

## 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

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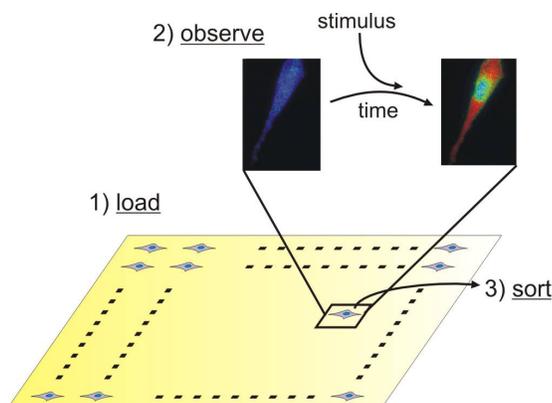
NIH NCRR  
NSF Graduate Research Fellowship

### Project Staff

Brian Taff, Andy Nnewihe

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



**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.

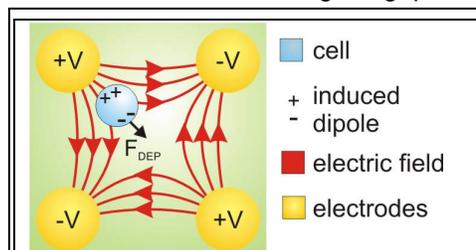
Our technology addresses two steps in any cell-based genetic screen: the observation of cells and the isolation of those cells exhibiting the desired phenotype. These two steps are inextricably linked, and the functionality available between them directly affects the types of screens that one can perform. 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.

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.

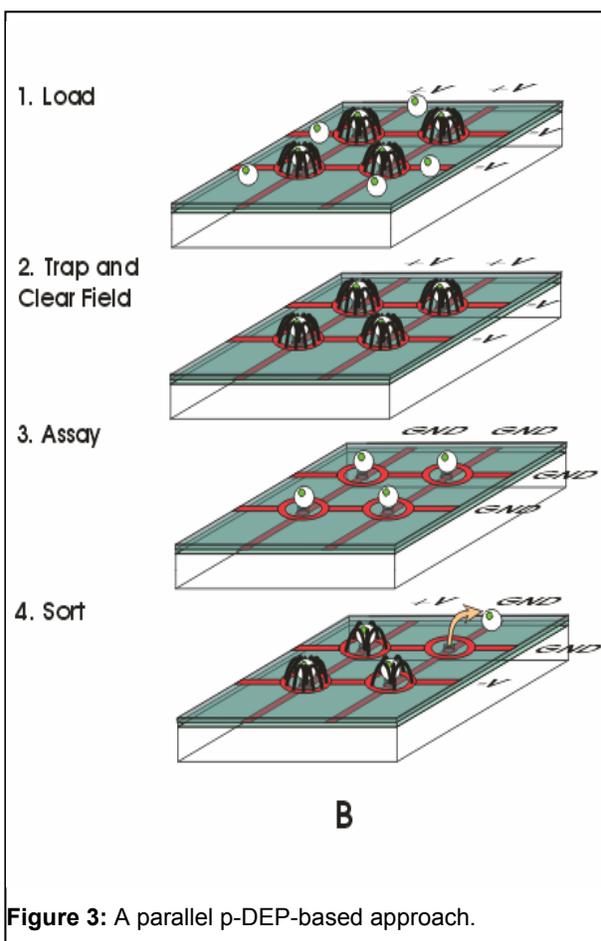
### Technology Background

*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.*

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.*



**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 ( $F_{DEP}$ ) towards the center, trapping the cell. A p-DEP trap, meanwhile, would draw the particle toward the electrodes.



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

### Current Research

We are currently developing a set of second-generation trap geometries that are scalable to 10,000 sites. At present we are testing 4x4 trap arrays to investigate how well our modeling pairs with the actual performance of the fabricated devices.

We have designed and fabricated both n-DEP and p-DEP based trapping geometries. Figure 3 shows a functional outline of the current p-DEP array design. We modeled the performance of individual pixels of the trap array (Figure 4) using our previously developed modeling software. These take the electric field associated with a trap geometry and use it to compute all of the forces on the particles in a trap for a given set of conditions. Such conditions can include applied voltages, drive frequencies, flowrates, chamber geometries, and electrical boundary conditions. The forces computed include multipolar dielectrophoretic forces, to account for high-order dielectrophoretic effects, as well as gravitational and fluid forces. From these forces the modeling tools can then compute the strength of the traps, either in absolute terms of piconewtons, or in terms of the applied flow needed to dislodge trapped cells.

