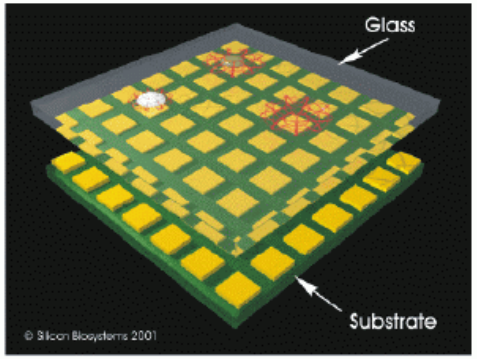
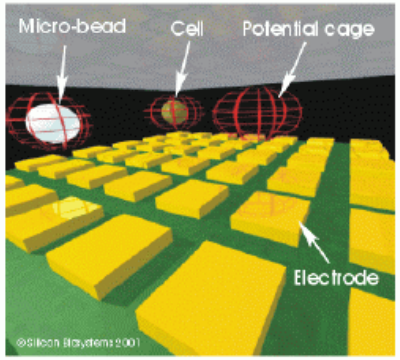
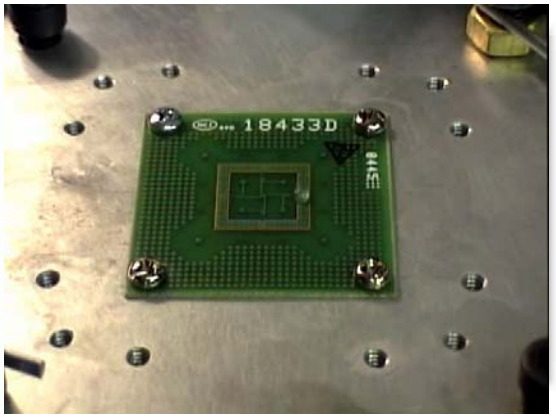
Automated Design of Microfluidics-Based Biochips

Connecting Biochemistry to Electronics CAD







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Acknowledgments

- Students: Tianhao Zhang, Fei Su, William Hwang, Phil Paik, Tao Xu, Vijay Srinivasan
- 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

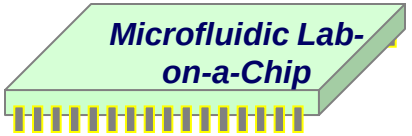
- Clinical diagnostics, e.g., healthcare for premature infants, point-of-care diagnosis of diseases
- "Bio-smoke alarm": environmental monitoring
- Massive parallel DNA analysis, automated drug discovery

CLINICAL DIAGNOSTIC APPLICATION

Conventional Biochemical Analyzer

Shrink

Lab-on-a-chip for CLINICAL DIAGNOSTICS

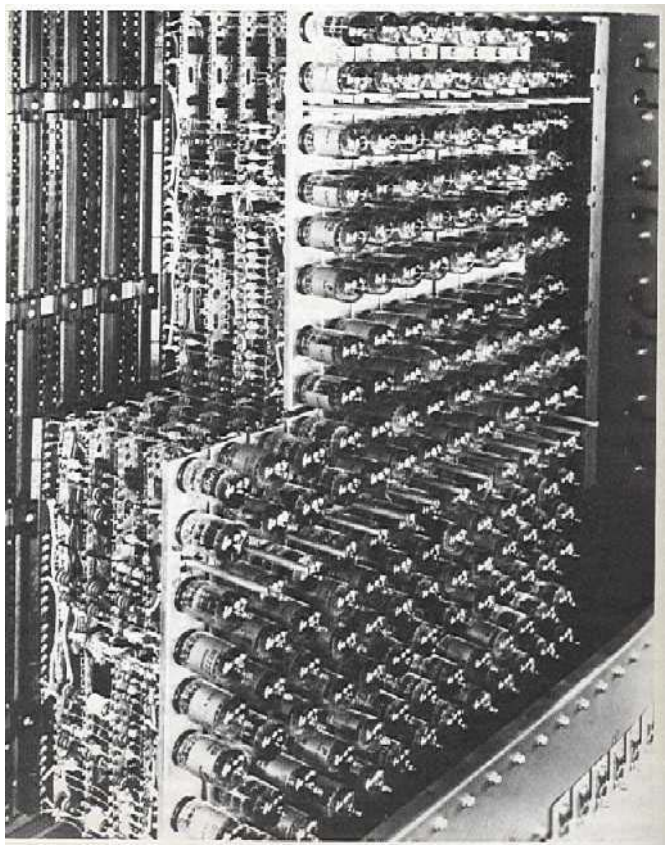


20nl sample

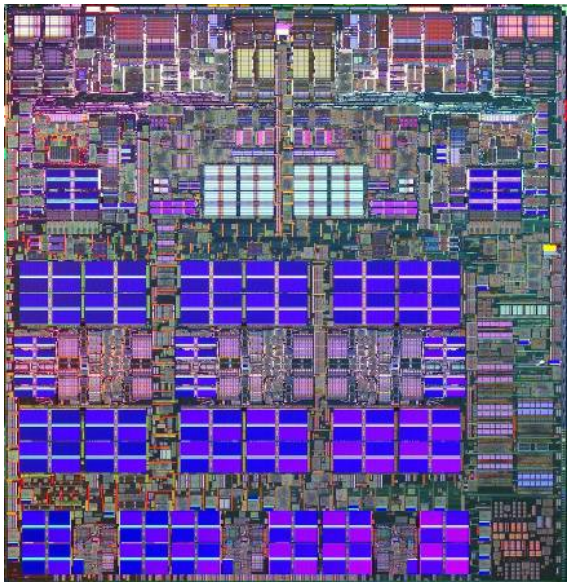
Higher throughput, minimal human intervention, smaller sample/reagent consumption, higher sensitivity, increased productivity

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

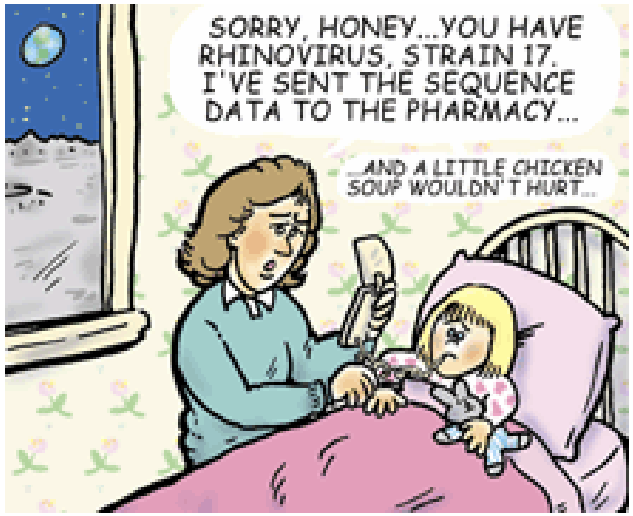
Agilent DNA analysis
Lab on a Chip (1997)

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"

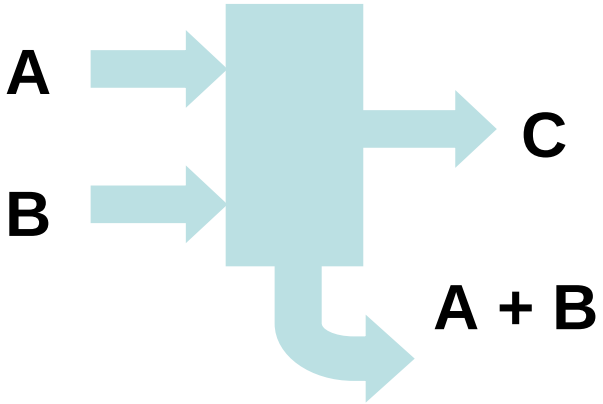
Spock with Tricorder Sensor + computer



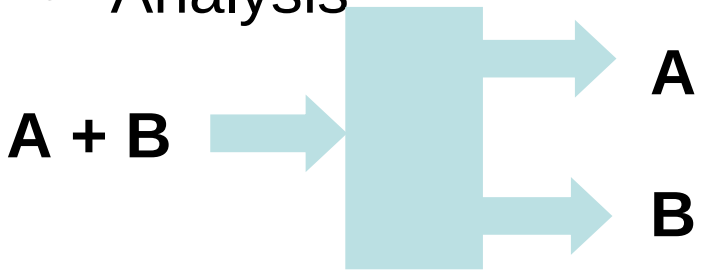
Burns Science 2002

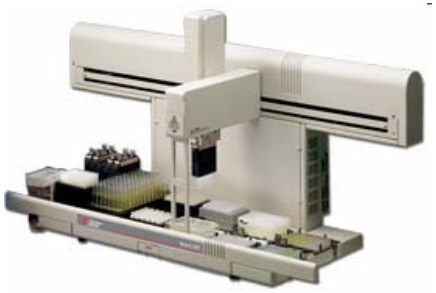
Typical Biological Lab Functions

- Synthesis



- Analysis





Mixing

Reaction

Separation

Motivation (Parallels with IC Design)

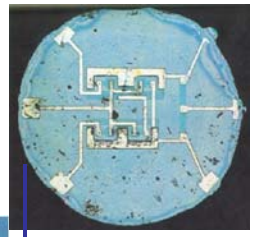
- Increasing application complexity and design complexity

First transistor

1947

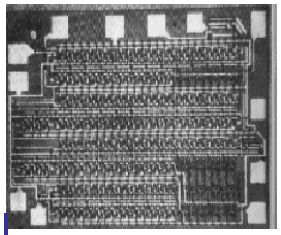
First IC (TI)

1958



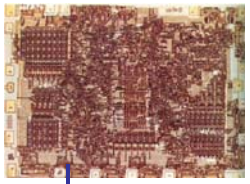
Fairchild

1961



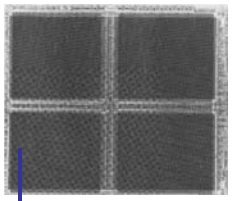
General Microelectronics

1964



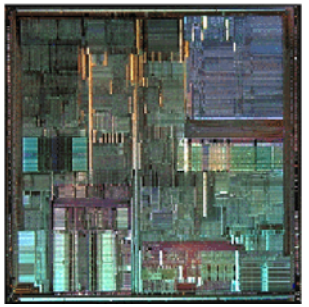
Intel

1971



Hitachi

1983



Intel

1996

IC maturation path

SSI (Small-scale Integration) 1~10^2#

MSI (Medium-scale Integration) 10^2 ~10^3#

LSI (Large-scale Integration) 10^3 ~10^5#

VLSI (Very-large-scale Integration) 10^5 ~10^6#

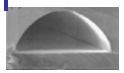
ULSI (Ultra-large-scale Integration) >10^6#

2000

Duke Univ.

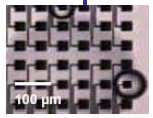
1998

UMich



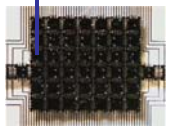
2001

Univ. of Texas

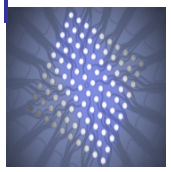


2001

Duke Univ.



Nanochip@ Nanogen

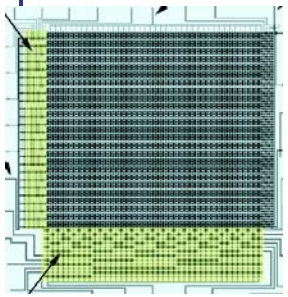


2002

Infineon

2002

CalTech.



Biochip maturation path

Silicon Biosystems

Caliper LS

Advanced Liquid Logic

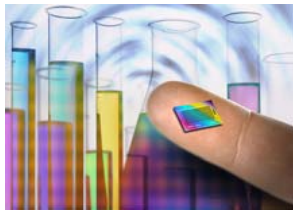
Agilent

Fluidigm, …

Talk Outline

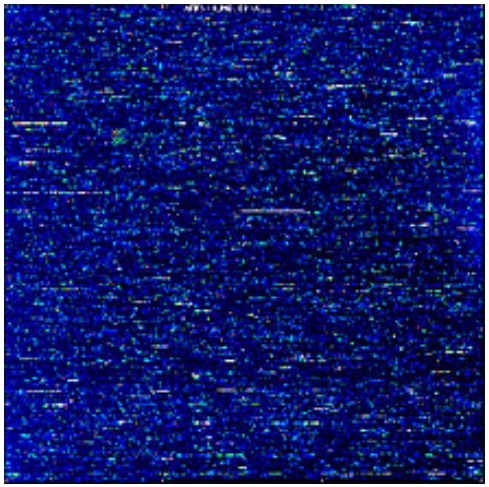
- Motivation
- Technology Overview
  - Microarrays
  - Continuous-flow microfluidics: channel-based biochips
  - “Digital” microfluidics: droplet-based biochips
- Design Automation Methods
  - Synthesis
  - Placement
  - Testing
  - Routing
- Conclusions

