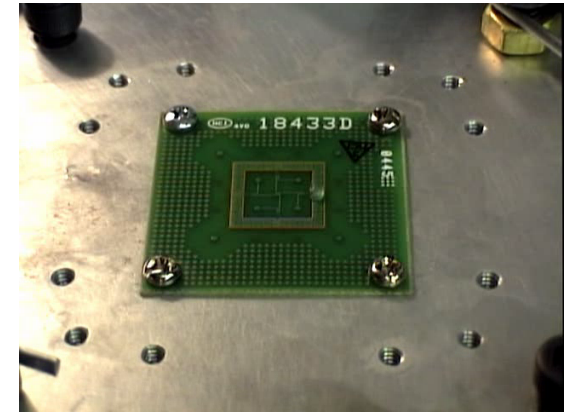
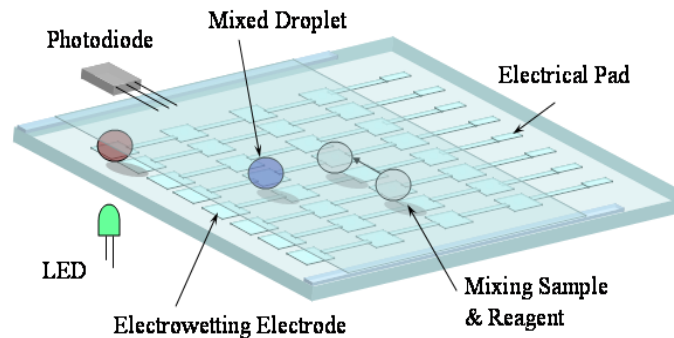


微流體生物晶片設計自動化



何宗易
Tsung-Yi Ho

Professor
Intelligent Design Automation Laboratory
Department of Computer Science
National Tsing Hua University
Hsinchu, Taiwan



Hans Fischer Fellow
Microfluidic Design Automation
Institute of Advanced Study
Technische Universität München
München, Germany

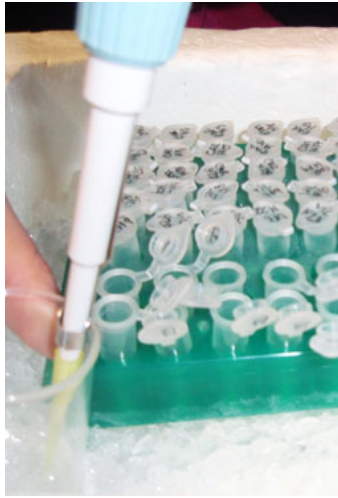


International Collaborators

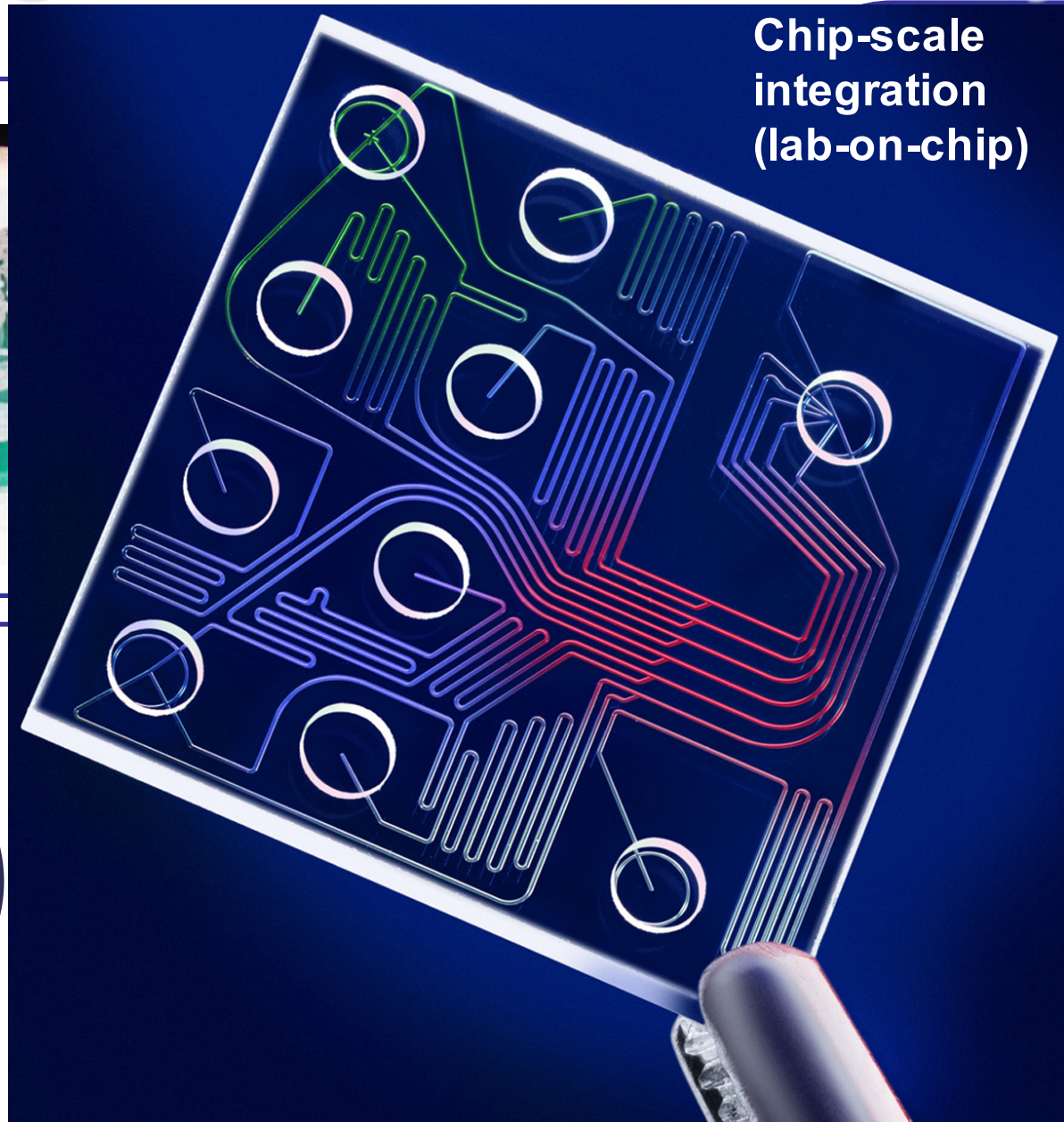
- Collaborators:
 - Prof. Bhargab Bhattacharya (Indian Statistical Institute)
 - Prof. Philip Brisk (University of California at Riverside)
 - Prof. Krishnendu Chakrabarty (Duke University)
 - Prof. Steven Quake (Stanford University)
 - Prof. Paul Pop (Technical University of Denmark)
 - Prof. Sudip Roy (Indian Institute of Technology Roorkee)
 - Prof. Ulf Schlichtmann (Technical University of Munich)
 - Prof. Rolf Dreschler (University of Bremen)
 - Prof. Shigeru Yamashita (Ritsumeikan University)
 - Prof. Hao Yu (Nanyang Technological University)
- Duke University's Microfluidics Research Lab
- Stanford Microfluidics Foundry



Paradigm Shift in Biochemistry

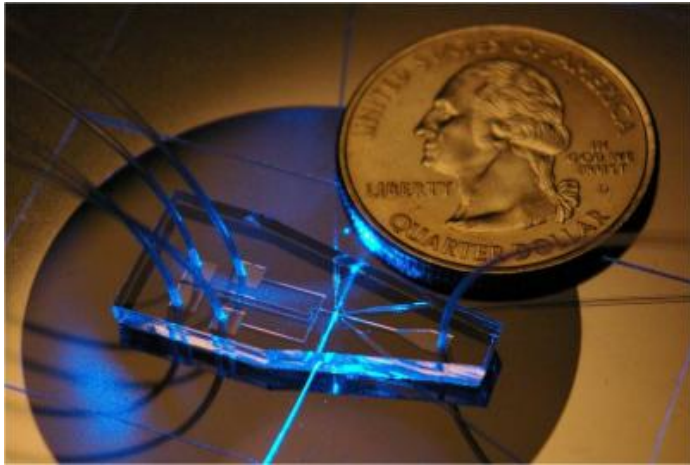


**Skilled
Technicians**

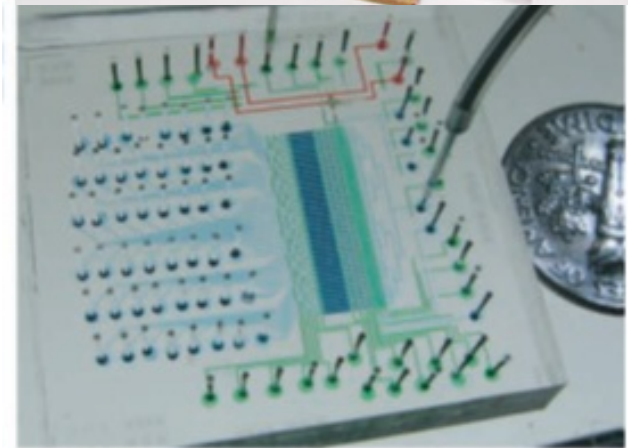


**Bulky
Equipment**

Why Biochips?



Small

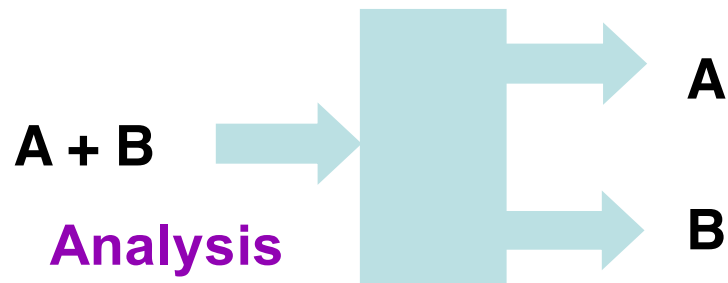
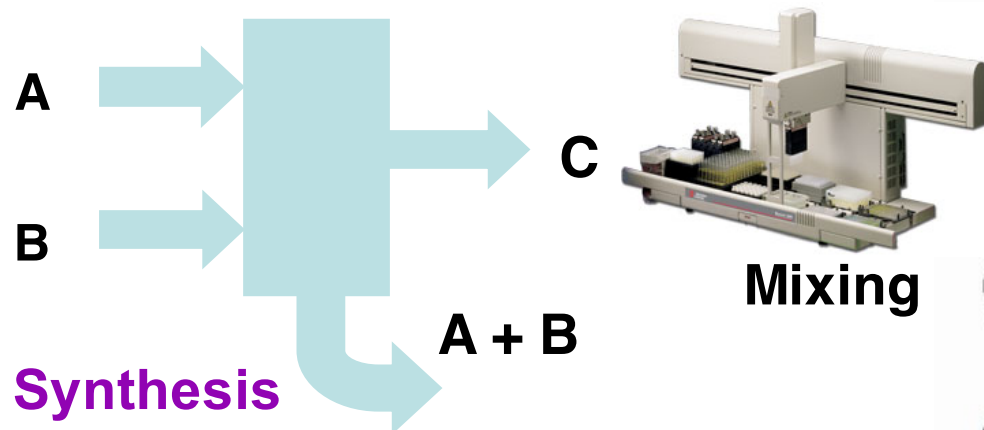


**High
Throughput**

Automation



Why is Biochemistry Difficult?



Reaction



Separation



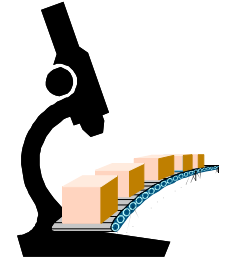
Why is Biochemistry Difficult?





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

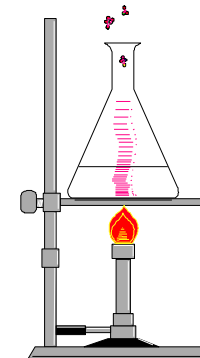
It's a miniature disposable for an
HTS - **H**igh-**T**hroughput **S**creening -
(bio)analytical instrument



what does it do?

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

dispensing,
mixing,
detecting,
discarding,-



just a lot cheaper and a lot faster than you did

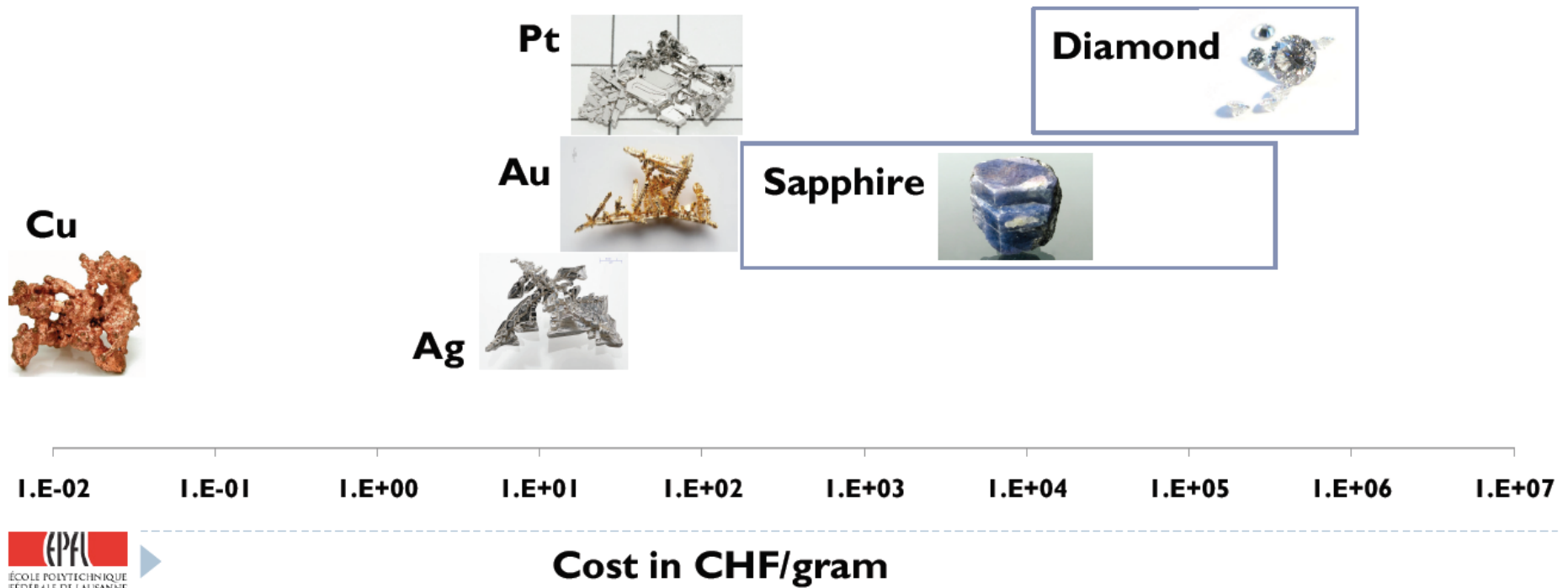


Why do chips have to be small?

High-**T**hroughput is why. If you do 10^6 assays in $10\mu\text{l}$ 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



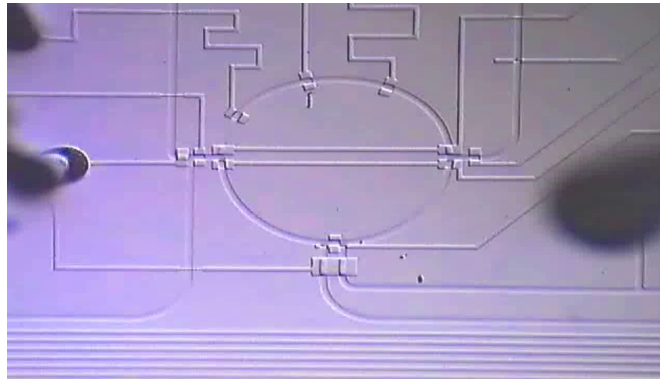
Statistics for High Throughput Screening

- 15 billion tests/year in 10 years
- 90% of cost and 95% of time today is associated with sample collection, transport, preparation

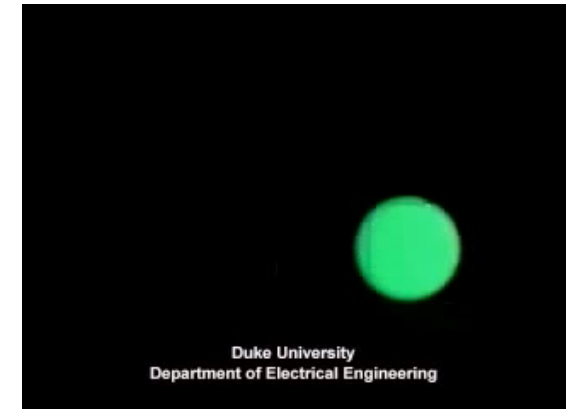
Mixing Operation



test tube



continuous microfluidics

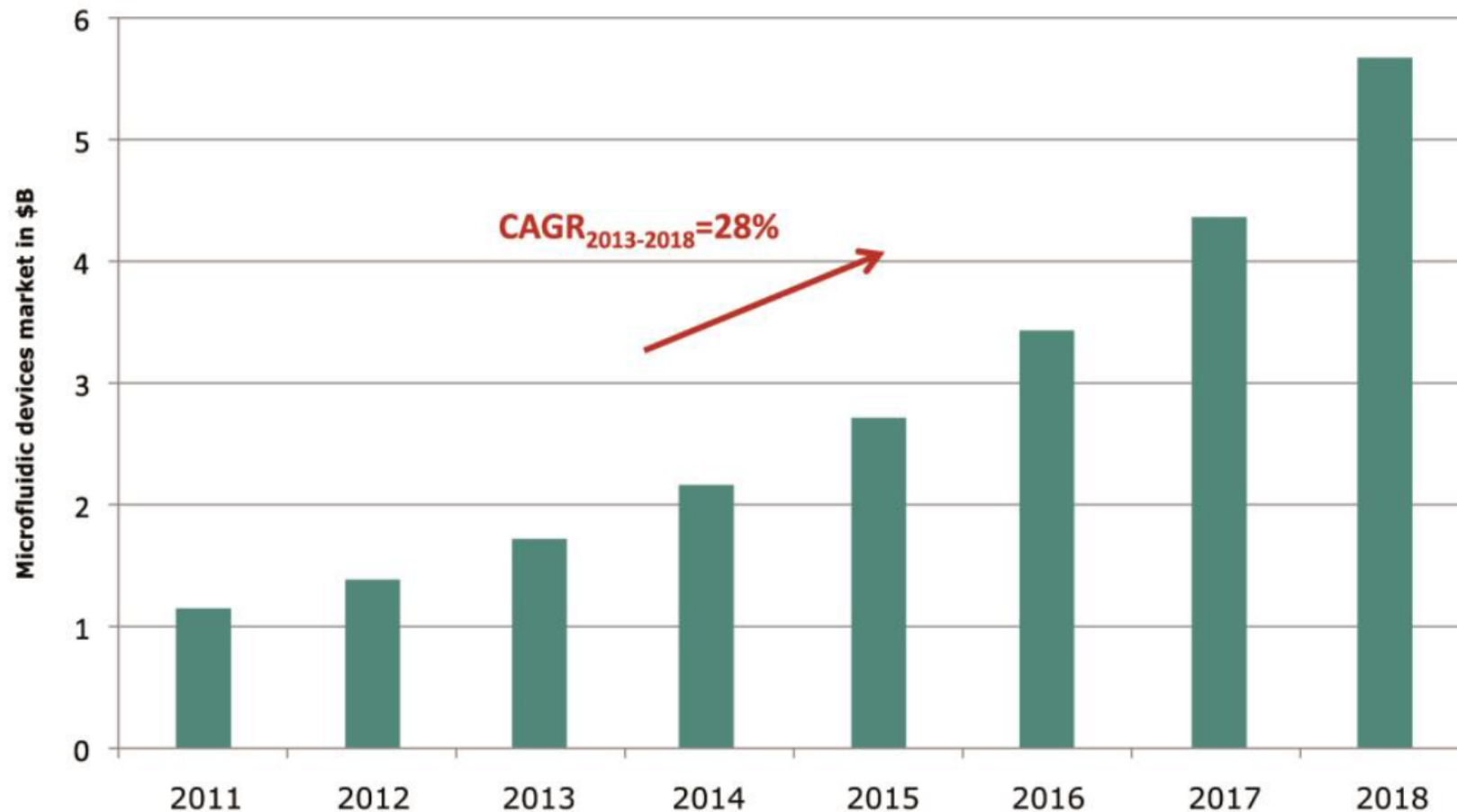


digital microfluidics

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

Microfluidic Device market and forecast from 2011 to 2018

Report includes detailed breakdown: General dispensing, drug delivery, accurate dispensing, analytical devices, clinical and veterinary diagnostics, point-of-care testing, industrial and environmental testing, pharmaceutical and life science, microreaction technology



“The microfluidic device market will grow swiftly, from \$1.4B in 2013 to \$5.7B by 2018”.

FUTURE OF PERSONALIZED MEDICINE

BETTER EVIDENCE
FOR DIAGNOSTICS
AND THERAPIES



NEED MORE
AGILE
REGULATORY SYSTEM

GENETIC COUNSEL
+
DECISION SUPPORT
+
GENETIC LITERACY

TRANSLATE RESEARCH...



...EMPOWER PATIENTS!

TAKE CARE OF
YOUR OWN HEALTH!



TEST BEFORE YOU
TREAT



GET TO THE
RIGHT DRUG



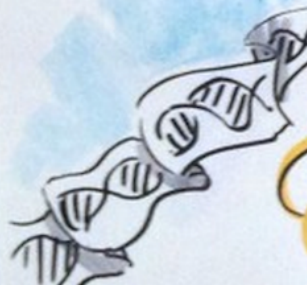
THE FIRST TIME!

