

toring the antiproton cloud. Instead, the -6-dB directional couplers are used to receive the signal that emanates from the antiproton cloud when the cloud interacts with the applied signal. The received signal can be routed to either a spectrum analyzer or a network analyzer.

This work was done by William H. Sims and James Martin of Marshall Space Flight Center, and Raymond Lewis of RLewis Co. Further information is contained in a TSP (see page 1).

This invention is owned by NASA, and a patent application has been filed. Inquiries

concerning nonexclusive or exclusive license for its commercial development should be addressed to the Patent Counsel, Marshall Space Flight Center, (256) 544-0021. Refer to MFS-31780.

Tumbleweed Rovers

Lightweight balls containing scientific instruments are propelled across terrain by wind.

NASA's Jet Propulsion Laboratory, Pasadena, California

Tumbleweed rovers, now undergoing development, are lightweight, inflatable, approximately spherical exploratory robotic vehicles designed to roll across terrain, using only wind for propulsion. Tumbleweed rovers share many features with "beach-ball" rovers, which were discussed in several prior *NASA Tech Briefs* articles. Conceived for use in exploring remote planets, tumbleweed rovers could also be used for exploring relatively inaccessible terrain on Earth.

A fully developed tumbleweed rover would consist of an instrumentation package suspended in an inflated two-layer (nylon/polypropylene) ball. The total mass of the rover would be of the order of 10 kg, the diameter of the ball when inflated would be 2 meters, and the minimum wind speed needed for propulsion would be about 5 m/s. The instrumentation package would contain

a battery power supply, sensors, a Global Positioning System (GPS) receiver, and a radio transmitter that would send the sensor readings and the GPS position and time readings to a monitoring station via a satellite communication system. Depending on the specific exploratory mission, the sensors could include a thermometer, a barometer, a magnetometer (for studying the terrestrial magnetic field and/or detecting buried meteorites), a subsurface radar system (for measuring ice thickness and/or detecting buried meteorites), and/or one or two diametrically opposed cameras that would take the part of sending two side-looking images out.

In the planned Antarctic field test, a prototype tumbleweed rover was released at a location near the South Pole. Using the global Iridium satellite network to send information about its position, the

rover transmitted temperature, pressure, humidity, and light intensity data to NASA's Jet Propulsion Laboratory. The rover reached speeds of 30 km per hour over the Antarctic ice cap, and traveled at an average speed of about 6 km per hour. The test was designed to confirm the rover's long-term durability in an extremely cold environment, with the goal being eventual use of the device to explore the Martian polar caps and other planets in the solar system. On future Antarctic exploratory missions, tumbleweed rovers might be used to acquire sensor data for studies of global warming, ozone depletion, and impacts of meteorites.

*This work was done by Alberto Behar, Jack Jones, Frank Carsey, and Jaret Matthews of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1).
NPO-40499*

Two-Photon Fluorescence Microscope for Microgravity Research

The benefits of two-photon fluorescence microscopy are realized at reduced cost.

John H. Glenn Research Center, Cleveland, Ohio

A two-photon fluorescence microscope has been developed for the study of biophysical phenomena. Two-photon microscopy is a novel form of laser-based scanning microscopy that enables three-dimensional imaging without many of the problems inherent in confocal microscopy. Unlike one-photon optical microscopy, two-photon microscopy utilizes the simultaneous nonlinear absorption of two near-infrared photons. However, the efficiency of two-photon absorption is much lower than that of one-photon absorption, so an ultra-fast pulsed laser source is typically employed.

On the other hand, the critical energy threshold for two-photon absorption leads

to fluorophore excitation that is intrinsically localized to the focal volume. Consequently, two-photon microscopy enables optical sectioning and confocal performance without the need for a signal-limiting pinhole. In addition, there is a reduction (relative to one-photon optical microscopy) in photon-induced damage because of the longer excitation wavelength. This reduction is especially advantageous for *in vivo* studies. Relative to confocal microscopy, there is also a reduction in background fluorescence, and, because of a reduction in Rayleigh scattering, there is a 4 \times increase of penetration depth.

The prohibitive cost of a commercial two-photon fluorescence-microscope sys-

tem, as well as a need for modularity, has led to the construction of a custom-built system (see Figure 1). This system includes a coherent mode-locked titanium:sapphire laser emitting 120-fs-duration pulses at a repetition rate of 80 MHz. The pulsed laser has an average output power of 800 mW and a wavelength tuning range of 700 to 980 nm, enabling the excitation of a variety of targeted fluorophores. The output from the laser is attenuated, spatially filtered, and then directed into a confocal scanning head that has been modified to provide for side entry of the laser beam. The laser output coupler has been replaced with a dichroic filter that reflects the longer-wavelength excitation

light and passes the shorter-wavelength fluorescence light. Also, the confocal pinhole has been removed to increase the signal strength.

The laser beam is scanned by a two-perpendicular-axis pair of galvanometer mirrors through a pupil transfer lens into the side port of an inverted microscope. Finally, the beam is focused by a 63-magnification, 1.3-numerical-aperture oil-immersion objective lens onto a specimen. The pupil transfer lens serves to match the intermediate image planes of the scanning head and the microscope, and its location is critical.

In order to maximize the quality of the image, (that is, the point spread function of the objective lens for all scan positions), the entire system was modeled in optical-design software, and the various free design parameters (the parameters of the spatial-filter components as well as the separations of all of the system components) were determined through an iterative optimization process. A modular design was chosen to facilitate access to the optical train for future fluorescence correlation spectroscopy and fluorescence-lifetime experiments.

