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An optical tweezer (OT) has been widely used to study the mechanical properties of microscopic living biological systems like red blood cells. These studies are based on measurement of deformations caused by a force exerted directly or indirectly by an optical trap. The trap is usually pre-calibrated using Stokes viscous force of the suspension fluids for the biological system which is directly proportional to the viscosity of the fluids. Therefore, calibration of the trap depends on the viscosity of the fluid which depends on temperature. In this work, we have demonstrated that OT can be used to precisely measure the viscosity of biological fluids affected by temperature. Using a an infrared laser trap which is calibrated using a 3.1micron silica sphere suspended in a distilled deionized water and measuring the power as function of escape velocity, we have measured the viscosities of a newborn and unborn bovine serum with a different concentration of antibodies. Comparative analysis of these measurements with the measurements carried out by direct use of a viscometer have revealed a significant effect of increase in temperature resulting from the intense beam of the laser trap.

1601-Pos Board B445
Line Scanning Flow Measurements
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We are developing a new technique to measure flow of micron scale particles using laser scanning. This technique will also detect complex flow patterns, identify stationary particles and determine particle size. In this method, a laser beam is raster scanned over an area containing a flowing liquid. Particles in the liquid scatter the laser. Detailed information about the flow can be obtained from analyzing the fluctuations in this scattered radiation.
Detailed flow information, such as can be provided by this technique, is valuable in medical applications. Blood cells can serve as the particles that scatter lights and the laser scanning can be applied to surgically exposed blood vessels in a patient or in a animal model. The information available with this method can help study or monitor conditions such as sickle cell anemia in which abnormal blood cells do not move smoothly through blood vessels or become stuck. It can also be used to study the formation of atheromatous plaques. One factor in the creation of these plaques on artery walls is the accumulation of platelets and leukocytes. Understanding what prevents blood cells from flowing normally and what causes them to accumulate would be a significant improvement in our understanding of vascular disease.
This work was supported by the National Center for Research Resources of the National Institutes of Health (PHS 5 P41-RR003155).

1602-Pos Board B446
Towards Growth Cone Guidance On Silicon Chips By Capacitive Stimulation Of Voltage Dependent Ca2+ Channels
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Hybrid systems of neuronal networks and microelectronic chips can be used to elucidate network processes like learning and memory. Systematic experiments on network dynamics require a well defined topology of the synaptic connections. We want to control the directional outgrowth of neurites directly from the chip. Intracellular Ca2+ concentration $[Ca^{2+}]_i$ of growth cones is known to play a decisive role in neuronal outgrowth. By capacitive stimulation of voltage dependent Ca2+ channels (VDCCs) we want to manipulate $[Ca^{2+}]_i$ to steer growth cone guidance.
To show the feasibility of capacitive opening of VDCCs, we used HEK293 cells expressing L-type VDCC Cav1.2. The capacitive gating of Cav1.2 was studied under whole cell voltage clamp and current clamp conditions. We detected the Ca2+ influx by Fura-2 fluorescence microscopy. We found that the cells $[Ca^{2+}]_i$ was greatly enhanced by repetitive capacitive chip stimulation.
In a next set of experiments, we stimulated VDCCs in large, nonmotile growth cones of A-Cluster neurons from fresh water snail *Lymnea stagnalis*. We monitored growth cone $[Ca^{2+}]_i$ by Fura-2 fluorescence microscopy and found that repetitive capacitive stimulation induced profound changes in $[Ca^{2+}]_i$. Observation of growth cone morphology before, during and after repetitive stimulation revealed significant structural reorganisation that relates to growth cone collapse and repulsion.
Our results provide a first step towards capacitive control of growth cone guidance on silicon chips. Further experiments with smaller, motile growth cones have to be performed to achieve chip-controlled directional neurite outgrowth.

1603-Pos Board B447
A Novel Protein Array Using Microbeads Aligned In A Microfluidic Chip
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Protein array is a powerful means to investigate protein-protein interactions. Yet current protein arrays are not versatile due to their low sensitivity (>1ng/ml) and cost-ineffectiveness.
In this study, we have developed a sensitive and cost-effective protein array using a commercial fluorescence microscope. The protein array has aligned antibody-immobilized microbeads (5 μm in diameter) inside a polydimethylsiloxane (PDMS) microfluidic chip. The minimum concentration requied for fluorescence detection was determined to be several tenths of pM (about 1 pg/ml) using fluorescently-labeled glutathione-S-transferase (GST) to the protein array having α-GST antibody immobilized microbeads.
Firstly, we tried detecting a recombinant protein expressed in cultured cells. We extracted cytoplasmic components of PC12 cell expressing green fluoresent protein (GFP) and labeled them with amino group reactive fluorescent dye. The labeled product was applied to the protein array having α-GFP antibody microbeads, α-β-actin antibody microbeads as positive control and α-IgE antibody microbeads as negative control. Only α-GFP and α-β-actin antibody microbeads were fluorescent, demonstrating that the protein array is capable of detecting a target protein in cytoplasmic extract containing a large number of other proteins.
At present, to test its applicability to endogenous proteins, we are trying to detect expression levels of transcription factors, c-Jun and c-Fos, in Hela cell by the method mentioned above with their counterpart antibody microbeads.

