Three-dimensional Hydrodynamic Focusing for Integrated Optofluidic Detection Enhancement

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***Abstract*—Integrated optofluidics are ideal for high sensitivity detection due to the size of device features being near the small scale of sample particles (10 microns). Novel integrated optofluidic designs enable single molecule detection through hydrodynamic focusing, making the approach ideal for low concentration biosensing. Detection was enhanced and device operation characterized. Further enhancement is promised with optimization of existing designs.**

Keywords—biosensor, hydrodynamic focusing, optofluidics

I. Introduction

The search for extra-terrestrial life requires technology ideal for space travel that is small, lightweight, low power, and has high detection sensitivity of bioparticles. The ARROW biosensor fills this order as shown in previous experiments [1]. Yet, without improving sensitivity it is possible that signs of life may be present but go undetected. Three-dimensional hydrodynamic focusing promises to enhance detection capabilities and provides the best chance of success [2].

II. Theory/Concepts

A. Chip Based Diagnostics

The emergent field of Lab-on-a-Chip (LOC) is the pursuit of shrinking laboratory functions to the size of a microchip, see Fig. 1. These functions can include any combination of sample processing such as mixing, separating, heating, cooling, or detecting. Typically a LOC is designed for use with liquid samples but can be used with gaseous samples as well. In either case, the ability to move the fluid around the chip in a controlled manner is key to its usefulness. Most commonly, a LOC is designed as a biosensor to perform diagnostic functions for the purpose of determining the contents of a biological sample [3].

Lab-on-a-Chip research and development is driven by some of the same motivations as integrated circuits [4]. Integrated circuits are commonplace in our world today in the form of consumer electronics and generally perform several electronic functions on a single small sized microchip. Some benefits of this include the size, as well as lower cost of production, low power consumption, and high speed performance. Researchers hope to derive the same benefits with medical devices.

Diagnostics and analysis of biological samples involves many different fields of expertise. This might include biology, chemistry, physics, engineering, and others. Some of these field-related functions of the LOC scale easily, such as biology and chemistry, while physics and engineering are more challenging to scale down.

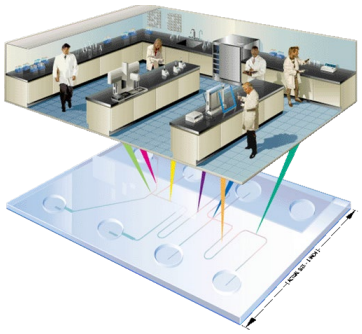

Fig. 1. "Lab-on-a-Chip" describes shrinking laboratory functions such as bio-sensing onto a microchip. https://www.gene-quantification.de/lab-on-chip.html

Fig. 2. Small scale fluidics exhibit different characteristics than large scale fluidics, represented here by surface tension. Image courtesy of Wikimedia.org

B. Microfluidics

Biological particles of interest are found in fluids. In order to test a sample to detect a bioparticle, we need to be able to move the particles around and analyze them in fluid. When shrinking fluids to the microscale we must consider that fluid behaves differently at the microscale than it does at the macroscale. This is most easily represented, as in Fig. 2, by a drop of water in which a small mass of liquid is of the size that it can hold its own weight by surface tension. A portion of water with greater mass does not form a drop.

At the scale of a Lab-on-a-Chip, the characteristics of water that dominate the system include fluidic resistance and laminar flow [5]. Fluidic resistance is determined largely by the dimensions of the fluidic channel and is considered when designing a chip. Laminar flow, as opposed to turbulent flow,

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describes the almost sheet like behavior of traveling fluid given certain conditions, including velocity and channel dimension. At the microscale, fluid travel almost always appears laminar. This highly predictable flow behavior makes engineering possible.

C. Optics and Photonics

A majority of sensitive diagnostics methods involves optics and fluorescence detection [6]. Fluorescence describes the use of light to identify a bioparticle. Often light is generated by a source, such as a laser, and shined on a target sample to energize the particles of interest, as seen in Fig. 3. When the particles return to their rest state by de-energizing, they release a photon of light which can be captured and detected.

Optics can be highly sensitive, requiring precision in use. At the macroscale, experienced technicians use expensive tools such as an optical table and special mounts to align the optical elements, represented in Fig. 4. At the Lab-on-a-Chip scale we can create integrated optics, similar to integrated circuits, where multiple light related functions can be performed on a single chip. Here the alignment of the optical elements is determined by design and fabrication. The critical characteristic of small scale optics is the optical mode which arises due to the wave properties of light, structure dimensions, material properties, and environmental factors [7].

