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TITLE OF THE INVENTION (500 characters max):
OPTICAL DETECTION OF SUSPENDED MICRO-OBJECTS USING ARRAY WAVEGUIDES

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ENCLOSED APPLICATION PARTS (check all that apply)

☐ Application Data Sheet. See 37 CFR 1.76

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☑ Yes, the name of the U.S. Government agency and the Government contract number are: Air Force Office of Scientific Research (AFOSR) Grant No. F49620-02-1-0288. U.S. Government has certain rights to this invention.

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U.S. PROVISIONAL PATENT APPLICATION

FOR

OPTICAL DETECTION OF SUSPENDED MICRO-OBJECTS USING ARRAY WAVEGUIDES

by

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OPTICAL DETECTION OF SUSPENDED MICRO-OBJECTS
USING ARRAY WAVEGUIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

STATEMENT REGARDING FEDERALLY
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[0001] This invention was made with United States Government support awarded by the following agencies: Air Force Office of Scientific Research (AFOSR) Grant No. F49620-02-1-0288. The United States Government has certain rights in this invention.

FIELD OF THE INVENTION

[0002] The present invention relates to techniques for detecting chemicals and, more particularly, to techniques that are used to detect biological materials such as cells and DNA including, for example, flow cytometry techniques.

BACKGROUND OF THE INVENTION

[0003] Over a period of nearly five decades, flow cytometry has evolved from a simple device to count suspended particles in fluid into a highly sophisticated and versatile instrument critical to clinical diagnosis and fundamental biomedical research. Early efforts in the development of flow cytometry have been focused on the attainment of a stable flow system able to transport particles without the disturbance by alien aerosol. These suspended particles (and cells) were then brought to regions of laser beam illumination for optical interrogation via fluorescence or light scattering. The standard approach for today’s flow cytometers is to create a laminar sheath flow in a transparent capillary tube to minimize noise due to fluctuations in position and propagation speed of the particles (cells). Besides the flow control, significant progress has also been made in the methods of cell preparation, new fluorescent dyes and new markers of cell properties.

[0004] These technological advances have led to the use of flow cytometers for analysis of white blood cells in AIDS patients. Other important applications of flow cytometers include cancer diagnosis and stem cell sorting, to name a few. Flow cytometer has been widely recognized as an important clinic and research tool. After nearly five decades of research and
development in flow cytometry, significant progress has been made both in the instrument and in the methods of cell preparation, fluorescent dyes, and cell markers. However, even though the size of a flow cytometer has been reduced from a piece of equipment occupying an entire room to a table top system with ever increasing functionality and performance, a flow cytometer that costs between $150K and $1M remains a tool affordable only by major medical centers and laboratories. Size and price reduction by orders of magnitude (e.g. 1000 times) would be necessary to make flow cytometer a prevailing diagnosis tool that can be afforded by more hospitals and medical practitioners around the world.

[0005] One area that holds much promise for miniaturization of flow cytometry has been microfabricated flow cells, enabled by the advances in microfluidics. Integrated microfluidic chips that perform a variety of functions for chemical analysis and biological screening have found wide applications in pharmaceutical industry and have accelerated the progress of research in biotechnology. Significant efforts have been made to integrate micro-optical and opto-electronic devices with microfluidic systems to provide on-chip fluorescence detection and biochemical sensing. Several research groups have demonstrated the ability to manipulate cells and micro-particles in microfluidic devices using the effects of fluidic pressure, dielectrophoresis, optical trapping, and electro-osmosis. Introducing microfabricated electrodes in the fluidic channels can facilitate the optical detection by controlling and manipulating the positions, angles, and populations of analytes in microfluidic channels, such as cells and DNAs, via dielectrophoretic effect.

[0006] There are several reasons that make these results particularly relevant to the development of compact flow cytometers. First of all, biological cell sizes fit well with the dimensions of the microfluidic devices that can be easily and precisely controlled via microfabrication techniques such as lithography and molding. Second, microfluidic devices tend to support laminar flow, making the flow control simpler and fluid transport highly efficient. Third, micro-scale integration allows more functionality (e.g. pumps, valves and switches) to be incorporated into the device. Finally, two-dimensional or even three-dimensional array structures can be fabricated to enhance the performance of the system and alleviate the limit of device throughput.

[0007] In contrast to the rapid progress in microfluidics, the scheme of optical detection in flow cytometry has experienced no major changes. Other than the availability of improved hardware such as more advanced lasers, more sensitive detectors, and superior optical mechanical components, there has been no paradigm shift in ways the optical detection is performed in flow cytometry. The expensive and bulky optical setup for fluorescence
detection will become the bottleneck for realization of compact, low cost flow cytometers. Additionally, the relatively high cost of lasers and light detectors for use in flow cytometry becomes further exacerbated when one reduces the size of the overall system.

For at least these reasons, it would be advantageous if an improved optical detector or optical detection scheme could be developed for use in performing flow cytometry and related techniques. More particularly, it would be advantageous if such an improved optical detector/detection scheme could be designed in which smaller (in terms of size and/or weight) optical components could be employed. Additionally, it would be advantageous if simpler, less expensive components could be employed for the purposes of generating and/or sensing light.

**BRIEF SUMMARY OF THE INVENTION**

The present inventors have recognized the desirability of achieving photonic designs and technologies that could provide a cost effective solution to the problem of fluorescence (and side scattering) detection in flow cytometry and, more particularly, have recognized the importance of at least one of (a) integrating optical components with the fluidic circuits to reduce the size and weight of the system, and (b) developing innovative architectures of photonic circuits to achieve superb sensitivity without the need for expensive components such as lasers and/or detectors.

To achieve such goals, the present inventors in at least some embodiments propose a microfluidic-photonic integrated circuit optical interrogation scheme that can be utilized as a microfabricated flow cytometer. The device includes a photonic circuit integrated monolithically with the microfluidic channels such that the optical interrogation zones are in the proximity of and well aligned to the optical waveguides that collect the fluorescence and/or scattering light signals. The use of waveguide approach to replace free-space optics eliminates the needs for lenses and precision mechanics for optical alignment, making significant size and weight reduction possible. The device can be fabricated using a fluidic-photonic integrated circuit (FPIC) process.

In at least some embodiments, multiple waveguides are employed to form an array waveguide structure so that, along the direction of flow, an object will pass a series of waveguide-defined optical interrogation zones, each producing a signal that is correlated in time and space to the others. In one such exemplary embodiment, an array of eight parallel waveguides is employed so that the signal produced by a single cell or particle will be detected eight times. At the detection end, an array of eight detectors can be employed or,
alternatively, it is possible to combine the eight waveguides into a single output waveguide and use only a single detector (or, also alternately, more than one but less than eight detectors could be employed). For the single detector approach, the signals from the eight waveguides are multiplexed in time domain, with a time delay equal to the transit time of the particle traveling between adjacent waveguides.

[0012] Although both the eight detector and single detector designs produce superior sensitivity in comparison with conventional detectors, the design with an integrated demultiplexer is preferred in at least some embodiments, since only one detector rather than multiple detectors in the form of a detector array is required. Also, in embodiments where a semiconductor avalanche photodetector (APD) or a photo-multiplier tube (PMT) is used, the detector bandwidth (e.g., > 1 MHz) is more than sufficient to support the sampling rate (e.g., eight times of the particle throughput) for unequivocal detection of each passing cell or particle. In at least some embodiments, the use of an 8-channel waveguide array as mentioned above also allows for other measurements to be made that can yield useful information. These include, for example, time-of-flight measurements and timing jitter measurements to monitor Brownian motion and flow effects. Such information makes it possible to track the behavior of each individual particle in the fluidic channel, producing insight into the particle properties and signals for downstream control.

[0013] In at least some embodiments, the present invention relates to a detector including a fluidic channel capable of conducting a fluid, a source of electromagnetic radiation arranged in relation to the fluidic channel so as to interact with at least a portion of the fluid conducted by the fluidic channel, and a plurality of waveguides having respectively a plurality of ends positioned along the fluidic channel.

[0014] Additionally, in at least some embodiments, the present invention relates to a fluidic-photonic integrated circuit that includes a microfluidic channel, a means for exciting a material within the microfluidic channel, and a waveguide means for detecting an attribute of the material.

[0015] Further, in at least some embodiments, the present invention relates to a method of manufacturing a fluidic-photonic integrated circuit. The method includes casting PDMS prepolymer onto a photo-lithographically patterned mold, thermally-curing the PDMS prepolymer, and demolding a PDMS piece from the mold. The method further includes bonding the PDMS piece to another piece of PDMS material to make a sealed fluidic channels, filling at least one additional channel with PDMS of a higher refractive index, and performing a
curing operation in relation to the filled additional channel to generate at least one waveguide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1 shows in schematic form an exemplary integrated fluidic-photonic device having a fluidic channel, an optical waveguide, and a dielectrophoretic (DEP) cage, in accordance with at least some embodiments of the present invention;

[0017] Fig. 2 is a microscopic photograph of an exemplary device in accordance with the schematic of Fig. 1;

[0018] Fig. 3 illustrates a process of manufacturing the device of Fig. 1 having DEP electrodes integrated with microfluidic channels;

[0019] FIGS. 4(a) and (b) respectively show a schematic view and a side cross-sectional view of an exemplary fluidic-photonic integrated circuit employed in an improved optical detector in accordance with at least some embodiments of the present invention;

[0020] Fig. 5 shows a side cross-sectional view of an alternate embodiment of the fluidic-photonic integrated circuit of Fig. 4 in accordance with at least some embodiments of the present invention, where the detector includes eight waveguide outputs;

[0021] Fig. 6(a) shows in schematic form an improved optical detector in accordance with at least some embodiments of the present invention, where the detector employs the fluidic-photonic integrated circuit of Fig. 4(a);

[0022] Fig. 6(b) is an exemplary image of fluorescent light emitting from a single output of a waveguide demultiplexer of the fluidic-photonic integrated circuit of Fig. 4(a);

[0023] FIGS. 7(a) and (b) are graphs showing exemplary output signals from a single output of the waveguide demultiplexer of the fluidic-photonic integrated circuit of FIGS. 4(a)-(b);

[0024] FIGS. 8(a)-(c) are graphs showing exemplary time variation of photon counts intensity generated from eight waveguide outputs of an alternate embodiment of the fluidic-photonic integrated circuit of Fig. 4 where the data in (a-c) were obtained from fluorescent beads of decreasing size and fluorescence intensity;

[0025] Fig. 9 is a schematic illustration of time domain cross-correlation; and

[0026] FIGS. 10(a)-(b) and 11(a)-(b) are graphs showing exemplary cross-correlated signals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0027] As described in detail below, the present invention is intended to encompass a variety of different embodiments of microfluidic-photonic integrated circuits that can be employed in
applications such as flow cytometry and other techniques, along with various methods of manufacturing such integrated circuits. In at least some embodiments, microfluidic-photonic-dielectrophoretic integrated circuits can be fabricated by way of a process involving micromolding, polymer bonding, and channel waveguides with capillary filling. At least some of the circuits described herein can be considered to represent a new class of circuits particularly attractive to lab-on-a-chip and biomedical applications.

[0028] Referring to Fig. 1, a schematic diagram shows a first exemplary microfluidic-photonic integrated circuit device 10. Although capable of being employed in various environments, the device 10 in at least some cases is intended to perform an on-chip optical detection of single cell(s) and/or small aggregations of DNA. Fig. 1 shows the device configuration. Two waveguides 12 and 14 perpendicular to a fluidic channel 16 perform fluorescent excitation and detection. Additionally, a dielectrophoretic electric cage 18 formed by 4 pairs of diagonally arranged electrodes 20, 22, 24 and 26, respectively, is designed to trap and rotate the target analytes electrically. Fig. 2 is a picture showing the integrated electrode pairs 20-26. Only the electrodes on the top surface are shown because the view of the bottom electrodes is blocked and out of focus.

[0029] A self-aligned process is employed to form microfluidic channels and waveguides. In order to incorporate the electrodes 20-28 as illustrated in Figs. 1-2 to form the electric cage 18, Figs. 3(a)-(c) show exemplary steps of an exemplary fabrication process. A significant step is the process of making the electrodes on both sides of the fluidic channel and of keeping the electrodes on different planes aligned to each other. After the Au electrodes on one side of the fluidic channel are formed, the handle wafer is delaminated from the structure, as shown in Fig. 3(b). Finally another electrode-patterned substrate is attached to the structure in 3(b) using an optical mechanical alignment tool such as a contact mask aligner or a wafer bonder to form a channel with 4 pairs of Au electrodes around it.

