Creation of micro-and nanochannels on the surface of silicon chips by lithography methods and investigation of ion transport in channel

Polina Afonicheva\textsuperscript{1}, Denis Lebedev\textsuperscript{1,2,3}, Anton Bukatin\textsuperscript{1,3}, Ivan Mukhin\textsuperscript{3,4} and Anatoly Evstratov\textsuperscript{1}

\textsuperscript{1}Institute for Analytical Instrumentation RAS, Ivana Chernykh 33A, 198095, St. Petersburg, Russia
\textsuperscript{2}Saint Petersburg State University, 7/9 Universitetskaya nab., 199034, St. Petersburg, Russia
\textsuperscript{3}St. Petersburg Academic University, 8/3 Khlopina Street, 194021, St. Petersburg, Russia
\textsuperscript{4}ITMO University, 9 Kronverksky pr., 197101, St. Petersburg, Russia

E-mail: polina.afonicheva@gmail.com

Abstract. We developed a technique for fabrication microfluidic silicon-glass chips with a system of nanochannels connecting two microchannel using traditional optical lithography and a focused ion beam. To investigate the transport phenomena in the nanochannels we experimentally studied their ion conductivity and using optical microscopy confirmed the existence of the diffusion flow through them. The developed method allows us to create systems of nanochannels with on-purpose geometry and controlled sizes. Devices with such nanochannels can be applied in the creation of biosensor devices and for genetic studies.

1. Introduction
Through development of methods and technologies for making micro-and nanostructures various processes studies became possible at the micro-and nanoscale [1]. Microfluidic chips (MFC) with integrated micro- and nanoscale structures are currently widely used for biological and medical application, such as analysis and selective detection of nucleic acids and proteins [2], DNA sequencing [3] and etc. Modern technologies allow one to integrate nanoscale structures (for example nanochannels, nanowhisker) into the microfluidic device (MFD) [4]. This approach allows us to study biophysical and biochemical processes at a qualitatively new level. Currently, the study of molecular and ion transport in microfluidic systems containing nanopores and nanochannels is very popular, especially in genetic study application [5]. These studies are in demand for the development of highly sensitive chemical and biochemical sensors [6-8], as well as for nucleic acid sequencing devices [9]. The creation of artificial (biomimetic) equivalent of living nanofluidic systems is promising, at that the study of the transport mechanisms in nanochannels is especially important [10].

Microfluidic devices also offer a unique solution to many of problems currently encountered in genetic analysis. Microfluidic systems have various benefits compared with their large-scale...
counterparts, including low cost, disposability, low reagent and sample consumption and possibility for automation to be implemented [11].

There are several main approaches that can be used for the formation of the artificial nanochannels in MFD [12, 13], such as conventional photolithography [14] and interference lithography [15], also nanoimprinting [16] or thin film deposition [17].

The first two methods allow us to fabricate nanochannels from different materials with on-purpose geometry, but due to the nature of processes it is complicated to get nanochannels with sizes less than 50 nm, limited by the wavelength of UV source used. A highly efficient techniques for obtaining single nanochannels are ion and electron beam lithography (especially focused ion-beam milling, FIB) [18]. Applying this technique, one can get channels of various geometries with a resolution of up to several nanometers [19].

The aim of the current study was to develop a comprehensive approach to the creation and research of a silicon-glass microfluidic chip containing a system of nanochannels.

2. Experiment and results
The MFD was made from silicon by a combination of optical lithography and the focused ion beam (FIB) local etching and was subsequently encapsulated by glass. We choose a X-shape topology for chip that consists of two microchannels and a thin membrane. Thin membrane separates the left and right parts of the chip, the nanochannels were formed in this membrane. The estimated membrane thickness is 10-15 µm. In each microchannel an inlet and an outlet holes needed for loading working solutions were formed in glass. Such system provides two-way flow in each channel.

Before the photolithography process the silicon surface underwent thermal oxidation in an oxygen medium at a temperature of 1100°C. The thickness of the obtained oxide layer was 200-300 nm though it generally depends on the oxidation time. The layer of silicon oxide is required on the surface, as it is a chemically and biologically inert material with well-studied surface properties in aqueous salt solutions.

To obtain a network of nanochannels and control the FIB etching process, a CrossBeam Neon40 (Carl Zeiss) crossed electron and an ion beam systems were used. We used gallium ions at an accelerating voltage of 30 kV. An ion beam current of 10 pA was used for etching the network of nanochannels. The etching was carried out along a segment with a length of more than 10 µ and a width of 1 pixel. The etching time of one channel was varied from 10 to 60 sec. If the etching time was less than 10 seconds, the channels depth was not reproducible. To investigate how the etching time influences the nanochannels width we used atomic force microscope (AFM) Bruker Bioscope Catalyst. Figure 1a shows the cross-section of a single channel obtained with an exposure time of 10 sec. It follows from the obtained image that the nanochannel has a wedge-shaped shape with a width at the base of 80 nm and a depth of 50-60 nm. It worth noting that the actual sizes of the nanochannel can be in fact smaller. This is because in AFM studies there is an effect of convolution of the probe profile with the surface profile. The dependence of the nanochannel width on the exposure time is shown on Figure 1b obtained by AFM. It can be seen that the dependence is linear, with the minimum channel width being 50-60 nm. The nanochannel depth in all cases was about 80 nm. The independence of formed channels depth on the etching time indicates that the material redeposition during the FIB etching indeed influences the process.

