Cellular BioMEMS

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Laboratory for Cellular BioMEMS – Research Themes

Our group performs research on BioMEMS, applying microfabrication technology to illuminate biological systems, especially at the cellular level. Specifically, we develop technologies that enhance or enable the acquisition of information from cells. Our research builds upon various disciplines: electrical engineering, microfabrication, bioengineering, surface science, fluid mechanics, mass transport, etc. We take a quantitative approach to designing our technology, using both analytical and numerical modeling to gain fundamental understanding of the technologies that we create. We then take our designs through microfabrication to packaging and testing and to biological assay.

1. A screening cytometer

Sponsors
NIH NCRR
NSF Graduate Research Fellowship

Project Staff
Salil Desai, Nick Mittal, Brian Taff

Overview

The overall goal of this research is the development of a microfabricated sorting cytometer that enables genetic screening for complex phenotypes—intracellular and/or dynamic behavior—in biological cells (Figure 1). We are addressing the challenges of creating a useable device capable of handling and sorting a sufficient number of cells for practical screens. Specifically, we are undertaking the significant evolution necessary to bring our existing technology to the functional level necessary for dissemination to the biological community. With this cytometer, biologists will be able to

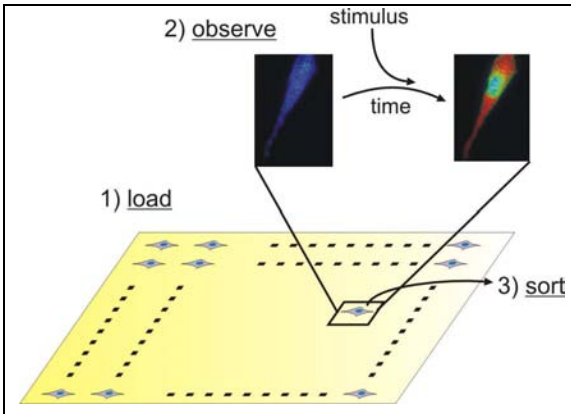

Figure 1: A sorting cytometer for screening complex phenotypes. The cytometer consists of a two-dimensional array of traps, each of which holds a single cell. After loading the traps (1), the array is optically interrogated (2), and cells with phenotypes of interest are sorted (3). In this case, the putative screen is for cells exhibiting altered kinase activation, resulting in changes in fluorescence over time and space.

isolate cells based upon dynamic and/or intracellular responses of fluorescent probes, enabling a new generation of genetic screens.

Our technology addresses two steps in any cell-based genetic screen: the observation of cells and the isolation of those cells exhibiting the desired phenotype. These two steps are inextricably linked, and the functionality available between them directly affects the types of screens that one can perform. For example, many screens involve fluorescent reporter proteins, and thus the search for desired phenotypes involves optical techniques. The premiere optical observation technique—microscopy—is, however, severely limited in its ability to isolate positive-responding cells. The premiere isolation technique—flow-assisted cell sorting (FACS)—is severely limited in its ability to observe cells. A technological gap thus exists between observation and isolation.

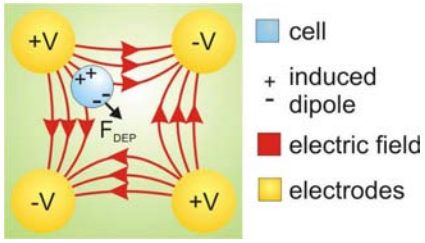

Figure 2: Dielectrophoresis. Four electrodes create an electric field that induces a dipole in a biological cell. The orientation of the induced dipole causes the cell to experience a negative DEP force (FDEP) towards the center, trapping the cell. A p-DEP trap, meanwhile, would draw the particle toward the electrodes.

On some level, this gap results from a lack of techniques to physically manipulate cells; microscopy and flow cytometry handle cells differently, leading to their different functionalities. We are thus developing a sorting cytometer that can hold cells in place and then release selected ones, combining the functionalities of microscopy and flow cytometry. We are realizing this by developing a massively parallel array of switchable traps that we will create using the electrical phenomena of dielectrophoresis.

