Microfluidic Hydrodynamics
Fields, Forces, and Flows at Low Reynolds Numbers

2.674 Labs 3, 4, 5
Spring 2016

Follow the directions from the laboratory instructors carefully. Wear personal protective equipment and follow general lab safety precautions as usual. In these experiments, there is a chance of liquid squirting out of pressurized tubing or devices, so care must be taken and goggles must be worn at all times. One of the labs involves the use of high voltage power sources. When attaching the electrodes to the microfluidic devices, always make sure that the power source is off.

General Overview

In the Microfluidic Hydrodynamics labs, we will prototype PDMS-based microfluidic devices and study some fundamental flow principles and methodologies in microchannels. Study of microfluidic flows will be spread out over three weeks (Labs 3, 4, 5). In Lab 3, we will bond the devices and study mass transport by diffusion and advection. We will investigate low Reynolds number flows in straight channel and square channel devices, and explore how mixing occurs in microfluidic channels. In Lab 4, droplet-generating microfluidic devices will be used to analyze the fundamentals of two-phase flow. These devices not only function as important tools for physicists and mathematicians to study principles such as surface tension and capillarity under highly-controlled conditions, but also function as useful platforms for chemistry and bioengineering in areas such as nanoparticle synthesis, measurement of reaction kinetics, and single-cell screening in nano- or picoliter volumes. In Lab 5, we will look at electrokinetics, i.e. the principles of using electromagnetic forces to manipulate small particles and molecules in microchannels, or to drive fluid flow in the channels. We will develop an understanding of how voltage can be applied to induce flow in microchannels, and investigate the flow dependency on parameters such as applied potential and the properties of immersed particles. We will also see how electrically driven flows can be used to separate molecules, which forms the basis of a widely used class of biological and chemical separation technologies.

Lab #3 – Mass Transport at Low Reynolds Numbers

I. Laboratory Objectives

In this section of the module, we will:

1) **Assemble** microfluidic devices for studying mass transport and droplet generation.

2) **Study** diffusion in parallel channel flows.

3) **Investigate** mixing using inertial effects in moderately low (<200) Reynolds number flows.

II. Device Fabrication

In Lab 2, you molded PDMS on two silicon wafers (Figure 1). Now, we will a) punch holes, b) clean, and c) bond the PDMS components to glass slides to complete assembly of the microfluidic devices. The devices will be ready once the PDMS elastomer component is sealed to a glass surface using plasma bonding. Before proceeding to punching, you can cut the PDMS into smaller pieces that will fit on the provided glass sides. As you cut, **be careful to leave at least 3-4 mm space around any device features!** Your lab instructors will show you how to cut the PDMS pieces.
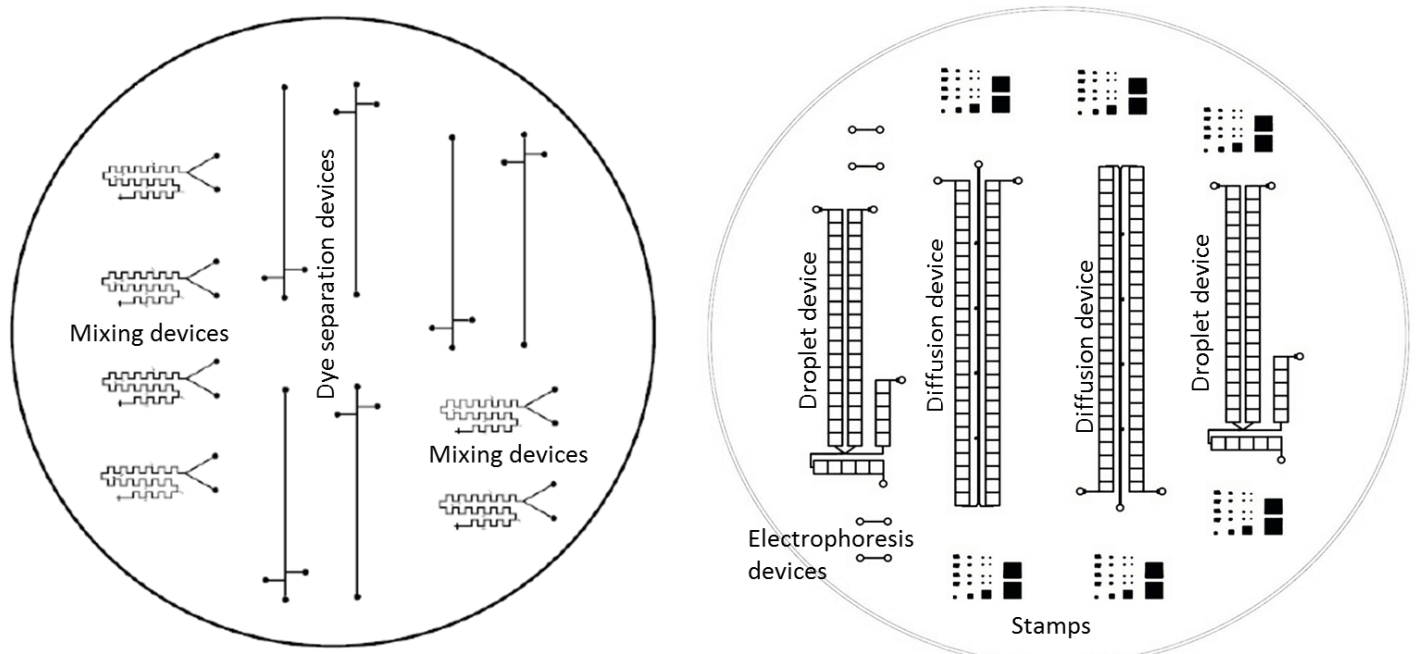

Figure 1. Schematic diagram of the two silicon wafer molds. Wafer on the left has large channel height devices, while that on the right has small channel height devices. Detailed geometries are provided in the appendix.

<u>II. A. Punching holes:</u>

First, the access ports to connect to the microchannels must be made before the bonding step. We will use a 0.5 mm diameter **blue** tissue sampling tool to punch a hole in the PDMS for diffusion, mixing, and droplet devices, and a 1.5 mm diameter **brown** tissue sampling tool for electrokinetics devices. No holes are necessary for the patterns to be used later for microcontact printing. **Caution: ask your instructor where to punch the outlet hole and which punch to use if you are not sure!**

- Place a PDMS device microchannel side up on the provided green punch mat, line up the punch stub over the end of each microchannel (normal to the molded surface), and gently push the stub through the PDMS until you reach the punch mat.

- Lift up the punch (with the PDMS piece hanging from it) and extrude the core using the button on the punch. Use the tweezers to subsequently remove the PDMS core.

- To remove the punch stub, place the PDMS down on the cutting mat, and <u>place the provided tweezers at the base of stub</u> on the PDMS surface, and pull out *without* touching the PDMS surface with your hands. Repeat until all holes are punched. **Make sure that none of the cores are left behind in the holes!**

<u>II. B. Cleaning:</u>

Next, we will clean the surfaces of the PDMS devices before bonding. This is an essential step to prevent dust, lint, etc. from getting in between the PDMS component and glass slide and ruining the devices. After punching all of the holes, clean the microfluidic devices to remove debris as follows:

- Stick scotch tape on the device surfaces and then peel it off. This should remove most large pieces of debris from the surface.

- Rinse with isopropyl alcohol in the chemical hood. This step will remove smaller debris as well as oils, etc. that may prevent bonding. This step can be skipped if the devices are not too dirty.

- Dry the devices with nitrogen gun using the tank with the hose adapter at ~20-30 psi pressure (not too much pressure or the devices will get knocked onto the floor). **It is better to hold the device in your hand using the sides of the device rather than use tweezers as many people tend to drop devices that they are holding with tweezers!**

- After drying, put the devices **channel side up** (placing them channel side down will negate your cleaning efforts!) in a covered Petri dish to protect from dust.