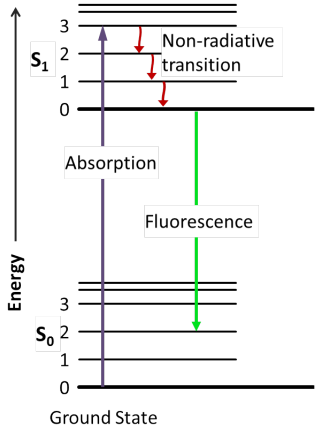

Fig. 3. Bio-sensors often use fluorescence for detection. Image courtesy of Wikimedia.org

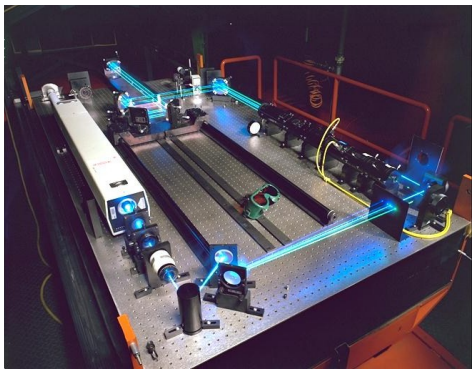

Fig. 4. Optics are highly sensitive, shrinking them down to the scale of a microchip has its challenges such as the optical mode. Image courtesy of Wikimedia.org

D. Optofluidics: Optics and Microfluidics

The combination of optics and fluidics has led to the development of interesting and useful technologies such as liquid mirror telescopes, displays, lenses, biosensors, molecular imaging, energy, molecular traps, and lab-on-a-chip devices. Here we combine them to create a biosensor for performing diagnostics. The device seen in Fig. 5 is a one centimeter by one centimeter silicon-based chip containing microfluidic channels and reservoirs as well as integrated optical elements. The key feature is the liquid-core waveguide which inherits all the challenges of both microfluidics and integrated optics.

As target particles pass through the liquid-core waveguide, laser light coupled to the chip through a fiber optic cable excites the sample, causing fluorescence. This signal light is guided to the detector and processed with a computer, the result seen in Fig. 6. The ARROW Biosensor has been used to successfully detect the presence of a variety of bacteria and viruses and has been proposed to detect the novel corona virus [1]. In each case, the target particle had a large number of fluorescent dye attached, making it easier to detect. Our goal is to detect single strands of DNA with few fluorescent beacons attached. This requires higher sensitivity than what the current ARROW Biosensor design can achieve.

E. Parabolic Flow Velocity

In the laminar flow regime represented in Fig. 7, particles flow side by side with the particles near the walls flowing slowest and the particles near the center flowing the fastest. This natural phenomenon creates a parabolic flow velocity profile [8]. Particle velocity variance introduces doubt in detection for a few reasons. One, the time spent in the excitation region where fluorescence occurs will be different for differently located particles. This translates to the amount of excitation light that the particles receives, and therefore the amount of fluorescence generated. This is seen in the signal plot as signal peaks of different height or intensity. It can be difficult to distinguish some of these peaks from the noise inherent in the system.

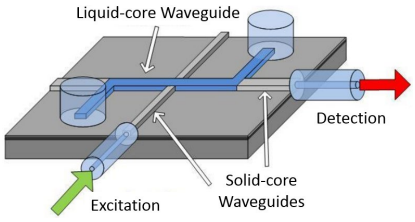

Fig. 5. The ARROW Biosensor is an integrated optofluidic device that combines optics and fluidics in solid-core and liquid-core waveguides.

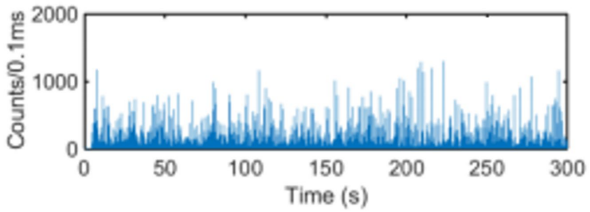

Fig. 6. Example signal intensity plot over time shows signal peaks representing particles with varying signal strengths.

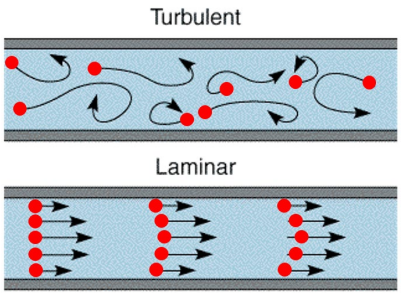

Fig. 7. Laminar flow found in our biosensor enables engineering.

The second reason particle velocity variance introduces doubt in detection occurs when multi-spot excitation is used. A biosensor chip can be designed such that the target particles pass by multiple spots of excitation light, generating fluorescence each time [1]. This is done to create a time delay constant that can be detected and used by a computer to magnify the signal out of the noise, greatly improving the signal to noise ratio. Particles at different positions in the channel will have different time constants, decreasing detection confidence. Narrowing the distribution of particle velocities would increase detection confidence. The predictability of the laminar flow exhibited by the microfluidic channels enables engineering. In theory, we could limit which paths the target particles would flow in to create a uniform particle velocity profile. It should be noted that choosing the center of the channel, where the velocity is the greatest, optimizes processing time.

F. Parabolic Excitation

Optical fiber functions by containing the light inside thin glass "wires". The light takes on a cross-sectional shape called an optical mode [7]. In our biosensor system, laser excitation light is coupled to the side of the chip through an optical fiber. On the chip, a solid-core waveguide guides the light in an optical mode shape from the side of the chip to the excitation region, represented in Fig. 8, where it meets the liquid-core waveguide. The optical mode exhibits a Gaussian intensity distribution which looks like a narrow oval mountain peak, with highest intensity of light in the center dropping off to the edges, represented in Fig. 9.

