

Lehigh University Center for Optical Technologies

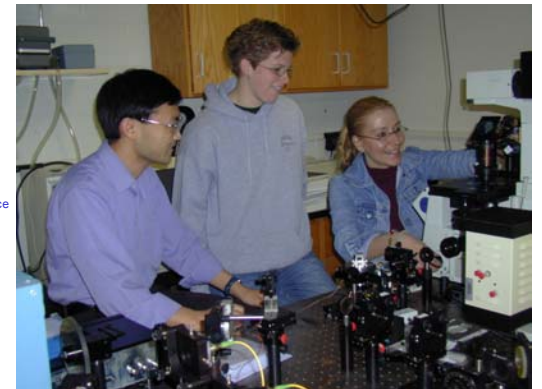
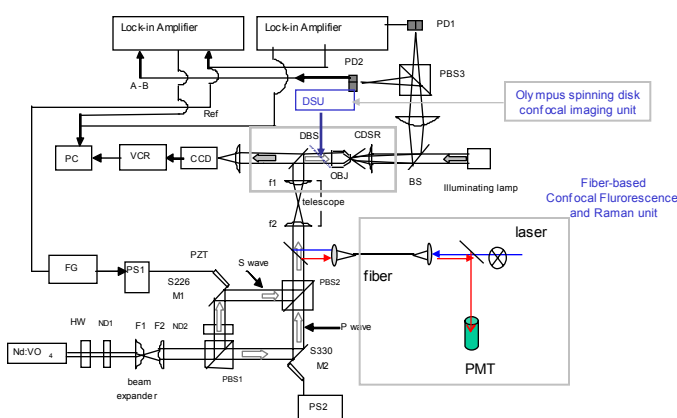
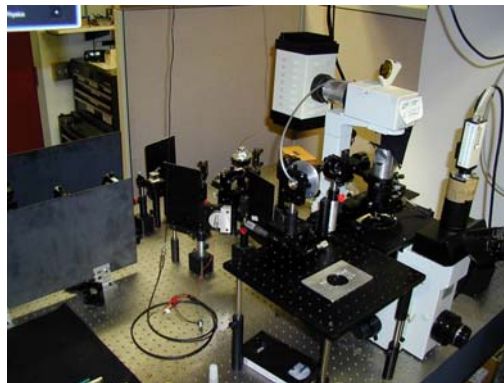
An Optical Tweezer-Based 5-D Photonics imaging System for Studying Cytoskeletal Dynamics in Living Cells

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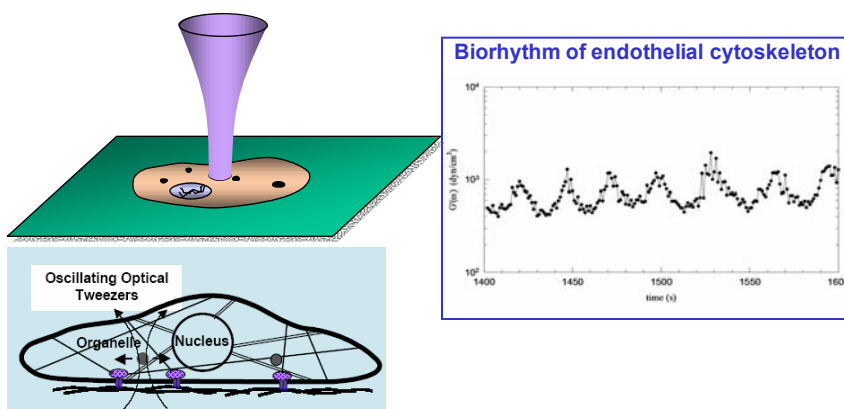
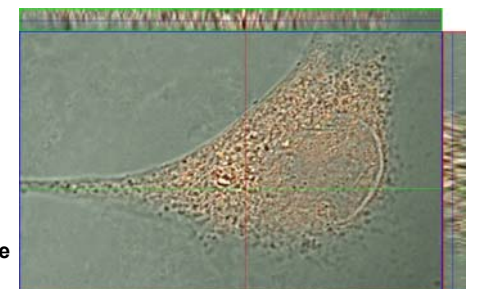
Abstract: Understanding the structural and mechanical properties of living cells is essential to gain insights to cellular signal transduction, intracellular molecular traffic and cell motility. We report the development of a new optical tweezers-based photonic sensor for studying cytoskeletal dynamics and mechanotransduction in living cells. We demonstrate how oscillating optical tweezers can be used to manipulate particles indigenous to the cell and, how the same laser that forms the tweezers is used to measure the motions of the optically trapped cellular structures from which the mechanical properties of the cytoskeleton are measured. Contrast to that of purified cytoskeletal protein networks, mechanical properties of living cells are highly dynamical, and some times rhythmic. To understand the cytoskeletal dynamics and how cytoskeletal structural changes take place, optical tweezers measurements and confocal imaging must be made *in situ*. Our goal is to develop an optical tweezer-based, state-of-the-art biophotonic 5-D imaging system that can simultaneously detect structural, chemical and mechanical properties of living cells. Fiber-based confocal fluorescence and Raman unit further enables the system with high temporal resolution needed for fast cellular dynamics.