ALL

OF THE DATA
FROM THE INTERNET
CAN BE STORED IN DNA
IN A SMALL TEST TUBE.



GIANT LEAPS IN
MEDICINE
ARE JUST AROUND
THE CORNER!



Next Generation Sequencing (NGS)

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](#).

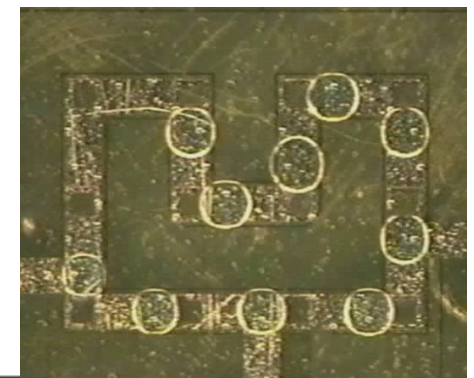
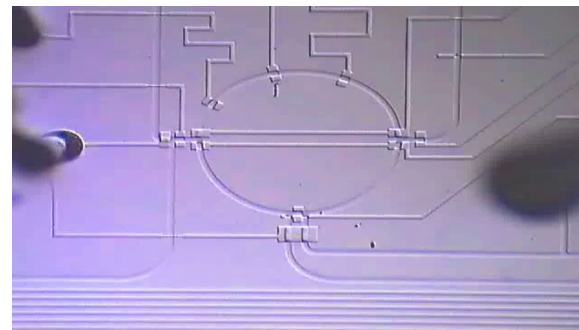
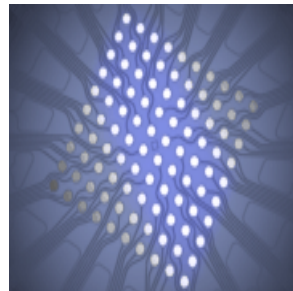
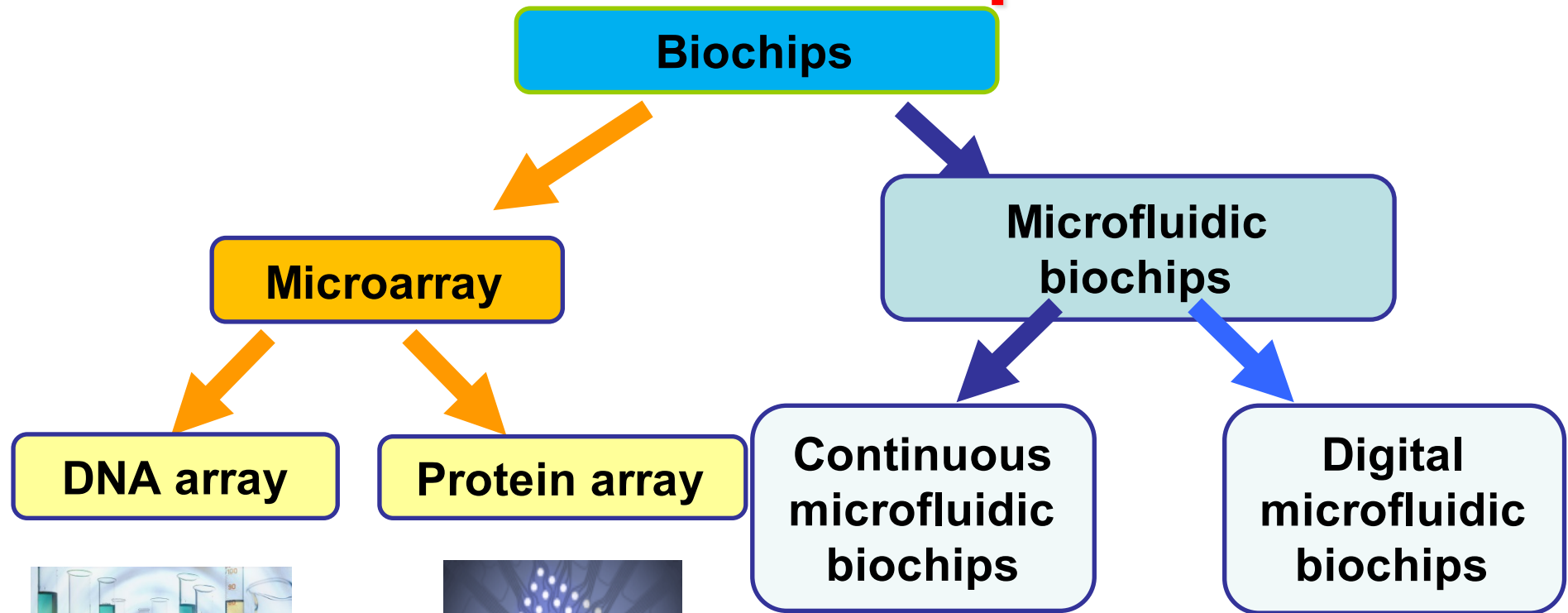
NCBiotech awarded the company more than \$468,000 in loans from 2005 to 2009 that helped it gain global attention in the field of microfluidics. ALL now holds hundreds of patents, and has launched its first gene-testing products into the research marketplace. It also raised millions of dollars in private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

Source: NIH

Classification of Biochips

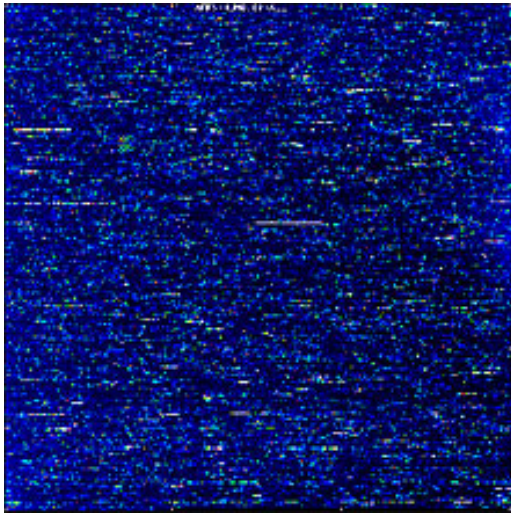


Passive (array)

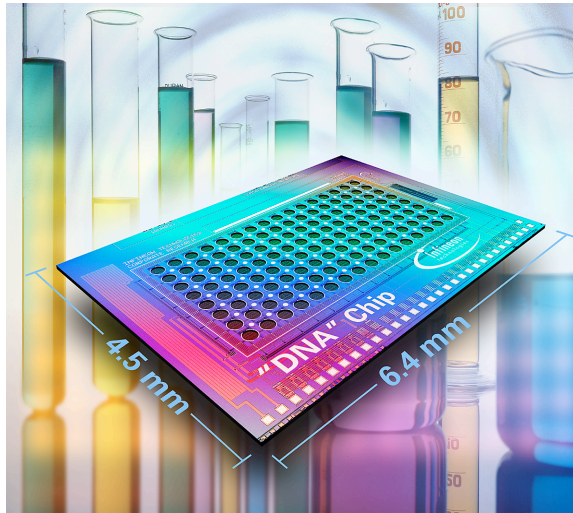
Active (lab-on-chip, μ -TAS)

Microarray

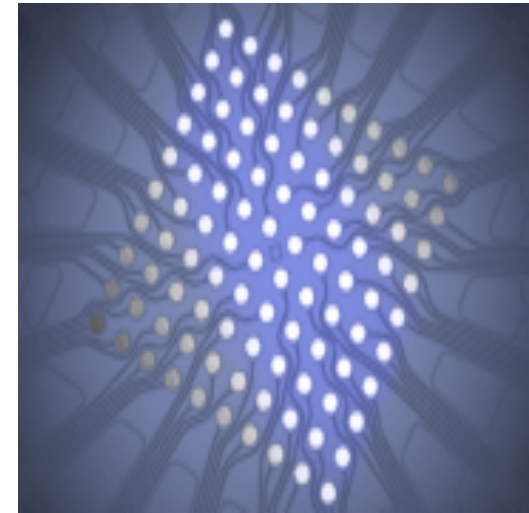
- DNA (or protein) microarray: piece of glass, plastic or silicon substrate
- Pieces of DNA (or antibodies) are affixed on a microscopic array
- Affixed DNA (or antibodies) are known as *probes*



GeneChip® DNAarray from
Affymetrix
<http://www.affymetrix.com>



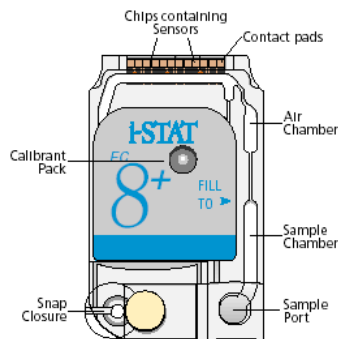
DNA microarray from
Infineon AG
<http://www.infineon.com>



NanoChip® microarray
from Nanogen
<http://www.nanogen.com>

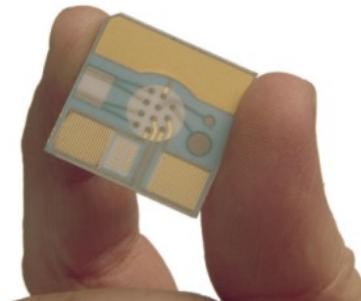
Microfluidic Biochip

- Unified Platform: Integration of multiple processes/functions
- Microchip: A microfluidic network fabricated on a substrate (glass, plastic, quartz etc.)
- “Micro”fluidics: At least one characteristic dimension in the micron range (1-100 μm)
- Miniaturization of biological separation and assay techniques
- Applications: Genomics/Proteomics; High-throughput screening (HTS); Drug discovery; biodiagnostics; environmental; Defense etc.



From <http://www.istat.com>

**i-STAT Biodiagnostic
microsystem**



<http://w4.siemens.de>

**Siemens Environmental Systems'
Water Quality Analysis Chip**



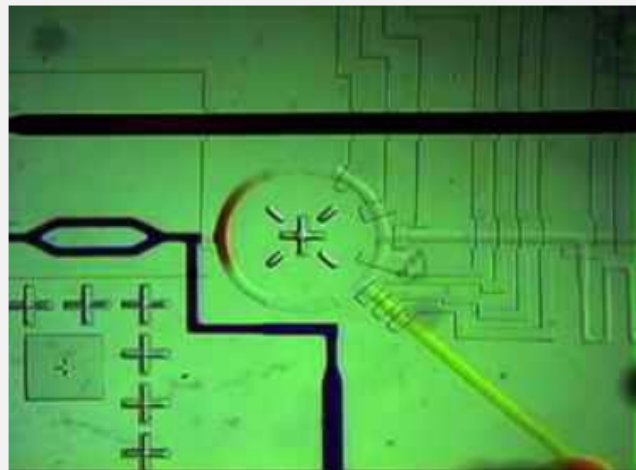
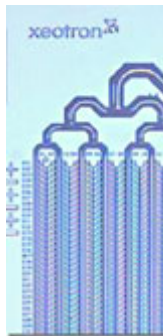
<http://www.agilent.com>

Agilent's Protein LabChip

Continuous-Flow Microfluidic Biochips

INVENTOR OF BIOCHIP THAT MAKES 10,000 SIMULTANEOUS MEASUREMENTS WINS “OSCAR FOR INVENTORS” – AND \$500,000

- Many contin



With thousands of valves and pumps, this microfluidic biochip has already been used to discover drugs and solve protein structures.

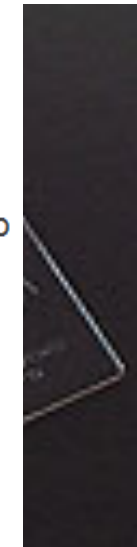
An inventor used the same principles of an integrated circuit to develop a 'biochip' that can perform nearly 10,000 independent simultaneous measurements – orders of magnitude above and beyond pipetting scientists in the lab. The chip is already being used to perform basic research and to discover new drugs. For his ingenuity, the chip's inventor was recently awarded the \$500,000 Lemelson-MIT prize for exceptional innovators.

The chip contains tens of thousands micromechanical pumps and valves that

XeoChi
Xeoti

move nanoliter volumes of liquid through microfluidic channels in a coordinated manner. With its multitude of working parts, an experiment that would normally take 18,000 steps and a week to perform could be completed with the chip in just three hours and in just 200 steps.

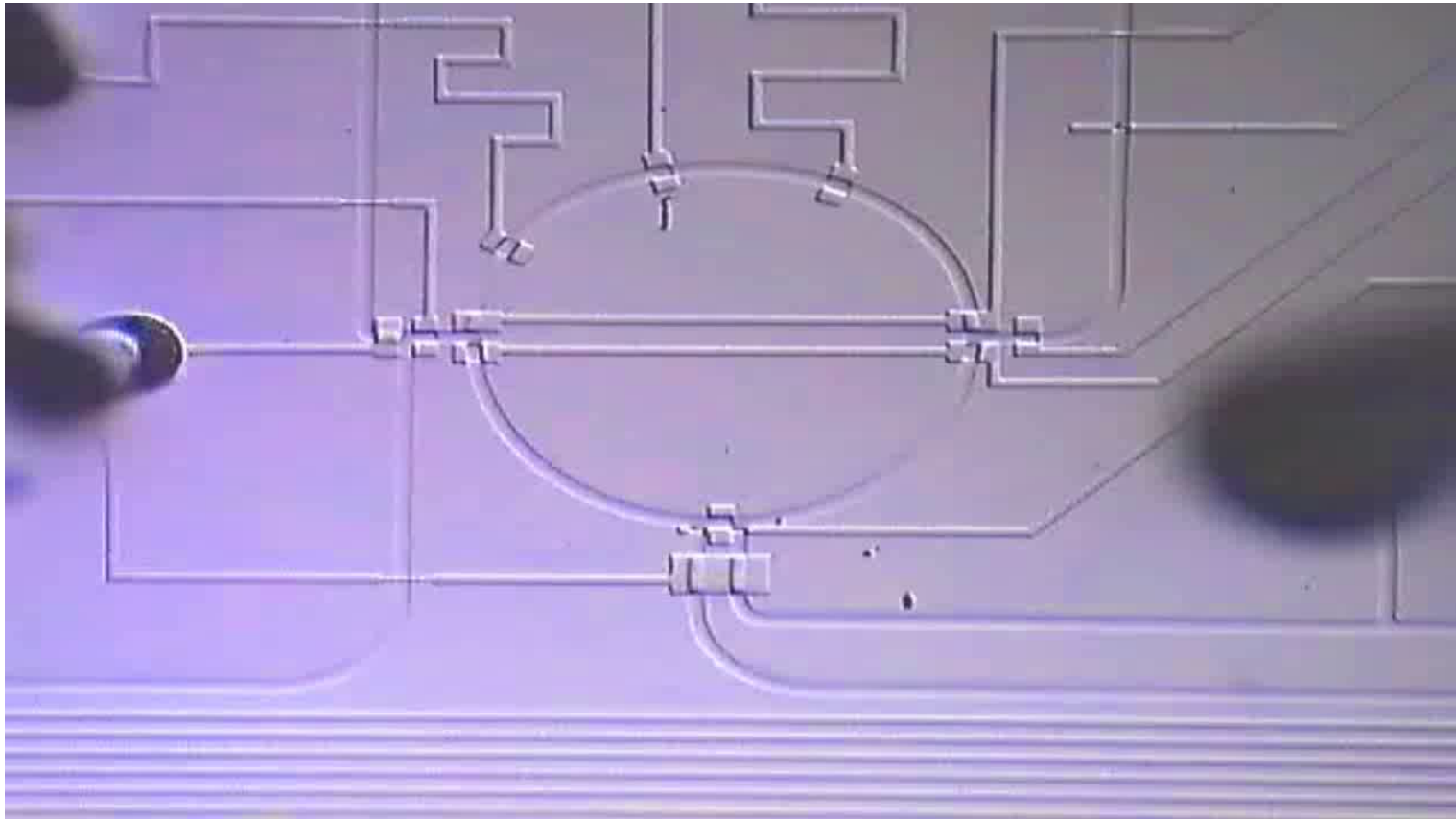
annels.



icrofluidic
om Aclara
ara.com

The inventor of the chip, Stephen Quake, is a professor of bioengineering and applied physics at Stanford University where he studies microfluidic large-scale integration. The

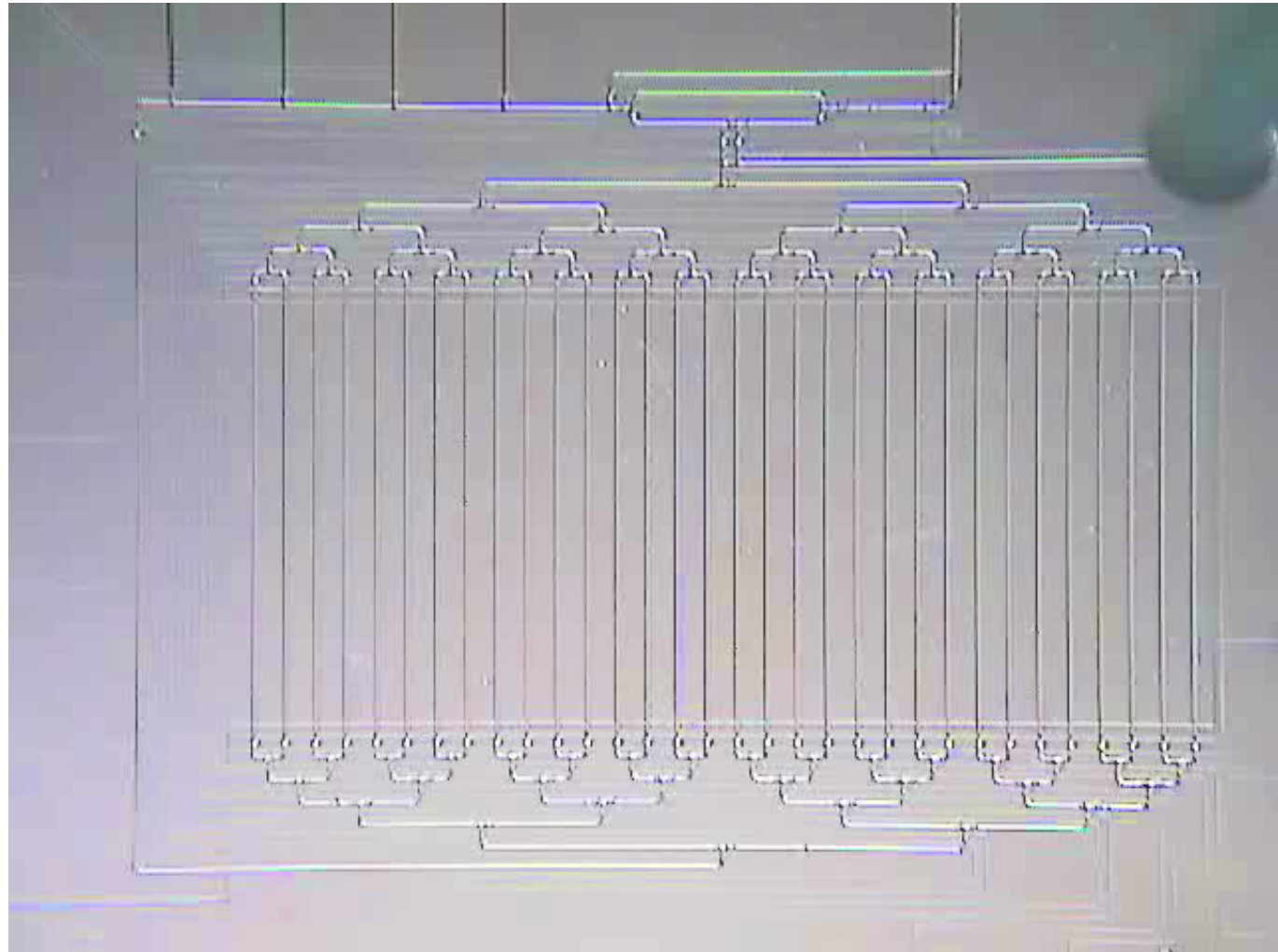
Microfluidic Mixer



<http://groups.csail.mit.edu/cag/biostream>

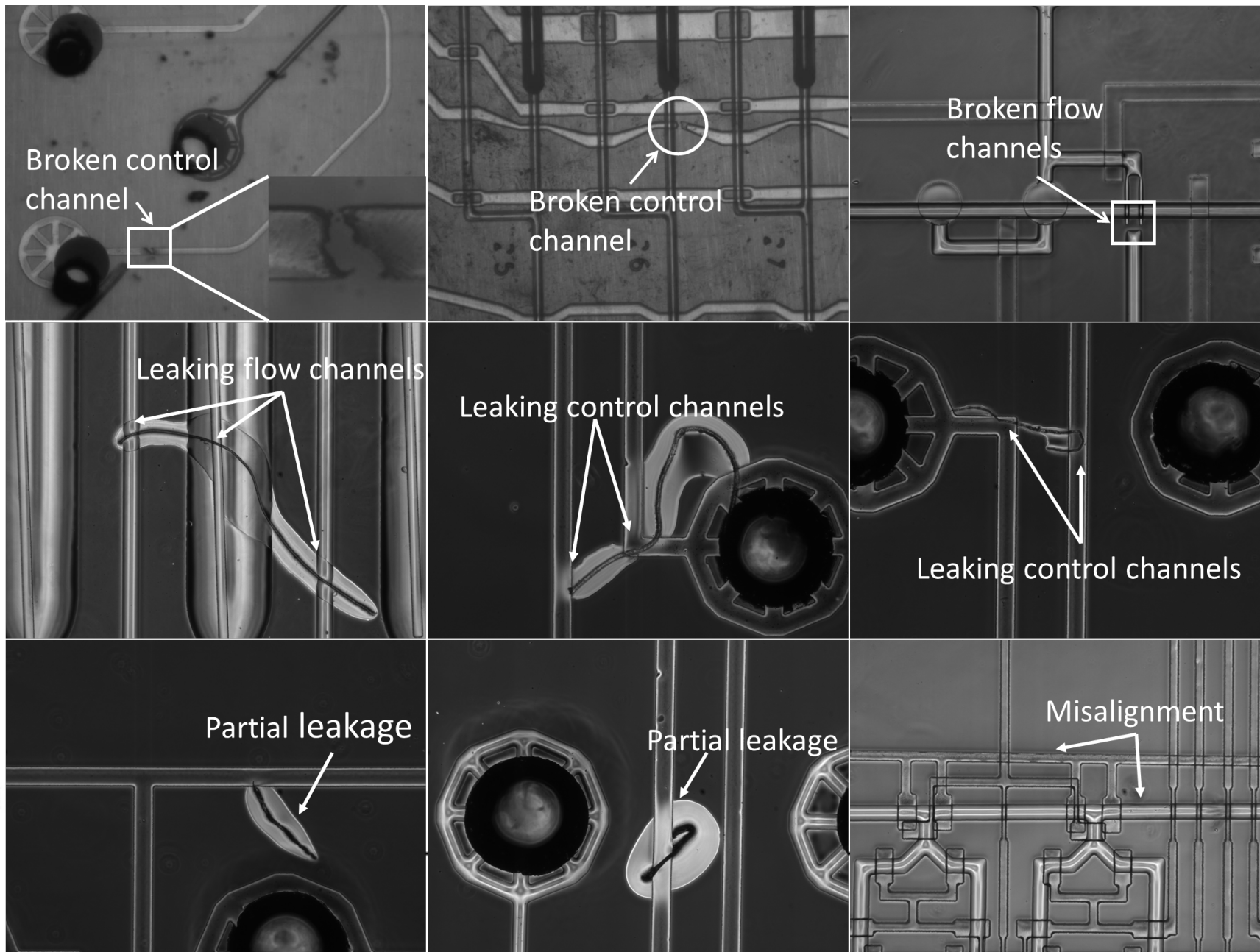
Components

- Mixer
- Detector
- Filter
- Heater
- Separator
- Storage Units
- Metering
- ...

