Digital Microfluidic Chips for Chemical and Biological Applications

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Outline of Presentation

- Background and motivation
  - Digital microfluidics
    - Chip architecture issues
    - Toolkit
- Applications of digital microfluidics
  - Sample preparation
  - DNA sequencing
  - Enzymatic assays
  - Cytotoxicity screening
- Summary and conclusions

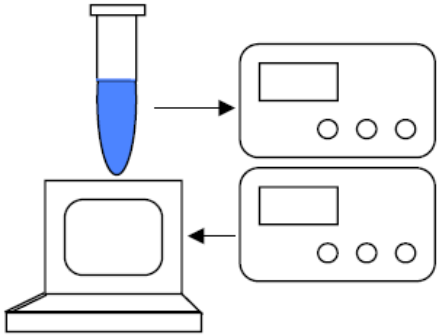
Microfluidic Functions

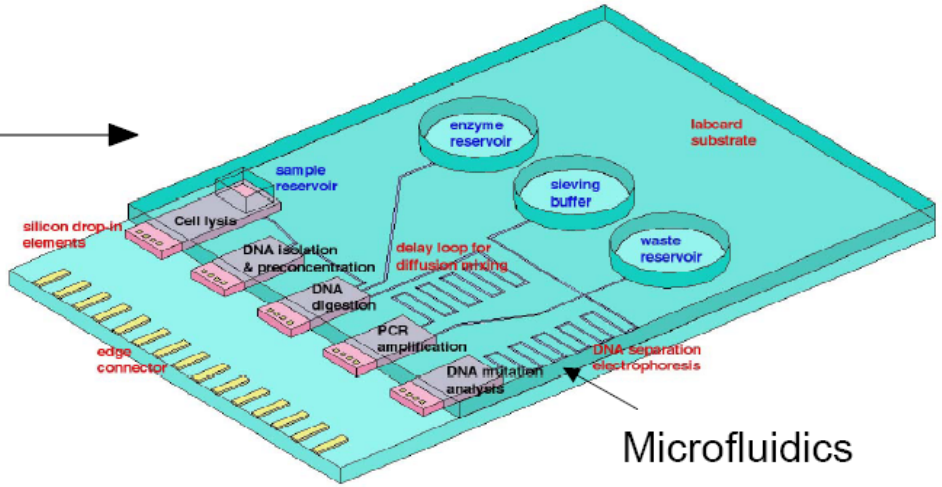
Benchtop Laboratory Techniques

Many manual steps

"Lab on a Chip"

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





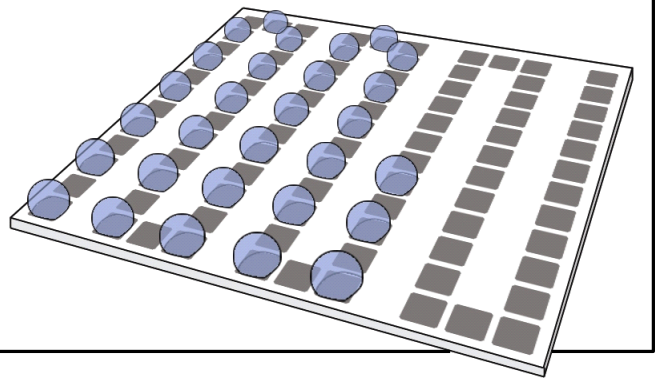
Background and Motivation

- 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 diagnostic 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

Digital Microfluidics

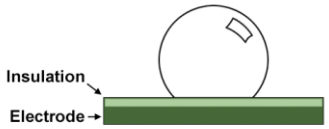
Features

- 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

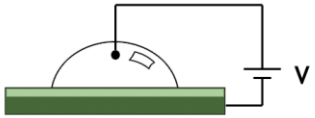


How It Works

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

Digital Microfluidic Toolkit

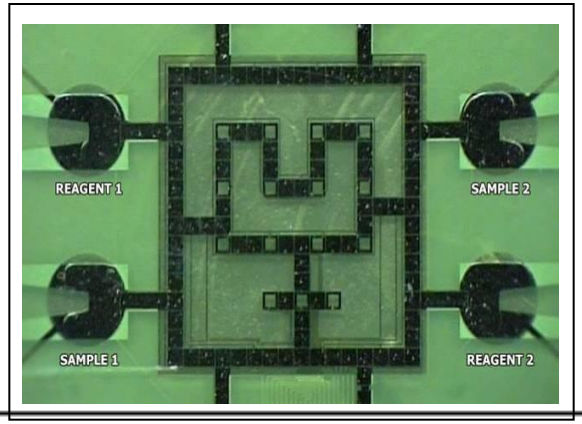
Implementing numerous applications on a elemental set of components:

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

Diagnostic Lab-on-a-Chip

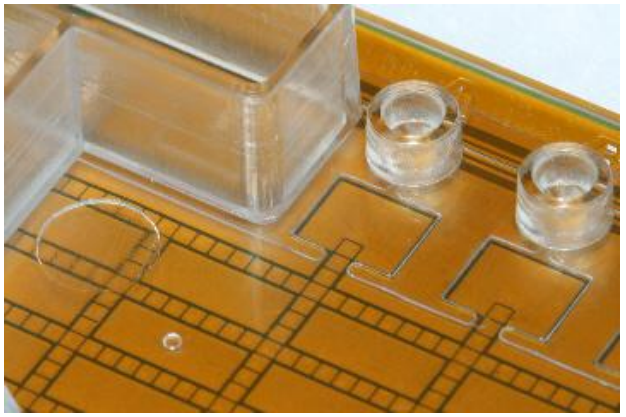
SAMPLE LOADING	DROPLET DISPENSING	DROPLET TRANSPORT	MIXING & REACTORS	DETECTION
Analog input	Analog-to-Digital	Digital	Digital-to-Analog	Analog

