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Poster Abstracts

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AES-1

Bioparticle Separation in An Insulator-Based Dielectrophoretic Microchannel

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A significant and wide-ranging body of work is focused on rapid bioparticle characterizations. Clinical diagnosis will be directly impacted by innovations in this field because it will enable rapid, on-site bioanalysis, improving the availability, accuracy, and scope for these tests. For bioparticle analysis, 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 a separations-based strategy for isolating and concentrating intact microorganisms within a single, continuous microchannel.

Using DC insulator-based dielectrophoresis in a converging, sawtooth-patterned microchannel creates multiple and distinct bioparticles traps. This channel design enables localized isolation and concentration of a wide variety of microbes and other particles based characteristic differences in their physical properties. Various targets have been captured and concentrated within the device, including human blood cells and mature amyloid protein fibrils. Recent work has focused on using this approach to differentiate very similar targets, such as different strains of a single bacterial species. This approach has demonstrated the ability to distinguish three serotypes of live Escherichia coli, indicating the potential this technique holds in terms of both separatory power and diagnostic applications.

AES-2

Dual-Electrode Electrochemical Detection for Microchip Electrophoresis: Voltammetric Identification of Chemically Labile Species

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Reactive nitrogen species such as nitric oxide (NO.) and peroxynitrite (ONOO-) are chemically labile species that participate in oxidative stress and nitration/nitrosylation in vivo. These species have been implicated in several cardiovascular and neurodegenerative diseases. The short life time of these molecules makes them difficult to detect, often requiring indirect methods of analysis. Microchip electrophoresis coupled to amperometric detection (ME-EC) offers fast separations and sensitive detection— allowing these species to be characterized before significant degradation. Amperometric detection normally utilizes migration time to identify analytes in a sample. For complex samples such as cell lysates, analyte identification solely utilizing migration time becomes problematic when contamination protrudes. Therefore, a ME-EC method with dual electrodes was developed for identification of analytes by voltammetric characterization. Voltammetric information for analytes was obtained through a current ratio generated by employing two working electrodes in a series configuration. The current ratio can be unique to analytes with different half-wave potentials and deviations from such can imply impurities. The electrodes were integrated into a 5 cm simple "T" microchip. In this setup, the first electrode is in in-channel configuration while the second electrode is in end-channel configuration. Nitrite, tyrosine and hydrogen peroxide standards were used to optimize the system. Current ratios for these standards were generated by correcting sensitivity differences between two working electrodes. Applying this to commercially available peroxynitrite samples, it was found that test samples were contaminated with hydrogen peroxide, which is used in peroxynitrite synthesis. This method will be employed to identify RNS production in bulk cell lysates. The ultimate goal of this project is to identify the heterogeneity of reactive nitrogen species production in single cells.

AES-3

An Ipad-Based Brownian Dynamics Simulator for Electrokinetics in the Classroom

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Tablet-based computing platforms offer enormous potential to provide immersive education experiences by virtue of their unique combination of a graphical environment and touch screen interface. Here we describe our efforts to exploit these features by porting a conventional Brownian dynamics simulation into an Apple iPad application that provides a visual representation of polymer coil size, relaxation phenomena, Brownian motion, and transport under an external driving force (i.e., electrophoresis).

Our app employs a coarse-grained bead-spring model of the polymer chain, enabling the user to customize the number of beads and their positions to define an initial polymer conformation. The user can choose between infinite and finite extension models for the connecting springs. The app's core simulation module provides two functions. First, in the free solution simulation mode, it mimics the polymer's Brownian evolution toward an equilibrium state. Key parameters continuously reported during the simulation include radius of gyration Rg, end-to-end length L, largest and smallest spring lengths, and contour length (determined from the average spring length). Secondly, the app is capable of simulating the polymer's response when a user moves individual beads by touching the screen, with force proportional to the speed of the user's finger swipe on the screen. This capability can be used to visualize the process of polymer stretching (single bead drag) or electrophoretic transport (whole polymer drag).
The app-based format makes it possible to visualize polymer dynamics in an appealing way and offers a useful tool to illustrate fundamental concepts involving polymer physics.

