

Greatly Enhanced Single Particle Fluorescence Detection Using High Refractive Index Liquid-Core Waveguides

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Abstract—High sensitivity and easy integration with microfabrication techniques has made silicon photonics one of the leading technologies used to build biosensors for diagnostic applications. Here we introduce a new silicon dioxide based optofluidic platform having a planar solid-core (SC) waveguide orthogonally intersecting a liquid-core (LC) waveguide with high refractive index ZnI₂ salt solution as core. This enables both more uniform collection of particle fluorescence by the core mode and its propagation to an off-chip detector. This approach results in ultra-high sensitivity performance, demonstrated by achieving 8X enhancement in signal-to-noise ratio, a 45x increase in detection efficiency, and a 100x lower detection limit of 80 aM of fluorescent nanobeads. This represents a key step towards an ultrasensitive biosensor system for analyzing pathogens at clinical concentrations.

Index Terms—Optofluidics, Integrated waveguides, Fluorescence, Signal enhancement, Limit-of-detection.

I. INTRODUCTION

EARLY stage diagnosis is very important to contain rapidly spreading infectious viral diseases such as Influenza or COVID-19. At the beginning of infection, patients have very small viral nucleic acid loads in their body in the range of 10⁴ copies/mL [1], [2] which corresponds to attomolar concentrations. Currently, polymerase chain reaction (PCR) is the go-to test used for sensitive diagnostics. PCR test involves target specific amplification of nucleic acids which allows detection limits down to 10² copies/mL [3] but it is a complex process typically requiring laboratory settings and trained professionals. Developing economical point-of-care diagnostic tools which are sensitive, precise and fast can replace the traditional diagnostic procedures and can make health care accessible to all

[4]. In recent years, integrated photonic approaches with Si based waveguides are increasingly being used as the basis for biosensing platforms [5]–[8]. One class of such sensors employs silicon photonics and is typically based on photonic microcavities or evanescent field coupling using interferometers, ring resonators, photonic crystals, Bragg gratings or surface plasmon resonators [9], [10]. These devices rely on direct interaction of the biomolecules with the photonic components of the chip (usually via surface functionalization) to trigger a very sensitive resonance wavelength shift in the microcavities [11]. Biosensors based on Mach-Zehnder interferometers have demonstrated detection of biomolecules with nanomolar detection limits [12]. Better performance down to the picomolar range is obtained with high quality factor microring resonators with subwavelength gratings. [13]. Sensors based on surface plasmon resonators rely on metallic nanoparticles which, when excited, have localized enhanced electric fields. The SPRs induce a very sensitive resonance wavelength shift upon interaction with biomolecules, enabling very low detection limits reaching the attomolar range [14], [15]. However, all these designs require precise fabrication steps and device performance varies greatly from chip to chip. Since silicon has very high loss in the visible spectrum, silicon photonics is unfit for conventional fluorescence-based detection of biomolecules. Thus, this technology cannot utilize the progress made in biotechnology on efficient labeling probes such as nano -strings, -barcodes, -beads and quantum dots [16], [17] with which femtomolar detection limits via direct probe counting has been achieved. Emerging ultra-sensitive fluorescence based diagnostic techniques such as CRISPR based specific high-sensitive enzymatic reporter unlocking (SHER-LOCK) promise sensitivities in the low attomolar range [18]. The true potential of many of these diagnostic techniques is often not exploited due to the limits of the microscopy systems used as the detection platform. This further drives the need to develop sensitive chip scale detection systems.

Dielectric materials such as oxides (SiO₂, Ta₂O₃) or nitrides (Si₃N₄) have low absorption in the visible spectrum and are good candidates to build fluorescence detection based photonic biosensor chips. Titanium dioxide (TiO₂) based photonic crystals and SiO₂ based ring resonators have demonstrated enhanced detection of fluorescent probes such as quantum dots and molecular beacons [19], [20]. SiO₂ is also used extensively

Manuscript received November 20, 2020; revised January 19, 2021; accepted January 21, 2021. Date of publication January 28, 2021; date of current version February 19, 2021. This work was supported by NIH under Grants 1R01EB028608 and 1R01AI116989. (Corresponding author: Gopikrishnan Gopalakrishnan Meena.)

