Multi-color DNA Analysis in an Optofluidic Chip

C. Dongre, H.J.W.M. Hoekstra, M. Hammer, and M. Pollnau

Sorting and sizing of DNA molecules by capillary electrophoresis (CE) within the human genome project have enabled the genetic mapping of various illnesses. By the use of miniaturized lab-on-a-chip devices, on-chip-integrated DNA sequencing and genetic diagnostics have become feasible. The inherent advantages of microfluidic CE separation of differently sized DNA molecules, high-speed operation and low reagent volumes, in combination with laser-induced fluorescence detection, result in optofluidic integration toward on-chip bio-analysis tools which aim at solving real-life challenges in medicine, e.g. identification of genomic deletions or insertions associated with genetic illnesses.

1. Optofluidic integration in an electrophoretic microchip

The lay-out of our optofluidic chip is presented in Fig. 1. Chips were fabricated in a two-step procedure. Firstly, the microfluidic channel network and microfluidic reservoirs were patterned photolithographically and wet-etched in fused silica glass and then sealed off by bonding another piece of fused silica glass on top (LioniX BV). The chip has dimensions of 55 mm × 5.5 mm × 1 mm and the microfluidic channels have a cross section of ~110 µm width and ~50 µm depth.

In a second step, the optical waveguide was inscribed into the bulk of such a fused silica chip by fs-laser writing using a Ti:Sapphire laser [1]. Employing astigmatic beam shaping, an elliptical cross section of the written waveguide was obtained, with a major diameter of ~50 µm in the vertical direction, in order to excite the maximum possible volume of the microfluidic channel, while the minor diameter in the horizontal direction is ~12 µm in order to retain a high spatial resolution along the direction of DNA flow and separation [2]. This 3-dimensional writing technique also allows for the fabrication of Mach-Zehnder interferometers in an optofluidic chip [3].

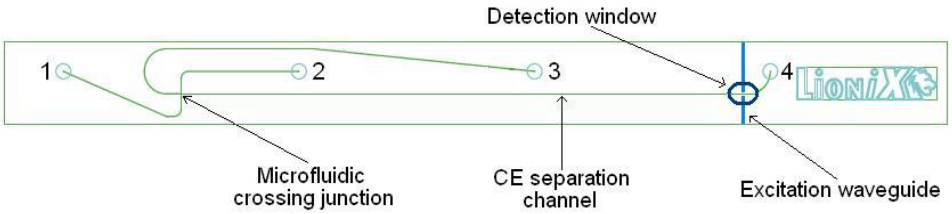

Fig. 1. Schematic of the optofluidic chip; indicated are reservoirs 1-4, sample injection channel (reservoir 1 → reservoir 2) and CE separation channel (reservoir 3 → reservoir 4), as well as the integrated optical waveguide and detection window [4].

2. Fluorescence monitoring of on-chip DNA separation

By applying integrated waveguide laser excitation to the optofluidic chip, fluorescence from static dye solutions [5] as well as migrating labeled DNA molecules [6] could be monitored through a microscope. Integrated waveguide excitation of the 12-µm narrow microfluidic segment (Fig. 2) provides a spatio-temporal resolution that would, in principle, allow for a 20-fold better accuracy than is currently supported by state-of-the-art electrophoretic separation in microchips, thereby demonstrating the potential of this integrated optical approach to fulfill the resolution demands of future electrophoretic microchips [4].

When optimizing the microchannel wall coating and sieving gel matrix, a DNA ladder consisting of molecules with 17 different base-pair sizes was separated by capillary electrophoresis with high

operating speed and low sample consumption of ~ 600 picoliters (Fig. 3, left). When detecting the fluorescence signals of migrating DNA molecules with a photomultiplier tube, the limit of detection was as low as 2.1 picomolar. In the diagnostically relevant size range of ~150–1000 base-pairs, the molecules were separated with reproducibly high sizing accuracy of > 99% (Fig. 3, right) [4]. When calibrating the system with a known set of DNA fragments, the sizing accuracy for an unknown sample could be improved to 4×10^4, which represents sub-base-pair resolution [7].

Numerical or mechanical lock-in amplification of the fluorescent signal from the labeled DNA molecules provided an improvement in signal-to-noise ratio by a factor of ~10, resulting in a record-low limit of detection of 210 fM, which equals merely 8 DNA molecules in the detection volume, thus approaching the goal of single-molecule detection [8]. Inscribing two parallel optical waveguides into the microfluidic chip allows for excitation and monitoring of differently labeled DNA molecules [9].

