signal representing a pixel at the focal point in the specimen. Scanning along the depth (Z) axis is accomplished by using the piezoelectric transducer to move the reference mirror closer to, or farther from, the light source. Scanning along the X and Y axes is accomplished by mechanical motion of the probe along X and Y.

The lower part of Figure 1 depicts a typical instrumental response (point-spread function) in the photodetector output obtained in scanning along the Z axis. The response includes oscillations attributable to interference between the light scattered from a point in the specimen and light scattered from the mirror. As the Z displacement increases, the contrast of the interference pattern is reduced by the loss of coherence. Usually, the envelope of the oscillations (in contradistinction to the oscillations themselves) is what is measured. In such a case, the width of the envelope and, thus, the depth resolution, is comparable to the coherence length of the light source.

Figure 2 includes a simplified schematic representation of the optical subsystem of a 3D OCT system. This system is based partly on the same principles as those of the prior system. However, there are several important differences:

- Light from the source is fed through a more-complex fiber-optic subsystem, not only to a photodiode but to three single-mode optical fibers on a probe. Light emerging from the tips of these three fibers illuminates the specimen and creates a 3D interference pattern in the specimen.
- Light scattered from the specimen is collected and sent to the photodetector by a wider, multimode optical fiber. The probe containing the illuminating single-mode fibers and the light-collecting multimode optical fibers is significantly smaller and more rugged, relative to a lens-containing probe in a prior OCT system.
- Instead of utilizing lenses and a piezoelectric actuation of a reference mirror to effect scanning in Z and focusing in conjunction with mechanical scanning in X and Y, the system utilizes a combination of (1) amplitude modulation of the light in the three illuminating optical fibers and of a portion of the source light sent directly to the photodetector, (2) nonlinear detection, and (3) an advanced signal-processing technique that, among other things, exploits the 3D nature of the interference pattern in order to obtain (4) a 3D point-spread function that affords localization in X, Y, and Z. In principle, because mechanical scanning is no longer necessary, it is possible to achieve scanning at a video frame rate.

This work was done by Mikhail Gutin, Xu-Ming Wang, and Olga Gutin of Applied Science Innovations, 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: Steve Fedor, Mail Stop 4–8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-18352-1.

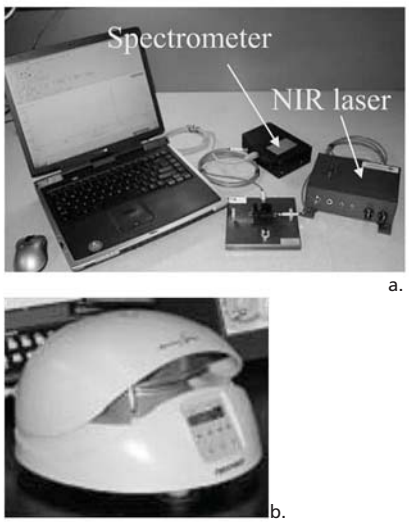
Benchtop Antigen Detection Technique Using Nanofiltration and Fluorescent Dyes

This technique can help to monitor the quality of water by testing for contamination at restaurants, water treatment plants, and food processing plants.

John H. Glenn Research Center, Cleveland, Ohio

The designed benchtop technique is primed to detect bacteria and viruses from antigenic surface marker proteins in solutions, initially water. This inclusive bio-immunoassay uniquely combines nanofiltration and near infrared (NIR) dyes conjugated to antibodies to isolate and distinguish microbial antigens, using laser excitation and spectrometric analysis. The project goals include detecting microorganisms aboard the International Space Station, space shuttle, Crew Exploration Vehicle (CEV), and human habitats on future Moon and Mars missions, ensuring astronaut safety. The technique is intended to improve and advance water contamination testing both commercially and environmentally as well. Lastly, this streamlined technique poses to greatly simplify and expedite testing of pathogens in complex matrices, such as blood, in hospital and laboratory clinics.

The approach relies on NIR fluorescent dyes derivatized to specific antibody sets that are selected to bind and differentiate microbial surface proteins, termed antigens. In a solution containing an antigenic slurry, NIR conjugated antibodies are added to the mixture, and will bind to the respective antigens if present. To eliminate any false positives, excess antibodies, i.e., those antibodies not bound to an antigenic protein or those with no respective antigen present, are removed via the nanofiltration process using a portable, table-top centrifuge. The remaining NIR dye/antibody and antigenic protein pairs left on the nanofilter are transferred to cuvette, excited by an NIR laser, and detected by spectrometer. Using simple computer software, the results are easily interpreted as intensity peaks at the appropriate NIR offset wavelength emission.



