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2952-Pos Board B722
High-Density Single Particle Tracking on the Plasma Membrane using High-Speed Hyperspectral Imaging
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We have developed a high-speed, line-scanning hyperspectral microscope (HSM) capable of acquiring 30 frames per second with 128 spectral channels per spatial pixel. The HSM allows simultaneous single particle tracking (SPT) of up to eight spectrally distinct quantum dots (QDs), improving the useable labeling density in SPT by nearly an order of magnitude as compared to single-color SPT. In addition, HSM imaging allows the spectral uniqueness of QDs within the same spectral family to be used for resolving ambiguities in trajectory reconstruction.
We describe our physical instrument as well as the required analysis for hyperspectral SPT of multi-color QDs. Our analysis extends multi-emitter fitting previously developed in our lab for accurate localization of single fluorophores at higher density [Huang, F. et al., Biomed Opt Express 2011;2(5):1377] to three dimensions (x, y, lambda). Using a finite pixel 3D Gaussian estimate for their combined point spread function and spectral features, simultaneous spatial localization and spectral identification of QDs is achieved. The algorithm is implemented in CUDA to take advantage of parallel processing of GPUs. Trajectories are constructed with spatial and spectral localization information using a cost matrix approach for global optimization. Finally, squared displacements from single particle trajectories are used to build viscosity maps of the plasma membrane.
We demonstrate high-density SPT by performing viscosity mapping of the plasma membrane, which provides important spatial and temporal information about local membrane effects on receptor mobility. We have applied HSM viscosity mapping to investigate the influence of the membrane environment on QD-IgE bound to FcεRI. Changes in mobility and confinement of FcεRI due to receptor crosslinking (receptor aggregation and signal initiation), latrunculin-beta treatment (induces actin depolymerization), and PMA treatment (induces actin polymerization) are examined.

2953-Pos Board B723
A Novel, Wafer-Level Method to Fabricate Zero-Mode Waveguides for Single Molecule Detection
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Zero-mode waveguides (ZMWs) are hollow, metal apertures that are substantially smaller than the wavelength of light typically used in fluorescence spectroscopy. Consequently, ZMWs restrict excitation illumination to sub-diffraction limited (zeptoliter-scale) volumes. Such small confinement volumes enable single molecule detection at micromolar concentrations of fluorescently-labeled target molecules, and hence offer a powerful tool for single molecule observations under near-physiological conditions. The primary limitation to the widespread use of ZMWs in modern biological laboratories is that well-defined nanoapertures currently require expensive fabrication methods to produce. Here, we present a novel method to create ZMWs that employs conventional, low-cost, wafer-level fabrication techniques. We first use conventional photolithography to pattern larger, microapertures (800-1000 nm diameter) into a gold film covering a glass microscope slide. The microapertures are then subjected to metal electrodeposition, where the metal-of-interest is plated onto all accessible metal surfaces. Consequently, the diameter of the nanoaperture array can be controlled by simply varying the electrodeposition time. As a proof-of-principle demonstration of the fabrication method, we fabricated waveguide arrays (300 μm x 300 μm) onto standard microscope cover glasses with diameters ranging from 70 ± 20 nm to 1000 ± 17 nm (mean $\pm$ std, as determined by SEM). To determine the attenuation of the excitation light within the ZMWs we characterized the fluorescence emission as a function of diameter (D) and found that it is proportional to $D^{3.4}$ in the zero-mode regime (where no modes can propagate), $D^{2.35}$ in the transition regime (modes increasingly propagate as a function of diameter), and $D^{\sim 2}$ in the super-wavelength regime (all modes exist). In addition, we have directly observed the diffusion of single molecules at a solution concentration of 1 μM. These results demonstrate that our fabrication method can produce usable waveguide geometries using low-cost processing techniques.

2954-Pos Board B724
Quantitative Fluorescent Labeling of Aldehyde-Tagged Proteins for Single-Molecule Imaging
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A major hurdle for single-molecule fluorescence imaging has been the lack of a general method for labeling a protein with a single fluorophore at high efficiency, specificity and speed. Limitations of existing techniques have prevented researchers from studying many important proteins at the ensemble and single-molecule levels. By incorporating an aldehyde motif genetically into proteins and improving the labeling kinetics significantly under physiologically relevant conditions, we achieve fast labeling of these proteins with ~100% efficiency at one location while maintaining their biological function. We then demonstrate that an unpurified protein in cell extracts can be labeled efficiently and specifically using our method and used for single-molecule pull-down analysis. We further show the applicability of our method in a series of studies on the transient interactions and switching between DNA polymerases, which was not possible with the techniques previously available.

