Integration of optical components on-chip for scattering and fluorescence detection in an optofluidic device

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Abstract: An optofluidic device is demonstrated with photonic components integrated onto the chip for use in fluorescence and scatter detection and counting applications. The device is fabricated by integrating the optical and fluidic components in a single functional layer. Optical excitation on-chip is accomplished via a waveguide integrated with a system of lenses that reforms the geometry of the beam in the microfluidic channel into a specific shape that is more suitable for reliable detection. Separate counting tests by detecting fluorescence and scattered signals from 2.5 and 6.0 μm beads were performed and found to show detection reliability comparable to that of conventional means of excitation and an improvement over other microchip-based designs.

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References and links

1. D. S. Boyle, K. R. Hawkins, M. S. Steele, M. Singhal, and X. Cheng, “Emerging technologies for point-of-care CD4 T-lymphocyte counting,” Trends Biotechnol. 30(1), 45–54 (2012).
2. H. Jiang, X. Weng, and D. Q. Li, “Microfluidic whole-blood immunoassays,” Microfluid. Nanofluid. 10(5), 941–964 (2011).
3. D. Heikali and D. Di Carlo, “A niche for microfluidics in portable hematology analyzers,” J. Assoc. Lab. Autom. 15(4), 319–328 (2010).
4. R. N. Zare and S. Kim, “Microfluidic platforms for single-cell analysis,” Annu. Rev. Biomed. Eng. 12(1), 187–201 (2010).
5. W. G. Lee, Y.-G. Kim, B. G. Chung, U. Demirci, and A. Khademhosseini, “Nano/Microfluidics for diagnosis of infectious diseases in developing countries,” Adv. Drug Deliv. Rev. 62(4-5), 449–457 (2010).
6. H. Yun, H. Bang, J. Min, C. Chung, J. K. Chang, and D.-C. Han, “Simultaneous counting of two subsets of leukocytes using fluorescent silica nanoparticles in a sheathless microchip flow cytometer,” Lab Chip 10(23), 3243–3254 (2010).
7. M. Ikeda, N. Yamaguchi, and M. Nasu, “Rapid on-chip flow cytometric detection of Listeria monocytogenes in milk,” J. Health Sci. 55(5), 851–856 (2009).
8. S. K. Hsiung, S. R. Lin, and C. H. Lin, “Micro flow cytometry chip device integrated with tunable microlens for circulating tumor cells detection and counting applications,” Jpn. J. Appl. Phys. 49(6), 060218 (2010).
9. M. Frankowski, N. Bock, A. Kummrow, S. Schädel-Ebner, M. Schmidt, A. Tuchscheerer, and J. Neukammer, “A microflow cytometer exploited for the immunological differentiation of leukocytes,” Cytometry A 79A(8), 613–624 (2011).
10. T. D. Chung and H. C. Kim, “Recent advances in miniaturized microfluidic flow cytometry for clinical use,” Electrophoresis 28(24), 4511–4520 (2007).
11. D. Huh, W. Gu, Y. Kamotani, J. B. Grothberg, and S. Takayama, “Microfluidics for flow cytometric analysis of cells and particles,” Physiol. Meas. 26(3), R73–R98 (2005).
12. B. R. Watts, T. Kowpak, Z. Zhang, C. Q. Xu, S. Zhu, X. Cao, and M. Lin, “Fabrication and performance of a photonic-microfluidic integrated device,” Micromachines 3(1), 62–77 (2012).