AES-4

Assembly of "Anisotropic" Colloidal Dimers and Spheres Under Applied Electric Fields

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Colloids possessing anisotropic interactions could potentially assemble into a much wider range of crystalline arrays and meso-structures than spherical particles with isotropic interactions. Here, we investigate the impact of geometric anisotropy on the assembly of colloidal dimers on conducting substrates under external electric fields. By systematically tuning the size ratio between two lobes on dimers, we have found that interactions between dimers strongly depend on their relative orientations. For example, the interaction between lying and standing dimers on the substrate is attractive. When all dimers stand on the substrate, the interaction between neighboring dimers with alternating orientations is also attractive. Otherwise, it is repulsive. Such kind of orientation-dependent interactions generate a good variety of new structures, such as chiral clusters and dimer crystals with alternating orientations. We will discuss the physical origin of those orientation-dependent interactions and the impacts of experimental conditions such as the ionic concentrations and surface charges. Our numerical model based on electrostatics agrees well with experimental observations and provide further insights on electric-field assisted assembly of anisotropic particles.
In addition, the assembly of isotropic spherical colloids under applied electric fields is investigated. By applying an external AC electric field, we show that the apparently isotropic particles experience "anisotropic" interactions. New types of sphere-packing have been observed within a previously unexplored experimental regime: low salt concentrations (<10-3M) and low frequency regime (100 Hz to 10 kHz). At low particles concentrations, a family of well-defined oligomers, ranging from 3 to 10 was observed. At high particles concentrations, the colloidal clusters will further assemble and connect themselves into a good variety of two-dimensional non-close-packed networks. We attribute these new types of non-planar structures to the competition among double layer repulsion, dielectrophoretic attraction, and dipolar interactions. The double layer and in-plane dipolar repulsion could make bottom

particles in the clusters separate from each other. While the out-of-plane dipolar attraction and particle-substrate dielectrophoretic attraction could be responsible for the formation of the clusters, i.e., the top central sphere is associated with the bottom spheres. The effects of salt concentration and frequency on the geometry of those colloidal molecules will be discussed. These non-close-packed structures could be used as building blocks for making photonic crystals and plasmonic structures.

AES-5

X-Ray and Raman Transparent Solvent Resistant Microfluidic Platforms to Screen Solid Forms of Pharmaceuticals

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We present microfluidic platforms for screening solid form (salts, co-crystals, and polymorphs) of pharmaceutical parent compounds (PC). Success in finding a crystalline solid form of a PC with optimized CMC properties using conventional screening procedures is limited due to the limitations of the number of experimental conditions that can be investigated while utilizing small quantities of PCs available (≈ 10 mg) in the early stages of drug development. Microfluidic technology allows screening with reduced sample volumes of PC and precipitants (salt or cocrystal formers or antisolvents) solutions [1] and thereby enable screening of multiple conditions using the limited amount of PC. This will enable early identification of all possible solid forms of PCs and help reduce the time and money invested in the solid form development.

To date, we have reported on hybrid polymer-based microfluidic platforms that permit combinatorial mixing of PC and precipitants solutions on-chip in arrays of 24/48, 50 to 200 nL wells, which is a drastic reduction in the volume of PC needed per condition compared to traditional approaches (~5 to 100 ul per condition [2]) [3-6]. These platforms were compatible with mild organic solvents and water, and enabled identification of the solid form crystallized on-chip via on-chip Raman spectroscopy. We screened for solid forms of PCs employing free interface diffusion (FID) mixing [3,5], antisolvent addition (AS) [4], and solvent evaporation [6] modes of crystallization employing the polymer-based platforms.

Here we present the design, fabrication, and application of solvent resistant microfluidic platforms for solid form screening of PCs. These newly developed hybrid polymer-based microfluidic platforms are compatible with a much wider range of solvents including chloroform, toluene, and hexanes, thereby enabling investigation of a much wider range of conditions on-chip as well as allow a better control on the supersaturation levels attained on-chip. We employed these platforms to screen for solid forms of model compounds including theophylline, piroxicam, and carbamazepine. On-chip Raman and X-ray analysis was employed to identify and distinguish different solid forms crystallized on-chip. These platforms have the potential to expedite the drug development process by enabling solid form screening at the early stages of drug development.

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AES-6

Brownian Dynamics Simulations of Electrophoretic DNA Separations in a Conducting Post Array

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We use Brownian dynamics simulations and computational fluid dynamics to exam the electrophoretic DNA separations through a conducting post array. Electrophoretic separation of DNA through a post array has been heavily investigated in both experiments and simulations. However, the posts were assumed to be either insulated or having the same conductivity as the buffer solution. In this study, we exam the idea that conducting posts may result in better DNA separation. Since the electric field lines concentrate around the more conductive posts, DNA molecules were expected to have higher probability to collide with the posts and to experience hooking and unhooking events more frequently, leading to better separation. In our simulations, we set the posts to have different degrees of conductivity higher than that of the buffer solution and analyze the average electrophoretic mobility and dispersion coefficient of lambda-DNA (48.5 kbp) and T4GT7-DNA (166 kbp) as they move through the array. However, the simulation results show that DNA separation has not been improved in spite of the higher collision rate. Moreover, in contrast to the observation in the insulated post array, T4GT7 DNA was found to move faster than lambda-DNA. We will explain these rather unexpected results with more detailed analysis.

