National Aeronautics and Space Administration

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

Principal Investigator: Wayne Schubert (352); Co-Investigators: James Lambert (389), Nicholas Tallarida (389)

Program: FY21 R&TD Topics **Strategic Focus Area: Remote/In Situ/Life Detection Sensors and Instruments**

Objectives: The objective of this RTD is to develop and demonstrate the rapid reagent-free detection and concentration of microscopic and potentially-biogenic particles contained in a raw water-ice sample via sorting flow cytometry. Additionally, the instrument will separate potentially-biogenic organic particles into a separate vessel, thereby isolating them from contaminates like inorganic mineral fines, increasing the biogenic concentration and purity. This customized flow cytometer builds upon state-of-the-art flight instruments, 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) differentiating mineral from organic particles using intrinsic fluorescence and fluorescence lifetime, and (4) implementing machine learning methods for cluster identification and characterization.

Background: Finding biogenic particles on icy worlds is not limited by the technology need to detect them but by our ability to get these particles to the detector. For example, detection of microbial life in ancient glacial ice on Earth is not easily amenable to the direct use of standard microscopy, Raman spectroscopy, or mass spectroscopy approaches because biogenic particles are present in very low concentrations. If life exists within the subsurface oceans of the icy moons, it is also likely to be extremely rare. In this RTD, we use flow cytometry with UV laser excitation to induce intrinsic fluorescence of individual particles as they rapidly flow through the nozzle, while performing high throughput, label-free screening and sorting in real time. This instrument is enabling because: (1) it is high throughput, sorting particles at 70,000 particles/sec in a sample volume of up to 1L/day, (2) it is label-free, and (3) allows automatic identification of clusters in multidimensional parameter space using forward and side scatter, fluorescence, and fluorescence lifetime (FLT) measurements. Thus, the system adaptively learns which classes of particle constituents within an icy sample are present, enabling intelligent and efficient sampling, sorting, and subsequent analysis by downstream instruments.

Approach and Results: In the RTD testbed, liquid samples and surrounding sheath fluid (saline) are forced through the flow cytometer's nozzle with a diameter of 70 microns using 90 psi of pressure from a nitrogen feed line producing hydrodynamic flow of the liquid sample such the particles exit the nozzle's tip in single file fashion. The nozzle's piezoelectric element vibrates in the vertical direction causing the stream to break up into small droplets a few millimeters from the nozzle tip. Just below the nozzle tip, 3 laser beams are focused on the fluidic stream where real-time measurements of forward scatter (FSC), side scatter (SSC), fluorescence (in up to 10 spectral bands), and fluorescence lifetime (FLT) are made for each particle present. These parameters are digitized to form a multidimensional hyperspace. Over time, clusters form in parameter space as the particle population passes through the instrument and are characterized. During sorting, a high voltage charge is imparted on the fluidic stream by the nozzle assembly at a point in time known as the "drop delay" where the first droplet separates from the fluidic stream. Upon separation from the stream, each droplet is charged at one of several voltages and is deflected by charged plates beneath to form collection and one waste streams that are directed into different collection vessels.

In this project, the collection and excitation optics of this Beckman Coulter MoFlo XDP instrument were both replaced with fused silica elements to allow deep UV excitation and collection (Figure 1). Additionally, photomultiplier (PMT) outputs from one fluorescence channel and the FSC where both digitized and processed using custom developed FPGA code to provide output pulses whose width correspond to the FLT of each particle. To the best of our knowledge, this is the only instrument in existence with both deep UV excitation and FLT measurement capabilities. These enhancements provide the instrument with the unique ability to distinguish UV intrinsic fluorescence from particles containing organic molecules, which fluoresce, and consequentially have short FLTs, from brightly luminescing minerals, which have much longer FLTs)[A]. However, size not-withstanding, relying just on intrinsic fluorescence may be sufficient for discrimination as microbes generally tend to fluoresce much more brightly than even the most luminescent minerals (Figure 2, 3). Microbes also tend to be smooth walled (diatoms a noted exception), whereas mineral particles are often rough, so their SSC signatures are different.

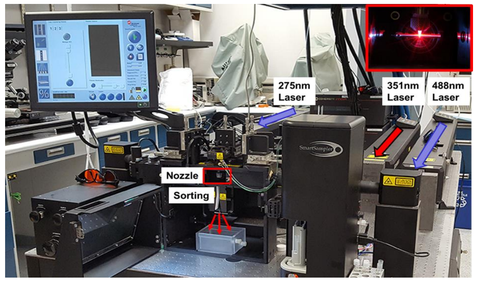

Figure 1: The MoFlo XDP Flow Cytometer was customized to allow Deep UV Excitation and Fluorescence Lifetime Measurement Capabilities

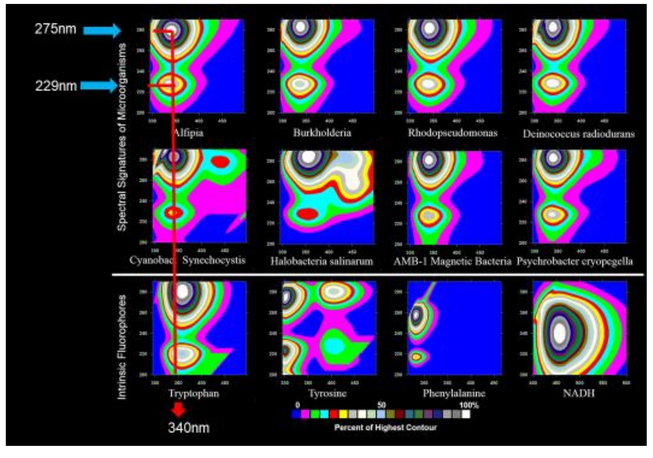

Figure 2: Excitation-Emission Matrices (EEM) of various extremophiles all show strong fluorescence under 229nm and 275nm excitation mostly due to tryptophan.

