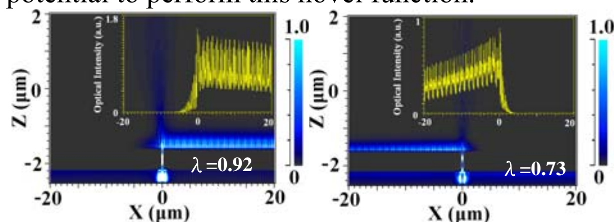
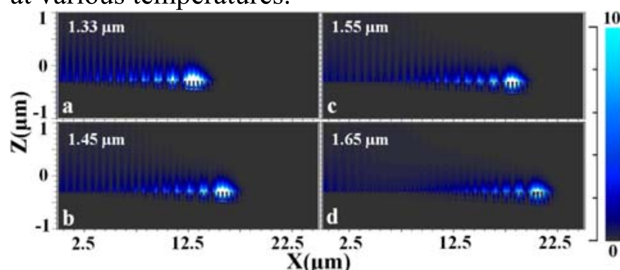
Center for Optical Technologies
Biophotonics Group Research Activities, October 13, 2008

Bidirectional surface wave splitter and trapped rainbow storage of light through nanoplasmonics: Q. Gan, Y. J. Ding, and F. J. Bartoli (fjb205@Lehigh.edu), *Lehigh University*. The primary goal is to understand the physics of surface plasmons and for application to integrated optics and bio-sensing. We designed two different architectures for integrated optics:

(1) Bidirectional surface wave splitter: We employ different surface waveguides on two sides of a central slit and guide waves at different frequencies in opposite predetermined directions on-a-chip, which is suitable for highly compact optical integration. Many schemes for coupling laser light into SPP modes have been demonstrated using prisms, gratings, and nanoscale defects (such as surface protrusions or holes). However, what has been missing in the past is an effective method for ensuring that the generated SPPs only travel in the desired directions from a subwavelength scale launching point. Consequently, our design has the potential to perform this novel function.



(2) Graded grating structure for trapped rainbow storage of light: We designed a device for slowing down surface electromagnetic waves, using metallic grating structures with graded depths, whose dispersion curves and cutoff frequencies are different at different locations. Since the group velocity of surface plasmons at the cutoff frequency is extremely low, light waves of different frequencies are actually stopped at different positions along the grating. The separation between stopped waves can be tuned by changing the grade of the grating depths. This structure offers the advantage of reducing the speed of the light over an ultra-wide spectral band, and the ability to operate at various temperatures.



Design and application of optical tweezers to quantify dielectrophoretic force: J. Junio, M.-T. Wei, H.D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. The dielectrophoretic force (DEP) on a dielectric particle in a non-uniform electric field is a function of the material property of the particle, the charge activities in the vicinity of the interface of the particle and the surrounding medium, as well as the property of the suspending medium itself. For colloidal particles in an aqueous suspension, the ionic mobility in the shear layer near the particle-water interface is a strong function of the field frequency, giving rise to a strong frequency dependent DEP force. More interestingly, in aqueous colloids, the force can change signs at a so-called cross-over frequency in the RF range. In order to use the DEP force for particle manipulation in a device such as a micro-fluidic chamber for particle sorting, an accurate calibration of the magnitude of the force and the crossover frequency is necessary; however, such a calibration has been missing so far. It is the lack of reliable experimental methods for characterizing the frequency dependent DEP force for comparison with theoretical predictions that impedes the progress in a fundamental understanding of the complex variables that attribute to the DEP effects at the microscopic level. Recently, using optical tweezers as force sensor we have successfully characterized the frequency dependent DEP force with a spatial resolution in the micron range and a force resolution of a fraction of 1pN. To achieve this, we used an AM modulation scheme to administer the oscillating electric field, so that we could monitor the phase and amplitude of the displacement of the particle while it was held by the optical tweezers and acted on by the DEP force. As the applied DEP field frequency approaches the cross-over frequency, the DEP force on the particle approaches zero but the force switches its direction from toward the high field to away from the high field or vice-versa. By the use of a lock-in phase sensitive detection method operating at the second harmonic of the AM frequency, we can determine precisely where the cross-over frequency is by monitoring where the phase of the particle motion relative to that of the AM field shifts 180 degrees while the magnitude of the particle displacement approaches zero. The figures below show the magnitude of the DEP force on a 1.5μm particle located in the field near the edge of a quadruple electrode. We are at a

position to apply this new technique to characterize DEP forces for particles of different size, composition and surface properties. Comparison with existing theories with our experimental results and exploration of potential applications of our method for medical diagnostics and sorting are in progress.

