Nanofluidic BioMEMS Research

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Overview of group
Nanofluidic BioMEMS group in RLE (Han group) is exploring various ways that the micro/nanofabrication techniques can be used for advanced biomolecule manipulation and separation applications. It is now possible to reliably fabricate nanofluidic gaps and filters that have regular, controllable structures, with near-molecular dimensions (10 -100nm). One can take advantage of these nanofluidic structures for advanced separation and manipulation of various biomolecules and bioparticles, including cell, cellular organelles, DNA, protein, and carbohydrates. The research of Nanofluidic BioMEMS group is currently actively designing, fabricating and testing the new kinds of molecular sieves and filters that can be essential for the next-generation biomolecule assays in the new era of genomics, proteomics and glycomics. At the same time, the subject of molecular stochastic motion and molecular interaction with nanostructure is actively studied, in order to provide firm theoretical and scientific ground for the development of novel nanofluidic molecular filters.

1. Biomolecule Separation in Nanofluidic Filters by Steric Hindrance Mechanism

Sponsors
Lincoln Laboratories (ACC 353)
National Science Foundation

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Micro/nanofluidic molecular sieving structures have great potential for a faster molecular separation. However, their uses have been largely limited to big molecules, since it is not trivial to fabricate structures with comparable molecular dimension. In this work, we present a novel separation method using nanofluidic filters, which is based on the steric hindrance effect of molecules (Fig. 1). When biomolecules are entering nanofluidic gaps with larger but comparable thickness, they are hindered from entering the gap due to the confinement entropy changes. This effect could be a basis for a new separation technique using regular nanofluidic filter structures.
To demonstrate this, we fabricated nanofluidic trap array devices consisting of alternating thin and thick regions in a microfabricated channel. The nanofluidic trap arrays were fabricated using photolithography and reactive ion etching (RIE) techniques on a Si substrate.

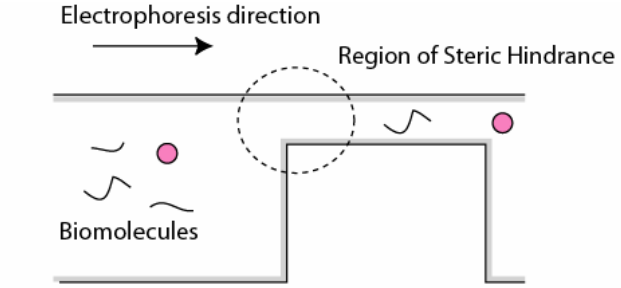

Figure 1. Cross-sectional schematic diagram of the nanofluidic biomolecule filter. When the sizes of DNA molecules are comparable to the thin channel depth, they are hindered from entering the filter due to the steric hindrance effect and their mobilities become size-dependent.

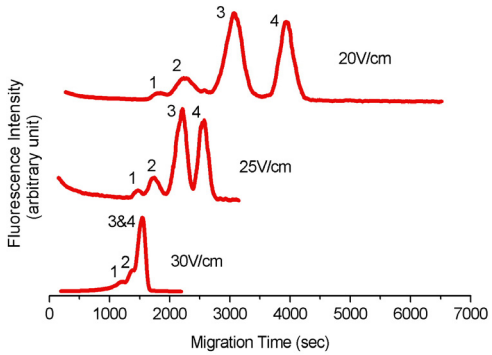

Figure 2. Separation of pBR322 DNA in the nanofluidic trap array device with 120nm thin regions, 500nm thick regions and 6μm channel period. Band assignment: (1) 121bp; (2) 383bp; (3) 929&1058bp; (4) 1857bp. Electropherograms were taken at 1cm downstream from the injection point under different electrical fields.

Double stranded DNA molecules between 100bp (~30nm extended length) and 2000bp were separated by dc electrophoresis and laser induced fluorescence detection, using devices with thin region thickness of 60nm and 120nm (Fig. 2). In all the experiments, smaller DNA molecules travel faster and have higher mobility, since smaller DNA molecules have more available space within the thin channel, and therefore they are more favored to enter the filter. Debye layer overlap is not a factor at the high buffer concentrations we used for this work, and we could not observe any such separation in the thin, flat nanofluidic channel with same dimensions. Also, separation resolution was lost when the driving electric field was increased above a certain value. These observations suggest that the separation mechanism is indeed due to molecular (entropy) energy barrier at the nanofluidic filter.

2. Muti-dimensional Microfluidic Biomolecule Separation

Sponsors
DARPA BIM (Bio:Info:Mirco)
MIT Ferry Fund
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One of the most important separation methods in proteomics is 2D-gel electrophoresis, the standard way so far to resolve over thousands of proteins before the mass spectrometry analysis. However, it is limited by its intrinsic labor-intensiveness and poor ability to resolve low abundance proteins. To identify low abundance proteins, it is necessary to load excessive amount of samples, which would sometimes overwhelm the separation instrument and make the detection even harder to achieve. In order to overcome these problems, we designed a microfluidic 2D separation system that can achieve rapid analysis with clear isolation between different separation steps.

