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Fabrication and interfacing of nanochannel devices for single-molecule studies

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Abstract

Nanochannel devices have been fabricated using standard micromachining techniques such as optical lithography, deposition and etching. 1D nanochannels with thin glass capping and through-wafer inlet/outlet ports were constructed. 2D nanochannels have been made transparent by oxidation of polysilicon channel wall for optical detection and these fragile channels were successfully connected to macro inlet ports. The interfacing from the macro world to the nanochannels was especially designed for optical observation of filling liquid inside nanochannels using an inverted microscope. Toward single-molecule studies, individual quantum dots were visualized in 150 nm height 1D nanochannels. The potential of 2D nanochannels for single-molecule studies was shown from a filling experiment with a fluorescent dye solution.

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1. Introduction

Recently, several single-molecule studies using nanochannels have been reported. For instance, single DNA fragments were confined and detected in 500 nm diameter silica capillaries [1]. Interactions of single DNA and protein molecules were studied in 120 nm × 150 nm fused silica channels [2]. Single rhodamine labeled cellulase enzyme was detected in 100 nm diameter glass nanochannels [3]. In single-molecule studies, individual molecules need to be distinguished and identified. Discrimination of single molecules [4] can simply be achieved by preparing extremely dilute solutions, containing in average only one molecule per detection volume. However, for biological applications this is undesirable because usually biomolecules are only functional at much higher concentrations, similar to those present in a cellular

environment [5]. Moreover, by extreme dilution the contribution of background signals from solvent molecules relative to the signals of the molecules of interest is enhanced. Another approach is to enable single-molecule studies by reduction of the detection volume. The detection volume can significantly be reduced by optical methods such as confocal fluorescence microscopy using small pinholes to minimize the detection of out-of-focus light [6]. Total internal reflection fluorescence microscopy (TIRFM) can also be used, where excitation only takes place in a limited field formed by evanescent waves [7]. Another option would be near-field scanning optical microscopy (NSOM) [8], where the detection volume is determined by the narrow aperture of an optical fiber probe. These optical techniques provide a detection depth as small as 100 nm. If nanochannels are used for sample confinement, the channel height can be reduced even further

Table 1. Overview of 1D nanochannel fabrication methods.

Nanochannel pattern	Materials	Etching/Deposition	Height	References
Optical lithography	Silicon	OPD solution, HF:NH ₄ F:H ₂ O	50 nm	[25–28]
Optical lithography	Silicon	BHF etching of SiO ₂ + local oxidation	70 nm	[29]
Optical lithography	Silicon	RIE	90 nm	[30]
Optical lithography	SiO ₂ , amorphous Si	BHF solution	150 nm	[31], this work
Optical lithography	SiO ₂	BHF + double thermal oxidation	75 nm	[9]
EBL	Silica	RIE (CHF ₃ /O ₂)	70 nm	[32]
Optical lithography	Fused silica	RIE	40 nm	[33, 34]
Optical lithography	Silicon, glass	RIE for silicon BOE for glass	20 nm	[35, 36]
Optical lithography	Pyrex	BHF	6 nm	[37]

Table 2. Overview of 2D nanochannel fabrication methods.

Nanochannel pattern	Materials	Etching/Deposition	Dimensions	References
FIB	Silicon (SiN), glass, quartz, fused silica	FIB	50 nm × 50 nm	[2, 12, 13]
EBL	Silicon (SiO ₂ , SiN), fused silica	RIE (CHF ₃ /O ₂ , CF ₄ :CHF ₃)	50 nm × 50 nm	[14–16]
NIL	Silicon (oxide), fused silica	RIE (CHF ₃ /O ₂)	10 nm × 50 nm	[17–19]
NIL+ diffraction gradient lithography	Silicon	RIE (CHF ₃ /O ₂)	10 nm × 50 nm	[38]
Interferometric lithography	Silicon	RIE (CHF ₃ /O ₂)	100 nm width & 500 nm height	[39]
Sacrificial etching	Silicon, silicon oxide, polymer	(B)HF, RIE, heating	30 nm height & 200 nm width	[3, 40], this work
Local oxidation	Silicon oxide	RIE (CH ₄)	150 nm × 200 nm	[41]
Electrochemistry	Silicon	KOH	30 nm diameter	[20]
Scanned coaxial electrospinning	Silica	Deposition	20 nm diameter	[21]
Thermo mechanical deformation & CO ₂ laser based puller	Polymer & silica glass capillaries	Pulling	400 nm diameter	[22, 23]
Chemical mechanical polishing	Silicon oxide	BOE	25 nm wide & 100 nm height	[24]
Nonconformal deposition	Polymer	Deposition	100 nm size	[42]

