Rapid isolation of blood plasma using a cascaded inertial microfluidic device

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Blood, saliva, mucus, sweat, sputum, and other biological fluids are often hindered in their ability to be used in point-of-care (POC) diagnostics because their assays require some form of off-site sample pre-preparation to effectively separate biomarkers from larger components such as cells. The rapid isolation, identification, and quantification of proteins and other small molecules circulating in the blood plasma from larger interfering molecules are therefore particularly important factors for optical blood diagnostic tests, in particular, when using optical approaches that incur spectroscopic interference from hemoglobin-rich red blood cells (RBCs). In this work, a sequential spiral polydimethylsiloxane (PDMS) microfluidic device for rapid (~ 1 min) on-chip blood cell separation is presented. The chip utilizes Dean-force induced migration via two 5-loop Archimedean spirals in series. The chip was characterized in its ability to filter solutions containing fluorescent beads and silver nanoparticles and further using blood solutions doped with a fluorescent protein. Through these experiments, both cellular and small molecule behaviors in the chip were assessed. The results exhibit an average RBC separation efficiency of $\sim 99\%$ at a rate of 5.2×10^6 cells per second while retaining 95% of plasma components. This chip is uniquely suited for integration within a larger point-of-care diagnostic system for the testing of blood plasma, and the use of multiple filtering spirals allows for the tuning of filtering steps, making this device and the underlying technique applicable for a wide range of separation applications. *Published by AIP Publishing.*

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I. INTRODUCTION

Sample filtration techniques for medical diagnostic testing typically require bulky equipment such as a centrifuge, high-performance liquid chromatography (HPLC), and/or the need for samples to be sent to a central laboratory for analysis.¹ This process can require hours or days for results to be available due to sample preparation, technical complexity, and logistic delays, possibly rendering the test results useless.² Separation of small biomarkers from cellular components in biological fluids is specifically critical for accuracy in diagnostic testing as cellular fractions can cause errors and inconsistencies.³ By progressing to an automated lab-on-a-chip type filtration device, results could be analysed at the patient's side, removing many of the sources of error associated with diagnostic blood testing, such as mislabelling of samples and improper sample preparation and transportation.⁴ The implementation of this filtration method could significantly enhance the realization of a number of developing point-of-care (POC) devices with a variety of applications such as remote and emergency health monitoring, pharmaceutical testing, academic research, or home test kits.

The characteristically small size of microfluidic technologies allows the volume of reagents used and samples taken from patients to be drastically reduced.⁵ Diagnostic blood tests typically require volumes of blood in ones to tens of millilitres.^{6,7} One of the most common examples of a microfluidic POC device is portable glucose monitors. These devices often utilize microfluidic

paper test strips that, for some models, only require 0.3 μl of blood.⁸ This miniaturization lends itself well to the generation and use of POC diagnostic devices.

Size separation in microfluidic channels has been investigated by several groups using various modalities,⁹ including dielectrophoresis,^{10–12} deterministic pillar arrays,^{13,14} pinched flow fractionation,^{15,16} inertial methods,^{17,18} acoustophoresis,¹⁹ centrifugal techniques,^{20,21} as well as some combined approaches.²² A comparison of the flow rates used as well as the separation efficiency of each of these methods can be found in Table I (p. 527) of a 2009 review comparing on-chip particle separation techniques specifically in regard to their potential for human plasma separation by Mukherjee *et al.*⁹ This table details the particular operating parameters for these methods, their separation efficiencies, as well as giving pros and cons for each of the methods. In brief, most highly efficient techniques suffer from difficulty or expense in miniaturization for translating these technologies from bench to bedside or, if they are easy to produce, suffer from issues with clogging or sample damage that result in large dilution requirements, thereby requiring pipetting or solvent packaging, and thus, a step back from the automatization these devices strive towards.

In one example, by taking advantage of differential electrical properties of different cells, cell separation by a dielectrophoretic approach demonstrated the potential to be very selective in separating the different components in blood.¹⁰ Applied voltages are used to create either attractive or repulsive areas in microchannels to selectively trap or repel particles with particular electrical properties. The use of electric fields in this application has been seen to generate irregular flow within the channels, which has the potential to damage cells.²³ The speed at which this technique is able to process fluid, in the $\mu\text{l}/\text{min}$ range, could lead to long processing times depending on the volume of fluid to be examined.

Deterministic lateral array devices utilize what are known as “bump arrays” to separate streamlines in a flow. Based on the diameters of the pillars in the array, particles of varying sizes will pass through the array in different streamlines and can be collected at distinct positions downstream in the channel. This technique can be tuned to separate particles differing in tens of nanometers in size, but the pump speeds at which this technique is applicable are also relatively slow, from 0.4 $\mu\text{l}/\text{min}$ to 1 $\mu\text{l}/\text{min}$. A drawback inherent to physical membrane filters, as well as these arrays, is the clogging that can occur when particle concentrations exceed a certain critical concentration for that array.¹⁴ This type of error results in low plasma retention and can increase the likelihood of the membranes bursting and leaking cells into the filtered outlet.

Pinched flow fractionation is a technique in which changes in channel diameter and flow rate determine the filtering and separation capabilities. The polydisperse fluid flows through a device, wherein a narrow section of the channel pinches the fluid into a smaller space. Upon release into a larger section of the channel, the particles separate in the flow to different positions based on size and can be collected at a point downstream in the channel. This technique has been tested using whole blood with fairly good efficiency, $\sim 96\%$, but once again this technique also suffers from relatively slow sample processing speed.¹⁶

The generation of acoustic standing waves in microchannels, known as acoustophoresis, is another method by which separation of microparticles has been explored.¹⁹ By altering the power supplied to the piezoelectric actuator, the focusing positions of particles of different sizes and densities in the channel can be altered. Depending on the power of the actuator, heating of the sample and hemolysis can be a concern; though in the referenced study by Petersson *et al.*,¹⁹ those factors were well controlled.

