ARTIST Summer School in Europe 2009

Autrans (near Grenoble), France

September 7-11, 2009

Design Automation Methods for Digital Microfluidic Biochips

Invited Speaker: Krishnendu Chakrabarty

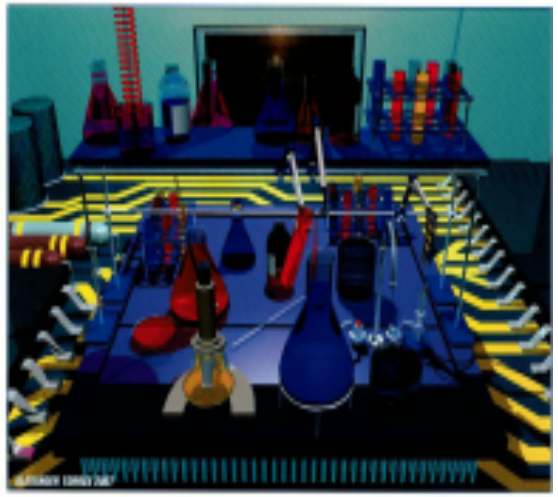
Professor of Electrical and Computer Engineering

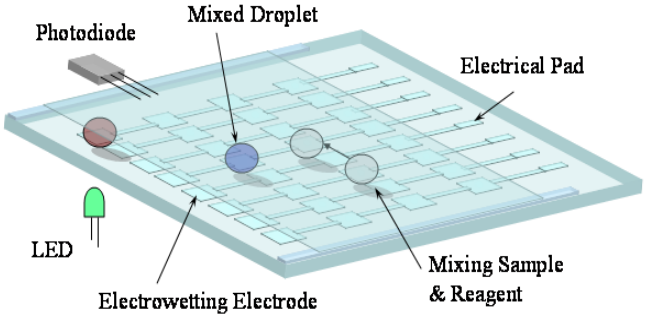
Duke University

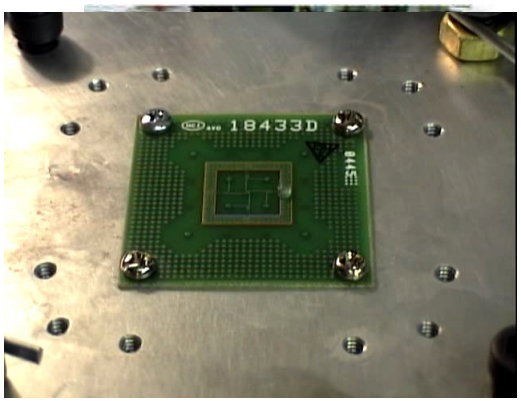
http://www.artist-embedded.org/

Design Automation Methods for Digital Microfluidics Biochips

Connecting Biochemistry to Electronics CAD







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Acknowledgments

- Students: Tianhao Zhang, Fei Su, William Hwang, Phil Paik, Tao Xu, Vijay Srinivasan, Yang Zhao
- Post-docs and collaborators: Dr. Vamsee Pamula, Dr. Michael Pollock, Prof. Richard Fair, Dr. Jun Zeng (Coventor, HP)
- Dr. S. (Krish) Krishnamoorthy, Baxter Healthcare Corporation
- 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

Advanced Liquid Logic, Inc.
nanoliter lab-on-a-chip powered by digital microfluidics

National Science Foundation
WHERE DISCOVERIES BEGIN

Talk Outline

- Motivation
- Technology Overview
  - Microarrays
  - Continuous-flow microfluidics: channel-based biochips
  - “Digital” microfluidics: droplet-based biochips
- Overview of Fabrication Method
- Design Automation Methods
  - Synthesis and module placement
  - Droplet Routing
  - Pin-Constrained Design
  - Testing and Reconfiguration
- Conclusions

Predict the Future

Slide adapted from Rob Rutenbar's ASP-DAC 2007 talk

Motivation for Biochips

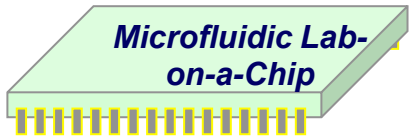
- Clinical diagnostics, e.g., healthcare for premature infants, point-of-care diagnosis of diseases
- “Bio-smoke alarm”: environmental monitoring
- Massive parallel DNA analysis, automated drug discovery, protein crystallization

CLINICAL DIAGNOSTIC APPLICATION

Conventional Biochemical Analyzer

Shrink

Lab-on-a-chip for CLINICAL DIAGNOSTICS



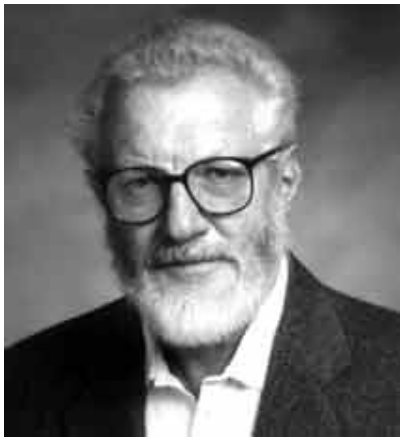
20nl sample

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

The Futility of Predicting Applications

Kroemer's Lemma of New Technology:

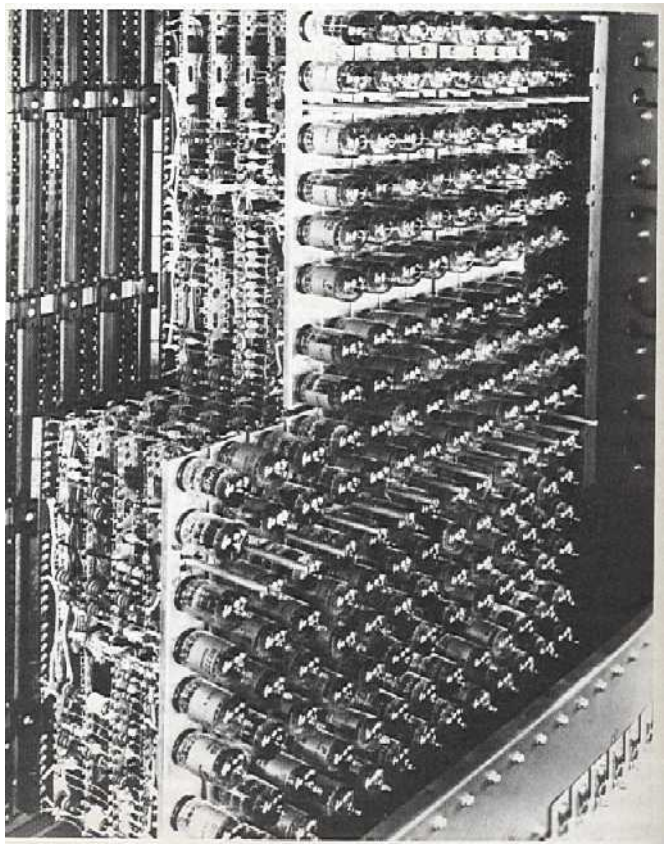
The principal applications of any sufficiently new and innovative technology have always been—and will continue to be—applications created by that technology.



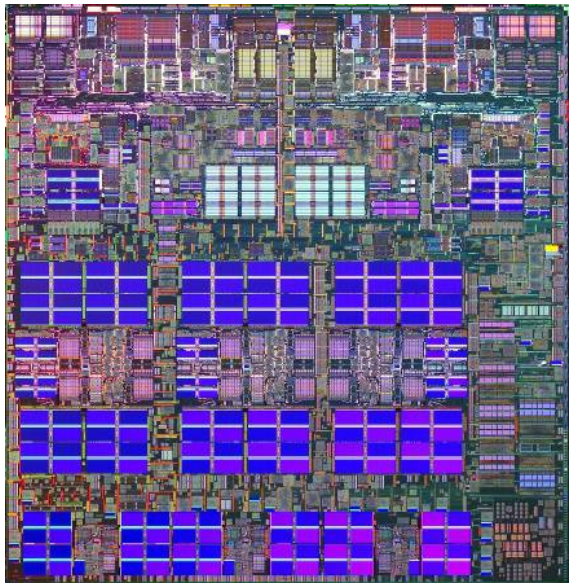
Herbert Kroemer, Department of Electrical and Computer Engineering, University of California at Santa Barbara
Nobel Prize winner for Physics, 2000

Tubes to Chips: Integrated Circuits

- Driven by Information Processing needs



IBM 701 calculator (1952)

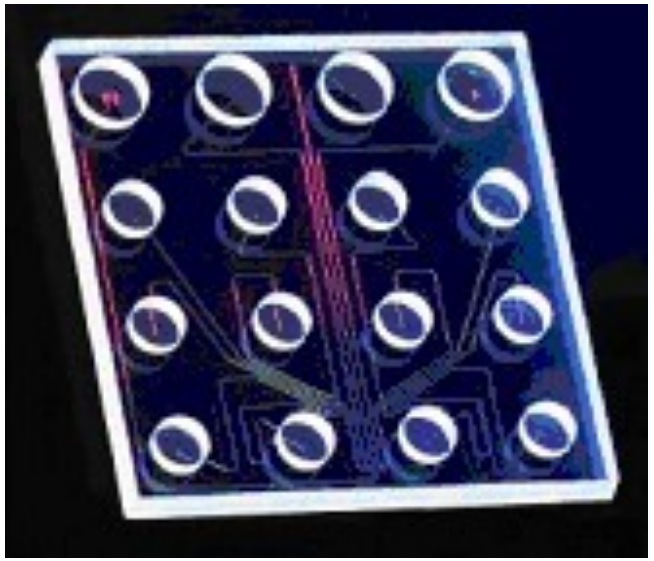


IBM Power 5 IC (2004)

Tubes to Chips: BioChips

- Driven by biomolecular analysis needs

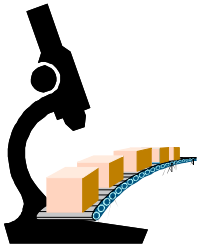
Test tube analysis



Agilent DNA analysis
Lab on a Chip (1997)

By the way, **what's a biochip?**

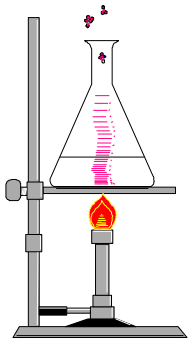
It's a miniature disposable for an
HTS - High-Throughput Screening -
(bio)analytical instrument



what does it do?

Essentially the same operations you did in high school chemistry class:

dispensing,
mixing,
detecting,
discarding,-



just a lot cheaper and a lot faster than you did

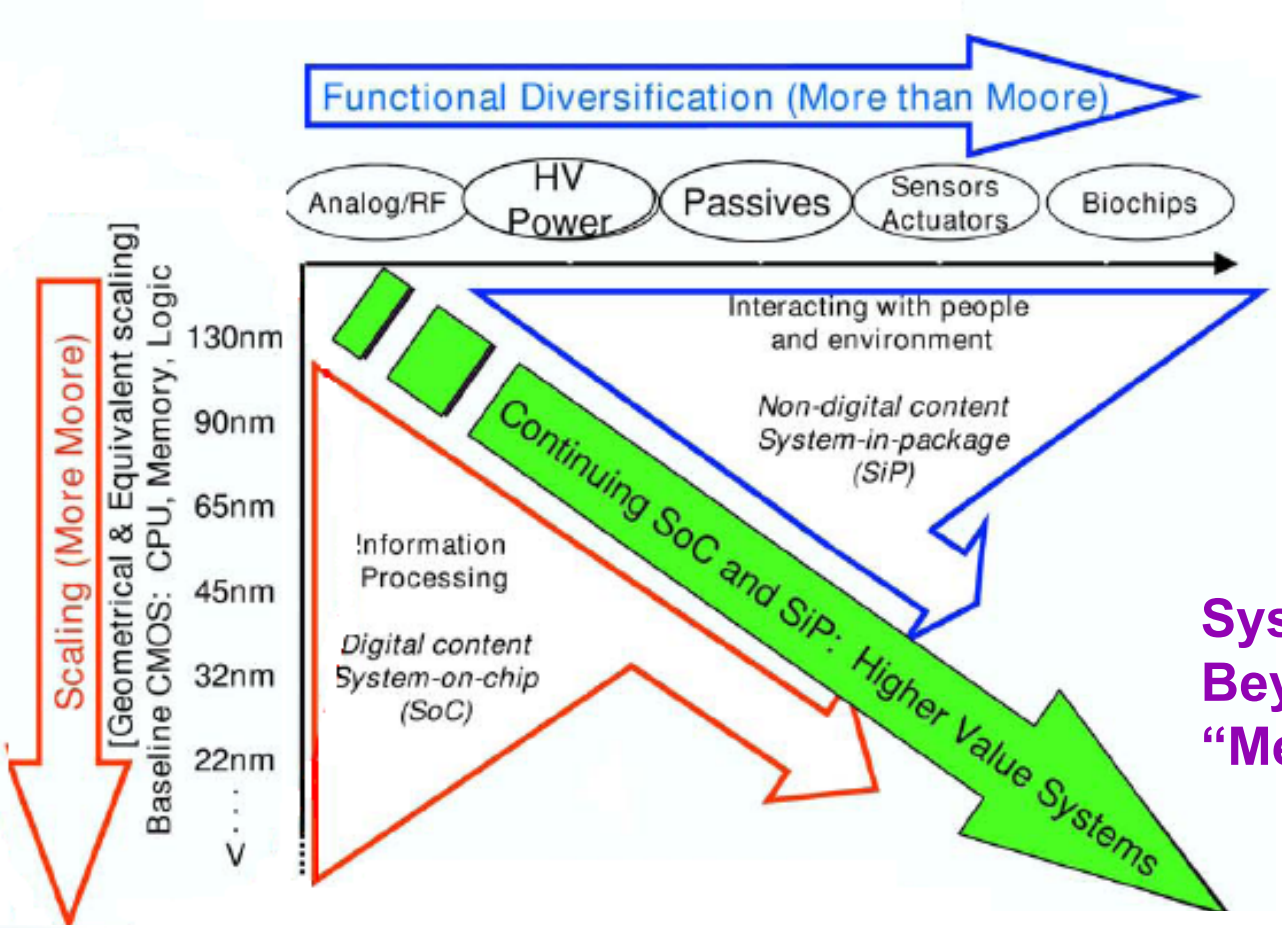
Why do chips have to be small?

High-**T**hroughput is why. If you do 10^6 assays in 10µl format, each time you do a reaction you'll need 10 liters of reagents. With the typical cost of biological reagents, even Big Pharma can't afford this.

By the way, why High-Throughput?

- Because you need a lot of raw data for many applications
- Because, with the currently available technology, to produce raw data that would keep a CPU busy for a few minutes (**$0.1**), you need a Ph.D. scientist and a couple of technicians for a month (**$10,000**)

Why Do We Care?



System Driver for Beyond 2009: "Medical"

ITRS 2007

Intel Research Day 2007: Biochip prototype demonstrated for point-of-care diagnostics and lab testing

Press Releases and News Items

NOVEMBER 10, 2008

Intel Takes Step Into Home Health Care

By DON CLARK

Article | Comments

Email | Printer Friendly | Share: Yahoo Buzz | Text Size + -

Intel Corp. is taking its next step in building a business in health care, introducing technology to help homebound patients with chronic medical problems.

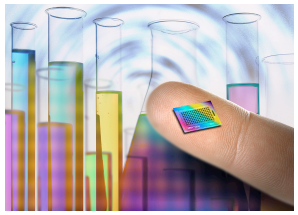
View Full Image

The Silicon Valley company, at a medical conference in New Orleans, announced a series of trials with health-care organizations of specialized hardware and software developed by the chip maker. The tests are designed to show whether the new tools bring improved results in treating conditions such as diabetes, hypertension and heart disease.

THE WALL STREET JOURNAL

A team of scientists at the Lawrence Berkeley National Laboratory won the Bronze for their work in developing a microchip that, by analyzing DNA, is able to identify thousands of different varieties of bacteria that might be present in air, water, soil, blood or tissue samples. The PhyloChip can detect potentially disease-causing bacteria without the lengthy process of growing cultures. And unlike other genetic-testing methods, it can distinguish thousands of different pathogens simultaneously.

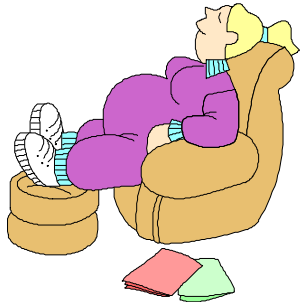
Classification of Biochips



What are the main types of biochips?

Passive (array):

all liquid handling functions are performed by the instrument. The disposable is simply a patterned substrate.

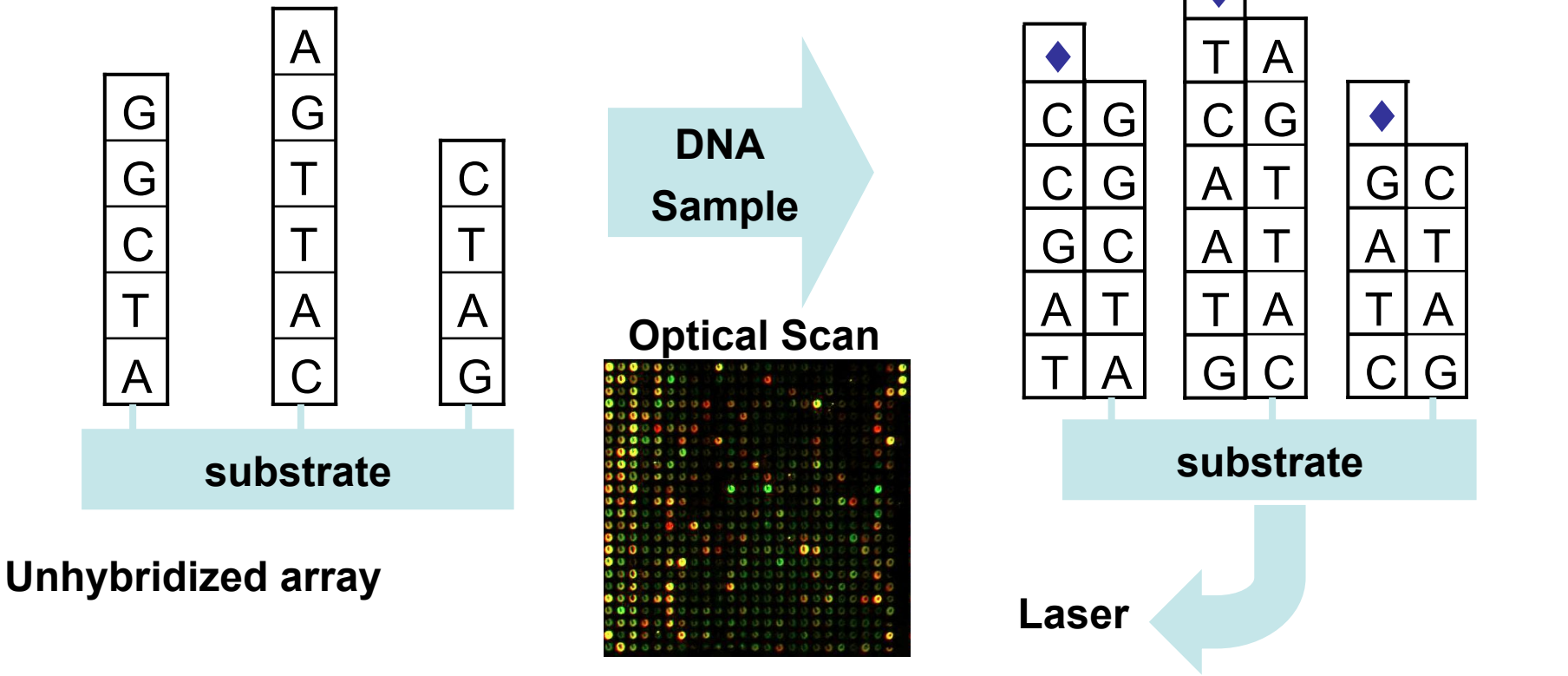


Active (lab-on-chip, μ-TAS):

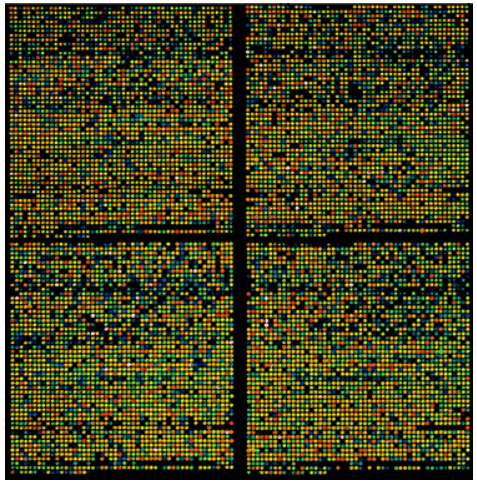
some active functions are performed by the chip itself. These may include flow control, pumping, separations where necessary, and even detection.

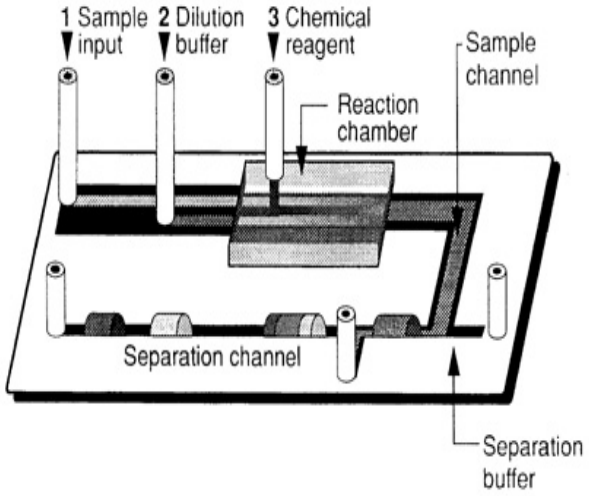
Microarrays

- 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*
- Only implement hybridization reaction



Types of Assays





DNA Probe Assays ← Immunoassays → Clinical Chemistry

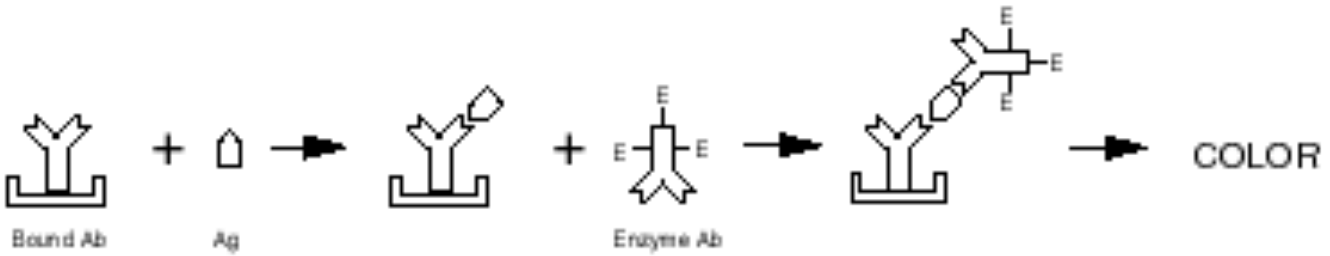
Cancer detection — Estrogens — Cortisol — Uric acid — Glucose

10 — 10^4 — 10^7 — 10^{10} — 10^{13} — 10^{16} — 10^{19} molecules/mL

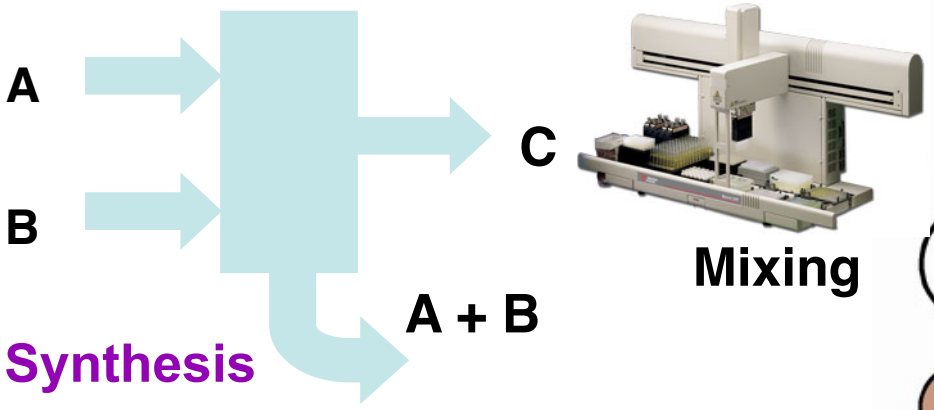
HIV in blood and concentrated airborne bioagents — Creatinine — Sodium — Cholestrol

Fluidic Steps in Immunoassays

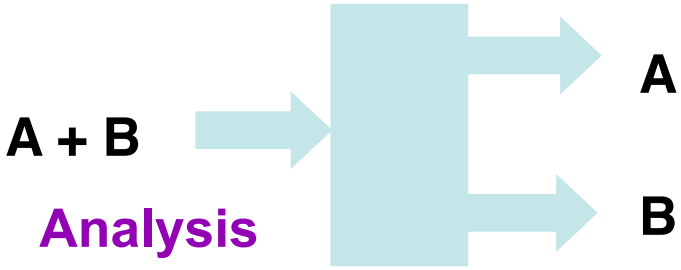
- Bind probe (antibody) to a substrate
- Wash substrate (removes unbound antibody)
- Add target solution to bind target (antigen) to probe (antibody)
- Wash substrate (removes unbound antigen)
- Add tagged antibody for detection

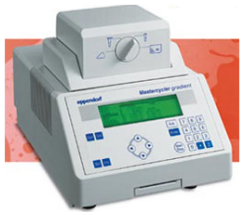


Why is Biochemistry-on-a-Chip Difficult?



Mixing



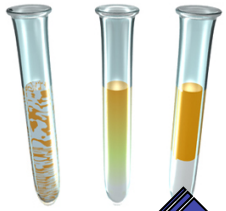


Reaction

Separation

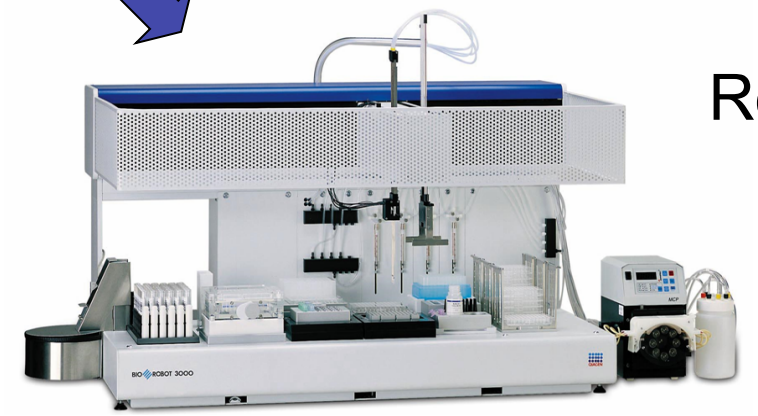
Why is Biochemistry-on-a-Chip Difficult?

