BioMEMS Case Study: Microdevices for PCR

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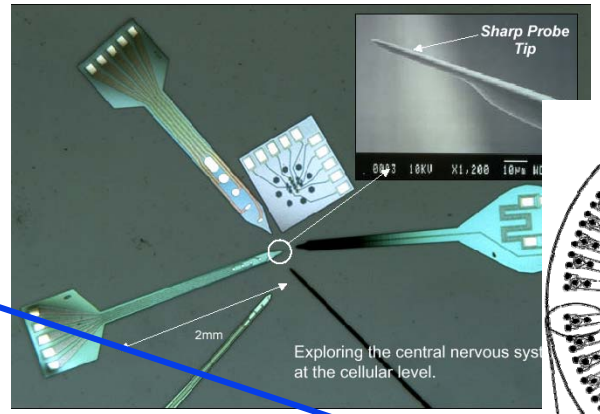
***with thanks to SDS**

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**



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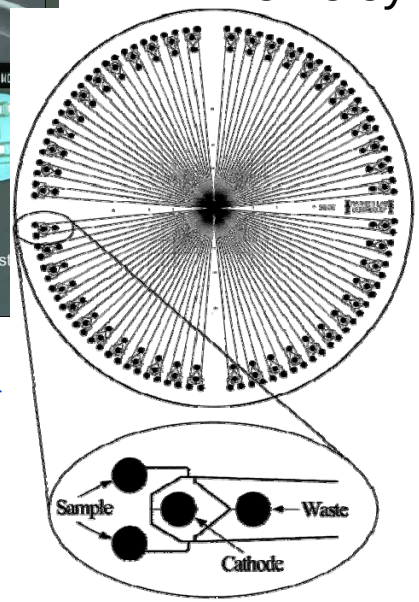
Berkeley



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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

Image removed due to copyright restrictions.
PCB-mountable pressure sensor for medical applications.

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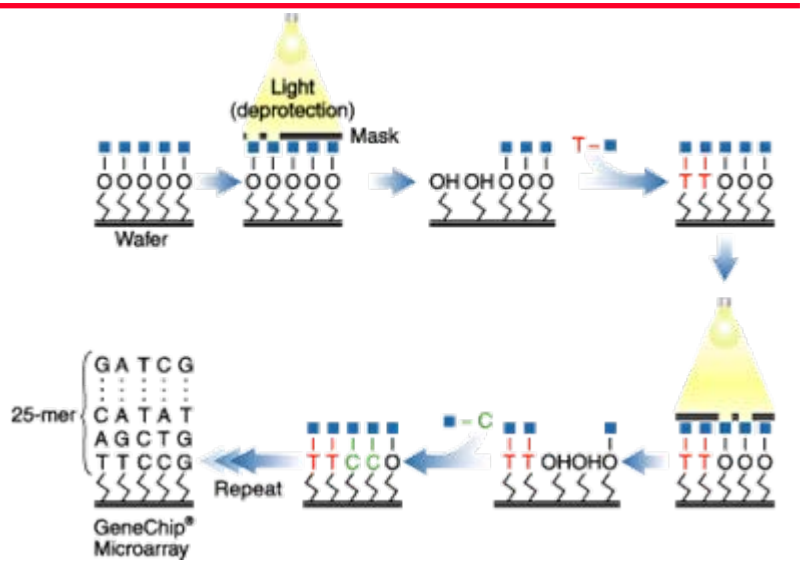

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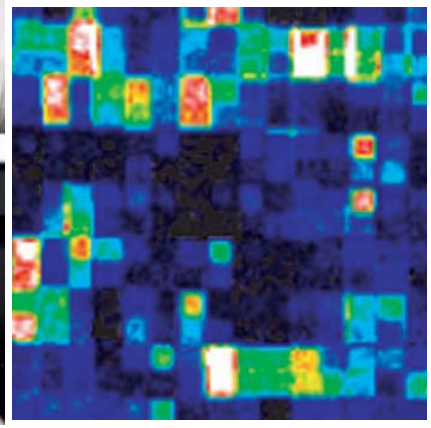

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BioMEMS success stories

- **In the commercial sector, there has been lots of hype**
  - **Success is uncertain**

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Lab-on-a-chip is about the size of a U. S. dime.

- **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**

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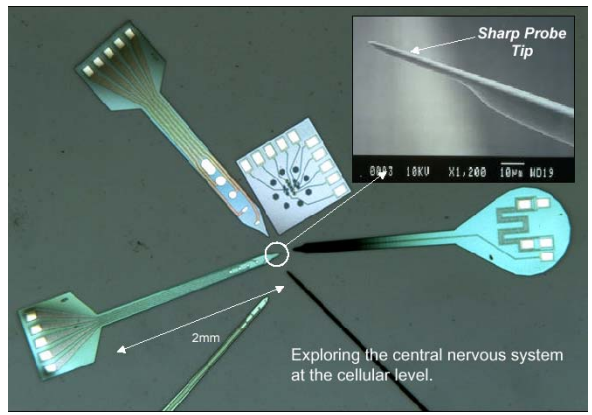
BioMEMS success stories

- Away from commercial sector and into basic science, more successes arise
- Success can be defined as having impact on the target audience



Univ. Michigan

- 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

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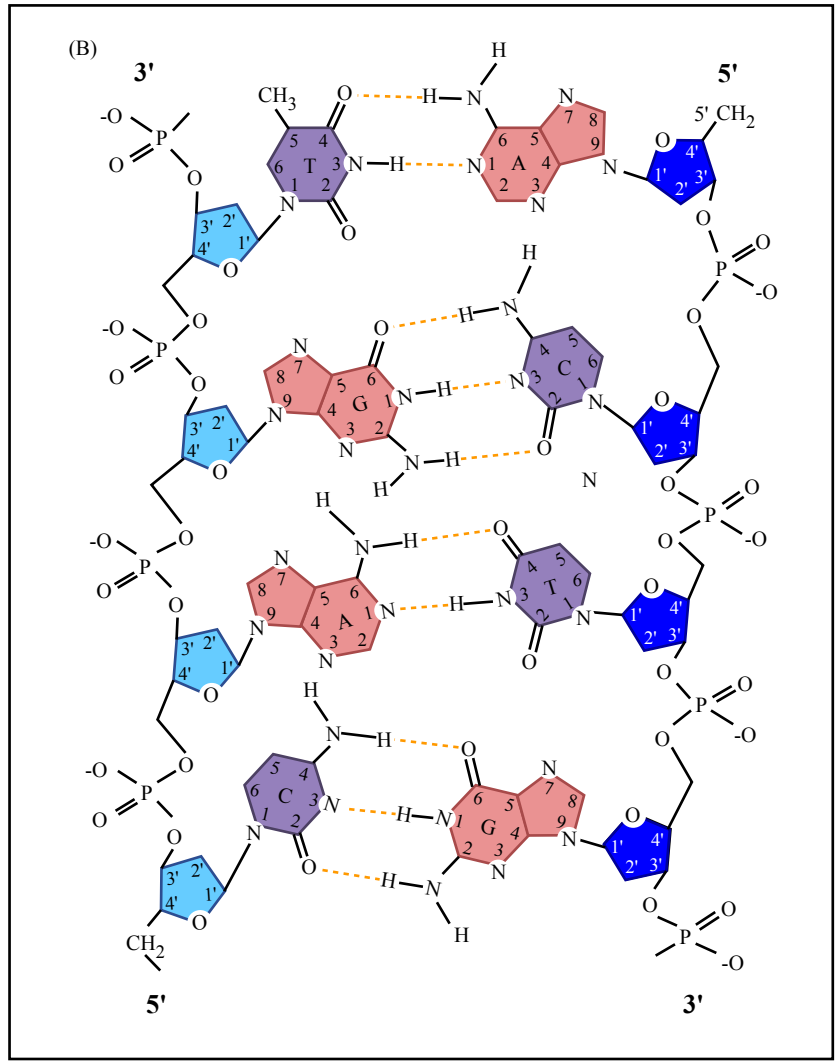
- Conclusions

