

# Intro What Is Microfluidics?

Microfluidic platforms, sometimes called “*lab on a chip*”, are small devices used to control small amounts of fluids (usually liquid) using tiny channels and chambers. You can think of a big chemical factory full of pipes and tanks; now make it smaller and smaller until everything shrinks and can now fit in the palm of your hand. Some microfluidic chips can fit in the tip of your finger! You have heard of *microprocessors* inside of computers: microfluidic chips are very similar, but instead of electricity they transport liquids in channels that can be as thin as the leg of an ant. We measure these channels in *micrometers* (one millionth of a meter!). To give you an idea of how small this is, one inch is 25,400 micrometers long, and the average cell in your body is around 15 to 20 micrometers in diameter. But why does anyone want to put liquid in such tiny devices? There are several reasons, but we will explore two of them here:

- 1) To work with small volumes or small things: imagine you want to measure molecules like sugar or antibodies in a patient’s blood. What if you can do many of these measurements in a small drop of blood instead of many tubes? Or imagine that you want to study how cells absorb nutrients or respond to vaccines. Would you rather work with them in big flasks or in small chambers that mimic the environment in which cells actually live?
- 2) Fluids and molecules do interesting things in small dimensions. Have you ever seen water defy gravity when it *climbs up* a piece of paper or a small tube (capillarity)? Could you pour lemonade and tea on a tall glass at the same time and keep them from mixing with each other? Inside of microfluidics chips, you can.

Scientists and engineers use the curious properties of the microfluidic world to analyze medical samples, synthesize chemical compounds, understand transport processes inside and outside of cells, test drugs in “*organs on a chip*” or to make nanoparticle vaccines, just to give a few examples.

**Microfluidics is the manipulation of fluids in channels and chambers at the micrometer scale**

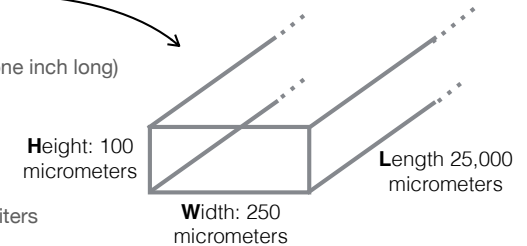


**Microfluidics** is the way of the future! Consider this:

A tube used to collect blood in a clinical laboratory can be filled with 10 milliliters of blood (=10,000 microliters). A channel in a microfluidic chip used to analyze blood has the following dimensions

$$\begin{aligned} \text{Volume} &= \mathbf{H \times W \times L} \\ \text{Volume} &= 100 \times 250 \times 25,000 \text{ micrometers (one inch long)} \\ \text{Volume} &= 750,000,000 \text{ micrometers}^3 \end{aligned}$$

Our channel is 750,000,000 micrometers<sup>3</sup>  
**How many microliters of blood fit in it?**



Each 1,000,000 micrometers<sup>3</sup> = 0.001 microliters

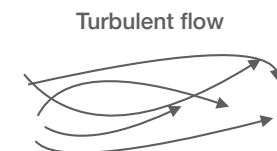
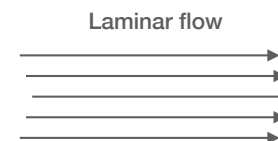
$$\frac{750,000,000 \text{ micrometers}^3}{1,000,000 \text{ micrometers}^3} = 750$$

750 x 0.001 microliters = **0.75 microliters ! That is a drop about this big**

This means that instead of collecting 10,000 microliters of blood, the nurse can collect a tiny (really tiny) drop of blood from a finger to run the analysis. Scientists can take advantage of this kind of difference in volumes (e.g. going from 10,000 to less than 1) to conduct hundreds of experiments using *smaller samples and less reagents*, obtaining more information with less expense and often in less time. It is also easier for the patient to donate one drop of blood instead of several tubes.

**Cool physics** in Microfluidics: **Laminar flow**

When you pour two liquids in a container they will mix (it is called convective mixing). However, in microfluidic channels you can have two or more liquids flow in the same channel, next to each other, without convective mixing! This kind of smooth or regular flow is called *laminar flow*, and it is opposed to *turbulent flow*. What is interesting and useful about laminar flow is that it can be used to carefully control the mixing of two liquids (to synthesize chemical compounds or separate molecules) or to generate layers of fluids with different concentrations (gradients).



What are actual applications of Microfluidics in our world today? Turn the page!

