Introduction to BioMEMS & Bionanotechnology
Lecture 3

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Key Topics

- Biochips/Biosensors and Device Fabrication
- Cells, DNA, Proteins
- Micro-fluidics
- Biochip Sensors & Detection Methods
- Micro-arrays
- Lab-on-a-chip Devices
Dielectrophoresis

Simplest approximation:

\[ F = 2\pi\varepsilon_0 \varepsilon_m r^3 \text{Re}\left[f_{CM}\right]\nabla|E_{RMS}|^2 \]

\[ f_{CM}(\varepsilon_p, \varepsilon_m) = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \]

\[ \varepsilon_p = \varepsilon(\omega) \]
Dielectrophoresis on Interdigitated Electrodes

Polystyrene beads: $\varepsilon_p < \varepsilon_m \rightarrow$ negative DEP

Cells: $\varepsilon_p < \varepsilon_m \rightarrow$ Negative DEP
Cells: $\varepsilon_p > \varepsilon_m \rightarrow$ Positive DEP

Interdigitated electrodes on a chip

A Dielectrophoretic Filter

Detection Chamber

Electrodes

Flow

Beads and bacteria

Schematic of the device cross-section
Forces on a particle in a micro-fluidic flow

1. DEP Force
2. Sedimentation Force
3. Hydrodynamic Drag Force:
4. Hydrodynamic lifting force

- Assume a parabolic laminar flow profile:
- Two orders of magnitude smaller than typical DEP lifting force
- Neglected here
X-component of DEP force at different heights

- Bead diameter: 0.7µm
- Bead conductivity: 2e-4 S/m
- Relative permittivity of bead: 2.6
- Bead density: 1.05 g/cm³
- Medium (DI water) conductivity: 2.5 S/m
- Relative permittivity of medium: 80
- Medium density: 1.0 g/cm³
- Voltage: 1Vrms
- Frequency: 580KHz
Y-component of DEP force at different heights
Trapping of beads (- DEP) and microorganisms (+ DEP)

Simulation
Experiments

2.38µm bead
5.44µm bead

0 0.2 0.4 0.6 0.8 1
Flow rate (µl/min)

0 50 100 150 200 250 300
Voltage^2 (V^2 p-p)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Flow rate (µl/min)

Vaccinia virus
Listeria innocua bacteria
B. Cereus spores
yeast cells

H. Li, Y. Zheng, D. Akin, R. Bashir, IEEE/ASME JMEMS, 2005
Dielectrophoretic Trapping of *Vaccinia* virus (positive DEP)

- Fluorescent imaging of nano-scale virus particles (*Vaccinia* virus and Human Corona Virus)
- Trapping of viruses in DEP filters
- Dual labeling of viruses with fluorescent dyes

Virus Size ~ 250x350nm
Picture taken at: 10Vpp, 1MHz, DI water ~1.5µS/cm, flow rate ~0.1μl/min

Release voltage vs. diameter for particle collecting the electrode edge, considering the Brownian motion

Equivalent external force due to Brownian motion is estimated to be $20kT/\theta d \sim 8.2 \times 10^{-15}$ N,

$k$ is Boltzmann constant, $T$ is the absolute temperature in Kelvin, and $\theta d$ is the trap width, assumed to be 10 $\mu$m

V_2^2 2\pi r^3 \text{Re}[f_{CM}] \nabla E^2 = 36\pi \eta kr \left( \frac{r}{h} - \frac{r^2}{h^2} \right) \approx 36\pi \eta \frac{kr^2}{h}$

$V = \sqrt{\frac{18\eta k}{r\nabla E^2 \text{Re}[f_{CM}]h}}$

Polarization factor=0.5,
flow rate 0.1 $\mu$m/min, in the channel with cross-section 350x11.6 $\mu$m$^2$,
interdigitated electrodes with 23 $\mu$m width and 17 $\mu$m spacing
Micro-fluidic Characterization

- Micro-Particle Imaging Velocimetry (µPIV)

Wereley, et al. Purdue

Gomez, et al. 2001
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Biochip Sensors

• Detect cells (mammalian, plant, etc.), microorganisms (bacteria, etc.), viruses, proteins, DNA, small molecules

• Use optical, electrical, mechanical approaches at the micro and nanoscale in biochip sensors
Sensing Methods in BioChips

Mechanical Detection

Surface Stress Change Detection

\[ \Delta z = 4 \left( \frac{l}{t} \right)^3 \frac{(1-v)}{E} (\Delta \sigma_1 - \Delta \sigma_2) \]

- \( \Delta z \) = deflection of the free end of the cantilever
- \( L \) = cantilever length
- \( t \) = cantilever thickness
- \( E \) = Young's modulus
- \( v \) = Poisson's ratio
- \( \Delta \sigma_1 \) = change in surface stress on top surface
- \( \Delta \sigma_2 \) = change in surface stress on bottom surface