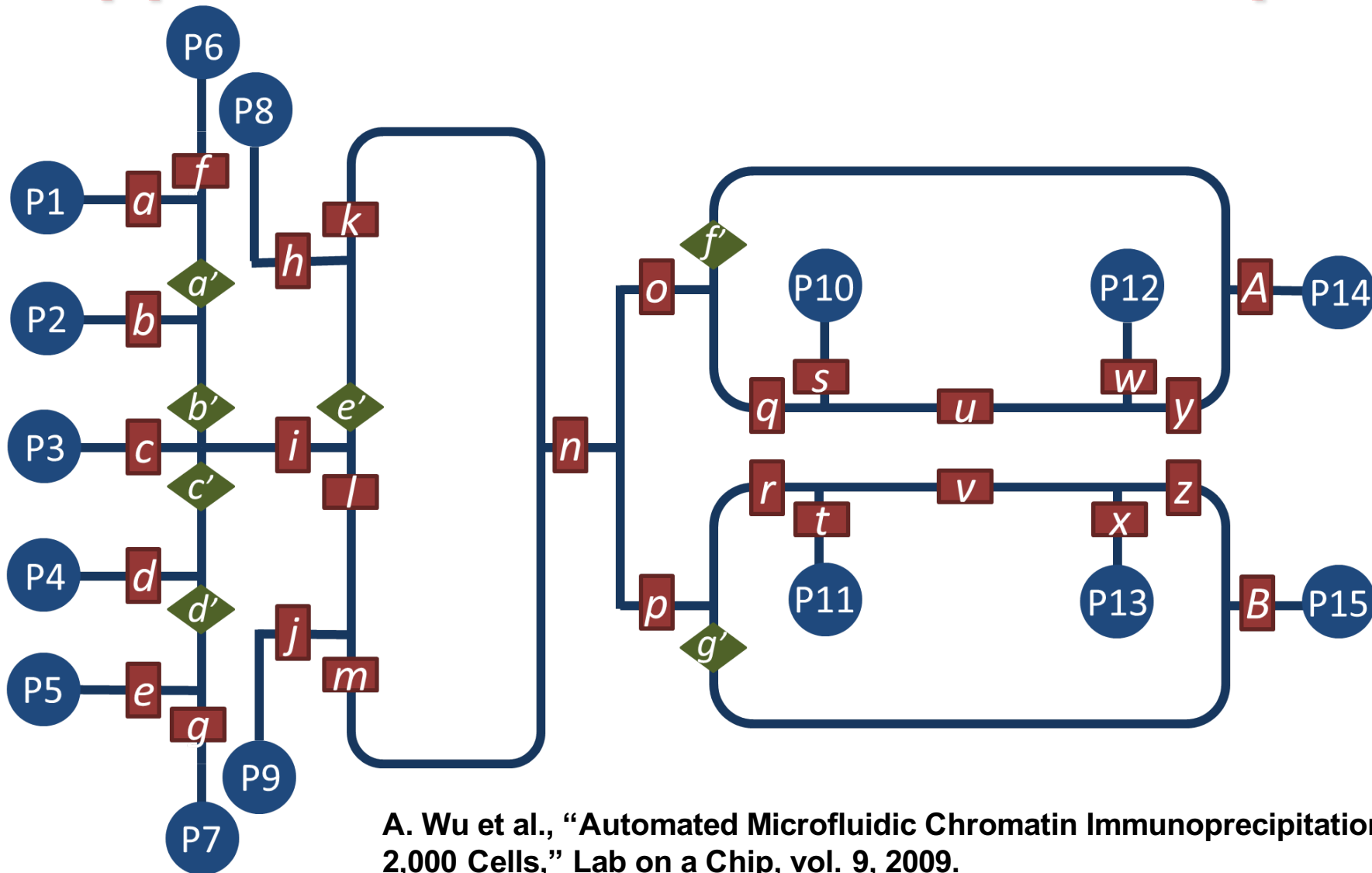


<http://groups.csail.mit.edu/cag/biostream>

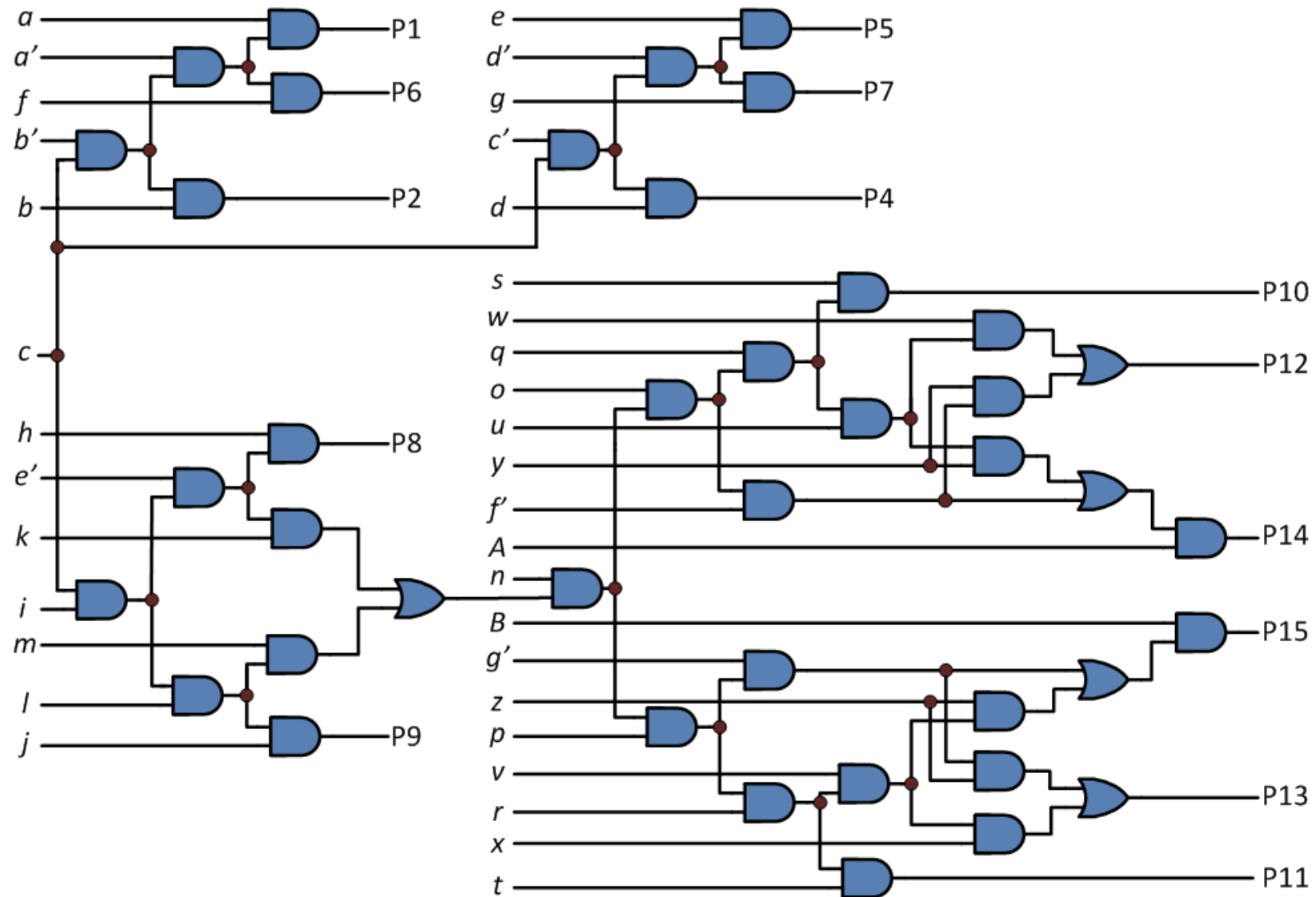
Defects in Flow-based Microfluidics



Application to Fabricated Biochip: I



Logic Circuit Model



Test Patterns after ATPG

30 test patterns and fault-free response for (100% fault coverage)

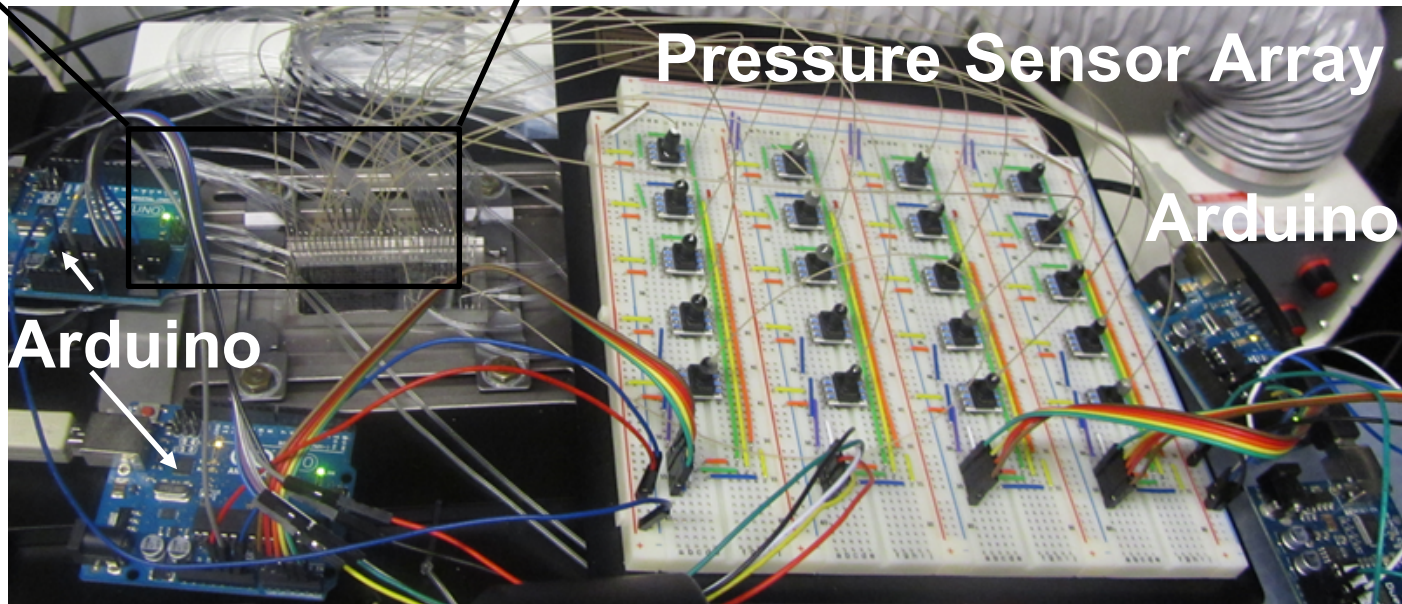
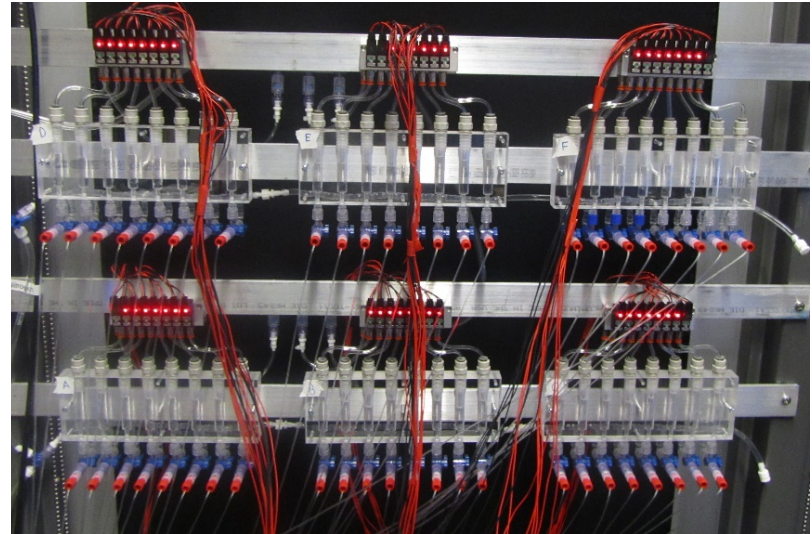
Stuck-at Faults				Bridging Faults		
	Test Pattern	Expected Response	Detected Faults		Test Pattern	Expected Response
1	1111010101 1000100101 10101000	1110100000 0000	12	1	1110111111 1101101010 10101110	1101111110 1010
2	0111001010 0101110101 00010011	0110010000 0000	10	2	1110011111 1111100110 01010111	1100111100 0010
3	1110011110 1001111001 11001111	1100111000 1111	13	3	1011110010 1011010101 10000101	1011100001 0101
4	1110010011 1011100011 10010011	1100100000 0010	4	4	1011000111 1101111001 11101100	1010001100 1100
5	0110100110 1011101000 11110100	0101001000 1000	5	5	1010011110 1011110100 01011000	1000111000 1100
6	1111011010 1001111101 11111000	1110110001 1100	5	6	1110001110 1001011100 10111000	1100011000 0000
7	0010101010 1101101011 01011001	0001010010 1000	2	7	0010111010 1101101100 10110000	0001110000 1000
8	1010011111 1101010100 10011100	1000111100 0100	4	8	1011011110 1111111110 01100100	1010111010 0100
9	0010011010 1101011101 00100010	0000110001 0000	1	9	0010010011 1111011011 10000100	0000100100 0100
10	0011010011 0111110100 00000011	0010100100 0011	1	10	1111100111 0011111100 01000001	1111001000 0000
11	0100101011 0101010100 01001101	0000000000 0000	1	11	1110110010 1001111110 00100000	1101100010 0000
12	1010100111 1000111000 01101011	1001001000 0000	1	12	1011111000 1101000111 00001110	1011110000 0000
13	1011010011 1101111100 00100001	1010100100 0001	1	13	1011001111 0101101010 11100001	1010011100 0000
14	1110001010 1101110100 10011001	1100010000 1001	1	14	0111100110 0111111110 01110000	0111001010 0100
15	1010011011 1111111010 11000100	1000110110 0100	1			
16	1011110111 1011110100 11001000	1011101000 1000	1			

Demo System

Biochip under Test



Solenoid valve array



IEEE Transactions on Computer-Aided Design Donald O. Pederson Best Paper Award

The IEEE Transactions on Computer-Aided Design Donald O. Pederson Best Paper Award is sponsored by the IEEE Council on EDA and recognizes the best paper published in the Transactions on Computer-Aided Design of Integrated Circuits and Systems publication.

The award is based on the overall quality, the originality, the level of contribution, the subject matter and the timeliness of the research. Anyone who is an author of a paper published in the Transactions on Computer-Aided Design of Integrated Circuits and Systems during the two calendar years preceding the award is eligible for nomination.



2015 Pederson Award Recipients at DAC 2015

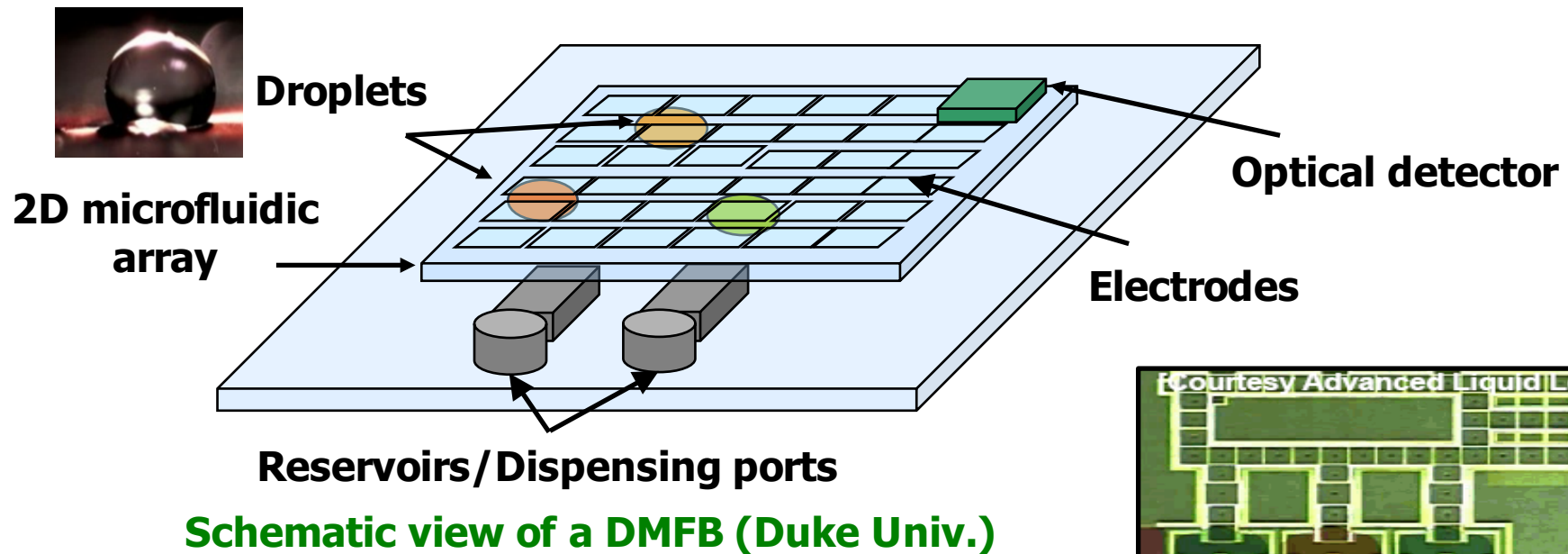
Award Recipients

2015 Title: **"Testing of Flow-Based Microfluidic Biochips: Fault Modeling, Test Generation, and Experimental Demonstration,"**
Authors: Kai Hu, Feiqiao Yu, Tsung-Yi Ho, Krishnendu Chakrabarty
Citation: IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems, Vol. 33, Issue 10, pp. 1463 - 1475, October 2014

Digital Microfluidic Biochips (DMFBs)

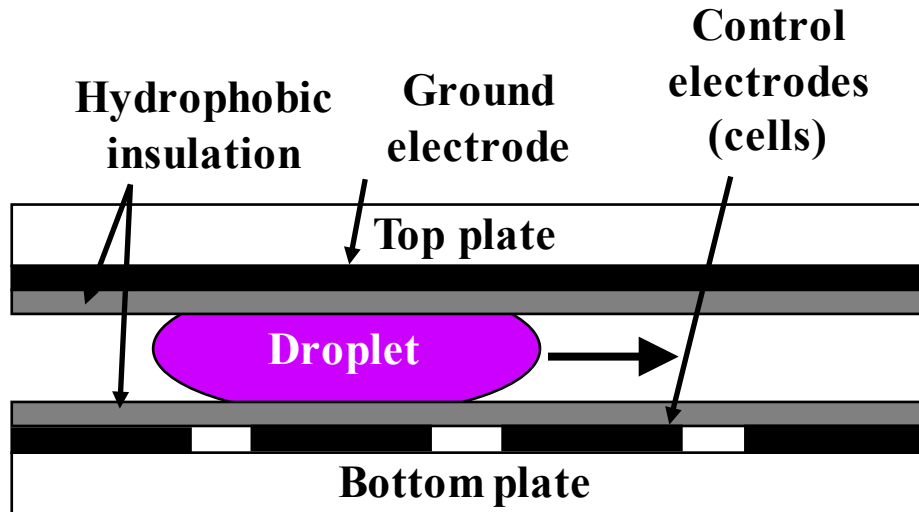
. Digital Microfluidic Biochips

- Droplets: biological sample carrier; basic units to perform the laboratory procedures on DMFBs
- 2D microfluidic array: set of electrodes for biological reactions
- Reservoirs/dispensing ports: for droplet generation
- Optical detectors: detection of reaction result

