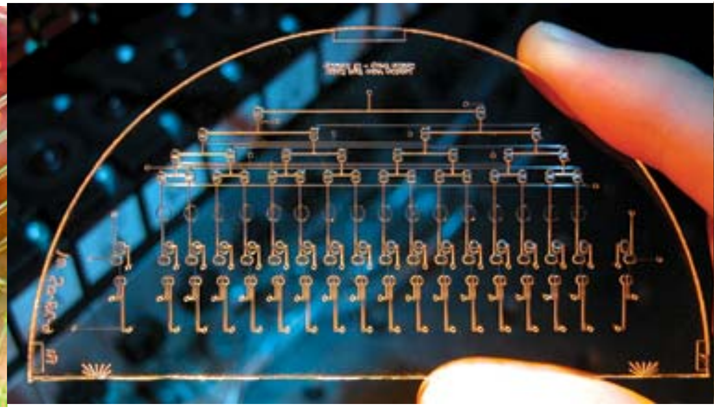
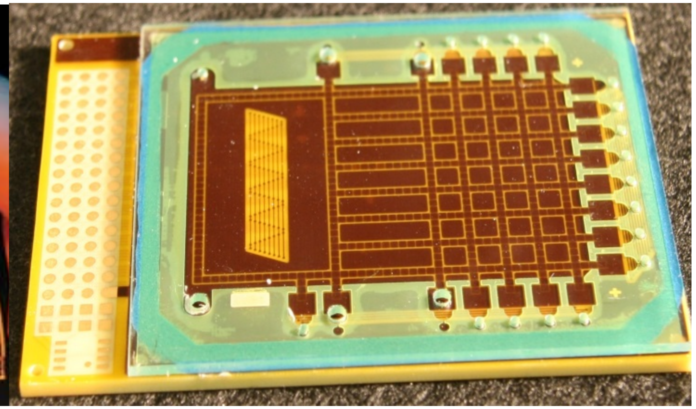
The Coming of Age of Microfluidics:
EDA Solutions for Enabling Biochemistry on a Chip

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  - The theory, analysis, design (computer aided design), and practical implementation of circuits, and the application of circuit theoretic techniques to systems and to signal processing. The coverage of this field includes the spectrum of activities from, and including, basic scientific theory to industrial applications.

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- CAS Mission

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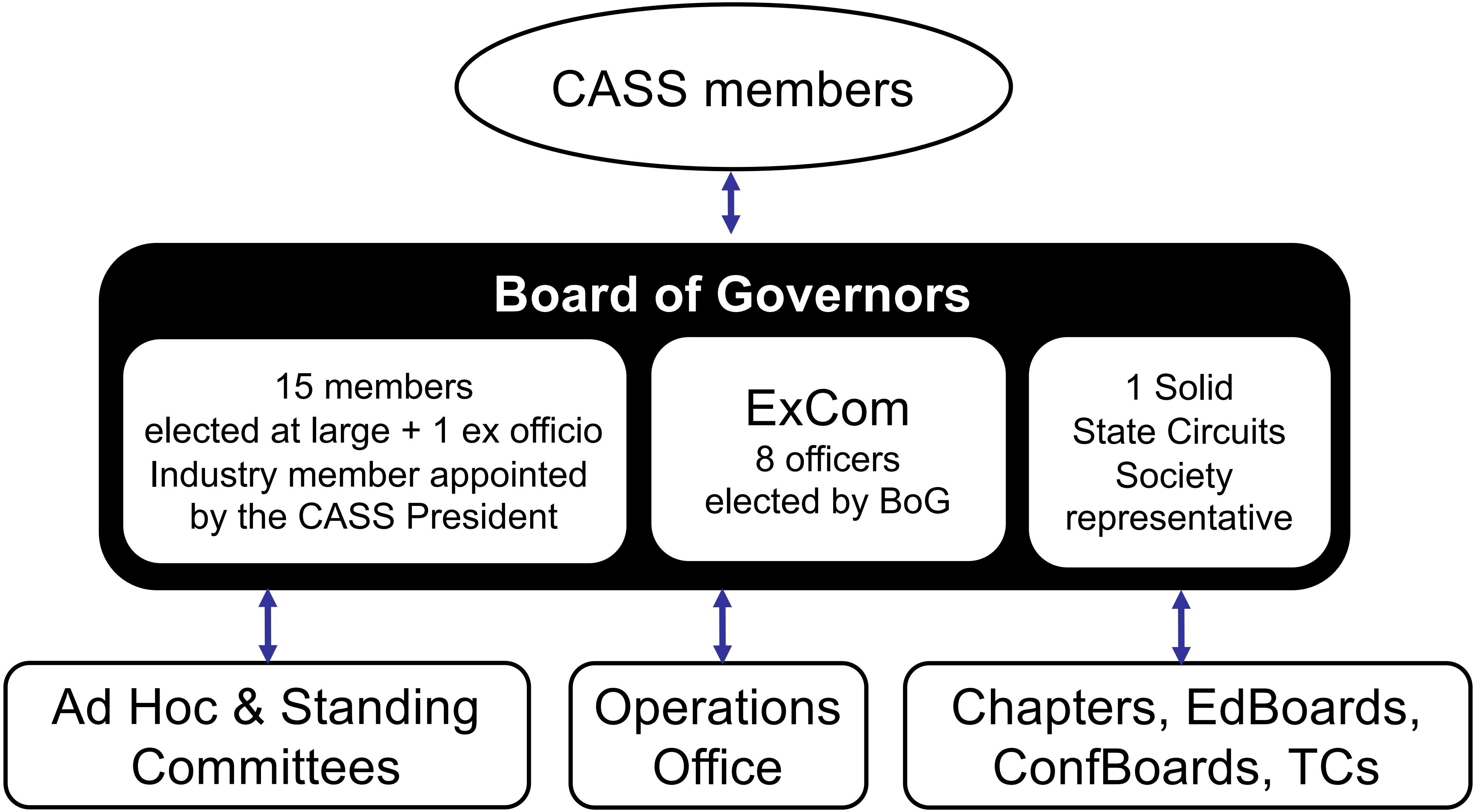

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  $22 / year (Student: $11 / year)
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  - free JETCAS (electronic)
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  - Digital Signal Processing
  - Multimedia Systems & Applications
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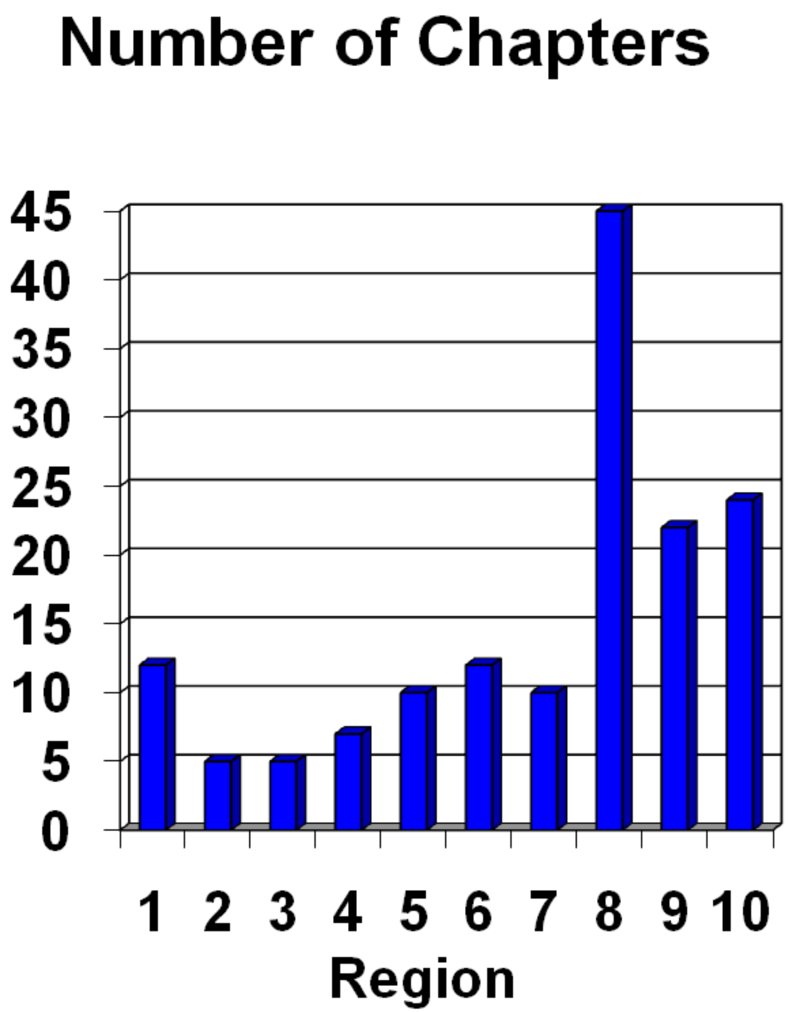
- Organize many local events



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  - Charles A. Desoer Technical Achievement
  - John Choma Education
  - Meritorious Service
  - Industrial Pioneer
  - Vitold Belevitch (every 2 years)
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  - Chapter of the Year – Regions 1-7
  - Chapter of the Year – Region 8
  - Chapter of the Year – Region 9
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    - BioCAS Best Paper
    - Darlington Best Paper
    - VLSI Best Paper
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  - Other conferences & workshops : ASP-DAC, BioCAS, ICCD, LASCAS, MPSOC, MWSCAS, etc.

- Visit our website ieee-cas.org/conferences for the full listing!

Acknowledgements

- Collaborators:
  - Prof. Bhargab Bhattacharya (Indian Statistical Institute)
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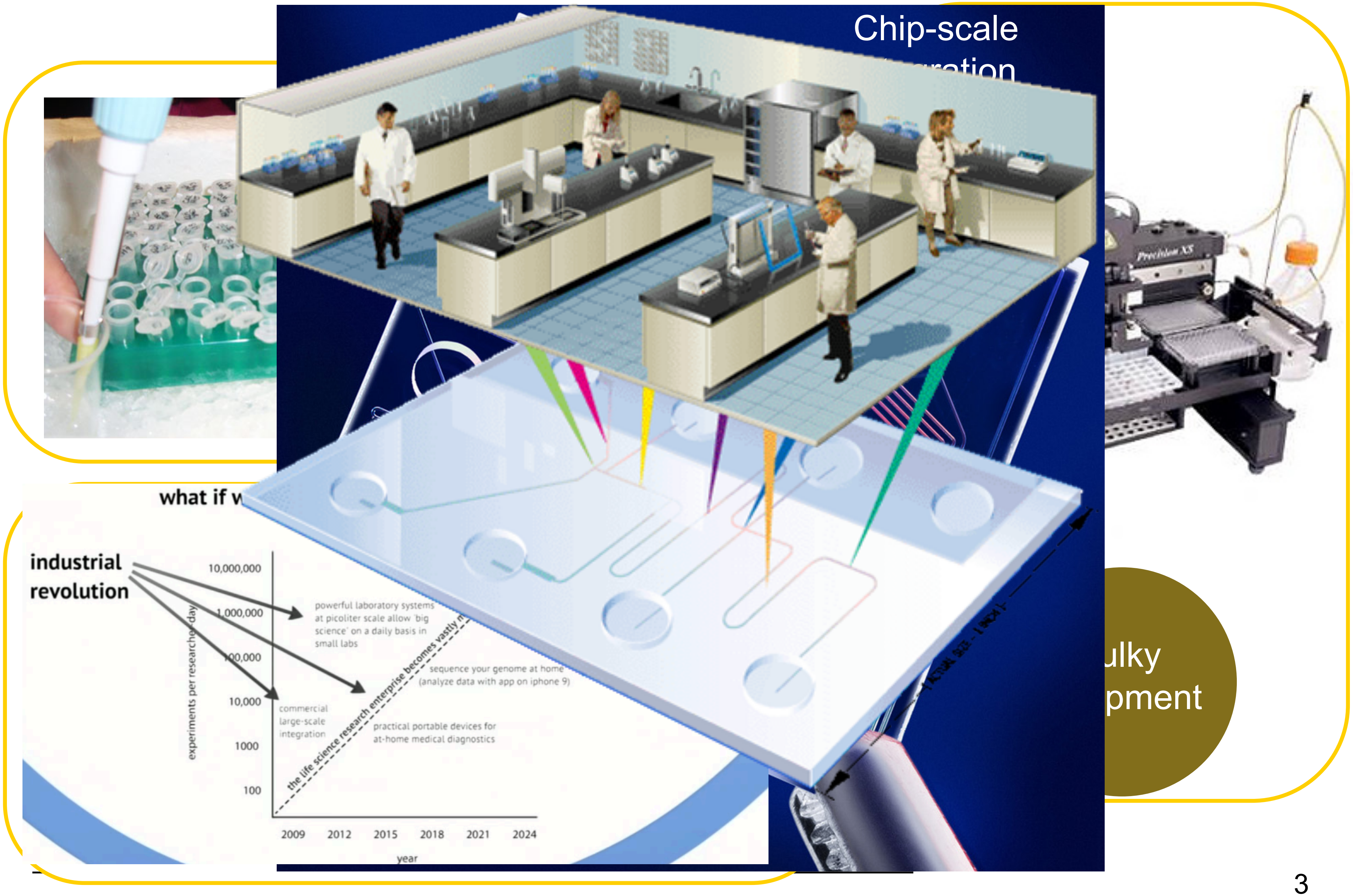
- Duke University's Microfluidics Research Lab
- Stanford Microfluidics Foundry
- Advanced Liquid Logic (http://www.liquid-logic.com/), now part of Illumina

Paradigm Shift in Biochemistry



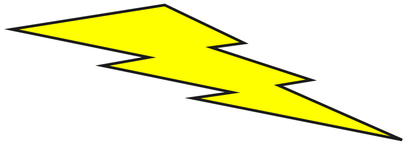
Motivation for Microfluidic Biochips

- 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*

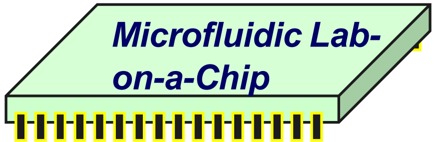
CLINICAL DIAGNOSTIC APPLICATION

Conventional Biochemical Analyzer

Shrink



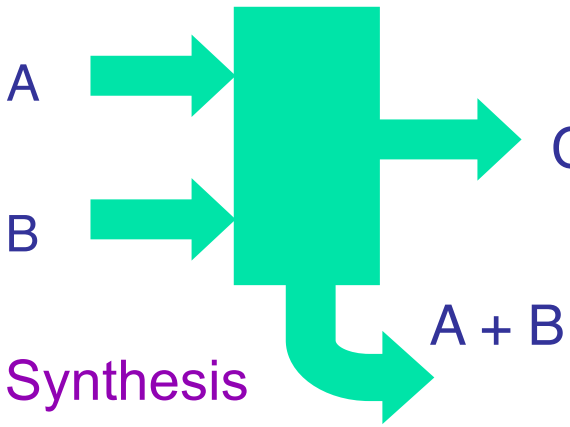
Lab-on-a-chip for CLINICAL DIAGNOSTICS

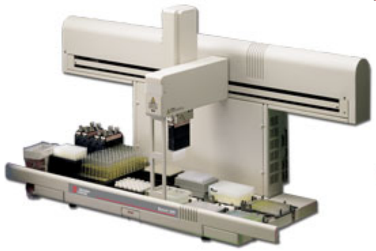


20nl sample

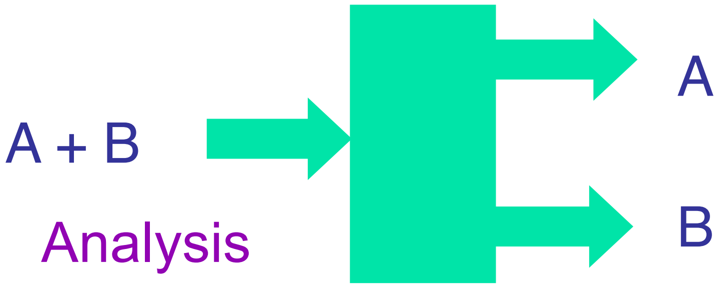
Higher throughput, minimal human intervention, smaller sample/reagent consumption, higher sensitivity, increased productivity

Why is Biochemistry Difficult?





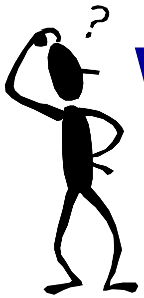
Mixing



Reaction

Separation

Why is Biochemistry Difficult?

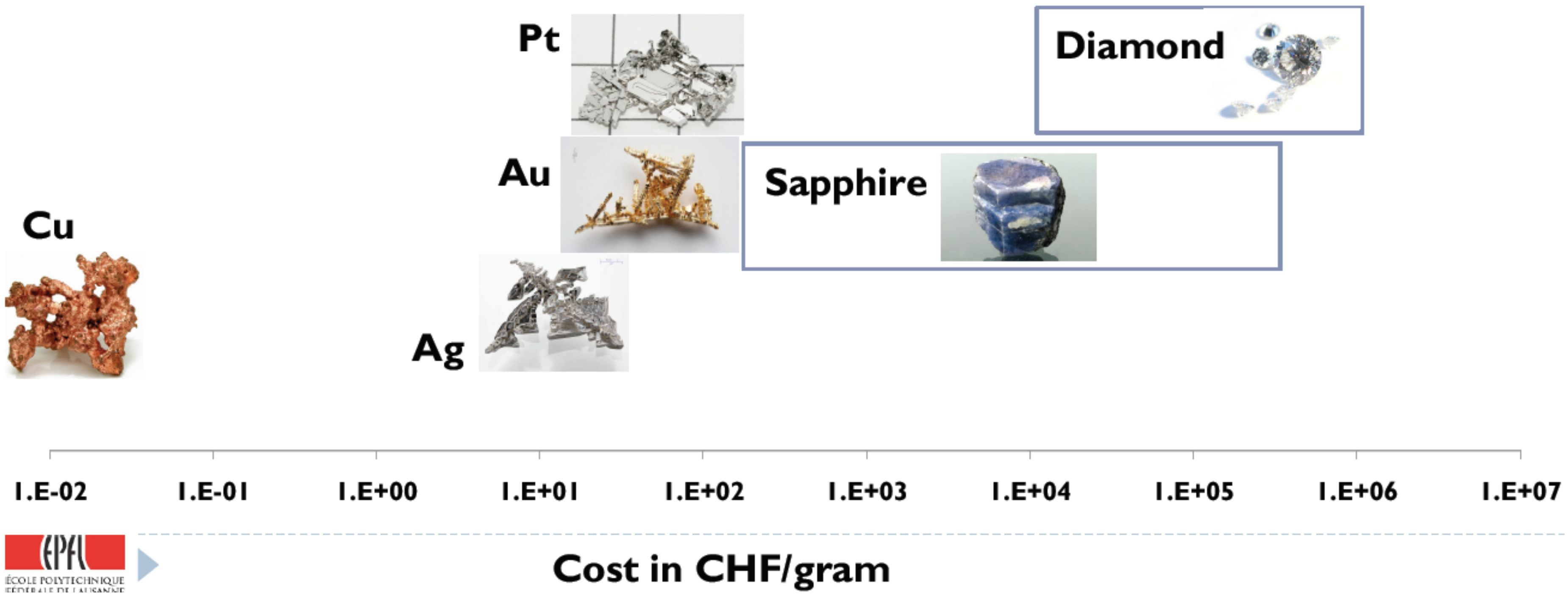


Why do chips have to be small?

