Integrated Digital Microfluidic Biochips

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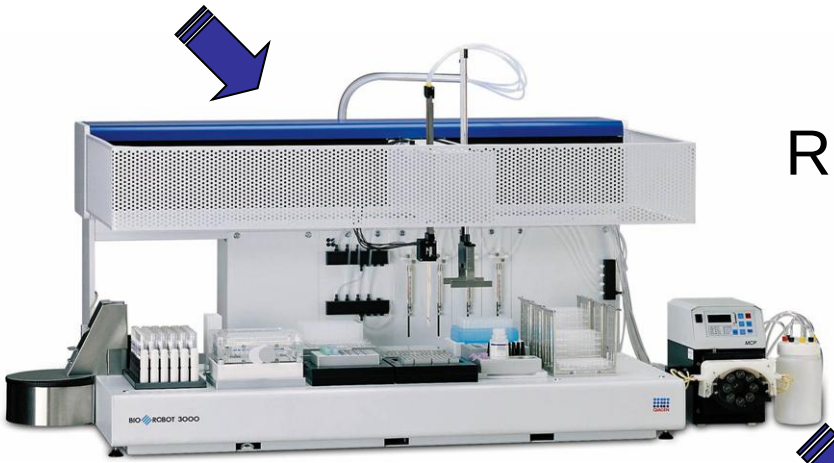
Outline of Presentation

- Background and motivation
  - Integrated disposable microfluidics
  - Integrated microfluidic systems: past and present
- Microfluidic integration issues
  - Architectural choices
  - Integrated detectors
- The digital microfluidic options and examples
  - Implications of droplet architecture
  - Examples of integration
    - Analog/Digital Hybrid Microfluidic Chip For DNA & RNA Analysis
    - Cytotoxicity Screening
    - Protein Crystallization
- Summary and conclusions

Background & Motivation

Test tubes

- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

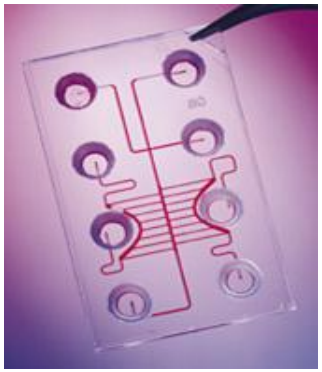


Robotics

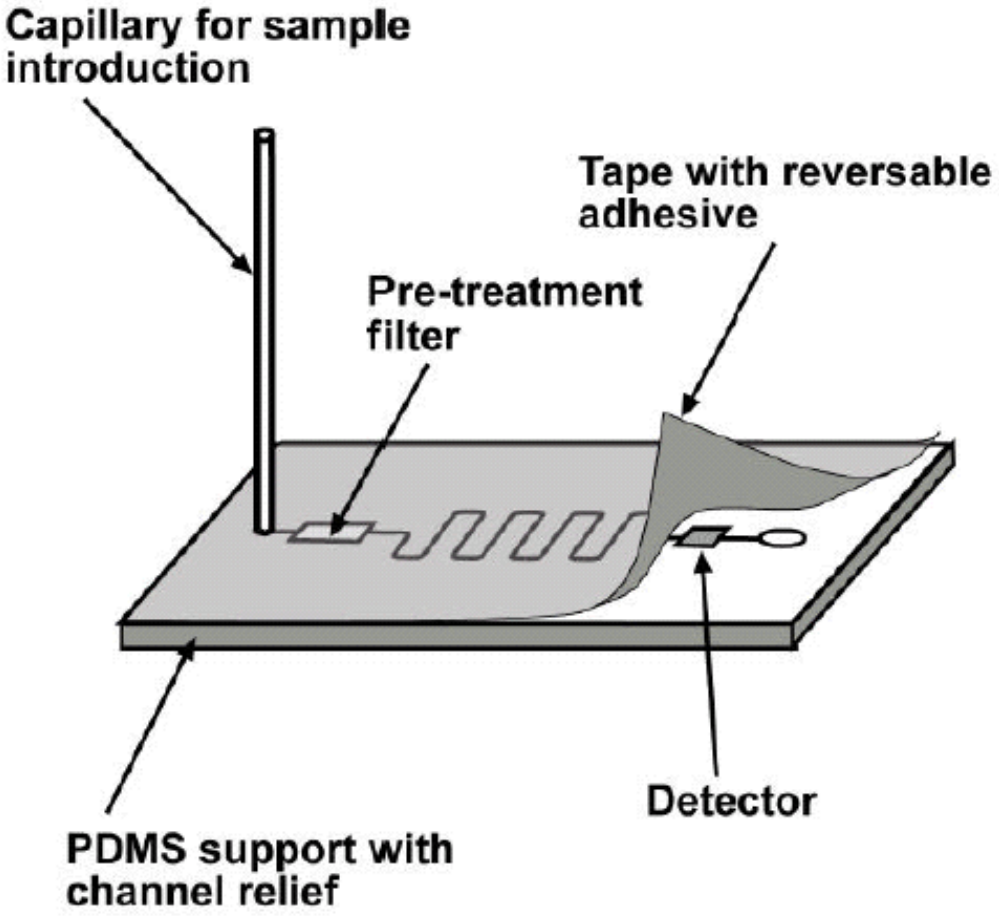
- ☑ Automation
- ☑ Integration
- ☐ Miniaturization

Microfluidics

- ☑ Automation
- ☑ Integration ?
- ☑ Miniaturization

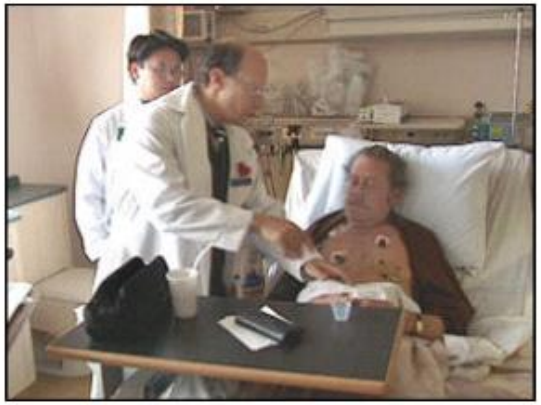


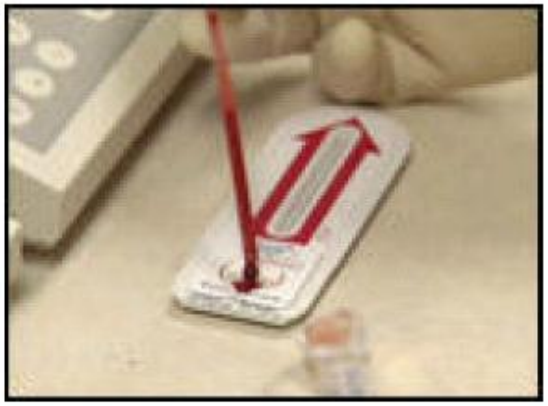
Start of the Art Commercial Disposable Microfluidics



BioSite Biochip

ANOTHER EXAMPLE OF A MICROFLUIDIC SYSTEM

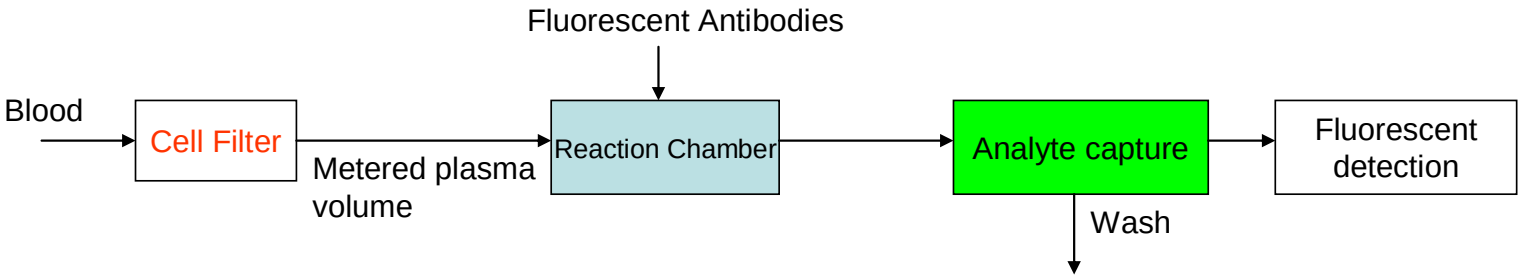


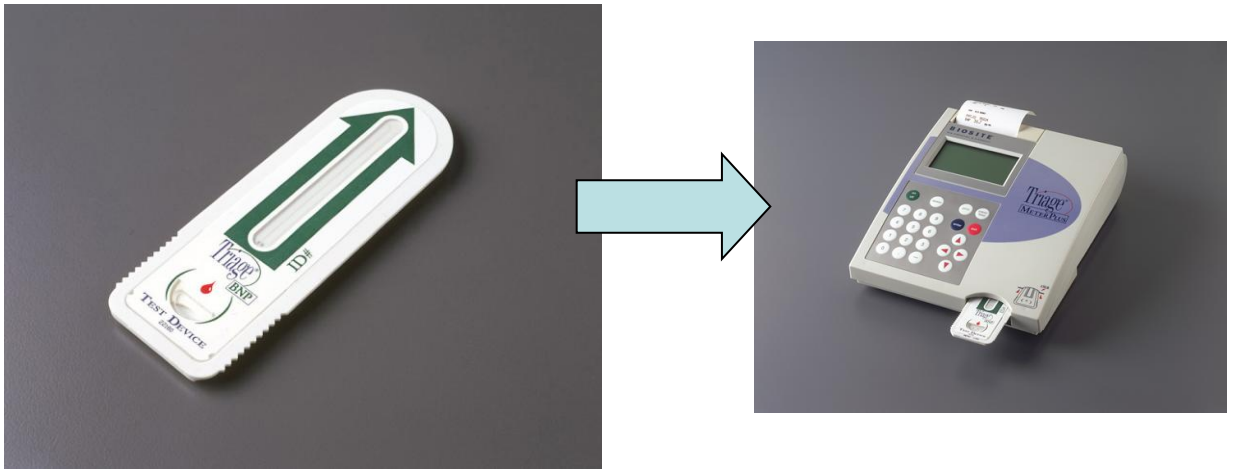


LAB-ON A CHIP BIOSITE

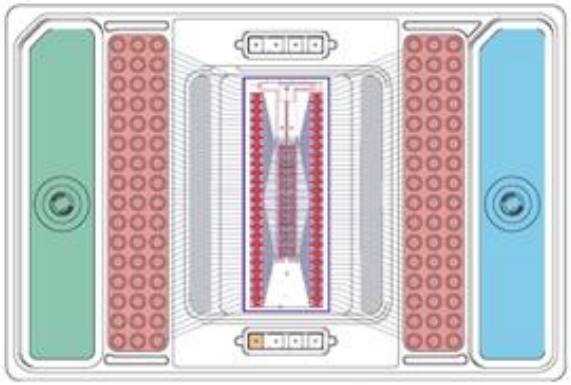
DIAGNOSES
HEART ATTACK
WITHIN 10 MN

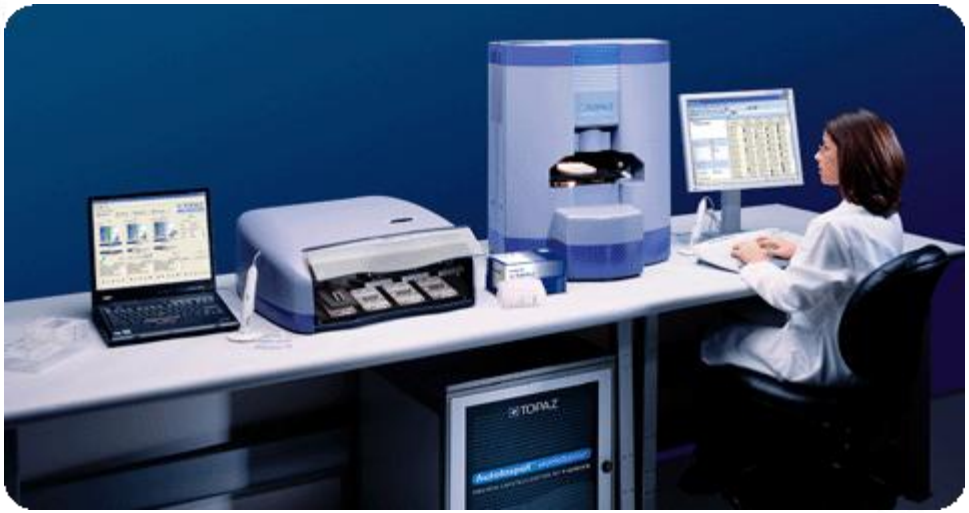
Disposable Chip Paradigm





Fluidigm 8.96 Screening Chip

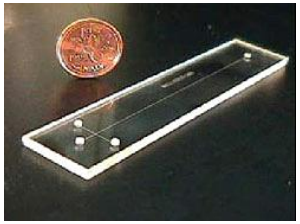


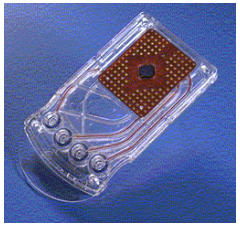


Concept of Disposable Integration

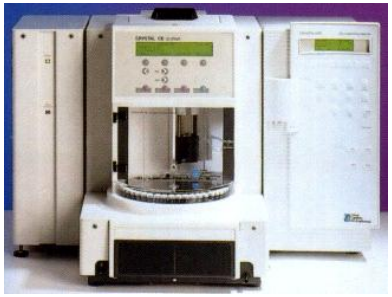
Application Devices

MICROFLUIDIC



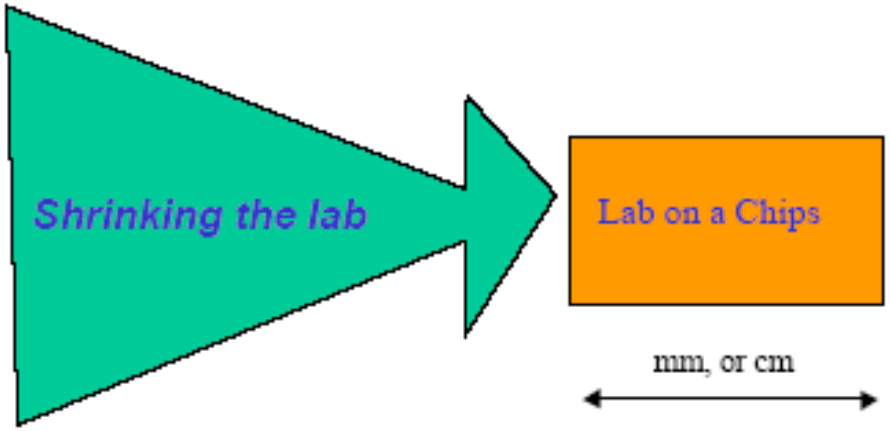


PROCESSING/ANALYSIS



Promise of Biochips

- Applications : Biotechnology (eg: high throughput screening , Diagnostics...)