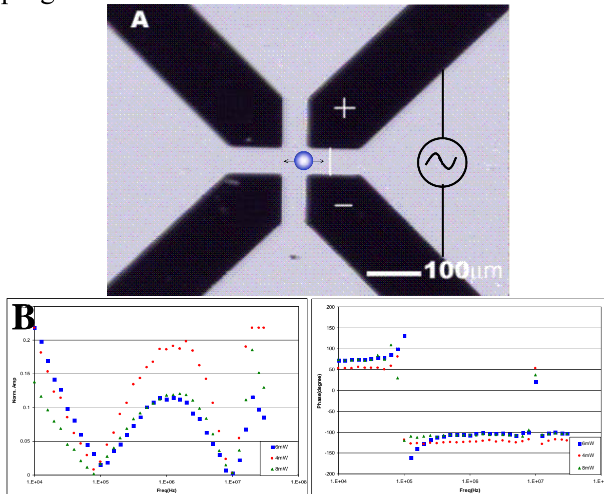


Fig. (A) Micrograph of a trapped particle in DEP chamber used to measure dielectrophoretic force in experiment. (B) The data displays the frequency dependent amplitude (top B) and phase (bottom B) recorded from a particle trapped in water under the influence of the DEP force field.

Probing intermolecular interactions in complex biosystems: Erin D. Sheets (eds11@psu.edu), *Penn State University*. During the past year, we continued our investigations into the dynamics of molecular interactions occurring in living cells and model systems mimicking biological processes, as measured with quantitative fluorescence microscopy and spectroscopy. In one project [1], we showed that in mammalian cells, enzyme complexes form transiently when required to form purines, which are essential for DNA and RNA synthesis and cell signaling. These protein complexes may become attractive therapeutic targets. In collaboration with Prof. Ahmed Heikal (Penn State Bioengineering) [2], we showed that the plasma membrane nanostructure of immune cells (mast cells) changes in response to stimulation by an antigen. Cholesterol-rich nanodomains in the biological membrane have been postulated to participate in a variety of cellular functions; however these nanodomains are too small and transient to resolve optically. We developed a quantitative and sensitive means of monitoring membrane nanostructure, based on ultrafast dynamics imaging that allows us to effectively beat optical resolution. Our work shows for the first time a direct correlation between changes in membrane nanostructure and cell function (that is, activation of mast cell signaling, which is critical in the allergic response) under physiological conditions and in real time. What makes this paper particular exciting is that it bridges the gap between many cell biological and biochemical studies and live cell imaging. We also developed an approach for patterning arrays of membrane microdomains on solid supports (e.g., glass) and showed that it is essential to characterize these complex biomimetic membranes for their use in biosensor and other applications [3]. We also developed a protein-free model system to mimic the interactions that occur between membrane systems prior to exocytosis (the regulated release of contents in response to neurotransmission, the allergic response, etc.), which is shown in the Figure [4]. We investigated the effects of lipid composition, ionic strength and pH on the dynamics of vesicles that are diffusing very close to another membrane, and our results suggest that vesicles may be spatially restricted by altering local physiological conditions may represent an additional mechanism for controlling fusion dynamics.

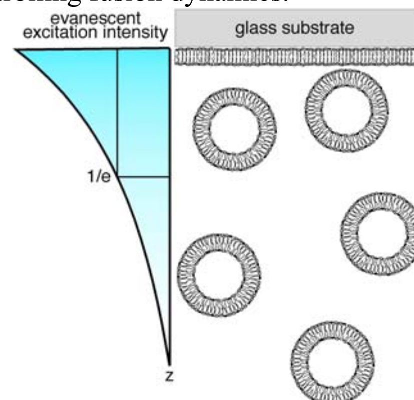


Fig. Small vesicles that mimic synaptic vesicles participating in neurotransmission (for example) interact with a planar target membrane of a different lipid composition, which mimics the plasma membrane. The dynamics of the fluorescently labeled vesicles that are diffusing within ~100nm of the target membrane are measured with total internal reflection fluorescence correlation spectroscopy.

Reference:

[1] An, S., R. Kumar, E.D. Sheets, and S.J. Benkovic. 2008. Reversible compartmentalization of de novo purine biosynthetic complexes in living cells. Science 320:103-106.

[2] Davey, A.M., K.M. Krise, E.D. Sheets, and A.A. Heikal. 2008. Molecular perspective of antigen-mediated mast cell signaling. J. Biol. Chem. 283:7117-7127.

[3] Vats, K., M. Kyoung, and E.D. Sheets. 2008. Characterizing the chemical complexity of patterned biomimetic membranes. Biochim. Biophys. Acta in press.

[4] Kyoung, M., and E.D. Sheets. 2008. Vesicle diffusion close to a membrane: intermembrane interactions measured with fluorescence correlation spectroscopy. Biophys. J. in press.

Three dimensional mapping of nanoparticles in localized fields: H. Y. Lin, H. D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. Dual beam fiber optical trapping of fluorescent nanoparticles was investigated by mapping the fluorescent image of the trapped particles by laser scanning confocal microscopy. A microfluidic channel was constructed using Polydimethylsiloxane (PDMS). The PDMS chamber includes two troughs for inserting two optical fibers so the fibers are facing each other and are at a variable distance. Each fiber was coupled to a 980 nm laser source. Due to the presence of the intense 980nm laser light, the radiation pressure generated by the laser caused an increase in the local number density of nanoparticles between the two opposing fibers. We are interested in measuring the trapping energy of the nanoparticles by the localized laser light and the interactions between the particles confined in the illuminated volume. We are also interested in investigating the cylindrical symmetrical periodicity of the optical fields along the optical axis caused by the interference of the two counter-propagating laser beams.

