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Fabrication of nanofluidic devices using glass-to-glass anodic bonding

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Abstract

In this work, we present a technology for fabrication of nanochannels created in glass with which bio-analysis can be performed in combination with fluorescence microscopy. The technology is based on a glass-to-glass anodic bonding process. In the bonding process, an intermediate layer (thin insulating film) is deposited on one of the two glass wafers. The channel is then defined, with one or two photo-patterning steps, in the intermediate layer. In our approach, a 33 nm thick amorphous silicon layer deposited by low-pressure chemical vapor deposition (LPCVD) was used as an intermediate layer. The depth of the channel is defined during the etching of this layer.

Keywords: Nanofluidic device; Nanochannels; Anodic bonding; Fluorescence; Separation device

1. Introduction

The fabrication of nanochannels has gained considerable attention in the last few years [1] because of the growing interest in detection and manipulation of single molecules. Recent advances in optical imaging and biomechanical techniques show a great potential for the investigation and observation of biological processes occurring at the molecular level. These techniques are finding an increasing use in both biological research and biomedical applications.

In biology, microfluidic systems have the potential for application such as separation of biomolecules [2], enzymatic assays, and immunohybridization reactions [3]. Possible biomedical applications of microfluidics (and nanofluidics) include areas such as drug discovery and high-throughput screening of patients [4].

As far as detection is concerned, fluorescence microscopy was proven to be a sensitive and reliable technique, and it is being used for a large number of quantitative applications [5,6]. However, in order to perform optical measurements, the nanostructure (or at least one side of it) must be transparent to light. This imposes further restrictions on the design and manufacturing of the nanochannel device.

We propose in this paper a new simple technology to fabricate nanochannels in glass, preserving the advantages of the fluorescence microscopy being used for optical detection. Therefore, the fluidic devices described here are suitable in both biological research and biomedical applications.

2. Fabrication

The fabrication process of the proposed nanochannels is shown in Fig. 1. For the glass-to-glass anodic bonding an intermediate layer (thin insulating film) has to be deposited on one of the glass wafers [7]. The nanochannels are defined in this intermediate layer by using a photo-patterning technique in one single step.

The glass wafers have to be rich on sodium oxide, which is required in order the anodic bonding to be successful. The top wafer may have pre-drilled (or lasered) holes at pre-defined positions as an access routes to the channels. In our experiments we used borofloat type glass wafers from Bullen Ultrasonics, USA, with 1 mm ultrasonically machined holes.

Next, a thin 33 nm amorphous silicon layer was deposited by low-pressure chemical vapor deposition (LPCVD) as an intermediate layer on the bottom glass wafer. Although, the bonding can be successfully performed using other intermediate layers, due to the low deposition rate amorphous silicon was chosen as it can be deposited to the required thickness,

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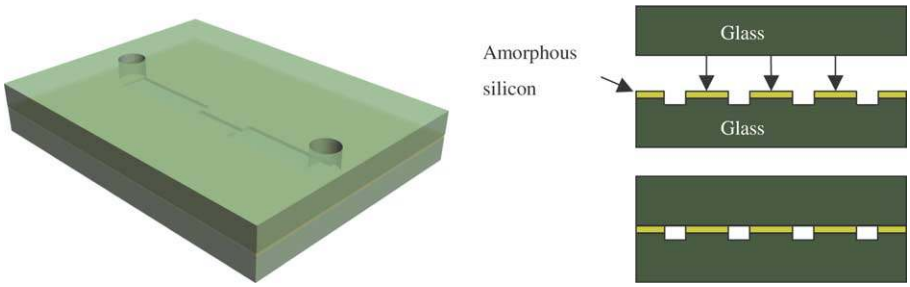

Fig. 1. Left: artist impression of a nanochannel in glass. Right: fabrication process for nanochannels in glass.