AES-7

Cell Lysis in Microfluidic Devices Employing DC Electric Currents

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Electrokinetics is a widely employed technique for particle manipulation in microdevices. Combination of electrophoretic, electroosmotic, and dielectrophoretic, among other forces are used with AC and/or DC electric fields inside microchannels for different applications. In this work, cell integrity of E. coli was evaluated in a microchannel after applying DC electric fields, as a method for cell lysis for DNA recovery. The use of surfactants along with electric field gradients, created by the inclusion of insulating posts in the

channel, caused cells to lose viability and release cytoplasmic material to the medium. This method can be used as an alternative for cell lysis and applied to protein, RNA, or DNA recovery.

AES-8

Fabrication of 3D Electrodes for Electrorotation Experiments

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Introduction

Electrorotation (ROT) is a powerful technique that has been used to characterize and differentiate different particles through the extraction of their electrical parameters. Through the study of a particle's response to an electric field at specific frequencies, it is possible to extract information such as the membrane integrity of biological particles. In order to achieve high accuracy of the extracted parameters, a rotating electric field with high linearity needs to be generated. For this purpose, Goater et al. introduced bone-shaped electrodes [1]. Most devices used for electrorotation consist of planar electrodes that result in electrical fields in z-direction, which causes out of focus movements and makes trapping and observation of the particles difficult. Different groups tried to overcome this problem by designing chips with octopole cages that trap and rotate cells at the same time [2] or by combining the ROT devices with laser tweezers for trapping [3]. We introduce a novel fabrication process for three-dimensional microelectrodes that can be used for electrorotation experiments. The advantages of our design are that the generated electric field does not have any z-component and thus no out-of-focal-plane movements are exerted on the particle. Moreover, the electrodes serve as a kind of mechanical trap for the particles. Further advantages of the presented fabrication process are that the microfluidic structures can be aligned with photolithographic precision and that it is possible to work with bulky and short-working distance lenses.

Simulation

The electrical fields that are generated by 3D and planar electrodes were simulated using Comsol Multiphysics. In both cases a bone shaped design of the electrodes was used for simulation. From the top views in Fig. 1 it can be seen that both designs lead to highly linear fields in the x-y plane, however, in the case of the planar electrodes the generated field lines have components in z-direction as well. A further advantage of 3D electrodes is that local heating effects can be reduced by about an order of magnitude, while keeping in the center the same field strengths as with the planar electrodes. This is due to the higher electrode surface that is used to apply the potential, which leads to a reduction of the current density.

Microfabrication

The microfabrication process is shown in Figure 2. We start with a silicon wafer passivated by Si3N4 and SiO2 layers with thicknesses of 200 nm each. First, a photoresist is applied by spin coating and is structured by photolithography. The oxide and the nitride layers are etched and the design is transferred 300 µm deep into the silicon by a Bosch process. Then, a 10 µm thick SU-8 layer is patterned by photolithography. For better adhesion of the metal on the SU-8, the resist is hardbaked at 135°C and plasma activation is done before sputtering a Ti/Pt layer onto the structures. Afterwards, a dry film resist is laminated on the wafer and patterned by photolithography. The resist serves as etch mask for plasma etching of the metal layer. After stripping of the photoresist, a second SU-8 layer to pattern the

microfluidic channels is applied. To planarize the SU-8 layer that is spun onto the high topographic surface, chemical mechanical polishing (CMP) is performed after the post exposure bake and before the development of the resist. This strategy is chosen that the undeveloped resist protects the access pads during CMP. Afterwards, the SU-8 is developed and the microfluidic accesses are opened up by etching the backside oxide and nitride layers and by grinding the wafer down to 300 µm from the backside. Finally, the wafer is diced and a coverslip is bonded onto the chips, where the SU-8 serves as adhesive. In Figure 3 we show a picture of such an electrorotation chip (a) and SEM images of (b) the 3D electrodes without glass coverage of the microfluidic channels and (c) the cross section of the whole chip with glass coverage.