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

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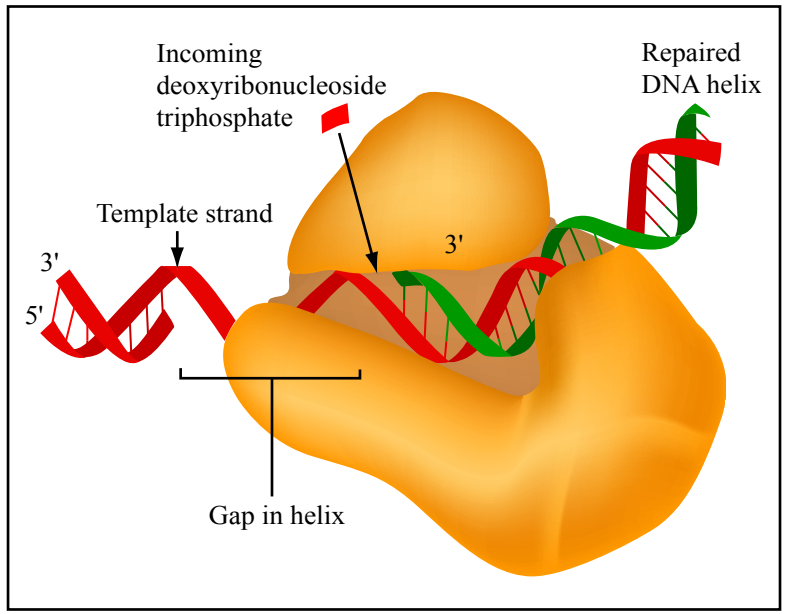
DNA Amplification

- 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



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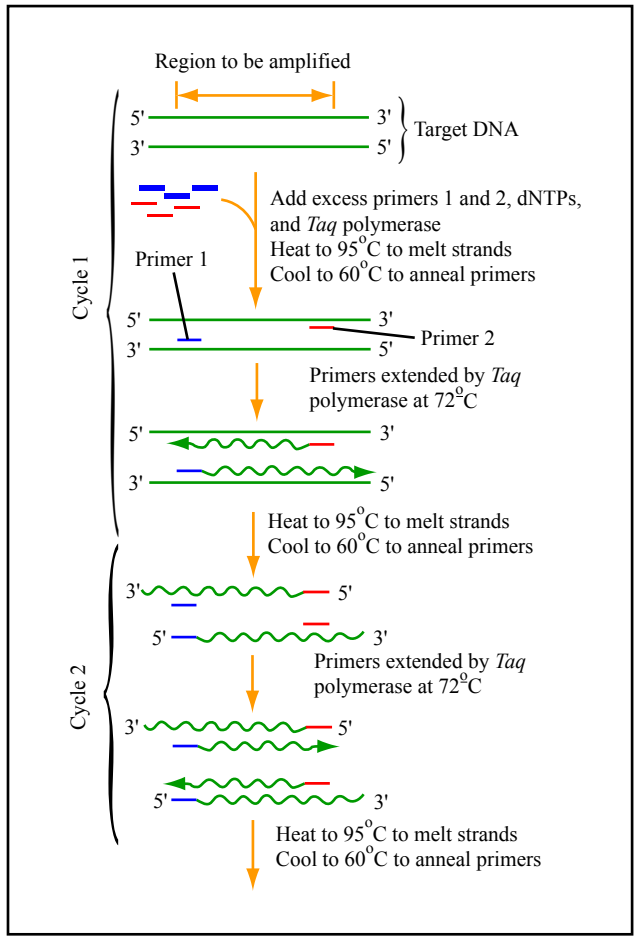
Alberts, MBC

Polymerase chain reaction (PCR)

> **Specifically amplify DNA starting from 1 double-stranded copy**



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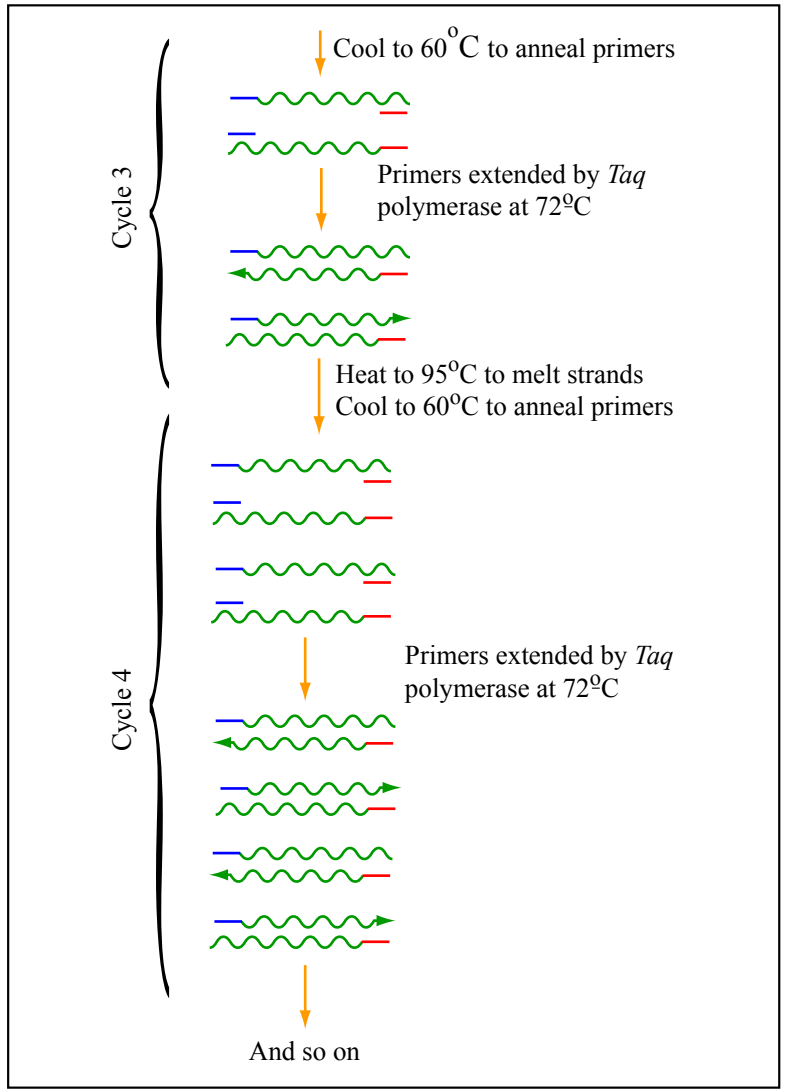
Polymerase chain reaction (PCR)



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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**