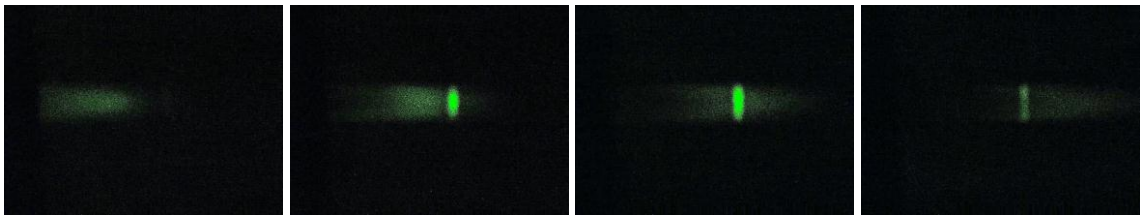

Fig. 2. Snapshots from a movie recorded with a CCD camera showing transient fluorescence from a molecule plug formed by CE separation of a DNA ladder as this plug passes by the point of integrated-waveguide laser excitation at 488 nm [4].

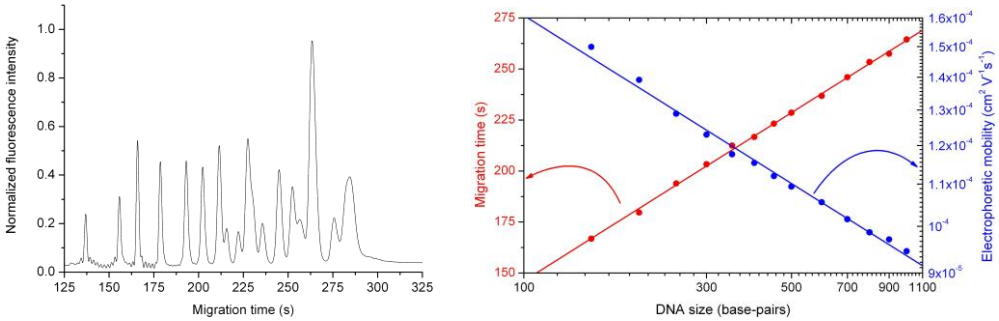

Fig. 3. (left) Electropherogram depicting the normalized fluorescence intensity vs. migration time from a DNA ladder consisting of 17 double-stranded molecules with integrated-waveguide laser excitation; (right) migration time (left-hand-ordinate) and electrophoretic mobility (right-hand ordinate) vs. base-pair size for the measured electropherogram [4].

3. Modulation-frequency encoded multi-color fluorescent DNA analysis

Then we introduced a principle of parallel optical processing [10] to our optofluidic lab-on-a-chip. During electrophoretic separation, the ultra-low limit of detection achieved with our set-up allowed us to record fluorescence from covalently end-labeled DNA molecules. Different sets of exclusively color-labeled DNA fragments – otherwise rendered indistinguishable by spatio-temporal coincidence – were traced back to their origin by modulation-frequency-encoded multi-wavelength laser excitation, fluorescence detection with a single ultrasensitive, albeit color-blind photomultiplier, and Fourier analysis decoding (Fig. 4). As a proof of principle, fragments obtained by multiplex ligation-dependent probe amplification from independent human genomic segments, associated with genetic predispositions to breast cancer and anemia, were simultaneously analyzed (Fig. 5) [10].

