MICROFLUIDIC PARTICLE TRACKING TECHNIQUE TOWARDS WHITE BLOOD CELL SUBTYPE COUNTING AND SERUM PROTEIN QUANTIFICATION

BY

TANMAY GHONGE

THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Bioengineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

Adviser:
Professor Rashid Bashir
Abstract

Microfluidic technologies have gained wide acceptance in the past decade as diagnostics tools in clinical setting world-wide. This is primarily due to the fact that microfluidic technologies enable rapid, quantitative assays from small amount of physiological sample in an easy-to-use, portable platform. In this work, we will describe a microfluidic technique that can be built upon to count white blood cell subtypes or serum protein from a drop of blood. Traditionally, researchers have counted white blood cell subtypes by capturing them. However, an elegant and more accurate way to do the same is by exploiting the transitory interactions between the antigen on the surface of the cell and a cognate antibody. Cells expressing the antigen of interest will take longer to traverse a microchannel which has been coated with a cognate antibody compared to the cells which don’t express that antigen. To our knowledge, no microfluidic assay exists which can rapidly count cells using this principle. Towards this end, we have developed a repeatable experimental technique to control the transit time and the order of particles in a microchannel. To least affect the uniformity of transit time, we have also optimized the geometry of pillars in the microchannel on which antibodies are functionalized.
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1. Introduction

Microfluidics has revolutionized clinical diagnostics in the past decade. The ability to rapidly and quantitatively determine biomarkers from small amount of physiological sample has accelerated medical research and patient care all over the world [1]. Part of the reason why microfluidics received wide acceptance as a diagnostic tool was the fact it enables hands-free and inexpensive tests. This makes it a particularly attractive technique for the developing world [2]. Developing parts of the world are resource poor in both capital and trained personnel. Only those techniques which will get around these constraints will ever have a chance of becoming viable in these parts of the world [3] [4]. It wouldn't be a hyperbole to say that microfluidics, in this respect, has surpassed these expectations.

There are numerous point-of-care microfluidic assays which are currently used on the field to test patients for disease biomarkers. The tissue that contains the largest representation of human proteome is blood [5]. Blood also contains white blood cells responsible for protecting human body against the invasion of thousands of microbes that attack human body every day. Quantitative understanding of variation of proteins during a disease can indicate much about the state of a disease and help save millions of lives every year. Similarly, tracking the concentration of white blood cells can provide invaluable insight to a doctor while treating a patient. A classic example of this the variation of CD4+ T cells in a patient infected with HIV (Fig. 1). HIV virus selectively kills CD4+ T cells as it is recognized by the CD4 receptors and subsequently internalized and then utilizes the cell’s transcriptional machinery to replicate itself. A CD4+ T cell count is routinely prescribed by physicians to track the efficacy of treatment [6].

The aim of this work is develop a technique that could be used to count both cells and quantify serum proteins. We will present the work to date on the basic technology develop and describe a framework which can be built upon to expand the work and begin assay testing and validation.

1.1 Flow Cytometry

Flow cytometry is the state of the art technique used to quantify concentration of proteins on the surface of a cell [7]. In this technique, the proteins on the surface of the cell are labelled with a fluorescent reporter or a fluorophore. A fluorophore is a special molecule which can emit light in a narrow range of frequency upon excitation by light of a specific frequency. Cells tagged with fluorophores are made to pass through a region illuminated by lasers (Fig. 2). Fluorophores emit light
which is detected by sensors. Intensity of light emitted by a fluorophore gives a quantitative idea about the amount of fluorophore on each cell. Using the intensity of light recorded by the sensor, the surface expression level of protein can be quantified.

However, this method of quantifying surface protein expression is not ideal for a lab-on-a-chip device. Firstly, this instrument requires a trained technicians to operate. In resource limited environments a trained technician may not always be readily available. Secondly, the equipment is prohibitively expensive. It costs upwards of $50,000, which makes it suitable only for centralized labs. Thirdly, the instruments is bulky and cannot be moved around once installed. All these factors make flow cytometers less than ideal choice for a hand-held lab-on-a-chip diagnostic device that can be used in the developing world

1.2 Microfluidic Coulter Counters

Coulter counters are instruments which can be used to count and size particles suspended in an electrolyte. At the core of this instrument is an orifice larger than the size of particle to be counted. Electric current across this orifice is constantly monitored in response to an applied potential difference. According to the coulter principle, particle flowing through the orifice produce a change in impedance of the orifice that is proportional to its volume [8]. The change in impedance causes a change in the electric current flowing through the orifice. The change in current is abrupt and looks like a sharp spike on electric current vs time plot (Fig. 3) [9]. Magnitude of the dip in the current across the orifice can be correlated with the size of the cell. Commercial coulter counters are routinely used in hematology labs across the world.