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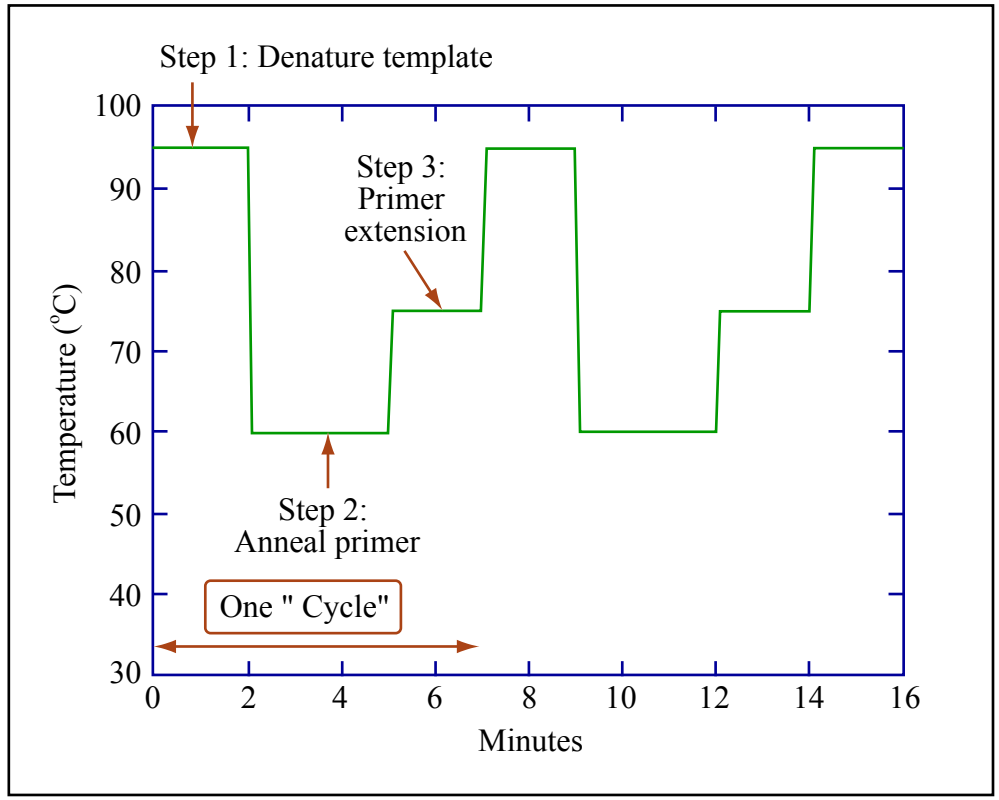
PCR machine by MJ Research.

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



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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**

Property	Spec
Temp range	5-105 ºC
Set-point accuracy	±0.25 ºC
Temperature uniformity	±0.4 ºC within 30 sec
Heating/cooling rate	~ 3 ºC/sec
Sample volume	50 μl
Number of samples	96
Power required	850 W

BioRad DNA Engine

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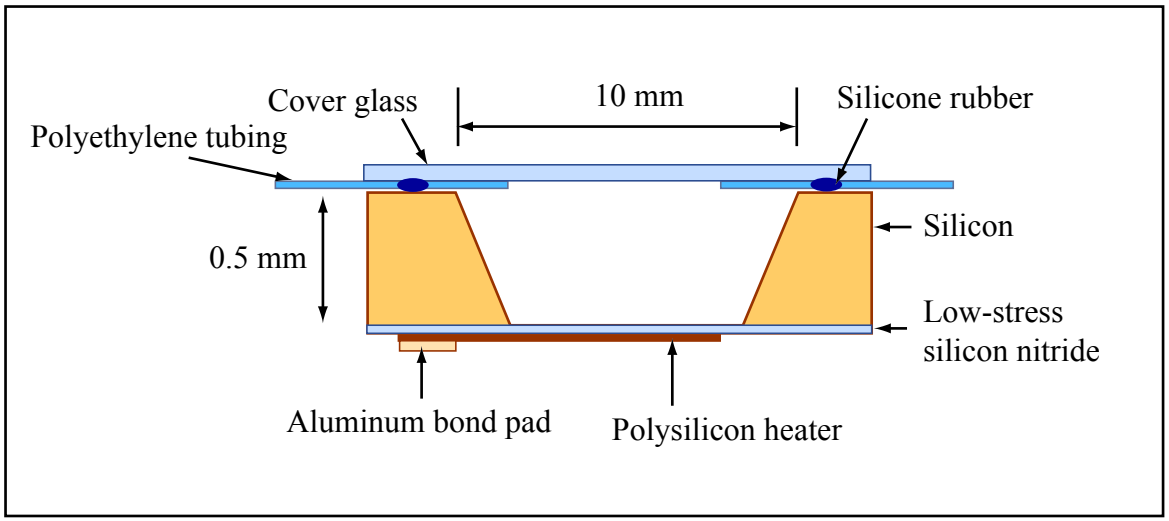
Two approaches to miniaturization

- 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 times

Batch PCR

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



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Adapted from Northrup, M. A., M. T. Ching, R. M. White, and R. T. Watson. "DNA Amplification with a Microfabricated Reaction Chamber." In *Transducers '93: the 7th International Conference on Solid-State Sensors and Actuators: digest of technical papers: June 7-10, 1993, PACIFICO, Yokohama, Japan*. Japan: Hiroyuki Fujita, 1993, p. 924-926. ISBN: 9784990024727.

Northrup et al., Transducers '93, p924

Batch PCR

> **Daniel *et al.* improved thermal isolation**

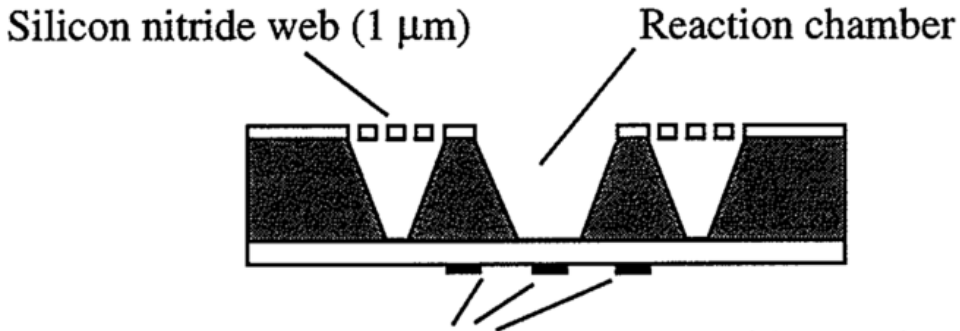

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Figure 5 on p. 84 in: Daniel, J. H., S. Iqbal, R. B. Millington, D. F. Moore, C. R. Lowe, D. L. Leslie, M. A. Lee, and M. J. Pearce. "Silicon Microchambers for DNA Amplification." *Sensors and Actuators A, Physical* 71, nos. 1-2 (November 1998): 81-88.

