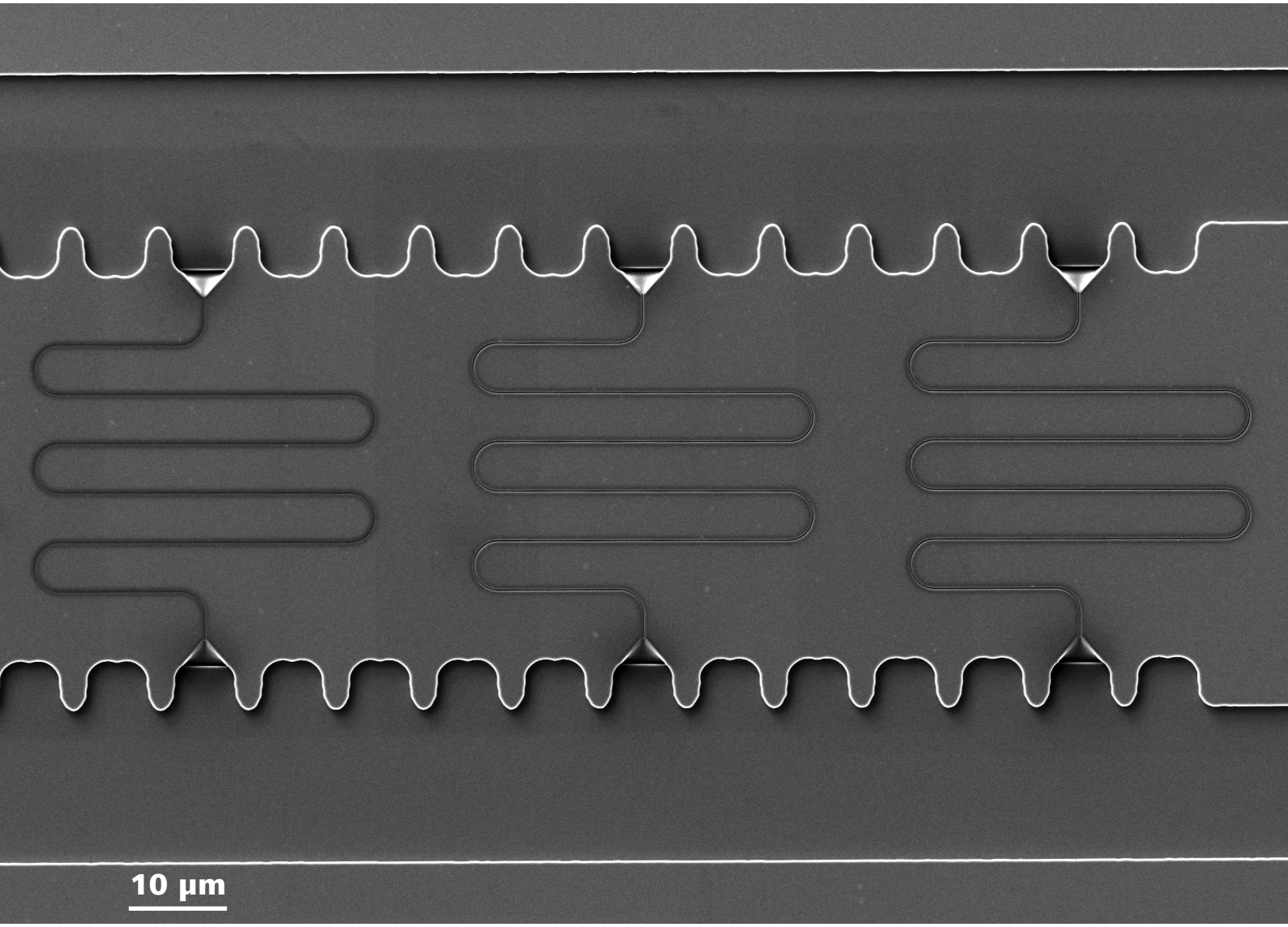
Application Note



Fabrication and Characterization of Nanofluidic Devices for DNA Optical Mapping

ZEISS

Seeing beyond

Fabrication and Characterization of Nanofluidic Devices for DNA Optical Mapping

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Nanofluidic lab-on-a-chip devices for the analysis of single DNA molecules were fabricated and characterized using FIB-SEM. Direct FIB nanopatterning of silicon master stamps allows fast prototyping of nanochannels of different shapes, cross sections and depths. Moreover, the fabrication of 3D structures, such as funnel-shaped inlets to connect the nanochannels to the much deeper and wider microchannels, is straightforward. This is essential to ensure an optimal DNA flow in the fluidic chip [1].
By means of UV nanoimprinting the pattern on the silicon master stamp is transferred to a polymer-on-glass stamp, which is used as a stamp in a second UV nanoimprint step, to create the actual chip.
In the FIB-SEM not only the master stamps can be machined, but the entire fabrication process can be monitored in a non-destructive way. ZEISS local charge compensation enables high-resolution SEM characterization of the highly insulating polymer-on-glass stamps and devices without any metal coating allowing us to use the devices after their inspection.

Introduction

Besides living habits and environmental factors, our genes determine our predisposition to suffer from certain diseases. On the other hand, the medical treatment of a disease can be more efficient if tailored to the genetics of the patient. Even though this new field of personalized medicine is still in an experimental phase, impressive results have been reported, like more effective cancer therapies [2,3], the cure of genetic diseases [4] and disease prevention by lifestyle changes [5]. These advances show that personalized medicine has the potential to revolutionize healthcare if fast and affordable DNA screening can be realized.

