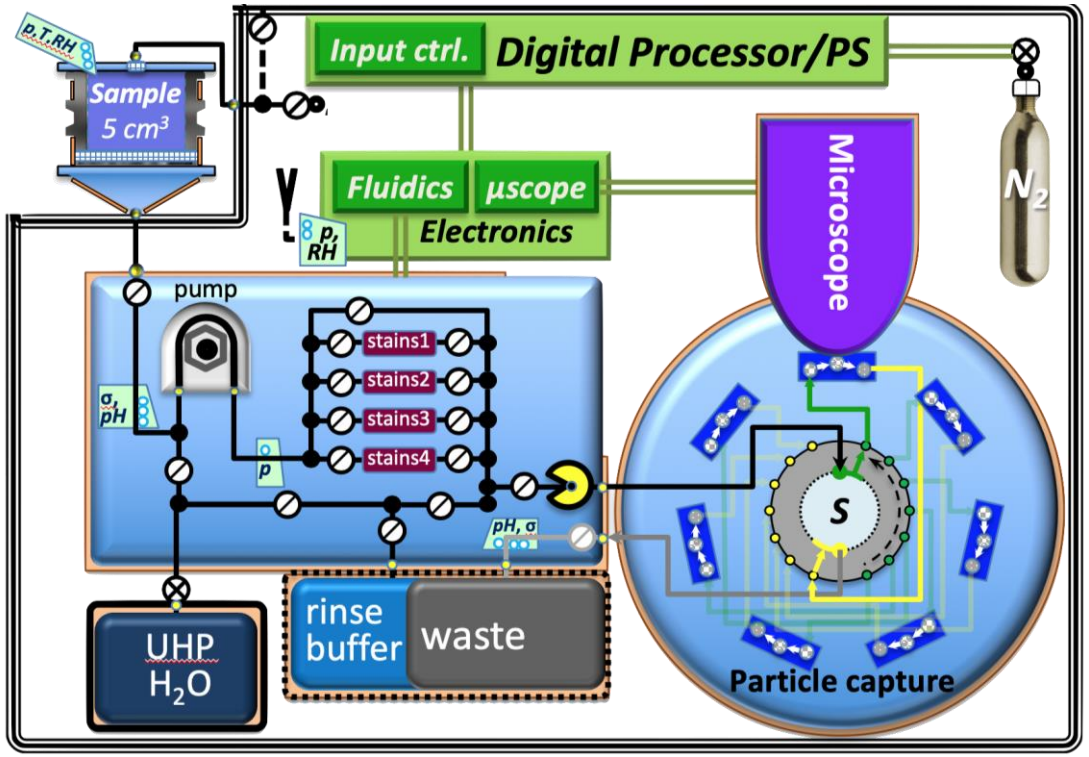


***SPLIce** Monolithic Manifold*

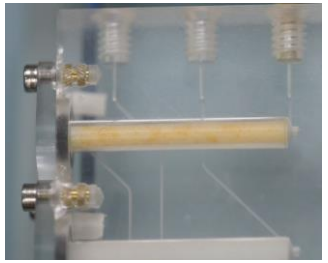
Figures courtesy of SPLIce/COLDTech/ICEE2 team at NASA Ames

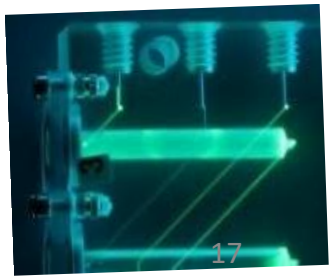
Europa Luminescence Microscope (ELM)





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





Figures courtesy of SPLIce/COLDTech/ICEE2 team at NASA Ames

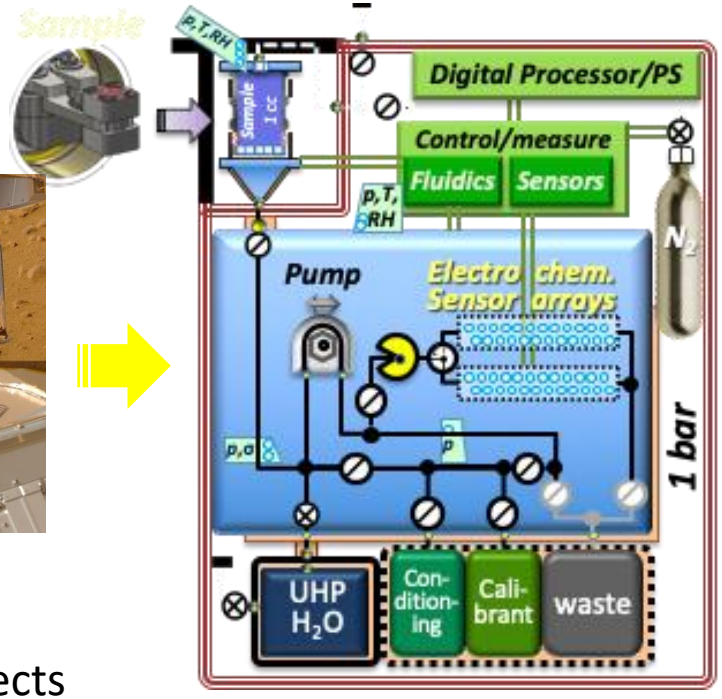
MICA: Microfluidic Icy-world Chemical Analyzer

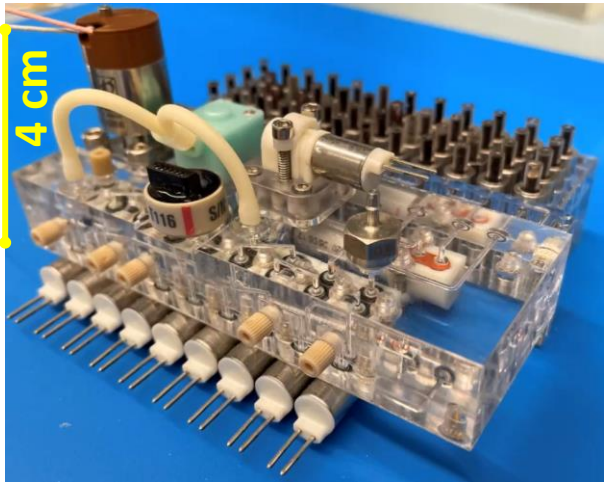
Lead: **ARC** Partners: **JPL, Tufts, MIT, U. of Alberta, Honeybee Robotics**

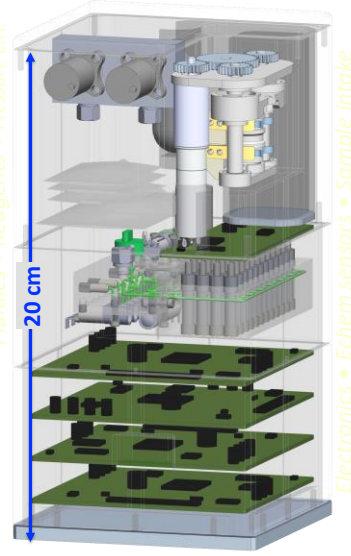
Key Measurements: pH, conductivity, ions, gases, ROS: Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, CO_3^{2-}, NH_4^+, NO_3^-, ClO_4^-, ClO_3^- PO_4^{3-}, SO_4^{2-}, SO_3^{2-} ; O_2, SO_2, O_2^-, H_2O_2; solution energetics: discrete & average of redox-active species (E_h)

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

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







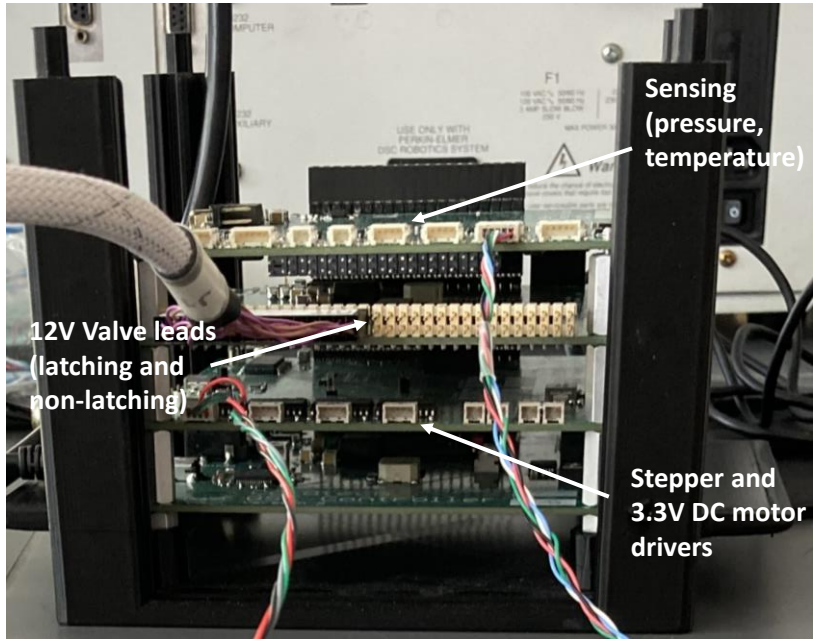
Figures courtesy of SPLIce/COLDTech/ICEE2 team at NASA Ames