In this project, we use microfluidic valves (shown in Figure 1) to create a peak transfer region to fulfill on-chip multidimensional separation. In this peak transfer region, we can isolate and transfer a group of protein (separated by the first dimension separation) to the next dimension separation, without cross-contaminating each separation channels by heterogeneous buffers used.

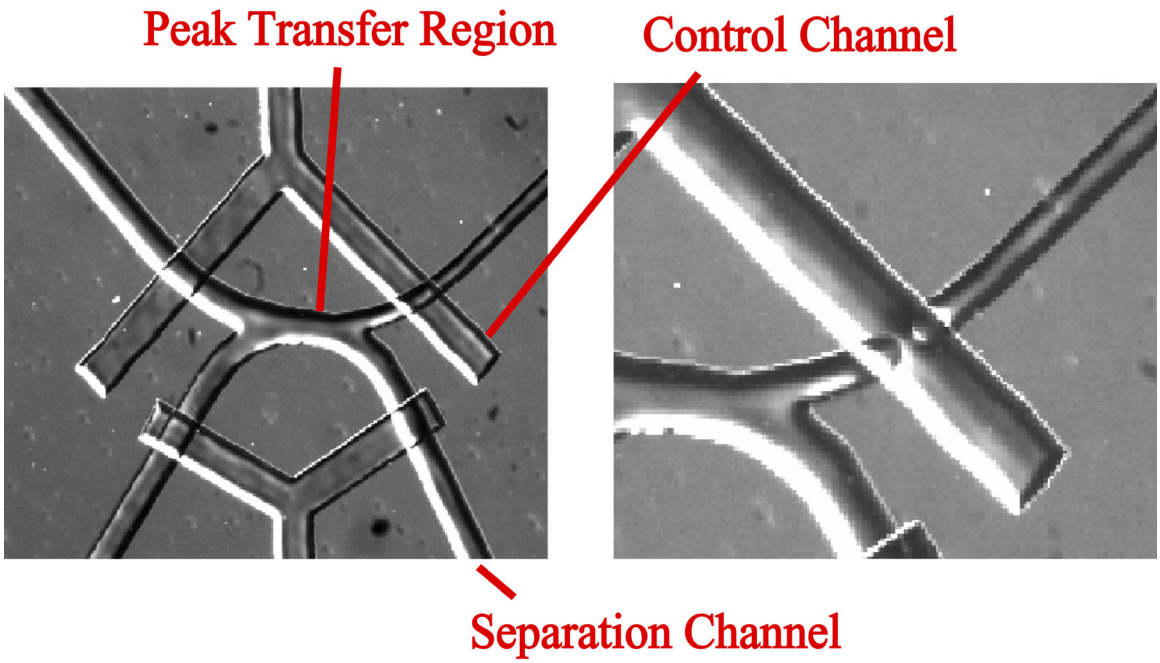

Figure 1. Pictures of microfludics valves: Multi-layer PDMS device showing two Y-shape control channels and two U-shape separation channels (LEFT). By pressurizing control channels, the separation can be isolated into distinct sessions (RIGHT).

Using this strategy, we successfully coupled microchip-based capillary isoelectric focusing (IEF, a charge separation technique) and capillary electrophoresis (a molecular weight separation technique) together. Compare to current 2D gel electrophoresis technique that could take a day, our system can acquire protein 2D separation on chip in 20 min by coupling post column labeling

technique into the device. In most conventional gel electrophoresis, many (~1000) protein peaks are separated on a slab gel but only a small number of tracer protein peaks are extracted from the slap gel and analyzed by mass spectrometry (MS). Our device is ideally suited to do just that, by selecting only the targeted protein groups (characterized by their isoelectric point) and transfer them to the next level analysis and detection system. Shown in Figure 2, the valve operation enables us to send either a group (GFP and Ovalbumin) or a single peak (GFP) from first dimension separation into second dimension separation for further analysis.

