Experimental Biophysics, Spring 2021

Lab 3:
Fluidics & Soft Lithography

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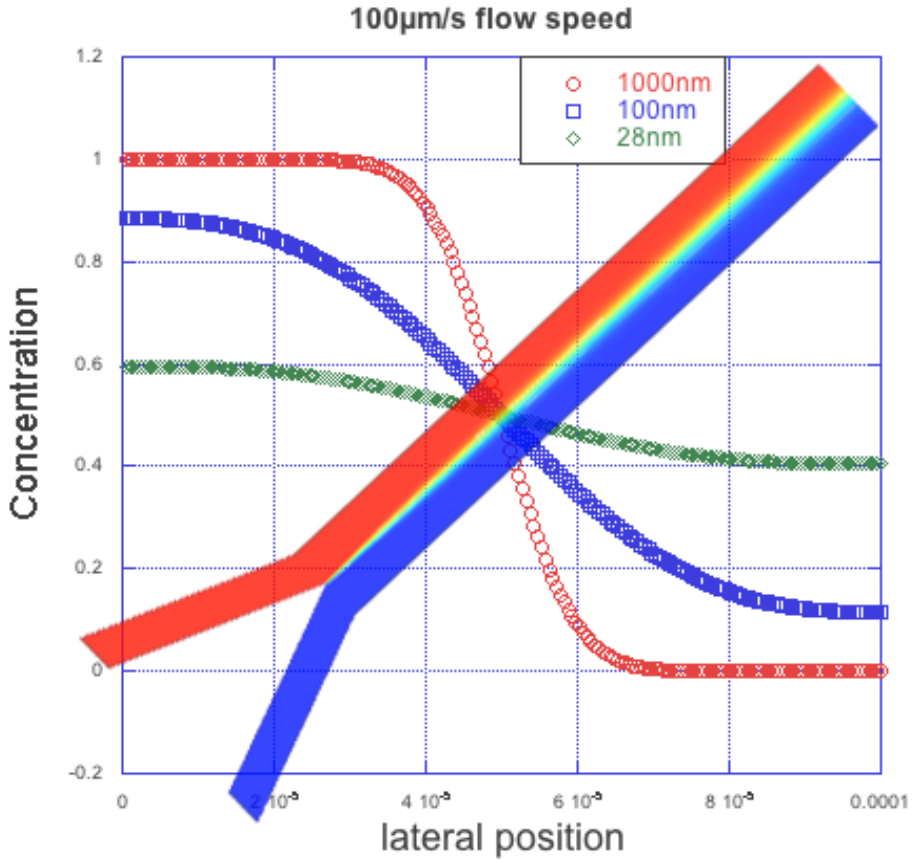


1 Introduction

Due to the COVID-situation, you will not be able to fabricate and run the devices yourselves. Instead, you will be guided through the process virtually.

The separation of particles by their differing properties, electrophoretic and dielectrophoretic mobilities, chemical affinity and size are among the most commonly performed analytical and preparative processes. Some of the methods that have gained widespread use are FACS, chromatography and mass spectrometry for the separation and analysis of cells, biomolecules and ions respectively.

In this lab we will look at a method of particle separation that was made possible by the advent of microfluidics. The H-filter separates particles based on their diffusion coefficients. We will also use COMSOL to build a model of particle separation/mixing that closely resembles the H-filter set-up. If time allows we will use this model to look at particles of different sizes and determine the mixing and separation of the particles within a single model.

2 Required Lab Preparation

2.1 Reading

In preparation for the lab the following three articles should be read. We will be fabricating our own H-filters for the lab in PDMS. The review by Whitesides (1) is an excellent introduction to PDMS and the concept of soft lithography and should be looked through with close attention being paid to the introduction and second section entitled 'the key elements of soft lithography'. The original H-filter article from 1997 by Brody and Yager (2) describes the device and gives a good introduction to the lab. The pdf provided in the COMSOL documentation (3) is a good introduction into the capabilities of this tool for microfluidics modeling and contains an example, which we will follow, to a certain extent, for generating our model:

1) Xia Y N & Whitesides G M (1998) Soft lithography *Annual Review of Materials Science* 28**:**153-184.
2) Brody J P & Yager P (1997) Diffusion-based extraction in a microfabricated device *Sensors and Actuators A* 58:13-18
3) COMSOL guide: ***Introduction to Microfluidics Module***. After downloading COMSOL you can find this pdf in the doc folder -> pdf folder -> Microfluidic Models folder. (Read only up to pg. 18 for a brief introduction).

2.2 Simulation

In addition to the articles, download and install COMSOL 5.5 (see below) and get as far as you can in making a model based on the experimental lab on the H-filter. You can work on your own or use the tutorial from COMSOL (link here). We will discuss the process together at the lab.

