High Throughput DNA Optical Mapping in Real-Time on 3D Nanofluidic Devices

Franziska M. Esmek[1*], Tim Erichlandwehr, Dennis H.B. Mors[1], Anton Kettner[1], Leon Seggerin[1], Irene Fernandez-Cuesta[1**]

[1] INF, Universität Hamburg, Institute of Nanostructure and Solid State Physics, HARBOR

*fesmek@physnet.uni-hamburg.de, **ifernand@physnet.uni-hamburg.de

DNA optical mapping is a powerful tool for analyzing long-range structures and the length of DNA in a reasonable time. It allows studying intact single DNA molecules in a cost-efficient way, with a variety of applications in many different fields. [1]We have developed a technology called laser-assisted DNA optical mapping (LADOM methodology) for analyzing the long-range structure of single DNA molecules.

Within the LADOM method, an optical map on single DNA molecules is generated and read out by a laser system. First, the molecules are stained with an intercalating dye and diluted in TBE buffer. A droplet of the solution is put on the nanofluidic device into the micro and nanochannel area. With the help of 3D inlets, the DNA molecules are guided in the nanochannels without any external forces like applying an electric field or pressure. The inlets also pre-stretch the molecules, which avoids clogging. A spot-like laser is focused in the center of a nanochannel. As the DNA is flowing through the laser spot, a photo detector measures the fluorescence emitted by the intercalating dyes. The obtained signal provides information about the length and barcode of the DNA molecule. It also gives information about the conformation of the DNA inside the nanochannel (e.g., hairpin and other folded variations).

We have designed and fabricated micro and nanofluidic structures in a multistep process in a silicon stamp, which is then transferred in a polymer stamp, which allows for the fabrication of single-use samples in a 2 minutes process.[2, 3] The polymeric chip consists of micro/nanostructured fluidic channels, and several inlet holes, to insert the liquid (Fig.1). The microchannels guide the DNA from the holes into the nanochannels, which differ in cross-section, length, and geometry. We have designed 3D inlets with nanopillars to uncoil the DNA and slow down the molecules in the nanochannel. In this configuration, it is possible to have spontaneous flow of single DNA molecules without applying pressure or an external field in nanochannels as small as 30 nm x 60 nm (Fig.2). This is a step forward to bringing the technology outside research labs.

With the presented LADOM we have successfully detected the barcode of different viruses (bacteriophages, and two different tumor virus). And we have also used it to analyze the length of fragmented DNA molecules, with sizes from 3kbp to 48kbp. To enhance the resolution, we studied the effect of the physical confinement, the topography and the ion condition in the DNA's environment.

For filtration of larger residues in the liquid sample (eg, proteins, cell residues, etc.), we can further integrate a filter system with circular- and diamond-shaped pillars ranging in distance from 200 nm to 10µm. This filter system could be used for cleaning up blood samples or human plasma before analyzing DNA molecules in the nanochannels. (Fig.3)

1. Reisner, W., *DNA confinement in nanochannels: physics and biological applications.* Reports on Progress in Physics, 2012. **75**(10): p. 106601.
2. Esmek, F.M., *Sculpturing wafer-scale nanofluidic devices for DNA single molecule analysis.* Nanoscale, 2019. **11**(28): p. 13620-13631.
3. Czech-Sioli, *High-resolution analysis of Merkel Cell Polyomavirus in Merkel Cell Carcinoma reveals distinct integration patterns and suggests NHEJ and MMBIR as underlying mechanisms.* PloS PATHOGENS, 2020. **16**(8): p. e1008562.

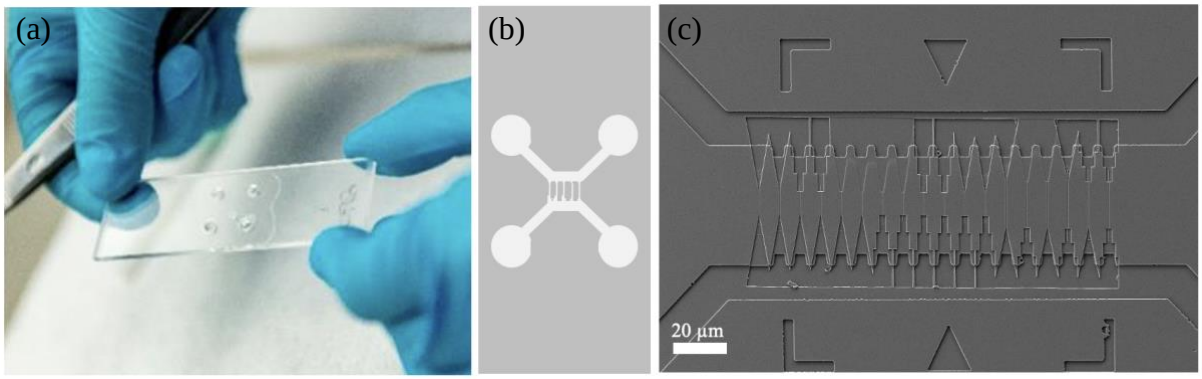

Figure 1: Nanofluidic device for DNA optical mapping. (a) Image of a polymer sample made in a 2 minutes process for single use. (b) Sketch of the nanofluidic device with four holes, two U-shaped microchannels and connecting nanochannel area. (c) SEM image of a silicon stamp with micro, nanochannels and 3D-inlets, fabricated in a multi-step process.

