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MICROFLUIDICS BOOTCAMP 2012

PRINCETON PHYSICAL SCIENCES-ONCOLOGY CENTER
PRINCETON, UCSF, JOHN HOPKINS, UCSC, SALK

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Part I

Lecture Notes

Lecture 1: Microfluidics, Fluid Mechanics and Scaling

Have you used a microfluidic device before? If you have used an ink-jet printer, you have used a microfluidic device. The ink-jet nozzle shoots picoliter (10^{-12} liter) volume drops of ink onto a page. Volumes this small fall within the ambit of microfludics. We can define microfluidics as the science and technology of controlling fluids (gases, liquids) at the micro-scale.

While printer cartridges are the most common microfluidic device, in this workshop, we are primarily interested in the bio-chemical applications of microfluidics rather than physical applications like printing[1]. And while picoliter volumes look small, they are still large in comparison to volumes common in biology and chemistry. For example, consider a bacteria like *Esherichia Coliform* (E.Coli) - nearly 10,000 E.Coli would fit in one picoliter. Scaling down into the world of proteins and atoms, over a million molecules of ribozyme can fit in one picoliter. And a 1 picoliter drop of water contains nearly 10^{13} molecules. Other volumes are more comparable: about 10 red blood cells would fit into 1 picoliter.

This leads us confronting the *central problem of microfluidics*: how to make machines and devices that manipulate cells, proteins and other biological materials, bridging the gap between everyday volumes we are familiar with to the micro-scale volumes common in biology. If successful, this plumbing could be used to miniaturize and automate

Conversion units:
$1000\mu m^3 = 1$ picoliter = Volume of a $10\mu m$ side cube
$10^6\mu m^3 = 1$ nanoliter = Volume of a $100\mu m$ side cube

[1] However, ink-jet printing is indeed used to make micro-arrays

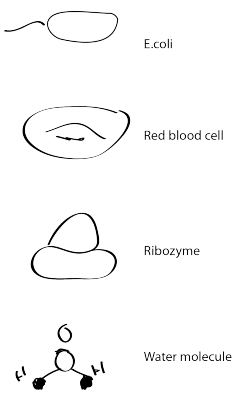


Figure 1: Volume of cells and proteins: 10000 E.coli = 10 RBC = 10^6 ribozymes = 10^{13} water molecules

sequencing, protein crystallization, immunoassays, cell cultures and many, many other applications[2].

[2] S.J. Maerkl. Integration column: Microfluidic high-throughput screening. *Integrative Biology*, 1(1):19–29, 2009

And, this is not all: perhaps we may find that physical laws at the micro-scale will allow machines and techniques that would simply be impossible at a larger scale. Indeed, many of the recent next generation sequencing schemes[3] would have been impossible if not for the way fluid mechanics behaves at the micro-scale.

[3] B.M. Paegel, R.G. Blazej, and R.A. Mathies. Microfluidic devices for dna sequencing: sample preparation and electrophoretic analysis. *Current opinion in biotechnology*, 14(1):42–50, 2003

A Tour of Fluid Mechanics

Despite several centuries of active work on fluids - far from being a sterile field where everything is understood, surprises hide at every corner. Still a formidable amount *is* known. Fluid mechanics is a large field, rich and deep. We will attempt a whirlwind tour of fluid mechanics in this section, proceeding in an approximately historic order. Of course, the reader will understand that this is not a genuine history, just a pedagogic method to quickly introduce basic concepts.

One way to think of fluid-mechanics is that it is a series of approximations to what a fluid is[4]. We build mathematical models that capture essential elements, while ignoring seemingly inconsequential or confounding complexities - hoping that they won't matter. Sooner or later, however, experiments will reveal the model fails against reality, and we need to add to our approximation as understanding increases with time. People have been trying to understand fluids for a long time and this process continues today. To start with there are three basic concepts that should be clearly understood - buoyancy, pressure and viscosity.

[4] A wonderful set of educational videos on fluid dynamics by prominent scientists can be found here `http://web.mit.edu/hml/ncfmf.html` for free (needs Real media player)

Figure 2: Archimedes' face on the Fields Medal in Mathematics. Archimedes was a military engineer and was successfully able to use his laws of buoyancy as well as his law of levers to overturn Roman ships that attacked his city.

[5] A good introduction to his life and times is at: `http://www.math.nyu.edu/~crorres/Archimedes/contents.html`

Buoyancy

Our starting point is the law of buoyancy discovered by Archimedes (280 to 212 BC, Syracuses, Sicily), supposedly while trying to solve the problem given to him by the king of finding out whether a crown was made of real gold (most historians think this story is mythical)[5]. Archimedes principle states that a body immersed in a fluid experiences a buoyant force equal to the weight of the fluid it displaces with its submerged volume. All bodies in any fluid experience this force. If an object was fully submerged, its apparent weight is given by:

$$W_{\text{fluid}} = W\left(1 - \frac{\rho_{\text{fluid}}}{\rho}\right) \tag{1}$$

Fluids exert a force on any body in them on the surface area that is

submerged. Where does this force come from? To understand this we need to understand the concept of pressure.

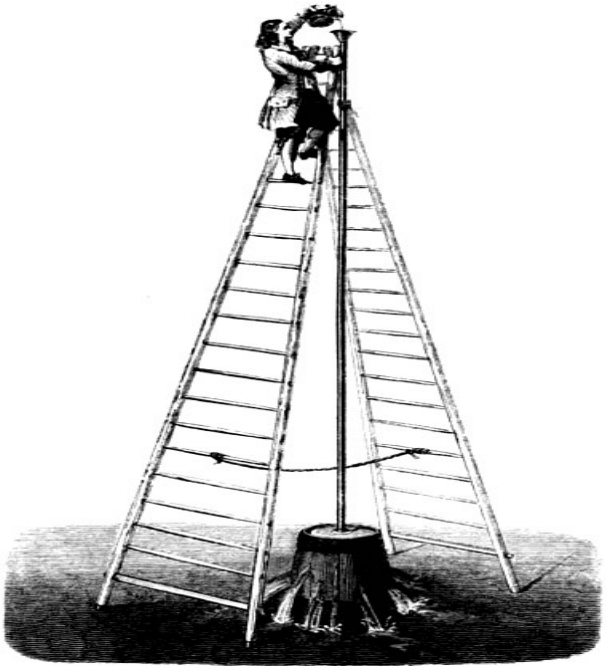
Figure 3: Blaise Pascal (1623-1662)

Pressure

The explanation for buoyancy lies in pressure exerted by the fluid on the solid body, which when integrated over the solid body surface area results in the buoyant force.

But what is pressure? Imagine that you put an small object in water - pressure is simply the force needed per unit area to keep water out. But this simple explanation hides the subtle nature of pressure - something that took many centuries to understand. Let's go back to our history to see why. After the Roman and Greek civilizations there was a long hiatus in scientific achievement in western Europe, though progress was being made elsewhere. It took the blossoming of the renaissance to clear the fogs of ignorance.

(a) Gasparo Berti's experiment



(b) Pascal bursting a barrell with pressure

In the 16th century it was observed that that suction piston pumps could only hold up a column of water about 10 meters long. Galileo, the world's modern scientist investigated this phenomena and came to the wrong conclusion that a vacuum force was responsible for holding up the column of water. At that time a controversy was raging on whether a vacuum could exists or not, and Galileo, bravely sticking his neck out

as usual, believed it did and it even exerted a force. Gasparo Berti, a follower of Galileo, tried to create a vacuum by using a long lead tube that he filled water, plugged both ends and then opened one end in a basin of water. Some water flows out but again a column of about 10.7 meters remains with a seemingly empty space above. Evangelista Torricelli replaced water with mercury resulting in a much smaller column, and of course, inventing the modern barometer. Finally, Blaise Pascal building on these mercury column experiments made a leap by proposing that it was weight of the air in the atmosphere that held up the column. This he proved by taking the barometer to a higher altitude and finding the column become shorter. This weight (=force) per unit area is the called pressure and is transmitted throughout the fluid in all directions.

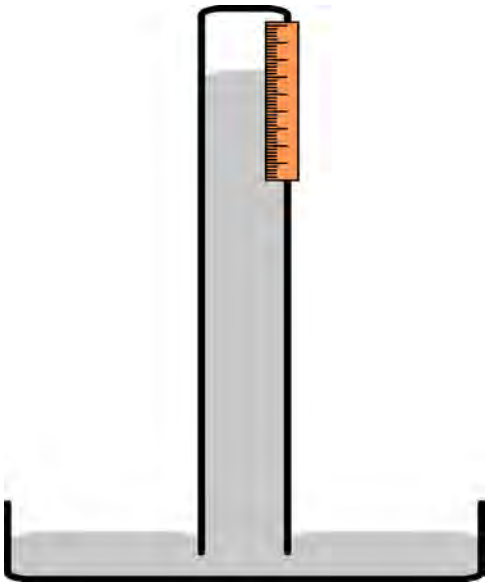


Figure 4: Mercury Barometer

At a given point, pressure in a fluid is the same in any direction - it is what physicists call a *scalar* quantity that does not have a direction in space associated with it. The SI unit of pressure is now called the Pascal (Pa) in Blaise Pascal's honor.

Consider a water tank standing undisturbed by itself. If you took a pressure sensor and measured the pressure you would find that the pressure depended linearly on depth given by:

$$p - p_0 = \rho g h \tag{2}$$

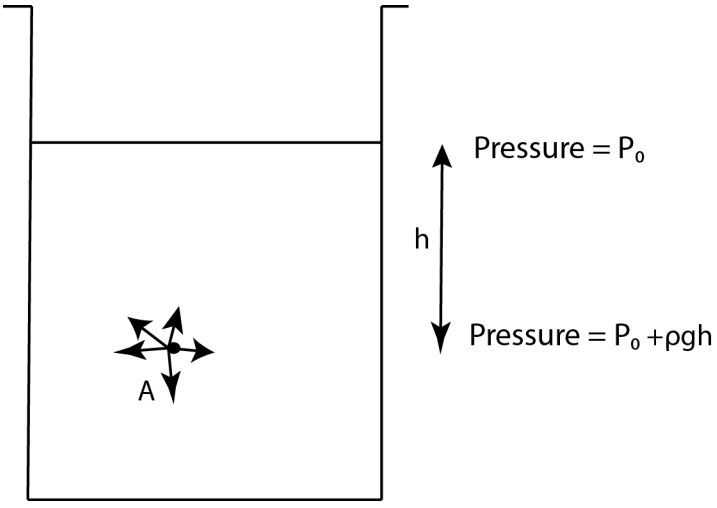


Figure 5: Pressure at a point A in a stationary fluid is the same in all directions and depends on the height of the column above it.

where p_0 is the atmospheric pressure (or reference pressure), ρ the density of the liquid, and h the height of liquid column. This equation can be derived from elementary considerations of force balances: the column of water is not accelerating, so from Newton's law its weight

must be balanced by the pressure exerted over any surface area chosen.

Pressure is measures in units of N/m^2 also called a Pascal(Pa). In practice, a variety of units are used - including bar ($= 10^5 Pa$) and Torr, named after Torricelli and referring to the mercury barometer. It is worth remembering the conversion factors between them. Atmospheric pressure is about 10^5 Pa. This being the US, we will must make things harder for ourselves by using psi (pounds per square inch) as our measuring unit in experiments! About 14.7 psi is equal to one atmospheric pressure as a point of reference.

Atmospeheric Presure
$1.01325x10^5$ Pascal (Pa)
1.01325 bar
1 atmosphere (atm)
760 torr
14.696 pound-force per square inch (psi)

A fluid has, at any given point, several physical properties - each point is associated with a pressure, temperature, a velocity etc. The job of a fluid mechanist is to try to predict, knowing some initial conditions and the geometry, what these values will be at a future time. As one knows from weather prediction, this is a miserable endeavor! Only in certain simple cases can complete prediction be made. Still, much information can be gleaned about the behavior in general, even if we cannot calculate everything. Let's continue with our history.

Viscosity

Next in the line of illustrious scientists is Issac Newton. Newton being rather perspicacious, grasped that fluids have a special property called viscosity. He describes an experiment: imagine two parallel plates separated by a distance d, and with a liquid in between. If one were to move the top plate forward, while keeping the bottom plate fixed- how much force is required? The force it turns out depends on the area of the plate, the velocity you want to move it, and the distance between the plates and a characteristic number for each fluid called the viscosity. We can write for the force.

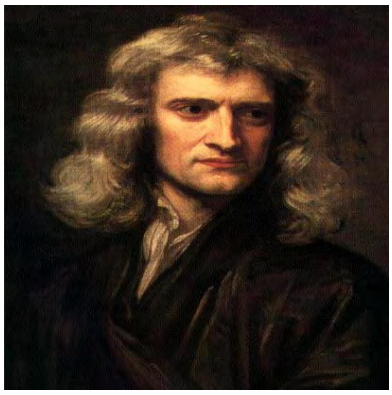


Figure 6: Issac Newton (1642-1727)

$$\frac{F}{A} = \mu \frac{v}{d} \tag{3}$$

Here F is the force, v the velocity of the plate, A the area of the plate and d the distance between the plates, and μ the viscosity. Newton realized that in contrast to solids,where the force to shear them depends on the distortion, in fluids the force depends on the *rate of distortion*. This is a fundamental difference between fluids and solids.

For many fluids the coefficient of viscosity is nearly independent of velocity. These are called Newtonian fluids. For some fluids, this is not the case and the viscosity can vary in many interesting ways depending on the velocity or past history. A classical example of this is silly putty.

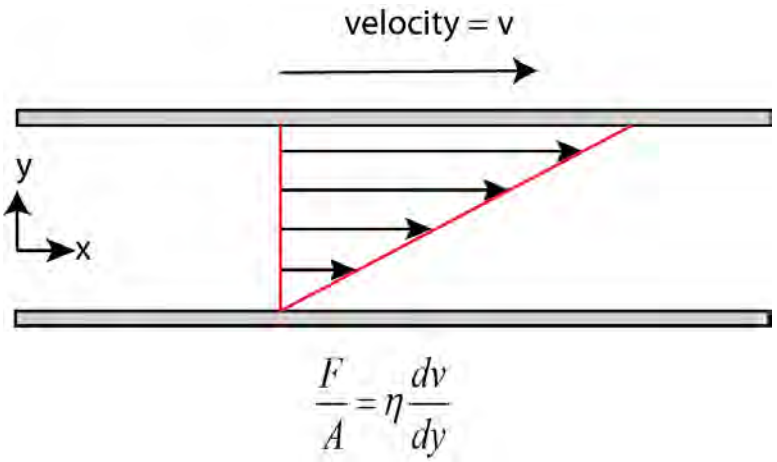


Figure 7: Viscosity determines the force needed to move the plate

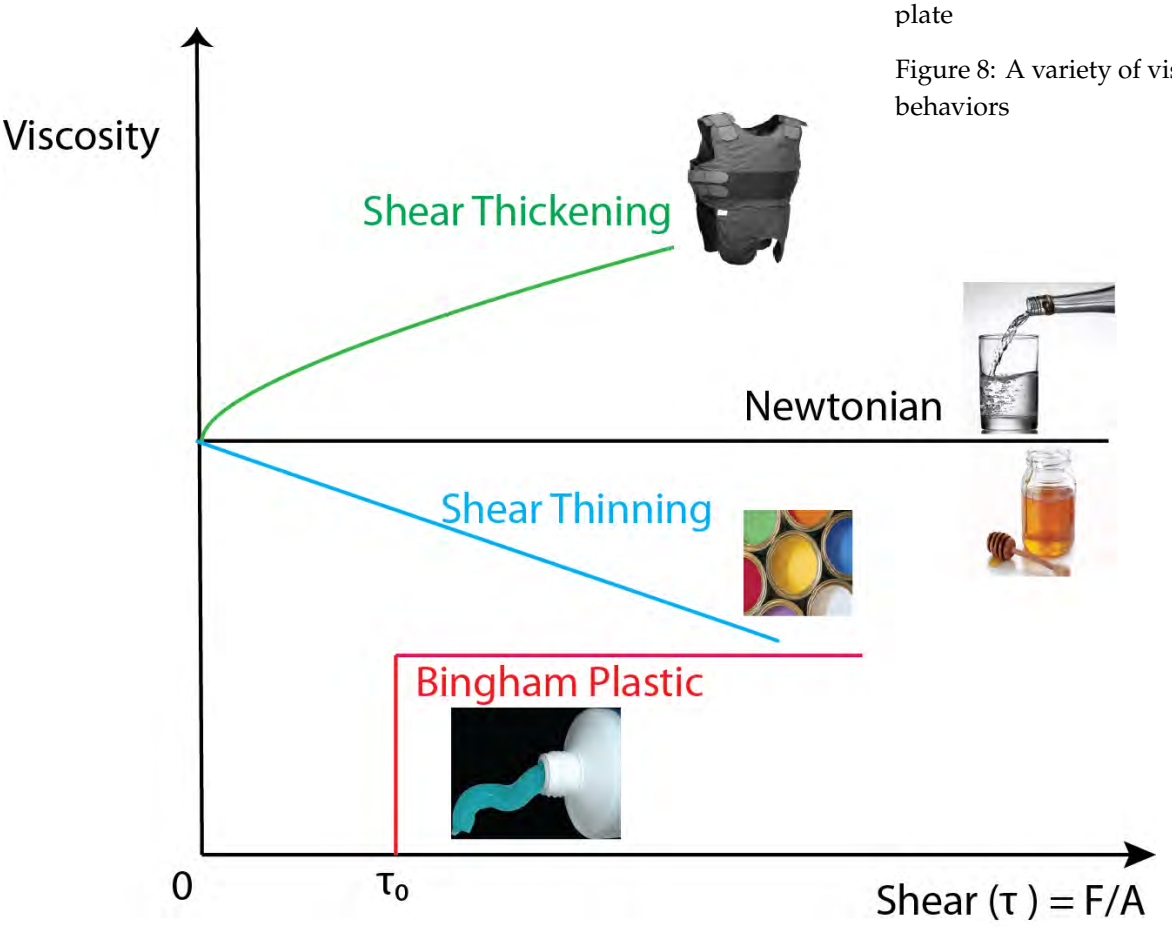


Figure 8: A variety of viscoelastic behaviors

Silly putty will move freely if you push on it slowly, but try impinging on it with a hammer and it will resist like a solid. This behavior is called viscoelastic - in-between what you could expect from a solid and a liquid. Figure 8 shows other kinds of behavior - for example dilatants (paints) and thickening agents (toothpaste). This is a large field or research in material science called rheology. Newton's equation is followed by many liquids - water, glycerin, alcohol, honey etc., but not all liquids. Fluids can be shear thinning or shear thickening, and can have complicated time (i.e history) dependent effects, many of which are not completely understood.

The units of viscosity are $kg \cdot m^{-1} \cdot s^{-1}$ or equivalently $Pa \cdot s$. Often other units are used: $1Pa \cdot s$ is equal to 10 Poise (P), and 1 Poise is equal to a 100 centi-Poise (cP). Occasionally, viscosity is divided by the density and this is called the kinematic viscosity or specific viscosity. Viscosity has a strong dependence on temperature.

Table 1: Viscosity

Viscosity values at 20^0 C		
Type	Viscosity (η) Ns/m^2	Specific Viscosity (ν) m^2/s
air	10^{-3}	10^{-6}
water	10^{-5}	15×10^{-6}

Surface and Body Forces

For any given object in a fluid two forces may act on it - surface forces that are localized to surfaces and body forces like gravity and electromagnetic forces that act at a distance and influence the whole body. The forces that act on a surface can take on two forms - either the force can be normal to the surface or tangential to it. These result in different consequences. The normal force per unit area - simply the pressure can compress or dilate the fluid, the tangential force called shear - tends to deform the fluid.

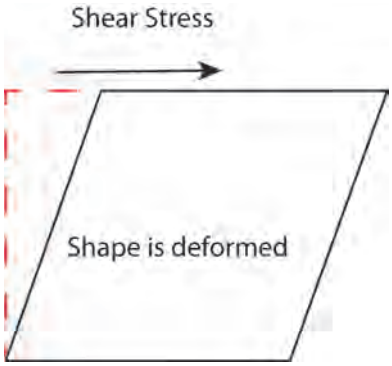


Figure 9: Shear Stress and Strain

In comparison to most solids, fluids are far more deformable. A element of fluid, as it moves in a stream, can be deformed in many ways. It translates - moves from one place to another. It can dilate or compress. And it can rotate. To visualize flows in a fluid experimentally we have to add tracers of some sort - bubbles, dyes, small particles etc. There are many possible visualization possibilities: we could follow a small element of fluid as it flows, and draw the line that joins these points. Or we could draw a line that is tangential to the velocity of the fluid particle etc. These possibilities are shown in Table 2.

Bernoulli's equation

Next consider the work of Venturi, Daniel Bernoulli and Giovanni Battista Venturi (the inventor of venturimeter used to measure velocities in aeroplanes etc.) They were the first to write down the equations of mass and energy conservation as applied to fluids. First let us write

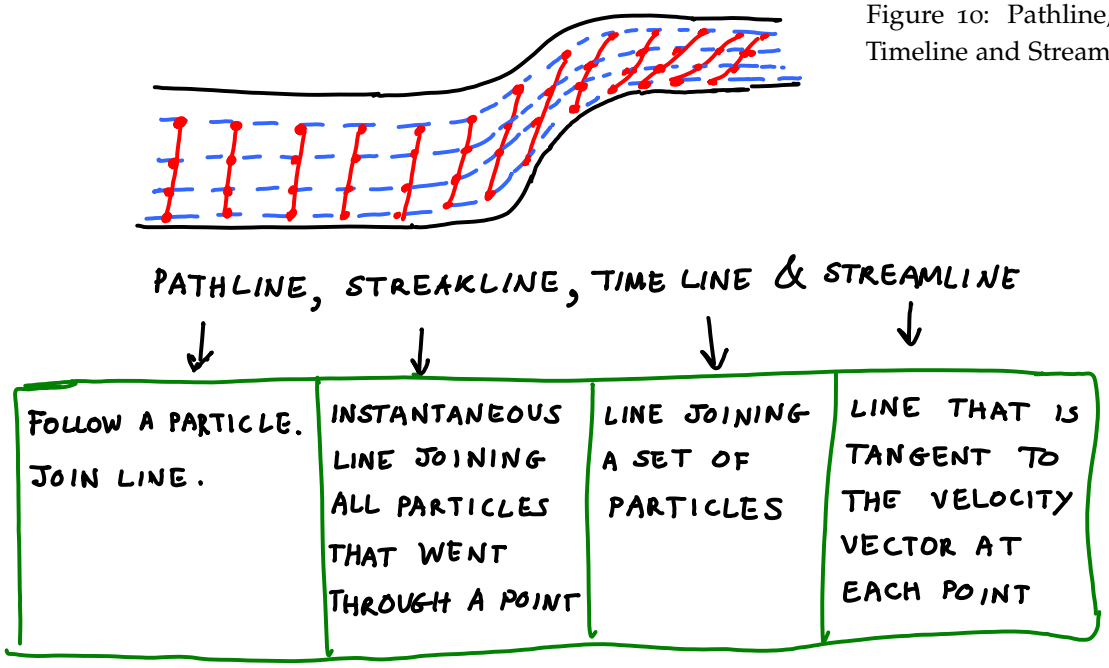


Figure 10: Pathline, Streakline, Timeline and Streamline

Type	Description	Comment
Path-line	Draw the path a small fluid element follows	
Streak-line	Draw the (instantaneous) line that joins all fluid elements that have passed through a given point	In steady flow path-lines and streak-lines are the same
Time-line	Draw a line joining a set of fluid elements and follow it with time	Generally, initially drawn perpendicular to the flow
Streamline	Draw the line joining the instantaneously tangent to the velocity vector of the flow	This is more sophisticated concept. Vector mathematics guarantees that if we have a continuous field of velocities - i.e. a velocity associated with every point in the fluid, then such streamlines exists and two of them don't intersect unless the velocity is zero

Table 2: Visualizing fluid motion

the equation of continuity for incompressible fluids. If Q is the mass flow rate

$$v_1 A_1 = v_2 A_2 = \frac{Q}{\rho} \tag{4}$$

Further, we have from Bernoulli's principle that

$$p_1 + \rho g h_1 + \frac{1}{2}\rho {v_1}^2 = p_2 + \rho g h_2 + \frac{1}{2}\rho {v_2}^2 \tag{5}$$

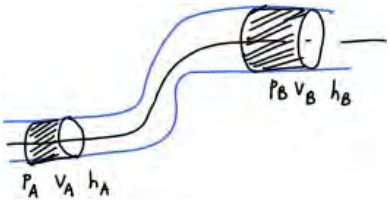
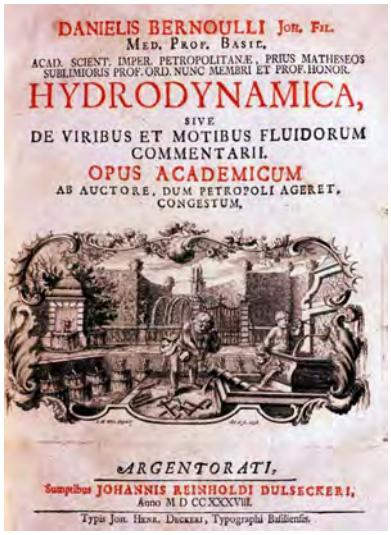


Figure 11: Fluid moving through a pipe

(a) Daniel Bernoulli (1700-1782)

(b) Venturi (1746-1822)



(c) Hydrodynamica by Bernoulli

Technically, Bernoulli equation is only valid along a streamline, for fluids of negligible viscosity. In irrotational flows (meaning that if you put a small particles in the flow it will not rotate) the equation is valid for all streamlines at once. It is safe to say that this is the most famous equation of fluid mechanics. Based on the Bernoulli's principle, a venturi-meter shown in Figure 12 can be used to determine flow speeds.