COMSOL 5.5 is available to all LTH students through the computer management group (DDG) at http://program.ddg.lth.se/. Please make sure that you have downloaded and installed COMSOL before coming to the lab!! If you have any questions regarding download please contact me immediately.

2.3 Lab questions

As well as reading the articles and making a model you should also answer the following questions in preparation for the lab. You will **turn in your answers (send a PDF to oskar.strom@ftf.lth.se)** on the day of the lab exercise. If they are not done you cannot participate in the lab.

1. What is the particle sorting? Choose one technique which interests you and explain briefly how it works.
2. What challenges could you face when making a PDMS device?
3. In order to achieve an average flow speed of 1mm/s (average speed of the parabolic flow) in the channel, what pressure difference is required? What volume flow rate does this equate to? See lecture notes.
4. What is the Reynolds number in the central channel at this flow rate?
5. The Péclet number is used to characterize diffusion. What is the Péclet number?
6. In this lab we will attempt to separate dye molecules with ($D \approx 4 \cdot 10^{-10}\mathrm{m}^2$), plastic beads of 200nm diameter and plastic beads of 7000nm diameter. What are the diffusion constants of such beads? How far would they diffuse in 1second in 1 dimension?
7. For the device dimensions and particle sizes specified above, what volume flow will we use to achieve separation using the H-filter?

2.4 COVID-Special

To gain some kind of practical experience with fluidics, I have made paper fluidic devices for you. Please have these ready for the lab together with the other material: some food dye, a Pasteur pipette and a USB-microscope (see figure 1). During the lab we will test these together.

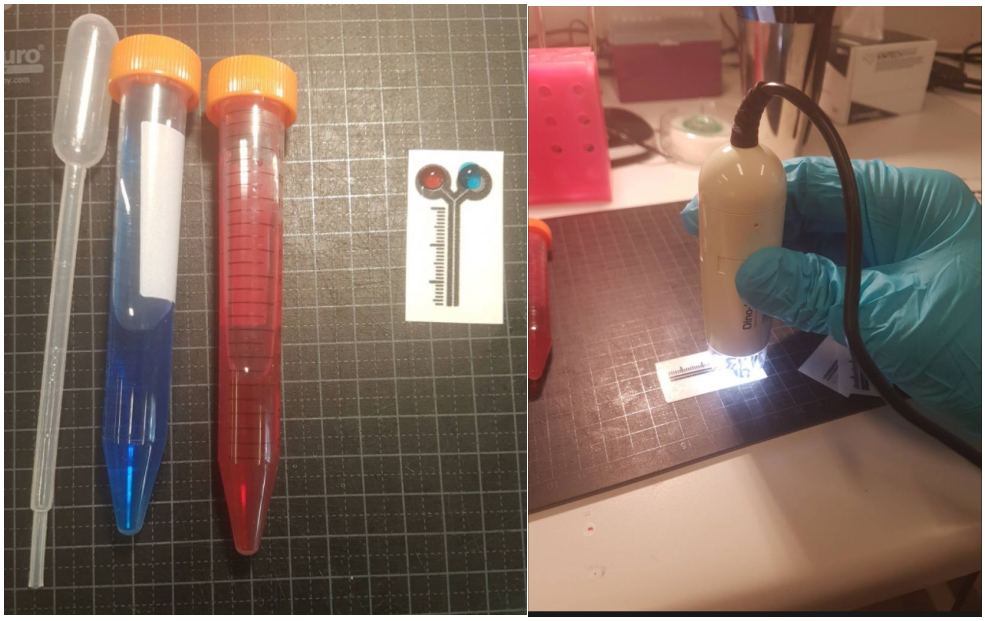


Figure 1. Equipment for the paper fluidics experiment. *It includes a Pasteur pipette, tubes containing food coloring (the tubes you will have are smaller), paper fluidic devices and a USB-microscope.*

3 Complementary Theory

A description of the H-filter can be found in the research paper mentioned above. As a complement to this, there follows a brief introduction to some common microfluidics terms.

3.1 *Re,* The Reynolds Number

$$\frac{F_{inertial}}{F_{viscous}} = \frac{\rho l v}{\eta} \equiv Re \quad (\mathbf{1})$$