toward detection depths as small as 5 nm [9, 10]. As said, the ultra small detection volume of nanochannels enables single-molecule experiments at relatively high concentrations. Furthermore, carrying out single-molecule experiments in nanochannels does not require immobilization of molecules and offers the possibility of exactly controlling and manipulating the sample conditions. In addition, the benefits of nanochannel devices may be exploited for high-throughput applications.

Nanochannels can be relatively easily fabricated using bulk, surface, mold and bond micromachining techniques [43]. Using bulk and bond machining, nanochannels are created by etching trenches in a substrate that are closed by bonding to another substrate [25, 36]. Surface channels can be formed on a substrate with deposited layers (sacrificial and structural layers) after selectively etching of the sacrificial layer [44, 45]. In mold/bond machining, a mold formed on a substrate is filled by a desired layer, and then the mold is removed to release channels which are closed by bonding the replica to another substrate (for a detailed review [11]). 1D nanochannels are created by etching shallow trenches after standard lithography, while 2D nanochannel patterns are generally obtained using nanolithographic techniques such as focused ion beam (FIB) lithography [2, 12, 13], electron beam lithography (EBL) [14–16] and nanoimprint

lithography (NIL) [17–19]. Although nanochannels with two dimensions down to 10 nm have been successfully fabricated with nanolithography techniques, drawbacks are the high costs, low throughput and pattern limitations. Alternatively, other techniques such as electrochemistry [20], electrospinning [21], mechanical deformation [22, 23] and chemical mechanical polishing [24] are also employed for 2D nanochannel fabrications. The latter techniques however have drawbacks such as precisely controlling channel sizes and integration with other fluidic components. Tables 1 and 2 give an overview of various methods applied for the construction of nanochannels.

In this work, we show that 150 nm height 1D nanochannels, created using the silicon oxide spacer layer method [46], can be bonded to blank thin glass wafers with suitable thickness for using high numerical aperture (NA) lenses. Fluidic filling holes for the 1D nanochannels were created on silicon wafers with nanochannel structures to enable optical observation using a microscope with inverted configuration. Without using expensive nanolithography, but by a combination of standard micromachining techniques such as optical lithography, deposition and selective etching, 2D nanochannels were created with well-controlled dimensions of 50 nm height and 400 nm width. Fabricated 2D nanochannels were integrated with inlet ports and made transparent for

Figure 1. Process outline for fabrication of 1D nanochannel devices.

optical detection by oxidizing the polysilicon channel wall. Deep reactive ion etching (DRIE) was used to fabricate inlet/outlet ports for 1D and 2D nanochannels. Toward single molecule applications individual quantum dots in a 12 nM concentration solution were visualized in 1D nanochannels; filling and observation of 2D nanochannels with a micromolar concentration of fluorescent solutions was shown.

