Modulation-frequency Encoded Multi-wavelength Fluorescence Analysis

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Abstract

We introduce a principle of parallel optical processing: modulation-frequency-encoded multi-wavelength laser excitation, fluorescence detection with a single detector, and Fourier analysis decoding. As an example, we demonstrate simultaneous detection of DNA fragments from different origins.

1. Introduction

Fluorescence sensing is an important detection technique in biochemical analyses. The biomolecules are either auto-fluorescent or deliberately labeled. Approaches to multi-wavelength fluorescence sensing usually apply a number of complicated bulk optical schemes to achieve an unambiguous separation of signals at different fluorescence wavelengths. We present an elegant approach by *a priori* encoding of the fluorescence originating in different biomolecules by intensity-modulating the light sources, followed by Fourier decoding of the detected biophotonic signals, thereby tracing back the origins of the biomolecules.

2. Modulation-frequency encoding/decoding

If an optical beam is modulated at a specific frequency, its temporal signature leads to a similar temporal excitation of, and emission by, the fluorescent molecules that absorb light at this wavelength, i.e., the fluorescence emitted by the molecules is encoded with the same frequency. If multiple analytes with distinct absorption bands are excited by optical beams with distinct modulation frequencies, the resulting fluorescence signals can be unambiguously addressed to the presence of the corresponding analyte molecule by Fourier analysis, each frequency peak corresponding to the specific modulation frequency of its exciting beam. By applying inverse Fourier transform, the individual time-domain signals are regenerated.

3. Experimental example

During electrophoretic separation, we record fluorescence from covalently end-labeled DNA molecules. Different sets of exclusively labeled DNA fragments are 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 [1]. 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, are simultaneously analyzed (Fig. 1).

Acknowledgements

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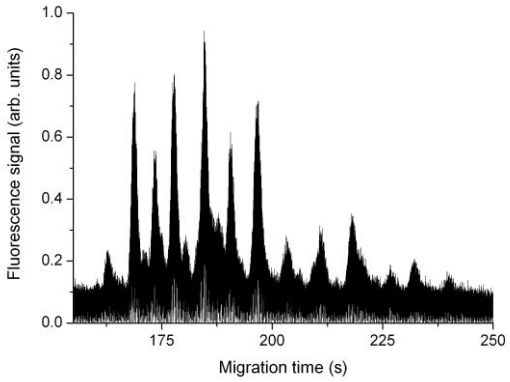
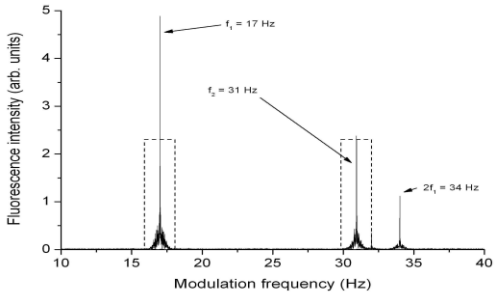
Reference

[1] 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, Lab Chip, accepted (2010).



(a)



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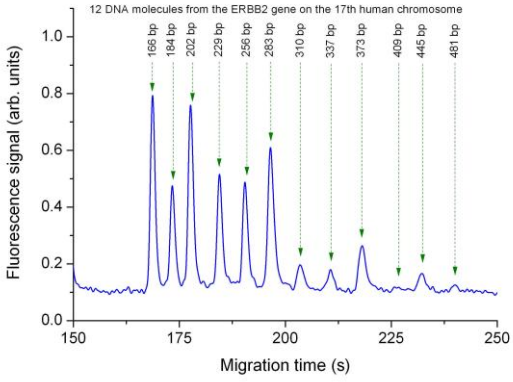
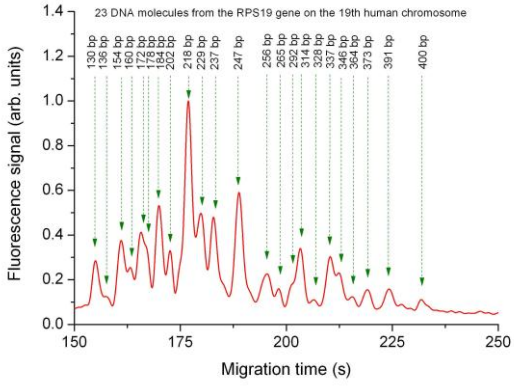


(c)



(d)

Figure 1. Multi-color fluorescence DNA analysis in an optofluidic chip [1]: (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.