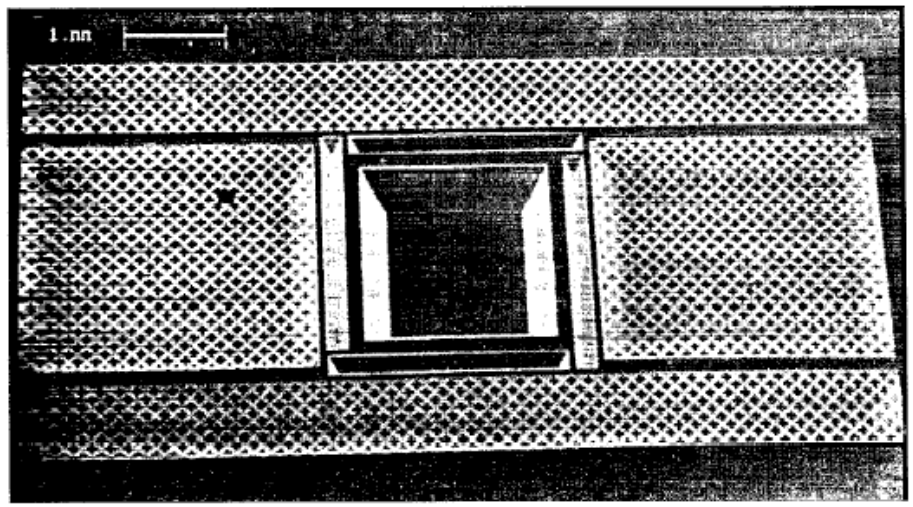

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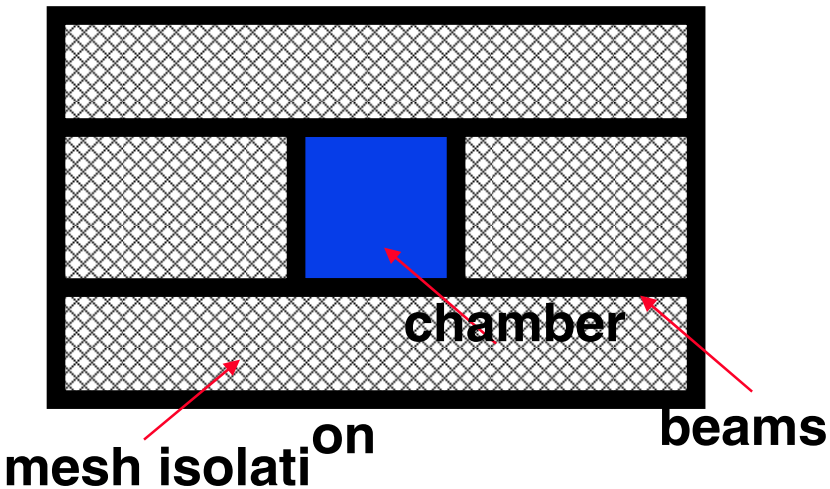
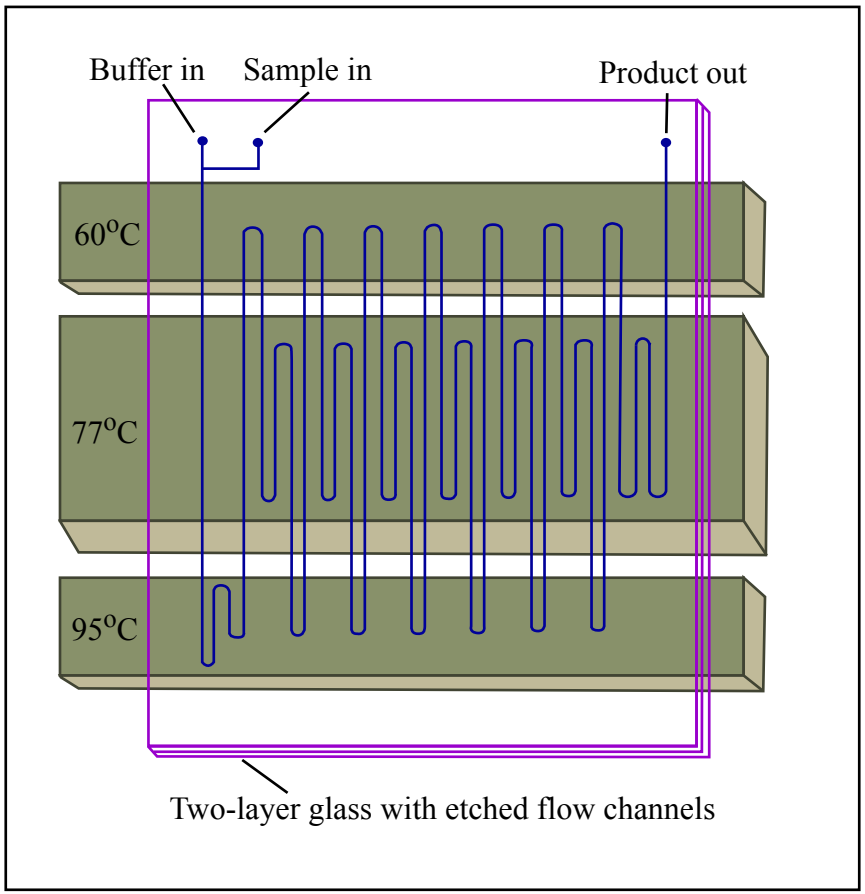
Figure 3 on p. 83 in: Daniel, J. H., S. Iqbal, R. B. Millington, D. F. Moore, C. R. Lowe, D. L. Leslie, M. A. Lee, and M. J. Pearce. "Silicon Microchambers for DNA Amplification." *Sensors and Actuators A, Physical* 71, nos. 1-2 (November 1998): 81-88.



Continuous-flow PCR

> Developed by Kopp *et al.* in 1998



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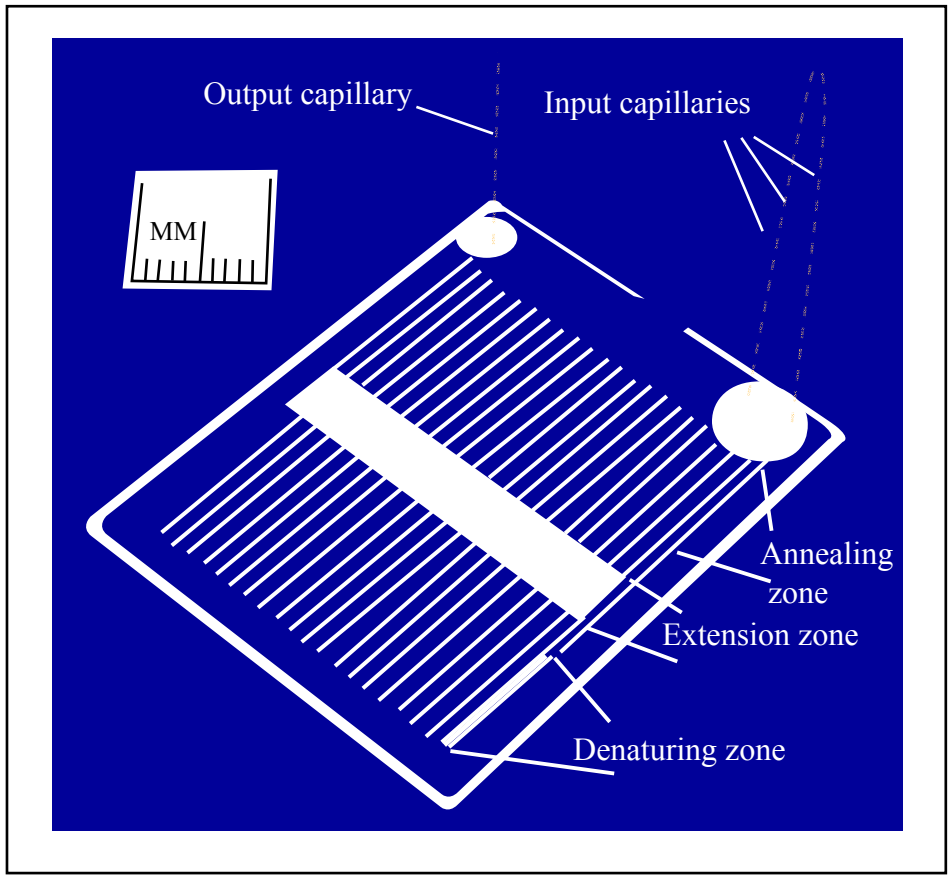