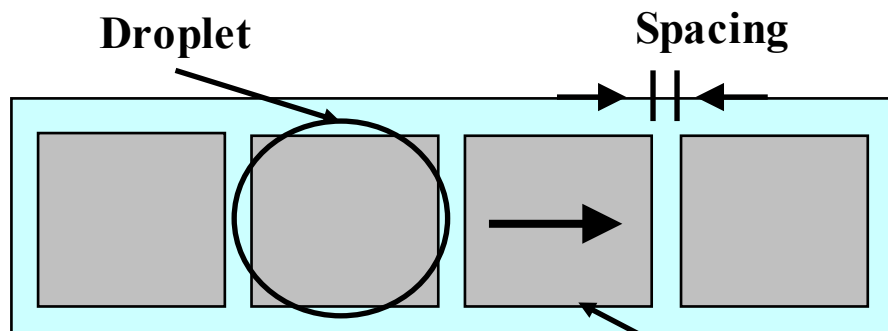


Droplet Movement on DMFBs

- Droplet movements are controlled by electrical manipulation

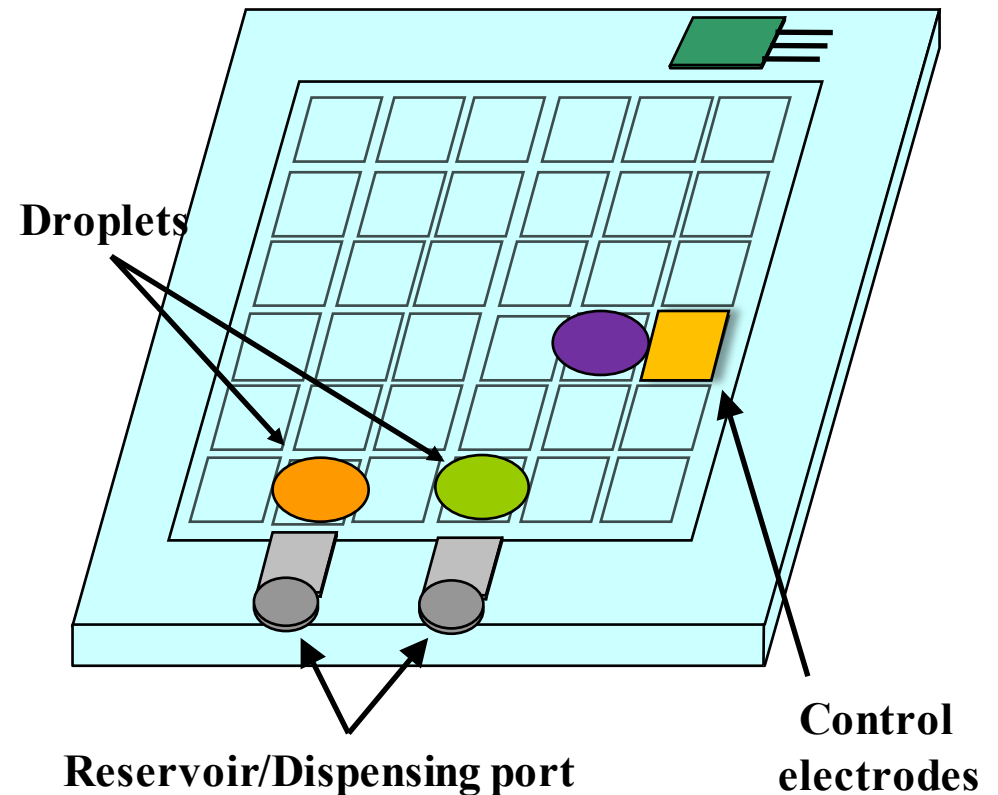


Side view



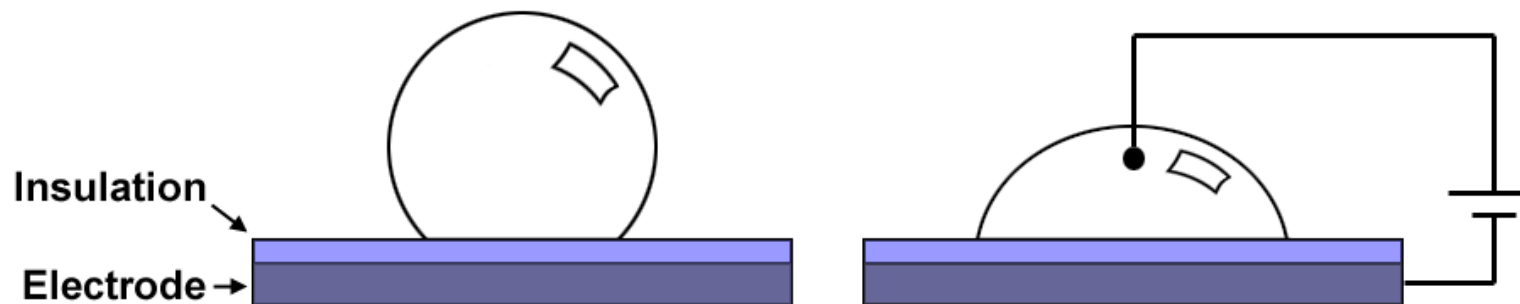
Top view

High voltage to generate an electric field



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.

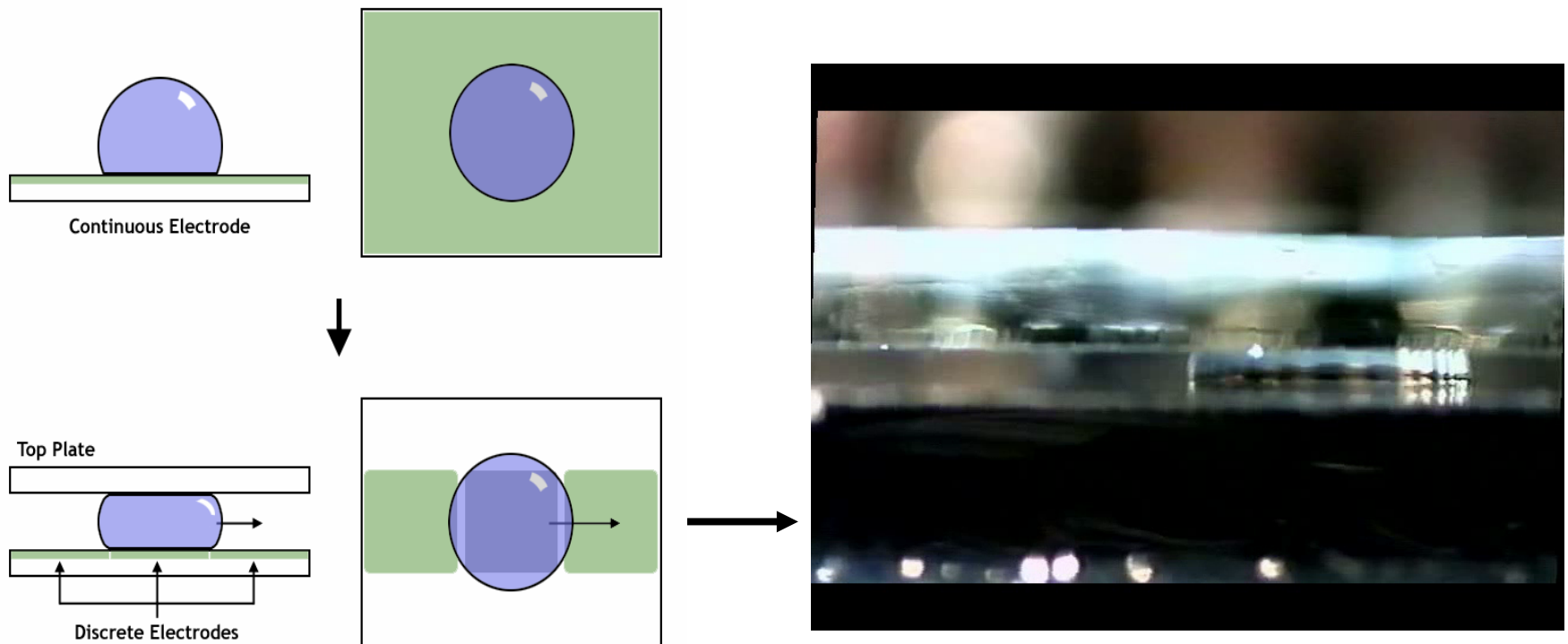
Applied Potential

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



What is Digital Microfluidics?

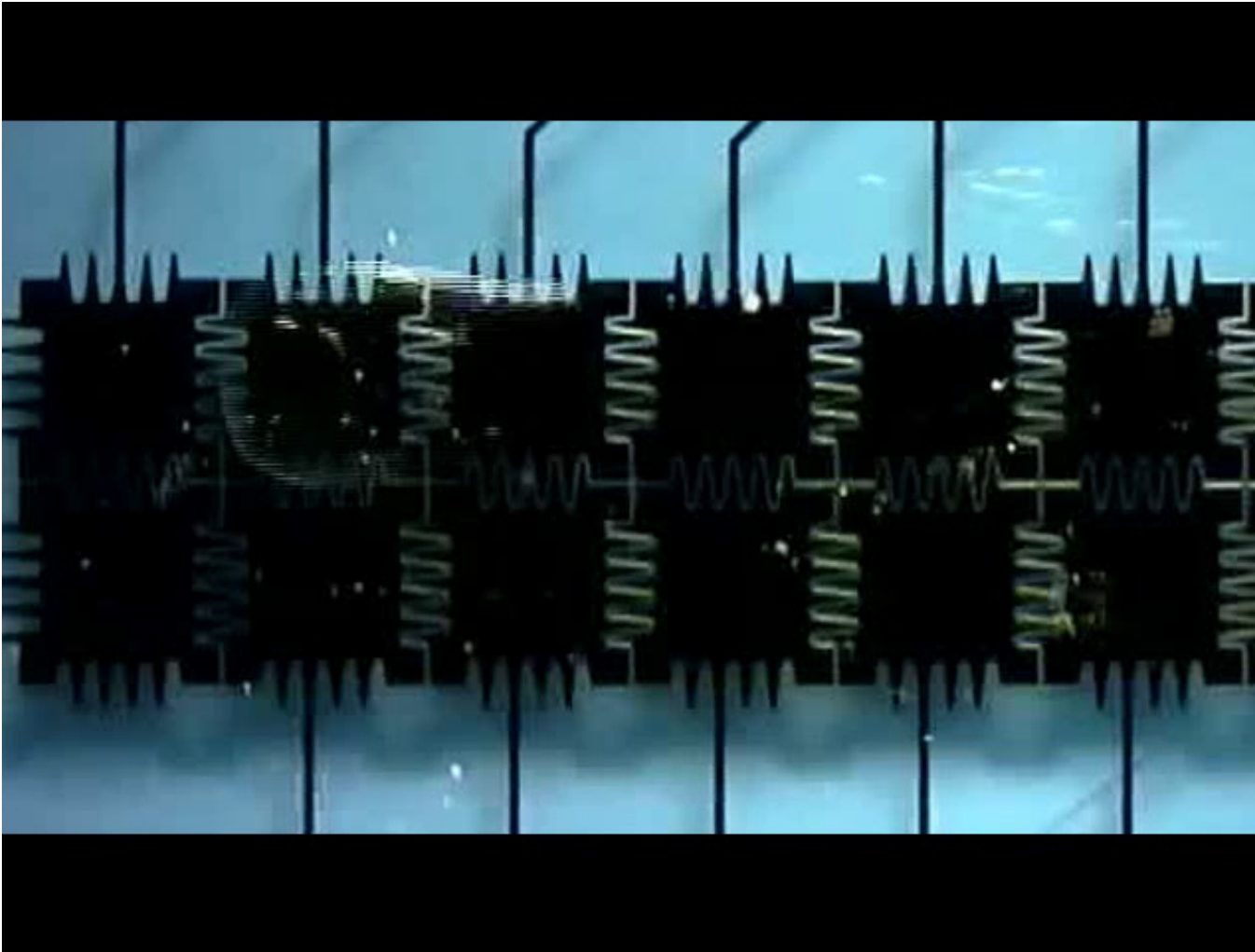
- Discretizing the bottom electrode into multiple electrodes, we can achieve lateral droplet movement



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

Droplet Transport (Side View)

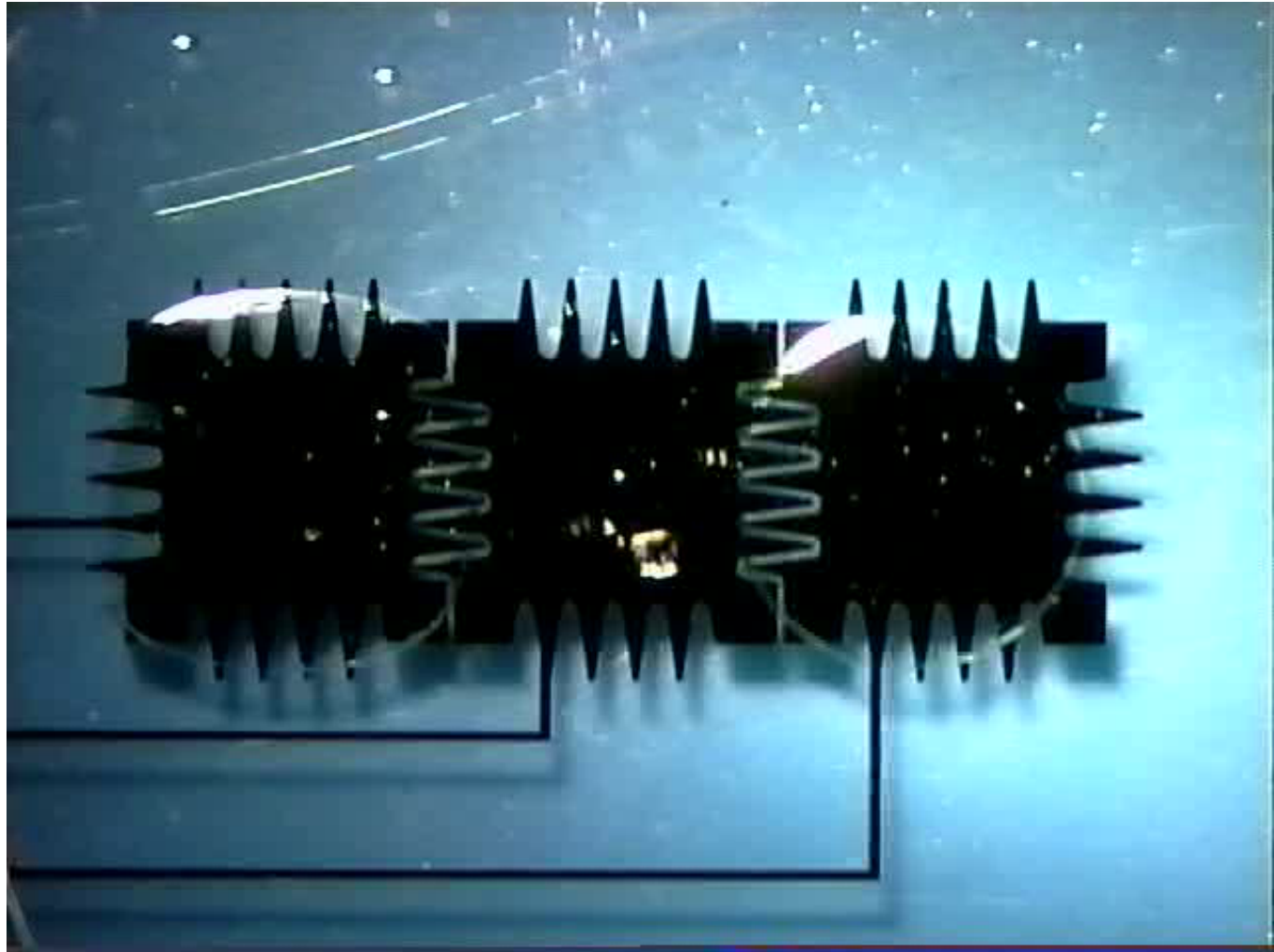
What is Digital Microfluidics?



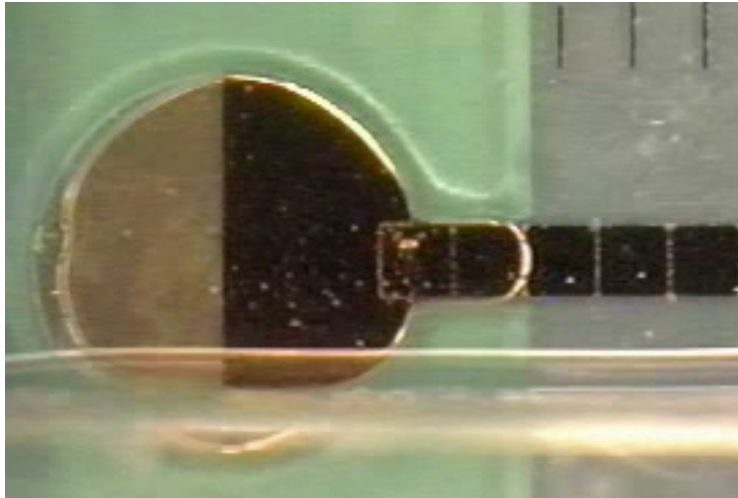
Transport
25 cm/s flow rates,
order of magnitude
higher than
continuous-flow
methods

What is Digital Microfluidics?