Motivation for Microfluidics



Test tubes

- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

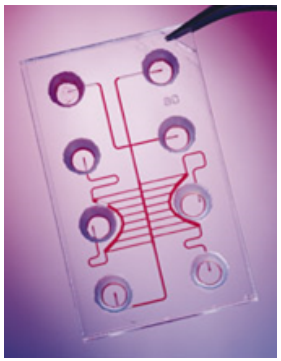


Robotics

- ☑ Automation
- ☑ Integration
- ☐ Miniaturization

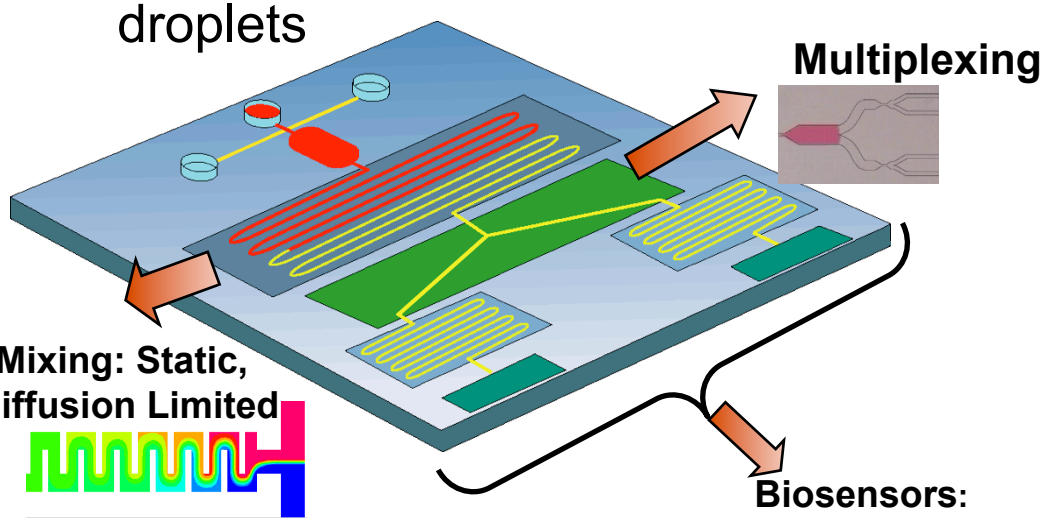
Microfluidics

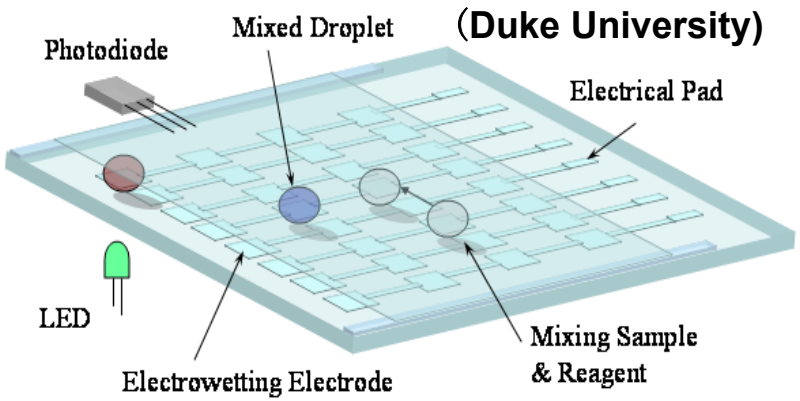
- ☑ Automation
- ☑ Integration
- ☑ Miniaturization



Microfluidics

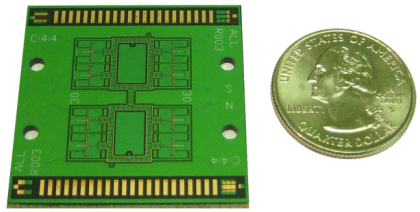
- Continuous-flow lab-on-chip: Permanently etched microchannels, micropumps and microvalves
- Digital microfluidic lab-on-chip: Manipulation of liquids as discrete droplets





Biosensors:
Optical: SPR, Fluorescence etc.
Electrochemical: Amperometric, Potentiometric etc.

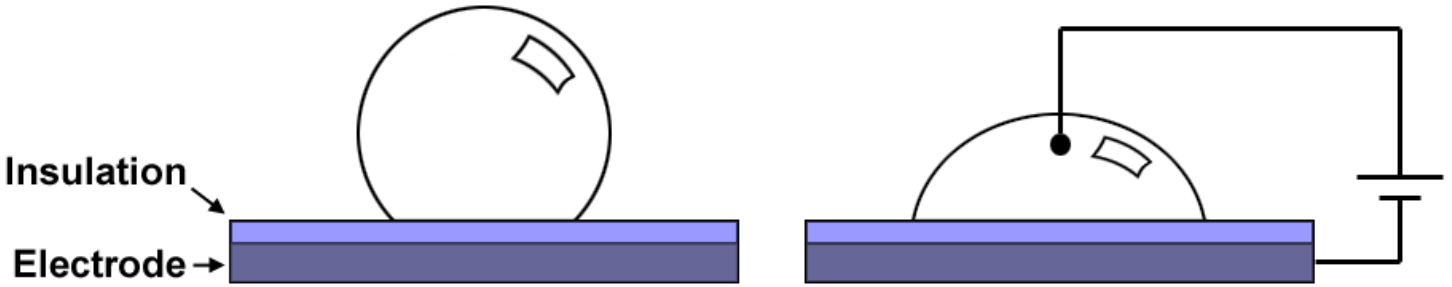
Printed circuit board lab-on-a-chip – inexpensive and readily manufacturable



Control electronics (shown) are suitable for handheld or benchtop applications

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

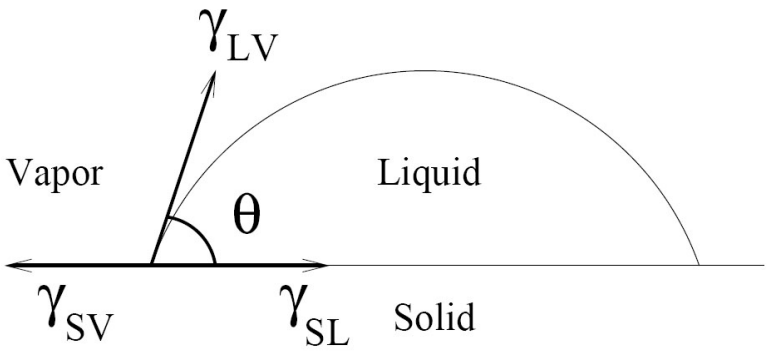


No Potential
A droplet on a hydrophobic surface originally has a large contact angle.

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

Electrowetting: Physical Principles (I)

- Motion of droplets is based on the differences between contact angles in the advancing and receding lines of a droplet.
- When a droplet rests on a non-wetting solid surface, the forces acting at the solid-liquid-vapor interface equilibrate and result in a contact angle θ between the droplet and solid, as described by Young's equation,

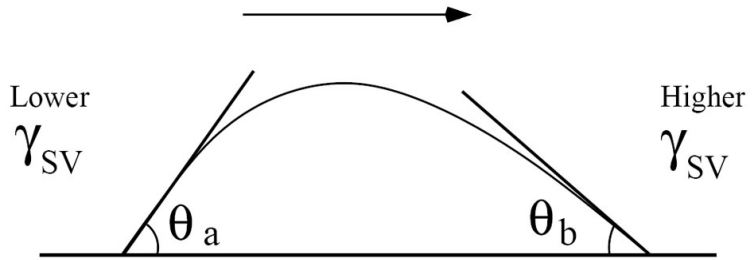


$$\gamma_{LV}\cos(\theta) = \gamma_{SV} - \gamma_{SL}$$

γ_{LV}, γ_{SV} and γ_{SL} are the liquid-vapor, solid-vapor and solid-liquid surface energies

Electrowetting: Physical Principles (II)

- When an imbalance in these surface energies occurs (as in the case of a droplet resting on a surface with a gradient surface energy), a net force is induced
  - Initiate droplet motion
- Imbalance can be induced by chemical, thermal, or electrostatic means
  - In the case of thermally-induced droplet motion, a surface tension gradient can be induced by differentially heating the ends of a droplet, since the surface tension of a liquid decreases with temperature.

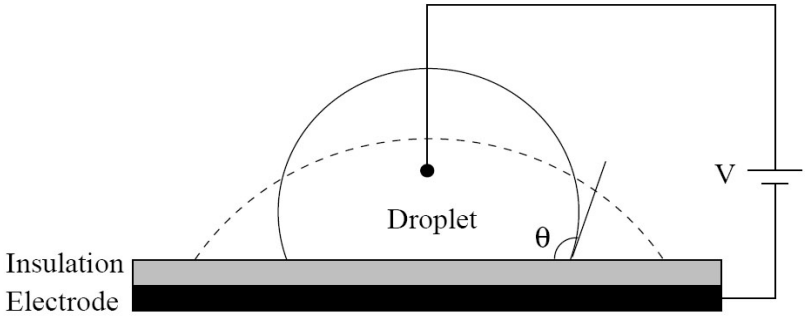


Electrowetting: Physical Principles (III)

- Electrowetting-based actuation of droplets: electrical fields used to induce surface tension gradients.
  - Electrowetting effect ⇒the surface energy can be directly modified by the application of an electric field
- Consider a droplet resting on a electrode separated by a hydrophobic insulator
  - A potential is applied between the droplet and the electrode, resulting in a capacitive energy *E* stored in the insulator. The resulting energy is:

$$E = \frac{\varepsilon_0 \varepsilon_r A}{2d} V^2$$

$$\Rightarrow \quad \gamma_{SL}(V) = \gamma_{SL}(0) - \frac{\varepsilon_0 \varepsilon_r A}{2d} V^2$$



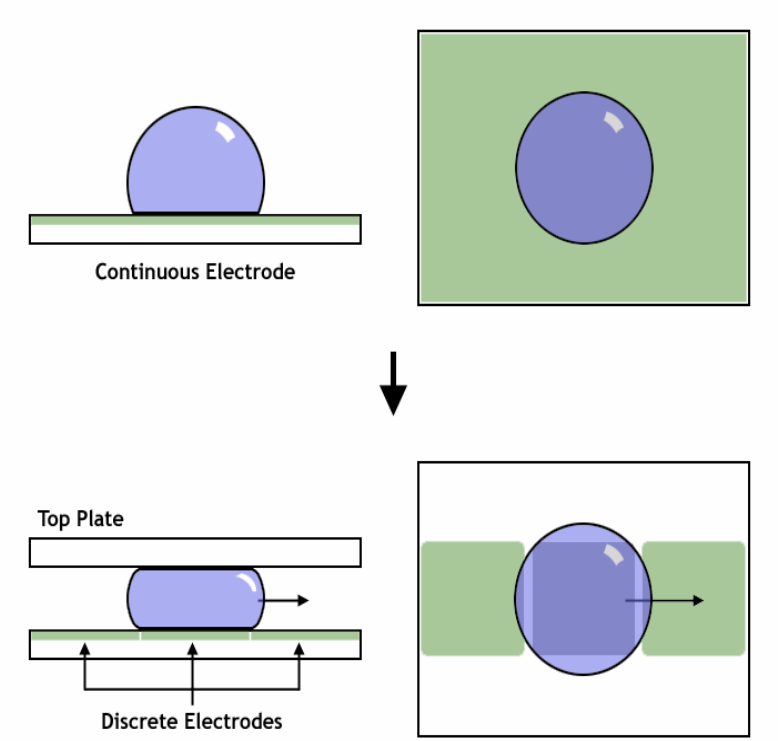
Contact angle change:

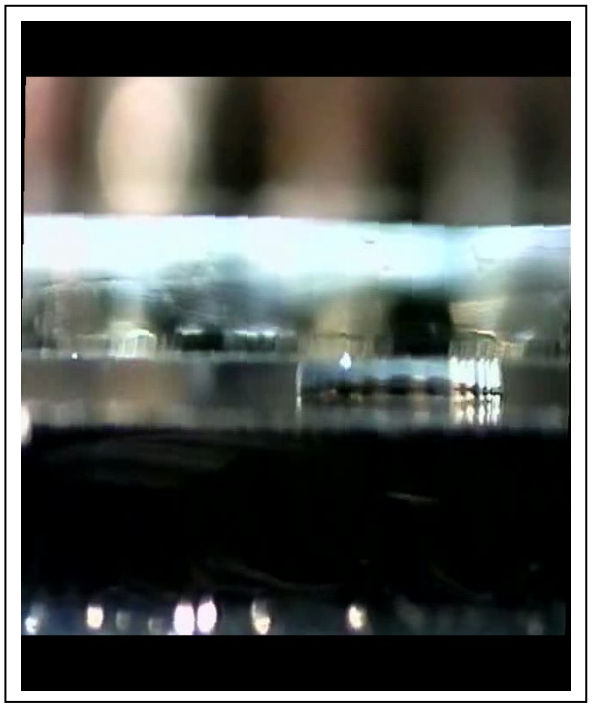
$$\cos\theta(V) = \cos\theta(0) - \frac{\varepsilon_0 \varepsilon_r A}{2d\gamma_{LV}} V^2$$

Reference: P. Y. Paik, V. K. Pamula and K. Chakrabarty, "*Adaptive Cooling of Integrated Circuits using Digital Microfluidics*", Artech House, Norwood, MA, 2007.

What is Digital Microfluidics?

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



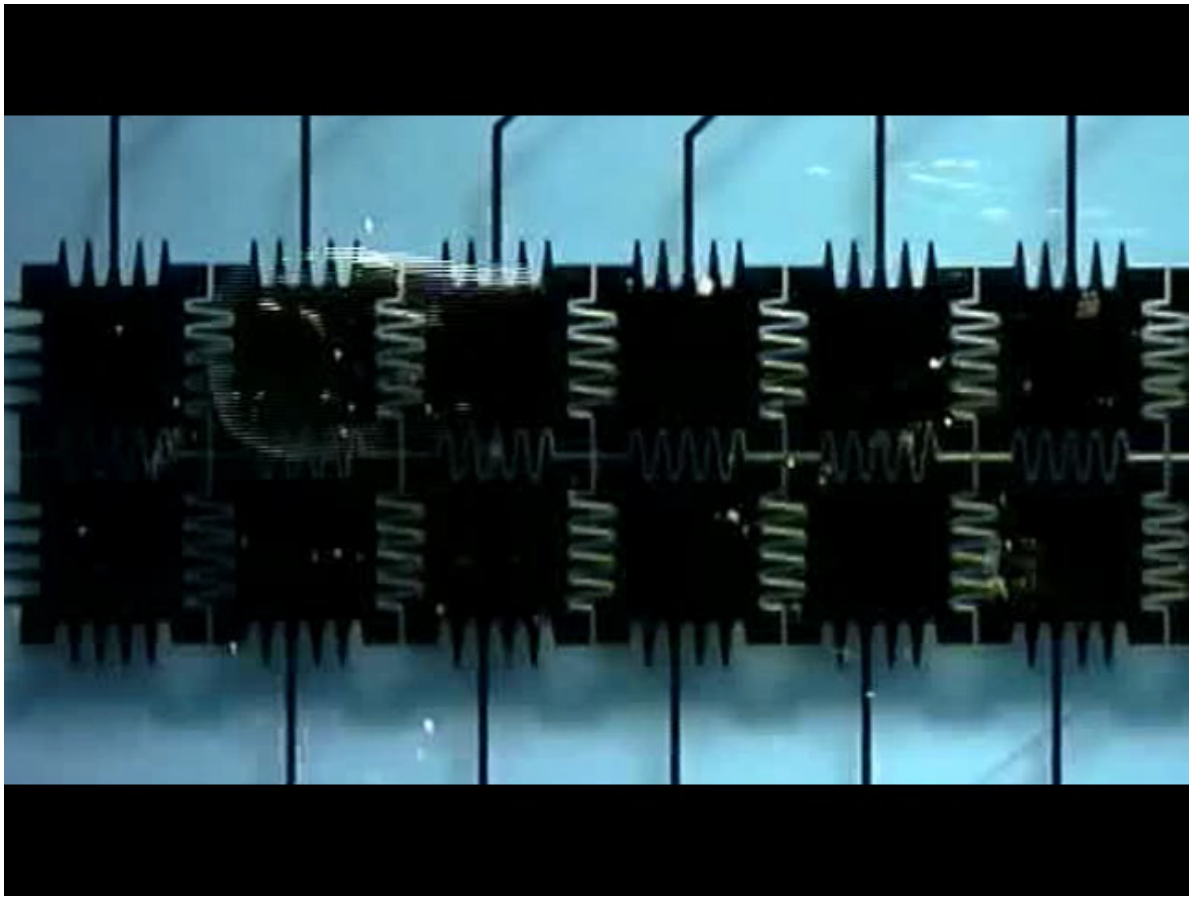


Droplet Transport (Side View)

Note: oil is typically used to fill between the top and bottom plates to prevent evaporation, cross-contamination

Pitch ~ 100 µm, Gap ~ 50 µm

What is Digital Microfluidics?

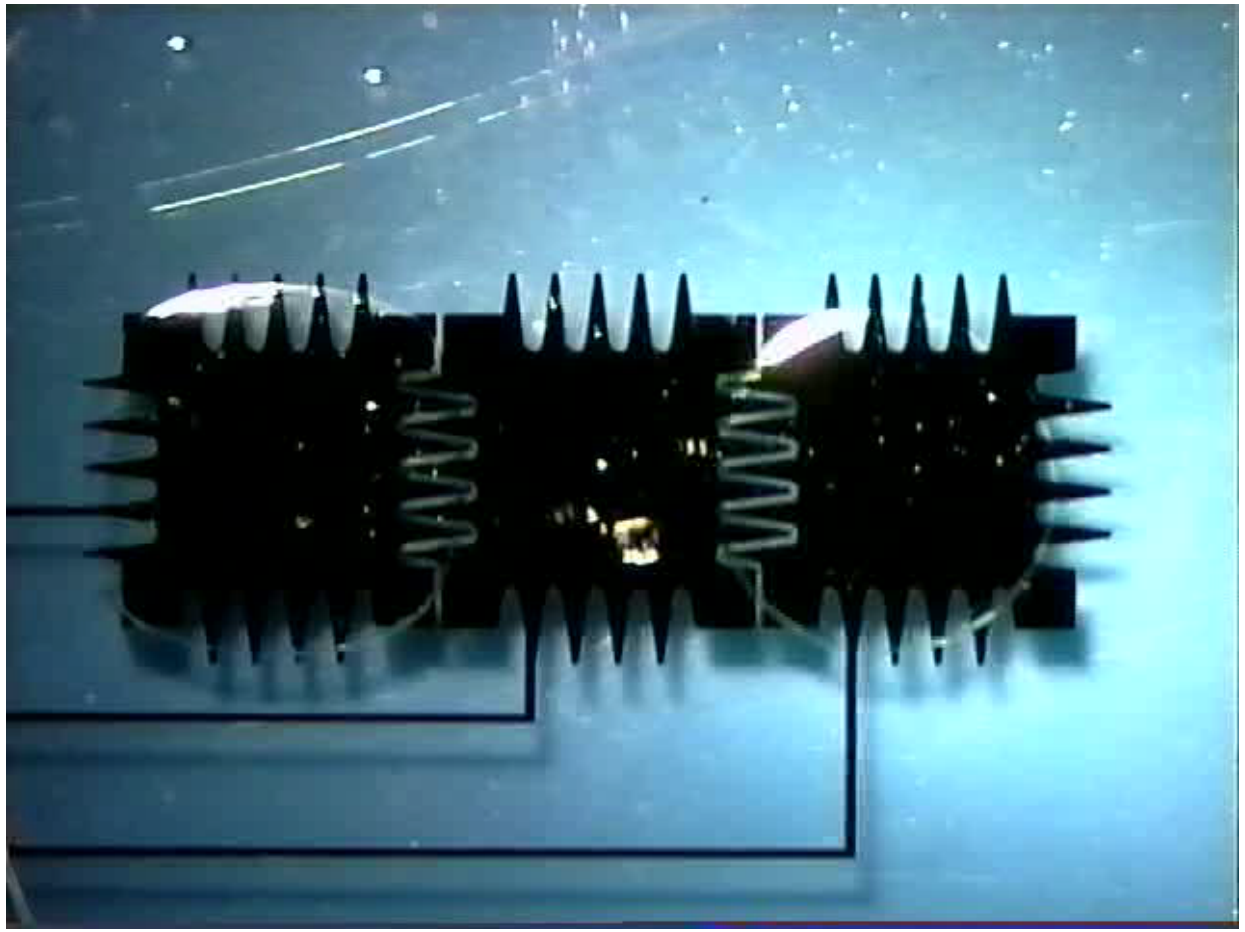


Transport
25 cm/s flow rates, order of magnitude higher than continuous-flow methods

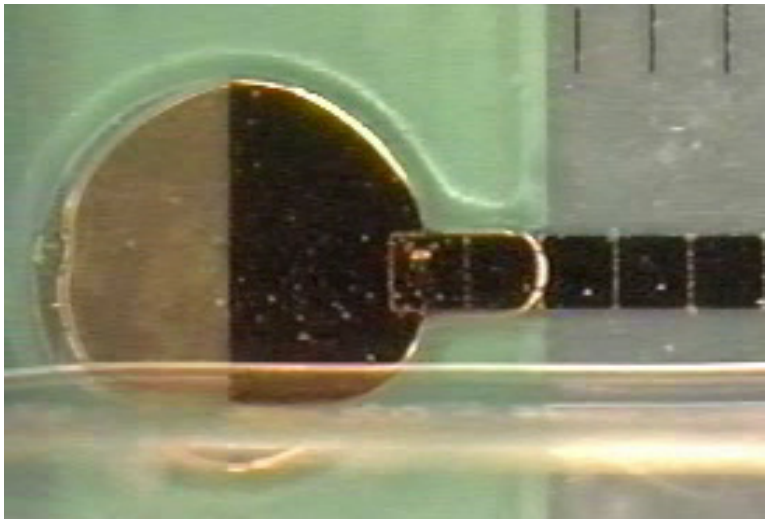
For videos, go to www.ee.duke.edu/research/microfluidics
http://www.liquid-logic.com/technology.html

What is Digital Microfluidics?

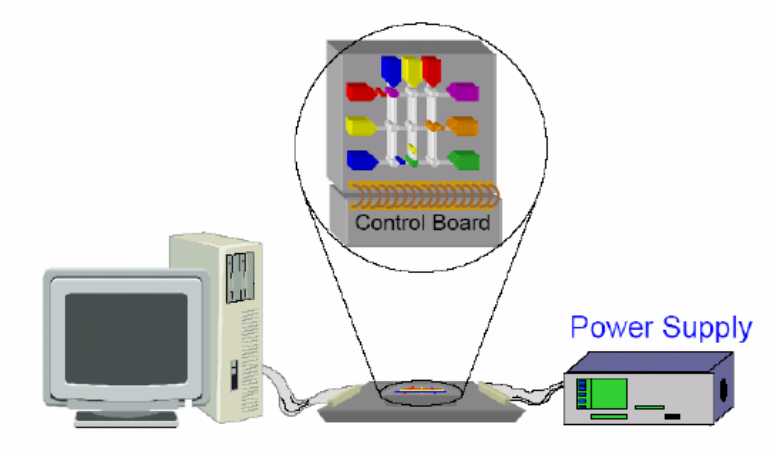
Splitting/Merging

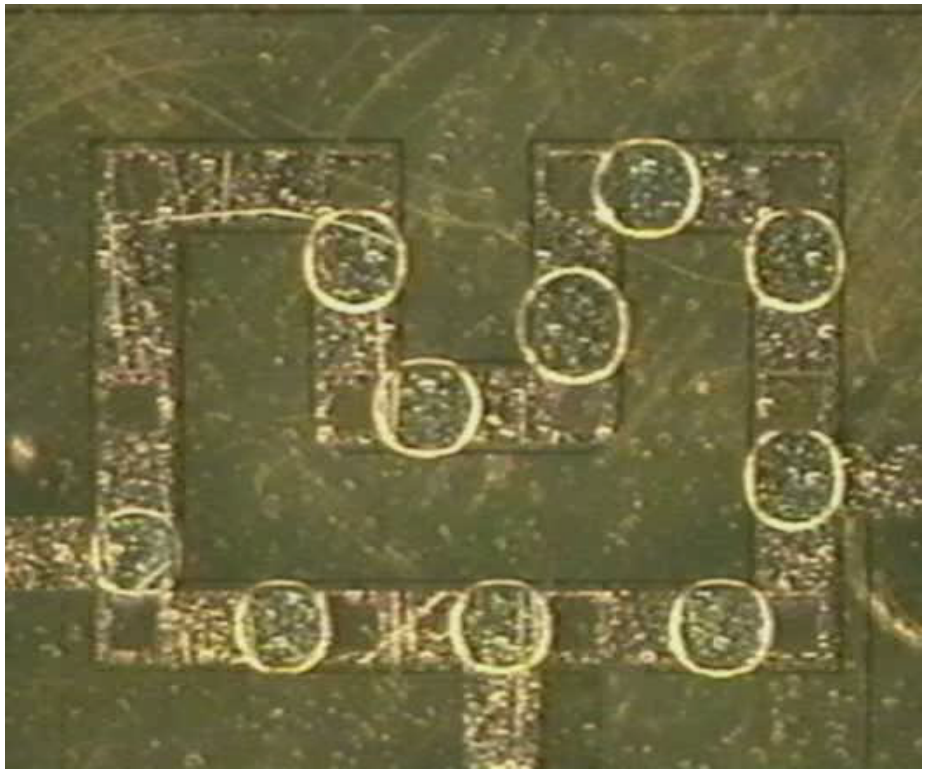


Demonstrations of Digital Microfluidics



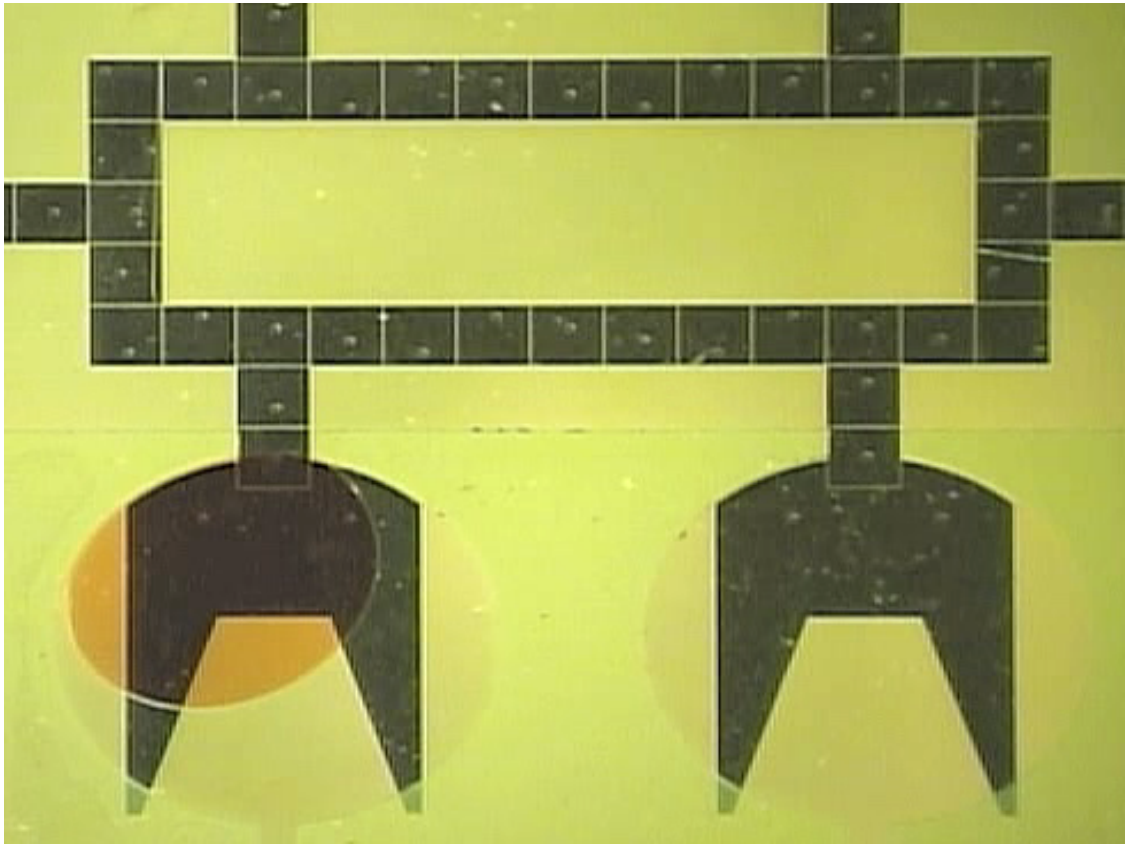
Droplet Formation





Synchronization of many droplets

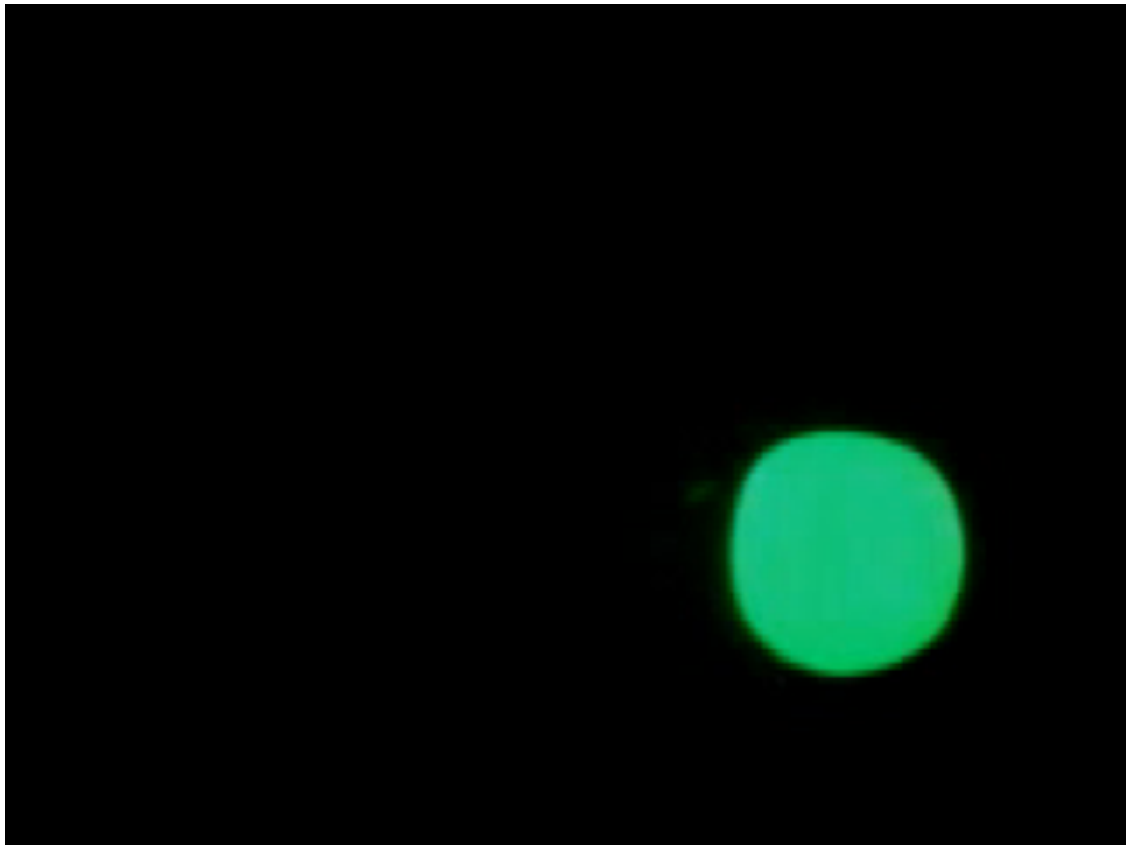
What is Digital Microfluidics?