Next, clean the glass slides (as many as required to fit your devices) with isopropyl alcohol and dry them (omit the scotch tape step).

Do not let the cleaned surfaces contact anything else- even tweezers, clean plastic, or gloves! Proceed to the plasma machine for bonding.


II. C. Plasma bonding:

The air plasma bonder is used to irreversibly bond the microfluidic devices to 25 x 75 mm glass slides. **Today we will only bond the mixer, diffusion, and droplet devices.** You can save the cleaned electrokinetics devices in a clean Petri dish for bonding later.

- Place one microfluidic device with **channel side up** on the provided tape-coated glass slide and one glass slide in the air bonder. **Do not place the PDMS in direct contact with any glass surface during plasma, since it will end up sticking!**

- Close the plasma bonder door, drop the pressure in the chamber down to ~600 mTorr, and expose to the plasma for 45 s on high (ask your instructors for the optimum plasma time). **Record** the plasma time and setting (low, medium, or high).

- After exposure, immediately align and place the channel-molded surface of the PDMS component in contact with the plasma-treated surface of the glass slide.

- Check whether the contact is good by looking at the reflection from the interface. If you observe that the contact is not good, **gently** push down with the tweezers (pushing hard will collapse the microchannels).

This process must be completed without delay (within about a minute) after plasma; else the bonding quality is severely compromised and the devices will leak.

- Bake the devices in the oven for **10 min**.

Plasma bonding can take hours to reach full bonding strength, though a fairly good bond is formed within a few seconds. Thus, **careful placing of the PDMS is important: once you place the PDMS on the glass slide after plasma treatment, you will not be able to move it without damaging the device**. Complete bonding can be accelerated by placing the devices in the oven. The bonding works as follows (Figure 2): 1) Oxygen radicals in plasma oxidize some of the PDMS to Si-OH (silanol) groups as shown. 2) The PDMS is brought into contact with glass surface, which typically also has Si-OH groups due to moisture in the air. 3) A condensation reaction takes place where Si-O-Si covalent bonds are formed between PDMS and glass by elimination of a water molecule. This method can also be applied for glass-glass bonding, but special processes are needed to bring two glass surfaces in good contact. The rubbery nature of PDMS allows it to easily conform to surfaces with molecular contact that facilitates bonding.
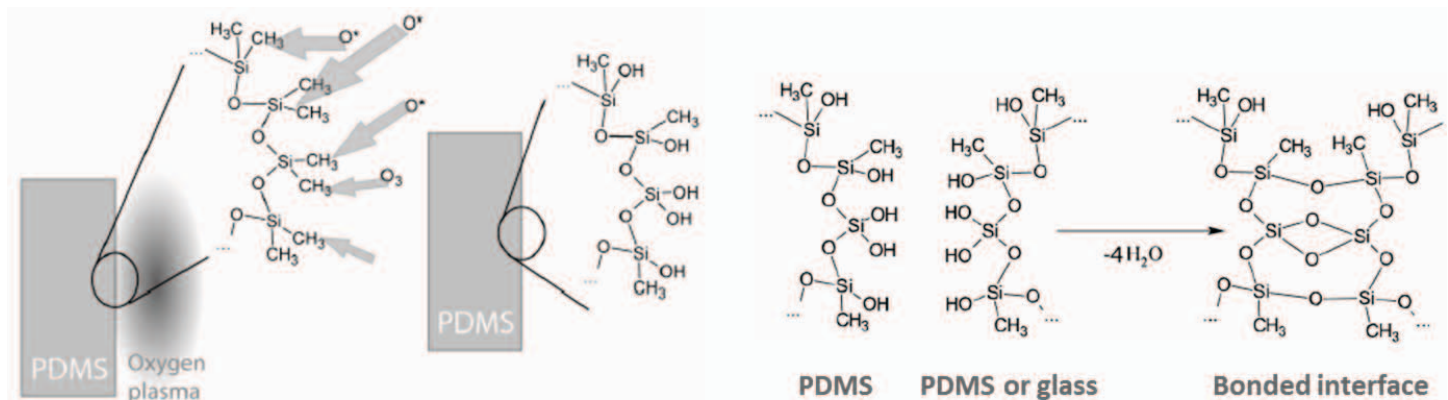
Figure 2. PDMS-glass bonding using oxygen plasma.

III. Diffusion and mixing at low Reynolds Numbers

In the second part of the lab, we will study diffusion and mixing behavior in microchannels using co-laminar streams of water, with one stream containing a food dye. At the microscope station, you will find gas-

tight vessels pre-assembled with Tygon tubing and stainless steel interconnects containing the following solutions:

- Water

- Food color solution

Bring the straight channel diffusion devices and square channel devices that you made (Figure 3) and two tubing sets to the inverted microscope lab station.
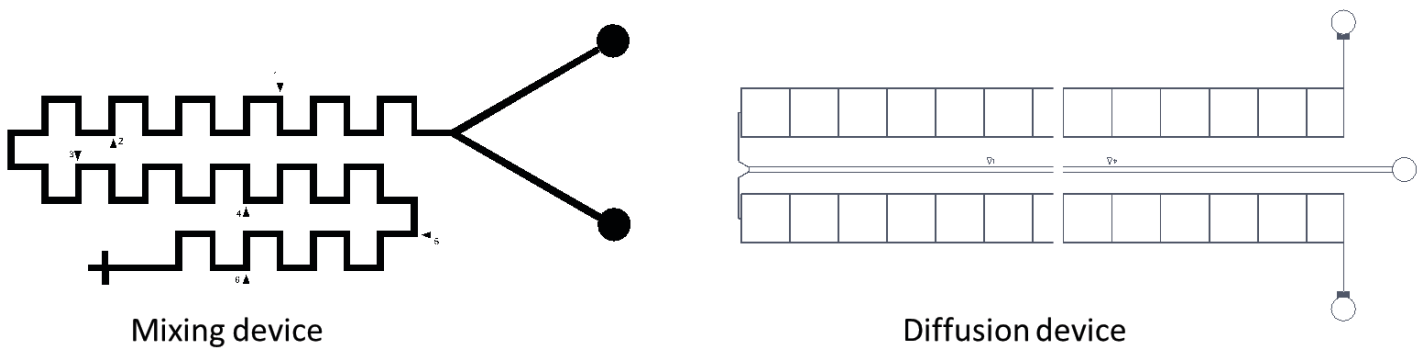

Mixing device   Diffusion device

Figure 3: Close-up of device designs for studying diffusion and mixing. Detailed device geometry is provided in the appendix.

III. A. Diffusion in a straight channel laminar flow

Setting up the flows:

First, let us look at the straight channel device. The pressure regulators should be turned completely anti-clockwise (zero flow). The attached pressure gauges should read near zero (± 0.5 psi, as they are not completely airtight at low flow rates). Connect the tubing to the outlet of the pressure vessels such that they are ready to be plugged into the device (they should terminate with a steel stub; ask your instructors before proceeding). Next, turn on the pressure valve at the base of the regulator assembly, and increase the pressure to ~1 psi so that all air bubbles are purged from the tubing. **This step is essential**. Next, plug the tubing in the device and pressurize each vessel using the corresponding regulators to get a gauge pressure reading of ~5 psi each. Looking at the device with the light microscope, you should observe a co-laminar flow stream develop after a few minutes.

After the co-flowing stream develops, balance the flows by adjusting the pressure regulators. **Record** the corresponding pressures.

In all experiments involving pressure-driven flow, be careful not to drive solutions into the pressure regulators!

Observing diffusion:

Using the microscope at low magnification (10x or 4x), **record** images of the blue dye at the inlet and at different points along the channel (marked by numbers indicating distance in cm on the device).

