Integrated Digital Microfluidic Functions for Chemical and Biological Applications

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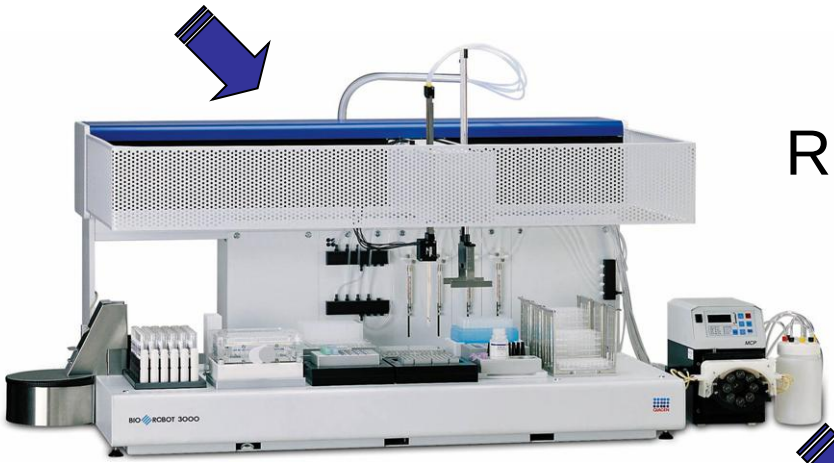
Outline of Presentation

- Background and Motivation
  - Issues in true lab-on-a-chip systems
- Lab-on-a-Chip toolkit
  - Sample loading
  - Dispensing
  - Sample transport
  - Mixing reactors
  - Detection
- Performance data
  - Statistics on glucose assays
  - Pyrosequencing
- Summary and conclusions

Background & Motivation

Test tubes

- ☐ Automation
- ☐ Integration
- ☐ Miniaturization

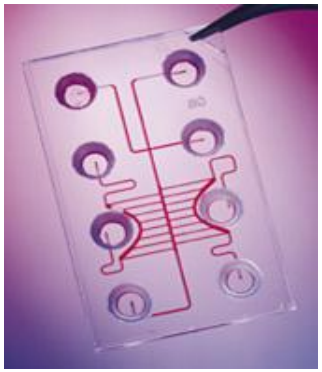


Robotics

- ☑ Automation
- ☑ Integration
- ☐ Miniaturization

Microfluidics

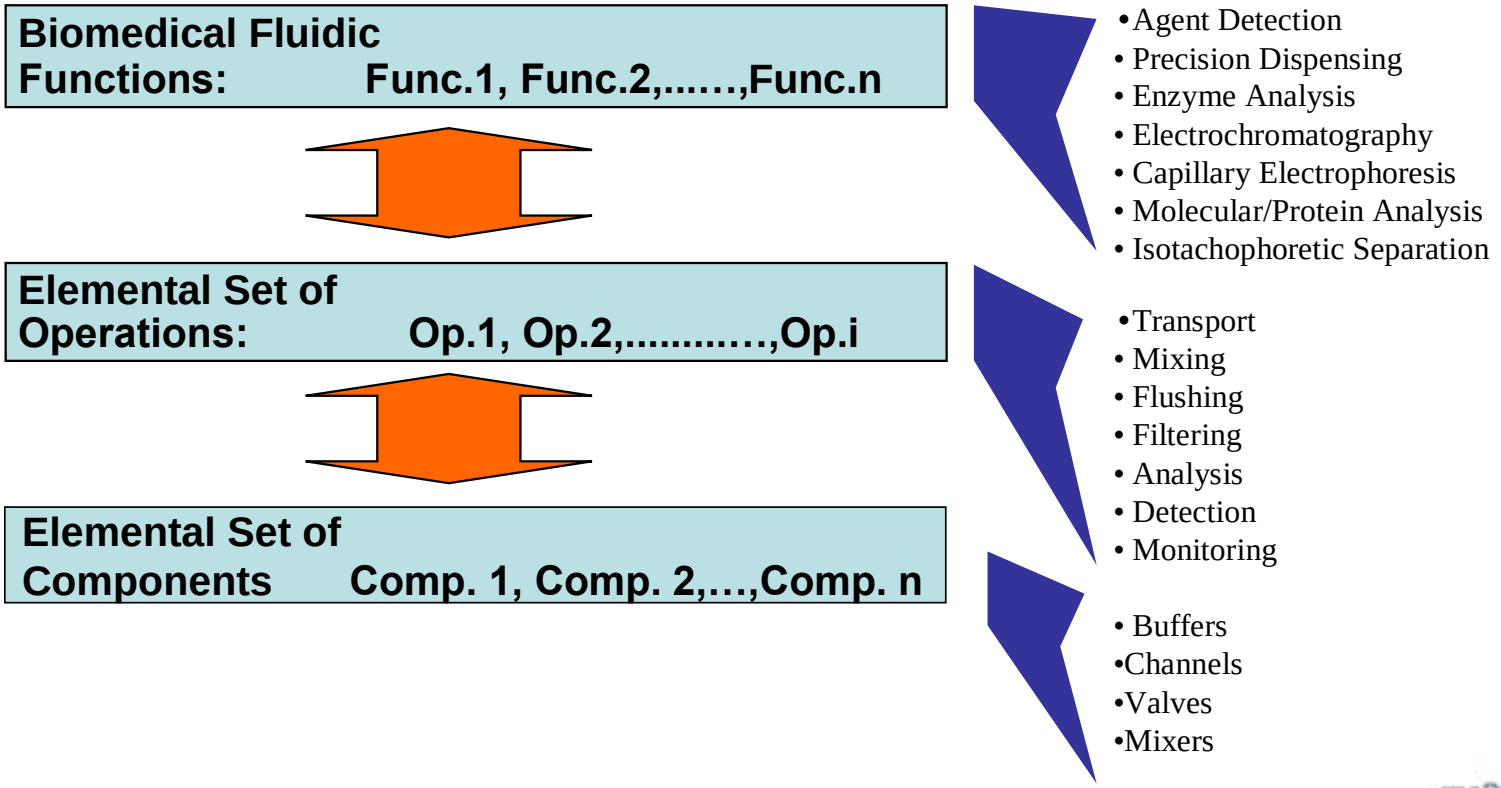
- ☑ Automation
- ☑ Integration
- ☑ Miniaturization



Background and Motivation

- 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 true lab-on-a-chip?
  - 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
  - Top-down design

Hierarchical Integrated Microfluidic Design



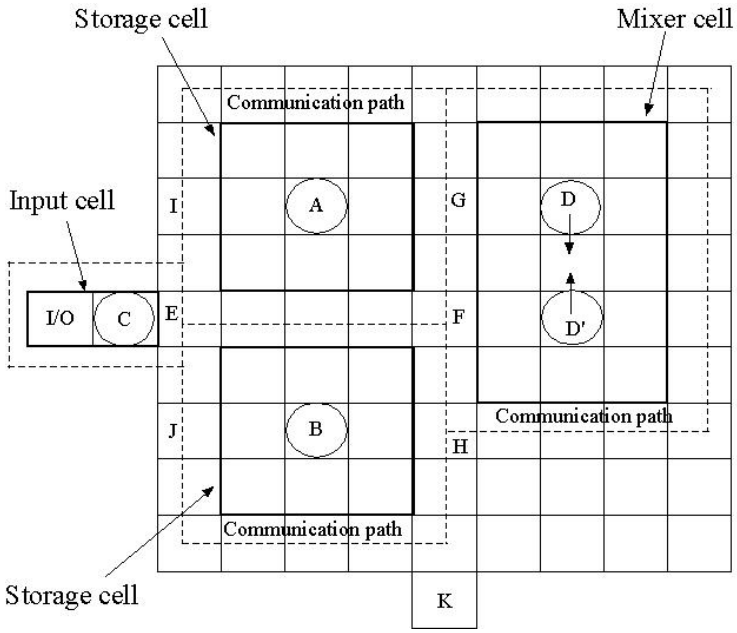
Digital Microfluidic Toolkit

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

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



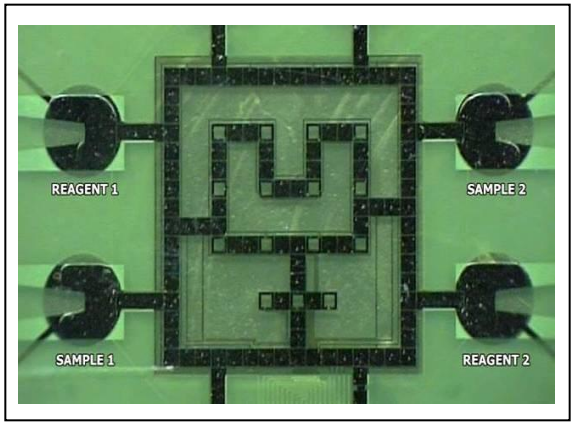
Approach

- Develop a digital microfluidic lab-on-a-chip for analytical applications
- Specific focus
  - Multiplexed assays
  - Compatibility with biologically relevant liquids
  - Nanoliter scale
  - Sample-in-result-out operation
- Test lab-on-a-chip for clinical application
- Apply concepts to on-chip DNA sequencing

