Experimental Biophysics, Spring 2020

Lab 3:
Fluidics & Soft Lithography

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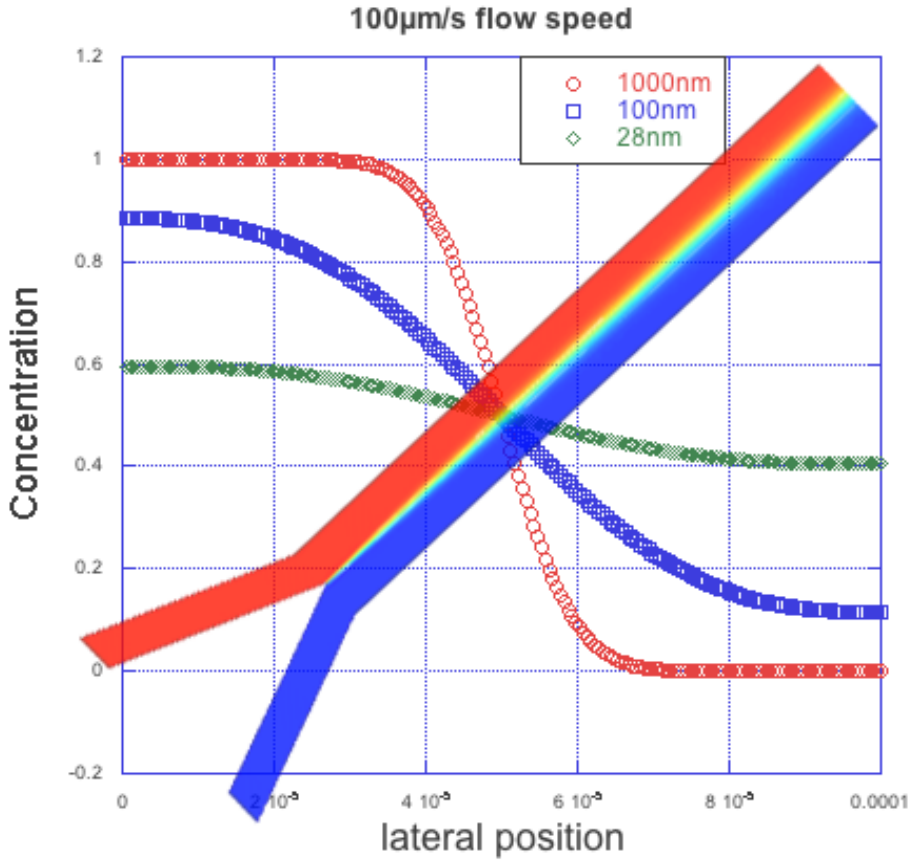


1 Introduction

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.

In preparation for the lab the following two articles should be read. We will be fabricating our own H-filters for the lab in PDMS. The review by Whitesides 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 describes the device and gives a good introduction to the lab.

Xia Y N & Whitesides G M (1998) Soft lithography *Annual Review of Materials Science* 28**:**153-184.
Brody J P & Yager P (1997) Diffusion-based extraction in a microfabricated device *Sensors and Actuators A* 58:13-18

2 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.

2.1 *Re,* The Reynolds Number

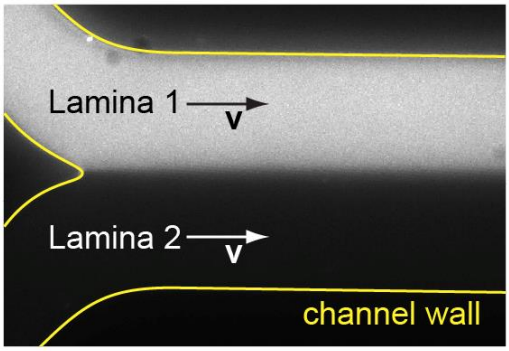
$$\frac{F_{inertial}}{F_{viscous}} = \frac{\rho l v}{\eta} \equiv Re \quad (\mathbf{1})$$

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.

2.2 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. 1*. Laminar flow occurs at low Reynolds numbers.



Figur 1. *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.*

2.3 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:

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

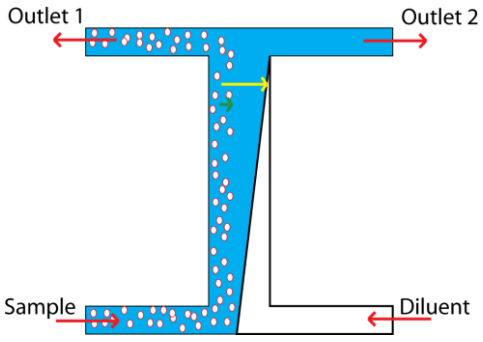
The diffusion coefficient is given by the Stokes-Einstein equation.

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

Here k_B is Boltzmann´s konstant, 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.

