Introduction to BioMEMS & Bionanotechnology
Lecture 4

R. Bashir
Laboratory of Integrated Biomedical Micro/Nanotechnology and Applications (LIBNA), Discovery Park
School of Electrical and Computer Engineering, Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana
http://engineering.purdue.edu/LIBNA
Key Topics

- Biochips/Biosensors and Device Fabrication
- Cells, DNA, Proteins
- Micro-fluidics
- Biochip Sensors & Detection Methods
- Micro-arrays
- Lab-on-a-chip Devices
Micro-fluidic Devices for Conductance Detection of Bacterial Metabolism

- Detection of Cell Growth by measuring their metabolic activity in micro-fluidic devices

![Image of micro-fluidic device with cavities, Pt electrodes, and input port]

Dielectric capacitance $C_{di}$

Electrolyte resistance $R_s$

Electrode-electrolyte interfaces $Z_w$

Electrode-Electrolyte Interface Model:

$$Z_w = \frac{1}{(j\omega)^n B}$$

- Commercially available Dynal magnetic beads with Listeria Antibodies
- *Listeria Monocytogenes* concentration of about $5 \times 10^6$ cfu/ml
- Beads concentration of about $3 \times 10^7$ beads/ml
- Capture done for 30 min
- About 80 beads captured inside the measurement chamber in the chip
- Total admittance (1/impedance) at 1kHz vs. time

4. Cell-Based Sensors/Biochips

- The transductions of the cell sensor signals maybe achieved by:
  - the measurement of transmembrane and cellular potentials,
  - impedance changes,
  - metabolic activity,
  - analyte inducible emission of genetically engineered reporter signals, and
  - optically by means of fluorescence or luminescence.

5. Micro/Nano-scale Coulter Counter

Trans chamber

Cis chamber

I

I

I

t

t

t
Micro-pore for cellular studies

- Micro-devices for single cell characterization – utilize the charge properties
- Micro-fabricate a pore where single entity can pass

Optical Picture of a Pore in a micro-fabricated filter

Cross section of micro-fabricated pore
Microscale Coulter Counter

I-T Diagram for Live Listeria, 1e8/ml, V = 40 V, 05112010

Velocity (cm/s) vs. Electrical Field (V/cm)
Live *Listeria innocua* with pore

- Mobility = -5e-7 cm²/V·s
- v = -5e-7E - 0.007; R² = 0.814

Electrical Field (V/cm)

Nanoscale DNA Coulter Counter

- α-hemolysin channel, a biological protein based-pore, was utilized.
- Pore size is 2.6 nm.
- Both RNA and DNA molecules were observed traversing the nanochannel.

Solid-state based nanopore. Made in silicon nitride membrane.
- Pore size: 3 nm and 10 nm.
- The relation among DNA lengths and translocation times and applied biases were determined.


TEM of Li’s nanopore. b. DNA measurement setup in Li’s work. From Li et. al. Nature Materials, 2003
DNA Translocation

Current fluctuations when DNA was passing through the pore

Histograms of relation among DNA lengths, translocation times and applied biases.

Li et. al. 2003
Silicon Based Nanopore

Start with a (100) 4 inch SOI wafer. Thickness: 525 μm. SOI: 250 nm, Buried oxide layer: 400 nm.

1. Grow thermal oxide on wafer surface and open etch window to etch through the handle layer. Etch stops on buried oxide layer.

2. Remove buried oxide layer and regrow 100 nm thermal oxide.

3. On SOI layer, open another etch window to etch through the SOI layer. Etch stops on buried oxide layer.

4. Remove buried oxide layer and regrow 100 nm thermal oxide.

5. Shrink the pore to 3–5 nm by TEM.
Pore shrinking and shape changing (After Thermal Oxidation, Oxide Thickness = 50 nm)

Slopes in the plot are the shrinkage rates. Different initial pore size had different shrinkage rates.


‘Nanopore Channel’ Sensors for Characterization of Single Molecule dsDNA

- 200bp DNA was used. Concentration of 0.3 mg/ml.
- Buffer solution : 0.1 M KCl, 2 mM Tris (pH 8.5)
- Ag/AgCl electrodes were utilized.
- Bias : 200 mV.
- Time sampling interval : 100 us

---

Explanation of Current Pulses

DNA induces extra potassium ions when passing through the nano-channel. The interface current of K ions thus increases. At the same time bulk currents decrease because of DNA blocking.
Integrated Optical Detection

1. **Fluorescence**: Markers that emit light at specific wavelengths and enhancement, or reduction (as in Fluorescence Resonance Energy Transfer) in optical signal can indicate a binding reaction.

2. **Chemiluminescence**: Generation of light by the release of energy as a result of a chemical reaction.
   - Light emission from a living organism is termed bioluminescence (sometimes called biological fluorescence),
   - light emission which take place by passage of electrical current is designated electrochemiluminescence.
DNA Hybridization in Microarrays

- Basis for detection of unknown nucleotides
- Example: Bio-chips for identification of DNA
  - Hybridization of an unknown, flourescently tagged strand with a many known strands - reaction will determine the sequence of the unknown (or vice versa)
  - Strands can be lithographically (Affymetrix) or electronically (nanogen) defined at a specific location
Electronic Placement of DNA Probes

(a) Metal Contact
Capture probes
Attachment layer

(b) Metal Contact
Capture probes
Attachment layer

(c) Metal Contact
Attachment layer
Capture probes
Target probe (w. fluo. Label)

(d) Metal Contact
Attachment layer
Capture probes

(e) Capture probes
DNA Biochips (Nanogen)

Technology Features:

- Biochips for DNA detection, antigen-antibody, enzyme-substrate, cell-receptor and cell separation techniques.
- Takes advantage of charges on biological molecules.
- Small sequences of DNA capture probes to be electronically placed at, or "addressed" to, specific sites on the microchip.
Hybridization.