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This article has supplementary material provided by the authors and color versions of one or more figures available at <https://doi.org/10.1109/JSTQE.2021.3055078>.

Digital Object Identifier 10.1109/JSTQE.2021.3055078

to build anti-resonant-reflecting-optical-waveguide (ARROW) based optofluidic biosensor chips [21]. An ARROW waveguide allows for guiding light in liquid core channels filled with low refractive index aqueous solutions. These devices use solid-core (SC) waveguides to excite and collect signals from fluorescently tagged particles flowed through an orthogonally intersecting liquid-core (LC) waveguide [22]. They have been used to demonstrate both singleplex and multiplex detection of viral pathogens, nucleic acids and proteins with single target sensitivity [23]–[25]. Despite the excellent performance, these fluorescence sensors still have the challenge of detecting low intensity signals due to the inherent properties of the ARROW design. The anti-resonant boundary condition of the waveguide makes the field intensity of the optical mode zero at the walls of the LC waveguide [26]. This lowers the efficiency of the coupling of fluorescence signals from broadly distributed targets near the wall with the tightly confined optical mode of the LC waveguide. This along with the small, but finite propagation loss of the LC waveguide limits the performance of the ARROW based biosensor in terms of signal-to-noise ratio and fraction of detected targets. This issue has been circumvented by hybrid integration of ARROW biosensors with microfluidic sample enrichment systems, allowing single molecule sensitive detection of viral nucleic acids down to attomolar concentrations [27], [28]. Another way to address this challenge is to use hydrodynamic focusing to push particles to the center of the LC waveguide where the optical mode is strongest [29], [30]. However, while this approach indeed improves performance, it dramatically increases the fluidic complexity of the device and is not practical for a point-of-care device.

Here, we introduce a new SiO_2 based optofluidic sensor chip that overcomes both limitations of non-uniform mode profile and finite propagation loss in the ARROW. It consists of an index guided SC waveguide that excites fluorescent particles flowing in a LC channel. Guiding of light in the LC channel is achieved via total internal reflection by using a high refractive index solution as the core. Ionic liquids [31] and choline derived ionic liquids [32] are organic solutions with refractive index higher than SiO_2 [33]. However, these solutions have very high viscosity, one or two orders more than water and so cannot be used for the current application [34], [35]. High refractive index salt solutions such as sodium or zinc iodides have been used in many applications such as in laser cavities, immersion lithography, ink jet printing and particle image velocimetry [36], [37]. These solutions are transparent and their viscosity is very close to water [38]. In this case, water soluble surface functionalized fluorescent probes such as nanobeads [39] and quantum dots [40] (Thermofisher) are a good choice of reporters as they are compatible with salt solutions and are not limited to a single target type [41]. Here, we use Zinc iodide (ZnI_2) solution as the core for the LC channel. The device geometry is designed for optimal coupling between SC and LC waveguides. The core refractive index of the LC waveguide is easily tuned by changing the salt concentration [42], [43] to optimize the optical mode for enhanced collection of signals from the fluorescent nanobeads broadly distributed in the LC channel while still maintaining a good confinement factor. Tuning the optical property rather than optimizing the fluidic

properties of the chip enables to effortlessly push the limit-of-detection (LOD) of the device to attomolar concentrations which is very relevant for testing clinical samples.