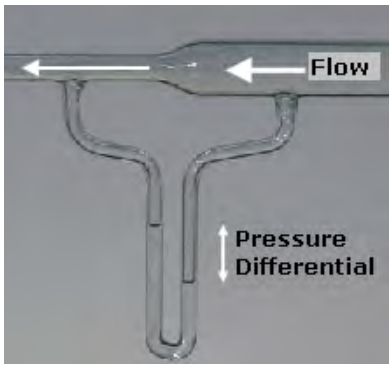


Figure 12: An venturimeter

Euler's equation

In the 18th century Leonard Euler (who was Bernoulli's student) wrote down equations for frictionless and non-compressible fluids - essentially "dry" water. Imagine a crowd of people moving: To follow what is going on we have two choices: either we can pick an individual and follow that person as she weaves through the crowd - ignoring everything else. The other possibility is to pick a location and study the people who pass through it. The first approach is called the Lagrangian method and the second Eulerian. In fluid mechanics we can do the same thing - concentrate of a small mass as watch as it moves or choose a point and watch the fluid whizzing by. The second approach is more

mathematically tractable. When Euler wrote down his equation, the idea of viscosity had still not caught on and so he missed a fundamental property. Nevertheless, he did get part of the idea right. In one dimension we can write:

$$\rho \frac{\partial v}{\partial t} + \rho v \frac{\partial u}{\partial x} = -\frac{\partial P}{\partial x} \tag{6}$$

The u and v are the x and y components of velocity. The complicated form of the derivatives on the left side of the equation is necessary to account for the Eulerian co-ordinate system.

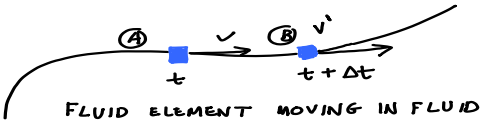
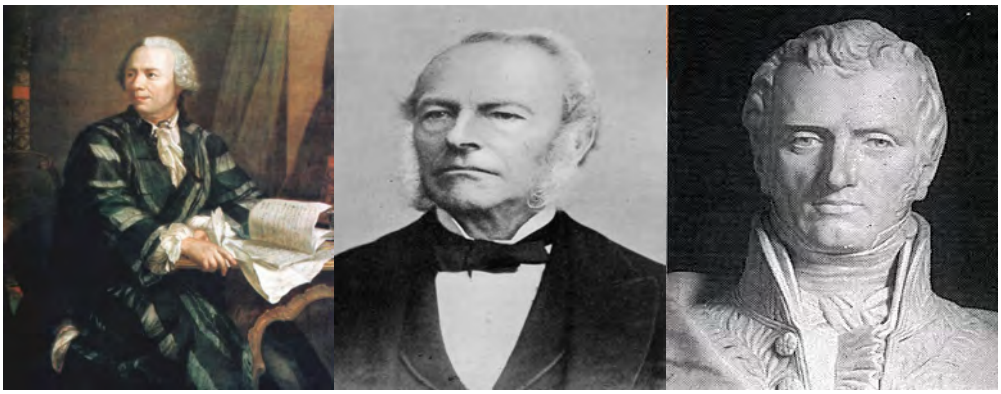


Figure 13: Velocity varies in time and space

Navier-Stokes equation



(a) Leonhard Euler (1707-1783) (b) George Gabriel Stokes (1819-1903) (c) Claude-Louis Navier(1785-1836)

Including viscosity and getting the complete and correct form of the equation right took the efforts of Navier and Stokes - two giants of fluid mechanics. The Navier-Strokes equation for fluid mechanics in the fundamental equation we use today to model fluidic behavior. However, it must be admitted that it is a nasty, nonlinear equation that defies complete understanding [6]. Here it is in it's full three dimesional g(l)ory

$$\rho(\frac{\partial v}{\partial t} + v \cdot \nabla v) = -\nabla p + \mu \nabla^2 v + f \tag{7}$$

(the equation written here is for the case of a non-compressible fluid). This equation is complicated but it is only a mathematical statement of force or momentum balance.

$$\overrightarrow{\text{Net Inertial Forces}} = \overrightarrow{\text{Net Pressure Force}} + \overrightarrow{\text{Net Viscous Force}} \tag{8}$$

In fluids one or more terms may become negligible simplifying the analysis. In particular, for most microfluidic devices the net inertial force term is negligibly small.

[6] Need a million dollars in a hurry? Find out if the Navier Strokes equations have "nice" solutions (in a certain mathematical sense): http://www.claymath.org/millennium/Navier-Stokes_Equations/

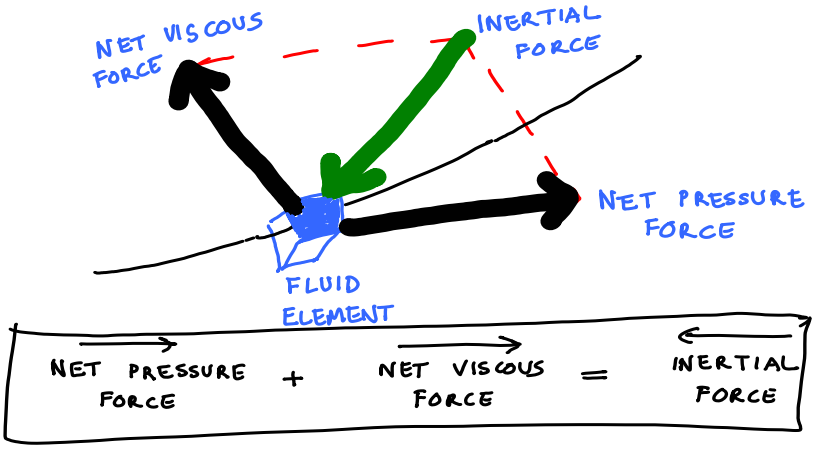


Figure 14: Force Balance on a Fluid Element

Scaling laws

Let us try to understand what happens as things become smaller. Consider a cube of lengthy L. The volume of this cube is L^3 and the surface area is $6L^2$. Assume that that it shrinks in size in each dimension by half. The volume of the cube shrinks by $1/8$ to $L^3/8$ and the surface area by $1/4$ to $1.5L^2$. Hence, the ratio of surface to volume has increased (from $6/L$ to $12/L$). The change is inversely proportional to the change in length. A smaller body has a much greater surface area to volume compared to a larger body. These sort of scaling arguments are extremely valuable when thinking about microfluidics. In many cases the scaling arguments can provide insight into whether something is feasible or impossible without having to go into a detailed analysis. For instance questions like can magnetic forces be used to actuate devices at the nanoscale? and what kind of pressures would be needed to push liquids though a nanoscale channel can be answered.

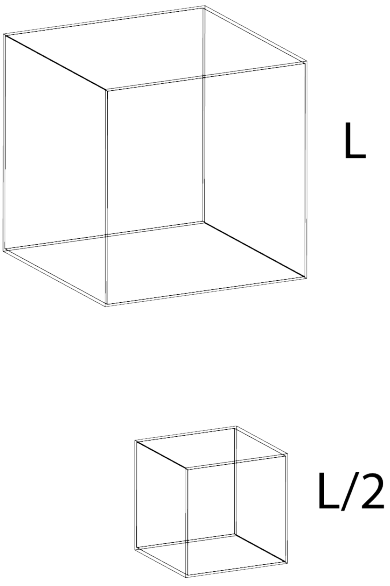


Figure 15: Scaling of Area and Volume

Scaling is widely used in fluid mechanics in the form of dimensionless numbers. Let's get to our first dimensionless number.

Reynolds' Number

As the full Navier Stokes equations are hard to solve in nearly every practical instance, engineers have to resort to other methods. The method of dimensionless number has been found to be extraordinarily useful. These numbers compare the ratio of two quantities of interest in a system. If in two experiments the the ratio is the same the

experiments are dynamically similar, and will show similar behavior. To avoid complicating things with measurement units the ratio is usually created so that all the dimensions cancel out and it is dimensionless.

Figure 16: Osborne Reynolds(1842-1912)

These handful of number can be defined in standard ways in different systems. Often engineers build smaller (or larger) scale models to perform experiments that would behave in the same way as the system we are interested in. The first dimensionless number we will consider is Reynolds number:

$$Re = \frac{\rho v L}{\mu} \tag{9}$$

We can gain some insight into *Re* by writing the equation in a slightly modified way; Think of a body of characteristic length L in a flowing fluid. The inertial forces are simply (mass $\times$ acceleration). The mass is (density $\times$ volume), which is ρL^3. Since a fluid particle abruptly comes to zero velocity at the surface, and the time it takes to do so is approximately L/v and the acceleration therefore is $v^2 L$. The viscous forces are proportional to the (area $\times$ viscous stress). Area can be approximated as L^2 and the viscous stress as $\mu v/L$. Putting this all together we have

$$\text{Inertial Forces/Viscous Forces} = \frac{\rho L^2 v^2}{\mu v L} = \frac{\rho v L}{\mu} = Re \tag{10}$$

The Reynolds number is really the ratio of inertial to viscous forces. The reader may wonder about what happens to bodies that have a complicated shape - how can one define L. In this case the answer is convention: We define L in some standard way for each geometry so that comparisons can be made. Comparing Re in two different systems requires two similar geometries - it is meaningless to compare Re in systems that are not geometrically similar.

Laminar and Turbulent flow

It turned out that Reynolds' number can be used to mark the change from laminar to turbulent flow in a particular geometry. It is easy to observe turbulence - just turn on the tap. Near the top, the fluid flows out smoothly, but beyond a certain height depending on the flow rate you can see a transition to another kind or flow regime - here the water flows in an irregular, chaotic way. In pipes with a circular cross section - at Re>4000 the flow is turbulent whereas below 2100 it is laminar. In between there is a transition regime. One consequence of turbulence is that mixing is much more efficient. Another consequence which we shall see in the next lecture is that the force opposing motion through a

fluid actually drops leading to some seemingly counter intuitive phenomena.

Most microfluidic devices operate at Re well below 5. This is easy to show with a calculation.

Example: Reynolds Number

Consider fluid flow through a circular channel or diameter $100\,\mu m$. Assume the flow is moving a $5\,\mathrm{mm/s}$ and the fluid is water (density $1000\,\mathrm{kg//}m^3$, viscosity $10^{-3}\,\mathrm{Ns/}m^2$

Solution:

Plugging in the number with appropriate dimensional conversions we have

$$Re = \frac{(100\mu m)(1m/1000000\mu m)(1000kg/m^3)(5mm/s)(1m/1000mm)}{(10^{-3}Nsm^{-2})} = 0.5 \tag{11}$$

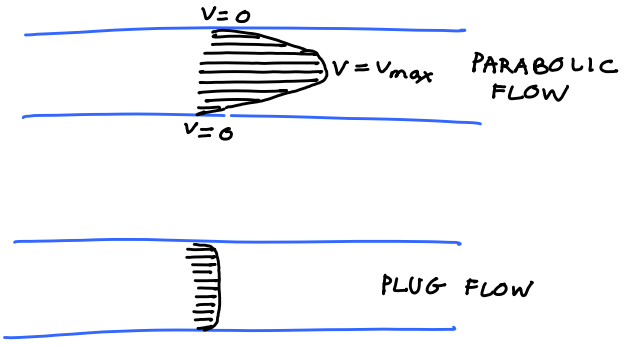


Figure 17: Parabolic and Plug Profiles

Re is much less than the transition number and flows are therefore laminar. In fact the flow in almost all microfluidic devices is laminar. Let us examine a few geometries and evaluate the flow there. For the case of a circular tube, at low Re the flow regime is known as *Hagen-Poisuelle flow*. The Navier Stokes equations can be solved in this case to reveal that the flow profile is parabolic with the velocity at the center being the highest and the velocity at the boundary being zero. This is called parabolic flow. Its counterpart is plug flow where the profile is flatter and this is seen in the turbulent regime and in certain electrical force driven flows. For a given mass flow rate Q, the pressure drop is

inversely proportional to the fourth power of the radius.

$$\Delta P = \frac{8\mu L Q}{\pi r^4} \tag{12}$$

Lecture 2: Drag, Diffusion, Gradients, and Mixing

Drag

Drag is the force that any body moving through a fluid experiences acting against the direction of motion. It is fluid friction. The drag is dependent only on the relative velocity - the body could be moving in a fluid or the fluid could be moving across it - only the relative velocity counts not the absolute velocity.

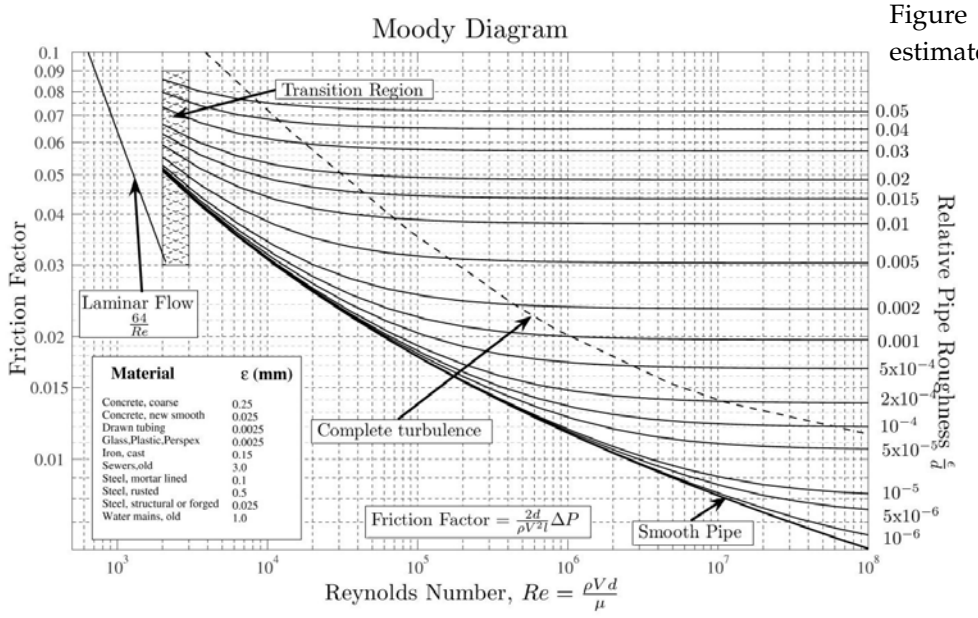


Figure 18: Moody Diagram to estimate friction factor

What does drag depend on? Drag is complicated function of flow

and body parameters and takes on different forms in different flow regimes. In the low reynolds number regime Strokes found that drag is directly proportional to the the viscosity, the relative velocity and size of body (BUT not the density of the fluid!)

$$Drag = C_d L \mu v \tag{13}$$

were C_d is a constant, the drag coefficient or friction factor. If you were an engineer and needed to calculate the friction factor you would use the Moody diagram for a pipe and add a geometric factor for other shapes. In the laminar regime the friction factor is a straight line of slope $64/Re$.

From the figure it is clear that behavior in the turbulent regime is complicated - in fact in that regime drag is a different function of velocity etc and depends on the density of the fluid unlike the laminar case.

$$Drag_{turbulent} = C_d v^2 \rho L^2 \tag{14}$$

It is interesting to note the non-intuitive fact that in either case the absolute pressure plays no role in determining the drag force. For a solid, frictional forces do depend on the normal component of force and hence on the pressure exerted by the solid on the surface. This is not the case for liquids. You would experience the same drag deep in the ocean or near the surface if the body shape and other parameters like density of water and size remain the same despite the higher pressure and similarly the drag experienced at higher altitudes is not different from that at lower altitudes if other parameters don't change.

Pressure, Flow Rate and Fluidic Resistance

We have seen earlier that the flow rate (Q) in microfluidic flows is generally proportional to the pressure difference (ΔP), with the constant of proportionality the fluidic resistance (R_F) of the system.

$$Q = \Delta P R_F \tag{15}$$

Microfluidic systems then behave very much like simple electrical circuits with pressure difference playing the role of voltage, flow rate playing the role of current and the fluidic resistance being like the electrical resistance.[7]

[7] The mathematical details for all these calculations and more can be found in Henrik Bruus' book Theoretical Microfluidics, Oxford University Press, 2007. Only recommended for readers who are mathematically sophisticated.

Channels in series behave like resistors in series:

$$R_{series} = R_1 + R_2 + R_3 + ... \tag{16}$$

Channels in parallel behave like resistors in parallel:

$$\frac{1}{R_{parallel}} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} + ... \tag{17}$$

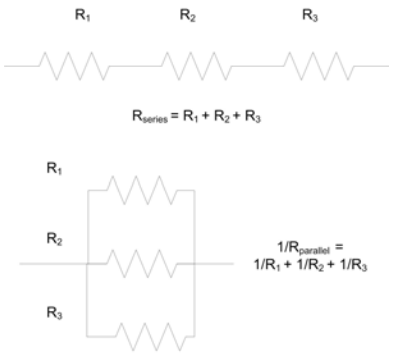


Figure 19: Series and Parallel

If the liquid flowing through is for most practical purposes incompressible (for instance water through a microfluidic device at low pressures) then the total flow rate must be conserved - what goes in must come out - just like the Kirchhoff's law for electrical currents

We earlier saw the resistance of a pipe with a circular cross section is given by (from Poiseulle's law):

$$R_F = \frac{8\mu L}{\pi R^4} \tag{18}$$

The resistance scales as the negative fourth power of the radius and can become enormously large very quickly as the radius is reduced. What about a square cross section - if the height is about the same magnitude as the width then it is appropriate to use that length instead of the radius. For a geometry where the width (w) is much larger than the height (h) the proportionality to the first order is wh^3

Diffusion

If we had a cube of sugar in tea and there was no other mixing process but diffusion - how long would it take the sugar to dissolve? Could you drink this tea? This section will allow use to answer this and other similar questions.

Figure 20: Robert Brown (1773-1858)

Diffusion is the process of random mass transport at the molecular scale. The first clue that this process can move things was the discovery of Brownian motion - observed by Robert Brown in 1827, incidentally a botanist. It is typically assumed that Brown found pollen grains to move around randomly in a drop of water observed under a microscope, but in fact pollen grains that Brown used were too big to show an effect and what he observed were smaller particles shed by the pollen grains. (Indeed, Brown may not have even been the first to observe diffusion, but in any case his name is forever associated with it). A fascinating modern reconstruction of this experiment can be found here:

`http://physerver.hamilton.edu/Research/Brownian/index.html`

We can hardly talk about diffusion without mentioning the name of the most famous person who ever lived at Princeton: Albert Einstein. As a 26 year old, Einstein published a paper in 1905 which connected viscosity, diffusion and temperature. His equation (Einstein-Stokes equation)[8]

[8] A. Einstein. *Investigations on the Theory of the Brownian Movement.* Dover Publications, 1956

$$D = \frac{k_B T}{6\pi\mu r} \tag{19}$$

Here k_B is the Boltzman constant, T the temperature, μ the viscosity and r the radius of the particles under consideration. A similar equation had been derived a little earlier by Smoluchowski using a different method.

Figure 21: Albert Einstein (1879-1954) in front of his house in Princeton

In the same paper Einstein showed that the average (root mean square) distance L, a particle would move in time t, is simply:

$$L = \sqrt{2Dt} \tag{20}$$

D is the diffusion coefficient. Note that the size dependence, viscosity and temperature is hidden here in the diffusion constant. The units of the diffusion constant are $[Length^2][Time^{-1}]$ and can be given in cm^2/s or in m^2/s.

The most important observation about this equation is that the length scales as the square root of time. To really understand where this relationship comes from we have to understand what a random walk is. Imagine you play a game - which involves tossing a coin - if heads you move a step d forward, if tails you move a step backwards. After n steps the distance traveled will be written as $L(n)$. Now L(n) is reached from L(n-1) and this could be one step ahead or back, so we may write

$$L(n) = L(n-1) \pm d \tag{21}$$

Now imagine that you did this many many times and took the average (or alternatively there were many people playing the same game and you take their average) represented by the symbol $\langle\rangle$

$$\langle L(n)\rangle = \langle L(n-1)\rangle \pm \langle d\rangle \tag{22}$$

Since the probability of a forward or backward step is the same, on average you get zero - the forward steps cancel out the backward steps.

So

$$\langle L(n)\rangle = \langle L(n-1)\rangle \tag{23}$$
$$\langle L(n-1)\rangle = \langle L(n-2)\rangle \tag{24}$$
$$\dots \tag{25}$$
$$\Rightarrow \langle L(n)\rangle = \langle L(0)\rangle = 0 \tag{26}$$

This also means we can work backwards all the way to the initial position and find that the average distance moved is zero! The forward and backward steps cancel out and you get nowhere! Or do you? Well, while in the long run you get nowhere, the walking does spread out if you take more steps. And if there are many walks, the average may be zero, but they will be more spread out. How can we characterize this? The trick is to consider the root-mean square, which gives a way to measure how spread out the path was. Here we average not $< L(n) >$ but the $< L(n)^2 >$ and then take its square root. We can write:

$$L(n)^2 = L(n-1)^2 \pm 2dL(n-1) + d^2 \tag{27}$$

Again taking averages, we find that the middle term on the right averages to zero, and since d is constant we can remove the average symbols to get:

$$\langle L(n)^2\rangle = \langle L(n-1)^2\rangle + d^2 \tag{28}$$

Now you can see the germ of the reason why the square root dependence comes from. As $L(0) = 0$, $\langle L(1)^2\rangle = d^2$ and $\langle L(2)^2\rangle = 2d^2$ and in general,

$$\langle L(n)^2\rangle = nd^2 \tag{29}$$

But n is simply the number of steps and if the movement is steady (equal number of steps per unit time), then n is proportional to t. But this means that on average the root mean square average distance is proportional to the square root of time as Einstein had noted:

$$\sqrt{\langle L^2\rangle} = \sqrt{2Dt} = L_{RMS} \tag{30}$$

This length is a useful quantity to know in many situations and is called the diffusion length. It can be used as ruler to compare other length scales and deduce the importance of diffusion to other processes.

Now with this information at hand we can answer the question posed at the beginning - sugar which is made of sucrose a small molecule like fluoresein. If you cup is approximately 10 cm , then nearly 10^6 seconds are needed to mix completely by diffusion - about 12 days!

Table 3: Diffusion constants

Diffusion constants		
Type	D (at 20^0 C)	$\sqrt{2Dt}$ (cm)
1 μm sphere	$2.5X10^{-9}cm^2/s$	$7x10^{-5}\sqrt{t}$
Protein (Haemoglobin)	$7X10^{-7}cm^2/s$	$1.2x10^{-3}\sqrt{t}$
Small Molecule (Fluorescein)	$5X10^{-6}cm^2/s$	$3.2x10^{-3}\sqrt{t}$

Laws of diffusion

In 1855, Adolf Fick derived the laws of diffusion that are still applied today. It is interesting to note that Fick was a physiologist, not a physicist. There are two basic laws. To understand them first consider a situation where are are two reservoirs connected by a channel. Let us assume that one reservoir has a chemical at a concentration c_1 and another at c_2. If we take a cross-section of the channel and ask how many molecules are diffusing through per unit area per unit time? This is called the flux (mass flux, or molecule flux, or molar flux depending on what you are working with) usually designated by the letter J. We have for the flux:

$$J = -D\frac{c_2 - c_1}{x_2 - x_1} = -D\frac{dc}{dx} \tag{31}$$

The flux depends on the change in concentration with distance and the negative sign indicates that transport occurs from a higher concentration to a lower one, in line with our intuition. Molecules diffuse from a region of higher to lower concentration. Note that there is no time dependence in this equation - it is just a static one-time picture. Fick's second law gives the full time dependent equation.

$$\frac{dc}{dt} = -D\frac{d^2c}{dx^2} \tag{32}$$

This is a partial differential equation of second order in the coordinate. Of course, we have only written it in one dimension x, and we can also write the same for y and z directions. We can derive it easily by combining the first law with the law of conservation of mass (try it!)

Figure 22: Adolf Fick (1829-1901)

As for all differential equation, given boundary conditions and initial conditions we can solve this to get the concentrations profiles with time. For the simple case where a tracer dye is released at a point instantaneously and spreads in one dimension the solution is a Gaussian. If instead it is released over a period of time the solution takes the form of a curve called the error function. For example if we had an interface with two different concentrations of a chemical - after a while the concentration profile appropriately scaled would look like Figure 23. Note that at the center the curve is almost linear - and this fact can be used to generate linear gradients.

Peclet number

In microfluidic devices there is competition between convectional flow and diffusion - two mass transport processes. Convectional flow tends to move matter in the direction of flow and diffusion tends to move mat-

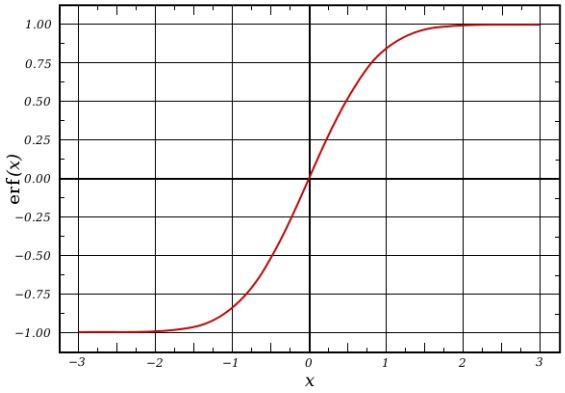


Figure 23: The error function

ter to equalize concentration gradients. We can write a dimensionless number called Peclet number, that compares the two tendencies

$$Pe = \frac{vL}{D} \tag{33}$$

where L is a characteristic length scale of the system, v the velocity of flow and D the diffusion constant. If Pe is large, gradients can exist, otherwise diffusion will smoothen any gradient out.

Mixing, Sorting, Dispersion

Depending on experimental needs we may need to mix two ingredients or sort them. Microfluidics designs offer a number of ways to do so. Often pure diffusional mixing is fast enough. In the course you will get to make a number of different mixers and gradient makers and understand their principle of operation.

Taylor-Aris dispersion

For flow in channels, the dynamics of flow makes the diffusion process more complicated. In laminar flow the effect is to increase the apparent diffusion constant in certain flow regimes leading to faster dispersion of a chemical. This phenomena is called Taylor-Aris dispersion after the discoverers[9]. In flow through a circular tube at low Reynolds number diffusion is occurring axially and radially.

[9] G. I. Taylor. Dispersion of soluble matter in solvent flowing slowly through a tube. *Proceedings of The Royal Society A: Mathematical, Physical and Engineering Sciences*, 219:186–203, 1953; and R. Aris. On the dispersion of a solute in a fluid flowing through a tube. *Proceedings of The Royal Society A: Mathematical, Physical and Engineering Sciences*, 235:67–77, 1956

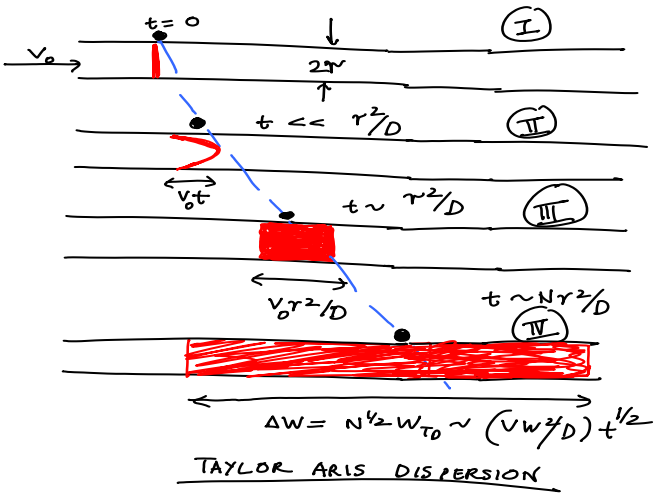


Figure 24: Taylor-Aris Dispersion (adapted from Squires and Quake, 2005)

Figure 25: GI Taylor (1886-1975)

Creating Gradients

Microfluidic devices excel at creating two dimensional spatial gradients. These spatial gradients can be generated either passively (with no flow) or dynamically (with flow)

Passive Gradients

A simple way to generate gradients is to have a source and sink reservoir connected by a smaller tube. Once all the pressure driven flows are balanced - diffusion creates more or less linear gradients that can last from a few minutes to several days. Cells can be put in the connecting chamber either by themselves (adherent cells) or in a gel.

