MEANDERING NANOCHANNELS FOR IMAGING OF ULTRA-LONG DNA MOLECULES

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ABSTRACT

A new chip design for handling and studying chromosomal DNA is described. Folding the nanochannels enables us to image an entire yeast chromosome in a single field of view. Together with existing mapping techniques our new design opens up the possibility of directly analyzing entire chromosomes in a single field of view using fluorescence microscopy. This would enable, for example, identification of microorganisms on a single-cell basis and the complete physical mapping of the human genome, chromosome by chromosome.

KEYWORDS: nanofluidics, genomics, chromosomal DNA, DNA analysis

INTRODUCTION

Direct visualization of DNA stretched in nanochannels has proven useful in several areas such as polymer physics and DNA mapping [1]. Observation of DNA in nano-structures improves our understanding of the behavior of semi-flexible polymers at different degrees of confinement [2]. The key benefit from a biological perspective stems from the ability to directly observe labels localized along the entire linear length of a DNA molecule with high optical resolution. For example, physical mapping can be performed based on the formation of a barcode-like pattern due to local denaturation along the DNA [3] (similar to chromosome banding but a 1000-fold better resolution). Also, by providing appropriate modification of the channel walls, proteins can be introduced and their location of binding along the stretched DNA can be determined. In this way copy-number variations, inversions and translocations can easily be detected along entire DNA molecules. However, to benefit even more from the simple nanochannel system for DNA analysis, one needs to increase throughput. Current device designs, based on linear channels, allow only limited segments of native genomic molecules to be observed in one single field of view. In our work we drastically decrease the number of frames that would be required to image large genomes by simply folding the nanochannels to form a meandering pattern.

EXPERIMENTAL

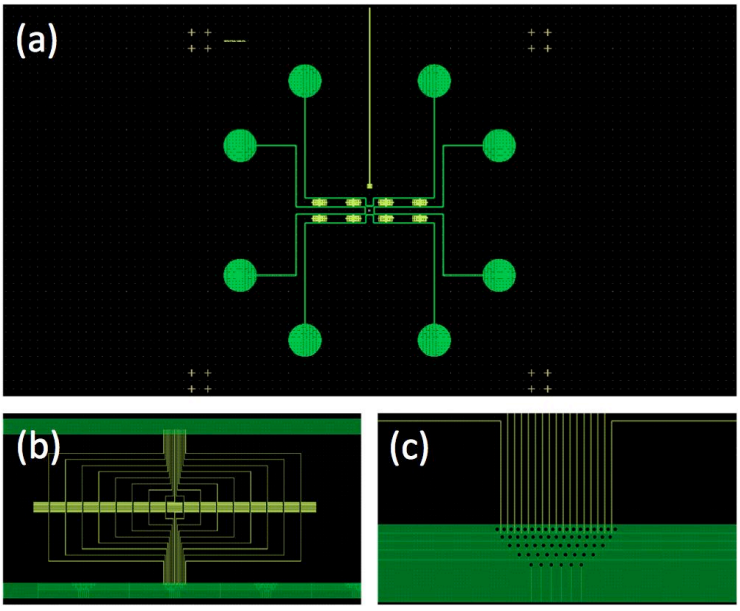

Figure 1. Design of meandering nanochannel devices. (a) Overview with eight sets of meanders. The total size of the device is 25 x 25 mm^2.The round features, corresponding to holes drilled in the device for connection to external reservoirs, are connected to micron-scale channels for easy fluid transport. (b) One set of 17 meanders joining two micron-scale channels. (c) The transition of the DNA from the microchannels to the nanochannels is facilitated by a pillar array for dis-entanglement and pre-stretching of the DNA as well as the collection of all nanochannels in close proximity.

We fabricated devices by patterning the nano- and microstructures into resist by electron beam and UV lithography, respectively and subsequently transferring them into the fused silica substrate using two consecutive steps of reactive ion etching. The design is laid out in *figure 1* and optical micrographs are shown in *figure 2*. In order to seal the fluidic systems, the structured substrates were bonded to thin cover slips of fused silica using thermal fusion bonding [1]. The meandering parts of the nanochannels are 1 mm long with bends designed to minimize stress on the molecule. One important challenge working with extremely long nanochannels is that the resulting fluid flow will be minute at reasonable pressures making it difficult to force the DNA into the nanochannels. Keeping the inlets of the parallel channels in close proximity to each other (*figure 1 (c)*) we ensure that the bulk flow rate into the nanochannels exceeds the diffusional rate and the drift in the microchannel of the DNA. In addition, a pillar array facilitating the introduction into the nanochannels by pre-stretching the DNA molecules is placed in the microchannel in close proximity to the nanochannel inlets. Here the long (as opposed to the short) DNA molecules are typically trapped and partially stretched as they are introduced into the nanochannels.

