Multi-point, Multi-wavelength Fluorescence Monitoring of DNA Separation in a Lab-on-a-chip with Monolithically Integrated Femtosecond-laser-written Waveguides

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Abstract: Electrophoretic separation of fluorescently labeled DNA molecules in on-chip microfluidic channels was monitored by integrated waveguide arrays, with simultaneous spatial and wavelength resolution. This is an important step toward point-of-care diagnostics with multiplexed DNA assays. © 2009 Optical Society of America
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1. Introduction

In a commercial lab-on-a-chip (LOC) for DNA analysis, capillary electrophoresis (CE) separation of DNA molecules in a microfluidic (MF) channel is typically monitored by means of UV absorption or confocal microscopy laser-induced fluorescence (LIF) , involving bulky, bench-top optical instrumentation for excitation/detection. This approach limits device portability and poses significant challenges to field applications. Furthermore, the measurement is typically performed at a single point and under single-wavelength excitation, leading to a loss of valuable spatial and spectral information. Here we report an integrated approach for simultaneously achieving spatial and wavelength resolution, based on optical waveguides integrated monolithically in a commercial LOC by femtosecond (fs) laser waveguide (WG) writing. Fs-laser post-processing allows for fast (few minutes/chip) and direct on-chip integration of optical WGs, thereby exploiting the mature technology for LOC mass production, while reducing system size, complexity, and cost [1,2].

2. Monolithic optofluidic integration in a lab-on-a-chip

An fs-laser-written WG array was monolithically integrated into a commercial fused-silica CE microchip (D8-LIF, LioniX BV) [2], intersecting the MF CE separation channel (cross-section: 100 μm x 50 μm) in plane at four different locations, see Fig. 1, at distances of 2 cm (WG1), 2.1 cm (WG2), 3.5 cm (WG3), and 3.6 cm (WG4), from the MF crossing junction at which the separation commences. The WGs were optimized for efficient light propagation, fiber-chip coupling, and direct coplanar WG-MF-channel intersection. They exhibit a circular cross-section. Upon laser excitation of a fluorescently labeled analyte in the MF channel through such a WG, a sharp fluorescent segment approximately 30 μm in width (matching well with the WG cross-section diameter) can be observed along the WG-MF-channel intersection. This enables high spatial resolution monitoring of different fluorescent analytes migrating in the MF channel and separated by CE [3]. A similar conventional approach would require intricate filtering of the wide-band excitation source, such as an Hg or Xe lamp illuminating the entire MF channel network, and repeated alignment of a pinhole in the detection path [4]. On the contrary, constant alignment of the excitation WG with the detection window renders our system more compact, faster to operate, and highly reproducible.
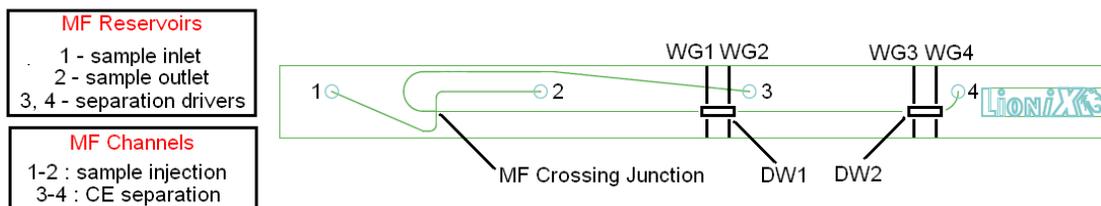

Fig. 1 Schematic of the MF chip with an integrated optical WG array (pitch not to scale)

