Fluorescence monitoring of capillary electrophoresis separation in a lab-on-a-chip with monolithically integrated waveguides

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***Abstract**: Femtosecond-laser-written optical waveguides were monolithically integrated into a commercial lab-on-a-chip to intersect a microfluidic channel. Laser excitation through these waveguides confines the excitation window to a width of 12 μm, enabling high-spatial-resolution monitoring of different fluorescent analytes, during their migration/separation in the microfluidic channel by capillary electrophoresis. Wavelength-selective monitoring of the on-chip separation of fluorescent dyes is implemented as a proof-of-principle. We envision well-controlled microfluidic plug formation, waveguide excitation, and a low limit of detection to enable monitoring of extremely small quantities with high spatial resolution.*

Introduction

Lab-on-a-chip (LOC) systems aim at miniaturizing and integrating functionalities of a biological/chemical laboratory into a microchip [1]. There is a continuously growing demand for the application of integrated optical sensing for monitoring in LOC devices [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. An important application which makes use of this sensing scheme is the separation of fluorescently labeled Deoxyribonucleic acid (DNA) molecules, which is implemented in a number of diagnostic bioassays, e.g. for the detection of chromosomal aberrations [3] in order to enable an early diagnosis of genetic disorders such as breast cancer. The most powerful technique for separation of DNA fragments is capillary electrophoresis (CE) [4], which is governed by differences in mobility of the to-be-separated species owing to the differences in their 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. The dependence on such instrumentation strongly limits device

portability and hinders the development of field applications. Compared to these experimental set-ups, 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 into lab-on-a-chip devices as investigated within this work is a novel, promising approach. It relies on non-linear absorption of a tightly focused ultra-short 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, which 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. In addition, it is a three-dimensional technique [7], allowing for the inscription of WGs at arbitrary positions with respect to a 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 post-processing 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 optical functionalities in LOC devices.

In this paper, we demonstrate on-chip-integrated WG excitation and detection of different types of fluorescent dye molecules during their microchip CE separation as a proof of principle [8].

Waveguide integration in a lab-on-a-chip

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 [9]. 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 [10], the WGs have a circular cross-section with a diameter of ~10 μm, a graded refractive index profile, 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 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 filterset (Olympus U-MWIB3). Integrated optical detection allows us to monitor passing plugs of, e.g., fluorescent dye molecules during on-chip CE separation.

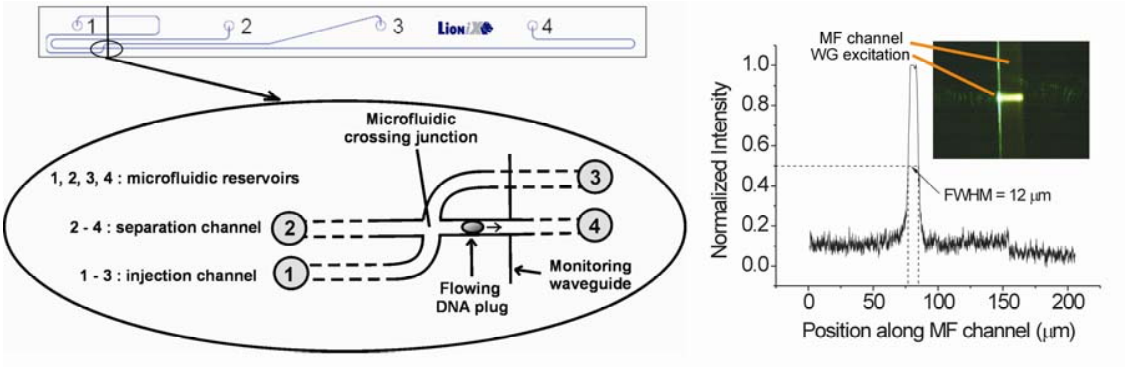

Fig.1. Layout of the MF chip with an integrated optical WG, schematic of the MF chip, with the fluorescent plug passage monitored by the integrated WG, and the resulting distribution of fluorescence intensity along the MF channel

Experimental protocol and results

The fluorescent dye molecules Rhodamine-B and Rhodamine-6G (absorption maximum at 530 nm and 540 nm, respectively) 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 molecules to flow into the CE injection channel from reservoir 1 to reservoir 3. Next, by switching the voltages at all four reservoirs simultaneously to suitable values, a well-confined (volume ~30 pl) plug of the fluorescent mixture is injected into the CE separation channel, from the MF crossing junction towards reservoir 4. The on-chip flow was controlled with a LabVIEW script and a MF control system (Capella, from CapiliX BV). The 543-nm line from a green He-Ne laser was coupled into the on-chip integrated WG. Distinct fluorescent segments gradually appear and fade off as the two fluorescent dyes migrate across the excitation WG, as shown in Fig. 2. Snapshots I (before arrival of the plugs), II-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-VI (passage of the Rhodamine-B plug), respectively, correspond to the significant stages of CE separation. Two distinct fluorescent plugs were thus observed owing to the ensuing separation during the passage.

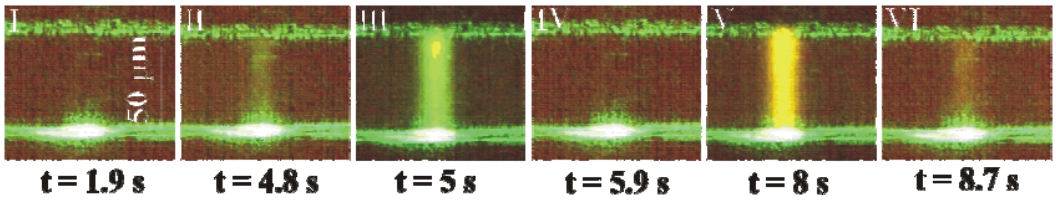

Fig.2. Passage of fluorescent dyes Rhodamine-6G and Rhodamine-B during CE separation in a MF channel, across an excitation WG

Conclusions and outlook

The CE-induced passage of fluorescent dye (Rhodamine-B and Rhodamine-6G) molecules along a MF channel has been analyzed with on-chip-integrated, fs-laser written WGs. The dimensions of the detection window have been considerably lowered by means of integrated WG excitation. The inherent mutual alignment of the excitation and detection windows renders the system more compact and faster to operate, making it more attractive for point-of-care applications. The current detection scheme makes use of a standard CCD camera in order to grant access to the spatial information and to enable better visualization. A real-world application involving CE separation of DNA molecules requires measurement of extremely low sample concentrations and demands lowering the limit of detection (currently 3 μM), e.g. by means of a photomultiplier tube as the detector. The integration of optical sensing in microchip CE may well pave the way for a new generation of compact and portable biophotonic devices to be used in point-of-care settings.

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