Technology Background

DEP refers to the force on a cell in a non-uniform electric field (Figure 2). Depending on the properties of cell, media, and applied electric field, DEP forces can propel cells toward field maxima (positive DEP or p-DEP) or minima (negative DEP or n-DEP), creating traps with either configuration. *DEP-based particle traps have several advantages* for manipulating micron-sized particles. First, because they are amenable to microfabrication they have the potential to be *arrayed and thus scale well*. Second, since they are active traps, *they can be turned off*, releasing particles and effecting sorting. Third, *they can be individually addressed* because they are electrical traps. Fourth, when designed and operated correctly, *they will trap all types of cells*. Finally, *they can trap cells of all relevant sizes*—sub-micron to tens of microns in diameter—depending on the trap geometry.

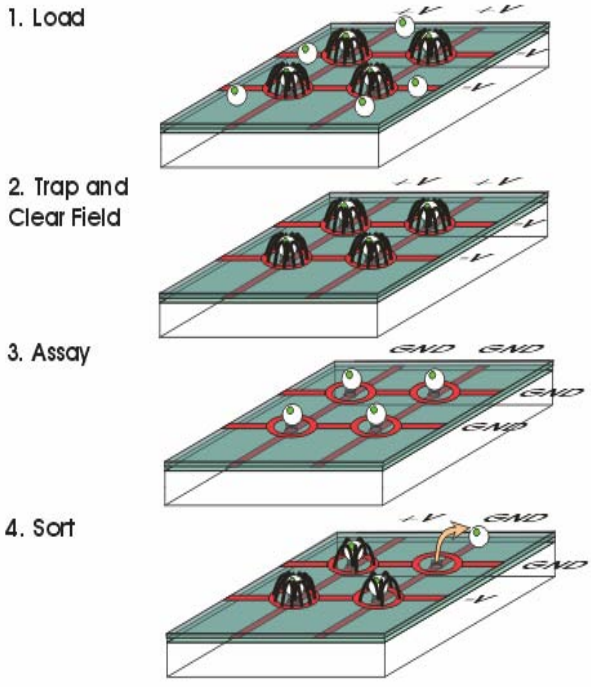

Figure 3: A parallel p-DEP-based trapping approach.

Most DEP-based traps use n-DEP because it positions cells at the lowest electric field and there are no concerns about cells sticking to electrodes (as can occur for p-DEP traps). Many electrode arrangements, operated in a suitable fashion, will make a rudimentary particle trap. *No scalable DEP-based trap exists that can robustly trap single cells and is amenable to high-throughput microscopy. Such a trap requires performance characteristics that can only be met through quantitative modeling. We are undertaking the design of just such a trap.*

Current Research

We have recently developed and functionally validated a p-DEP trap geometry oriented as a

"bull's eye" (see Figure 3) for future integration in larger (e.g., 10,000 site) screening arrays. This trap is fabricated using a two-level metal process on a silicon substrate. The planar design easily maps into a series of row and column linked control lines that enable expansion to larger array sizes. This row/column format enables electrical control over the trapping behaviors of all sites in the array and presents demands for chip-to-world electrical connections that scale with the square root of the number of traps in the array. Our strategy for addressing individual sites in the array is outlined in Figure 4. Using a simple 4x4 trapping grid we have empirically shown holding characteristics for individual beads and HL60 mammalian cells that agree well with predicted simulation-based device behaviors. Additionally, using beads, we have demonstrated the row/column addressing operations necessary for releasing trapped particles from specific sites within an overarching grid (see Figure 5).