INTEGRATE



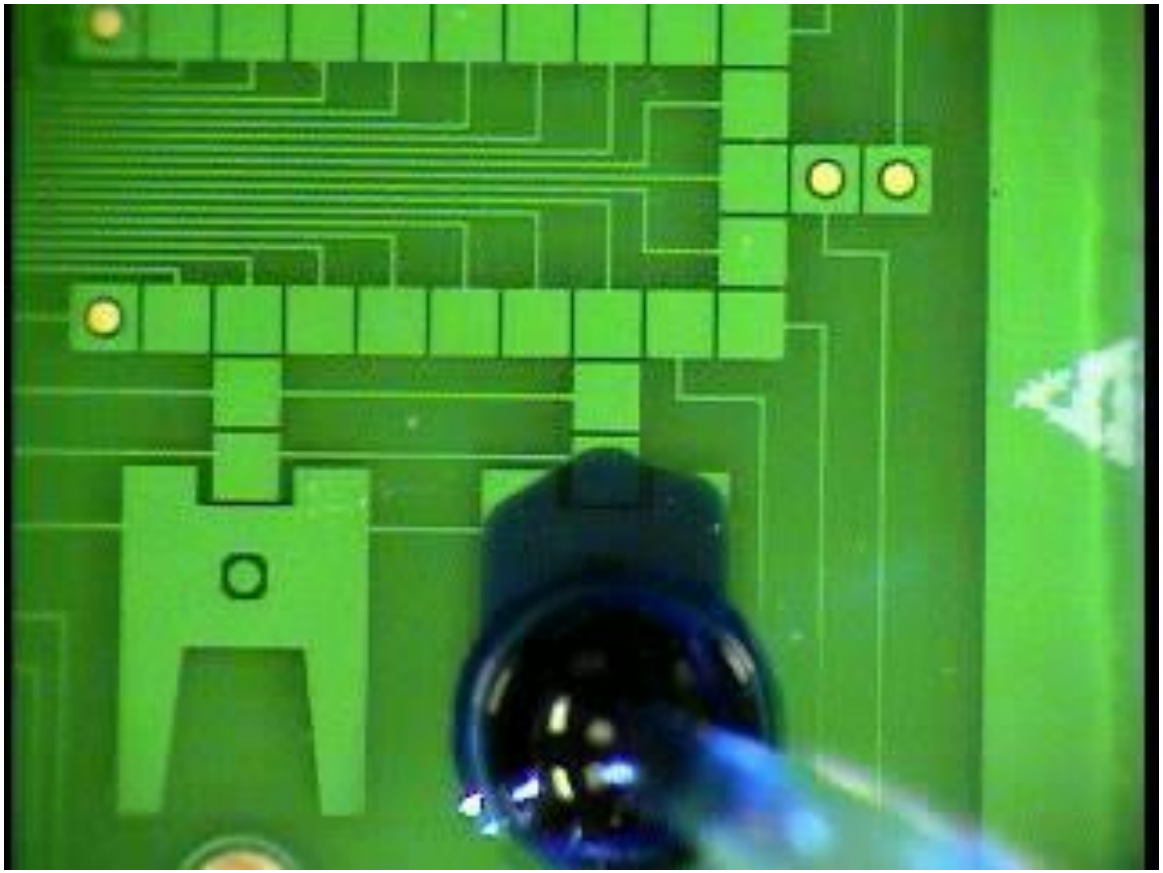
Digital microfluidic lab-on-a-chip

World to Chip Interface

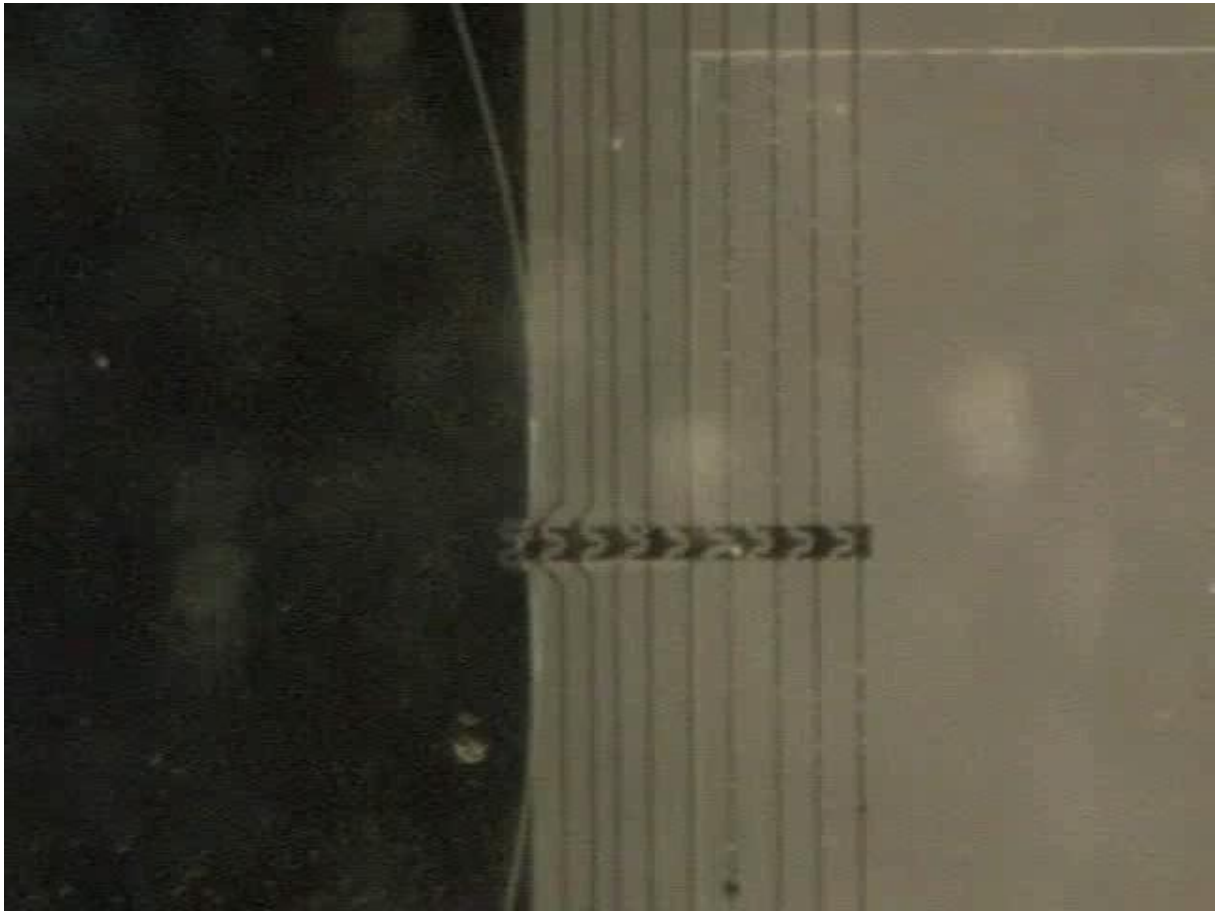


- well plate interface
  - 384 well spacing
  - reservoir inputs from microliters to milliliters