Mass Change Detection

\[ f = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \]

\[ \Delta m = \frac{k}{4\pi^2} \left( \frac{1}{f_0^2} - \frac{1}{f_1^2} \right) \]

- \( k \) = spring constant
- \( m \) = mass of cantilever
- \( f_0 \) = unloaded resonant frequency
- \( f_1 \) = loaded resonant frequency

Electrical Detection

Conductometric Detection

Amperometer Detection

Potentiometric Detection

Optical Detection

DNA detection on chip surfaces

Protein detection on chip surfaces

Cell detection on chip surfaces

(a)  
(b)  
(c)
1. Microcantilever Stress Sensors

Mechanical Detection

Surface Stress Change Detection

\[ \Delta z = 4 \left( \frac{l}{t} \right)^2 \frac{1-\nu}{E} \left( \Delta \sigma_1 - \Delta \sigma_2 \right) \]

- \( \Delta z \) = deflection of the free end of the cantilever
- \( L \) = cantilever length
- \( t \) = cantilever thickness
- \( E \) = Young’s modulus
- \( \nu \) = poison’s ratio
- \( \Delta \sigma_1 \) change in surface stress on top surface
- \( \Delta \sigma_2 \) change in surface stress on bottom surface

0.2µm thick, 100µm long, silicon cantilevers
IBM Zurich Research: DNA Detection

Detection of PSA, Prostate Specific Antigen (cancer marker protein in blood)

- PSA ~ 30kDa ~ 30 x 1e3 x 1.66e-24gm
- In 1ng/ml ~ 2e10 molecules/ml
- Area of 20um x 60um, each protein 10nm x 10nm → ~1e8 proteins

Wu et al., Nature Biotechnology, 19, September 2001

Deflections have been measured with a resolution of 0.4x10^-12 m.*
Polymer/Silicon Cantilever Sensors

- Environmentally sensitive micro-patterned polymer structures on cantilevers
- Hydrogel patterned on cantilever and then exposed to varying pH

- $\Delta p$H = 1-10e-5
- pH = 6.5 $\rightarrow$ ~ 1.9e5 H$^+$ in 1000$\mu$m$^3$
- $\Delta p$H = 5e-4 $\rightarrow$ change of ~ 150 H$^+$

J. Zachary Hilt, Amit K. Gupta, Rashid Bashir, Nicholas A. Peppas  Biomedical Microdevices, September 2003, Volume 5, Issue 3, 177-184
2. Microcantilever Mass Sensors

Unloaded Resonant Frequency:

\[ f_0 = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \]

Spring constant for a rectangular shaped cantilever beam:

\[ k = \frac{Et^3w}{4l^3} \]

Loaded Resonant frequency:

\[ f_1 = \frac{1}{2\pi} \sqrt{\frac{k}{m + \delta m}} \]

\[ \Delta m = \frac{k}{4\pi^2} \left( \frac{1}{f_1^2} - \frac{1}{f_0^2} \right) \]

- \( k \) = spring constant
- \( m \) = mass of cantilever
- \( f_0 \) = unloaded resonant frequency
- \( f_1 \) = loaded resonant frequency
Detection of Bacterial Mass

\[ \omega_0^2 = \frac{k}{m} \]

Detection of Listeria Cell Mass

Non-specific binding of *Listeria innocua* bacterial cells to a cantilever beam

\[ m^* = \frac{x}{L} m \]


Frequency change after binding of 180 dry bacteria cells

Loaded Resonant Frequency (84.7 kHz)

Unloaded Resonant Frequency (85.6 kHz)
Minimum Detectable Mass

The frequency measurement is limited by thermo-mechanical noise on the cantilever beam.

**Minimum Detectable Frequency,**
\[ f_{\text{min}} = \frac{1}{A} \sqrt{\frac{f_0 k_B T B}{2\pi kQ}} \]

**Minimum Detectable Mass,**
\[ m_{\text{min}} = \frac{1}{A} \sqrt{\frac{4 k_B T B}{Q} m_{\text{eff}}^{5/4} k^{3/4}} \]

- \( k_B \) = Boltzmann constant
- \( T \) = Temperature in Kelvin
- \( B \) = Bandwidth measurement, (~ 1 kHz)
- \( Q \) can increase by 100X by driving the cantilevers

\[ \text{Width of cantilever beam} = 1 \, \mu\text{m} \]
\[ \text{Thickness of cantilever beam} = 10 \, \text{nm} \]

Roukes, et al.
Fabrication Process Flow

Materials Legend

- Silicon
- Silicon dioxide
- PECVD Silicon dioxide

Cross-sectional view

Top view

Etch Window

Bottom of channel
SEM Pictures of Cantilevers

Frequency Shift vs. No. of Particles

- 1 kHz frequency shift for 160 ag
- Sensitivity ~ 6.3 Hz/ag

- Average mass of Vaccinia Virus ~ 9.5fg
- Work on going to integrated concentration elements
- Integrated Abs on cantilevers

Specific Capture of Virus Particles

Virus capture experiment: $\omega_0$ decreases with Ab attachment and with antigen capture.

