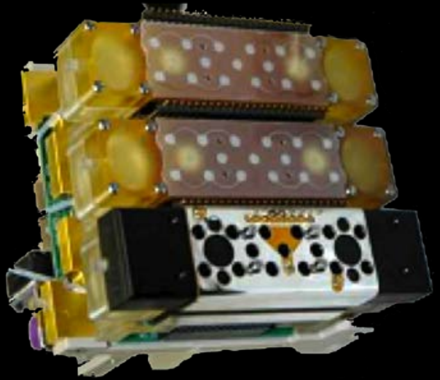
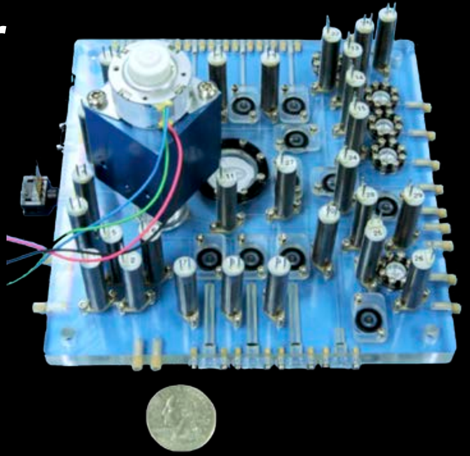
Development of Life-Detection Technologies & Instruments at NASA-Ames Research Center



Tony Ricco

Richard Quinn, Mary Beth Wilhelm and Jessica Koehne

NASA Ames Research Center



with special thanks to

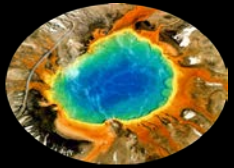
ARC's *Lifemarker Exploration Instruments & Analysis* (LEIA) and *Small Payloads* Teams

and the astrobiologists at ARC + everywhere who

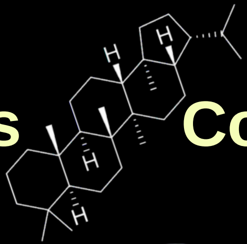
Define the Science that Defines the Technology

Searching for Indicators of Life

Basic Understanding:

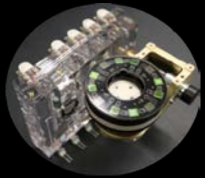
Life's origins  Analog research

Search Strategy:



Diverse targets Complementary measurements

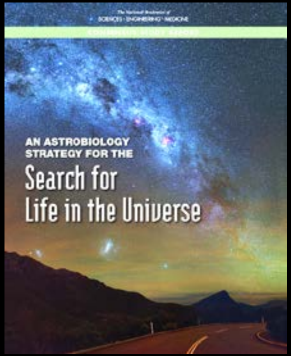
Tech Development:

Process samples  Develop / enable instruments

Plan Missions:

Choose destination  Develop concept

The Center for Life Detection



2019 NASEM Astrobiology Strategy Report:

"NASA should support the community in developing a comprehensive framework for assessment of in situ and remote biosignatures"

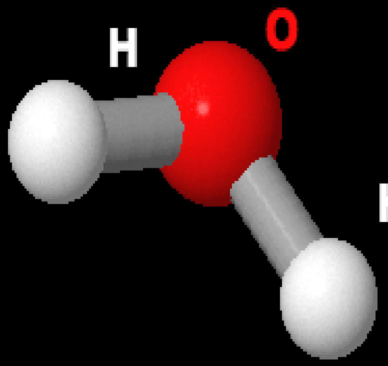
The **Center for Life Detection**, a collaboration among scientists and technologists from **Ames & Goddard**, is supported by the astrobiology program to catalyze this process:

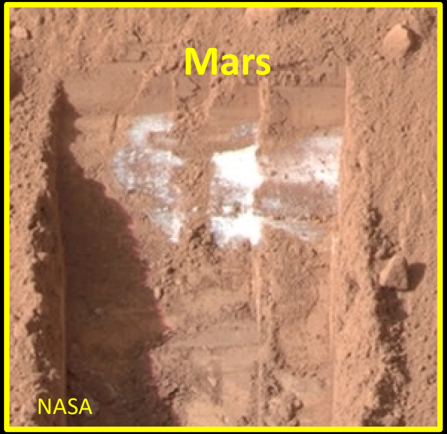
- **Develop the conceptual basis for an evaluative framework** by facilitating community-level activities (e.g., workshops).

- From this basis, **develop** a web-based platform, **"The Life Detection Forum," to centralize and capture the exchange of knowledge, ideas, and dialog** as life detection science & technology evolve.

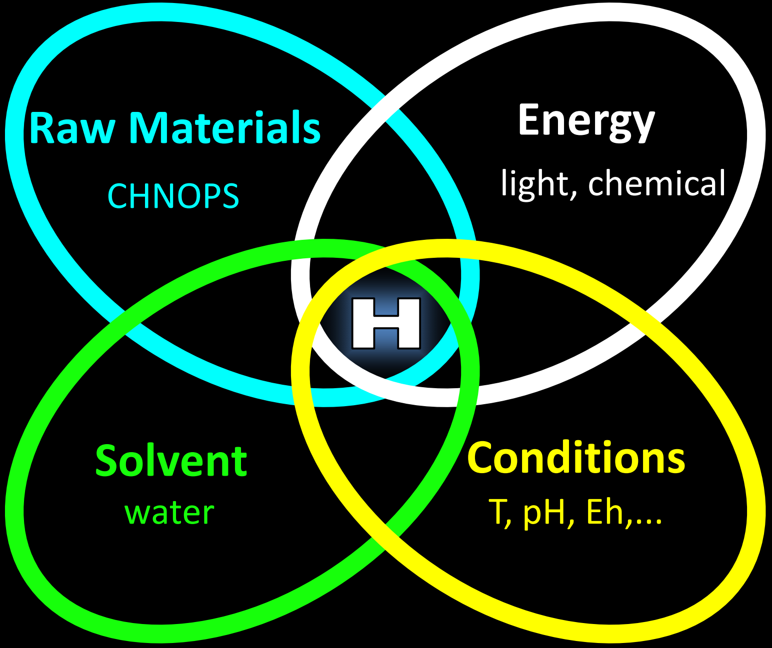
  - The LDF is envisioned as a **resource for science definition, program and mission planning, and identification of priorities** for research and technology development to support the life detection endeavor.

Life-Search Primary Exploration Targets



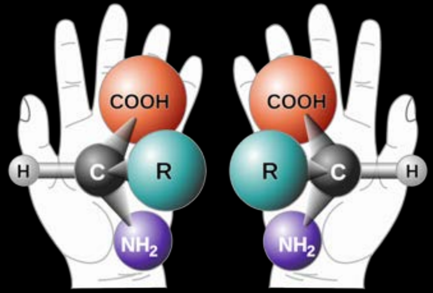


Habitability: *Indispensable Context for Life Search*

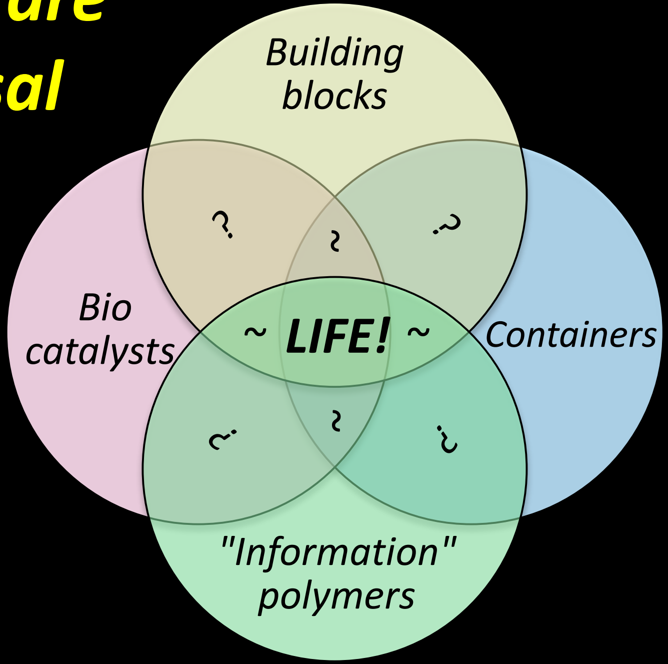


Past vs. Present: *Signature may differ*

H = Habitable conditions



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

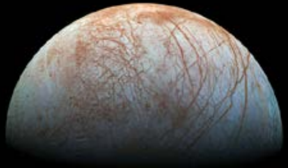


- Versatile chemical building blocks
- Complex multimeric biomolecules
- Containment structures
- Function-specific molecules

