Fluorescence monitoring of microchip capillary electrophoresis separation with monolithically integrated waveguides

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Received July 18, 2008; accepted August 22, 2008; posted September 23, 2008 [Doc. ID 98733]; published October 24, 2008

Using femtosecond laser writing, optical waveguides were monolithically integrated into a commercial microfluidic lab-on-a-chip device, with the waveguides intersecting a microfluidic channel. Continuous-wave laser excitation through these optical waveguides confines the excitation window to a width of 12 µm, enabling high-resolution monitoring of the passage of different types of fluorescent analytes when migrating and being separated in the microfluidic channel by microchip capillary electrophoresis. Furthermore, we demonstrate on-chip-integrated waveguide excitation and detection of a biologically relevant species, fluorescently labeled DNA molecules, during microchip capillary electrophoresis. Well-controlled plug formation as required for on-chip integrated capillary electrophoresis separation of DNA molecules, and the combination of waveguide excitation and a low limit of detection, will enable monitoring of extremely small quantities with high spatial resolution. © 2008 Optical Society of America


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 DNA molecules, is implemented in a number of diagnostic bioassays, e.g., for the detection of chromosomal aberrations [3]. The most powerful technique for separation of DNA fragments is capillary electrophoresis (CE) [4], governed by differences in 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 contradicts many advantages of an LOC system by strongly limiting device portability and hindering the development of field applications. Compared with these experimental setups, direct integration of optical waveguides (WG) into a commercial LOC device offers several advantages by reducing system size, complexity, and cost. Several approaches have been reported in the literature describing WG fabrication by silica on silicon, ion exchange in soda-lime glasses, photolithography in polymers, and liquid-core WGs [5]. Femtosecond (fs) laser WG writing relies on nonlinear absorption of a tightly focused ultrashort pulse in a 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 an MF channel. In addition, it is a three-dimensional technique [7], allowing the inscription of WGs at arbitrary positions with respect to an MF channel, thereby enabling complex photonic structures, such as splitters and interferometers in sensing schemes. Furthermore, by use of this technique, optical WGs can be integrated directly by postprocessing in commercial LOC devices, which complements the mature technologies for the mass production of LOCs. This is an important value addition, making fs-laser WG writing a true enabling technology for implementing functionalities in LOC devices.

In this Letter, we demonstrate on-chip-integrated WG excitation and detection of different types of dye molecules as well as a biologically relevant species, fluorescently labeled DNA molecules, during their migration and separation in an MF channel by CE.

The optical WGs were written at a speed of 20 µm/s into a fused-silica LOC device (Fig. 1) 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 [8]. 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 [9], the WGs have a circular cross section with a diameter of \(\sim 10 \mu m\), a graded refractive index profile, and a maximum effective index increase of \(1 \times 10^{-3}\), and they are single mode for wavelengths ranging from 400 to 650 nm. The near-field mode profile matches well with that of a well-aligned single-mode optical fiber, ensuring efficient fiber-to-chip coupling. 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. 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 incorporated in an inverted microscope (Olympus IX-71) through a filter set (Olympus U-MWIB3). Integrated optical detection allows us to monitor passing plugs of, e.g., dye or fluorescently labeled DNA molecules during on-chip CE.

The DNA used in our experiments was synthesized as amplicon having a length of \(\sim 150\) base pairs, by polymerase chain reaction (PCR) targeting a specific, diagnostically relevant region of a template DNA under analysis, and subsequently labeled with the intercalating fluorescent dye SYBR Green I. The absorption and emission maximum is at the wavelength of 494 and 535 nm, respectively, with the emission spectrum extending to \(\sim 640\) nm. The label exhibits its fluorescence property only when intercalated along a DNA fragment.