2. Experimental section

2.1. Fabrication of 1D nanochannels by wafer bonding

Fabrication of 1D nanochannels was based on the approach of Haneveld [46] (figure 1). The process was started on a $\langle 1\ 1\ 0 \rangle$ silicon wafer (Okmetic) with 380 μm thickness and 100 mm diameter (step 1). First standard cleaning was applied to the wafer (10 min in fuming (100%) HNO_3 , 10 min in boiling (69%) HNO_3). A 150 nm thick silicon oxide layer was grown by thermal dry oxidation with oxygen flow of 4 l/min at 950 $^\circ\text{C}$ in 7 h (Amtech Tempress Omega Junior, step 2). The thickness of the silicon oxide was measured by an ellipsometry (Plasmos SD 2002). Nanochannel structures with a 20 μm width were created by a standard lithography procedure (step 3) consisting of a dehydration step (5 min, 120 $^\circ\text{C}$), spin coating of a HMDS adhesion promotor and Olin 907/12 photoresist (20 s, 4000 rpm), soft-bake (1 min, 95 $^\circ\text{C}$), exposure (3 s using a 12 mW cm^{-2} Electro Vision exposure apparatus (EVG 620)), post-exposure bake (1 min, 120 $^\circ\text{C}$) and development (1 min in an OPD 4262 developer). The structures were transferred to the silicon oxide layer (step 4) by wet chemical etching (3 min) in a buffered hydrofluoric acid (BHF) solution (Merck). Using this silicon oxide spacer layer method [46], the channel height was controlled by the thickness of the silicon oxide layer and by the time to completely etch this layer. For silicon oxide etching, BHF or 1% HF solutions can be used. In the case of channel heights larger than 50 nm, the BHF

solution is preferred because of its 'resist friendly' properties. However, BHF also etches silicon, although only at a very low rate [10]. Therefore, if the channel heights are below 20 nm, the 1% HF solution is selected due to its very high selectivity between the etch rates of silicon and silicon oxide. This means that, when using the 1% HF, the etching stops exactly on the silicon/silicon oxide interface, and the channel height is more precisely controlled.

To create fluidic interfacing to the nanochannels, microchannels were created on the wafer with nanochannel structures. After resist lithography (step 5), the microchannel structure was transferred to the silicon oxide layer by wet chemical etching in a BHF solution (4 min, step 6), then to the silicon layer by Reactive Ion Etching (RIE, step 7) (Oxford Plasmalab 100). The main etching parameters were a power of 600 W, 120 sccm SF_6 flow, $-110\ ^\circ\text{C}$ substrate temperature, 10 mTorr process pressure and a time of 40 s for 2 μm depth. Next, the resist was removed (step 8) for further processing. For use on an inverted microscope (figure 2(a)), inlet ports were also fabricated on the silicon wafer from the backside, connected to the microchannels (details of inlet-hole fabrication in part 2.3).

For optimal collection of fluorescent signals, high NA water immersion lenses are commonly used, optically corrected for use with 170 μm thick cover glasses. Therefore we covered the 1D-nanochannels by bonding them to special, 170 μm thin, blank glass wafers (Borofloat, Mark Optics). Hence, using an inverted microscope, observation of the nanochannels from the bottom and filling of the channels through inlet ports from the top was possible. Before bonding, the channel height was measured by a mechanical surface profiler (Veeco Dektak 8). Both wafers were cleaned by standard cleaning and Piranha cleaning (20 min, 120 $^\circ\text{C}$, solution of $\text{H}_2\text{SO}_4\text{:H}_2\text{O}_2 = 3\text{:}1$) to obtain clean hydroxylated surfaces before fusion bonding. The final cleaning step was extremely difficult because the thin glass wafer is very fragile. The cleaning was performed in a rinsing bath in which water flow-up and nitrogen bubbles could be reduced. For drying a spinner at low speed or a nitrogen spray gun was used. Broad plastic tip tweezers were preferred to handle this thin wafer.

The silicon wafer with all structures was directly bonded to the blank thin glass wafer (step 13). Then the bonded wafers were annealed in a Nabertherm C 250 furnace with a program controller (4 h, 400 $^\circ\text{C}$) to enhance the bond between the silicon and the glass wafer. The bonded wafer was diced (step 15) into smaller sized chips with a protection step (step 14).