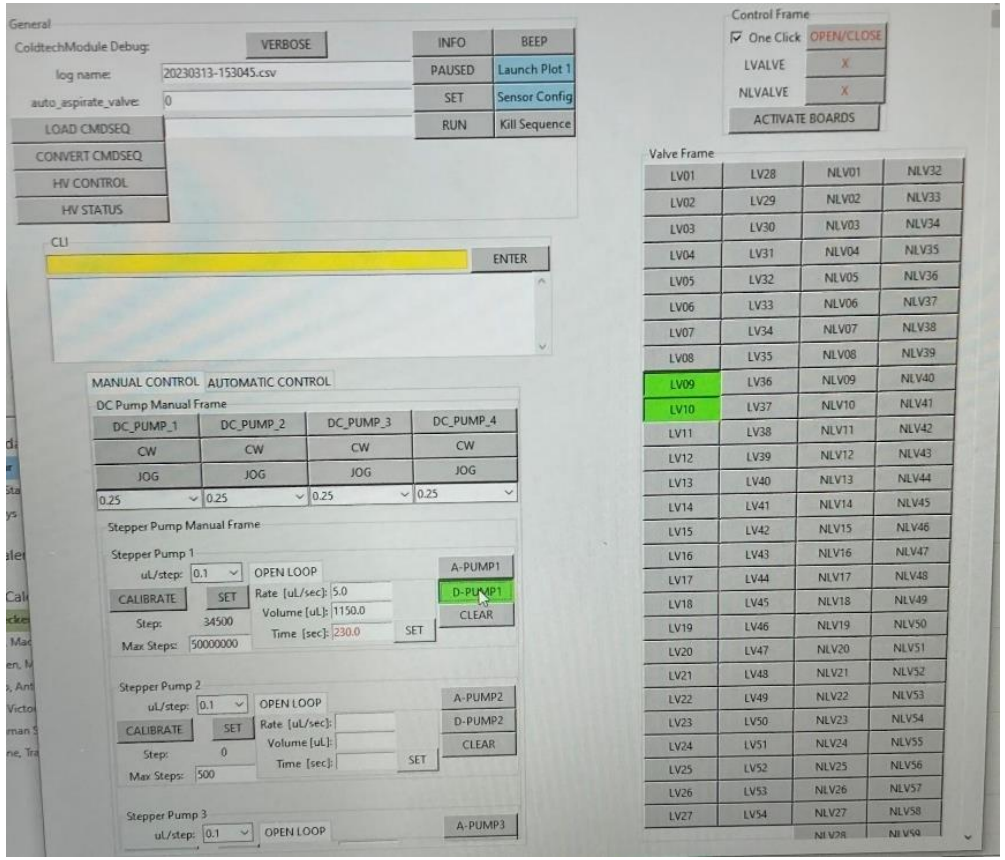
An Incomplete List of Fluidic Capabilities Demonstrated

- Receive samples from collector
- Concentrate/dilute, prepare/deliver solutions
- Salt removal, pH adjustment
- Fluorescent labeling, anhydrous reagent storage
- Bubble removal and generation
- Electrolysis
- Rotary valve and rotating stage for microscope
- Sensor interfacing and measurement
- Closed-loop pump control
- Control temperature & pressure

Plug-n-Play GSE Stacks

- During ICEE2/COLDTech, stackable PCs board that control valves, pumps and sensors
  - GUI for manual actuation
  - Closed-loop pump control
  - Accepts scripted command sequences

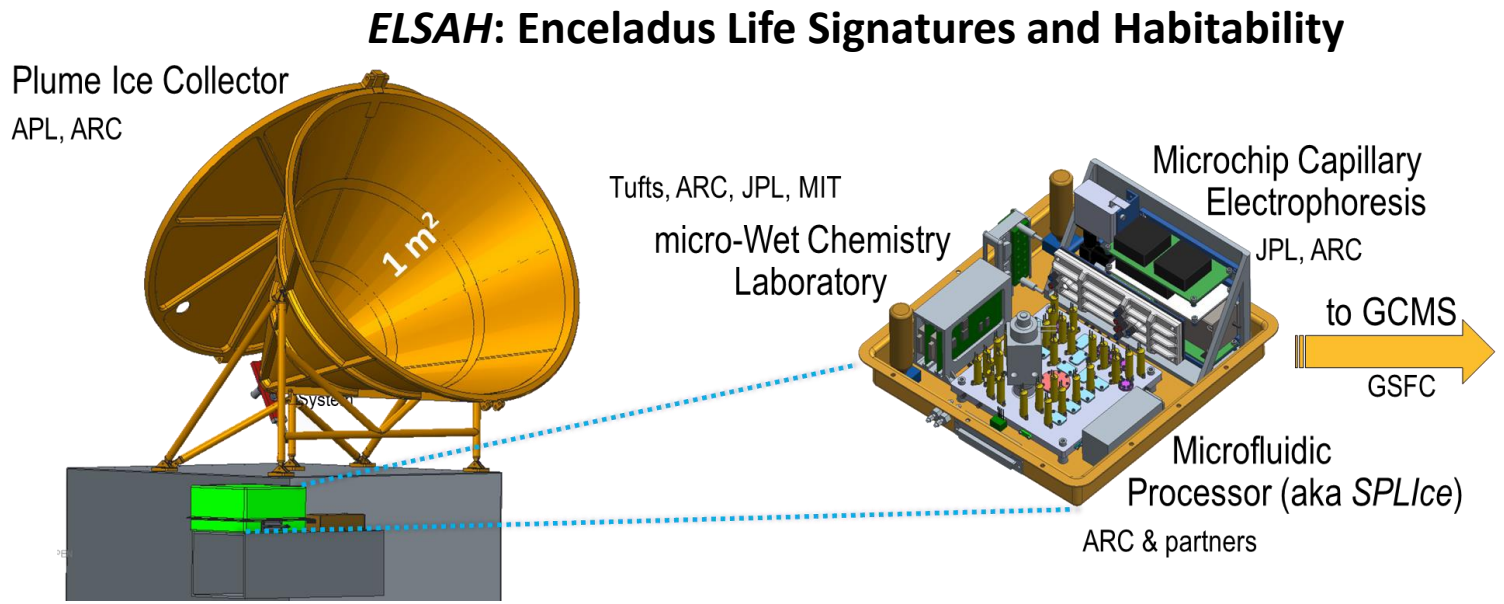




Figures courtesy of NASA Ames

Current Status Life Detection Manifolds

- Continued development and technology maturation
  - LIfE Luminescence Imager for Exploration

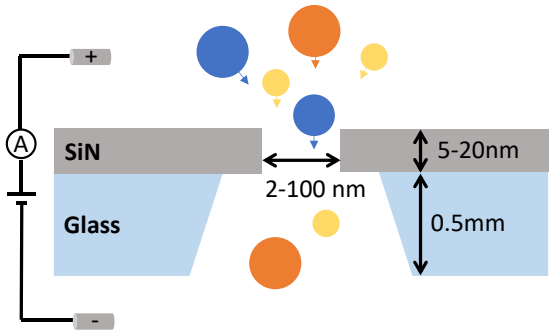


µCAFE: microChemical Analyzer of Fluids for Exobiology
(SPLIce is the core of a Life-Search System for Enceladus)

Figures courtesy of NASA

FLOW (Fluidics for Life detection on Ocean Worlds) & ChIPPS (Charged Information-storage Polymer Preparation System)

- IRADs looking to detect polyelectrolytes as information storage biosignature
  - DNA, RNA, but want to be more general
- Isolate polyelectrolytes from sample, concentrate, characterize with nanopore



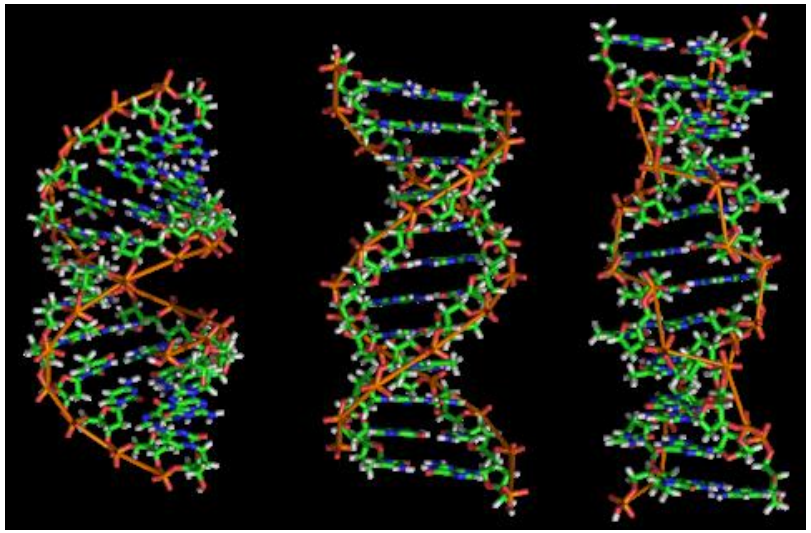


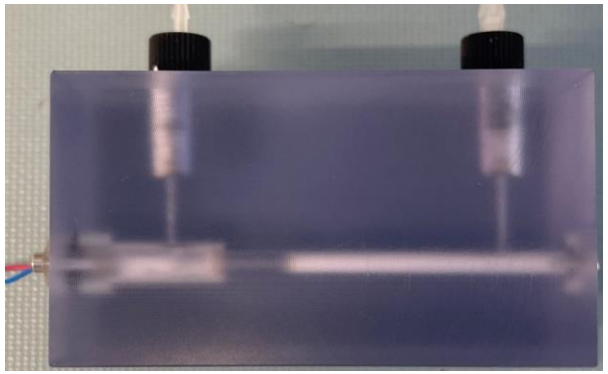
Figure 1. Structural representations of various possible polyelectrolytes. Linear molecules that can hold their shape due to a repeating charge backbone are required for information encoding and allow Darwinian Evolution – the defining process of life. (Photo credit: mytoba.ca)

