

Multi-color fluorescent DNA analysis in an optofluidic chip

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

Integrated Optical Microsystems Group, MESA+ Institute for Nanotechnology,
University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands
email: m.pollnau@ewi.utwente.nl

Summary

Modulation-frequency-encoded fluorescence excitation enables the identification of end-labeled DNA samples of different genetic origin during their electrophoretic separation, opening perspectives for intrinsic size calibration, malign / healthy sample comparison, and exploitation of multiplex ligation-dependent probe amplification.

Introduction

By capillary electrophoresis (CE) in miniaturized lab-on-a-chip devices, integrated DNA sequencing and genetic diagnostics have become feasible. We introduce a principle of parallel optical processing to significantly enhance analysis capabilities.

Discussion

In a commercial microfluidic chip (LioniX BV), a plug of DNA molecules with a volume of ~605 pl was injected and the DNA molecules were CE-separated with a high relative sizing accuracy of > 99% [1]. Through an optical waveguide inscribed by femtosecond-laser writing [2] a laser was launched perpendicularly into the microfluidic channel. A photomultiplier collected the fluorescence signals from a small detection window with a limit of detection of ~8 DNA molecules [3].

In our approach (Fig. 1), different sets of exclusively end-labeled DNA fragments are unambiguously identified by simultaneously launching several continuous-wave lasers, each modulated with a different frequency, detection of the frequency-encoded signals at different fluorescence wavelengths by a single ultrasensitive, albeit color-blind photomultiplier (Fig. 2a), and Fourier-domain frequency decoding (Fig. 2b) [4]. As a proof of principle, fragments from independent human genomic segments, associated with genetic predispositions to breast cancer and anemia, are simultaneously analyzed in a single flow experiment (Figs. 2c and 2d) [4].

Such multiple optical identification of biomolecules opens new opportunities in genetic diagnostics. (i) One can obtain intrinsic molecule-size calibration by adding a standard reference (several end-labeled molecules of standardized sizes) to a diagnostic DNA sample. (ii) One can flow an unknown, potentially malign sample of DNA molecules together with their healthy counterparts, thus providing unprecedented resolution. (iii) One can exploit multiplex ligation-dependent probe amplification of samples from different genomic regions, each exclusively color-end-labeled, to simultaneously investigate several diagnostically relevant regions.

Conclusions

This approach enables parallel optical detection in a lab-on-a-chip. Fluorescent labels with narrow absorption bands will allow for much higher degrees of spectral multiplexing, thereby fully exploiting this extra dimension in biomolecule detection.

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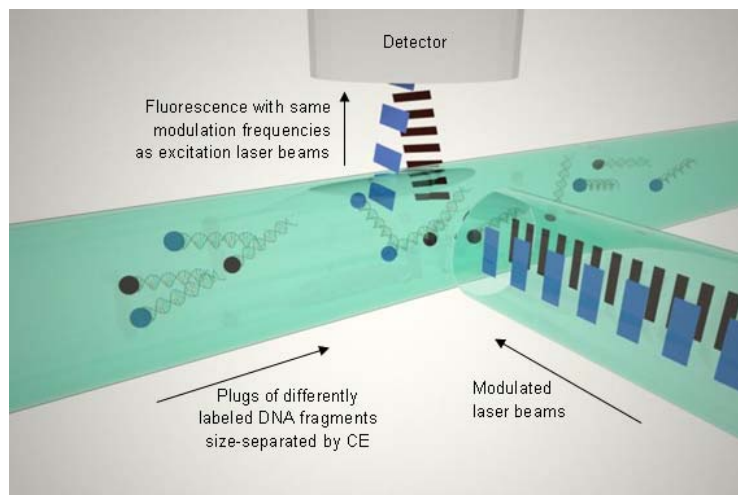


Fig. 1. Modulation-frequency encoded multi-wavelength sensing: Schematic showing plugs of exclusively fluorescence-labeled molecules migrating through the microfluidic separation channel, intersecting the excitation waveguide that guides laser light of different wavelengths and modulation frequencies, 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 [4].

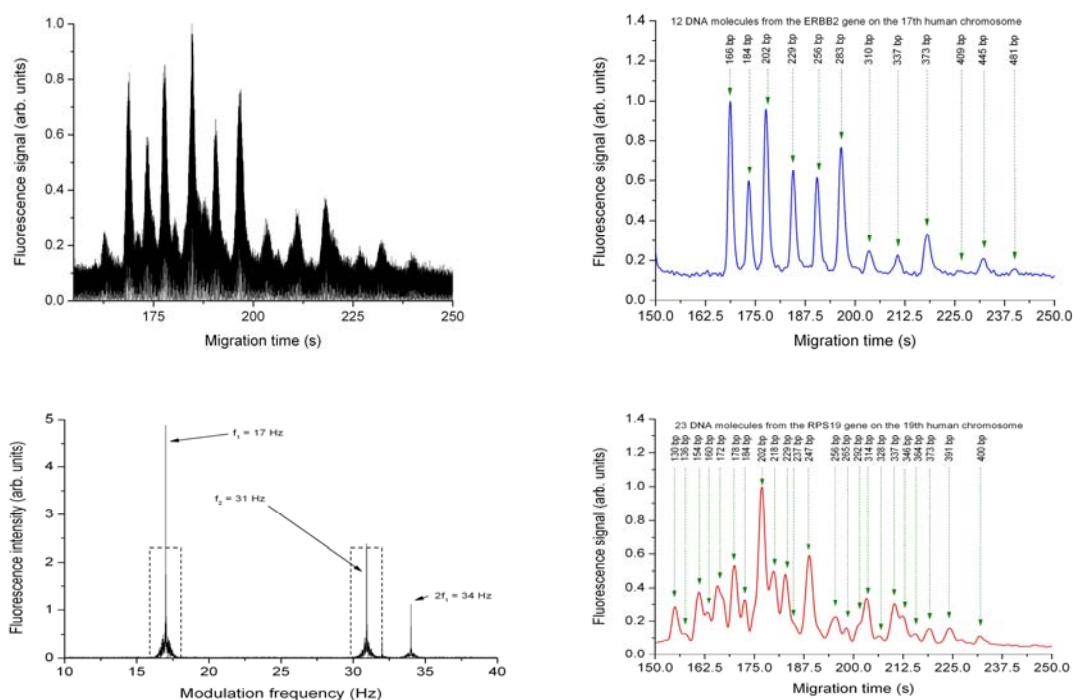


Fig. 2. (a) Fluorescence signal from 35 end-labeled DNA molecules vs. migration time, as detected by a color-blind photomultiplier. (b) Fourier spectrum of the fluorescence signal and applied transfer functions (dashed lines). Individual signals separated by Fourier analysis of (c) 12 DNA molecules from a breast cancer gene and (d) 23 DNA molecules from a Diamond-Blackfan anemia gene [4].

References

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