2.2. Fabrication of transparent surface 2D nanochannels

2.2.1. Channel fabrication. In this work, we adapted and extended our previously reported surface-micromachining procedure to create transparent 2D nanochannels [43]. Figure 3 shows a brief process flow to realize the nanochannels. A starting substrate is a $\langle 1\ 0\ 0 \rangle$ silicon wafer with a 525 μm thickness (step 1). The wafer is thermally dry oxidized (4 l/min O_2 flow, 950 $^\circ\text{C}$, 2 h) to realize a 50 nm silicon oxide sacrificial layer (step 2). Afterward, a standard

Figure 2. (a) Schematic of the experimental setup. (b) An artist drawing of a 1D nanochannel device. (c) SEM cross section of a 20 μm width nanochannel bonded between a silicon wafer and a thin glass wafer. Inset C1: channel wall morphology of the nanochannel formed by wet chemical etching. Inset C2: SEM cross section of a 150 nm height nanochannel.

Figure 3. Process outline for fabrication of 2D nanochannel devices.

optical lithography was carried out to create 14 μm wide lines in an Olin 907–12 photoresist layer (step 3). Then this pattern was transferred into the silicon oxide layer by RIE etching (step 4, 5 min) using a 75 W power, 25 sccm CHF_3 flow and 10 mTorr pressure. After photoresist stripping, the patterned oxide layer was capped by a 20 nm thick polysilicon layer deposited by low-pressure chemical vapor deposition (LPCVD) with 50 sccm SiH_4 flow at 590 $^\circ\text{C}$ and 200 mTorr pressure in 7 min (Amtech Tempress Omega Junior, step 5). After a second lithographic step (6), a second RIE etching (100 W power, 30 sccm SF_6 , 7 sccm CHF_3 , 11 sccm O_2 , 100 mTorr pressure, 2 min etching time) was carried out to open windows (step 7). Then through these windows, the sacrificial oxide layer was etched away in a 50% HF solution for 2 min (step 8). The etching time was short because the sacrificial layer was removed sideward. In this etching step, the sacrificial layer was partly or completely

removed to give different possibilities for the shape of the nanochannels (figure 5). Subsequently, the wafer was spin dried (step 9), and during these crucial steps, the capping polysilicon layer was pulled down and adhered to the bearing silicon substrate, thus forming closed nanochannels. Then, the wafer was thermally annealed (step 10) at 1150 $^\circ\text{C}$ in N_2 environment (1 l/min flow) for 2 h to strengthen the bond between the polysilicon and the substrate, realizing better sealed channels. Afterward, the channels were completely oxidized (step 11) to make the capped polysilicon layer optically transparent and to create a 100 nm silicon oxide layer as a stop layer underneath the channels.

2.2.2. Controlling of channel-fabrication process

2.2.2.1. Selection of materials. 2D nanochannels were formed by adhesion of the capping layer to the substrate after removing the sacrificial layer. In sacrificial layer etching technique, silicon oxide and polysilicon layers are a common combination for sacrificial and capping layers because wet chemical etching of silicon oxide using a HF solution has high selectivity over silicon [47]. Additionally in our work, the sacrificial silicon oxide layer is preferred because of its smoothness. This leads to smooth bottom surface of the polysilicon layer which serves as the top nanochannel surface. Also, the high uniform surface of the silicon oxide layer defined the smoothness of the silicon, which forms the bottom nanochannel surface. As the nanochannels are formed by deformation and adhesion of the polysilicon film to the silicon substrate, the highly smooth surface of the used layers is a crucial factor to create strong bonding between the two materials composing the channels, creating completely sealed nanochannels.

Figure 4. SEM cross-sectional images of 2D nanochannels fabricated with different thicknesses of the sacrificial layer, corresponding to the initial gap between the capping layer and the substrate of (a) 30 nm and (b) 50 nm.

2.2.2.2. Channel dimensions after fabrication. Nanochannels were formed by the elastic deformation and adhering of the capping layer to the substrate after the removal of the sacrificial layer. Therefore, both the channel height and width are strongly determined by the thickness and mechanical properties of the used sacrificial and capping layers. First, the height of the channels was exactly equal to the thickness of the sacrificial layer. We observed channels with 27 ± 3 nm height (figure 4(a)) and 48 ± 3 nm (figure 4(b)) height, in correspondence with to the initial gap of 30 nm and 50 nm (measured by ellipsometry) between the capping layer and the substrate.