Coulter counters have an advantage over flow cytometers when it comes to its adaptability to lab-on-a-chip devices. Coulter counters can be easily miniaturized and hence are an ideal candidate for cell counting for a portable, lab-on-a-chip inspired device. Researchers have utilized coulter counters in microfluidic assays to perform cell sizing and differentiation of white blood cells from whole blood. Holmes et al have demonstrated the capability of such microfluidic counters where they not only counted white blood cells but also differentiated them into three distinct categories (viz. monocytes, neutrophils and lymphocytes) [10].
1.3 Cell Capture Techniques

Cell capture is a popular way of enumerating cells in a sample. Immunocapture of CD4+ T cells from whole blood was demonstrated by Watkins et al as a viable technique to enumerate CD4+ T cells from whole blood. In this technique, red blood cells are selectively digested using a lysing buffer. The lysing buffer has a different osmolality than blood which causes the red blood cells to burst due to high osmotic pressure. In the second stage of the device, quenching buffer is introduced to prevent the white blood cells from bursting (Fig. 4). The white blood cells then flow through a coulter counter where they are counted. Following the first coulter counter, the white blood cells flow in the capture chamber which is functionalized with antibody conjugate to CD4 protein. Remaining white blood cells, devoid of CD4 T cells, are counted again using a coulter counter. Number of CD4 T cells in the sample is simply the difference of counts of the two counters.

Researchers have utilize mechanical obstruction based methods to capture circulating tumor cells clusters [12]. In this study cancer cell clusters, owing to their larger size were trapped by triangular posts (Fig. 5). Captured cells were eluted and further analysis was performed.

An important shortcoming of cell capture assays is that they have low sensitivity. Watkins et al report 20 cells/µL as the lower limit of CD4 cells that can be counted with cell capture technique. Nonspecific capture was attributed as one of the leading causing of low sensitivity of their device.

1.4 Transient Interactions between Cell and Receptor

Instead of capturing the cells, one can potentially utilize the transitory bond formation between the cell surface receptor and an antibody to count or sort cells. Leukocytes are recruited to the inflammation site in the body by a process called leukocyte extravasation. During this process macrophages release cytokines such as IL-1, TNFα and C5a to cause the endothelial cells of blood vessels of infection to express cellular adhesion molecules like selectins [13]. Ligands on the surface of white blood cells recognize these molecules which causes the leukocytes to slow down and roll on the surface of the vessel wall. Eventually the cell firmly adheres to the cell wall (Fig. 6).

Assays which utilize the transitory bond formation have been exploited by researchers to sort cells. Choi et al patterned selectins on the bed of an asymmetric corrugated PDMS to separate HL60 cells from K562 cells [14]. HL60 are known to express high levels of P-selectin glycoprotein ligand-1 and exhibit rolling on P-selectin coated surfaces. The direction of rolling is different from the direction of fluid flow.
which causes separation (Fig. 7). A similar yet a more clinically more relevant sorting technique was demonstrated by Bose et al where lateral migration of neutrophils on P-selecting modified gold pattern led to sorting of neutrophils in the channel [15].

Techniques based on transient interaction between receptor-ligand offer an attractive advantage over cell capture assays that cells are fully recovered at the end of the assay for downstream analysis. They are expected to be more specific and sensitive as compared to cell capture assays. Such a technique has not been used to count cells to the best of our knowledge.

The fundamentals of this technique is depicted in Figure 8. Let’s say we are interested in enumerating CD4+ T cells in a sample. We coat the surface of the microchannel with anti CD4 antibodies. When CD4+ T cells are flow through the microchannel they interact with antibodies which slows them down. As compared to a non CD4+ T Cell, a CD4+ T cell takes longer to traverse the microchannel. The two coulter counters at the entrance and exit of the microchannel are used to time each cell. The histogram of transit time of all the cells in the sample is expected to have two peaks – one corresponding to CD4+ T cells (on the right) and the other corresponding to all other cells. Gating techniques can then be used to enumerate CD4+ T cells in the sample.