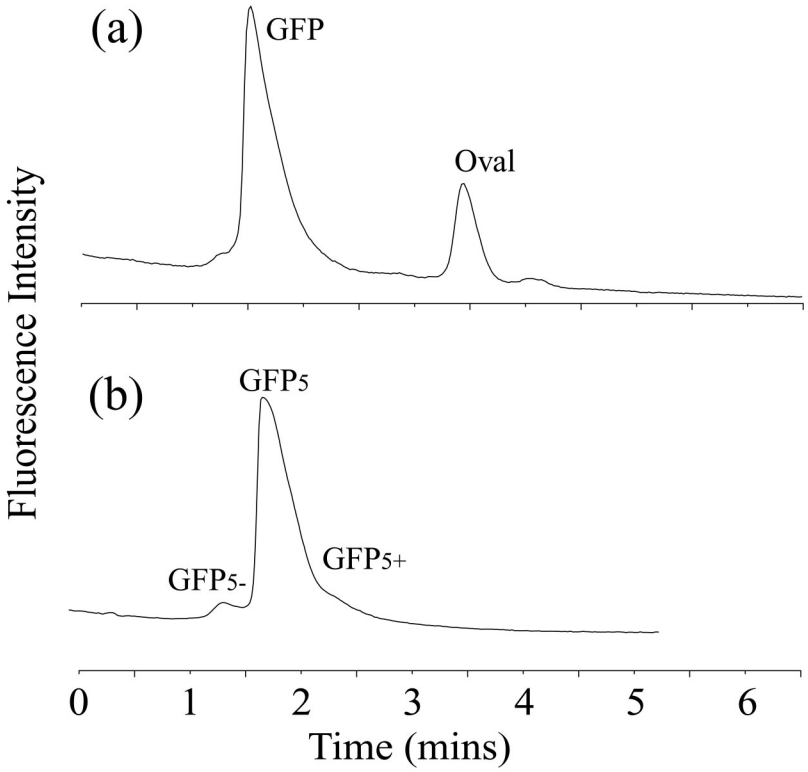

Figure 2. Selectivity experiment with free solution CE: By preparing the same ampholyte solution, we can capture different target protein (proteins) and execute second dimension
free solution CE, Capillary Gel Electrophoresis (CGE), or other separation technologies like chromatography.(a) Only GFP being trapped in IEF separation and sent into second dimension CE with 200 mM, pH 8.0 Tris-Cl and 0.1 % Methyl cellulose. (b) Use the same ampholyte mixture and second dimension CE buffer, but captured GFP and Ovalbumin in IEF. Note: Ovalbumin (45K) has higher mobility because it has similar mass with GFP (26K) but required more charges than GFP in pH 8.0 buffers.

The successful completion of this project allows various kinds of advanced separation techniques coupled together to perform complex protein mixtures separation on chip. Future research directions include realization of complete 2D mapping of proteins, as well as coupling to mass spectrometry detection systems.

3. Investigation of Molecular Stochastic Motion in Nanofluidic Channels Using Fluorescence Correlation Spectroscopy (FCS)

Sponsors
National Science Foundation (NSE)

Project Staff
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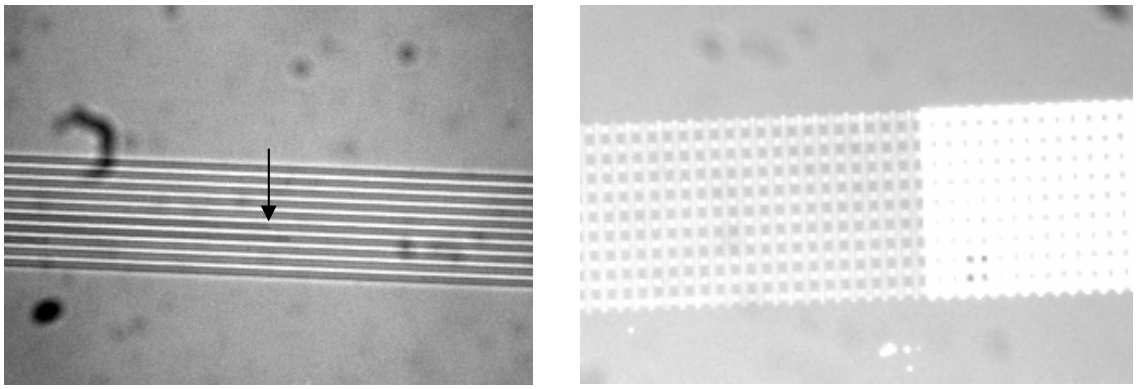
The objective of this research is to investigate the stochastic motion of biomolecules or small particles in a nanofluidic channel for the purpose of developing novel concepts for molecular sorting and manipulation technology. Regular nanofluidic channels with the known shapes and depths (10~3000nm) will be fabricated on silicon, glass or polymer substrate to confine molecular motion at least in one dimension, and the stochastic motion of single molecules will be followed



(**A**) (**B**)

Figure 1. (**A**) Optical micrograph of 26nm deep Si nanofluidic channels being filled with TBE buffer solutions. Due to a strong capillary force, buffer solution spontaneously filled the entire nanofluidic channel without any external driving force. Interface between air and buffer was designated with arrows. The width of individual channels is 3μm. (**B**) PDMS nanofluidic channels with alternative thin and thick regions with the period of 6μm (1.4μm in thick region and 110 nm in thin region). Thin region consists of 3μm lines spaced by 3μm. The left part was filled with DI water while the right part was not filled yet.

by the fluorescence correlation spectroscopy (FCS). The successful completion of the project would contribute to the development of new kinds of molecular filters and separation sieves, as well as to better understanding of molecular diffusion process in the nanostructures or confined spaces (such as intracellular spaces).

