Lab-on-a-Chip

Professor Abraham “Abe” Lee
Departments of Biomedical Engineering
Mechanical & Aerospace Engineering
University of California at Irvine

Outline

- Molecular diagnostics
- Scaling of microfluidics
- Microfluidic systems
- BioMEMS
- Future directions

Micro: From MEMS to BioMEMS

- Top down microfabrication by photolithography
Motivation: The awesome scale of potential molecular testing

- >6 billion people on our planet needing all forms of molecular diagnosis including cancer screening, risk profiling and detection
- Potential pathogens drawn from:
  - ~1.5M species of fungi
  - ~800,000 species of bacteria
  - All possibly with drug resistance genes
- Food, water, agricultural and environmental testing needs

Perhaps the need for >15 billion molecular tests per year
<table>
<thead>
<tr>
<th>Lab-On-A-Chip: Size, Speed, Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Today</strong></td>
</tr>
<tr>
<td>Hours to days</td>
</tr>
</tbody>
</table>

Time from sample to "CORRECT" answer
Can We Change the Current Healthcare Paradigm?

Microfluidics: A technology that walks on water!

- Miniaturized channels and reservoirs
  - High surface to volume ratio
  - Low Reynolds number
  - Increase speed of reaction
  - Reduce cost of reagents
  - Reduce power consumption
  - Precise mixing/dosage and heating
- Integration
  - Reduce cost of manufacture
  - Minimize dead space, void volume
  - Minimize sample carryover
  - Multiplex capability: increased number of parameters monitored per assay

Source: U Cincinnati

Microscale Flow

Source: U of Washington

Source: UTMDACC
Create the Interface Between the Microworld and the Nanoworld

Scaling with Length Scale

- Mechanical Examples: Linear Oscillators and Dissipative Phenomena
Impact Dynamics and Impact Microactuation

Scaling with Length Scale

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Small Scale</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density ρ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass m</td>
<td>= ρV</td>
<td></td>
</tr>
<tr>
<td>Inertial effects</td>
<td></td>
<td>minimal</td>
</tr>
<tr>
<td>Surface to volume</td>
<td>K -1</td>
<td></td>
</tr>
<tr>
<td>Flow rate Q</td>
<td>K 3</td>
<td>minute sample volumes</td>
</tr>
<tr>
<td>Diffusion time</td>
<td>K 2</td>
<td>rapid</td>
</tr>
<tr>
<td>Pressure drop ∆P</td>
<td>L1.5</td>
<td>(turbulent)</td>
</tr>
<tr>
<td>∆P is independent of size</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Droplets

- Did you ever wonder why there aren’t droplets as big as a baseball?
Dimensional and Dimensionless

<table>
<thead>
<tr>
<th>Physical Variable</th>
<th>Dimensionless</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal conductivity, k</td>
<td>L²T⁻²Θ⁻¹</td>
</tr>
<tr>
<td>Specific heat at constant pressure, Cₚ</td>
<td>ML⁻¹T⁻²</td>
</tr>
<tr>
<td>Modulus Elasticity, E</td>
<td>ML⁻³</td>
</tr>
<tr>
<td>Mass density, ρ</td>
<td>MT⁻²</td>
</tr>
<tr>
<td>Surface tension, γ</td>
<td>ML⁻¹T⁻¹</td>
</tr>
<tr>
<td>Dynamic viscosity, µ</td>
<td>ML²T⁻²</td>
</tr>
<tr>
<td>Energy (work), E</td>
<td>ML⁻¹T⁻²</td>
</tr>
<tr>
<td>Pressure, P; Stress, s</td>
<td>ML⁻¹T⁻²</td>
</tr>
<tr>
<td>Acceleration, g</td>
<td>LT⁻²</td>
</tr>
<tr>
<td>Velocity, v</td>
<td>M</td>
</tr>
<tr>
<td>Mass, m</td>
<td>T⁻¹</td>
</tr>
<tr>
<td>Frequency, f</td>
<td>L²</td>
</tr>
<tr>
<td>Area, A</td>
<td></td>
</tr>
<tr>
<td>Force, F</td>
<td>T</td>
</tr>
<tr>
<td>Time, t</td>
<td></td>
</tr>
<tr>
<td>Length, l</td>
<td></td>
</tr>
</tbody>
</table>

MLT (or MLT Θ) Physical Variable

Dimensionless groups help us understand the physics at different scales. They can be either constant values like the pendulum (T²g/L = k), or ones that scale with physical properties like the Reynolds number (uL/µ).

