Fluorescence sensing with femtosecond laser written waveguides in a capillary electrophoresis chip for monitoring molecular separation

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Abstract: *Fluorescence detection is one of the most sensitive among different optical sensing techniques. This work focuses on integrated optical excitation/detection of fluorescence originating from dye molecules during on-chip capillary electrophoresis. Excitation occurs via femtosecond laser written waveguides intersecting the microfluidic channels both integrated in a commercial capillary electrophoresis chip.*

Introduction

Recent advances in microchip fabrication technology and microfluidics have enabled the implementation of a large number of biochemical processes in miniaturized lab-on-chip systems. Many of these systems largely depend on conventional bench-top measurement instrumentation for monitoring. On-chip integration of biochemical sensors therefore continues to be a challenging field of research, empowering the existing lab-on-chip systems with ever more functionalities while also making them a more cost-effective option. In particular, integrated optical detection has emerged as an attractive tool to fulfill the requirements of such an on-chip *in-situ* probing strategy [1]. Current detection schemes mostly depend on the hybrid integration of an existing microfluidic system with external detection optics. The work presented in this paper focuses on integrating both functionalities in a single substrate viz. glass. Parallel to the progress in glass-based on-chip microfluidics, femtosecond (fs) laser writing has emerged as an interesting technique to inscribe optical WGs in bulk glass substrates [2]. The combination of these two developments to integrate a detection system of fs laser written WGs in a glass microfluidic chip is a novel approach, forming the key focus of this work [3]. In this paper we describe the experimental progress in integration of optics and microfluidics in a capillary electrophoresis (CE) chip, the concept of on-chip CE, the integrated detection scheme to monitor the same, and concluding with an experimental demonstration.

Waveguide fabrication and characterization

Fs laser irradiation of glass leads to localized melting in the focal volume of the beam followed by re-solidification upon removal of the beam. The corresponding localized densification leads to a refractive index variation. WGs are written by a continuous transverse movement of the substrate with respect to the writing beam. In principle a suitable movement of the substrate during writing could create any desired 3D waveguiding structure hence making this technique extremely attractive [2]. The WGs under investigation were fabricated by means of a Ti:sapphire laser at a repetition rate of 1 kHz, with typical pulse energies of 1 μJ, pulse durations up to 200 fs, and writing speeds of 100 μm/s, in a commercial fused silica microfluidic chip aimed at on-chip CE applications. The WGs were in turn thoroughly characterized [4]. The writing

process was optimized to obtain single-mode WGs with a circular cross-section (diameter: 10 μm) to ensure low fiber-chip coupling losses (~4 dB/end-facet). Propagation losses were found to be as low as 0.5 dB/cm. Cross-sectional refractive index profiles were evaluated and the maximum refractive index change was measured to be 1×10^{-3}.

Micro-opto-fluidic integration

A fused silica CE chip (commercially available from LioniX BV) was empowered with fs laser written WGs. Such a chip layout can be seen in figure 1.

Fig. 1 Left: CE chip with integrated WGs crossing the microfluidic channels
Right: Side view image of WG array – microfluidic channel intersection

The chip consists of 4 fluidic reservoirs (volume ~ 400 nl, each of them numbered and denoted by circles in Fig. 1) connected to each other by means of mf channels (cross section dimensions: 50 μm × 12 μm). The vertical lines along the breadth of the chip denote WGs crossing the mf channels in their plane. As a preliminary test of the quality of integration, the entire mf channel network in such a chip was suction-filled with Rhodamine-B which is a strongly fluorescent dye. Light from a green He-Ne laser (λ = 543.5 nm) was coupled into the WGs by means of an in-house-assembled fiber array unit glued to the chip end-facet. An on-off switchable Hg arc lamp served as background illumination for monitoring the entire chip. Detection was performed with an inverted microscope (Olympus IX-71). A combination (from Chroma Technology Inc.) of band-pass filters and a dichroic mirror was used to pass only the fluorescence signal while cutting off all other wavelengths including the scattered laser light and background illumination. A sharp fluorescent segment (breadth ~ 13 μm) was then observed at the WG - mf channel intersection, with and without a background illumination, as shown in figure 2. This result demonstrates that the desired quality of micro-opto-fluidic integration has been achieved.

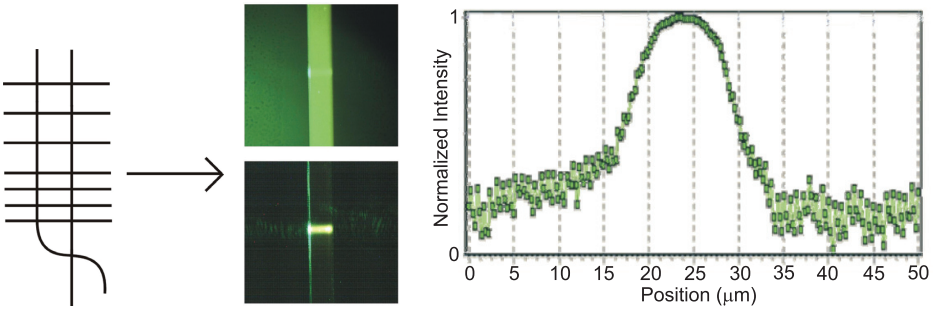

Fig. 2 WG excitation of Rhodamine-B at 543.5 nm and corresponding distribution of fluorescence intensity along the microfluidic channel

Fluorescence imaging of microchip capillary electrophoresis (MCE)

