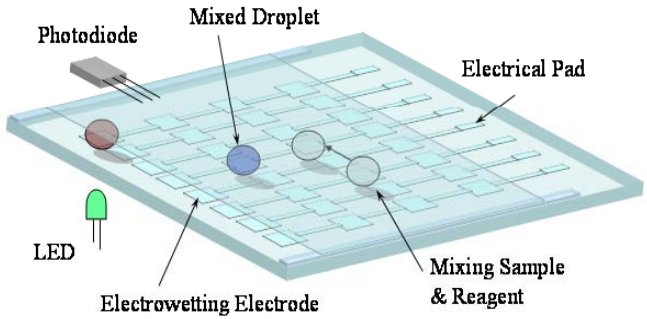
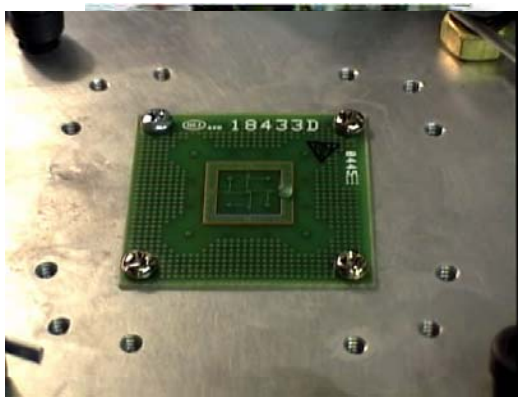
Design of Microfluidics-Based Biochips

Evangeline F.Y. Young





Slides partly adopted from Krishnendu Chakrabarty of Duke University

Motivation for Biochips

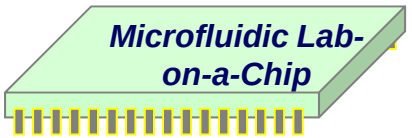
- Clinical diagnostics, e.g., healthcare for premature infants, point-of-care diagnosis of diseases
- “Bio-smoke alarm”: environmental monitoring
- Massive parallel DNA analysis, automated drug discovery, protein crystallization

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

Motivation for Biochips

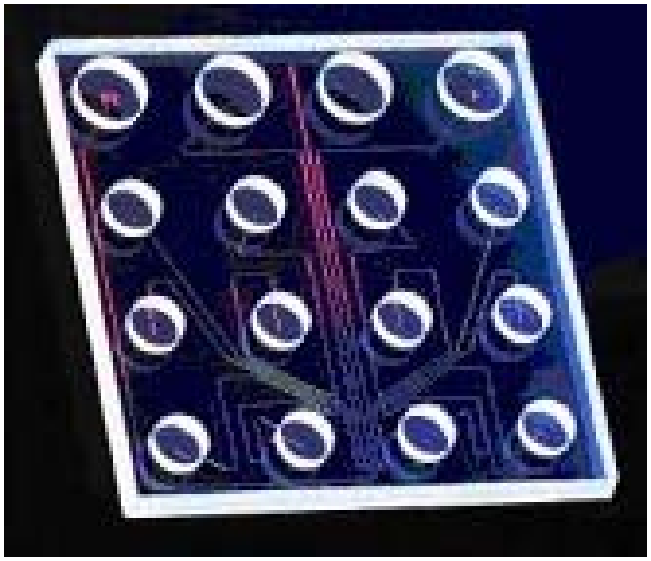
- **Disease related mortality is the No. 1 bottleneck for Aquaculture industry.**
- **Biochip can be used for fish disease testing.**

	Laboratory	Biochip
Sample Processing	1 hr 30 min	5 min
PCR Response	3 hr	1 hr 15 min
Analysis	30 min	10 min
Total	5 hr	1 hr 30 min

Tubes to Chips: BioChips

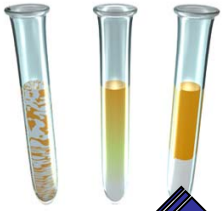
- Driven by biomolecular analysis needs

Test tube analysis



Agilent DNA analysis
Lab on a Chip (1997)

Motivation for Microfluidics



Test tubes

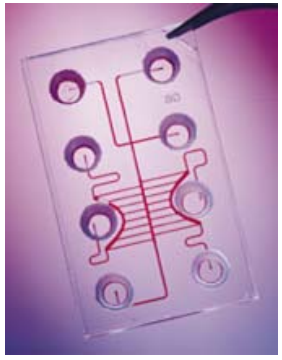
- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

Robotics

- ☑ Automation
- ☑ Integration
- ☐ Miniaturization

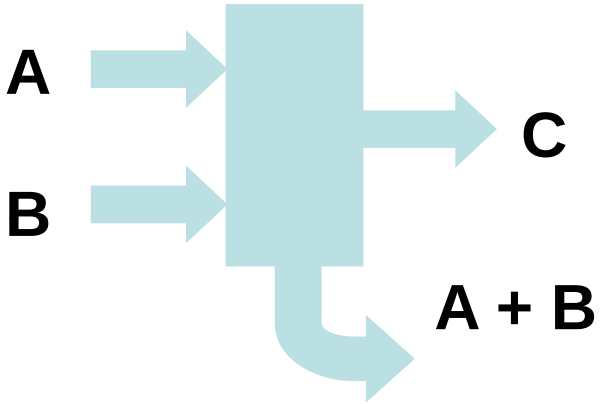
Microfluidics

- ☑ Automation
- ☑ Integration
- ☑ Miniaturization

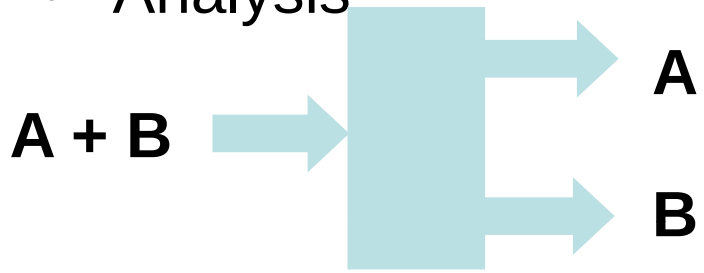


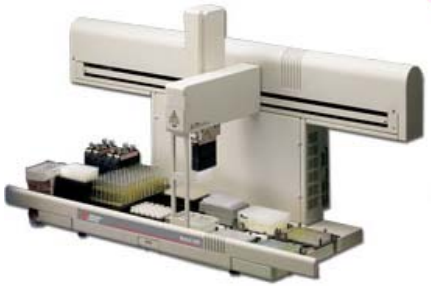
Typical Biological Lab Functions

- Synthesis



- Analysis





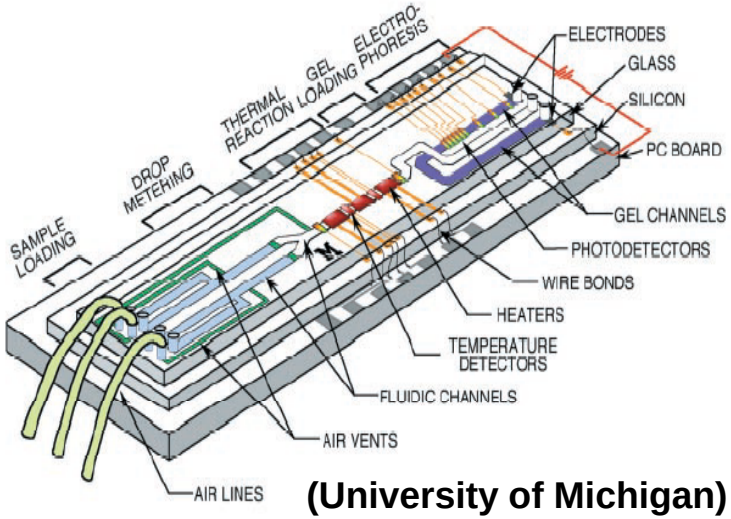
Mixing

Reaction

Separation

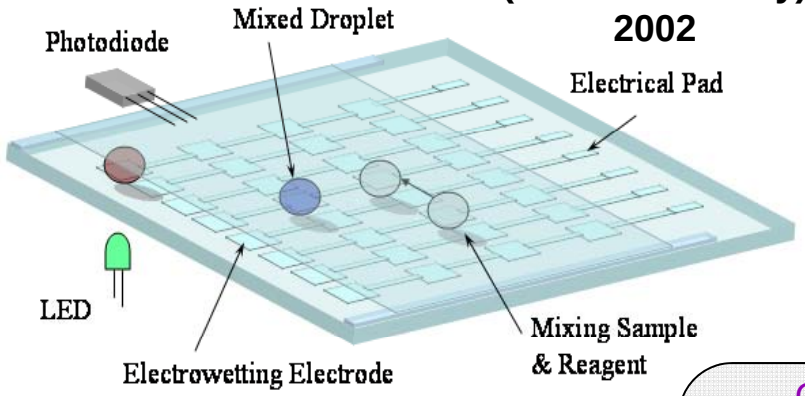
Microfluidics

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

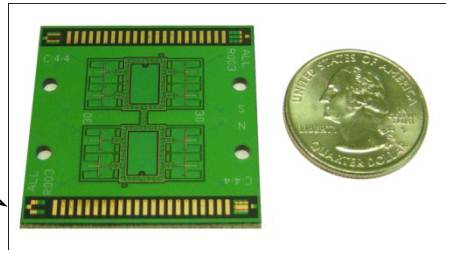


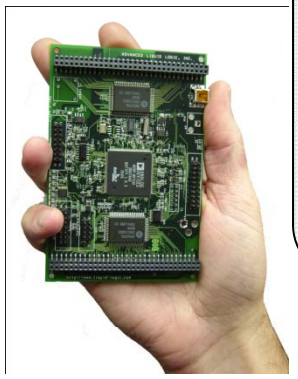
(University of Michigan) 1998

(Duke University) 2002



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

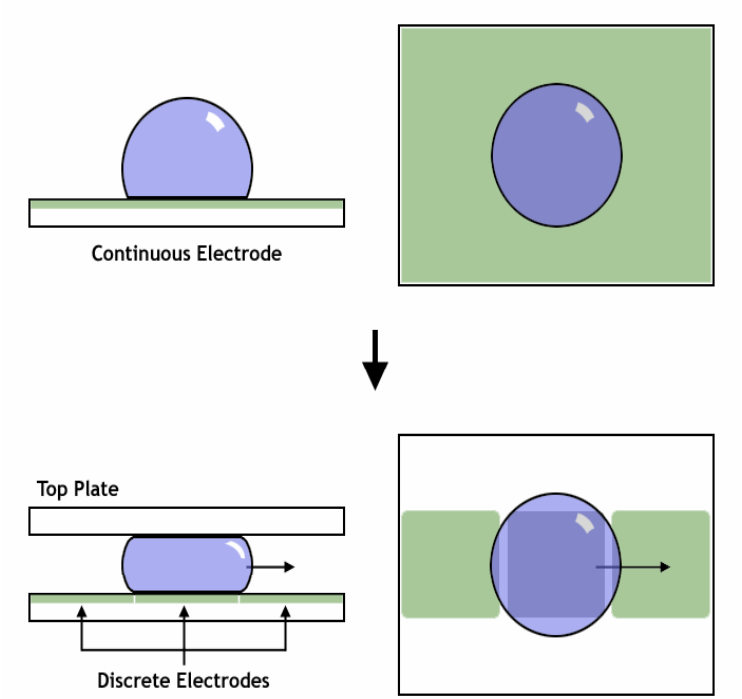


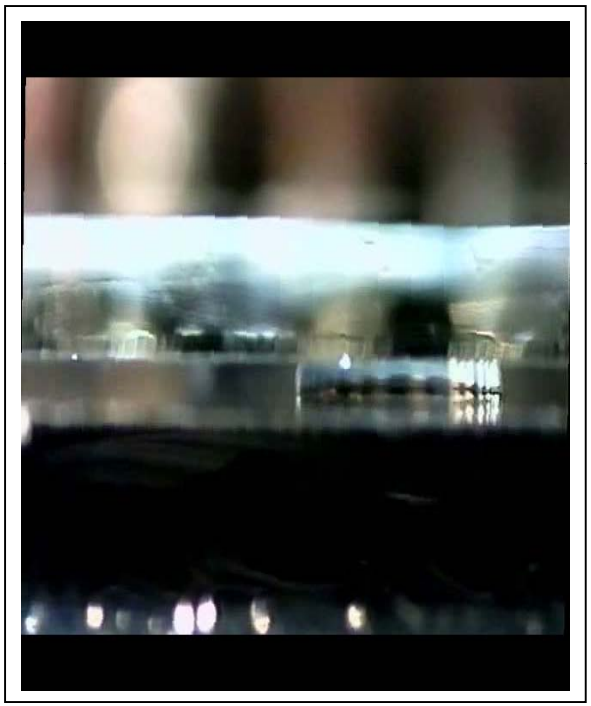


Control electronics (shown) are suitable for handheld or benchtop applications

What is Digital Microfluidics?