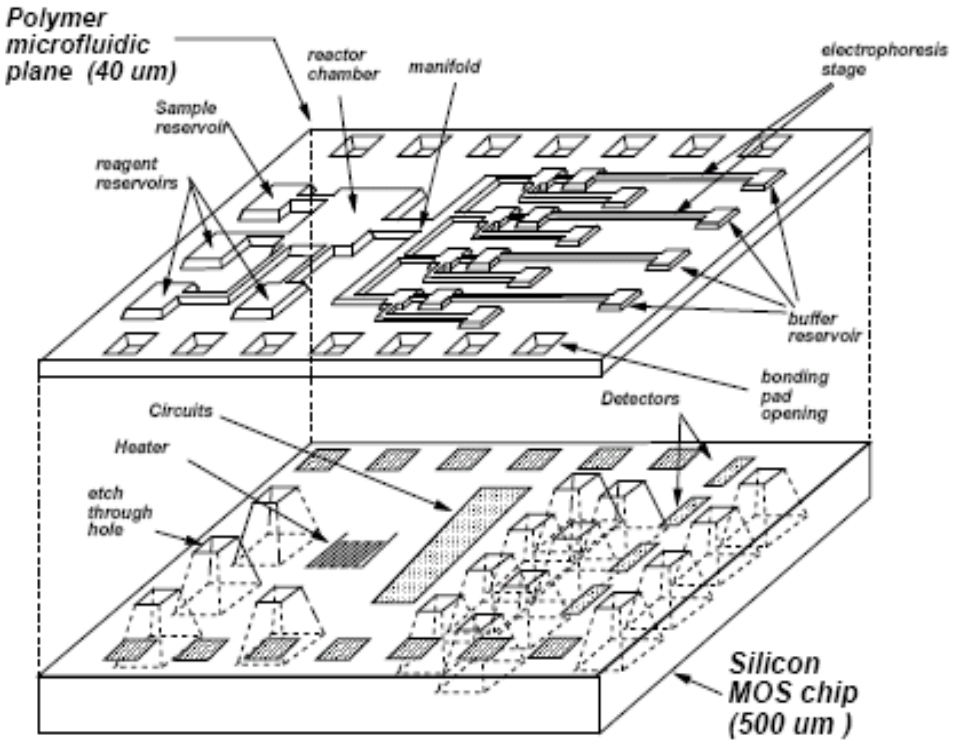


How important is a fully integrated chip?

Historical Electronic/Fluidic Integration

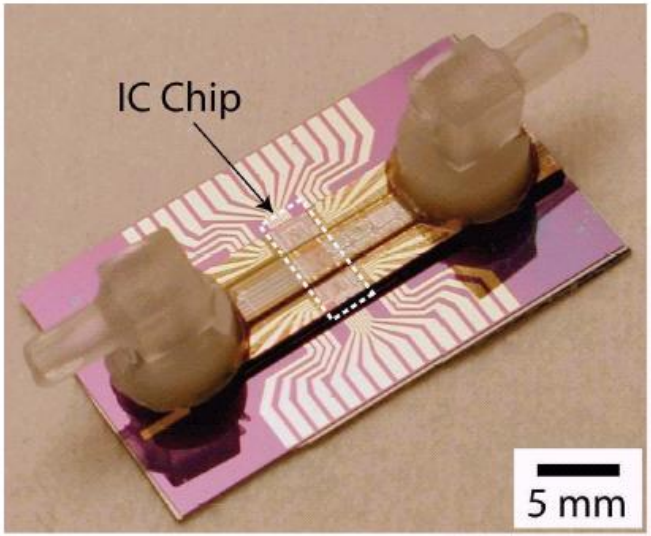
- Trend has been to integrate the fluidics on the electronics

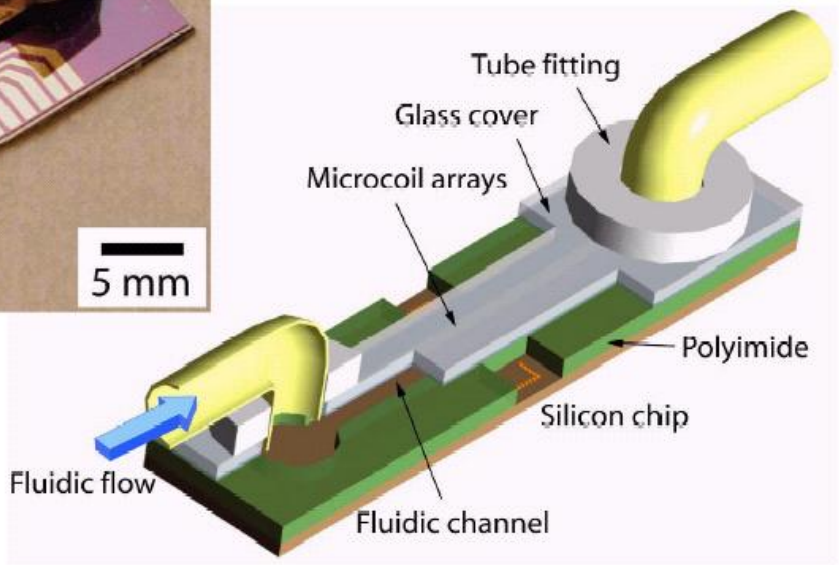
Man, 1997, UM



Current Integrated Microfluidic Devices

IC / Microfluidic Hybrid Prototype



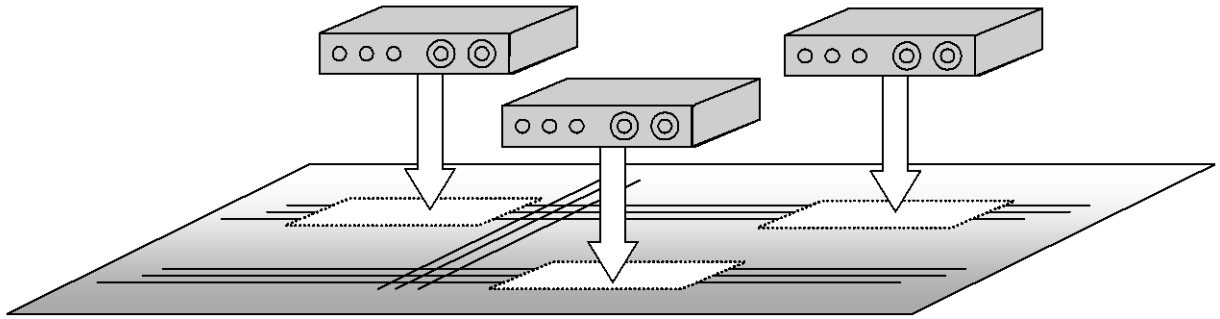


Lee *et al*, *ISSCC* 2005.

Hybrid Integration

Semicustom Multi-Chip-Module Implementation

- Pilot key architecture components to access manufacturability
- Microelectrofluidic “printed circuit board”
  - Common footprints - device interoperation
  - Precursor to future monolithic “shrink”



Integration Issues

- Can only integrated simple fluidic functions on an IC!
- Option: integrate the electronics on the fluidic platform (Motorola, 2004)

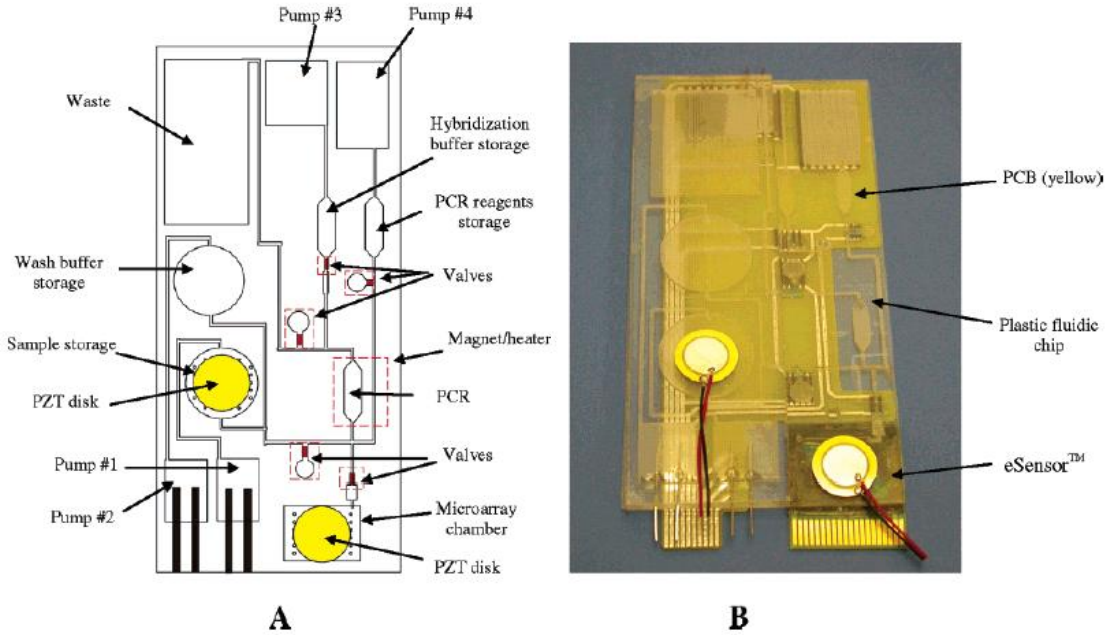
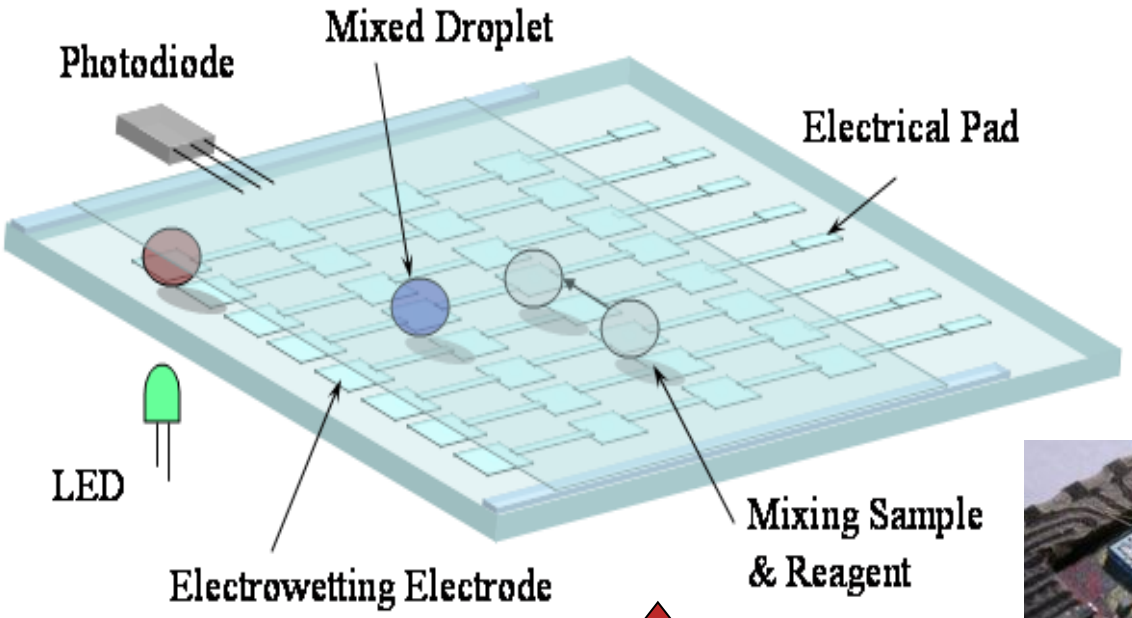
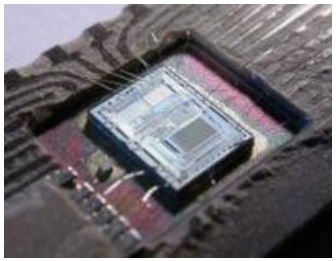


Figure 1. (A) Schematic of the plastic fluidic chip. Pumps 1–3 are electrochemical pumps, and pump 4 is a thermopneumatic pump. (B) Photograph of the integrated device that consists of a plastic fluidic chip, a printed circuit board (PCB), and a Motorola eSensor microarray chip.