Arguably, all are required for life

In combination, these indicators could provide ***conclusive evidence of life***

Translating basic research and analog fieldwork into *requirements-driven, mission-enabling development*

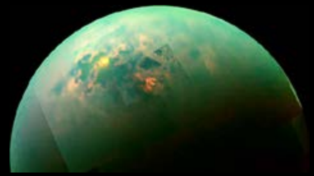


Tech. focus: Automated *Liquid Processing Systems* integrated/interfaced with:

- Chemical/Environmental Sensors; Microanalytical Systems
- Microscopy; Optical & Vibrational Spectroscopy
- Separations Methods (capillary electrophoresis; gas & liquid chromatography)
- Mass Spectroscopy (often with front-end separations)
- ➢ Including analytical **instruments developed by partners**, e.g. GSFC & JPL

Technology Lineage and Heritage:

- SmallSat Space Biology and Astrobiology Missions
- Mars Exploration



Current and Emerging Applications:

- Astrobiology Life-Search Missions
- Gateway Science Payloads and Environmental Monitoring
- Robotic and Human Lunar Exploration

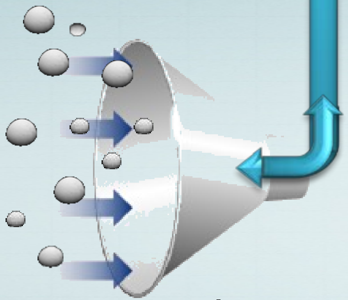
Targets, Rationale, Instruments for Life Search

Measurement Target	Observed Parameter	Life Detection Rationale *examples*	Analytical Approach
Molecular building blocks	Chirality	**Enantiomeric excess** enables biochemistry *amino acids, saccharides*	*Capillary Electrophoresis* *Mass Spec*
Functional molecules	Catalysis	**Biochemical processes; electron transfer** *kinases; quinones*	*Electrochemical BioSensors* *Mass Spec*
Biogenic organic polymers	'Structural' polymers	**Containers, energy, biochemistry** *lipids*	*Mass Spec* *Capillary Electrophoresis*
	'Information' polymers	**Information storage and transfer** *poly nucleic acids*	*Sequencing & Sizing* *Mass Spec*
Containers	Morphology	**Containers, structures, barriers** *cells, membranes*	*Fluorescence Microscopy with staining*
Ions, Energy molecules	Concentration, redox potential	**Habitability incl. energy** *specific ions, redox species*	*Ion-Specific Electrodes* *Cyclic Voltammetry*

Fluidically Enabled Instrument Suite

Fluidics Processor

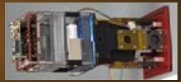
- Deliver extraction solution
- Retrieve sample with particles
- Separate particles
- Add dyes
- Degas / de-bubble
- Adjust ionic strength
- Remove interfering ions
- Adjust pH or solvent polarity
- Adjust solvent polarity
- Concentrate samples
- Store & reconstitute reagents
- Provide calibration standards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



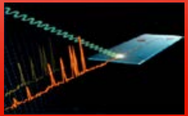
Sample Input

Instrument Suite Candidates

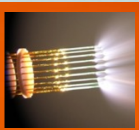
Fluorescence microscopy



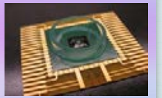
Microchip capillary electrophoresis with laser-induced fluorescence



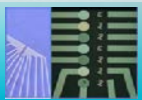
Mass spectrometry w/ electrospray, GC, or (MA)LDI "front end"



Electrochemical biosensors

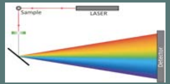


Ion-selective electrodes [Habitability, energy]



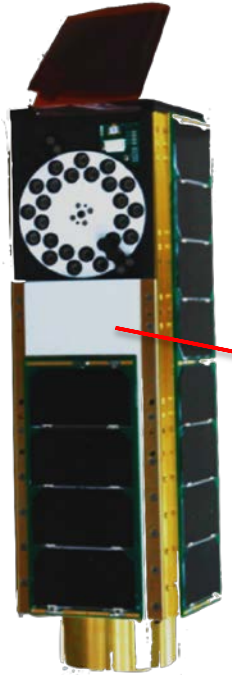
Ion chromatography [Habitability]

Vibrational spectroscopy



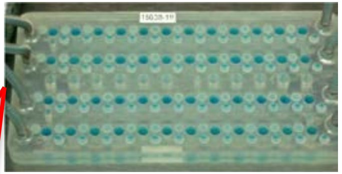
Ames Research Center

ARC's Nanosatellite (Astro)Biological Missions: Enabling Technologies for Life Detection

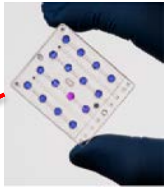


O/OREOS

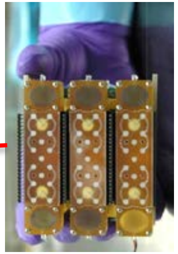
E. Coli *GeneSat-1* (**2006**/3U): ***gene expression***
EcAMSat (**2017**/6U): ***antibiotic resistance***

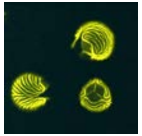


S. Cerevisiae *PharmaSat* (**2009**/3U): ***drug dose response***
BioSentinel (**2021**/6U): ***DNA break/repair***



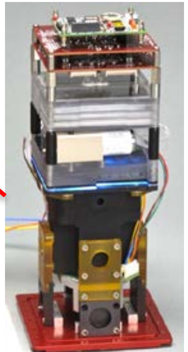
B. Subtilis *O/OREOS** (**2010**/3U): ***survival, metabolism***
*ADRoIT-M*** (6U): ***mutations / lithopanspermia***

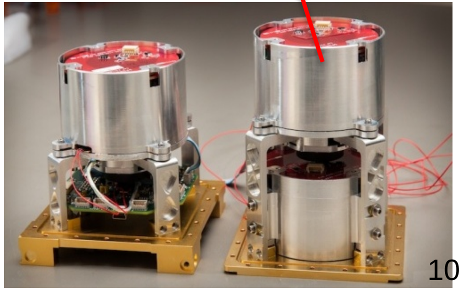




Ceratopteris Richardii *SporeSat-1* (**2014**/3U): **ion channel sensors, µ-centrifuges**
SporeSat-2 (3U): ***plant gravity sensing threshold***

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

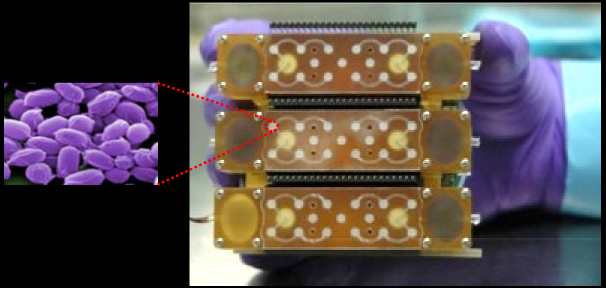


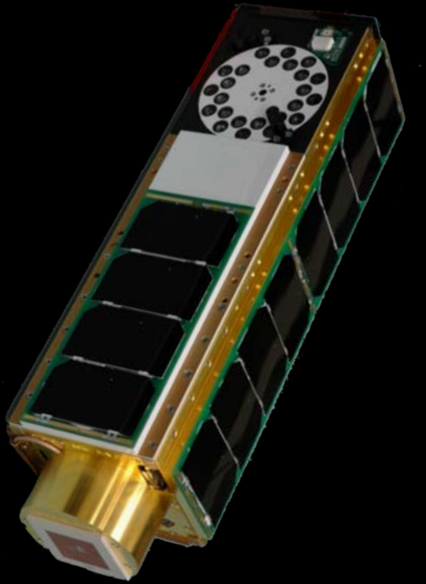


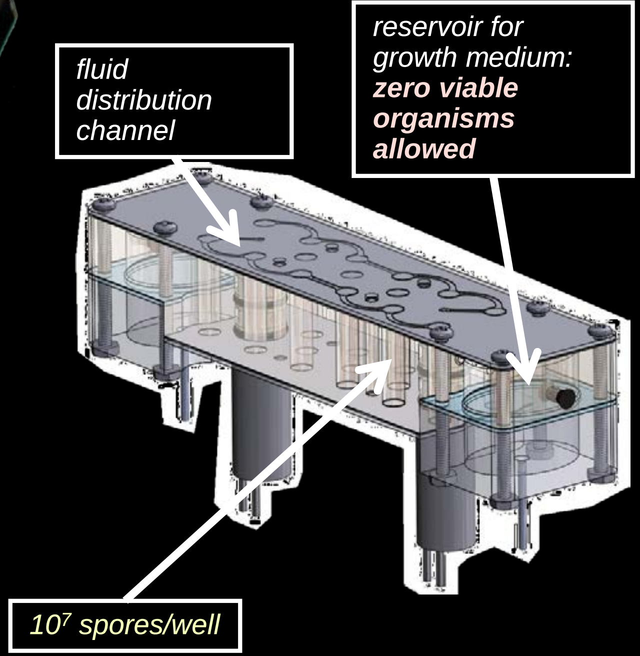
**Organism/Organic Exposure to Orbital Stresses*
***Active DNA Repair on Interplanetary Transport of Microbes*

