Experimental Biophysics, VT 2021

Lab 4:
Droplets on a chip

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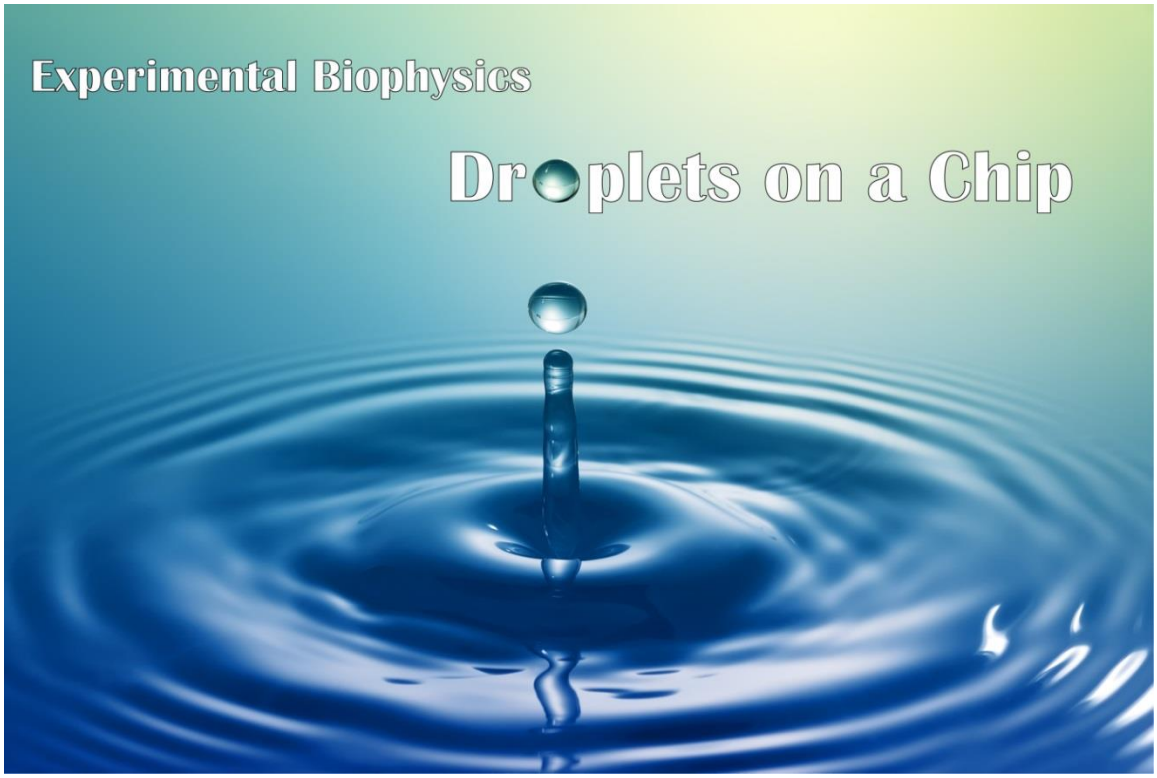
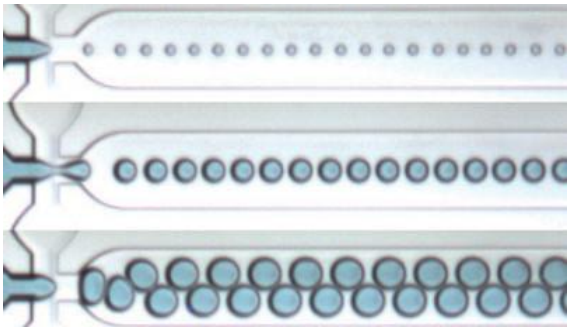
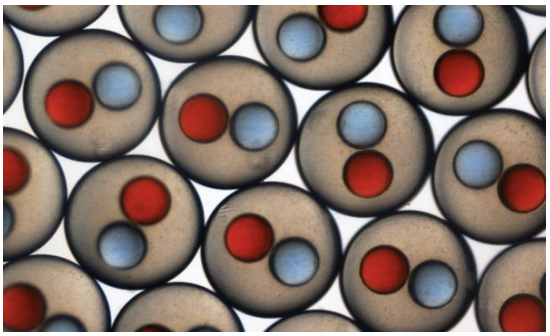
Solid State Physics