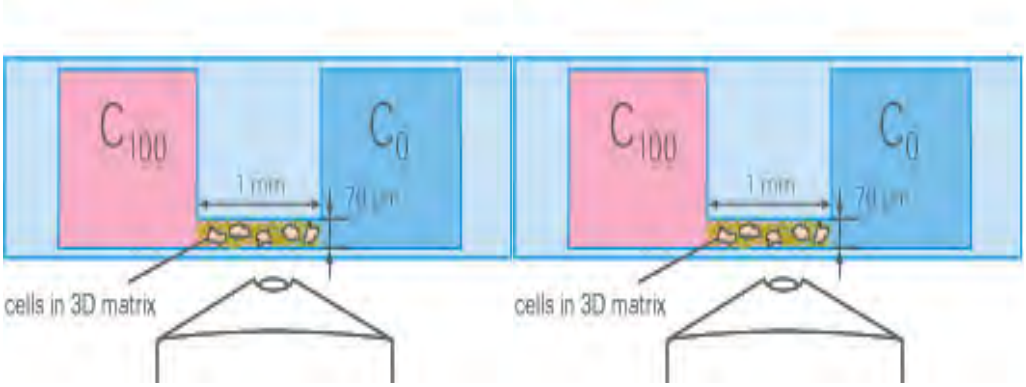
Dynamic Gradients

In this case flow is used to create the gradient allowing gradients to be kept for as long as flow is possible and also changing them on the fly. The first such gradient generator was described by Jeon et al [10]. We will make a similar mixer in our experiments.

Recently a arbitrary gradient mixer was described that uses a gel and channels in PDMS. these devices are suitable for bacterial studies [11] - for mammalian cells the conditions to maintain gradients, while also keeping cell happy are much more stringent. At the Princeton PS-OC

[10] Noo Li Jeon, Stephan K. W. Dertinger, Daniel T. Chiu, Insung S. Choi, Abraham D. Stroock, and George M. Whitesides. Generation of solution and surface gradients using microfluidic systems. *Langmuir*, 16(22):8311–8316, 2000

[11] Tanvir Ahmed, Thomas S. Shimizu, and Roman Stocker. Microfluidics for bacterial chemotaxis. *Integr. Biol.*, 2:604–629, 2010



(a) Cell in a gel (b) Adherent cells

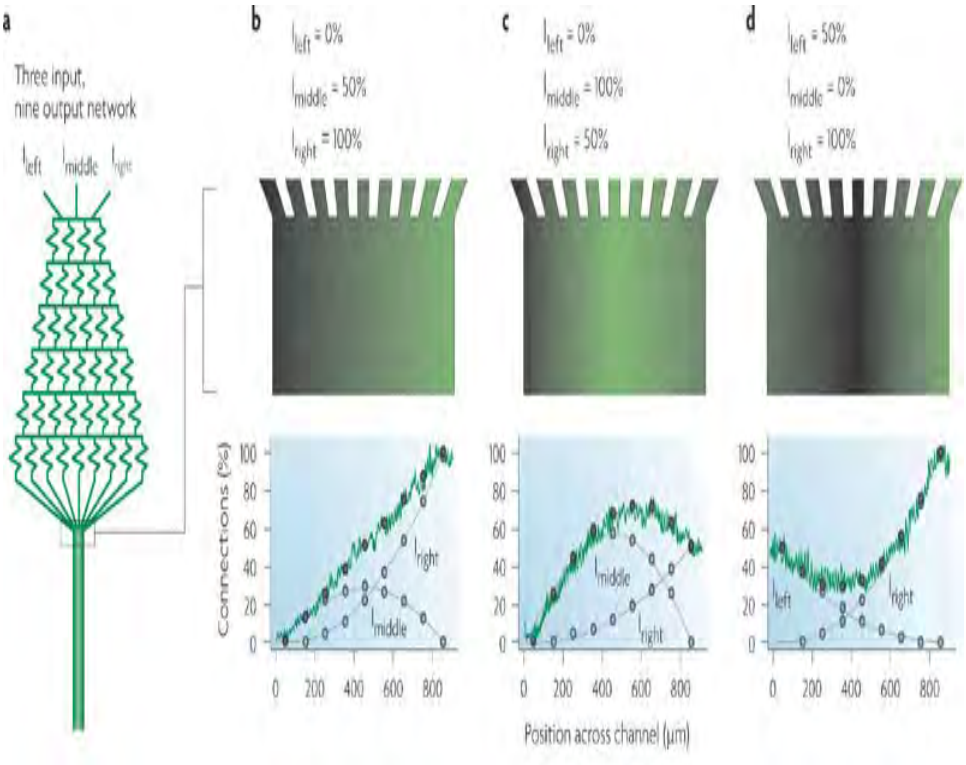


Figure 26: Dynamic Gradient Generator

we have several students working on Silicon devices for gradients. The idea here is to use a set of posts to separate out the growth chamber from the source and sink channels. The separation between the posts is small enough that most cells don't escape. The posts protect the cells from direct flow and hence preserve cell growth factors and other chemicals inside the growth chamber for longer.

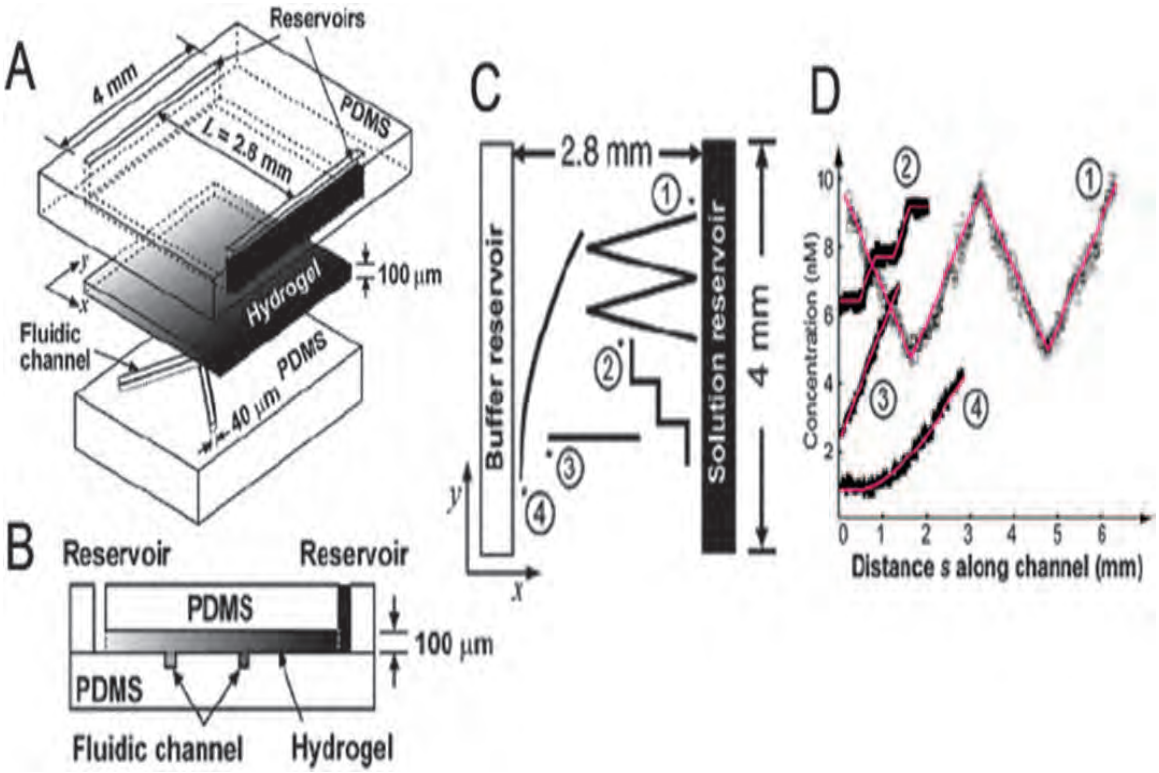


Figure 27: Arbitrary Gradient Generator

This kind of gradient control can be used to make micro-habitats or ecological niches where food concentration are varied. An Example of this device is the micro-habitat patch developed at Princeton University Figure 28. In the figure each square alternates with posts or no posts creating regions of food and starvation. These devices are unique in the sense that they are neither batch cultures nor chemostats, instead cells stay in place but nutrients diffuse in and out.

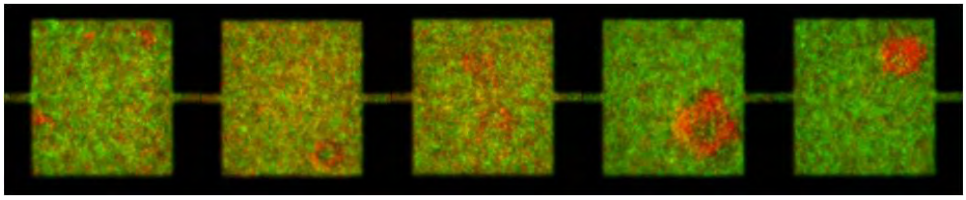


Figure 28: Microhabitat Patch

Lecture 3: Micro/Nano Fabrication Technology, Fluidic Circuit Components

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In this lecture we will learn about various manufacturing processes, materials used and their advantages and disadvantages and finally various fluidic components that can be used in a design.

Microfluidic Rules of Thumb

Let us write down some rules of thumb:

Re number is small

The Reynolds number in all microfluidics devices is below the turbulent limit. Flow is linear and laminar.

Flow velocity at surfaces is zero

The flow velocity at the surface is zero. This is peculiar, non-intuitive fluid phenomena. In almost everything we do this is the case and this is known as the no-slip boundary condition. For solids this is not true as you well know from motion of cars etc.

Density is constant for liquids

Most liquids are not very compressible and their density remains constant inside our devices. This also means that waves, particularly of small wavelengths, cannot be sustained inside the liquid, because they require density changes. Large wavelengths on the other hand can are needed and indeed this is the principle of submarine sonar and whale songs - however these are irrelevant for most microfluidic devices.

Basics of Micro/Nano Technology

Wafers

Much of the processing capability we have in electronics is dependent on having high quality crystalline silicon wafers that range in size from 1" to 18" in diameter. These wafers are the substrates on which all processing is done. They are sold by several manufacturers and come in different qualities. In this course we care only for surface smoothness, and we use test quality wafers. Typically, prime wafers are used in many MEMS processes. Even higher grade wafers are available for specialized manufacturing.

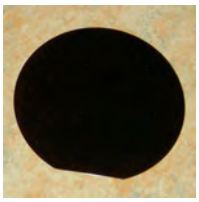

Figure 29: Silicon Wafer: The flat indicates crystalline orientation and doping

Photoresists

Photoresists are UV sensitive chemicals. They are viscous enough to be spun on a wafer. The spin speed detemines the height of the features. When exposed through a mask to UV light a chemical reaction takes place that changes the solubility of the exposed region. Then a solvent (called developer) can be used to remove the exposed (or unexposed area). When the exposed area is more soluble, we have a positive resist (e.g. SPR, AZ series). When the exposed area is less soluble, we have a negative resist (eg SU8 series). The chemistry of the resists is optimized to ensure it has the right viscosity, adhesion to wafer surface, and matches the exposure system. Extensive development work has gone on to make suitable photoresist for the elctronics industry. In microfluidics it is common to use more viscous resists that give taller heights than those used in electronics.

The dependence of spin speed on the height of resist is complicated and generally can only be detemined empirically. Photoresists are examples of non-newtonian fluids.

Masks

A mask is pattern on a transparent substrate. UV light passes through the mask to hit a wafer coated with photoresist allowing transfer of the pattern from the mask to the photo-resist coated wafer. A transparency mask, which as the name suggests is printed on a transparency, is suitable for designs that have patterns 15 micron or larger, whereas a chrome (or ferric oxide) mask is better for smaller patterns (1 micron) For features smaller than 1 micron you would have to resort to e-beam lithography, steppers and other specialized equipment.

Name	Location and Contact
CAD/Art Services, Inc.	87509 Oberman Lane, Bandon, OR 97411 (541)-347-5315 (phone) (541)-347-6810 (fax) cas@outputcity.com
Fine Line Imaging	4733 Centennial Blvd., Colorado Springs, CO 80919 (719)-268-8319 (719)-359-5497 (fax) plotting@fineline-imaging.com

Table 4: List of companies making transparency masks

Making transparency masks generally involve using a high quality laser printer (from 5000 to 40000 dots per inch) to print on a transparency. This is the most economic option for micro-fluidic designs with features over 15 microns. Chrome masks are the next step up - these masks have a coating of chrome on soda lime or quartz glass plates with a layer of photo-resist on top. A laser writer is used to write a pattern on the resist. Finally, developing the resist, etching the chrome and dissolving away the remaining resist gives the mask plate. For certain kinds of multi-layer designs having a more transparent mask helps in alignment - and this is done by using a ferric oxide coating instead of chrome coating. Ferric oxide is more transparent in the visible light spectrum compared to chrome. Ideally, all kinds of masks should be made inside clean-rooms to avoid any errors due to dust particles.

Mask Aligners

Mask Aligners are machines that have a vapor lamp (mercury, xenon, deuterium) to expose photoresist through a mask with a pattern. They have precision stages and optics that allows control of alignment and contact. A mask fits into the mask holder of such a aligner. Vacuum is used to hold both the mask and the aligner. Contact aligners involve direct contact between the mask and photoresist. Proximity aligners allow a small separation between the phototresist and mask (which can protect the mask from damage but could also reduce the resolution.

Soft-lithography

Soft-lithography is a technique invented at Harvard by Duffy, Whitesides and co-workers. It involves pouring uncured PDMS over a mold and curing it in an oven[12].

[12] The two common PDMS vareities used are RTV 615 made by GE and Sylgard 184 made by Dow Corning

Name	Location and Contact
Photo Sciences, Inc.	2542 W 237th St. Torrance CA. 90505 (310)-634-1500 (phone)
HTA photomask Inc.	1605 Remuda Lane San Jose, CA 95112 (408)-452-5500 (phone) (408)-452-5505 (fax) sales@htaphotomask.com (sales) kenc@htaphotomask.com (technical)
Advance Reproductions Corp.	100 Flagship Dr. North Andover, MA 01845 (978)-685-2911 (phone) sales@advancerepro.com (sales) drobinson@advancerepro.com (technical)
Fine Line Imaging	4733 Centennial Blvd. Colorado Springs, CO 80919 (719)-268-8319 (phone) (719)-359-5497 (fax) plotting@fineline-imaging.com, cust.service@fineline-imaging.com

Table 5: List of companies making chrome masks

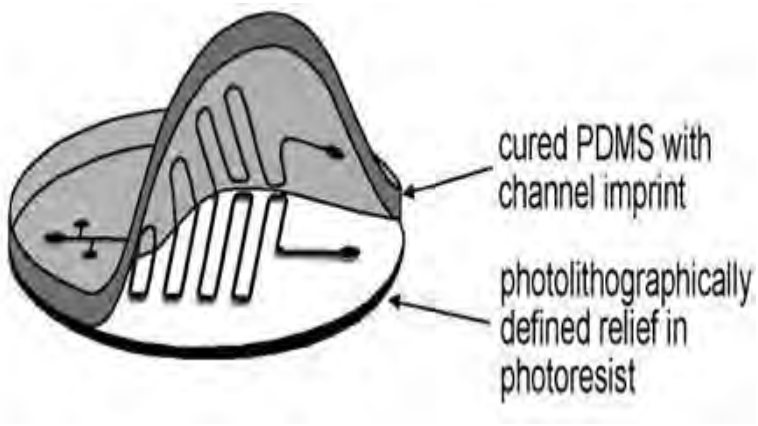

Figure 30: Soft-Lithography

Fluidic Components

We have now seen a few ways to make fluidic devices. Just like in electronics, fluidic devices can be considered to be made of various components. These can be put together to form a "fluidic circuit". Let us consider the elements one by one. We will primarily concentrate on components made with soft-lithography because this is where the most progress in integrating components has been made.

Channels

The basic element of a circuit is a channel. In microfluidics the channel depth is the order or few tens or microns, whereas the width is of the order or few hundreds of microns. The aspect ratio is typically limited by the materials use - PDMS need an aspect ration of 1:15 or less, otherwise it will sag under its own weight.

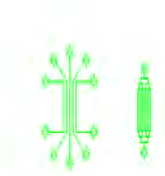

Figure 31: Connecting channels in parallel and serial

The channels can be connected in a variety of ways - in parallel or in series. In recent years there have also been demonstrations of 3-D geometries. Further the cross section may be square, rounded or some other shape. The resistance of a such a channel to fluid flow depends on the the geometry, the roughness of walls, and on the size. For example, we showed earlier that for a round cross section, the resistance scales inversely as the forth power of the radius.

3D channels

In the course we will have the opportunity to build channels that have a step change in height using multi layer photolithography. These kind of geometries are useful for trapping cells. However, building microfluidic devices that use the third dimension is hard because of the need for alignment. Alternatives include certain 3D printers that use meltable waxes may be used for millimeter sized 3D geometries. Another option is to use laser ablation to ablate out 3D channels and features. In this workshop we will also use plastic adhesive sheets to build things layer by layer.

Valves

Two channels separated by a thin membrane can be used to create a pneumatic valve[13]. The deflection of the membrane when the control channel is pressurized closes the channel. The geometry of the flow channel can be important in determining pressures needed to actuate

[13] M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, and S. R. Quake. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science*, 288:113–116, 2000

- rounded channels help lowering pressures because the sharp edges make it hard to close.

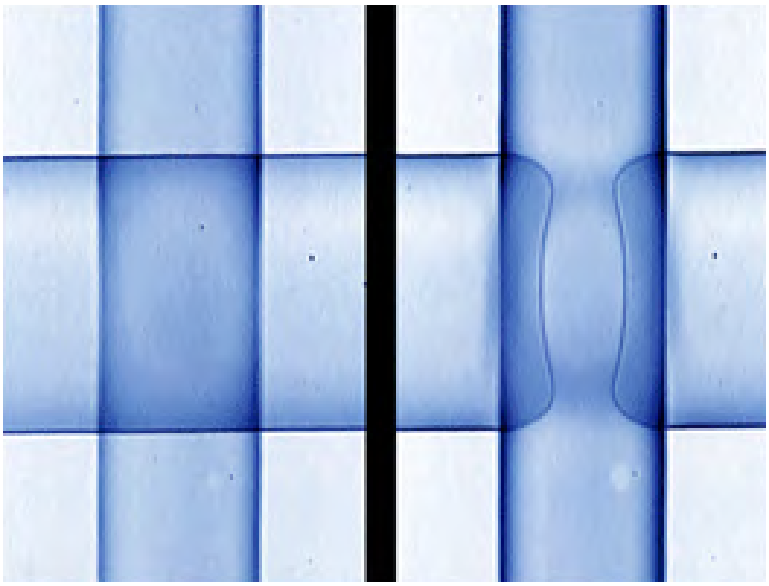

Figure 32: Pneumatic valve closing a channel

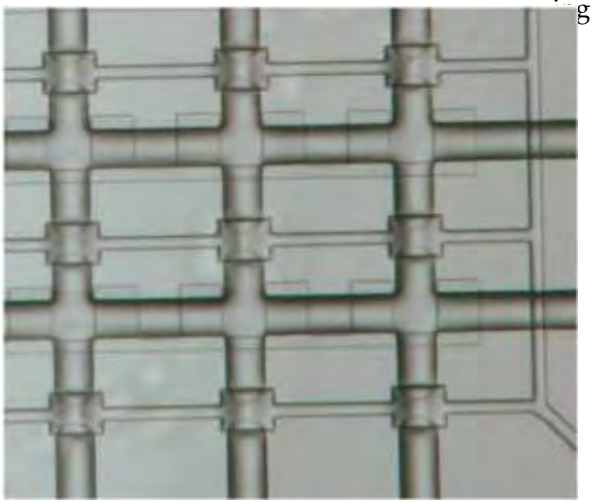

Figure 33: Many valves operated simultaneously

The design for a valve can be push down or push up as shown in Figure 34. In a push up design, lower actuation pressures are needed but the material in the channel is surrounded by PDMS. In the push down case the material in a channel can have one side in contact with a cover glass - an advantage if surface chemistry needs to be controlled. Many valves can be connected to a single control pressure line and this feature is extremely useful when many valves need to be opened and closed at the same time(Figure 33)

Cross-over geometry

If the control channel is too narrow the flow channel will not close. Thus if you want a control channel to lie over a flow channel due to topological contraints but not have a valve there this is one way to do it. Cross over channels become necessary in complicated designs where many channels criss-cross each other. They are also needed to make multi-plexers which we will dicuss in a future section.

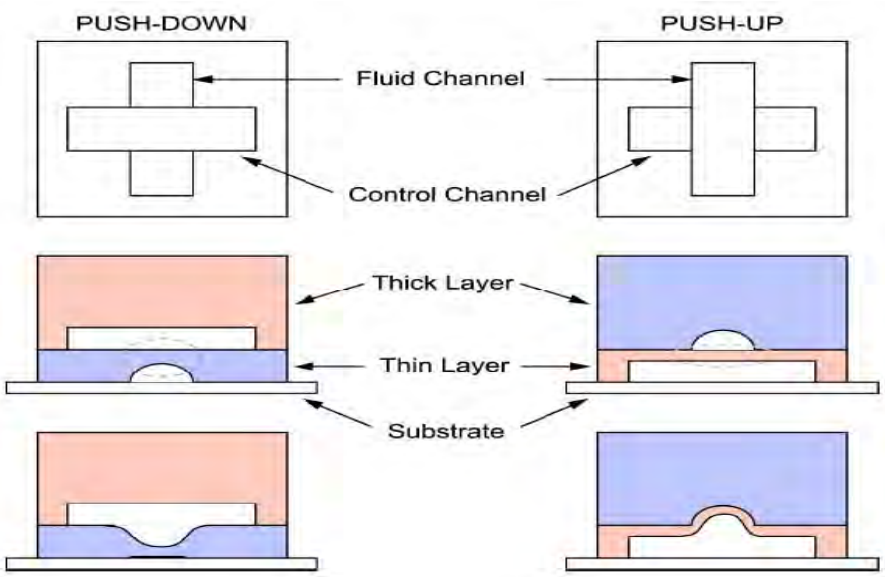

Figure 34: Push-up and push down valves

A variety of attempts have been made to make electrical-on-chip valves - including the use of braille pins, electroactive polymers, capacitive and magnetic valves, electro-rheological valves, shape memory alloys , electrolysis, gels, torque screws (including by the author himself!). However, none of them match the versatility and ease of manufacturing of plain pneumatic ones yet. Electronic valves are desirable because we can then miniaturize the control system and integrate it with the fluidics.

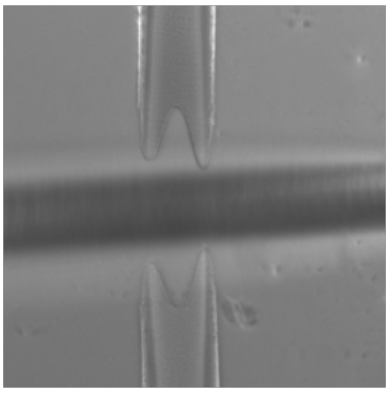

Figure 35: Shape memory alloy valve

Mixers and Pumps

A simple on-chip peristaltic pump can be made using 3 valves in a row that operate in the perstaltic sequence (101,100, 110,010,011,001) continuously. This pump operates by squeezing the liquid forward (or backward) like you would squeeze a toothpaste tube. One nice feature is that the number of cycles precisely determines the amount

of fluid dispensed. This pump will only work with incompressible fluids (i.e. liquids). Peristaltic pumps can also be made with four valves.

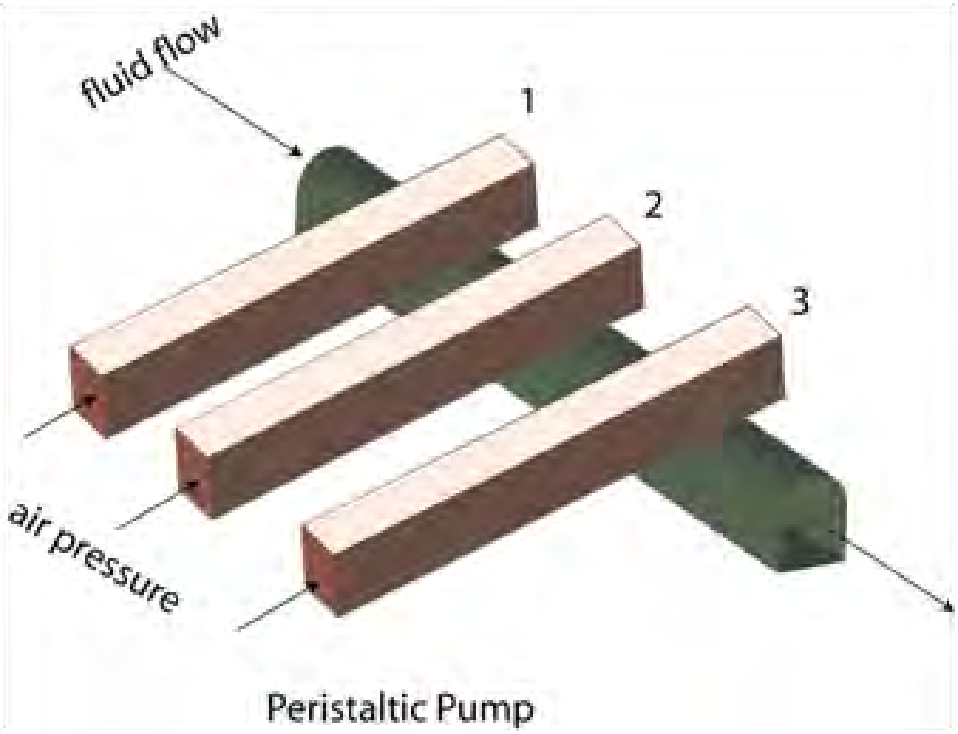

Figure 36: Three valves can create a peristaltic pump

In laminar flow based devices mixing by diffusion can be so slow that it is often a problem and specialized mixers are needed. A rotary peristaltic mixer involves a closed off circle and a a peristaltic pump as shown in Figure 37

Another kind of mixer is the herringbone mixer shown in Figure 38. This was first introduced in 2002 [14]. This type of mixer is based on chaotic advection - something we will discuss more in the next lecture.