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

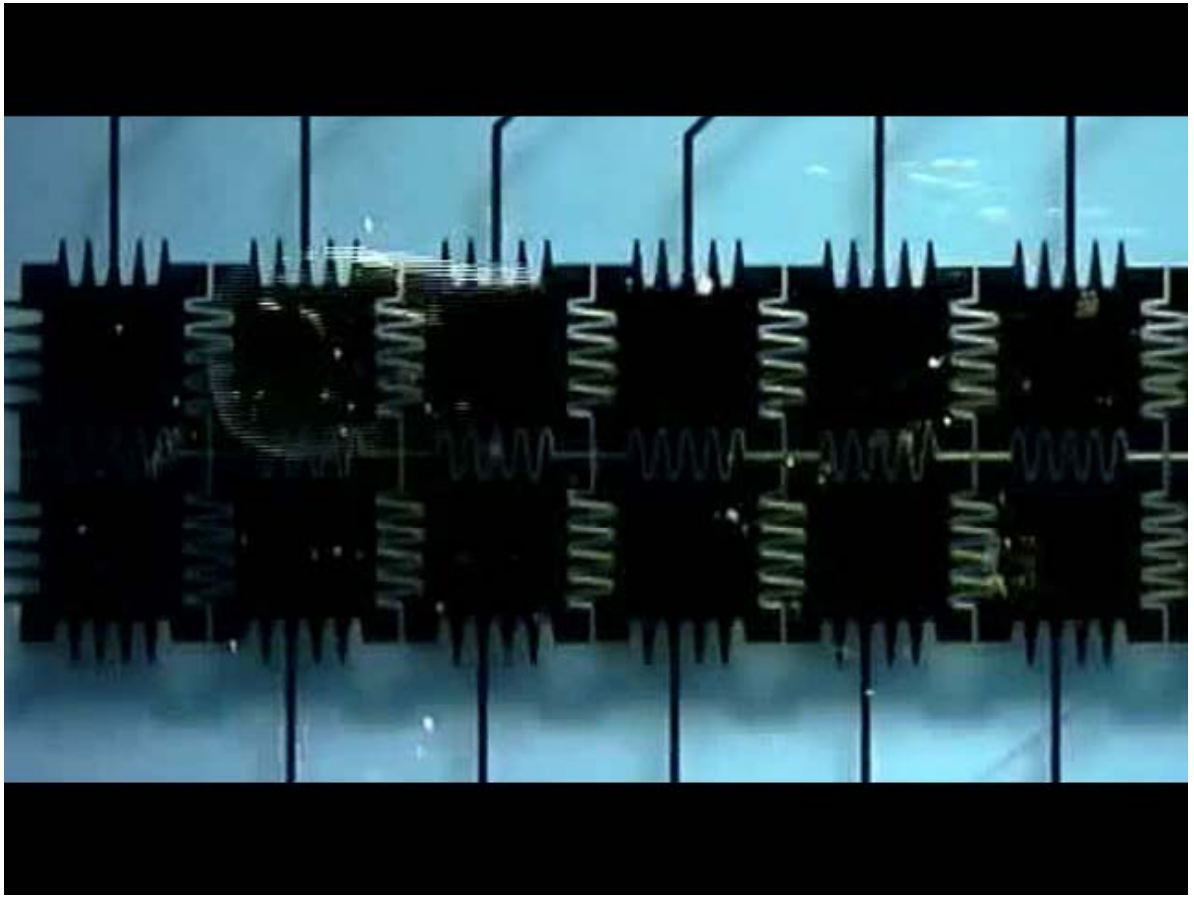




Droplet Transport (Side View)

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

What is Digital Microfluidics?

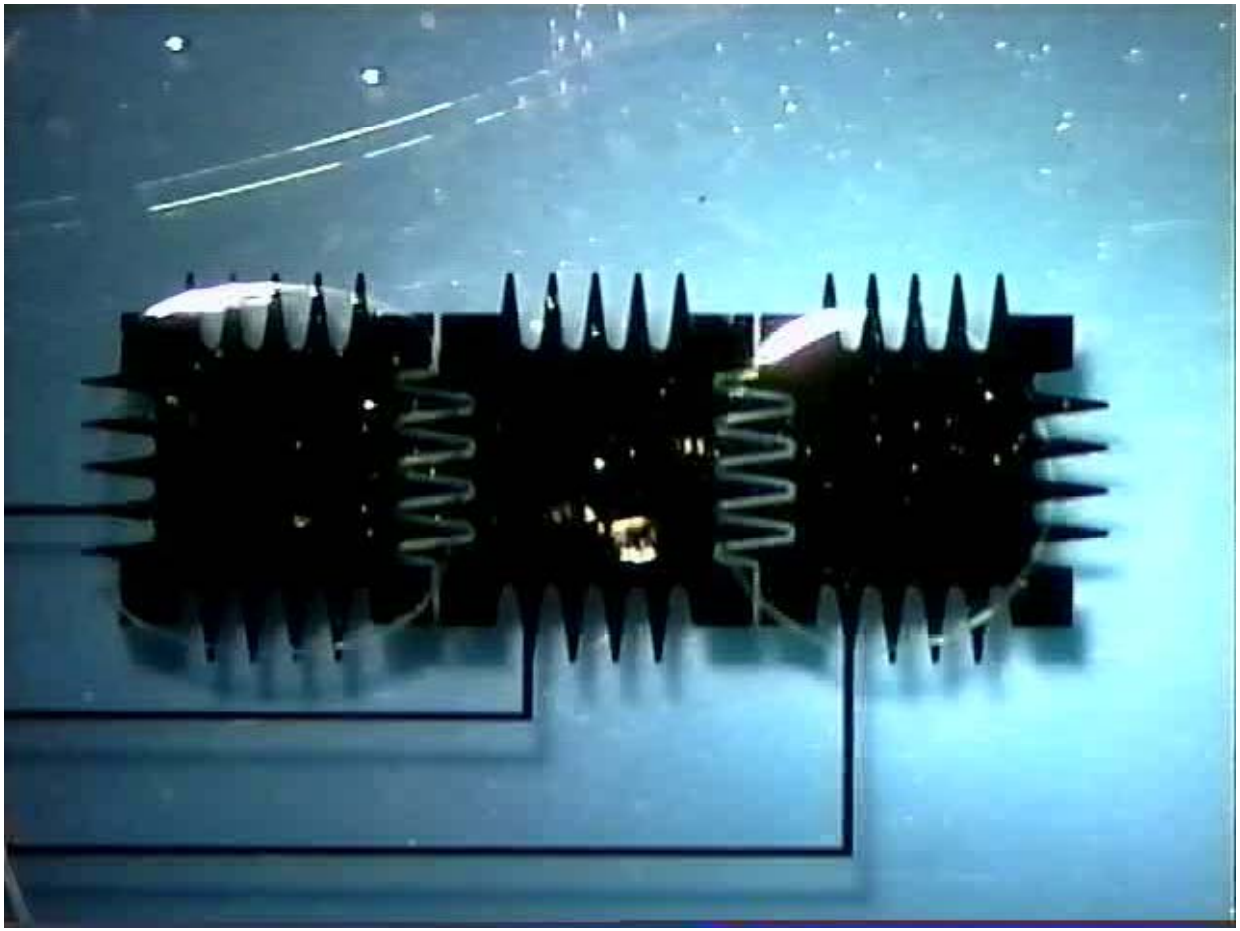


Transport
25 cm/s flow rates, order of magnitude higher than continuous-flow methods

For videos, go to www.ee.duke.edu/research/microfluidics

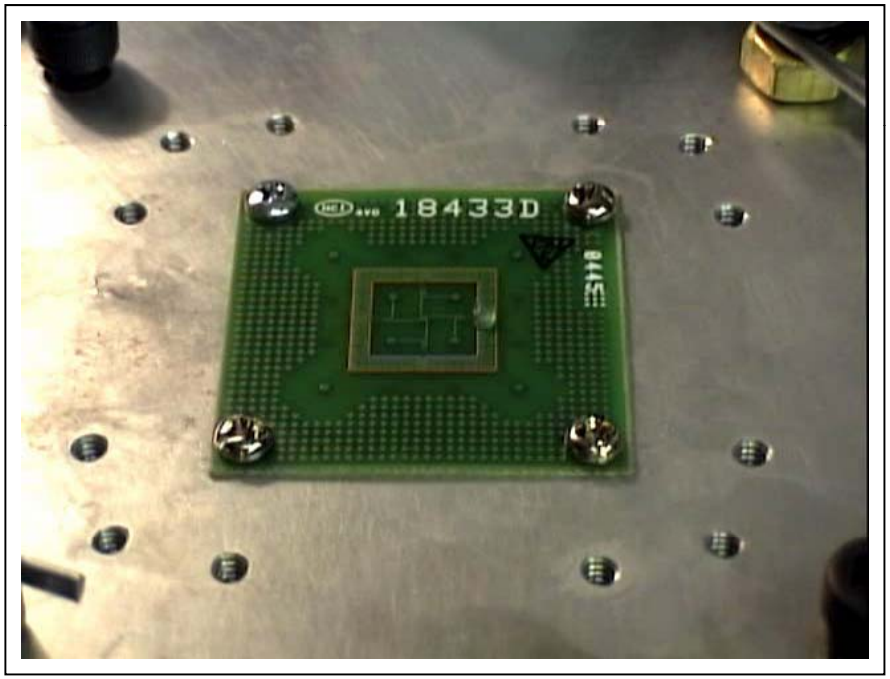
What is Digital Microfluidics?

Splitting/Merging



Advantages

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



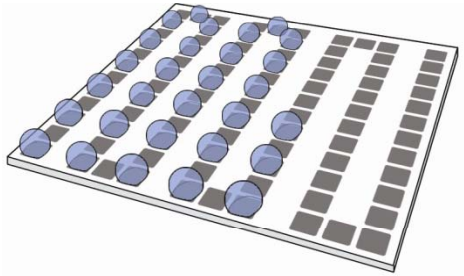
Droplet Transport on PCB (Isometric View)

- 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

Advantages of Digital Microfluidics

Digital Microfluidics

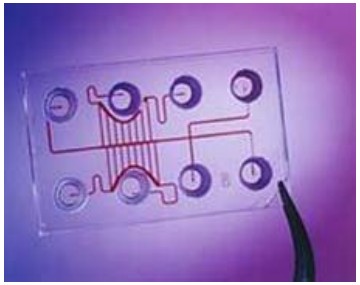
- Very accurate droplet volumes
  - Droplet sizes in the 1 nanoliter to several microliter range; droplet dispensing volume variation ~1%
- Programmable, software-driven electronic control
  - No moving parts, tubes, pumps or valves
- More efficient use of samples and reagents
  - No liquid is wasted priming channels
- Extremely energy efficient
  - Nanowatts of power per single step of actuation
- Development cycles are short, and assays can be implemented with software changes
- Compatible with live biologic and most other materials



•Droplets moved in "virtual channels" defined by electrodes
•Programmable electrodes directly control discrete droplet operations

Other Microfluidic Technologies

- Pump fluids through channels
- Must adapt assays to channel-based format
- Complex or multiplexed assays become a plumber's nightmare
- Off-chip pumps and valves mean large, expensive equipment and low reliability
- Expensive, time consuming, up-front investments required for most chip developments
- Designs are fixed in the development process

