Automated Design of Microfluidics-Based Biochips

Connecting Biochemistry to Electronics CAD

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Acknowledgments

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• Post-docs and colleagues: Dr. Vamsee Pamula, Dr. Michael Pollock, Prof. Richard Fair, Dr. Jun Zeng (Coventor, Inc.)

• Duke University’s Microfluidics Research Lab (http://www.ee.duke.edu/research/microfluidics/)

• Advanced Liquid Logic (http://www.liquid-logic.com/): Start-up company spun out off Duke University’s microfluidics research project
Motivation for Biochips

- Clinical diagnostics, e.g., healthcare for premature infants, point-of-care diagnosis of diseases
- “Bio-smoke alarm”: environmental monitoring
- Massive parallel DNA analysis, automated drug discovery

Conventional Biochemical Analyzer

Lab-on-a-chip for CLINICAL DIAGNOSTICS

Higher throughput, minimal human intervention, smaller sample/reagent consumption, higher sensitivity, increased productivity
Tubes to Chips: ICs

• Driven by Information Processing needs

IBM 701 calculator (1952)

IBM Power 5 IC (2004)
Tubes to Chips: BioChips

- Driven by biomolecular analysis needs

Test tube analysis

Agilent DNA analysis Lab on a Chip (1997)
Portable Analysis

- New knowledge of molecular basis of biology
  - e.g. Human Genome Project
  - Massively parallel analysis infrastructure
- Integration and miniaturization will drive biomolecular analysis instrumentation

Biomolecular “mainframes”

Spock with Tricorder Sensor + computer

Burns Science 2002
Typical Biological Lab Functions

- **Synthesis**
  - A + B → C

- **Analysis**
  - A + B → A + B

**Equipment Diagrams**

- **Mixing**
- **Reaction**
- **Separation**
Motivation (Parallels with IC Design)

- Increasing application complexity and design complexity

SSI (Small-scale Integration) $1 \sim 10^2$

MSI (Medium-scale Integration) $10^2 \sim 10^3$

LSI (Large-scale Integration) $10^3 \sim 10^5$

VLSI (Very-large-scale Integration) $10^5 \sim 10^6$

ULSI (Ultra-large-scale Integration) $>10^6$

First transistor 1947

Fairchild 1961

General Microelectronics 1964

Intel 1971

Hitachi 1983

First IC (TI) 1958

General Microelectronics 1964

Intel 1971

Hitachi 1983

IC maturation path

Biochip maturation path

Silicon Biosystems
Caliper LS
Advanced Liquid Logic
Agilent
Fluidigm, …
Talk Outline

• Motivation

• Technology Overview
  – Microarrays
  – Continuous-flow microfluidics: channel-based biochips
  – “Digital” microfluidics: droplet-based biochips

• Design Automation Methods
  – Synthesis
  – Placement
  – Testing
  – Routing

• Conclusions
Classification of Biochips

Biochips

Microarray

DNA array

Protein array

Microfluidic biochips

Digital microfluidic biochips

Continuous-flow biochips

Chemical methods

Thermal methods

Acoustical methods

Electrical methods
**Microarray**

- DNA (or protein) microarray: piece of glass, plastic or silicon substrate
- Pieces of DNA (or antibodies) are affixed on a microscopic array
- Affixed DNA (or antibodies) are known as *probes*

GeneChip® DNAarray from Affymetrix
[http://www.affymetrix.com](http://www.affymetrix.com)

DNA microarray from Infineon AG
[http://www.infineon.com](http://www.infineon.com)

NanoChip® microarray from Nanogen
[http://www.nanogen.com](http://www.nanogen.com)
DNA Arrays

- Gene Chips
- Only implement hybridization reaction

Unhybridized array

<table>
<thead>
<tr>
<th>DNA Sample</th>
<th>Optical Scan</th>
<th>Hybridized array</th>
</tr>
</thead>
<tbody>
<tr>
<td>A T C G G</td>
<td></td>
<td>G T A A C</td>
</tr>
<tr>
<td>G G T T T</td>
<td></td>
<td>T A G C C</td>
</tr>
<tr>
<td>C T A A C</td>
<td></td>
<td>A T A T A</td>
</tr>
<tr>
<td>G C A T T</td>
<td></td>
<td>G C A T T</td>
</tr>
<tr>
<td>T A G C G</td>
<td></td>
<td>T A G C G</td>
</tr>
</tbody>
</table>

