A method for detecting forward scattering signals on-chip with a photonic-microfluidic integrated device

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Abstract: A photonic integrated microfluidic device is demonstrated to perform optical excitation and forward scatter collection all on-chip in a planar format. Integrated on-chip optics formed a tailored beam geometry for optimal excitation of particles while a special design modification allowed for on-chip forward collection with the beam shaping capabilities. A notch was placed in the lens system that caused a dark spot on the facet of a collection waveguide while not affecting the beam geometry at the point of interrogation. The modified device with the ability to form a 10 μm beam geometry was demonstrated to detect the forward scatter from blank 5 μm diameter polystyrene beads. Free-space collection of side scatter signals was performed simultaneously with the on-chip collection and the designs demonstrated and enhanced SNR while the reliability of detection was determined to be appropriate for many applications. Excellent performance was confirmed via a false positive rate of 0.4%, a missed events rate of 6.8%, and a coincident rate of 96.3% as determined between simultaneously performed free-space and on-chip detection schemes.

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1. Introduction

Microchip-based flow cytometers – a microfluidic version of their conventional counter-part – form a very important and specialized segment of Lab-on-a-chip (LOC) devices that has been receiving a great amount of attention with examples proliferating throughout the literature [1–6]. Many functioning devices have been demonstrated in many diverse applications [7,8]; though particular importance has been stressed on point-of-care (POC) medical applications [1,2,9–12]. Due to the small size of the channels, finer manipulation of smaller samples and the associated dyes is possible. Furthermore, parallel processing and automation of chemical processes is a viable solution that allows for a higher level functionality on-chip with fewer handling steps and associated errors and costs. These advantages became a reality due to the advances in manufacturing processes based on conventional photolithography or micromolding [13,14].

Optical detection techniques are the standard method for interrogation in the biological assays performed in flow cytometry [15]. Many analysis techniques rely on fluorescence detection due to the very high level of specificity afforded by targeted labeling with fluorescent dyes [9–11]. However, the importance of scatter detection cannot be ignored – especially due to the inherent advantage that it does not require an expensive dye or the associated labeling step. Forward scattered light is typically defined as scattered light that deviates from the illuminating beam axis by an angle of 0.5° to 5° [15]. This parameter can yield important information such as the size of the specimen under illumination and the viability of the cell – sometimes a singular defining characteristic in an assay [15].

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For microchip-based cytometers to achieve feasible operation in LOC-based and POC applications, a higher level of functionality must be integrated onto the chip. This relieves the dependence of these devices on bulky and expensive external components resulting in devices that are truly portable and inexpensive; perfectly suited for POC and remote applications [16,17]. Recent work on microchip-based flow cytometers has demonstrated the integration of optical components on the chip to perform the optical interrogation of particles [2–7,13,18–23]. Waveguides and focusing elements have been demonstrated on-chip with varying degrees of success and performance. Our earlier works have demonstrated on-chip beam shaping optics to form ideal excitation beams for uniform optical particle excitation, and thereby, reliable detection [21]. With these devices we determined that the beam geometry must be specifically shaped according to the targeted particle of interest [23]. With properly deployed devices, we showed excellent fluorescent performance demonstrated by CVs of 8.5% and 15.9% for 2.5 µm and 6 µm fluorescent beads, respectively [23], while side scatter detections yielded CVs of 15.8% and 20.4% for 2.5 µm and 6 µm beads, respectively [22] – all using on-chip excitation optics. Integration of optics onto the chip allowed the creation of devices that could serve a plug-and-play function as the specific optical calibration is done on-chip and the dependence on specific or special external equipment is reduced or eliminated leading to cheap and easy procedures using reusable devices. However, with on-chip beam shaping excitation it is still impossible insofar to achieve forward scatter detection with an on-chip detection scheme, even though a forward scatter function should be incorporated into the device to satisfy the broader need of bio-detection assays.

Forward scatter has been a difficult technique to integrate on-chip – though several successful attempts have been demonstrated. One device integrated narrow waveguides on-chip with specific waveguide numerical apertures (NA) to limit the collection window within the channel and reduce noise [20]. A second device inserted fibres to deliver a low divergent single-mode beam [19] allowing the nearly collimated beam to be eliminated from the carefully place collection waveguide. Both devices showed excellent results but have subtle shortcomings that hamper design flexibility or that limit feasibility in true LOC applications. In the former device, the collection angle limits the magnitude of the signal, while the latter device relies on a low-divergent beam from an expensive single-mode light source that creates difficult coupling to the device. Both methods are also not compatible with beam shaping – something that both of the works mentioned would improve performance [19,20] and a technique that we identified is crucial for optimal device performance [23].