Nanopore schematic courtesy of Goeppert/NASA Ames

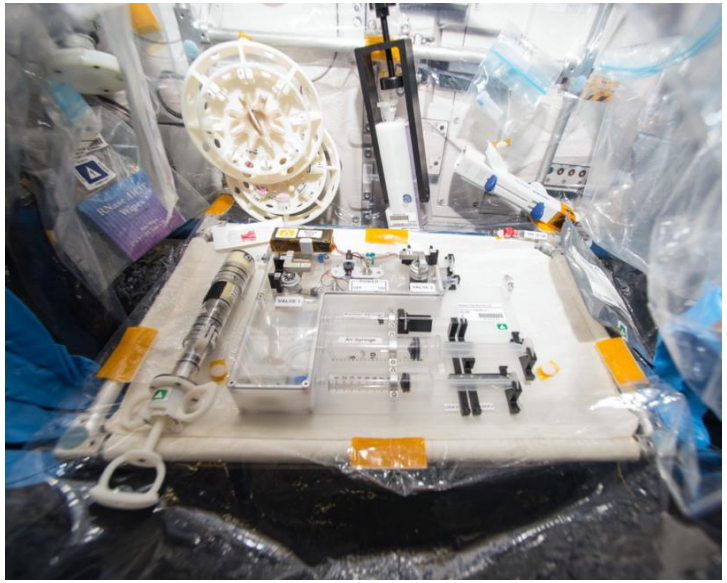
Adapting Wetlab-2 Hardware

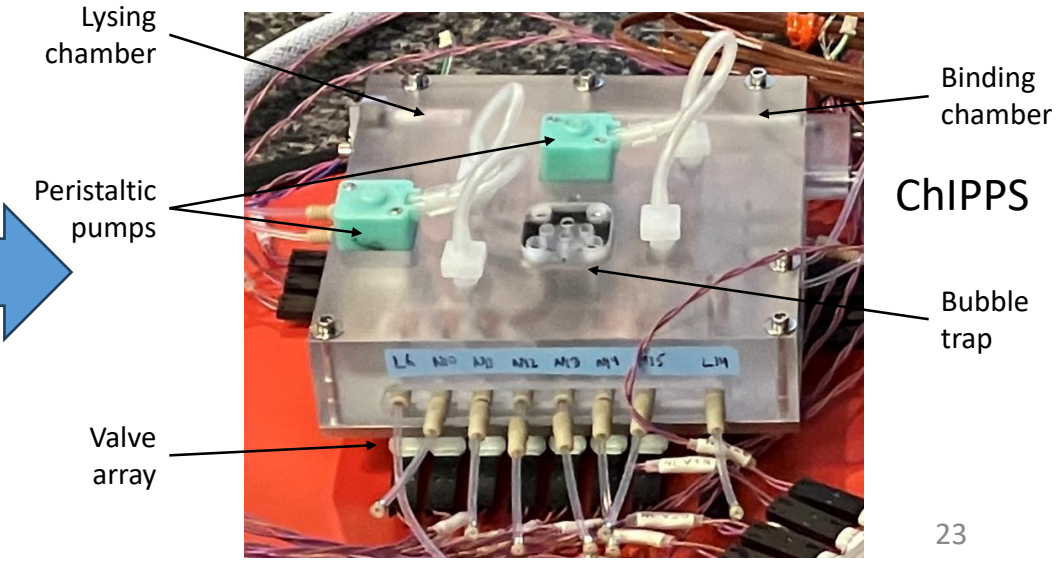
- Wetlab-2 used Claremont BioSystems OmniLyse system to extract RNA on ISS
  - Pre-loaded syringes, hands-on processing by astronauts
- Automate process, integrate Omnilyse into manifold
- Extraction test block to optimize parameters

Test Block



Wetlab-2

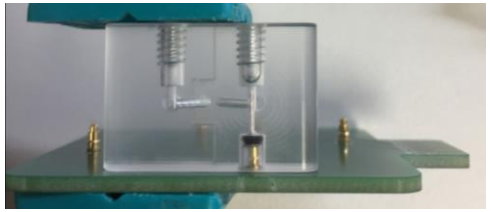


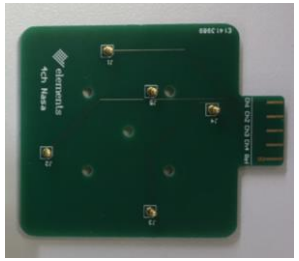


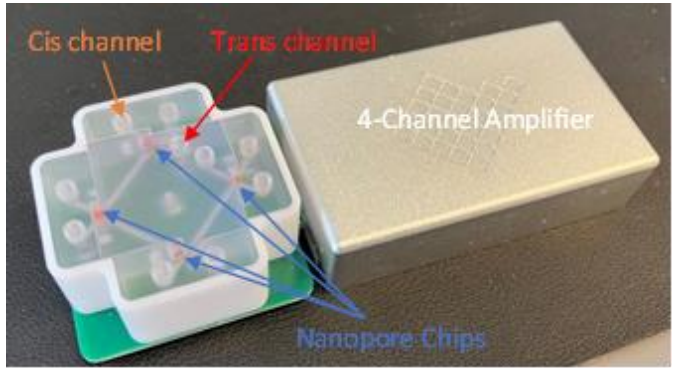
Parra, et al. PLoS One 2017

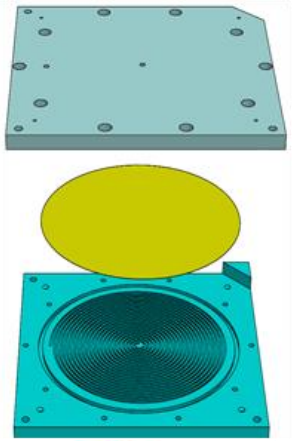
Dialysis, Concentration and Nanopore Measurements

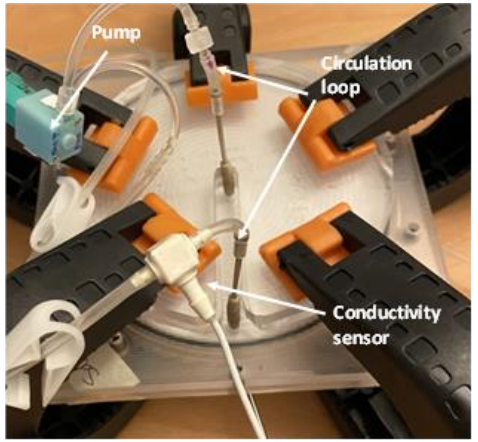
- Low expected concentration in any life detection mission
  - Dialysis to remove salt
  - Vacuum concentration
- Flow cell w/ 4 solid state nanopore chips
  - Cis channel (sample side)
  - Trans channel (shared, buffer side)

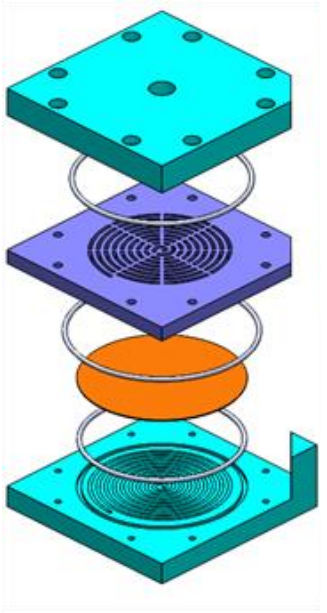


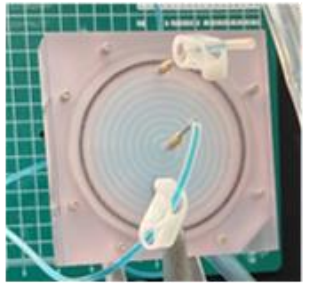






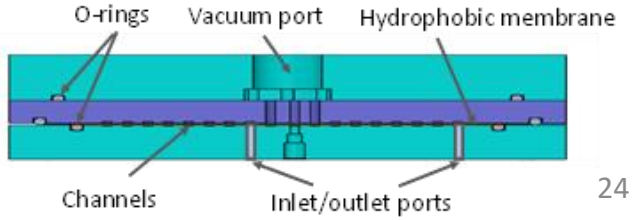






(Top)

