Formation and characterization of an ideal excitation beam geometry in an optofluidic device

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Abstract: An optimal excitation beam shape is necessary to perform reliable flow cytometric analysis but has so far not been implemented in a photonic-microfluidic integrated (i.e. optofluidic) device. We have achieved this feature by integrating a 1D lens system with planar waveguides and microfluidic channel on a substrate using one patterning material via a one-shot process. In this paper, we report the method of design and the performance of specifically formed excitation regions shaped to be ideal for reducing double detections, improving SNR, and for reliable detection in a flow cytometry application. Demonstration of different sizes via changes to lens design shows the ability to control the width of the shaped beam according to a targeted detection.

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1. Introduction

Flow cytometry is a very well-established and powerful analysis technique with high throughput [1,2] and multi-parametric (multicolour) analysis [3,4] capabilities. It is used for many important medical diagnosis and analysis applications in fields such as HIV/AIDs [5], hematology [2], oncology [2], rare event detection [2], and drug development [6]. A conventional flow cytometer is usually limited to large scale facilities permitting access from a large group of users as extensive capital investment, and the associated equipment, maintenance, and operation costs, are extremely high.

A microchip-based device is obtained by replacing the glass capillary tube of the flow cell with a microfluidic channel. This reduces sample sizes – thus reducing costs of associated labelling dyes and antibodies - while permitting precise control of samples. Existing micro-fabrication techniques can allow integration with other devices on the same substrate to increase overall device capabilities, reduce overall testing time, and increase sensitivity and productivity of the system. These types of devices are part of the larger field of micro total analysis systems (μ TAS), where entire analysis procedures can be completed in a single, integrated microchip-based system (i.e. lab on a chip) with no, or very limited, external requirements. μ TAS devices have the potential to make a huge impact as a powerful and inexpensive screening and diagnostic tool for epidemics in destitute and remote locations as a

point-of-care (POC) medical application [7–9]. Microchip-based devices have demonstrated comparable detection efficiencies (94% detection of *E. coli*) [10], multiple-parameter (scatter) detection (able to discern between 2 and 9 μm sized particles) [11], and monolithic integration via a microchip-based a cell sorter with sort speeds of 44 cells/s and a recovery rate (percent success in removing target cell from the general population) of 50% [12] and high speed applications achieving sort speeds of 12000 cells/s and an enrichment-factor (boost in the sequestered fluid's target cell concentration) of 100-fold [13]. These devices, however, still have a reliance on free-space optics for either excitation, detection, or both, and thus are still associated with the conventional systems that are bulky, costly, and rigidly fixed.

The integration of photonic elements onto the chip via monolithic integration offers a means to eliminate the reliance on free-space optics - thus removing the dependence on bulky and expensive external equipment - and make the associated system truly portable and inexpensive. Integration of photonic components onto the microfluidic chip, resulting in what is termed an optofluidic device, have demonstrated waveguides [14,15] to increase proximity and alignment to the samples, sources and detectors [16–18] to increase portability and functionality, apertures [19] and filters [17,20] to decrease noise, and focusing elements to increase power for excitation [15,19,21–25]. Despite recent advancements, these optofluidic devices are still not yet suitable in a flow cytometry application. In flow cytometry it is of paramount importance that the machine be able to detect each cell individually while being able to discriminate between positive and negative events, as well as to ensure that the fluorescence signal can be reliably quantified. Conventional cytometry employs the procedure of beam shaping to help remedy this problem. Beam shaping focuses the input light beam to a narrow region in the centre of a microfluidic channel to optimize the detectable signals from the cells by increasing the SNR and by limiting the number of cells that may fit within the beam at one time - guaranteeing that each cell is individually interrogated - even when travelling back-to-back single-file in the flow stream. Devices that incorporate a lens into the structure aim to curb the effect of the light diverging from an incorporated fibre or waveguide and have demonstrated great success. Single 1D lenses have shown to limit divergence and increase the level of detection by 3-fold for fluorescence spectroscopy [21] and boost excitation intensity by 1.67x [24] and 10x [19] in a capillary electrophoresis (CE) application. One work noted that beam intensity was shown to vary by 13% across the flow stream of beads and generated a large difference in detected signal intensities [25]. Fabrication of a compound lens system showed improvement to excitation intensity by 7x over lensless, and 4x over single lens designs by eliminating beam degrading aberrations. These improvements, however, have focused on improving only the intensity of the beam at the particle to boost detection signals and make more efficient use of the light source – a suitable result for some analysis schemes – however, flow cytometry requires a beam waist whose size is targeted towards the desired cells in order to accurately discern between single and double events. Such improvements are important as they increase the signal-to-noise ratio (SNR) by exciting only the cell and not multiple cells or debris simultaneously. A secondary requirement of beam shaping is that the beam form a region of uniform and focused power intensity across the cell flow stream to ensure repeatable and reliable signal generation for accurate population discrimination - despite slight deviations of the cell from the flow centre [26]. Conventional flow cytometry regularly applies beam shaping to ideally shape the excitation region via free-space optics to achieve large coefficients of variation (CV) – such ideal illumination has not been shown in a fully photonic-integrated fashion. In this paper we present results from our recent progress in integrating beam shaping components onto the chip in a photonic-microfluidic integrated (i.e. optofluidic) device [27] that has been inspired from a need from previous work in microchip-based flow cytometry [28]. Through integrating a waveguide and a 1D lens system together with a microchannel into a single device, and by controlling the aberrations through precise control of the surfaces in the lens system, we have been able to shape the beam from an input waveguide into a pre-determined shape with a defined beam waist while retaining excellent intensity uniformity across the sample flow, .

