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Use of Negative Dielectrophoresis for Selective Elution of Protein-bound Particles

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Abstract

In this paper with the aid of negative dielectrophoresis force in conjunction with shear force and at an optimal sodium hydroxide concentration we demonstrated a switch-like functionality to elute specifically-bound beads from the surface. At an optimal flow rate and sodium hydroxide concentration, negative dielectrophoresis turned on results in bead detachment, whereas when negative dielectrophoresis is off, the beads remain attached. This platform offers the potential for performing a bead-based multiplexed assay where in a single channel various regions are immobilized with a different antibody, each targeting a different antigen. To develop the proof of concept and to demonstrate the switch-like functionality in eluting specifically-bound beads from the surface we looked at two different protein interactions. We chose interactions that were in the same order of magnitude in strength as typical antibody-antigen interactions. The first was Protein G-IgG interaction, and the second was the interaction between anti-IgG and IgG.

Introduction

In the past, protein analysis has been performed in a singleplex format¹, analyzing one protein at a time, which can be a slow and time consuming process when one wants to analyze a complex biological system. For applications such as systems biology², biomarker discovery³, drug discovery⁴, high throughput multiplex analysis of proteins simultaneously is necessary. In systems biology, in order to gain a complete understanding of various metabolic pathways, the study of protein-protein interactions⁵, protein-glycan interactions⁶, protein-small molecule interactions⁷, protein-nucleic acid interactions⁸, nucleic acid-nucleic acid⁹, and even protein-cellular interactions¹⁰ is necessary. There are various ways of performing these assays, including mass spectrometry and microarray technology. Each approach has its' own advantages and disadvantages. Mass spectrometry allows for very high throughput analysis without the need for any surface immobilization techniques, yet is expensive and can be time consuming. Array format assays allow for high throughput detection as well, however require surface immobilization, however are less time consuming and less expensive. Microarrays generally require expensive fluorescent scanners. In general, sensitivity of the assay is limited by the dynamic range and background fluorescence of the non-targeted interactions. In both mass spectrometry and microarray technology the cost of the detector is high. Fluorescent microarrays require an excitation source and a detector which scans the entire surface of the array and detects the optical signal point by point.

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Microfluidics technology offers the advantage of greater sensitivity and reduction in sample volume and the amount of reagents used¹¹ which is of great importance in protein-protein interaction studies, where the cost and time required for protein purification is high.

Here we aim to capitalize on the advantages offered by microfluidics and microarray technology. This would allow for the minimal use of reagents and high throughput analysis at the same time. Additionally, one way to reduce the cost of the detector is to perform the detection at a single point, rather than requiring scanning across the whole surface of the array.

Here we envision performing a bead-based multiplexed assay for analyzing protein-protein interactions where in a single channel an array of proteins to be studied is patterned. Similar to a protein array, the idea is to study the interaction of that array of proteins against a single protein (Figure 1). For multiplexed analysis, we need to selectively elute the specifically-bound beads from each individual region one at a time for further downstream quantification and analysis. With this format, the detection of the beads will occur downstream, simply requiring a single detector for the whole system, without the need for scanning the whole surface. The challenge lies in finding a method suitable for selectively eluting beads from a desired element of the array with minimal disturbance to beads attached to other elements of the array. Effectively this requires a smart surface.

The ability to manipulate the flow of fluids and bioparticles in an integrated micro total analysis system (microTAS) continues to be a challenging problem. In order to develop a true high throughput microfluidic bioanalysis platform, an important requirement is the ability to independently control the movement of fluids and various other bioparticles in an addressable manner, similar to the ability to control the movement of current in an integrated electronic circuit. To date, several well established methods have been developed, each with its' own advantages and disadvantages, including on-chip pneumatic valves¹², various electrokinetic methods¹³, and also magnetic manipulation^{14, 15}. Each of the above listed methods may be appropriate for a set of applications, while being inappropriate for others. On chip pneumatic microfluidic valves for example are very versatile and rapid and have proven quite successful, however the disadvantage is that the requirement for a large number of tubes for controlling the flow required can become a plumber's nightmare as the number of control channels becomes large. Thus, these types of valves may not be suitable for clinical applications requiring low cost handheld devices.