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I. Introduction

Microchip based devices have the potential to expand the scope of applications for diagnostic and clinical procedures to point-of-care settings and remote and on-line sensing situations. This is largely due to the miniaturization and integration capabilities afforded by microfabrication techniques. Thus, very complex biological assay schemes can be completed automatically on-chip with little to no handling via an external operator. Devices exploit cost savings in two ways: via economies of scale from large volumes of production of simple and inexpensive devices for very specific applications, and from reductions in required samples and associated dyes for fluorescent labeling. These lab-on-a-chip (LOC) devices have been demonstrated in point of care (POC) medical and remote or on-site applications ranging from diagnosing and monitoring HIV infections [1], immunoassays [2], portable hematology
Flow cytometry performed with a microchip based device is a niche application within LOC. As each cell in the sample is individually interrogated, flow cytometry is a very powerful analytical tool that allows an exact picture of the sample population and detection of rare specimens. Application on a microchip based device creates the possibility for flow cytometric detection in portable and remote uses. Typically, the interrogation of particles is performed by optical means and has relied on bulky and expensive free-space optics to detect multiple scattered and fluorescent signals [6–11]. Free-space optical detection methods, however, eliminate the possibility for these devices to become truly inexpensive and portable for on-site and remote application. The diversity of microchip based devices has been demonstrated in applications such as counting leukocytes [6], detecting bacterial contamination in milk [7], detecting tumor cells [8], the immunological differentiation of leukocytes [9], among many others [10,11]. Successful commercialization of these devices will rely on the ability to integrate components on chip—specifically the optical excitation and detection components. This will simplify the system while reducing the device’s cost, and increase portability and durability. Devices will be manufactured to complete a specific analytical application that requires as little time, training, or cost as possible to operate.

To accomplish this, new photonic-microfluidic integrated devices have appeared recently that incorporate the required optical components for excitation and/or collection on-chip. This creates devices that—although they have limited flexibility—are inexpensive, shock resistant, and truly portable. Waveguides [12–20], lenses [12–14,19,20], and even sources and detectors [21] have been demonstrated on-chip with varying success. As optical components are optimized through simulations and designed into a photomask for production, the result is a pre-calibrated device that is able to quickly and inexpensively perform a specific analysis. Despite these recent successes, however, there have still not been any truly optical devices that have found their way into any widespread commercial application. This has partly been due to the reliability of detection still being too inadequate for meaningful results to be interpreted.

Our work has been focused on integrating the optical components for excitation onto the chip to allow reliable detection without the need for free-space optics. The process of beam shaping is a common conventional cytometry practice where the objective is to create a uniform volume of intensity across the sample flow stream via free-space optics. This ensures uniform detection of particles regardless of slight deviations within the flow stream while reducing the incidence of double detections (DD). It has the added benefit of boosting the optical intensity for excitation, and therefore, the detection signal given off by the specimen. Our previous research showed that by integrating a waveguide and a lens system on chip, it is possible to form an optical excitation region within the microfluidic channel to achieve this goal [13]. This work demonstrates excellent performance of the developed devices through fluorescence and scatter detections via a completely guided optical excitation approach.

2. Device design and fabrication

Devices were fabricated in a planar fashion by patterning all devices necessary for fluidic control, and optical excitation of samples, into a single SU-8 layer as shown in Fig. 1(a). The details of device design and construction, using SU-8 2025 and 3000 from Microchem, PDMS Sylgard 184 from Dow Corning, and Pyrex 7740 glass wafers from University wafers, can be found in our previous works [12–14,22]. Essentially, the SU-8 device layer was patterned onto a Pyrex substrate and sealed with a soft PDMS cap for a completely conforming seal. These choices of materials ensure optical and fluidic confinement within the device layer, stable and rigid optical structures, easy interconnection with external fluidic control, and simple and inexpensive fabrication that can be scaled up for mass production. The resulting
Device layer is only 30 μm thick—allowing the device’s microchannel to handle a variety of specimen sizes from many biological applications.

Device designs integrate the input from a waveguide with a lens system and a microchannel. The SEM image in Fig. 1(b) shows the resulting device structures while details of the design, simulation, and characterization of the lens system design are found in a previous work [13]. A compound lens system is used to allow for greater beam shaping capabilities of the multimodal input through control of image aberrations. The geometry of the beam shape is carefully tailored in order to produce reliable signal detection through uniform optical excitation. This is accomplished by ensuring an optical excitation region with an approximate uniform intensity profile in both the cross-channel and fluid-flow directions. Non-uniform beam intensity has been cited as a main contribution of signal degradation in other microchip devices and conventional devices [23–26]. Control of the beam width for specimens to traverse also limits the chances for double detections due to two particles occupying the beam area at the same time—a common occurrence at large specimen concentration. All these enhancements serve to try to lower the device’s coefficient of variation (CV)—the measure of a cytometer’s performance. The CV—expressed as a percent—is defined as the standard deviation divided by the mean from a population of identical specimens. Identical specimens should yield identical signals—an ideal cytometer will have as small a CV as possible. Obviously, imperfections with the device performance will lead to variations in signals and the method employed in this work aims to limit this introduced variation from integrated excitation optics on a microchip based device.

