Answers to Question Set 4

Applications in fluidics

Note that this time the number of articles is quite high. The purpose is to give you an opportunity to train in extracting the essential information. It is not necessary to read the papers beyond what is indicated by the questions and what is covered by the lecture notes.

Remember to draw figures to explain your answers. This is often more efficient than just using words.

If you have any questions feel free to send an email: jonas.tegenfeldt@ftf.lth.se.

Questions about applications

The questions below relate to the challenges that can be addressed by microfluidics in different contexts.

1. (F) What is Global Health? Explain the requirements underlying the acronym ASSURED.

Global Health typically refers to health issues in the developing world, in resource-deprived settings.

ASSURED (WHO) – affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users

2. (F) Give examples of types of devices suitable for Global Health applications.

Paper fluidics; self contained devices; sample in answer out; …

3. (F) Discuss differences between different health-care markets. Veterinary medicine, medicine in High-income countries, medicine in Medium and low-income countries.

Veterinary medicine – less paperwork; higher price sensitivity

High-income countries – more paperwork; lower price sensitivity

Low-income countries – very high price sensitivity; requirements for simplicity;

Questions about nanochannels

The questions below relate to stretching of DNA in nanochanels. Most answers can be found in a review paper[1].

1. What is the underlying mechanism that forces the DNA molecule into a stretched conformation in a nanochannel?

The conformation of DNA in solution is determined by a balance between entropic effects, that serve to minimize the volume of the DNA coil, and excluded volume effects between interacting segments of the DNA causing the coil to expand.

$$\frac{r}{L} \propto \left(\frac{w_{\text{eff}} P}{D_{av}^2}\right)^{1/3}$$

2. What type of information can be gained using devices for stretching of DNA? What types of disease can be identified?

Overall structure of the genome at a resolution of approximately 1kbp.

Structural variations (copy number variations, deletions, insertions, translocations, inversions)

Not the detailed sequence, though.

Genetic disease, bacterial infections, other types of infections (viruses, parasites, …).

Note that antibiotic resistance can be detected using DNA mapping.

Questions for the "bumper" papers

The questions below relate the bumper array (a.k.a. deterministic lateral displacement, DLD) described in the original paper by Richard Huang and coworkers at Princeton[2]. Specifically, we will focus on two papers: one on sorting trypanosomes from blood based on morphology [3] and one characterizing different variants of red blood cells by their morphology and deformability [4].

4. (F) What is the basic principle governing the size separation in bumper arrays?

The key method is deterministic lateral displacement.

The basic principle of the separation method is to make use of the laminar flow of fluid lanes through a regular array of obstacles where each row is displaced a small distance compared to the preceding row. Small particles (< critical size~d/N), as compared to the distance between the obstacles (~d), follow the flow pattern. N is the number of rows required to come back to the original position. Larger particles (> critical size) are pushed by the steric interaction with the posts and therefore cannot follow the laminar flow in those cases where the lane passes close to an obstacle. They are pushed into a neighboring lane. This results in a zigzag transport of the smaller particles, while the larger particles keep moving in the centre between the obstacles, thus having a constant displacement for each row of obstacles. The small particles thus move along the overall flow direction while the large particles move in the direction of the array. Varying the obstacle/channel size varies the critical size.

To summarize, without any force interactions the particles would simply follow the flow. In the bumper array, the force is simply the steric force preventing a bead from going inside a post. In other devices, the force could be electric, optical or of any other origin.

In addition to the steric force, there are stochastic forces that spread out the beads randomly.

5. (F) What is hydrodynamic chromatography? How is it related to the paper?

In a flow with a non-uniform flow profile such as pressure driven flow with a parabolic flow-profile, the position of a particle radially determines its speed. All particles are excluded from a space corresponding to one radius from the channel wall. Large particles are thus moved further from the wall than smaller particles. Since the velocity of the fluid has a maximum at the center of the channel, the larger particles experience a larger average speed than the smaller particles. Running a mixture of large and small particles in a small channel will therefore result in a segregation of the large and small particles, with the larger particles exiting the channel first, followed by the smaller particles. Note that lateral diffusion ensures that the small particles will have a small dispersion.

One key point in the idea of the bumper array is that large beads are excluded from the posts more than small so that they experience a different set of flow streams, in this case for sufficiently large particles (size > critical size) flow streams that lead the larger particles in a different direction in the device.