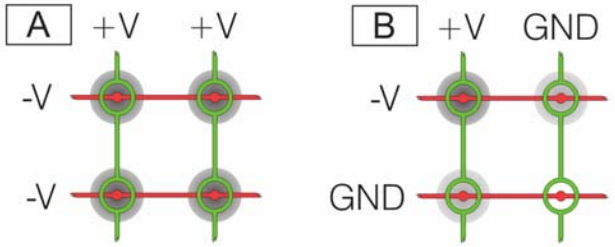

Figure 4: Here we demonstrate the row/column addressing scheme. **(A)** shows a 2x2 grid where all sites are in the "on" state. **(B)** shows the same array with the lower right trap set to the "off" state. This condition is realized by grounding the associated row and column electrodes. Traps on the same row and column are "on" but activated with half the strength of other "on" state traps.

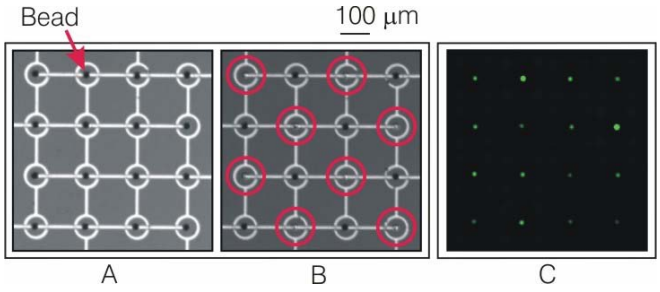

Figure 5: (A) shows a 4x4 p-DEP array loaded with metal-coated beads. **(B)** displays a selection pattern enabled by sequential row/column addressing routines. Circles depict release sites. **(C)** shows trapped HL60 cells.

Currently, we are working to enhance the reliability of this p-DEP trap design by examining different device surface coatings and assessing their impact on cell/substrate adhesion characteristics. Ideally, our cytometer chip surfaces will enable cell attachment such that normal cellular process can take place, yet the attachment will not be so severe as to overwhelm the potential for extracting cells requiring sorting.

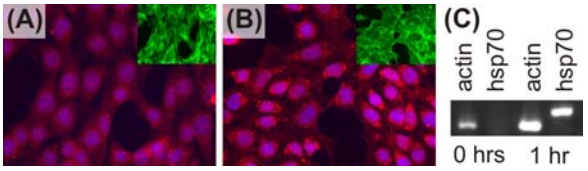

Figure 6: Heat-shock analysis. (A-B) Immunofluorescence of hsp70 (red) before and after heat shock, showing nuclear translocation. Also shown are DNA stain (blue) and an inset actin stain to outline the cells. **(C)** RT-PCR of hsp70 mRNA, showing upregulation after heat shock, along with actin control.

To perform a successful genetic screen we must understand the effects of DEP trapping on cell health. All cellular manipulations alter the phenotype of the cells under study, from trypsinization to trituration to conventional flow cytometry to optical tweezers. We wish to investigate, at a molecular level, the interactions between our system and cells. Our initial investigations have been into the heat-shock response, induced either because of elevated temperatures or direct electric-field interactions

We have developed assays to examine the effects of elevated temperatures on cells, specifically assaying activation of hsp70 via RT-PCR and immunofluorescence (IF). We have initially focused on conventional heat shock to validate our assays. We have been able to observe both nuclear translocation and upregulation of hsp70 mRNA upon exposing NIH 3T3 cells to 43 ºC heat shock for 0-180 min (in 30 min intervals) as detailed in Figure 6 These assays can now be used as positive controls for trapping experiments performed at the micro scale.

2. Bio-process device for *c*ell culture and *in situ* assay

Sponsors
NIH NCRR
NSF Graduate Research Fellowship

Graduate Students
Lily Kim, Adam Rosenthal, Maia Mahoney, Katya Puchala

Overview

Living cells in culture are powerful biological models for many different processes, ranging from fundamental issues in biocomplexity to developmental or disease processes. For these reasons, intense effort is being focused on characterizing and manipulating cells in culture, identifying culture conditions that can propagate new cell types, and controlling their phenotype in culture. However, despite the impressive progress made on these fronts, the techniques and technology used are predominantly based in traditional culture techniques that are labor-intensive and qualitative. *Our long-term objective is to streamline the ability to manipulate cells in vitro while automating the extraction of quantitative in situ phenotypic data.*