Table 1
Results provided for various layers, as an intermediate layer, for the glass-to-glass anodic bonding (+ indicates strong bonding, and − indicates no strong bonding obtained)

Layer 2	Layer 1					
	None	Polysilicon	Nitride	Oxide	Amorphous silicon	Carbide
None	−	−	−	−	−	−
Polysilicon	+	−	−	+	−	−
Nitride	+	−	−	+	−	−
Oxide	−	−	−	−	−	−
Amorphous silicon	+	−	−	+	−	−
Amorphous silicon/nitride	+	−	−	+	−	−
Carbide	+	−	−	+	−	−
Nitride/oxide	+	−	−	+	−	−
Amorphous silicon/oxide	+	−	−	+	−	−

which still allows a successful bonding and makes it impossible to separate the bonded wafers without breaking them. Bonding results, provided for different intermediate layers has been published elsewhere. Table 1 gives an overview of the results achieved with different combinations of intermediate layers [7]. The layer indicated as "layer 1" is deposited on the top wafer (cathode wafer) and the layer, indicated as "layer 2" is the layer on the bottom wafer (anode wafer). The layer coating on the bottom glass wafer can also be a combination of two layers. Successful bonding has been achieved with one (layer 2) or two intermediate layers (layers 1 and 2). However, no good results were achieved when there is no intermediate layer on the bottom wafer.

After the deposition, the intermediate layer is patterned using a photoresist mask. Fifty nanometer deep channels were created by dry etching through the amorphous silicon layer and slightly into the substrate glass wafer in an Alcatel GIR 300 fluoride etcher. The plasma parameters used for the etching process were: $CF_4 : SF_6 : O_2 = 70/10/10$ sccm, power −60 W and pressure 50 μbar. The etching time was 30 s. Afterwards the photoresist mask was stripped in an oxygen plasma.

Prior to the bonding the wafers are cleaned in HNO_3 solution. This cleaning step is essential for successful bonding and making the surface of the glass hydrophilic. The bonding is performed on EVG501 wafer bonding system. To guarantee a good bonding the wafers are preheated for 2 h at 400 °C and bonded at the same temperature at 1000 V for 1 h [8]. Few conditions have to be fulfilled for successful glass-to-glass anodic bonding. Beside the requirements on the glass composition and the intermediate layer, the glass wafer surface should be kept free from particles, have superior finish, and be parallel to enable the anodic bonding. The glass wafers used in this experiment have a surface roughness less than 30 Å and are parallel within 9 μm. Although these requirements are very strict, they are much lower than those for the fusion bonding process.

Photos of typical fabricated channels with the described technology are shown in Figs. 2–4. Figs. 3 and 4 show holes in the upper glass plate, which can be used for filling the channels with liquid.

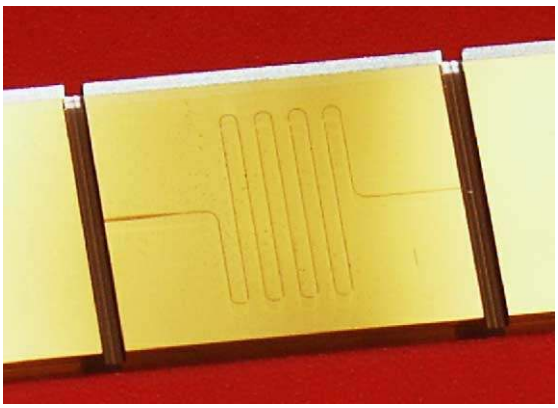

Fig. 2. Photo of a fabricated channel. The channel is 40 μm wide and 50 nm deep.

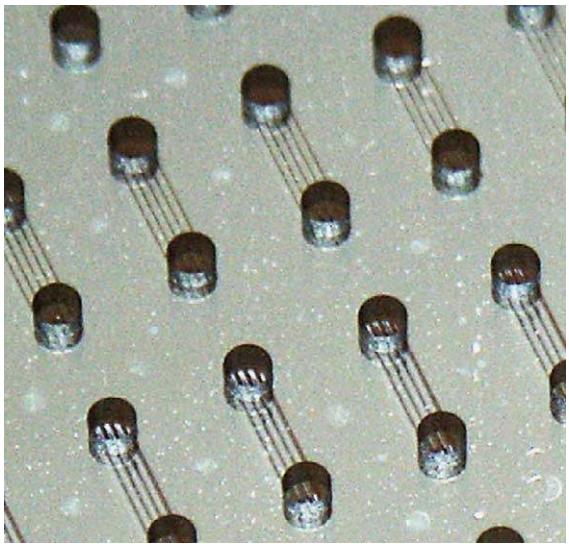

Fig. 3. Photo of fabricated channels that are 3 mm long and 50 nm deep. The smallest fabricated channel width is 2 μm and the largest 100 μm.