Astro/Biological Space Missions: providing Enabling Technologies

O/OREOS







<u>Relevant payload technologies</u>

- Handling biological specimens in space
- Payload: perfect sterility (flight-proven)
- Ultra-low organic contamination
- Biocompatible materials
- Fly dry, then wet-out fluidics in reduced gravity
- Manipulate µL volumes
- Functional in high-radiation environments

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

Microfluidic technology development for Enceladus & Europa life search

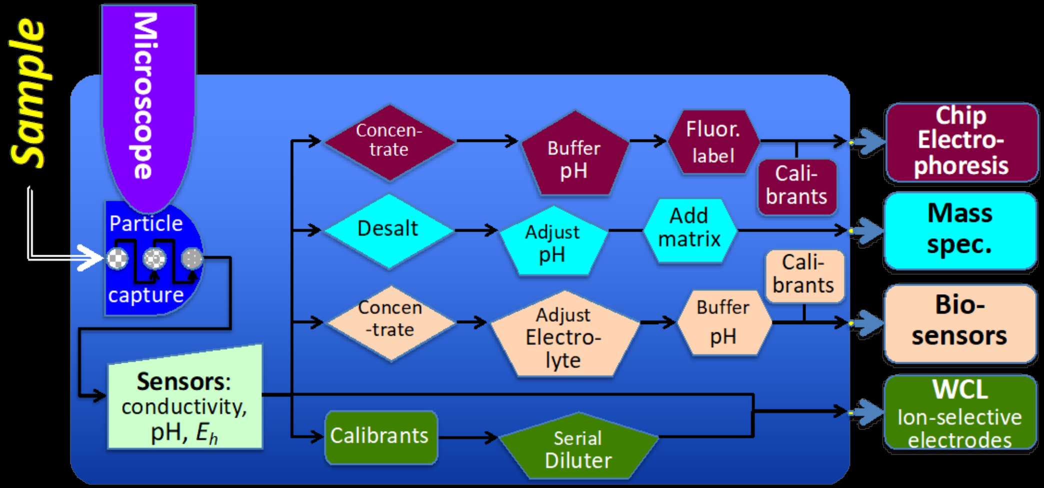
Multifunctional sample processing hub for science payload integration

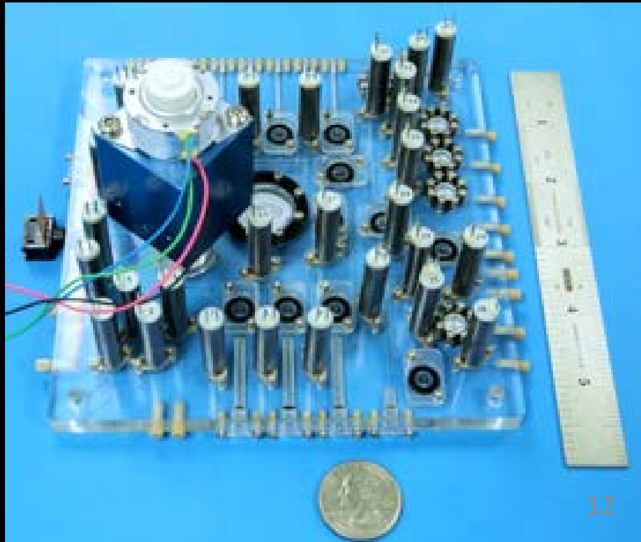
Lead: **ARC** Partners: **APL, GSFC, JPL, MIT, Tufts, MIT** Funding: **COLDTech**

PI/Co-I's: A Ricco, R Quinn; S Getty, W Brinckerhoff; P Willis, A Noell; E Adams, R Gold; S Kounaves; M Hecht

Infusion: **Three ICEE-2's → Europa Lander; Enceladus fly-by & orbi-lander** mission concepts

