Progress in Reconfigurable Microfluidic Systems for Evolvable BioChips

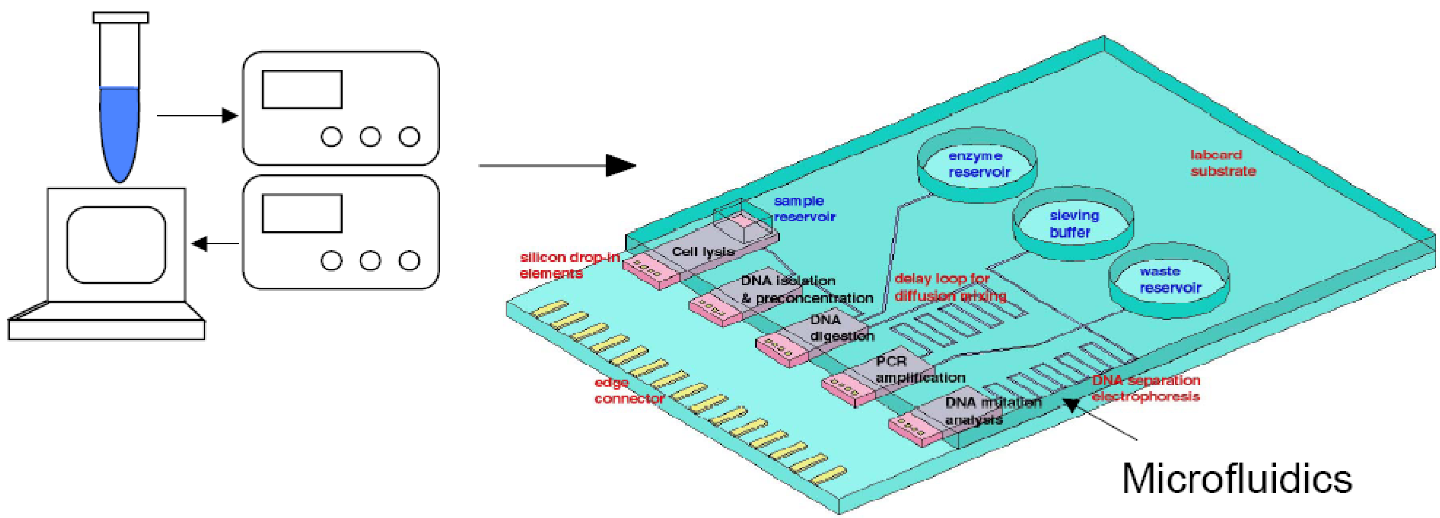
R.B. Fair
Department of Electrical and Computer Engineering
Duke University
Durham, N.C.

Outline of Presentation

- Background and motivation
  - The single-application-chip paradigm
  - Is there a technology for integrated, reconfigurable, evolvable microfluidic devices?
- Evolvable microfluidic devices
  - Architectural choices
  - Digital microfluidics
- Digital microfluidic architecture concepts
  - Implications of droplet architecture
  - Examples of reconfigurable devices
    - Cytotoxicity screening
    - DNA sequencing
- Summary and conclusions

Microfluidic Functions

Benchtop Laboratory Techniques		"Lab on a Chip"
Many manual steps	→	Integrated sampling, chemical reactions, mixing, separation, detection, data processing

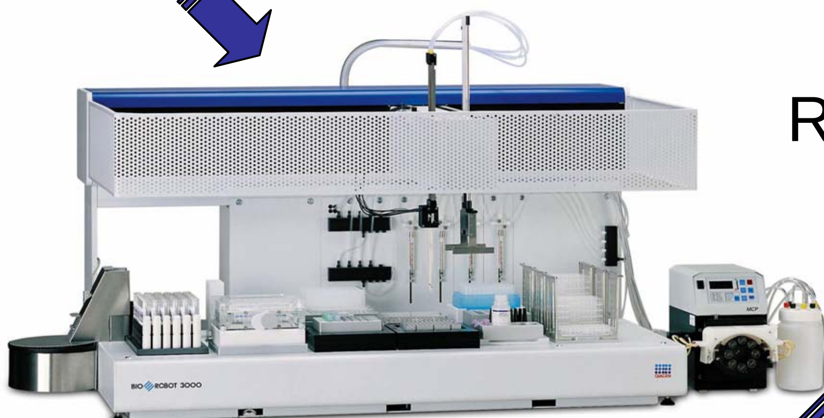


Department of Electrical and Computer Engineering

Background & Motivation

Test tubes

- [ ] Automation
- [ ] Integration
- [ ] Miniaturization
- [x] Reconfiguration

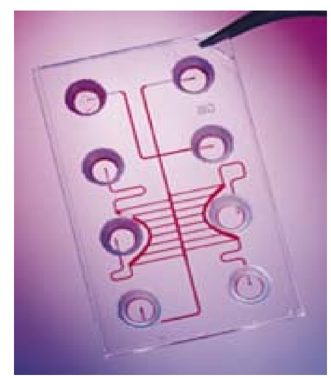


Robotics

- [x] Automation
- [x] Integration
- [ ] Miniaturization
- [x] Reconfiguration

Microfluidics

- [x] Automation
- [x] Integration ?
- [x] Miniaturization
- [ ] Reconfiguration



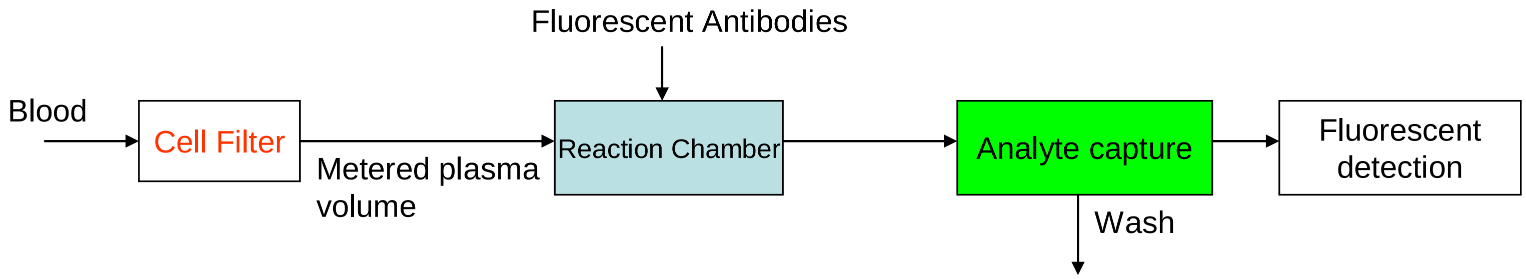
Start of the Art Commercial Disposable Microfluidics

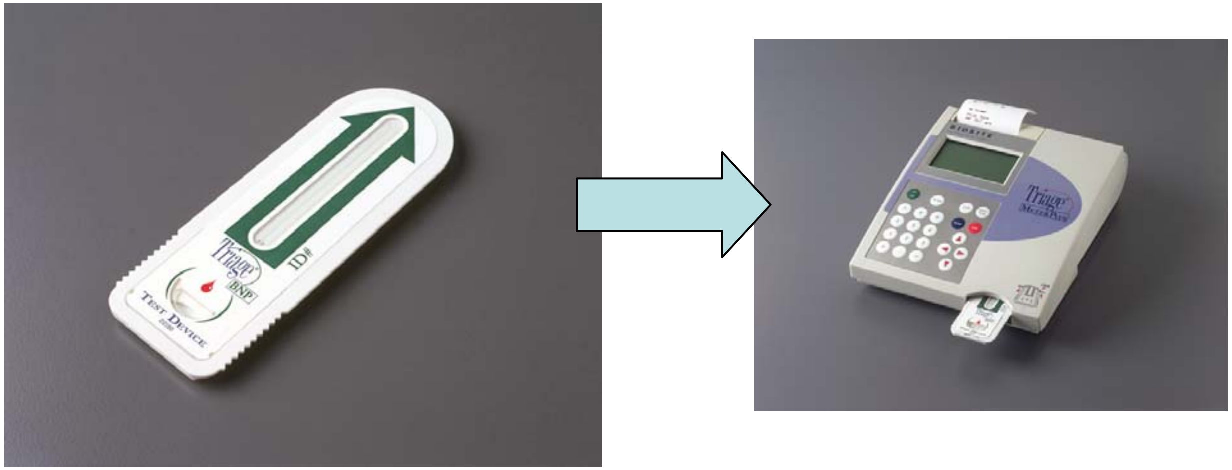
ANOTHER EXAMPLE OF A MICROFLUIDIC SYSTEM

LAB-ON A CHIP BIOSITE

DIAGNOSES HEART ATTACK WITHIN 10 MN

Single Application Chip Paradigm

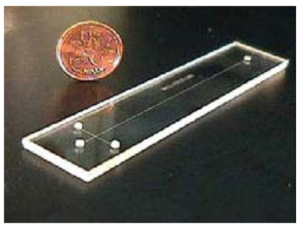


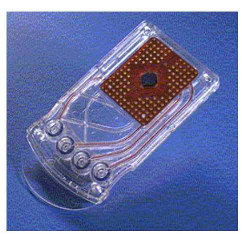


Concept of Disposable Integration

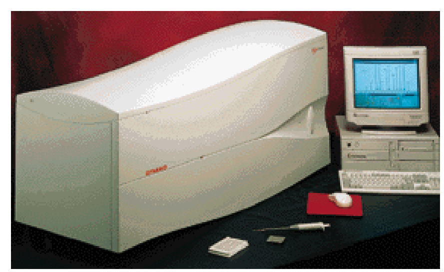
Application Devices

MICROFLUIDIC

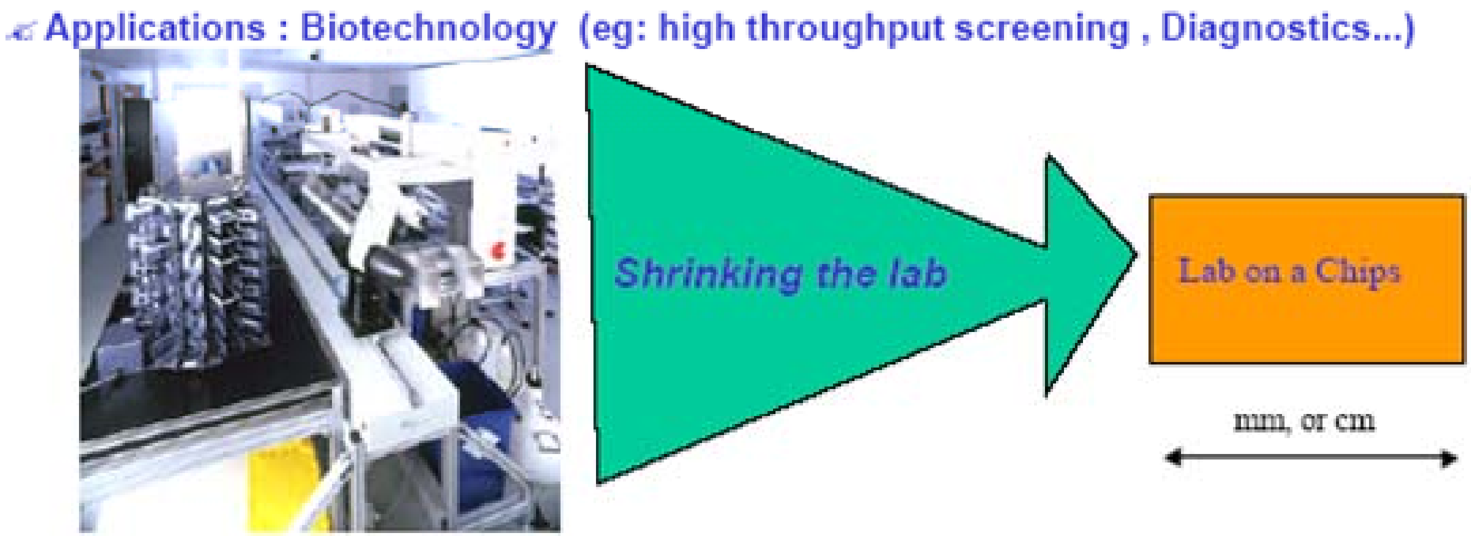




PROCESSING/ANALYSIS



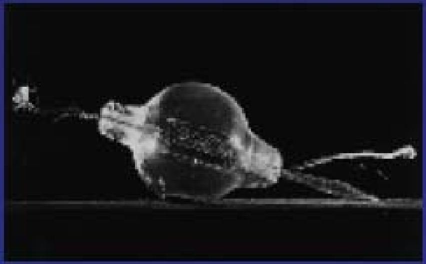
Promise of Biochips

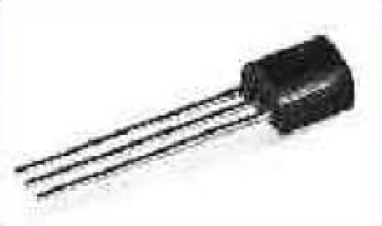


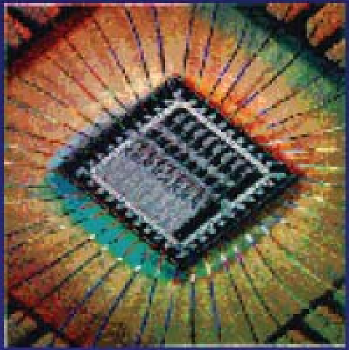
• Can a lab-on-a-chip be as versatile as the macro lab it replaces?
• Can it be programmed to evolve?

Department of Electrical and
Computer Engineering

The Electronics Revolution



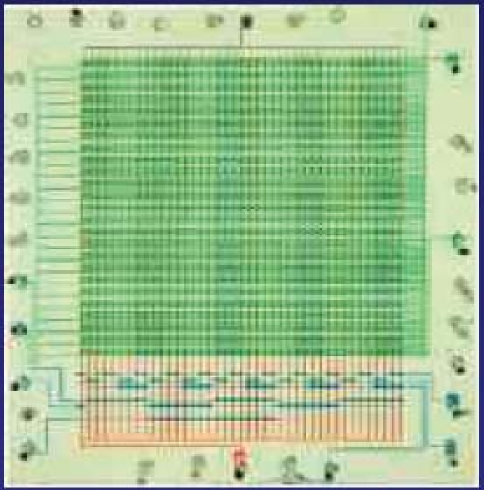




(After T. Thorsen, 2004)

The Fluidic Revolution

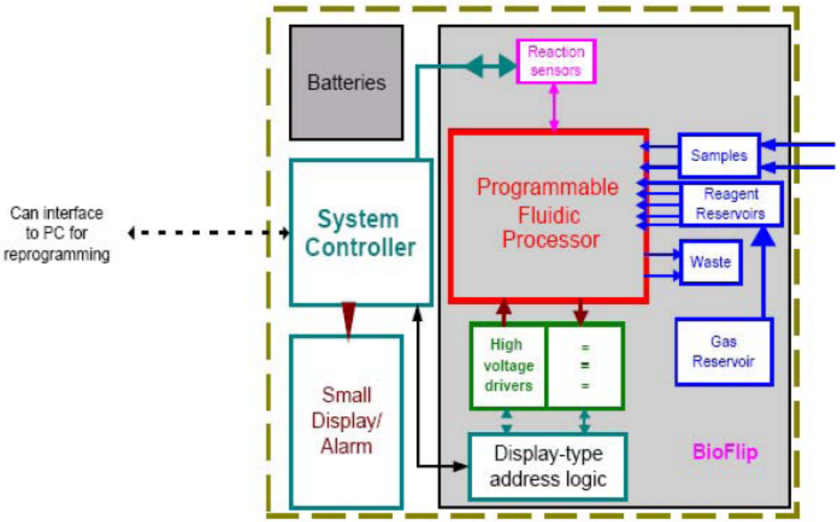


(After T. Thorsen, 2004)

Evolable Microfluidic System Questions

- Is there a fluidic equivalent to the electronic FPGA?
- Is it possible to manipulate fluidic inputs to accomplish hardware optimization?
- Is it possible to have a common fluidic platform that can evolve to run multiple applications?
- What special requirements do fluidic systems have beyond electronic systems?