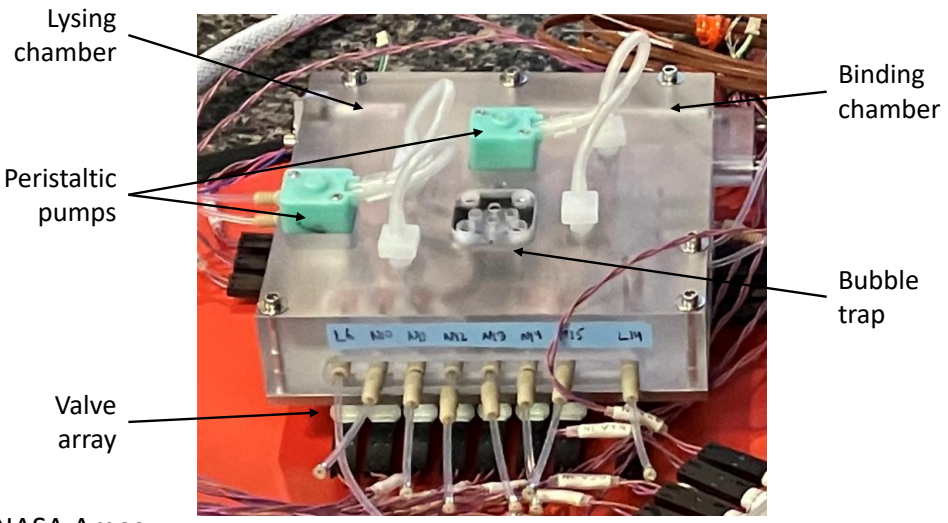
(Side)



Figures courtesy of NASA Ames

Spaceflight Autonomous Multigenerational Microbial Sequencer (SAMMS)

- Polaris Project – early career led by Aubrie O'Rourke (KSC)
- Automated, CubeSat payload to sequence microbial genomes over time to look for changes in different space environments
- ChIPPS manifold to extract DNA
  - Manifold design modified to accommodate requirements from SAMMS and ChIPPS
- Sequencing performed using Oxford Nanopore MinION



ChIPPS image courtesy of NASA Ames

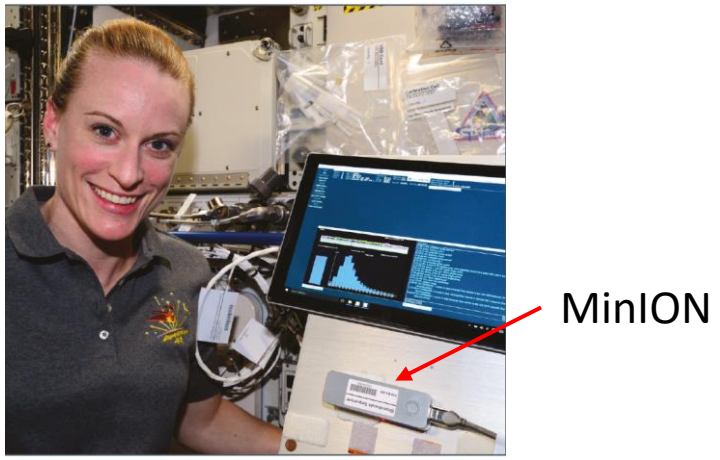
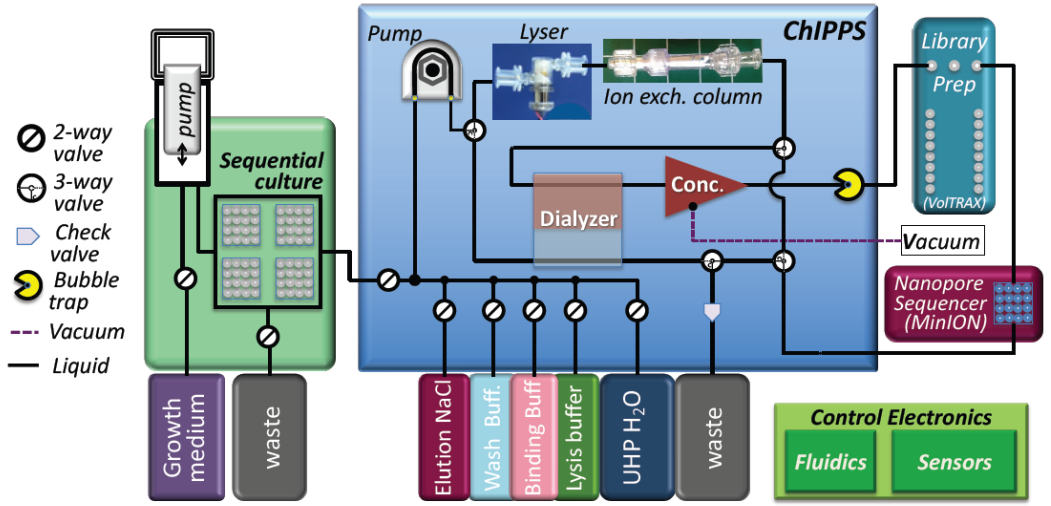
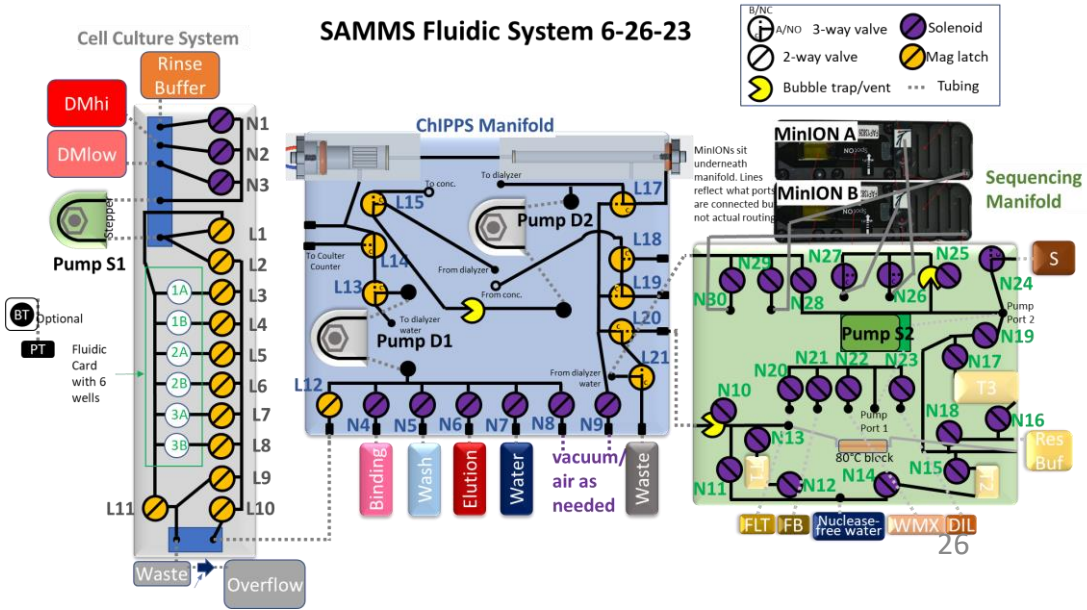


Fig. 1 Astronaut Kate Rubins on the ISS
Image from Oxford Nanopore

Evolution of the SAMMS Fluidic Diagram

- Requirements defined
- Initial layout
- Iterations, concept of operations (Conops) development
- CAD starts
  - Test blocks
- Conops vs. CAD checks
- Manifold fabrication
  - Assembly and leak testing
  - Dye runs to visualize steps
  - Finalize conops and scripting

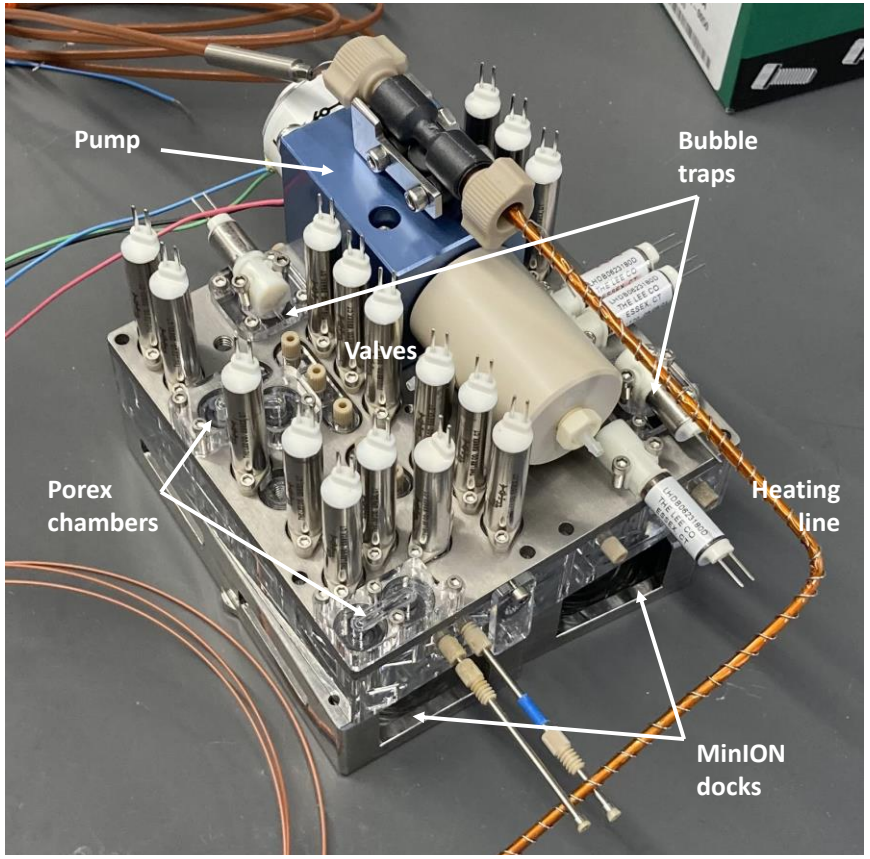




Figures courtesy of NASA Ames

Library Prep for MinION/Sequencing Manifold

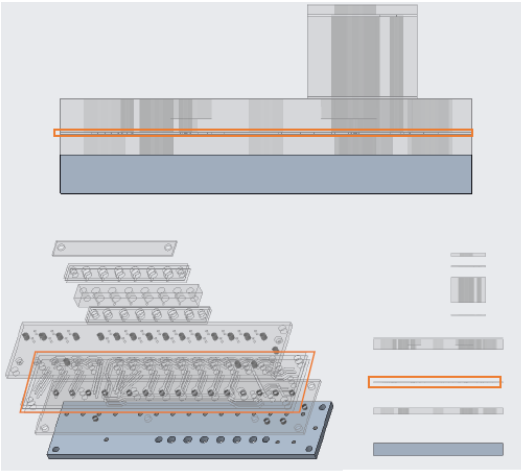
- Voltrax ruled out
- Field sequencing kit
  - Lyophilized reagents, fewer heating steps
- Sequencing manifold
  - Store lyophilized reagents in Porex
  - Interface to 2x MinION flow cells
    - Bubble-free connection
  - Deliver buffers to MinION
  - Mix ~10 µL sample with reagents, heat to 80C
  - Deliver prepared sample, sequence
  - Wash and reuse MinION