This technique can be extended to counting cells expressing multiple antigens of interest (Fig. 9).
Fig 1. Representative plot of the variation of CD4+ T cell count and HIV virus concentration in an infected patient. CD4+ T cell count needs to be continuously monitored to assess the efficacy of the treatment. An HIV positive patient is said to have AIDS when CD4 cell count falls below 200/µL (red tail of the plot CD4 cell count)

Fig. 2 Schematic showing a flow cytometer in action. Cells conjugated with fluorophore are focused in a single file. Fluorophore on the surface of a cell is excited using lasers of appropriate frequency. Intensity of light emitted by the fluorophore can be correlated with amount of protein of interest on the surface of the cell. This image originally appears here: https://www.semrock.com/flow-cytometry.aspx
Fig. 3 Figure shows signal generated by a bead in a coulter counter. a) Figure shows the pulse train of many beads detected by the counter. b) Figure zooms into an individual event. Adapted from [9]
Fig. 4 Overview of CD4 capture technique. A) Schematic of microfluidic design and sample processing. (i) Infusing 10 µL of blood in the chip. (ii) On-chip erythrocyte lysis. (iii) Counting all white blood cells using a coulter counter. (iv) The capture chamber immobilized with anti CD4 antibody. (v) counting remaining white blood cells using a coulter counter. (B) Image of microfluidic chip used in the experiment. (C) Two device design explored in the study. (D) Graphical layout of set-up and acquisition system. (E) Typical bipolar pulses obtained as cells flow over the electrodes. Adapted from [11].
Fig. 5 Representative images of CTC clusters isolated from patients with metastatic cancer. Adapted from [13]
Fig 6 Diagram showing white blood cells rolling using weak interactions with selectin along the vascular endothelium. Image originally appears in [16]
Fig 7 Schematic of a microfluidic channel comprising of focusing ridges. HL60 cells roll laterally to the direction of flow of fluid and hence get sorted. Adapted from [14].
Fig. 8 Schematic shows working principle of the technique. CD4+ T cells interact with anti CD4 antibodies and consequently take longer to traverse the microchannel as compared to a non CD4+ T cells.
Fig. 9 Technique can be extended to assay for cells expressing multiple biomarkers. A cell expressing one biomarker slows down in one half of the microchannel whereas a cell expressing both biomarkers slows down in both halves.
2. Materials and Methods

2.1 Hydrodynamic Focusing

We know from the knowledge of fluid mechanics that the velocity of fluid flowing in a microchannel is not uniform. The velocity profile, in fact, across a cross section is parabolic (Fig. 10). Fluid in a rectangular microchannel travels the fastest at the geometric center of the cross section and the slowest near the walls. Particles randomly injected from the inlet are be randomly distributed at any cross-section. As a result the particles travel at different speeds. Any assay which attempts to measure the transient interactions of biomolecules by linking it to the time taken by a particle must be able to take this effect into account. It is for this reason that focusing the particles in a tight stream becomes mandatory. The particles focused in a narrow tube will travel at same speed and traverse approximately the same distance in the microchannel. This causes the particles to travel in first-in first-out fashion which allows us to identify all the particles on both counters uniquely.

Researchers have used a number of physical forces to focus particles such as inertial [17], dielectrophoretic [18], or acoustic [19]. Because of the elaborate experimental set-up required or the need for the flow rates to be prohibitively high, these techniques cannot be used to focus the particles. For example, the flow rate required to hydrodynamically focus particles of the size of white blood cells is of the order of 100 µL/min [20]. In a typical microchannel, which is 50 µm tall and 100 µm wide, the shear stress generated at the walls is 400 dynes/cm². This is two orders of magnitude higher than the shear stress conducive for antibody-antigen interaction reported in the literature [21].