Droplet Formation
8 droplets in 3.6s

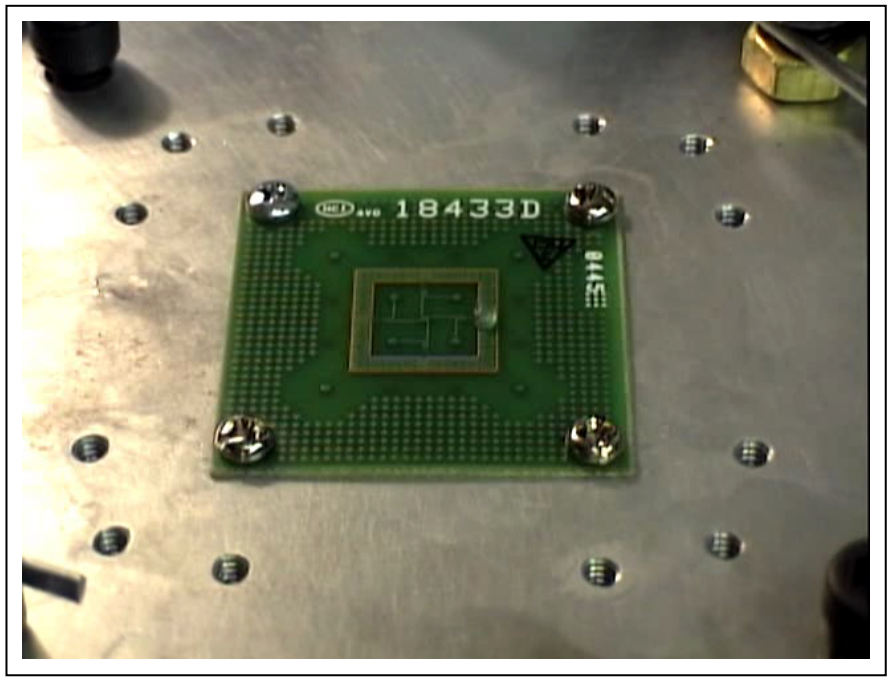
What is Digital Microfluidics?

Mixing



Advantages

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

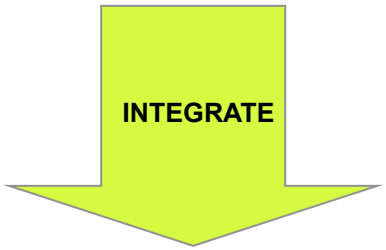


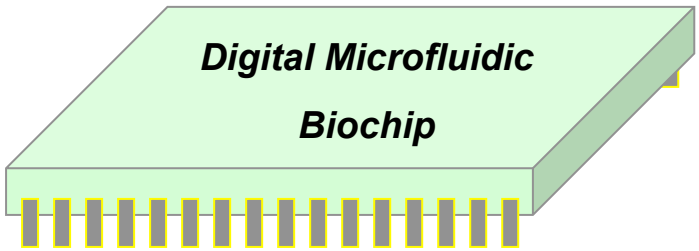
Droplet Transport on PCB (Isometric View)

- 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

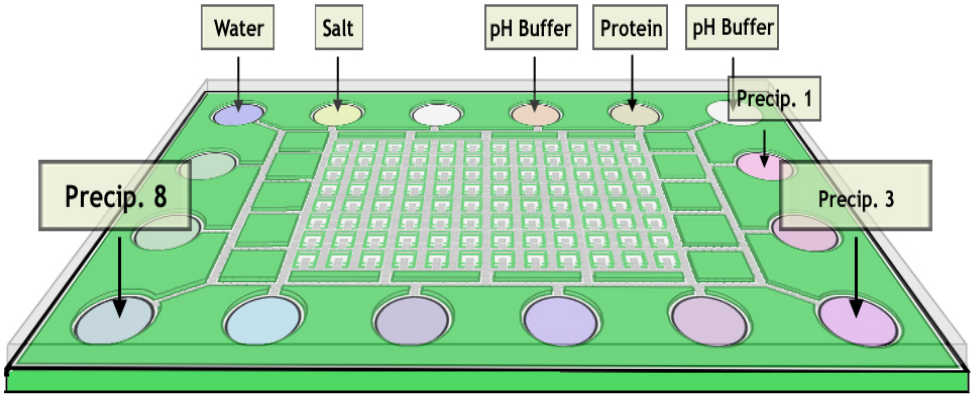
Capabilities

- Digital microfluidic lab-on-chip

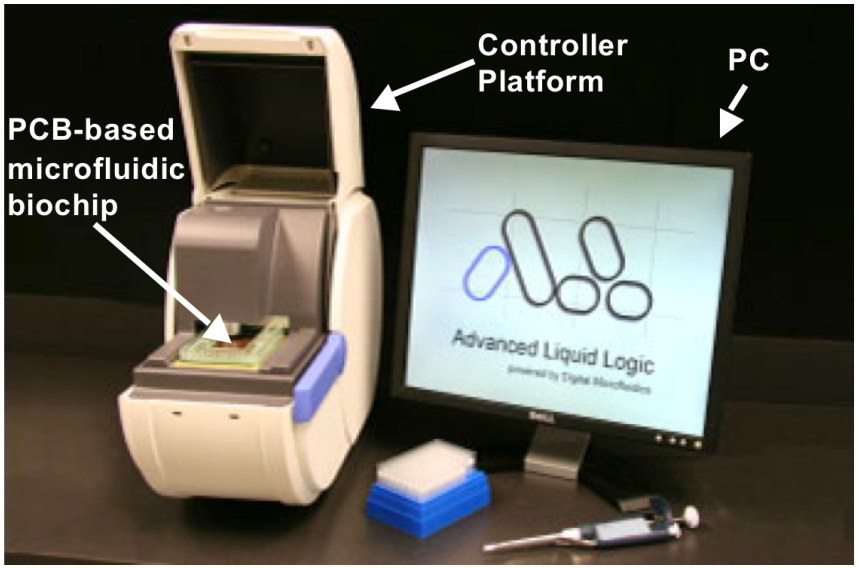


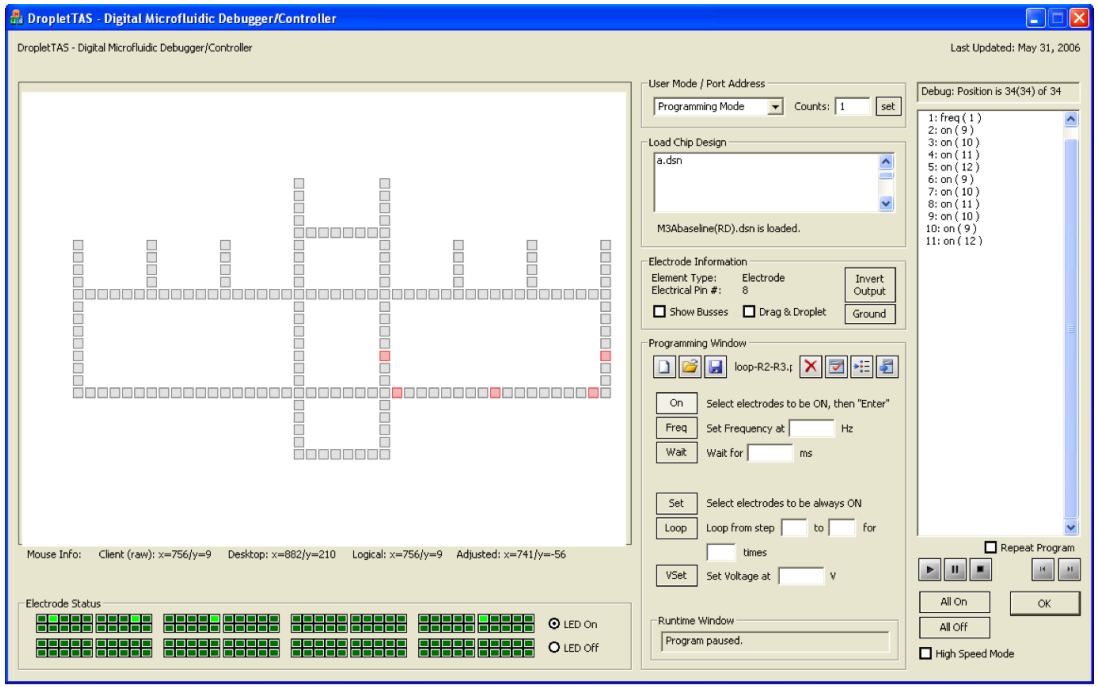


- Basic microfluidic functions (transport, splitting, merging, and mixing) have already been demonstrated on a 2-D array
- Highly reconfigurable system



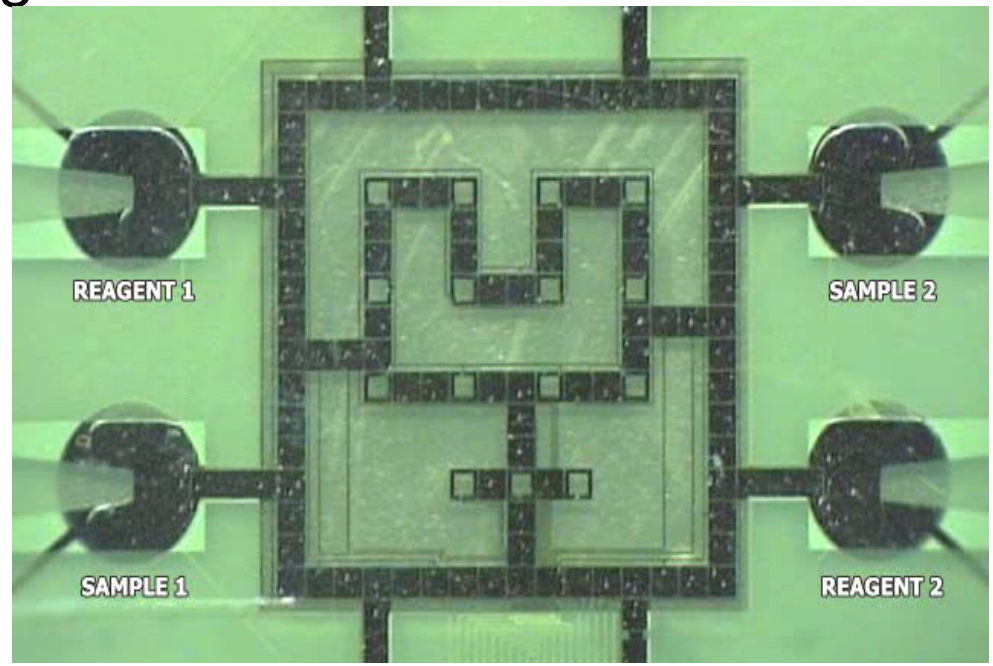
Protein crystallization chip
(under development)


Controller Platform
PC
PCB-based microfluidic biochip
Advanced Liquid Logic


DropletTAS - Digital Microfluidic Debugger/Controller
DropletTAS - Digital Microfluidic Debugger/Controller
Last Updated: May 31, 2006
User Mode / Port Address
Programming Mode
Counts: 1
set
Load Chip Design
a.dsn
M3Abaseline(RD).dsn is loaded.
Electrode Information
Element Type: Electrode
Electrical Pin #: 8
Invert Output
Show Busses
Drag & Droplet
Ground
Programming Window
loop-R2-R3.
On
Select electrodes to be ON, then "Enter"
Freq
Set Frequency at
Hz
Wait
Wait for
ms
Set
Select electrodes to be always ON
Loop
Loop from step
to
for
times
VSet
Set Voltage at
V
Runtime Window
Program paused.
Debug: Position is 34(34) of 34
1: freq (1)
2: on (9)
3: on (10)
4: on (11)
5: on (12)
6: on (9)
7: on (10)
8: on (11)
9: on (10)
10: on (9)
11: on (12)
Repeat Program
All On
OK
All Off
High Speed Mode
Mouse Info: Client (raw): x=756/y=9 Desktop: x=882/y=210 Logical: x=756/y=9 Adjusted: x=741/y=-56
Electrode Status
LED On
LED Off

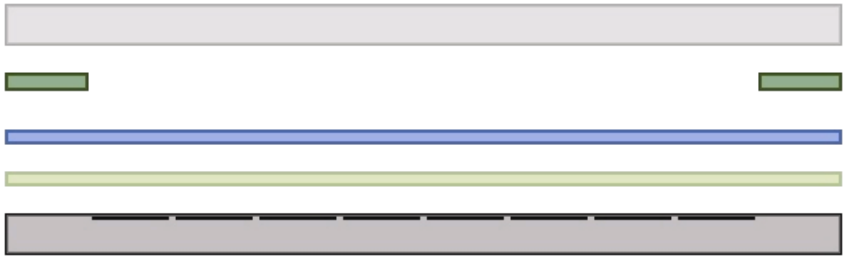
An Example

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



Pipelining of fluidic operations in fabricated microfluidic array

Glass Chip Platform Development



Top Plate (Optional) (i.e. glass or plastic)

Gasket Layer (100 to 600 µm) (proprietary)

Hydrophobic Layer (50 nm) (i.e. Teflon dip coated)

Insulator Layer (1 to 25 µm) (i.e. parylene)

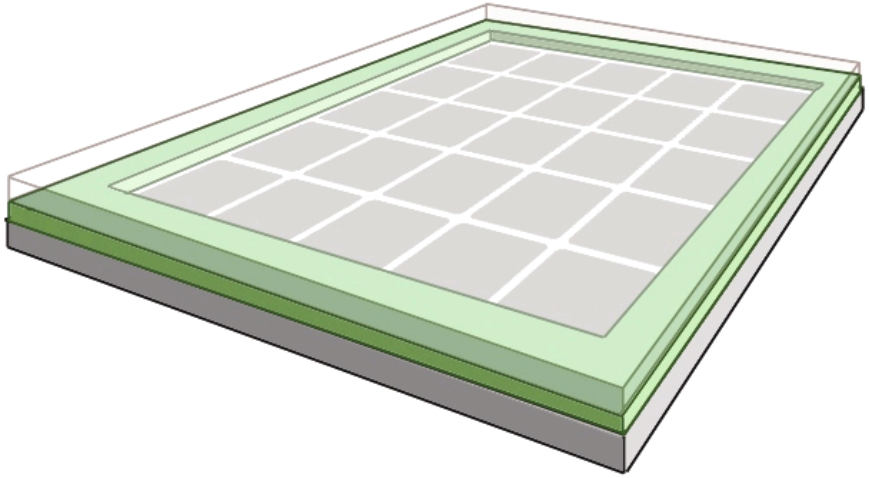
Patterned Metal on Substrate (i.e. chrome on glass via lift-off process)

Chip Assembly

Top plate is either glued or fixed in place by pressure

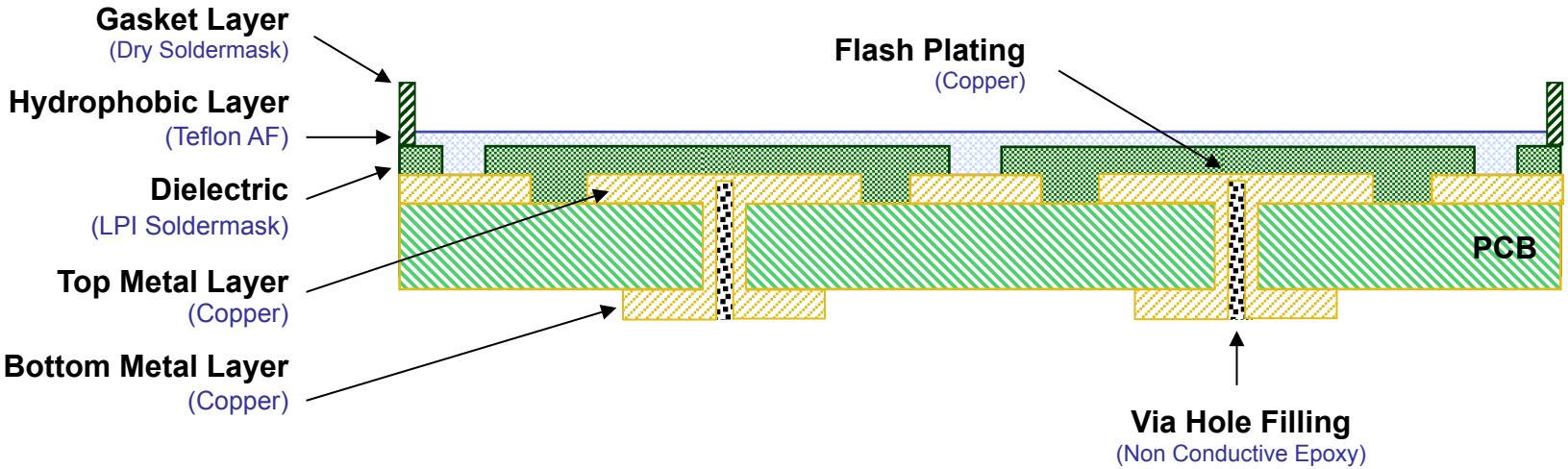
Contacts are made either through the top or bottom

Droplets are either dispensed by hand or formed from on-chip reservoirs



PCB Chip Platform Development

Fabrication Process



- PCB Material – Mitsui BN300 – 64 mil
- Top Metal Layer (Electrodes) – Cu – 15µm
- Bottom Metal Layer (Contacts) – Cu – 15µm
- Dielectric – LPI Soldermask – 25 µm
- Via Hole Filling – Non-conductive Epoxy
- Hydrophobic Layer – Teflon AF – 0.05 to 1.0 µm
- Gasket (spacer) – Dry Film Soldermask (Vacrel 8140) – 4 mils (~95µm after processing)

Computer-Aided Design: Vision

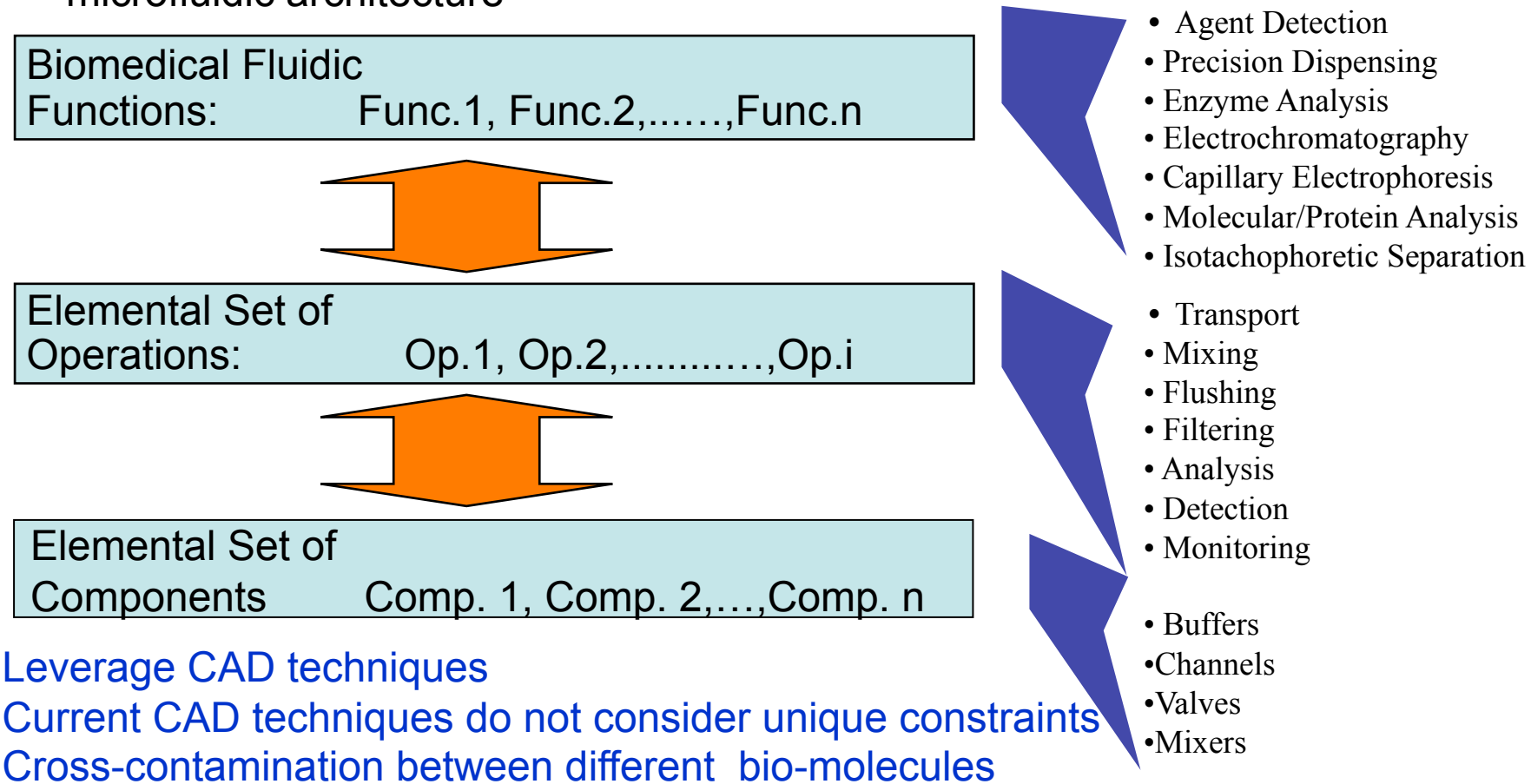
- Automate labor-intensive tasks, reduce burden on chip users
  - Map bioassays to a fabricated chip: schedule fluidic operations, determine droplet flow pathways, configure fluidic modules dynamically, etc.
  - Monitor the chip for defects that require remapping of bioassays
- Role of computer-aided design (CAD) tools
  - Reduce setup time, ...
  - Allow automatic reconfiguration of a faulty chip and remap the remaining steps of bioassay.
  - Develop capabilities that mirror compiler and operating system support provided to software programmers
  - Obviate the need for tedious remapping of assays to the chip by hand for each target application.
- **Similar to an FPGA?**

But, there are important differences

Logic ⟷ Interconnects

Manageable Design Approach

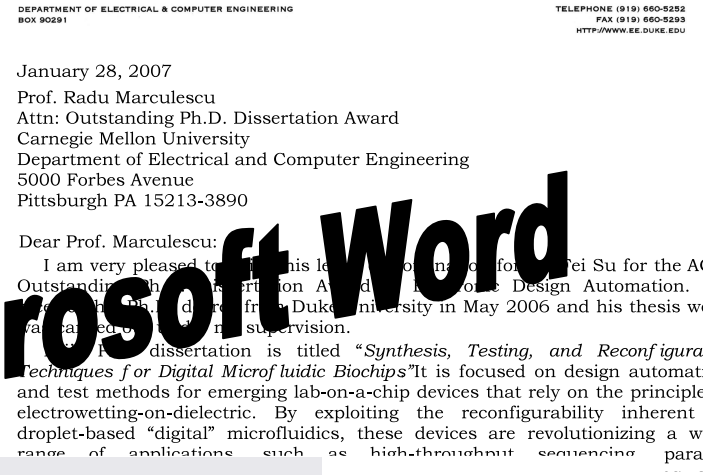
- Diverse biotechnology functions major source of requirements for microfluidic architecture



- Leverage CAD techniques
- Current CAD techniques do not consider unique constraints
- Cross-contamination between different bio-molecules
- Limited availability of stock solutions for use in assay protocols

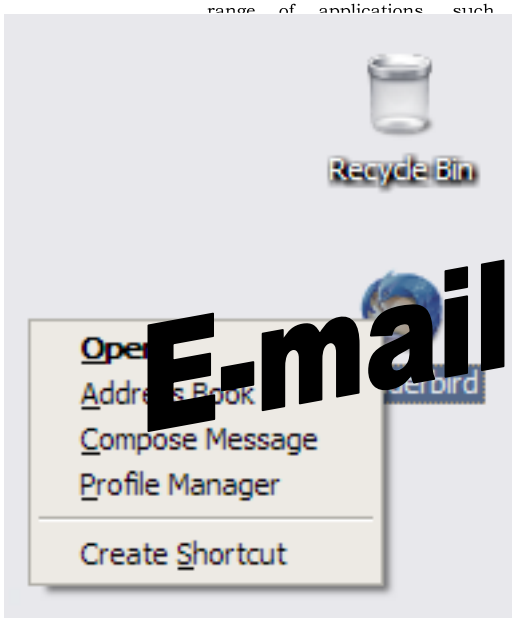
Similar to Concurrency in a PC!

