A micro fluorescent activated cell sorter for astrobiology applications

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ABSTRACT

A micro-scale Fluorescent Activated Cell Sorter (μFACS) for astrobiology applications is under development. This device is designed to have a footprint of 7 cm x 7 cm x 4 cm and allow live-dead counts and sorting of cells that have fluorescent characteristics from staining. The μFACS system takes advantage of microfludics to create a cell sorter that can fit in the palm of the hand. A micron-scale channel allows cells to pass by a blue diode which causes emission of marker-expressed cells which are detected by a filtered photodetector. A small microcontroller then counts cells and operates high speed valves to select which chamber the cell is collected in (a collection chamber or a waste chamber). Cells with the expressed characteristic will be collected in the collection chamber. This system has been built and is currently being tested. We are also designing a system with integrated MEMS-based pumps and valves for a small and compact unit to fly on small satellite-based biology experiments.

Keywords: Microfluidics, cell sorting, cell counting, live-dead staining, fluorescence

1. INTRODUCTION

A novel miniature instrument to assess in-situ cell viability as a function of exposure to microgravity and the space environment is under development The instrument is a microfluidics-based fluorescent activated cell sorter (μFACS) that is small enough to be flown aboard a free-flying CubeSat or micro-satellite to carry out viability studies of frozen anaerobic and aerobic bacterial cells.

This device is designed to have a footprint of 7 cm x 7 cm x 4 cm and to allow live-dead bacterial counts and sorting of cells that have characteristics that respond to BacLight™Live/Dead bacterial green fluorescent stain (e.g., SYTO 9 for live cells or SYTOX for dead cells) or molecular probes such as the BacLight™ Bacterial Membrane Potential probe. Since these molecular probes use nucleic acid stains, they can distinguish live or dead biological cells from abiotic particles. The μFACS system takes advantage of microfluidics to create a miniaturized cell sorter that can fit in the palm of the hand. This staining system has been previously demonstrated with extermophilic bacteria.[1]

The detection of living Pleistocene bacteria in the Fox Tunnel and Antarctic Ice[2] suggests that ice encased bacteria might survive for long periods in microgravity and the space environment. This enhances the feasibility of the transfer of microorganisms by impact ejection phenomena. If cross-contamination of Solar System bodies might have resulted from natural processes, perhaps some of the Planetary Protection Protocols that must be implemented for future spacecraft could be relaxed at additional cost savings to NASA. This device can be used to determine the viability of this hypothesis in a small nanosatellite or CubeSat system by carrying samples of bacteria in ice exposed to space.

The test μFACS System could also be used to determine if living or dead bacteria are present in the upper crust (2-5 meters) of Europa. This would be much cheaper than developing a cryobot to penetrate the 3-4 km thick ice to reach the liquid water oceans.

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2. SYSTEM DESIGN

A micron-scale channel allows study of cells stained with a mixture of SYTO 9 green fluorescent (excitation/emission maxima of 480/500 nm) and the red propidium iodide (excitation/emission maxima of 490/635) nucleic acid stains. The SYTO 9™ stain penetrates through both intact cell membrane (live cells) and damaged cell membranes (dead cells) whereas the propidium iodide reacts only with the damaged cell membrane and enters into the dead cells resulting into red fluorescent cells.

Since it does not react with the intact membrane, live cells fluoresce green. The stained cells enter the microfluidics capillaries and pass by the blue light emitting diode (LED), which causes the stained live cells to fluoresce green and the dead cells to fluoresce red. The fluorescence emitted by cells is detected by a filtered photodetector. A small microcontroller then operates high speed valves to divert the cell to the collection chamber (live cells) or the waste chamber (dead cells). We take advantage of prior experience with micro-scale valve technology to create a system with integrated MEMS-based pumps and valves for a small and compact unit to fly on small satellite based biology experiments. Figure 1 shows a schematic of current system and Figure 2 shows the integrated prototype electronics and microfluidics assembly.