Named after Osborne Reynolds, the Reynolds number, Eq.1, is ubiquitously used to predict whether inertia or viscosity dominates the behavior of a fluid. Systems with large spatial dimensions (l), high densities (ρ), large velocities (v) or small viscosities (η) are characterized by large Reynolds numbers. It is such flow, known generally as turbulent flow that we are well acquainted with on the macro scale. The flow of coffee around the inside of a cup is dominated by inertia (stop stirring and see what happens) and is therefore in general turbulent, exhibiting eddies and vortices that are characteristic of turbulent flow. The onset of turbulence depends on the geometry of the system and occurs only when inertia totally dominates, becoming increasingly more likely for Re>1500. For Reynolds numbers spanning the range on the order 1 to 1000, non-turbulent inertial effects may occur. Low Reynolds numbers (<1) characterize systems with sufficiently small dimensions, low densities, low velocities or high viscosities. In microfluidic systems with water as the fluid, characteristic channel dimensions in the range 10^{-6} m to 10^{-3} m and characteristic velocities in the range 10^{-6} m s^{-1} to 10^{-3} m s^{-1} *Re* ranges from 10^{-6} to 1 and viscosity dominates. Under such

conditions when the inertial terms can be neglected. Microfluidics systems are almost always characterised by low Reynolds numbers.

3.2 Laminar Flow

The word lamina means, "thin layer", and laminar means, "consisting of thin layers". In fluidics these thin layers consist of non-mixing parallel flows, often visualized by particles or dye molecules, see Fig. 2. Laminar flow occurs at low Reynolds numbers.

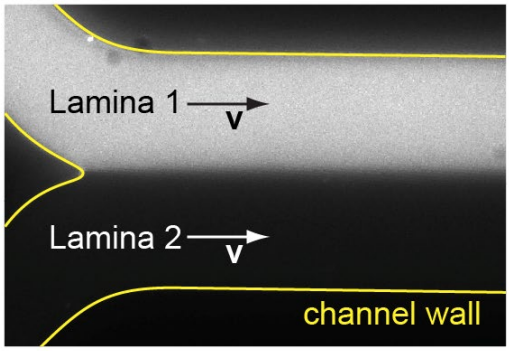


Figure 2. Two streams, one containing fluorescent beads, meet but continue as if still confined by channel walls. These non-mixing streams are referred to as lamina and such flow is called laminar flow.

3.3 Diffusion

The molecules in a fluid are constantly moving due to their thermal energy. A 1µm particle suspended in water will be bombarded by water molecules somewhere on the order of 10^{12} times a second from all directions. Each collision imparts some inertia to the particle. Because the collisions are coming from all directions the average position of the particle does not change. Random fluctuations in the number of molecular collisions coming from each direction however mean that the mean square of the distance, *r*, at which the particle can be found from the mean position, grows with time. This phenomenon is known as Brownian motion. The mean square distance a particle diffuses over time *t* is given by the following equation:

$$\langle r_k \rangle^2 = 2kDt \quad (\mathbf{2})$$

where *k* gives the number of dimensions 1,2 or 3 and *D* is the diffusion coefficient.

For three dimensional systems we get:

$$\langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle = \langle (r_3)^2 \rangle = 6Dt \quad (\mathbf{3})$$

The diffusion coefficient is given by the Stokes-Einstein equation.

$$D = \frac{k_b T}{6\pi\eta R_H} \quad (4)$$

Here k_B is Boltzmann´s constant, T is the temperature, η the viscosity and R_H the hydrodynamic radius of the particle. Because of the quadratic dependence of the distance d, diffusion becomes highly relevant in micro-fluidics devices. An example of this is haemoglobin in water for which the diffusion coefficient D=7*10^{-7}cm^{2}s^{-1}. A molecule of haemoglobin can diffuse a distance of 10µm in only 1 second but it would take about 3 months for the same molecule to diffuse a distance of 1cm.

3.4 Separation in an H-filter

Two streams, one containing a mixture of particles to be separated and another containing a dilutant (water in our case) are introduced into an H-shaped channel, *see below*, under low Reynolds number conditions. As the particles follow the flow towards the outlets they will diffuse from the sample stream into the dilutant stream. We choose conditions such that a large fraction of the smaller particles with a high diffusion coefficient will diffuse into the dilutant stream and exit through outlet 2 whereas the larger particles do not have time to diffuse and are mostly contained in the sample stream exiting through outlet 1. Note that, since we operate at low Reynolds number, the streams will not mix (apart from the mixing caused by diffusion).

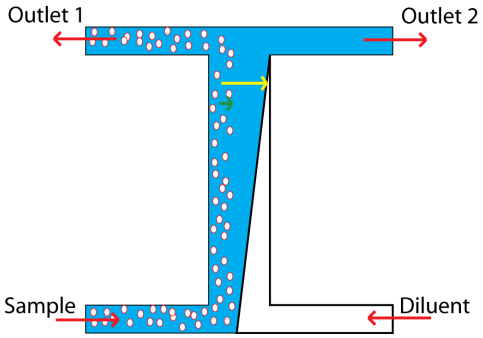


Figure 3. Small particles (blue) diffuse into the dilutant stream due to their large diffusion coefficient (yellow arrow) whereas larger particles don't diffuse as far (green arrow) and remain in the sample stream.

