OPTOFLUIDIC DEVICE FOR HIGH RESOLUTION VOLUME REFRACTIVE INDEX MEASUREMENT OF SINGLE CELL

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ABSTRACT

This paper reports a label-free biosensor combining microfluidics and optical detection based on a Fabry-Perot refractometer. The device monolithically integrates waveguides coupled to distributed Bragg mirrors and also a hydrodynamic particle injector enabling cells to enter the sensor cavity. Recent developments in sub-populations identification based on volumetric refractive index measurements of single glass beads are discussed. A statistically significant difference between two sub-populations of glass beads was found when particles flow through the detection region.

KEYWORDS

Optofluidic, Refractometer, Fabry-Perot, Microfluidics, MEMS

INTRODUCTION

Miniaturization and integration of optical and microfluidic structures on a single platform, in order to perform particles count and differentiation, are of great interest for life sciences research. We reported previously an all-silicon volume refractive index sensor for homogenous liquids [1] with a resolution of 1.7×10^{-5} RIU (Refractive Index Unit), which we now apply for particle (e.g. cells) characterization. Other research groups previously used similar detection principles for trapped cells refractive index measurement with coated optical fibers [2], or for characterization of immobilized cells in an out of plane resonant cavity configuration [3]. Compared to these works [2, 3], the novel BioMEMS presented in this paper allows a significantly higher throughput (up to several counts per second) for the characterization of large population of cells. Our device therefore offers the potential to become a complementary analysis tool for flow cytometers and haematology analyzers by allowing the detection of small variation in sub-populations based on their refractive indices.

OPERATION PRINCIPLE

The optofluidic device integrates, on a single Silicon On Insulator (SOI) platform (11 µm thick device layer), microfluidic channels in a hydrodynamic focusing configuration [4] (Fig. 1) and an optical detection system based on a Fabry-Perot resonant cavity with curved waveguides. The Fabry-Perot cavity was designed using the formalism presented in [5] to achieve optimal parameters for refractive index detection. The dimensions of the waveguides were simulated using the beam propagation method (RSoft) to provide the highest optical fiber-waveguide coupling by conserving the shape of the optical mode. Figure 1 (a) shows the working principle of the device, while Fig.1 (b) presents the microsystem in a 3D schematic view. When a glass bead enters the cavity, the effective volume refractive index changes, inducing a shift of the Fabry-Perot resonance peaks. At a fixed wavelength, in the slope of the resonance peak, the measured transmission changes proportionally to the size, refractive index and velocity of the particle.

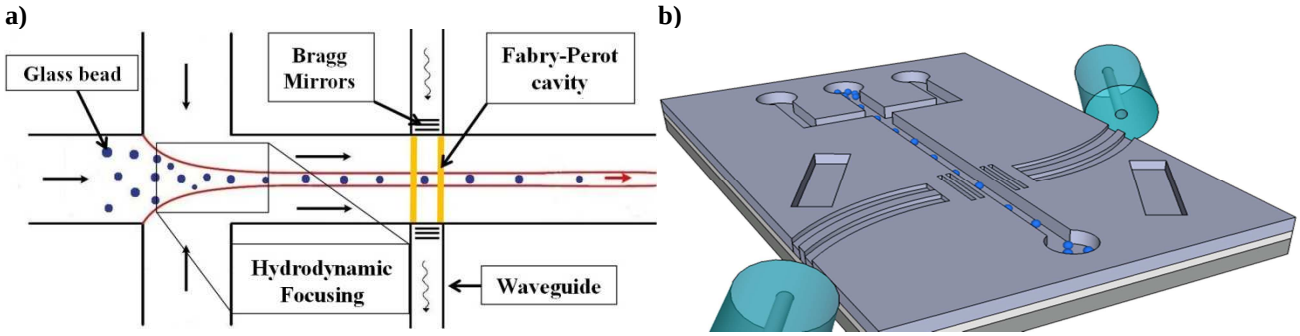

Figure 1. (a) Schematic view of the device working principle. (b) 3D schematic view of the device.

EXPERIMENTAL

Microfabrication of the device is divided in two steps, both comprising regular photolithography and DRIE (Deep Reactive Ion Etching). In the first step, microfluidic channels and distributed Bragg mirrors are defined and etched down to the buried SiO_2 layer. The second fabrication step defines and etches rib waveguides, which will couple light from SMF28 optical fibers to the Fabry-Perot cavity. The SOI wafer is subsequently cut into small pieces using a dicing saw (ADT, Provectus 7100) and an optimized process that yield diced facets of optical quality. The measured roughness on waveguide facets was 5.5 nm, which provides optical coupling with negligible scattering losses. Finally, the microchannels are sealed with a Pyrex top layer by anodic bonding [6]. Figure 2 presents a scanning electron micrograph

of a part of the optofluidic device including the microfluidic channel, the two Bragg mirrors forming the Fabry-Perot cavity and a portion of the waveguides.