In the last decade, sequencing techniques have developed dramatically. However, they are still too slow and expensive to make personalized therapies accessible to the public. One promising alternative for fast, simple, and affordable analysis of single DNA molecules is optical mapping [6]. This methodology can benefit from nanofluidic chips produced by UV-nanoimprint lithography (UV-NIL) [7,8]. These devices have nanochannels, where the DNA molecules can be stretched and observed for analysis.

In this research work, the nanochannels of silicon master stamps were fabricated by focused ion beam (FIB) direct lithography with ZEISS Crossbeam 550. Using this same instrument, the subsequent, different byproducts of the UV-NIL process could be characterized by high-resolution scanning electron microscopy (SEM). A local charge compensation technique was used for the imaging of insulating materials.

Master Stamp Fabrication by FIB

Figure 1 shows an SEM image of a master stamp after FIB nanopatterning. The master stamp is made of silicon. The micron-sized fluidic structures (with dimensions of 15 µm and above) were patterned by photolithography and reactive ion etching. Much narrower channels were patterned directly into the silicon master stamp by FIB. These nanochannels are less than 100 nm wide and 38 µm long. They connect top and bottom microchannels.

In the optical mapping experiment, whole molecules or fragments of DNA are driven through the narrow channels by electrophoresis, i.e. by applying an electric potential difference between the microchannels. Since DNA has a net charge, it is driven into the nanochannels to pass from one microchannel to the other. Inside the nanochannels the DNA molecules are uncoiled and elongated, which enables the optical mapping measurement [9].

The threading and prestretching of the DNA into the channel is facilitated by the funnel-shaped inlet (see inset) [1].

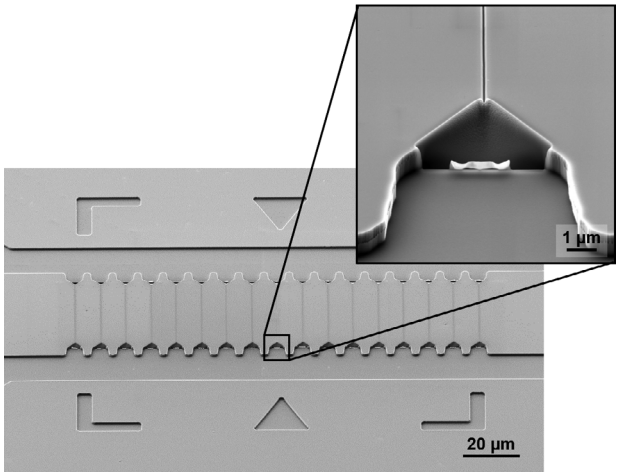

Figure 1 *SEM image of a silicon master stamp with straight nanochannels after FIB patterning. The inset shows the tapered nanochannel inlet. The sample is tilted 54°.*

