DNA separation and fluorescent detection in an optofluidic chip with sub-base-pair resolution

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100-word summary:
A microfluidic chip was post-processed by femtosecond-laser writing of an optical waveguide. 12 blue-labeled and 23 red-labeled DNA fragments in the diagnostically relevant range of 150–1000 base-pairs were separated in size by capillary electrophoresis, each set excited by a laser power-modulated at a specific frequency, their fluorescence detected by a photomultiplier, and blue/red signals distinguished by Fourier analysis. Different calibration strategies were tested. Choosing a single, suitable dye label combined with reference calibration and sample investigation in consecutive experiments results in a sizing accuracy of 4×10^{-4}, thereby enabling detection of single base-pair insertions/deletions associated with genetic illnesses in a lab-on-a-chip.

250-word abstract:
DNA sequencing in a lab-on-a-chip aims at providing cheap, high-speed analysis of low reagent volumes to, e.g., identify genomic deletions or insertions associated with genetic illnesses. Detecting single base-pair insertions/deletions from DNA fragments in the diagnostically relevant range of 150–1000 base-pairs requires a sizing accuracy of $S < 10^{-3}$. Here we demonstrate $S = 4\times10^{-4}$.
A microfluidic chip was post-processed by femtosecond-laser writing of an optical waveguide. 12 blue-labeled and 23 red-labeled DNA fragments were separated in size by capillary electrophoresis, each set excited by either of two lasers power-modulated at different frequencies, their fluorescence detected by a photomultiplier, and blue/red signals distinguished by Fourier analysis. Different calibration strategies were tested: a) use either set of DNA molecules as reference to calibrate the set-up and identify the base-pair sizes of the other set in the same flow experiment, thereby eliminating variations in temperature, wall-coating and sieving-gel conditions, and actuation voltages; b) use the same molecular set as reference and sample with the same fluorescence label, flown in consecutive experiments; c) perform cross-experiments based on different molecular sets with different labels, flown in consecutive experiments.
From the results we conclude: Applying quadratic instead of linear fit functions improves the calibration accuracy. Blue-labeled molecules are separated with higher accuracy. The influence of dye label is higher than fluctuations between two experiments. Choosing a single, suitable dye label combined with reference calibration and sample investigation in consecutive experiments results in $S = 4\times10^{-4}$, enabling detection of single base-pair insertion/deletion in a lab-on-a-chip.