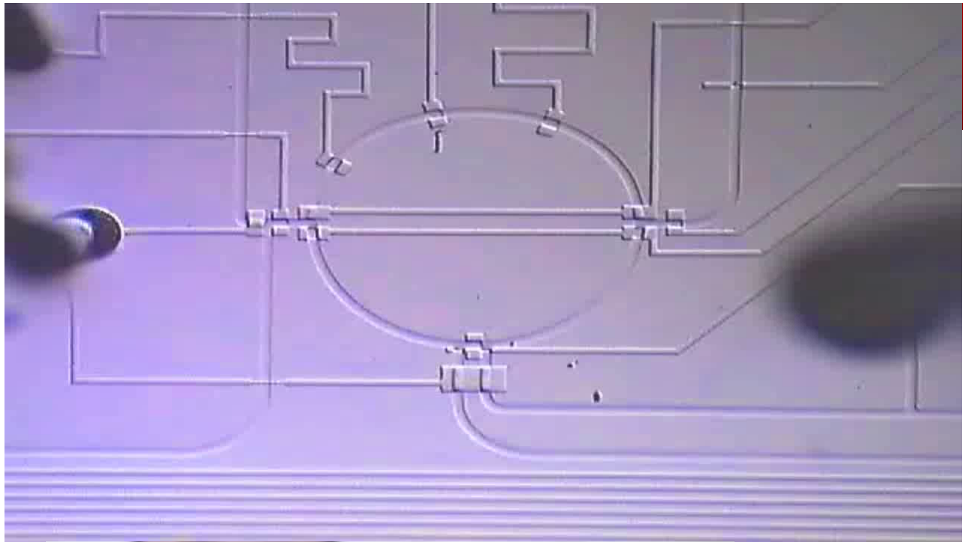
High-**T**hroughput is why. If you do 10^6 assays in 10µl format, each time you do a reaction you'll need 10 liters of reagents. With the typical cost of biological reagents, even Big Pharma can't afford this.

How much does the biological reagent cost?

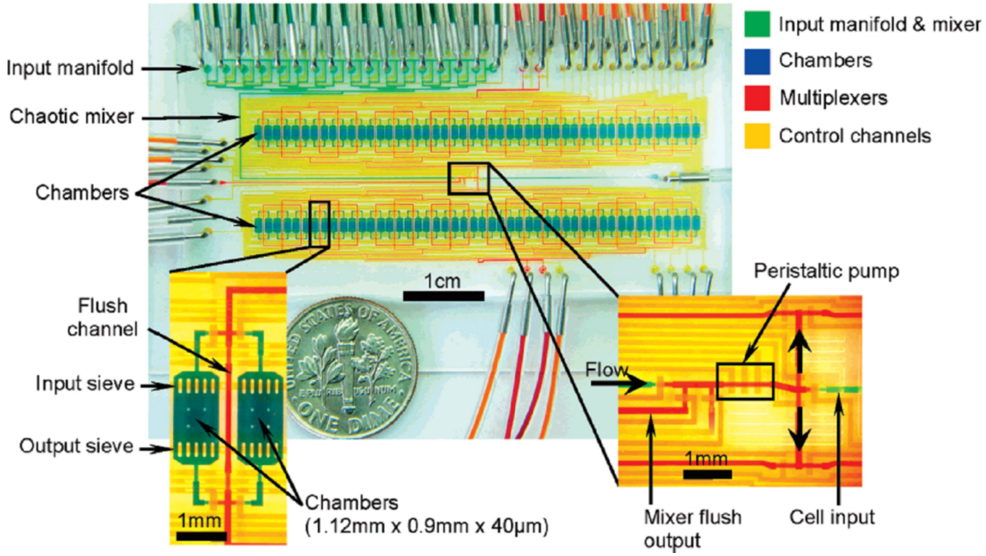
Cost of Biological Reagents

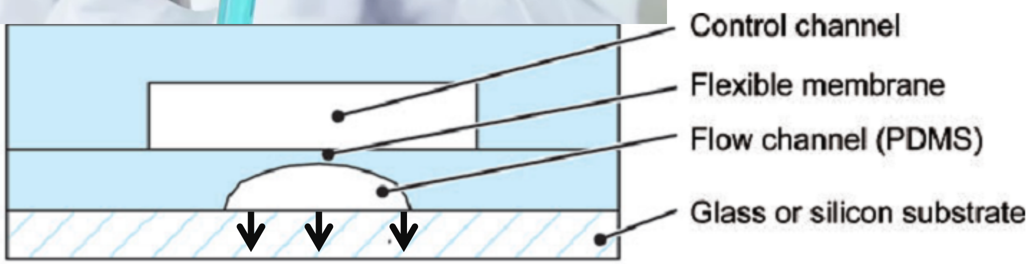


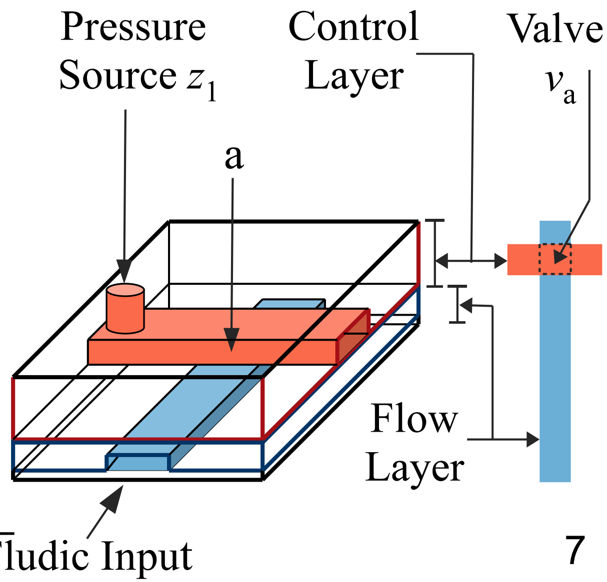
Demonstration of Flow-Based Microfluidics



STANFORD UNIVERSITY | STANFORD MICROFLUIDICS FOUNDRY

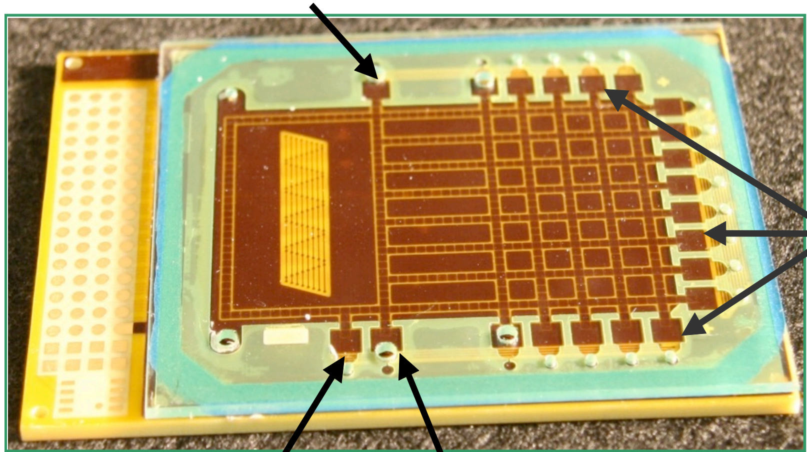


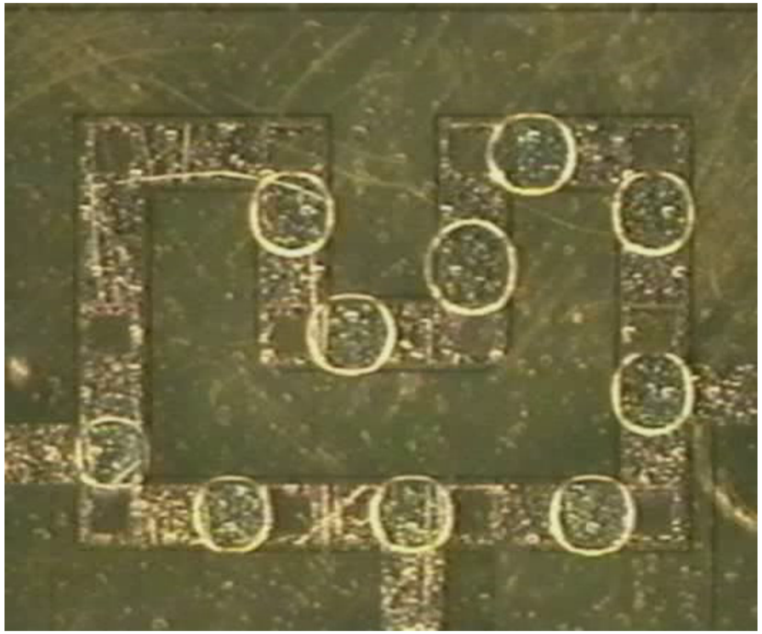




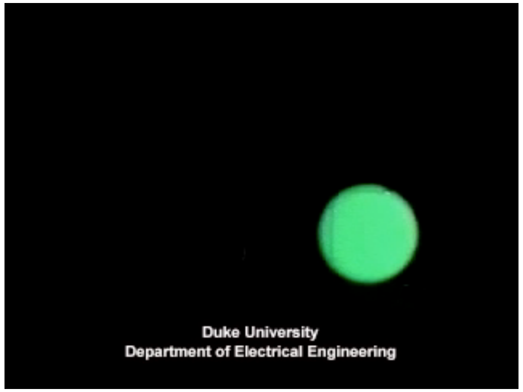
T. Thorsen, S. J. Maerkl, and S. R. Quake, "Microfluidic Large-scale Integration," Science, Oct. 2002.

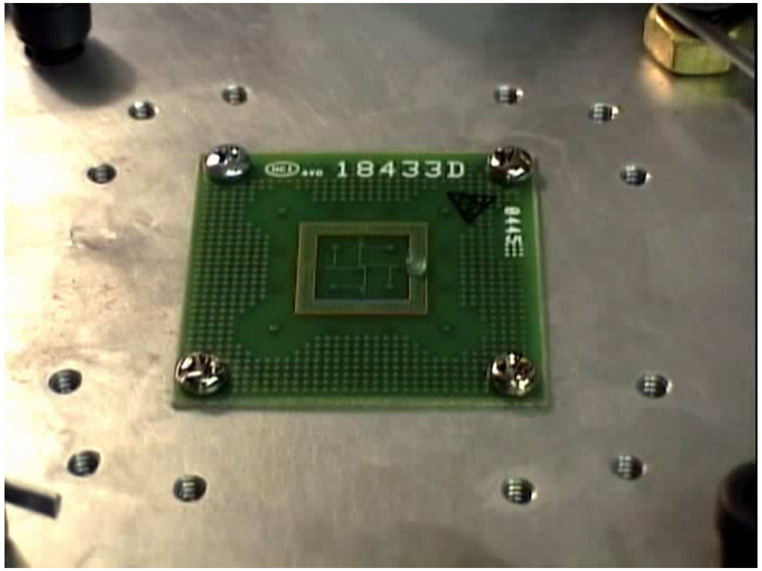
Demonstration of Droplet-Based Microfluidics





EWOD: Electrodewetting on Dielectric



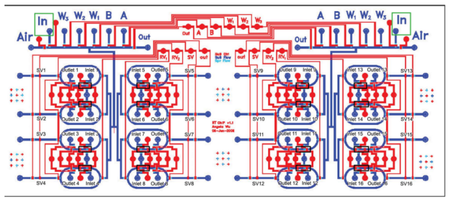


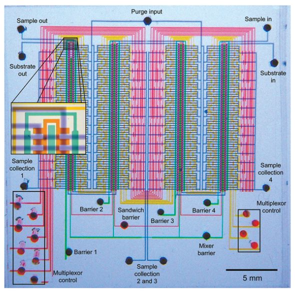
Source: Advanced Liquid Logic (now Illumina) and Duke Univ.

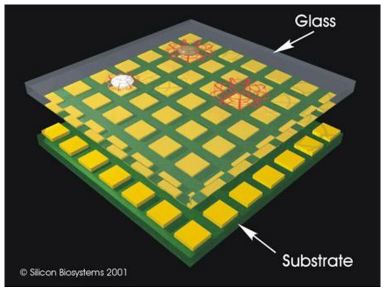
Microfluidic Very Large Scale Integration (mVLSI)

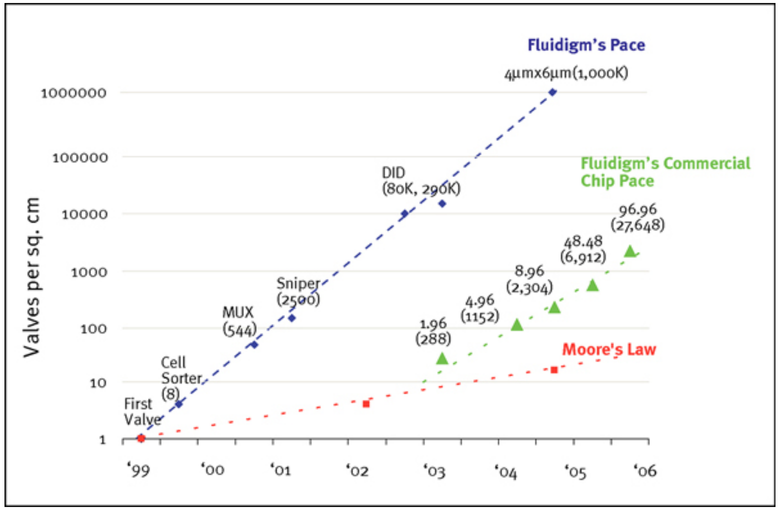
- Applications become more complicated
  - Large-scale bioassays
  - Multiple and concurrent assay operations on a biochip

- Design complexity is increased
  - Moore's Law of Microfluidics: Valve Density Doubles Every 4 Months.
  - 25,000 valves for 9,216 PCR*
  - 600,000 electrodes for tumor cell analysis**









Source: Fluidigm

* Perkel, Science, 2008

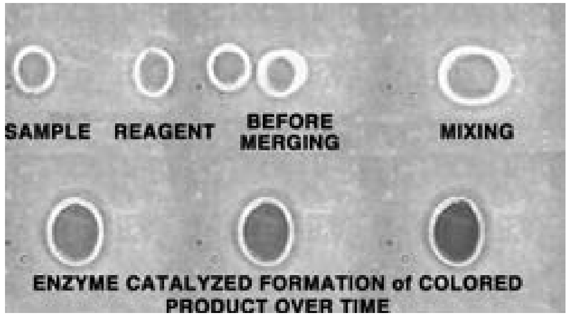
** Silicon Biosystems, http://www.siliconbiosystems.com/applications/webwork/DEPArray.page

Application model: from this…

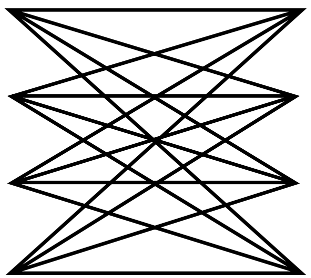
$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{TOPS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Trinder’s reaction, a colorimetric enzyme-based method



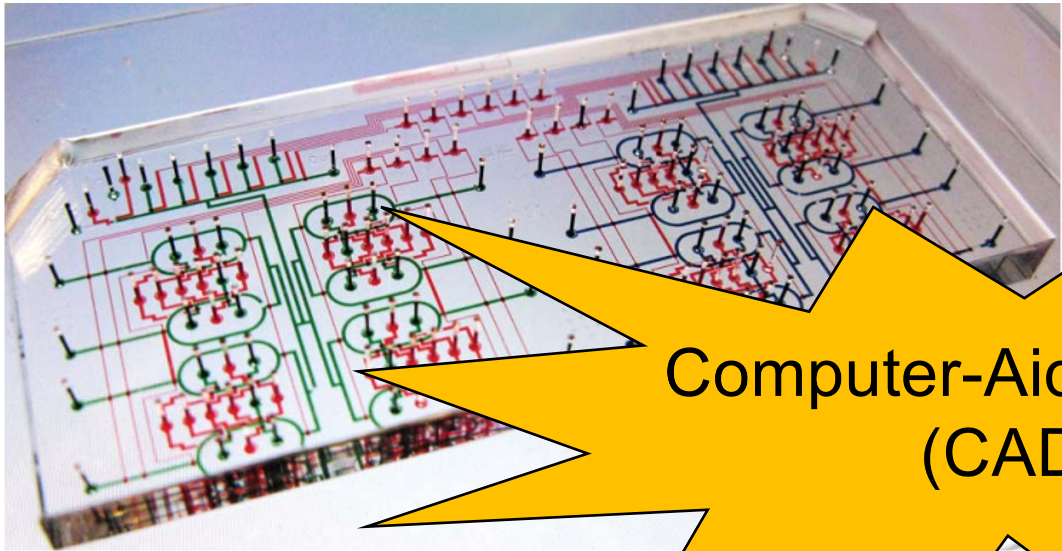
Glucose assay steps on the biochip



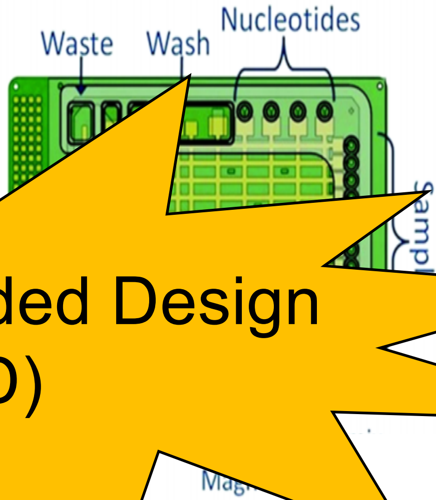
Sample	Reagent	Enzymatic Assay
Plasma: S_1	R_1	Glucose Measurement
Serum: S_2	R_2	Lactate Measurement
Urine: S_3	R_3	Pyruvate Measurement
Saliva: S_4	R_4	Glutamate Measurement

Several such reactions assays in parallel:
“in-vitro diagnostics” application

Application model: …to this—a biochip

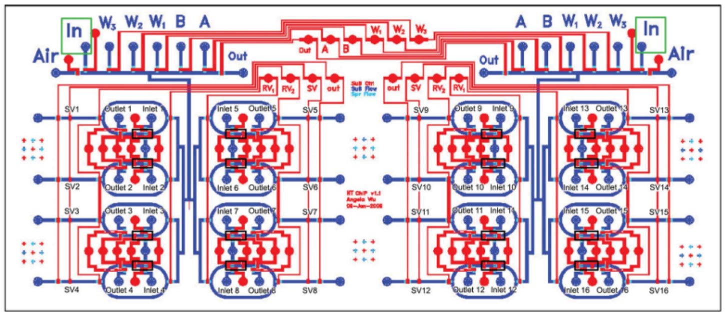


Wu et al., Lab on a Chip, 2012.

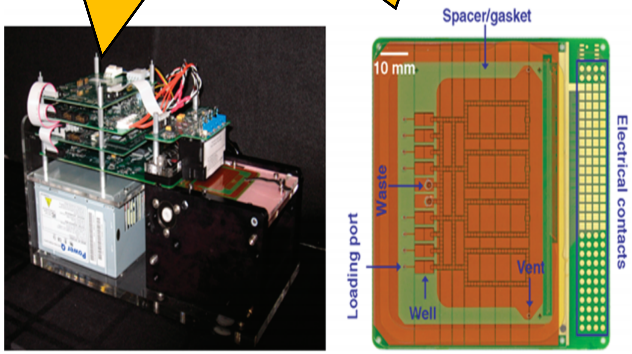


Boles et al., *Analytical chemistry*, 2011.