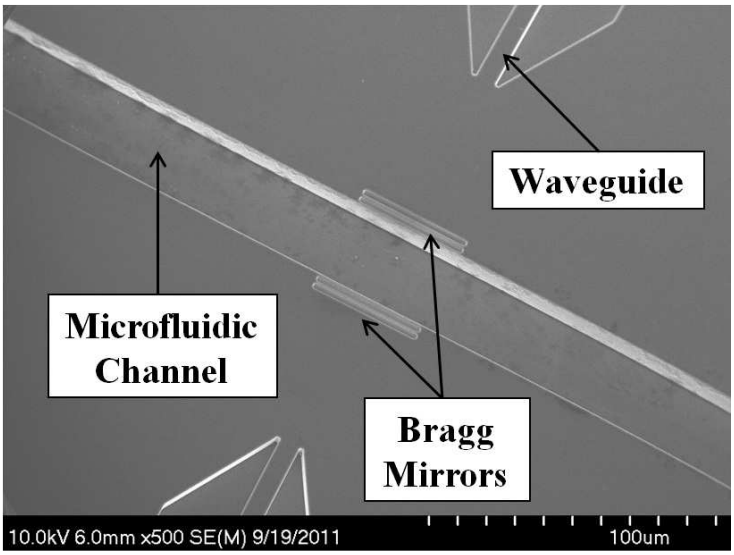

Figure 2. SEM image of the interferometer

Prior to particle injection and detection, a broadband light source (Newport, BBS-430) and an Optical Spectrum Analyzer (Agilent, 86142A) were used for initial optical characterization of the interferometers. Certified refractive index oils (Cargille Laboratories, Series AA) were used to test the sensitivity of the device. A linear relation was established between the displacement of the resonance peak's maximum and the refractive index of the certified RI fluids, yielding a 555 nm/RIU sensitivity. Once the resonance peaks were located (Fig. 3 (a)), a tunable laser source (Agilent, 81600B) was fixed at a wavelength close to the resonance peaks. A fast InGaAs detector (ThorLabs, DET01CFC) connected to an oscilloscope was used to measure the collected output light intensity. Glass beads of 3.5µm and 6.1µm diameter (Bangs Laboratory, SS05N/5903 and SS06N/10061) were mixed in deionized water in a final concentration of $3x10^6$ beads/ml. The expected refractive index difference between the two groups of glass beads, when in the center of the cavity, is around $\Delta n \sim 0.01$ (considering an effective optical mode diameter of 7.5 µm). Syringe pumps (New Era, NE-300 and NE-4000) were used to flow the centered sample solution and the side focusing solutions (see Fig. 1 (a)) at a rate of 1.0 µl/min and 2.89 µl/min respectively. The measured signals of hydrodynamically focused beads flowing through the cavity were statistically analyzed to differentiate the two sub-populations.

RESULTS AND DISCUSSION

Figure 3 (a) presents the transmission spectra of the interferometer with and without a glass bead inside the optical cavity. Figure 3 (b) presents a typical intensity signal obtained at a fixed wavelength, i.e. on the red arrow in Fig. 3 (a), while a bead is flowing through the sensor. The two sub-populations differentiation statistics, based on the FWHM of the recorded signals (see Fig. 3 (b)), are reported in Fig. 4 and in Table 1.

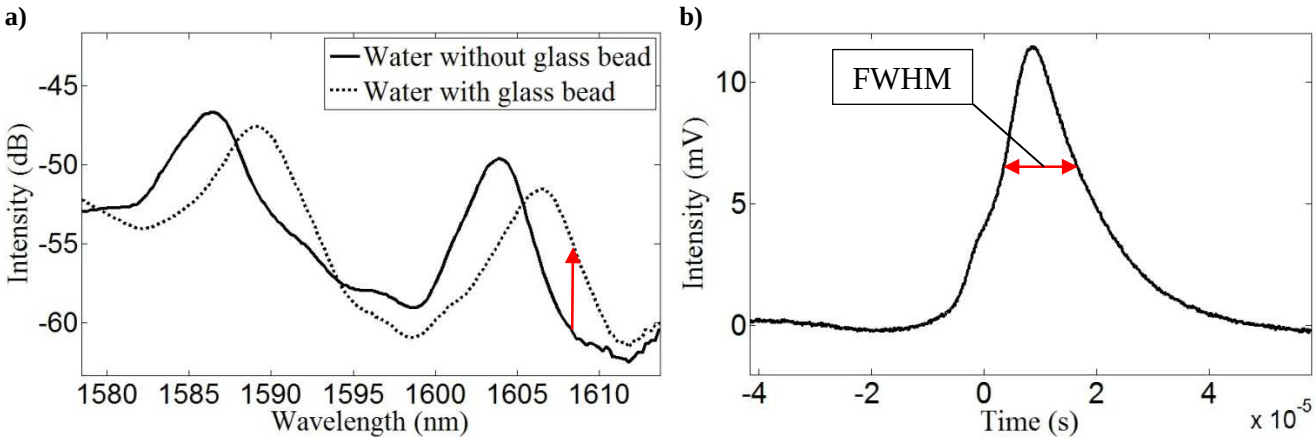

Figure 3. (a) Transmission spectra in water with and without a glass bead inside the cavity. The red arrow indicates the chosen fixed wavelength for high throughput measurements. (b) Typical intensity signal recorded at the chosen fixed wavelength during the passage of a glass bead in the Fabry-Perot cavity. The red double arrow represents the Full Width at Half Maximum (FWHM).