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



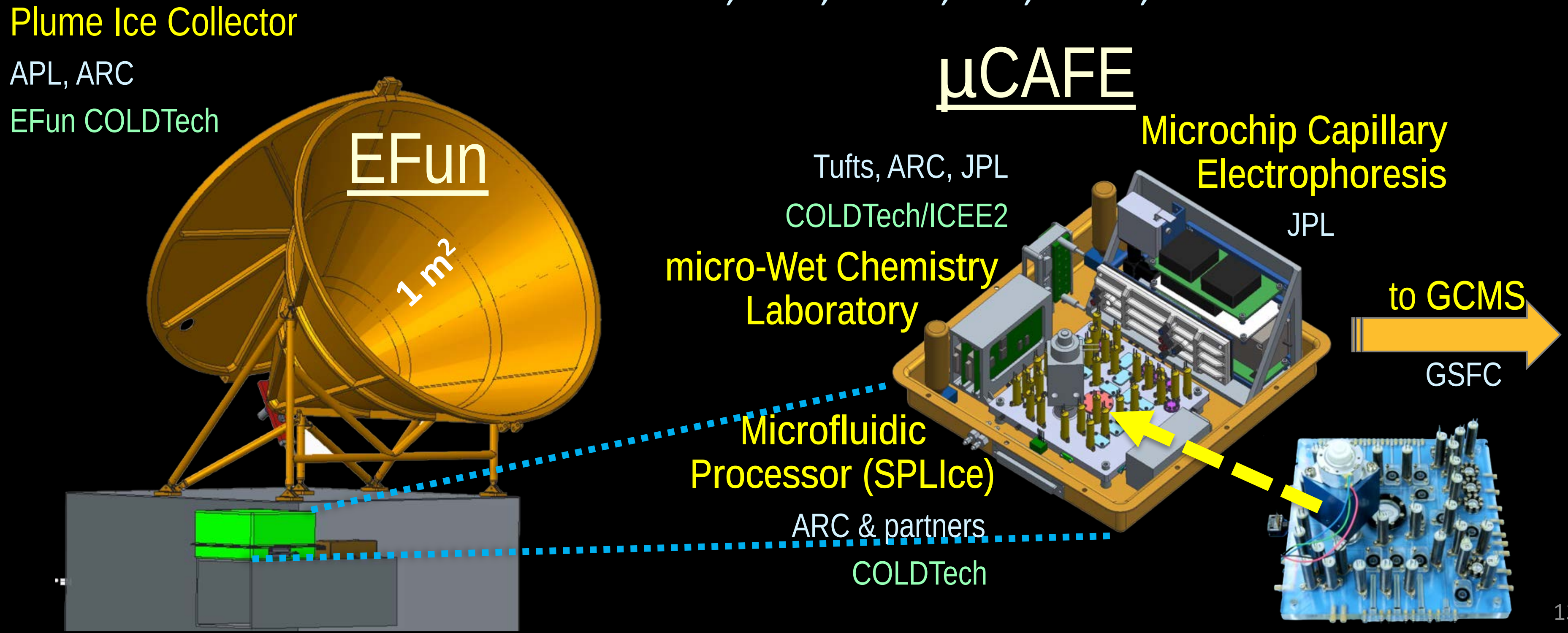


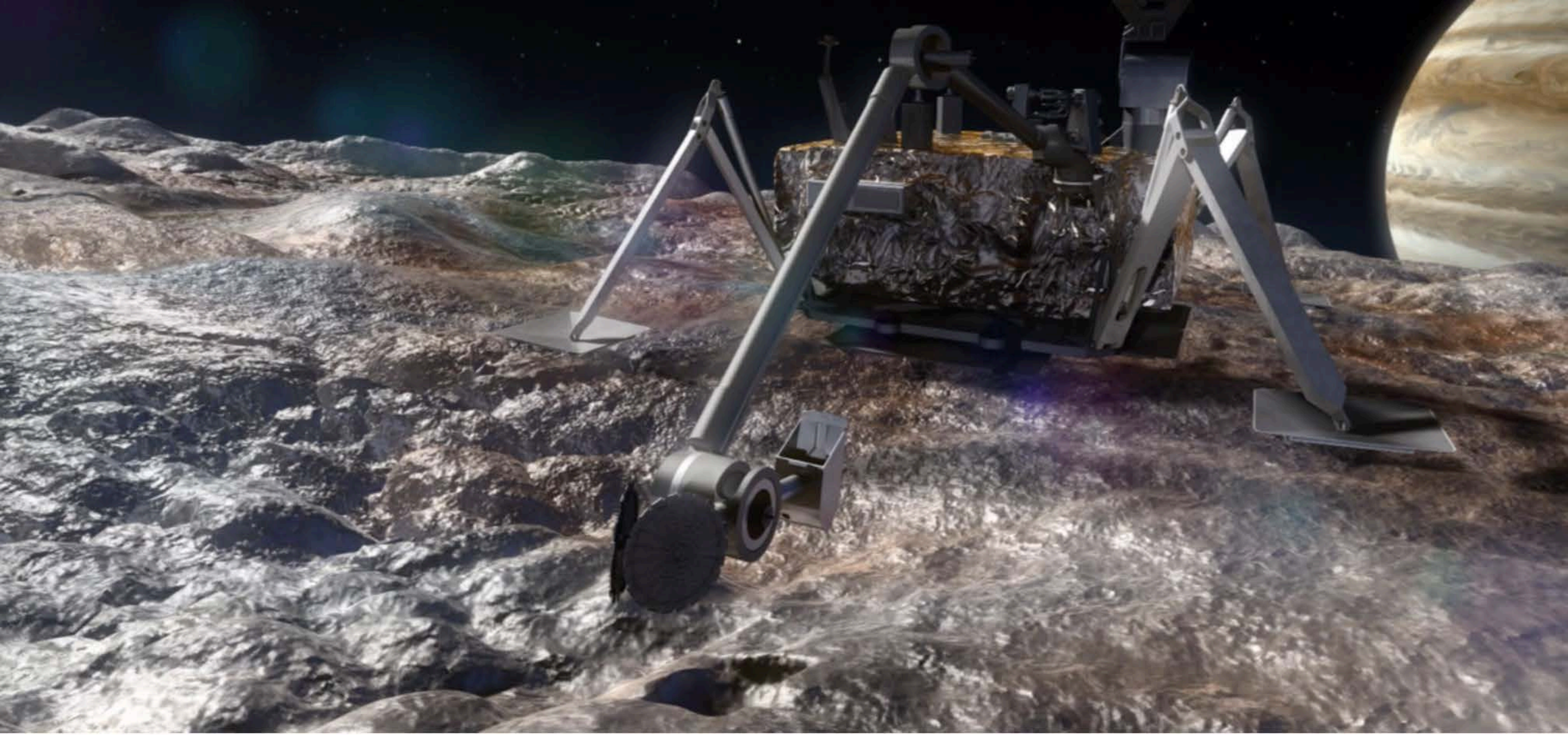
μCAFE: microChemical Analyzer of Fluids for Exobiology

Microfluidic system of *ELSAH*: Enceladus Life Signatures and Habitability

***NF4* submission;** C.P. McKay, PI (Cat. 2, selected for tech. dev.)

Partners: ARC, APL, GSFC, JPL, Tufts, MIT





Europa/Ocean Worlds Lander Mission Concept

Europa Lander Pre-Project Science and Engineering teams May 14th, 2020

Jet Propulsion Laboratory
California Institute of Technology

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

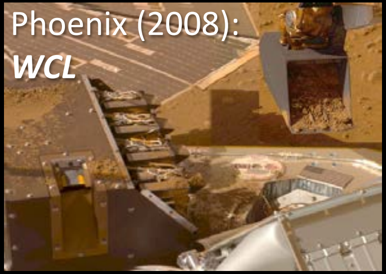
Fluidically Integrated Habitability Assessment for Icy Worlds

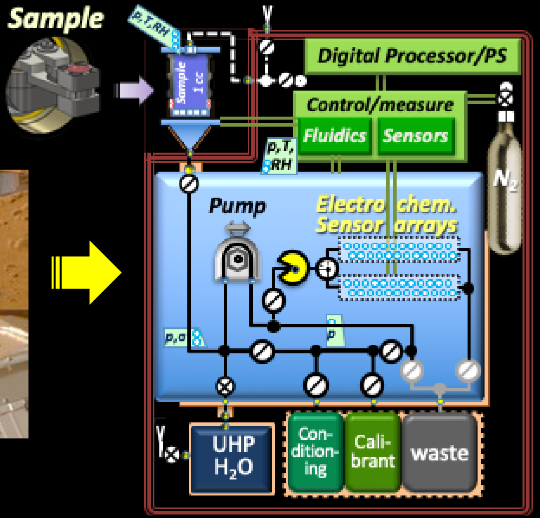
Heritage: Phoenix Wet Chemistry Lab (WCL) + COLDTech, ICEE-2 projects

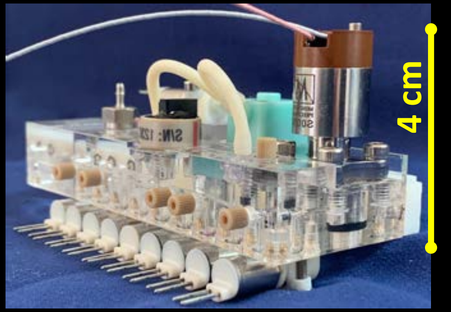
Partners**: ARC, JPL, Tufts, MIT, U. of Alberta, Honeybee Robotics**

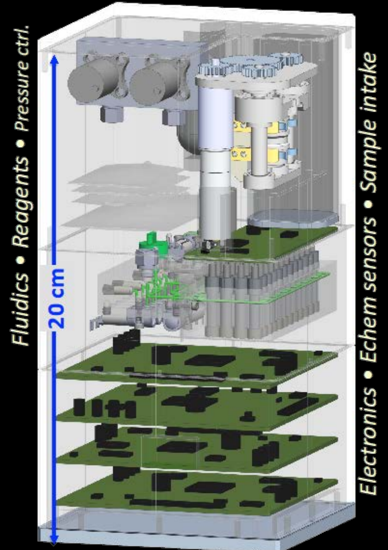
Key Measurements: pH, conductivity, ROS, gases, ions: Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, CO_3^{2-}, NH_4^+, NO_3^-, ClO_4^-, ClO_3^- PO_4^{3-}, SO_4^{2-}, SO_3^{2-} ; O_2, SO_2, O_2^- , H_2O_2; solution energetics: discrete & average pot'ls. of redox-active species (E_h)

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





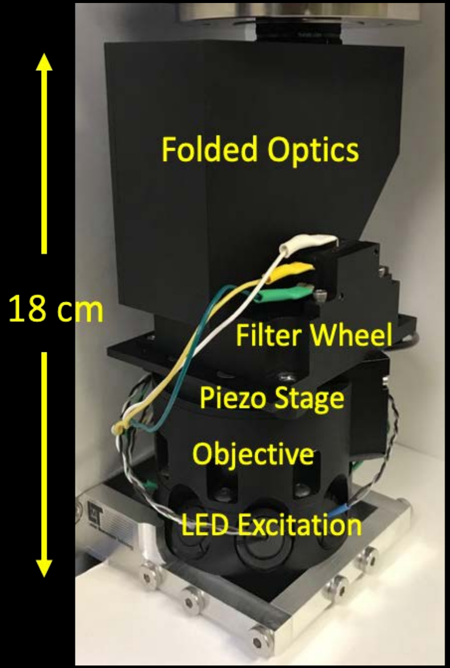




Ames Research Center Microscopes for Life Detection

LIfE: Luminescence Imager for Exploration (NASA COLDTech Program)
ELM: Europa Luminescence Microscope (NASA ICEE-2 Program)

PI/Co-I's: R.C. Quinn[1]; A.J. Ricco[1], N. Bramall[2], J. Forgione[1], L. Timucin[1], K. Zacny[3]