Lund University







Two- phase microfluidic flows, C. Zhao, Chemical Engineering Science, 2010

http://www.sciencedirect.com/science/article/pii/S0009250910005130

Zoom link: https://lu-se.zoom.us/j/63022438803

Please answer preparatory questions and do a home experiment as described in the end of this manual before the lab.

Introduction

Droplet microfluidics is a rapid growing interdisciplinary research field which has received great interest due to its potential in, among other things, high-throughput drug screening, material synthesis and toxicology screening. Droplet microfluidics allows for exact control of droplet volumes and generation frequency and offers tools for individual droplet manipulation such as droplet splitting, droplet merging, rapid content mixing by chaotic advection and droplet sorting. This, together with fast analytical tools allows for precise control of droplet contents and reactions or cell analysis to take place under well-defined conditions. Further, the introduced physical and chemical contrast with the outer medium introduces effects which can be exploited for novel microfluidic devices such as those based on dielectric constant and interfacial tension. [1-2]

Droplet generation

Droplets can be generated by external actuation, such as in ink-jet printers, but a simpler and more common approach (for lab-on-a-chip applications) is through passive techniques. These microfluidic droplet generators utilize the shear field of the surrounding fluid to deform the interface, and can often produce droplets in the kHz range while the polydispersity index (PDI) is down to a few percent.

$$PDI = \frac{\sigma}{\pi} \quad (1)$$

where σ is the standard deviation and μ the mean droplet diameter. (σ/μ). The ultimate cause for droplet breakup depends on the droplet regime present and, consequently, different mathematical models are used to describe the process. (see The droplet regime depends on several factors including the flow regime, viscosities, flow rates, interfacial tension in combination with the device geometry. More on this is found in the reading material at the end of this lab manual. However, during the droplet breakup process, the size of the generated droplets is a function of opposing forces. Capillary pressure (due to surface tension) act to resist deformation while shear stress and pressure due to external flow act to promote deformation and, consequently, enhance droplet generation. The relative importance of these forces is expressed by the Capillary number, see below, here η and U is the viscosity and flow velocity of the continuous phase respectively while γ is the interfacial tension. Another dimensionless parameter is the flow quota, q see **Eq. 2**, here expressed as the inlet flow rate fraction of the total flow rate.

$$Ca = \frac{viscous\ forces}{interfacial\ tension} = \frac{\eta U}{\gamma} \quad (\mathbf{1})$$

$$q = \frac{Q_d}{Q_d + Q_c} \quad (\mathbf{2})$$

where Q_d is the flow rate of the dispersed phase while Q_c is the flow rate of the continuous phase.

