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A microfluidics-based technique for automated and rapid labeling of cells for flow cytometry

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Abstract

Flow cytometry is a powerful technique capable of simultaneous multi-parametric analysis of heterogeneous cell populations for research and clinical applications. In recent years, the flow cytometer has been miniaturized and made portable for application in clinical- and resource-limited settings. The sample preparation procedure, i.e. labeling of cells with antibodies conjugated to fluorescent labels, is a time consuming (∼45 min) and labor-intensive procedure. Microfluidics provides enabling technologies to accomplish rapid and automated sample preparation. Using an integrated microfluidic device consisting of a labeling and washing module, we demonstrate a new protocol that can eliminate sample handling and accomplish sample and reagent metering, high-efficiency mixing, labeling and washing in rapid automated fashion. The labeling module consists of a long microfluidic channel with an integrated chaotic mixer. Samples and reagents are precisely metered into this device to accomplish rapid and high-efficiency mixing. The mixed sample and reagents are collected in a holding syringe and held for up to 8 min following which the mixture is introduced into an inertial washing module to obtain 'analysis-ready' samples. The washing module consists of a high aspect ratio channel capable of focusing cells to equilibrium positions close to the channel walls. By introducing the cells and labeling reagents in a narrow stream at the center of the channel flanked on both sides by a wash buffer, the elution of cells into the wash buffer away from the free unbound antibodies is accomplished. After initial calibration experiments to determine appropriate 'holding time' to allow antibody binding, both modules were used in conjunction to label MOLT-3 cells (T lymphoblast cell line) with three different antibodies simultaneously. Results confirm no significant difference in mean fluorescence intensity values for all three antibodies labels ($p < 0.01$) between the conventional procedure (45 min) and our microfluidic approach (12 min).

Keywords: rapid cell labeling, flow cytometry, automated sample preparation

(Some figures may appear in colour only in the online journal)

Introduction

Flow cytometry has transitioned from a research tool primarily used in laboratories to a powerful tool for the clinical diagnosis of various diseases and conditions [1]. Today, flow cytometry is used extensively for hematopathology [2, 3], clinical microbiology [4, 5], cancer diagnostics [6] and the monitoring of host response to various treatments including immunosuppression therapy [7] and drugs [8]. Flow cytometers are still large instruments typically housed in diagnostic labs where samples collected from various clinical sites are transported for analysis. Despite the growing list of new flow cytometry-based diagnostic and prognostic assays,

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its use is somewhat limited to clinics in the proximity of a diagnostic lab with a flow cytometer. The most common sample analyzed via flow cytometry is blood which contains cellular populations that are best analyzed almost instantly. Transportation and extended delays in processing adversely affect the cells in blood causing unnecessary and artifactual activation that can compromise the quality of information attained during analysis. To fully exploit the potential and capabilities of the flow cytometer, it is best that the instrument be available at the clinical site for instantaneous analysis of samples. This will also be of immense benefit in resource-limited settings where access to expensive flow cytometers is not available. To address this issue, all major flow cytometry manufacturing companies including BD Biosciences, Beckman Coulter, Applied Biosciences and Millipore have developed smaller portable versions. Several research groups have also worked to develop microfluidic versions of the flow cytometer for point-of-care applications [6, 9–11].

Another important aspect of flow cytometry that is often overlooked is sample preparation. Typically, blood samples obtained for analysis are depleted of erythrocytes or red blood cells using a selective lysis process. The remaining leukocytes or white blood cells (WBCs) are then labeled with fluorescently labeled antibodies specific to WBC phenotype and activation markers prior to analysis. The process of labeling WBCs is a time consuming process that requires several steps including pipetting, mixing, extended incubation time and multiple wash steps that require the use of a centrifuge. This entire process can take longer than 45 min and is not point-of-care compatible. Microfluidics-based approaches have great potential to automate and significantly reduce processing time for sample preparation. However, unlike significant interest in miniaturizing the instrument itself, very few groups have focused on automating flow cytometry sample preparation using microfluidics [12–14].

Microscale systems have inherent advantages over conventional macroscale systems in their ability to precisely control fluid flow and unique flow phenomena that can be exploited to accomplish new devices for automated sample preparation. Two techniques that can be integrated together to enable automated sample preparation for flow cytometry are chaotic mixing in microchannels [15] and inertial focusing [16]. In microfluidic channels, mixing is diffusion limited due to predominantly laminar flows within the channels. In conventional macroscale systems, the labeling typically involves vortex mixing and incubation for extended periods (∼30–45 min) to ensure complete mixing and cell labeling. Stroock *et al* [15] elegantly demonstrated that the introduction of microstructures in the form of staggered double herringbone structures within on the floor of a microchannel can accomplish high-efficiency chaotic mixing. Cells and antibodies can be introduced within a microfluidic channel with an integrated chaotic mixer to rapidly mix, placing antibodies in the proximity of cells and thereby eliminating the need for vortex mixing and significantly reducing the duration of incubation in the labeling step. Inertial focusing is another technique that can be used to automate the washing step in sample preparation. Inertial focusing exploits shear gradients that develop in rectangular high aspect ratio channels to focus cells at equilibrium positions close to the channel walls. Gossett *et al* [12] have previously shown that this focusing effect used in conjunction with a wash buffer stream can be used to move cells from the sample solution into the buffer solution. This approach eliminates the need for a centrifuge and ensures the isolation of cells into a wash buffer, whereas the unbound labels (antibodies) remain in the sample stream.