Computer-Aided Design (CAD)



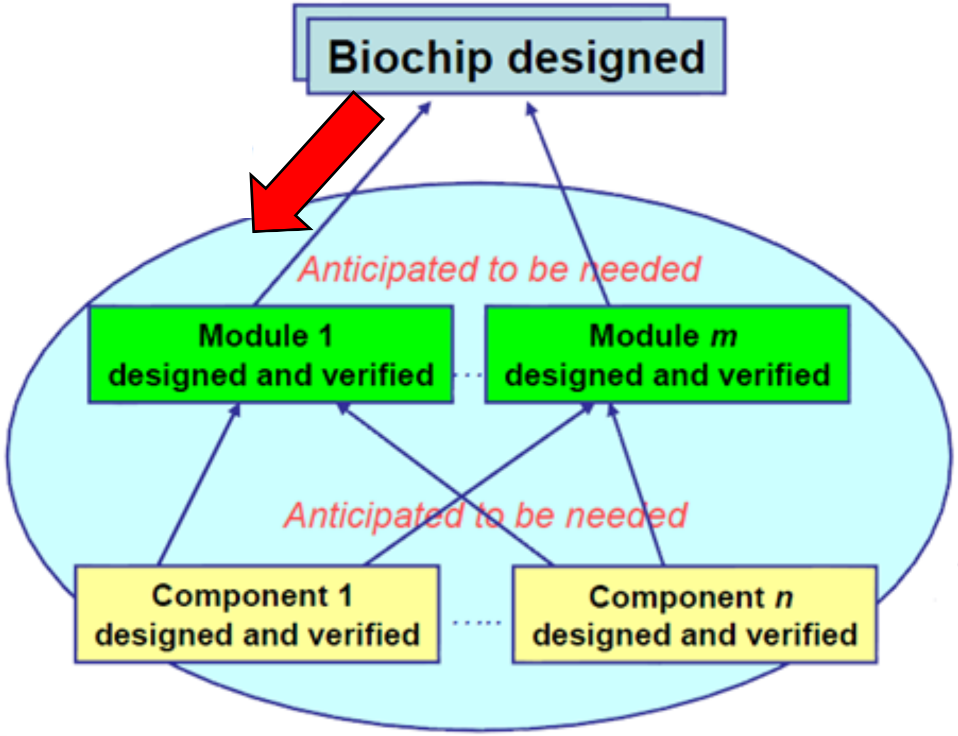
Wu et al., Lab on a Chip, 2012.



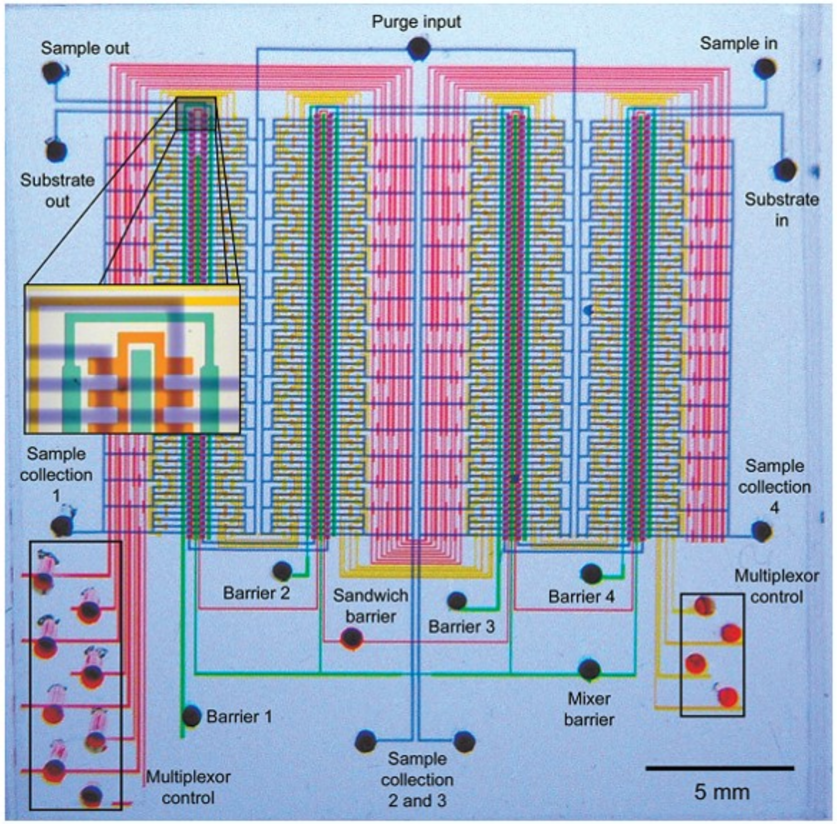
Hua et al., *Analytical chemistry,* 2010.

Current Design Methodology

- CAD tools are in their infancy
  - Bottom-up design methodology
  - Most groups use Matlab or AutoCAD
  - Limited automation; every line drawn by hand; manual control



Bottom-up design method



The BioCoder Language*

* Developed by MIT CSAIL

- BioCoder is a protocol language for reuse & automation
  - Independent of chip or laboratory setup
  - Initial focus: molecular biology
- Implemented as a C library
  - Used to express 65 protocols, 5800 lines of code

Example: Plasmid DNA Extraction

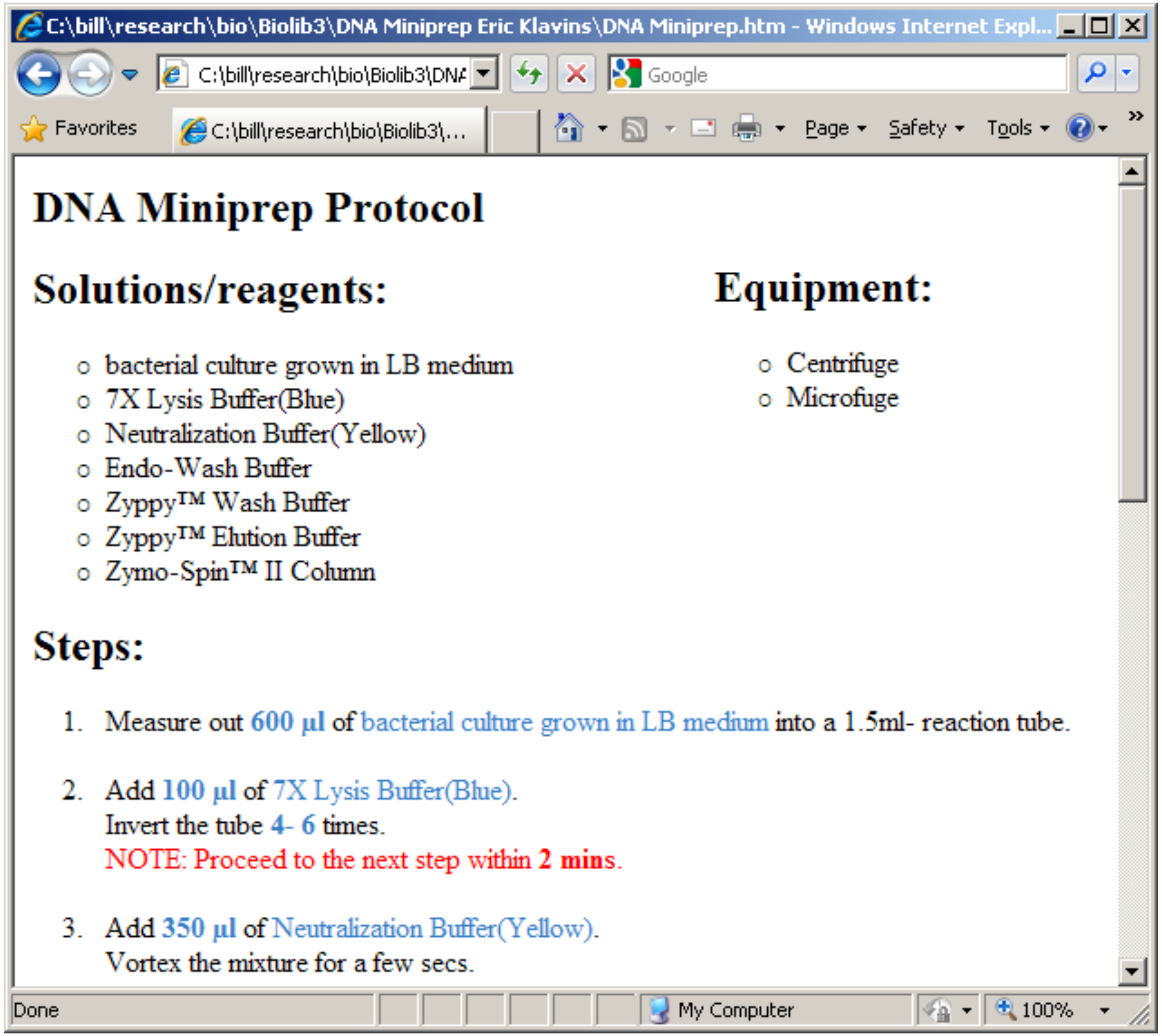
I. Original protocol *(Source: Klavins Lab)*

Add 100 ul of 7X Lysis Buffer (Blue) and mix by inverting the tube 4-6 times.
Proceed to step 3 within 2 minutes.

II. BioCoder code

```
FluidSample f1 = measure_and_add(f0, lysis_buffer, 100*uL);
FluidSample f2 = mix(f1, INVERT, 4, 6);
time_constraint(f1, 2*MINUTES, next_step);
```

Example: Plasmid DNA Extraction



DNA Miniprep Protocol

Solutions/reagents:

- bacterial culture grown in LB medium
- 7X Lysis Buffer(Blue)
- Neutralization Buffer(Yellow)
- Endo-Wash Buffer
- Zyppy™ Wash Buffer
- Zyppy™ Elution Buffer
- Zymo-Spin™ II Column

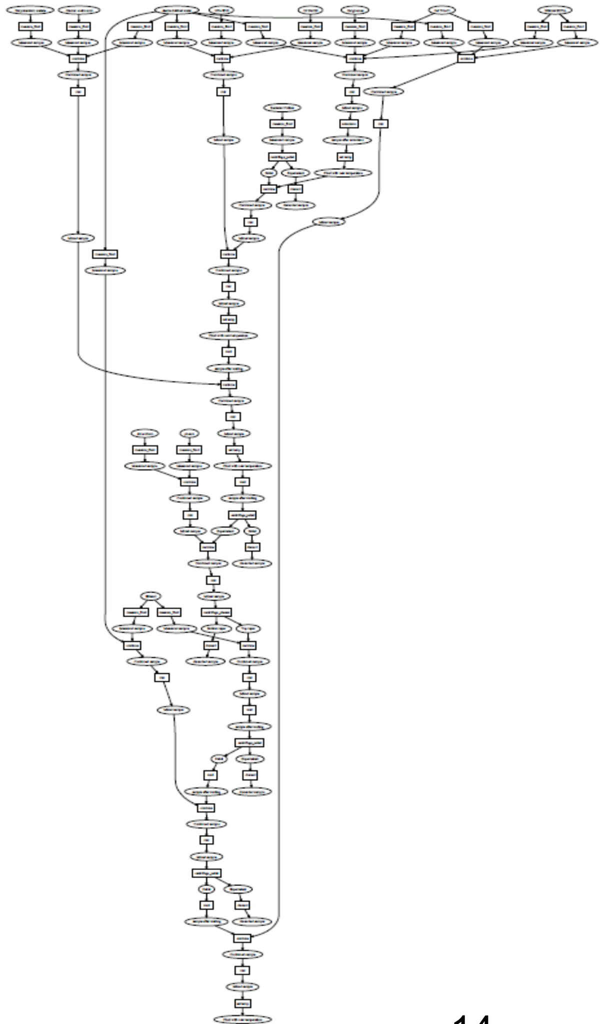
Equipment:

- Centrifuge
- Microfuge

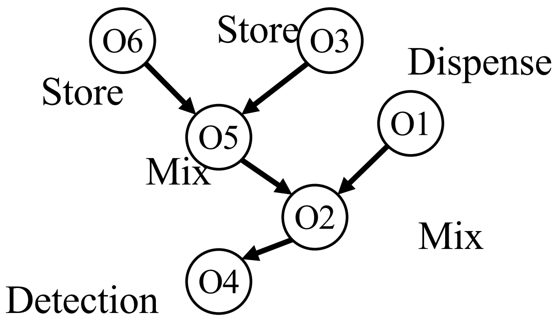
Steps:

1. Measure out **600 µl** of bacterial culture grown in LB medium into a 1.5ml- reaction tube.
2. Add **100 µl** of 7X Lysis Buffer(Blue).
   Invert the tube **4- 6** times.
   NOTE: Proceed to the next step within **2 mins**.
3. Add **350 µl** of Neutralization Buffer(Yellow).
   Vortex the mixture for a few secs.

Auto-Generated Dependence Graph



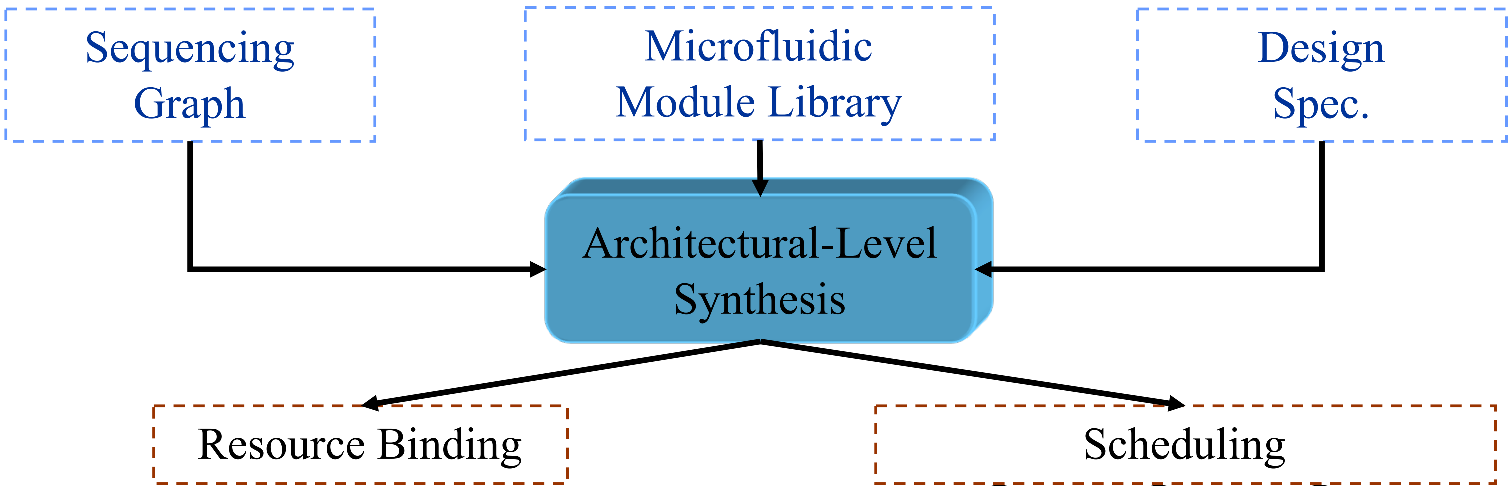
Architecture-Level Synthesis



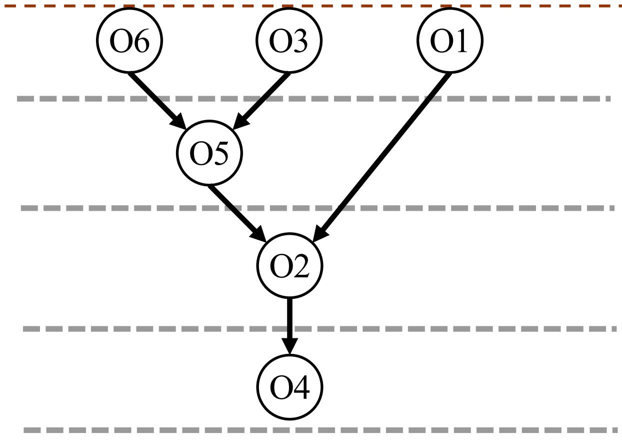
Resource	Area	Time
Mixer	2x2-array	7
Mixer	1x3-array	4
LED	1x1 cell	10
Storage	1x1 cell	N/A

Max. Area: 5x5 array

Max. Completion Time: 50 seconds

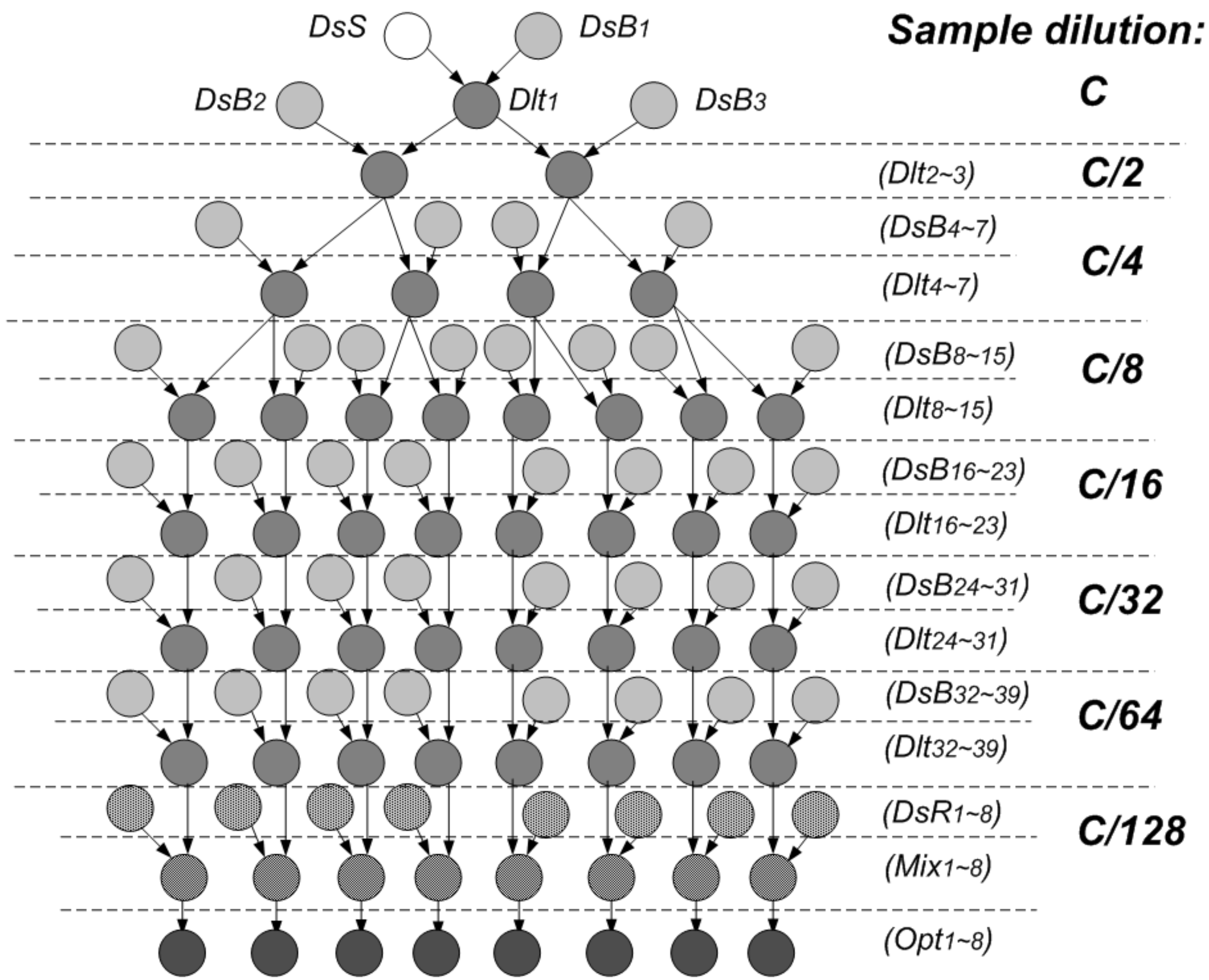


Operation	Resource
O1	On-chip
O2	2x2-array
O3	1x1 cell
O4	LED
O5	1x3-array
O6	1x1 cell