Buckingham's theory (1914): number of dimensionless groups is m - n, where m is the number of physical variables defined by n independent fundamental quantities (e.g., mass, force, length, time, etc.).

Soap bubble: P/γ = (L²T⁻²γ⁻³)/R, a = -1, b = 1, if derived P = 4γ/πR, small bubbles make louder sound.

For liquid-solid interface:

For liquid-vapor interface:

P = aπRbR

θ is angle between liquid and the surface. It is radius of curvature of the interface.

Reference: On Size and Life by McMahon and Bonner.

Scales of BioMEMS

Laminar Flow: Low Reynolds Number

In microfluidics Reynolds number (Re) << 2000 → viscous forces dominate

\[ \text{Re} = \frac{uL}{\eta} \]

\( \eta \) = viscosity (Pa·s or kg/(m·s))

\( u \) = flow velocity (m/s)

\( L \) = channel diameter

\( \rho \) = density (kg/m³)

Mixing – Is a Challenge in Low Reynolds Number Flow

Steady state flow - same even after 1 hour. Flow is mixed 2 seconds after PZT is turned on showing that the yellow fluid has mixed with red.

Some basic microfluidics concepts

- Continuity (Kn<0.3) and low Reynolds number
- Motion is reciprocal if it is reversed
- Cannot swim by inertia
- For incompressible flow (most liquid microfluidics) density variation is negligible

Stoke’s Law

\[ F_D = 6\pi \eta ru \]

\[ \nabla^2 u = \frac{P}{\eta} \]

\[ Kn = \frac{\lambda}{r} \]

\( Kn \): Knudsen's number
\( \lambda \): mean free path of fluid

Examples of Low Reynolds Numbers

<table>
<thead>
<tr>
<th>Examples</th>
<th>Reynolds numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterium swimming</td>
<td>0.000001</td>
</tr>
<tr>
<td>Pollen grain falling, sperm swimming</td>
<td>0.01</td>
</tr>
<tr>
<td>Fruit fly in flight</td>
<td>100</td>
</tr>
<tr>
<td>Small bird flying</td>
<td>100,000</td>
</tr>
<tr>
<td>Squid fast jetting</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Large whale swimming</td>
<td>200,000,000</td>
</tr>
</tbody>
</table>
### Microfluidics

#### Shear by Viscosity
- Shear stress = shear modulus x shear strain
- Shear stress = viscosity x shear rate = \( \eta u / h \)

#### Capillary Viscous Resistance
- Analogous to electrical resistance relating pressure drop (voltage) to flow rate (current)
- For high aspect ratio rectangular channels \((h>>w\text{ or } w>>h)\), \(r\) can be approximately

#### Poiseuille's Law

\[
\frac{Q}{R} = \frac{AP}{8\eta L}
\]

### Other Microfluid Dimensionless Numbers

- **Froude number** (Fr) = \( \frac{u^2}{gL} \)
  - inertial force/gravitational force
  - Similar to Reynolds Number

- **Capillary number** (Ca) = \( \frac{\eta c G}{\gamma eq / r} \)
  - viscous stress/Laplace pressure
  - Significant in droplet emulsions
  - \( G\): shear rate
  - \( \gamma eq\): equilibrium interfacial tension
  - \( r\): droplet radius
  - \( \eta c\): viscosity of continuous phase

