Virtual Research Presentation Conference

Isolation and Concentration of Biogenic Samples via Flow Cytometry for Icy Moons

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Program: Topic

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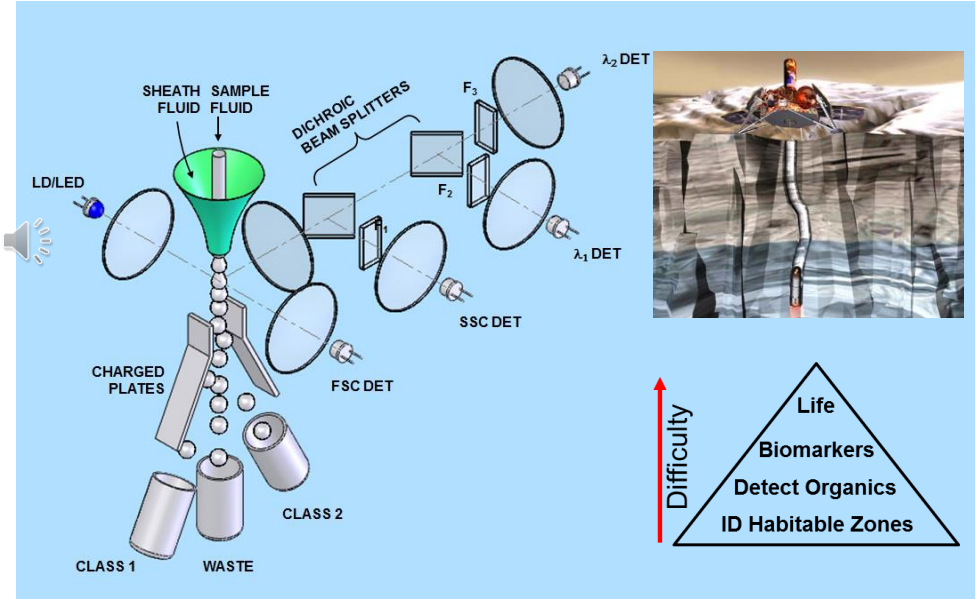
Tutorial Introduction

Abstract

The objective of this work is to develop and demonstrate the rapid reagent-free detection and concentration of microscopic potentially-biogenic particles contained in a raw water-ice sample via sorting flow cytometry. Further, these potentially-biogenic organic particles are to be physically isolated from contaminates like microscopic inorganic particles, increasing the sample's organic concentration. This customized flow cytometer builds upon the state-of-the-art, like the ISS-proven Microflow1 used to conduct biological analyses on-orbit. It does this by (1) foregoing the need to stain or fluorescently tag the sample, (2) integrating a unique capability of differentiating and sorting organic from inorganic particles in real-time, (3) optimizing the detection and processing of small particles and microbes (down to 0.2 µm), and (4) utilizing new low-power lasers.



Problem Description

NASA is striving to find life outside of Earth: Europa and Enceladus?

If life exists on such Icy Worlds, it is likely:

rare < 300 cells/mL [1]
microscopic 0.2 – 3.0 um [1,2]
hidden 80-98% of these particles are abiotic. [2]

Microscope-based instruments must fight against the background.
Mass Spectrometers and **Capillary Electrophoresis** usually do not leave cells intact, small sample volumes might not have cells.

Cytometers are used to detect rare particles (from sub-glacial cells to biohazard aerosols) and have been flow to the ISS.

Deployed to an Icy World, a cytometer could:

- process large volumes of surface melt (1 liter/day), isolating bacterial/organic content.
- produce a scientifically-rich, pre-characterized surface sample, enabling previously difficult downstream analysis.

Methodology

Innovations

UV laser (275 nm) excitation: detect bacteria via intrinsic fluorescence, no reagents/dyes required.

Fluorescence Lifetime measurements: differentiates inorganic (>1 µs) from organic (< 10 ns) particle fluorescence.

Sub-micron detection/isolation: optimized to detect particles down to 0.2 um.

(1) Detect a particle, 0.2 to 40 um in size.

(2) Fluorescence, scattered light, and FLT measurements characterize particle.

(3) Predefined (automatically or manually) setpoints determine particles identity.

(4) Particle is sorted into designated “bucket” at up to 70,000 particles/sec.

Original Milestones for FY20

	Milestone	Status
0	Calibrate FLT measurements	Complete
1	Detect Bacteria in simulant	Partially Complete
2	Isolate Bacteria in simulant	Paused
3	Isolate Bacteria in ice-core sample	Paused
4	Integrate compact low-power lasers	Partially Complete

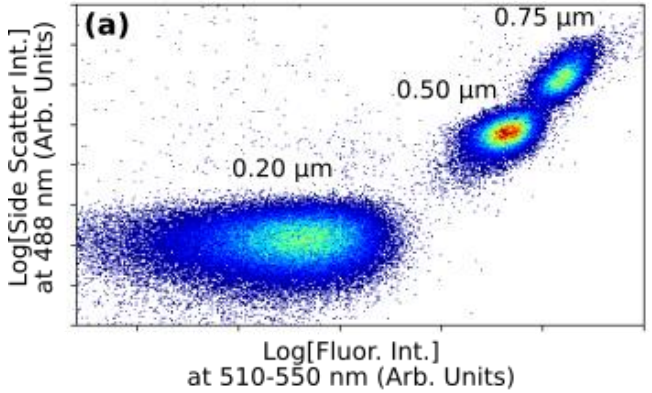
Progress has been delayed due to COVID Shutdown.

