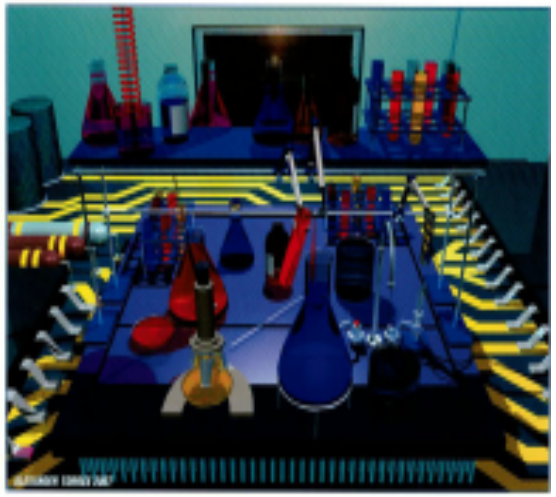
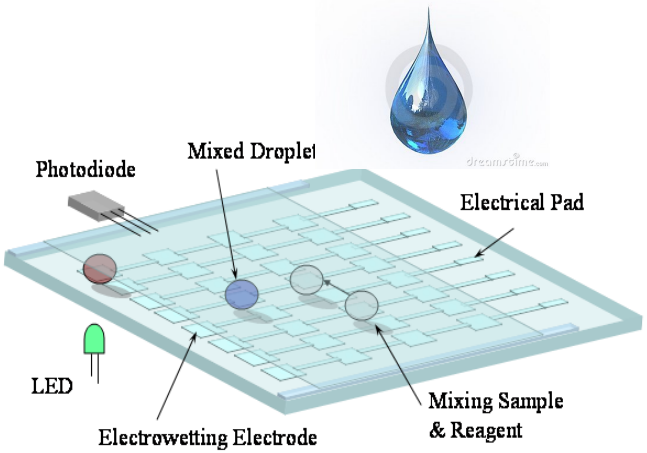
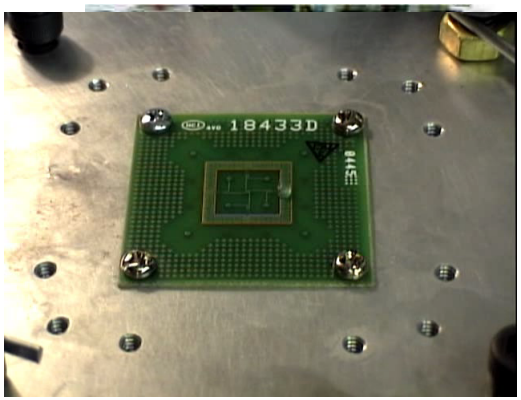
Digital Microfluidic Biochips: Towards Functional Diversity, More than Moore, and Cyberphysical Integration







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- Post-docs and collaborators: Dr. Vamsee Pamula, Dr. Michael Pollock, Prof. Richard Fair, Prof. Nan Jokerst
- Duke University's Microfluidics Research Lab
- Pioneering work at U. Toronto (A. Wheeler), UCLA (C. J. Kim), Univ. Grenoble (J. Berthier), U.C. Irvine, NTU-Taiwan (S.-K. Fang),…
- 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

Outline

- Motivation
- Technology Overview
  - Microarrays and channel-based microfluidics
  - “Digital” microfluidics: droplet-based biochips
- Design and Optimization Methods
  - Synthesis and module placement
  - Droplet routing
  - Pin-constrained design
- Cyberphysical Integration
  - Experimental demonstration
- Conclusions

Predict the Future

Motivation for Biochips

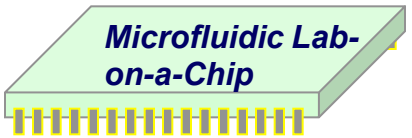
- Clinical diagnostics, e.g., healthcare for premature infants, point-of-care diagnosis
- "Bio-smoke alarm": environmental monitoring
- Massive parallel DNA analysis, automated drug discovery, protein crystallization
- *Functional diversification*, *More than Moore*

CLINICAL DIAGNOSTIC APPLICATION

Conventional Biochemical Analyzer

Shrink

Lab-on-a-chip for CLINICAL DIAGNOSTICS

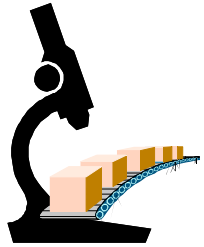


20nl sample

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

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

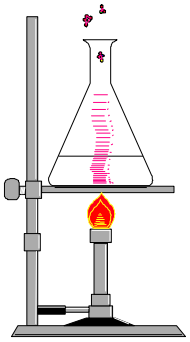
It's a miniature disposable for an
HTS - High-Throughput Screening -
(bio)analytical instrument



what does it do?

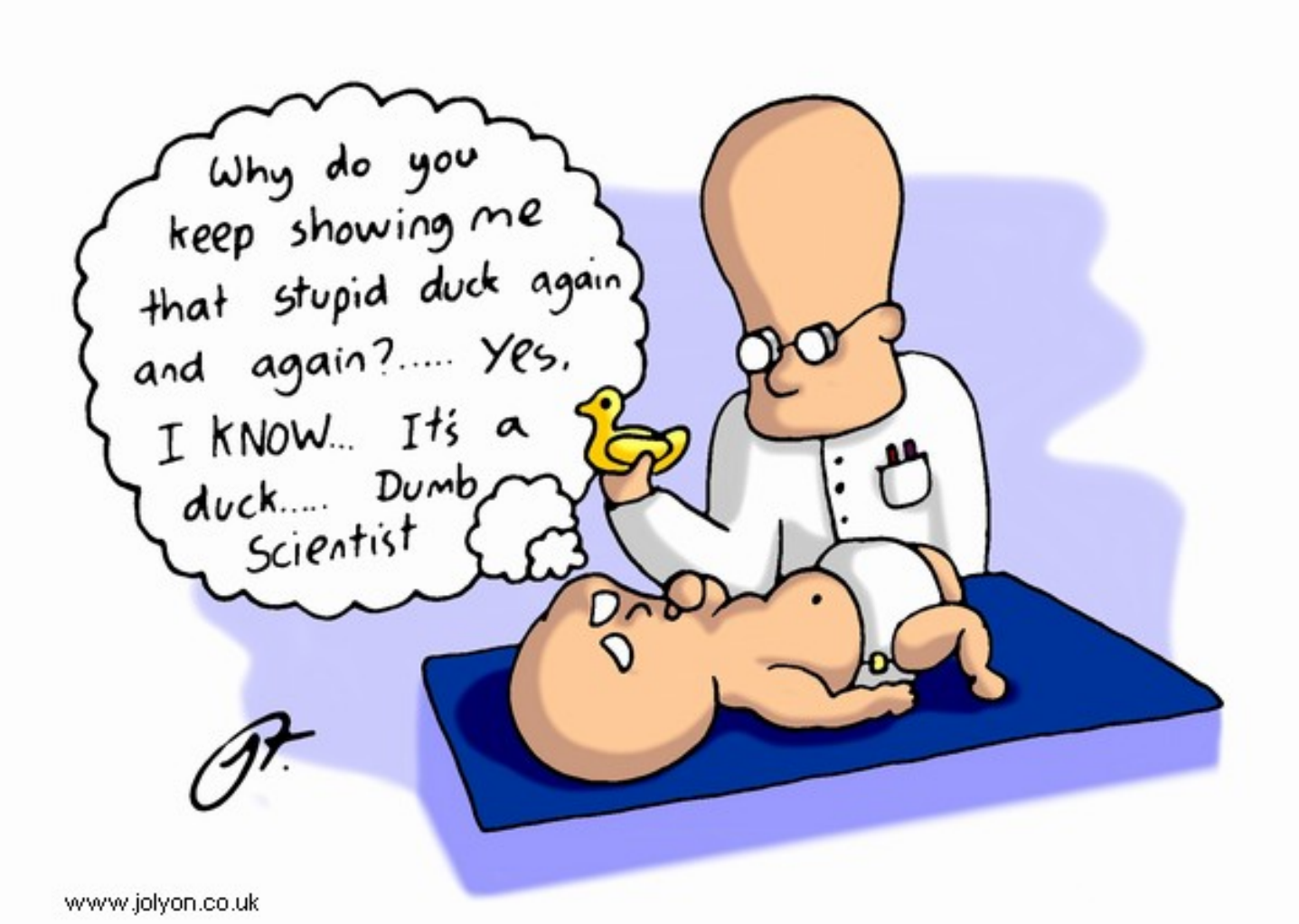
Essentially the same operations you did in high school chemistry class:

dispensing,
mixing,
detecting,
discarding,-



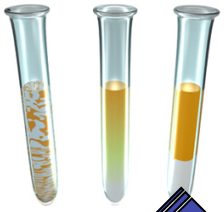
just a lot cheaper and a lot faster than you did

Why is Biochemistry-on-a-Chip Difficult?



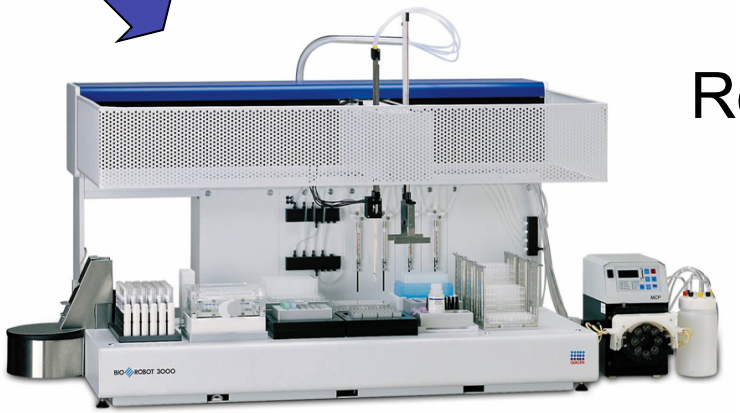
www.jolyon.co.uk

Motivation for Microfluidics



Test tubes

- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

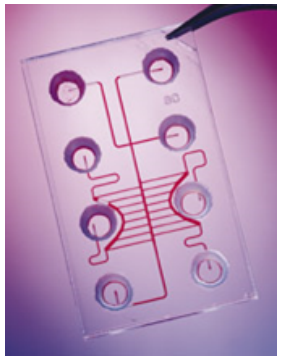


Robotics

- ☑ Automation
- ☑ Integration
- ☐ Miniaturization

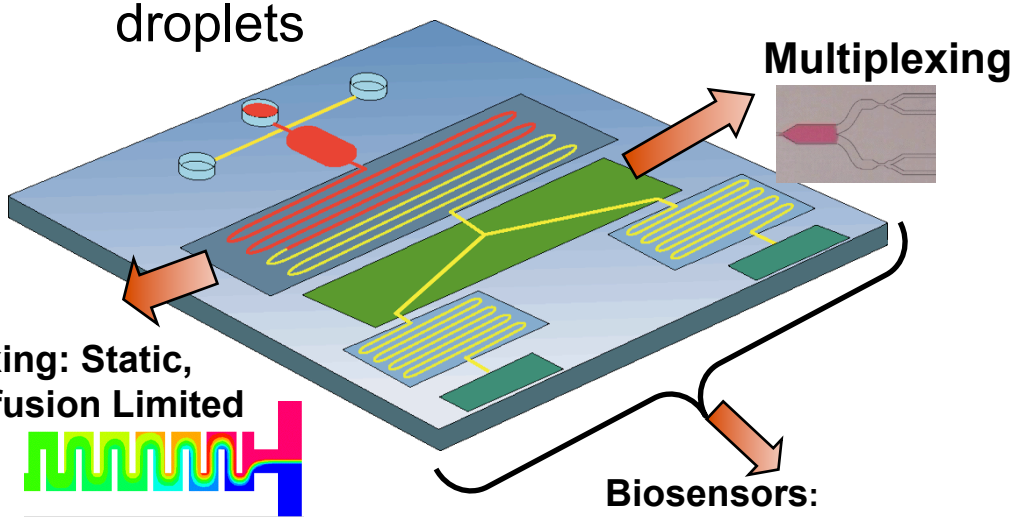
Microfluidics

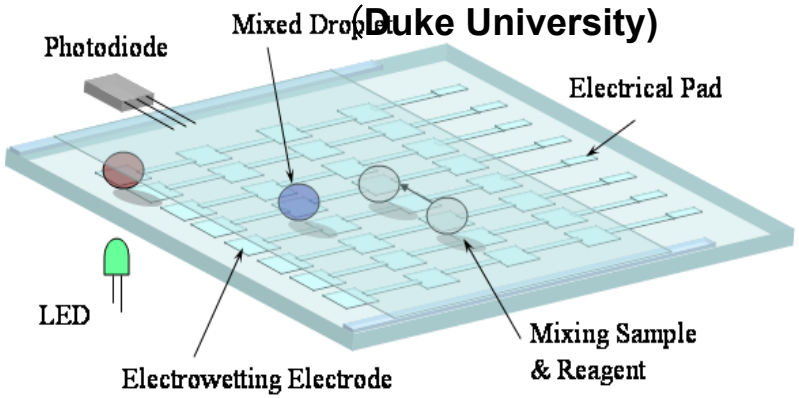
- ☑ Automation
- ☑ Integration
- ☑ Miniaturization



Microfluidics

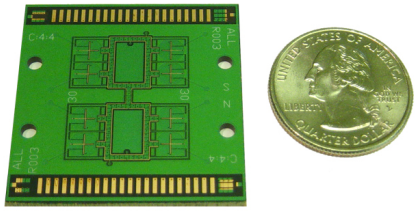
- Continuous-flow lab-on-chip: Permanently etched microchannels, micropumps and microvalves
- Digital microfluidic lab-on-chip: Manipulation of liquids as discrete droplets

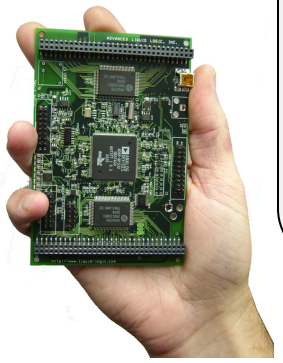




Biosensors:
Optical: SPR, Fluorescence etc.
Electrochemical: Amperometric, Potentiometric etc.

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



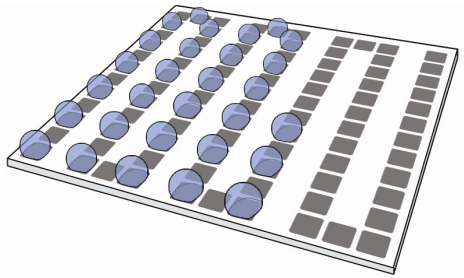


Control electronics (shown) are suitable for handheld or benchtop applications

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

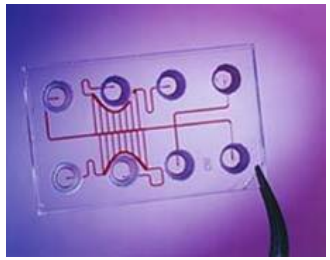


•Droplets moved in "virtual channels" defined by electrodes

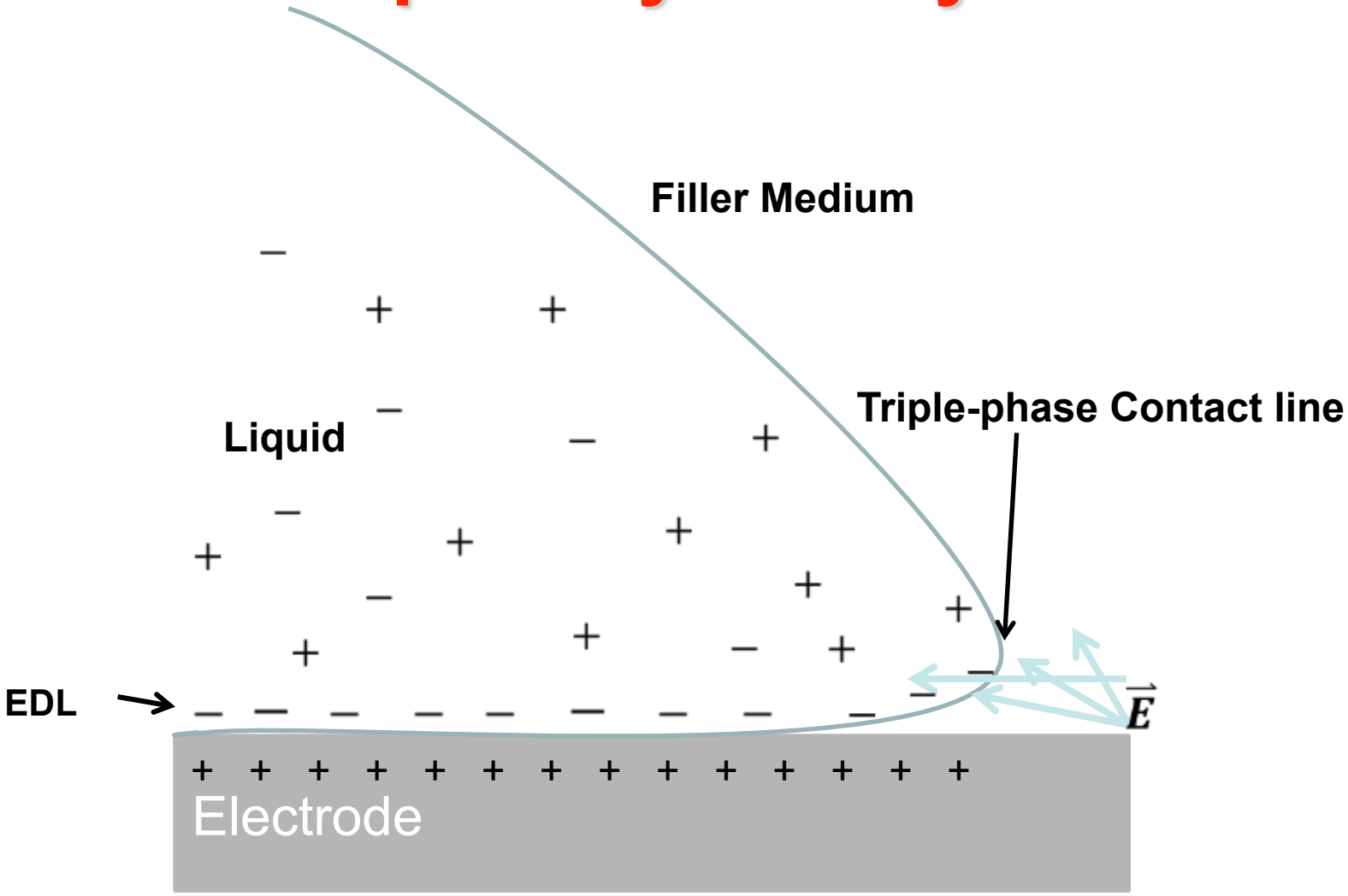
•Programmable electrodes directly control discrete droplet operations

Other Microfluidic Technologies

- Pump fluids through channels (priming and dead volumes)
- 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