The spatial resolution of the microscope at an excitation wavelength of 780 nm was measured by scanning a 170-nm-diameter fluorescent bead throughout the focal region and found to be 320 nm in the transverse direction and 740 nm in the longitudinal direction. The sectioning capability of two-photon microscopy is demonstrated in Figure 2, which depicts a human bone specimen as imaged by use of two-channel spectral detection.

The two-photon microscope is now being employed to study osteoblast and osteoclast bone cells in cultures, including the effects of fluid flow and other environmental stimuli. Ultimately, these studies will be used to investigate and develop effective countermeasures to the bone loss experienced in a reduced-gravity environment. In addition, a variant of this instrument is being considered as an add-on module to the Light Microscopy Module, which will be deployed on the International Space Station.

This work was done by David G. Fischer and Gregory A. Zimmerli of Glenn Research

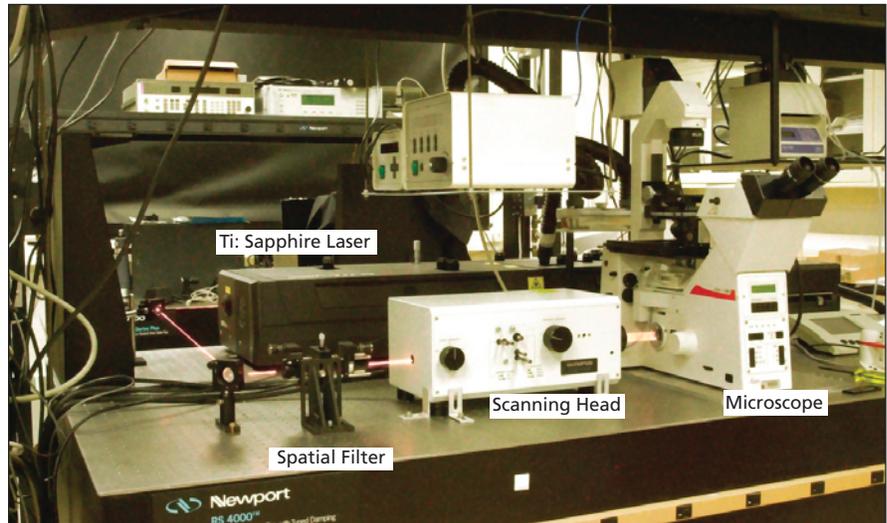


Figure 1. This Optoelectronic System is a custom-built system for two-photon fluorescence microscopy.

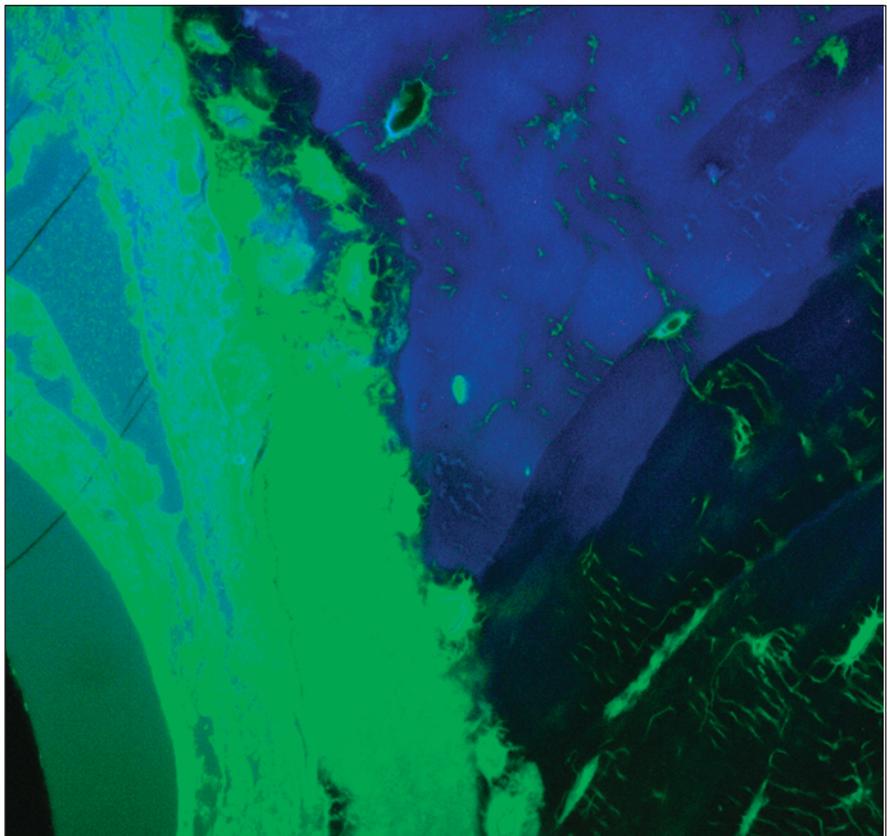


Figure 2. This Two-Photon Image of a human femur section shows several areas of remodeled bone. The field of view is 200 μm . The specimen in this image was provided by the Cleveland Clinic Foundation.

Center and Marius Asipauskas of the National Center for Microgravity Research.

Inquiries concerning rights for the commercial use of this invention should be addressed

to NASA Glenn Research Center, Commercial Technology Office, Attn: Steve Fedor, Mail Stop 4-8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-17573-1.