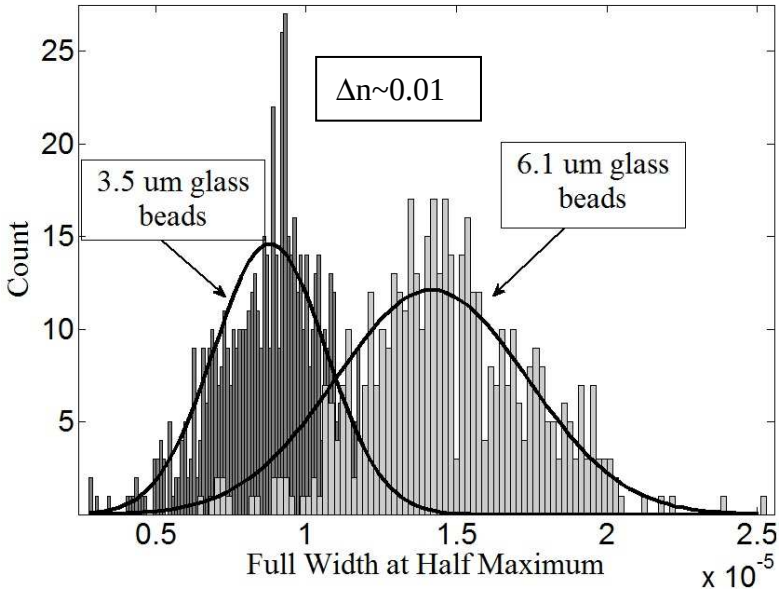

Figure 4. Histogram of glass beads populations differentiation based on an ANOVA analysis.

The ANOVA analysis is based on the null hypothesis that all samples are randomly picked from populations possessing the same mean. In our experiment, main statistics values provided by the ANOVA analysis (Table 1) are helpful in order to determine if the two beads populations have a statistically significant difference based on the measured FWHM of the fixed wavelength signals. The degree of freedom (number of populations minus one) reports the number of ways the variability can be tested against the null hypothesis. The F statistic is the ratio of the mean squares between the groups, over the mean squares within the groups. Finally, the p value is calculated using a cumulative F-distribution and corresponds to the percentage of shaded area under the curve for values higher than the F statistic. The reported p value, indicating the level of significance, is inferior to 0.01 which is a common limit to reject the null hypothesis. Accordingly to these values, it is acceptable to conclude that there is a statistically significant difference between the two beads populations, thus confirming the sub-populations identification capability of our optofluidic device.

Table 1. Main values of ANOVA analysis.

Degree of freedom	Nb. of samples	F statistic	P value (Sig.)
1	1087	1871.08	<0.01 (2.19×10^{-238})

CONCLUSION

We presented a novel optofluidic microsystem capable of measuring, at high throughput, refractive index variations caused by the presence of particles in a Fabry-Perot cavity. A mixed solution of two sub-populations of glass beads having different diameter was analyzed with the device and yielded a statistically significant difference based on the duration of the recorded single particle signals. Upcoming work includes the characterization of single living cells in a repeated manner, which could be integrated with flow cytometers as complementary measurement tool for life sciences researchers.

REFERENCES

[1] R. St-Gelais, J. Masson and Y.-A. Peter, *All-Silicon integrated Fabry-Pérot cavity for volume refractive index measurement in microfluidic systems*, Applied physics letters, Vol. 94, Issue 24, 243905 (2009).
[2] W. Z. Song, X. M. Zhang, A. Q. Liu and C. S. Lim, *Refractive index measurement of single living cells using on-chip Fabry-Pérot cavity*, Applied Physics Letters, Vol. 89, Issue 20, 203901 (2006).
[3] W. Wang, D. W. Kisker, D. H. Thamm, H. Shao and K. L. Lear, *Optofluidic Intracavity Spectroscopy of Canine Hemangiosarcoma*, IEEE Transactions on Biomedical Engineering, Vol. 58, Issue 4, pp. 853-860, (2011).
[4] G.-B. Lee, C.-C. Chang, S.-B. Huang and R.-J. Yang, *The hydrodynamic focusing effect inside rectangular microchannels*, J. Micromech. Microeng., Vol. 16, No. 5, pp. 1024-1032, (2006).
[5] R. St-Gelais, A. Poulin and Y.-A. Peter, *Advances in Modeling, Design, and Fabrication of Deep-Etched Multilayer Resonators*, Journal of Lightwave Technology, Vol. 30, No. 12, pp. 1900-1908, (2012).
[6] A. Cozma and B. Puers, *Characterization of the electrostatic bonding of silicon and Pyrex glass*, J. Micromech. Microeng., Vol. 5, No. 2, pp. 98-102, (1995).

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