REVIEW ARTICLE

Advancements in Flow Cytometry Fluidics: A Hypothesis

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Abstract

Flow cytometry is an invaluable tool to unravel the complexities of cell signaling. This article discusses the flow focusing principle behind most conventional cytometers and proposes an optimal Reynolds number range supported by published literature for increasing the accuracy of the data obtained by reducing doublets in the data. A description of the implementation of the microstructures in microfluidic cytometry chips for two-dimensional focusing along with an overview of its advantages has been presented along with how the proposed hypothesis can benefit this particle focusing scheme. Methods to test the given hypothesis have also been discussed.

Keywords: Cytometry chips, Flow cytometry, Fluidics, Microfluidic Reynolds number, Two-dimensional focusing.

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Introduction

The flow cytometer is an extremely powerful clinical diagnostic tool that has been made possible through the knowledge of the realm of microfluidics. Efforts continue to be made to optimize the working of the flow cytometer and applying our knowledge of the physics of microfluidics will result in important improvements to this diagnostic tool. From initially being able to count 1,000 particles per second to today's impressive 5,000 particles per second count,[1] the principles behind flow cytometry have come a long way. This article aims to highlight hypothetical advancements that can be made to flow cytometry applying knowledge of microfluidic systems.

Optimizing hydrodynamic Focusing

Most flow cytometers use the principle of hydrodynamic focusing to obtain a thin channel of the sample where the cells can be detected effectively. The sample is sandwiched between two layers of sheath fluid and the dimensions of the channel can be adjusted by adjusting the flow rates and therefore the pressure of the three adjacent channels. The flow stream which is introduced at a higher flow rate will occupy a larger part of the channel, than a flowing stream at a lower flow rate, which will force the slower stream into a smaller cross-sectional area.[2] Hydrodynamic focusing minimizes the effect of the characteristic parabolic flow profile and maintains consistent linear velocities of the flowing particles to enhance the precision of measurements.

A detailed study of hydrodynamic focusing is incomplete without the knowledge of Reynolds number[3] which is a dimensionless parameter used to predict flow patterns in different flow situations. Generally laminar (smooth) flow is characterized by a low Reynolds number and as the Reynolds number increases—it leads to more chaotic and turbulent flow—which causes a decrease in the accuracy of measurements made. In the most general case, the expression for Reynolds number is given by:

$$R = \frac{\rho v D}{\mu}$$

R—Reynolds number
ρ—fluid density
v—flow velocity
D—diameter of channel
μ—fluid viscosity

Deformation of the Sample Stream Leading to Less Accurate Measurements

A lot of work has been done in applying the principle of hydrodynamic focusing; however, little is known about it at a basic level. It is a complex phenomenon that is difficult to model. However, detailed three-dimensional investigations conducted using confocal laser scanning microscopy (CLSM) show that hydrodynamically focused streams are usually deformed in two ways:[4]

- Non-uniform distribution of the width of the stream.
- Curvature and deviation of the focused stream from the axis of the channel due to the side streams (sheath fluid).

The sudden change in flow direction at the point of intersection of sheath and sample fluid produces a secondary fluid flow which forces the fluid to move toward the walls of the channel.[4] This leads to the mixing of sheath and sample fluids and reduces the flow focusing efficiency.

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Relevance of Reynolds Number to the Width and Shape of Focused Flow Streams

On analyzing the data, the flow profile for the focused stream of sample fluid[4] falls broadly into four categories based on the Reynolds number:

- At $5 < R < 8$ a flat focused plane of sample fluid with nearly constant width can be obtained.
- At $R < 5$ the focused stream acquires a slightly convex shape.
- At $R > 10$ a double concave shape is present.
- As the R approaches complete layering of the focused stream on the sides of the channel takes place.

Effect of Side Stream Ratio on the Position of the Focused Stream in the Outlet Channel

Q_a, Q_b—flow rates of focusing streams

$\frac{Q_a}{Q_b}$ —side stream ratio

A significant aspect in the designing and operation of flow cytometers is determining the position of the focused stream in the channel for given flow conditions.