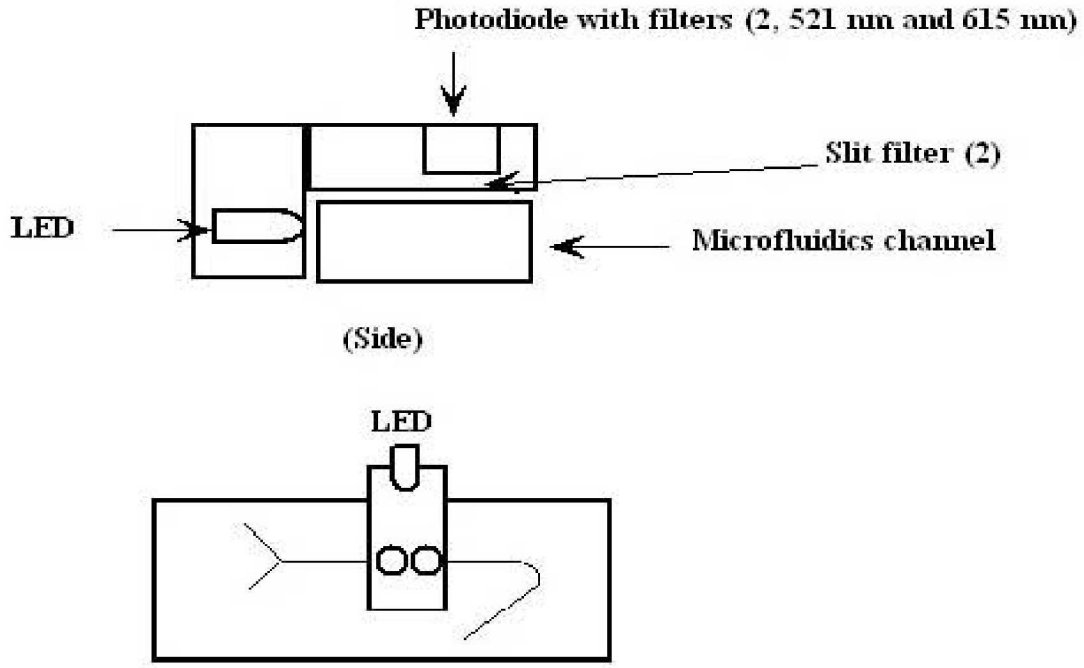

Fig. 1. Microfluidics Channel Design, Showing Flow Channel, Cells Enter on Right, Optics and Split Channel for Cell Sorting (Live/Dead Sorting)

A basic microfluidics channel and electronics control system has been developed and is undergoing testing. The next phase of this project will take the microfluidics channel and integrate valving and controls to allow cells to be automatically fed into the system and then sent to one of the two chambers (one for live cells and one for dead cells as indicated by staining).

Photodiodes with appropriate wavelength filters observe the flow channel and associated electronics count the cells and sort them using a microcontroller-based circuit board. A breadboard design has already been built and tested, but the current system is manually fed and only counts cells and provides live-dead ratios. We are currently evaluating how we can integrate other micromechanical components into the microfluidics system design. For instance microvalves,

microlenses and micropumps could be incorporated into the μFACS system design to make the system more compact and efficient. A variety of microoptics systems are also under evaluation.