1604-Pos Board B448
Evoking and Resolving Quantal Neurotransmitter Release on a Microchip
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A microchip that facilitates *in-vitro* electrical and electrochemical measurements of individual cells and cell clusters was fabricated using surface micromachining and thick film technologies. In the present study, the device was applied towards the detection of exocytotic events from electrically stimulated rat pheochromocytoma (PC12) cells. Using device microfluidics, cells were positioned in a recording chamber over a 5 μm × 10 μm gold working electrode (WE). Channel dimensions (10 μm deep × 10 μm wide) ensured a tight fit for the ~12 μm diameter PC12 cells in the chamber resulting in direct contact of the cells with the WE. This proximity allowed for quantal resolution of catecholamine release events from the cells and corresponding analysis of release kinetics and quantal size. Cells were stimulated through the application of sinusoidal voltage waveforms across axially-positioned, extracellular electrodes. In this manner, patterned extracellular gradients were generated across the cell thereby resulting in membrane depolarization. To facilitate interpretation of the stimulating electric field in relation to the cell and subsequent dopamine release, quasi-static electromagnetic FEM models were generated using COMSOL Multiphysics software. Upon depolarization, simultaneous chronoamperometric recordings at the WE confirmed stimulus-triggered dopamine release from the cells with a small subset of cells exhibiting release that modulated with the depolarizing cycle of the sinusoidal stimulus. It is anticipated that such a chip could provide a semi-automated alternative to the conventional, labor-intensive carbon fiber electrode (CFE) approach to neurotransmitter measurement.
This work was supported in part by the National Institutes of Health, NIDCD R01 DC04928 and by National Science Foundation, IGERT NSF DGE-9987616.

1605-Pos Board B449
Silicon Chip Patch-clamp Electrodes Integrated With Pdms Microfluidics
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We have developed an integrated planar patch-clamp system for the acquisition of ion channel activity from single cells. The system consists of a pore within a suspended silicon oxide membrane integrated with PDMS microfluidics. The silicon electrodes have enabled the achievement of gigaohm seals in high yield and the electrical nature of the cell/wafer seal has been characterized for several pore geometries. The PDMS microfluidics allow the placement of a single cell directly over the silicon pore hydrodynamically within PDMS microfluidic channels, without user input. Furthermore, the microfluidic channels permit the use of low solution volume and very rapid extracellular and intracellular solution exchange. This device enables a real-time, *multi-parameter* analysis on high-density arrays of single cells in distinct physiological environments.

1606-Pos Board B450
Automated Reactor For Extraction And Manipulation Of Sub-Megabase Fragments Of Genomic DNA With Flow-Focusing
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Long DNA fragments (0.1-1 Mb) are required in many polymer physics studies, especially implementing single-molecule approaches. Previously we presented a membrane reactor designed to produce samples of sub-megabase DNA fragments. This reactor is capable of extracting and purifying high quality genomic DNA and additionally perform various reactions such as restriction enzyme digestion, intercalation with fluorescent dyes, and labeling with sequence-specific tags. This 125 μl volume reactor performs preparations significantly faster than routine procedures and is completely automated.
To extend the ability of the reactor to work with smaller bacterial loads (10^6 cells vs. 10^8 cells), we recently introduced an axisymmetric flow focusing mode. In this mode, flow fields created within the chamber focus the bacterial cells to a small area in the center of the membrane. This arrangement helps to limit the interaction of deformable DNA coils with membrane nanopores which leads to decreased sample losses. It also enables elution of the sample in a smaller volume and at 5-10-fold higher concentration. Implementation of flow splitting during the elution process helps to further increase the DNA concentration by an order of magnitude.
This work focuses on experimental and numerical characterization of the flow fields employed in the reactor. Experimental study was performed with 160 kb DNA and 240 kDa proteins. Numerically, the semidilute DNA solution on the membrane is modeled with deGennes' reptation model. The models are used to estimate flow fields necessary to carry out reactions and purification of genomic DNA on the membrane without shear degradation.
As an illustration of reactor performance, we demostrate successful extraction of genomic DNA from *E. coli* cells, its purification, specific digestion with NotI restriction enzyme, and intercalation with POPO-1. The largest eluted DNA fragment was nearly 1 Mb-long.

1607-Pos Board B451
Using A Natural Material For Bacteria Concentration and Removal From Water
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In the last decade an extraordinary amount of research and development has focused on alleviating problems associated with contaminated water. With the majority of the World's population living on the brink of illness due to bacterial contamination in town water supplies, much of this attention has been focused on bacteria removal and sensors. Many current decontamination techniques are too technologically advanced for less developed countries, often resulting in their rejection by the societies they serve. Sensor work has also come across problems including poor sensitivity making it difficult to detect microorganisms at low concentrations. We have been testing a material extracted from the Opuntia ficus-indica cactus which could possibly address both of these problems in conjunction with one another. This material, referred to as cactus mucilage, has proven itself in the past as a viable flocculating agent for use in water contaminated with sediments and heavy metals. Flocculation tests, now focused on Bacillus cereus and Escherichia coli, have also given insight on the mucilage's ability to gather and concentrate bacterial contaminants from ion-rich water supplies. In columns with bacteria suspended in hard and soft water, flocculation begins immediately and is complete in approximately five to ten minutes with concentration rates of up to 99%. In addition to cleaning the water, the flocs formed with the mucilage could be removed from the water for sensor use. Cactus mucilage is an ideal material for water treatment and assessment because it is a naturally occurring, low cost material that is easy to obtain, process and use. Using this type of green chemistry, not only are bacteria concentrations significantly lowered in contaminated water, but also a highly concentrated volume of bacteria is produced that could potentially aid in biosensors.