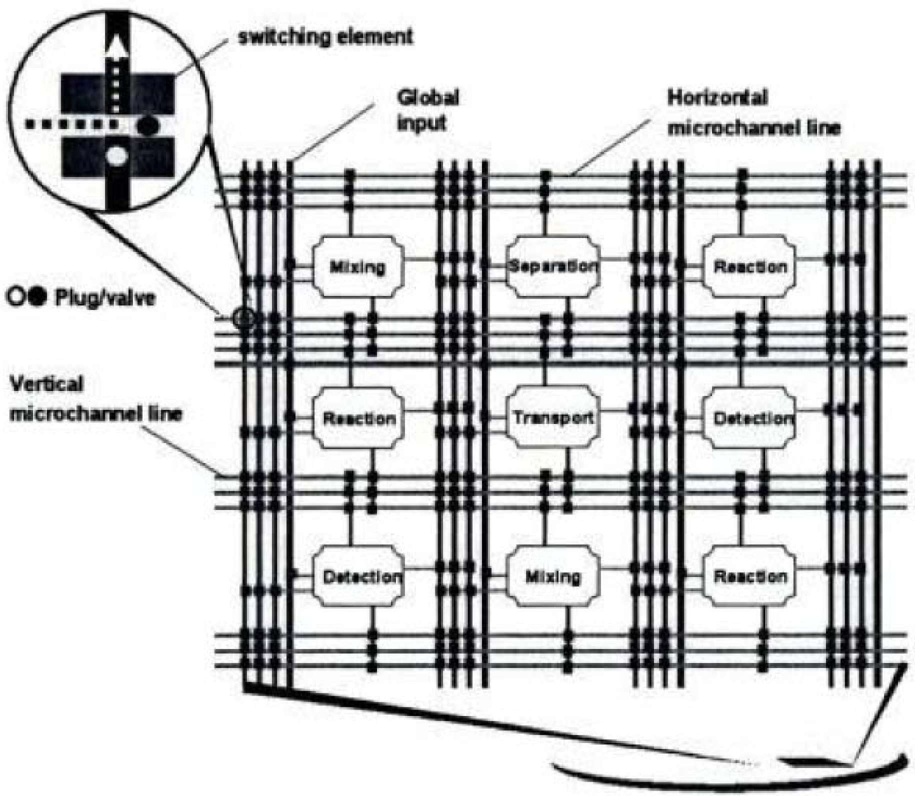


Evolvable Microfluidic Systems

- Goal: bridge world of biofluidic operations and reconfigurable hardware
- Approaches:
  - Fixed fluidic processors and reconfigurable fluidic connections
    - Dynamically switchable fluidic connections require 1000's of simple valves
  - Reconfigurable fluidic processors and fixed fluidic connections
    - Dynamically reconfigurable processors need to support numerous fluidic operations with a common set of reusable components
  - Reconfigurable processors and connections and programmable control layer

Fixed fluidic processors and reconfigurable fluidic connections

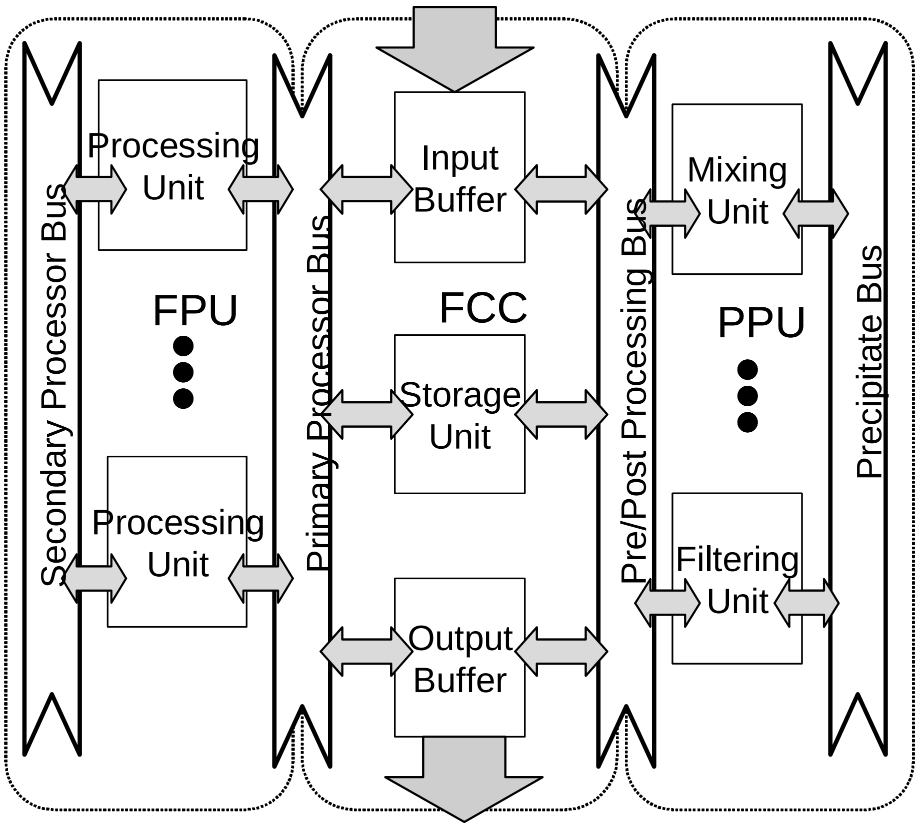
- McCaskill and Wagler (2000):



Department of Electrical and Computer Engineering

Monarch Microfluidic Architecture (Duke University – 2000)

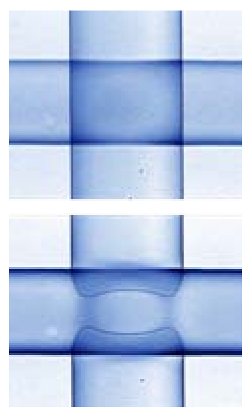
- Continuous Flow
- Segregated Processing Units
- Shared Bus Architecture
- I/O to/from outside world
- Pressure driven
- Rigid functional units defined at assembly



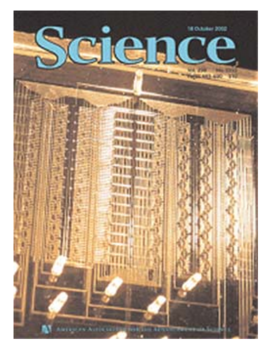
Department of Electrical and Computer Engineering

Reconfigurable Continuous Flow

- Difficult to implement in continuous flow microfluidic systems
  - Need for many valves and external pneumatic control box
  - Difficult to stage samples
- Field was ripe for breakthrough technology
- Membrane microvalves (Quake et al. Cal Tech)



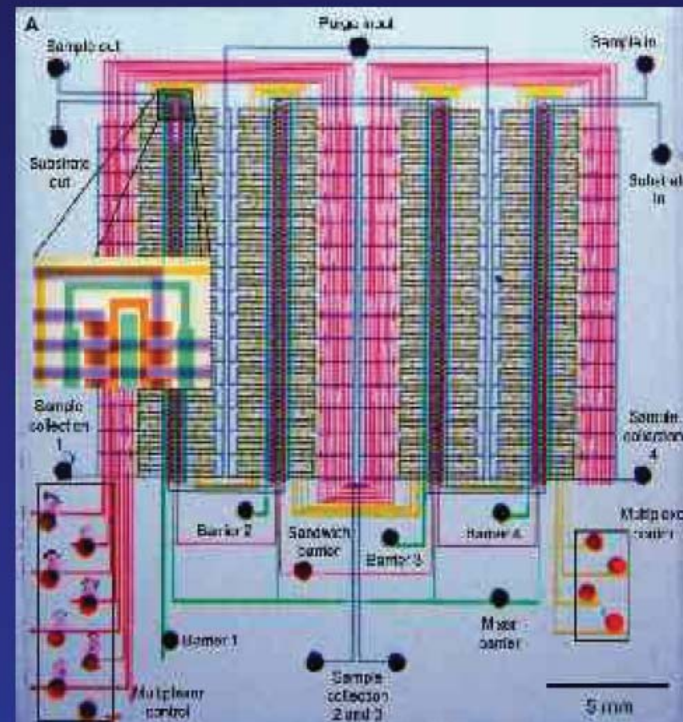
Shown here is a magnified close-up of a NanoFlex™ valve. The image on top shows the overlap of a control channel and a fluid channel. The image below shows the membrane fully deflected into the fluid channel, effecting a tight seal.



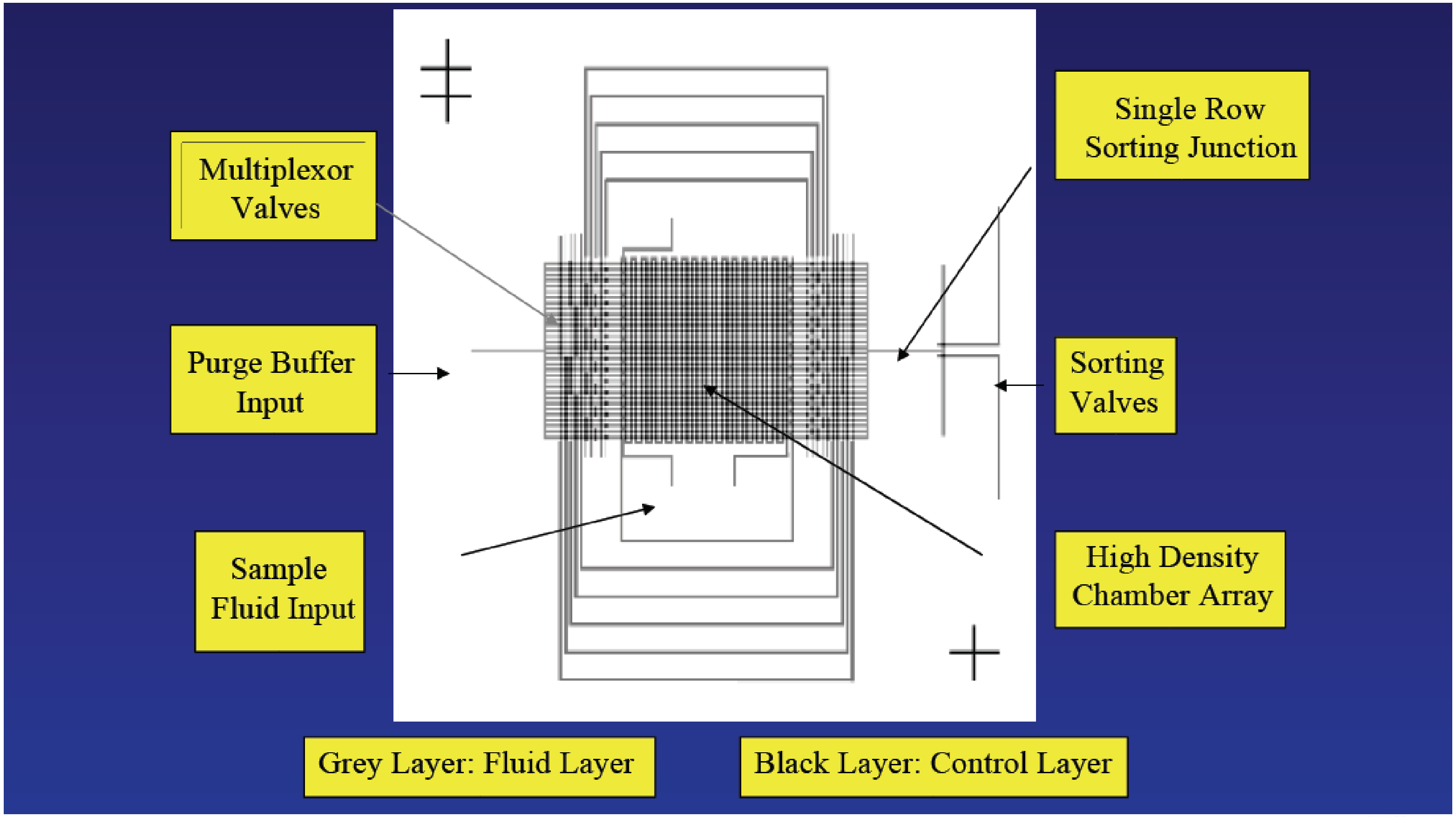
Oct. 18, 2002

Flex Valve Allows Complexity
(Cal Tech-2002)

- Elastomeric Valves in Multiplexed format can be used to construct sophisticated chips
- Dual sample chip with mixing functionality



Cal Tech Multilayer Fluidic Chips



Department of Electrical and Computer Engineering

Fluidigm Protein Crystallization Chip

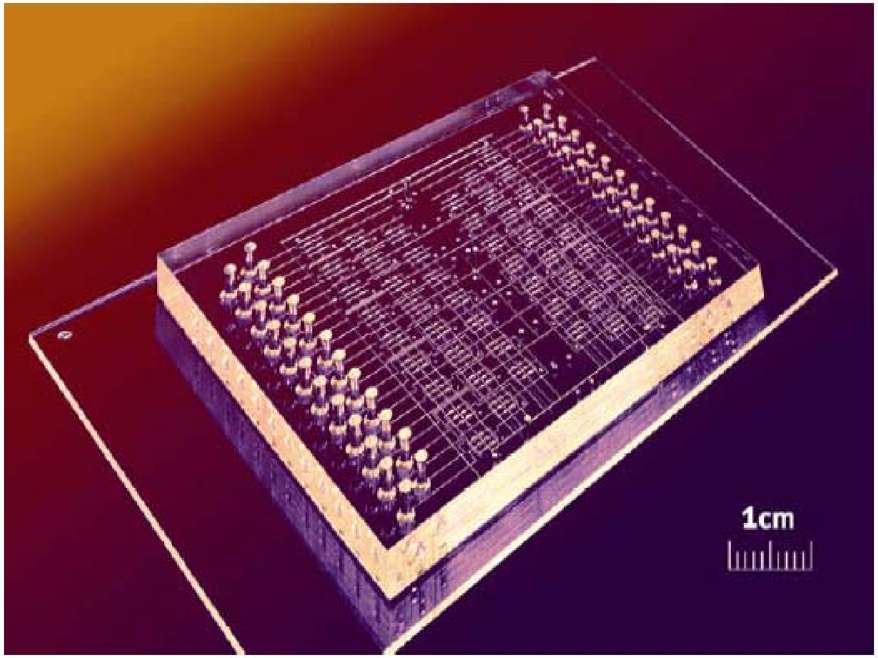
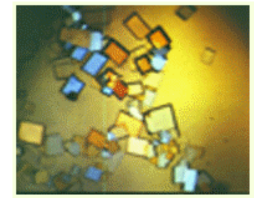
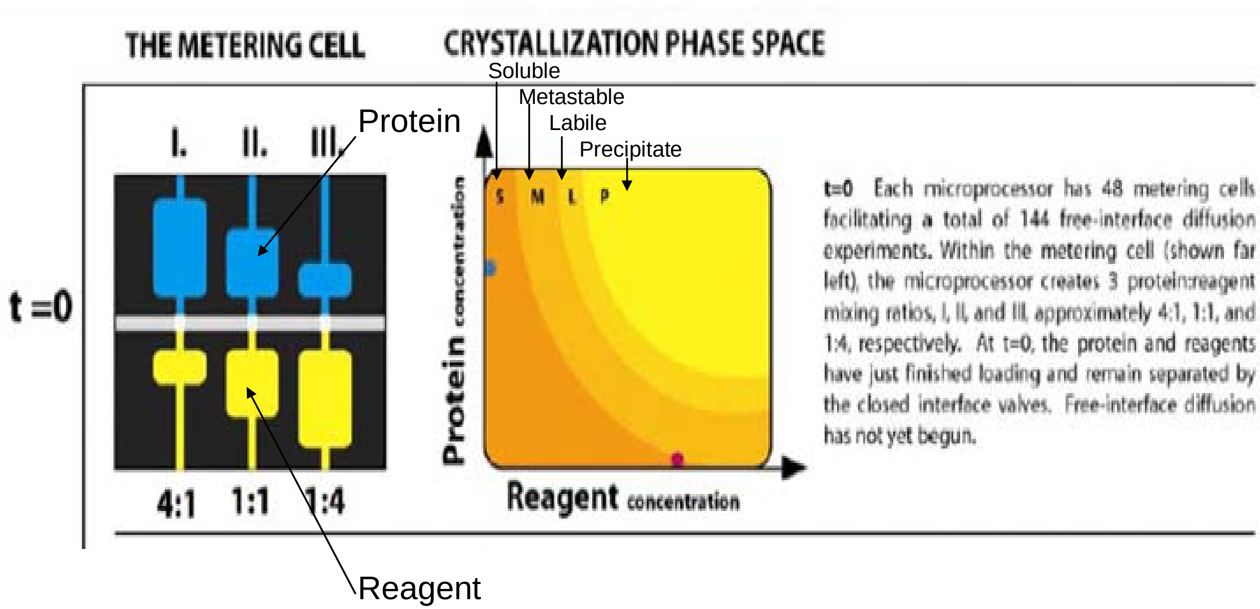


Figure 1: The Topaz™ Microprocessor. The microprocessor consists of layers of molded elastomer that are bonded to an etched-glass substrate. Reagents are loaded into 48 inlets and protein sample into a single inlet. The fluids are dispersed, via pressurized air, into 48 metering cells. The valves do the work of containing fluids within each cell and of isolating sample from the reagent until the user opens the interface line to begin free interface diffusion.