The technique that can be integrated with our cell counting set-up and easy to implement is called hydrodynamic focusing. In this technique, fluid streams are used to focus the fluid stream containing particles of interest in a narrow tube [22]. The particles contained in this narrow tube flow in an orderly fashion parallel to the channel walls in the direction of flow because the flow is laminar. Fluid flow is called laminar where there are no cross currents perpendicular to the direction of flow. It is characterized by low Reynolds number (Re<2000, see Eq. 1 below). Such a flow is commonly observed in microchannels at typical flow rates.

\[ \text{Re} = \frac{\rho v L}{\mu} \]

Where \( \rho \) and \( \mu \) are the density and viscosity of fluid respectively, \( v \) is velocity, \( L \) is the characteristic length scale of microchannel.
The stream carrying the particles or beads is focused in a narrow tube of flow of uniform velocity as shown in figure 10. This technique was previously described by Simonnet et al to focus nanoparticles [23]. Buffer carrying beads is injected through port A in microchannel of height 15 μm. Focusing buffers from port B and C are introduced in a channel of height 100 μm and 15 μm respectively. Buffers flowing from port A and C (15 μm height) can be effectively thought to be injected from the bottom of the channel since the height of these channel is 15% of the height of the microchannel where transit time is measured (connected directly to port B). Fluid flowing from port B and C serve to sandwich fluid flowing from port A. This step provides out-of-plane focusing for particles. Fluid flowing from port D pinches the flow further. This provides in-plane focusing for fluid. From this point forth, stream containing the beads is tightly focused and the beads are expected to travel at uniform speed in the microchannel. Motion of beads in the microchannel was captured using a high speed camera (Vision Research, Phantom v310)

2.2 Experimental Set up

Syringe pumps (Harvard Biosciences) are used to inject beads and focusing buffer (PBS) in the microfluidic channel. Beads flow over electrodes connected to a lock-in amplifier supplying an AC signal at 303 kHz (1 V peak to peak). Current flowing between the electrodes is fed to a current amplifier (Zurich Instruments, Switzerland) to boost the signal-to-noise ratio. Output from this instrument is fed back to the lock-in amplifier to extract the signal. Noise free data generated at this step is recorded at 50 kHz by PCI-6351 DAQ card (National Instruments, Austin, TX). Data is stored on the hard drive for further analysis.

2.3 Recipe for fabricating electrodes

a) Piranha clean (Sulfuric Acid:Hydrogen Peroxide::1:1), 15 minutes; rinse 10 minutes; nitrogen dry.

b) Dehydration bake @ 110 C, 5 minutes minimum; 5 minutes cool-off.
c) Spin LOR 3A (put on enough LOR 3A to cover entire wafer before spinning; this helps chip yield greatly) a. 0 to 500 rpm in 1 s (acceleration setting) b. hold at 500 rpm for 2 s c. 500 to 3000 rpm in 2 s (acceleration setting) d. hold at 3000 rpm for 35 s

d) Soft bake: 5 min. @ 183 C (directly on hotplate, not with metal holders); 5 minutes cool off 5. Spin S1805 (put on enough S1805 to cover entire wafer, not just 4 mL) a. 0 to 500 rpm in 1 s (acceleration setting) b. hold at 500 rpm for 5 s c. 500 to 4000 rpm in 3 s (acceleration setting) d. hold at 4000 rpm for 40 s

e) Soft bake, 90 s @ 110 C; 5 minutes cool off (important before exposure to prevent wafer from sticking to mask)

f) Exposure energy: 28 mJ/cm2: a. 3.7 s exposure time with MNTL’s Quintel (currently 7.5 mW/cm2 power output) b. 2.6 s exposure time with MNMS’ Mask Aligner (currently 21.4 mW/cm2 power output)

g) Post Exposure Bake: 60 s @ 110 C (wafer directly on hotplate)

h) Develop in CD-26 for 18 s a. CD-26 underneath developer hood, on right b. Let wafer sit in developer (no agitation, swirling, etc.; this can cause features to break off of wafer)

i) Rinse in DI water for 2 minutes in a dish with DI water gently flowing into it or simply swirling the wafer gently in a static dish of DI water

j) Examine wafer under microscope.

k) Perform 20 s oxygen plasma on wafer(s) before placing in CHA for metal deposition

l) Evaporation: 250 Angstroms of titanium + 750 Angstroms of gold per wafer 14. Metal lift-off (do this in the biolab) 56 a. Place wafers in Remover PG at 70 C for 1 hour+ b. Dump liquid into non-halogenated solvent waste container c. Wipe gently with a cleanroom wipe to remove any stubborn metal

2.4 Recipe for Fabricating PDMS Channels

a) Use lithography to described above to pattern alignments on the silicon wafer

b) Spin 5ml SU8-10 at 2100 rpm for 30 s.

c) Prebake @ 65 C for 2 min. Softbake @ 95 C for 5 min. Turn-off hotplate. Let it cool to room temperature on hot plate itself.