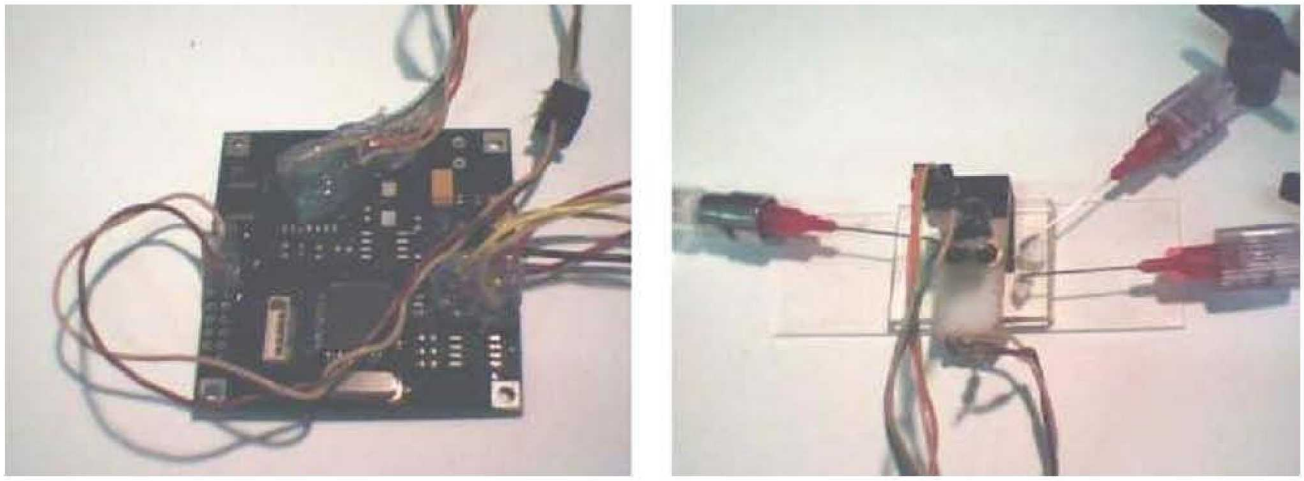

Fig. 2. Protype System (Electronics, left and Microfluidics, right).

The end goal will be to create a complete astrobiology lab-on-a-chip system to sample bacteria, count the live-dead ratio over time and then sort the bacteria for further analysis as seen in Figure 3. This system integrates microfluidics, optics, control electronics and valves in a package that can easily fit into a 3U CubeSat with multiple systems to study different types of bacteria and/or different bacteria exposures. The system can also be adaptable to different excitation LEDs and filter wavelengths for detection.

Overall system mass is 100 grams within the footprint of 7 cm x 7 cm x 4 cm. It uses under 100 mA of power to process a bacterial sample. The LED has emission centered at 485 nm. The optics system consists of micro lenses with filters optimized for green and red emission for SYTO 9 and red propidium iodide, respectively. A photodiode measures emission intensity. Simple green/red ratios can be measured using the two channels to determine percent live bacteria or more sophisticated analysis can take place and bacteria samples separated based upon viability. Figure 3 shows the final system design.