[0030] Turning to Figs. 4(a)-(b) and 5, more sophisticated guided wave photonic circuits than that of Figs. 1-2 can also be fabricated with microfluidic channels to deliver new functions including (for example) functions relating to the performance of flow cytometry, which is the workhorse for many biomedical applications. One concern in miniaturized flow cytometry is signal reliability, which can be compromised by the non-uniformity of biological samples and complex flow patterns. Figs. 4(b) and 5 show microscopic pictures of first and second exemplary devices 40 and 60, respectively, that are capable of addressing this issue, while Fig. 4(a) shows the device 40 of Fig. 4(b) in schematic form.
[0031] As shown, in each of the embodiments of Figs. 4(a)-(b) and 5, in addition to two excitation waveguides 42, 44 and 62, 64, respectively, near the ends of vertical sections 48, 68 of the respective fluidic channels 50, 70, two arrays 52, 54 and 72, 74 of 8 waveguide channels are arranged on opposite sides of the vertical sections 48, 68. These waveguides 52, 54, 72, 74, which can be considered detection waveguides, are each fabricated perpendicular to the vertical sections 48, 68 of the respective fluidic channels 50, 70 for optical interrogation. Although each array 52, 54, 72, 74 of waveguides includes 8 waveguide channels, in the embodiment of Fig. 5 the receiving array 74 includes an 8×1 demultiplexer 76, such that all of the waveguides of that array eventually are merged to form a single output waveguide. It should be understood that, while Fig. 5 only shows a portion of the demultiplexer (since Fig. 5 only shows the eight waveguides are first merged into four waveguides), the demultiplexer additionally involves the merging of those four waveguides into two waveguides and then subsequently into the single output waveguide.

[0032] These waveguide arrays 54, 74 provide 8 detection points for the same target flying by, resulting in improved signal-to-noise ratio and suppressed randomness caused by Brownian motions. In addition, the waveguides can perform time-of-flight measurement and multi-label fluorescent detection with wavelength filters. Compared with conventional single-point detection, the data with multiple sampling points contain rich information about the properties of the object, its interplay with the fluid, and the statistic behaviors under Brownian forces.

[0033] This fluidic-photonic integrated circuit (FPIC) devices 40, 60 of Figs. 4(a)-(b) and 5 in at least some embodiments are made of polymer entirely, and are fabricated by way of micro-molding and waveguide capillary filling. Details of the micro-molding process that can be utilized to create the devices 40, 60 of Figs. 4(a)-(b) and 5 are illustrated in Fig. 6(a). The mold master was made from photo-lithographically patterned SU-8-50 (MicroChem, Inc.) photoresist on a 4" silicon wafer (Silicon Quest). Polydimethylsiloxane or PDMS (Gelest OE 41, Gelest, Inc.) pre-polymer was cast onto the mold master. After thermo-curing at 65°C for 4 hours, the PDMS layer was peeled from the mold master, transferring the pattern to the PDMS layer. In order to make enclosed microfluidic channels, the layer with the channel patterns was bonded to another PDMS layer of the same refractive index of 1.407. A short treatment (10 seconds) with high power (100 Watts) oxygen plasma (Technics 500II Asher) was used to activate the PDMS surfaces to facilitate permanent bonding, thus completing the fabrication of microfluidic channels (see Fig. 1b).
[0034] Similar molding techniques can also be used to fabricate ridge waveguides with a chosen polymer of a proper refractive index. One technique the can be employed to make the waveguides is a channel-waveguide filling process. To make channel waveguides, some chosen channels are filled with polymer of higher refractive index. PDMS (Gelest OE 42, Gelest Inc.) can be chosen as the core material. Pre-polymer is introduced into the channels through the inlets. Pre-polymer completely fills the channels in 20 minutes. Then, after the same thermo-curing procedure as mentioned before, the core material is solidified with refractive index of 1.42.

[0035] For the purpose of realizing monolithic fluidic-photonic integrated circuits (FPICs), several integration schemes can be utilized. For high sensitivity chemical sensors that require long interaction length, we demonstrated an integrated structure having a single-mode ridge waveguide inside the microfluidic channel so that the light wave propagates in the same direction as the flow. For applications where on-chip optical processing or contact-free detection is needed, we also demonstrated a stacking structure in which waveguides and fluidic channels were located at different planes so that they can be routed without crossing. Another manner of integrating waveguides and fluidic channels is a self-forming technique that utilizes a capillary effect and immiscibility between liquids inside microfluidic channels to create waveguides that intersect the microfluidic channels. Such an integration scheme is particularly suitable for highly localized fluorescent excitation and detection. In at least some embodiments of FPICs to be used for flow cytometry, a waveguide/channel integration structure similar to but simpler than what is used in the self-forming technique can be employed.

[0036] The particular fluidic-photonic integrated device is made of PDMS (polydimethylsiloxane). As shown in Figs. 4(b) and 5, in the center of the devices 40,60 are the respective microfluidic channels 50,70 delivering the liquid-suspended samples (e.g. cells or micro-particles). In the present embodiments, the dimension of each channel cross-section is 50x50 \( \mu m^2 \), although other cross-sectional dimensions are also possible. The two waveguides 42,44 and 62,64 near the ends of the respective vertical sections 48,68 of the respective fluidic channels deliver optical power for fluorescent excitation. The cross-section of the waveguides in the present embodiment also 50x50 \( \mu m^2 \), although other dimensions are also possible. In at least some embodiments, it is desirable that the waveguides have substantially the same cross-sections as the corresponding fluidic channels. In the present embodiments of Figs. 4(b) and 5, both the excitation waveguides 42,44,62,64 and the detection waveguides 52,54,72,74 are multi-mode devices with a numerical aperture of 0.3.
The excitation light can come from various directions including from above and below the plane (e.g., as shown in Figs. 4(a)-(b) and 5) as well as, in alternate embodiments, from other directions. Also, the excitation waveguides (42, 44, 62, 64) integrated on the device provide convenient access of the excitation light at chosen wavelengths. The well-defined excitation directions by the waveguides also facilitate the measurements of forward, side, and back scatterings of light by the analytes.

[0037] As discussed above, on one side of the respective vertical sections 48,68 of the respective fluidic channels 50,70 is a respective array of eight waveguides with separated outputs 52,72. On a second side of the respective vertical sections 48,68 is, in the case of Fig. 4(b), a second array of eight waveguides 54 and, in the case of Fig. 5, a second array of eight waveguides 74 that are then combined by way of the demultiplexer 76. The purpose of the parallel arrays in each embodiment is to perform time-of-flight measurement to determine the velocity of particles flying by.

[0038] More particularly, in the case of Fig. 5, the 8x1 waveguide combiner or demultiplexer 76 receives time-multiplexed signals from eight detection zones of the array 74. Using the information obtained from the array 74, the 8x1 demultiplexer 76 is able to restore the time domain signal chain. To be discussed later, the detection sensitivity can be enhanced with increasing number of channels for both the parallel array and the space-demultiplexed waveguide structure (likewise, in the case of the device 40 of Figs. 4(a)-(b), the detection sensitivity can be enhanced with increasing the number of channels from 8 to a higher number of channels).

[0039] Indeed, the present invention is intended to encompass a variety of fluidic-photonic integrated circuits (and other combination fluidic-photonic devices) that have any number of detection waveguides corresponding to the arrays of waveguides 52,54,72,74, including embodiments having more than eight or less than eight waveguides in each array (or even only one waveguide on each side of the fluidic channel). Likewise, although the waveguides of the arrays 52,54,72,74 are perpendicular to the vertical sections 48,68 of the fluidic channels, in alternate embodiments, the waveguide(s) can approach the fluidic channels at oblique or other angles. A curved waveguide surface can also be formed to create the light focusing effect to either increase the numerical aperture of the waveguides or to move the waveguides further away from the fluidic channel.

[0040] Nevertheless, in the present embodiments of Figs. 4(a)-(b) and 5, with a total of eight waveguide detection zones, the detection sensitivity can be enhanced by nearly 1,000 times, compared to a single channel device. This manifests the advantage of the FPIC approach over
the free-space optical set-up found in conventional flow cytometry because for the latter, the number of interrogation zones is severely limited by space and cost. It should further be noted that, while in at least some embodiments such as those discussed above, the fluidic-photonic integrated circuit devices such as those shown in Figs. 4(a)-(b) and 5 are PDMS (polydimethylsiloxane)-based microchips, in alternate embodiments the fluidic-photonic integrated circuit devices can be made from other materials and via other processes than are used to develop PDMS-based microchips.

[0041] Referring to Figs. 6(a), an exemplary flow cytometry device 80 employing a FPIC of the type shown in Figs. 4(a)-(b) (e.g., without any demultiplexer) is shown in schematic form. In this embodiment (contrary to that of Figs. 4(a)-(b)), only one of the waveguide arrays 52,54 (namely, the waveguide array 54) is employed and, as a result, signals are only detected along one side of the microfluidic channel 50. The inlet and the outlet of the microfluidic channel 50 are connected to a pumping unit 82 and the waste beaker (not shown), respectively. As part of the standard microfluidic device fabrication and packaging process, appropriate connectors can be fabricated to connect the plastic tubing to the microfluidic channel on the chip without leakage. In at least some embodiments, a syringe pump can be used as the pumping unit 82 to deliver liquid samples. Also, in the present embodiments, laser excitation light from a source 84 is coupled to the two waveguides 44,42 facing either end of the vertical section of the fluidic channel using multi-mode optical fiber 86. In the present embodiment, to secure the connection between optical fiber and waveguide, a multi-mode fiber is inserted into the channel before the core material is solidified.

[0042] After PDMS curing, the encapsulated fiber-waveguide structure shows low insertion loss (< 0.3 dB) and negligible interface reflection, as well as mechanical robustness. Using an objective lens 88, the outputs of the array of detection waveguides 54 form their near-field image on a CCD camera screen of a CCD camera 90 (note that, in the embodiment of Fig. 6, the waveguides 52 are not shown). Fig. 6b shows an exemplary still image of exemplary waveguide output from a single waveguide output terminal (e.g., from the device of Fig. 5) caught by the CCD camera. The striations in Fig. 6b were caused by waveguide cutting and can be removed by polishing. Facet polishing does not appear to be necessary because the striations do not appear to disturb the measurements.

[0043] As mentioned before, the purpose of the 8X1 demultiplexer 76 of Fig. 5 is to restore the time-domain signal chain from the noise-masked data. Figs. 7(a)-(b) shows the directly detected signals from the single output of an exemplary 8X1 demultiplexer. Fig. 7(a) in
particular shows data for a sample containing 5 μm fluorescent microbeads, while Fig. 7(b) in particular shows data for a sample containing 1 μm fluorescent microbeads. The data represents the combination of the signals from 8 separate channels (e.g., the combination of the waveguides 74 or 54). When the fluorescent signal is weak, the raw data shown in Figs. 7(a) and (b) can be largely corrupted by noise due to stray light and the electronic noise of the CCD camera. Nevertheless, in spite of the potentially poor signal quality, it is possible to perform the following algorithm to restore the signal chain in the time domain, utilizing the property that the signals detected at the 8 sequential detection zones are time-correlated. Specifically,
\[ S(t) = F_1(t) \cdot F_2(t-T) \cdot F_3(t-2T) \cdot F_4(t-3T) \cdot F_5(t-4T) \cdot F_6(t-5T) \cdot F_7(t-6T) \cdot F_8(t-7T) \]  \hspace{1cm} (1)
where \( S(t) \) is the time-dependent signal and \( T \) is the time interval for a particle to pass through two adjacent waveguide channels (e.g., to pass from one of the waveguides 74 to a neighboring one of the waveguides 74). The value of \( T \) can be obtained from the time-of-flight measurement.

[0044] Equation (1) represents only one of several possible methods to restore the signal from the noisy measurements, based on the concept of time-correlation among the detection waveguides. Although Eq. (1) provides a method that is mathematically simple, a more robust method to restore the real signal produced by each passing analyte would be through the formal cross-correlation calculation to be discussed with respect to Eqs. (2) and (3) below. With respect to Equations (2) and (3), it is not necessary to assume that every analyte travels at exactly the same speed as in Eq. (1), since the application of Equations (2) and (3) involves the calculating of the travel velocity and time delay of each passing analyte. Therefore, although the method to be discussed in Eqs. (2, 3) is more computation heavy, it alleviates the requirement for keeping all the analytes in the streamline of a constant velocity, thus greatly simplifies the design and processing complexities of fluidic channels.

[0045] The array of 8 parallel waveguides is designed for the time-of-flight measurement. In one exemplary embodiment, 10 μm-diameter fluorescent beads can be used to perform the time-of-flight measurement. When a bead travels through the interrogation region (e.g., within the vertical section 68 of Fig. 5), its fluorescence is detected by each of the 8 waveguides 74 sequentially. The output intensity of each of the 8 waveguides is recorded by the CCD camera 90. Fig. 8(a) shows the intensities of 8 waveguide-channels as functions of time. The center-to-center difference between intensity peaks is the time period when a particle travels across two adjacent waveguides. Therefore, the velocity of the particle can be
easily obtained. In the case of 10 \( \mu m \) fluorescent beads, images of high signal-to-noise ratio can be obtained directly from the output of any single waveguide.