For our multiplexed bead-based assay, we propose the use of negative dielectrophoresis (nDEP) in conjunction with shear force, which can provide the switch-like behavior necessary to achieve this goal. At an optimal flow rate, nDEP turned on results in bead detachment, whereas when nDEP is off, the beads remain attached. Once the beads have been eluted, they can be quantified and analyzed. At this point, the viability of the proteins on the bead is no longer important, since the goal is to quantify the beads.

Over last few years the dielectrophoresis (DEP) has found use in cell capture, microfluidic pre-concentration, cell trapping, and biomolecular analysis. Computer-aided design tools are now utilized to model the electric field gradients generated by various electrode designs. Moreover, the advances in fabrication technology resulted in development of sophisticated electrode designs and microfluidic devices that are amenable to biomedical applications. Fabrication of microfluidic devices in PDMS (polydimethylsiloxane) by soft lithography offered a faster and less expensive method to create microfluidic devices¹⁶. Recently, DEP devices that can be deployed for manipulation and separation of biological particles were fabricated using rapid and low cost microfabrication technologies¹⁷.

Several groups have developed and characterized DEP-based platforms geared towards characterization and manipulation of biological particles and interactions. The holding force on a single-particle in dielectrophoretic trap was determined¹⁸. Besides, the holding forces for DEP traps on an array of interdigitated electrodes were characterized and modeled¹⁹, and the relationship of the geometry of the design and the magnitude of the DEP force was analyzed²⁰.

Previously, dielectrophoretic crossover measurements and analysis were used to characterize the dielectric properties of antibody-coated submicrometer Latex spheres²¹. Also, the use of DEP in an integrated dielectrophoretic chip was reported for continuous filtering, focusing, sorting, trapping, and detection of the bioparticles²². In addition, based on nDEP, generated by an array of interdigitated electrodes, a rapid and separation-free assay was performed²³. Similarly, using nDEP, in an interdigitated microarray electrode, different cell types were patterned, without any special pretreatment of a culture slide²⁴. Baek et al.²⁵ developed an array of interdigitated electrodes to quantify chemical and biological interactions. Gadish et al.²⁶ used a combination of interdigitated electrodes and a chaotic mixer to achieve high throughput particle concentration and capture system. Such efforts on interdigitated electrodes illustrate the convenience of utilizing this form of geometry for a DEP system to manipulate and characterize biological particles. Thus, we chose this geometry to illustrate the microfluidic switch-like functionality of our DEP-based platform for the purpose of this work.

Theoretical Background

Particles that are bound to the surface experience three dominating forces, the gravitational force, the hydrodynamic drag force, and the dielectrophoretic force. The sedimentation force that acts on the particle is governed by the following equation:

$$F_{sed} = \frac{4}{3}\pi r^3 (\rho_b - \rho_m) g \quad (1)$$

where g is the gravitation constant, ρ_b is the bead density, ρ_m is the medium density, and r is the particle radius. For an 8 μm diameter polystyrene bead, this comes to approximately 100 fN, which is at least an order of magnitude smaller than the other forces in our system.

Dielectrophoresis Force

Dielectrophoresis is the motion of dielectric particles as a result of polarization force produced by non-uniform electric fields. This net force is due to the difference in the magnitude of the force experienced by the charges at each end of the induced dipole when a spatially non-uniform electric field is applied. The first order time-average DEP force acting on a dielectric sphere is given by:

$$F_{DEP} = 2\pi\epsilon_0\epsilon_m r^3 \text{Re}\{f_{CM}\} \nabla |E_{RMS}|^2 \quad (2)$$

where ϵ_m is the relative permittivity of the surrounding medium, r is the particle radius, and E_{RMS} is the root mean square value of the electric field. f_{CM} in the above equation is the Clausius-Massotti factor which is related to the effective polarizability of the particle with respect to that of the medium, and is of the form

$$f_{CM} = \frac{s_p^+ - s_m^+}{s_p^+ + 2s_m^+} \quad (3)$$

where ε_p^* and ε_m^* are the relative complex permittivities of the particle and the medium respectively.

