We direct cells by acoustic streaming excited by a surface acoustic wave which deflects the fluid independently of the contrast in material properties of deflected objects and the continuous phase; thus the device underlying principle works without additional enhancement of the sorting by prior labelling of the cells with responsive markers such as magnetic or polarizable beads. Single cells are sorted directly from bulk media at rates as fast as several kHz without prior encapsulation into liquid droplet compartments as in traditional FACS. We have successfully directed HaCaT cells (human keratinocytes), fibroplasts from mice and MV3 melanoma cells. The low shear forces of this sorting method ensures that cells survive after sorting.

1005-Pos

High Frequency Chemical Stimulation of Living Cells

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Fundamental and applied biomedical research increasingly relies on the quantitative analysis of the response of individual cells to controlled stimulations. The advent of microfluidics has revolutionized the methods to tailor the chemical environment of live cells, allowing stimulations to be applied with high spatio-temporal control and using minute amounts of reagents. It has been shown that Dictyostelium Discoideum cells (D.D.) can sense cAMP gradients on scales smaller than their size (a few tens of microns) and can adapt to very rapid concentration changes on sub second timescales. Classical methods to apply chemical stimulations, which are often based on the diffusion of solutes released by micropipettes, suffer from a poor spatial and temporal resolution and cannot discriminate the different timescales involved in the gradient sensing mechanism of D.D. Here we take advantage of a recently developed microfluidic toolbox based on microfluidic stickers made out of stiff polymers to stimulate adherent D.D. cells within microchannels. Microfluidic stickers allow for periodic chemical stimulation at the subcellular scale at frequencies up to 10Hz.We will present the dependence of the gradient sensing response as a function of both the temporal and spatial frequency. We will show how the microfluidic framework used in this study overcomes several experimental limitations and thus considerably extends previous investigations on the chemotaxis of D.D. by giving a new insight on previously proposed gradient sensing models.

1006-Pos

Ionflux: A Microfluidic Approach to Ensemble Recording and Block of Whole-Cell Current from Voltage-Gated Ion Channels

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Automated patch clamp (APC) addresses a need for high throughput screening of chemical entities altering ion channel function. Systems that can produce pharmacologically relevant data rapidly and consistently find considerable utility in the pharmaceutical industry, but also increasingly in academic laboratories. Here we present data obtained with a novel APC platform utilizing a well-plate microfluidic design. Unlike existing devices, the IonFlux system incorporates no internal robotic liquid handling, and features continuous recording from cell ensembles during rapid solution switches with a bench-top footprint resembling a conventional plate reader.

True whole cell voltage clamp was applied to linear arrays of up-to thirty cells in parallel, utilizing fully-featured 16 or 64 channel voltage-clamp amplifiers under computer control. Laminar flow of solutions in a microfluidic network delivered cells in suspension to the recording sites and enabled fast exchange of bathing solutions via an electro-pneumatic interface, on either 96 or 384 well-plate formats. Electrophysiological characterization was achieved for K_V 2.1 and hERG potassium channels, and examples of Na_V sodium channels. Our results show the voltage-dependence of these currents and their block by pharmacological agents. The recordings also demonstrate the potential for microfluidics-enabled, exceptionally fast superfusion and washout of candidate drugs. Incorporation of multiple experiments per well-plate enables many investigations to be performed in parallel, especially if multiple compounds are applied to each individual cell ensemble recording array. Data on recording success rates, throughput and assay reproducibility show that high throughput experiments can be performed with enhanced reliability.

1007-Pos

Nanoelectrodes for Neuron Recording and Stimulation

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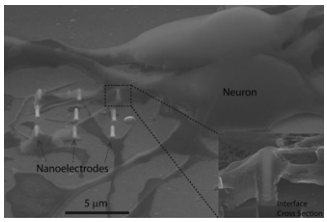
Effective electronic neuron interface requires tight attachment between electronic devices and neuron cells. However, it is the nature of living cells to maintain an extracellular cleft between their membrane and the substrate which they adhere to, and the cleft contribute to most of the signal leakage. Nano-scale electrodes could enable tight attachment to cell membrane and thus form good electrical coupling with cultured neuron cells. We fabricate nano-electrodes by focused ion beam (FIB) Pt deposition. The electrodes are vertical aligned, and with diameter of 200 nm and height of 1 um. We cultured cortical neurons on the substrates with nanoelectrode arrays. Scanning electron microscopy (SEM) analysis shows that neuron cells engulf nano-electrodes readily. The tight engulfment provides good electrical coupling between neuron cells and nano-electrodes. We also examine action potential recording and stimulation with nano-electrode arrays. Image 1 shows SEM images of neuron nano-electrodes interface. The cross section view of the interface is milled by FIB.



1008-Pos

8-Parallel Bioparticle Sorter with a Multilayer PDMS Chip

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A major challenge in organelle proteomics is purifying the organelle of interest from cell lysate. We have been developing a fluorescence-activated microfluidic sorter for organelle purification. Flow switching mechanism of the sorter is based on the sol-gel transition of a thermoreversible gelation polymer (TGP). The TGP is liquid at low temperature and turns to a gel immediately upon heating. The TGP solution is mixed with bioparticles and introduced into a mirochannel that branches into waste and collection channels. When the fluorescence signal from a target particle is detected, the sol-gel transition of the TGP is induced at the entrance of the waste channel by heating with an infrared laser. This operation allows a fluorescent target to flow into the collection channel. In this study, eight parallel sorters were integrated in a microchip to achieve high-throughput sorting. The microchip consists of a glass cover plate and a PDMS block. The PDMS block has a multilayer structure with two inlets for sample and buffer solutions and two outlets for collection and waste solutions. Its structure makes it possible to form a sheath flow, i.e., sample-solution flow is focused by neighboring two buffer-solution flows in each sorter. We confirmed that sheath flows were formed in the chip. We demonstrated that fluorescent microspheres and *E. coli* cells were detected and sorted in each channel. A recovery ratio of 90% and a throughput of 20 particles/s were achieved. Future studies will allow us to integrate 16 or 32 channels into a single chip. The parallel sorter is a promising device for purifying large amount of bioparticles.

1009-Pos

Capture & Release of Single Cells on a Microfluidic Chip Via Conical Nanopores

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In modern biology, it is often assumed that populations of cells are composed primarily of average cells; cells that do not deviate significantly from an observational mean. This assumption is empirically convenient and until recently was necessary due to technological limitations. However, it is possible that ignorance of cellular individuality may lead one to draw incorrect conclusions, especially when the population under study is heterogeneous. Cells that exhibit significant deviation from the mean behavior can reveal important information which would be normally obscured by ensemble averaging techniques.

We have developed an array of microfluidic analytical techniques capable of studying the biochemistry of single cells [1,2,3]. Our current effort focuses on the development of a device which can capture a significantly large number of unicellular organisms resolved at the single-cell level. In our microchip, *Synechococcus* cells are captured via electroosmostic flow on a thin polycarbonate membrane that is populated by conical nanopores [4]. Cells can be efficiently captured and released via control of the flow's directionality. Applications of this chip, including those for high-throughput selective sorting and on-chip culturing, are being explored.

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