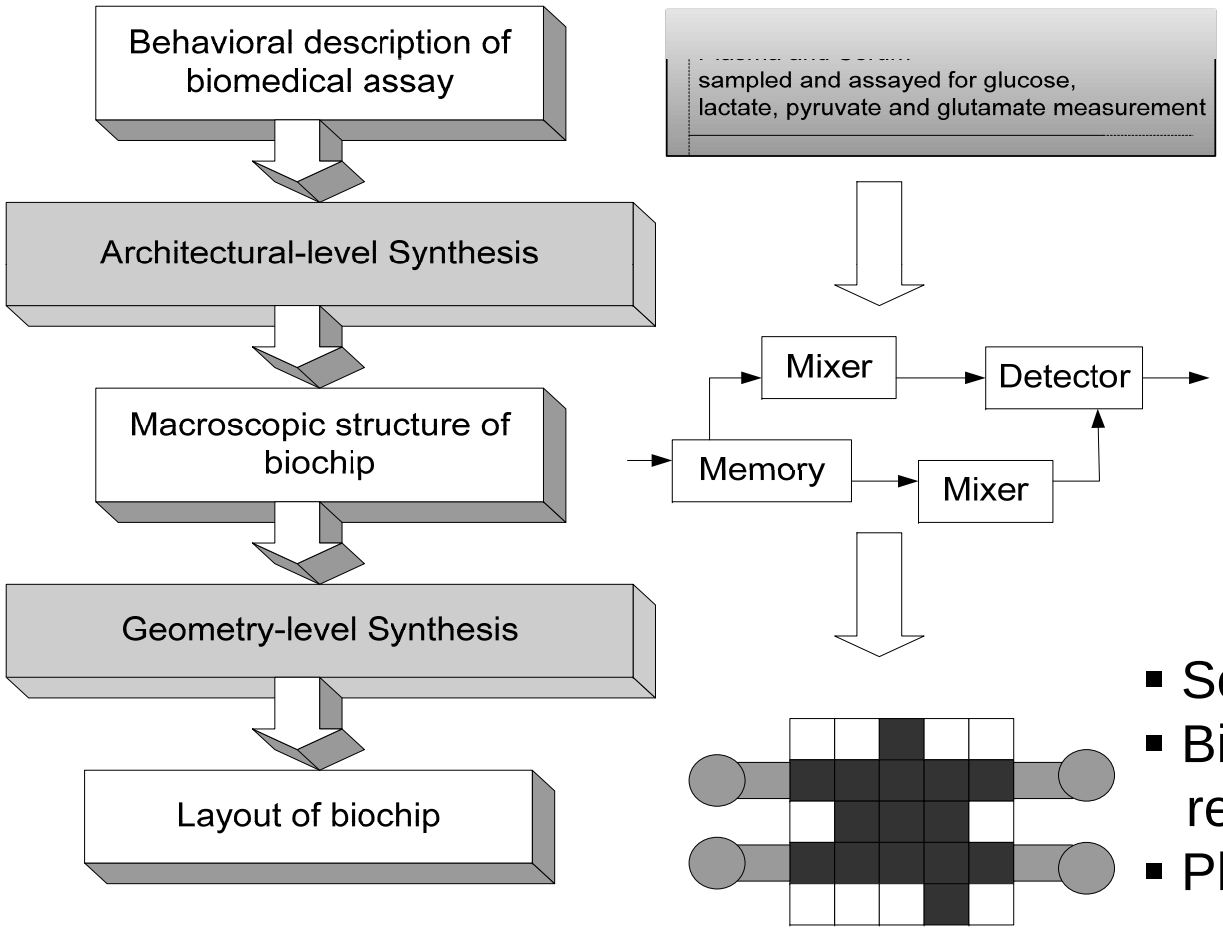


Applications of Digital Microfluidic Biochips

- Drug discovery and biotechnology
  - Proteomics
  - High-throughput screening
  - Genomics
- Medical diagnostics and therapeutics
  - Clinical chemistry
  - Immunoassays
  - Nucleic acid tests
- Environmental and other applications
  - Micro-optics
  - Countering bioterrorism
  - Air/water/agro food monitoring

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

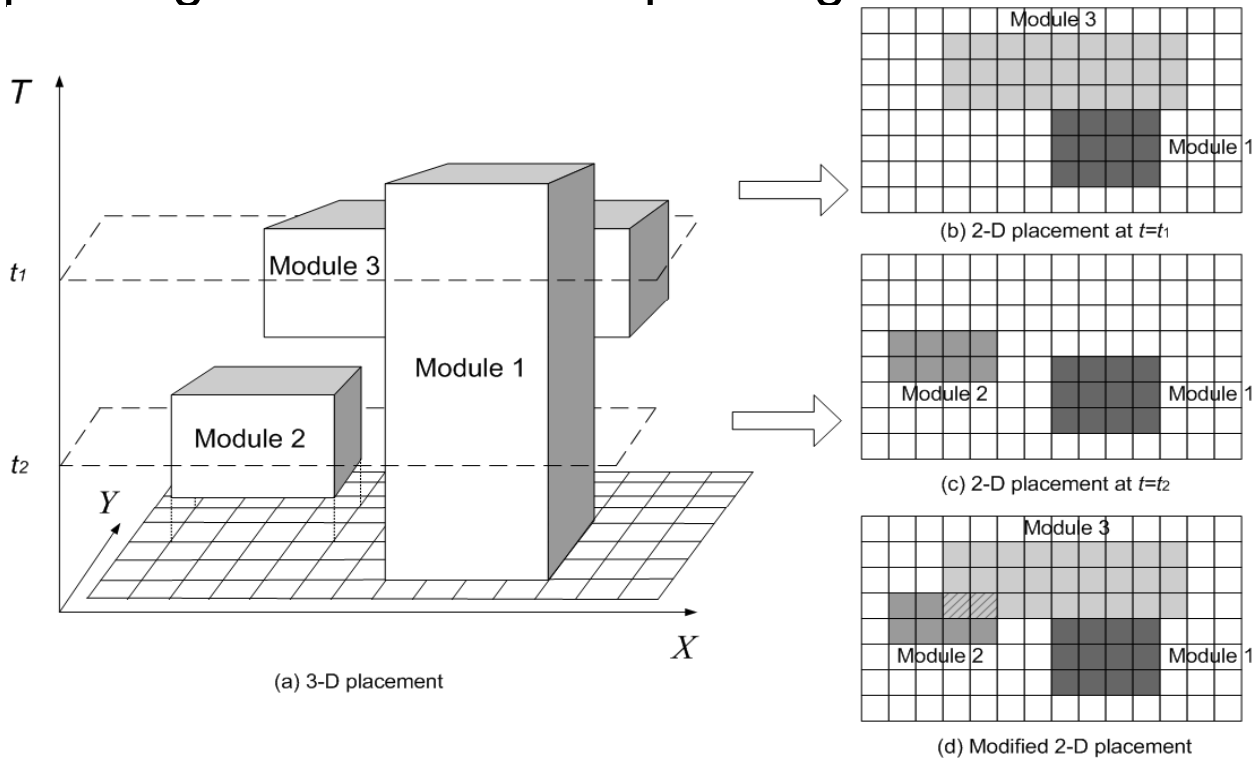
Assay1: Glucose assay,
Assay2: Lactate assay,
Assay3: Pyruvate assay,
Assay4: Glutamate assay

*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

Physical Design: Module Placement

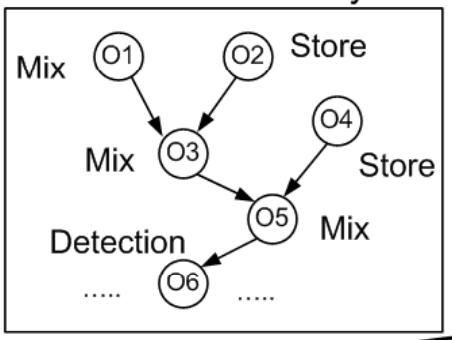
- Placement determines the locations of each module on the microfluidic array in order to optimize some design metrics
- High dynamic reconfigurability: module placement → 3-D packing → modified 2-D packing



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

Unified Synthesis Methodology

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
Detectors		
LED+Photodiode	1 cell	30 s

Design specifications

Maximum array area ***Amax*****:** 20x20 array

Maximum number of optical detectors: 4

Number of reservoirs: 3

Maximum bioassay completion time ***Tmax*****:** 50 seconds

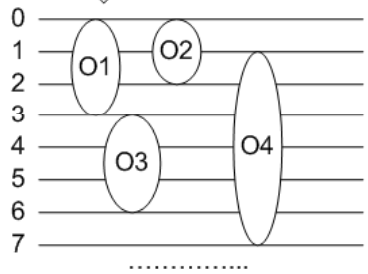
Unified Synthesis of Digital Microfluidic Biochip

Output:

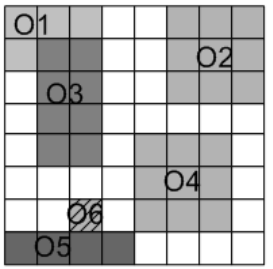
Resource binding

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

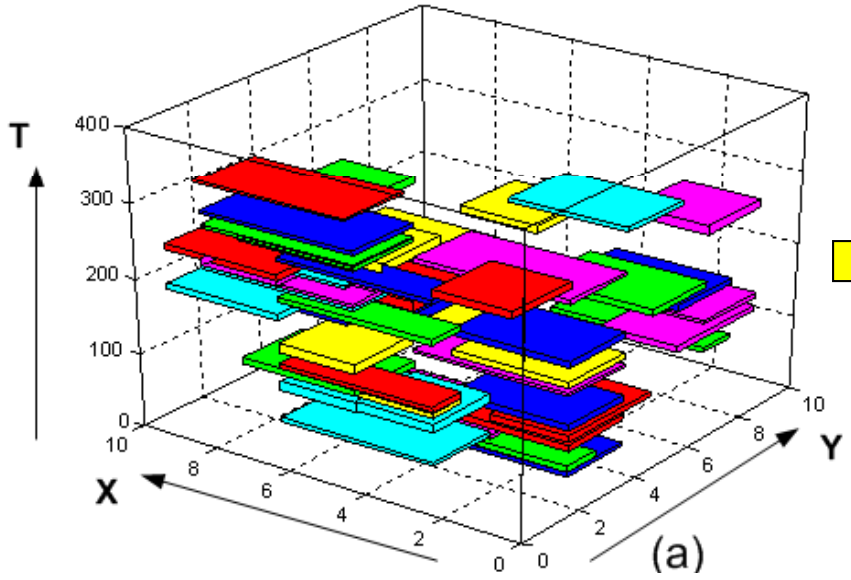


Placement



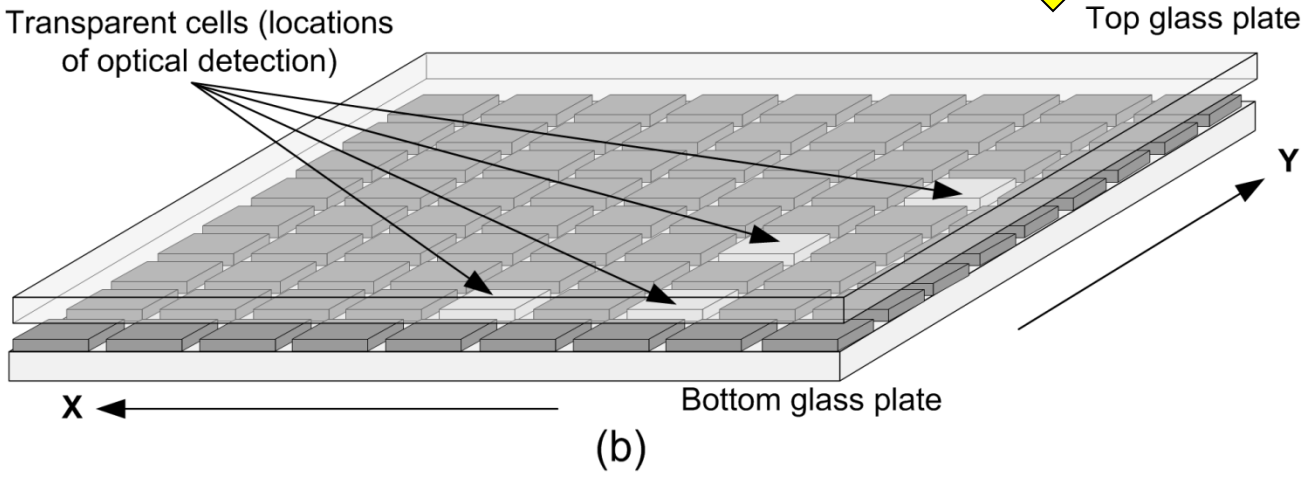
Biochip design results: **Array area:** 8x8 array **Bioassay completion time:** 25 seconds

