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Poster Abstracts

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AES-1

Combination of Electroosmotic Flow and Dielectrophoretic Trapping of Particles in a Contactless-Dielectrophoretic Device

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Microfluidics is a rapidly growing field that offers great potential for many biological and analytical applications. There are important advantages that miniaturization has to offer, such as portability, shorter response times, higher resolution and sensitivity. There is growing interest on the development of microscale techniques. Among these, electrokinetic phenomena have gained significant importance due to their flexibility for handling bioparticles. Dielectrophoresis (DEP), the manipulation of particles in non-uniform electric fields due to polarization effects, has become one of leading electrokinetic techniques. DEP has been successfully employed to manipulate proteins, DNA and a wide array of cells, form bacteria to cancer. Contactless-DEP (cDEP) is a novel dielectrophoretic mode with attractive characteristics. In cDEP nonuniform electric fields are created by using insulating structures and external electrodes that are separated from the sample by a thin insulating barrier, this prevents bioparticle damage, and makes cDEP a technique of choice for many biomedical applications. In this study, a combination of cDEP generated with AC potentials and electrokinetic liquid pumping generated with DC potentials is employed to achieve highly controlled and selective particle trapping and manipulation, allowing for lower applied potential than those used in traditional insulator-based DEP, and making for a simpler system that does not require the use of an external pump. This is the first demonstration of electrokinetic (EK) pumping in which the driving electrodes are not in direct contact with the sample fluid. Multi-physics simulations were used to aid with the design of the system and predict regions of particle trapping. Results show the advantages of combining AC-cDEP with DC EK liquid pumping for dynamic microparticle trapping, release and enrichment.

AES-2

ABO Membrane Antigens Alter Dielectric Properties of Red Blood Cells

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Human blood is of incredible diagnostic interest because it is easily obtainable and can provide a plethora of information about a person's general health such as protein, iron, vitamin and electrolyte levels. The goal of this research is to expand blood diagnostic capabilities by using dielectrophoresis as a means to provide an ABO-Rh blood type classification from blood. Prior results from our lab suggest that the dielectric properties (permittivity and conductivity) of human erythrocytes change dependent upon ABO-

Rh antigen expression. The Clausius-Mossotti factor is directly dependent upon the dielectric properties and thus alternating current dielectrophoresis is a mechanism by which ABO-Rh blood types can be identified by quantifying red blood cell behaviors. Additional prior results with β(1-3) galactosidase treatment demonstrated cleavage of the ABO antigens without harming the erythrocyte and verification of the galactose residues in the supernatant. Treatment yields immunologically bare erythrocytes whose membrane surface is bereft of the ABO antigens. Previous experiments done in a 0.9S/m conductivity buffer have shown that the range over which the crossover frequency occurs for ABO-Rh blood types in their native state is 17MHz, whereas the range over which the crossover occurs for the modified samples is 5MHz. This uniformity in cross over frequency is an indication that the ABO antigens influence crossover frequency of the red blood cells. Further experiments at lower medium conductivities of 0.01S/m and 0.1S/m have been conducted over a large frequency range In addition to measuring the crossover frequencies of blood samples, the dielectrophoretic spectra as a function of frequency have been constructed using a novel intensity profile approach. This approach allowed typical dielectrophoretic traces to be plotted versus frequency for each ABO-Rh blood type, both native and modified samples. In this manner, not only could the crossover frequencies of the blood types be compared, but also the curvature of their traces, giving a more accurate method of distinguishing between blood types.

AES-3

Electrostatics Potential in Annular Geometry

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Flow through an annular geometry has many applications in chemical, environmental, mechanical and bio-mechanical engineering [1]. A number of researchers have proposed combining electroosmotic flow (EOF) and pressure-driven flow as a means of controlling the motion and separation of bioparticles in an annular geometry to sort particles using electrophoresis [2-3]. We present here a systematic investigation of the electrostatic potential distribution in an annular geometry. Our objective in this contribution is present a mathematical model for the electrostatic potential distribution in both straight and divergent annular geometry. The analytical solutions for the electric potential profile in the annulus are obtained by solving the 2D Poisson–Boltzmann equation with both long channel and Debye– Huckel approximations. This result is in preparation to the derivation of the Electrohydrodynamic Velocity profile. The ultimate goal of this research is to understand the role of capillary geometry in determining biomolecular separations. As a result of this investigation, one can assess the behavior of the electrostatic potential inside of annular channel. Three key parameters have been identified to describe the electrostatic potential behavior: the angle ($\propto$), ratio of up wall potential to the linear combination of both wall potentials, R, which handles the symmetrical/non-symmetrical aspects of the electrostatic potential, and the ratio of the width to the length (γ) that controls the "shape" of the channel section. Results of this study are illustrated by using a series of portraits that capture the key behaviors of the electrostatic potential with respect to the three parameters described above.