In this paper, we present an integrated microfluidic device that consists of a labeling module and a washing module. Samples and labeling antibodies are automatically metered into the labeling module using syringe pumps where they interact with the double herringbone structures and are mixed rapidly and efficiently. Samples are then collected in a holding syringe where they are held for a time sufficient for antibody binding. The samples are then flowed along with the washing buffer into an inertial focusing device where the labeled cells are separated from unbound antibodies at a concentration suitable for direct analysis using the flow cytometer. The entire process can be automated and is accomplished in <12 min from sample to analysis.

Materials and methods

Microfluidi device fabrication

Devices were fabricated by using standard soft-lithographic techniques, as described previously [17]. In brief, master molds were prepared by spin coating a negative photoresist, SU-8 100 (Microchem, Newton, MA) on 4″ silicon wafers. The negative photoresist was exposed to ultraviolet light through a bright field mask of a transparency layout. Uncross-linked photoresist was removed by washing the wafer with SU-8 developer. The negative replicas were used to mold polydimethylsiloxane (PDMS) devices. The PDMS was prepared by mixing Sylgard 184 silicone elastomer base (Dow Corning; Midland, MI) with Sylgard 184 silicone elastomer curing agent (Dow Corning, Midland, MI) in a ratio of 10:1 followed by baking in an oven at 70 °C for 3 h. Molded PDMS devices were removed from the silicon wafer and trimmed, and inlet and outlet holes were opened out using syringe needles (Luer Hub 20G × 1/2″, Smallparts, Logansport, IN). To form the channels, PDMS structures and standard glass slides (Fisher Scientific; Waltham, MA) were exposed to O_2 plasma treatment in a Plasma Asher (Nordson March Instruments, Amherst, OH) and both pieces were placed together against each other and heated at 115 °C for 10 min. Inlet and outlet tubing (Smallparts, Logansport, IN) were introduced and fitted into the holes prior to use.

Microfluidi device design

The labeling module used is similar to that used by our group previously [17–19] and has two inlets and two outlets (figure 1(*A*)). The sample loading end has two inlets, for the injection of cells and antibodies using syringe pumps. The antibody solution is divided into two streams that flank the suspended cell stream leading into the serpentine mixing channel. At the flow rates used, the cells are in contact

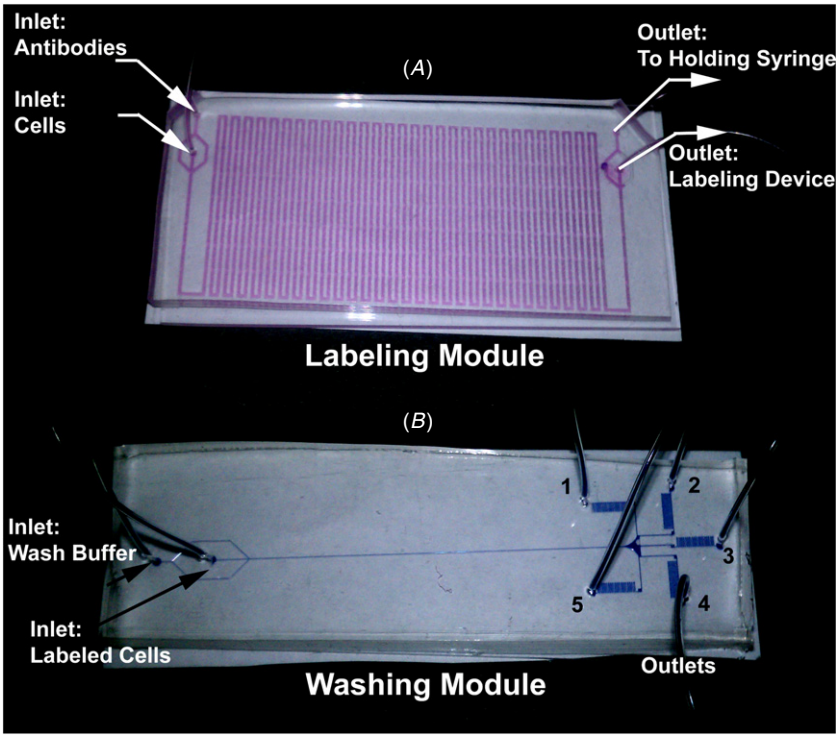

Figure 1. Pictures of the two modules used to accomplish automated labeling and washing of cells for flow cytometry: (*A*) labeling module and (*B*) washing module.

with the antibody mixture for ∼40 s. For rapid and uniform mixing of cells with the antibodies, the bottom of the channels are patterned with staggered double herringbone structures, which generate non-uniform flow resistance, thus effecting two counter rotating vortices within the channel. In addition, the variable ridge length and their arrangement produce immediate chaotic mixing that promotes the even distribution of the cells and antibodies within the channel. The channels are 160 cm long with a cross section of 500 $\times$ 200 μm. Ridges are 25 μm high and 20 μm wide. Internal volume is 140 μL. The overall footprint of the device is 3.5 cm $\times$ 7.5 cm.