Synthesis Results



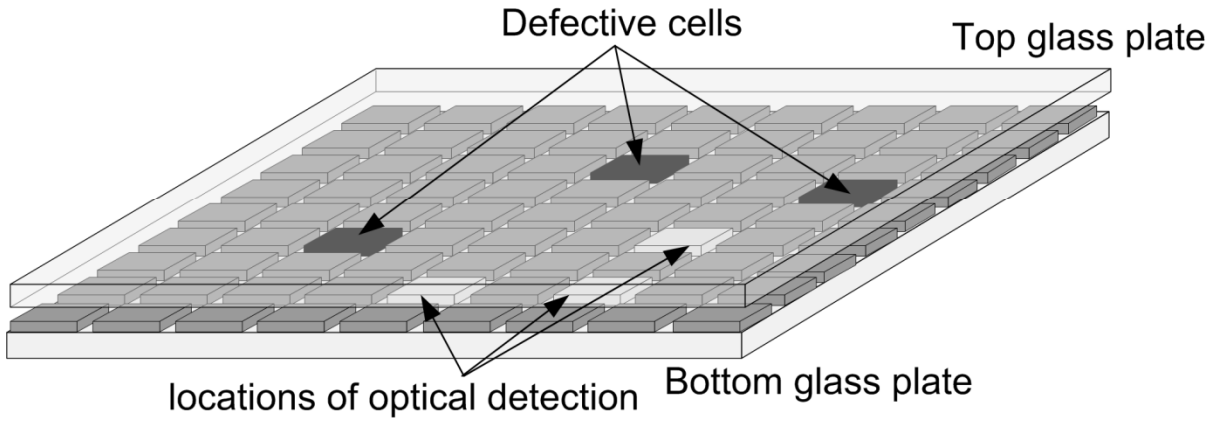
Bioassay completion time *T*: 363 seconds

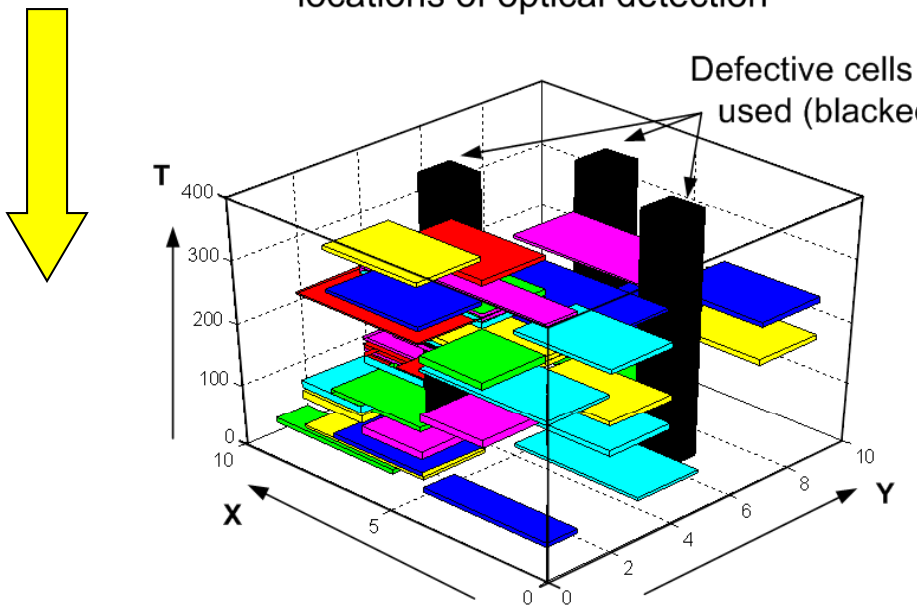
Biochip array: 9x9 array



Synthesis Results (Cont.)

- Defect tolerance





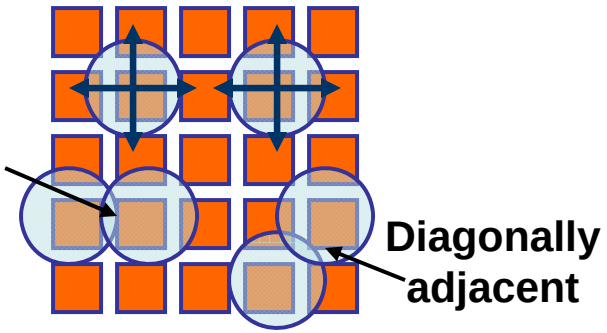
Bioassay completion time *T*: 385 seconds (6% increase)

Droplet Routing

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

Fluidic Constraints

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



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.

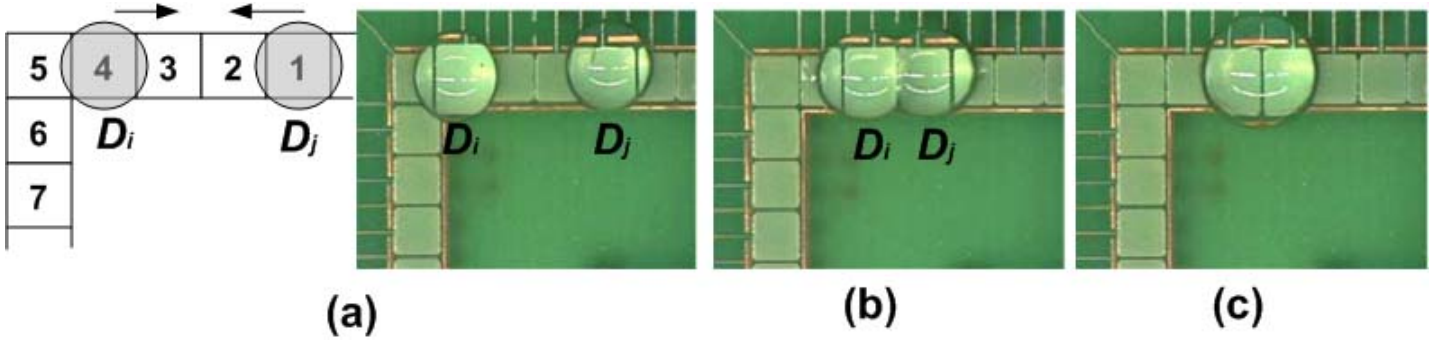
Rule #2: $|X_i(t+1) - X_j(t)| \geq 2$ or $|Y_i(t+1) - Y_j(t)| \geq 2$, i.e., the activated cell for D_i cannot be adjacent to D_j.

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

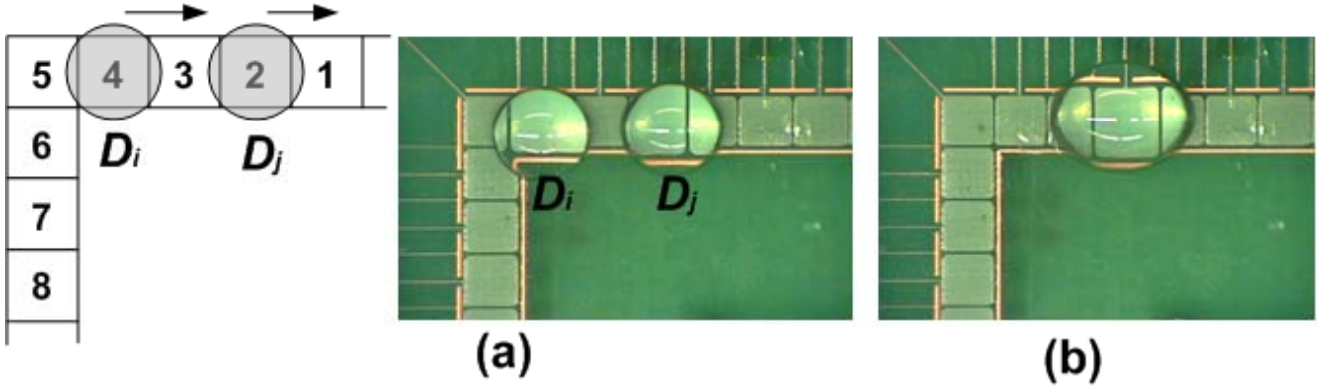
Static fluidic constraint

Dynamic fluidic constraints

Experimental Verification

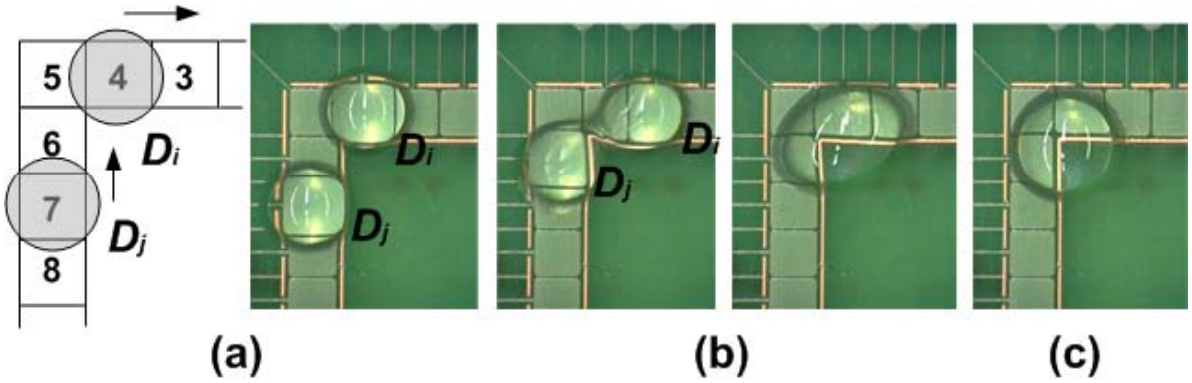


(a) Experimental verification of Rule #1: droplets begin on electrodes 1 and 4; (b) Electrodes 2 and 3 are activated, and 1 and 4 deactivated; (c) Merged droplet.



(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.

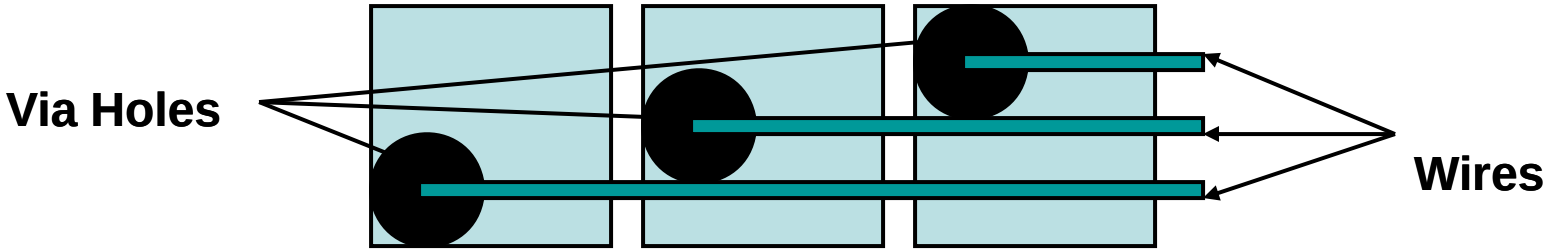
- To demonstrate that adherence to Rule #1 is not sufficient to prevent merging. Both Rule #2 and Rule #3 must also be satisfied during droplet routing.
- These rules are not only used for rule checking, but they can also provide guidelines to modify droplet motion (e.g., force some droplets to remain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

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

Cross-referencing

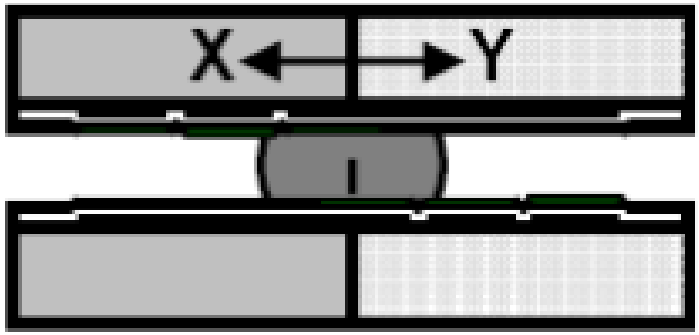
Orthogonally placed pins on top and bottom plates

