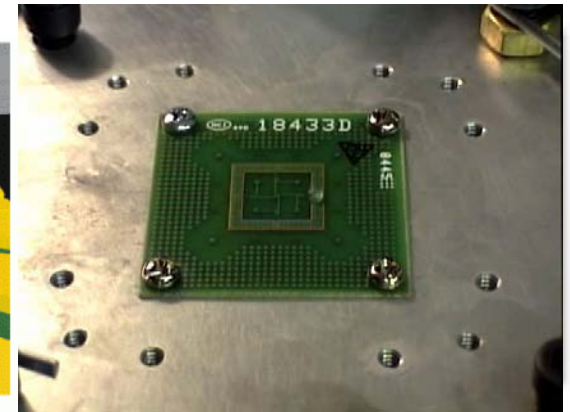
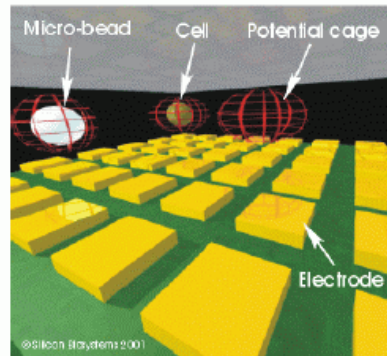
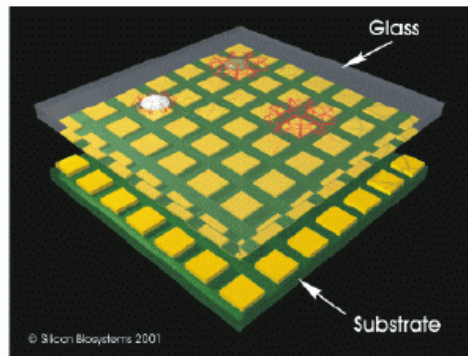


# Automated Design of Microfluidics-Based Biochips

*Connecting Biochemistry to Electronics CAD*



**Krishnendu Chakrabarty**

Department of Electrical and Computer Engineering  
Duke University  
Durham, North Carolina  
USA



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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



National Science Foundation  
WHERE DISCOVERIES BEGIN

# 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

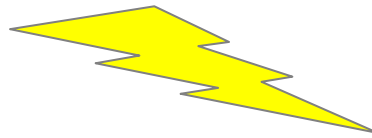


CLINICAL DIAGNOSTIC  
APPLICATION

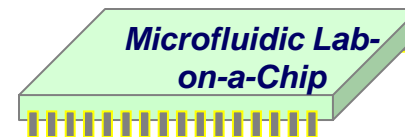


**Conventional Biochemical Analyzer**

*Shrink*



Lab-on-a-chip for  
CLINICAL DIAGNOSTICS



20nl sample

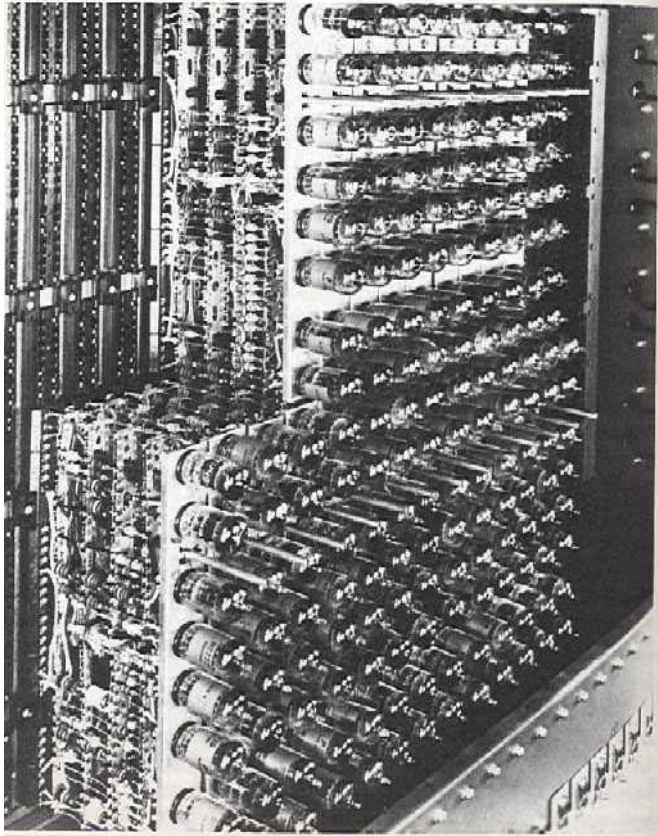


Higher throughput, minimal human intervention,  
smaller sample/reagent consumption, higher  
sensitivity, increased productivity

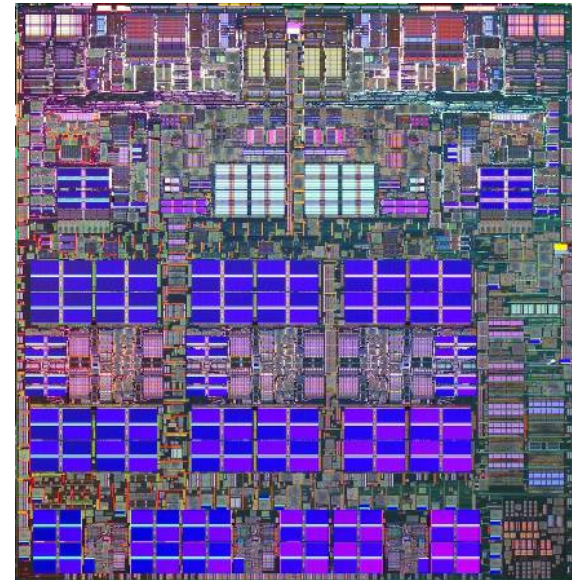
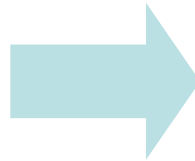
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# Tubes to Chips: ICs

- Driven by Information Processing needs



**IBM 701 calculator (1952)**



**IBM Power 5 IC  
(2004)**



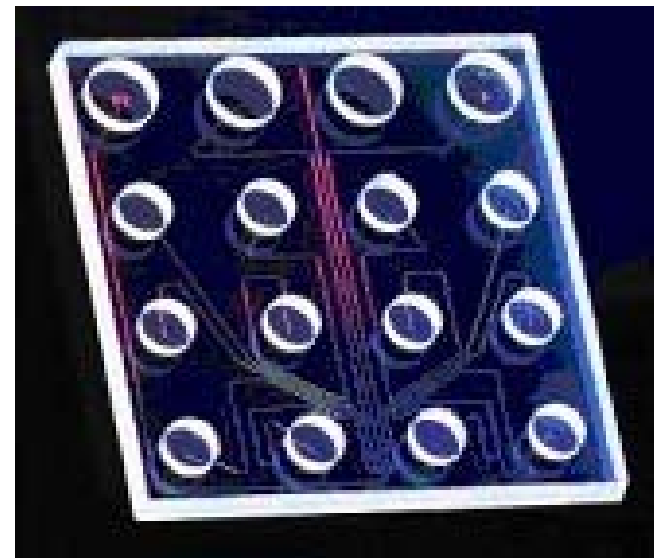
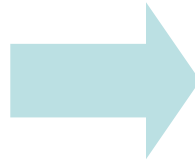
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# Tubes to Chips: BioChips

- Driven by biomolecular analysis needs



**Test tube analysis**



**Agilent DNA analysis  
Lab on a Chip (1997)**

---

# Portable Analysis

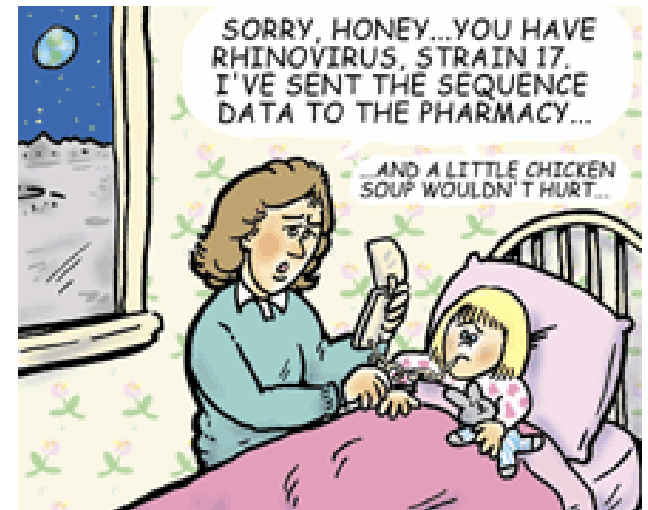
- New knowledge of molecular basis of biology
  - e.g. Human Genome Project
  - Massively parallel analysis infrastructure
- Integration and miniaturization will drive biomolecular analysis instrumentation



**Biomolecular  
“mainframes”**



**Spock with Tricorder  
Sensor + computer**

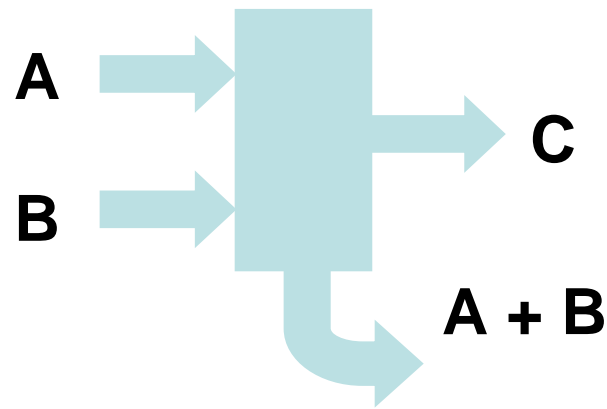


**Burns  
Science 2002**

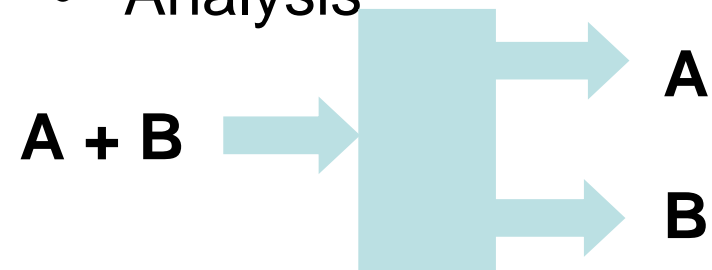
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# Typical Biological Lab Functions