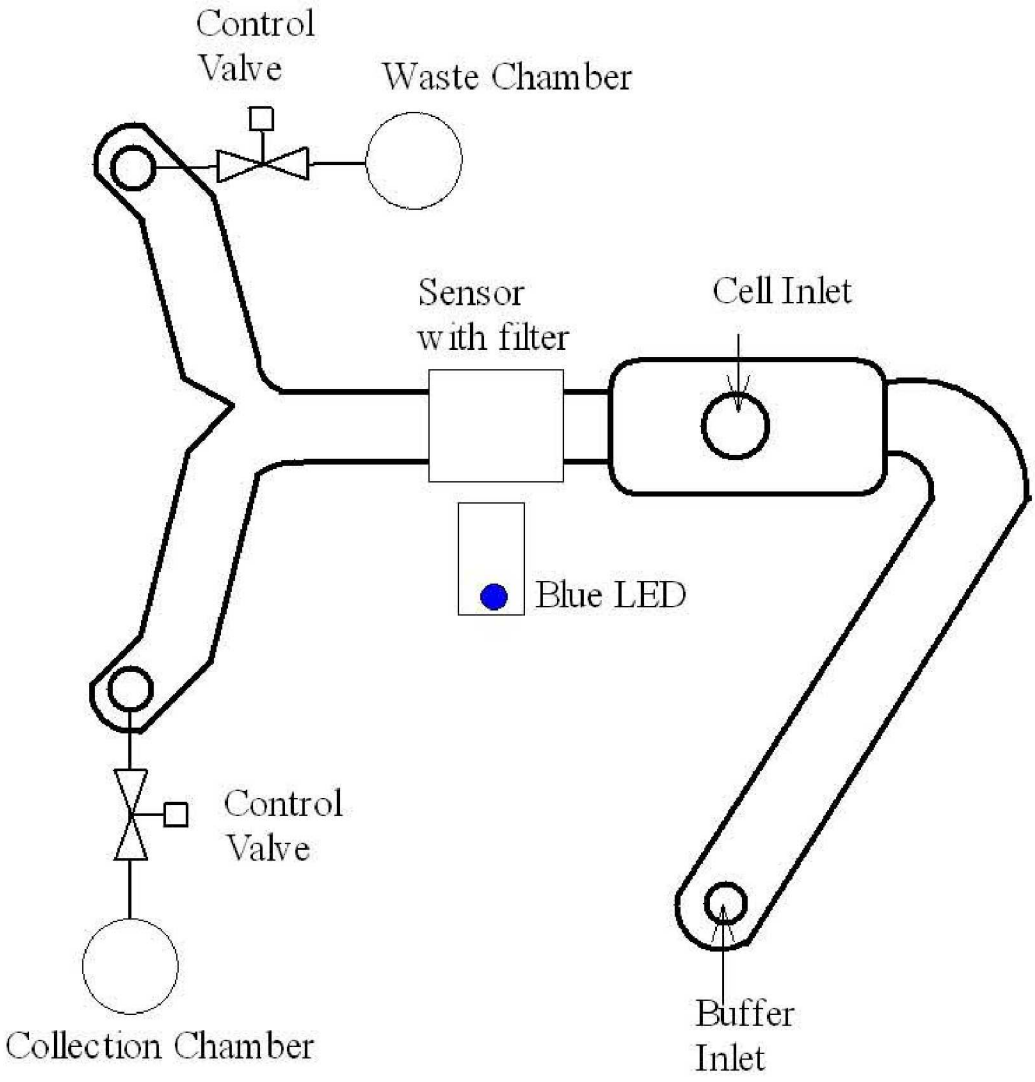

Fig. 3 System Design Schematic Show the Microfluidcs Flow Channel with Sheathing Buffer Inlet and Cell Inlet, Blue Illumination LED (for emission) and red/gree filtered photodetector output. A microcontroller counts cells and controls valves for collection.

A sheathing flow is used to hydrodynamically focus the cells. Wolff *et al*. implemented a "smoking chimney" focusing technique that is used in the system described in this paper.[3] An important factor will be the required relative flow rate of cell-suspended fluid compared to the buffer fluid flow. Increasing the ratio of sheath flow to sample flow increases the focusing of the system. The transverse injection of the cell-suspension fluid should provide the proper level of mixing even in a microgravity environment. With microcontroller-based control of sheathing flow and sample flow, the system can be optimized for various conditions and focusing can be done "on the fly". This focusing flow is illustrated in Figure 4.

Other focusing techniques require microfabrication of electrodes on both the bottom and top of the channel, precision alignment of these electrodes, electronics for generating electric AC field, and conductivity of the focusing liquid. These additional complications are eliminated in this design allowinga smaller, more compact design ideal for small satellite systems.