As the lens construction is limited to two dimensions, the beam focusing is one dimensional (in the direction of the flow) and thus, the vertical direction—perpendicular to the plane of the device—is left untouched. The beam profile in the vertical direction is assumed nearly uniform as the input beam is heavily multimodal and channel height is only 30 μm. Although the beads traverse a volume of beam intensity, as the devices only have control of the beam in the lateral direction of the beam, the 2D control of the beam shape will be referred to as an “area” instead of a “volume.” The uniform regions discussed herein are

Fig. 1. Design and realization of the device. (a) Concept drawing of the device showing the three layer construction. (b) SEM image of the device showing details of the lens systems and channel integration. (c) Picture of the packaged device. (d) Schematic for the experimental setup used for bead detection.
defined as the area formed by the FWHM in the sample flow direction (beam waist), and by the length where the intensity decreases to 5% of the maximal beam intensity in beam axis direction (perpendicular to flow). This uniform region—although it won’t cover the entire channel width—should be large enough to easily cover the entire sample stream formed through hydrodynamic focusing while being narrow enough to limit double detections.

Packaged devices were connected to fluids via a novel sealing technique designed to handle large pressures [27]. The final packaged device is shown in Fig. 1(c). Optical excitation light was introduced to the device via butt-coupling a fiber to the waveguide facet that was freed by dicing the wafer within a few microns from the facet. The waveguides used here were 50 μm wide by 30 μm tall and ensure a heavily multimodal propagation. This relaxes the coupling requirement and allows simple coupling done with a commercial passive alignment process. Furthermore, because a multimodal beam is shaped into a specific geometry in the channel, the restrictions on the quality of the excitation light source can also be relaxed, reducing costs and complexities of future integrated devices.

3. Device testing

Construction and packaging of the device allows signal detection to be performed via a typical free-space configuration—Fig. 1(d)—which allows simple and rapid prototyping of the integrated excitation optics. The free-space optics for excitation is replaced with the designed on-chip guided optical scheme while keeping free-space detection. This will allow a better comparison with a complete free-space system and the efficacy of the integrated optics. Once the integrated excitation optics are proven to be feasible and functional, the free-space collection scheme can be replaced with on-chip collection waveguides—which are included in current designs—as seen in Fig. 1—but not used in this particular work.

3.1 Beam visualization

To visualize the beam and characterize the shape, the microchannel was filled with a fluorescent dye (Exciton Inc., 690 Perchlorate) with a 630 nm peak excitation and 660nm peak emission. The dye was mixed with ethanol as a solvent at a concentration of 0.1 mol/mL. A narrow band pass filter (Newport, 10BPF10-660) was used to block the laser light and allow only imaging of the fluoresced light. This intersection of the dye and the laser beam was then imaged using a CCD camera (Lumenera, Infinty 2-3c) placed where PMT is in the schematic in Fig. 1(d). Beam analysis was then conducted via simple intensity analysis—as detailed in a previous publication [13]—to determine the width of the beam and the depth of focus.

