Intro to Lab: Modeling a Microvascular Network on a Chip

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Lab Objectives

- To characterize simple model of microvascular system using microfluidic device
- To introduce you to microfluidics
 - Measure flow velocities within model network before and after a blockage (occlusion or clot of a blood vessel - stroke)

particle velocimetry

- Show how EE concepts transfer to the design and analysis of a microfluidic network
- Lab will be accompanied by an assignment
 - theoretically calculate pressure and velocity in each channel before and after blockage
 - compare calculated pressure and velocities with empirical velocities

What is Microfluidics: What's in a name?

- Infer: manipulation of minute quantities of fluids (L and G) ... inside very small devices
- Applies ultra-precise fab technology to conventionally "messy" fields like biology (dishes, plates) and chemistry (vats, reaction vessels)
 - Emerged early 90s: Andreas Manz, George Whitesides, but idea has been around much longer:
 - Richard Feynman "There's Plenty of Room at the Bottom" 1959
 - Why?
 - Unique physics at microscale permits novel creations
 - Permits more orderly, systematic approach to bio-related problems, reduces physical effort:
 - drug discovery
 - cytotoxicity assays
 - protein crystallization

Background

- Aims to do for chemistry and biology what Kilby's and Noyce's invention of the IC did for the field of electronics:
 - dramatic reduction in size and cost
 - increase in performance and portability
 - lab on a chip: goal to have all stages of chemical analysis performed in integrated fashion:
 - sample prep.
 - rxns
 - analyte sep.
 - analyte purification
 - detection
 - analysis
 - μTAS, POCT, decentralized
- Benefits: integration of functionality, small consumption of expensive reagents, automation, high throughput, reproducibility of results, parallelization, disposable

Defining Characteristics – Surface Area

Compare surface area to volume ratio of cubes 1 cm³, 100 μm³, and 10 μm³, 100nm³, and 10nm³ on a side:

side length (I)	side area (l ²)	surface area (6l ²)	volume (l ³)	SA/V ratio
0.01	0.0001	0.0006	0.000001	600
0.0001	0.0000001	0.0000006	1E-12	60000
0.00001	1E-10	6E-10	1E-15	600000
0.0000001	1E-14	6E-14	1E-21	6000000
0.0000001	1E-16	6E-16	1E-24	60000000

Defining Characteristics – Surface Area

- Surface effects become dominant:
 - Surface tension
 - Disparity in intermolecular forces among molecules in bulk and at interface – leads to formation of droplets or bubbles
 - Capillary action
 - Wicking of fluid through a capillary tube (microchannel) when adhesive attraction for walls > than cohesive forces among liquid molecules



Droplets on hydrophobic surface: cohesive forces > adhesive forces fluid beads into a droplet

Note: More pronounced in smaller tubes

Defining Characteristics – Laminar Flow

Compare surface area to volume ratio of cubes 1 cm³, 100 μm³, and 10 μm³, 100 nm³, and 10 nm³ on a side. Calculate the gravitational force acting on each cube assuming it is made of silicon with a density of 2.33 g/cm₃:

side length (I)	side area (l ²)	surface area (6l²)	volume (l³)	SA/V ratio	volume in cm ³	mass (g)	mass (kg)	F = ma (N)
0.01	0.0001	0.0006	0.000001	600	1	2.33	0.00233	0.022834
0.0001	0.0000001	0.0000006	1E-12	60000	0.000001	0.0000233	2.33E-09	2.2834E-08
0.00001	1E-10	6E-10	1E-15	600000	0.00000001	2.33E-09	2.33E-12	2.2834E-11
0.0000001	1E-14	6E-14	1E-21	6000000	1E-15	2.33E-15	2.33E-18	2.2834E-17
0.00000001	1E-16	6E-16	1E-24	60000000	1E-18	2.33E-18	2.33E-21	2.2834E-20

Conclusion: gravitational force is negligible at microscale when compared to other forces (like surface forces)

Defining Characteristics – Laminar Flow

Reynolds number – compares relative strengths of different forces (inertial vs. viscous)