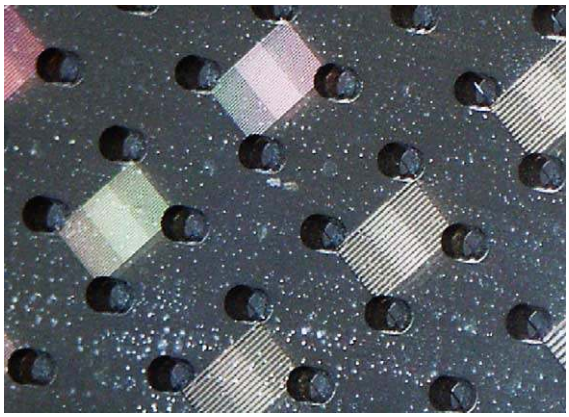

Fig. 4. Photo of the fabricated channels that are 50 nm deep and have different widths ranging from 2 to 100 μm.

If after the first patterning and plasma etching of the intermediate layer a second photo-patterning step is applied followed by plasma etching then it is possible to create channels with two different depths as shown in Fig. 5. Such device with many entropic traps can be used, for an example, as a nanofluidic separation device of long DNA molecules [9]. Photos of fabricated channels with two different depths are shown in Figs. 6 and 7. Main limitation for the maximum channel depth that can be etched is the selectivity of the masking layer. Using a photoresist mask we were able to fabricate 500 nm deep channels.

3. Results and discussion

The quality of the channel cross section was inspected by SEM (Figs. 8 and 9). In order to overcome the minimum inspection size limits, we have enlarged the openings of the channels by etching the glass-amorphous silicon–glass interface in a buffered HF 1:7 solution for 7 min and 15 s. The size of the channel openings was increased then from 50 to 250 nm. The SEM images showed no obstructions in the channels.

Further, fluorescence microscopy measurements were conducted to ensure that the channels were hydrophilic. The openings on one side of the channels were filled with a fluorescence dye.

The fluorescence measurements were performed on a standard epi-fluorescent microscope (Leica, Wetzlar, Germany) with a matching filter cube for each fluorescent dye (i.e. both excitation and emission were performed from the top side of the sample). The signal was detected through a 20× (NA = 0.45) objective with a large working distance and a high-performance CCD (ORCA-ER, Hamamatsu, Japan) and a Xe light source was used for the various excitations. The signal was usually bright enough and relatively short exposure times were required for detection (typically 20–100 ms). Therefore, movies at the rate of few images per second were easily recorded with high enough signal-to-noise.

No pushing or sucking force was applied at the channel opening during the measurements. Slow penetration of the liquid by capillary forces into the channels was observed. After a short time the fluorescence reached the opposite side of the 3 mm long channels and appeared in an access hole as shown in Fig. 10.

After testing different fluorescence dyes we found that Rhodamine 6G was the best candidate for our optical detection (excitation and emission peaks are at 530 and 560 nm, respectively).

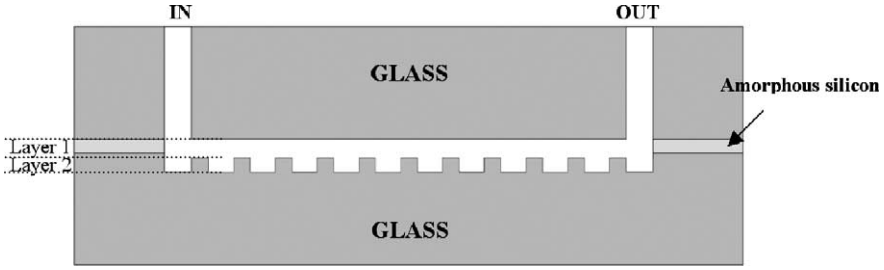

Fig. 5. Sketch of a cross-section of a device consisting of many entropic traps. Layer 1 is formed during the first plasma etching and layer 2 is formed during the second plasma etching.