Micro & Nanotechnology: Nanopores II

2955-Pos Board B725
Deconstructing Structural Transitions of Biomolecules
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Structural transitions appear everywhere: proteins fold, nanotubes collapse, DNA denatures, ice melts, and so on. In biology, these transitions play a role in processes such as transcription and also determine protein function. Yet, at the same time, they give examples of highly nonlinear processes that are challenging to model and understand. I will discuss one such transition - the denaturation of DNA, where its double stranded form unravels into two single strands. There are many models that can describe certain aspects of this transition equally well, such as the fraction of bound base pairs versus temperature. I will show, however, that two well-known models yield drastically different predictions for thermal transport. The latter can then be used to ''peek inside'' DNA and understand what is happening during the denaturation transition. Thus, on the one hand, thermal transport gives a method to probe structural transitions in biological molecules and other materials. On the other hand, molecular systems and materials with nonlinear structural transitions also give opportunities for developing novel thermal devices.

2956-Pos Board B726
A Multichannel Electrophysiology Workbench for Single Ion Channel Screening
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Ion channels are involved into a large amount of processes of the cell life, regulating ion exchange between intra and extra cellular environment. Several diseases are affected by unregulated protein behavior and the possibility to test target drugs with single ion channels will open new approaches for the drug discovery and screening. This could be accomplished by testing single ion channels into artificial lipid membranes arrays.
Currently, it is possible to monitor ionic current flowing through ion channels embedded into BLMs, cells or giant lipid vesicles, by characterizing the electrical transmembrane protein behaviour using laboratory techniques and low noise equipment for signal acquisition. State-of-the-art equipments offer high precision recording amplifiers that are able to sense single ion channels. However, these techniques require sophisticated manual skills. On the other hand, High Throughput Screening systems require high data volumes implemented with automatic procedures. At the moment, they are available only for patch-clamp techniques. We present a multichannel electrophysiology system for single ion channel recording, based on a modular system embedded into a hybrid architecture that is able to record single ion channels on an arbitrary number of spots. The system is composed of:
• an array of disposable microfluidic chambers with embedded Silver/Silver-Chloride electrodes, for manual or automatic formation of BLMs to host single ion channel experiments, tightly interfaced with low noise electronics front-end;
• an array of low noise integrated microelectronic interfaces for signal amplification and analog to digital conversion;

• digital data elaboration and link to PC;
• a graphical user interface for data display and storage.
The flexibility of the proposed architecture allows different configurations, ranging from a limited number of data channels to be monitored, suitable for manual experiments, to thousands of data channels, coping with automatic HTS requirements.

2957-Pos Board B727
Dynamic Modulation of Multi-Cellular Clusters by Repetitive Microscope Projection Photolithography using Bio-Friendly Photoresist
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Multicellular interactions are critical for many essential cellular functions under various physiological/pathological circumstances including embryonic development, cancer metastasis, and immune responses. Recent development of surface chemistry and microfabrication has provided new opportunities to better understand behavior of multicellular clusters. Surface micro-patterning of cell-adhesion ligand (e.g. fibronectin, RGD peptide) islands surrounded by cell-repelling backgrounds (e.g. poly(ethylene glycol), bovine serum albumin, etc.) can be used to create multicellular clusters with different sizes and shapes to examine various biophysical and biochemical factors critical for multicellular interactions. However, the number of cells occupying identical size of islands within a surface may vary depending on local cell density and kinetics of cell adhesion/spreading. Variation in number may not be a serious issue for multicellular clusters composed of tens of hundreds of cells, but would be critical for small multicellular clusters composed of less than ten cells. Dynamic modulation of micropatterned cells were typically achieved by electrochemical or photochemical removal of cell-repelling moieties on the surfaces and subsequent adsorption of cell-adhesion proteins, therefore cellular responses tended to be delayed until enough cell-adhesion proteins were deposited.
Here, we developed a new technique that enables us to control the size/shape/composition of multicellular clusters composed of small numbers of cells, and to spontaneously modulate the shape and migratory behavior of multicellular clusters. Microscope projection photolithography (MPP) based on a bio-friendly photoresist poly(2,2-dimethoxy nitrobenzyl methacrylate-r-methyl methacrylate-r-poly(ethylene glycol) methacrylate) (PDMP) previously developed for the micropatterning of multiple proteins and cells with precise registration was extended. Using this technique, we successfully monitored actin, E-cadherin dynamics and collective migration of multicellular clusters.