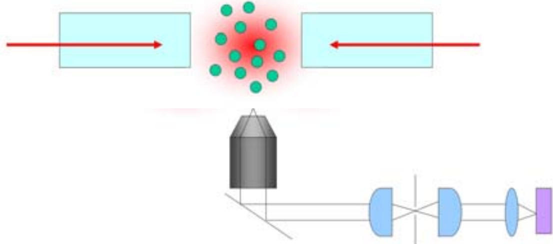


Fig. A schematic of measurement of the light field between two counter fibers.

References:
[1] M. Kawano et al., Opt. Exp. **16**, 9306 (2008).
[2] S. A. Tatarkova et al., Phys. Rev. Lett. **89**, 283901 (2002).
[3] W. Singer et al., J. Opt. Soc. Am. B **20**, 1568 (2003).

Measurements of the compressibility of colloidal suspensions by radiation pressure: J. Junio, H.D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. This project reports an experimental study of the interparticle interactions present in a model colloid system composed of fluorescently labeled 100nm diameter polystyrene particles in aqueous suspension. By independently measuring the fluorescence intensity as a function of particle number density, we were able to determine the relationship between the radiation pressure generated by the optical trap and the resulting number density increase, yielding the calculation of the isothermal compressibility of the colloid system. Optical trapping was made by a tightly focused and periodically blinking IR laser beam. A green laser beam, aligned co-linearly with the IR laser, was used as the fluorescence excitation light. The fluorescence signals from particles trapped by the blinking IR laser were measured by a lock-in amplifier to improve the signal to noise ratio required to detect the changes in local particle density induced by optical trapping. The use of confocal detection ensured that the fluorescent signals measured were only from the diffraction-limited focal region of the two laser beams.

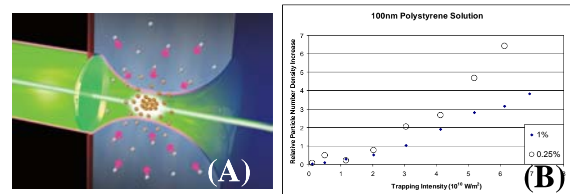


Fig. (A) A conceptual diagram of the optical trapping of multiple particles by IR laser and excitation by green laser (B) The plot shows the relative increase in particle number density in the focal region as a function of trapping laser intensity for two concentrations by mass, 1% and .25%. Notice the lower (.25%) concentration has a higher relative increase in particle number density, signifying the higher compressibility of a less concentrated solution. Once the compressibility is determined as a function of concentration, the interactions present in the system can be calculated.

References:
[1] A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, "Observation of a single-beam gradient force optical trap for dielectric particles," Opt. Lett. **11**, 288 (1986).
[2] J. Junio, E. Blanton, H. D.Ou-Yang, "The Kerr effect produced by optical trapping of nanoparticles in aqueous suspensions," SPIE 2007.

Determination of the Kerr coefficients of a nanoparticle suspension by intensity dependent transmittance: H. Y. Lin, C. Y. Lin, J. Y. Wang, H. D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. The objective of this research is to develop a new method for determining the Kerr coefficients of nanomaterials in suspension. DNA-wrapped single wall carbon nanotubes [(DNA-SWCNTs, a mixture of (n,m) = (7,5) and (6,5)] in aqueous suspension are selected as a model system for this project. Because these SWCNTs have absorption that is peaked at 990nm, a 980nm laser beam was used for this study. As shown in the figure below, the laser beam is focused on the DNA-SWCNT solution through a 10x objective (NA =0.3). Due to the radiation pressure generated by the focused laser beam, the DNA-SWCNT particles are pulled toward the focal point of the beam. In the region of the focal point, the increased concentration of DNA-SWCNTs reduces the relative optical transmittance as the laser intensity is increasing. We

have developed a theoretical model to calculate the Kerr coefficients from the laser intensity dependent transmittance. From the Kerr coefficients, we are able to determine the trapping energy per SWCNT as well as the interaction between the particles.

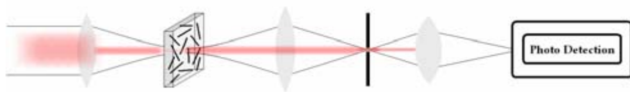


Fig. A schematic of the experimental setup for determine the Kerr coefficient of the aqueous suspension of DNA-SWCNTs.

A comparative study of living cell micromechanical properties by oscillatory optical tweezers: M.-T. Wei, A. Zaorski, H. C. Yalcin, J. Wang, M. Hallow, S. N. Ghadiali, A Chiou, and H. D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. Micromechanical properties of biological cells are crucial for cells functions. Despite extensive study by a variety of approaches, an understanding of the subject remains elusive. We conducted a comparative study of the micromechanical properties of cultured alveolar epithelial cells with an oscillatory optical tweezers. In this study, the frequency dependent viscoelasticity of these cells was measured by oscillatory optical tweezers of either an endogenous intracellular organelle (intra-cellular) or a 1.5μm silica bead attached to the cytoskeleton through trans-membrane integrin α_V receptors (extra-cellular). Both the storage modulus and the magnitude of the complex shear modulus followed weak power-law dependence with frequency (f = 1Hz ~ 1,000Hz), which is consistent with the soft glassy behavior of cellular materials. The intracellular oscillation of endogenous organelles may be more useful in investigating intracellular heterogeneity and temporal fluctuations.