- A test sample can be analyzed for the presence of target DNA molecules by determining which of the DNA capture probes on the array bind, or hybridize, with complementary DNA in the test sample.

- Fluorescence output

www.nanogen.com
Light Directed DNA Synthesis on a chip (Affymetrix)

Light Directed DNA Synthesis on a chip (Affymetrix)

Table 1. Combinatorial synthesis of polynucleotide probe arrays

<table>
<thead>
<tr>
<th>Probe Length</th>
<th>Chemical Steps</th>
<th>Number of Possible Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>16</td>
<td>256</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>65,536</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
<td>16,777,216</td>
</tr>
<tr>
<td>16</td>
<td>64</td>
<td>~4.3 × 10^9</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>~1.1 × 10^12</td>
</tr>
</tbody>
</table>

Light Directed DNA Synthesis on a chip (Affymetrix)

- Fluorescence detection
- Ultimately will limit size of pixel in array

Applications:
- Polynucleotide array
- HIV resequencing
- mRNA expression monitoring
Protein Arrays

- Protein-Protein Interactions
- Protein small molecule interactions
- Derivatized substrates – glass, plastics
- High Throughput screening of chemical compounds

Note: Sensor Arrays

- Any of the individual sensors described earlier can be used in an array format to make micro/nano sensor arrays.
- The sensors in the array need addressing.
- Each sensor can be functionalized with different bio-receptor molecule to detect different entities.
- Examples, cantilever array, electrochemical detection in electrode arrays, cellular arrays for chemical detection, etc.
Lab-on-a-Chip/Integrated Devices

- Single chip device for DNA electrophoresis
- Sample loading and metering
- PCR on a chip (faster temperature cycling due to reduced thermal mass)
- Gel electrophoresis on chip

CD Format Biochips

- Micro-fluidic devices on a CD type platform using centrifugal and capillary forces for liquid transport
- Cheap plastic CDs
- Optical detection systems

Cellular Analysis on Chip

- Plastic biochips using hydrodynamic transport of cells
- Electric field mediated lysing
- Fluorescence detection (off-chip detectors)
- Analysis time of about 10 cells/minute

Polymer µSensor and Actuator

Process flow for the preparation of a hydrogel valve.

Hydrogel valve designs (2D and 3D)

A biomimetic valve based on bistrip hydrogel.

DNA Capillary Electrophoresis

Design of the 96-channel CAE microplate and radial scanner. Mask pattern used to form the 96 straight channel radial microplate on a 150-mm diameter wafer.
Integrated Systems for Study of Microorganisms and Cells

Fluidic Ports
- On-chip Dielectrophoresis
- Ab-based Capture
- Micro-scale Impedance Spectroscopy
- Nano-probe Array
- Cantilevers, NanoFETs, Nano-pores

Conc. Sorting
- Selective Capture
- Viability Detection
- Cell Lysing
- Mech/Elect. Detection DNA, protein

“Lab on a Chip” for Enabled by BioMEMS and Bionanotechnology
Micro-fluidic Polymer Devices for Culture Bacteria and Spores

- Growth of bacteria inside a micro-fluidic polymer chip
- Rapid detection and reduced time to result

Woo-Jin Chang, Demir Akin, Miroslav Sedlek, Michael Ladisch, Rashid Bashir, “Hybrid Poly(dimethylsiloxane) (PDMS)/Silicon Biochips For Bacterial Culture Applications”, Biomedical Microdevices 5:4, 281-290, 2003,
Future Directions

• Integrated device for analysis of single cells – applications and fundamental science
• Building cell by cell/tissue engineering using micro and nano fabrication techniques
• Integrated diagnostics and therapeutics (drug delivery)
• Tools for genetic manipulation of microorganisms and viruses – synthetic biology
Acknowledgements

Research Scientists/Post-docs:
• Dr. Demir Akin
• Dr. Dallas Morisette
• Dr. Rafael Gomez

Graduate Students:
• Sangwoo Lee
• Haibo Li
• Amit Gupta
• Hung Chang
• Yi-Shao Liu
• Samir Iqbal
• Oguz Elibol
• Angelica Davilia
• Kidong Park

Industries:
• BioVitesse, Inc. Co-Founder

Funding Agencies
• US Department of Agriculture (Food Safety Engineering Center)
• NASA Institute on Nano-electronics and Computing
• NSF, NSF Career Award
• National Institute of Health
• DARPA Nanotechnology Research
• Discovery Park at Purdue University

Faculty Collaborators
– Prof. D. Bergstrom (Med Chem)
– Prof. A. Bhunia (Food Science)
– Prof. M. Ladisch (Ag& Bio Engr)

Special Thanks
– Prof. S. Broyles (BioChem)
– Profs. D. Datta, D. Janes (ECE, NASA INAC), J. Cooper (BNC)