Lab-on-a-chip Toolkit

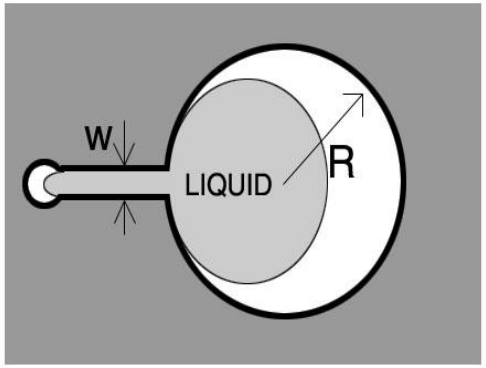
SAMPLE LOADING	DROPLET DISPENSING	DROPLET TRANSPORT	MIXING & REACTORS	DETECTION
Analog input	Analog-to-Digital	Digital	Digital-to-Analog	Analog

INTEGRATE

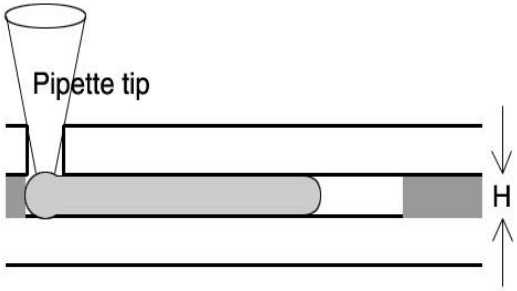


Digital microfluidic lab-on-a-chip

Sample Loading



TOP VIEW

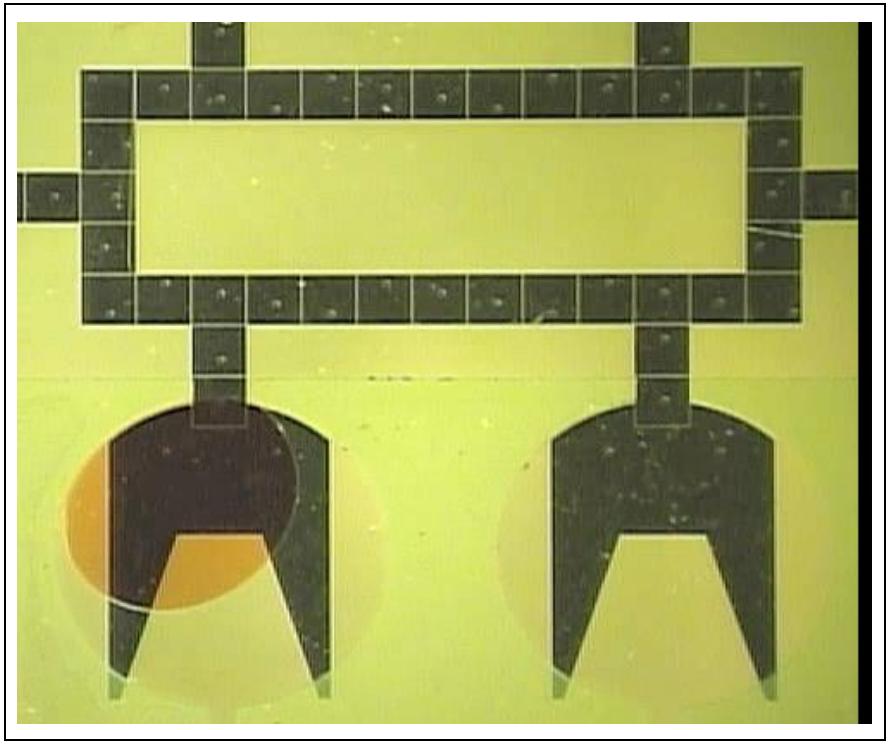


SIDE VIEW

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

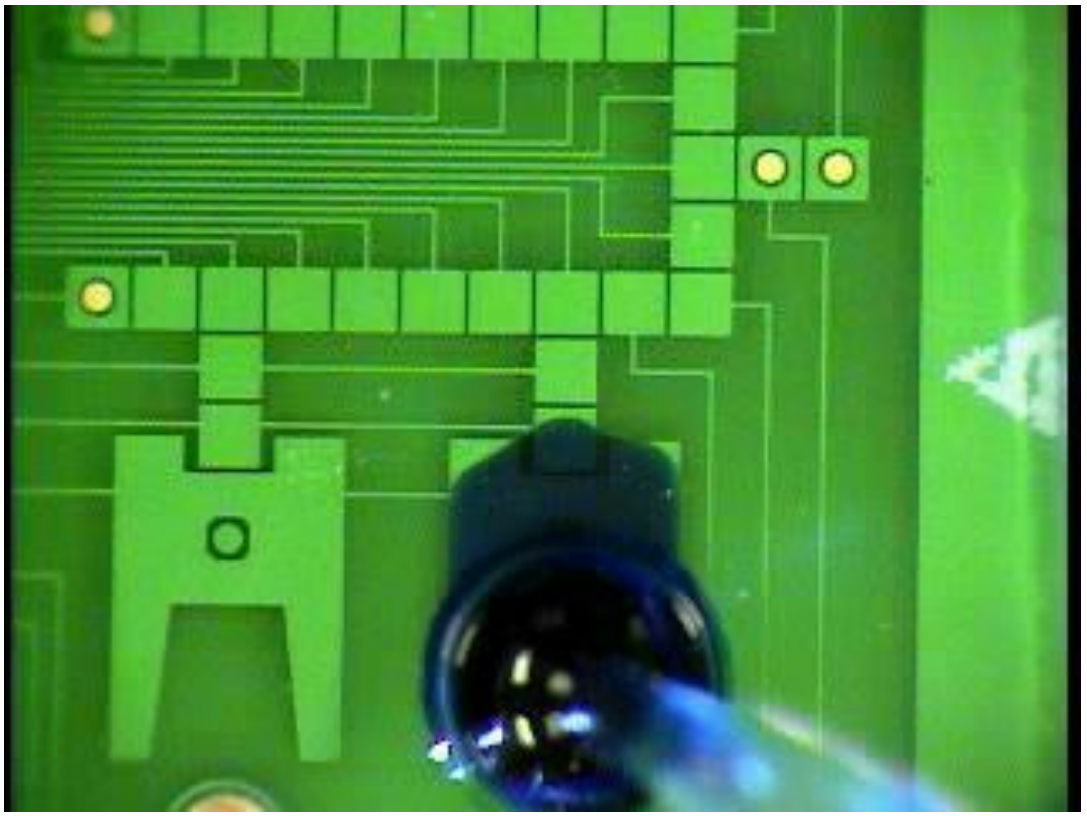
Droplet Dispensing

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



- High speed dispensing
- 8 droplets in 3.7 seconds
- Droplet volume ~ 20nL

High Speed Continuous Droplet Dispensing



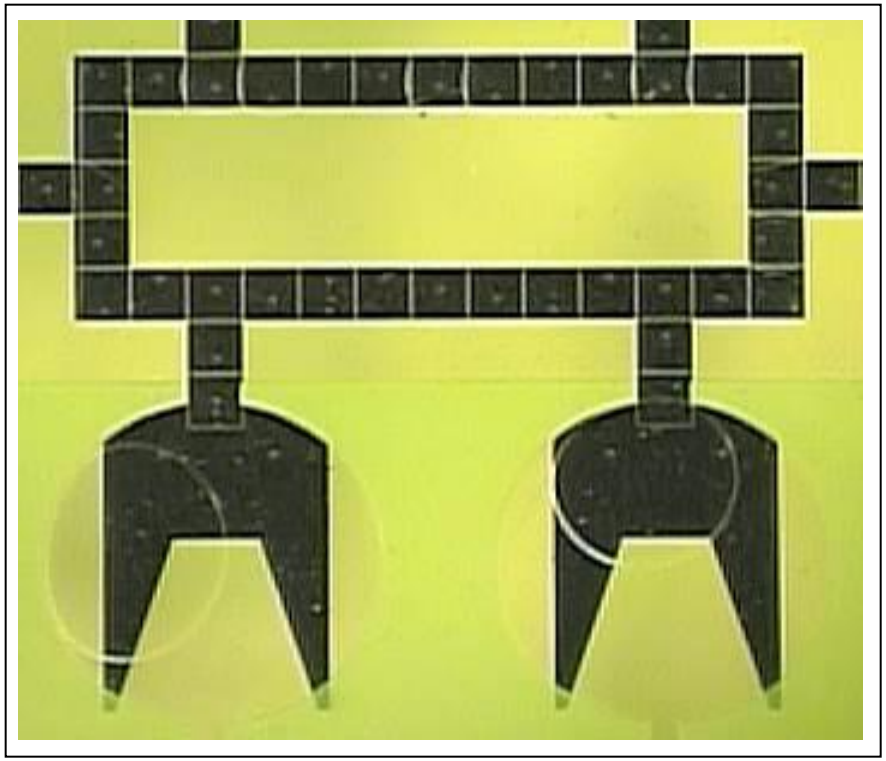
Droplet Dispensing

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

- Dispensing of proteins
  - Up to 1mg/mL BSA dispensable
  - Smaller than transportable concentrations
  - More adsorption due to larger surface area in the reservoir
- Dispensing of physiological fluids
  - Serum and plasma dispensable
  - Whole blood NOT dispensable
  - Use pressure-assisted dispensing