High Speed Continuous Droplet Dispensing

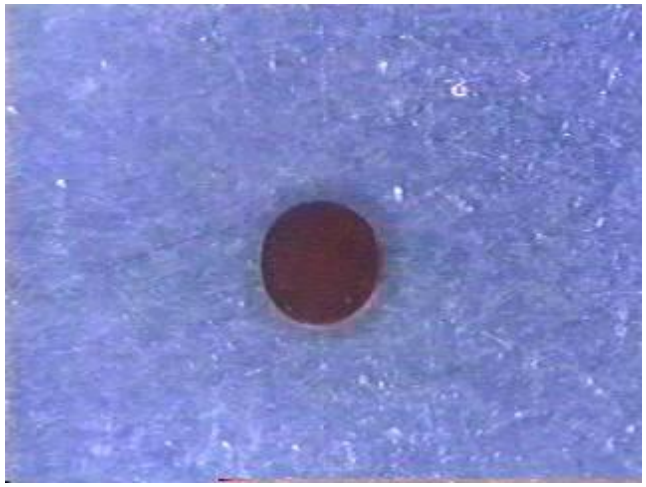


35 Picoliter Droplet Dispensing

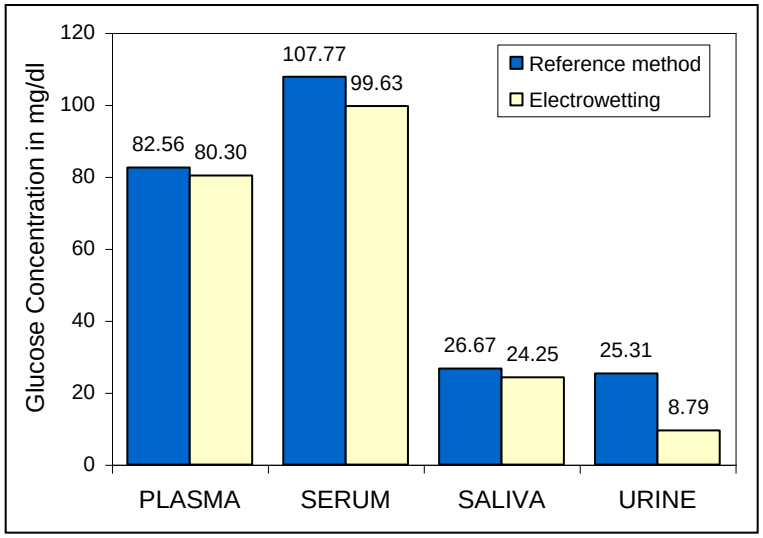


On-Chip Processing

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

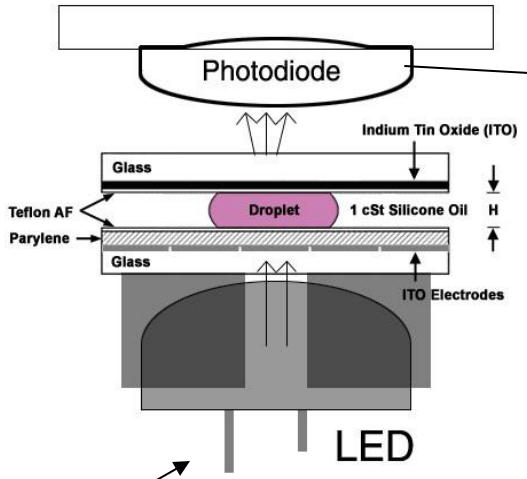
Droplet Mixing

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

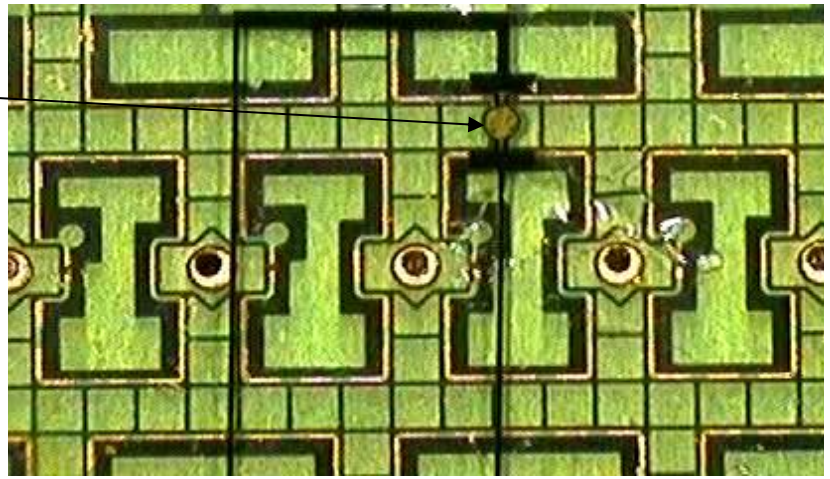
- Mixing in ~5 seconds by shuttling on linear array for 1µL (1.5mm scale) droplets
- Shuttling reverses flow causing un-mixing
  - unidirectional motion is preferred
- Scaling down volume decreases mixing time
  - mixing of two 25nL droplets was complete in 0.8 seconds at 10Hz switching @ 50V

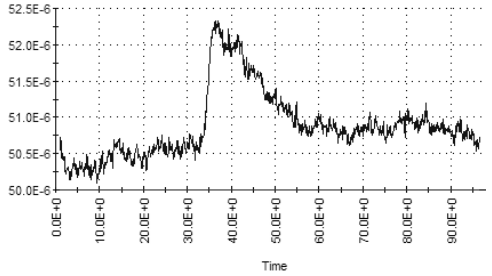
Detection Methodology

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



Opaque solid





Chemoluminescence underneath the TeflonAF coated photodetector

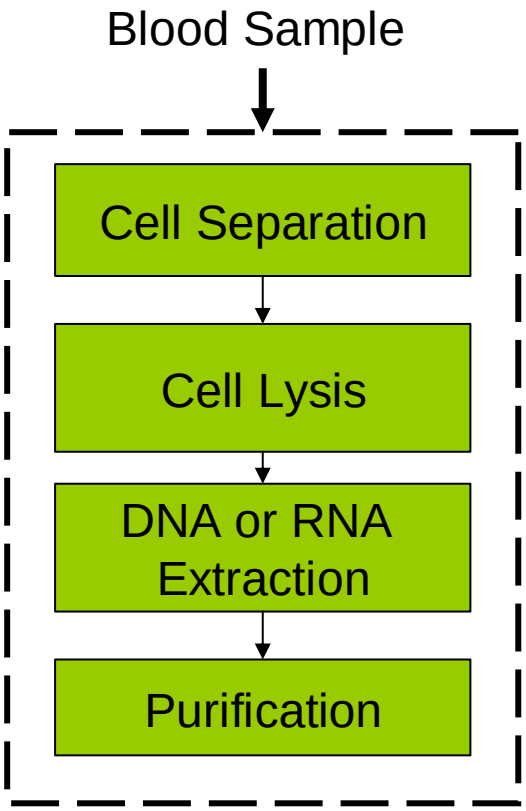
Reconfigurable Lab-on-a-Chip Status

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

Microfluidic Operations

- How are key operations performed on chip?
  - Sample preparation (fluidic function)
    - Cell separation
    - Cell lysis
    - DNA/RNA extraction
    - Purification