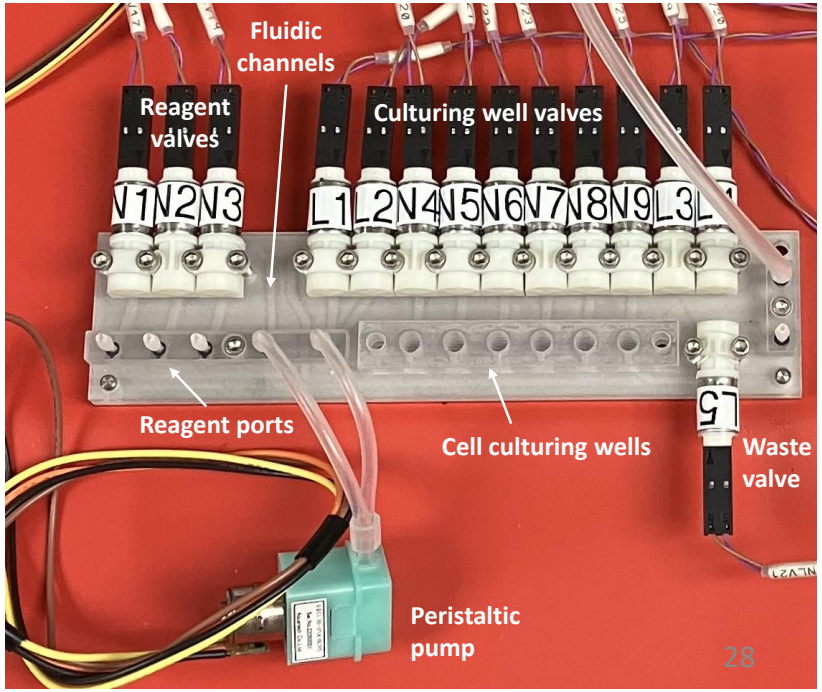
Figures courtesy of NASA Ames

SAMMS Cell Culture System

- Maintain cell growth and density for several days before sampling higher OD growth and sequencing
- Card and manifold assembled and sterilized separately
  - Load cells in card wells, attach card to manifold
  - LED/detector system to measure OD



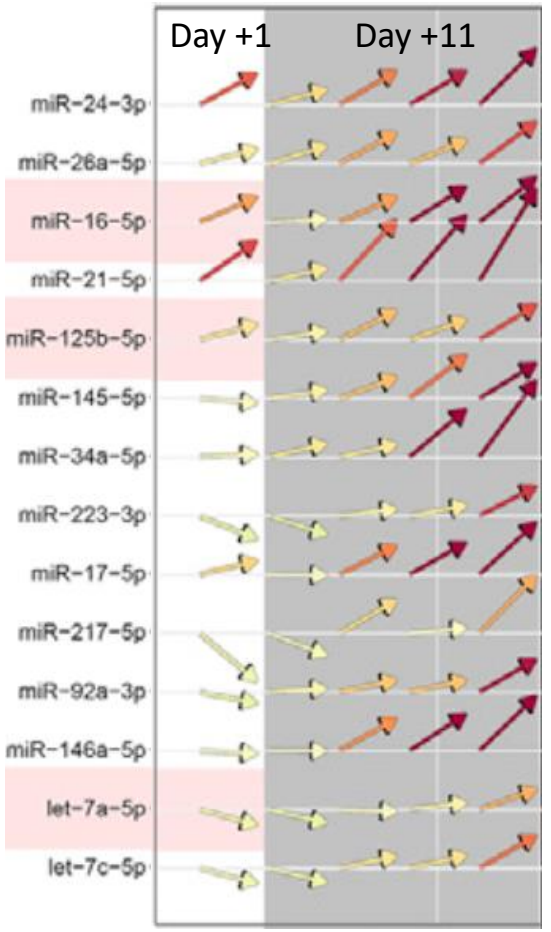
PowerCell LED/detector boards



Figures courtesy of NASA Ames

Radiation Assessment DuRing Exposure And long Duration Spaceflight (RADREADS)

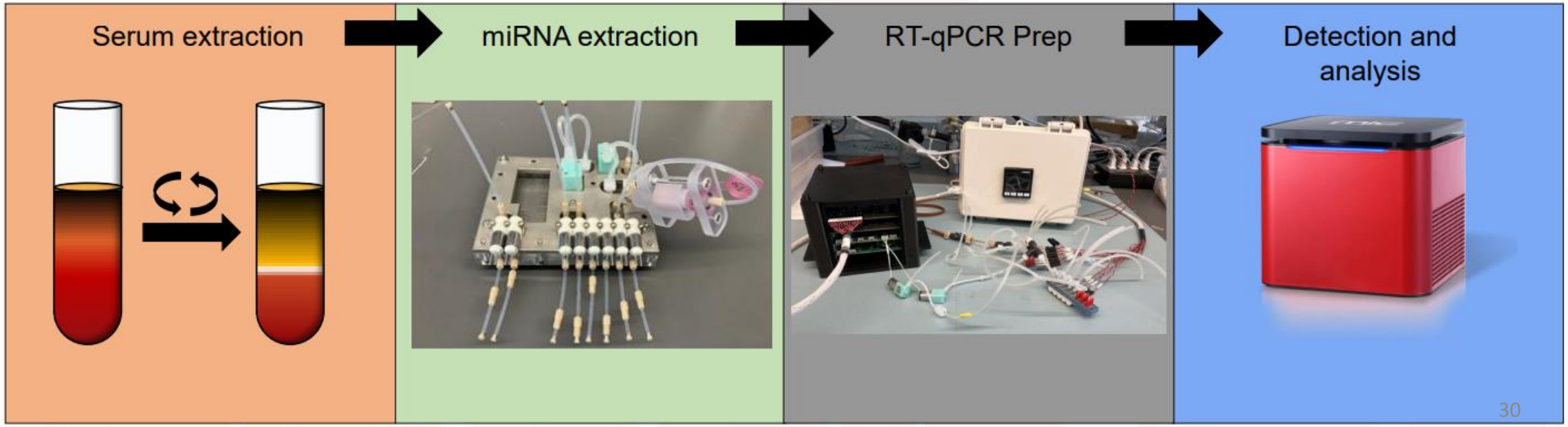
- Polaris Project – early career led by Yasaman Shirazi and Victor Yeh
- Automated extraction and preparation for quantification of miRNA biomarkers
- Deep space travel -> more radiation exposure
  - Exposure can manifest in variety of symptoms
  - Physical dosimetry techniques only measure radiation dose
  - Want to understand individual response to personalize treatment



