ME 141B: The MEMS Class
Introduction to MEMS and MEMS Design

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BioMEMS Case Study: Microdevices for PCR

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Outline

- What is hard about BioMEMS
- BioMEMS success stories
- DNA amplification and PCR
- Two designs
  - A static PCR themocycler
  - A flow-thru design
  - Comparison
- Design evolution of static approach
- Conclusions
Applications of microsystems to bioscience

- Neural probes
- Capillary electrophoresis
- Drug delivery
- Cellular engineering
- Tissue engineering
What is hard about BioMEMS

• The biological system is poorly defined
  ➢ We fundamentally understand physics
  ➢ We DON’T fundamentally understand biology
  ➢ Thus, only part of system can be truly predicatively designed

• “Intrinsic” biological limitations can dictate system performance
  ➢ Protein-protein interaction kinetics
  ➢ Polymerase error rates

• The materials (and thus processes) are often NOT silicon (and thus harder)
  ➢ We must move away from the most established fabrication technologies
BioMEMS success stories

- Depending on definition, there are very few
- Commercial successes
  - Blood pressure sensors
    - Low cost “widget” allows devices to be disposable
  - Affymetrix DNA microarrays
    - Vastly decreases time and cost for analyzing nucleic acids
    - But these are not really bioMEMS
BioMEMS success stories

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In the commercial sector, there has been lots of hype
- Success if uncertain
- Caliper/Aclara
  - Lab-on-a-chip
- I-stat
  - Portable blood analyzer
  - Uses ion-selective electrodes, conductivity, etc. to measure salts, glucose, etc.
  - Introduced ~1997
  - Purchases by Abbott
Away from commercial sector and into basic science, more successes arise.

Success can be defined as having impact on the target audience.

Ken Wise’s neural probes:
- CMOS + bulk micromachining
- Puts op-amp right near neural recording sites \( \rightarrow \) amplifies and buffers weak (~uV) signals
- These are being used by neuroscientists in actual experiments.
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DNA

- DNA → deoxyribonucleic acid
- DNA contains the genetic information (genotype) that determines phenotype (i.e., you)
- It consists of two antiparallel helical strand
  - Read 5’ to 3’
  - A sugar-phosphate backbone
  - Specific bases (A,C,T,G) that contain genetic code
  - This code determined the sequence of amino acids in proteins
DNA amplification

• The bases pair specifically
  ➢ A with T
  ➢ C with G

• Specific enzymes (DNA polymerases) can add complementary nucleotides to an existing template + primer
  ➢ This is done in vivo in DNA replication

• Was capitalized in vitro in polymerase chain reaction
  ➢ Invented in 1985, Nobel Prize in 1993
PCR Intro

double-stranded DNA → T≈95°C denaturation → T≈55°C annealing of primers → T≈72°C extension by polymerase → etc.
Polymerase Chain Reactions (PCR)

- Specifically amplify DNA starting from 1 double-stranded copy
Polymerase Chain Reactions (PCR)

Cycle 3
- Cool to 60°C to anneal primers
- Primers extended by Taq polymerase at 72°C
- Heat to 95°C to melt strands
- Cool to 60°C to anneal primers

Cycle 4
- Primers extended by Taq polymerase at 72°C

And so on
PCR

- Key technological improvement was use of polymerase that could withstand high temperatures
  - Isolated from Thermus aquaticus (Taq)
  - Don’t have to add new polymerase at each step
- The device is a simple thermocycler
- Allows amplification and detection of small quantities of DNA
PCR cycles

- *Taq* extension rate ~60nt/sec

- PCR products are typically a few hundred bases
  - Need ~5 sec for extension
  - Plus time for diffusion

- Typical protocols
  - ~25-35 cycles at 1-3 min/cycle
  - ~30 cycles → 75 minutes
Cycle time is dominated by ramp times due to thermal inertia
   - Usually much longer than kinetically needed

Transient and steady-state temperature uniformity limits cycle time & specificity

<table>
<thead>
<tr>
<th>Property</th>
<th>Spec</th>
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<tbody>
<tr>
<td>Temp range</td>
<td>5-105 °C</td>
</tr>
<tr>
<td>Set-point accuracy</td>
<td>±0.25 °C</td>
</tr>
<tr>
<td>Temperature uniformity</td>
<td>±0.4 °C within 30 sec</td>
</tr>
<tr>
<td>Heating/cooling rate</td>
<td>~ 3 °C/sec</td>
</tr>
<tr>
<td>Sample volume</td>
<td>50 µl</td>
</tr>
<tr>
<td>Number of samples</td>
<td>96</td>
</tr>
<tr>
<td>Power required</td>
<td>850 W</td>
</tr>
</tbody>
</table>