d) UV expose - 150 mJ/cm2 in Soft Contact, Constant Dose mode
e) Bake at 95 C for 1 min starting from room temperature. Turn-off hotplate. Let it cool to room
temperature on hot plate itself.

f) Spin 5 ml SU8-50 at 2000 rpm for 30 s

f) Prebake @ 65 C for 10 min on a hotplate. Softbake @ 95 C for 30 min hotplate. Turn-off
hotplate. Let it cool to room temperature on hot plate itself.

h) UV expose – Exposure energy = 450 mJ/cm2 in Soft Contact, Constant Dose mode

i) Post Exposure Bake 1 - @ 65 C for 1 min

j) Post Exposure Bake 2 - @ 95 C for 10 min. Turn-off hotplate. Let it cool to room temperature on
hot plate itself.

k) Develop in SU8 Developer for 10 min (shake well)

l) Rinse with IPA. Nitrogen Dry. (If you see white film during rinse then it is underdeveloped.
Develop in SU8 again for 3-4 mins)

m) Hard bake @ 150 C for 5 min. Turn-off hotplate. Let it cool to room temperature on hot plate
itself.

n) Silanize the mold with MPTMS.

2.5 Soft Lithography

a) Pour PDMS over the SU8 mold (1:10 :: cross linker : PDMS)

b) Place the petri dish in desiccator for 15 min to remove air bubbles

c) Bake in oven at 65 C for at least two hours

2.6 Validating Coulter Counter

In order to measure the transit time of a bead it is very important that the electrical sensor has a high
fidelity. It should not only miss beads entering the counting region and it should not generate false
positives. Towards this end, experiments were conducted to measure the accuracy of this counter.
Beads were injected at 5 μL/min in the microchannel which looped on one electrode. Beads were
sensed twice on the same electrode which were sensed twice, at the entrance and at the exit. The
number of beads entering the microchannel should be equal the number beads exiting the
microchannel. Figure 11 shows the schematic of the microchannel in which this experiment was
performed.
2.7 Measuring Transit Time

The assembled microfluidic devices were infused with beads from inlet port A at 3 μL/min. Focusing buffer was injected from inlet port B, C, and D at 9 μL/min, 4.5 μL/min and 9 μL/min respectively. The signal generated by beads flowing over entrance was saved separately from the signal generated by beads flowing over exit counter. At the end of the experiment, collected data was processed offline. Since focusing enables the beads to travel in first-in first-out fashion transit time can be very easily calculated. Transit time of a bead through the channel is simply the difference in the timestamps of a bead in over the entrance counter and exit counter. Figure 12 shows the schematic of the microchannel in which this experiment was performed.

We also calculate a figure of merit, \( \phi \), which is the probability that a bead will be passed by another bead which entered the microchannel just after it. The smaller the value of \( \phi \) the higher the percentage of beads that traversed the microchannel in first-in first-out fashion. The probability of passing is function of average rate at which beads enter the microchannel \( \lambda \) and the spread of the histogram of transit time of beads \( \sigma \). The derivation of the analytical expression of \( \phi \) is outlined in the appendix. The final expression is reproduced below

\[
\phi = \frac{1}{2} \left( 1 - e^{-\frac{\lambda^2}{\sigma^2}} \text{erfc} \left( \frac{\sigma \lambda}{\sigma^2} \right) \right)
\]

2.8 Optimizing Pillar Arrangement

Beads flowing at uniform speed bump into pillars coated with conjugate proteins. These pillars introduce gradients in the velocity field altering the speed of focused particles. Although this defeats the very purpose of focusing beads, having pillars in the microchannel cannot be avoided. Pillars provide the necessary surface for immobilizing proteins.

The gradients in the velocity profile would disrupt the first-in first-out motion of the beads in the microchannel. In order to avoid this, one must be able to exploit these velocity gradients such that average speed of particles remains uniform.

