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
MIT, NSF Graduate Research Fellowship, MIT Presidential Graduate Fellowship Health Sciences and Technology MEMP Fellowship

Project Staff
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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.

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.

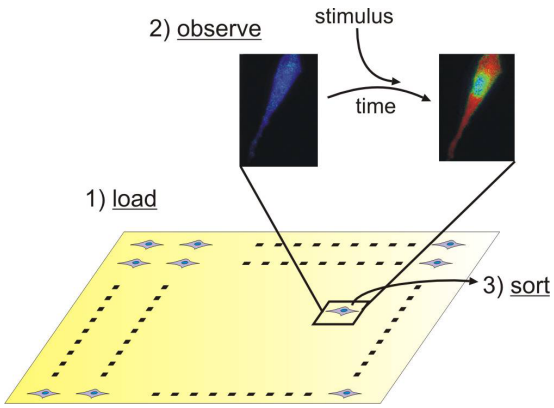

Figure 1: A sorting cytometer for screening complex phenotypes. The cytometer consists of a two-dimensional array of traps, each of which holds a single cell. After loading the traps (1), the array is optically interrogated (2), and cells with phenotypes of interest are viably sorted (3). In this case,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

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.

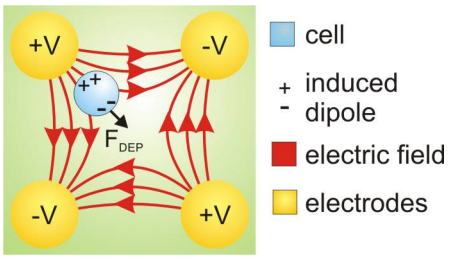

Figure 2: Dielectrophoresis. Four electrodes create an electric field that induces a dipole in a biological cell. The orientation of the induced dipole causes the cell to experience a negative DEP force (F_{DEP}) towards the center, trapping the cell. A p-DEP trap, meanwhile, would draw the particle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

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 are currently developing a second-generation trap geometry that addresses the limitations of our current generation (Figure 3). This trap will be scalable to 10,000 sites, will robustly trap only single cells, and will work with adherent and non-adherent cells.

This new trap array will use n-DEP (Figure 4), and operates as follows. The array is loaded by first flowing media with cells across the array and letting the cells settle into the wells (Figure 4A). The wells are sized so that on average only a single-cell is trapped in each site. Next, all the electrodes are excited, turning on all the traps. The application of flow in conjunction with the DEP cage causes all the cells on the substrate surface to be swept away while capturing the cells in the wells. The cells are then allowed

to attach, after which the electrodes are turned off, and the assay proceeds (Figure 4B). Sorting proceeds by turning off the row and column lines of the sorted cell (Figure 4C).