Droplet Transport

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



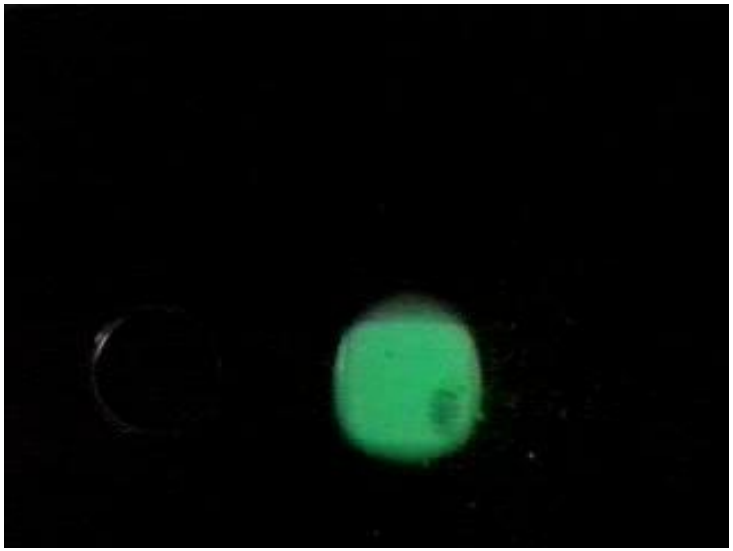
- High-speed transport
- 50Hz switching frequency
  - 2.5cm/sec speed
- 50V operation

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

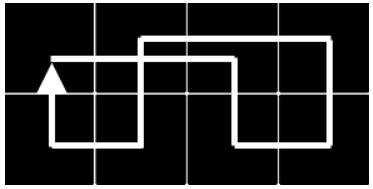
Rapid Droplet Mixing



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



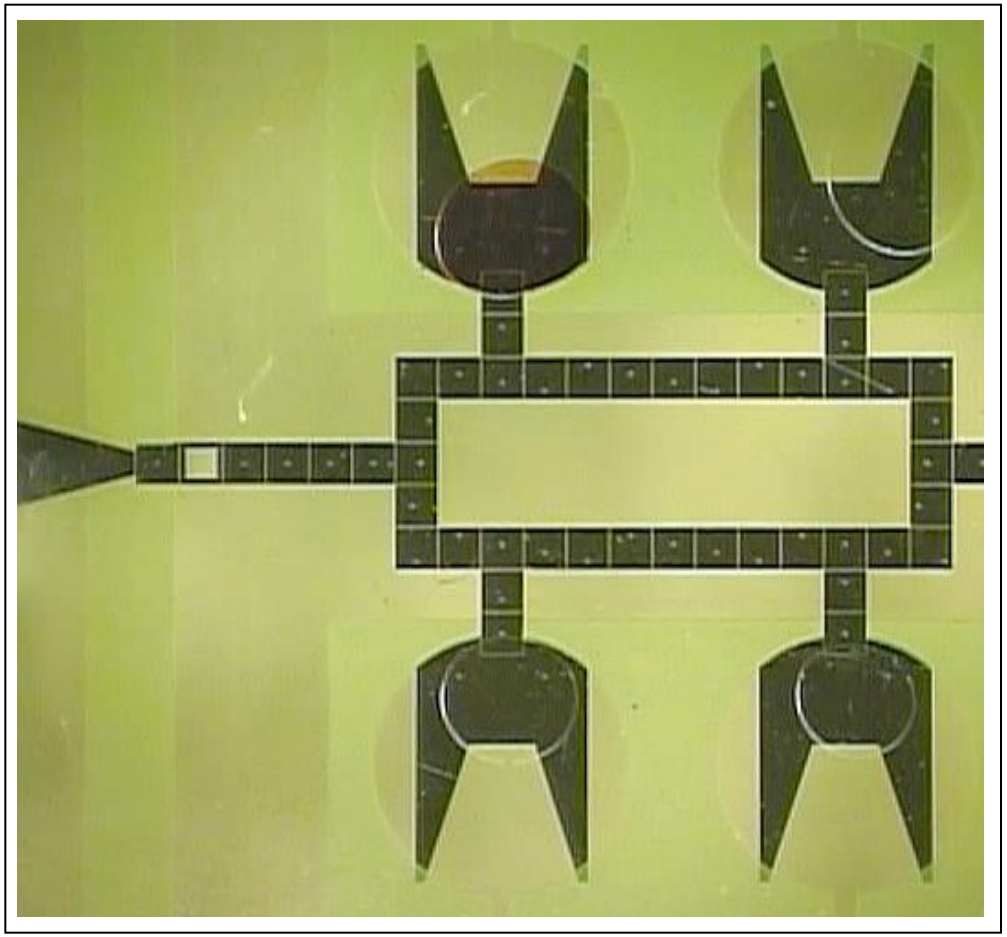
- Droplets completely mix in 2.8 seconds
- 30 times faster than the diffusion-only passive mixing case

Chemistry on Chip

SAMPLE LOADING	DROPLET DISPENSING	DROPLET TRANSPORT	MIXING & REACTORS	DETECTION

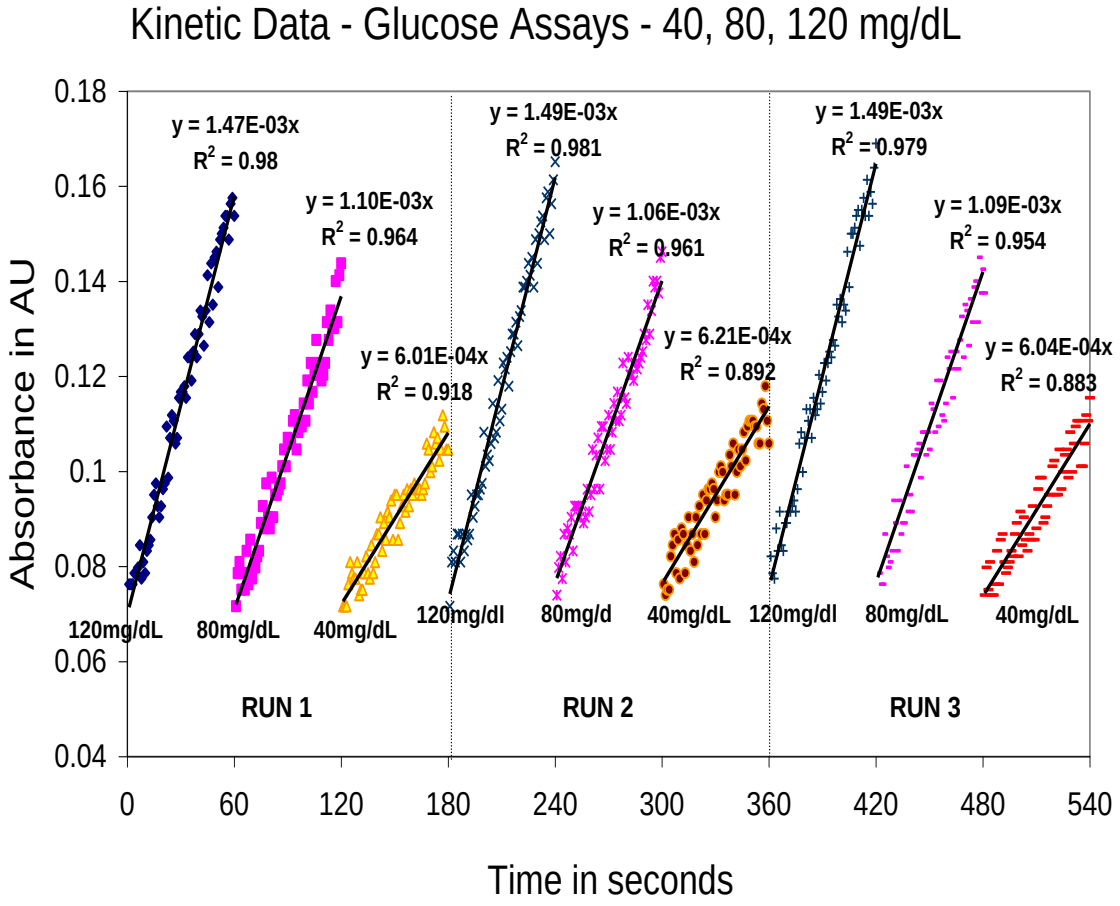
- Droplets are containerless chemical reactors
- Large and arbitrary dilutions are challenging and difficult to implement
  - Affects linearity of enzyme kinetic assays
  - Assays are sensitive to the sample composition
  - Possibility of interferences is much higher
  - Reactions requiring oxygen are affected
- Dilution factor of 2 (1 sample droplet + 1 reagent droplet) is most easily implemented