Student \_\_\_\_\_

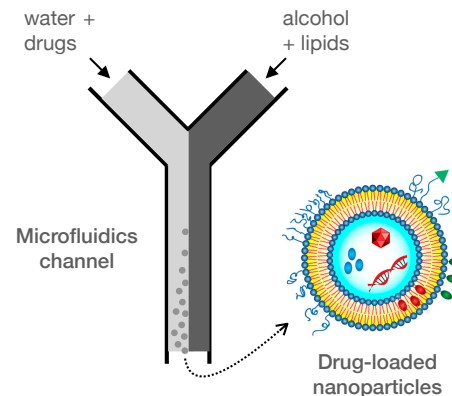
# Applications of Microfluidics

We have learned that microfluidic technologies allow us, for instance, to reduce the volume of biological samples that are required to conduct clinical analyses. A great example is the personal device used by diabetic patients to measure glucose in blood. A thin cartridge featuring microfluidic channels directs the patient's blood from their finger to the reader inside the device. Here are other applications:



Did you know that some people apply engineering principles to biological systems? They are called Bioengineers. They can use microfluidic devices to measure how cells respond to stretching, or compression. They also use them to understand how cells communicate with each other and how they can self-assemble into tissues and organs. Controlling fluids and other materials at the micro scale allows them to study biology in a whole different way!

Because of laminar flow in microfluidic devices scientists can make tiny particles that are the size of a virus. These particles are easily absorbed by the body and can carry drugs to attack tumors, vaccine components to help the body fight infectious diseases, or even new genes for cells so they can fabricate new proteins. This is possible because inside microfluidic channels mixing occurs by *diffusion*, meaning that single molecules move randomly from one type of fluid to the other. When lipid (fat) molecules move into water, they all get together forming tiny particles and capturing a little bit of water (and whatever is in it) inside of them.



## Quiz

1. Microfluidic devices are similar to \_\_\_\_\_ inside computers but instead of electricity they usually carry liquids.
2. There are one million micrometers in a meter. If you are 1.7 meters tall (about 5' 8"), how many micrometers tall are you? \_\_\_\_\_
3. \_\_\_\_\_ and \_\_\_\_\_ are two advantages of conducting experiments in microfluidic devices instead of using standard methods.
4. Turbulent flow leads to \_\_\_\_\_ mixing. In contrast, laminar flow allows for gradual or controlled mixing by \_\_\_\_\_.
5. \_\_\_\_\_ and \_\_\_\_\_ are examples of processes or applications made possible by laminar flow in microfluidic devices.
6. Imagine a channel with the height of a human hair (100 micrometers), triple the width, and 1.5 inches in length. How many microliters are needed to fill that channel?

Volume =  $H \times W \times L$  | 1 inch is ~25,000 micrometers |  $1,000,000 \text{ micrometers}^3 = 0.001 \text{ microliters}$

Bonus question: Bioengineers use physics and mathematics to understand and also to mimic and even improve biological systems. Can you think of a health or environmental problem that could be solved using bioengineering? How? Discuss with your instructor and classmates.

# How do I run my Microfluidic devices?

The goal is to run liquid through those channels! The liquid in your experiments is usually water plus food coloring so you can observe what the fluid does. Infusing, introducing, pumping (all the same thing) liquid into the devices and then observing what happens might be all you need to do. But first you have to have your pump and your devices ready to go:

## Tubing

The tubing should be firmly attached to the pump (burette or syringe). If you are using a gravity pump, attach tubing before filling the pump with liquid and either close the valve (burette) or clamp the tubing (open syringe). Follow the instructions for the **manual pump** in a separate insert.

## Set up your “pump”

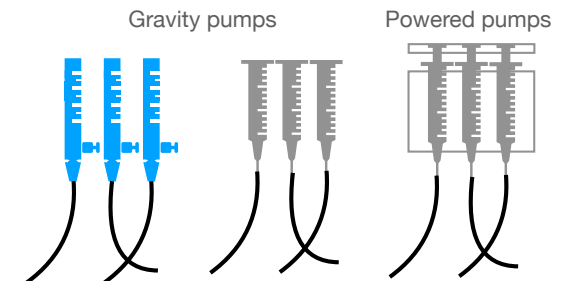
Whether you use gravity (burettes or open syringes) or a powered (manual or motorized) syringe pump, prepare colored water (food dye is great) and fill up the pump. For gravity pumps the higher the volume the better the flow. Purge the air in the tubing by testing the flow before connecting to the device (have a container or paper towels at hand). Check the pump **set up** instructions.

## Attach the bumper inlets to your device

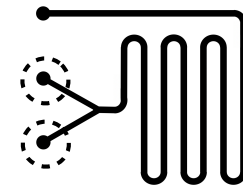
Take one of the bumpers and then feel with your finger the holes/openings of the device. That is the side (front) you want to apply the bumper on. We have a diagram that shows you how to do this! Press hard to attach the bumper firmly. This is the **number one mistake** we sometimes make, attaching the bumper to the wrong side of the device, where there is no opening!! If you fail to see flow when you start the pump, the bumper is probably placed in the wrong side of the device.