Consider figure 14, in this figure the horizontal distance (\( \varepsilon \)) between subsequent rows is such that the pattern of rows repeats after every 5 rows. Here, \( \varepsilon = \frac{\lambda}{n} \), where \( \lambda \) is the horizontal spacing between the center of two pillars. The vertical distance between the centers of pillars is also \( \lambda \). The total fluid flux
between two pillars can be divided into 5 flow streams carrying equal amount of fluid as shown in figure 14. Since the velocity profile (mapped horizontally) between any two pillars is parabolic, at the first row, fluid stream 3 flows the fastest. Fluid flows slower in stream 2 and the slowest in stream 1 (similar argument can be made for stream 4 and stream 5). Notice that the fluid streams shift their positions cyclically at each row and after 5 rows (i.e. at the 6th row) they assume their original positions. Therefore, the speed of fluid streams also changes cyclically. This means that over a distance of 5 rows all the fluid streams have same average speed. Hence, all particles below a critical diameter will travel at a uniform average speed over a distance which is an integral multiple of $5\lambda$ [24]. This is the minimum distance over which the average speed of particles remains uniform. This minimum distance can change depending on how many rows it takes for the cyclic pattern of rows to repeat. We call the number rows after which the cyclic pattern of rows repeats as the period (denoted by letter $n$) of the pillar geometry.

We want to find out minimum possible period where all particles bump into at least one pillar per cycle. Motion of beads was recorded using a high speed camera in staggered geometry with period 2, 3, 4, 5 and, 7.
Fig. 10 Parabolic velocity profile of a laminar flow in a rectangular microchannel. The axes are normalized to the channel height, channel width and maximum velocity. Adapted from [25]
Fig. 11 Schematic of hydrodynamic focusing. This figure originally appears in [23]
Fig. 12 Schematic of the microfluidic coulter counter used for validation. Beads are injected at 5 µL/min from the inlet port. One bead is counted four times, twice on each electrode. Four measurements are taken on each bead to make the validation robust.
Fig. 13 Graphic drawing of the mask used to make the microchannel. This geometry was used to test the efficacy of hydrodynamic focusing.
Fig. 14 Schematic showing flow streams around staggered pillars in a microchannel. Horizontal and vertical distance between any two closest pillars is $\lambda$. Horizontal shift between two subsequent rows of pillars is $\epsilon$. 
3. Results, Discussion, and Future Work

3.1 Characterizing Coulter Counter

As described in earlier section, beads were injected in a microchannel with two coulter counters, one at the entrance and the other one at the exit. See figure 12 for a schematic. This microchannel was connected to another channel in series which looped on a separate coulter counter. The beads flowing over the counter generate bipolar pulses as seen in figure 15. A sinusoidal pulse signifies a bead entering the channel whereas a reverse sinusoidal pulse signifies exiting the microchannel. Figure 16 shows the total count of beads entering and exiting both micro channels at two different flow rates. The entrance and exit counts on both micro channels are very close to each other. This shows that the counter neither misses a bead nor generates false positives.

The entrance and exit signatures of a bead were also examined. Ideally a bead should generate bipolar pulses of equal magnitude as it enters and exits the microchannel. Figure 17 shows the difference in amplitudes of a bead at the entrance and at the exit. Mean percentage deviation is -2.5% in channel 1 and -2.9% in channel 2. The distribution is quite narrow for a coplanar coulter which experiences several drawbacks compared to top-bottom type configuration.

Figures 16 and 17 prove that the coulter counter is quite accurate when it comes to detecting events making it attractive for lab-on-a-chip applications.

High fidelity of a counter such as this one makes it an attractive choice for inexpensive lab-on-a-chip applications.

3.2 Measuring Transit Time of a Bead in a microchannel

Transit time of a focused bead in the microchannel was measured using the coulter counters. Figure 18 shows the histogram of transit time for three separate but identical microchannels. Particle concentration was 4/µL, 6/µL and, 20/µL in fig 16 a, b, c respectively. Mean and variance of transit time are denoted by \( \mu \) and \( \sigma \) respectively. The three plots have identical metrics (\( \mu \) and \( \sigma \)). This shows that hydrodynamic focusing can be reliably used for focusing particles.
3.3 Optimum period of staggered geometry of pillars in a microchannel

It was found that geometry with period equal to two did not let all beads travel at uniform speed. This is because once a bead is trapped in a streamline it never escapes it. Although the speed of individual bead remains constant, all the beads don’t have uniform speed.

The smallest period which satisfied all the constraints is 3 (fig 19)

3.4 Conclusion and Future Work

We have described a microfluidic technique that can used to focus beads to achieve uniformity in the transit time of beads in a microchannel. We used hydrodynamic focusing to focus beads in a narrow tube of particles. This narrow tube of particles traverses the microchannel at uniform speed. We saw that this technique was repeatable and provided good control over transit time of particles.