[0046] Referring to Figs. 8(b)-(c), to demonstrate the ability of sensitivity enhancement with the array waveguide structure, time-of-flight measurements were performed using fluorescent beads of smaller diameters: 5 \( \mu m \) and 1 \( \mu m \). Because these fluorescent micro-beads have fluorescent dye doped over the entire volume, their fluorescent intensity is proportional to the volume of the particle, making the fluorescence intensity 8 times and 1000 times weaker than the 10 \( \mu m \) beads. Figs. 8(b) and 8(c) show that the directly detected signals at the output of each waveguide channel were masked by the noise. Such output from any single channel, analogous to the signal obtained from a conventional flow cytometer using a low power excitation source and a low sensitivity detector, cannot produce any meaningful signal. For that reason, conventional flow cytometry systems require high power lasers and photomultiplier tubes with photon counting sensitivity, which are expensive and non-scalable in size.

[0047] However, in accordance with embodiments of the present invention, by taking advantage of the array waveguide architecture, the signal-to-noise ratio can be significantly enhanced using the multi-channel detection technique explained next. With the knowledge that the true signals were time-correlated whereas the noise were not, we employed cross-correlation analysis on the raw data of Figs. 8(b) and 8(c). The concept of cross-correlation is further illustrated graphically in Fig. 9. As long as signals from two channels are time correlated, we can obtain the time delay between two signals by calculating the cross-correlation function \( R(\tau) \) defined in Eq. (2).

\[
R(\tau) = \int f_1(t) * f_2(t - \tau) \, dt \tag{2}
\]

where \( f_1 \) and \( f_2 \) are normalized intensity functions of two individual channels and \( \tau \) is a time domain variable. \( R(\tau) \) maximizes as \( \tau \) equals to the time-delay between two signals. Since in the present embodiment, the device has eight waveguide-channels, the above analysis is extended to calculate the 8-channel cross-correlation, as follows:

\[
R(\tau) = \int f_1(t)f_2(t\tau)f_3(t-2\tau)f_4(t-3\tau)f_5(t-4\tau)f_6(t-5\tau)f_7(t-6\tau)f_8(t-7\tau) \, dt \tag{3}
\]

This 8-term multiplication would further amplify the signal and suppress the noise.

[0048] This feature demonstrates the superior detection sensitivity for the FPIC because it can accommodate essentially any number of detection channels without increasing the cost and complexity of the system substantially. After the cross-correlation calculation performed
according to Equation (3), the raw data of Figs. 8(b)-8(c) can be converted into cross-correlation data as shown in Figs. 10(a)-(b). Clearly the signals have been completely restored as manifested by the pronounced peak of $R(t)$. The maximum of $R(t)$ occurs at 0.3 sec and 0.08 sec for 5μm and 1μm beads, respectively. These are the durations for the respective particles to travel across two waveguide channels. For a center-to-center channel spacing of 100μm, the velocities of particles in each case are 333μm/sec and 1250μm/sec respectively. Therefore, the 8-channel waveguide array shows its superb ability of signal enhancement to allow for time-of-flight measurement on even extremely weak fluorescent beads. The velocity obtained in this way is a direct measurement of particle speed and can be used for in-situ calibration of the fluidic system.

[0049] For particle detection and sorting, it is further desirable to measure signals in real time as an intensity signal chain. Such results are shown in Figs. 11(a)-(b), obtained from the 8x1 demultiplexed outputs. To generate the signals shown in Figs. 11(a)-(b) (which respectively pertain to the 5 μm and 1 μm bead data), the noise-masked raw data in Figs. 7(a)-(b) is processed through Equation (1) with the value of T obtained from the previous time-of-flight measurement. Distinctive peaks with side-lobes represent the passing beads in real time. From the results in Figs. 11(a)-(b), one can find that the peak values of 5 μm beads are many orders of magnitude greater than those of 1 μm beads, due to the fact that Eq. (1) is a product of 8 correlated signals and therefore significantly magnifies the difference in signal intensity. What the results suggest is that the scheme of multi-channel detection not only improves the signal-to-noise ratio but also enhances the ability to distinguish signals of slightly different intensity, an important merit for flow cytometry.

[0050] For detection and sorting in some conventional flow cytometry systems, emission intensity is used to identify targets of different characteristics. Although cell sorting by intensity is the predominant and simplest method, it is not as reliable and accurate as desired when the intensity difference between normal and targeted samples is small. As shown in Figs. 11(a)-(b), the peaks produced by passing beads show considerable differences in their magnitude even though these beads belong to the same group within a variation of a few percents in their size and shape. These large intensity variations of these “similar” beads suggest the ability of detection schemes in accordance with at least some embodiments of the present invention to distinguish samples of only small difference, thus, making cell detection/sorting by intensity more reliable and accurate.
One adverse effect that desirably would be eliminated is the variation of the distance of the bead passing each waveguide, causing signal intensity change due to the variation of light coupling efficiency. Optical detection from the opposite side of the fluidic channel is proposed to eliminate the effect as any positional variation of the bead is supposed to produce an anti-correlation signal between the oppositely located waveguides. On the other hand, the intrinsic property variations of the bead will produce a positive signal correlation. In addition, improving the flow channel design such as using multiple stream laminar flows could also suppress such undesirable effect. Another noticeable feature for the signal peaks in Figs. 11(a)-(b) is the occurrence of the side-lobes. In an ideal case when the background noise is small, high main-lobe-to-side-lobe ratio should be obtained due to the algorithm in Eq. (1). However, when the signal of each waveguide channel is comparable to the noise, the nonzero background would raise the magnitude of the side-lobes. The existence of side lobes becomes a key concern when the bead population increases to the extent that the signals produced by neighboring beads interfere with each other through their side lobes.

It should be noted that the occurrence of side lobes or multiple peaks for a single passing analyte or bead is the result of using the simplest algorithm in Eq. (1). Should Eq. (3) be employed such that a sliding time scale is used to define the lower and upper limit of integration time interval, there will be no side lobes and the signal appears to be a sequence of peaks similar to those in Fig. 10 in time domain. In that situation, the specific time for each peak represents the arrival time of the analyte (or bead) to the first waveguide. To avoid crosstalk, the flow rate needs to be controlled so that there will be no two analytes (beads) passing the array at any given time, which will set the limit of the device throughput for each fluidic channel. The system throughput is the product of the throughput of each fluidic channel and the total number of channels.

In at least some embodiments, to verify the counting accuracy, a CCD connected microscope can be placed on top of the sample device to simultaneously monitor the events happening in fluidic channel. By comparing the monitoring video and channel data in the same period of time, it is possible to address the correspondence between individual beads and peaks. The error rate determined by this method is only valid when the flow rate is slow, due to the restriction of the speed of the monitoring CCD. On the other hand, the verified period of time is limited, because the image acquisition and process are executed by PC. The ultimate solution for this problem is to incorporate the real-time signal processing and sorting functions with the current detection architecture, so that targeted particles can be collected for counting and calibration. To approach this solution, we have been working on the design of
signal processing circuits that will trigger the sorting mechanism in real-time when events are detected. On the other hand, for the sorting part, a new sorting mechanism using acoustic wave can also be introduced. It is intended that, in at least some embodiments, a flow cytometer with complete functions of detection, signal processing and sorting will be integrated in a single chip platform.

[0054] Although not limited to applications relating to flow cytometry, at least some embodiments of the present invention can offer significant cost, size, and performance advantages to revolutionize the flow cytometry techniques. The technology and the architecture design of fluidic-photonic integrated circuits in accordance with at least some embodiments of the present invention significantly enhance the detection sensitivity through multi-point detections, hence opening up the possibility of using low cost light sources (e.g. LEDs and lamps) and detectors (e.g. semiconductor APDs) to replace mainframe lasers and PMTs. It also offers new functions such as measurements of particle velocity, quantum efficiency fluctuation, signal difference between similar samples, etc. that could produce new insight to biosensing. Finally, the FPIC platform offers a natural path to form array structures for parallel processing, which makes up the possible throughput reduction due to the lower flow rate of microfluidic circuits. The FPIC is made of polymer material in a simple yet controllable method (i.e. micro molding and capillary channel filling), and the circuit can be readily transferred to semiconductor or silica substrates for integration with optoelectronic and electronic devices.

[0055] A significant purpose of at least some embodiments of the fluidic-photonic integrated circuit devices of the present invention when implemented for on-chip flow cytometry applications is to enhance the sensitivity of fluorescent detection using the architecture of array waveguides that provides multiple detection zones for objects traveling through the fluid channel. In addition, the waveguide arrays can perform time-of-flight measurement and multi-label fluorescent detection with wavelength filters. Such micro-scale fluidic-photonic integrated circuits can be entirely made of polymer using micro-molding and waveguide filling techniques reported earlier. The monolithic integration of waveguides with microfluidic channels is the first and a significant step to explore the rich asset of photonic ICs to realize flow-cytometry-on-a-chip. Similar methodologies can also be employed to incorporate more functional optical waveguide devices, such as multiplexers/demultiplexers, power splitters, filters, polarizers, etc. to further enhance and expand the detection and analysis functions.
As mentioned above, the present invention is not limited to applications relating to flow cytometry but rather is intended to encompass a variety of embodiments of devices, systems and processes that can be utilized in a variety of biomedical, biochemical, and other sensing applications. Also, while in at least some embodiments of the present invention, one or more arrays of eight detection waveguides are arranged along one or more sides of a fluidic channel, in alternate embodiments, lesser or greater numbers of waveguides than eight waveguides could be employed (indeed, in at least some embodiments, only one detection waveguide would be positioned along one or both sides of the fluidic channel). The waveguides can be oriented in a perpendicular manner relative to the fluidic channel as discussed above, but in alternate embodiments can be oriented in any particular manner relative to the fluidic channel.

Additionally, while the detection waveguides can be rectangular in cross-section as shown, in alternate embodiments the waveguides can take on alternative cross-sectional shapes (e.g., circular cross-sections). Although the sections 48,68 of the waveguides can be vertical as shown in Figs. 4(a)-(b) and 5, the sections need not be vertical but rather could be horizontal or oriented in another manner and, in certain embodiments, could also be curved. Further, although the excitation waveguides 42,44,62,64 shown in Figs. 4(a)-(b) and 5 are aligned with the vertical sections 48,68, respectively of the fluidic channels, in alternate embodiments, any of a variety of other types of light sources (including simply light bulb(s)) could be utilized to illuminate the fluid flowing within the channels. In such other embodiments, it would not be necessary that the light be shined through/along the lengths of the fluidic channels as shown in Figs. 4(a)-(b) and 5; indeed, in certain embodiments, the light could be directed toward the fluidic channels from any direction.

Additionally, while the fluidic channels (e.g., the channels 50,70) of the devices described above are microfluidic channels, the present invention is further intended to encompass other embodiments of devices and systems that employ combinations of fluid channels and waveguides and/or electrodes even where the fluid channels are not "microfluidic channels". For example, the present invention is intended to encompass devices having fluid channels having dimensions substantially greater than those considered as being "microfluidic" channels, e.g., channels having cross-sectional dimensions of greater than micrometers or millimeters. Further, it is intended that the present invention encompass methods of constructing photonic-fluidic integrated circuit devices that involve conventional techniques for manufacturing microfluidic channels, and then supplement those conventional
techniques with additional steps to integrate photonic components (e.g., waveguides) with those channels/carriers.

[0059] It is specifically intended that the present invention not be limited to the embodiments and illustrations contained herein, but include modified forms of those embodiments including portions of the embodiments and combinations of elements of different embodiments as come within the scope of the following claims.
High-Sensitivity Cytometric Detection Using Fluidic-Photonic Integrated Circuits with Array Waveguides

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We demonstrate a new detection scheme for a microfabricated flow cytometer. The fluidic-photonic integrated circuits (FPIC) that perform flow cytometric detection possess new functionality such as on-chip excitation, time-of-flight measurement, and above all, greatly enhanced fluorescence detection sensitivity. Using the architecture of space-division waveguide demultiplexer and the technique of cross-correlation analysis, we obtained high detection sensitivity with a simple light source and a detector, without high power laser excitation and lock-in amplifier (or photomultiplier tube (PMT)) detection. Besides improving cytometric detection, the technology of integrating microfluidic circuits with photonic circuits into the fluidic-photonic-integrated-circuit (FPIC) presents a new platform for sophisticated biomedical sensing devices with significant cost, size, and performance advantages.