## Let the pump work for you

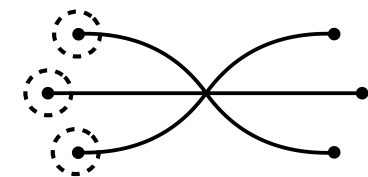
Arrange everything so the device remains as flat as possible (you can use tape to secure the device down if needed). Set your device on top of a paper towel or sheet of paper and get the pump going! The white background will help you see the colored liquid run through the device. Observe the behavior of the liquid and be ready with a paper towel to remove the liquid that comes out from the outlet. You can observe the flow with the naked eye, but if your instructor allows it, you can use a phone with a camera to zoom in and take pictures or video of the channels. Check out laminar flow in action, it is really cool! Record what you see in the worksheet and explain your observations.



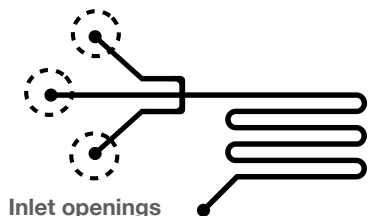
FLOW →



Inlet openings



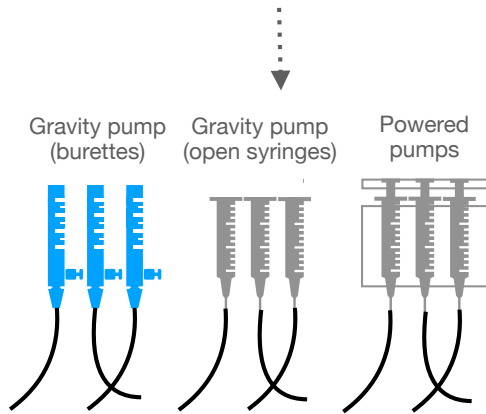
Inlet openings



Inlet openings

# Set up: Pumps and Inlet Bumpers

Manual pump and bumpers video:  
[petfluidics.com/education](http://petfluidics.com/education)



## Fill the pump with colored solution

For gravity pumps the more solution the better the flow. **Concentrate** the color; small amount of liquid in channels can be hard to see unless color is concentrated. For powered pumps, (manual or motorized) the flow should be really slow. Do not attempt squeezing syringes individually by hand; the pressure is too high (it can delaminate devices) and it is hard to make flow even in every channel.

**1**

**Identify the front side of the PETL device.**

The front side has open access to the channels.

Feel the indentation of the channel opening with your fingers to identify the front side.

**2**

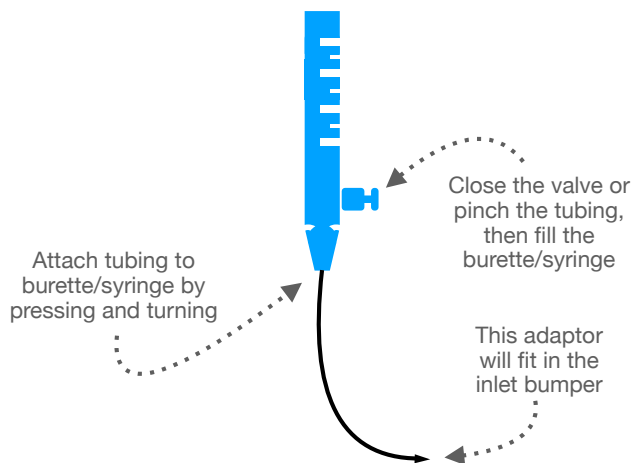
**Use a paper clip to guide the bumper onto the channel opening.**

Slide the bumper down a bent paper clip onto the device. The perforation in the bumper should be centered directly over the channel opening.

**3**

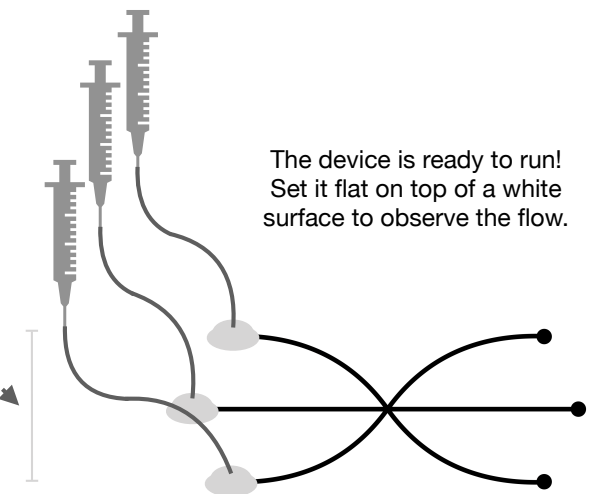
**Press firmly to attach and seal the bumper to the device.**

Continue applying bumpers to channel openings (*inlets*). Bumpers may also be attached to *outlet* openings if sample recovery is desired.