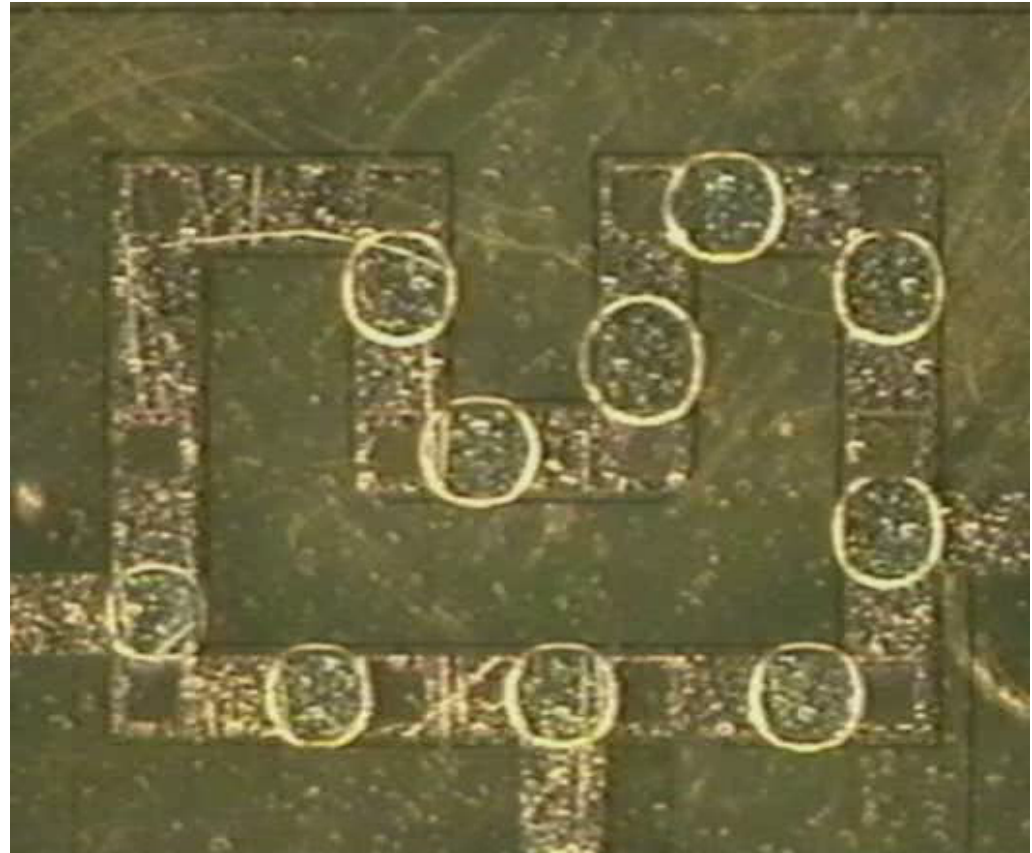
Splitting/Merging



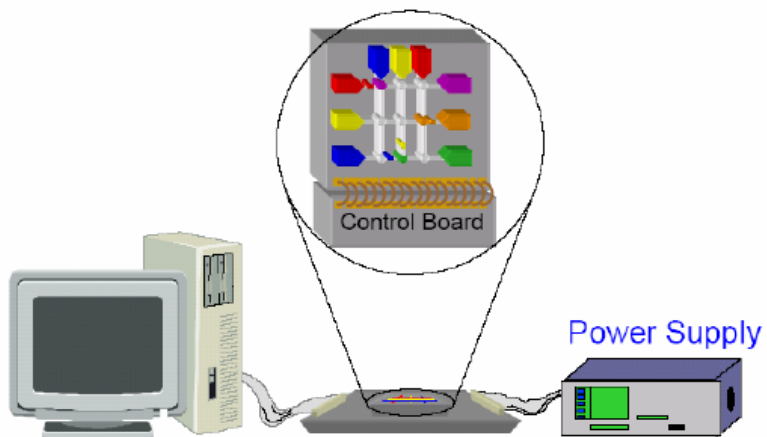
Demonstrations of Digital Microfluidics



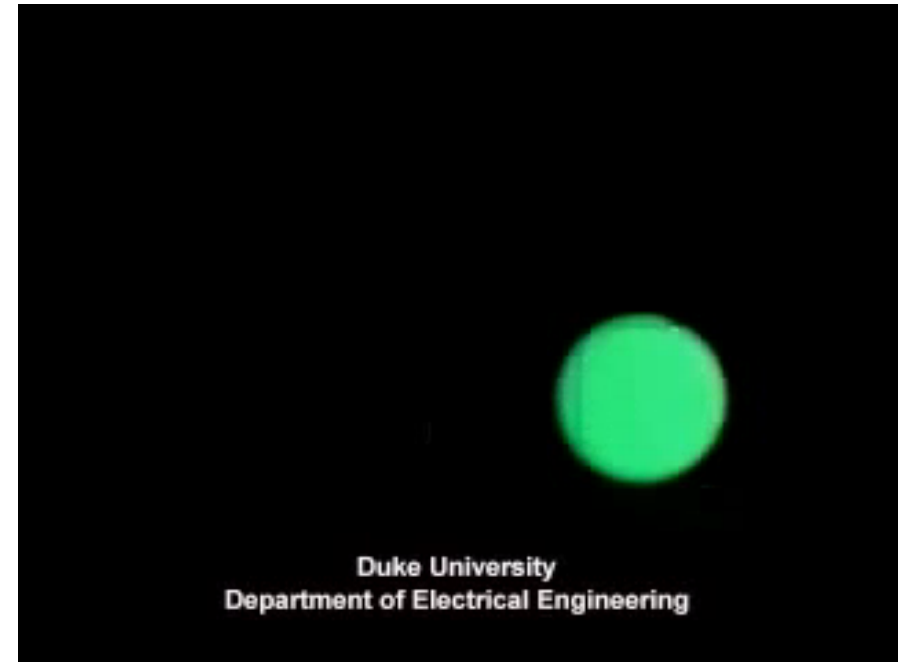
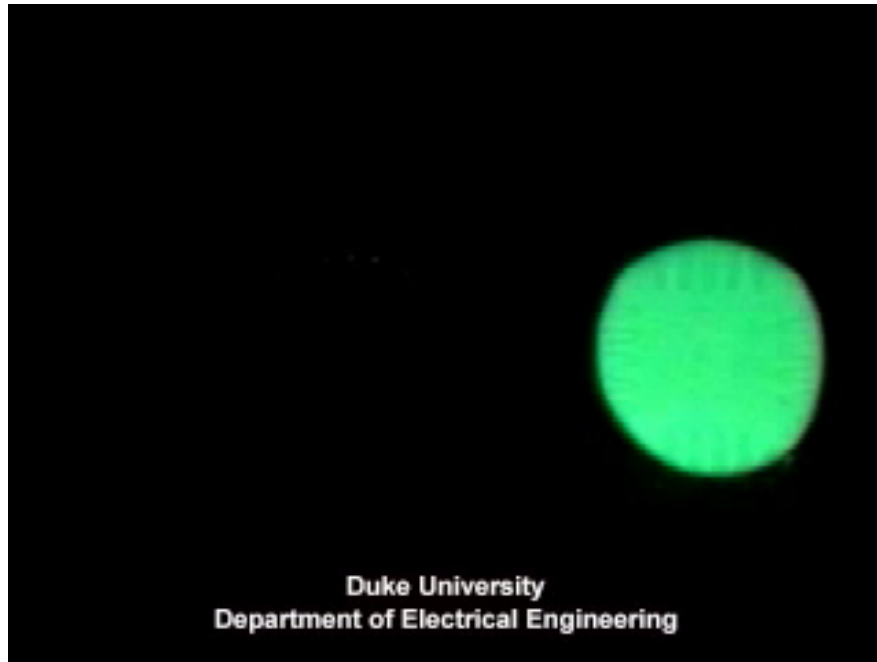
Droplet Formation



Synchronization of many droplets



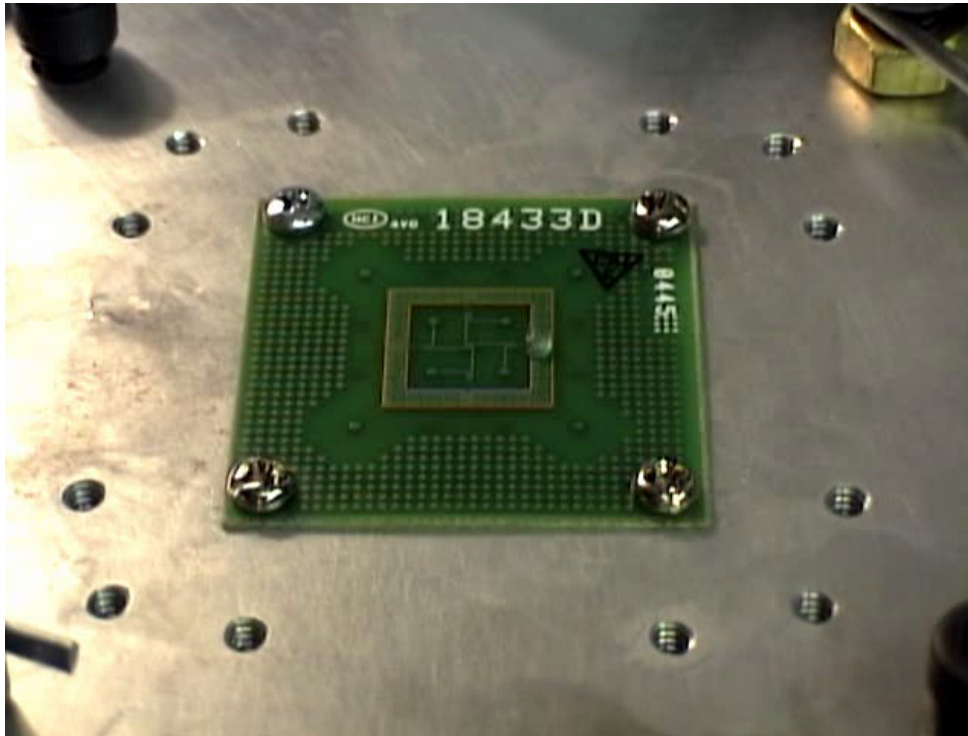
What is Digital Microfluidics?



Mixing

Advantages

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



Droplet Transport on PCB (Isometric View)

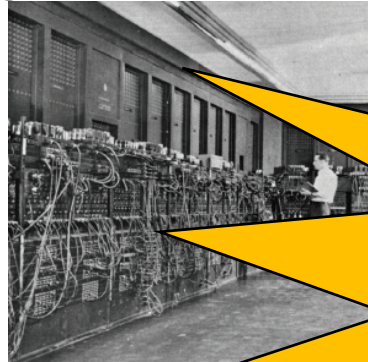
- Standard low-cost fabrication methods can be used
 - Continuous-flow systems use expensive lithographic techniques to create channels
 - Digital microfluidic chips are possible using solely PCB processes

The Need of CAD Support

1924: “Multi-core” processing



1946: ENIAC I



Vacuum Tube

1953: Transistor Computer

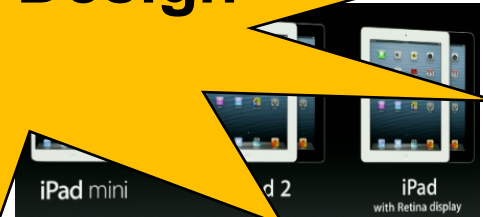


1976: Apple-I



**Computer-Aided Design
(CAD)**

- Design complexity is increased
 - Large-scale bioassays
 - Multiple and concurrent bioassay operations on a biochip



Laboratory Instruments



Biochemical Analyzer



Robotics
Cybersystem

Microfluidic Biochip



Micro-valve
Electrowetting on Dielectric
Multilayer Soft Lithography

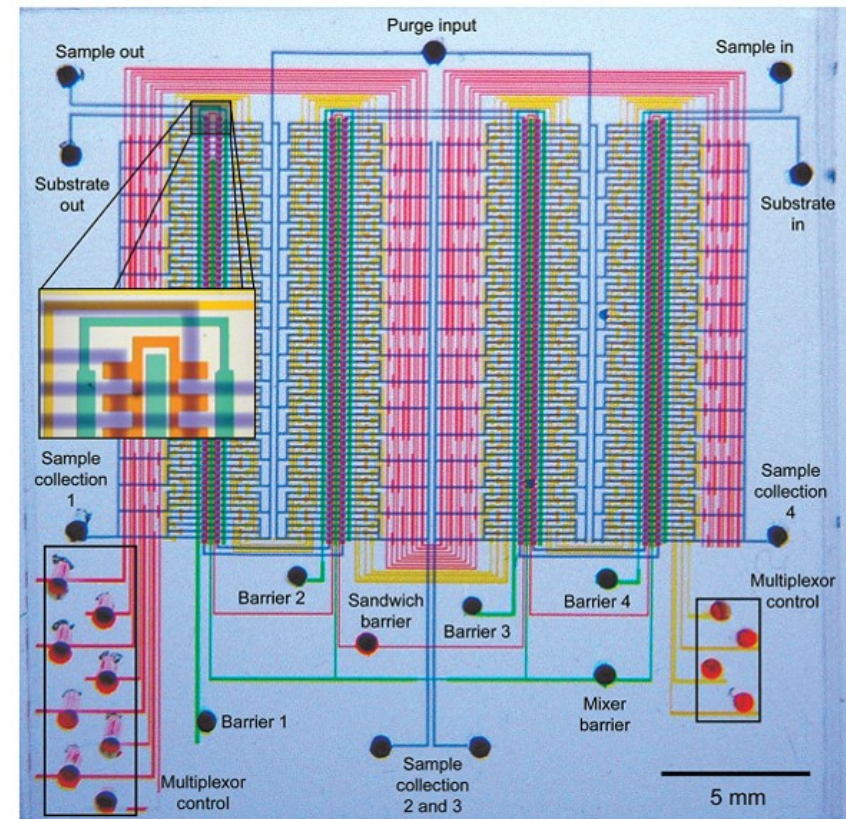
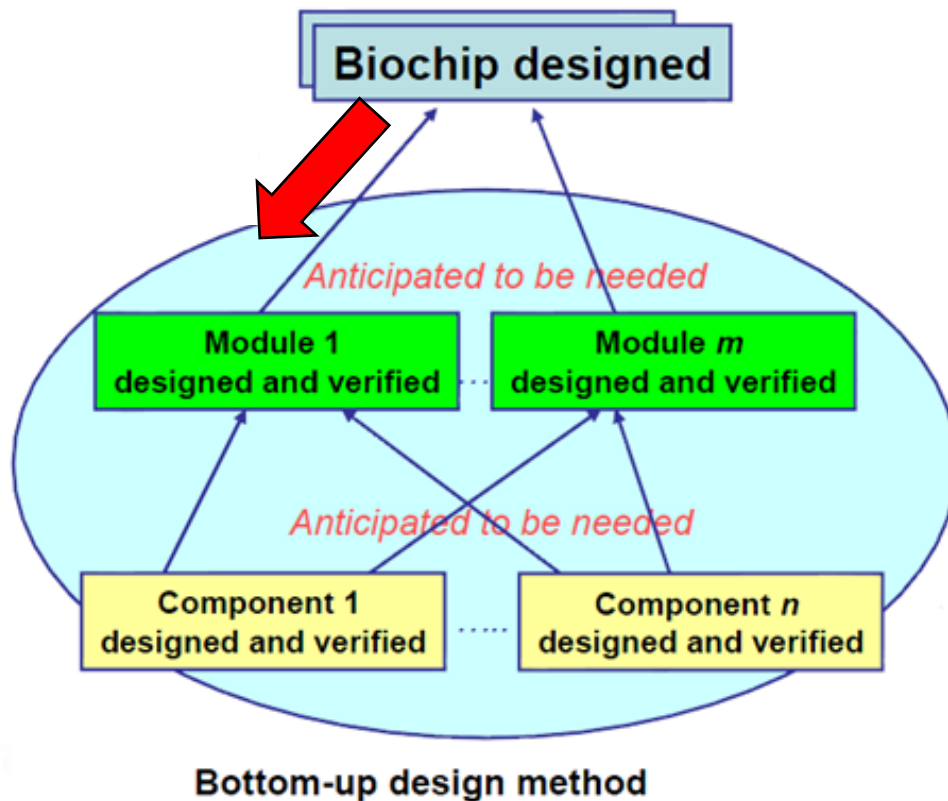
Microfluidic Large
Scale Integration (MLSI)



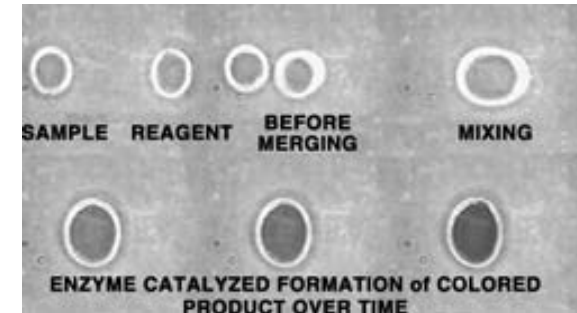
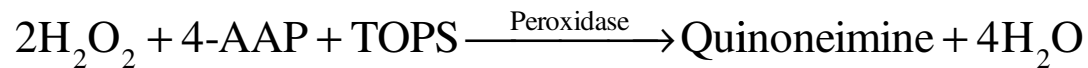
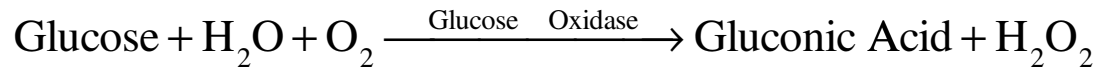
Agilent LabChip

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



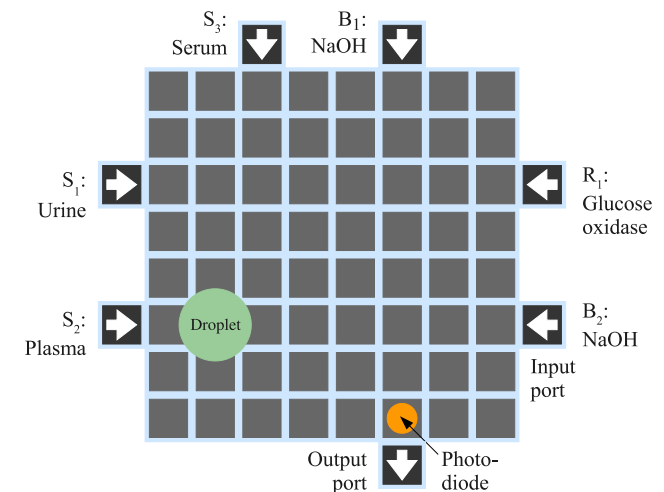
Application model: from this...



Trinder's reaction, a colorimetric enzyme-based method

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

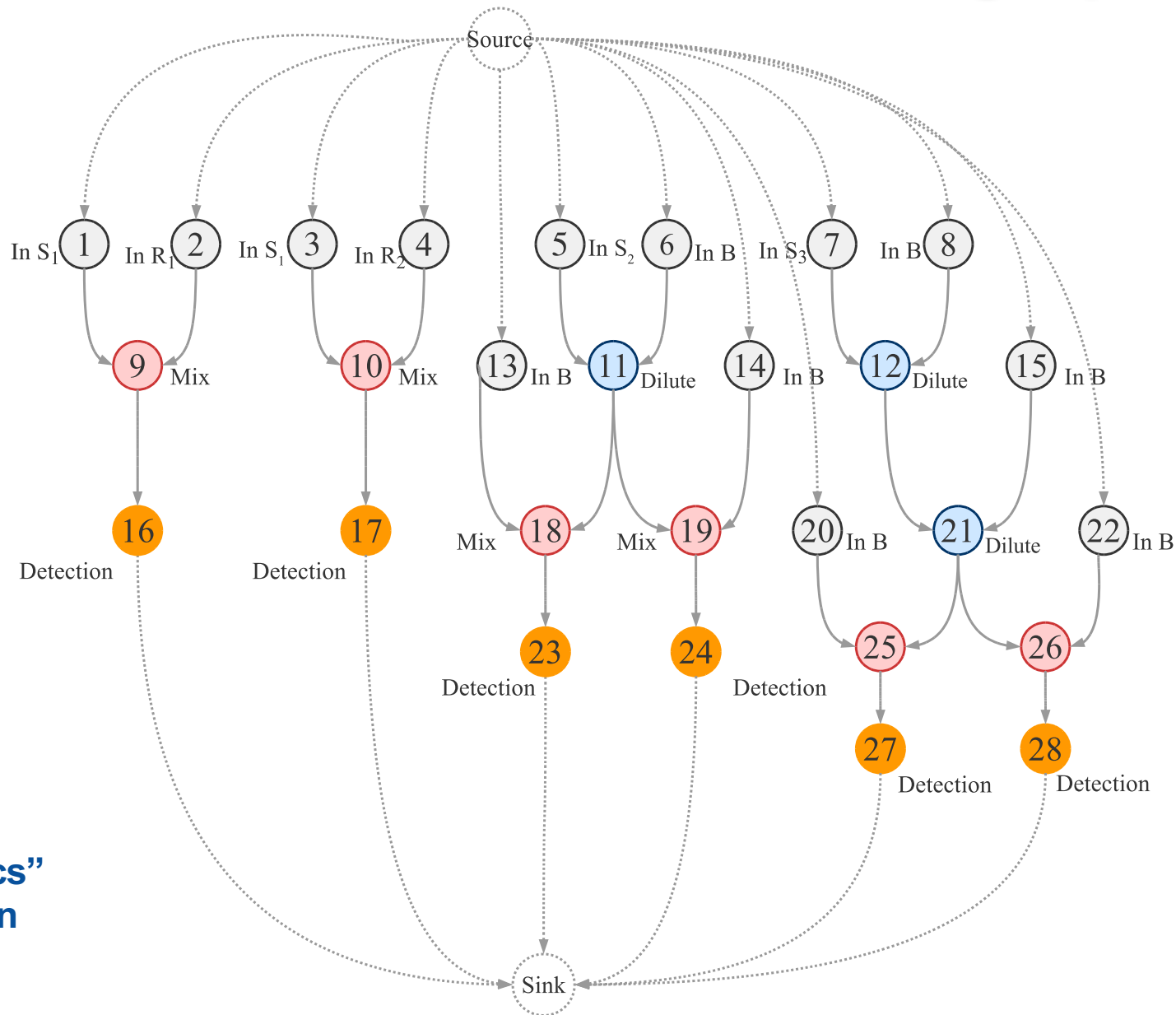
Glucose assay steps on the biochip



Reconfigurable architecture

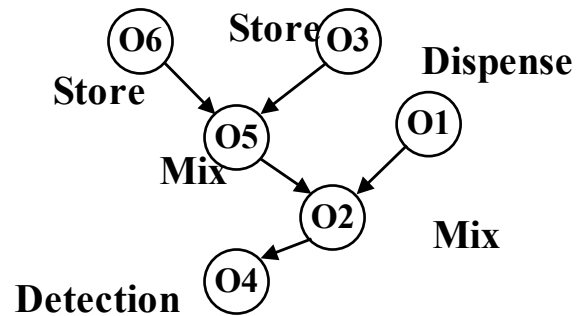
Several such reactions assays in parallel:
“in-vitro diagnostics” application

Application model: ...to this—a graph



“in-vitro
diagnostics”
application

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**

**Sequencing
Graph**

**Microfluidic
Module Library**

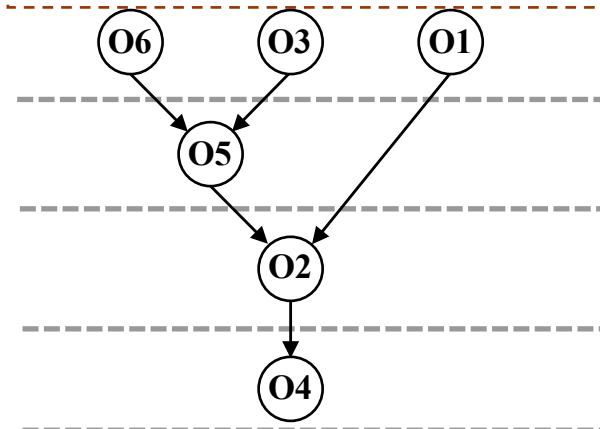
**Design
Spec.**

**Architectural-Level
Synthesis**

Resource Binding

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

Scheduling

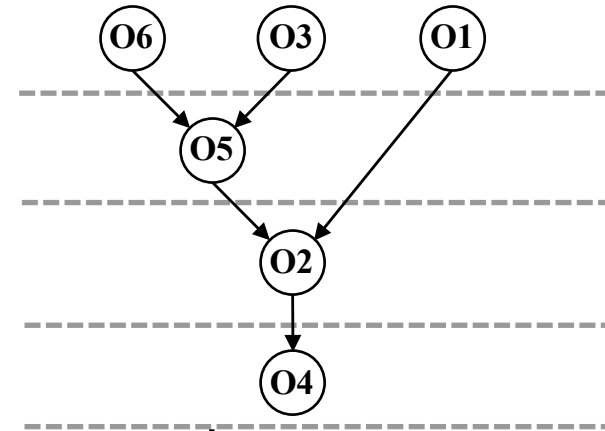


Physical-Level Synthesis

Resource Binding

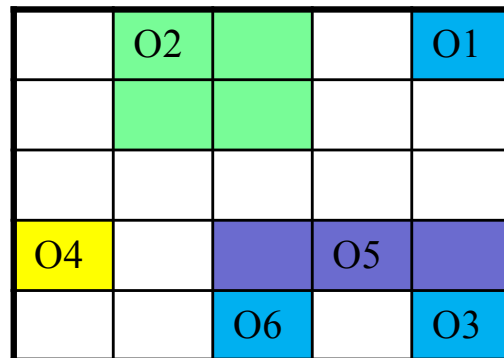
Operation	Resource
O1	On-chip
O2	2x2-array
O3	1x1 cell
O4	LED
O5	1x3-array
O6	1x1 cell