Centrifugal techniques paralleling the traditional use of the centrifuge have also been developed that utilize microfluidic channels.^{20,21} These devices utilize the same rotational aspects of the traditional centrifuge, though they exchange the centrifuge tubes for sophisticated, microfluidic channels designed to automate the separation of blood into its component parts. With well-designed disks, these filters are very good at separating cellular components from plasma²¹ or separating specific cell types,²⁰ though they are run on a sample-by-sample basis, and are therefore more difficult to parallelize.

Particle focusing and separation in curved channels come as a result of secondary flows in the fluid. This inertial technique is explored herein, and a more detailed explanation of the fluid behavior is provided. The filtering properties of these devices, arising from secondary flows, are heavily dependent on the geometry of the channels and flow rates used. While the separation quality of these types of devices has been relatively lower than the others discussed, the flow rates used, in the range of milliliters per minute, allow for rapid processing of samples. In the case of these inertial filters, for proper filtering to occur, the fluid introduced to the channels must behave as a Newtonian fluid. In the case of whole blood, with a hematocrit of $\sim 45\%$, the higher concentration of particles has been seen to disrupt focusing positions.²⁴ For this reason, the implementation of these channels requires a dilution of the whole blood. This dilution reduces the analyte concentration in the blood, making detection of those molecules of interest more difficult. Previous work utilizing spiral channels has shown effective filtering performance, $>99\%$ separation efficiency, with a $100\times$ dilution of whole blood.²⁵ Through the use of a multistage, spiral microfluidic device, an improvement of the filtering quality, as well as a 5-fold reduction in the required dilution of the whole blood, of these inertial filters is shown herein. While other spiral devices have included multiple spirals,²⁶ these spirals have been used to increase the flow rate and bulk filtration rate by using several single spirals in parallel. The improvements offered by the device presented herein are through the use of two cascaded spirals, which perform two filtering steps. This alternate method of the application of the spirals allows for a greater initial hematocrit with similar filtering results. The potential improvement in separation efficiency through the use of multiple spirals paired with high flow rates makes this microfluidic device a unique advancement for the development of disposable lab-on-a-chip devices, bringing spiral fluidics one step closer to rapid, reagent-free whole blood filtration and plasma retention at the POC.

A. Theory of inertial spiral microfluidics

In the case of inertial spiral microfluidics, the effectiveness arises in its parallelism to the gold standard for plasma extraction: the centrifuge. The microfluidic channel's laminar flow is disrupted upon introduction of the spiral geometry, which induces rotational components into the flow known as Dean vortices.¹⁷ As a polydisperse fluid approaches the curved channels, the inertia of the fluid at the inner wall of the channel carries it toward the outer wall of the channel and shifts the Poiseuille flow profile in a manner that the maximum velocity of the fluid now occurs closer to the outer wall of the channel. This shift introduces the circulation of fluid near the outside of the channel back toward the inner wall of the channel. These "whirlpools" push larger particles toward the inner wall of the channels, allowing them to be extracted. The strength of these vortices can be quantified in the form of the Dean number (De), a dimensionless parameter that is calculated as a factor of the hydrodynamic diameter of the channel (D), the radius of curvature of the spiral (R), and the Reynolds number (Re). The Re is another dimensionless parameter quantifying the flow regime in the channel, whether turbulent or laminar.²⁷ The expression for the Dean number is shown in Equation (1)¹⁷

$$De = Re \sqrt{\frac{D}{2R}}. \quad (1)$$

In microchannels with a square cross-section, particles in the flow will position themselves in the centers of each of the faces of the channel, giving 4 focusing positions.¹⁷ When the cross-section is altered into a rectangular configuration, only two of the focusing positions remain, seated on the centers of the two longest faces of the rectangle. The introduction of Dean vortices shifts these focusing positions to a favorable location off center in the channel, allowing for size separation of particles by selective sectioning of the channel downstream from the spiral. The final focusing position can be tuned by altering the strength of the secondary Dean flow²⁸ and effectively the filtering capability of the spiral. It is noted that an increase in the Dean number will not necessarily result in better particle focusing, as the profile of the

Dean vortex varies with an increasing Dean number and above a certain limit becomes counter-productive to focusing.²⁹ The filtering capabilities of these microchannels are also affected by the proportionality of the particle size to the diameter of the channel.²⁸ For particles to be effectively filtered, they must fulfill the condition given in Equation (2), requiring them to be larger than a minimum fraction of the microfluidic channel

$$\frac{a_p}{D} \gtrsim 0.07. \quad (2)$$

For this equation, a_p is defined as the diameter of the particle of interest, and D is the hydrodynamic diameter of the channel. This relation gives a measure of the cross sectional footprint of a particle in the channel. For particles below this size, the forces acting on the particle due to the vortices do not sum in a manner that keeps them confined to a particular domain in the channel, as happens with larger particles.^{17,27} The length of the channel also plays a role in the quality of filtering of these spiral channels. To allow for the hydrodynamic forces in the channel to move particles to their final focusing positions, the total length of the channel must meet a certain minimum value, given by Equation (3)¹⁶

$$L_f = \frac{\pi \mu W^2}{\rho U_m a_p^2 f_L}, \quad (3)$$

where μ is the viscosity of the fluid, W is the width of the channel, ρ is the density of the fluid, U_m is the maximum velocity of the fluid in the channel, and f_L is a coefficient of lift that is a function of Re and position in the channel.