6. (F) How does the DLD technique differ from the H-filter and the pinched flow separation techniques? First describe the H-filter and the pinched flow separation techniques.

H-filter – see fig 10 in ref [5]. Sorting based on diffusion. A fluidic arrangement shaped like an H. A sample solution is run from one side to the other in one pair of reservoirs. In the other reservoirs plain buffer is run. The plain buffer will pick up particles in the sample stream if the particles diffuse sufficiently. The result is one stream with large and small particles and one stream with small particles. The H-filter is thus useful for removing large particles from a sample.

The bumper array is deterministic and does not rely on diffusion. It can therefore be run at high speeds without any loss in performance (as long as laminar flow conditions are maintained).

Pinched flow – see ref [6]. Two flow streams (sample and buffer) are squeezed through a narrow constriction and then expanded. Any particles in the sample stream with radii larger than the minimum width of the sample flow stream will migrate over to the buffer stream.

The bumper arrays is based on the exclusion of beads with radii larger than a critical size from one flow stream, just like in pinched flow. On the other hand, in the bumper array the size selection happens multiple times as opposed to in the pinched-flow device where it happens just once.

7. (F) What is the key mechanism used for morphological separation?

By ***controlling the orientation*** of the particles as they pass through the device, the dimension can be chosen based on which the trajectory of the particles is determined.

8. (F) What is the key mechanism used for deformability-based separation?

As the particles are run through the device, the shear causes the particles to deform and to appear smaller. ***By running the devices at different speeds and monitoring the size of the particles, the deformability of the particles can be characterized.*** The effective size of highly deformable particles quickly decreases with increasing speed, whereas for completely rigid particles the effective size remains essentially constant.

9. What are the key performance advantages/drawbacks of DLD as compared to other microfluidic techniques for particle and cell sorting?

The device results in a *continuous* and *spatial* separation of both solid particles and DNA. Diffusion is not necessary in the device. This makes it possible to run the device at high speeds and provided the particles are stable, the device can be run with arbitrarily high resolution.

The chip is capable of separation of large DNA molecules in 10 min as compared to several hours for standard methods and comparable resolution.

Important parameters are: resolution, throughput, theoretical plates (Péclet number).

10. How is the critical size affected by the speed of the sorting? What could the underlying mechanisms be?

See the supplementary material of the paper by Johan Davis[7] and the paper by Brian Long[8] (and the lecture notes). There are at least two regimes:

Large soft particles are increasingly deformed as the speed increases. This makes them appear smaller with increasing speed. This is equivalent to an increasing apparent critical size.

Small particles diffuse readily. At high enough speeds diffusion does not have any influence. At lower speeds, diffusion will act on the particles. However, diffusion will act asymmetrically. The large particles (size > critical size) will change trajectory due to diffusion only between the rows, while the small particles (size > critical size) may change their trajectories due to diffusion anywhere.

The effect of diffusion (visible at low speeds) is thus a lowering of the critical size.

On top of that, inertial effects (at sufficiently high flow rate) will push the particles away from the walls making them appear larger than they are or conversely in effect decrease the critical size.

Very large speeds require high applied pressures. This may deform the device and change the critical size.

Inertial effects may influence the trajectories at very high speeds. The wall may then move particles away from the posts, thereby increasing the effective size of the particles.

11. Why is it interesting to move particles across streams with different chemical composition? What are the requirements for it to work?

A standard labeling reaction involves mixing *e.g.* the cells with a dye, incubation and finally washing of the cells to get rid of the dye molecules. Using a bumper array with a critical size smaller than the particles of interest, the particles can readily be moved from a dye rich medium to a clean buffer. The buffer conditions can thus be changed for the particles without any active action from the user, no valves or complicated network of channels is needed. The process can therefore be done not only much simpler but also much faster.

Requirements for it to work could be *e.g.:* size of beads > Rc; laminar flow; high Péclet number; low Reynolds number; involved chemical should not react (violently or producing disturbing products such as precipitations or gas).

Questions for DNA sorting

Most of the questions can be answered by reading the paper by Richard Huang from Austin's group. Make sure to look at the supplementary material that comes with this paper (on the website of the Journal). There are more detailed descriptions as well as movies.

1. (F) What is the basic mechanism for sorting of ultralong (>100 kbp) DNA? How does it work in a gel (PFGE)?