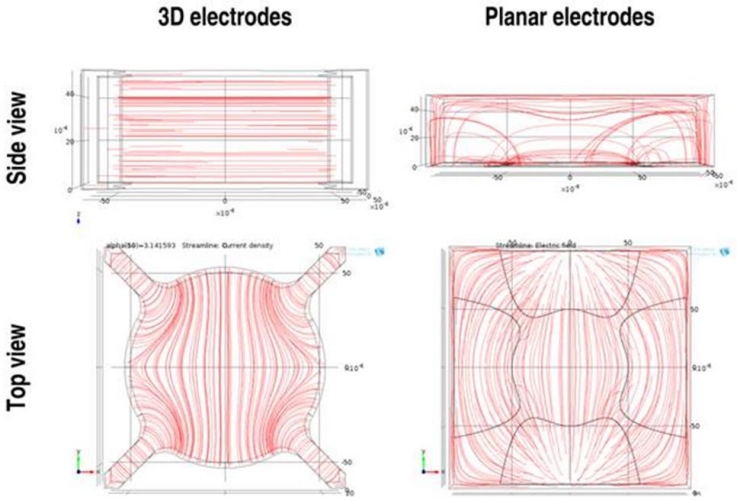

Figure 1: Comsol simulations of the electric field for planar and 3D electrodes

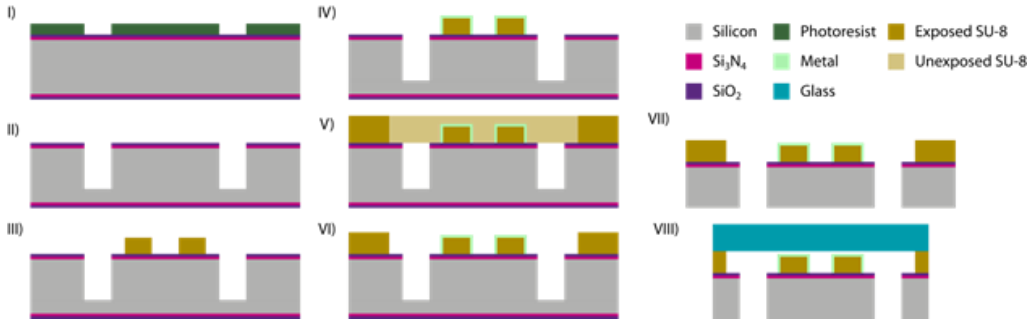

Figure 2: Fabrication process of the electrorotation chips with 3D electrodes

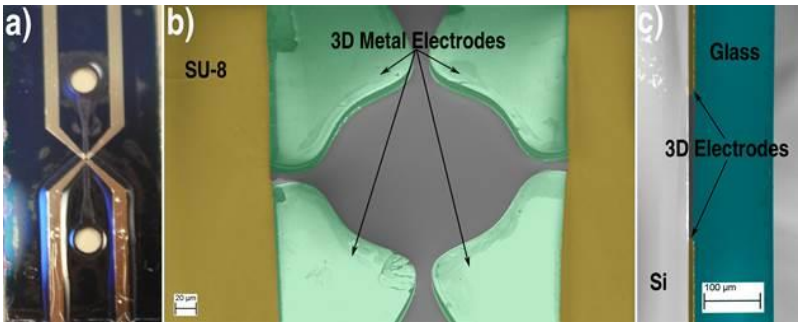

Figure 3: (a) Electrorotation chip; (b) SEM of the 3D metal electrodes; (c) SEM of the cross-section of a chip with the glass coverage.

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AES-9

Electrokinetic Behavior of Large Polystyrene Particles in Insulator Based Dielectrophoresis

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Dielectrophoresis is a microscale electrokinetic technique that has been successfully employed for the manipulation and assessment of a wide array of bioparticles, from proteins, to DNA to cells. This work is focused on insulator-based dielectrophoresis (iDEP), a technique where dielectrophoretic forces are generated by creating non-uniform electrify fields using insulating structures embedded between two external electrodes. It was recently observed that "larger" (>5 μm diameter) polystyrene spheres did not exhibit a clear negative dielectrophoresis (DEP) behavior when working with a microfluidic channel with insulating posts in insulator-based dielectrophoresis (iDEP) experiments. DEP theory predicts that larger particles will experience much stronger trapping than smaller particles, which have been easily manipulated under identical conditions. Cells in the 5-10 μm range have also been successfully manipulated using iDEP. Understanding the deviation of large polystyrene particles from predictions of current DEP theory may lead to novel applications and methods for DEP based manipulation and separation of particles.