The DNA 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 (1–2 kV) at the MF reservoirs with integrated platinum electrodes causes the DNA 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 DNA molecules—with a volume of \(\sim 30 \mu l\) 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, from Capilix BV). The 488 nm line from an argon laser was coupled into an on-chip integrated WG \(\sim 1.5\) mm away from the MF crossing junction toward reservoir 4. Distinct fluorescent segments gradually appear and fade off as the DNA plugs pass across the excitation WG, as shown in Fig. 2(a) (Media 1). Snapshots I (before arrival of the plugs), II and III (appearance and passage of the DNA 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), respectively correspond to the significant stages during CE separation. The distribution of fluorescence intensity along the MF channel in snapshot III possesses a FWHM of 12 \(\mu m\), which corresponds well with the WG cross-section dimensions, and a signal-to-noise ratio of \(\sim 20\) dB, thereby emphasizing the high quality of micro-opto-fluidic integration at the WG–MF-channel interface. The appearance of the second plug is attributed to a relatively small quantity of the original DNA template remaining in the PCR product.

In a second experiment, a sample containing two different fluorescent dyes, Rhodamine-6G and Rhodamine-B (absorption maximum at 530 and 540 nm, respectively), was used to demonstrate CE separation. The experimental procedure remained similar to the one described above. A plug containing a mixture of the two fluorescent dyes was injected into the separation channel. The 543 nm line from a green He–Ne laser was coupled into the on-chip integrated WG. Again, distinct fluorescent segments gradually appear and fade away as the two fluorescent dyes migrate across the excitation WG, as shown in Fig. 2(b) (Media 2). Snapshots I (before arrival of the plugs), II and III (appearance and passage of Rhodamine-6G), IV (transient period where the Rhodamine-6G plug has passed and the Rhodamine-B plug is yet to appear), and V and VI (passage of the Rhodamine-B plug), respectively correspond to the significant stages during CE separation.

In both cases, two distinct fluorescent plugs were thus observed owing to the ensuing separation dur-
ing the passage. The passage of the DNA plugs (Media 1) as well as that of the fluorescent dyes (Media 2) was captured in the form of a movie, where each frame consisted of $640 \times 512$ color (RGB) pixels. A region of interest was selected to span over $80 \times 40$ pixels, with its center at the bright fluorescent segment (Fig. 2) corresponding to the WG–MF-channel intersection, and the intensities of all pixels in this region were integrated at time intervals of 300 ms to obtain the corresponding fluorescence intensity data points, plotted as an electropherogram (Fig. 3). The two distinct peaks in Fig. 3(a) correspond to the CE-separated DNA (amplicon and residual template) plugs, while those in Fig. 3(b) correspond to the CE-separated Rhodamine-6G and Rhodamine-B plugs. The roman numerals (I–VI) in Figs. 3(a) and 3(b) denote the data points resulting from the analysis of the corresponding snapshots (I–VI) in Figs. 2(a) and 2(b), respectively. The plate heights for these electropherograms are estimated to be only up to 550, owing to the short distance of $\sim 1.5$ mm between the MF crossing junction and the position of the excitation/detection window.

In conclusion, the CE-induced passage of DNA molecules along an MF channel has been analyzed with on-chip-integrated, fs-laser written WGs. As an additional experiment, the passage of the fluorescent dyes Rhodamine-6G and Rhodamine-B during on-chip CE separation was monitored. Integrated WG excitation enables exact definition of the dimensions of the detection window down to 12 $\mu$m, 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 $\geq 400 \mu$m) in the optical path toward the detector [10], 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 3 $\mu$M. Further improvements in the LOD are necessary for real applications, which will be achieved by use of an electron-multiplying CCD camera or a photomultiplier tube to detect the fluorescence signal. Better-controlled DNA plug formation by coating the inner walls of the MF channel to suppress diffusion-induced plug broadening will allow for on-chip integrated CE separation of DNA molecules and the detection of extremely small quantities with high 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 DNA analysis, to be used in clinical and point-of-care settings, for the diagnosis of a variety of exogenous and endogenous diseases.

This work was supported by the European Commission, FP6 Project Contract IST-2005-034562 [Hybrid Integrated Biophotonic Sensors Created by Ultrafast Laser Systems (HIBISCUS)]. The authors thank Marcel Hoekman (Integrated Optical MicroSystems/LioniX BV) for his help in developing the image analysis software.

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