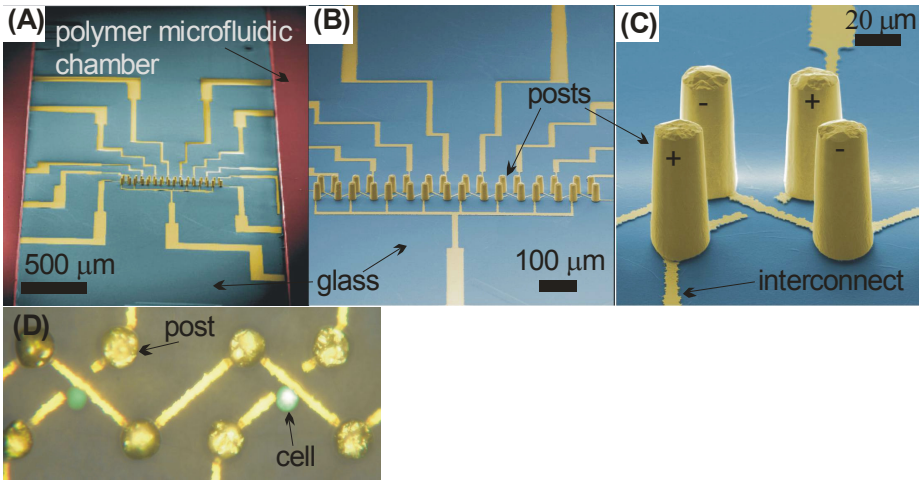

Figure 3: (A-C) Pseudo-colored electron micrographs of DEP traps for holding single cells. Each trap (C) consists of four post electrodes made of gold. An array of 8 traps (B) is situated in a microfluidic chamber (A). **(D)** Top-down view of two viably stained HL-60 cells trapped in the dielectrophoretic traps.

We are currently modeling the performance of individual pixels of this trap (Figure 5) using our previously developed modeling tools. These modeling tools work by taking electric field computations provided by any other method (analytical or numerical) and computing all the forces on the particles in a trap for a given set of conditions, including voltage, frequency, flowrate, chamber geometry, electrical properties. 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.

As examples of our modeling thus far, Figure 5 contains a schematic of the trap geometry, derived from the cartoon in Figure 4. Also shown are computed electric-field magnitude along the central axis of the trap, as well as the total force in the trap with and without a cell. The results show that cells below approximately 30 microns will be pushed downward into the trap and held with over 25 pN of force.

We are currently finalizing the modeling to ensure that heating is minimized and that the cells do not experience too high an electrical stress. Following this we will begin microfabricating the traps at MIT.

Concurrently, we are addressing the issue of using adherent cells in our devices. Adherent cells are cells that prefer to attach to surfaces, rather than remain free-floating. In mammals, this refers to all cells except blood cells. We have performed experiments on attachment times of NIH 3T3 cells onto polystyrene dishes with either uncoated, serum-treated, or fibronectin-coated surfaces. Results on un-coated plates indicate that cells will attach within one hour (Figure 6), and we are confident that this can be reduced by pre-coating the substrates. We are following up these results with experiments on detachments times.

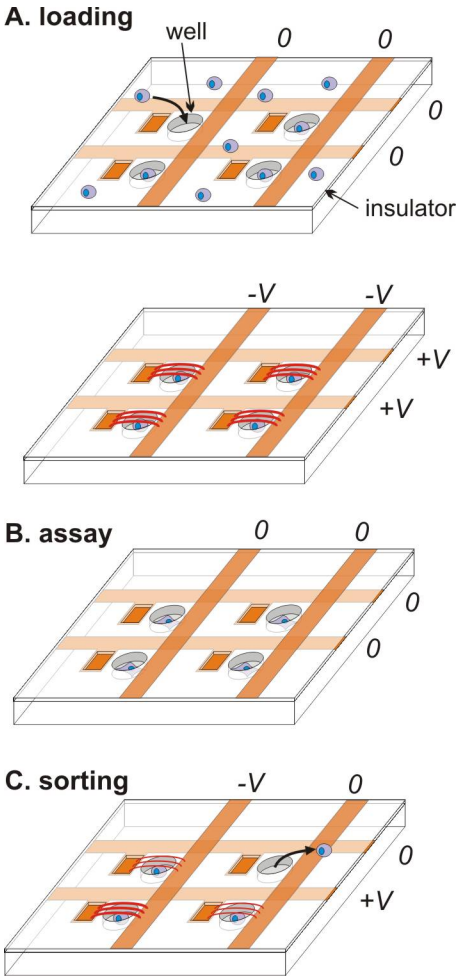

Figure 4: A n-DEP—based scalable trap.