BioRad DNA Engine
Outline

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Two approaches to miniaturization

• Decrease size of chamber
  ➢ Vary temperature in time

• Use a flow-thru approach
  ➢ Vary temperature in space (and therefore time)

• In both cases, the device is a thermal MEMS device and the key is to reduce thermal response time
Batch PCR

- First reported by Northrup et al. in 1993
  - Essentially a miniaturized thermal cycler
Batch PCR

- Daniel et al. improved thermal isolation

Silicon nitride web (1 μm)  Reaction chamber

Platinum resistors on 3 μm silicon nitride membrane
Continuous flow PCR

- Developed by Kopp et al. in 1998
Microfluidic PCR
What is ITP?
Microfluidic PCR

Denaturant → DNA template

ITP-focused DNA template

Bulk flow

LE / PCR mix

Electromigration

GND

+HV

denaturation

annealing and extension

GND

+HV

5/11/09
Batch PCR

- Daniel reactor
  - SiN mesh structure, undercut with KOH
    - Made hydrophobic to keep water in chamber during loading
  - Platinum heater resistors heat up beams
  - Two temperature sensing resistors
    - One on beams for feedback control
    - One of membrane to sense “liquid” temp
  - Use oil drop on top of liquid to prevent evaporation
Thermal modeling – batch system

- Three steps
- Model chamber
- Model beams
- Combine the two, with heating at beams
Chamber model
Assume rectangular cross-section
Assume dominant heat loss through beams
\[ \Rightarrow \text{2D heat flow problem} \]
- Neglect conduction along top and bottom
- Temperature does not vary in z
Interested in dominant time constant
Thermal modeling – batch system

- Obtain lumped heat capacity by weighing over model volume
- Extract thermal resistance from time constant
- Thermal resistance same as zeroth-order model suggests

\[
C_f = \rho_m \tilde{C}_m \int_{-L/2}^{L/2} \int_{-L/2}^{L/2} \int_0^H \cos \left( \frac{\pi x}{L} \right) \cos \left( \frac{\pi y}{L} \right) dx \, dy \, dz
\]

\[
C_f = \rho_m \tilde{C}_m \frac{2L}{\pi} \frac{2L}{\pi} H
\]

\[
C_f = \frac{4 \rho_m \tilde{C}_m L^2 H}{\pi^2}
\]

\[
\tau_f = R_f C_f \Rightarrow R_f = \frac{1}{8\kappa H}
\]

- For L=2 mm, t=1.4 s
- H = 400 um, volume = 2uL
Lumped elements for beams
- Include beam capacitance

Two circuits to model beams
- Capacitor in center
- Capacitor at edge

Both circuits contain same energy in capacitor at steady state
- Capacitor at edge is simpler

\[ R_b = \frac{1}{4} \frac{1}{\kappa} \frac{\text{length}}{\text{area}} \]
\[ C_b = \rho m \tilde{C} m \text{volume} \]
Thermal modeling – batch system

- Lumped circuit model of reactor
- First-order lag between wall and fluid temperature

\[ T_f = \frac{1}{1 + \tau_f s} T_w \]

\[ \tau_f = \frac{L^2}{2\pi^2 D} \]

- Making L smaller reduces lag
• Simulink proportional control circuit
• Saturation needed for maximum +/- voltage swing
  ➢ Set to 0 to 15 V
Simulation response

- Wall temperature can be controlled very quickly
- Wall to fluid heat transfer limits performance
- Sensing fluid temperature marginally reduces response times
  - But creates high-temperature regions at chamber wall
Continuous Flow device

• Systems partitioning: put heaters off-chip
• Etch channels in glass, bond glass cover
Thermal model of continuous flow device

- Wall-to-fluid time constant is same as in batch device
  - $L_1 = 40 \, \mu m$, $L_2 = 90 \, \mu m$
  - $D = 1.4 \times 10^{-7} \, m^2/s$
  - $\tau_f = 1.2 \, ms$
  - 1000x faster than batch device!