- Synthesis



- Analysis



**Mixing**



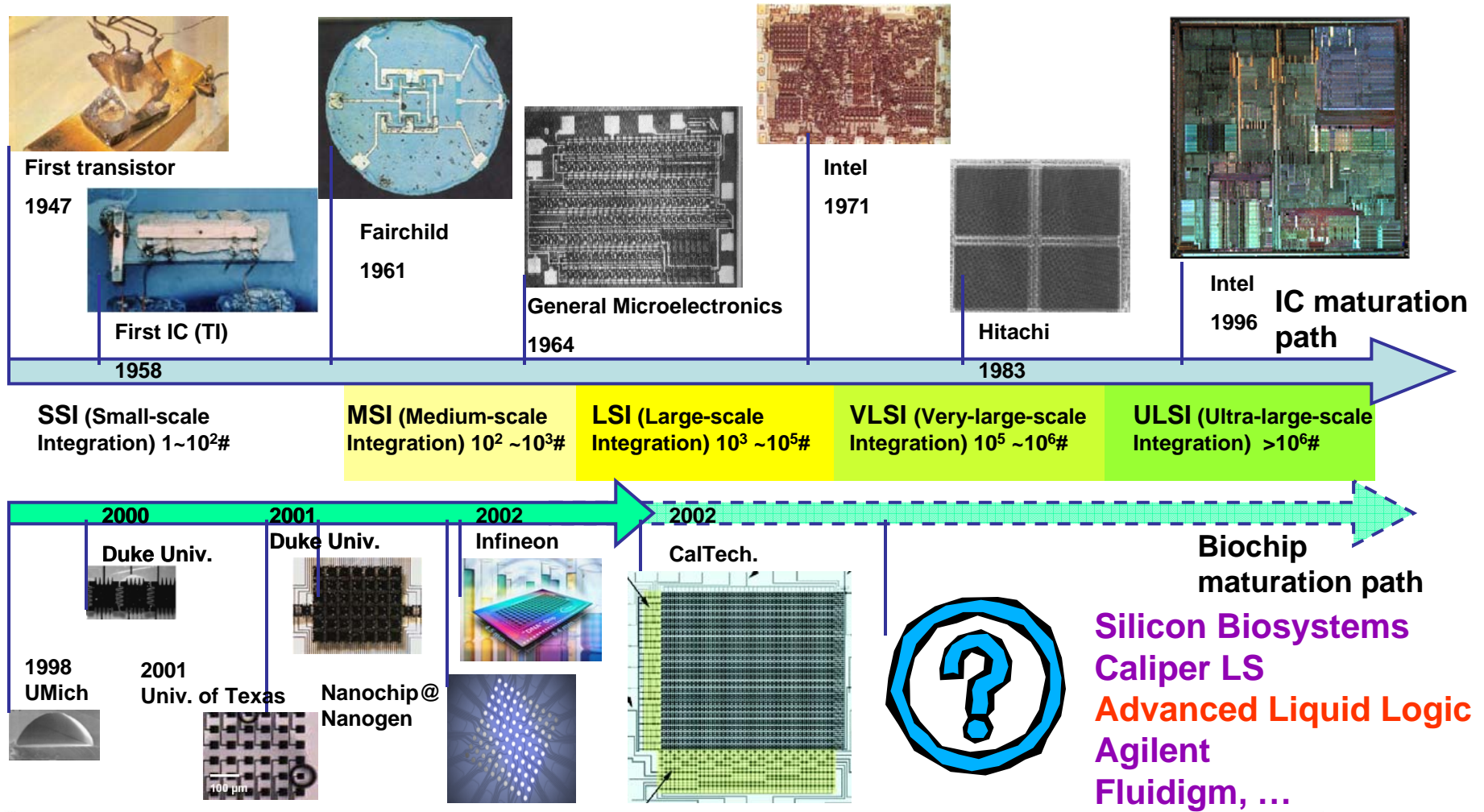
**Reaction**



**Separation**

# Motivation (Parallels with IC Design)

- Increasing application complexity and design complexity



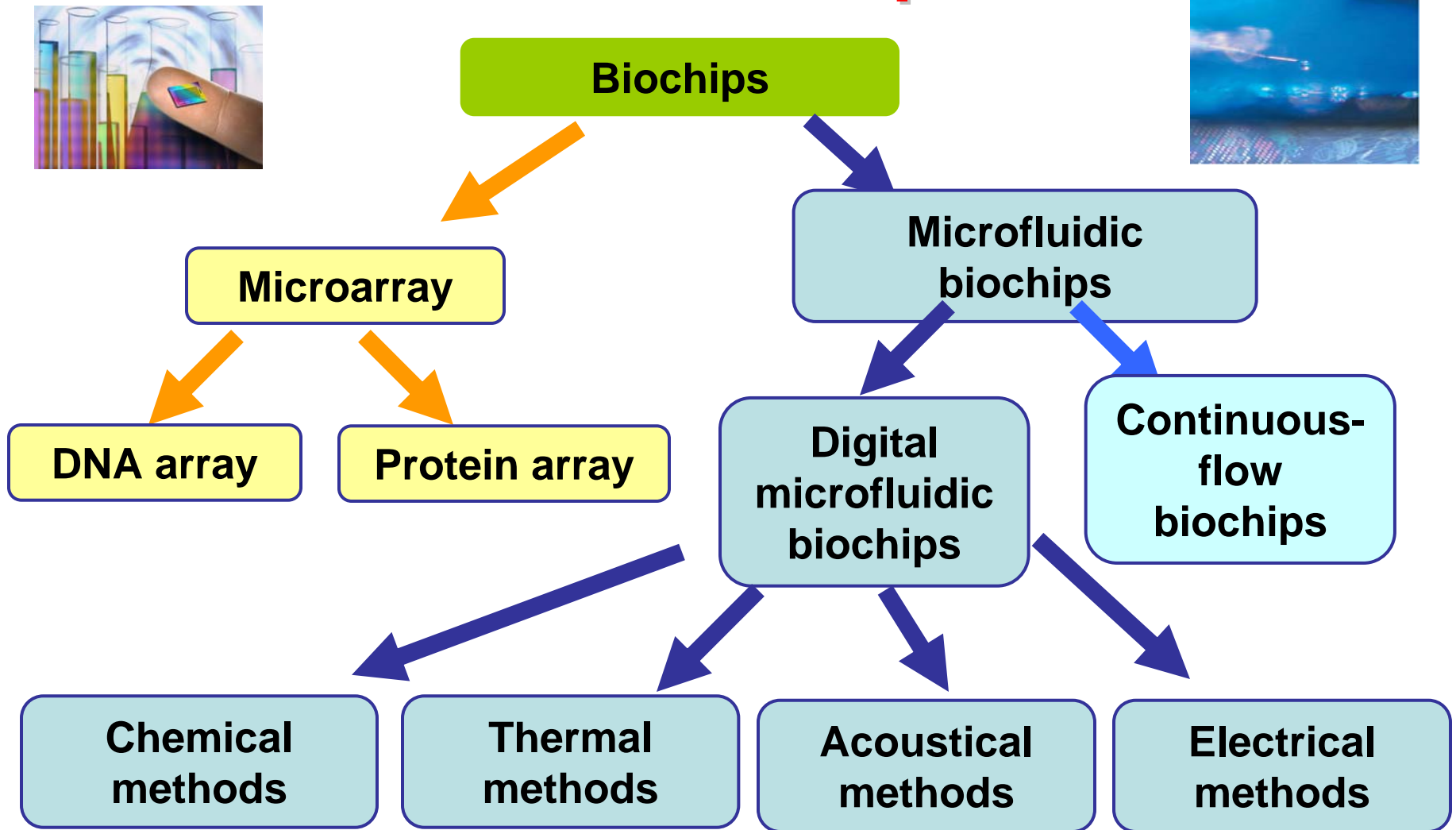


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# Talk Outline

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

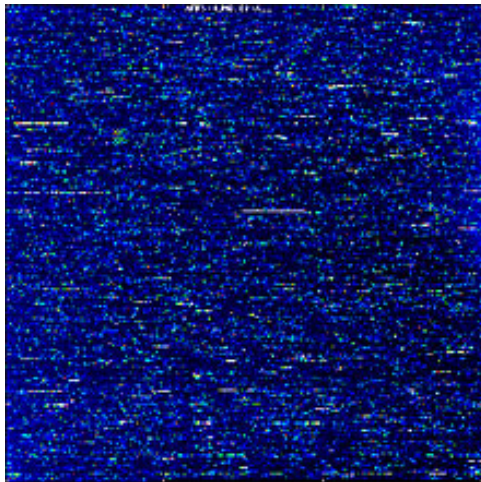
# Classification of Biochips



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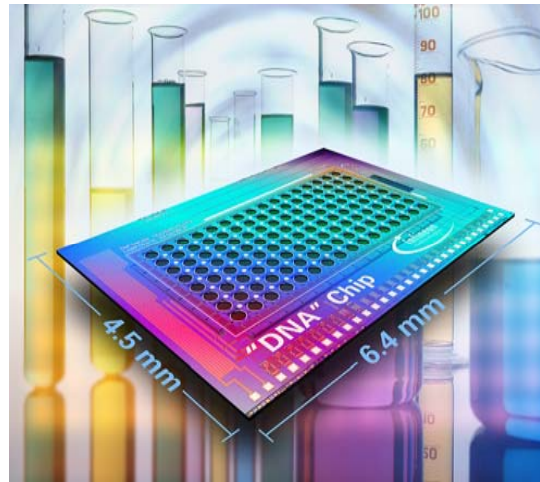
# Microarray

- 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*



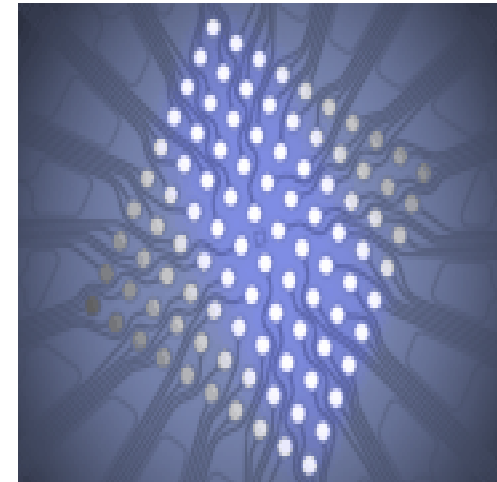
**GeneChip® DNAarray**  
from Affymetrix

<http://www.affymetrix.com>



**DNA microarray from**  
**Infineon AG**

<http://www.infineon.com>



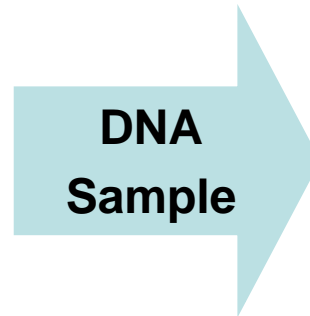
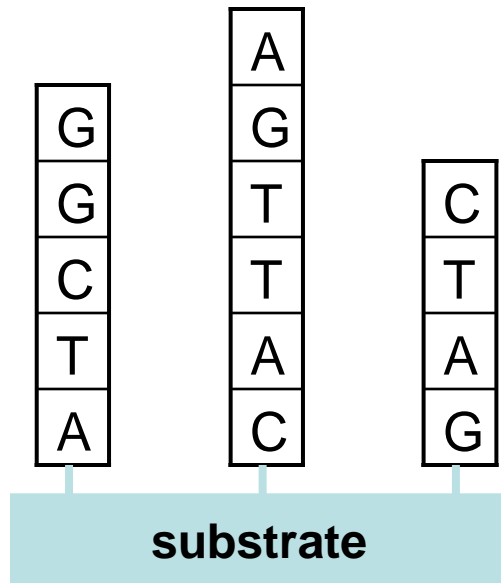
**NanoChip® microarray**  
from Nanogen

<http://www.nanogen.com>

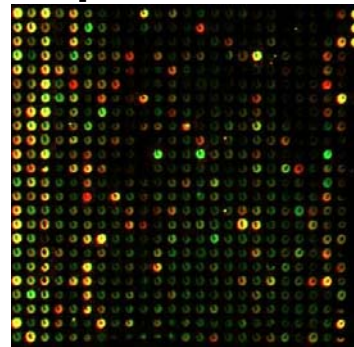
# DNA Arrays

- Gene Chips
- Only implement hybridization reaction

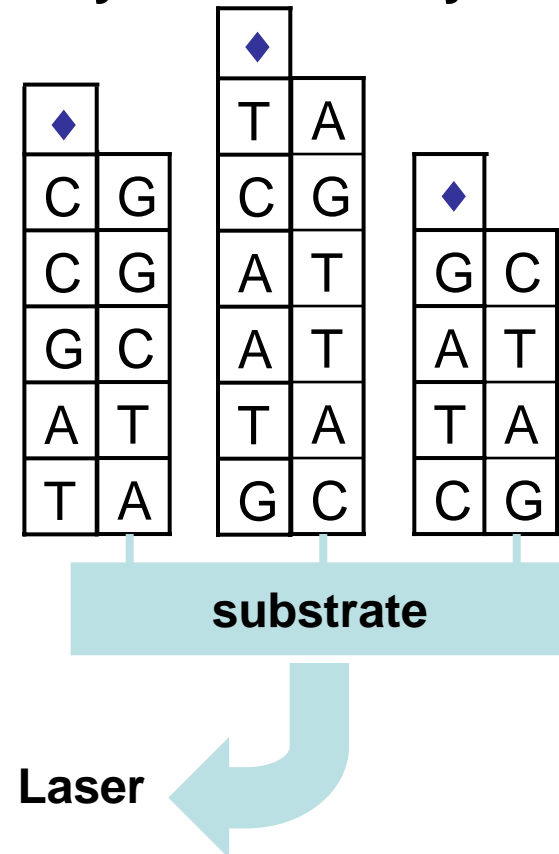
Unhybridized array



Optical Scan



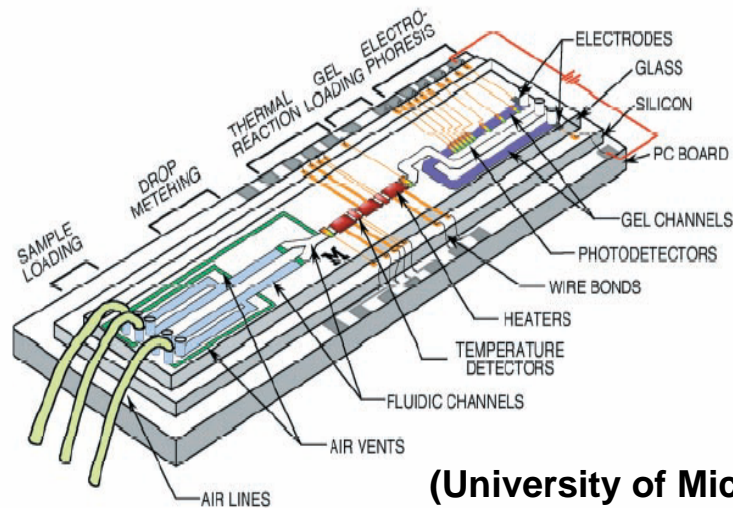
Hybridized array



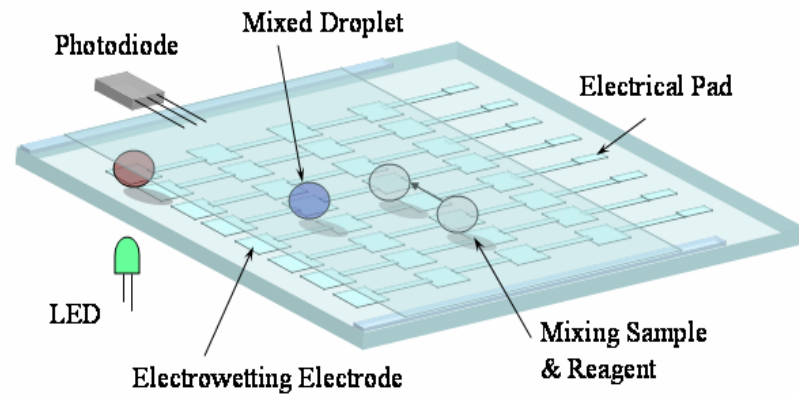


# Microfluidics

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

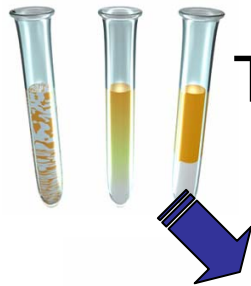


(University of Michigan)  
1998



(Duke University)  
2002

# Motivation for Microfluidics



Test tubes

- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

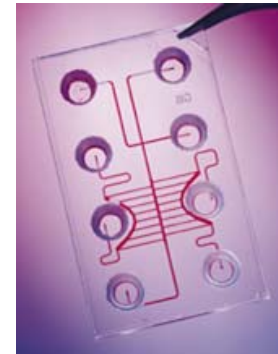


Robotics

- ☒ Automation
- ☒ Integration
- ☐ Miniaturization

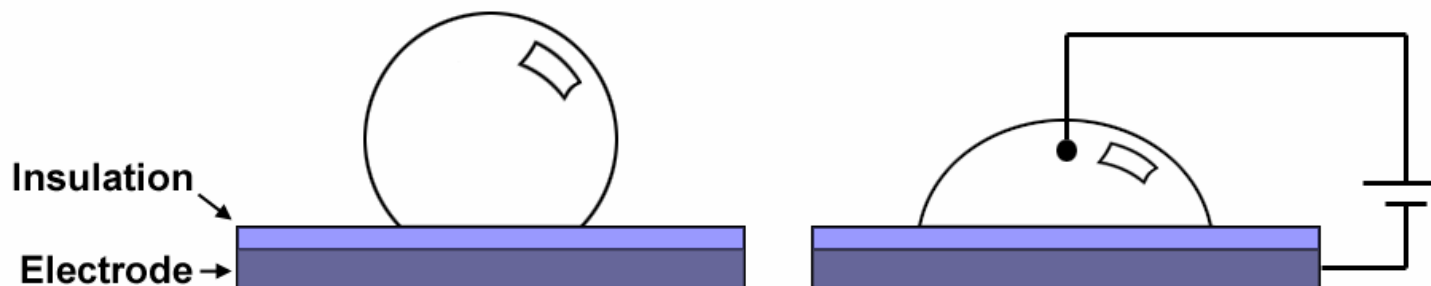
Microfluidics

- ☒ 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.