Malkani 2021 Fig 7D

RADREADS Process Flow

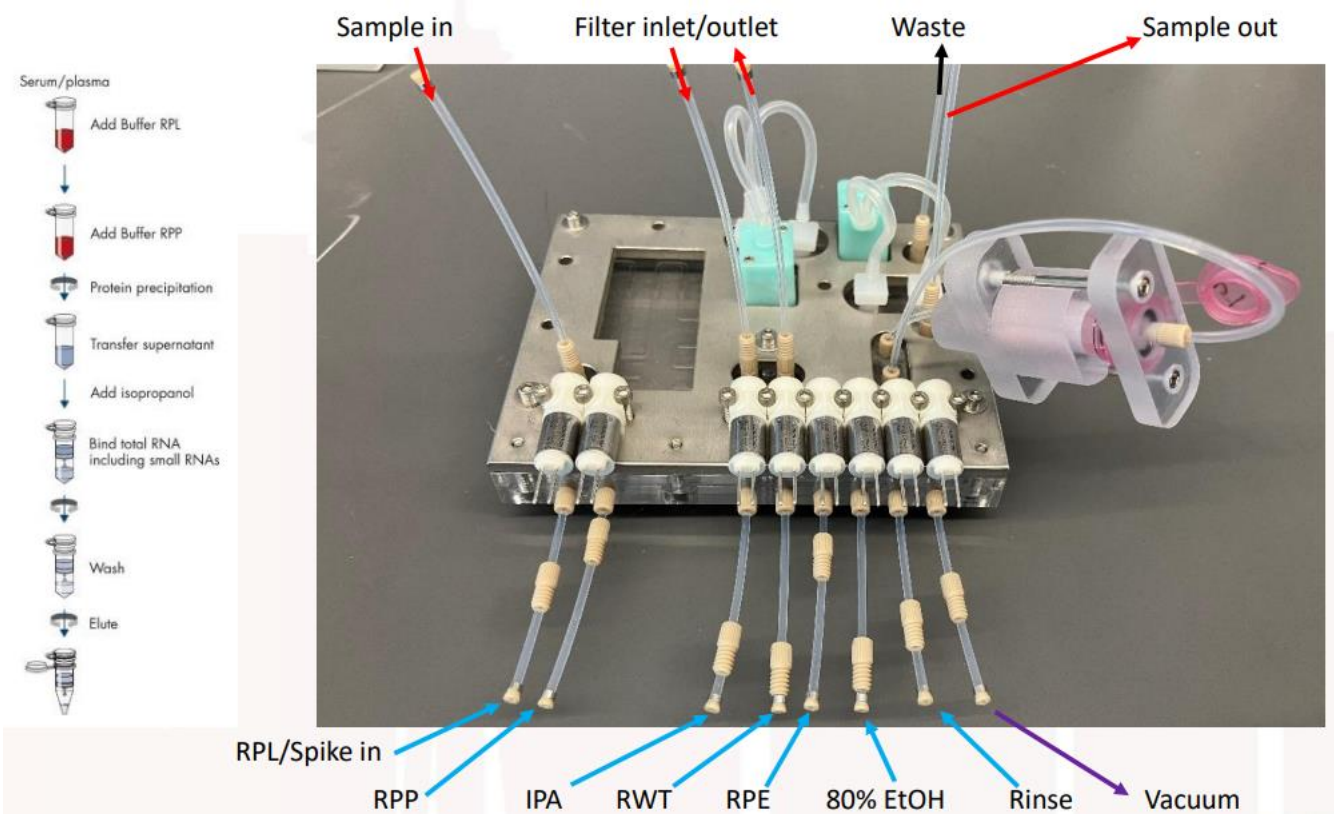
- Separated development of each functional block
- MicQ PCR machine
  - Uses rotor to spin, should avoid bubble issues
  - Works upside down and on its side



Figures courtesy of NASA Ames

RADREADS miRNA Extraction Block

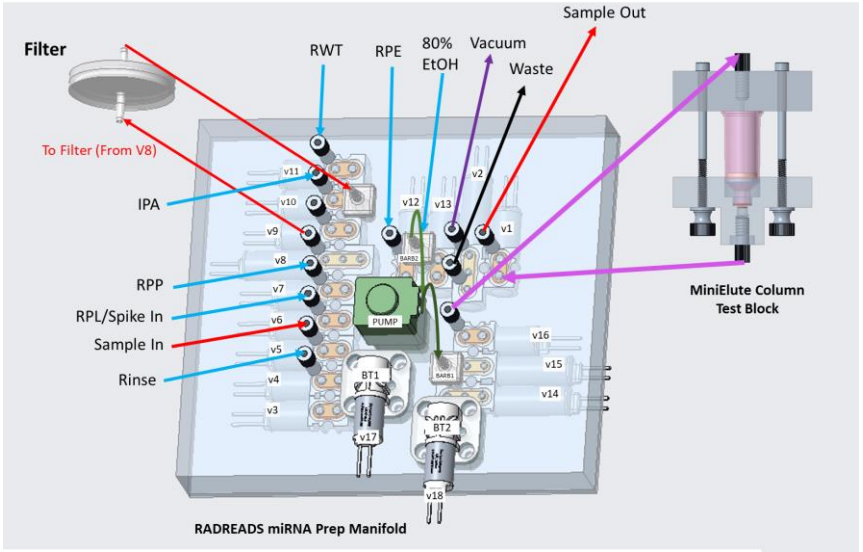
- Not a fused PC manifold!
  - 3 PC layers, 1 layer of PSA
- miRNeasy Advanced kit
  - Mix sample and reagents, filter out precipitated protein
  - Mix with IPA and deliver to COTS MinElute column
    - 3D printed holder/interface
  - Series of washes, vacuum dry, elute in water
- Adapting for Taqman ABC magnetic bead protocols adapted

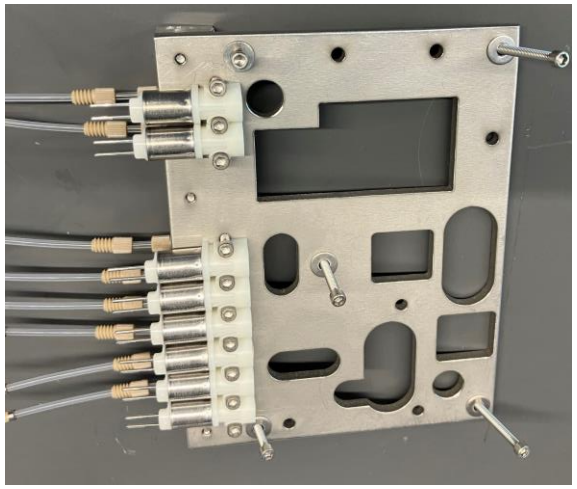


Figures courtesy of NASA Ames

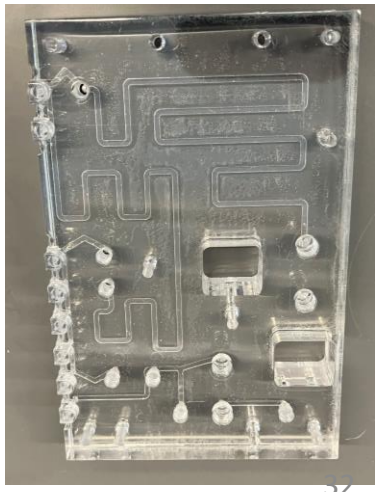
Semi-consumable design of extraction block

- How do we clean between samples?
- Split manifold in 2 between valve ports
  - Reagents only in valves
  - Sample remains in channels, never in mixing pump or valves
- Demonstrated repeatable sealing to valve interface





