

C O R N E L L
R E S E A R C H

Researchers produce images showing neurotransmitter in live cells

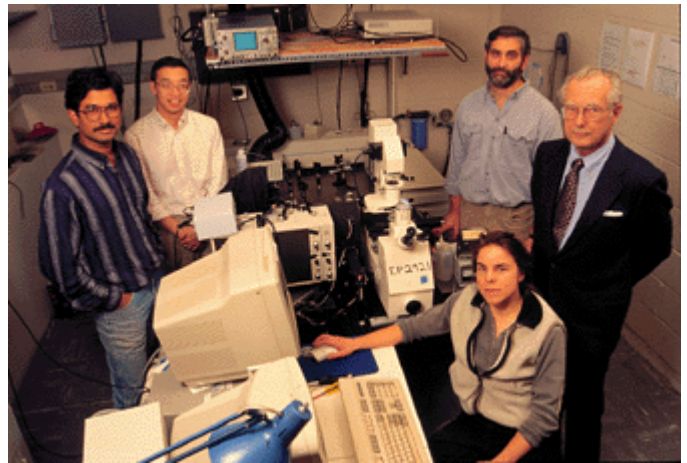
Watt W. Webb, professor of applied and engineering physics, far right, poses with his research team, from left: Sudipta Maiti, postdoctoral associate; Chris Xu, postdoctoral associate; Rebecca Williams, graduate student; and Warren Zipfel, research associate.

Frank DiMeo/University Photography

By Larry Bernard

Cornell researchers, using non-linear laser-microscope technology developed at Cornell, have produced images displaying the neurotransmitter serotonin in live cells in real time, and they have for the first time measured the concentration of serotonin in secretory granules.

The microscope, which uses pulsed lasers for excitation, can record ultraviolet (UV) fluorescence images of live cells without using UV illumination to detect and image cellular activity.



"This technique caught serotonin granules in the act of releasing the substance, without damaging the cell or changing the process and without requiring any external fluorescent marker," said Watt W. Webb, Cornell professor of applied and engineering physics, who led the work. "It is a new way of doing three-dimensional UV microscopy in functioning cells and the best way we know of for doing UV microscopy in thick tissues," Webb said.

The studies were reported in a paper, "Measuring serotonin distribution in live cells with three-photon excitation," published in the journal *Science* (Jan. 24, 1997) by Webb and Sudipta Maiti, a postdoctoral associate; Jason B. Shear, former National Science Foundation postdoctoral fellow now at the University of Texas at Austin; Rebecca M. Williams, physics graduate student; and Warren R. Zipfel, a research associate, all at Cornell.

Serotonin is a neurotransmitter that is becoming increasingly important as medical science learns of its role in a host of human disorders. It has been implicated in central nervous system disorders such as anxiety, depression, obsessive-compulsive disorder, schizophrenia, stroke, obesity, pain, hypertension, vascular disorders, migraine and even nausea. Serotonin is synthesized in brain neurons and is released upon a nerve impulse, where it interacts with receptors. The antidepressant Prozac (fluoxetine) is thought to treat depression by inhibiting re-

uptake of serotonin into cells from which secretion occurs.

The Cornell technology may be useful in gaining an understanding of a broad spectrum of physiological and psychological effects of this -- and other -- neurotransmitters. The technology could be useful, then, for researchers in designing more effective drugs for a host of disorders.

Previous efforts to image neurotransmitter secretory granules have not directly detected the neurotransmitter content or allowed visualization of secretory processes. But the Cornell technology, called non-linear laser scanning microscopy, can detect and image the serotonin and measure its concentration and the total neurotransmitter content of individual granules in intact cells.

Here is how it works: A laser in the 700 to 750 nanometer wavelength (infrared) fires photons bunched in very short pulses (each 10-13 seconds or a 10 millionth of a millionth of a second), which are focused by the microscope so that there is a high probability that three photons arrive at the same time (in about 10-16 seconds) at the same molecule near the focus. Molecules such as serotonin and tryptophan, which can normally be excited only with deep ultraviolet (~250nm) illumination, are now excited by simultaneously absorbing three infrared photons and, subsequently, fluorescence in the UV. These photons are collected as the laser is scanned through the specimen, and the resulting 3-D image can be viewed and analyzed on a computer monitor. For these studies, the researchers used basal leukemia cells from rats.

"This three-photon excitation produces very high energy corresponding to shorter absorption wavelengths than was possible before, without killing the cells," Webb said. "All you could see before this technique was diffuse brightness increase attributable to serotonin. But now we can see individual granules and we have a way to measure the serotonin in active cells."

Maiti said that serotonin absorbs wavelengths below 250 nanometers, a wavelength that is far shorter than the human eye can see or than can penetrate tissue without damage. So quantitative measurement of serotonin in live cells had not been possible. "There are molecules that act like pumps. They concentrate serotonin within a cell. We found very high concentrations, about 50 millimolar, in the secretory granules," he said.

Williams is following the release of serotonin from a cell as it occurs after allergic stimulation. "The larger granules secrete faster. Once the serotonin is secreted it is available in the tissue to stimulate swelling and fluid release," she said. The researchers used a pollen-like antigen to add to the solution that turned the signal on in the cell. As that was happening, the laser was illuminating the process to visualize the granules.

Webb said he hopes to use the technology to look for serotonin secretions deep inside the brain. The technology could be used for studies of broad range of areas.

Webb invented the technology for scanning laser microscopy in 1989 with Winfried Denk, now at AT&T Bell Labs, and Jim Strickler, now at McKinsey Co., and has been using it since for biophysical investigations with pre- and postdoctoral students. Cornell holds a patent on the technology, which recently has been licensed to Bio-Rad Laboratories of California.

Webb directs Cornell's Development Resource for Biophysical Imaging and Optoelectronics, funded by the National Institutes of Health and National Science Foundation. For 3-D image reconstruction, the researchers used the IBM SP2 supercomputer at the Cornell Theory Center, part of the NIH Parallel Processing Resource for Biomedical Scientists.

| [Cornell Chronicle Front Page](#) | | [Table of Contents](#) | | [Cornell News Service Home Page](#) |