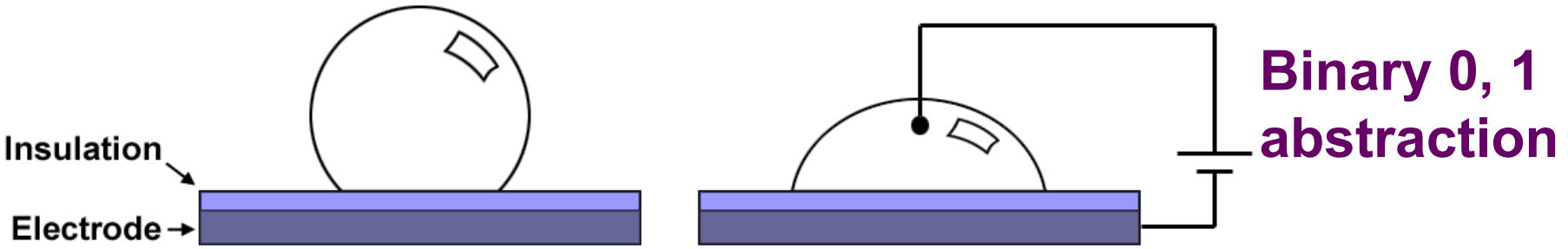


Electrocapillarity Theory



Electrowetting

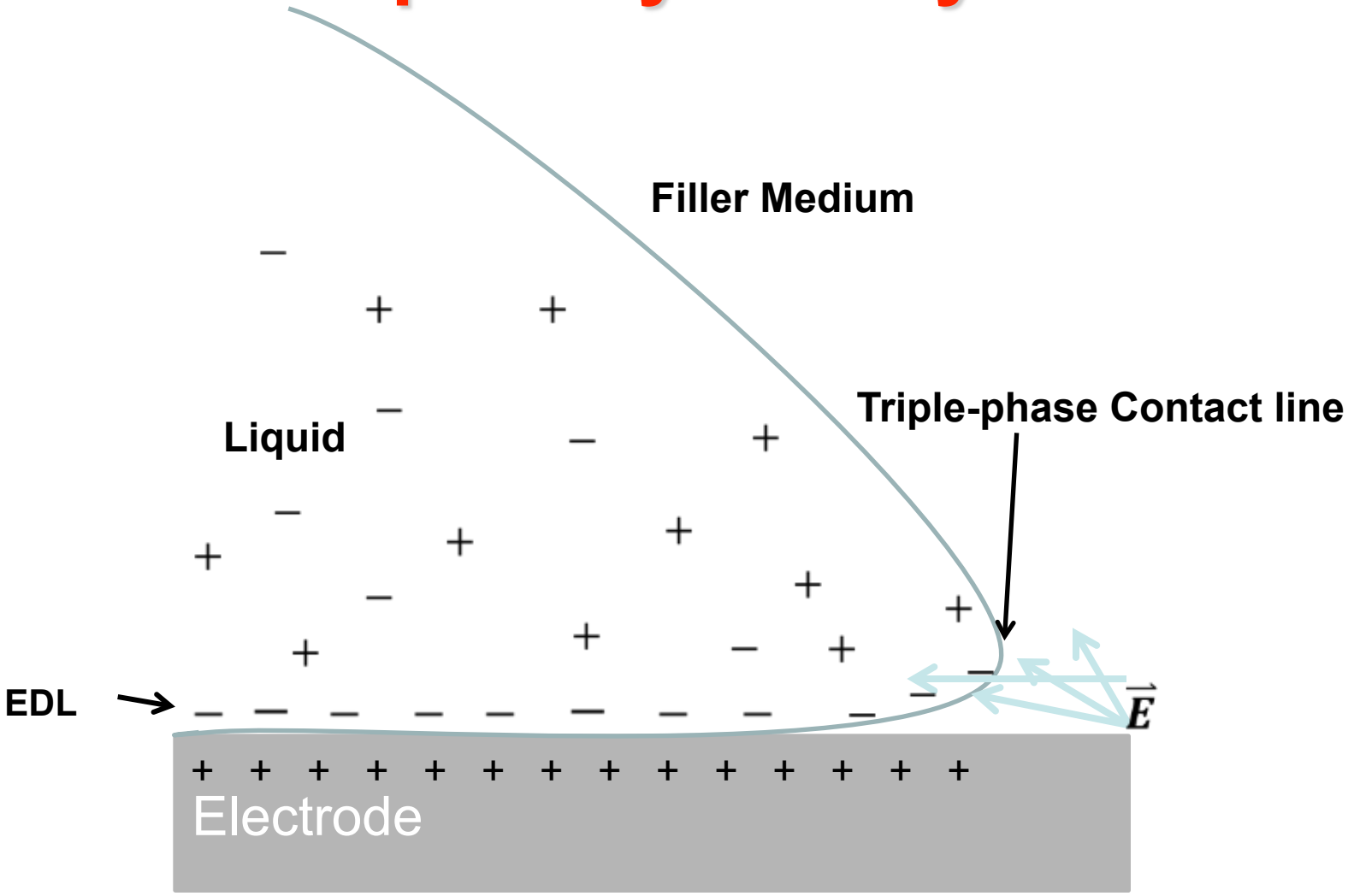
- Novel microfluidic platform invented at Duke University
- Droplet actuation is achieved through an effect called *electrowetting*
  - Electrical modulation of the solid-liquid interfacial tension



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.

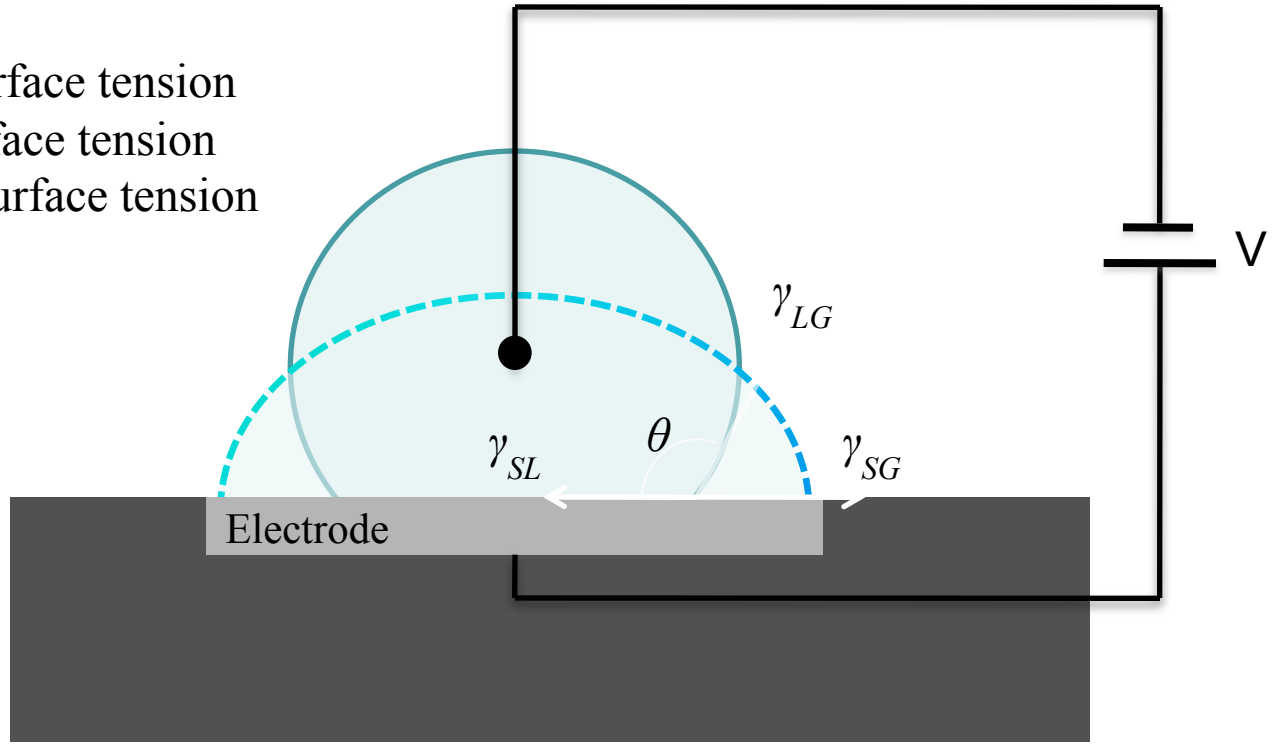
Electrocapillarity Theory



Interfacial Tensions

- γ_{LG} and γ_{SG} remain constant
- γ_{SL} varies with the applied voltage
- All three interfacial tensions always keep an equilibrium at the contact line.

γ_{LG}: liquid-gas surface tension
γ_{SG}: solid-gas surface tension
γ_{SL}: solid-liquid surface tension
Θ : contact angle



J. Berthier, *Microdrops and digital microfluidics*, 2008.

Electrowetting Theory

- Lippmann's Equation:

$$\gamma_{SL,V} = \gamma_{SL,0} - \frac{1}{2} c_E V^2$$

- Young's Equation:

$$\gamma_{SL} = \gamma_{SG} - \gamma_{LG} \cos\theta$$

- Lippmann-Young's Equation:

$$\Rightarrow \quad \cos\theta_V = \cos\theta_0 + \frac{c_E}{2\gamma_{LG}} V^2$$

– EDL cannot sustain > 1Volt

c_E: the relative capacitance per unit area between liquid and electrodes

M. G. Lippmann, *Ann. Chim. Phys.*, 1875

Electrowetting-on-Dielectric (EWD)

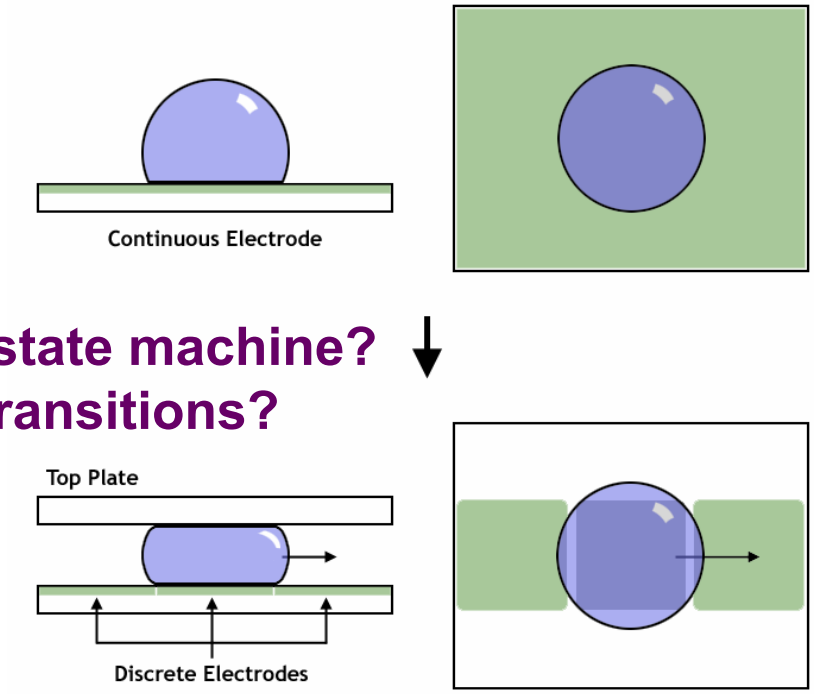
- To have significant contact angle change and avoid electrolysis
  - A thin insulating layer is needed between liquid and electrode.
  - The dielectric layer is in series with the capacitance between liquid and solid surface, and dominates.

➡ Lippmann-Young's Equation:

$$\cos\theta_V = \cos\theta_0 + \frac{\varepsilon_r \varepsilon_0}{2\gamma_{LG} t} V^2$$

What is Digital Microfluidics?

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

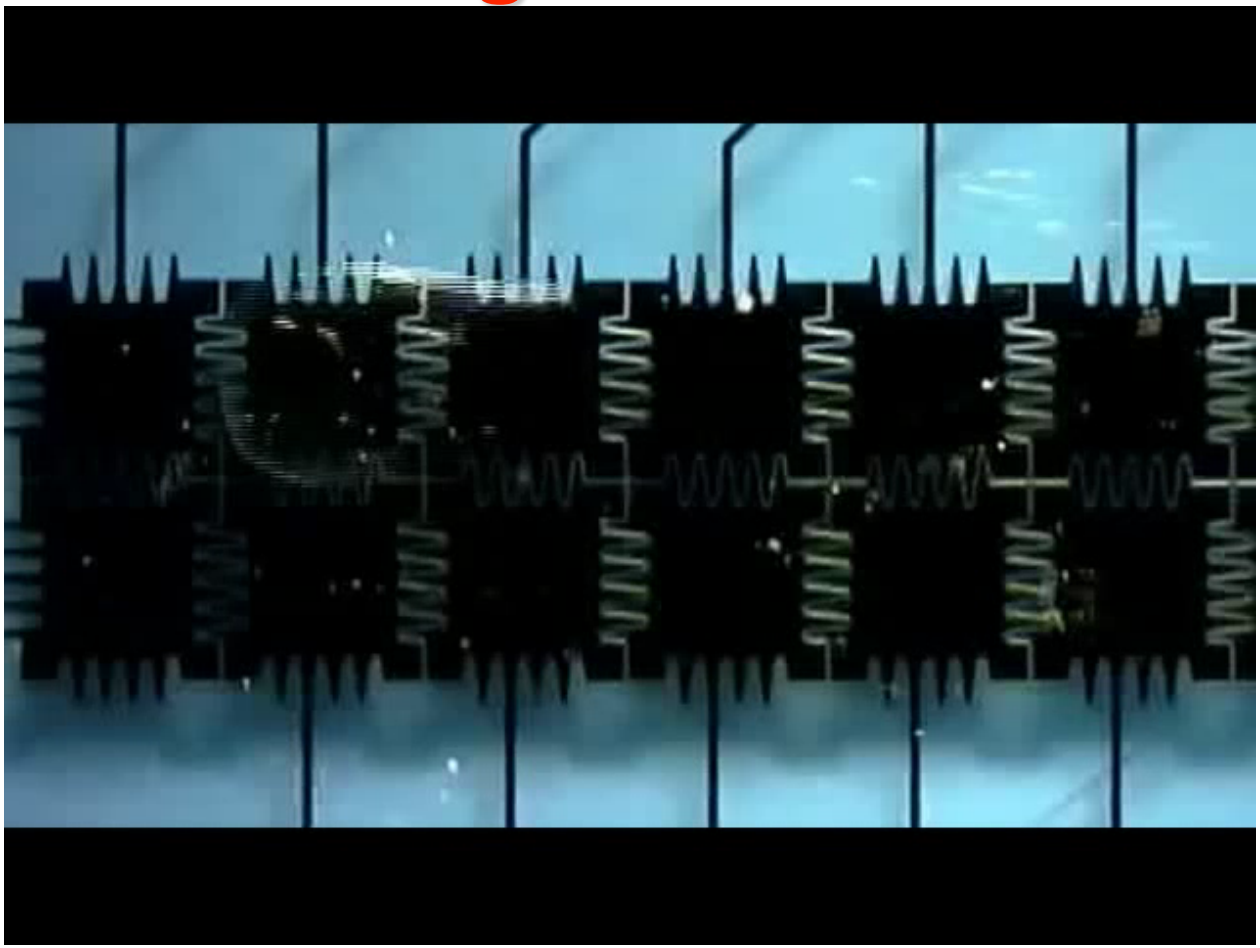


Finite-state machine?
State transitions?

Droplet Transport (Side View)

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

What is Digital Microfluidics?

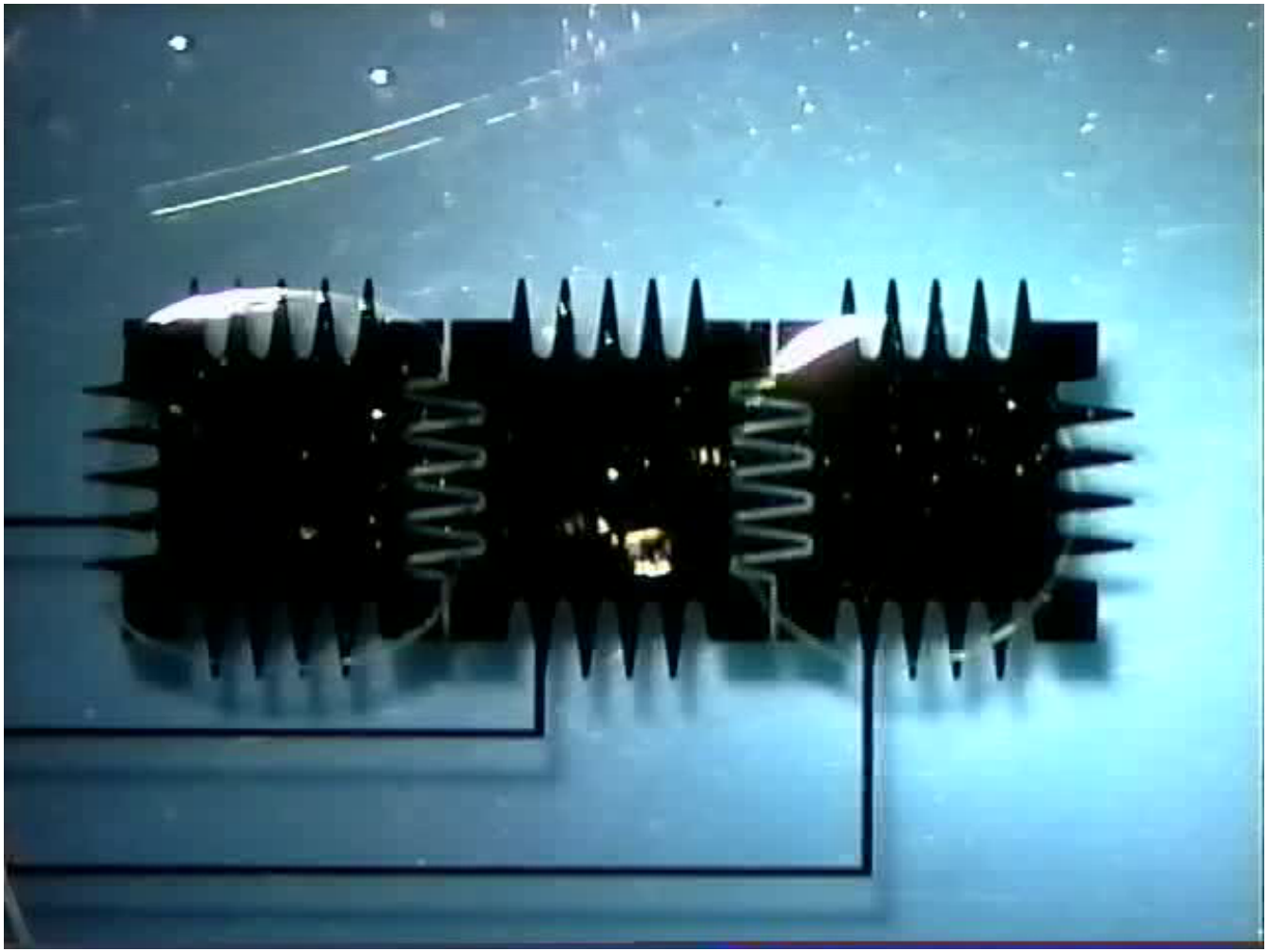


Transport
25 cm/s flow rates, order of magnitude higher than continuous-flow methods

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

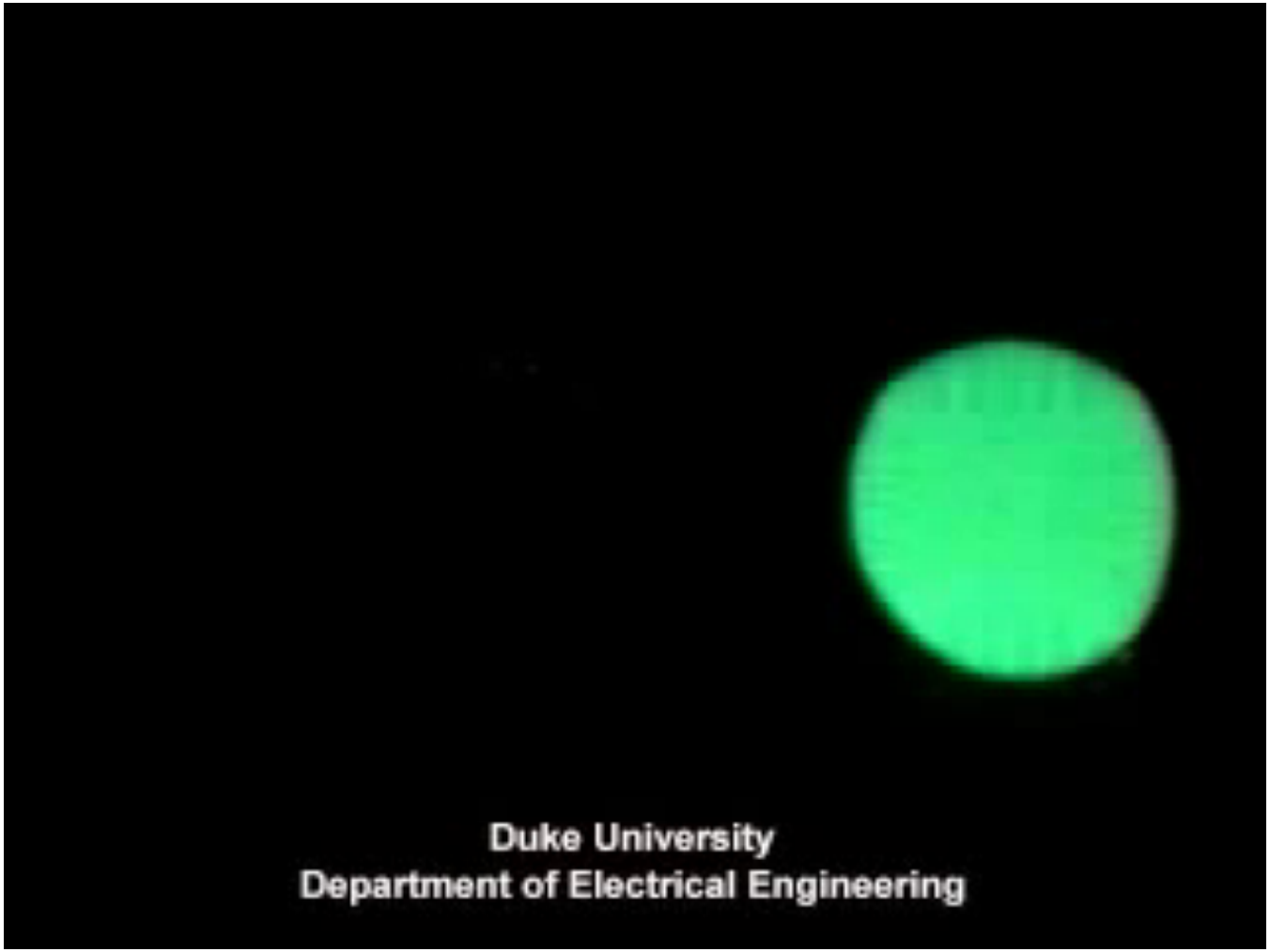
What is Digital Microfluidics?

