



Purifying, Separating, and Concentrating Cells From a Sample Low in Biomass

This fluorescence-activated cell-sorting-based approach has applications in operating room cleanliness validation assays, and in pharmaceutical development and quality assurance.

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Frequently there is an inability to process and analyze samples of low biomass due to limiting amounts of relevant biomaterial in the sample. Furthermore, molecular biological protocols geared towards increasing the density of recovered cells and biomolecules of interest, by their very nature, also concentrate unwanted inhibitory humic acids and other particulates that have an adversarial effect on downstream analysis.

A novel and robust fluorescence-activated cell-sorting (FACS)-based technology has been developed for purifying (removing cells from sampling matrices), separating (based on size, density, morphology), and concentrating cells (spores, prokaryotic, eukaryotic) from a sample low in biomass.

The technology capitalizes on fluorescent cell-sorting technologies to purify and concentrate bacterial cells from a low-biomass, high-volume sample. Over the past decade, cell-sorting detection systems have undergone enhancements and increased sensitivity, making bacterial cell sorting a feasible concept. Although there are many unknown limitations with regard to the applicability of this technology to environmental samples (smaller

cells, few cells, mixed populations), dogmatic principles support the theoretical effectiveness of this technique upon thorough testing and proper optimization. Furthermore, the pilot study from which this report is based proved effective and demonstrated this technology capable of sorting and concentrating bacterial endospore and bacterial cells of varying size and morphology.

Two commercial off-the-shelf bacterial counting kits were used to optimize a bacterial stain/dye FACS protocol. A LIVE/DEAD BacLight Viability and Counting Kit was used to distinguish between the live and dead cells. A Bacterial Counting Kit comprising SYTO BC (mixture of SYTO dyes) was employed as a broad-spectrum bacterial counting agent. Optimization using epifluorescence microscopy was performed with these two dye/stains. This refined protocol was further validated using varying ratios and mixtures of cells to ensure homogenous staining compared to that of individual cells, and were utilized for flow analyzer and FACS labeling.

This technology focuses on the purification and concentration of cells from

low-biomass spacecraft assembly facility samples. Currently, purification and concentration of low-biomass samples plague planetary protection downstream analyses. Having a capability to use flow cytometry to concentrate cells out of low-biomass, high-volume spacecraft/facility sample extracts will be of extreme benefit to the fields of planetary protection and astrobiology.

Successful research and development of this novel methodology will significantly increase the knowledge base for designing more effective cleaning protocols, and ultimately lead to a more empirical and "true" account of the microbial diversity present on spacecraft surfaces. Refined cleaning and an enhanced ability to resolve microbial diversity may decrease the overall cost of spacecraft assembly and/or provide a means to begin to assess challenging planetary protection missions.

This work was done by James N. Bernardini, Myron T. La Duc, and Rochelle Diamond of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1). NPO-48086

Virtual Ultrasound Guidance for Inexperienced Operators

This audio/video system provides real-time help to inexperienced ultrasound operators in remote environments.

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Medical ultrasound or echocardiographic studies are highly operator-dependent and generally require lengthy training and internship to perfect. To obtain quality echocardiographic images in remote environments, such as on-orbit, remote guidance of studies has been employed. This technique involves minimal training for the user, coupled with remote guidance from an expert. When real-time

communication or expert guidance is not available, a more autonomous system of guiding an inexperienced operator through an ultrasound study is needed. One example would be missions beyond low Earth orbit in which the time delay inherent with communication will make remote guidance impractical.

The Virtual Ultrasound Guidance system is a combination of hardware and

software. The hardware portion includes, but is not limited to, video glasses that allow hands-free, full-screen viewing. The glasses also allow the operator a substantial field of view below the glasses to view and operate the ultrasound system. The software is a comprehensive video program designed to guide an inexperienced operator through a detailed ultrasound or