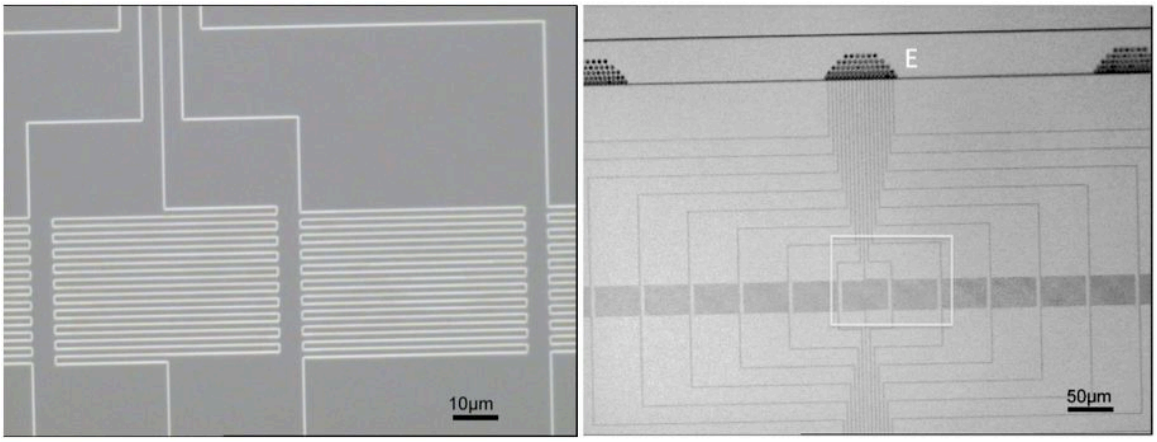

Figure 2: Bright field optical microscopy images of 200 x 200 nm^2 channels. (LEFT) Zoom of area indicated by rectangle in the RIGHT image. (RIGHT) Overview with the collection of the nanochannels and the pillar array at the entry area (E) in the microchannel to facilitate the transfer of the DNA into the nanochannels.

The chip design can easily be slightly modified to accommodate a wide range of applications, for example upstream cell handling and lysis functionalities or structures for the change of buffer locally along the DNA.

RESULTS AND DISCUSSION

Each meander was designed to hold 6 Mbp of DNA stretched to 50% extension (1 mm) and to be imaged within one field of view. As a proof of principle we show an entire chromosome (5.7 Mbp) from *Schizosaccharomyces pombe* (*figure 3*). In *figure 4* we show a smaller (1Mbp) segment subjected to melting mapping [3]. The molecules in both images were stained with YOYO-1 to a ratio of 1 dye molecule to 6 bp. With an optimized denser design we expect to be able to hold at least 20 mm of DNA per field of view. At 50% stretching, this would allow us to image the entire human genome with a mere 25 frames.

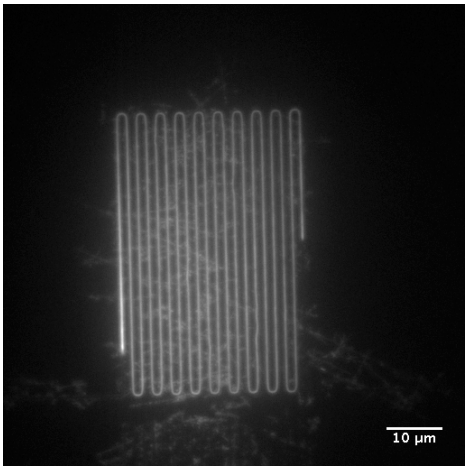

Figure 3: 5.7Mbp long S. pombe chromosome in 250 x 250 nm^2 meander channels imaged using a fluorescence microscope. The DNA molecule is stained with YOYO-1.

We expect that meandering nanochannels will contribute to several application areas where long-range genomic and epigenomic information is important. The physical mapping of genomes from single cells will enable the characterization of the genomic heterogeneity among, for example, cancer cells in a solid tumor. Mapping of entire chromosomes will be useful for identification of the large genomic rearrangements and copy number variants associated with a number of medical conditions [4]. Finally, physical (*e.g.* melting) signature mapping will lead to the identification of single microorganisms, which will be important for rapid pathogen diagnosis and metagenomic studies without the need for culturing of the cells nor DNA amplification.

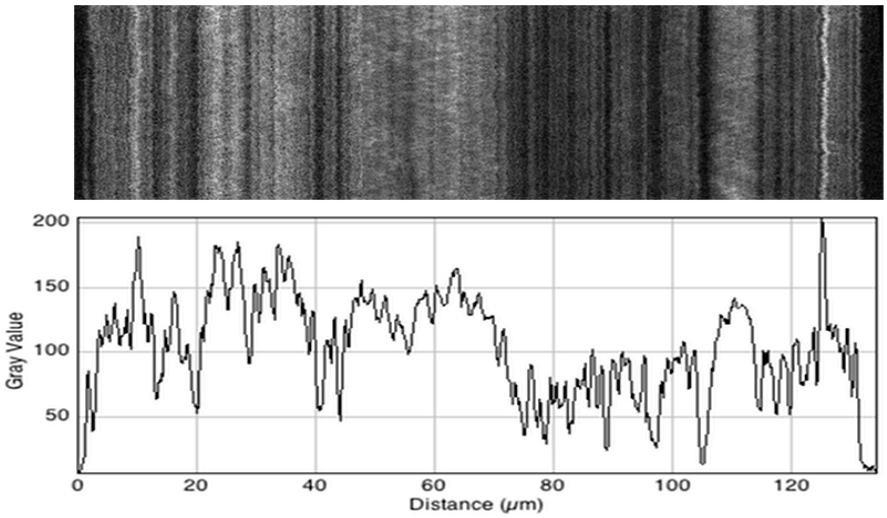

Figure 4: TOP Timetrace of a part of an S. pombe chromosome. The DNA is stained with YOYO-1 to a ratio of 1dye to 6bp. BOTTOM Profile plot of the partially melted S. pombe chromosome.

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REFERENCES

1. Persson, F. and J.O. Tegenfeldt, *DNA in nanochannels - directly visualizing genomic information.* Chemical Society Reviews, 2010. **39**(3): p. 985-999.

2. Persson, F., et al., *Confinement Spectroscopy: Probing Single DNA Molecules with Tapered Nanochannels.* Nano Letters, 2009. **9**(4): p. 1382-1385.

3. Reisner W., et al., *Single-Molecule Denaturation Mapping of DNA in Nanofluidic Channels*, PNAS 2010, **107**(30): p. 13294-13299.

4. Stankiewicz P and Lupski JR, *Genome architecture, rearrangements and genomic disorders*, Trends in Genetics, 2002. **18**(2): p. 74-82.

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