Keywords/Indexing Terms -- Biomedical instruments, Microelectromechanical devices, Optical waveguide components, Fluidics, Fabrication
I. INTRODUCTION

Over a period of nearly five decades, flow cytometry has evolved from a simple device to count suspended particles in fluid into a highly sophisticated and versatile instrument critical to clinical diagnosis and fundamental biomedical research. Early efforts in the development of flow cytometry have been focused on the attainment of a stable flow system able to transport particles without the disturbance by alien aerosol. These suspended particles (and cells) were then brought to regions of laser beam illumination for optical interrogation via fluorescence or light scattering. The standard approach for today's flow cytometers is to create a laminar sheath flow in a transparent capillary tube [1] to minimize noise due to fluctuations in position and propagation speed of the particles (cells). Besides the flow control, significant progress has also been made in the methods of cell preparation, new fluorescent dyes and new markers of cell properties [2]. These technological advances have led to the use of flow cytometers for analysis of white blood cells in AIDS patients. Other important applications of flow cytometers include cancer diagnosis and stem cell sorting, to name a few. Flow cytometer has been widely recognized as an important clinic and research tool [3, 4]. However, even though the size of a flow cytometer has been reduced from a piece of equipment occupying an entire room to a table top system with ever increasing functionality and performance, a flow cytometer that costs between $150K and $1M remains a tool affordable only by major medical centers and laboratories. Size and price reduction by orders of magnitude (e.g. 1000 times) would be necessary to make flow cytometer a prevailing diagnosis tool that can be afforded by more hospitals and medical practitioners around the world.

One area that holds much promise for miniaturization of flow cytometry has been microfabricated flow cells, enabled by the advances in microfluidics [5]. Several research groups have demonstrated the ability to manipulate cells and micro-particles in microfluidic devices using the effects of fluidic pressure, dielectrophoresis, optical trapping, and electro-osmosis [6-10]. There are several reasons that make these results particularly relevant to the development of compact flow cytometers. First of all, biological cell sizes fit well with the dimensions of the microfluidic devices that can be easily and precisely controlled via
microfabrication techniques such as lithography and molding. Second, microfluidic devices tend to support laminar flow, making the flow control simpler and fluid transport highly efficient. Third, micro-scale integration allows more functionality (e.g. pumps, valves and switches) to be incorporated into the device. Finally, two-dimensional or even three-dimensional array structures can be fabricated to enhance the performance of the system and alleviate the limit of device throughput.

In contrast to the rapid progress in microfluidics, the scheme of optical detection in flow cytometry has experienced no major changes. Other than the availability of improved hardware such as more advanced lasers, more sensitive detectors, and superior optical mechanical components, there has been no paradigm shift in ways the optical detection is performed in flow cytometry. The expensive and bulky optical setup for fluorescence detection will become the bottleneck for realization of compact, low cost flow cytometers. This motivates us to look into innovative photonic designs and technologies that could provide a cost effective solution to the problem of fluorescence (and side scattering) detection in flow cytometry. The objectives are (a) to integrate optical components with the fluidic circuits to reduce the size and weight of the system, and (b) to develop innovative architectures of photonic circuits to achieve superb sensitivity without the need for expensive lasers and detectors.

To achieve the above goals, we propose and demonstrate an innovative optical interrogation scheme for microfabricated flow cytometer. The device comprises a photonic circuit integrated monolithically with the microfluidic channels such that the optical interrogation zones are in the proximity of and well aligned to the optical waveguides that collect the fluorescence and/or scattering light signals. The use of waveguide approach to replace free-space optics eliminates the needs for lenses and precision mechanics for optical alignment, making significant size and weight reduction possible. Another major advantage resulting from the approach of microfluidic-photonic integrated circuits is the availability of the array waveguide structure so that along the direction of the flow, the object will pass a series of waveguide-defined optical interrogation zones, each producing a signal that is correlated in time and space to the others. For our device, we have fabricated an array of eight parallel waveguides so the signal produced by a single cell or particle will be detected eight times. At the detection end, we have the option to use an array of eight
detectors or to combine the eight waveguides into a single output waveguide and use only a single detector. For the latter approach, we essentially multiplex the signals from the eight waveguides in time domain, with a time delay equal to the transit time of the particle traveling between adjacent waveguides. As to be shown later, although both designs produce superior sensitivity, we prefer the design with an integrated demultiplexer since it needs only one detector instead of a detector array. When a semiconductor avalanche photodetector (APD) or a photo-multiplier tube (PMT) is used, the detector bandwidth (> 1 MHz) is more than sufficient to support the sampling rate (i.e. eight times of the particle throughput) for unequivocal detection of each passing cell or particle.

The 8-channel waveguide array also allows us to make other measurements that could yield useful information. These include time-of-flight measurement and timing jitter measurement to monitor Brownian motion and flow effects. Such information enables us to track the behavior of each individual particle in the fluidic channel, producing insight into the particle properties and signals for downstream control. The device was fabricated using a generic fluidic-photonic integrated circuit (FPIC) process we have developed earlier.

II. FLUIDIC-PHOTONIC INTEGRATED CIRCUITS

This FPIC was made of polymer entirely, and the key fabrication techniques were micro-molding and waveguide capillary filling. The details of the micro-molding process are illustrated in Fig. 1a. The mold master was made from photo-lithographically patterned SU-8-50 (MicroChem, Inc.) photoresist on a 4” silicon wafer (Silicon Quest). PDMS (Gelest OE 41, Gelest, Inc.) pre-polymer was cast onto the mold master. After thermo-curing at 65°C for 4 hours, the PDMS layer was peeled from the mold master, transferring the pattern to the PDMS layer. In order to make enclosed microfluidic channels, the layer with the channel patterns was bonded to another PDMS layer of the same refractive index of 1.407. A short treatment (10 seconds) with high power (100 Watts) oxygen plasma (Technics 500II Asher) was used to activate the PDMS surfaces to facilitate permanent bonding, thus completing the fabrication of microfluidic channels (see Fig. 1b). As demonstrated in our previous work, the similar molding techniques can also be used to fabricate ridge waveguides with a chosen polymer of a proper refractive index [11]. The technique
we used in this work to make waveguide is the channel-waveguide filling process. To make channel waveguides, some chosen channels are filled with polymer of higher refractive index. In this work, we chose to use PDMS (Gelest OE 42, Gelest Inc.) as the core material. Pre-polymer was introduced into the channels through the inlets. Pre-polymer completely filled the channels in 20 minutes. Then, after the same thermo-curing procedure as mentioned before, the core material solidified with refractive index of 1.42. For the purpose of realizing monolithic fluidic-photonic integrated circuits (FPIC), we have developed several integration schemes in our previous work [11]. For high sensitivity chemical sensors that require long interaction length, we demonstrated an integrated structure having a single-mode ridge waveguide inside the microfluidic channel so that the light wave propagates in the same direction as the flow. For applications where on-chip optical processing or contact-free detection is needed, we also demonstrated a stacking structure in which waveguides and fluidic channels were located at different planes so that they can be routed without crossing. The most intriguing way of integrating waveguides and fluidic channels is probably the self-forming technique reported in our earlier paper [12]. It utilizes the capillary effect and immiscibility between liquids inside microfluidic channels to create waveguides that intersect the microfluidic channels. Such integration scheme is particularly suitable for highly localized fluorescent excitation and detection. In this work, for the purpose of demonstrating a new sensing technique most promising for flow cytometry, we have chosen a waveguide/channel integration structure (Fig. 2) similar to but simpler than what is discussed in [12].

The particular fluidic-photonic integrated device is made of PDMS (polydimethylsiloxane). As shown in Fig. 2, in the center of the device is the microfluidic channel delivering the liquid-suspended samples (e.g. cells or micro-particles). The dimension of the channel cross-section is 50x50 μm². Two waveguides near the ends of the vertical section of the fluidic channel deliver optical power for fluorescent excitation. The cross-section of the waveguides is also 50x50 μm². Both excitation and detection waveguides are multi-mode devices with a numerical aperture of 0.3. On one side of the fluidic channel is an array of eight waveguides with separated outputs. The purpose of this parallel array is to perform time-of-flight measurement to determine the velocity of particles flying by. On the other side of the fluidic channel is an 8x1 waveguide combiner producing time-multiplexed signals from eight detection zones. Together with the
information obtained from the parallel array, the 8X1 demultiplexer is able to restore the time domain signal chain. To be discussed later, the detection sensitivity can be enhanced with increasing number of channels for both the parallel array and the space-demultiplexed waveguide structure. With a total of eight waveguide detection zones, the detection sensitivity can be enhanced by nearly 1,000 times, compared to a single channel device [13, 14]. This manifests the advantage of the FPIC approach over the free-space optical set-up found in conventional flow cytometry because for the latter, the number of interrogation zones is severely limited by space and cost.

III. Results and analysis

A. Experimental set-up

The device characterization setup is shown in Fig. 3a. The inlet and the outlet of the microfluidic channel were connected to the pumping unit and the waste beaker, respectively. Special connectors were fabricated to connect the plastic tubing to the microfluidic channel on the chip without leakage. In this work, we used a syringe pump to deliver liquid samples. Laser excitation light was coupled to the two waveguides facing either end of the fluidic channel using multi-mode optical fiber. To secure the connection between optical fiber and waveguide, a multi-mode fiber was inserted into the channel before the core material was solidified. After PDMS curing, the encapsulated fiber-waveguide structure shows low insertion loss (< 0.3 dB) and negligible interface reflection, as well as mechanical robustness. With an objective lens, the outputs of the detection waveguides form their near-field image on the CCD camera screen. Fig. 3b shows the still image of the waveguide output caught by the CCD camera. The striations in Fig. 3b were caused by waveguide cutting and can be removed by polishing. We did not do end facet polishing because the striations did not seem to disturb our measurements.

B. Results and analysis

As mentioned before, the purpose of the 8X1 demultiplexer is to restore the time-domain signal chain from the noise-masked data. In Fig. 4 are the directly detected signals from the single output of the 8X1 demultiplexer. The data is equivalent to the combination of the signals from 8 separate channels. When the fluorescent signal is weak, the raw data shown in Figs. 4(a) and (b) are severely corrupted by noise due to stray light and the electronic noise of the CCD camera. In spite of the very poor signal quality, we can
perform the following algorithm to restore the signal chain in time domain, utilizing the property that the
signals detected at 8 sequential detection zones are time-correlated.

\[ S(t) = f_1(t) \ast f_2(t-T) \ast f_3(t-2T) \ast f_4(t-3T) \ast f_5(t-4T) \ast f_6(t-5T) \ast f_7(t-6T) \ast f_8(t-7T) \]  

(1)

where \( S(t) \) is the time-dependent signal and \( T \) is the time interval for a particle to passes through two
adjacent waveguide channels. The value of \( T \) can be obtained from the time-of-flight measurement.

The array of 8 parallel waveguides is designed for the time-of-flight measurement. We first used 10 \( \mu \)m-
diameter fluorescent beads to perform the time-of-flight measurement. When a bead traveled through the
interrogation region, its fluorescence was detected by each of the 8 waveguides sequentially. The output
intensity of each of the 8 waveguides was recorded by the CCD camera. The center-to-center difference
between intensity peaks is the time period when a particle travels across two adjacent waveguides.

Therefore, the velocity of the particle can be easily obtained. In the case of 10 \( \mu \)m fluorescent beads,
images of high signal-to-noise ratio can be obtained directly from the output of any single waveguide. To
demonstrate the ability of sensitivity enhancement with the array waveguide structure, we perform the
time-of-flight measurements using fluorescent beads of smaller diameters: 5 \( \mu \)m and 1 \( \mu \)m. Because these
fluorescent micro-beads have fluorescent dye doped over the entire volume, their fluorescent intensity is
proportional to the volume of the particle, making the fluorescence intensity 8 times and 1000 times weaker
than the 10 \( \mu \)m beads. The directly detected signals at the output of each waveguide channel were masked
by the noise. Such output from any single channel, analogous to the signal obtained from a conventional
flow cytometer using a low power excitation source and a low sensitivity detector, cannot produce any
meaningful signal. This explains why today’s flow cytometry system requires high power lasers and
photomultiplier tubes with photon counting sensitivity, both being expensive and non-scalable in size.

Taking advantage of the array waveguide architecture, we demonstrate that the signal-to-noise ratio can be
drastically enhanced using the multi-channel detection technique explained next. With the knowledge that
the true signals were time-correlated whereas the noise were not, we employed cross-correlation analysis
on the raw data. As long as signals from two channels are time correlated, we can obtain the time delay
between two signals by calculating the cross-correlation function \( R(\tau) \) defined in Eq. (2).

\[ R(\tau) = \int f_1(t) \ast f_2(t-\tau) \, dt \]  

(2)
where \( f_1 \) and \( f_2 \) are normalized intensity functions of two individual channels and \( \tau \) is a time domain variable. \( R(\tau) \) maximizes as \( \tau \) equals to the time-delay between two signals. Since our device has eight waveguide-channels, we extend the above analysis to calculate the 8-channel cross-correlation:

\[
R(\tau) = \int f_1(t) f_2(t-\tau) f_3(t-2\tau) f_4(t-3\tau) f_5(t-4\tau) f_6(t-5\tau) f_7(t-6\tau) f_8(t-7\tau) dt
\]  

This 8-term multiplication would further amplify the signal and suppress the noise. This feature demonstrates the superior detection sensitivity for the FPIC because it can accommodate essentially any number of detection channels without increasing the cost and complexity of the system substantially. After the cross-correlation calculation, the raw data can be converted into the cross-correlation data in Fig. 5. Clearly the signals have been completely restored as manifested by the pronounced peak of \( R(\tau) \). The maximum of \( R(\tau) \) occurs at 0.3 sec and 0.08 sec for 5\( \mu \)m and 1\( \mu \)m beads, respectively. These are the durations for the respective particles to travel across two waveguide channels. For a center-to-center channel spacing of 100\( \mu \)m, the velocities of particles in each case are 333\( \mu \)m/sec and 1250\( \mu \)m/sec respectively. Therefore, the 8-channel waveguide array shows its superb ability of signal enhancement to allow for time-of-flight measurement on even extremely weak fluorescent beads. The velocity obtained in this way is a direct measurement of particle speed and can be used for in-situ calibration of the fluidic system.