3.2 Bead detection

To determine the CV of the devices, a population of uniform microspheres was run through the device. Solutions were prepared using fluorescent 2.5 μm and 6.0 μm beads (AlignFlow and AlignFlow Plus, Invitrogen) and 2.0 μm blank (Polysciences) beads with bead concentrations of 5x10⁷/mL for the fluorescent beads and 20x10⁷/mL for the blank spheres. The selected concentrations are typical of what is found in blood/immunology procedures and allow characterization of the devices to be performed quickly. Fluorescent beads had manufacturer calibration CVs determined to be 3% and 5% for the 2.5 and 6 μm beads, respectively, while the blank 2 μm beads had a CV of 5%. The beads were hydrodynamically focused in the microchannel by using a 1:10 and 1:5 sample to sheath ratios to produce 3 μm and 7 μm wide sample stream widths, respectively. Figure 1(d) shows a picture of the experimental setup for excitation (on-chip) and detection (free-space). This detection scheme is similar to what is used in conventional cytometry and is detailed in another work [13]. Essentially, a collection objective (Mitutoyo, 10x NT46-144) focuses light onto a PMT (Oriel, 77341) after passing through a spatial filter and a band pass filter centred on either the scattered wavelength (Newport, 10BPF10-630) or the fluorescent wavelength (Omega Opical,
660DF20). The signal was converted to a voltage via a current to voltage amplifier (Keithley, model 428) and digitized via a DAQ board (National Instruments, NI USB-6211) and analyzed with a custom LabView program to properly determine intensity bursts and subsequently log them. Optical excitation was accomplished by butt-coupling a fiber from a pigtailed 635nm laser diode (Meshtel, MFS-635-50M) with a 50 μm core. This choice of light source creates an all-guided optical excitation scheme while introducing a low-quality multimodal signal. This choice of light source is low-quality as there is no control over which modes propagate and thus, the intensity profile is noisy with ‘holes’ burned into the profile while being very highly divergent.

4. Results and discussion

4.1 Beam quality

Confirmation of beam formation according to simulation predictions is shown in Fig. 2. Figure 2(a) shows an overhead picture of the device with an overlay of a sample of the optical ray trace performed from lens design software (ZEMAX). Details of beam optimization and lens design are covered in previous work [13]. This particular device is designed to take a 50 μm wide multimodal input and form a 6 μm wide beam in the channel with uniform optical properties over the beam axis and flow axis simultaneously.

An image of the formed beam is shown in Fig. 2(b). The FWHM of this beam was measured to be approximately 7 μm and agrees with the spot size formed by the ray trace done through simulation and thus, confirms accurate design replication in the formed devices.

Fig. 2. Beam shaping performance of the device. (a) Image of the device with an overlay of the simulated ray trace to show the beam formed within the channel. (b) Image of the beam formed in the channel. (c) Contour plot showing the intensity of the beam as a function of position within the channel.
The beam also demonstrates a large depth of focus which will ensure uniform illumination of the beads despite deviation from the center of sample stream. A large uniform intensity will also allow the device to permit a range of bead sizes without much detriment to CV performance, increasing the versatility and reliability of the device.

The beam image is converted to a contour plot in Fig. 2(c) to easily show the uniform region of intensity formed by the beam. The beam forms a region covering the sample flow stream 7 μm wide (vertical direction in Fig. 2(c)) by 10 μm long (horizontal direction in Fig. 2(c)). The latter dimension (10 μm) describes an area where intensity deviates by less than 5% of the peak intensity. This region only covers a small portion of the microchannel due to the limitations of the lens system to reshape a very divergent input from the waveguide. However, the region formed by the beam ensures that the particles in the flow stream all traverse the beam in this area of high quality illumination. The beam is of high quality for excitation as bead deviation in the sample stream will receive little fluctuation in excitation intensity, and thus, produce more reliable detected scatter or fluorescence signal. Furthermore, the narrow beam waist in the direction of fluid flow will limit the instances of double detections leading to more reliable population characteristics—especially in large sample concentrations. A trade-off has been noted that a smaller beam waist results in a narrower depth of focus and thus, a smaller overall region of illumination [13]. This is of little concern as smaller specimens demand the use of narrower hydrodynamic focusing and thus, the necessary region for shaped illumination also decreases.

An extra consideration has to be given to the light source used. Many reported devices utilize a high quality single mode laser beam and expand it to cover the channel using only a narrow near-uniform portion of the center of the Gaussian shaped mode to ensure reliable excitation [18–20,23–25,28–30]. As the devices in this work can take an input of lower quality beam (multimodal in this case) and shape it to a high quality interrogation region, the possibility of inexpensive sources is feasible. These devices create the possibility of removing the dependency of analysis on a high quality laser source—a necessary requirement for device in LOC and POC medical applications.

