Multi-wavelength fluorescence sensing with integrated waveguides in an optofluidic chip

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***Abstract*—Femtosecond-laser-written integrated waveguides enable multi-wavelength fluorescence sensing of flowing biomolecules in an optofluidic chip. Fluorescence from differently labeled biomolecules with distinct absorption wavelengths, encoded by uniquely modulating each excitation beam, is detected by a color-blind photodetector, and the origin of each signal is unraveled by Fourier analysis.**

Keywords-optofluidics; biophotonics; fluorescence sensing

I. INTRODUCTION

Optical sensing, specifically fluorescence sensing, is the preferred detection technique in a number of biochemical analyses due to the high sensitivity that is achievable. Most systems consist of a single excitation wavelength in order to induce fluorescence in the labeled analytes. While a few approaches report multi-wavelength fluorescence sensing [1] for bioanalysis, these tend to apply complicated bulk optical schemes to separate signals at different fluorescence wavelengths, which is critical to any multi-wavelength approach.

We present an elegant approach to achieve multi-wavelength sensing in an optofluidic chip with integrated optical waveguides, by means of *a priori* encoding of the fluorescence originating in different biomolecules by intensity-modulating the excitation light sources, followed by Fourier decoding of the detected biophotonic signals, thereby tracing back the origins of the biomolecules. We implement capillary electrophoresis (CE) as a powerful method to separate differently sized biomolecules in a tiny microfluidic (MF) lab-on-a-chip (LOC) device, where high-speed operation and low reagent volumes pave the way to solving real-life challenges in medicine. We post-processed integrated optical waveguides (WGs) into such commercially mass-produced MF LOCs on a fast (few minutes / chip), flexible, chip-by-chip basis by femtosecond-laser micromachining [2]. This approach allows the exploitation of the existing mature MF fabrication infrastructure, while achieving integrated-WG laser-induced fluorescence (LIF) monitoring during on-chip migration and separation of biomolecules [3].

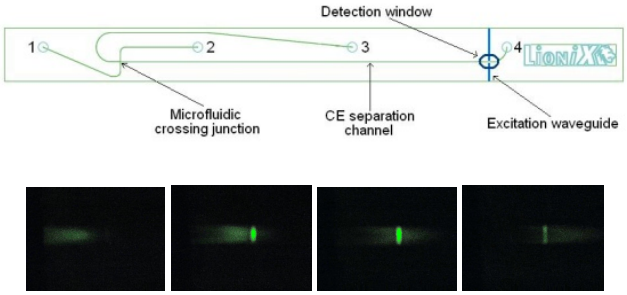

Fig. 1. (a) Schematic of the optofluidic chip showing MF reservoirs, injection (reservoir 1 → reservoir 2) and CE separation (reservoir 3 → reservoir 4) channels, integrated optical WG and detection window; (b) movie recorded with a CCD camera showing transient fluorescence from several molecule plugs formed by CE separation of the DNA ladder as these plugs pass by the point of integrated-WG laser excitation at 488 nm.

II. OPTOFLUIDIC INTEGRATION

The integrated optical WG intersecting the MF channel perpendicularly in plane was written at a speed of 20 µm/s into a fused-silica LOC device (Fig. 1a) 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 WGs were tuned by astigmatic beam shaping to have an elliptical cross section with a width of ~12 µm and a height of 50 µm (identical to the MF channel height, to maximize the excitation volume). They have a graded refractive index profile, and a maximum refractive index increase of 1×10^{-3}. Propagation losses were measured to be in the range of 0.5–0.9 dB/cm at a wavelength of 543 nm. Initial tests in this setup indicated that fluorescently labeled DNA molecules could be flown and detected (Fig. 1b) as bright fluorescent segments appearing at the WG-MF-channel interface, with a sizing accuracy of 99% (comparable to the state of the art in DNA sequencing). The current spatial resolution of this setup is limited by the MF plug width (>248 µm), while the integrated-WG width (12 µm) would allow for a 20-fold better resolution, demonstrating that integrated optics can handle the resolution demands of future MF-chip generations.