Finally, in Figure 5 we provide a set of fabrication pictures displaying the plan view of the final n-DEP and p-DEP trap layouts as seen through a Nomarski DIC objective.

## 2. Bio-process device for cell culture and *in situ* assay

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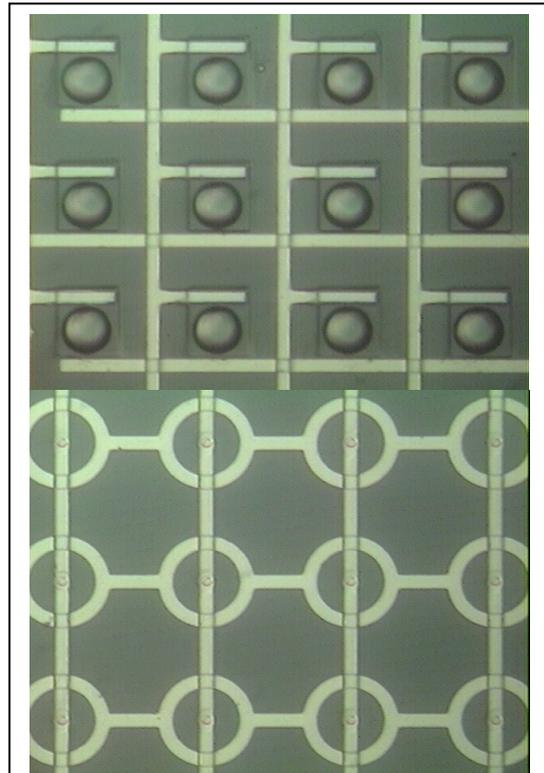
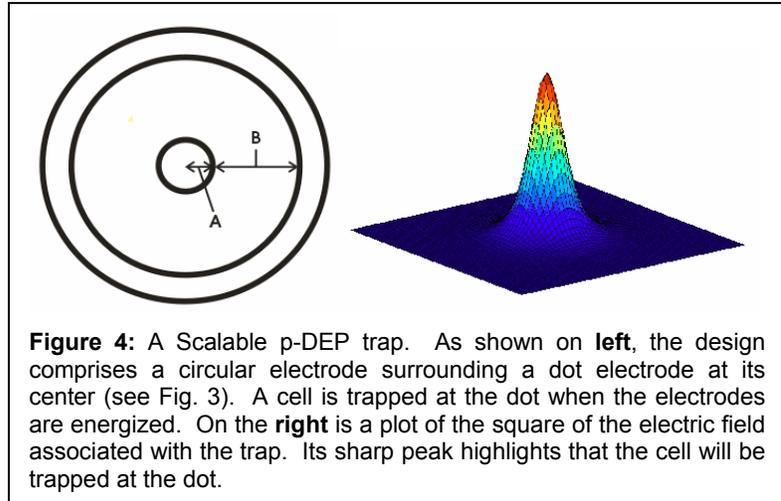
NIH NCRR  
NSF Graduate Research Fellowship  
MIT Presidential Graduate Fellowship  
Health Sciences and Technology MEMP Fellowship

### Project Staff

Adam Rosenthal, Lily Kim, Joseph Kovac, Andy Nnewiwe, Katya Puchala, Colin Weltin-Wu

### Overview

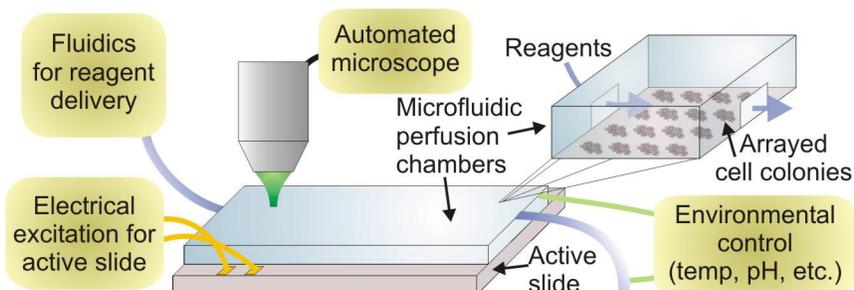
Living cells in culture are powerful biological models for many different processes, ranging from fundamental issues in biocomplexity to developmental or disease processes. For these reasons, intense effort is being focused on characterizing and manipulating cells in culture, identifying culture conditions 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*