2. Design and simulations

2.1 Beam shaping

In conventional cytometry, a laser beam is shaped by utilizing a sequence of two cylindrical lenses that have their long axes crossed at 90° with each other and the optical axis. This allows independent control of beam focusing in two directions perpendicular to sample flow and thus, the ability to form an elliptical beam shape from a circularly shaped source and visa versa. Ideally a beam is very narrow in the direction of cell flow - improving the SNR and preventing the simultaneous excitation of back-to-back cells causing doublet detections that skew final count numbers - while uniform in the directions perpendicular to the fluid flow as it is very important for reliable detection. Beam uniformity across cell flow is of consequence for detection because, typically, the beam intensity across the flow stream is of Gaussian shape. If the beam is shaped across a channel such that a slight deviation in the flow stream results in a deviation in the Gaussian beam by 0.67 standard deviations, the cell will thus receive only 78% of the maximum intensity compared to an identical particle that is in the centre of the sample flow. This difference in intensity leads to a large CV – the measure of performance in a flow cytometer [26].

In a microchip-based device utilizing a waveguide for light injection, the beam diverges according to the numerical aperture (NA) of the waveguide as it exits the waveguide facet into the free propagation region defined by the channel wall and the channel itself. We propose to use an integrated 1D lens system with the waveguide and microchannel to design an ideal beam shape in the channel through precise control of aberrations in the lens system. The lens system consists of vertical cylindrical lenses at the end of the waveguide to shape the beam in the plane of the integrated device while utilizing the upper and under cladding to vertically confine the beam within the lenses on the device. Such 1D beam shaping in the plane of the device allows the beam shape to be controlled in the fluid-flow direction by tailoring the beam waist to the desired size - minimizing the chance of double detection – and in the cross-channel direction by controlling the uniformity of the beam perpendicular to the fluid flow - limiting the differences in excitation intensity due to cell deviation. Furthermore, an increase to excitation intensity via beam focusing allows more efficient use of the light source power for detection compared to a system without beam shaping, with the same source power. Targeted beam size, plus increases to uniformity of the beam in the interrogation region meets the requirements of flow cytometry as discussed above.

2.2 Theory

The sample fluid in the microfluidic channel is designed to be focused co-planarly in 1 dimension (1D) only; therefore, our targeted beam shaping is also co-planar, one dimensional. A 1D lens system is proposed to achieve this goal. As only cylindrical lenses are used, the beam is assumed to be roughly uniform in the vertical direction – an adequate assumption and consequence when uniform illumination across the channel is desired. Careful formation of the beam must be considered for formation of an optimal beam width and therefore, design of the lens system must be done with the end result in mind.

A waveguide core (index of refraction, n_{core} , 1.59 at 635nm) and air side cladding (index of refraction, n_{clad} , 1.00) will yield a waveguide NA of 1.236 corresponding to a beam divergence half angle of 68.02° in a propagation medium of water ($n = 1.333$). This large NA will also allow a very divergent source to be coupled into the waveguide with minimal effort – relaxing the condition for the source that can be used. The NA of input beam should be smaller than the input NA of the lens system to ensure that losses are minimized. A magnified beam will have an altered image NA according to the magnification of the system. The image NA will determine the shape of the beam within the channel, specifically, the divergence of the beam.