Integration Compatibility Issues





Fluidic Platform

IC Platform

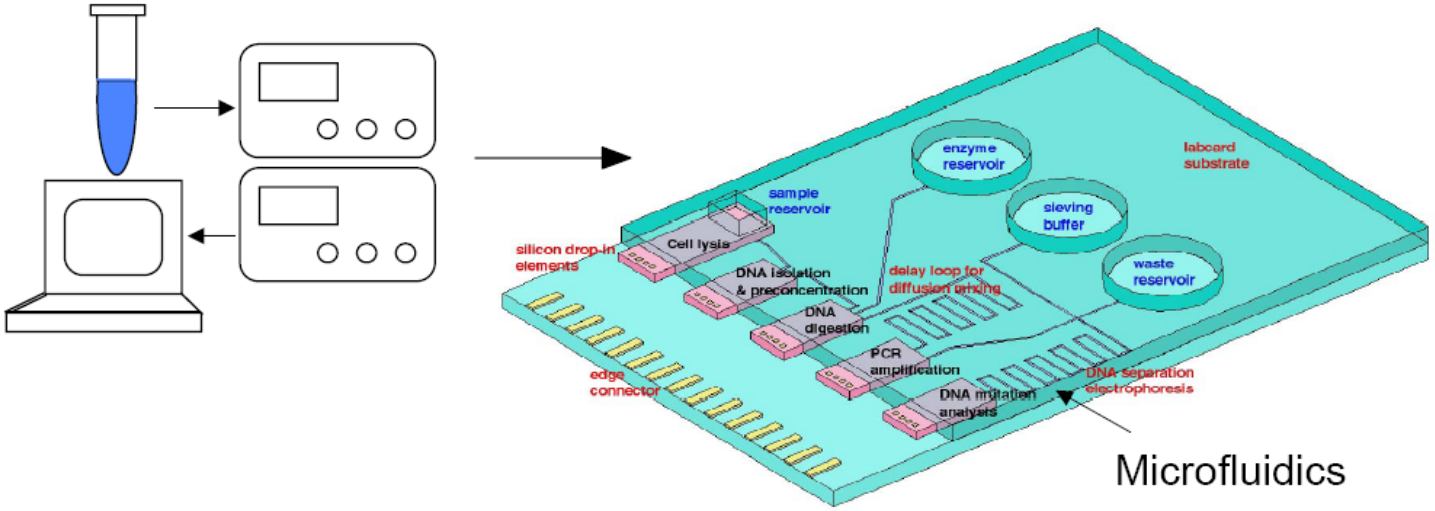
Microfluidic Functions

Benchtop Laboratory Techniques

Many manual steps

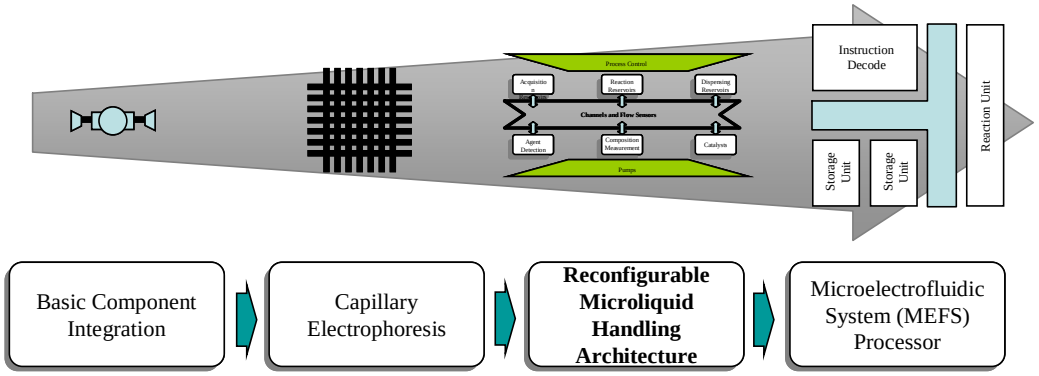
"Lab on a Chip"

Integrated sampling, chemical reactions, mixing, separation, detection, data processing

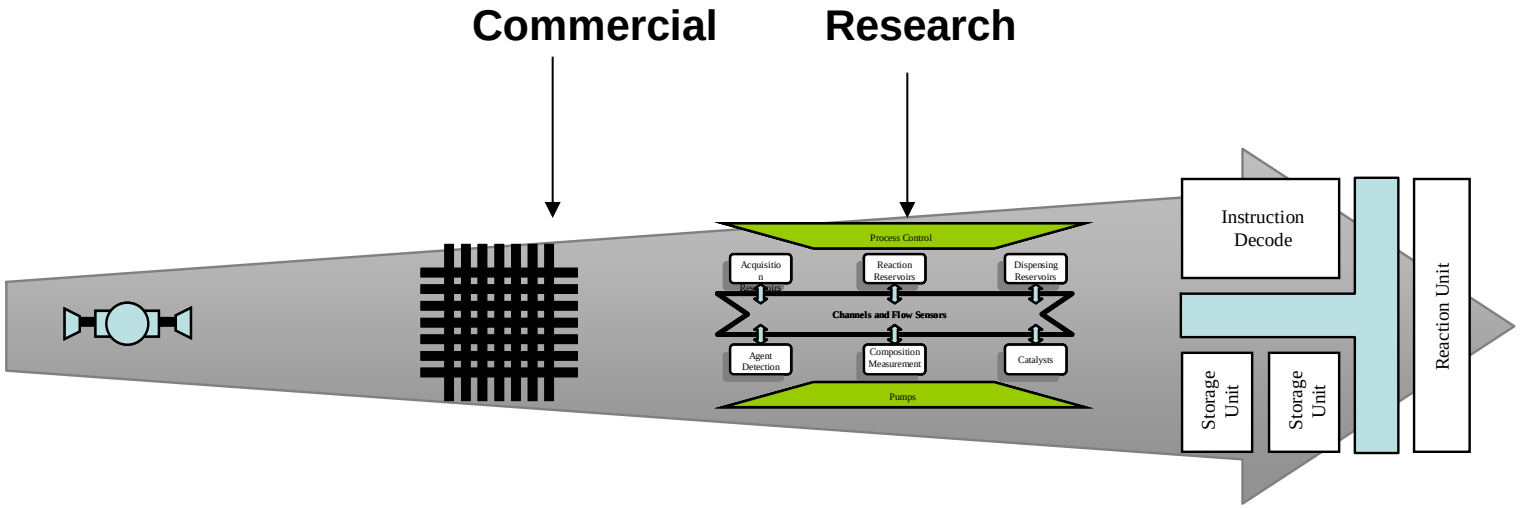


Architectural Choices

- Fixed data path (application specific)
- Reconfigurable (multiple applications)
  - Shared elemental operations
  - Microfluidic instruction set
  - Programmable
  - Reusable



Where Are We?



Basic Component Integration → Capillary Electrophoresis → **Reconfigurable Microliquid Handling Architecture** → Microelectrofluidic System (MEFS) Processor

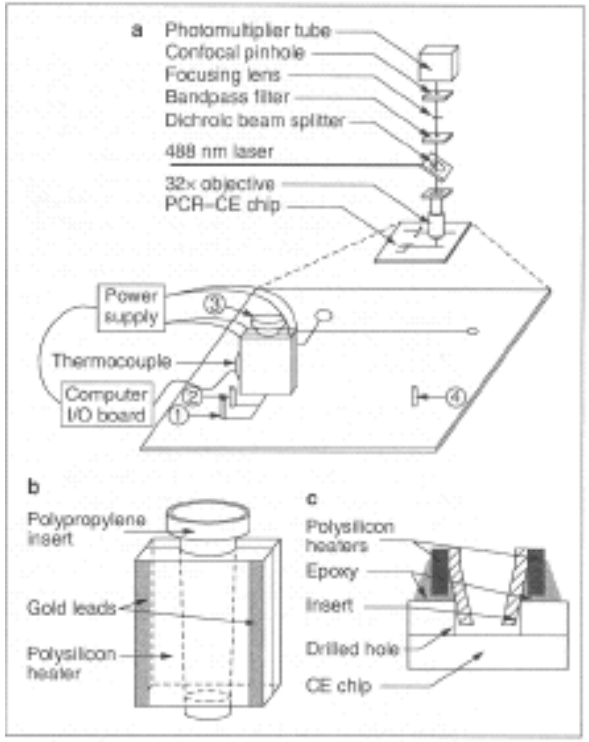
Present Status Summary

- 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 integrated 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
  - Integrated fluidic I/O
  - Integrated low voltage CMOS control incompatible with current fluidic operating voltages and footprints
  - Detector integration a priority

PCR Integrated System

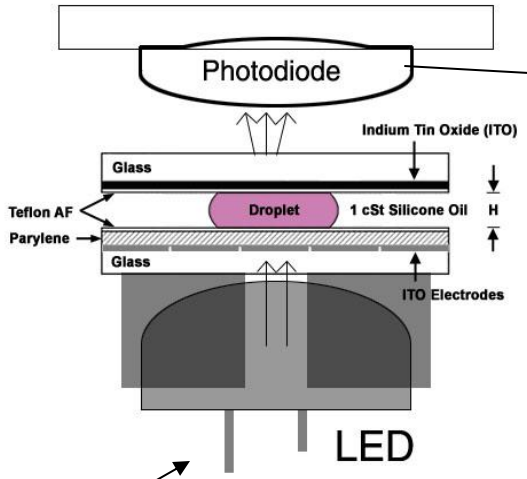
Woolley, Mathies and Northrup et al., 1996