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AES-4

Micron-Scale Ion Concentration Gradients in Nonuniform AC Electric Fields

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Erythrocytes were observed to first swell slightly and then shrink substantially over time in phosphate buffer saline in a two dimensional nonuniform AC dielectrophoretic field. We hypothesized that this cell behavior was due to changing osmotic pressure between the cell and the local medium as propagation of an ion concentration gradient occurs in the electric field area. Cells are known to respond rapidly to the tonicity of the surrounding media; isotonic media yields an erythrocyte typical biconcave shape while hypotonic will cause erythrocytes to swell and hypertonic cause cells to shrink. Experimental results were examined to describe the cell shrinkage behavior in initially isotonic buffer solution under nonuniform AC DEP field. Results have shown that cell shrinkage can be observed from 180 seconds close to the higher field density region and will expand to the lower field density region under high frequency (1 MHz) AC field. Also, cell shrinkage starting time will change with the change of applied signal frequency and peak-to-peak potential. Ion behaviors were examined with COMSOL 'Transport of Dilute Species' physics to simulate diffusive, convective and electromigration transport under experimental conditions. Results have shown that high ion concentration increased in the regions adjacent to the electrode over 100 cycles of the AC field. These results have important implications in the field. Research in this field has, until now, assumed polarization of dielectrics in dielectrictrophoretic fields occurs in relatively constant medium conditions. The Debye layer comprises tens to hundreds of nanometers from the dielectric particle surface or electrode particle surface; ion behaviors in this layer have been studied. This work elucidate the formation and transportation of waves of ions tens to hundred of microns from the electrode surfaces in non-uniform AC electric fields and corresponding experimental results suggest complex interaction between the dynamic ion distribution in the medium and the particle's induced dipole response.

AES-5

Multi-Frequency Impedance Method for the Rapid Detection of Viable Micro-Organisms

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We present a novel culture-based electrical method for detecting presence of viable micro-organisms in suspensions that is significantly faster than existing methods. In our previous work, we have used our method to detect presence of viable bacteria in various samples, and in this paper, we extend our work to detect viable yeast and mold.

The principle underlying our method of detection is the polarizability of viable microbial cells. Cell membranes of viable microbial cells do not, in general, allow charge-carrying ions to pass through. So, in

the presence of an alternating electric field, there occurs a build-up of charge at the membrane, causing the cells to act like capacitors. As they multiply, there occurs a corresponding increase in the charges thus stored in the interior of the suspension (its "bulk capacitance"), and this increase in bulk capacitance serves as our "signature" indicating the presence of live micro- organisms. The bulk capacitance is estimated by first confining an aliquot drawn from the suspension of interest into a long microfluidic channel with electrodes at either end, measuring the electrical Impedance (Z) measurements at multiple frequencies (ω) ranging from 1 KHz to 100 MHz, and then analyzing the Zvs ω data to obtain an estimate of the bulk capacitance. Both the microfluidic geometry and the high frequencies used serve to overcome the effects of interfacial (double-layer) capacitances at the electrodes, which usually make it difficult to measure bulk capacitance.

The results obtained are used to demonstrate the ability of our method to extend it to detect viable yeasts and molds. We show that, for slow growing yeast and mold (doubling time of 6-8 hrs), our method is able to detect ~5, -10, of *Dekkera anomala and Aspergillus brasiliensis* in growth media in 60 and 45 hrs, respectively, with a threshold concentration of detection of ~5000-8000 CFU/ml.

AES-6

Microfluidic Characterization of the Dielectric Properties of Human Mesenchymal Stem Cells, Adipocytes, and Osteoblasts

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Degenerative diseases such as diabetes and Parkinson's disease combined affect over 28 million people. Human mesenchymal stem cells (hMSCs) can be used to autologously treat such diseases through stem cell purification. hMSCs are ideal because they are easily isolated from bone marrow and they have the ability to self-renew while undergoing differentiation. hMSCs have the potential to differentiate into osteoblasts, adipocytes, chrondrocytes, astrocytes, and myoblasts based on environmental promoters. One issue associated with the differentiation of hMSCs is cell purification. In order for hMSCs and their differentiated progeny to be useful in cell therapies, the cells need to be separated after differentiation occurs. Currently, magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) are the separation techniques employed. FACS and MACS use unique cell-surface antigens or other recognition elements to tag the target cells. This 'labeling' of the cells alters cellular function which is not desirable for the treatment of degenerative diseases. Another disadvantage of FACS and MACS is that it takes days or event weeks for cells to be purified. These approaches require expensive raw materials, are labor intensive, and have seen limited adaptation to other stem cells. Therefore a label-free, one-step cell purification technique that can rapidly sort all types of stem cells without altering cellular function is needed.

Dielectrophoresis (DEP) is a separation technique that has the potential to overcome the short-comings of FACS and MACS. DEP utilizes nonuniform electric fields to polarize cells based on the polarizability and dielectric properties of their membrane, cytosol, and other structurally dominant organelles. Based on these properties, cells will exhibit either positive DEP force, cells move to areas of stronger electric field strength, or negative DEP force; cells are repelled from areas of stronger electric field strength. DEP has been used to study other cell systems such as red blood cells, breast cancer cells, leukemia cells, cervical cancer cells, white blood cells, and yeasts cells. Based on evidence from these cell studies, DEP can be used to determine the dielectric properties of subtle cellular changes, and we hypothesize that DEP can discern hMSCs and morphological changes within its differentiated progeny.

In this work, a microfluidic device with gold quadrupole electrodes spaced 50 microns apart within a microfluidic channel and chamber device is used to quantify the DEP response of hMSCs that were differentiated into osteoblasts and adipocytes. The hMSCs were characterized from 100 kHz to 80 MHz frequency at a rate of 0.67 MHz/sec for 120 seconds in dextrose media at 0.01 S/m to 0.90 S/m conductivities. Narrower frequency sweeps were used to map out ranges with positive, negative, or cross-over frequency DEP behavior of the hMSCs. The positive DEP response of adipocytes and bone marrow derived hMSCs were found in a range of 70-80 MHz and 30-80 MHz respectively. COMSOL simulations and MATLAB were used to fit the data to the multishelled spherical and multishelled ellipsoidal DEP polarization models in order to calculate the structural polarizability and conductivities of hMSCs, adipocytes, and osteoblasts. These preliminary results suggest that DEP is a sufficient method to distinguish different cell types. Electrokinetically identifying differences in stem cells have broad implications in purification and control for tissue engineering and cell therapies for degenerative diseases.

