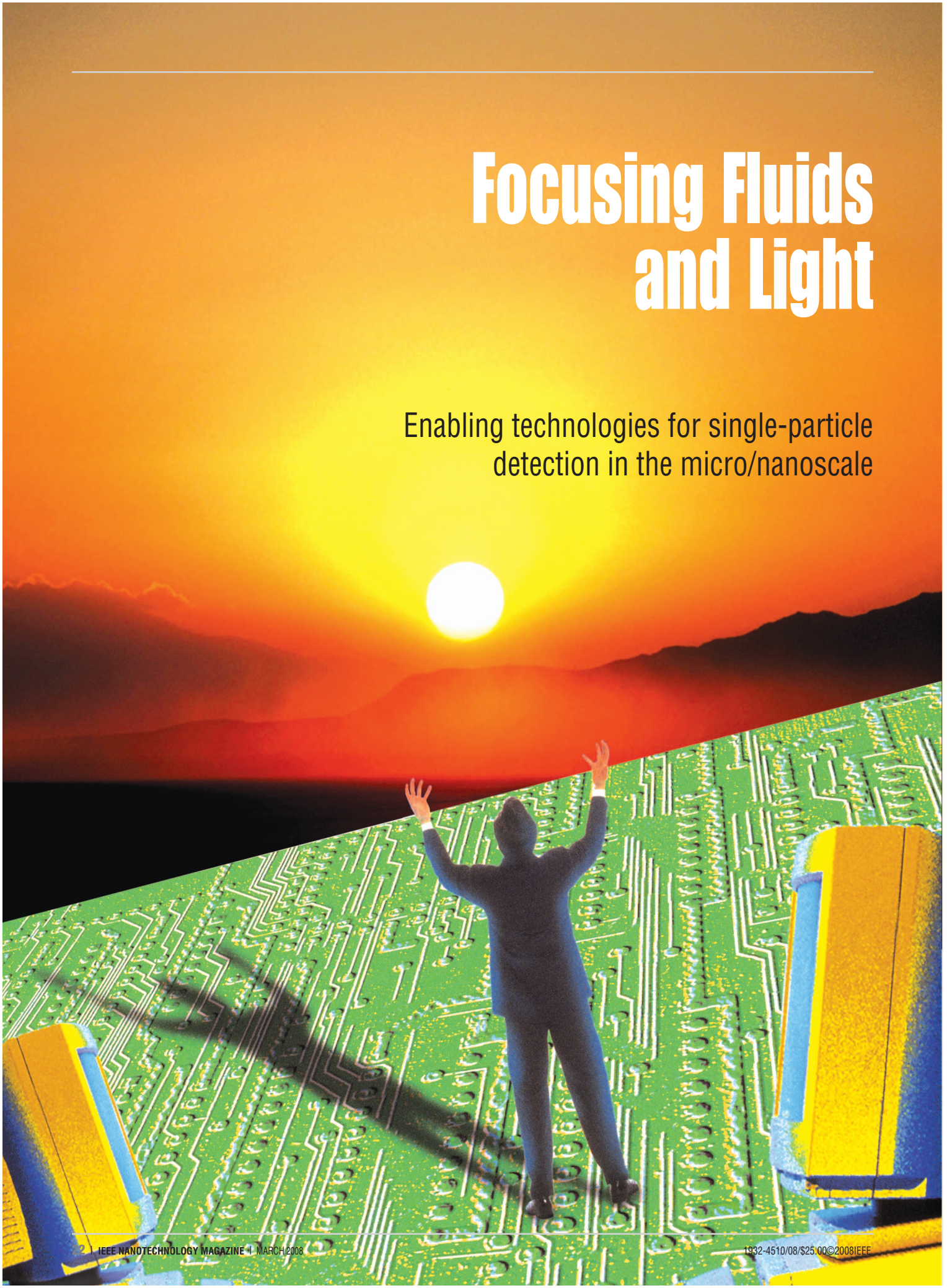
Focusing Fluids and Light

Enabling technologies for single-particle detection in the micro/nanoscale



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MANY BIOMEDICAL AND CLINICAL applications rely on registering the passing of micro- and nano-sized single particles and measuring their characteristics. Flow cytometry and single-molecule detection are among the most prominent examples. Flow cytometry is used to measure the optical and fluorescence characteristics of single cells while cells pass through an optical interrogation area (with dimensions on the order of several micrometers to tens of micrometers) that is defined by a focused laser beam [1]. Various fluorescent cell markers are now available to allow a wide range of applications such as cell counting, cell sorting, and disease diagnosis [2]. Single-molecule detection, the second example, is an emerging yet highly promising technology. It resembles flow cytometry in the sense that target particles are detected one at a time. In this method, a micrometer-scale, diffraction-limited laser focal volume (with dimensions on the order of submicrometer to a few micrometers) is created to excite individual molecules. The quantitative study of rare biological events at the single-molecule level has enabled ultrasensitive disease diagnostics and has proven useful in various biomedical studies such as gene expression and drug discovery [3].

There is much interest in the development of a fully integrated, single-particle detection system, one that utilizes the advances of microfluidics and microfabrication techniques. The major motivation is to reduce the operational cost and complexity of the system, with the possibility of developing a portable device for field applications [2], [3]. Microfluidic devices can dramatically reduce the consumption of precious samples. Their flow chambers, made of inexpensive polymers such as polydimethylsiloxane (PDMS) that are patterned through soft-lithography techniques [4], can serve as an alternative to the costly precision-machined quartz flow curvettes that are currently used in bench-top equipment. Miniature lasers and waveguides [5]–[8] can be fabricated in situ on the device to generate and guide the light that is used to illuminate the sample. Such miniature components can replace the currently used expensive and bulky free-space optics, allowing seamless and maintenance-free optical-fluidic integration.