According to equations (2) and (3) depending on whether the real part of the Clausius-Massotti factor is positive or negative, the particle is attracted to (positive DEP, pDEP) or repelled from (negative DEP, nDEP) a region of high electric field strength. This in turn depends on the frequency of excitation and the particle's permittivity and conductivity values with respect to that of the medium. For the purpose of this work we needed to elute polystyrene beads from the surface. Hence, we had to choose a design space that would provide us with negative value for the real part of the Clausius-Massotti factor, corresponding to nDEP. Figure 2 illustrates the real part of the Clausius-Massotti factor vs. frequency for a range of medium conductivity. Therefore, in order to achieve nDEP independent of the conductivity of the medium we chose to operate at the frequency of 10 MHz. Based on our COMSOL (COMSOL, Stockholm, Sweden) simulations, and previous work in the literature using micron sized interdigitated electrodes, it is possible to provide DEP forces ranging from 10–100pN easily¹⁹.

Hydrodynamic Drag Force

The hydrodynamic drag force of a bead attached to the surface of the channel can be approximated with the following equation¹⁹:

$$F_{\text{Drag}} = 6\pi r k \eta v_f \quad (4)$$

where r is the bead radius, k is a factor accounting for wall effects ($k \approx 1.7$) which has no dimension, η is the dynamic medium viscosity, and v_f is the fluid velocity.

Previously²⁷, we showed that using shear force spectroscopy we are able to quantify the binding forces of various biomolecular interactions including DNA-DNA interactions, antigen-antibody interactions, and protein-protein interactions. According to this study, we showed that flow rates ranging from 10 nl/min to 10 μ l/min, provides drag forces between 0.5 pN to 500 pN respectively. Based on our experimentation, a typical antigen-antibody interaction is roughly on the order of 400 pN, therefore the total force that must be exerted to detach specifically bound beads from the surface should be greater than this value.

Experimental Section

Fabrication

Standard evaporation and lift-off processing techniques were used to fabricate the microfluidic biochip. The device consisted of an array of interdigitated Au/Cr electrodes on a glass substrate. Two designs of interdigitated electrodes were fabricated for this work. In the first design, each metal line in the electrodes was 14 μ m wide and 10 μ m apart, and in the second design both the width and the spacing of electrodes were 7 μ m. The microchannel with 200 μ m width, 50 μ m height, and 1 cm length was fabricated in PDMS (polydimethylsiloxane). The master mold for the microchannel was patterned onto a silicon substrate using SU-8 photoresist. PDMS (10:1 prepolymer:curing agent) was poured onto the master mold and allowed to cure at 75° overnight. Once the PDMS channel was formed, it was removed from the mold. Then, two holes of diameter 3 mm were punched, one at each end, to serve as the channel's inlet and outlet ports. The glass chip and the PDMS microchannel were aligned and then bonded together after oxygen plasma treatment¹⁶.

Sample Preparation and Surface Chemistry

To develop the proof of concept and to demonstrate the switch-like functionality in eluting specifically-bound beads from the surface we looked at two different protein-protein interactions. We chose interactions that were in the same order of magnitude in strength as typical antibody-antigen interactions. The first was Protein G-IgG interaction, and the second was the interaction between anti-IgG and IgG.