[14] Abraham D. Stroock, Stephan K. W. Dertinger, Armand Ajdari, Igor MeziÄĞ, Howard A. Stone, and George M. Whitesides. Chaotic mixer for microchannels. *Science*, 295(5555):647–651, 2002

Filters, Sieves, Button valves

In many fluidic devices having a way to trap particles or cells while having fluid flow by is useful. There are multiple ways to do this - one could simply introduce a series of posts, separated by a distance smaller than the particles themselves.

Alternatively one could design a pneumatic valve which would only partially close - usually done by simply not rounding the channel, but

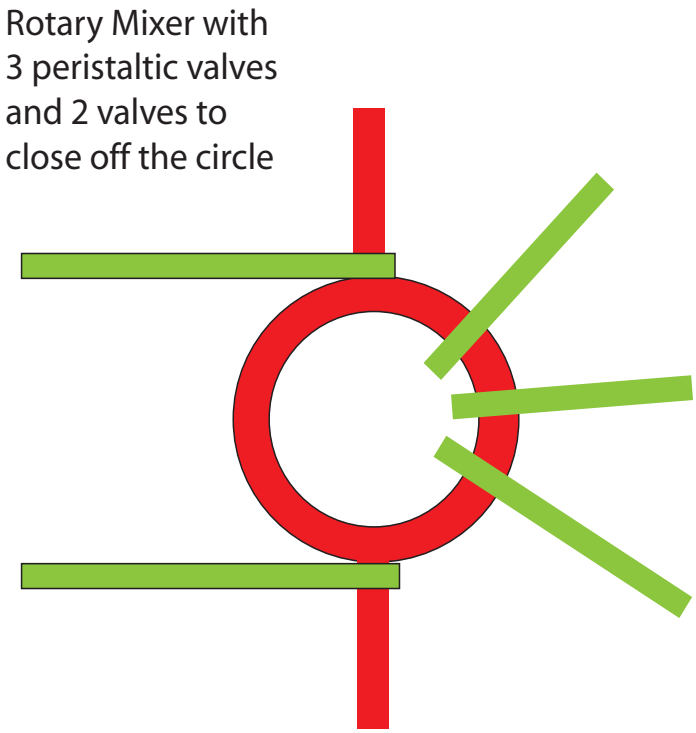

Figure 37: Rotary Peristaltic

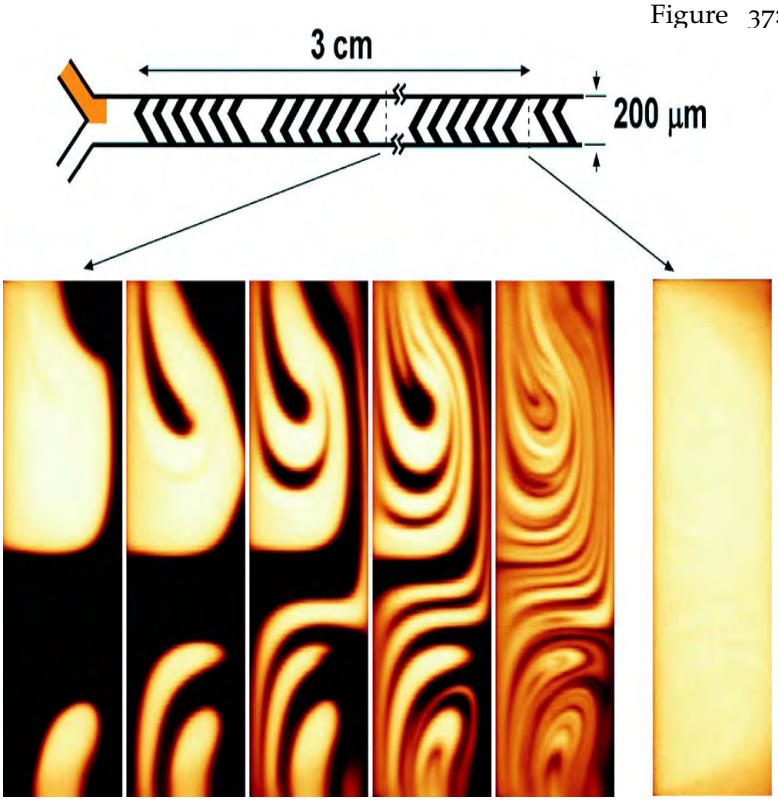

Figure 38: Herring Bone mixer from reference Stroock et al 2002

leaving it square. This allows trapping of particles.

Figure 39: Seive Valve with beads trapped

Another kind of specialized valve is the button valve. It allows isolation of a region on a surface with a valve - removing any material non-specifically bound.

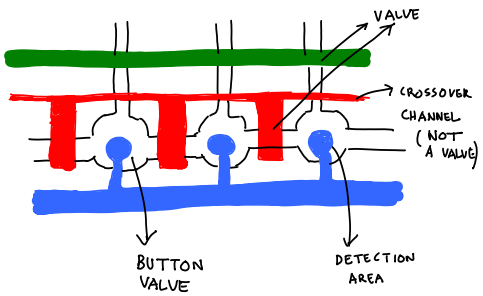

Figure 40: Button Valve

Multiplexers

A multiplexer in electronics selects out one output from a several input lines. It is mainly used to increase the amount of data transmitted. For instance a cell phone tower may have to send out the information from several calls in a given amount of time. One way to do this is to divide that time into smaller units and select one line for every small unit

of time, switching very quickly to another call. If the system is fast enough you would never notice the difference - this is simply a form of timesharing. A demultiplexer does the opposite - it takes one input and splits it into many outputs

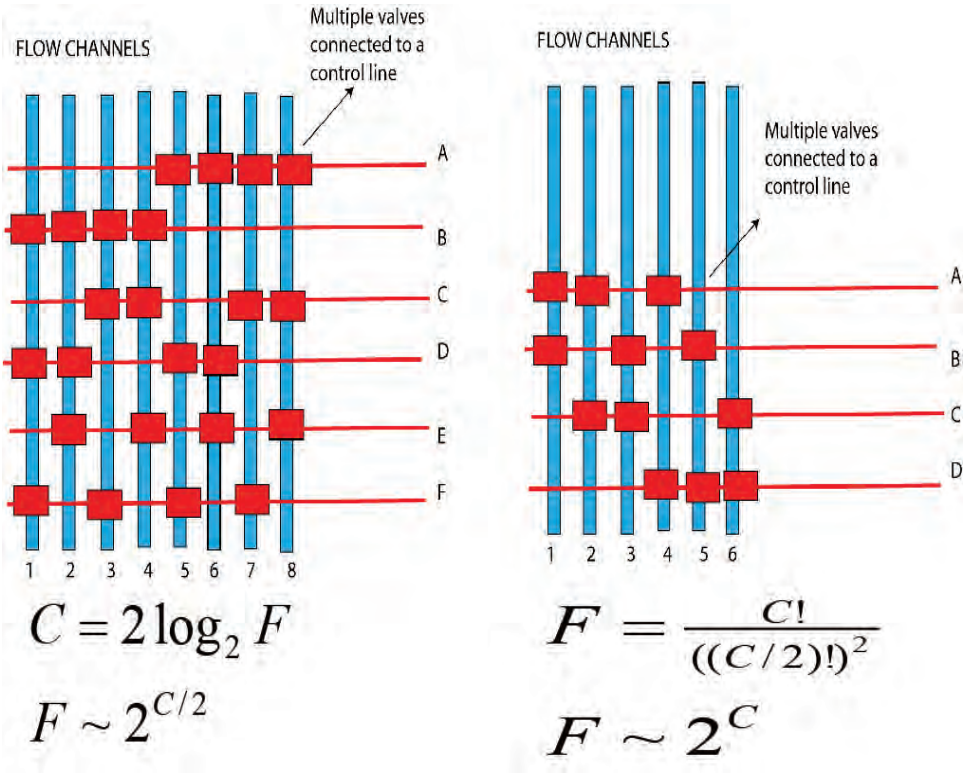

Figure 41: Logarithmic and Combinatorial Mulltiplexer

In fluidics, it is possible that there are several channels that supply reagents and you need to switch from one to the other. We can build a fluidic multiplexer to do this. Two multiplexers logarithmic and combinatorial - shown in Figure 41. In these multiplexers a set of control lines are used to select out one flow line from a larger set of flow lines. This allows savings in number of control lines required. If each flow line had its own valve the number of control lines would be equal to the number of flow lines. If however, for the operation of the chip, only a single line needed to be used at a give time, we could save on control lines by putting in a multiplexer. In this case a number of control lines are activated and they select out a particular flow line - for instance in the combinatorial mixer - turning on lines A and D selects flow line 3. Another example: selecting control lines F, D and A results in flow line 2 being activated in the logarithmic multiplexer.

It must be understood that there is no free lunch - certain combinations of lines are impossible. For instance if I wanted BOTH 1 and 2

flow lines to be on, and all other lines closed - no combination of valves would do (in either type). Multiplexers are useful only if at a given time only one input line is needed.

The reader may wonder how these multiplexers are designed - the design is based on elementary principles of logic and combinatorics. A combinatorial multiplexer is more efficient then a logarithmic one, but a logarithmic multiplexer can also be used in a binary tree design which makes it more versatile in some situation where cross-contamination between lines has to be minimized

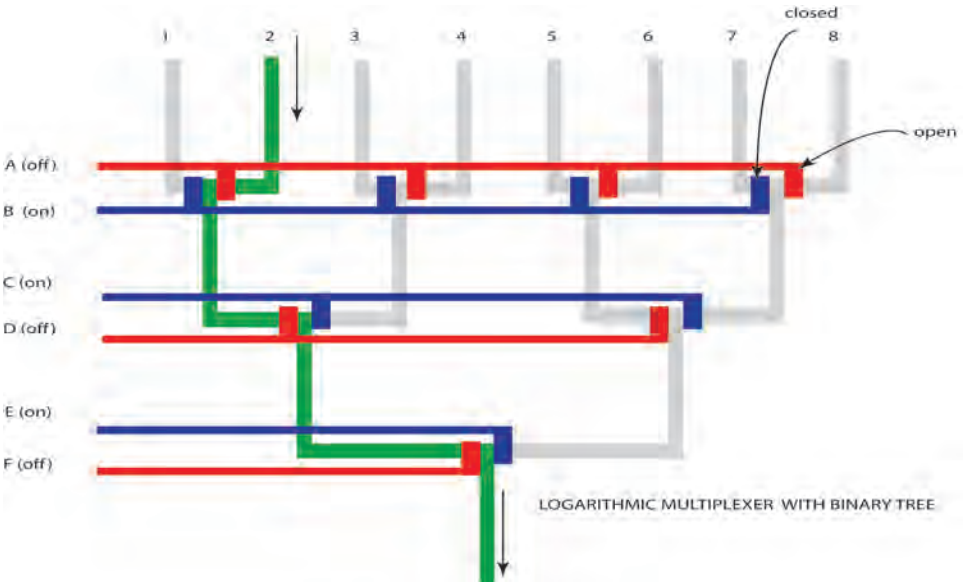

Figure 42: Binary Tree multiplexer

It's worth mentioning that even more sophisticated possibilities exist. Valves may be of latching variety - sending signal turns them on or off and lets them remain that way. Further, vacuum may be used in conjunction with pressure to make three state systems. The multiplexer can itself be used to run another set of channels that in turn control valves on a chip - so the multiplexer selects out an instruction or state of a chip rather than a simple line. This design called deconvolver was proposed by the author. The possibilities for control are endless - but we are running ahead of things here - our fabrication technologies have not reached the level of reliability to routinely use these options and unless the process actually needs such control, there is no point in making a device with such complicated controls.

All the component described can be combined to produce a device that has many capabilities - exactly in the same way as you would com-

bine electronic components into a circuit. We shall see such examples in our experiments.

Building a Control System

The valves and pumps on the chips are actuated with pneumatic solenoids connected to a pressure supply. At least two pressures are needed - a low pressure for flow channels (1-5 psi) and a higher one (10-25psi) for actuating valves. We built our 24 valve controller based on a design from Raphael Gomez-Sjoberg. The printed circuit board for the controller was obtained from the Stanford Microfluidic foundry (for free!) and the matching for some of the fixtures was done at the Princeton Physics departmental workshop by Jason Puchalla.

To make these you need a little familiarity with basic electronics. You can find all the details on his website, with the latest designs for controllers and the parts you will need:

`https://sites.google.com/a/lbl.gov/microfluidics-lab/valve-controllers`

Ordering a Completed Device

It is possible to avoid any fabrication and just order in a device after providing the design. Of course, this will cost you. Two foundries exist on the west coast and more are likely to spring up at other universities.

Name	Location and Contact
Caltech Microfluidic Foundry,	Kavli Nanoscience Institute 1200 E. California Blvd., California Institute of Technology, Pasadena, CA 91125, USA (e-mail) foundry@caltech.edu
Stanford Microfluidic Foundry	Stanford University, Palo Alto, CA 94315, USA (e-mail) su-foundry@lists.stanford.edu

Table 6: List of Microfluidic Foundries. These foundries will generally accept a design or a mask and provide you a PDMS chip for a fee.

Comparison of materials for microfluidics

A variety of materials have been used for microfluidics. A partial listing includes: silicon, elastomers (PDMS etc), plastics, metals, glass, ceramics, paper, gels, wax, adhesive tapes, epoxies, parylene, fabrics. In this course we will see plastic films, PDMS, adhesive films and silicon being used in experiments.

Each material has advantages and disadvantages. The most popular materials in academia are PDMS and Silicon/Glass. PDMS is inexpensive, transparent and tolerates aqueous solutions (aqueous acids, bases etc) well. It is easy to work with and seals gaps very well (being elastomeric). PDMS is air permeable which is helpful in dead-end filling and reduction in bubbles, but harmful for any kind of anaerobic culture and maintaining osmolarity. PDMS does not age well - PDMS after a few years may have very different properties than what it started out with. Most organic solvents tend to partition into PDMS and swell it - making chemical reactions that involve such solvents difficult. This rules out DNA or peptide synthesis which need solvents like aceto-nitrile. A class of fluorinated elastomers - teflon-like have been developed (the author was involved with some of that work) to overcome these problems - however they are expensive and difficult to work with at the moment[15].

[15] R.M. Van Dam. *Solvent-resistant elastomeric microfluidic devices and applications*. PhD thesis, California Institute of Technology, 2006

Silicon and glass on the other hand are much more inert and resistant to chemical attack. However, working with them requires harsh chemical conditions making processes expensive. Bubbles can be a huge problem as they cannot diffuse through the material of the chip. With silicon, due to the electronics industry, well developed processes allow careful control of etching - and very small nanometer sized features can be built. Silicon and glass tend to age well, unlike PDMS. Due to their hardness, valving can be a difficult proposition - some sort or gaskets or diaphragms are needed. Silicon and gals tend to withstand higher pressures than PDMS. For very high pressure applications, steel and other metal alloys are used.

Plastics (polythene, polycarbonate etc) are the most common materials in industry - injection molding is used to make inexpensive parts by the millions. Currently, there no well developed processes to make very small features reliably, so plastics are used for larger parts and manifolds.

Lecture 4: Surface Tension, Two Phase flows, Cells in Devices

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Anyone who has played with soap bubbles will know that surface tension leads to many beautiful and unexpected phenomena. In microfluidics, as we go down to smaller and smaller size scales - surface tension keeps increasing relative to other forces since it only scales as the inverse power of length (as opposed to the inverse fourth power for pressure for instance). It becomes overwhelming the largest force at small length scales. In this lecture we will study some of these consequences.

Surface Tension

Simply stated, surface tension is the energy needed to create more surface area. Why does creating a surface need more energy? imagine a water molecule deep inside a water glass - it feels attractive forces from all sides. From elementary chemistry we are aware that these bonds allow it to rest in a energetically favorable state. Compare this with one at a surface. Approximately, half it's interactions are now missing and energetically it is an unhappy molecule. Creating a surface requires energy. The energy is approximately half the average energy of a molecules (kT). The table at the side shows some surface tension values for a number of liquids.

Table 7: Surface Tension

at 20^0C	
Type	$\gamma(Nm^-1 \times 10^{-3})$
Water-air	73
Mercury-air	485
PDMS-air	19
Ethanol-air	22

There is a another complementary way to think of surface tension - as a the force exerted along a boundary. Since the molecules at a surface are energetically in a unfavorable position, they exert a force to reduce the surface area - to close a boundary if possible.

Laplace Pressure

Fluid surfaces that are curved cause an increase in surface area - which must be balanced by a change in pressure to balance out the forces.

This excess pressure is given by the Young-Laplace equation written as:

$$\Delta P = \gamma(\frac{1}{R_1} + \frac{1}{R_2}) \tag{34}$$

(a) Thomas Young (1773-1829) (b) Pierre-Simon Laplace (1789-1827

Here R_1 and R_2 are the principle radii of curvature of a surface, γ the surface tension, and ΔP the pressure difference. Each surface has two principle radii of curvature at every point - they are both equal to the radius for a sphere, for a cylinder one is infinity and the other - the radius of the cylinder

Capillary forces are responsible for the rising of liquids in small capillaries. An elementary force balance calculation shows that the meniscus of an liquid has an height given by

$$h = \frac{2\gamma cos\theta}{\rho g r} \tag{35}$$

where r is the radius, θ the contact angle, γ the surface tension, *rho* the density. This height can be substantial - several inches for sub-millimeter bore capillaries.

Several diagnostic devices use capillary action to take in liquids - examples of this include diabetes test strips, and lateral flow kits for pregnancy testing. Recently several groups developed methods to produce simple microfluidic devices with paper and photo-resist - creating hydrophilic and hydrophobic areas and using wicking to move liquids [16]. This technology is being commercialized by "Diagnostics for All" `www.dfa.org`. The main attraction for this technology is the low cost of fabrication and autonomous operation (no external control needed).

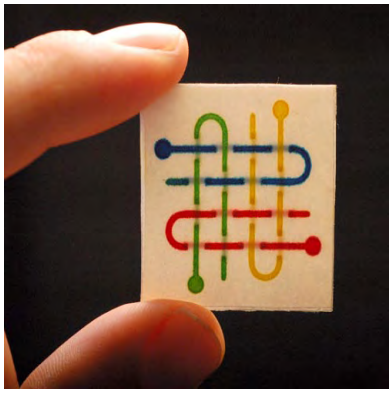


Figure 43: Paper Microfluidic Device

[16] Andres W. Martinez, Scott T. Phillips, George M. Whitesides, and Emanuel Carrilho. Diagnostics for the developing world: Microfluidic paper-based analytical devices. *Analytical Chemistry*, 82(1):3–10, 2010. PMID: 20000334; and Elain Fu, Barry Lutz, Peter Kauffman, and Paul Yager. Controlled reagent transport in disposable 2d paper networks. *Lab Chip*, 10:918–920, 2010

Capillary Number

The Capillary number is the dimensionless scaling number that governs the behavior of systems involving surface tension. It is given by:

$$Ca = \frac{\mu v}{\nu} \tag{36}$$

The capillary number compares viscous forces to surface tension forces. For values $>> 1$ viscous forces dominate and vice-versa. The capillary number will show up in most microfluidic phenomena involving bubbles and emulsions.

Wetting and De-wetting

If you own a waterproof raincoat you may have noticed that water droplets tend to ball up on the surface of the raincoat. The surface is hydrophobic or non-wetting. When three phases of matter (solid, liquid and gases) meet at an interface they create an angle that is the most energetically favorable - this is known as the contact angle.

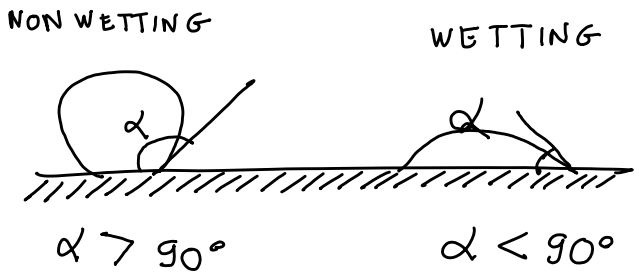


Figure 44: Contact angle

Wetting and de-wetting properties of aqueous solution have been used to create microfluidic devices in a field called "digital microfluidics". In these devices the surface energy is changed using electrical fields and the difference in surface energy provides the force for droplets to be moved. While very promising, at the moment this is very much a niche area with only a few groups working on it. A review is available for those interested [17]. This technology was first developed at Duke University and is being commercialized at Advanced Liquid Logic `http://www.liquid-logic.com/`

[17] R. B. Fair. Digital microfluidics: is a true lab-on-a-chip possible? *Microfluidics and Nanofluidics*, 3:245–281, 2007

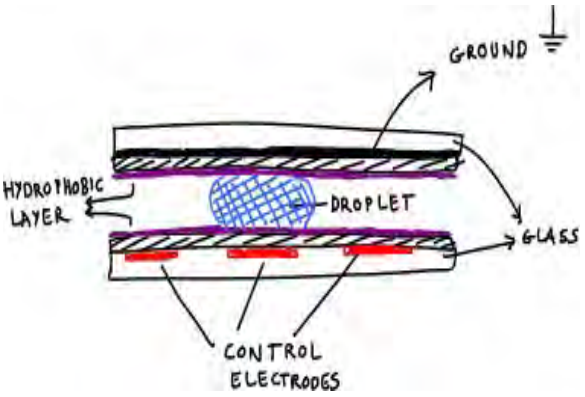


Figure 45: Design of electrowetting devices

Two Phase Devices

Up to now we have only considered single phase devices. Adding an extra phase makes things much more complicated and interesting. There is great interest in using water droplets in oil as tiny reaction chambers, with the idea of increasing throughput drastically. In some sequencing techniques emulsion PCR - done in drops of aqueous reagents suspended in oil is used. In these cases droplets acts as independent compartments. It is also possible to use air as the continuous phase, separating plugs of liquids.

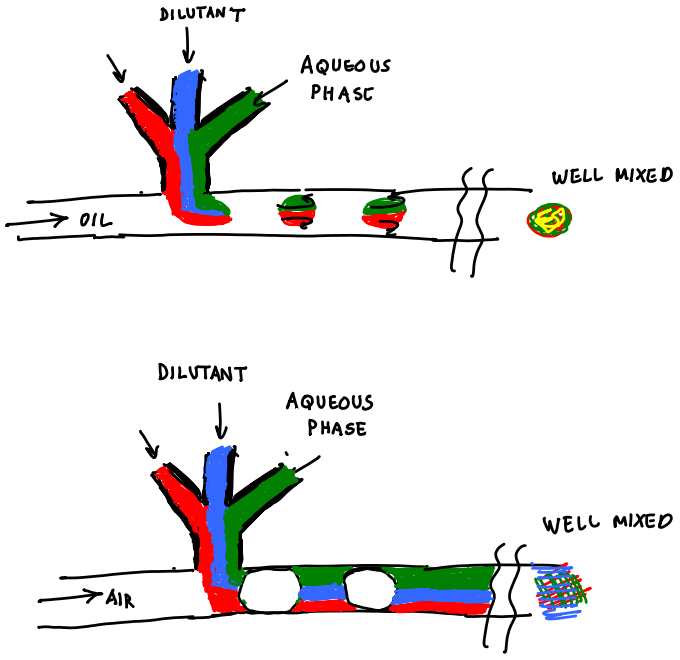


Figure 46: Creating isolated picoliter compartments

Another two phase flow involves solids in liquids. In this case a

parameter called Stokes number is useful

Stokes Number

Stokes number is the ratio between the particle relaxation time to the fluid relaxation time. Let us assume that particles of diameter d are in a fluid and encounter an object of size L. The fluid velocity far away from the obstacle is taken to be v

$$St = \frac{\tau v}{L} \tag{37}$$

where τ is the relaxation (also called response time) for the particle. This can be calculated as

$$\tau = \frac{d^2 \rho_p}{18\mu} \tag{38}$$

Putting these two together we have

$$St = \frac{\rho_p d^2 v}{18\mu D} \tag{39}$$

A low Stokes number indicates particles that can easily follow the fluid flow. A high (»1) indicates particles that cannot follow the flow and will tend to bump into obstacles. This principle can be used to make a separating device as shown in Figure 47[18]

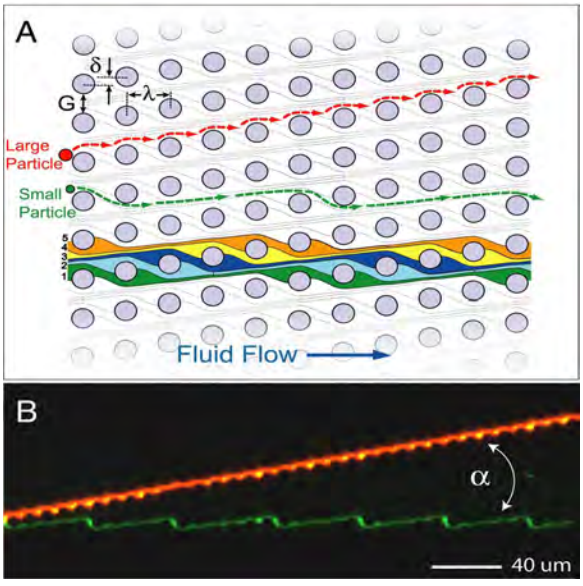


Figure 47: A set of posts at an angle to the flow can sort particles by size

[18] Keith J. Morton, Kevin Loutherback, David W. Inglis, Ophelia K. Tsui, James C. Sturm, Stephen Y. Chou, and Robert H. Austin. Hydrodynamic metamaterials: Microfabricated arrays to steer, refract, and focus streams of biomaterials. *Proceedings of the National Academy of Sciences*, 105(21):7434–7438, 2008

Surfactants

Surfactant are chemicals that are active at surfaces. They tend to lower the surface energy (hence surface tension). Surfactants can be neutral, anionic, cationic, amphoteric etc. For microfluidic two-phase devices that involve droplets, surfactants are critical to get appropriate physical and chemical properties and influence everything from the physical properties of emulsion to the denaturing of proteins at interfaces.