Classification of Biochips



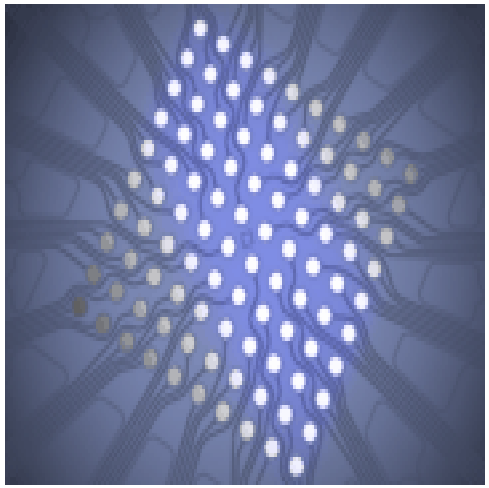
Microarray

- 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

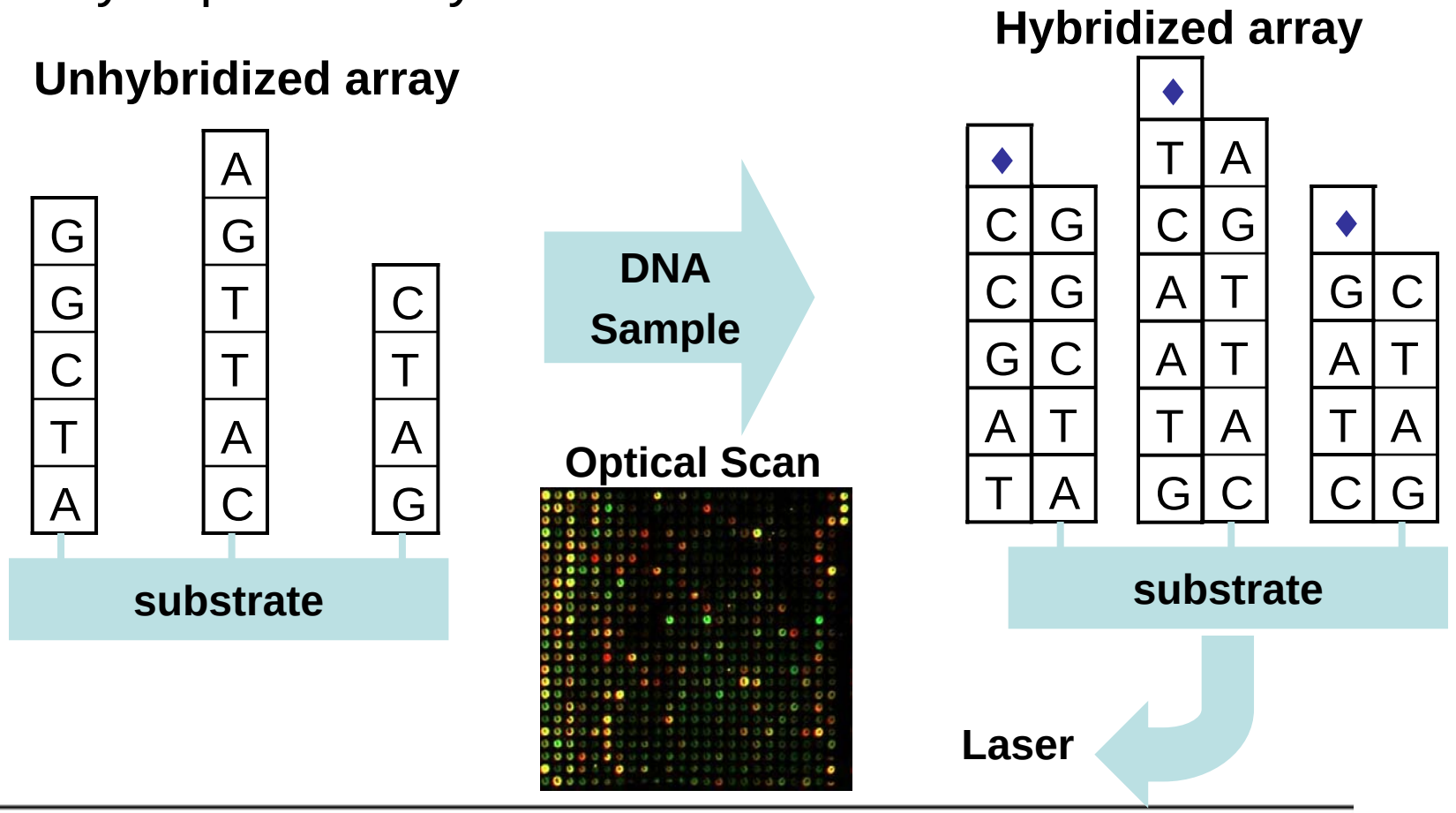
DNA microarray from Infineon AG
http://www.infineon.com



NanoChip ® microarray from Nanogen
http://www.nanogen.com

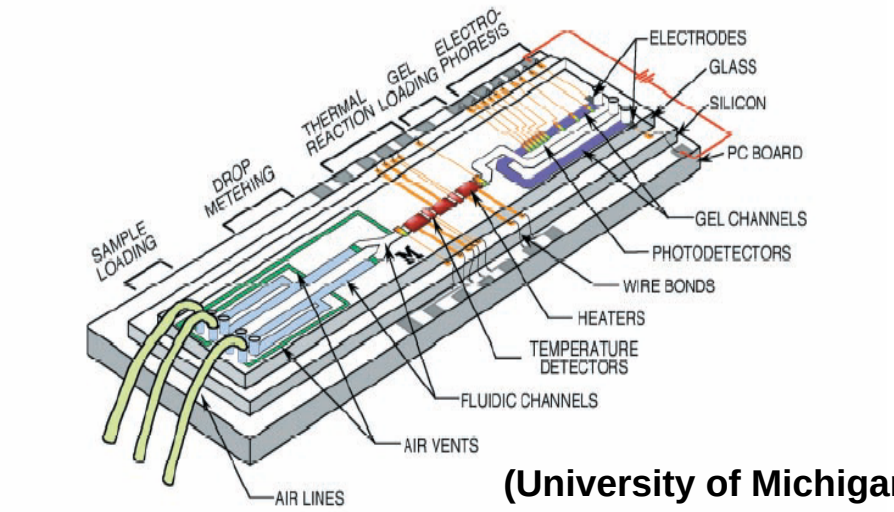
DNA Arrays

- Gene Chips
- Only implement hybridization reaction

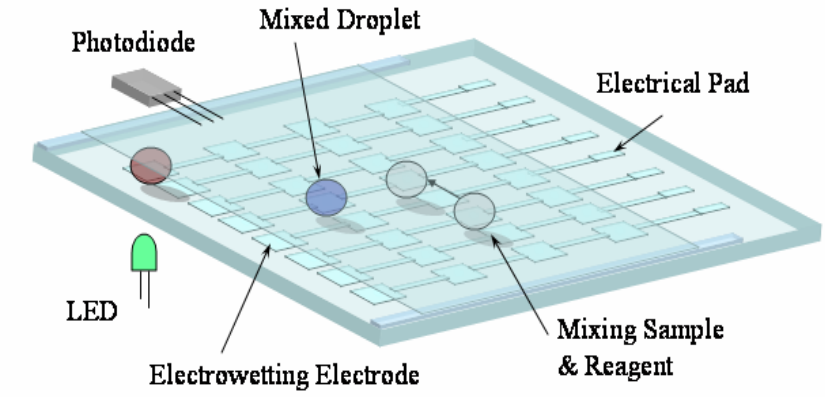


Microfluidics

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

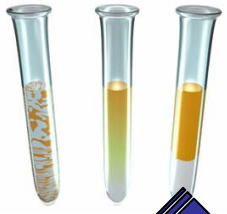


(University of Michigan)
1998



(Duke University)
2002

Motivation for Microfluidics



Test tubes

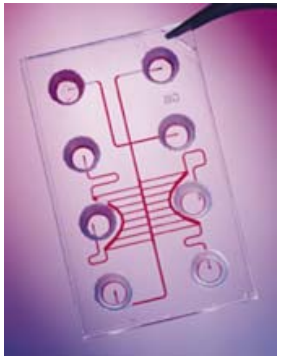
- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

Robotics

- ☑ Automation
- ☑ Integration
- ☐ Miniaturization

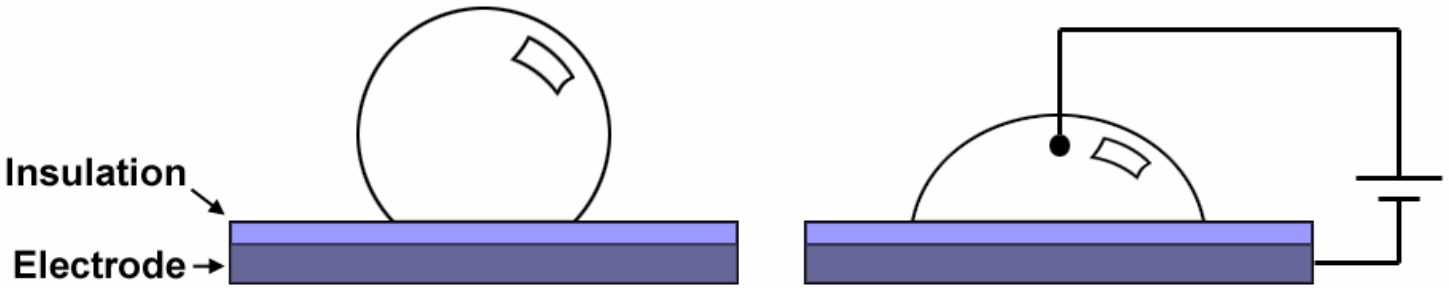
Microfluidics

- ☑ 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

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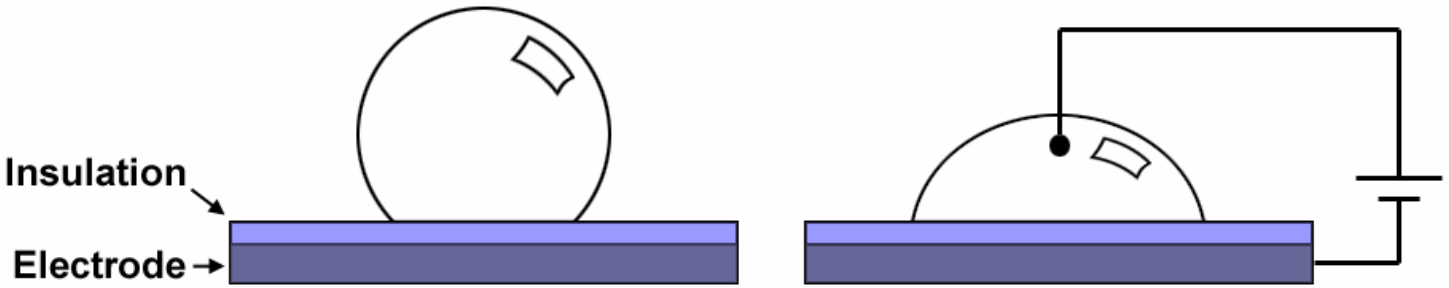

No Potential
A droplet on a hydrophobic surface originally has a large contact angle.

Applied Potential
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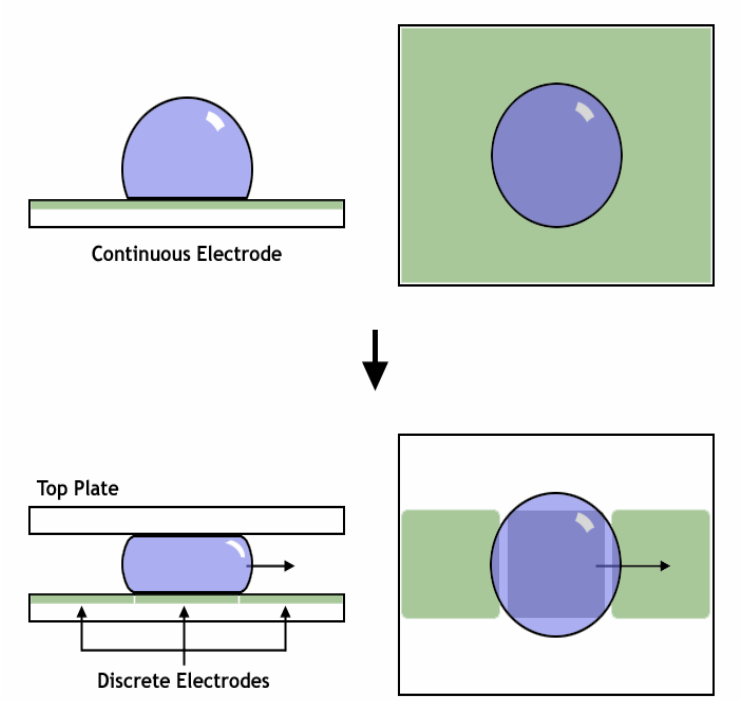
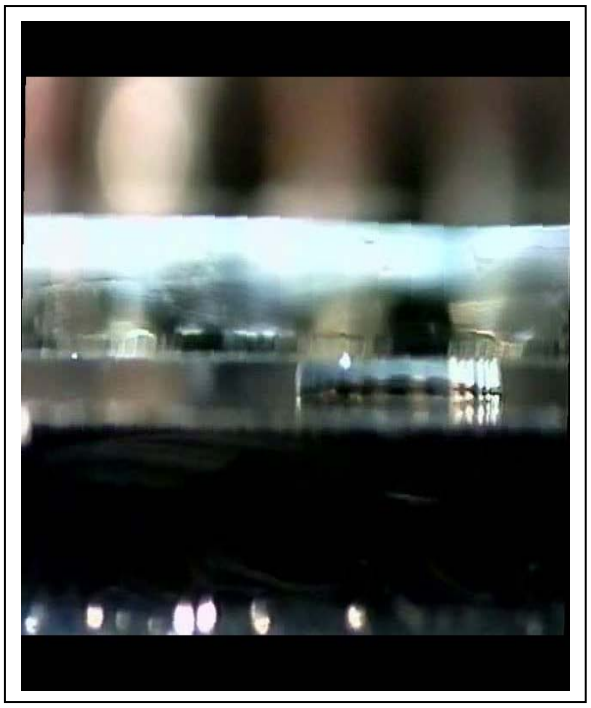
What is Digital Microfluidics?