Web Browser



Microsoft Word

Acrobat Reader

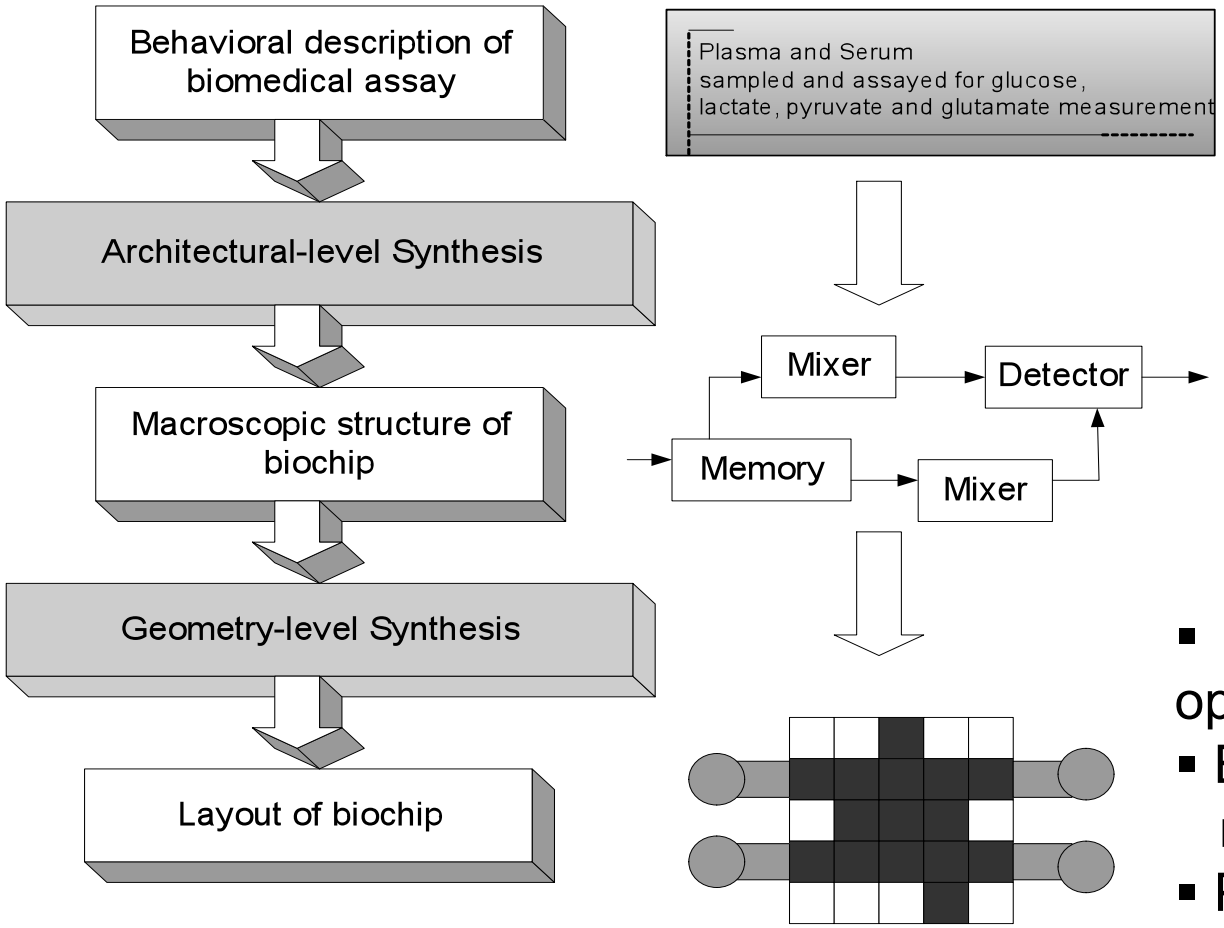


E-mail client

The operating system manages complexity, allows multi-tasking!

Design Automation: Biochip Synthesis

- Full-custom bottom-up design → Top-down system-level design



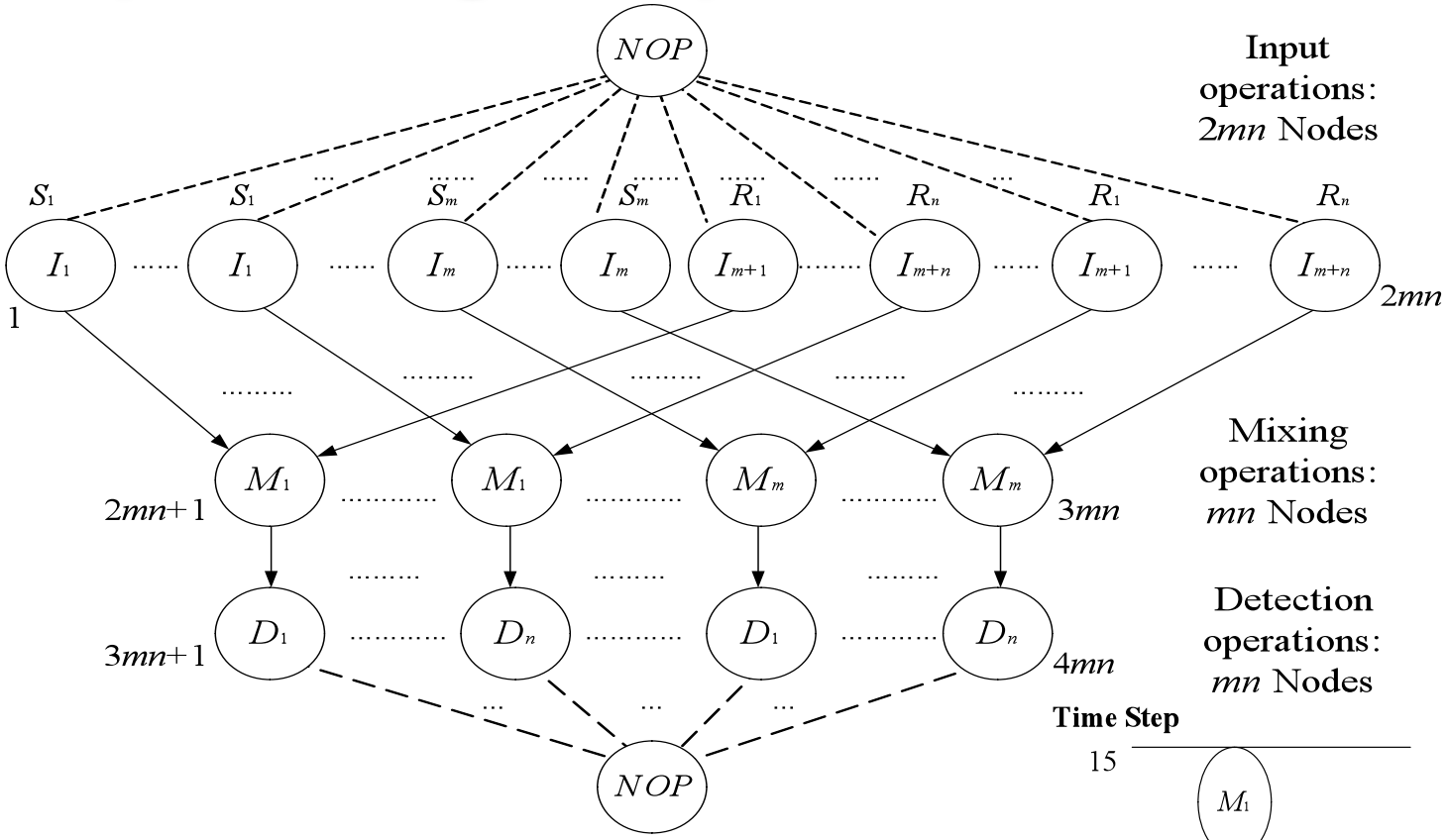
*S*1: Plasma, *S*2: Serum, *S*3: Urine, *S*4: Saliva

Assay1: Glucose assay,
Assay2: Lactate assay,
Assay3: Pyruvate assay,
Assay4: Glutamate assay

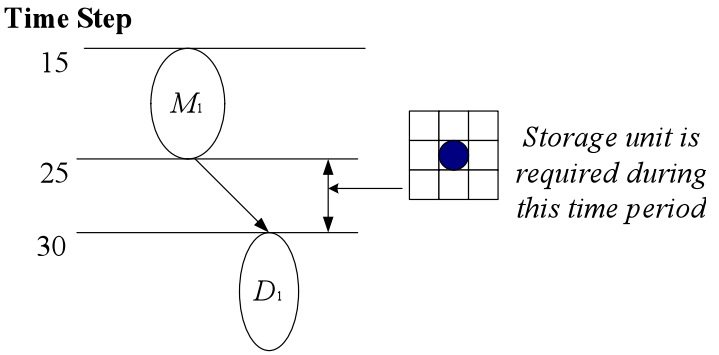
*S*1, *S*2, *S*3 and *S*4 are assayed for Assay1, Assay2, Assay3 and Assay4.

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

Sequencing Graph Model



Sequencing graph model for multiplexed bioassays



Mathematical Programming Model

Objective

- First define a binary variable

$$X_{ij} = \begin{cases} 1 & \text{if operation } v_i \text{ starts at time slot } j. \\ 0 & \text{otherwise} \end{cases}$$

- Starting time of operation v_i :

$$St_i = \sum_{j=1}^{T} j \times X_{ij}$$

- Completion time of operation:

$$C = \max \{St_i + d(v_i) : v_i \in D_1, \ldots, D_n\}$$

- Objective function:

minimize C

Constraints

- Dependency constraints

$St_j \geq St_i + d(v_i)$ if there is a dependency between v_i and v_j

- Resource constraints
  - *Reservoirs/dispensing ports*

Nr reservoirs/dispensing ports assigned to each type of fluid ($Nr = 1$)

$$\sum_{i:v_i \in I_1} X_{ij} \leq 1, \quad \cdots \quad \sum_{1 \leq i:v_i \in I_n \leq m} X_{ij} \leq 1$$

  - *Reconfigurable mixers and storage units*

$$Nmixer(j) + 0.25\, Nmemory(j) \leq Na \quad 1 \leq j \leq T$$

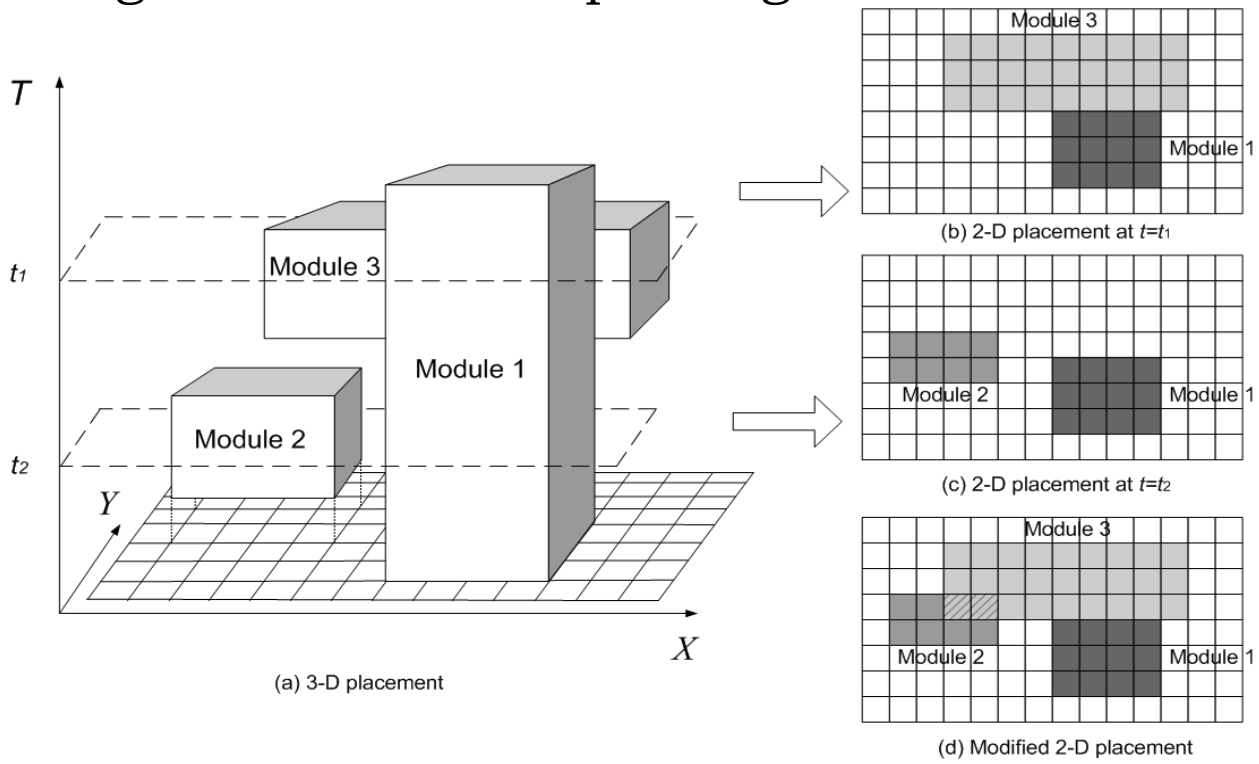
  - *Optical detectors*

Nd detectors are assigned to each bioassay ($Nd = 1$)

$$\sum_{i:v_i \in D_1} \sum_{l=j-d(v_i)}^{j} X_{ij} \leq 1, \quad \cdots \quad \sum_{1 \leq i:v_i \in D_n \leq n_1} \sum_{l=j-d(v_i)}^{j} X_{ij} \leq 1$$

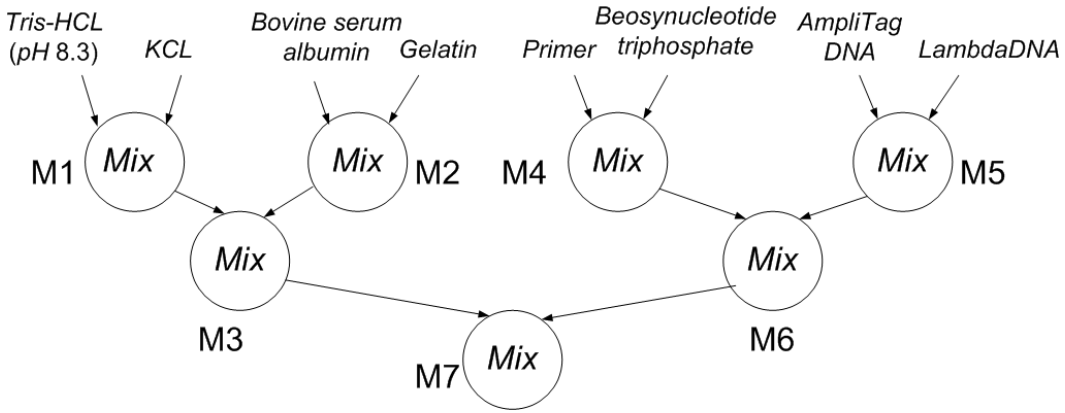
Physical Design: Module Placement

- 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

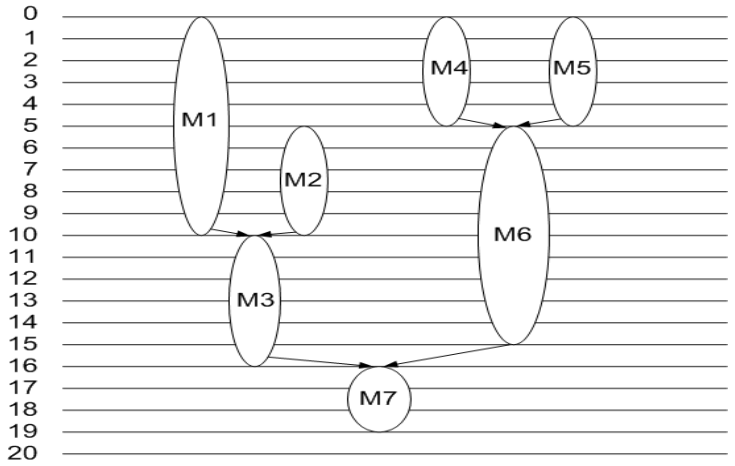


Reduction from 3_D placement to a modified 2-D placement

Application to PCR



Protocol of PCR (mixing phase)



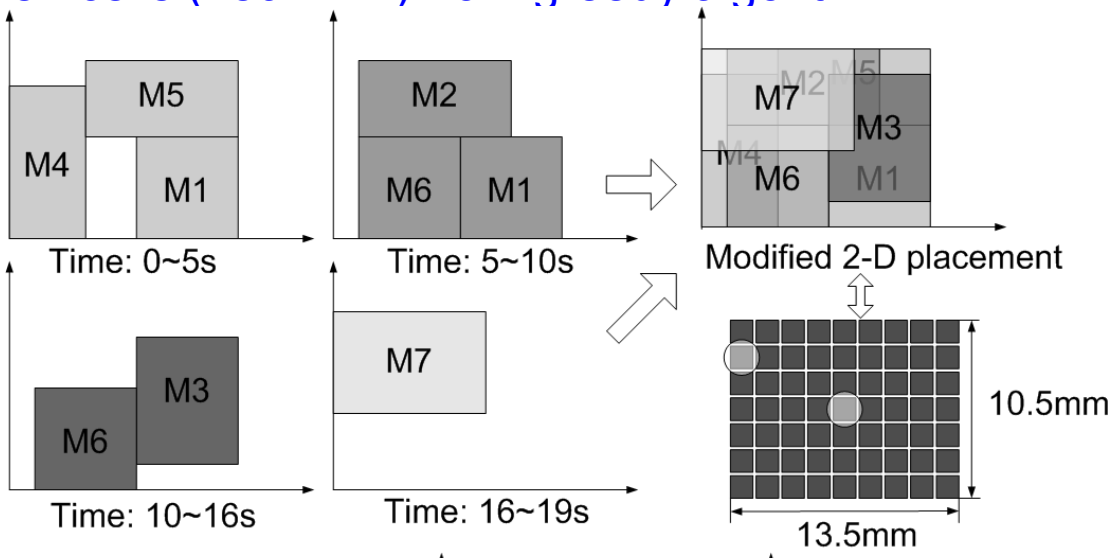
Schedule of PCR

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

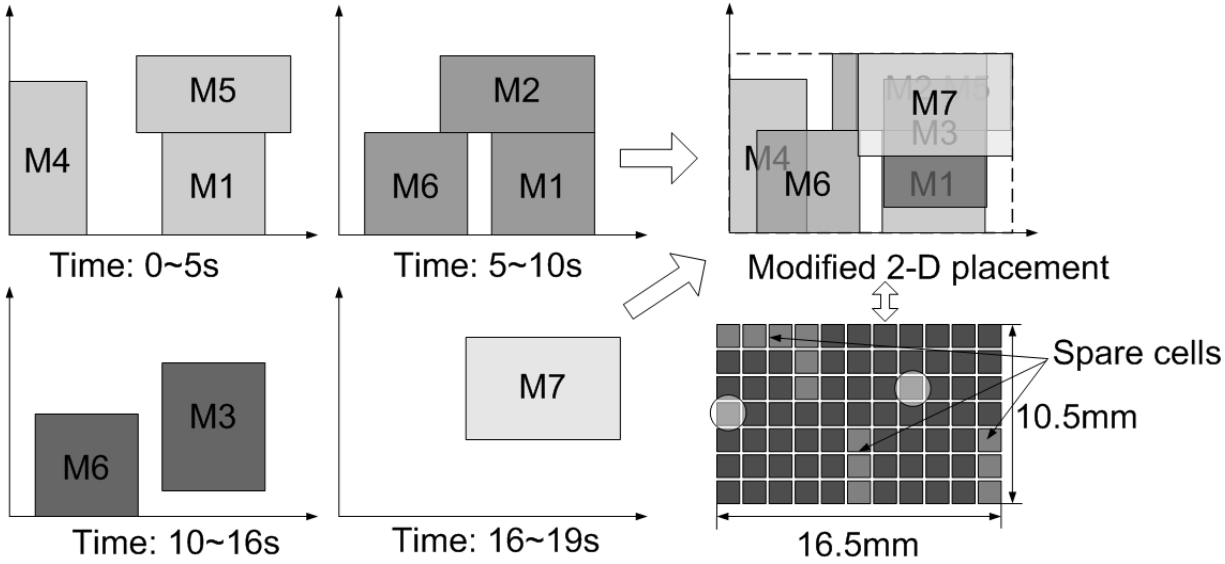
Area: 7x9=63 cells

FTI: 0.1270

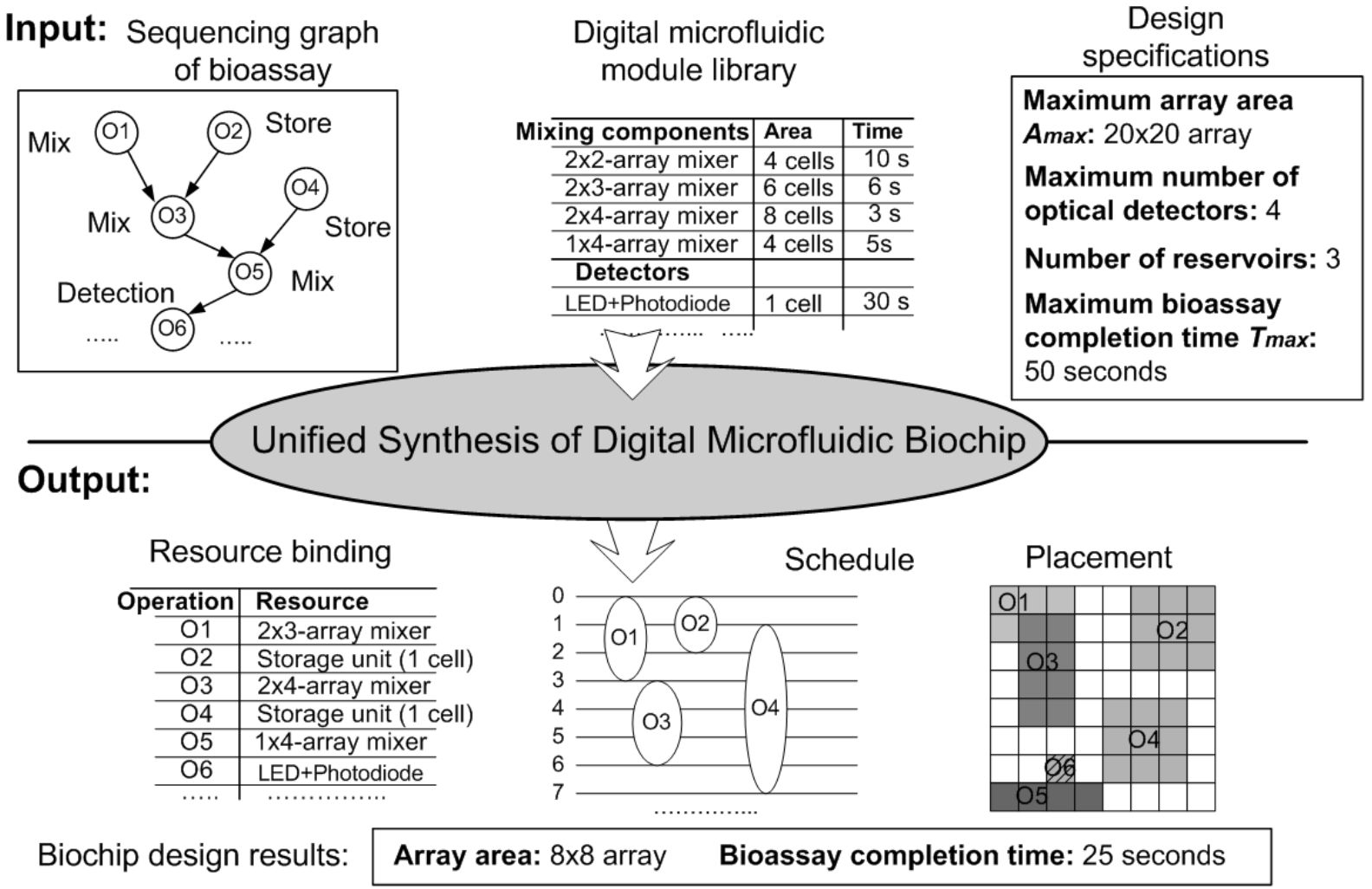
Placement from enhanced module placement procedure

Area: 7x11=77 cells

FTI: 0.8052

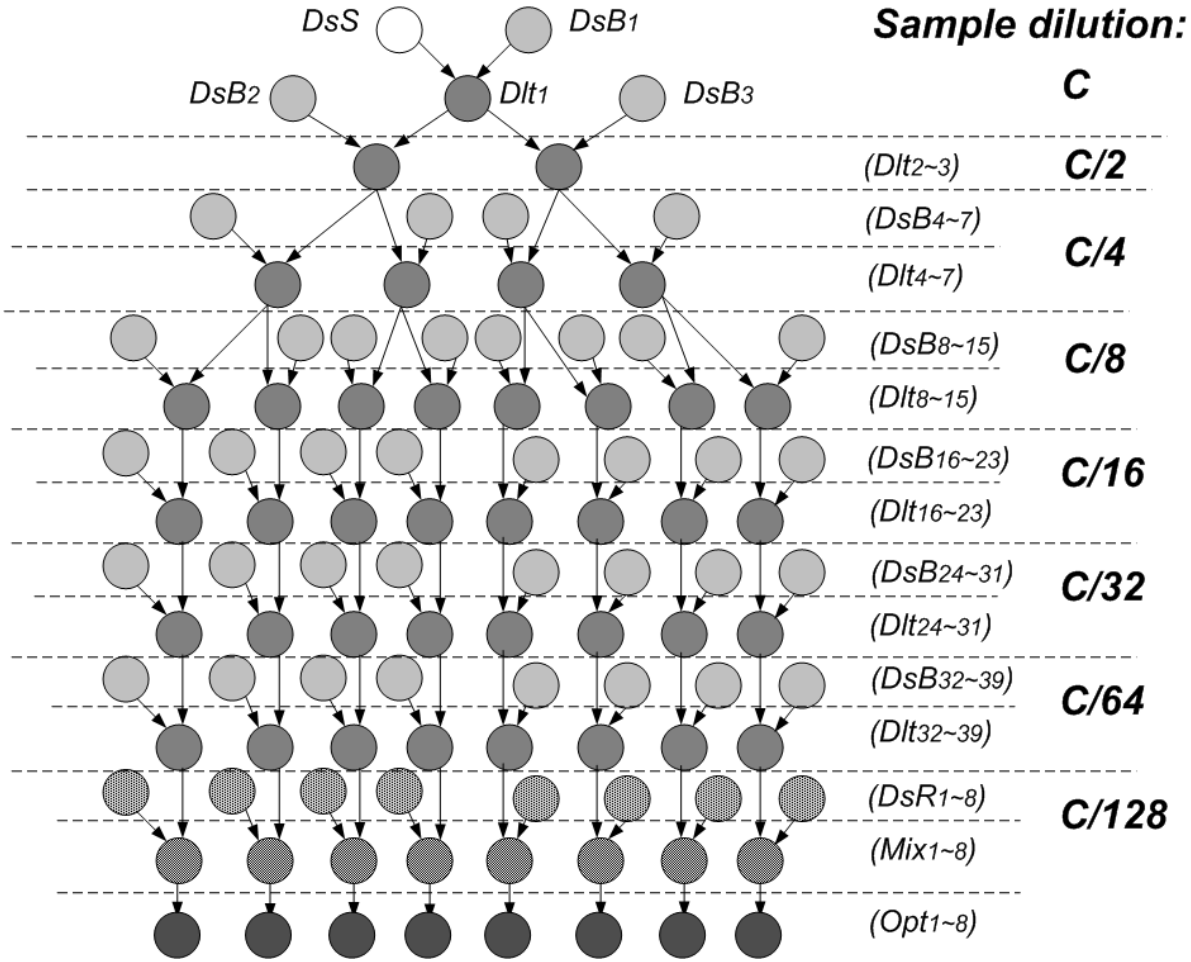


Unified Synthesis Methodology



Protein Assay

Sequencing graph model



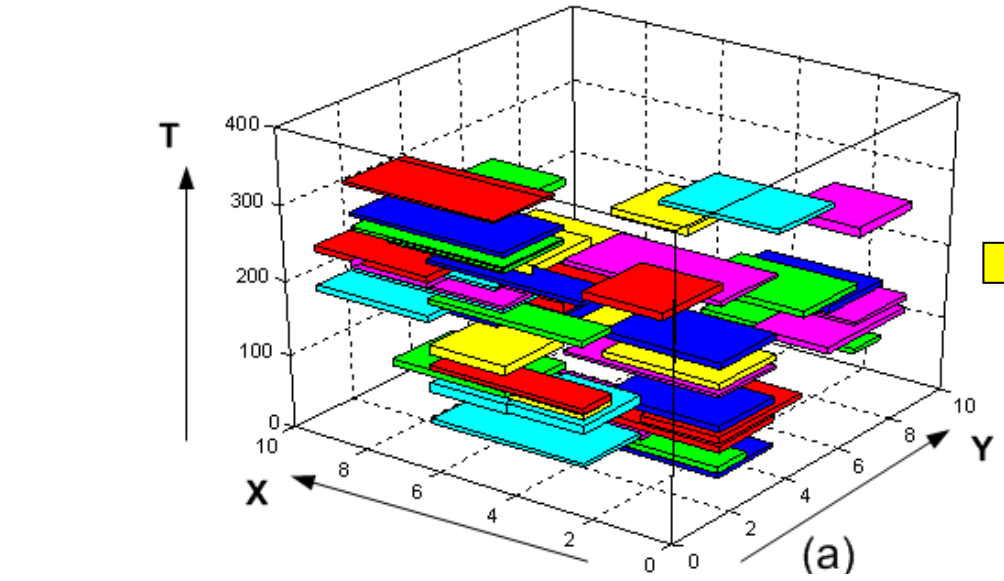
- Maximum array area: *10x10*
- Maximum number of optical detectors: *4*
- Reservoir number: *1 for sample; 2 for buffer; 2 for reagent; 1 for waste*
- Maximum bioassay time: *400 s*

Protein Assay (Cont.)