Stays with valves

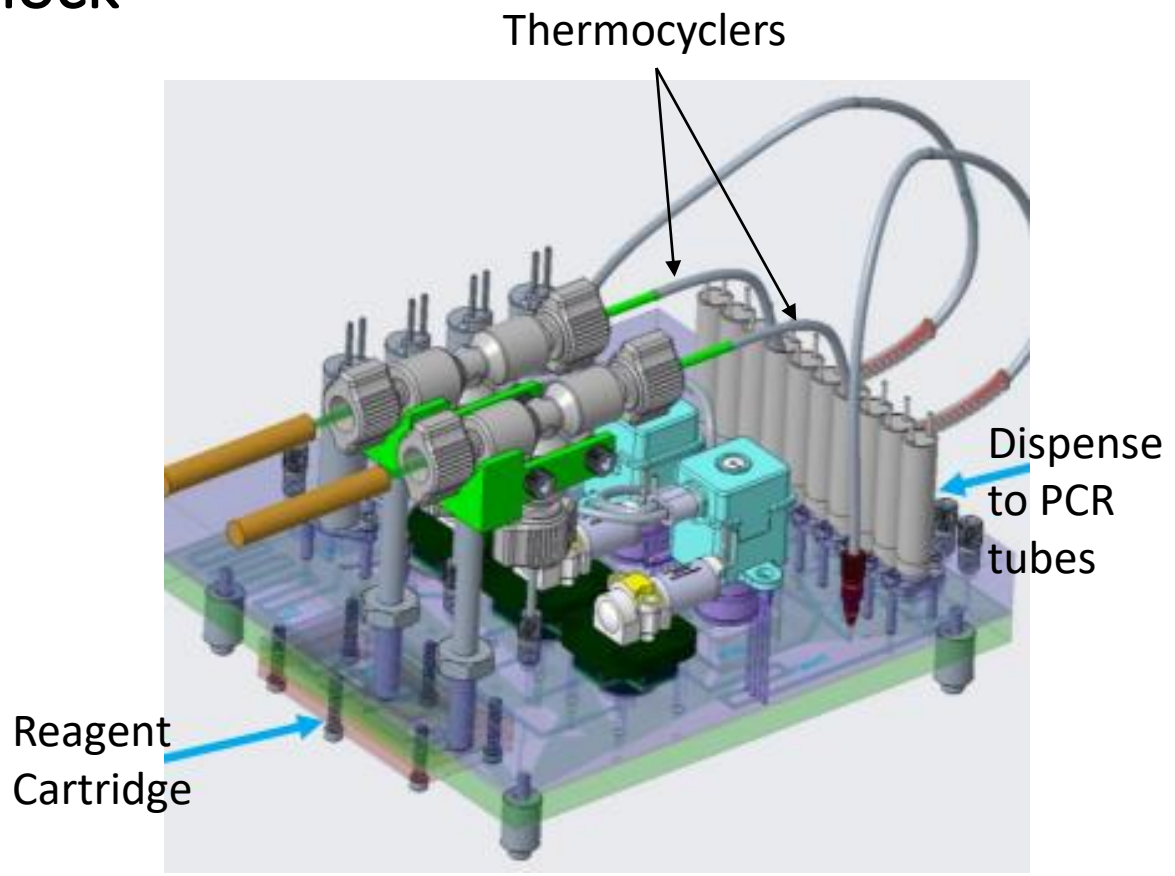


Removed and replaced

Images courtesy of NASA Ames

PCR Prep Block

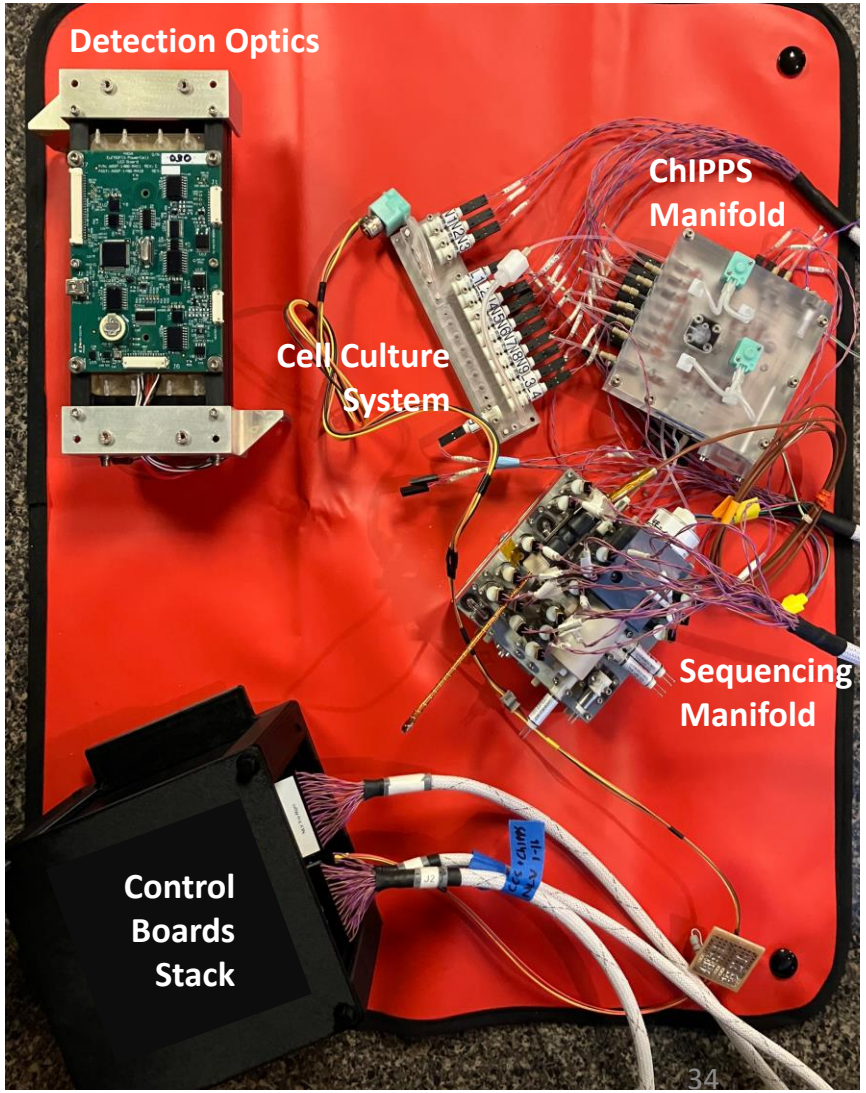
- Reverse transcriptase (RNA -> cDNA) step required before delivering to PCR tubes with dried reagents
  - Reagents stored separately in cartridge in freezer
  - Mix with sample, split solution to mix with 2 sets of primers
  - Heaters (adapted from SAMMS) to perform thermocycling
  - Interface with PCR tubes with dried PCR reagents on outlet to deliver 10-20 µL



Figures courtesy of NASA Ames/ Tori Chinn

ChIPPS, SAMMS, and RADREADS Status

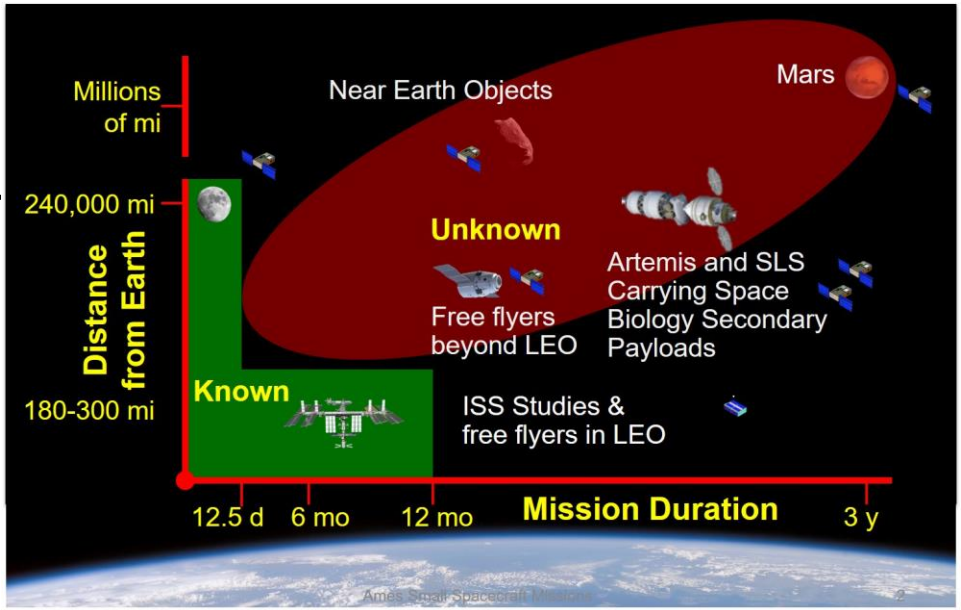
- ChIPPS
  - Follow-on proposal FISHNChIPPS to be submitted any day now
- SAMMS
  - Refining/optimizing steps for each subsystem
  - Preparing to grow *E. coli* in CCS w/ optical feedback
  - Working towards integration of all three subsystems, RPM testing
- RADREADS
  - Refining/optimizing processing steps
  - qPCR prep block CAD finalizing
  - Integration of Extraction -> PCR Prep -> PCR

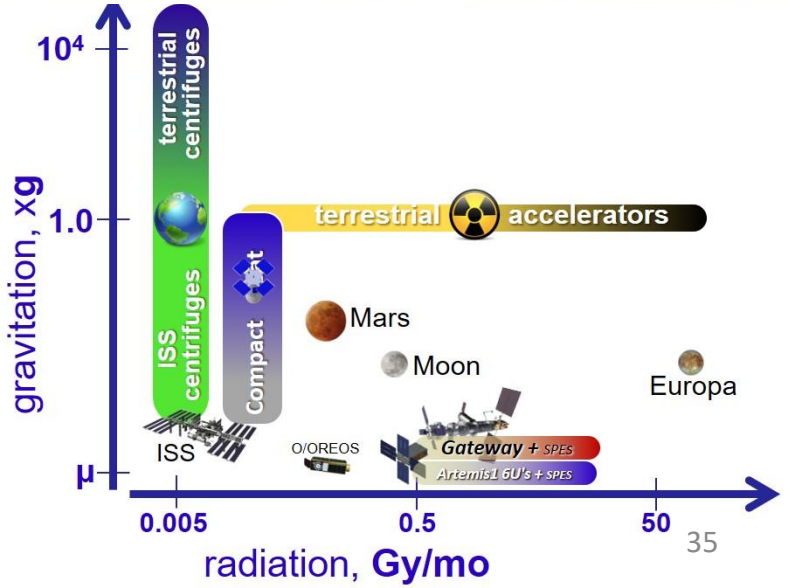


Figures courtesy of NASA Ames

What's Next? Bio-Mapping the Solar System

- Fluidic technologies developed at Ames can be used for array of biology and/or life-detection instruments sent to various space environments
  - Impact of environment on terrestrial microbes
  - Where life does/could exist in solar system
- Needed to refine safety mitigations/ counter-measures for astronaut health
- Need *in situ* data for combinatorial effects of:
  - Deep space radiation
  - Long-term exposure to reduced gravity





Figures courtesy of NASA Ames

Acknowledgements

- Thanks for the figures

Travis Boone, Tori Chinn, Chris Espinoza, Tristen Head, Macarena Parra, Tony Ricco

- Code RET

Macarena Parra, Walter Alvarado, Tori Chinn, Chris Espinoza, Tristen Head, Jessica Koehne, Julie Schoenfeld, Kanch Sridhar, Victor Yeah

- SAMMS

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- RADREADS

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- SPLIce/COLDTech/ICEE2

Tony Ricco (PI), Richard Quinn (PI)

- BioSentinel

Matt Napoli (PM), Sergio Santa Maria (Proj Scientist)