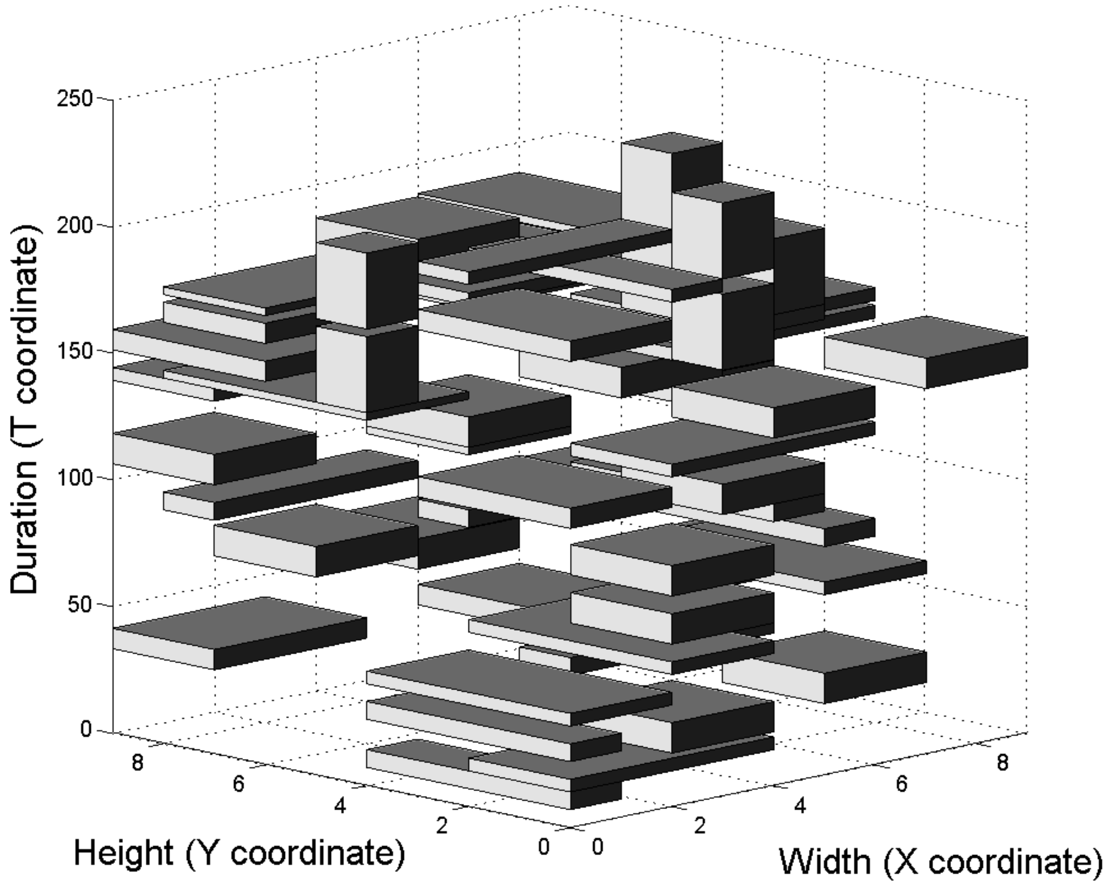
Protein Assay

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*

Resulting Placement of the Protein Assay

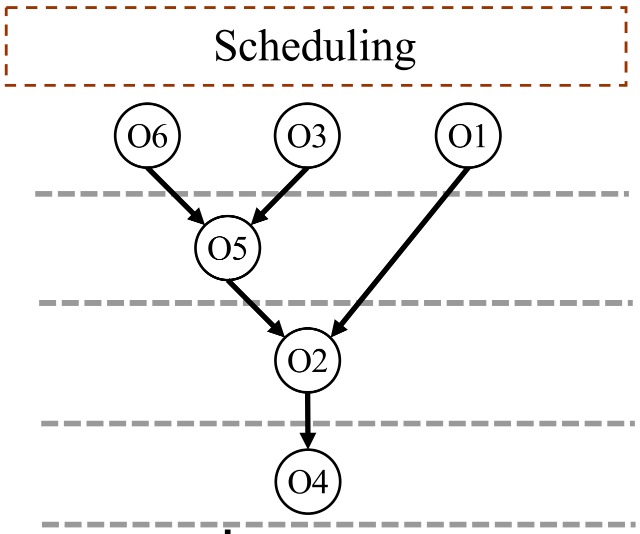


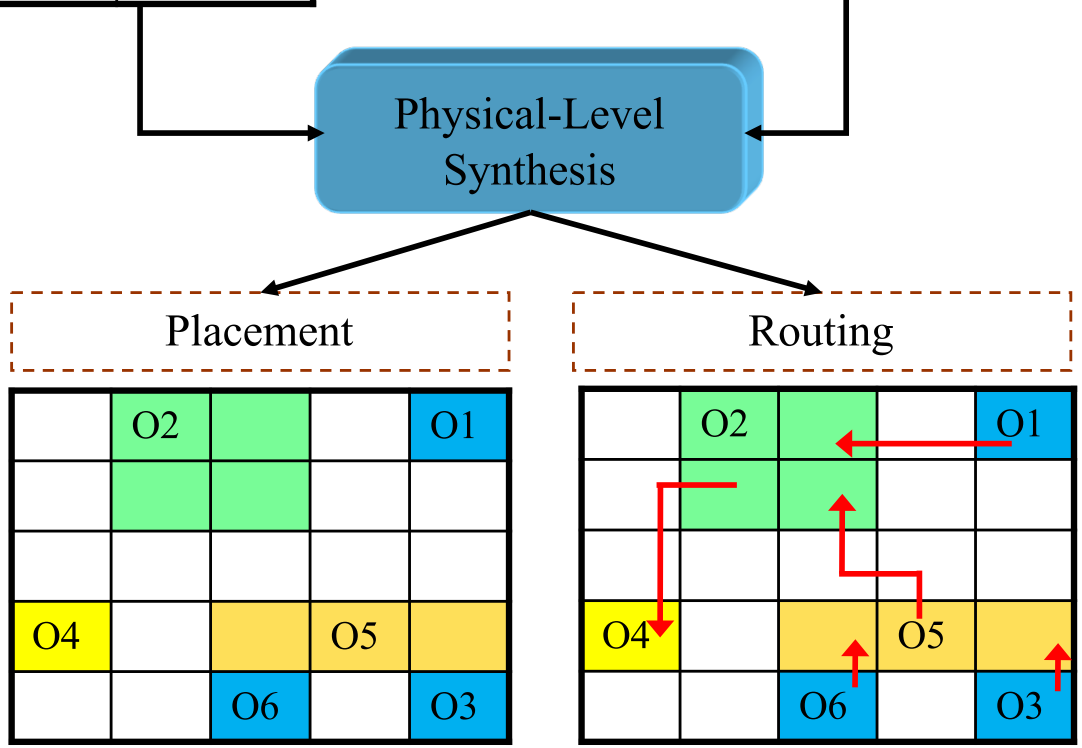
Volume = 9x9x241 (10x10x400 fixed-cube constraint)

Physical-Level Synthesis

Resource Binding

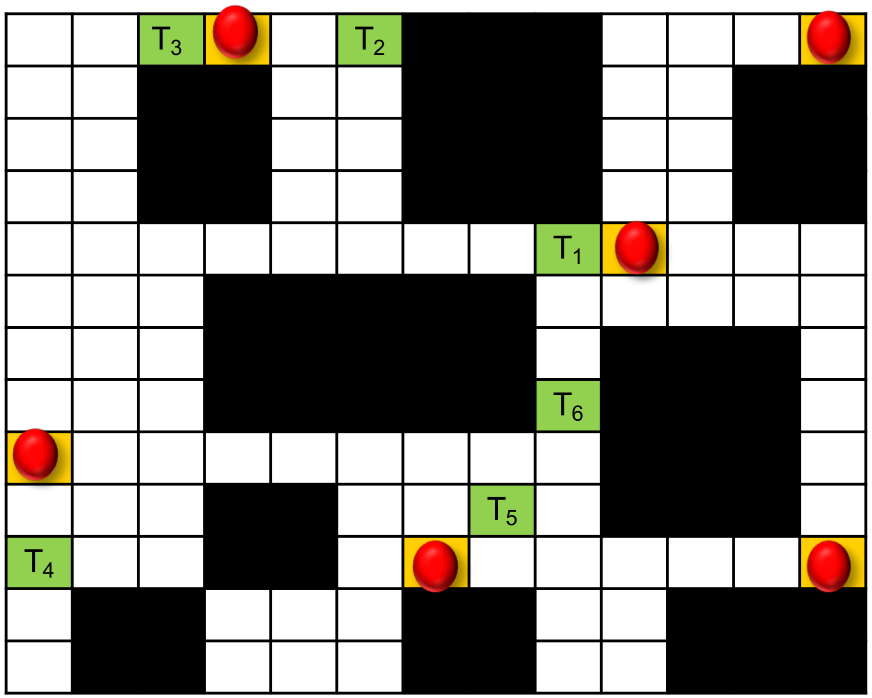
Operation	Resource
O1	On-chip
O2	2x2-array
O3	1x1 cell
O4	LED
O5	1x3-array
O6	1x1 cell



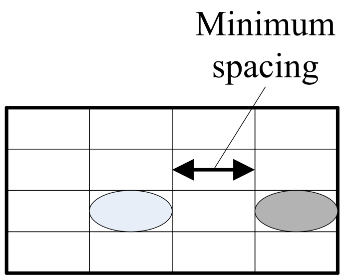


Droplet Routing

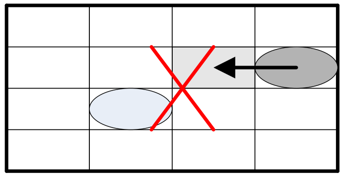
- **Input:** A netlist of *n droplets* $D = \{d_1, d_2, \ldots, d_n\}$, *the* locations of m blockages $B = \{b_1, b_2, \ldots, b_m\}$, and the timing constraint T_{max}.
- **Objective:** Route all droplets from their sources to their targets.
- **Constraint:** Both fluidic and timing constraints are satisfied.