AES-7

Supporting Electrolyte Gradients in a 1 Mm DC Electric Field Microchannel

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When an electric field is applied across an electrolyte solution, solute anions and cations electrophoretically migrate to the anode and cathode, respectively. As a concentration gradient starts to form, diffusional ion transport begins to counter electrophoretic motion, although this is potentially negligible since diffusional ion mobilities are an order of magnitude or more smaller than the electrophoretic mobilities achieved in microfluidic devices. The electric field catalyzes reactions at the electrodes in an aqueous medium; H^+ and OH^- ions are generated at anode and cathode surfaces, respectively. The association/dissociation of solute species is another mechanism that affects ionic concentrations at a given locality. This especially becomes important when the abovementioned electrode reactions cause a pH gradient. In that case, the extent of buffer species dissociation varies spatially, in accordance with the pH gradient. Thus, the pK values of the solute species is a factor in determining the ion concentration gradients in microfluidic devices under an electric field. Microdevices typically contain <10 nL volumes such that movements of only 1 nanomole of ions can cause significant shifts in local concentrations. Recent results revealed that the H^+ concentrations in a phosphate buffer can go from pH 8 to pH 3 in under 10 mins in a 100 V/cm electric field across a 1 cm microfluidic channel designed for protein iDEP, and under 1 min in a 3000 V/cm field in the same system. Such concentration changes can have a profound effect; for instance, it was discovered that the reported pH changes enabled protein iDEP by increasing particle size through protein aggregation.

The study presented here aims to complement the abovementioned work by elucidating the K^+ and Cl^- concentration behaviors in similar electrokinetically driven microdevices. K^+ is present as a supporting electrolyte and buffer ion in the phosphate buffer saline while Cl^- is present only as a supporting electrolyte. Therefore, due to the buffer association/dissociation mechanism mentioned above, it is expected that the changes in K^+ and Cl^- concentrations do not mirror each other. K^+ and Cl^- concentrations were quantified with fluorescence microscopy using potassium green and MQAE (n-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide), respectively. The electric fields were applied across a 1 mm gap between Pt thin film electrodes in a microfluidic channel. Fluorescence intensity

profiles across the gap were analyzed at 535 (Potassium green) nm and 460 (MQAE) nm, then correlated to concentration profiles. 0.1 S/m phosphate buffer solutions were spiked with KCl at concentrations ranging from 0 to 100 mM and the ion concentration gradient formation was observed under 100 and 3000 V/cm electric fields. The ion concentration gradient formation behavior provides insight into the transient responses of microfluidic devices, the buffering reactions occurring dynamically within the devices, and underscores the substantial differences between the solutions loaded into devices and the rapidly changing solutions within electric fields.

AES-8

Dielectrophoretic Differentiation of Bioparticles in a Sawtooth Microchannel

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Rapid bioparticle characterization, as a research goal, has fueled a significant and wide-ranging body of work. Much has been accomplished, but new techniques and applications continue to emerge. Innovations in this field may soon enable the development of rapid, on-site bioanalysis devices that improve the availability, accuracy, and scope of clinical diagnosis. Microfluidic electrokinetic approaches provide unique advantages, including short analysis times, microliter sample and reagent volumes, potentially low cost, and portability. The work presented here explores the use of a single, continuous microchannel in which opposing electrokinetic and dielectrophoretic forces create multiple and distinct bioparticle traps. Specifically, it focuses on capture of bioparticles using DC insulator-based dielectrophoresis in a converging, sawtooth-patterned microchannel. The channel design enables localized isolation and concentration of specific particles based on differences in their physical properties. Various targets with disparate characteristics have been captured and concentrated within the device, including human blood cells and mature amyloid protein fibrils. In each case, reproducible capture occurred at specific locales within the sawtooth channel. Differentiable capture of three live *Escherichia coli* serotypes has also been demonstrated, indicating the potential this technique holds for diagnostic applications.

AES-9

Investigating the Electrical Properties of Prostate Cancer Cell Lines Using Contactless Dielectrophoresis

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Prostate cancer is the most common malignancy in men and the second cause of cancer death among men in the United States. An early diagnosis of this cancer is crucial to decrease the mortality rate due to this cancer. Investigating the electrical and mechanical properties of cancer cells may lead to finding novel techniques to diagnose cancer in early stages and new cancer treatment methods. Dielectrophoresis (DEP), the polarity induced motion of particles in a non-uniform electric field, is a successful technique for

characterization and separation of cells. The non-uniform electric field is traditionally generated by patterning metal electrodes onto the bottom of a microfluidic channel. While this technique has been tremendously successful, electrode delamination and fouling are a challenge. We have developed a relatively new technique, known as contactless dielectrophoresis (cDEP), in which metal electrodes are exchanged for conductive fluid electrode channels. These fluid electrode channels are isolated from a main sample channel by means of a thin insulating membrane. The absence of direct contact with metal electrodes provides a permissive environment for the cells and allows for studying their electrical properties.

We previously characterized the dielectrophoretic response of prostate tumor initiating cells (TICs) of a prostate cancer cell line (PC3) utilizing cDEP. An electric field generated within the cDEP microdevice using applied voltages between 0 and 300 V_{RMS} with frequencies between 200 and 600 kHz. We have shown that the required voltage to completely trap TICs is different from that of non-TICs. This data was then used to find the optimal parameters to sort then culture enriched TIC populations. We observed that the TIC-identified cells, sorted using cDEP produced significantly more spheroids than control. Additionally, the average size of the cDEP enriched TIC spheroids was about 4 times larger than control, and about 17 times larger than cDEP enriched non-TICs spheroids.