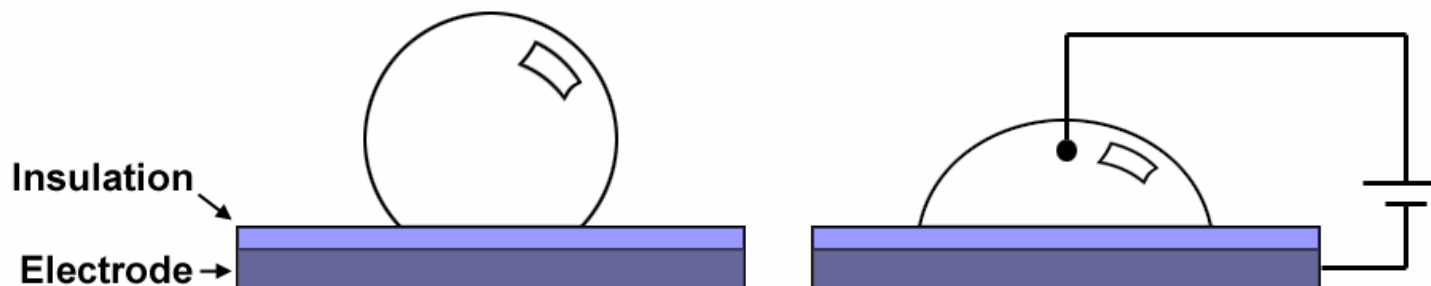
## Applied Potential

The droplet's surface energy increases, which results in a reduced contact angle. The droplet now wets the surface.



# Electrowetting

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## Applied Potential

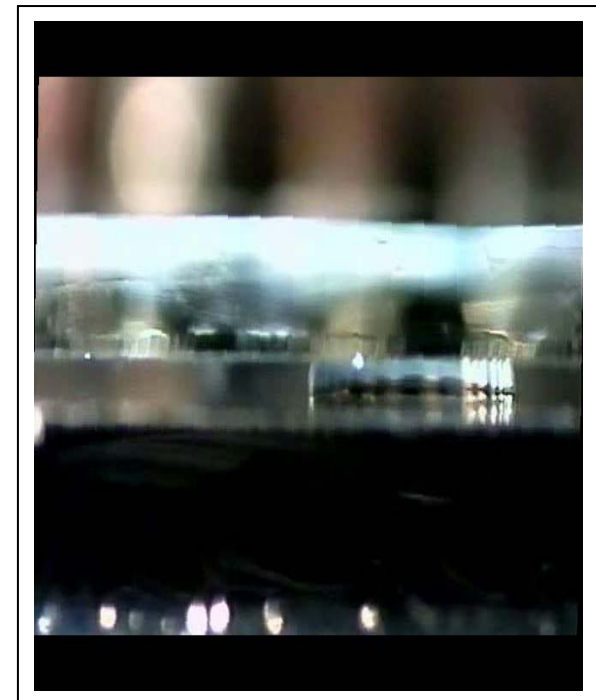
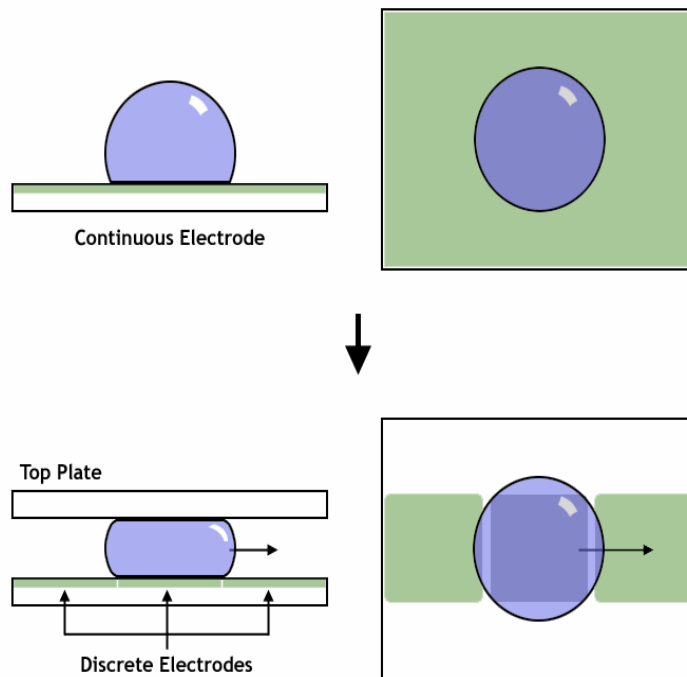
The droplet's surface energy increases, which results in a reduced contact angle. The droplet now wets the surface.





# What is Digital Microfluidics?

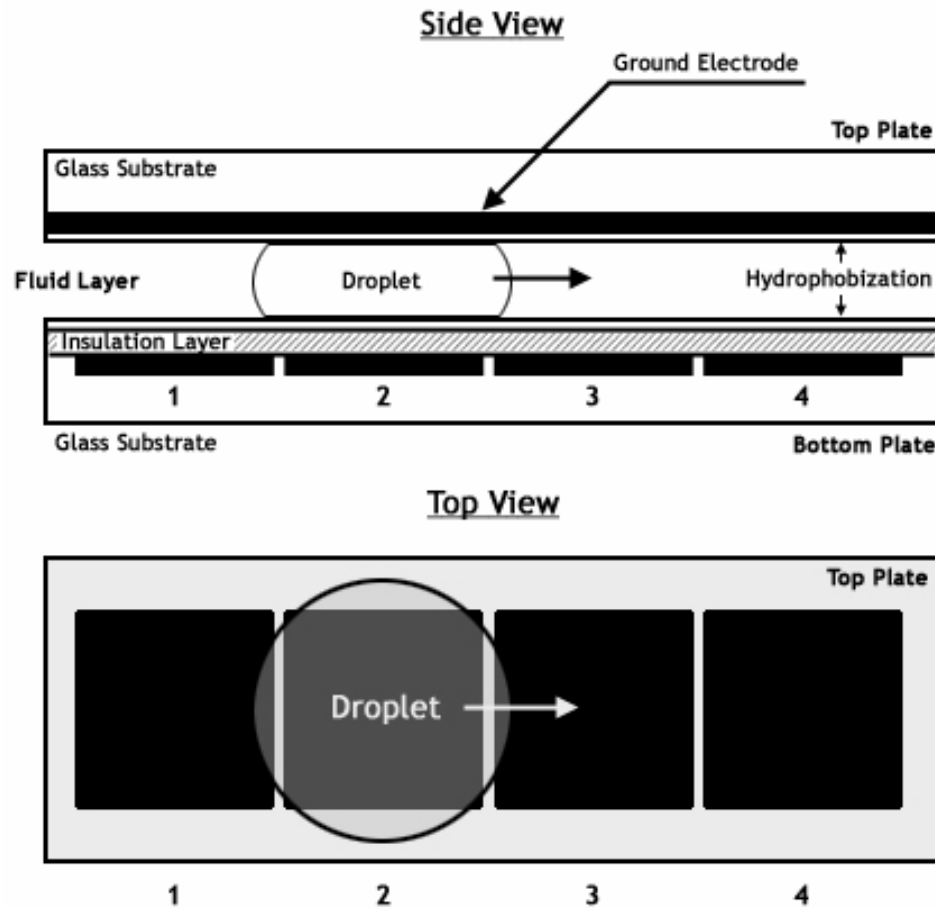
- Discretizing the bottom electrode into multiple electrodes, we can achieve lateral droplet movement



**Droplet Transport (Side View)**

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

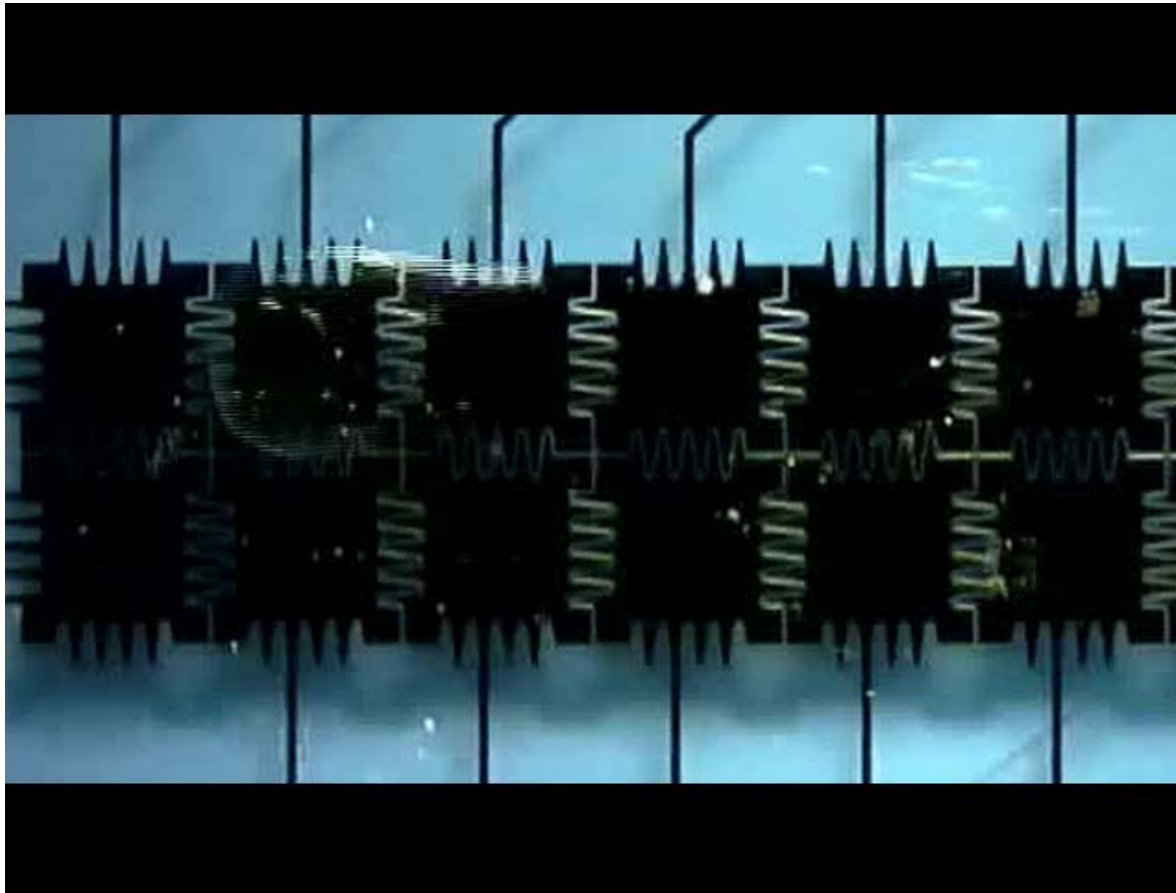
# What is Digital Microfluidics?



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?