For particle detection and sorting, one would like to measure signals in real time as an intensity signal chain. Such results are shown in Fig. 6, obtained from the 8x1 demultiplexed outputs. To generate the signal in Fig. 6, the noise-masked raw data in Fig. 4 were processed through equation (1) with the value of \( T \) obtained from the previous time-of-flight measurement. Distinctive peaks with side-lobes represent the passing beads in real time. From the results in Fig. 6, one can find that the peak values of 5 \( \mu \)m beads are many orders of magnitude greater than those of 1 \( \mu \)m bead, due to the fact that Eq. (1) is a product of 8 correlated signals and therefore significantly magnifies the difference in signal intensity. What the results suggest is that the scheme of multi-channel detection not only improves the signal-to-noise ratio but also enhances the ability to distinguish signals of slightly different intensity, an important merit for flow cytometry.
IV. DISCUSSION

For detection and sorting in flow cytometry, emission intensity is often used to identify targets of different characteristics. Although cell sorting by intensity is the predominant and simplest method, it is not as reliable and accurate as desired when the intensity difference between normal and targeted samples is small. As shown in Fig. 6, the peaks produced by passing beads show considerable differences in their magnitude even though these beads belong to the same group within a variation of a few percents in their size and shape. These large intensity variations of these "similar" beads suggest the superb ability of the detection scheme to distinguish samples of only small difference; and therefore make cell detection-sorting by intensity more reliable and accurate. However, more research is required to quantify the ability of the technique to detect small differences between samples. One adversary effect that needs to be eliminated is the variation of the distance of the bead passing each waveguide, causing signal intensity change due to the variation of light coupling efficiency. Optical detection from the opposite side of the fluidic channel is proposed to eliminate the effect as any positional variation of the bead is supposed to produce an anti-correlation signal between the oppositely located waveguides. On the other hand, the intrinsic property variations of the bead will produce a positive signal correlation. In addition, improving the flow channel design such as using multiple stream laminar flows [15] could also suppress such undesirable effect.

Another noticeable feature for the signal peak in Fig. 6 is the occurrence of the side-lobes. In an ideal case when the background noise is small, high main-lobe-to-side-lobe ratio should be obtained due to the algorithm in Eq. (1). However, when the signal of each waveguide channel is comparable to the noise, the nonzero background would raise the magnitude of the side-lobes. The existence of side lobes becomes a key concern when the bead population increases to the extent that the signals produced by neighboring beads interfere with each other through their side lobes.

To verify the counting accuracy, we placed a CCD connected microscope on top of our sample device to simultaneously monitor the events happening in fluidic channel. By comparing the monitoring video and channel data in the same period of time, we were able to address the correspondence between individual beads and peaks. The error rate determined by this method is only valid when the flow rate is slow, due to the restriction of the speed of the monitoring CCD. On the other hand, the verified period of time is limited, because the image acquisition and process are executed by PC. The ultimate solution for this problem is to
incorporate the real-time signal processing and sorting functions with the current detection architecture, so that targeted particles can be collected for counting and calibration. To approach the final solution, we have been working on the design of signal processing circuits that will trigger the sorting mechanism in real-time when events are detected. On the other hand, for the sorting part, we introduce new sorting mechanism using acoustic wave. The results will be published elsewhere. Eventually, we hope to construct a flow cytometer with complete functions of detection, signal processing and sorting all integrated in a single chip platform.

V. CONCLUSION

We demonstrated that the technology and the architecture design of fluidic photonic integrated circuit (FPIC) create a paradigm shift for a new generation of flow cytometry system. The attractive features of the FPIC approach include (a) enhancement of the detection sensitivity to allow the use of low-power excitation light source (e.g. LEDs, mercury lamp) and detectors (e.g. APDs), and (b) orders of magnitude reduction in size, weight, and cost of the system. The new approach also offers functions highly valuable yet not achievable by the existing flow cytometry system, such as measurements of particle velocity, quantum efficiency fluctuation, etc. Besides flow cytometry, the FPIC approach provides a technology platform for integration of optical, fluidic, electrical, and even mechanical components for biomedical applications. The materials and process techniques discussed in this paper can be adaptive to silicon/silica substrates, on which semiconductor optoelectronic/electronic devices can be built. The work reported here is considered as a stepping stone towards the research of fluidic photonic integrated circuit as an emerging area that could significantly benefit flow cytometry as well as many other applications.

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Figure 1

(a) Soft lithography process of fabricating microfluidic channels; (b) Oxygen plasma or UV-ozone treatment for surface activation to facilitate permanent PDMS bonding. The picture in the bottom-right corner shows the cross section of a finished 50 μm wide channel.
Figure 2. Micrograph of a fluidic-photonic integrated circuit. The 8x1 waveguide demultiplexer has eventually a single output (not shown in the photo).
Figure 3. (a) Schematic illustration of experimental set-up; (b) Fluorescent light emitting from the single output of the waveguide demultiplexer.
Figure 4. The outputs of 8x1 demultiplexer (a) for sample containing 5 μm fluorescent microbeads; (b) for sample containing 1 μm fluorescent microbeads.
Figure 5.

Figure 5. Cross-correlated signal for samples containing 5 µm and 1 µm fluorescent micro-beads.
Figure 6. Signal chain after treating signals in Fig. 4 with Eq. for samples containing 5 μm and 1 μm fluorescent micro-beads. Each group of peaks (including the side lobe) represents a passing micro-bead. Note that the signals detected from 5 μm beads and 1 μm beads differ by 17 orders of magnitude, manifesting the great potential of the technique to detect very small differences between samples. The fluctuations in the detected signal intensity could be partly attributed to the non-uniformity of the beads.
Microfluidic-photonic-delectrophoretic integrated circuits for biophotonic sensing

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Integrated microfluidic chips that perform variety of functions for chemical analysis and biological screening have found wide applications in pharmaceutical industry and have accelerated the progress of research in biotechnology. Significant efforts have been made to integrate micro-optical and opto-electronic devices with microfluidic systems to provide on-chip fluorescence detection and biochemical sensing [1-4]. On the other hand, introducing microfabricated electrodes in the fluidic channels can facilitate the optical detection by controlling and manipulating the positions, angles, and populations of analytes in microfluidic channels, such as cells and DNAs [5], via dielectrophoretic effect. In this work, we demonstrate microfluidic-photonic-delectrophoretic integrated circuits fabricated using a novel technology involving micromolding, polymer bonding, and channel waveguides with capillary filling. This represents a new class of circuits particularly attractive to lab-on-a-chip and biomedical applications.

The first device is intended to perform an on-chip optical detection of single cell and small aggregation of DNAs. Figure 1 shows the device configuration. The two waveguides perpendicular to the fluidic channel would perform fluorescent excitation and detection. The dielectrophoretic electric cage formed by 4 pairs of diagonally arranged electrodes is designed to trap and rotate the target analytes electrically. In our previous publication, we have demonstrated a self-aligned process of forming microfluidic channels and waveguides [6]. The new challenge in device fabrication is to incorporate the electrodes as illustrated in Fig. 1 to form the electric cage. Figure 2 is the picture of the integrated electrode pairs. Only the electrodes on the top surface are shown because the view of the bottom electrodes is blocked and out of focus. Figure 3 shows the fabrication process flow. The key step of the process is making the via-channel. The details of device fabrication will be presented in the conference.

![Fig. 1 - Integrated fluidic-photonic device with a dielectrophoretic (DEP) cage.](image)

![Fig. 2 - Microscopic photograph of the device illustrated in Fig. 1. The width of the fluidic channel (vertical) and the waveguide (horizontal) is 50 μm.](image)

![Fig. 3 - Process flow of integrating DEP electrodes with microfluidic channels.](image)
Beyond simple waveguide structure shown above, sophisticated guided wave photonic circuits can also be fabricated with microfluidic channels to deliver new functions including flow cytometry, the workhorse for many biomedical applications. One critical concern in miniaturized flow cytometry is signal reliability, which may be compromised by the non-uniformity of biological samples and complex flow patterns. Figure 4 shows the microscope picture of the device to address this issue. In addition to the two excitation waveguides near the ends of the vertical section of the fluidic channel, an array of 8 waveguide channels (right) and an 8x1 DMUX (left) were fabricated perpendicular to the fluidic channel for optical interrogation. These waveguide arrays provide 8 detection points for the same target flying by, resulting in improved signal-to-noise ratio and suppressed randomness caused by Brownian motions. In addition, these waveguides can perform time-of-flight measurement and multi-label fluorescent detection with wavelength filters. Figure 5 shows representative data produced by 5.5um beads flowing through the microfluidic channel. The same waveform of fluorescent signals was obtained by all 8 waveguides. In Figure 5, the upper trace shows the signal produced by a single bead traveling at high speed and the lower trace shows the signal produced by two beads at lower speed. In this experiment, the speed of the beads was primarily controlled by a syringe pump. The experiment demonstrates the basic functionality of the device. Compared to conventional single-point detection, the data with multiple sampling points contain rich information about the properties of the object, its interplay with the fluid, and the statistic behaviors under Brownian forces. Detailed analysis of the data on different samples will be presented in the conference.

![Photo-picture of integrated arrayed WG devices](image1)

![Testing data of micro-flow-cytometer](image2)

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References:
Fluidic Photonic Integrated Circuit
For In-line Detection

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We present a microfabricated fluidic photonic integrated circuit (FPIC) performing the
detection function for flow cytometry. This device was entirely made of polymer using
micro-molding and capillary filling techniques. An array waveguide design was chosen to
achieve superb sensitivity and the time-of-flight measurement for each particle flowing by.
With multi-channel sampling and cross-correlation analysis, the results show significant
enhancement of detection sensitivity.
Since the first cell counter was invented in 1950s and commercialized in 1970s, flow cytometry has become an essential tool for clinical diagnosis and biomedical researches [1]. The worldwide annual market of flow cytometry instruments and reagents is estimated to be $700 million currently and continues to grow. As an essential tool in medicine, flow cytometry is needed worldwide, even in the developing countries with limited funds for healthcare [2]. One critical application for flow cytometry is white blood cell analysis for AIDS patients. Other important applications include cancer diagnosis and stem cell sorting [3]. After nearly five decades of research and development in flow cytometry, significant progress has been made both in the instrument and in the methods of cell preparation, fluorescent dyes, and cell markers [4]. However, even though the size of a flow cytometer has been reduced from a piece of equipment occupying an entire room to a table top system with ever increasing functionality and performance, a flow cytometer that costs between $150K and over $1M each remains to be a tool afforded only by major medical centers and laboratories. Significant size and price reduction by orders of magnitude is necessary to make flow cytometer a prevailing tool that can be afforded and used by hospitals and medical practitioners around the world, particularly in the developing countries. To reach such an ambitious goal, technological breakthroughs are required to fundamentally change the way flow cytometers are designed and fabricated.

Flow cytometry sorts different kinds of cells via detection of light scattering and/or fluorescent labeling. This requires multiple units of high-power lasers, photo-multiplier tubes (PMTs), and complex optical mechanical systems, which become the road blocks for reduction of system cost and size. Furthermore, the high hardware cost severely limits the number of optical interrogation (detection) channels the system can possess and therefore prevents the system from improving its signal-to-noise ratio and error rate. We believe that the most promising solution to these problems resides on the approach of integrating the fluidic system with the optical detection system using microfabrication techniques. The ultimate goal is to combine the technologies of microfluidics, photonics, and micro machining to realize flow-cytometry-on-a-chip.

Over the last decade, great accomplishments have been made in photonic integrated circuits (PICs) [5-7] as well as in microfluidics [8] by two separate communities. The progress in microfluidics turns out to be more relevant to flow cytometry. For example, microfluidic devices have demonstrated the potential to
replace conventional hydrodynamic flow cells in flow cytometers. Several research groups have also demonstrated the manipulation of cells and micro-particles in microfluidic devices using such mechanisms as liquid pressure, dielectrophoresis, optical trapping, and electro-osmosis [9-13]. The relevancy between microfluidics and flow cytometry has become rather obvious because of the following reasons. First, the size of cells fits well with the dimensions of the microfluidic devices that can be easily and precisely controlled. Secondly, microfluidic devices tend to support laminar flow, making the flow control simpler and flow transport efficient. Last but not least, micro-scale integration offers a cost-effective approach to incorporate more functionality (e.g. integrated pumps, valves, etc.) and to form an array structure to enhance the performance of the system. In contrast, the development of photonic integrated circuits (PICs) has mostly been tangential to the development of flow cytometry. Besides the fact that the development was made by a rather different society for different applications (i.e. telecommunications), it is also true that there has been limited effort until recently showing a viable integration process that can combine PICs with microfluidic devices to yield the desired functions and performance [14-18]. The work presented in this paper depicts the first demonstration that shows how an integrated fluidic photonic integrated circuit (FPIC) can drastically enhance the detection sensitivity of fluorescent signals, as the first step towards the goal of flow-cytometry-on-a-chip.