**Figure 5:** Fabricated traps. **Top** shows a Nomarski image of the n-DEP trap configuration in plan view, while **bottom** shows the ring-dot geometries of the p-DEP layout.

*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 6). 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 6:** Multiplex culture and *in situ* assay system.

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.

### Technology Background

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*Controlling liquid flows at the microscale is well-developed technology.* At the small size scales (<1 mm) and typical flow rates ( $\sim\mu\text{l}/\text{min}$ ) 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 ( $\sim<\text{sec}$ ) 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  $>100\times$  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*

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 planar nDEP trap that is simple, scalable, strong, well-suited for cells, inexpensive to fabricate, and displays size-selective trapping.

The electrode traps (Figure 7) are formed by patterning gold onto a glass slide. The minimum feature size of the traps is  $>10\ \mu\text{m}$ , which allows the use of inexpensive transparency masks for photolithography.

To demonstrate the strength of our traps, we measured the maximum flow rate that trapped beads could withstand before getting pushed out of the trap and compared this to predictions generated by our modeling program. We note that the predictions include no fitting parameters. The typical deviation between the predictions and experiments was 0-14% (Figure 7).

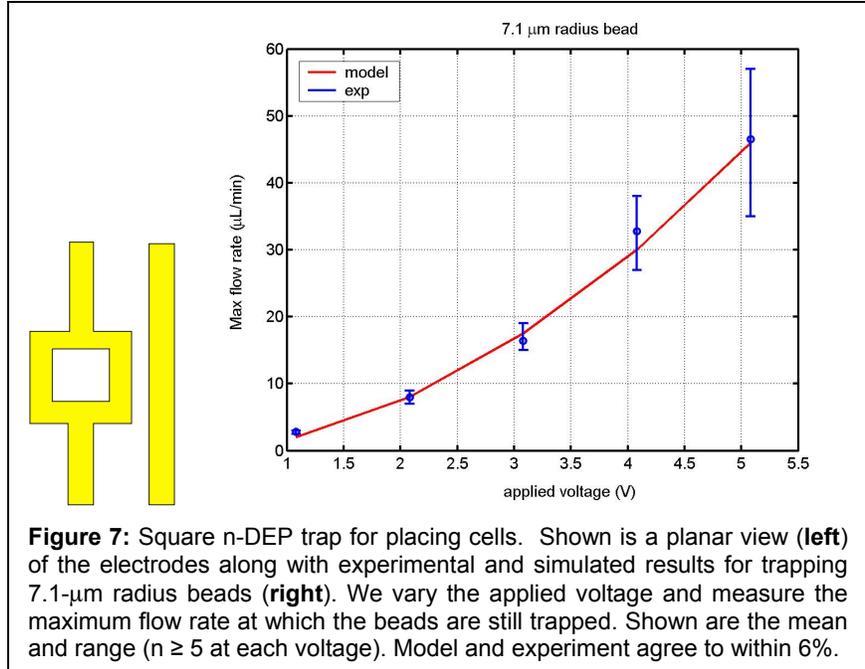
These traps also display interesting peak size-selectivity behavior, with peak holding for  $6\text{-}\mu\text{m}$  beads (Figure 8). This size selectivity can be used to tailor traps for specific cells sizes or other system parameters. We are currently outlining the design rules for these traps.

Finally, to demonstrate use with cells, traps were used to array single mouse fibroblasts (Figure 8).