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Despite these exciting advances, there exist two major technical challenges when developing on-chip single-particle detection systems: focusing the particles and focusing the light. In single-particle detection, fluorescently labeled targets are excited by a laser that is tightly focused in a region much smaller than the cross section of the channel. Such a small excitation volume reduces the detection effectiveness, as not all particles in the solution can be excited. Therefore, the microparticles need to be "focused" to constrain their distribution in the channel so that all particles pass through the detection region. Second, the light exiting from the integrated waveguide is divergent. The lack of a well-defined optical interrogation region affects the optical detection. Therefore, on-chip light focusing is needed to shape the excitation beam. These issues pose significant challenges for developing integrated on-chip single particle detection systems. We summarize our recent attempts to solve the on-chip particle/light focusing problems, attempts that integrated fluidics, acoustics, and optics in microfabricated devices.

ON-CHIP PARTICLE FOCUSING

MICROFLUIDIC DRIFTING-BASED THREE-DIMENSIONAL HYDRODYNAMIC FOCUSING

In conventional single-particle detection, particle focusing is often accomplished by compressing the inner particle suspension flow with an outer sheath flow (hydrodynamic focusing) using a pair of coaxial capillaries. Such coaxial structures, however, are difficult to fabricate using standard lithography-based fabrication. As a result, many integrated devices developed so far facilitate only two-dimensional (2-D) focusing [9], [10]. This is done by horizontally compressing the inner sample flow into a thin "sheet" between two sheath flows injected from both sides. Consequently, the particles are not focused in the Z direction (out-of-plane), and many of them pass undetected through the detection regions.

Recently there have been many efforts to develop three-dimensional (3-D) hydrodynamic focusing techniques as alternatives to coaxial focusing. In 3-D hydrodynamic focusing, sheath flows are injected from both vertical and horizontal directions. These techniques focus much like coaxial focusing and are more practical to implement using lithography-based fabrication. On-chip 3-D hydrodynamic focusing of particles has been demonstrated in several cases [11]–[14]. However, in these studies, complex 3-D multilayer microfluidic devices are necessary to deliver sheath fluids from both the

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vertical and horizontal directions. The complexity that accompanies these 3-D devices compromises the applications of microfluidic systems; thus, it is highly desirable to obtain 3-D focusing with a standard 2-D device. Our group recently developed a novel fluid manipulation technique named microfluidic drifting [15]. This technique enables 3-D hydrodynamic focusing with a simple single-layer planar microfluidic device fabricated via standard soft lithography.

> We have studied the manipulation and focusing of micro/nanoparticles using surface acoustic waves generated on a piezoelectric substrate.

