



### 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.**

*NASA's Jet Propulsion Laboratory, Pasadena, California*

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.**

*Lyndon B. Johnson Space Center, Houston, Texas*

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

echocardiographic study without extensive training or guidance from the ground. The program contains a detailed description using video and audio to demonstrate equipment controls, ergonomics of scanning, study protocol, and scanning guidance, including recovery from sub-optimal images.

The components used in the initial validation of the system include an Apple iPod Classic third-generation as the video source, and Myvue video glasses. Initially, the program prompts the operator to power-up the ultrasound

and position the patient. The operator would put on the video glasses and attach them to the video source. After turning on both devices and the ultrasound system, the audio-video guidance would then instruct on patient positioning and scanning techniques.

A detailed scanning protocol follows with descriptions and reference video of each view along with advice on technique. The program also instructs the operator regarding the types of images to store and how to overcome pitfalls in scanning. Images can be forwarded to

the ground or other site when convenient. Following study completion, the video glasses, video source, and ultrasound system are powered down and stored. Virtually any equipment that can play back video can be used to play back the program. This includes a DVD player, personal computer, and some MP3 players.

*This work was done by Timothy Caine and David Martin of Johnson Space Center. Further information is contained in a TSP (see page 1). MSC-24800-1*

## Beat-to-Beat Blood Pressure Monitor

**This invention is applicable to all segments of the blood pressure monitoring market, including ambulatory, home-based, and high-acuity monitoring.**

*Lyndon B. Johnson Space Center, Houston, TX*

This device provides non-invasive beat-to-beat blood pressure measurements and can be worn over the upper arm for prolonged durations. Phase and waveform analyses are performed on filtered proximal and distal photoplethysmographic (PPG) waveforms obtained from the brachial artery. The phase analysis is used primarily for the computation of the mean arterial pressure, while the waveform analysis is used primarily to obtain the pulse pressure. Real-time compliance estimate is used to refine both the mean arterial and pulse pressures to provide the beat-to-beat blood pressure measurement.

This wearable physiological monitor can be used to continuously observe the beat-to-beat blood pressure (B3P). It can be used to monitor the effect of prolonged exposures to reduced gravitational environments and the effectiveness of various countermeasures.

A number of researchers have used pulse wave velocity (PWV) of blood in the arteries to infer the beat-to-beat blood pressure. There has been documentation of relative success, but a device that is able to provide the required accuracy and repeatability has not yet been developed. It has been demonstrated that an accurate and repeatable blood pressure measurement can be obtained by measuring the phase change (e.g., phase velocity), amplitude change, and distortion of the PPG waveforms along the brachial artery. The approach is based on comparing the full PPG waveform between two points along the

artery rather than measuring the time-of-flight. Minimizing the measurement separation and confining the measurement area to a single, well-defined artery allows the waveform to retain the general shape between the two measurement points. This allows signal processing of waveforms to determine the phase and amplitude changes.

Photoplethysmography, which measures changes in arterial blood volume, is commonly used to obtain heart rate and blood oxygen saturation. The digitized PPG signals are used as inputs into the beat-to-beat blood pressure measurement algorithm. The algorithm consists of the following main components:

- First harmonic isolation bandpass filters take the raw PPG signals and separate out the first harmonics.
- Three harmonic lowpass filters take the PPG signal and filter out all spectral components outside the first three harmonics. The first three harmonics are used for regeneration of the pulse pressure waveforms.
- Phase analysis engine takes the first harmonics of the PPG signals and computes the phase difference between them in real time using a cross-correlation-based algorithm. The phase difference is to the first order correlated to the MAP (mean arterial pressure).
- Compliance estimation engine takes information on the general shape of the waveforms and the phase delay to compute the local compliance of the artery. The higher the arterial pres-

sure, the higher the Young's modulus and thus the lower the compliance.

- MAP computation engine obtains the phase delay and compliance information and provides the mean arterial pressure.
- Waveform analysis engine takes the PPG signal containing the first three harmonics and provides the signal processing needed for compliance (elasticity) estimation and pulse pressure computation.
- Pulse pressure computation engine takes the filtered PPG signal and an estimate of the arterial compliance to regenerate the pulse waveform.
- B3P computation engine takes the MAP and the pulse pressure computations and combines them with a blood pressure model and calibration data to produce the final signal of interest — the beat-to-beat blood pressure.

*This work was done by Yong Jin Lee of Linea Research Corporation for Johnson Space Center. Further information is contained in a TSP (see page 1).*

*In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:*

*Linea Research Corporation  
1020 Corporation Way  
Suite 216  
Palo Alto, CA 94303*

*Refer to MSC-24601-1, volume and number of this Medical Design Briefs issue, and the page number.*