Splitting/Merging

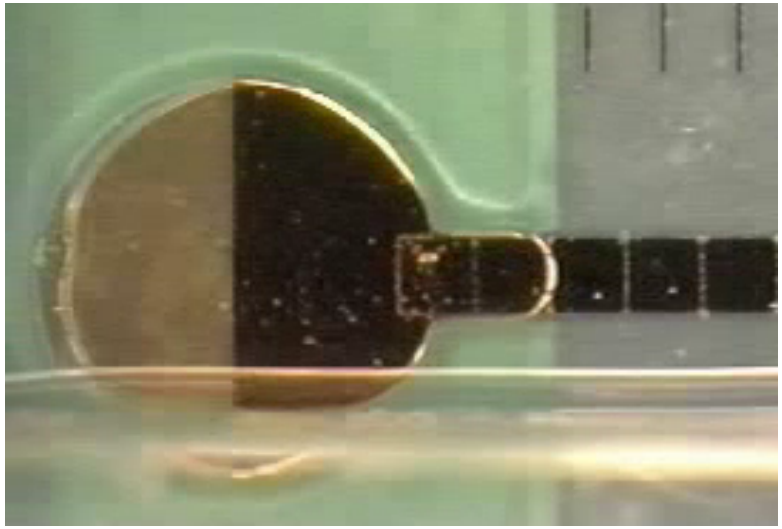


What is Digital Microfluidics?

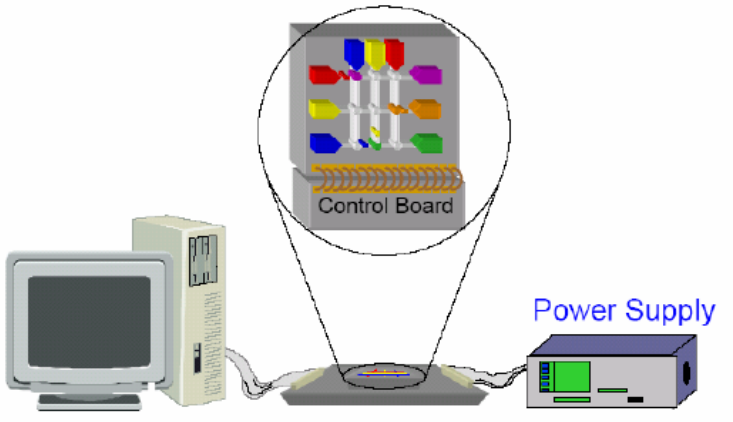
Rapid
Mixing

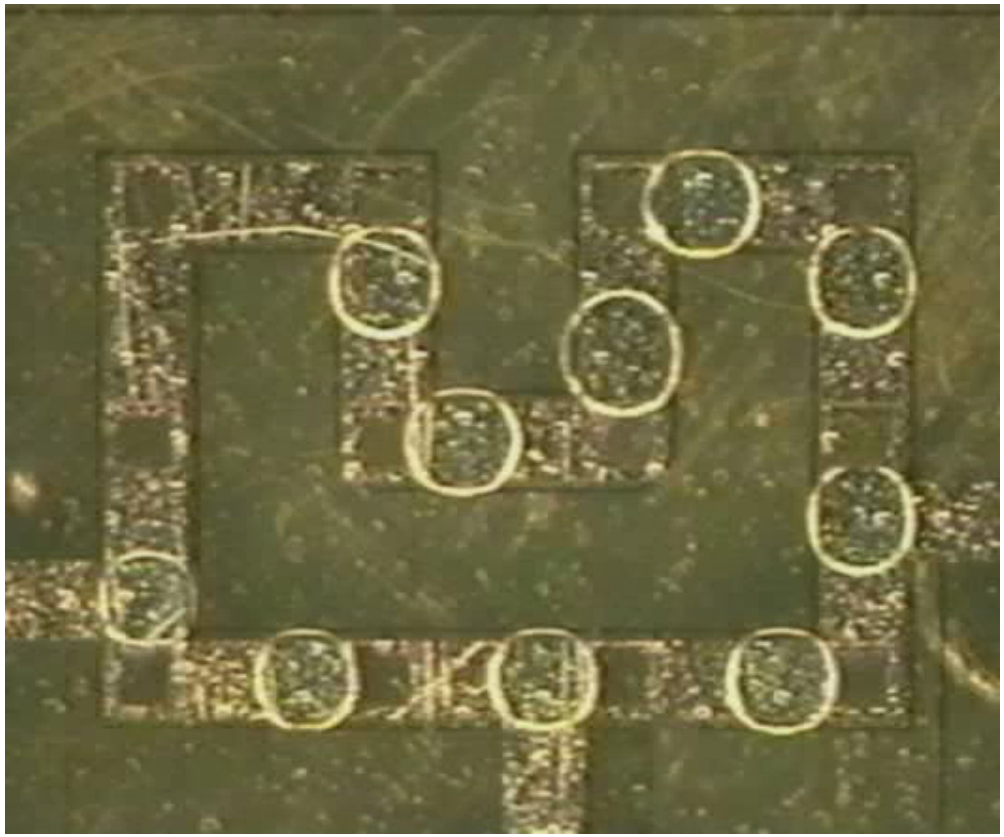


Demonstrations of Digital Microfluidics



Droplet Formation





Synchronization of many droplets

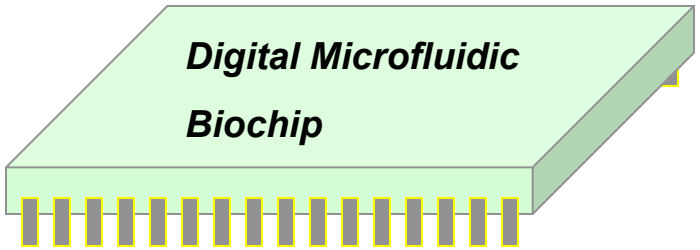
Capabilities

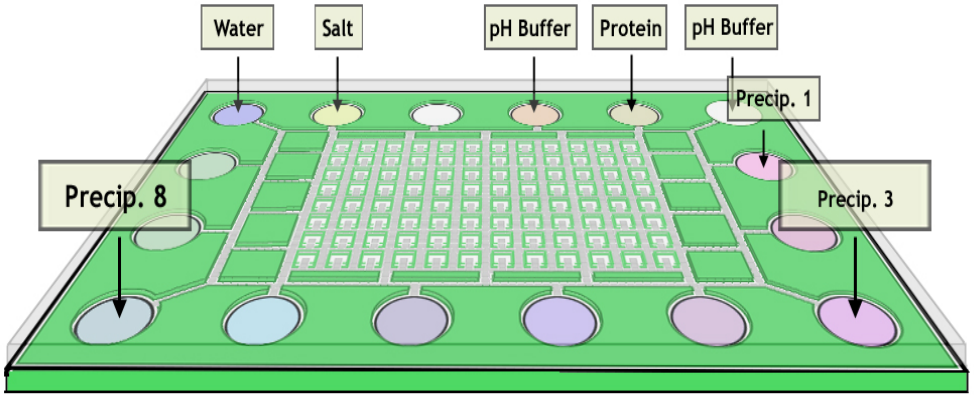
- Digital microfluidic lab-on-chip

TRANSPORT	DISPENSING	MIXERS	REACTORS	DETECTION

INTEGRATE

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

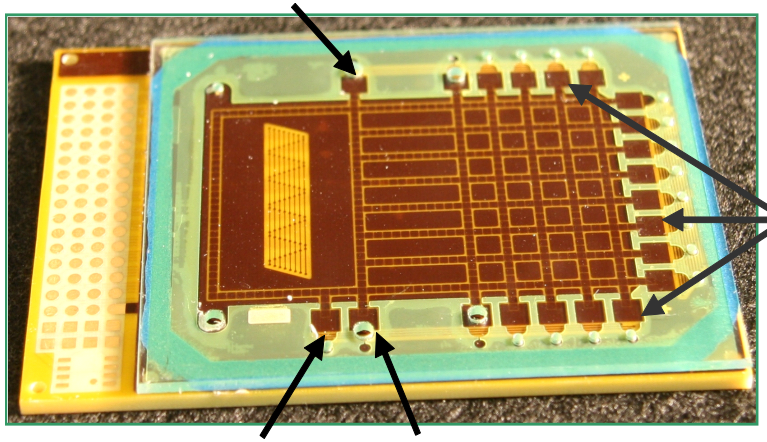




Protein crystallization chip

Commercial Biochip (Product)

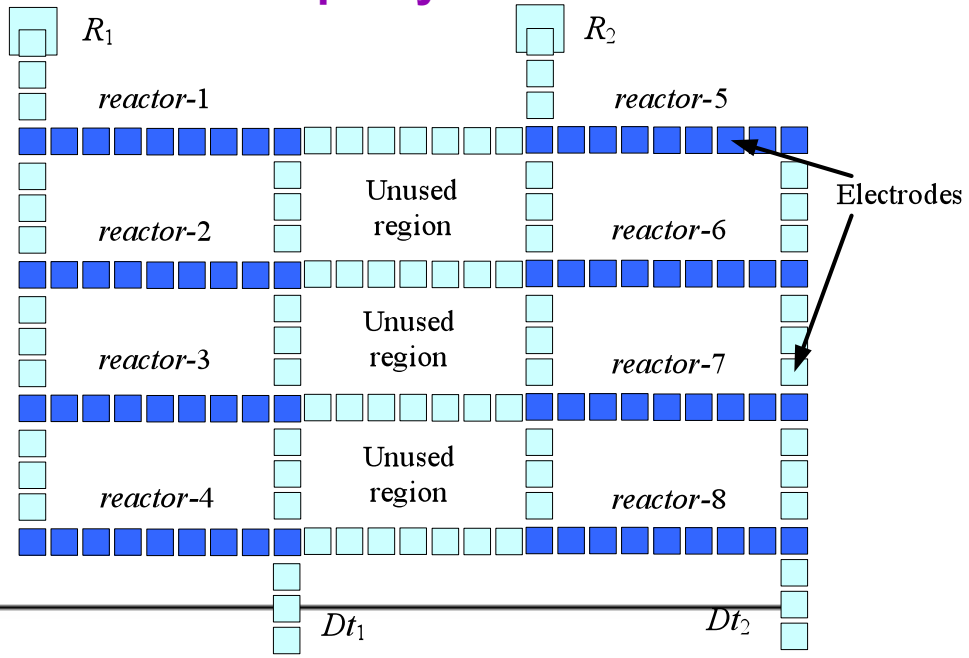
(Advanced Liquid Logic, Inc.,)

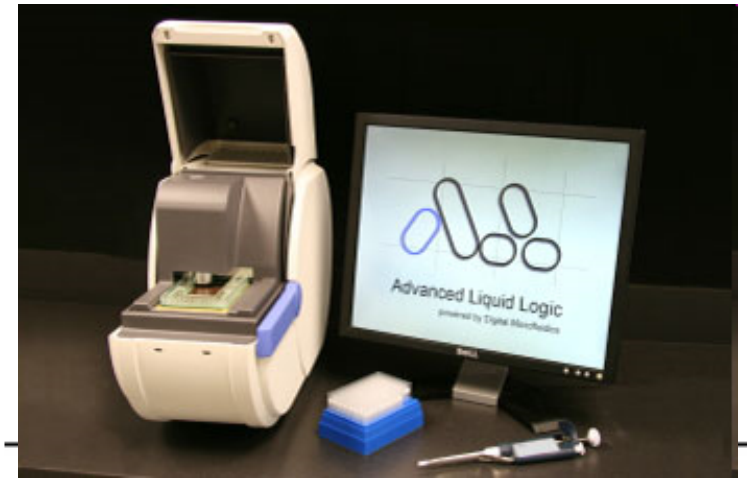


Fabricated Chip

- Pitch: 1.5 mm; Height: 0.475 mm Actuation voltage: 50 V
- Fabricated on PCB board
- 1140 electrodes, 64 pins
- Used for *n*-plex bioassay

Part of Chip Layout

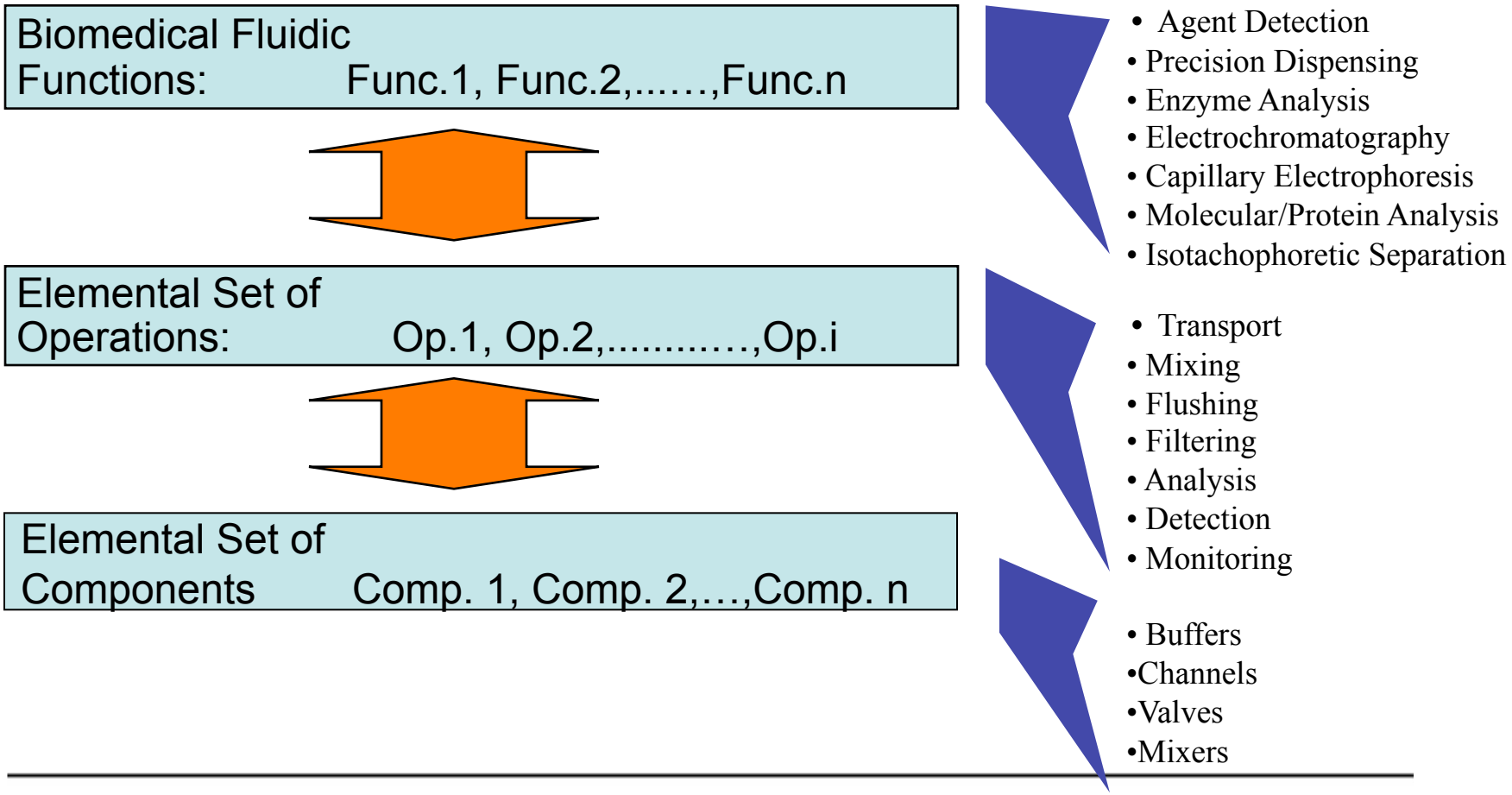




Application Platform

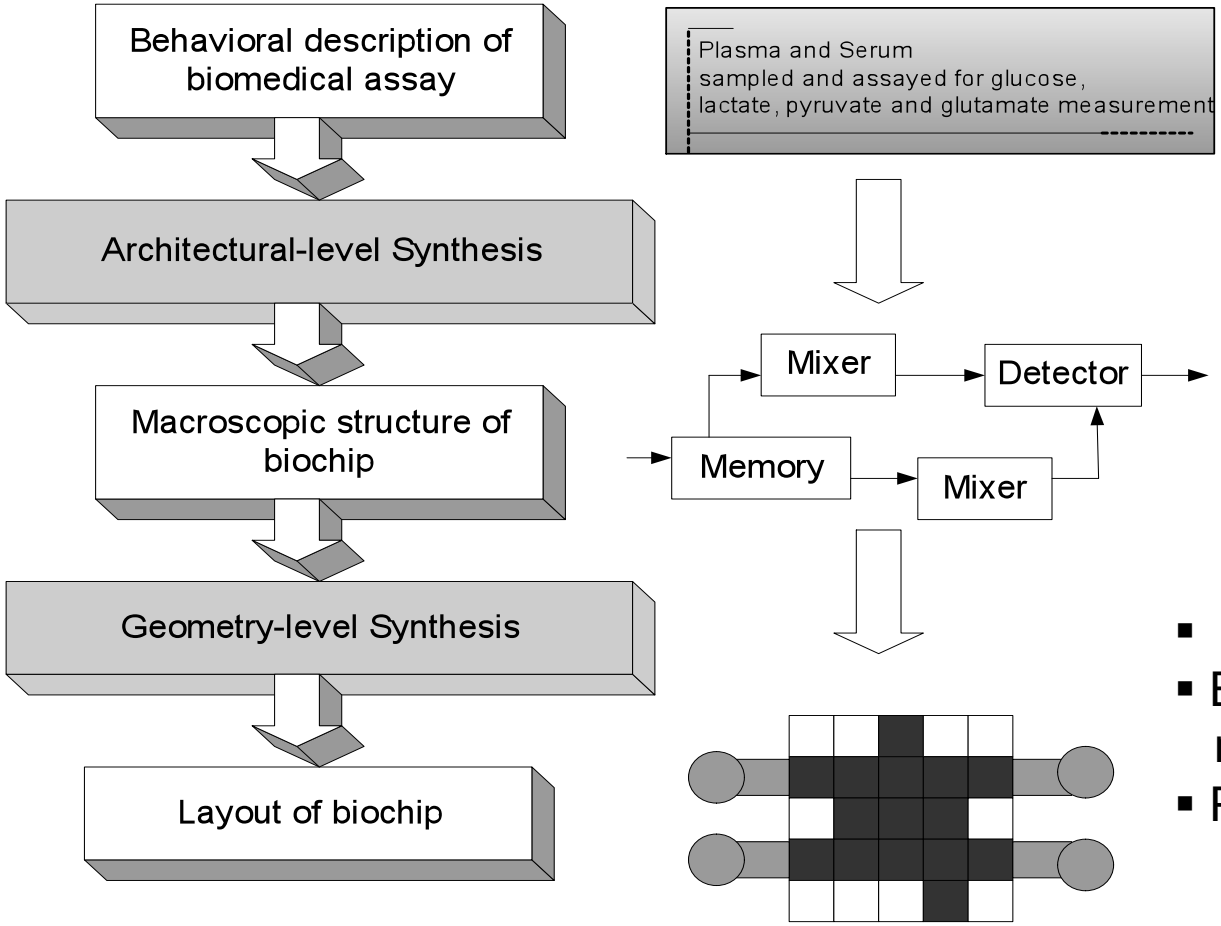
Manageable Design Approach

- Diverse biotechnology functions provide requirements for microfluidic architecture