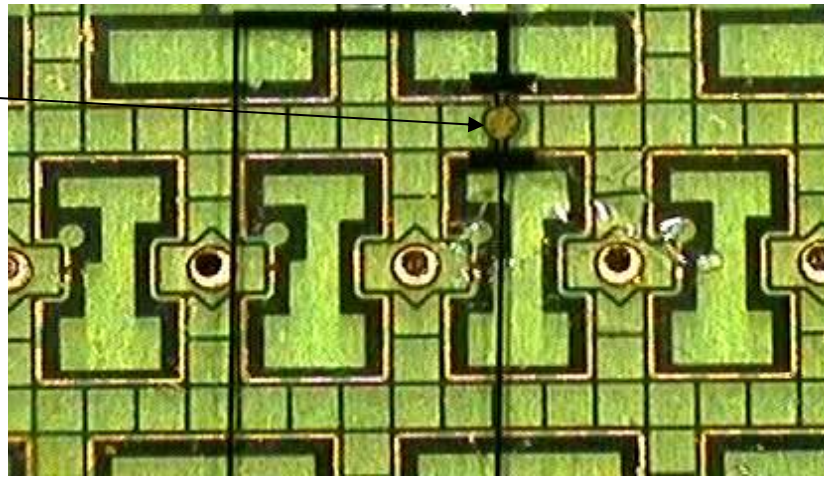
Cepheid, Sunnyvale CA

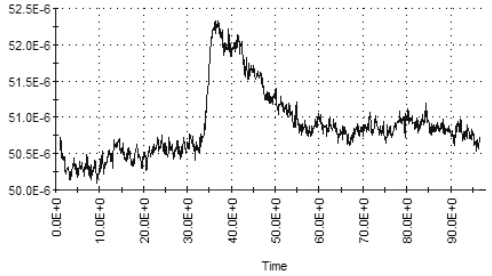
Detection Methodology

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



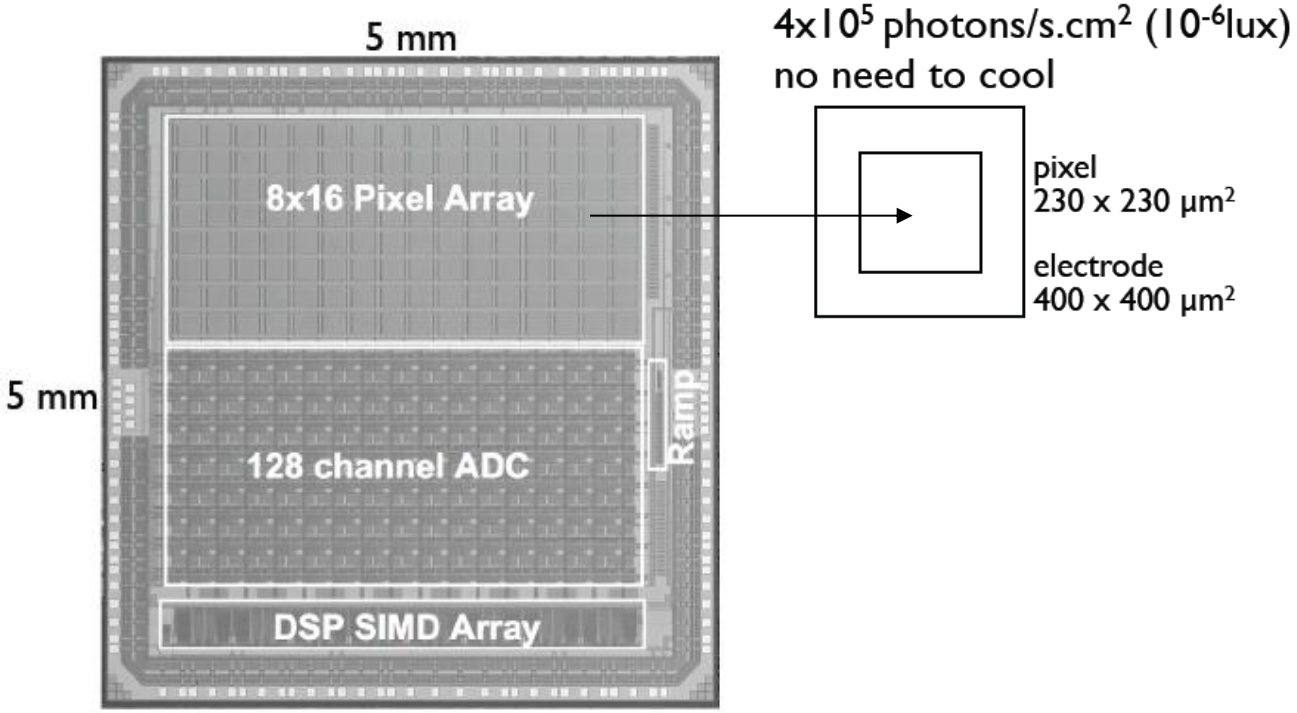
Opaque solid





Chemoluminescence underneath the TeflonAF coated photodetector

Detector Integration



Integrated Microdisk Sensor

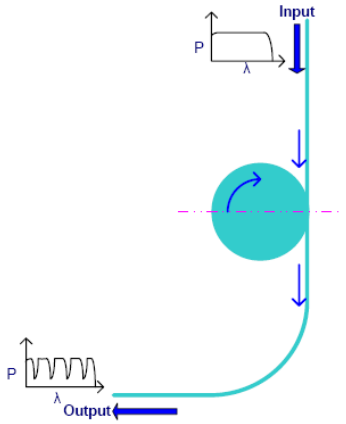


Fig. 1 Schematic of a vertically coupled microdisk resonator showing an input broad linewidth optical signal, and resultant output signal [37]

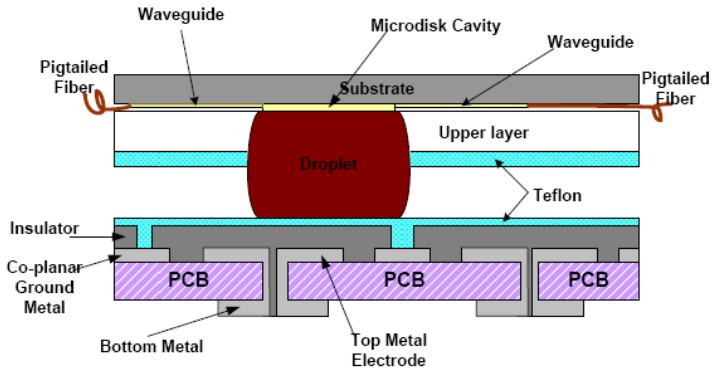
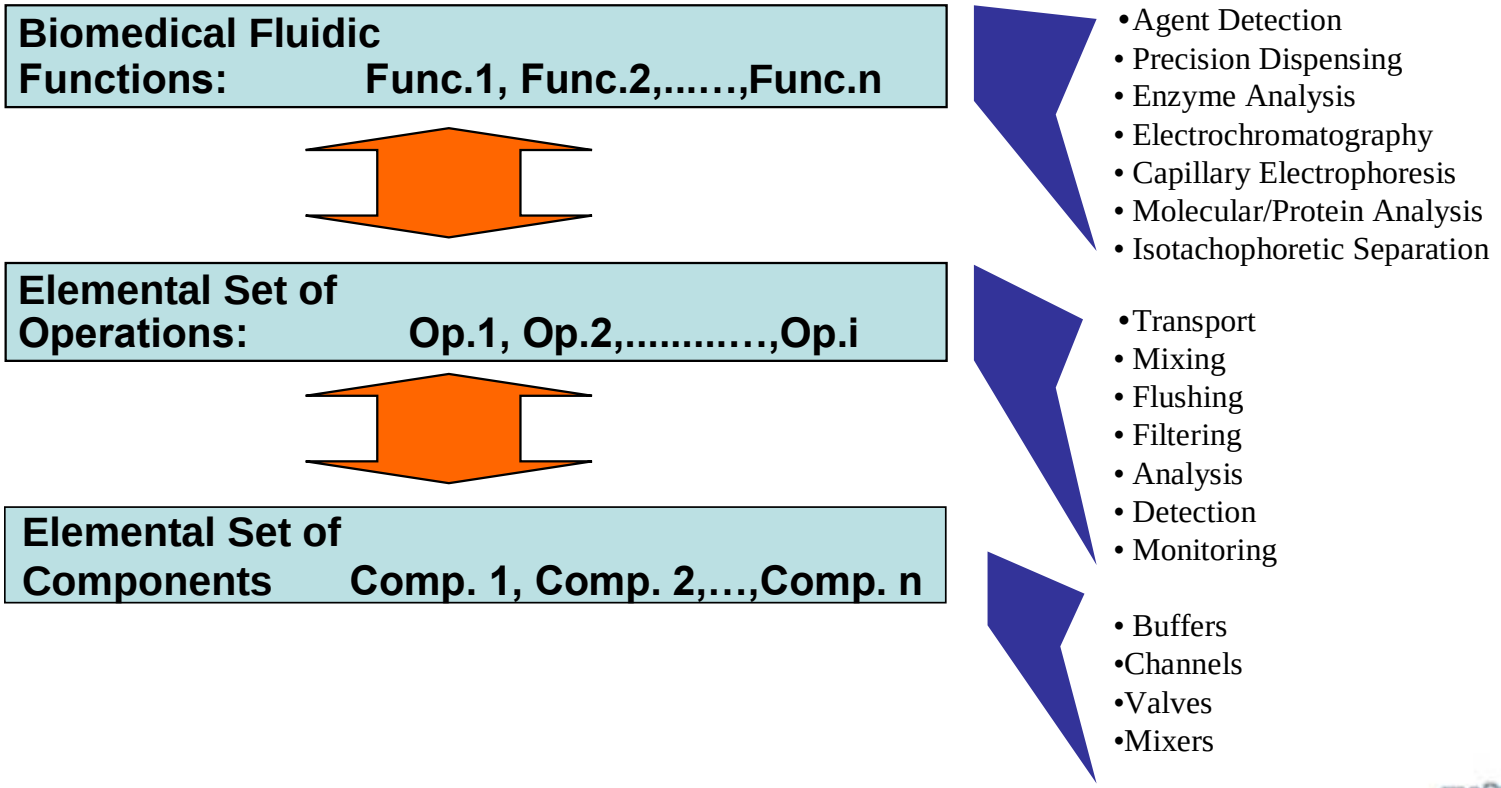


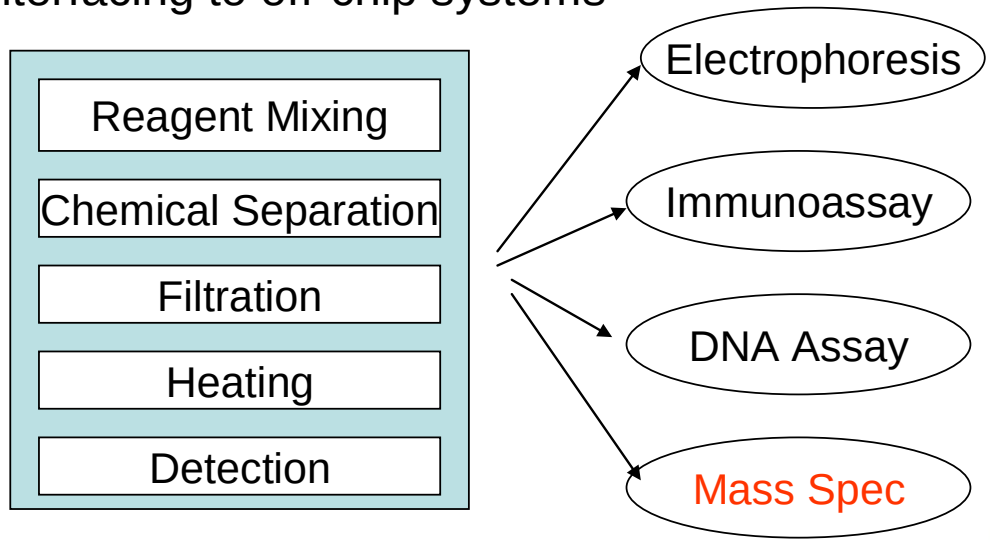
Fig. 16 Side view of an integrated glucose optical microdisk sensor integrated with an electrowetting chip.

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



Microfluidic Architecture

- Extensive biomedical analysis technology base needs to be leveraged by expanding integration of microfluidic operations into a complete system
  - Key is integration of sample preparation processes on chip. Hybrid integration option possible.
  - Alternative: interfacing to off-chip systems

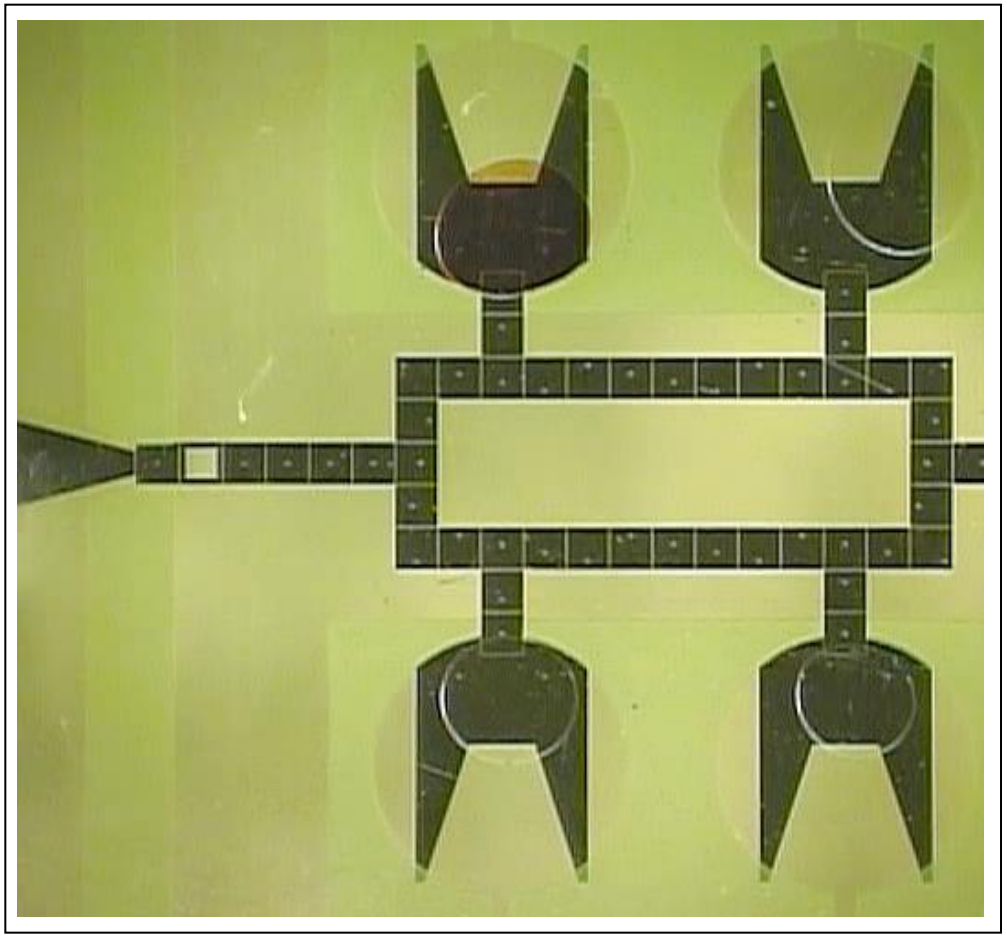


Digital Microfluidic Toolkit

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

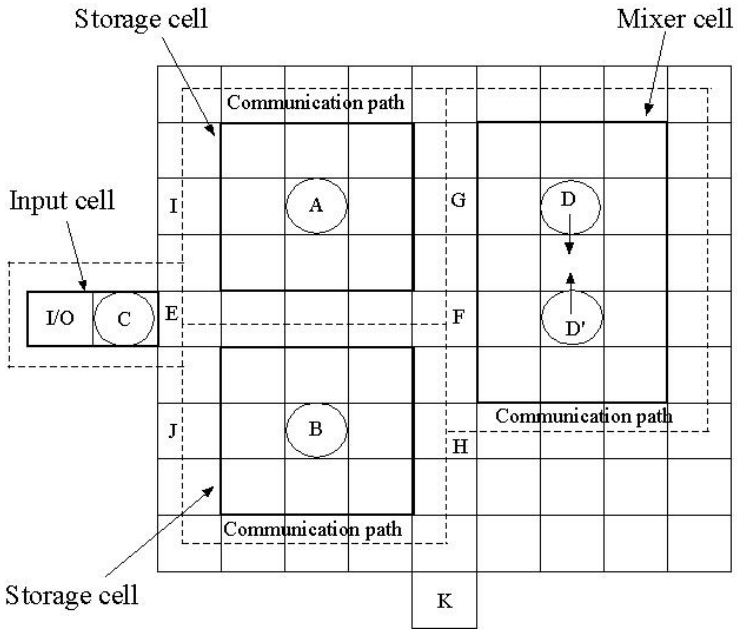
Integrated Operation - Serial



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

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 common set of elemental components
- Array can be partitioned into “cells” that perform fluidic functions
- Functional cells dynamically reconfigured at least once per clock cycle