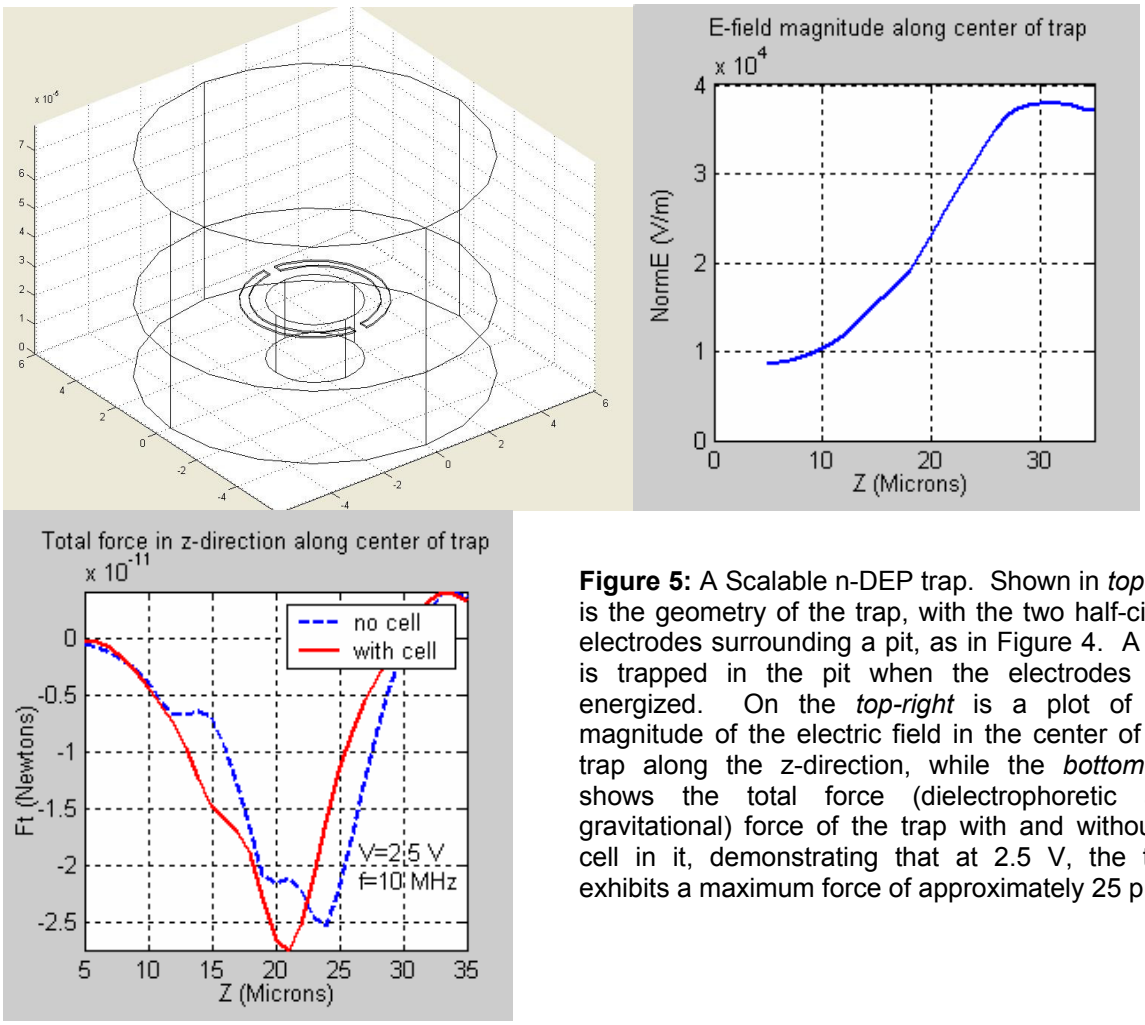

Figure 5: A Scalable n-DEP trap. Shown in *top-left* is the geometry of the trap, with the two half-circle electrodes surrounding a pit, as in Figure 4. A cell is trapped in the pit when the electrodes are energized. On the *top-right* is a plot of the magnitude of the electric field in the center of the trap along the z-direction, while the *bottom-left* shows the total force (dielectrophoretic and gravitational) force of the trap with and without a cell in it, demonstrating that at 2.5 V, the trap exhibits a maximum force of approximately 25 pN.