Nanofluidic channels with the well-controlled depths are fabricated by using silicon-based standard photolithography and reactive ion etching techniques. Nanofluidic channels as thin as 26nm have been achieved easily and filled with biomolecules. Figure 1 (A) shows the fabricated 26nm nanofluidic channels and the filling of the channel with buffer solution. We are still in the process of testing the ultimate minimum thickness one could achieve using this fabrication technique.

On the other hand, polydimethylsiloxane (PDMS) material and replica molding technique have also been investigated to make very thin nanofluidic channels. Both Sylgard 184 PDMS (Dow Corning) and hard PDMS (h-PDMS) developed by Schmid at al. are tested and compared. Table 1 summarizes the results of achieved features with 184 and h-PDMS. PDMS is too flexible to fabricate nanofluidic channels thinner than 100nm required in this work. We are developing new fabrication methods to make thin channels on glass or plastic substrate.

A new two-photon FCS setup has been built in the So lab, and the schematic diagram of the setup is shown in Figure 2. For the initial characterization of the FCS setup, we observed the correlation curves of simple dye molecules in a bulk solution. The samples we are currently using are, small fluorescent dyes (less than 1nm in diameter), phycoerythrin protein (3-5nm in diameter), 18 base pair double stranded DNA oligomer (~6nm, stretched). We obtained characteristic correlation curves of theses molecules in the bulk solution. The measurement of diffusion constants of biomolecule samples in a nanofluidic channel is under way.

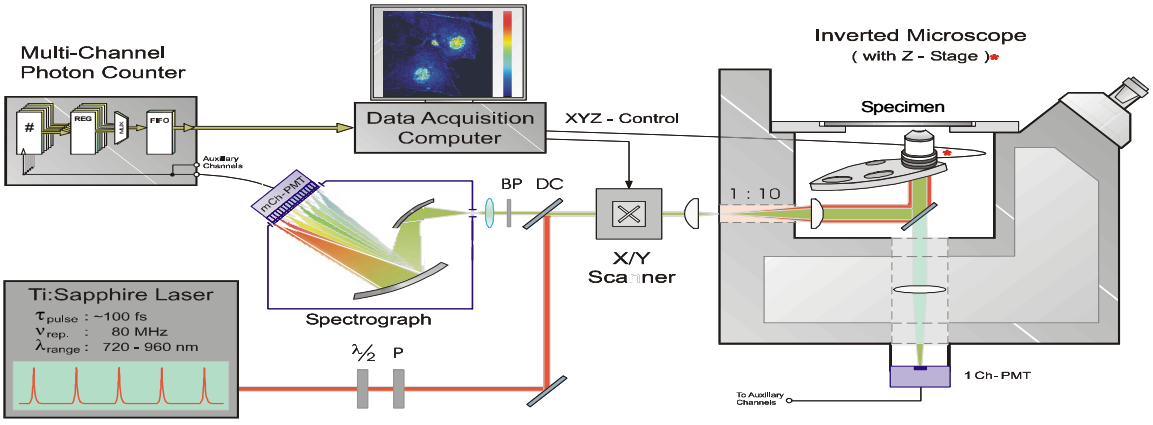


Figure 2. Schematic diagram of two-photon FCS setup for measurement of diffusion coefficient in nanofluidic channels.

Publications

Journal Articles, Submitted for Publication

Y.-C. Wang, M. H. Choi, and J. Han, "Two-Dimensional Protein Separation with Advanced Sample and Buffer Isolation Using Microfluidic Valves", submitted to Anal. Chem.

Book/Chapters in Books

J. Han, "Nanofluidics", in *Introduction to Nanoscale Science and Technology,* eds. M. Di Ventra, S. Evoy, and J. R. Heflin (Kluwer Academic Publishers, 2004).

Meeting Papers, Published

Y.-C. Wang, M. H. Choi, and J. Han, "On-Chip IEF Peak Manipulation for 2D Protein Separation and MS Coupling", Proceedings of the International Conference on Micro Total Analysis Systems, Squaw Valley, California, USA, October 5-9, 2003

Theses

Y.-C. Wang, On-chip Multi-dimensional Biomolecule Separation Using Multi-layer Microfabricated Valves, S.M. thesis, Department of Mechanical Engineering, MIT, 2004