- Microfluidic module library for synthesis

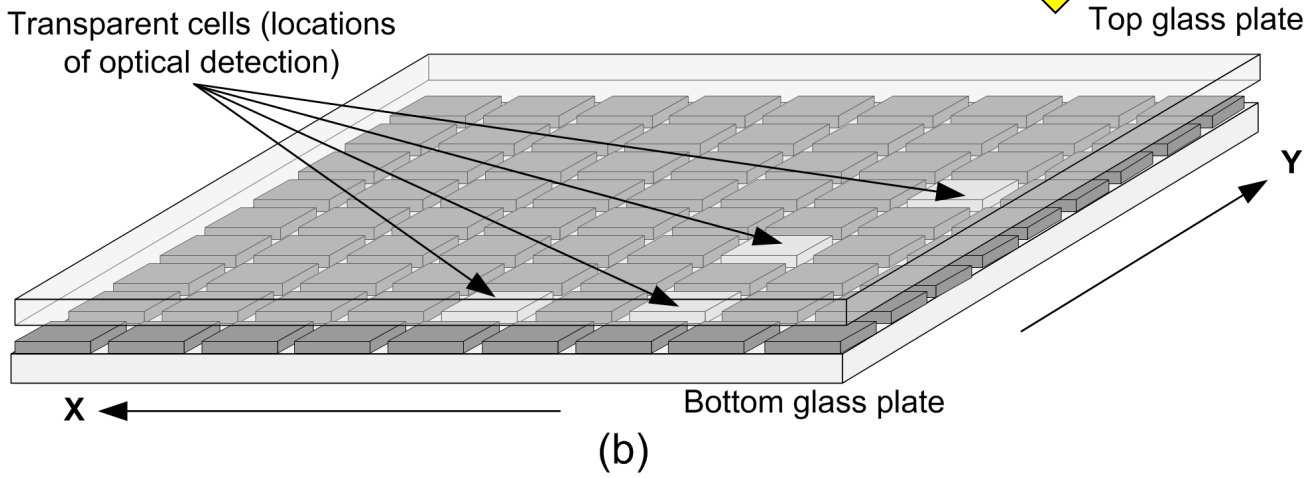
Operation	Resource	Operation Time (s)
DsS; *DsB*; *DsR*	On-chip reservoir/dispensing port	7
Dlt	2x2-array dilutor	12
	2x3-array dilutor	8
	2x4-array dilutor	5
	4-electrode linear array dilutor	7
Mix	2x2-array mixer	10
	2x3-array mixer	6
	2x4-array mixer	3
	4-electrode linear array mixer	5
Opt	LED+Photodiode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

Synthesis Results



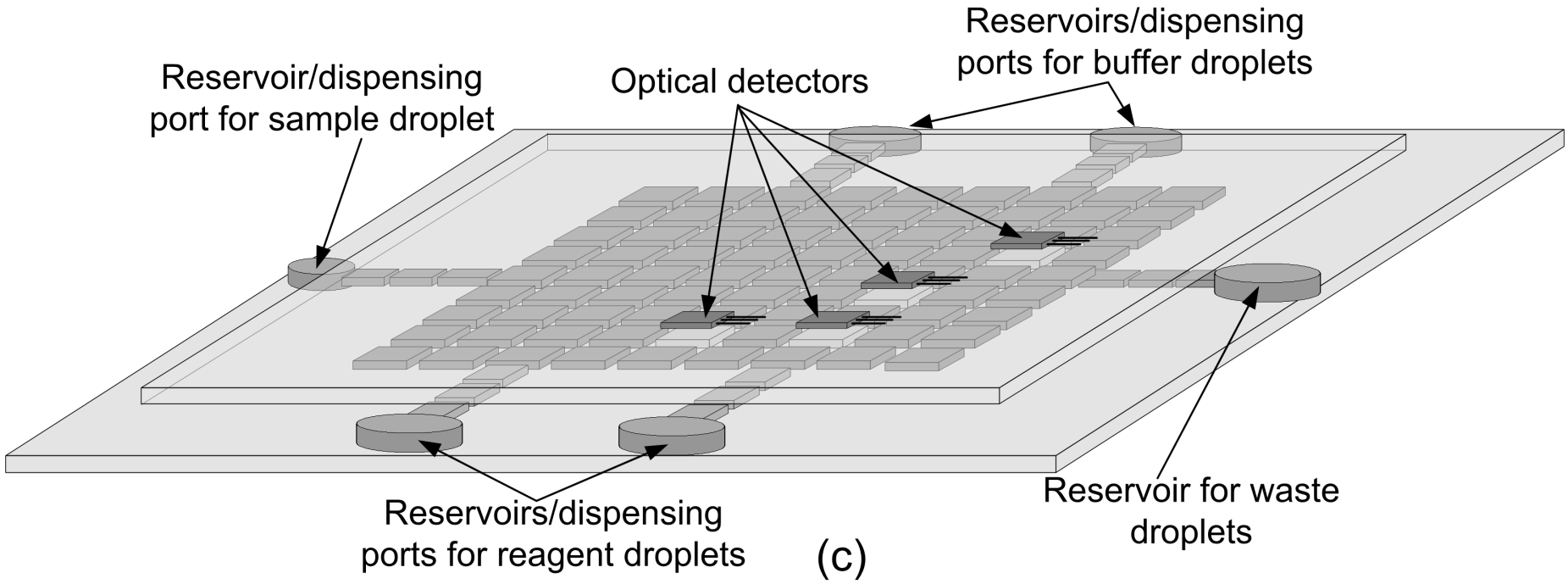
Bioassay completion time *T*: 363 seconds

Biochip array: 9x9 array



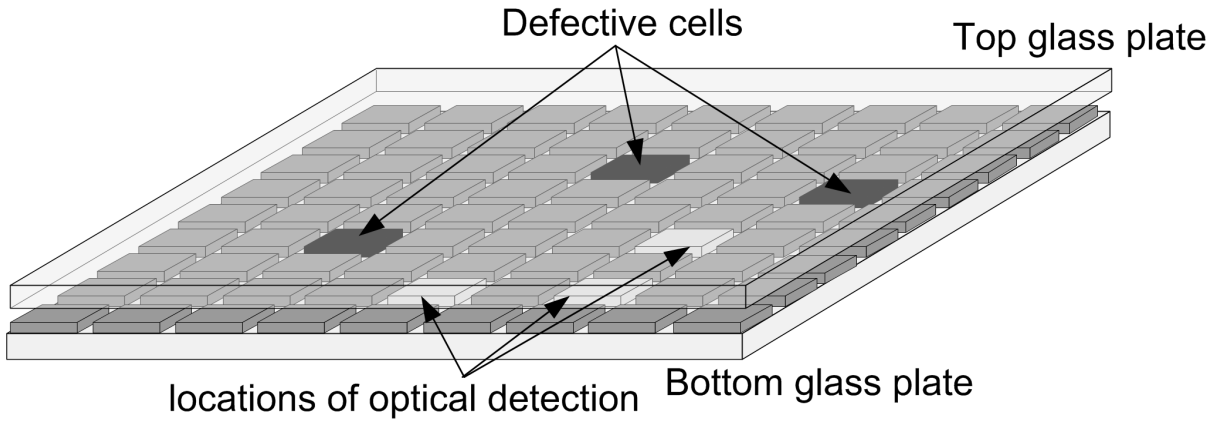
Experimental Evaluation (Cont.)

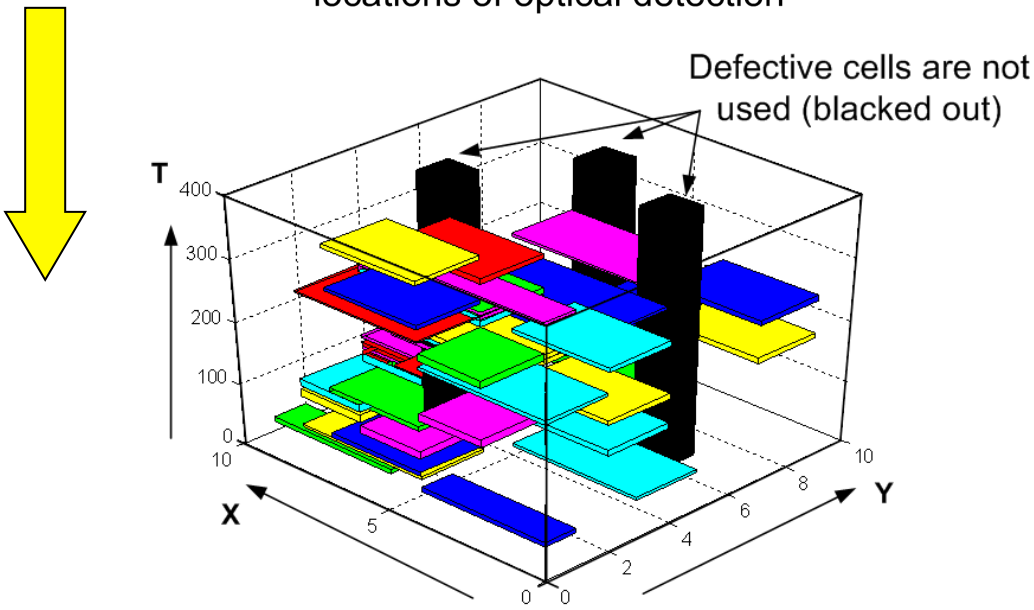
- Results of the unified synthesis method



Experimental Evaluation (Cont.)

- Defect tolerance





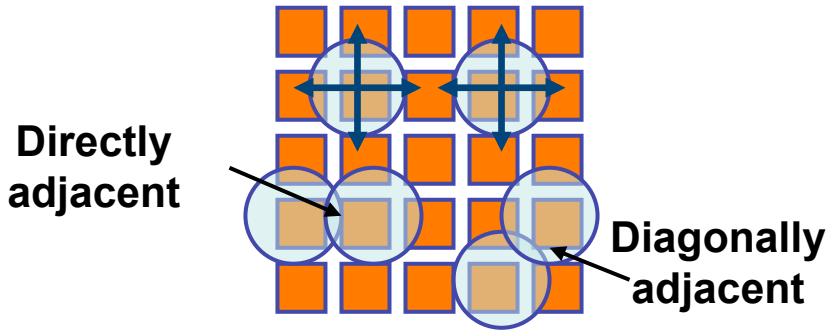
Bioassay completion time *T*: 385 seconds (6% increase)

Droplet Routing

- design problem fo dic
- s from architectu s and
  ent:
  - let pathways using t the
    ay; these routes are plets
    es, or between mod orts
    on-chip reservoirs)
- outes with minim
  he minimization of th 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



How to select the admissible locations at time t +1?

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.

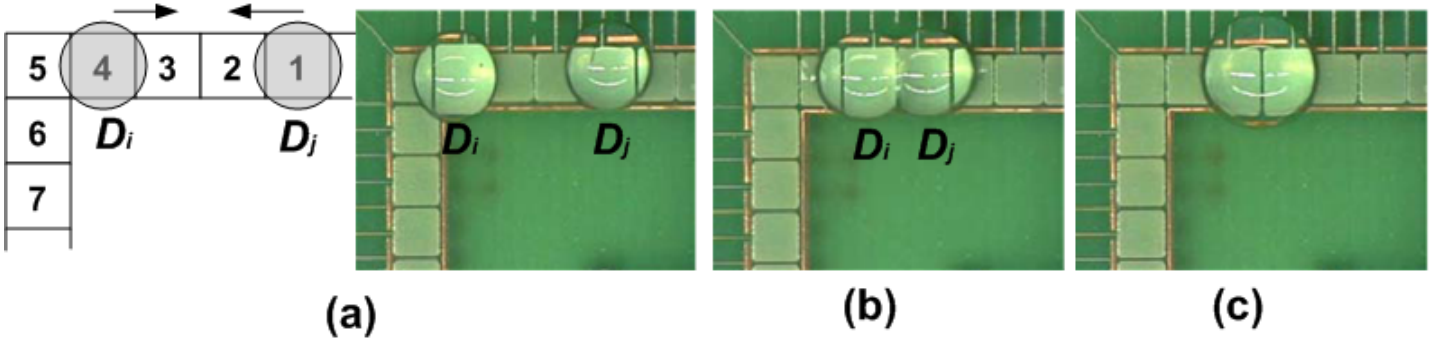
Rule #2: $|X_i(t+1) - X_j(t)| \geq 2$ or $|Y_i(t+1) - Y_j(t)| \geq 2$, i.e., the activated cell for D_i cannot be adjacent to D_j.

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

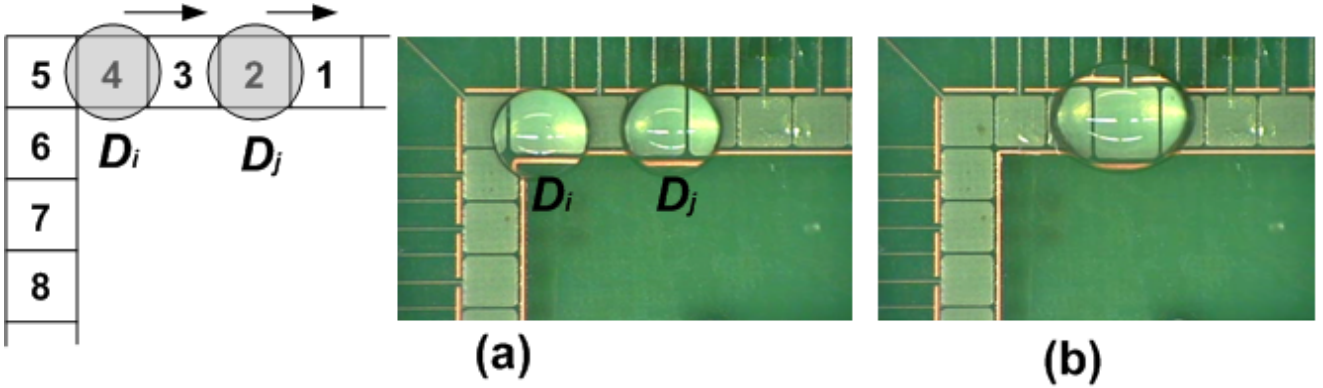
Static fluidic constraint

Dynamic fluidic constraints

Experimental Verification

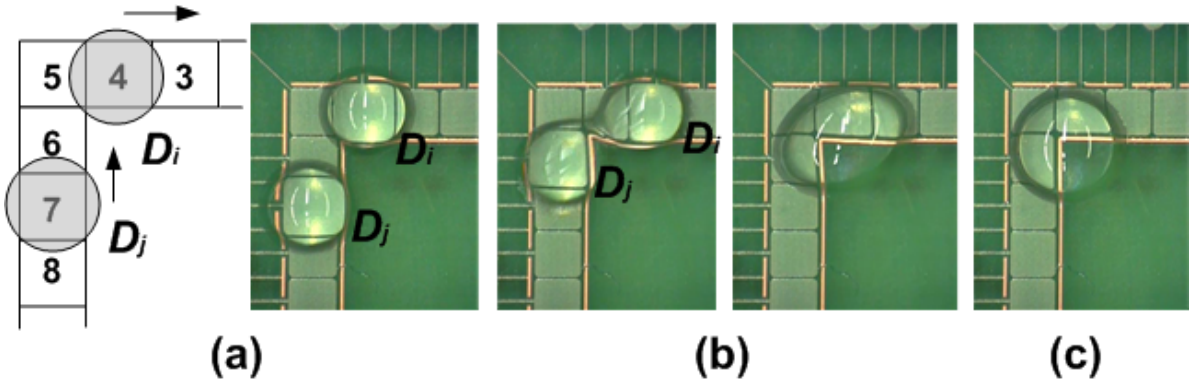


(a) Experimental verification of Rule #1: droplets begin on electrodes 1 and 4; (b) Electrodes 2 and 3 are activated, and 1 and 4 deactivated; (c) Merged droplet.



(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.

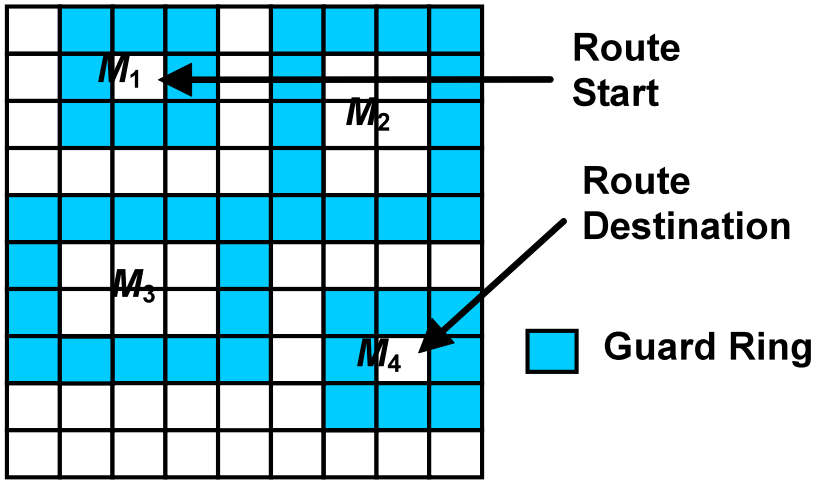
- To demonstrate that adherence to Rule #1 is not sufficient to prevent merging. Both Rule #2 and Rule #3 must also be satisfied during droplet routing.
- These rules are not only used for rule checking, but they can also provide guidelines to modify droplet motion (e.g., force some droplets to remain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

Unified Synthesis

- PRSA-based unified synthesis method
  - genetic algorithms using *Boltzmann trials* during evolution
- Goal:
  - Carry out scheduling, resource binding, and module placement under design specifications
- Cost function
  - $(\alpha \times A/Amax + (1-\alpha) \times T/Tmax)$

Drawback of Unified Synthesis Method

- Routing-oblivious synthesis
  - No guarantee of feasible routing pathways
- Requires powerful post-synthesis routing tool
  - Time-consuming method

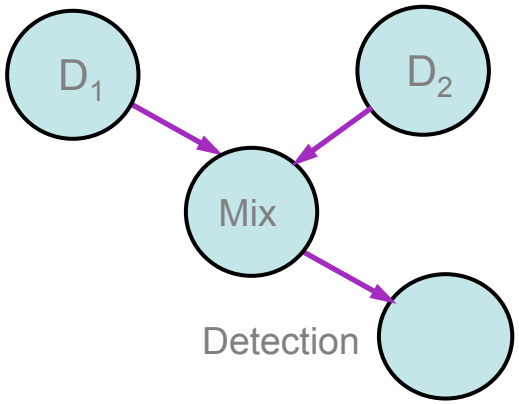


No pathway exists between M_1 and M_4

Routing considerations needed for synthesis!

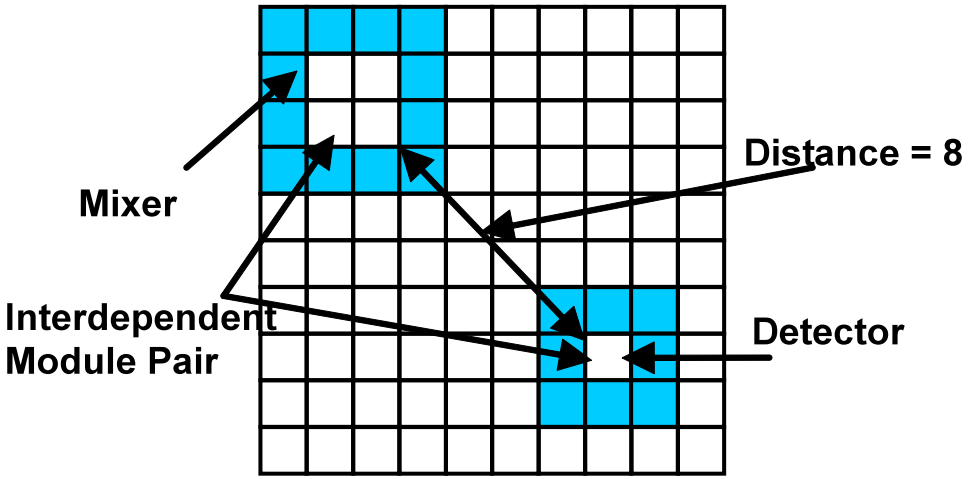
Routing-Aware Synthesis

- **Routability estimation**
  - Interdependent modules
  - Distance between interdependent modules



Interdependent operations

Distance = 0 for overlapping interdependent modules



Routing-Aware Unified Synthesis

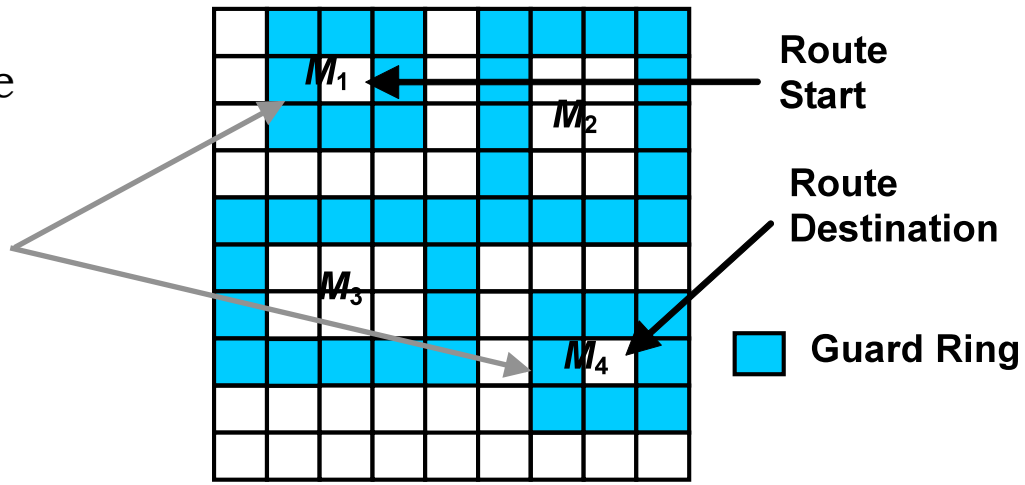
- **Routing distance**

  Average distances between all the interdependent module pairs

$$D(G) \approx \sum D(M_i, M_k) \; / N_{int}$$

  - $\{M_i, M_k\}$ — interdependent module pair
  - N_{int} — # of interdependent module pairs in a given design G

Synthesized results with high routing distance are likely to have *unroutable interdependent module pairs*



Routing-Aware Unified Synthesis

- **Routability**

  $R(G) = -D(G)$

- **Integrate into unified synthesis method**

  for every chromosome design (layout) do routability estimation
  Add to cost function

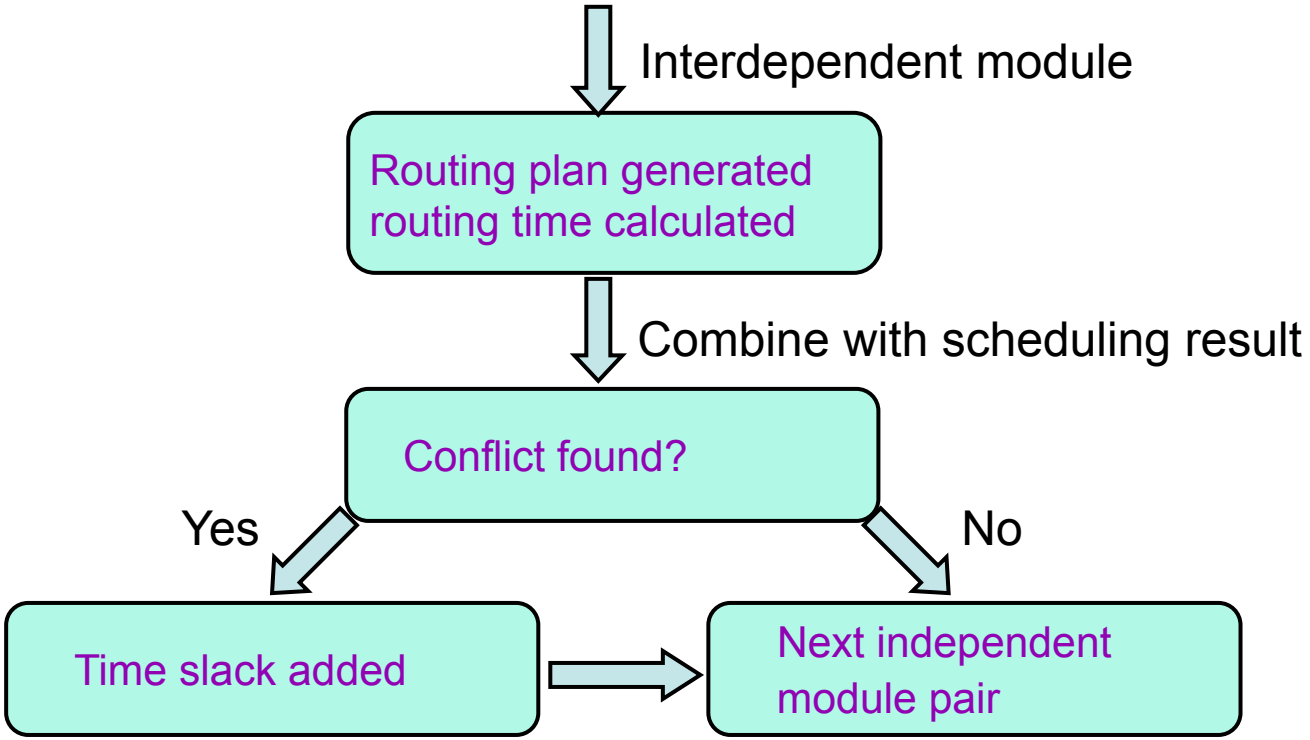
  $Fitness = \alpha Area + \beta Time + \gamma Routability$

  α,β,γ *are weights that can be fine-tuned according to different design specifications*

- **Candidate designs with low routability are discarded during evolution**

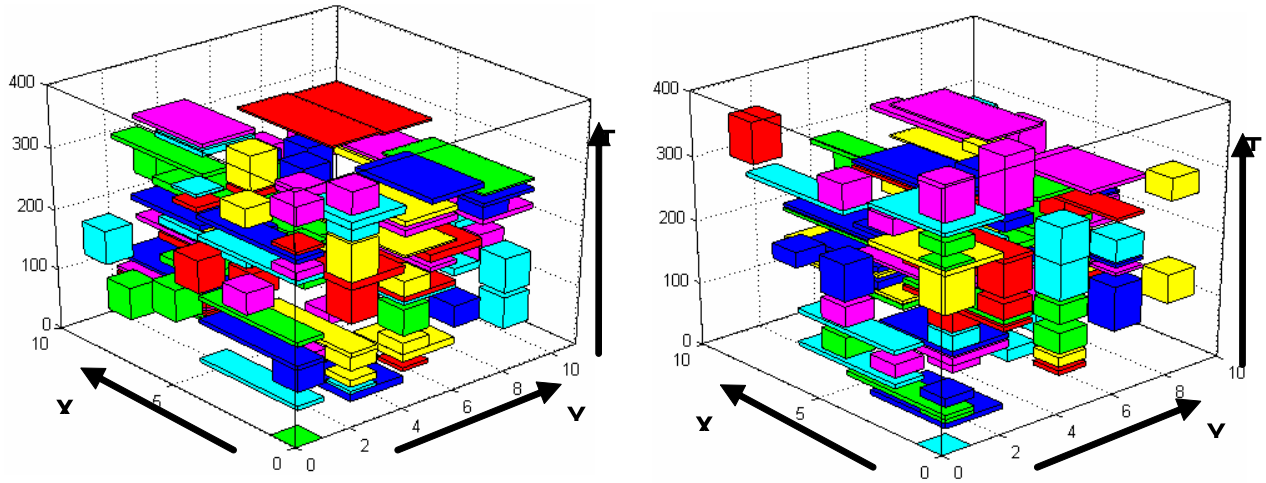
Routing-Aware Unified Synthesis

- **Adjusted completion time**
  - Non-negligible droplet routing time for routing algorithm from Su et al. (DATE 2006)
  - Time-slack method