Integrated Operation - Serial



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

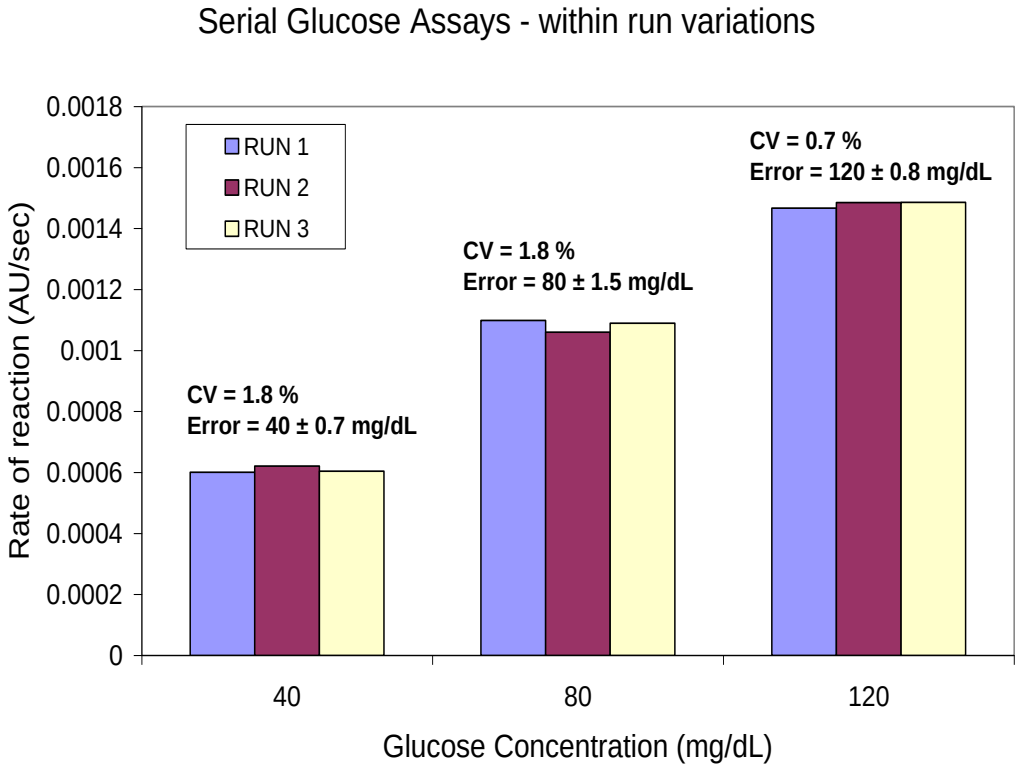
Multiple Glucose Assays



- 9 consecutive assays
- 3 glucose concentrations
- 60 seconds absorbance measurement at same spot

Data more noisy for 40 and 80mg/dL

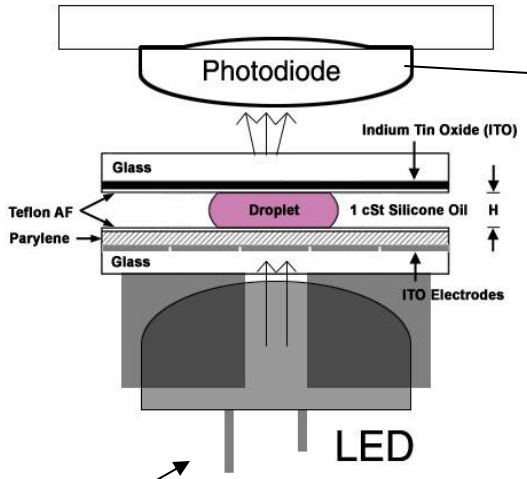
Multiple Glucose Assays



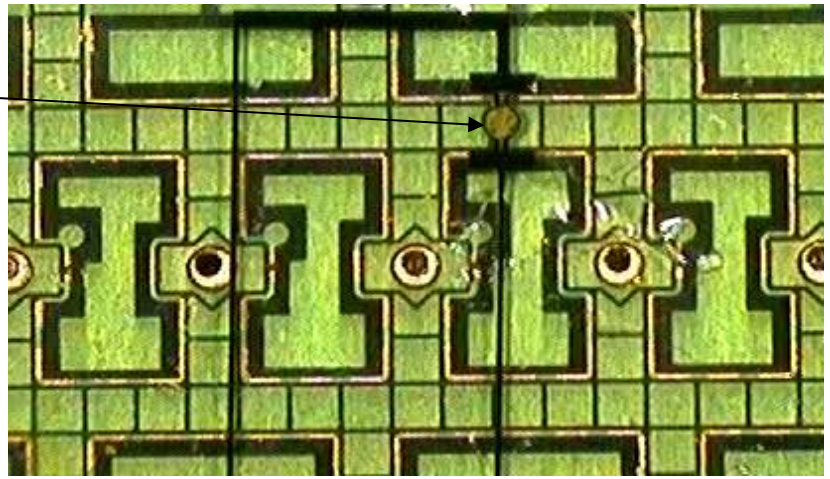
- *Potential* error sources: volume variation, cross contamination, measurement errors
- Low CV indicates good volume reproducibility
- No trend in the error indicates no cross contamination

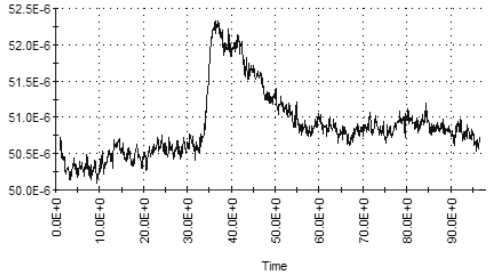
Detection Methodology

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



Opaque solid

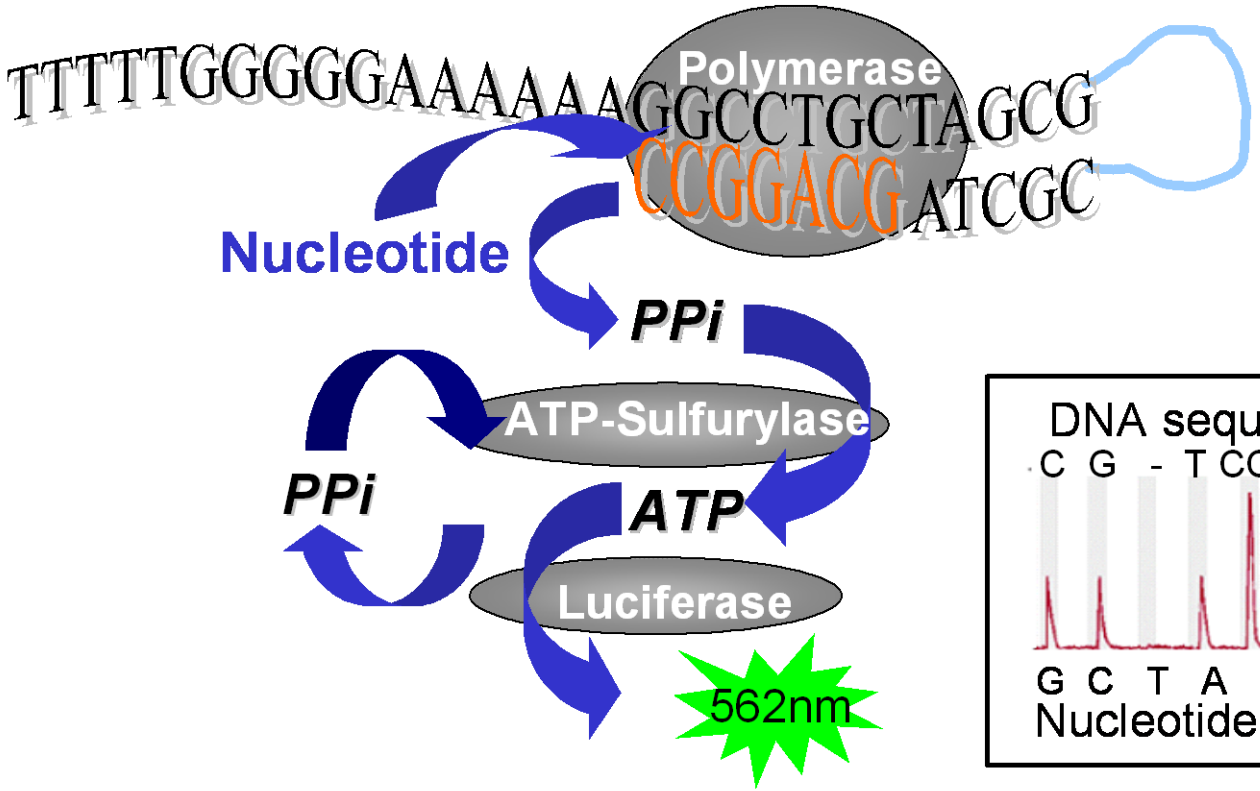




Chemoluminescence underneath the TeflonAF coated photodetector

On-Chip Sequencing

- The promise of digital microfluidics to analyze large genome populations…
  - Miniaturized sequencing by synthesis using droplets
  - High throughput, massively parallel on chip reactions
  - Avoidance of by-product build-up that limits read lengths
  - Programmable operation
- Eliminating on-chip bottlenecks
  - Decouple the synthesis and detection steps
  - Introduce optimized concentrations of clean reagents for optimized times
  - Chemically amplified signal detection proceeds at leisure at a remote array site
- Potential to sequence an array of genomic segments each 10,000 to 100,000 bases long