## Add bumper inlets and insert tubing

Follow the 3 steps above to add the bumper inlets to your device. When using a gravity pump make sure the device is below the tip of the burette or syringe. Insert the tubing adaptor into the inlets; move the pump so the device lays flat over a white surface. You may want to use some tape, but do not block the view of the channels. Have some paper towels at hand, use them to remove liquid from the outlet. Run one device at a time.



# Laminar Flow & Fluidic Resistance

One of the main characteristics of flow in microfluidic channels is laminar flow, in which viscous forces are sufficiently strong that cannot be ignored and may become dominant. Laminar flow occurs when fluids move in parallel layers, and is distinct from turbulent flow, where motion is characterized by chaotic changes in pressure and velocity. To determine whether flow is laminar in a given channel system, one can calculate the **Reynolds number**, a non-dimensional parameter that represents the ratio of the inertial force to the viscous force:

$$Re = \rho u D_h / \mu$$

$\rho$  = density (water = 997 kg/m<sup>3</sup>)

$u$  = velocity (m/s)

$D_h$  = hydraulic diameter (m)

$\mu$  = fluid viscosity (water dynamic viscosity @20°C = 0.001N\*s/m<sup>2</sup>)

A flow regime with a  $Re < 2300$  is considered laminar. A number that will be useful here, and to make other calculations later, is the **hydraulic diameter** ( $D_h$ ). For a closed rectangular duct where width ( $w$ )  $\gg$  height ( $h$ ), the hydraulic diameter can be approximated using:

$$D_h = 2 w h / w + h.$$

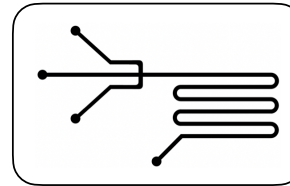
The channels in PETL chips are rectangular, usually 150 $\mu$ m in height and 750 $\mu$ m in width. We will assume a velocity ( $u$ ) of 0.1m/s for water @20°C.

(1) Calculate  $Re$ . Is the flow laminar?

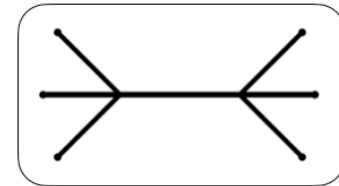
(2) What do you predict will occur when 3 different dyes flow along the central channel in the serpentine and focusing chips?

Flow in microchannels is used in a variety of applications in chemical synthesis, cell biology, diagnostics and others. Devices with channel architectures as simple as the ones used here can be used to manufacture nanometer-sized lipid particles called liposomes, used for cosmetics but also for cancer therapies and vaccines.

Serpentine chip



Focusing chip



Now that we know whether the flow is laminar, we can discuss fluidic resistance ( $R$ ). Simple solutions to the **Navier-Stokes** equations for unidirectional flows lead us to Poiseuille and ultimately to the well known **Hagen-Poiseuille** equation:

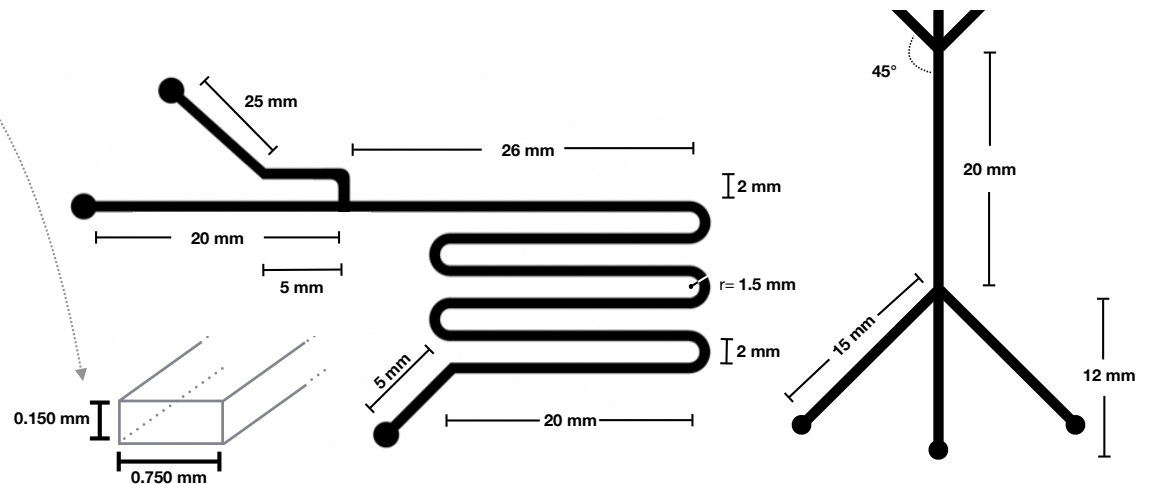
$$Q = \Delta P / R$$

... which will be useful to describe resistance, flow rate and pressure ( $P$ ) drops across microchannels. For rectangular channels where width ( $w$ )  $\gg$  height ( $h$ ), we can approximate the **fluidic resistance** ( $R$ ) using:

$$R = 12 \mu L / wh^3$$

Where  $L$  is the length of the channel. Choose a PETL chip and calculate the fluidic resistance.