Our approach is to develop a *microfabricated culture system* that can precisely, accurately, and automatically deliver nanoliter to microliter volumes of reagents to a multiplexed array of millimeter-sized perfusion chambers containing cells, and couple this to *automated light microscopy to extract images* of cells in the chambers (Figure 7). We will keep the system sufficiently low cost to be accessible to a single-investigator laboratory and disseminate its plans and protocols in an open-source format to ensure wide distribution. *Our initial goals are to develop the technology base for such a system.*

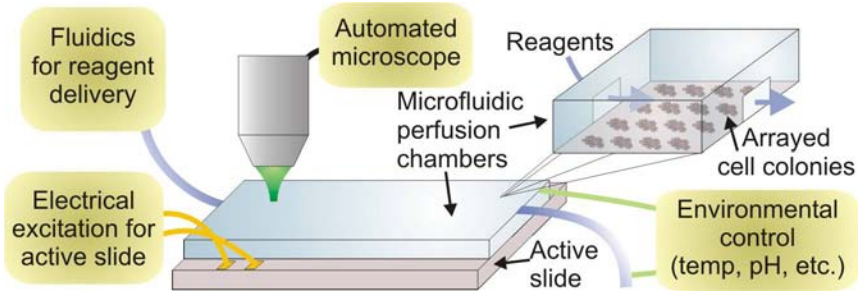

Figure 7: Multiplex culture and *in situ* assay system.

Technology Background

Our system uses two main microtechnologies to achieve our aims. First, we use microfluidics to precisely deliver combinatorial reagents to a large array of chambers, and second, we use dielectrophoresis (Project 1) to position cells in those chambers.

Controlling liquid flows at the microscale is well-developed technology. At the small size scales (<1 mm) and typical flow rates (~μl/min) encountered in these systems, the *fluid flow is laminar and well controlled*. Mixing occurs only by diffusion, which means that one can force two liquids to mix quickly (~<sec) or stay separate by changing geometries and flow rates. Using accepted technologies, one can make arrays of microfluidic chambers containing cells, and "hard-wire" different concentrations and combinations of input reagents to each of these wells, allowing one to run many experiments in parallel.

The other technology that we are using is dielectrophoresis (DEP, described in Project 1). With DEP we can precisely position cells in the chambers. *This critically addresses one functionality missing with current technology: the ability to independently pattern cells and the extracellular matrix (ECM) to which they attach*. Cells introduced onto patterned ECM distribute themselves randomly; given an expected colony size after culture, one must plate at a low enough concentration to ensure adequate distance between progenitor cells. Even then, the distance between any two cells will vary, creating variations in

diffusible signaling. According to our calculations, ensuring that most (90%) of randomly arrayed cells are far enough apart (e.g., twice the eventual colony radius) at the start of culture requires >100x times the chamber area that would be necessary if the cells were actively placed at twice the eventual colony radius. *Thus, efficient use of space requires active cell placement and gives the benefit of being able to control for the effects of colony distance.*

Current Research

Our current research is focused on two areas: cell placement via DEP traps and microfluidic cell-culture systems.

Cell placement via DEP traps

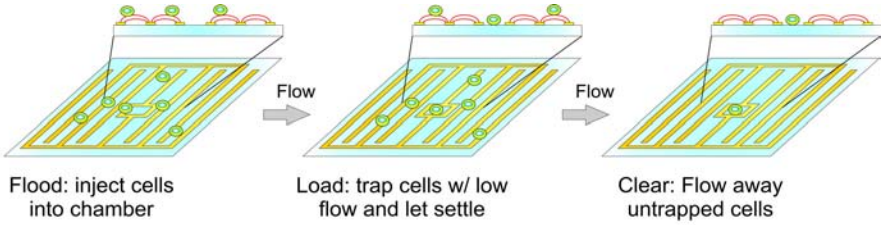

Figure 8: Operation of the DEP microwell: flooding the trapping area with cells (**left**), loading the traps (**middle**), and clearing away untrapped cells (**right**).