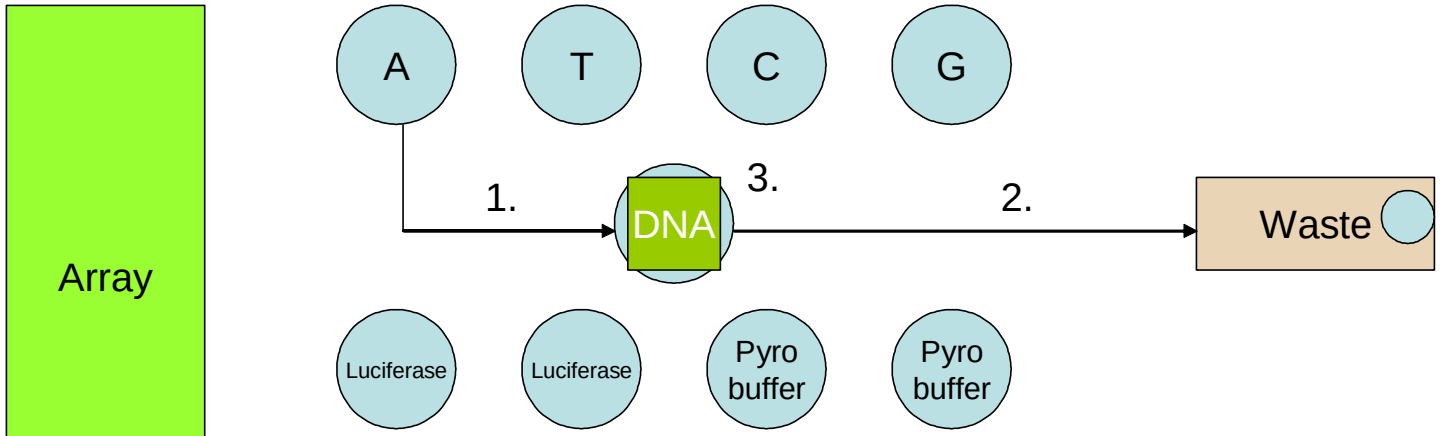
$$DNA_N + dNTP \xrightarrow{\text{Polymerase}} DNA_{N+1} + PPi$$

Coupled Reactions

$$PPi + APS \xrightarrow{\text{Sulfulylase}} ATP$$

$$ATP + O_2 + \text{Luciferin} \xrightarrow{\text{Luciferase}} AMP + PPi + CO_2 + \text{Oxyluciferase} + \text{Light}$$

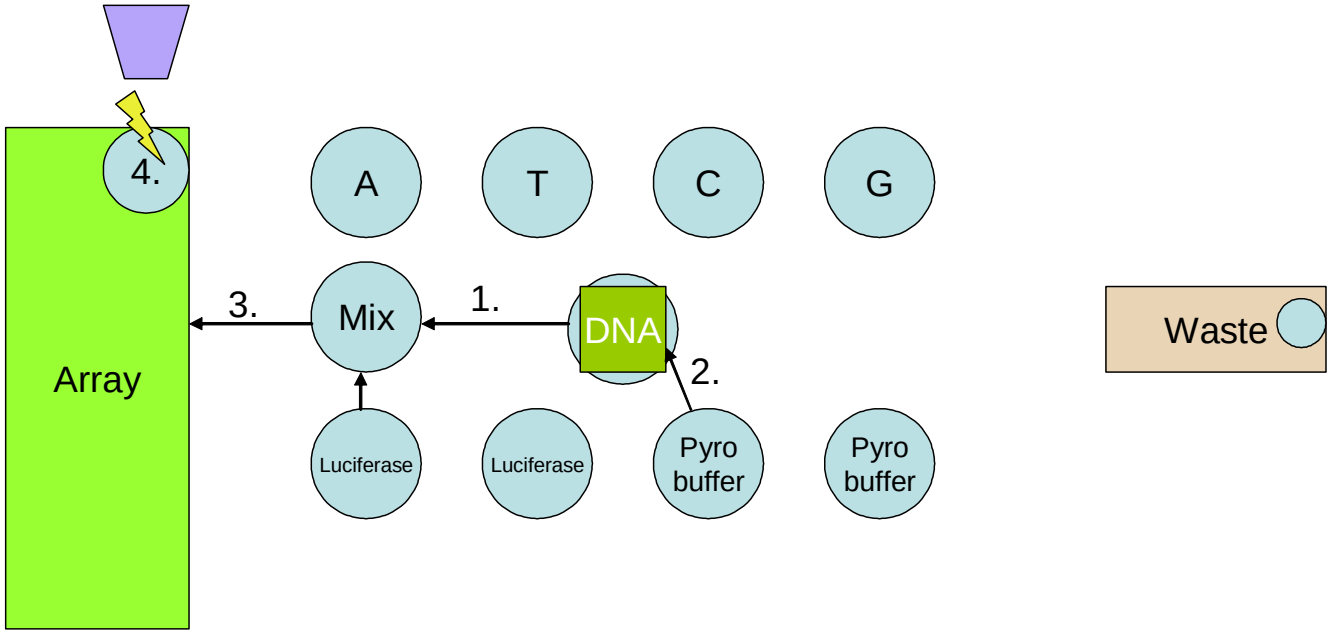
Droplet-Based Pyrosequencing



Steps:

1. Move “A” nucleotide drop to DNA site and replace pyrobuffer drop
2. Move pyrobuffer drop to waste
3. Incubate incorporation reaction

On-Chip Pyrosequencing

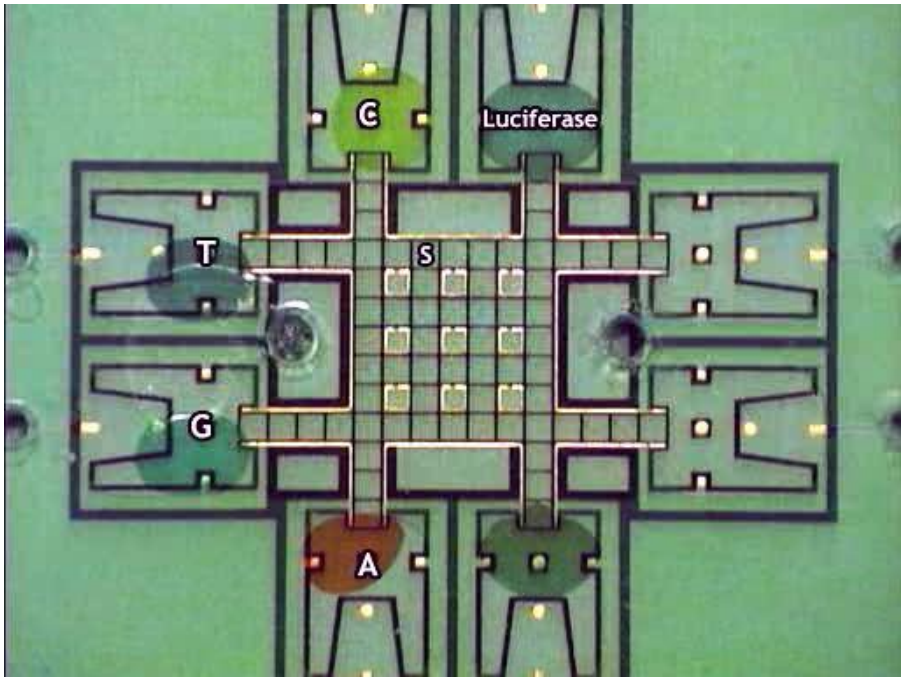


Steps:

1. Move incubated droplet and mix with luciferase
2. Move pyrobuffer drop and wash DNA (may be repeated)
3. Move combined droplet to array
4. Detect pyrophosphate generated light

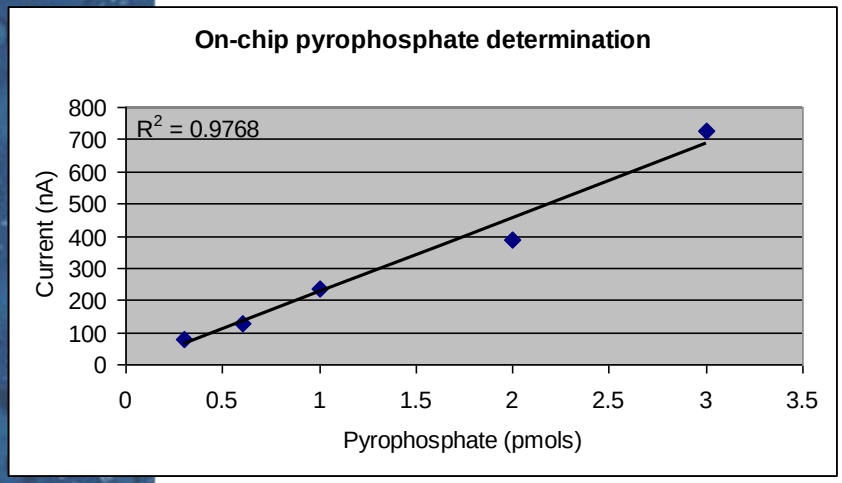
Example Fluidic Protocol for DNA Sequencing