Estimating the flow velocity:

Now, let us estimate the average flow velocity in the channel. Unplug the pressurized gas inlet tubing of the blue dye solution to stop the flow of dye. As soon as the blue dye clears from the channel, plug it back in to resume the flow. You should be able to see the blue dye make its way along the channel. **Record** the time taken by the blue dye to traverse the channel and use it to estimate the flow velocity. **Repeat thrice** to get mean and standard deviation.

Effect of flow velocity:

Now, in addition to 5 psi, **repeat the observation of diffusion at inlet pressures of 1 psi and 15 psi**. For these points we can skip the flow velocity measurements and assume that it scales linearly with the pressure (e.g. at 1 psi it is $1/5^{th}$ of what you measured at 5 psi).

III. B. Mixing using weak inertial effects

Now, let us look at fluid flow in the device with a square serpentine channel. This device has a larger channel, consequently allowing for the Reynolds number to reach moderate values such that inertial effects can be seen although the flow is not turbulent. This is one of the tricks used to mix fluids in microchannels.

As before, connect the water and dye solutions and set the inlet pressures to ~**1 psi**. Connect tubing at the outlet to collect the fluid exiting the device. Balance the two flows, adjusting the flow width of the blue dye to approximately 1/2 of the channel width, and **record** the pressures. Next, **image** the dye distributions at the inlet junction, outlet, and along the channel (numbers indicating downstream distance in centimeters are marked along the channel on the device). Also **image** the first 4-5 turns and any other locations that you find interesting (you may want to note down which image corresponds to which location). Next, collect the outlet flow in the provided small petri dishes or vials for a fixed time interval (say 1 min or 5 min), and weigh the collected fluid (you will have to subtract the dry weight of the petri dish or vial). **Record** the collection time and weights. Assuming a density of 1000 kg/m^3, the volumetric flow rate can be obtained. **Repeat the experiment with inlet pressures around 20 psi.**

See the appendix at the end of this lab manual for detailed dimensions of the devices.

Lab #3 write up questions:

Show your work and the equations used in answering the questions below.

Diffusion in straight channel:
1. (*1 point*) What is the flow velocity (mean±std. dev.) measured at 5 psi inlet pressure? What are the estimated velocities at the two other driving pressures (1 & 15 psi)? Calculate the Reynolds numbers based on hydraulic diameter or channel height for the three inlet pressures. Present your results as a table listing actual pressures at the two inlets, the flow velocities, and Reynolds numbers.
2. (*1 point*) Present images at the junction of the dye and water streams and two downstream positions for the lowest and highest inlet pressures. Include scale bars. How does the 'width' of the interface vary with the velocity of the flow and with the distance along the channel?

Analysis of diffusion:
3. (*1 point*) Use ImageJ (available free at http://rsbweb.nih.gov/ij/) or any image analysis software of your preference to plot the intensity *vs*. distance across the channel width at the junction and at each channel marker position, for each of the three pressures. To do this select the line draw button, and then draw a line at the desired location, then Analyze→Plot Profile. Avoid crossing the channel walls or bright/dark debris in your line profiles. Then click on "Copy" to obtain the intensity numbers to export into a spreadsheet. You may use a single line profile, or average over multiple line profiles or areas to decrease the noise in intensity. You may also use the blur function to smooth the image before analysis (Process→Filters→Gaussian Blur). Use the pixel conversion chart to convert pixels to distance.
4. (*2 points*) If the diffuse interface does not approach the channel walls, and if the channel height << channel width, the solution to the diffusion equation for our experiment is very similar to that of transient heat conduction between two semi-infinite slabs where the time and distance coordinates map

on to each other given by time t (in heat transfer) = downstream distance l ÷ flow velocity U (in diffusion experiment). The solution for our diffusion problem follows as:

$$\frac{c - c_0/2}{c_0/2} = erf\left(\frac{w}{\sqrt{4Dt}}\right) = erf\left(\frac{w}{\sqrt{4Dl/U}}\right)$$

Here, c is dye concentration on the water side and $c_0/2$ is the dye concentration at the interface, which is half the input dye concentration c_0. D is the diffusivity, and w is the distance from the interface in the channel width direction. The error function is plotted below.
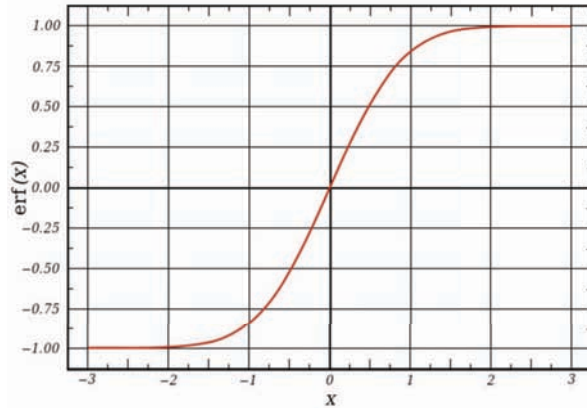
$$erf(x) = \frac{2}{\sqrt{\pi}} \int_0^x \exp(-t^2)dt$$

$$\frac{d}{dx}erf(x)_{x=0} = \frac{2}{\sqrt{\pi}}$$

Since we are looking at transmitted light, it turns out that the change in light intensity is approximately equal to the concentration of the dye, at least for solutions that transmit much of the light. Further, note that the function $erf(x)$ goes from -0.5 to +0.5 when x goes from -0.477 to +0.477, *i.e.* 50% of the change in intensity in the vicinity of the interface occurs when x changes by 0.954. So, if we measure the length w_{50} over which 50% of the intensity change occurs, we have:

$$\frac{w_{50}}{\sqrt{4Dl/U}} = 0.954,$$ from which we can obtain the diffusivity D.

Pick 3-4 good intensity profiles that you have (preferably for different downstream distances l and for different flow velocities, where the profile is not too sharp) and extract the diffusivity for each curve. What is the diffusivity of the dye (mean ± std. dev.)?

Mixing device:
5. (*1 point*) What are the actual pressures, estimated flow velocities in the channel, and the corresponding Reynolds numbers for the two cases (1 psi and 20 psi)? Present your results as a table. Does the flow rate scale linearly with the driving pressure?
6. (*1 point*) Present images of the dye distribution across the channel at 3 different locations in the two cases (1 psi and 20 psi). Include scale bars.

Analysis of mixing:
7. (*2 points*) Open each image in ImageJ and draw an area box to select a region within the channel that represents the dye distribution (avoid glare/channel edges). Go to Analyze→Histogram to obtain the standard deviation and mean intensity in the selected area. Plot the normalized standard deviation (*i.e.* std. dev. divided by the mean intensity) as a function of downstream distance in the device for the two cases (make sure that you include the inlet and first few turns as well as the marked locations). The normalized standard deviation is a metric for the degree of mixing: when mixing is complete, we expect the standard deviation to remain constant down the channel.
8. (*1 point*) Estimate the mixing time in the 20 psi case. You may assume that mixing is complete when the plot in question (7) approaches the mixed value within 10% of the difference between inlet and mixed value. E.g. if the normalized std. deviation goes from 0.3 to 0.1, then assume that mixing is complete when the value is 0.1 + 0.1x(0.3-0.1) = 0.12.

Lab #4 – Droplet Microfluidics

I. Laboratory Objectives

This week, we will utilize the mixing devices and PDMS droplet generating devices you made last week as tools (a) to emulsify water in light mineral oil, creating monodisperse droplets, and (b) to investigate the mixing behavior of molecules (food dye) and particles (~2 µm polystyrene beads) in two-phase droplet flows.