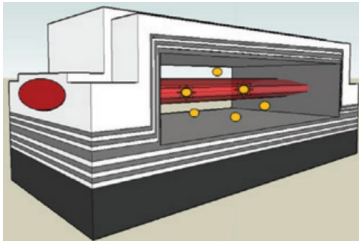

Fig. 8. A cross section of the liquid-core waveguide at the excitation region shows the optical mode interacting with the particles. D. Ozcelik, H. C. Cai, K. D. Leake, A. R. Hawkins, H. Schmidt, Nanophotonics 6 (4) 647-661 (2017).

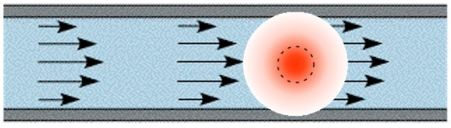

Fig. 9. Gaussian shaped intensity profile overlaid on parabolilc flow velocity profile informs design decisions. Altered image courtesy of Wikimedia.org

Just as the parabolic particle velocity profile caused by laminar flow introduces doubt in detection, the Gaussian shaped excitation light intensity distribution introduces doubt in detection. The variance in the intensity of light that a particle might experience as it travels through the excitation region can result in signal intensity peaks of varying height. Even worse, there might be particles near the floor or ceiling of the channel that go undetected because the intensity at the edge is so low.

If we could limit the position of the particles to the vertical center of the channel, we could ensure that each particle would experience the highest intensity of excitation light possible, thus creating optimal signal. This would also narrow the signal intensity distribution, further increasing detection confidence. One final consideration is that not all photons generated are captured by the waveguide. The collection efficiency of the waveguide is highest at the center or axis of the liquid-core waveguide [8]. The goal then is to center the sample stream inside the channel both left and right, up and down, or in other words horizontally and vertically.

G. Implications of Optofluidics: Hydrodynamic Focusing

The natural distribution of particles inside a fluidic channel causes very uneven excitation profiles for several reasons outlined previously. Controlling the position of particles in fluid is called hydrodynamic focusing. Forcing particles into a limited cross-sectional area as represented in Fig. 10 would enhance detection by narrowing the particle velocity distribution, excitation intensity distribution, and signal intensity distribution. Focusing the particles to the center of the channel would optimize signal intensity and collection efficiency as well as processing time. This would appear in the resulting signal intensity plot as more uniform peak heights, lower noise floor, higher signal to noise ratio, and a narrower right-shifted signal distribution as shown in Fig. 11.

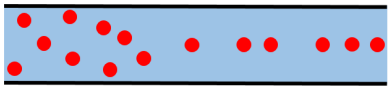

Fig. 10. Forcing particles into a small region is called hydrodynamic focusing.

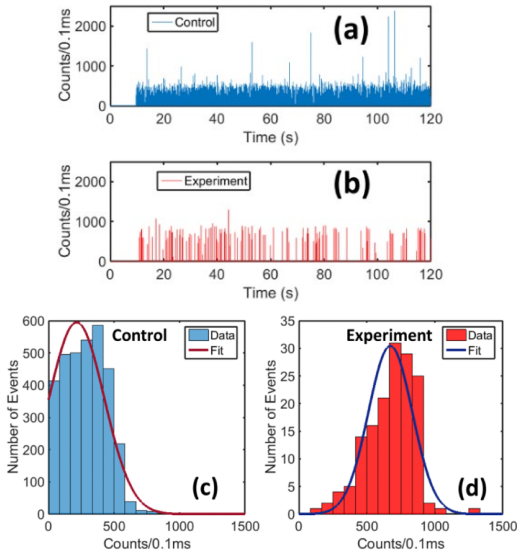

Fig. 11. Example plots of how hydrodynamic focusing improves detection [9].

III. Methods/Implementation

A. Hydrodynamic Focusing

Forcing sample particles to a limited region of a fluidic channel is almost always achieved by occupying the remaining space with a buffer fluid. This increases the complexity of the device fabrication and operation by requiring additional fluid channels and reservoirs for the buffer fluid. Perhaps more importantly, because the buffer fluid occupies channel volume, the processing time required for a sample increases. Yet, in the case of biosensors for example, the high sensitivity benefit outweighs the drawbacks as it ensures that target pathogens don't go undetected. This translates to low concentration detection abilities which means for example, a subject can be treated during early stages of an illness before symptoms may have manifested. This is crucial for preventing outbreaks. In another case, low concentration detection is ideal for the search for extra-terrestrial life.

B. Existing Hydrodynamic Focusing Methods

1) 2D Focusing: The sample stream is sheathed from the sides by buffer fluid as seen in Fig. 12 but the vertical position of the sample particles remains unrestricted. This method is easy to implement on a wide variety of device materials and structures and improves performance to some degree. Enveloping the sample stream on all sides, known as 3D hydrodynamic focusing, would further improve performance but is more difficult to implement.

2) Dielectrophoresis: The fluidic channel is fabricated with tapered electrodes which induce a non-uniform electric field on the particles as they flow (see Fig. 13). This exerts a net force, even on uncharged particles, that can move the particles to the center of the channel, effectively 3D focusing them. However, this method adds complexity to fabrication and significant difficulty to operation as it requires high voltage.