Such 3D structures can be fabricated easily by FIB direct lithography with the right choice of parameters. For this application, this is a key advantage as compared to other possible nanopatterning techniques. Another advantage of FIB direct patterning is that it allows for quick prototyping. Thanks to this, the layout of the nanochannels can be easily tailored to match the requirements of the experiment. As an example, Figure 2 shows meander channels with a total length of almost 200 µm for the analysis of larger DNA fragments. The channel width decreases from left to right as a result of FIB milling dose variation.

With ZEISS Crossbeam direct control of the nanochannel FIB milling was possible thanks to live SEM imaging at the instrument's best resolution [10]. Thus, milling recipes could be optimized efficiently.

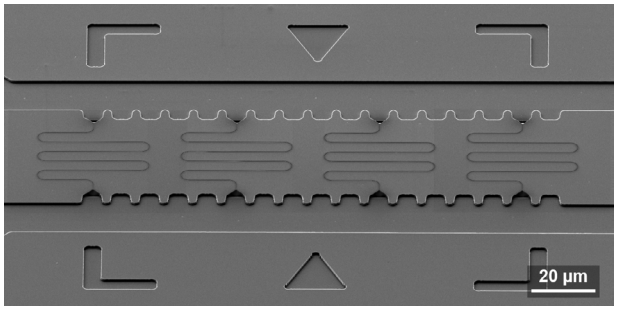

Figure 2 *SEM image of a silicon master stamp with meander nanochannels. The sample is tilted 54°.*

Process Control with Charge Compensation

The actual devices for the DNA optical mapping experiment are fabricated by means of two-step UV-NIL (see Figure 3) [7,8]. In a first step, the FIB fabricated silicon master stamp is imprinted into a polymer layer on a glass substrate, the polymer-on-glass stamp. In a second step, the polymer-on-glass stamp is replicated again in the same polymer-on-glass system. Thus, after the two steps the original geometry of the silicon master stamp is recovered on the final device. Both, the master stamp and the polymer-on-glass stamp can be reused, which makes the technique suitable for cheap mass production of nanofluidic chips.

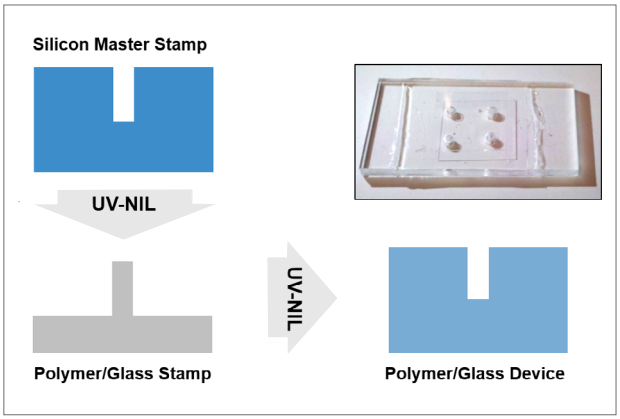

Figure 3 *Schematic of the two-step UV-NIL process for the mass fabrication of devices for DNA optical mapping.*

While SEM characterization of the silicon master stamp is straightforward, the polymer-on-glass system is challenging to image. Figure 4a shows the polymer-on-glass device imaged at 1.31 keV electron landing energy. In the SEM and even for low primary beam energies and currents, severe charging of the polymer-on-glass stamps and samples was observed.

Therefore, we studied these samples using local charge compensation. This is a charge neutralization option available on ZEISS electron microscopes.

In short, a gas injection needle is placed near the sample and dry nitrogen injected through the needle (Figure 4c). The primary beam ionizes the gas and the ions neutralize any excess charge that has built up in the sample in a self-sustaining manner. Because the nitrogen injection is local, the raise in chamber pressure is below 10^{-2} mbar and Inlens SE detection is still possible. Additionally, the short gas path length minimizes detrimental scattering effects. The result is a clear and crisp SEM image as shown in Figure 4b.