The beam used in this device will be of a Gaussian form and will therefore deviate from geometric optics predictions. The Gaussian beam will be characterized by three parameters: depth of focus, d , divergence, θ_0 , and beam waist, w_0 . Figure 1 illustrates a Gaussian beam

propagating through a focusing element and the effect on the beam shape. The depth of focus, conventionally defined as the axial distance between points on the beam where cross-sectional area of the beam is twice that of the cross-sectional area at the beam waist, is applied for only the planar direction. The Rayleigh length, z_R , holds the same definition of $\sqrt{2}w_0$. The beam cannot be focussed down to an infinitesimally small point, but rather to a defined width w_0 .

Fig. 1. Diagram depicting Gaussian beam focusing.

The beam waist, w_0 , is altered by a lens to a size w_1 defined by the lens parameters given by Eq. (1) [29]:

$$w_1^2 = \frac{f^2}{z_o^2 + (d_o - f)^2} w_o^2 \quad (1)$$

where f is the focal length of the lens system, z_o is the Rayleigh parameter of the input beam, d_o is the distance from the lens to the waist in object space, and w_o is the waist of the beam in object space. For the complex lens system used, the effect of the total system on the propagating wave is described by applying Eq. (1) for each focusing element in a matrix method fashion. A carefully designed lens system will allow control of the beam in the cell flow direction and cross-channel direction through control of the beam width and depth of focus, respectively. Focusing a Gaussian beam through a lens system with a magnification of m has an effect on all the parameters of the beam. The waist radius is changed by a factor of m , $w_1 = m \cdot w_o$, the divergence is scaled by a factor of $1/m$, $\theta_1 = \theta_o/m$, and the depth of focus is scaled by a factor of m^2 , $z_{R1} = m^2 \cdot z_R$ [29]. There is a trade-off on decreasing the beam waist while increasing the divergence and decreasing the depth of focus.

To correct for image aberrations that will distort the image and create non-ideal shapes, it is necessary to warp the surface contour of the lens structure far from the axis. Equation (2) shows the method to form a curved surface that allows a warping parameter, the conic constant, to change the surface from a spherical shape.

$$z = \frac{cr^2}{1 + \sqrt{1 - (1+k)c^2r^2}} \quad (2)$$

Equation (2) defines the sag of the lens surface, z , via c , the lens curvature (inverse of the radius), r , the radial coordinate, and k is the conic constant changing the surface to elliptical or hyperbolic.

2.3 Device design and simulation

These devices will integrate the beam shaping components and optical waveguide in the same plane as the microfluidic channel. The devices will use an epoxy photoresist (SU-8, MicroChem) to simultaneously fabricate both the waveguide cores (50 μm width), optical lenses, and microfluidic channels (50 μm width) on a glass substrate – which will also serve as a bottom cladding region – in order to achieve rapid fabrication to facilitate quick design prototyping and testing. Poly(dimethylsiloxane) (PDMS) will be used to simultaneously act as the upper cladding of the waveguides and to seal the fluid channels. Air regions formed by removing material from either side of a waveguide core region will form the side cladding

(100 μm wide) of the waveguides. The developed waveguides have a large size to match the channel height, and are multimodal at the design wavelength, 635 nm.

The ridge waveguide will terminate at a system of vertical cylindrical lenses that have been designed to focus the input beam from the waveguide into a determinable size in the middle of the microfluidic channel. These designs must respect a couple guidelines in order to form feasible devices. Devices must form a narrow beam in the middle of the microfluidic channel (width of 50 μm), allow a distance between the last optical element and the channel so that sufficient material is left to form a barrier for the fluid, and have a minimum device layer thickness to allow sufficient fluid flow. Lenses were designed to utilize two materials, SU-8 and air.

Fig. 2. Device design and layout. (a) 3D concept drawing. (b) Photomask design showing microfluidic integration with optical elements. (c) Detailed schematic of the lens system.

Figure 2 shows a schematic structure of the proposed device [Fig. 2(a)] with a picture of one of the designed devices from the photomask [Fig. 2(b)] and a blow up of the region next to the channel where the beam shaping elements are placed [Fig. 2(c)]. The device is based upon a previous device that changed the size of the beam in order to concentrate power using an all integrated method [15]. The excitation beam will first enter the lens system and then pass through the microfluidic channel. On the opposite side of the channel, two waveguides without any lens system are placed; one of them will be used to form an unfocused beam in the channel to serve as a control to compare improvements in this study.