The mechanism of microfluidic drifting-based 3-D focusing is illustrated using a computational dynamic (CFD) simulation model shown in Figure 1(a). The focusing is accomplished in a two-step sequence. The first step focuses the sample flow in the vertical direction by passing the sheath fluid (slice 1) and sample fluids (fluorescent dye, slice 2) through a channel with a 90° curve. Due to the centrifugal effect, a pair of transverse counter-rotating secondary Dean vortices [inset of Figure 1(a)], positioned in the upper and lower portion of the channel cross-sectional plan, are induced in the curved microchannel. The secondary vortical flow perturbs the fluidic interface, pulling the sample flow toward the outer channel wall and sweeping the vertical focusing sheath fluid toward the inner channel wall (slices 5–8). At certain flow-rate conditions, the sample flow can be literally "stretched" into a thin horizontal flow and vertically focused between the split vertical focusing sheath flows (slice 8). Further focusing of sample fluids in the horizontal direction (slices 8–10) is conducted with two horizontally focusing sheath flows (slices 3 and 4), which further compress the vertically focused sample flow from both sides. The combined effects of these two focusing steps result in a hydrodynamically focused, 3-D sample flow in the center of the microfluidic channel.

The 3-D structure of the sample flow was constructed using confocal microscopy. Figure 1(b) depicts the 3-D image of the sample flow. The image reveals the microfluidic drifting in the curve as well as the final 3-D-focused flow. Figure 1(c) is a CFD simulation obtained with the same flow conditions. Strong agreement is evident between the confocal microscopic image and the simulated result. A recent study of ours (not shown) has revealed that 3-D focusing can be easily adapted to effectively focus nano/microparticles. Our devices do not