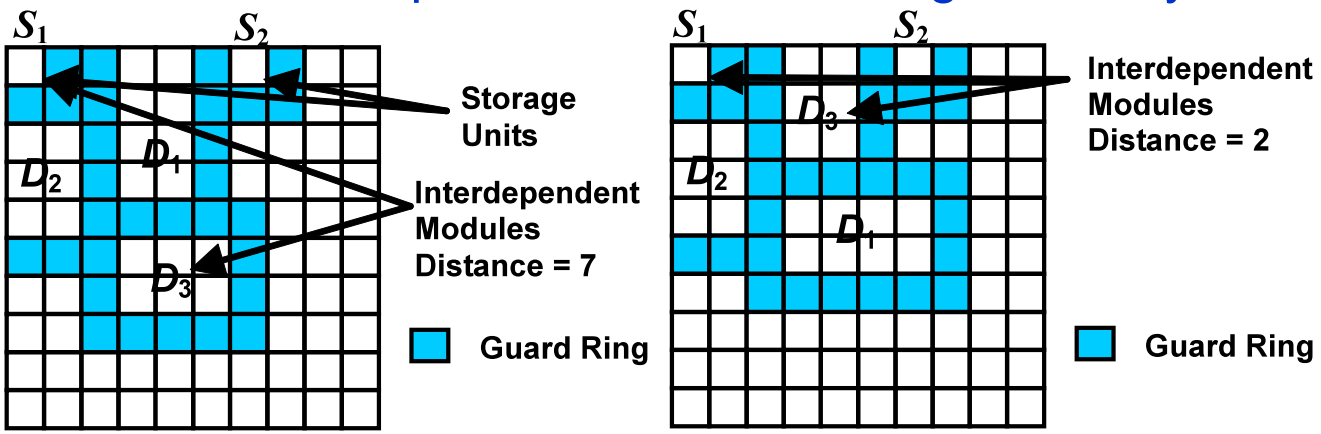


Experimental Evaluation (Protein Assay)

- Routing-oblivious method versus routing-aware method



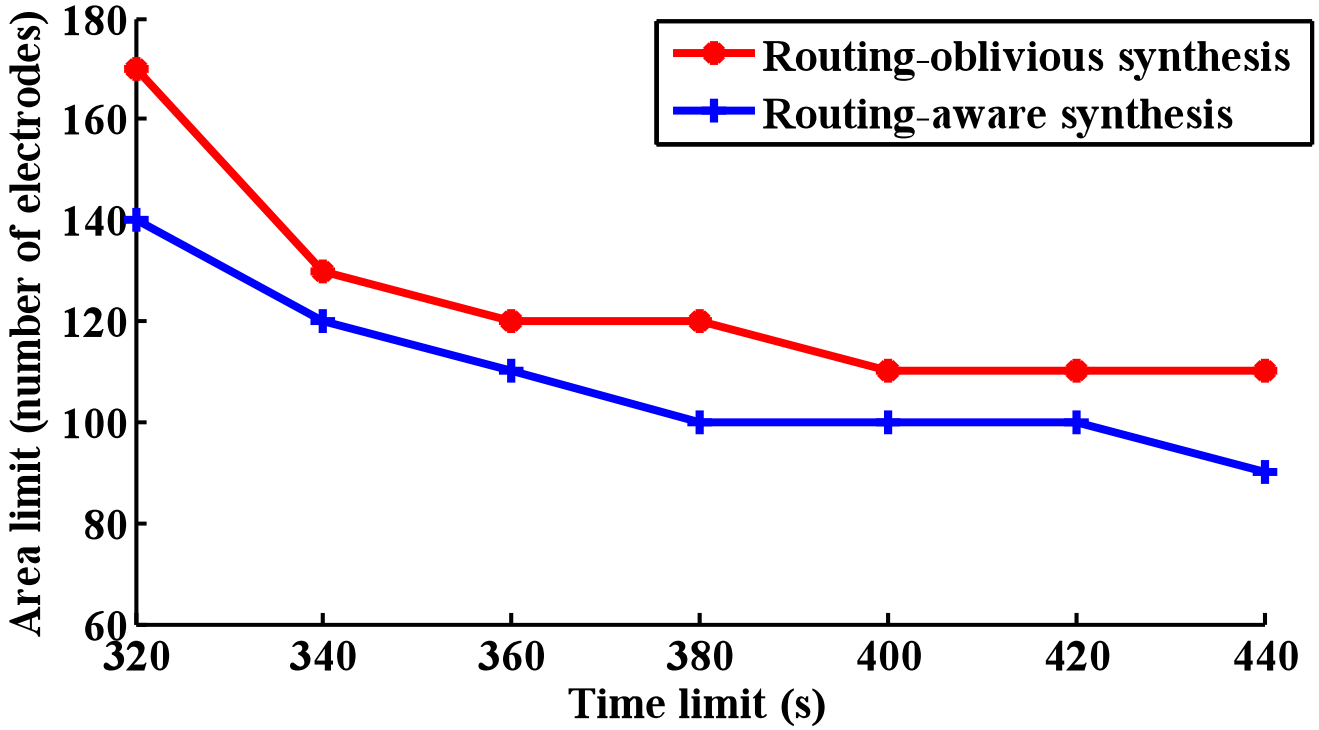
Interdependent modules are placed closer in routing-aware synthesis



Experimental Evaluation

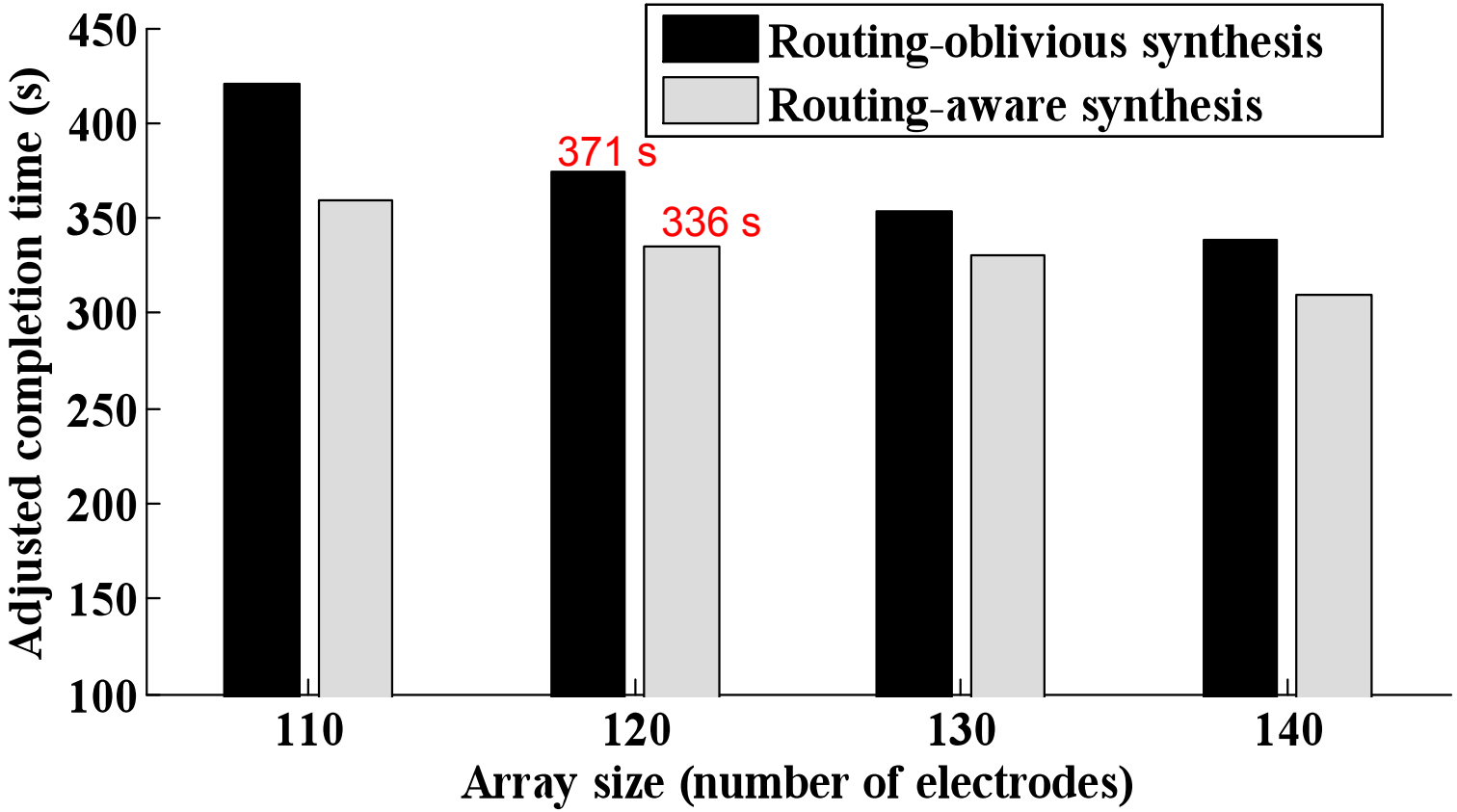
- Feasible design region
  - Feasibility boundary point:
    no other points (T_m, A_n) such that G_{ij} is routable and $T_m < T_i$, $A_n < A_j$.
  - Feasibility frontier

Feasible design region – area above the feasibility frontier



Experimental Evaluation

- Adjusted completion times (includes droplet routing time)



Direct Addressing: Problems

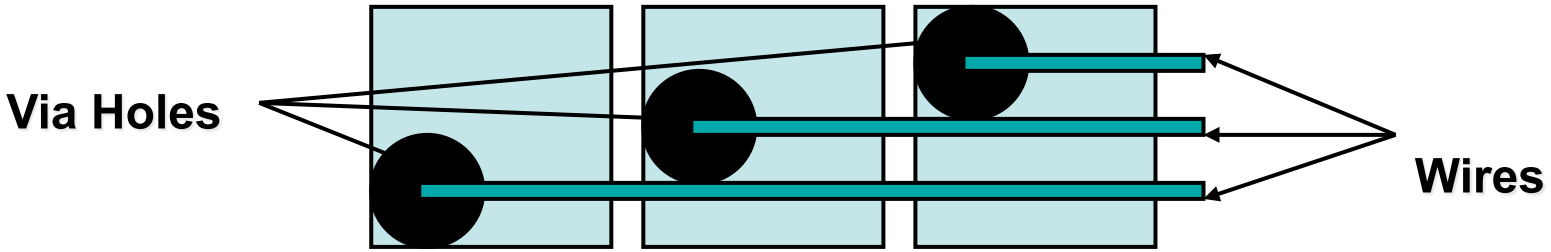
- Most design and CAD research for digital microfludic biochips has focused on directly-addressable chips
  - Suitable for small/medium-scale microfluidic electrode arrays (e.g., with fewer than 10 x 10 electrodes)
- For larger arrays (e.g., > 100 x 100 electrodes), multi-layer electrical connection structures and complicated routing solutions are needed
  - Product cost: major market driver due to disposable nature of devices
  - Multiple metal layers for PCB design: reliability problems, higher fabrication cost
- Goal: Reduce number of independent control pins
  - Reduce input bandwidth between electronic controller and microfluidic array while minimizing any decrease in performance

Design of Pin-Constrained Biochips

Direct Addressing

- Each electrode connected to an independent pin
- For large arrays (e.g., > 100 x 100 electrodes)
  - Too many control pins ⇒ high fabrication cost
  - Wiring plan not available

PCB design: 250 um via hole, 500 um x 500 um electrode



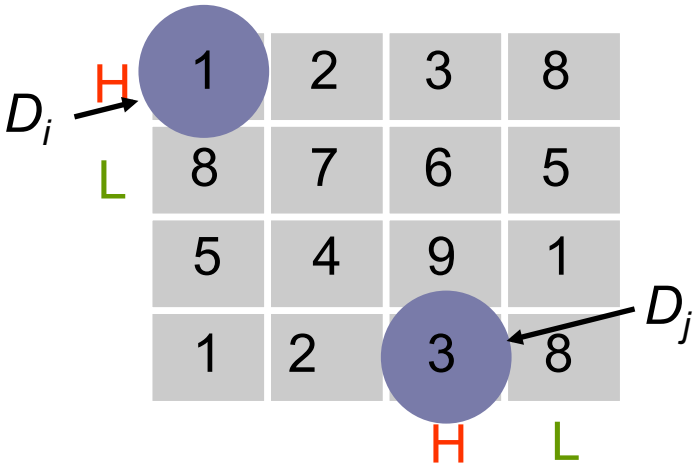
Nevertheless, we need high-throughput *and* low cost:

DNA sequencing (10^6 base pairs), Protein crystallization (10^3 candidate conditions)

Disposable, marketability, $1 per chip

Pin-Constrained Biochip Design

- Pin-constrained array design
  - **Advantage**: Reduce number of independent pins for *n* x *m* array from *n x m* to $k \leq n \times m$
    - $k = 5$ is fewest # of control pins to control single droplet
  - **Disadvantage**: Potential for unintentional interference between multiple droplets: no way to concurrently move D_i to position (1,2) and D_j to position (4,4)
- Solution
  - Single droplet: Addressing each electrode and its neighbors with distinct pins
  - Multiple droplets: Partition the chip
- Need for stall cycles?



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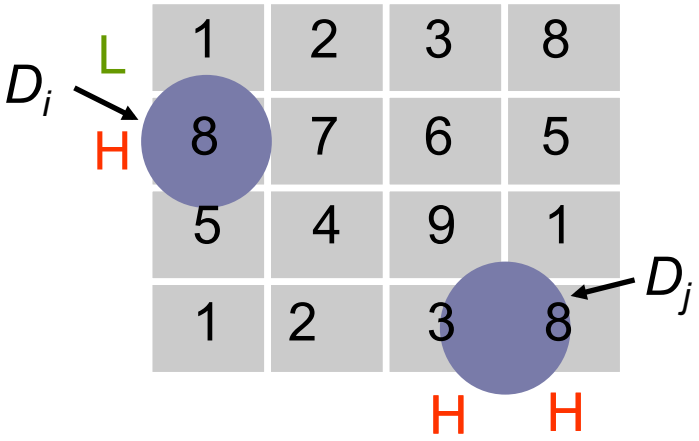

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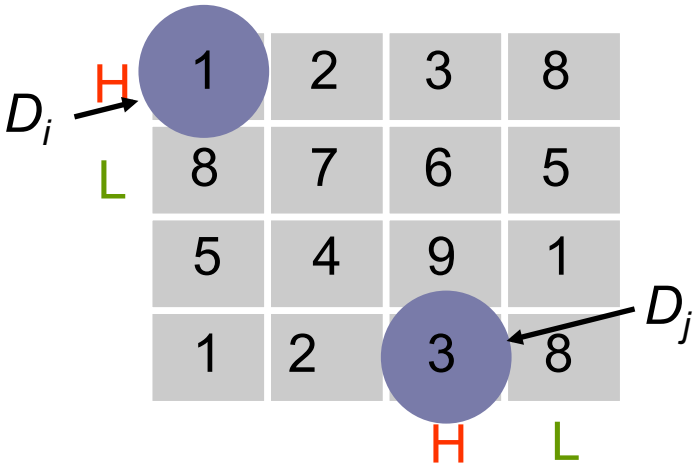

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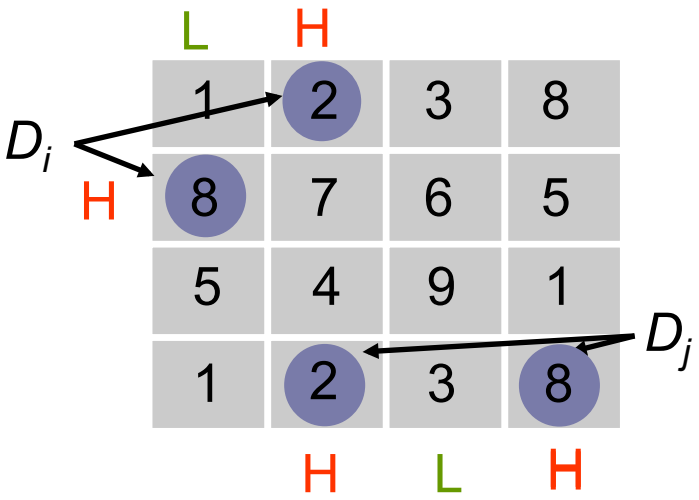


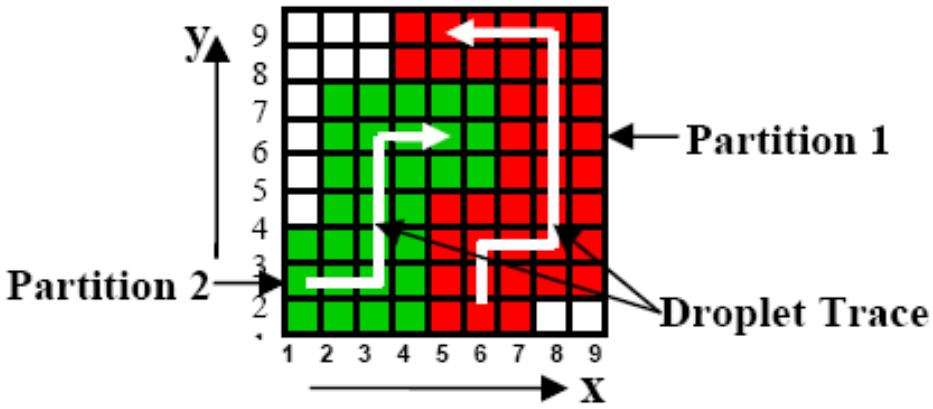
Partitioning for Pin-Constrained Designs

- Goal

  Each partition has exactly one droplet in it

	Detector1(x,y)	Detector2(x,y)	Detector3(x,y)
Droplet 1	(8, 3)	(8, 9)	(5, 9)
Droplet 2	(3, 2)	(3, 6)	(5, 6)

- ***Droplet Trace***
  - All the cells visited by a droplet in its lifetime
  - Can be derived from synthesis output: scheduling, droplet routing, and placement

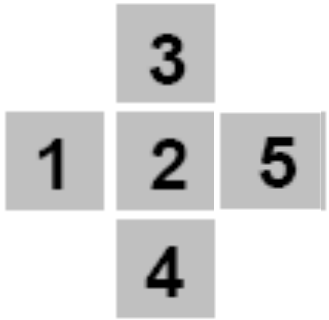


- Partition

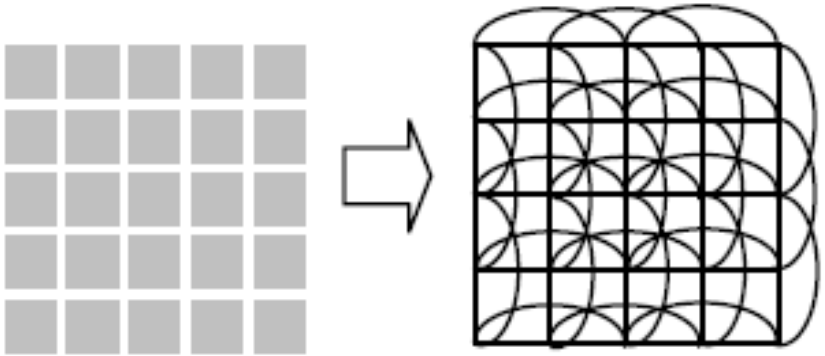
  = Droplet Trace + “Guard ring” (to avoid inadvertent mixing)

Pin Assignment in Each Partition

- Goal
  - Addressing each electrode and its neighbors with distinct pins (“cross” constraint)
  - 5 pins is minimum



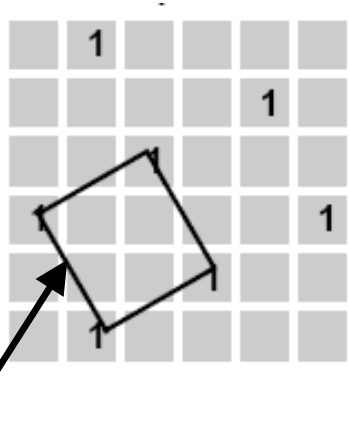
- Problem formulation
  - Vertex coloring problem from graph theory



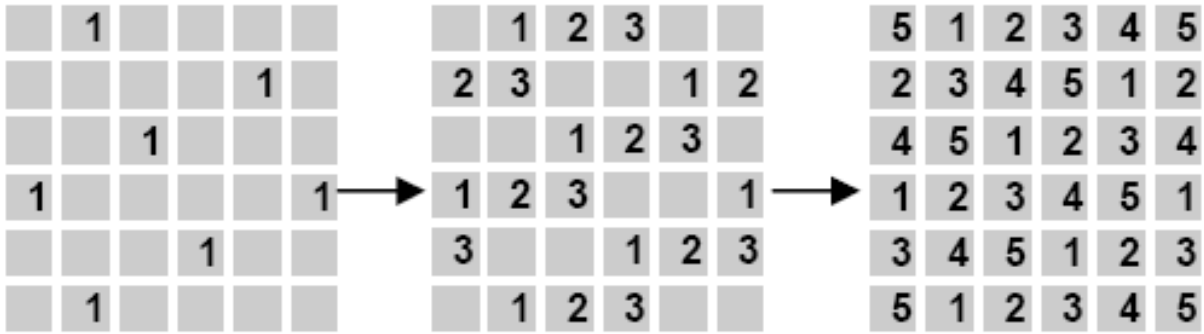
Pin Assignment in Each Partition

- *5 pins (colors) are sufficient for each partition!*
- *Connect-5* algorithm
  - Bagua structure
  - Tiling the Bagua structure
  - Implementation

    shifting a ordering by 2



Bagua structure and its repetition in a square partition

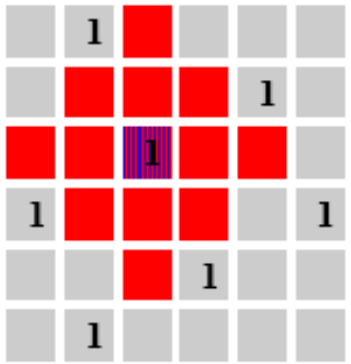


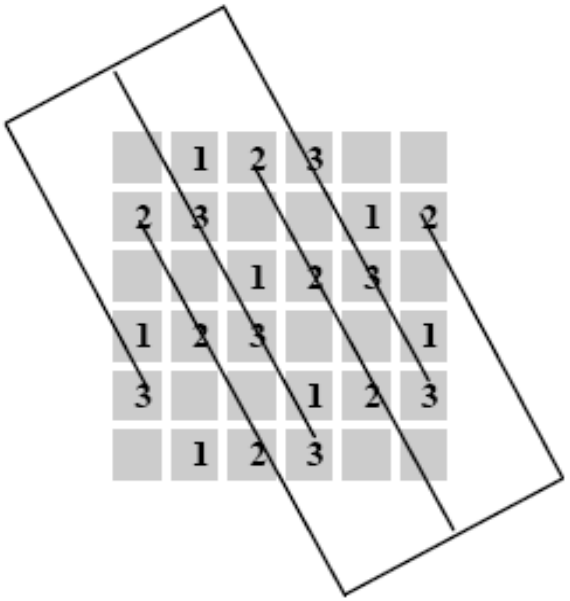
Pin Assignment in Each Partition

- Number of pins need

  = 5×number of partitions

- Cross constraint is not violated
  - Not violated in Bagua repetition
  - Not violated in the partition
- Easy for wiring
  - 2 pins can be wired on one layer of PCB without intersection
  - $\lceil 5/2 \rceil$ = 3 PCB layers is sufficient for wiring all the pins

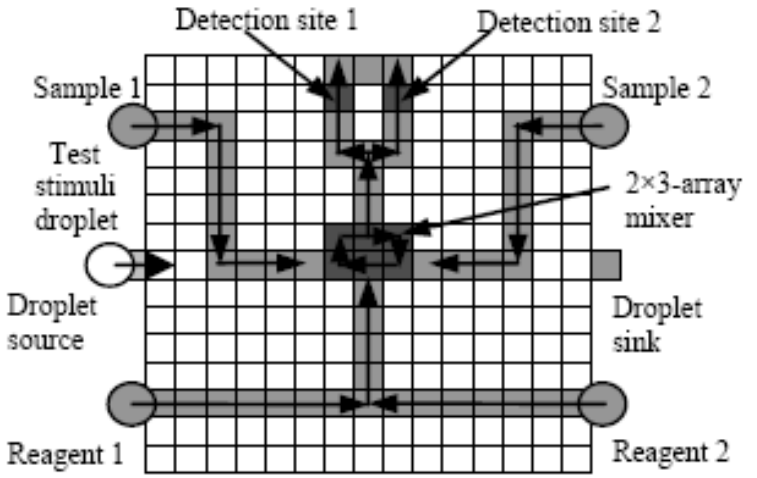




An Example

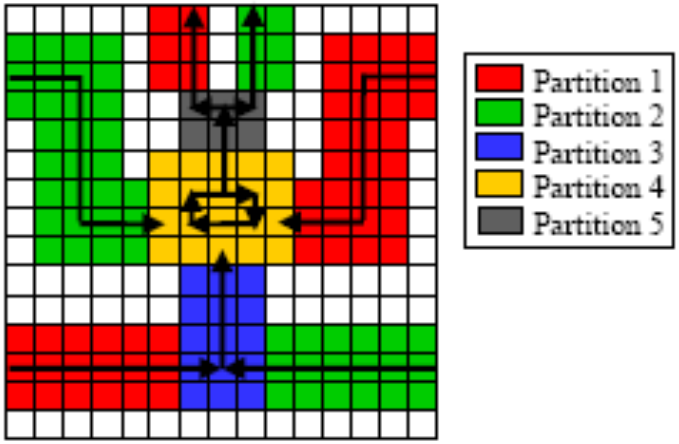
Schedule for a multiplexed bioassay

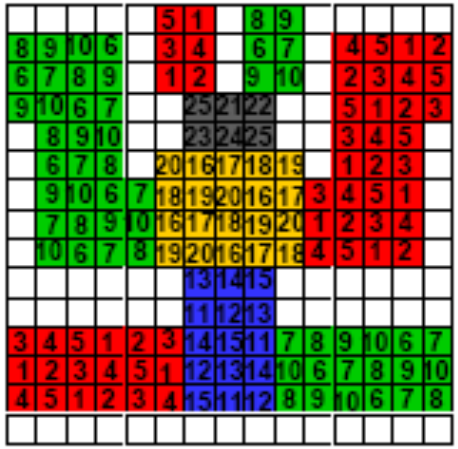
Step/Time Elapsed (s)	Operation
Step 1 / 0	Sample 2 and Reagent 2 start to move towards the mixer.
Step 2 / 0.8	Sample 2 and Reagent 2 begin to mix together and turn around in the 2×3-array mixer.
Step 3 / 6.0	Sample1 and Reagent 1 start to move towards the mixer. Sample 2 and Reagent 2 continue the mixing.
Step 4 / 6.8	Sample 2 and Reagent 2 finish the mixing and product 2 leaves the mixer to optical detection location 2. Sample 1 and Reagent 1 begin to mix in the 2×3-array mixer.
Step 5 / 12.8	Sample 1 and Reagent 1 finish the mixing and product 1 leaves the mixer to the optical detection location 1. Product 2 continues the absorbance detection.
Step 6 / 19.8	Product 2 finishes optical detection and leaves the array to the waste reservoir. Product 1 continues the absorbance detection.
Step 7 / 25.8	Product 1 finishes optical detection and leaves the array to the waste reservoir. One procedure of the multiplexed bioassays ends.