- small Re: laminar flow (<2300)</p>
- Iarge Re: turbulent flow (>2300)
- Re in most µfluidic devices << 1</p>
 - Laminar flow is both an asset and hindrance:
 - Transport of objects is predictable
 - Mixing is difficult two parallel streams will mix only across the interface via Fickian diffusion



Examples of Microfluidics



LSI



Body on chip



Concentration gradient



Filtering Without Membranes



Traction force microscopy



Portable medical diagnostics

Examples of Microfluidics





Cantilever-based biosensors



Biomimesis

Lab on a CD / Centrifugal Microfluidics



Microneedle Array for Painless Drug Delivery



Microfabrication

> Droplet Microfluidics

Field Overview

- Very low tech to very complex
- Single layer lithography to multiple layers requiring alignment
- Minimalistic (paper-based) vs. sophisticated
- Components vs. Systems (hierarchical)
- Devices vs. Applications

Components → Systems

- Wide array of components: components together make up systems:
 - µreactors
 - µmixers
 - µheaters (PCR, cell culture)
 - µfilters cell capture
 - µvalves
 - µpumps

Everything at macroscale has counterpart at microscale, but microscale physics permits novel components



Application 1: motile sperm sorter

- Membraneless H-filter
 - low-Re number so mixing occurs by diffusion
 - Two streams of fluid will flow alongside each other
 - Exploit this to separate particles or cells based on differences in diffusivity or motility





Application 2: laminar flow to study individual cell movement

- Cellular response to stimuli can be probed
- Trypsin disrupts cell-substrate adhesion
 - causes cell to lose its hold and recoil to the trypsin-free stream



Application 3 – drug screening

- Each droplet can serve as a reaction vessel to study a cell's response to antibiotic
 - Encapsulate bacterium into droplet
 - Droplet containing viability indicator is merged with bacterium containing droplet
 - Droplet containing antibiotic is merged with this droplet, incubated, and intensity of fluorescent agent measured







Commercialization

- Despite being subject of ongoing research, microfluidics is being commercialized (startups, spinoffs, lucrative extensions to established)
- Microfabrication-related industries (MEMS, inkjet heads, accelerometers, gyroscopes) much more mature and profitable
 - No standards (DIP), competing paradigms, diff. materials
- Research has spawned microfluidics companies
 - high-throughput low-cost DNA sequencing
 - portable diagnostic tools
 - novel displays for E-readers

Commercialization

Company	Assigned microfluidic and microarray patents (US only)
Caliper Lifesciences	215
Agilent	97
Affymetrix	73
Nanostream	34
Nanogen	20
GE Healthcare (formerly Amersham)	19
Zyomyx	16
Illumina	15
Gyros	14
Tecan	13
Honeywell	11
Advion	10
Cepheid	9
Beckman Coulter	8
Biacore	8
Micronics	8
Fluidigm	7
Qiagen	6
Sequenom	6
Aurora Biosciences	4
Micralyne	4
Bioforce Nanosciences	3
Eksigent Technologies, LLC	3
Evotec Technologies	3
Genomic Solutions	3
Genetix	3
Molecular Devices	3
Randox Laboratories	3
Innovadyne	2
Telechem International	2
Amnis Corporation	1
BioDot	1
Bio-Rad	1
BioTrove	1
Cellectricon	1
Combimatrix	1
Perkin Elmer	1
Shimadzu	1

Table 1 Companies assigned microfluidic and microarray patents in the USA

Connection to EE

- Basic concepts have direct analogs to EE (voltage, current, resistance)
- Design and analysis of devices uses same methods used to analyze circuits (KCL, KVL, Ohm's Law)
- Fabrication of devices uses tools and techniques borrowed from semiconductor industry
- Entire electronic devices have counterparts in microfluidics:
 - shift register
 - voltage divider (drop pressure)
 - current divider (split flow)
- Control is effected in same way:
 - multiplexing
 - truth tables

Fabrication: How Are Microfluidic Devices Made?

- Semiconductor fabrication methods
- Pattern is defined on surface (2D) and then transferred into vertical plane
- Process flow depicted as a cross-sectional view, each step showing execution of one step (addition of layer, exposure, removal of layer, etc.)
- Detailed process to create master, replicas created inexpensively

Fabrication: How Are Microfluidic Devices Made?