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Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California

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Approach and Results (con't): Given the great difference between fluorescent mineral and organic FLTs, it is easy to distinguish them, but we have also demonstrated the ability to distinguish among fluorescent particle populations containing different organic compounds (Figure 4). Using deep UV intrinsic fluorescence and side scatter (SSC) we demonstrated the ability to distinguish between different microbial populations. In Figure 5, we show a dot plot of SSC (which informs as to the texture of the particle) vs intrinsic fluorescence of three different microbial species. Finally we have also shown that machine learning can identify and characterize the number of clusters present in the sample (Figure 6).

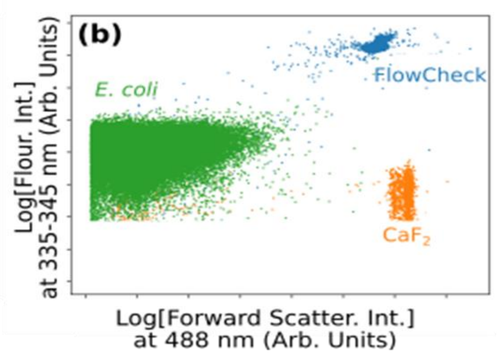

Figure 3: Discriminating between mineral and organic fluorescence using Deep UV laser excitation (275nm).

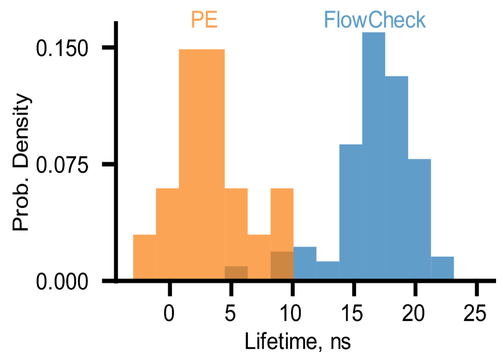

Figure 4: FLT discrimination between two particle populations containing two different fluorescent organic compounds

Significance/Benefits to JPL: The results of this project highlight the potential of developing a flight instrument to create a highly concentrated, pre-characterized, and scientifically-rich Icy Worlds samples. FLT analysis demonstrated in this project provides an means of unequivocally discriminating between intrinsically fluorescent organic particles (with ps-ns FLTs) from intrinsically fluorescent inorganic particles (with us-ms FLTs). The flow cytometer can automatically identify for clusters in a multiparameter space of FSC, SSC, fluorescence, and FLT to "learn" the major types of particle species present without any a priori knowledge. The cytometer can then sort and thereby concentrate these populations into individual aliquots for detailed analysis by downstream instruments using, for example, capillary electrophoresis and mass spectroscopy. The concentration of an in-situ sample containing bacteria that can be theoretically increased by 4 orders of magnitude, from the anticipated concentration of 102 cells/mL to over 106 cells/mL, while simultaneously removing all non-organic particles. Thus, this instrument optimizes sample acquisition and analysis, both of which are essential for short-duration lander missions like the proposed Europa Lander. This technology would improve the scientific output of the instrument payload as well as maximize the scientific return for each analyzed sample.

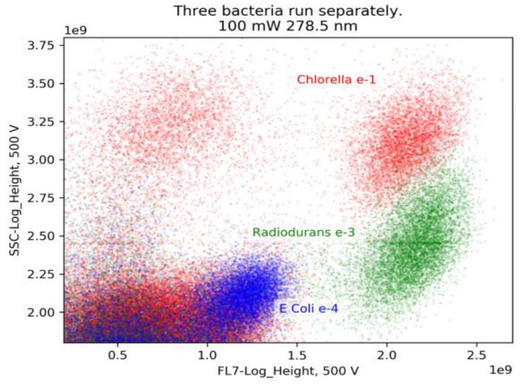

Figure 5: Deep UV excitation (275nm) and SSC allow microbial species to be differentiated without the need for stains, antibodies, or reagents.

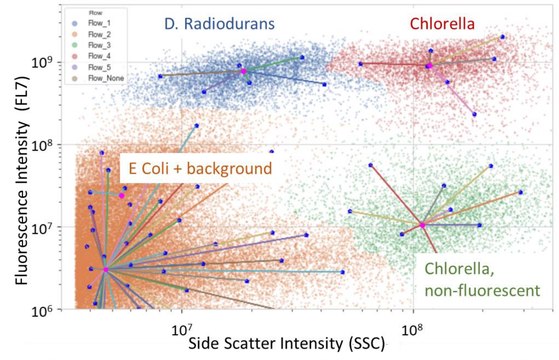

Figure 6: Unsupervised learning of the sample population of 4 microbial species. JPL software automatically determines which clusters are present and their bounds using K-means and DBSCAN machine learning techniques.

Publications: Nicholas Tallarida, Wayne Schubert, and James Lambert "Isolation, Concentration, and Characterization of Icy World Simulants using Flow Cytometry, *Europa Lander Workshop, Pasadena, CA,* 4/28-30/2020

PI/Task Mgr Contact
Email: Wayne.W.Schubert@jpl.nasa.gov

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