We then optimized the arrangement of pillars in the microchannel so as to least affect the transit time of focused beads while ensuring that particles still bump into the pillars.

At this point we are all set to immobilize the microchannel with protein of interest and observe the modulation of the transit time caused by transitory bond formation. We are going to test for streptavidin-biotin interaction in our experiment. Since biotin-streptavidin is the strongest non-covalent interaction found in nature, we believe that it is the ideal starting point.
3.5 Figures

Fig. 15 a) Pulse train of beads flowing past the coulter counter. b) Figure zooms into individual bead and shows inter pulse duration (or transit time of bead in microchannel). Solution was diluted so that at most bead was present in the channel.
Fig. 16 a) Bar plot shows the number of beads counted by two coulter counters in two micro channels connected in series. a) Beads injected at 5 µL/min are counted by co-planar coulter counters. The counts on both counters as well entrance and exit counts on the same counter agree very closely. b) Identical counting experiment is performed on the same coulter counters at 10 µL/min. Counts match very closely.
Fig. 17 Histogram shows percentage difference in amplitude of a bead when detected at the entrance and at the exit of a microcannels. Two microchannels, Channel 1 and Channel 2, are connected in series with beads being injected in Channel 1 first. See figure 12 for the schematic a) Beads are flowing at 5 µL/min. b) Same as a) but beads are flowing at 10 µL/min.
Figure 18 (cont.)

Chip 29, A=3, B=9, C=4.5, D=9

- $\mu = 1.49$ s
- $\sigma = 0.013$ s
- $\lambda = 0.2$ s$^{-1}$
- $\phi = 0.001$
- $N = 321$

Figure 18 (cont.)

Chip 30, A=3, B=9, C=4.5, D=9

- $\mu = 1.48$ s
- $\sigma = 0.014$ s
- $\lambda = 0.3$ s$^{-1}$
- $\phi = 0.002$
- $N = 594$
Fig. 18 a), b), and c) Histograms of transit time of hydrodynamically focused bead in three different microchannels
Figure 19 Schematic showing the optimum of arrangement of pillars which maintains the first-in first-out motion of beads.
References


Appendix

A.1 Finding the probability of passing

In this section we are going to find the probability, given the distribution of transit time of beads, of a bead passing another bead which entered the microchannel just before it.

We assume the following

i) No two beads enter the microchannel at exactly the same time. There is a finite time difference between the entrance events of two beads

ii) Transit time of beads is normally distributed

Consider bead 1 which takes time $t_1$ to traverse the microchannel. Another bead, bead 2, enters the microchannel after time $w$ and takes time $t_2$ to traverse the microchannel. So, bead 2 will pass bead 1 if

$$t_1 > w + t_2$$

We have assumed that $T_1$ and $T_2$ are normally distributed. Hence, $T_1 \sim N(\mu, \sigma)$ and $T_2 \sim N(\mu, \sigma)$.

Since that the rate at which the beads enter the microchannel is poisson distributed, the time difference between entrances of two bead entering the channel consecutively is exponentially distributed. Hence, $W \sim \text{Exp}(\lambda)$, where $\lambda$ is average rate at which beads enter the microchannel.

We have to find the probability $P(t_1 > t_2 + w)$ or $P((t_1 - t_2) - w > 0)$. Let us declare a variable $t$, where $t = t_1 - t_2$. Let us declare a variable $\nu$, where $\nu = t - W$. Now, we have to find $P(\nu > 0)$

Since $T_1$ and $T_2$ are normally distributed with mean $\mu$ and standard deviation $\sigma$, $T_1 - T_2$ is normally distributed with mean 0 and standard deviation $\sqrt{2}\sigma$. Symbolically,

$$T \sim N(0, \sqrt{2}\sigma)$$

Now, let us calculate $P(\nu > 0)$

$$P(\nu > 0) = 1 - P(\nu < 0) = 1 - \int_{-\infty}^{0} f_{\nu}(\nu)d\nu$$

The conditional probability distribution $f_{\nu/w}(\nu | W = w)$ can be calculated as
\[ F_{v|w}(v | W = w) = f_{v|w}(v \leq v | W = w) = f_{T|w}(T \leq w + v | W = w) = f_{T}(T \leq w + v) \]

(since T and W are independent random variables)

