National Aeronautics and Space Administration

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

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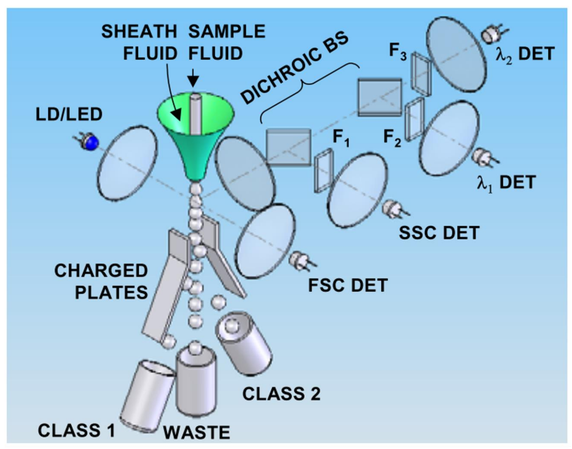
Project Objectives

Life on Icy Worlds, if it exists, is likely:		Detection and Analysis requires:		Flow Cytometry
rare, < 300 cells/mL		high-throughput concentration		50 mL/hr, up to 10,000x concentration enhancement
microscopic, 0.2 – 3.0 µm	→	optical sensing	→	UV laser excitation, scattered light and fluorescence detection.
hidden amongst a large background (80-98% abiotic particles)		composition-specific sorting		Sorts by particle size/shape and organic/inorganic composition

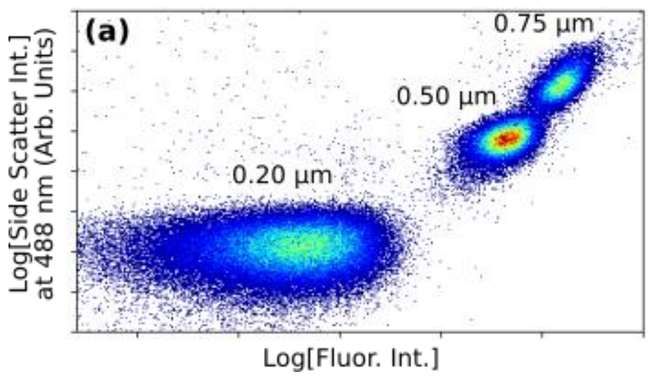
The mission is to develop and demonstrate the rapid reagent-free detection and concentration of microscopic potentially-biogenic particles contained in a raw water-ice sample via sorting UV flow cytometry.



Flow Cytometry irradiates a jet of liquid sample, containing particles, with laser light. Fluorescence and scattered light intensity from each particle is used to classify and sort (via charged plates) the particles in real time.

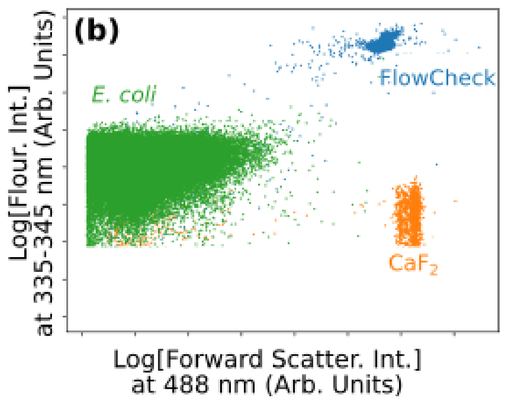
FY19 Results

Cytometer can detect and distinguish sub-micron particles



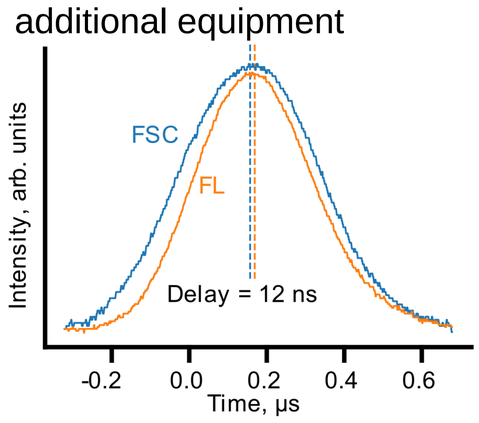
The 0.20 µm particle size requirement was verified. The instrument successfully detected and distinguished populations of 0.2, 0.5 and 0.75 µm particles.

Bacteria can be distinguished from raw minerals with flow Cytometry



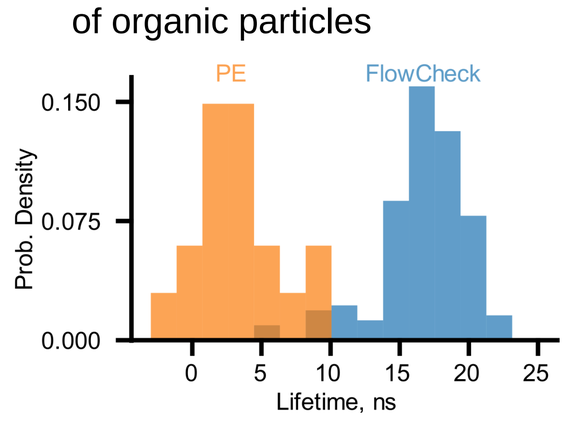
As a step towards the processing of realistic icy worlds simulants, a mixture of E. coli, calibration beads (FlowCheck), and raw powdered fluorite (CaF_2) were processed sequentially. Distinct populations are observed with both the scattered light (488 nm excitation) and the fluorescence channel (275 nm excitation, 340 ± 5 nm emission).

Fluorescence lifetime (FLT) can be measured without additional equipment



While scattered light, e.g., FSC, is produced instantaneously, fluorescence is a chemical process taking a finite amount of time, defined as its lifetime. Measuring the delay in the arrival times of the two signals at the PMTs gives a estimate of this lifetime.

Cytometry-based FLT can distinguish different populations of organic particles



Microspheres dyed with fluorescence standards can be distinguished solely based off of this lifetime measurement. Our Beckman Coulter MoFlo XDP has a FLT resolution of roughly 5-10 ns.

Inorganic particles have longer lifetimes (>10^{-6} s) than organics (< 10^{-8} s). This difference can be used to identify and isolate organics from inorganics.

Benefits to NASA and JPL (or significance of results):

Key Missions

Targets containing large amounts of water:

- Europa Lander
- Lunar/Martian Polar lander
- Enceladus lander

Unique Capabilities

- UV excitation enables detection and characterization without the need for biological/chemical staining. Concentrated sample remains in its native chemical state.
- Particle by particle fluorescence lifetime measurements
- Acts as a sample concentrator and filter, lowering detection requirements for the fellow lander instruments.
- Enables the processing of large volumes of dilute sample in order to find the very rare, scientifically-rich, and potentially biogenic particles.

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Conferences:
AbsSciCon 2019, Poster 142-182
LPSC 2019, Poster 1471

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