Fundamentals of Microfluidics with Applications in Biological Analysis and Discovery

Harvard Extension School
E155
Dr. Anas Chalah
03.22.16
E155 Roadmap (2016)

- Microarrays
- Microfluidics for Detection and Separation
- Droplet Microfluidics
- Paper Microfluidics
- Microfluidics for Drug Discovery
- Lab on Chip (L.O.C.) Devices
- Microfluidic platforms for point of care applications
Office Hours

By appointment – Send an email to:

achalah@seas.harvard.edu
Homework - Communications - Questions

Send by Emails to:
achalah@seas.harvard.edu
pduane@seas.harvard.edu
E155 Course Objective (Reminder)

E155 introduces:
Physical concepts of fluid mechanics, fabrication of microfluidic systems and Biomedical applications in biological detection and analysis as well as many other clinical applications

You will not become an expert - but you will be able to take more advanced courses and complement your knowledge

Discussion is important - Ask questions
E155 Section 2 Homework

Research papers - Read and understand the paper

Do not write a summary of the paper

Read and “dissect” the paper very carefully

Consider yourself a “reviewer” - Paper is a “Grant Proposal”

Evaluate the work (answer five specific questions)

Decide whether it is worth publishing it
Homework Questions

Present your opinion in your own words and no more than one page

(1) **Main goal of the paper:** The question the paper tried to answer
(2) **The application:** Describe the microfluidics application
(3) **Strong & weak points:** Was that the best approach for such a study
(4) **Your suggestions:** What would you do differently
(5) **Possible “novel” future applications:** As described by the author(s)
(6) **Your final decision:** Was it worth publishing – Grant or No Grant
Assignments should be typed in **size 12 Arial** font

Pages should be **numbered**

Your **name** must appear on each and every page

Send all documents ONLY in a **.PDF** format
Notes about E155 Homework

Adhere to the question and answer

Be precise with your analysis

Don’t be afraid to “grill” a paper just because it was published in NATURE

Do not write more than one page

Bring a copy of your homework to class

Online students are encouraged to send questions

Just because you are online students does not mean you can not participate
When is E155 Homework due

You will get a paper homework on **Tuesday**

Email your homework no later than **Monday midnight**

In class discussion **one week** from the day you get the paper

**Grades** will be sent the **following week**

Late homework = Full grade reduction
Where to send E155 Homework

EMAIL your homework to BOTH addresses

achalah@seas.harvard.edu
pduane@seas.harvard.edu
E155 2016 FINAL Project

The final project will cover Prof. Chalah’s Section ONE project - Individual work (No teams)

The exercise is to come up with a microfluidic chip design for a biomedical application
Final Project Format

Choice of The Analysis:
Rational behind each test/analysis you picked - Possible links between different tests - Already known tests and solutions

The Fabrication of The Chip:
Platform fabrication (Micro valves, Pumps, Mixers, Channels, Heater, reservoirs, etc.)

The Detection Methods ( SIGNALS): 
Combine and detection of different signals

Possible Applications and Marketing:
Describe the use of designed device in real life - targeted applications and market
E155 CLASS GRADE FORMULA

Homework are worth 40% of the course grade

Midterm Exam is worth 30% of the course grade

Final Project is worth 30% of the course grade
Fundamentals of Microfluidics with Applications in Biological Analysis and Discovery

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03.22.16
Microfluidics refers to the set of technologies used to control and manipulate the flow of few microliters of a fluid sample (liquid or gas) in a miniaturized microfluidic device.
Microfluidic Devices by Definition:

**Architecture:**
3D network of channels and other components (pumps, valves, heaters, reservoirs, ..etc)

**Dimension:**
Has one or more flow channel component with at least one dimension less than 1mm

**Function:**
Provide a high level of fluid control required for a certain application
Scope of Applications

Microfluidics are very powerful techniques in controlling and measuring chemical reactions and physical and biological processes at the microscale.