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

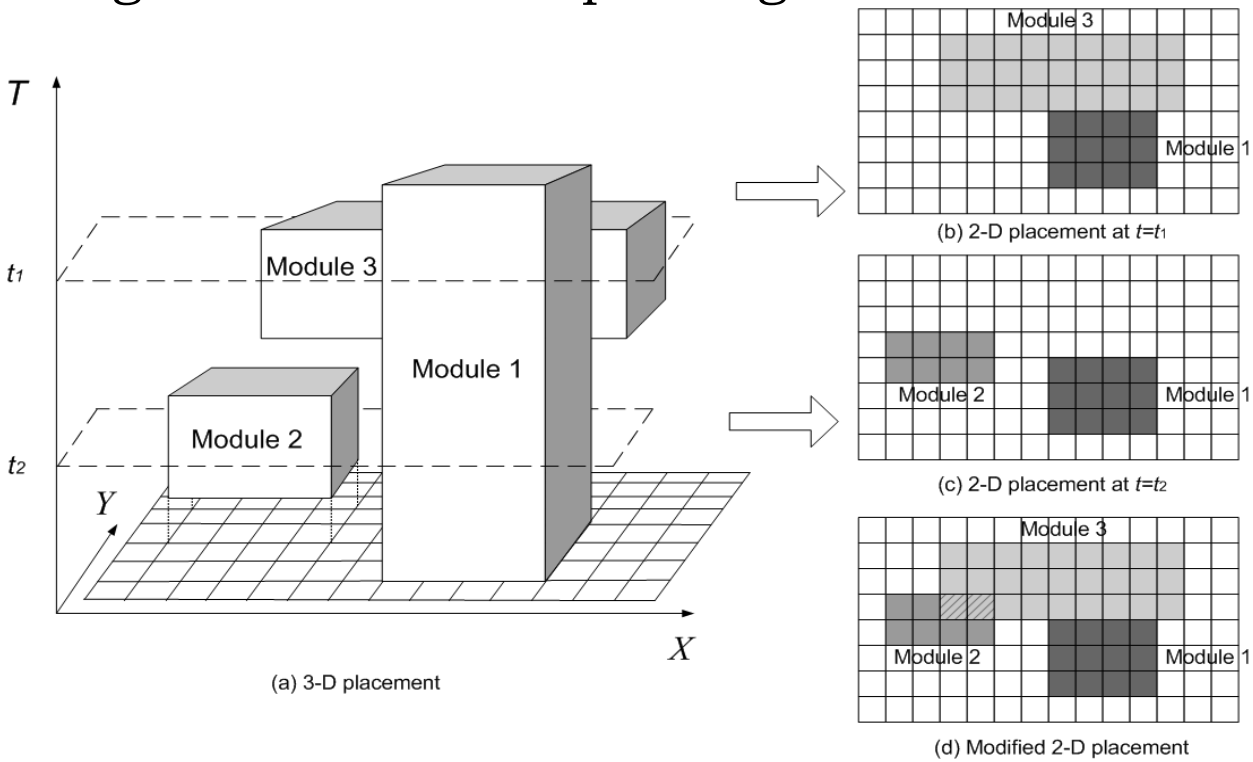
Assay1: Glucose assay,
Assay2: Lactate assay,
Assay3: Pyruvate assay,
Assay4: Glutamate assay

*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

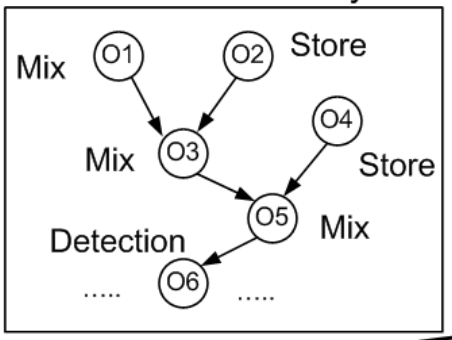
- Placement determines the locations of each module on the microfluidic array in order to optimize some design metrics
- High dynamic reconfigurability: module placement → 3-D packing → modified 2-D packing



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

On-Chip Biochemistry Synthesis

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

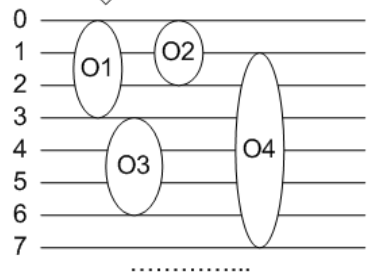
Unified Synthesis of Digital Microfluidic Biochip

Output:

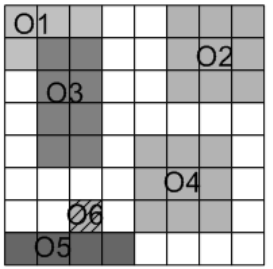
Resource binding

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



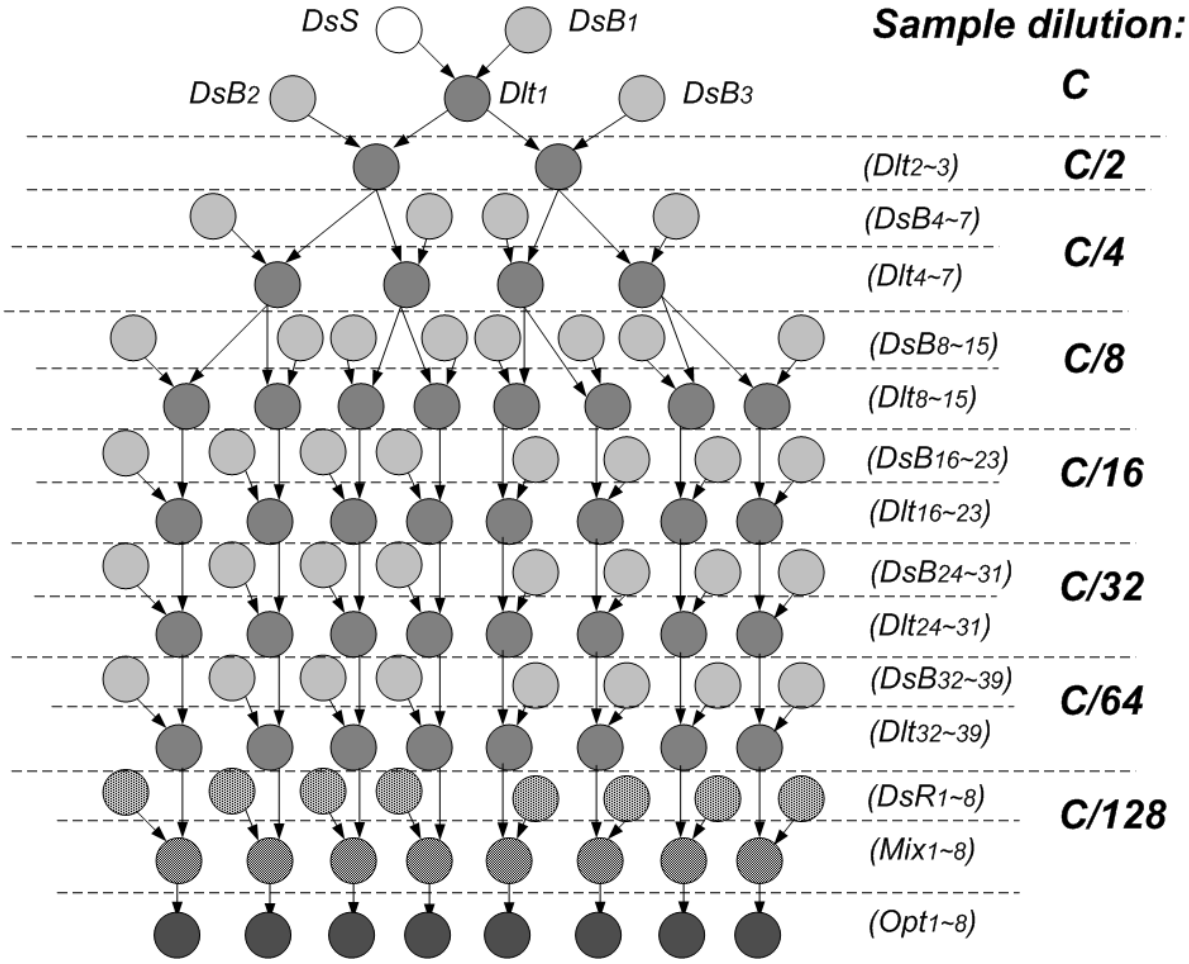
Placement



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

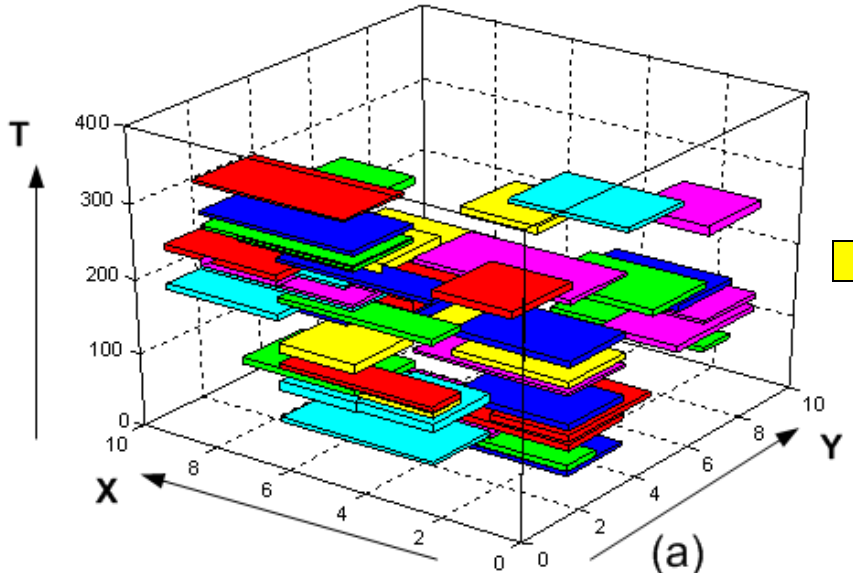
Protein Assay

Sequencing graph model



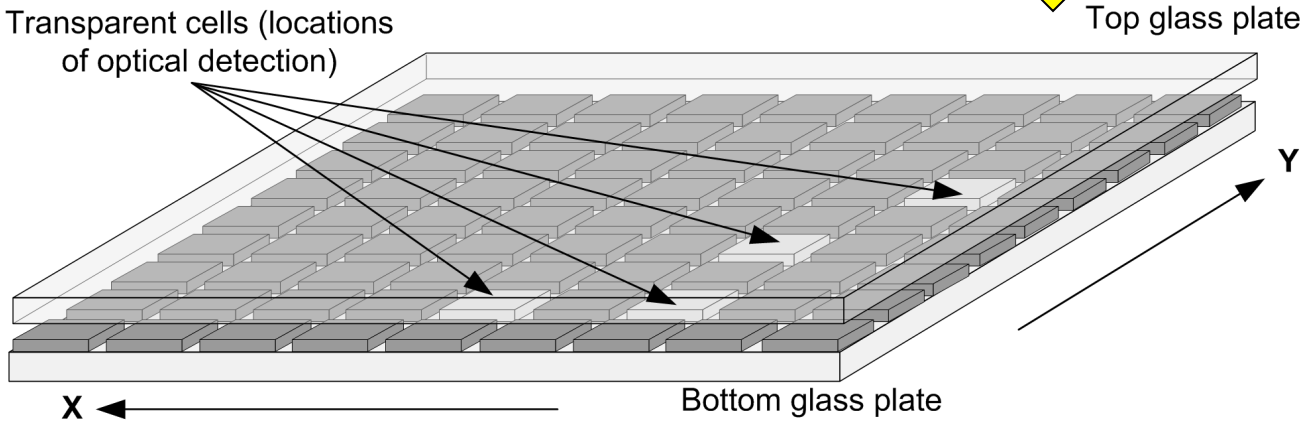
- Maximum array area: *10x10*
- Maximum number of optical detectors: *4*
- Reservoir counts: *1 for sample; 2 for buffer; 2 for reagent; 1 for waste*
- Maximum bioassay time: *400 s*

Synthesis Results



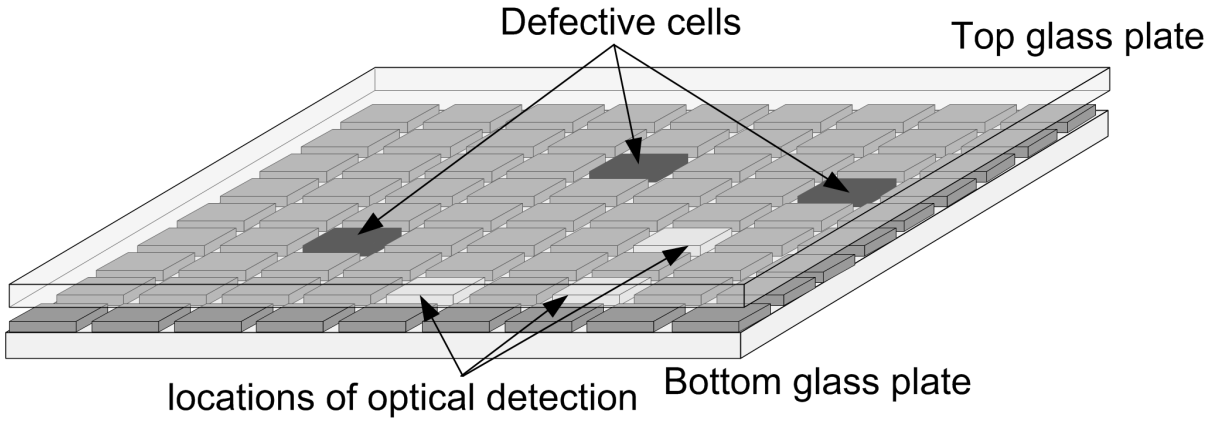
Bioassay completion time *T*: 363 seconds

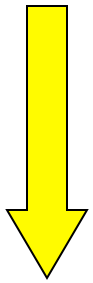
Biochip array: 9x9 array

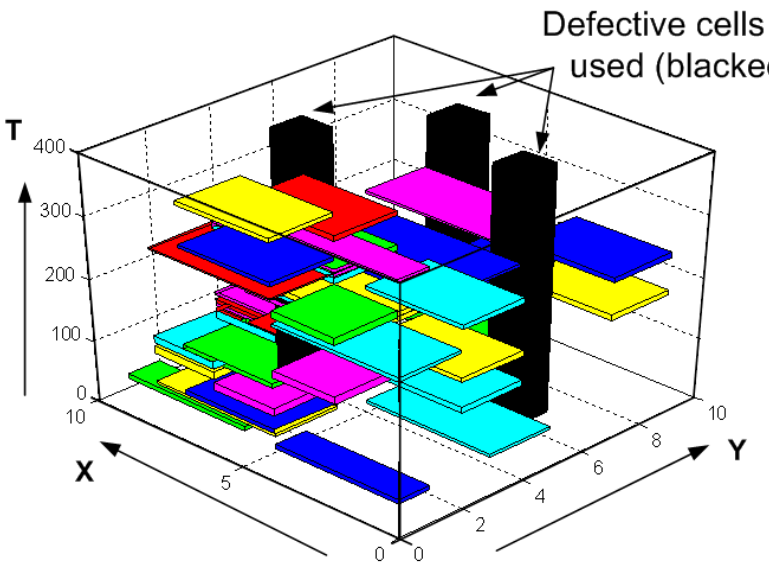


Experimental Evaluation (Cont.)

- Defect tolerance







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

Droplet Routing

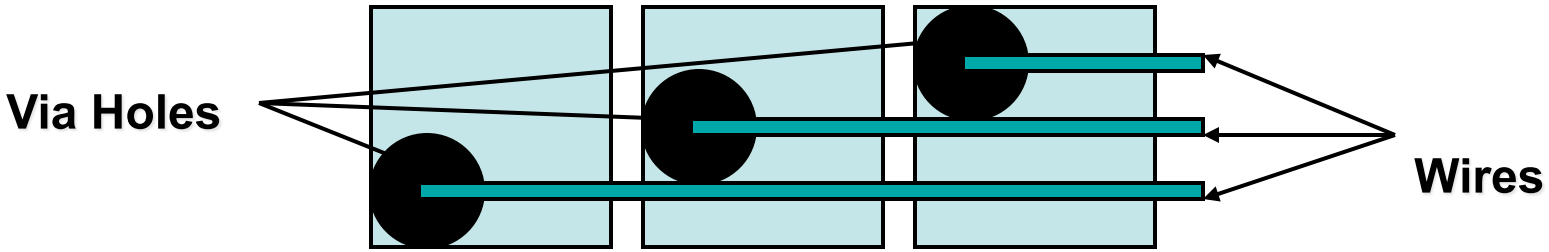
- design problem fo dic
- ts from architectu s and ent:
  - let pathways using t the ay; these routes are plets les, or between mod orts on-chip reservoirs)
- routes with minim he minimization of th VLSI routing
- Need to satisfy critical constraints
  - A set of fluidic constraints
  - Timing constraints: (delay for each droplet route does not exceed some maximum value, e.g., 10% of a time-slot used in scheduling)

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

- **Cross-referencing**

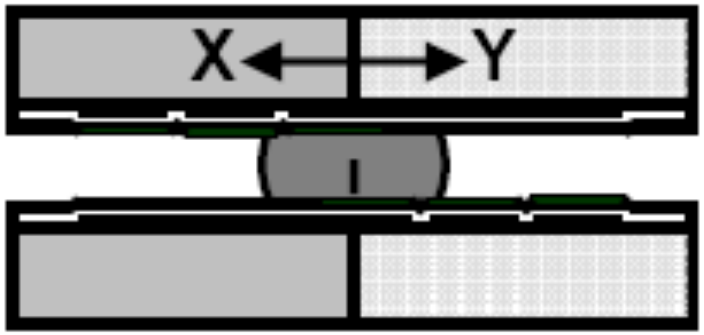
  Orthogonally placed pins on top and bottom plates

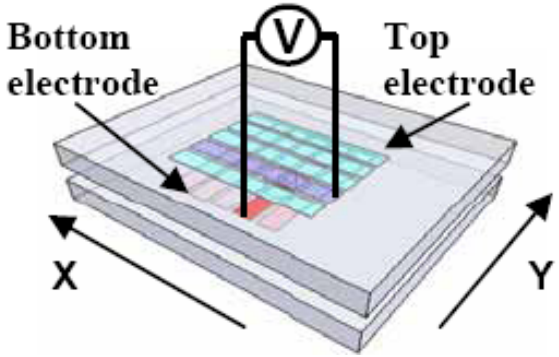
  Advantage

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

  Disadvantage

  Suffers from *electrode interference*



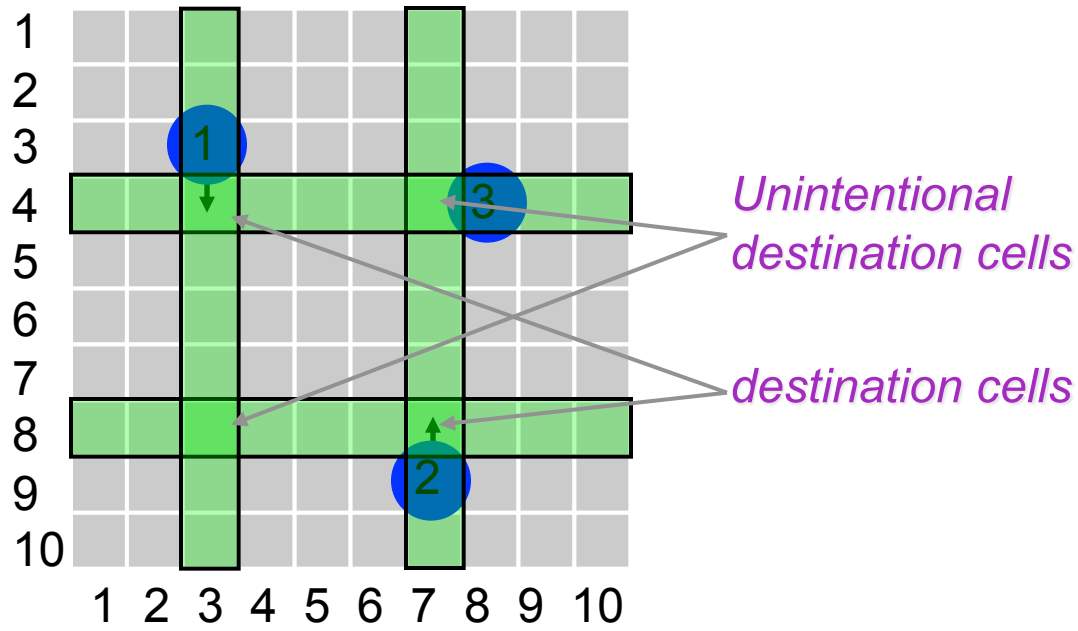


Electrode Interference

- **Unintentional Electrode Actuation**

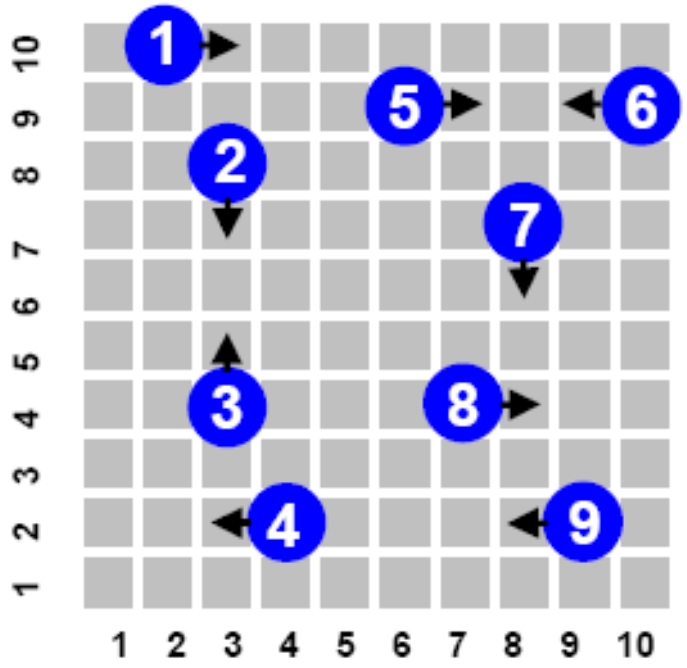
  Selected column and row pins may intersect at multiple electrodes