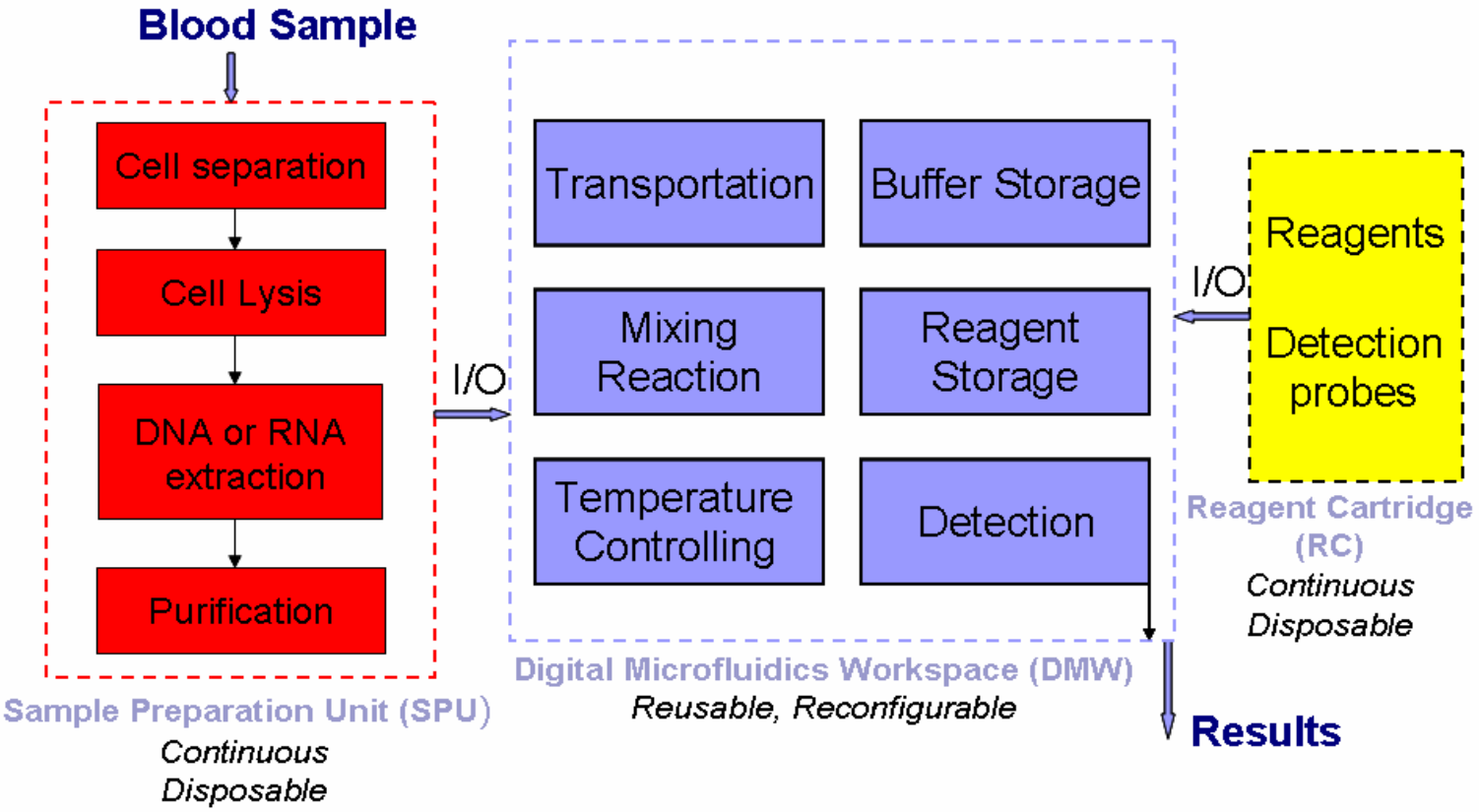


Integrated Lab-on-a-Chip Systems

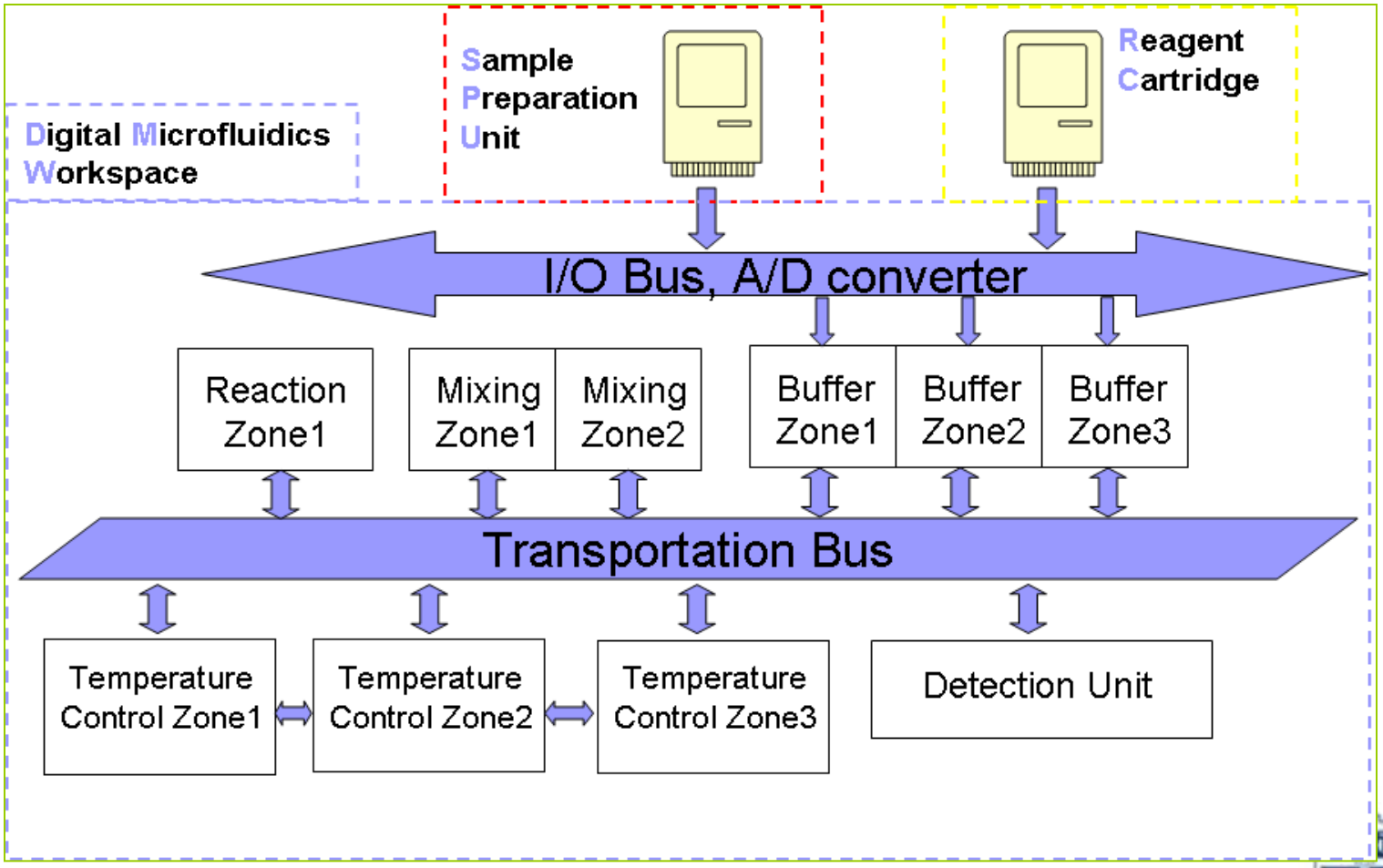
- Digital microfluidic toolkit demonstrated
- Can digital microfluidics deliver a true integrated lab-on-a-chip technology that is adaptable to numerous applications?
- Examples from ECE299 (Duke Univ. Fall 2006/2007)
  - Analog/Digital Hybrid Microfluidic Chip For DNA & RNA Analysis
  - Cytotoxicity Screening
  - Protein Crystallization

Analog/digital hybrid biochip

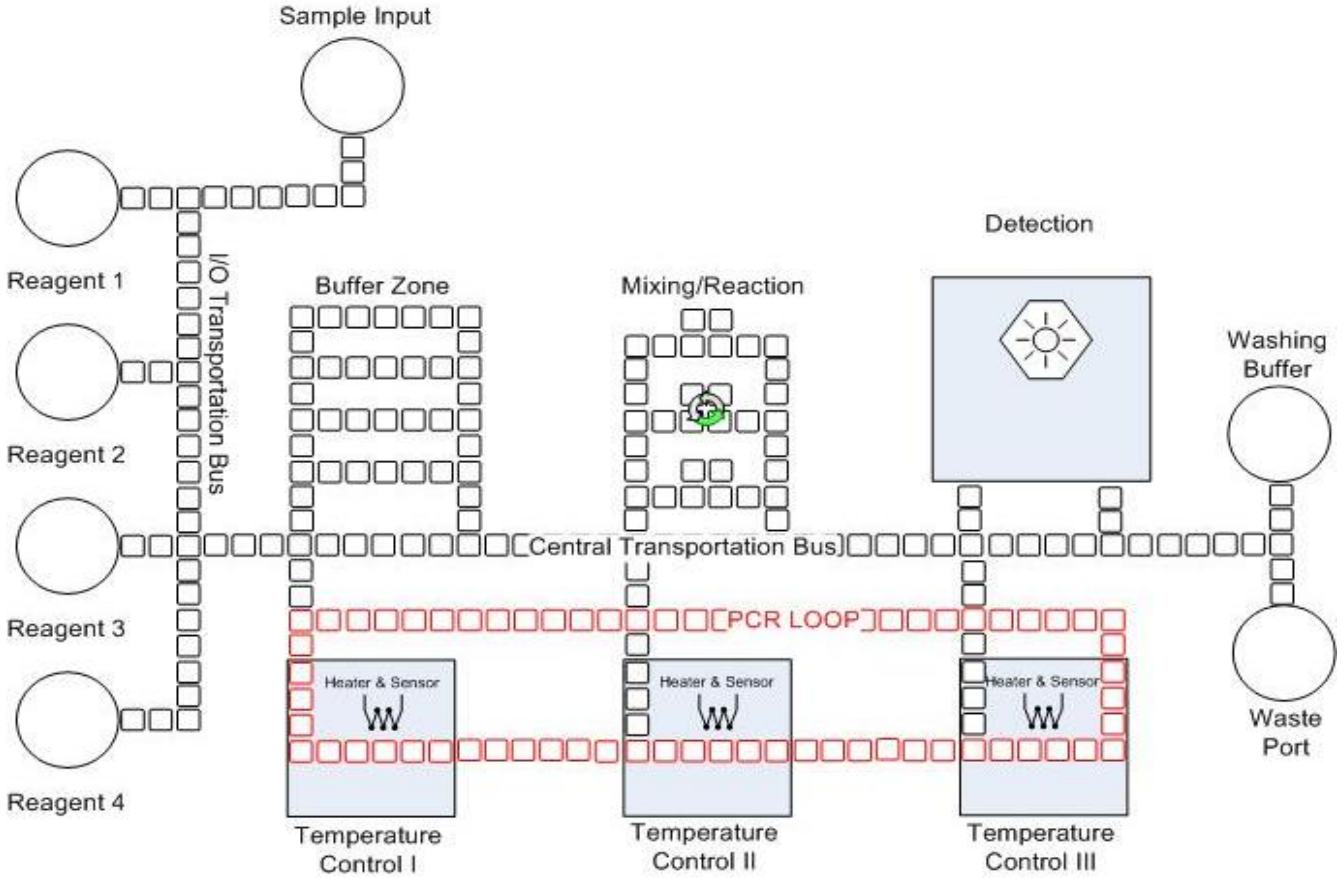
(A. Garcia, G. Pan, J. Zhang)



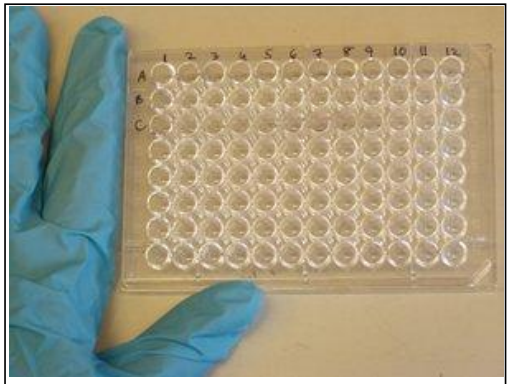
Fluidic Platform



Floor Plan of the DMW

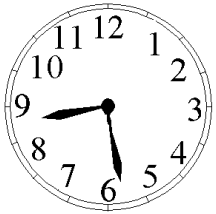


On-chip Dilution Tree for Cytotoxicity Screening (Y. Zhao, A. Wang, Y. Yamanaka)