AES-10

Size Based Separation of Lipid Droplets Using Insulator-Based Dielectrophoresis

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Cells store neutral lipids in cytosolic lipid droplets, which are hypothesized to form from the endoplasmic reticulum. Droplets are often "called obesity" organelles because they occupy the vast majority of the volume of adipocytes in white adipose tissue. Much information on the roles of lipid droplets has been obtained by proteomic and lipidomic analysis of isolated droplets. However, lipid droplets harvested from

cultured cells can only be analyzed in aggregate. Here, we present size based sorting of lipid droplets using insulator-based dielectrophoresis (iDEP). Submicron lipid droplets from yeast cells and lipid cells from human hepatocytes and human adipocytes in the 1-10 μm range were sorted according to their sizes in a microchannel with insulating posts using insulator-based dielectrophoresis (iDEP). DEP refers to the movement of particles, due to polarization effects when particles are exposed to non-uniform electric fields. In particular, in iDEP, these electric field gradients are generated by employing insulating structured between two external electrodes. By carefully selecting operating conditions and microdevice design, it was possible to concentrate and sort a sample of lipid droplets.

AES-11

Using Low Frequency Electrical Signals for Particle Separations With Dielectrophoresis

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In insulator-based dielectrophoresis (iDEP), particles are trapped and immobilized when the dielectrophoretic force acting on them dominates over other transport mechanisms, such as by electroosmotic flow (EOF). Thus, particles are released from the "dielectrophoretic" raps when EOF dominates over the DEP. Cyclical electrical signals were used to reduce and fine tune EOF transport by allowing iDEP separations using lower applied electric potentials than reported before. The cyclical signals reduced net EOF since EOF direction depends on the electric field direction. DEP direction does not change with the direction of the electric field, and was not weakened by a cyclical signal. Polystyrene spheres of different sizes were separated in a microchannel with cylindrical insulating structures 450 μm diameter and spaced 500 sym center to center. Initially, a sinusoidal signal between +400 and -400 V was applied, and minimum value was gradually increased, while the maximum value was kept constant at +400 V. This method achieves iDEP separations at lower applied voltages than those reported in previous work for similar particle mixtures.

AES-12

The Zeta Potential Of PMMA Surfaces In Contact With Electrolytes Of Various Conditions: Theoretical and Experimental Investigation

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The miniaturization of fluidic systems has developed into versatile technologies such as Lab-on-a-Chip which aims at the integration of all tasks that are performed in a (bio-)chemical laboratory on a single microfluidic chip. Typical tasks comprise unit operations such as sample enrichment, mixing, temperature

cycling, and species separation with subsequent detection. A multitude of these unit operations can be conveniently realized by using electrokinetic phenomena since they can be controlled by an external electrical field induced in the micro-structures. Generally, electrokinetic phenomena are related to the presence of electrical surface charges of substrates -e.g. micro-channel walls or dispersed particles- in contact with a liquid. The origin of the surface charges can be manifold and their impact on the electrokinetic phenomena is also determined by the liquid parameter. Since surface charges cannot be easily measured, the zeta potential is considered as a relevant parameter for electrokinetic phenomena instead.
Poly(methyl methacrylate) (PMMA) is an attractive microfluidic substrate since micron size features can be manufactured by employing cost effective and rapid techniques such as hot embossing. However, the surface charge mechanisms of PMMA surfaces with respect to acidic and alkaline environments are not very well understood and the data found in literature shows significant discrepancies. In this work, the zeta potential of PMMA surfaces with respect to ionic strength, pH, temperature and the nature of co- and counter-ions of aqueous electrolytes are investigated both theoretically and experimentally. In terms of theoretical investigation, we numerically solve the Poisson-Boltzmann equation for a cylindrical coordinate system to obtain surface potential gradients for various electrolyte conditions. The assumption of a solely diffusive double layer configuration (i.e the Gouy-Chapman model) implies an absence of surface reactions and that the zeta potential is influenced by pure shielding. In case of low to moderate ionic strengths, the computational results predict a linear correlation with the logarithm of the ionic strength and a minor influence of temperature and electrolyte composition. Experimentally, we engage electrophoretic light scattering to infer zeta potentials of PMMA particles dispersed in various aqueous electrolytes. In detail, negative zeta potential values are observed at all conditions which imply negatively charged surfaces. It is observed that the magnitude of the zeta potential values decreases linearly as the ionic strength of the aqueous electrolytes increases. If we normalize the zeta potential values with the logarithm of the ionic strength, a collapse of the data to a single curve is observed. The pH dependency of the PMMA zeta potential values can be explained as two distinct regions. We find that in an acidic to neutral pH range a negligible zeta potential variation while significant pH-dependency can be observed for an alkaline milieu. Further to our study on temperature dependency of zeta potentials, we find that parameter-corrected viscosities and permittivities play a vital role for the correct interpretation of the results. Finally, we observe that the electrolyte's counter-ions have more contribution in the determination of zeta potential in comparison with the electrolyte's co-ions. The data are used to infer an empirical correlation which can be used for modeling of electrokinetic phenomena and to derive design guidelines for electrokinetic unit operations for microfluidic applications.

