Nanofluidics and Plasmonics for In-Line DNA Optical Mapping

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On-chip DNA optical mapping allows studying intact individual molecules with higher throughput than conventional sequencing techniques. In optical mapping, typically a "fluorescent barcode-like" pattern is first created, then the DNA molecules are elongated and the fluorescent signal is read out to get information about the genomic structure. Here, we explore different techniques to selectively label the DNA molecules with quantum dots, and/or organic fluorophores as well as by competitive binding. The DNA molecules are then stretched in nanofluidic devices made by nanoimprint lithography and the signal is read out in real time using a focused laser and a photon counter.

We investigated different methods to create a sequence-dependent optical barcode. For instance, selectively labeling single DNA molecules with quantum dots or fluorophores (ATT0647 and Cy5) at sticky ends and/or at specific locations and then staining the DNA molecules with intercalating dyes to track the molecules. We are also exploring competitive binding using Netropsin to create a barcode that is dark in AT-rich regions and bright in GC-rich regions [1,2] and read out in the time scan to demonstrate the sequence.

To stretch the molecules, we developed a wafer-scale fabrication process, based on nanoimprint lithography [3], fabricating the complete fluidic devices in one single step, only 120 seconds long. The all-transparent polymeric devices have complex structures with multiple levels and multiple dimensions: the microchannels and 3D tapered inlets guide the molecules, and the nanochannels stretch them to their full contour length (Fig. 1 & Fig. 2a-e)

We perform in-line detection of DNA molecules as they pass through the nanochannels with a focused laser as point excitation and a photon counter, without using a camera. In this configuration, the molecules are detected as step-like peaks in time scans, allowing for real time read-out, with high throughput (Fig. 2f). Peak analysis (as intensity and duration) gives information about the molecule length, as well as its sequence-dependent barcode. Here there is no limitation with the length of the molecules, or the resolution due to the thermal drift and molecule movement.

Different types of DNA molecules (λ-Bacteriophage and Kaposi's sarcoma herpesvirus) were stained with intercalating dyes, stretched and successfully detected by our in-line detection method. The molecules are ~48.5 kbp and ~150 kbp long respectively and show step-like fluorescent signals with duration of 5 ms and 15 ms respectively (Fig. 3 a). With this method, we can also detect continuous unbroken ultra-long molecules (megabase pair range) with duration of about hundreds of ms. To optimize the resolution beyond diffraction limit, our goal is to combine the nanochannels with plasmonics. Therefore, we integrated bowtie plasmonic nanoantennas adjacent to the nanochannel (Fig. 1).

KEYWORDS: DNA Optical Mapping, Single Molecule, Nanoimprint, Nanochannels, Plasmonic Antenna, Sensor

[1] Østergaard et al, J. Analyst, 138, 1249–1255(2013)

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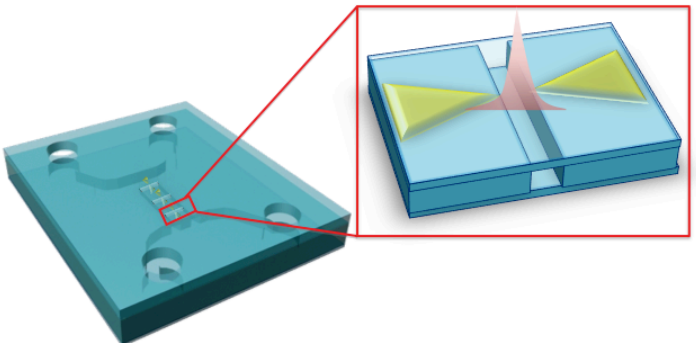

Fig. 1. Micro- and nanofluidic device with parallel nanochannels. A nanochannel has a gold bowtie nanoantenna that can create a hot spot inside. The nanochannel can be used to stretch DNA molecules and deliver them through the hot spot for read-out. The nanochannel is part of a complete fluidic system for total liquid control.