- Fluidic constraint



Static fluidic constraint



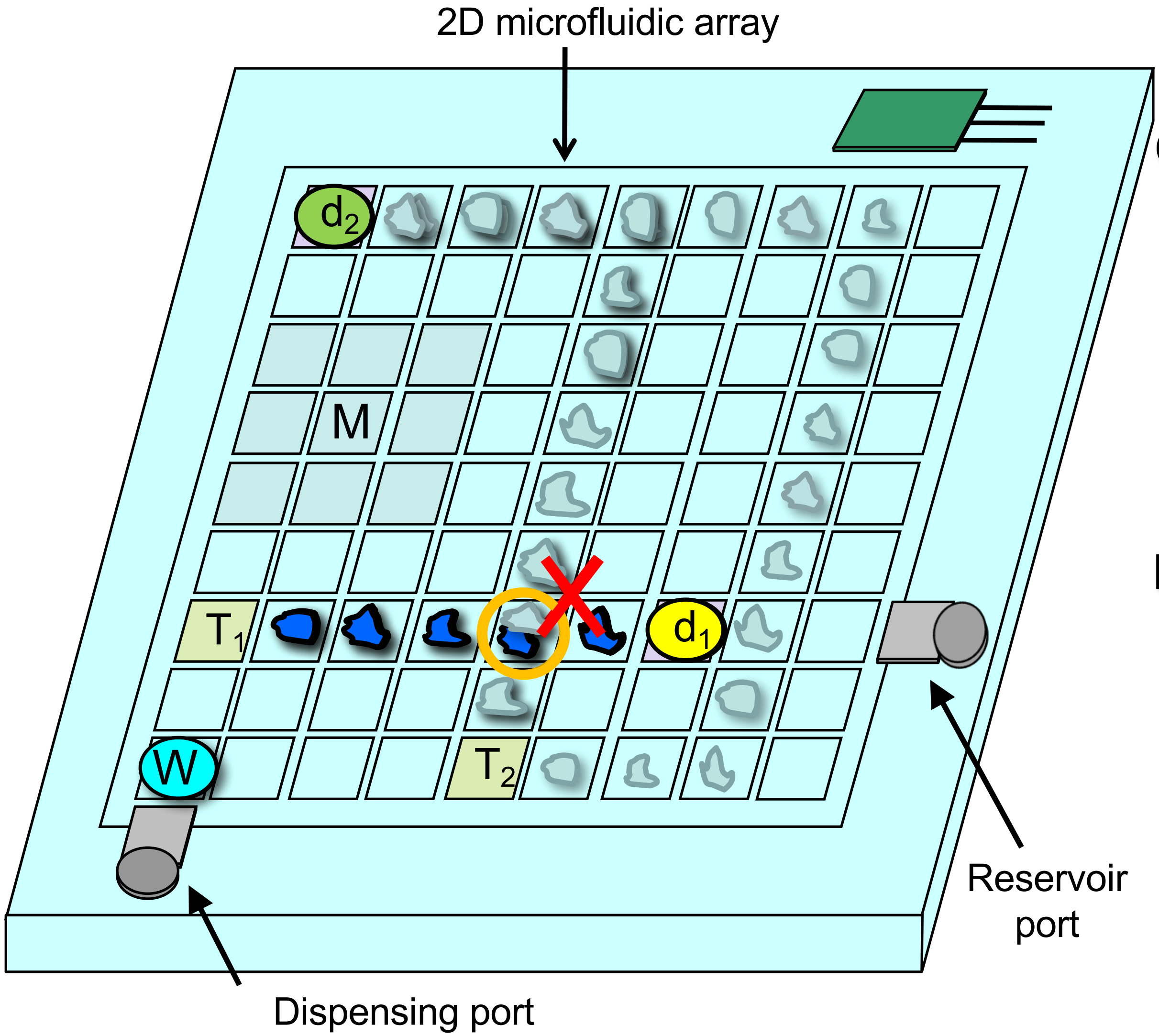
Dynamic fluidic constraint

- Timing constraint

Droplet   Blockage S_i Source of droplet *i* T_i Target of droplet *i*

Contamination Problem

- Contamination problem



Contamination problem

↓

Disjoint routes

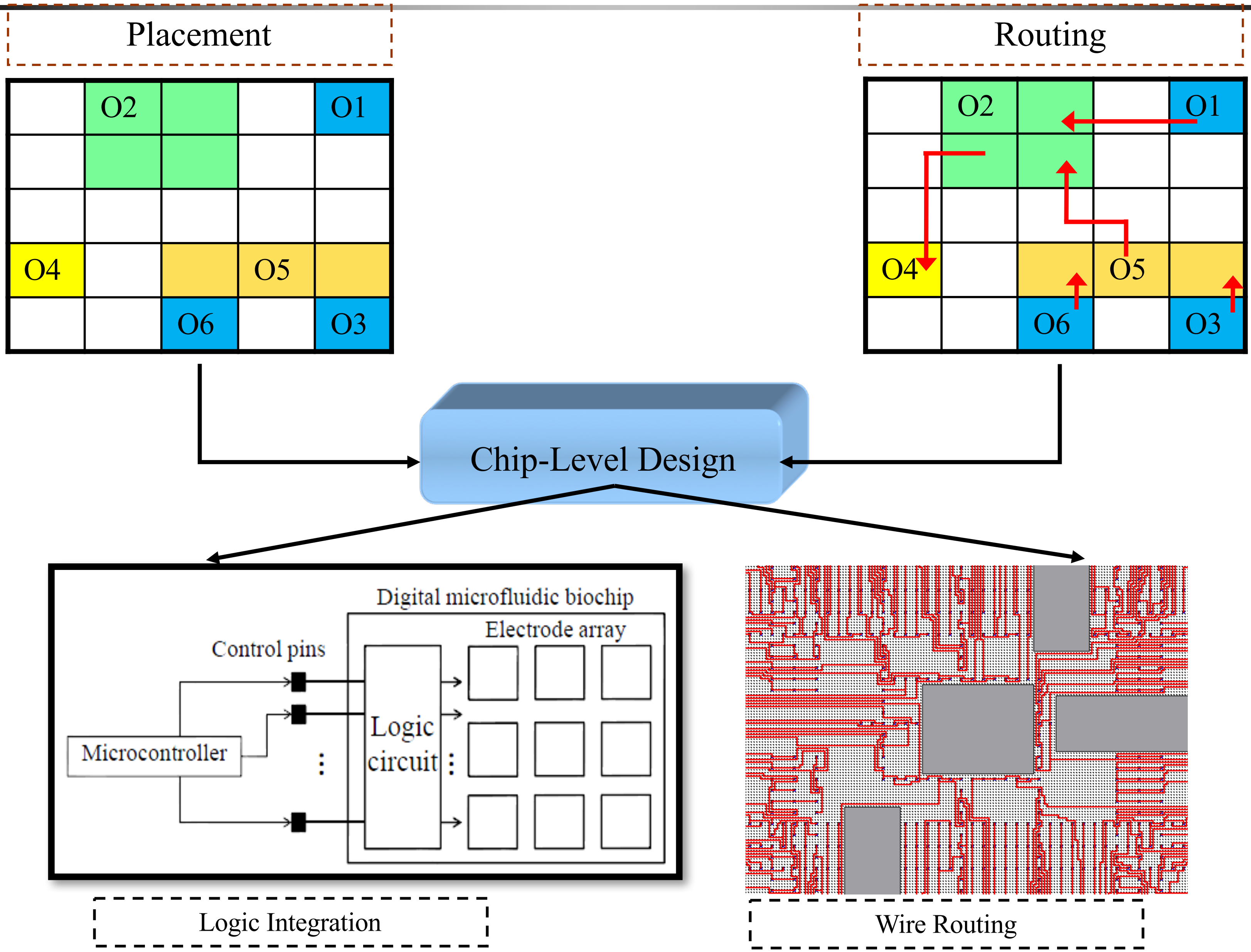
↓

Routing with the wash droplet

(1) separately

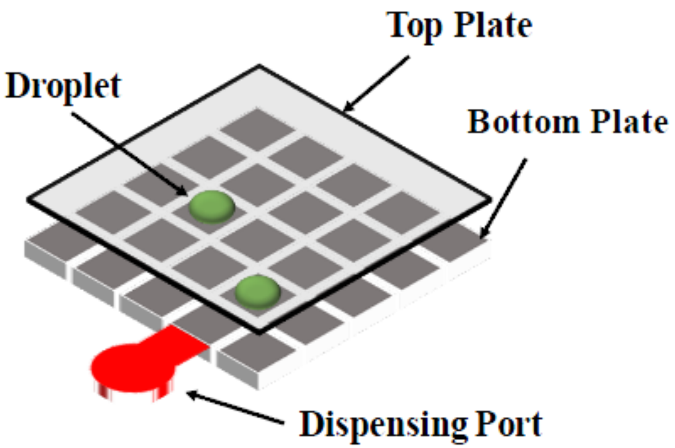
(2) simultaneously

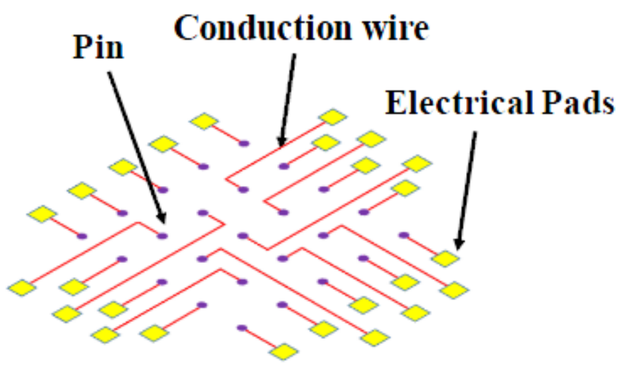
Chip-Level Design



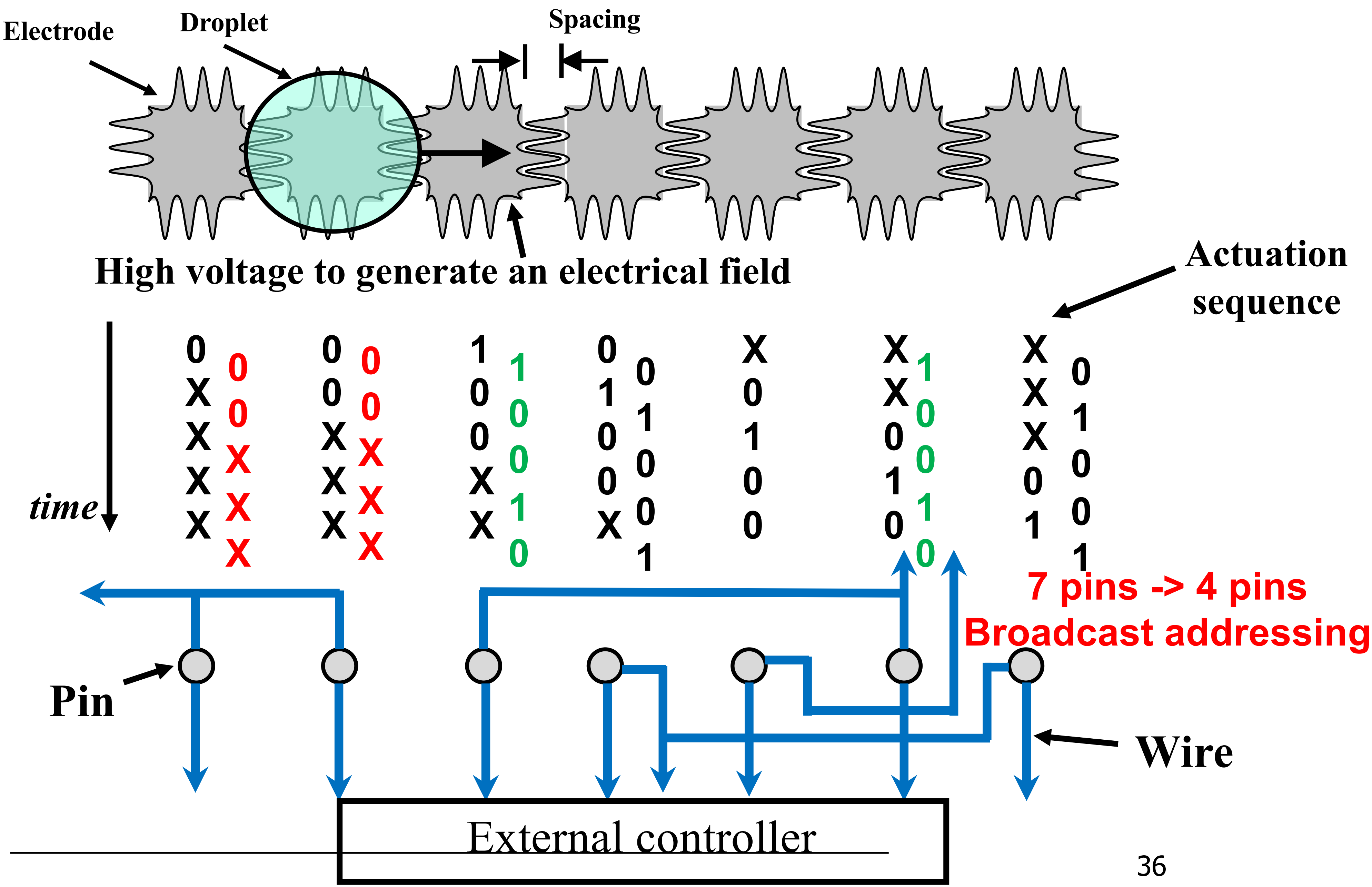
Chip-Level Co-Design

- 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



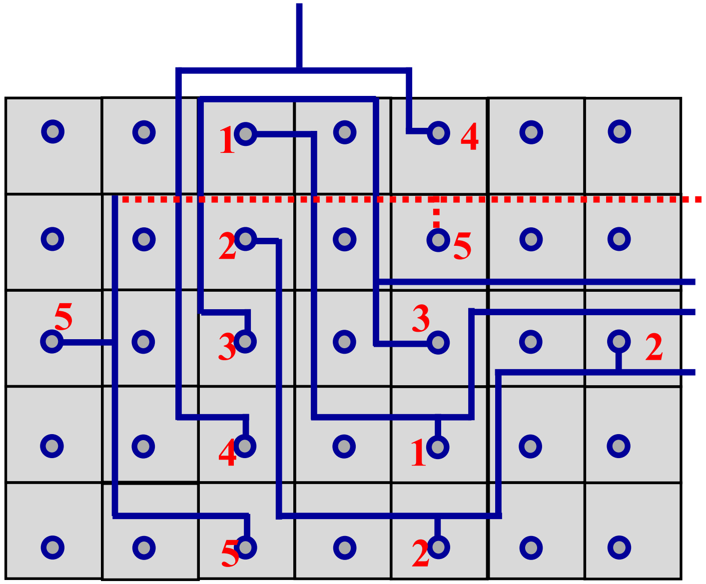


Broadcast Electrode Addressing Scheme

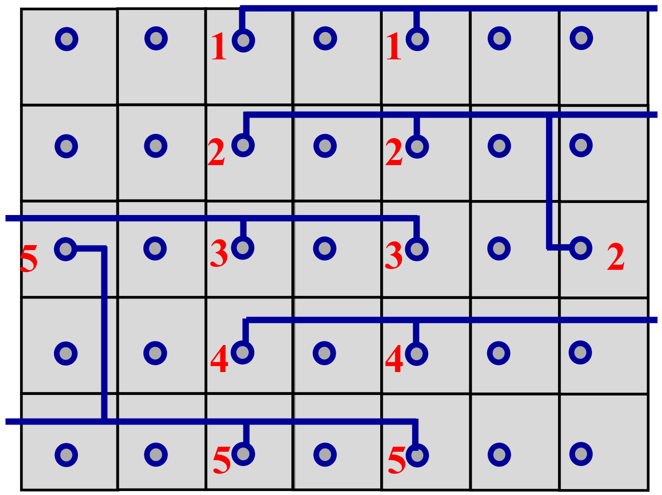


Impacts on PCB Routing

5 control pins



(a) Infeasible routing solution

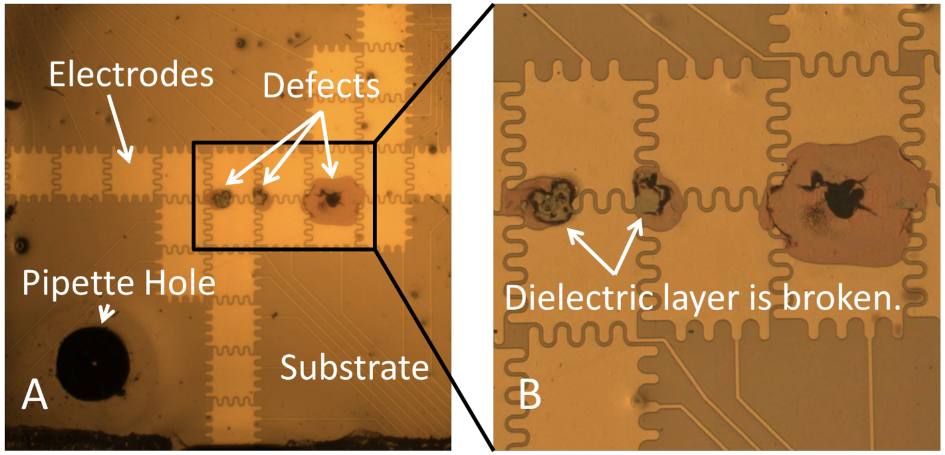


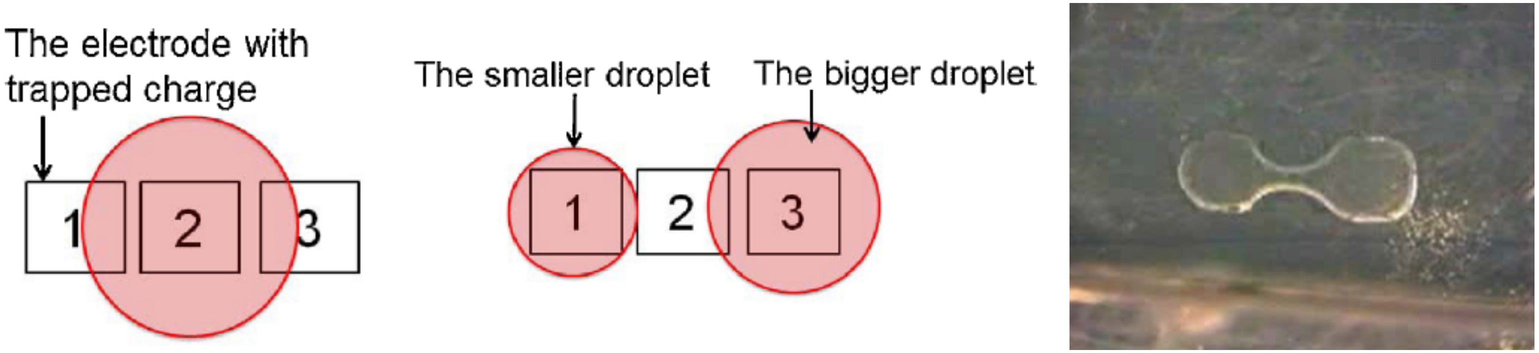
(b) Feasible routing solution

T.-W. Huang, S.-Y. Yeh and T.-Y. Ho, "A Network-Flow Based Pin-Count Aware Routing Algorithm for Broadcast Electrode-Addressing EWOD Chips," *Proceedings of IEEE/ACM ICCAD* 2010 (IEEE TCAD 2011).

Testing

- A microfluidic biochip can fail due to following reasons
  - Dielectric degradation
  - Irreversible charge concentration
  - Misalignment of parallel plates
  - Non-uniform dielectric layer
  - Grounding failure
  - Broken connection to the control source



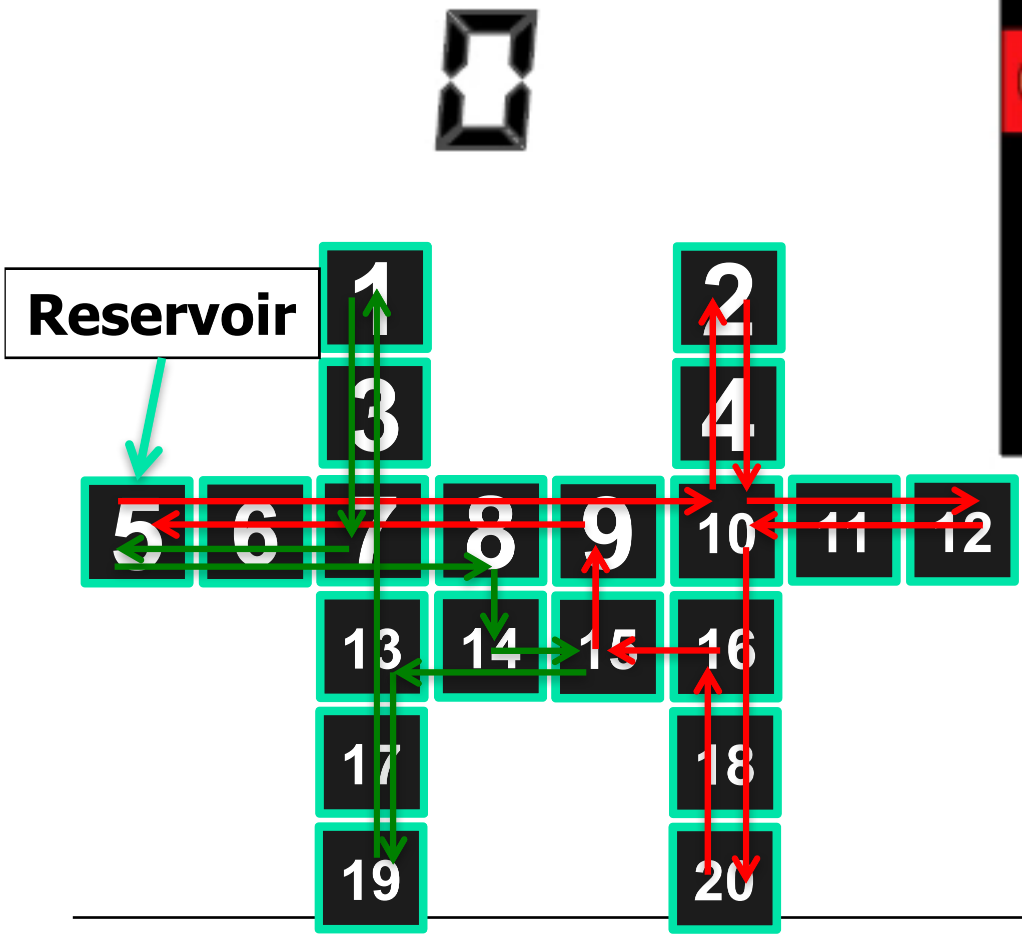


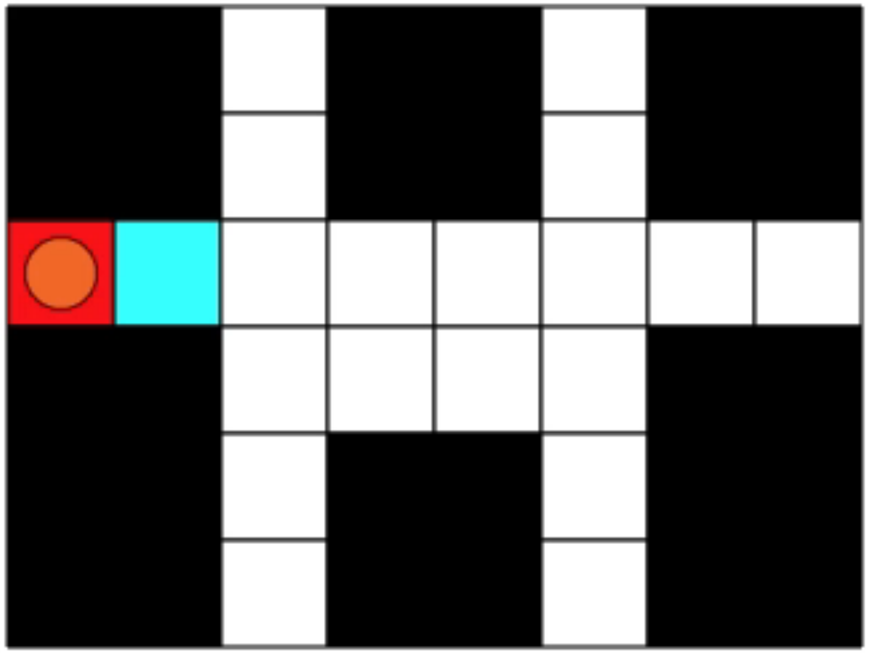
(a) (b) (c)

(a) Error caused by residual-charge problem; (b) Splitting operation with droplets of unbalanced volumes; (c) Imperfect split operation

Test on Arbitrary Layouts

2 test droplets





Amino Acid Synthesis

min-max K Chinese Postmen Problem

INPUT

- An undirected graph G = (V, E)
- A starting node v_{start}
- The number of Postmen: K

OUTPUT

- A set of K circuits starting and ending at v_{start}

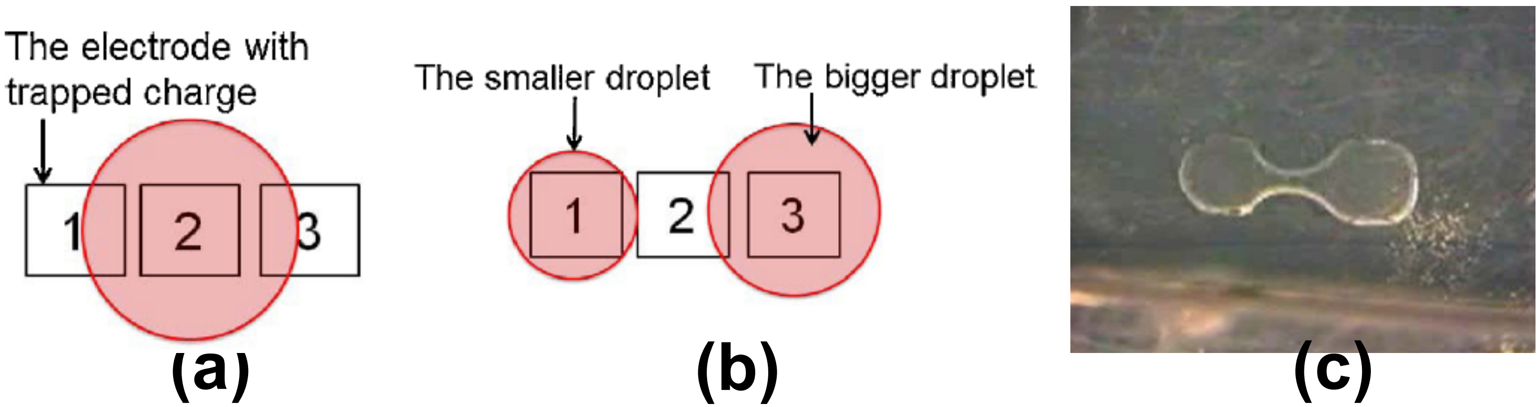
OBJECTIVES

- Every edge is traversed by **at least** 1 circuit
- The length of the longest circle is **minimized**

T. A. Dinh, S. Yamashita, T.-Y. Ho, and K. Chakrabarty, "A General Testing Method for Digital Microfluidic Biochips under Physical Constraints," *Proceedings of IEEE International Test Conference (**ITC-2015**)*, pp. 1-8, Anaheim, CA, October 2015.