Sorbitan monooleate (Span 80) and Polysorbate 80 (Tween 80) are two common surfactants used in many microfluidic oil/water devices. One problem with these is that material tends to be exchanged between droplets which destroys the isolation needed for many applications; proteins can denature at the interface - damaging enzymatic capabilities and properties of droplets can change with time. More recent devices tend to use perfluoro oils (e.g. Fluoinert FC-75, FC-40 etc) as the inert carrier and fluoro-surfactants that have been chemically modified[19]. In the commercial space RainDance Technologies is commercializing droplet based processes `http://www.raindancetechnologies.com/`

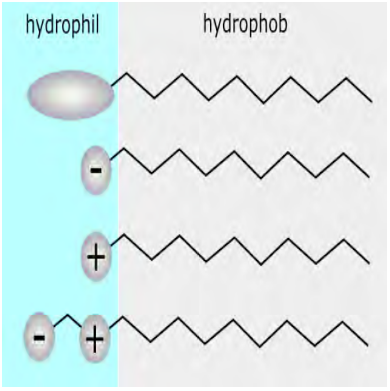


Figure 48: Neutral, Anionic, Cationic and Amphoteric surfactants

[19] Jenifer Clausell-Tormos, Diana Lieber, Jean-Christophe Baret, Abdeslam El-Harrak, Oliver J. Miller, Lucas Frenz, Joshua Blouwolff, Katherine J. Humphry, Sarah KÃűster, and Honey Duan. Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms. *Chemistry and Biology*, 15:427–437, 2008; C. Holtze, J. J. Agresti, J. B. Hutchison, C. H. J. Schmitz, H. Duan, K. J. Humphry, R. A. Scanga, J. S. Johnson, D. A. Weitz, and D. Pisignano. Biocompatible surfactants for water-in-fluorocarbon emulsions. *Lab on A Chip*, 8, 2008; and Eric Brouzes, Martina Medkova, Neal Savenelli, Dave Marran, Mariusz Twardowski, J. Brian Hutchison, Jonathan M. Rothberg, Darren R. Link, Norbert Perrimon, and Michael L. Samuels. Droplet microfluidic technology for single-cell high-throughput screening. *Proceedings of the National Academy of Sciences*, 106(34):14195–14200, 2009

Mixing by Chaotic Advection

Mixing in droplets occurs due to a process called chaotic advection and serpentine channels are good at inducing it. The process is best shown in picture format in Figure 49. We will make a serpentine mixer in our labs that will make the process clearer.

Cells in Chips

We can subdivide the methods to put cells into devices into four possibilities as shown in the Table **??**.

Name	Cells move in-out	Nutrients move in-out
Batch	✖	✖
Chemostat	✔	✔
Microhabitat patch	✖	✔
(no name -ecology?)	✔	✖

Table 8: Types of systems

Bacterial cells in microfluidic chips is much easier than dealing with mammalian cells. Mammalian cells need precise control of gas concentrations, surface chemistry and osmolarity and this can be challenging to achieve especially on longer time scales (days and weeks). Various types of incubators have been designed to overcome the challenges.

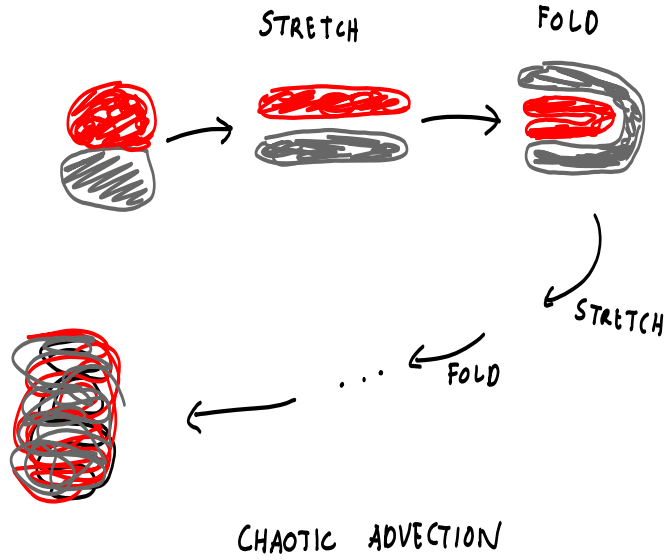
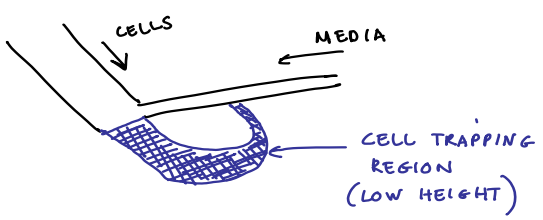
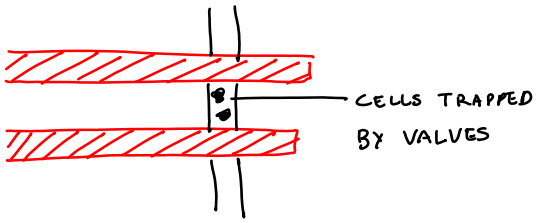


Figure 49: Clay model of chaotic advection

A large number of different ways have been designed to trap cells in microfluidic devices for imaging purposes. A prison for cells can be created with valves - and here as little as a single cell or a millions of them could be trapped. Posts and height differences can also be used for the same purpose.

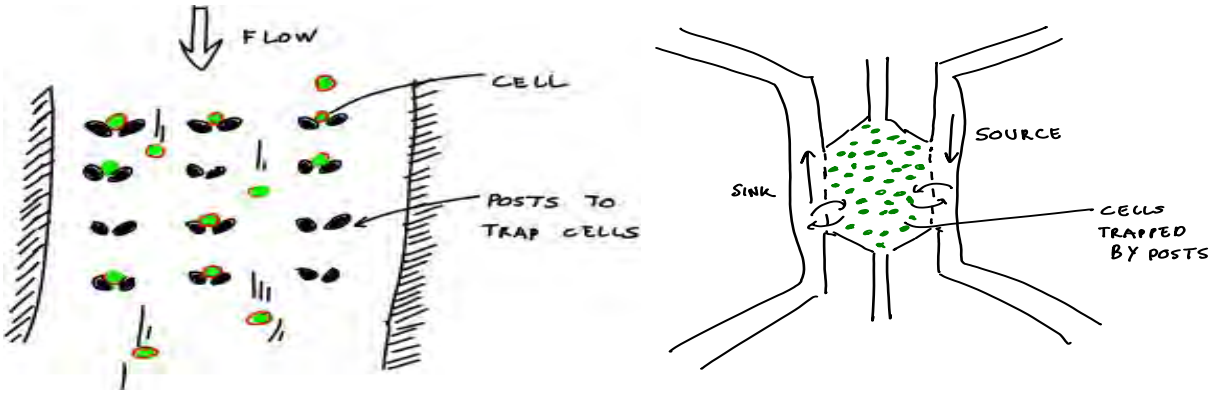


(a) Tesla chemostat: using differencial height



(b) Valvetrap: using valves

Figure 50: Capturing cells



(a) Post trap: using posts

(b) Post trap: using posts in another way

Figure 51: Capturing cells

Small Organisms in Chips

Besides cells it is also possible to work with small organisms like C. elegans, zebra fish, planaria. In particular in the case of C.elegans, microfluidic chips have proved very versatile and will find widespread use [20]. To take one such instance, C. elegans worms can be trapped in channels and its nose exposed to odorants, and the neural pathways that are activated studied. This was shown in a paper a few years ago [21]. We will in the experimental part of the course attempt a entertaining project with C.Elegans

[20] S. Elizabeth Hulme and George M. Whitesides. Chemistry and the worm: Caenorhabditis elegans as a platform for integrating chemical and biological research. *Angewandte Chemie International Edition*, 50(21):4774–4807, 2011

[21] Sreekanth H. Chalasani, Nikos Chronis, Makoto Tsunozaki, Jesse M. Gray, Daniel Ramot, Miriam B. Goodman, and Cornelia I. Bargmann. Dissecting a circuit for olfactory behaviour in caenorhabditis elegans. *Nature*, 451:102–102, 2008

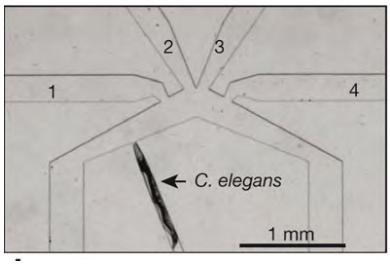


Figure 52: C.Elegans trapped in a device and exposed to odors

Lecture 5: Conclusion

Future of Microfluidics

We would like microfluidics to be a practical technology that can be used by a large number of people. Ink cartridges remains the biggest market for these devices - biomedical application being far behind. In the biomedical field, simple devices for the consumer market - like pregnancy tests and glucose testing devices are well known. Recently niche application like sequencing are pushing the limits of what is currently possible.

There are several challenges that limit the widespread adoption of microfluidics The first is the barrier to learning and doing things - and this course, and others like it, are designed to reduce that threshold. There are other problems that need solutions; we mention three. A breakthrough in any of them will have a major impact:

Standardization

The connectors and designs for microfluidic devices are not standardized. In electronics we have many different standards - USB, serial ports, that allow easy interchangeability. Other than 96-well plates this kind of standards do not exist for fluidics at the micro scale, making it harder to use them.

Control and Detection

Building smaller chips is not enough - the control and detection systems like microscopes and solenoids also need to be miniaturized. This macro to micro problem was solved in electronics with techniques like wire-bonding and specialized packaging.

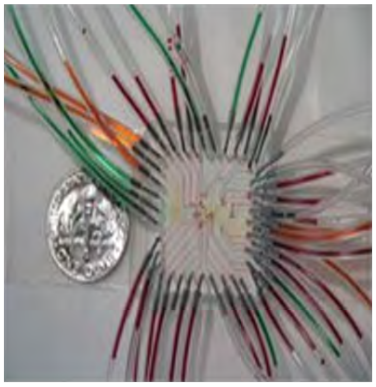


Figure 53: Chips may be small...tubing, control and microscopes are large

Universal platform

Unlike electronics where PCB boards and silicon wafers are industry wide standards, there are many competing platform technologies in microfluidics. It is not clear which ones will become universal standards. In academics, the "it's no good, because it was not invented here" syndrome prevents quick adoption of technology. In industry, patents and trade secrets create a barrier to diffusion of know-how.

PDMS devices in recent years have really taken off - but they are largely ignored by industry because of problems with gas diffusion, leaching of chemicals and poor aging characteristics. Silicon devices which were very popular in the 90s have waned because of the difficulties in getting simple devices made without access to clean-rooms. They may yet stage a comeback because of silicon's desirable chemical characteristics.

What was left out

Microfluidics has grown so large that is impossible to cover or even do justice to many parts of it. So the author apologizes in advance to anyone offended that their favorite technology was not included. This omission is not a judgment on the importance of a topic, but a reflection of the author's ignorance.To mitigate this let me go through a few things we have not encountered at all in this workshop.

We have barely scratched the surface of flows influenced by electrical and magnetic fields. This is a very important part of microfluidics with many applications. Capillary electrophoresis is what got microfluidics started. We did not cover any kind of heat transfer. In reactions like PCR, temperature cycling is required. A variety of new effects and scaling numbers are needed to understand thermal effects in fluids.

Also left out several technologies - for instance hydrogels microfluidics and microfluidics on spinning disks. No mention was made of nanofluidics - the technology where we try to go down even further in size scale. For most industrial application the most cost effective way to make devices is via injection molding which we have also not covered. There is also considerable interest in implantable devices, which we have not had much of a chance to talk about.

Conclusion

We have attempted to give a broad, simplified introduction to microfluidics. Our hope is to reduce the intimidation that a novice faces in making and using these devices, and enable the student to exercise scientific judgment on whether it makes sense to use these tools in their specific research problem or project. Microfluidics is a young field, and there much scope for making new discoveries and we hope that some of you will.

Part II

Experiment Manual

Lab 1: Creating Static Gradients

Summary:

Haploid cells of S. cerevisiae come in two mating types, Mat a and Mat alpha. Haploid cells release mating pheromones (termed a and alpha-factor) into the media. When a cell of the opposite mating type senses this mating pheromone is "shmoos" (forms a projection) towards the mating partner. If two haploid cells shmoo close enough towards each other, they will fuse and form a zygote, from which diploid cells will bud.

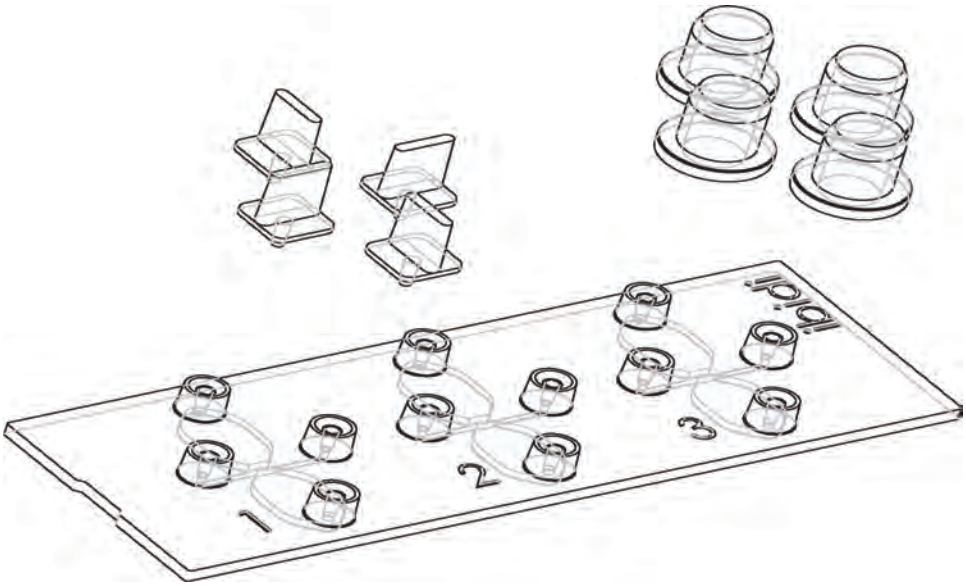


Figure 54: Ibidi 2D Chemotaxis plastic slide

The Ibidi 2D Chemotaxis slide establishes a gradient of a desired factor across a cell observation area. We will use this device to setup a gradient of alpha-factor across yMM736 cell which are Mat a. We can measure the response of these cells to the gradient in two ways: 1) we can analyze cell morphology, and look for shmooing and the direction

of the shmoo polarization and 2) yMM736 has a mating-response promoter (FUS1) tagged with GFP so that cells will produce GFP as they respond to pheromone.

Reagents:

Ibidi 2D Chemotaxis Îij-Slide
Low fluorescence yeast media
yMM736 yeast strain (YPH499 Mat a STL1promoter::HA-tdtomato::leu2::ADE2 FUS1promoter::HA-eGFP::leu2::ADE1 bar1::KanMX ura3-52)
1mg/ml alpha-factor in DMSO
1ug/ml sulfur rhodamine
2mg/ml concanavalin A solution

You will also need the manual for the Ibidi chip - a copy will be available during the experiment and there is also a file in the papers folder on our website

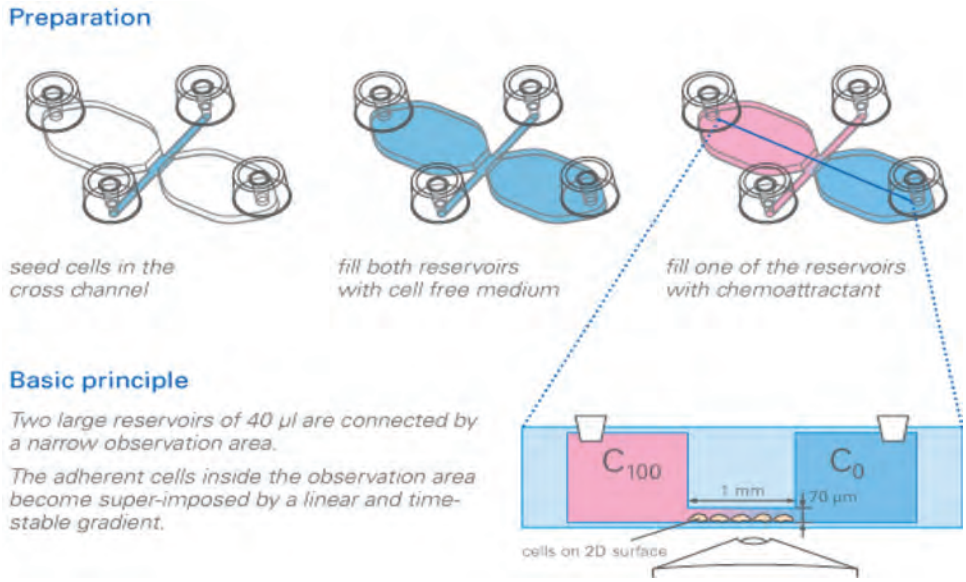


Figure 55: Experimental Protocol

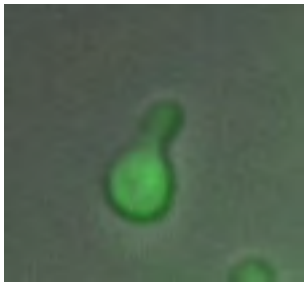
Protocol

1. Cell Preparation
a. Inoculate a single colony of strain yMM736 overnight into low fluorescence yeast media (2 mls in a glass test tube). Grow overnight at 30^0C.

b. In the morning, set back the yeast in 5 1:100 in 5mls of fresh media. Let grow for approximately 5 hours at 30^0C (timing is not crucial for this experiment, cells just need to be happily growing to respond to alpha-factor).

2. Chamber preparation
a. Prime the chamber with concanavalin A solution (this will affix yeast cells to the chamber, so that they do not float around during imaging). To coat the chamber, follow instructions 4A in the manual.
b. Allow this solution to incubate in the chamber for 5 minutes.
c. Aspirate the solution.
d. Fill the observation area with cell culture, following step 5A in the manual. Allow cells to settle for at least 5 minutes (you can set them on the microscope and watch the cells settle down. Look for cells that are not wiggling around - when you have a desired amount of these you are in good shape).
e. Fill the chamber, being careful to avoid air bubbles, with 80ul of low fluorescence cell media following step 5C in the manual. To wash out excess cells you can flow an extra 80ul of media.

Figure 56: Yeast cell shmoo



f. Add your chemoattractant. This will be given to you as a 1ng/ul rhodamine, 100ng/ul alpha-actor solution dissolved in low fluorescence media. Follow instructions 5d in the manual to add chemoattractant. You should be able to see one chamber take on a faint pinkish color due to the rhodamine.

3. Imaging
a. Pick a field of view. Set up a timecourse with GFP and transmitted to image cells in time. Within 1 hour you should see visible shmoos and cells nearest the mating factor should be turning very green.

Lab 2: Easy Microfluidics I: Shrinky-Dink Devices

In the first few experiments we will make inexpensive microfluidic devices and use them. If you want to make something quickly without being too worried about the exact dimensions, these techniques will work. Of course, there is a 'price' to pay in the tolerances, and hence what is possible.

About the experiment

This experiment is based on 2007 paper by Michelle Khine (`http://shrink.eng.uci.edu/`) and collaborators[22]. The design we will make is a H-filter first conceived of by Brody et al[23]. More information on this design can be found here:

`http://faculty.washington.edu/yagerp/microfluidicstutorial/hfilter/hfilterhome.htm`

We use a children's toy called shrinky-dink. Shrinky dinks are sheets of plastic (poly-olefins for the plastic-geeks) that shrink when heated. When you shrink a film with something drawn on them, the ink particles become raised and squished leading to intense colors. You can buy these films from a hobby craft store or on-line[24].

The raised ink particles create a mold with the height of features depending on the particular shrink factor and the ink used (printing multiple times can help in getting taller features). This is microfluidics at its simplest - just a printer, some shrink film, an oven and some PDMS is needed.

[22] Anthony Grimes, David N. Breslauer, Maureen Long, Jonathan Pegan, Luke P. Lee, and Michelle Khine. Shrinky-dink microfluidics: rapid generation of deep and rounded patterns. *Lab Chip*, 8:170–172, 2008

[23] James P. Brody, Thor D. Osborn, Fred K. Forster, and Paul Yager. A planar microfabricated fluid filter. *Sensors and Actuators A-physical*, 54:704–708, 1996

[24] Some places to get them `http://www.shrinkydinks.com`, `http://www.grafixplastics.com/shrink.asp`

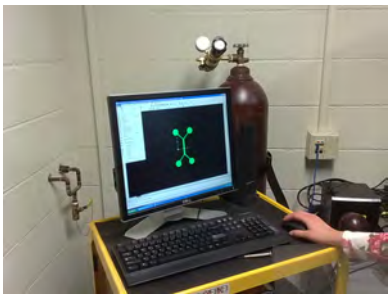


Figure 57: H filter design

Procedure

Follow these steps:

1 Open the Hfilter.dwg file on the computer.

2 Insert a laser transparency in the laser printer (manual tray 2) and plot the file on the printer. We have set the printer to print on a laser transparency. Don't change this setting. If all goes well the design will show up in the middle of the transparency.

3 Wearing gloves and using the printed design as a guide, place a 2 in x 2in shrinky film to cover the design and tape all 4 sides with scotch tape.

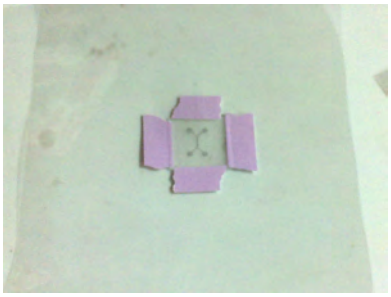


Figure 58: Tape shrink film

▷Note	Tape on all 4 sides is necessary to prevent curling due to the toner heat

4 Reprint the design - this time it will be printed on the shrink film.

5 Now cut out the shrink films and discard the laser transparency

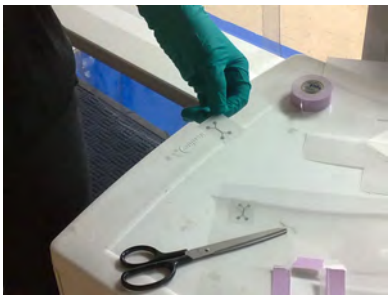


Figure 59: Cut out the printed film

6 Take the film to our toaster oven. Place the film on a large glass slide inside the oven. Press "CONVECTION BAKE". Press the temperature button and adjust the temperature to 225 F. Press START. After a few minutes the oven will beep and display temperature ready. Now count down to 5 minutes.

▷Note	The shrinky dink will first curl and then flatten - don't be alarmed!

7 Press temperature again and adjust to 275 F. Again like the last step bake for 5 minutes.

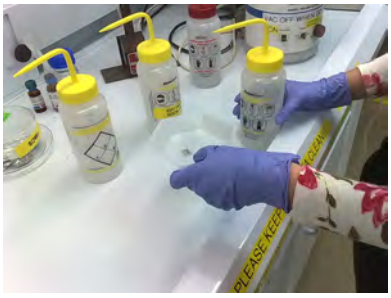


Figure 60: Wash with water and isopropanol

8 Remove from oven carefully -it's hot. If not completely flat, you can carefully flatten it while hot

9 Take this design to the white room and using a plastic dish wash with isopropanol and water

Figure 61: PDMS mixer

10 Now keep the chip apart. Let's make our PDMS mixture. Take a white plastic 150 ml container and mix the PDMS A and B in a 1:10 ratio. Only about 25g is needed - so add about 2.5 g of curing agent

and about 25g of main mixture. Weigh the container. Add the weight of the holder and balance the machine. Run the standard program.

11 Put your shrink mold in a small petri dish. Pour the PDMS mixture on top of the shrinky dink and let it cure in the curing oven at 60^0 centigrade for 30 minutes (until solid)

Figure 62: Pour PDMS

12 Remove the PDMS carefully and cut out the design.

13 Take a cover glass and the PDMS to the plasma machine and treat with plasma for <10 s. Your TA will show you how to use this machine. Bond the two. Put in the curing oven for 30 minutes.

(a) Plasma (b) Toaster Oven

Figure 63: Microwave Plasma and Toaster Oven

14 Remove and insert bent hollow steel pins into ports (0.025 in OD and 0.017 ID, New England Small Tube, MA). Using the syringe and tubing (Tygon microbore tubing, 0.020in ID x 0.060in OD, 100, Cole Parmer EW 06418-02) attached to it - suck a solution of micro-spheres in one and distilled water in another.

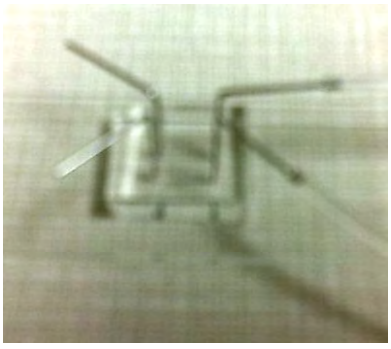


Figure 64: Insert pins into chips

15 Flow through you chip using the syringe pumps provided and observe the flow pattern. Start with a low pumping rate (in micro-liters per hour).

16 We will provide you with a solution of fluorescence micro-spheres and another solution of water. Flow them through the chip and collect the two solutions that emerge for the outlet ports. Device a protocol to count the number of micro-spheres in each. What do you observe?

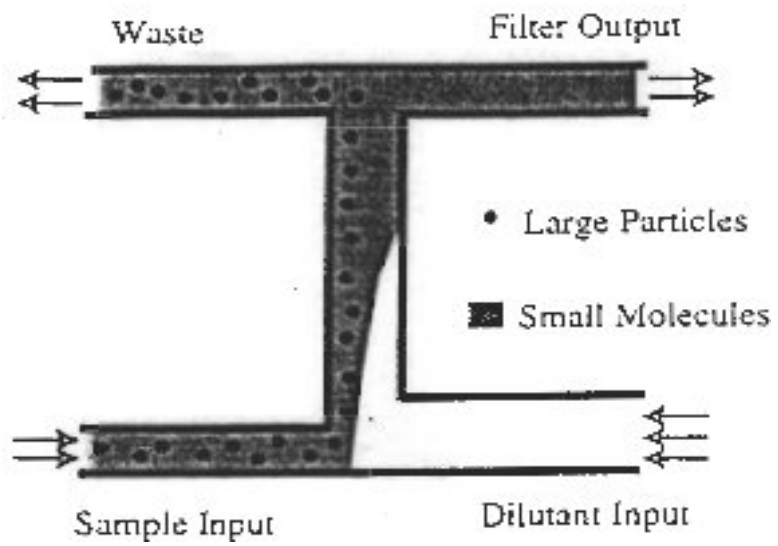


Figure 65: The original H filter design

Further Followup:

1) Using Shrink film to Culture Embyroid Bodies - `http://www.jove.com/details.php?id=692`

2) Shrink-film microfluidic education modules: Complete devices within minutes, Diep Nguyen, Jolie McLane, Valerie Lew, Jonathan Pegan, and Michelle Khine Biomicrofluidics 5, 022209 (2011); doi:10.1063/1.3576930

3) Diffusion-based extraction in a microfabricated device J P Brody, P Yager Sensors and Actuators A: Physical (1997) Volume: 58, Issue: 1, Pages: 13-18

Lab 3: Easy Microfluidics II: Adhesive Tape Devices

This experiment uses a craft cutting printer to cut shapes in pressure sensitive adhesive films. For simple microfluidic devices, the method is attractive because cutting printers are inexpensive and a variety of materials can be used, including paper, adhesive tapes, plastic and PDMS films. Their shortcoming is the inability to make things at high resolution, but they work well for channels whose size is of the order of millimeters . In industrial application plastics are used to make most microfluidic devices due to available standard processing and cheap raw materials.