2.4 Separation in an H-filter

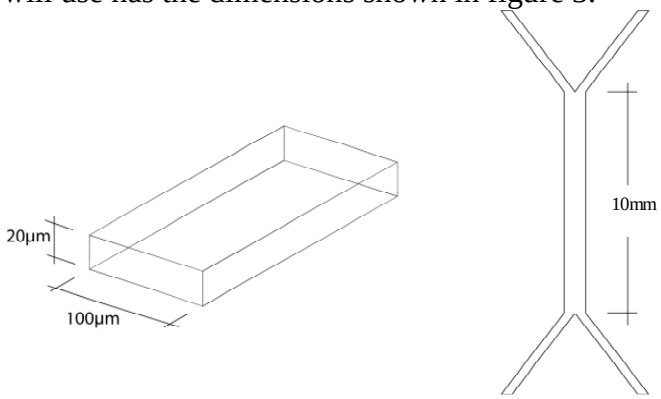
Two streams, one containing a mixture of particles to be separated and another containing a dilutant (water in our case) are introduced into an H-shaped channel, *see below*, under low Reynolds number conditions. As the particles follow the flow towards the outlets they will diffuse from the sample stream 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).



Figur 2. *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.*

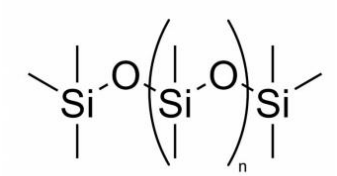
3 Design and Fabrication

The H-filter we will use has the dimensions shown in figure 3.



Figur 3. *The dimensions of the H-filter*

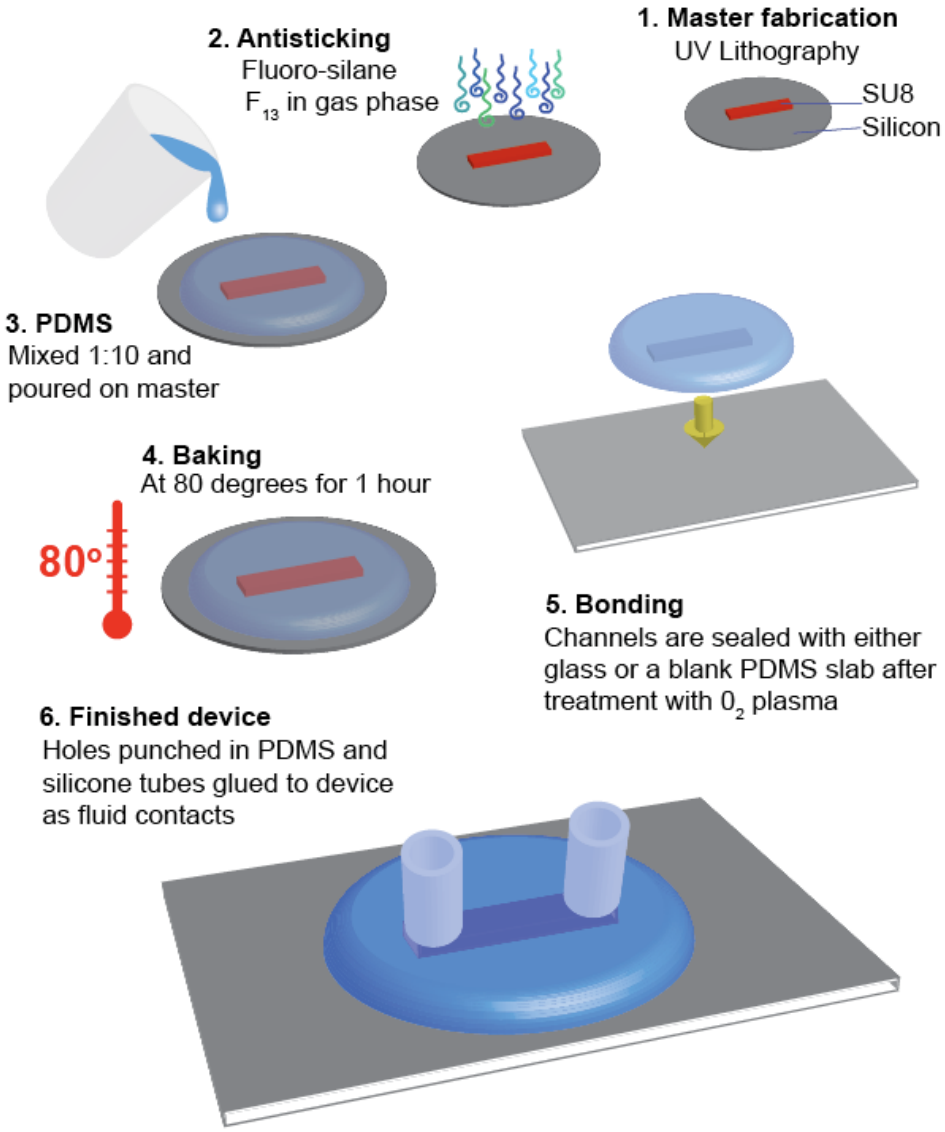
3.1 Soft Lithography



Figur 4. *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 4. 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.



Figur 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 glas*

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.

4 The Challenge

The 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. You will work together with the supervisor on the device described in this lab manual. The supervisor will then give you second H-filter with different dimensions and as a group you will work independent 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 28nm, 250nm and 5000nm beads. 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?

As well as reading the articles you should answer the following questions in preparation for the lab. You will **turn in your answers** on the day of the lab exercise. If they are not done you cannot participate in the lab. These questions will prepare you for the experiment with the second device in the lab.

1. What is the particle sorting? Choose one technique which you interest in and explain briefly how it works.
2. What challenges could you get 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 plastic beads of 28nm, 250nm and 5000nm diameters. 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?

5 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