- **Unintentional Droplet Manipulation**



Efficient Droplet manipulation Method

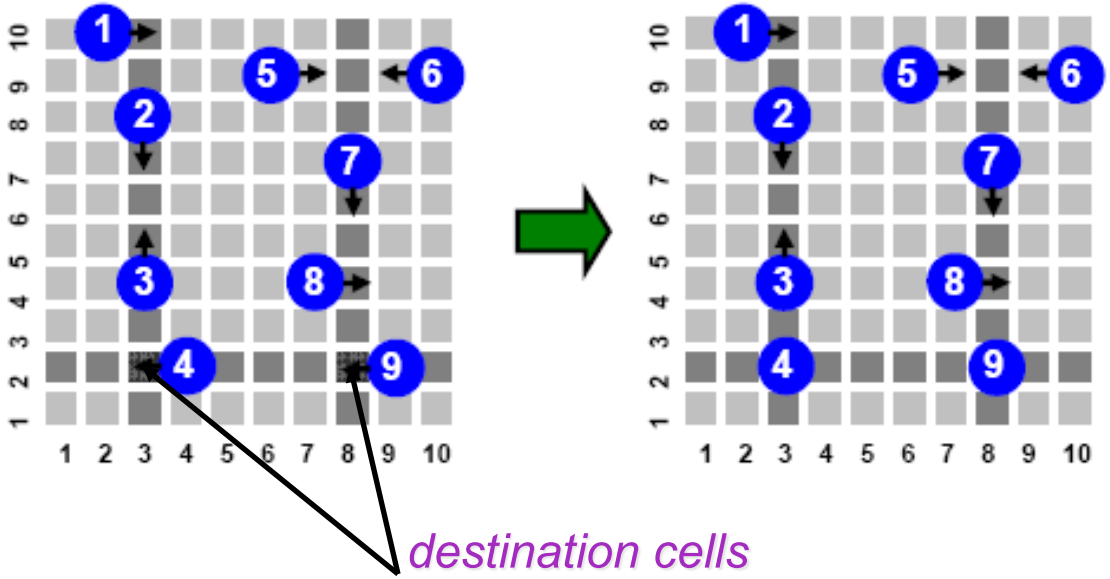
- **Goal**
  - Improve droplet manipulation concurrency on cross-referencing-based biochips.



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

Efficient Droplet Manipulation Method

- **Observation**
  - Droplet manipulations with *destination cells* in same column/row can be carried out without electrode interferences



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

Goal: Find the optimal grouping plan (minimum number of groups)

Efficient Droplet Manipulation Method

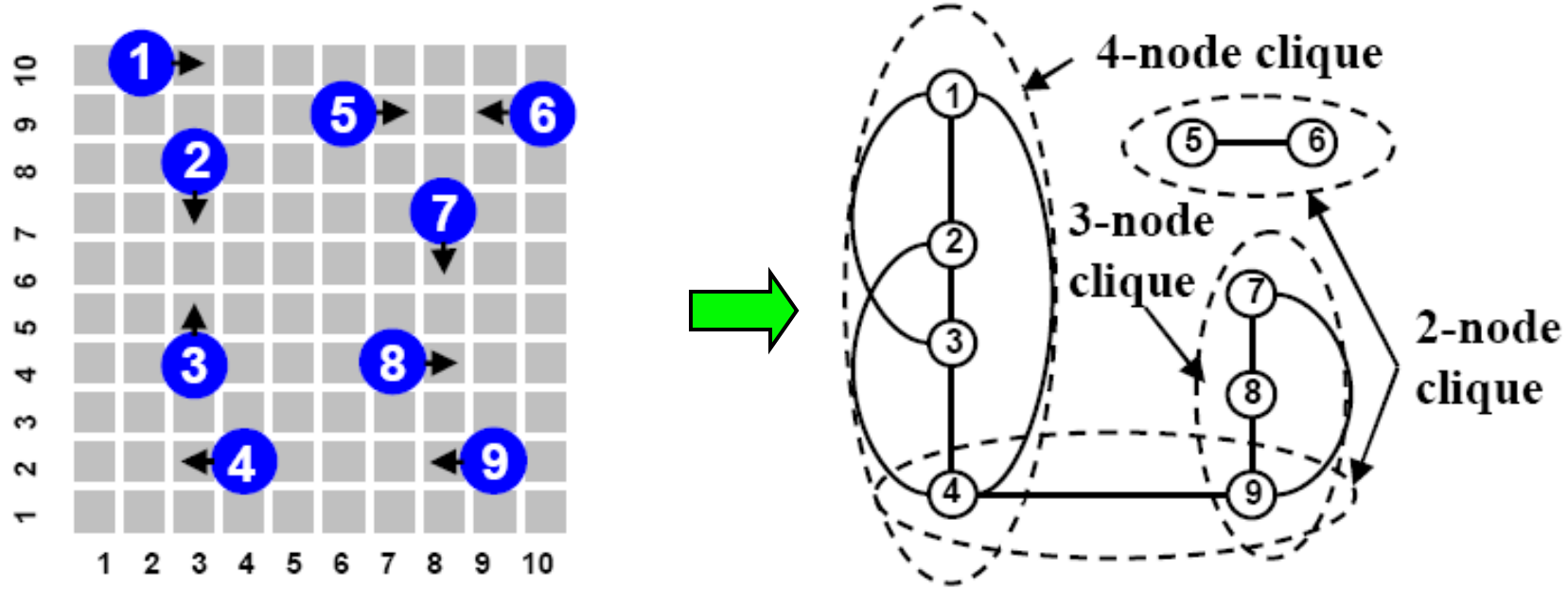
- **Problem formulation**

  Destination cells → Nodes

  Destination cells in one column/row → a Clique

  Grouping → Clique partitioning

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



Broadcast Electrode-Addressing

- **Observation**

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

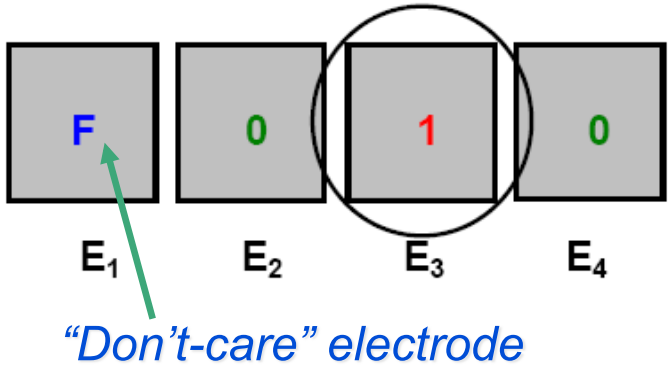
  Electrode control inputs: 3 values

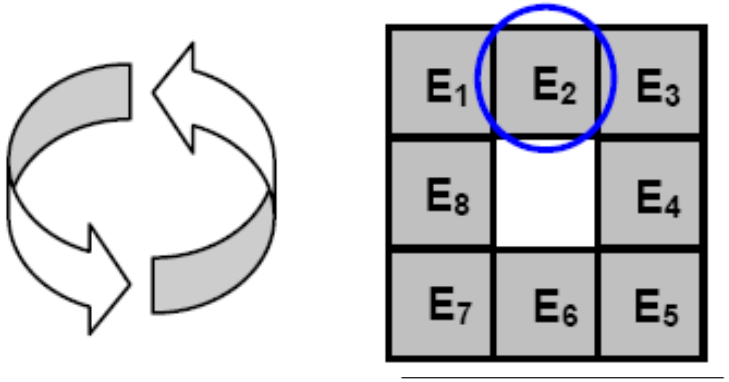
  "1" –- activated

  "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

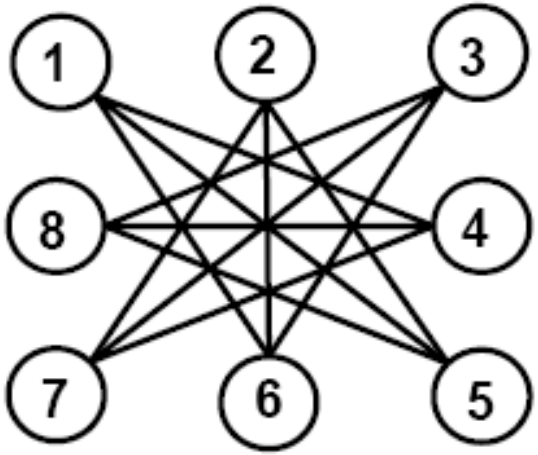
- **Idea**
  - Combining compatible sequences to reduce # of control pins
- **Clique partitioning based method**

  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



Theoretical Results (General-Purpose Biochips)

- An *m x n* digital microfluidic array
- *M* pins
- A lower bound on *M* is given by:

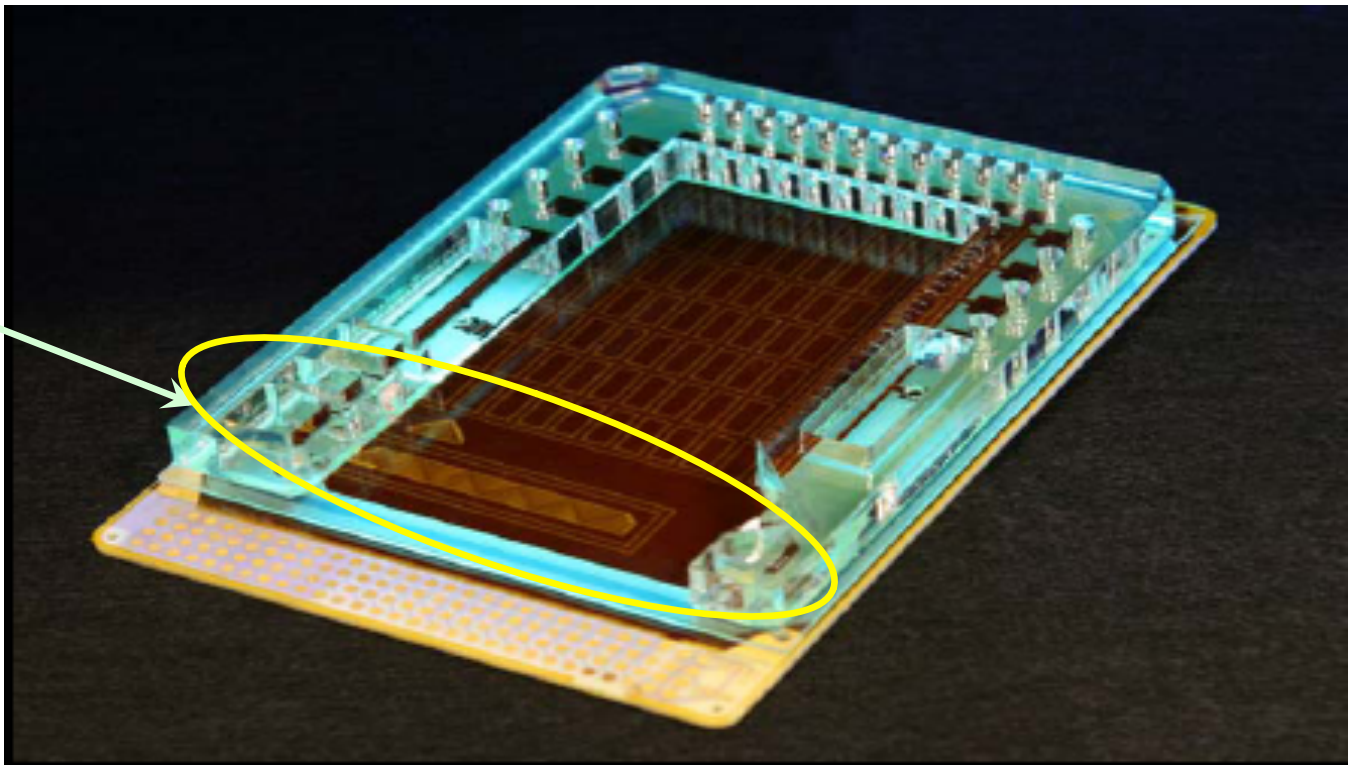
$$\binom{M}{2} \geq 6mn - 5m - 5n + 2$$

- For large values of *m* and *n*: $M_{min} \approx 2\sqrt{3mn}$
- If *m* = *n* = *t*: $M_{min} \approx 2\sqrt{3}t$

Case Study

- Fabricated platform
  - 1140 electrodes*; 64* input pins; 12 reactors
- 3-plex assay: diagnosis of acute myocardial infarction
  - Sample: serum
  - Assays: troponin-I, myoglobin, and creatine kinase-MB

Detection Region

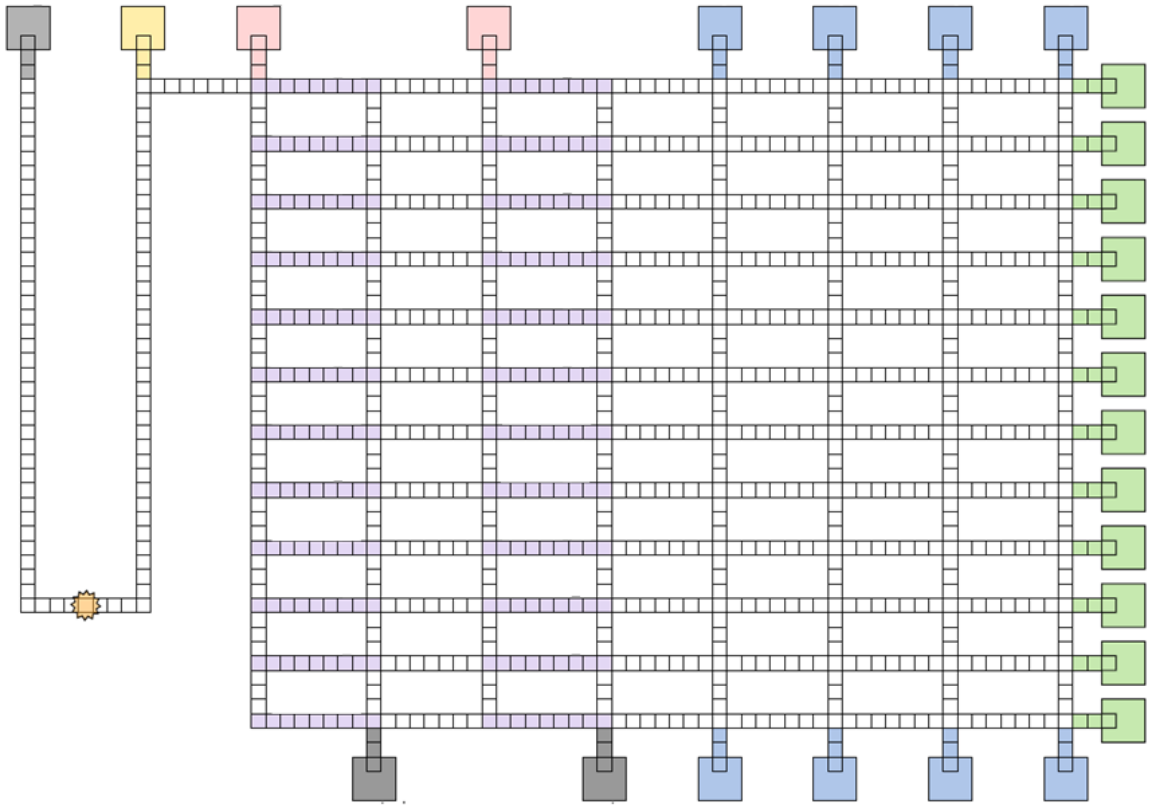


(Product of Advanced Liquid Logic, Inc.)

Evaluation using Commercial Chip

- Commercial chip for *n*-plex immunoassay: 1140 electrodes, 64 input pins;
  - Three regions: routing, reaction, and detection

Reaction and detection region Routing region

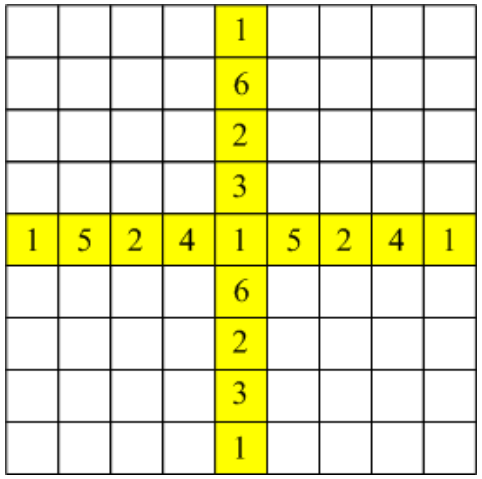


Objective: Minimize number of pins and time needed for droplet movement

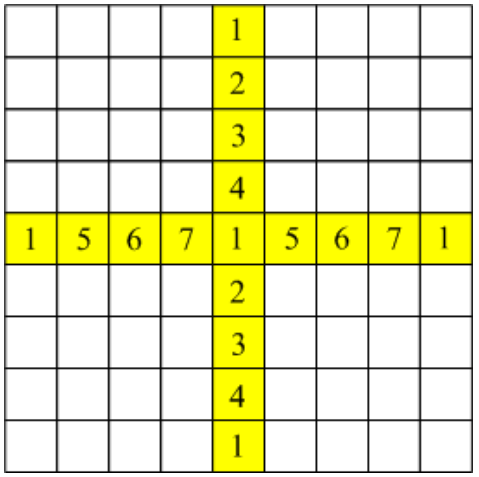
Evaluation using Commercial Chip

- Compare proposed ILP model with baseline design

Routing region: New pin-assignment using ILP (6 pins)