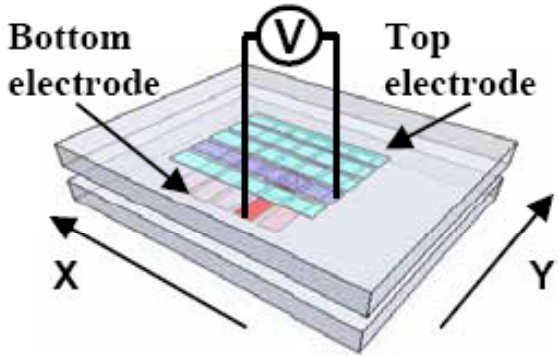
Advantage

k = n x m pins → n + m pins for an n x m microfluidic array

Disadvantage

Suffer from *electrode interference*



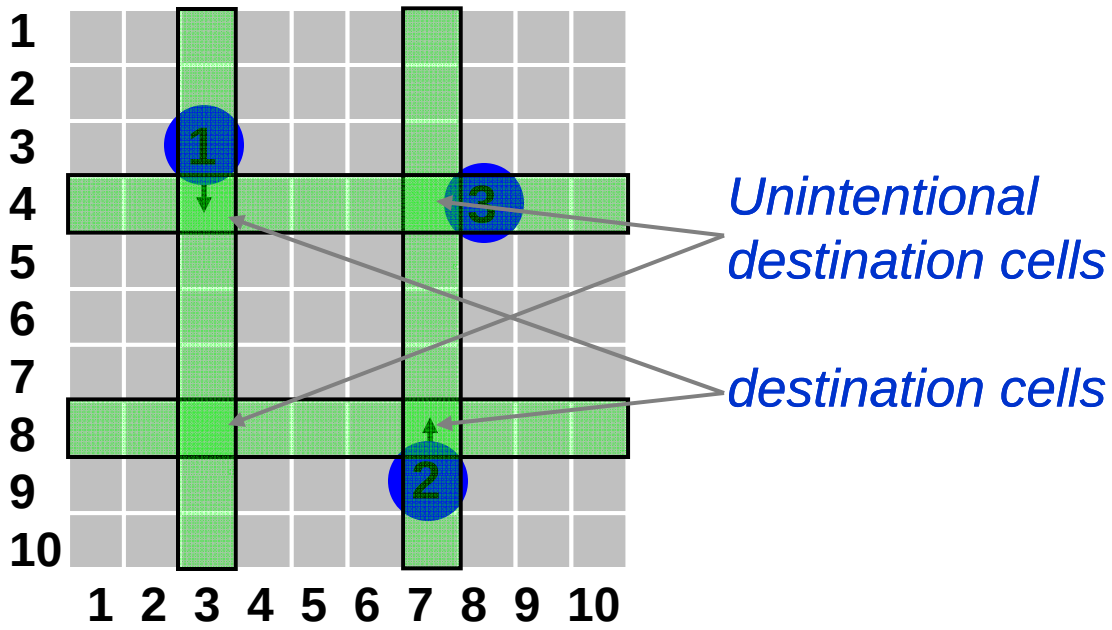


Electrode Interference

- **Unintentional Electrode Actuation**

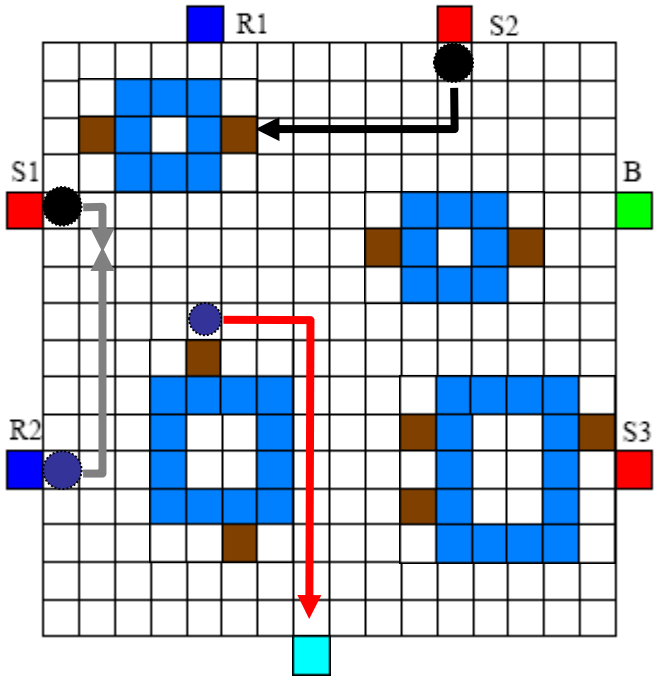
  Selected column and row pins may intersect at multiple electrodes

- **Unintentional Droplet Manipulation**



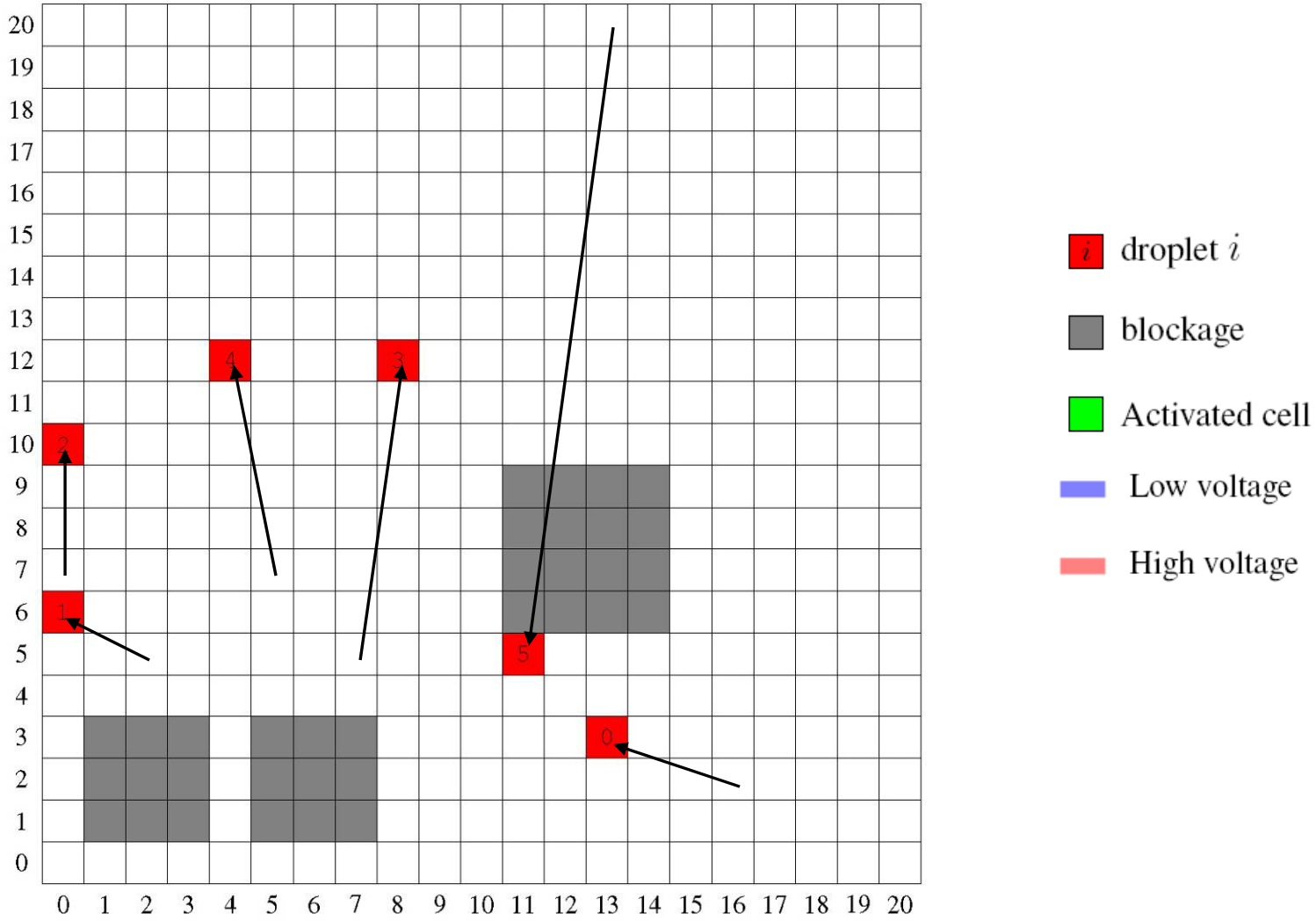
Droplet Routing for Cross Referencing Chip - CrossRouter

- Input:
  - A *WxH* 2D array
  - *K* blockages
  - Waste disposal location *WR*
  - A netlist of *N* nets, either 2-pin or 3-pin
  - Time limit *T*
- Output:
  - A *schedule of voltage assignment* for each time step
- Objective:
  - *Route all droplets to their destinations* without violating constraints
  - Minimize arrive time and # cell used
- Constraints:
  - Timing constraint
  - Fluidic constraints
  - Electrode constraint



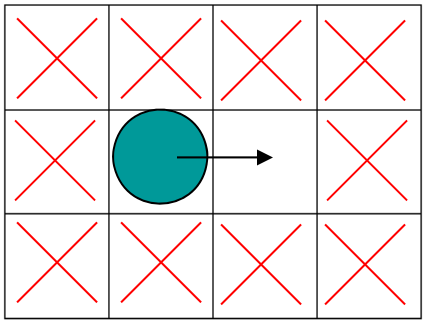
Time Limit: 20 units

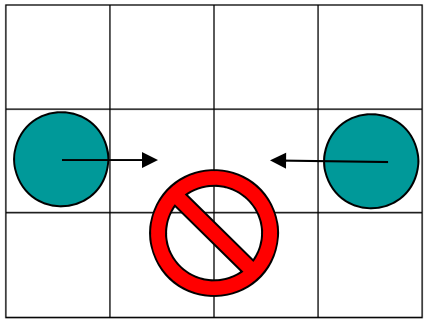
Example of Droplet Movement



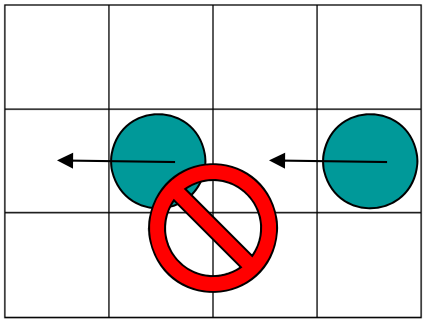
Constraints

1. Fluidic Constraint
   - A minimum spacing of one cell to avoid unexpected mixing
2. Timing Constraint
   - All droplets should be moved to sinks within time limit *T*
3. Electrode Constraint (major problem)
   - No interference is caused while activating electrodes simultaneously.