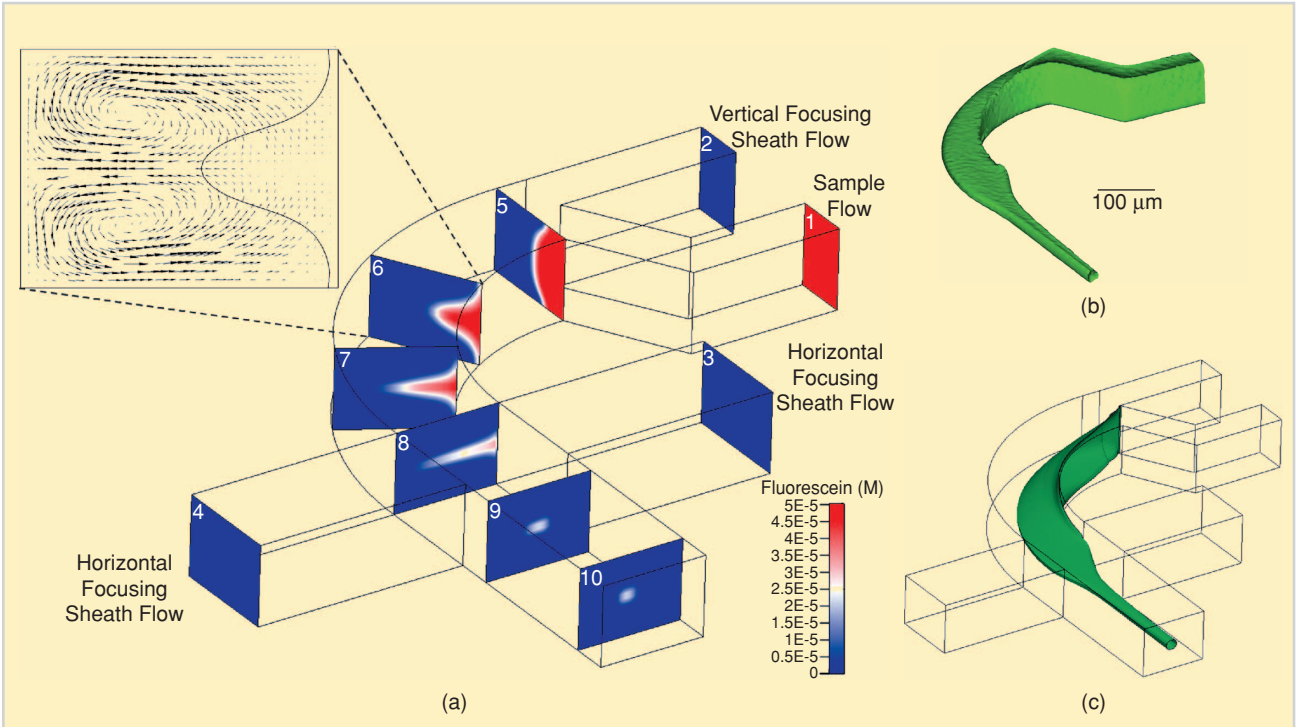

FIGURE 1 (a) CFD simulation of the microfluidic drifting-based 3-D hydrodynamic focusing process. Slices 1–10 are the cross-sectional profiles of the fluorescein dye concentration. Inset: simulated secondary Dean vortices. (b) The 3-D profile of the focused flow by confocal microscopy and (c) the CFD simulation performed under the same flow conditions. Reproduced with permission from The Royal Society of Chemistry.

require fabrication techniques other than standard soft lithography. These microdevices can be easily functionalized by exploiting novel fluidic phenomena in the micro/nanoscale.

FOCUSING OF MICRO/NANOPARTICLES USING STANDING SURFACE ACOUSTIC WAVES

Aside from hydrodynamic focusing, much research has focused on utilizing a variety of electrokinetic methods [16], such as electrophoresis (EP) [17] and dielectrophoresis (DEP) [18], which can directly generate forces on particles to eliminate the need for sheath fluids. However, there are certain limitations of such methods. For example, electrophoretic focusing is only for charged species, and DEP focusing relies on the polarizability of the particles as well as the surrounding medium. Recently we have studied the manipulation and focusing of micro/nanoparticles using surface acoustic waves (SAWs) [19] generated on a piezoelectric substrate. The leak of SAWs through the surface results in pressure gradients in a liquid; these gradients can be used to manipulate suspended particles [20]. The advantage of acoustic particle manipulation is that it does not depend on the charge or polarity of the particles. As such, the method can be applied to virtually any type of particles.

The schematic of the standing surface acoustic wave (SSAW)-focusing device is depicted in Figure 2(a). The device includes a pair of interdigital transducers (IDTs) that are deposited on a lithium niobate substrate, and microparticle suspensions are introduced through a PDMS channel bonded between two IDTs. A radio-frequency signal is applied to each IDT, which then generates a SAW that propagates toward the microchannel. The interference of the SAWs results in the formation of a SSAW on the substrate. With fluids filling the microchannel, the SSAW leaks into the liquid medium and induces pressure waves in the medium. Such pressure waves are also standing waves because of the periodic distribution of their pressure nodes (minimum pressure amplitude) and anti-nodes (maximum pressure amplitude) [21]. Their periods are determined by their origin—the SSAW on the piezoelectric substrate. These pressure waves result in acoustic radiation forces that act on the particles, forcing them to move toward either the pressure nodes or antinodes. Our channel was designed in such a way that only one pressure node (or antinode) was located within the center of the channel. As a result, the particles were in this case focused toward the channel center while they traveled along with the fluids.

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Figure 2(b) depicts the distribution of the fluorescent microparticles during the focusing process at four different regions (I to IV). Region I was outside the SSAW propagation area. The particles at this position did not experience acoustic forces and were distributed uniformly across the width of the channel [Figure 2(b), I]. As the particles entered region II within the SSAW propagation area, they started to experience acoustic forces and began to migrate toward the center of the channel [Figure 2(b), II]. The focusing was completed by the time the particles exited from region II. The duration of the focusing process was on the order of a few milliseconds. In region III [Figure 2(b), III] where the focused stream was well stabilized, the width of the stream was determined to be approximately 5 μm, which was less than three times the diameter of a single particle, 5% of the SSAW wavelength, and 10% of the channel width. We also noticed that the particles remained focused even after they entered region IV [Figure 2(b), IV], which is outside the SSAW propagation area, due to the laminar nature of the flow.

