Micro & Nanotechnology

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An Integrated Multifunctional Lab-on-a-Chip Platform for High Throughput Optical Mapping of DNA

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Direct Linear Analysis (DLA) technology employs continuous microfluidic elongational flow to stretch and optically map individual DNA molecules. Sequence specific tagging of DNA molecules with bisPNAs enables genomic differentiation between species. We report on the operation of a microfluidic lab-on-a-chip platform with the integrated functionality of sample concentration, fractionation, and high throughput optical mapping. Integration of the components on a single chip enables high throughput analysis of sub-nanogram samples. Previously described DLA devices [1] had throughput rates that were directly proportional to the initial sample concentration. Integrated on chip concentration in conjunction with tunable sample delivery flow rates to the DLA component allows for dynamic optimization of throughput to 12,000 kbp/s, independent of the initial sample concentration. In addition, fractionation of the sample enhances information throughput by discarding shorter fragments with lower information content prior to the optical mapping step. This integrated microfluidic device has been demonstrated in conjunction with a macrofluidic upstream sample preparation chamber in an automated system. This research was supported by the Department of Homeland Security Science and Technology Directorate.

[1] Phillips et al, Nucleic Acids Research, 2005, 33 (18), 5828.

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Development Of A Cell Sorting Device Based On The Integration Of Porous Poly(Dimethylsiloxane) (PDMS) Membranes Into Layered Microfluidic Devices

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Layered microfluidic devices integrated with semi-porous membranes have been widely used for mass transport control, immunoassays, and blood cell sorting. The placement of a semi-porous membrane at the interface of two channel layers is crucial to minimize unwanted crossover of fluid flows between microchannels while allowing diffusive mixing of reagents. Several methods have been reported to seal off the crevices inevitably generated because of the thickness of the membrane. For example, the application of PDMS pre-polymer as a mortar layer could prevent the leakage along the membrane. This method provides robust and reliable bonding between two PDMS layers. However, in the case of thicker membranes and/or narrower channels, the mortar layer can clog the channels easily. We introduce an alternative strategy of directly using PDMS as a porous membrane itself to fabricate monolithic microfluidic devices. In this case, the integration of a porous PDMS membrane can be completed without clogging microchannels. To prepare porous PDMS membranes, a photoresist is utilized as posts on a silicon wafer. Therefore, a thin film of porous PDMS can be prepared by spin coated on the wafer. This method allows varying sizes of pores on a single membrane, compared to commercially available porous membranes with a fixed pore size. In addition, the wafer can be repeatedly used to create porous membranes. We demonstrate the use of this method to fabricate a cell sorter where a porous PDMS membrane between two layers of microchannel. This porous membrane has two porous regions: 10 μm and 20 μm regions. Thus, a cell sample (lymphocytes) loaded from the top microchannel can be filtered into small or lager size of cells through the porous membrane, and collected from the bottom microchannels.

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3-D Microfluidic Technique for Patterning Cells

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We developed a technology that can pattern single cells in two-dimensional space, where each cell is placed in a well-defined spot. The basis of our technique is a microfluidic system that can simultaneously capture a large number of cells from solution, position each with sub-micron precision, and transfer the patterned cells to a substrate. The critical component of the system is a polymer membrane containing a pattern of microscopic holes, whose diameters are slightly smaller than that of a single cell. One side of the membrane is immersed in a solution of cells, and gentle suction is applied from the other side. The resulting fluid flow carries cells toward the holes of the membrane. When a cell reaches the membrane, it blocks a hole. Thus, the fluid flow, along with the residual cells, is redirected toward the remaining unblocked holes, until each hole is plugged by exactly one cell. Once the membrane is laden with cells, it is brought into contact with an adhesive surface. The suction is released to transfer the cells. Repeated application of this patterning process can yield complex structures of cells of different types.
Such spatial control of single cells will allow the study of intercellular interactions, as in embryogenesis and cancer. With the ability to program cell-cell interactions within and amongst multiple cell types via spatial localization, we will study how the interplay between geometry and genetics affects observed phenotype. This technique can also be used to create cellular microarrays for high-throughput data acquisition. Ultimately, we hope to build three-dimensional tissues *de novo,* patterned slice by slice, and cell type by cell type.

