Modulation-frequency encoded multi-color fluorescent DNA analysis in an optofluidic chip

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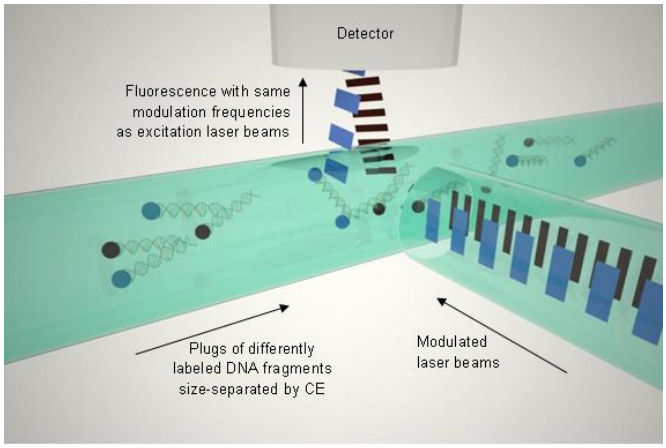
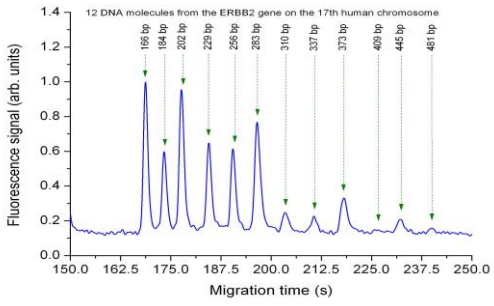
By capillary electrophoresis (CE) in miniaturized lab-on-a-chip devices, integrated DNA sequencing and genetic diagnostics have become feasible. We introduce a principle of parallel optical processing to significantly enhance analysis capabilities.

In a commercial microfluidic chip, a plug of DNA molecules 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, left), 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, and Fourier-domain frequency decoding [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 (Fig. 1, right) [4].

This novel method of modulation-frequency-encoded fluorescence excitation opens new opportunities in genetic diagnostics. It 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.





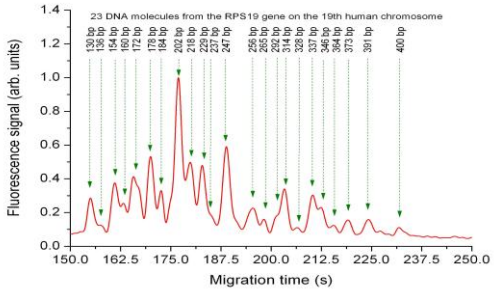


Fig. 1. (top left) The principle of modulation-frequency-encoded multi-color DNA analysis in an optofluidic chip, as pioneered in our research group: A sample volume of only 600 pl is separated in size by capillary electrophoresis and in color by modulation of the excitation lasers and subsequent Fourier analysis, resulting in the simultaneous analysis of 12 fragments from a breast cancer gene (top right) and 23 fragments from an anemia gene (bottom right).

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[2] R. Martínez Vázquez, R. Osellame, D. Nolli, C. Dongre, H. H. van den Vlekkert, R. Ramponi, M. Pollnau, and G. Cerullo, Lab Chip **9**, 91-96 (2009).

[3] C. Dongre, M. Pollnau, and H. J. W. M. Hoekstra, Analyst **136**, 1248-1251 (2011).

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