Scheduling

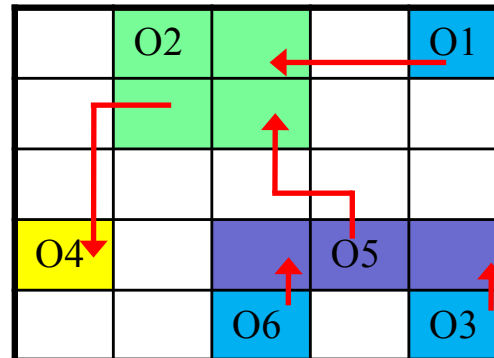


Physical-Level Synthesis

Placement

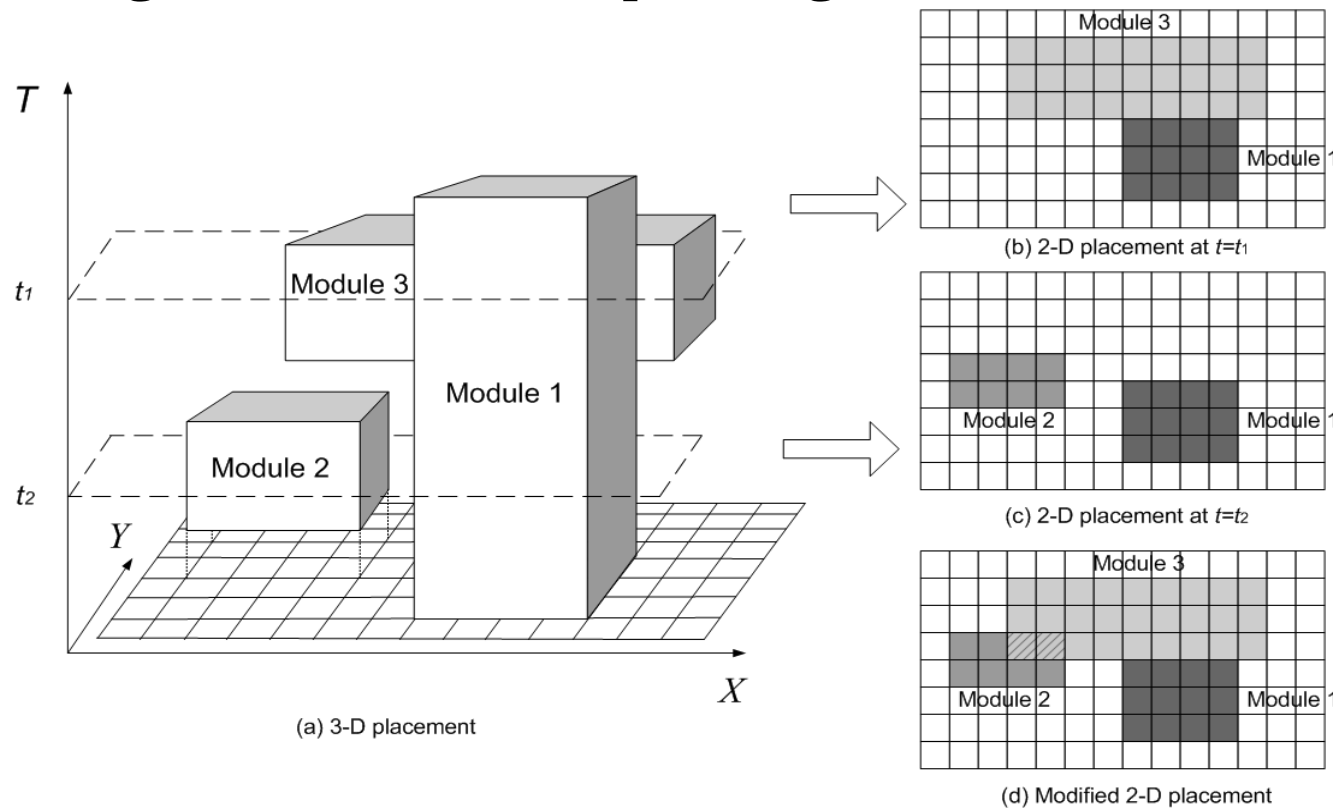


Routing



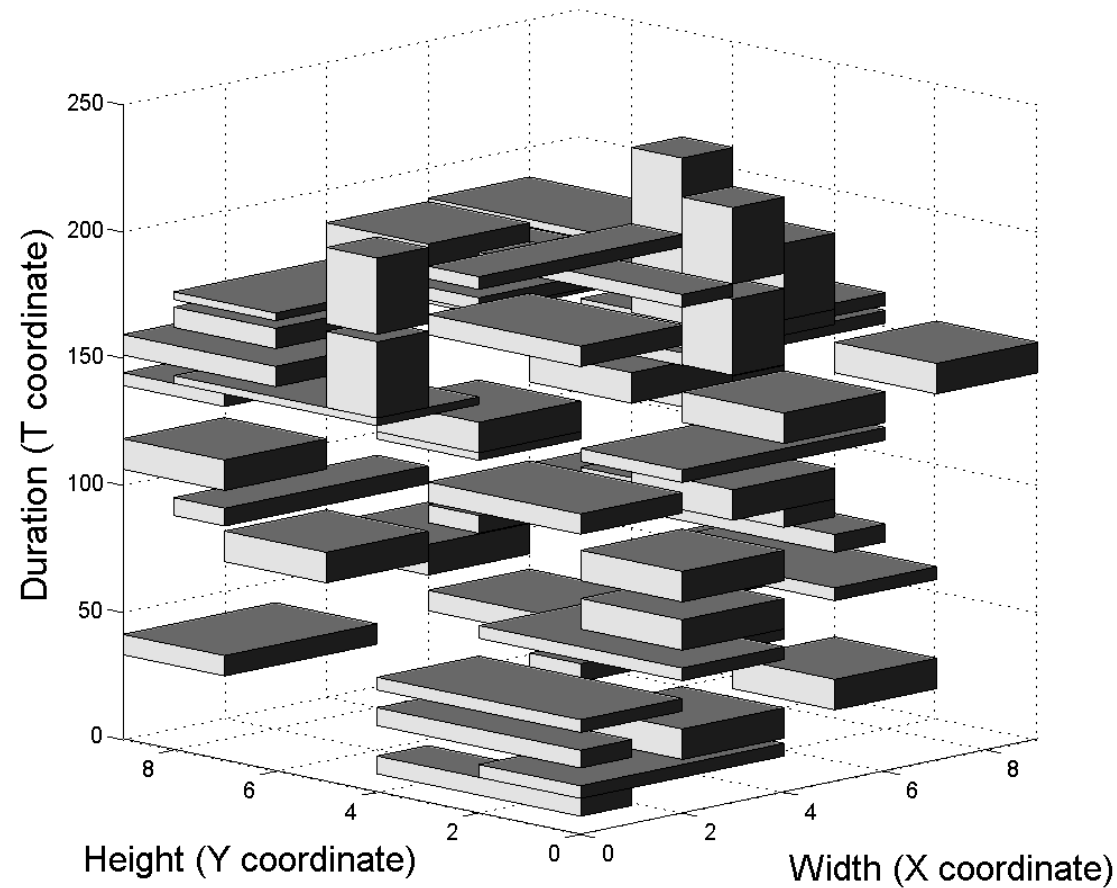
Physical Design: Module Placement

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



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

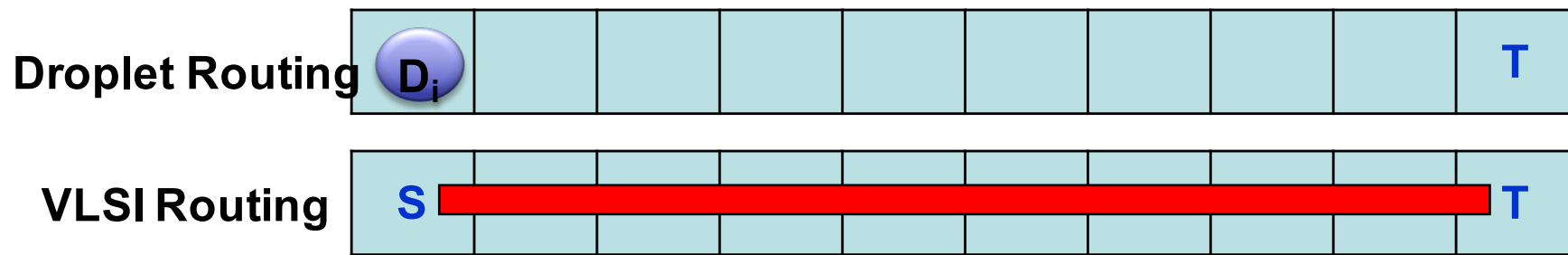
Resulting Placement of the Protein Assay



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

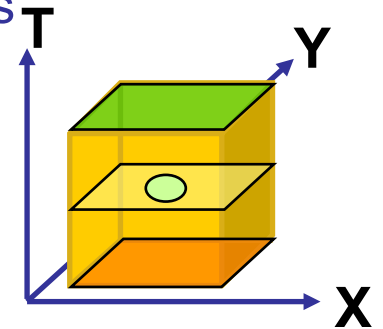
Physical Design: Droplet Routing

- Droplet routing
 - Droplets transportation from one location to another for reaction
- Difference from traditional VLSI routing
 - Cells can be **temporally** shared by droplets - no **permanent** wires on a biochip
 - Droplet routing and scheduling; **scheduling** is to determine droplets' locations at each time step
 - Unique **fluidic properties** for correct droplet movement

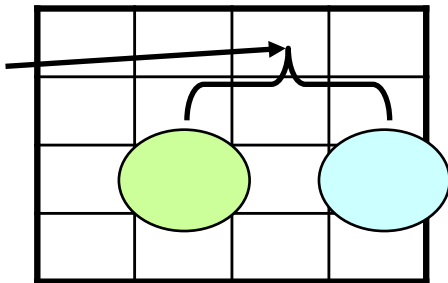


Routing Constraints

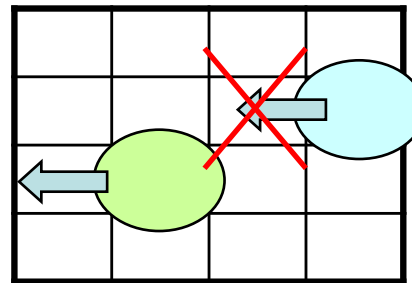
- Fluidic constraint
 - For the correctness of droplet transportation
 - No unexpected mixing among droplets of different samples
 - **Static** and **dynamic** fluidic constraints
- Timing constraint
 - Maximum transportation time of droplets



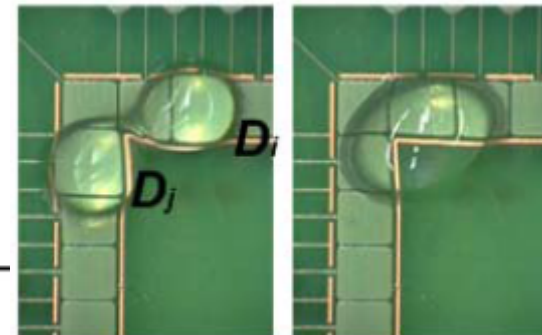
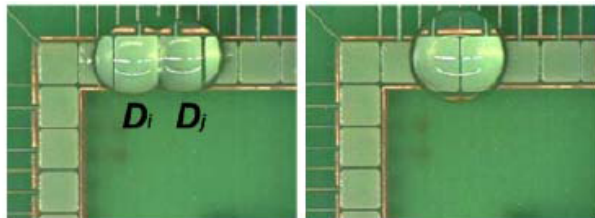
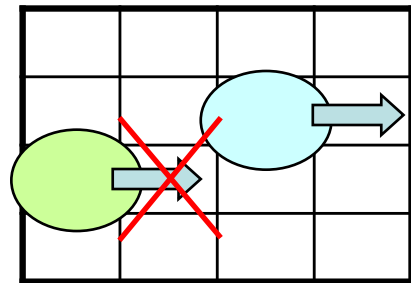
Minimum spacing