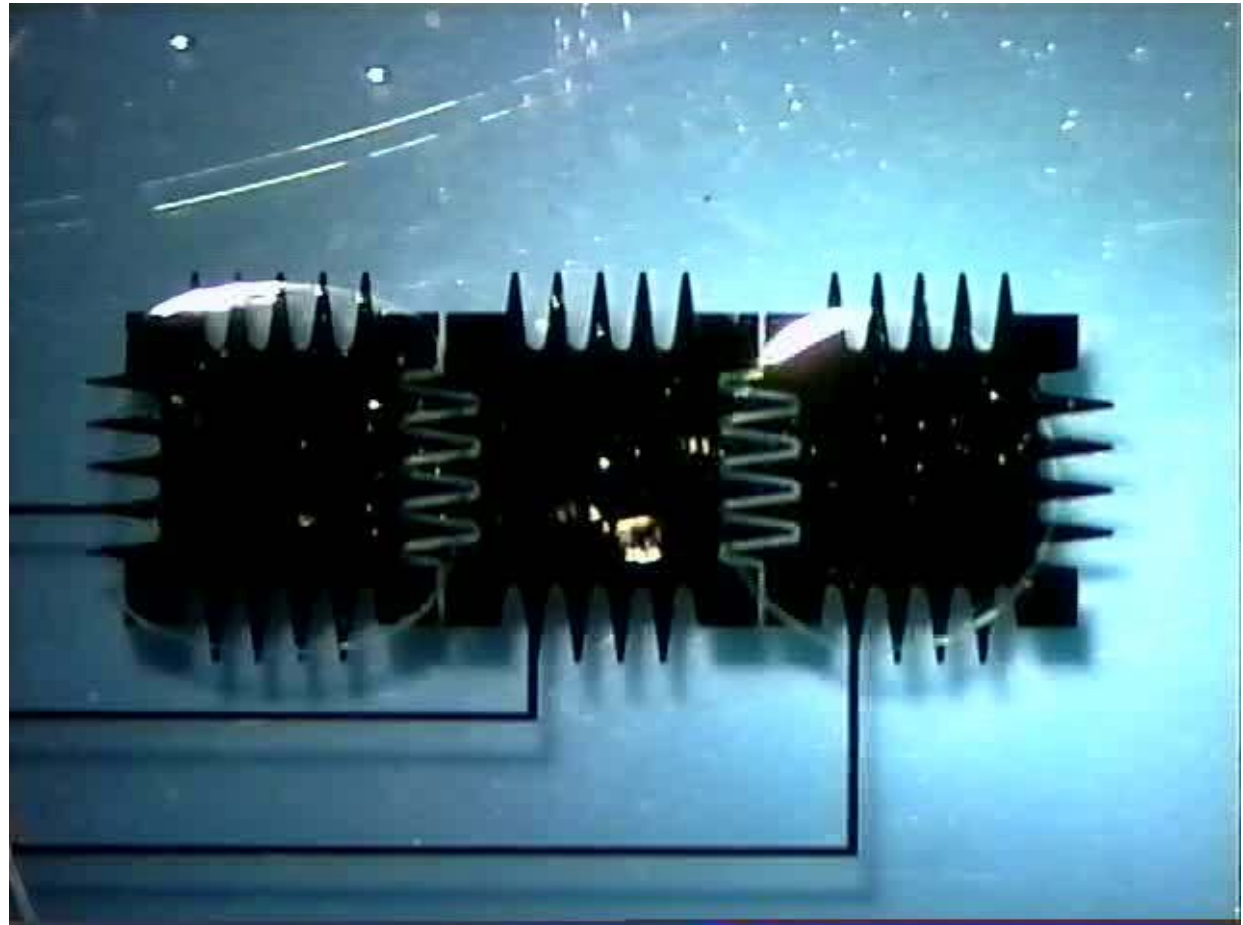


Transport  
20 cm/s flow rates

---

# What is Digital Microfluidics?

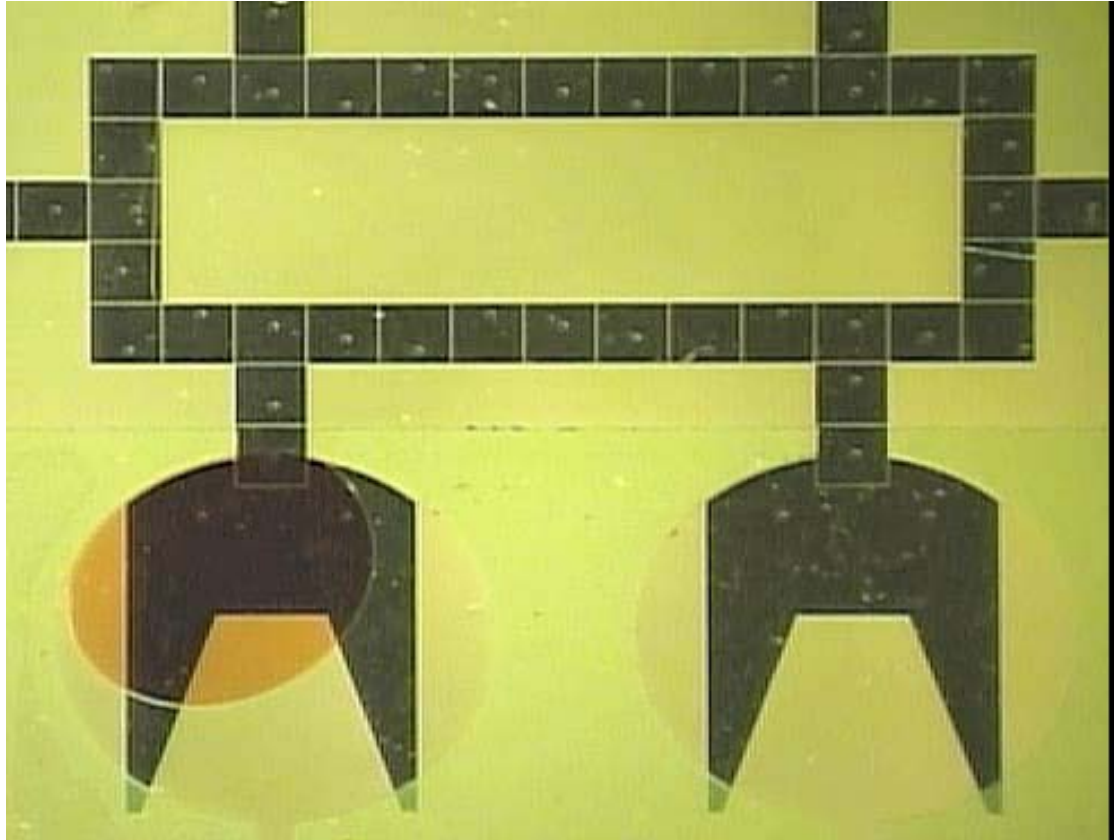
Splitting/Merging





---

# What is Digital Microfluidics?



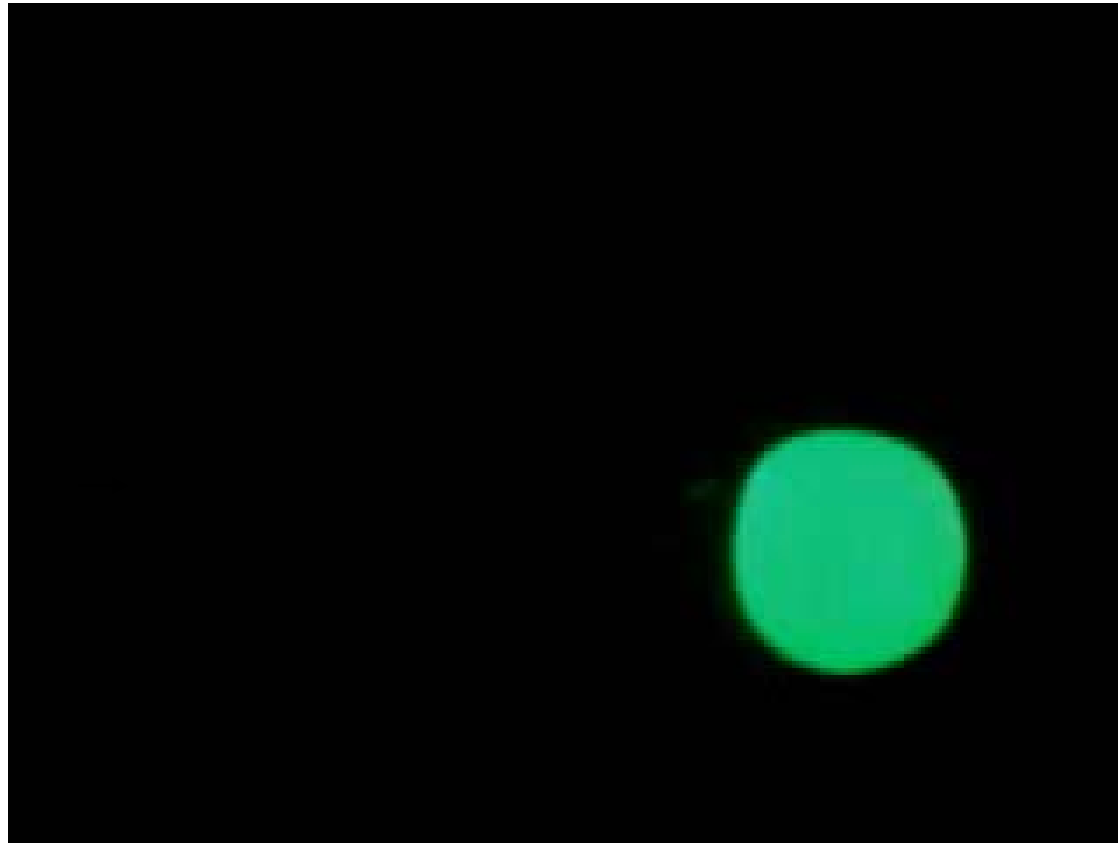
## Droplet Formation

8 droplets in 3.6s

---

# What is Digital Microfluidics?

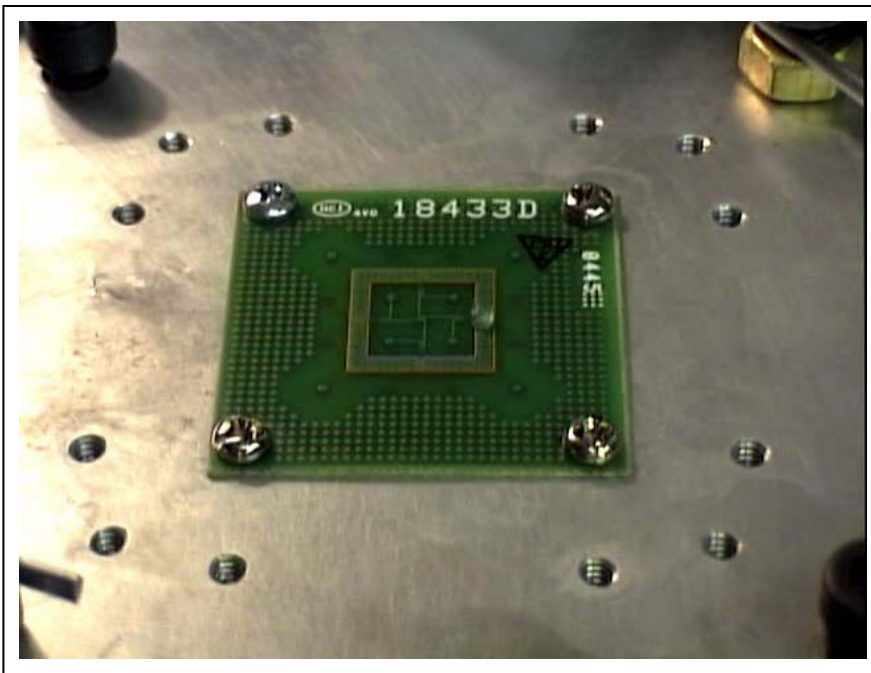
Mixing



---

# Advantages

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

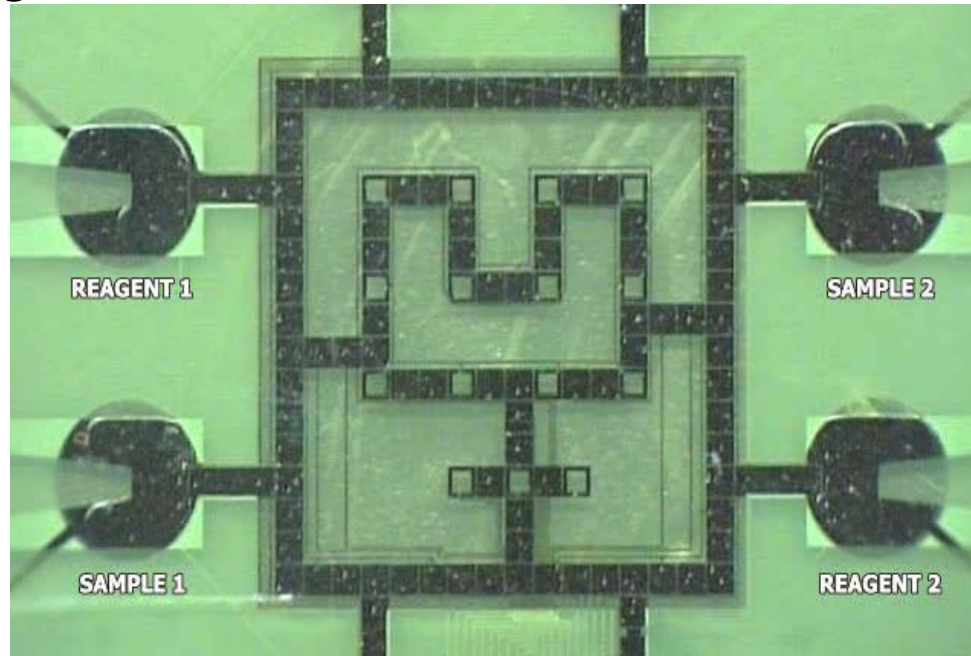


**Droplet Transport on PCB (Isometric View)**

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## An Example

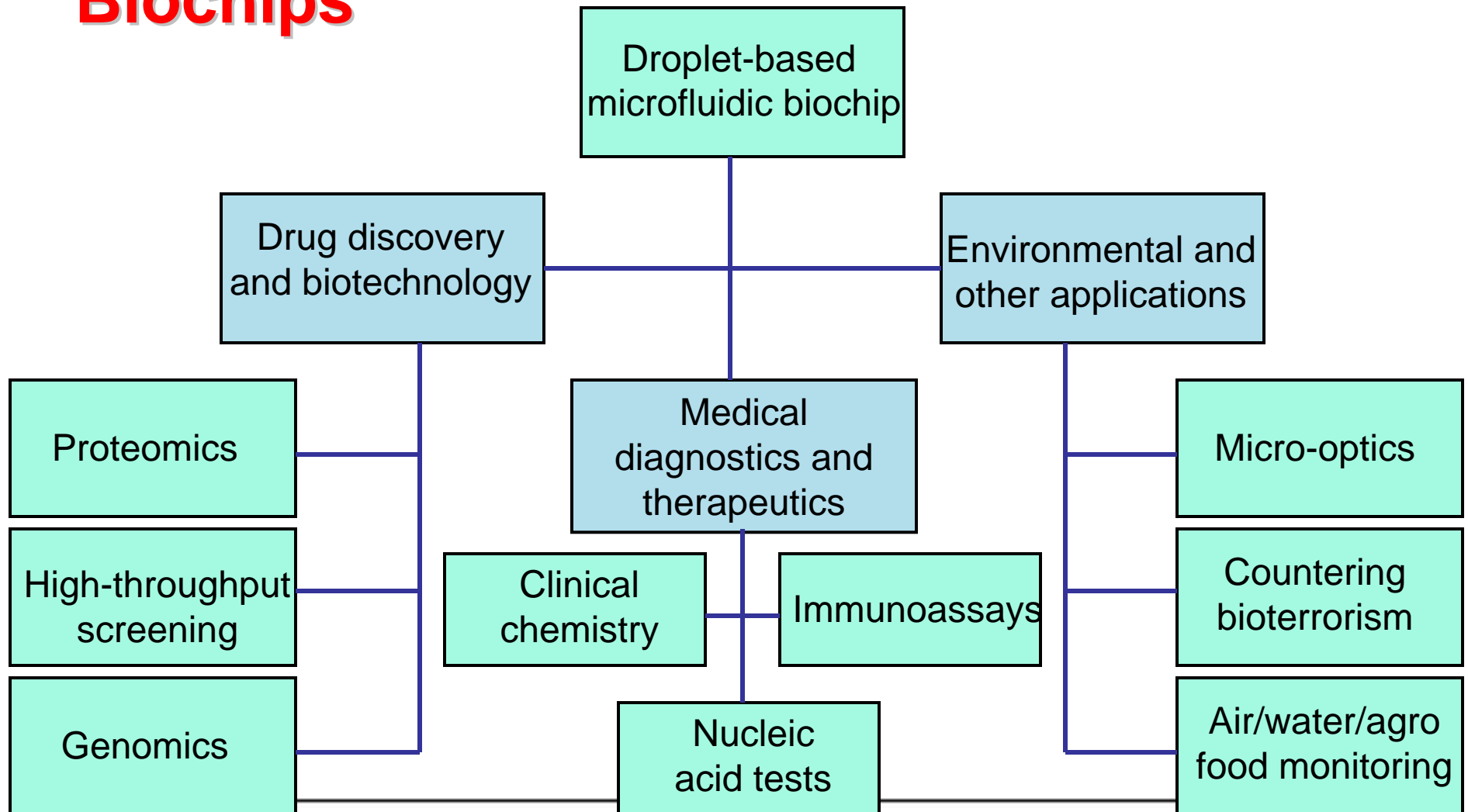
- Detection of lactate, glutamate and pyruvate has also been demonstrated.
- Biochip used for multiplexed in-vitro diagnostics on human physiological fluids