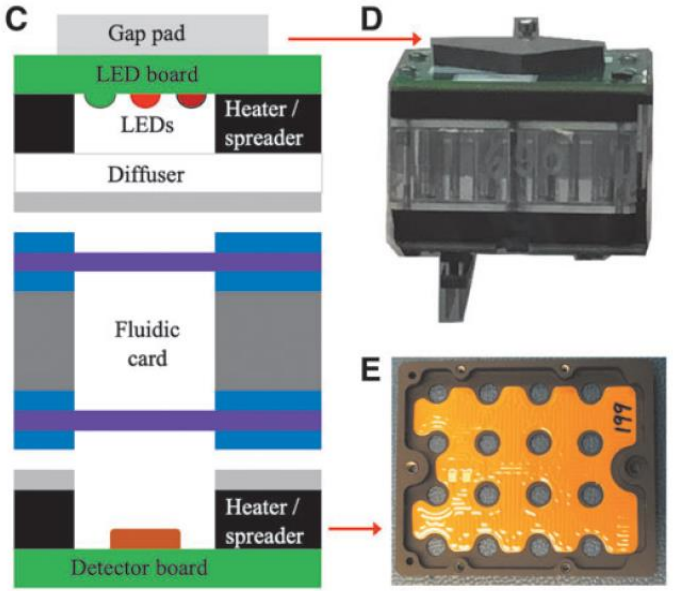
- LEIA

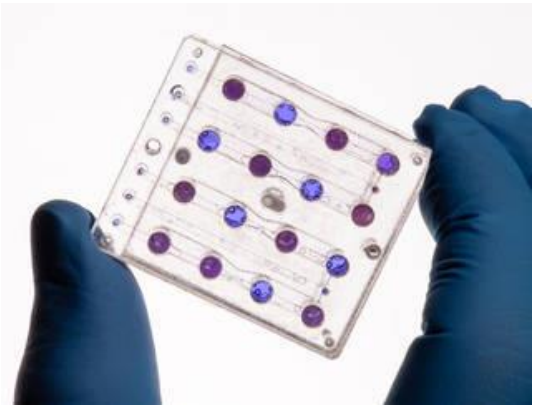
Dave Pletcher (PM), Mark Settles (PI)

Backup

BioSentinel Experiment

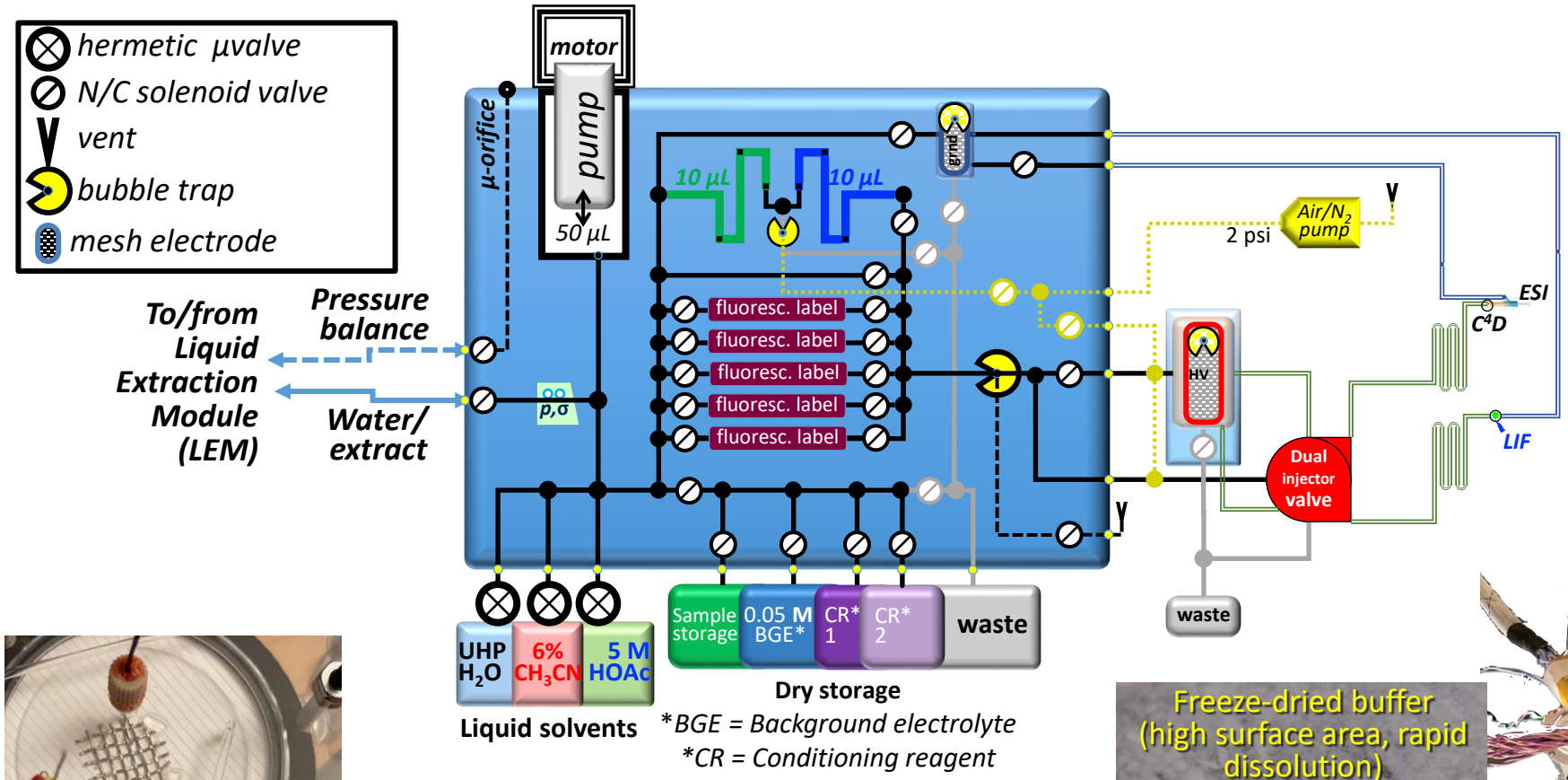
- Wild type and radiation-sensitive mutant strain of yeast loaded onto sidewalls of card wells
  - 570/630/850 nm LEDs, light intensity detector
- Cards filled with growth medium and alamarBlue
  - Metabolism causes aB to go blue -> pink -> clear
  - IR used to monitor optical density of cells
- 2 cards activated at the same time (32 wells/time point), 9 time points

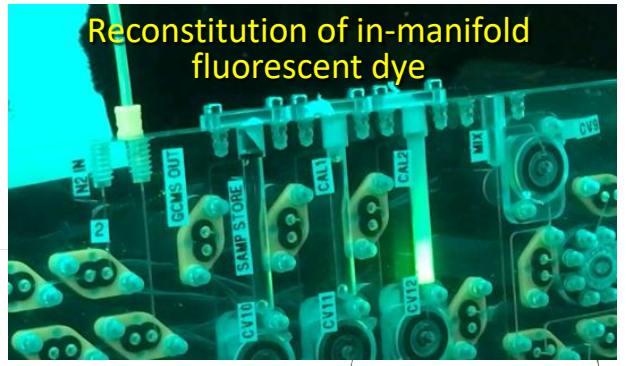




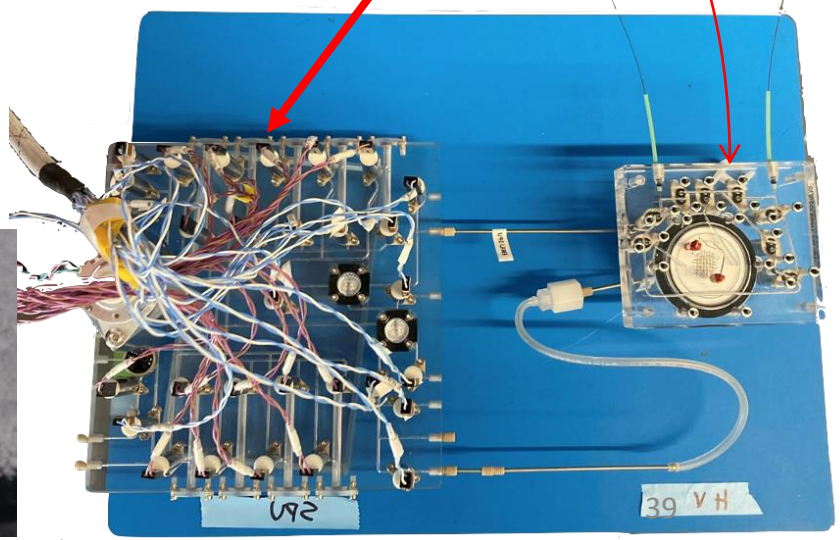
Images courtesy of NASA Ames

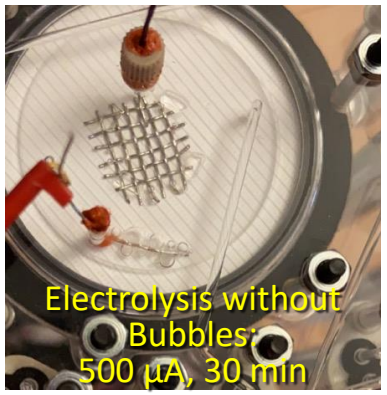
EMILI: Europan Molecular Indicators of Life Investigation



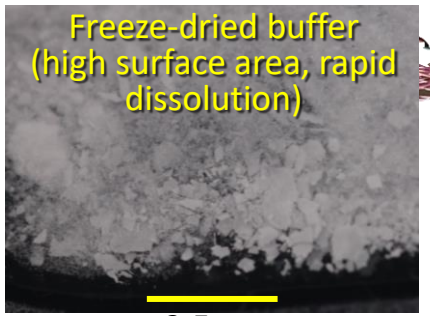


ARC HV test manifold interfaced to ARC SPU manifold





In partnership with GSFC and JPL



Figures courtesy of SPLIce/COLDTech/ICEE2 team at NASA Ames