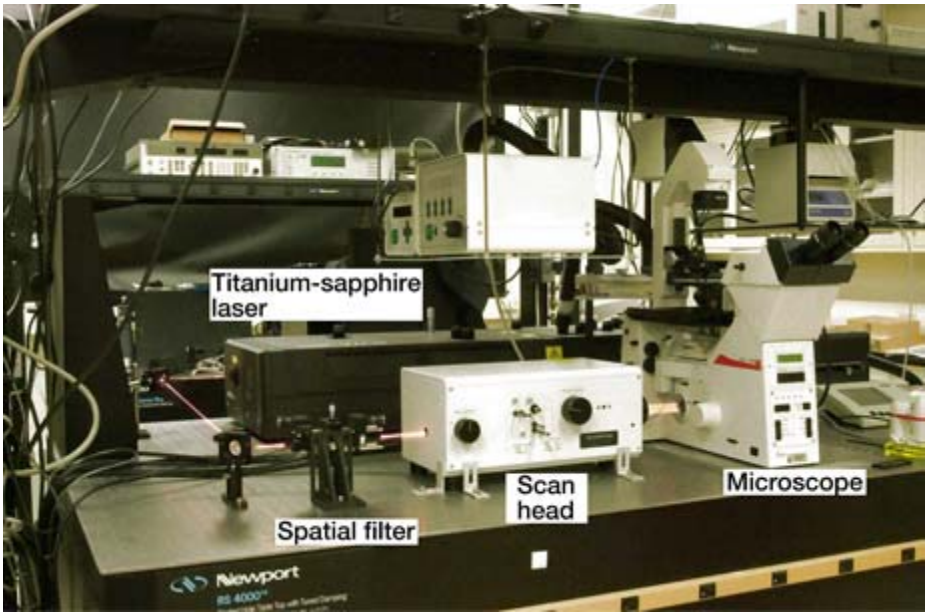
Two-Photon Fluorescence Microscopy Developed for Microgravity Fluid Physics

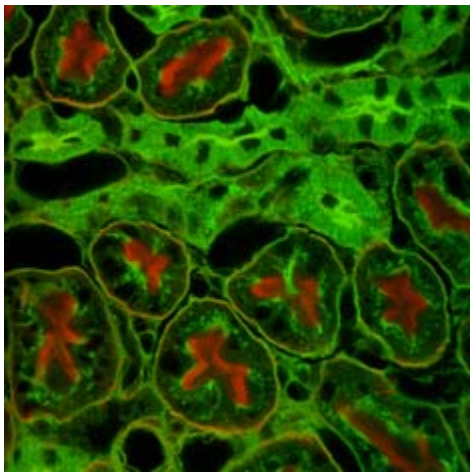
Recent research efforts within the Microgravity Fluid Physics Branch of the NASA Glenn Research Center have necessitated the development of a microscope capable of high-resolution, three-dimensional imaging of intracellular structure and tissue morphology. Standard optical microscopy works well for thin samples, but it does not allow the imaging of thick samples because of severe degradation caused by out-of-focus object structure. Confocal microscopy, which is a laser-based scanning microscopy, provides improved three-dimensional imaging and true optical sectioning by excluding the out-of-focus light. However, in confocal microscopy, out-of-focus object structure is still illuminated by the incoming beam, which can lead to substantial photobleaching. In addition, confocal microscopy is plagued by limited penetration depth, signal loss due to the presence of a confocal pinhole, and the possibility of live-cell damage.

Two-photon microscopy is a novel form of laser-based scanning microscopy that allows three-dimensional imaging without many of the problems inherent in confocal microscopy. Unlike one-photon microscopy, it utilizes the nonlinear absorption of two near-infrared photons. However, the efficiency of two-photon absorption is much lower than that of one-photon absorption because of the nonlinear (i.e., quadratic) electric field dependence, so an ultrafast pulsed laser source must typically be employed. On the other hand, this stringent energy density requirement effectively localizes fluorophore excitation to the focal volume. Consequently, two-photon microscopy provides optical sectioning and confocal performance without the need for a signal-limiting pinhole. In addition, there is a reduction in photodamage because of the longer excitation wavelength, a reduction in background fluorescence, and a ×4 increase in penetration depth over confocal methods because of the reduction in Rayleigh scattering.



Optical system for two-photon fluorescence microscopy.

For these obvious advantages, a two-photon scanning microscope system was selected for development. The prohibitive cost of a commercial two-photon system, as well as a desired modularity, led to the construction of a custom-built system. It employs a Coherent mode-locked titanium:sapphire laser (Coherent, Inc., Santa Clara, CA) emitting 120 fsec pulses over a tuning range of 700 to 980 nm, allowing the excitation of a variety of targeted fluorophores. The ultrafast laser was interfaced with an Olympus (Tokyo, Japan) confocal scan head and a Leica Microsystems AG (Wetzlar, Germany) inverted microscope (shown in the preceding photograph), and optimal coupling of the hybrid system involved the design and optimization of both beam conditioning and transfer optics. In addition, an integrated software environment was developed for *x*-*y*-*z* scanning control as well as image acquisition and processing. A modular design was chosen to allow easy 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 fluorescent bead throughout the focal region. The resolution was found to be 320 nm in the transverse direction and 740 nm in the longitudinal direction. The improved resolution and depth-sectioning capability of two-photon microscopy is illustrated in the following photograph, which shows a multiple-stained mouse kidney section that was imaged using two-channel spectral detection.



Multiple-channel two-photon fluorescence image of mouse kidney cells. The field of view is 134 μm square.

Glenn contacts: Dr. David G. Fischer, 216-433-6379, David.G.Fischer@nasa.gov; and Dr. Gregory A. Zimmerli, 216-433-6577, Gregory.A.Zimmerli@nasa.gov
Authors: Dr. David G. Fischer, Dr. Gregory A. Zimmerli, and Marius Asipauskas
Headquarters program office: OBPR
Programs/Projects: Microgravity Science