3) Nanoneedle: Fig. 14 shows a nanoneedle formed to extend inside a buffer stream, effectively releasing the sample stream inside the center of the buffer stream. The fragility of the structure as well as the complexity of the fabrication make this method unapproachable.

4) Tilted Lithography: Similar to the nanoneedle, the sample stream is released into the buffer stream center by a nozzle. Then the entire fluid stream is shaped by angled channel walls, seen in Fig. 15, to complete the focusing. Again, the complexity of fabrication makes this method difficult to consider. However, it does appear more robust than the nanoneedle.

5) Focused Laser Etching: This design is formed by exposing points inside a block of light-sensitive resin, then developing out the reacted portion to leave hollow channels. This makes it possible to easily fabricate sheathing channels all around the sample stream to achieve 3D focusing as Fig. 16 shows. However, the ARROW biosensor is silicon based and this method is incompatible. It also requires a femtosecond laser which can be cost-prohibitive.

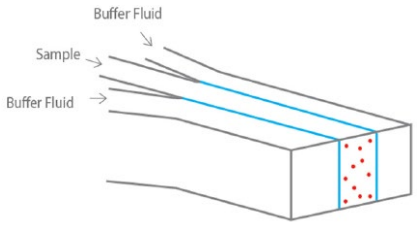

Fig. 12. Focusing is achieved by sheathing the sample stream with buffer fluid from the sides. M. A. Olson, BYU Scholars Archive, All Theses and Dissertations. 4071 (2014).

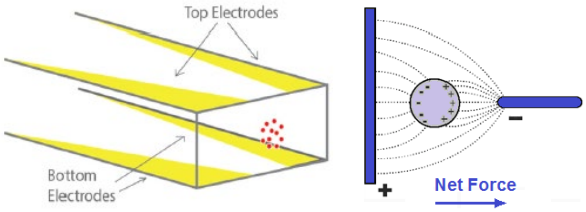

Fig. 13. High voltage electrodes exert a net force on uncharged particles of interest. M. A. Olson, BYU Scholars Archive, All Theses and Dissertations. 4071 (2014).

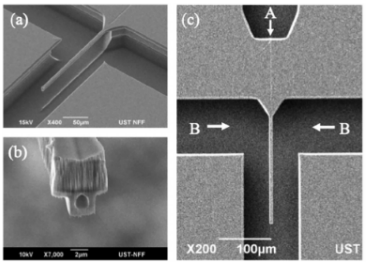

Fig. 14. A nanoneedle releases the sample stream into the buffer stream. Y. Liu, Y. Shen, L. Duan, L. Yobas, Applied Physics Letters 109 (14) (2016).

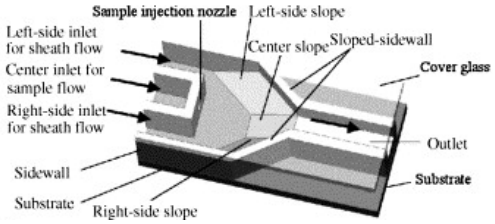

Fig. 15. Fluid channel walls sculpt the sheath stream around the sample stream. R. Yang, D. L. Feeback, W. Wang, Sensors and Actuators A: Physical 118 (2), 259-267 (2005).

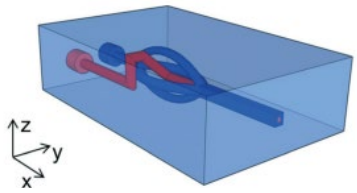

Fig. 16. Complex channel geometries can be formed in a block of material. P. Paie, F. Bragheri, R. M. Vazquez, R. Osellame, Lab Chip 14, 1826-1833 (2014).

6) PDMS Stacks: PDMS is a popular microfluidic material because it is relatively easy to work with and the design can be iterated quickly. Complex structures can be formed by creating individual layers containing a part of the desired fluidic system and then bonding the layers together. In this case, the five layers seen in Fig. 17 form a stack which results in 3D focusing. However, we want to integrate the focusing element into the ARROW biosensor and this will not work. It also looks complicated to operate due to the high number of fluid channels.

7) Vortex Generation: The most appealing existing approach can be implemented with a single layer as shown in the next three designs. Each makes use of vortex generation which sculpts the sample stream in relation to the buffer stream and results in 3D focusing. However, vortex generation requires high velocity fluid flow which is impossible at the scale of the ARROW biosensor.

a) Contraction Expansion Array: The combined stream is contracted before coming upon a wider space which makes the fluid spread out, causing vortexing. After a few of these expansion chambers, the sample stream has been surrounded by buffer as shown in Fig. 18.

b) Pillar Induced Stream Sculpting: Again, the combined stream is incident on a structural feature which generates a vortex shaping the stream. In this case pillars, seen in Fig. 19, are placed inside the fluidic channel and the spacing and placement of the pillars can be designed to produce 3D focusing.

c) Curve Design: Fig. 20 shows the method sometimes referred to as microfluidic drifting because it occurs around a curve, this method of vortex generation is achieved by flowing the combined stream around a 90 degree bend which causes vortexes to draw the sample stream out horizontally, accomplishing vertical focusing before horizontal sheathing completes the 3D focusing.