- **Peclet number** (Pe) = \( \frac{u^2}{gL} \)
  - advective flux/diffusive flux
  - Significant in dispersion in DK flow

- **Mirelle number** (Je) = \( \frac{\gamma L}{\rho L^3 g} \)
  - surface tension force/gravitational force
  - Determine whether can walk on water
  - \( \gamma\): surface tension
  - \( L\): perimeter of wetted surface

- **Diffusion**

\[
J(x,t) = -D \frac{\partial C}{\partial x}
\]

Time to diffuse \( L \) is:

\[
T = \frac{L^2}{D}
\]

### Fick's Law

#### Diffusion Constant

<table>
<thead>
<tr>
<th>Substance</th>
<th>( D ) [cm^2/sec]</th>
<th>Time to diffuse 10um</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle</td>
<td>0.2 ( \eta )</td>
<td>200 sec</td>
</tr>
<tr>
<td>55 ( \mu m ) particle</td>
<td>0.5 ( \mu m/sec )</td>
<td>1 sec</td>
</tr>
<tr>
<td>Protein (hemoglobin)</td>
<td>1 ( \mu m/sec )</td>
<td>200 sec</td>
</tr>
<tr>
<td>Small molecule (ammonium)</td>
<td>200 ( \mu m/sec )</td>
<td>0.2 sec</td>
</tr>
</tbody>
</table>

From Brody & Yager Hilton Head 1996
**Mixing in Low Reynolds Number Fluid**

- Time for transport by stirring
  \[ t_s = \frac{L}{v} \]
  
  - L: length of transport
  - v: velocity of stirring

- Time for transport by diffusion
  \[ t_d = \frac{L^2}{D} \]
  
  - D: diffusion constant

- **S(stirring number)** = \[ \frac{t_s}{t_d} = \frac{Lv}{L^2/D} \]

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**Surface Forces in Microfluidic Channels**

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**Droplets**

- The movie *Antz*
**Controlled Femtoliter Volume**

- Confinement creates new biomolecular events that better mimic biology
- Vesicles – hybridization of biomolecules is reduced from 10 minutes to 2 seconds

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**Wettability**

Water & PTFE

\[ \gamma_{SL} = \gamma_{SV}^{oo} + \gamma_{LV} \cos \theta \]

Hydrophilic surface

Hydrophobic surface

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**Example: A Passive Micropump by Surface Tension Forces**

Photograph of water droplet on COC surface

Walker and Beebe, Lab Chip, 2002, 2, 101
**Marangoni Effect**

- Motions of the surface of a liquid are coupled with those of the subsurface fluid or fluids, so that movements of the liquid normally produce stresses in the surface and vice versa. The movement of the surface and of the entrained fluid(s) caused by surface tension gradients is called the Marangoni effect.
- Marangoni effect can be induced by:
  - Temperature
  - Electrowetting
  - Chemical gradients

**Surface Tension Control by Self-Assembled Monolayers (SAMs)**

SAMs: A monolayer of ordered molecular assemblies formed by the adsorption of an active surfactant on a solid surface.

**Detection Limits of Microfluidic Assays**

\[ V = \frac{1}{\eta N A C_i} \]

- \( V \): sensor efficiency
- \( \eta \): Avogadro's number
- \( C_i \): concentration of analyte i

Courtesy Dr. Kurt Petersen, Cepheid, Inc.
The Power of Miniaturization

1. Agent attacks cell and disrupts dielectric properties
2. Agent binds to surface properties
3. UTMACC/LYNNTech

From Breadboard Microfluidics to Integrated Microfluidics

A Generic Microvalve

Common Features
- Inlet
- Outlet
- Valve Seat
- "Membrane"
- Hinge section
- Boss

**Microfluidic Components**

- Glass substrate
- Silicon nitride
- Silicon dioxide
- Titanium/Au/Ni
- Polyimide 1
- Polyimide 2
- Polyimide 3
- Ground plane electrode
- Phosphorous doped
- Ground plane

(a) (b) (c)