Laser
Microfluidics

- Continuous-flow biochips: Permanently etched microchannels, micropumps and microvalves
- Digital microfluidic biochips: Manipulation of liquids as discrete droplets

(Unciversity of Michigan) 1998
(Duke University) 2002
Motivation for Microfluidics

- Test tubes
  - Automation
  - Integration
  - Miniaturization

- Robotics
  - Automation
  - Integration
  - Miniaturization

- Microfluidics
  - Automation
  - Integration
  - Miniaturization
Electrowetting

- Novel microfluidic platform invented at Duke University
- Droplet actuation is achieved through an effect called *electrowetting*
  - Electrical modulation of the solid-liquid interfacial tension

**No Potential**
A droplet on a hydrophobic surface originally has a large contact angle.

**Applied Potential**
The droplet’s surface energy increases, which results in a reduced contact angle. The droplet now wets the surface.
Electrowetting

- Novel microfluidic platform invented at Duke University
- Droplet actuation is achieved through an effect called *electrowetting*
  - Electrical modulation of the solid-liquid interfacial tension

![Diagram of electrowetting](image)

**No Potential**
A droplet on a hydrophobic surface originally has a large contact angle.

**Applied Potential**
The droplet’s surface energy increases, which results in a reduced contact angle. The droplet now wets the surface.
What is Digital Microfluidics?

- Discretizing the bottom electrode into multiple electrodes, we can achieve lateral droplet movement

Note: oil is typically used to fill between the top and bottom plates to prevent evaporation.
What is Digital Microfluidics?

A droplet can be transported by removing a potential on the current electrode, and applying a potential to an adjacent electrode.
What is Digital Microfluidics?

Transport
20 cm/s flow rates
What is Digital Microfluidics?

Splitting/Merging
What is Digital Microfluidics?

Droplet Formation
8 droplets in 3.6s
What is Digital Microfluidics?

Mixing
Advantages

• No bulky liquid pumps are required
  – Electrowetting uses microwatts of power
  – Can be easily battery powered

• Standard low-cost fabrication methods can be used
  – Continuous-flow systems use expensive lithographic techniques to create channels
  – Digital microfluidic chips are possible using solely PCB processes
An Example

• Detection of lactate, glutamate and pyruvate has also been demonstrated.

• Biochip used for multiplexed in-vitro diagnostics on human physiological fluids

Fabricated microfluidic array used for multiplexed biomedical assays
Applications of Digital Microfluidic Biochips

Droplet-based microfluidic biochip

Drug discovery and biotechnology

Environmental and other applications

Medical diagnostics and therapeutics

Proteomics

High-throughput screening

Genomics

Clinical chemistry

Immunoassays

Nucleic acid tests

Micro-optics

Countering bioterrorism

Air/water/agro food monitoring
Synthesis Methodology

- Full-custom bottom-up design $\rightarrow$ Top-down system-level design
- (Su & Chakrabarty, ICCAD 04)

- Scheduling of operations
- Binding to functional resources
- Physical design

- Behavioral description of biomedical assay
- Architectural-level Synthesis
- Macroscopic structure of biochip
- Geometry-level Synthesis
- Layout of biochip

Plasma and Serum sampled and assayed for glucose, lactate, pyruvate and glutamate measurement