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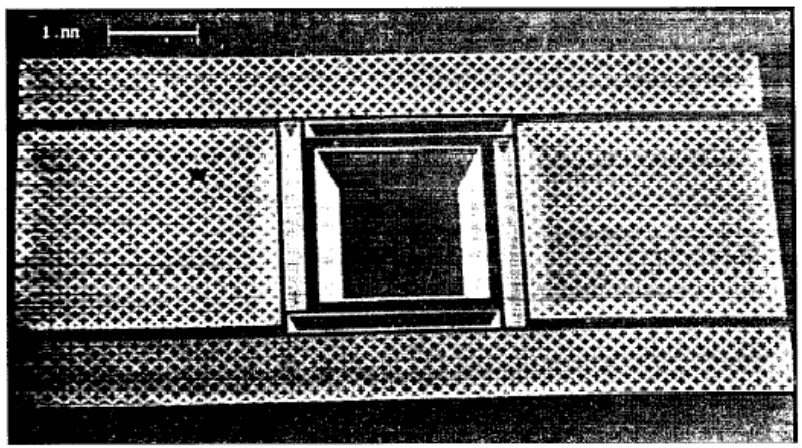
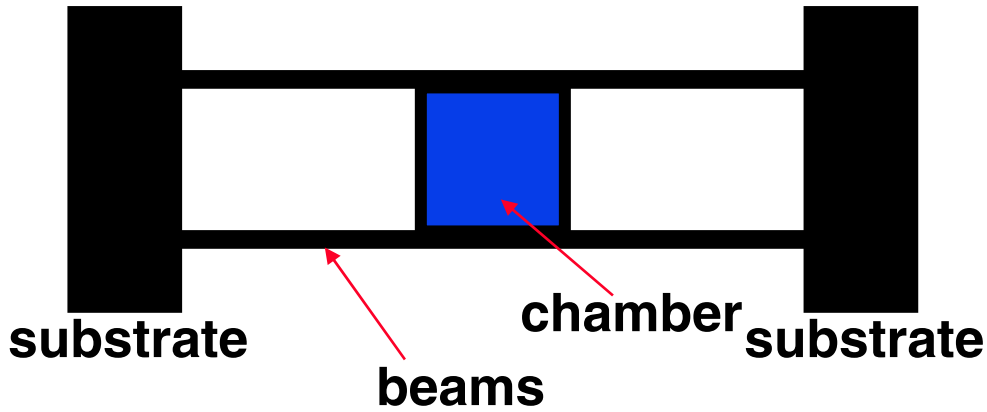
Batch PCR

- **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**



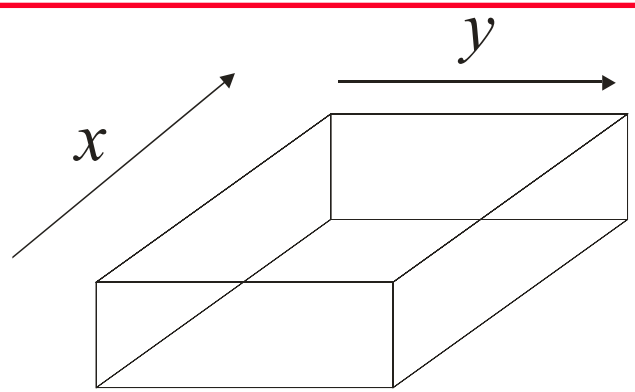
Thermal modeling – batch system

- 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



Eigenfunction expansion solution to heat-flow eqn.

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

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

Lowest mode

$$\tau_f = \frac{1}{\alpha_1} = \frac{L^2}{2\pi^2 D} = \frac{L^2 \tilde{C}_m \rho_m}{2\pi^2 \kappa}$$

Thermal modeling – batch system

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

$$C_f = \rho_m \widetilde{C}_m \int_{-L/2}^{L/2}\int_{-L/2}^{L/2}\int_{0}^{H} \cos\left(\frac{\pi x}{L}\right)\cos\left(\frac{\pi y}{L}\right) dx\,dy\,dz$$

$$C_f = \rho_m \widetilde{C}_m \frac{2L}{\pi}\frac{2L}{\pi} H$$

$$C_f = \frac{4\rho_m \widetilde{C}_m L^2 H}{\pi^2}$$

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

$$R_f = \frac{1}{\kappa}\frac{length \cdot 1/4}{area} = \frac{L/2\ 1/4}{\kappa L H} = \frac{1}{8\kappa H}$$

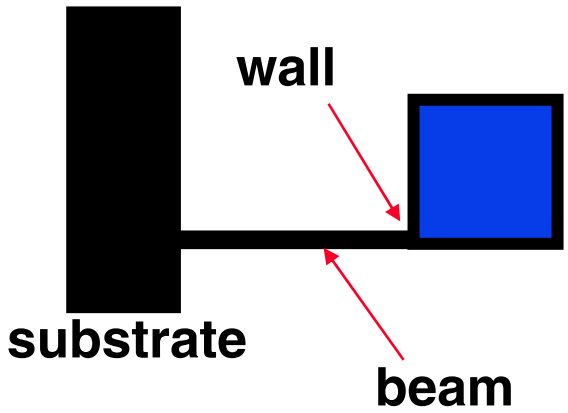
- For L=2 mm, τ=1.4 s
- H=400 μm, volume = 2 μl

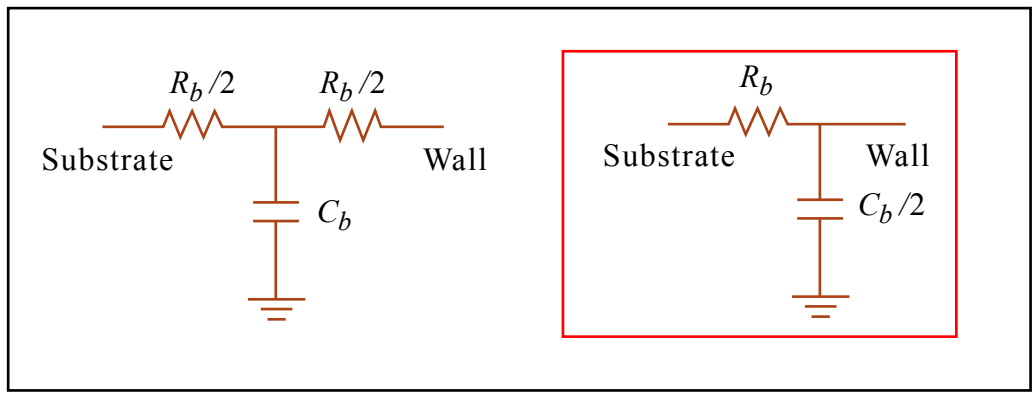
Thermal modeling – batch system

- **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**

$$R_b = \frac{1}{4}\frac{1}{\kappa}\frac{length}{area}$$

$$C_b = \rho_m \tilde{C}_m volume$$





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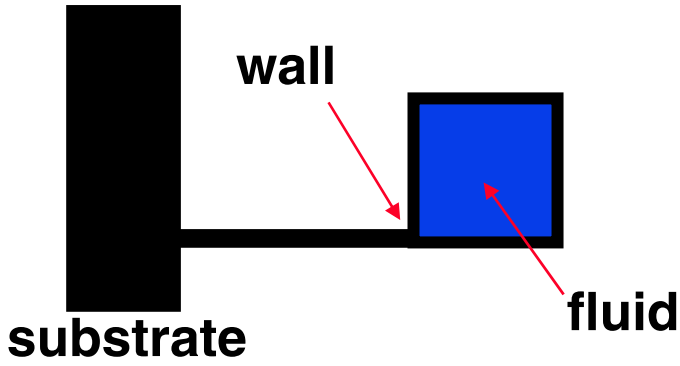
Thermal modeling – batch system