2958-Pos Board B728
Automation of "Classical" Patch Clamp Experiments Featuring Multi Channel Recordings, Optical Cell Selection and Ultra Fast Compound Application
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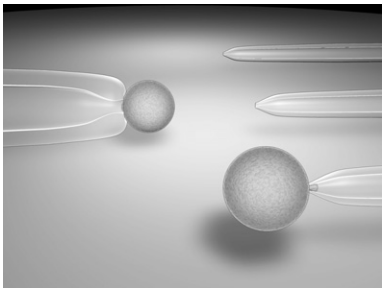
We present a new approach of automating the ''classical'' patch clamp experiment based on cost efficient standard patch pipettes. Built on top of a standard patch clamp setup, the system enables recording of multiple cells in parallel. Pipette positioning, as well as seal and whole cell formation are automated. Unlike concurrent approaches utilizing ''planar'' chip designs, the method retains important features of the classical patch clamp methodology, including the possibility to optically select individual cells to be patched. This enables the use of transiently transfected cells qualified by fluorescent markers. The system also offers enhanced throughput for experiments which so far could only be addressed by tedious ''manual'' patch clamp. This includes piezo-driven, ultrafast application of compounds to the cell membrane with millisecond exchange- and exposure times which is crucial to investigate the kinetics of fast inactivating ligand gated ion channels. Thus, the new method offers a cost efficient approach to significantly enhanced throughput in areas of neurobiological and neuropharmaceutical research which so far were not amenable to automation.



2959-Pos Board B729
Intracellular Recording of Cardiomyocyte Action Potentials by Nanoelectrode Arrays
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Recent years have seen numerous applications of nanoelectronic devices for cell electrophysiology measurements. Here we present vertically aligned Pt and Au nanopillar arrays for both extracellular and intracellular recording of HL-1 cardiomyocytes. The small footprint of our nanopillar arrays holds the advantage of high spatial resolution recording. We discover that the tight cell membrane-electrode interface allows recording of a large extracellular signal despite the small detection area. After local electroporation of the cell membrane around the pillars, we demonstrate intracellular recording of action potentials. Because this method is minimally invasive, we are able to record action potentials from the same cell over a span of three days.

2960-Pos Board B730
A Micro-Fluidic Power Generation using an Electrical Double Layer
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Any solid in contact with a liquid acquires some immobilized charge on its surface. The immobile charge on the surface attracts counter ions. This structure is so-called electrical double layer (EDL). This is a kind of capacitor, an electrical double layer capacitor (EDLC). We investigated an EDLC in a liquid droplet bridge between two parallel solids under vertical vibration. The solid substrate is an Indium tin oxide (ITO) and the upper solid plate has teflon coating on the ITO surface. It changes that the contact area of the solid-liquid interface at the upper plate when the lower plate vibrates vertically. During this process it repeats that the EDLC is charged and discharged. The results of this experiment can be useful for constructing an micro-fluidic power generation.

2961-Pos Board B731
Altering the Cellular Morphology Results in the Mechanical Regulation of Nuclear Shape and Functions by Central Actin Filaments
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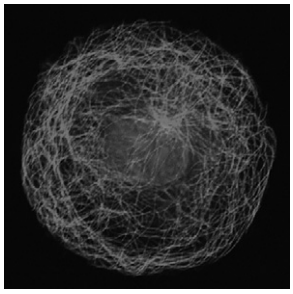
Growing evidence suggests that cytoplasmic actin filaments are essential players in the modulation of nuclear shape and functions. However, the mechanistic understanding of the internal orchestration between cell and nuclear shape is still lacking. In this communication, we shape-engineered single endothelial cells to quantitatively and non-invasively assess the nuclear morphology and the intracellular force balance in response to large-scale cell elongations. Our study reveals for the first time that nuclear orientation and deformation are regulated by lateral compressive forces driven by tension in central actomyosin stress fibers. We show that tension in central stress fibers is gradually generated by anisotropic force contraction dipoles as the cell elongates and strongly dependent on the cell spreading area. Our findings indicate that large-scale cell shape changes induce a chromatin condensation and dramatically affect cell proliferation. On the basis of these findings, we propose a simple mechanical model that quantitatively accounts for our experimental data and provides a conceptual framework for the mechanistic coordination between cell and nuclear shape.



2962-Pos Board B732
Electrophysiology Methods to Investigate Molecular Interactions Between Nanoparticles and Lipid Bilayers
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A diverse range of molecular interactions can occur between engineered nanomaterials (ENM) and cell membranes, some of which could lead to toxic outcomes. The ability to measure, predict, and control these molecular interactions would enable ENM to be designed to be both effective and safe. While some classes of ENM-membrane interactions involve participation of proteins or other membrane-associated macromolecules, ENM can also interact directly with the membrane's lipid bilayer. This latter class of interactions can be characterized using electrophysiology methods, in which transient ionic currents