In this study, we investigated the dielectrophoretic properties of three different prostate cancer cell lines which have previously been evaluated in the study of prostate cancer progression: PC3, DU145, and LNCaP. The LNCaP cell line is established from human prostate adenocarcinoma and derived from the lymph node metastasis. The PC3 cell line has a high metastatic potential and was derived from the bone metastasized prostate and the DU145 cell line was derived from the brain metastasized prostate cancer.

A low frequency cDEP device was used to find the crossover frequency of these cell lines. A silicon master stamp was first fabricated using typical photolithography techniques and deep reactive ion etching. The device was then cast in PDMS using a 10:1 ratio of monomers to curing agent. The polymer replicates were bonded to glass slides using air plasma after access holes were punched. The cells from three lines were independently suspended in a low conductivity buffer and driven through the device using a syringe pump at a flow rate of 0.005 mL/hour. A 200 VRMS signal was then applied across the device for frequencies between 5 and 100 kHz. The distribution of cells within the device was recorded and processed to determine the first crossover frequency.

We found that the crossover frequency for PC3, DU145, and LNCaP are 23.23 ± 0.51, 22.68 ± 0.64, and 31.22 ± 1.30 kHz, respectively. Crossover frequencies of PC3 and DU145 were not statistically different but both of them were significantly different from LNCaP ($p<0.001$ and $p<0.005$, respectively). We hypothesize that being derived from different types of metastasis could be the main reason for difference in their DEP response.

AES-10

Dielectrophoretic Response of Polystyrene Particles and Perfluorocarbon Oil-Core, Chitosan/Poly-L-Lysine/CaPO4 Shell Nanoparticles

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Dynamic (dis)assembly of nanoparticles into three-dimensional, packed structures would be useful for drug delivery, nanoparticle films and diagnostics. Dielectrophoretic (DEP) microdevices can rapidly assemble, discriminate, and manipulate particles based on a polarizability and medium interactions within non-uniform

electric fields. DEP has been used to discern micrometer scale cells and particles, but the manipulation of nanometer scale objects such as viruses, proteins, and nanoparticles has seen less exploration due to the lower forces on these smaller particles. In this work, we examine dielectrophoretic behaviors of spherical core-shell nanoparticles (CSnp) in 2D and 3D particle-assemblies in order to determine dielectric properties (permittivity and conductivity) of the core and shell. Three types of CSnp were custom synthesized with a perfluorocarbon oil liquid core within a phospholipid micelle templating for three shell materials (chitosan, poly-L-lysine, and $CaPO_4$). The synthesis procedures for all three biocompatible shell materials are established and demonstrate a range of permittivities (chitosan= $7.1*10^{-10}$-$4.4*10^{-9}$ F/m, poly-L-lysine= $6.2*10^{-10}$-$8.0*10^{-10}$ F/m, $CaPO_4$= Unknown) and conductivities (chitosan= $3.0*10^{-4}$- $3.5*10^{-1}$ S/m, poly-L-lysine, $CaPO_4$= Unknown) based on concentrations and frequencies. We report the frequency-dependent and medium conductivity-dependent responses of ~250 nm CSnps with ~10 nm shells for all three shell types. Experiments were conducted within a 100 nl chamber housing 100 um wide Ti/Au quadrupole electrodes spaced 25 um apart. Frequencies from 100kHz to 80MHz at a fixed local field of $10V_{pp}$ were tested and the frequency-dependent DEP responses of the nanoparticles were quantified by tracking optical intensity profiles via video microscopy. Average velocity of particles inferred from intensity profiles moving up the electric field gradient (positive DEP) and down the electric field gradient (negative DEP) were compiled as a function of frequency and medium conductivity, then compared with spherical core-shell models for DEP polarization in order to calculate core and shell dielectric permittivity and conductivity for comparison with the literature. This data is useful for dynamic assembly applications for these biocompatible nanoparticles.

AES-11

A Combinatorial Microfluidic Approach for Point-of-Care Applications

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We describe a microfluidic platform with optimized normally closed valves for high throughput quantitative analyses of stimulus driven responses in E. coli cells [1]. Specifically, we designed and fabricated a 4x6-array microfluidic chip capable of confining cells and reagents in sub-micron volume compartments. Leak-free isolation of contiguous compartments is achieved with elastomeric valves that are closed at rest [2]. Actuation of valves with negative pressure permits controlled exposure of cells confined in ~2 nL volume compartments to various chemical stimuli loaded in adjacent compartments. The device design is convenient for biological assays due to increased detection sensitivity, thereby enabling analysis at single cell resolution. Additionally, the device facilitates portability and exhibits multiple advantages as a miniaturized biological assay, including low sample and reagent volumes for analysis and integration of assay steps in an on-chip format. The overall goal of this work is to develop a diagnostic platform for screening antibiotic resistant phenotypes in infected clinical samples.

In this work, we employ the microfluidic platform to investigate antibiotic susceptibilities of E. coli cells to several commonly used antibiotics. Furthermore, we explore the synergistic and antagonistic effects of antibiotic combinations on E. coli cell proliferation. Specifically, we investigate the effects of ampicillin, tetracycline, chloramphenicol, cephalexin, ciprofloxacin, and combinations thereof on actively growing E. coli cells. The results were used to determine effective bactericidal and bacteriostatic concentrations for the aforementioned antibiotics (e.g., > 100 μg/mL for ampicillin, > 10 μg/mL for tetracycline). We also observed and characterized synergistic effects of certain antibiotic combinations. For example, although ampicillin at 10 μg/mL and tetracycline at 1 μg/mL are ineffective individually, together they exhibit significant bactericidal activity when used in combination. Finally, we extended the platform to characterize antibiotic resistance in a model pathogen, Pseudomonas aeruginosa.