C. Modeling with ANSYS Fluent

Before integrating a 3D hydrodynamic focusing design into the ARROW platform, it was validated and optimized using computational fluid dynamics software ANSYS Fluent. ANSYS is a finite element analysis (FEA) tool that enables modeling of a structure and input of physical parameters to predict physical interactions. It has its limitations as it is not optimized for the microscale and its use is often limited by the length of fluid channels. This was observed as particle stream lines abruptly ending inside channels or being unresolved at boundaries. However, it functions well enough to develop fluidic designs.

The use of a FEA tool improves design speed by accelerating iterations and enabling the user to make changes without fabricating and testing a device. These changes can include layout, dimensions, or operational parameters such as fluid flow velocity and pressure drop values.

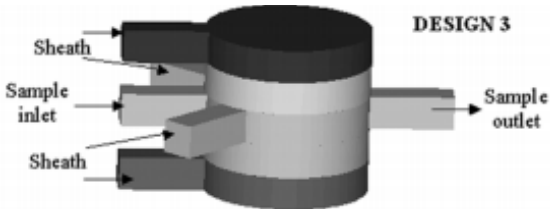

Fig. 17. Bonded layers form a fluidic manifold. N. Sundaranrajan, M. S. Pio, L. P. Lee, A. A. Berlin, Journal of Microelectromechanical Systems 13 (4) 559-567 (2004).

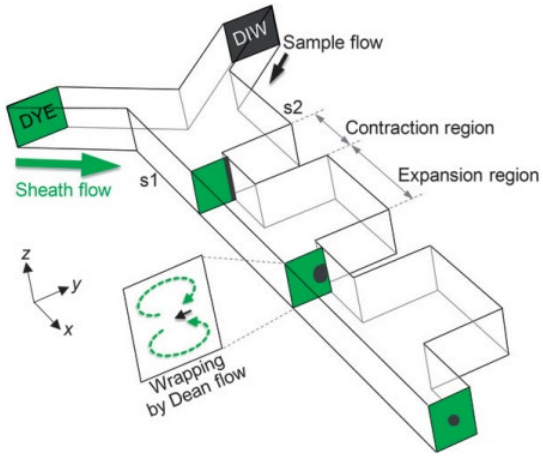

Fig. 18. Vortexes cause the sample stream to be sheathed. M. G. Lee, S.Choi, J. Park, Lab Chip 9, 3155-3160 (2009).

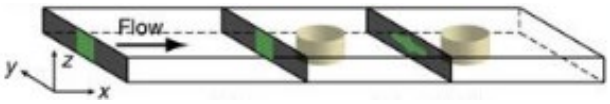

Fig. 19. Pillars induce the vortexes to sculpt the sample stream. H. Amini, E. Sollier, M. Masaeli, Y. Xie, B. Ganapathysubramanian, H. A. Stone, D. D. Carlo, Nature Communications 4, 1826 (2013).

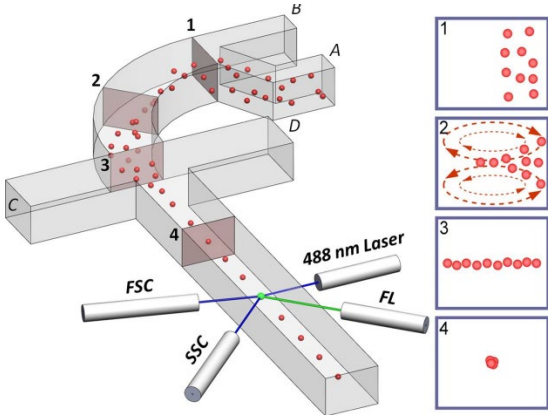

Fig. 20. A curved channel induces a vortex, shaping the sample stream. X. Mao, A. A. Nawaz, S. S. Lin, M. I. Lapsley, Y. Zhao, J. P. McCoy, W. S. El-Deiry, T. J. Huang, Biomicrofluidics 6, 024113 (2012).

D. Fabrication

3D focusing designs for integration in the ARROW biosensor must be compatible with the existing device and possible to fabricate using the same technology, namely photolithography and other cleanroom processes. As part of this, an experiment was run that confirmed that consecutive SU8 layers planarized as seen in Fig. 21, enabling interesting fluidic channel structure design [10]. In addition, new SU8 layer height recipes were developed.

The general fabrication process for the ARROW biosensor is shown in Fig. 22. It begins with a blank silicon wafer on which layers of tantalum oxide and silicon oxide are deposited to form the ARROW layers necessary for liquid-core guiding. SU8 photoresist is spun on, patterned, and baked. A pedestal is etched, over which oxide is deposited and etched to define the waveguides. A final oxide layer is deposited to protect the structures and the SU8 is etched out leaving the liquid-core hollow.

E. Testing

After completing fabrication on the silicon wafer, the wafer is cleaved into chips just one centimeter by one centimeter. Fluid reservoirs are attached to each channel port. Then, negative pressure is applied at the outlet by vacuum, which draws fluid through the chip. Additionally, laser light is coupled to the excitation waveguide through a fiber optic cable, and a detector is placed at the perpendicular side to collect the fluorescence, seen in Fig. 23. This all happens underneath a camera which aids in chip alignment and enables recording and analysis of fluid flows.