Static fluidic constraint

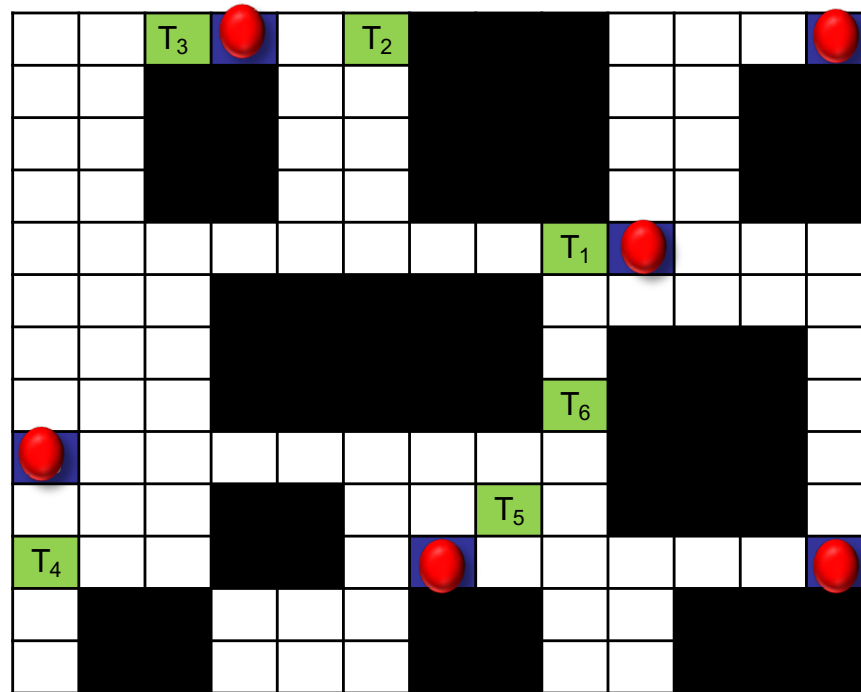


Dynamic fluidic constraint



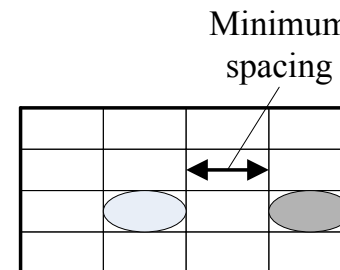
Problem Formulation

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

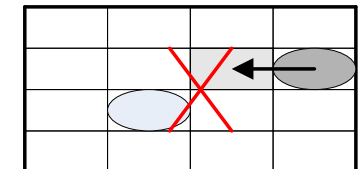


 Droplet  Blockage  Source of droplet i  Target of droplet i

• Fluidic constraint



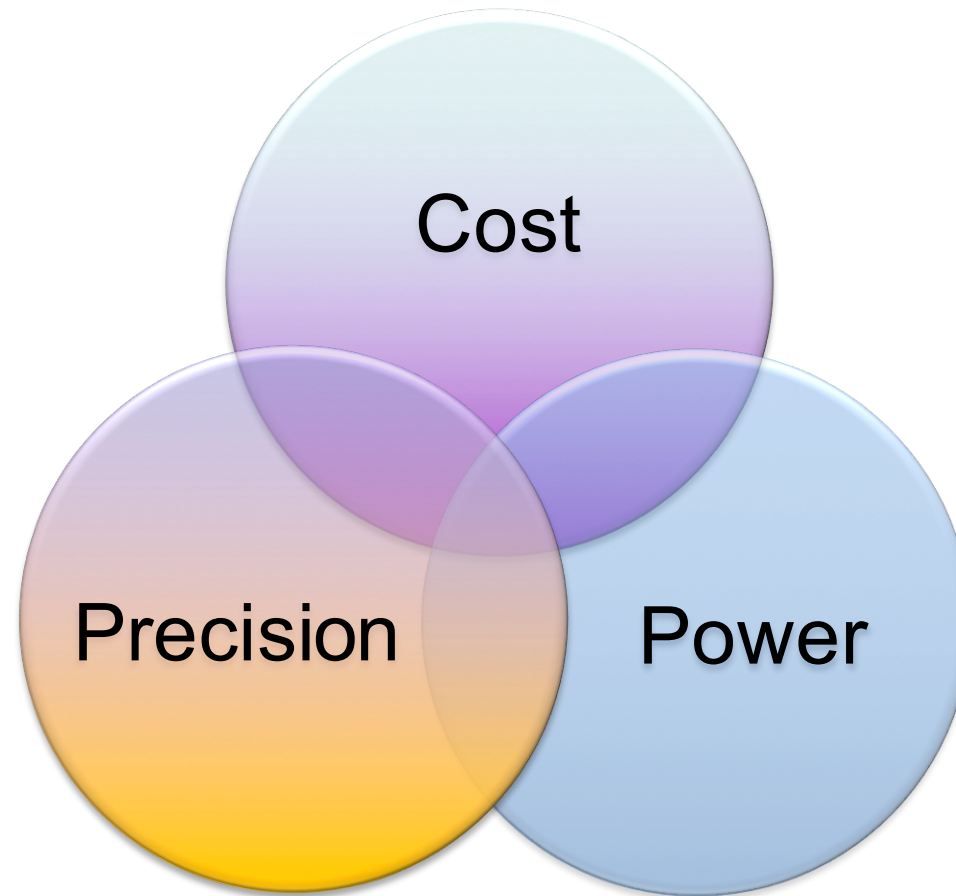
Static fluidic constraint



Dynamic fluidic constraint

• Timing constraint

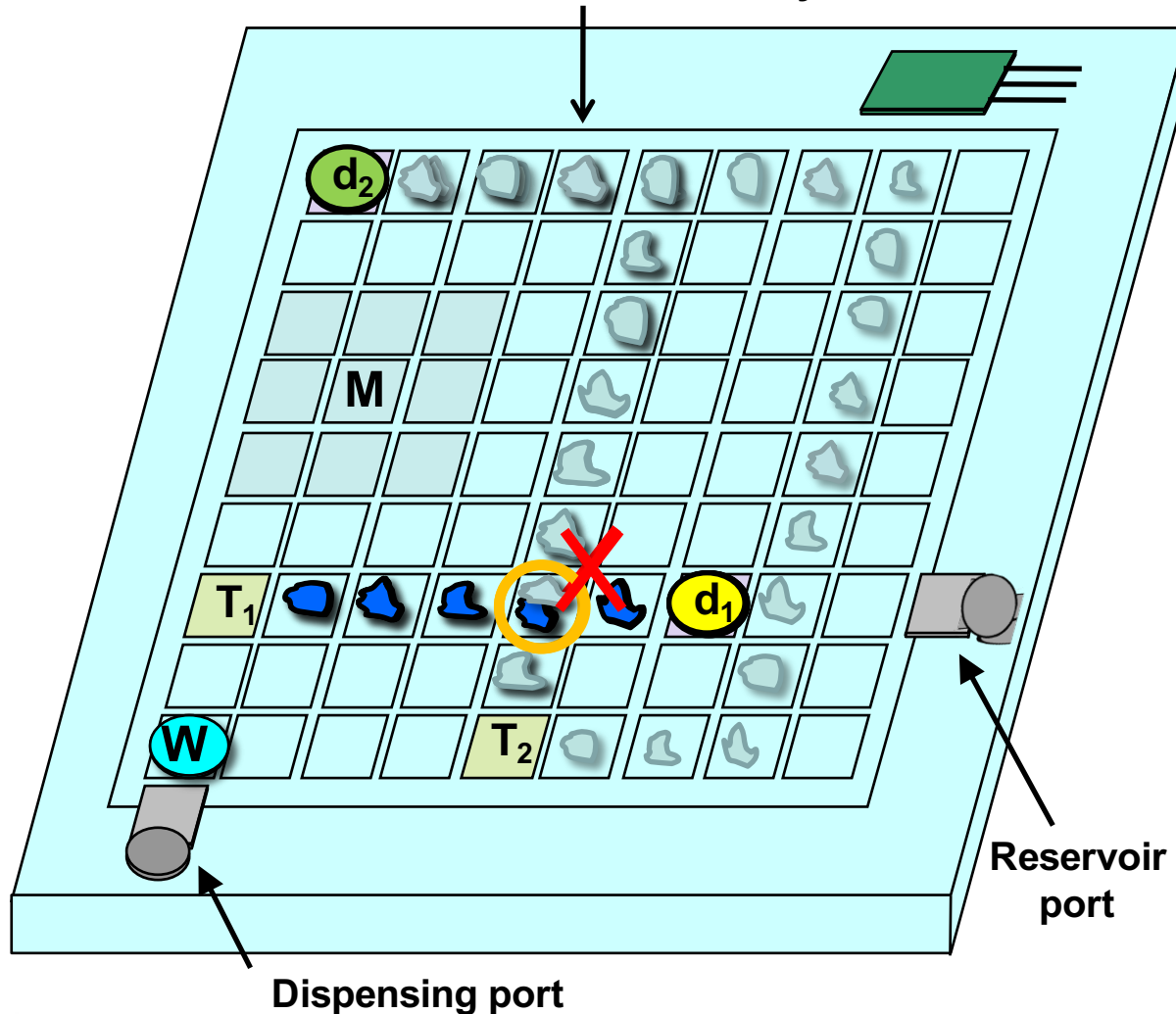
Optimization Goals for DMFBs



Contamination Problem

- Contamination problem

2D microfluidic array



Contamination problem



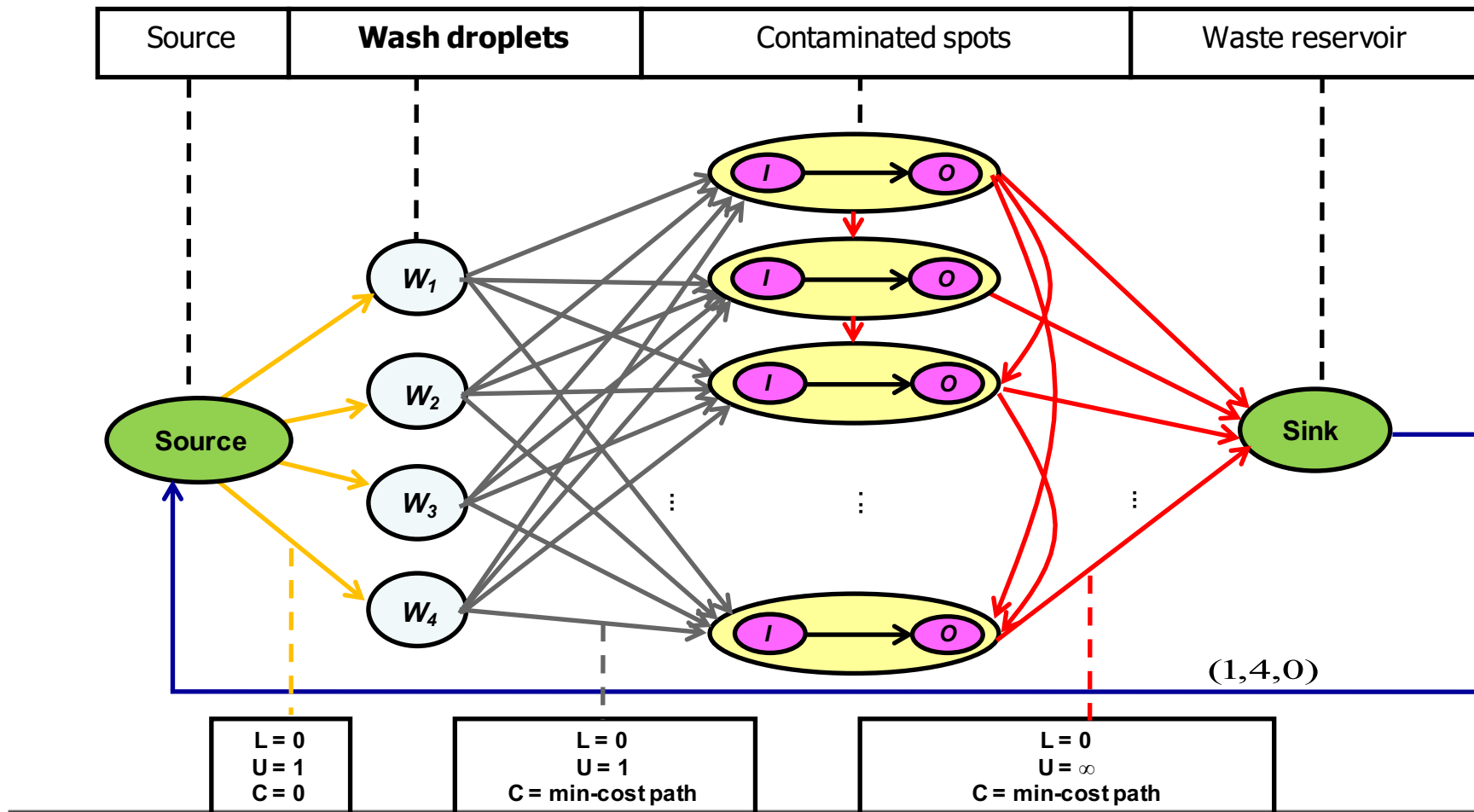
Disjoint routes



Routing with the wash droplet
(1) separately
(2) simultaneously

Scheduling Wash Operations by Minimum Cost Circulation flow network (MCC)

- Construction of the MCC flow network
 - Four major phases in the MCC formulation



成大資工男 美國國際研究賽奪冠

【聯合報/記者修瑞瑩／台南報導】

成功大學資訊工程系四年級學生黃琮蔚，參加美國計算機學會舉辦的國際學生研究競賽，擊敗來自各國名校100多名優秀的碩、博士生拿到冠軍，是獲獎者中唯一大學生，連競賽主席頒獎時都稱讚「從來沒見過這麼厲害的大學生」，為台灣爭光。

黃琮蔚得獎後第一個要感謝的是指導他的資工系助理教授何宗易，他說，如果沒有老師的帶領，可能現在還不知未來方向。

嘉義高中畢業後考上成大，黃琮蔚形容自己高中時期功課只算中上等，但「大學後遇到對的老師，彷彿開竅一般。」他的研究論文已多次發表在國際知名期刊上。

暑假過後將升上碩士班一年級的黃琮蔚，是以「設計數位微流體生物晶片液珠繞線演算法」，獲得6月間在美國舉辦的「國際學生研究競賽」首獎。

他指出，生物晶片在近幾年已發展到「微流體」的階段，更為輕薄短小、效率高，但缺點包括成本高及不同的檢體易交叉感染等問題，他以新的運算方法設計，不僅降低成本達3成，並排除交叉感染，而且能正確讓晶片進行生化反應。

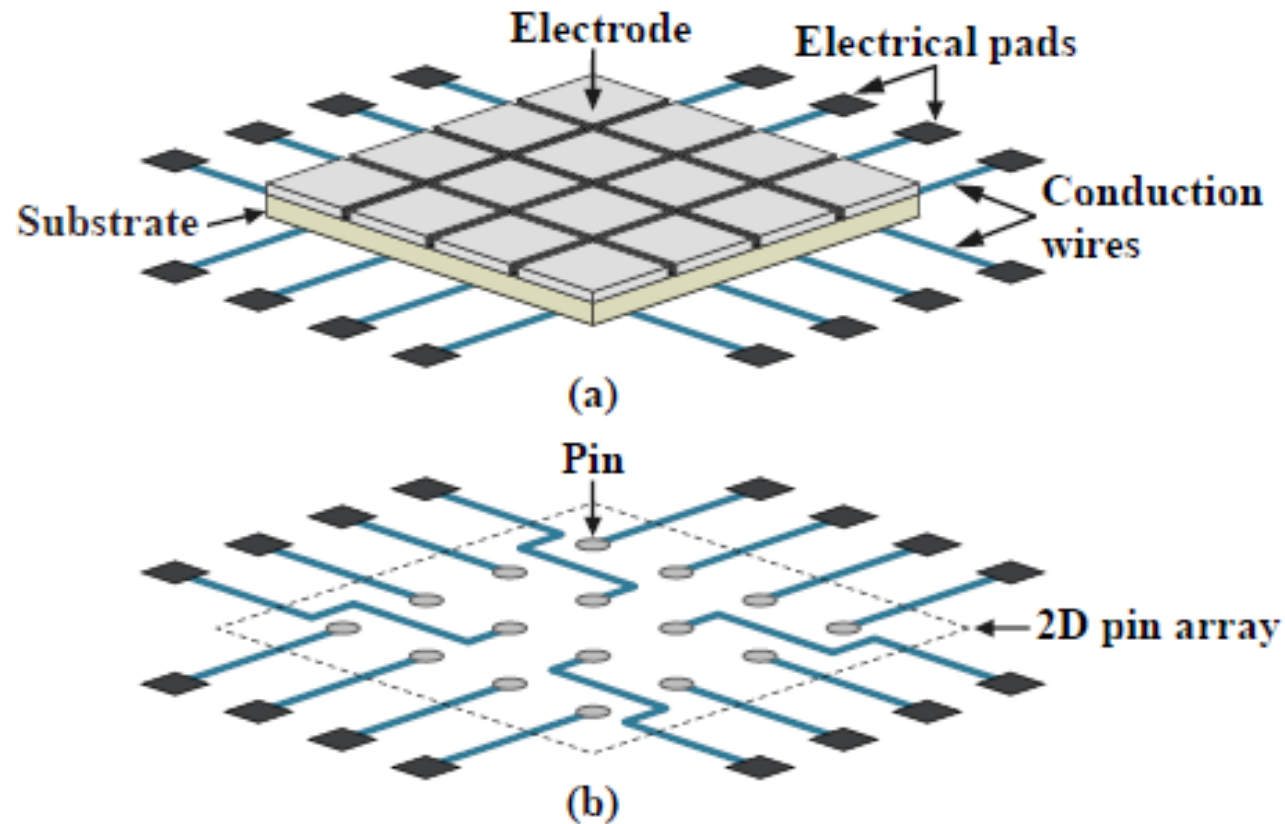
這套運算方法一路過關斬將，連續打敗來自伊利諾大學香檳分校、加州大學柏克萊分校等名校的碩、博士生；同時也有產業界對他的設計感興趣，計畫合作。

黃琮蔚因為想出國深造，大二就開始跟著何宗易做研究，希望做出成績為申請學校加分，當別的大學生在玩樂時，他經常熬夜埋首在實驗室才有好成績。

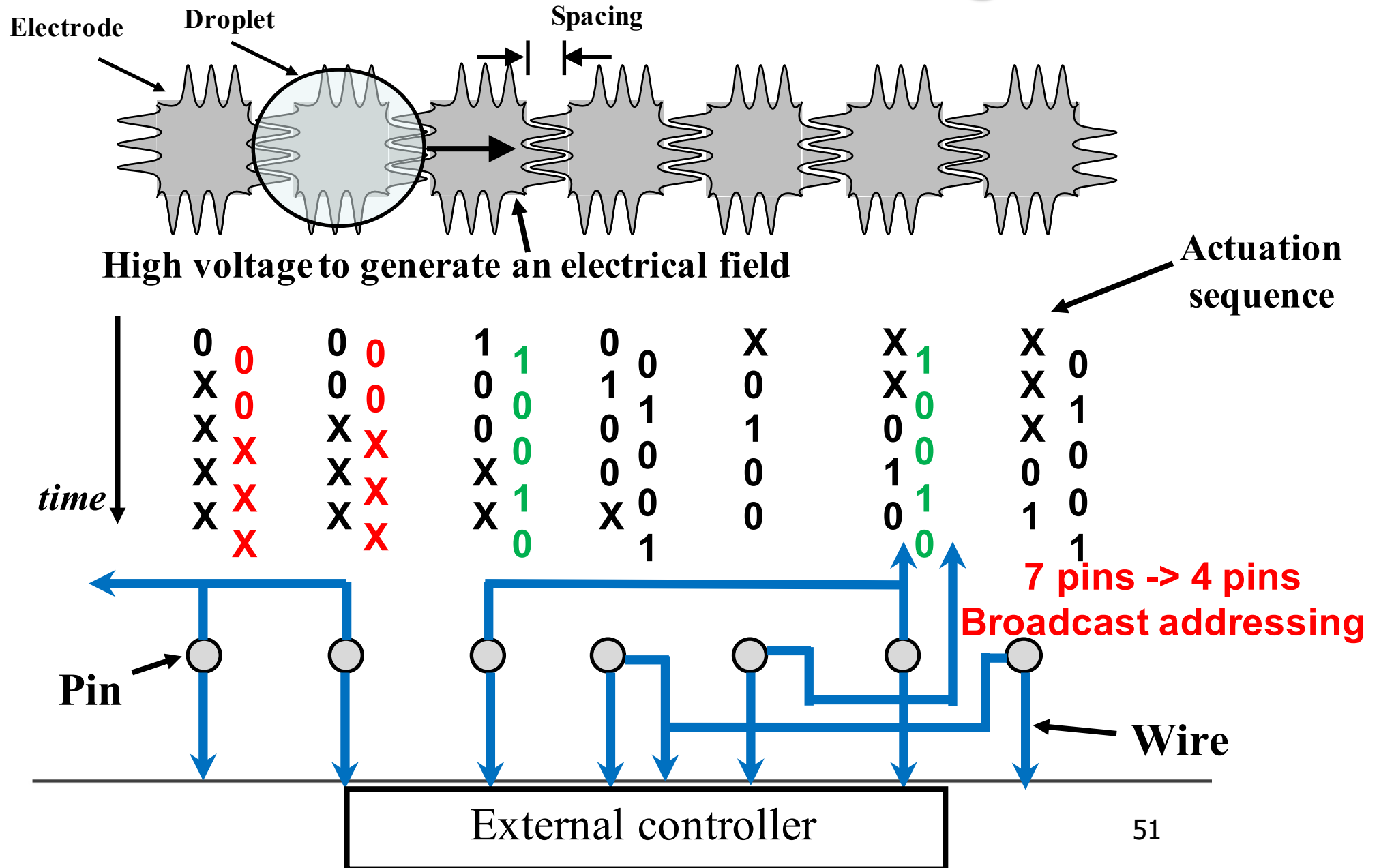


成大資工系學生黃琮蔚（右）在「國際學生研究競賽」中拿到首獎，他認為是恩師何宗易的啟發。記者修瑞瑩／攝影

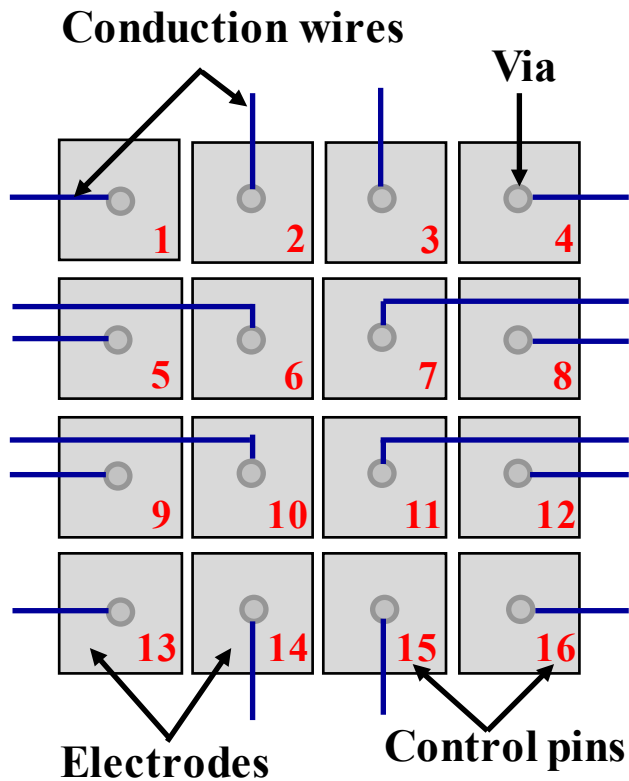
Co-Design for DMFB and PCB



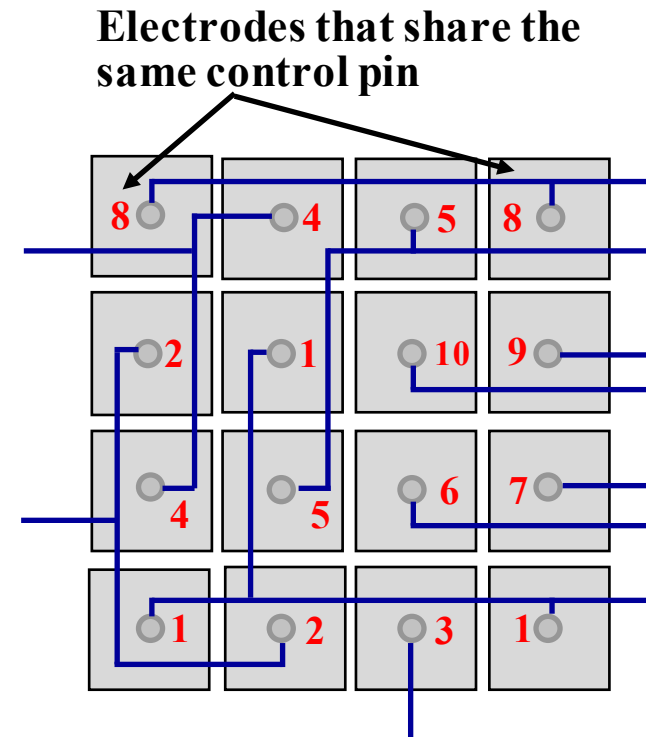
Broadcast Electrode Addressing Scheme



Impacts on PCB Routing



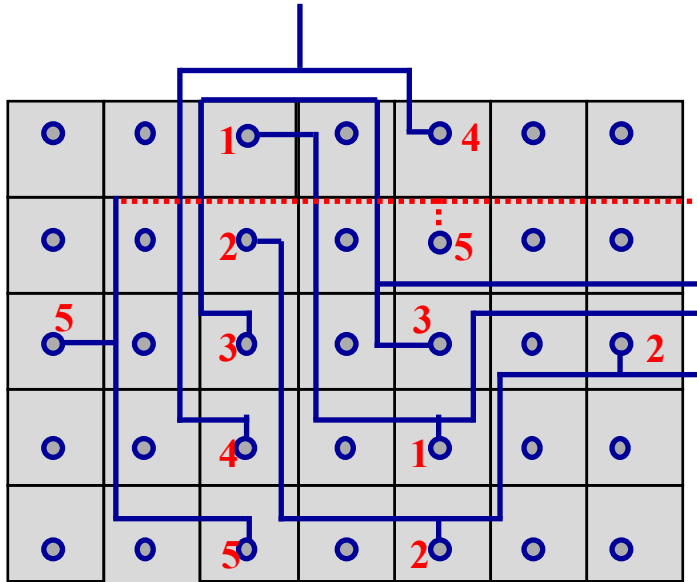
(a) Direct Addressing
Control pins: 16



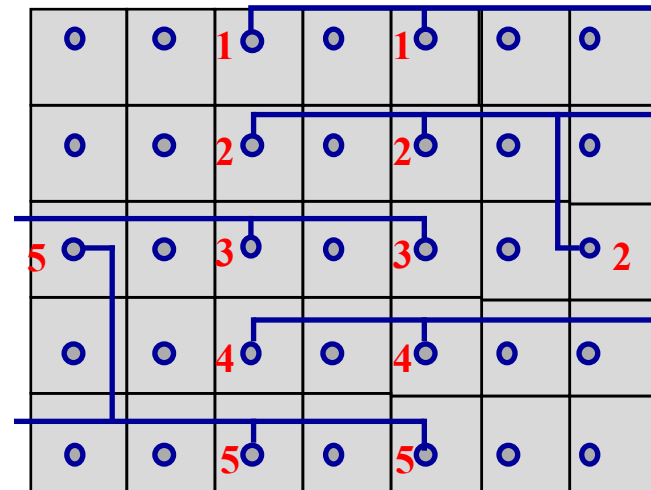
(b) Broadcast addressing
Control pins: 10

Impacts on PCB Routing

5 control pins

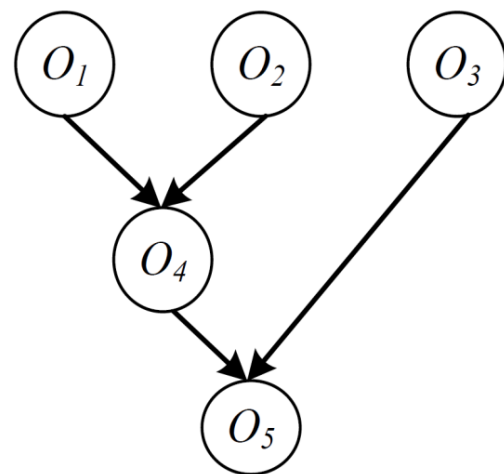


(a) Infeasible routing solution



(b) Feasible routing solution

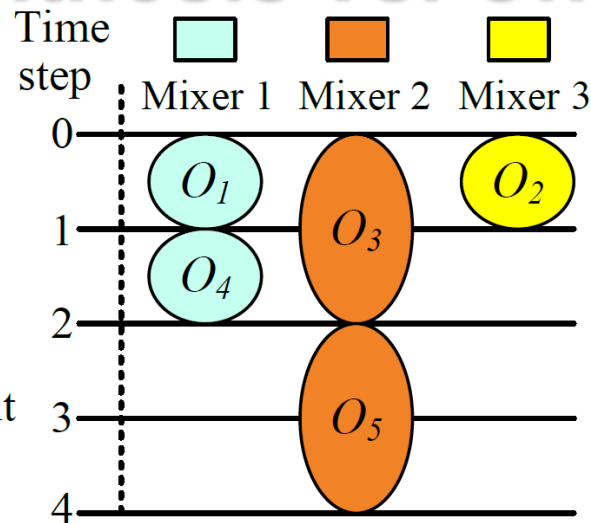
Fluidic-Level Synthesis vs. Chip-Level Design