The use of single spirals and variations on single spirals has been explored by other groups^{28,30,31} and works well when particle concentrations do not exceed a certain limit. Successful filtering and separation of particles were performed when the particles made up 0.05% of the volume of the fluid. Some of the difficulties in filtering particles such as cells from undiluted solutions with inertial filters can be seen as a product of cell-to-cell interactions.²⁵ When the channels are overfilled, large particles begin filling focusing positions and steric interactions jostle particles in the flow to less optimal positions. These particle-to-particle interactions limit the capabilities of these spirals to effectively separate the microparticles from the sample. In a clinical setting, this means that fluids introduced into the channel would need to be significantly diluted prior to filtering, which will significantly decrease the concentrations of analytes of interest that are already in micro and nanomolar concentrations.³² One potential solution is the use of multiple filtering spirals in series to allow for separation of particles in multiple steps, effectively reducing the dilution required to come to the same diagnostic result with the benefit of having higher concentrations of biomarkers with less initial sample required. Herein, the effectiveness of single and cascaded spiral microchannels is compared, as shown in Figure 1 for filtering out large cellular components of blood. The efficiency of this design is also demonstrated for biosensors in terms of its ability to isolate and retain a fluorescently labeled protein from a 50 μ l sample of whole blood.

II. EXPERIMENTAL

A. Design considerations

In the design of the single spiral, channel dimensions of 500 μ m for width and 60 μ m for height were selected to allow for the filtration of particles of size 7.5 μ m, using Equation (2). The use of five loops in the spiral ensured that the channel met the focusing length requirement. The dimensions of the secondary spirals, reduced to 250 μ m by 60 μ m, allow for the filtration of particles with a diameter of 6.78 μ m. The number of spirals was selected to fulfill the length requirement given by Equation (3). Fluid properties, viscosity, and density were estimated as a weighted average of saline and whole blood, using a ratio of 20:1. While this method of inertial filtration relies on a shift in the flow profile to estimate max flow velocity in the channel, the

FIG. 1. (a) Drawings of the single (top) and dual (bottom) spiral filters tested herein. The use of two secondary spirals was to balance the fluidic resistance at the bifurcation. With comparable resistances, the two secondary spirals receive fluid velocities similar to those in the initial filtering spiral, leading to the appropriate Dean number being reached for appropriate filtering in the secondary spirals, (b) schematic drawing of the single spiral channel with outlets indicated by the alphanumeric combinations a1, a2, and a3, and (c) schematic drawing of the double spiral channel with outlets indicated by the alphanumeric combinations b1, b2, and b3; these outlets are referenced later in the paper.

assumption of a flow without secondary Dean flows was used to calculate the maximum fluid velocity. From these calculations, the required channel length for focusing was found to be 108.5 mm, which for the channels used herein required five spirals. The use of two secondary spirals was selected to balance the flow speed for the second filtering spiral to maintain flow conditions similar to those in the initial filtering spiral.

B. Fabrication of PDMS chips

A single microfluidic spiral channel and a sequential spiral microfluidic channel were each drawn in Solidworks. Photolithography transparency masks were purchased from CAD/Art services and printed with a resolution of 2,500 DPI. The initial spiral of the sequential channel and the single spiral channel were designed to have a channel width of 500 μm , whereas the secondary spirals in the sequential channel have widths of 250 μm . Master molds were generated on silicon wafers with a SU-8 2050 photoresist using traditional soft photolithography techniques for a feature height of 60 μm . Polydimethylsiloxane (PDMS, Sylgard 184) was mixed in a 10:1 ratio of pre-polymer to the curing agent and poured over the molds. The polymer mixture was degassed for 1 h and baked at 85 $^{\circ}\text{C}$ for 20 min to cure. PDMS chips were removed from the molds and affixed to glass slides by oxygen plasma treatment and subsequently baked at 85 $^{\circ}\text{C}$ for 20 min to achieve a more patent bond.

C. Separation efficiency quantification

Optimization of the flow rate in the channel was performed using the single spiral micro-channel since the initial filtering spiral has the same dimensions for both designs. A syringe pump (New Era Pump Systems, Inc.) was used to pump fluid into the central inlet of the channel, and fluid from each of the three outlets was collected. Adult bovine whole blood (Lampire Inc.) was purchased and diluted in a 1:20 ratio with physiologic saline, resulting in a hematocrit of $\sim 2\%$. Flow rates were varied from 0.75 ml/min to 1.5 ml/min in increments of 0.25 ml/min. Fluids collected at the outlets were analyzed using UV/Vis spectroscopy with a TecanI spectrometer (TECAN Inc.) to characterize the separation of red blood cells (RBCs) from the diluted blood via their hemoglobin spectra.³³

Volumetric measurements were also made to determine the distribution of fluids to the outlets over the course of 1 min. After analysis of the flow rate dependent blood cell separation, further testing was performed to determine the separation quality of the sequential spiral channel. With similar geometry in mind, the optimum flow rate determined in the single spiral channel was used in the sequential spiral channel. Visual examination of filtered fluids was performed at the time of filtering, a comparison between the filtered results of the two chips can be seen in Figure S1 ([supplementary material](#)). In addition to UV/Vis absorbance measurements from 450 nm to 650 nm of the outlet fluids of both designs, red blood cell-counting studies were performed to further quantify the cell separation performed by the sequential spiral channel. These were performed using a hemocytometer and a brightfield microscope, seen in Figure 2. Additionally, a magnified (20 $\times$) image of the cell counting square for blood diluted at a 1:20 ratio can be found in Figure S2 ([supplementary material](#)).