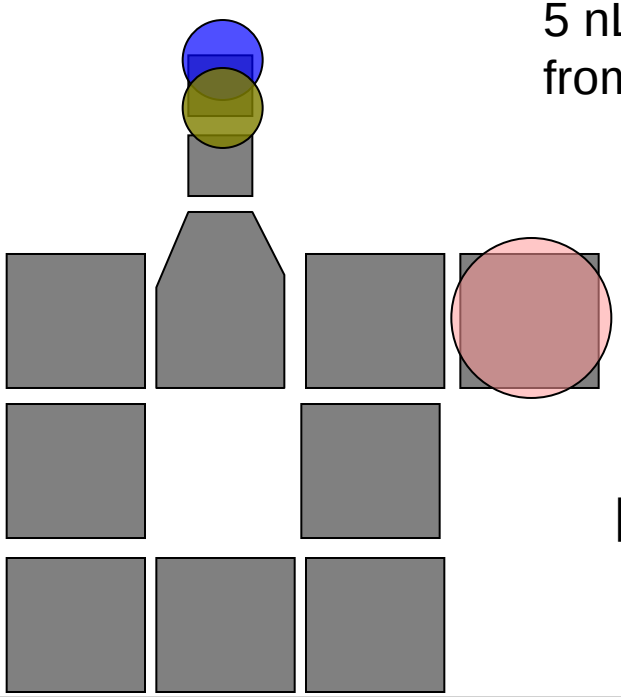
    Sample loading
    Transport
    Mixing
    Dilution/washing
    Filtering
    Separation
  - Mixing and transport
  - Sample/reagent storage
  - PCR (fluidic function)
    - Mixing
    - Temperature cycling
    - Transport
    - Detection



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 which lyses 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.

Red – blood
Blue – Rose buffer
Yellow – mag. beads

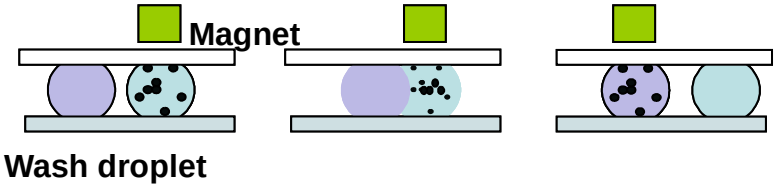


[click for example]

Magnetic Separation

Washing Solvent

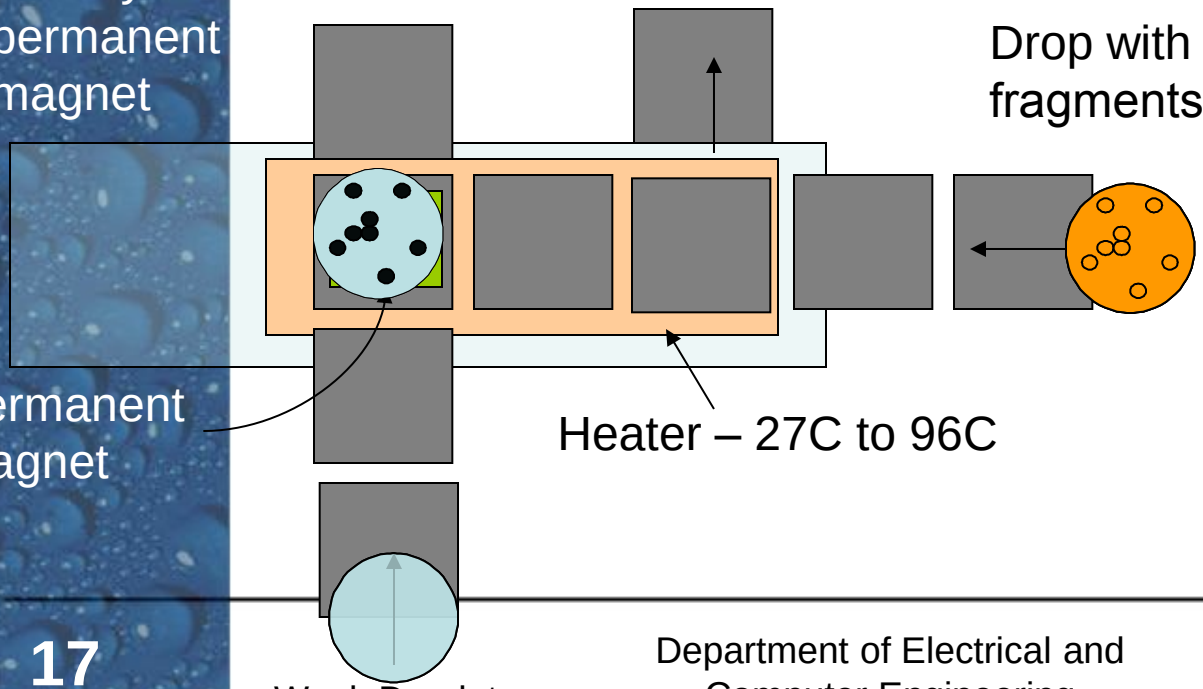
DNA Extraction



Wash droplet

Final droplet enters, pauses, temperature increases, releasing A'B' DNA, and droplet splits and moves on…

Magnetic Beads held by permanent magnet



Drop with magnetic beads, cell fragments, dna, etc… enters

Input: Droplet containing dna strands, cell fragments, magnetic particles attached to A'B' dna