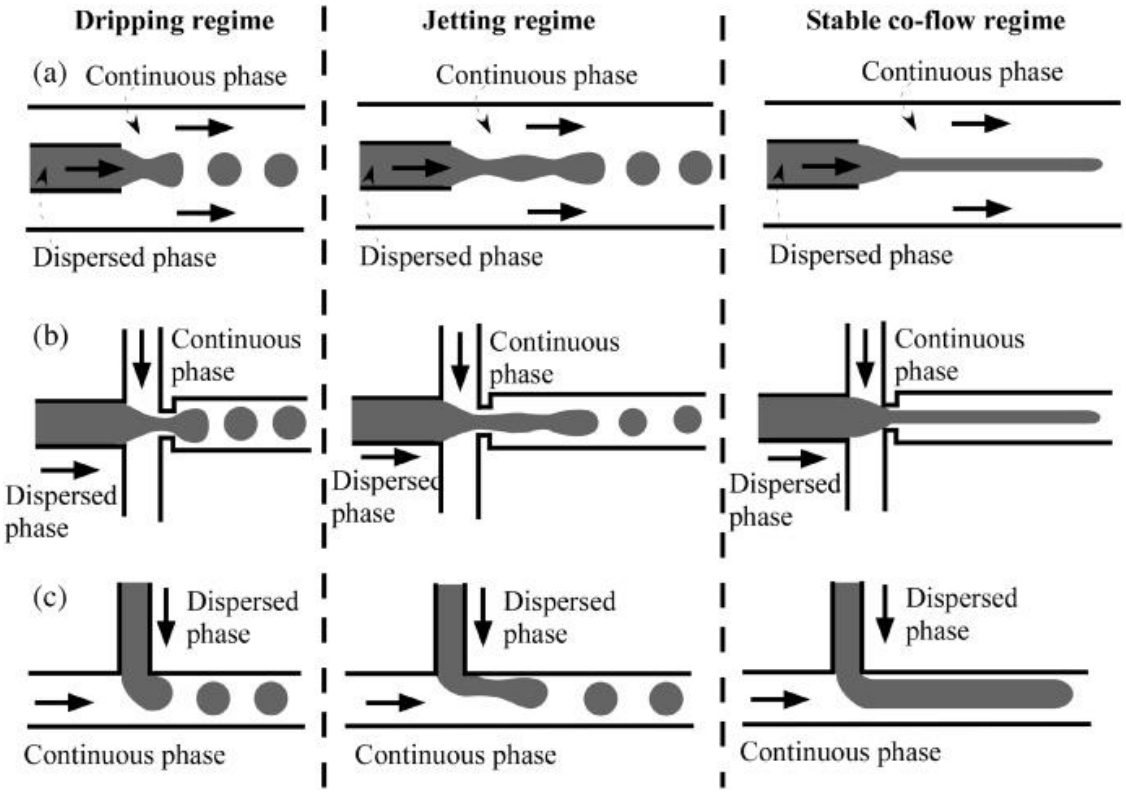


Figure 1 *Droplet microfluidic devices operate in different droplet regimes, which depend on factors such as flow rates, viscosity and surface tension in combination with the device design. Due to the great interest in droplet application a clear understanding of the break up process and the transition between droplet regimes are needed and has been studied extensively. Different flow regimes can be advantageous depending if the final result is for example to obtain monodisperse droplets, droplets of minimal size (satellite droplets) or to produce fibers instead of droplets. In the case of fibers, the inertial forces play a major role, as indicated by a higher Weber number: $We=\rho U^2 l/\gamma$. Different mathematical models are used to describe the droplet breakup process depending on the droplet regime present. Further information is available in reference 11, where also the figure is adapted from.*

As discussed above, the generation of droplets is significantly influenced by the interfacial tensions. For our system the water to oil interfacial tension is 52.07 ± 0.66 mN m^{-1}, [4] a relatively high value for oil/water systems. However this will be altered during the lab by the use of amphiphilic molecules, ie surfactants. These are molecules which have both a polar and a non-polar part. The molecule is often characterized by the HLB (hydrophilic-lipophilic balance) which is given by

$$HLB = 20 * Mh/M \quad (\mathbf{3})$$

where *Mh* is the molecular mass of the hydrophilic portion of the molecule and *M* the molecular mass of the whole molecule. Note that this is Griffin's method of calculating the HLB; other methods exist but this one is the most widespread. The resulting HLB is used to predict the behavior in an emulsion, see Table 1. Values for a wide range of surfactants are available in the literature. It should also be noted that the theoretical value of the surfactant may sometimes differ slightly from the actual value acquired from the equation above.