ON-CHIP FOCUSING OF LIGHT

In the development of on-chip, single-particle detection systems, the focusing of light is at least as important as the focusing of particles. Thus far, integrated polymer (PDMS) cylindrical microlenses have been demonstrated to focus light in the X-Y plane [6], [22]–[25]. Such lenses

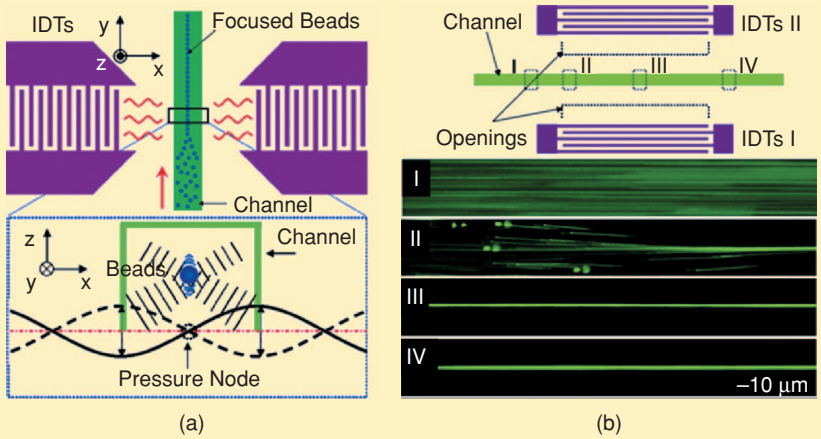

FIGURE 2 (a) Schematic of the SSAW focusing mechanism, and (b) distribution of the particles at chosen sites (I–IV) for monitoring the focusing process. Reproduced with permission from The Royal Society of Chemistry.

