BioMEMS Case Study: Microdevices for PCR

Joel Voldman*
Massachusetts Institute of Technology
*with thanks to SDS
Outline

> What is hard about BioMEMS

> BioMEMS success stories

> DNA amplification and PCR

> Two designs
  • A static PCR thermocycler
  • A flow-thru design
  • Comparison

> Design evolution of static approach

> Conclusions
BioMEMS

> Applications of microsystems to bioscience
  - Neural probes
  - Capillary electrophoresis
  - Drug delivery
  - Cellular engineering
  - Tissue engineering

Courtesy of Kensall D. Wise.
Used with permission.

Courtesy of Richard A. Mathies.
Used with permission.

Image removed due to copyright restrictions.
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 predictively 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 the 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

> Depending on the 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

Courtesy of Affymetrix, Inc. Used with permission.
BioMEMS success stories

> In the commercial sector, there has been lots of hype
  • Success is 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
  • Purchased by Abbott
BioMEMS success stories

> 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 → amplifies and buffers weak (~μV) signals
   - These are being used by neuroscientists in actual experiments

Univ. Michigan

Courtesy of Kensall D. Wise. Used with permission.
Outline

> What is hard about BioMEMS

> BioMEMS success stories

> DNA amplification and PCR

> Two designs
  • A static PCR thermocycler
  • A flow-thru design
  • Comparison

> Design evolution of static approach

> Conclusions
DNA (deoxyribonucleic acid)

> DNA contains the genetic information (genotype) that determines phenotype (i.e., you)

> It consists of a two antiparallel helical strands
  - Read 5’ to 3’
  - A sugar-phosphate backbone
  - Specific bases (A, C, G, T) that contain genetic code

> This code determines the sequence of amino acids in proteins

Lodish, MCB

Image by MIT OpenCourseWare.
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 (PCR)
  • Invented in 1985, Nobel Prize in 1993

Image by MIT OpenCourseWare.

Albers, MBC
Polymerase chain reaction (PCR)

> Specifically amplify DNA starting from 1 double-stranded copy

Image by MIT OpenCourseWare.

Lodish, MCB
Polymerase chain reaction (PCR)

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

Primers extended by Taq polymerase at 72°C

And so on

Image by MIT OpenCourseWare.

Lodish, MCB
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 ~60 nt/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

Image by MIT OpenCourseWare.
PCR

> 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>
</tr>
</thead>
<tbody>
<tr>
<td>Temp range</td>
<td>5-105 ºC</td>
</tr>
<tr>
<td>Set-point accuracy</td>
<td>±0.25 ºC</td>
</tr>
<tr>
<td>Temperature uniformity</td>
<td>±0.4 ºC within 30 sec</td>
</tr>
<tr>
<td>Heating/cooling rate</td>
<td>~ 3 ºC/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

> What is hard about BioMEMS

> BioMEMS success stories

> DNA amplification and PCR

> Two designs
  - A static PCR thermocycler
  - A flow-thru design
  - Comparison

> Design evolution of static approach

> Conclusions
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 times
Batch PCR

> First reported by Northrup et al. in 1993

• Essentially a miniaturized thermal cycler


Northrup et al., Transducers ’93, p924
Batch PCR

> Daniel et al. improved thermal isolation

Silicon nitride web (1 μm)  Reaction chamber

Platinum resistors on 3 μm silicon nitride membrane


Cite as: Joel Voldman, course materials for 6.777J / 2.372J Design and Fabrication of Microelectromechanical Devices, Spring 2007. MIT OpenCourseWare (http://ocw.mit.edu/), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].

Continuous-flow PCR

> Developed by Kopp et al. in 1998

Two-layer glass with etched flow channels

Output capillary

Input capillaries

Annealing zone

Extension zone

Denaturing zone


Image by MIT OpenCourseWare.
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 on 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
Thermal modeling – batch system

> Chamber model

> Assume rectangular cross-section

> Assume dominant heat loss through beams

  ➔ 2-D heat flow problem

  • Neglect conduction along top and bottom

  • Temperature does not vary in \( z \)

> Interested in dominant time constant

\[
T(x, y, t) = \sum_n \sum_m A_{n,m} \cos \left( \frac{n\pi x}{L} \right) \cos \left( \frac{m\pi y}{L} \right) e^{-\alpha_{n,m} t}
\]

\[
T_1(x, y, t) = A_1 \cos \left( \frac{\pi x}{L} \right) \cos \left( \frac{\pi y}{L} \right) e^{-\alpha_1 t}
\]

\[
\tau_f = \frac{1}{\alpha_1} = \frac{L^2}{2\pi^2 D} = \frac{L^2 \bar{C}_m \rho_m}{2\pi^2 \kappa}
\]
Thermal modeling – batch system