4 Design and Fabrication

The H-filter we will use has the dimensions shown in figure 4.

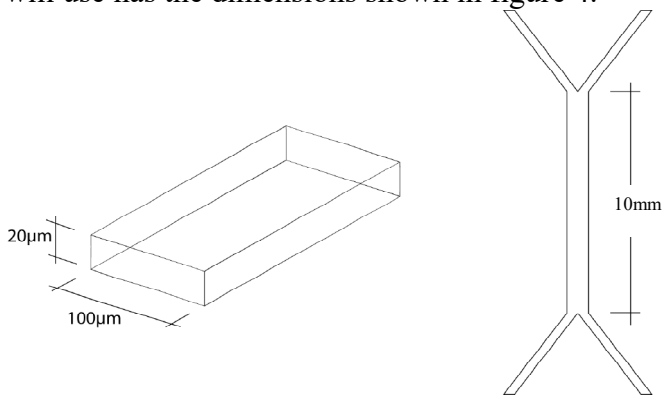


Figure 4. The dimensions of the H-filter

4.1 Soft Lithography

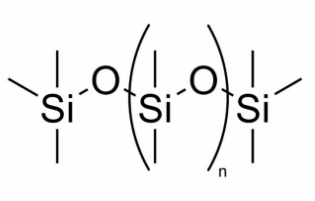


Figure 1. The chemical structure of PDMS

PDMS is relatively cheap, compatible with many methods of optical detection due to its transparency between 230nm and 1100nm, biologically compatible, permeable to gases and impermeable to water. These properties, combined with its low price, make PDMS an ideal candidate for microfluidic devices.

Another of the advantages of PDMS is the speed at which designs can move from the drawing board to prototype, working devices using the technique known as replica molding, see fig 5. UV lithography is first used to make bas-relief masters in SU-8 spin coated onto silicon wafers. After treating the masters with fluorinated silanes to prevent irreversible bonding, un-polymerized PDMS is poured on the master, cured and removed.