- Top-down vs. bottom-up
- Bottom-up:
 - μcontact printing for SAM
- Top-down:
 - Bulk (subtractive)
 - Isotropic etching
 - wet
 - Anisotropic etching
 - dry
 - Laser ablation
 - CMP
 - Surface (additive structural and sacrificial layers)
 - spin coating
 - electroplating
 - oxidation
 - chemical vapor dep. evaporation
 - physical vapor dep. sputtering



lsotropic (HF)



Directional Crystal planes (KOH)



Anisotropic (RIE)

Process Flow: Soft-Lithography



4) Apply Anti-stick monolayer



5) Cast PDMS



6) Plasma treat + bond



Example: Serial Dilution Gradient Generator

- Works on laboratory practice of serial dilution:
 - Create a number of solutions, each with a unique concentration, by using a small amount of the more concentrated solution to create the next less concentrated solution:
 - More reliable
 - Log base 10 Example:
 - Stock solution consists of 1mL of solute in 9mL of solvent = 1X
 - 1mL of this into 9mL solvent yields 0.1X
 - 1mL of this into 9mL solvent yields 0.01X
- In a microfluidic implementation, the concentration of each step is determined by the ratio of two merging flow rates at the junction of the buffer and the agent
- After the chemical to be diluted is merged with the buffer at the intersection, the combined solution is passed through the mixing region
- Some portion of the diluted solution is collected at the outlet and remainder is passed on to the next intersection



Serial Dilution: Log Base 10 Profile

```
System of Equations to Solve (42 equations in 33 variables):
1. v_1/rL + (v_1 - v_2)/r_4 + (v_1 - v_4)/r_2 + (v_1 - v_2)/r_2 + (v_1 - v_3)/r_4 - I_2 = 0
                                                                                                       22. v_7 = i_4 r_7
2. (v_2 - v_1)/r_4 + (v_2 - v_5)/r_{miv} + (v_2 - v_3)/r_5 = 0
                                                                                                       23. v_7 - v_6 = (i_{10} + I_1 - i_6 - i_5 + i_9 - i_4) * r_{split}
                                                                                                       24. v_6 - v_9 = (i_{10} + I_1 - i_6 - i_5 + i_9 - i_4 + i_8) * r_{mix}
3. -I_1 + (v_2 - v_2)/r_1 + v_2/rR = 0
                                                                                                       25. v_0 = i_2 r_6
4. (v_4 - v_1)/r_3 + (v_4 - v_5)/r_{solit} + (v_4 - v_7)/r_{mix} = 0
                                                                                                       26. v_{9} - v_{8} = (i_{10} + i_{1} - i_{5} - i_{5} + i_{9} - i_{4} + i_{8} - i_{3}) * r_{solit}
5. (v_5 - v_4)/r_{snlit} + (v_5 - v_2)/r_{mix} + v_5/r_8 = 0
                                                                                                       27. v_8 = i_2 r_{mix}
6.(v_6-v_1)/r_2+(v_6-v_7)/r_{snlit}+(v_6-v_9)/r_{mix}=0
                                                                                                       28. i_2 = i_7 + i_{10} + i_1 - i_6 - i_5 + i_0 - i_4 + i_0 - i_2
7. (v_7 - v_6)/r_{snlit} + (v_7 - v_4)/r_{mix} + v_7/r_7 = 0
                                                                                                       29. i_1 = i_2
                                                                                                       30. i_1 = i_2
8. (v_8 - v_1)/r_1 + (v_8 - v_9)/r_{snlit} + v_8/r_{miv} = 0
                                                                                                       31. i_1 = i_4
9. (v_9 - v_8)/r_{solit} + (v_9 - v_6)/r_{mix} + v_9/r_6 = 0
                                                                                                       32. i_1 = i_5
10. I_0 + I_1 = i_1 + i_2 + i_3 + i_4 + i_5 + i_6
                                                                                                       33. i_1 = i_6
11. i_{10} = I_0 - i_1 - i_7 - i_8 - i_9
                                                                                                       34. 0.0001 = ((i_2 - i_7)/i_2) * 0.001
12. v_1 - v_2 = i_{10} * r_4
                                                                                                       35.\ 0.001 = ((i_{10}+I_1-i_6-i_5+i_9-i_4)/(i_{10}+I_1-i_6-i_5+i_9-i_4+i_8))*0.01
                                                                                                       36. 0.01 = ((i_{10}+I_1-i_6-i_5)/(i_{10}+I_1-i_6-i_5+i_9))*0.1
13. v_1 - v_4 = i_0 * r_3
                                                                                                       37. 0.1 = ((I_1 - i_6)/(i_{10} + I_1 - i_6))*1
14. v_1 - v_6 = i_8 r_2
                                                                                                       38. r<sub>4</sub> = 10000
                                                                                                                                                Node Voltage Equations
15. v_1 - v_8 = i_7 r_1
                                                                                                       39. r<sub>5</sub> = 2500
                                                                                                                                                KCL
16. v_1 = i_1 rL
                                                                                                       40. r<sub>split</sub> = 2500
                                                                                                                                                Ohm's Law
17. v_2 - v_2 = (I_1 - i_6) * r_5
                                                                                                                                                Equations stipulating flow rates
                                                                                                       41. r<sub>mix</sub> = 31446.54
                                                                                                                                                of all six concentrations be
                                                                                                       42. I_0 = 1
18. v_2 - v_1 = (i_{10} + I_1 - i_6) * r_{mix}
                                                                                                                                                equal
19. v_5 = i_5 r_8
                                                                                                                                                Equations specifying
                                                                                                                                                concentrations
20. v_5 - v_4 = (i_{10} + I_1 - i_6 - i_5) * r_{split}
                                                                                                                                                Selected values
21. v_4 - v_7 = (i_{10} + I_1 - i_6 - i_5 + i_9) * r_{mix}
```