The field of microfluidics combines:

(1) **SKILLS**: Fabrication methods at the micro and nanoscale
(2) **KNOWLEDGE**: Behavior of fluids at the microscopic level
Examples of Biomedical Applications

- Microarrays & DNA Hybridization
- Gene analysis, Isolation and Amplification
- PCR Integrated Microsystems
- Surface Assembly & Biomolecular anchoring techniques
- Immunosensing
- Chemical Separation & Chromatography
- Electrophoresis (DNA & Protein)
- Surface Plasmon Resonance (SPR)
- Cell Sorting & Flow cytometry
- Micro NMR
- Lab-on-a-chip Fabrication
- Disposable microfluidic devices
- Paper microfluidic chips
- Microdroplet formation and biological applications
- Blood, Glucose, Protein and pathogen analysis on LOC devices
- Microchips for drug discovery: Microfluidic gradient devices
- High-throughput drug screening on microfabricated chips
- 3D cell culture vs. single cell analysis
- Microchips for Cancer metastasis and cell deformation
A microfluidic device is defined as a device that has one or more flow channel component with at least one dimension less than 1mm.
It Is All About The Scale

- Human hair diameter 25-100 µm
- Red blood cell diameter 6-8 µm
- Red blood cell thickness ~ 2 µm
- Cellular components (range 10-30 µm)
- Nucleus size ~ 3-10 µm
- Mitochondria size ~ 0.5-1.5 µm
- Virus diameter 10-400 nm
- DNA Alpha helix structure diameter is 2nm
- Artificial Nanosystems are in the range of 10-100 nm
- Atomic limit 0.1-0.5 nm
Driving Force for Device Miniaturization

- $$\text{ }$$
- Time
- Volume Limitation of Biomaterial
- Point of Care Approach

How do we translate these driving forces into device Design Requirements?
Driving Force ➔ Design Requirements

- $$$ ➔ Understand the device market
- Time ➔ Device Integration
- Volume Limitation of Biomaterial ➔ Amplification
- Point of Care Approach ➔ Manufacturing ➔ Platform Selection
Market for Microfluidic Devices

“The microfluidic device market will grow swiftly, from $1.4B in 2013 to $5.7B by 2018”

“This impressive 27% growth will be fueled mainly by Pharmaceutical research and Point-of-Care applications”

Source: www.researchandmarkets.com
More Market Data Points

BioMEMS and microsystems for life science market (in $M)
Including: pressure sensors, silicon microphones, accelerometers, gyroscopes, optical MEMS and image sensors, microfluidic chips, microdispensers for drug delivery, flow meters, infrared temperature sensors, emerging MEMS (RFID, strain sensors, energy harvesting)  
(Source: BIOMEMS report, Yole Development, February 2013)

$ 6.5B by 2018

$ 3.9B by 2016
MEMS Market: What Is The Emphasis On?

$21B by 2017
Patent and Publications in Microfluidics

Compare 2013 to 2003 then to 1993
Google Hits (Microfluidics)

MEMS
• Micro Electro Mechanical Systems
• Technology of very small devices
• Merges at the nanoscale into nanoelectromechanical systems (NEMS)

μTAS
• Micro Total Analysis Systems
• Integration of the total sequence of lab functions to perform a chemical analysis

L.O.C.
• Lab-on-a-chip
• Device that integrates one or several lab functions on a single chip of few cm$^2$ in size
BioMEMS / μTAS / L.O.C.