After turning on the laser and aligning the fiber to the chip, a test is performed by filling each reservoir with either sample fluid or buffer fluid, and activating the vacuum. Flow is observed as fluorescence is detected in the camera and in the detector. A control experiment can be performed by filling both the sample and buffer reservoirs with sample fluid (fluorescent) and comparing the signal distributions to that of an experiment.

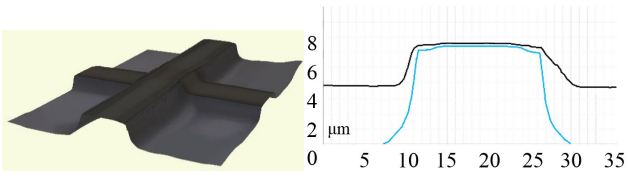

Fig. 21. Consecutive SU8 layers planrize, enabling interesting fluid channel designs.

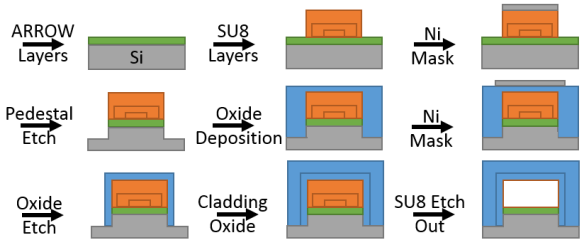

Fig. 22. Silicon-based ARROW Biosensor device fabrication flow chart.

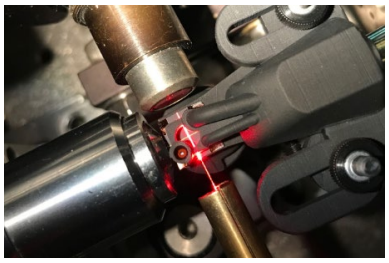

Fig. 23. Test apparatus showing the microchip connected to fluidic manifold and aligned to the laser light and objective lenses.

IV. Results

A. Curve Design

The vortex generation designs were the most appealing existing focusing methods for integration with the biosensor due to their ease of fabrication and operation. Each was modeled and simulated at the reported dimensions. However, due to the cascade nature of the contraction expansion array and the pillar design, the software had a difficult time resolving the large models. The compact nature of the curve design allowed the software to manage it. The size of the design is also appealing for chip real-estate. After validating the reported scale design, it was shrunk by an order of magnitude to the scale of the ARROW biosensor and optimized as shown in Fig. 24 a).

Our partners at the University of California Santa Cruz fabricated and tested a suboptimal design by soft lithography in PDMS seen in Fig. 24 b) and c). The results included some good news and some bad news [11]. We discovered that the velocity required for the optimal focusing was too great for this scale. The physical dimensions of the fluidic channel result in high fluidic resistance, restricting flow velocity and limiting the amount of vortexing possible. The device was run at 1/100th the simulated velocity. Running the simulation at this velocity shows little vertical focusing. Despite this, a comparison of the control and experimental data shown in Fig. 25 revealed an improvement in the coefficient of variation by a factor of 4 as it dropped from 0.98 to 0.24. Detection of H1N1 viruses enhanced by 1.64 times. All this with a suboptimal design, and operation at flow velocity two orders of magnitude lower than designed.

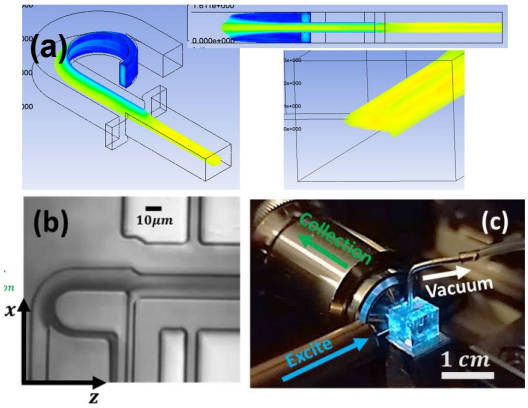

Fig. 24. a) Oblique, side, and outlet views of the optimal curve design, b) top view of a suboptimal fabricated device and, c) image of testing in progress.

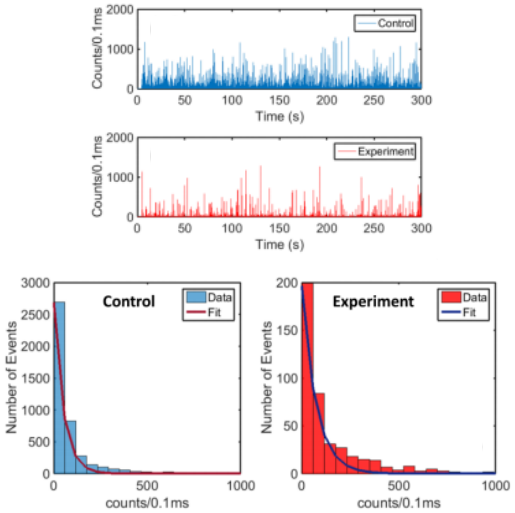

Fig. 25. Control and experimental signal intensity plots and signal intenity distributions for H1N1 virus in the suboptimal curve design. The coefficient of variation dropped from 0.98 to 0.24. Detection enhanced by 1.64 times.