We can now determine **flow rate** ( $Q$ ) for a fluid in a given channel architecture when the pressures are known or obtained by other means (can be given to you by your instructor). The instructor may also ask you to make other calculations using any of these microchannel configurations.





# INSTRUCTOR SHEET

This worksheet is meant to start the laboratory session with a conversation on laminar flow and some of the calculations that can be done using the supplied chips. The students may start with calculating the predicted  $Re$  number for the focusing chip and then go on to observe flow in it. Following the first observation they may start thinking about flow rates and pressure; approximate pressures may be given by the instructor and/or other equations may be brought up to be applied to the channel architectures. They might then move on to the serpentine channel; this is a good transition to the Diffusion Worksheet, since the focusing chip will show no mixing but the serpentine device will feature some passive diffusion and mixing. Although regular food coloring is enough to visualize flow and mixing, the use of a pH indicator + pH solution (e.g. phenol red + pH8+ solution), if available, does a great job at making students think about the interphase between liquids, contact time and molecular diffusion. The chips offer the instructor the opportunity to customize the laboratory experience (addressing topics from lecture) or simply ask the students to execute the work as directed.

## Useful sources:

Beebe DJ, Mensing, GA & Walker GM. 2002. *Annu Rev Biomed Eng* 4, 261-286  
 White FM. *Viscous Fluid Flow*, 2Ed. McGraw Hill.  
 Sharp KV, Adrian RJ, Santiago JG & Molho JI. *Liquid Flow in Microchannels*. CRC.

## Laminar Flow & Fluidic Resistance

Student Worksheet

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(1) Calculate  $Re$  (25). Is the flow laminar?

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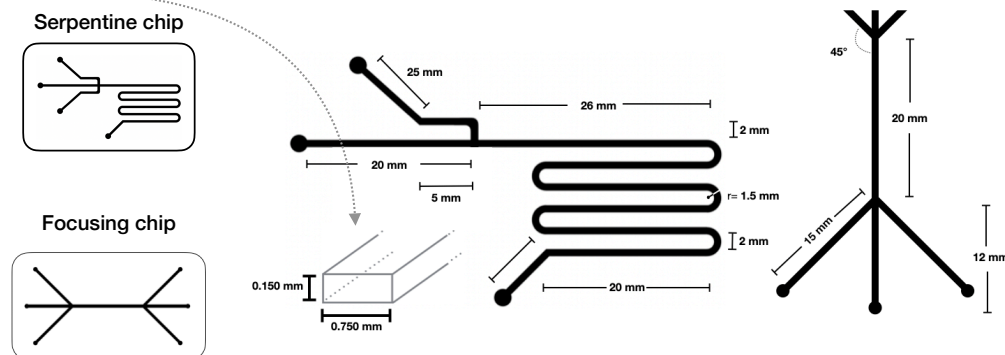
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Where  $L$  is the length of the channel. Choose a PETL chip and calculate the fluidic resistance.

We can now determine **flow rate** ( $Q$ ) for a fluid in a given channel architecture when the pressures are known or obtained by other means (can be given to you by your instructor). The instructor may also ask you to make other calculations using any of these microchannel configurations.



# Diffusion

One consequence of the characteristic low Reynolds laminar flow in simple microchannels is the absence of convective mixing. This is useful for many applications, like the flow rate-controlled generation of nanoliposomes\*, which depends on diffusive mixing. Generating adjacent layers of fluids with varying concentrations (gradients) is another common application. This is possible because diffusion is very slow, since it varies to the square power of the distance:

$$d^2 = 2Dt$$

d = distance the particle moved

t = time

D = diffusion coefficient of particle in solvent.

- (1) Calculate the distance travelled in 1 second by a molecule of dye (in water) with a  $D = 1.2 \times 10^{-9} \text{ m}^2/\text{s}$

In some instances however, the lack of convective mixing is a “problem” found in microfluidic systems that has resulted in hundreds of scientific articles addressing potential solutions. Whether mixing is beneficial or not for a given application, it is useful to assess the degree of diffusive mixing expected for a particular channel configuration. The Péclet number, which provides us with the relative magnitude of diffusion in a given system can be calculated as follows:

$$Pe = U \ell / D$$

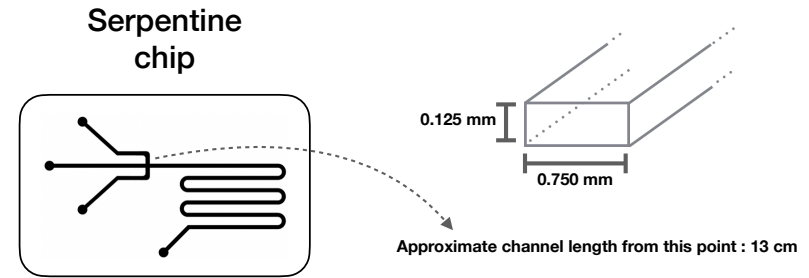
U = velocity of stream (*Poiseuille flow*)

$\ell$  = diffusion length

In a channel with two or more laminar streams,  $\ell$  is transversal to the flow, since it is the distance the particle must travel by diffusion.