Designs were completed using commercial lens design software (ZEMAX) and several designs were generated to focus the input from the 50 μm wide waveguide with various NA into specific beam widths. Beam widths of 3.6 μm and 10 μm were selected to demonstrate the variability of the design and the ability to apply beam shaping effectively in a optofluidic device. As many biological cell sizes vary from 1 μm to 10 μm , these selected beam widths demonstrate applicability to the variety of sizes of specimens of interest common in cytometry. Figure 3 shows the designs used for the 3.6 μm and 10 μm lens systems. Also included are the detailed ray diagrams showing how the paraxial rays and extreme rays will focus into a narrow width at the centre of the channel. A trade-off between the beam width and the input NA limits the power density of the beam shape as a smaller beam width limits the amount of collected power by the input of the lens system.

Fig. 3. Simulation results for 3.6 μm lens system and 10 μm lens system.

3. Experimental

3.1 Device fabrication

Two sequential SU-8 layers were deposited on cleaned 4" pyrex wafers – the first one was 600 nm thick as an immediate layer for bonding improvement, and the second one was 27 μm thick as the device layer [27]. SU-8 3000 and SU-8 2025 (Microchem) were used for the deposition respectively. The device layer was patterned under the designed mask using a high performance mask aligner and exposure system from ABM Inc. and subsequently post baked and developed with a developer (Microchem) to generate the waveguide cores, microfluidic channels, and curved lens surfaces simultaneously. The devices were then diced and the waveguide facets were polished, first with a grit of 1 μm and then with a grit of 0.3 μm . Devices were then bonded with a 1.5 mm-thick layer of PDMS using our recently developed polyaddition bonding method [30].

3.2 Device testing

To confirm the correct formation of beam shape within the device, the beam in the channel was imaged. Light from an 8.2mW HeNe laser (Spectra Physics) was coupled via a 0.8NA objective lens (Olympus) into the end facet of the waveguide to provide light for the integrated lens systems to form a shaped beam in the channel. This free-space coupling scheme was employed to facilitate easy testing and it will be easily replaced by standard pigtailed fibers when the devices meet all the technical specifications. A fluorescent dye, diluted to 10^{-1} mol/ml in ethanol, was used to illuminate the entire beam in the channel (Nile Blue 690-Perchlorate, Exciton Inc.) with an absorption peak of 630nm and an emission peak of 660nm. A CCD camera, positioned above the device, was used to image the region where the beam and the channel intersected. The camera (Lumenera Infinity2-3) was connected to a 10x objective (Mitutoyo) via a 12x zoom lens (Navitar) and images were taken using the camera equipped software (Lumenera Infinity Analysis). To eliminate the laser wavelength from the images, a 660nm bandpass filter (Newport 660-10) was affixed to the end of the camera objective. Figure 4 shows a schematic and picture of the testing setup.

Fig. 4. Schematic of testing setup (top) with a picture of actual testing setup (bottom).

3.3 Testing and analysis

To properly analyze the beam, each picture was converted to a text file by simply creating a matrix that held the value of each pixel converted into a numerical value that was proportional to its intensity. For example, a pixel in the complete absence of light was assigned a value of 0 while a pixel that was completely saturated was given a value of 255. To avoid artificial flattening of the beam peak by pixel saturation, the exposure time on the camera was adjusted so that the maximum pixel value was below saturation. Once the matrix was created, it was separated into rows (which represent horizontal slices on the picture) and each was individually analyzed by determining the FWHM from the difference between the beam pixel value and the background dark intensity. From here, it was possible to compare with the simulations and comment on the level of uniformity across the channel, such as how the width of the beam changed as a function of position across the channel, and how the beam width, divergence, and depth of focus changed via the lens system.

4. Results and discussion

Figure 5 shows a fabricated device. Its effective area, including all the ports for input and output liquids and facets for light coupling, is smaller than a nickel - less than 20 mm x 20 mm [Fig. 5(a)]. In the optical interrogation area, a thin SU-8 wall is applied to separate the microfluidic channel with the optical features and retain the fluid within the channel [Fig. 5(b)]. The whole SU-8 device layer is sandwiched between glass (substrate) and PDMS with strong bonding [Fig. 5(c)] and the devices were tested to be able to hold a fluidic pressure of over 0.9 MPa [30]. The sharply developed waveguide ridge is essential for its optical performance.