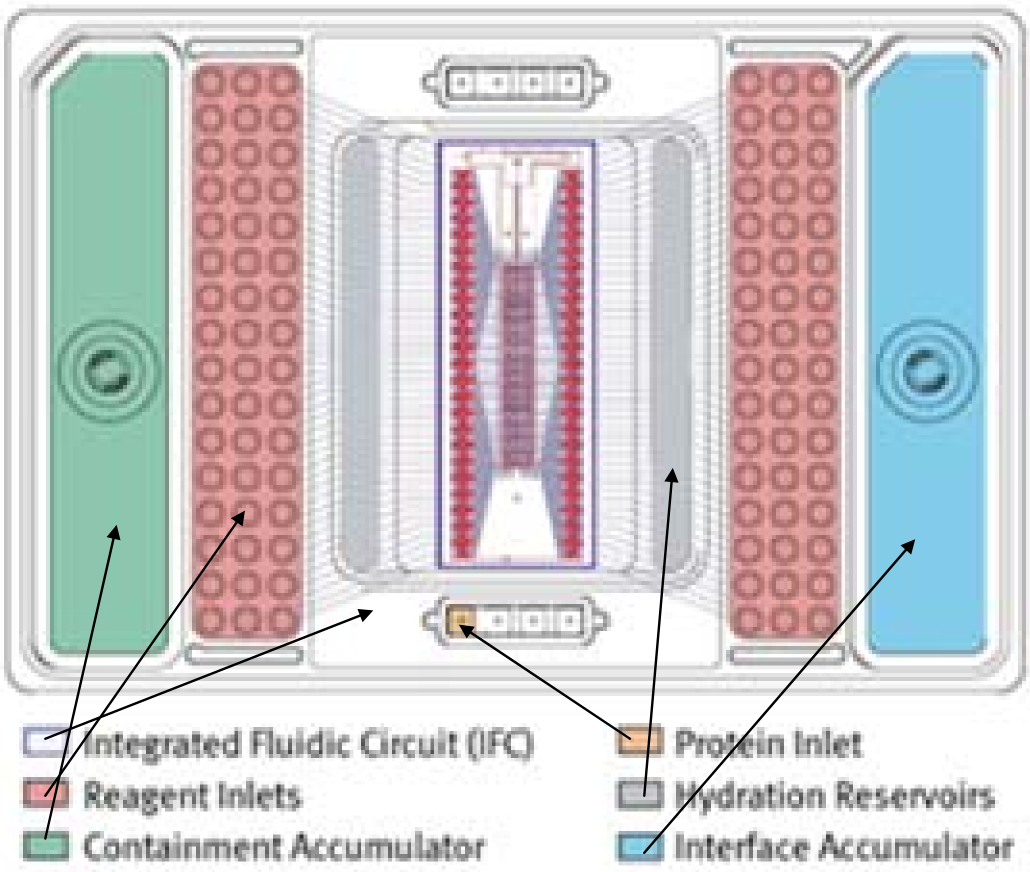
Protein Crystallization



- Proteins form “jelly” crystals
  - Need to find critical combinations of protein solutions and reagent concentrations for crystal formation
  - Use combinatorial approach



Fluidigm 8.96 Screening Chip



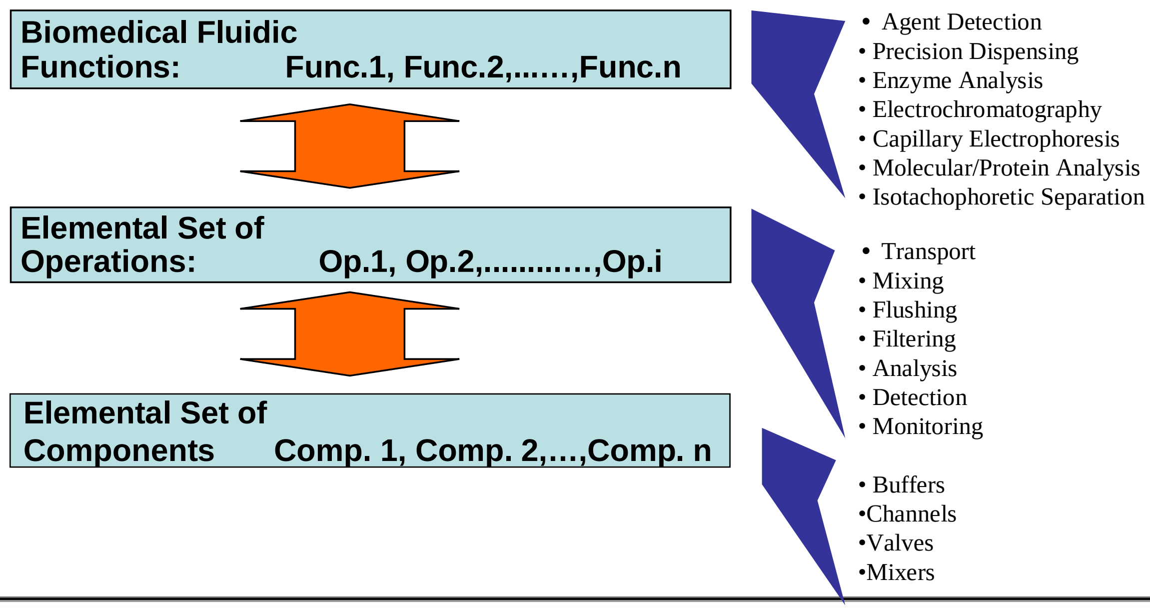
Department of Electrical and
Computer Engineering

Topaz System

- Topaz systems automates fluid delivery to 15nl reaction chambers, allowing combinatorial reactions for protein crystal growth.
- Fixed fluidic processors/reconfigurable connections
- Not sufficient for evolvable hardware system

Evolvable Microfluidic Architecture Requirements

- Evolvable microfluidic hardware can change its architecture and behavior dynamically and autonomously by interacting with its environment.
- Elements of a evolvable microfluidic architecture
  - Shared elemental components among multiple operations to support multiple microfluidic functions:



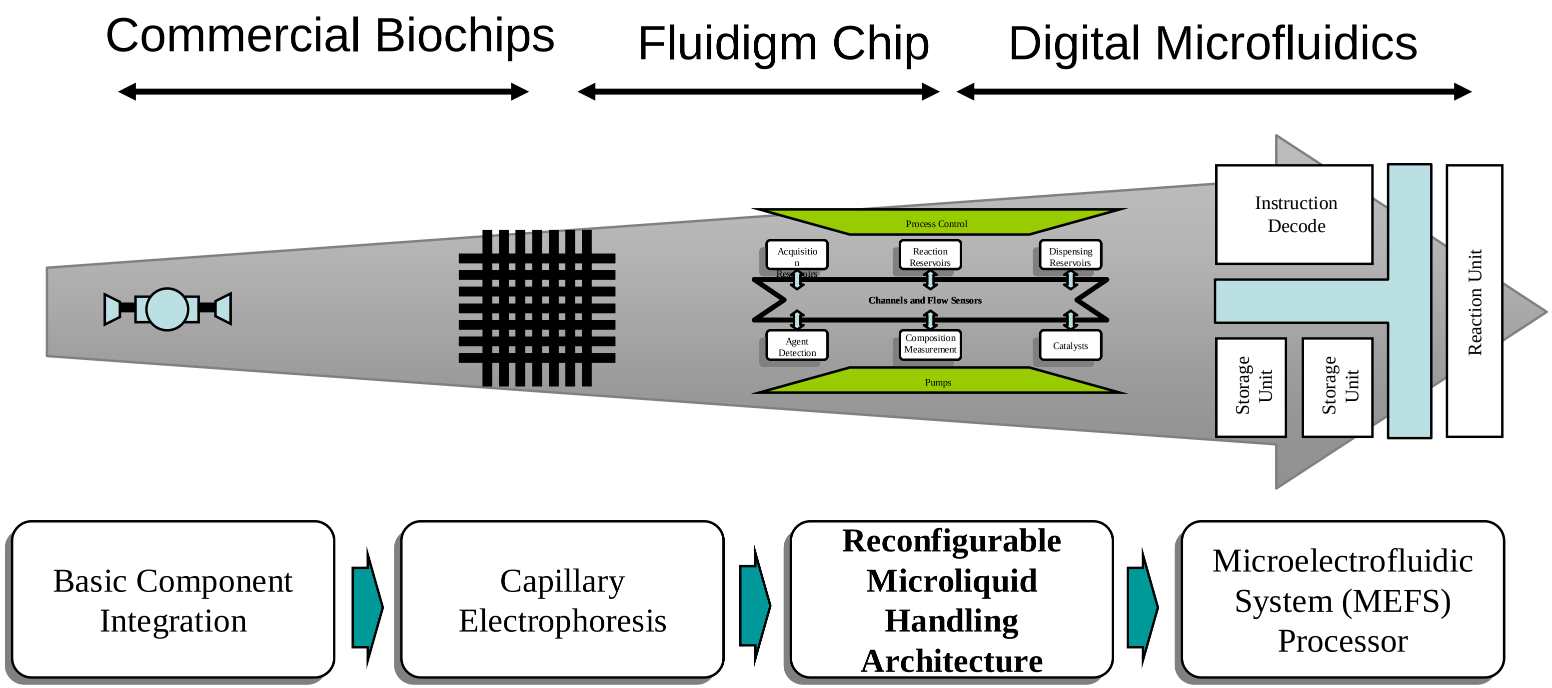
Department of Electrical and Computer Engineering

Evolvable Microfluidic Architecture Requirements

- Integration on single chip
- Reconfigurable
  - Programmable at electronic control level
  - Fluidic operations performed on “assembled”, configured components, not fixed components
    - Requires elemental components that can be assembled under electronic control to perform a fluidic operation
    - Reconfigurable fluidic processors and fluidic connections
  - No molecular cross-contamination of components
    - Reusable components
  - Multitasking
  - Adapts around processing bottlenecks
- Integrated sensing for adaptive behavior

Road to Microfluidic Evolvable Systems

- Where are we?



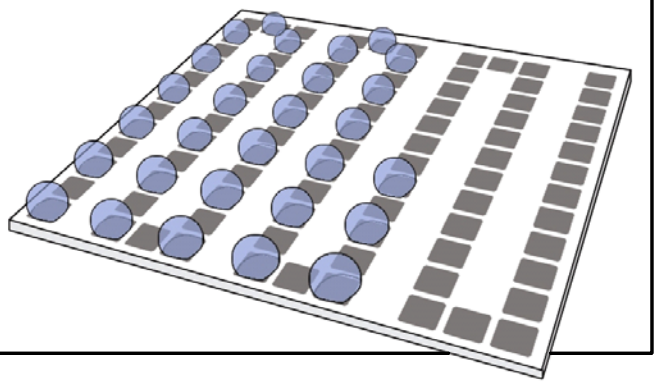
Digital Microfluidics

- Idea: *Microfluidics by repeated use of a small set of basic "instructions" on "unit" sized liquid volumes*
- Advantages:
  - Simplified metering and control
  - Larger dynamic range
  - Simplified design and analysis
  - Scalable
  - Reconfigurable and flexible

Digital Microfluidics

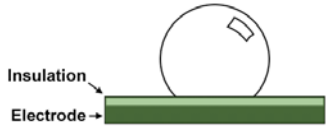
Features

- Droplet-based microfluidic devices
  - Droplets are moved in "virtual channels" defined by electrodes
  - Programmable electrodes in an array directly control discrete droplet operations – dispense, transport, mix, split, incubate – to perform any liquid-based test

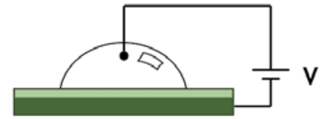


How It Works

Voltage Off:

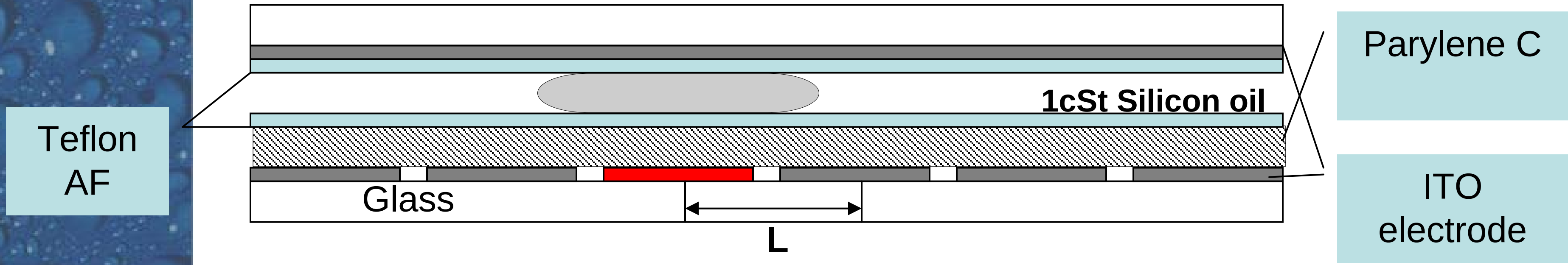


Voltage On:

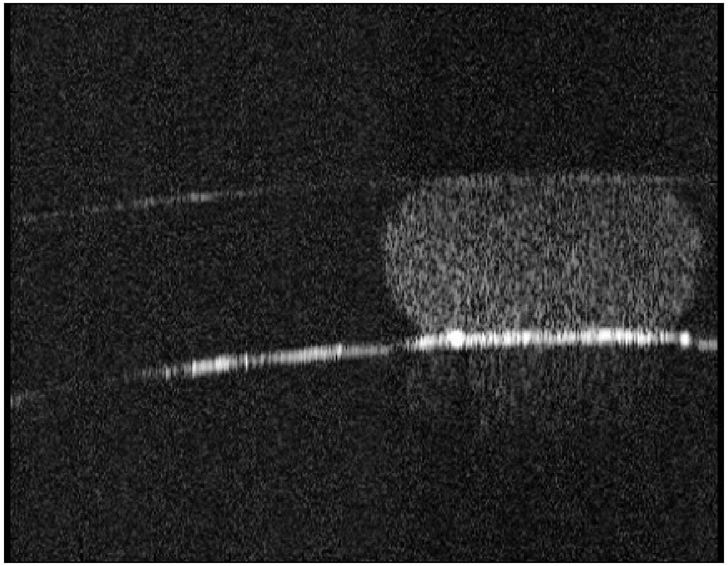


- Electrowetting
  - Modulation of solid-liquid interfacial tension by the application of an electric field
- Works with or without a top plate
  - Newly developed coplanar electrowetting method