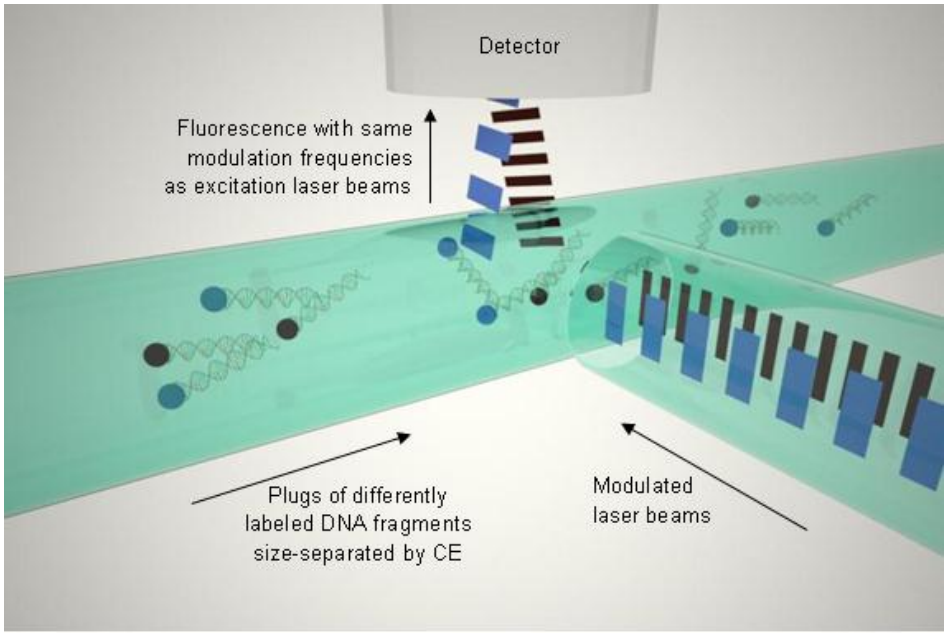

Fig. 4. Modulation-frequency encoded multi-wavelength sensing [10]: Schematic showing plugs of exclusively fluorescence-labeled molecules migrating through the microfluidic separation channel, intersecting the excitation waveguide that guides laser light of different wavelengths and modulation frequencies, and a plug containing DNA molecules with two different labels emitting fluorescence with the signatures of the two modulation frequencies while crossing the excitation waveguide.