2U LIfE Microscope (20x10x10cm)

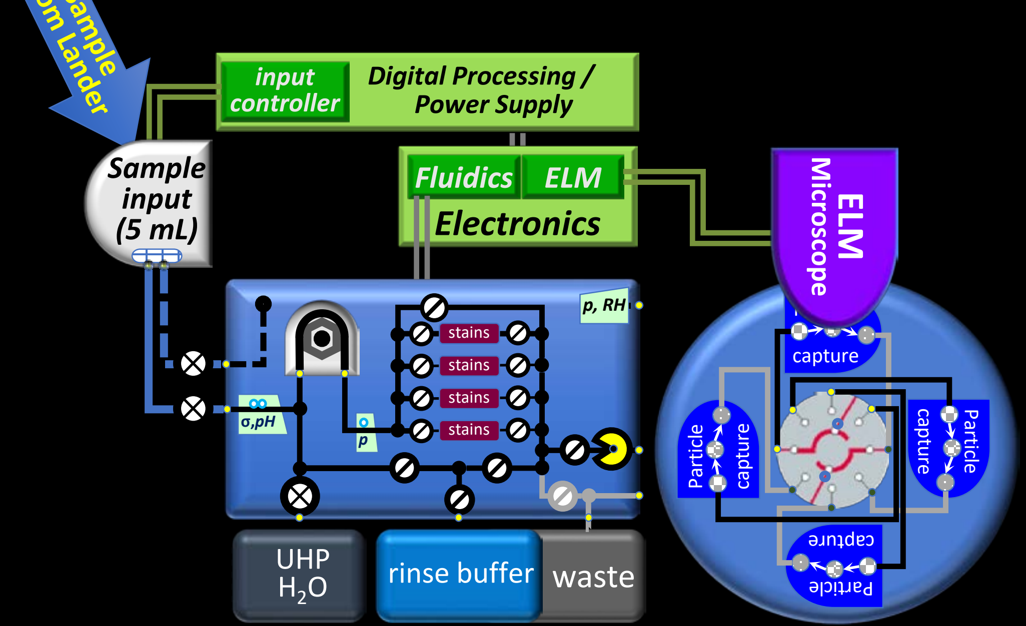
- Visible light imaging of organic and inorganic structures with submicron resolution.
- Sample is autonomously manipulated to chemically stain key molecular and structural indicators of microbial life (proteins, lipids, nucleic acids) for fluorescence microscopic detection.

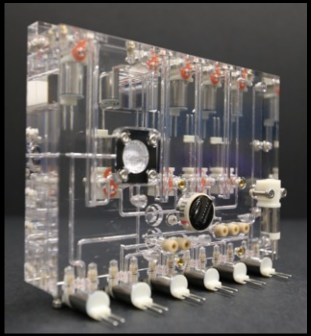
1U Sample Processor (10x10x10 cm)

- Uses DUV and visible excitation of native luminescence; mineralogical and biological.
- In-line filter sets capture successively smaller particle sizes on 10, 1.0, and 0.1 µm pore-size filters for sample imaging.

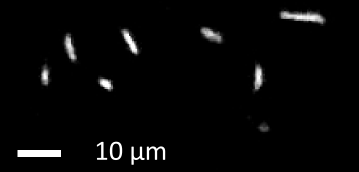
[1]NASA ARC, [2]Leiden Measurement Technology, [3]Honeybee Robotics

ELM Block Diagram and Prototype

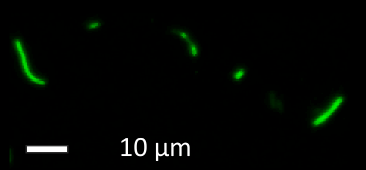




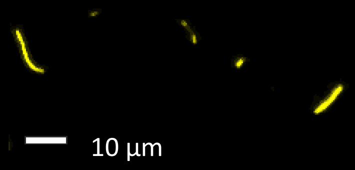
Kaolinite Clay
Automated z-stacked image



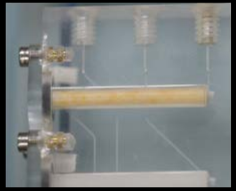
B. megaterium
DUV excited autofluorescence

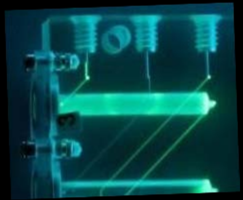


B. megaterium
Alexa Fluor 488 (protein stain)



B. megaterium
Vibrant DiI (lipid stain)





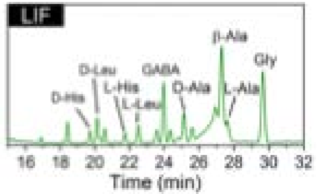
Fluorescent stains: Porous-polymer supports for anhydrous storage, aqueous rehydration

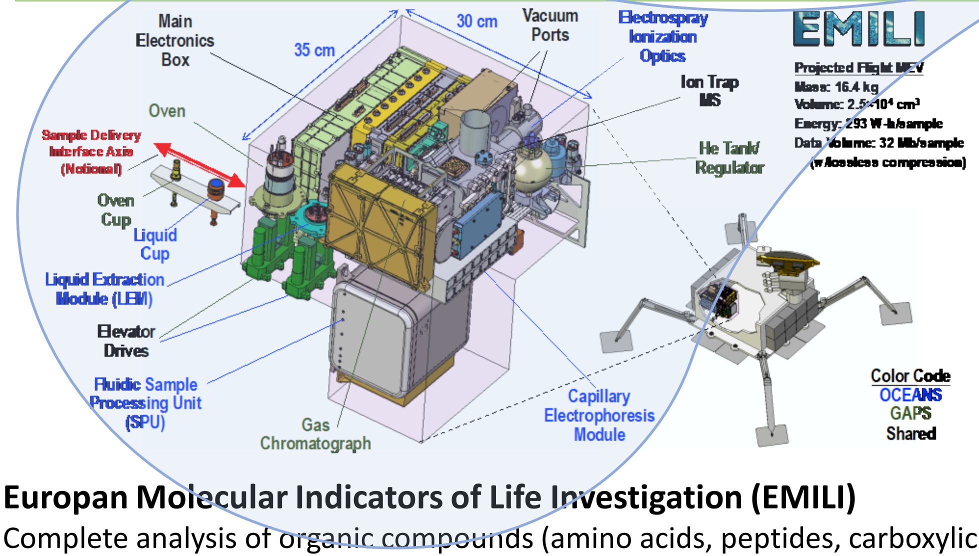
EMILI: High-Precision In-Situ Molecular Biosignature Analysis on Enceladus, Europa, & Beyond

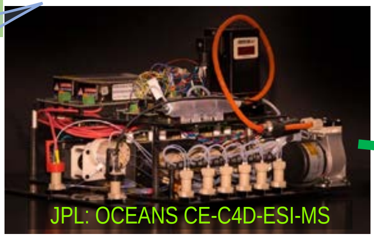
A Life Detection Technology Partnership between Goddard, JPL, Ames, Honeybee Robotics, and Others [POC: W Brinckerhoff, GSFC]

Newly-developed and demonstrated (COLDTech, ICEE-2) technology combines complementary liquid and gas sample processing with exquisitely sensitive, precise, and broad molecular characterization methods (mass spectrometry, capillary electrophoresis/laser-induced fluorescence, capillary electrophoresis/conductivity)

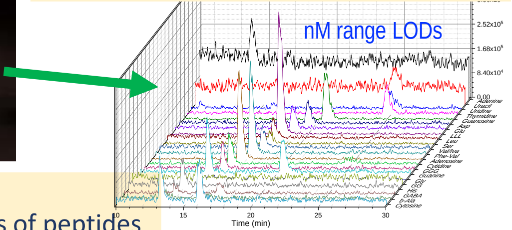
Demonstrated
1-5 nM amino acid limit of detection (LOD) with CE-LIF