To demonstrate the Protein G-IgG elution from the surface, we used 6.7 μm -diameter Protein G covered polystyrene beads initially in 0.5% w/v suspension. 250 μL of this solution was washed with PBS (Phosphate Buffered Saline with 110 mM NaCl concentration) and was resuspended in 40 μL of PBS to achieve the desired concentration. Then, 4 μL of 1.3 mg/mL Biotinylated rabbit IgG antibody was added to the sample. Next, the solution was placed in the rotator for 45 minutes to rotate at 36 rotations/min (at room temperature). The beads were then extensively washed with PBS and 0.01% Tween (2000:1). At this point, 5 μL Streptavidin was added to the solution, and the sample was once again placed in the rotator for 45 minutes to rotate at 36 rotations/min. Finally, the beads were washed again with PBS and Tween and were resuspended in 40 μL of PBS and Tween. The washing steps explained above were done by centrifuging the sample three times at 14000 rpm. For the control experiment to test for the specificity of bindings, a separate sample was prepared following the above steps, except the addition of Streptavidin, to eliminate the link between the Biotin on the sample beads and the Biotin on the surface. This effectively eliminated the possibility of attachment of beads to the surface of through specific-bindings.

To prepare the channel surface for Protein G-IgG interaction, the device with electrode width of 14 μm and spacing 10 μm was used. Biotinylated BSA (BBSA) was physically adsorbed on the channel surface, by first injecting PBS/Tween/BBSA (9:0.0045:1) in the channel and allowing for the solution to incubate for 20 minutes. This was followed by introducing 3% non-fat milk with Tween in the channel to cover the regions of the surface that were not covered by BBSA, and hence to eliminate non-specific binding (attachment of beads to the gold surface). The channel was then flushed and filled with PBS and Tween (2000:1) as the channel buffer.

To demonstrate the interaction between anti-IgG and IgG, 7.4 μm -diameter goat anti-mouse IgG covered polystyrene beads (initially in 0.5% w/v suspension) were used. 250 μL of this solution was washed with PBS and Tween similar to the previous preparation and were resuspended in 80 μL of PBS and Tween. Initially, we resuspended the final sample in 40 μL , but we had to dilute it $\times 2$ further to achieve a more uniform and spread out distribution of specifically-bound beads, which in turn helped with the quantification process.

To prepare the channel surface for this interaction, the device with electrode width and spacing of 7 μm was used. Mouse IgG (originally 2 mg/mL, diluted by $\times 100$) was pipetted in and physically adsorbed on the channel surface, by allowing for the solution to incubate for 20 minutes. This was followed by introducing BSA in the channel to eliminate non-specific bindings. The channel was then flushed and filled with PBS and Tween similar to the previous preparation. For the control experiment to test for the specificity of bindings, on a separate chip the above steps were performed to prepare the surface, except the addition of IgG, which effectively eliminated the possibility of attachment of beads to the surface of through specific-bindings.

Experiment Setup

We used a function generator (Agilent 33220A) to provide electrical excitation to the device. Electrodes were excited with sine wave at 10 MHz. A syringe pump (Harvard Apparatus) was used to control the flow rate through the device.

Experimental Procedure

We first flushed the channel with PBS and Tween to eliminate the air bubbles. Then, the prepared sample beads were injected into the channel by directly pipetting them into the inlet well. The syringe was attached to tygon tubing which was connected to the outlet well. Negative pressure was applied to the syringe to pull the solution. With the aid of a syringe and the syringe pump the flow was controlled. The beads were allowed to settle for 15 minutes so that they had sufficient time to bind to the surface.

For each experiment, flow rate and hence drag force was increased gradually until all beads detached for an applied voltage level. Applying voltage to the electrodes resulted in establishing a non-uniform electric field necessary to produce nDEP force. The upward nDEP force caused the specifically-bound beads to be pushed away from the surface, and eventually at the proper flow rate to be detached fully. At each flow rate, we quantified the percentage of detached beads relative to total number of initially captured beads (at rest).