II. DEVICE FUNCTION AND DESIGN

Figure 1(a) presents the overall experimental setup and layout of the device which preserves the planar optical geometry of previously reported LC-ARROW devices [21]. Fluorescent particles such as nanobeads are flowed through the LC channel. The SC waveguide, butt coupled to a laser via a fiber, excites the particles at the SC-LC intersection. The fluorescence signal is collected by the LC and sent to an avalanche photo detector (APD, Excelitas) off chip via a collection SC waveguide and an objective. Any scattered excitation light is filtered out off-chip using an optical band pass filter (Semrock). The device is fabricated on a silicon (Si) wafer using standard microfabrication techniques (See Supplementary material 1. Device fabrication). Figure 1(b) shows the cross-section of the SC waveguide (xy plane) and the SC-LC intersection (yz plane, black dotted line in Fig. 1(a)). The SC waveguide has a $2.4\ \mu\text{m}$ thick and a $3.4\ \mu\text{m}$ wide SiO_2 core of refractive index 1.508 and is surrounded by a cladding with SiO_2 of low refractive index 1.451. The bottom of the SC waveguide has two layers of cladding. The first “bottom cladding” layer is common for both the SC and LC waveguide and prevents leakage of light into the Si substrate. The second layer (marked as black dotted line in Fig. 2(b)) allows to align the SC waveguide in position with the LC waveguide and also acts as the cladding material for the other three sides of the LC waveguide. Thus, the LC waveguide is surrounded by low refractive index SiO_2 in all four sides. The thickness of this alignment layer ($1.3\ \mu\text{m}$) and that of the LC waveguide ($5.2\ \mu\text{m}$) are designed so that the SC fundamental mode aligns with the middle of the LC waveguide and have at least 70% or more coupling efficiency from the SC to LC waveguide (PhotonD). This ensures that the fluorescent particles flowing in the LC waveguide are efficiently excited by the SC waveguide. Dimensions are checked using SEM/FIB cross section images (Fig. 1(c)) and show a mismatch in the alignment by $0.1\ \mu\text{m}$ due to imperfections in fabrication. This mismatch is negligible compared to the micron size dimensions of the waveguides and has an insignificant effect on the coupling.

To have high detection sensitivity it is important to couple the fluorescence signals from the particles efficiently into a waveguide mode and guide it off the chip to the APD with minimal loss. The index of ZnI_2 solution ranges from 1.4 to 1.6 by varying the salt concentration in water (index 1.33) as shown in Fig. 2(a). The LC waveguide is filled with a ZnI_2 solution of refractive index higher than the surrounding cladding layer (1.451) to support an optical mode guided via total internal reflection. Figure 2(b) shows the simulated fundamental mode in the LC waveguide filled with water and ZnI_2 , respectively. The modes are calculated by numerical solutions of Maxwell's equations with appropriate boundary conditions using the Finite Difference Method (FDM) (PhotonD simulation package). In the case of water, the channel supports a leaky mode with field radiating out of the core whereas the index guided mode