Electrowetting Actuator

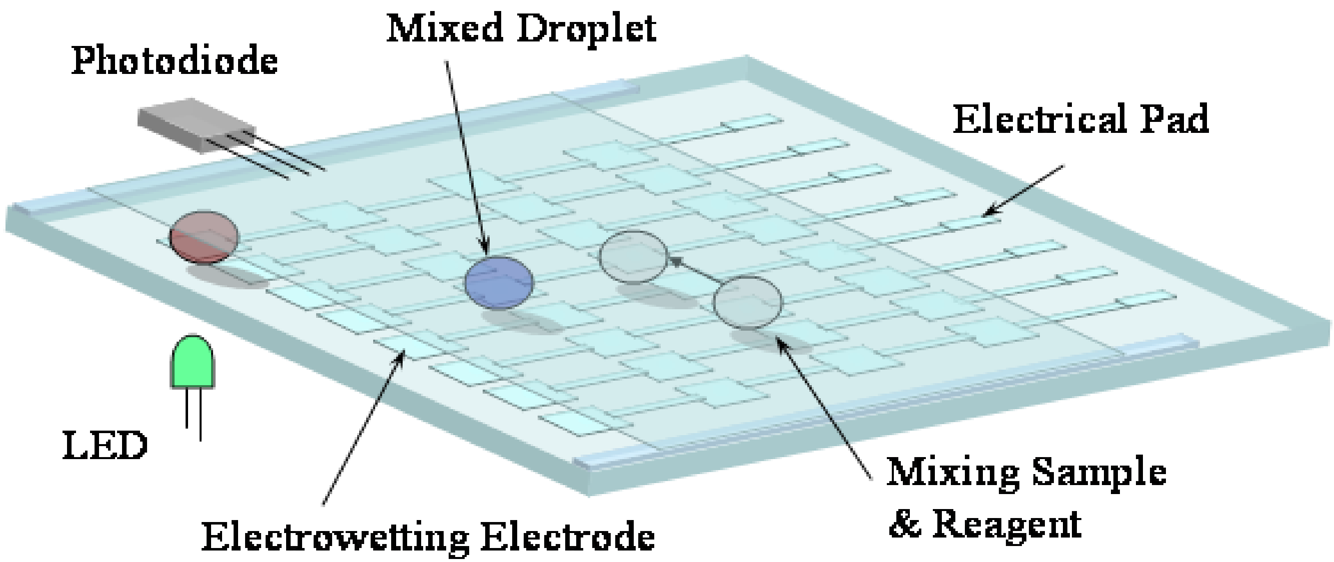


Optical side view

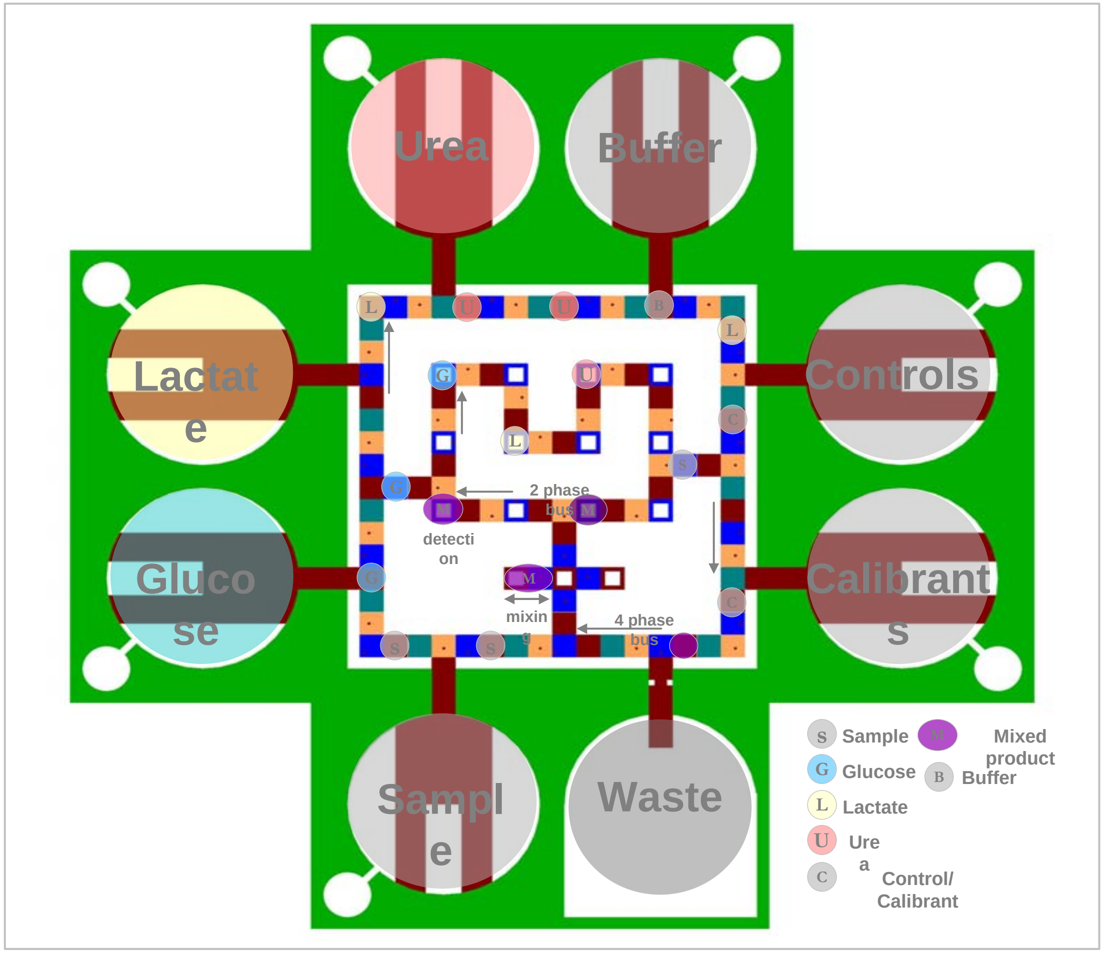


OCT Image

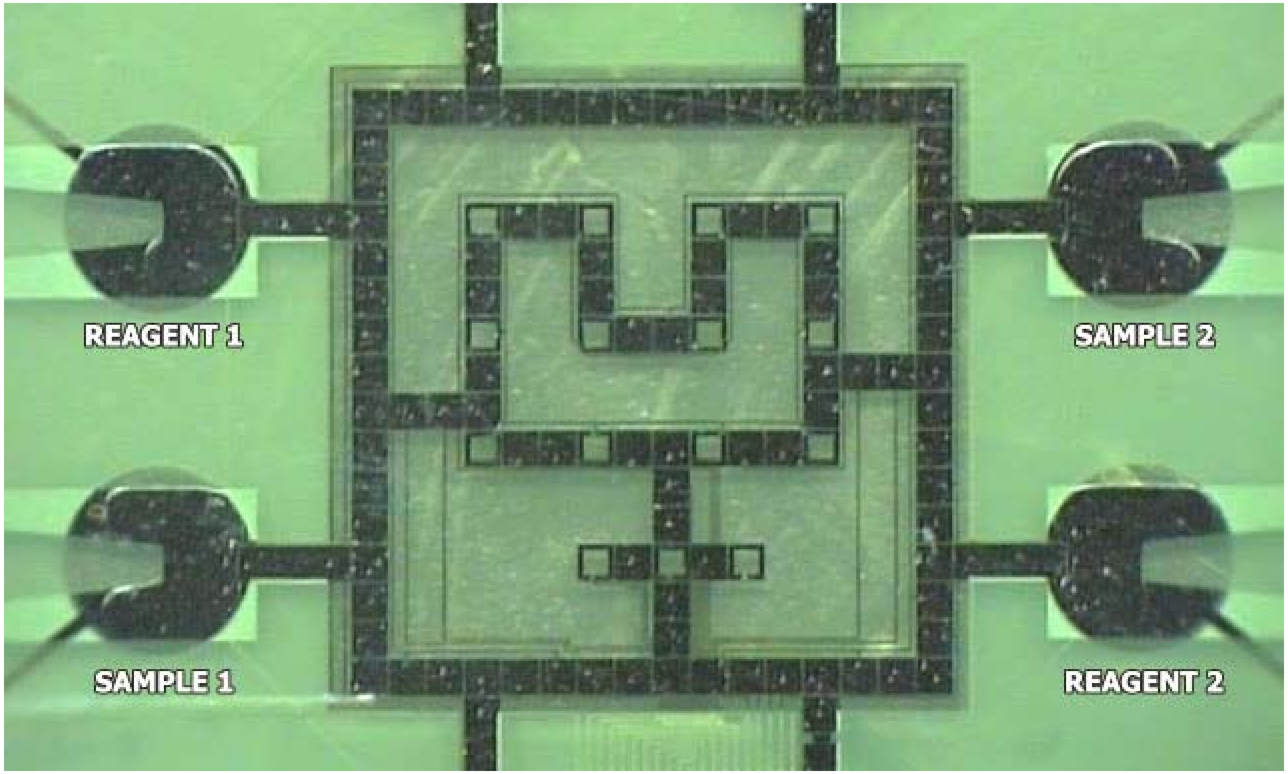
Integrated Microfluidic Functions on Set of Shared Components



Multiplexed Assay Chip



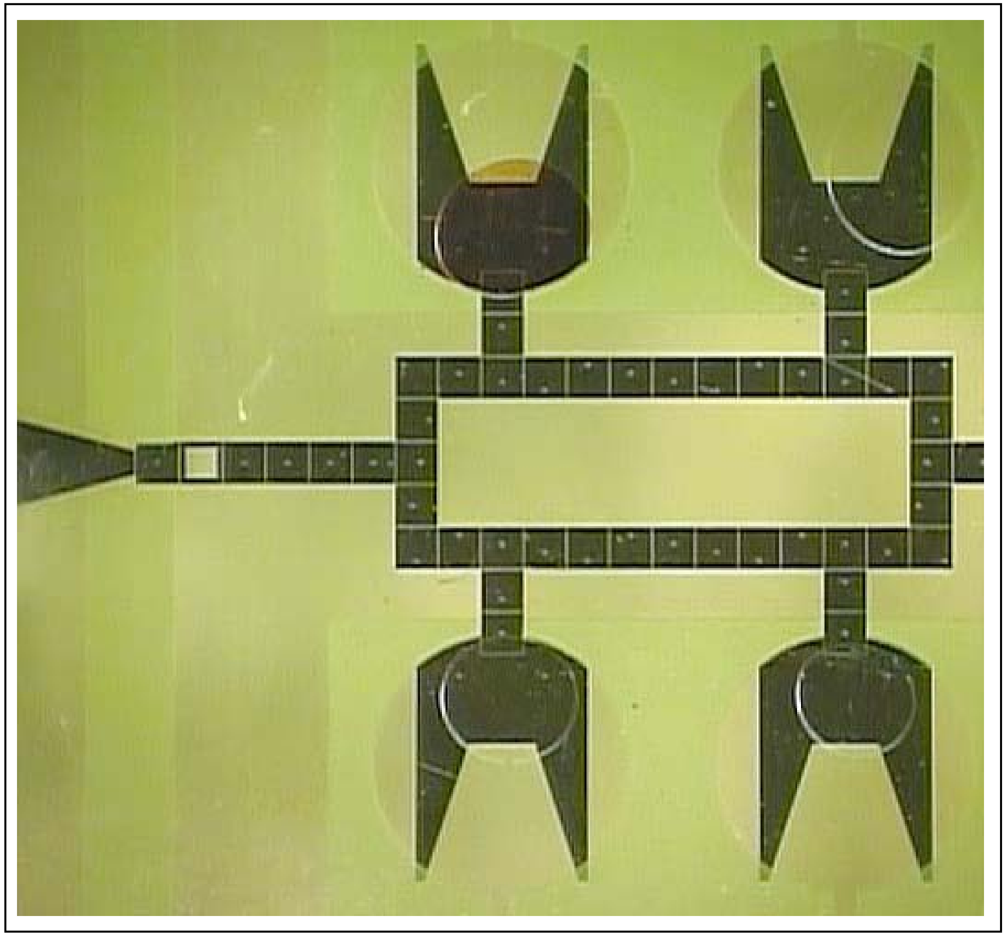
Integrated Operation-Pipelined Glucose Assays



Multiplexed Glucose Assay (Fast Forwarded by 4x)

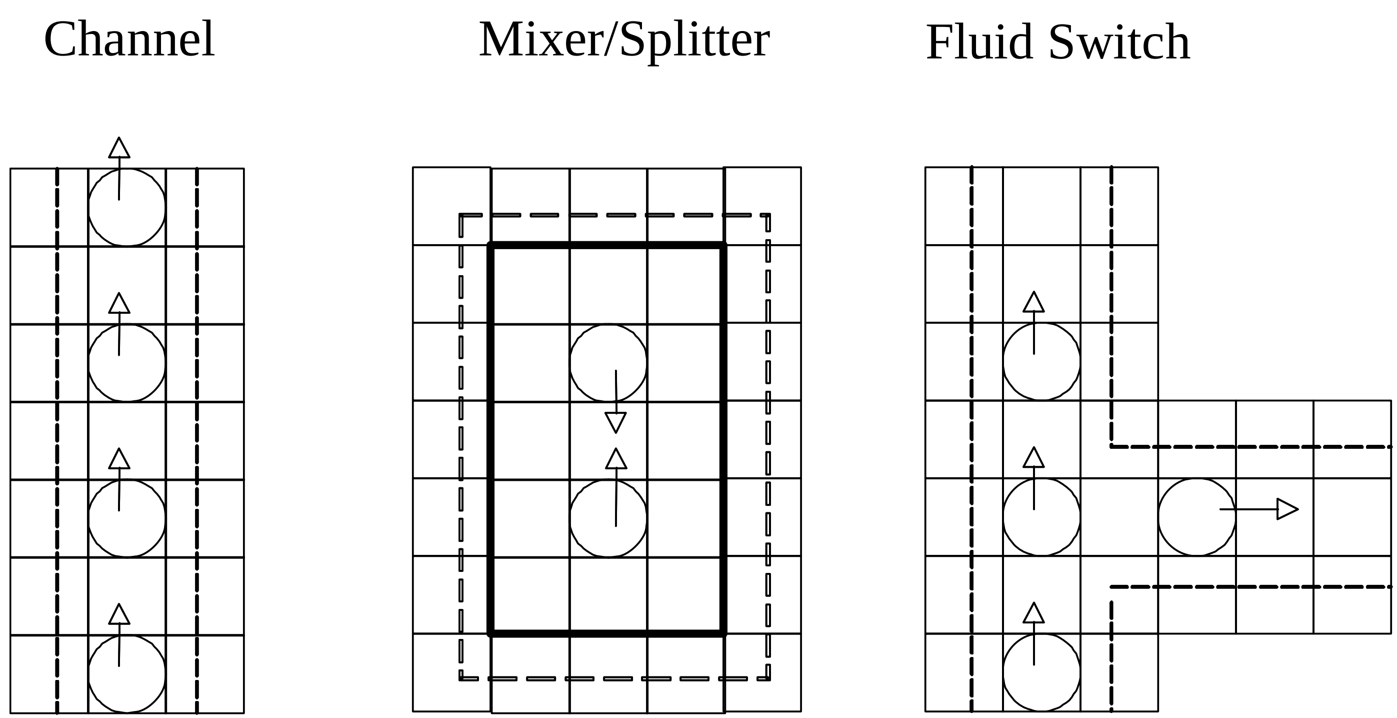
Department of Electrical and Computer Engineering

Integrated Operation - Serial



- Serial protocol
- One glucose assay at a time
- Much simpler
- Does not require detector multiplexing

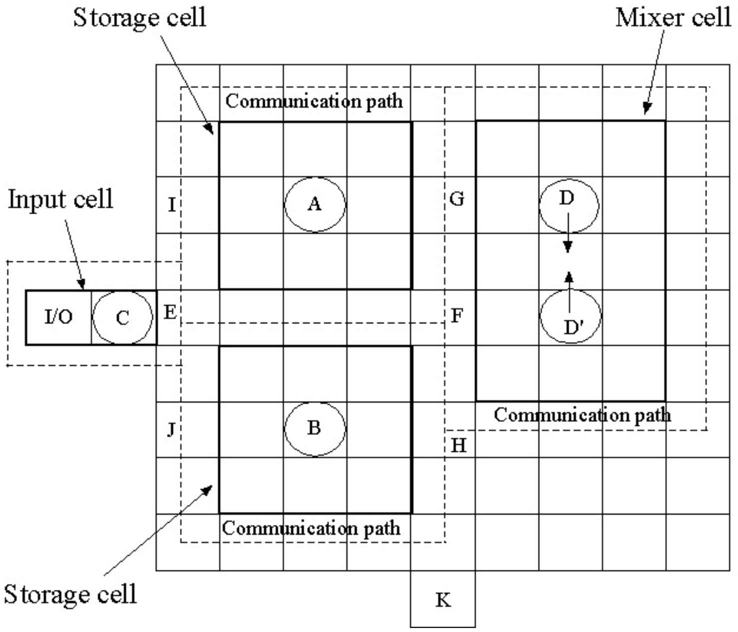
Digital Microfluidic Operations



Department of Electrical and
Computer Engineering

Implications of Droplet Architecture

- Droplets allow microfluidic functions to be reduced to a set of basic operations
- Numerous elemental fluidic operations can be accomplished with a <u>common set of elemental components</u>
- Array can be partitioned into “cells” that perform fluidic functions
- Functional cells dynamically reconfigured at least once per clock cycle

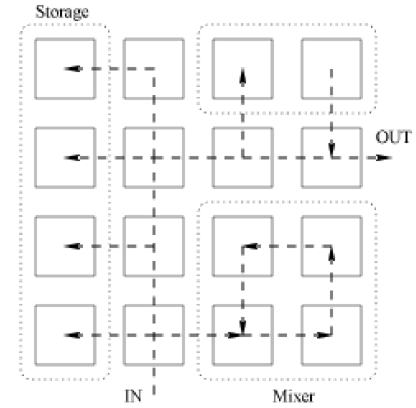


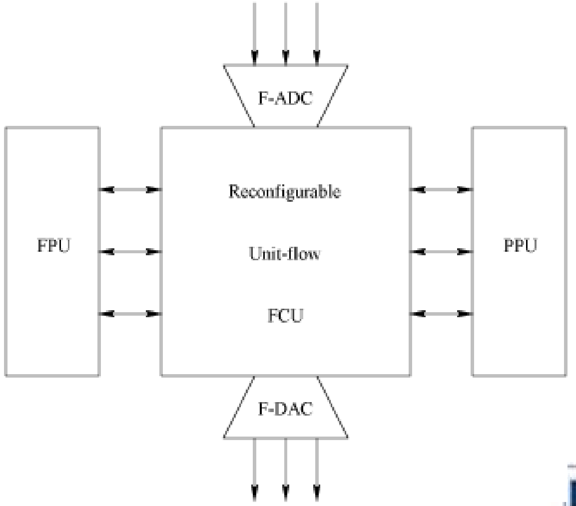
Department of Electrical and Computer Engineering

Architecture for Droplet Flow

- Key technology - Unit flow
  - Capitalize on the flexibility of a unit flow grid array
  - Allow for wholly new operations
  - Allow for dynamic routing
  - Allow for ‘volatile’ functional units

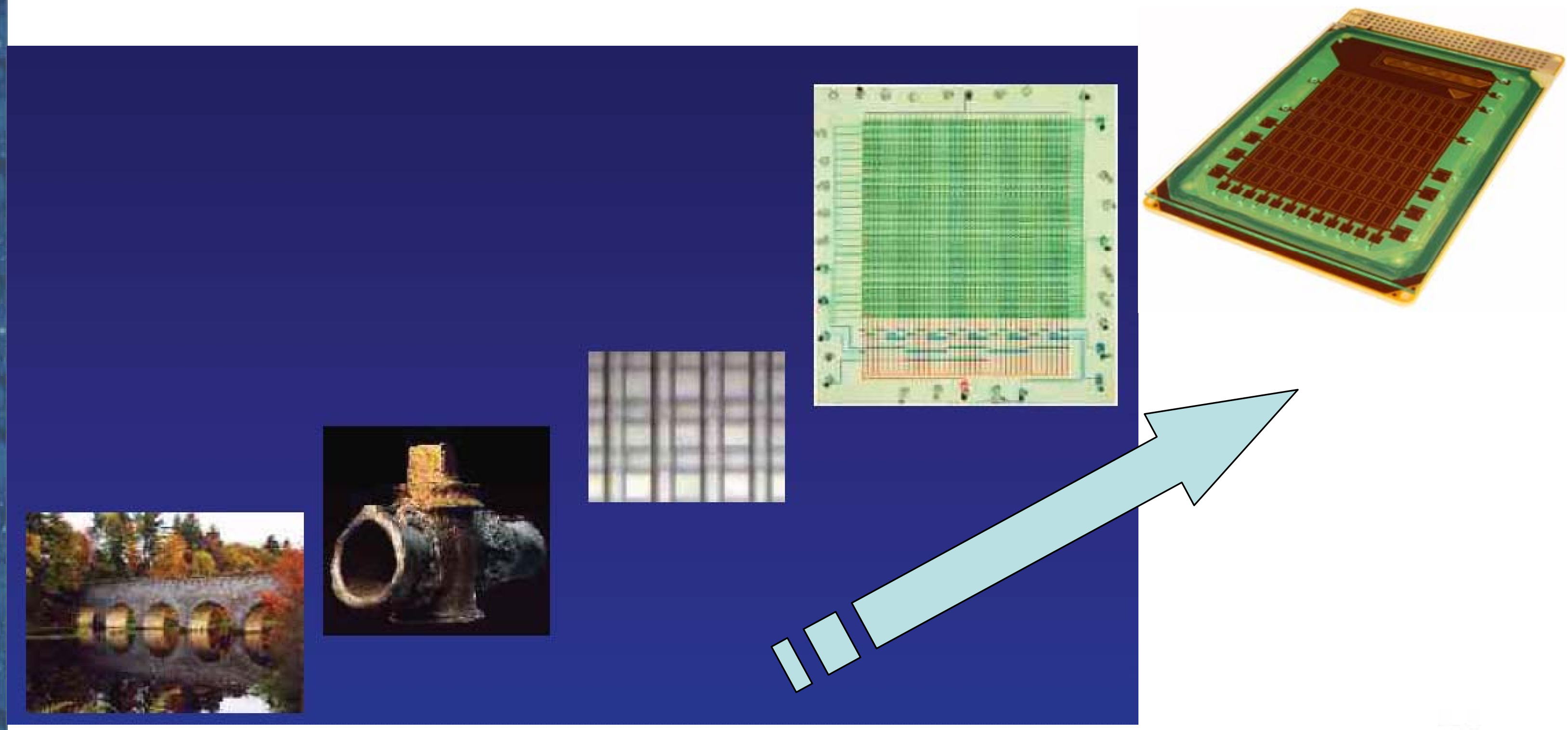
- Idea
  - Merge flexible droplet-based technology with v1.0 concept
  - Leverage flexibility of unit flow in central controller/router