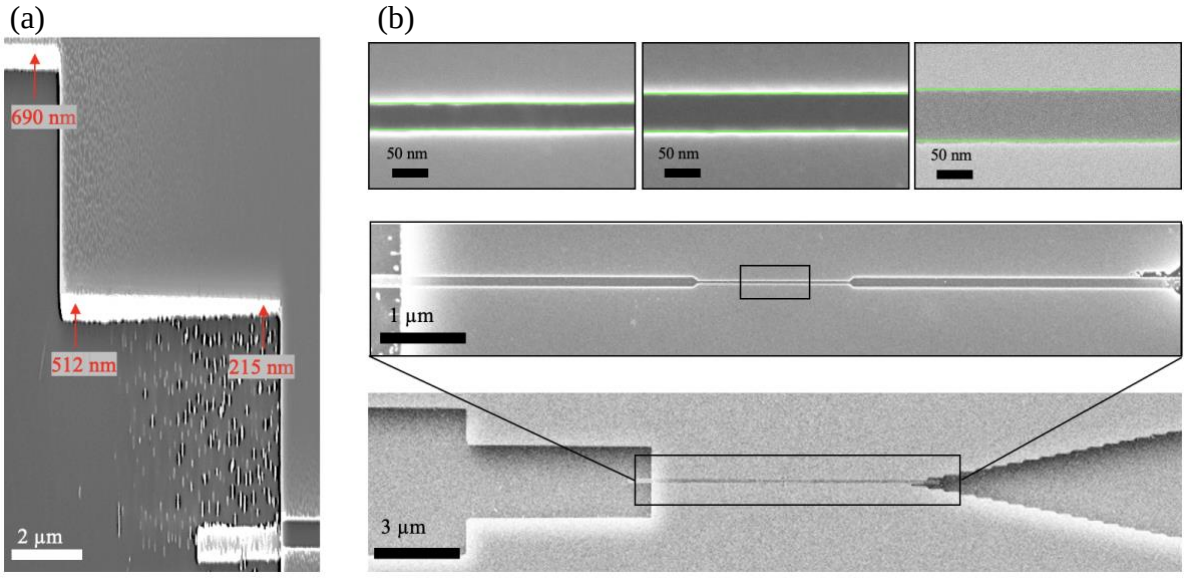

Figure 2: SEM images of the fabricated silicon stamp showing the nanostructured area. (a) 3D inlet with an integrated pillar system and a gradient from 690 nm to 215 nm helping the DNA to uncoil and to enter the nanochannel. (b) Fabricated nanochannels with different width ranging from 30 nm to 70 nm.

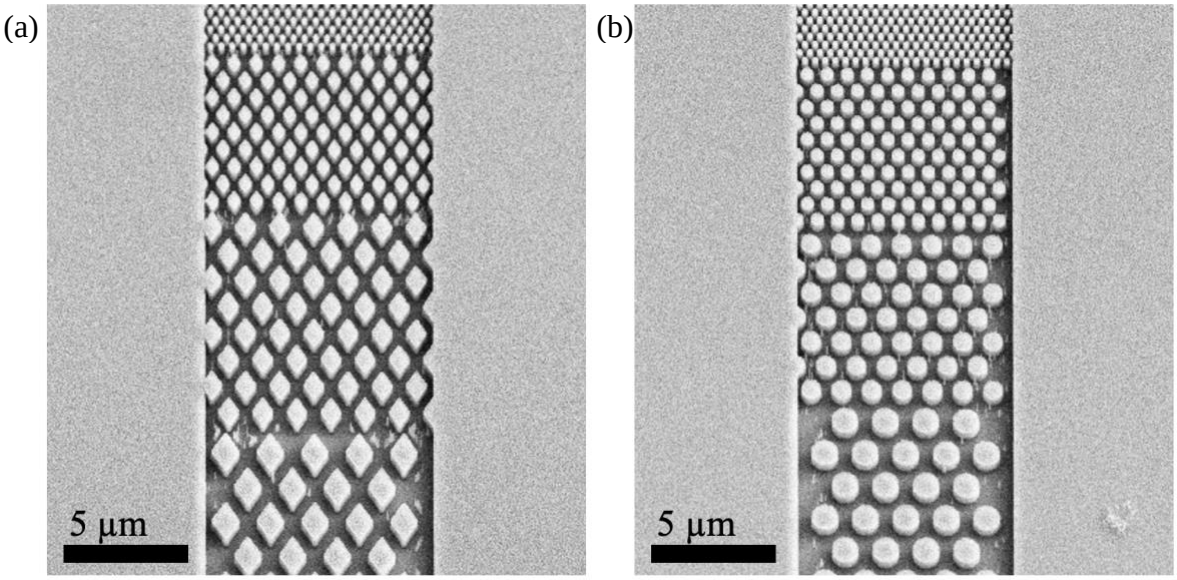

Figure 3: SEM images of (a)diamond and (b)circular-shaped filter systems for DNA purification.