- (2) Calculate  $Pe$  for a dye-in-water system with  $U = 4 \text{ cm/s}$  in which half the channel width is  $\sim 400 \mu\text{m}$ .

\*A simple way to capture molecules (e.g. mRNA) inside lipid particles (e.g. liposomes) is to dissolve lipids in an organic solvent that flows in a microchannel along water carrying the desired molecule; nano-sized vesicles are formed as Brownian motion transports lipids to the water phase, encapsulating the water-soluble molecules.



Large  $Pe$  numbers (higher than 100) indicate slow mixing and are found in most microfluidic systems. Interestingly, we can use  $Pe$  to determine the distance along the channel that is required for mixing since the diffusion time is proportional to  $\ell^2 / D$ , which multiplied by  $U$  is:

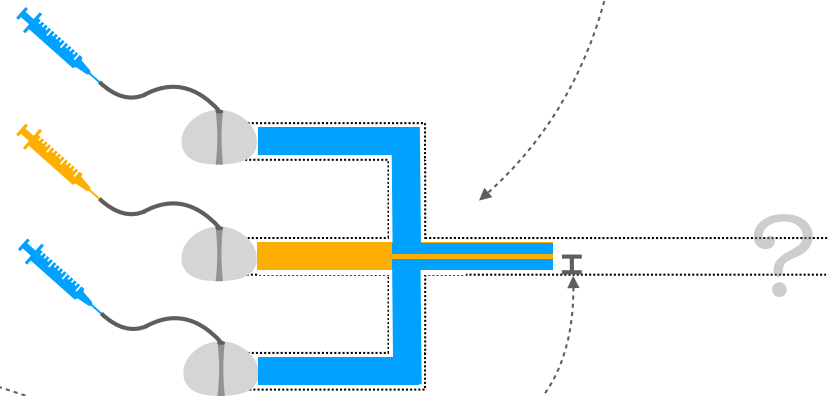
$$Pe \ell$$

Run dye-in-water solutions through the serpentine chip if you haven't yet (go very slow if using manual pump, see instructions). Choosing colors wisely will aid in visualization. Do you expect to see mixing in this chip?

- (3) Calculate the length of a channel required to observe mixing the dye-in-water system used in (1) and (2).

- (4) Compare your empirical observations for the serpentine and the focusing chips. Are there differences in mixing? Explain.

- (5) How could one maximize diffusive mixing in laminar flow systems like this?



# INSTRUCTOR SHEET

After discussing laminar flow, completing the first worksheet and running the focusing chip (where there is a lack of observable mixing), the instructor may direct students to approach the concept of diffusion. Because of the lack of turbulence in microfluidics channels, most mixing occurs due to diffusion, which represents a challenge for applications in which mixing is desired. The worksheet guides students through empirical examples of diffusion and the use of the Péclet number to determine whether significant mixing will occur in a given channel configuration. The comparison between the focusing chip and the serpentine chip (where the length of the channel and the triple stream facilitate significant diffusive mixing) will be useful to illustrate controlled diffusion (e.g. varying flow rate) and a point of entry to discuss gradient formation, particle synthesis, controlled release, gas exchange or other applications.

## Useful sources:

Stroock et al. 2002. *Science* 295, 647-651  
Takayama et al. 2001. *Nature* 411, 1016.  
Walker GM & Beebe DJ. 2002. *Lab Chip* 2, 131-134.

Remind students to use the pump slowly, about 1 revolution / 10 seconds;  
the actual volume of most channel configurations is usually less than 10 $\mu$ l.

## Diffusion

(Student Worksheet)

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(2) Calculate  $Pe$  for a dye-in-water system with  $U = 4 \text{ cm/s}$  in which half the channel width is  $\sim 400 \mu\text{m}$ .

## Notes:

A good color combination for this chip is yellow in the central channel, flanked by blue dye. Mixing will be evident when solution turns green.

Answers:

(1)  $\sim 50 \mu\text{m}$

(2) 13,333.33

(3) 5.3 cm

The focusing chip does not display mixing, the main reason being that the length of the channel shared by all 3 streams is much shorter than in the serpentine chip.

Reducing the distance particles need to travel improves diffusive mixing. Introducing features that cause chaotic flow (not turbulent) along the channels is one often-used solution.