D. Flow and filtration visualization

Visualization of the two bifurcations, 1-following the initial filtering spiral and 2-following the secondary filtering spiral in the sequential-spiral channel, was performed using a lab-built “macroscope” video system. The 1.25 $\times$ magnification epifluorescence imaging system was built to image the bifurcations in the full field of view. A 530 nm green LED (light-emitting diode) (Thorlabs) was used to illuminate the microfluidic chip. A 531 ± 20 nm excitation filter, a 562 nm longpass dichroic mirror, and a 630 ± 34.5 nm emission filter (Semrock) were chosen to provide the appropriate spectral separation. The objective was a 60 mm focal length doublet used in conjunction with a 75 mm focal length doublet tube lens (Thorlabs) to image the sample onto a USB camera (OptixCam). The 8 mm $\times$ 6 mm field of view is determined by the focal lengths of the objective lens and tube lens and the camera sensor size. The macroscope was used in both the epifluorescence-imaging mode with all the filters in the system and the reflectance mode upon the removal of the emission filter. The dual imaging functionality allows for the visualization of fluid flowing through the channels in reflectance mode, and imaging of the behavior of fluorescent particles present in the flow in fluorescence mode. Videos at 8 frames per second and still images were captured to show (1) the separation of 10 μ m diameter red fluorescent polystyrene beads (Molecular Probes) from 40 nm diameter silver colloid and (2) the filtration of 1 ml of a 1:20 diluted blood solution doped with fluorescently labeled albumin. The stock solution of red fluorescent microbeads was mixed with 40 nm silver nanoparticles to obtain a concentration of 4.8×10^5 particles/ml³⁴ and the solution of blood cells contained 2.5×10^8 cells/ml. The examination of the

FIG. 2. Cells were counted in the eight 4×4 grids, and cells counted were averaged to give a more reliable number of cells per unit volume. (a) Original diluted blood solution at 10 $\times$ magnification, in the unfiltered case, the cells were too densely packed to count. (b) Filtered sample at 10 $\times$ magnification where individual cells can be identified.

second bifurcation to outlets b2 and b3 was only performed with the blood solution, as the channel was able to filter all of the beads at the stock concentration of particles.

E. Protein behavior examinations

To examine protein interactions with the channel walls and quantify analyte loss due to protein adsorption onto PDMS, a solution of bovine serum albumin tagged with AlexaFluor590 was flowed through the channel at 1.25 ml/min. Fluorescence measurements of the channel outlets were measured using a TecanI spectrometer, exciting at 590 nm and measuring fluorescence emission at 617 nm.

The possible negative effects that the cellular components of the blood might have on the distribution and concentration of proteins were also examined. A solution of diluted blood was prepared and doped with the AlexaFluor590 labeled bovine serum albumin (BSA). This solution was flowed through the channel, and fluorescence measurements of the inlet and outlet fluids were taken to quantify the protein distribution. Protein concentrations are reported as a ratio of the outlet solution's concentration to the concentration of the inlet solution.

III. RESULTS AND DISCUSSION

A. Determination of optimal flow rate and dean force analysis

The UV-Vis spectra from initial flow rate studies performed on the single spiral chip are shown in the left panel of Figure 3. Observing the oxyhemoglobin absorbance peak at 540 nm, it can be seen in the inset plot in Figure 3 that the absorbance measurement observed as a function of flow rate yielded a local minimum of 1.25 ml/min, and thus, this was used as the optimal flow rate for the separation of cellular components from blood solutions.

This range of flow rates, yielding a range of Dean numbers, was used to examine the achievable range of focusing positions in the channels, described by Kuntaegowdanahalli *et al.*,²⁸ that were found to be a function of the Dean number. Quantification of these focusing positions was determined by examination of the contents of the outlet solutions. Figure 4(a) shows the Dean number at the minimum and maximum radii of curvature in the channels, 2.25 mm and 6 mm, for the different flow rates. The Dean numbers experienced at the higher flow rates do not quite approach the extremes expressed by Schönfeld and Hardt,²⁹ but the changes in separation

FIG. 3. (Left) Absorbance profiles of outlet a2 for each flow rate examined; (right) Maximum filtration determined to be at 1.25 ml/min based on oxyhemoglobin peak absorbance at 540 nm.

FIG. 4. (a) Changes in the Dean number for tested flow rates at different focusing points in the channel; (b) Dean number is maximized when the spiral's radius of curvature is at a minimum.

efficiency shown here are consistent with the alterations in the focusing position observed by Kuntaegowdanahalli *et al.*²⁸ The fluid focusing in the inertial chip is also known to change throughout the length of the channel, and as mentioned in the theory section, an increase in the Dean number will not necessarily result in better particle focusing as the profile of the Dean vortex also varies with an increasing Dean number. With changes in the radius of curvature, the strength of the secondary flow is altered as well. In our design, the dual spiral channel benefits from this increasing strength of secondary flow as the fluid travels toward the outlet, a benefit not seen in the initial filtering spiral or in the single spiral channel. In those channels, the strength of the secondary flow decreases in transit to the outlet. Figure 4(b) contains the radius dependent change in the Dean number for the 1.25 ml/min flow rate. For subsequent flow experiments, 1.25 ml/min was used as it showed the best separation efficiency.