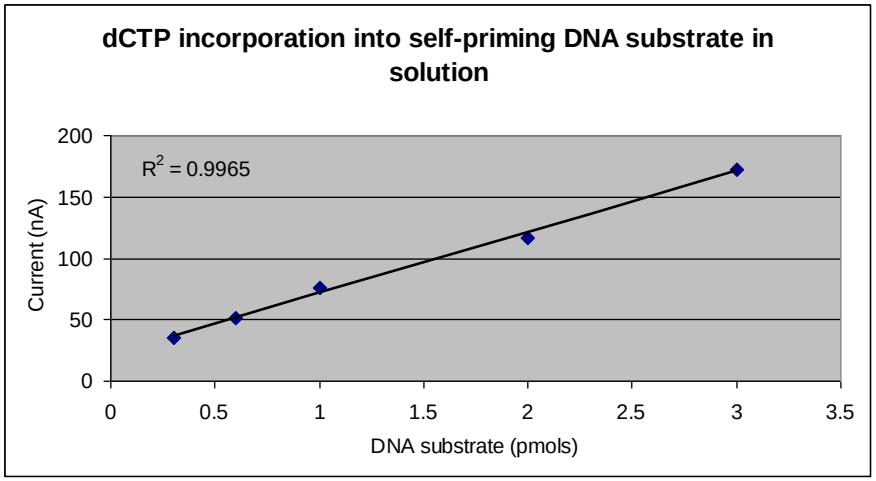
- Dispense droplets of each dNTP
- Transport droplets to synthesis reaction site and allow to react
- Transport droplets to storage area
- Mix each dNTP droplet with light producing droplet
- Transport combined droplets to detector site

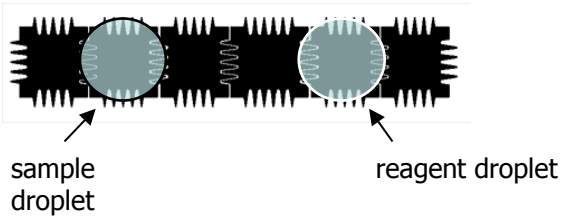


Dyed liquids represent pyrosequencing reagents, droplet volumes are 50 – 100 nL.

On-chip Assay Development







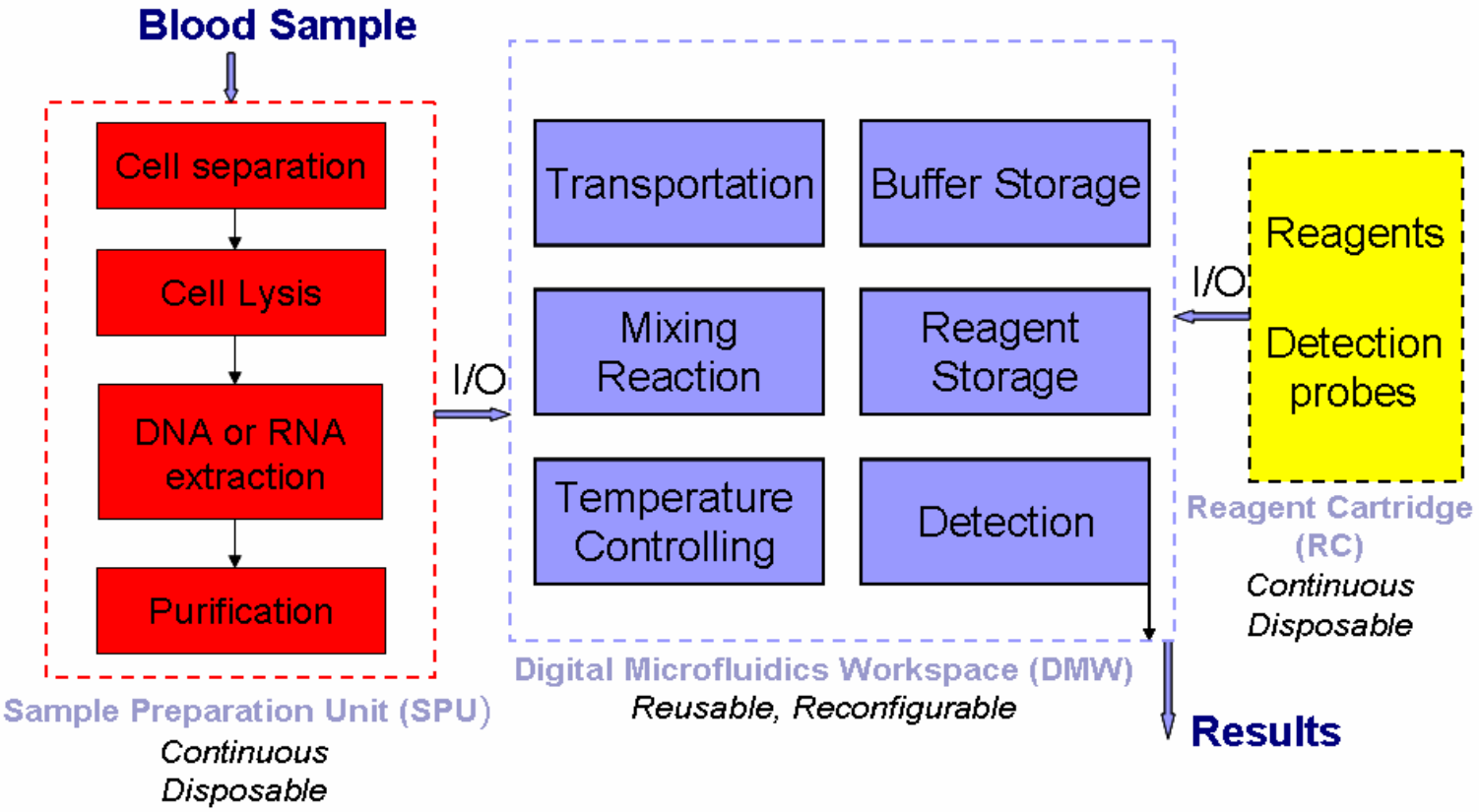
- Protocol: 1µL sample droplet mixed with 1 µL pyrosequencing reagent droplet on-chip
- Pyrophosphate concentration is linearly correlated to luminescent peak height 0.3-3 pmol
- Self-primed DNA substrate + first complementary base (dCTP) is also linearly correlated
- Approximately 1 pmol DNA can be reliably detected with the current system

Lab-on-a-Chip Systems

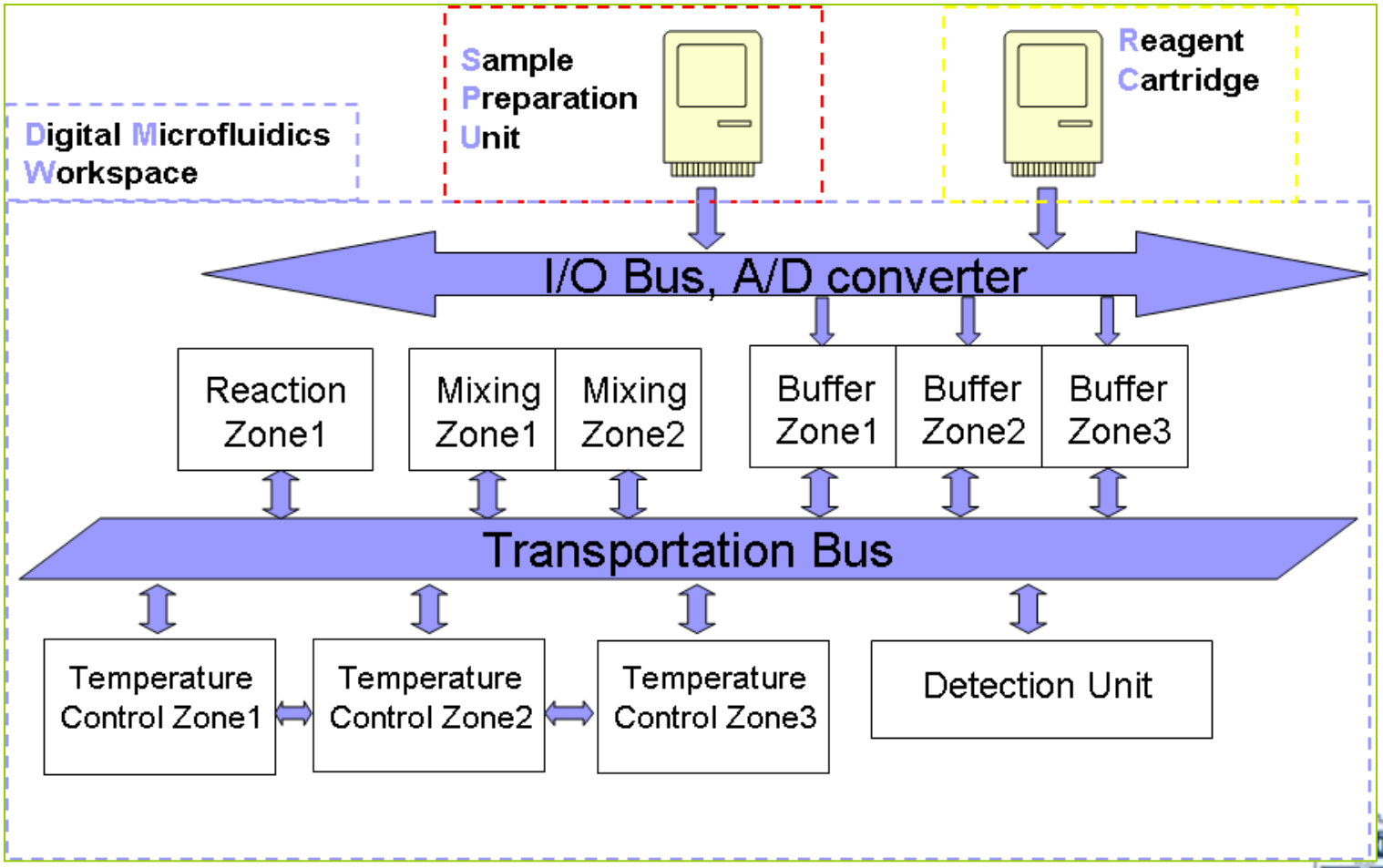
- Digital microfluidic toolkit demonstrated
- Can digital microfluidics deliver a true lab-on-a-chip technology that is adaptable to numerous applications?
- Examples from ECE299 (Duke Univ. Fall 2006)
  - **Analog/Digital Hybrid Microfluidic Chip For DNA & RNA Analysis**
  - **Detection of DNA Using Fiber Optic Spectroscopy**