Virus capture experiment: $\omega_0$ increases with Ab attachment and decreases with antigen capture.

Power Spectral Density Plot at Various Stages of Biosensor Analysis

- Vaccinia virus
- Biotinylated antibody
- Streptavidin
- BSA and Biotinylated-BSA
- Chip

Resonant Frequency after Antibody Attachment, 2.556 MHz
Resonant Frequency after Virus Capture, 2.751 MHz
Unloaded Resonant Frequency, 2.427 MHz

Unloaded Resonant Frequency, 1.256 MHz
Resonant Frequency after Antibody Attachment, 1.889 MHz
Resonant Frequency after Virus Capture, 1.757 MHz
To probe the amount of thiolate binding to the Au contacts, we have measured the frequency spectra before and after the thiolate self-assembly. Figure 14 shows the measured shift in the resonant frequency for DNP-PEG4-C11thiol binding on 50- and 400-nm-diam Au contacts. The measured frequency shifts were 125 Hz and 1.10 kHz, corresponding to calculated masses of 6.3 and 213.1 ag, respectively.

FIG. 14. Experimentally measured frequency spectra before (solid line) and after (dashed line) the adsorption of the thiolate on (a) 50- and (b) 400-nm-diam Au contact. Rectangular beam dimensions were $l=10 \, \mu m$, $w=1 \, \mu m$, and $t=250 \, nm$. 
Electrical/Electrochemical Detection

1. amperometric biochips, which involves the electric current associated with the electrons involved in redox processes,

2. potentiometric biochips, which measure a change in potential at electrodes due to ions or chemical reactions at an electrode (such as an ion Sensitive FET), and

3. conductometric biochips, which measure conductance changes associated with changes in the overall ionic medium between the two electrodes.
1. Amperometric Detection

Glucose Oxidase

\[ \beta-D-Glucose + O_2 + H_2O \rightarrow D-gluconic\ acid + H_2O_2 \]

hydrogen peroxide is reduced at -600mV at Ag/AgCl anode reference electrode.

- Detection of Glucose, Lactate, Urea, etc.
- Enzyme entrapped in a gel
- Surface regeneration and sensor reusability

Ref: Perdomo, et al., 2000
Detection of DNA Hybridization

- Capture probes are attached to electrodes.
- Target DNA binds to complementary probes.
- DNA sequences, called signaling probes, with electronic labels attach to them (ferrocene-modified DNA oligonucleotides, E1/2 of 0.120 V vs. Ag/AgCl, act as signaling probes).
- Binding of the target sequence to both the capture probe and the signaling probe connects the electronic labels to the surface.
- The labels transfer electrons to the electrode surface, producing a characteristic signal.

Drummond, Hill, Barton, Nature Biotech, v21, n10, Oct 2003, p1192
http://www.motorola.com/lifesciences/esensor/tech_bioelectronics.html
2. Potentiometric Sensors

- ISFETs, ChemFETs, etc.
- Potential difference between the gate and the reference electrode in the solution
- Change in potential converted to a change in current by a FET or to a change in capacitance in low doped silicon
- Gate material is sensitive to specific targets
- pH, Ions, Charges
Nanoscale pH Sensors

- Label Free !!
- Detection of pH change
- Detection of protein binding

Integrated Silicon Nanowire Sensors

Objectives:
- Bio-sensors with electronic output
- Capability of dense arrays integrated with ULSI silicon
- Direct Label Free Detection of DNA and Proteins

• Plate Size ~ 20nm X 1μm X 3μm
• Wire Size ~ 20nm X 20nm X 3μm

Electrical response of the device upon exposure to oxygen (red dotted lines) and nitrogen (blue solid lines)

Field Effect Sensing of DNA

3. Conductometric Biochips

- Conductometric sensors measure the changes in the electrical impedance between two electrodes, where the changes can be at an interface or in the bulk region and can be used to indicate biomolecular reaction between DNA, Proteins, and antigen/antibody reaction, or excretion of cellular metabolic products.
Nanoparticle Mediated DNA Detection

- Au nanoparticles assemble between two electrodes if DNA is hybridized
- Silver staining of the Au nanoparticles
- Conductance changes between micro-scale electrodes indicate DNA hybridization
- Sensitivity of $5 \times 10^{-13}$ M shown