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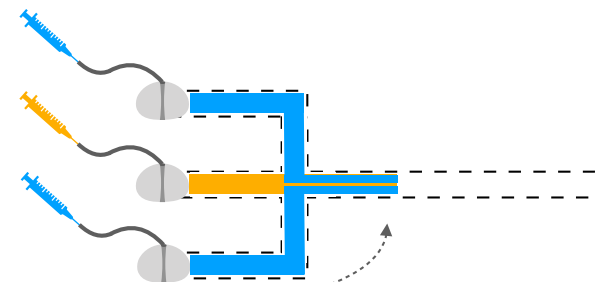
$Pe \ell$

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# Surface tension

There are several different ways to **pump** fluid through microfluidic devices. Finding simplified pumping methods facilitates the use of microfluidics platforms in a variety of settings. Here you will explore a method that utilizes the pressure found inside a drop of liquid to pump fluid through a microchannel.

The amount of pressure within a spherical drop of liquid at an air/liquid interface can be found using a simplified **Young-Laplace** equation:

$$\Delta P = 2\gamma / R$$

Where  $\Delta P$  is the difference between the atmospheric pressure and the pressure inside the drop,  $\gamma$  is the surface free energy of the liquid and  $R$  is the radius of the sphere.

Now consider individual drops of the same liquid placed at two ports connected by a flooded channel. One of the drops is very small (with a radius approaching the radius of the port), while the other is much larger. (1) What happens to the pressure at each of these ports?

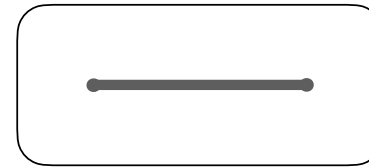
(2) What do you predict will happen to the volume of the small drop?

The movement of fluid can be observed using water and dye. Using the straight channel chip you can experiment with drops of different volume and may even roughly quantify the amount of fluid displaced per unit of time.

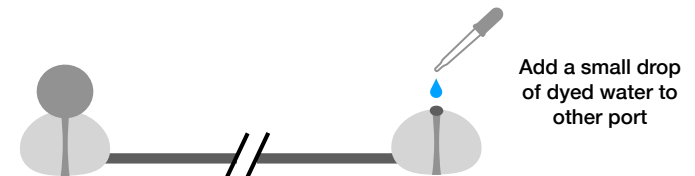
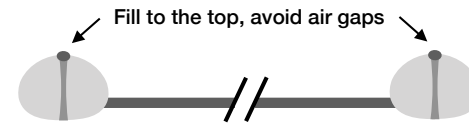
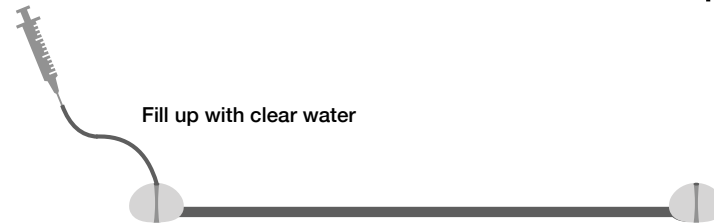
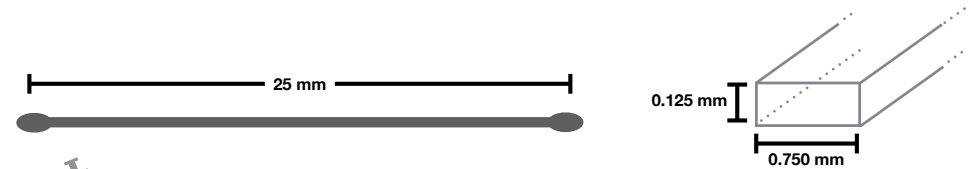
Back to the equation, as time goes by and the radius of a drop is diminished, (3) will the rate at which the volume change stay constant?

The radii of the drops has a direct effect on the pumping rate. If you are concerned with maximizing the pressure within a drop, (4) how will the hydrophobicity of the port's surface affect this rate?

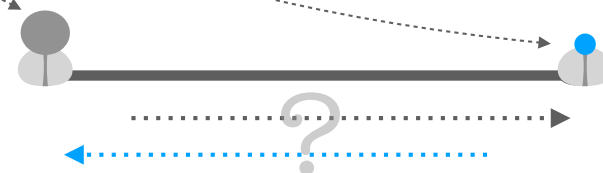
Your instructor may ask you to use other equations to predict parameters and relationships like linear pressure drops, the effect of contact angles and flow rates as a function of the height of the drop.



Straight channel chip



The bumper on this side may be removed for this experiment (see video on petl microfluidics channel)



# INSTRUCTOR SHEET

The surface tension exercise illustrates the use of surface energy in a passive pumping device and makes use of the Young-Laplace equation. Because the pressure inside a large drop is almost negligible, flow can be observed and measured moving from the small (higher pressure) drop across the channel. The materials are hydrophobic (no capillarity) and diffusion can be ignored over the short term. The questions in the worksheet are simple enough, but the instructor may wish to ask students to determine the radius of the drops or determine the volumetric flow rate across the channel (see sources suggested below). Although water and food coloring are indicated here, other solvents may be used to illustrate how changes in viscosity affect pumping rates.