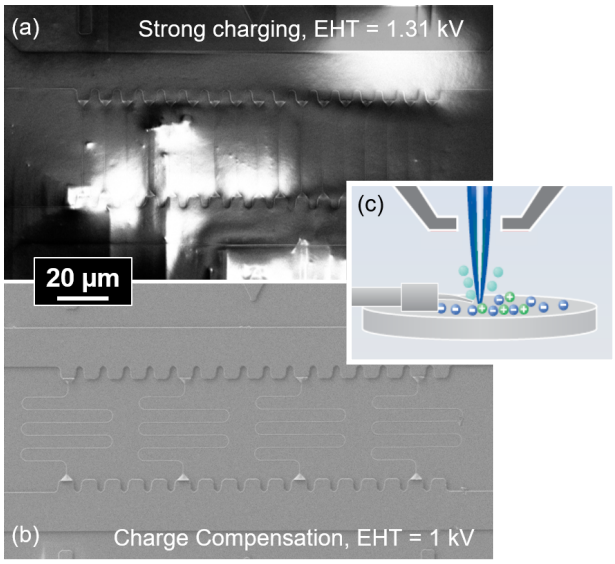

Figure 4 *SEM images of a polymer-on-glass stamp without (a) and with charge compensation (b). The inset (c) shows the principle of local charge compensation*

With local charge compensation, we could compare the polymer-on-glass stamp with the corresponding original structure in silicon. An example is shown in Figure 5. Because the polymer-on-glass stamp was not coated for SEM observation, it could be reused in later experiments.

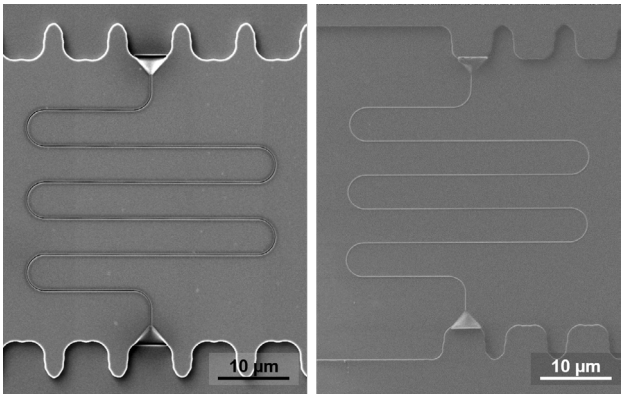

Figure 5 *SEM images of a silicon master stamp with a meander nano-channel (left) and the same structure in the corresponding polymer-on-glass stamp (right).*

Local charge compensation on the polymer-on-glass samples proved very efficient. Important details of the nano-channels could be studied with high resolution (see Figure 6), in particular, the continuity and profile of the nanochannel and the 3D shape of its two inlets.

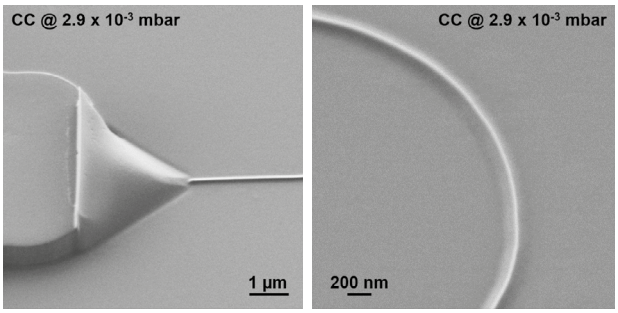

Figure 6 *Close-up images of the inlet (left) and bend (right) of a polymer nanochannel using local charge compensation.*

Conclusion

ZEISS Crossbeam is an extremely useful tool for research in the field of nanofluidics and single molecule detection. The application described in this note is a good example.

On the one hand, FIB direct lithography allowed fast and flexible prototyping of finest nanoscale structures, including 3D shapes, in the silicon master stamps. On the other hand, SEM with the same instrument allowed optimizing and monitoring of the entire nanoimprint process without interfering with it. With local charge compensation, there was no need to sputter coat the insulating polymer-on-glass stamps, and they could be reused after their quality assurance.

References:

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