**MEMS**
- Micro Electro Mechanical Systems

**μTAS**
- Micro Total Analysis Systems
- Integration of a sequence of lab functions to perform a chemical analysis

**L.O.C.**
- Device that integrates one or several lab functions on a single chip
Fields of Microfluidic Impact

- General Accurate Dispensing
- Drug Delivery
- Analytical Devices
- Clinical Diagnostics
- Point Of Care (P.O.C) testing
- Industrial & Environmental Testing
- Pharmaceutical and Life Science
- Micro-reaction Technology
Quick Exercise:

The Challenge:

Design a Microfluidic Chip for a Simple Cell Sorting Task

- How do you go about this design?
- Start by asking the right questions
Microfluidic Device

**General Design Requirements**

1. Minimized volume of solvents, reagents and chemicals
2. Limited Biological sample volume (critical for HTS)
3. Short reaction & assay time
4. Fluid control
5. Parallel operation & testing = INTEGRATION
6. Signal processing
7. Low energy consumption
8. Portability of device
9. Storage
10. Reusable vs. disposable
11. Low production cost
Microfluidic Device

**Specific Design Requirements**

Regulations and Ethical Considerations

For In-vitro Diagnostic Devices, Regulations and Ethical Considerations Need To Be Addressed
In-vitro Diagnostics Regulation
FDA vs. CLIA

**FDA**: Food and Drug Administration
**CLIA**: Clinical Laboratory Improvement Amendments

**FDA** regulates manufacturers and devices intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease, to make sure they are *reasonably* safe and effective.

**CLIA** program regulates laboratories that perform testing on patient specimens in order to ensure accurate and reliable test results.
Bio Device Design Early Challenges

Source: Prof. Dr. Thomas Otto Fraunhofer ENAS, Germany
Bio Device Specific Design Challenges

Source: Fraunhofer ivD-Platform (www.ivd.fraunhofer.de)
In Summary:

Advantages of Microfluidics Compared to Conventional Technologies

- Low cost
- High parallelization
- Ease of use and compactness
- Reduction of human error
- Faster response time and diagnosis
- Low volume samples
- Real time process control & monitoring increase sensitivity
- Expandability
Early Microfluidic Technology
InkJet Printers

Milestones in inkjet technology

1979
- Thermal inkjet technology is invented at HP laboratories.

1984
- HP ThinkJet printer is developed.
- The disposable cartridge is introduced.

1986
- New HP QuietJet/QuietJet+ printers offer 192 dpi resolution through near letter quality (NLQ) printing.

1987
- First color printer, the HP PaintJet, provides full-color graphic printing and quickly becomes the market leader.

Source: www.hp.com
(A) Simulation: Inkjet nozzle ejects a “bubble” that separates from the main body and leaves behind a residual tail.

The simulation models the ink surface tension and characterizes the air-ink boundary to track movement of the fluid drop.

(B) High-speed photos: Images echo the simulation

Source: US Department of Energy

Continuous vs. Drop-on-Demand

Continuous inkjet printers

A mechanical pump forces ink at high pressure through a nozzle in a print-head, while adding an electrostatic charge to each droplet.

The resulting stream of charged ink droplets can be steered by applying a variable voltage to electrodes.

If no ink is required, the ink is directed into a gutter and collected for reuse.

Example:
Stamping date-codes on drink cans.
Continuous vs. Drop-on-Demand

Drop-on-demand inkjet printers

Device has an array of chambers

Each chamber is linked to an individual nozzle

An electrical signal generates a pressure pulse that ejects a droplet of ink onto the substrate

Example:
Graphic printing applications
Creating Images with InkJet

Ink-jet printers have several hundred nozzles, each is 20-40 µm in diameter.

Printer ejects series of ink droplets on a substrate.

In color printing, every dot of the image is formed from several different color droplets.

When viewed from a distance, these dot appear to have a single color.

Dots are built from four separate colors: Black, Cyan, Magenta, Yellow.
Example: Protein Printing: Arrayjet printing technology

(A) Flushing the print heads: Preparation for sampling

(B) Print head is moved to the wells containing the biological samples

(C) Print head is moved across the microarray slides, depositing samples as it travels

Source: http://www.arrayjet.co.uk
“Arrayjet” print head contains **126 nozzles** in a linear arrangement

The print head is suitable for printing **viscous** sample including **cell lysate**

Could handle up to **20cP** (centipoise) in fluid viscosity (1cP is equivalent to the viscosity of water)

For protein stability, printing is done at **low temperature**

**Source:** http://www.arrayjet.co.uk
Inkjet Printing for Oligonucleotide Synthesis

Inkjet printing enables the delivery of extremely small, accurate volumes (picoliter $10^{-12}$ range) of the chemicals.