Example 1

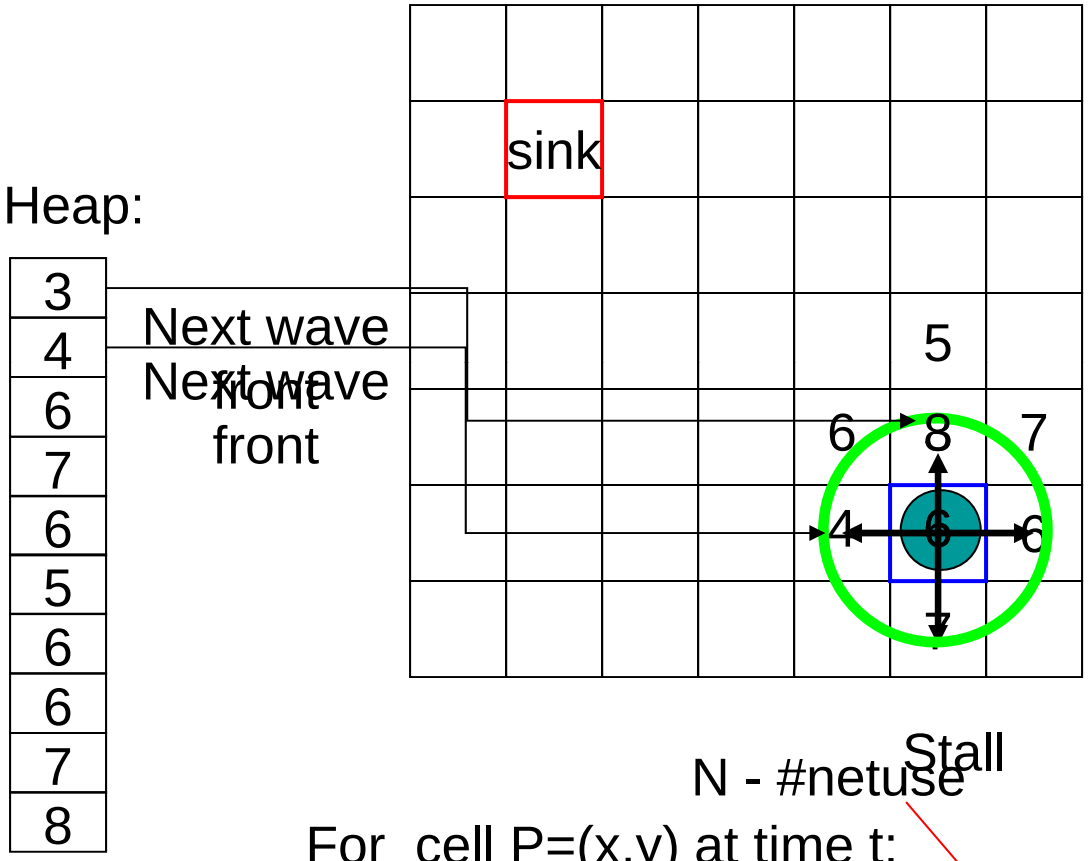


Example 2

CrossRouter

1. **Net ordering**: for net *i* and net *j*, route *i* first if:
   - src(*i*) is in the bounding box of net *j*, **or**
   - *Manhattan_Distance (i) > Manhatten_Distance (j)*
2. **Maze routing**: route each net while considering those already routed nets
   - Modified Lee's algorithm as basic framework
   - Handle constraints during this step to consider those already routed nets
3. **Rip-up & Re-route**: identify bottleneck regions, rip off some already routed nets and re-route the failed net

Propagation



Note: push into heap only if no constraint is violated for a movement

For cell P=(x,y) at time t:

N - #netuse

Stall

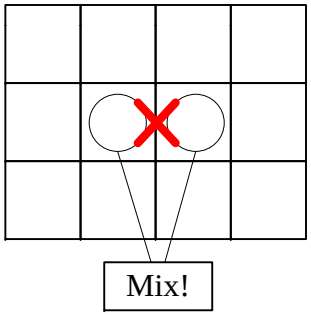
Current path length

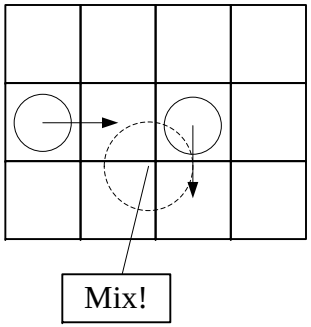
```
Weight(P,t) = t + MD(P,sink) + Use(P) + Len(P,t)
```

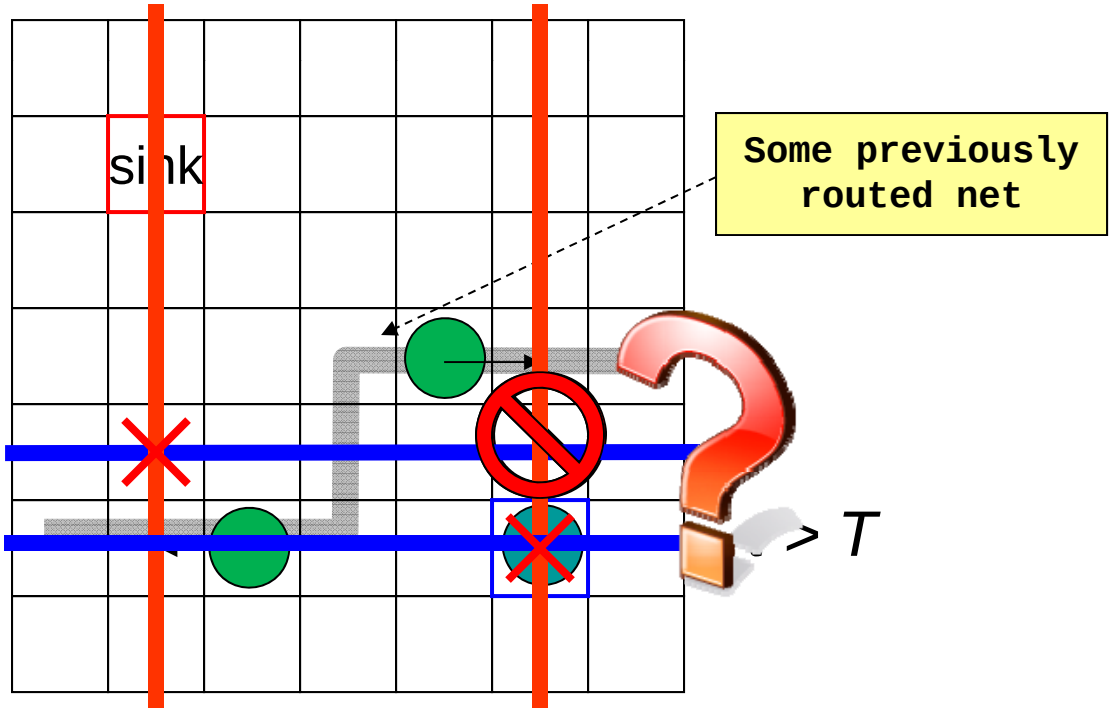
Constraint Check

1. Timing constraint
2. Fluidic constraint – avoid unexpected mixing
3. Electrode constraint

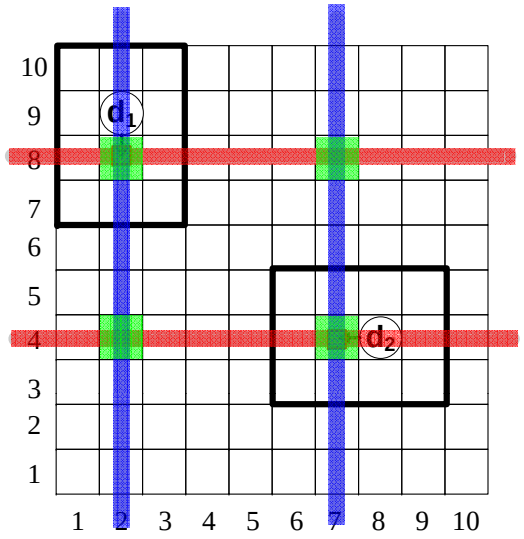
Any valid voltage assignment?

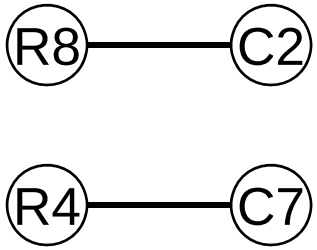


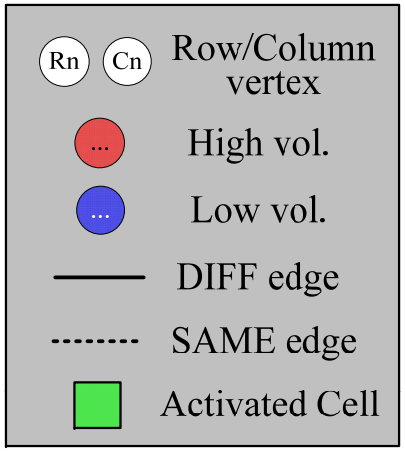




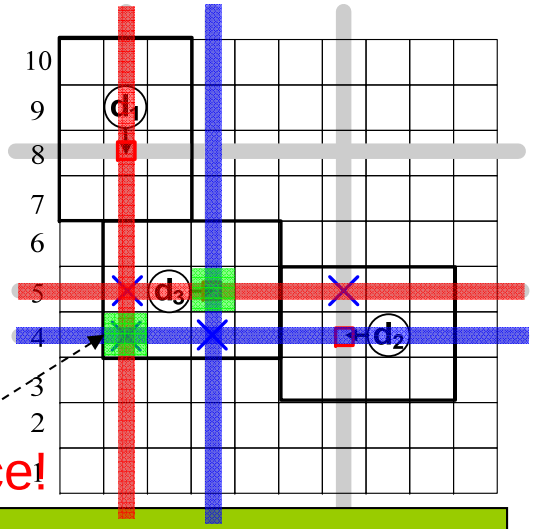
Constraint Check by Graph





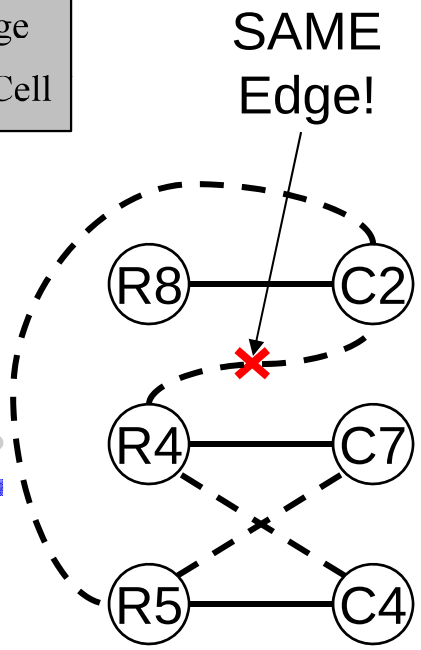


Scenario 1: d_1 routed, checking a movement of d_2



Scenario 2: d_1 & d_2 routed, checking a movement of d_3

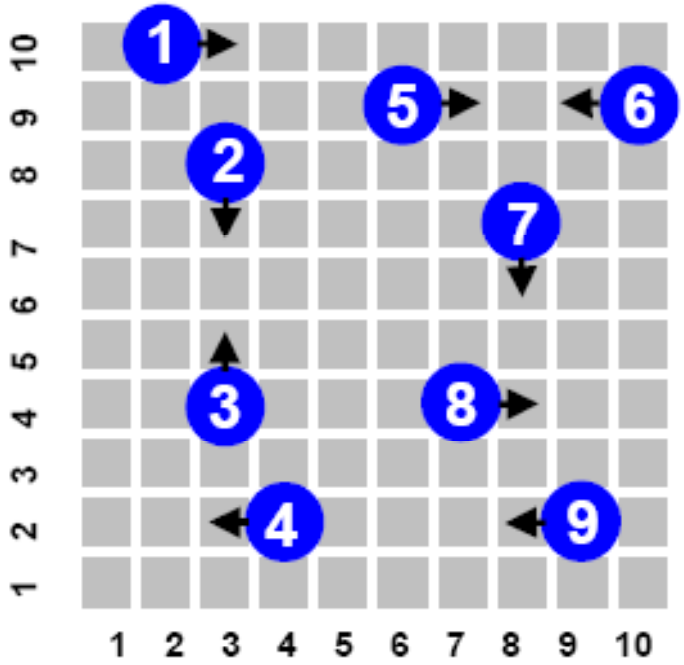
Interference!



NO valid assignment for these movements!

Efficient (Concurrent) Droplet Manipulation

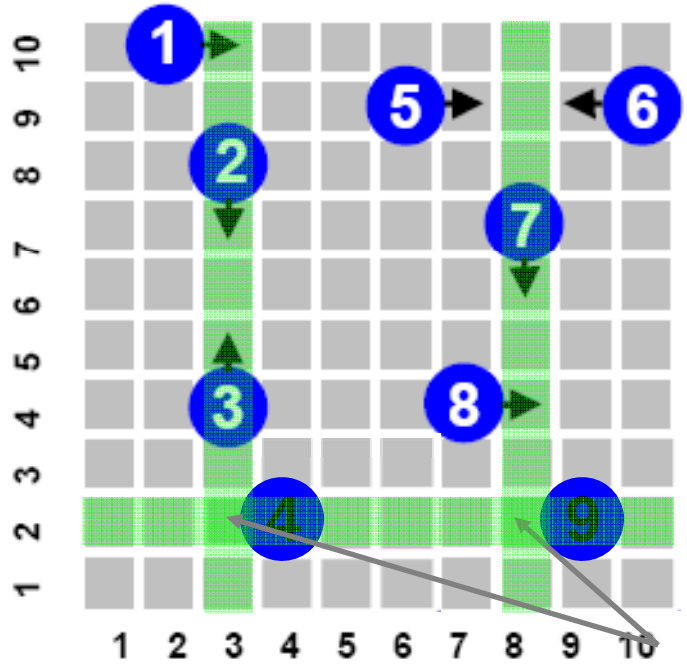
- **Goal:** Improve droplet manipulation concurrency on cross-referencing-based biochips.