In this work we propose a new design – based on conventional techniques – to allow on-chip collection of forward scattered light while allowing for beam shaping and the use of a low quality, highly divergent, and inexpensive light source. The design blocks transmitted light in the paraxial regime while allowing large collection of forward scattered light. Furthermore, the designs do not hamper the ability of other detection methods to be simultaneously integrated with the device. The designs lower the complexity and cost of devices while allowing customization of the device function to suit a specific application through the on-chip beam shaping. The method is adaptable to many different lens designs and will allow a fully optically integrated device suitable for true POC and remote sensing applications.

2. Device design

As mentioned earlier, forward scattered light is the light whose direction deviates from the initial ray direction by a tiny amount; typically 0.5 to 5 degrees. This mode of detection is problematic when the beam is divergent, as pictured in Fig. 1(a). A particle situated in a divergent beam is subjected to many different ray directions - for example, the directions illustrated by rays 1 and 2 in Fig. 1(a). The forward scattered light generated by ray 1 is obscured by the transmitted light from rays in the direction of ray 2 (and visa-versa). Simply blocking light in the direction of ray 2 will eliminate it as a source of noise and allow a larger
SNR for rays scattered from direction 1. Blocking rays in the immediate (angular) vicinity of ray 2 will block much of the noise that interferes with the forward scattered rays in the (angular) vicinity of ray 1. Two caveats when implementing this design are that blocking these rays will cause a slight drop in overall interrogation intensity; as well the annulus of rays defined around the direction of ray 1 cannot be too large as side scatter from extreme rays will begin to interfere with those in the forward scatter direction.

Fig. 1. a) A particle in a focused beam has the forward scattered light masked by transmitted light from other divergent rays. b) Conventional techniques insert a field stop in the incoming light to ensure a dark field in the image space where collection takes place [15].

Conventional cytometry uses a physical obscuration in the central (axial) portion of the beam to create a dark region in an image coplanar with a photon detector, Fig. 1(b). The imaging system is able to focus the off-axis light to a narrow region in the interrogation region and carefully shape the beam geometry for ideal optical excitation. Forward scattered light rays will deviate from the imaging system’s simulated train of rays and instead land in the dark region. This effect boosts the SNR and is a very reliable method as conventional techniques can achieve very small CVs – 2-5% [19,20].

A planar on-chip solution for forward scatter light is difficult to achieve, however some successful chip-based solutions have been demonstrated. Employing a single-mode beam allowed the use of an inserted single-mode fibre and a very low divergent beam to avoid coupling to inserted detection fibres [20]. Another work used very narrow collection waveguides to block coupling of light from all but a very small portion from a beam with a small NA [19]. These methods, however, rely on low divergence beams from expensive sources and are not adaptable to beam shaping and the large divergent beam that can result from low quality sources. Our previous works have shown beam shaping to be an important tool for improving reliable detection and that quality of detection depends on the geometry of the beam used with the specimen size [21–23]. Employing integrated optics on-chip to perform a beam shaping function has had the best performance in fluorescence and scattering detection so far. Enabling on-chip forward scatter detection capabilities in the same device as one that employs beam shaping would alleviate complex, expensive, and bulky optics and allow device performance and requirements to better suit market requirements.

The beam shaping function on-chip is achieved by integrating a waveguide and a 2D lens system with a microfluidic channel on chip [21,24]. Due to the divergent beam issue, as described above in Fig. 1, normal on-chip collection via a waveguide has difficulties obtaining reliable signal in a forward scattering direction. Figure 2(a) shows a picture of a planar device where on-chip beam shaping excites particles within a microchannel – using no other design considerations for forward scatter. Light is injected via a 50 µm wide by 30 µm tall waveguide that supports a heavily multimodal and divergent beam. The lens system focuses the divergent light into a specific beam geometry with a tailored beam waist and depth of focus. Seven surfaces comprise the designed lens system: #1 being the input from the
waveguide facet, #2-5 being the surfaces (curved or not) forming the lens elements, #6 being the microchannel wall on the side of the lens system, and #7 being the plane that is in the centre of sample flow. Immediately opposite the channel from the input lens system is a waveguide for collection of forward scattered signal while side scatter can be collected out the top or bottom of the device via a free-space detection scheme to confirm forwards scatter events. In this configuration forward signal is obscured by transmitted light and results in a low SNR. This is confirmed by the raw data shown in Fig. 2(b) that contrasts simultaneous free-space scatter events with on-chip forward collection with a waveguide and the divergent beam formed by the on-chip lens system. Some events will be lost in the noise leading to poor performance.