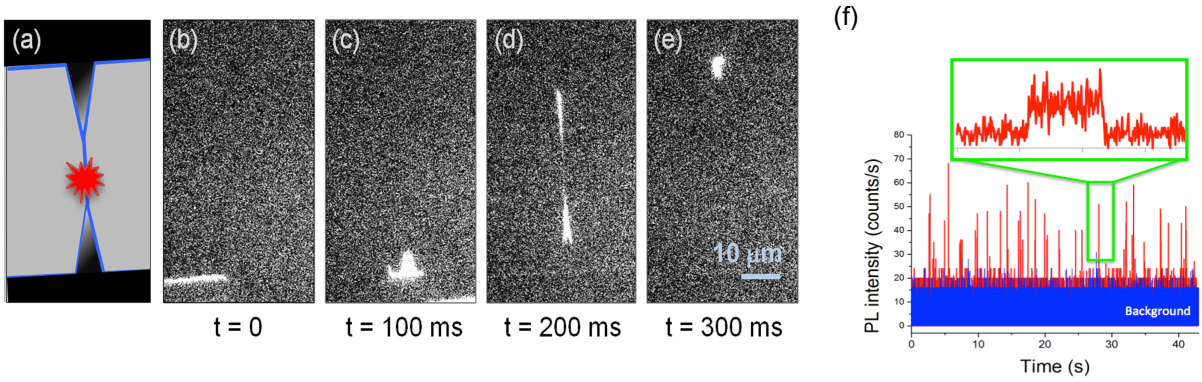

Fig. 2: (a) Sketch of the micro and nanofluidic structures of the imprinted, polymeric device, and (b-e) time sequence fluorescence images of a single λ-DNA molecule (stained with TOTO-3 (5:1)) passing through the nanochannel, derived by electrophoresis. (f) In-line photoluminescence time scan acquired by focusing a laser spot at the nanochannel shown in (a-e) and by recording the signal with a photon counter. Each peak from the red line corresponds to the translocation event of an individual DNA molecule through the nanochannel. The blue signal was recorded without electrophoretic voltage (so no DNA flows through).

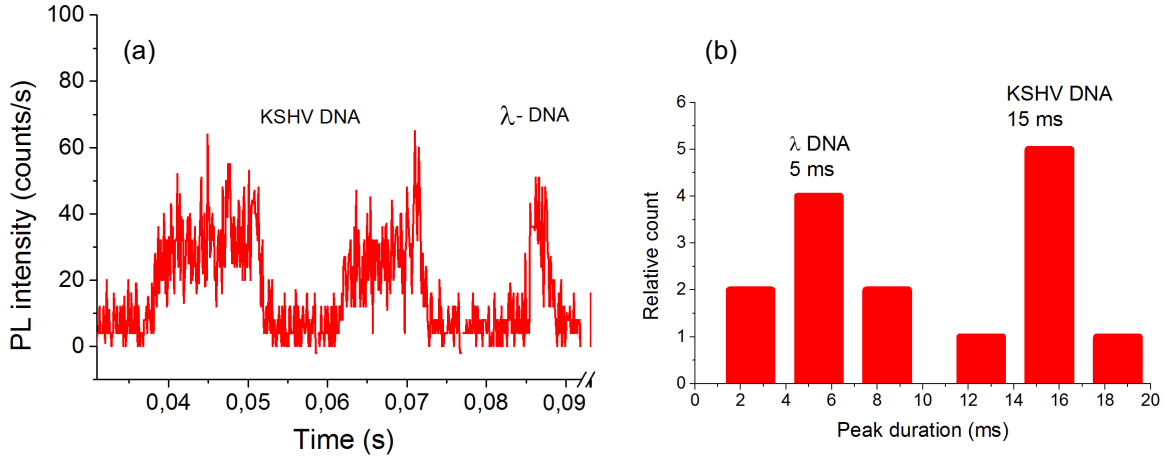

Fig. 3: λ-Bacteriophage and Kaposi sarcoma herpesvirus DNA molecules are stained with intercalating dyes and stretched in the nanochannels. (a) Fluorescence time scans obtained in real time. The molecules are ~48.5 kbp and ~150 kbp long respectively and show step-like fluorescent signals with duration of 5 ms and 15 ms respectively, as we obtain for the histogram in (b).