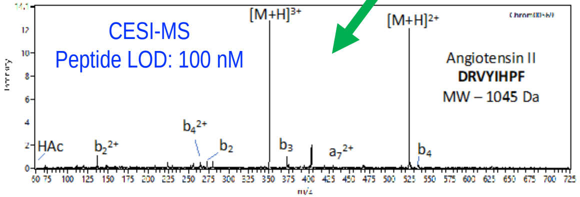


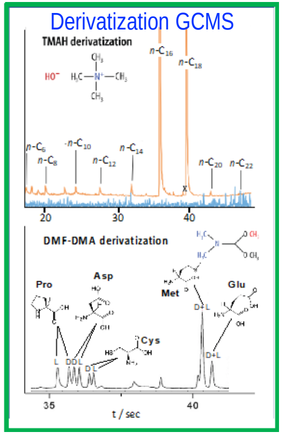


Demonstrated
Integrated & automated CE-C4D-ESI-MS



Demonstrated
Wide mass range ESI-MS analysis of peptides





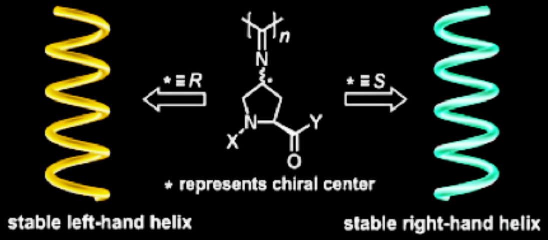
Europan Molecular Indicators of Life Investigation (EMILI)

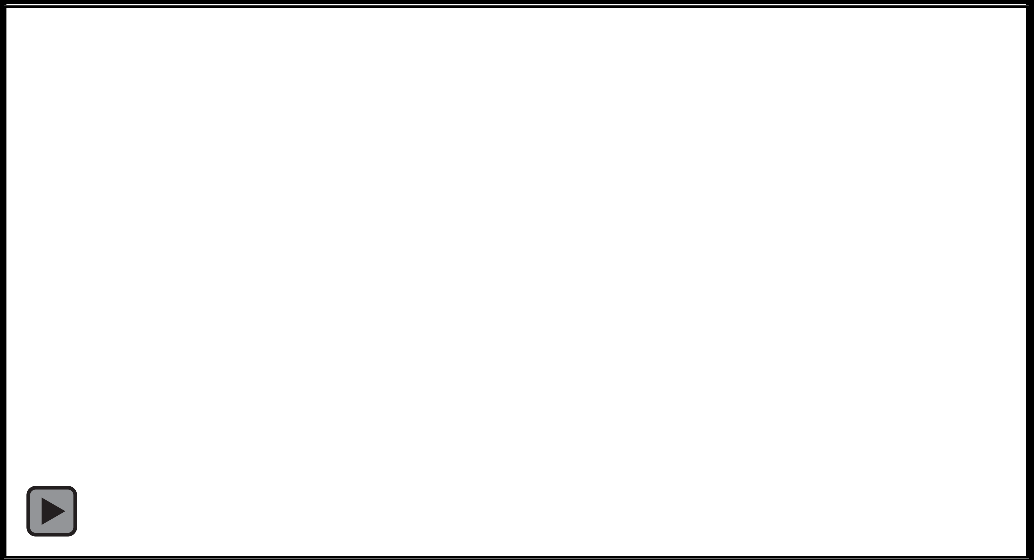
Complete analysis of organic compounds (amino acids, peptides, carboxylic acids, aromatics, …), light volatiles, and salts in ocean world samples. Merges Capillary Electrophoresis (CE), Laser Induced Fluorescence (LIF), Gas Chromatography (GC), and Ion Trap Mass Spectrometry (ITMS) on track to TRL 5/6 meeting all Europa SDT objectives and **fully adaptable to Enceladus**.

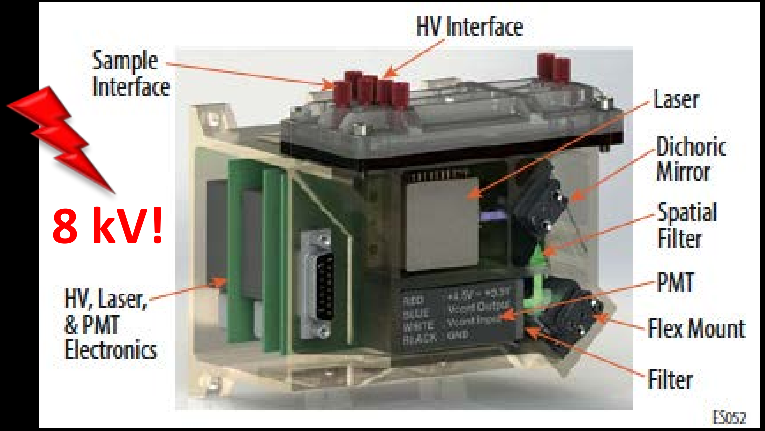
Microchip Capillary Electrophoresis (JPL, ARC)

POC for MCE: P Willis, JPL

- Chiral separations, e.g. amino acids (JPL: field demos.)
  - ➢ Ultrasensitive laser-induced-fluorescence detection
- ARC sample-processing "front end" (COLDTech/ICEE2)
- ➢ Combination bubble trap / air-gap generator

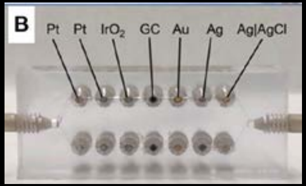


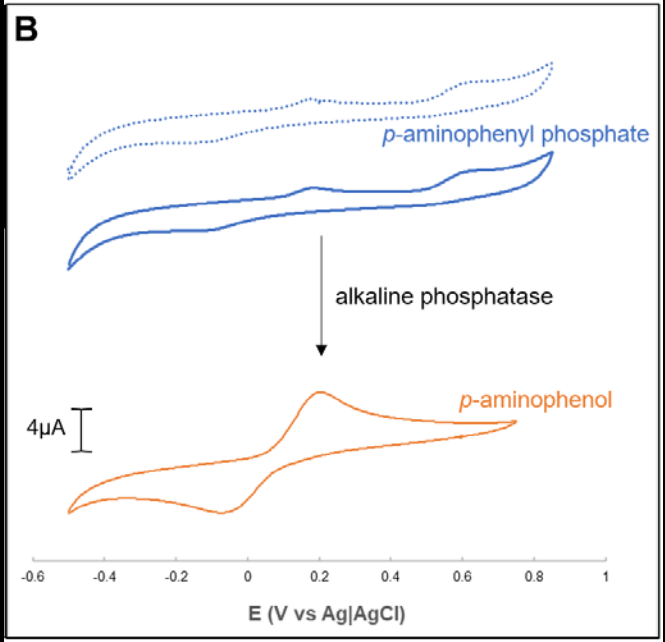


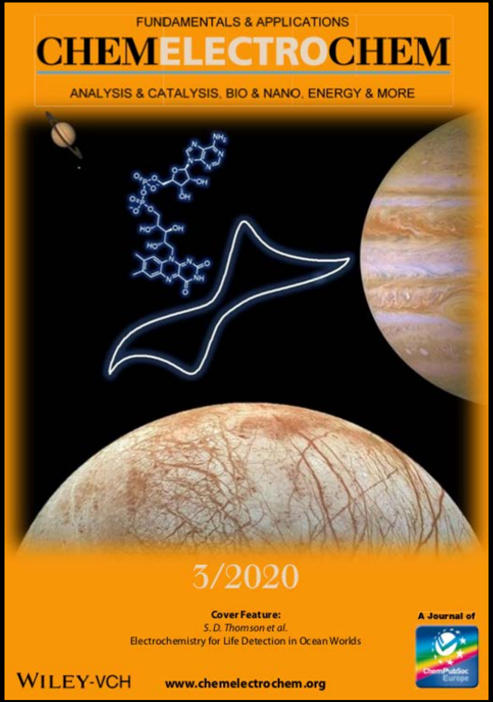


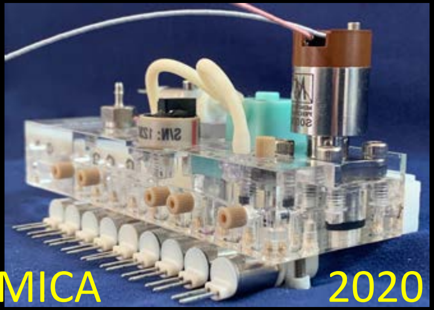
Electrochemical Detection of Catalysts as Signatures of Life