Additionally, for the anti-IgG and IgG interaction we experimented with the NaOH concentration of the buffer as one of the contributing factors in determining the strength of the binding. This was done by varying the concentration of Sodium hydroxide (NaOH) in the washing step. Here, the binding of the anti-mouse IgG beads to the mouse IgG coated surface was done in PBS. After sufficient binding occurred we injected varying concentrations of NaOH at a controlled flow rate. Our goal was to establish an optimal NaOH concentration for the buffer that would facilitate the desired switch-like functionality. Ideally, NaOH concentration had to be sufficiently high in order to make the binding weak enough to enable the elution of the bound beads from the surface when applying a force that is in the same order of magnitude as nDEP. On the other hand, NaOH concentration should not be too high, as it would make the bindings too weak to allow for the beads to stay attached to the surface in absence of the flow (drag force) or nDEP, preventing us from proceeding with the experiment.

Results and Discussions

Representative results for the Protein G-IgG interaction are shown (Figure 3) for three cases of 0 V (nDEP-off), 10 V_{pp} and 20 V_{pp} (10 MHz). For the case of 20 V_{pp} all beads detached as flow rate reached 0.35 μLmin^{-1} , whereas when DEP was off more than 90% still remained attached. With nDEP off, the flow rate had to be increased to 0.95 μLmin^{-1} in order to remove the majority (70%) of the beads. Therefore, for this interaction, the flow rate range within which switch-like behavior necessary for detachment of specifically-bound beads can be exhibited is between 0.4 to 0.6 μLmin^{-1} . The illustrated data represents the overall outcome of our experiments, totaling to 1900 beads that were carried out for the cases of nDEP off, 10 V_{pp} and 20 V_{pp} independently. For each case the total number of beads (detached and remained at each flow rate) from all the corresponding experiments were quantified and normalized. The corresponding control experiment for this interaction showed no attachment of the sample beads to the surface in absence of Streptavidin, indicating no occurrence of non-specific binding in our assay.

One thing to note in this set of experiments (the protein G – IgG interaction) was that the beads tended to form aggregates, which was likely due to cross linking of the Streptavidin and biotinylated antibodies on the different beads. This cross linking of the beads helped the

DEP force in dislodging the beads off of the surface. This is due to the fact that the aggregate of beads effectively acts as one larger particle, which results in experiencing higher nDEP force. However, for the purpose of performing multiplexed assay we had to revise our chemistry to achieve the interaction that would allow us to test the effect of DEP on a distribution of beads that are individually spread out. As a result, we switched to a new chemistry, i.e. testing the binding between anti-mouse IgG and mouse IgG instead, which indeed provided us with the desired distribution. Henceforth, we focused the experiments and the corresponding data analysis on the new distribution that is a better representative of and more relevant to our overall system.

As mentioned above, for the new anti-IgG and IgG interaction the beads were attached to the surface individually rather than as aggregates and clumps. This in turn reduced the effect of the DEP force in dislodging the beads when we performed the elution with PBS alone. This required the use of a stronger elution buffer during our wash step to weaken the binding sufficiently such that DEP forces would help in pushing off the beads, yet weak enough such that beads would remain firmly bound while DEP was off.

Thus, we examined the effect of using varying concentrations of NaOH on the detachment of beads from the surface both with and without nDEP. Figure 4 illustrates the flow rate up to which 50% of the originally attached beads got eluted (the switching point) for the range of different NaOH concentrations and for the cases of nDEP on and off. The top and bottom error bars for each vertical bar indicate the flow rate up to which 80% and 20% of the attached beads got eluted respectively. An optimal NaOH concentration is the one that would provide a wide gap between the 50% detachment flow rates for the cases of nDEP on and off; a gap wide enough to eliminate the overlap between the 20%–80% ranges between the cases of nDEP on and off to minimize the switching error.