Overall, these results emphasize the utility of the microfluidic platform for rapidly characterizing antibiotic susceptibilities over a wide range of concentrations. The platform improves on existing diagnostic methods in terms

of assay time, ease-of-use, sensitivity, and sample volumes [3]. The described microfluidic platform is expected to expedite elucidation of treatment regimens necessary for combating infections with multi-antibiotic resistant "superbugs" that represent an emerging and acute global health concern.

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AES-12

Assembly of Anisotropic Particles Under Electric Fields

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Anisotropic colloids can possess anisotropic properties in geometry, chemical composition or surface functionality. As such, they can recognize each other and assemble via directional interactions. By tuning the directional interactions between particles, anisotropic particles can assemble into diversified types of crystalline and non-crystalline structures. This poster will focus on the electric-field directed assembly of two types of anisotropic particles -rigid dimers and flexible trimers under electric fields.
For colloidal dimers with rigid bonds, we recently observed rich patterns in both crystalline and non-crystalline forms, such as clusters with chiraities, and dimer crystals with alternating orientations, which depend on the anisotropies in geometry and surface charges. Our modeling demonstrated that the competition and balance between electrostatic interactions and electrohydrodynamic interactions bring a variety of phases and assembly pathways between dimers. The close-packed crystal of dimers provides promise for fabricating three-dimensional photonic crystals based on non-spherical particles.
In addition, we have investigated colloidal trimers with flexible bonds, assembled from spherical colloids under electric fields. At low particle and salt concentrations, we observed a family of well-defined clusters ranging from 3 to 9 particles, with non-trivial high populations for trimers, tetramers, hexamers, and nonamers. At higher particle and salt concentrations, the colloidal clusters with flexible angles can further assemble and connect themselves into several hierarchical structures, including non-close-packed networks that have not been observed before. The dynamic assembly process of colloids resembles the chemical reactions between real molecules, which could open a new realm for making complex "colloidal molecules" from simple particles with prescribed assembly pathways.

AES-13

Solvent Compatible Polymer Based Microfluidics for Applications in Pharmaceutical Industry

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Poly(dimethylsiloxane) or PDMS has been the prime material of choice in microfluidics mainly because PDMS enables simple, inexpensive fabrication using soft-lithographic technique, which facilitates rapid prototyping of devices. Other advantages, such as biocompatibility, optical transparency, low young's modulus, have led to the use of PDMS-based microfluidics for several applications in chemistry and biology. However, PDMS suffers from several limitations, including poor solvent compatibility, small molecules absorption, and limited compatibility with analytical techniques, low mechanical rigidity, and issues with large-scale fabrication. Traditionally, silicon and

glass have addressed some of PDMS' deficiencies, but the complex and expensive fabrication procedures, material fragility, and incompatibility with a wide range of analytical techniques have limited their applications.
Polymeric materials that enable simple, inexpensive fabrication while addressing PDMS limitations are emerging as attractive candidates for developing microfluidic devices. Since no single polymer will satisfy all the requirements of the end application, we have developed hybrid devices comprising multiple layers of different polymers to overcome the deficiencies resulting from using single polymers. Our hybrid-microfluidic platforms, ranging from simple single channel devices to complex multilayer devices, comprise thin layers of different polymers, viz. cyclic olefin copolymer (COC), Teflon FEP, perfluoropolyether (SIFEL), thiolene, and PDMS.
Here, we demonstrate the application of these devices in the pharmaceutical industry for two specific examples. In the first example, we developed microfluidic platforms for screening solid forms (salt, cocrystal and polymorph) of pharmaceutical compounds with different additives (e.g., salt or cocrystal formers) employing several modes of crystallization. These platforms allow for solid form screening much early in the drug development cycle when only small quantities of drug (~10 mg) are available thereby aiding in expediting the drug development process. The platforms consist of multiple layers of COC-PDMS-PDMS-COC-PDMS-thiolene and COC-PDMS-SIFEL-SIFEL-Teflon-SIFEL-thiolene. In the second example, we have developed devices for liquid-liquid extraction, where (a) thiolene-glass devices were used to evaluate distribution coefficient of drugs in octanol-water mixture for in-vitro drug uptake studies and (b) COC-SIFEL-SIFEL-glass devices were used to purify radioisotopes with applications in synthesis of cancer imaging agents. The material choice was guided by several considerations, including compatibility with solvents (e.g., alcohols, toluene, DMSO, chloroform) and analytical techniques (X-ray, Raman), the strength of bond between heterogeneous polymers bonded via surface activation by plasma or chemical treatment, amenability for high-resolution fabrication, and mechanical robustness as well as reliability of the assembly for long-term experiments.