II. Introduction

Controlling droplet size in emulsions is an important part of the manufacturing process for a wide variety of items, including food products[1], cosmetics[2], and topical drugs[3]. Droplet monodispersity is critical for the long-term stability of emulsions, which coarsen over time through mechanisms like Oswald ripening, creaming, and flocculation[4-6]. Reported techniques for producing monodisperse emulsions include membrane emulsification[7,8], in which the discontinuous phase is forced through a microporous membrane to form droplets in an insoluble bulk phase, shear rupturing by Couette flow[9,10], crossflow in polymer-based microfluidic devices[11-13] and extrusion through silicon-based microarrays[14,15].

Microfluidic devices, consisting of micron-scale channels molded into soft polymers, are ideal tools to study the emulsification process of complex fluids. Microemulsification has the potential to generate droplets for a wide range of applications, from drug delivery to chemical engineering to screening of biomolecules. The small internal volume of the droplets, tunable from attoliters (10^{-18} L) to picoliters (10^{-12} L), also creates an ideal platform for self-assembly of ordered nanostructures. The nature of flows within droplets also makes them an excellent platform for rapidly mixing reactants and probing reaction kinetics of chemical or biological molecules. In these droplet-based devices, pressurized immiscible fluids are introduced through microconduits, utilizing viscous shear to emulsify the discontinuous phase and generate monodisperse droplets (variation < 5%) (Figure 4). By modifying the relative pressures of the two fluids and the channel dimension, the size of the

formed droplets can range from sub-micron to hundreds of microns in diameter, with potential applications in areas ranging from drug delivery to chemical engineering and synthetic lubricant formulation[16-19].

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Please see Figure 1 in http://authors.library.caltech.edu/6871/1/THOprl01.pdf
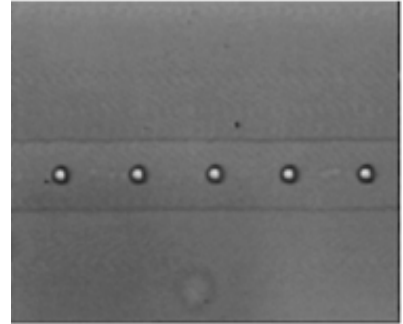
Figure 4: Microfabricated channel dimensions at the point of crossflow and optical micrograph of the disperse water phase introduced into the continuous oil phase containing oil-soluble surfactant (hexadecane with 2% Span 80). Dashed rectangle indicates area in center micrograph. Droplets become spherical as they transition from the high velocity shear region to the wider 60 μm outlet channel (right). From *Thorsen et al. Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device, PRL (2001)*.

Flow instability is generally not present in microfluidic devices because the length scales are small enough that inertial effects in the fluid can be neglected. As most microfluidic devices operate at low Reynolds number, the governing Navier-Stokes equation for fluid flow becomes linear and the flow is laminar. However, in presence of surfactant, a system consisting of two pressurized immiscible fluids becomes unstable, causing droplet formation. Under conditions where the water pressure is lower than the oil pressure, monodisperse separated water droplets are formed. As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream. At water pressures that exceed the oil pressure, complex, organized patterns begin to emerge in the stream, ranging from helical-like structures to coherent ribbon motifs (Figure 5).

Figure 5: Droplet patterns in rounded channels at different water and oil/surfactant pressures (psi - noted in figure) and the corresponding phase diagram depicting the relationship between the oil and water pressure differences and droplet morphology. Solid lines are used to define approximate boundaries between the following droplet states (top to bottom): solid water stream, ribbon layer, pearl necklace, single droplets, and solid oil stream. Optical micrographs show 60 μm channel regions downstream of the point of crossflow. *From Thorsen et al. Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device, PRL (2001).*

Microfluidic devices function as excellent controlled environments to study the physics of droplet formation, reagent compartmentalization, and mixing at small length scales where flow is laminar and surface tension effects are dominant. An array of flow conditions can be tested as a function of the relative pressures and viscosities of the two immiscible solutions, the nature and concentration of the surfactant, and the microchannel dimensions and geometry.

III. Experimental

We will look at droplet formation in the droplet generation devices using Phosphate-buffered saline (PBS) as our dispersed phase, and mineral oil mixed with a non-ionic detergent, Span 80 (1% vol/vol), as our bulk oil phase. Our devices have two water inlets (Figure 6). We can just block one inlet to make a single-inlet device, or we can flow fluids through both inlets to mix two different solutions.
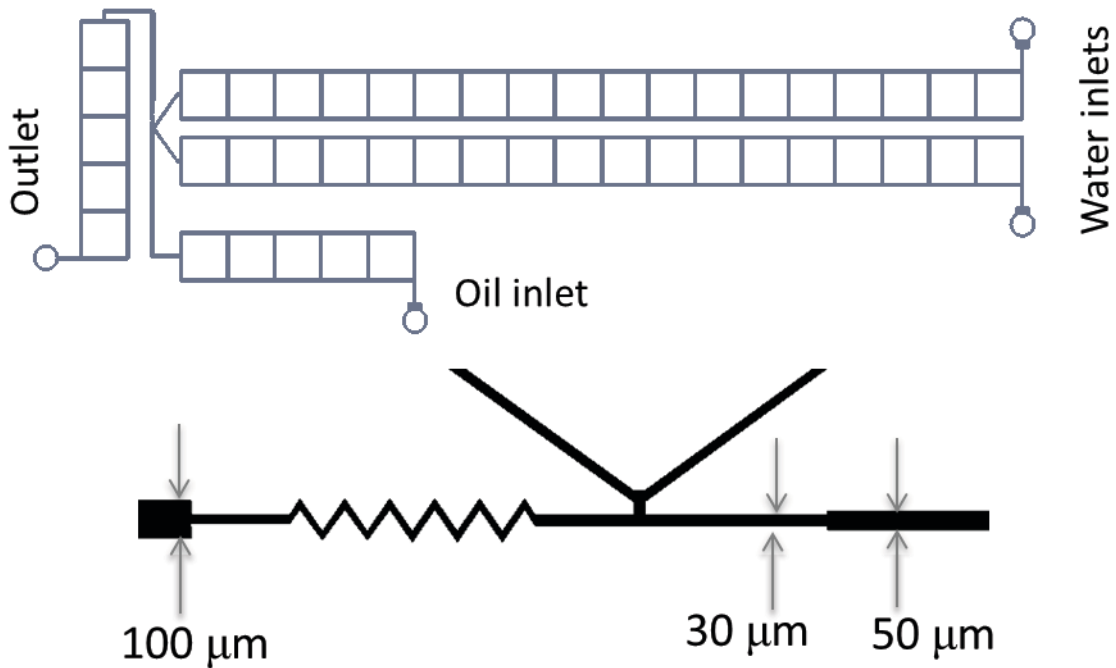
Figure 6: Device for generation of droplets. Magnified view of the junction is shown at the bottom. Detailed dimensions are provided in the appendix to this manual.

The oil/surfactant syringe/Tygon tubing assembly has been pre-filled and should be on your lab bench. Take the following items to the inverted microscope:

- Syringe/Tygon tubing pre-loaded with oil surfactant

- Two empty syringe/Tygon tubing assemblies

- 1.5 mL tube of PBS (Phosphate-buffered saline)

- 1.5 mL tube of PBS mixed with 1 μm yellow fluorescent beads

- Microfluidic devices

Device assembly

You should have already assembled the droplet devices in the previous lab. If not, ask the instructors.