Mixer
Memory
Detector

- Mixer
- Memory

26
Simulation Experiments (Cont.)


<table>
<thead>
<tr>
<th>Example</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Example 1**  
$(Nr=Nd=1, Na=3) \ m=2, n=2$ | $S_1$ and $S_2$ are assayed for Assay1 and Assay2. |
| **Example 2**  
$(Nr=Nd=1, Na=4) \ m=2, n=3$ | $S_1$, and $S_2$ are assayed for Assay1, Assay2, and Assay3. |
| **Example 3**  
$(Nr=Nd=1, Na=5) \ m=3, n=3$ | $S_1$, $S_2$, and $S_3$ are assayed for Assay1, Assay2, and Assay3. |
| **Example 4**  
$(Nr=Nd=1, Na=7) \ m=3, n=4$ | $S_1$, $S_2$, and $S_3$ are assayed for Assay1, Assay2, Assay3 and Assay4. |
| **Example 5**  
$(Nr=Nd=1, Na=9) \ m=4, n=4$ | $S_1$, $S_2$, $S_3$ and $S_4$ are assayed for Assay1, Assay2, Assay3 and Assay4. |
Physical Design: Module Placement
(Su and Chakrabarty, DATE’05)

- Placement determines the locations of each module on the microfluidic array in order to optimize some design metrics
- High dynamic reconfigurability: module placement → 3-D packing → modified 2-D packing
## Application to PCR

### Resource binding in PCR

<table>
<thead>
<tr>
<th>Operation</th>
<th>Hardware</th>
<th>Module</th>
<th>Mixing time</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2x2 electrode array</td>
<td>4x4 cells</td>
<td>10s</td>
</tr>
<tr>
<td>M2</td>
<td>4-electrode linear array</td>
<td>3x6 cells</td>
<td>5s</td>
</tr>
<tr>
<td>M3</td>
<td>2x3 electrode array</td>
<td>4x5 cells</td>
<td>6s</td>
</tr>
<tr>
<td>M4</td>
<td>4-electrode linear array</td>
<td>3x6 cells</td>
<td>5s</td>
</tr>
<tr>
<td>M5</td>
<td>4-electrode linear array</td>
<td>3x6 cells</td>
<td>5s</td>
</tr>
<tr>
<td>M6</td>
<td>2x2 electrode array</td>
<td>4x4 cells</td>
<td>10s</td>
</tr>
<tr>
<td>M7</td>
<td>2x4 electrode array</td>
<td>4x6 cells</td>
<td>3s</td>
</tr>
</tbody>
</table>

### Protocol of PCR (mixing phase)

#### Schedule of PCR

- M1
- M2
- M4
- M5
- M6
- M7

### Diagram

- Tris-HCl (pH 8.3)
- KCl
- Bovine serum albumin
- Gelatin
- Primer
- Deoxynucleotide triphosphate
- AmpliTg
- DNA
- LambdaDNA

Mix M1 → M2 → M4 → Mix M5 → Mix M7 → M3 → M6
Application to PCR (Cont.)

Baseline: 84 cells (189mm²) from greedy algorithm

- Area: 7x9=63 cells
- FTI: 0.1270

Placement from the simulated annealing-based procedure

Placement from enhanced module placement procedure

- Area: 7x11=77 cells
- FTI: 0.8052
Unified Synthesis Methodology

Su and Chakrabarty (DAC 2005)

Input: Sequencing graph of bioassay

Digital microfluidic module library

<table>
<thead>
<tr>
<th>Mixing components</th>
<th>Area</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x2-array mixer</td>
<td>4 cells</td>
<td>10 s</td>
</tr>
<tr>
<td>2x3-array mixer</td>
<td>6 cells</td>
<td>6 s</td>
</tr>
<tr>
<td>2x4-array mixer</td>
<td>8 cells</td>
<td>3 s</td>
</tr>
<tr>
<td>1x4-array mixer</td>
<td>4 cells</td>
<td>5 s</td>
</tr>
</tbody>
</table>

Detectors

<table>
<thead>
<tr>
<th>Detector Type</th>
<th>Area</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED+Photodiode</td>
<td>1 cell</td>
<td>30 s</td>
</tr>
</tbody>
</table>

Design specifications

- Maximum array area: $A_{max}$: 20x20 array
- Maximum number of optical detectors: 4
- Number of reservoirs: 3
- Maximum bioassay completion time $T_{max}$: 50 seconds