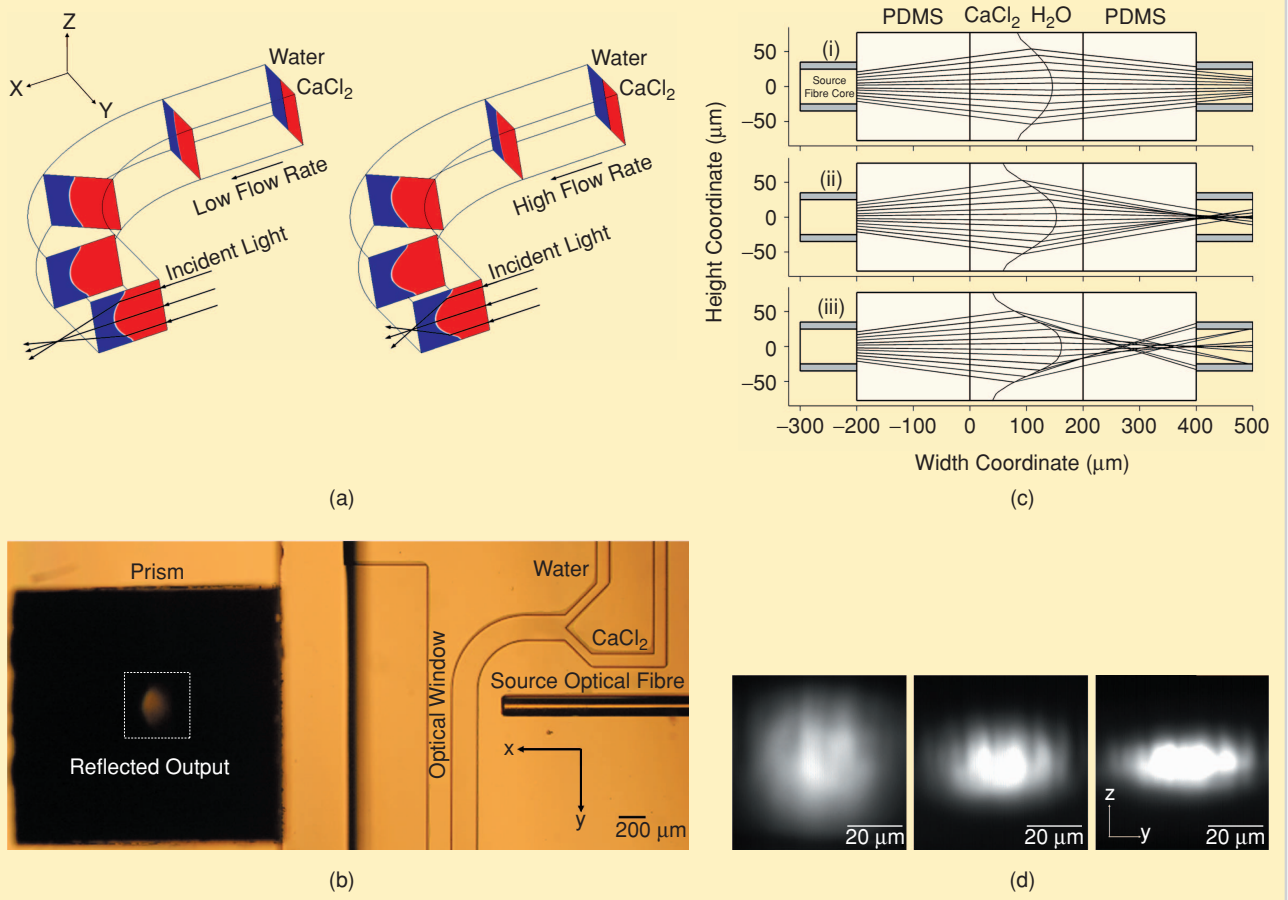

FIGURE 3 (a) Working mechanism of the tunable optofluidic cylindrical microlens; (b) the microscopic image of the device setup; (c) ray-tracing simulation of optofluidic lens at different flow rates (i–iii: low flow rate to high flow rate), and (d) direct visualization of output light spot (left to right: no focusing, under focusing, and well focusing). Reproduced with permission from The Royal Society of Chemistry.

increase the sensitivity of single-particle detection systems. However, most of these lenses are of fixed geometry and thus lack tunability, making it difficult to adjust the size and profile of the light beam. More importantly, few cylindrical lenses that focus light in the X-Z plane have been reported because it is challenging to fabricate such a lens structure by lithography. The limits of lithography can be overcome by the adaptation of an optofluidic (fusion of microfluidics and optics) approach [26]. Recently we demonstrated a tunable, cylindrical optofluidic microlens that focuses the light in the X-Z plane [27]. The mechanism of the microlens is shown in Figure 3(a). The lens utilized the optically smooth, curved fluidic interface and the refractive contrast between the fluids [28] to realize the focusing. The principle of generating the curved fluidic interface is similar to that used in 3-D hydrodynamic focusing described in the previous section. The cylindrical microlens was constructed using two fluids of different refractive indices, a 5-M $CaCl_2$ solution ($n_D = 1.445$) and deionized (DI) water ($n_D = 1.335$). We simultaneously injected the two fluids into a 90° microfluidic curve. Due to the laminar nature of the flow at the micro/nanoscale, the two fluids formed an optically smooth, nearly vertical interface. Upon entering the curve, however, the fluids experienced a centrifugal effect. Consequently, the originally flat fluidic interface is perturbed and bows outward, creating a cylindrical microlens.

Microfluidic drifting enables 3-D hydrodynamic focusing with a simple single-layer planar microfluidic device fabricated via standard soft lithography.

Figure 3(b) shows a device for creating and characterizing the optofluidic, tunable cylindrical microlens. This device

includes an optical fiber connected to a light source for coupling the input light. The fiber is aligned to the exit of the 90° curve where the cylindrical lens will be formed. The image of the focused light spot can be observed from the other side of the channel [29]. Figure 3(c) depicts the ray-tracing simulation of the focusing effect. The fluidic interface profile was obtained from a CFD simulation. The profile indicates that the formation of a curved fluidic interface can be used to focus the light and that the lens profile and focal length can be conveniently tuned by changing the flow rate. Figure 3(d) is a demonstration of variable light focusing using the tunable optofluidic microlens. Three light spots are shown for three cases (no focusing, under focused, and well focused). The focusing effect is evident from the observed changes in the shape and intensity of the light spot.

CONCLUSION

In this tutorial we introduced several techniques recently developed in our group that are relevant to the development of on-chip single-particle detection systems. In the aforementioned studies, we solved on-chip focusing issues for both particles and light. Such issues are major technical hurdles for implementing single-particle detection in microfabricated planar devices. Our methods rely on implementing multidisciplinary (fluidics, acoustics, and optics) approaches, as well as exploiting novel phenomena in the micro/nanoscale to circumvent the limitations of micro/nanofabrication techniques. All components in our study can be conveniently fabricated using a standard lithography technique with minimum procedures, and the components are highly compatible for future device integration. Such methodology is simple, cost-effective, and can potentially be applied in many other fields for the development of functionalized micro/nanodevices.

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