A 15×15 array used for multiplexed bioassays

Partition and pin assignment results for the multiplexed bioassay





Pin-Constrained Biochip Design

- **Cross-referencing**

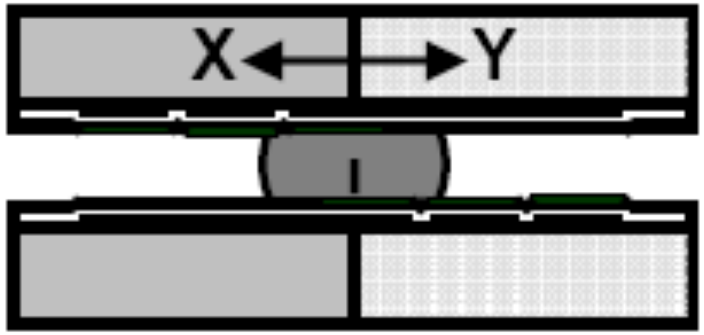
  Orthogonally placed pins on top and bottom plates

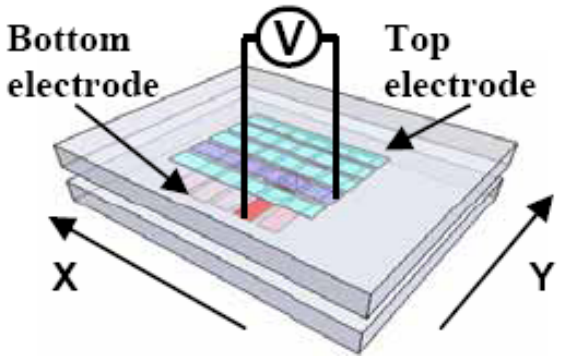
  Advantage

  k = n x m → n + m for a n by m microfluidic array

  Disadvantage

  Suffer from *electrode interference*



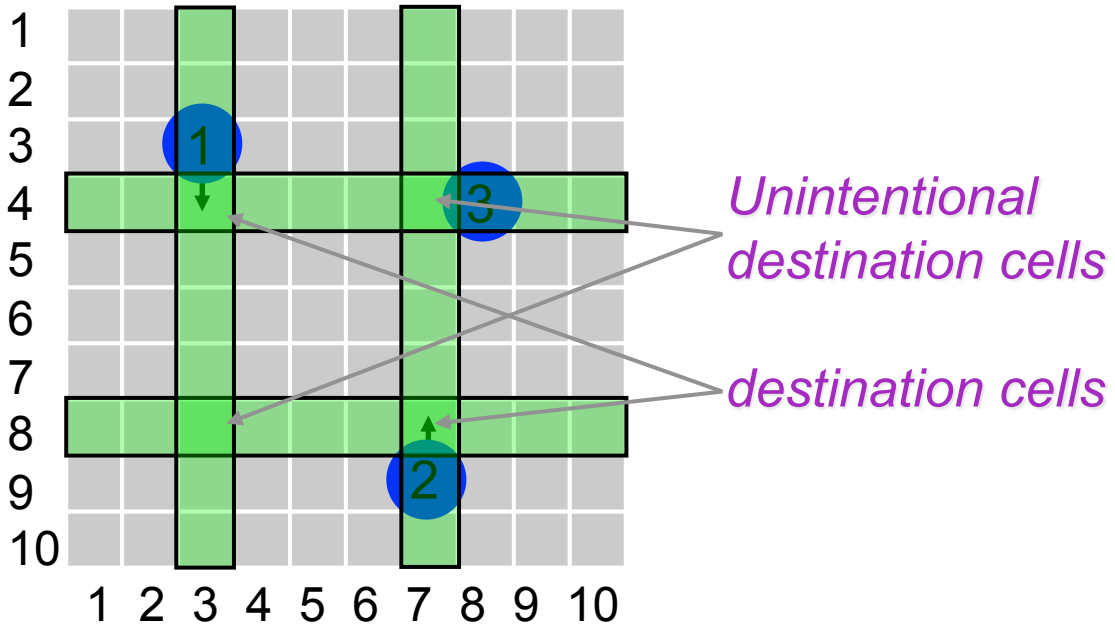


Electrode Interference

- **Unintentional Electrode Actuation**

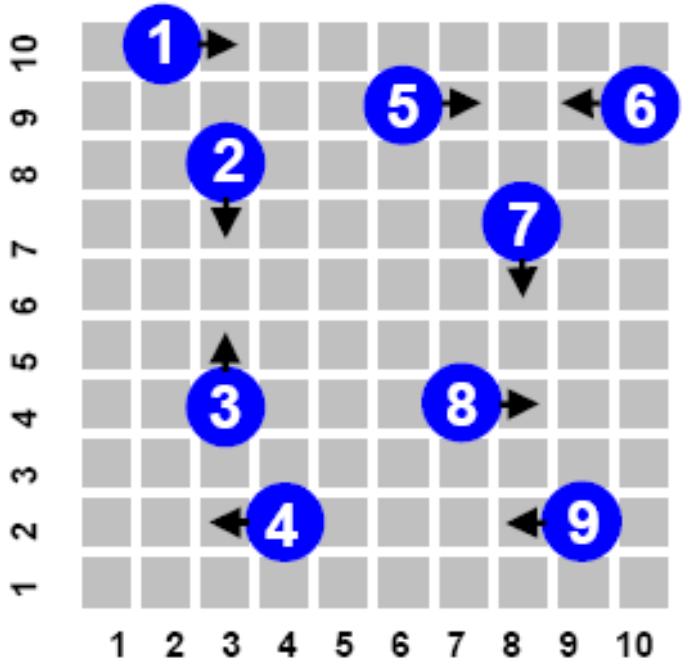
  Selected column and row pins may intersect at multiple electrodes

- **Unintentional Droplet Manipulation**



Efficient Droplet manipulation Method

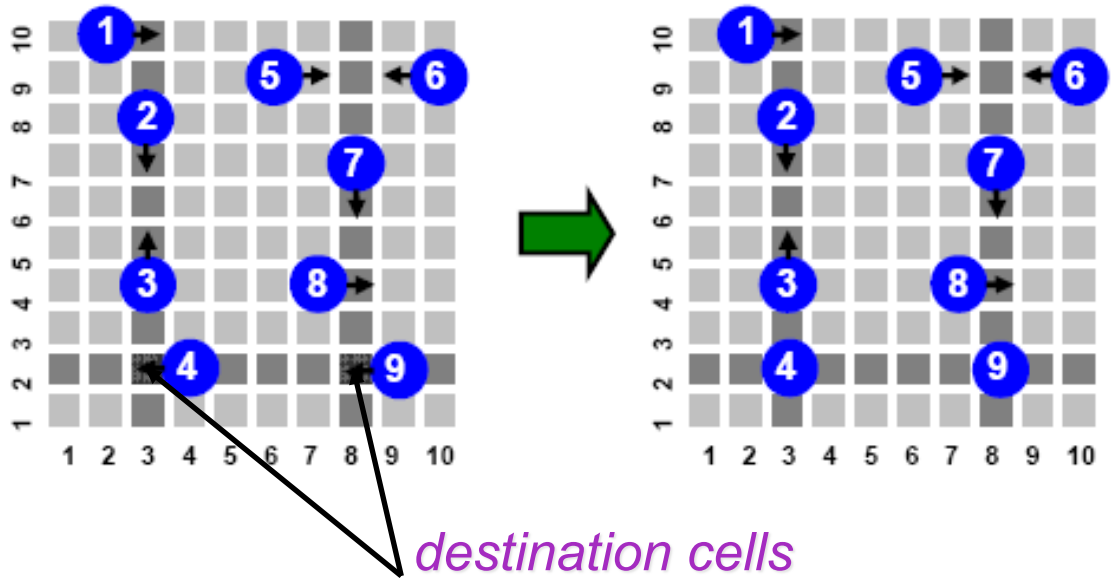
- **Goal**
  - Improve droplet manipulation concurrency on cross-referencing-based biochips.



9 steps needed if moving one droplet at a time (Too slow)

Efficient Droplet Manipulation Method

- **Observation**
  - Droplet manipulations whose *destination cells* belongs to the same column/row can be carried out without electrode interferences as long as fluidic constraints are not violated.



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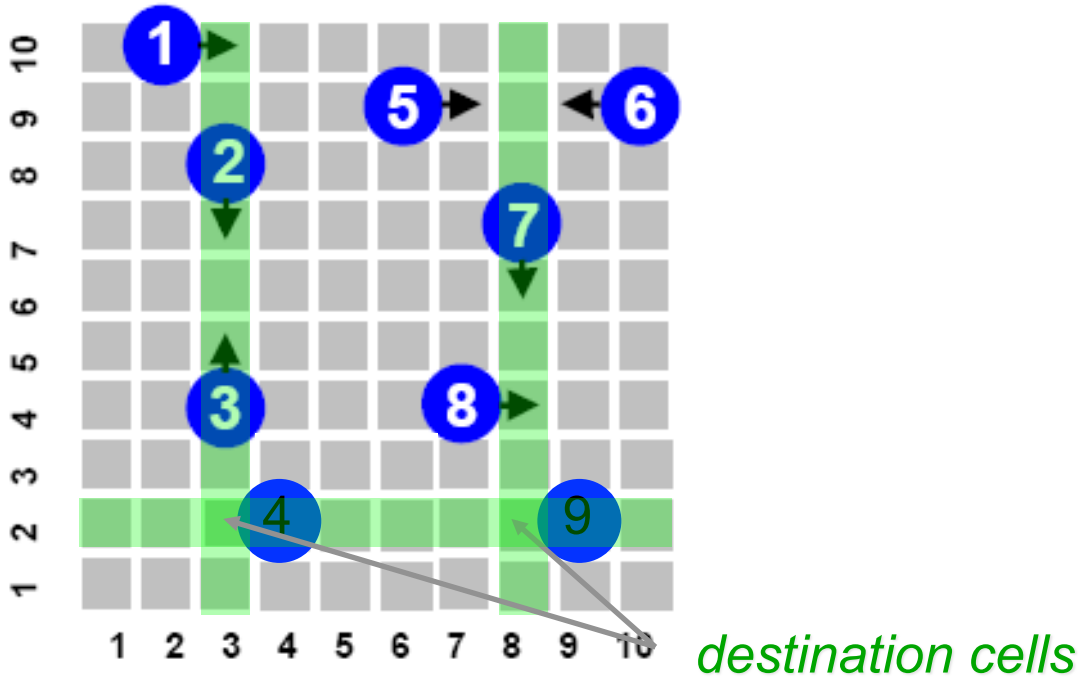


Efficient Droplet Manipulation Method

- **Methodology**
  - Group droplet manipulations according to their *destination cells*
  - All manipulations in a group can be executed simultaneously

The goal is to find the optimal grouping plan which results in the minimum number of groups.

Efficient Droplet Manipulation Method

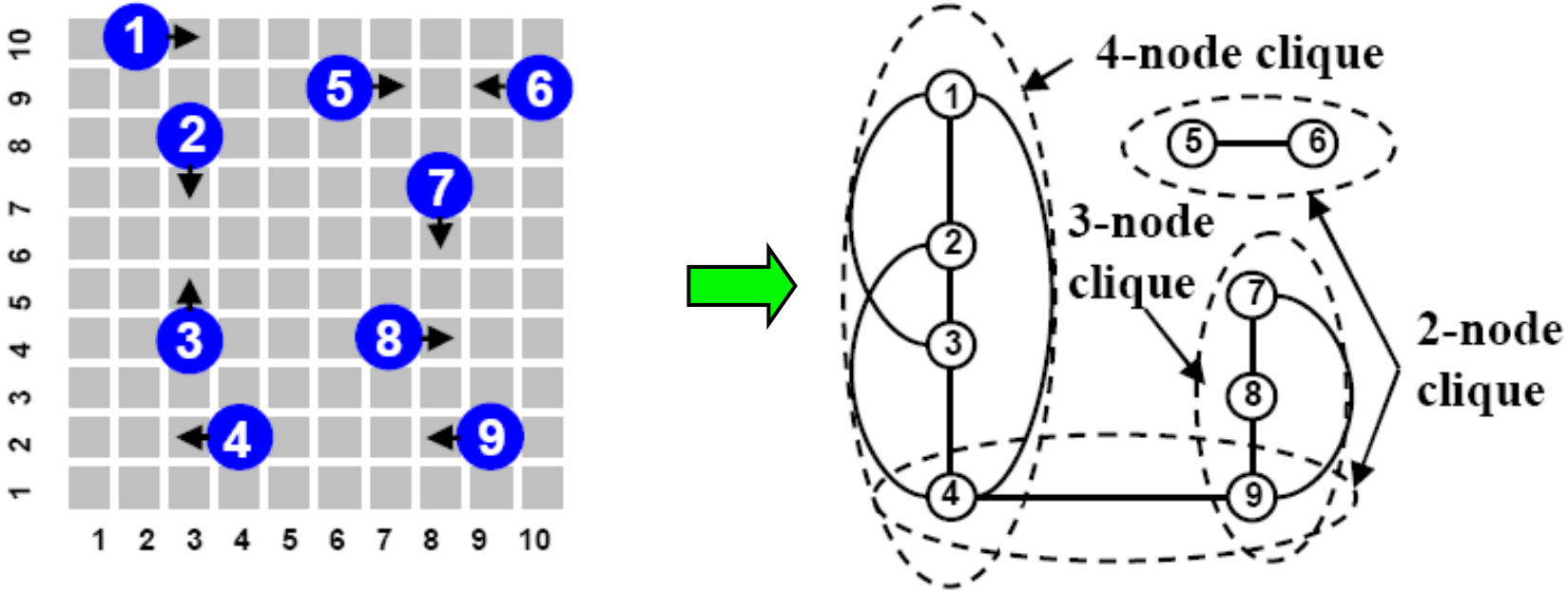
- **Problem formulation**

  Destination cells → Nodes

  Destination cells in one column/row → a Clique

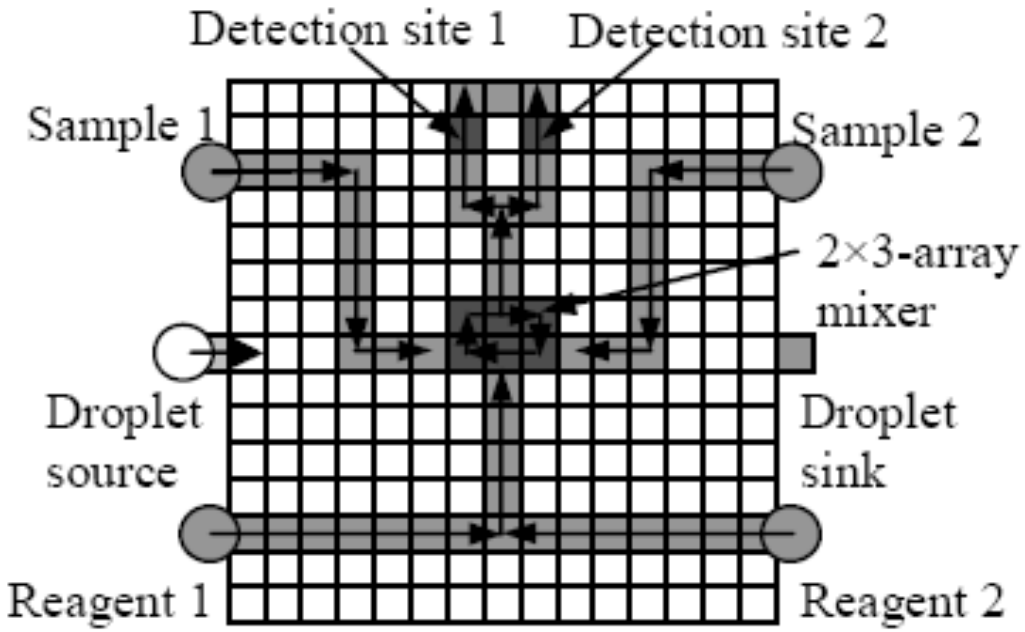
  Grouping → Clique partitioning

  Optimal grouping → Minimal clique-partitioning *(NP-Complete)*



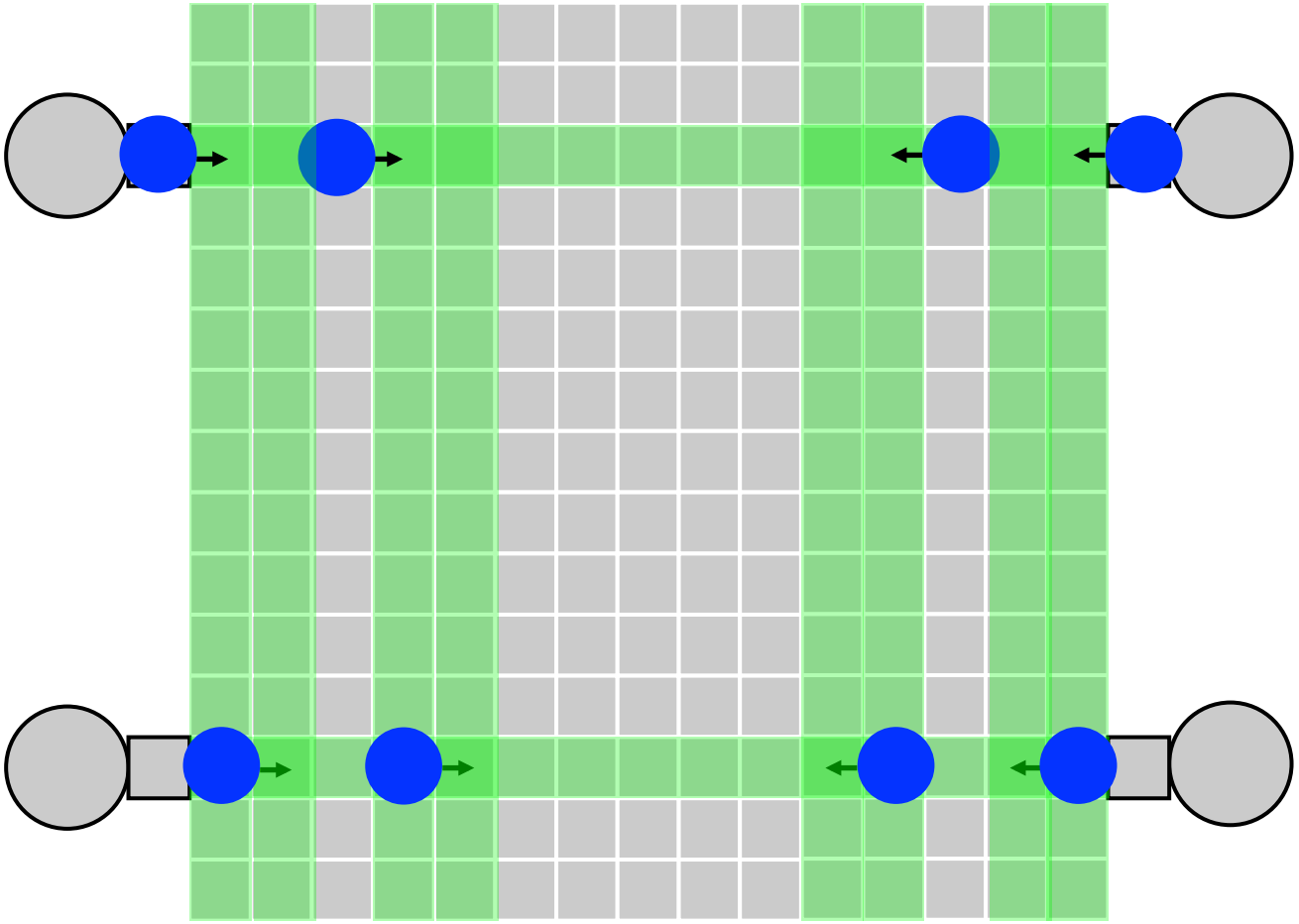
An Example

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



Synthesized microfluidic array used for multiplexed biomedical assays

An Example



- **Significant reduction of manipulation time**
  from 35 seconds (moving one droplet at a time) to 15 seconds !

Broadcast Electrode-Addressing

- **Observation**

  "Don't-Cares" in Electrode-Actuation Sequences

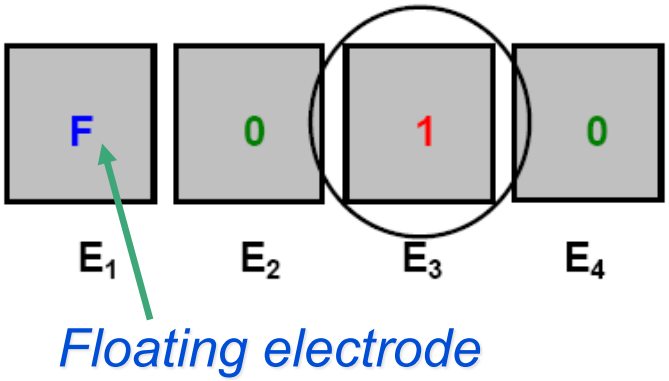
  Electrode control inputs: 3 values

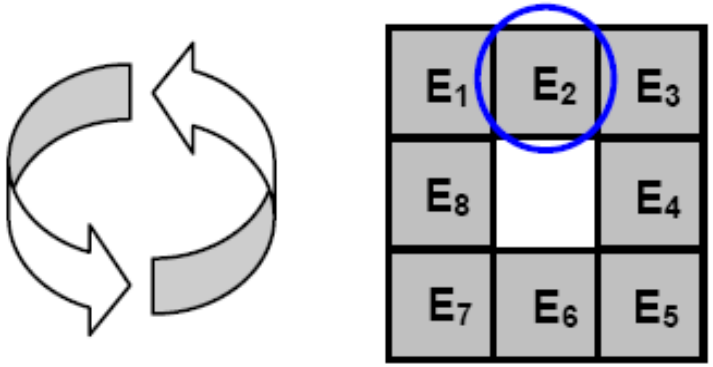
  "1" –- activated

  "0" –- deactivated

  "x" –- can be either "1" or "0"

  Therefore, activation sequences can be combined by interpreting "x"





Example: A droplet routed counterclockwise on a loop of electrodes

Electrode	1	2	3	4	5	6	7	8
Activation Sequence	0	1	0	0	X	X	X	X
	1	0	0	X	X	X	X	0
	0	0	X	X	X	X	0	1
	0	X	X	X	X	0	1	0
	X	X	X	X	0	1	0	0
	X	X	X	0	1	0	0	X
	X	X	0	1	0	0	X	X
	X	0	1	0	0	X	X	X

Corresponding electrode activation sequences

Solution Based on Clique Partitioning

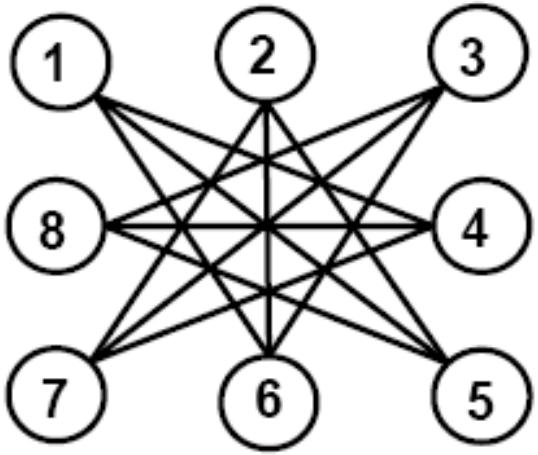
- **Idea**
  - Combining compatible sequences to reduce # of control pins
- **Clique partitioning based method**

  Electrodes → Nodes

  Electrodes with compatible activation sequences → a clique

  Optimal combination → Minimal clique-partitioning

Electrode	1	2	3	4	5	6	7	8
Activation Sequence	0	1	0	0	X	X	X	X
	1	0	0	X	X	X	X	0
	0	0	X	X	X	X	0	1
	0	X	X	X	X	0	1	0
	X	X	X	X	0	1	0	0
	X	X	X	0	1	0	0	X
	X	X	0	1	0	0	X	X
	X	0	1	0	0	X	X	X



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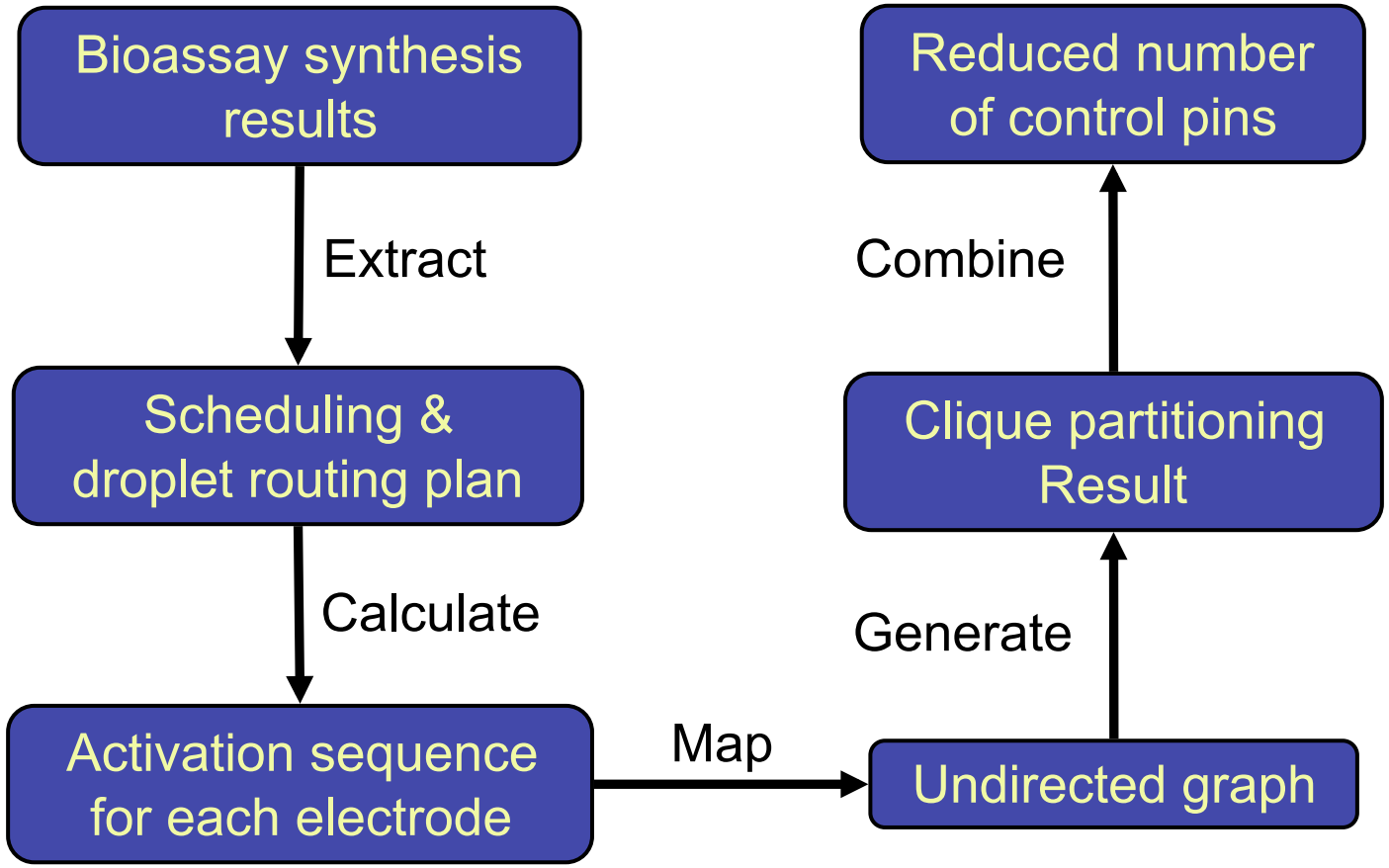
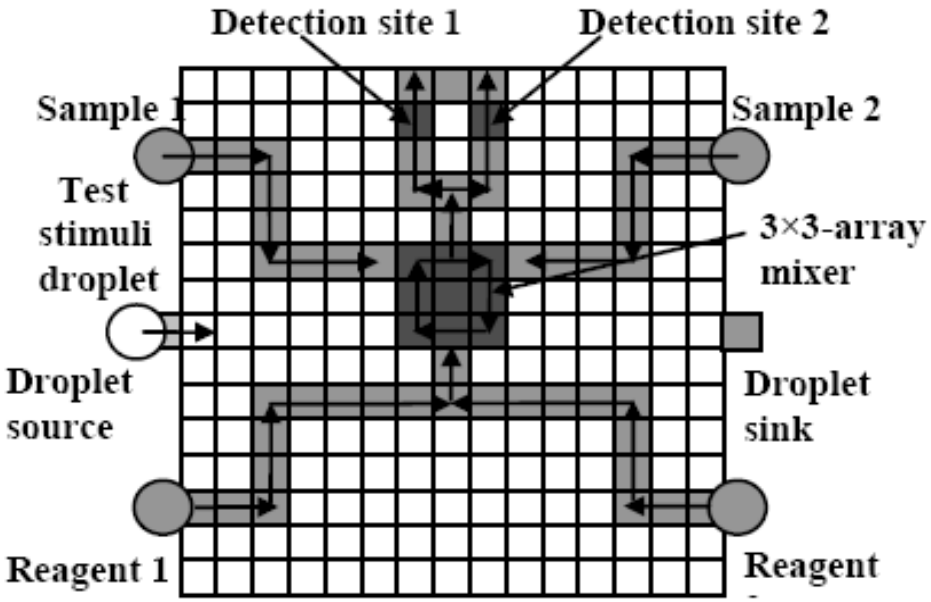
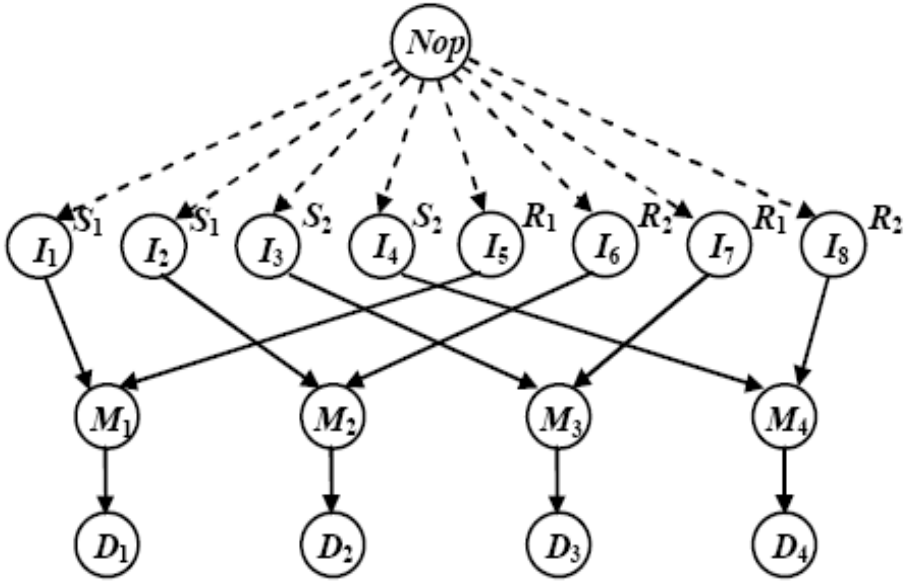