Reliability Concerns in Digital Microfludics

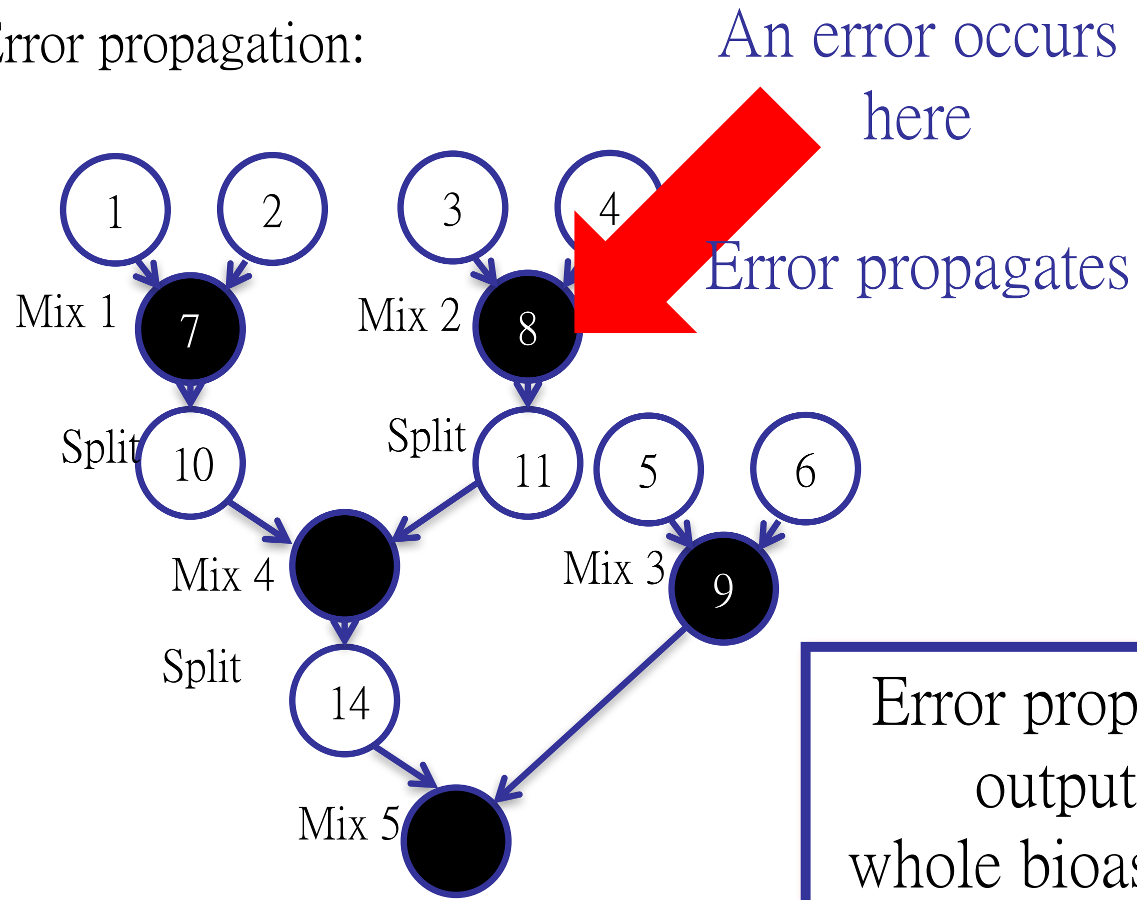
- Residual-Charge Problem
  - Electrode 1 has the residual-charge problem. High voltages are applied on electrode 1 and 3 for splitting operation
  - Trapped charge on electrode 1 will reduce the EWOD force
  - Droplet split by unequal force, and two resulting droplets may have unequal volumes



(a) Error caused by residual-charge problem; (b) Splitting operation with droplets of unbalanced volumes; (c) Imperfect split operation

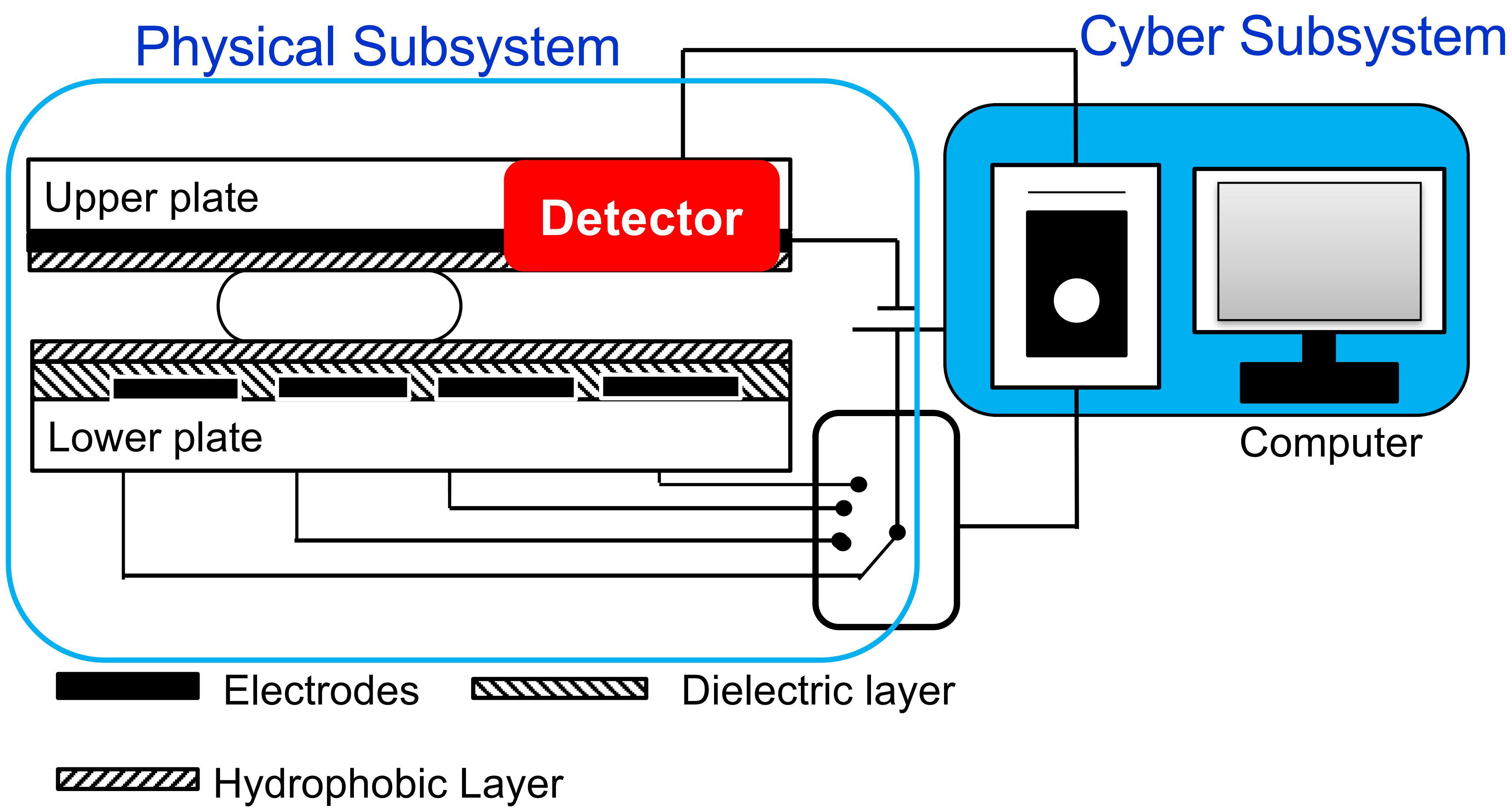
Errors and Error Propagation

Error propagation:



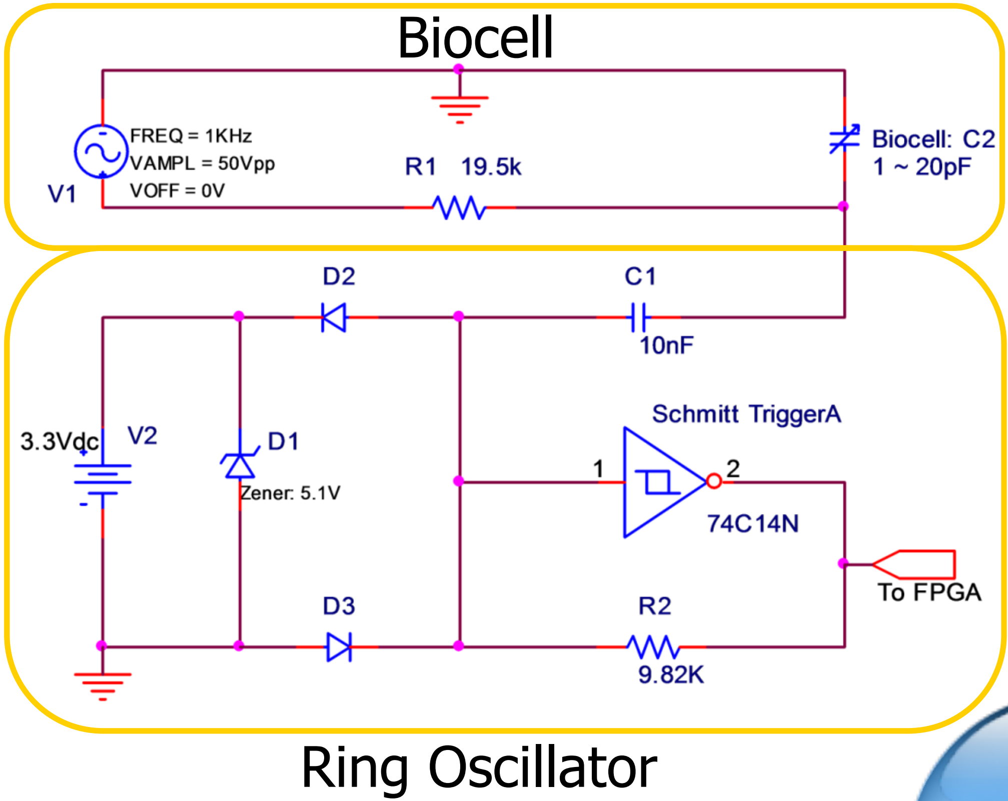
Error propagates to the output, and the whole bioassay has to be re-executed!

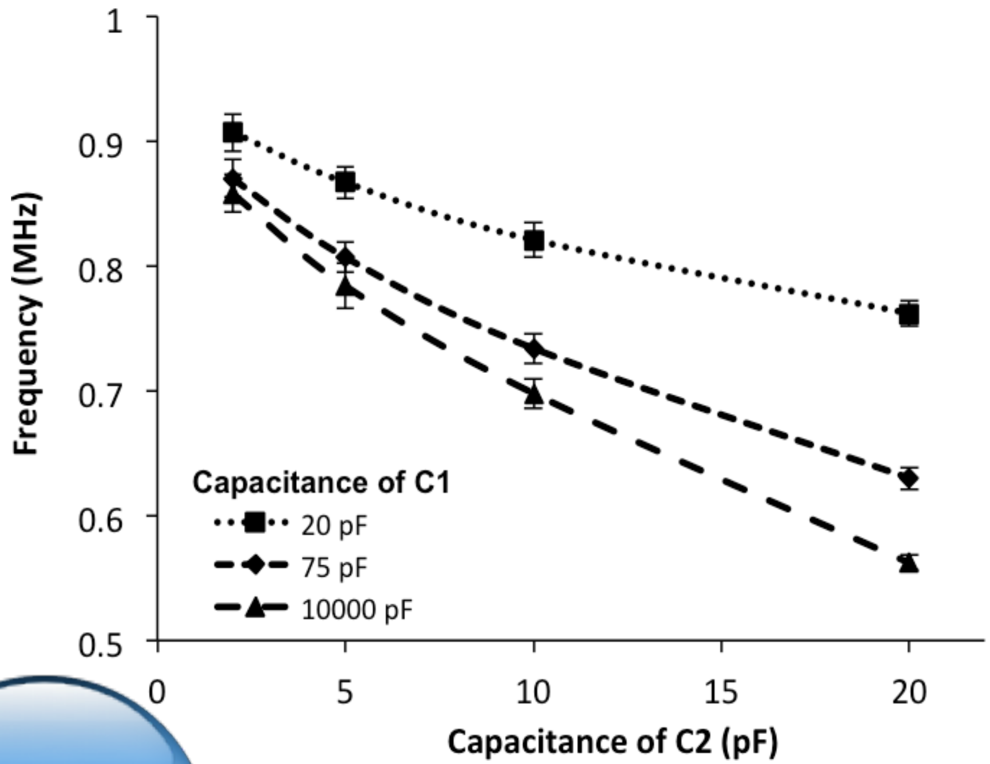
Cyber-Physical System Integration



Capacitive Sensing

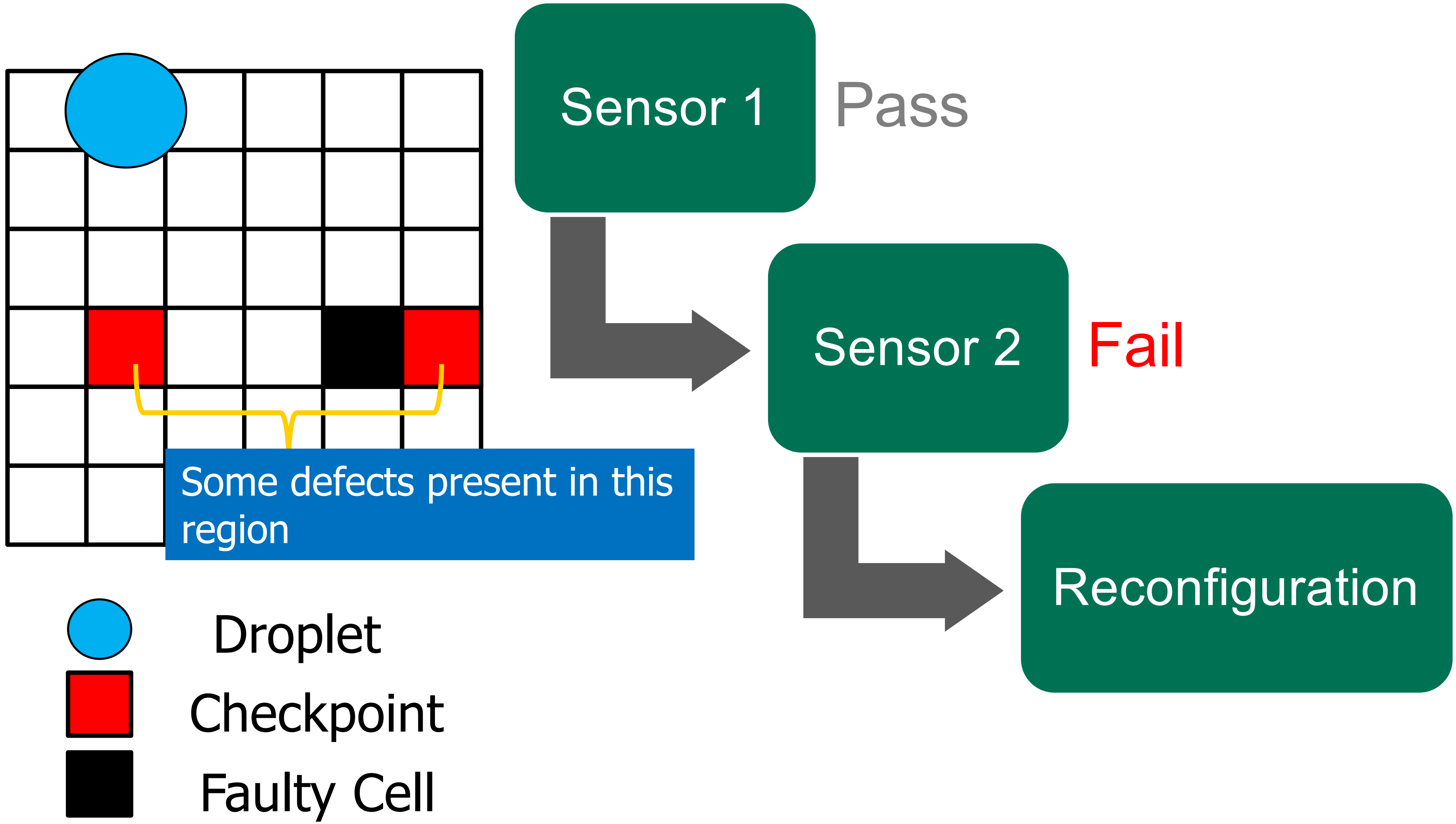
Monitor capacitance change → **Test for droplet presence**