The channel width is depending on the thicknesses of both layers. Because the nanochannels were created due to deformation of the capping layer, the channel width also depends on mechanical properties of the capping layer as well as the adhesion energy of the capping layer to the substrate. The channel width x is found by energy minimization [48]:

$$x = \sqrt[4]{\frac{3}{2} \frac{Et^3g^2}{\gamma}}, \quad (1)$$

where E is Young's modulus of the capping layer, t is the thickness of the capping layer, g is the thickness of the sacrificial layer, and γ is the adhesion energy. From equation (1), one can see that the thinner layers create more narrow nanochannels. From a fabricated channel (figure 5(c)) with width $x = 375 \pm 15$ nm, thickness $g = 44 \pm 5$ nm, thickness $t = 21 \pm 3$ nm, the adhesion energy $E = 150$ GPa, γ of the bond between the capping layer and the substrate was about 0.2 J m^{-2} , which is calculated from equation (1) and confirmed to be in the range of the adhesion energy of silicon–silicon bonds [43, 49].

2.2.2.3. Preservation of channel features after post-processes. For optical detection, transparent channels are required therefore the fabricated channels were oxidized to transform the polysilicon layer which forms channel walls to a transparent silicon oxide layer. It is desired that features of the fabricated channels such as shape and sizes are preserved after post-processes. Therefore, an investigation of the oxidation of the fabricated channels was carried out. The

polysilicon capping layer was partly oxidized and figure 5(e) shows the capping layer with an interface (indicated by white dots) between oxidized polysilicon (14 ± 3 nm thick) and the remaining polysilicon (18 ± 3 nm thick) layers with a total thickness of 32 ± 3 nm. To prove this observation, the oxidized polysilicon layer was removed by HF 50% to reveal the remaining polysilicon layer (20 ± 3 nm thick in figure 5(g)). Furthermore, we observed that the capping layer was pushed up due to the volume expansion during transformation of polysilicon to silicon oxide. It was indicated by an increase in channel height from 44 ± 5 nm (before oxidation in figure 5(c)) to 84 ± 5 nm (after oxidation in figure 5(h)). During oxidation, the capping polysilicon layer (from point A to point B in figures 5(c), (h)) was elongated from 370 ± 15 nm to 374 ± 15 nm. Also, the capping layer became thicker, from 21 ± 3 nm to 53 ± 3 nm. From its thickness and length expansion, the volume ratio of the capping layer after and before oxidation was determined to be about 2.6, which is in the same range as the ratio in bulk-silicon oxidation [50]. Surely, the most important observation is the oxidized polysilicon that hangs over channel areas rather than collapsing or blocking the channels, which confirms the preservation of the fabricated channels.

For integration of the fabricated nanochannels to the outer world, a thicker layer such as silicon oxide or silicon nitride was deposited on top of the channels. This layer mechanically protects for the fragile channels from damage. Figure 7(a) shows the fabricated channels with a deposited silicon oxide layer of 500 nm thickness without any collapse.

2.3. Fabrication of fluidic inlet/outlet ports

In nanochannel devices interfacing from macro inlet ports to the nanochannels is necessary for proper delivery of liquid into the nanochannels. Inlet ports can be created by different processes, such as powder blasting and DRIE etching [51]. For 1D bonded nanochannels, powder blasting is commonly selected to form inlet ports on glass wafers [25]. In order to use an inverted microscope, inlet ports formed on silicon wafers containing the nano-/micro-channel structures, DRIE etching was a preferred alternative method to avoid damage of nanochannels. Protection of the nanochannels against damage was crucial. The fabricated channels were protected by coating

Figure 5. Cross-sectional SEM images of 2D nanochannels during processing with two etching possibilities of the sacrificial layer. Left images: remained silicon oxide and right images: completely etched silicon oxide. (a), (b) Sketched finished process steps. (c), (d) Nanochannels just after formation. (e), (f) Nanochannels after partly oxidized the polysilicon capping layer. (g) A nanochannel after the oxidized polysilicon capping layer was removed. (h), (i) Nanochannels after completely oxidizing the polysilicon capping layer.