Based on Figure 4 the desired NaOH concentration was 0.2 M. At this concentration 80% of the beads got detached at zero flow rate for nDEP on while it took at least the flow rate of $0.5 \mu\text{Lmin}^{-1}$ to detach 20% of the beads for nDEP off. NaOH concentration of 0.1 M provided a fairly good switching behavior as there was $1.4 \mu\text{Lmin}^{-1}$ gap between the 50% detachment flow rates for the cases of nDEP on ($2.2 \mu\text{Lmin}^{-1}$) and off ($3.4 \mu\text{Lmin}^{-1}$). However, at this concentration, there was an overlap between the ranges specified by the error bars for nDEP on and off, indicating that at least 20% of beads with nDEP off got eluted before reaching the flow rate necessary to detach 80% of the beads with nDEP on. As can also be seen from Figure 4, increasing NaOH concentration beyond 0.2 M is not desirable. For the NaOH concentrations of 0.25 M and 1 M 100% of the beads got detached at zero flow rate independent of having nDEP on or off, indicating that the high NaOH concentration made the bindings too weak to allow for the beads to stay attached to the surface. The results from each experiment were verified through repeating the process for each case independently (totaling to 5200 beads), and finally a quantification procedure similar to that of the first interaction was followed.

The switching behavior of our system for the interaction between anti-IgG and IgG can be better visualized in Figure 5(a) (derived from the same data summarized in Figure 4), which shows in more details the process of bead detachment (depicted as percentage distribution) at a given flow rate and at the optimal NaOH concentration of 0.2 M. The corresponding control experiment showed some non-specific binding of the beads to the surface in the absence of the IgG. Those non-specifically bound beads got eluted at flow rates higher than $3 \mu\text{Lmin}^{-1}$ for NaOH concentration of 0.2 M. We believe that the small bumps that can be observed in the detachment distributions beyond $3 \mu\text{Lmin}^{-1}$ (Figure 5(a)) are in fact as a result of elution of such non-specifically bound beads. This in turn shows another advantage of our method which allows for distinction of non-specifically bound beads and only

detaches the beads that were specifically bound to the surface as a result of the interaction between anti-IgG and IgG; the major detachment peaks for both DEP on and off occurred at flow rates less than $3 \mu\text{Lmin}^{-1}$.

To further demonstrate our device's functionality as a switch (ideally zero detachment for the period of time that the switch is off and 100% detachment upon turning the switch on), at the NaOH concentration of 0.2 M, the flow rate was set to $0.15 \mu\text{Lmin}^{-1}$ and allowed to run for a total of 10 minutes, then the nDEP was turned on ($20 V_{pp}$) at the end of the 5th minute. Figure 5(b) presents the bead detachment time profile for this process. As flow is applied, beads slowly get detached and then the unbinding finally levels off after 4 minutes. At the end of the 5th minute, once the nDEP is turned on, the detachment of beads reinitiates. The corresponding captured snapshots from the video recorded experiment are shown by Figure 5(c–e). As can be seen from the results, the rate of the bead detachment increased by a factor of 3.6 upon turning nDEP on, resulting in the total elution of 90% of the originally attached beads at the end of the 10th minute.

Together our results demonstrate the successful switching operation of our microfluidic device in eluting the beads that were originally attached (through protein-protein interactions) to the surface. Our future work on improving the performance of the device will be focused on reducing the switching time that is required to fully detach the specifically-bound beads, and also reducing the number of beads which become detached while DEP is still off. This will involve attacking the problem from two separate angles. The first is to increase the strength of the DEP force by optimizing the electrode design, and also applying higher voltages. The second approach involves experimenting with different elution conditions such as salt concentration, chelating agents, and chaotropic salts.

Conclusions

We have designed, fabricated, and experimentally tested a novel microfluidic platform that demonstrates the ability of nDEP to elute specifically-bound beads with a switch-like behavior. We used nDEP in conjunction with shear force, at an optimal NaOH concentration (which sets the pH level of the buffer) to illustrate this behavior in a singleplex assay. At an optimal flow rate and NaOH concentration, turning nDEP on results in bead detachment, whereas when nDEP is off, the beads remain mostly attached. This platform offers the potential for performing a bead-based multiplexed assay where in a single channel various regions are immobilized with a different antibody, each targeting a different antigen. Because of the advances made in VLSI in routing wires and electrodes on a silicon chip, the use of electrical actuation offers great potential in realizing a true high throughput microfluidic device without requiring large number of tubes and external valves for controlling the channels.