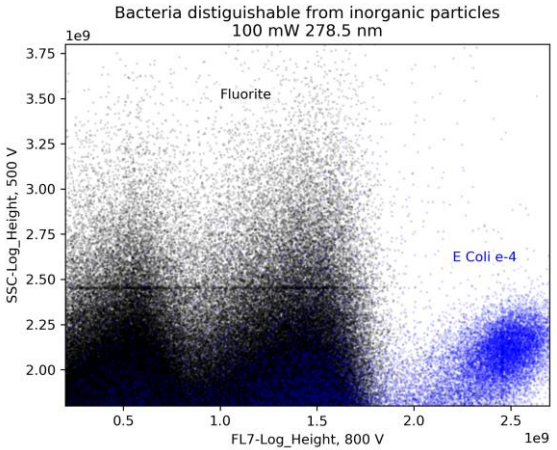
Results and Significance

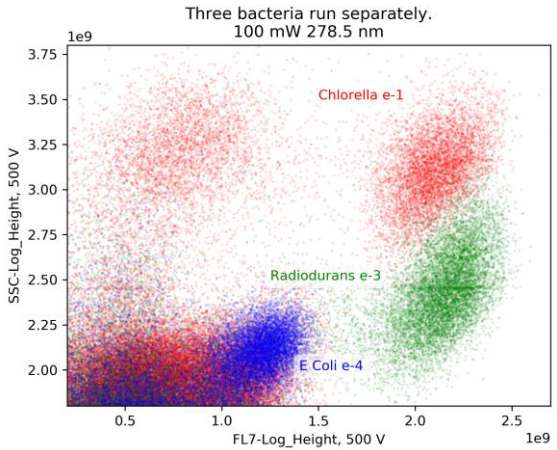
Demonstrated particle detection and distinguishing down to 0.2 um
Meets specifications requested by Europa Lander Science Definition Team for size detection limits [1].
Milestone 1

Verified uncertainty (1 σ) of FLT measurements to be < 10 ns.
Allows fluorescence from inorganics (> 1us) to be distinguished from organics (<10 ns)
Milestone 0

Demonstrated ability to distinguish 3 types of bacteria from each other and mineral containment.
Demonstrates potential to separate bacteria from unwanted particle background.
Milestone 1





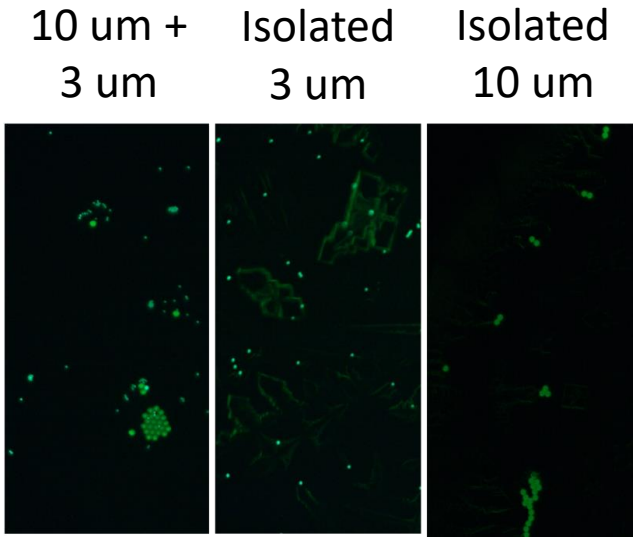


Results and Significance

Demonstrated ability to identify and sort particles by size and fluorescence intensity.
Reduces risk associated with the next step: sorting bacteria from mineral particles.
Milestone 2/3

Same technique was used to demonstrate ability to concentrate isolated particles by at least 100x.
Demonstrates progress onto major objective: concentration of desired particles.
Milestone 2/3

Assembled and tested small frame lasers (532 nm), acquired small frame 488 nm lasers.
Enables upcoming integration into instrument.
Milestone 4



UV Sorting Flow Cytometry

Can detect and distinguish particles by their natural properties

- Size
- Intrinsic Fluorescence

No need for stains or reagents

Initial life-detection method

Bacteria concentrator for other on-board instruments.

Publications and References

Conferences:

AbsSciCon 2019, Poster 142-182

Lunar Planetary Science Conference (LPSC) 2019, Poster 1471

References:

[1] K.P Hand et. al, “Report of the Europa Lander Science Definition Team”, *NASA* (2017). JPL D-97667.

[2] Christner, B. C., Skidmore, M. L., Priscu, J. C., Tranter, M., & Foreman, C. M. (2008). Bacteria in Subglacial Environments. In *Psychrophiles: from Biodiversity to Biotechnology* (pp. 51–71). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-74335-4_4