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

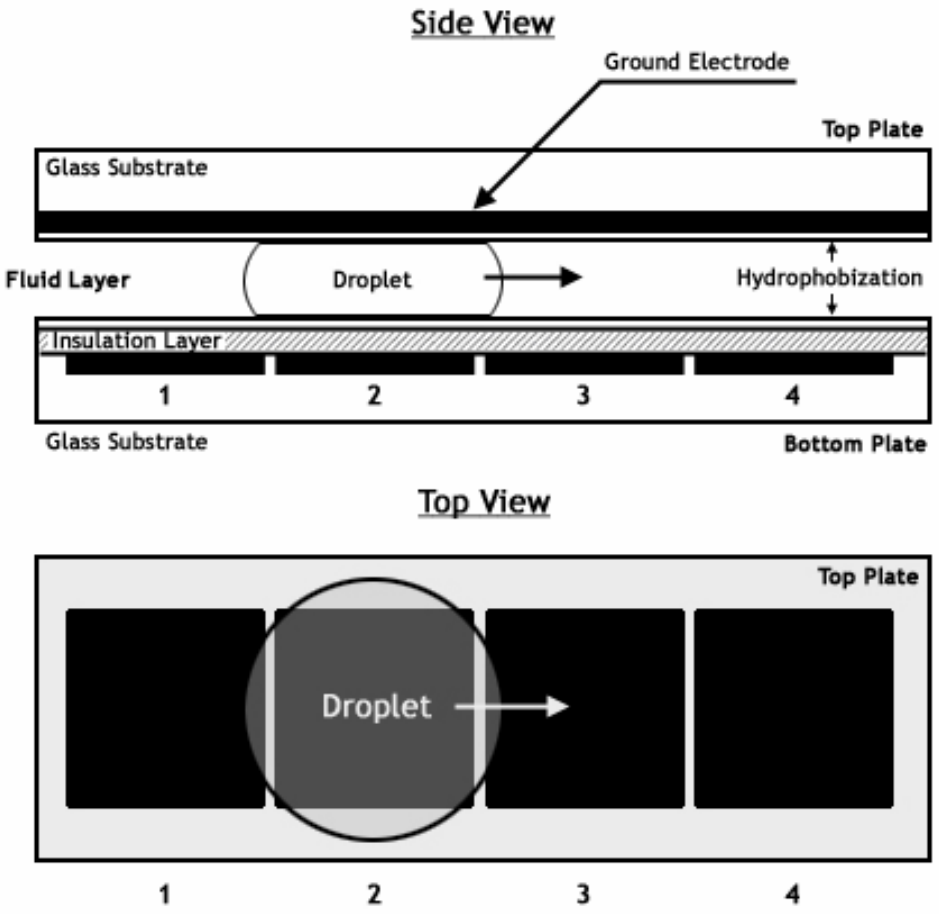




Droplet Transport (Side View)

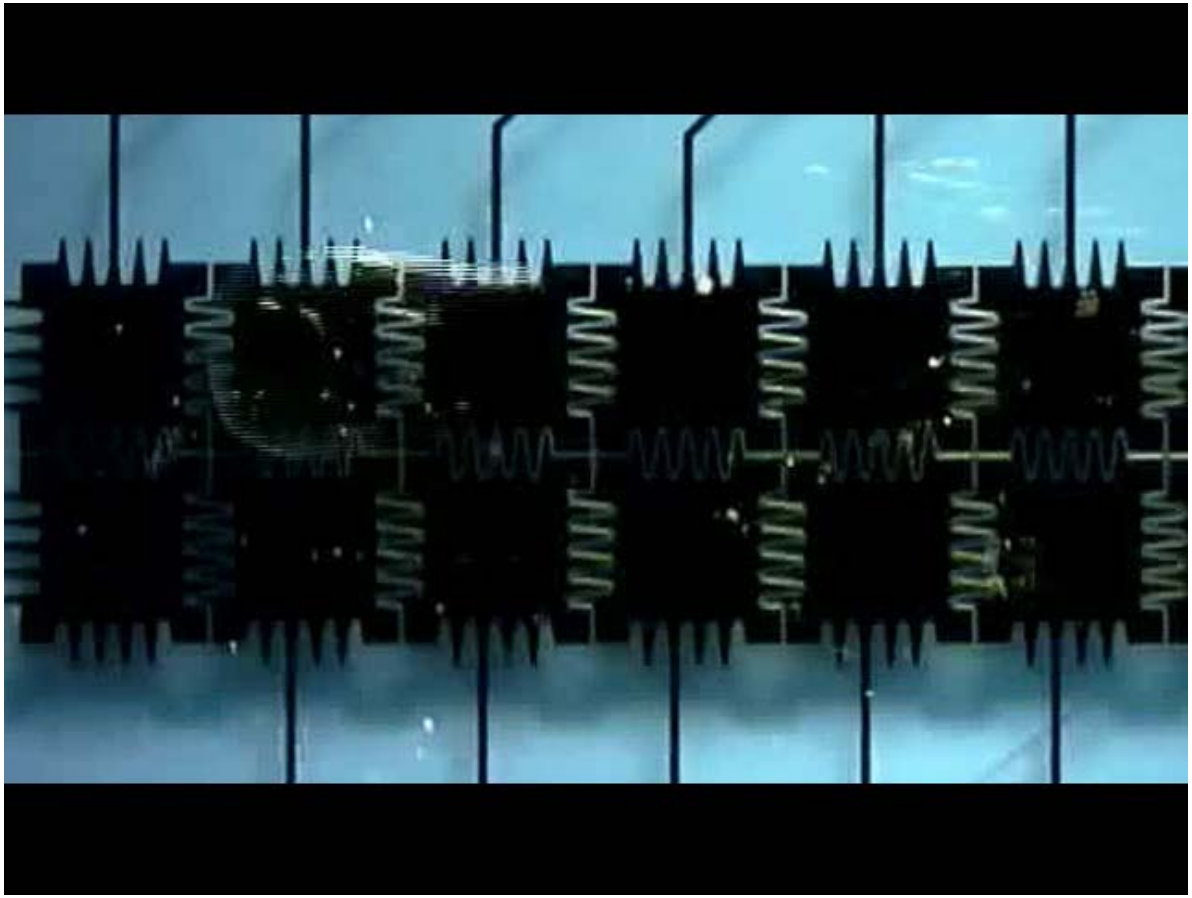
Note: oil is typically used to fill between the top and bottom plates to prevent evaporation.

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.

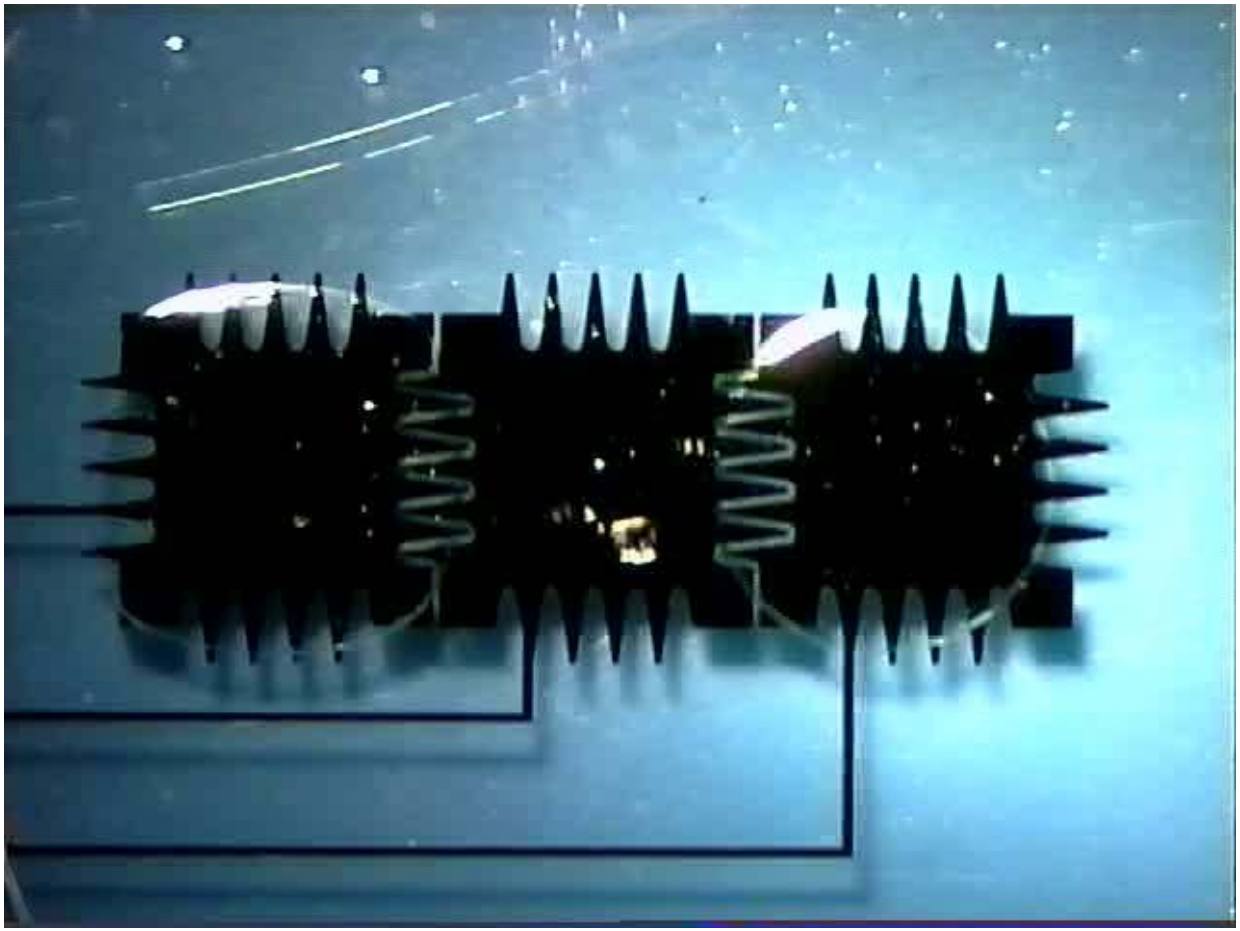
What is Digital Microfluidics?



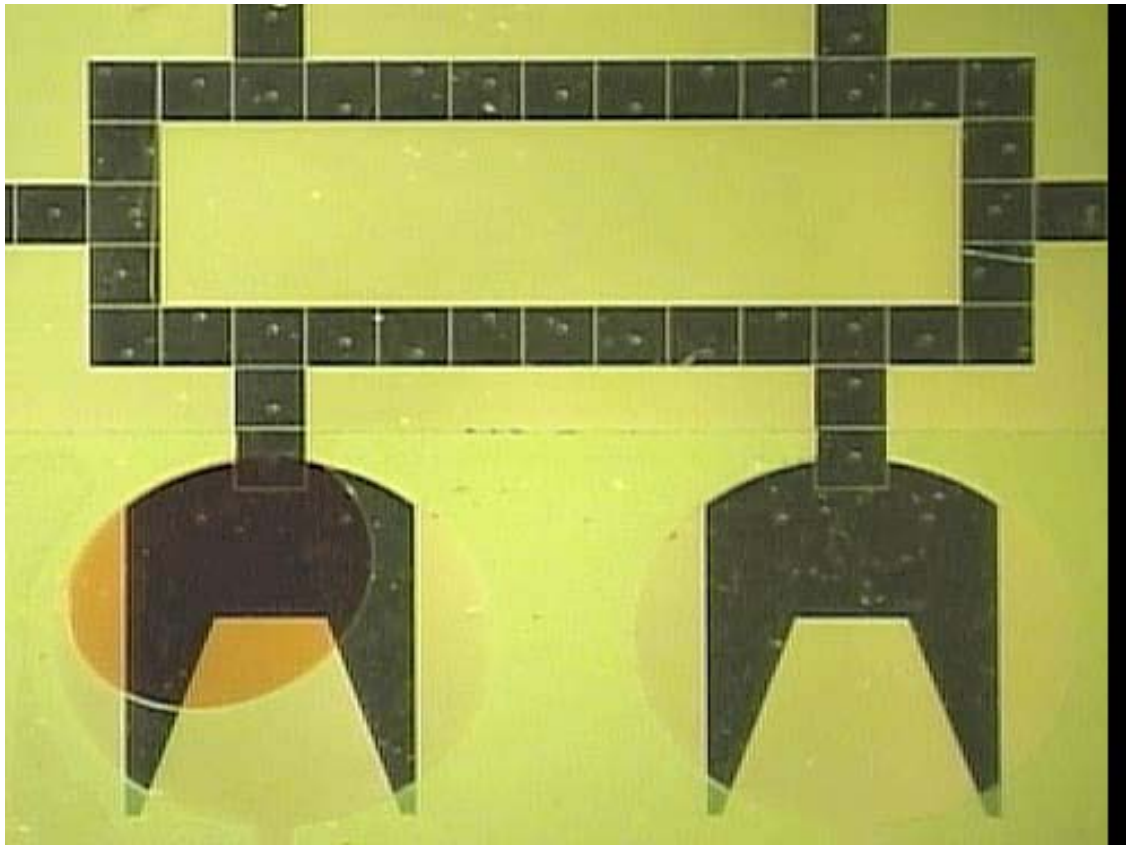
Transport
20 cm/s flow rates

What is Digital Microfluidics?

Splitting/Merging



What is Digital Microfluidics?