Getting droplet formation

Fill one of the empty syringe assemblies with oil as follows, while wearing protective gloves. **Make sure you introduce oil into the device before introducing any aqueous solution**. First, remove the plunger from the syringe, and introduce the oil into the syringe while taking care not to trap air. Next, make sure that the tube is completely filled with oil; this can be done by making use of gravity. Next, insert the steel stub into the device, and then connect the oil syringe to the pressure regulator interconnects next to the microscope, making sure that the pressure regulators are turned completely clockwise (zero flow). Now turn up the pressure in the oil syringe to 0.5 – 1 psi. **Connect the pressure sources to the syringes only after the corresponding tube is inserted into the device**. Next, block one of the water inlets with closed-ended tubing provided by your instructors. Fill another syringe with the yellow bead solution in the same manner. **You will need to be extra careful about the relative heights of the solution in the syringe and tubing; gravity can quickly empty the solutions if you are not careful.** Connect the yellow beads syringe to the water inlet of the device, and the oil/surfactant syringe to the oil inlet of the device. Now, connect the syringes to the pressure inlets and put the device on the microscope stage and focus on the water/oil junction under low bright field magnification (10x). Due to the viscosity differences between the oil/ water solutions, device filling takes some finesse to get to remove air bubbles from the microchannels and balance the oil/ water streams. We are looking to "balance" the oil and water streams, bringing the water stream up to the junction, but keeping the pressure just low enough to keep the water from flowing into the oil/surfactant stream going to the outlet. To prime, adjust the pressure regulator for the oil syringe to ~8 psi and the buffer regulator to ~1 psi. Watch the creep of the oil toward the junction as it enters the device. If it backflows into the water channels (upstream of the junction), increase the driving pressures of the aqueous solutions until the buffer flows into the device. It may take a few minutes to see the water solutions enter the device, due to air in the tubing, so have a little patience. With assistance from the instructor, continually adjust the oil/ aqueous solution pressures until you get droplets at the oil/water junction as the aqueous solutions (disperse phase) break off into the oil stream (continuous phase).

In the first part of this experiment, we are just going to look at droplet formation and ignore the droplet content. Once the oil/water streams are balanced, *slowly* lower the oil pressure (by a few tenths of a psi), so that

the buffer just enters the microchannel and droplets are generated with a **low frequency (~1 Hz or lower)**. Make three sets of observations as follows for oil pressures of about 6, 10 and 15 psi: Under the conditions where droplets are formed, **record** the oil/water pressures and **sketch/image** the evolution of the water-oil interface geometry at the junction as the droplets are formed. **Record** a video of droplet formation for each condition of droplet formation. **Record** data to estimate the droplet flow velocity (ask your instructors).

Now let us look at flow *inside* the droplets. Use a slow driving pressure (~6 psi or lower) for best visualization. Ask the instructors to set up the fluorescence imaging filters to observe trajectories of the beads in the droplets. Observe how the beads move within the droplets. **Record** images of the bead trajectories at the droplet formation junction. R**ecord** images (and video, if possible) of bead trajectories in the wider channel downstream of the wiggly channel.

*Rapid mixing of two solutions in droplets*

Now unplug the unused water intet and introduce the bead solution in one inlet and blue dye in the other water inlet. Perform the experiment at about 15-20 psi oil inlet pressure, and try to balance the bead/dye streams. Once the streams are balanced, observe flow patterns and mixing in the droplets at the junction and in the wiggly channel. **Record** images of the dye and beads in the droplets at the junction and in the wiggly channel. **Record** data to estimate the droplet velocity (ask your instructors).

Detailed device dimensions are provided in the appendix at the end of this module.

Lab #4 write-up questions:
Show your work including equations and values of parameters used.

Droplet formation:
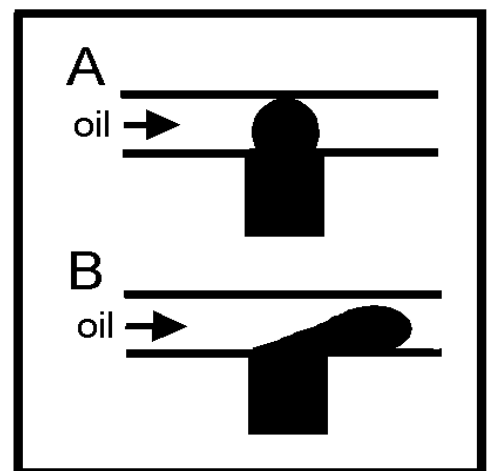
1. (*2 points*) The formation of droplets arises due to interplay between surface tension and shear stress. Just as Reynolds number compares inertial forces to viscous forces, a non-dimensional Capillary number $Ca = \mu U/\gamma$ compares the viscous forces with interfacial tension forces. Using viscosity of the oil-surfactant mixture as $\mu \sim 1.5 \times 10^{-2}$ Pa.s and the interfacial tension between water and oil/Span 80 mixture $\gamma \sim 2 \times 10^{-2}$ N/m, estimate the range of Capillary numbers in your experiments at the three different pressures. You may assume that the measured droplet velocities are a good approximation for flow velocity U. Present your results in a table.

2. (*2 points*) Plot the estimated droplet diameter (approximate by comparing droplet size to the channel width) vs. oil input pressure. Is there a trend in your plot? Generate a hypothesis explaining why you would expect this behavior. The reference papers may be useful to help you answer the question. *Hint:* Think about the Capillary number.

Flow inside droplets:
3. (*2 points*) The droplets are not purely translated by the oil down the microchannels. They are separated from the microchannel walls by a thin layer of oil, and experience some friction with the walls. For the wider downstream channel, sketch/show images of the trajectories of the beads in the droplets, in a frame of reference fixed with respect to the translating droplet (top view). Given the dimensions of the wider channel, what is the diameter and what is the height (measured perpendicular to line of sight) of the droplet? Explain the motion of the beads in terms of the relative velocities of the droplet and oil that may be expected for this flow.

4. (*2 points*) Sketch or show image of beads depicting their motion in the aqueous phase at the junction as a droplet is being formed. Explain the motion of the beads in terms of the relative velocities of the aqueous solution and oil that may be expected in this situation.

Mixing of two streams:
5. (*2 points*) Estimate the time taken to mix the two solutions in the mixing experiment.

References for Further Reading

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Lab #5 – Fields, Forces, and Flows

I. Laboratory Objectives

In Lab 5, we will use our microfluidic devices to study electrokinetics. In particular, we would like to investigate:

(a) How microspheres (beads) move in response to electric fields in microfluidic channels.

(b) How we can separate dyes and analyze samples by electrophoresis.

II. Background and History: Microfluidic Electrophoresis and Electroosmosis

Electrophoresis and electroosmosis are electrokinetic phenomena. "Electrokinetics" implies motion induced by applied electric field. Electrophoresis refers to migration of a charged molecule or particle in a solution under the influence of an electric field. On the other hand, the solution or fluid itself can be made to move with respect to a fixed charged surface (e.g. channel wall), which is called electroosmosis.

The development of some of the earliest bio-inspired microfluidic devices in the last decade originated as an extension of MEMS-based technology for analytical chemistry applications. The miniaturized 'total chemical analysis system' (µTAS) proposed by Manz and Widmer in 1990[1], incorporating automated flow injection and electrophoresis provided the foundation for the chip-based analysis of chemicals and biomolecules. Using photolithography and established chemical etching techniques, the first microfluidic devices consisting of a network of micron-scale channels etched in planar glass substrates, created an enabling platform for the manipulation and characterization of small biomolecules at a fraction of the cost of conventional automated systems.

Some of the earliest microfluidic devices developed in the 1990s used free-flow (i.e. molecules moving freely in solution as opposed to within a gel) capillary electrophoresis (CE) devices to separate small molecules like fluorescent dyes and amino acids[2]. Glass was routinely used as a substrate for these chips for several reasons. The excellent power dissipation characteristics of planar glass microfluidic devices allowed separations to be carried out using higher electrical fields while minimizing Joule heating, resulting in faster separation times than those achieved using air-cooled cylindrical capillaries. The surface chemistry at the glass/ buffer

surface in the microchannels is also optimal for CE. Silanol groups (Si-OH) present on the glass surface interact with free hydroxyl ions in the water to give the glass surface a net negative charge, creating an electric double layer with the surrounding hydrogen ions whose migration induces electroosmotic flow (EOF) in the presence of an applied filed. The combination of EOF with electrophoretic separation of the samples in the microchannels gave researchers unprecedented control over the sample injection process, minimizing both loading times (ms) and volumes (pL) while virtually eliminating dead volume. In addition to glass, other materials have been used to fabricate free-flow microfluidic CE devices, including hard plastics and elastomers[3]. While plastics and elastomers often require surface chemistry modifications for EOF, their rapid prototyping characteristics, cost, and sealing characteristics have increased their popularity as substrates for microfluidic electrokinetic devices.