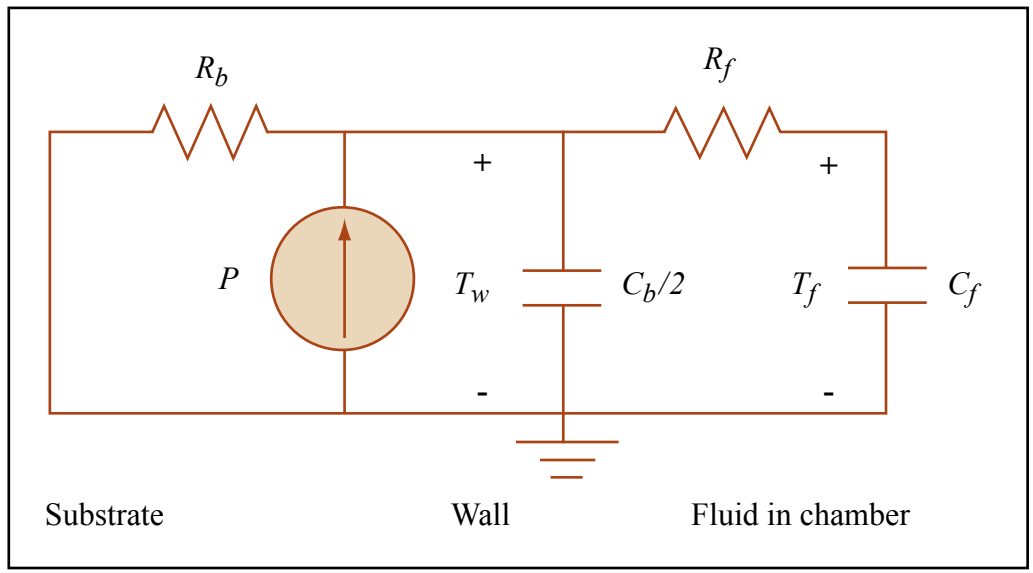
- Lumped circuit model of reactor
- First-order lag between wall and fluid temperature

$$T_f = \frac{1}{1+\tau_f s} T_w$$

$$\tau_f = \frac{L^2}{2\pi^2 D}$$

- Making *L* smaller reduces lag





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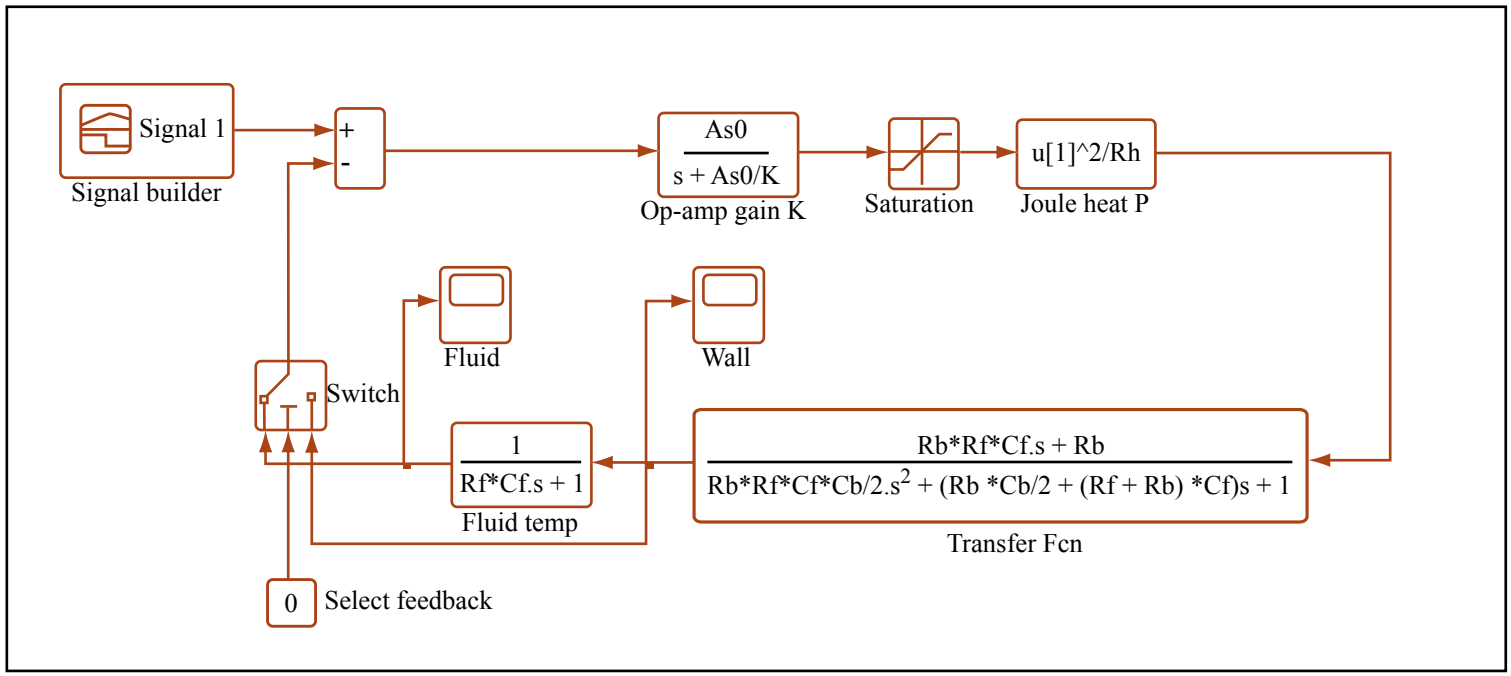
Thermal modeling – batch system

