Fabrication of 1D nanochannels with thin glass wafers for single molecule studies

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ABSTRACT

1D nanochannels are fabricated by direct bonding of a silicon wafer, containing nanochannels, microchannels and access holes with a 170 μm thick and 100 mm diameter glass wafer. The thin glass wafers are used as channel covers because of their appropriate thickness for use with coverglass corrected water immersion lenses. As the nanochannel chips are used on an inverted microscope, access holes are created in the silicon wafer. It was found that polyimide is a good material for protection of the channel structures during deep reactive ion etching of the macro holes. Fabricated nanochannels with 50 nm depth were successfully filled with low concentrations of Alexa 488 solution which could be observed by fluorescence microscopy. These nanochannels are promising for mobility studies at the single molecule level.

Keywords: nanochannels, fabrication, single molecule, fluorescence

1   INTRODUCTION

In the pas few years the field of nanochannel fabrication has rapidly developed. 1D nanochannels can be fabricated by shallow etching of trenches in the silicon substrate wafer, followed by bonding of a glass cover wafer to form enclosed nanochannels [1-2]. 2D nanochannels are fabricated using different lithographic techniques such as focused ion beam [3], electron beam lithography [4], nano-imprinting [5] and edge-lithography [6]. In the latter technique 2D nanochannels are formed at the side walls of steps, which can be created by conventional lithography.

Single molecule studies have applications in many fields such as molecular dynamics, DNA and protein analysis. Nanochannels have been used because of their extremely small volume [7-8]. The small observation focal volume enables single molecule experiments at relatively high concentrations. Furthermore, nanochannels have essential advantages such as little samples consumption and reduced of background signal in fluorescence applications due to their ultra shallow depth.

This work presents bond-micromachining based fabrication of 1D nanochannels with thin glass wafers

suitable for fluorescence applications and compatible with the configuration of an inverted fluorescence microscope equipped with high numerical (NA) water immersion lenses. The design of the nanochannels together with the applied highly sensitive fluorescence microscope eventually will enables mobility studies of individual molecules.

2   FABRICATION

Fabrication of 1D nanochannels with thin glass wafers (Borofloat, Mark Optics) of 170μm thickness is based on the approach of Haneveld [9]. It is started on a <110> DSP wafer (Okmetic) with 380 μm thickness and 100 mm diameter. Oxidation is followed in Tempress Systems Furnace to control the thickness of the silicon oxide spacer layer which then consequently is patterned to define the depth of nanochannels by etching in 1% HF solution (Merck) after standard lithography. In order to perform long-time etching in the HF solution, vapor Hexamethyldisilizane (HMDS) is applied to obtain good adhesion between the silicon substrate and the photoresist layer. This prevents the attack of photoresist from the HF solution. Microchannel structure is created on the photoresist layer on the nanochannel wafer after the second
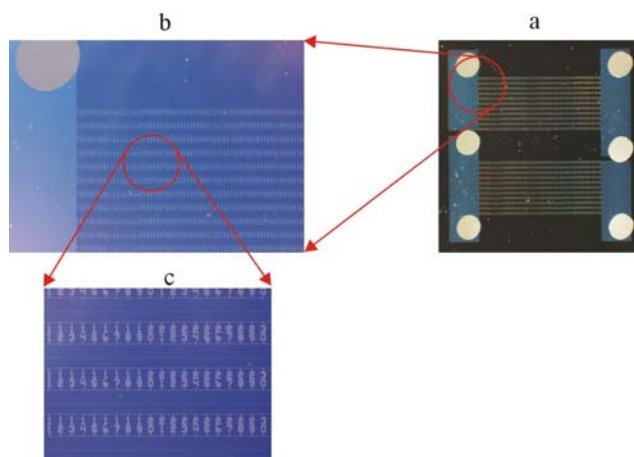