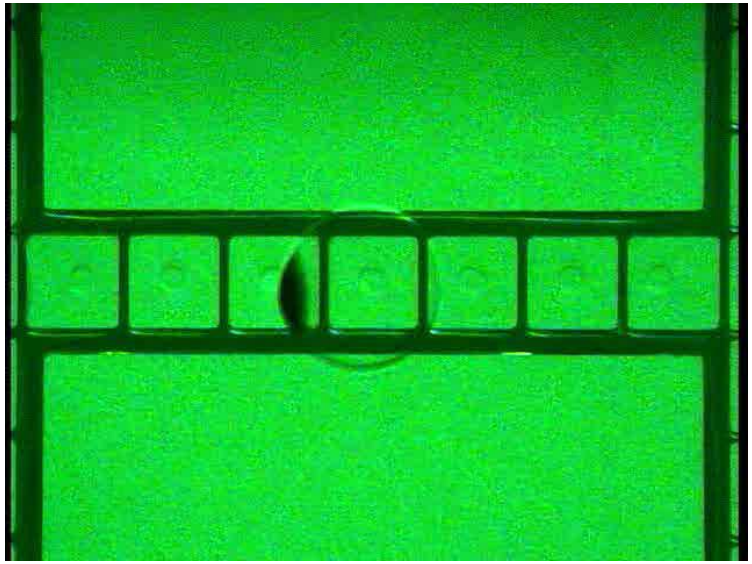
Output: Droplet containing A'B' dna

Permanent Magnet

Heater – 27C to 96C

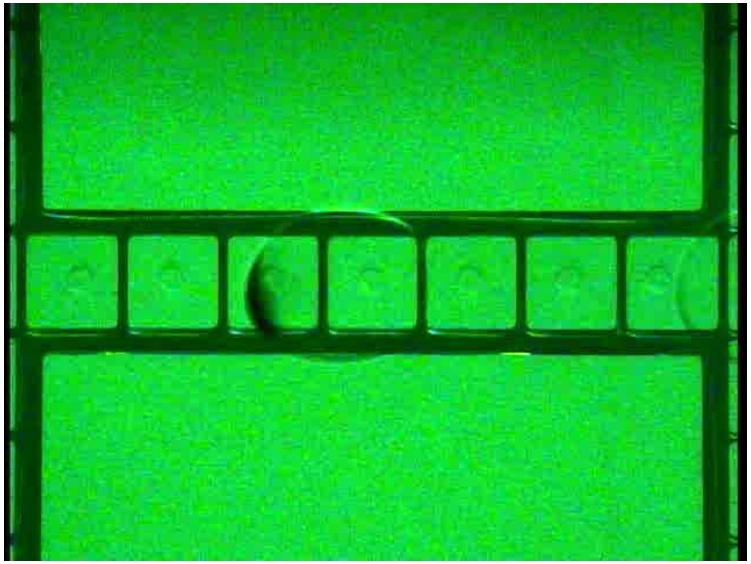
Wash Droplets

Magnetic Bead Washing



One complete wash cycle

1X speed video of first wash cycle



800 wash cycles (condensed to 26 s)

Still images taken after completion of each wash cycle are played at 30 fps

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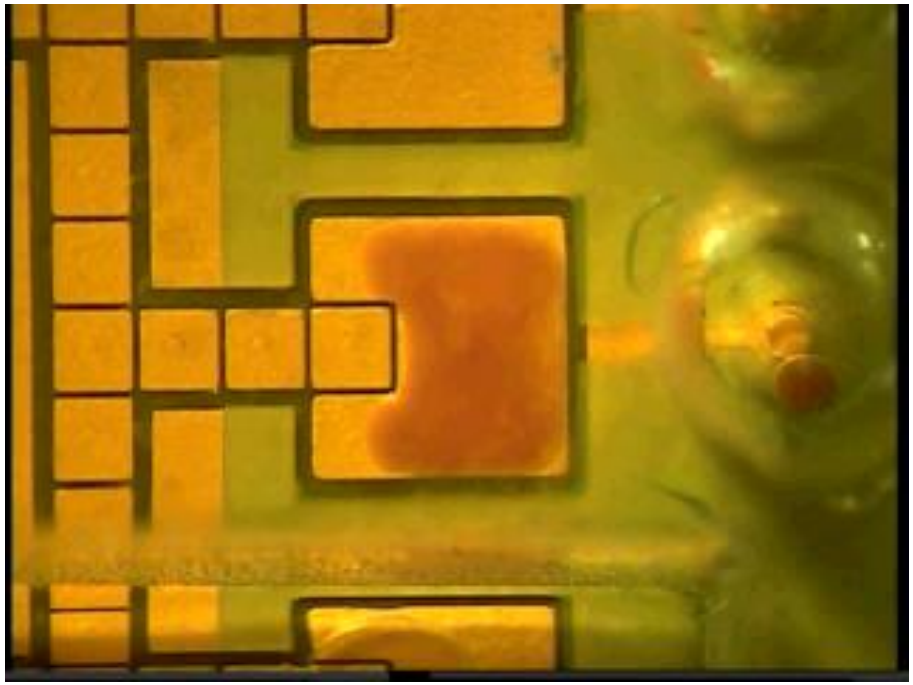
Advanced Liquid Logic
powered by Digital Microfluidics

On-Chip Pyrosequencing Protocol

Steps involved

- Dispense beads (sample)
- Wash beads
- Add dNTP mix to beads
- Add enzyme mix to beads
- Mix & Detect



