avenues of AFM study - has previously only been achieved in highly isolated non-biological environments (i.e., cryogenic temperatures, ultra-high vacuum). We adapted techniques originally developed by the optical trapping microscopy community, and have constructed an ultra-stable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci in three dimensions. Recently, we have extended ultra-stable AFM to common biological imaging conditions (tapping mode in aqueous buffer solution) and have exploited local observation of the three dimensional (3D) tip trajectories to yield 3D interaction force components in a direct manner. In this talk I will discuss these developments in the context of addressing central questions in membrane biophysics.

836-Pos Board B616

Fast and Accurate Photodiode-Based Detection of Multiple Trap Optical Tweezers with Crosstalk-Elimination

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Multiple trap optical tweezers have proven instrumental for a variety of exciting biophysical discoveries, e.g., from shedding light on molecular motor mechanisms to revealing intricate details of DNA-protein interactions. This 'second' generation of optical tweezers allows for advanced trapping geometries beyond single traps, however, these increased manipulation capabilities typically come at the price of more challenging position and force detection. The accuracy of position and force measurements is often compromised by crosstalk between the detected signals, this crosstalk leading to systematic and significant errors on the measured forces and distances. We developed an easy-to-implement simple method that enables simultaneous three-dimensional tracking of several individual objects in dual trap optical tweezers [1] and multi-trap holographic optical tweezers [2,3]. The method is based on spatial filtering and is highly compatible with standard back-focal-plane photodiode-based detection offering unrivaled temporal and spatial resolution with minimal crosstalk. This fast and accurate photodiode-based detection offers distinct advantages over camera-based solutions and opens the possibility for a variety of new biophysical assays. The reported technique significantly improves the accuracy of force-distance measurements, e.g., of single molecules, and hence provides much more scientific value for the experimental efforts.

Publications:

[1] D. Ott ,S.N.S. Reihani, & L.B. Oddershede, ''Crosstalk elimination in the detection of dual-beam optical tweezers by spatial filtering,'' Review of Scientific Instruments,(2014).
[2] D. Ott ,S.N.S. Reihani, & L.B. Oddershede, ''Simultaneous three-dimensional tracking of individual signals from multi-trap optical tweezers using fast and accurate photodiode detection,'' Optics Express (2014).

Filed Patent:

[3] L.B. Oddershede, D. Ott , S.N.S. Reihani, ''A detection system for an optical manipulation system for manipulating micro-particles or nano-particles of a sample by means of at least two optical traps,'' PA 2014 70097, Denmark.

837-Pos Board B617

Precise Partition of Micro/Nanoparticles in an Electro-Optofluidic Platform

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Integrated optofluidics, when controlled electro-optically, can facilitate the high-throughput on-chip manipulation and detection of biomolecules and micro/nanoparticles. Previously we demonstrated stable trapping and precise manipulation of trapped microspheres, with positions of trapped microspheres dictated by antinodes of a standing wave formed along a nanofabricated waveguide [1]. Using this nanophotonic platform, we now report a method of generating an optical binding effect on-chip. We show that an array of microspheres can be stably trapped and uniformly spaced with a travelling wave. The direction of the travelling wave is rapidly switched using the thermo-optic effect via an integrated electric microheater which modulates the phase of a wave in a waveguide in a Mach-Zehnder interferometer. Our theoretical work suggests that the periodic trapping potential is a result of multiple-interference of the wave due to its interactions with the microspheres. Such a demonstration of on-chip optical binding provides a novel mode of manipulation and spacing of trapped micro/nanoparticles on an optofluidic platform.

[1] M. Soltani, J. Lin, R. A. Forties, J. T. Inman, S. N. Saraf, R. M. Fulbright, M. Lipson & M. D. Wang, Nanophotonic trapping for precise manipulation of biomolecular arrays. *Nature Nanotechnology* **9**, 448-452 (2014).

838-Pos Board B618

Transverse Magnetic Tweezers for Direct DNA Extension Measurements

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Magnetic tweezers are an important tool used to stretch and twist single DNA molecules. While longitudinal magnetic tweezers have been widely used for DNA extension and rotation measurements, transverse MTs have found more limited applications due to the lack of a method to directly measuring DNA extension, even though they are better suited for visualization of events taking place on DNA. Here, we present a transverse magnetic tweezers setup that can directly measure DNA extension. This method should provide a more accurate determination of the anchoring position of the DNA on the substrate. It simplifies the measurement of the DNA extension and is potentially useful for studying short DNA constructs. In addition, our external lateral magnet can rotate, allowing us to twist torsionally constrained DNA at a constant force to generate DNA supercoils.

839-Pos Board B619

Human Red Blood Cell Adhesion to Laminin Measured by Atomic Force Microscopy

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Introduction: Red blood cells (RBCs) express several surface adhesion receptors which modulate cellular function and mediate cell-cell interactions, or adhesion, on both healthy and pathological RBCs. In this study, we established an in vitro single-cell force spectroscopy (SCFS) technique to study human RBC adhesion to substrates coated with laminin protein. Additionally, in order to establish the sensitivity of the assay, we investigated changes in the detachment force between single RBCs and the laminin substrate in the presence of pharmacological modulators of the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway.

Methods and Results: Using blood obtained from healthy human subjects, we recorded adhesion forces from single RBCs attached to AFM cantilevers as the cell was pulled-off of substrates coated with laminin. SCFS records force-distance curves of an approach/retraction cycle between the RBC probe to and from the laminin-functionalized substrate. We found that an increase in the overall cell adhesion measured via SCFS is correlated with an increase in the resultant total force measured on 1 μm2 areas of the RBC membrane via single-molecule force spectroscopy (SMFS). Finally, RBCs were treated with the PKA activator-forskolin (FSK), PKA inhibitor-KT5720 and epinephrine to study the variations in the adhesion of BCAM/Lu to laminin due to pharmacologic modulation of the cAMP signaling pathway.

Conclusion: We established that SCFS can detect variations in the detachment force of RBCs to laminin based primarily on the number of active BCAM/Lu receptors, which was modulated via biochemicals affecting the cAMP-PKA pathway. This study shows important implications for AFM-based SCFS measurements in understanding and evaluating the pharmacologic response of adhesion receptors on RBCs. Further, this method can easily be employed to measure the adhesive interactions of various cell types with functionalized substrates or other cells.

840-Pos Board B620

Atomic Force Microscopy (AFM) Analysis of the Bacterial Polar Protein Popz

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Structural studies of filament-forming biomolecules have classically relied on optical imaging methods such as fluorescence and electron microscopy. While these methods have yielded novel insights into mechanisms of macromolecular assembly, they often require perturbative labeling or staining procedures that can affect the assembly process. In contrast, Atomic Force Microscopy (AFM) has emerged as a powerful imaging tool to directly study native nanostructures at high resolution without labeling. Unlike optical methods, AFM utilizes a nano-scale cantilever to generate a topography map of surface-immobilized molecules in air or in aqueous environments, allowing studies under more physiological or dynamic conditions. However, like all imaging methods, most biological samples require electrostatic or covalent interaction