Fluidic Revolution

Continuous Flow Fluidics | Discrete Flow



Droplet Architecture Concepts

- **Fluid Volumes**
  - Fluid quanta
    - The fundamental unit/resolution of fluid that characterizes the microfluidic system
  - Fluidic Packet
    - A grouping of fluidic quanta that is more convenient and efficient to manipulate/operate on
    - The necessary electronic data associated with the fluidic packet
- **Reusability**
    - The component is cleaned via action taken by the control system -washing
    - The component cleanses itself automatically or when given a signal by he control system
    - The droplet is immersed in a filler medium that prevents contamination
- **Input/Output**
  - Input
    - Retrieve/Receive fluid packets from large semi-permanent reservoirs or one-time sample sources
  - Output
    - On-chip detection; sample goes to waste
    - Export/Deliver fluid packets to large semi-permanent waste reservoirs or product collection

Architecture Assembly

- Fluidic Component
  - The most fundamental level of fluidic device
  - Cannot be broken down into other fluidic components
- Functional Unit
  - A device assembled from fluidic components that performs a specific basic useful operation on a fluidic packet
    - e.g. mixer, filter, pump, I/O, etc.
- Fluidic Bus
  - A special type of functional unit that is capable of transporting a fluidic packet from an arbitrary entry point on the bus to an arbitrary exit point on the bus
  - Abstracted; independent of implementation method
  - Entry and exit points will typically be coupled to a functional unit
- Processing Unit
  - Subsystem of a fluidic architecture composed of a collection of functional units
  - Communicates fluidic packets to/from rest of system via a shared bus
  - May contain internal buses to enhance communication internally

Application Execution

- Fluidic Operation
  - Fundamental/Canonical operation that can be performed on a fluidic packet
  - Typically has 1-to-1 association with a functional unit designed to perform that specific operation
  - Analogous to assembly language operations
- Fluidic Program
  - Sequence of fluidic operations that performs the steps of a larger application procedure
  - Analogous to source code for electronic program
- Advanced Fluidic Programming
  - 'Compiler' optimizations
    - Optimize a fluidic program by processing with a fluidic program 'compiler' that can identify inefficiencies and opportunities for parallelism
  - Scheduling optimizations
    - Some optimization performed by above 'compiler'
    - Additional scheduling optimizations/algorithms incorporated into hardware and control system

Complexity of Diverse Applications Reduced to a Manageable Set of Fluidic Operations

Biomedical Fluidic Functions: **Func.1, Func.2,……,Func.n**

- Agent Detection
- Precision Dispensing
- Enzyme Analysis
- Electrochromatography
- Capillary Electrophoresis
- Molecular/Protein Analysis
- Isotachophoretic Separation

Elemental Set of Operations: **Op.1, Op.2,…………,Op.i**

- Transport
- Mixing
- Flushing
- Filtering
- Analysis
- Detection
- Monitoring

Elemental Set of Components **Comp. 1, Comp. 2,…,Comp. n**

- Buffers
- Channels
- Valves
- Mixers

Technology Advantages

Digital Microfluidics

- Very accurate droplet volumes
- Droplet-based digital microfluidics is functionally more similar to bench protocols
  - Assays more easily adapted
- 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
  - Suitable for low power and portable applications
- Development cycles are short, and assays can be tuned with software changes
- Low cost, production-ready lab-on-a-chip on printed circuit board substrate

Other Microfluidic Technologies

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

Digital Microfluidic Toolkit

Implementing numerous applications on a elemental set of components:

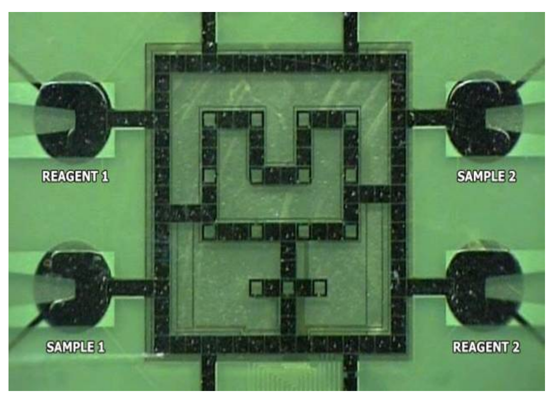
Reservoirs ➡ droplets
Dispensers ➡ electrode sets
Pumps ➡ electrode sets
Valves ➡ electrode sets
Reaction vessels ➡ droplets
Mixers ➡ electrode sets
Collection ➡ scanning droplet

Department of Electrical and
Computer Engineering

Diagnostic Lab-on-a-Chip

SAMPLE LOADING	DROPLET DISPENSING	DROPLET TRANSPORT	MIXING & REACTORS	DETECTION
Analog input	Analog-to-Digital	Digital	Digital-to-Analog	Analog

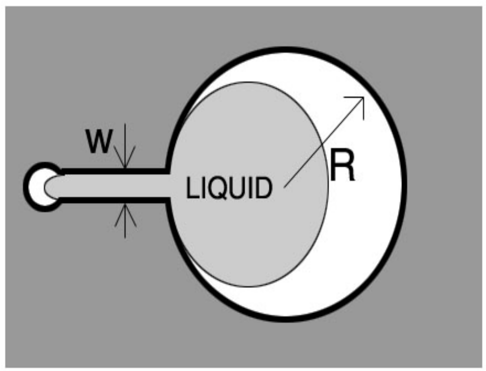
INTEGRATE



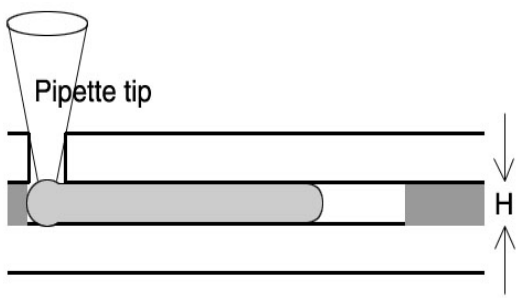
Digital microfluidic lab-on-a-chip

Sample Loading

SAMPLE LOADING → **DROPLET DISPENSING** → **DROPLET TRANSPORT** → **MIXING & REACTORS** → **DETECTION**



TOP VIEW



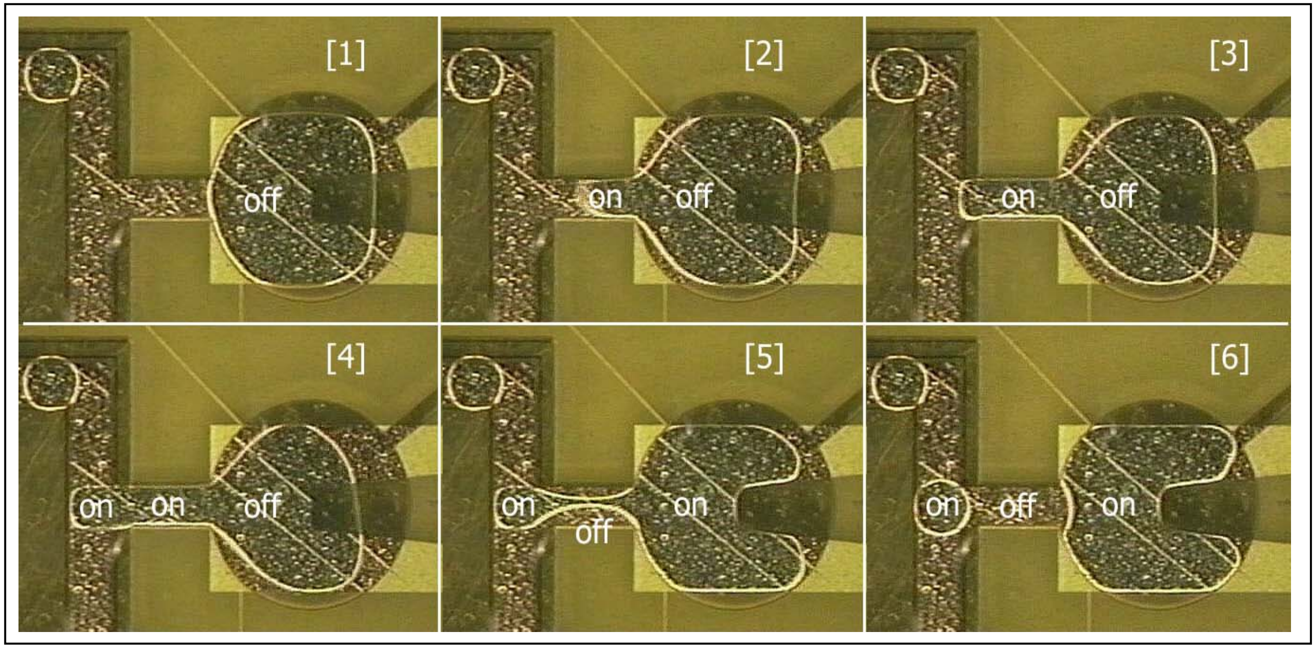
SIDE VIEW

- World-to-chip interface
- Loading using small volume pipette (<2µL)
- W<<R ensures that liquid stays in reservoir after loading

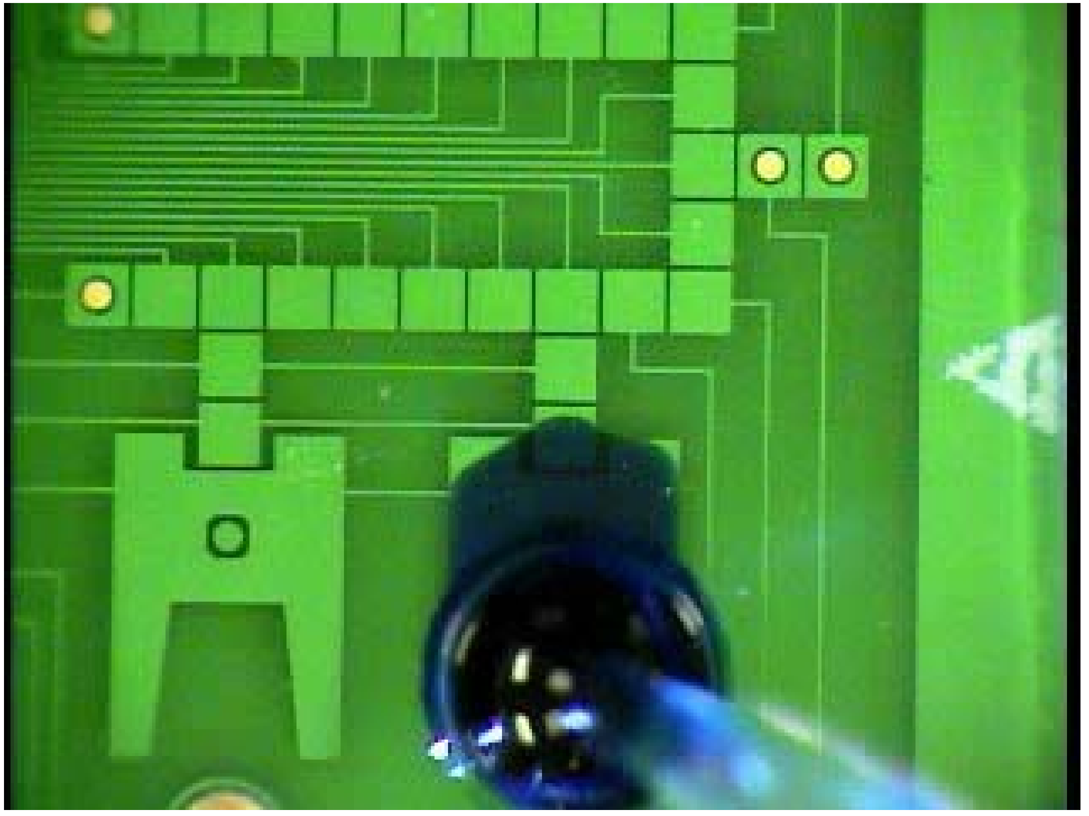
Dispensing

SAMPLE LOADING | **DROPLET DISPENSING** | DROPLET TRANSPORT | MIXING & REACTORS | DETECTION

From on-chip reservoir without external pumps



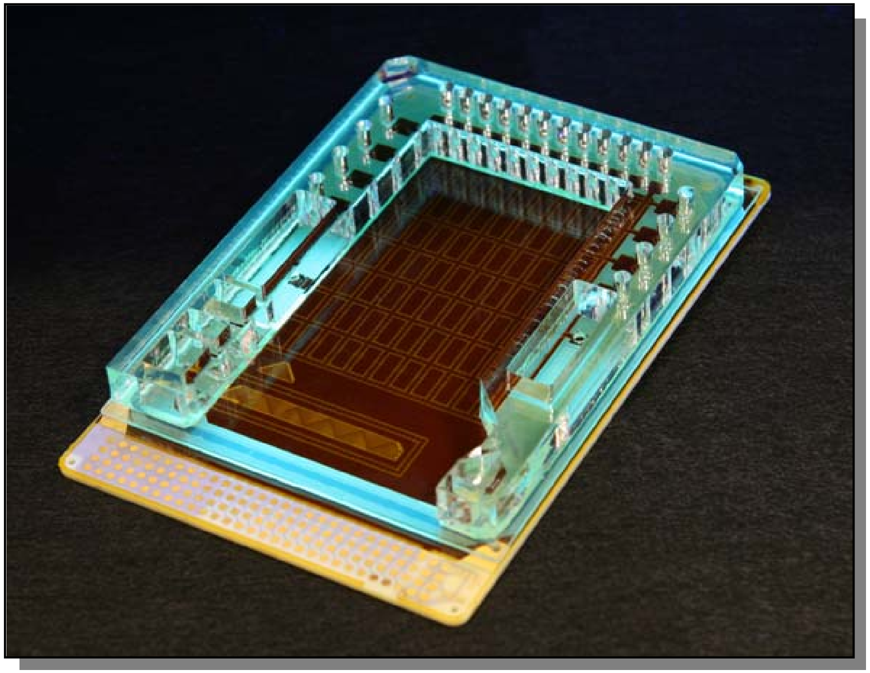
High Speed Continuous Droplet Dispensing



STANFORD
UNIVERSITY

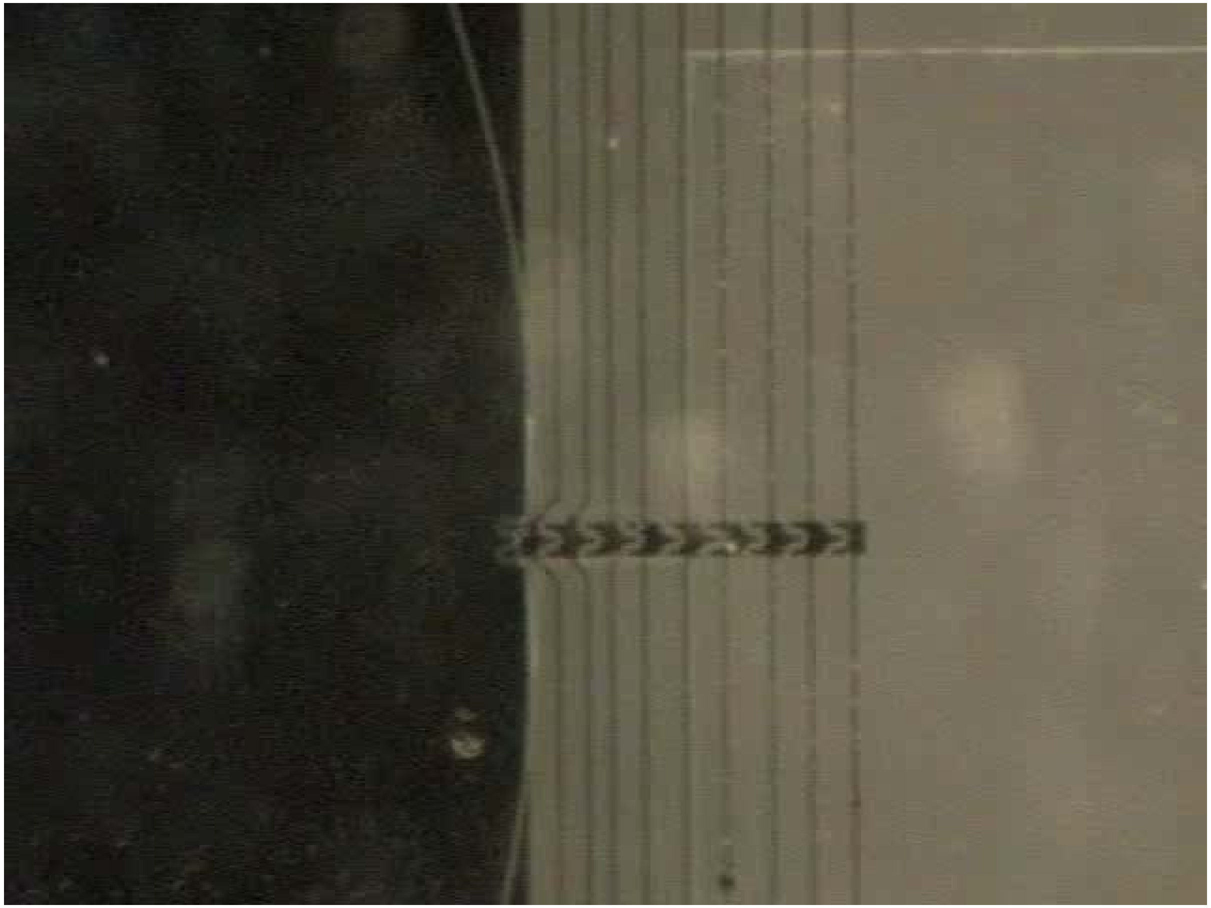
Advanced Liquid Logic, Inc.