Reflection and scatter losses are introduced to the beam path by the formation of the lens surfaces. The losses from this reflected light are given by the standard Fresnel reflection equation from normal incidence on a surface: The indices used for air and SU-8 are 1.0 and 1.59 respectively. Each lens surface will reflect approximately 5.2% of the incident light. After passing through the four surfaces introduced by the lens system, the total losses from the introduction of the lens system will be 19.3%. This means that 80% of the remaining light will be available for optical interrogation. However, decreasing the 50 μm waveguide input to a 40 μm spot will increase the intensity at the particle by 5/4—due to a magnification of 4/5—and will balance out the losses from the lens system. As our designs all incorporate a smaller beam waist (some by almost 10x factor), the lens systems actually increase the intensity of light available for excitation.

4.2 Bead detection

Results from a scatter detection counting test performed with 2.0 μm blank beads in a device that formed a 6.0 μm beam waist are shown in Fig. 3. Figure 3(a) shows 1 second of raw data clearly illustrating detection bursts. Each burst is clear, resolved from other bursts around it, and there is a limited range in the peak intensity of the detected bursts. A histogram of all the detected bursts in the 100 second test is shown in Fig. 3(b). The CV of the device is calculated as 18.5% by a Gaussian fit to the histogram. This value is acceptable for many applications [31,32]. There is a low incidence of double detections as there are very few detections outside the obvious population in the histogram plot. The small number of detections at intensities below the main histogram are from debris or from beads that escape into the sheath fluid and traverse the beam in the much less intense regions near the channel wall.
A fluorescence detection counting test was subsequently performed using 2.5 μm fluorescent beads—the results of which are shown in Fig. 4. Figure 4(a) shows 2 seconds of raw data from the test showing, again, clear individual bursts with a near uniform intensity. Despite some bursts appearing to happen very close together, they are resolved as separate events. The histogram from the entire 100 second run, Fig. 4(b), shows a very narrow peak of event intensities. The CV is calculated to be 11.4%—a very good performance indication as separate populations can be accurately resolved and as it is near the value necessary for DNA quantification applications; requiring a CV of 8% [33]. Double detections are minimal as evidenced by the lack of large intensity bursts outside the main histogram peak.

To test the versatility of the devices and designs, a device that forms a 10.0 μm beam waist was tested with 2.5 μm and 6.0 μm fluorescent beads—scatter and fluorescent signals were measured separately. Measurements of CV, signal-to-noise ratio (SNR) and double detections (DD) were determined and tabulated in Table 1 along with the results from the tests presented in Fig. 3 and Fig. 4.

Double detections are very rare in all cases except one—when 2.5 μm beads were run with the 10 μm device in a scatter detection scheme. This is due to a beam much larger than the bead permitting more than one bead within the beam simultaneously. The double detections for the fluorescence test are not quite as large due to a noted settling of the beads in solution leading to a lower bead concentration. The actual tolerance of DD allowed in an analysis will vary depending on the application, however, 1% is an acceptable value for many applications. In fact, referring back to the raw data shown in Fig. 3(a) and Fig. 4(a), there are peaks that are observed very close together. The tight control of the beam waist in the direction of sample flow limits the event that multiple particles will occupy the beam space simultaneously. A
smaller beam size limits the probability of this event occurring. These types of detection can greatly affect results in analyses that depend on quantifying the intensity of detected light to discern population characteristics. In the most extreme of situations, double detection will still occur when two particles are stuck together.

Table 1. Scatter and fluorescence detection performance of beads using devices with two sizes of beam waists

| Beam waist (μm) | Beads (μm) | Detection Type | CV (%) | DD (%) | SNR  |
|----------------|------------|----------------|--------|--------|------|
| 6              | 2.0        | Scatter        | 18.5   | 1.4%   | 11.2 |
| 6              | 2.5        | Fluorescence   | 9.0    | 0.4%   | 41.1 |
| 10             | 2.5        | Scatter        | 15.8   | 3.0%   | 13.0 |
| 10             | 6.0        | Fluorescence   | 11.4   | 1.8%   | 395.3|
|                |            | Scatter        | 20.4   | 1.0%   | 8.8  |
|                |            | Fluorescence   | 15.9   | 1.8%   | 86.6 |

The SNR of the average intensity detected in each run is shown in Table 1. A SNR of 3 is the minimum value in order to reliably resolve intensity bursts. Fluorescence signals all show a much larger SNR than signals from scatter tests—a result contrary to what is a general rule in conventional cytometry and to what has commonly been demonstrated in the literature. This is due to two factors: a very steep bandpass filter (an optical density difference greater than 6 between 635nm and 660nm) that cuts out large amounts of excitation light and boosts the fluorescence SNR, and the small channel width causing larger than normal noise on scatter tests due to the close proximity of scattering off channel walls. Devices with a larger beam waist have a larger SNR due to the larger collection NA of the lens system and, thus, larger intensity at the interrogation region.