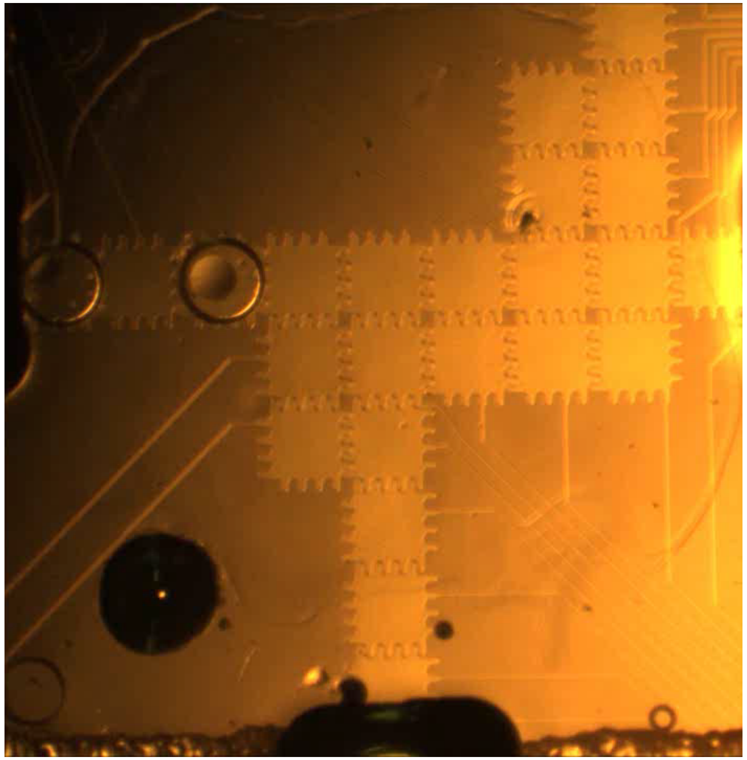
$$f \propto \frac{1}{C2}$$

Cyperphysical Error Recovery

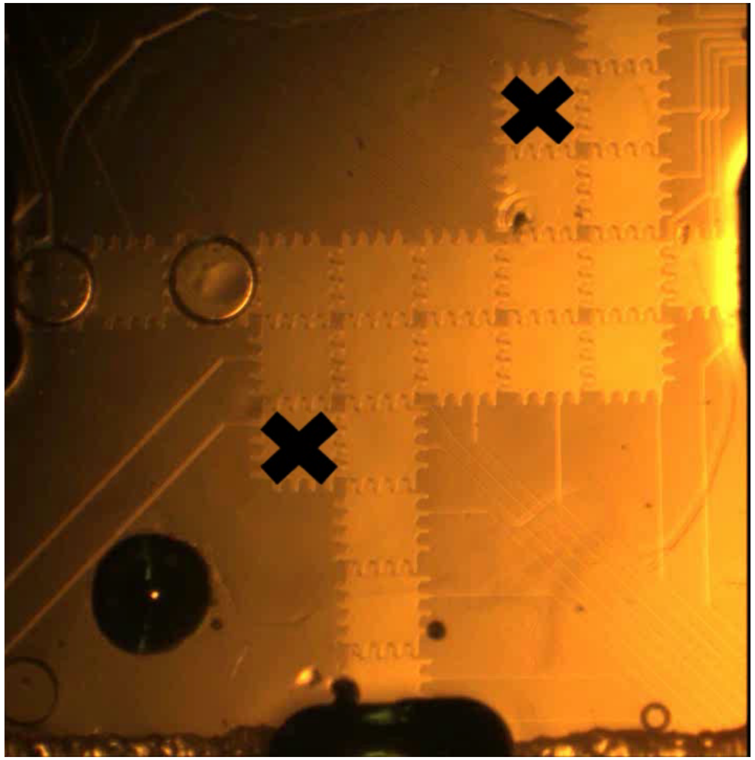


Cyber-Physical Error Recovery

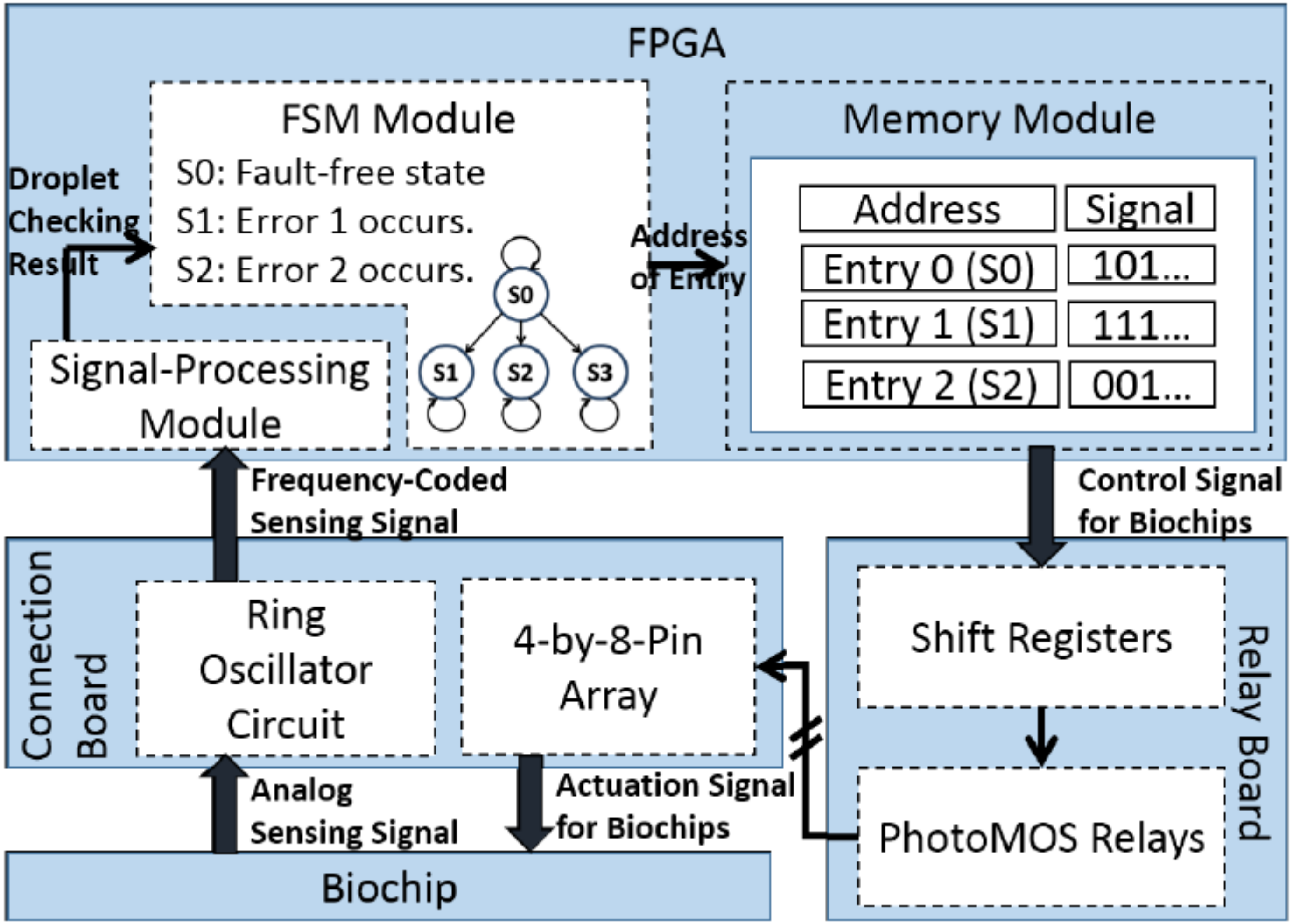
Fault Free



Defects in Region A & B

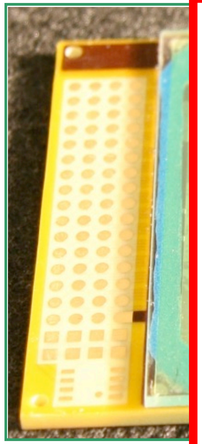


Integrated System for Error Recovery Using FPGA



Chip Sample from Advance Liquid Logic

Waste handling reservoir



Target genera

'5 mm;

Illumina's $96M Buys RTP's Advanced Liquid Logic

SUBMITTED BY JSHAMP ON TUE, 2013-07-23 17:18

Advanced Liquid Logic (ALL), a groundbreaking Research Triangle Park company built from Duke University research with North Carolina Biotechnology Center support, has been purchased for "up to $96 million" by San Diego gene-testing powerhouse Illumina.

The sale is the culmination of a bioscience success story with origins in the lab of Duke scientist Richard Fair, Ph.D.

NCBiotech awarded Fair a $50,000 Collaborative Funding Grant in 2003. He used the money to hire Vamsee Pamula, Ph.D., as a postdoc to work in Fair's lab with Michael Pollack, Ph.D. Pollack had been working since 1998 on developing and commercializing a tiny "lab" on a microchip, using a process known as microfluidics.

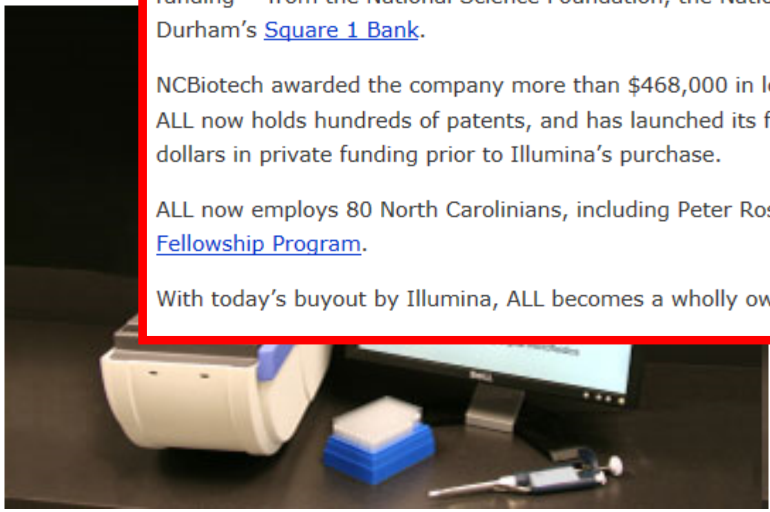
The following year, Pamula and Pollack formed ALL to advance the groundbreaking platform technology for analyzing complex genetic data, precisely manipulating small droplets within a sealed disposable cartridge.

Pamula says the early NCBiotech grant not only helped keep the research program alive, but it also led to millions of dollars in subsequent funding -- from the National Science Foundation, the National Institutes of Health and, more recently, a term loan and revolving credit from Durham's Square 1 Bank.

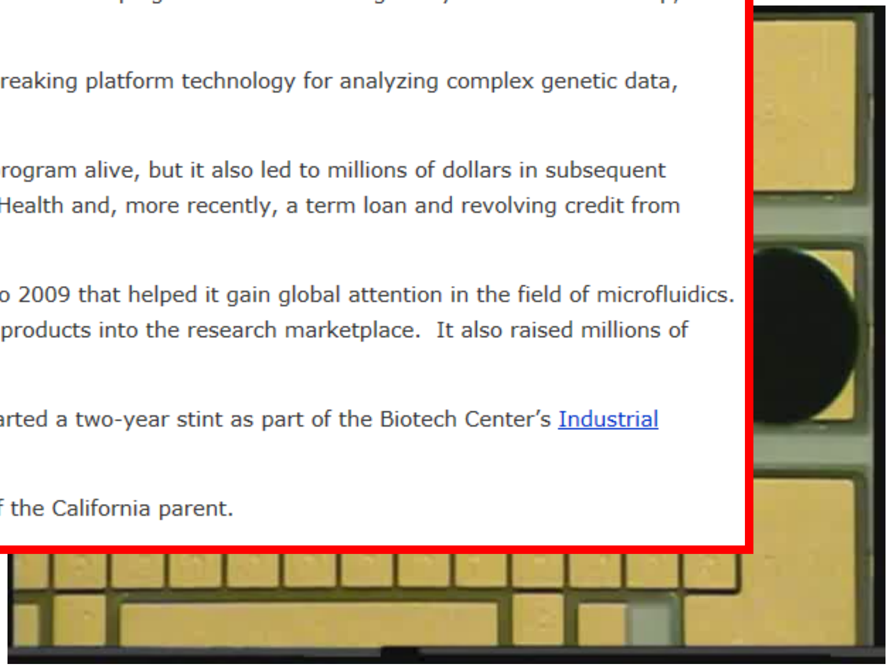
NCBiotech awarded the company more than $468,000 in loans from 2005 to 2009 that helped it gain global attention in the field of microfluidics. ALL now holds hundreds of patents, and has launched its first gene-testing products into the research marketplace. It also raised millions of dollars in private funding prior to Illumina's purchase.

ALL now employs 80 North Carolinians, including Peter Ross, Ph.D., who started a two-year stint as part of the Biotech Center's Industrial Fellowship Program.

With today's buyout by Illumina, ALL becomes a wholly owned subsidiary of the California parent.



Application Platform



NeoPrep Library Prep System

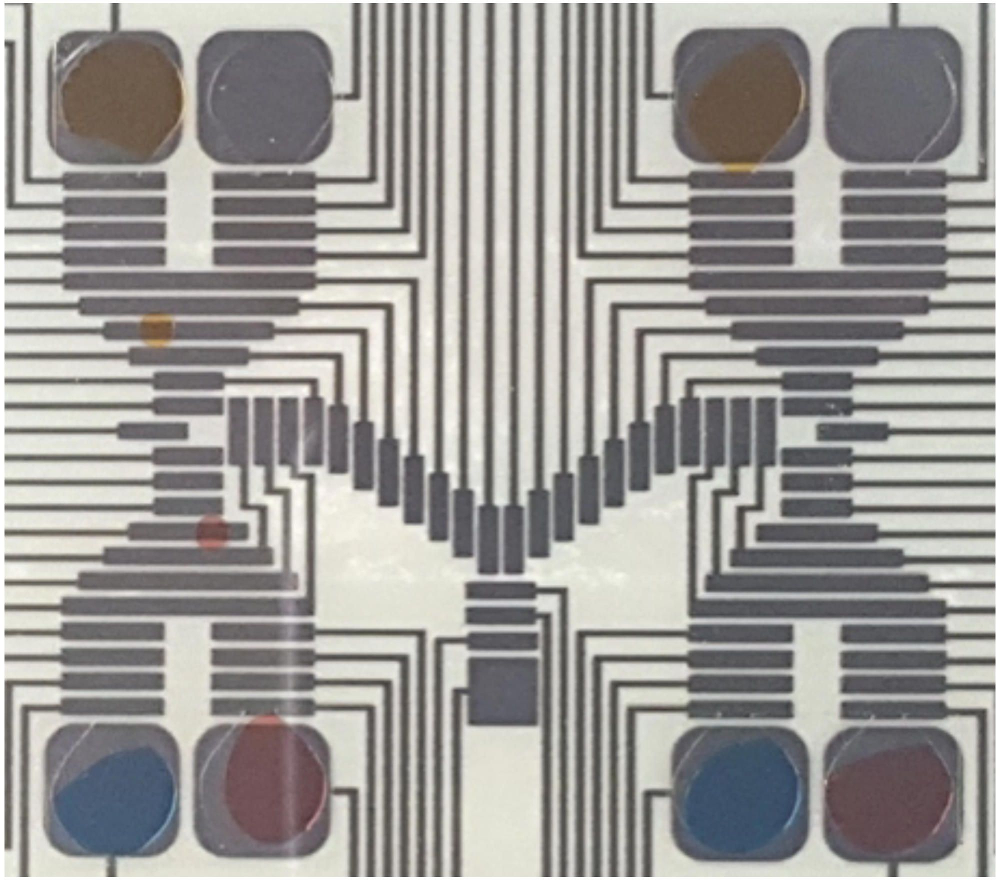
"Next Generation Sequencing Technology"

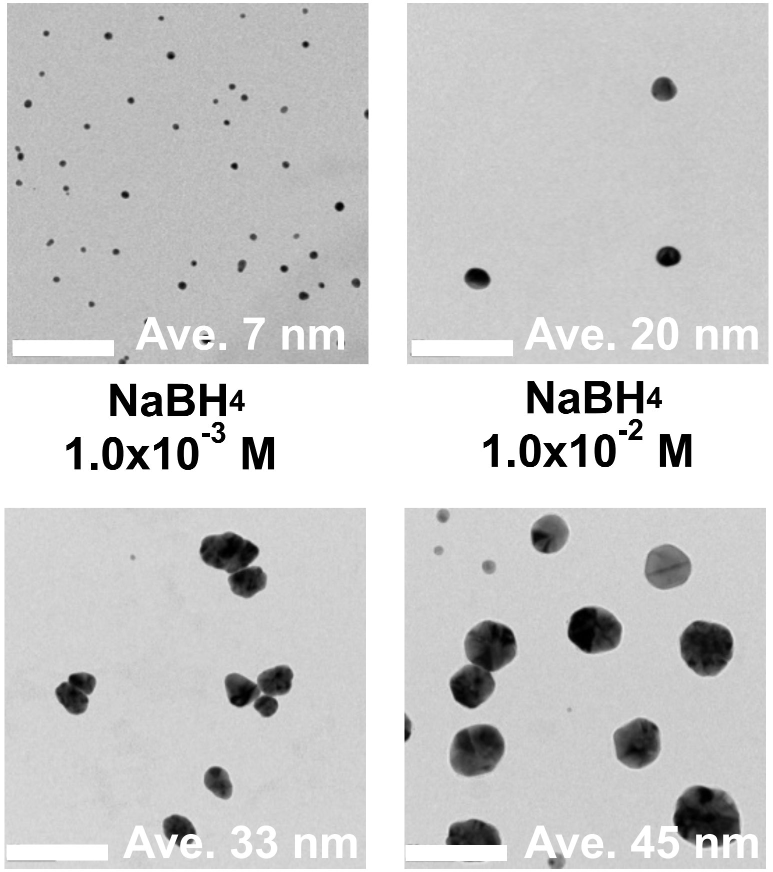
Paper-Based Digital Microfluidics

Source: Sogang Univ.

Synthesis of AuNPs on paper reactors

CTAB + $HAuCl_4$ $\xrightarrow{NaBH_4}$ AuNPs (Seed Solution)





$NaBH_4$ 1.0×10^{-3} M

$NaBH_4$ 1.0×10^{-2} M

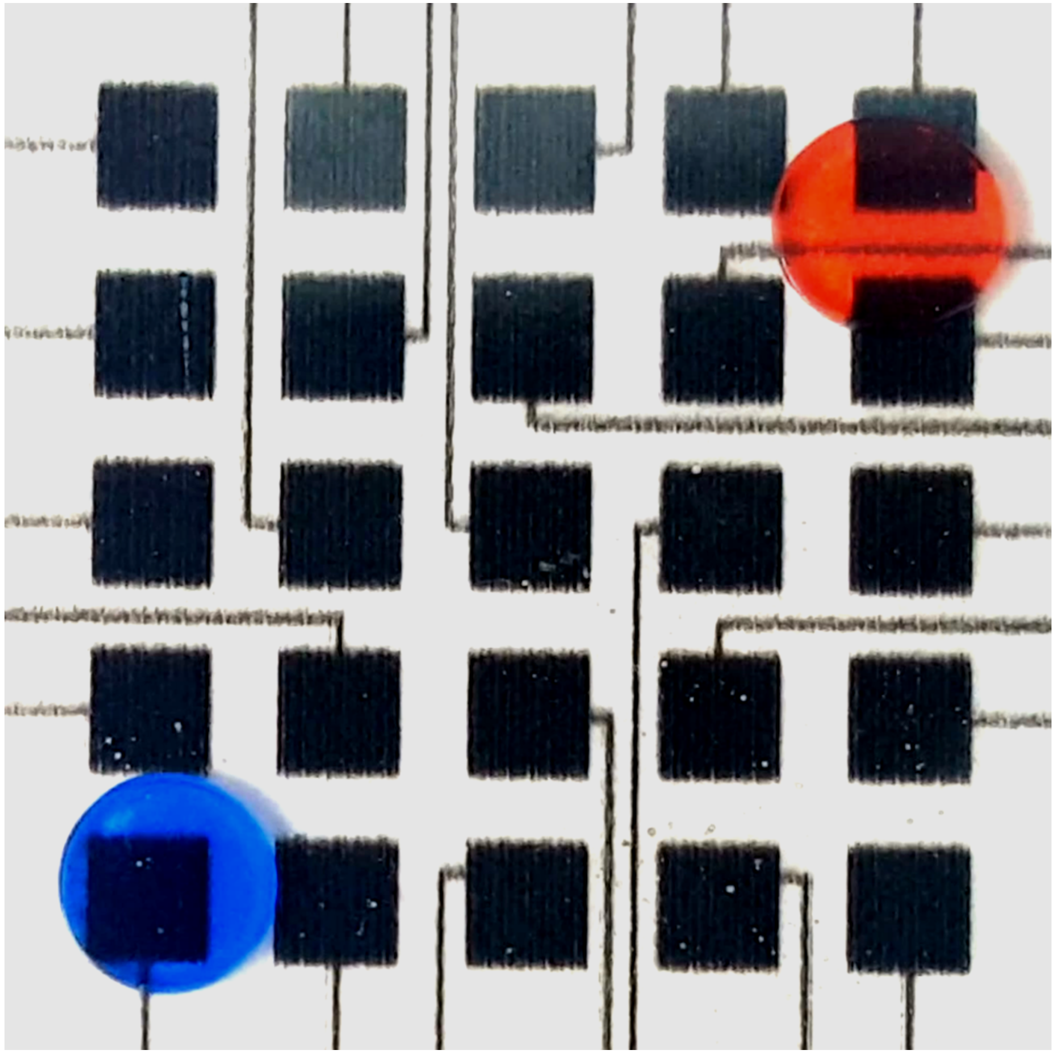
$NaBH_4$ 1.0×10^{-1} M

$NaBH_4$ 1.0×10^{0} M

Scale bar: 100nm

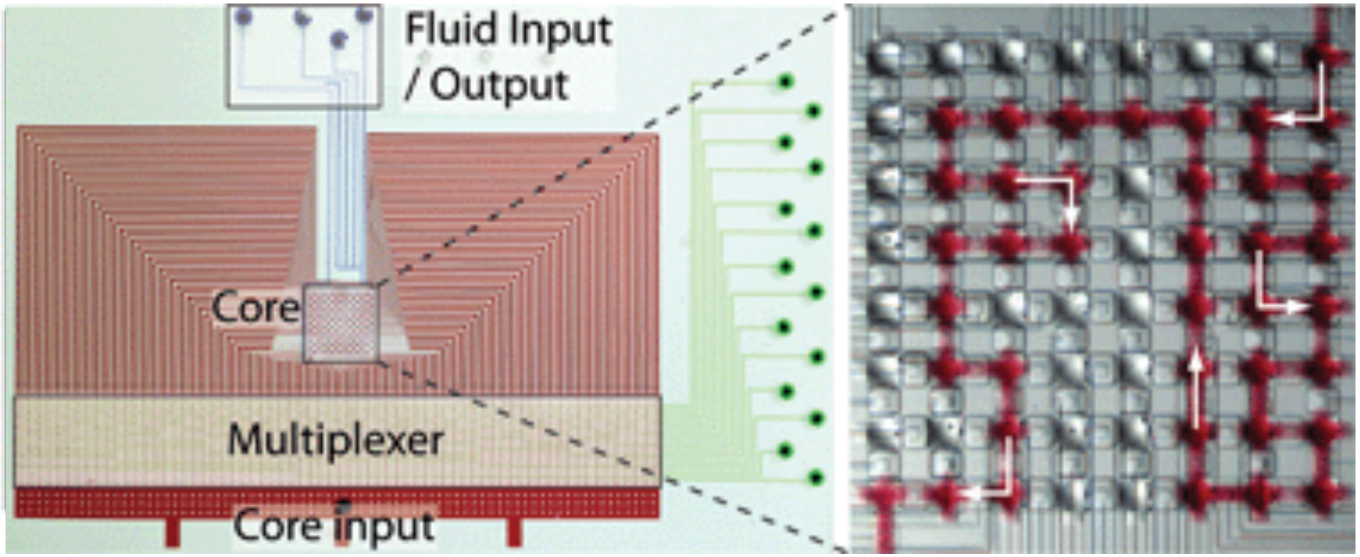
Design Challenge

- Control interference issue



Software-Programmable Microfluidic Devices (PMDs)