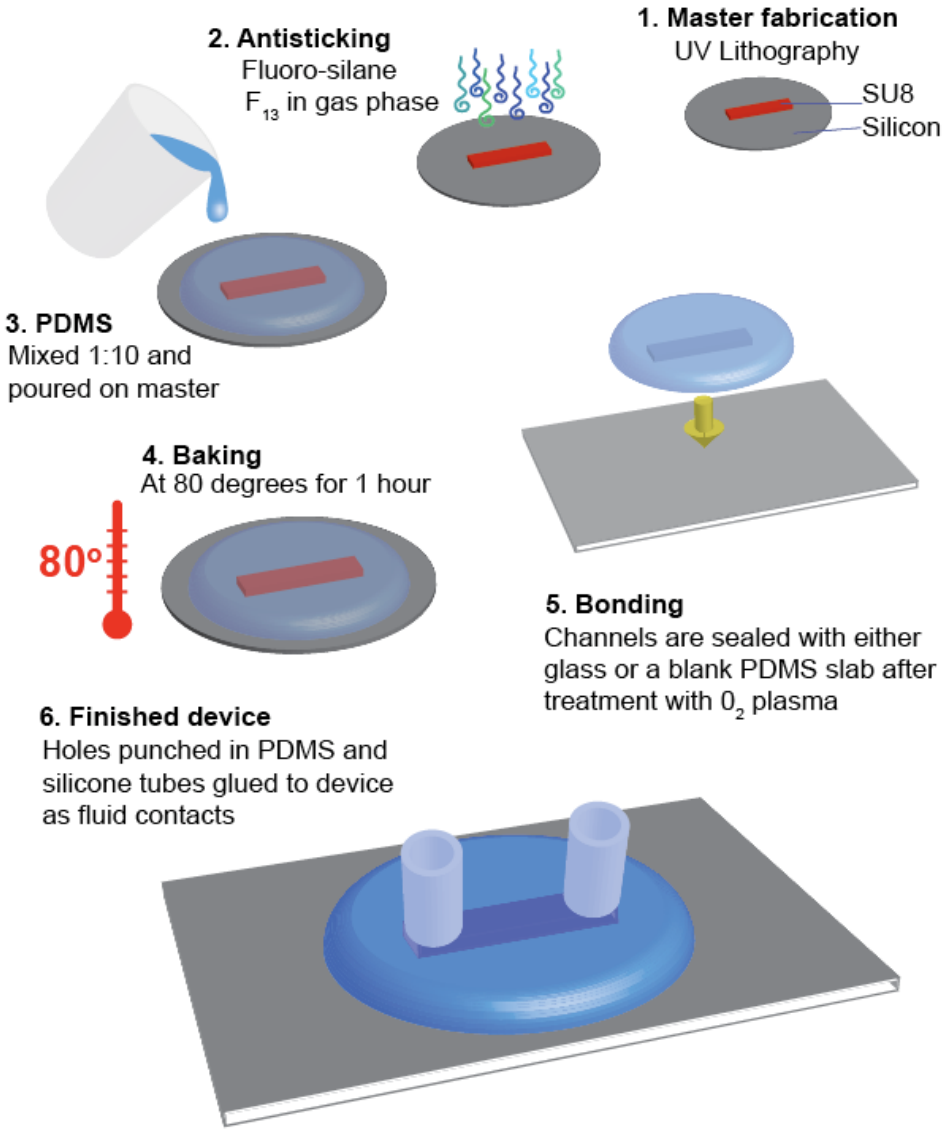


Figure 5. . The photoresist is lithographically patterned (1) and an anti-sticking layer is applied (2) PDMS is mixed and poured onto the master (3). After curing at 80°C (4) access holes are punched through the PDMS, which is plasma treated and sealed with a glass slide.

PDMS is hydrophobic. It can be very difficult to introduce aqueous solutions into hydrophobic channels without trapping air bubbles that disrupt flow, block channels and lead to devices that function poorly if at all. The surfaces of PDMS devices are easily made hydrophilic by treating them with oxygen plasma. Such oxygen plasma treatment also makes possible the bonding of PDMS to PDMS or other Si-based materials such as glass via O-Si-O covalent bonds, in order to achieve closed channels.

5 Simulation

Within all fields of science and technology, there is a common need to generate reliable models, which either aid in the next generation of new devices, or evaluates the performance of existing ones. A variety of modeling tools exist for such studies. The ubiquity of COMSOL as a modeling tool is clearly seen within the field of microfluidics and more generally within micro- and nanotechnology. Here we make use of COMSOL for generating a realistic model for the H-filter device used in our experimental lab. We will generate a model, which closely resembles the device, including the particles and settings used for separation, but will also see how COMSOL can be easily used to transfer a set of basic steps into a widely applicable area of microfluidics. While modeling tools, such as COMSOL, can be used to aid in the investigation of experimental techniques, caution must also be used when making claims about the results. Just because we get an answer, for example, in the specific questions asked within this lab, does not implicitly guarantee that our answer is correct or that it will correspond to what we will see in the experiments.

5.1 Model Design

The H-filter we will model has the dimensions shown in figure 6. Here we will model the bottom half of the device along the symmetry axis.

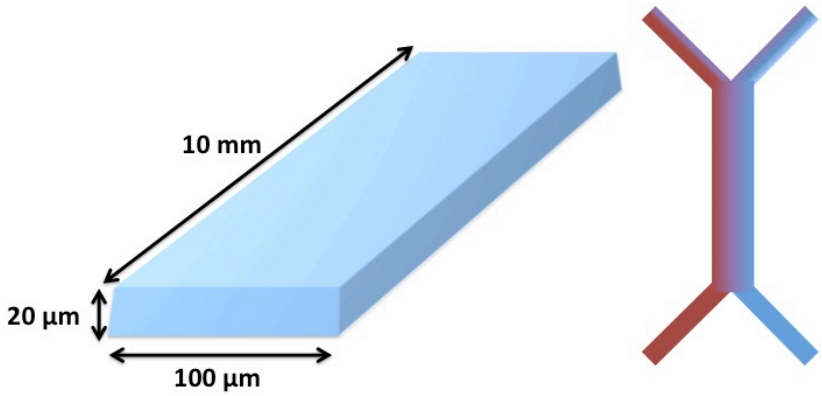


***Figure 6. The dimensions of the H-filter**. Right: height, length and width of the main transport channel. Left: example sketch of h-filter device model.*

5.2 Finite Element Simulation

Finite element simulations can be very useful as an aid to visualize fluid flow in a fluidics system. Using COMSOL, we can simulate both fluid flow and the effects of diffusion on different sized particles at varying flow rates in our H-filter. Figure 7 shows, in blue a stream containing no particles, i.e. water or buffer solution with initial concentration $c_0 = 0$, and in red a stream containing particles with initial concentration $c_0 = 1$ (or 1 molar). Here the initial concentration of the particles doesn't matter, as we only input particles into one inlet. As the two flows move side by side through the center channel the particles diffuse from the area of high concentration (red) to the area of low concentration (blue), which can be seen as the mixing of the colors (light blue and yellow). The amount of mixing that is seen at the outlets (or at the end of the transport channel) will depend on the initial conditions that we set in our model, including channel length, diffusion coefficients, pressure at the inlets, and flow rate.

