

NANOFLUIDIC DEVICES FOR HIGH-THROUGHPUT ANALYSIS OF SINGLE FLUORESCENT MOLECULES

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ABSTRACT

Single-molecule-fluorescence studies are often hampered by limited throughput and limited time resolution. We developed a novel fluidic device for TIRF microscopy that allows for individual detection of non-immobilized fluorescent molecules. In our devices, molecules are flowing through an array of nanochannels, which confine the diffusional movement of the molecules in the z-direction along the optical axis of the microscope. This geometrical confinement does not only increase the observation time, but also allows us to track molecules as they move. One of the designs contains a number of parallel channels for high-throughput analysis of a single molecular species; another design is a T-shaped channel for precise mixing of two different species allowing to continuously trigger enzymatic reactions such as DNA synthesis. We tested our devices by flowing small DNA hairpins (labelled with a donor and acceptor dye for FRET) through the channels. By fine-tuning flow rate, sample concentration, illumination conditions and our detection algorithms, we were able to detect changes in the FRET efficiency corresponding to conformational changes in the DNA. We plan to extend the assay to include (labelled) polymerases and visualize DNA synthesis in real time inside the channels.