Table 1

HLB Range	Use
4-6	W/O emulsifiers
7-9	Wetting agents
8-18	O/W emulsifiers
13-15	Detergents
10-18	Solubilizers

Table adapted from [5]

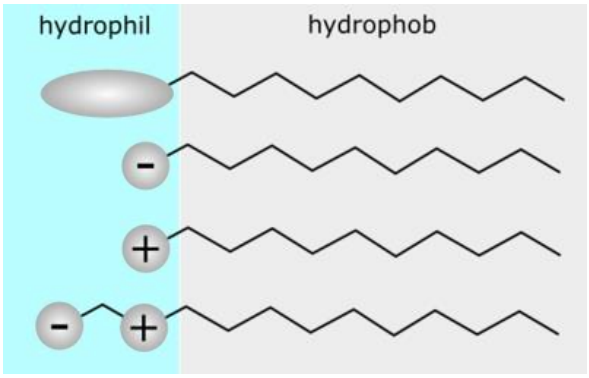


***Figure 2**, different surfactants. The non-ionic molecule is often prefered for biological studies due to its increased biocompatibility. Image from Wikipedia, used under CC.*

Poisson distribution

For single cell studies in droplets it is essential that each droplet contains a single cell. This can be ensured by either droplet sorting or the use of techniques such as inertial focusing of cells[6,10] or hydrodynamic sorting of droplets[9]. Under normal droplet generation conditions, without the help of these techniques, the distribution of cells in each droplet is given by the Poisson distribution.

The Poisson distribution is a discrete probability distribution that expresses the probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event. Besides giving us the distribution of cells in each droplet, it is also usefull in other situations. For example it could be used to express the number of photons emitted from a light source in a specific time interval (which fluctuates over time). Given that we know the average photon emission in this time interval.

The probability mass function of the discrete stochastic variable X with Poisson distribution is given by

$$f(k,\lambda) = \mathrm{p}(\mathrm{X} = \mathrm{k}) = \frac{\lambda^{\mathrm{k}} e^{-\lambda}}{k!} \quad (\mathbf{4})$$

For k=0, 1, 2..
With E(X) = λ.
The maximum for the different k is acquired when k equals λ. For example, the maximum value on k=1 is given when λ=1. In this case, p(X=1) = 0.37.

Device manufacturing

The flow-focusing device which we will use in this lab has been manufactured by the common UV- and soft lithographic method which also was used in the H-filter lab. This includes UV-lithography of an SU-8 mold which subsequently was used to cast the features of the device. This PDMS sheet, together with a blank PDMS slab was exposed to oxygen plasma and bonded together to form a sealed device. Thereafter, inlet and outlet reservoirs were attached using silicone glue. However, in comparison with the previous H-filter device, this device requires an additional step. Namely, surface treatment with vaporized 1H,1H,2H,2H-Perfluorooctyltrichlorosilane (PFOTS) in a dehumidified atmosphere.

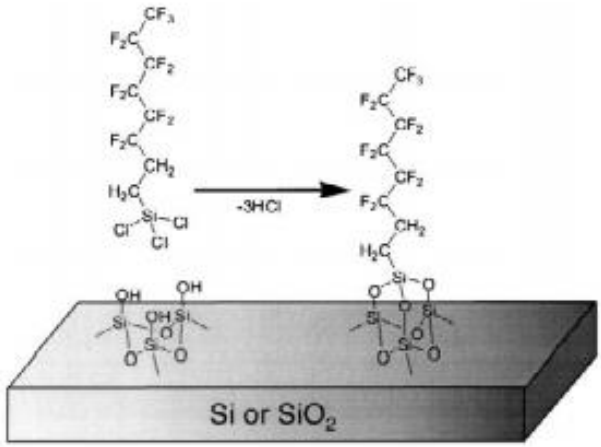


Figure 4 *The devices were subjected to PFOTS for a minimum time of 12 hours, resulting in a condensation reaction where hydrogen chloride is produced. Picture from reference 8.*