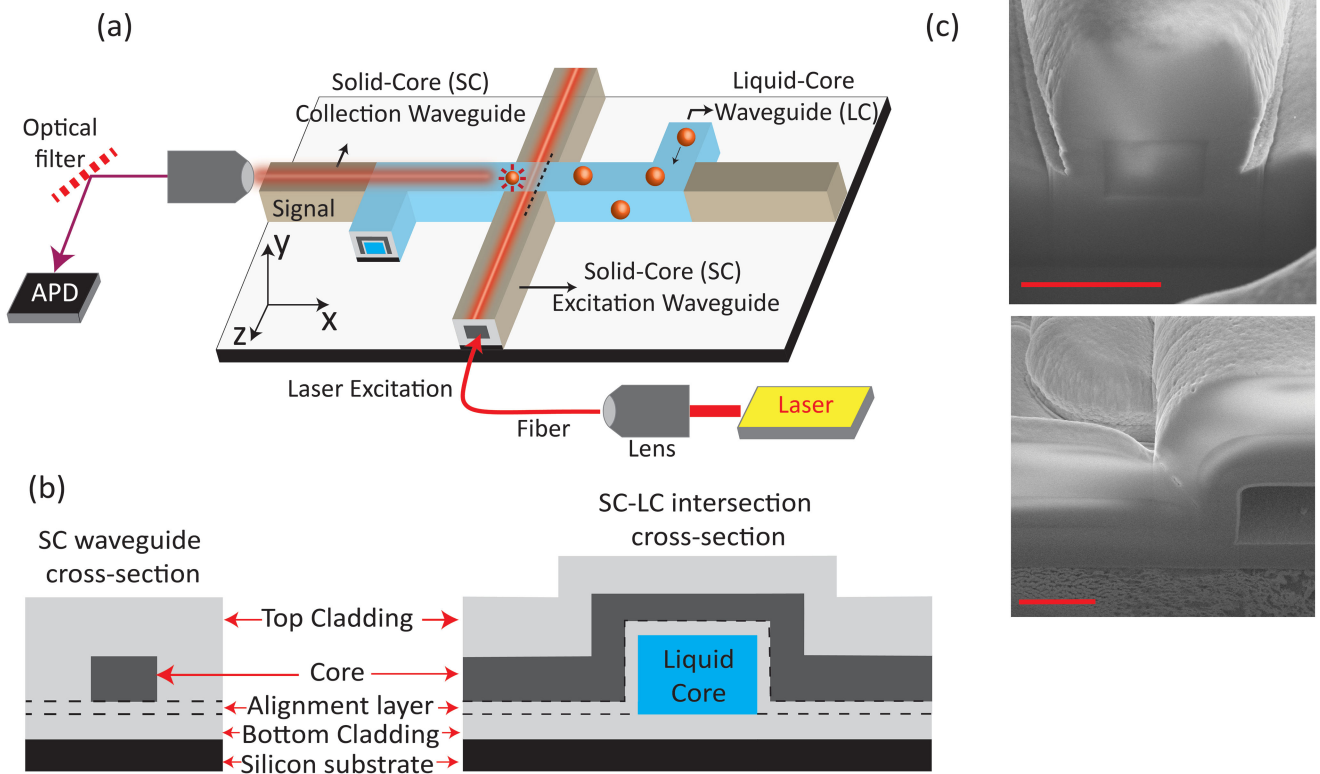


Fig. 1. Device description. (a) Schematic view of the optofluidic sensor chip. Fluorescent particles flow through the LC waveguide and are excited by the SC excitation waveguide. Signals are sent to an APD by the SC collection waveguide. (b) Cross-section of SC waveguide and SC-LC intersection. The SC waveguide has a high refractive index SiO₂ ($n = 1.508$) surrounded by low index SiO₂ (1.451) cladding. The bottom cladding layer and the “alignment layer” surrounds the LC waveguide with low index SiO₂. (c) SEM images of the SC waveguide and SC-LC intersection cross sections (Scale bars: 5 μm).

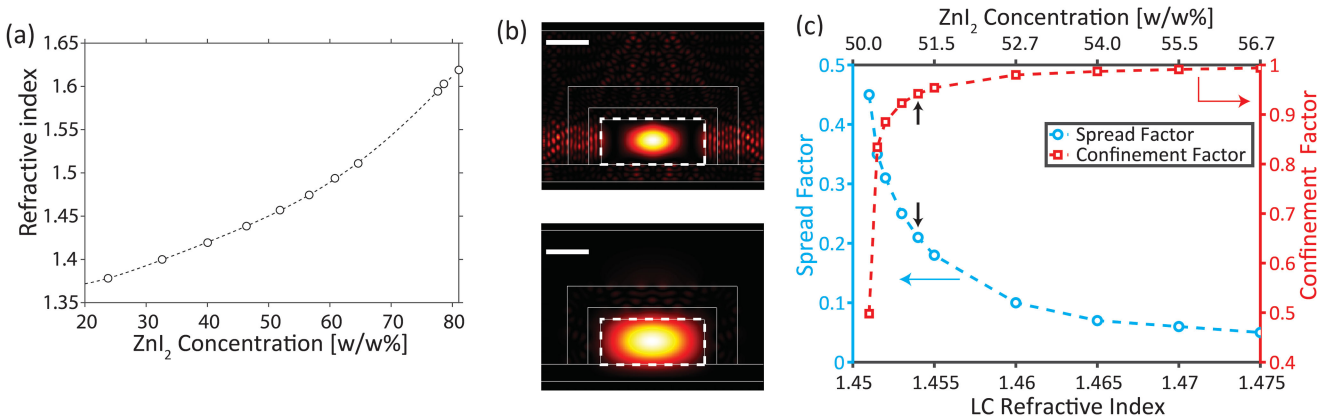


Fig. 2. Device design. (a) Refractive index of ZnI₂ solution (symbols: data after [42]; line: polynomial fit). (b) LC fundamental mode when filled with water (top) and ZnI₂ solution of refractive index 1.4515 (bottom, Scale bar 5 μm). The white dashed line indicates the boundary of the LC waveguide (c) LC fundamental mode Spread Factor and Confinement Factor. The colored arrows point towards the y axis for each plot. The black arrow indicates the ZnI₂ concentration (51%-52%) used for detecting fluorescent beads.