AES-14

Frequency Dependence of Protein Dielectrophoresis Probed with Insulator Based Devices

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Dielectrophoresis (DEP) occurs when polarized particles in a non-uniform electric field move towards (positive DEP) or away (negative DEP) from high electric field gradients. When applied to biomolecules, DEP has great potential as a bioanalytical tool for pre-concentration, fractionation, and separation. However, in contrast to well-characterized biological cells, the mechanism of protein DEP is not well understood limiting its potential for bioanalytical applications. The use of insulator posts (i-DEP) is a novel method that generates inhomogenous electric field gradients within a microchannel. In order to enhance the DEP force on proteins, we combine optical lithography with focused ion beam milling (FIBM) to build an array of nanostructured insulator posts in a microfluidic channel and study the DEP behavior of immunoglobulin G (IgG) molecules under DC and AC conditions. Under DC conditions, IgG molecules move according to positive DEP as reported previously [1]; however, utilizing AC, we observed a change from positive to negative DEP. Our preliminary results show that at 1Hz, IgG molecules concentrate in regions of high electric field gradients indicating positive DEP, however above 5Hz, the particles move away of these regions showing negative DEP behavior. This study indicates that such a novel fabrication process has the potential to improve applications for dielectrophoretic separation, concentration, and fractionation of biomolecules.

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AES-15

Impedance Analysis of Endothelial Cells in Development of an Orbital Shear Platform

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Impedance analysis of adherent cells on top of an electrode can reveal information about cell morphology, monolayer permeability, and other physiological parameters. This data provides insights into the structure and functionality of the endothelium in real-time. Because the cell monolayer is subjected to wall shear stresses that oscillate due to the pulsatile flow of the cardiovascular system it is necessary to investigate the behavior of endothelial cells under conditions that simulate the environment in vivo. The aim of this study is to develop a microfluidic impedance platform for the characterization of endothelial barrier function during hydrodynamic stress. For this purpose a device was fabricated that contains a circular region for cell monolayer growth that can be used to produce a rotational oscillatory fluid flow when the device is placed on an orbiting table. Human Umbilical Vein Endothelial Cells (HUVECs) were cultured on the surface of the device, which contains patterned electrodes for recording impedance. At various time points the impedance of the endothelial cells was recorded between 40Hz and 1.1MHz using AC of amplitude 10 millivolts. A high-precision impedance analyzer (Agilent 4294A) and impedance probe (Agilent 42941A) were the instruments used for data acquisition. In order to develop an appropriate circuit model to describe the behavior of the device the impedance data from static cell cultures was curve-fit using impedance analysis software (Scribner Associates ZView). The results of the circuit model curve-fit demonstrated that cell monolayer resistance increases and capacitance decreases as the fraction of the device surface area that is covered by cells increases. These changes in the electrical characteristics of the cell monolayer both contribute to higher impedance as reflected in the data when compared to the impedance of the device with media and attachment factor alone. The future direction of this study is to analyze the impedance of the endothelial cells when they are subjected to hydrodynamic shear stress with the expectation that endothelial barrier function will become more permeable.

AES-16

Nano-Bioparticle Separations Based On Frequency Response for Double-Layer Polarization

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Diagnosis of diseases and detection of their pathogenesis requires the quantification of a spectrum of closely related biomarkers. However, these closely related biomarkers are present in extremely small quantities (~ng-pg/mL) and need to be detected in complex biological fluids, such as blood serum, which contain several other proteins at million to billion-fold higher concentration levels (mg/mL). To address this need for separations and selective enrichment of closely-related biomarkers over other proteins in the bio-fluid media, we herein explore frequency-selective methods for polarization of the electrical double-layer around the bio-particles. Dielectrophoresis (DEP) enables highly selective translation of polarized bio-particles based on the characteristic frequency response of the dielectric permittivity of the bio-particle versus that of the medium, and it has been extensively applied towards

sorting of somewhat similar sized biological cells with differing dielectric frequency response. However, its application to nanoscale bio-particles, such as ss-DNA, proteins, and nanostructures requires micro- or nano-device geometries to enhance the local field to offset the steep fall in dielectrophoretic trapping forces with particle size. Herein, through utilizing a microfluidic electrode-less DEP device with 1000-fold lateral constrictions to enhance the localized field, we demonstrate that the relaxation time constant for polarization of the electrical double layer around nanoscale bio-particles is highly dependent on its size and charge density. The double layer around nanoscale bio-particles is completely formed at ~kHz frequencies and the ensuing screening of the external electrical field by the double layer causes negative DEP behavior. On the other hand, at 0.1-1 MHz frequencies where the double layer is not fully formed, the bio-particles exhibit positive DEP due to interfacial polarization. We demonstrate that this crossover from negative to positive DEP is highly sensitive to small differences in size and charge density of bio-particles. Bio-particles of smaller size and/or higher charge density exhibit substantially faster relaxation frequencies, thereby extending field screening and negative DEP to higher frequency ranges. This enables more effective bio-particle separations, based on the magnitude as well as direction of the DEP force. We envision the application of this methodology towards separations for biomarker discovery.

AES-17

AC and DC Protein Streaming and Trapping with Insulator-Based Dielectrophoretic Devices

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Microfluidic devices offer several experimental advantages over macroscale methods such as speed, efficiency, and high throughput giving them great potential for rapid and reliable separation and analysis of proteins. Here we propose to manipulate proteins rapidly and reliably with a novel migration technique utilizing dielectrophoresis (DEP). Our study demonstrates protein DEP responses under DC and AC conditions using a variety of insulator-based DEP (iDEP) devices and proteins including immunoglobulin G (IgG), lysozyme, and β-galactosidase. Even though DEP has been extensively employed as a separation, fractionation, and pre-concentration technique for large biological objects such as cells and DNA, protein DEP behavior is not well understood. Our detailed study of protein DEP provides novel information for future optimization of this protein migration method for pre-concentration and other analytical techniques.