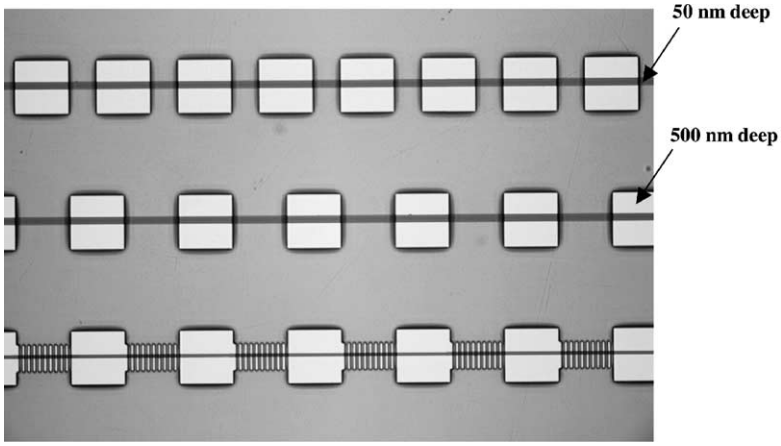

Fig. 6. Photo, taken with an optical microscope of the top of the nanofluidic separation device (the channel depths are, respectively 50 and 500 nm).

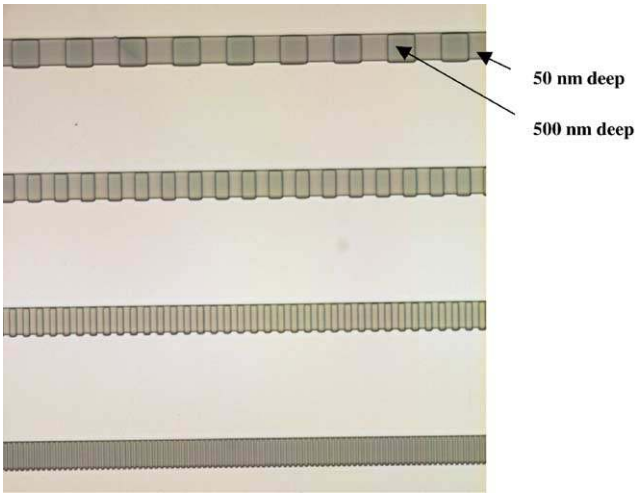

Fig. 7. Photo, taken with an optical microscope of the top of the nanofluidic separation device (the channel depths are, respectively 50 and 500 nm).

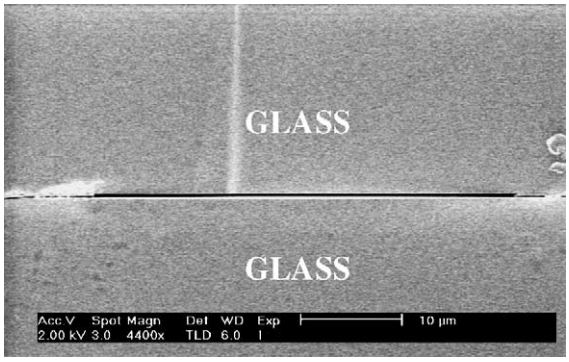

Fig. 8. SEM cross-section of the channel (50 nm × 40 μm). The channels have been treated in BHF 1:7 for 7 min and 15 s in order to improve the contrast between the glass wafer and the amorphous silicon, and to enlarge the opening of the channels. Due to the selectivity between glass and amorphous silicon, the etching in BHF resulted in wider channels only in the vertical direction (approximately 250 nm on the SEM image).

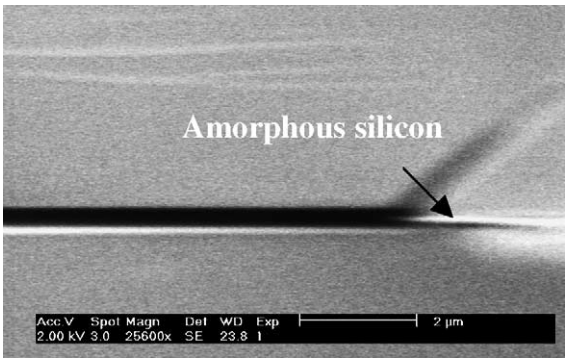

Fig. 9. SEM close-up on the right side of the opening of the same channel, shown in Fig. 8. The amorphous silicon layer has a thickness of 33 nm.