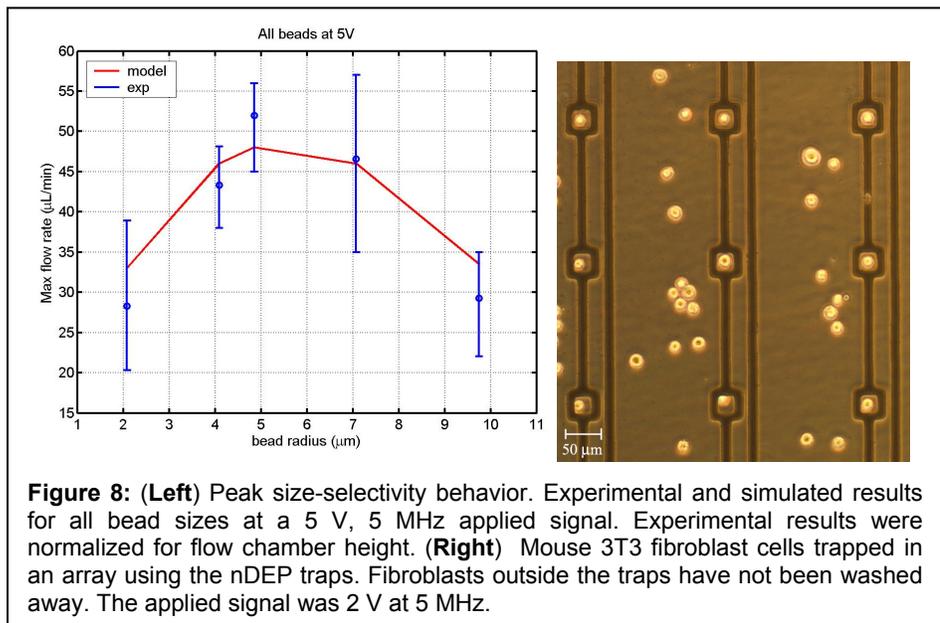
#### Microfluidic cell culture and assay

We are also developing microfluidic systems that form the array of cell culture chambers and the fluidic channels connected to them. The system is composed of three sets of channels: 1) Loading channels that deliver the cells to initially seed the chambers. 2) A

serial diluter to mix two input fluids in logarithmic ratios which get delivered to the cells 3) Flow-restricting channels that specify the flow rates (ranging from  $\sim 10^{-4}$  uL/min to  $\sim 0.1$  uL/min) through each chamber. The three sets of channels require the device to have two layers of interconnected channels. We have successfully fabricated these systems using multi-level polydimethylsiloxane (PDMS) patterned using SU-



**Figure 7:** Square n-DEP trap for placing cells. Shown is a planar view (left) of the electrodes along with experimental and simulated results for trapping  $7.1\text{-}\mu\text{m}$  radius beads (right). We vary the applied voltage and measure the maximum flow rate at which the beads are still trapped. Shown are the mean and range ( $n \geq 5$  at each voltage). Model and experiment agree to within 6%.

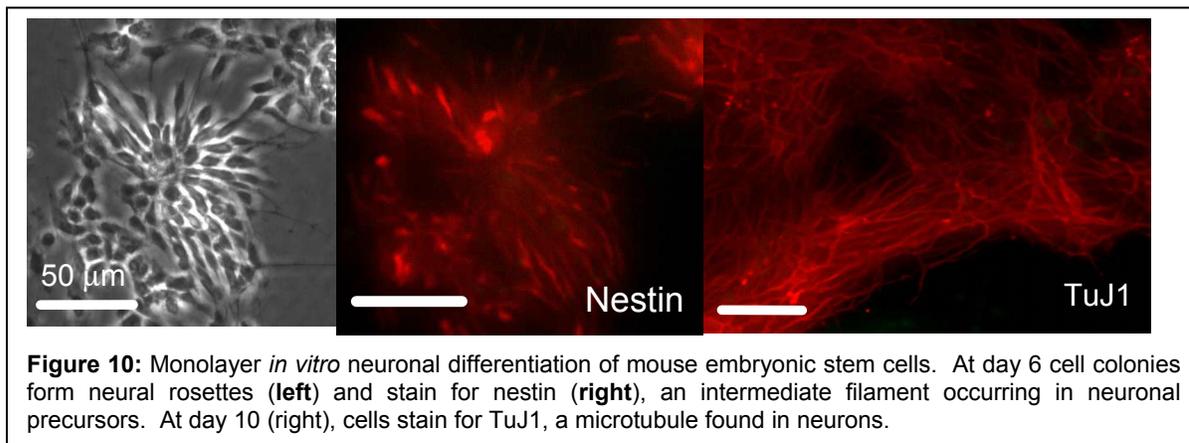
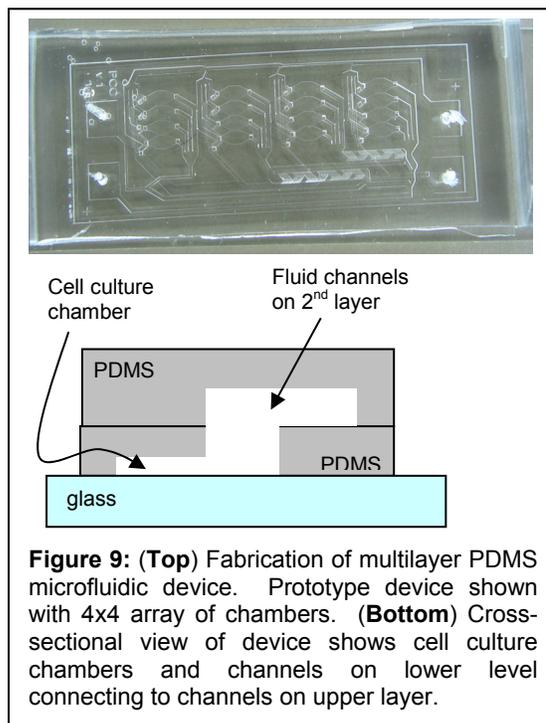