B. *Stacked Channel Design*

It became apparent from the testing of the curve design that we would need a velocity independent design that would generate 3D focusing even at low flow speeds. The Stacked Channel design shown in Fig. 26 is a novel design that operates based on flow volume ratio [10]. It has a key injection feature that raises the sample stream into the center of the buffer stream. It is made of three layers of SU8 resist which form the channels before being etched out. Sheath outlets are included in an attempt to limit the amount of buffer fluid processed through the chip as a way to optimize the sample processing time. The design was developed iteratively in simulation before being fabricated on silicon as shown in Fig. 27. The simulations suggest excellent focusing. However, the complexity of the design led to cracks in the material preventing proper operation.

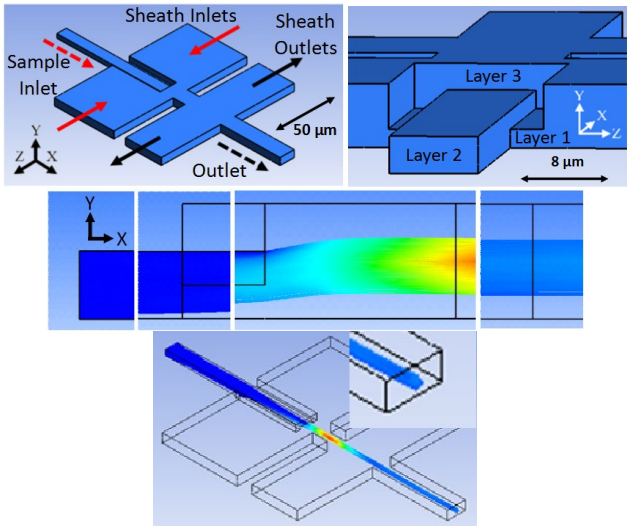

Fig. 26. Schematic views of the fluidic junction region from an oblique angle, detailing operation and toward the outlet, showing layers. Views of sample stream shown from the side and from an oblique angle, exhibiting 3D focusing.

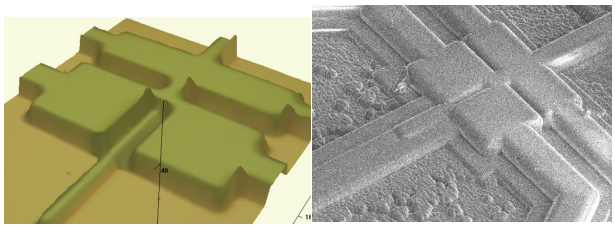

Fig. 27. Oblique views of fabricated fluidic junction by a 3D profilometer showing layers and by an SEM showing the oxide.

C. *Trench Design*

Allowing etching of the substrate enabled a more symmetric design requiring just two layers of photoresist [12]. Additionally, the buffer to sample fluid volumetric flow ratio required to perform the focusing effect was greatly reduced along with fluid handling complexity. Fig. 28 shows the trench design which uses a prismatic plus-shaped fluid junction to surround the sample stream with buffer fluid.

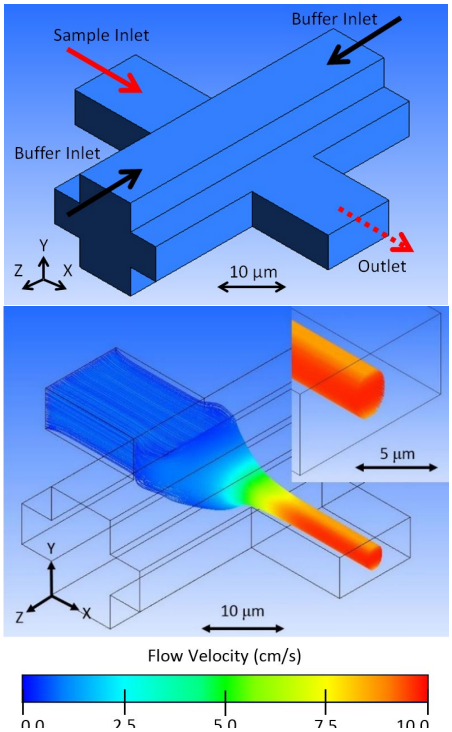

Fig. 28. The trench design creates a more symmetrically focused sample stream due in part to more symmetric fluid channel geometry. The volumetric flow ratio of buffer fluid to sample fluid is also equal.

The modeling suggests a much more desirable stream shape. This design was used to observe three-dimensional focusing and to characterize diffusion in ten-micron scale fluid channels. Most importantly, particles of interest, such as one thousand kilobase pair single-stranded DNA, were predicted to exhibit negligible diffusion and remain focused through the entire length of the liquid-core waveguide channel. However, the nub portion of the fluid junction which was formed with a second layer of photoresist was fragile and led to low device yield.

D. *Pit design*

Constraining fluid channel design to a single layer of photoresist makes for a more robust device and improved device yield. The pit design shown in Fig. 29 is formed with one layer of photoresist which fills voids etched into the substrate [13]. The key feature is an inline pit into which the sample fluid drops while simultaneously covered by buffer fluid. This moves the sample stream to the floor before a trench feature raises the sample fluid and envelopes it for a three-dimensionally focused stream.