The CVs demonstrated here are all well within acceptable limits to resolve population characteristics. This indicates that this method of integrated lens system is feasible for use in a specific biological detection applications mass produced in order to meet the inexpensive and portable requirements for LOC and POC application. It should be noted that there is better performance of the 6 μm device than the 10 μm with respect to the 2.5 μm beads indicating that the device performance is dependent on the choice of beam geometry. This is something that is explored in another work—the findings of which indicate that the beam geometry, specimen size, and performance are inherently linked. Careful consideration of the detection species must be taken into account when designing the lens systems.

Currently, microchip based devices demonstrated in the literature have used a variety of techniques to lower the CVs of detection. Free-space based optical excitation and collection schemes demonstrated scatter detection CVs of 25% to 32.1% for beads ranging from 3.2 μm to 10.2 μm in diameter [34]. Devices that incorporate on-chip waveguides for optical excitation and collection have shown similar results, yielding scatter CVs of 26.6% to 29.7% for bead sizes ranging from 2.8 μm to 9.1 μm [30], and 37.5%, 34.2%, and 27.7% for 5 μm, 10 μm, and 15 μm beads, respectively [17]. The inclusion of on-chip 3D hydrodynamic focusing has improved results as fluorescence detection of 15% to 17% for 6 μm beads [18], and 32.9%, 19.1%, and 8.4% for 4 μm, 8 μm, and 12 μm beads, respectively [19], has been demonstrated. A device that performs simultaneous 2-parameter detection using an on-chip lens system with a single beam waist of 71 μm and 3D hydrodynamic focusing demonstrated fluorescence detection CVs of 17.1% and 23.8% for 15 μm and 31 μm beads, respectively and scatter detection CVs of 38.4%, 44.8%, 12.0%, and 20.1% for 7 μm, 10 μm, 15 μm, and 31 μm beads, respectively.

By contrast, the CV’s from devices demonstrated in this work were lower while relying on a simpler method to lower the CV and—importantly—while all the tests were performed with smaller beads—2.0 μm, 2.5 μm, and 6.0 μm—which are not as easily detected as large particles. On-chip beam shaping improves the performance and avoids complex free-space optics for excitation and other 3D hydrodynamic flow techniques to improve detection. The
method of on-chip beam shaping demonstrated in these devices increases the reliability of detection while allowing compact and inexpensive devices to be fabricated for specific detection analysis.

5. Conclusions

The ability to form a custom shaped beam in the center of a microchannel for use as a source of optical excitation in a flow cytometric application is demonstrated. Optical components to accomplish this are fabricated in the same functional layer as the fluidic components forming a more robust and stand-alone LOC device. The method for integrating excitation optics and the process of careful beam shaping demonstrated in these devices are suited for applications in POC and remote settings where conventional cytometry methods for excitation are too expensive or cumbersome for efficient operation. The formed beams had been designed and characterized to produce a more reliable signal in the form of a low CV (<15%) from particles via a region of uniform intensity with a narrow beam waist to limit the instance of double detections. This method eliminates the need for free-space optics or a high quality laser source or on-chip 3D hydrodynamic focusing; simplifying and creating more inexpensive devices. Detection of signals from microbeads, both fluorescence from 2.5 and 6.0 μm beads and scattering from 2.0, 2.5, and 6.0 μm beads, were analyzed and the reliability of the device was found to be satisfactory. Instances of double detections were very rare, and the SNR was found to be excellent for fluorescence tests and well above the minimal requirement for scattering. It was noted that the selection of the beam geometry must be taken into account depending on the bead size used and the method of detection.

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