The design of the wash module used was similar to that developed previously by our group [20] (figure 1(*B*)). In brief, the device has two inlets and five outlets connected with a long and narrow channel. The width, length and height of the channel were 40 μm, 4.5 cm and 95 μm, respectively, to ensure maximum cell separation from the unbound antibody based on inertial lift forces. The overall footprint of the device is 1.5 cm $\times$ 7.5 cm.

Characterization experiments

To perform the characterization of the labeling module to efficiently mix and the washing module to separate cells from unbound antibodies, the devices were characterized with a fluorescent protein Fluorescein (Sigma Aldrich, St. Louis, MO) with a diffusion coefficient of 4.25 $\times$ 10^{-6} cm^2 s^{-1} at a concentration of 1 mg mL^{-1}. In comparison, antibodies have a diffusion coefficient of 3.9 $\times$ 10^{-7} cm^2 s^{-1} and are typically used at a concentration of 10–500 μg mL^{-1}.

To characterize mixing in the labeling module, a fluorescein solution was introduced via the cell inlet and 1X phosphate buffered saline (PBS) was introduced via the antibody inlet such that a stream of the fluorescein was flanked on both sides by 1X PBS. The ratio of fluorescein to 1X PBS was 1:1 and the overall flow rate was 200 μL min^{-1} which is the same as the conditions used for all experiments. Mixing was evaluated qualitatively via fluorescence microscopy. Images were taken at the inlet and at subsequent locations along the length of the channel. To characterize diffusion of antibodies in the washing module, fluorescein solution was introduced at cell inlet with 1X PBS in the buffer inlet such that the fluorescein solution was flanked on both sides by 1X PBS. The ratio of fluorescein to 1X PBS was 1:2 and the overall flow rate was 400 μL min^{-1} which was identical to flow rates used for all experiments. The diffusion of fluorescein was observed at the inlet and the expanding region of the outlet via fluorescence microscopy.

Experimental setup for automated sample preparation

The experimental setup for automated sample preparation is illustrated in figures 2(*A*) and (*B*). The two modules were connected in series as shown in figure 2(*A*). Cell samples and antibody solutions were introduced via the two inlets into the labeling module at a ratio of 1:1 and an overall flow rate of 200 μL min^{-1} and collected at the outlet into a holding syringe. Fluid flow through the second outlet leading to the washing module was stopped using a valve (figure 2(*A*)). The sample was held in the holding syringe for a predetermined amount of time. Once the sample was ready to be washed, the setup was adjusted to that shown in figure 2(*B*). The valve connecting the labeling and washing modules was opened, and flow out of