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References

1. W. Neal B. "Western Blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Analytical Biochemistry*. 1981; 112(2):195–203. [PubMed: 6266278]
2. Kitano H. Systems Biology: A Brief Overview. *Science*. 2002; 295(5560):1662–1664. [PubMed: 11872829]

3. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotech.* 2006; 24(8):971–983.
4. Sevecka M, MacBeath G. State-based discovery: a multidimensional screen for small-molecule modulators of EGF signaling. *Nat Meth.* 2006; 3(10):825–831.
5. Zhu H, Snyder M. Protein arrays and microarrays. *Current Opinion in Chemical Biology.* 2001; 5(1):40–45. [PubMed: 11166646]
6. Song X, Lasanajak Y, Xia B, Heimbürg-Molinaro J, Rhea JM, Ju H, Zhao C, Molinaro RJ, Cummings RD, Smith DF. Shotgun glycomics: a microarray strategy for functional glycomics. *Nat Meth.* 2011; 8(1):85–90.
7. MacBeath G, Koehler AN, Schreiber SL. Printing Small Molecules as Microarrays and Detecting Protein–Ligand Interactions en Masse. *Journal of the American Chemical Society.* 1999; 121(34): 7967–7968.
8. Horak, CE.; Snyder, M. ChIP-chip: A genomic approach for identifying transcription factor binding sites. In: Christine, G.; Gerald, RF., editors. *Methods in Enzymology.* Vol. Volume 350. Academic Press; 2002. p. 469-483.
9. Schena M, Shalon D, Davis RW, Brown PO. Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray. *Science.* 1995; 270(5235):467–470. [PubMed: 7569999]
10. Beske OE, Goldbard S. High-throughput cell analysis using multiplexed array technologies. *Drug Discovery Today.* 2002; 7(18):S131–S135. [PubMed: 12546879]
11. Whitesides GM. The origins and the future of microfluidics. *Nature.* 2006; 442(7101):368–373. [PubMed: 16871203]
12. Unger MA, Chou H-P, Thorsen T, Scherer A, Quake SR. Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography. *Science.* 2000; 288(5463):113–116. [PubMed: 10753110]
13. Chuan-Hua C, Santiago JG. A planar electroosmotic micropump. *Microelectromechanical Systems, Journal of* 2002. 11(6):672–683.
14. Kose, AR.; Fischer, B.; Mao, L.; Koser, H. Label-free cellular manipulation and sorting via biocompatible ferrofluids; *Proceedings of the National Academy of Sciences*; 2009.
15. Tsai, KL.; Pickard, D.; Kao, J.; Yin, X.; Leen, B.; Knutson, K.; Kant, R.; Howe, RT. Magnetic nanoparticle-driven pumping in microchannels. *Solid-State Sensors, Actuators and Microsystems Conference, 2009. TRANSDUCERS 2009. International*; June 21–25. 2009 p. 2274-2277. 2009
16. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu H, Schueller OJA, Whitesides GM. Fabrication of microfluidic systems in poly(dimethylsiloxane). *ELECTROPHORESIS.* 2000; 21(1):27–40. [PubMed: 10634468]
17. Rajaraman S, Noh H.-s. Hesketh PJ, Gottfried DS. Rapid, low cost microfabrication technologies toward realization of devices for dielectrophoretic manipulation of particles and nanowires. *Sensors and Actuators B: Chemical.* 2006; 114(1):392–401.
18. Voldman J, Braff RA, Toner M, Gray ML, Schmidt MA. Holding Forces of Single-Particle Dielectrophoretic Traps. *Biophysical Journal.* 2001; 80(1):531–542. [PubMed: 11159423]
19. Li H, Yanan Z, Akin D, Bashir R. Characterization and modeling of a microfluidic dielectrophoresis filter for biological species. *Microelectromechanical Systems, Journal of* 2005. 14(1):103–112.
20. Crews N, Darabi J, Voglewede P, Guo F, Bayoumi A. An analysis of interdigitated electrode geometry for dielectrophoretic particle transport in micro-fluidics. *Sensors and Actuators B: Chemical.* 2007; 125(2):672–679.
21. Hughes MP, Morgan H. Dielectrophoretic Characterization and Separation of Antibody-Coated Submicrometer Latex Spheres. *Analytical Chemistry.* 1999; 71(16):3441–3445.
22. Cheng I. An integrated dielectrophoretic chip for continuous bioparticle filtering, focusing, sorting, trapping, and detecting. *Biomicrofluidics.* 2007; 1(2):021503.
23. Lee HJ, Yasukawa T, Shiku H, Matsue T. Rapid and separation-free sandwich immunosensing based on accumulation of microbeads by negative-dielectrophoresis. *Biosensors and Bioelectronics.* 2008; 24(4):1000–1005. [PubMed: 18815025]
24. Suzuki M, Yasukawa T, Shiku H, Matsue T. Negative dielectrophoretic patterning with different cell types. *Biosensors and Bioelectronics.* 2008; 24(4):1043–1047.