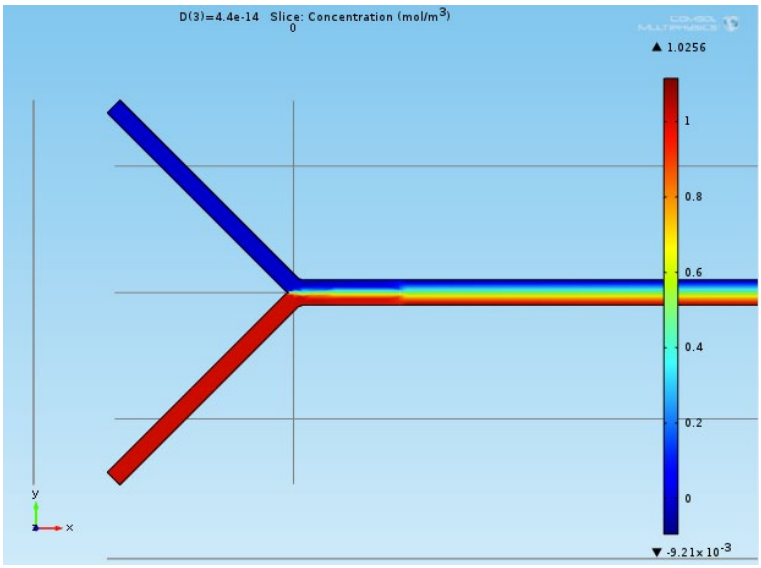


Figure 7. Example model of how particles diffuse between flow streams in the H-filter. *Red is a concentration of 1 molar and blue is a concentration of 0. The software simulates the effects of diffusion, which can be seen as the mixing of the two colors in the center.*

We will use the Tutorial example: A controlled Diffusion Micromixer as a template for generating our model, but will make changes so that it closely resembles the H-filter used in the experimental lab.

5.3 Measurements

After generating our model, we can perform measurements on the device to see how the flow is changing along the channel, how the concentration of dilute species changes and, if time permits, how different boundary conditions can affect our results.

An example of such measurements is shown in Figure 8. Here we see the cross sections of the concentrations of the three different particles at different points along the channel. In Figure 5a the two streams are mixed well at the end of the channel, as the

diffusion coefficient of the particle is high (i.e. small particles modeled) compared to 8b-c. Increasing the particle size, and thereby decreasing the diffusion coefficient in the model by a factor of 2 (Fig. 8b), we see that the mixing at the end of the channel is reduced. Further increase in particle size, corresponding to an increase in the diffusion coefficient by a factor of 10, shows that the mixing at the end of the channel is much further reduced, (Fig. 8c). Figures 8d shows an example of the velocity profile for the flow in such a device. Regions of higher velocity are shown in red, whole regions of low velocity are shown in blue. In this cross-section along the z-axis, we see that the channel is symmetric through the center. In some devices, a deviation in the distribution along the height of the channel will change the profile, i.e. if the material is not similar (through surface chemistry or otherwise) and there will be changes in the boundary conditions. Figure 9 shows how the concentration can be measured along a cutline in the device. Selecting a different position for the cutline in Figure 9a will change the measurement, in this example for larger particles, and thus the perceived mixing capabilities.