Analog/digital hybrid biochip

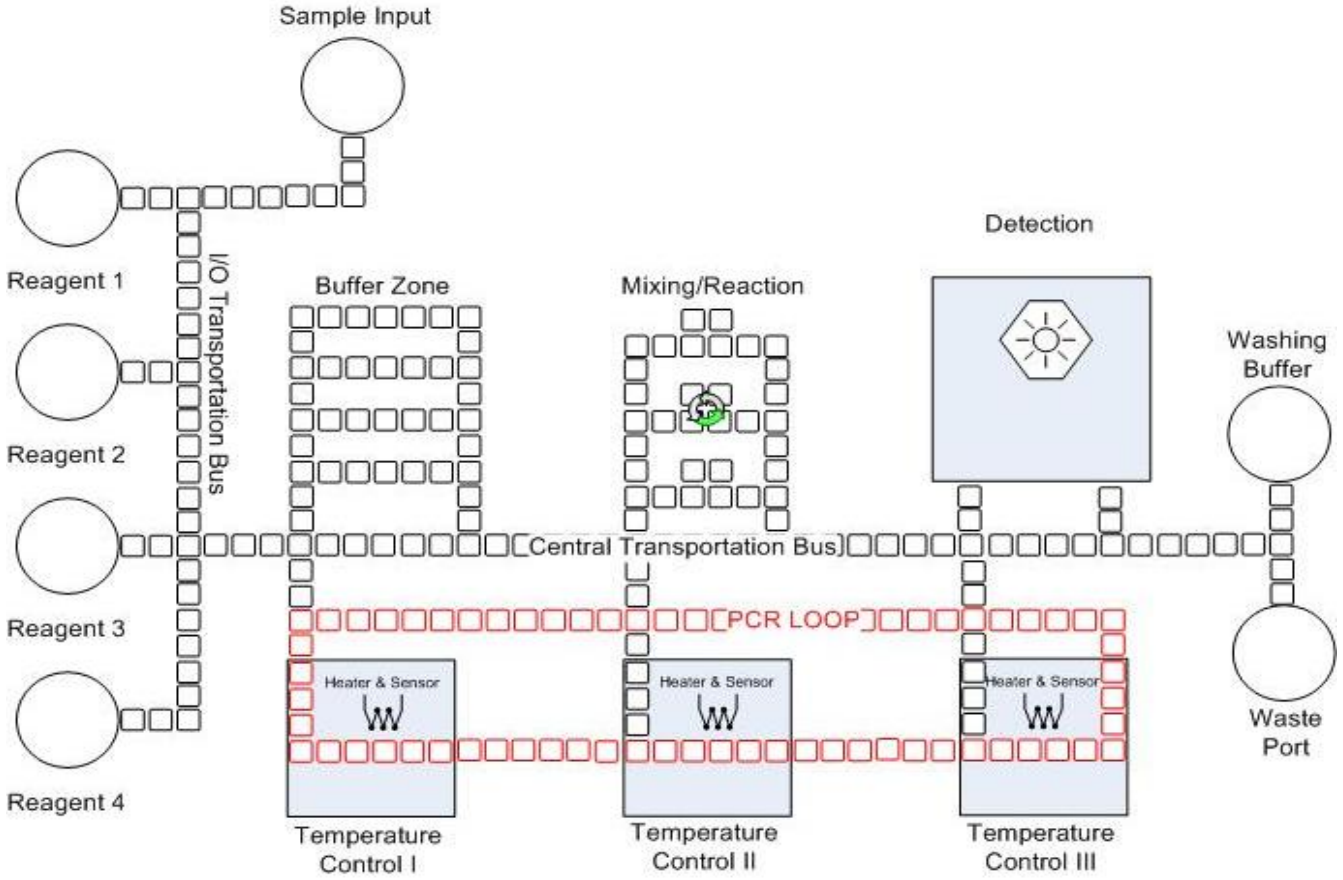
(A. Garcia, G. Pan, J. Zhang)



Fluidic Platform



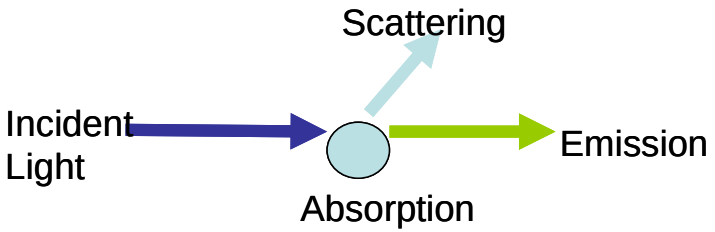
Floor Plan of the DMW

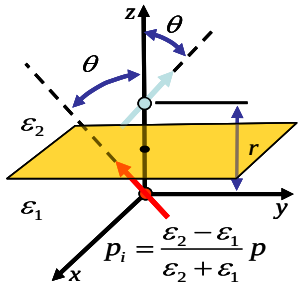


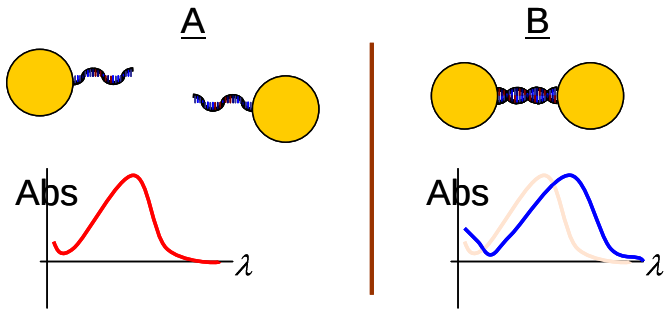
Spectroscopic DNA Detection

(A. Vijay, N. Mathew, R. Erb)

- System that can detect the presence of specific DNA strands inside a blood sample in an entirely self-contained and hand-held system.