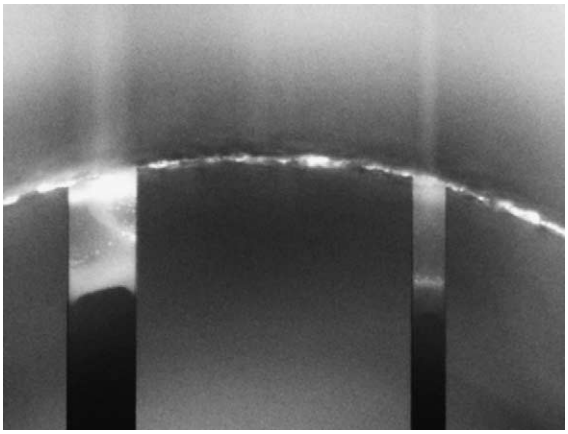

Fig. 10. Image of the fluorescence that has come out on the other side (opening) of the channels. The channels on the photo are, respectively 25 and 50 μm wide, 3 mm long, and 50 nm deep.

During our filling experiments of the channels we measured the change of the fluorescence intensity from the top. We detected a very interesting phenomena, as recorded in Fig. 11: once the channels were already filled (first phase), in less than a minute we detected a second spontaneous wave of filling of the channels. This second phase of filling of the channels was recorded as a sudden increase in the fluores-

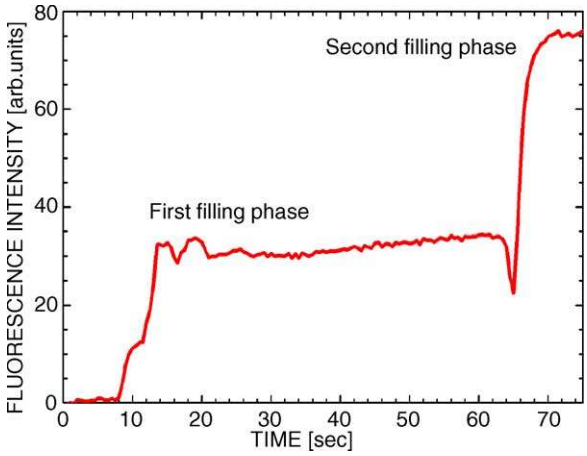

Fig. 11. Fluorescence during channel filling.

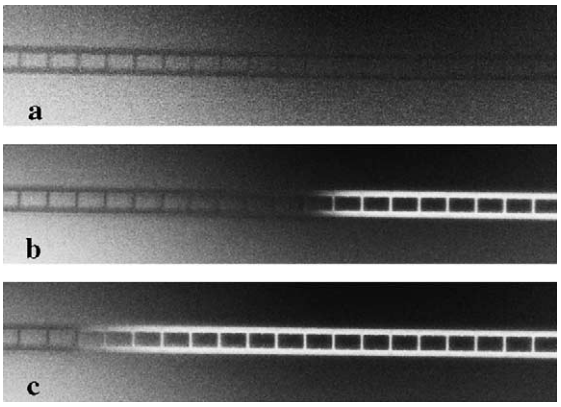

Fig. 12. Sequence of images taken at intervals of several seconds showing the first phase of the filling of the channels. The first image shows completely empty channels, which become filled with Rhodamine 6G during the next couple of seconds. The channels are, respectively 2 and 5 μm wide, and 50 nm deep.

cence intensity due to the increase in the fluorescence dye layer thickness.

In Fig. 12 we show how the filling of ladder type channels occurs in time. The fabricated fluidic devices used for this experiment are shown in Fig. 4, while Fig. 13 presents the sketch of one of those devices. The first image in Fig. 12 (Fig. 12a) shows a channel which is empty and therefore no fluorescence intensity can be observed. On the next image (Fig. 12b) it is shown that the fluorescence has already penetrated into almost half of the channel while on the last image (Fig. 12c) the fluorescence has almost reached the end of the channel.