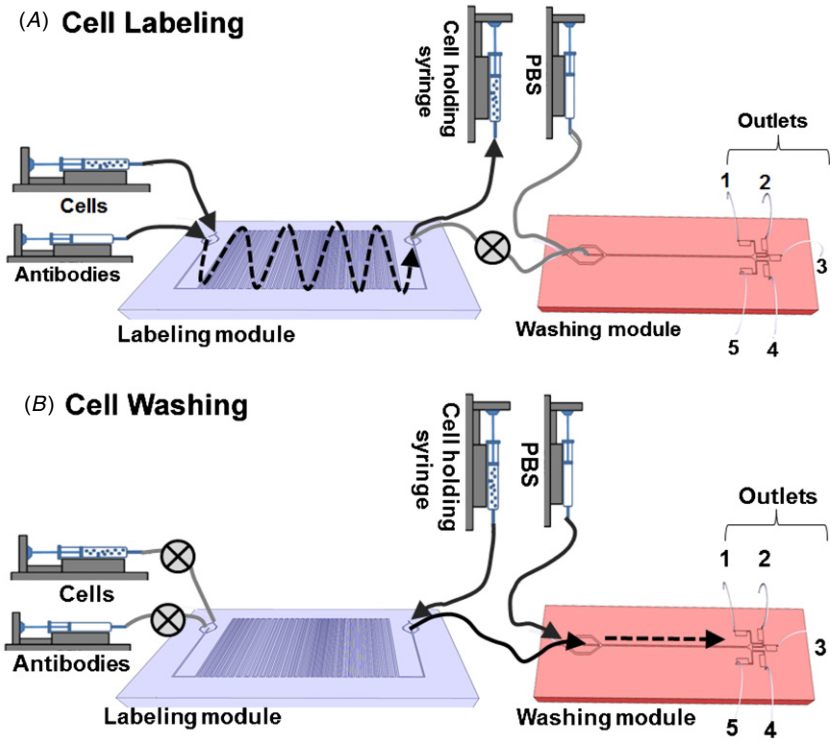

Figure 2. (*A*) Schematic of setup used to accomplish the labeling of cells using the labeling module. Cells and antibody solution are flowed into the labeling module where they are rapidly mixed and collected in a holding syringe for 8 min to complete the labeling process. A valve ensures separation from the washing module. (*B*) Schematic of the setup used to wash cells and remove unbound antibodies following labeling. Two valves are used to prevent outflow from the labeling module into the sample delivery syringes. The valve used to separate the labeling and washing modules is opened and the wash buffer is delivered via a second syringe to ensure laminar flow of cells flanked on both sides by the wash buffer. Inertial focusing in the washing module ensures separation of cells from unbound antibodies(arrows indicate direction of fluid flow).

the labeling module inlets was stopped using two valves. The sample in the holding syringe was introduced into the labeling device via a syringe pump in conjunction with 1X PBS wash buffer. The samples briefly transit through the labeling module into the washing module and are focused at the center of the channel flanked on both sides by 1X PBS wash buffer. The ratio or samples to 1X PBS was 1: 2 and the overall flow rate was 400 μL min^{-1}. Once the samples transit through the focusing channel, the cells are collected via outlets 2 and 4, whereas the unbound antibodies are collected via outlet 3. Excess buffer is collected via outlets 1 and 5. The resistances of the outlet channels are varied such that 50% of the fluid flows via the center outlet, 30% of the fluid flows via outlets 1 and 5 and 20% of the fluid flow via outlets 2 and 4.

Cell culture

MOLT-3 cells (peripheral blood, leukemia, T cell) were purchased from American Type Culture Collection (ATCC; Manassas, VA) and were cultured in RPMI-1640 medium (Thermo scientific; Waltham, MA) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Mediatech Inc. Cellgro, Waltham, MA). Cells were incubated at 37 °C in a humidified atmosphere containing 5% (v/v) CO_2. After 48 h, the cells were centrifuged, washed with 1X PBS and resuspended in 1X PBS containing 4% paraformaldehyde and incubated for 15 min at room temperature. Cells were centrifuged, washed and resuspended in the wash buffer (1% bovine serum albumin in 1X PBS) for further experiments.

Staining and washing procedure

For staining using the standard protocol, conjugated antibodies were added to 300 μL of cell suspension and incubated for 45 min at 4 °C. Samples were centrifuged for 5 min at 250 $\times$ g, washed two times, centrifuged again and resuspended in 400 μL of 1X PBS for analysis by flow cytometry.

Evaluation of MOLT-3 cells by fl w cytometry

Approximately 20 000 MOLT-3 cells were used in each experiment. For this, approximately 300 μL of cell suspension containing 30 000 cells was used. By nature of continuous flow-through from device to device without pipetting steps, minimal cell loss is expected. However, considering the brief centrifuge step and transfer to flow cytometry tubes, 30 000 cells were used to ensure the processing of at least 20 000 cells. Cells simultaneously stained with anti-CD50-FITC, -CD162-PE and -CD45-PerCP (BD Biosciences, San Jose, CA) using standard procedures or within the microdevices, as described above, were analyzed using flow cytometry (BD FACS Calibur, San Jose, CA). Results were normalized to isotype controls and analyzed using flow cytometry data analysis software (WinMDI and BD Cell Quest Pro). Paired Student's *t*-tests

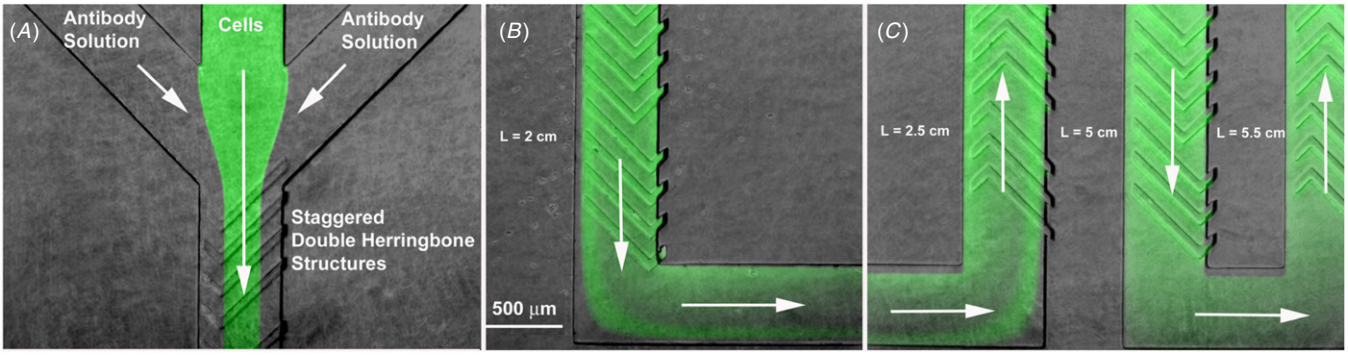

Figure 3. Fluorescence microscopy images to visualize mixing in the labeling module. (*A*) Fluorescein solution was introduced via the center inlet flanked on both sides by phosphate buffered saline (PBS). The double herringbone structures arranged in a staggered fashion induce fluid rotation and chaotic mixing. (*B*) Image at a location 2 cm from the inlet showing partial mixing and (*C*) image at $L = 5$ and 5.5 cm from the inlet showing complete mixing of fluorescein and PBS. Note. This image uses fluorescence imaging to visually demonstrate mixing in the labeling module and does not contain any cells or antibodies.