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The Toxic Effects of Quantum Dots on Embryogenesis in Caenorhabditis elegans

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Quantum dots have the potential to be used in medical applications such as tumor targeting and directed drug delivery. Their high molecular brightness, tunable emission spectra and photostability are just a few of the properties that make these nano-sized semiconductor particles attractive candidates for such applications. However, before quantum dots can be employed for therapeutic or diagnostic purposes, their potential toxicity needs to be thoroughly investigated. Such toxic effects may arise from their size or from the inherent toxicity of the materials that make up their core. In this study, the toxic effects of quantum dots on embryogenesis have been explored using the *Caenorhabditis elegans* (*C. elegans*) embryo as a model organism. Quantum dots are introduced into this nematode by microinjection and are then incorporated into its developing oocytes. Preliminary results suggest that the level of toxicity on embryo development is strongly dependent on the composition of the nanoparticles, on its coating, and on its propensity to aggregate. Additionally, it has been observed that the embryo uses a technique to package the quantum dots into isolated aggregates during development. The dynamic behavior of quantum dots in the *C. elegans* oocyte has also been studied using fluorescence correlation spectroscopy.

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Collaboratory for Structural Nanobiology (CSN), Nanoparticles Database

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Nanotechnology is the science and engineering field of functional systems at the molecular scale. This covers the manufacture of functional materials, devices, and systems through control of matter over the nanometer scale (1 to 100 nanometers) and the study of novel properties and phenomena developed at that scale. Many existing technologies depend on processes that take place in the nanoparticles, components that have very high surface areas, making them ideal for using in composite materials, reacting systems, drug delivery, developed cancer treatment and energy storage and many others. Due to the significant progress of this discipline, the initiative to develop a web-service database emerges, with structural and experimental information about nanoparticles. This project has been created as a result of a multidisciplinary team effort. Here you will find downloadable structural files, related research data, and resources for visualization of different nanoparticles such as dendrimers, buckyballs, nanotubes and metallic particles.

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A Nano-assay To Measure Modification Of Cysteine Residues In GST-fusion Proteins

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Cysteine residues are the target of numerous posttranslational modifications and play important roles in protein structure and enzymatic function. Because of this, much research on the biochemistry of proteins is dependent on understanding the activity and state of these residues. Many current methods for measuring modified and unmodified cysteine residues in proteins are cumbersome and often lack sensitivity, requiring large amounts of protein. We have developed a highly sensitive and simple assay that accurately measures the relative amounts of free cysteine residues in GST-fusion proteins using 96 well glutathione-coated plates. Free-unmodified cysteines are labeled and visualized using biotin and HRP-conjugated streptavidin. Our assay can be used to quantify the extent of reactions targeting -SH groups in proteins. We demonstrate this

assay using full-length and truncation mutants of the SNARE proteins: syntaxin-1A, SNAP-25B and synaptobrevin, which have 0-4 cysteines. With this assay we are able to quantitatively measure the number of cysteine residues modified in reactions such as palmitoylation and oxidation. This assay is as simple as running an ELISA or western and should allow greater elucidation of the chemistry of cysteine residues in proteins cysteine due to its high resolution.

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A Microfluidic Platform for the Culture & Analysis of Single Cells

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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 microfluidic device capable of sustaining a cell culture of a unicellular microorganism, Synechococcus, which can be resolved at the single-cell level. In our microchip, cells are captured hydrodynamically via a pressure-driven cross-flow of nutrient media. With efficient manipulation of the cellular microenvironment, the individuality of the cells' adaptive responses to stress conditions such as nutrient deprivation can be studied quantitatively using fluorescence microscopy. The design of imaging system with controlled illumination source as well as the use of different pumping mechanisms is described.

1. Wheeler, A.R., Throndset, W.R., Zare, R.N. et al. Anal Chem 75, 3581-3586 (2003).
2. Wu, H., Wheeler, A. & Zare, R.N. Proc Natl Acad Sci U S A 101, 12809-12813 (2004).
3. Huang, B., Wu, H., Zare, R.N. et al. Science 315, 81-84 (2007).

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A Pulse Width Modulated Microfluidic Diluter

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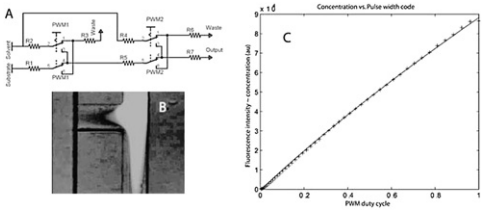
The preparation of solutions of different concentrations is often an essential part of chemical or biological assays. We have developed a pulsed microfluidic dilution concept, suitably for flexible programming of an accurate output solution concentration. Only minute amounts of chemicals are needed and dilution series with high resolution can be generated. The concept of our dilution chip is similar to digital analog conversion in electronics, using pulse width modulation (PWM).