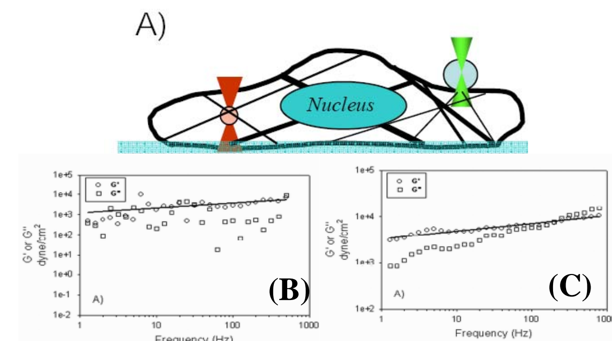


Fig. (A) A sketch of optical tweezer-based cytorheometer. Optical tweezers were used to manipulate an intracellular granular structure (lamellar body, left circle), or an extracellular anti-body coated glass bead (right circle); Experimental data obtained with human lung cells by (B) using intracellular organelles as probes (C) probing with anti-integrin conjugated silica beads attached to the plasma membrane: G'(ω) and G"(ω). Solid lines represent power-law fits to G'.

References:

[1] B. Fabry, G. N. Maksym, J. P. Butler, M. Glogauer, D. Navajas, and J. J. Fredberg, “Scaling the microrheology of living cells,” Phys. Rev. Lett. **87,** 148102 (2001).

[2] M.-T. Wei, A. Zaorski, H. C. Yalcin, J. Wang, M. Hallow, S. N. Ghadiali, A. Chiou, and H. Daniel Ou-Yang, “A comparative study of living cell micromechanical properties by oscillatory optical tweezers,” Opt. Express **16,** 8594 (2008).

Probing mechanotransduction in living cells with optical tweezers and fluorescent image: M.-T. Wei, L. Lowe-Krentz, C. Shu and H. D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. The ability to study how cells respond to mechanical stimulation and the dynamical micromechanical properties of living biological cells is crucial for understanding cells functions. To study the local cell signaling through lipid raft formation, caveolin transport, or structure transformation of Src proteins etc., in response to localized stresses, we are developing an experimental protocol that uses membrane adhering micro-beads to which a localized mechanical stress can be administered by optical tweezers. Endocytosed intracellular microbeads or endogenous intracellular structures are then used as probes for temporal fluctuations in local viscoelasticity. The dynamical responses of the signaling molecules and the intracellular viscoelasticity to mechanical stimulation are used to investigate mechanotransduction of living cells.

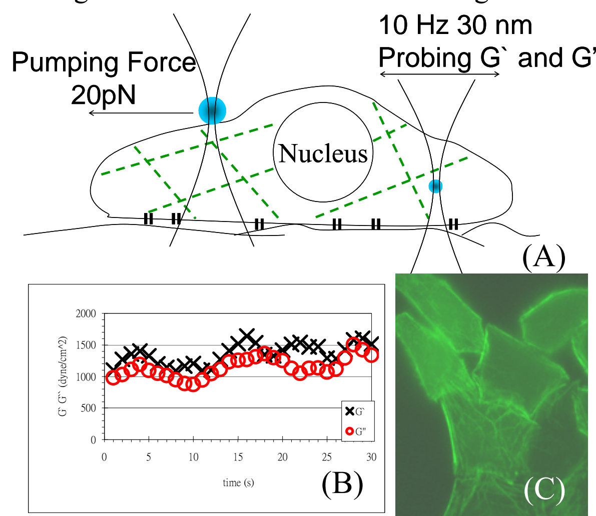


Fig. (A) A schematic diagram of a protein-coated bead adhered to the plasma membrane of a living cell (bead size not to scale). (B) Experimental data obtained from probing Hela cells with the use of the intracellular beads as probes shown as G' and G“ v.s. time. (C) A fluorescence image of Hela cells containing myosin-GFP.

Response of mitotic HeLa cells to mechanical perturbations: Yi Hu, M.-T. Wei, K. M. Weber, D. Vavylonis, and H. D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. The cortical actomyosin layer below the membrane of dividing cells and the astral microtubules of the mitotic spindle are coupled through internal feedback mechanisms that regulate their local and global mechanical properties. We developed an experiment to perturb mitotic HeLa cells with controlled localized forces and used fluorescence imaging to follow the cellular response. Anti-integrin coated microbeads were attached to HeLa cells expressing MRLC-GFP. We applied external forces to the beads by optical tweezers and tracked the fluorescence of the myosin-II in order to study the dynamics of the mitotic spindle during cell division. Experimental results showed that the mitotic spindle appeared to move to an asymmetric post perturbation position. This observation is consistent with the expected coupling between force dependent cortical flow and spindle position and will be useful in more detailed studies of the response of actin and microtubules to external or internal forces.