Output:
Unified Synthesis of Digital Microfluidic Biochip

Resource binding

<table>
<thead>
<tr>
<th>Operation</th>
<th>Resource</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>2x3-array mixer</td>
</tr>
<tr>
<td>O2</td>
<td>Storage unit (1 cell)</td>
</tr>
<tr>
<td>O3</td>
<td>2x4-array mixer</td>
</tr>
<tr>
<td>O4</td>
<td>Storage unit (1 cell)</td>
</tr>
<tr>
<td>O5</td>
<td>1x4-array mixer</td>
</tr>
<tr>
<td>O6</td>
<td>LED+Photodiode</td>
</tr>
</tbody>
</table>

Schedule

Biochip design results:

Array area: 8x8 array  
Bioassay completion time: 25 seconds
Protein Assay

Sequencing graph model

- Maximum array area: 10x10
- Maximum number of optical detectors: 4
- Reservoir number: 1 for sample; 2 for buffer; 2 for reagent; 1 for waste
- Maximum bioassay time: 400 s
Protein Assay (Cont.)

- Microfluidic module library for synthesis

<table>
<thead>
<tr>
<th>Operation</th>
<th>Resource</th>
<th>Operation Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$DsS; DsB; DsR$</td>
<td>On-chip reservoir/dispensing port</td>
<td>7</td>
</tr>
<tr>
<td>$Dlt$</td>
<td>2x2-array dilutor</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2x3-array dilutor</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2x4-array dilutor</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4-electrode linear array dilutor</td>
<td>7</td>
</tr>
<tr>
<td>$Mix$</td>
<td>2x2-array mixer</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2x3-array mixer</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2x4-array mixer</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4-electrode linear array mixer</td>
<td>5</td>
</tr>
<tr>
<td>$Opt$</td>
<td>LED+Photodiode</td>
<td>30</td>
</tr>
<tr>
<td>$Storage$</td>
<td>Single cell</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Design for Protein Assay

- Baseline techniques
  - Full-custom design
  - Architectural-level synthesis

\[ T = 560 \text{ s} > T_{\text{max}} = 400 \text{ s} \]

5x8 + 14 < 10x10 (satisfies the resource constraint in architectural-level synthesis)

Fail to meet the design specification!
Experimental Evaluation (Cont.)

- Results of the unified synthesis method

Bioassay completion time $T$: 363 seconds

Biochip array: 9x9 array
Experimental Evaluation (Cont.)

- Defect tolerance

Bioassay completion time $T$: 385 seconds (6% increase)
Testing of Microfluidics-Based Biochips

- Defect types
- Test stimuli generation
- Test response observation
- Test planning, scheduling
- Concurrent testing
Classification of Faults
(Su et al., ITC’04)

Catastrophic Faults:

- Open in the metal connection between the electrode and the control source
- Short between two adjacent electrodes
- Breakdown of the insulator
- Dielectric breakdown

Manufacturing

 Operational

Parametric Faults:

- Geometrical parameter deviation
- Degradation of the insulator
- Change in the viscosity of the droplet and the filler medium

Manufacturing

 Operational
Example of Electrode Degradation
Unified Detection Mechanism

- Detection mechanism
  - minimally invasive
  - easy to implement
  - fault effect should be unambiguous

If there is a droplet, output=1; otherwise, output=0

Fault-free: there is a droplet between electrodes
Faulty: there is no droplet.

Capacitive changes reflected in electrical signals (Fluidic domain to electrical domain)
Defect-Oriented Testing and Diagnosis (Su et al, ITC’05)

- Defect-Oriented Experiment
  - To simulate the effect of an electrode short on microfluidic behavior

![Diagram](https://via.placeholder.com/150)

(a)  
(b) The first step
(c) The second step
Experimental Results and Analysis

Experimental results and analysis for the first step

(a) Test droplet stuck during its motion
(b) Electrode Short

Experimental results and analysis for the second step

(a) Electrode Short
(b) Test droplet not stuck
Testing for Electrode-Short Faults