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

Efficient Droplet Manipulation

- **Observation**
  - Droplet manipulations whose *destination cells* belongs to the same column/row can be carried out without electrode interferences.



Efficient Droplet Manipulation

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

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

Efficient Droplet Manipulation

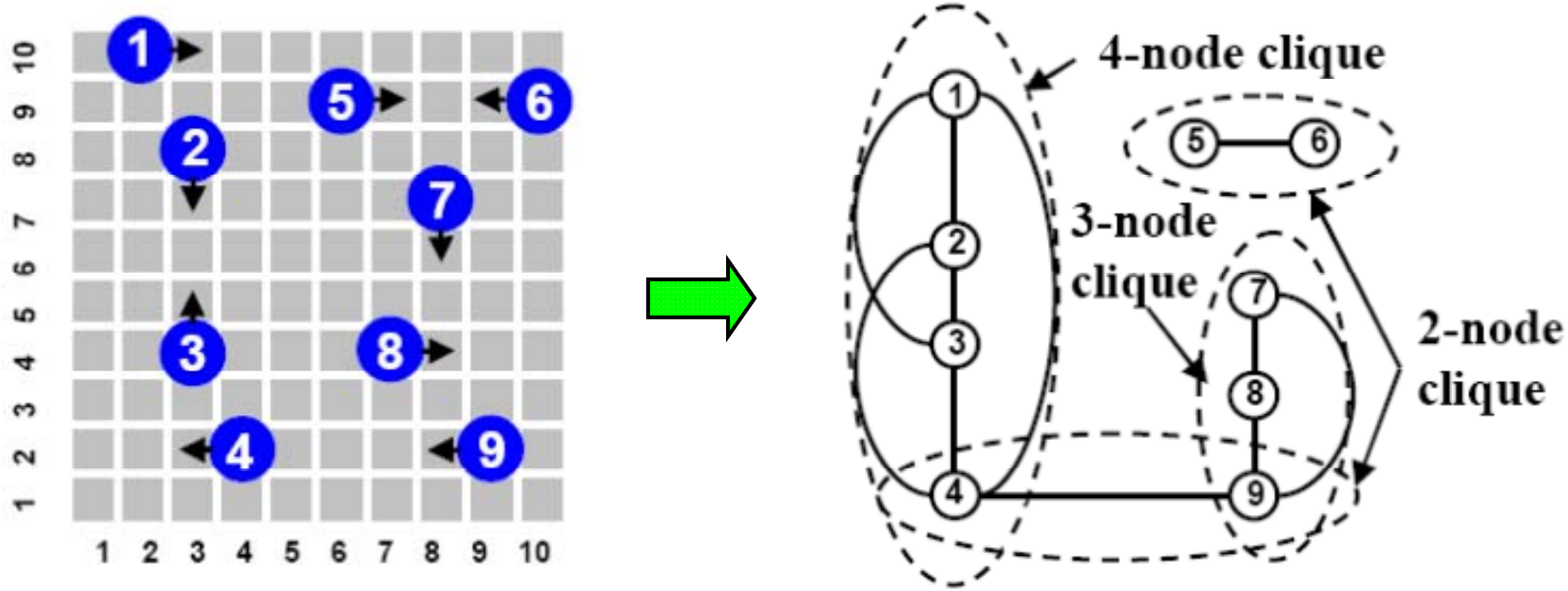
- **Problem formulation**

  Destination cells → Nodes

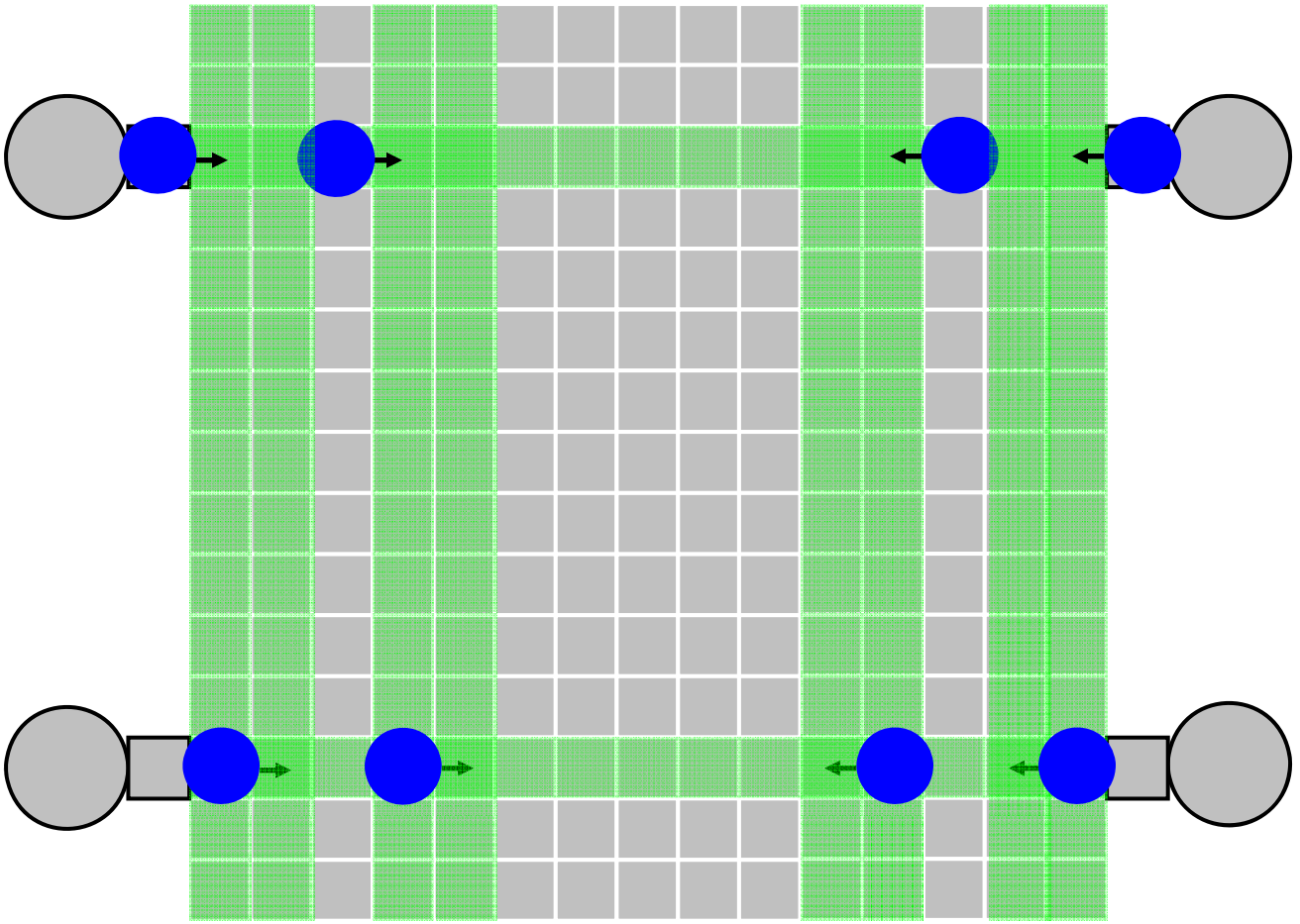
  Destination cells in one column/row → a clique