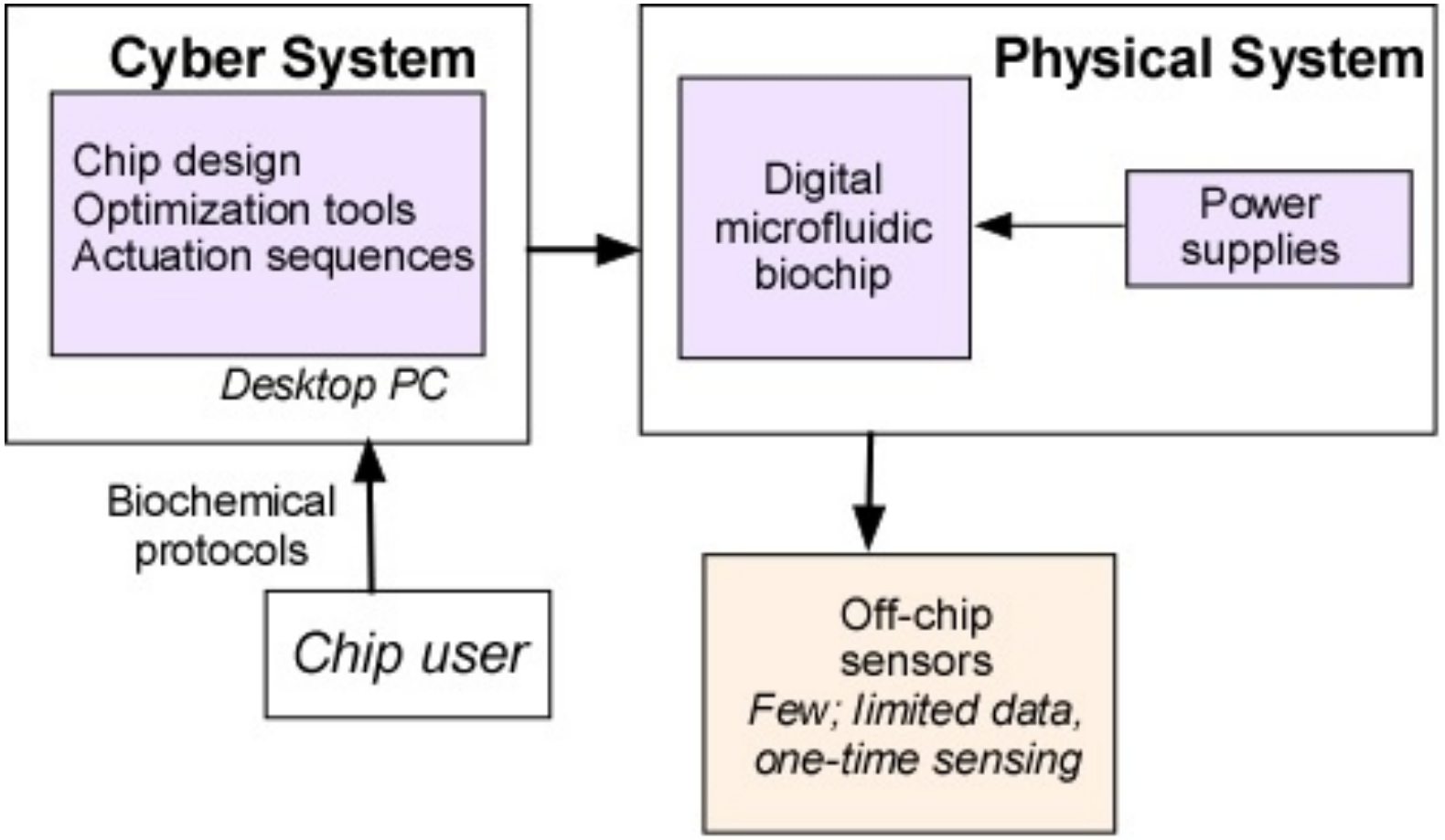


Routing region: Existing pin-assignment (7 pins)



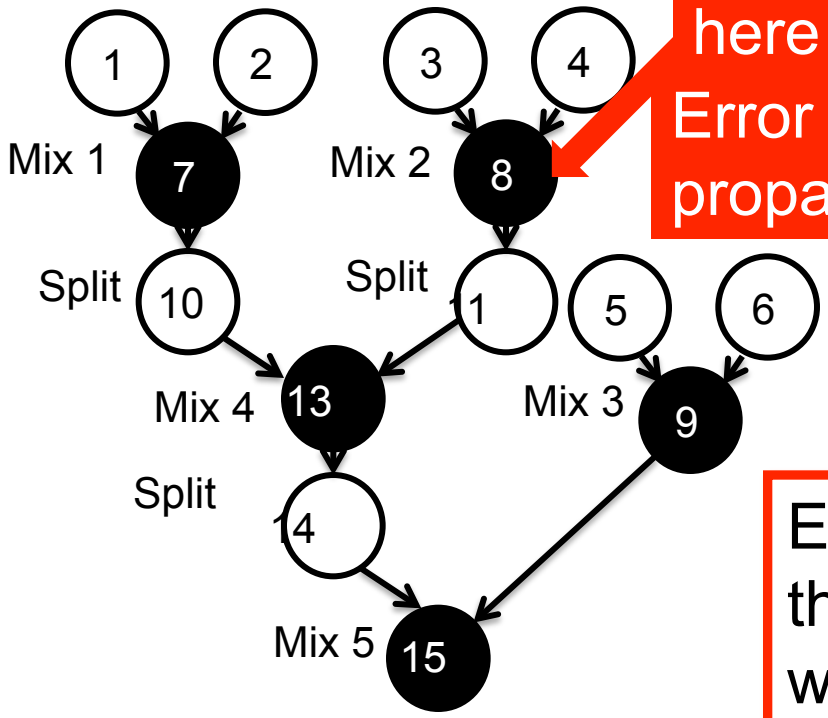
	No. pins (existing design)	No. pins (ILP model)
Routing region	7	6
Reaction region	19	13
Detection region	8	4
Total	34	23

Today's Digital Microfluidic Biochips



Errors and Error Propagation

Error propagation:

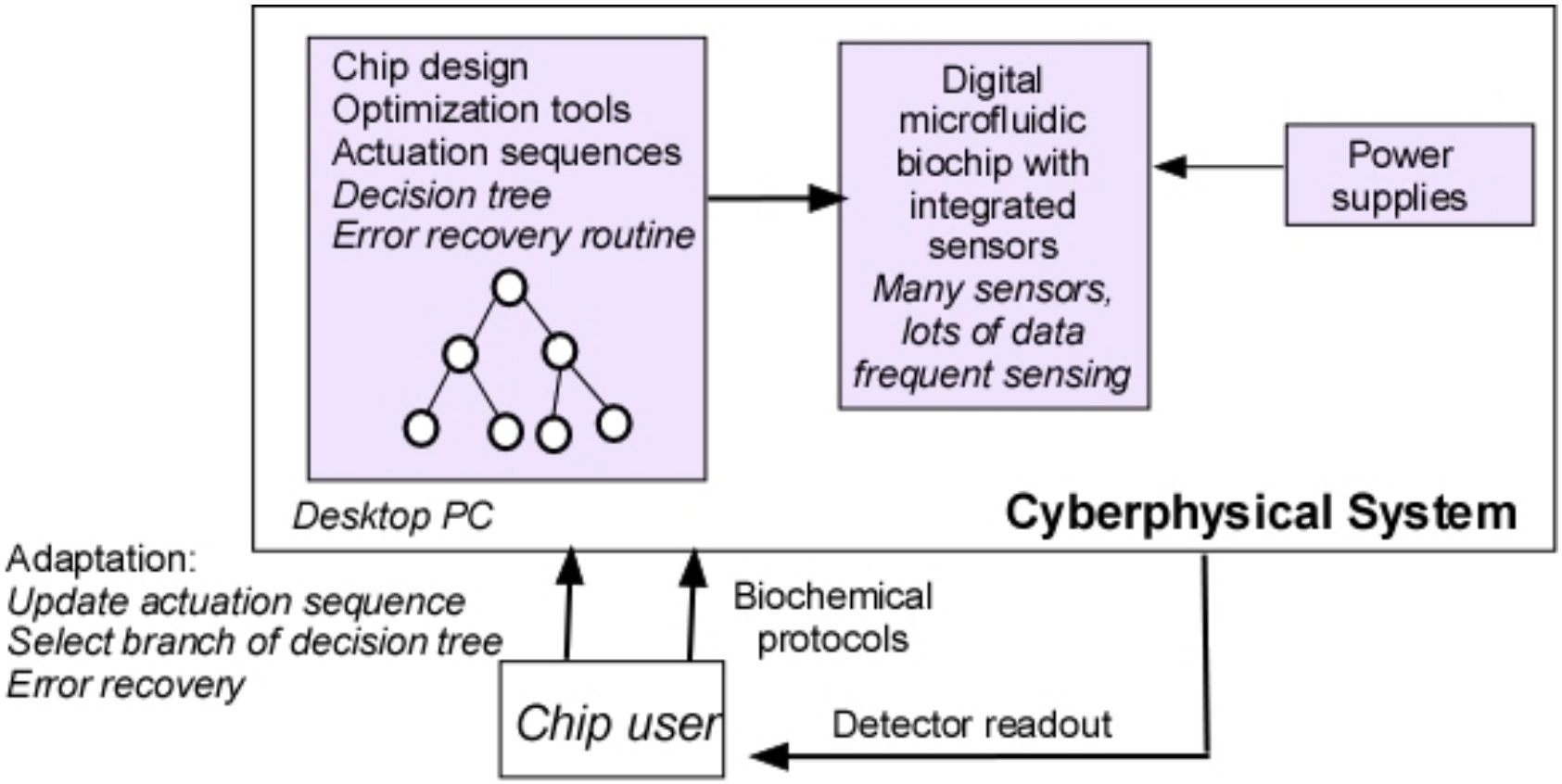


An error occurs here

Error propagates

Error propagates to the output, and the whole bioassay has to be re-executed!

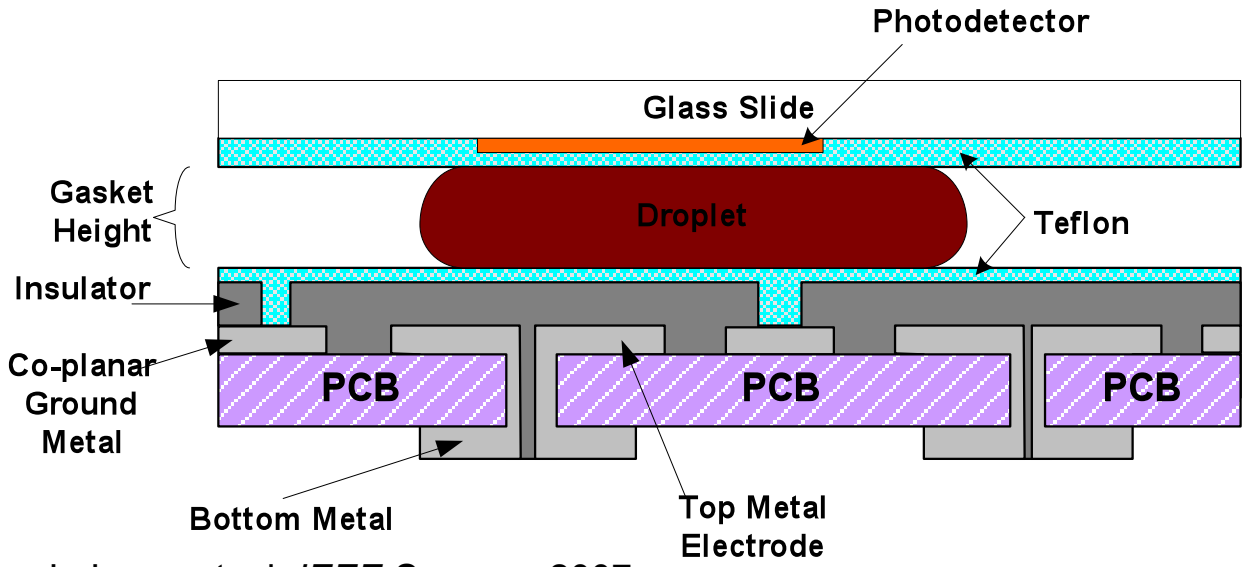
Tomorrow's Digital Microfluidic Biochips



Integrated Photodetectors in Digital Microfluidic Platforms

Structure of Integrated Photodetector in a Digital Microfluidic Platform

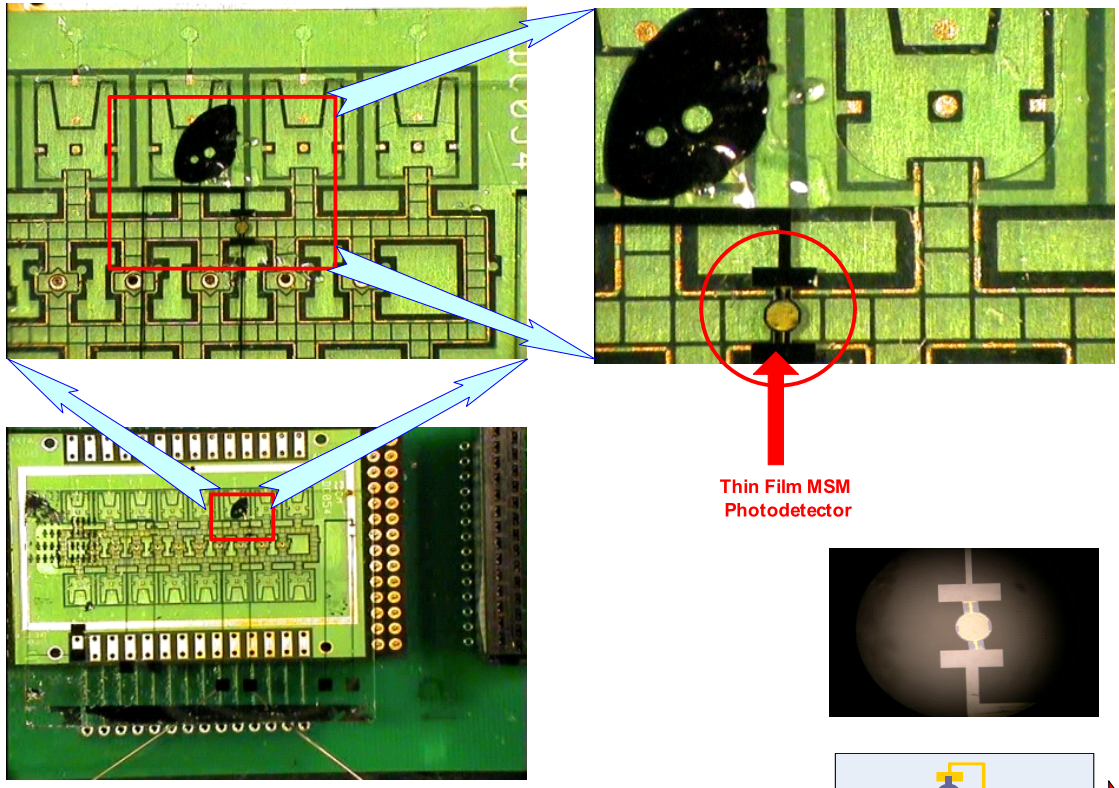
Lab-on-a-chip (LoC) technology
Integration and operation of an active optical device with a microfluidic system
First step toward integration of optical sensing systems with microfluidic systems



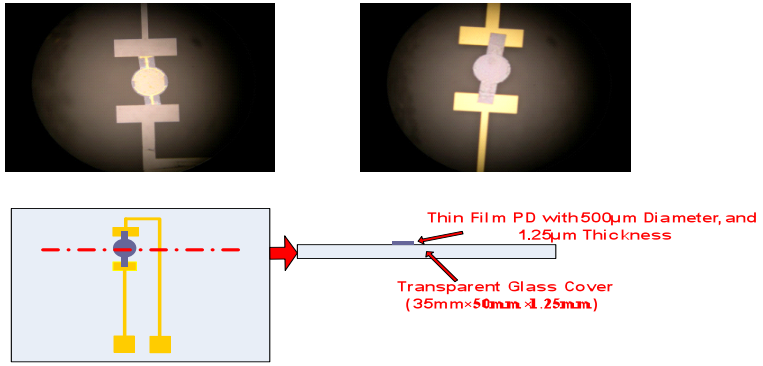
Side view of a coplanar electrowetting chip integrated with a thin film photodetector

