DNA separation and fluorescence monitoring by integrated waveguides in an optofluidic chip

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We report on the monolithic integration of optical waveguides and microfluidics in a fused-silica lab-on-a-chip. Labeled biomolecules such as double-stranded DNA are flown and separated in the microfluidic channel by capillary electrophoresis and their fluorescent labels are excited by a continuous-wave laser beam through femtosecond-laser-written integrated waveguides. In this context, desirable features such as high spatial resolution (~12 μm) and a low limit of detection (~ 6 nano-molar) have been experimentally demonstrated. The proof of concept is being extended to real-world diagnostic samples for on-chip diagnosis of genetic diseases, e.g. breast cancer.

Introduction

Lab-on-a-chip (LOC) systems aim at miniaturizing and integrating functionalities of a biological/chemical laboratory into a microchip [1]. The use of integrated optical sensing for monitoring in LOC devices has seen a continuously growing demand [2]. Laser-induced fluorescence (LIF) is one of the most sensitive and widely used among different optical sensing techniques, especially in biological applications, owing to the wide availability of different fluorescent labeling schemes, which can selectively impart fluorescent properties to certain species of biomolecules. One important application, the separation of double-stranded DNA (dsDNA) molecules, is implemented in a number of diagnostic bioassays, e.g. for the detection of chromosomal aberrations. The most preferred technique for such diagnostic separation of dsDNA fragments is capillary electrophoresis (CE) [3], governed by differences in the electrophoretic mobility of the concerned species according to the fragment size and electric charge. CE separation and analysis performed in an on-chip-integrated microfluidic (MF) channel typically rely on bulky, bench-top optical excitation and detection instrumentation. This is in contrast with many advantages of an LOC system by strongly limiting device portability and is hindering the development of field applications. Compared with these experimental setups, direct integration of optical waveguides (WG) into a commercial LOC device, as presented in this paper, offers several advantages by reducing system size, complexity, and cost. Several approaches have been implemented for such optofluidic integration, based on WG fabrication by silica on silicon, ion exchange in soda-lime glasses, photolithography in polymers, and liquid-core WGs [4]. In this work we shall focus on the unique monolithic approach based on femtosecond-laser material processing [5].

Optofluidic Integration

Fs-laser WG writing relies on nonlinear, multi-photon absorption from a tightly focused ultrashort pulse in an otherwise transparent material to selectively deposit its energy in the focal volume, thus inducing a permanent material modification and a refractive index increase [6]. It is a cost-effective, direct fabrication technique that avoids the use of photolithography. This technique enables the precise definition of the location and the dimensions of highly confined excitation/detection windows along a MF channel. Furthermore, by use of this technique, optical WGs can be integrated in a straight-forward, fast way by post-processing in commercial LOC devices [7], which complements the mature cleanroom-based technologies for the mass production of LOCs. This is an important value addition, making fs-laser WG writing a true enabling technology for implementing optical sensing functionalities in LOC devices. In this work, the optical WGs were written at a speed of 20 μm/s into a fused-silica LOC device, see Fig. 1(a), by translating it perpendicular to a focused Ti:sapphire laser beam consisting of 150-fs, 4-μJ pulses emitted at a repetition rate of 1 kHz and a wavelength of 800 nm. The typical length of the WGs being of the order of a few millimeters, the processing time per chip is of the order of a few minutes. Higher repetition rates may allow higher translation speeds, thus further reducing the processing time. Thanks to the use of astigmatic beam shaping, the WGs have a tunable circular cross section with a typical width of ~10 μm, a graded refractive index profile, and a maximum refractive index increase of 1×10^{-3}, and they are single mode for wavelengths ranging from 400 to 650 nm. The near-field mode profile matches well with that of a single-mode optical fiber, ensuring efficient fiber-to-chip coupling after proper alignment. Propagation losses were measured to be in the range of 0.5–0.9 dB/cm at a wavelength of 543 nm.
The WG crosses the MF channel in plane, as shown in Fig. 1. Launching laser light of an appropriate wavelength into the WG leads to the excitation of fluorescent molecules as they pass through the MF channel at the WG–MF-channel intersection. Detection of the fluorescent light emitted in a direction perpendicular to the WG–MF-channel plane is performed by means of a CCD camera for visualization or a photomultiplier tube (PMT) for quantitative analysis. Both are incorporated in an inverted microscope with a combination of filters for selectively detecting the fluorescence signal while suppressing the scattered excitation light. As an example Fig. 1(b) shows a fluorescent segment at the intersection of the MF channel filled with Fluorescein, and a WG carrying light with a wavelength of 488 nm. Such optofluidic integration allows us to monitor passing plugs of, e.g., fluorescently labeled dsDNA molecules during on-chip CE [8].