with two tailed significance set at $p \leqslant 0.01$ were used to quantify differences in expression of various markers ($n = 3$).

Results

Characterization of mixing in the labeling module

Our prior work using a device identical to the labeling module confirms that cells are uniformly distributed within the device at ~5 cm form the inlet [17]. In order to confirm the efficiency of mixing within the device, a fluorescein solution and 1X PBS were flowed into the labeling module at a 1:1 ratio at a combined flow rate of 200 μL min^{-1} as described in the methods section. The staggered double herringbone structures induce chaotic mixing, and enable complete mixing of the fluorescein across the cross section of the channel. Fluorescence microscopy images taken at the inlet and at 2.5, 5 and 5.5 cm from the inlet show the formation of counter rotating vortices, which ensures complete mixing ~5 cm from the inlet (figure 3). Fluorescein has a diffusion coefficient comparable to that of antibodies used in subsequent experiments and fluorescence microscopy images confirm that uniform mixing of fluorescein occurs close to the inlet. The fluid continues mixing as it transits through the remaining 123 cm of the labeling channel prior to exiting the device. At the flow rate used, the transit time for the sample is ~40 s. Uniform distribution of antibodies occurs within 1–2 s; however, longer time periods are required for uniform redistribution of cells within the channel, and this can take ~10–40 s based on the concentration of cells introduced within the channel.

Characterization of diffusion in the washing module

The primary purpose of the washing module is to isolate labeled and unlabeled cells into the wash buffer without the unbound antibodies. Antibodies within the sample stream do not experience inertial forces and their mixing into the sample stream is diffusion limited. To determine the diffusion of free antibodies within the washing module, fluorescein was introduced at the center, flanked on both sides by 1X PBS as described in the methods section. The ratio of fluorescein solution to 1X PBS was 1:2 and the total flow rate was 400 μL min^{-1}. Fluorescence microscopy images taken at the inlet and the expanding region of the outlet (figure 4) show that laminar flow ensues within the channel. The fluorescein solution is confined to the center stream with buffer on both sides. At the outlet, there is some diffusion of fluorescein into the buffer stream but the fraction of fluid containing fluorescein does not overlap with the equilibrium focusing position of cells. Focusing of cells close to the outer walls is denoted by dashed lines as verified previously by our group [21]. The outlet flow resistances were adjusted by either cutting short the length of the outlet tubing or gently crushing the soft outlet tubing using screw-type clamps to isolate ~50% of the fluid via outlet 3 (all unbound antibodies). The outlet flow resistances were also tuned to ensure ~30% is isolated via outlets 1 and 5 and only 20% of the fluid is isolated via outlets 2 and 4. All cells focus at their equilibrium positions and the adjustment of the resistances ensures that cells exit via outlets 2 and 4. This was verified via the rectangular chambers at each outlet that enable the visualization of cells exiting the device. The concentration of cells collected at outlets 2 and 4 is high enough to be used directly for flow cytometry (slightly less than 1×10^5 cells mL^{-1}).

Determination of optimal holding time for labeling

The labeling device accomplishes highly efficient mixing (even distribution of cells and antibodies) in <2 min. However, this time is not sufficient for all antibody binding events to occur. To ensure sufficient time for antibody binding to occur after uniform distribution of cells and labeling antibodies, cells were collected in a holding syringe for a fixed amount of time prior to flowing through the washing module. MOLT-3 cells mixed with CD45-PerCP in the labeling module were collected in a holding syringe for 2, 3, 4, 5 and 8 min, respectively, prior to washing. Binding of CD45-PerCP to targets on the surface of MOLT-3 cells was quantified by calculating the mean fluorescence intensity (MFI) in each cell which is a direct measure of antibody binding. Results were compared to unstained controls and cells stained with anti-CD45-PerCP using the standard 45 min incubation protocol.