L. Luan, et. al, *IEEE Sensors*, 2007

Integrated Photodetector in a Digital Microfluidic Platform

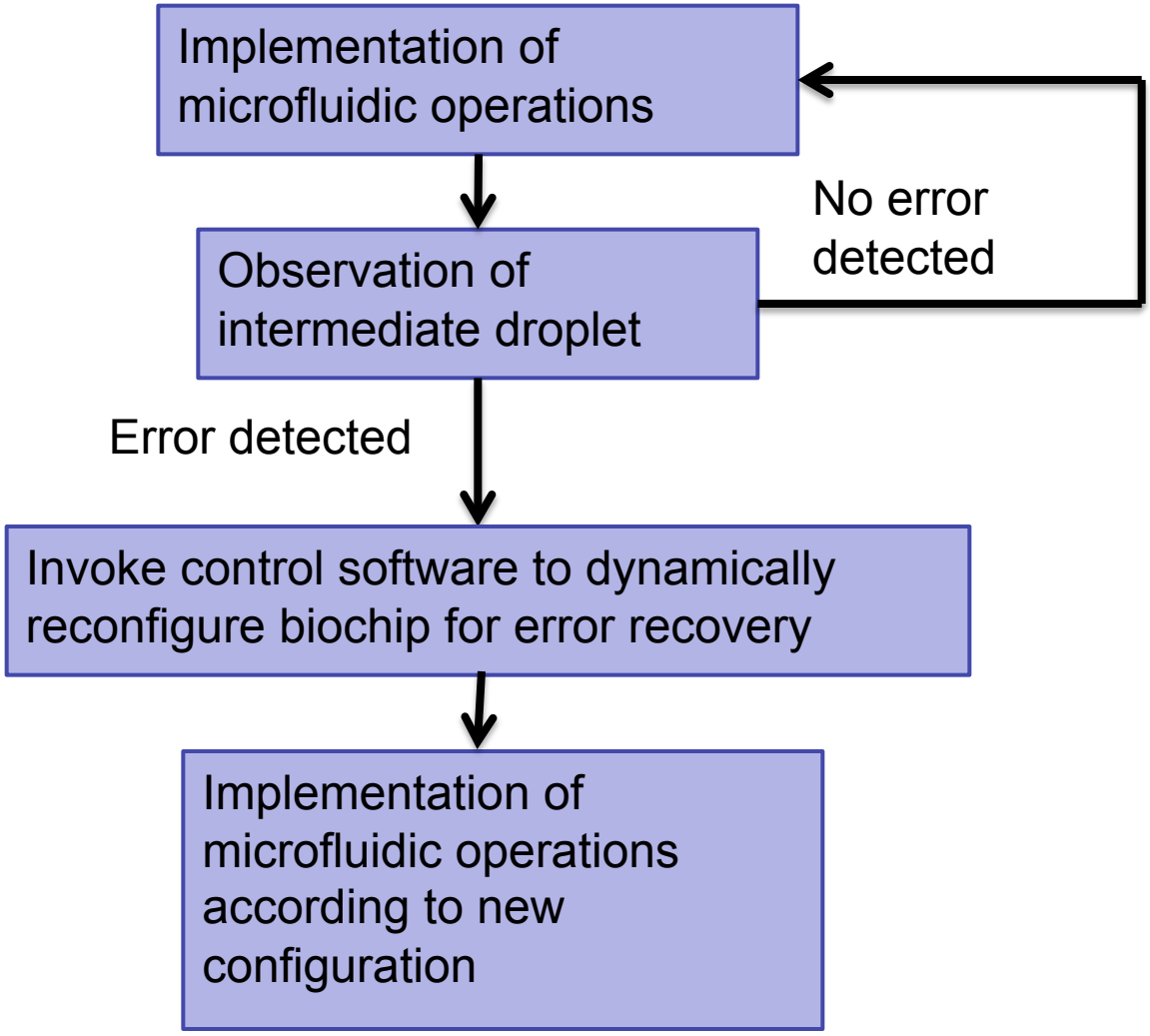


Top views of the electrowetting chip with a thin film MSM photodetector

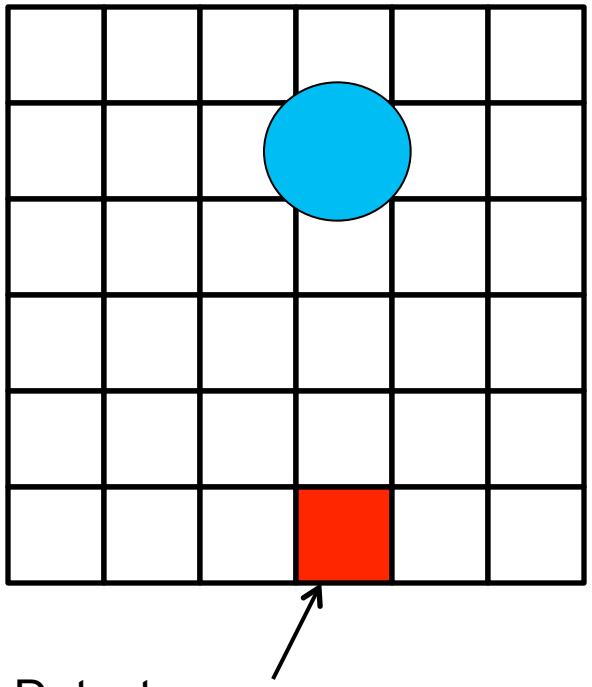


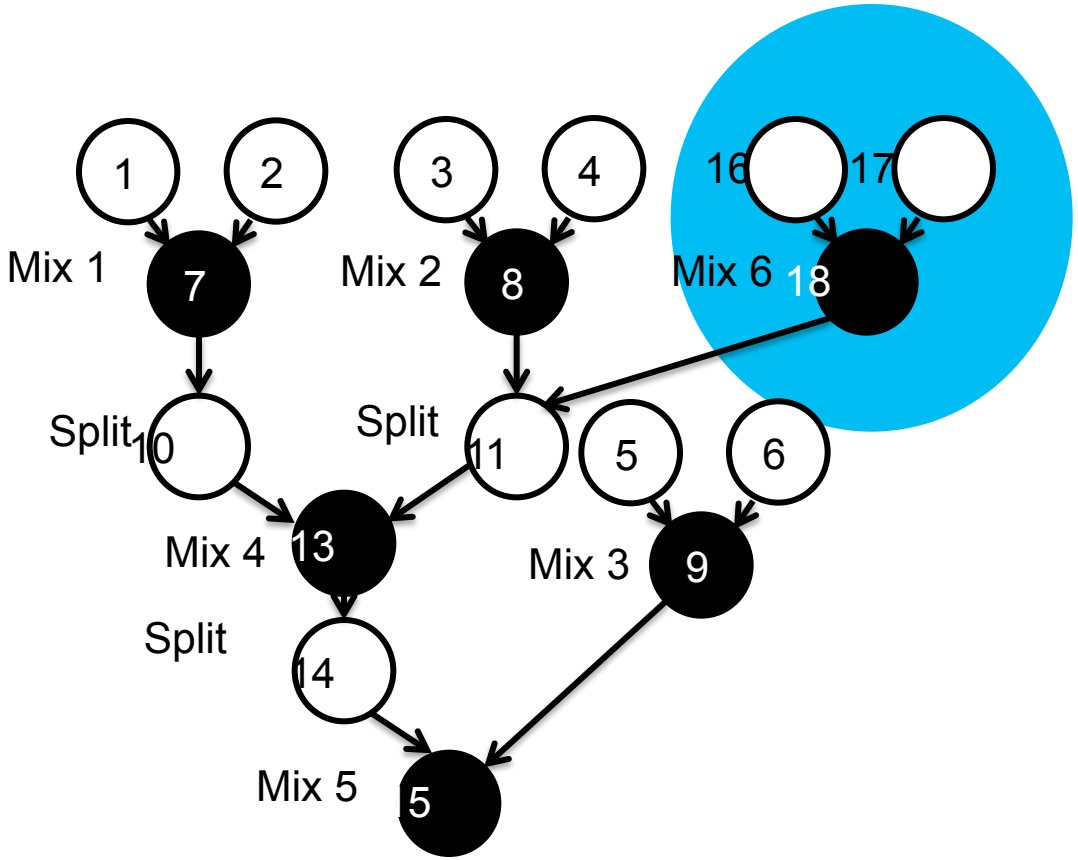
L. Luan, et. al, *IEEE Sensors*, 2007

Resynthesis-based Recovery



Cyberphysial System Integration

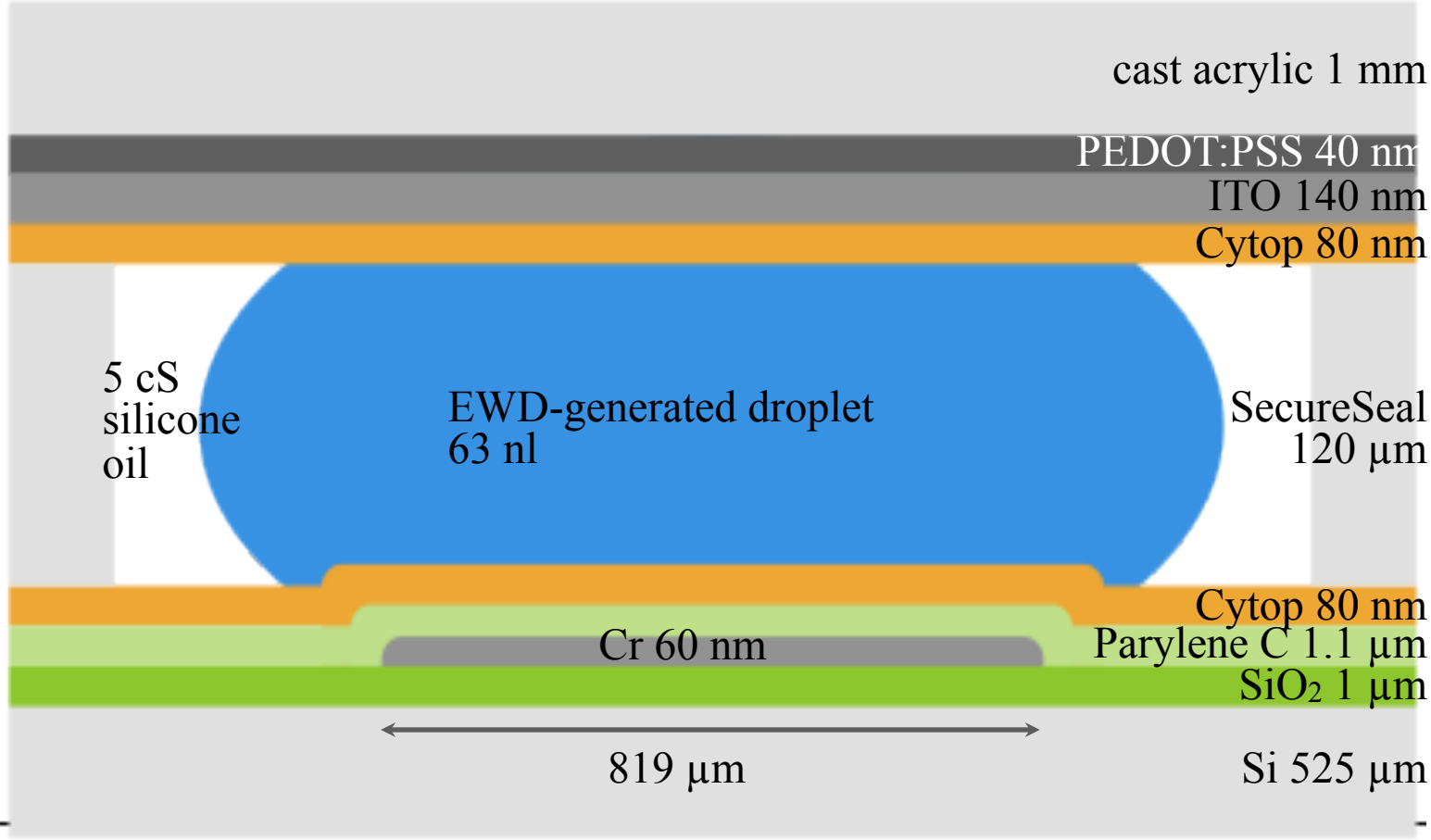




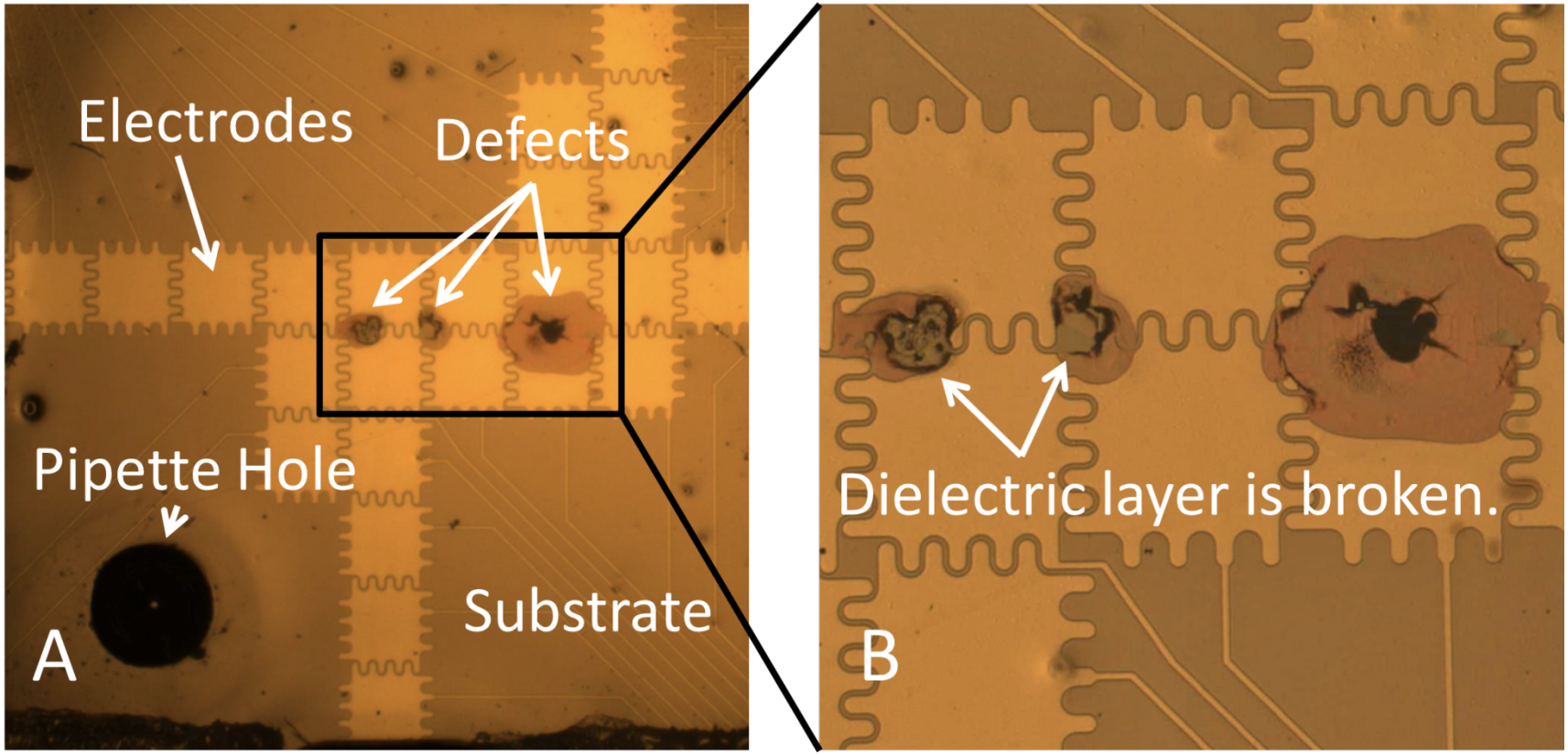
If the result indicates an error has occurred, the sequencing graph will be adjusted

Experimental Demonstration

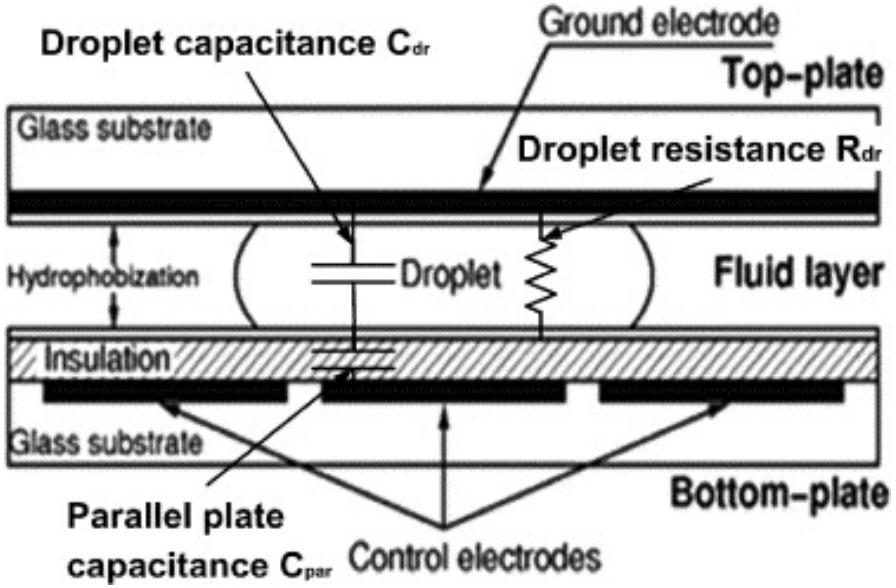
- Cyberphysical adaptation: hardware/software interaction
- Adaptive error recovery: fully automated



Biochip with Defects



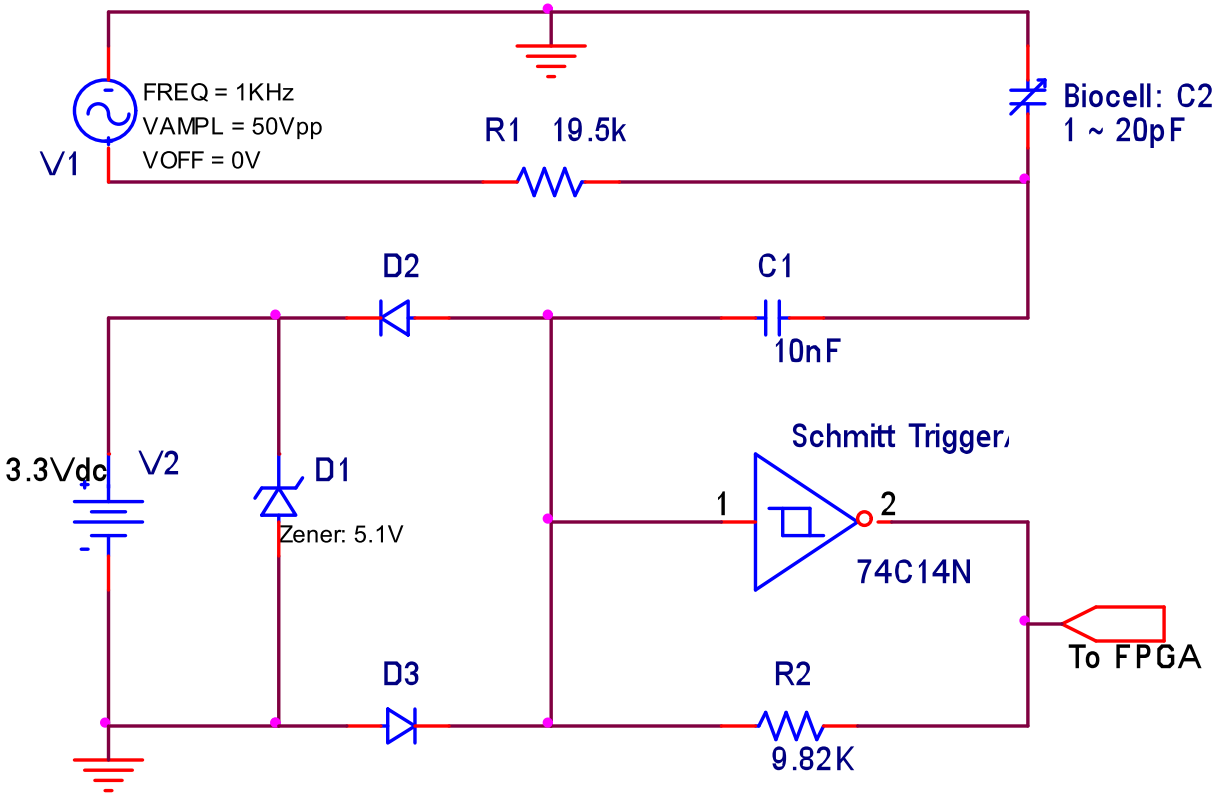
Capacitance Sensing: Physical Principle



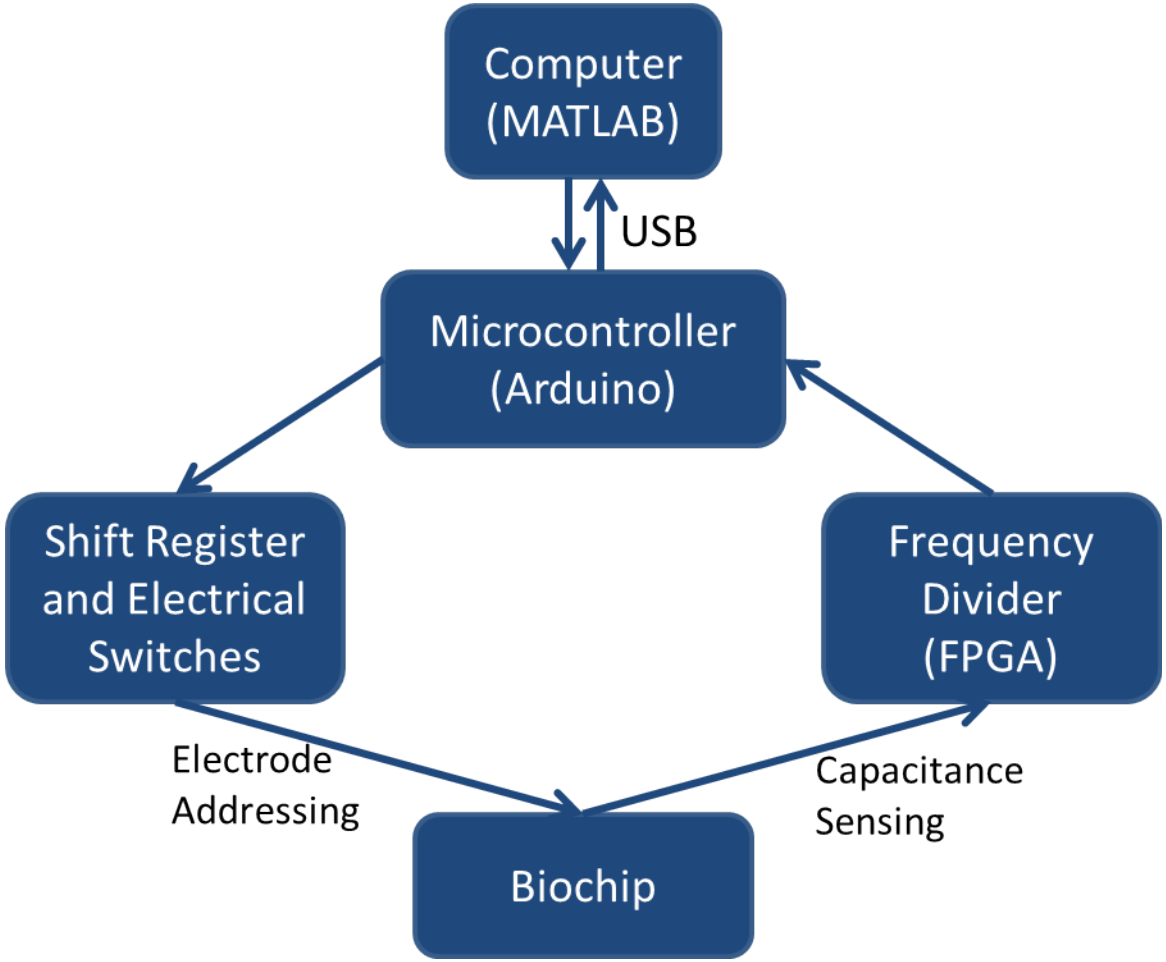
- Droplets are sandwiched by ground plane and control electrodes
- The difference of permittivity between silicon oil and droplets causes capacitance variance, which indicates whether droplets are present

Capacitance Sensing: Circuit Design

- C2: capacitance of a checking biocell
  - Droplet present: ~15pF
  - Droplet is absent: <1pF
- C1: isolate capacitance sensing circuit from driving circuit
- Schmitt Trigger: ring oscillator encoding C2 into the frequency of output signal