The use of electric fields to both induce flow and separate molecules are popular laboratory techniques that have been widely adapted to microscale devices for the separation of nucleic acids and proteins[4-12], e.g. for DNA sequencing, analysis of chemicals and microbes in water, and identification of proteins. An extensive body of work has been done to study intracellular contents of single cells by electrophoresis inside nanofluidic channels[13-20]. The majority of applications have focused on the detection of small molecules and metabolites[14,16-18], as well as DNA content[15]. Electrophoretic detection of specific protein expression has been accomplished in nanofluidic devices in immunoassay-based formats[21] and has been integrated into microfluidic devices for rapid detection of multiple proteins in small volumes in an immunoassay format[22].

III. Experimental

Measuring the electrokinetic motion of microspheres:

In the first experiment, we are going to look at the motion of microspheres under the influence of electric fields in microchannels. We will use the short channel devices for this experiment. The devices have a ~30 μm channel height to minimize the effect of surface tension-driven flows. We will look at the flow of **one** of the following beads with micron-scale diameters with a) carboxylate (-COOH), b) sulfate (-SO$_4$), or c) amine (-NH$_3$) surface groups suspended in water. Electrokinetic motion is affected by the diffuse layer and surface

19

charge, which may change with type of surface and buffer conditions. Therefore, we will also investigate the effect of a surface coating on electrokinetic flow of the beads. *Ask your lab instructors about diameters and concentration of the beads and the salt/buffer used and* **record** *it in your lab notebook.*

On your bench, you should have 1.5 mL tubes of different bead solutions. You should also have an aqueous solution of 2% Polyvinylpyrrolidone (PVP) with 1.3 million molecular weight. PVP adsorbs to the channel surfaces and minimizes any electroosmotic flow[23].

- We will complete plasma bonding and baking of the devices as in previous labs.

- We will coat the microchannels with PVP.

- Then, we will introduce the beads (select <u>one</u> of your liking).

- We will then study the effect of voltage on bead motion.

- Finally, we will compare the motion of beads in a channel that has not been treated with PVP.

Device assembly:

Bond the electrokinetics devices using the same procedure as in Lab 3. If you have already cleaned the devices, you do not need to clean them again. Ask your instructors about the 10 min baking step.

Surface treatment of the microchannels and filling with beads:

- Using the provided syringe with Luer needles, load the punched holes of the device with the PVP solution. Check whether the solution has wicked into the channel. Be careful not to overflow the solution into adjacent channels!

- If the channel is hydrophobic and the solution does not flow in, you can attach a pipette tip to the syringe and force the solution in. Ask your instructors if you need to do this.

- Wait for 5 min, then use Kimwipes to absorb the excess PVP solution.

- Take an empty "waste" syringe with luer stub, and remove any remaining liquid from the punched holes.

- Add buffer into the holes using another clean syringe. **Label the syringes to avoid confusion!**

- Wipe off and remove the buffer from the holes using the "waste" syringe.

- Repeat the process a couple of times to remove any free PVP as this may coat the bead surfaces and interfere with the experiment.

- Then, introduce the bead solution using another syringe.

- After priming the microdevices with bead solutions, we are ready to apply voltage.

Applying voltage across the channels:

- Place the device on the inverted microscope stage, tape the device down on the stage, and inspect using white light and a 20x objective to make sure the microchannel is in focus. ***Be very careful when handling the leads for the high voltage source. The power should be off and the positive lead disconnected from the source for all setup procedures.***

- Attach a small piece of the wire to the end of the positive (red) and negative (black) leads. Insert one wire into each of the two reservoirs of the channel.

- Secure the leads to the microscope stage using scotch tape to prevent movement of the leads during the experiment. **Use a glass slide to ensure that the leads do not contact the microscope stage, which is conductive!**

- After inserting the electrodes in a microdevice (**record** which solution you are using for the experiment), connect the positive lead to the power source, making sure the voltage is dialed down to its minimum setting. Turn the power source on.

- You should see the beads moving due to any pressure-driven flow due to the two menisci in the punched holes and random Brownian motion if the flow is slow. If the water level is below the top of the punched holes (i.e. no droplet on top), the flow will settle down to a slow flow rate after a few minutes as the pressure at the two ends balances out (*can you think of any reason for why it takes much longer if there are droplets on the top of the two holes?*).

- Slowly turn up the voltage until you can see directional flow. This should occur on the order of a hundred volts or less. If you have turned up the supply to a few hundred volts and still see no flow, something is wrong, and you should turn the voltage down to its minimum setting, power off, disconnect the positive lead, and troubleshoot.

- **Measure the velocity for 4-5 different applied voltages starting with 0 V (reference) and then up to ~500 V.** When you are not acquiring images, **switch off the voltage.** Applying voltage for longer periods will almost certainly lead to formation of bubbles. To quantify the bead velocity, set a fixed (long) exposure time and measure the length of the resulting streaks. **Note whether the beads move towards the positive or negative electrode.**

- After you finish your experiment, use the calipers to **measure** the distance between the punched holes for calculating the electric field in the channel.

Observing pressure-driven flow:

After you finish measuring bead velocities under applied electric field, remove the electrodes and create a pressure gradient by adding a little fluid in one of the inlets. **Record** streak images of the beads.

Repeat the experiment in a channel without the PVP coating:

In this case, record a video or streak images for only a couple of voltages. You **only need to quantify the velocities for one of the applied voltages**.

__Separation of molecules using electrophoresis:__

In this experiment, we will implement electrophoresis to actually separate two chemicals (food color dyes) by electrophoresis. Since fluid flow by electroosmosis or undesired pressure gradients can easily disrupt separation, gels are often used to suppress fluid flow. The gels form a porous structure that allows molecules to pass through, but impedes fluid flow. The molecule-gel interactions can also enhance separation. Here we will

use an agarose gel to inhibit convection. **For this experiment, we will use the staggered cross channel device bonded on glass slides.** The stagger enables more dye to be introduced into the long channel for separation.

Assembly of the injection syringe:

Take one 1 mL syringe and attach a 200 μL pipette tip (the top part needs to be cut with a razor blade for this purpose), making sure that it fits tightly to the syringe. Now, cut a little piece off the tip of the pipette tip to make it fit into one of the punched holes (This will ensure that the syringe will drive the gel through the device by applying pressure).

Agarose gel preparation and injection procedure:

To prepare 5 mL agarose gel, we need:

- 0.05 g of Agarose

- 5 mL of 0.5x TBE (Tris-buffered EDTA buffer)

Agarose gel is already prepared for you. However, we outline the procedure here for your information. First, measure 0.05 g of agarose using the balance. Next, measure 5 mL of 0.5x TBE using a 5 mL syringe. Then, warm up the TBE using a microwave or a hot plate in the hood till it is close to boiling. Now that the TBE is hot, mix the TBE and the agarose and shake thoroughly. Since agarose takes some time to dissolve, the gel is prepared in advance for you.

While the gel is still hot, load the syringe making sure that no air bubbles are trapped. Insert the syringe with pipette tip into the punched hole at the far end of the cross and gently apply pressure to the gel and drive it till it comes out of the other three ports. Once the solution cools, agarose will gel in place inside the microfluidic channels.