When the flow rates of the side streams are identical, i.e., $Q_a = Q_b$, then then the sample stream is focused at the center of the outlet channel.[4] As the side stream ratio increases, the focused stream is pushed away from the axis of the channel and toward the wall.[4] Experimental data obtained from plotting the deviation of the focused stream to the side stream ratio of channels with different dimensions also reveals that although the displacement of the stream from the central axis is independent of the channel dimensions, the curvature of the focused stream does depend on the dimensions of the channel and the curvature increases for smaller microchannels.

It can be inferred from the data that decreasing the size of the microchannel can reduce the loss of accuracy of data due to the curvature of the focused stream.

Cytometry Using Integrated Microfluidic Chips

The field of microfluidics is touted to be the future of flow cytometry, with the several components of cytometry being integrated on a single chip. Recent developments in microfluidics and microfabrication will allow flow cytometry to take place in challenging areas. There is also enormous potential for mass production and reducing the cost of conventional cytometers.[5]

Implementation of Microweirs, Chevron Grooves, and Other Microstructures for Effective Particle Focusing

Most conventional cytometers and microfabricated chips employ hydrodynamic focusing in one dimension, i.e., across the width of the channel. However, there is also a need for two-dimensional focusing of the flow stream not only from side to side but also from top to bottom to be able to accurately predict and detect the particles and prevent particle coincidences.

Advantages of Implementing Microstructures

The integration of simple groove and channel-like structures to adjust the flow profile of the sample is an elegant and relatively simple approach to ensure the two-dimensional focusing of the flow stream.[2] The use of these structures provides an effective alternative to applying sheath fluid both above and below the stream by redirecting the fluid from the sides to the top and bottom offering two-dimensional focusing. Studies by Golden et al.[2] have shown promising results with a microfluidic system implementing chevron grooves that can distinguish phytoplankton ranging from 1 to 80 µm.

The application of such grooves also solves another significant problem related to microflow cytometers which is the requirement of large volumes of sheath fluid. This can be a significant disadvantage in certain environments. The chevron grooves[2] have the unique property of reversing or "unsheathing" the focusing effect of the flow, returning the core and sheath streams to their initial positions. This property allows us to reuse the relatively undiluted sample fluid and uncontaminated sheath fluid. This can have enormous applications for automated and unattended microfluidic cytometry systems. Lee et al.[6] have also shown that sequential microweir structures induce vertical separation of particles making them flow in a straight line which is a relatively cost-effective solution to improve particle focusing.

Overall, the implementation of microstructures during microfabrication of channels is a viable and elegant solution to effectively focus particles from multiple directions which can easily be implemented in most microfluidic chips. It also does not require the use of multiple flows streamlines and its simplicity allows its widespread application.

Hypothesis

Based on the above-mentioned experimental data relating the Reynolds number and uniformity of width of the focused stream, it can be hypothesized that maintaining the Reynolds number in a small range from 5 to 8 (by adjusting the concentration of the sample, width of microchannel side stream velocity, and sample fluid velocity) will give optimal accuracy of data. The likely outcome of maintaining the width of the flow stream is the reduction of any abnormalities in the shape of the flow stream, prevention of particles acquiring lateral velocities, and an overall reduction in particle coincidences.