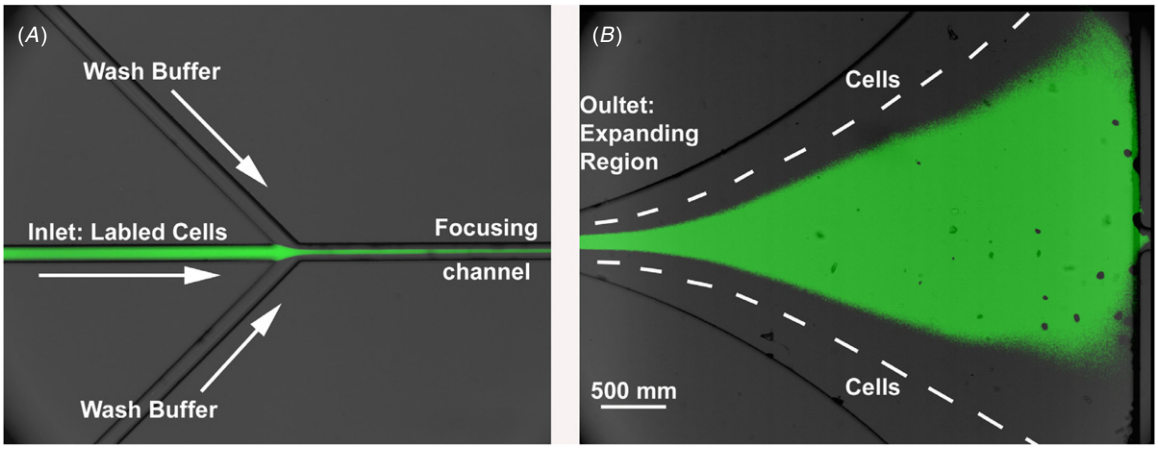

Figure 4. Fluorescence microscopy images to visualize laminar flow and diffusion of fluorescein which mimics free antibodies in the washing module. (*A*) A fluorescein containing solution was introduced at the center inlet flanked on both sides by the wash buffer at a ratio of 1:2 fluorescein: wash buffer at a combined flow rate of 220 μL min^{-1} and (*B*) an image of the expanding region of the outlet confirms that the fluorescein introduced at three orders of magnitude higher concentration is restricted to the center portion of the channel and does not reach the regions where MOLT-3 cells focus (dotted lines), enabling isolation via separate outlets and adjustment of outflow resistances. Note. This image uses fluorescence imaging to visually demonstrate diffusion in the washing module and does not contain any cells or antibodies.

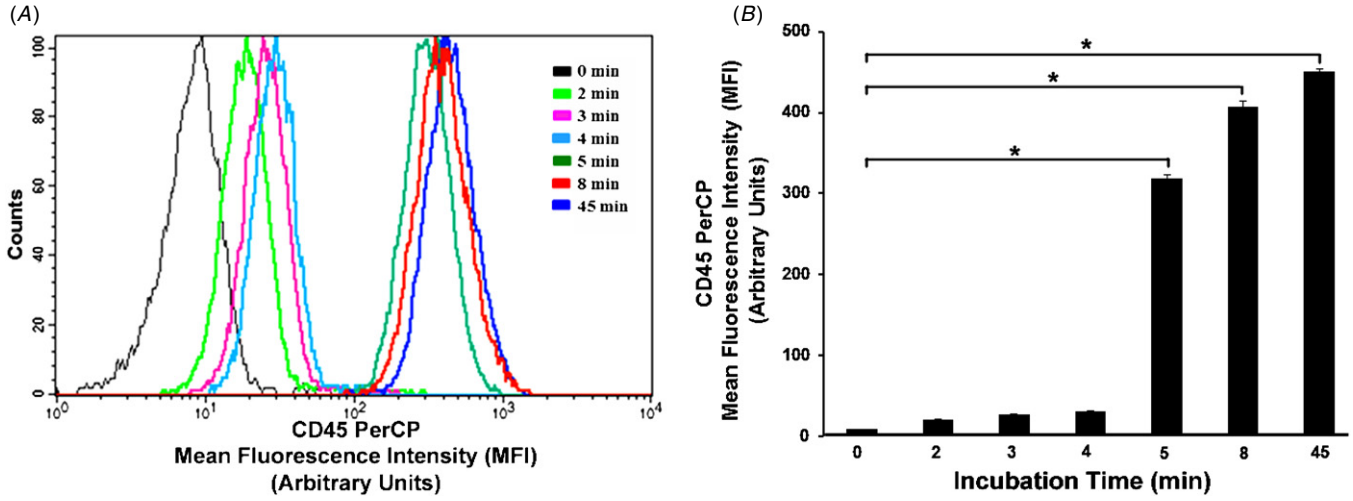

Figure 5. (*A*) Flow cytometry analysis of MOLT-3 cells labeled with anti-CD45-PerCP using both the microfluidic protocol (incubation times of 0, 2, 3, 4, 5 and 8 min) and using the standard 45 min procedure. (*B*) Bar plots summarizing the flow cytometry data. Sample size was $n = 3$ (three independent experiments).

Results quantified in figure 5 clearly show that there is a time-dependent increase in antibody binding to MOLT-3 cells. Holding times of 5 and 8 min show statistically significant ($p < 0.01$) differences in MFI values in comparison to 2, 3 and 4 min. The 5 and 8 min holding time did not show statistically significant ($p < 0.01$) differences between the conventional 45 min staining protocol. These results indicate that a holding time of >5 min is sufficient for a majority of the antibody binding to occur following efficient mixing in the labeling module. The 8 min holding time was used for subsequent experiments.