The rationale for the generation and testing of both the single and sequential channels comes from the need to quantify the filtration state of the fluid being passed to the secondary filtering spirals. By specifically analyzing the filtered fluid of the one spiral channel, though it is acknowledged that it has more differentiated outlets, the hematocrit of the passed fluid from the first to the second filter in the dual spiral design can be approximated. However, it was theorized that this fluid that is passed to later filtering steps would still not be quite as cleanly separate as with the single filter chip, a foreseeable consequence of the reduction in the number of outlets and widening of the outlets.

B. Evaluation of cell filtration efficiency

To first visualize and verify the separating capabilities of the dual spiral channel, 10 μ m fluorescent polystyrene beads were imaged flowing at the first bifurcation. A superimposed bright field image of the channel and an image of the red/orange fluorescence observed from the particles are shown in Figure 5. Video S1 shows the sequential filter separating these fluorescent beads. Beads only focus in the presence of the driven flow and can be seen to be distributed throughout the channel when the pump is turned off, and when 1.25 ml/min flow is resumed (0:06), the beads refocus near the inner wall of the channel in approximately 3 s. This initial time required to focus the particles may be the factor leading to the very small number of beads present in the final filtered outlet solution. Discarding the fluid from the first 3 s of operation may improve the filtering result. Assuming spherical particles with the given diameter, the polystyrene beads made up 0.025% volume of the inlet solution. This inlet proportion falls below the proportion of 0.05% by volume, explored by other groups,^{28,30} in which filtering with a single spiral was seen to separate the particles from the solution.

FIG. 5. Image of the first bifurcation of the microchannel. 10 μm beads are seen to be very well ordered near the inner wall of the channel.

To test this chip against the single spiral, diluted blood solution was also flowed through the dual spiral channel to judge its filtering efficiency. Visual inspection of the outlet fluids was performed, depicted in Figure S1 in the [supplementary material](#), showing qualitatively that the subsequent filtering design performed much better than the first iteration single spiral design. The oxyhemoglobin absorbance peak at 540 nm was once again used to compare the filtering efficiencies of the channels. Figure 6 displays the results of the comparative filtering studies, demonstrating that the usage of subsequent filtering spirals can produce a nearly 30-fold reduction in the number of cells remaining in the filtered fluid over the single spiral design. The single spiral device was determined to remove 55% of the cells from the inlet solution, whereas the double spiral device removed $\sim 99\%$ of cells. Although the filtering of this single spiral is not sufficient at this dilution, at $100\times$ dilution the single spiral is able to maintain a similar efficiency of $\sim 99\%$.

Brightfield images of the two bifurcations, seen in Figure 7, further display the effectiveness of the secondary filtering spiral. In Figure 8(b), the presence of cells entering the secondary filtering spiral can be observed as a dark streak in the straight channel. This is expected since even plasma outlet a3 in the single spiral device was populated with blood cells.

FIG. 6. Filtered outlet absorbance at 540 nm—demonstrating the filtration efficiency of the second spiral compared to the single spiral design, as well as a comparison to the starting diluted blood solution. The single spiral chip removed $\sim 55\%$ of the cells in the input solution, and the two spiral chip removed $\sim 99\%$ of the cells in the input solution.

FIG. 7. Brightfield images of the channel while filtering blood. (a) Solidworks model of the spiral channel indicating areas of imaging. (b) Image of the first bifurcation with blood cells not completely filtered; the shadow boxed in red shows the cells passing the bifurcation to be filtered again by the second spiral. (c) The second bifurcation where the cells passed by the first spiral are removed to the b3 waste outlet.

FIG. 8. Calibration curve of diluted sample absorbance at 540 nm compared to the cell concentration in millions of cells per milliliter, $r^2 = 0.99093$. The inset shows the performance of several chip filtering trials' outlet absorbance data plotted against red blood cells per ml (RBC/ml), with an average remaining concentration of $3.17 \pm 2.8 \times 10^6$ RBC/ml.

However, as depicted in Figure 7(c), the outer walls of the channel at the end of the second spiral are apparently clear of RBCs, resulting in a highly filtered fluid. Videos of the channel at the first and second bifurcations can be seen in Videos S2 and S3, respectively. Experimental results were visually confirmed in that the filtering seen at the first bifurcation is not complete, as evidenced by the “shadow” of RBCs seen flowing in the filtered outlet. Beginning with an empty channel, the filtering can be seen to reach the steady state approximately 6 s after the fluid is first seen at the bifurcation (0:02). In Video S3 of the second bifurcation, the flow was halted (0:06), and defocusing of the cells can be visualized. When the flow is resumed (0:16), the cells refocus in the same time frame as observed in Video S1.

To further quantify the cell separation achieved with the sequential filter design, the filtered samples produced were examined under a microscope, and the cells present in the solution were counted. An absorbance calibration curve was generated for known dilution ratios of blood. To ensure the proper estimations of hematocrit in filtered fluids, a cell counting study of the most dilute sample of the known dilutions was performed. These counting studies were not performed for more concentrated dilutions of the fluid because multilayer stacking of cells in some regions limited the accuracy in cell counting. Figure 8 contains the calibration curve for absorbance as a function of cell concentration, extrapolated from cell counts of diluted blood. Here, the 1:20 diluted solution is seen approximated as 2.5×10^8 cells/ml, which would correspond to an undiluted blood solution containing 5×10^9 cells/ml, agreeing with known human cell counts.³⁵ The inset of Figure 8 contains data from cell counting studies across four different filtering trials plotted alongside the calibration curve.