Differentiating both sides w.r.t. \( v \)

\[
\frac{d}{dv} F_{v|w}(v) = \frac{d}{dv} \int_{-\infty}^{w+v} f_{T}(w+v) dt
\]

\[
f_{v|w}(v) = f_{T}(w+v) \frac{d}{dv}(w+v)
\]

Now,

\[
f_{v}(v) = \int_{0}^{\infty} f_{v|w}(v) f_{w}(w) dw
\]

\[
= \int_{0}^{\infty} f_{T}(w+v) f_{w}(w) dw
\]

\[
= \left[ e^{-\frac{(w+v)^2}{2(\lambda \sigma^2)}} \right]_{0}^{\infty} \int_{0}^{\infty} \lambda e^{-\lambda w} dw
\]

\[
= \frac{\lambda e^{-\frac{\nu^2}{4\sigma^2}}}{2\sigma\sqrt{\pi}} \int_{0}^{\infty} e^{-\left[\frac{w^2}{4\sigma^2}\right] + \left[\frac{\lambda w}{4\sigma^2}\right]} dw
\]

\[
(\text{Using} \int e^{-ax^2-bx} dx = \frac{\sqrt{\pi} e^{-\frac{b^2}{4a}}}{2\sqrt{a}}, \text{here } a = \frac{1}{4\sigma^2}, b = \frac{\lambda}{2} + \frac{\nu}{4\sigma^2})
\]

\[
f_{v}(v) = \frac{\lambda}{2} e^{-\frac{\nu^2}{4\sigma^2}} e^{\left[\frac{(\lambda+\frac{\nu}{2\sigma})^2}{2\sigma}\right]} \text{erfc}(\frac{\lambda \sigma + \frac{\nu}{2\sigma}}{2\sigma})
\]

(One can integrate \( f_{v}(v) \) from \(-\infty\) to \( \infty \) and find that the value of this integral 1. This satisfies one of the criteria for a legitimate probability density function)
With an analytical expression of \( f_v(v) \) in hand, we can calculate 

\[
\int_{-\infty}^{0} f_v(v) \, dv = \frac{\lambda e^{\lambda^2 \sigma^2}}{2} \int_{-\infty}^{0} e^{\lambda \sigma v + \frac{v^2}{2\sigma}} \text{erfc} \left( \frac{v}{2\sigma} \right) \, dv
\]

let \(-\lambda \sigma + \frac{v}{2\sigma} = z\)

\[
\int_{-\infty}^{0} f_v(v) = \frac{\lambda e^{\lambda^2 \sigma^2}}{2} (2\sigma) \int_{-\infty}^{\lambda \sigma} e^{-(z+\lambda \sigma)(2\sigma)} \text{erfc}(z) \, dz
\]

\[
\int_{-\infty}^{0} f_v(v) = \frac{\lambda e^{\lambda^2 \sigma^2}}{2} (2\sigma) e^{2\lambda^2 \sigma^2} \int_{-\infty}^{\lambda \sigma} e^{2\sigma z} \text{erfc}(z) \, dz
\]

\[
\therefore \frac{1}{b} b^z \text{erfc}(az) + \frac{1}{b} e^{2\sigma z} \text{erf}(az - \frac{b}{2a})[1]
\]

here, \(a = 1\) & \(b = 2\sigma \lambda \)

\[
\int_{-\infty}^{0} f_v(v) = \frac{1}{2} (1 + e^{\sigma^2 z^2} \text{erfc}(\sigma \lambda))
\]

Therefore, the probability of passing, \(\phi\), is

\[
\phi = 1 - \int_{-\infty}^{0} f_v(v) \, dv = \frac{1}{2} (1 - e^{\sigma^2 z^2} \text{erfc}(\sigma \lambda))
\]

The probability of passing, \(\phi\), as a function of \(\lambda\) and \(\sigma\), is plotted in fig. A1. \(\phi\) increases monotonically with both \(\lambda\) and \(\sigma\). \(\phi\) levels off at 0.5 for large values of \(\lambda\) and \(\sigma\). This is expected because either at high particle concentration (consequently large \(\lambda\)) or when the time taken by beads to traverse the microchannel is spread over a large range of values (consequently large \(\sigma\)), a passing event is equally probable to the event when the bead does not pass the bead in front of it.
Fig. 20 3D plot shows the variation of probability of passing, $\phi$, with the average rate at which beads enter the microchannel per second ($\lambda$) and the standard deviation of transit time of beads ($\sigma$) in the microchannel.

References