Figure 66: Cutting printer

Cutting

In this experiment we make a 3D serpentine mixer first described in 0.2in]

Passive mixing in a three-dimensional serpentine microchannel Liu, R.H. Stremler, M.A. ; Sharp, K.V. ; Olsen, M.G. ; Santiago, J.G. ; Adrian, R.J. ; Aref, H. ; Beebe, D.J. Journal of Microelectromechanical Systems, Volume: 9 , Issue: 2,

190 - 197 (2000)

The device works on the principle of chaotic advection in 3 dimensions. The paper describes it thus:

Dynamical systems theory shows that chaotic particle motion can occur when a velocity field is either two-dimensional and time-dependent or three-dimensional (with or without time dependence). The occurrence of chaotic advection typically indicates rapid distortion and elongation of material interfaces. This process significantly increases the area across which diffusion occurs, which leads to rapid mixing.

They built that system with silicon micromaching - an expensive and lengthy process - but the only technology known then. We will work instead with a cutting machine - the kind used to cut craft stickers and use double sided adhesive tape. Our ideas are based on another paper:

Low-cost rapid prototyping of flexible microfluidic devices using a desktop digital craft cutter Po Ki Yuen and Vasiliy N. Goral Lab Chip, 2010,10, 384-387

Reagents

2 color food dye (to visualize mixing)
2 syringe pumps with 5 or 10 ml syringes, injection luer stubs and tubing
P-200NX flangeless ferrules (IDEX coprporation)
Transparency sheets
Double sided adhesive tape (product 92712, clear polyester tape 0.5 mil - double sided acrylic biocompatible adhesive. Obtained from ARcare contact: Benjamin Medows)

Procedure

1) Our machine is a Craft Robo Pro CE-5000 machine, but any machine that can cut films will do. The exact cutting condition have been previously determined and saved. We will only use them. Attach a film on the carrier sheet

2) Load the carrier sheet and let the cutting printer initialize. It will move the carrier sheet forward and backward and try to sense how

long it is and then finally home the cutting blade

▷Caution	The cutting blade is sharp - don't bring your fingers close to it while the machine is operating

3) Select the program Robo Cut Master Pro and select the design files. Cut the design out.

▷Caution	Make sure you press origin and let the machine know where the origin is. Otherwise unpredictable cuts can happen. The machine can be stopped by using the "Pause" button

A diagram of the device from the paper is shown in Figure 67. The design files are saved on the computer. Your TA wil lshow you how to access them and use the cutiing printer to print them.

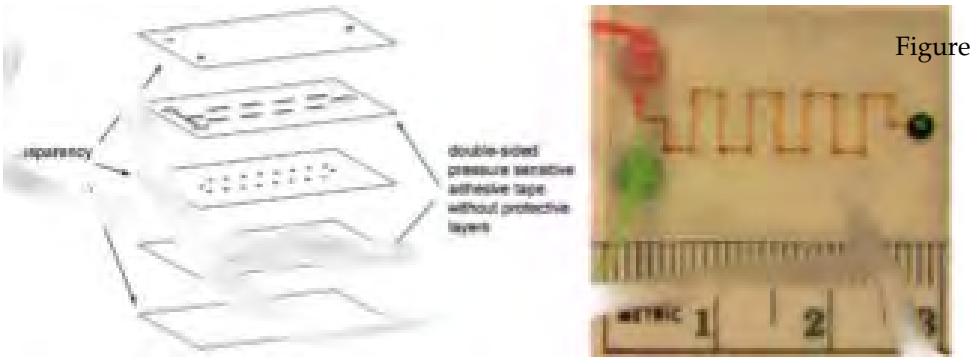


Figure 67: 3D serpentine channel

3) Assemble the layers carefully - wear gloves to avoid dust.

4) Set up syringe pumps and flow food coloring - adjust the rates so that both colors have the same flow rate.

5) Use the microscope to image the mixing process down he serpentine channel.

Lab 4: Photo-lithography

In this laboratory we make all the photo-resist molds needed for future labs using transparency masks. This is will involve negative and positive photoresists, aligning negative to positive layers, negative to negative layers as well as a rounding step where we round the photoresist profile. There are eight layers of photoresist that you will spin and in the end you should have 6 photoresist molds.

Three resists will be used SU8 (negative), SPR (positive) and AZ (positive). With negative resists the UV exposed area remains on the wafer, whereas the areas not exposed stay on for positive resists after developing. Further information about resists will be available in Lecture 3.

The molds we make include:

1) Simple single layer molds (SU8 2010)

2) Two layer molds (SPR 220-7 for flow with rounding step and SU8 2010 for control)

3) Project 1 mold (SU8 2025 at two heights - and a negative to negative alignment)

4) Project 2 mold (SU8 2005+AZ 50XT for flow mold - needs positive to negative alignment and rounding steps and SU8 2050 for control layer)

The basic protocol to follow is very similar for each resist.

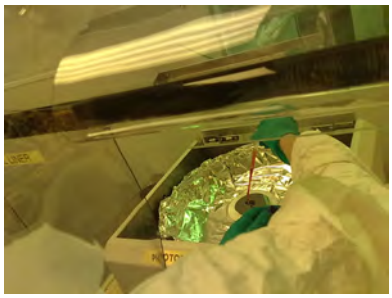


Figure 68: Spinning resist

Procedure

Follow these steps:

1) Coat your wafers with HMDS (hexamethyldisilazane). One way

to do this is to put a few drops of HMDS in a closed container and leave your wafer in there for > 2 minutes. Your TA will set this up for you. This step is optional for SU8 molds.

▷Note	Silicon wafers are brittle and break easily. Handle with care

2) Spread resists (flow mold) on the wafer, covering 1/4 to 1/2 the surface. Spin wafer to get a uniform coat. SPin speed determines the height

3) Soft-bake on a hot-plate usually at a lower temperature first followed by a higher temperature

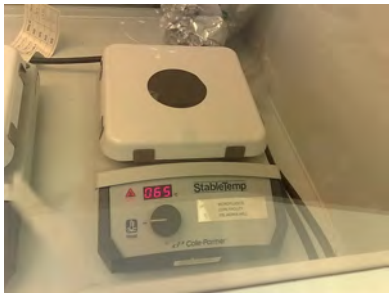


Figure 69: Baking wafer on hot-plate

4) Expose the wafers in a mask aligner using a transparency or chrome mask. Exposure time may vary depending on thickness of photoresist film and condition of the lamp.

▷Caution	Wear protective glasses of look away when UV exposure is being done

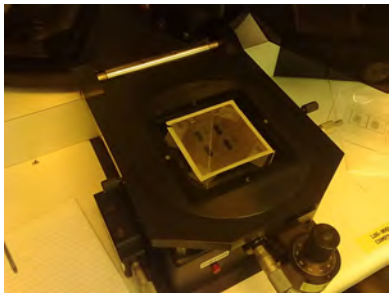


Figure 70: Using the Mask Aligner

6) Some resist like SU8 need a post expose bake.

7) Develop the appropriate developers. Discard into chemical waste once finished with developing.

8) (optional) Round your mold profile by baking on a hot plate (for SPR and AZ) or Hard bake your mold by leaving wafer on a hot plate (for SU8)

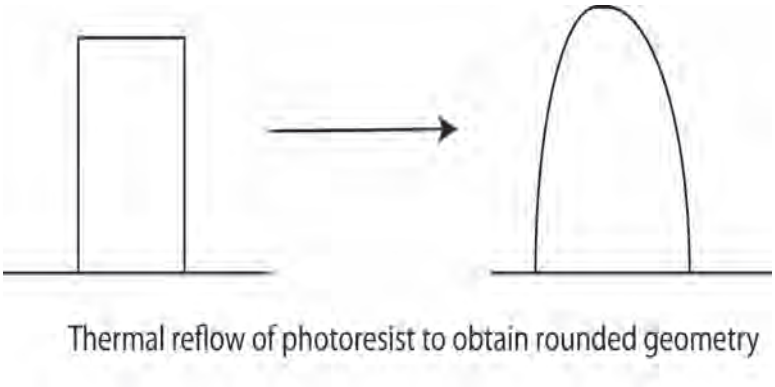


Figure 71: Rounding a channel

9) Inspect wafer under stereoscope. Measure the height profile with a profilometer. Store wafer safely away from dust.

Timing

Since time is limited, we like to perform the photolithography in the order that saves most time, with processes running in parallel. We recommend doing the two SU8 molds that need to be further aligned first, followed by remaining SU8 molds and finish with the AZ and and SPR molds. Here are the details of the recipes (these are dependent on the specific machine used):

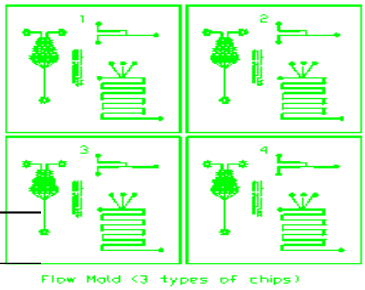


Figure 72: Single layer devices (SU8 2010)

Flow mold channels of lower height for yeast chambers (SU8 2005)	
Spin speed	2500 rpm
Soft bake	1 minute at 65^0C, 2 minutes at 95^0C
Exposure time	15 s
Developer	SU8 developer
Developing time	few minutes
Post Expose Bake Temperature	120^0 C
Post Expose Bake Time	about 30 minutes minutes

Flow mold channels of lower height for Project 1 (SU8 2025)	
Spin speed	depends on height needed - try 2000 or 2500 rpm
Soft bake	3 minute at 65^0C, 5 minutes at 95^0C
Exposure time	15 s
Developer	SU8 developer
Developing time	few minutes
Post Expose Bake Temperature	120^0 C
Post Expose Bake Time	about 30 minutes minutes

Control mold for Project 2 (SU8 2050)	
Spin speed	2000 rpm
Soft bake	>3 min at 65^0C, >5 minutes at 95^0C
UV exposure time	ask!
Post expose bake	>3 min at 65^0C, >5 minutes at 95^0C
Developer	SU8 developer
Developing time	few minutes
Hard Bake temperature	120^0C for >30 minutes

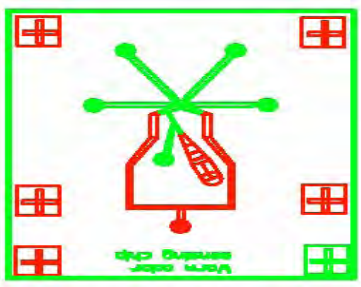


Figure 73: Project 1 (SU8 2025 twice)

> ▷Note The hotplates may not be at the temperature you read in the panel. At the end of soft-bakes always check the edge of the wafer for wetness - a sign that the soft-bake is not done. Also, place the wafer at the center of the hotplate.

Control mold channels for Valve, Pump, Multiplexer (SU8 2010)	
Spin speed	2000 rpm
Soft bake	1 minute at 65^0C, 3 minutes at 95^0C
Exposure time	15 s
Developer	SU8 developer
Developing time	few minutes
Post Expose Bake Temperature	120^0 C
Post Expose Bake Time	about 30 minutes minutes

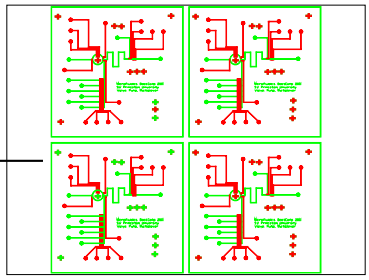


Figure 74: Valve,Pump, Multiplexer (SU8 2010, SPR 220-7)

Flow mold Single layer device (SU8 2010)	
Spin speed	2000 rpm
Soft bake	>1 min at 65^0C, >1 minutes at 95^0C
UV exposure time	15 s
Post expose bake	>1 min at 65^0C, >1 minutes at 95^0C
Developer	SU8 developer
Developing time	few minutes
Hard Bake temperature	120^0C for >30 minutes

Now we do our first negative to negative alignment. Use the windows at the edge of the design for alignment.

Flow mold channels of taller height for Project 1 ALIGNMENT (SU8 2025)	
Spin speed	Ask TA
Soft bake	3 minute at 65^0C, 5 minutes at 95^0C
Exposure time	15 s
Developer	SU8 developer
Developing time	few minutes
Post Expose Bake Temperature	120^0 C
Post Expose Bake Time	about 30 minutes minutes

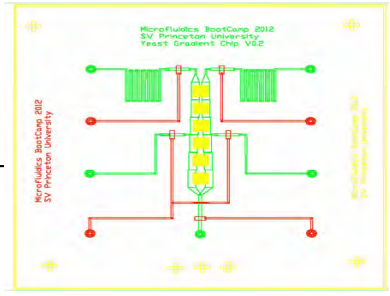


Figure 75: Project 1 (SU8 2005 alignes to AZ50XT, Su8 2050)

> ▷Note Align the layers by first rotating the design so that lines are parallel to each other acrss the wafer and then using the x and y adjustment to get the alignment right. Human eyes are very good at x-y alignment but not at judging angles. This alignment can be hard the first time you do it!

This is followed by the positive to negative alignment. AZ is a difficult resist to work with because channels made of it tend to crack under thermal stress.

Flow channel taller structures for Project 2 (AZ 50XT) ALIGNMENT	
Spin speed	1500 rpm
Soft bake	2 minute at 85^0C, 4 minutes at 115^0C. Leave covered for an hour at room temperature
Exposure time	15 s
Developer	AZ400K developer diluted with excess water 1:3 ratio
Developing time	few minutes
Rounding Step Ramp	10 minutes each at 50^0 C, 80^0 C, 100^0 C, 30 minutes at 125^0 C
Rounding Step cooling	Bring down to 80^0C and then shut down the hot plate for a gentle cool down

Flow mold for Valve, Pump, Multiplexer (SPR 220-7)	
Spin speed	2000 rpm
Soft bake	1 minute at 65^0C, 2 minutes at 95^0C
Exposure time	15 s
Developer	CDK 351, dilute with DI water 1:3
Developing time	few minutes
Rounding temperature	105^0 C
Rounding time	>30 minutes

> ▷Note SPR and AZ do not require a post expose bake, SU8 does!

Once done you could measure the heights of the channels - and note them down.

Lab 5: Multi-layer Soft-Lithography

Soft-lithography

We now take our molds to perform soft-lithography. Soft lithography was a technique invented around 1998 by researchers in the Whitesides group at Harvard - and since then it has become a routine technology used in laboratories worldwide. We will work with single and dual layer PDMS devices.

Single layer Devices

Follow these steps:
1) Expose your molds to TMCS (Chlorotrimethyl silane). One way to do this is to put a few drops of TMCS in a closed container and leave your wafer in there for > 2 minutes. This must be done inside a chemical safety fume-hood. This step is optional for molds that will have a thick film of PDMS

2) Mix PDMS in 1:10 ratio with 1 part of B, and 10 parts of A by weight. Use the weighing machine. Your TA will explain how to use

(a) Puncer (b) Mixer (c) Curing Oven

Figure 76: Punching, Curing, Mixing

the machine. Mixing can also be done by using a spatula and vigorouly shaking - but this also introduces bubbles with need de-gasing.

3) Put the wafer in a springform pan[25] (and place this a large petri dish) and pour PDMS on it carefully. Wait for 10-15 minutes until most bubbles burst. Move away any remaining bubbles with a toothpick. You can also use the degasing vacuum chamber to remove air bubble.

[25] Using springform pans is a Princeton speciality. Thank Jason Puchalla for this idea.

4) Put in a 60-80 degree oven for 10-30 minutes until it is no more liquid.

5) Remove from oven and release from springform pan Remove any PDMS behind the wafer first. Release the wafer. Using a razor blade cut the design out.

> ▷Note Keep the PDMS covered and avoid touching the design area at all - even with gloves.

6) Punch through holes for the flow layer. Clean with Scotch Tape. Wash with Ethanol.

7) Bond the chip to a glass slide either using a plasma. Put in the oven for a few hours.

Multi layer Soft Lithography

This involves some extra spinning, alignment and punching steps that make it more challenging. Timing can be critical if good bonding strength is required. We will use the push-down geometry so the control layers of the designs will be thick pieces of PDMS and the flow layers will be thin layers that we spin on. Multi-layer lithography was invented in 1999 by the groups of Stephen R Quake and Axel Scherer at Caltech.

Procedure

Follow these steps:
1) Instead of using the standard 1:10 ratio of PDMS A and B parts we will use 1:20 for the flow layer and 1:5 for the control layer. Make two

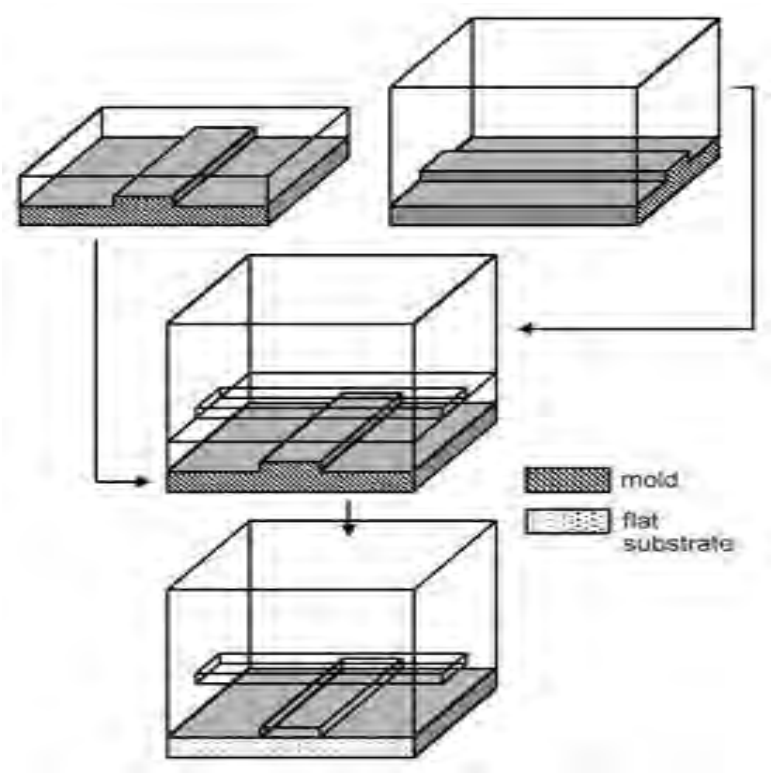


Figure 77: Multi-layer Soft-lithography

containers of PDMS with these ratio. You need about 35-60 g for each control layer and about 10-20g for each flow layer.

2) Treat the control and flow molds with TMCS for >2 minutes in the fume-hood

> ▷Note TMCS makes the wafer less sticky to PDMS allowing easier peel off. If even greater non-stick properties are needed - fluorinated silanes can be also used

3) Put the wafer in a springform pan (in a large petri dish) and pour PDMS on it carefully. Wait for 10-15 minutes until most bubbles burst. Move away any remaining bubbles with a toothpick. You can also use the degasing vacuum chamber to remove air bubble.

4) Put in a 60-80 degree oven for 10-30 minutes until it is no more liquid.

5) Remove from oven and release from springform pan. Remove any PDMS behind the wafer first to make life easy. Relaese the wafer. Using a razor blade cut the design out.

6) Place inside the caring oven and wait until it is just solid (15-30minutes). Now take it out and peel off the PDMS carefully just as for the single layer device.

7) Using the punching tool punch holes in the control layer. Make certain that the design side faces the punch.

> ▷Note The alignment and punching must be done as quickly as possible, the bond quality decreases with time - you have only about 30 minutes to 1 hour after you remove the thick layer from the oven.

8) While punching spin the 1:20 PDMS on the flow layer at about 2000-2500 rpm and place in the curing oven.

9) Remove 1:20 layer and align the control layer to the flow layer using a stereoscope. It is helpful to cut out the chip instead of doing them all at once. Avoid touching the surface of the design as far as possible and use a new pair of gloves. Use scotch tape to remove any dust. The control layer pieces can be washed with ethanol if they are very dirty.

> ▷Note PDMS stretches and compresses. You can use this to your advantage while aligning. Lift the PDMS from one end partially, while the rest is still stuck, and move the wafer slightly against it - so that it gets aligned - this can help getting elements on one part of the design aligned.

There are many repeated designs on each mold and since this may the first time you are making these devices many are likely to fail. This happens commonly because of poor bonding, mis-alignment and

breaking of the molds. Hopefully you will have a few working devices to test out.

Lab 5: Silicon devices and manifolds for silicon chips

This experiment is more a demonstration than a full experiment. Here we will understand how to use Silicon devices. The silicon devices has been etched and made ready for use. Because our lab has no silicon etching facilities, we did the etching at the Micro Nano Fabrication laboratory (MNFL) at Princeton University which you will have a chance to visit during the workshop.

Silicon Etching

The etching is done using a plasma etcher. The particular etcher used is the SAMCO-800 ICP-RIE (Inductively coupled Plasma Reactive Ion Etching). It uses fluorine gas chemistry to rapidly etch silicon structures.

The details of this machine are available here:

`http://www.princeton.edu/mnfl/the-tool-list/samco-rie800ipb/`

Briefly, the procedure involves coating a Silicon wafer with photo-resist, exposing the wafer (done on a MA6 mask aligner) and developing the design followed by silicon etching and removal of the any remaining resist.

Figure 78: Silicon etcher

Silicon devices are different from PDMS in that they are more resistant to chemicals, allow for smaller size features and do not allow diffusion of gases through them. However, they are harder to fabricate, generally more expensive to make and require access to a clean room and etching bays or tools, outside the range of an average biologist. Nevertheless, for certain projects, silicon is the only good option. Commercially, several foundries have decades of experience in silicon devices and processes, and will allow you to do process runs for large scale manufacturing.

Figure 79: Karl Suess MA6 mask

Making a manifold

(a) Sandblasting Machine (b) Protecting features with resist

Figure 80: Using a manifold

To go with a silicon device we made a manifold out of acrylic and steel. This manifold was made at the Princeton Physics department workshop. The design is shown in the picture on the right. It consists of 6 ports to connect to the device. The silicon device itself had holes etched through it using a sand blasting machine. The procedure involves coving the sensitive portion of the design with thick photo-resist and baking on a hot plate. Then the wafer is taken to a sandblasting system which uses particles of alumina to drill through the ports. The resist is removed with iso-propanol and acetone, and the device is cleaned to dislodge any remaining particles. This process was also done for you by our teaching assistants.

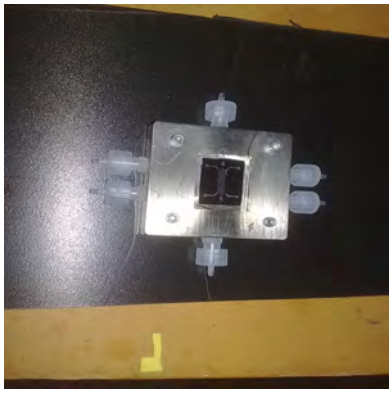
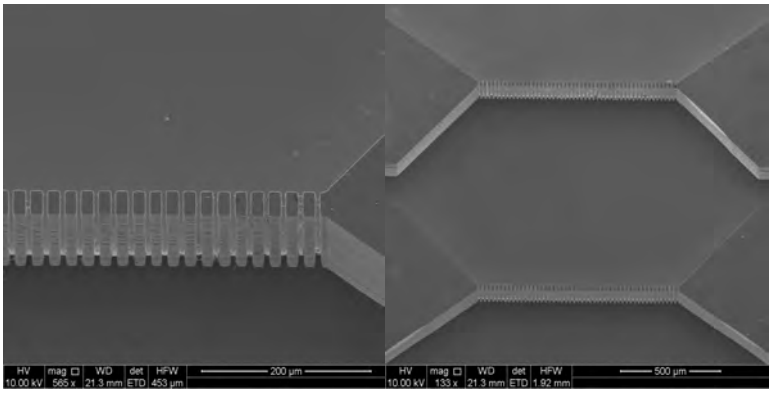


Figure 81: Silicon chip inside jig



(a) Silicon Chip - etched seives (b) Silicon Chip - growth chamber

Figure 82: Scanning electron microscope images of device

The silicon chip is inserted into the jig and a thin film of PDMS is used to cover it. This PDMS cover is topped with a cover glass. The glass and PDMS provide a transparent barrier allowing the use of a microscope to take images of the channel. O-rings are placed at the

ports and the entire jig is tightened with screws. Using luer stub fittings we can connect the chip to tubing of our choice.

Lab 7: AutoCAD tutorial

This section teaches you how to make your own designs. We will get you to draw one of the designs of the class. This will be done in AutoCAD, but the procedure is similar for other CAD software, and you can quickly adapt to other software.

Design Dimensions

The first thing to consider is the dimensions you need for your system. Depending on the biology involved you may need the channels to be a certain height or width (for e.g. so that you cells won't get squished). *The CAD design only determines the width of a channel or feature* - the height will depend on the photo-lithography step. However, the two are inter-related: if the aspect ratio (height to width ratio) of a channel in PDMS exceed 1:15, a sagging of of the PDMS can be expected to close off the channel. In this case you may have to add pillars to support the channel. Similarly, you must consider what kind of aspect ratios are achievable for silicon based devices and any other materials you will use to prevent structural failure.

The second thing to consider is the size of silicon wafer you will work with - the design must fit within the wafer with adequate margins. It is good practice to draw a margin and write in the mask the details of who made it, when it was made, design details etc. A few months later all designs start to look the same, and you will have a hard time knowing what changes were made or whay a particular feature was added. Trust us on this.

