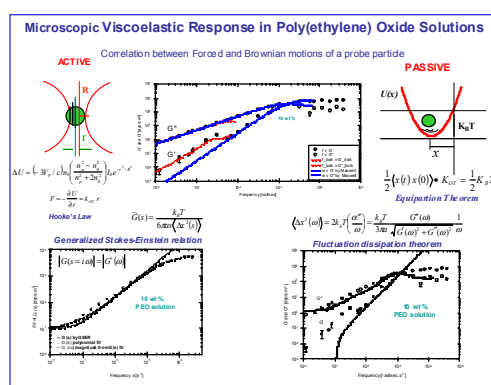


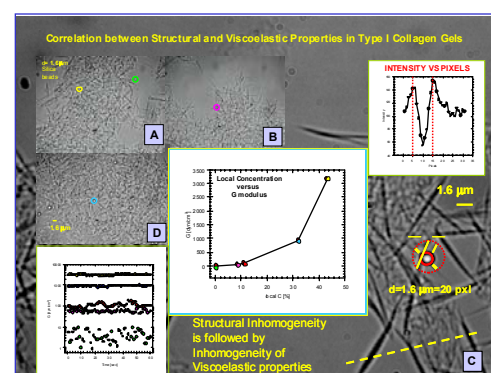
## Biophotonics Studies of Soft and Biological Materials at Cellular and Molecular Levels

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**Abstract:** Optical tweezers technique is relatively new, powerful and non-invasive technique for investigating local viscoelastic properties of medium, such as soft materials, fluids and biological materials. Using tightly focused laser beam, displacement and phase shift of trapped particle's motions can be used to calculate the elastic and the viscous moduli as a function of frequency. Unlike traditional microrheology, optical tweezers-based microrheology allows us to probe viscoelasticity of a medium in a wide frequency range. Technique requires only a minute amount ( $\mu\text{L}$ ) of the samples, important in working with biological materials, and optical tweezers gives us the opportunity not only in the studies of local viscoelastic properties, but also in the studies of non-local viscoelastic properties of the medium. An IR laser setup, available now in our lab, is less damaging to living cells. Combining confocal microscopy and optical tweezers allows us to investigate the relationship between the structural and mechanical properties of biological materials.



For determining material's viscoelastic properties, we propose a combined passive-active approach. In the passive measurements, the mean square displacement and power spectral density of Brownian motions of a probe particle are used to obtain the viscoelastic modulus of poly(ethylene) oxide solutions by the generalized Stokes-Einstein relation and the fluctuation dissipation theorem. In the active measurements, the probe particle is set into forced oscillations by oscillating optical tweezers, and the viscoelastic moduli of the medium are obtained from the displacements and the phase shifts of the particle's motion.

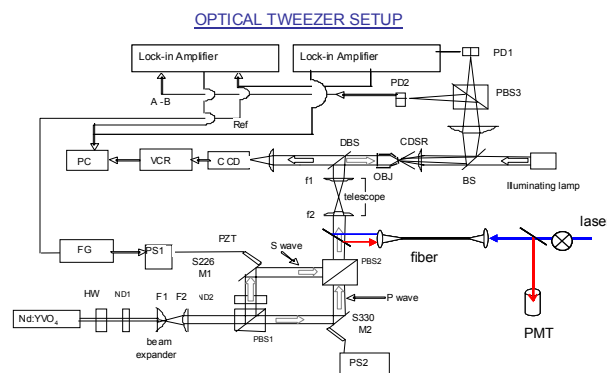


In order to characterize the structure of the collagen fiber matrix, we study the material structure and micromechanical properties, simultaneously. Directly measured (using optical tweezers technique), local viscoelastic properties in Type I Collagen gels were correlated with the local population of collagen fibers in the close vicinity of a probe particle. Our goal was to determine the range of gel's structural and micromechanical inhomogeneity. We establish a relationship between the local structural and micromechanical properties in collagen gels.

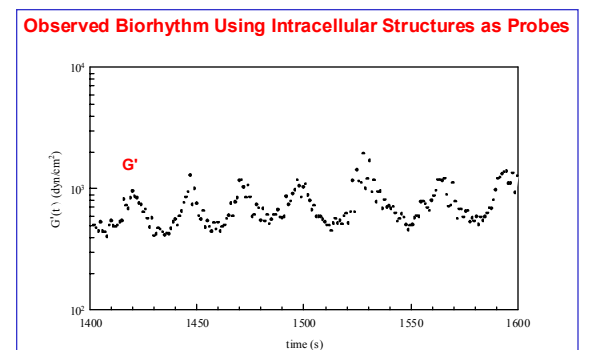
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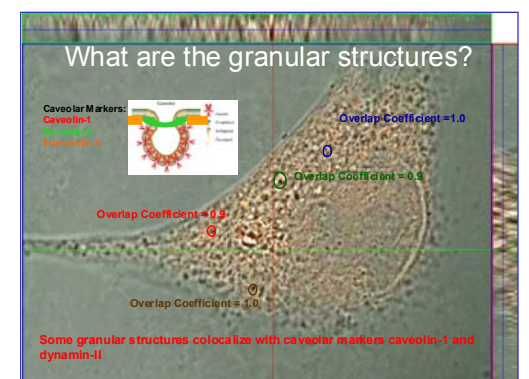


We use a Spectra-Physics Millennum Nd:YVO<sub>4</sub> laser at 532 nm wavelength in one, and IR 1064 nm wavelength in another setup. Creation of two polarized beams is allowed by splitting the initial beam with a beam splitter (PBS1). The first beam, P polarized, is steered by a piezo-controlled mirror S226-M2 (Physik Instrumente). The second beam, S polarized, is steered by a high frequency piezo-driven mirror S226 (PI). Two beams are recombined at the second PBS2, before going to the microscope (Olympus IX-70 and IX-81). The split-photodiodes PD1 and PD2 are used to determine the position of the trapped particles from the intensities of the forwardly diffracted laser light from the particles. Lock-in amplifier measures the displacement and the phase shift of the particle motions from which micromechanical properties of the material are determined in the close vicinity of the particle.



Optical tweezers are used to measure intracellular mechanical properties in the vicinity of the granular structures of the bovine endothelial cells. The figure above shows surprising rhythmic behavior of the cytoplasm elasticity,  $G'(t)$ . Periods of the oscillations are in the range of 20- 40 sec. This newly discovered rhythmic behavior is found only in living cells. We do not understand the origin for these oscillations.

**Biophotonics Imaging, Sensing and Manipulation**  
**SCIENTIFIC CHALLENGES:**  
 To establish a correlation between Cellular and Molecular Structures with Local Micromechanical Properties and Biological Functions



In order to test the hypothesis that the granular structures, trapped by optical tweezers in the study mentioned above, are the part of transcytosis machinery (caveolae), we label three protein markers: Caveolin-1, Dynamin-II, and Intersectin-1, for fluorescence confocal microscopy study. The figure above shows co-localization of three proteins with bovine serum albumin (BSA). To study transcytosis in bovine aorta endothelial cells (BAECs), we transfected cells with Caveolin-1-GFP and introduced fluorescently labeled albumin (BSA), a protein known to be transported across the cell through caveolae. We tracked BSA transport through granular structures that co-localize with our GFP marker as shown on Figure below.