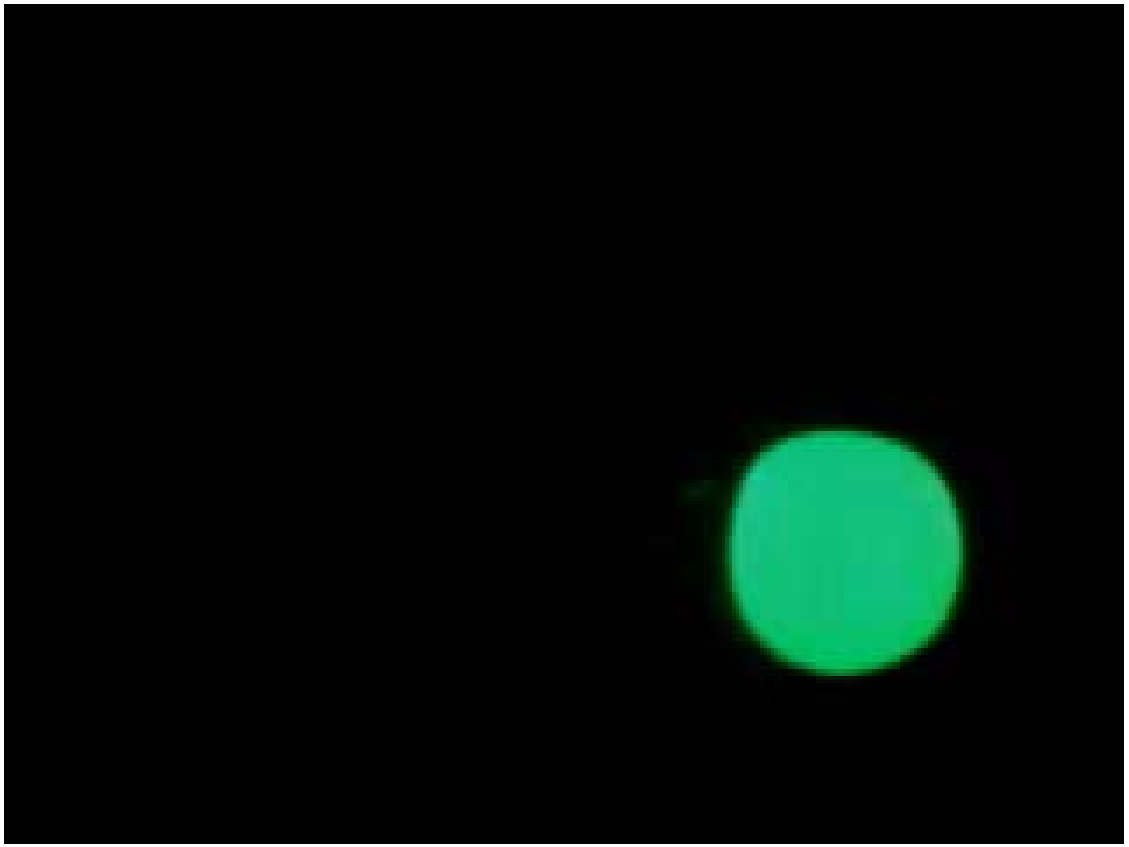


Droplet Formation

8 droplets in 3.6s

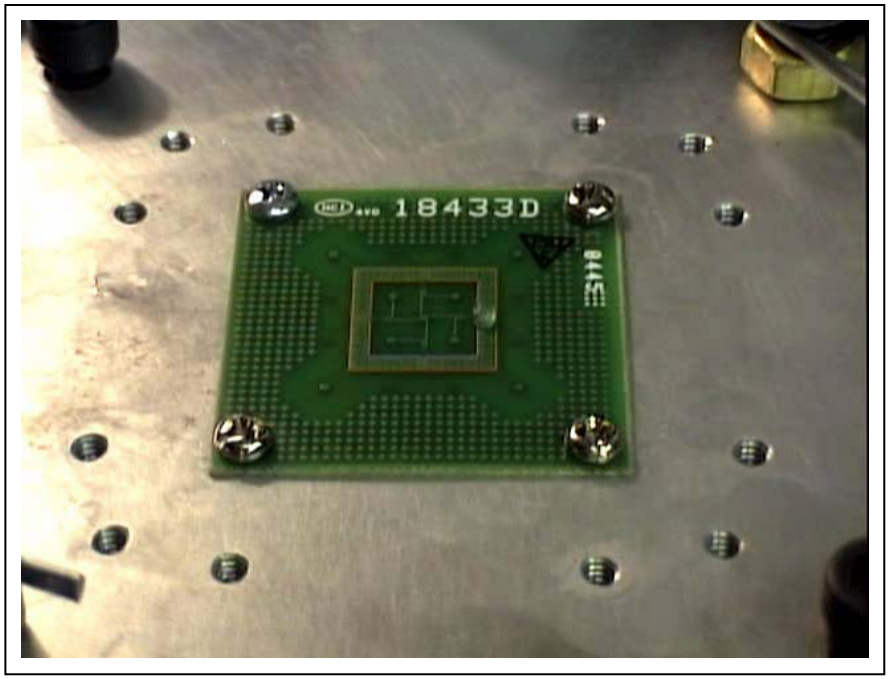
What is Digital Microfluidics?

Mixing



Advantages

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

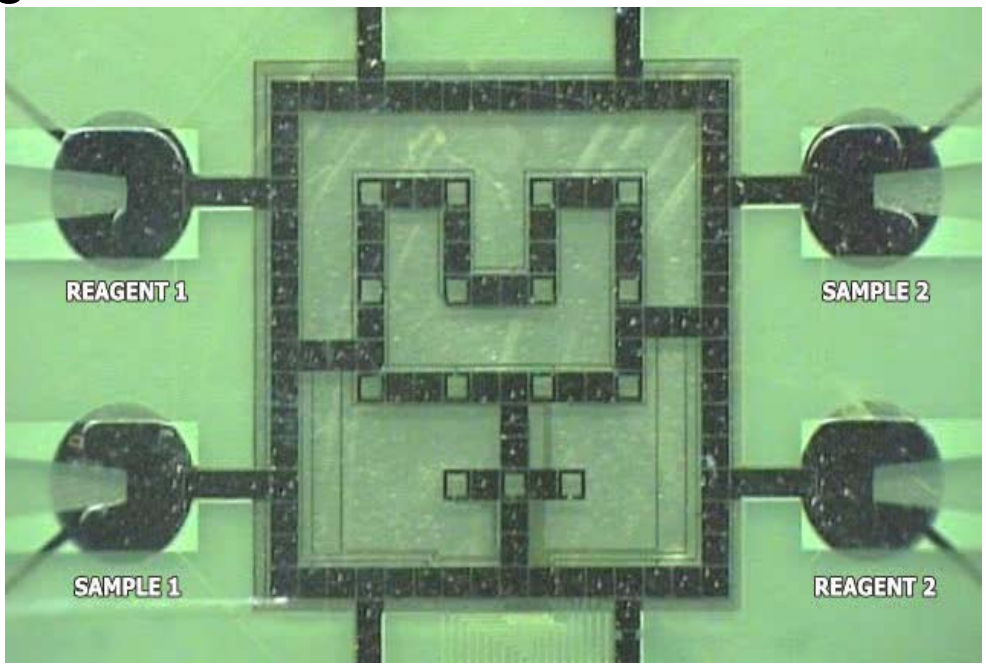


Droplet Transport on PCB (Isometric View)

- 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

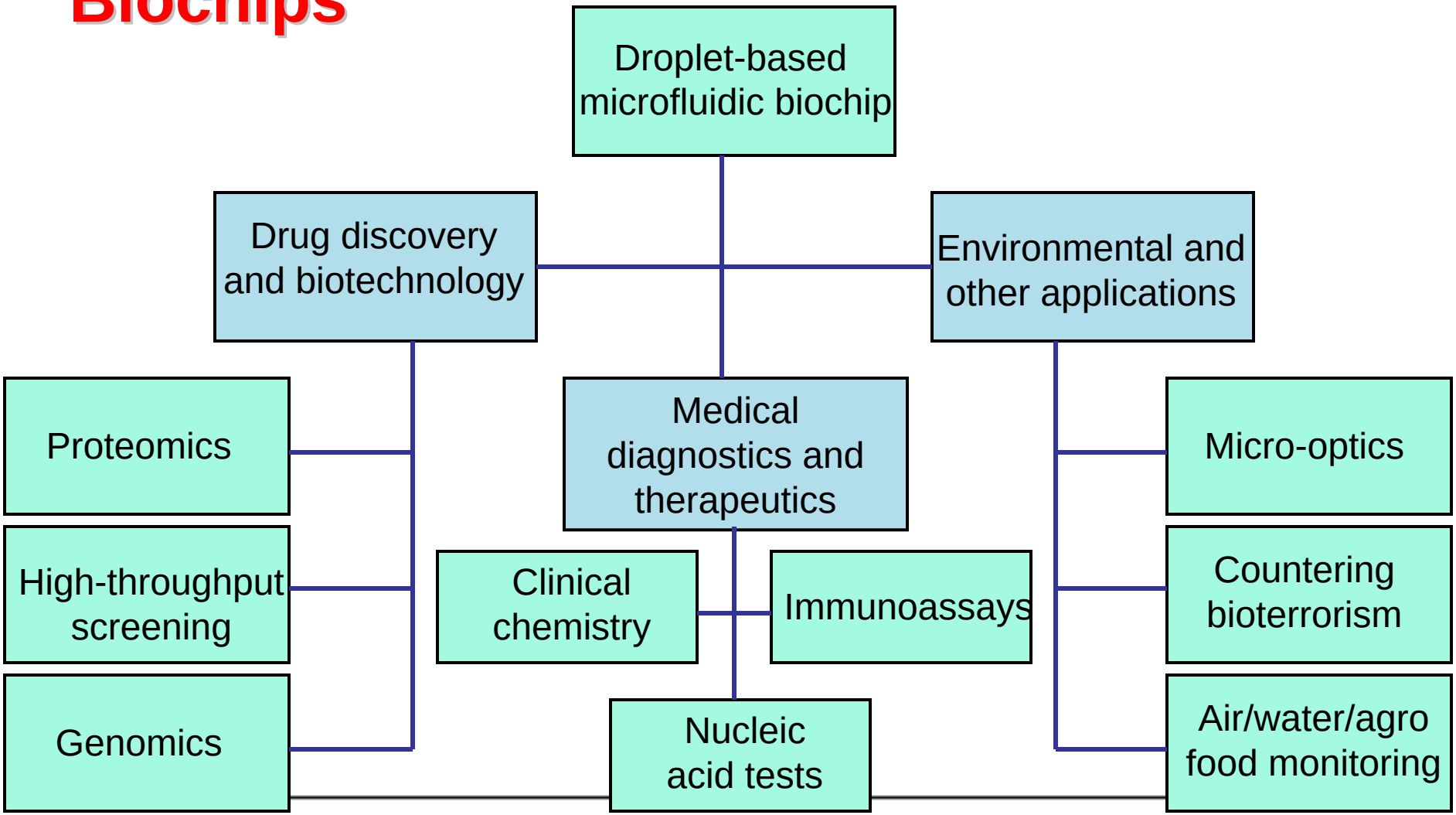
An Example

- Detection of lactate, glutamate and pyruvate has also been demonstrated.
- Biochip used for multiplexed in-vitro diagnostics on human physiological fluids



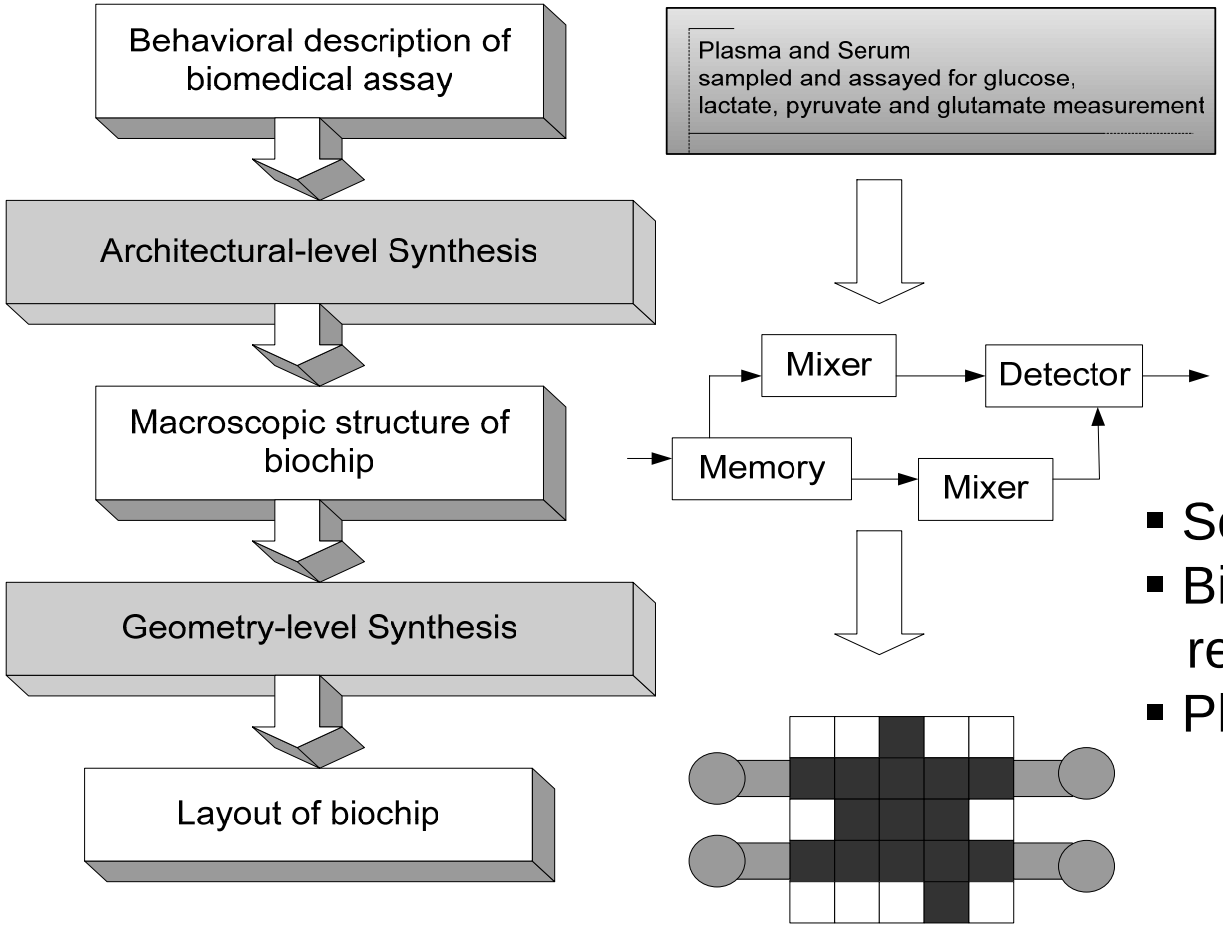
Fabricated microfluidic array used for multiplexed biomedical assays

Applications of Digital Microfluidic Biochips



Synthesis Methodology

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



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

Simulation Experiments (Cont.)