Method

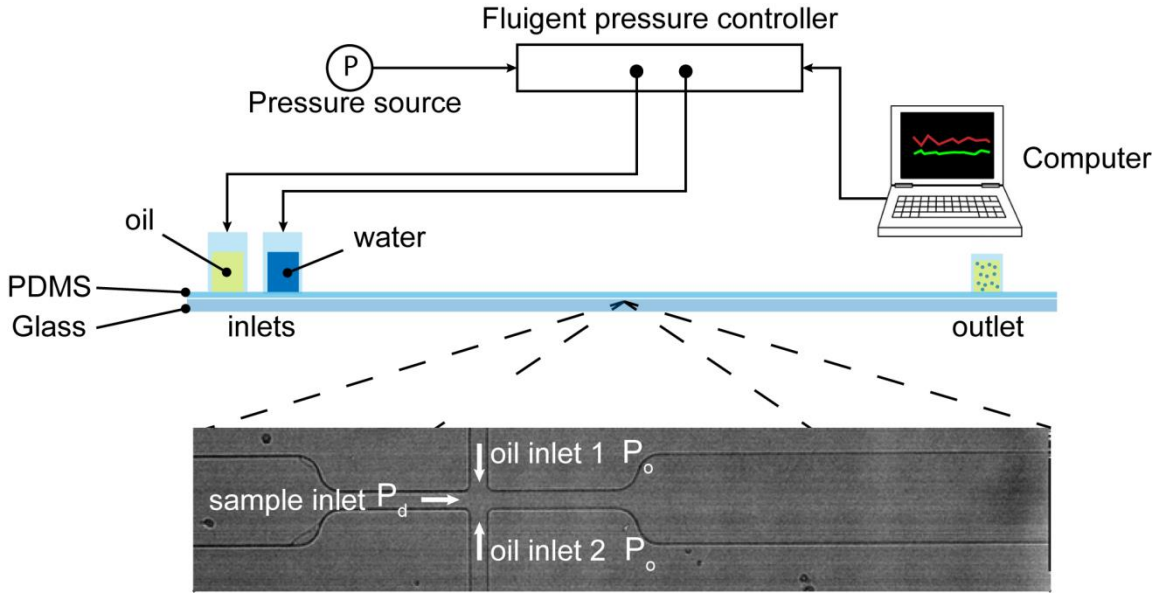


Figure 5 *Setup during the lab. Two individually controlled syringe pumps are used to induce flow in the device. They contain the continuous phase fluorocarbon oil and the sample which will constitute the droplets respectively. After the junction where the droplet breakup occurs the Oil and droplets travel to the outlet reservoir for storage and observation.*

In the lab we will be using the fluorocarbon oil Fluorinert FC-40(Sigma F9755) as the continuous phase. The oil is often used in microfluidics due to its good solubility of gases often necessary for cell work and due to its low solubility in PDMS which result in lower swelling of the device (and hence decreased performance) than many other oils. We will study the behavior of the system both with and without surfactants. A high speed camera will be used which allows for detailed imaging of the droplet breakup process.

Experimental work

During the lab we will investigate two different water phase pressure and both of these pressures we will analyze the droplet generation at three different oil pressures. **Steps 1-2 will be done by your lab**

teacher. For the next steps, you will access the lab computer remotely. For that, please download the configuration file here: https://lu.box.com/s/zwl1hamw4tk38ccm6myqbvmq2huql1ms

1. First, fill the water inlet up with MilliQ water so that a bit of space is left for inserting a pump connector, make sure thereis no air bubbles at the inlet bottom, and position the stage so that the crossing is visible. Slowly increase pressure to 20 mBar and further if needed, until the water has reached the crossing; when it happens, immediately switch off the pressure.

2. Now, add the oil with surfactants to the oil inlet. Set both pressures to the value adjusted earlier and wait until the flow has stabilized. When setting two pressure at the same time (like we need to do later in the lab) make sure you unclick “direct adjustment” before setting the new pressure values for both channels before clicking “direct adjustment” again and the pressures will simultaneously change.