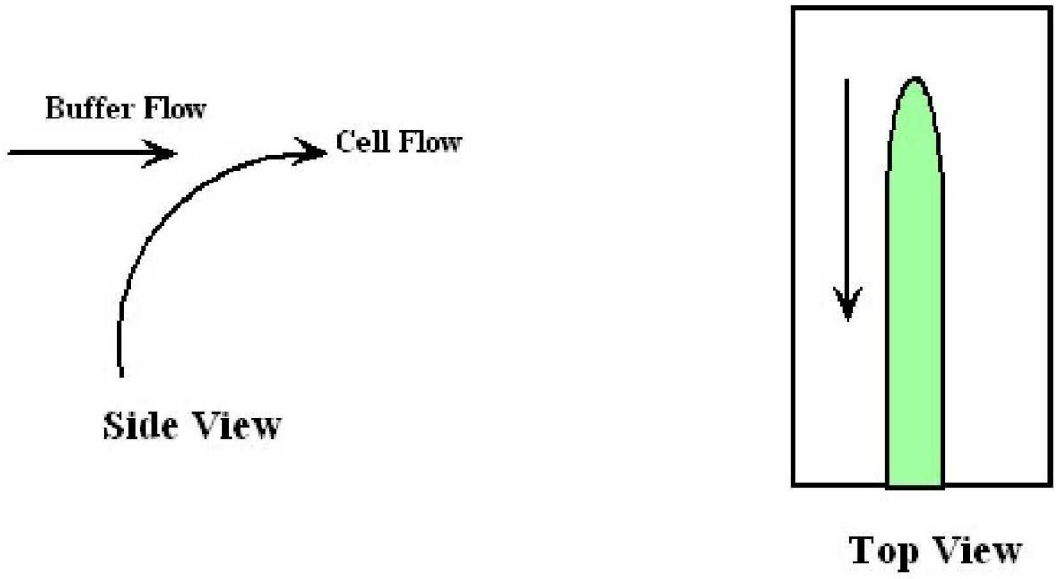

Fig. 4 Microchannel sheathing (buffer) flow and focusing of the introduced bacterial fluid. Arrows indicate flow direction.

Figure 5 shows BacLight™ Live/Dead stain of Spirochaeta americana pure culture from Owens Lake, CA showing live cells (green) and dead cells (red). This illustrates the ease in distinguishing between viable and dead bacteria with this staining technique. New high intensity, yet miniature LEDs with emissions around 470 to 490 nm allows this technology to be implemented in a microfluidics system.

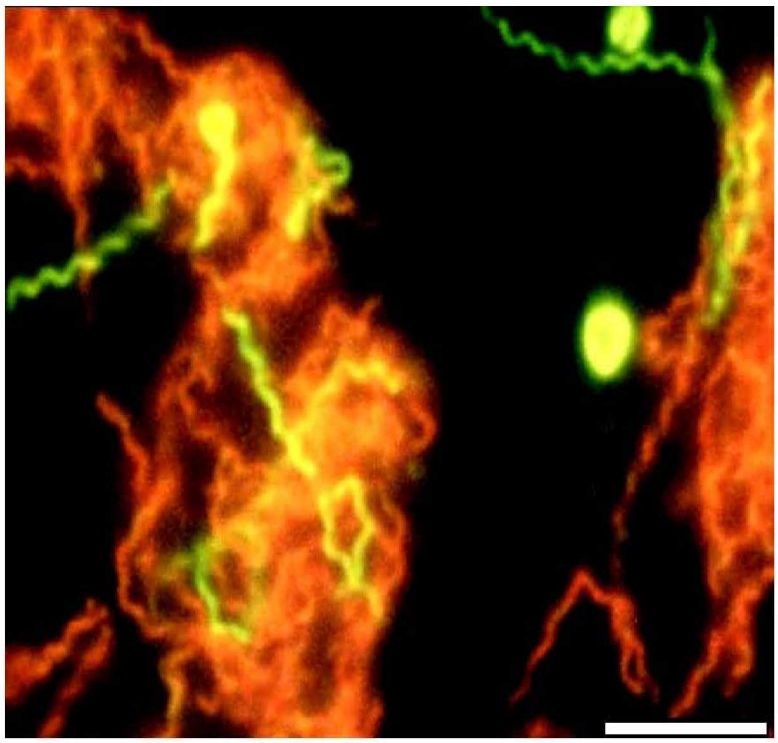

Fig. 5 *Bac*Light™ Live/Dead stain of *Spirochaeta americana* pure culture from Owens Lake, CA showing live cells (green) and dead cells (red).

