Integrated Digital Microfluidic Biochips

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

Blood → Cell Filter → Reaction Chamber → Analyte capture → Fluorescent detection

- Metered plasma volume
- Fluorescent Antibodies
- Wash
Fluidigm 8.96 Screening Chip
Concept of Disposable Integration

Application Devices

MICROFLUIDIC

PROCESSING/ANALYSIS
Promise of Biochips

Applications: Biotechnology (e.g., 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

- 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

Photodiode

Mixed Droplet

Electrical Pad

LED

Electrowetting Electrode

Mixing Sample & Reagent
Microfluidic Functions

Benchtop Laboratory Techniques
Many manual steps

“Lab on a Chip”
Integrated sampling, chemical reactions, mixing, separation, detection, data processing

Microfluidics
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

Commercial

Research

Instruction Decode

Storage Unit

Reaction Unit

Process Control

Pumps

Acquisition

Reaction Reservoirs

Dispensing Reservoirs

Channels and Flow Sensors

Agent Detection

Temperature Measurement

Catalysts

Catalysts

Acquisition

Reaction Reservoirs

Dispensing Reservoirs

Channels and Flow Sensors

Agent Detection

Temperature Measurement

Catalysts

Catalysts

Commercial

Research

Where Are We?
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**

Chemoluminescence underneath the TeflonAF coated photodetector
Detector Integration

4x10^5 photons/s.cm^2 (10^-6 lux) no need to cool

8x16 Pixel Array

128 channel ADC

DSP SIMD Array

5 mm

pixel 230 x 230 µm^2

electrode 400 x 400 µm^2
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

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


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

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

• Transport
• Mixing
• Flushing
• Filtering
• Analysis
• Detection
• Monitoring

• Buffers
• Channels
• Valves
• Mixers
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

- Reagent Mixing
- Chemical Separation
- Filtration
- Heating
- Detection

- Electrophoresis
- Immunoassay
- DNA Assay
- Mass Spec
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

Digital Microfluidics Workspace

Sample Preparation Unit

Reagent Cartridge

I/O Bus, A/D converter

Transportation Bus

Reaction Zone1

Mixing Zone1

Mixing Zone2

Buffer Zone1

Buffer Zone2

Buffer Zone3

Temperature Control Zone1

Temperature Control Zone2

Temperature Control Zone3

Detection Unit
Floor Plan of the DMW
On-chip Dilution Tree for Cytotoxicity Screening (Y. Zhao, A. Wang, Y. Yamanaka)

1. Grow cells in 96 well plate
2. Add various concentrations of compound to be tested to cells
3. Wait specified length of time
4. Add Cytotoxicity Assay reagent 1, incubate, add reagent 2
5. Use plate reader to measure color intensity (proportional to survival)
Previous Work

Toxicology in Vitro 2007, 21, 535–544
Lab on a Chip 2007, 7, 740-745
Architecture

1. Dispense buffer and compound droplets, mix.

Assay Reagent

Media

Compound to Test

Cells + Media

To Waste
1. Dispense buffer and compound droplets, mix.

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

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.
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**

![Graph showing transmission vs. wavelength for different concentrations of solution.](Graph)

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

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

- **Cytotoxicity assay result**

**Color intensity detector**

<table>
<thead>
<tr>
<th>Most cells alive</th>
<th>Most cells dead</th>
</tr>
</thead>
</table>

**Output:**

- # cells alive in droplet
- # cells dead in droplet

- If not in range, send back.
Protein Crystallization on an Array
(H. Fang, M. Shafir, T. Xu)

Glucose isomerase crystals on chip – 20x
Proteinase K crystals on chip – 40x
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
Fig. 1. Schematic illustration of a protein crystallisation phase diagram.
Integrated Array Chip Layout
Implementation

- Multi-well-plate
Sample Droplet Splitting and Dilution Scheme

Protein Stock Solution

Well Electrode

Crystallization Reagents

Route one droplet to an adjacent well / We can split the droplets to a uniform volume

Incubate in well / Dilution can be performed by routing further water droplets
Architectural Block Diagram

Sample Injection

Sample Dilution

Sample Mixing

Sample Incubation

Detection

Waste Handling
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 a 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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