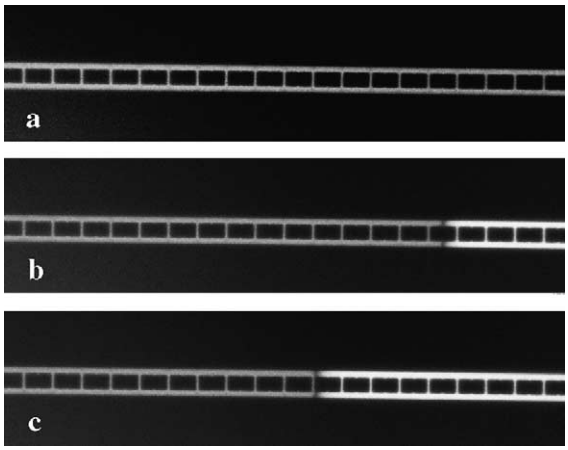

Fig. 14. Sequence of images taken at intervals of several seconds showing the second phase of the filling of the channels. The first image shows already filled channels with Rhodamine 6G, while in the next couple of seconds a second wave of Rhodamine 6G, filling the channels completely can be observed. The channels are, respectively 2 and 5 μm wide, and 50 nm deep.

Fig. 14 shows the filling during the second phase. The first image in this figure (Fig. 14a) illustrates the same channel from Fig. 12, which is already filled with fluorescence. The next image (Fig. 14b) shows the second wave of the filling of the channel with fluorescence, which has penetrated about one quarter of the way along the channel. On the last image (Fig. 14c), the second wave has penetrated almost to the middle of the channel.

The second phase of filling of the channels is most likely due to the nature of the channel surface. As it is shown in

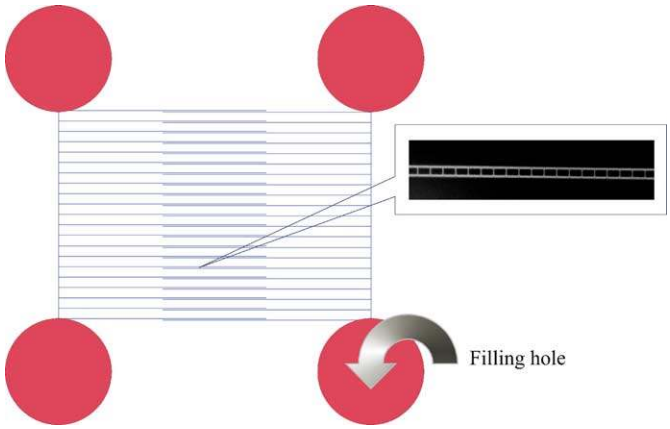

Fig. 13. Sketch of the fluidic device, shown in Fig. 4.

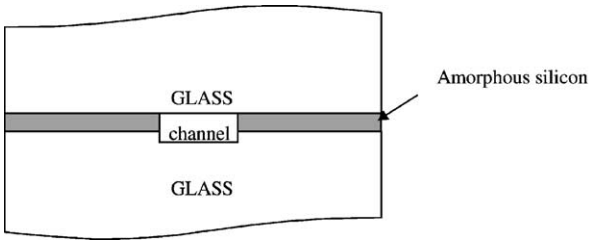

Fig. 15. Sketch of a cross-section of the tested in Figs. 12 and 14 nanochannels. The thickness of the amorphous silicon is 33 nm, while the channel depth is 50 nm.

Fig. 15 the sidewalls of the channel consist of two different surfaces: glass and amorphous silicon. In this measurement the tested channels are 50 nm deep, but the amorphous silicon layer is 33 nm, which is 66% from the channel height. The glass in our case is hydrophilic (wettable) and it imparts a high affinity for water molecules, while the amorphous silicon is hydrophobic and it repels the water molecules. The top and the bottom of the channels are made of glass and therefore they are hydrophilic. We believe that due to the wetting properties of the channel the fluid might fill first the bottom part of the channel which is hydrophilic and after some short time, when the fluid reaches the top of the channel, it completely fills the channel and this was recorded in our experiments as a second wave.