Figure 1: A nanochannel device. a: a channel chip; b: close-up of an interconnect position between a microchannel and nanochannels; c: close-up of nanochannels.
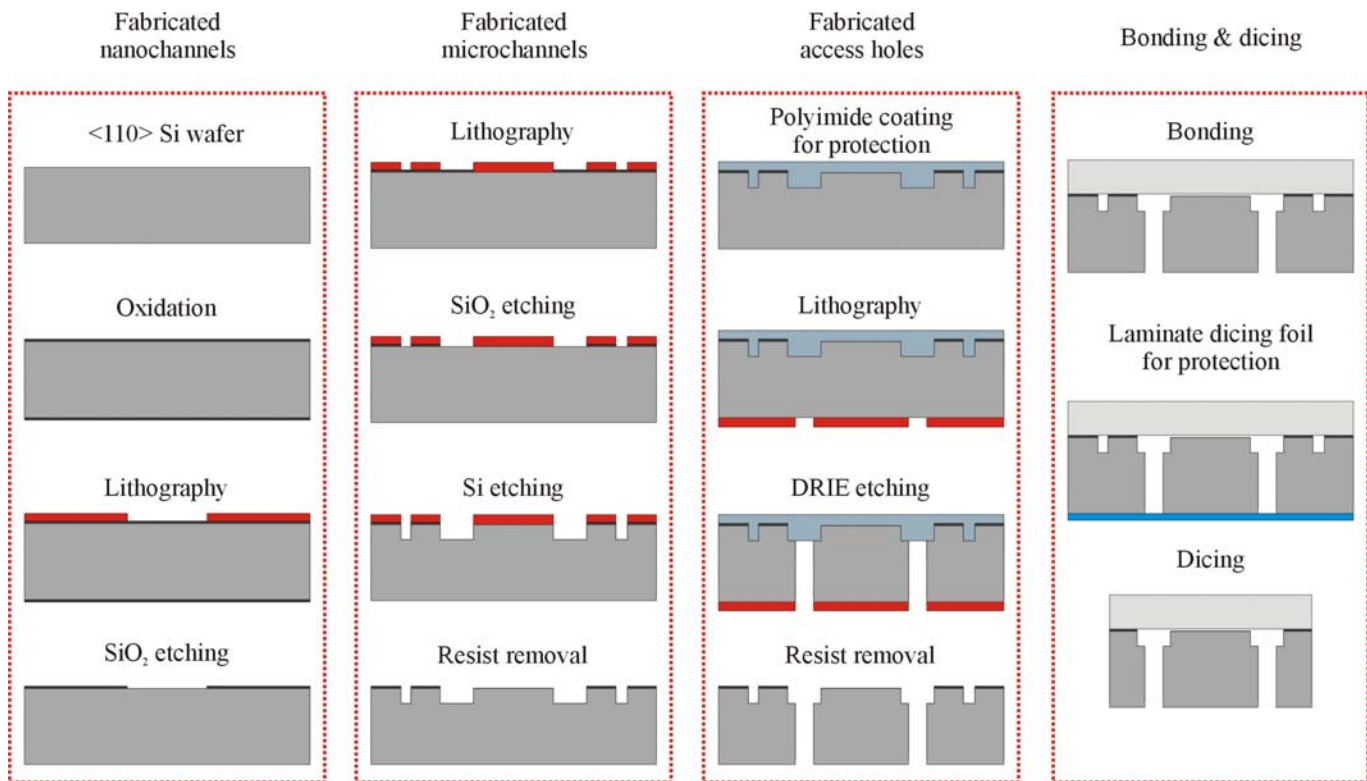
Figure 2: Process outline of 1D nanochannels with thin glass wafers.

lithographic step and transferred to the silicon oxide layer and the silicon layer by wet etching in BHF solution and Reactive Ion Etching (RIE), respectively.

As the chips will be used on an inverted microscope (figure 3), macro holes are also fabricated from the back side of the silicon wafer connected to the microchannels. To avoid damage to the micro-, nano-channels and the fabricated side as well in this step, the protection of the front side becomes important. Several materials such as TI35, SU-8 (Micro Chemicals) have been tested but they are cracked and peeled-off during cryogenic deep RIE etching (Oxford plasma Lab 100 machine). Durimide 7500 series polyimide (Arch) successfully protected the fabricated structures during the cryogenic deep etching through the wafer because it exhibits the good combination of thermal stability and mechanical toughness. After etching, the polyimide layer could be removed by Piranha cleaning. With the polyimide protection from the front side, macro holes are etched through the wafer from the back side using a cryogenic process with main parameters: power of 600 W, SF_6 flow of 200 sccm, O_2 flow of 1.0 sccm, substrate temperature of -115˚C and process pressure of 10 mtorr. Connection is established from the outside world to the nanochannels through microchannels and then macro holes.

The thin glass wafers are used as channel covers as they have the appropriate thickness for the use with coverglass corrected water immersion lenses. Nanochannels are closed by direct bonding between the silicon wafer containing the nanochannel, microchannel, access-hole structures and the blank glass wafer. Then the bonded wafer is annealed at 400˚C for 4h to attain the permanent bond between the silicon and the glass wafer. Before bonding, channel height is measured by a mechanical surface profiler (Veeco Dektak 8). Both wafers are cleaned by standard cleaning in HNO_3 solutions and Piranha solution ($H_2SO_4 : H_2O_2 = 3:1$) to obtain clean hydroxylated surfaces for fusion bonding. Handling of extremely fragile glass wafers needs to be very cautious. A complete nanochannel device and all detailed steps are shown in figure 1 and 2.