First, we performed numerical simulations to estimate electric field strength (E) as well as electric field gradients, ∇E^2 within our microdevices. Based on the calculated E and ∇E^2, we simulated protein concentration profiles considering electrokinesis, protein diffusion, and DEP to predict protein migration behavior. Simulation results indicated that under both positive and negative DEP conditions, proteins concentrated as streamlines between the post arrays. With IgG, we experimentally observed protein streaming behavior due to positive DEP with a maximum concentration of 70 %. This concentration profile was in excellent qualitative agreement with numerical simulations for a monomeric IgG species. Moreover, using improved nanoconstriction devices, we successfully trapped proteins under both AC and DC conditions. Our study thus provides valuable information to develop novel protein iDEP devices for separation, pre-concentration, and fractionation.

AES-18

A Chaotic Map of Accelerated DNA Replication in Microscale Rayleigh-Bénard Convection

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We have previously shown that chaotic advection in micro-scale flow geometries can be harnessed to greatly enhance the rate of thermally activated biochemical reactions (e.g., the polymerase chain reaction (PCR)). Here we present a 3D analysis of coupled flow and reaction that provides a time-resolved mapping of the DNA replication process as it unfolds. Chemical reaction kinetics are incorporated into the 3D flow model, enabling us to obtain a measure of global DNA replication rate within the entire reactor volume, expressed in terms of a characteristic doubling time. Mass action kinetics were assumed for the denaturing, annealing, and extension steps. Annealing and extension were modeled as bimolecular reactions to capture the contribution of the primers and dNTPs. Reversibility of denaturing and annealing reactions was also taken into account. Reaction models of varying complexity were compared via addition of side reactions. individual stream traces were examined for different geometries, expressed in terms of their aspect ratio (height/diameter, h/d). The residence time of a fluid element in a particular reaction zone and the number of times that fluid elements pass through the zones were determined for 300 randomly chosen stream traces yielding a distribution of residence times and frequencies of ingress in each of the reaction zones. At larger values of h/d we find that individual fluid elements on average spend more time in each temperature zone. But the number of times a fluid element passes through each zone is much larger in wider geometries (lower h/d), owing to the chaotic characteristics of the flow field. Since our experiments show much faster DNA replication at low h/d, the number of excursions into each reaction zone appears to play a more important role than the transit time through each temperature range. Finally a reaction map was constructed to determine the cylindrical cell geometries and other parameters that should be used to enhance the efficiency of microscale Rayleigh Bénard convection PCR. These results highlight the importance of the flow field's 3D characteristics.

AES-19

Particle Deposition and Potential Resuspension Studies Inside Microchannels by Reflection Interference Contrast Microscopy

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Different deposition scenarios are expected when micron-size particles are deposited on a surface depending upon environmental conditions (relative humidity, temperature), deposition medium (air, liquid), and time effects. For instance, when using a liquid as deposition medium, a liquid meniscus can form between the particles and the substrate at the end of the drying process; depending on the conditions, the meniscus can dry out completely or partially, and the particles might undergo deformation due to capillary forces, in addition to regular adhesion forces, creating a variety of deposition scenarios. Reflection Interference Contrast Microscopy (RICM) offers a unique and convenient view of the deposition phenomenon as the minimum separation distance between particle and substrate, contact area, and particle contour can be accurately quantified when looking at the sample from below using monochromatic light. For particles deposited inside a properly setup microfluidic chamber, experiments can be

performed to study the effect of an increasing shear rate that eventually produces unbinding of particles adhered onto the substrate under controlled conditions.

AES-20

Rapid Electrokinetic Patterning (REP) of Hydrosol Colloids at a Planar Electrode Surface

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Rapid Electrokinetic Patterning (REP) is a relatively new technique for controlling hydrosol colloids at a planar electrode surface. REP uses AC electric fields and a heat source to generate micro-scale vortices for manipulating trapped particles. An infrared laser was used as a heat source to characterize aggregate groups of particles. A device was constructed using an indium-tin oxide (ITO) coated glass slide and cover slip. The glass slide and cover slip were separated by 50 µm double-sided tape with a small region removed. One-micron polystyrene spheres were inserted into a solution of water and KCl. The infrared laser was focused on and partially absorbed by the ITO on the surface of the cover slip. This generated a thermal gradient in the fluid medium, which generated a gradient in the electrical properties of the medium. The AC electric field acted on this gradient to produce a net force on the medium away from the surface of the electrode. Particles trapped at the electrode surface by the AC field were then corralled into a group.

Particles collected on the surface of an electrode were manipulated to vary the particle-particle spacing. Aggregations were stretched into lines as well. The spacing of particles was characterized in order to study the crystallinity of the aggregation. The spacing was characterized in dot-shaped aggregations by laser power, AC frequency and voltage, medium conductivity, and number of particles in the aggregation.

More complicated geometries, such as ellipses, are shown. Particles were aggregated in a dot-shaped aggregation, then stretched into a line-shaped aggregation, and then into an elliptical shape. Intersecting and colliding geometries are also discussed, such as two line-shaped aggregations brought into contact with each other.

AES-21

Protein Structure Determination Using X-RAY Compatible Microfluidic Platforms

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Microfluidics has found extensive use in fields as diverse as energy conversion, medical and clinical diagnostics, point-of-care medical devices, proteomics and protein crystallization. Microfluidics allows for ease of sample preparation, small sample sizes, ease of scale-up, as well as integration of various sample analysis techniques on a single platform[1,2].