Procedure

Open AutoCAD (any version beyond AutoCAD 2000). The following rules must be followed:

1) Select microns as the unit with 0 precision `Format>Units`

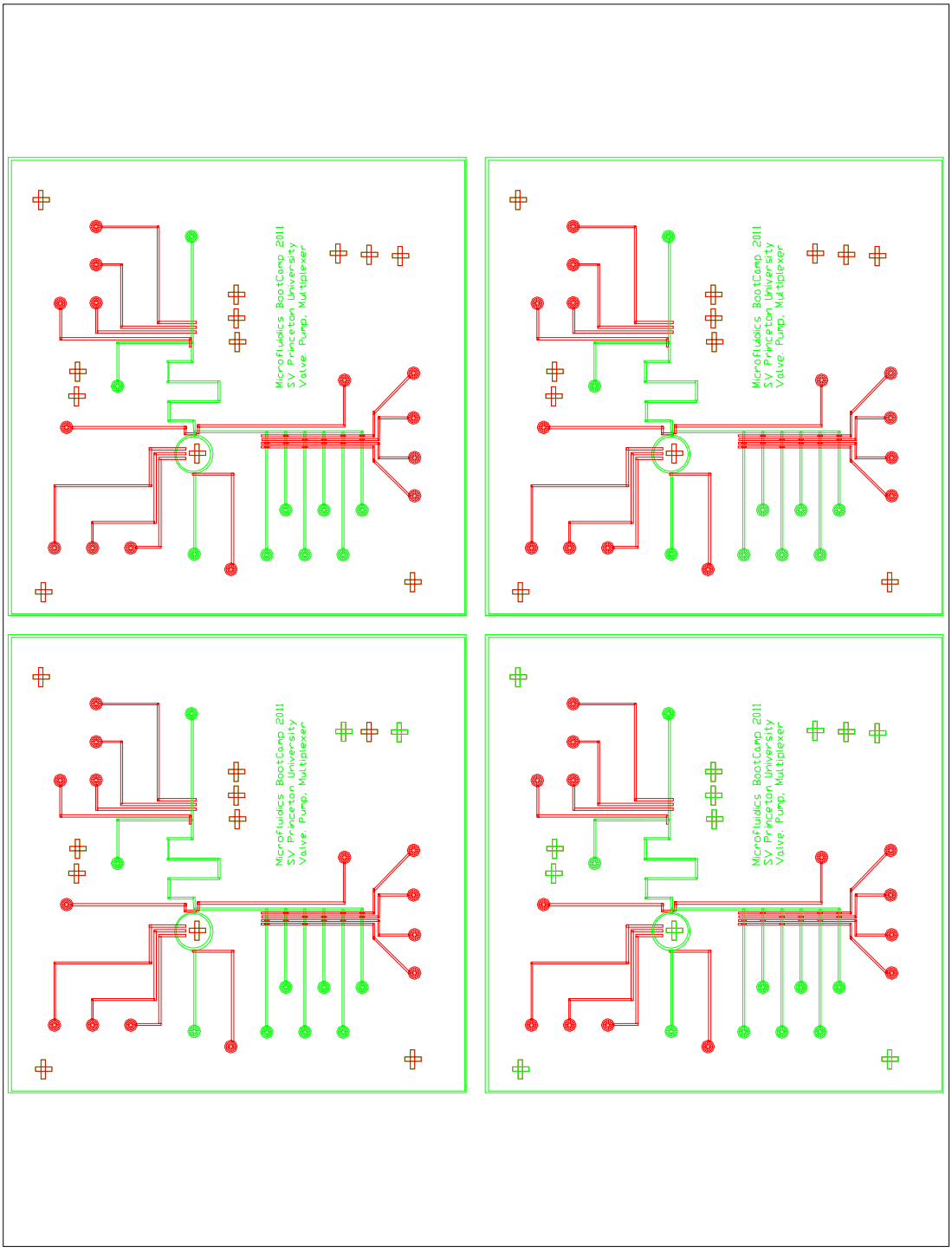


Figure 83: Two Layer PDMS chip design meant to fit a 3" wafer

2) Everything must be drawn using CLOSED POLYLINES. A closed polyline can be drawn by using the polyline command and then drawing the last line by typing the command `CLOSE` in the command line. The conversion software needs close polylines because it has to recognize the inside and outside of a figure - and only closed figures have an inside and outside region.

3) Draw everything on a layer different from ZERO layer. Select a new layer and use it for drawing `Format>Layer`. Let us call the layer "FLOW". Choose a color for the layer.

4) Three functions may be useful for your drawing ORTHO, SNAP and GRID. Find out about them. A circle or a square or a polygon is a closed polyline in AutoCAD. Closed polylines can overlap - and there are various possibilities for conversion of overlapping figures. Most conversions program will do b and c as shown in Figure 84 and Figure 85

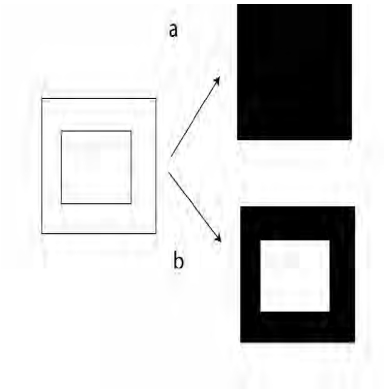


Figure 84: Complete encapsulation

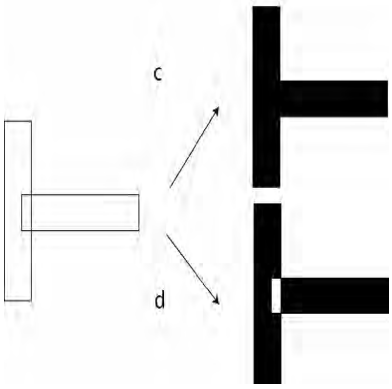


Figure 85: Partial overlap

5) It is good practice to draw a border around the diagram. Let us make a boundary of 24000 micron x 24000 micron (fitting an inch square). We use a double square because otherwise the conversion program may get confused (see 2)

6) Also, a good practice is to put identification marks and notes on the chip that tell who made it, when it was made etc as we mentioned earlier. This writing will also help identify which side was printed which can be hard to tell on a transparency

7) Note that because the lines are fairly thin we will need to put some ports at the end of the line where hollow metal pins can poke through the PDMS and connect put fluids into and out of the chip. That is the larger structure at the end of each line. We put in some triangular posts to prevent collapse.

8) We can also get started on the Control layer by opening a new layer as before (use a different color). Note how the valves are larger where they need to be activated. For the multiplexer note the cross-over channels used. When making the control layer you must consider the needs of alignment - make the figure as simple to align as possible. Put in many alignment marks - which can be crosses, or other kinds of figures. The mask aligner allows rotation and x,y translations so your alignment mark must distinguish these movement (hence a circular alignment mark is a bad idea since you won't get rotational alignment

9) It is essential that the ports be separated and as far as possible from each other and other channels - at least 2 mm away from all other channels (except the one it connect to of course!) and ports is a good rule of thumb. Since we need to poke pins into the ports and there is an element of error involved, this minimize the chance of the pin rupturing another channel or port and ruining your device.

Preparing for Printing

10) Once the figure is done you need to prepare it for printing. This is done by creating a new file and separating out the layers. need to separate the control and flow layers, make allowances for polymer shrinkage and arrange them to be put on a wafer. We use 3 inch silicon wafer so 4 chips can fit in. So we put them on separate files. Here we will plan to make the flow layer to be the thin layer. This is called the top down geometry, because the valve functions by a thin membrane moving downwards. The other geometry is the bottom up geometry with the control channel below the flow channels.

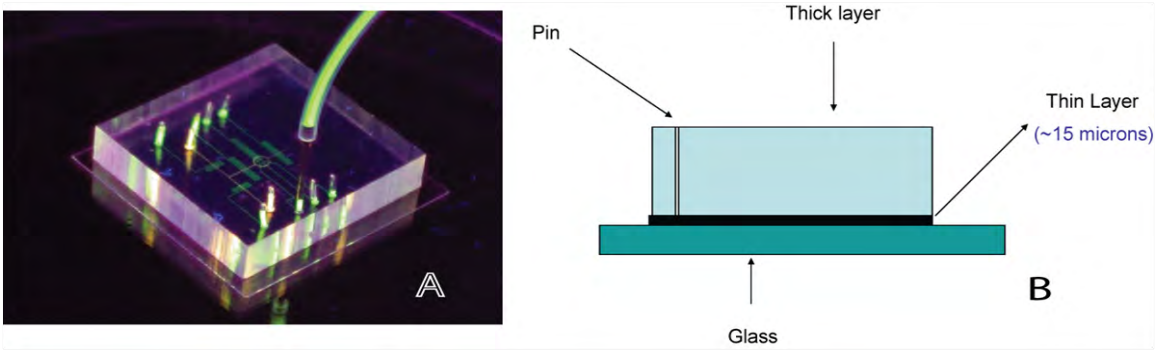


Figure 86: Two Layer PDMS chip top-down

11) The thick layer (control) must be EXPANDED by 1.6% to compensate for the shrinkage in the fabrication. Expand the control layer using the SCALE command, selecting the entire chip and expanding by 1.016 from the center.

12) Save the files in AutoCAD 2000 .dwg or .dxf format and now you can send these off for printing.

Positive and Negative, Emulsion side up or down

You will also have to decide whether you design is to be printed negative or positive. If printed positive it will be used with positive

photo-resists, and if negative it will use a negative photo-resist. (Negative means that the channels you made will be transparent and the rest will be dark like a negative of a photograph). In our case the flow layer must be printed positive - since only positive DNQ-Novalac resists can be thermally re-flowed to create rounded channels. Emulsion up makes any writing readable when the printed layer faces you. Emulsion down is the opposite - now the writing is mirror reversed. You can use either - but make sure you know which is which, as it will help when you attach the transparency to the glass plate in photo-lithography.

Lab 8: Mixer, Two phase flows

Our single layer devices consist of three devices. One is a mixer - the remaining two devices are meant for generation of droplets.

Gradient Maker

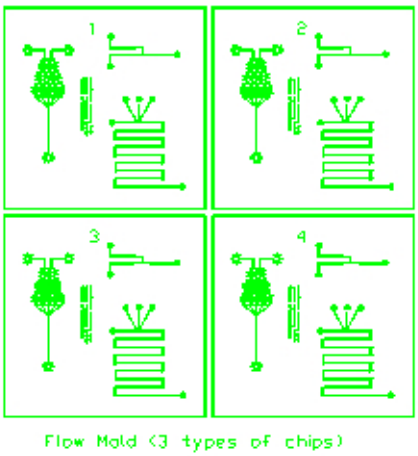


Figure 87: Single layer devices

The flow gradient mixer allows the generation of gradients from two inputs. Our first device is a gradient generator developed by Jeon et al. [26]

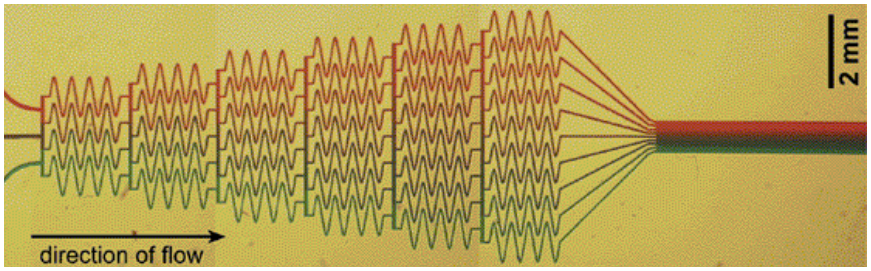


Figure 88: A flow gradient maker

[26] Noo Li Jeon, Stephan K. W. Dertinger, Daniel T. Chiu, Insung S. Choi, Abraham D. Stroock, and George M. Whitesides. Generation of solution and surface gradients using microfluidic systems. *Langmuir*, 16(22):8311–8316, 2000

1) Set up your device on the microscope carefully.

2) Suck in food dye coloring in tygon tubing you have used preciously and attach to a syringe pump at one end and the chip at the other end using hollow steel pin connectors.

3) Try to get flow rates to achieve the gradients shown in Figure 89. (We are using a simpler version of that device with only two inputs)

4) Vary the flow rates and observe the changes

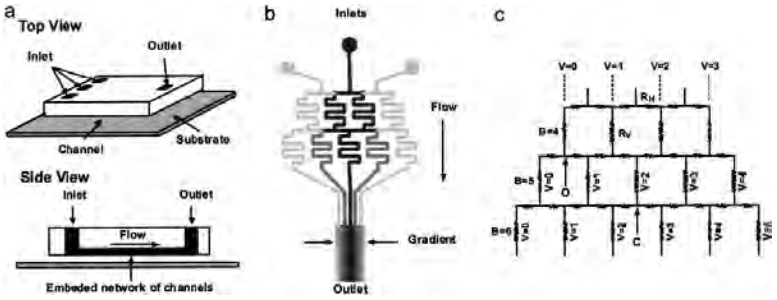


Figure 89: Gradient generator (from the original mixer paper by Jeon et al 2000)

This simple device allows the generation of a gradient using two inputs. These dynamic gradients depend only on the flow and diffusion rates and can be controlled easily.

Oil and water

The remaining two devices deal with oil and water. Our experiments are based on a number of papers that have appeared in recent years that use droplets to speed up analysis[27]. Picoliter sized droplets can be generated at millisecond rates and in parallel in a microfluidic device. Using two phase flow with sorting objects embedded inside droplets thus provides one route to achieving very high throughput.

However, before this is possible, a whole slew of tools have to be developed that will allow manipulation of droplets - creating, combining, splitting them for instance. Further, more work has to be done on surface chemistry and surfactants to ensure proteins don't get denatured at

[27] Helen Song, Delai L. Chen, and Rustem F. Ismagilov. Reactions in droplets in microfluidic channels. *Angewandte Chemie International Edition*, 45(44):7336–7356, 2006

the oil water interface, which presents an enormous surface to volume ratio. In recent years much progress has been made - techniques like emulsion PCR have been invented, sorting of cells and small organisms has been shown as proof of concept and methods have been developed to have precise control over droplets.

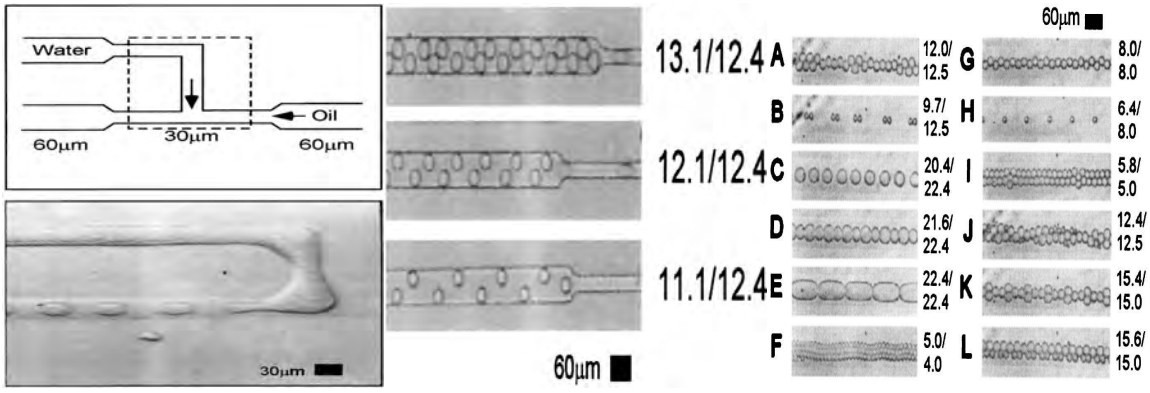
Procedure

Follow these steps:

1) As in the previous case set up your device on the microscope carefully. Suck oil and water into the tubing before you attach it to the chips. You should now be familiar with this procedure from the previous experiments.

2) First let us examine the device on the top right - it consists of perpendicular channels with 3 ports. Flow oil and water through perpendicular channels and observe the junction. The device on the bottom left is based on a paper published in 2001 [28] and some images from the paper are shown in Figure 90. Here we can try to dynamically generate different phases that depend on the pressure differential. Try to produce the patterns that were seen in that experiment.

[28] Todd Thorsen, F. H Roberts, Frances H. Arnold, and Stephen R. Quake. Dynamic pattern formation in a vesicle-generating microfluidic device. *Physical Review Letters*, 86:4163–4166, 2001



(a) Device design (b) Oil/water phases (c) Water/Oil phases

Figure 90: Oil and water

3) The final design on the bottom right of the wafer is a design that allows the creation of aqueous droplets with mixtures of different components [29]. Here, we will use food dyes to create droplets of different colors and observe the mixing as the drops snake down the channels. Repeat the same procedure as before, but this time flow in

[29] Joshua D. Tice, Helen Song, Adam D. Lyon, and Rustem F. Ismagilov. Formation of droplets and mixing in multiphase microfluidics at low values of the reynolds and the capillary numbers. *Langmuir*, 19:9127–9133, 2003

three different food dyes through the aqueous inlets (see Figure 91).

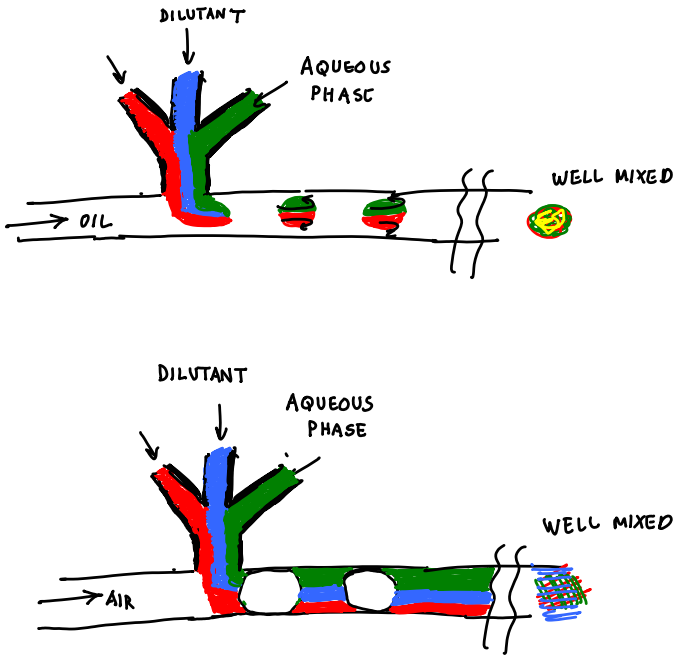


Figure 91: Creating isolated picoliter compartments

4) If you have time you can try to add surfactants like span 80 and tween 80 and observe the difference in behaviour.

Further Follow up

1) Microfluidics Using Spatially Defined Arrays of Droplets in One, Two, and Three Dimensions Rebecca R. Pompano, Weishan Liu,Wenbin Du, and Rustem F. Ismagilov Annu. Rev. Anal. Chem. 2011. 4:59âĂŞ81

2) Generation of Solution and Surface Gradients Using Microfluidic Systems Noo Li Jeon, Stephan K. W. Dertinger, Daniel T. Chiu, Insung S. Choi, Abraham D. Stroock, and George M. Whitesides*, Langmuir 2000, 16, 8311-8316

Lab 9: Valve, Pump, Multiplexer

In this experiment we will test our multilayer device. The design is meant to test the various fluidic circuit components: valves, pump, multiplexer.

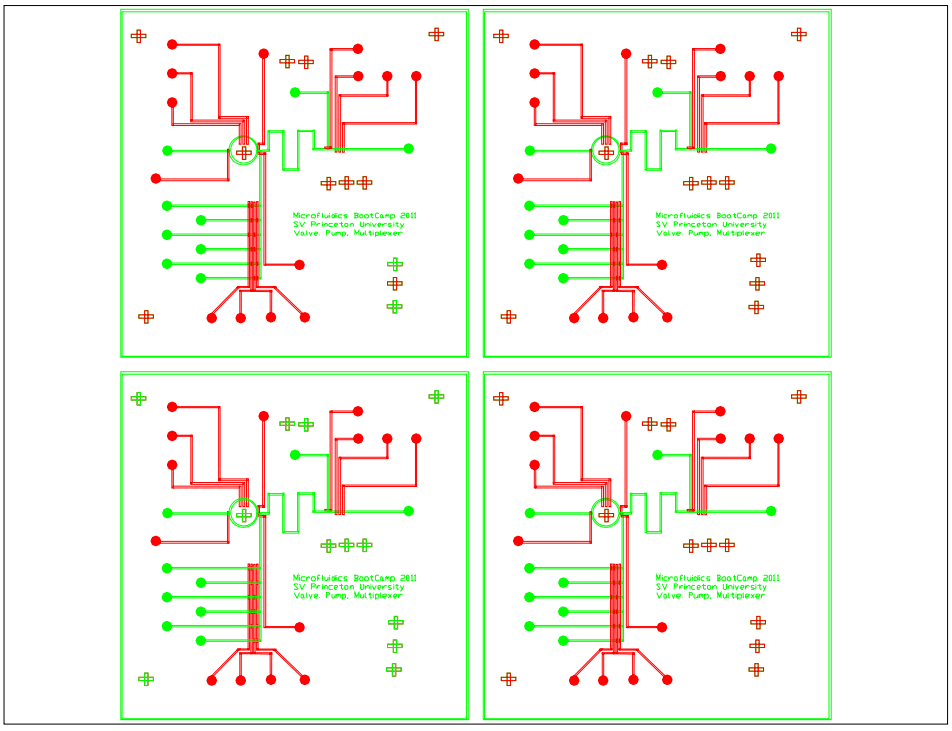


Figure 92: Simple two layer PDMS device with a rotary mixer and combinatorial multilexer

Figure 92 shows a design that contains a combinatorial multiplexer on the left bottom that allows multiplexing of 6 input lines. these line fill up an chamber that has a peristaltic pump backing allowing precise metering of fluids into a rotary mixer.

Reagents and Materials

Chips
Pins and tubing
Syringes and injection luer stubs
Water and dye solution

Procedure

1) To run this chip one needs a pressure manifold run by a controller. The controller runs off a Labview program. It looks complicated with all the wires and tubes! Don't panic!! All you need to know is that the program 'bootcamp' operates the valves. You need to click on a button to actuate a valve and you can also create a pump with any three valves of your choice. A little red light indicates a valve is on.

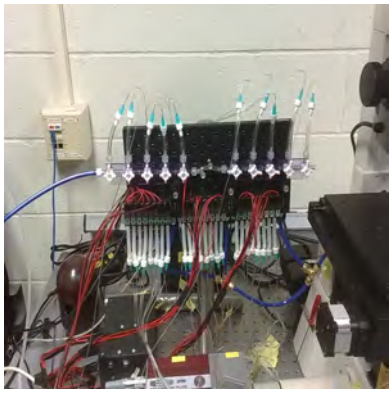


Figure 93: Pneumatic Control Manifold

2) Fill tubing with water and attach to the tubing of the manifold (the manifold with the solenoid valves). Test your valves and pumps by attaching the tubing and flowing in water. Check if the valves work - it is possible to have collapses, delaminations etc- and in that case you have to do triage to see what portions of the design are still usable. Using liquid in the flow lines can help prevent some problems with as diffusion and maintaining salinity. Typical pressures to run valves range from 10-25 psi.

> ▷Note Typical Pressures: 1-5 psi for flow channels; 10-25 psi for control channels; 25-35 psi for chip delamination!

3) Suck in reagents into tubing and attach one end to the chip and the other end to the manual manifold kept at lower pressure (1-5psi). The simple chip design allows metered insertion of up to 6 different reagents (controlled by a combinatorial multiplexer) into a rotary mixer. The reagents are flown in into the serpentine channel, Then this is isolated and the pump is used to push liquid (metering by using a known number of cycles) in the rotary mixer. Reagents can be mixed in the rotary mixer. This design gets you to test valves, pumps, mixers and multiplexers.

4) Fill the chip with water at the beginning to remove any air in the system. Then test whether you can flow a metered amount of solution (selecting the solution with a multiplexer) and using a given number of peristaltic cyles to meter a known known amount of liquid into the rotatry mixer. After putting multiple solution into the rotary mixer, operare the mixer and see how fast things mix.

Suggested Follow-up

1) Melin J, Quake SR. "Microfluidic Large-Scale Integration: The Evolution of Design Rules for Biological Automation" Annu. Rev. Biophys. Biomol. Struct. 36:213-31

Lab 10: Project 1 Calcium imaging in C.Elegans

Our projects are day long experiments that are deisgned to give as close an experience of realistic research experiments with the time we have. Because these are closer to reserach projects we expect a high rate of failure. So if things don't work out - don't be too disheartened. The experiments in real life would have taken several months to carry from start to finish and we are compressing it all into a day.

Each group is expected to present a short 15-30 minute talk the next day about their experimnents to the other group so take plenty of pictures and notes.If you do manage to finish a project early to your satifaction you are welcome to try the other one.

Introduction

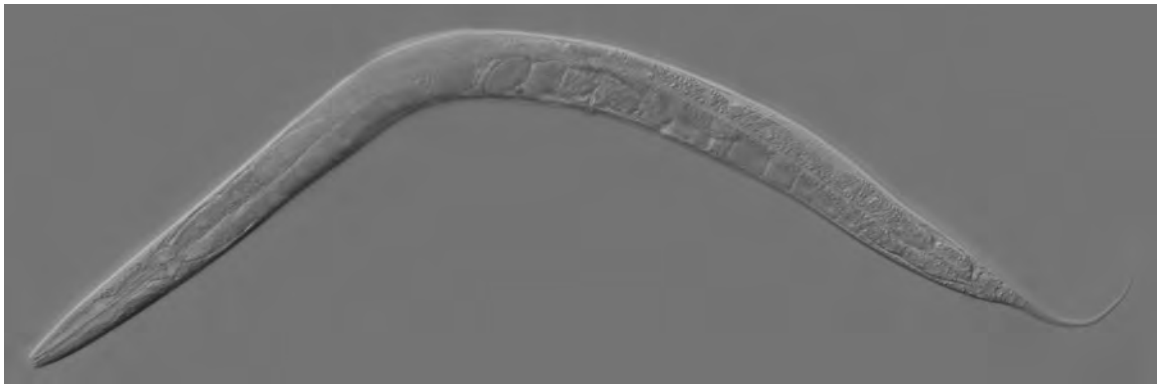


Figure 94: Adult C. elegans

Besides working with cells and molecules, microfluidics is a versatile tool for working with small creatures like C. elegans, planaria, and zebra fish. The size scale of microfluidic devices is well suited to manipulating these animals. In particular, high throughput sorting of mutants is an area receiving much attention.

Our experiment is based on the following paper:

Chokshi T.V., Bazopoulou D. and Chronis N., An automated microfluidic platform for calcium imaging of chemosensory neurons in Caenorhabditis elegans, Lab Chip. 10(20):2758-63 (2010).