generated with ZnI₂ solution is well confined. It should be noted here that even for the case of an ARROW waveguide, the LC channel only has the anti-resonant reflective layers in the bottom to prevent leakage of light into the Si substrate and the other three sides are surrounded with SiO₂ [22]. Therefore, when filled with water, the LC-ARROW waveguide supports a mode which is only reflected from the bottom and behaves similar to

the above described leaky mode. Transmission measurements along the liquid channel (x-direction in Fig. 1) show a 9.4x increase at 633nm when filled with ZnI₂ solution compared to water. This demonstrates improved light guiding which permits the LC waveguide to guide the fluorescence signals from the particles to the APD with minimal loss thus improving the signal-to-noise-ratio (SNR) of the signals.

An equally critical component for optimized signal collection is to couple fluorescence signals from all the excited particles to the LC waveguide optical mode. This becomes very significant at ultra-low concentrations where very few particles might be present in the whole sample volume. Since the particles are broadly distributed over the LC waveguide, the optical mode field has to cover the whole LC cross section to couple in the signals. When the LC is filled with water, the mode only overlaps with the particles near the middle of the LC and excludes those further away (Fig. 2(b) top, white dotted line depicts the border of the core). By nearly matching the refractive index of the core of the LC waveguide with the cladding, an index guided optical mode with much more uniform field distribution all the way up to the channel walls can be generated. This maximizes the LC cross section area the mode field covers and overlaps the mode with particles even at the edges of the LC [Fig. 2(b) bottom]. The refractive index of the ZnI_2 solution in the LC is tuned by varying the salt concentration. To quantify how well the mode field covers the LC cross section, a “Spread Factor” is defined as the ratio between the mode field intensity at the boundary of the LC and the highest mode intensity value. Figure 2(c) shows the spread factor and the confinement factor - defined as the fraction of the mode energy density confined in the core of the waveguide - of the simulated fundamental mode of the LC waveguide for varying salt concentrations. The higher the confinement factor, the less lossy the waveguide is. As the refractive index of the core approaches that of the cladding, the spread factor increases but the modes confinement factor decreases. Since both parameters need to be maximized for good coupling and collection of the fluorescence signals, ZnI_2 solution of optimum refractive index range of 1.454-1.455 [51%-52% of salt concentration, arrow mark in Fig. 2(c)] is chosen. The refractive index of the ZnI_2 solution is very sensitive to the salt concentration. 50% salt concentration has a lower refractive index (1.45) than the cladding SiO_2 (1.451). Therefore, an optimum salt concentration of 51% to 52% is used after taking into consideration errors that could come up from solution preparation such as mass and volume measurements. The ZnI_2 solution with the optimized refractive index increases the mode spread factor by 33x as compared to the leaky mode with water. Assuming that particles are evenly spread across the LC cross section, the larger spread factor of the mode with ZnI_2 brings 47% more particles within the mode field diameter ($1/e^2$). This refractive index range also increases the confinement factor by 38% (also confirmed by optical mode images) compared to leaky mode guiding in water. Thus, optimizing both these parameters will greatly enhance the sensitivity of the biosensor.