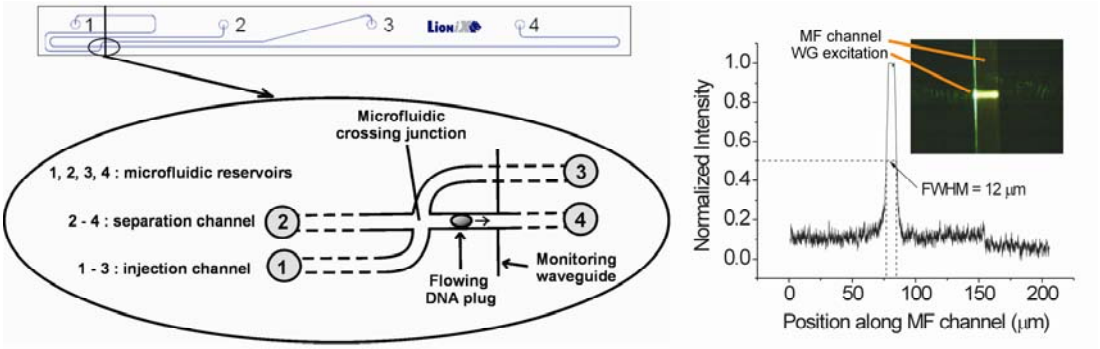

Fig. 1. (a) Layout of the integrated optofluidic chip; (b) integrated WG for localized fluorescence excitation of a MF channel filled with Fluorescein

On-chip DNA Separation: Experimental Protocol

The dsDNA molecules used in our experiments were synthesized as an amplicon having a length of ~150 base pairs, by polymerase chain reaction (PCR) targeting a specific, diagnostically relevant region of a template dsDNA being tested for breast cancer, obtained from the ERBB2 gene on the 17th human chromosome, and subsequently labeled with the intercalating fluorescent dye SYBR Green I. The absorption and emission maximum occur at the wavelengths of 494 and 535 nm, respectively, with the emission spectrum extending to ~640 nm. The label exhibits its fluorescence property only when intercalated along a dsDNA fragment. The dsDNA fragments were introduced into reservoir 1 of the CE chip (Fig. 1) The MF channels were filled with a buffer (20 mM MES/20 mM His, pH 6.2). Application of optimized high voltages of ~ 1–2 kV at the MF reservoirs with integrated platinum electrodes causes the dsDNA molecules to flow into the CE injection channel from reservoir 1 to reservoir 3. By switching the voltages at all four reservoirs simultaneously to well-chosen, optimized values, a well-confined plug of dsDNA molecules—with a volume of ~30 picoliters at the crossing junction of the two MF channels—is injected into the CE separation channel, from the MF crossing junction toward reservoir 4. The entire on-chip flow was controlled with a Labview script steering an MF control system (Capella, Capilix BV).

Fluorescence Monitoring: Results and Discussion

The 488-nm line from an argon laser was coupled into an on-chip integrated WG ~ 1.5 mm away from the MF crossing junction toward reservoir 4. Distinct fluorescent segments gradually appear and fade off as the dsDNA plugs pass across the excitation WG. Figure 2(a) shows snapshots corresponding to the significant stages during CE separation: I) before arrival of the plugs, II) and III) appearance and passage of the dsDNA amplicon plug, IV) transient period where the first plug has passed and a smaller, less bright second plug starts to appear, and V) and VI) passage of a second plug, which could be attributed to a relatively small quantity of the original dsDNA template remaining in the PCR product.. The distribution of fluorescence intensity along the MF channel in snapshot III possesses a FWHM of 12 μm, similarly as in Fig. 1(b), which agrees well with the WG cross-section dimension, and a signal-to-noise ratio of ~ 20 dB, thereby once again emphasizing the high quality of optofluidic integration at the WG–MF-channel interface.