Please look through this paper before starting.

C. Elegans

C.Elegans is a small worm that was introduced as a biological model by Sydney Brenner in 1974. Its genome and cell lineage are completely known. Because it is so easy to handle and grow, and mutants can be frozen and kept for years, it is as close as possible to an ideal biological model. Recently, evidence emerged that starvation can prolong the life of these worms - this exciting finding applies to many organisms and may also be relevant to humans. Surprisingly, it was found that mutations that lead to longer life spans reduce tumor formation [30] which is am intriguing connection. Many genes in C. Elegans share homologues with human genes, making genetic manipulation with C.elegans relevant to human disease. In fact, C.Elegans seems to have many genes related to to human oncogenes. Studies on this worm have reveled many insights into apoptosis (programmed cell death) and RNA interference mechanisms[31].

[30] Julie M. Pinkston, Delia Garigan, Malene Hansen, and Cynthia Kenyon. Mutations that increase the life span of c. elegans inhibit tumor growth. *Science*, 313(5789):971–975, 2006

[31] Gino Poulin, Ramkumar Nandakumar, and Julie Ahringer. Genome-wide rnai screens in caenorhabditis elegans: impact on cancer

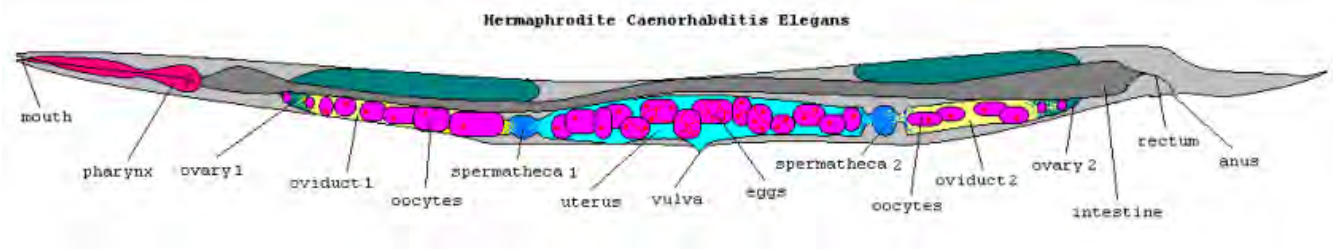


Figure 95: Anatomy of C.elegans worm

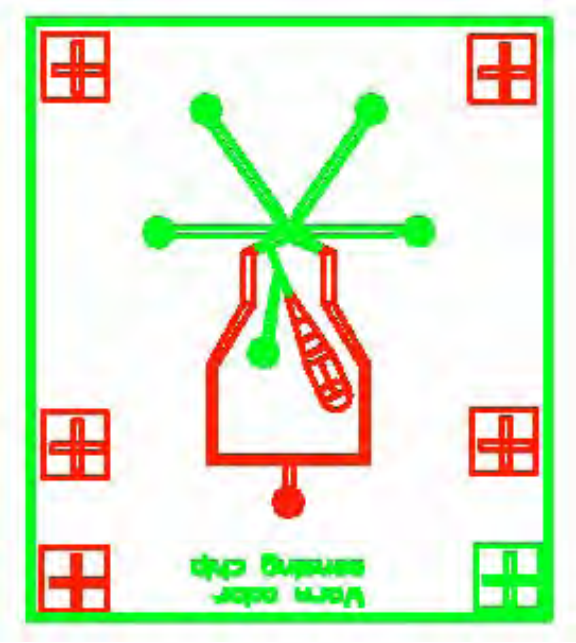


Figure 96: Dynamic Gradient Chip

Calcium Imaging in C. elegans

You will be observing calcium responses in vivo, in the nervous system of Caenorhabditis elegans. C. elegans are able to detect a variety of olfactory cues through amphid chemosensory neurons. One pair of chemosensory neurons, the AWC neurons, detect several attractive odors, including benzaldehyde, isoamyl alcohol, and butanone, and are required for chemotaxis to these odors (Bargmann, 2006). In this experiment you will monitor the odor response of one AWC neuron using a transgenic C. elegans which expresses G-CaMP using an AWC-selective promoter (pstr-2::GCaMP2). Previous work has shown an increase in G-CaMP fluorescence in response to removal of isoamyl alcohol, benzaldehyde, and OP-50 medium(Chalasani 2007). Worms were grown and maintained under standard methods (Brenner, 1974) on HG medium at 20C on OP-50 E. coli. Synchronized animals will be tested on Day 1 of adulthood.

Bargmann, C. I. Chemosensation in C. elegans (October 25, 2006), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.123.1, http//www.wormbook.org.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics, 77(1), 71-94.

Chalasani, S. H., Chronis, N., Tsunozaki, M., Gray, J. M., Ramot,

D., Goodman, M. B., et al. (2007). Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans. Nature, 450(7166), 63-70.

Reagents and Supplies

Chips with associated pressure manifold Worms (1-3 day old) Buffer (SBasal) Control color (Bromophenol blue or xylene cynol) Odorant Conical Tubes and rubber caps with two holes for holding liquids Tygon tubing and pins

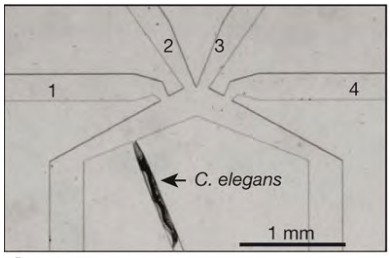


Figure 97: C.Elegans trapped in a device and exposed to odors

Procedure

Follow these steps:
1) Set up the chip such as shown so that the stimulus, control, buffer, worm and flush channels are connected to the appropriate tubes and solenoid valves. Use low pressures of 1-3 psi so as to not stress the worm

2) Let worms enter your device and use the flush channel to position the worm correctly and prevent any other worm from entering.

3) If you get the conditions right - the size of the channel will trap the worm while you can take time lapse fluorescent images of its neuron.

4) Flush the worm and repeat for as many worms as you can

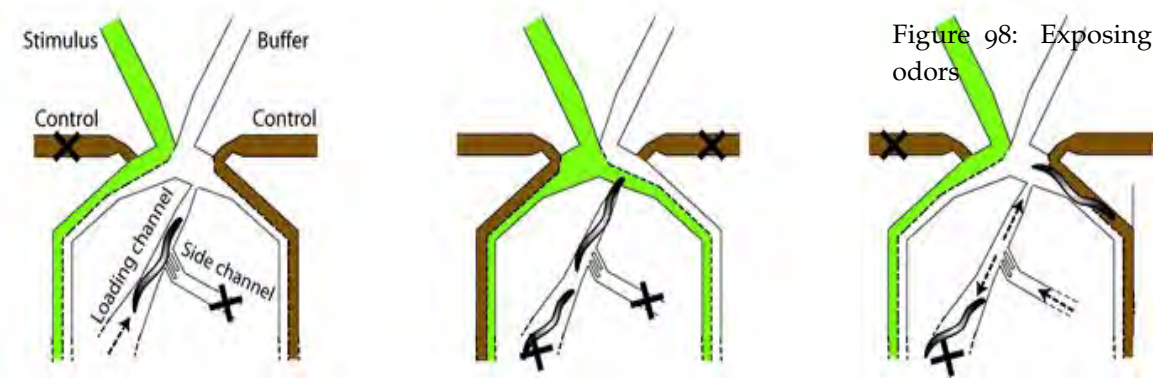


Figure 98: Exposing worm to odors

Further Follow-up:

1) "Chemistry and the Worm: Caernorhabditis elegans as a Platform for Integrating Chemical and Biological Research", Hulme, S.E., and Whitesides.G.M., Angewandte Chemie International Edition, 2011, 50, 4774-4807

2) Bargmann, C.I. Chemosensation in C. elegans, WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/ wormbook.1.123.1, http://www.wormbook.org

Lab 11: Project 2 - Yeast in Dynamic Gradients

Our projects are day long experiments that are designed to give as close an experience of realistic research experiments with the time we have. Because these are closer to research projects we expect a high rate of failure. So if things don't work out - don't be too disheartened. The experiments in real life would have taken several months to carry from start to finish and we are compressing it all into a day.

Each group is expected to present a short 15-30 minute talk the next day about their experimnents to the other group so take plenty of pictures and notes.If you do manage to finish a project early to your satifaction you are welcome to try the other one.

In this project we will attempt to use microfluidic chips to create dynamic gradients. Yeast cells will be inserted into chambers of varying length that are isolated from the main flow. Due to the differing lengths, steepness of gradients can be varied and single cell behavior studied in them at the same time. Our design and experiment is based on the following paper:

MAPK-mediated bimodal gene expression and adaptive gradient sensing in yeast, Saurabh Paliwal, Pablo A. Iglesias, Kyle Campbell, Zoe Hilioti, Alex Groisman and Andre Levchenko Nature 446, 46-51 2007

The paper is available in the paper folder online. Please look through the paper before starting the experiment. The supplementary section provides the details of the fluid flow in this design. The design which you have made in the previous labs looks like Figure 99. The green

lines are the flow channels - these are connected to the yellow channel - which are the yeast chambers of varying length. The red lines are the control channels used to operate valves.

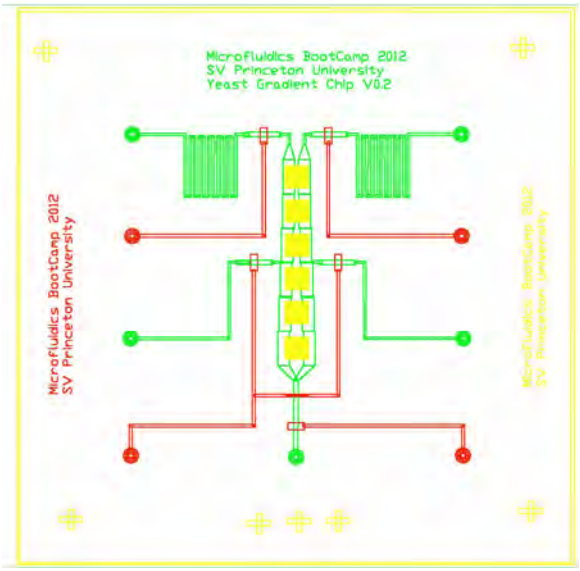


Figure 99: Dynamic Gradient Chip

This technique can be applied to any type of cell as provided provided the geometries are adjusted to accommodate the cell. Your aim in this project is to get the cells running and run osmolarity and pheromone gradients, while imaging single cells or cluster of cells and taking multi-dimensional images using the fluorescent microscope. The same methods used in previous labs with multilayer chips can be used here.

Reagents and Materials

Chips, pressure manifold, tubes, pins etc.
Yeast cells (exact type will be specified on the day of the experiment)
Solutions of pheromone and high osmolarity
Dye solution (to visualize gradients)

Procedure

Follow these steps:
1) Like in the previous experiments use 1-5 psi for flow channels and 10-25 psi for the control channels.

2) Fill the control lines with water and test the valves on the chip.

3) Isolate the yeast chambers with valves and flow in cell till they fill up the chambers and spread out.

4) Create a variety of gradient profile by flowing in solutions in the two large channels.

5) Set up the imaging on the microscope. Image and graph the behavior of cells are various locations in the gradient

Take many images and you will use theses images to prepare your presentation at the end of the workshop.

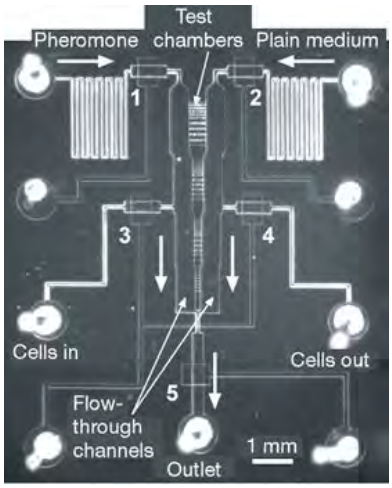


Figure 100: Diagram of chip

Follow-up

Single cell imaging is a rapidly expanding field. Two recent reviews are:

Microfluidic Platforms for Single-Cell Analysis Richard N. Zare and Samuel Kim Annual Review of Biomedical Engineering Vol. 12: 187-201 (Volume publication date August 2010)

Microfluidic single cell analysis: from promise to practice Veronique Lecault, Adam K White, Anupam Singhal, Carl L Hansen Current Opinion in Chemical Biology, Available online 21 April 2012, ISSN 1367-5931, 10.1016/j.cbpa.2012.03.022.

Suggested References

The biggest lament we hear from students is that mathematical sophistication needed to read books on fluid mechanics and microfluidics can be scary. Somehow this message has not reached the writers - unfortunately, almost all of them assume some familiarity with vector calculus. I wish someone would write a sophisticated *and* non-mathematical book. For the time being, anyways, the resources I would recommend are:

1) Introduction to microfluidics, Patrick Tabeling ; translated by Suelin C, 2005, Oxford University Press

While the field is rapidly evolving, and will likely make this book obsolete in a few years, currently I believe it constitutes the most balanced introduction, and whose chapters can be read independently of each other. The book does require a moderate amount of mathematical sophistication.

2) Shape and Flow: The Fluid Dynamics of Drag, Ascher H Shapiro, 1964 Heinemann

This now out-of-print book is the best non-mathematical introduction to the basics of fluid mechanics.

3) National Committee for fluid mechanics Films `http://web.mit.edu/hml/ncfmf.html`

A set of freely available videos that are simply brilliant. they have aged very well even though it's now over 50 years since they were made. Just don't treat mercury in the same way they handle it!

4) Feynman Lectures on Physics , Volume 2, Chapters 40 and 41 "The Flow of Dry water" and "The Flow of Wet Water", Feymann, Leighton,

Sands, Addison Wesley

If you can tolerate a little mathematics, see a master physicist in action. These lectures were meant for freshman undergraduates in Physics.

5) Random Walks in Biology, Howard C Berg, Princeton University Press(1993)

Howard Berg is most famous for working on the motility of bacteria. In this slim book (the kind of book I like), he tackles diffusion and related phenomena. The book has mathematics, but it does not feel like he bludgeons you with it.

6) The science of soap films and soap bubbles, Cyril Isenberg, Dover Publications (1992)

A good introduction to surface tension and associated phenomena, with plenty of pretty pictures

7) Microfabrication Techniques for Biologists: A Primer on Building Micromachines, Douglas Chinn (Chapter 1 in Microengineering in Biotechnology, Methods of Molecular Biology, MP Hughes, KF Hoettges (editors) Humana Press 2010)

If you want to fabricate a device and need to use a nano/micro fabrication facility this would be an excellent place to start. It explains the various processes, tells you what you could expect, and how to prepare so that you can seem reasonably intelligible to the staff there.

Appendix 1: Microfluidics and Cancer Research

Applying the tytpe of microfluidics we have learnt in this course to cancer research is a fairly new area of work. There is interest in extracting circulating tumor cells, studying evolution of resistance and metasthesis, and any number of designs to perform biomarker tests to detect cancer. A few early reviews of this kind of work have emerged and provide the newcomer an introduction to the field.

Microfluidic approaches for cancer cell detection, characterization, and separation, J Chen , J Li and Y Sun Lab Chip, 2012,12, 1753-1767

Microfluidics: Emerging prospects for anti-cancer drug screening D Wlodkowic and Z DarzynkiewiczWorld J Clin Oncol. 2010 November 10; 1(1): 18âĂŞ23. Published online 2010 November 10. doi: 10.5306/wjco.v1.i1.18 PMCID: PMC3095457

Nanotechnology and cancer, J Heath, M Davis, Annual Review of Medicine [0066-4219] yr: 2008 vol: 59 iss: 1 pg: 251

Microfluidic platforms for the study of cancer metastasis J Ng, Y Shin and S Chung Biomedical Engineering Letters Volume 2, Number 2 (2012), 72-77, DOI: 10.1007/s13534-012-0055-x

Admittedly, any review in a rapidly developing field should be taken with a grain of salt. Nevertheless, it is useful to know what has already been done before starting a new project

A large set of papers also exist and the next table gives a sampling of the papers with one example paper in each sub-field.

Type	Comment	Representative Example
Diagnostic Screening	Devices take advantage of small volume handling. Also relevant for drug testing.	Rong Fan, Ophir Vermesh, Alok Srivastava et. al. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. Nature biotechnology, 26(12):1373âĂŞ1378, December 2008p
Extracting Circulating tumor cells	Replacement for biopsies? Testing therapies?	Sunitha Nagrath, Lecia V. Sequist, Shyamala Maheswaran, et al Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature, 450(7173):1235âĂŞ1239, December 2007.
Chemotaxis and Motility Assays	These assays tells us more about the mechanism of metastasis. Microfluidic devices can be used to generate a variety of gradients. Sizes of channels can mimic blood capillaries	Liu, Liyu, Bo Sun, Jonas N. Pedersen, Koh-Meng, Robert H. Getzenberg, Howard A. Stone, and Robert H. Austin. "Probing the invasiveness of prostate cancer cells in a 3D microfabricated landscape." Proceedings of the National Academy of Sciences (2011)
Mechanical Signaling	Cancers cells may have altered mechanical responses	"Matrix Crosslinking Forces Tumor Progression by Enhancing Integrin Signaling" Kandice R. Levental, Hongmei Yu, Laura Kass, Johnathon N. Lakins, Mikala Egeblad, Janine T. Erler, Sheri F.T. Fong, Katalin Csiszar, Amato Giaccia, Wolfgang Weninger, Mitsuo Yamauchi, David L. Gasser, Valerie M. Weaver Cell - 25 November 2009 (Vol. 139, Issue 5, pp. 891-906)

Table 9: Examples of Microfluidics and Cancer Research applications

Appendix 2: A Very Brief History of Microfluidics

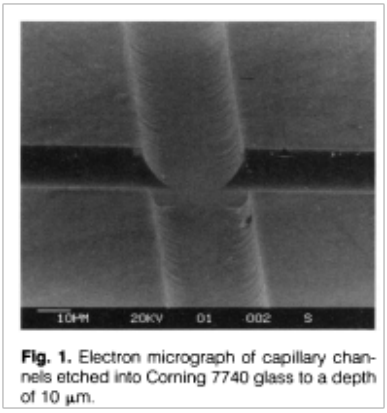


Fig. 1. Electron micrograph of capillary channels etched into Corning 7740 glass to a depth of 10 μm.

Figure 101: A channel in a capillary electrophoresis device built in early 90s

Historically, microfluidic devices in the form they can be recognized today started being developed in the 80's. Prior to that in the 50's and 60's there was an extensive effort to make fluidic circuits for defense application in the US as fluidic circuits would be able to outlast the impact of the electromagnetic pulse of a nuclear detonation. During that time while miniaturization for making electronics and circuits had tremendous success, fluidic devices had long been used in control systems proved problematic to miniaturize and this effort largely petered out. A prescient and much beloved talk by Richard Feynman from that era (`http://calteches.library.caltech.edu/archive/47/02/1960Bottom.pdf`) including speculation about machines for biological applications is worth reading even today [32].

A few decades later the increasing needs of small volumes for chemical analysis lead to work on miniaturizing sensors. Perhaps the first microfluidic devices were gas sensors made in the 70s. But another starting point can be taken to be the particularly noteworthy work done by Andreas Mann and co-workers at Imperial College, London miniaturizing devices to perform chemical analysis[33]. They called their devices Micro-Total Analysis Systems. These are the precursors to modern capillary electrophoresis equipment that you may use in a biological laboratory today. More researchers making devices borrowing lithography and etching techniques from electronics (this field is called Micro Electro Mechanical Systems (MEMS) today) became involved and several innovative devices made out of silicon and other hard materials were demonstrated in the 80's and 90's and indeed new devices continue to be designed even today. Robert Austin, the senior PI of the Princeton PS-OC and his collaborators designed a number of innovative nano-scale sorting devices in the early 90's. This work is summarized in a review in 1997 [34], which also recounts the challenges

[32] R.P Feynman. There is plenty of room at the bottom. *Engineering and Science*, 23(5):22–36, 1960

[33] A. I. Manz. Miniaturized total chemical-analysis systems - a novel concept for chemical sensing. *Sensors and actuators. B, Chemical*, 1(1-6):244–248, 1990. 0925-4005

[34] J.P. Brody, P. Yager, R.E. Goldstein, and R.H. Austin. Biotechnology at low reynolds numbers. *Biophysical Journal*, 71(6):3430 – 3441, 1996

and opportunities for small scale fluidic biotechnology. A few MEMS foundries have now over 30+ years experience in making microfluidic devices for customers for both biological (eg. loading reagents into a machine) and physical applications (pressure sensors used in cars)

Related Names:
Lab-on-a-chip (LOC)
Micro-Total Analysis Systems (μTAS)
Micro-Electro-Mechanical-Systems (MEMS)
Bio-microfluidics
Bio-mimetics

In the late 90's Duffy, Whitesides and co-workers invented soft-lithography[35] and demonstrated the technique with an transparent elastomer PDMS (poly dimethyl siloxane) and this finally brought microfluidics to the masses. The ease of fabrication and low overhead made PDMS a very popular material. Within a few years complicated multi-layer devices with hundred of valves per square inch became feasible and a shift towards softer elastomeric materials caught steam[36] that continues today.

[35] D. C. Duffy, J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Analytical Chemistry*, 70(23):4974–4984, 1998

[36] S. R. Quake and A. Scherer. From micro- to nanofabrication with soft materials. *Science*, 290(5496):1536–1540, 2000

In the last few years, the push for faster sequencing machines and single cell analysis has resulted in a host of new highly parallel fludic devices and integrated detectors and this is the driving force moving the density of fluidic components further. Several new startup companies in the last decade have focused on making microfluidic products including Ibidi, Dolomite, Fludigm, Raindance, Bio-nanomatrix among many others. And over 2000+ papers are published in the field every year. Microfluidics is no more a obscure topic of research, but a thriving field with dedicated journals and conferences, and nearly every large research university in the US has multiple groups working in the field.

A recounting of the factors that lead to the development of microfluidics can be found in a (somewhat sober) review by Whitesides [37]. This particular issue of Nature contains a special supplement to microfluidics which has many broad review articles `http://www.nature.com/nature/journal/v442/n7101/` and a good starting point if you want to start research in the field.

[37] George M. Whitesides. The origins and the future of microfluidics. *Nature*, 442:368–373, 2006

Appendix 3: Exercises and Extras

Here are a few questions and extra materials. The papers referred to can be found on our website. Unfortunately, due to copyright reasons they need a password. But the papers are mostly well known and you should be able to access them from other places too.

Lecture 1

1) If I had a helium balloon in a car and the car suddenly stopped - would the balloon move forward or backward? Why? (to watch this (and many other) demonstrations see Walter Lewin perform in inimitable style(shortened url):
`http://goo.gl/GEKLN`

2) Do you think a fluid without any viscosity can exist? Why or why not?

3) Reading Material (papers will be available in a zip file that you can access) "Life at low Reynold's numbers" by Edward Purcell.
Osborne Reynold's paper

4) Watch videos from the National Committee for Fluid Mechanics films
`http://web.mit.edu/hml/ncfmf.html`
a) Eulerian Lagrangian Description
b) Deformation of Continuous Media c) Flow Visualization
d) Low Reynolds Number Flow
e) Rheology and Fluid Mechanics

5) Try this experiment: tie a string across a cup of water. Tilt the cup and start to pour while holding the the string over the top of the cup and at an angle. The water will seem to stick to the string! You can also see the same effect if you brought the round side of a spoon near a water stream pouring from a faucet - the water stream will be attracted

to the spoon. The spoon itself will seem stuck to the stream. Why?

6) For your entertainment: `http://news.bbc.co.uk/2/hi/science/nature/227572.stm`

7) How does a siphon work? (Hint:Bernoulli's Principle). See figure 102

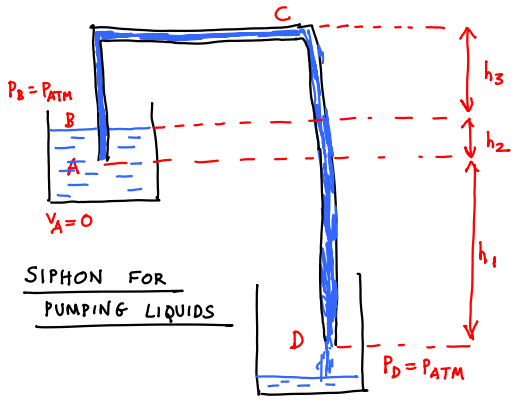


Figure 102: Siphon

Lecture 2

1) What does it mean when we say a body is streamlined? Will streamlining increase or decrease drag in micro-fluidic devices?

2) Read Einstein's original 1906 paper on diffusion.

3) Read the article titled: Half a century of Diffusion by Jean Philibert and/or the Physics Today 2006 historical article by T. N Narsimhan

4) Watch videos from the National Committee for Fluid Mechanics films
`http://web.mit.edu/hml/ncfmf.html`
a) Fluid Dynamics of Drag Part I
b) Fluid Dynamics of Drag Part II
c) Fluid Dynamics of Drag Part III
d) Fluid Dynamics of Drag Part IV

Lecture 3

1) How does a rotary mixer physically work? Can you explain it to someone else in a few sentences.

2) Read Trimmer's paper "Microbots and Micromechanical Systems"
3) Read "There is plenty of room at the bottom" (1960) - Richard Feynman

Lecture 4

1) The mixing time in droplets is often given a $log_e(Pe)$ Where do you think the log factor comes from?
2) Watch videos from the National Committee for Fluid Mechanics films
`http://web.mit.edu/hml/ncfmf.html`

Surface Tension in Fluid Mechanics

3) Pouring Salt water into salt water results in more bubbles than poring fresh water in fresh water. Why? (From Jearl Walker's The Flying Circus of Physics)

4) Read "Snow, Rain, and the Stokes Number", by Daniel Cromer and Lynn Pruisner

5) Read the Bump array paper

6) Read Microfabrication Techniques for Biologists: A Primer on Building Micromachines, Douglas Chinn (Chapter 1 in Microengineering in Biotechnology, Methods of Molecular Biology, MP Hughes, KF Hoettges (editors) Humana Press 2010)

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[7] Jenifer Clausell-Tormos, Diana Lieber, Jean-Christophe Baret, Abdeslam El-Harrak, Oliver J. Miller, Lucas Frenz, Joshua Blouwolff, Katherine J. Humphry, Sarah KÃűster, and Honey Duan. Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms. *Chemistry and Biology*, 15:427–437, 2008.

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poly(dimethylsiloxane). *Analytical Chemistry*, 70(23):4974–4984, 1998.

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