DNA Separation in a Nanofluidic Device

Elizaveta Davies, Travis Del Bonis-O'Donnell and Dr. Sumita Pennathur
Department of Mechanical Engineering, University of California, Santa Barbara, USA

Motivation:

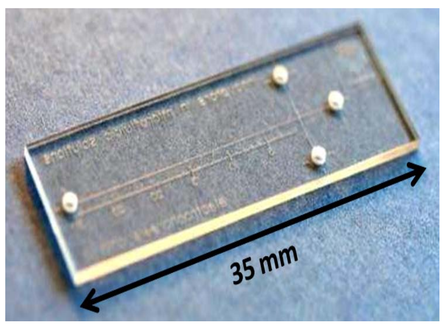
There has been an increasing interest in developing "lab-on-a-chip" devices, which would allow for DNA analysis for medical and forensic applications. The goal is to quantitatively characterize the separation capacity of nanofluidic devices leading to the development of a portable platform for DNA analysis.

Current method (electrophoresis)

- Bulky
- Time consuming
- Requires trained laboratory technicians
- Uses excess carcinogenic reagents

Nanoscale method (Lab-on-a-chip)

- Fast
- Portable
- Inexpensive
- Reagent/sample conservative

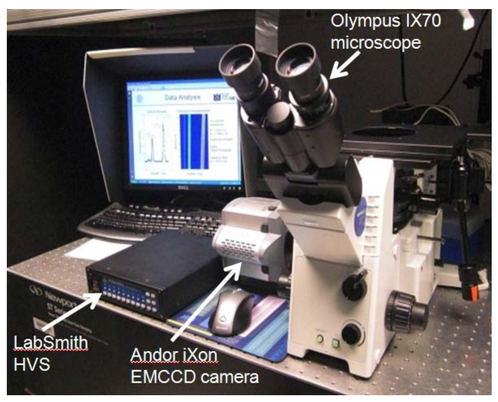


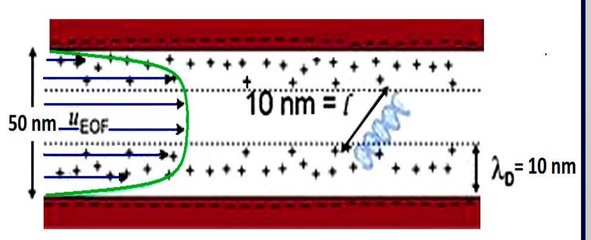
Lab-on-a-chip (height of the channel is about 100nm)

Background:

Nanofluidic separation

- Less ions in the channel (~ no Joule heating)
- Flow occurs at low voltages
- EDL thickness is large compared to the channel dimensions
- Surface and intermolecular interactions are very important (possible to separate DNA)

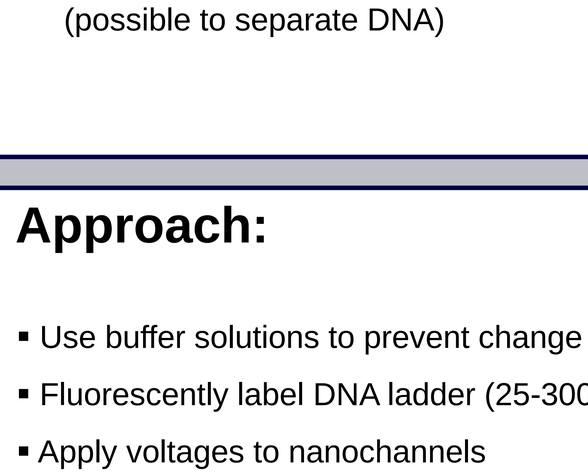




Electrophoresis of rod-like oligonucleotide in a nanochannel. The depth of the channel (~50nm), the length of the dsDNA *(l)*, and the Debye length (ìD) are important length scales.

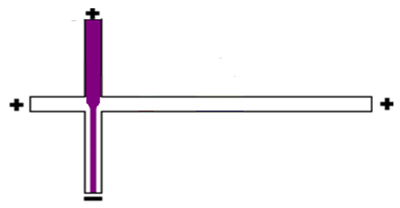
Approach:

- Use buffer solutions to prevent change in pH
- Fluorescently label DNA ladder (25-300bp)
- Apply voltages to nanochannels
- Drive and separate DNA in a solution
- Use fluorescence microscopy
- Observe electrophoretic movement
- Record electrophoretic movement
- Analyze data using Matlab



Electropherogram of DNA separation in a nanochannel
(Michael G. Kattah, Jonathan B. Steinman, and Paul J. Utz, *Anal. Chem.*, 2007, *79* (21), pp 8316–8322)

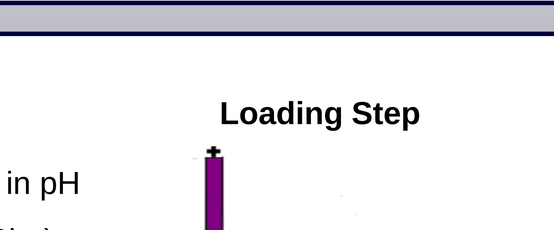
Loading Step



Voltages applied at N, E and W wells.