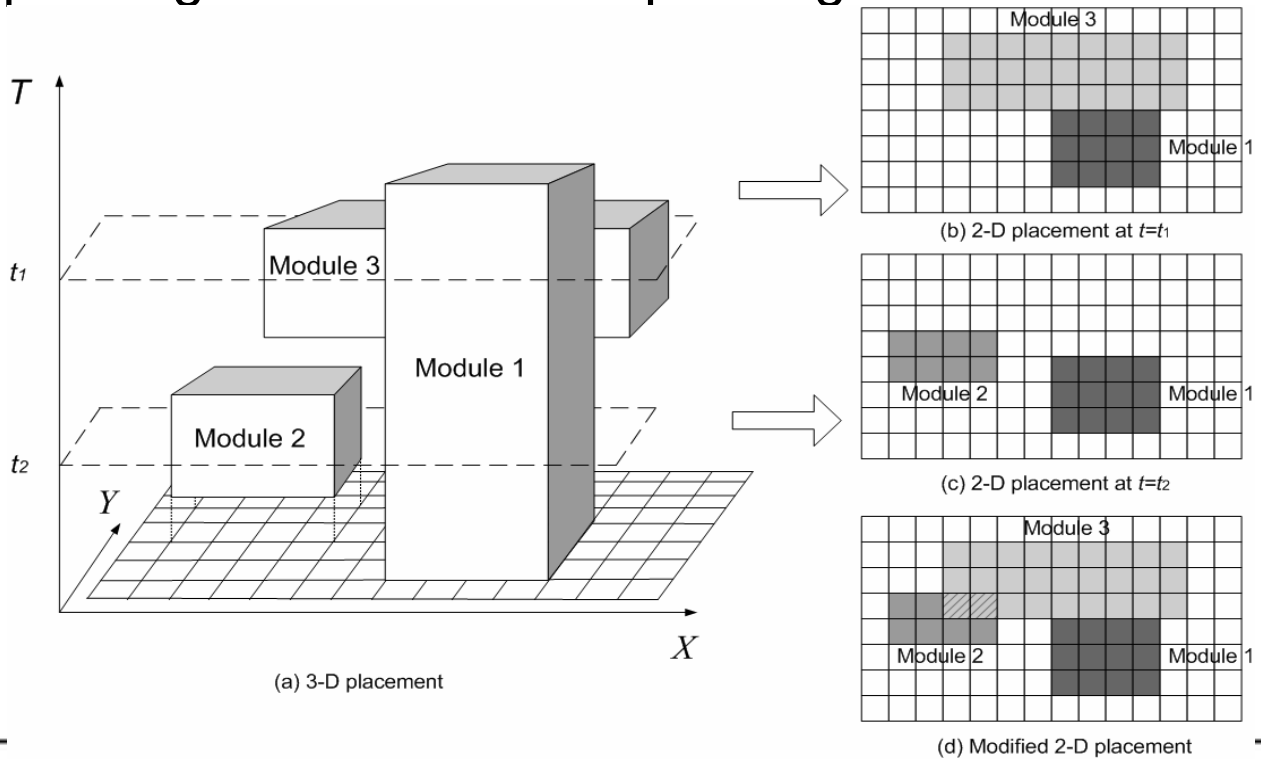
- **Five examples (four samples)** S_1: Plasma, S_2: Serum, S_3: Urine, S_4: Saliva, Assay1: Glucose assay, Assay2: Lactate assay, Assay3: Pyruvate assay, Assay4: Glutamate assay

Example	**Description**
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

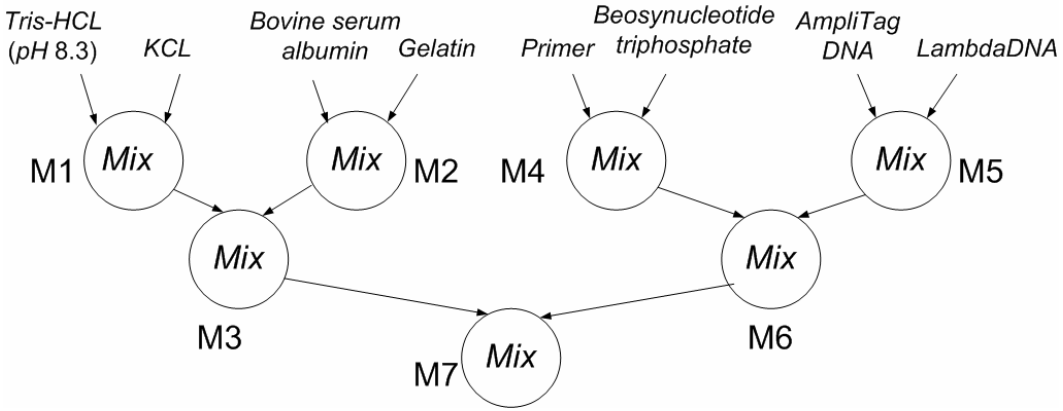
(Su and Chakrabarty, DATE'05)

- 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

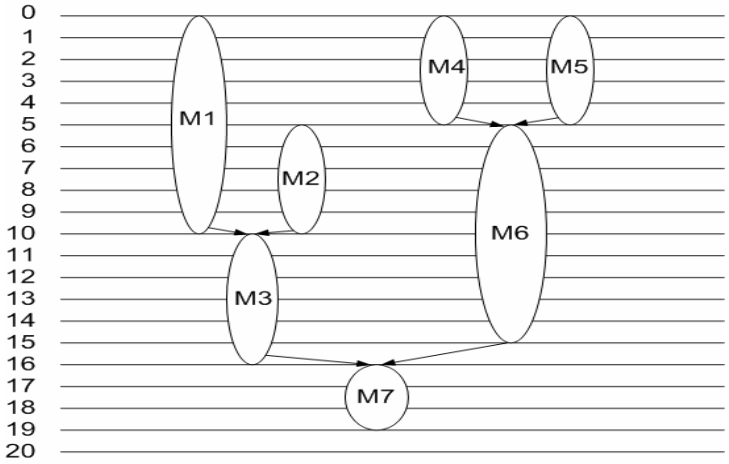


Reduction from 3_D placement to a modified 2-D placement

Application to PCR



Protocol of PCR (mixing phase)



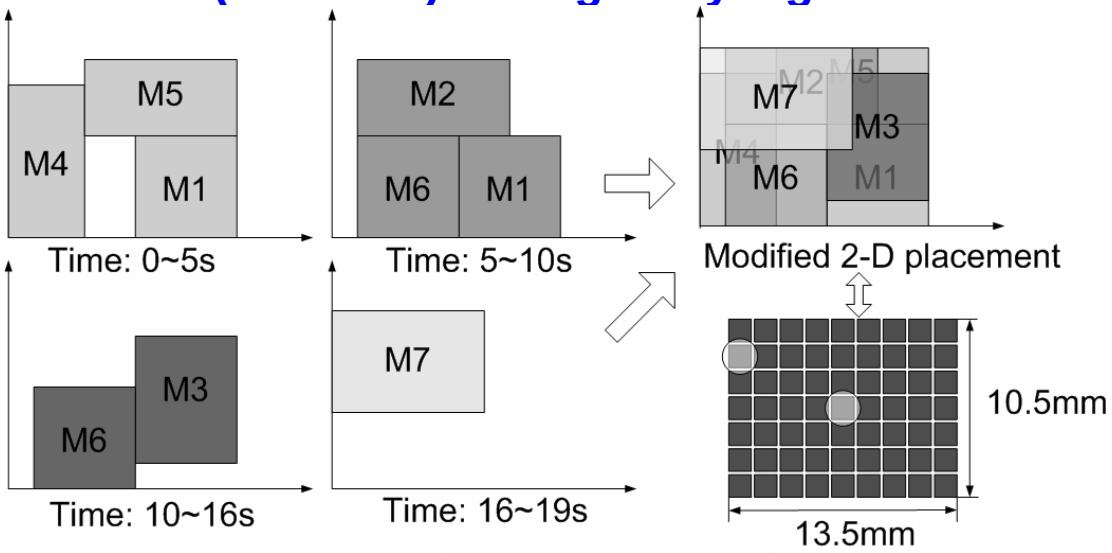
Schedule of PCR

Resource binding in PCR

Operation	Hardware	Module	Mixing time
M1	2x2 electrode array	4x4 cells	10s
M2	4-electrode linear array	3x6 cells	5s
M3	2x3 electrode array	4x5 cells	6s
M4	4-electrode linear array	3x6 cells	5s
M5	4-electrode linear array	3x6 cells	5s
M6	2x2 electrode array	4x4 cells	10s
M7	2x4 electrode array	4x6 cells	3s

Application to PCR (Cont.)