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







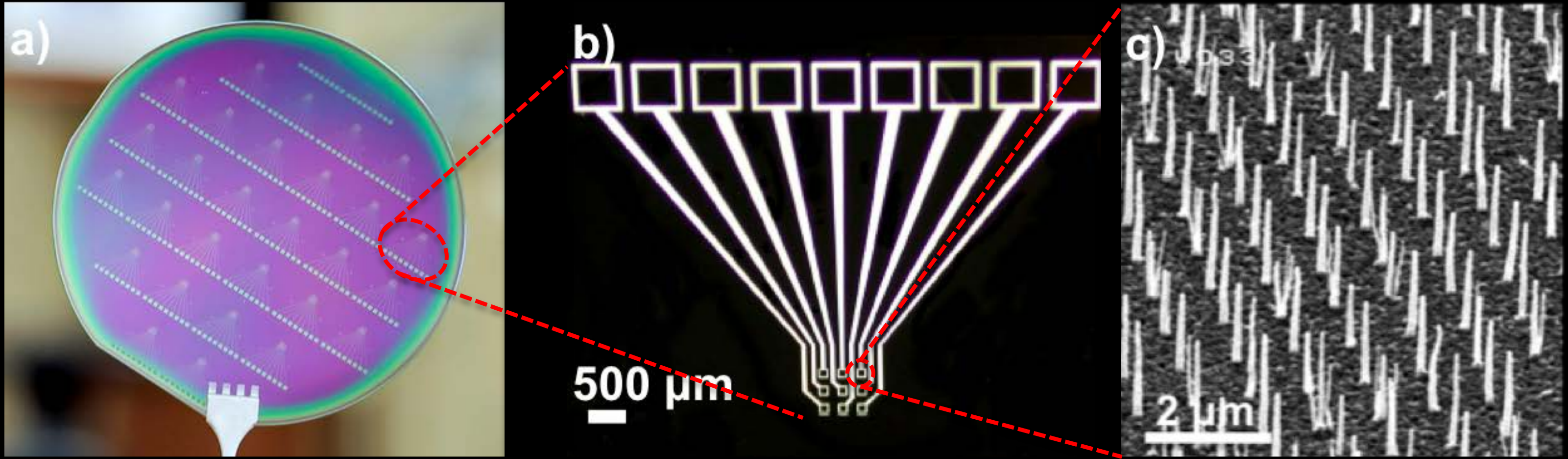


Ames, JPL, Tufts U., MIT

Thomson SD, Quinn RC, Ricco AJ, Koehne, JE.
ChemElectroChem **2020**

Miniaturized Multiplexed Electrochemical Sensors

- 30 sensor chips per wafer; 9 sensing nanoelectrode arrays per chip
- Nanoelectrode arrays provide high signal to noise ratios
- One sensor chip can characterize habitability, energetics, biocatalysis
- Manifold integration straightforward



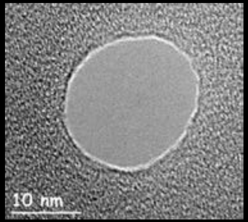
Gupta RK, Pandya R, Sieffert T, Meyyappan M, Koehne, JE. *J Electroanal Chem* **2016**

Solid-State Nanopore Life-Detection Technology

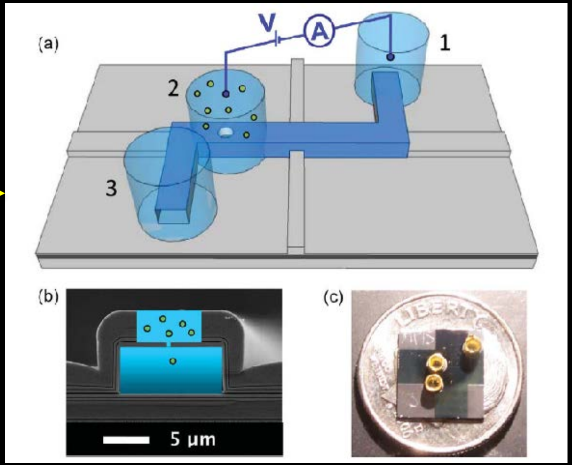
- Robust silicon nitride nanopore membranes for spaceflight missions
- Detection of multiple types of charged polymers and small charged particles (20 nm – 20 µm): sizing, sequencing
- Ames' fluidic processor front-end prepares samples:
  - extract/isolate; de-salt, concentrate

ARC, UC Santa Cruz

Biological nanopore



Choi et al., PLOS ONE **2018**

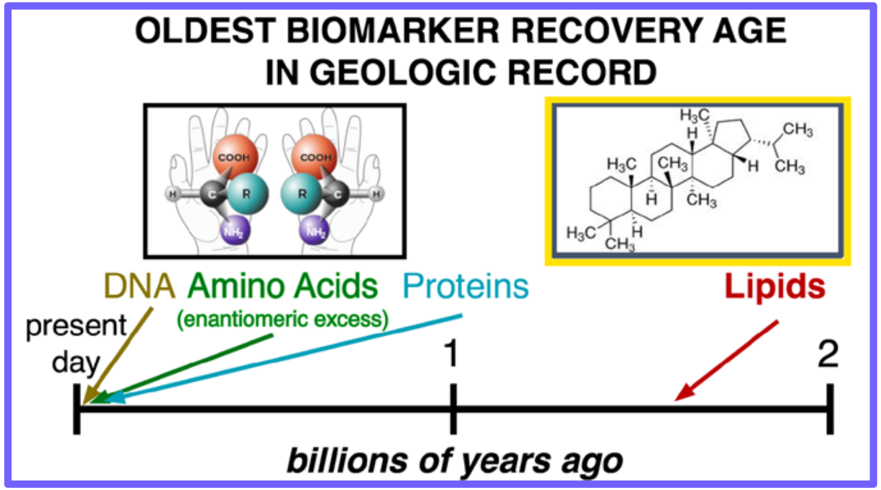


ExCALiBR: Extractor for Chemical Analysis of Lipid Biomarkers in Regolith

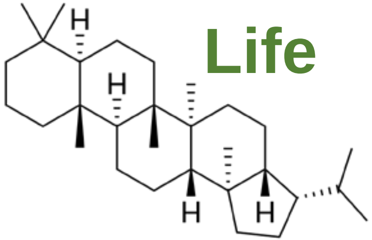
MB Wilhelm (PI), AJ Ricco, M Chin, J Eigenbrode, L Jahnke, M Furlong, D Buckner, T Chinn, K Sridhar, T McClure, T Boone, L Radosevich, A Rademacher, T Hoac, M Anderson, S Getty, A Southard, R Williams, X Li, T Smith, L Friend, W Alvarado

Organic solvent-based extraction system integrates & automates laboratory **sample preparation** to optimize instrumental analysis of organics in regolith

Why lipids?

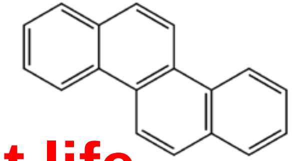


1. **Ubiquitous** in both "life as we know it" & abiotic organic matter
2. **Stable** for eons in the geological record
3. **Diagnostic** of their origin: biotic or not



Life

vs.