World to Chip Interface



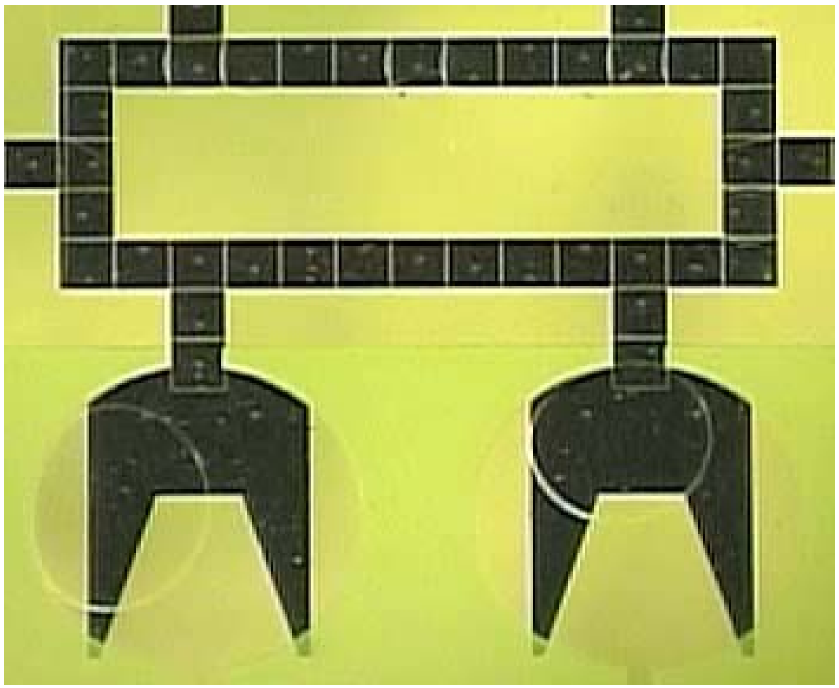
- Well-plate interface
  - Easy and familiar loading
  - 384-well spacing
  - Inputs from microliters to milliliters
- Wash/waste reservoirs support 48+ tests
  - Load and go

35 Picoliter Droplet Dispensing



Droplet Transport

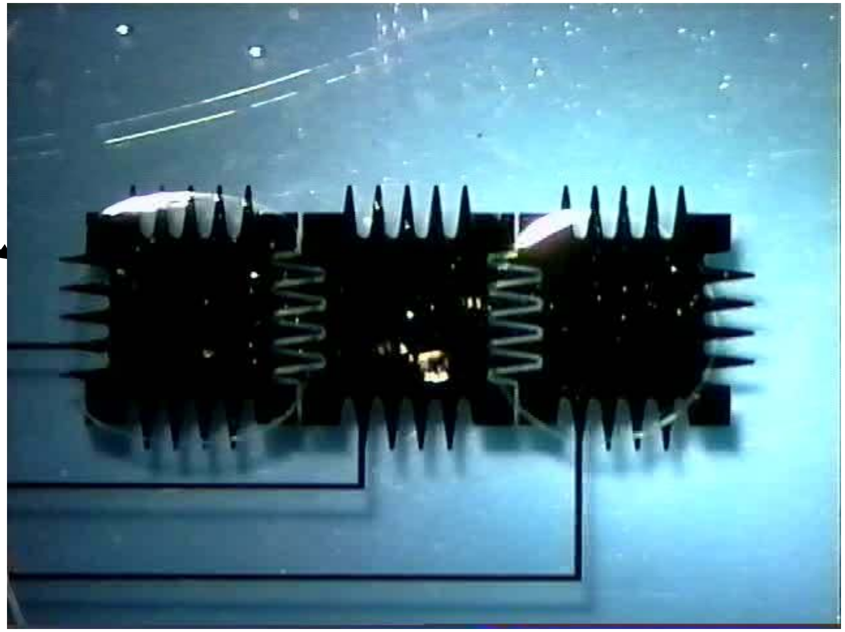
SAMPLE LOADING	DROPLET DISPENSING	DROPLET TRANSPORT	MIXING & REACTORS	DETECTION



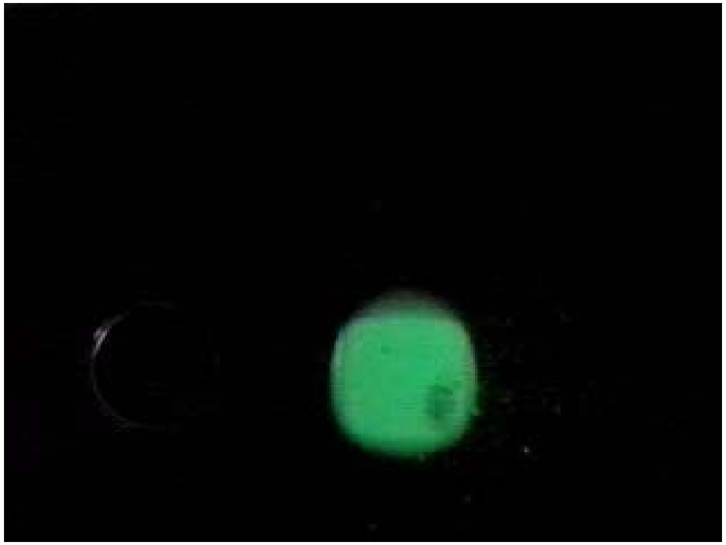
- High-speed transport
- 50Hz switching frequency
  - 2.5cm/sec speed
- 50V operation

Digital Microfluidic Operations

- Splitting
  - **Cycles: 1**
- Dispensing
  - **Cycles: 1**
- Transport
  - **Cycles: 1**
- Merging
  - **Cycles: 1**
- Mixing
  - **Cycles: passive mixing >1000 @16 Hz**
  - **Cycles: active mixing < 50 @16 Hz**



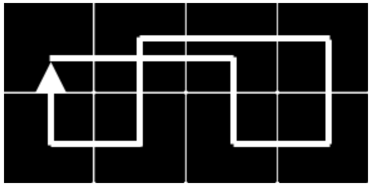
Rapid Droplet Mixing



2x4 Array Droplet Mixing

Droplet Mixing on a
2x4 Electrode Array

Frequency:	16 Hz
Voltage:	50 V
Gap Height:	600 µm
Volume (each):	1.40 µl



- Droplets completely mix in 2.8 seconds
- 30 times faster than the diffusion-only passive mixing case

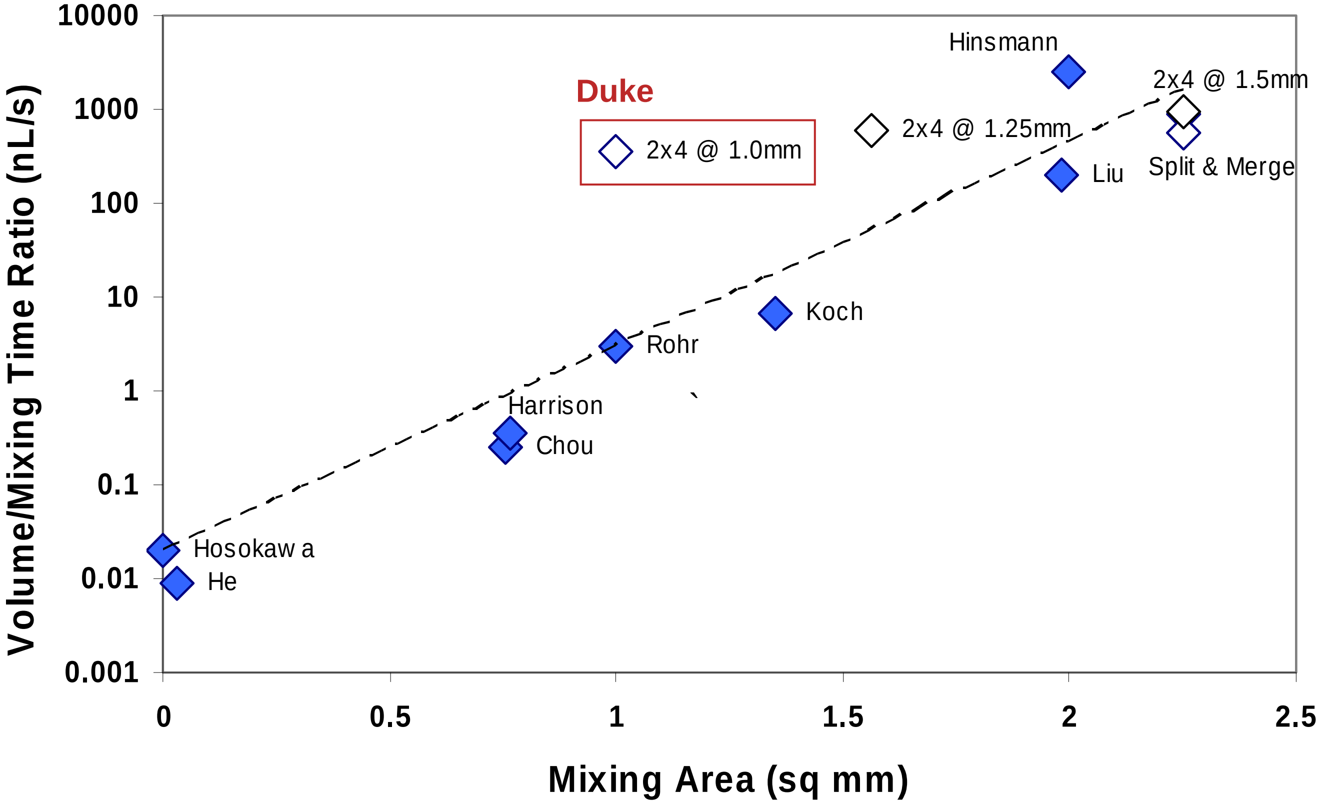
Droplet Mixing

SAMPLE LOADING	DROPLET DISPENSING	DROPLET TRANSPORT	MIXING & REACTORS	DETECTION

- Mixing in ~5 seconds by shuttling on linear array for 1µL (1.5mm scale) droplets
- Scaling down to 0.5mm will decrease mixing time
- Shuttling reverses flow causing un-mixing
  - unidirectional motion is preferred
- Mixing of two 25nL droplets was complete in 0.8 seconds at 10Hz switching @ 50V

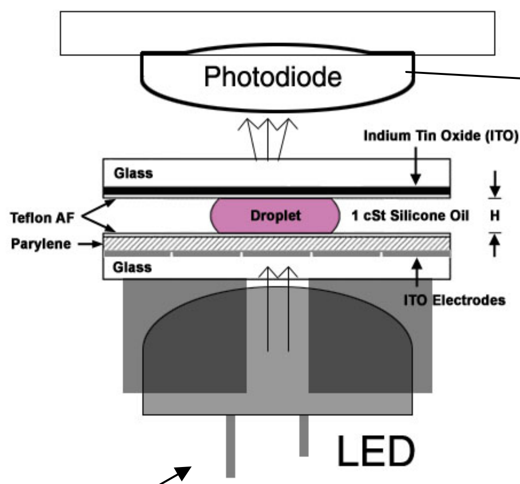
Department of Electrical and
Computer Engineering

Performance Comparisons

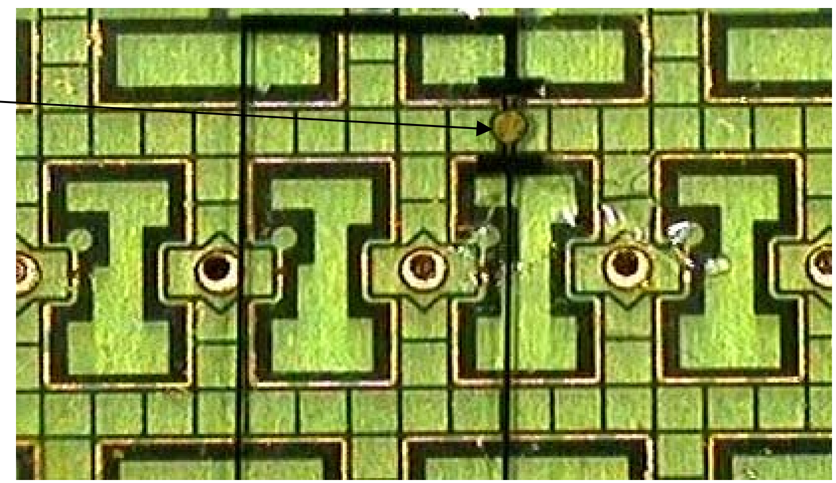


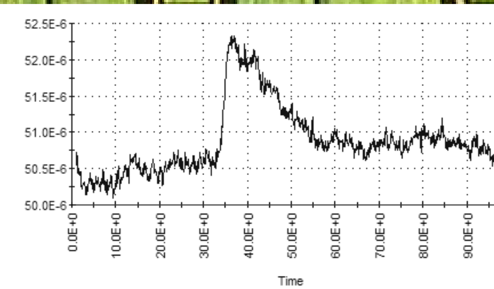
Detection Methodology

SAMPLE LOADING → DROPLET DISPENSING → DROPLET TRANSPORT → MIXING & REACTORS → **DETECTION**



Opaque solid

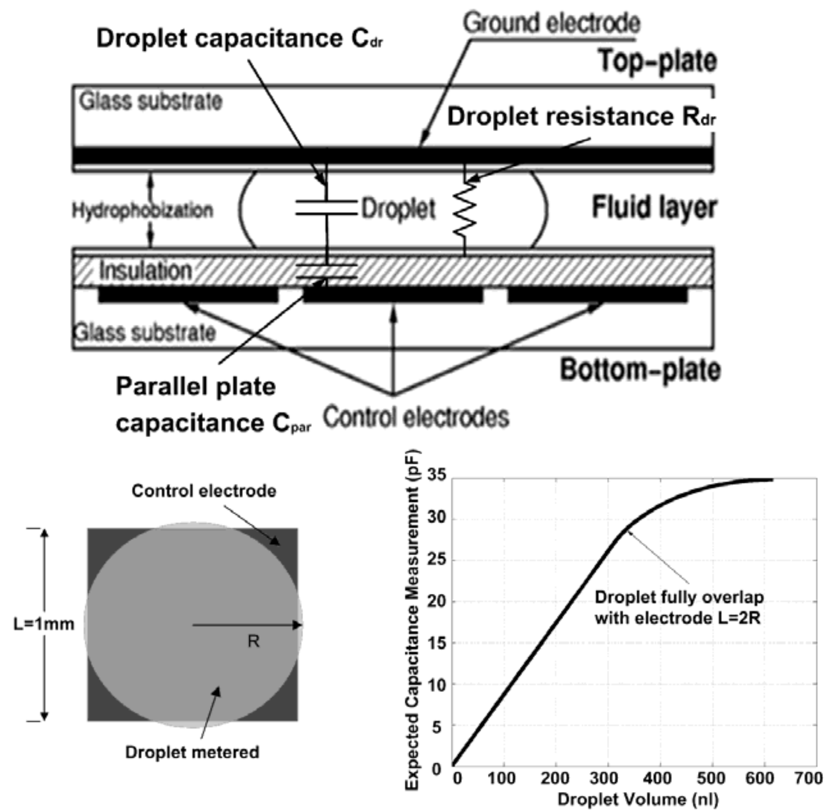




Chemoluminescence underneath the TeflonAF coated photodetector

Electronic Sensing

- Capacitance feedback control



(a) (b)

Integrated Microdisk Sensor

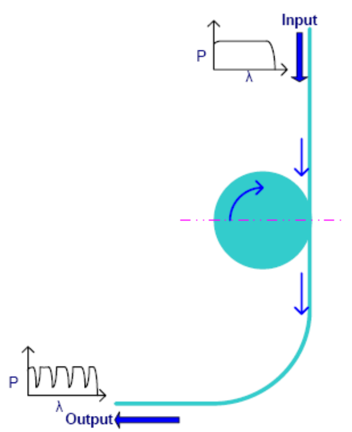


Fig. 1 Schematic of a vertically coupled microdisk resonator showing an input broad linewidth optical signal, and resultant output signal [37]

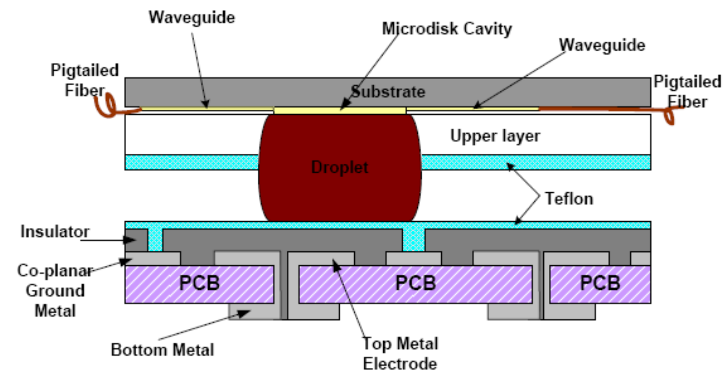
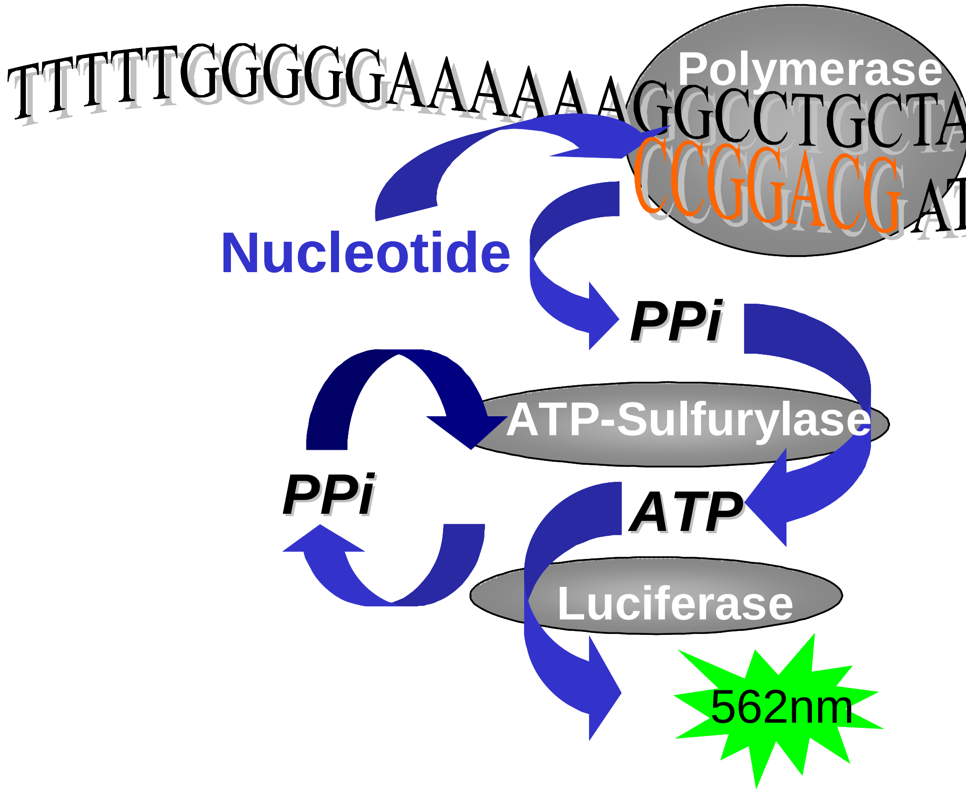
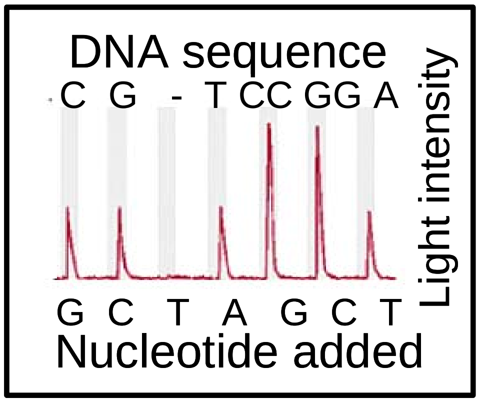


Fig. 16 Side view of an integrated glucose optical microdisk sensor integrated with an electrowetting chip.