The main purpose of our fluidic photonic integrated circuit (FPIC) for on-chip flow cytometry is to enhance the sensitivity of fluorescent detection using the architecture of array waveguide that provides multiple detection zones for objects traveling through the fluid channel. In addition, the waveguide array can perform time-of-flight measurement and multi-label fluorescent detection with wavelength filters. This micro-scale fluidic-photonic integrated circuit (FPIC) was entirely made of polymer using micro-molding and waveguide filling techniques reported earlier. The monolithic integration of waveguides with microfluidic channels is the first and a significant step to explore the rich asset of photonic ICs to realize flow-cytometry-on-a-chip. We can apply the similar methodology to incorporate more functional optical waveguide devices, such as multiplexers/demultiplexers, power splitters, filters, polarizers, etc. to further enhance and expand the detection and analysis functions.
The fluidic/photonic-integrated device is a PDMS (polydimethylsiloxane)-based microchip (Fig. 1). In the center of the device is the microfluidic channel delivering the liquid-suspended samples (e.g. cells or micro-particles). The dimension of the channel cross-section is 50x50 µm². Two waveguides near the ends of the vertical section of the fluidic channel deliver optical power for fluorescent excitation while the arrays of 8 waveguide-channels perpendicular to the fluidic channel are for optical interrogation. The cross-section of waveguides is also 50x50 µm². Both excitation and detection waveguides are multi-mode devices with a numerical aperture of about 0.3. The device was fabricated using the micro-molding process, which is also known as soft lithography. Starting with a photo-lithographically patterned mold, the PDMS pre-polymer was cast onto the mold and thermally cured. The PDMS piece was then demolded from the mold master and bonded to another piece of PDMS layer to make sealed fluidic channels. To make waveguides, some chosen channels were filled with PDMS of higher refractive index. Capillary force drove the PDMS pre-polymer liquid into the channels till they were filled completely. Although occasionally there were air bubbles trapped in the PDMS prepolymer, these bubbles eventually disappeared due to the air permeability of surrounding PDMS. Finally, the entire device was hot baked to cure the core of the waveguides.

The experimental setup for device testing is shown in Fig. 2a. The inlet and outlet of the microfluidic channels were connected to the pumping unit and the waste reservoir respectively. Specially fabricated connectors held the plastic tubing on the chip without leakage. The two excitation waveguides were coupled to optical fiber to deliver excitation light from the laser. The outputs of eight detection waveguides were focused by an objective lens to form an image on the CCD camera. Fig. 2b shows the image of the output waveguide facets on the CCD camera screen. The striations in Fig. 2b were caused by waveguide cutting and can be removed by polishing. We did not do the end facet polishing because the striations did not disturb the measurements.

To test the device, we first used fluorescent micro-beads with diameters of 10µm. When fluorescent beads travel through the array waveguide interrogation regions, excited fluorescence lighted up the eight waveguides sequentially. The sequential image was recorded by the CCD camera and the intensity of each channel was measured. Fig. 3a shows the intensities of 8 waveguide-channels as functions of time. The side
pictures show the image of each individual waveguide-channel at the moment of the maximum intensity. The time difference between the intensity peaks is the time of travel for a particle to pass two adjacent waveguide-channels. Therefore, the velocity of the particle can be obtained by simply dividing the channel spacing (100 μm in our device) by the peak-to-peak time interval.

In the case of 10μm fluorescent beads, clear image and data of high signal-to-noise ratio were obtained due to the strong fluorescent intensity. To demonstrate the ability for sensitivity enhancement with the array waveguide structure, we used fluorescent micro-beads with smaller diameters. These fluorescent micro-beads have fluorescent dye doped over the entire volume; therefore the fluorescent intensity is proportional to the volume of the particle. We chose to use fluorescent beads with diameters of 5μm and 1μm, having their fluorescent intensities 8 times and 1,000 times lower than that of 10μm beads. As shown in Fig.3b and 3c, the directly detected signals at the output of each waveguide channel were masked by the noise. Because of the extremely poor signal-to-noise ratio, output from any single channel, as being used in most conventional flow cytometers, cannot generate any meaningful signal. That explains why today’s flow cytometry system requires high power lasers and photomultiplier tubes with photon counting sensitivity, both being expensive and non-scalable in size. Instead of following the traditional approach of increasing laser power and using ultra-sensitive detectors, we took advantage of the array waveguide architecture to drastically increase the signal-to-noise ratio using the multi-channel detection technique explained next.

The multi-channel detection technique is based on the fact that the signals contained in each channel are correlated with a delay of inter-channel transit time whereas the noise is uncorrelated. Therefore, the correlation function \( R(\tau) \) between any two channels, as defined in Eq.1 should display a peak when \( \tau \) is equal to the inter-channel transit time.

\[
R(\tau) = \int f_1(t) * f_2(t-\tau)dt
\]  \hspace{1cm} (1)

where \( f1 \) and \( f2 \) are intensities of two individual channels. Extending Eq.1 to the case of 8 channels, we can write the 8-channel correlation function as

\[
R(\tau) = \int f_1(t)f_2(t-\tau)f_3(t-2\tau)f_4(t-3\tau)f_5(t-4\tau)f_6(t-5\tau)f_7(t-6\tau)f_8(t-7\tau)dt
\]  \hspace{1cm} (2)
Figure 4 shows the results of the 8-channel correlation function. Superb signal-to-noise ratio is obtained for both 5 μm and 1 μm fluorescent beads although the signal intensity of the latter was only one thousands of that of the 10 μm bead. This result demonstrates how photonic integration enabled by fluidic photonic integrated circuit (FPIC) technology can drastically improve the signal quality even with simple excitation sources and detectors.

As shown in Fig. 4, the maximum of \( R(r) \) occurs when \( d \) is equal to 0.3 sec and 0.08 sec for 5 μm and 1 μm beads, respectively. For a channel spacing of 100 μm, this yields a particle velocity of 333 μm/sec for 5 μm bead and 1250 μm/sec for 1 μm bead. In the plots of Fig. 4, the amplitude of the cross-correlation function was normalized to the maximum. From their absolute values, one can find that the absolute value of 5 μm bead is 21 orders of magnitude greater than that of 1 μm bead, due to the fact that the correlation function contains a product of signals in 8 channels that magnify the difference in signal intensity. What the result suggests is that the scheme of multi-channel detection not only improves the signal-to-noise ratio but also enhances the ability to distinguish signals of slightly different intensity, an important merit for flow cytometry. At this moment, the flow rate being tested is limited by the speed of the CCD that operates at 30 frames per second. When the flow rate increases such that the inter-channel travel time is shorter than the response time of the CCD camera, one can find peaks of the correlation function with a negative delay, the so called aliasing effect. In the future, semiconductor avalanche photodetectors (APDs) will be used to replace the CCD so that the signal bandwidth can reach as high as 100 MHz.

We demonstrated that, among its many promising applications, the technology and the architecture design of fluidic photonic integrated circuit (FPIC) could offer significant cost, size, and performance advantages to revolutionize the flow cytometry system. The FPIC significantly enhances the detection sensitivity through multi-point detections, hence opens the possibility of using low cost light sources (e.g. LEDs and lamps) and detectors (e.g. semiconductor APDs) to replace mainframe lasers and PMTs. It also offers new functions such as measurements of particle velocity, quantum efficiency fluctuation, signal difference between similar samples, etc. that could produce new insight to biosensing. Finally, the FPIC platform offers a natural path to form array structures for parallel processing, which makes up the possible
throughput reduction due to the lower flow rate of microfluidic circuits. The FPIC is made of polymer material in a simple yet controllable method (i.e. micro molding and capillary channel filling), and the circuit can be readily transferred to semiconductor or silica substrates for integration with optoelectronic and electronic devices. The work reported here is considered as stepping stone towards the research of fluidic photonic integrated circuit as a new discipline that will benefit flow cytometry as well as many other biomedical and sensing applications.

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FIGURE CAPTIONS

Figure 1. The illustration and photograph of fluidic-photonic integrated circuit (FPIC). The central fluidic channel is 50 μm wide. All the waveguides are 50 μm wide, multi-mode.

Figure 2. (a) Schematic illustration of experimental set-up; (b) Cross-section of 8-channel waveguide array.

Figure 3. Fluorescent intensity from eight channel based on samples containing (a) 10 μm; (b) 5 μm; (c) 1 μm micro beads.

Figure 4. Cross-correlation of signals of the arrayed waveguides for microbead detection. (a) for a 5 μm fluorescent microbead; (b) for a 1 μm fluorescent microbead.
Figure 2

(a) Diagram showing fluidic tubing, sample device, objective, and excitation laser source.

(b) Image or diagram not clearly visible.
Figure 3

10um fluorescent beads

(a)
Figure 4

(a)

(b)
Microfluidic-photonic-delectrophoretic integrated circuits for biophotonic sensing

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Integrated microfluidic chips that perform a variety of functions for chemical analysis and biological screening have found wide applications in pharmaceutical industry and have accelerated the progress of research in biotechnology. Significant efforts have been made to integrate micro-optical and opto-electronic devices with microfluidic systems to provide on-chip fluorescence detection and biochemical sensing [1-4]. On the other hand, introducing microfabricated electrodes in the fluidic channels can facilitate the optical detection by controlling and manipulating the positions, angles, and populations of analytes in microfluidic channels, such as cells and DNAs [5], via dielectrophoretic effect. In this work, we demonstrate microfluidic-photonic-delectrophoretic integrated circuits fabricated using a novel technology involving micromolding, polymer bonding, and channel waveguides with capillary filling. This represents a new class of circuits particularly attractive to lab-on-a-chip and biomedical applications.

The first device is intended to perform an on-chip optical detection of single cell and small aggregation of DNAs. Figure 1 shows the device configuration. The two waveguides perpendicular to the fluidic channel would perform fluorescent excitation and detection. The dielectrophoretic electric cage formed by 4 pairs of diametrically arranged electrodes is designed to trap and rotate the target analytes electrically. In our previous publication, we have demonstrated a self-aligned process of forming microfluidic channels and waveguides [6]. The new challenge in device fabrication is to incorporate the electrodes as illustrated in Fig. 1 to form the electric cage. Figure 2 is the picture of the integrated electrode pairs. Only the electrodes on the top surface are shown because the view of the bottom electrodes is blocked and out of focus. Figure 3 shows the fabrication process flow. The key step of the process is making the via-channel. The details of device fabrication will be presented in the conference.

Fig. 1 - Integrated fluidic-photonic device with a dielectrophoretic (DEP) cage.

Fig. 2 - Microscopic photograph of the device illustrated in Fig. 1. The width of the fluidic channel (vertical) and the waveguide (horizontal) is 50 µm.

Fig. 3 - Process flow of integrating DEP electrodes with microfluidic channels.
Beyond simple waveguide structure shown above, sophisticated guided wave photonic circuits can also be fabricated with microfluidic channels to deliver new functions including flow cytometry, the workhorse for many biomedical applications. One critical concern in miniaturized flow cytometry is signal reliability, which may be compromised by the non-uniformity of biological samples and complex flow patterns. Figure 4 shows the microscope picture of the device to address this issue. In addition to the two excitation waveguides near the ends of the vertical section of the fluidic channel, an array of 8 waveguide channels (right) and an 8x1 DMUX (left) were fabricated perpendicular to the fluidic channel for optical interrogation. These waveguide arrays provide 8 detection points for the same target flying by, resulting in improved signal-to-noise ratio and suppressed randomness caused by Brownian motions. In addition, these waveguides can perform time-of-flight measurement and multi-label fluorescent detection with wavelength filters. Figure 5 shows representative data produced by 5.5um beads flowing through the microfluidic channel. The same waveform of fluorescent signals was obtained by all 8 waveguides. In Figure 5, the upper trace shows the signal produced by a single bead traveling at high speed and the lower trace shows the signal produced by two beads at lower speed. In this experiment, the speed of the beads was primarily controlled by a syringe pump. The experiment demonstrates the basic functionality of the device. Compared to conventional single-point detection, the data with multiple sampling points contain rich information about the properties of the object, its interplay with the fluid, and the statistic behaviors under Brownian forces. Detailed analysis of the data on different samples will be presented in the conference.

