

**Life Detection in Water-Ice Matrices.** J. L. Lambert, J. Morookian and A.M. Fisher, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA 91109 ( james.l.lambert@jpl.nasa.gov).

**Introduction:** Periodic changes in tilt angle of the polar axis of Mars (obliquity) occur approximately every 124,000 years and cause significant climatic change. Temperatures in the polar regions may increase from the current maximum of  $-67^{\circ}\text{C}$  to temperatures potentially above  $0^{\circ}\text{C}$  during periods of high obliquity<sup>[1]</sup>. Water is known to exist as thin films surrounding particulates entrained in ice at temperatures as low as  $-20^{\circ}\text{C}$ . Hence, putative microbial communities trapped in the dust-laden Martian polar ice may have opportunities to perform DNA repair and growth during periods of high obliquity, potentially allowing them to survive millions of years. The Mars polar regions may represent one of the few habitable zones accessible for robotic exploration since water trapped in groundwater may be deep and/or so heavily laden with regolith that drilling operations at lower latitudes would be impractical. In order to screen melt-water for microorganisms in melt water, a specialized flow cytometer has been developed and demonstrated using four strains of microorganisms.

**Emission-Excitation Matrices:** In order to determine how laser induced fluorescence might best distinguish between microorganisms, bioremnants, organic molecules and geologic rock and mineral fines, we used a Jobin-Yvon SPEX FluoroMax Fluorometer to collect a series of excitation-emission matrices (EEM) across a diverse set of microbial species as well as from various Mars mineral analogs. EEMs were developed over excitation and emission wavelength ranges of 200-290nm and 295-495nm, respectively, using 2 nm steps for both parameters. Eight different types of microorganisms were tested: *Alfipia*, *Burkholderia*, and *Rhodospseudomonas* (cultured from soils of the dry valleys of the Atacama desert), *Halobacteria salinarum*, *Deinococcus radiodurans*, and *Psychrobacter cryopegella*, (extremophiles adapted to conditions of high salinity, dryness & radiation, and cold temperatures, respectively), AMB-1 (a strain of bacteria containing intracellular magnetite), and *Cyanobacterium synechocystis* (a photosynthetic oxygen producing bacteria). EEMs for tryptophan and a psychrophilic bacteria (representative of all the EEMs acquired for microorganisms tested) are shown in Figure 1. Two peaks were universally observed in the microorganisms tested, with emission and excitation wavelength coordinates of (340nm, 282nm) and (340nm, 227nm) corresponding to the intrinsic fluorescence EEM of membrane-bound tryptophan. All of these microorganisms had a dominant tryptophan component in their EEM.

In contrast, EEM data from a set of eight Mars mineral analogs, each produced a fluorescence contour map quite distinct from any of the microbial species tested. Although EEMs of the minerals were quite variable, none resembled tryptophan. A variety of intrinsic fluorophores are present in microorganisms including NADH, FAD, nucleic acids and aromatic amino acids. Based on extinction coefficient and quantum yield measurements, tryptophan has a brightness that is greater than all of these other biomarkers. Since tryptophan is both ubiquitous and bright, it serves as an excellent biomarker for earth-centric life.

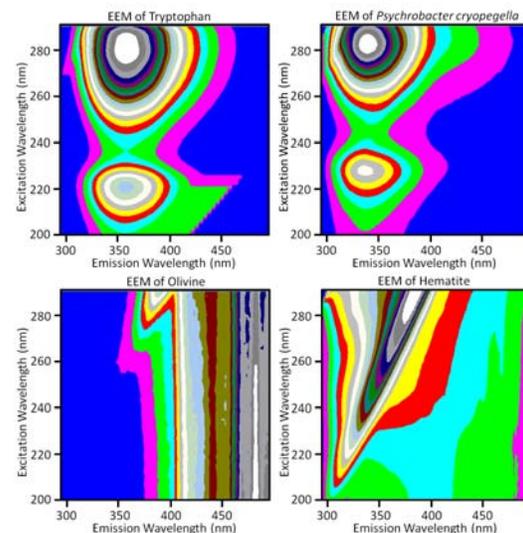


Figure 1: Tryptophan, bacterial, and mineral EEMs

**Detection Strategy:** We have developed a fluidic-based particle analyzer that uses the two-stage approach shown in Figure 2 for detecting microbes or bioremnants from melt-ice based on a specially modified flow cytometer<sup>[2]</sup>. In Step 1, forward scatter and intrinsic tryptophan fluorescence are used as selection criteria. Those particles that meet these threshold-based selection criteria for forward scatter, temporally coincident with intrinsic tryptophan fluorescence, are isolated and concentrated through cytometrically controlled electrostatic sorting. Once isolated, the presumably biogenic particles are characterized by first incubating the isolates with dyes or conjugated surface-specific ligands, and then cytometrically processed again. The advantage of this two-phased approach is that reagents are only required in Step 2, so potentially large volumes of melt-ice are reagentlessly screened first, and then analyzed only after put-

ative biota have been detected, isolated, and concentrated. The isolates can be characterized by downstream *in situ* instruments in lieu of, or in adjunct to, the Step 2 protocol.