Reconfigurable Lab-on-a-Chip Status

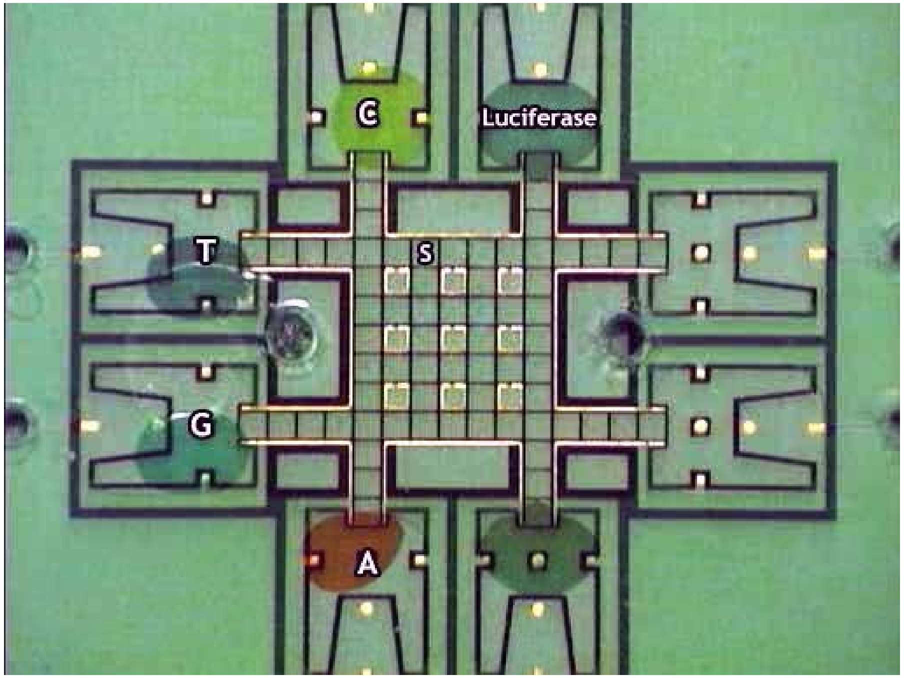
- Digital microfluidic toolkit demonstrated
  - All fluidic functions demonstrated
  - Lacking molecular separation method
- Commercial prototypes available (ALL)
- Examples from current research
  - DNA sequencing by synthesis
  - Enzyme assays
  - Cytotoxicity screening


TTTTTGGGGGAAAAAAGGCCTGCTAGCG
Polymerase
CCGGACG ATCGC
Nucleotide
PPi
ATP-Sulfurylase
PPi
ATP
Luciferase
562nm


DNA sequence
C G - T CC GG A
G C T A G C T
Nucleotide added
Light intensity

Example Fluidic Protocol for DNA Sequencing

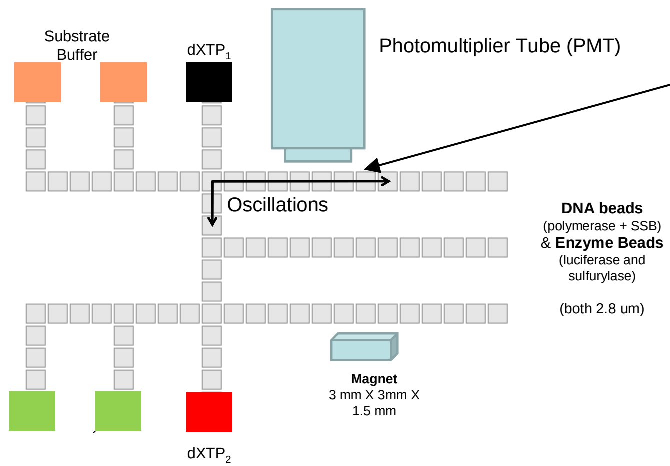
- Dispense droplets of each dNTP
- Transport droplets to synthesis reaction site and allow to react
- Transport droplets to storage area
- Mix each dNTP droplet with light producing droplet
- Transport combined droplets to detector site

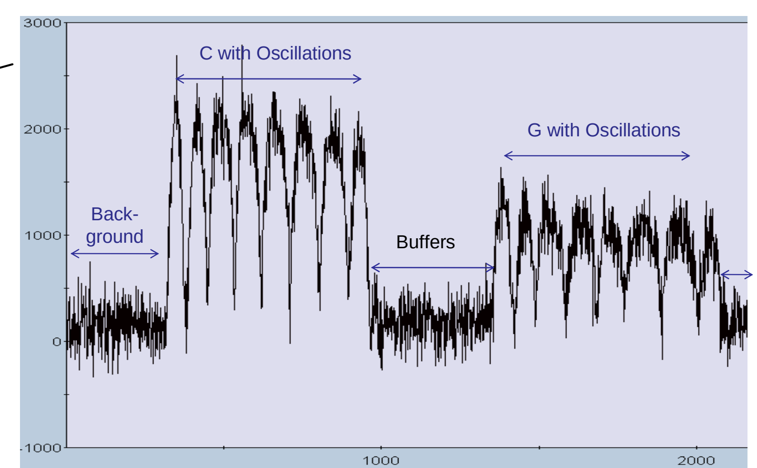


Dyed liquids represent pyrosequencing reagents, droplet volumes are 50 – 100 nL.

On-Chip Pyrosequencing

- Pyrosequencing protocols run at Stanford on ALL platform having six reservoirs and three transport lanes.
- Magnet under bottom lane immobilizes DNA and enzyme beads
- After incorporation, reaction products transported to PMT

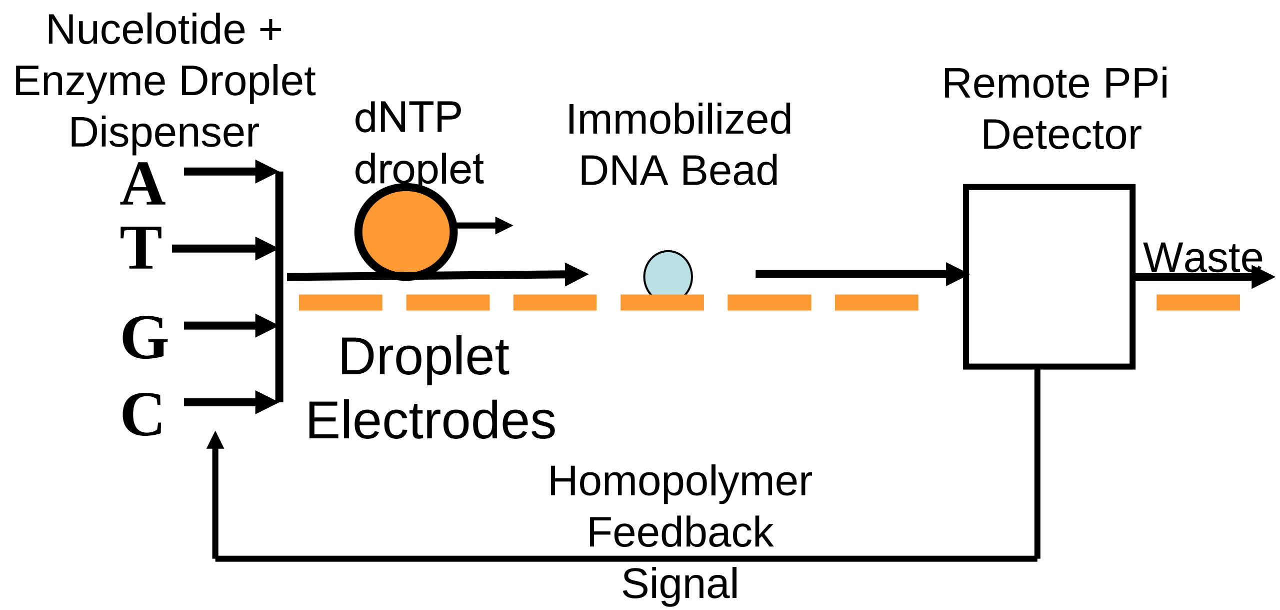




STANFORD UNIVERSITY

Advanced Liquid Logic, Inc.

Feedback Controlled Nucelotide Addition For High Fidelity Homopolymer Sequencing



- Deliver dNTP droplet
- If excess light is detected (homopolymer), add more of same dNTP
- Continue adding same dNTP until full incorporation detected
- Else, deliver next nucleotide

STANFORD UNIVERSITY

Advanced Liquid Logic, Inc.

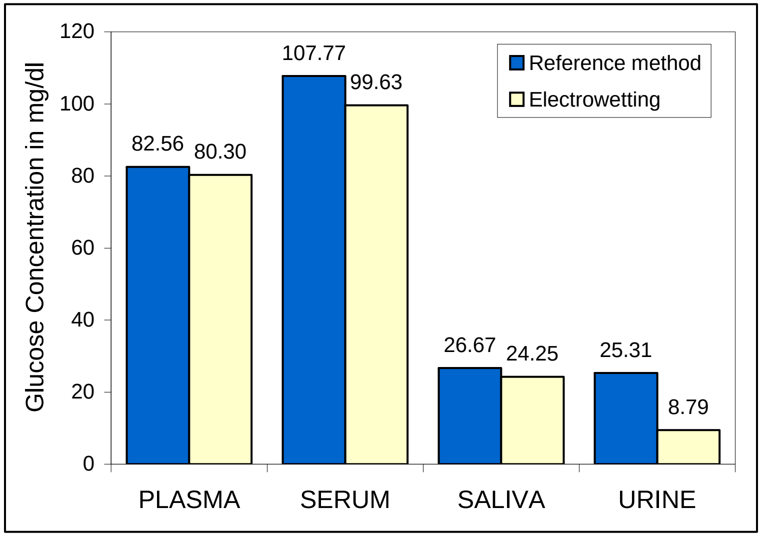
Benefits of Reconfiguration in Pyrosequencing

- Feedback-controlled nucleotide addition for sequencing through homopolymer regions of DNA
- Look-ahead sequencing and voting schemes possible for reliable and high throughput sequencing
- Other benefits of digital microfluidic platform:
  - Continuous droplet dispensing
  - Scalable

On-Chip Processing

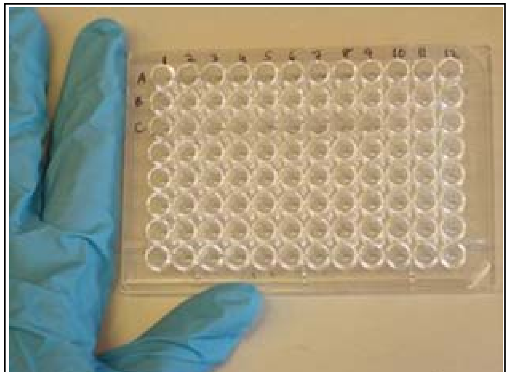
Clinical Diagnostics

Whole blood transport @ 20 Hz



- Demonstrated transport of whole blood, plasma, serum, urine, saliva, and sweat.
- Colorimetric glucose assay on Plasma, Serum, Saliva and Urine

On-chip Dilution Tree for Cytotoxicity Screening (Y. Zhao, A. Wang, Y. Yamanaka)

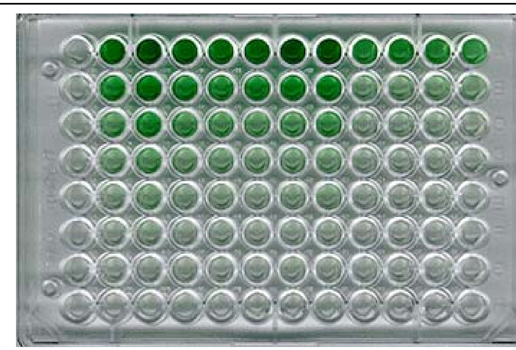


Grow cells in 96 well plate

Add various concentrations of compound to be tested to cells

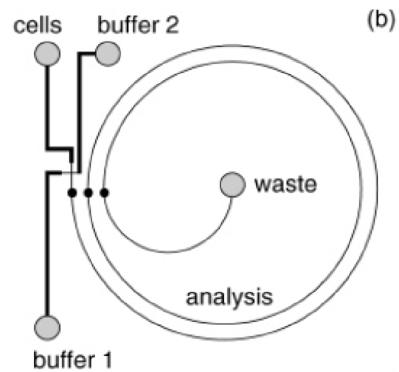
Wait specified length of time

Add Cytotoxicity Assay reagent 1, incubate, add reagent 2

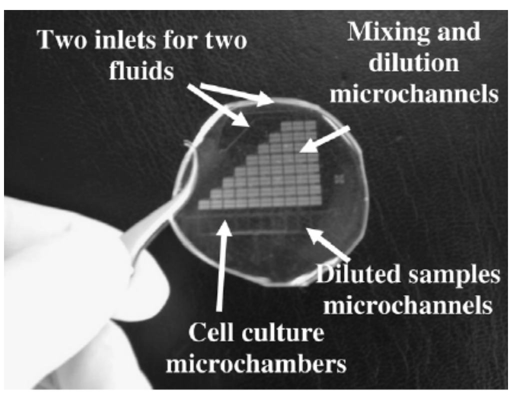


Use plate reader to measure color intensity (proportional to survival)