3. APPLICATIONS

This system relies on technology based on Live/Dead Molecular probes and microfluidics capillary to permit in-situ bioanalytical assessment of the viability of selected anaerobic bacteria and archaea thawed out after different periods of exposure to the space environment. This work has drwan on expertise in microfluidics-based system combined with recent discoveries of survivability of microbial extremophiles entrapped in the ancient ice from the Fox Tunnel of Alaska, Deep Vostok Ice Cores as well as more modern ice from the Anuchin Glacier, Ice Sculptures and Ice-Bubbles studied in-situ during the 2008 Antarctic Expeditions.

This instrumentation can have valuable commercial applications and permit significant cost reductions to future Space Missions. A miniature, lightweight, low-power instrument that can distinguish live bacteria from dead bacteria and bacterial cells in ice from abiotic particles could be incorporated into future SMD Planetary Science Missions. Verification that microbial life entrained in ice can remain viable suggests that trans-planetary transfer of intact microbiota may have occurred during great cosmic impacts.

Recently there was a discovery of living Pleistocene microorganisms, (Carnobacterium pleistocenium, sp. nov.) that had been frozen in the ice of the Fox Tunnel of Alaska for 32,000 years and was still alive and expressed a motility immediately as the ice thawed. This finding implies that ancient living bacteria may be crypopreserved in frozen water ice glaciers at the Polar Caps of Mars. It also means that it may not be necessary to drill through several kilometers of ice to penetrate the crust of icy moons such as Europa or Enceladus to search for life. Living microbes may be present within the first few centimeters or meters of the ice crust, if they are protected from the UV and radiation environment by the ice sheet. These icy moons have become of great interest to Astrobiology due to indications of liquid water oceans beneath their icy crusts. However, viable microorganisms have been discovered in the permafrost and frozen thermokarst ponds of Siberia and Alaska (Carnobacterium pleistocenium). During Antarctic Expeditions, living and physiologically active microorganisms were observed in abundance in freshly thawed ice from the Anuchin glacier and ice bubbles of Lake Untersee and the frozen ice sheets covering the Schirmacher Oasis lakes (unpublished data). These facts expand the possibility of surviving of microbial extremophiles on the Polar Ice Caps of Mars, and the crusts of other frozen bodies of our Solar Systems. Understanding the nature and distribution of microbial life in the glaciers and perennially ice covered lakes of Antarctica should provide meaningful and valuable data required to develop and evaluate instruments and mission operational techniques to search for and recognize evidence of extant or extinct life during future Mars Missions or during explorations of other Solar System bodies. This compact device can be used to analyze bacteria in-situ in these environments. Combining this mFACS with a compact imaging instrument would create a complete field laboratory that is transportable in a backpack.

The possibility that viable microorganisms entrapped in ice may survive exposure to microgravity and the space environment enhances the feasibility of transfer of microorganisms via impact ejection phenomena. If ice-entrapped microorganisms remain viable in the space environment and can be revived on melting then it is feasible to search for living bacteria in the uppermost 2-5 meters of the crust of Europa or Enceladus using the μFACS system. This is much less expensive than developing robotic systems to penetrate a 3-4 km thick ice shell to reach liquid water. Ice is an excellent absorber of high energy particles – only 20 cm of ice reduces the flux of 100 MeV protons to 1/e. Demonstrating the feasibility of cryo-panspermia could have implications to the NASA Origins Program concerning distribution and Evolution of Life in the Cosmos and the Origin of Life on Earth as well as the NASA Planetary Protection Program. If cross-contamination of Solar System bodies can feasibly occur by natural impact ejection transfer of ice containing viable microbes, it may be possible to relax Planetary Protection Protocols at significant cost savings to NASA.

Potential commercial applications of the device include lab-on-a-chip systems, field-based medical instruments and astronaut health monitoring systems to measure blood counts or pathogen activity on long duration space missions. Since the system can be tailored to counting other cell-types and particles; these devices may be used for counter-bioterrorism efforts by detecting potentially deadly microbiological agents in their intact states. Very small, portable medical research devices may be used for pathogen diagnostics in field settings in the rural US and developing countries. Their low cost, ease of use, small footprint and low power consumption could bring state of the art medical care to areas of the world which have not previously had such services. A main issue previously has been the large size, complex support equipment, and cost associated with many FACS systems.