with various materials such as TI35, SU-8 (Micro Chemicals), unfortunately all resulting in cracking and peeling-off during the cryogenic DRIE step. Durimide 7500 series polyimide (Arch) successfully protected the fabricated structures during etching through the wafer, exhibiting the proper combination of thermal stability and mechanical toughness. Polyimide was coated (figure 1, step 9) on the front side containing nanochannel structures by a lithography procedure (step 9): dehydration (5 min, 120 °C), spin coating (20 s, 6000 rpm), soft-bake (1 min, 95 °C), flood exposure (3 s), post-bake in Leybold Heraeus vacuum oven (1 h, 350 °C, 2 mbar). After a standard lithography step (10), inlet-port patterns were created on the back side of the wafer. Next, these patterns were transferred to the silicon substrate by DRIE etching (step 11) with an etch rate of $6 \mu\text{m min}^{-1}$. The main etching parameters

were a power of 600 W, 200 sccm SF_6 flow, 1.0 sccm O_2 flow, -110°C substrate temperature, 10 mTorr process pressure and 65 min etching time. After etching, the polyimide layer was removed (step 12) by Piranha cleaning.

For the enclosed 2D nanochannels, DRIE was absolutely preferred over powder blasting. In powder blasting the high-pressure flow of aluminum oxide will damage the fragile channels. Therefore, DRIE was used to create inlet ports for 2D nanochannels. The fabricated nanochannels were protected by a 500 nm thick silicon oxide layer deposited at 700 °C and 400 mTorr in 70 min (Tempress Systems Furnace) from TEOS (tetra-ethoxy-silane) and nitrogen (figure 3, step 12). This layer served as a protective layer during further processing and provided a transparent layer for optical observation as well. Inlet ports were integrated with the

Figure 6. Blinking behavior of individual quantum dots visualized inside 1D nanochannels (150 nm height, 20 μm width). Interfacing of the nanochannels is indicated by the white arrow. ‘On’ and ‘off’ states were observed in the quantum dot marked B (inside the white circle) whose fluorescence disappeared at the 4 s frame then reappeared in the 5 s frame. This behavior can be more clearly seen in Movie 1 (supporting material) stacks.iop.org/JMM/19/065017.

Figure 7. (a) A preserved nanochannel with 500 nm TEOS protection layer (b) A confocal fluorescence image of 4 μM Alexa Fluor 488 solution in 50 nm height and 400 nm width 2D nanochannels. Image size is 43.4 $\mu\text{m} \times 43.4 \mu\text{m}$. (c) An artist drawing of a 2D nanochannel device including inlet holes connected to channels for filling. (d), (e) Optical microscope image of 0.9 mm diameter inlet holes, observed on top of the inlet hole and above the channels.

channels (figure 7(b)) from the back side to avoid interference with the objective, a problem may occur when filling and observation takes place at the same side. A standard lithographic step (13) was applied to form patterns of inlet ports on the back side of the wafer and to coat the front side with a photoresist layer to protect the nanochannel structures. Next, the inlet-port patterns were transferred to the oxide layer by wet chemical etching in a BHF solution (10 min, step 14). Inlet ports were etched almost through the wafer by

the DRIE etching (90 min, step 15) using the same parameters as presented for fabrication of the 1D nanochannels. Etching of the silicon was stopped on the stop layer introduced by the oxidation step (step 11) after fabrication of the channels. The stop layer was removed by RIE etching in 3 min and the channels were partly opened in the inlet-port area (step 16). The photoresist layers from both sides were removed (step 17) by oxygen plasma (20 min, Tepla 300E). In a final step (18), thermal dry oxidation at 950 $^{\circ}\text{C}$ in 15 min the surfaces and

side walls of the inlet ports were made hydrophilic to facilitate filling of the channels.