3. Fluorescence monitoring of DNA separation

Single-stranded DNA (ssDNA) molecules with sizes of 15 and 20 nucleotides (nt) were labeled with fluorescent dyes Alexa Fluor 647 (AF647) and Cyanine 3 (Cy3), respectively. The inner walls of the entire MF channel network were pre-coated with an epoxy-poly-(dimethylacrylamide)-based polymer [5], and filled with a gel matrix consisting of hydroxypropyl-cellulose [6], dissolved (3% wt./vol.) in 20 mM MES / 20 mM His buffer (pH 6.2). Red He-Ne (λ = 633 nm) laser beams were coupled into WG1 and WG3, while green He-Ne (λ = 543 nm) laser beams were coupled into WG2 and WG4. A cooled photomultiplier tube (PMT) (H7421-40, Hamamatsu Photonics K.K.) was built onto the output port of an inverted microscope (DMI5000M, Leica Microsystems GmbH), and was optimally aligned to collect light from two detection windows, DW1 and DW2, which are located in the regions where WG1/WG2 and WG3/WG4, respectively, intersect the MF CE separation channel. An appropriate multi-band filter (XF57, Omega Optical, Inc.) ensured that only the desired fluorescence signals reached the PMT.

Initially, only a single species (15-nt-ssDNA-AF647) was CE-flown through the MF separation channel with both lasers turned off and in the absence of any other source of illumination. The corresponding baseline detector signal was measured. This experiment was repeated twice, firstly with only the red He-Ne laser turned on, in which case a strong signal peak was observed from DW1 and DW2 when the fluorescently labeled ssDNA plug crossed WG1 and WG3, respectively, and secondly with only the green He-Ne laser turned on, in which case the signal measured from both DW1 and DW2 matched with the baseline detector signal. This procedure was then repeated for the other species (20-nt-ssDNA-Cy3), leading to the opposite result. Thus, it was experimentally confirmed that cross-excitation between the two species was, for all practical purposes, absent.

The two species were then mixed and the resulting polychromatic ssDNA mixture was separated by CE into the individual monochromatic ssDNA components in the MF CE separation channel. The flow protocol was based on driving voltages of up to 1.2 kV, delivered by Pt electrodes integrated into the MF reservoirs, with the help of a LabVIEW script to steer the microfluidic control system (Capella, CapiliX BV). The volume of a typical ssDNA mixture plug upon injection into the MF CE separation channel was approximately 275 picoliters.

Figure 2 (a) depicts an electropherogram based on the fluorescence signal measured from DW1. The measurement commenced when a plug of the ssDNA mixture was injected into the MF CE separation channel. The separation between the two individual ssDNA plugs amounts to a delay of approximately 4 s between the fluorescence peaks of the two plugs. Since cross-excitation is absent, the two peaks correspond to the two separated species, each being excited by the corresponding laser wavelength of 633 nm (from WG1) or 543 nm (from WG2), respectively. Similarly, Fig. 2 (b) depicts an electropherogram based on the fluorescence signal measured from DW2, where the separation between the fluorescence peaks of the two plugs now amounts to a larger delay of approximately 11 s, because of the larger distance of DW2 from the starting point, namely the MF crossing junction.
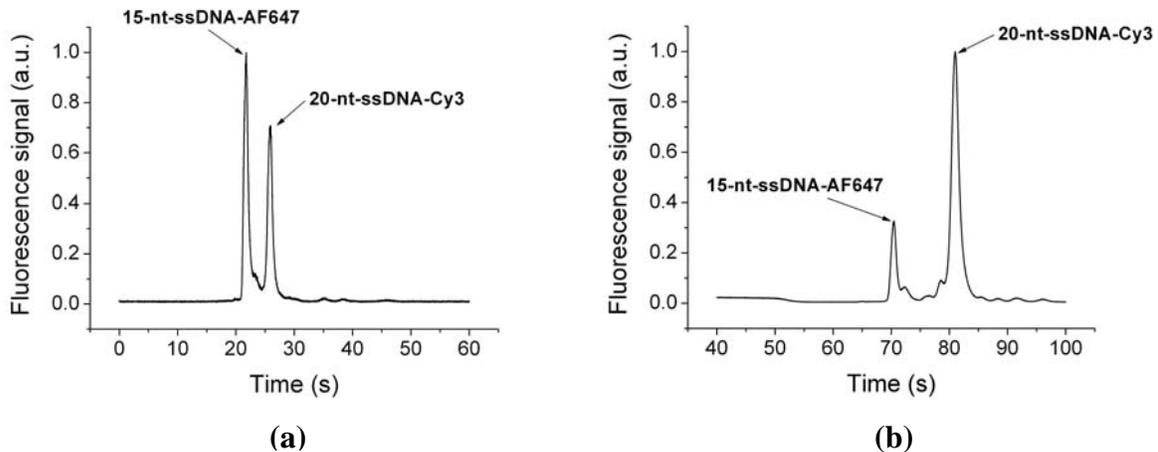