Figure 6 shows captured images from the camera where the fluorescent dye in the microchannel has been excited by the differently shaped input beams to produce a visual image of the beam shape in the microchannel. Clear field images show the device structure along with the fluorescent beam shape [Fig. 6(a), 6(c)], while magnified images of the beam within the channel reveal detail of the beam shape that covers the entire width of the channel [Fig. 6(b), 6(d)]. With a device that employs no beam shaping in the microfluidic channel [Fig. 6(a), 6(b)], the width of the beam within the channel appears wider than the input waveguide. Furthermore, it appears to spread as it traverses the channel and intensity diminishes as a result. Devices that incorporate waveguides with no lens [13,14,20,31] feature this result in the microchannel, with the beam width dependent on waveguide dimension and NA of the waveguide. This is undesirable for a flow cytometer application since the wide beam waist could illuminate back-to-back cells in the cell sample flow and excite them simultaneously rather than each one individually resulting in a false counts. Meanwhile, beam expansion is associated with a lower power and varied intensity distribution across the flow stream yielding a lower signal/noise ratio and large CV, respectively. With a device that incorporates a lens system to form a 3.6 μm beam width within the channel [Fig. 6(c), 6(d)], it is easy to see the effect of beam shaping within the channel and the optimal effect on beam width and depth of focus in the centre of the channel. There is a region of narrow intense light

density in the centre of the channel where sample flow occurs with excellent contrast in the direction of fluid flow – this will allow detection signals to form sharp on/off pulses. The formed plateau of intensity across the centre of the channel is near uniform with respect to beam waist with peak intensity in the centre and low intensity near the channel walls. This shaped beam is narrow enough to excite only one cell at a time while producing near-uniform excitation for all particles in the flow stream – regardless of position in the sample flow.

Fig. 5. Picture of the fabricated device with fluid tubes (a), close image of the integrated lens area (b) and cross-section of the device (c).

Our designs allow individual beam shaping on each device to be controlled separately through slight changes to the lens system in each design, using aberrations to warp the image to simultaneously adjust beam waist and control the beam intensity across the flow stream. By changing the lens system's surface parameters, we were able to shape the beam to various dimensions, ranging from 3.6 to 50 μm for the beam width, to meet the requirements for various applications covering the analysis of bacterial cells (less than 1 μm in dimension) and human cells (5-30 μm). The size of the beam waist should be adjusted for each targeted detection as the beam must ensure that the aligned cells are detected in a one-by-one fashion, while the depth of focus should be adjusted to cover the entire width the sample fluid flow in a near uniform fashion to permit uniform detection so that throughput can be maximized through slightly relaxed hydrodynamic focusing.

Our comparisons of the obtained beam shapes within the channel to the designed simulation results showed that designs accurately predict observed results. Figure 7 shows the resulting beam shape of a device with a lens system designed to form a 10 μm beam width with the simulation results superimposed next to the beam shape. The lens system used was similar to the lens system that formed the 3.6 μm -wide beam and forms a beam shape similar to the one achieved with a 3.6 μm lens system [Fig. 6(d)], however, it is wider in both the cell flow direction and the beam direction – to compensate for a larger cell size. The comparison (Fig. 7) shows the simulated results of both the NA and beam width of the beam match well with the actual beam shape formed; thus, a beam width of desired shape can be formed within the centre of the channel through careful optical design and simulation.

Fig. 6. Captured images of fluorescence in the microchannel. a) Device without beam shaping with back light (without filter). b) Detail of beam from device without lens system (with filter). c) Device with 3.6 μm lens system and back light (with filter). d) Detail of beam from device with 3.6 μm lens system (with filter).

Fig. 7. Comparison between simulation and actual results for 10 μm device.