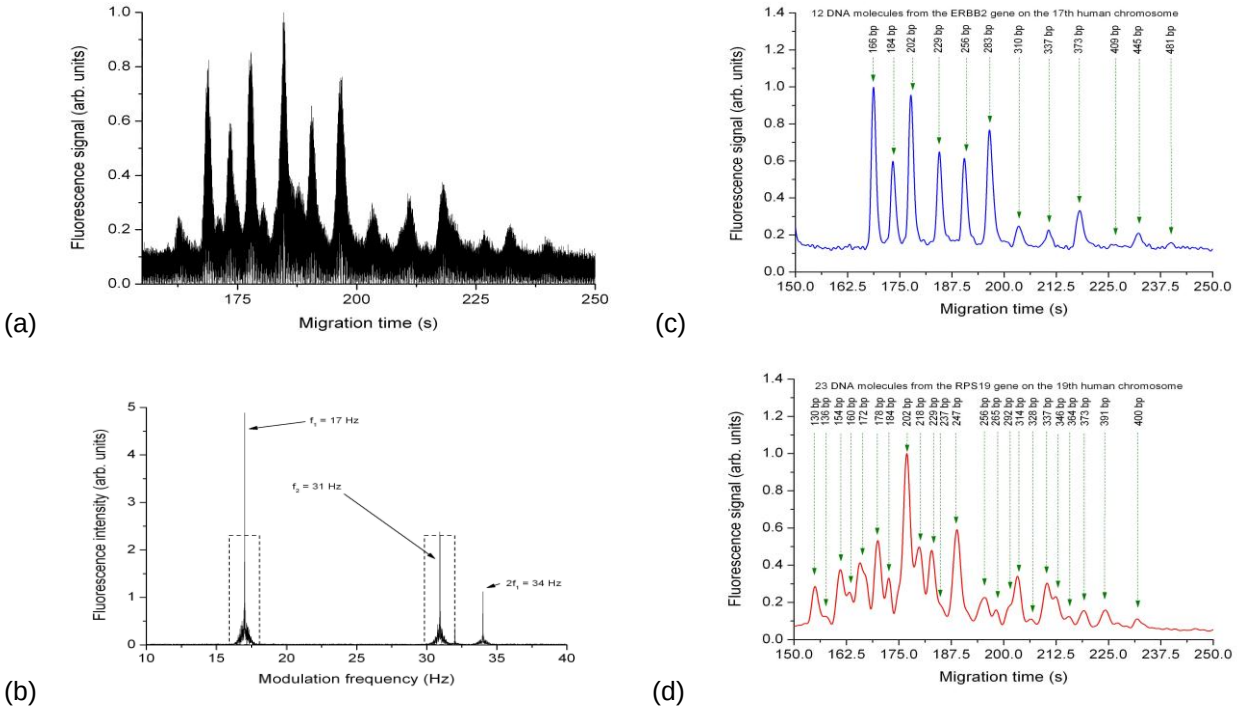

Fig. 5. Multi-color fluorescence DNA analysis in an optofluidic chip [10]: (a) fluorescence signal from 35 end-labeled DNA molecules (consisting of 12 and 23 DNA molecules from two chromosome regions) *vs.* migration time, as detected by a color-blind photomultiplier. (b) Fourier spectrum of the fluorescence signal and applied transfer functions (indicated by the dashed line). Individual signals separated by Fourier analysis of (c) 12 DNA molecules from a breast cancer gene and (d) 23 DNA molecules from a Diamond-Blackfan anemia gene. Several fluorescence peaks are below the noise level in (a), but are resolved in (c) or (d) by Fourier analysis.

Collaborations

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2. LioniX BV, Enschede, The Netherlands.
3. Zebra Bioscience BV, Enschede, The Netherlands.

References

[1] R. Martínez Vázquez, R. Osellame, D. Nolli, C. Dongre, H.H. van den Vlekkert, R. Ramponi, M. Pollnau, and G. Cerullo, *"Integration of femtosecond laser written optical waveguides in a lab-on-chip"*, Lab Chip **9** (1), 91-96 (2009).

[2] R. Osellame, H.J.W.M. Hoekstra, G. Cerullo, and M. Pollnau, *"Femtosecond laser microstructuring: an enabling tool for optofluidic lab-on-chips"*, Laser Photonics Rev. **5** (3), 442-463 (2011). Invited Paper.

[3] A. Crespi, Y. Gu, B. Ngamson, H.J.W.M. Hoekstra, C. Dongre, M. Pollnau, R. Ramponi, H.H. van den Vlekkert, P. Watts, G. Cerullo, and R. Osellame, *"Three-dimensional Mach-Zehnder interferometer in a microfluidic chip for spatially-resolved label-free detection"*, Lab Chip **10** (9), 1167-1173 (2010).

[4] C. Dongre, J. van Weerd, G.A.J. Besselink, R. van Weeghel, R. Martínez Vázquez, R. Osellame, G. Cerullo, M. Cretich, M. Chiari, H.J.W.M. Hoekstra, and M. Pollnau, *"High-resolution electrophoretic separation and integrated-waveguide excitation of fluorescent DNA molecules in a lab on a chip"*, Electrophoresis **31** (15), 2584-2588 (2010).

[5] R. Martínez Vázquez, R. Osellame, M. Cretich, M. Chiari, C. Dongre, H.J.W.M. Hoekstra, M. Pollnau, H. van den Vlekkert, R. Ramponi, and G. Cerullo, *"Optical sensing in microfluidic lab-on-a-chip by femtosecond laser written waveguides"*, Anal. Bioanal. Chem. **393** (4), 1209-1216 (2009).

[6] C. Dongre, R. Dekker, H.J.W.M. Hoekstra, M. Pollnau, R. Martínez Vázquez, R. Osellame, G. Cerullo, R. Ramponi, R. van Weeghel, G.A.J. Besselink, and H.H. van den Vlekkert, *"Fluorescence monitoring of microchip capillary electrophoresis separation with monolithically integrated waveguides"*, Opt. Lett. **33** (21), 2503-2505 (2008).

[7] M. Pollnau, M. Hammer, C. Dongre, and H.J.W.M. Hoekstra, *"Sub-base-pair resolution during DNA separation in an optofluidic chip"*, submitted (2015).

[8] C. Dongre, M. Pollnau, and H.J.W.M. Hoekstra, *"All-numerical noise filtering of fluorescence signals for achieving ultra-low limit of detection in biomedical applications"*, Analyst **136** (6), 1248-1251 (2011).

[9] C. Dongre, J. van Weerd, N. Bellini, R. Osellame, G. Cerullo, R. van Weeghel, H.J.W.M. Hoekstra, and M. Pollnau, *"Dual-point dual-wavelength fluorescence monitoring of DNA separation in a lab on a chip"*, Biomed. Opt. Express **1** (2), 729-735 (2010).

[10] C. Dongre, J. van Weerd, G.A.J. Besselink, R. Osellame, R. Martínez Vázquez, G. Cerullo, R. van Weeghel, H.H. van den Vlekkert, H.J.W.M. Hoekstra, and M. Pollnau, *"Modulation-frequency encoded multi-color fluorescent DNA analysis in an optofluidic chip"*, Lab Chip **11** (4), 679-683 (2011).