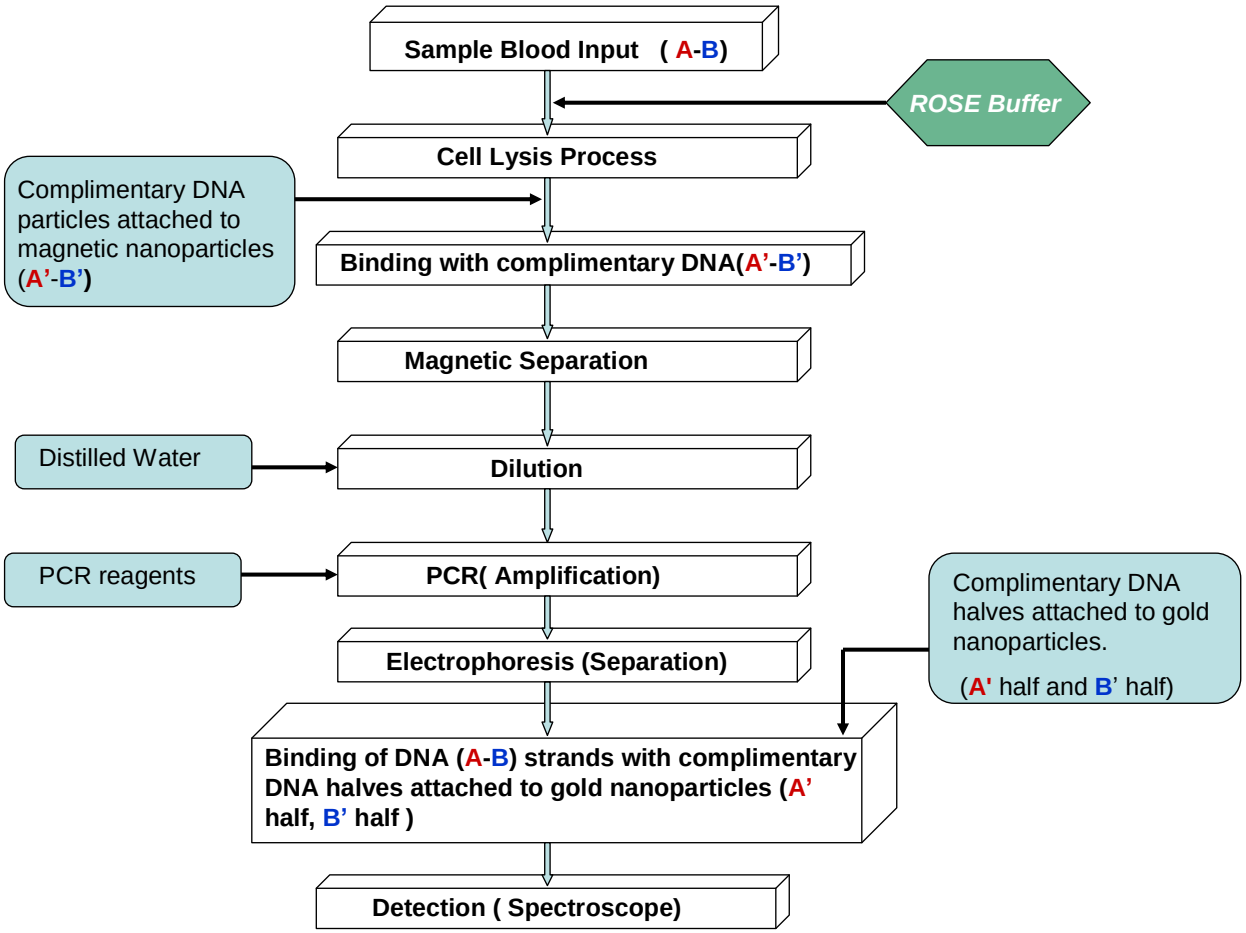






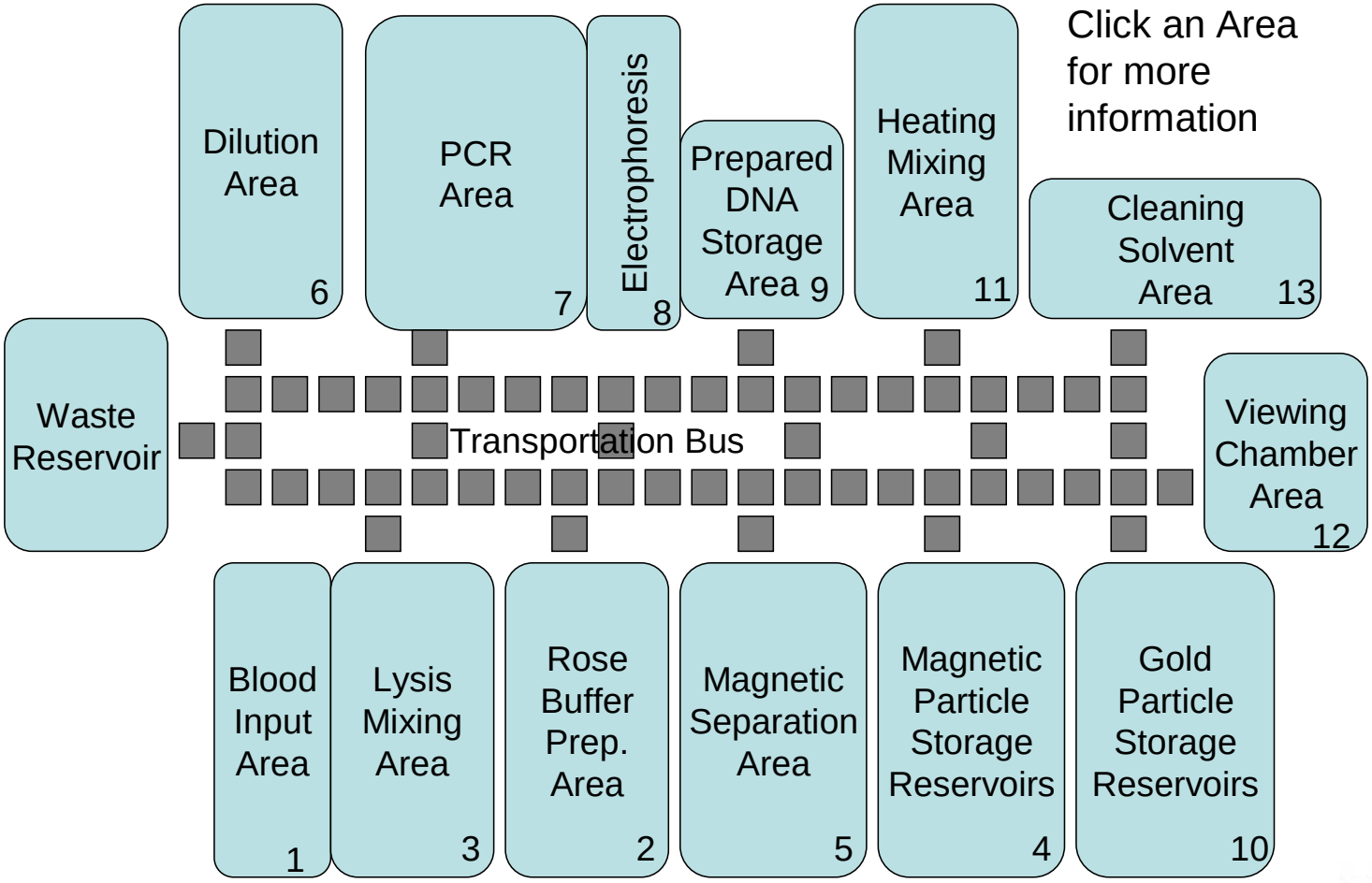
Aggregation is detectable!

Fluid Level Flow Chart



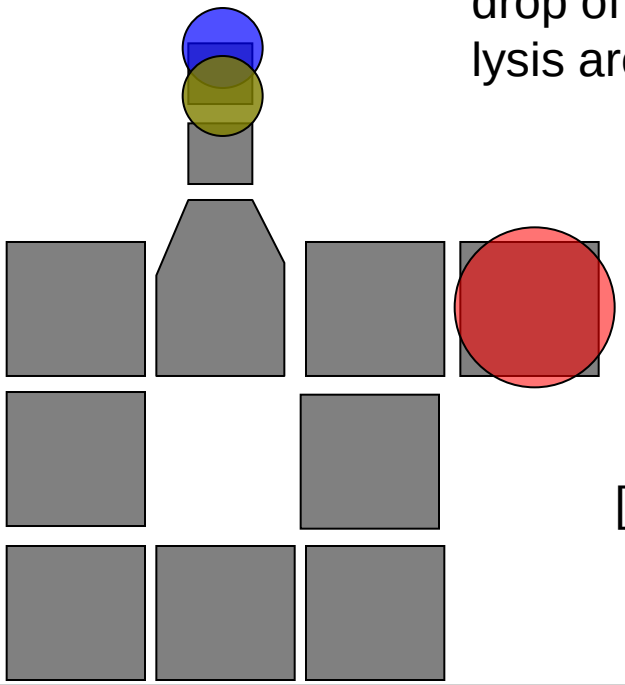
Overview | Flow Chart | Block Diag. | Timing | Disposability | Power Req. | Chip Area

Block Diagram for Fluid Level



Cell Lysing Area

In the Cell Lysis Area, Blood will be inputted in 25 nL sized droplets from the blood storage reservoir. This will be mixed with a 5 nL drop of rose buffer solution to lyse the cells. The end solution will be mixed with a 5 nL drop of magnetic beads. Then a 5 nL drop of solution will be pulled from the lysis area.



From Blood Input

Red – blood
Blue - rose
Yellow – mag.

[click for example]

Remarks on Applications

- Support concept that extensive biomedical application base can leveraged 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

- Basic functionality of a true lab-on-a-chip demonstrated
  - Sample in/result out integration and automation achieved
- Electrowetting toolkit demonstrated
  - Automated droplet operations
  - Catalog of compatible reagents
  - Demonstrations of a few important biological assays
- Open issues:
  - On-chip sample preparation
  - System integration and interfacing to other laboratory formats and devices
    - Capillary electrophoresis
  - On-chip dilution still a problem
  - Scalable, compatible detector technology needed

Acknowledgements

- NSF
- NIH
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- ECE299 students