- Entrance length for thermal equilibration
  \[ L_e \approx 3v_f \tau_f \]

- Average flow velocity
  \[ v_f = \frac{Q_f}{area} = 20 \, mm/s \]

- Thermal Pe number is $\sim 10^{-3}$

This is much smaller than zone lengths

\[ L_e \approx 60 \, \mu m \]
Continuous-flow device

- What about Taylor dispersion
- Pressure-driven may cause multiple samples to coalesce
- Hydrodynamic radius of 1 kb DNA ~ 50nm
- Dispersivity is dominated by convection
  - Samples will spread out a lot, limited usefulness for multiple samples

\[
D = \frac{k_BT}{6\pi\eta R}
\]

\[
Pe = \frac{LU}{D} = \frac{(40 \mu m)(0.02 m/s)}{4.4 \times 10^{-8} cm^2/s} \sim 2 \times 10^5
\]

\[
K = D \left(1 + \frac{Pe^2}{210}\right) f \left(\frac{L_1}{L_2}\right)
\]

\[
\frac{L_1}{L_2} \sim 0.4 \rightarrow f \left(\frac{L_1}{L_2}\right) = 4
\]

\[
K \sim 8 \times 10^8 \cdot D \sim 35 cm^2/s
\]
Thermal Lessons

• Wall temperature in most microsystems can be quickly controlled
• Limiting step is wall-to-fluid heat transfer
• Solution is to minimize fluid characteristic length for heat diffusion
Microfluidic PCR

- Temperature is held constant in space and time
- Focus particles using ITP
- Drive DNA sample with ITP through denaturant concentration zones
- High/Low concentrations since denaturant in neutral
Microfluidic PCR

[Diagram showing the process of PCR with labels for Denaturant, DNA template, LE / PCR mix, TE, ITP-focused DNA template, Electromigration, GND, denaturation, and annealing and extension.]
Microfluidic PCR

- Can use thermostable polymerase
- Reduces power consumption

Fluorescent Intensity (AU)

40 denaturant cycles at 55°C

194 bp DNA ccPCR product
Detecting PCR products

• Speeding up amplification is only half the battle
• DNA is not normally visible
• In conventional PCR, detect products by separating stained DNA using electrophoresis in gel sieving matrix
  ➢ This can take 0.5-2 hours
Detecting PCR Products

- Newer techniques allow real-time detection
  - “real-time PCR”
- Integrate illumination/detection optics, thermal cycler, and chemistry
## Comparison of thermo

<table>
<thead>
<tr>
<th>Continuous flow</th>
<th>Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faster thermal response</td>
<td>Slower</td>
</tr>
<tr>
<td>No temp overshoot</td>
<td>Depends on control system</td>
</tr>
<tr>
<td>Static protocol</td>
<td>Can change protocol easily</td>
</tr>
<tr>
<td>Taylor dispersion effects, and sample carryover</td>
<td>Sample carryover only</td>
</tr>
<tr>
<td>Optical detection more complicated</td>
<td>Simpler optical detection</td>
</tr>
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</table>
Materials issues

- Reactor surface must be compatible with PCR reagents
  - DNA, nucleoside triphosphates, polymerase, buffers
- Decreasing length scale and increasing SA/V hurts here!
  - More molecules start to interact with surface
- Bare Si or SiN inhibits PCR
  - Probably due to denaturing of polymerase at surface
  - Silanizing or depositing/growing SiO$_2$ helps
  - Add carrier protein (e.g., BSA) to “block” surface
- Kopp uses glass, silanization, surfactant, and buffer!
- Northrup used deposited SiO$_2$ plus BSA
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Evolution of chamber device

- Initial device introduces in 1993

- 1995-1996
  - Two heater chambers
  - Improved surface coating
  - Fan for cooling
  - Camber volume 20uL
  - 20 sec cycle time
  - Real time PCR coupled to electrophoresis
  - Cepheid formed

- 1998
  - Same two-heater chambers
  - Portable application
• 2001 to present
  - Abandoned silicon → moved to plastic and NOT microfabricated
  - Tubes are disposable thin-wall 50um plastic that expands upon introduction
  - Tube is flat to decrease thermal response
  - ~30 sec cycle time
  - Ceramic chamber with thin film heater
  - Thermistor temp sensor
Conclusions

- BioMEMS commercial successes are still not here
- Designing the engineered part is often routine
- Interfacing with biology is where it gets hard
- Sometimes the right solution is to NOT microfab