Loading the dyes into the separation device:

You should have the following solutions:

- 0.5x TBE

- Blue V calcium salt and amaranth in 0.5x TBE

After loading the gel, fill the dye mixture into another syringe with luer stub attached. Using the syringe, load **one** of the inlet ports that lead to the **short** channel with the dye mix (Blue and Red mix). (The pattern is a cross, made of a long channel and a perpendicular short channel). Fill the remaining three ports with 0.5x TBE. Be sure to inject the solutions at the **bottom** of the punched wells and not on top: **It is very important not to trap any air bubbles in the device, and equally important to make sure that the solution is close to the microchannel entrance at the bottom of the hole!**

Separating the dyes:

Always wear protective equipment (GLOVES) and verify grounding of the equipment!

ASK IF YOU DON'T KNOW. Better a busy instructor than a electrically shocked student!!

We will use an electrokinetic method of cross-injection to load a plug of the dye mixture into the long separation channel:

- Make sure the power supply is off. Connect the negative end of the power supply to the provided electrode wire, and that wire to the well into which the dye mix was added.

- Connect the positive end to a steel wire, and the wire to the opposite outlet to the dye mix.

- Connect the power supply and apply ~100 V to drive the dye mix till it crosses the long channel and flows into the opposite punched hole.

- If you don't see any movement turn off everything, unplug the power supply, reconnect and turn it on again.

It is important to move on quickly to the next step as the dyes will start diffusing in the channels!

After the dye has been loaded into the device, unplug the electrodes and apply 500 V voltage between the extremes of the long channel. **Ground is applied to the nearest inlet to the short filled channel, and positive**

voltage is applied to the farthest inlet. You should see the dyes flowing along the channel and separating. After the dyes separate, switch off the voltage. Separation is sometimes easy to miss, so discuss with your TA/instructor before you begin this step. **Record** the time required for each dye to move along the length of the separation channel, and the distance traveled by each dye. **Record** an image of the separated dyes with a camera, and also **record** the approximate size of the dye bands. You can repeat the dye injection and separation steps in the same device.

Exploration:

Electrophoresis, and separation methods in general, are used for analysis. On your bench, you have "**mystery dye solutions**" to be analyzed. The problem is to identify how many components and of what color are present in the solution. Follow the procedure above, but load the mystery solution instead of the calcium blue and amaranth. Can you correctly determine the contents?

In reality, the speed at which different compounds move is calibrated beforehand. Knowing the time it takes for a particular mystery component to run though the channel, it can be identified using the calibration.

Lab #5 write-up questions:

Electrokinetic motion of beads (PVP coated channel):
1. (*1 point*) Present 2-3 images of the streaks that you obtained at different voltages, stating the type of bead and direction of motion (towards + or -). Include scale bars.
2. (*2 points*) Plot the velocity of the beads as a function of electric field in the channel. Plot each point as mean±std. dev. by measuring at least three representative bead velocities. Fit a line to your plot and estimate the electrophoretic mobility of the beads (= velocity/electric field) in SI units.

Comparison of electrophoretic and pressure-driven flows:
3. (*2 points*) For <u>one</u> of the applied voltages and the pressure-driven flow experiment without any applied voltage, for each case, measure and list the velocities of at least 10 beads (or as many as you can) in order of increasing velocity. Now normalize the velocities by the mean velocity, and normalize the bead rank by the total number of beads. E.g. Suppose there are 12 beads with a mean velocity of 80 μm/s. If bead number 5 has a velocity of 72 μm/s, its normalized velocity is 72/80 = 0.9 and its normalized rank is 5/12 = 0.417. Plot the normalized velocity vs. normalized bead rank for the two cases on the same plot. What conclusion can you draw from this plot?

Effect of PVP coating:

4. (*2 points*) What qualitative differences do you observe between the motion of beads with and without PVP? Prepare the same plot as in Question (3) for one of the applied voltages and compare. Form a hypothesis to explain the results.

Dye separation:
5. (*1 point*) Present an image of the device with dyes that you separated. From the final positions of the dyes when the voltage was turned off, and the distance traveled, estimate the mobilities of the two dyes in SI units. Compare your results with literature (see paper[24]). If you observe discrepancies, can you think of reasons why the results don't agree with the literature?
6. (*1 point*) You will have noticed that the dyes form broadening bands as they separate. This broadening effect limits the separation resolution. Given this "width" and the channel length, estimate the resolution of separation defined as the "width" divided by the distance traveled by the dye. The inverse of the resolution is an indicator of the maximum limit of the number of dyes that the device can potentially separate. E.g. if the band is 2 cm in size has traveled 80 cm, the resolution is 2/80 = 1/40, and a maximum of ~40 different dyes may be separated out. For this calculation, use data for the dye that travels farthest in the channel.
7. (*1 point*) Diffusion is one of the many factors that can cause this "band broadening". We can estimate the length of the band as $\sim(Dt)^{1/2}$, where D is dye diffusivity and t is separation time. The length traveled by the dye during separation is related to separation time t as $l = \mu Et$, where μ is the dye mobility and E is the electric field. If we wanted to improve the resolution by 4x in a case where the "band broadening" is dominated by diffusion, how much longer does the channel need (in terms of original length) to be for the same μ, E, and D?
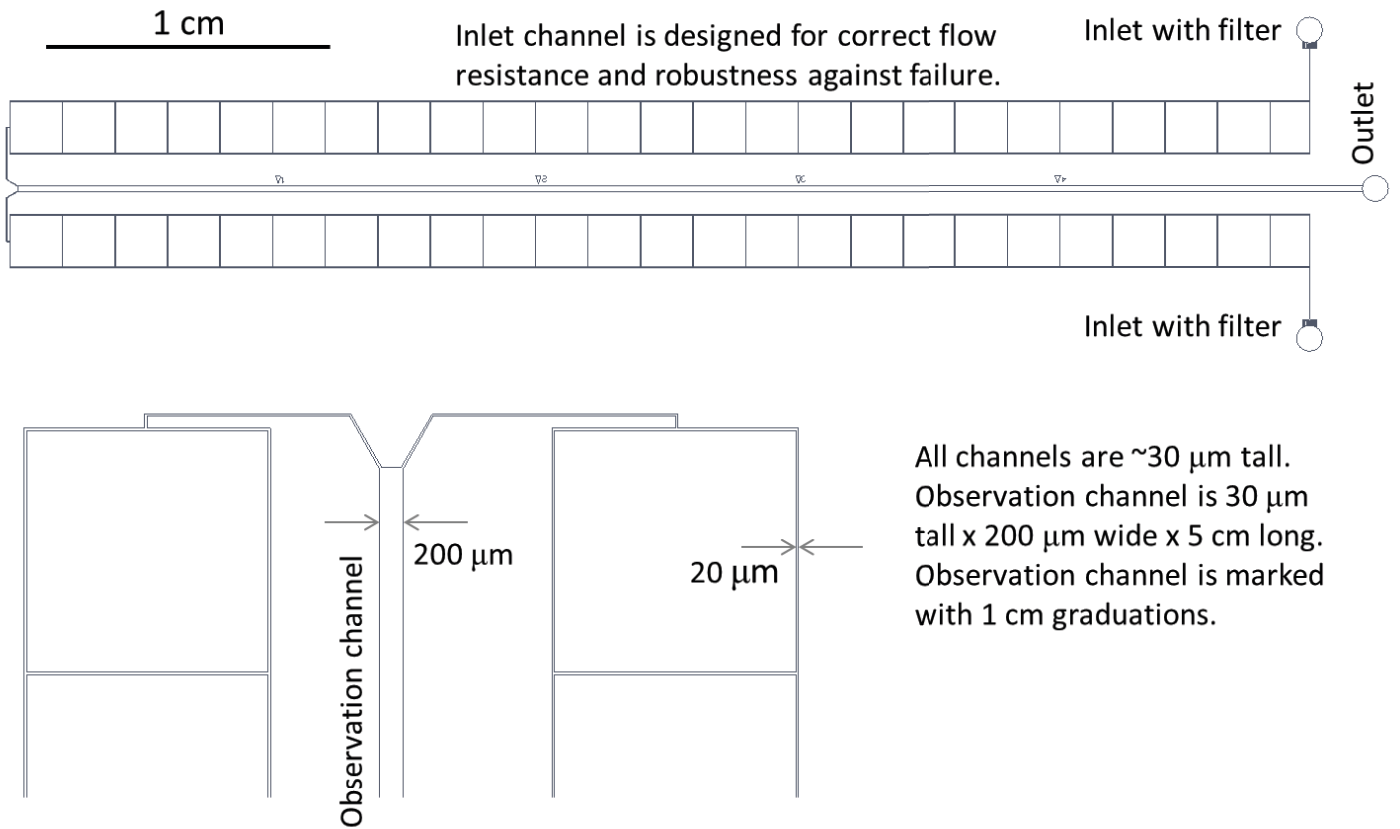
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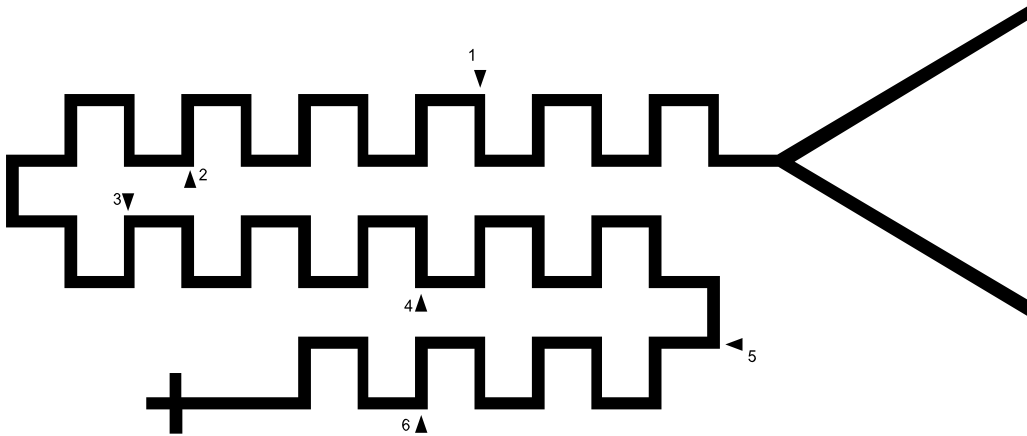[13] McClain, M.A. et al., Microfluidic devices for the high-throughput chemical analysis of cells, Anal. Chem., 75, 5646, 2003.