Serial Dilution: Log Base 10 Profile

MATLAB Results:

Having specified:

$$R_{mix} = 31446.54$$

$$R_{split} = 2500$$

$$R_{4} = 10000$$

$$R_{5} = 2500$$

$$I_{o} = 1$$

$$C's = 1,0.1,0.01,0.001,0.0001,0$$

Yields:

$$I_1 = 0.2273$$

component	length (um) mul	tiple of r _{mix}
rL	146682.00	4.66
rR	136960.75	4.36
r1	128039.40	4.07
r2	81206.33	2.58
r3	45258.86	1.44
r4	10000.00	0.32
r5	2500.00	0.08
r6	31696.54	1.01
r7	66562.73	2.12
r8	101745.89	3.24
rmix	31446.54	1.00
rsplit	2500.00	0.08

Serial Dilution: Log Base 10 Profile CAD Image



Experimental Results: Log Base 10 Profile





Grayscale fluorescent image of three most concentrated solutions, 40x magnification, ISO400, 10s, 35uM fluoroscein solution,1uL/min buffer flow rate

Biological Relevance

Stroke

- Interruption in blood supply to the brain caused by ischemia (insufficient blood flow) due to a blockage (blood clot, thrombus, embolism)
- Atherosclerosis
 - Narrowing of blood vessels due to build-up of fatty deposits
 - Leads to hypertension, which in turn exacerbates atherosclerosis
 - High blood pressure causes distension of vessels, which damages endothelium lining, which in turn attracts more fatty deposits

Microfluidic analogs of vascular system

- 2008 Harvard investigated process of vasoocclusion (in sickle cell anemia patients) looking at geometrical, physical, chemical, bi ological factors that contribute
 - Channel width, total pressure difference across network, oxygen levels
 - Timed how long before an occlusion occurred
- 2008 Cornell created 3D vascular network using strands of molten sugar (cotton candy)
 - Sugar as sacrificial layer



Your Microfluidic Device



Particle Velocimetry

- Technique to qualitatively visualize and quantitatively assess fluid flow
 - Small tracer particles (beads) are added to fluid
 - Velocity of beads assumed to be same as velocity of surrounding fluid
 - Special buffer solution used to prevent beads from aggregating / adhering to channel surfaces

Note on Bead Motion

- In pressure-driven flow, velocity profile of fluid is parabolic
- v max in center
- v min near walls
- Be systematic: observe same location or take same number of beads from each location before and after blockage!