  Grouping → Clique partitioning

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



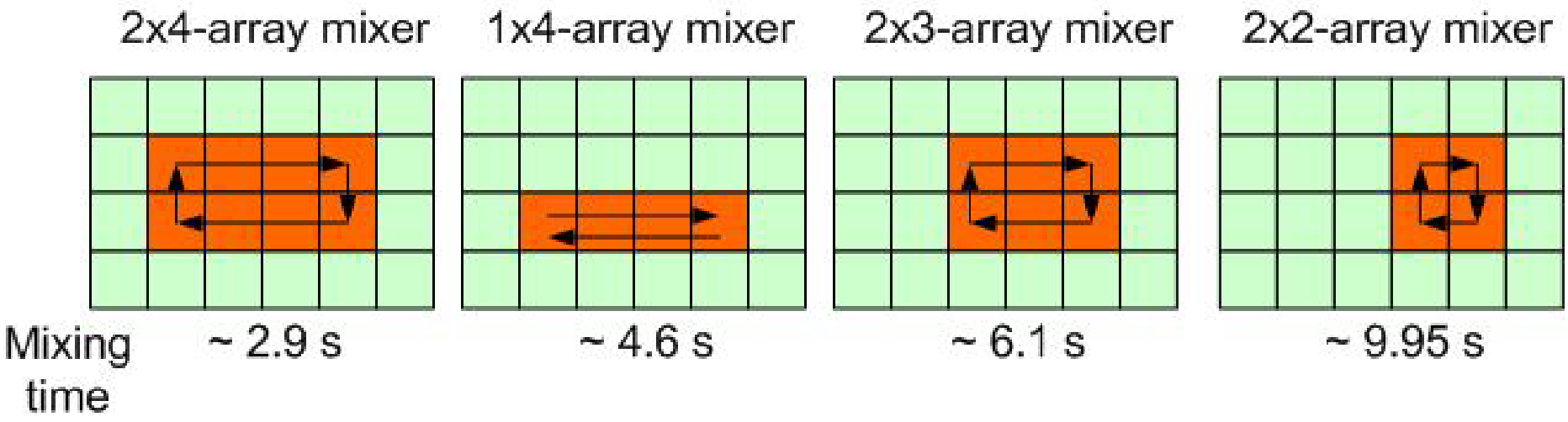
An Example



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

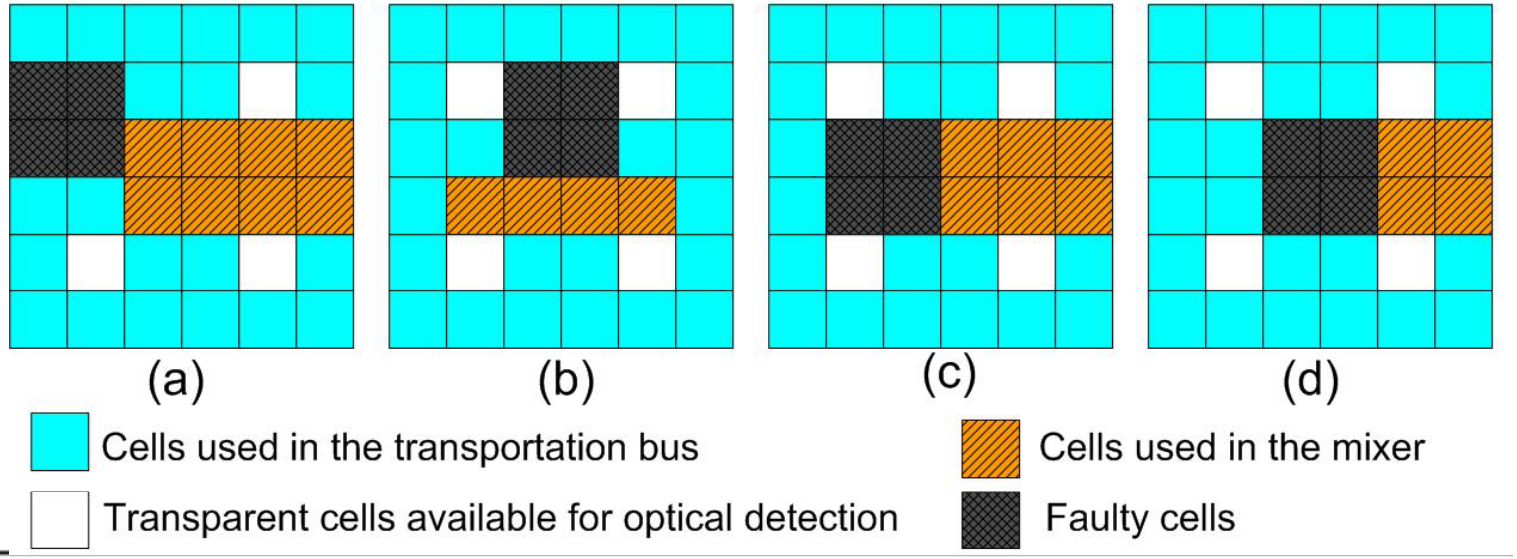
Reconfigurability

- 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



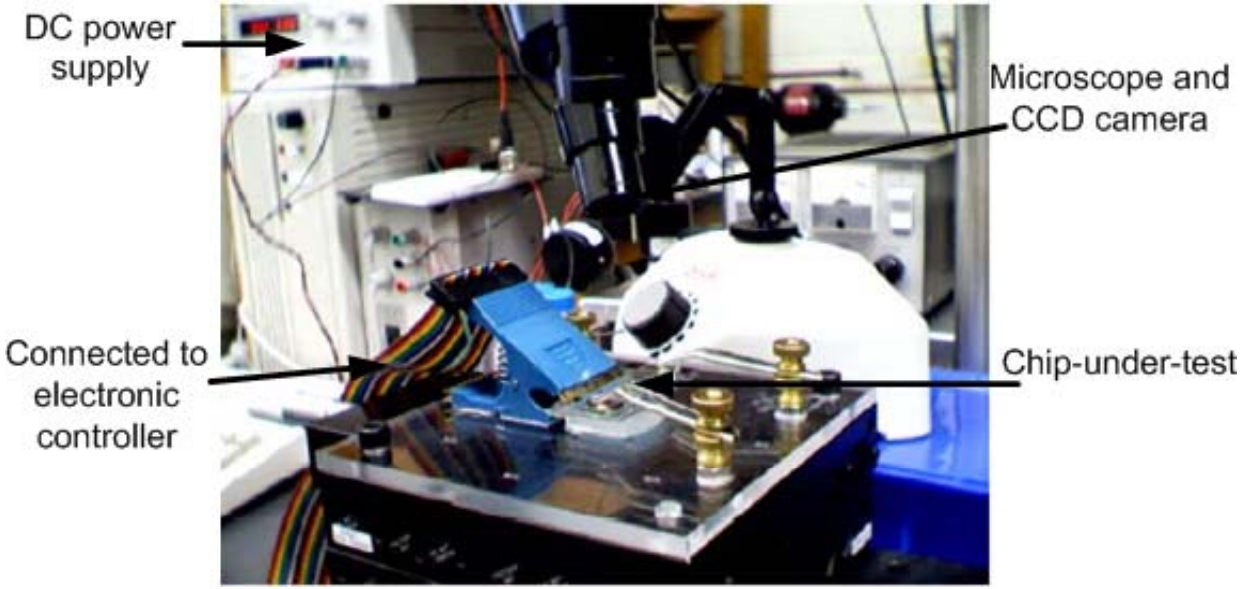
Reconfiguration and Graceful Degradation

- 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)
-



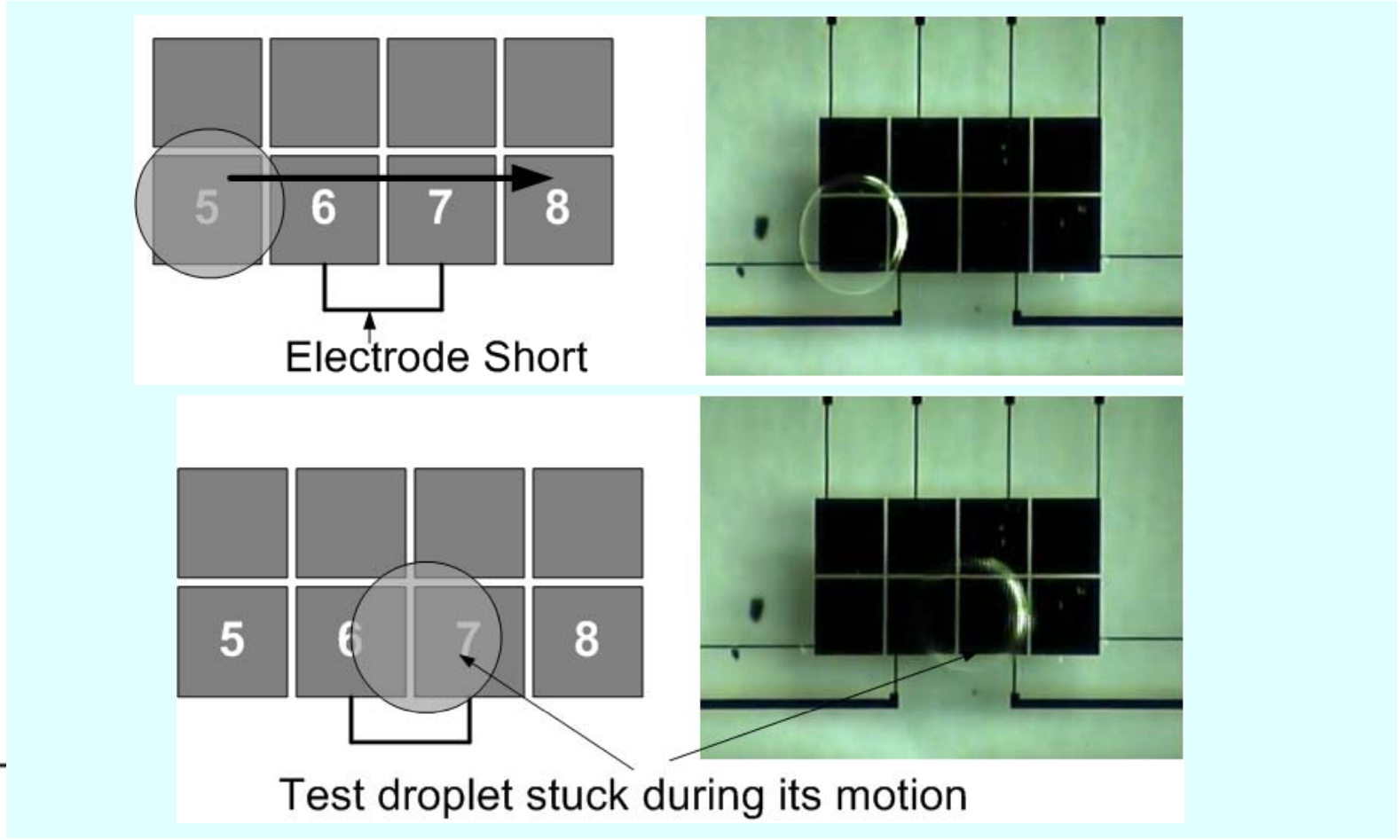
Defect-Oriented Experiment

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



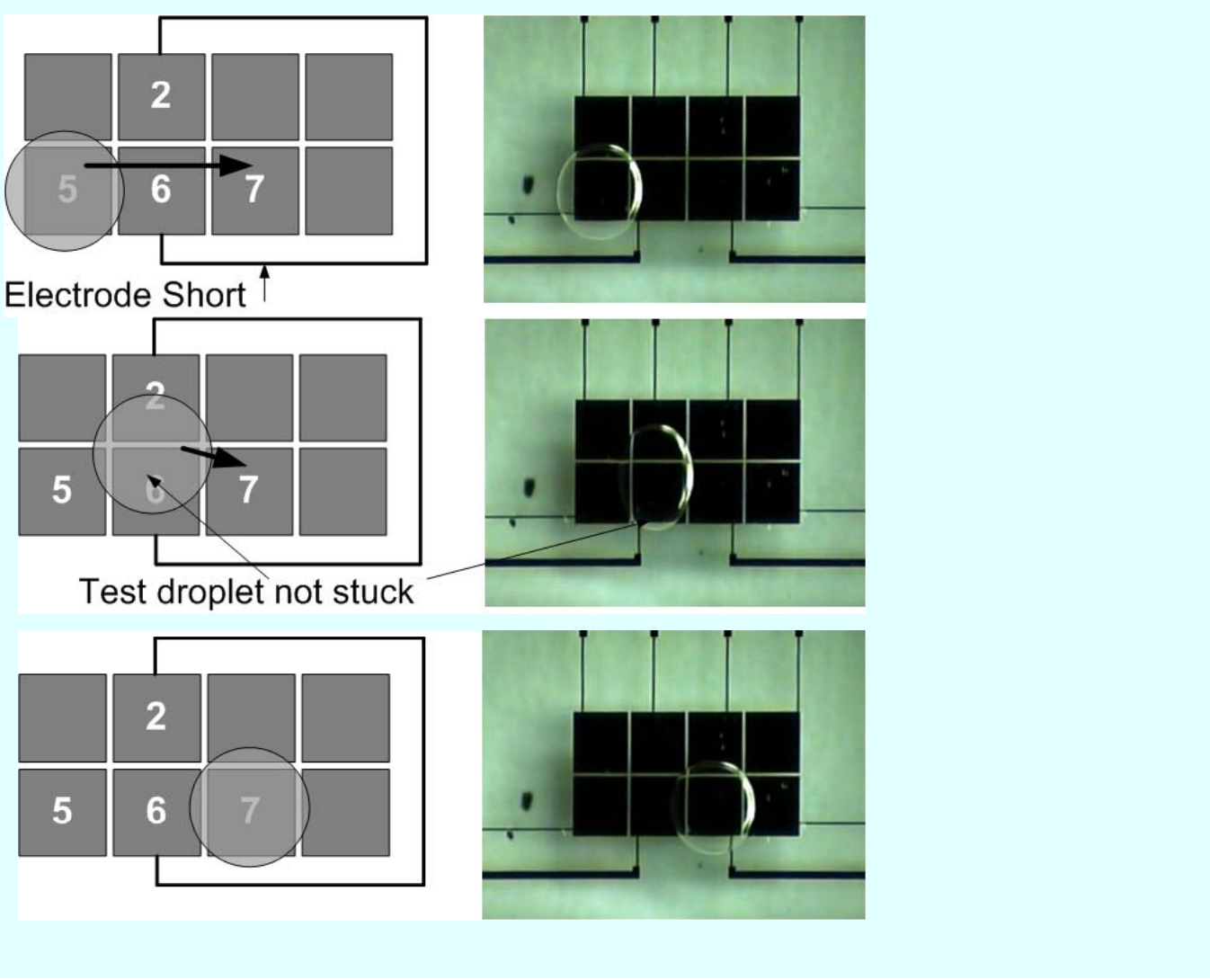
Defect-Oriented Experiment (Cont.)

- Results and Analysis
  - Experimental results and analysis for the first step.



Defect-Oriented Experiment (Cont.)

- Experimental results and analysis for the second step.



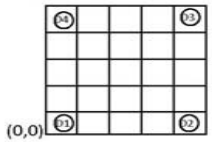
Conclusions

- Digital microfluidics offers a viable platform for biochips for clinical diagnostics and biomolecular recognition
- Design automation challenges
  - Automated synthesis: scheduling, resource binding, module placement; droplet routing; testing and reconfiguration
- Bridge between different research communities: bioMEMS, microfluidics, electronics CAD and chip design, biochemistry
- Growing interest in the electronics CAD community
  - Special session on biochips at CODES+ISSS'2005 (appears in CFP now)
  - Special issue on biochips in *IEEE Transactions on CAD* (Feb 2006)
  - Workshop on biochips at DATE'06
  - Tutorial on biochips at DATE'07, VDAT 2007, embedded tutorial at VLSI Design 2005
  - Special Issue of IEEE Design & Test, Jan/Feb'07

Homework

Droplet Routing for Microfluidic Biochip

Consider a 5×5 cross referencing microfluidic biochip:



Suppose now you have 4 droplets to route:
D1: from (0,0) to (4,0)
D2: from (4,0) to (4,4)
D3: from (4,4) to (0,4)
D4: from ((0,4) to (0,0)
Show how you can move all the droplets from their sources to their destinations by applying high/low/don't care voltages to the columns and rows. Illustrate your route properly by showing at each time step $t = 0..., T$ the following, where T is the length of time your route takes:
(1) Positions of the droplets at time t (before you apply the voltages as described in (2)).
(2) Voltages applied at each row and column.
(3) The activated cells.
What is the total time T taken?