**Fabricated microfluidic array used for multiplexed biomedical assays**

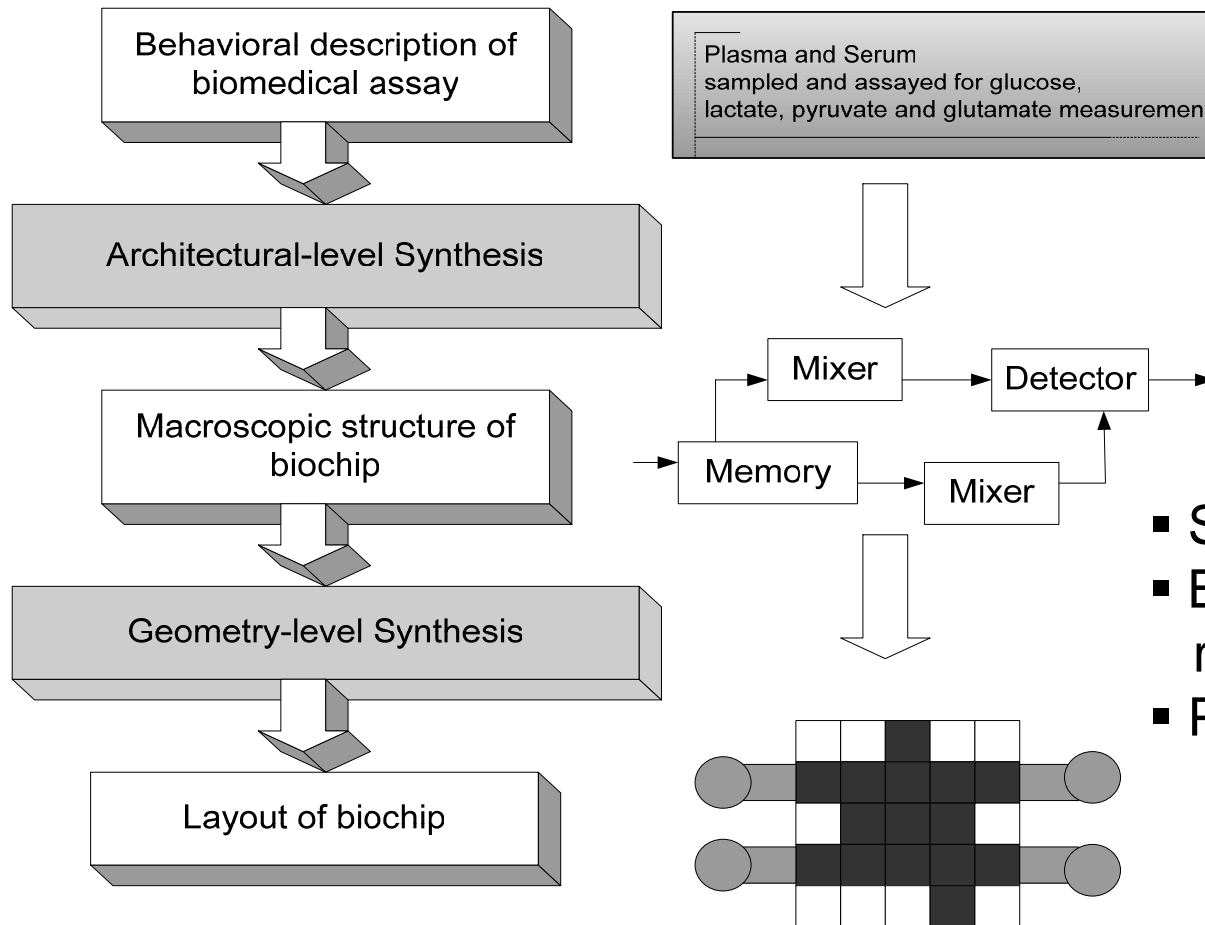


# Applications of Digital Microfluidic Biochips



# 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.)

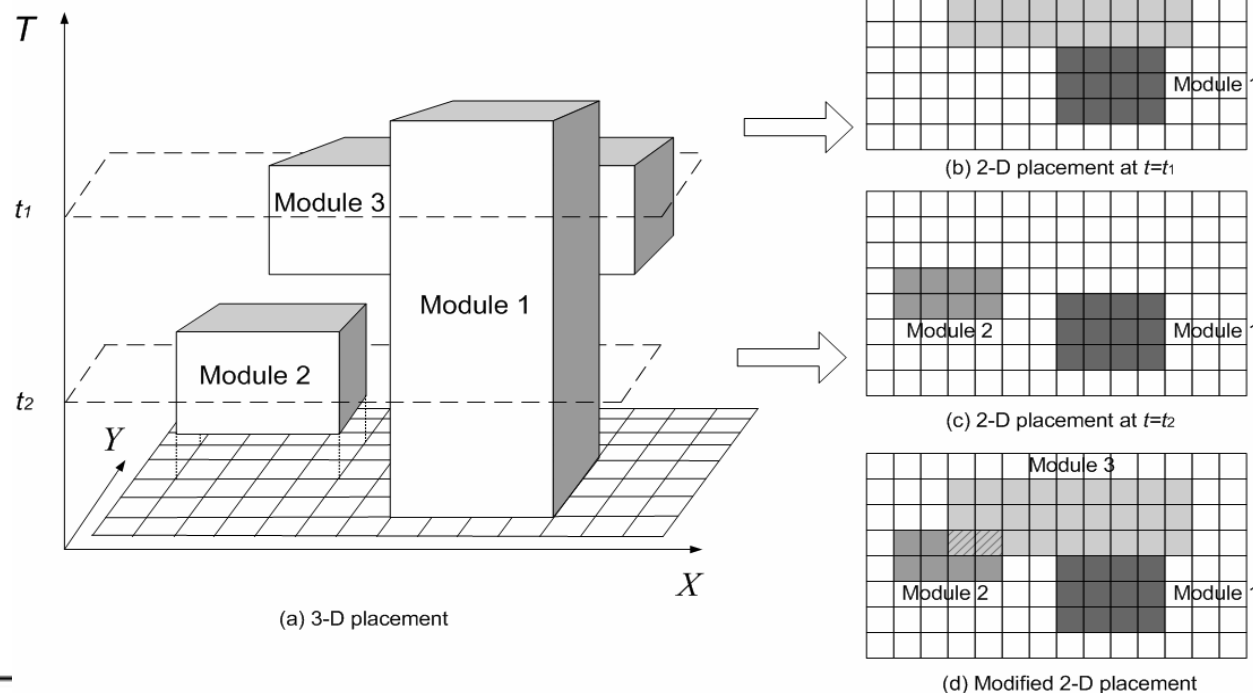
- **Five examples (four samples)**  $S_1$ : Plasma,  $S_2$ : Serum,  $S_3$ : Urine,  $S_4$ : Saliva, Assay1: Glucose assay, Assay2: Lactate assay, Assay3: Pyruvate assay, Assay4: Glutamate assay

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

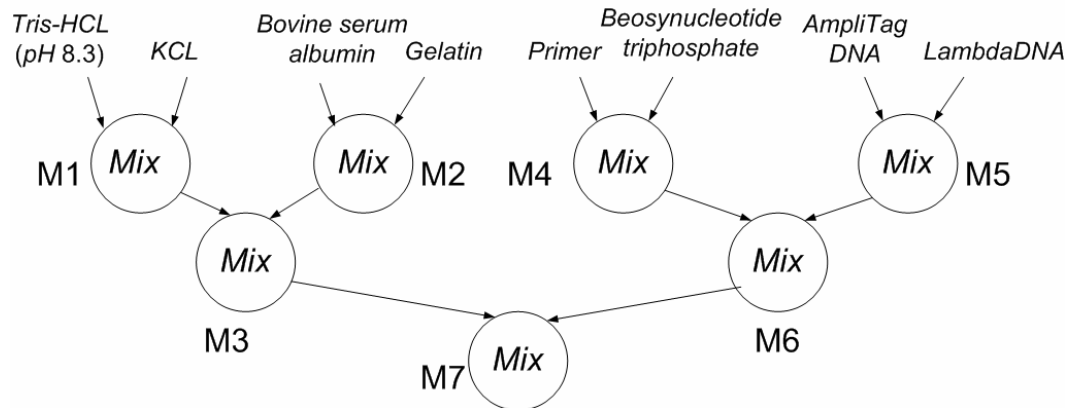
(Su and Chakrabarty, DATE'05)

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

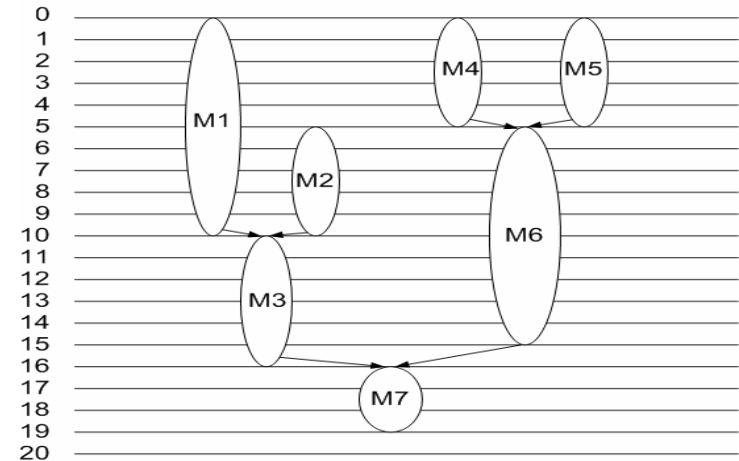


**Reduction from  
3\_D placement  
to a modified  
2-D placement**

# Application to PCR



Protocol of PCR (mixing phase)



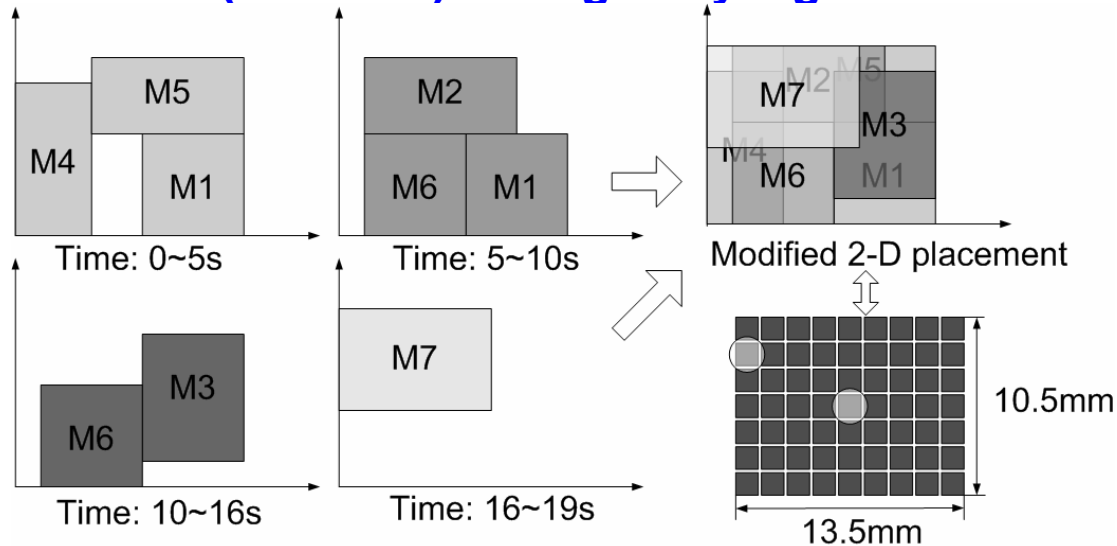
Schedule of PCR

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 ( $189\text{mm}^2$ ) from greedy algorithm



Placement from  
the simulated  
annealing-based  
procedure

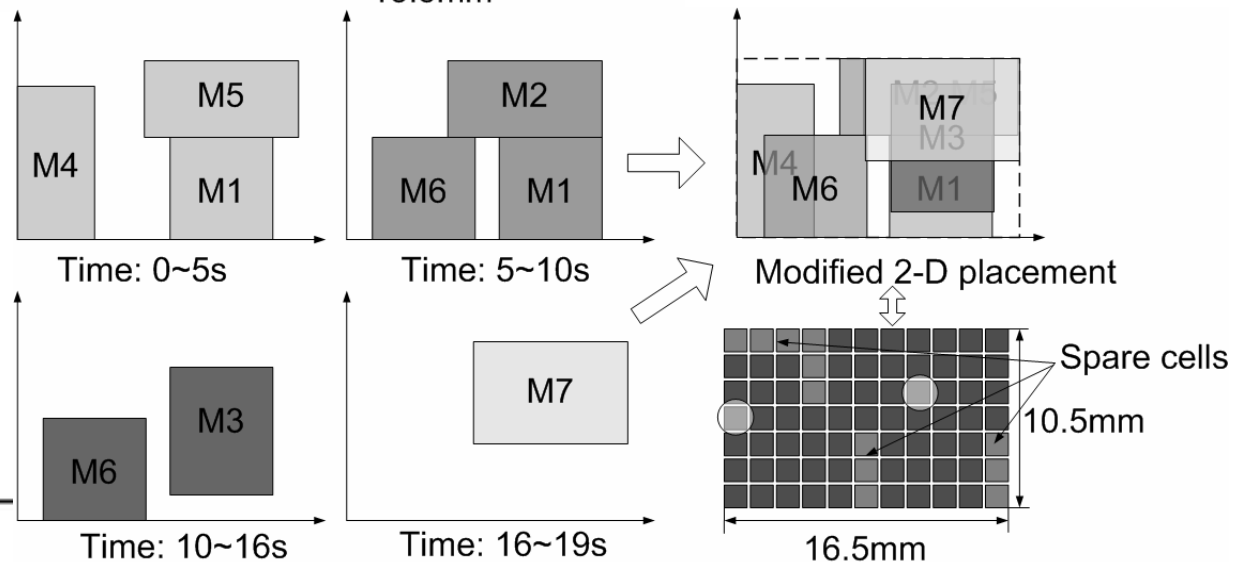
Area:  $7 \times 9 = 63$  cells

FTI: 0.1270

Placement from  
enhanced module  
placement  
procedure

Area:  $7 \times 11 = 77$  cells

FTI: 0.8052

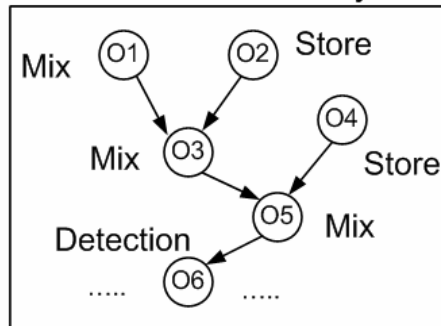




# Unified Synthesis Methodology

Su and Chakrabarty (DAC 2005)

**Input:** Sequencing graph of bioassay



Digital microfluidic module library

Mixing components	Area	Time
2x2-array mixer	4 cells	10 s
2x3-array mixer	6 cells	6 s
2x4-array mixer	8 cells	3 s
1x4-array mixer	4 cells	5s
<b>Detectors</b>		
LED+Photodiode	1 cell	30 s

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

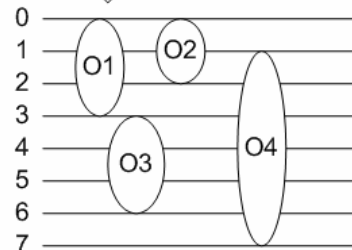
Unified Synthesis of Digital Microfluidic Biochip