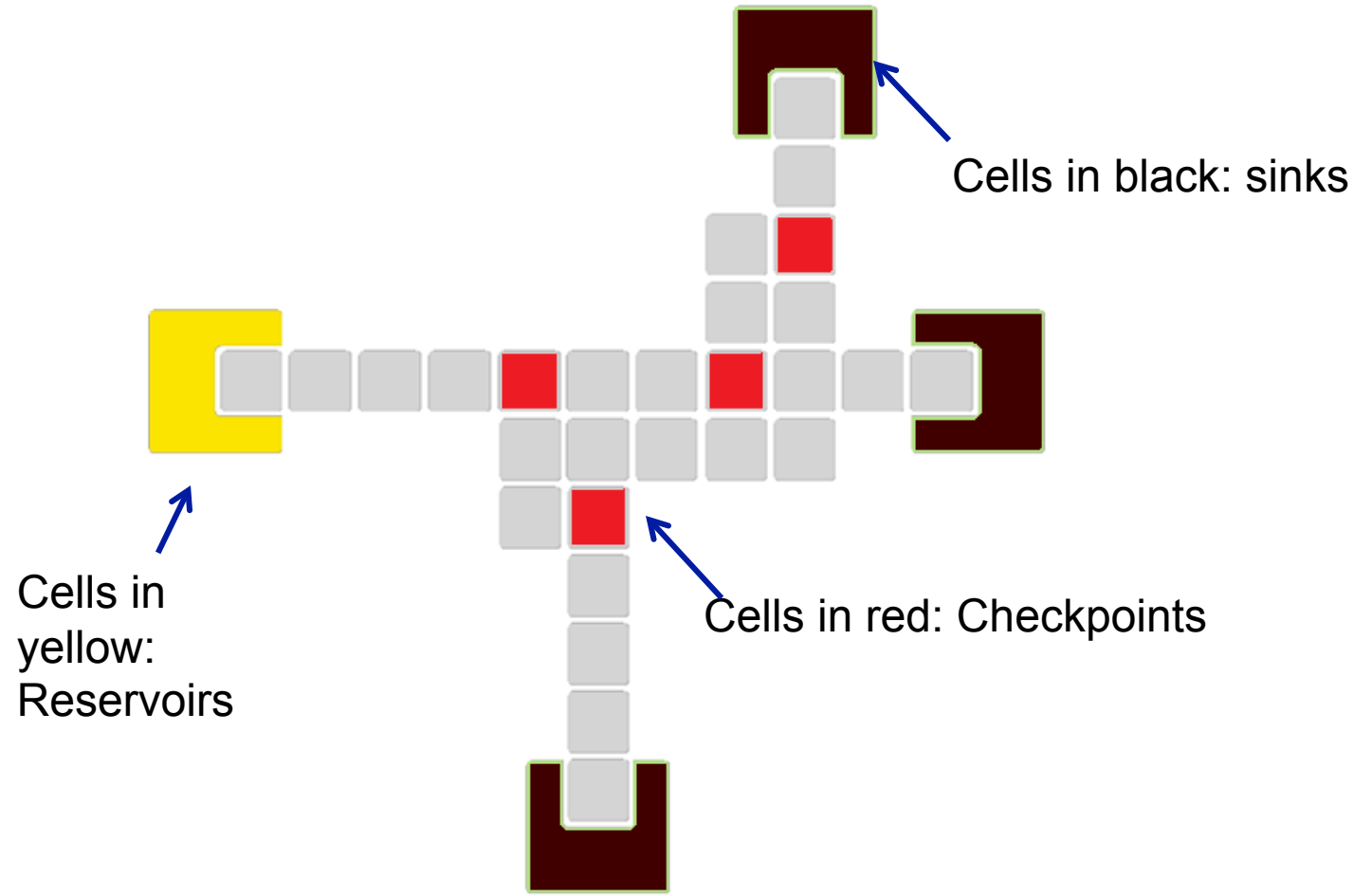


Hardware/Software Interface

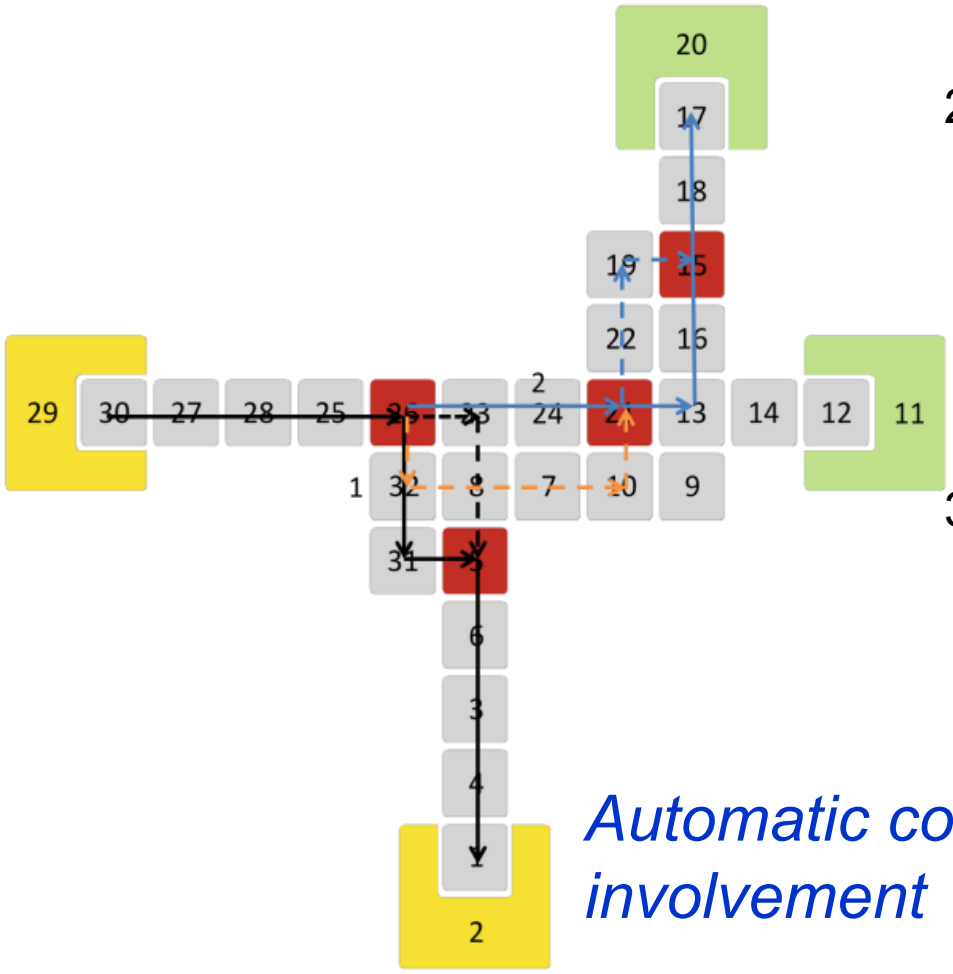


- MATLAB: experiment plan generation & decision making
- Microcontroller: directly communicate with computer
- Shift register: serial input from microcontroller and parallel output for electrode addressing

Chip Design


Cells in black: sinks
Cells in yellow: Reservoirs
Cells in red: Checkpoints

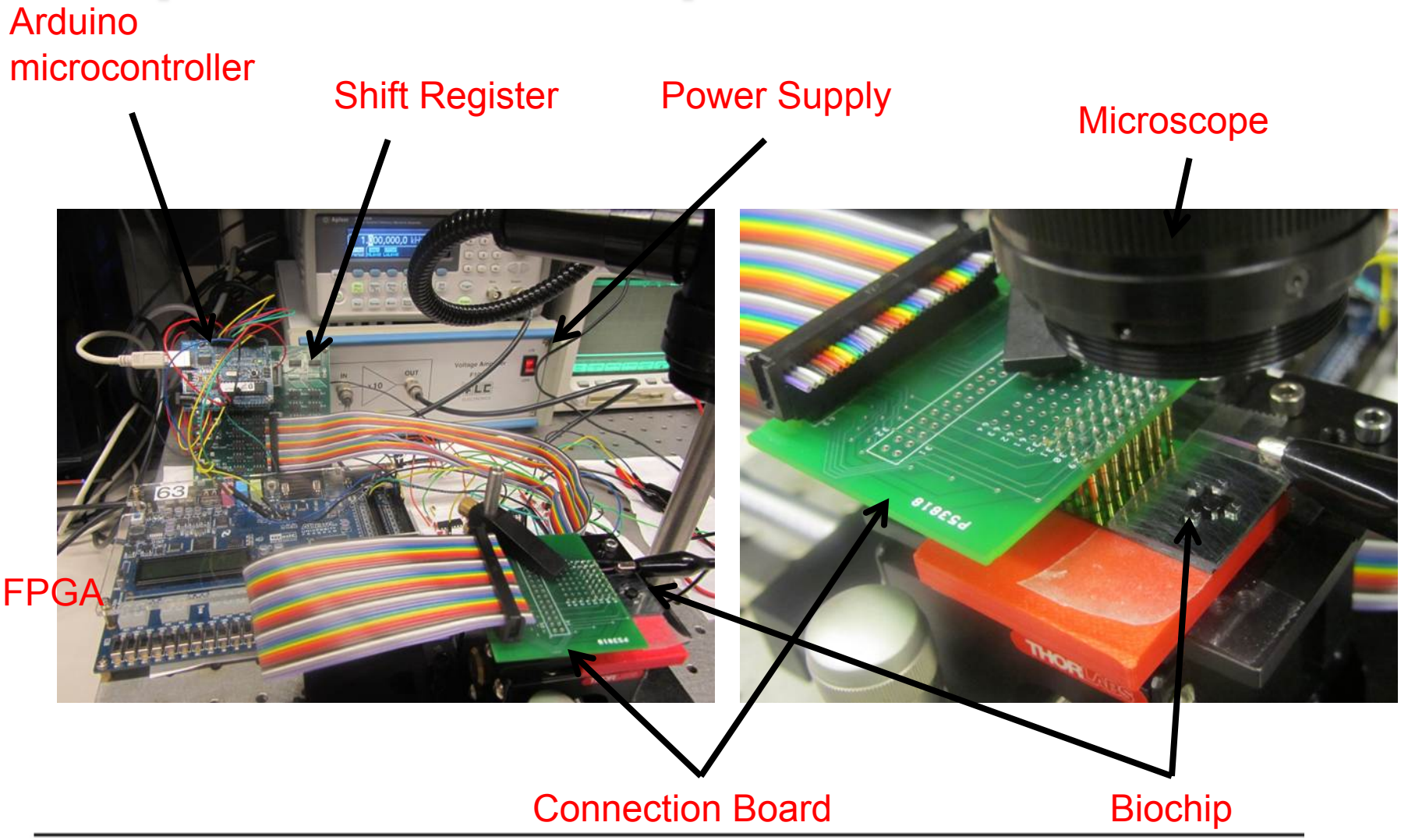
Experiment Plan



1. Two droplets transported simultaneously
2. Once a droplet reaches a checkpoint, capacitance sensing carried out
   - Signal frequency compared with preset threshold to make decision whether droplet is present
3. Self-recovery: a backup plan is activated to bypass faulty cells if necessary (dashed line)

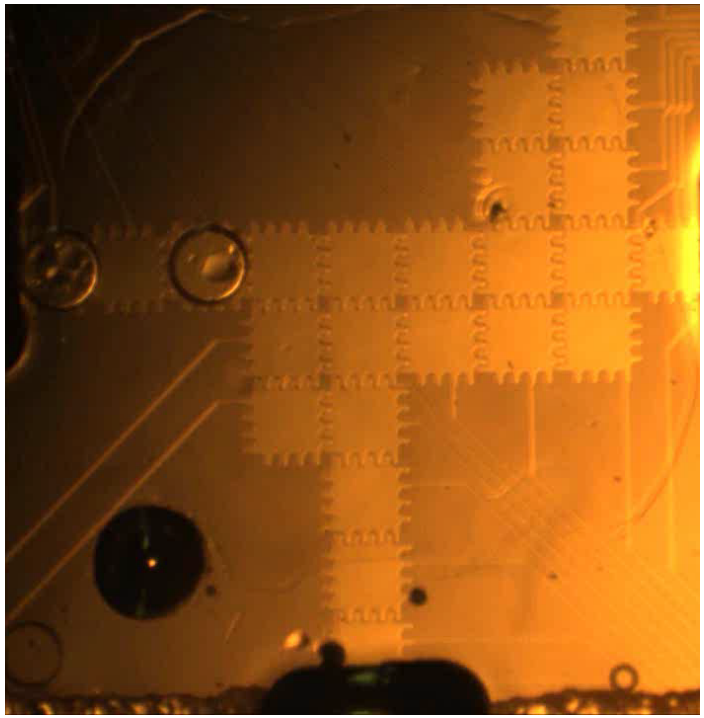
Automatic control without any human involvement

Experiment Set-up

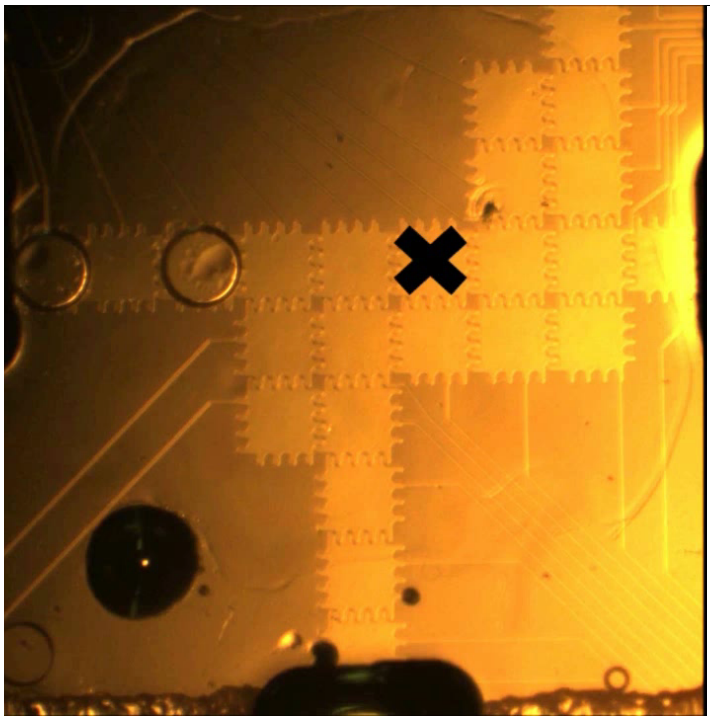


Results and Videos

Fault Free

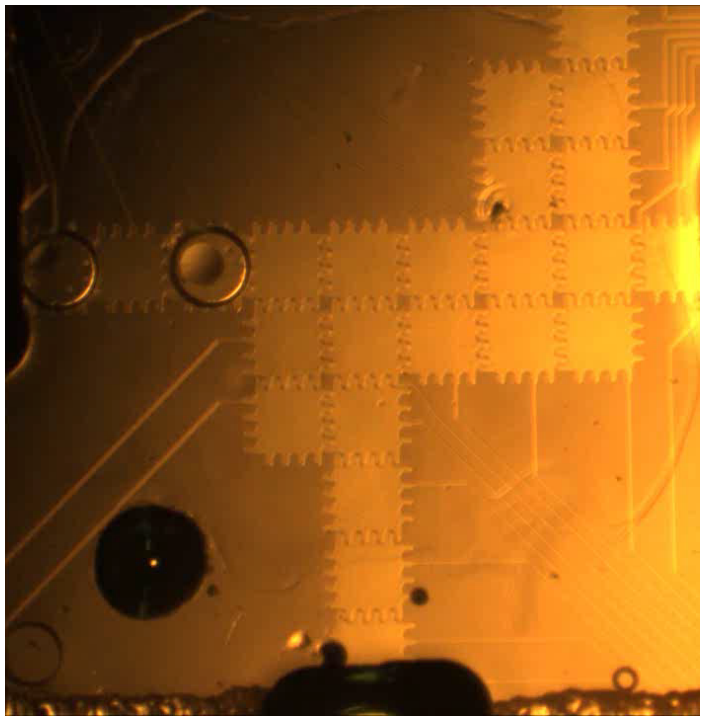


Defect in Region B

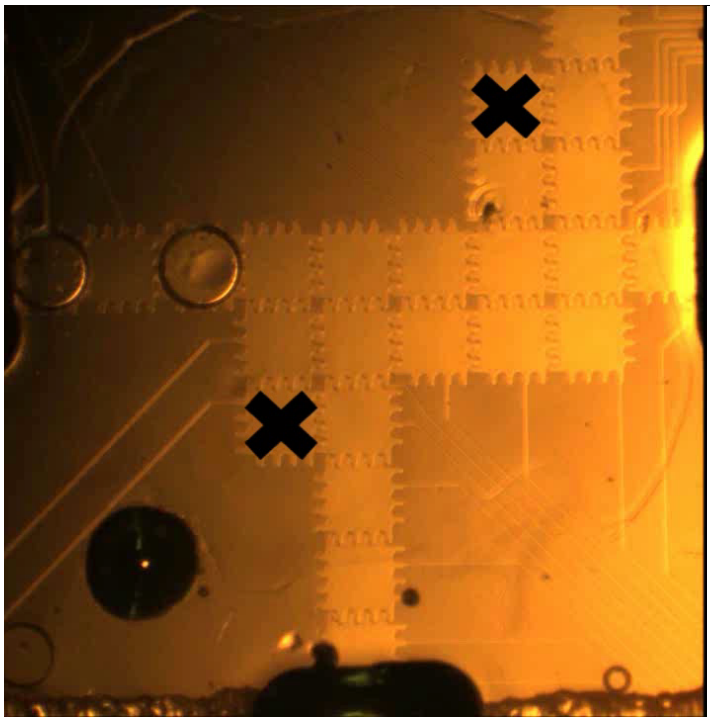


Results and Videos (Contd.)

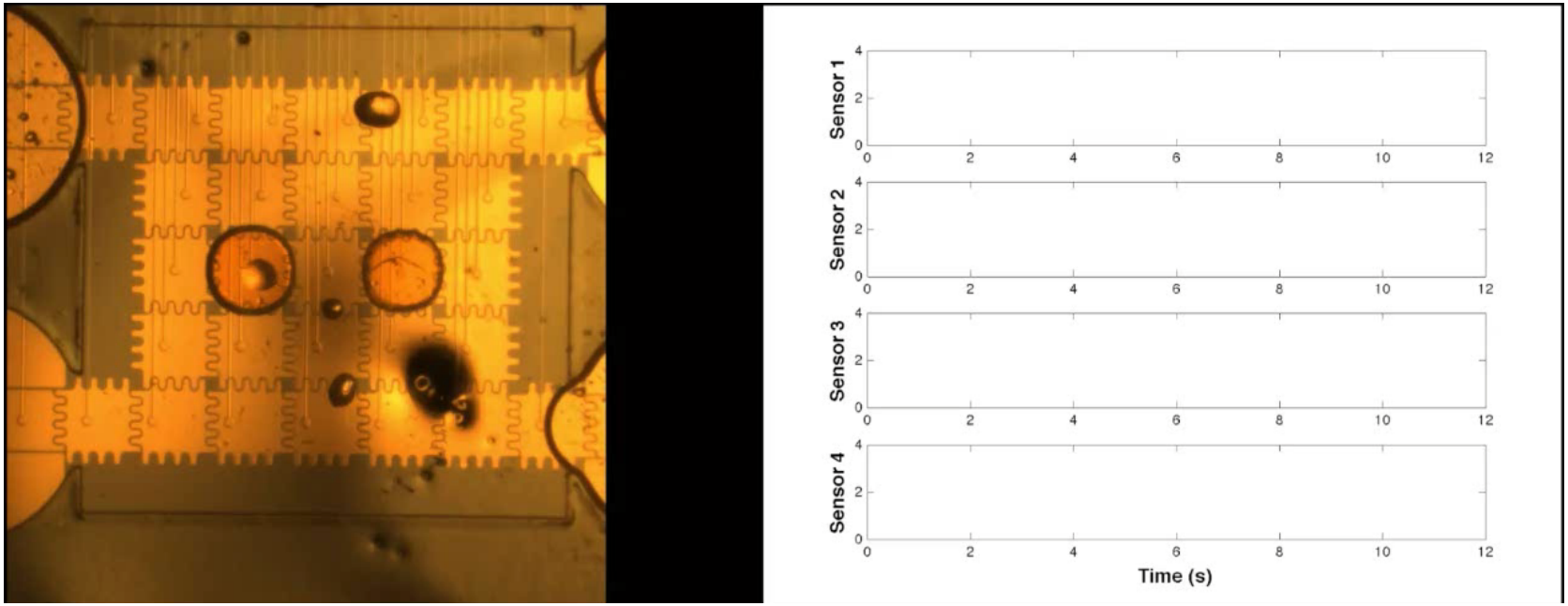
Fault Free



Defects in Region C & D



Demonstration for Capacitive Sensing



Conclusions

- Digital microfluidics offers a viable platform for lab-on-chip for clinical diagnostics and biomolecular recognition
- Design and integration challenges
  - Automated synthesis: scheduling, resource binding, module placement; droplet routing.
  - Sensors and real-time feedback processing
- Bridge between different research communities: bioMEMS, sensors, microfluidics, algorithms and optimization, electronics CAD and chip design, biochemistry
- Cyberphysical system design
- Closed-loop and sensor feedback-driven biochip operation under program control
  - Use sensor data at intermediate checkpoints to dynamically reconfigure the biochip
  - Recovery errors "seamlessly" without interruption of other operations

The Numbers…

- 300pl droplet on 95µm electrode EWD devices (*lowest voltages*)
  - Dispensed from 140nl reservoir with 11.4V
  - Actuation voltage as low as 7.2V
  - Ta_2O_5 + Parylene C provide more robust structures
- Device dimensionality scaling (*smallest chips, volumes*)
  - 12pl droplets can be dispensed and split on 33µm electrode EWD devices
  - 5pl droplets can be dispensed on 21µm electrode EWD devices