Application to a Multiplexed Bioassay



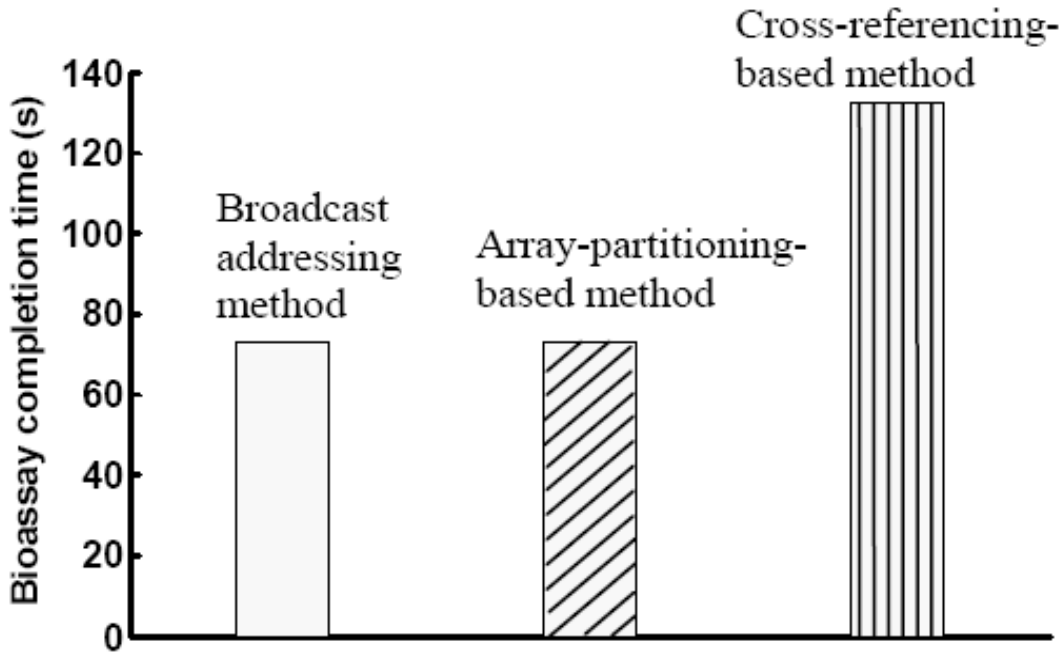
A biochip target execution of a multiplexed assay



Sequencing graph model of the multiplexed assay

- A glucose assay and a lactate assay based on colorimetric enzymatic reactions
- 4 pairs of droplets – {S1, R1}, {S1, R2}, {S2, R1}, {S2, R2}, are mixed in the mixer in the middle of the chip, the mixed droplets are routed to the detector for analysis

Results



Comparison of bioassay completion time using different addressing methods

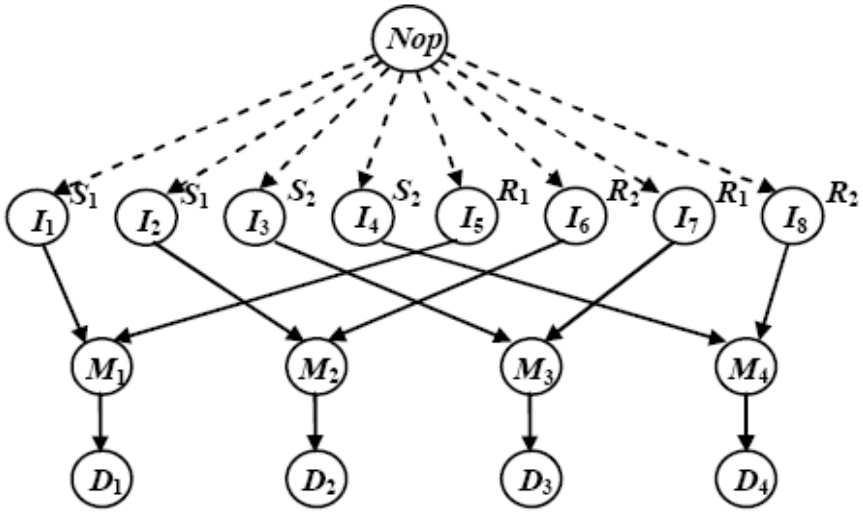
Addressing methods	Broadcast addressing	Array-partitioning-based method	Cross-referencing-based method
# of control pins	*25*	*35*	*30*

Application to Multi-functional Chip

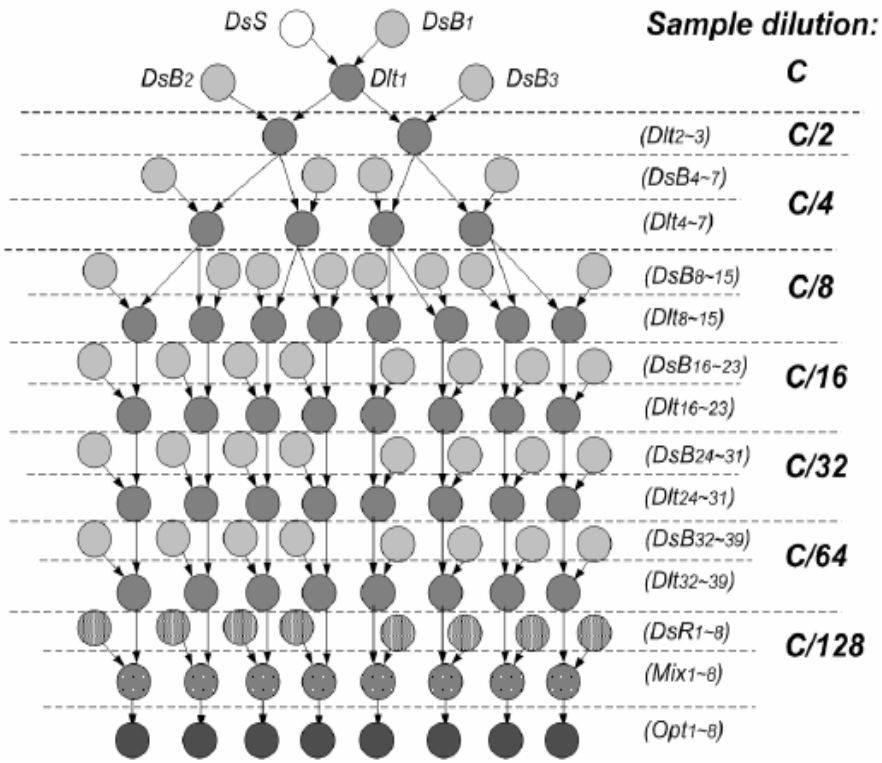
- Multi-functional Chip
  - biochips targeting the execution of a set of (multiple) predetermined bioassays
- Application of Broadcast Addressing to Multi-functional Chips

  Key idea: treat the union of the target bioassays as a single bioassay
  - Collect droplet routing and schedule information for each target bioassay
  - Calculate activation sequences for each bioassay
  - Merge the activation sequences from the different assays and obtain a collective activation sequence for each electrode
  - Note that merging of activation sequences can be carried out in any arbitrarily-chosen order

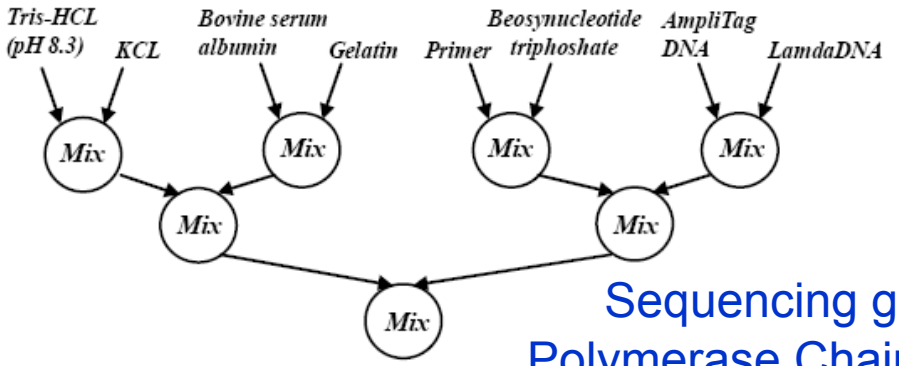
Addressing Results



Sequencing graph model of the multiplexed assay

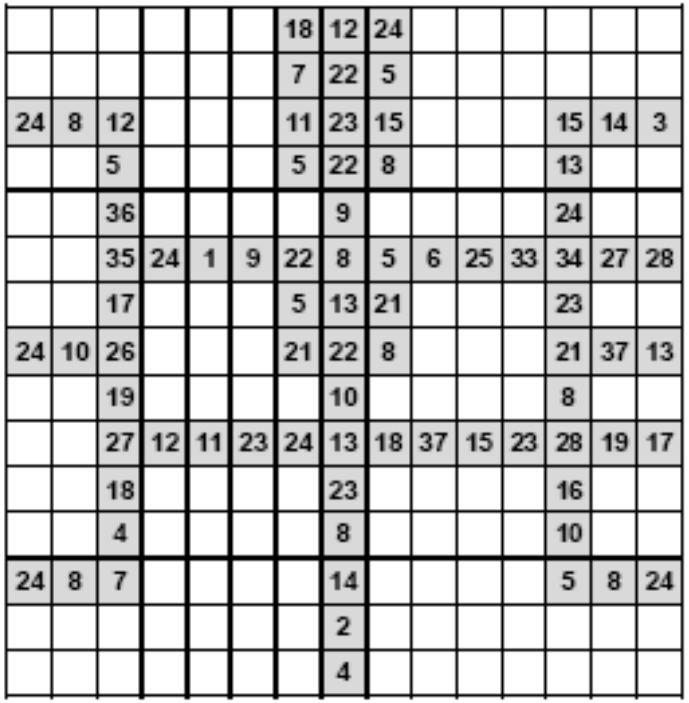


Sequencing graph model of protein dilution



Sequencing graph model of Polymerase Chain Reaction (PCR)

Addressing Results



						18	12	24						
						7	22	5						
24	8	12				11	23	15				15	14	3
		5				5	22	8				13		
		36					9					24		
		35	24	1	9	22	8	5	6	25	33	34	27	28
		17				5	13	21				23		
24	10	26				21	22	8				21	37	13
		19					10					8		
		27	12	11	23	24	13	18	37	15	23	28	19	17
		18					23					16		
		4					8					10		
24	8	7					14					5	8	24
							2							
							4							

Chip layout and broadcast-addressing result for the multi-functional chip for

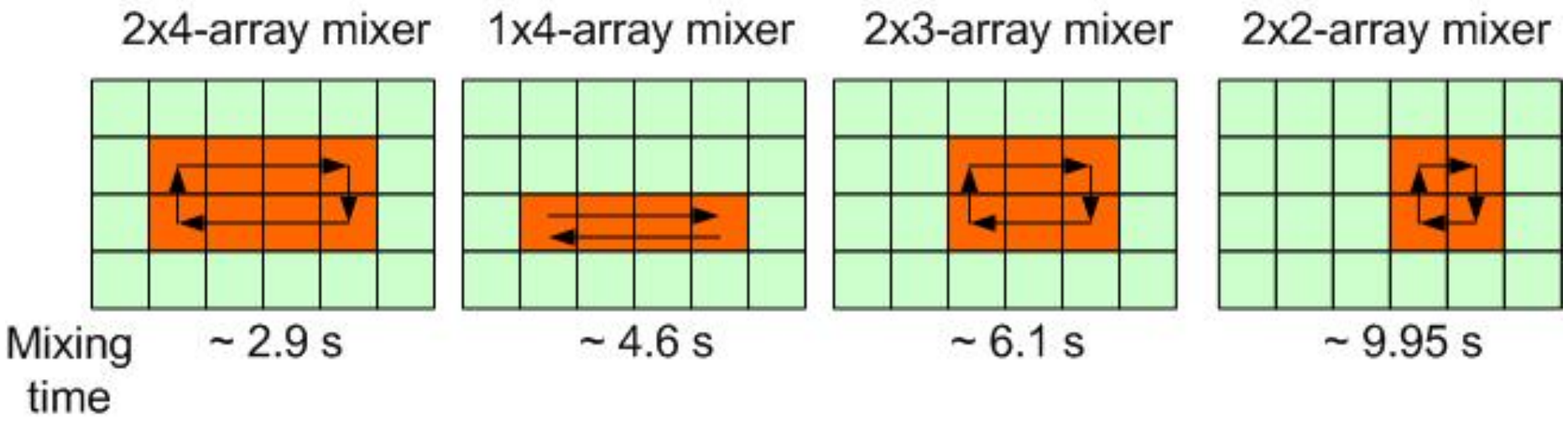
1. Multiplexed assay
3. PCR assay
3. Protein dilution assay

Total number of control pins: 37

The addition of two assays to the biochip for the multiplexed assay leads to only 13 extra control pins

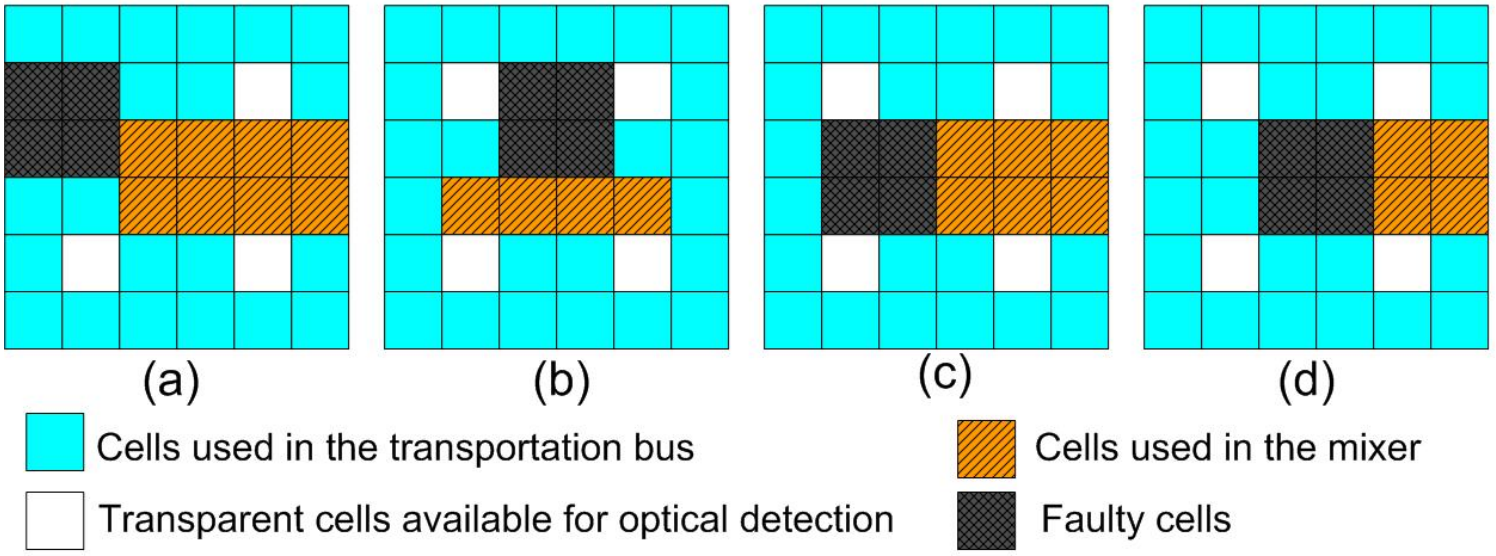
Reconfigurability

- 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



Reconfiguration and Graceful Degradation

- Reconfigure the faulty module
  - Avoid defects (faulty cells)
- Reconfiguration: bypass faulty cells
  - No spare cells; use fault-free unused cells
    - Defect tolerance in design procedure (increase in design complexity)
  - Incorporate physical redundancy in the array
    - Spare cells replace defective cells (local reconfiguration, application-independent)
-



Testing of Digital Microfluidics Biochips

Stimuli: Test droplets; **Response**: Presence/absence of droplets

Cause of defect	Defect type	No. cells	Fault model	Observable error
Excessive actuation voltage applied to electrode	Dielectric breakdown	1	Droplet-electrode short (short between the droplet and the electrode)	Droplet undergoes electrolysis; prevents further transportation
Electrode actuation for excessive duration	Irreversible charge concentration on electrode	1	Electrode-stuck-on (electrode remains constantly activated)	Unintentional droplet operations or stuck droplets
Excessive mechanical force applied to chip	Misalignment of parallel plates (electrodes and ground plane)	1	Pressure gradient (net static pressure in some direction)	Droplet transportation without activation voltage
Coating failure	Non-uniform dielectric layer	1	Dielectric islands (islands of Teflon coating)	Fragmentation of droplets and their motion is prevented

More Defects in Digital Microfluidic Biochips

Cause of defect	Defect type	No. cells	Fault model	Observable error
Abnormal metal layer deposition and etch variation during fabrication	Grounding failure	1	Floating droplets (droplet not anchored)	Failure of droplet transportation
	Broken wire to Control source	1	Electrode open (actuation not possible)	Failure to activate the electrode for droplet transportation
	Metal connection between adjacent electrodes	2	Electrode short (short between electrodes)	A droplet resides in the middle of the two shorted electrodes, and its transport cannot be achieved
Particle contamination or liquid residue	Particle connects two adjacent electrodes	2	Electrode short	
Protein absorption during bioassay	Sample residue on electrode surface	1	Resistive open at electrode	Droplet transportation is impeded.
			Contamination	Assay results are outside the range of possible outcomes

Electrical Detection Mechanism

- Minimally invasive
- Easy to implement (alleviate the need for external devices)
- Fault effect should be unambiguous

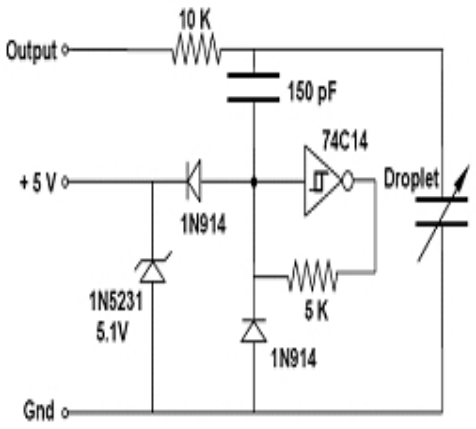
Electrically control and track test stimuli droplets

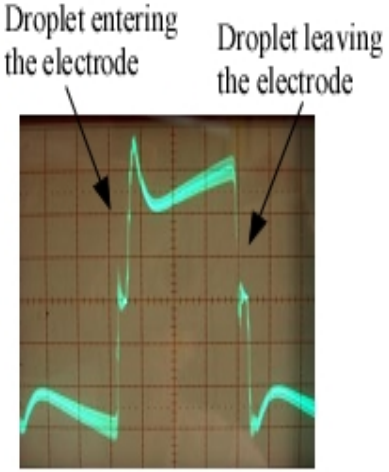
Periodic square waveform

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 sink electrodes

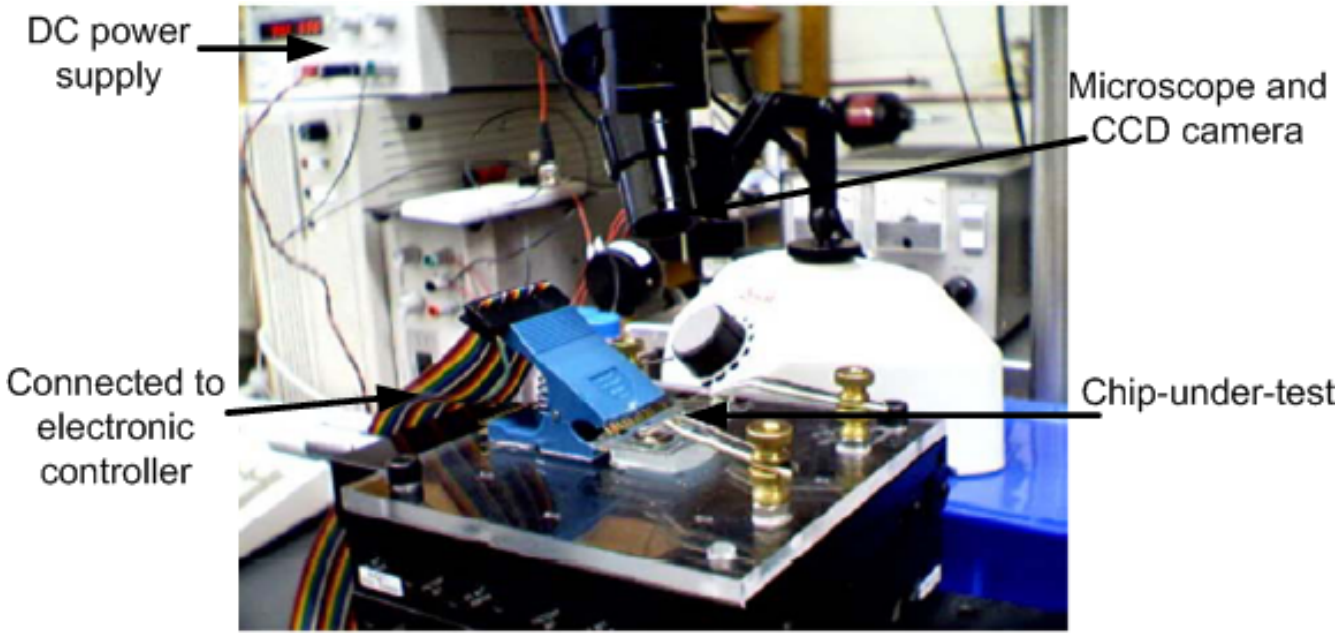
  Faulty: there is no droplet.





Experimental Platform

- Understand the impact of certain defects on droplet flow, e.g., *for short-circuit between two electrodes*
  - To evaluate the effect of various defects on microfluidic behavior



Topics Not Covered

- Testing and fault diagnosis (Yang, Xu and Chakrabarty, ITC 2008; Xu and Chakrabarty, TBCAS 2007)
- Droplet routing to avoid cross-contamination (Zhao and Chakrabarty, DATE 2009)
- Design and optimization of a protein crystallization chip (Xu, Chakrabarty and Pamula, ICCAD 2008)
- Optimization of solution preparation (Xu, Pamula and Chakrabarty, BioCAS Conf., 2008)

Conclusions

- Digital microfluidics offers a viable platform for lab-on-chip for clinical diagnostics and biomolecular recognition
- Design automation challenges
  - Automated synthesis: scheduling, resource binding, module placement; droplet routing; testing and reconfiguration
- Bridge between different research communities: bioMEMS, microfluidics, electronics CAD and chip design, biochemistry
- Growing interest in the electronics CADand circuits/systems communities
  - Special session on biochips at CODES+ISSS'2005 (appeared in CFP now)
  - Special issue on biochips in *IEEE Transactions on CAD* (Feb 2006), *IEEE Design & Test of Computers* (Jan/Feb'07), invited papers in TCAD 2009, TCAS-I 2009
  - Workshop on biochips at DATE'06
  - Tutorials on digital microfluidic lab-on-chip at DATE'07, ISCAS'08, ISCAS'09, VDAT 2007; embedded tutorials at VLSI Design'05, ISPD'08
  - Other notable activities in digital microfluidics: University of California at Los Angeles, University of Toronto, Drexel University, IMEC (Belgium), Freiburg (Germany), Philips (Netherlands), Fraunhofer Institute (Berlin, Germany), National Taiwan Univ., Tech. Univ. Denmark, Univ. Texas, and many more….

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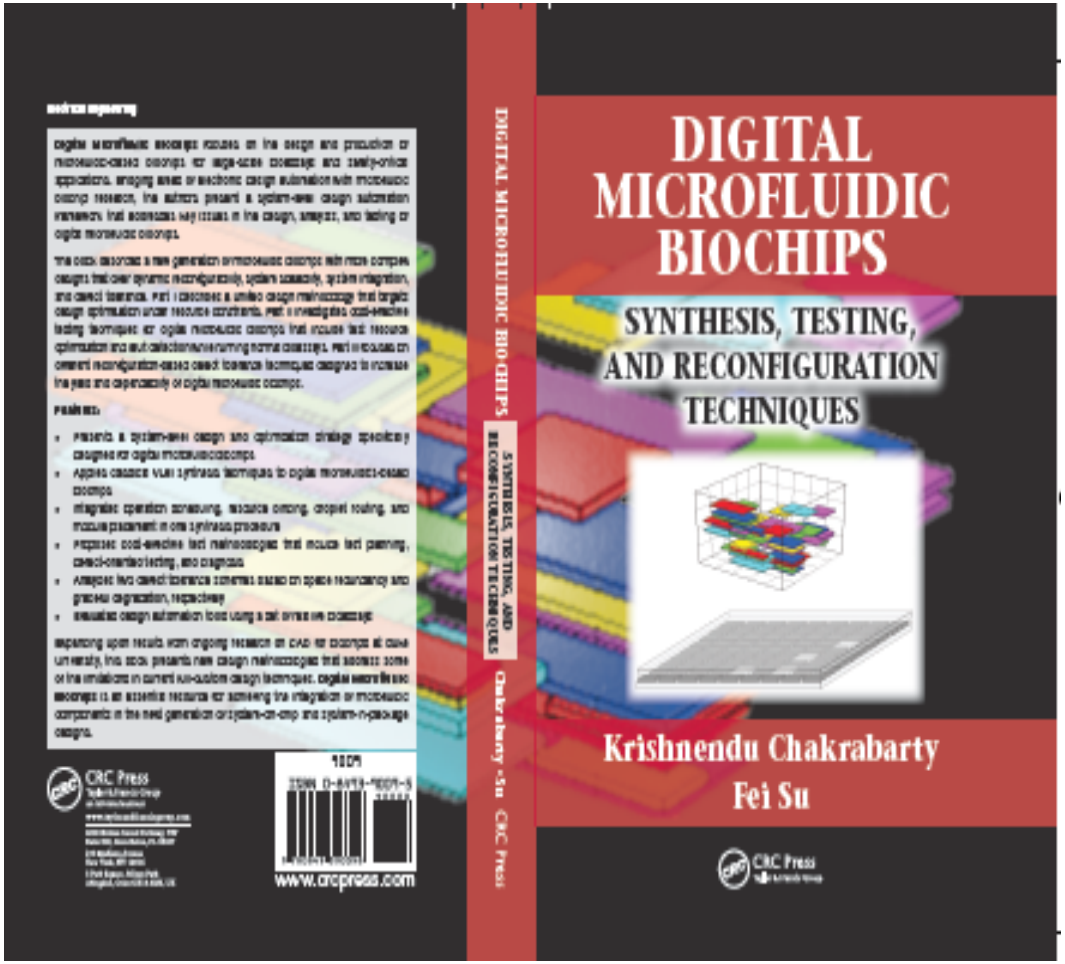

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Additional Reading Materials

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