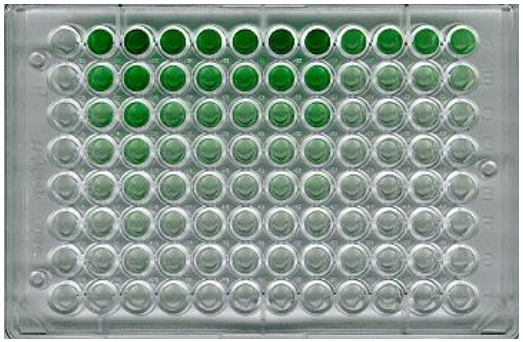
Grow cells in 96 well plate

Add various concentrations of compound to be tested to cells



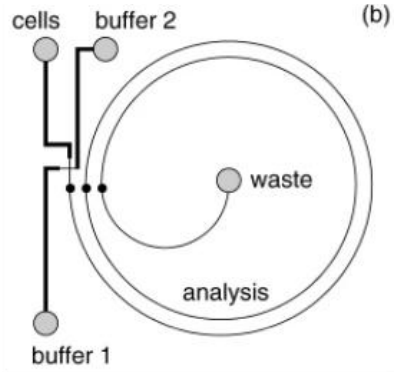
Wait specified length of time

Add Cytotoxicity Assay reagent 1, incubate, add reagent 2

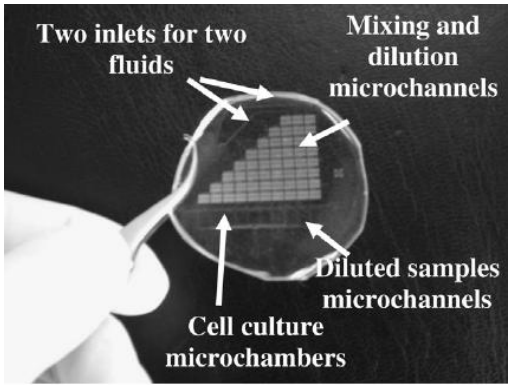


Use plate reader to measure color intensity (proportional to survival)

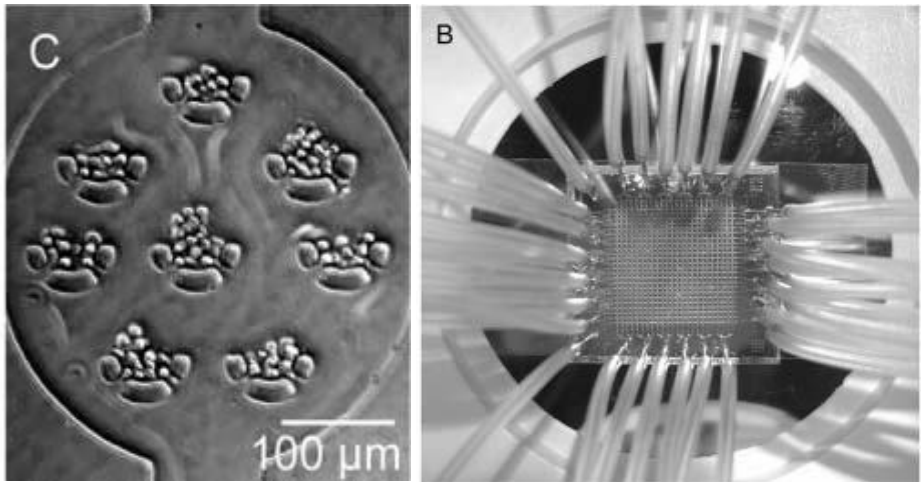
Previous Work

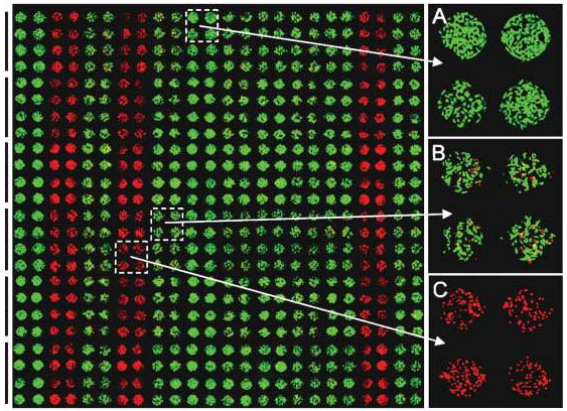


Anal. Chem. **2005,** 77, 667-672



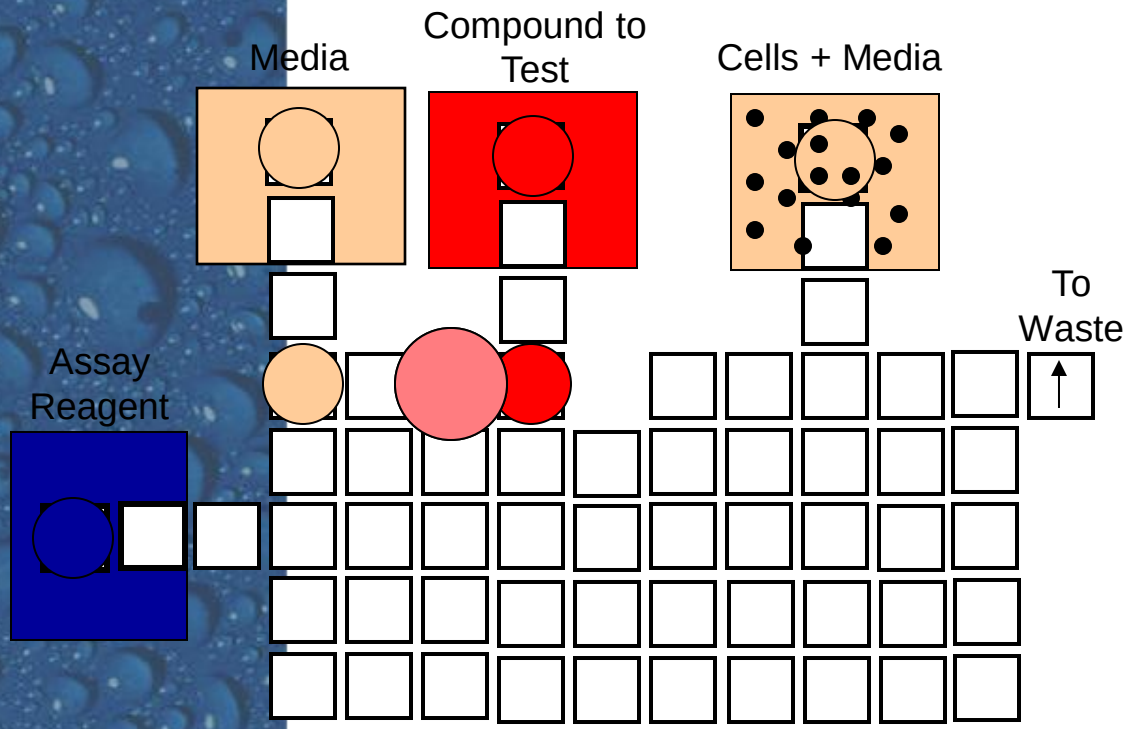
Toxicology in Vitro **2007,** 21, 535–544





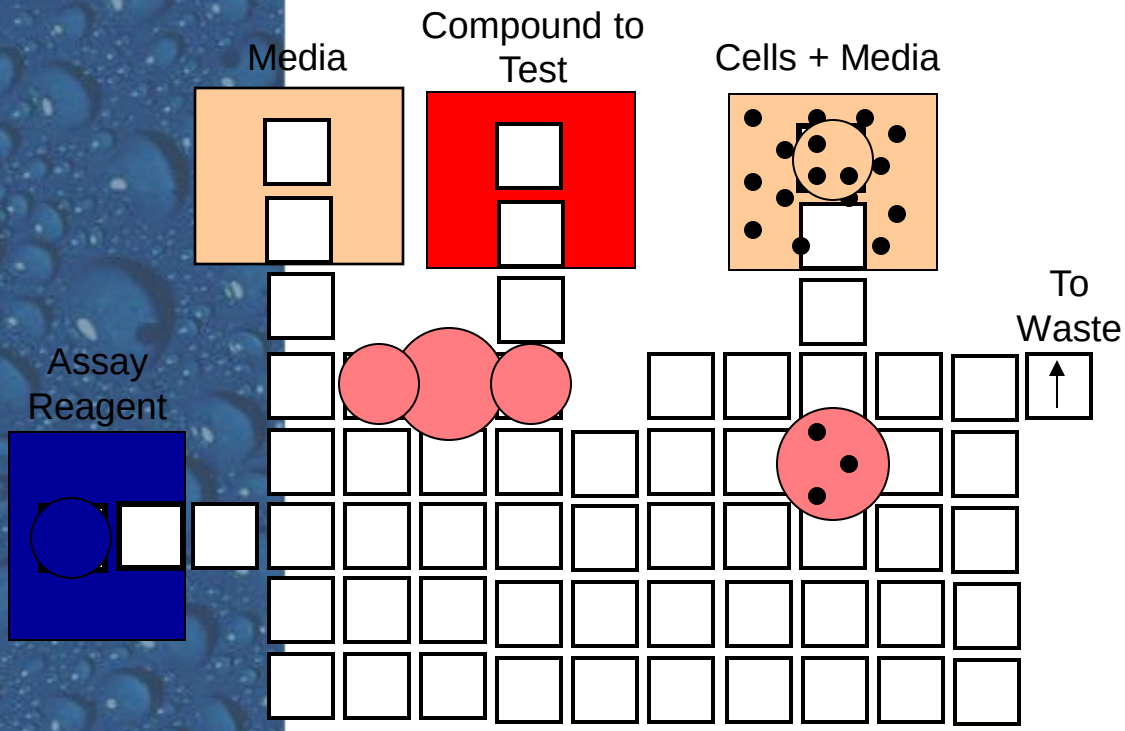
Lab on a Chip **2007,** 7, 740-745

Architecture



1. Dispense buffer and compound droplets, mix.

Architecture

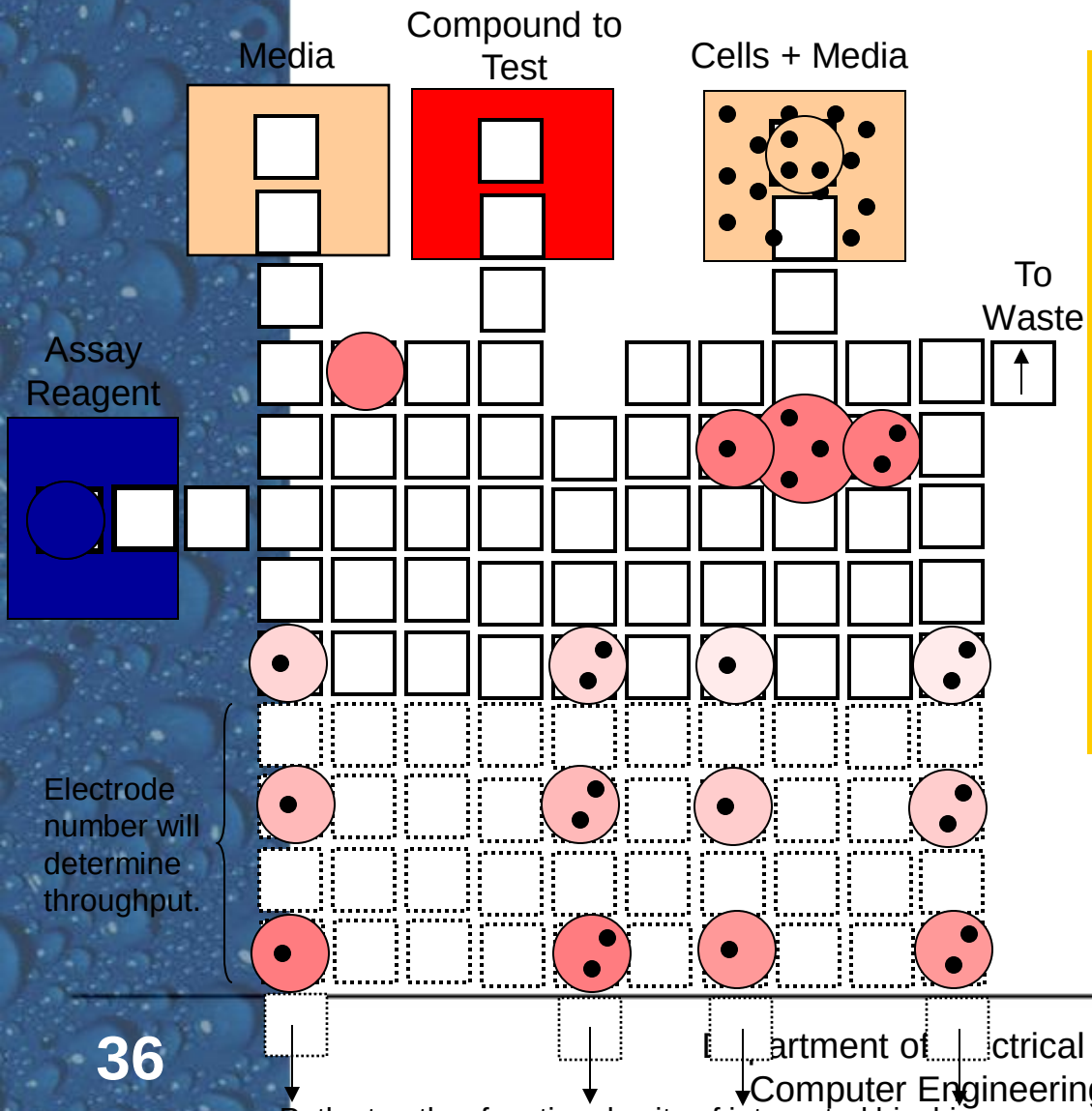


1. Dispense buffer and compound droplets, mix.

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.