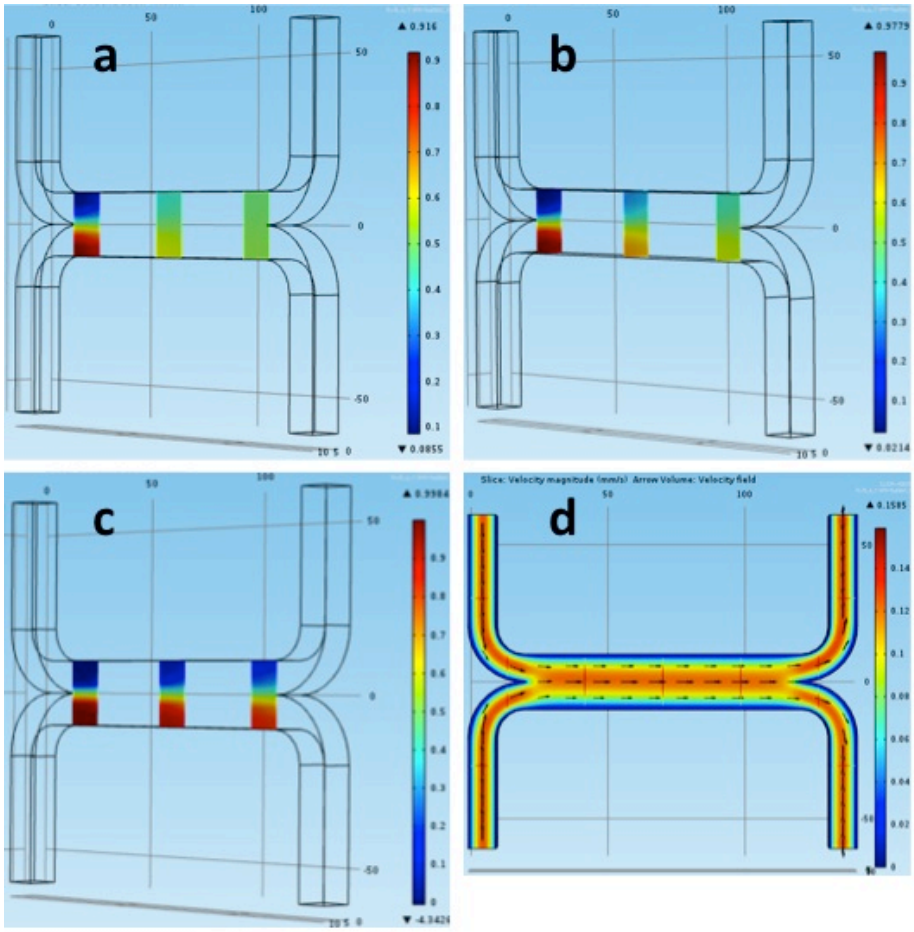


Figure 8. (a-c) Example model of how particles of different size, and therefore different diffusion coefficients, diffuse between flow streams in the H-filter. *Red is the highest concentration (near c_0 or 1M), while blue is the lowest (near zero).* ***(d) Velocity profile (z-plane) of H-filter with particles of one size.*** *Red indicates areas of highest velocity, while blue indicates regions of lowest velocity. Arrows indicate weighted velocity in the device.*