III. ENHANCED DETECTION OF FLUORESCENT NANOBEADS

For fluorescence detection, streptavidin coated nanobeads of known bead concentration of 800 fM (≈ 20 nm in diameter, Thermofisher) are flowed through the LC by applying a negative pressure at the outlet. The SC waveguide is excited with a 633 nm He-Ne laser. Based on the flow speed of the nanobeads and the excitation volume at the SC-LC intersection of the device, the number of beads that are simultaneously excited by

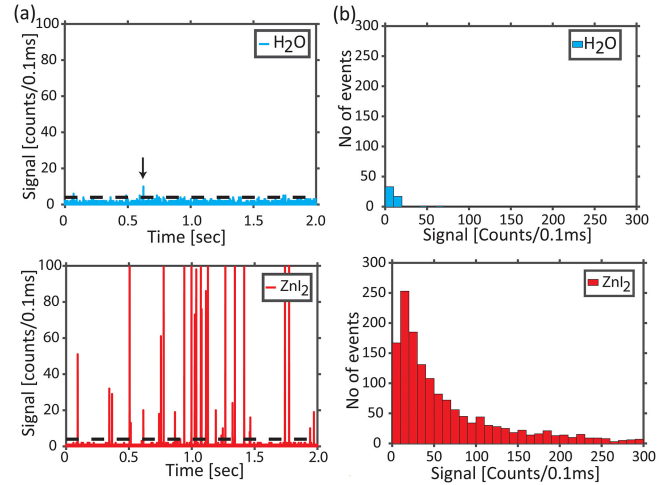


Fig. 3. Nano-Bead fluorescence signals. (a) Fluorescence signals from individual nanobeads detected when the LC is filled with water (top) and ZnI_2 solution (bottom). The arrow marks the signal of a nanobead detected above the background noise threshold (black dashes line). (b) Distribution of nanobead signals from a 100 s long fluorescence trace.

the intersecting solid-core waveguide can be determined. For sub-picomolar concentrations, only one bead is excited at a time, allowing for digital counting of these reporters. For picomolar concentrations or above, a bulk signal in the analog regime, whose average value increases linearly with concentration, is observed [27]. Fluorescence signals of individual nanobeads detected from the biosensor are presented in Fig. 3(a). The black dotted line depicts the maximum threshold of 5 counts/100 μs from the background photoluminescence of the chip. Each spike above the threshold is signal from an individual nanobead. The black arrow marks a signal from the nanobead. When the channel is filled with water, events with an average signal strength of 10 counts/100 μs were detected with an average SNR (average signal strength divided by maximum background threshold) of 2. The same experiment with water was repeated with a standard LC-ARROW chip, which showed the same performance. Events detected when the waveguide is filled with ZnI_2 solution clearly show dramatically better collection of signals, both in amplitude and number, by the index guided mode as compared to water. Signals with an average strength of 80 counts/100 μs are detected corresponding to an 8x increase in SNR which is in very good agreement with the 9.4x improvement seen in the LC waveguide transmission measurement (Section II).

Figure 3(b) shows the distribution of the signal strength of the events observed in the fluorescence trace and reveals a dramatic difference between the two approaches. The variation in signal strength is due to the broad distribution of the position of the nanobeads in the LC cross section [30], [44]. For the ZnI_2 filled channel, the liquid-core mode fills the channel cross section very well (Fig. 2b), and, therefore, signals from beads distributed all over the waveguide, even from those near the wall couple to the mode. On the other hand, when the LC is filled with water, the mode field has lower spread factor and couples in only the signals of the beads located around the middle of the channel. Over a 100 s long experiment, we detect

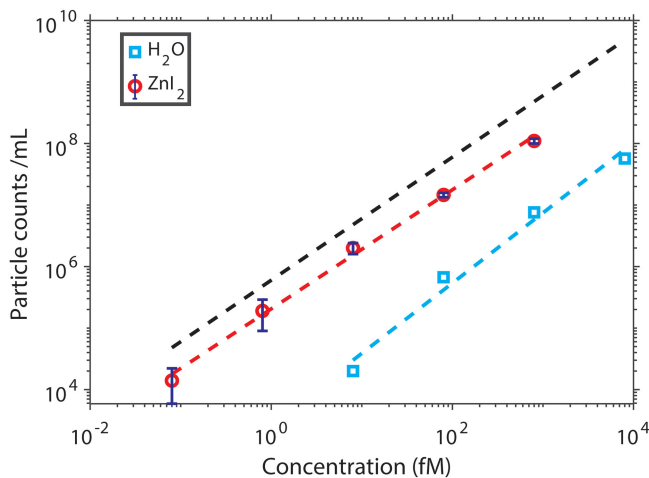


Fig. 4. Concentration dependent nanobead particle counts per volume when LC is filled with water (blue) and ZnI₂ (red). The black dashed line shows the maximum particle counts per unit volume that can be there for each concentration.

