

# Fabrication of Nanochannels with Funnel-like Inlet Structures for the Analysis of Single DNA Molecules

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## Summary:

A new fabrication method for realizing fluidic nanochannels with funnel-like inlet structures in silicon technology is presented. Nanochannels are a useful tool for DNA analysis since the molecules must first be linearized to enter the nanochannels and the linearization is a prerequisite for DNA mapping or sequencing. Inlet structures, like funnels, help guiding the DNA and bringing it into the channels. The nanochannels demonstrated in this work are realized using standard MEMS processes and photolithography, enabling cheap and scalable production for a wide range of biotechnology applications.

**Keywords:** Nanochannel, Inlet structure, Funnel, MEMS Fabrication, DNA Analysis

## Background and Motivation

Micro-biosensors bring many benefits through their miniaturization [1]. Micro- and nanoscale features allow manipulation of single cells or even molecules and the integration of their analysis on lab-on-chip systems. The sequencing and mapping of single DNA molecules is made possible by the linearization of single DNA molecules inside of a nanochannel [2]. When the dimensions of a structure are smaller than the persistence length of DNA, the DNA is forced to extend instead of the entropically-favorable coiled state, as sketched in figure 1. The negatively charged DNA can be pulled into a nanochannel by an electric field. Nevertheless, inserting the DNA into the channels is challenging. To change its conformation from coiled to linear the so-called entropic barrier has to be overcome [3].

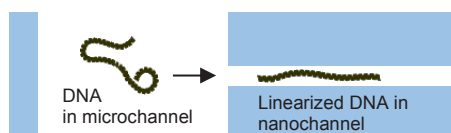


Fig. 1. Schematic of linearized DNA inserted into a nanochannel

Adding a funnel-like inlet to the nanochannel creates a gradient between the volume in front of the nanochannel and the channel itself, which makes the overcoming of the entropic barrier easier and lowers the needed electrical force [3]. A funneled inlet can also increase the capture rate of molecules in the channel and avoid hairpin formation and clogging of nanochannels [2,4].

Therefore, creating nanochannels with funnel-inlets with a cheap and scalable process is vital for the fabrication of DNA analysis systems. However, the fabrication of nanostructures poses challenges. Since the standard fabrication processes for microsystems are limited by the wavelength of the light used for photolithography structures smaller than 1  $\mu\text{m}$  are difficult to achieve. There are methods to overcome this, like deep-UV lithography or electron beam lithography. A different method is focused ion beam milling (FIB) to directly create the nanostructures or a stamp to use in nanoimprint lithography (NIL). These mentioned methods are expensive and often not scalable [1]. To circumvent these expensive methods and the limit of photolithography, certain workarounds can be used to create nanostructures only with standard fabrication processes. For example, nanochannels can be fabricated by thin-film evaporation and etching of a sacrificial layer [5]. But the addition of funnel inlet structure to the channels is only reported in systems fabricated with NIL or FIB [2,3,4], and not for structures fabricated using only standard processes. Such a process was investigated for the first time and will be discussed here.

## Description of the New Fabrication Method

The fabrication of long nanochannels with horizontal funnel-shaped inlet structures to facilitate the analysis of DNA molecules is shown schematically in Figure 2. The nanochannel is

created by selectively under-etching a sacrificial layer under a top layer. Therefore, the channel dimensions depend on the thickness of the sacrificial layer and the under etch time. The channel is then closed by a capping layer. Microchannels, that contact the nanochannels, are etched with an anisotropic process. To fabricate the funnel-shaped inlets of the channels, an additional etch step is added, which etches the material next to the nanochannel faster at the entrance than the inside of the channel. This step creates a horizontal funnel inlet.

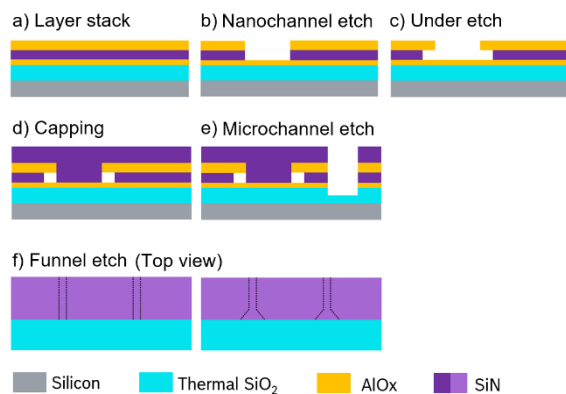


Fig. 2. Fabrication scheme of nanochannel (a-d), microchannel (e) and funnel-inlet (f).