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Advanced Liquid Logic
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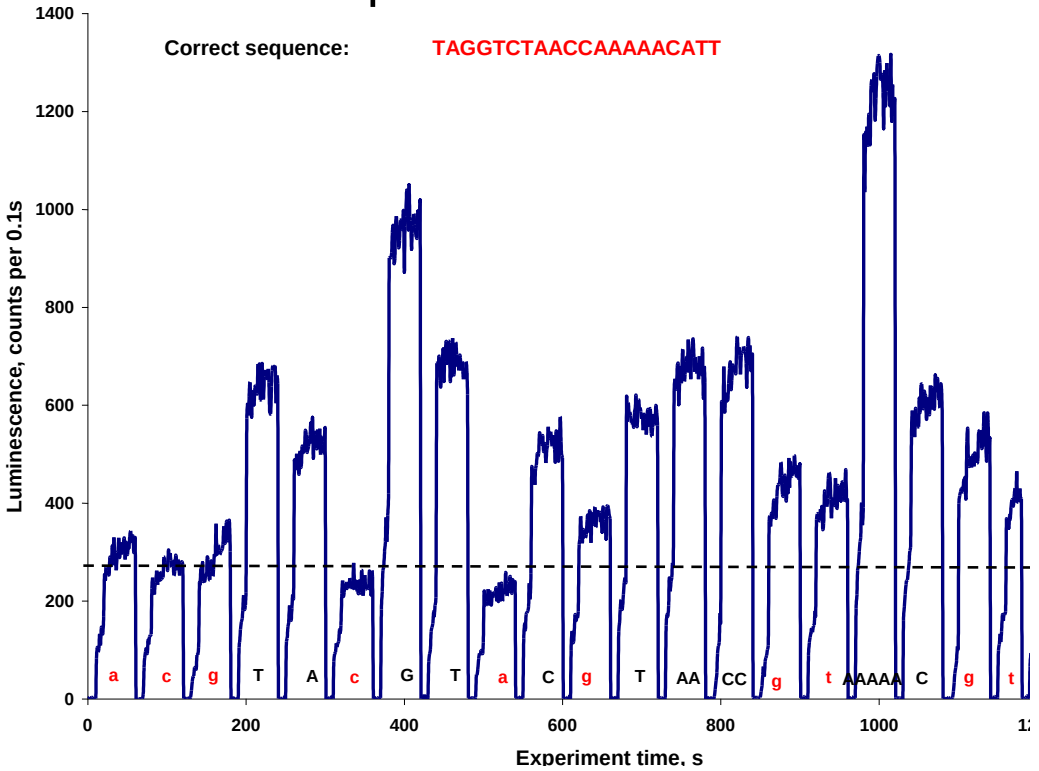
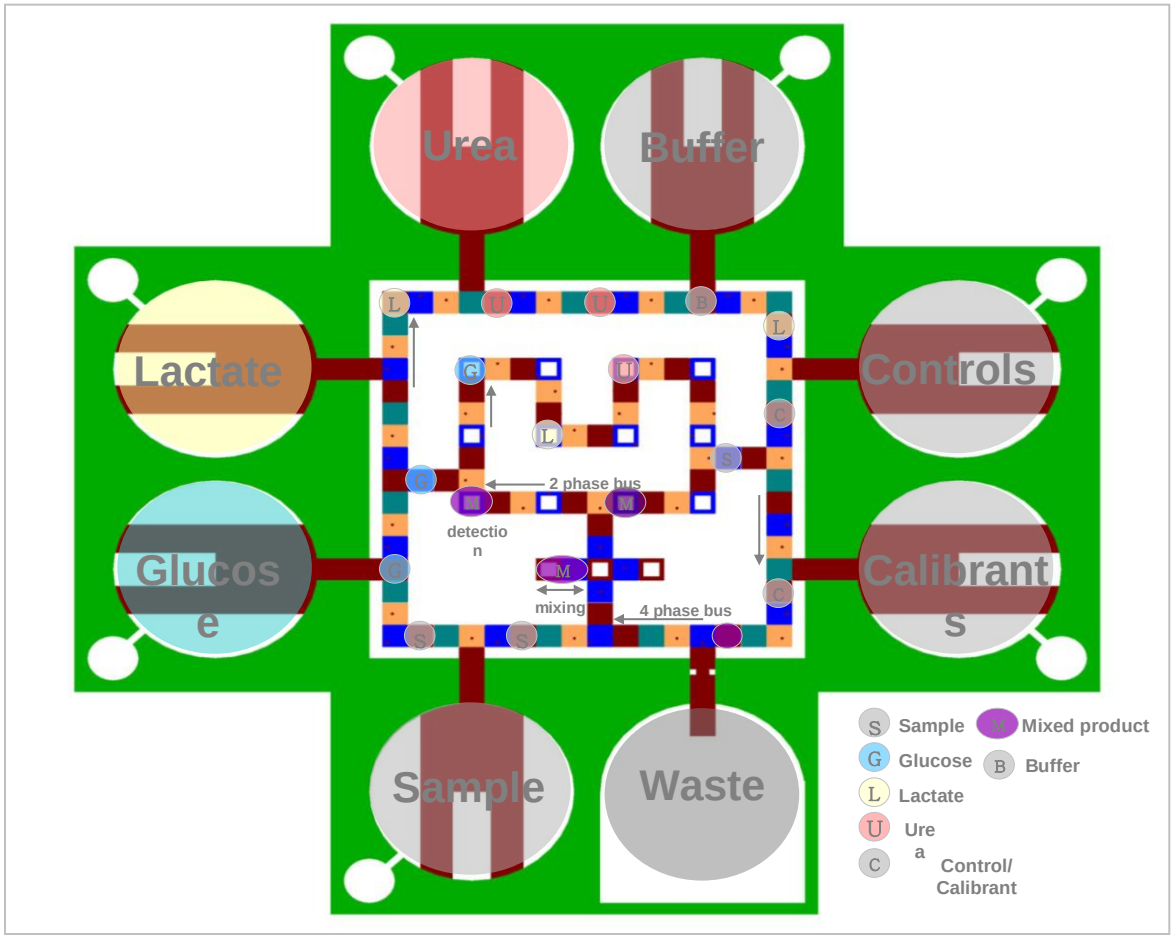
Results

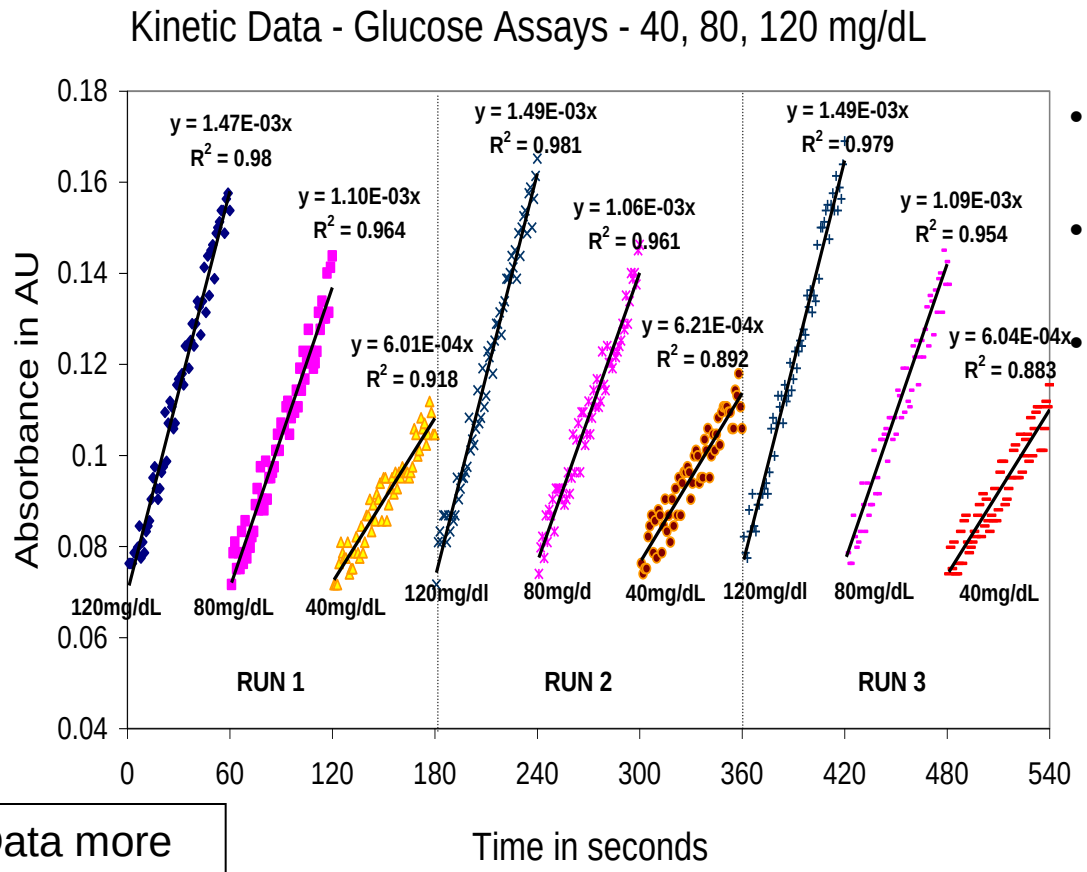
- 17-bp sequencing of 211-base long C.albicans DNA template with 20-base primer attached