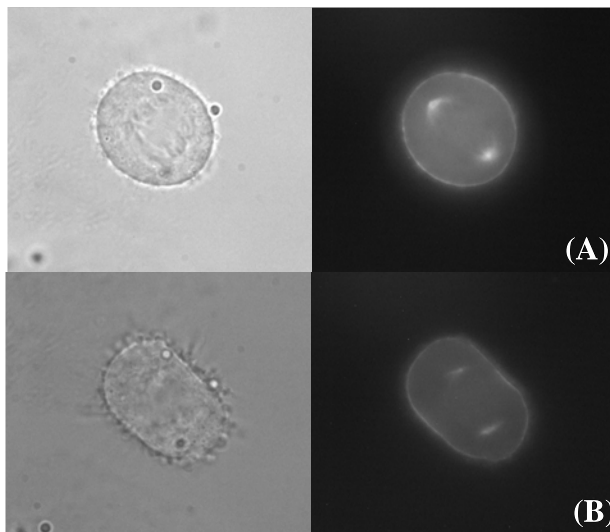
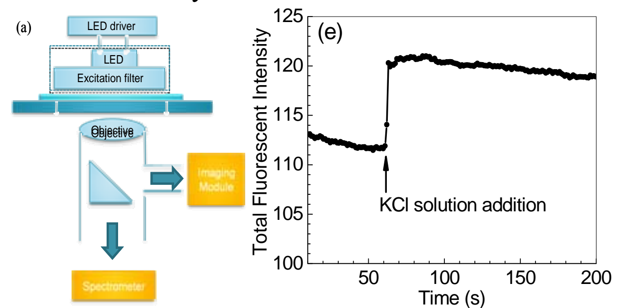


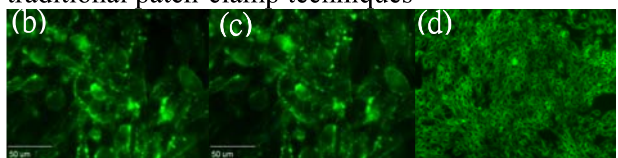
Fig. Bright field and fluorescence images of mitotic Hela cell expressing MRLC-GFP (A) a bead was attached to the cell and an oscillatory force was exerted on the bead for a few minutes. (B) Post-perturbation image of the cell has meanwhile progressed into cytokinesis. One of the poles of the mitotic spindle appears to have moved toward the bead.

References:
[1] Sanjay Kumar, et. al.," Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization, and extracellular matrix mechanics," Biophy. J. **90** 3762 (2006).
[2] Dimitrios Vavylonis, et al., "Cytokinesis by fission yeast assembly mechanism of the contractile ring for cytokinesis by fission yeast," Science **319**, 97 (2008).

Miniaturized fluorescence microscope for optical probing of membrane potential of in vitro neurons: Y. Wang, Y. Sun, Q. Gan, S. Perry, S. Tatic-Lucic, and F. Bartoli(fjb205@Lehigh.edu), *Lehigh University*. Integrated optical biosensors have significant advantages for applications requiring small size, low cost, and portability. For example a miniaturized fluorescence sensing system designed to probe patterned arrays of neurons should aid in research on memory, neurodegenerative diseases such as Alzheimer's, and neuropharmacology, and facilitate the screening of chemical libraries for drug discovery. We addressed current barriers to achieving this goal by significantly reducing the size of optical excitation subsystem through the proximal stacking of a small light-emitting diode (LED) source, excitation filtering, and cell-culturing substrate. This modified microscope, with a miniaturized LED illumination unit, can be used to effectively probe cells in a neuronal network. In our experiment, the modified microscope (shown in Fig.a) was used to image immortalized GT1-7 mouse hypothalamic neurons stained with voltage-sensitive fluorescent dyes and to probe their plasmas membrane potential change, which is directly responsible for signal propagation in the nervous system.



The system obtained high-quality of fluorescence images (Fig.b) comparable to those obtained by a conventional fluorescence microscope (Fig.c) and detected chemically membrane potential change of a monolayer (Fig.d) of neurons induced by chemical (KCl) stimulation (Fig.e). Such optical probing is also non-invasive and advantageous over traditional patch-clamp techniques



Characterization of bone cell proliferation and attachment on nano-macro porous glass scaffolds: Himanshu Jain (h.jain@lehigh.edu), *Lehigh University*. One of the key challenges in today's medicine is the treatment of bone loss. We

have recently developed two novel methods for fabricating bioactive glasses with dual nano-macro porosity based on (I) a conventional Melt-Quench method followed by selective heating and etching and (II) a novel Sol-Gel procedure with polymerization-induced phase separation. A macro porous structure of the glass scaffolds is necessary to obtain good implant incorporation through rapid vascularization and bone ingrowth, while the nanopores simulate the natural extracellular environment. We have fabricated such nano-macro dual-porous glass bone-replacement scaffolds and then tested their biocompatibility and bioactivity using immunofluorescence and confocal microscopy techniques. Specifically, the colonization and growth of MC3T3 and MG63 cells on the scaffolds have been monitored for cell attachment, proliferation, and differentiation using appropriate fluorescence-based cell detection methods. The results are helping us determine the most promising scaffolds and are thus expected to provide basis for further *in vivo* testing by our partners at Tissue Engineering Laboratory, Faculty of Dentistry, Alexandria University, Egypt.