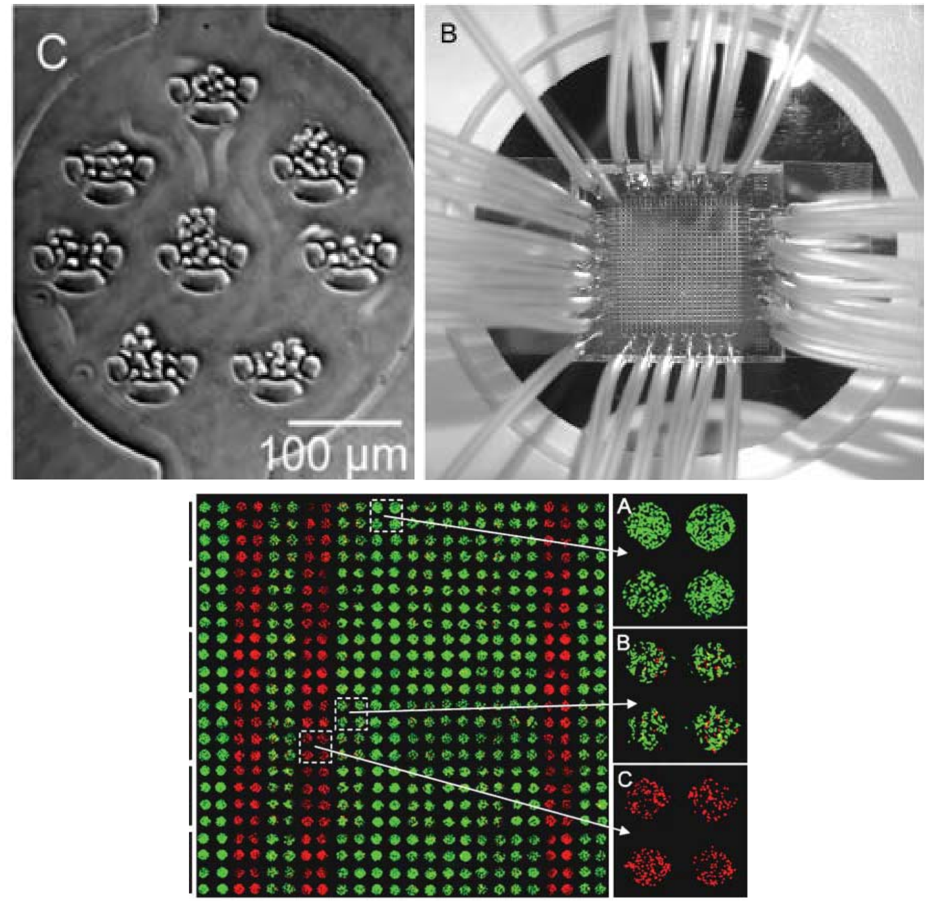
Previous Work



Anal. Chem. **2005,** 77, 667-672

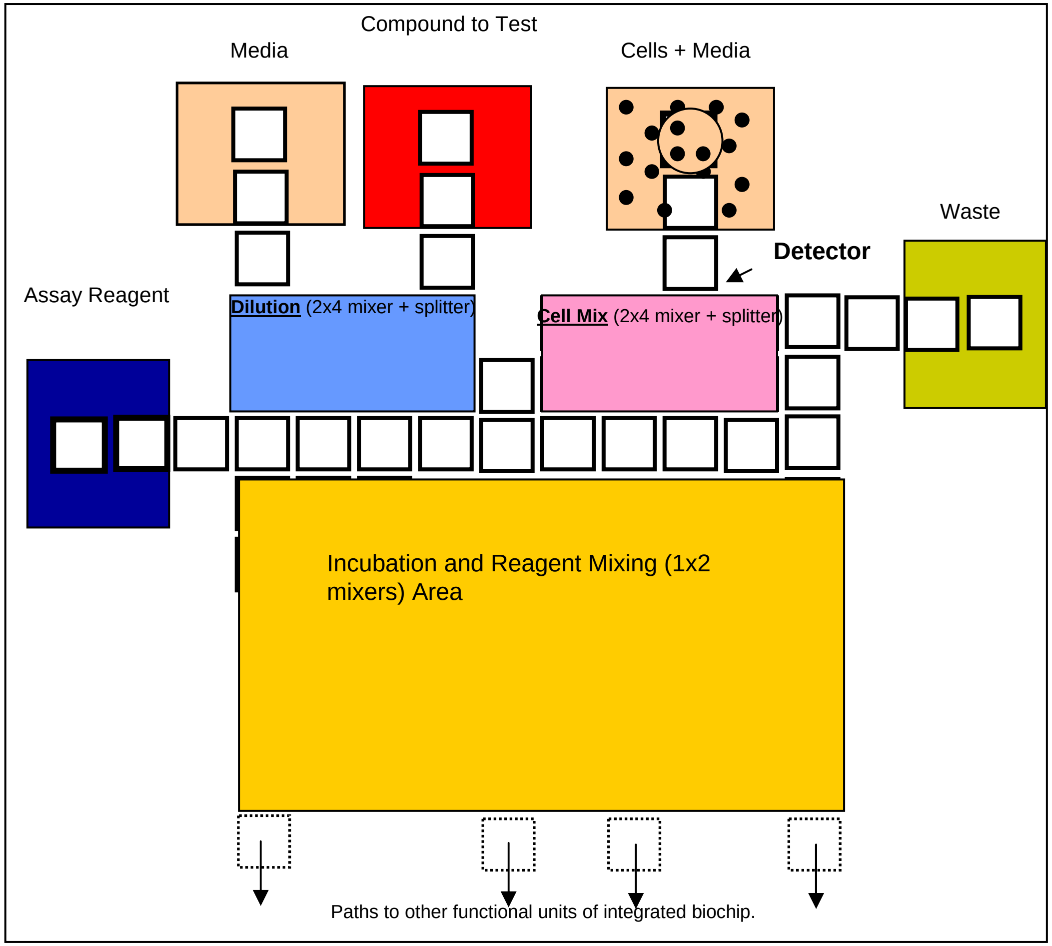


Toxicology in Vitro **2007,** 21, 535–544



Lab on a Chip **2007,** 7, 740-745

Digital Microfluidic Screener

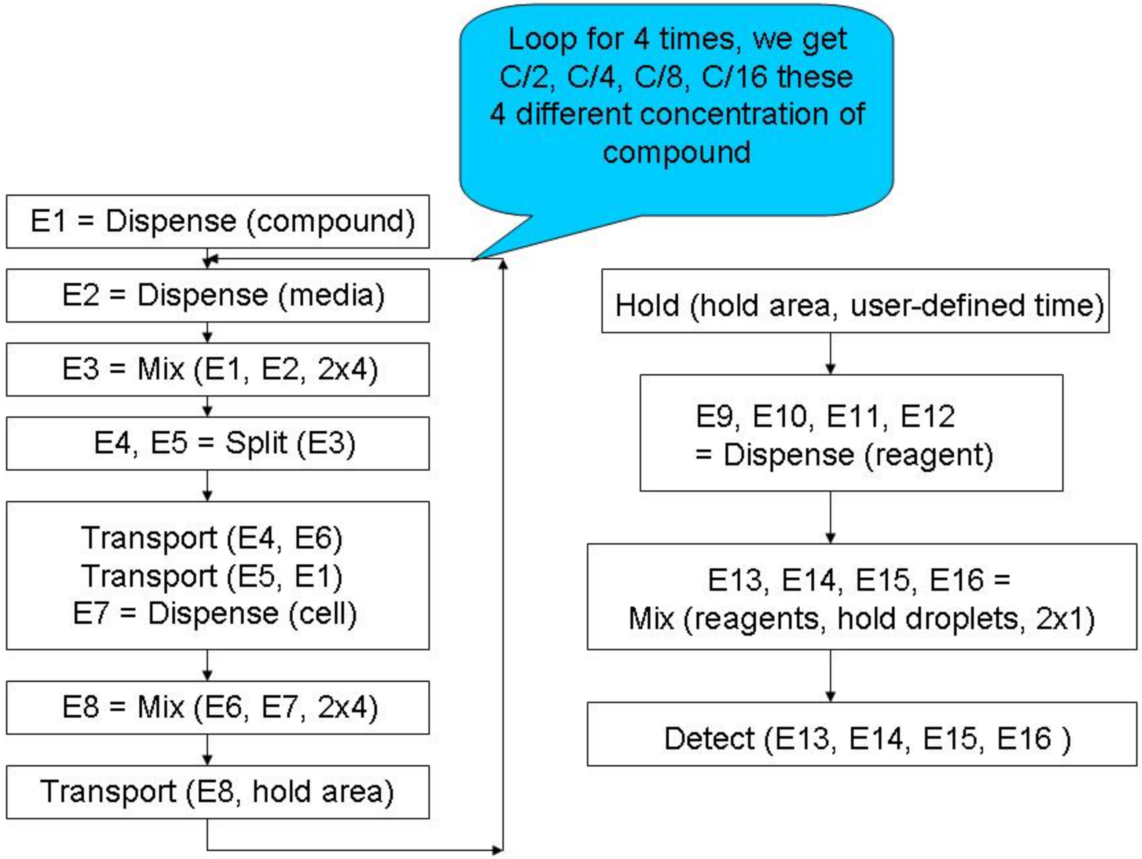


Department of Electrical and
Computer Engineering

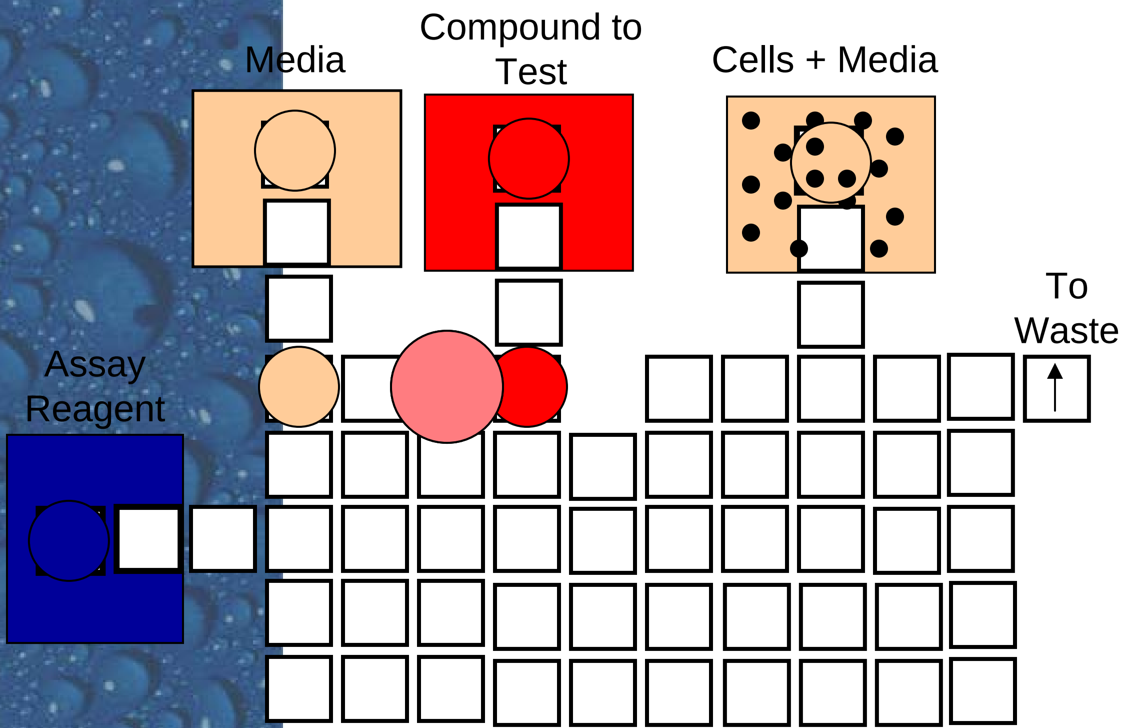
Algorithms and Programming

- Cytotoxicity screening implemented on platform using basic microfluidic operations
  - Transporting, merging, mixing, and splitting
  - Requires on-chip binary dilution
- Functional control requires abstraction layer between protocols and microfluidic operations
- Abstraction layer translate protocols into programming control statements
  - Dispense, transport (a,b), mix (a,b, type), split (a), detect (a)

Programming flow for dilution and cell injection

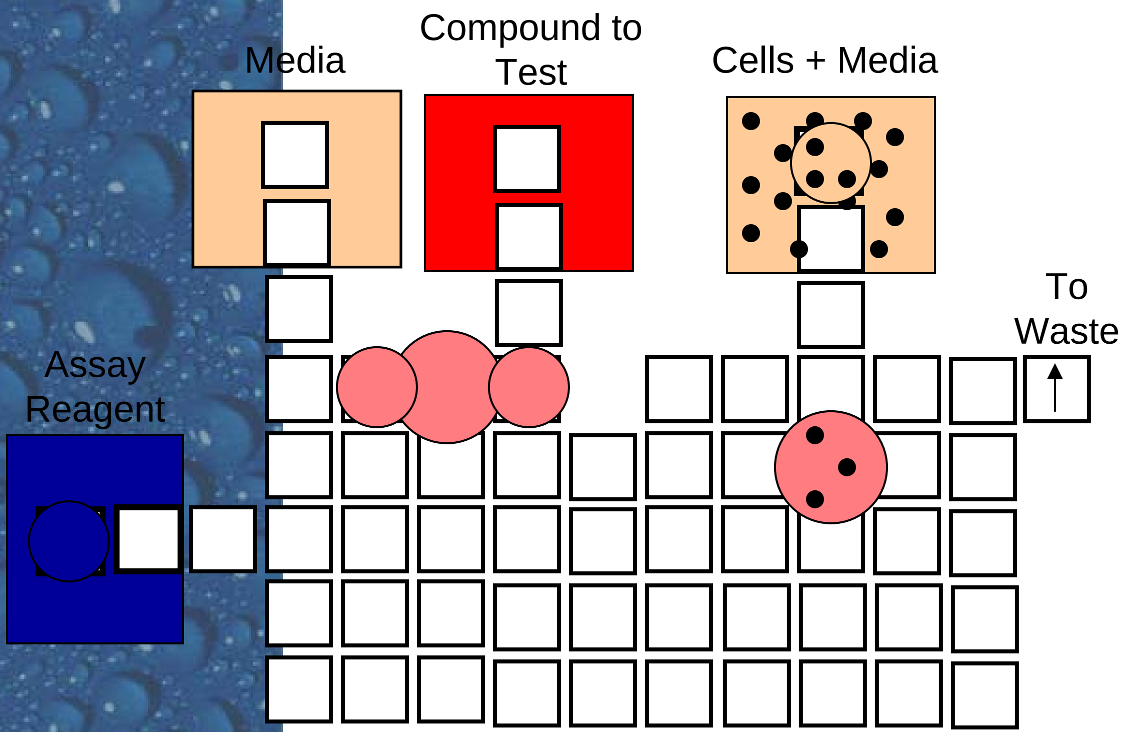


Architecture



1. Dispense buffer and compound droplets, mix.

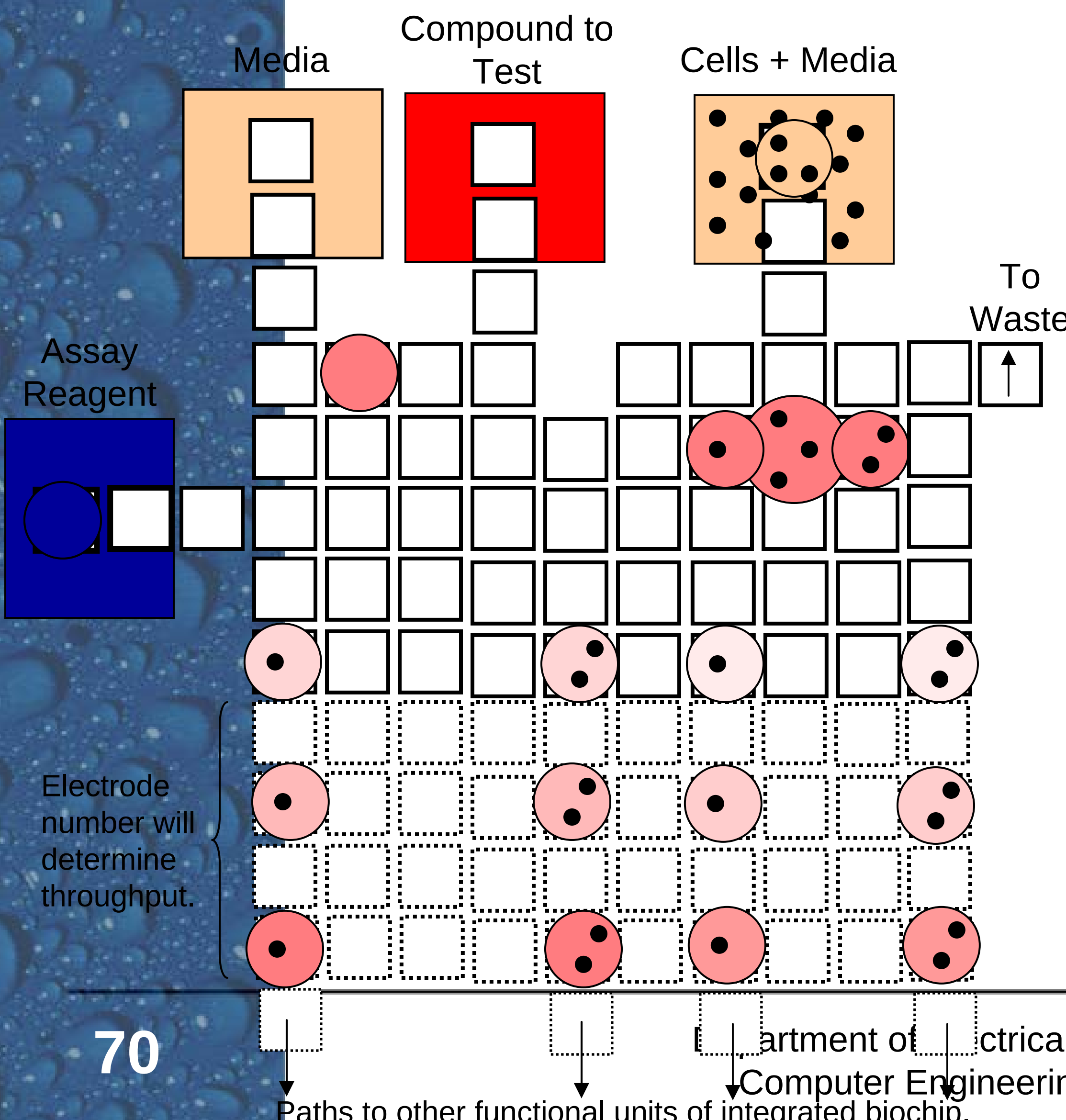
Architecture



1. Dispense buffer and compound droplets, mix.
2. Split. One droplet stays for further dilution, one droplet gets mixed with cells.
3. Dispense cell solution. Optical absorbance check of concentration (optional). Mix with diluted compound droplet.

Department of Electrical and Computer Engineering

Architecture



REPEAT to test multiple dilutions

1. Dispense buffer ~~and compound droplets~~, mix. with previous dilution drop.

2. Split. One droplet stays for further dilution, one droplet gets mixed with cells.

3. Dispense cell solution. Optical absorbance check of concentration (optional). Mix with diluted compound droplet.

4. Split. Both droplets go to holding.

5. Incubate desired length of time.

6. Transport droplets to integrated on-chip functions (lysis, PCR, etc)

Remarks on Applications

- Extensive biomedical application base can leverage microfluidic operations in an electrowetting system.
- Based on:
  - Shared elemental fluidic operations
  - Reconfigurable functional units and programmable control
  - No cross-contamination/wash droplets
  - Multi-tasking
  - Recongfigures around bottlenecks
- Wide diversity of applications can be parsed into manageable components and assembled into a programmable, reconfigurable and reusable architecture.

Present Status Summary

- The reality of current lab-on-a-chip technologies...
  - Highly application specific
  - Commercial trend: simple, disposable devices that interface with expensive control boxes
  - Disposable devices may perform limited set of steps
- What is required for a reconfigurable microfluidics?
  - Leverage devices into multiple applications
  - Complexity of diverse applications reduced to a manageable set of fluidic operations
  - Modular architecture gives flexibility of choosing fundamental operations
- What is required for evolvable digital microfluidics?
  - Programmable controllers available running fluidic programs
  - Need to implement sensing and feedback control

Acknowledgements

- NSF
- NIH
- ECE299 students