AES-13

High Dynamic Range Imaging Of Polyacrylamide Gels For Proteomic Exploration

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The range of protein concentrations in living cells is vast, approximately 100,000-fold. This poses significant problems to comparative proteomic studies where the goal is to detect protein differences between cells across different conditions. We have developed an imaging device, referred to as Structured

Illumination Gel Imager (SIGI), that is designed to detect proteins over a million-fold concentration range in an electrophoretic gel. Scientific-grade, Peltier-cooled, 16-bit CCD cameras can distinguish 65,535 gray levels, or ~10,000-fold protein concentration range. Beyond this range gel images saturate, where brightest pixels have a value of 65,535 regardless of true brightness, making longer exposures uninformative and severely limiting dynamic range. SIGI extends dynamic range through structured illumination: an LCD projector projects a binary mask where regions of the gel with low-abundance proteins receive full illumination while regions with high-abundance proteins receive no illumination, preventing high-abundance spots from saturating the CCD camera, thus enabling longer exposures. Applied iteratively, structured illumination allows SIGI to capture exposures many times longer than typical single-exposure images taken without masking. In our experiments SIGI detected proteins from 10 picograms to 10 micrograms in the same image of a typical polyacrylamide gel, a dynamic range of 1,000,000 fold.

SIGI's projected masks also reduce light scatter, making it possible to visualize low-abundance proteins close to high-abundant proteins in 2DE gels. Quantification of 2DE gels using SIGI as compared to conventional fluorescence imaging shows a more than 50% increase in the number of detected protein spots. These results show SIGI's potential for peering more deeply into the proteome than previously possible.

AES-14

The Effect Of Cell Detachment Methods On The Electrical Properties Of The Cells

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Dielectrophoresis (DEP) has gained significance in recent years in various biological applications. This technique is able to detect the dielectric properties of cells, including the conductivity and permittivity of the membrane and cytoplasm by employing non-uniform AC electric fields. Whilst DEP has increasingly been used for cell characterisation, questions have arisen about the validity of cell membrane measurements where the membrane is modified by the protocol, for example in the detachment of adherent cells.

In order to asses this; we have evaluated the DEP-derived properties using several detachment methods. In this study, seven different methods including six different cell detachment reagents (Trypsin-EDTA 0.25x, Trypsin-EDTA 1x, Dissociation Fluid, Dissociation Buffer and Accutase) and the scraping method were examined through DEP experiments. 3-D electrode microwell DEP chips developed at the University of Surrey was employed in this research to investigate the electrical properties of the cells, and the conductivity and permittivity of membrane and cytoplasm were determined. HN5 cells were cultured in RPMI medium in standard cell culture conditions.

The results revealed that scraping made the most significant effect on cell properties causing a remarkable reduction in membrane conductivity values. However, the membrane conductivity values remained unchanged using the other detachment methods. Trypsin EDTA produced minimum effects on membrane conductivity and permittivity of the cells relative to other cell detachment methods.

AES-15

Using Gradient Insulator-Based Dielectrophoresis to Concentrate Small Molecular Weight Proteins

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Dielectrophoresis (DEP) has the potential to serve as a separation and concentration technique for small volume samples. Dielectrophoresis has typically been applied to particles or cells, but recently analytes with small molecular weights such as proteins, have been targeted. Protein dielectrophoresis has been demonstrated, with the first observations of protein DEP trapping about twenty years ago by Washizu et. al. (IEEE Trans. Ind. Appl., 1994, 30, 835-843). The smallest proteins to be captured thus far using techniques similar to ours are the proteins BSA at 66 kDa and streptavidin at 60 kDa.

Proteins of smaller molecular weights have been studied, and even successfully manipulated using dielectrophoresis resulting in streaming (Electrophoresis, 2013, 34, 1085-1096), but discrete isolation in a pseudo steady state has not been shown. Here, we extend the range of capture down to 14.3 kDa by capturing lysozyme from chicken egg white, along with other small molecular weight proteins.

Dielectrophoresis is carried out on a microfluidic device consisting of an insulating sawtooth-patterned microchannel such that an inhomogeneous electric field is induced in the channel when a DC potential is applied across the device. The gradient of dielectrophoretic forces in our device arises from the varying distances of each successive gate within the device: as the gates become narrower, the ratio of the dielectrophoretic force to the electrophoretic force increases. When the dielectrophoretic force is great enough to counteract all other forces a protein experiences within the channel, immobilization and concentration occurs.