While a few cells are still present in the filtered fluid, with an average 3.17×10^6 cells/ml, the number is reduced by nearly 80 times relative to the original sample, which contained 2.5×10^8 cells/ml. To put things into perspective for biosensor sensing capabilities, this reduction is sufficient to reduce the number of cells in a voxel of fluid $100 \mu\text{m} \times 100 \mu\text{m} \times 50 \mu\text{m}$ to about 2 cells (as opposed to 160 that would have been in the diluted blood inlet solution). Overall, the channel was seen to exhibit an average separation efficiency of a $\sim 99\%$ reduction of cells from the initial fluid. This separation efficiency equates to the removal of over 3×10^8 cells per minute, a large increase in the filtering capacity of these sorts of spiral channels.²⁷

C. Evaluation of plasma retention efficiency

In the dual spiral channel that contains two separate bifurcations leading to waste outlets, theoretically, the volume of fluid recovered from the second outlet should be about 25% of the input fluid. However, in the case of this channel, the fluid distribution behavior is not so simply estimated due to the addition of secondary Dean flows. The experimentally determined distribution of the fluid recovered at the outlets of the dual chip filter can be seen in Figure 9.

A slight change in the distribution of fluid is seen when blood cells are added to the solution. After fluid passes the first bifurcation, a slight volume increase is seen at the first waste outlet compared to the saline control. This increase in fluid corresponds to a decrease in the volume of filtered fluid collected at outlet 2, while the volume of fluid traveling to outlet 3 remains relatively unchanged compared to the saline control. This uneven distribution of fluid in the second bifurcation leads to further speculations of the filtering phenomena. In addition to secondary flows caused by the spirals, this flow inequality between the two outlets allows for further strengthening of the filtering mechanisms by the Zweifach-Fung effect,³⁶ a behavior seen in which particles presented with a bifurcation will prefer to travel through the channel with a higher flow rate. The effect of this flow rate difference can be estimated by comparing the ratios of the volumes of fluid travelling toward different channels at the bifurcations.³⁷ Comparing the volume collected at b1 and the sum of the volumes collected at b2 and b3, the contribution from the Zweifach-Fung effect at the first bifurcation can be estimated to be $\sim 4\%$. The effect can also be judged at the second bifurcation by comparing the volume collected at b3 to the volume collected at b2. Here, it can be seen to have a contribution of $\sim 10\%$. This combined presence of Dean force and unequal flow rates leads to an improved filtering of the sample. Performing further analysis on the channel, using the Solidworks model of the channel,

FIG. 9. Viscosity dependent volume distribution of filtered fluids across the outlets of the sequential filtering channel: blood compared to saline. The volume of filtered fluid made up 18% of the 1 ml used for these experiments.

the volume that would be lost unless air was passed to remove the remaining fluid was found to be $12.22\ \mu\text{l}$; although the loss found in these experiments was seen to be consistently $50\ \mu\text{l}$, most likely due to the fluid dead volume contained within the tubing used.

While particles of a certain size are filtered in these spiral channels, the fate of other smaller components, such as proteins and small molecules, is less deterministic. Circulated by the Dean vortices present in the channel, these molecules should be collected in equal concentrations at each of the outlets with no focusing behavior observed. Solutions of fluorescently labeled BSA, with and without the addition of diluted blood, were used to investigate the behavior of small molecules and proteins within spiral microfluidics. Protein retention is a factor often overlooked or unreported in the literature on on-chip blood filtration, yet could be useful in determining the device's capability to serve as a multi-functional optofluidic chip for use in conjunction with cell staining or ELISA (enzyme-linked immunosorbent assay) kits. Results of the flow studies in the form of percent of input solution fluorescence, representing the relative concentration of the protein recovered from the outlets, are shown in Figure 10.

The mean concentrations from each of the outlets are seen to be only slightly below the concentration of the input solution in both cases. It is possible that, since a hydrophobic PDMS material was used, protein adsorption to the channel walls or the syringe may have contributed to this slight decrease in each of the outlets.³⁸ There was also a slightly higher reduction in concentration for all three outlets for the case where the solution contained blood cells. The reduction in protein retention when the labeled protein was doped into blood is most likely due to side interactions within the blood sample occurring before or during the filtering process. The differences in the concentration of protein at each of the outlets may also be affected by the flow rates of the fluid through each of the secondary spirals. With an increased fluid velocity in the channel leading to outlet b1, which can be inferred from the greater volume of fluid collected at b1 as compared to the sum of volumes collected at b2 and b3, the protein has less time to adhere to the walls, perhaps contributing to the higher protein concentration. While a concentration loss is present at outlet 2, the filtered plasma outlet, the mean reduction is minimal, with the range of values expected being reduced by roughly 5% in both cases. This relatively unaffected concentration allows for the accurate testing of filtered fluids produced by this

FIG. 10. Distribution of fluorescently labeled albumin across the sequential chip outlets, with retention rates of 95.7% and 92.0% for free protein in saline solution and in diluted blood, respectively.

channel design. To reduce the loss of protein due to the hydrophobic nature of the PDMS, groups have modified the surface to be more hydrophilic, thereby reducing the adsorption of protein to the surface of the channel.³⁸