4. FUTURE WORK

The newly developing field of CubeSat-based science missions is the perfect setting to test the μFACS system and the viability of bacteria submerged in ice in the space environment. NASA has previously flown Genesat and Pharmasat 3U CubeSats with total spacecraft masses under 5 kilograms.[4] This system can be flown on a similar CubeSat platform for astrobiology research into bacterial viability in space. A simple 3U CubeSat concept is shown in Figure 6.

An exposure section can be configured on the bottom and top surfaces of the spacecraft (the circle in Figure 6). This exposure area would then interface to a valve manifold system to allow bacterial samples to be made at desired intervals during the mission. For instance, each week or month a sample could be taken and bacterial viability analyzed using the μFACS device.

A series of ground tests will also be conducted with various extremophile bacteria to gain a system baseline and for calibration purposes. The system can also be tested with bacteria in extreme Earth environments such as the polar regions and radiation chambers.

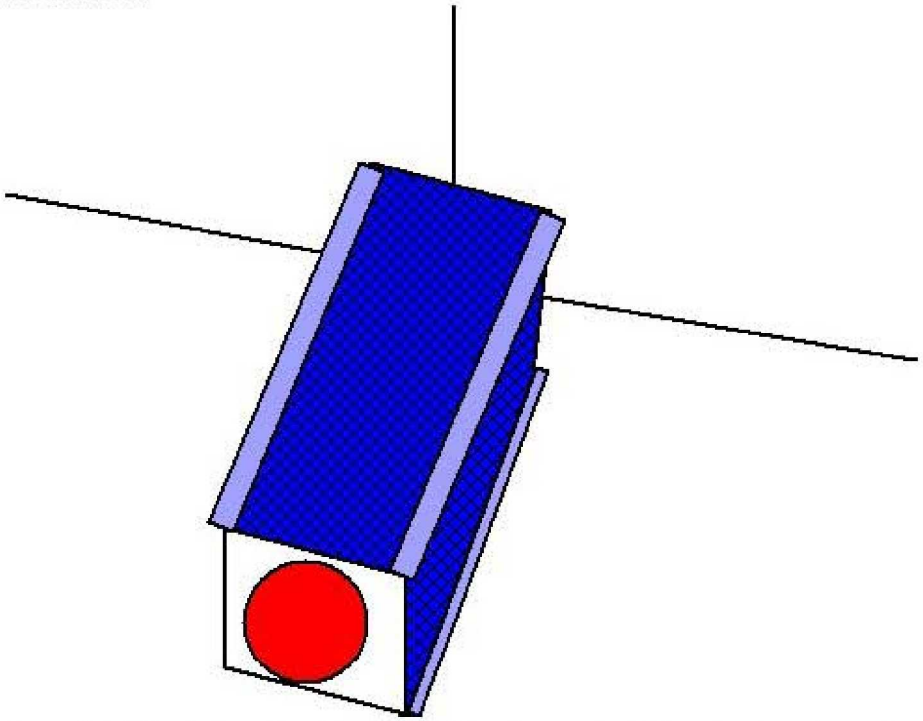

Figure 6 A 3U CubeSat with an Exposure Platform (bottom circle) for an ice-bacteria sample

5. CONCLUSION

The mFACS system offers a low cost and responsive solution for astrobiology missions to analyze bacterial viability in space as well as detect life on other solar system bodies and analyze bacteria in remote locations on Earth. The design is based upon the fundamental principles that a microfluidic-μFACS system in a CubeSat format that would function in conjunction with a well established commercially available fluorescent dye-based microbial detection technology that allows specific identification of the live cells from dead. This overall system makes a low-cost, low-mass and low-power astrobiology lab-on-a-chip.

We are working towards the development of a flight instrument prototype that could form the basis for future flight instruments to address the most fundamental question of Astrobiology: Is life restricted to Planet Earth or is it distributed throughout the Cosmos?

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