Procedure: Before Clot

- PDMS devices made for you
- Fill syringe with bead (100m dia.) solution
- Connect needle to syringe
- Connect tubing to needle
- Place syringe in syringe pump
- Flow @ 500L/min to flush out bubbles
- Reduce flow rate to 2uL/min
- Capture series of images to track beads in each channel of device

V = d/t

Procedure: Clot

- Disconnect device from pump
- Using empty syringe, introduce air to dry device
- Punch hole in desired channel with punch
- Remove plug of PDMS
- Inject sealant to block channel
- Cure @ 60C for 10min
- Repeat process: track beads in each channel

How Many Channels?



Circuit representation of microvascular chip



Results and Data Analysis Expected

- Average velocity for each channel before blockage
- Average velocity for each channel after blockage
- Prediction of flow pattern after channel blockage
- Bead Motion Analysis:
 - Want to track at least 10 beads in each channel
 - Track beads in each channel over several frames
 - Not interested in instantaneous velocity of beads (changes from frame to frame)
 - Interested in average velocity (total distance traveled / time observed)
 - Bin data to generate histogram (data partitioned into intervals and frequency of occurrence plotted)

Report Guidelines – SHEN/BRUCE

Background

- In analyzing a microfluidic device, it is useful to make an analogy to an electrical circuit:
 - Pressure → Voltage (Ground = reference potential → same pressure)
 - Flow Rate → Current
 - Hydraulic Resistance → Electrical Resistance (each channel gets represented by resistor)
 - R=C_{geometry}*L/A²
 - For channel with square cross section:
 - R = 120L/[(w^2)(h^2)]

Special cases:

Open circuit = infinite resistance

Short circuit = zero resistance

In this lab, blocking a channel is equivalent to an open circuit = zero flow

Analysis: Circuit Analog

- Can employ circuit analysis techniques and rules like Ohm's law, KCL, KVL to facilitate analysis
- Ohm's Law:
 - V = iR
 - Pressure = Volumetric Flow Rate * Channel Resistance
 - Volumetric flow rate = average linear velocity * crosssectional area of channel
- KCL:
 - Conservation of charge \rightarrow conservation of mass:
 - Flow rate into a node MUST equal flow rate out of that node
 - Nothing collects at node
- KVL:
 - Conservation of energy
 - Sum of voltage drops around a closed loop equals zero



Convention: Define flow into node as + and flow out of node as –

$$I_1 + I_2 - I_3 = C$$

 $I_3 = I_1 + I_2$

Pressure Drop Along Channel Equation valid only if w >>h

 $\Delta P = \left(\frac{-12\mu QL}{wh^3}\right) \mapsto rectangular \ channel$

 $\Delta P - pressure \ drop$ $\mu - dynamic \ viscosity$ $Q - volume \ flow \ rate$ $L - channel \ length$ $w - channel \ width$ $h - channel \ height$

Circuit Analysis

 Resistances are either in series or parallel, except for when they're not

Resistors in Series



 $R_T = R_1 + R_2 + R_3 =$ 1000+2000+3000 = 6000 ohm

Sum is ALWAYS greater than any single resistance

Same current through each one

Resistors in Parallel

R_T = 1 / (1/R₁ + 1/R₂ + 1/R₃) = 1/ (1/1000 + 1/2000 + 1/3000) = 545 ohm

Sum is ALWAYS less than any single resistance

Special Case: 2 in parallel: $R_T = R_1 * R_2 / (R_1 + R_2)$

Same voltage across each one



Example: Resistance Network







1. R1 and R2 in series = 6k





2. R3 and R4 in series = 500 ohm



3. 500 ohm in parallel with 1500 ohm = 375 ohm



Remaining resistors in series = 6000 + 375 + 250 + 400 = 7025 ohm

Special Case: Delta-to-Wye (Δ-toY) Transformation

- Used when resistors are neither in series nor parallel
- How to identify this case:
 - Sometimes two resistors look like they are in parallel but they really aren't
 - Ask: do they share nodes at both terminals?
 - If yes, they are in parallel
 - If no, they aren't