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)

Inputs, Outputs, and On-Chip Function

- **Inputs**
  - (1) Cell suspension, (2) Cell media for dilutions, (3) Solution of compound to be tested for cytotoxicity, (4) Reagents for the cytotoxicity assay
  - If portable: include Lithium ion battery
- **On-chip functions**
  - Create droplets of input liquids, split and mix droplets, incubate droplets for programmed length of time, detect intensity of droplet color or presence of stained cells.
- **Outputs**
  - Color intensity of droplets or presence of stained cells.

- **Cell concentration after dispensing**

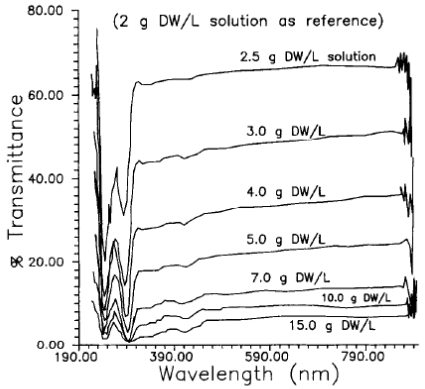
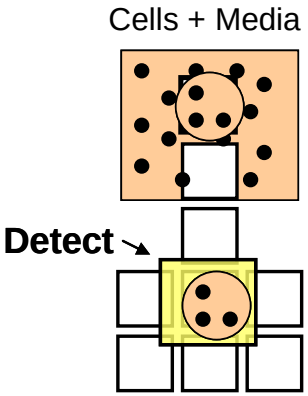


Figure 2. Absorption spectra of different concentrations of *S. cerevisiae*.

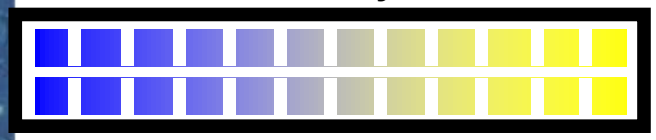
Biotechnology and bioengineering, Vol 38, Iss. 9, 1007-1011.



If not in range, send back.

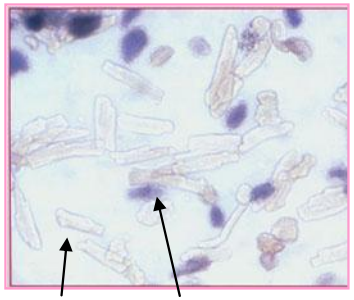
- **Cytotoxicity assay result**

Color intensity detector



Most cells alive — Most cells dead

OR



Alive cell — Dead cell

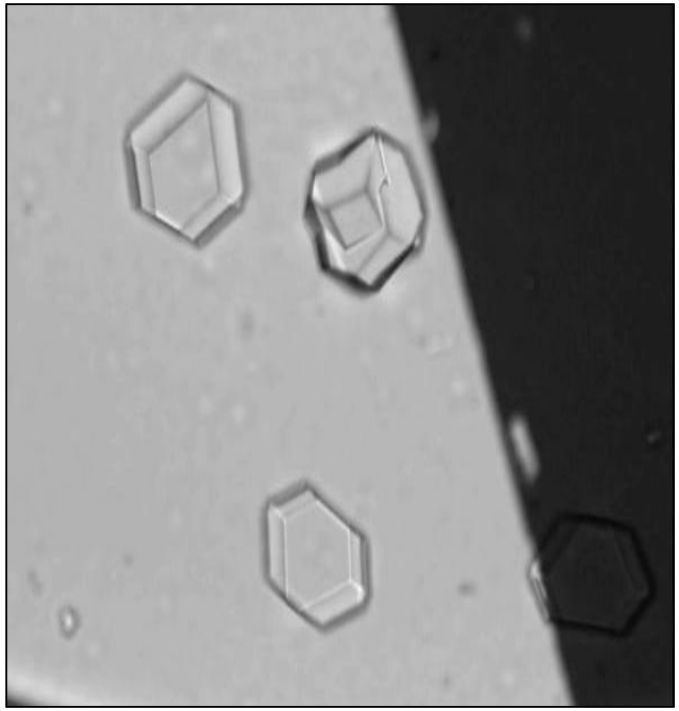
Image acquisition and processing →

Output:

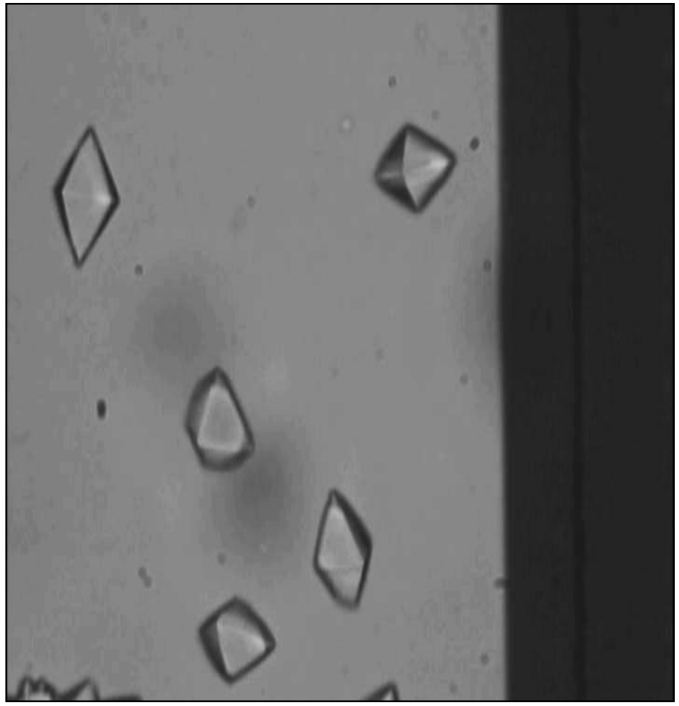
cells alive in droplet
cells dead in droplet

Protein Crystallization on an Array

(H. Fang, M. Shafir, T. Xu)



Glucose isomerase crystals on chip – 20×



Proteinase K crystals on chip – 40×

Protein Crystallization

- Major applications of proteins crystallization
  - Structural biology and drug design
  - Bioseparations
  - Controlled drug delivery
- Requires large number of experiments to get the correct parameters for the crystallization of proteins

Phase Diagram

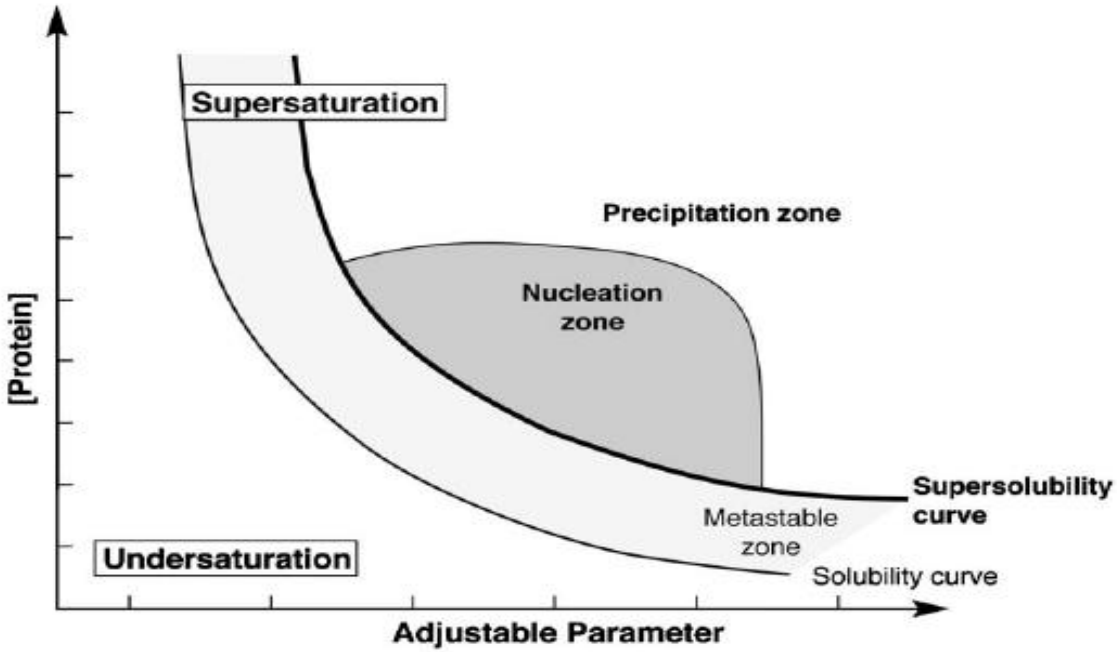
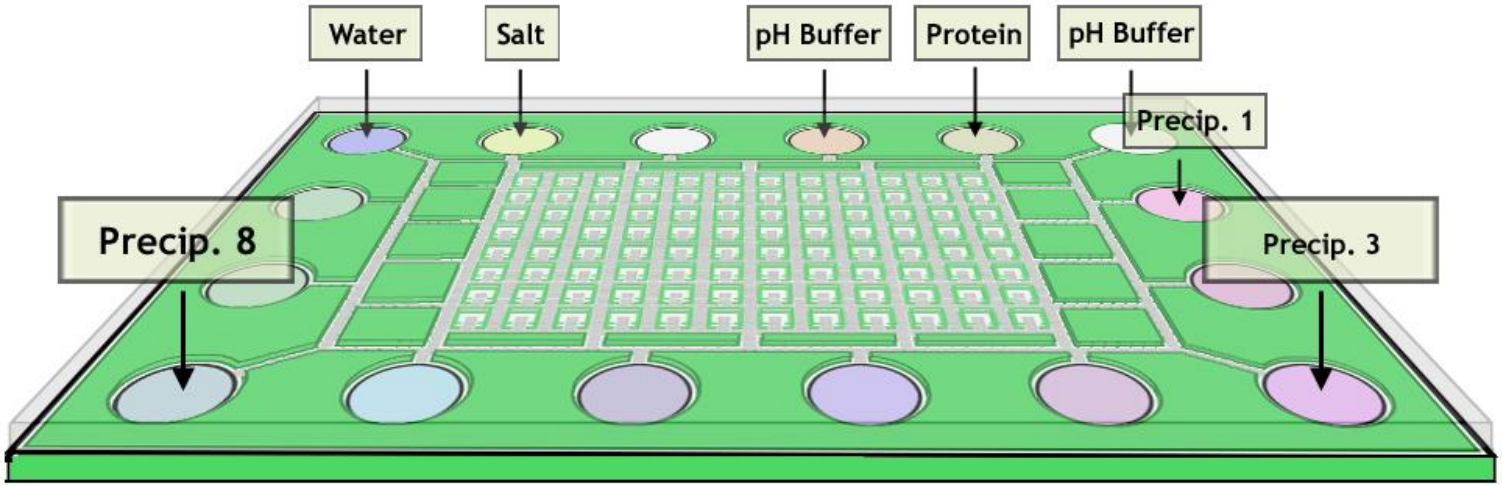


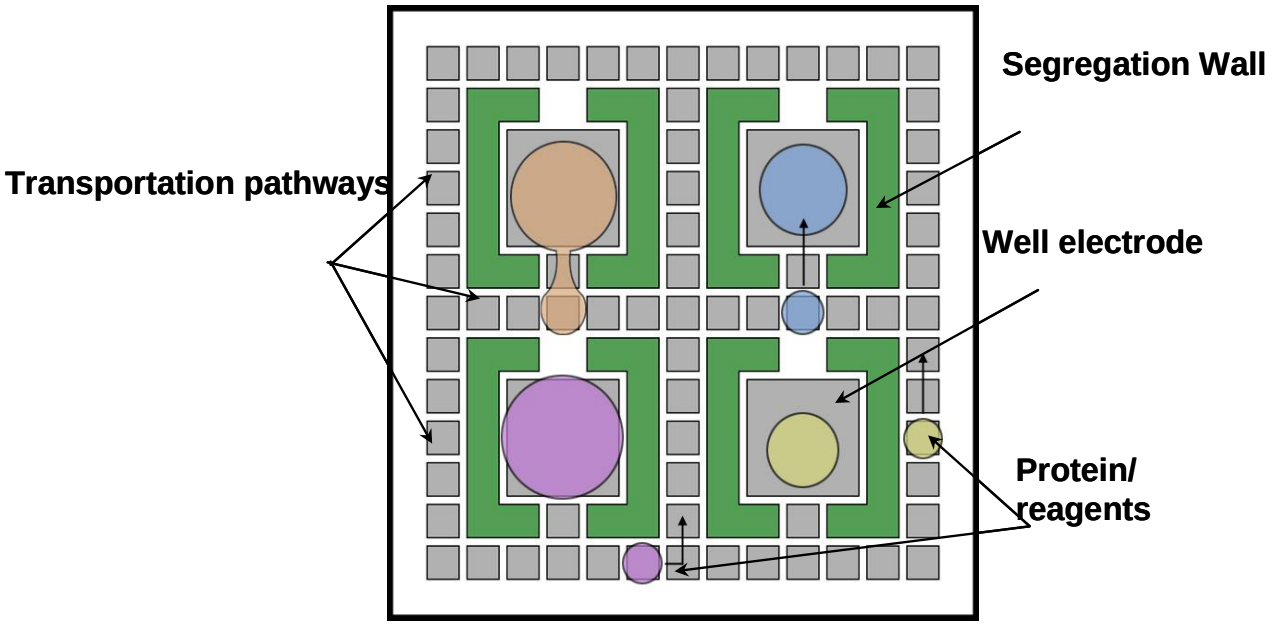
Fig. 1. Schematic illustration of a protein crystallisation phase diagram.