By means of PDMS replica molding, a multi-stages PWM diluter has been constructed. Fluorescence imaging protocols with microscope/SLR camera as well as electrochemical probing with microelectrodes were employed for characterization and calibration, showing that the design allows for accurate dilution over 2 orders of magnitudes with high controllability, and at the same time minimal external components. This device concept can be applied in stand-alone diluter circuits or as a component in more sophisticated fully integrated analytical devices.



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Controlled Near Infrared Laser-Activated Liposome Release

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Liposomes have been evaluated as drug delivery vehicles for decades. However, it is hard to prepare liposomes to both balance enhanced drug retention and rapid and targeted content release. The challenge is to initiate the release of encapsulated drugs at the diseased site at a controlled rate. We recently developed a novel photo-activated approach by which near-complete contents release from liposomes can be completed within seconds by irradiating encapsulated or tethered hollow gold nanoshells (HGN) with a near infrared (NIR) pulsed laser. The rapid heating of the gold nanoshells leads to unstable microbubble formation and collapse, the same type of cavitation events associated with ultrasound. Our approach is conceptually analogous to the use of optically triggered nano-''sonicators'' deep inside the body for drug delivery. We demonstrate that even though the local temperature surrounding HGNs can be very high, the bulk temperature of the solution only rises by ~1°C. Results from electrophoresis and quantitative PCR all show no damage to DNA molecules mixed with HGNs after NIR irradiation. These results confirm the potential of using this optical approach to permeabilize lipid membranes and facilitate the cellular uptake of DNAs for gene therapy. Since DNAs are relatively robust macromolecules, we also investigated the more delicate dye molecule, carboxyfluorescein (CF), which contains a double bond; liquid chromatography followed by mass spectral analysis shows that ~95% of CF molecules are intact. These results agree well with our hypothesis that only an few nanometer thick layer surrounding HGNs reach temperatures above the explosive boiling temperature (~ 650 K), so that only CF molecules close to HGNs are damaged. Other NIR responsive materials, such as carbon nanotubes and solid gold nanoparticles were used as triggering agent for liposome release, and their release efficiencies are compared with those of HGNs.

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The Collaboratory for Structural Nanobiology

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Manufactured nanobiomaterials exploit the unusual properties of nanomaterials to develop new forms of intervention in biological systems. Nanobiotechnology is the field of science focused on the design, synthesis, characterization and application of nanomaterials and nanodevices to biological and biomedical problems. The success of nanobiotechnology hinges on our ability to characterize, predict, and control the biological properties of nanobiomaterials. Nanoinformatics is a collection of multi-disciplinary approaches to catalog, correlate, and model nanomaterial properties. CaNanoLab (http://cananolab.abcc.ncifcrf.gov) is an early example of a nanobioinformatics portal dedicated to foster the rapid dissemination of nanobiological information across the scientific community. Nanobioinformatics studies are complex because they must simultaneously deal with the large dispersion of chemical formulations of nanobiomaterials (ranging from polymer to metal oxide particles), the lack of a common language across contributing disciplines, and the lack of a low level language that can be used across nanoparticles. We could argue that, in lieu of a sequence space, similar to that available to bioinformatics studies of peptides and nucleotide sequences, we could build a structure based annotation and analysis of nanobioparticles that could help us cross-analyze their properties. Computer characterization of nanobioparticles is key to build a structure-based nanoinformatics infrastructure. We are in the process of building a nanobioinformatics service dedicated to the collection, curation, and correlation of structural, physico-chemical, and biological, and biomedical data: the Collaboratory for Structural Nanobiology (CSN http://csn.ncifcrf.gov). We have used CSN to explore nanobioparticles data storage, retrieval, and analysis in the context of nanobiological studies. This work has been funded in part with funds from the NCI-NIH (Contract No. NO1-CO-12400 and HHSN261200800001E). The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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Tunable Delivery Of Chemical Gradients Over Large Cell Culture Substrates Using Microfluidic Stacked Flows

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Biomolecular gradients play an essential role in studying various biological phenomena such as development, cancer, inflammation, and wound healing. This paper reports a novel microfluidic device for generating tunable biomolecular gradients over large areas on cell culture surfaces. Laminar streams are stacked above the surface to generate a steady-state gradient via diffusion in the direction orthogonal to the flow and to the surface. Finite-element modelling was used to predict negligible shear forces at the range of gradients possible by tuning flow rates. The surface gradients were characterized with fluorescence microscopy; image analysis verified the presence of a one-dimensional gradient across a 2x2 mm area. Fig. 1 shows a variety of surface gradients obtained simply by changing the inlet configuration and pressure settings. Superimposed onto these images are the linescans taken across 4 different regions of the device which demonstrate lateral uniformity (excluding edge