Delta-to-Wye (Δ-toY) Transformation



Delta-to-Wye (Δ-toY) Transformation



Equations for the transformation from Δ -load to Y-load

$$R_1 = \frac{R_a R_b}{R_a + R_b + R_c},$$
$$R_2 = \frac{R_b R_c}{R_a + R_b + R_c},$$
$$R_3 = \frac{R_a R_c}{R_a + R_b + R_c}.$$

Equations for the transformation from Y-load to Δ -load

$$\begin{split} R_{a} &= \frac{R_{1}R_{2} + R_{2}R_{3} + R_{3}R_{1}}{R_{2}}, \\ R_{b} &= \frac{R_{1}R_{2} + R_{2}R_{3} + R_{3}R_{1}}{R_{3}}, \\ R_{c} &= \frac{R_{1}R_{2} + R_{2}R_{3} + R_{3}R_{1}}{R_{1}}. \end{split}$$

Example





Example



3. See that (R2+R8), (r5+R6), an d R4 are in a delta configuration and that further analysis can be promoted by converting to wye configuration



4. See R1 in series
with R1 and R3 in
series with R2 and
R3 in series with R7



Example



Voltage Divider

- Key Idea: The voltage that appears across a resistor – "dropped across it' – is proportional to the resistance
- Intuitive: If two resistors are in series, we expect that the one with more resistance will see the majority of the voltage
 - Since resistors in series have the same current through them, we can expect the component with greater resistance will "consume" most of the voltage

Voltage Divider

Voltage Divider Equation:



$$V_{R_2} = V_{in} * R_2 / (R_1 + R_2) =$$

12*3/(3+1) = 12V*3/4 = 9V

$$V_{R_1} = V_{in} - V_{R_2} =$$

 $V_{in} * R_1 / (R_1 + R_2) =$
 $12V * 1/(1+3) = 12V * 1/4 = 3V$

Could also solve using KVL

simply a proportion!

Voltage Divider = Pressure Divider

- In a microfluidic network, same rule holds:
 - Channels with high resistances (shallow, narrow, long) will have large pressure drop across them
 - Channels with low resistances (tall, wide, short) will have small pressure drop across them

Current Divider

- Key Idea: The current that flow through a resistor is inversely proportional to the resistance
- Intuitive: If two resistors are in parallel, we expect that most of the current will pass through the one with less resistance – "follow the path of least resistance"

Current Divider



Current Divider Equation:

$$i_{R_1} = I_{i_1} * R_2 / (R_1 + R_2) = 12A * 3/(1+3) = 9A$$

 $i_{R_2} = I_{i_1} * R_1 / (R_1 + R_2) = 12A * 1/(1+3) = 3A$

Could also solve using KCL: $i_{R_2} = I_{in} - i_{R_1}$

Current Divider = Flow Divider

- In a microfluidic network, the same rule holds:
 - Channels with high resistances (shallow, narrow, long) will have small fluid flow through them
 - Channels with low resistances (tall, wide, short) will have large fluid flow through them

Composite Example



Find current through each resistor and voltage drop across each resistor

Solution: First, reduce to Rtotal



Assignment

- Several questions related to your lab and to lecture content
- Remember: linear velocity = volumetric flow rate / cross-sectional area of channel

Solution: Second, Find i_T

- Ohm's Law:
- V=iR
- I_T = V/R_T = 10V/5833.33Ω = 0.00171A =
 1.71mA

Solution: Third, find I and V for each resistor

Observation: Current through $R_1 = i_T$

Ohm's Law: V=iR VR₁= i_T *R₁ = 1.71mA*5k Ω = 8.57V

Observation: V across $(R_2+R_3) = V$ across R_4 **KVL:** $V_{R4} = 10V-8.57V = 1.43V$ **Ohm's Law:** $i_{R4} = V_{R4}/R_4 = 1.43V/1k\Omega = 1.43mA$

Observation: $i_{R2} = i_{R3}$ **KCL:** $i_{R2} = i_{R3} = i_{R1} - i_{R4} = 1.71 \text{mA} - 1.43 \text{mA} = .28 \text{mA}$ **Ohm's Law:** $V_{R2} = .28 \text{mA} + 2k\Omega = .56V$ **Ohm's Law:** $V_{R3} = .28 \text{mA} + 3k\Omega = .84V$