- Nickel
- Voltage (V)
- Pressure (psi)

**Micropumps**

**Categories of Active Microfluidic Pumps**

- Pressure Driven
  - Membrane Pumps
  - Rotary Pumps
  - Diffuser Pumps
  - Ultrasonic pumps
- Electro-driven
  - Electroosmotic/ Electrophoretic pumps
  - EHD (electro-hydrodynamic) pumps
  - MHD (magneto-hydrodynamic) pumps
- Other
  - Venturi principle
  - Osmotic
- Air-bursting detonators as alternative on-chip power source and pumps
**Membrane Pump - Principle**

- Membrane Micropump
- **Membrane**
- **Actuator**
- **Inlet Passive Valve**
- **Outlet passive valve**

- Reciprocating motion of membrane
- Needs inlet and outlet valves (active or usually passive)
- Actuation mechanisms —
  - Most of the actuation schemes covered for microvalves can be used to actuate the membrane
Membrane Pump - Principle

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Sample Preparation

Current BioChips for Drug Development Require Extensive Sample Preparation

- 65% of the cost is in sample preparation
- 95% of the time is for sample transportation and preparation

Micro Arrays
Electrokinetic Microfluidics

Basic Samples

- Tissue
- Blood
- Cells
- DNA
- Protein
Basic Sample Preparation Procedures

- Extraction
- Purification
- Separation
- Amplification
- Sorting

H-Filter: Diffusion based separation

Time to diffuse L is:
\[ T = \frac{L^2}{D} \]

- The normalized probability density function, \( y(x) \), for the 1-D distance, \( x \), which a particle diffuses in time \( \tau \):
\[ y(x) = \frac{1}{\sqrt{4\pi D\tau}} \exp\left(\frac{x^2}{4D\tau}\right) \]

<table>
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<tr>
<th>Particle</th>
<th>( d ) (20°C)</th>
<th>Time to diffuse 10 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 µm sphere particle</td>
<td>0.5 µm/sec</td>
<td>200 sec</td>
</tr>
<tr>
<td>Protein (hemoglobin)</td>
<td>10 µm/sec</td>
<td>1 sec</td>
</tr>
<tr>
<td>Small molecule (fluorescein)</td>
<td>200 µm/µsec</td>
<td>2.5 sec</td>
</tr>
</tbody>
</table>


DEP Separation of Bacterial and Blood Cells on Microelectronic Array

**On-Chip Microfluidic System for Biological Detection**

- Magnetic Beads
- Antibodies
- Filterless Bio-Separator
- Micropump and Microvalve
- Immonosensor

**Magnetic Bead-based Biofilter as Biocell Sampler**

- Core component of a microfluidic bio/chemical detection system
- To detect micro-organisms from concentrated liquid samples
  - Immobilized antibodies or cells on magnetic beads
  - Sampling and capturing of target antigens or cells
- Magnetic bead as a carrier and a substrate
  - Separation and manipulation of magnetic beads marked with antibody or enzyme

**Magnetic Beads**

- Fe₂O₃ nano-clusters (superparamagnetic nanoparticles)
- Polyelectrolyte
- Bio-affinity coated on surface such as streptavidin

- Magnetic beads in liquid suspension
**Biofilters with Planar Electromagnet Surfaces**

- Magnetic force on a magnetic bead
  \[ F_{mag} = \nabla \cdot \mathbf{H} \times \mathbf{χ} \nabla \times \mathbf{B} \]
  \[ \mathbf{χ} \] Susceptibility of a magnetic bead

- Drag force on a magnetic bead due to the fluid flow
  \[ F_{drag} = 6\pi\eta R \]
  \[ \eta \] Viscosity of the fluid
  \[ R \] Radius of a magnetic bead
  \[ v \] Velocity of the fluid

**Biofilter Characteristics**

- Magnetic beads separation in continuous fluid flow

**Sampling Procedures**

(a) Injection of magnetic beads
(b) Separation and holding of beads
(c) Flowing samples
(d) Immobilization of target antigen
(e) Release to bioreactor or biosensor
**Designed Filterless Bio-Separator**