Baseline: 84 cells (189mm^2) from greedy algorithm



Placement from the simulated annealing-based procedure

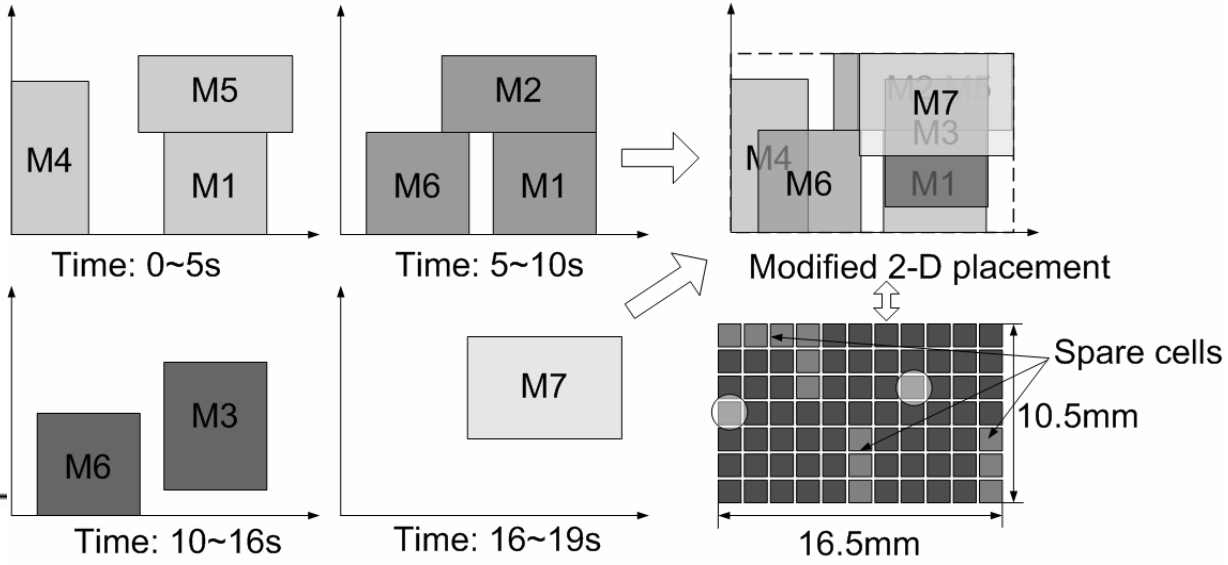
Area: 7x9=63 cells

FTI: 0.1270

Placement from enhanced module placement procedure

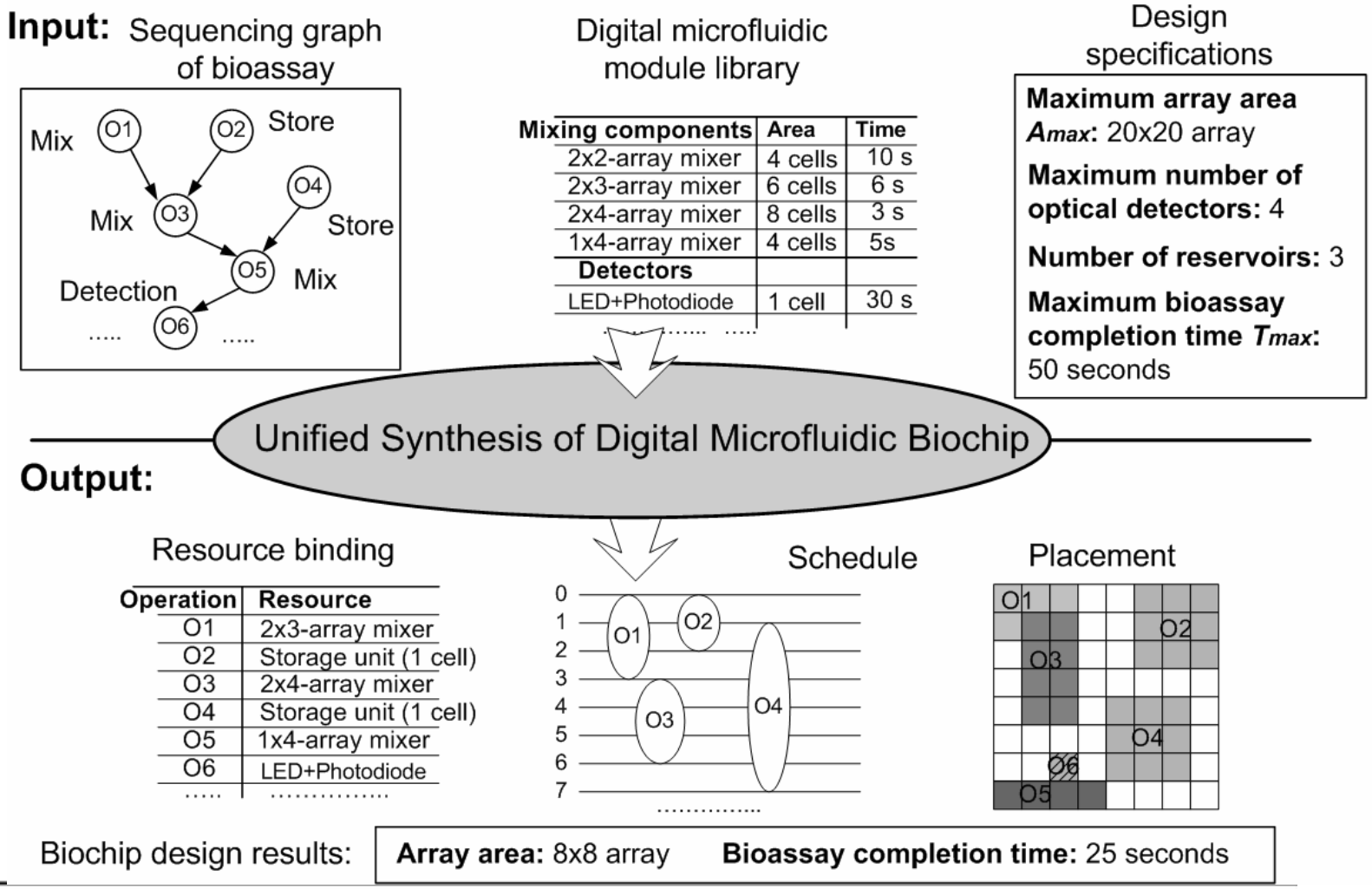
Area: 7x11=77 cells

FTI: 0.8052



Unified Synthesis Methodology

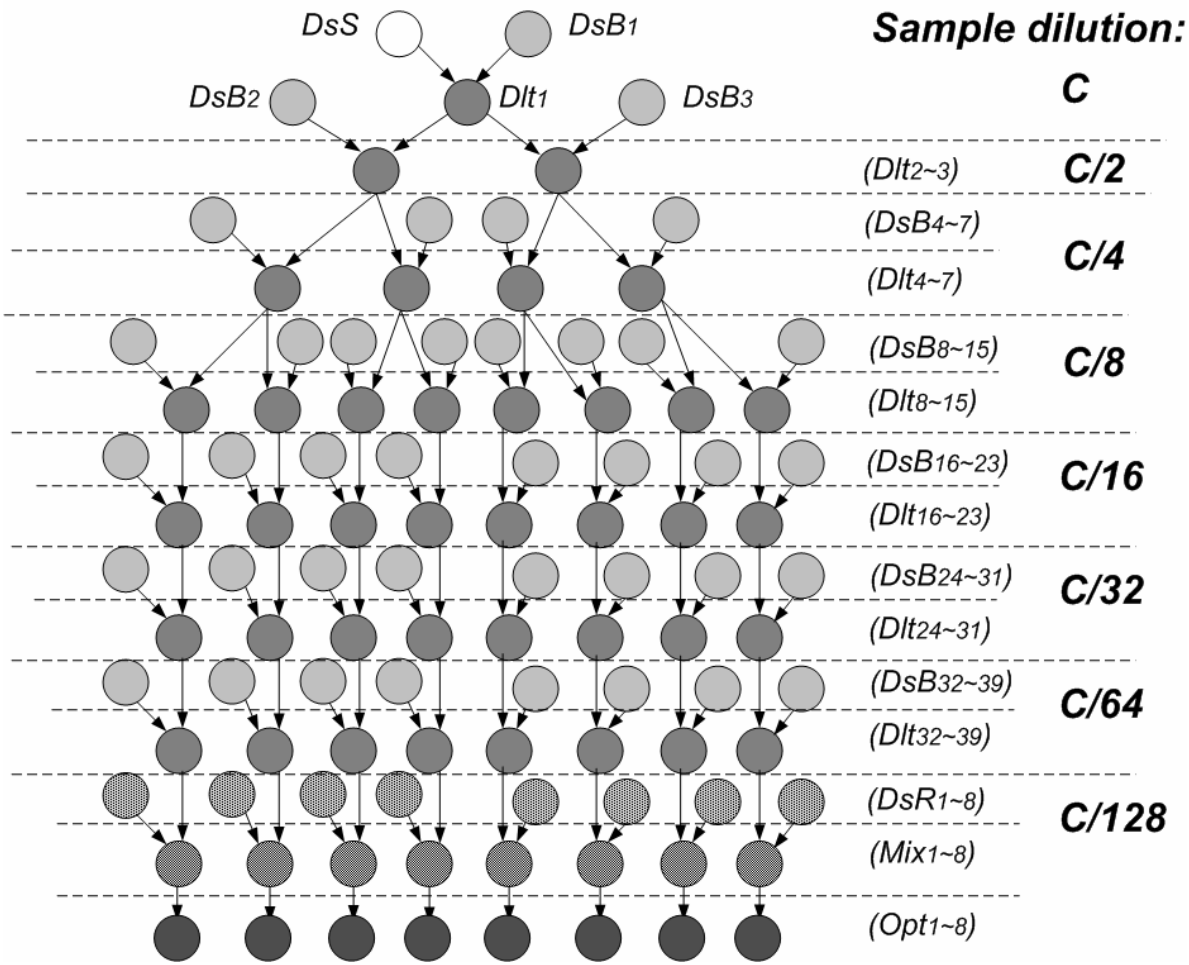
Su and Chakrabarty (DAC 2005)



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***

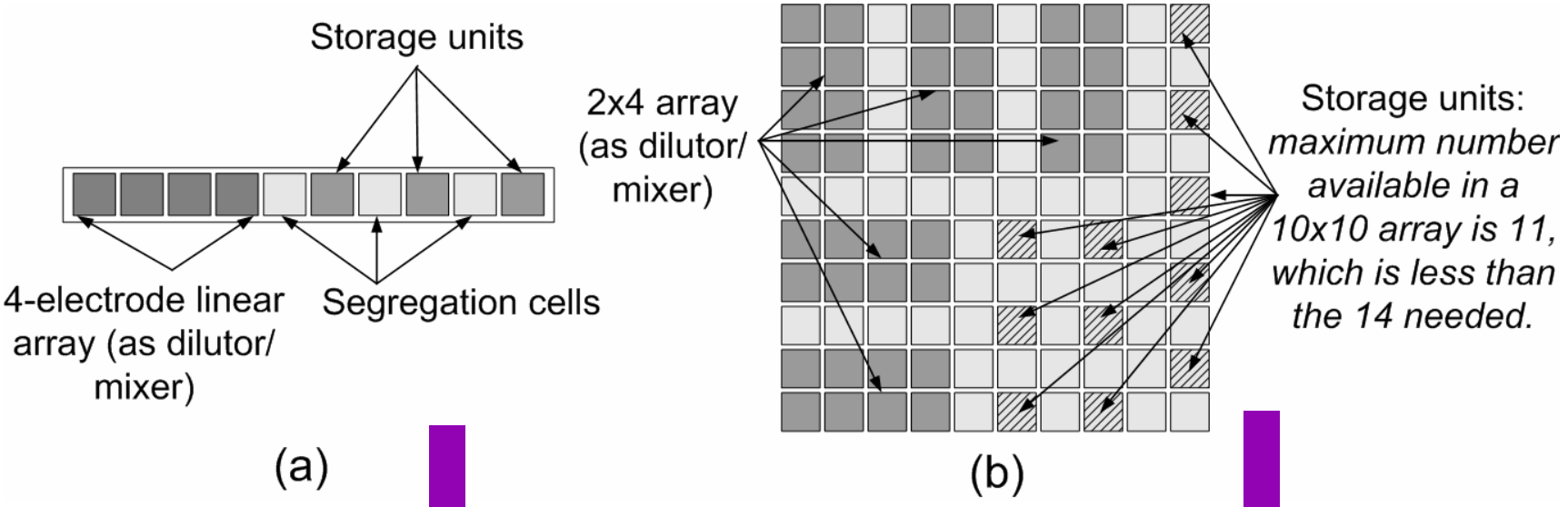
Protein Assay (Cont.)

- Microfluidic module library for synthesis

Operation	**Resource**	**Operation Time (s)**
DsS; DsB; DsR	On-chip reservoir/dispensing port	7
Dlt	2x2-array dilutor	12
	2x3-array dilutor	8
	2x4-array dilutor	5
	4-electrode linear array dilutor	7
Mix	2x2-array mixer	10
	2x3-array mixer	6
	2x4-array mixer	3
	4-electrode linear array mixer	5
Opt	LED+Photodiode	30
Storage	Single cell	N/A

Design for Protein Assay

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



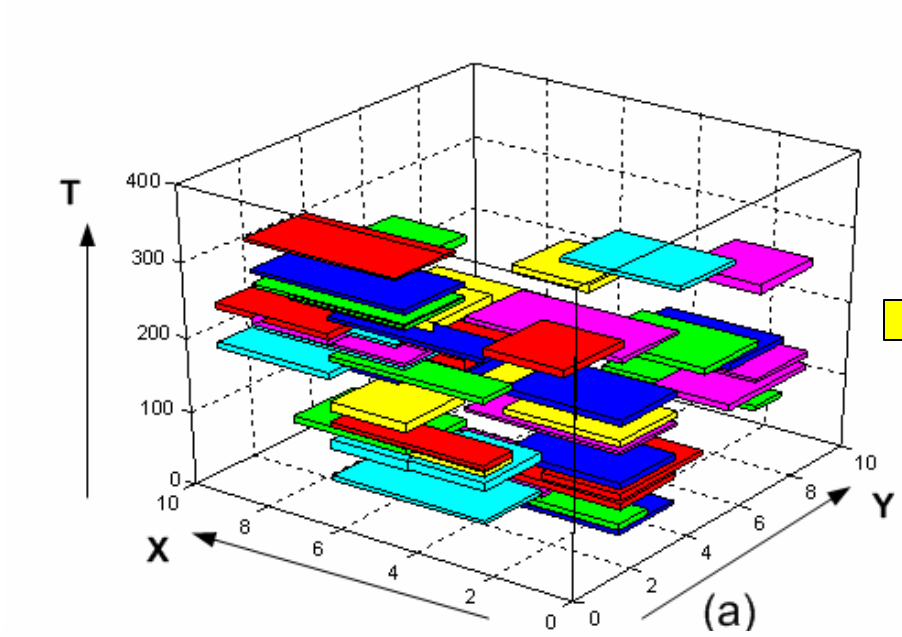
T = 560 s > T_{max} = 400 s

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

Fail to meet the design specification!

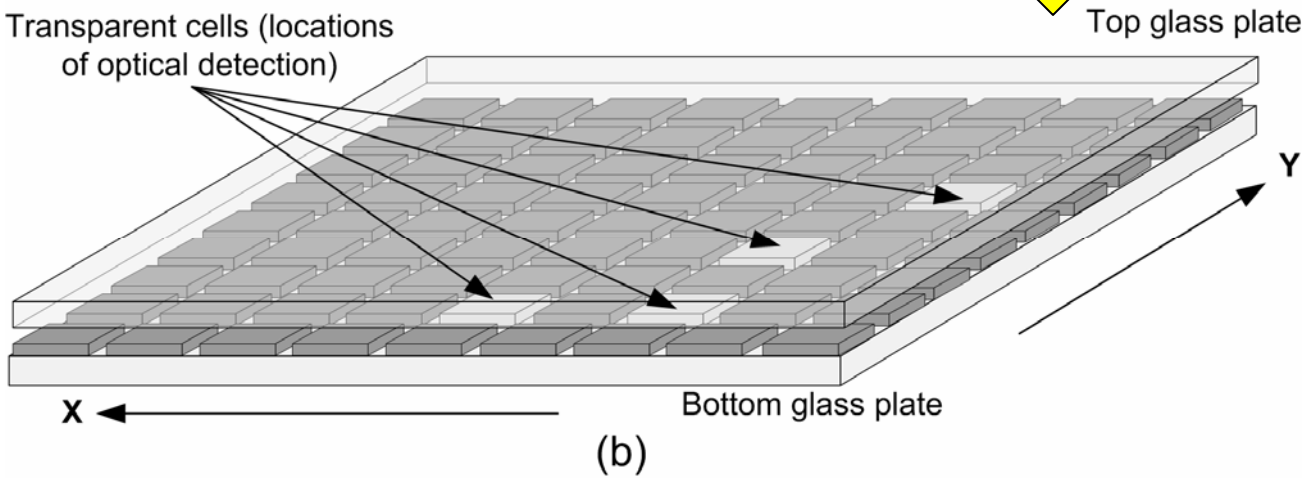
Experimental Evaluation (Cont.)

- Results of the unified synthesis method



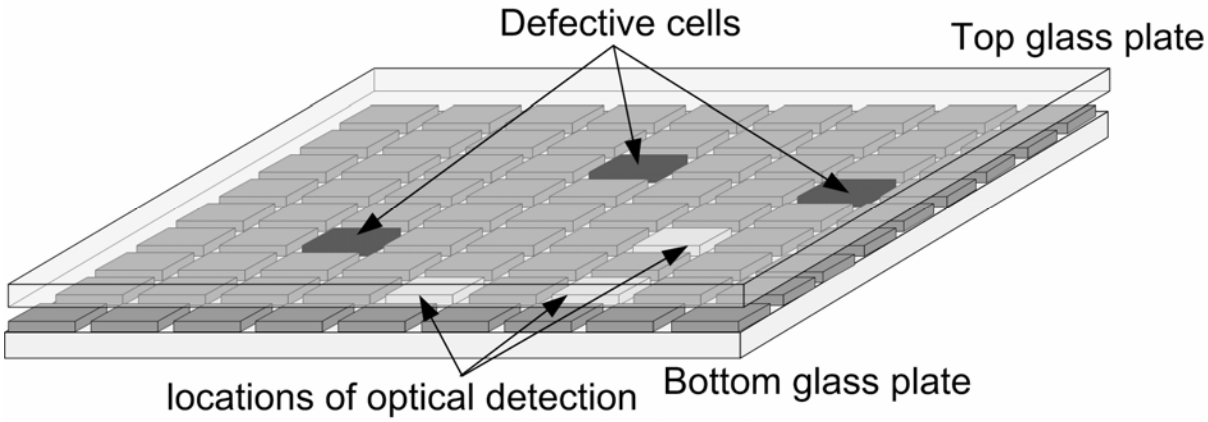
Bioassay completion time *T*: 363 seconds

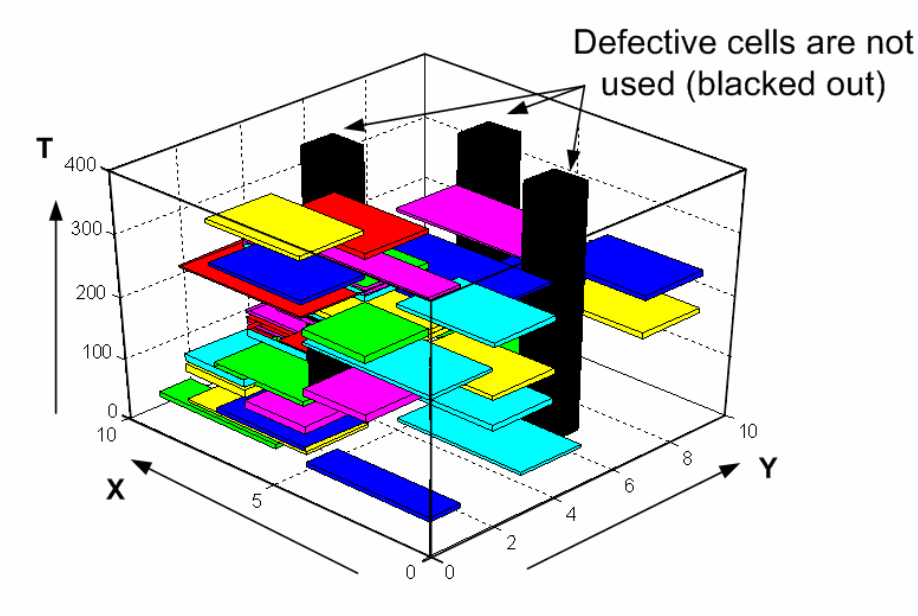
Biochip array: 9x9 array



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)