> Simulink proportional control circuit

> Saturation needed for maximum +/- voltage swing

- Set to 0 to 15 V



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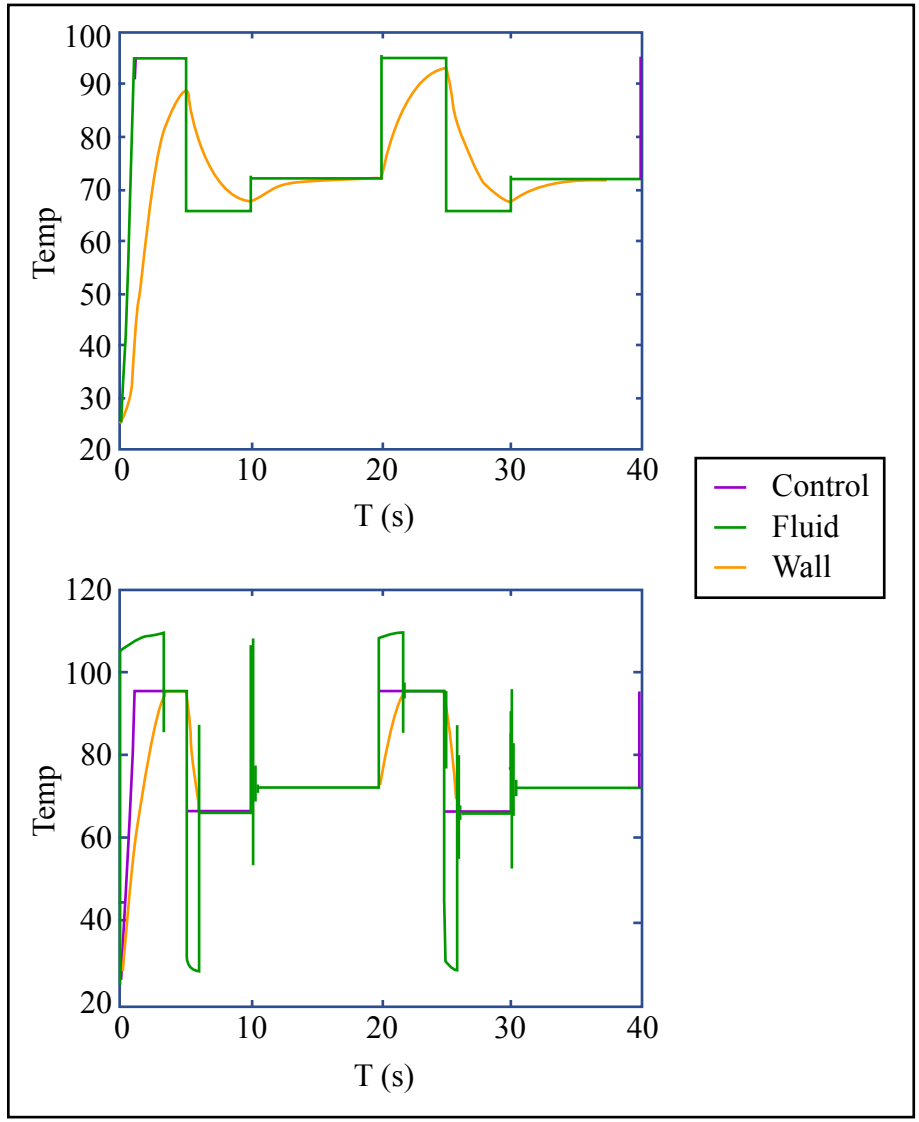
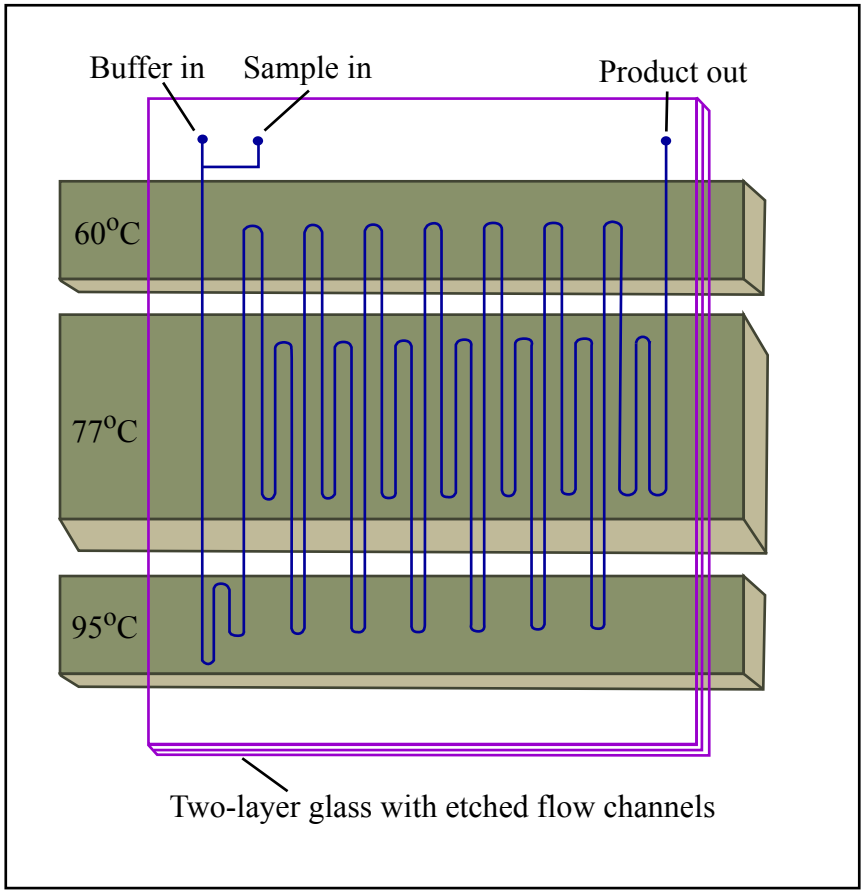
Simulation response

- 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



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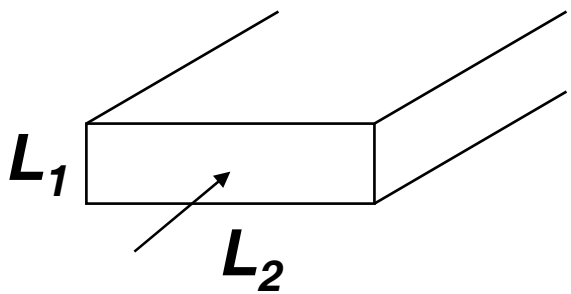
Thermal model of continuous-flow device

- Wall-to-fluid time constant is same as in batch device
- L_1=40 μm, L_2=90 μm
- D=1.4x10^{-7} m^2/s
- τ_f= 1.2 ms
- 1000× faster than batch device!

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



- Entrance length for thermal equilibration

$$L_e \approx 3 v_f \tau_f$$

- Average flow velocity

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

$$L_e \approx 60 \ \mu\text{m}$$

This is much smaller than zone lengths

- Thermal Pe number is ~10^{-3}

Continuous-flow device

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

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

$$Pe = \frac{LU}{D} = \frac{(40\mu m)(0.02 m/s)}{4.4x10^{-8} cm^2/s} \sim 2x10^5$$

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

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

$$K \sim 8x10^8 \cdot D \sim 35\, cm^2/s$$

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

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

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Loading gel in electrophoresis apparatus.

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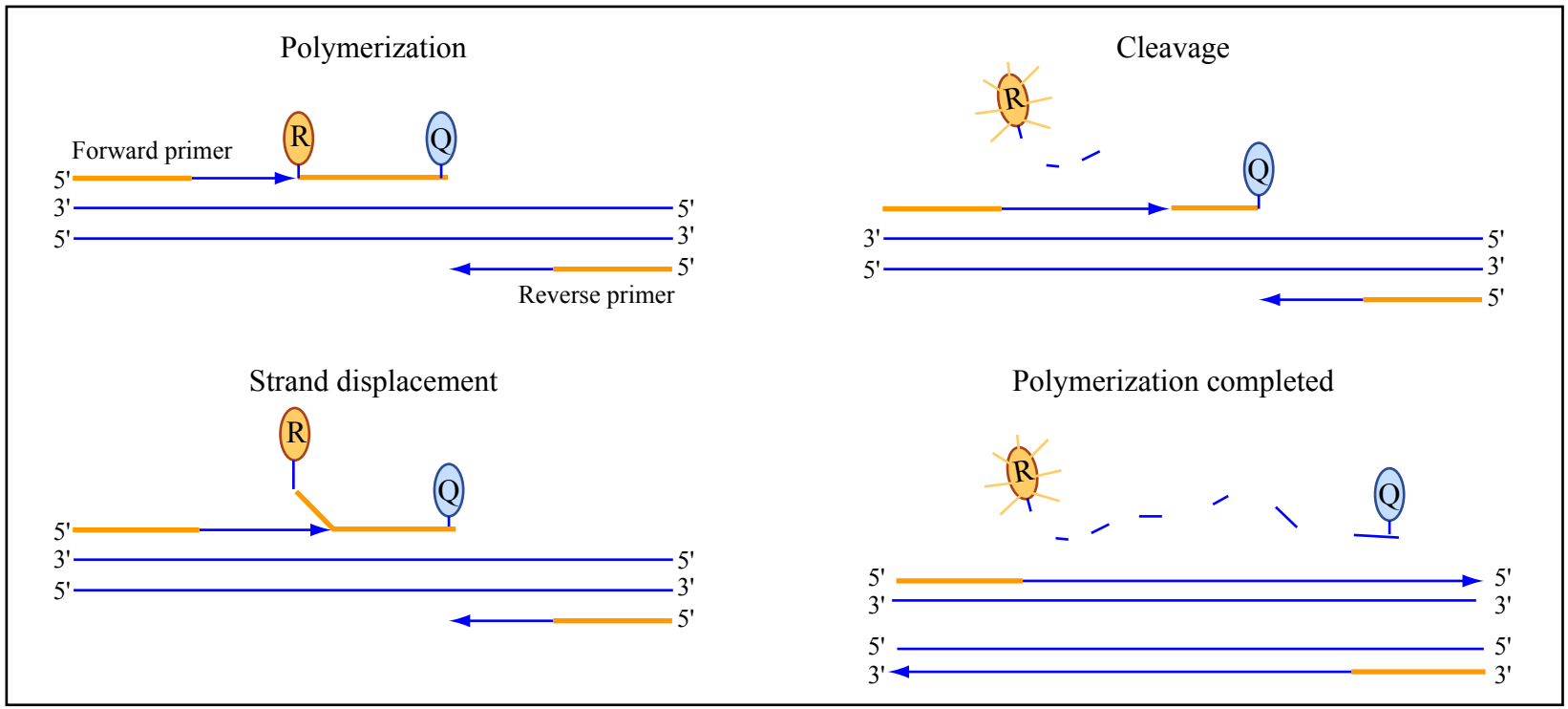
Photograph of an illuminated gel after electrophoresis.