![Cut view of the separator with the magnetic flux along the microfluidic channel](image)

**Concept of Magnetic Bead-Based Sandwich Immunoassay**

![Magnetic Bead-Based Sandwich Immunoassay](image)

**Magnetohydrodynamic (MHD) Microfluidic Devices**

- **Principle of operation**
  - $\mathbf{F} = I \times \mathbf{B} \times \mathbf{w}$
  - $P = I B / h$

- **AC current avoids electrolysis**
  - Higher maximum current threshold with higher frequency
  - Electromagnet must be synchronized to current
  - Pump speed and direction vary with magnet phase

- $F = B w \int \sin \omega t \sin (\alpha t + \phi) d\phi$
- $P = \frac{B V}{\mu} \int \frac{\sin \omega t \sin (\alpha t + \phi)}{\mu} d\phi$
- $P = Q R$
- $R = \frac{2 \pi d (\text{perimeter}^2)}{\text{cross-sectional area}}$
- $Q = \frac{B w h^2}{8 \mu d (w + h)^2}$
MHD Integrated Microfluidics Enables Multi-functional components on a single microfabrication platform

Arm 1
Arm 2
Arm 3

MHD electrode pairs

Flow

Vertical Electrodes in Microchannels

Biological and Chemical Applications:
Modular Interconnect or Integrated Chip

Integrated Sample Preparation

NaCl solution
Bg spores in lysing solution

DNA Extractor

Integrated Sample Preparation

Modular Switch
Flow Cytometer

To Detection System
Reagent A
Reagent B

Combinatorial Tests

Waste
Abraham P. Lee, Ph.D.

Cell and Bead Sorting On-Chip

Sorting of Neural Stem Cells for Controlled Differentiation
(to be lectured in more detail on Week 7)

Cell Flow Method

Flow driven admittance spectroscopy

Principle of Flow Induced Admittance Gain

Diffusion perpendicular to the flow and convection along flow

\[ E_x + D_y \frac{\partial [A]}{\partial x} - v_y \frac{\partial [A]}{\partial y} = 0 \]

Under steady state flow

\[ i_l = 0.925nF[A]_{cell} D^{1/3} Q^{1/3} W \cdot \sqrt{v_x^2 / h^2 d} \]


**Flow Induced Admittance**

Depends on
- Concentration
- Frequency
- Voltage
- Conductivity
- Dielectricity


**Cell Sensing**

**Droplets in Microfluidic Arrays**
**Encapsulation of Cells**

The cells are initially sheared off at a T-junction. They proceed to an area that simultaneously focuses them to the center of the channel and stabilizes the downstream, droplet-generation process. Some channel lengths have been modified to better balance the pressures and flows in the channel.

**Device Design**

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**Encapsulation Region**

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Cell Encapsulation

The Cell – Biomolecular Systems

- Specified by
  - $4 \times 10^{9}$ base pairs
  - $10^5$ proteins
- Features
  - High sensitivity and specificity
  - Regulation & adaptation
  - Hierarchical self-assembly
  - Repair and maintenance
  - Parallel processing
  - “Just-in-time” processes
  - Non-linear dynamics
  - Asynchronous control and signalling
  - Multicellular systems
  - Scalability

Current Biomolecular Transducers

- Single molecule detection
- Gene and Protein array chips
- Sequencing of genes
- Expression of genes
- RNAi
- Other molecular binding detection techniques
Limitations of Current Technologies

- Ensemble of interactions
- Singular sensing modality
- Parallel and not sequential
- Observation of single event
- No direct link to the biomolecular event
- No ability to control the functions

Complexity of Biomolecular Networks

- Internet
- Computers
- IC chips/machines
- Bioinformatics

MEMS/NEMS (Bridging the gap)

- Future Payoffs
- Survivability in combat
- Origin of disease
- Engineering vaccines
- Identify hard targets