**Figure 8:** (Left) Peak size-selectivity behavior. Experimental and simulated results for all bead sizes at a 5 V, 5 MHz applied signal. Experimental results were normalized for flow chamber height. (Right) Mouse 3T3 fibroblast cells trapped in an array using the nDEP traps. Fibroblasts outside the traps have not been washed away. The applied signal was 2 V at 5 MHz.

8 photoresist molds on silicon wafers. The PDMS layers are then bonded to a glass substrate as shown in Figure 9.

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. A micro-culture and assay system using monolayer cell culture provides a more controlled environment for stem cell experiments and reduces experiment time. We have demonstrated the ability to culture embryonic stem cells *in vitro* in a monolayer and to induce neuronal differentiation, shown in Figure 10.

We are also working on the control systems to run these chips, including environmental and electrical control. Research is being undertaken to control the medium temperature on-chip, using integrated temperature sensing resistors and heaters, as well as control of pH and dissolved oxygen.



### 3. Dielectrophoretic micro-organism concentrator

**Sponsors**  
Draper Laboratory

**Project Staff**  
Rikky Muller

#### Overview

This project focuses on the development of a micro-organism concentrator. Pathogen detection, particularly MEMS based detection, is often limited by sample concentration. The proposed concentrator will interface with a pathogen detector. This type of pathogen concentrator can be useful for many kinds

of applications including water purification systems, medical applications and biological warfare agent detection. Due to the nature of these applications, the concentrator must be able to operate under real-world conditions and be robust to particulates and variations in conductivity.

The concentrator will be an active filter that uses n-DEP to concentrate bacteria in solution. As shown in Figure 14, the concentrator sets up an electric field barrier that guides cells toward a concentrated outlet flow path while the bulk of the fluid, which goes through the electric field barrier, is sent to a waste outlet.

Current Research

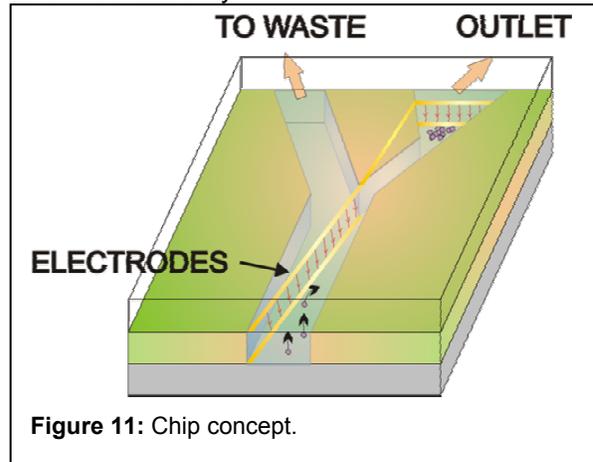
We have designed a device optimized to specifically concentrate *E.coli*. We model the strength of the concentrator to set the design specifications. As an example, Figure 13 shows a plot used to verify the strength of the  $\mu$ -concentrator. Here we plot the trajectories of *E.coli* using calculated forces from finite element models (FEM). These trajectories use extracted fields from the FEM to calculate a DEP force which are then used to show the bacterial trajectories for various flowrates. The graphs clearly show that at a volumetric flowrate of 10  $\mu\text{L}/\text{min}$ , the bacteria are sufficiently deflected away from the electrode to be concentrated.