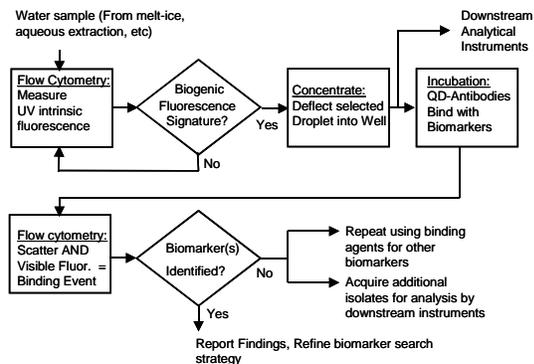


Figure 2: Two-Step Microbial Detection Scheme

**Instrument Testing:** We demonstrated detection of single microorganisms using intrinsic fluorescence with a flow cytometer for the first time. An argon laser was used to provide UV light with an excitation wavelength of 275nm corresponding close to the peak absorption band tryptophan. A second argon laser was used to provide 488nm light to measure forward and side scatter. A Beckman-Coulter XDP Moflo flow cytometer was extensively modified to provide beam routing, focusing, and collection optics in the UV.

**Intrinsic Fluorescence (Step 1):** Isolation of different strains of microorganisms was successfully demonstrated using a UV flow cytometer with intrinsic fluorescence as a selection criterion with forward and side scatter (Figure 3).

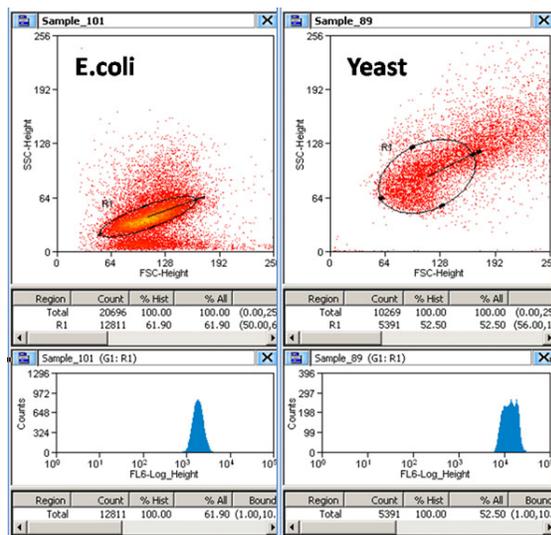


Figure 3: Plots of forward scatter vs side scatter (top) and intrinsic fluorescence (bottom) for *E. coli*

**Characterization (Step 2):** To perform cytometric characterization of the isolates, the particles acquired from Step 1 are incubated with (1) conventional biological stains or (2) with CdSe quantum dots (QD's) chemically linked to antibodies or molecule with the desired binding specificity. Testing using cellular dyes and QD's were all acquired from Invitrogen, Inc. Dye-based cytometric characterization included Syto-16-green which fluoresces only when bound to nucleic acid, Oregon Green-488 DHPE (1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine) that stains cell membranes, liposomes, and lipid monolayers, and Syto-9 (green) and propidium iodide (red) nucleic-acid dyes which were used in combination for a live/dead assay. Each of these dyes were incubated with microbial isolates and then the treated isolates were analyzed cytometrically.

Testing with CdSe QD's was also performed; however, finding commercially available antibodies to nonpathogenic bacterial strains proved problematic. We instead linked QD's to DHPE (a membrane binding molecule) through a biotin-streptavidin linkage. The conjugated DHPE-QD complex was incubated with *B. subtilis* bacteria to complete the labeling process.

The advantage of using CdSe QD's as spectral tags is that all absorb in the ultraviolet while fluorescing in a narrowband spectral regions at central wavelengths proportional to their diameter (Figure 4). Thus a set of QDs of different diameters conjugated to different ligands can be used to characterize isolates in a multiplexed fashion using the same laser employed for detection through UV intrinsic fluorescence.

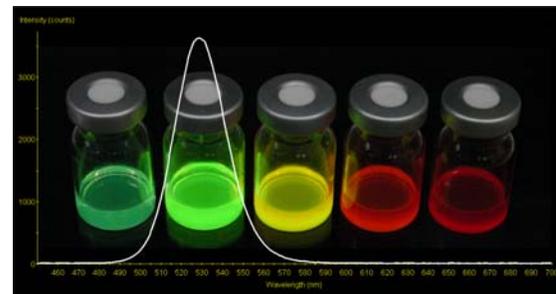


Figure 4: Quantum Dots under UV excitation

**Summary:** Reagentless flow cytometry has been shown to isolate bacteria based on the use of tryptophan intrinsic fluorescence. A two-step method has been advanced to first detect and then to characterize microorganisms from melt-ice.

**References:**[1] Jakosky B.M, Neilson K.H, Bakermans C, et. al. (2003) *Astrobiology*, 3, pp. 343-349., [2] Lambert, J, Fisher A.M, Borchert M, U.S. Patent pending 11/463,834.