![Image](image1.png)

Fig. 2. a) Picture showing a planar device with on-chip waveguides to collect scattered light showing that a forward scatter waveguide collects transmitted light. b) Graph showing 200ms of raw data from a simultaneous free-space and on-chip scatter detection and showing poor SNR from the forward scatter from a simple on-chip waveguide – detection algorithms will miss such events.

![Image](image2.png)

Fig. 3. a) Simulation results showing the function of the notch at the lens surface as it deflects central rays away while leaving far radial rays intact. b) simulation of the notch in the lens system and the image plane of surface two with a hole burned in the image from the notch (physical drawing of notch omitted for clarity).

To solve this low SNR problem, an obscuration in the lens system of the device in Fig. 2(a) is introduced that accomplishes a similar function as the conventional method, as outlined
in Fig. 1(b). This allows an all-planar design for an all-guided on-chip optical solution such as is accomplished by the form of the device depicted in Fig. 2(a). The obscuration is introduced via an angled notch in surface #2 of the lens system, as shown in Fig. 3(a). The function of the notch is to reflect light away from the lens system via total internal reflection (TIR), thus removing axial rays from the beam. Figure 3(b) shows the design from Fig. 2(a) depicting an identical lens system with the added notch on surface #2 and the resulting ‘hole’ formed in the transmitted rays. Design of the lens system still allows the remainder of the beam to be focused to a narrow beam waist while the image of surface #2 is formed 100 µm behind the channel. This notch-induced dark spot coincides with a 10 µm wide waveguide facet. This allows low background noise and close proximity to the particle to allow for greater collection efficiency.

3. Experimental

3.1 Device fabrication

Devices herein were fabricated following a standard photolithographic procedure, as detailed in our previous work [24–26]. Pyrex wafers served as the substrate that facilitated bonding to the device layer and optically confined light from below into the device layer. Pyrex is also optically transparent in the spectrum of interest. These wafers were sequentially spun with a 600nm thick layer of SU-8 3015 and a 30µm thick layer of 2025 to form an intermediate layer for bonding and then a device layer, respectively. SU-8 allows planar fabrication of optical and fluidic components simultaneously, as well as being optically transparent in the spectrum used for biological detection. Cured SU-8 forms optically smooth and rigid walls for device structures. Each layer was baked and exposed through a photomask containing the designed device features to form rigid polymer layers. A post-exposure bake was done to the device layer to cross-link material and form permanent structures while a development step washed away material to form voids. Devices were then sealed via a nitrogen plasma bonding method [26]. PDMS was chosen as it is optically transparent in optical spectrum and confines light in the device layer from above. It allows a completely conforming contact to the device layer to ensure complete sealing everywhere on the device [24]. To chemically bond the PDMS to SU-8, the PDMS cover slips were exposed to a nitrogen plasma to introduce a chemical reaction with the residual epoxy groups on the SU-8 device layer to form a permanent bond between the PDMS and SU-8. To facilitate fluidic interconnection to external devices, the PDMS layer had holes punched in it. Interconnection sealing was accomplished via a new method [27]. A dicing step was done to free the waveguide facets and allow optical coupling to the device.

3.2 Device testing

Testing was done via a simple forward scatter detection and counting test. A suspension of 5 µm diameter beads (Polybead Microspheres, Polysciences Inc.) at a concentration of 20x10⁶/mL was hydrodynamically focused in device as they flowed past the excitation beam (10 µm wide) formed by the on-chip lens system described above. Light was introduced to the device via a fibre butt-coupled to the device. The fibre had a 50 µm core diameter with light provided from a pigtailed diode laser with a 635 nm wavelength (Meshtel MFS-250-50). The forward scatter light was collected by the on-chip waveguide and the output was butt-coupled to another fibre and delivered to a PMT. Side scatter light was simultaneously collected out the top of the chip via a free-space optical scheme and was used to confirm forward scatter events. Data was collected via a custom LabView program to determine events and log data over a 100 second period. Approximately 2400 events were logged in that time-space; enough to draw accurate conclusions about device performance. Events were binned and a histogram had a Gaussian curve fit to it to determine the coefficient of variation (CV) of the detection method; the standard deviation divided by the mean and expressed as a percent. This is the standard measurement for device performance in flow cytometry.
4. Results and discussion

Device fabrication produced excellent quality devices with smooth side walls, as shown in the SEM of Fig. 4(a). Voids were clean and clear of debris and unwanted material. Comparison of the lens system in Fig. 2(a) shows the slight difference via the notch in surface #2. Figure 4(a) shows the notch was well defined. Paraxial light was removed from the propagating beam and reflected away, however, the residual material left in the small corner of the notch permitted a small portion of on-axis light to reach the forward scatter waveguide and resulted in a source of noise in detection.