One protein of great interest in the medical field is Aβ (1-40) amyloids. Aggregates of Aβ amyloid and other amyloids have been implicated in numerous human diseases, including Alzheimer's disease.

While it is known that the fully-developed Aβ amyloid fibrils are related to this disease, recent research has suggested the smaller oligomers and protofibrils are more prevalent in disease pathogenesis and are more cytotoxic. The proteins for our research were chosen based on their small molecular weights such that their capture conditions might be comparable to small oligomers of Aβ amyloid proteins. Future work includes studying the behavior of small Aβ amyloid oligomers in our device.

AES-16

Microchannel Isoelectric Focusing (IEF): Elucidating the Separation Performance Impacts of Chemical Mobilization

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Introduced in the 1960's, isoelectric focusing (IEF) remains a powerful electrophoresis technique. In particular, IEF finds a dominant role in modern day analyses of proteins by isoelectric point (pI), making the assay critical for

detection of post-translational modifications and in 2D separations. Capillary IEF enables rapid separations, but pH zones must be mobilized after IEF if a sensitive single-point detector is used (i.e., photomultiplier tube) [1]. Chemical mobilization is the technique of choice, as this mechanism leads to less shear-flow broadening (compared to pressure driven mobilization) and is independent of surface chemistry. **Intriguingly, despite prior chemical mobilization optimization efforts and recent numerical simulations, our understanding of chemical mobilization and its implications on analytical performance remain limited** [2]. Previous studies have tested conditions for mobilization, but are incomplete regarding the quantitative performance assessment and detailed mechanistic descriptions connecting performance with theory [3, 4]. Given this gap in the current literature, in this as of yet not reported study we: **1) detail experimental disagreements with previously proposed theoretical descriptions, 2) highlight areas where there is incomplete theoretical understanding, and 3) provide quantification performance metrics for different mobilization schemes.**

We measure the effect of mobilization solution pH, the differences between ionic and zwitterionic mobilization, and the impact of diffusion as a source of zone broadening. To this end, we define quantitative metrics and utilize microchannel IEF to assess the performance of different typically used mobilization schemes. Leveraging the planar format of glass microfluidic chips that allows easy interfacing with microscopy equipment, we compare the location of the pH gradient using fluorescent pH markers before and during mobilization. Performing our separations in the microfluidic format enables rapid and automated separations, thus enabling multiple conditions to be quickly tested with technical replicates with minimal experimental variation. Additionally, we polymerize cross-linked polyacrylamide gels within the device channels to remove the effects of electroosmotic flow and be able to isolate distortions stemming from mobilization.

From our results, **we quantify performance of the different tested mobilization schemes including the efficiency of mobilization across the entire pH range, the distortion of the focused zones resulting from zone broadening, and changes in pH gradient linearity**. Our results will inform future assay design for IEF and 2D separations as well as help elucidate the physical mechanisms for the varying performance of different mobilization schemes.

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AES-17

Effects of Intravenous Immunoglobulin Treatment on the Alzheimer's CSF Proteome

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Intravenous immunoglobulin (IVIg) therapy has shown promise in the treatment of Alzheimer's disease (AD). In this study, serial cerebrospinal fluid (CSF) samples from a group of subjects with AD undergoing IVIg immunotherapy are analyzed to identify IVIg-related changes. CSF samples from eight subjects were collected before therapy, after treatment, and after a drug washout period. Samples were analyzed by 2-DE and using two

different statistical approaches. The first analysis identified nearly seventy proteins that showed a considerable and consistent change during IVIg treatment. The second analysis identified more than two dozen proteins that changed significantly after six months of treatment, and then the change was either sustained or reversed during the washout period. These results describe an interesting group of CSF proteins that may be associated with the treatment of AD, as well as the potential use of IVIg as an AD immunotherapy.

AES-18

Riboswitch screening enabled by microfluidic electrophoretic mobility shift assays (EMSAs)

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Riboswitches are RNA cis-regulatory genetic control elements in prokaryotic cells. The molecular conformation of riboswitch encodes important information regarding its metabolic function and is closely tied to ligand binding in the cells. Upon binding, riboswitch conformation changes, in turn controlling gene expression at the transcriptional level. In exciting new research directions, riboswitch binding interactions have been targets for antibacterial drug development. A key aspect of screening involves compound library assessment to identify effective binders for riboswitches and subsequent binding affinity characterization.