One common basic mechanism is based on an electric field that changes between two directions, typically separated by 120 deg. The DNA is stretched during the process and is entangled around obstacles. See the images in the lecture notes.

As the electric field is shifted the longer DNA must retract much more than the shorter DNA. Therefore, the overall mobility of the long DNA becomes shorter. If the length exceeds some maximum length, the long DNA will not change the obstacle that it is entangled around. Instead it will just move back and forth.

The principle is typically implemented in a gel where the DNA molecules are entangled around the gel molecules. Here the separation takes several hours to several days to complete. By instead creating an artificial gel based on an ordered (often hexagonal) array of obstacles in a microfluidic channel the separation can be performed much faster, around a minute or so depending on the size of the molecules.

2. (F) How can the approach be modified to sort the DNA in space rather than in time?

In standard pulsed field electrophoresis, the molecules are moved in two directions separated by 120 deg. This can be viewed as two vectors of equal size pointing in two directions. The net motion is then the vector sum of these two vectors. Since the two vectors are equal in size the resulting vector sum is always pointing in the same direction independent on the size of the vectors.

To achieve a resulting vector that has a direction that is a function of the size of the vector, one of the two vectors can be increased by a constant amount. This can be done by an asymmetric pulsed field: first one pulse in one direction and then one longer or stronger pulse in the other direction.

Questions for inertial focusing and sorting

Most of the questions can be answered by reading the paper by Dino di Carlo from Toner's group[9]. Make sure to look at the supplementary material that comes with this paper (on the website of the Journal). There are more detailed descriptions as well as movies. Quake's review could also be useful[10].

1. (F) What is the basic principle of the device? What is a Dean vortex? What happens at various speeds? What relationship does it have to centrifugation?

Focusing of the streams is the result of three interacting components: inertial lift due to the "wall effect" from the wall; inertial lift due to the shear gradient from the center towards the wall; Dean vortices. An asymmetrically curved channel will result in the focusing of one single stream. The focusing is strongly dependent on the size of the particles so that by careful extraction of the focused stream size-based sorting can be implemented.

A Dean vortex is formed in a curved channel (radius of curvature R) where the inertial forces act on the fluid. Since we have a parabolic flow profile, the centrifugal force will not be equal for each part of the fluid. The fluid at the center will move at the highest speed and so will experience the greatest centrifugal force. See fig 4 in Squires' and Quakes' review paper[10].

Dean vortices are formed because of the centrifugal force on the *fluid*. Remember that the centrifugal acceleration is $\sim v^2/r$, where r is the radius of curvature of the tube in this case. The inertial effects refer to effects of the fluid, not on the particles. This was shown by running particles in buffer solutions of different densities where the result was the same independent on whether the particles were denser or less dense than the surrounding fluid.

Centrifugation on the other hand relies on the sedimentation in a (artificial) gravitational field. Denser particles fall down whereas particles lighter than the buffer solution move up. This obviously happens in the curved channels, but the net motion due to sedimentation will be zero on average since the curves are going both left and right.

2. (F) What are the requirements to focus to one single stream?

See above. There exists an optimum speed, above which Dean vortices dominate, mixing the particles, and below which inertial lift dominates focusing to four streams in a rectangular channel. Very strong function on bead size: ratio scales with volume of the particle.

3. (F) In which way can the technique be used to sort cells? How are the particles sorted?

The involved forces are dependent on the volume of the particles; some particles are focused, the rest are spread out; run the device in series to achieve high purity.

Run at De>1. For De<1 all particles are focused to four streams or not focused. For De>1 particles with diameters $a<D_h$ are all focused to four streams or not focused, whereas for $a>D_h$ particles are predominantly focused to one stream. See fig 4 in [9].

4. (C) What is the benefit of this technique as compared to the bumper array? ... as compared to conventional techniques? In general and specifically in terms of speed and throughput?

Less clogging and larger throughput due to larger channels for a given set of particle sizes. The cells are focused and not in contact with the walls. On the other hand: lower precision and lower concentration. ***The sample must often be diluted for the inertial focusing to work.*** For applications where the sample is already very diluted (safety test of drinking water) this is of course not a problem.

Ordering of cells in flow cytometry. No two cells close to each other. Aligned non-spherical cells for reproducible scattering measurements.

One example: 1.5mL/min flow rate of 1% particle concentration giving 1 g per hour using a 1.6 cm^2 device. The bumper array used for blood separation was run at 30mg per hour for a device of size $15cm^2$.