Continuous Monitoring and Control of Physiological Status through Access to Body Fluids

- Chips as the Bridge from the Host Response to in vivo Molecular Signatures
- Two-way transdermal technologies
- 1. Sampling
- 2. Drug Delivery
- Sweat
- ISF
- Blood
- Saliva
- Urine

Body Fluids - Portals of Entry

- Sweat
- ISF
- Blood
- Saliva
- Urine

- WBC sorting
- Protein cytokines
- Distribution
- Metabolic processes
- Rapid gene expression
- Biochemical signals
- (metabolite, hormones)
Reverse Iontophoresis – the
GlucoWatch by Cygnus (Redwood City)

- GlucoWatch biographer are:
  - frequent,
  - automatic,
  - non-invasive,
  - time-averaged measurements
  - of transdermally extracted glucose
  - that are calibrated to blood glucose
- Able to detect trends and track patterns in glucose levels.
- The result is more information.
- Something that people with diabetes, and the professionals who care for them, have been seeking for many years.
- Still needs to be calibrated by blood acquired by finger pricking every 12 hours.

Microneedles

- Have been fabricated out of single crystalline silicon (selective etching), polycrystalline silicon (micromolding), Pd (electroplated)
- Can create complex geometries
- Can be used for new protein-based, DNA-based, and other therapeutic compounds produced by modern biotechnology since it avoids GI tract and first-pass effects of the liver associated with oral delivery or pain and inconvenience of IV.

Microneedles for Transdermal Drug Delivery

- Permeability of calcein was increased by 3-4 orders of magnitude
- There is a time dependence on increased permeability
- Reactive ion etching to fabricate both solid and hollow tubes
- Tests were carried out using human skin from autopsy and plastic surgery procedures (epidermis)
- In vivo tests showed no "ouches"

Collection Using Reverse Iontophoresis

SAMPLING

Source: www.cygn.com

Source: Rosemary Smith, UC Davis

Sample extraction is obtained by means of capillary and extracellular fluid osmotic forces.

Henry et al., J. Pharm. Sci. (1998), vol. 87, no. 8, p. 922

26 gauge needle

Same magnification

12.5 µm

12.5 µm

length 150mm, radius <1mm
Figure 1. Warfighter Physiological Status Monitoring (WPSM)

USAMRMC/Military Operational Medicine Research Program/Ft. Detrick, MD
POC: COL John P. Obusek (508) 233-4811/LTC Karl E. Friedl (301) 619-7301
Graphics: Janet G. Reese

**SENSORS/MEASUREMENTS**
- Headband EEG and Oximetry
- Acoustic
- Voice Stress and Content Analysis
- Dead Reckoning Module
- EKG, EMG, and Thoracic Impedance Cardiography
- Body Core and Skin Temperature
- Environmental and Other Parameters
- Tissue pH, Glucose, and Lactate
- Near-Infrared (or Other) Technology
- Wrist-Worn Actigraph
- Foot Contact (Weight/Locomotion)
- Wireless Inter-Module Communication

**PHYSIOLOGICAL CONSEQUENCES OF CONCERN**
- Hypothermia
- Hyperthermia
- Hypoxia
- Metabolic Fatigue
- Vigilance Lapses
- Dehydration
- Psychological Stress
- Inadequate Restorative Sleep
- Desynchronization of Circadian Functions
- Jolt, Blast, and Repeated Impact Exposure
- Toxic Substance Exposure

**Tool Kit** to Understand Warfighter Physiology
- Specifications for Minimal Sensor Set to Predict Warfighter Physiology

**CURRENT**

**FUTURE**
* Concept

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Monitoring the Warfighter’s Health Parameters

Bio Defense Applications
- Combat casualty care, triage
- Battle readiness
- Human responses during testing
- Controlled drug delivery
- Presymptomatic, early detection of infections
- Distributed, covert deployment of bio-detectors
- Rapid indication of CBW Incident

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