## Useful sources:

Walker GM & Beebe DJ. 2002. *Lab Chip* 2, 131-134.  
 Berthier E & Beebe DJ. 2007. *Lab Chip* 7, 1475-1478.  
 Chen IJ & Lindner E. 2009. *Anal Chem* 81, 9955-9960.

## Surface tension (Student Worksheet)

There are several different ways to **pump** fluid through microfluidic devices. Finding simplified pumping methods facilitates the use of microfluidics platforms in a variety of settings. Here you will explore a method that utilizes the pressure found inside a drop of liquid to pump fluid through a microchannel.

The amount of pressure within a spherical drop of liquid at an air/liquid interface can be found using a simplified **Young-Laplace** equation:

$$\Delta P = 2\gamma / R$$

Where  $\Delta P$  is the difference between the atmospheric pressure and the pressure inside the drop,  $\gamma$  is the surface free energy of the liquid and  $R$  is the radius of the sphere.

Now consider individual drops of the same liquid placed at two ports connected by a flooded channel. One of the drops is very small (with a radius approaching the radius of the port), while the other is much larger. (1) What happens to the pressure at each of these ports?

(2) What do you predict will happen to the volume of the small drop?

The movement of fluid can be observed using water and dye. Using the straight channel chip you can experiment with drops of different volume and may even roughly quantify the amount of fluid displaced per unit of time.

Back to the equation, as times goes by and the radius of a drop is diminished, (3) will the rate at which the volume change stay constant? Smaller radius=higher pressure

The radii of the drops has a direct effect on the pumping rate. If you are concerned with maximizing the pressure within a drop, (4) how will the hydrophobicity of the port's surface affect this rate? Hydrophilic material=larger radius=less pressure

Your instructor may ask you to use other equations to predict parameters and relationships like linear pressure drops, the effect of contact angles and flow rates as a function of the height of the drop.

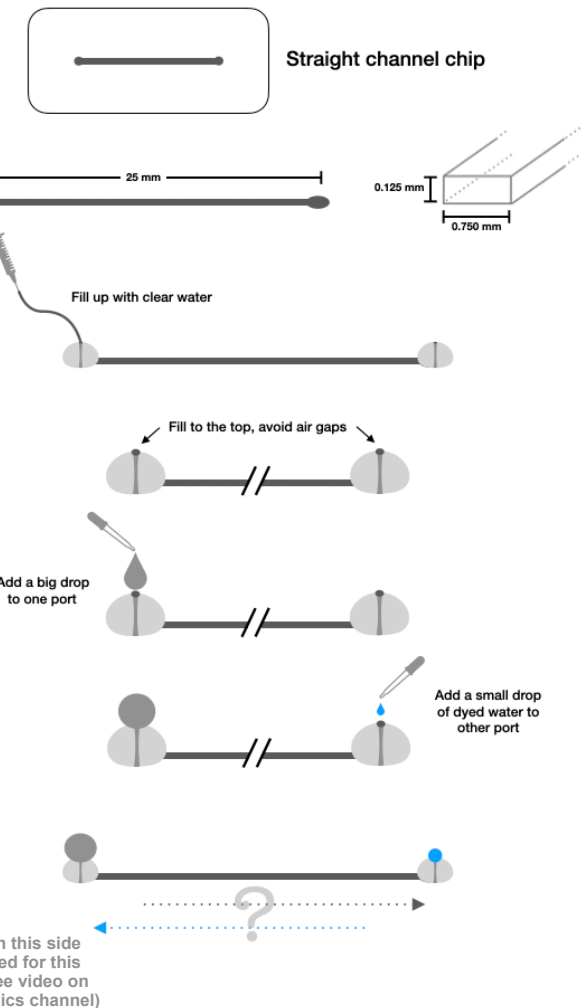
## Notes:

Students are given the channel dimensions, which may be used to determine flow rates and other parameters. The radius of the port opening is  $\approx 0.75$  mm.

The dye will travel from the small drop (pumping drop) to the large drop (reservoir drop). The pumping drop may be replenished continually until the reservoir drop collapses.

(Optional) Both bumpers and chip are made of hydrophobic materials (PVC and PET) which help to maintain sphericity (bead) of the drop (and prevent capillarity to a large extent). After channel is manually filled, the bumper(s) may be removed to test drops on a flat surface.

Students might need to go through trial and error to find optimal drop volumes to better observe flow. The channel may be emptied and refilled repeatedly.



The bumper on this side may be removed for this experiment (see video on petfluidics channel)