IV. CONCLUSIONS

The microfluidic device presented here has the capability to address the need for effective blood filtration that can be coupled to a POC microfluidic system. Specifically, this research demonstrated the filtration capabilities of a multi-stage inertial dual spiral microfluidic chip. The chip was fabricated from cheap, disposable materials (PDMS and glass slides) and can be reused, though the efficiency is reduced by $\sim 1\%$ after each use. The effects of Dean vortices introduced by curved channel geometries were analysed and quantified through the separation of diluted blood solutions. With the optimal configured flow rate of 1.25 ml/min, this micro-channel exhibited a cell filtration rate of just over 5×10^6 cells per second, with an average separation efficiency of $\sim 99\%$ and a plasma protein retention efficiency of 95%. The high filtering efficiency in the removal of blood cells from diluted blood samples using this rapid throughput inertial microfluidic technique provides a unique opportunity for it to perform as a front-end filtering chip for developing diagnostic systems aimed at monitoring blood plasma at the POC. While the $20\times$ dilution of whole blood may make the detection of molecules already at low concentrations more difficult, the dilution used is an improvement on the $100\times$ dilution required previously to achieve similar separation efficiency.^{18,25} Further optimization of the channel dimensions could be performed to enhance the chip's ability to filter higher hematocrit samples in order to compensate for the loss of sparse analytes. Ultimately, it would be preferred that no dilution be required at all in order to facilitate global health applications, but this is limited by the width of the particle streams at higher cellular concentrations. Additionally, this device could be improved by: altering surface chemistry of the channel walls to prevent adhesion of proteins, using magnetic particles coated with capture agents for isolating specific biomarker analytes from the blood, automating cell counting using a phone app for use in remote

settings, allowing for rapid processing of samples for testing in blood banks/emergency relief, and other environments.

Overall, the high reliance of inertial filtration efficiency on the spiral channel geometry of these microfluidic devices allows one to tailor the filtration and collection of desired particles present in polydisperse samples by simply altering the channel dimension. The multi-staged inertial filtration presented here also provides a unique method by which particles in a fluid could be separated across large differences in particle size by the integration of more sub sequential spirals in series. Although not explored thoroughly in this work, alterations to the primary and secondary filtering spirals themselves also present an opportunistic approach for tuning these channels for filtration of particles present in biological samples as well as those in drinking water and wastewater.³⁹ Last, the transparent packaging allows this device to be easily integrated with other lab-on-a-chip devices, specifically for miniaturizing flow cytometry, POC cancer cell imaging, spectroscopic assays, and others. By removing complicated actuating components used in other techniques as well as the requirement of a trained user, the improvements brought about through this work not only simplify the implementation of these lab-on-a-chip technologies but also display a large improvement in their efficacy, further reducing the gap in the development of improved POC diagnostic devices.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the images of the filtered samples, the enhanced magnification of the sample viewed in Figure 2(a), and the videos of the channel during operation as described in the text.

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- ¹M. M. Laboratory, http://www.metromedlab.com/SiteContent/Documents/File/ReferenceGuide/additional/CLG_5_CentrifugeProcedure.pdf for Centrifugation of Blood Specimens.
- ²See <https://www.labcorp.com/wps/portal/patient/mytest> for LabCorp My Test; accessed 29 May 2016.
- ³S. F. Green, "The cost of poor blood specimen quality and errors in preanalytical processes," *Clin. Biochem.* **46**(13), 1175–1179 (2013).
- ⁴M. Plebani, "Errors in clinical laboratories or errors in laboratory medicine?," *Clin. Chem. Lab. Med.* **44**(6), 750–759 (2006).
- ⁵S. K. Sia and L. J. Kricka, "Microfluidics and point-of-care testing," *Lab Chip* **8**(12), 1982–1983 (2008).
- ⁶Amherst, <http://www.umass.edu/uhs/services/laboratory/faq> for U. o. M. Laboratory FAQs; accessed 10 July 2016.
- ⁷See https://www.labcorp.com/wps/portal/ut/p/c1/04_SB8K8xLLM9MSSzPy8xBz9CP0os_hACzO_QCM_IwMLXyM3AyNjMycDU2dXQwN3M6B8JG55AwMCuv088nNT9QtyI8oBPK_DWA!!/dl2/d1/L0IDU0NTQ1FvS1VRIS9JSFJBQUlnb0FNeUtiBTZiL1ICSkp3NDU0a3N1eWx3ISEvN19VTRTMuk5MzBPR1MyMEITM080TJONjY4MC92aWV3SXRlbQ!!/?itemId=408 for LabCorp Introduction to Specimen Collection; accessed 25 May 2016.
- ⁸S. Alva, "FreeStyle lite—A blood glucose meter that requires no coding," *J. Diabetes Sci. Technol.* **2**(4), 546–551 (2008).
- ⁹S. Mukherjee, T. G. Kang, Y. Chen, and S. Kim, "Plasma separation from blood: The 'lab-on-a-chip' approach," *Crit. Rev. Biomed. Eng.* **37**(6), 517–529 (2009).
- ¹⁰S. Chang and Y.-H. Cho, "A continuous size-dependent particle separator using a negative dielectrophoretic virtual pillar array," *Lab Chip* **8**(11), 1930–1936 (2008).
- ¹¹P. Gascoyne, C. Mahidol, M. Ruchirawat, J. Satayavivad, P. Watcharasit, and F. F. Becker, "Microsample preparation by dielectrophoresis: isolation of malaria," *Lab Chip* **2**(2), 70–75 (2002).
- ¹²C. Szydzik, K. Khoshmanesh, A. Mitchell, and C. Karnutsch, "Microfluidic platform for separation and extraction of plasma from whole blood using dielectrophoresis," *Biomicrofluidics* **9**(6), 064120 (2015).
- ¹³L. R. Huang, E. C. Cox, R. H. Austin, and J. C. Sturm, "Continuous particle separation through deterministic lateral displacement," *Science* **304**(5673), 987–990 (2004).
- ¹⁴J. A. Davis, D. W. Inglis, K. J. Morton, D. A. Lawrence, L. R. Huang, S. Y. Chou, J. C. Sturm, and R. H. Austin, "Deterministic hydrodynamics: Taking blood apart," *Proc. Natl. Acad. Sci.* **103**(40), 14779–14784 (2006).
- ¹⁵M. Yamada, M. Nakashima, and M. Seki, "Pinched flow fractionation: continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel," *Anal. Chem.* **76**(18), 5465–5471 (2004).