95 and 1535 nanobeads when the channel is filled with water and ZnI₂, respectively. Due to the viscosity of ZnI₂ solution [42] being higher than water, the velocity of the particles is a bit lower (by two times) in the salt solution compared to water. Thus, improvement in particle count rate needs to be calculated with respect to the total tested sample volume rather than time. Using the dimensions of the excitation volume of the chip and the average time duration of the signals, a concentration of 7.6×10^6 particles/mL is estimated from the fluorescence trace with water and 1.1×10^8 particles/mL is detected with ZnI₂ (800 fM corresponds to 4.8×10^8 particles/mL). This corresponds to an improvement by over an order of magnitude and enables detection of sub-femtomolar concentrations of particles.

Figure 4 shows the number of counts per volume detected by the sensor from six orders of serial dilutions of the nanobeads. The black dashed line corresponds to the maximum particle count per unit volume that would be observed if every single bead were detected (for example: 1fM concentration corresponds to 6.023×10^5 particles/mL). With water in the LC, very few nanobeads, two orders lower than the expected counts are detected per unit volume, resulting in an LOD of ~ 8 fM (The limit of detection is the lowest starting concentration of nanobeads that is passed through the chip and from which fluorescence signal can be detected). Some beads produce signals below the detection threshold because they flow close to the channel walls and do not fluoresce as brightly [23]. By enhancing the sensitivity of the device using ZnI₂ solution signals from almost all the beads are picked up. An average of 45X increase in particle counts per volume is observed. The measured concentrations are now very close to the expected value. A few of the particles are still missed because there is more room to lower the core refractive index and match it close to the cladding (Δn is 0.004 at 51% salt concentration bringing 88% of the particles within the mode field diameter). By matching the refractive index of the core even closer to the cladding, more particles would fall within the LC optical mode, but adjusting

the concentration of the salt at such precision is very difficult and limits the performance of the device. Enhanced detection using ZnI₂ solution has enabled to lower the LOD of the device by two orders of magnitude to reach clinically relevant concentrations of 80aM. Further improvements of this limit of detection are possible by increasing the assay time or preconcentrating the nanobeads before detection. The high sensitivity of the sensor allows to digitally count nanobeads even at very low sample concentrations. This enables the sensor to have a linear response function with a one-to-one correspondence between the detected particle count rate to sample concentration. This linear response allows the sensor to differentiate sample concentrations over six orders of dilution, even down to the attomolar range.

IV. CONCLUSION

In summary, we have developed a SiO₂ based high-sensitive optofluidic sensor using SC and LC waveguides for diagnostic applications. Enhanced fluorescence detection is achieved by tuning the LC refractive index to support an index guided optical mode that effectively collects signals from particles distributed across the LC cross section. This is accomplished by using an optimized concentration of high refractive index ZnI₂ salt solution in the LC to closely match the core refractive index with the cladding. The device is used to detect nanometer size streptavidin coated fluorescent beads. Through optimizing the optical performance of the device, eight times increase in SNR and a 100x improved LOD in the attomolar range are reached. The ability of the device demonstrated in this work to detect ultra-low concentrations of such fluorescent probes is directly applicable to diagnostic assays [45] and points towards development of a highly sensitive, portable and accurate diagnostic platform.

DISCLOSURES

A.R.H and H.S have financial interest in Fluxus Inc. which is developing optofluidic devices.

ACKNOWLEDGMENT

The authors also acknowledge fruitful discussions with Dr. J.W. Parks and Dr. Tom Yuzvinsky for assistance with electron microscopy and focused ion beam milling and the W.M. Keck Center for Nanoscale Optofluidics for use of the FEI Quanta 3D Dual beam microscope.

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