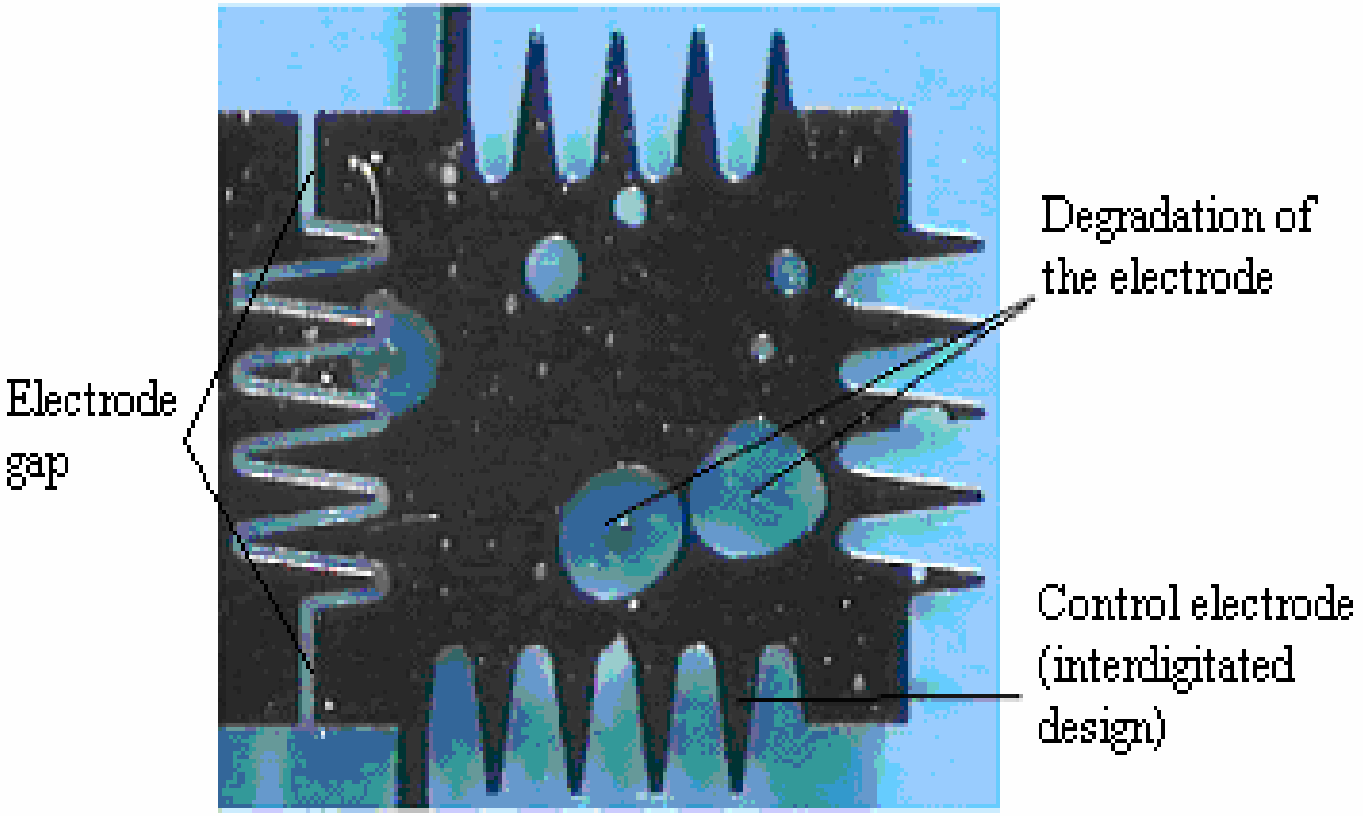
Catastrophic Faults:

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

Parametric Faults:

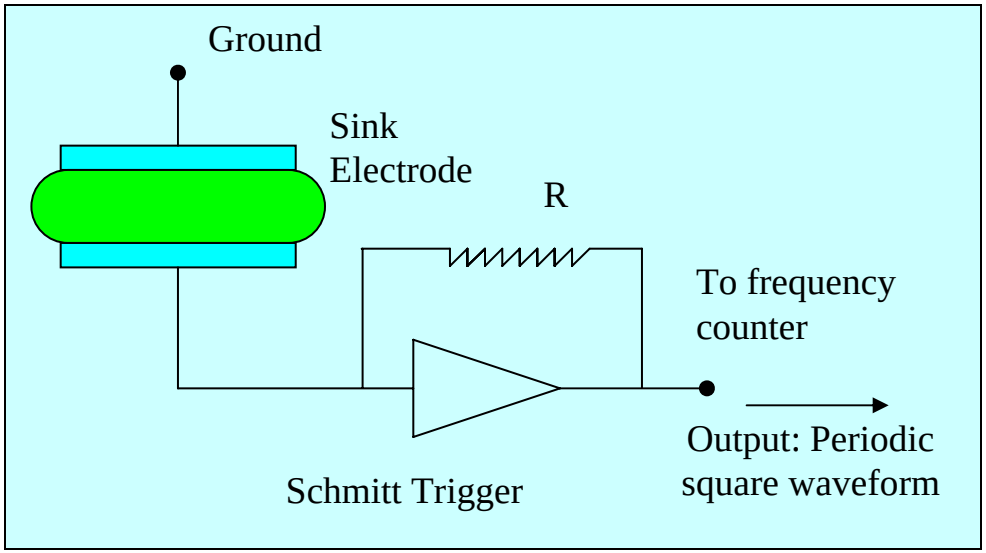
- Geometrical parameter deviation — **Manufacturing**
- Degradation of the insulator — **Manufacturing**
- Change in the viscosity of the droplet and the filler medium — **Operational**

Example of Electrode Degradation



Unified Detection Mechanism

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



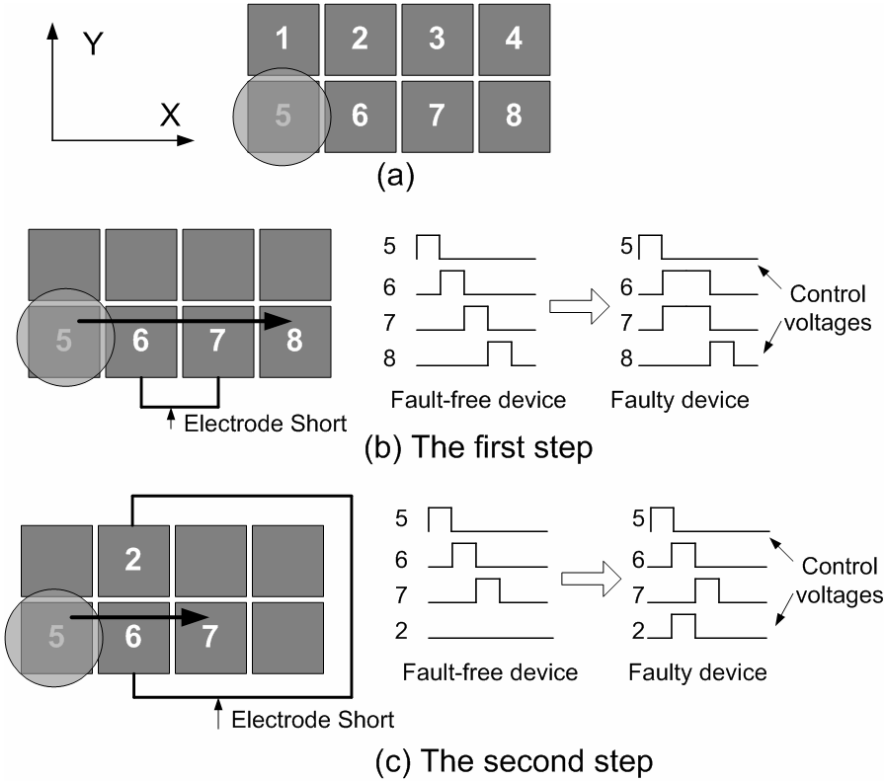
Capacitive changes reflected in electrical signals (Fluidic domain to electrical domain)

- If there is a droplet, output=1; otherwise, output=0
- Fault-free : there is a droplet between electrodes

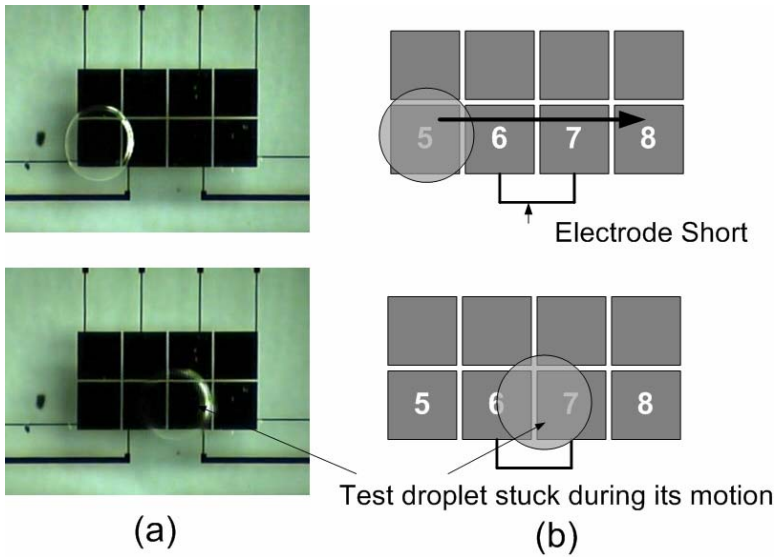
Faulty: there is no droplet.

Defect-Oriented Testing and Diagnosis (Su et al, ITC'05)

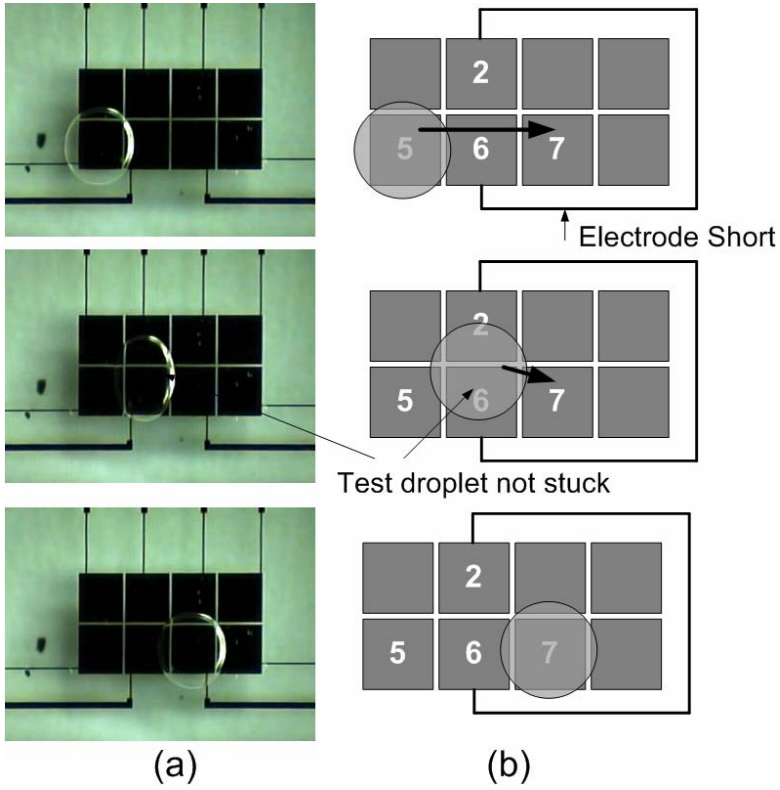
- Defect-Oriented Experiment
  - To simulate the effect of an electrode short on microfluidic behavior



Experimental Results and Analysis



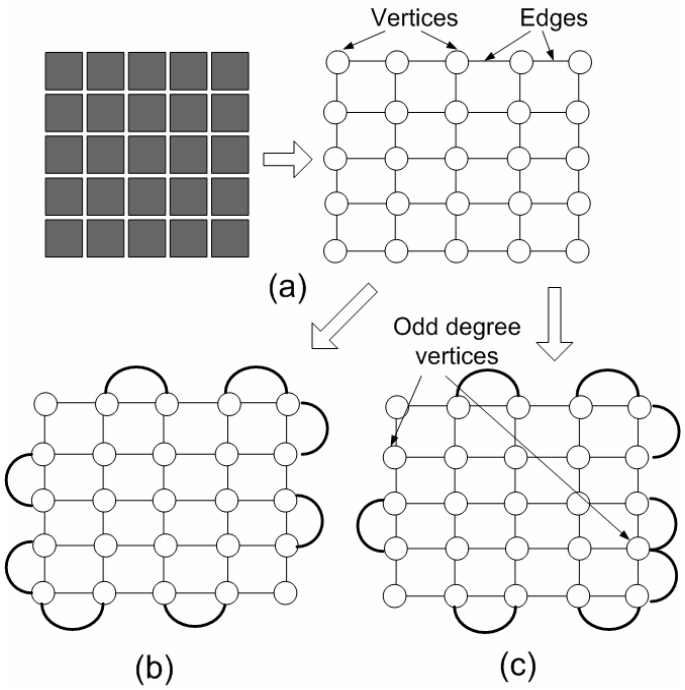
Experimental results and analysis for the first step



Experimental results and analysis for the second step

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;

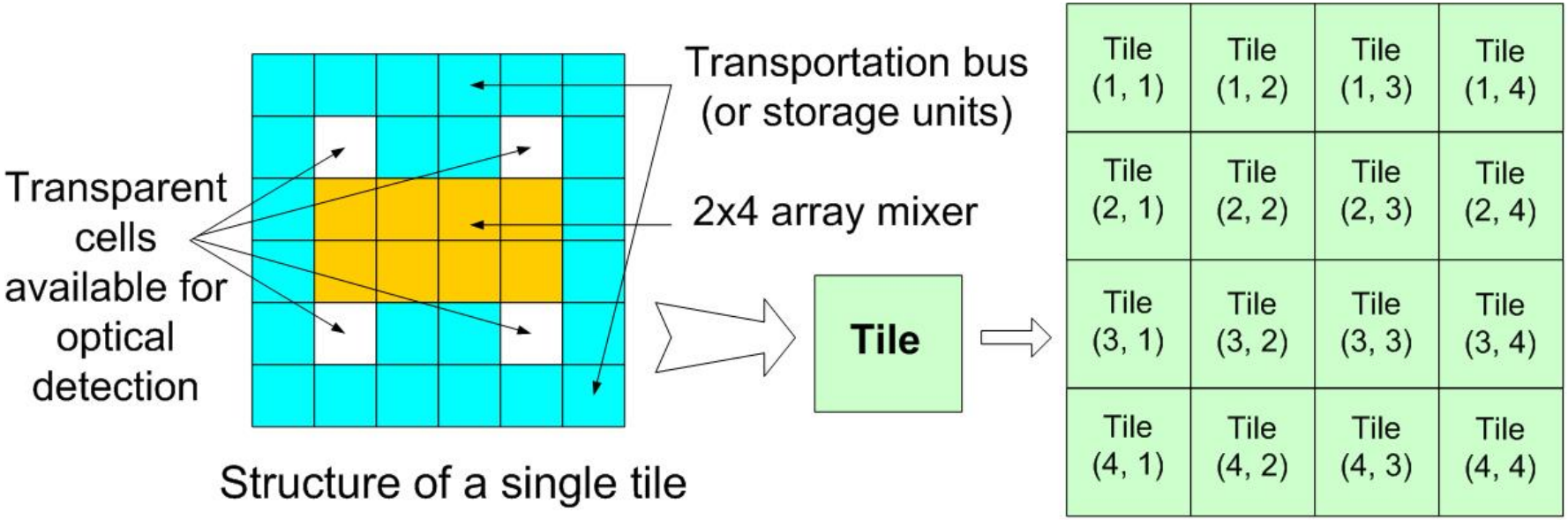
(b) eulerized graph containing an Euler circuit;

(c) eulerized graph containing an Euler path.