Here, we focus on the application of microfluidic platforms for membrane protein crystallization. Membrane proteins (MPs) play a crucial role in many important biological processes including energy and material transduction across cellular membranes, molecular recognition and immune response. They are amphiphilic, structurally diverse, and extremely responsive to the surrounding environment. Efforts to understand the structure-function aspects of these proteins through X-ray crystallography have been hampered by difficulty in obtaining high quality crystals[3]. Identifying the optimal crystallization condition(s) requires systematic screening of a huge

chemical space (salts, buffers and precipitants), which requires a lot of protein sample that is generally not available. Additionally, current methods of structure determination are not optimized for handling small and fragile crystals that MPs tend to form.
We present an array-based, X-ray compatible microfluidic platform comprised of cyclic olefin copolymer (COC) and a thin polydimethylsiloxane (PDMS) membrane needed for valve actuation. The array-based design allows for screening for upto 96 conditions using < 6μL of protein solution. After screening, a similar chip is used for structure determination of the protein by merging data from multiple crystals grown on-chip. The use of X-ray compatible materials allows on-chip analysis, thus preventing damage to fragile protein crystals that might occur during manual harvesting and mounting of crystals. As proof of concept, we have screened crystallization conditions for a soluble protein, phosphonoacetate hydrolase(PhnA). After obtaining the optimal condition and using anomalous data collected solely on-chip, wedetermined its structure to a resolution of 1.99 Å.
We are now in the process of validating our platform by screening and solving the structure of two MPs - photosynthetic reaction center (RC) and cytochrome ba3-type oxidase. We have collected data on RC to 3.0 Å and are currently optimizing the crystallization conditions and chip design to get higher quality data.

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AES-22

Temperature Measurement in a Microfluidic Platform for Insulator-Based Dielectrophoretic Protein Manipulations

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Direct current (DC) insulator-based dielectrophoresis (iDEP) has been used with cells and biomolecules such as DNA and protein for separation, pre-concentration, and fractionation. Unlike other existing analytical techniques, DEP response is governed by a particle's polarizability in an inhomogeneous electric field. This additional parameter space facilitates improved separation in a gel-free environment which is of particular importance for more complex samples such as disease markers found in bodily fluids. DC iDEP has potential to be used as an alternative to AC iDEP since DC iDEP does not require electrokinetic and/or pressure pumps necessary in AC iDEP experiments. Despite this advantage, the application of large DC voltage in iDEP results in heat generation known as Joule heating within the microfluidic device. This phenomenon is of great interest due to its influence on protein migration and stability and has not been thoroughly investigated experimentally under iDEP conditions. In this work, we present a means to measure fluid temperature in microfluidic systems by implementing fluorescence microscopy with dual color detection via a beam splitter and CCD camera. We have demonstrated a way to quantify the fluorescence emission ratio of a temperature sensitive dye, Rhodamine B (RhB), and a temperature insensitive dye, Rhodamine 110 (Rh110), using the same device developed for our protein DEP experiments. Our preliminary experiments show that there is no significant temperature rise either within the channel or reservoir when low conductivity buffers are used. This experimental finding is in agreement with numerical simulations.
In addition to DC iDEP, our scope can be further expanded to temperature measurements within a channel with smaller structures and constrictions (i.e. nano-posts). In combination with AC iDEP, nanostructures can provide larger DEP forces to immobilize proteins via DEP trapping making it important to measure temperature fluctuations due to the expected increase in Joule heating effects in small constrictions. Our study provides valuable information about the micro- and nano-environment in which protein iDEP experiments are performed leading to a more profound understanding of protein iDEP.

AES-23

Manipulation of Nanoparticles Using Electro-Kinetics Generated by Nano-Needles

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Electro-kinetics is the motion of a fluid or a suspended particle due to the effects of an electric field on the fluid and particle. There are three types of electro-kinetic forces generated by a nano-needle that can manipulate nearby particles. These are dielectrophoresis, AC electroosmosis, and electrothermal fluid flow.
Dielectrophoresis is the movement of a neutral particle through an electric field gradient due to an imbalance in induced charge forces on the particle. Dielectrophoresis is considered positive when the particles are attracted to the location of the highest electric field gradient, and it is considered negative when the particles are repelled. The magnitude of the dielectrophoretic force is directly proportional to both the electric field gradient, and the volume of the particle. The electric field gradient needed to capture or repel particles is inversely proportional to the size of the particle because other forces begin to dominate particle motion as particle size decreases. AC electroosmosis (ACEO) is the electro-kinetic pumping of ionic fluids caused by an AC electric field. Charges inside the double layer are acted upon by the tangential component of the AC electric field. The region influenced by ACEO is close to the electrode surface, and is dependent upon the dielectric properties of the surrounding fluid. ACEO typically dominates when the AC field has a low frequency and when the fluid has low conductivity. Electrothermal fluid flow occurs when an electric field acts upon fluid dielectric gradients caused by a non-uniform temperature field. Electrothermal fluid flow typically dominates when the AC field has a high frequency and when the fluid has high conductivity.

When an AC signal is applied using a nano-needle and a planar surface as electrodes, sharp electric field gradients form in the surrounding fluid at the tip of the needle. These gradients cause the above electro-kinetic forces to act on the fluid and any nearby particles. The dominating electrokinetic mechanism is a function of the conductivity of the fluid, the frequency of the AC field, the location of the particle, and the diameter of the particle.

The nano-needles used were made from siver gallium (Ag2Ga) and were manufactured at room temperature on the end of a probe pulled from a glass capillary tube. The nano-needles had a diameter less than approximately 500 nm and were up to 25 μm long. The nano-needles were typically positioned less than 40 μm above the planar electrode. Using these electrodes and supported by numerical simulation, these electrokinetic mechanisms were demonstrated in low conductivity solutions using particles of various sizes and materials.