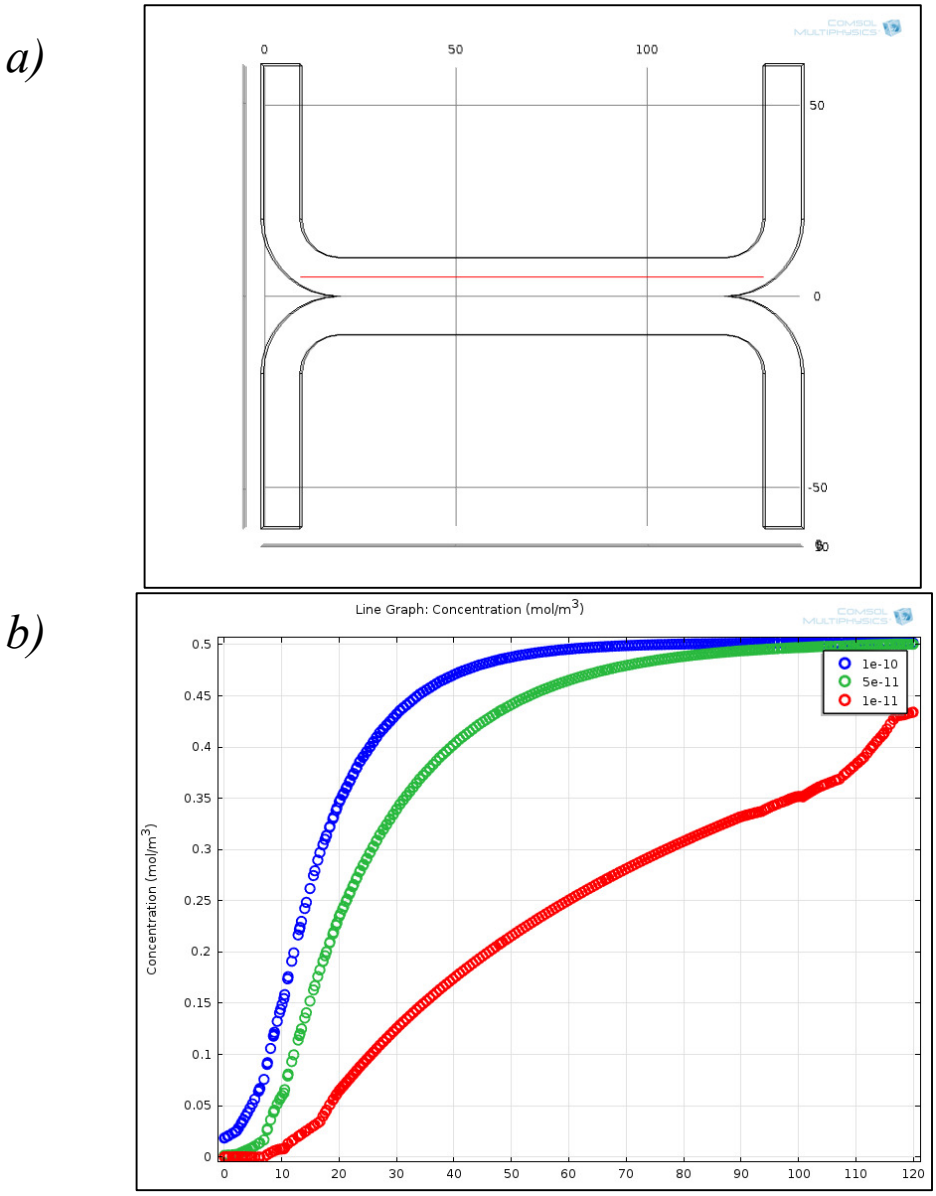


Figure 9. Example of diffusion between flow streams in the H-filter. *a) Cut line placed at upper half of channel where initial concentration at the inlet is zero. b) Plot of concentration along cut line in (a). Here the particles with the largest diffusion coefficient (blue circles) are well mixed as in Fig. 5a, having a final concentration at the upper outlet of 50% of the initial concentration. The largest particles with the smallest diffusion coefficient (red circles) do not mix well with the upper channel stream but do eventually diffuse across the middle of the channel reaching the upper outlet, see also Fig. 5c.*

6 The Challenge

The usual challenge in this laboratory exercise is to fabricate an H-filter device, connect it to pumps, place it in a microscope and attempt to observe the separation of particles by size. Due to the COVID-19 situation, you will be guided through all the steps in the fabrication. You will also be given two paper fluidics devices
The supervisor will place a second H-filter device under the microscope and you will then as a group work independently of the lab supervisor to achieve particle separation.

The first step will be to make the devices. The masters (moulds) for the soft lithography is already fabricated so we will begin by mixing PDMS, pouring onto our master and baking it in an oven. We will then need to bond the PDMS to a glass cover and glue on fluidic connections, see fig 5. The next exercise will be to connect the fluidics and try, by changing the flow speeds in the two entrance channels, to achieve separation of the dye molecules ($D \approx 4 \cdot 10^{-10}\mathrm{m}^2$), plastic beads of 200nm diameter and plastic beads of 7000nm diameter. The lab supervisor will give further information about the fluidics setup.

Note: *We will be observing the beads using fluorescence microscopy so it is essential that you have taken the introductory microscopy lab.*

At the end of the lab we will discuss the following points:

- Is the H-filter an effective method of separation?
- What are the limitations of the method?
- The H-filter utilizes diffusion in order to separate particles but in many other situations diffusion has a negative effect on results. How?
- How could we improve the design of our device?
- Is COMSOL an effective tool for modeling the H-filter?
- What are the limitations of the method?
- Are there alternative methods, which would produce faster/more accurate results?
- How could we improve the model of our device?

7 Take-home message

From this lab and the reading material you should after the lab have acquired knowledge about:

Soft lithography

- How and why the processing steps to construct our H-filters were carried out
- Advantages and limitations of this technique and how it possibly could be improved

H-filter

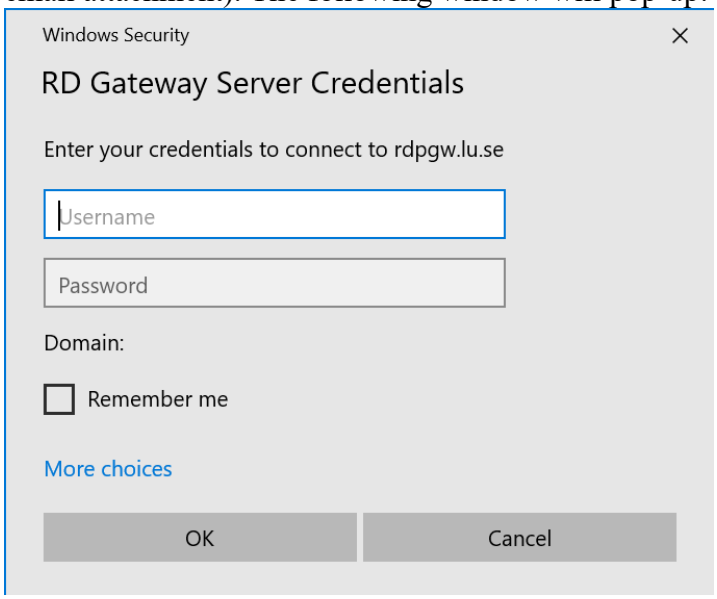
- What the H-filter can be used for
- How the separation technique works
- What dimensionless numbers, such as Re and Pe, can tell you about how the device performs

Simulation

- Basics of working with Finite Element Method Simulation using COMSOL
- Limitations and benefits of simulating experiments

8 How to access the lab computer

If you are using Windows: Open the file "bioplneo.rdp" (this file has been sent as an email attachment). The following window will pop-up:



Use the following login credentials:

Username: <nanolab\bio-plneo admin> (note the spacing)
Password: <labfaraway>

You are now able to control the lab computer. Please wait for further instructions by the lab supervisor.