III. MULTI-WAVELENGTH FLUORESCENCE SENSING

Two sets of fluorescence-labeled biomolecules, each set consisting of two differently sized molecules, m_1 and m_2, which migrate with different speeds served as analytes. One set was red-labeled, the other blue-labeled. Two laser beams at the absorption wavelengths of the labels, intensity-modulated at frequencies f_1 and f_2, respectively, were launched into the excitation/detection area via a single integrated optical WG. For fluorescence detection, a cooled PMT was aligned to collect the fluorescence signal from the excitation/detection area. The modulation of each laser induced an accordingly time-varying fluorescence signal emitted by those dye molecules absorbing this specific laser wavelength, and the resulting time-modulated fluorescence signal at both wavelengths was simultaneously recorded by the ultrasensitive, albeit color-blind PMT, as shown in the upper part of Fig. 2. Fourier analysis was implemented for post-processing the recorded total fluorescence signal, resulting in the frequency spectrum of Fig. 3, which consists of signals at the different modulation frequencies. Only a narrow band of frequencies around each modulation frequency (f_1 = 17 Hz, f_2 = 31 Hz) was selectively passed by a flat-top transfer function and extracted back into the time domain by an inverse Fourier transform, leading to the blue and red colored graphs in the lower part of Fig. 2. This scheme enables an unambiguous extraction of the signals corresponding to specific excitation wavelengths and the regeneration of corresponding individual time-domain signals.

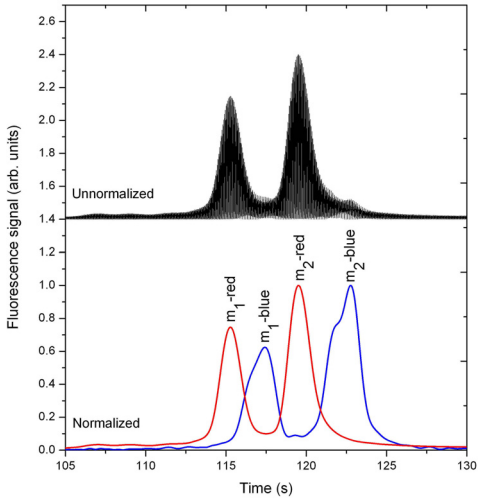

Fig. 2. Fluorescence intensity vs. time as measured by a color-blind PMT during the migration of two sets of blue- and red-labeled biomolecules through the integrated optical excitation/detection area under modulated excitation: cumulative signal from all four molecules (upper part) and corresponding individual signals resolved by Fourier decoding (lower part).

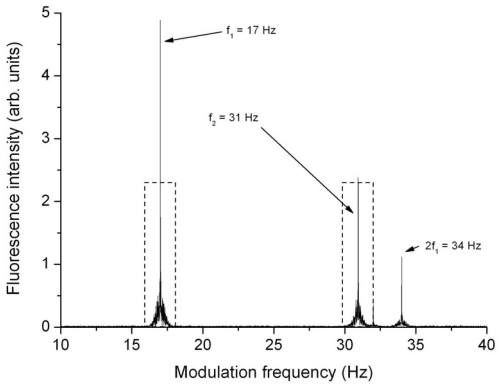

Fig. 3. Fourier transform of the cumulative signal showing signals at the individual modulation frequencies of the excitation laser beams.

Extracting specific, narrow frequency bands in the Fourier domain back into the time domain also resulted in an enhanced signal-to-noise ratio, leading to a limit of detection of 130 femto-molar (corresponding to merely 5 molecules in the excitation volume), which is a record-low value as far as on-chip-integrated fluorescence monitoring is concerned.

IV. CONCLUSIONS

The proposed integrated optical detection scheme enables exciting possibilities whenever wavelength multiplexing or ultrasensitivity can add value to an existing biophotonic system. We are currently building upon the example described in this paper making simultaneous investigation of several diagnostically relevant regions of a gene become feasible in a single optofluidic experiment, based on exclusive wavelength labeling of the different genetic origins and encoding of their fluorescence by different modulation frequencies, thereby leading to multiplex genetic diagnostics in an integrated optofluidic chip. Efforts are also in progress to further enhance the sensitivity, allowing us to eventually reach the Holy Grail of single molecule detection in an on-chip-integrated dynamic migration experiment.

ACKNOWLEDGMENT

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REFERENCES

[1] R. Iten, "Apparatus for emitting and detecting light in a nucleic acid amplification reaction," European Patent 1962084, 2007.

[2] R. Martinez Vazquez, et al, "Integration of femtosecond laser written optical waveguides in a lab-on-chip," Lab Chip, vol. 9, pp. 91–96, January 2009.

[3] C. Dongre, et al, "Fluorescence monitoring of microchip capillary electrophoresis separation with monolithically integrated waveguides," Opt. Lett., vol. 33, pp. 2503-2505, November 2008.