> Obtain lumped heat capacity by weighing over mode volume

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

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

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

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

> Extract thermal resistance from time constant

> Thermal resistance same as zeroth-order model suggests

\[ R_f = \frac{1}{k} \frac{\text{length} \cdot \frac{1}{4}}{\text{area} \cdot \frac{1}{4}} = \frac{L/2}{\kappa L H} = \frac{1}{8kH} \]

> For \( L = 2 \text{ mm}, \ \tau = 1.4 \text{ s} \)

> \( H = 400 \mu\text{m}, \ \text{volume} = 2 \mu\text{l} \)
Thermal modeling – batch system

> 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

\[
R_b = \frac{1}{4} \frac{1}{\kappa} \frac{length}{area}
\]

\[
C_b = \rho_m \bar{C}_m \frac{volume}{\text{beam}}
\]

Image by MIT OpenCourseWare.
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

Image by MIT OpenCourseWare.
Thermal modeling – batch system

> Simulink proportional control circuit

> Saturation needed for maximum +/- voltage swing

- Set to 0 to 15 V

Image by MIT OpenCourseWare.

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-temp regions at chamber wall

Continuous-flow device

- System partitioning: put heaters off-chip
- Etch channels in glass, bond glass cover

Two-layer glass with etched flow channels

Image by MIT OpenCourseWare.
Thermal model of continuous-flow device

Wall-to-fluid time constant is same as in batch device:

\[ \tau_f = 40 \mu m, L_2 = 90 \mu m \]

\[ D = 1.4 \times 10^{-7} m^2/s \]

\[ \tau_f = 1.2 \text{ ms} \]

1000× faster than batch device!

\[ \tau_f = \frac{L_1 L_2}{2\pi^2 D} \]

\[ L_e \approx 60 \mu m \]

This is much smaller than zone lengths

Entrance length for thermal equilibration:

\[ L_e \approx 3v_f \tau_f \]

Average flow velocity:

\[ v_f = \frac{Q_f}{area} = 20 \text{ mm/s} \]

Thermal Pe number is \( \sim 10^{-3} \)
Continuous-flow device

> What about Taylor dispersion?

> Pressure-driven may cause multiple samples to coalesce

> Hydrodynamic radius of 1 kb DNA \( \sim 50 \text{ nm} \)

> Dispersivity is dominated by convection
  
  • Samples will spread out a lot, limiting usefulness for multiple samples

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

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

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

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

\[
K \sim 8 \times 10^8 \quad D \sim 35 \text{ cm}^2/s
\]
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
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 hrs
Detecting PCR products

> Newer techniques allow real-time detection
  • “Real-time PCR”

> Integrate illumination/detection optics, thermal cycler, and chemistry

[Diagram of PCR process]

ABI Taqman assay

Image by MIT OpenCourseWare.
Detecting PCR products

- Real-time thermocycler
- 1.5 mm-wide capillaries
- $\tau \sim 2-3$ sec up and down

Images removed due to copyright restrictions.
Schematic of the thermal chamber for the LightCycler(R) 2.0.
Comparison of two microfluidic PCR approaches

<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>
</tbody>
</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 SiO₂ helps
  - Add carrier protein (e.g., BSA) to “block” surface

- Kopp uses glass, silanization, surfactant, and buffer!

- Northrup use deposited SiO₂ plus BSA
Outline

> What is hard about BioMEMS

> BioMEMS success stories

> DNA amplification and PCR

> Two designs
  • A static PCR thermocycler
  • A flow-thru design
  • Comparison

> Design evolution of static approach

> Conclusions
Evolution of chamber device

> Initial device introduced in 1993

> 1995-6

- Two-heater chambers
- Improved surface coating: silanize+BSA+polypro insert
- Fan for cooling
- Chamber volume 20 μl
- 30 sec cycle time
- ~10 °C/s up, 2.5 °C/s down
- “Real time”-PCR via coupled electrophoresis
- Cepheid formed

Evolution of chamber device

> 1998

> Same two-heater chambers

> Portable application

> Up to 30 °C/s up, 4 °C/s down

> 17 sec minimum cycle time

> Usually use 35+ sec
Evolution of chamber device

> 2001 to present

> Abandoned Silicon ➔ Move to plastic and NOT microfabricated

> Tubes are disposable thin-wall (50 μm) 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

Image by MIT OpenCourseWare.
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