The developed technique made it possible to create a chip with two microchannel systems separated by nanochannels. The depth of the microchannels ranged from 5 to 10 microns. The geometric parameters (depth) of the nanochannels varied from 20 to 100 nm.

In addition, the sealed MFD can be reused after cleaning the channels. Polydimethylsiloxane (PDMS) were used as fittings to attach the supply capillaries and electrodes to the MFD (Figure 2a). In figure 2b scheme of the microfluidic silicon-glass chips is shown, the flow directions are indicated by arrows. COMSOL Multiphysics® was used for simulation of the velocity distribution.
To study the transport properties of the formed nanochannels, the method of measuring the ionic conductivity was used. Using a two-channel Harvard Apparatus PHD 2000 syringe pump, an aqueous KCL solution of a given concentration was injected into both halves of the microfluidic system. For all experiments Milli-Q deionized water was used. After the solution was infused into the channels, the chip was left to soak in the solution for at least half an hour. Then silver-chloride electrodes were installed in the chip by PDMS fittings. It is worth mentioning that the surface of the silver electrodes was refreshed by chlorinating the silver wires in 1M KCl solution before each experiment.

Before the measurements, the MFC was placed in a Faraday cage (a metal box). The nanochannel ion conductivity was determined by measuring the voltammetric curve in the potentiostatic mode. It is important to note that at low electrolyte concentrations (less than 0.01 M) hysteresis was observed. This hysteresis is related to the capacitance component of the nanochannel resistance, which is caused by the accumulation of counter ions at the entrance and exit of the channel during rapid potential unfolding. To eliminate this effect, we increased the measurement time. In this way we obtained a
The linear I–V curve in all cases. In the same time the linear character of the I–V curve was remained even at high bias voltages (-1 ... + 1 V).

The typical period of measuring the nanochannels conductivity was 1 week. During this period the electrolyte concentrations was varied from lower to higher values. Between experiments the chip was kept filled with clean water in a sealed box at a humidity close to 99%.

Figure 3 shows the typical dependence of nanochannels ionic conductivity on the solution concentration for chips with different nanochannel etching times. The experimental points were measured on a chip with 15 parallel channels. At low concentrations of working solution, the double electrical layers overlap inside the pores, so the conductivity changes slightly with increasing concentration. However, at high concentrations the thickness of the double layers decreases and the overlap disappears, so in that case the conductivity increases.

In addition to the experimental measurement of the ion conductivity we also analyzed the properties of nanochannels using optical microscopy. To study the solutions diffusion through nanochannels we used an experimental setup, which consists of a Leica TCS SL confocal laser scanning microscope needed for observation in the transmitted light mode and a Harvard syringe pump needed for the supply of liquids at constant speeds. A 0.1M KCl solution was injected into one half of the microfluidic chip, and a fluorescein (FITC) solution with a concentration of $10^{-5}$ mol/l in 0.1M KCl was infused into the second half. After the solutions were loaded into the channels, the MFC was left for some time for soaking. The image obtained from the microscope (Fig. 4) shows that through the nanochannels the KCl solution evenly spreads out in all directions into the channel where the FITC solution was located. Hence we confirmed the diffusion of fluids through nanochannels in a fabricated MFD.

![Figure 3. The dependence of ionic conductivity of the nanochannels on the concentration of the working solution](image1)

![Figure 4. Diffusion of the KCl flow (left part of the image) through nanochannels into a microchannel (cell) with FITC (right part of the image).](image2)

3. Conclusion

In this work, we developed a technique for fabricating microfluidic silicon-glass chips with a system of nanochannels connecting two independent microchannel(microchamber) using traditional optical lithography and a focused ion beam. The MFD were sealed with glass plates using an anode bonding technique. This approach makes it possible to extend the service life of such chips up to a year without significant change of its properties. The nanochannels ion conductivity was studied experimentally, and the effective diameter of the nanochannels was determined to be in range of 20-50 nm. The surface charge density inside the channel was about 1.5 mC/m$^2$. We also carried out the
experiments using optical microscopy and confirmed that there is a flow of liquid through the formed nanochannels.

The proposed method allows us to create systems of nanochannels with on-purpose geometry and controlled sizes. Devices with such nanochannels can be widely used in the study of the ion transport and various molecules through nanostructures, as well as in the creation of highly sensitive biosensor devices.

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