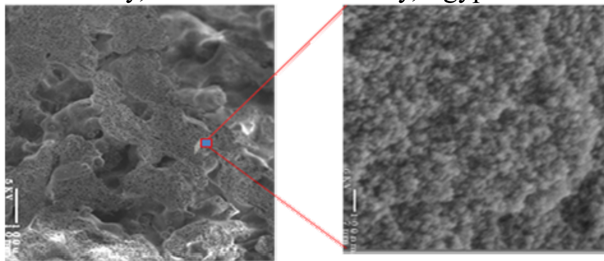


Fig. Macro (left) and nano (right) pores in glass developed at Lehigh for bone scaffold applications.

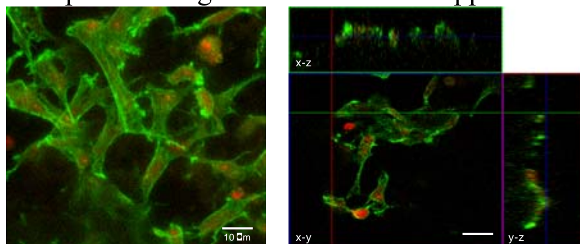
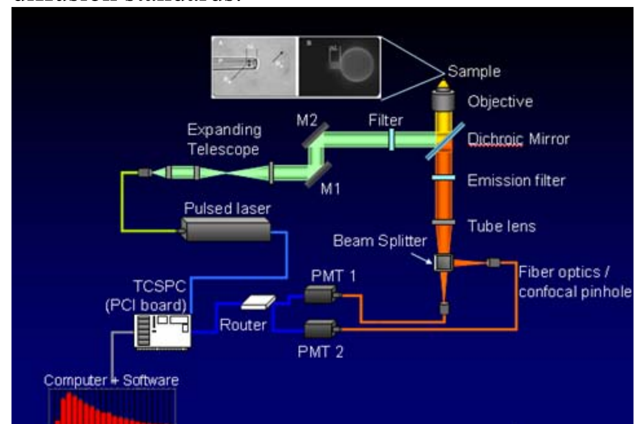


Fig. Confocal fluorescence microscopy of bone forming cells showing actin fibers and nuclei. The picture on the right shows three orthogonal views of the cells that had migrated into the pores of a nano-macro porous glass prepared by modified sol-gel method. F-actin was stained with Phalloidin Alexa 488 (green) and nuclei were stained with Propidium Iodide (red).

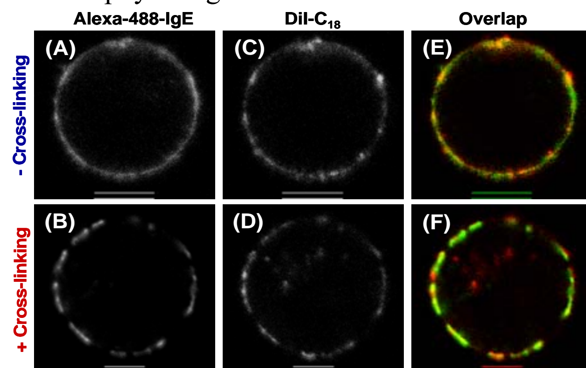
Description of TCSPC integrated with multimodal microscopy: Peter J. Butler (pjbbio@engr.psu.edu), *Penn State University*. Our multimodal confocal molecular dynamics microscope (CMDM) allows high spatial- and temporal-resolution optical interrogation of spectroscopic parameters in fluorescent samples in a temperature-controlled environment during micropipette aspiration (see figure). This CMDM consists of an Olympus IX71 microscope, a pulsed 532nm YAG laser (High-Q, Boston), a piezo, 3-axis stage for sample positioning (Mad City Labs, WI), dichroic mirrors, a pinhole and a polarizing beam-splitter, and two high speed, high quantum efficiency PMTs (Hamamatsu). The system is interfaced with a 1.25MHz 64 Channel A/D board for controlling the mirror and z-focus. For photon detection, the PMTs are interfaced with a picosecond, high-speed single photon counting (SPC) module (Becker-Hickl, Germany) capable of single molecule detection. Photon data analysis is accomplished using Fluofit software (Picoquant, Germany). All other data collection and experiment control are accomplished using LabView software running on a Dell Precision Workstation 530. This system has been calibrated using lifetime and diffusion standards.