Tile-Based Architecture for Reconfiguration

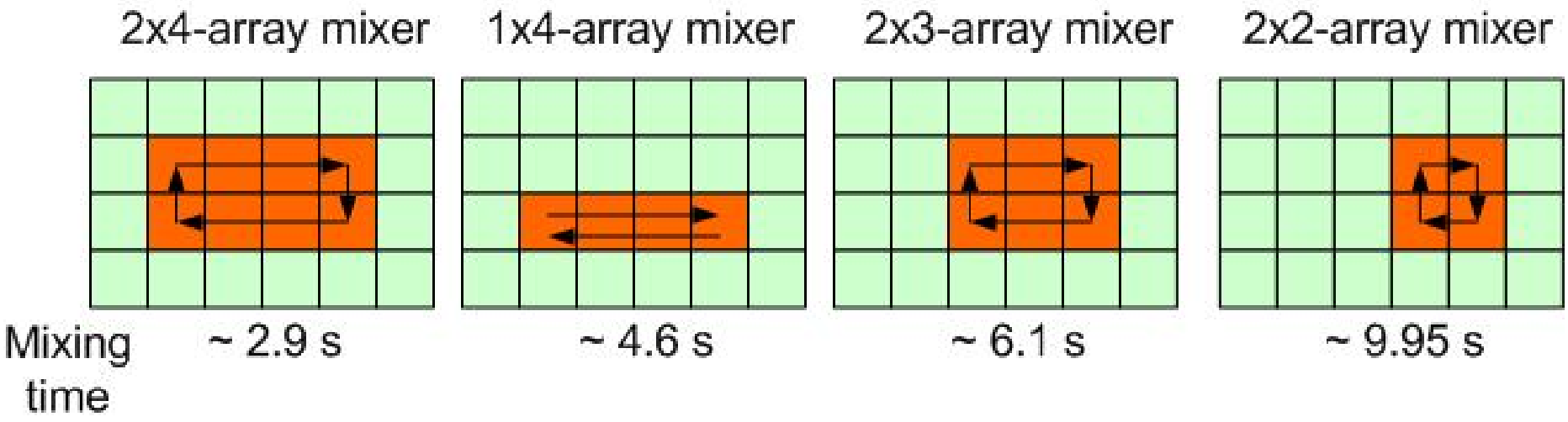
(Su and Chakrabarty, VTS'05)

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



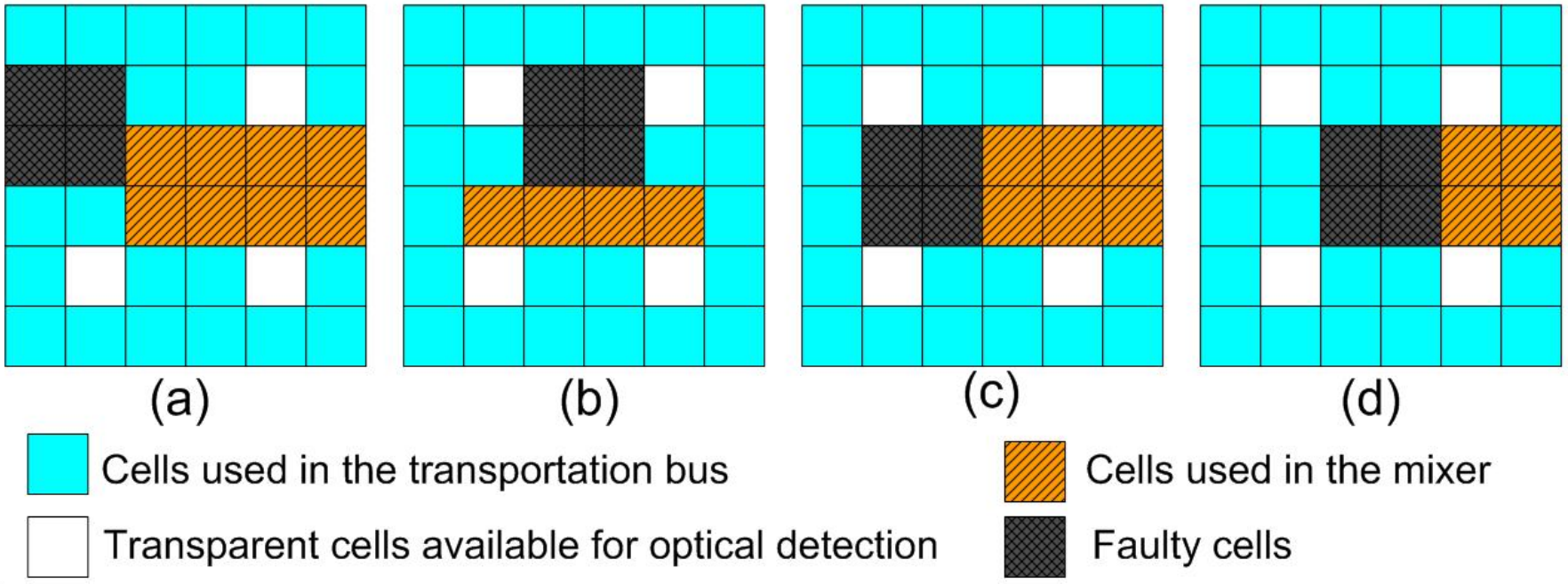
Reconfigurability

- 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

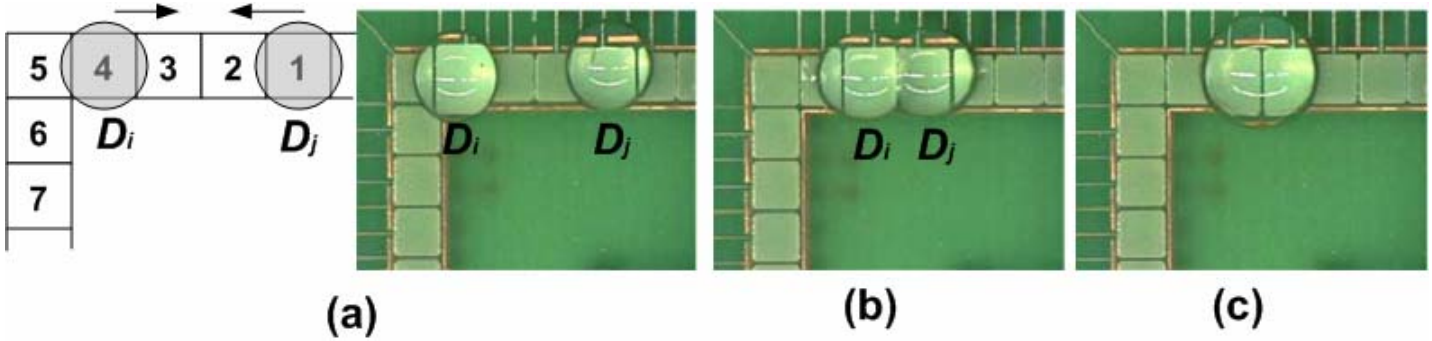
Assume two given droplets as D_i and D_j, and let $X_i(t)$ and $Y_i(t)$ denote the location of D_i at time t

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.

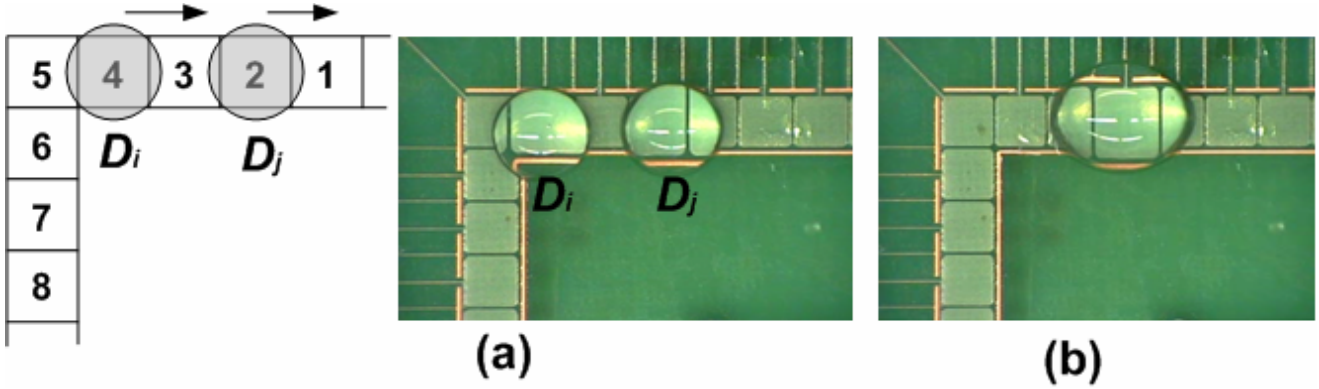
Rule #2: $|X_i(t+1) - X_j(t)| \geq 2$ or $|Y_i(t+1) - Y_j(t)| \geq 2$, i.e., the activated cell for droplet D_i cannot be adjacent to droplet D_j. Otherwise, there is more than one activated neighboring cell for D_j, which may leads to errant fluidic operation.

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

Experimental Verification

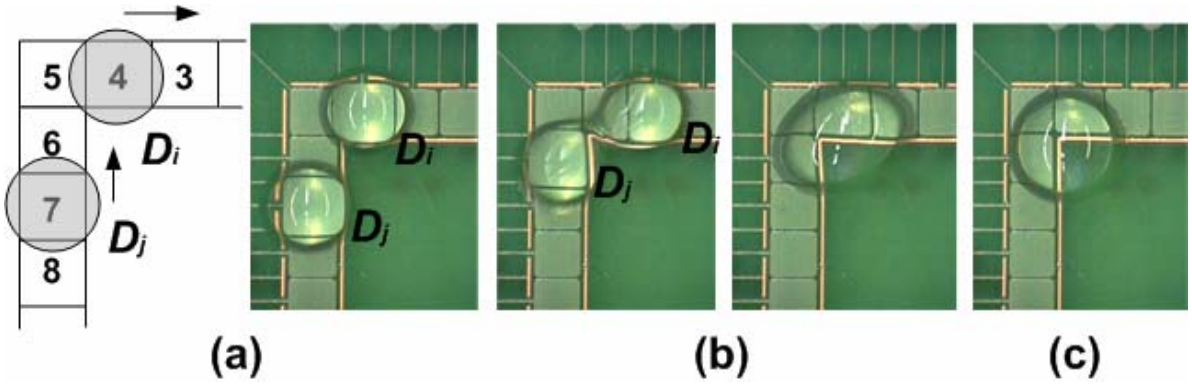


(a) Experimental verification of Rule #1: droplets begin on electrodes 1 and 4; (b) Electrodes 2 and 3 are activated, and 1 and 4 deactivated; (c) Merged droplet.



(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.)

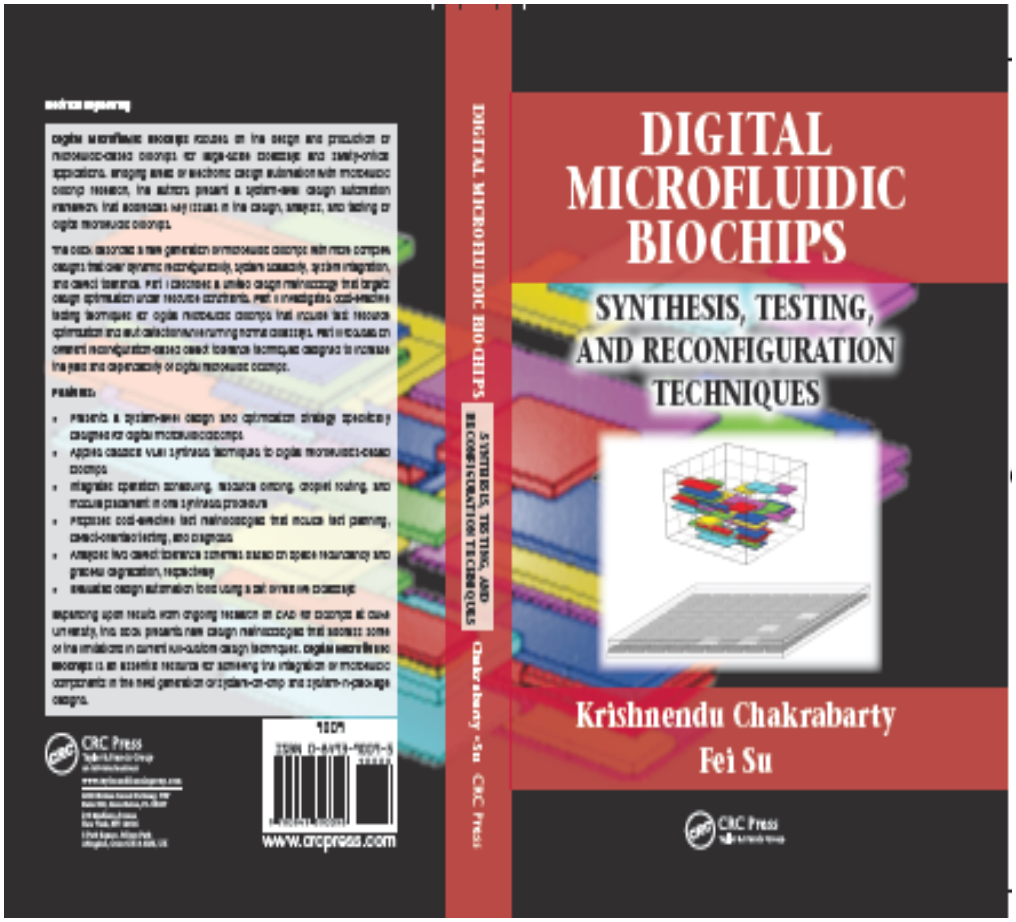


(a) Experimental verification of Rule #3: droplets begin on electrodes 4 and 7; (b) Electrodes 3 and 6 are activated, and 4 and 7 deactivated; (c) Merged droplet.

- 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



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