Digital Multiplexed Assay Chip



Multiple Glucose Assays



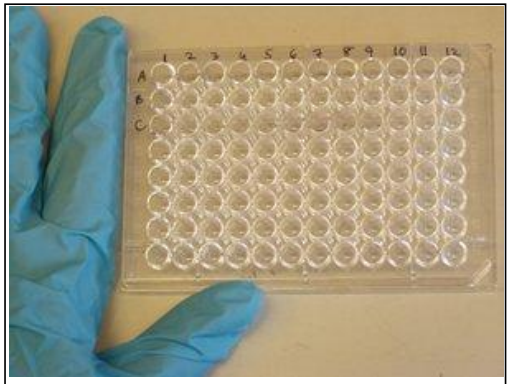
- 9 consecutive assays
- 3 glucose concentrations
- 60 seconds absorbance measurement at same spot

Data more noisy for 40 and 80mg/dL

Cytotoxicity Screening

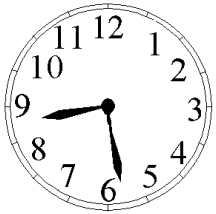
- Large market: Drug discovery, product testing, experimental research, and more.
- Researchers and companies must determine how their drug or other compound effects cells before animal and human trials can begin.
- Limited supply (1-10 mg) of compound to test, many compounds (up to 1 million) need tested Miniaturization and automation provided by biochips is attractive.