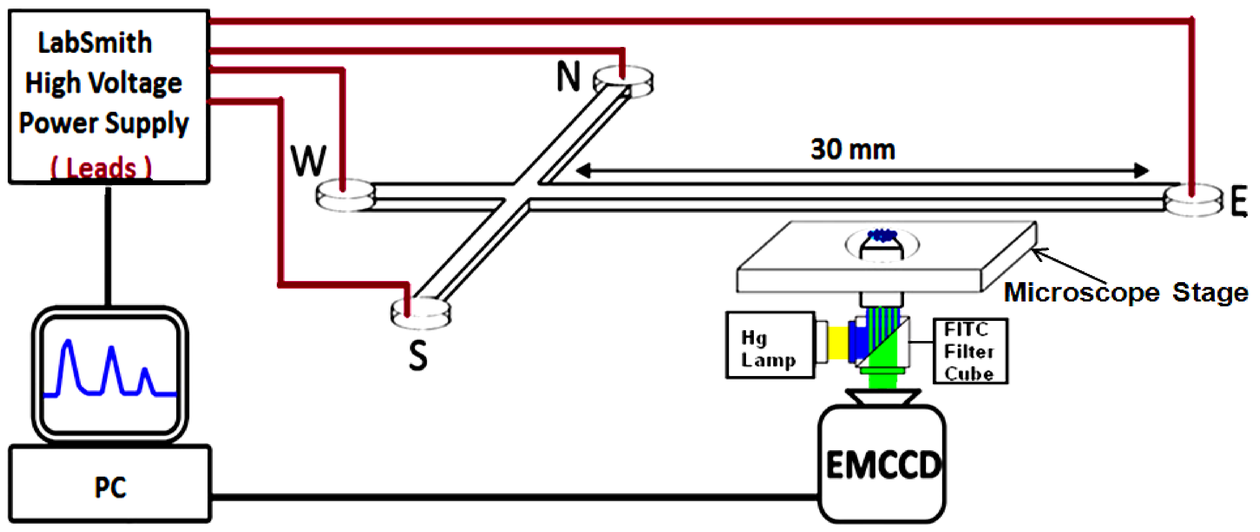
Injection Step

Voltages applied at N, S and W wells.



Electrophoretic movement of fluorescent "plug"

Experimental Setup:



Modified image from Jess M. Sustarich, Brian D. Storey, and Sumita Pennathur, **Phys. Fluids**, 2010, **22**/11, p.2003-2024

Equipment and Materials

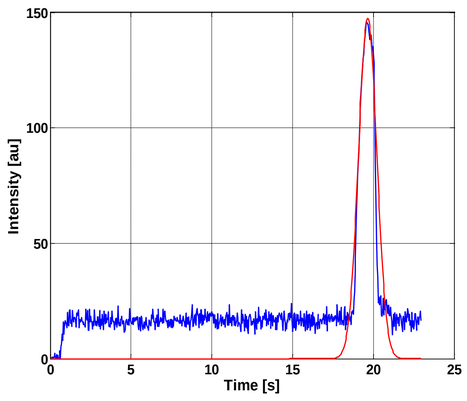
- Inverted microscope
- High Voltage Power Supply
- Mercury Bulb Illumination
- EMCCD Camera
- 60X 1.0NA Water Objective
- FITC Filter Cube
- Micro/nanochannels (cross-channels)
- Fluorescently labeled 25bp DNA ladder
- Tris/EDTA buffer solution

Nanofluidic chip mounted on to the microscope stage

Preliminary results:

Control Experiments

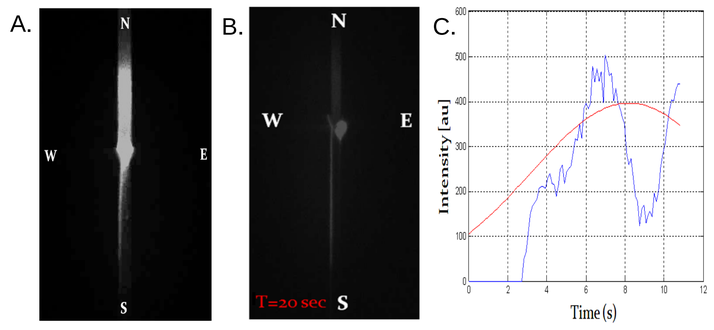
- Ran injections with Fluorescein
- Observed/recorded plug movement
- Analyzed recorded data



MatLab generated electropherogram of Fluorescein/Phosphate buffer sample. Tall and narrow peak (red fit) proves the sample to be well concentrated. Blue is background noise.

Experiments With DNA

- Ran injections with YoYo-1 labeled DNA
- Observed/recorded electrophoretic movement
- Analyzed recorded data



- Fluorescently labeled DNA adsorbs to channel walls (A)
- Accumulates at the East channel entrance (B)
- No defined Gaussian fit for 25bp DNA ladder injection 5mm down the East channel (C)

Future Work:

We plan to run our experiments using:

- Hydrophilic neutral silane coated channels
- 25bp and 10bp DNA ladders
- Optimized voltages and DNA concentration
- Ideal buffering conditions

Andersen et al, Journal of Colloid and Interface Science Volume 353, Issue 1, 1 January 2011, Pages 301-310

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References:

[1] D. Huber et al., Lab Chip, 2009, 9, 2933-2940.

[2] S. Pennathur et al., Anal. Chem. (2007) 79, 8316-8322.