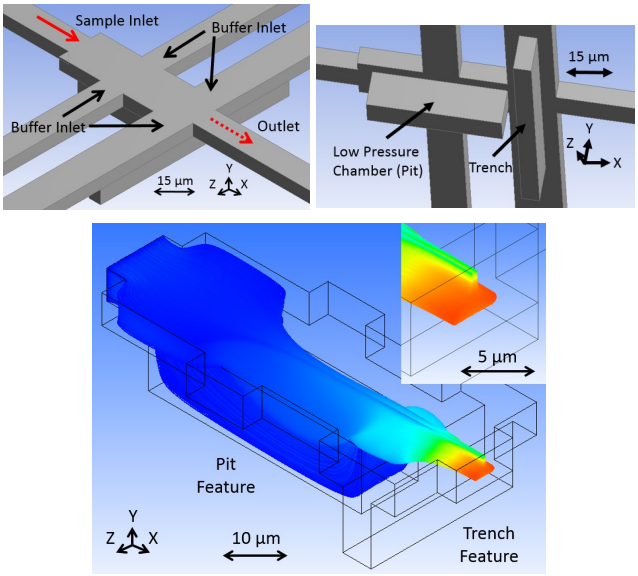

Fig. 29. The pit design is formed with a single layer of sacrificial photoresist, making for improved device yield. The key is the inline pit etch feature.

A more consistent signal distribution was observed, representing enhanced detection, and used to discover reservoir effects in the system. This is a result of backpressure induced on the fluid channels due to gravity acting on the fluid in reservoirs. Such an effect is nonnegligible at low operation pressures but is overcome when high vacuum pressures dominate the effect. Note however that the sample stream shape seen in Fig. 29 is not ideal. This was a result of optimizing the design geometry using incomplete stream profile information arising from a low finite element resolution. This can be overcome in the present geometry by simply increasing the buffer volumetric flow while holding the sample flow rate constant, as represented in Fig. 30.

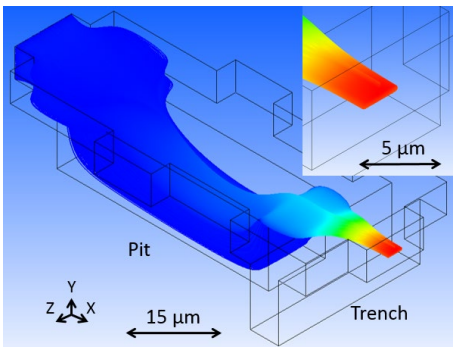

Fig. 30. Increasing the buffer flow ratio in the exisiting geometry provides for amore desirable sample stream shape.

Alternatively, the geometry could be again optimized using the high-resolution finite element analysis to bring the buffer to sample flow ratio back to one to one. However, this requires additional design work, retooling, and total fabrication of new devices whereas simply increasing the buffer flow can be done with exiting devices.

V. Discussion

A. *Hydrodynamic Focusing Fabrication Equipment*

New cleanroom equipment was required for the fabrication of these novel devices. This includes a plasma enhanced chemical vapor deposition machine (PECVD) with the ability to deposit low stress oxide. Theoretically, this enables robust fluidic channels made of the oxide, which are less likely to crack or break. The author installed, characterized, and developed recipes for the PECVD and was responsible for the repair and maintenance of it. A 3D profilometer was also required to measure and tune the oxide recipes and to aid in device characterization and optimization. The author was responsible for the repair and maintenance of it. Together, these machines were foundational to this work as well as that of many other researchers at BYU.

B. *Downsides to 3D Hydrodynamic Focusing*

While the result of hydrodynamic focusing promises to improve device sensitivity dramatically, there are some disadvantages that might make a designer reconsider adding it to their design. As seen, it can be difficult to develop a design compatible with the other functions of your device. Additional equipment may be required which adds to upfront cost. What's more, fabrication is complex which leads to higher costs and sometimes fragile elements. Operation is often more complex due to the inclusion of sheathing fluidics which also take up chip real-estate. For some applications such as pathogen detection, the increase in processing time caused by the use of buffer fluid may be unacceptable.

C. *Future Work*

Each of the novel methods discussed here is performed by using fluid channel layout to sheath the sample stream with buffer fluid. Other methods exist which use fluid properties to cause focusing, including inertial focusing and viscoelastic focusing [14, 15]. It may be of interest to look into those. Alternatively, using an over the channel reservoir approach may enable parallel processing of sample fluid, speeding testing times and opening up chip real estate [16].

VI. Conclusion

Hydrodynamic focusing narrows the sample particle velocity distribution, optimizes excitation intensity and narrows signal intensity distribution, and optimizes collection efficiency. Together this improves signal consistency and detection sensitivity, enabling positive identification of low concentration and single molecule samples. This makes it ideal for the search for extra-terrestrial life. Various novel microfluidic designs were presented and discussed, showing more consistent signal and representing foundational findings in ten-micron scale hydrodynamic focusing.

Acknowledgment

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