We have fabricated these devices and show some results in Figure 14. Here we use polystyrene beads in fluids of known conductivity to measure the throughput of the device for full concentration. The left part of Figure 14 shows beads being collected along the electrode when there is a voltage applied to the electrodes and then being released in the direction of the flow when the voltage is turned off. The right part of Figure 14 shows fluorescent beads collecting along the electrode against flow. We are currently working on quantifying the throughput and concentration factor of the device and matching it to the modeled data.

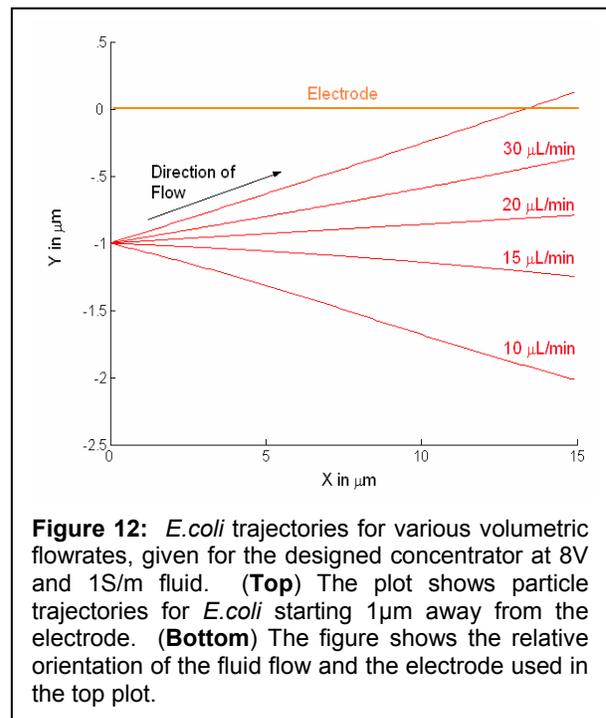
**Publications**

**Journal Articles, Published**

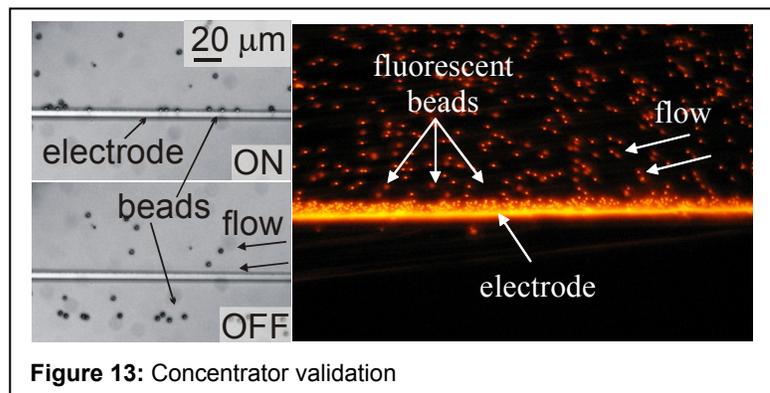
D. S. Gray, J.L. Tan, J. Voldman, and C.S. Chen. "Dielectrophoretic registration of living cells to a microelectrode array," *Biosensors and Bioelectronics* 19: 771-780 (2004).



**Figure 11:** Chip concept.



**Figure 12:** *E.coli* trajectories for various volumetric flowrates, given for the designed concentrator at 8V and 1S/m fluid. (Top) The plot shows particle trajectories for *E.coli* starting 1 $\mu\text{m}$  away from the electrode. (Bottom) The figure shows the relative orientation of the fluid flow and the electrode used in the top plot.



**Figure 13:** Concentrator validation