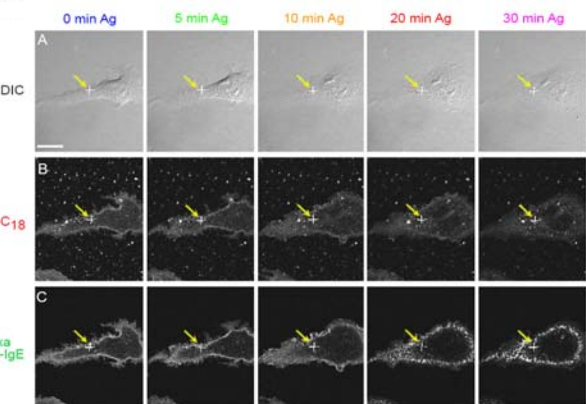


Biological applications of time-correlated single photon counting: Peter J. Butler (pjbbio@engr.psu.edu), *Penn State University*. Blood flow-related shear stress induces biochemical and physiological changes in vascular endothelial cells (ECs), a process termed mechanotransduction. These forces and the resultant biochemical reactions are highly localized and regulated by the cell. Thus there exists a need for spatial and temporal maps of mechanotransduction events on the subcellular and molecular level. We develop new engineering analyses and perform experimental studies of single EC mechanotransduction. Central to our approach is the novel use of multimodal microscopy including DIC, TIRFM, confocal fluorescence imaging, time-resolved fluorescence, and optical trap rheometry, all on a single platform. This infrastructure provides experimentally determined inputs to advanced 3-D image processing algorithms, computational fluid dynamics solvers, and finite element (FE) solid

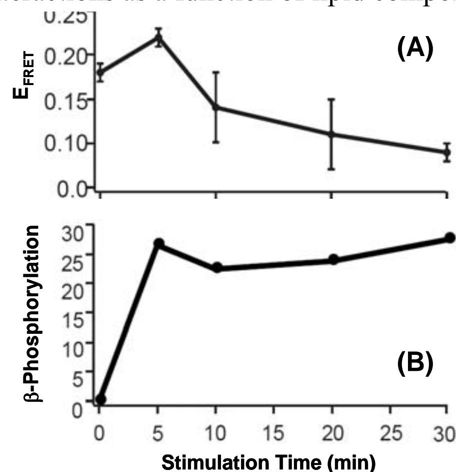
mechanics models enabling time- and position-dependent correlations of cell stresses with signal transduction. Such research may lead to new molecular level interventions for endothelial cell dysfunction and provide new tools for intelligent development of novel biomaterials, tissue engineered blood vessels, and drug screening modalities.

Fluorescence Dynamics Imaging of IgE Receptor Signaling in RBL Mast Cells: Davey, A.M., K. Krise. E.D. Sheets, and A.A. Heikal (aah12@psu.edu), *Peen State University*. Bio-membranes are complex, heterogeneous and dynamic systems that regulate numerous biological processes such as cell signaling, endocytosis and exocytosis, and protein trafficking. Cholesterol-rich microdomains (or lipid rafts) within the plasma membrane have been hypothesized to exist in a liquid-ordered phase and play important roles in cell signaling. While cholesterol-rich lipid microdomains have been observed in model systems under a controlled environment, these rafts defy detection using conventional imaging in living cells under physiological conditions.





Here, I will discuss our recent results on IgE receptor signaling in RBL mast cells, under both extensive antibody crosslinking and physiological antigen stimulation, using a newly developed fluorescence dynamics assay. Central to our approach are multiscale, integrated biophotonics techniques that include two-photon fluorescence lifetime imaging (FLIM), fluorescence polarization anisotropy imaging, and image processing algorithms. Changes in the nanostructure of the plasma membrane of RBL cells will be correlated with crosslinking of the high affinity receptor for IgE (FcεRI), which triggers co-redistribution of molecules associated with cholesterol-rich microdomains. Comparative studies on engineered lipid bilayers (namely, freely suspended giant unilamellar vesicles GUVs), as a model system, will serve as a testing ground for lipid analogue interactions as a function of lipid compositions.



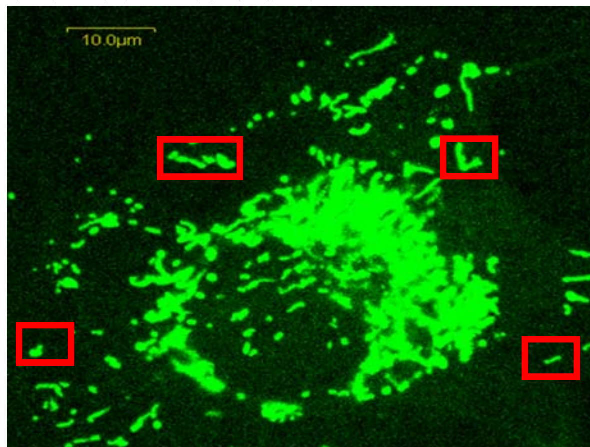
Reference:

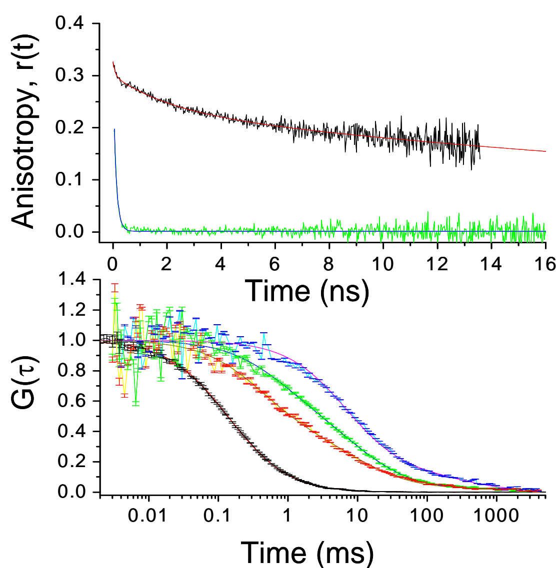
[1] Davey, A.M., K. Krise. E.D. Sheets, and A.A. Heikal. Molecular perspective of antigen-mediated mast cell signaling. J. Biol. Chem., 283:7117-7127 (2008).