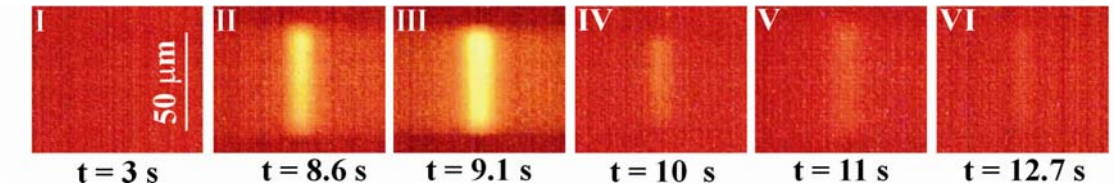

Fig. 2. Fluorescence snapshots of the microchip capillary electrophoresis separation of dsDNA molecules in a MF channel, across an excitation WG, in an optofluidic chip

Conclusions and Outlook

In conclusion, the CE-induced passage of dsDNA molecules obtained along a MF channel has been analyzed with on-chip-integrated, fs-laser written WGs. Integrated WG excitation enables exact definition of the dimensions of the detection window down to 12 μm and its inherent alignment with respect to the MF channel. This presents an

important advantage compared to conventional approaches for lowering the dimensions of the LIF detection window, e.g., the use of a pinhole (typical dimensions ~ 400 μm) in the optical path toward the detector, since it achieves an inherently constant mutual alignment of the excitation and detection windows. This renders the system more compact, faster to operate, and highly reproducible, making it very attractive for field applications. Besides, it promises to increase the spatial and temporal resolution with which the consecutive peaks in an electropherogram can be distinguished from each other. A conservative estimate of the current limit of detection (LOD) leads to a value of approximately 6 nano-molar. Better controlled dsDNA plug formation by coating the inner walls of the MF channel to suppress diffusion-induced plug broadening, and by using an optimized sieving gel matrix is currently under investigation. It is foreseen that a much larger number of dsDNA molecules can be separated and monitored with high sensitivity and spatial resolution. The integration of optical sensing in microchip CE may well pave the way for a new generation of compact and portable biophotonic devices for dsDNA analysis, to be used in clinical and point-of-care settings, for the diagnosis of a variety of genetic as well as infectious diseases.

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References

[1] A. Manz, N. Graber, and H.M. Widmer, “Miniaturized total chemical analysis systems: A novel concept for chemical sensing,” Sensors and Actuators B: Chemical, vol. 1, 244, 1990.

[2] E. Verpoorte, “Chip vision – optics for microchips,” Lab on a Chip, vol. 3, 42, 2003.

[3] J.P. Landers, “Molecular diagnostics on electrophoretic microchips,” Analytical Chemistry, vol. 75, 2919, 2003.

[4] B. Kuswandi, Nuriman, J. Huskens, and W. Verboom, “Optical sensing systems for microfluidic devices: a review,” Analytica Chimica Acta, vol. 601, 141, 2007.

[5] K.M. Davis, K. Miura, N. Sugimoto, and K. Hirao, “Writing waveguides in glass with a femtosecond laser,” Optics Letters, vol. 21, 1729, 1996.

[6] R. Gattass and E. Mazur, “Femtosecond laser micromachining in transparent materials,” Nature Photonics, vol. 2, 219, 2008.

[7] R. Martinez Vazquez, R. Osellame, D. Nolli, C. Dongre, H.H van den Vlekkert, R. Ramponi, M. Pollnau, and G. Cerullo, “Integration of femtosecond laser written optical waveguides in a lab-on-chip,” Lab on a Chip, vol. 9, 91, 2009.

[8] C. Dongre, R. Dekker, H.J.W.M. Hoekstra, M. Pollnau, R. Martinez Vazquez, R. Osellame, R. Ramponi, G. Cerullo, R. van Weeghel, G.A.J. Besselink, and H.H. van den Vlekkert, “Fluorescence monitoring of microchip capillary electrophoresis separation with monolithically integrated waveguides,” Optics Letters, vol. 33, 2503, 2008.