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References:
High-Sensitivity Cytometric Detection Using Fluidic-Photonic Integrated Circuits with Array Waveguides

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We demonstrate a new detection scheme for a microfabricated flow cytometer. The fluidic-photonic integrated circuits (FPIC) that perform flow cytometric detection possess new functionality such as on-chip excitation, time-of-flight measurement, and above all, greatly enhanced fluorescence detection sensitivity. Using the architecture of space-division waveguide demultiplexer and the technique of cross-correlation analysis, we obtained high detection sensitivity with a simple light source and a detector, without high power laser excitation and lock-in amplifier (or photomultiplier tube (PMT)) detection. Besides improving cytometric detection, the technology of integrating microfluidic circuits with photonic circuits into the fluidic-photonic-integrated-circuit (FPIC) presents a new platform for sophisticated biomedical sensing devices with significant cost, size, and performance advantages.
I. INTRODUCTION

Over a period of nearly five decades, flow cytometry has evolved from a simple device to count suspended particles in fluid into a highly sophisticated and versatile instrument critical to clinical diagnosis and fundamental biomedical research. Early efforts in the development of flow cytometry have been focused on the attainment of a stable flow system able to transport particles without the disturbance by alien aerosol. These suspended particles (and cells) were then brought to regions of laser beam illumination for optical interrogation via fluorescence or light scattering. The standard approach for today's flow cytometers is to create a laminar sheath flow in a transparent capillary tube [1] to minimize noise due to fluctuations in position and propagation speed of the particles (cells). Besides the flow control, significant progress has also been made in the methods of cell preparation, new fluorescent dyes and new markers of cell properties [2]. These technological advances have led to the use of flow cytometers for analysis of white blood cells in AIDS patients. Other important applications of flow cytometers include cancer diagnosis and stem cell sorting, to name a few. Flow cytometer has been widely recognized as an important clinic and research tool [3, 4]. However, even though the size of a flow cytometer has been reduced from a piece of equipment occupying an entire room to a table top system with ever increasing functionality and performance, a flow cytometer that costs between $150K and $1M remains a tool affordable only by major medical centers and laboratories. Size and price reduction by orders of magnitude (e.g. 1000 times) would be necessary to make flow cytometer a prevailing diagnosis tool that can be afforded by more hospitals and medical practitioners around the world.

One area that holds much promise for miniaturization of flow cytometry has been microfabricated flow cells, enabled by the advances in microfluidics [5]. Several research groups have demonstrated the ability to manipulate cells and micro-particles in microfluidic devices using the effects of fluidic pressure, dielectrophoresis, optical trapping, and electro-osmosis [6-10]. There are several reasons that make these results particularly relevant to the development of compact flow cytometers. First of all, biological cell sizes fit well with the dimensions of the microfluidic devices that can be easily and precisely controlled via microfabrication techniques such as lithography and molding. Second, microfluidic devices tend to support laminar flow, making the flow control simpler and fluid transport highly efficient. Third, micro-scale
integration allows more functionality (e.g. pumps, valves and switches) to be incorporated into the device. Finally, two-dimensional or even three-dimensional array structures can be fabricated to enhance the performance of the system and alleviate the limit of device throughput.

In contrast to the rapid progress in microfluidics, the scheme of optical detection in flow cytometry has experienced no major changes. Other than the availability of improved hardware such as more advanced lasers, more sensitive detectors, and superior optical mechanical components, there has been no paradigm shift in ways the optical detection is performed in flow cytometry. The expensive and bulky optical setup for fluorescence detection will become the bottleneck for realization of compact, low cost flow cytometers. This motivates us to look into innovative photonic designs and technologies that could provide a cost effective solution to the problem of fluorescence (and side scattering) detection in flow cytometry. The objectives are (a) to integrate optical components with the fluidic circuits to reduce the size and weight of the system, and (b) to develop innovative architectures of photonic circuits to achieve superb sensitivity without the need for expensive lasers and detectors.

To achieve the above goals, we propose and demonstrate an innovative optical interrogation scheme for microfabricated flow cytometer. The device comprises a photonic circuit integrated monolithically with the microfluidic channels such that the optical interrogation zones are in the proximity of and well aligned to the optical waveguides that collect the fluorescence and/or scattering light signals. The use of waveguide approach to replace free-space optics eliminates the needs for lenses and precision mechanics for optical alignment, making significant size and weight reduction possible. Another major advantage resulting from the approach of microfluidic-photonic integrated circuits is the availability of the array waveguide structure so that along the direction of the flow, the object will pass a series of waveguide-defined optical interrogation zones, each producing a signal that is correlated in time and space to the others. For our device, we have fabricated an array of eight parallel waveguides so the signal produced by a single cell or particle will be detected eight times. At the detection end, we have the option to use an array of eight detectors or to combine the eight waveguides into a single output waveguide and use only a single detector. For the latter approach, we essentially multiplex the signals from the eight waveguides in time domain,
with a time delay equal to the transit time of the particle traveling between adjacent waveguides. As to be shown later, although both designs produce superior sensitivity, we prefer the design with an integrated demultiplexer since it needs only one detector instead of a detector array. When a semiconductor avalanche photodetector (APD) or a photo-multiplier tube (PMT) is used, the detector bandwidth (> 1 MHz) is more than sufficient to support the sampling rate (i.e. eight times of the particle throughput) for unequivocal detection of each passing cell or particle.

The 8-channel waveguide array also allows us to make other measurements that could yield useful information. These include time-of-flight measurement and timing jitter measurement to monitor Brownian motion and flow effects. Such information enables us to track the behavior of each individual particle in the fluidic channel, producing insight into the particle properties and signals for downstream control. The device was fabricated using a generic fluidic-photonic integrated circuit (FPIC) process we have developed earlier.

II. FLUIDIC-PHOTONIC INTEGRATED CIRCUITS

This FPIC was made of polymer entirely, and the key fabrication techniques were micro-molding and waveguide capillary filling. The details of the micro-molding process are illustrated in Fig. 1a. The mold master was made from photo-lithographically patterned SU-8-50 (MicroChem, Inc.) photoresist on a 4” silicon wafer (Silicon Quest). PDMS (Gelest OE 41, Gelest, Inc.) pre-polymer was cast onto the mold master. After thermo-curing at 65°C for 4 hours, the PDMS layer was peeled from the mold master, transferring the pattern to the PDMS layer. In order to make enclosed microfluidic channels, the layer with the channel patterns was bonded to another PDMS layer of the same refractive index of 1.407. A short treatment (10 seconds) with high power (100 Watts) oxygen plasma (Technics 500II Asher) was used to activate the PDMS surfaces to facilitate permanent bonding, thus completing the fabrication of microfluidic channels (see Fig. 1b). As demonstrated in our previous work, the similar molding techniques can also be used to fabricate ridge waveguides with a chosen polymer of a proper refractive index [11]. The technique we used in this work to make waveguide is the channel-waveguide filling process. To make channel waveguides, some chosen channels are filled with polymer of higher refractive index. In this work, we
chose to use PDMS (Gelest OE 42, Gelest Inc.) as the core material. Pre-polymer was introduced into the channels through the inlets. Pre-polymer completely filled the channels in 20 minutes. Then, after the same thermo-curing procedure as mentioned before, the core material solidified with refractive index of 1.42. For the purpose of realizing monolithic fluidic-photonic integrated circuits (FPIC), we have developed several integration schemes in our previous work [11]. For high sensitivity chemical sensors that require long interaction length, we demonstrated an integrated structure having a single-mode ridge waveguide inside the microfluidic channel so that the light wave propagates in the same direction as the flow. For applications where on-chip optical processing or contact-free detection is needed, we also demonstrated a stacking structure in which waveguides and fluidic channels were located at different planes so that they can be routed without crossing. The most intriguing way of integrating waveguides and fluidic channels is probably the self-forming technique reported in our earlier paper [12]. It utilizes the capillary effect and immiscibility between liquids inside microfluidic channels to create waveguides that intersect the microfluidic channels. Such integration scheme is particularly suitable for highly localized fluorescent excitation and detection. In this work, for the purpose of demonstrating a new sensing technique most promising for flow cytometry, we have chosen a waveguide/channel integration structure (Fig. 2) similar to but simpler than what is discussed in [12].

The particular fluidic-photonic integrated device is made of PDMS (polydimethylsiloxane). As shown in Fig. 2, in the center of the device is the microfluidic channel delivering the liquid-suspended samples (e.g. cells or micro-particles). The dimension of the channel cross-section is 50x50 μm². Two waveguides near the ends of the vertical section of the fluidic channel deliver optical power for fluorescent excitation. The cross-section of the waveguides is also 50x50 μm². Both excitation and detection waveguides are multi-mode devices with a numerical aperture of 0.3. On one side of the fluidic channel is an array of eight waveguides with separated outputs. The purpose of this parallel array is to perform time-of-flight measurement to determine the velocity of particles flying by. On the other side of the fluidic channel is an 8x1 waveguide combiner producing time-multiplexed signals from eight detection zones. Together with the information obtained from the parallel array, the 8X1 demultiplexer is able to restore the time domain signal chain. To be discussed later, the detection sensitivity can be enhanced with increasing number of
channels for both the parallel array and the space-demultiplexed waveguide structure. With a total of eight waveguide detection zones, the detection sensitivity can be enhanced by nearly 1,000 times, compared to a single channel device [13, 14]. This manifests the advantage of the FPIC approach over the free-space optical set-up found in conventional flow cytometry because for the latter, the number of interrogation zones is severely limited by space and cost.

III. RESULTS AND ANALYSIS

A. Experimental set-up

The device characterization setup is shown in Fig. 3a. The inlet and the outlet of the microfluidic channel were connected to the pumping unit and the waste beaker, respectively. Special connectors were fabricated to connect the plastic tubing to the microfluidic channel on the chip without leakage. In this work, we used a syringe pump to deliver liquid samples. Laser excitation light was coupled to the two waveguides facing either end of the fluidic channel using multi-mode optical fiber. To secure the connection between optical fiber and waveguide, a multi-mode fiber was inserted into the channel before the core material was solidified. After PDMS curing, the encapsulated fiber-waveguide structure shows low insertion loss (< 0.3 dB) and negligible interface reflection, as well as mechanical robustness. With an objective lens, the outputs of the detection waveguides form their near-field image on the CCD camera screen. Fig. 3b shows the still image of the waveguide output caught by the CCD camera. The striations in Fig. 3b were caused by waveguide cutting and can be removed by polishing. We did not do end facet polishing because the striations did not seem to disturb our measurements.

B. Results and analysis

As mentioned before, the purpose of the 8X1 demultiplexer is to restore the time-domain signal chain from the noise-masked data. In Fig. 4 are the directly detected signals from the single output of the 8X1 demultiplexer. The data is equivalent to the combination of the signals from 8 separate channels. When the fluorescent signal is weak, the raw data shown in Figs. 4(a) and (b) are severely corrupted by noise due to stray light and the electronic noise of the CCD camera. In spite of the very poor signal quality, we can perform the following algorithm to restore the signal chain in time domain, utilizing the property that the signals detected at 8 sequential detection zones are time-correlated.
\[ S(t) = f_1(t) \cdot f_2(t-T) \cdot f_3(t-2T) \cdot f_4(t-3T) \cdot f_5(t-4T) \cdot f_6(t-5T) \cdot f_7(t-6T) \cdot f_8(t-7T) \]  

(1)

where \( S(t) \) is the time-dependent signal and \( T \) is the time interval for a particle to pass through two adjacent waveguide channels. The value of \( T \) can be obtained from the time-of-flight measurement.

The array of 8 parallel waveguides is designed for the time-of-flight measurement. We first used 10 \( \mu \)m-diameter fluorescent beads to perform the time-of-flight measurement. When a bead traveled through the interrogation region, its fluorescence was detected by each of the 8 waveguides sequentially. The output intensity of each waveguide was recorded by the CCD camera. Fig. 5a shows the intensities of 8 waveguide-channels as functions of time. The center-to-center difference between peaks is the time period when a particle travels across two adjacent waveguides. Therefore, the velocity of the particle can be easily obtained. In the case of 10 \( \mu \)m fluorescent beads, images of high signal-to-noise ratio can be obtained directly from the output of any single waveguide. To demonstrate the ability of sensitivity enhancement with the array waveguide structure, we perform the time-of-flight measurements using fluorescent beads of smaller diameters: 5 \( \mu \)m and 1 \( \mu \)m. Because these fluorescent micro-beads have fluorescent dye doped over the entire volume, their fluorescent intensity is proportional to the volume of the particle, making the fluorescence intensity 8 times and 1000 times weaker than the 10 \( \mu \)m beads. As shown in Fig.5b and 5c, the directly detected signals at the output of each waveguide channel was masked by the noise. Such output from any single channel, analogous to the signal obtained from a conventional flow cytometer using a low power excitation source and a low sensitivity detector, cannot produce any meaningful signal. This explains why today’s flow cytometry system requires high power lasers and photomultiplier tubes with photon counting sensitivity, both being expensive and non-scalable in size. Taking advantage of the array waveguide architecture, we demonstrate that the signal-to-noise ratio can be drastically enhanced using the multi-channel detection technique explained next. With the knowledge that the true signals were time-correlated whereas the noise were not, we employed cross-correlation analysis on the data in Figs. 5(b,c).