**Output:**

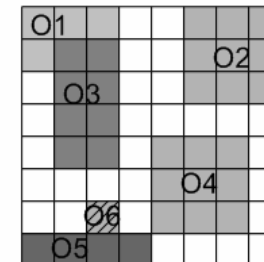
Resource binding

Operation	Resource
O1	2x3-array mixer
O2	Storage unit (1 cell)
O3	2x4-array mixer
O4	Storage unit (1 cell)
O5	1x4-array mixer
O6	LED+Photodiode

Schedule



Placement



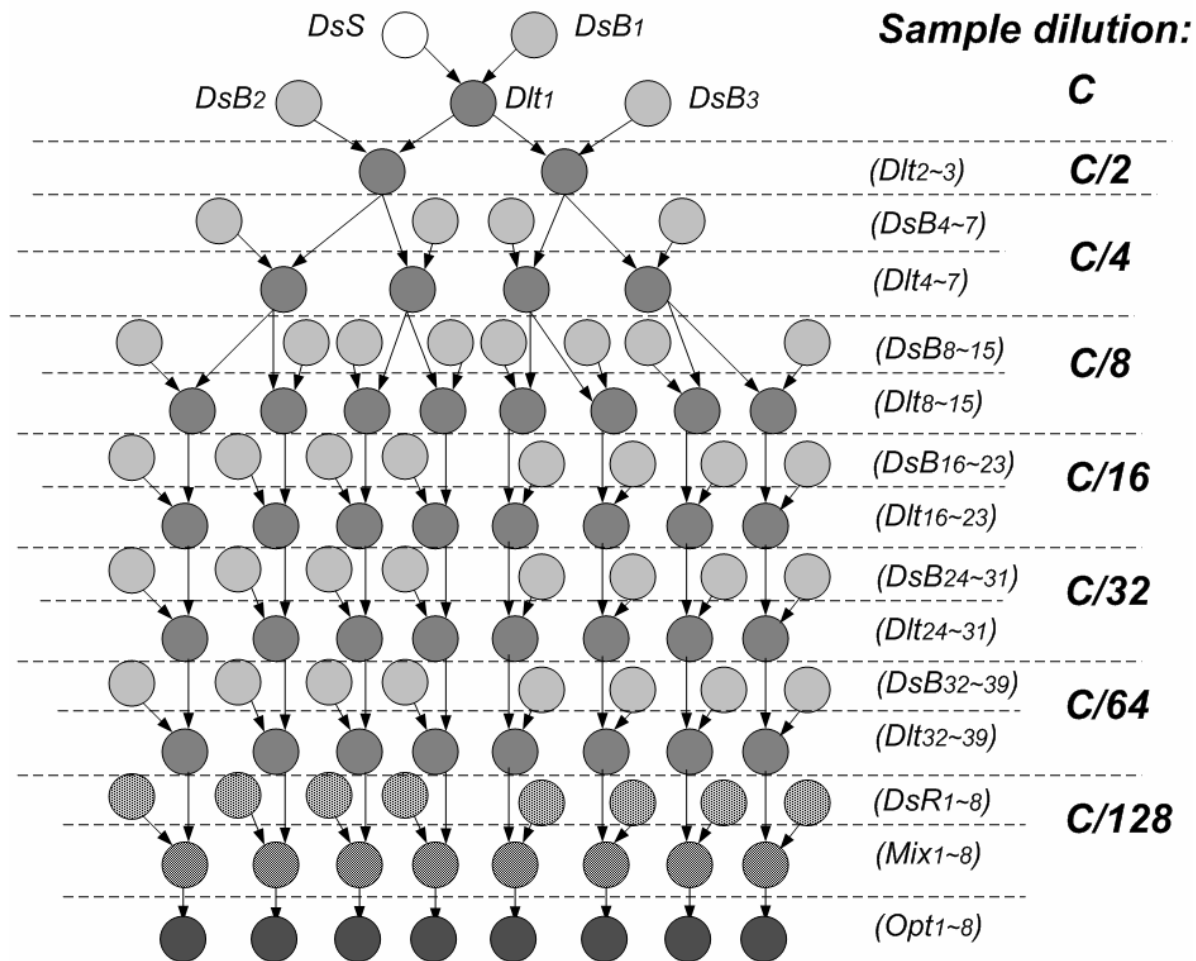
Biochip design results:

**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**

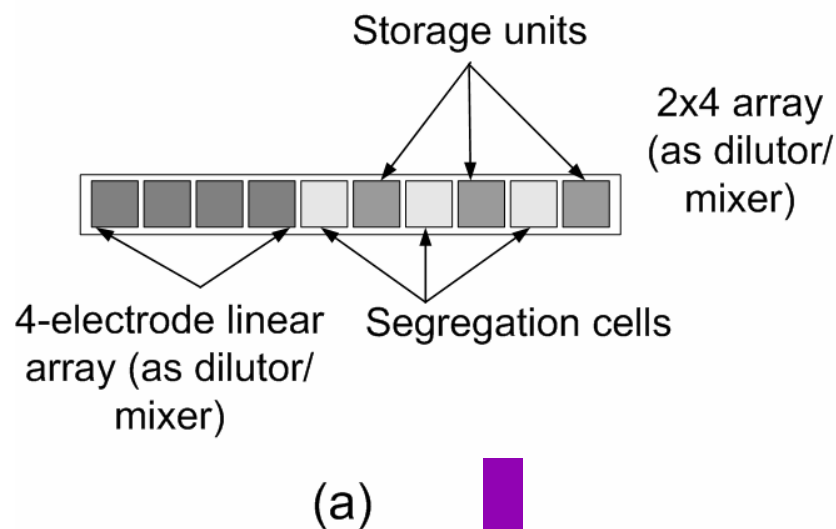
## Protein Assay (Cont.)

- Microfluidic module library for synthesis

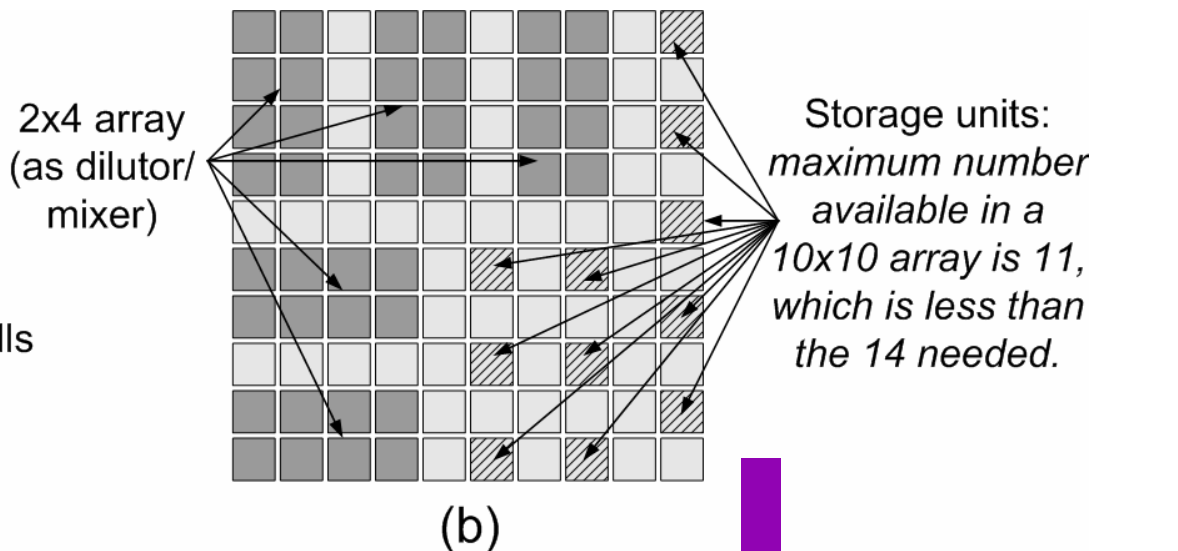
Operation	Resource	Operation Time (s)
<i>DsS; DsB; DsR</i>	On-chip reservoir/dispensing port	7
<i>Dlt</i>	2x2-array dilutor	12
	2x3-array dilutor	8
	2x4-array dilutor	5
	4-electrode linear array dilutor	7
<i>Mix</i>	2x2-array mixer	10
	2x3-array mixer	6
	2x4-array mixer	3
	4-electrode linear array mixer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

# Design for Protein Assay

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



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

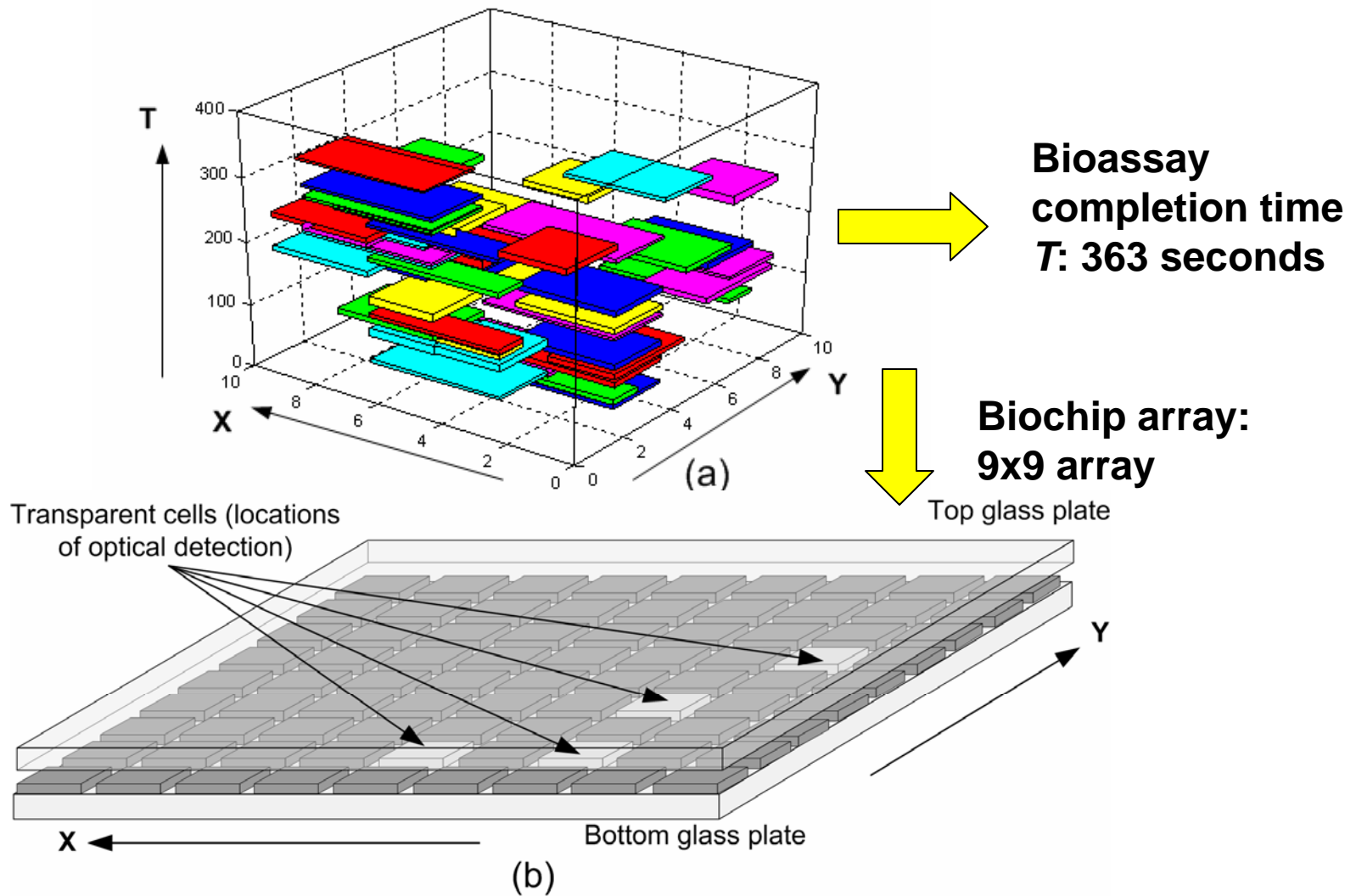


$$5 \times 8 + 14 < 10 \times 10 \text{ (satisfies the resource constraint in architectural-level synthesis)}$$

***Fail to meet the design specification!***

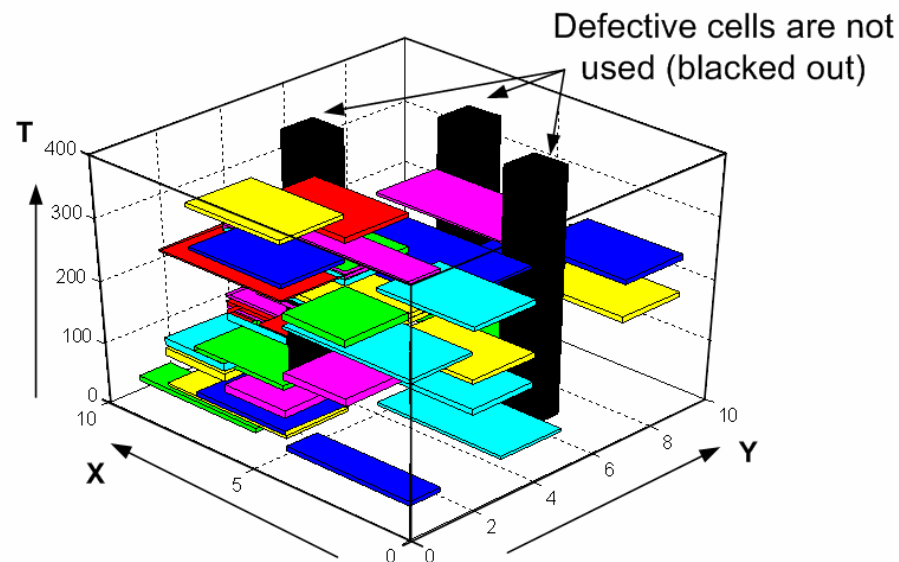
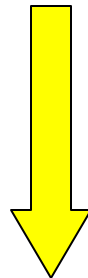
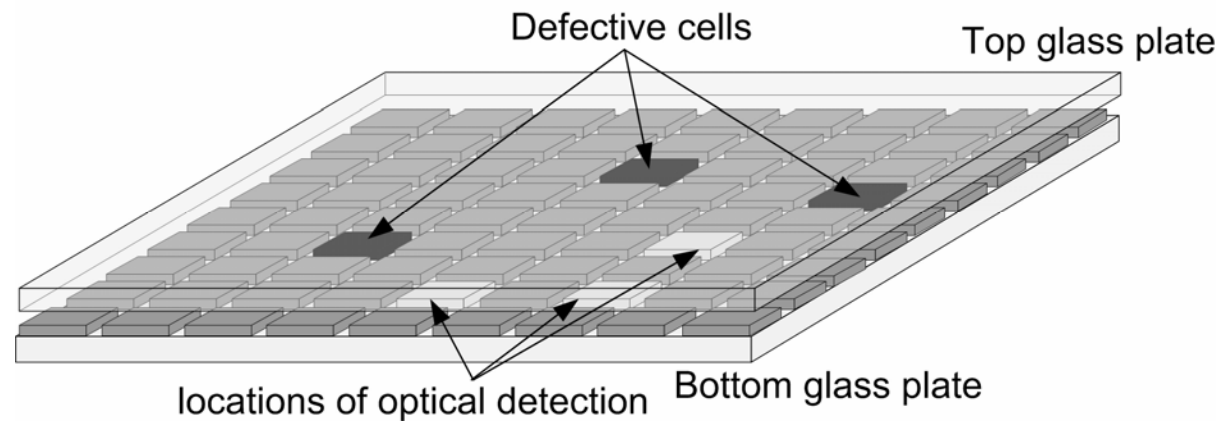
# Experimental Evaluation (Cont.)