3. Adjust the region of interest in the camera. Smaller image allows for higher framerate, and we will need at least 2000 fps. Draw a small rectangle around the crossing and a small distance of the outlet channel.

4. Set both pressures to the first chosen water pressure. Remember unclicking “direct adjustment”. Now change the oil pressure up and down to see how it affects the droplet generation and frequency. Choose three different oil pressures which shall be analyzed further.

5. Start capturing by clicking on record and wait around two seconds until clicking on stop. Step back in time in the captured sequence to ensure that the frame rate is high enough to see every single droplet being generated. If not, then increase it and start again. If the frame rate is high enough then save the file into the designated folder. Name it with the water and oil pressures and export only 1000 frames. Click “apply” before clicking on “OK”.

6. Repeat this process for the second water pressure.

7. After capturing the six different movies at high enough frame rate, open ImageJ and start the analysis. Frequency, diameter of 10 droplets and the PDI shall be produced.

8. Plot the results in two different graphs, one for each water pressure. X-axis is oil pressure while the Y-axis is both diameter and frequency. Use different colors to mark these.

9. What can be said about the two graphs? Why do the diameter curve and the frequency curve look the way they do?

10. How would the curves look like if we used syringe pumps (changing flow instead of pressure)?

Reading Material

G F Christopher et al 2007 *J. Phys.D:Appl.Phys.* **40** R319 doi:10.1088/0022-3727/40/19/R01 **Chapter 1-3**
Ralf Seemann *et al* 2012 *Rep. Prog. Phys.* **75** 016601 doi:10.1088/0034-4885/75/1/016601 **Chapter 1-4, 7**

Preparative questions – be prepared to discuss these before the lab:

Experimental task to do before the lab – take images with a USB microscope (if not available, use a camera)

- Take some cold water, add vegetable oil, and stir the mixture vigourously. What do you see?
- Do the same with hot water. What is the difference and why?
- After stirring the oil-water mixture, add some vinegar to it. What does it do?
- Now add soap/detergent (on the knife's tip should be enough). What happens and why?

Theory questions:

1. **Advantages**

   Give…
   - .. five advantages with using droplet microfluidics over "continuous microfluidics"
   - .. five examples of applications to droplet microfluidics.
2. **Droplet generation**
   - How are droplets created? Why do they get a specific size? What forces balance each other during droplet generation?
   - In the lab we will create W/O (water-in-oil) droplets, what can we change to get O/W droplets instead.
   - What would happen if we if we change the temperature while producing droplets?
3. **Poisson Distribution**
   - What is the Poisson distribution, when can it be used?
   - What is the theoretical maximum fraction of droplets containing two cells given Poisson distribution?
   - If we want the fraction from question 4, what concentration should the sample have if we aim to produce droplets with a diameter of 20 µm.
   - If we use this calculated concentration value but end up making 30 µm droplets, how many single celled droplets could we expect in that case?
4. **Surfactants**
   - What effect should the surfactants have on our droplet system, what should the HLB of them be, why?
   - We have two Eppendorf tubes which both contain oil and water at a ratio of 10:1. The second one however has surfactants with an HLB of 5 added. We shake both Eppendorfs with the same speed, how would the generated droplets compare? Why?

References

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(9) Chabert et al. Microfluidic high-throughput encapsulation and hydrodynamic self-sorting of single cells. PNAS March 4, 2008 vol. 105 no. 9 3191-3196
(10) Di Carlo et al. Continuous inertial focusing, ordering, and separation of particles in microchannels. PNAS 2007 104 (48) 18892-18897
(11) J K Nunes *et al*, Dripping and jetting in microfluidic multiphase flows applied to particle and fibre synthesis . 2013 *J. Phys. D: Appl. Phys.* **46** 114002

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http://greenlifeinsocal.com/tag/southwest-birders/

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