1608-Pos Board B452
A Microfluidic Device For Concentrating High Molecular Weight DNA
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Direct Linear Analysis (DLA) technology obtains high content sequence information by optically mapping sequence specific fluorescent tags bound to elongated genomic DNA molecules in shear flows.[1] To facilitate sensitivity and throughput, we have implemented a high molecular weight DNA concentrating system by photopatterning a semi-permeable membrane inside the microfluidic device. This minimizes the fluid volume in which the DNA molecules reside prior to optical mapping leading to decreased read times. The membrane is selectively permeable to buffer ions but not high molecular weight DNA molecules allowing enhanced sample concentration at the membrane surface during electrokinetic transfer. In addition, the semi-permeable membrane allows electrophoretic sample transfer into and throughout the microfluidic device avoiding hydrodynamic induced shear forces that can degrade the integrity of large DNA molecules. The device employs novel microfluidic channel geometries to limit the electric field strength to appropriate levels near the membrane surface to minimize both sample and membrane degradation while maintaining a sufficiently high electric field for rapid sample transfer. Additionally, ion polarization across the membrane due to selective membrane permeability is addressed by active buffer replenishment through devoted channels behind the membrane. This architecture is amenable to integration into electrophoretic systems requiring rapid sample concentration, positioning, and transfer between microfluidic components. This research was supported by the Department of Homeland Security Science and Directorate Technology.
[1] Chan et al., Genome Research, 2004, 14:1137.

1609-Pos Board B453
Single Microtubule Orientation on Patterned Non-fouling Surfaces
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Microtubule (MT) configuration and assembly are essential to cytoskeletal reorganization and vesicle transport. Through physical manipulation of microtubules we seek to investigate these sub-cellular processes and to fabricate lab-on-a-chip diagnostic tools. We have developed a straightforward method for on-demand orientation of single microtubules on lithographically patterned electrodes. A poly(ethylene glycol) self-assembled monolayer (SAM) passivates the electrodes to MT adsorption prior to inducing MT migration through application of an electrostatic potential. The nonfouling layer allows MTs to adsorb and orient on the patterned electrodes while preventing adsorption in the surrounding regions. In this way single microtubules can be coaligned to arbitrarily shaped submicron electrodes. This method has advantages over those which make use of kinesin, antibodies or biotin/streptavidin to bind microtubules as it is capable of on-demand adsorption and produces patterns of MTs without requiring subpatterns of these other biomolecules. In addition we present a facile method for producing the nonfouling SAM which prevents microtubule adsorption on silicon and gold surfaces, eliminating the need for casein, bovine serum albumin or other passivating treatments.

1610-Pos Board B454
Planar Lipid Bilayer Formation on a Laser-Drilled Quartz Substrate
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Quartz substrates are attractive platforms for ion channel research, owing to their improved dielectric properties over currently used substrates. Further, the piezoelectric properties of quartz make it an ideal candidate for probing mechanosensitive ion channels. Here we present evidence of planar lipid bilayer formation on a laser-drilled quartz substrate in transport measurements. Bilayer formation is evidenced by the incorporation of voltage-gated ion channels in the membrane.

1611-Pos Board B455
Exploring The Dynamic Actions Of Cellulolytic Enzymes In A Heterogeneous System With Micro-cantilever Technology
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The cellulolytic enzyme degradation process suffers from low efficiency and high cost because of the low activity of cellulases against their natural substrates cellulose, which is insoluble and crystalline in its native form. As a result, the degradation of crystalline cellulose becomes the rate-limiting step in the overall scheme of biomass conversion to ethanol. To address this problem, the development of a highly efficient and cost-effective cellulase has become one of the top priorities of the Advance Energy Initiative. Such an effort requires a thorough understanding of the mechanisms of cellulolytic enzyme actions including interactions between the cellulases and their native substrates. The current technologies such as ellipsometry and quartz crystal microbalance have not been able to provide with the level of sensitivity and resolution required for detailed characterization of the cellulolytic enzyme actions, especially the interaction between cellulase and glucan chains of cellulose, and the impact of such interaction on overall cellulose structure. To define cellulase actions in such a heterogeneous system, we focus on investigating the initial interaction between cellulase and its native substrate, crystalline cellulose by taking advantage of emerging micro-cantilever technology. We have constructed a micro-cantilever with a cellulose coating which allows us to detect the actions of cellulolytic enzymes in real time.