Four nucleotides are applied:

(A) The first layer of nucleotides is deposited on the activated microarray surface.

(B) Growth of the oligos is shown after multiple layers of nucleotides have been deposited.

(C&D) Close-up of one oligo as a new base is being added to the chain.

Source: www.Agilent.com
Inject Oligonucleotide Synthesis

Building blocks are sequentially coupled to the growing oligonucleotide chain to produce the desired sequence.

Once complete, the chain is released from the solid phase to solution.

Oligo synthesis process has been fully automated since the 70’s.

Limitations:
The occurrence of side reactions & sequence errors → limits for the length of synthesized oligos (~200 nucleotide residues).
Building An Advanced Microfluidic Device

**Question:**
What the factors you need to study/understand to be able to build an advanced microfluidic device?
General Anatomy of a Microfluidic Device

1. Fluid Control
2. Interface (Electric, Mechanic, Optic, etc..)
3. Integration
4. Signal = Sensing
Understanding the Flow

There are two main methods for driving the flow of fluids in micro channels:

(1) **Pressure-driven flow:**
Also known as hydrodynamic flow:
- Negative pressure - vacuum at the outlet
- Positive pressure - pump at the inlet

(2) **Electrokinetic flow:**
Molecules move in an electric field due to their charges:
- **Electrophoresis:** motion of charged molecule in an electric field
- **Electroosmosis:** a layer of **positively charged molecules** forms at the surface of **negatively charged silanol groups** of the channel wall; an electric field drives the layer of cations towards the negatively charged cathode and transfers the motion to the rest of the liquid
Understanding the Probe

WHAT IS THE (biological) PROBE?

Understanding probe Stability, Storage, Transfer, and Surface attachment

- DNA microarrays can be printed onto a range of surfaces with a range of buffers = need to understand the structure of DNA
- Protein microarrays require printing buffers, in some cases specific for each individual protein
- Protein samples must be kept at low temperatures during printing
- Protein’s complex 3-D structure must be maintained (preserve structure & activity)
Understanding the Fluid

- Whole blood samples
- Bacterial cell suspensions
- Mammalian cell suspension
- Whole cell lysate
- Protein or antibody purified solutions
- Various buffers
Understanding Device Integration
Understanding the Signal & Interface

PCR
Electrophoresis
Mass Spec
SPR
FACS
Microscopy
Microarray

Microarrays are broadly defined as tools for paralleled ligand binding assays

Biomolecules (oligonucleotides, protein fragments) are placed on a solid support (glass, PDMS slides) at high density

Goal: Recognizing a complex mixture of target molecules
Microarray Design

In general…

A microarray is a **DEVICE** that consists of different **PROBES** chemically attached to a **SUBSTRATE** and designed to detect a target molecule.
Microarray Technology

Microarray technology allows for:

(1) **Estimation** of target abundance
(2) **Detection** of biological interactions

Both at the molecular or cellular level
Building a Microarray Chip
“Design Thinking”

Application → Components → Fabrication

↓

Revise ← Testing
Microarray Design Components

**Device:** Fabrication - Design - Signal - Interface

**Probe:** Bio materials (DNA, RNA, Protein, Cells, Tissue)

**Substrate:** Chemical Platform (Polymers: Glass, Silicon, PDMS)

**Buffer:** The fluid

**Surface Fabrication:** Binding Chemistry

**Signal:** Test outcome

**Interface:** Collecting/analyzing the signal
Types of Microarrays (Probes)

There are various types of microarrays with different probe materials:

- DNA/RNA and oligonucleotides
- Soluble proteins
- Membrane proteins
- Peptides
- Carbohydrates
- Small molecules
- Transfection live cells
- Tissue TMA