[2]Davey, A.M., R.P. Walvick, Y. Liu, A.A. Heikal, and E.D. Sheets. Membrane order and molecular dynamics associated with IgE receptor crosslinking in mast cells. Biophys. J., 92:343-355 (2007).

Integrated Biophotonics Approach for Quantitative and Multiscale Analysis of Biological Systems: Qianru Yu, Michael Prioa, Ahmed A. Heikal (aah12@psu.edu), *Penn. State University*. Fluorescence-based techniques are powerful research tools in biomedical engineering and life sciences. Fluorescence microscopy allows for visualization of sub-cellular structures with a diffraction-limited spatial resolution, but without molecular dynamics information that underlying the majority of cellular processes. We have developed a multimodal and integrated biophotonics for gaining molecular information over a wide range of spatial and temporal resolution. These biophotonics techniques include DIC, confocal and two-photon (2P) microscopy, 2P-fluorescence lifetime imaging microscopy, fluorescence polarization anisotropy imaging, fluorescence resonance energy transfer and different modalities of fluorescence correlation spectroscopy; in a single platform. In addition to concentration imaging of biomolecules in living cells, this approach yields valuable information on the rotational and translational diffusion of a

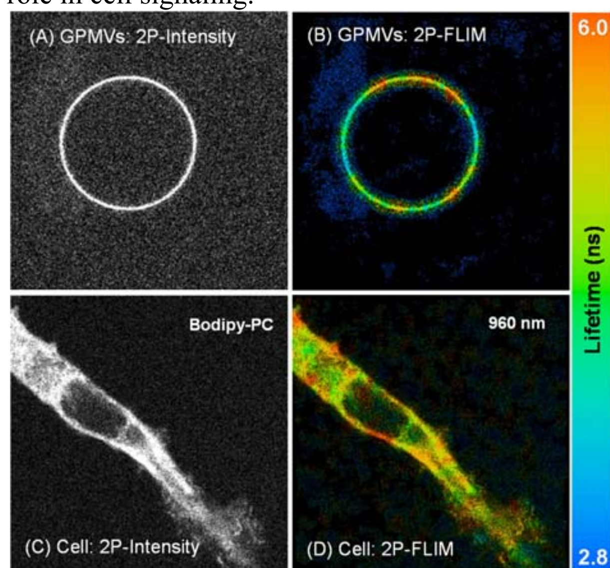
fluorophore, which are exquisitely sensitive to cell environment and physiology. Complementary to these techniques, image processing algorithms are being developed for biophysical analysis and modeling. As a proof of principle, we use rhodamine-123-stained mitochondria in breast cancer (Hs578T) cells as a model system to investigate biophysical properties of the inner membrane of mitochondria.





Reference:
[1] Yu, Q., M. Proia, and A.A. Heikal. Integrated biophotonics approach for noninvasive and multiscale studies of biomolecular and cellular biophysics. *J. Biomed. Optics*; *July/August Issue*, 13(4):041315 (2008).

Membrane fluidity and lipid order in synthetic and natural membrane vesicles: Florly S. Ariola and Ahmed A. Heikal(aah12@psu.edu). *Penn. State University*. Biomembranes in living cells are complex, heterogeneous and dynamic systems that regulate numerous biological processes such as cell signaling, endocytosis and exocytosis, and protein trafficking. Cholesterol-rich lipid domains have been hypothesized to exist in a liquid-ordered phase and play an important role in cellular functions. While these domains have been observed in synthetic model systems, they defy detection in living cells under physiological conditions using conventional imaging. Here, we present biophysical studies on an engineered cell-like membrane model, giant unilamellar vesicles (GUVs), under controlled thermodynamic perturbations. Of particular interest here are liquid-ordered, cholesterol-rich domains that are believed to be biologically relevant. To assess the influence of membrane proteins on membrane fluidity and diffusion of lipid analogs, we compared these GUVs results with giant plasma membrane vesicles (GPMVs) that are isolated from the plasma membranes of live cells. Our experimental approach is an integrated, multiscale fluorescence-dynamics assay that includes two-photon fluorescence lifetime imaging and diffusion (both rotational and translational). Our comparative studies on GUVs and GPMVs serve as a platform to test our understanding of lipid-lipid and lipid-protein interactions in biomembranes under controlled thermodynamic perturbations. These studies also allow for basic understanding of the biophysical and nanostructural properties of the in vivo membranes and, ultimately, their functional role in cell signaling.



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