## Results

Nanochannels with a length of 50  $\mu\text{m}$  have been fabricated, but channels with lengths up to several millimeters are possible with this fabrication method since the length is not the limiting factor in the fabrication. A cross section of a channel with a width of 250 nm and a height of 40 nm is displayed in figure 3.

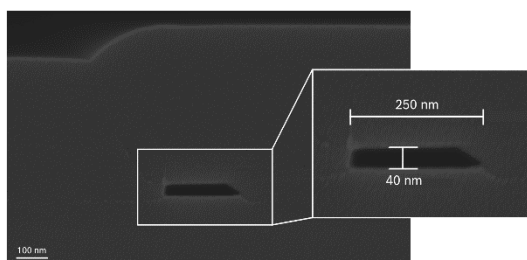


Fig. 3. Electron microscope picture of a nanochannel cross section with its dimensions.

The existence of a continuous nanochannel can be demonstrated with the help of a fluorescent solution. The solution is introduced in the upper microchannel, then fills the nanochannels by capillary forces and reaches the lower microchannel (see figure 4).

Different funnel inlet sizes can be created by varying the duration of etch step, with the funnel inlet getting larger and the angle shallower with increasing time, as seen in figure 5. The inlet size widens from roughly 200 nm nanochannel width to 1  $\mu\text{m}$ . The influence of other parameters of the etch step on the funnel shape, like

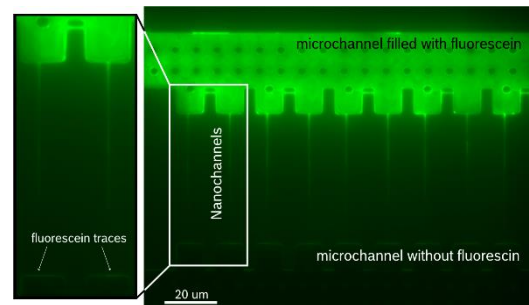


Fig. 4. Picture of a microchannel with several nanochannels. The fluorescein solution added in the upper microchannel got through the nanochannels into the lower microchannel.

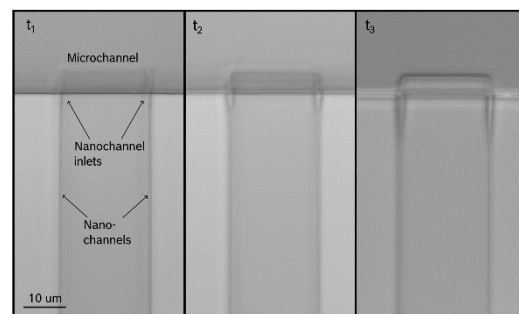


Fig. 5. Picture of funnel inlet shapes after different etching times ( $t_1 < t_2 < t_3$ ).

pressure and flow rate of the etchant will be tested to create inlets with arbitrary shapes.

In conclusion, it can be said that nanochannels with inlet structures of different sizes have been successfully fabricated. The size of the inlets can be varied by the duration of the etch step. Inlet sizes of 1  $\mu\text{m}$  have been created for a 200 nm wide channel.

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## References

- [1] J. O. Tegenfeldt *et al.*, Micro- and nanofluidics for DNA analysis, *Anal. Bioanal. Chem.* 378 (7), 1678–1692, (2004), doi: 10.1007/s00216-004-2526-0
- [2] J. Wu *et al.*, Engineering inlet structures to enhance DNA capture into nanochannels in a polymer nanofluidic device produced via nanoimprint lithography, *Micro Nano Eng.* 21 (2023), doi: 10.1016/j.mne.2023.100230
- [3] J. Zhou *et al.*, Enhanced nanochannel translocation and localization of genomic DNA molecules using three-dimensional nanofunnels, *Nat. Commun.* 8 (2017), doi: 10.1038/s41467-017-00951-4
- [4] F. M. Esmek *et al.*, Sculpturing wafer-scale nanofluidic devices for DNA single molecule analysis, *Nanoscale* 11, 13620–13631 (2019), doi: 10.1039/C9NR02979F
- [5] H. T. Hoang *et al.*, Wafer-scale thin encapsulated two-dimensional nanochannels and its application toward visualization of single molecules, *J. Colloid Interface Sci.* 367, 455–459, (2012), doi: 10.1016/j.jcis.2011.10.00