We are currently focusing on the development of a dielectrophoretic trap that can be used to array single cells in flow chambers. Prior single-cell DEP traps are strong but are either not appropriate for patterning cells, would not allow unobstructed cell division, are difficult to package or fabricate, or cannot be used with normal cell culture media. The challenge is fabricating a strong trap that allows single-cell patterning, is planar, and uses nDEP. To meet this challenge, we have designed a novel method of trapping called a dielectrophoretic microwell. The DEP microwell allows cells in the trap to settle while cells outside the traps are levitated and flowed away (Figure 8). We have used our previously validated modeling software to identify operating conditions that minimize cell heating and induced transmembrane voltage and maximize trap strength.

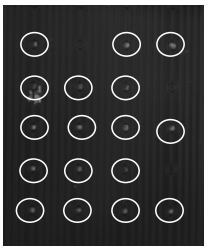

Figure 9: Trapping of murine ES cells in a 4x5 array.

We fabricated the DEP microwells by patterning gold electrodes on a glass slide using a simple metal lift-off process. The DEP traps are then surrounded with a PDMS gasket and covered by a glass lid to form the flow chamber. Liquid flow is controlled by a syringe pump. We have used these traps to position single murine embryonic stem (ES) cells in a 4x5 array with 85% efficiency (Figure 9).

Microfluidic cell culture and assay

We are also developing microfluidic systems for culturing adherent cells over a range of flowrates and reagent concentrations. Such systems enable greater control over the cell culture microenvironment by controlling media composition via continuous perfusion. Specifically we have designed and fabricated a device for performing adherent cell culture over a logarithmic range of flowrates. Logarithmic ranges are commonly used in experimental biology to explore a large parameter space but to-date have been lacking in microscale cell culture. The device, shown in Figure 10, sets the flowrates through each culture chamber using

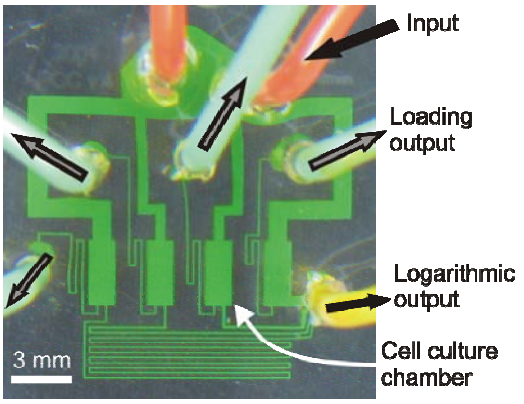

Figure 10: Photograph of 4-chamber logarithmic perfusion device filled with fluorescein to illustrate channels. Two orange input tubes are shown. The yellow tube shows the joint logarithmic output. Four green tubes (gray arrows) show individual loading outputs.

syringe-driven flow and a network of fluidic resistances. Each cell culture chamber has one input channel and two output channels. When cells are loaded, one set of output channels creates the same flowrate through all four chambers. After loading and cell attachment, the other set of output channels creates a logarithmic range of flowrates across the device. The design is easy to fabricate, scalable, and consists of a single-layer network of channels in PDMS bonded to a glass substrate to which cells adhere. We have also designed and fabricated a 4x4, multilevel microfluidic array that combines logarithmic flowrates with logarithmic reagent concentrations—this device includes an on-chip diluter to generate a range of concentrations.

To better understand the design requirements for a cell culture and *in situ* assay system, we are focusing on mouse embryonic stem cells as an example biological system. Stem cells are particularly sensitive to their microenvironment and are slow to culture using traditional techniques. We have successfully cultured embryonic stem cells in continuous, logarithmically scaled perfusion for 4 days using a device with the same design as in Figure 10. Results are shown in Figure 11. Perfusion rates varied >300x across the array, with poor proliferation at lower flowrates and healthy colony morphology at higher flowrates. We are also working on the control systems to run these chips, including environmental and electrical control.