MCE corresponds to the separation of species with different charge density and mobility as they flow along an electric field applied at the ends of a microcapillary (in this case at the mf reservoirs at the ends of the mf channels). In order to perform this experiment the entire mf channel network was suction-filled with MES/His buffer (pH: 6.2). A mixture of species to be separated was added to reservoir 1, which in this proof of principle consisted of strongly fluorescent dyes Fluorescein and Rhodamine-B. In a practical application, the mixture would typically consist of biologically relevant species pre-labeled with fluorescent dye molecules. The mixture is transported to reservoir 3 via the injection channel by means of electro-osmotic flow on application of optimized, strong electric potentials (in the order of 10^3 V) at the reservoirs using integrated platinum electrodes. A continuous influx of the fluorescent mixture into reservoir 1 ensures that the injection channel (volume ~ 40 nl) connecting reservoir 1 and 3 is always filled with the mixture. Pinching potentials applied at reservoirs 2 and 4 prevent the mixture from leaking into the separation channel connecting reservoirs 2 and 4, resulting in the formation of a well-defined plug (volume: 30 pl) at the channel junction. Next, by suitably switching the potentials the plug is launched into the separation channel resulting in separation of the individual species. This series of events is captured and depicted in figure 3.

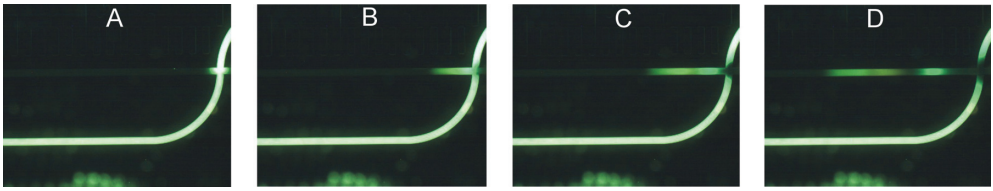

Fig 3. a) Formation, b) launching and c-d) MCE separation of a 30 pl plug consisting of Fluorescein and Rhodamine-B along a microfluidic channel

On-chip integrated dynamic fluorescence monitoring

The approaches described in the previous two sections have further been combined to demonstrate the monitoring of MCE using integrated WGs. As described earlier, a plug consisting of Fluorescein and Rhodamine-B was ejected into the separation channel. Light from a green He-Ne laser (λ = 543.5 nm) was coupled into a WG crossing the separation channel 3 mm away from the mf channel junction. Movement of the separated plugs was followed with Hg lamp background illumination. It was observed that only the Rhodamine-B plug showed a sharp increase in fluorescence intensity as it flowed across the excitation WG owing to the additional excitation signal. Wavelength selectivity is therefore demonstrated by the observation that the Fluorescein plug showed no response to WG excitation, owing to the absence (in the present experiment) of a light source corresponding to the absorption spectrum of Fluorescein, which extends up to λ = 525 nm. The observed series of events is depicted in figures 4 and 5. Rhodamine-B plug leads the flow while the Fluorescein plug follows. With the first and the last images in fig. 5 corresponding to the lowest Fluorescein concentration in the channel, it is still possible to detect along the WG a negligible localized fluorescence originating from residual Rhodamine-B particles sticking to the channel walls from inside, .

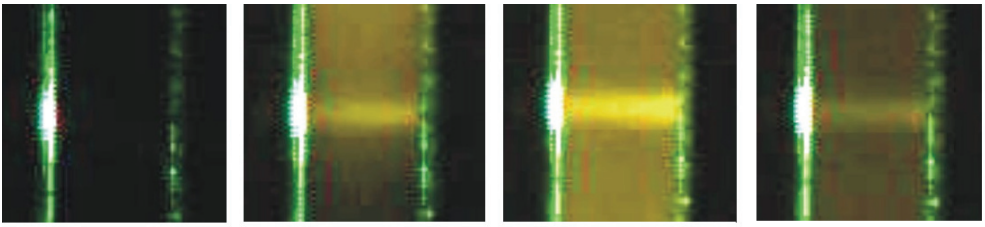

Fig. 4 Flow of Rhodamine-B (leading) plug across the excitation WG after CE separation

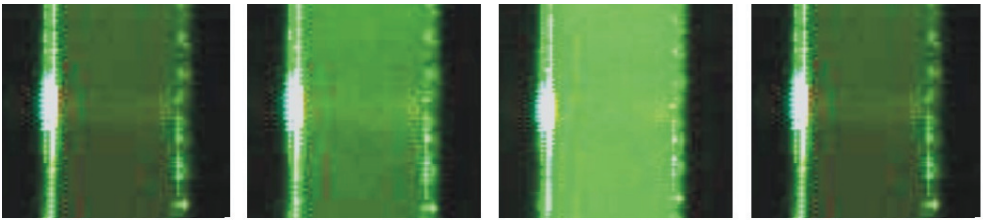

Fig. 5 Flow of Fluorescein (lagging) plug across the excitation WG after CE separation

Concluding Remarks

The paper presents recent progress in the field of monitoring on-chip capillary electrophoresis, using fluorescent molecules excited via integrated femtosecond laser written optical waveguides. It has been demonstrated that Fluorescein and Rhodamine-B molecules, separated by capillary electrophoresis, can be detected with the described set-up in a wavelength selective manner. Experiments are in progress to demonstrate integrated optical sensing for monitoring CE separation of fluorescence-labelled DNA fragments which would be an important diagnostic application of the presented work.

Acknowledgements

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