3 RESULTS AND DISCUSSION

Nanochannels of 50 nm in height were successfully filled with low concentration solutions of Alexa 488 in water and the fluorescence could be observed with an inverted fluorescence microscope. For excitation the 488 nm Argon laser line was focused into the nanochannels by using a 100 x magnification, NA 1.20, water-immersion
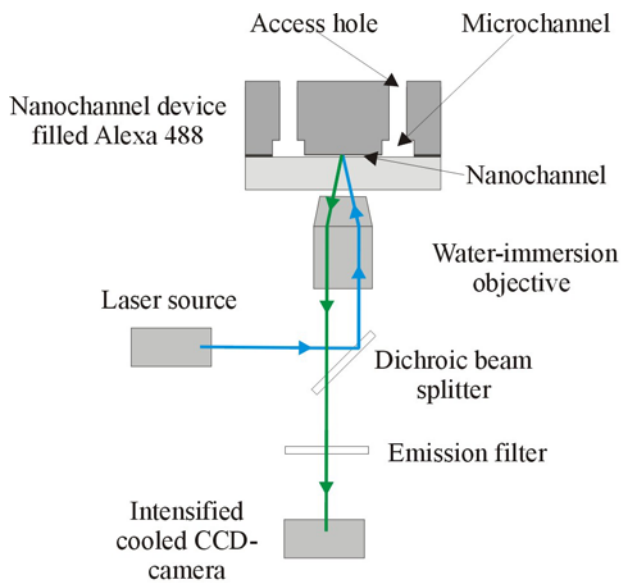
Figure 3: Schematic representation of the measurement configuration.

Figure 4: 1D nanochannels filled with different concentrations of Alexa 488 in water. Exposure time was 200 ms. Image size is 50 µm x 50 µm with 512 x 512 pixels.

objective (Leica Planachromat) (figure 3). For detection of fluorescence intensities the microscope was equipped with an air cooled and intensified CCD camera (Pentamax, Princeton Instruments). Emission was detected with a 500-550 nm band pass filter and an exposure time of 200 ms. Images of 50 µm x 50 µm and 512 x 512 pixels were recorded by using the WinSpec 32 program (Roper Scientific).

The nanochannels were filled with solutions of Alexa 488 (Molecular Probes) in water. We started with a high concentration of 160 µM Alexa 488, and gradually diluted this solution. Figure 4 shows that a concentration as low as 1.6 µM has been observed in the nanochannels. A simple calculation shows how far the concentrations have to be reduced to reach the single molecule level. Assume we have one molecule in a channel volume $V = h$ x A, where h (m) is the channel height and A (m^2) is the lateral channel area occupied by the molecule. The required molarity is then given by $C = 1/ (N_a$ x V x $1000)$. N_a is Avogadro number ($N_a = 6.02 \times 10^{23}$/mole). For $A = 10$ µm^2 and $h = 50$ nm this results in $C = 3$ nM. This means that a further reduction by three orders of magnitude is required compared the lowest concentration (1.6 µM) achieved in our imaging experiments. One way to make the concentration requirement less stringent is the use of 2D-confined nanochannels. Technologically it is possible to reduce the channel width and thus A by a factor of 100. Another possibility is to detect the fluorescence not by using an imaging method, but by applying fluorescence correlation spectroscopy (FCS) [10]. FCS measurements can be performed with confocal fluorescence microscopy, where the fluorescence is detected from individual m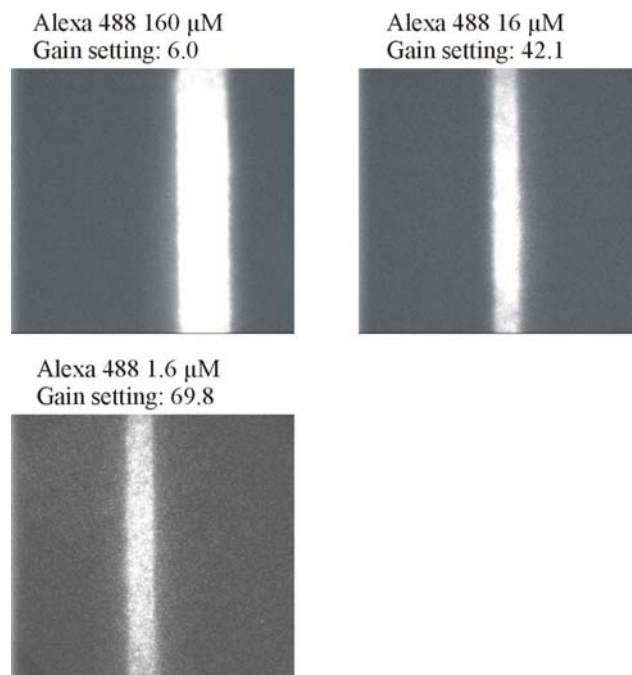olecules during passage through a sub-femtoliter confocal volume. The application of 1D nanochannels will reduce the detection volume even further and enable FCS measurements at physiologically relevant concentrations.

4   CONCLUSIONS

1D nanochannels with 50 nm height, integrated to microchannels and access holes were successfully fabricated by bond- and bulk- micromachining. We have shown that nanochannels closed by a thin 170 µm thickness glass wafer can be filled with fluorescent solutions and observed by a high NA objective in combination with an intensified CCD camera. Fluorescent solutions with µM concentrations were visualized inside the fabricated nanochannels. These nanochannels have potential for mobility studies on individual molecules like fluorescently labeled DNA and protein.

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