- Results of the unified synthesis method



# 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:*

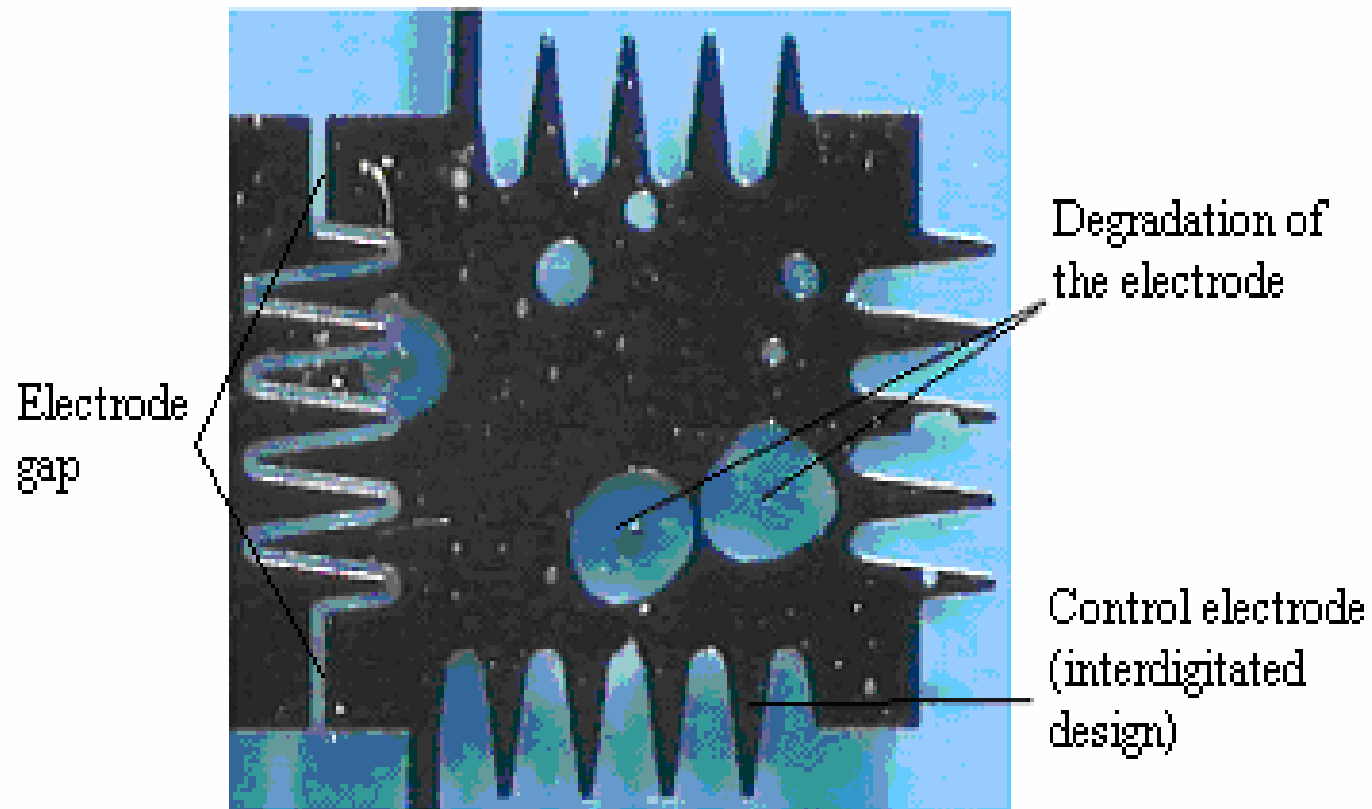
- Geometrical parameter deviation
- Degradation of the insulator
- Change in the viscosity of the droplet and the filler medium

**Manufacturing**

**Operational**

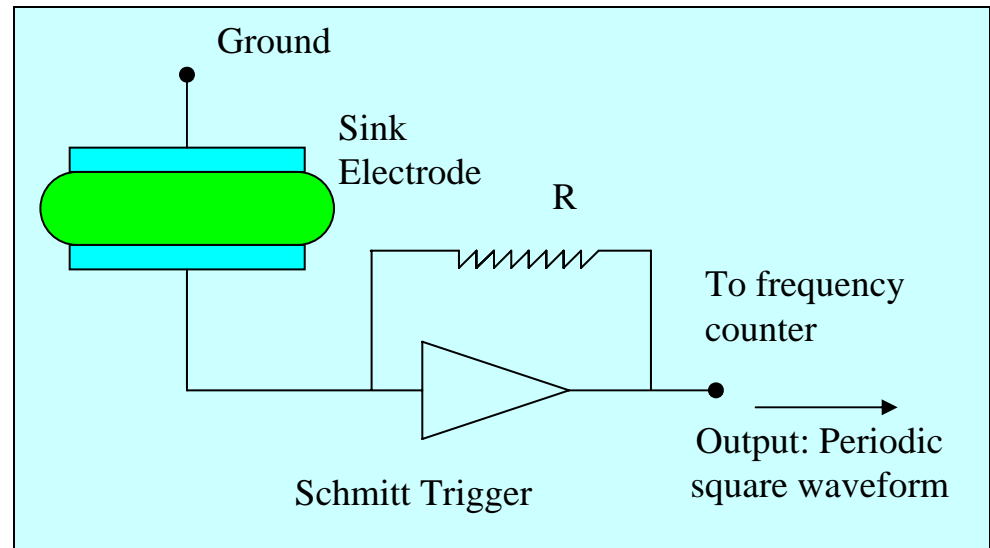
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## Example of Electrode Degradation



# Unified Detection Mechanism

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

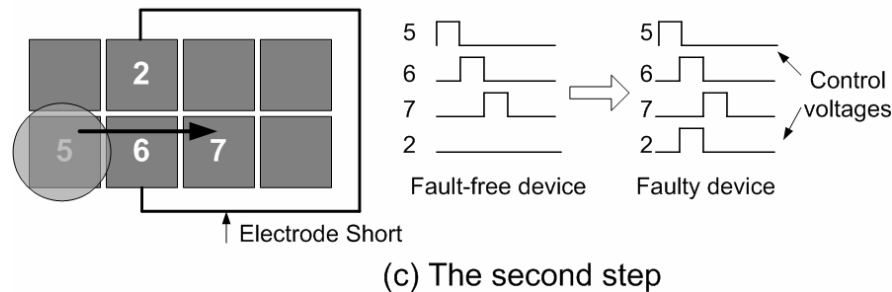
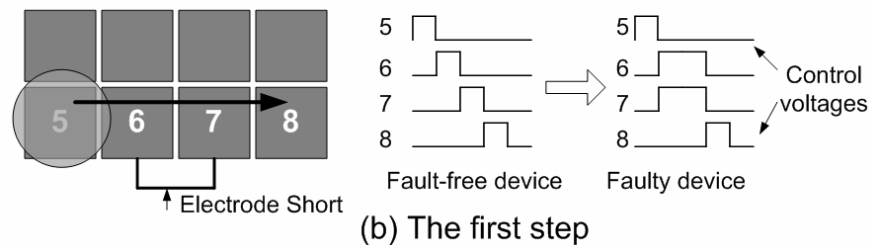
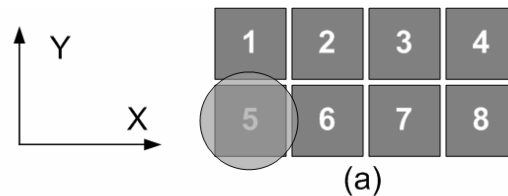


Capacitive changes reflected in electrical signals (Fluidic domain to electrical domain)

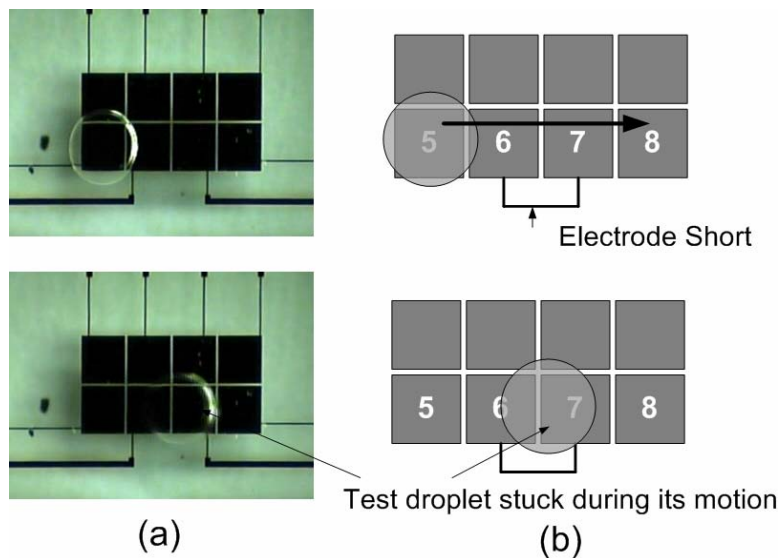
- If there is a droplet, output=1; otherwise, output=0
- Fault-free : there is a droplet between electrodes
- Faulty: there is no droplet.

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

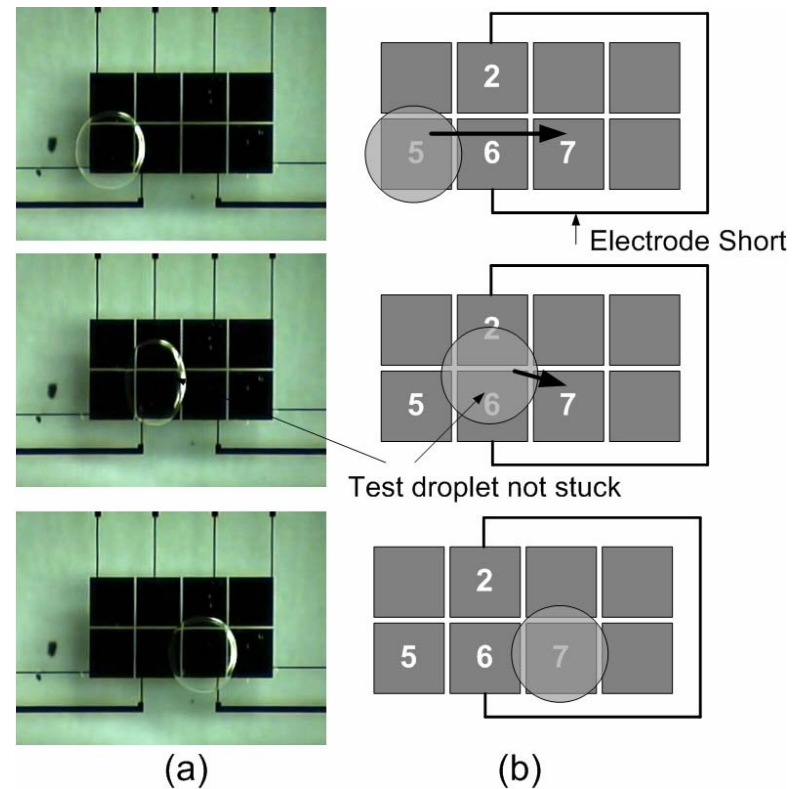
- Defect-Oriented Experiment
  - To simulate the effect of an electrode short on microfluidic behavior



# Experimental Results and Analysis



**Experimental results and analysis for the first step**

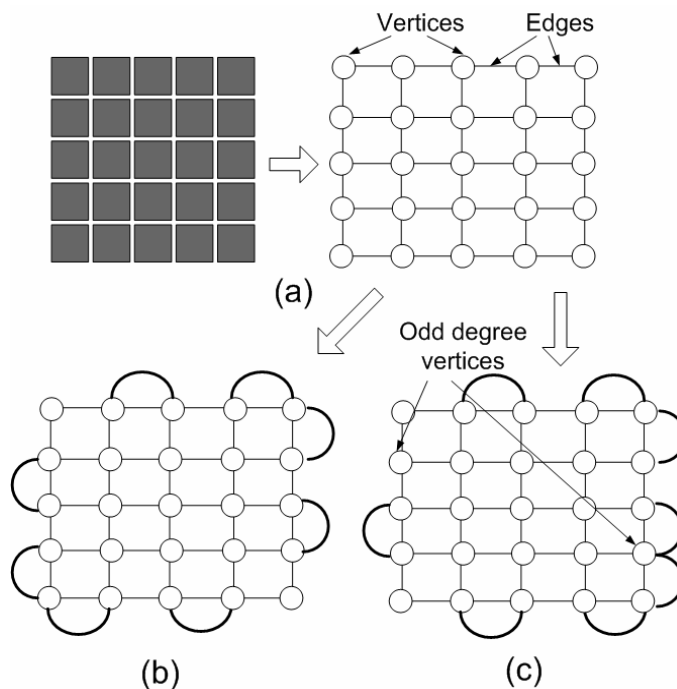


**Experimental results and analysis for the second step**



# 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 5x5 microfluidic array;**

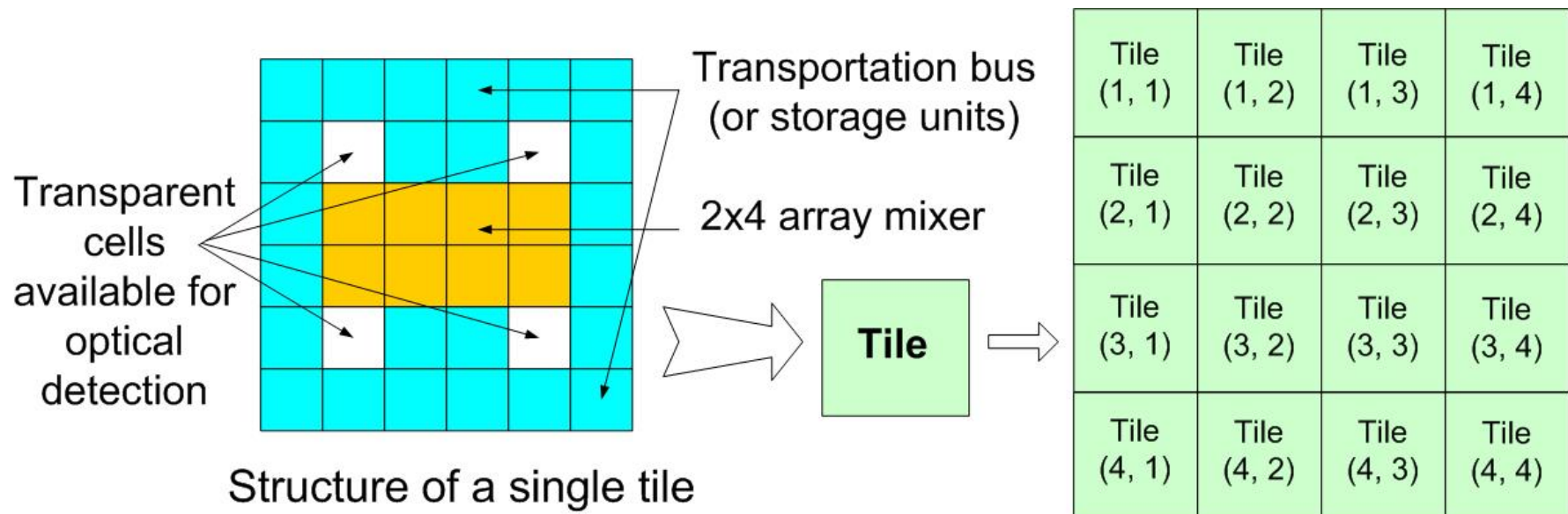
**(b) eulerized graph containing an Euler circuit;**

