

Middle Tennessee State University, Murfreesboror, TN, USA.

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 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

Molly J. Rossow, William W. Mantulin, Enrico Gratton.

University of California Irvine, Irvine, CA, USA.

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

Kerstin Scheidt, Peter Fromherz.

Max Planck Institute of Biochemistry 82152 Martinsried, Germany.

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 [Ca²⁺]_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 [Ca²⁺]_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 [Ca²⁺]_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 *Lymnaea stagnalis*. We monitored growth cone [Ca²⁺]_i by Fura-2 fluorescence microscopy and found that repetitive capacitive stimulation induced profound changes in [Ca²⁺]_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

Yoshihiro Ooe¹, Yasuhiro Sasuga², Osamu Ohara^{3,4}, Yoshie Harada⁵.

¹The University of Tokyo, Tochigi, Japan, ²The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, ³Kazusa DNA Reserch Institute, Kisarazu, Japan, ⁴RIKEN, Yokohama, Japan, ⁵Kyoto University, Kyoto, Japan.

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 required 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 fluorescent 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

Gregory M. Dittami, Sameera S. Dharia, Jeffrey J. Wyrick, Andras Pungor, Richard D. Rabbitt.

University of Utah, Salt Lake City, UT, USA.

A microchip that facilitates *in-vitro* electrical and electrochemical measurements of individual cells and cell clusters was fabricated using surface micro-machining 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

John M. Nagarath, James R. Heath.

Caltech, Pasadena, CA, USA.

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

Vishal A. Patil, Patrick S. Noonan, Dylan A. Bulseco, Rudolf Gilmanshin.

U.S. genomics inc., Woburn, MA, USA.