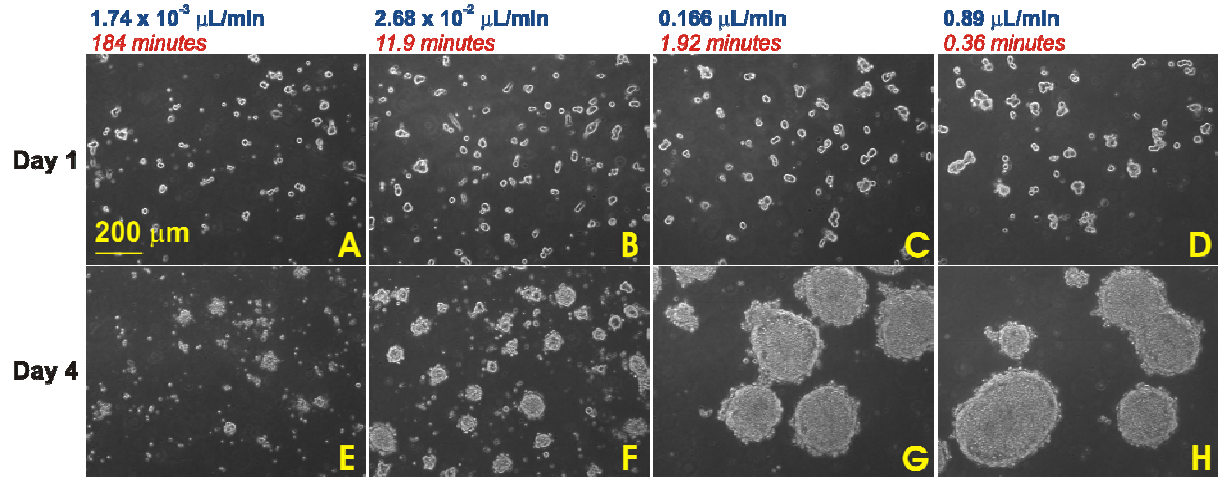

Figure 11: Perfusion culture of ABJ1 mouse embryonic stem cells over a logarithmic range of flowrates. Cells were loaded on Day 0 and each culture chamber was perfused at the same rate throughout the experiment. Perfusion rates are listed above each column in bold. The average time to replace one culture chamber volume is italicized. **A-D)** Typical colonies at Day 1 for each flowrate. **E-F)** Typical colonies at Day 4 for each flowrate. Round, healthy colony morphology is seen at higher flowrates (G-H). ABJ1 cells were generously donated by George Daley.

3. A Combined Microfluidic/Dielectrophoretic Microorganism Concentrator

Sponsors
Draper Laboratory
Siebel Scholarship

Project Staff
Nitzan Gadish

Overview
This project focuses on the development of a microorganism concentrator for pathogen detection applications. A common problem in microfluidic systems is the mismatch between the volume of a sample and the volume that a device, such as a detector, can process in a reasonable amount of time.

Concentrators can therefore be used in pathogen detection and other microfluidics applications to reduce sample sizes to the micro-scale without losing particles of interest.

The concentrator, illustrated in Figure 11, is an active filter that uses dielectrophoresis to concentrate bacterial spores in low-conductivity solution. Dielectrophoresis uses spatially nonuniform, alternating electric fields to move particles by polarizing them and then acting on the induced dipole. This concentrator uses positive dielectrophoresis, pulling particles toward electric field maxima.