**(c) eulerized graph containing an Euler path.**

# Tile-Based Architecture for Reconfiguration

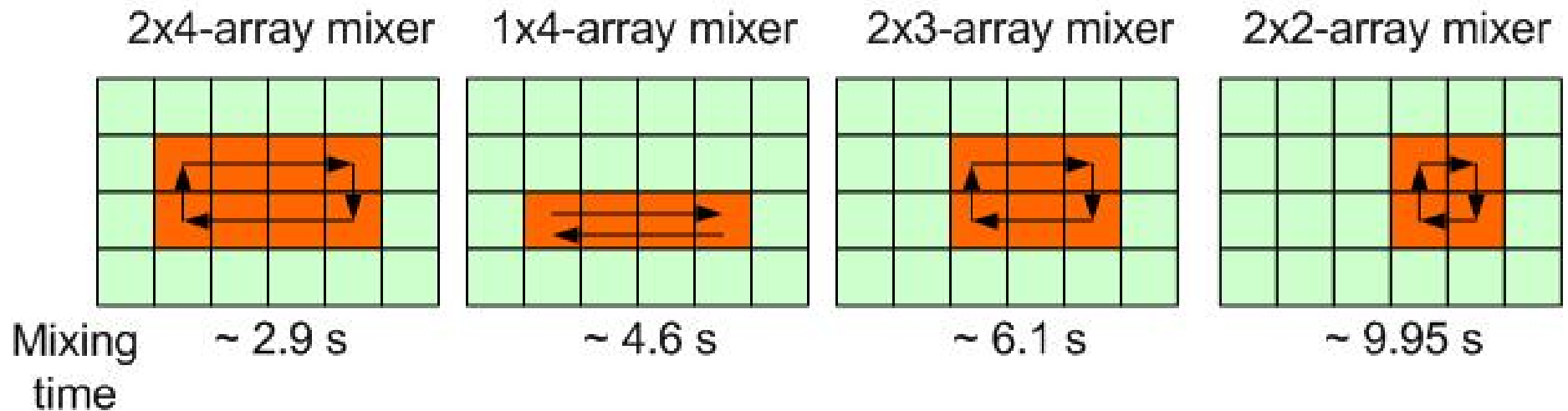
(Su and Chakrabarty, VTS'05)

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



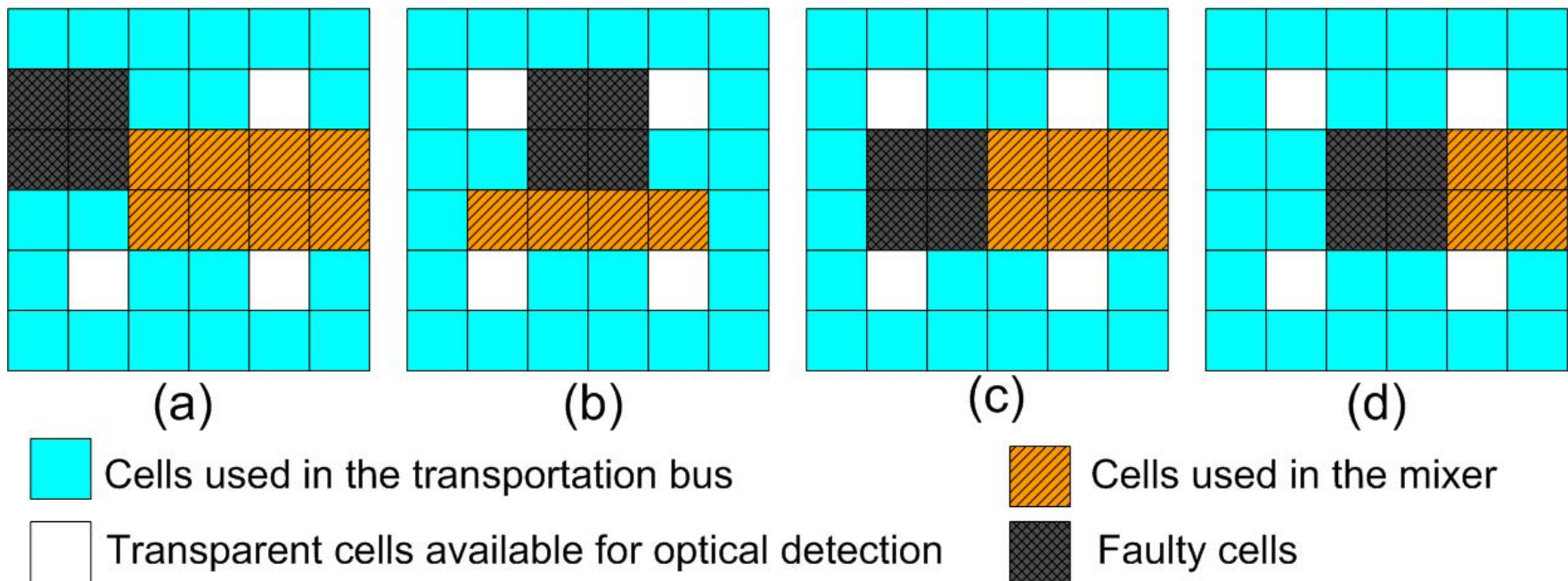
# Reconfigurability

- 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)



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# 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)

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# 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)



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# Fluidic Constraints

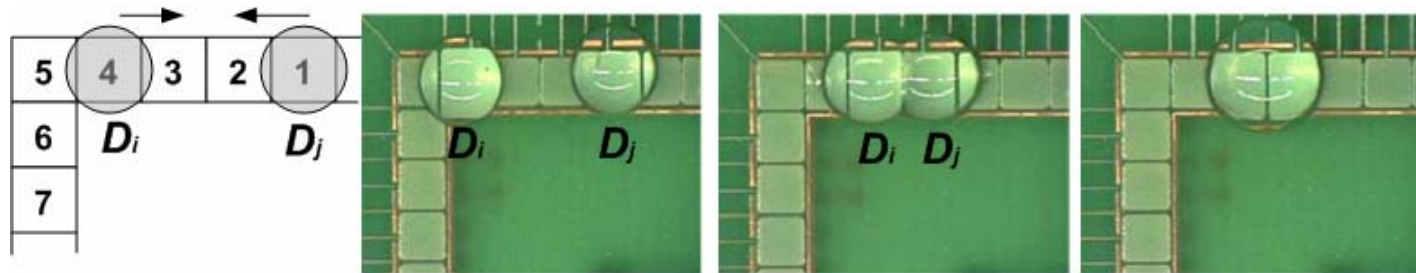
Assume two given droplets as  $D_i$  and  $D_j$ , and let  $X_i(t)$  and  $Y_i(t)$  denote the location of  $D_i$  at time  $t$

**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

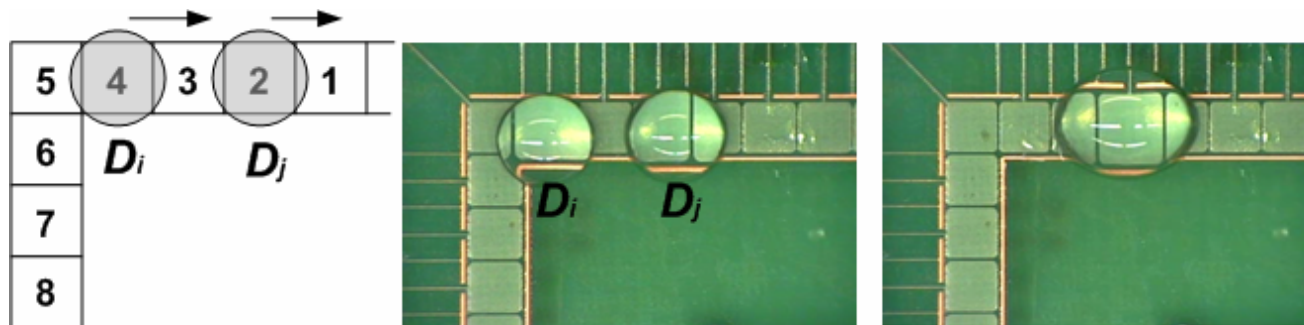


(a)

(b)

(c)

(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.

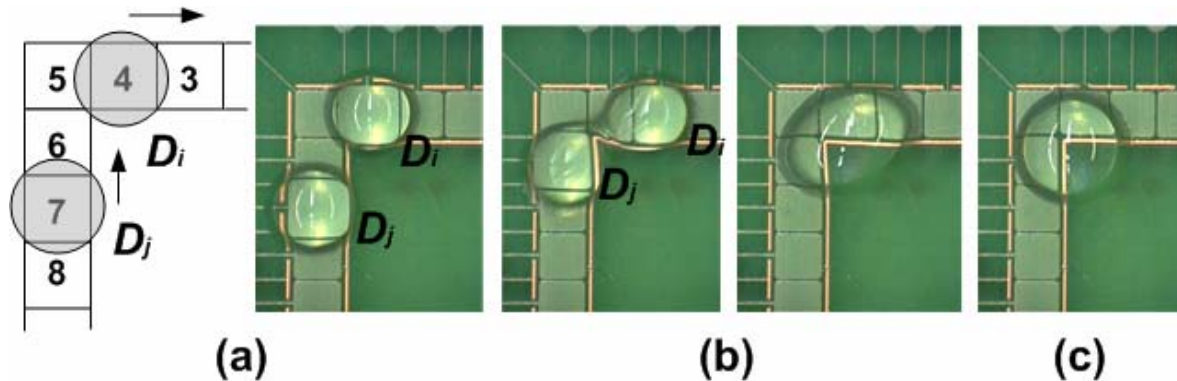


(a)

(b)

(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

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# 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

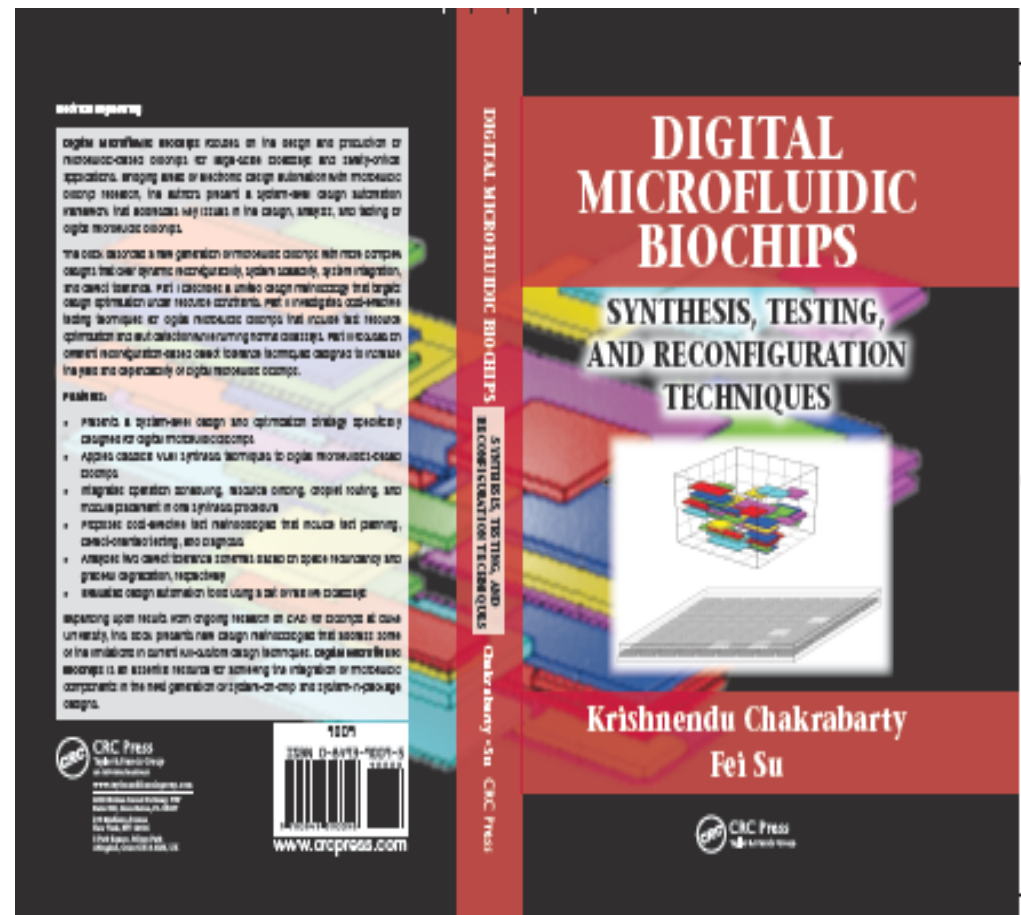
Krishnendu Chakrabarty  
Jun Zeng

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