Integrated Array Chip Layout

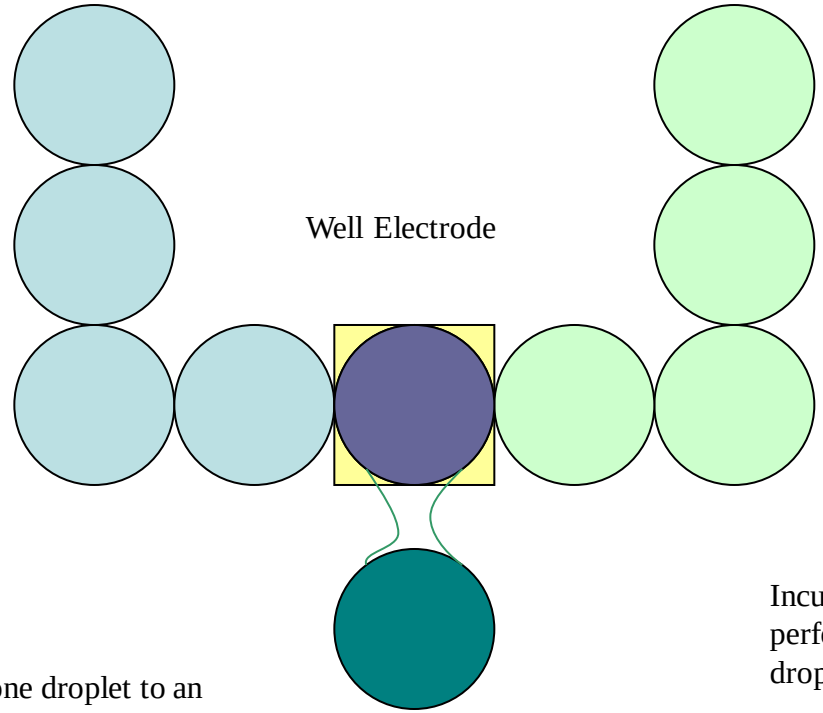


Implementation

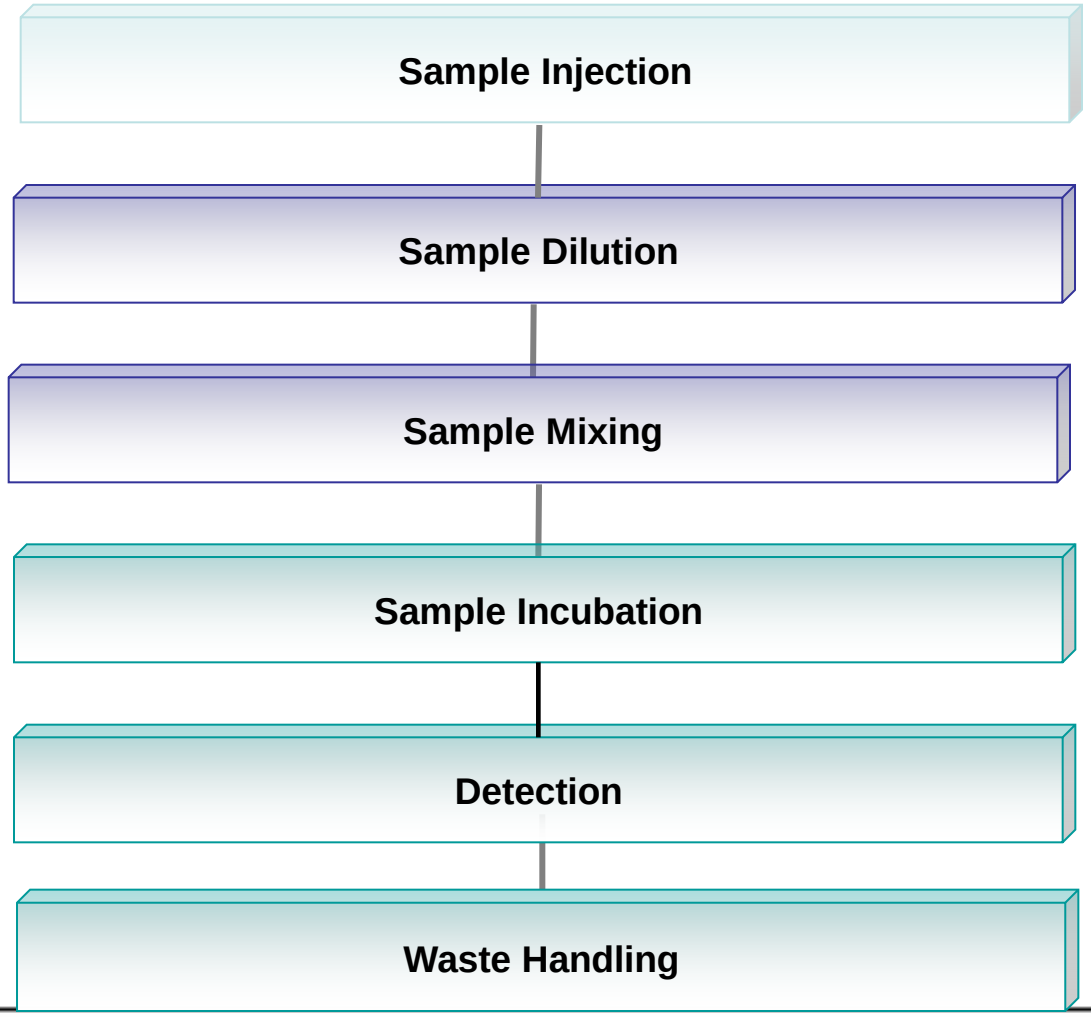
- Multi-well-plate



Sample Droplet Splitting and Dilution Scheme

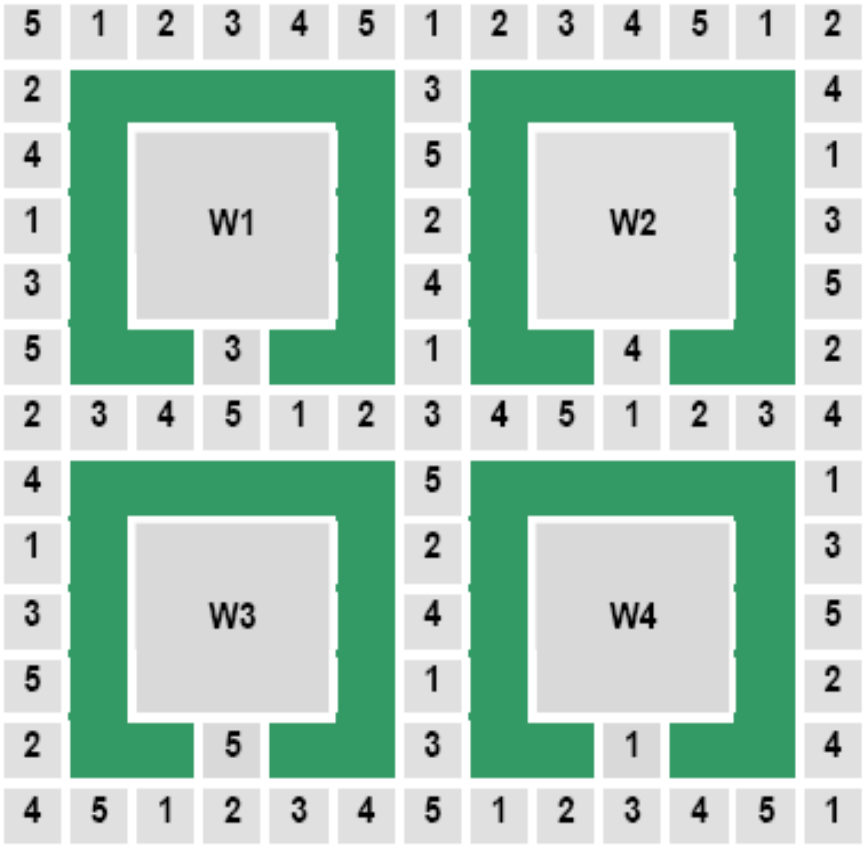


Architectural Block Diagram



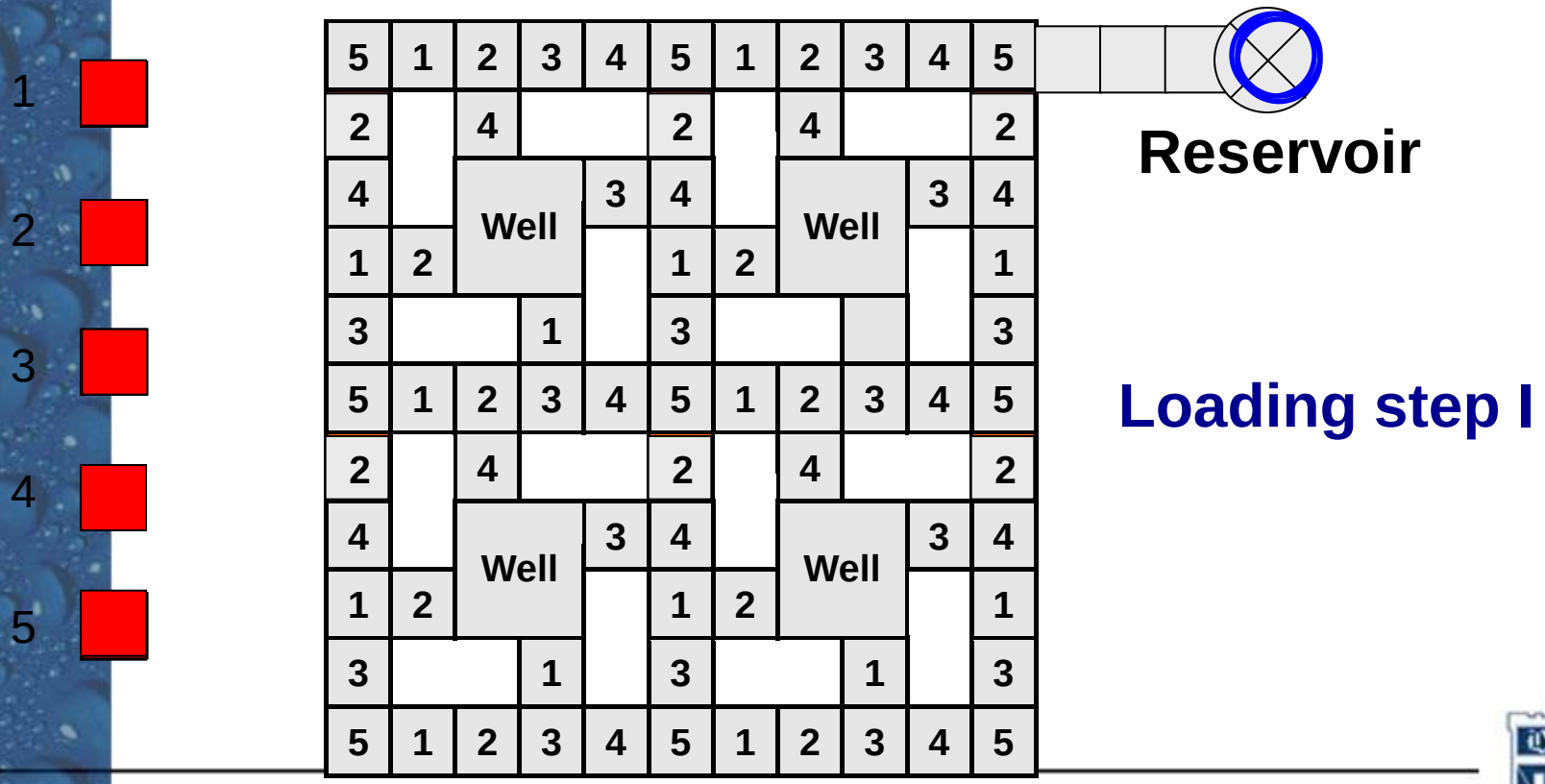
Pin-constrained Design

- 1284 pins→133 pins



Efficient loading of condition solutions

- Shuttle-passenger-like well-loading



Remarks on Applications

- Extensive biomedical application base can leverage microfluidic operations in an electrowetting system.
- Based on:
  - Shared elemental fluidic operations
  - Reconfigurability
  - No cross-contamination
  - Multitasking by components
  - Few bottlenecks.
- Wide diversity of applications can be parsed into manageable components and assembled into a programmable, reconfigurable and reusable architecture.

Summary and Conclusions

- Integration of lab-on-chip microfluidics on IC's may happen at the femtoliter scale (1μm)
  - Requires sample in/result out integration
  - High sensitivity detector
- Electrowetting-based digital microfluidics is good candidate for multifunctional microfluidics
  - Programmability
  - Reconfigurability
  - Multifunctional
- Open issues:
  - On-chip sample preparation
  - Lack of a molecular separation method
    - Capillary electrophoresis
  - Accurate on-chip dilution an open issue
  - Scalable, compatible detector technology needed

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- NIH
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- ECE299 students