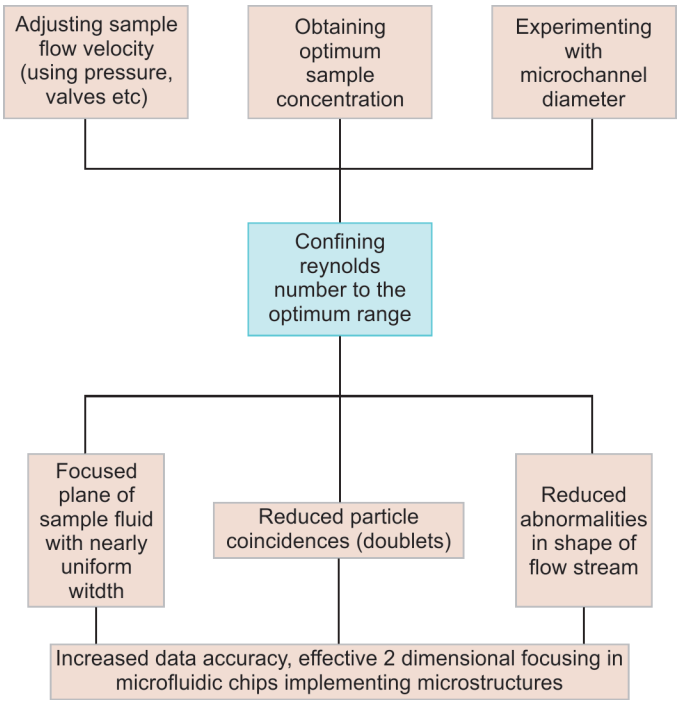
The implementation of this optimum Reynolds number range to microfluidic systems involving chevron grooves and microweir structures can ensure the effective ensheathment of the flow sample by the sheath fluid leading to better two-dimensional focusing of the sample. As the width of the sample stream will remain constant, it will allow the accurate placement of microstructures to allow effective focusing. Maintaining uniform width for the focused sample will also aid in the "unsheathing" or reversibility of the focusing effect of the flow whose advantages have been discussed earlier.

Moreover, if the experimental data comply with the hypothesis, an elementary computer program can be written which will recommend certain sample preparation criteria and values for flow velocities to ensure optimum accuracy of data based on Reynolds number range. It can also serve as a guideline while designing further microfluidic systems and obtaining an optimum value for microchannel diameter for various microfluidic chips. An optimal Reynolds number range also has immense potential for automated microfluidic cytometers where a computer can monitor the velocities of the sheath and sample streams ensuring that they remain confined to a particular range. Flowchart 1 summarizes the proposed hypotheses.

Testing the Hypothesis

The testing of the hypothesis would involve the microfabrication of several microfluidic chips with varying microchannel sizes with

Flowchart 1: Summary of the proposed hypotheses



and without the inclusion of microstructures such as chevron grooves and microweires. The flow profiles and structures of the streams can be monitored using microparticle image velocimetry (micro-PIV)[7] and CLSM[8] to obtain high lateral and axial resolution. Computational fluid dynamics (CFD)[9] simulations can also be used to predict the flow behavior in various microchannels. The width of the focused stream can be analyzed for various Reynolds number cases arising by varying the microchannel diameter and flow velocities.

The next natural step would be to test and analyze the viability of producing this required Reynolds number range for a real-world sample used in an integrated microfluidic chip and plotting the side and forward scatter data for various cases, these data can be used to analyze whether the optimum range reduces particle coincidences ("doublets") by plotting forward scatter (FSC) height vs FSC area.

It is expected that the optimal range for the Reynolds number suggested will be sufficiently tweaked after experimental analysis.

Conclusion and Clinical Significance

Doublet Discrimination

A doublet (particle coincidence)[10] occurs when two particles in the flow sample pass the laser interrogation point so close to each other that the cytometer is unable to distinguish them and it is registered as a single event. Doublets have many adverse effects in cytometry such as affecting the purity of the sorted sample in cell sorting, incorrectly identifying subpopulation of cells during analysis, and affecting staining patterns. Doublets are generally dealt with at the data analyzing stage by a process known as doublet discrimination, where the FSC height and are plotted.

Confining the Reynolds number to a particular range is expected to reduce the doublets and therefore increase the accuracy of the data garnered by flow cytometry.

Effective Two-dimensional Focusing

A standard Reynolds number range for integrated microfluidic chips implementing microstructures can greatly boost the effectiveness of this focusing method.

Developing a commercially accessible flow cytometer by implementing the above-mentioned techniques will also allow the entry of this technology into everyday clinics where disease progression of AIDS[11,12] and other conditions in countries such as Africa can be monitored with relative ease.

The realm of microfluidics continues to be an active area of research and the future of cytometry lies in effectively integrating all the components of a cytometer into the palm of our hand.

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