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

Sponsors
MIT

Project Staff
Brian Taff, , Joseph Kovac, Andy Nnewihe

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.*

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.

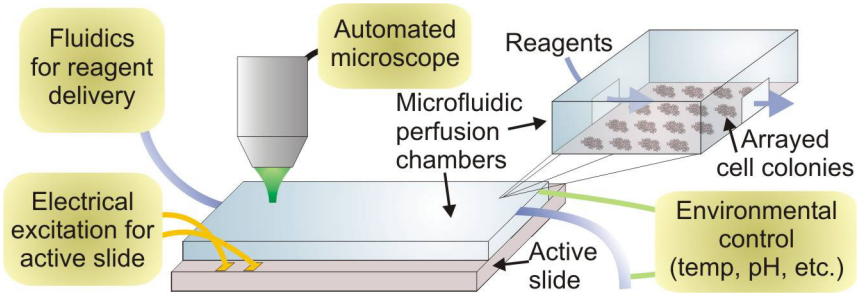

Figure 7: Multiplex culture and *in situ* assay system.

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

We are currently focusing on the development of a dielectrophoretic trap that can be used to array cells in chambers. We are designing both n-DEP and p-DEP candidate traps for our system, as each has its own advantages. The p-DEP trap geometry is shown in Figure 8, along with holding of different-sized cells against flows. Our current n-DEP geometry is shown in Figure 9 along with electric fields and simulated holding characteristics with cells. This D-shaped geometry in particular is notable for several reasons. First, it uses n-DEP yet pushes trapped cells *against* the substrate, rather than levitating them. Second, it provides strong confinement with a planar electrode geometry. Third, it provides size-specific trapping that has a tunable maximum, meaning that one can select, by scaling the geometry, the optimum size cell that one wishes to trap.

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. In addition, we are undertaking the design of a robust electrical control circuit for the DEP traps, capable of providing 80 MHz signals at >10 Vpp into these capacitive loads, and being able to be computer controlled.