Sequence graph

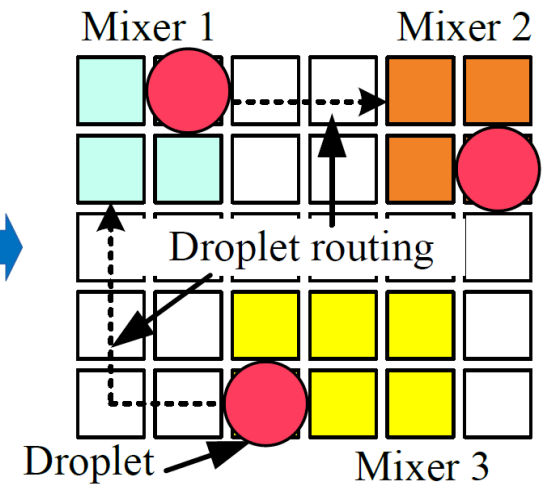
Device
binding

Time-step
assignment



Scheduling result

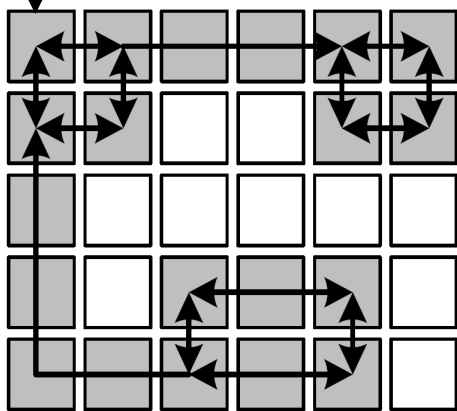
Chip
layout



Placement & droplet routing

Fluidic-level synthesis

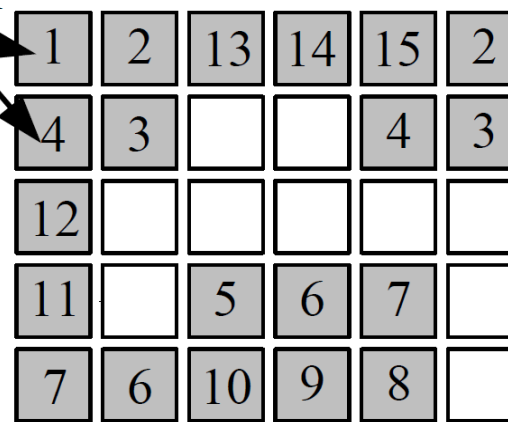
Chip-level design



Used electrodes

Signal
merging

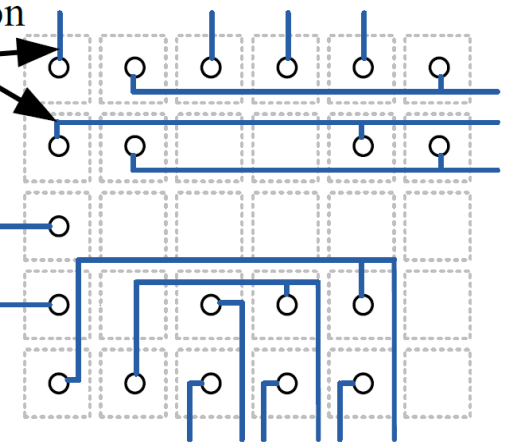
Control
pins



Electrode addressing

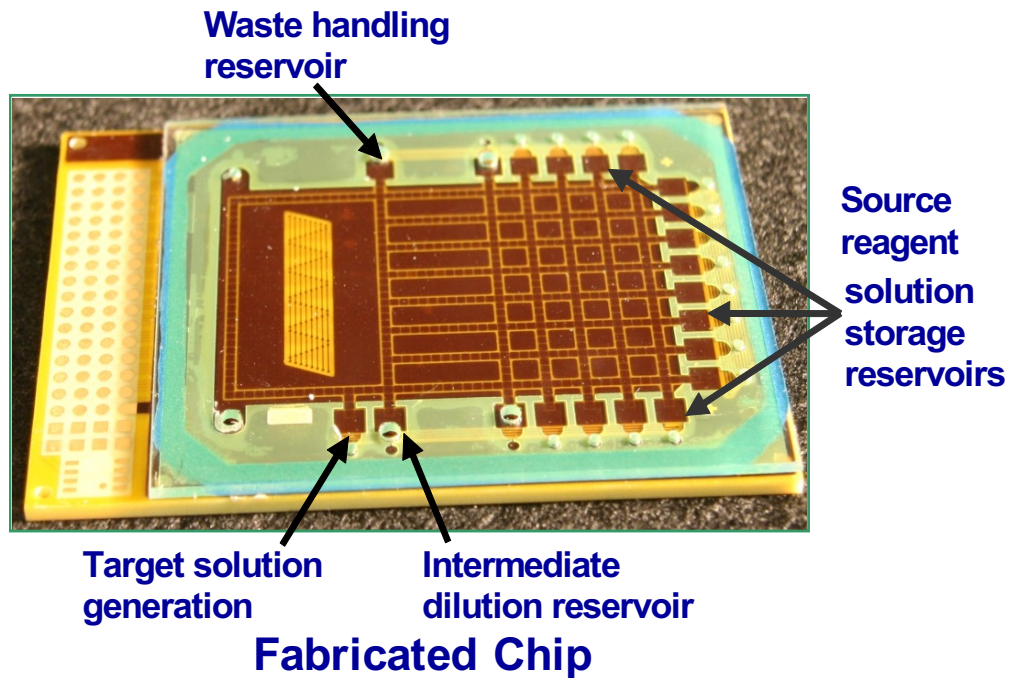
Conduction
wires

Escape to
I/O pads



Wire routing

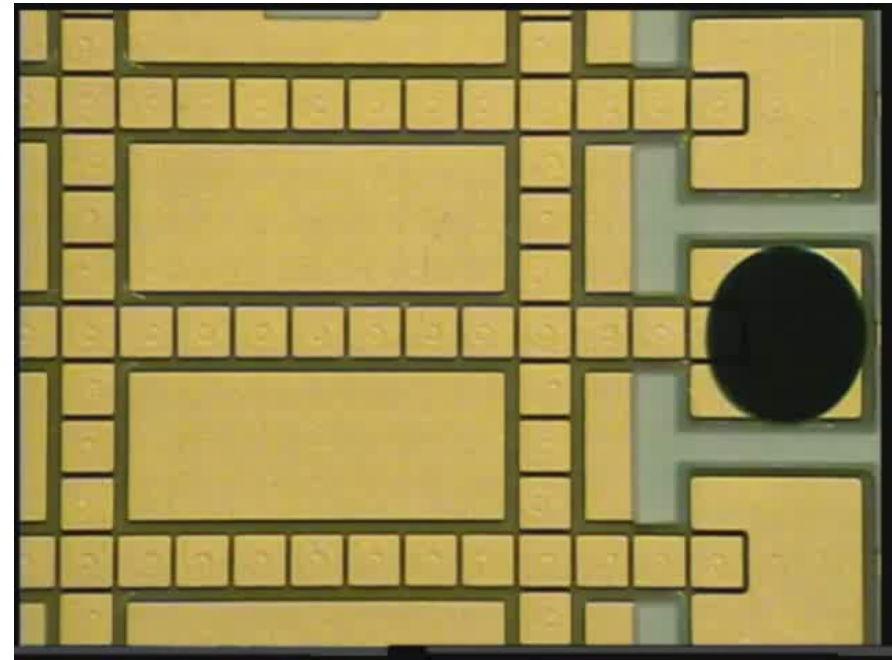
Chip Sample from Advance Liquid Logic



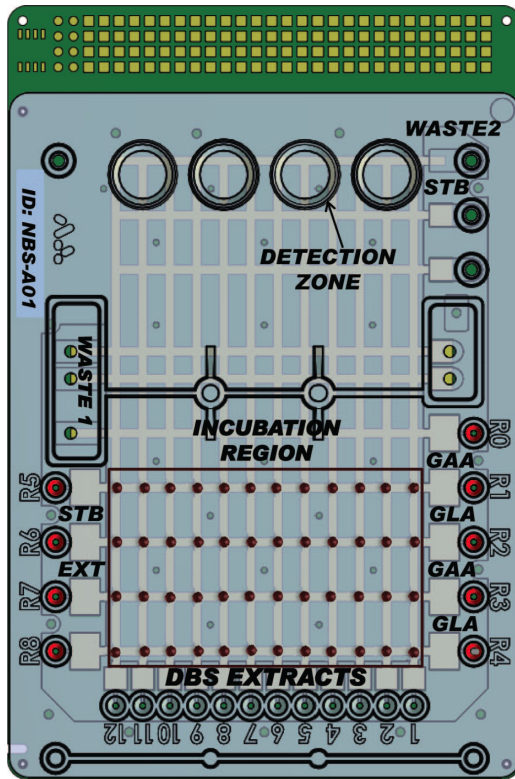
- Pitch: 1.5 mm; Height: 0.475 mm; Actuation voltage: 50 V
- Fabricated on PCB board
- 1140 electrodes, 64 pins
- Applied for *n*-plex bioassay



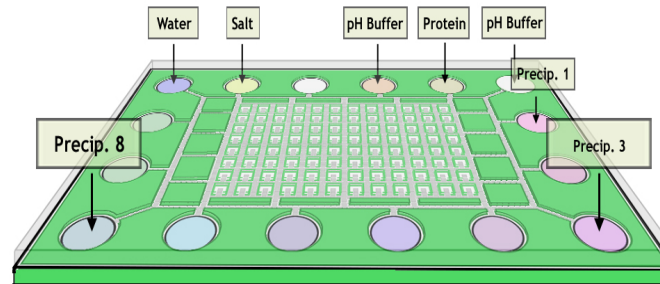
Application Platform



Other Successful Collaborations



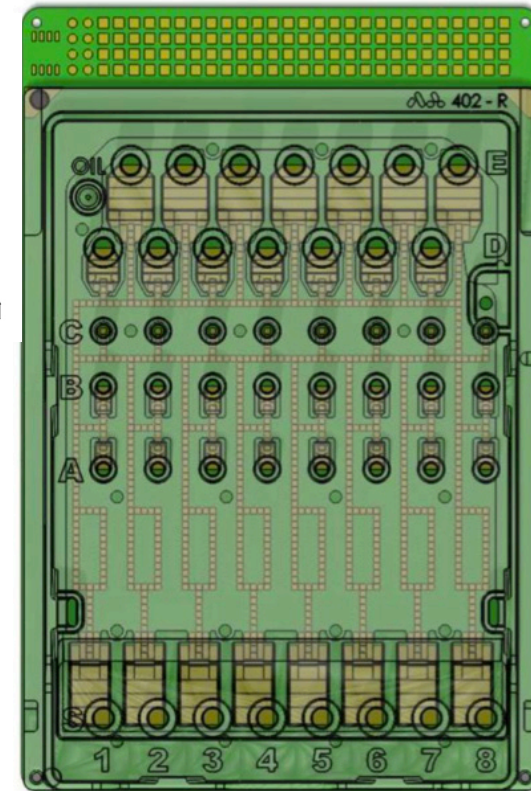
Biochip for newborn screening
(Advanced Liquid Logistics, Inc.)



Protein crystallization chip

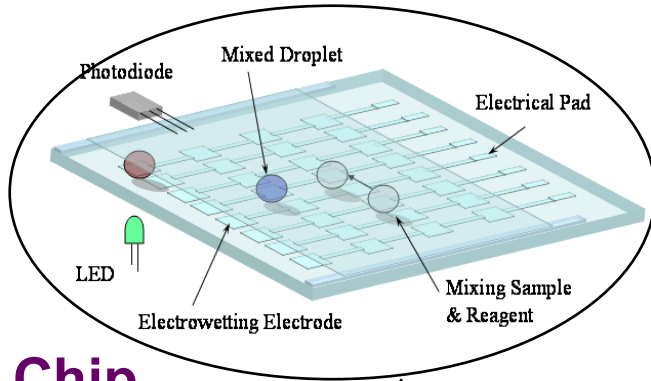


Hemophilia

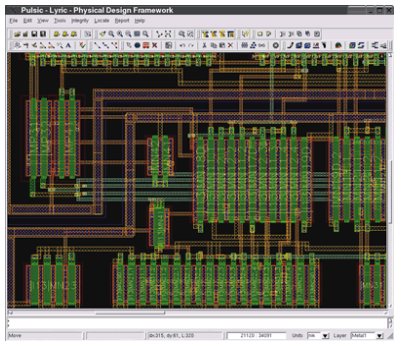
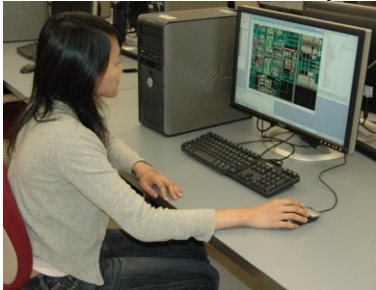


Biochip for sample preparation
(Nugen Technologies, Inc.)

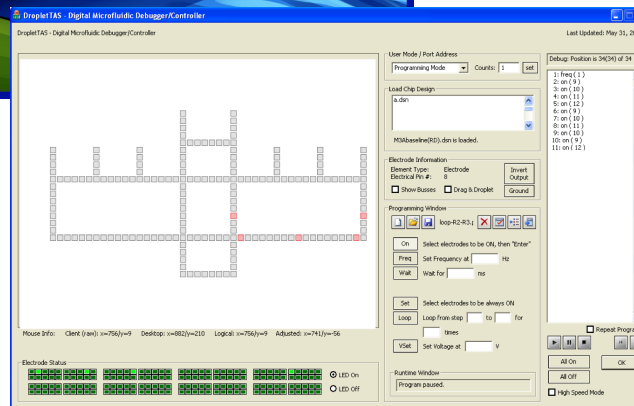
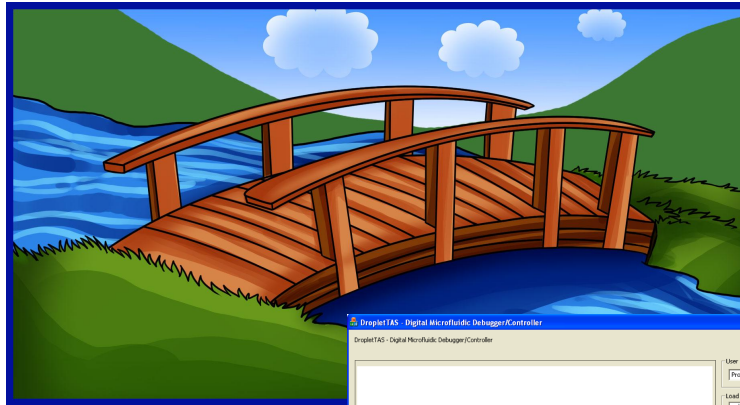
The Biochip Ecosystem



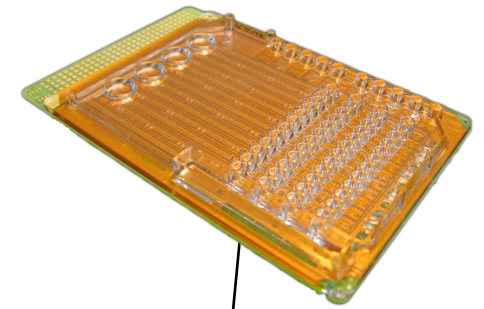
Chip
Designers



Control software



Chip Users



**Thank You for
Your Attention!**

CAREER-DEVELOPMENT LESSONS

- **So, you are a researcher or engineer climbing the career ladder**
- **Here are three lessons to help you succeed**

LESSON 1

A crow was sitting on a tree, doing nothing all day.



A small rabbit saw the crow, and asked him, *"Can I also sit like you and do nothing all day long?"*

The crow answered: *"Sure, why not."*

So, the rabbit sat on the ground below the crow, and rested.



All of a sudden, a fox appeared,

Jumped on the rabbit... and ate it.



LESSON 1

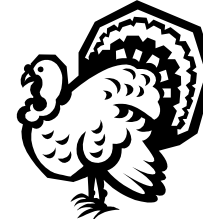
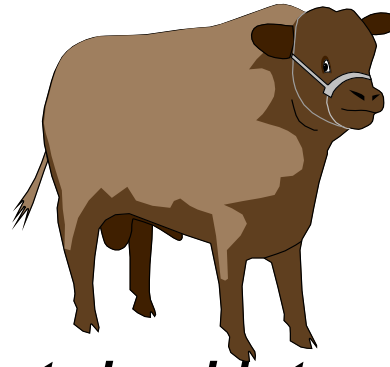
Moral of the story is....

To be sitting and doing nothing,
you must be sitting very, very high up.



LESSON 2

A turkey was chatting with a bull.



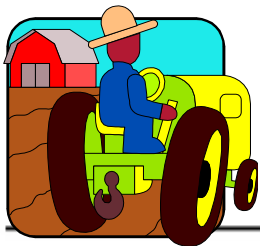
"I would love to be able to get to the top of that tree," sighed the turkey, "but I haven't got the energy."

"Well, why don't you nibble on some of my droppings?" replied the bull. "They're packed with nutrients."

The turkey pecked at a lump of dung and found that it gave him enough strength to reach the first branch of the tree.

The next day, after eating more dung, he reached the second branch.

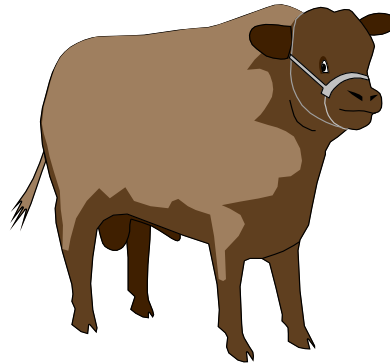
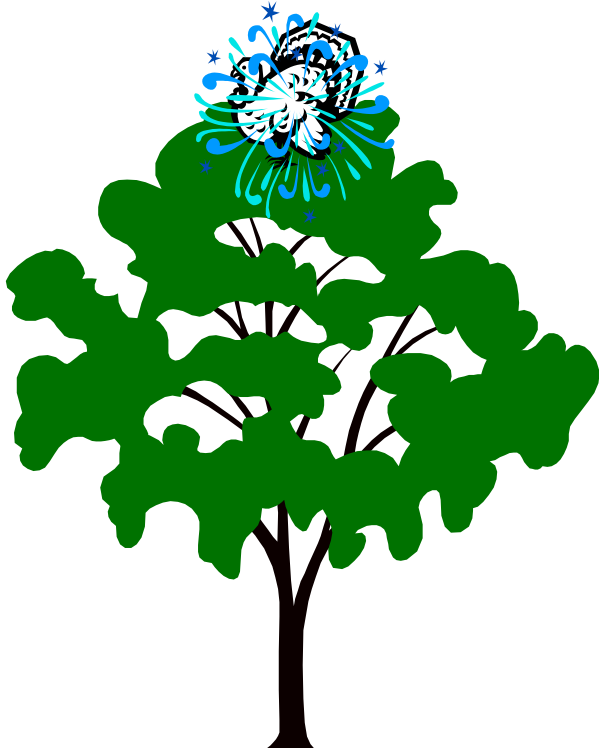
Finally after a fortnight, there he was proudly perched at the top of the tree



Soon he was spotted by a farmer

Who promptly shot the turkey out of the tree.

LESSON 2



Moral of the story:

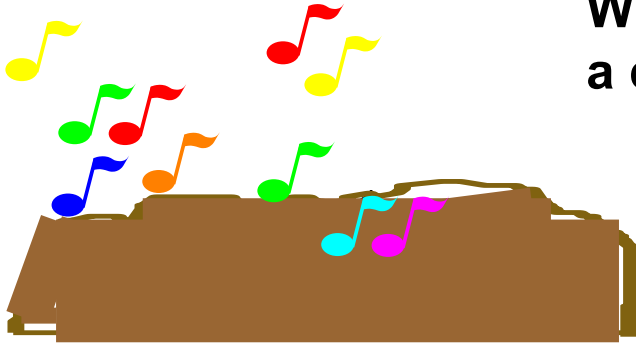
Bullst might get you to the top,
but it won't keep you there.**

LESSON 3

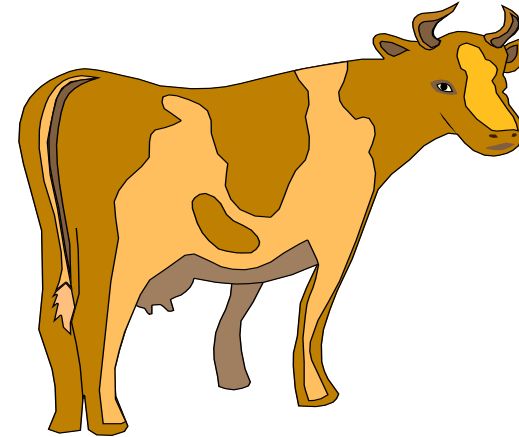
A little bird was flying south for the winter.



It was so cold, the bird froze and fell to the ground in a large field.



While it was lying there,
a cow came by
and dropped some
dung on it.



As the frozen bird lay there in the pile of cow dung, it began to realize how warm it was. The dung was actually thawing him out!

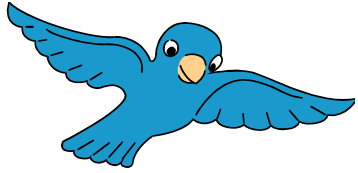


He lay there all warm and happy, and soon began to sing for joy.

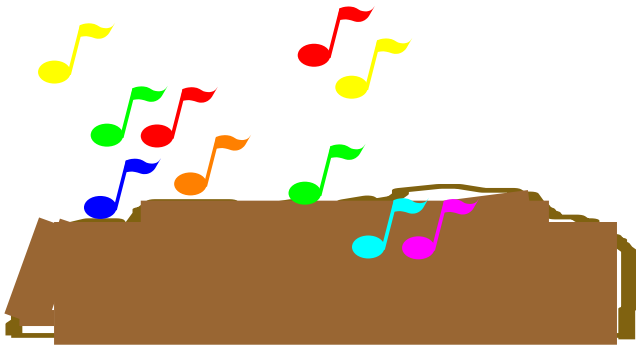
A passing cat heard the bird singing and came to investigate.

Following the sound, the cat discovered the bird under the pile of cow dung, and promptly dug him out and ate him!

LESSON 3



The morals of this story are:



- 1) Not everyone who drops s**t on you is your enemy.
- 2) Not everyone who gets you out of s**t is your friend.
- 3) And when you're in deep s**t, keep your mouth shut.