Electrophoretic mobility shift assays (EMSAs) are the most common approach for interaction screening studies. Generally, the electrophoretic mobility depends on the size and charge of a molecule and EMSAs report analyte mobility shifts as species migrate through the nanoporous sieving medium, in turn enabling probing of molecular binding, reflecting the conformation induced molecular size change. However, conventional slab-gel polyacrylamide electrophoresis (Slab-PAGE) based EMSAs struggle in quantifying conformation based small shifts in mobility due to long durations required to electrophoretically resolve these species. In addition, the standard slab-gel format is not amenable for scale-up, which hinders the development for high-throughput screening. To fill this technical gap, we build on our recently developed multiplexed free-standing polyacrylamide gel (*fs*PAG) microsystem [1] to introduce quantitative, robust and rapid EMSAs for assessment of small riboswitch conformation change, with up to 500-fold increase in throughput.

In this as of yet not reported study, we investigate key factors contributing to separation performance in the new *fs*PAG format and utilize engineering approaches to optimize for a reproducible and quantitative EMSA. In particular, **we detail (1) PAG structural non-uniformity resulting from UV photopatterning and (2) moisture evaporation due to the open gel environment.** We then demonstrate the EMSA on a single-plex *fs*PAG with cyclic-di-GMP riboswitch. EMSA on *fs*PAG format provides substantially higher throughput than slab-PAGE. We observe a 200 fold reduction in required separation time and 30 fold reduction in separation distance. A subsequent EMSA on a 96-plex *fs*PAG provides quantitative measurement of the binding affinity of the riboswitch binding pair. The ability for *fs*PAG EMSAs to offer

tight control of conditions enables statistical testing of observed shifts. The EMSA *fs*PAG device offers a multiplexed format with promise for high-throughput binding screening for a range of applications.

Reference:
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AES-19

Crystallographically Templated Topologies: Toward Novel Packings for Microfluidic Separations

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We describe a novel method to imprint complex surface topographies on biodegradable substrates by exploiting the sensitivity of enzymatic activity to a substrate's degree of cyrstallinity. This process is illustrated in an enzyme/substrate system involving proteinase K and poly(lactic acid) (PLA), where a strong etch rate selectivity to PLA crystallinity is observed. By establishing a laminar flow of the enzyme solution through a template microchannel, morphological features associated with the substrate's crystalline domains can be embedded into the sidewalls. The PLA crystalline morphology is governed by its thermal history (annealing time and temperature, cooling rate) and material properties (molecular weight), enabling the size and density of the imprinted features to be manipulated. We identify conditions under which post-like arrays of tunable size can be produced, making it possible to embed a variety of complex architectures within a micro- or nano-scale fluidic channels in a lithography-free manner. The simplicity and robustness of this approach may offer advantages for producing barrier or packing structures relevant for filtration and chromatography applications. The highly-specific nature of the governing interactions and wide range of enzyme/substrate combinations lays a foundation for broad control over the templated nano-scale morphologies.

AES-20

Microfluidically-Enabled Nanoparticle Monitoring and Characterization

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A variety of characterization tools are available to sensitively analyze collected nanoparticles (e.g., condensation particle counters, differential mobility analyzers, scanning mobility particle sizers (SMPS), ion chromatography, mass spectrometry, and electron microscopy). But these methods often require a dedicated laboratory infrastructure to operate optimally, and are therefore not straightforward to adapt for online use. We have developed a new microfluidic-based approach that overcomes many of these limitations by harnessing the inherently steep chemical gradient established at the interface between co-flowing streams containing a nanoparticle-laden suspension and a fluorescent dye. This sharp mismatch acts to localize adsorptive dye-nanoparticle complexation interactions within a narrow interfacial zone,

instantaneously producing an intense and easily detectable fluorescence signature. These interactions are inherently dominated by phenomena occurring at the nanoparticle surface, introducing the exciting possibility to extract information about particle size and morphology from the fluorescence profile. We demonstrate this capability experimentally using ZnO and TiO_2 nanoparticles, and introduce a reaction model that enables interfacial fluorescence to be predicted so that kinetic parameters associated with the underlying surface complexation can be quantified. We also demonstrate potential for integrated sampling and detection by using a low cutpoint WWC to collect Al_2O_3 nanoparticles aerosolized from test suspensions and dispersed into an environmental chamber. These results indicate that the microfluidic approach is capable of detecting Al_2O_3 nanoparticles in the ultra-fine size range (4 – 160 nm) at environmental concentrations below 200 $\mu g/m^3$, in the vicinity of established toxicity limits. Our approach offers potential for continuous operation that is particularly attractive in manufacturing settings where robust on-line characterization tools are needed.