- ¹⁶A. I. Rodríguez-Villarreal, M. Arundell, M. Carmona, and J. Samitier, "High flow rate microfluidic device for blood plasma separation using a range of temperatures," *Lab Chip* **10**(2), 211–219 (2010).
- ¹⁷D. Di Carlo, "Inertial microfluidics," *Lab Chip* **9**(21), 3038–3046 (2009).
- ¹⁸X. Wang, J. Zhou, and I. Papautsky, "Vortex-aided inertial microfluidic device for continuous particle separation with high size-selectivity, efficiency, and purity," *Biomicrofluidics* **7**(4), 044119 (2013).
- ¹⁹F. Petersson, L. Åberg, A.-M. Sward-Nilsson, and T. Laurell, "Free flow acoustophoresis: Microfluidic-based mode of particle and cell separation," *Anal. Chem.* **79**(14), 5117–5123 (2007).
- ²⁰A. Lee, J. Park, M. Lim, V. Sunkara, S. Y. Kim, G. H. Kim, M.-H. Kim, and Y.-K. Cho, "All-in-one centrifugal microfluidic device for size-selective circulating tumor cell isolation with high purity," *Anal. Chem.* **86**(22), 11349–11356 (2014).
- ²¹D. Jens, H. Stefan, L. Sascha, P. Sarah, F. V. Stetten, and Z. Roland, "The centrifugal microfluidic bio-disk platform," *J. Micromech. Microeng.* **17**(7), S103 (2007).
- ²²Z. Geng, Y. Ju, W. Wang, and Z. Li, "Continuous blood separation utilizing spiral filtration microchannel with gradually varied width and micro-pillar array," *Sens. Actuators, B* **180**, 122–129 (2013).
- ²³S.-Y. Tang, W. Zhang, R. Soffe, S. Nahavandi, R. Shukla, and K. Khoshmanesh, "High resolution scanning electron microscopy of cells using dielectrophoresis," *PLoS One* **9**(8), e104109 (2014).
- ²⁴J. M. Martel and M. Toner, "Inertial focusing in microfluidics," *Annu. Rev. Biomed. Eng.* **16**, 371–396 (2014).
- ²⁵N. Nivedita and I. Papautsky, "Continuous separation of blood cells in spiral microfluidic devices," *Biomicrofluidics* **7**(5), 054101 (2013).
- ²⁶M. E. Warkiani, A. K. P. Tay, G. Guan, and J. Han, "Membrane-less microfiltration using inertial microfluidics," *Sci. Rep.* **5**, 11018 (2015).
- ²⁷A. Karimi, S. Yazdi, and A. Ardekani, "Hydrodynamic mechanisms of cell and particle trapping in microfluidics," *Biomicrofluidics* **7**(2), 021501 (2013).
- ²⁸S. S. Kuntaegowdanahalli, A. A. S. Bhagat, G. Kumar, and I. Papautsky, "Inertial microfluidics for continuous particle separation in spiral microchannels," *Lab Chip* **9**(20), 2973–2980 (2009).
- ²⁹F. Schönfeld and S. Hardt, "Simulation of helical flows in microchannels," *AIChE J.* **50**(4), 771–778 (2004).
- ³⁰A. A. S. Bhagat, S. S. Kuntaegowdanahalli, and I. Papautsky, "Continuous particle separation in spiral microchannels using dean flows and differential migration," *Lab Chip* **8**(11), 1906–1914 (2008).
- ³¹J. M. Burke, R. E. Zubajlo, E. Smela, and I. M. White, "High-throughput particle separation and concentration using spiral inertial filtration," *Biomicrofluidics* **8**(2), 024105 (2014).
- ³²L. Babuin and A. S. Jaffe, "Troponin: The biomarker of choice for the detection of cardiac injury," *Can. Med. Assoc. J.* **173**(10), 1191–1202 (2005).
- ³³S. Prahl, See <http://omlc.org/spectra/hemoglobin/summary.html> for Tabulated Molar Extinction Coefficient for Hemoglobin in Water; accessed 17 July 2015.
- ³⁴R. Smith, *Certificate of Analysis* (Molecular Probes, Eugene, OR, 2014).
- ³⁵L. Dean, "Blood and the cells it contains," in *Blood groups and red cell antigens* (National Center for Biotechnology Information, 2005).
- ³⁶V. Doyeux, T. Podgorski, S. Peponas, M. Ismail, and G. Couplier, "Spheres in the vicinity of a bifurcation: Elucidating the Zweifach–Fung effect," *J. Fluid Mech.* **674**, 359–388 (2011).
- ³⁷S. Yang, A. Ündar, and J. D. Zahn, "A microfluidic device for continuous, real time blood plasma separation," *Lab Chip* **6**(7), 871–880 (2006).
- ³⁸K. Boxshall, M. H. Wu, Z. Cui, Z. Cui, J. F. Watts, and M. A. Baker, "Simple surface treatments to modify protein adsorption and cell attachment properties within a poly (dimethylsiloxane) micro-bioreactor," *Surf. Interface Anal.* **38**(4), 198–201 (2006).
- ³⁹M. Jimenez, B. Miller, and H. L. Bridle, "Efficient separation of small micro particles at high flowrates using spiral channels: Application to waterborne pathogens," *Chem. Eng. Sci.* **157**, 247–254 (2015).