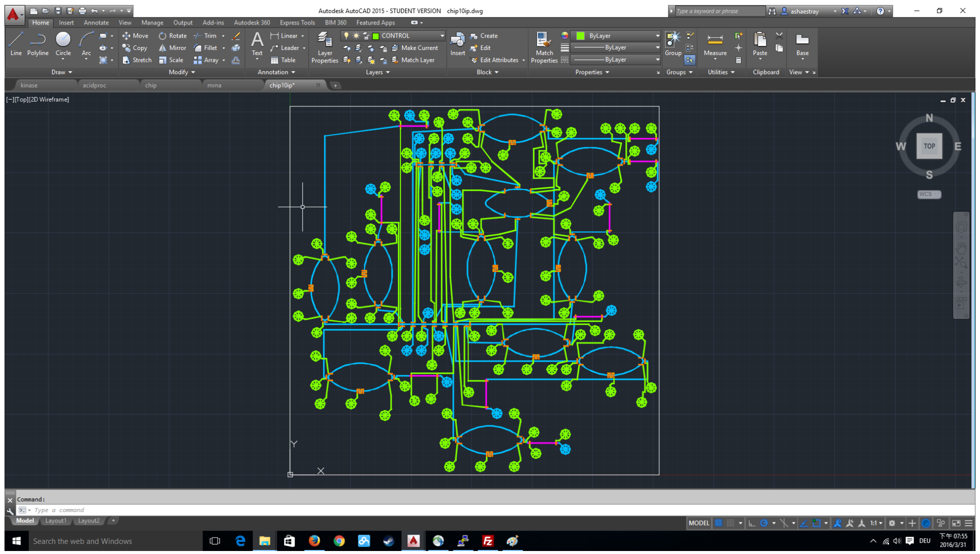
- Achieve multitude functions without hardware modifications
- Control chips with pure software programs
- Lower cost
- Higher throughput



* L. M Fidalgo, and S. J. Maerkl, A software-programmable microfluidic device for automated biology," *Lab on Chip*, 11(9): pp. 1612-1619, 2011.

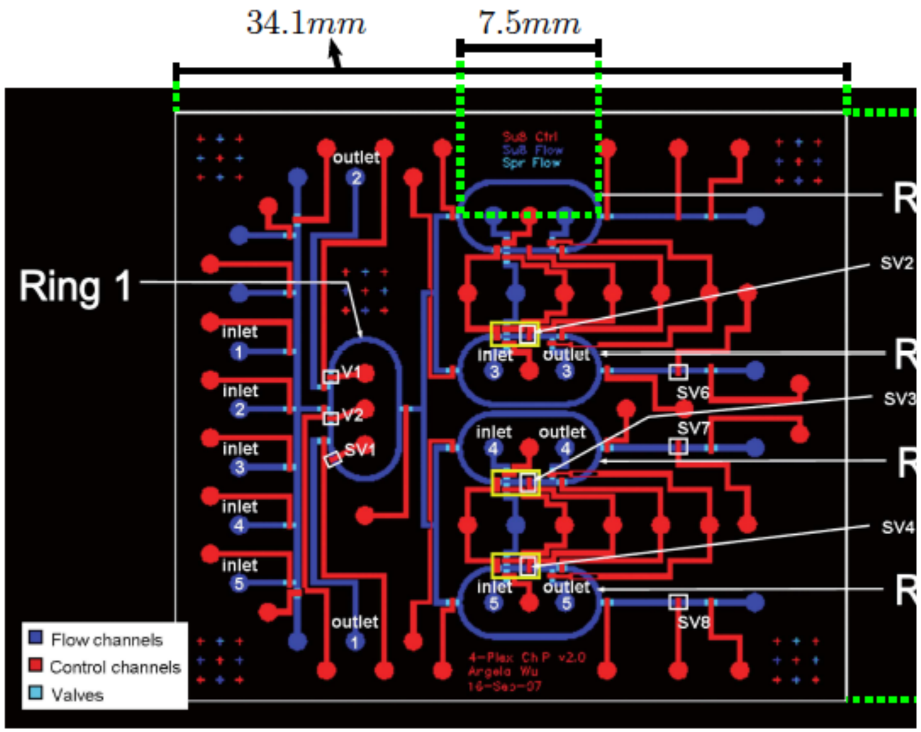
Automatic Layout Synthesis

A. R. Wu, J. B. Hiatt, R. Lu, J. L. Attema, N. A. Lobo, I. L. Weissman, M. F. Clarke, and S. R. Quake, "Automated Microfluidic Chromatin Immuno Precipitation from 2,000 Cells," Lab on a Chip, 9:1365–1370, 2009.

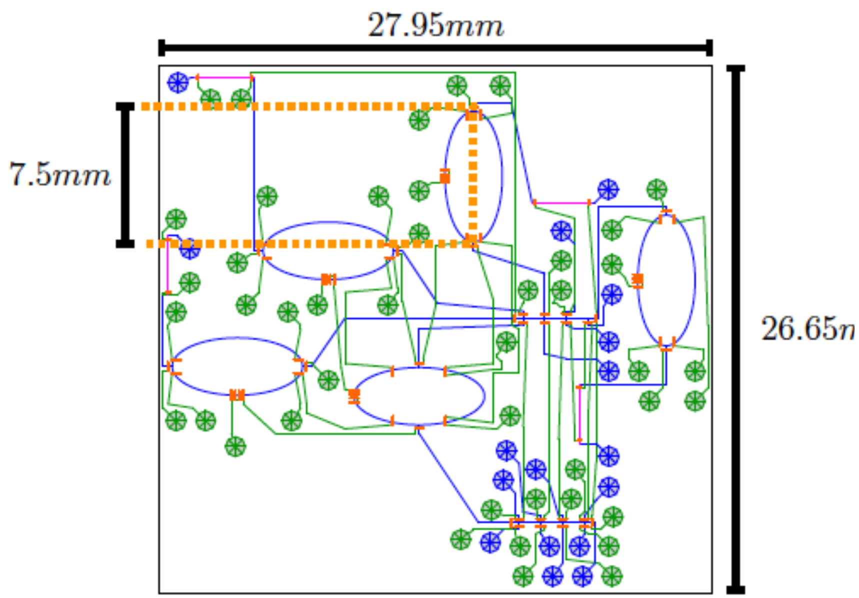


AutoCAD Compatible

T.-M. Tseng, M. Li, B. Li, T.-Y. Ho, and U. Schlichtmann, "Columba: Co-Layout Synthesis for Continuous-Flow Microfluidic Biochips," *Proceedings of ACM/IEEE Design Automation Conference (DAC-2016*, pp., Austin, TX, June 2016

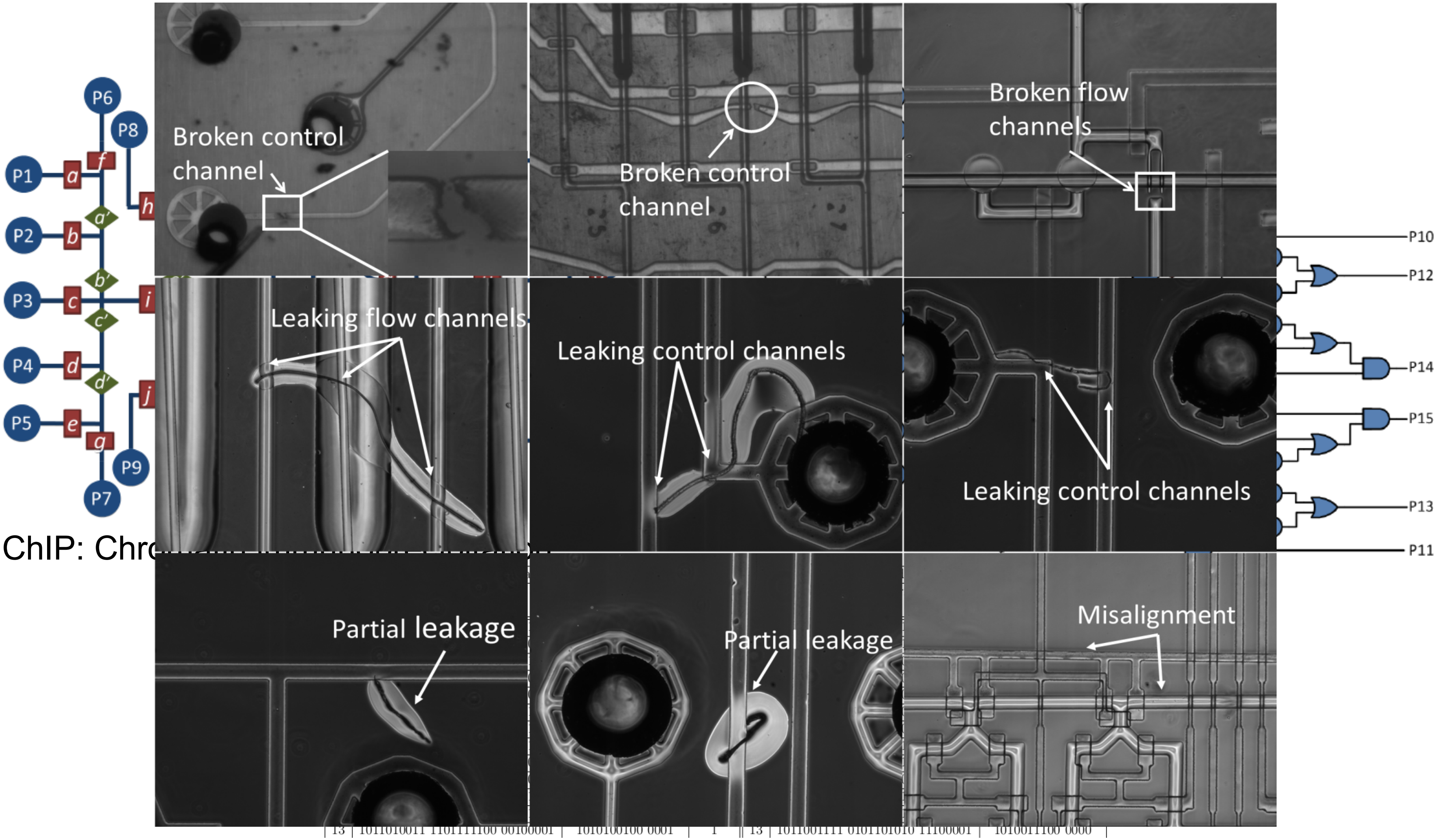


(a)



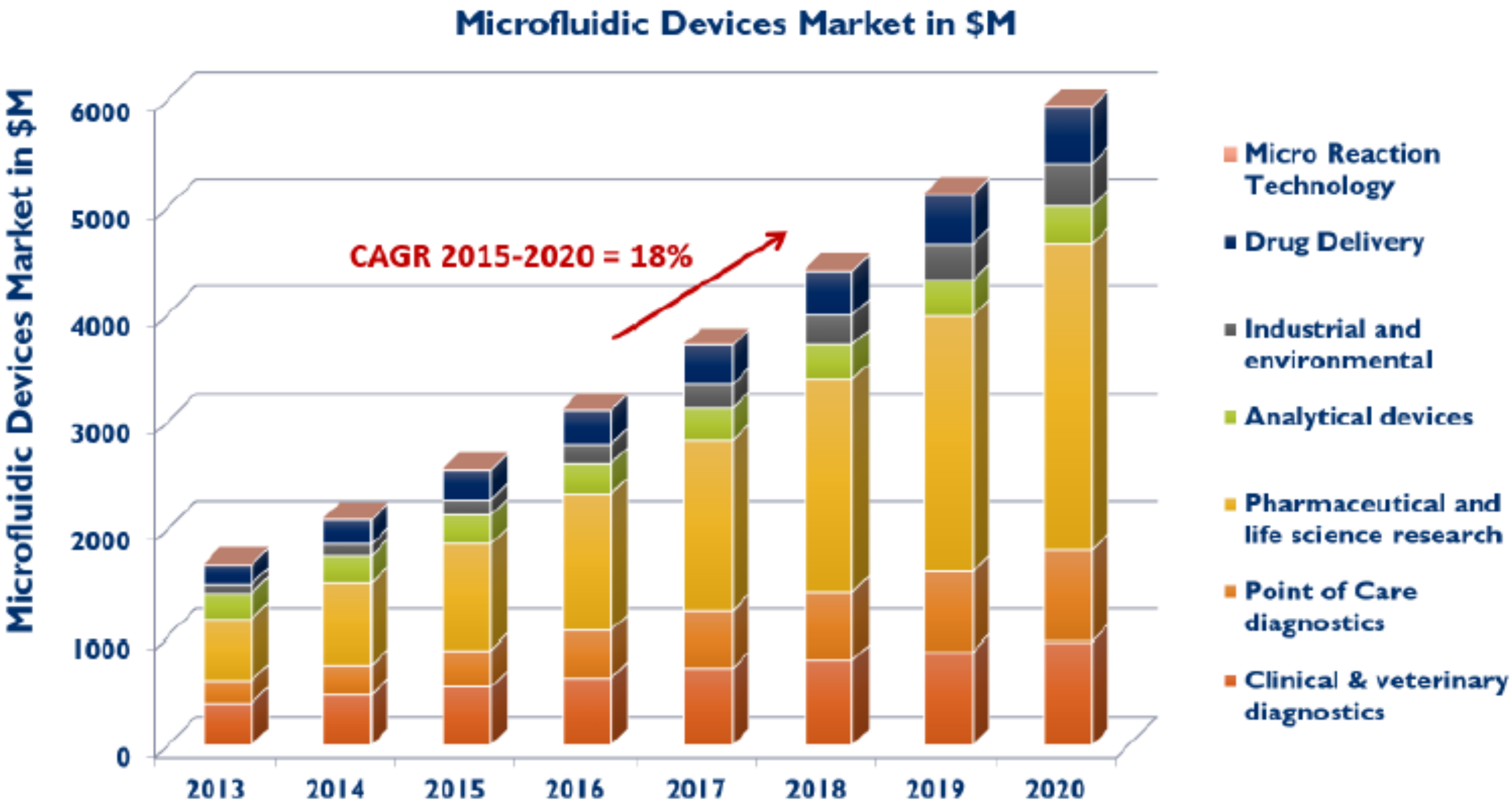
(b)

Testing



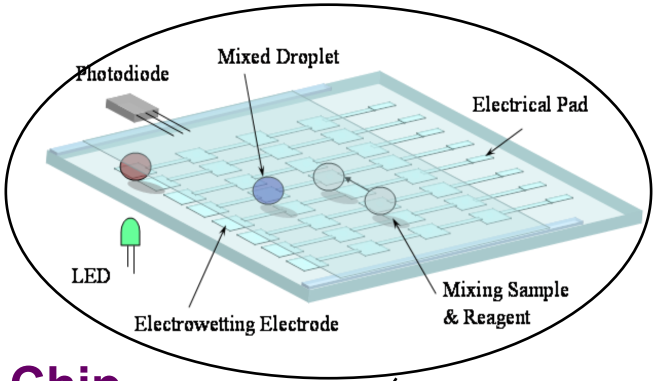
K. Hu, F. Yu, T.-Y. Ho, and K. Chakrabarty, "Testing of Flow-Based Microfluidic Biochips: Fault Modeling, Test Generation, and Experimental Demonstration," *IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems* (***IEEE TCAD***), vol. 33, no. 10, pp. 1463-1475, October 2014 (**Donald O. Pederson Best Paper Award 2015**).

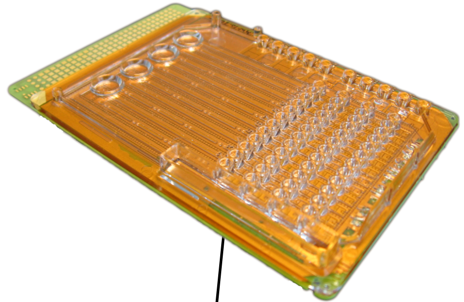
Microfluidic Market



Source: Yole Development

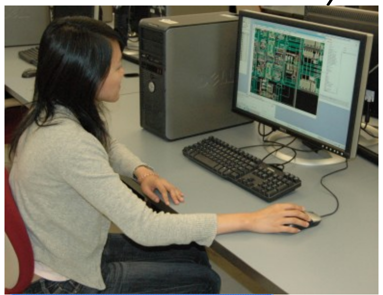
The Biochip Ecosystem

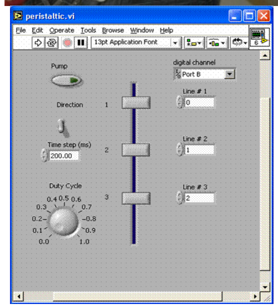


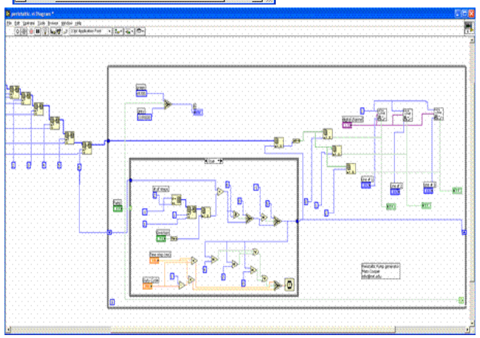


Chip Users

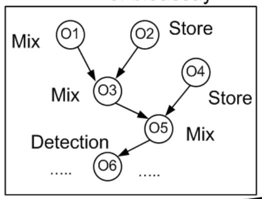
Chip Designers







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

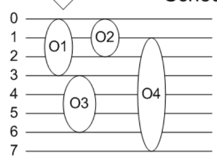
Unified Synthesis of Digital Microfluidic Biochip

Output:

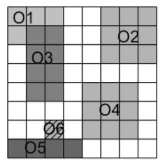
Resource binding

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

Thank You for Your Attention!