(a) (b)

Fig. 2 Electropherograms based on the fluorescence signal generated by multi-point, multi-wavelength excitation, as measured from (a) DW1 and (b) DW2

In additional experiments, an identical MF CE separation was performed twice. In the first case, only the red He-Ne laser was switched on. The corresponding fluorescence data measured independently from the two detection windows are plotted as the red colored electropherograms in Figs. 3 (a) and 3 (b), respectively. In the second case, only the green He-Ne laser was switched on, and the corresponding fluorescence data are plotted as the green colored electropherograms in Figs. 3 (a) and 3 (b), respectively.
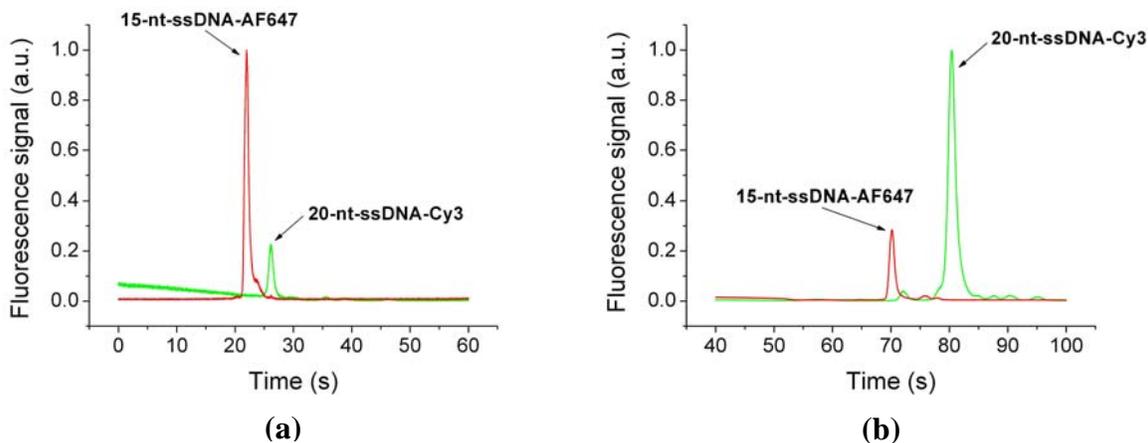

(a) **(b)**

Fig. 3 Electropherograms based on the fluorescence signal generated by multi-point, single-wavelength excitation by the red and green He-Ne lasers, respectively, and temporally superimposed on one another, as measured from (a) DW1 and (b) DW2

Comparison of the electropherograms in Figs. 2 (a) and 3 (a) as well as those in Figs. 2 (b) and 3 (b) yields identical temporal positions of the fluorescent peaks, hence proving that successful multi-point, multi-wavelength fluorescence monitoring of the MF CE separation of ssDNA molecules has been achieved.

4. Conclusions

CE separation of fluorescently labeled ssDNA molecules along a MF channel has been analyzed with on-chip-integrated, fs-laser-written WG arrays. Contrary to conventional LIF detection, the excitation/detection windows are now inherently mutually aligned, and have considerably smaller dimensions, thanks to the integrated WG approach. Multi-point, multi-wavelength excitation/detection has been demonstrated as a promising tool to empower on-chip DNA analysis with essentially tunable spatial and wavelength resolution. The on-chip integration of optical sensing with DNA analysis as presented here bears the potential of leading to a new generation of fast, compact, and portable biophotonic devices with increased functionality for use in point-of-care diagnostics.

5. Acknowledgment

6. References

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