Multi-color labeling of cells using the microfluidi labeling protocol

To demonstrate that this microfluidic protocol can be used for more complex flow cytometry sample preparation like multi-color labeling, the staining of MOLT-3 cells with three different antibodies was accomplished and compared to unstained controls and the conventional 45 min staining procedure. MOLT-3 cells were introduced into the labeling module and stained simultaneously with CD50-FITC, CD162-PE and CD45-PerCP, held in the holding syringe for 8 min and then washed in the washing module prior to analysis using the flow cytometer. Scatter plots simultaneously evaluating MFI values of different combinations of antibody binding in figure 6 confirm that the results obtained using the microfluidic protocol for MOLT-3 cells appear identical to results obtained using the standard 45 min procedure. These results confirm that high-quality multi-color staining can be accomplished in <12 min of overall processing time using the described microfluidic protocol without any qualitative or quantitative differences.

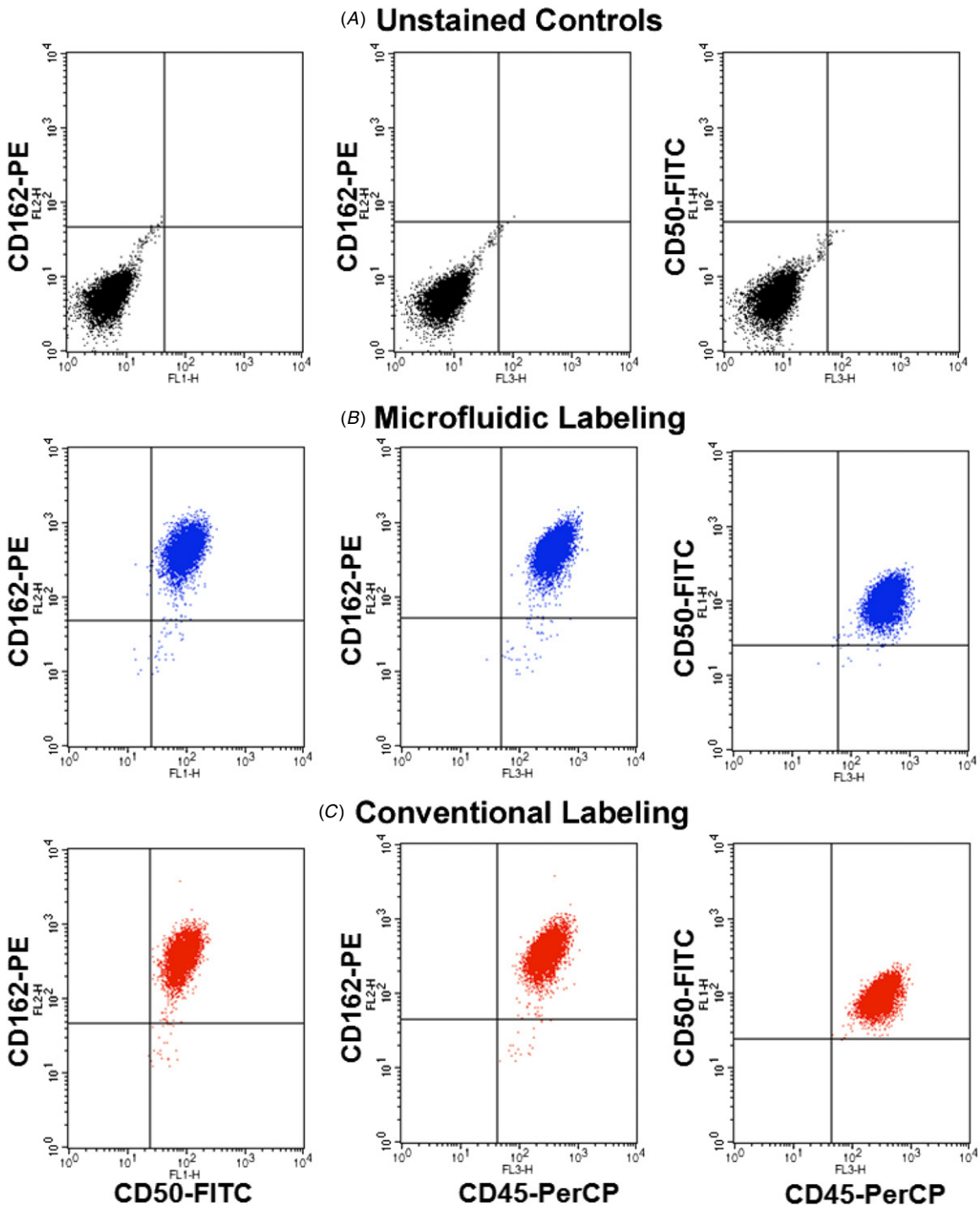

Figure 6. Multi-color flow cytometry analysis of MOLT-3 cells labeled simultaneously with anti-CD50-FITC, -CD162-PE and -CD45-PerCP using (*A*) unstained controls, (*B*) microfluidic labeling (8 min incubation in a holding syringe) and (*C*) conventional labeling (45 min incubation in a 15 mL tube).