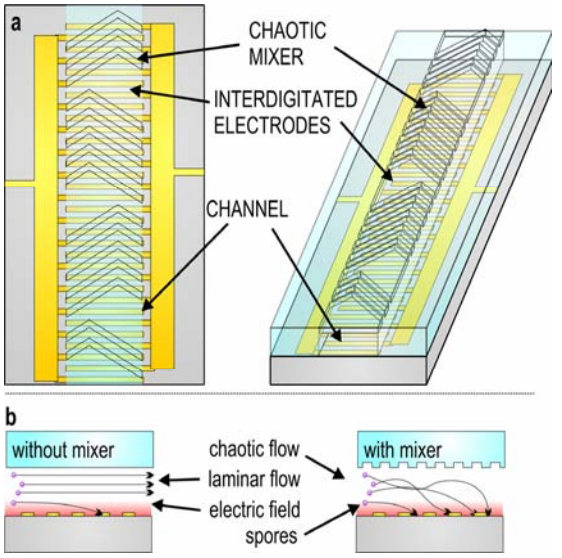

Figure 11: Chip concept.

In operation, we set up the electric fields by lining the bottom of the channel with interdigitated electrodes. We combine a passive mixer with these electrodes to enable trapping at high flowrates: the mixer circulates the liquid, bringing particles to the bottom of the channel where they are trapped by the electrodes. When enough particles have been collected, they are all released at once in a small volume, thereby producing a concentrated sample.

Current Research

We have designed a device to concentrate *B. subtilis* spores. We use our in-house modeling software to predict the behavior of the concentrator. Based on our simulation results, we specify the dimensions and operating parameters for the device.

We have fabricated and tested the concentrator and present some representative results here. The microscope images in Figure 12 show a time sequence of a concentration experiment. In the top row, from left to right, beads flow through the device and are trapped on the electrodes. In the bottom row, from right to left, beads are released from the electrodes into a concentrated sample. Figure 13 shows a plot of output concentration over time as a sample of beads is released. The plot was produced by sampling discrete droplets at the output of the device and measuring their bead concentration using a spectrophotometer. This result shows a concentration enhancement of 40× between the input (C_0) and output (Drop #5) concentrations.

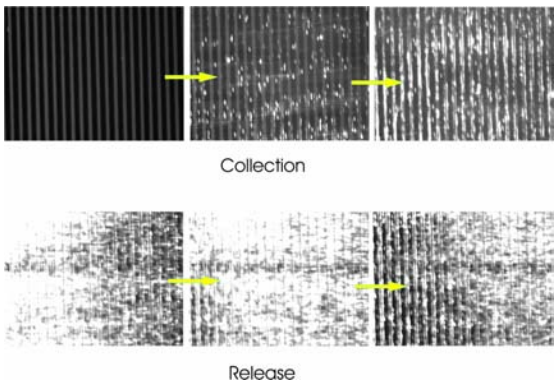

Figure 12: Concentration experiment

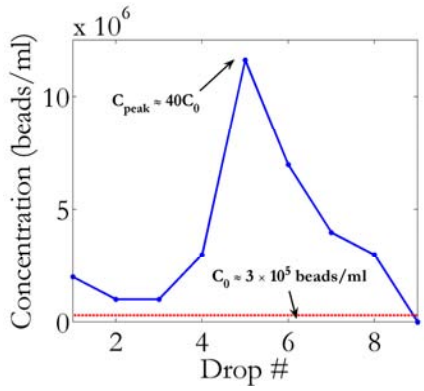

Figure 13: Experimental data of concentration enhancement of beads.

Publications

Journal Articles, Published

Rosenthal, A. and Voldman, J. Dielectrophoretic traps for single-particle patterning. Biophys. J. 88: 2193-2205 (2005).

Meeting Papers, Published

Rosenthal, A. and Voldman, J., "Simple, Strong, Size-Selective Dielectrophoretic Traps for Single-Cell Patterning", Micro Total Analysis Systems '04. (eds. T. Laurell, J. Nilsson, K. Jenseon, D.J. Harrison, and J.P. Kutter), pp. 228-230 (2004).

Theses

R. Muller, *A microfabricated dielectrophoretic micro-organism concentrator*, S.M.thesis, Department of Electrical Engineering and Computer Science, MIT, 2004

B. M. Taff, *Design and fabrication of an addressable MEMS-based dielectrophoretic microparticle array*, S.M.thesis, Department of Electrical Engineering and Computer Science, MIT, 2004