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APPENDIX: Device Dimensions

Diffusion Device:

1 cm

Inlet channel is designed for correct flow resistance and robustness against failure.

Inlet with filter

Outlet

Inlet with filter

Observation channel

200 μm

20 μm

All channels are ~30 μm tall. Observation channel is 30 μm tall x 200 μm wide x 5 cm long. Observation channel is marked with 1 cm graduations.

Mixing Device:

*Actual channel height may vary depending on the photolithographic process. While 200 μm is the designed height, actual height in the current devices is ~170 μm.

Droplet Device:
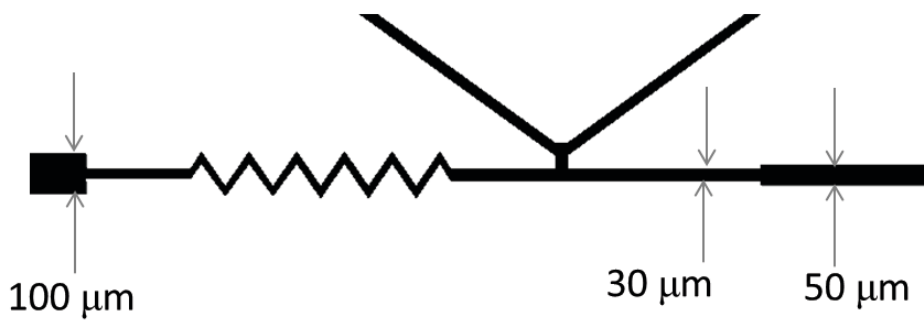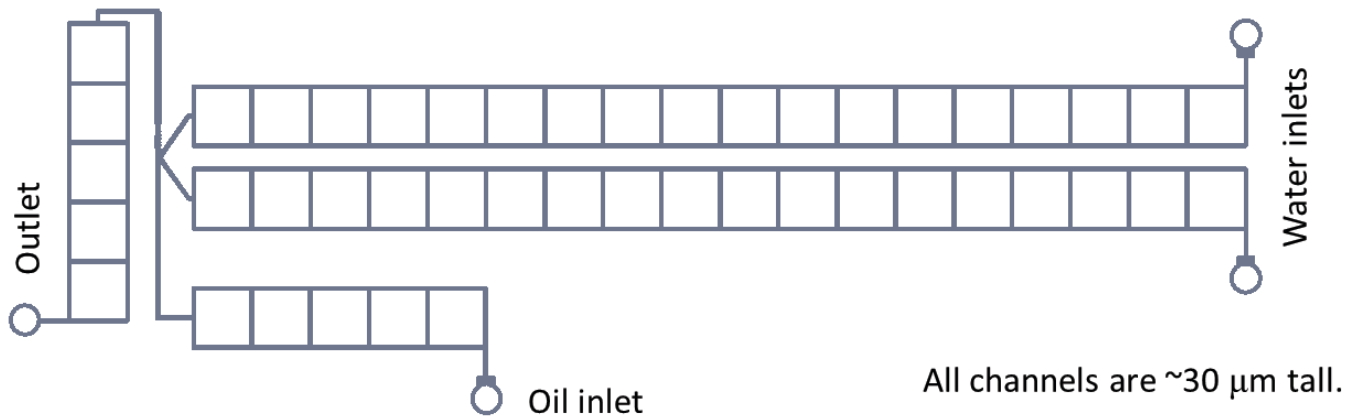
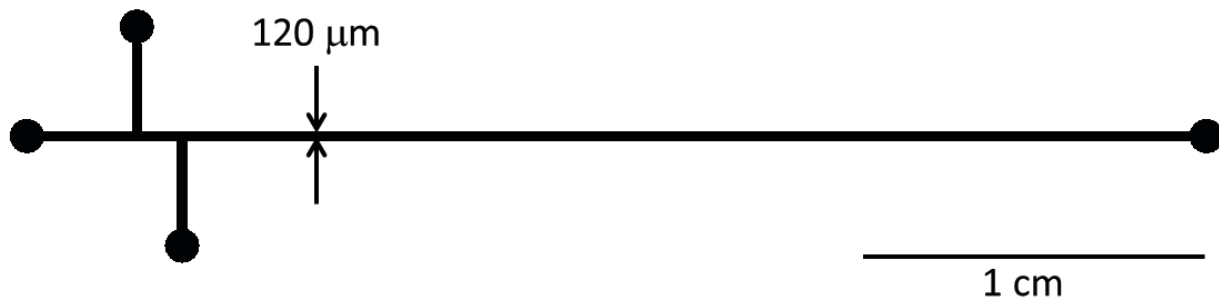**Bead electrophoresis device:**

20 μm
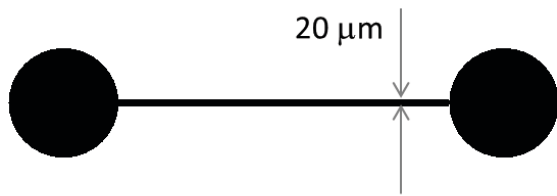
Electrokinetics channel is ~30 μm tall, 20 μm wide, and 3 mm long.

Dye separation device:

120 μm

1 cm

200 μm tall channels (Actual height ~170 μm)

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