Discussion

Miniaturization of flow cytometry has been a topic of great interest over the past few years. Several groups and companies have worked toward miniaturizing and automating the flow cytometer for point-of-care in clinical and research settings. However, to enable portable flow cytometry, sample preparation protocols also need to be suitably adapted. Currently, flow cytometry sample preparation is time consuming (>45 min) and requires at least two centrifugation steps and skilled personnel to process samples. In its current state, the labeling protocol is not compatible with clinical or resource-limited settings. Therefore, the development of a faster automated sample preparation protocol without the need for centrifugation is essential to enable point-of-care flow cytometry.

To accomplish rapid and automated sample preparation for flow cytometry, we exploit scaling effects unique to

microfluidics. Samples and reagents can be introduced in precise amounts into microfluidic channels and mixed almost instantaneously using microstructures (staggered double herringbone structures). This ensures that cells and antibodies are evenly distributed in a confined space (microchannel), which not only accomplishes the even distribution of cells and antibodies but also places antibodies in the proximity of their binding targets on the surface of the cell. Our results with fluorescein confirm that uniform mixing can be accomplished very close to the inlet (∼5.5 cm from inlet). However, to enhance mixing and ensure the even distribution of labeling reagents and cells, chaotic mixing continues throughout the entire length of the microchannel (128 cm). Samples mixed in such a fashion require significantly smaller incubation times as every cell experiences identical conditions, and heterogeneity of conditions in large test tubes is overcome. Unlike vortex mixing which enhances mixing in test tubes, microfluidic mixing using the double herringbone structure accomplishes mixing by redirecting fluid streamlines, and since the cells are in flow, the effect of shear stress is minimal, thereby eliminating undue stress on cells. Therefore, gentle back-and-forth pipetting is the preferred method of mixing in the macroscale. Once samples are mixed efficiently in the microfluidic channel, the sample is collected in a holding syringe for the subsequent washing step. Collection into a syringe minimizes transfer steps and samples can be seamlessly transferred to the washing module. Despite rapid and efficient mixing within the microchannel, antibody binding events require extended periods of time to accomplish proper orientation and binding. Our results suggest that this process requires at least 5 min to ensure statistically significant differences ($p < 0.01$) between labeled cells and unlabeled controls. An 8 min incubation time did not show any statistically significant differences in comparison to cells labeled using the conventional 45 min protocol. Therefore, microfluidic labeling using the labeling module ensures rapid and efficient mixing and labeling of cells with antibodies. Moreover, the devices are readily reusable after a quick rinse with DI water and 1X PBS. This device represents proof-of-concept demonstration. Multiple devices can be operated in parallel for the processing of large numbers of samples.

Centrifugation is an important aspect of cellular sample preparations. Centrifugation steps typically subject cells to ∼250–350 *g* for 5 min. Microfluidic approaches can be used to eliminate centrifugation. Two examples of microfluidic alternatives to centrifuges have been demonstrated [12, 13]. The technique that is compatible with flow through systems exploits inertial focusing effects to move cells from the sample stream to a buffer stream, thereby eliminating contaminating non-target materials in the sample stream. This approach was slightly modified and used in conjunction with microfluidic mixing to accomplish the wash step following labeling to remove unbound antibody labels. Introducing the sample stream at the center of a high aspect ratio flow channel flanked on both sides with wash buffer allows cells to focus at equilibrium positions close to the outer walls (within the wash buffer) while the smaller unbound antibodies remain confined to the center sample stream due to viscous effects. This step essentially replicates the centrifuge in a continuous flow through fashion. At the exit of the flow channel, five outlets were used to fractionate the cells and unbound antibodies. By adjusting the flow resistances, the volumes of fluid exiting via each outlet can be controlled. The flow resistances were modulated to not only ensure that all unbound antibodies exited via the center outlet, but also to fractionate excess wash buffer and obtain cells at concentrations that are suitable for direct analysis using flow cytometry.

Finally, the ability to label cells with multiple antibodies using this method was also evaluated. Results confirm that microfluidic labeling combined with an 8 min holding step and inertial washing results in cell labeling that is similar to that obtained with the conventional 45 min procedure accomplished in a test tube with two centrifugation steps. This approach can therefore be used as a portable and automated alternative to conventional flow cytometry sample preparation.

Conclusions

In summary, we developed a technique that can accomplish sample preparation for flow cytometry in a rapid and automated fashion. The whole process from start to analysis using flow cytometry takes <12 min and can accomplish staining using one or multiple antibodies. No quantitative or qualitative differences were found when compared to the conventional 45 min procedure. This technique can be adapted for point-of-care sample preparation for flow cytometry.

Acknowledgments

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