Detailed information about the intensity and shape of the beams from fabricated devices can be seen when images are converted and displayed as contour (and 3D contour) plots (Fig. 8). The unshaped beam [Fig. 8(a), 8(b)] confirms the beam spreading in the free-propagation region of the channel wall and the channel, and a resultant intensity decrease across the channel. The large waist across the channel is detrimental to detection, as discussed earlier. On the other hand, the shaped beams [Fig. 8(c)–8(f)] show clearly that there is a narrow and distinct region of increased intensity in the middle of the channel while maintaining a large depth of focus that forms a plateau of near-uniform intensity for a significant width of the channel. Figure 9 shows the beam full-width-half-max (FWHM) and maximum pixel intensity of the shaped beams as a function of the position across the channel. It can be seen that each beam is focused near the channel center (25 μm), creating a small plateau where the FWHM is a minimum while the intensity is maximum for a designed width of the channel. The minimum FWHM (the width of the formed plateau region) of each beam is approximately 6 μm for the 3.6 μm -based device and 11 μm for the 10 μm -based device. Far from the region, the beam width grows as its power is spread over a larger distance. The length of the plateau, described earlier by the depth of focus, is around 26.2 μm for the beam focused with the 10 μm lens system and 14.1 μm for that focused with the 3.6 μm lens system. By considering a region where intensity is slightly more uniform – the distance where the maximum beam intensity will vary by less than 5% from the maximum – these plateau lengths decrease to 10.4 μm and 6.2 μm for the 10 μm and 3.6 μm devices, respectively. These regions indicate a region of more uniform excitation. In a flow cytometry application, the plateau regions will

cover the trajectory of every cell through the interrogation region as hydrodynamic focusing will ensure that every cell passes the interrogation point in the defined plateau region and not outside of it.

Fig. 8. Contour plots of the beam within the channel for a no lens system (a), the 3.6 μm lens system (c) and the 10 μm lens system (e) along with the 3D representation of the beam intensity profile within the channel for no lens (b), 3.6 μm (d), and 10 μm (f) lens systems.

There is a trade-off between the power density and dimensions of the obtained beams. The 3.6 μm lens system has a smaller input NA and thus collects less light, but can focus the light to a smaller region than the 10 μm lens system. The smaller beam waist means that the plateau of near-uniform intensity (or the depth of focus) is also decreased, as evidenced by the narrower peak for the 3.6 μm device shown in Fig. 9. Another trade-off occurs with respect to the depth of focus and the beam waist.

Fig. 9. Plot showing the maximum intensity (open points) and FWHM (closed points) of each beam as a function of cross-channel position.

Ideally, a shaped beam would form a narrow and long region through the whole channel to ensure that all cells will pass the beam in a one-by-one fashion and be exposed to uniform excitation power - no matter the position in the cross-sectional area of the channel. Our method of beam shaping shows formed excitation beam shapes that most closely resemble the ideal geometry for excitation in a photonic-microfluidic integrated flow cytometer. The beam width achieved in the shaped beams using the 3.6 μm and 10 μm lens systems meets the requirement for our targeted detection of bacterium (around 1 μm in dimension) and some regular cells (5-10 μm). Also, the shaped region, i.e. the plateau in Fig. 9, although not large enough to cover the entire width of the channel, is large enough to cover the entire sample stream ensuring that every passing cell receives uniform excitation - regardless of the cell's deviation from the exact centre of the sample flow. For the first time, such a beam shaping feature required in flow cytometry has been achieved in a photonic-microfluidic integrated flow cytometer, solving one of the three major problems, as described earlier, in these devices. In addition to flow cytometry, the beam shaping can be used in other biological applications. For instance, the technology is used in optics-based cell sorting, as mentioned earlier [11], because the narrowly shaped beam with high optical power density is in favour of sorting efficiency. It may also serve as an important element in various photonic-microfluidic integrated devices that are potentially low-cost and portable.

5. Conclusions

The optofluidic devices demonstrated here have promising applications in flow cytometry but still faces some technical challenges. Current microchip-based devices still rely on conventional free space optics to mimic conventionally cytometry. It is important that photonic-based devices can also mimic conventional cytometry methods – shaping of the excitation beam in this case. We have been able to reproduce beam shaping in an optofluidic integrated device via the formation of a system of 1D cylindrical lenses integrated with a waveguide and microchannel through a single step using photolithography. Our work successfully demonstrated a couple of devices that were able to form a beam of defined shape in the centre of a microchannel using careful design and simulation methods to control image aberrations. Though a range of beam widths and shapes are possible, we demonstrated devices that were able to form beam width of 6.5 μm and 11.7 μm for designs of 3.6 μm -based and 10 μm -based devices respectively with depths of focus of 14.1 μm and 26.2 μm , respectively. The ability to shape a beam in a photonic-integrated device for a flow cytometer application has implications that will make portable POC device more feasible through better detection capabilities via a lower CV. A formed excitation beam, with a waist narrow enough to eliminate double detection events and with a depth of focus large enough to facilitate deviations of sample flow from the centre of the channel, will produce more uniform and

repeatable detections. Furthermore, detection capabilities will be improved as the power density of the excitation beam was shown to be increased from the density of an unshaped beam.

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