Green Optical Tweezers: A schematic of the optical tweezers (right photo above) is shown in the middle figure. We use a Spectra-Physics Millennium Nd:VO₄ laser (frequency doubled) at 532 nm wavelength. Splitting the initial laser beam with a polarizing beam splitter (PBS1) creates two laser beams. The first laser beam, P polarized, is steered by a piezo-controlled mirror S330-M2 (PI, Physik Instrumente) and forms the stationary optical tweezers. The second laser beam, S polarized, is steered by a high frequency piezo-driven mirror S226 (PI), and forms the oscillating optical tweezers. The two beams recombine at the polarizing beam splitter PBS2 before going into the microscope (Olympus IX-70, U-Plan-Apo high N.A. objective). Each beam will trap a micro particle. Since each beam can be steered independently, the distance between particles can be varied. The split-photodiodes PD1 and PD2 are used to determine the position of the particle from the intensities of the forwardly diffracted trapping laser light from each of the particles in the trap. The optical signals detected by PD1 and PD2 are fed into a lock-in amplifier, which measures the magnitude and the phase shift of the displacement of the particle from which mechanical properties of the materials in the vicinity of the probe particles are measured.

New IR Optical Tweezers integrated with spinning disk confocal imaging and fiber-based fluorescent and Raman spectroscopy detection: The new system (above) uses a Spectra Physics IR Nd:YAG laser at 1060 nm wavelength (less phototoxicity to biological systems). Built on a new fully automated Olympus IX-81 inverted microscope, the platform permits simultaneous operation of optical tweezers and a spinning disk (DSU) confocal imaging. The DSU can provide 5-D (xyz, wavelength and time lapse) imaging with approximately 100 nm spatial resolution at 5 – 15 frame per second. The fiber-based confocal fluorescent and Raman unit afford high temporal resolution due to the use of a high sensitivity PMT photon detector and the lock-in signal processing technique. Also in the photo are visiting research scientist Dr. Chuan Pu (left), graduate students Elizabeth Rickter (center) and Olga Latinovic.

Right: To identify of the structures trapped by the optical tweezers, a confocal fluorescent 3-D image of an endothelial cell was made, showing caveolin-1 & dynamin I/II co-localization with structures similar to those trapped by the tweezers. In the past, confocal images of the cell structures were taken by a confocal microscope different from the optical tweezers, it is difficult to make a firm conclusion of the identities of the trapped objects.



Above: the figures on the left show the top and side views of how optical tweezers are aligned to granular structures indigenous to endothelial cells and imparting oscillating mechanical forces to the trapped granule with minimal invasion. The same optical tweezers are used to detect motions of the granule with the sub-nanometer resolution. Figure on the right shows surprising rhythmic behavior of the cytoplasm elasticity. The newly discovered rhythmic behavior is found only in living cells (Biophotonics International, January 2004).

Opportunities and Challenges: Combining optical tweezers, photonics and optical imaging, we have open doors to discover new and exciting biological phenomena at the cell and molecular levels unimaginable only a decade ago. With the development of the new optical tweezer-based 5-D photonic imaging system, we will have unprecedented capabilities for measuring simultaneously the structural, chemical and mechanical properties of cells *in situ*. Fiber-based confocal fluorescence and Raman unit will further enable the system with high temporal resolution needed for fast cellular dynamics. The challenges are the availability of high efficiency fluorescent labels and optical imaging with the even better spatial resolution. Continuous advancements in the fluorescent protein gene construct techniques, high efficiency biocompatible fluorescent quantum dots and the multiphoton confocal microscopy will open new possibilities for photonic imaging that may be adoptable for *in vivo* applications.

Acknowledgments:

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