![Fig. 4. Pictures of the fabricated device: a) SEM image of the lens system with notch, b) packaged device, c) photomask design of the device showing the microfluidics integrated with the optical interrogation scheme.](image)

Figure 4(b) shows the packaged device after sealing, interconnection, and dicing. Diced ends are opposite one another and allow easy coupling to both inputs and outputs of the device. A clear window above the interrogation region allowed free-space detection. Figure 4(c) shows a photomask design of the device depicting the microfluidics and optical excitation and detection scheme. The microfluidic channel employs very simple 2D hydrodynamic focusing that confines particles to a 6 \( \mu \)m wide vertical strip in the channel to limit the deviation of particles in the optical beam and limit the variation of the detected signal [19,20]. Vertical deviation of particles in the channel will have a limited amount of variation in signal due to the more even intensity pattern from the Super-Gaussian shape of the multimodal input, this variation can be mitigated via a 3D hydrodynamic focusing scheme [19], however this work only demonstrates the incremental advantage of employing a modified beam shaping scheme for forward scatter.

4.1 On-chip detection

Figure 5(a) shows a short burst of raw data from a run where forward scatter was collected from the notch modified lens system. The bursts are clear, distinct from others around it, and are well above the noise level. Bursts are of a fairly uniform and regular intensity – a desirable feature as it is from a uniform population of spheres. SNR of detection is approximately 3 which is the minimum requirement for reliable detection. Sources of noise in detection are from diffraction of the notch image, scattering from channel walls and lens walls, and cross-coupling from the input side of the device via leaked modes into the PDMS cover and Pyrex substrate due to the 50 \( \mu \)m fibre and 30 \( \mu \)m waveguide geometry mismatch. Figure 5(b) shows the histogram from many events in the full 100 second long scatter test. The histogram shows a distinct population with few double detection events. The CV is measured to be 29% – a decent result for a microchip-based device, though this is still far below the capabilities of conventional methods. Free-space detection yielded a CV of 20.4% for similarly sized beads in a side scatter detection scheme [22] and 18.3% using the free-space detection with the on-chip excitation optics performed simultaneously with the on-chip detection scheme.
Of note is the apparent negative burst around the positive forward scatter burst. This was due to the particle blocking the source of light regarded as noise as the particle moved into the edge of the excitation beam spot. This means that performance (i.e. SNR) can be further improved by eliminating these sources of noise and bring the baseline down to a level approximate with the bottom of those negative peaks.

Fig. 5. a) Raw data from 50ms of bead flow showing bursts from forward scatter light collected on-chip using the new designs showing clear identifiable bursts, b) histogram from the on-chip scatter detection.

4.2 Confirmation with free-space detection

To confirm proper operation, events from free-space scatter detection were detected and logged simultaneously with events logged from the on-chip forward scatter design. This allows the correlation of positive events, false positives, and missed events.

Fig. 6. Simultaneous free-space side and on-chip forward scatter detection. a) Plot of raw data showing the simultaneous bursts. b) Scatter plot of on-chip and free-space data showing an excellent correlation between events, including the double detections, missed events, and noise events.

Figure 6(a) shows a comparison of raw data from both the free-space and on-chip forward scatter detection techniques. Both sets of data show bursts that are clear, resolved, and coincide well with one-another. One significant difference between free-space and on-chip raw signals are the negative pulses before (and after) the positive burst for on-chip signals. As noted above, this is due to a bit of the input light making it to the collection waveguide. As the particle moves into the interrogation region, it blocks the extreme edges of the beam and thus, some of the light from reaching the detection waveguide. As the particle moves into the centre of the interrogation region this light is no longer blocked, while the forward scatter light is directed to the detection waveguide. The negative burst on the latter end of the pulse is the same phenomena as the particle exits the beam. This negative burst could be removed with subsequent design and improvements to the lens system. Free-space detection performed
simultaneously does not have this problem as it is side scatter and thus orientated 90 degrees to the chip and does not pick up any stray light from the beam and will not have any light blocked by the bead as it traversed the interrogation region. Figure 6(b) shows a scatter plot comparing the on-chip and free-space scatter detection techniques. The on-chip and free-space thresholds were set just above the noise level at 0.013 and 0.12 a.u., respectively; no upper gating was used as only one size of spheres were used. A clear population is resolved, while a sparse double detection population is visible, though kept under control due to the narrow beam waist used in the device, limiting the number of beads within the beam at one time. Very few noise events were detected as evidenced by the lack of a free-space event, while missed events are demonstrated by the lack of an on-chip event; a more common event as the SNR of the on-chip detection is quite low.