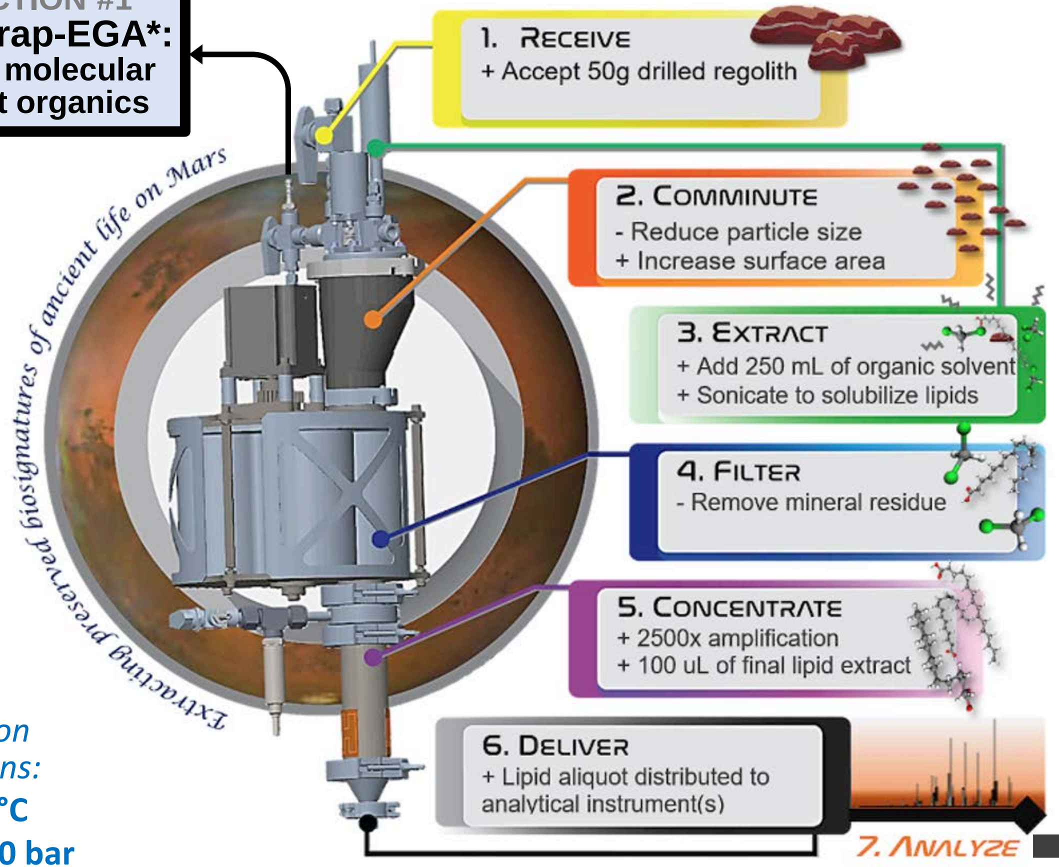


Not life

STMD Early Career Initiative, ARC internal funding

ExCALiBR CONCEPT OF OPERATIONS

FRACTION #1
Cold Trap-EGA*:
"Low" molecular weight organics



Extraction conditions:

0 – 120 °C

0.01 – 10 bar

FRACTION #2
GC-MS:
"Moderate" molecular weight /non-polar lipids

FRACTION #3:
Derivatize + GC-GPF*:
"Moderate" molecular weight, low/moderate polarity lipids

FRACTION #4
Thermochemolysis + GC-MS:
Soluble macromolecular matter

FRACTION #5
LDI*-MS:
"High" molecular weight lipids

*EGA = evolved gas analysis;
GPF = gas-phase fluorescence;
LDI = laser desorption/ionization

Summary: Enabling Life Detection with Fluidic Integration

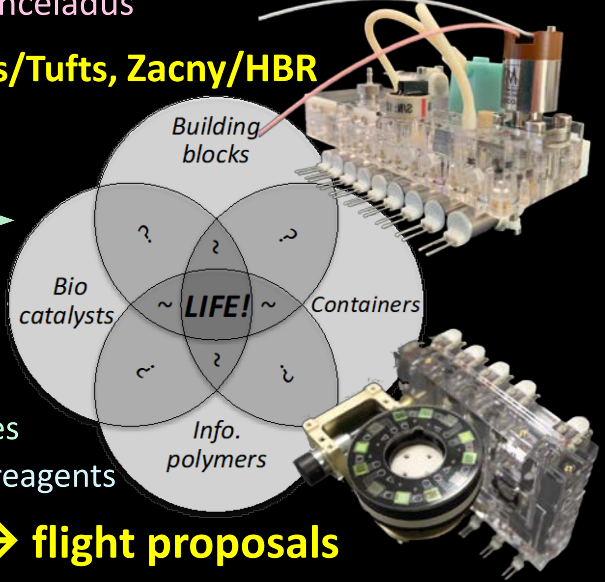
ARC / GSFC / JPL / APL Collaborative activity spans multiple ICEE-2, COLDTech, ECI Projects:

EMILI: Europan Molecular Indicators of Life Investigation; ***MICA***: Microfluidic Icy-world Chemical Analyzer;
ELM: Europa Luminescence Microscope; ***ExCALiBR***: Extractor for Chemical Analysis of Lipid Biomarkers in Regolith
LIfE: Luminescence Imager for Exobiology; ***mWCL***: Microfluidic Wet Chemistry Laboratory;
SPLIce: Sample Processor for Life on Icy worlds; ***EFun***: Plume Sampling System for Enceladus

NASA PIs: Brinckerhoff, Quinn, Ricco, Wilhelm, Willis; Adams/APL; Kounaves/Tufts, Zacny/HBR

Guiding Principles to Search for Indicators of Life

1. Follow the water: ***Enceladus, Europa; Mars*** (ancient life)
2. Seek multiple**, diverse indicators**: ***dispel ambiguity*** →
3. Employ a **suite** of complementary **instruments: *extraordinary proof***



A Fluidically Integrated Analytical Suite will:

- Maintain ***molecular*** *and* ***structural integrity*** of life indicators
- Extract, process, filter, concentrate, de-bubble, label, aliquot, and deliver samples
- ***Optimize instrument performance:*** meter samples, blanks, controls, calibrants, reagents

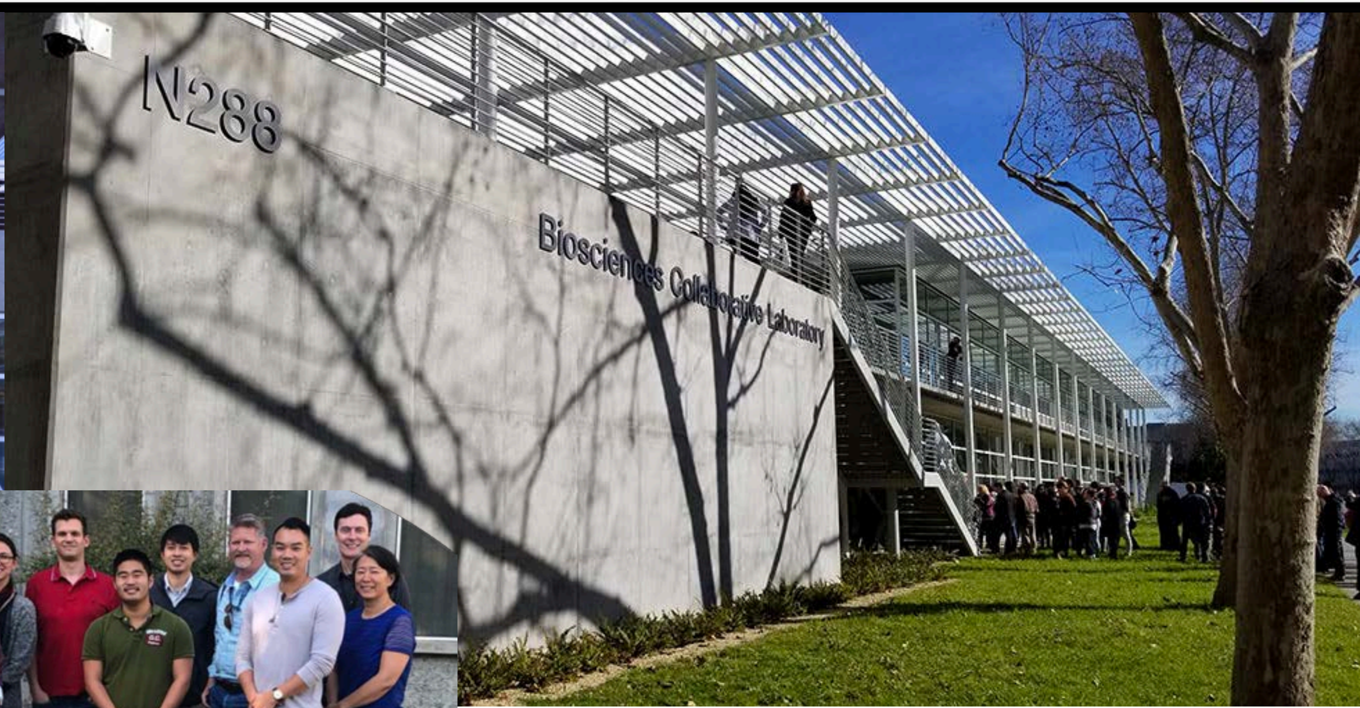
NOT a matter of "IF" but "WHAT'S REQUIRED": TRL 5-6 systems → flight proposals

- Enabling fluidics technology has ***matured dramatically*** thru repetitive design, development, integration, spaceflight
- Achieving this powerful combination of measurements on a planetary mission is ***NO LONGER A HIGH-RISK PROPOSITION***

P Willis, WB Brinckerhoff, A Ricco, et al. **2018**, *A Universal Approach in The Search for Life at the Molecular Level*, 42nd COSPAR Assy., Paper F3.6-0005-18

ARC Biosciences Collaborative Facility

(opened February 2020)



<u>Support</u>: NASA SMD ICEE-2 & COLDTech

NASA STMD Early Career Initiative

NASA ARC Internal R&D