25. Baek SH, Chang W-J, Baek J-Y, Yoon DS, Bashir R, Lee SW. Dielectrophoretic Technique for Measurement of Chemical and Biological Interactions. *Analytical Chemistry*. 2009; 81(18):7737–7742. [PubMed: 19663393]
26. Gadish N, Voldman J. High-Throughput Positive-Dielectrophoretic Bioparticle Microconcentrator. *Analytical Chemistry*. 2006; 78(22):7870–7876. [PubMed: 17105182]
27. Javanmard, M.; Babrzadeh, F.; Davis, RW. Microfluidic force spectroscopy for characterization of biomolecular interactions with piconewton resolution. Vol. 97. AIP; 2010. p. 173704

Figure 1. Bead-based multiplexed assay. Applying voltage V_2 turns nDEP on, resulting in elution of beads from the surface of the second set of interdigitated electrodes.

Figure 2.

Real part of the Clausius-Massotti factor vs. frequency for a range of medium conductivity (particle dielectric constant: 2.5 and particle electrical conductivity: 0.2 S/m). To detach the specifically-bound beads from the surface, we need to operate in the design space where the real part of the Clausius-Massotti factor is negative (nDEP).

Figure 3. Percentage of beads remaining on the surface (for Protein G-IgG interaction). Buffer for washing beads was Phosphate Buffer Saline with the salt concentration of 110 mM NaCl.

Figure 4.

Flow rate up to which 50% (switching point) of the originally attached beads (through anti-IgG and IgG interaction) got eluted for the range of different NaOH concentrations and for the cases of nDEP on (20 V_{pp}) and off. The top and bottom error bars for each vertical bar indicate the flow rate up to which 80% and 20% of the attached beads got eluted respectively. The desired NaOH concentration is 0.2 M. At this concentration 80% of the beads got detached at zero flow rate for nDEP on while it took at least the flow rate of 0.5 μLmin^{-1} to detach 20% of the beads for nDEP off.

Figure 5.

(a) Percentage distribution of detached beads at NaOH concentration of 0.2 M. Upon turning nDEP on about 80% of beads got detached. Detachment of beads beyond 3 μLmin^{-1} is due to elution of non-specifically bound beads (verified by control experiment). (b) Bead detachment time profile at flow rate of 0.15 μLmin^{-1} and NaOH concentration of 0.2 M. The corresponding video snapshots of the beads distribution (c) Initially (d) Immediately before turning nDEP on (5th minute) (e) After 10 minutes (i.e. nDEP was on for 5 minutes). The beads that are circled (red) are the ones that were stationary and bounded to the surface.