The concept of cross-correlation is illustrated in Fig. 6. As long as signals from two channels are time correlated, we can obtain the time delay between two signals by calculating the cross-correlation function \( R(\tau) \) defined in Eq. (2).

\[ R(\tau) = \int f_1(t) \cdot f_2(t-\tau) \, dt \]  

(2)
where $f_1$ and $f_2$ are normalized intensity functions of two individual channels and $\tau$ is a time domain variable. $R(\tau)$ maximizes as $\tau$ equals to the time-delay between two signals. Since our device has eight waveguide-channels, we extend the above analysis to calculate the 8-channel cross-correlation:

$$R(\tau) = \int f_1(t)f_2(t-\tau)f_3(t-2\tau)f_4(t-3\tau)f_5(t-4\tau)f_6(t-5\tau)f_7(t-6\tau)f_8(t-7\tau)dt$$

This 8-term multiplication would further amplify the signal and suppress the noise. This feature demonstrates the superior detection sensitivity for the FPIC because it can accommodate essentially any number of detection channels without increasing the cost and complexity of the system substantially. After the cross-correlation calculation, the data in Figs. 5(b,c) can be converted into the cross-correlation data in Fig. 7. Clearly the signals have been completely restored as manifested by the pronounced peak of $R(\tau)$. The maximum of $R(\tau)$ occurs at 0.3 sec and 0.08 sec for 5 \(\mu\)m and 1 \(\mu\)m beads, respectively. These are the durations for the respective particles to travel across two waveguide channels. For a center-to-center channel spacing of 100\(\mu\)m, the velocities of particles in each case are 333\(\mu\)m/sec and 1250\(\mu\)m/sec respectively. Therefore, the 8-channel waveguide array shows its superb ability of signal enhancement to allow for time-of-flight measurement on even extremely weak fluorescent beads. The velocity obtained in this way is a direct measurement of particle speed and can be used for in-situ calibration of the fluidic system.

For particle detection and sorting, one would like to measure signals in real time as an intensity signal chain. Such results are shown in Fig. 8, obtained from the 8x1 demultiplexed output. To generate the signal in Fig. 8, the noise-masked raw data in Fig. 4 were processed through equation (1) with the value of T obtained from the previous time-of-flight measurement. Distinctive peaks with side-lobes represent the passing beads in real time. From the results in Fig. 8, one can find that the peak values of 5 \(\mu\)m beads are many orders of magnitude greater than those of 1 \(\mu\)m bead, due to the fact that Eq. (1) is a product of 8 correlated signals and therefore significantly magnifies the difference in signal intensity. What the results suggest is that the scheme of multi-channel detection not only improves the signal-to-noise ratio but also enhances the ability to distinguish signals of slightly different intensity, an important merit for flow cytometry.
IV. DISCUSSION

For detection and sorting in flow cytometry, emission intensity is often used to identify targets of different characteristics. Although cell sorting by intensity is the predominant and simplest method, it is not as reliable and accurate as desired when the intensity difference between normal and targeted samples is small. As shown in Fig. 8, the peaks produced by passing beads show considerable differences in their magnitude even though these beads belong to the same group within a variation of a few percents in their size and shape. These large intensity variations of these “similar” beads suggest the superb ability of the detection scheme to distinguish samples of only small difference; and therefore make cell detection-sorting by intensity more reliable and accurate. However, more research is required to quantify the ability of the technique to detect small differences between samples. One adversary effect that needs to be eliminated is the variation of the distance of the bead passing each waveguide, causing signal intensity change due to the variation of light coupling efficiency. Optical detection from the opposite side of the fluidic channel is proposed to eliminate the effect as any positional variation of the bead is supposed to produce an anti-correlation signal between the oppositely located waveguides. On the other hand, the intrinsic property variations of the bead will produce a positive signal correlation. In addition, improving the flow channel design such as using multiple stream laminar flows [15] could also suppress such undesirable effect.

Another noticeable feature for the signal peak in Fig. 8 is the occurrence of the side-lobes. In an ideal case when the background noise is small, high main-lobe-to-side-lobe ratio should be obtained due to the algorithm in Eq. (1). However, when the signal of each waveguide channel is comparable to the noise, the nonzero background would raise the magnitude of the side-lobes. The existence of side lobes becomes a key concern when the bead population increases to the extent that the signals produced by neighboring beads interfere with each other through their side lobes.

V. CONCLUSION

We demonstrated that the technology and the architecture design of fluidic photonic integrated circuit (FPIC) create a paradigm shift for a new generation of flow cytometry system. The attractive features of the FPIC approach include (a) enhancement of the detection sensitivity to allow the use of low-power excitation light source (e.g. LEDs, mercury lamp) and detectors (e.g. APDs), and (b) orders of magnitude
reduction in size, weight, and cost of the system. The new approach also offers functions highly valuable yet not achievable by the existing flow cytometry system, such as measurements of particle velocity, quantum efficiency fluctuation, etc. Besides flow cytometry, the FPIC approach provides a technology platform for integration of optical, fluidic, electrical, and even mechanical components for biomedical applications. The materials and process techniques discussed in this paper can be adaptive to silicon/silica substrates, on which semiconductor optoelectronic/electronic devices can be built. The work reported here is considered as a stepping stone towards the research of fluidic photonic integrated circuit as an emerging area that could significantly benefit flow cytometry as well as many other applications.

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REFERENCES


Figure 1

(a) Soft lithography process of fabricating microfluidic channels; (b) Oxygen plasma or UV-ozone treatment for surface activation to facilitate permanent PDMS bonding. The picture in the bottom-right corner shows the cross section of a finished 50 μm wide channel.
Figure 2

Figure 2. Micrograph of a fluidic-photonic integrated circuit. The 8x1 waveguide demultiplexer has eventually a single output (not shown in the photo).
Figure 3. (a) Schematic illustration of experimental set-up; (b) Fluorescent light emitting from the single output of the waveguide demultiplexer.
Figure 4. The outputs of 8x1 demultiplexer (a) for sample containing 5 μm fluorescent microbeads; (b) for sample containing 1 μm fluorescent microbeads.
Figure 5. Fluorescent intensity from individual eight channels for samples containing (a) 10 μm; (b) 5μm; (c) 1μm micro beads.
Figure 6

\[ R(\tau) = \int f_1(t) \ast f_2(t - \tau) dt \]

Figure 6. Schematic illustration of the concept of time domain cross-correlation.
Figure 7. Cross-correlated signal of a single bead (a) for sample containing 5 μm fluorescent microbeads; (b) for sample containing 1 μm fluorescent microbeads.
Figure 8

(a) Restored Signal Chain for Sample of 5um Fluorescent Beads

(b) Restored Signal Chain for Sample of 1um Fluorescent Beads

Figure 8. Signal chain after treating signals in Fig. 4 with Eq. (1). (a) for sample containing 5 µm fluorescent micro-beads; (b) for sample containing 1 µm fluorescent micro-beads. Each group of peaks (including the side lobe) represents a passing micro-bead. Note that the signals detected from 5 µm beads and 1 µm beads differ by 17 orders of magnitude, manifesting the great potential of the technique to detect very small differences between samples. The fluctuations in the detected signal intensity could be partly attributed to the non-uniformity of the beads.
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CLAIMS

WE CLAIM:

1. A detector comprising:
   a fluidic channel capable of conducting a fluid;
   a source of electromagnetic radiation arranged in relation to the fluidic channel so as
   to interact with at least a portion of the fluid conducted by the fluidic channel; and
   a plurality of waveguides having respectively a plurality of ends positioned along the
   fluidic channel.

2. The detector of claim 1, wherein the fluidic channel is a microfluidic channel and
   includes at least one of a substantially linear section and a substantially curved section.

3. The detector of claim 2, wherein the microfluidic channel includes the substantially
   linear section, and wherein the substantially linear section is aligned substantially with a
   vertical axis.

4. The detector of claim 1, wherein the source is arranged in a manner that is aligned
   with the substantially linear section.

5. The detector of claim 1, wherein the source includes at least one of an electrode, an
   additional waveguide, a light bulb, a laser, and an additional source of electromagnetic
   radiation.

6. The detector of claim 5, wherein the source includes the electrode and a plurality of
   additional electrodes, and wherein the electrodes form a dielectrophoresis cage.

7. The detector of claim 5, wherein the source includes the additional waveguide, and
   wherein the additional waveguide is aligned with a substantially linear section of the fluidic
   channel and positioned along a first end of the substantially linear section and extending
   away therefrom.
8. The detector of claim 7, wherein the detector includes a further waveguide that is positioned along a second end of the substantially linear section.

9. The detector of claim 1, wherein the plurality of waveguides is a first array of waveguides, and wherein each of the ends of the waveguides of the first array is positioned at a different location along a length of the fluidic channel.

10. The detector of claim 9, wherein each of the waveguides of the first array extends substantially perpendicularly away from the fluidic channel.

11. The detector of claim 9, wherein the plurality of waveguides of the first array includes eight waveguides.

12. The detector of claim 9, further comprising a demultiplexer by which signals from the plurality of waveguides are combined.

13. The detector of claim 9, wherein at least one signal from the plurality of waveguides is received by at least one of a CCD camera and another sensor.

14. The detector of claim 9, further comprising an additional plurality of waveguides, wherein the additional plurality of waveguides are arranged along a first side of the fluidic channel opposite a second side along which are arranged the plurality of waveguides.

15. The detector of claim 1, wherein a first of the waveguides extends away from a first side of the fluidic channel and a second of the waveguides extends away from a second side of the fluidic channel, and wherein the first and second waveguides are substantially in alignment with one another.

16. A flow cytometry system comprising the detector of claim 1.

17. A fluidic-photonic integrated circuit comprising:
   a microfluidic channel;
   a means for exciting a material within the microfluidic channel; and
   waveguide means for detecting an attribute of the material.
18. The fluidic-photonic integrated circuit of claim 17, wherein the waveguide means includes at least one waveguide arranged along the microfluidic channel.

19. The fluidic-photonic integrated circuit of claim 17, wherein the integrated circuit is a PDMS (polydimethylsiloxane)-based microchip.

20. A method of manufacturing a fluidic-photonic integrated circuit, the method comprising:
   - casting PDMS pre-polymer onto a photo-lithographically patterned mold,
   - thermally-curing the PDMS pre-polymer;
   - demolding a PDMS piece from the mold;
   - bonding the PDMS piece to another piece of PDMS material to make a sealed fluidic channels;
   - filling at least one additional channel with PDMS of a higher refractive index; and
   - performing a curing operation in relation to the filled additional channel to generate at least one waveguide.
OPTICAL DETECTION OF SUSPENDED MICRO-OBJECTS USING ARRAY WAVEGUIDES

ABSTRACT

A new detection scheme for a microfabricated flow cytometer is disclosed herein. The fluidic-photonic integrated circuits (FPIC) that perform flow cytometric detection possess new functionality such as on-chip excitation, time-of-flight measurement, and above all, greatly enhanced fluorescence detection sensitivity. Using the architecture of space-division waveguide demultiplexer and the technique of cross-correlation analysis, high detection sensitivity is obtainable using a simple light source and a detector, without high power laser excitation and lock-in amplifier (or photomultiplier tube (PMT)) detection. Besides improving cytometric detection, the technology of integrating microfluidic circuits with photonic circuits into the fluidic-photonic-integrated-circuit (FPIC) presents a new platform for sophisticated biomedical sensing devices with significant cost, size, and performance advantages.
Figure 6

(a) Schematic illustration of experimental set-up; (b) Fluorescent light emitting from the single output of the waveguide demultiplexer.
Figure 4. The outputs of 8x1 demultiplexer (a) for sample containing 5 μm fluorescent microbeads; (b) for sample containing 1 μm fluorescent microbeads.
Fig. 9

Schematic illustration of the concept of time domain cross-correlation.
Fig. 8 (b)

Fig. 8 (c)

Fluorescent intensity from individual eight channels for samples containing (a) 10 μm; (b) 5μm; (c) 1μm micro beads.
Cross-correlated signal of a single bead (a) for sample containing 5 µm fluorescent micro-beads; (b) for sample containing 1 µm fluorescent micro-beads.
Signal chains after treating signals in Fig. 4 with Eq. (1). (a) for sample containing 5 μm fluorescent micro-beads; (b) for sample containing 1 μm fluorescent micro-beads. Each group of peaks (including the side lobe) represents a passing micro-bead. Note that the signals detected from 5 μm beads and 1 μm beads differ by 17 orders of magnitude, manifesting the great potential of the technique to detect very small differences between samples. The fluctuations in the detected signal intensity could be partly attributed to the non-uniformity of the beads.