4. Conclusions

We have demonstrated a simple process to fabricate nanochannels in the intermediate layer, deposited on one of the two bonded glass wafers. This intermediate layer is not limited only to the use of amorphous silicon, but also other thin layers can be successfully used.

We have successfully fabricated, using standard UV lithography, and tested by filling with fluorescence solution channels, which are 50 nm deep and having a width starting from 2 up to 100 μm. We have successfully fabricated also nanofluidic separation devices with many entropic traps, which could be used for separation of long DNA molecules.

The channels have a rectangular shape and the channel depth is defined only by controlling the etching time. The channel width is restricted by the physical limitations of the lithography step but smaller structures (even around 50 nm wide) are feasible by using electron beam lithography.

The channels are transparent which makes them a very good candidate for nanoscale devices in which to conduct biochemical experiments, while the data can be collected via optical detection.

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Biographies

Vladimir Kutchoukov was born on 18 February 1973 in Sofia, Bulgaria. He received M.Sc. degree in Microelectronics from the Technical University of Sofia in 1996. During his last year in the university he spent 3 months at the Technical University of Ilmenau, Germany where he wrote his Master's thesis on "Electroplating of Copper in Silicon Wafers". After his compulsory military service in 1998 he joined the Laboratory of Electronic Instrumentation at the Delft University of Technology, The Netherlands, where he worked towards his Ph.D. degree in the field of wafer level packaging. In 2002, after his Ph.D., he became a postdoc at the same laboratory working on a project for nanoscale electrophoresis for biomolecular technologies.

Frederic Laugere was born in Angouleme, France, on April 16, 1974. In 1992, he started to study electronics at the Technical University of Kourou, French Guyana. He moved to Bordeaux, France, in 1994 to follow his studies at the Sciences University of Bordeaux. In 1998 he received his Master Science degree at the National School of Electronics, Informatics, and Radioelectricity from Bordeaux (ENSEIRB) with a specialization in microelectronics. He joined the Electronic Instrumentation Laboratory as a Ph.D. student in august 1998, and he worked on a development of a conductivity detector for application in capillary electrophoresis. In 2003 he obtained his Ph.D. degree.

Wim van der Vlist was born on June 9, 1948. He started in September 1964 in the service group of the Department of the Electrical Engineering,

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Lukasz Pakula was born in Jelenia Gora, Poland, in 1976. He studied at Wroclaw University of Technology, Poland, in Faculty of Electronics, where he received his M.Sc. degree in Electronics and Telecommunication in 2000. His scope of education was Microelectronics and Electronics Devices. Subject of his M.SC. thesis was "Thick Film Heating Elements Made in LTCC technology". Since August 2000 he is a Ph.D. student in Electronic Instrumentation Laboratory at Delft University of Technology where he works on "Development of Post-Processing Micromachining Modules for Integrated Sensors and Actuators Applications".

Yuval Garini received his BsC, Master of arts and Ph.D. degrees at the Physics Department, Technion, Haifa Israel and graduated in 1992. From 1993 to 2001 he was employed at Applied Spectral Imaging, Israel where he developed novel bio-physical imaging methods for various life sciences applications. In 2002 he joined the Imaging Science and Technology department of the Applied Sciences Faculty at the Delft University, The Netherlands as an Assistant Professor. His research interests are mainly in optical biophysics and include the development of high resolution optical microscopy, single molecule detection, and the application of imaging methods to genetic studies.

Andre Bossche was born in Rotterdam, The Netherlands, in 1956. He received the MSc degree in Electrical Engineering with honors in 1983, and the PhD degree in Electrical Engineering in 1988, both from Delft University of Technology, The Netherlands. He is Associated Professor at the Department of Electrical Engineering, Mathematics and Computer Science (EEMCS) of the Delft University of Technology. His project group is engaged in research work on the subjects of Integrated Sensors, Nano-Fluidic Devices and Packaging Technology at the Laboratory of Electronic Instrumentation of the Delft Institute of Micro-Electronics and Submicron Technology (DIMES). He is author or co-author of two books and more than 75 scientific papers in journals and conference proceedings. He is also associated editor of IEEE Sensors Journal.