- Based on Euler circuit and Euler path theorems
- Modified Fleury’s algorithm
- On-line testing/off-line testing

(a) Graph model for a 5×5 microfluidic array;
(b) eulerized graph containing an Euler circuit;
(c) eulerized graph containing an Euler path.
Tile-Based Architecture for Reconfiguration
(Su and Chakrabarty, VTS’05)

- Array of tiles
- Each tile is configurable (mixer, transport bus, etc.)
- Constraints (performance and array size)
Reconfigurability

- Common microfluidic operations
  - Different modules with different performance levels (e.g., several mixers for mixing)
  - Reconfiguration by changing the control voltages of the corresponding electrodes
Graceful Degradation

• Reconfigure the faulty tile
• Avoid defects (faulty cells)
Droplet Routing
(Su et al, DATE’06)

• A key physical design problem for digital microfluidic biochips

• Given the results from architectural-level synthesis and module placement:
  – Determine droplet pathways using the available cells in the microfluidic array; these routes are used to transport droplets between modules, or between modules and fluidic I/O ports (i.e., boundary on-chip reservoirs)
Droplet Routing: Objective Function

- To find droplet routes with minimum lengths
  - Analogous to the minimization of the total wirelength in VLSI routing
- Need to satisfy critical constraints
  - A set of fluidic constraints
  - Timing constraints: (the delay for each droplet route does not exceed some maximum value, e.g., 10% of a time-slot used in scheduling)
Fluidic Constraints

Assume two given droplets as $D_i$ and $D_j$, and let $X_i(t)$ and $Y_i(t)$ denote the location of $D_i$ at time $t$

**Rule #1:** $|X_i(t+1) - X_j(t+1)| \geq 2$ or $|Y_i(t+1) - Y_j(t+1)| \geq 2$, i.e., their new locations are not adjacent to each other.

**Rule #2:** $|X_i(t+1) - X_j(t)| \geq 2$ or $|Y_i(t+1) - Y_j(t)| \geq 2$, i.e., the activated cell for droplet $D_i$ cannot be adjacent to droplet $D_j$. Otherwise, there is more than one activated neighboring cell for $D_j$, which may leads to errant fluidic operation.

**Rule #3:** $|X_i(t) - X_j(t+1)| \geq 2$ or $|Y_i(t) - Y_j(t+1)| \geq 2$. 
Experimental Verification

(a) Experimental verification of Rule #1: droplets begin on electrodes 1 and 4; (b) Electrodes 2 and 3 are activated, and 1 and 4 deactivated; (c) Merged droplet.

(a) Experimental verification of Rule #2: droplets begin on electrodes 2 and 4; (b) Electrodes 1 and 3 are activated, and 2 and 4 deactivated.
Experimental Verification (Cont.)

(a) Experimental verification of Rule #3: droplets begin on electrodes 4 and 7; (b) Electrodes 3 and 6 are activated, and 4 and 7 deactivated; (c) Merged droplet.

- To demonstrate that adherence to Rule #1 is not sufficient to prevent merging. Both Rule #2 and Rule #3 must also be satisfied during droplet routing.
- These rules are not only used for rule checking, but they can also provide guidelines to modify droplet motion (e.g., force some droplets to remain stationary in a time-slot) to avoid constraint violation if necessary.
Conclusions

• Digital microfluidics offers a viable platform for biochips for clinical diagnostics and biomolecular recognition
• Design automation challenges
  – Automated synthesis: scheduling, resource binding, module placement
  – Testing and reconfiguration
  – Droplet routing
• Bridge between different research communities: bioMEMS, microfluidics, electronics CAD, biochemistry
• Growing interest in the electronics CAD community
  – Special issue on biochips of IEEE Transactions on CAD (Feb 2006)
  – Special session on biochips at CODES-ISSS’2005
  – Special session on bioMEMS at DAC’04
  – Invited talk at ICCAD’05, embedded tutorial at VLSI Design 2005
  – Workshop on biochips at DATE’06
  – Two books on biochips CAD to be published in 2006
  – Special Issue of IEEE Design & Test, Jan’07