The **Benchtop Analysis System** (a) consists of a medium-power, class IV laser, a four-port cuvette sampler holder, a spectrometer, laptop and software, and fiber-optic cables. (b) The microcentrifuge used in nanofiltration of the complexes.

Initial data reveal the assay sensitively identified antigens at intensity counts of 100 IC or higher (or roughly 36 pW) with an accuracy of 85 percent for 2-

hour incubations and 75 percent for 3-hour incubations. Interestingly, samples incubated for less time (2 hours vs 3 hours) produced an increased percentage of antigen detection. Further testing at incubation times such as 1 hour or lower could potentially increase positive predictability based on the study's results. Also encouraging were negative control experiments with nonspecific antigens, beta galactosidase and thyroglobulin, which showed results of 100 percent accuracy, with no false positive detection.

This work was done by Maximilian C. Scardelletti and Vanessa Varaljay of 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: Steve Fedor, Mail Stop 4–8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-18387-1.

Isolation of Precursor Cells From Waste Solid Fat Tissue

Lyndon B. Johnson Space Center, Houston, Texas

A process for isolating tissue-specific progenitor cells exploits solid fat tissue obtained as waste from such elective surgical procedures as abdominoplasties ("tummy tucks") and breast reductions. Until now, a painful and risky process of aspiration of bone marrow has been used to obtain a limited number of tissue-specific progenitor cells.

The present process yields more tissue-specific progenitor cells and involves much less pain and risk for the patient. This process includes separation of fat from skin, mincing of the fat into small pieces, and forcing a fat saline mixture through a sieve. The mixture is then digested with collagenase type I in an incubator. After centrifugation tissue-specific progenitor cells are recovered and placed in a tissue-culture medium in flasks or Petri dishes. The tissue-specific progenitor cells can be used for such purposes as (1) generating three-dimensional tissue equivalent models for studying bone loss and muscle atrophy (among other deficiencies) and, ultimately, (2) generating replacements for tissues lost by the fat donor because of injury or disease.

This work was done by Diane Byerly of Johnson Space Center and Marguerite A. Sognier of Universities Space Research Association. Further information is contained in a TSP (see page 1). MSC-23775-1

Identification of Bacteria and Determination of Biological Indicators

Identifying mechanisms of micro-organisms can prevent forward contamination in space missions and can help in developing new antibiotics and amino acids.

NASA's Jet Propulsion Laboratory, Pasadena, California

The ultimate goal of planetary protection research is to develop superior strategies for inactivating resistance-bearing micro-organisms like *Rummelibacillus stabekisii*. By first identifying the particular physiologic pathway and/or structural component of the cell/spore that affords it such elevated tolerance, eradication regimes can then be designed to target these resistance-conferring moieties without jeopardizing the structural integrity of spacecraft hardware. Furthermore, hospitals and government agencies frequently use biological indicators to ensure the efficacy of a wide range of sterilization processes. The spores of *Rummelibacillus stabekisii*, which are far more resistant to many of such perturbations, could likely serve as a more significant biological indicator for potential survival than those being used currently.

Numerous surveys of the contaminant microbial diversity housed within spacecraft assembly facilities over the past six years have resulted in the recurrent isolation of spore-forming bacteria belonging to the *Bacillus* genus. As *Bacillus* species are capable of existing as metabolically inactive, extremely hardy spores, many lineages exhibit remarkable resilience to varying modes of bioreduction/sterilization aimed at their eradication (UV and gamma radiation, oxidizing disinfectants, etc.). The microorganism *Rummelibacillus stabekisii sp. nov.* was isolated from the surfaces of the cleanroom facility in which the Mars Exploration Rovers (MER) underwent assembly. This bacterium has not been previously reported, and shows no close relation to any previously described species (as is assessed via 16S rRNA gene sequence comparison). This unique isolate, and the *Bacillus* species most genetically similar to it, were subjected to a multitude of biochemical tests in order to thoroughly characterize its taxonomic position based on physiological and phylogenetic evidence. The results clearly show that this bacterium is significantly different from its nearest relatives.

The microbial colonization of spacecraft and cleanroom assembly facility surfaces is of major concern to NASA and others commissioning modern-day space exploration. The search for life elsewhere in the solar system will rely heavily on validated cleaning and sterility methods. It would be devastating to the integrity of a mission directed at pristine environments such as the Europa's subsurface ocean or the Martian polar caps to be compromised as a result of terrestrial microbial contamination. To this end, planetary protection policies are in place to ensure the cleanliness and sterility of mission-critical spacecraft components in order to prevent forward or backward contamination.

Spores of *Bacillus subtilis*, a model spore-forming laboratory strain that demonstrates higher susceptibility to ultraviolet and gamma radiation than