Detecting PCR products

- Newer techniques allow real-time detection
  - "Real-time PCR"
- Integrate illumination/detection optics, thermal cycler, and chemistry



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ABI Taqman assay

Detecting PCR products

- **Real-time thermocycler**
- **1.5 mm-wide capillaries**
- **τ~2-3 sec up and down**

Roche LightCycler

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Schematic of the thermal chamber for the LightCycler(R) 2.0.

Comparison of two microfluidic PCR approaches

Continuous flow	Batch
Faster thermal response	Slower
No temp overshoot	Depends on control system
Static protocol	Can change protocol easily
Taylor dispersion effects, and sample carryover	Sample carryover only
Optical detection more complicated	Simpler optical detection

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 use deposited SiO_2 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 ºC/s up, 2.5 ºC/s down**
  - **"Real time"-PCR via coupled electrophoresis**
  - **Cepheid formed**

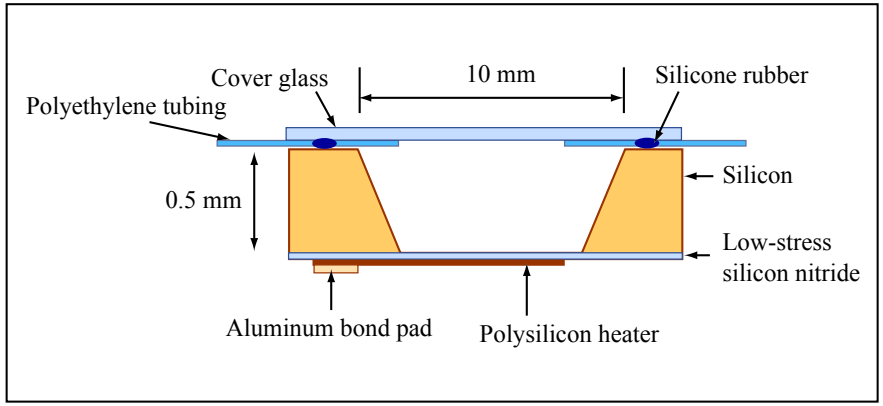

Image by MIT OpenCourseWare.

Adapted from Northrup, M. A., M. T. Ching, R. M. White, and R. T. Watson. "DNA Amplification with a Microfabricated Reaction Chamber." In *Transducers '93: the 7th International Conference on Solid-State Sensors and Actuators: digest of technical papers: June 7-10, 1993, PACIFICO, Yokohama, Japan*. Japan: Hiroyuki Fujita, 1993, p. 924-926. ISBN: 9784990024727.

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Evolution of chamber device

- 1998
- Same two-heater chambers
- Portable application
- Up to 30 ºC/s up, 4 ºC/s down
- 17 sec minimum cycle time
- Usually use 35+ sec

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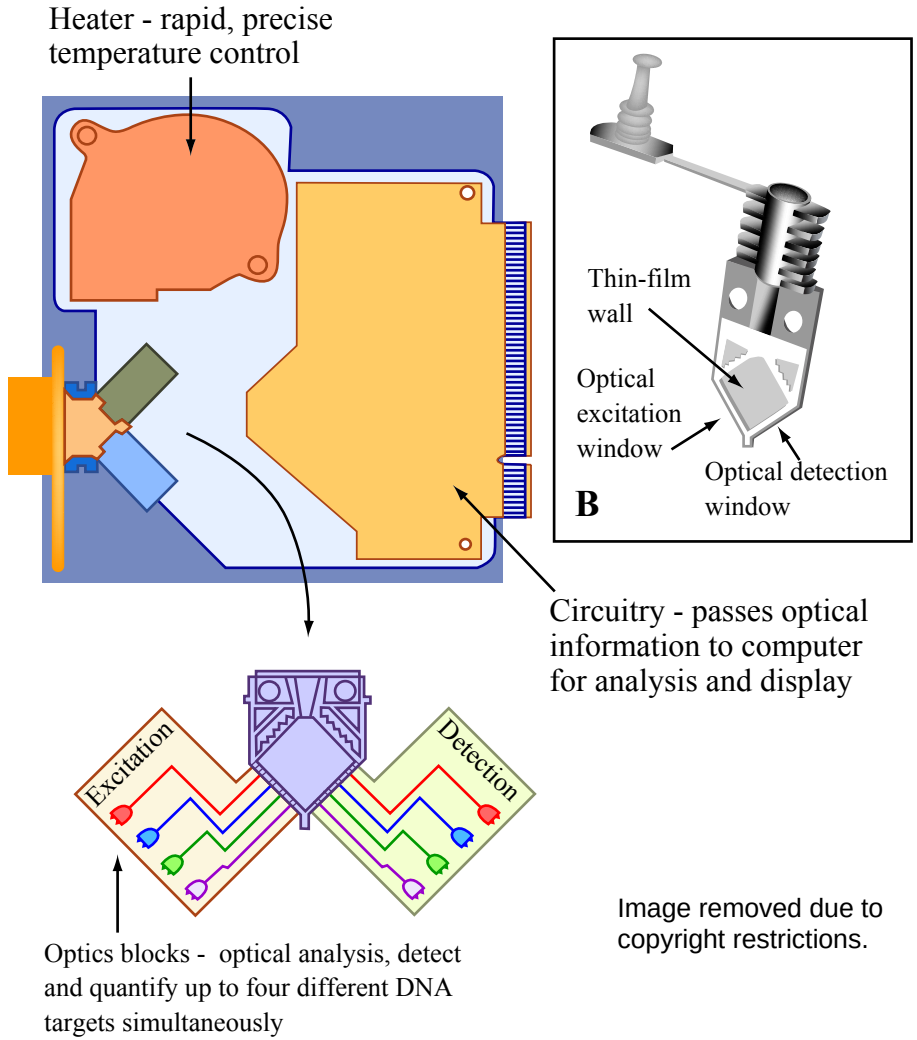
Evolution of chamber device

- 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



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Conclusions

- BioMEMS commerical 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