3. Single-molecule mobility studies

The 150 nm high 1D nanochannels (figure 2(c)) were used for observation of single quantum dots. The experiments were carried out on an upgraded inverted fluorescence microscope (Zeiss, Axiovert) (figure 2(a)) [52]. For excitation 488 nm argon laser light was focused into the nanochannels by using a 100 $\times$, 1.20 NA, water-immersion objective (Leica Planachromat). For detection of fluorescence intensities the microscope was equipped with an air-cooled intensified CCD camera (Pentamax, Princeton Instruments). Emission was detected with a 610/75 nm band pass filter and a notch filter was used to remove residual excitation intensities. Images of 50 $\mu\text{m} \times 50 \mu\text{m}$ (512 $\times$ 512 pixels) were recorded by using the WinSpec 32 program (Roper Scientific). Series of 40 sequential images were acquired at a rate of 5 frames per second (200 ms exposure time).

By capillary force the nanochannels were filled with a 12 nM quantum dot solution (Evitag Fort Orange, type T2-MP, carboxyl, CdSe/ZnS, ~ 25 nm diameter, emission 600 ± 10 nm, Evident Technologies, NY). Imaging was started only after the equilibrium state was reached indicated by an absence of flow inside the channels. Quantum dots performed the well-known Brownian motion that a quantitative analysis is in progress. In the Brownian motion of single quantum dots, the average of its displacements tends to zero value which means there is no flow. Individual quantum dots could be visualized by virtue of the nano-confinement in the channels in combination with the high NA objective and the sensitive detection camera. The sequences of images (figure 6) identified the presence of individual quantum dots from the instantaneous appearance and disappearance of fluorescence, a demonstration of the well-known blinking behavior, characteristic for single fluorescent molecules. In every image of the series, dots could be recognized that were already present in the first image (dots A, B, C) and could be followed over several consecutive images (2 s-, 3 s-images); other dots disappeared (dot B in 4 s-image) and reappeared (dot B in 5 s-image) during imaging. For more detail, a movie of these sequence images is shown in the supporting information (Movie 1), stacks.iop.org/JMM/19/065017. Note that the number of quantum dots in the observation window is two orders of magnitude smaller than the calculated number corresponding to the original quantum-dot solution. Therefore, the observed concentration corresponds to ~ 0.01 nM.

The performance of the 2D nanochannels was only tested regarding their filling capability. For visualization a home-built scanning confocal fluorescence microscope was used [53, 54]. Briefly, 488 nm Ar/Kr laser light was used for excitation in combination with a 63 $\times$, 1.20 NA water immersion objective, a 525/50 nm band pass emission filter and an avalanche photodiode detector. Application of 4 μM Alexa Fluor 488 (Molecular Probes, Eugene, OR) solution confirmed that filling of the 2D nanochannels indeed can properly be accomplished (figure 7(c)). The concentration

of 4 μM was still three orders higher than the concentration required for the single-molecule level¹. The observation of single fluorescent dye molecules inside 2D nanochannels is part of work in progress.

4. Conclusions

We fabricated 1D nanochannels using thin glass wafers as cover especially for observation with high NA lenses. Inlet ports for these 1D channels were constructed on silicon wafers for use in an inverted microscope configuration. 2D fragile nanochannels were successfully fabricated and made transparent for optical observation by oxidation then they were integrated to macro world by through-wafer inlet ports. Inlet/outlet ports integrated with the fabricated nanochannels were created using deep reactive ion etching at cryogenic temperature. The performance of the 1D nanochannels in single-molecule fluorescence detection was demonstrated from visualization of single quantum dots from a 12 nM solution. These results indicate promising prospects for utilization of the nanochannels for various applications, such as e.g. single-molecule position tracking at physiologically relevant concentrations. Testing of the 2D nanochannels showed adequate performance in filling with a fluorescent solution.

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¹ In a 2D channel volume $V = h \times w \times l$, where h , w , l are the height, width and length of the channel; $h = 50$ nm, $w = 400$ nm, $l = 50 \mu\text{m}$. In order to have one molecule in this volume, concentration C of solution should be $C = 1/(1000 \times V \times N) = 1.7$ nM. N is Avogadro number ($N = 6.02 \times 10^{23}$ /mole).

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