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



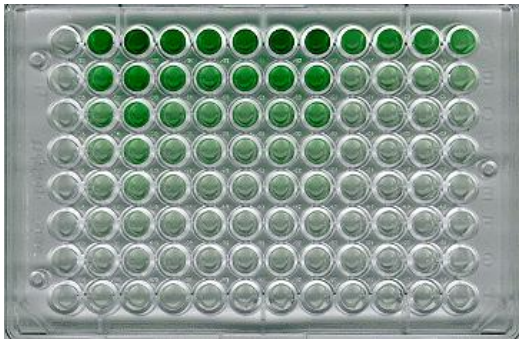
Grow cells in 96 well plate

Add various concentrations of compound to be tested to cells



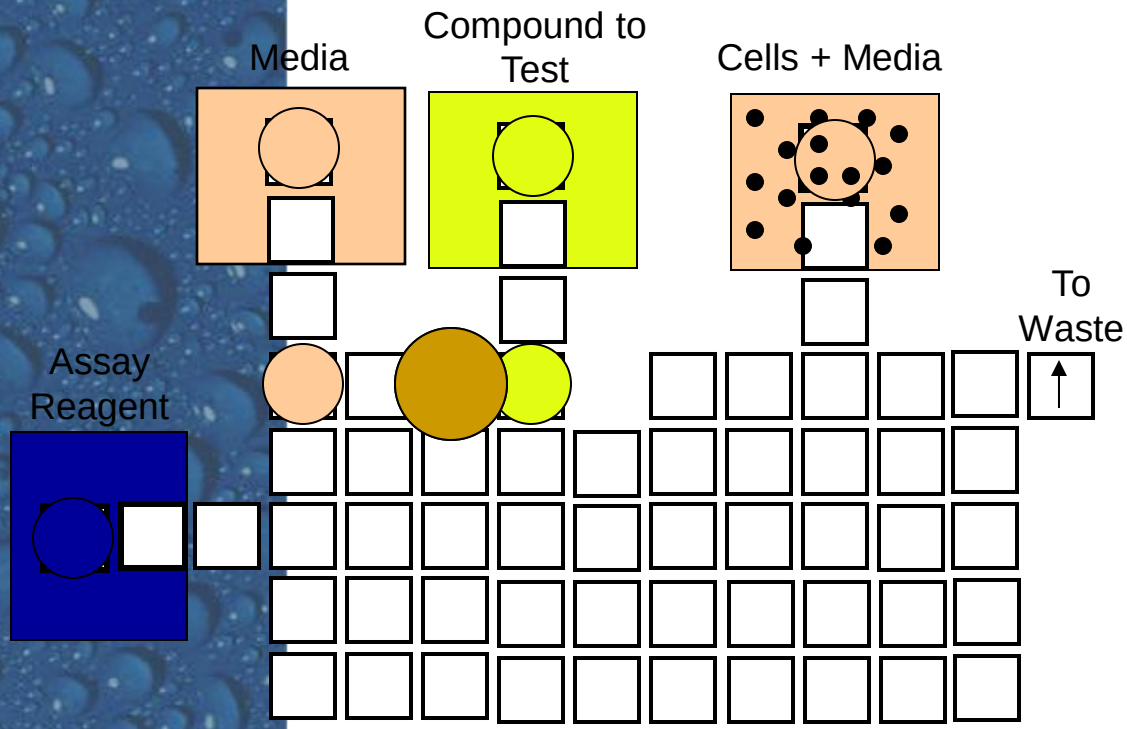
Wait specified length of time

Add Cytotoxicity Assay reagent 1, incubate, add reagent 2



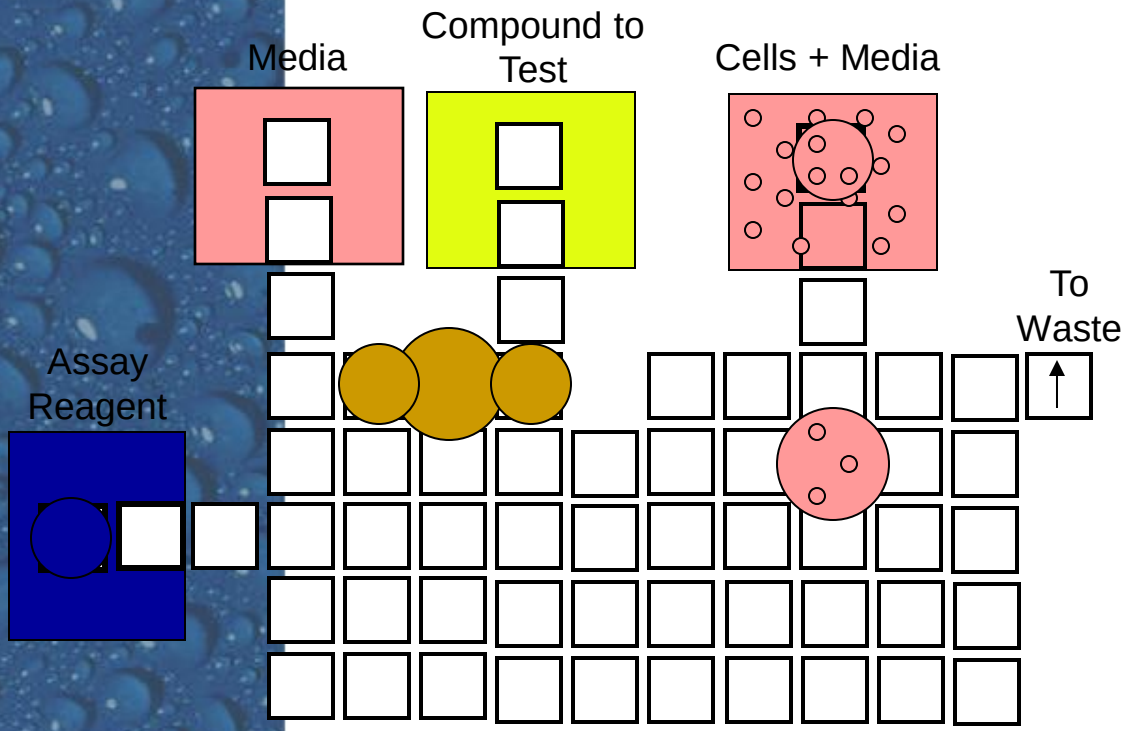
Use plate reader to measure color intensity (proportional to survival)

Architecture



1. Dispense buffer and compound droplets, mix.

Architecture

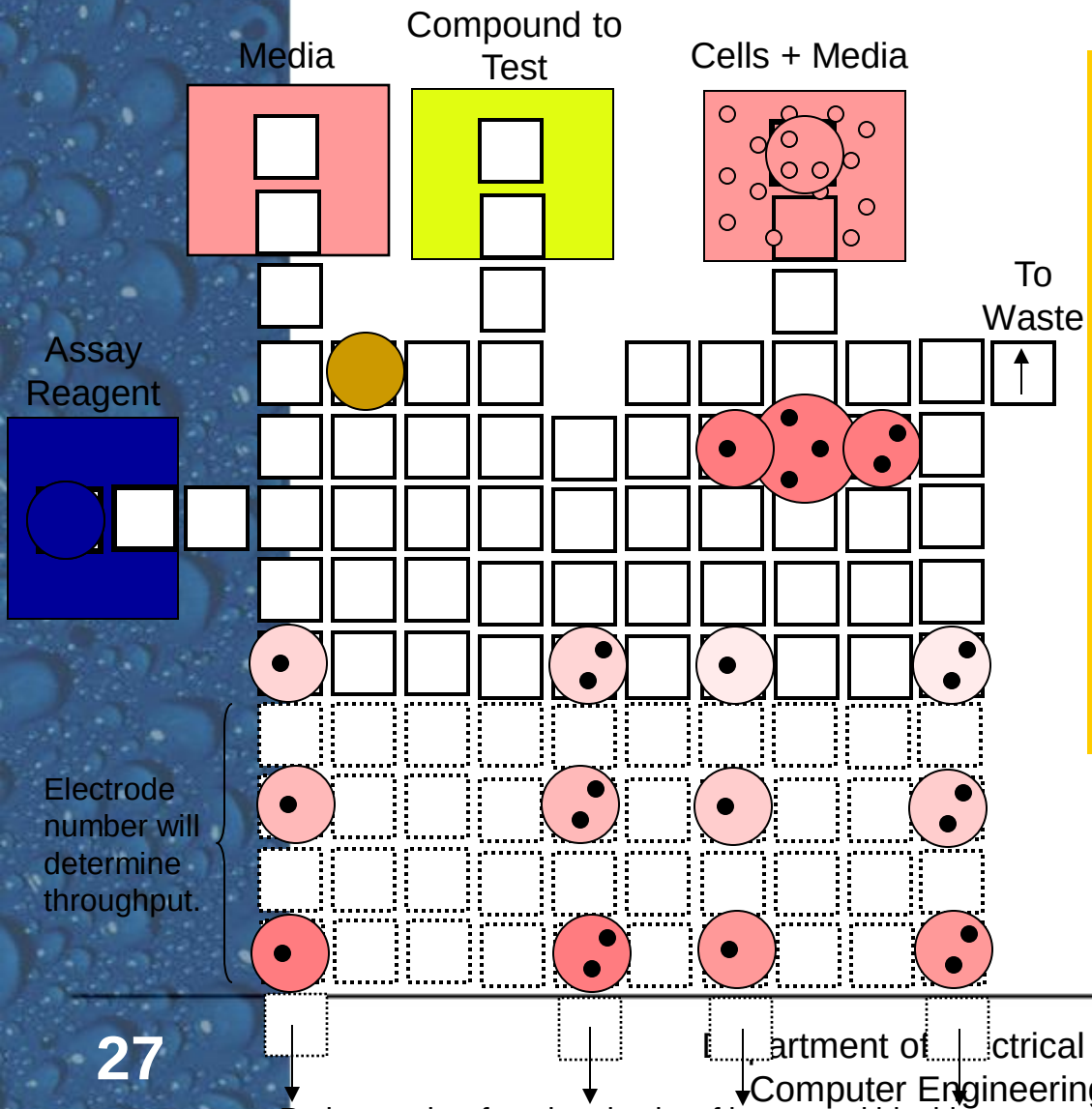


1. Dispense buffer and compound droplets, mix.

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)

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.

Summary and Conclusions

- Electrowetting-based digital microfluidics is good candidate for multifunctional microfluidics
  - Programmability
  - Reconfigurability
  - Multifunctional reliable EWD actuator operation if $V \leq V_{sat}$
- 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