A valuable technique for applications where volumes are high but particle densities are low.

Questions single-cell measurements

Most of the questions can be answered by reading the paper from Luke Lee's group[11]. (There is also an overview paper from the same group[12]. A similar paper has also been published in Lab on a Chip[13]. At the website, a movie is available showing trapped individual cells in an array.)

1. (F) What is the basic idea of the single-cell trapping approach? In which way does the design ensure that only one cell is trapped in each trap?

The basic component is the small U-shaped traps that are designed such that they do not quite reach the bottom of the device. Some of the flow is thus going "straight" through the trap, dragging a cell into the trap. Once the trap is filled with one cell, the fluid flow is changed due to the trapping of the cell such that other cells are dragged around the trap, ensuring only one cell in each trap.

2. (F) Why is this approach better than the alternatives? What are the alternatives? How can droplet fluidics be used for studying single cells? What are the challenges?

Simpler, perturbs cells less, higher throughput, possible to follow the time evolution of a cell.

To trap cells: DEP, holographic tweezers arrays, arrays of containers, 1536-well plates, microarrays (see work by Helen Andersson-Svahn and coworkers at KTH).

Laser scanning cytometry. Cells are immobilized and can be interrogated repeatedly over time.

Automated microscopy. Can be used for any cell sample that can be imaged using microscopy, including obviously Dino di Carlo's & Luke Lee's arrays.

Flow cytometry. The cells are interrogated once.

Some examples in ref [12]!

Droplets are useful to trap small number of cells. However, the number of cells is normally Poisson distributed. This can be avoided using inertial fluidics due to the longitudinal ordering. Another challenge (or benefit depending on the circumstances) is that the cells are isolated causing buildup of rest products and depletion of nutrients.

3. (C) Why is it interesting to study single cells?

Several reasons (in analogy to single-molecule biophysics): heterogeneity within a cell population, time evolution of single cells, correlation between parameters, detection of rare cell types. Another important point is that single-cell techniques allows us to distinguish between the case where all cells change their behavior and the

case where some cells change their behavior. In bulk these two cases are indistinguishable.

Some examples in ref [12].

References

1. Persson, F. and J.O. Tegenfeldt, *DNA in nanochannels - directly visualizing genomic information*. Chemical Society Reviews, 2010. **39**(3): p. 985-999.
2. Huang, L.R., et al., *Continuous Particle Separation Through Deterministic Lateral Displacement*. Science, 2004. **304**(5673): p. 987-990.
3. Holm, S.H., et al., *Separation of parasites from human blood using deterministic lateral displacement*. Lab on a Chip, 2011. **11**(7): p. 1326-1332.
4. Beech, J.P., et al., *Sorting cells by size, shape and deformability*. Lab on a Chip, 2012. **12**(6): p. 1048-1051.
5. Weigl, B.H., R.L. Bardell, and C.R. Cabrera, *Lab-on-a-chip for drug development*. Advanced Drug Delivery Reviews, 2003. **55**(3): p. 349-377.
6. Takagi, J., et al., *Continuous particle separation in a microchannel having asymmetrically arranged multiple branches*. Lab on a Chip, 2005. **5**(7): p. 778-784.
7. Davis, J.A., et al., *Deterministic hydrodynamics: Taking blood apart*. Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(40): p. 14779-14784.
8. Long, B.R., et al., *Multidirectional sorting modes in deterministic lateral displacement devices*. Physical Review E, 2008. **78**(4).
9. Di Carlo, D., et al., *Continuous inertial focusing, ordering, and separation of particles in microchannels*. Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(48): p. 18892-18897.
10. Squires, T.M. and S.R. Quake, *Microfluidics: Fluid physics at the nanoliter scale*. Reviews of Modern Physics, 2005. **77**: p. 977-1026.
11. Di Carlo, D., N. Aghdam, and L.P. Lee, *Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays*. Analytical Chemistry, 2006. **78**(14): p. 4925-4930.
12. Di Carlo, D. and L.P. Lee, *Dynamic single-cell analysis for quantitative biology*. Analytical Chemistry, 2006. **78**(23): p. 7918-7925.
13. Di Carlo, D., L.Y. Wu, and L.P. Lee, *Dynamic single cell culture array*. Lab on a Chip, 2006. **6**(11): p. 1445-1449.