The demonstrated method increases the overall intensity of detection – as compared to the conventional free-space method – based on two method improvements: the collection waveguide has a much closer proximity to the sample, and a much more intense excitation intensity was created due to the focusing of the excitation beam. Close proximity allows a larger collection NA from the particle, while the lens system focuses the light at the interrogation region. The notch in the device only blocks small portion of light at the interrogation region. The measured detection signals are over 300 times more intense than the light intensity detected by the simultaneous free-space detection method – mainly due to proximity.

Evaluation of the performance of the forward scatter detection with the new designs is done by assuming the free-space detection is perfect. The total number of free-space events that occurred was 2088, while the total number of on-chip events that occurred was 1953. 1945 free-space and on-chip events happened simultaneously leaving 143 free-space and 8 on-chip events that happened in isolation. Assuming that the side scatter free-space detection techniques is perfect, this allows false positives and missed events to be discerned from the isolation events. Alone free-space events are missed events by the forward scatter method and are calculated to be 6.8%. Alone on-chip events are false positive by the forward scatter method and are calculated to be 0.4%. The coincidence coefficient – calculated by dividing the simultaneous events by the average of the on-chip and free-space events – was 96.3% - a very good performance of the on-chip notched forward scatter design.

Other works have demonstrated excellent forward scatter results using all-guided optical solutions. One group used inserted waveguides with a single-mode high-quality laser and 3D hydrodynamic focusing to achieve a CV of 9.1% using 7.32µm diameter beads [20]. Another group used 3D hydrodynamic focusing and specially shaped excitation and collection waveguides with limited collection NA to reject much of the incident light [19]. This work achieved 18.3% CV with 5µm beads and did not use on-chip beam shaping optics. It demonstrated a wide variation of performance of CV with different bead sizes. Furthermore, free-space side scatter values from this device were large and both forward and side scatter results could be corrected with beam shaping [28]. Again, as stated earlier, the important improvement our device makes over these two previous devices is that our method will allow for the integration of beam shaping optics onto a device that will also facilitate forward scatter collection. Device performance could be improved through design refinements. Furthermore, the designs do not affect the designs of simultaneous multiple fluorescence or side scatter detection designs as the beam shaping still maintains a uniform region for excitation while leaving room for multiple collection waveguides.

Our method has a greater CV than those quoted in the literature, due mainly to a fairly low (~3) SNR. Variation from these reported values could be due to the method of calculating CV in this paper being based on pulse height values, not pulse area [19]. Using only pulse heights as a measure to create histograms is a method that is susceptible to flow instabilities from pump driven fluid, leading to larger CV values. Device performance in this work can be simply improved with modifications to beam geometry to find the optimal match of beam
geometry and bead size. This work also doesn’t use any 3D hydrodynamic focusing – a feature that does improve detection, though, it also complicates devices and is difficult to control. The devices in here have demonstrated to have very reliable detection of events, that is, the likelihood of a measured event actually occurring is very reliable. Our method also does not rely on single-mode or very narrow and non-divergent beams allowing the elimination of expensive and cumbersome sources. This is a must in true point-of-care and remote sensing applications.

Future improvements to the performance of this device will come from some slight design and device fabrication improvements. For instance, the removal of all material from the notch formed in lens surface. The largest source of noise is thought to be due to the fact that output coupling is directly opposite the input coupling allowing leaky or uncoupled light to cross the device and couple to the output. As the waveguide is only 30µm high, a significant amount of light from the 50µm fibre core diameter is coupled into the PDMS or Pyrex. A source of noise to be corrected is to the notch and collection waveguide; diffraction from the notch allows light to appear in the dark region. Lastly, in future devices a significant improvement can replace lens system entirely with a single diffractive optical element (DOE) that forms a beam geometry in the channel and a dark spot on the waveguide facet with high efficiency.

5. Conclusions

A design for forward scatter detection via an all on-chip planar guided-wave method was demonstrated. Operation was compared to a conventional method, and a low false positive rate of 0.4%, a low missed events rate of 6.8%, and a correlation of 96.3% was calculated. The CV of detection was calculated to be 29% while the free-space detection yielded 18% CV. The CV is a bit high but can be improved via slight improvements to coupling and manufacturing. The demonstrated design works well with integrated beam shaping optics and is adaptable to any design of lens system while not interfering with any other on-chip structures for fluorescence or side-scatter detection and allows fully planar and portable solution.