ures features of reflected and transmitted ultrasound signals, and correlates these signals with bone structure metrics such as bone mineral density, trabecular spacing, and thickness, etc. The techniques used to determine these various metrics require measurements over a broad range of ultrasound frequencies, and therefore, complete characterization requires the use of several narrowband transducers.

This is a single transducer capable of making these measurements in all the required frequency bands. The device achieves this capability through a unique combination of a broadband piezoelectric material; a design incorporating multiple resonator sizes with distinct, overlapping frequency spectra; and a micromachining process for producing the multiple-resonator pattern with common electrode surfaces between the resonators.

This device consists of a pattern of resonator bars with common electrodes that is wrapped around a central mandrel such that the radiating faces of the resonators are coplanar and can be simultaneously applied to the sample to be measured. The device operates as both a source and receiver of acoustic energy. It is operated by connection to an electronic system capable of both providing an excitation signal to the transducer and amplifying the signal received from the transducer. The excitation signal may be either a wide-bandwidth signal to excite the transducer across its entire operational spectrum, or a narrow-bandwidth signal optimized for a particular measurement technique. The transducer face is applied to the skin covering the bone to be characterized, and may be operated in through-transmission mode using two transducers, or in pulse-echo mode.

The transducer is a unique combination of material, design, and fabrication technique. It is based on single-crystal lead magnesium niobate lead titanate (PMN-PT) piezoelectric material. As compared to the commonly used piezoceramics, this piezocrystal has superior piezoelectric and elastic properties, which results in devices with superior bandwidth, source level, and power requirements. This design necessitates a single resonant frequency. However, by operating in a transverse length-extensional mode, with the electric field applied orthogonally to the extensional direction, resonators of different sizes can share common electrodes, resulting in a multiply-resonant structure. With carefully sized resonators, and the superior bandwidth of piezocrystal, the resonances can be made to overlap to form a smooth, wide-bandwidth characteristic.

This work was done by Yu Liang and Kevin Snook of TRS Technologies, Inc. for Glenn Research Center. Further information is contained in a TSP (see page 1).

Inquiries concerning rights for the commercial use of this invention should be addressed to NASA Glenn Research Center, Innovative Partnerships Office, Attn: Steven Fedor, Mail Stop 4–8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-18842-1.

Fluorescence-Activated Cell Sorting of Live Versus Dead Bacterial Cells and Spores

Commercial applications include hospital operating room cleanliness validation assays, pharmaceutical development, and semiconductor development.

NASA's Jet Propulsion Laboratory, Pasadena, California

This innovation is a coupled fluorescence-activated cell sorting (FACS) and fluorescent staining technology for purifying (removing cells from sampling matrices), separating (based on size, density, morphology, and live versus dead), and concentrating cells (spores, prokaryotic, eukaryotic) from an environmental sample.

Currently, the state of the art is limited to the sorting of larger eukaryotic cells (e.g., yeast, mammalian). Over the past decade, cell sorting technologies have evolved significantly and sensitivity levels have increased remarkably, rendering bacterial cell sorting a feasible concept. In parallel, optimized protocols for broad-spectrum fluorescence staining of bacterial cells and spores have been established, most of which are based on nucleic acid-intercalating dyes.

Smaller DNA-intercalating dyes, such as SYTO-9, permeate the intact membrane of living, viable cells and spores and upon excitation with white light, emit a detectable signal such as the green spectra emitted by DNA-bound SYTO-9. A larger DNA-intercalating dye such as 7- amino actinomycin (7-AAD), which is unable to permeate the membranes of healthy, viable cells and spores and thus only able to access the DNA of dead or dying cells and spores through compromised membranes, is also applied to the sample. This larger dye is engineered to fluoresce red spectra upon excitation. Ergo, the membranes of healthy, viable bacterial cells and spores preclude the infiltration of the larger red dyes (which have a greater affinity for DNA than the smaller green dyes) and as a result, their DNA fluoresces green. The DNA of dead or dying cells and spores fluoresces red as a result of the high-affinity binding and of the larger red dyes. This motif makes possible the ability to sort and segregate live from dead bacterial cells and spores via fluorescence staining.

This technology directly contributes to NASA missions as it focuses on the separation, purification, and concentration of cells or spores from a given spacecraft or associated facility sample. Coupling live/dead fluorescence dyes and flow cytometry enhances the resolving power of any attempt at predicting the microbial genetic that actually poses a forward contamination threat. The capability to provide an account of the living organisms present on spacecraft surfaces, to the exclusion of the expired population, will facilitate much more accurate predictive risk assessments of forward contamination on missions with challenging planetary protection issues. A specific account of only the living microbial population will also allow for immediate feedback to a project as to the success of cleaning, microbial reduction, and general housekeeping processes.

This work was done by James N. Benardini, Myron T. La Duc, Rochelle Diamond, and Josh Verceles of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1). NPO-48176