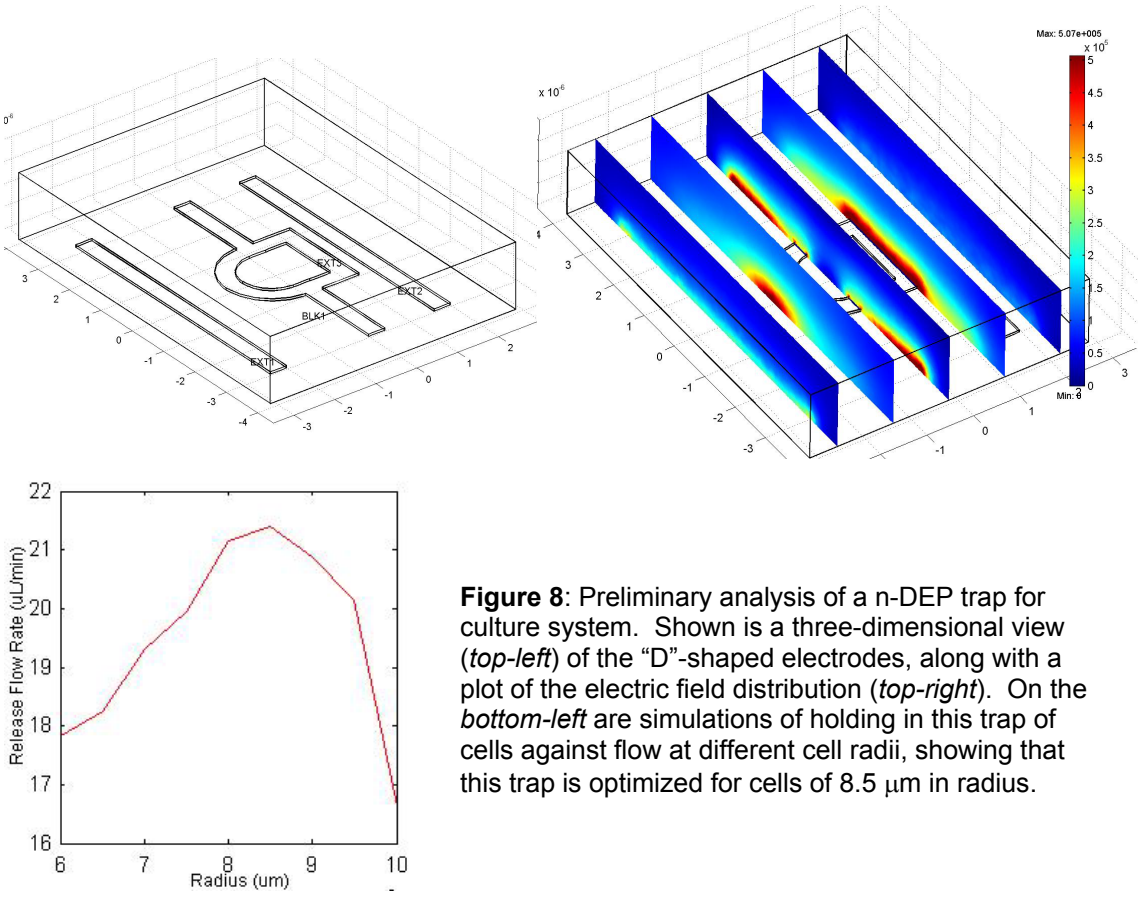

Figure 8: Preliminary analysis of a n-DEP trap for culture system. Shown is a three-dimensional view (*top-left*) of the "D"-shaped electrodes, along with a plot of the electric field distribution (*top-right*). On the *bottom-left* are simulations of holding in this trap of cells against flow at different cell radii, showing that this trap is optimized for cells of 8.5 μm in radius.

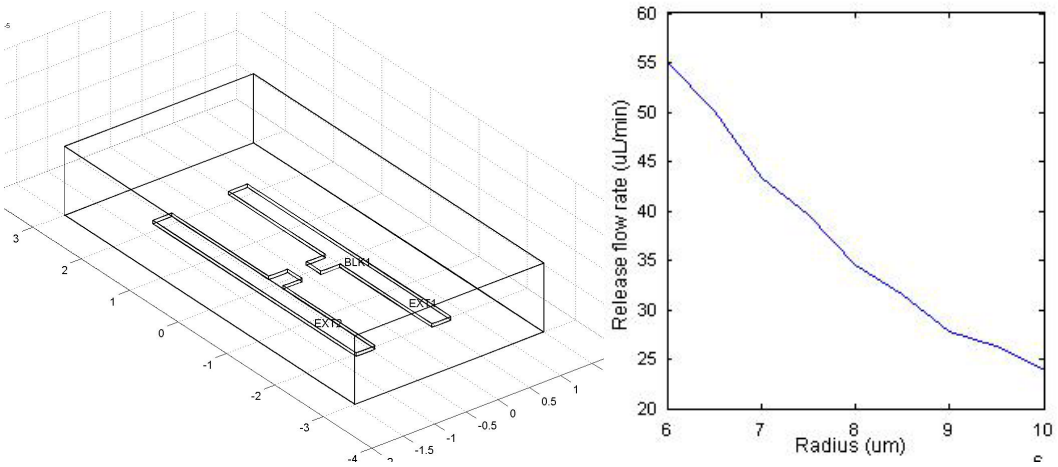

Figure 9: Preliminary analysis of a p-DEP trap for culture system. Shown is a three-dimensional view of the "H"-shaped electrodes. On the right are simulations of holding in this trap of cells against flow at different voltages, showing that this trap is optimized for holding smaller cells.