Materials and Processes for Medical Devices (MPMD) Conference and Exposition, September 23-25, 2007

Fabrication and Characterization of Nanofluidics Device Using Fused Silica for Single Protein Molecule Detection

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Abstract

Fabrication of nanofluidic devices was carried out and the devices were characterized. These devices will be used to trap, manipulate and detect single protein molecules in nanometer size channels in a laser fluorescence spectroscopy process to investigate dynamical and photophysical behavior of single molecules. On the substrate of fused silica (SiO_2) glass wafers, Electron Beam Lithography (EBL) was used to pattern nanochannels, and normal photolithography methods were used to fabricate microchannels to link nanochannels with the source molecular solution through syringes and tubing. The microchannels and nanochannels on a glass chip were sealed by a blank chip in a fusion bonding process to complete the chip level device. Un-bonded microchannels and nanochannels were characterized using SEM. Processing parameters including electron dose and etch conditions were elucidated for their impact on channel dimensions. Preliminary laser fluorescence results are also presented to demonstrate that unclogged channels were fabricated successfully in the nanofluidics devices.

Introduction

During the past decade, there has been significant and sustained advance in experimental and modeling work of Lab-On-A-Chip devices [1-4]. Lab-On-A-Chip is widely studied and has broad applications in analytical chemistry, biology, medical research and genomics. Lab-On-A-Chip refers to miniaturized devices based on integration of multiple small size parts including fluidic channels, electrodes, sensors and actuators elements into one chip with a size on the order of square centimeters or less. A subset of Lab-On-A-Chip regime, microfluidics and nanofluidics are chip-size devices with embedded fluidic channels of micrometer and nanometer dimensions. Microfluidics and nanofluidics are commonly fabricated from a polymer polydimethylsiloxane (PDMS) and glass materials because they are transparent, biocompatible, electrically insulating and simple to implement. They are used for experiments to handle extremely small fluid volumes down to less than picoliters. The general applications of microfluidics are molecule and cell separation and DNA bioassays in fields of life science [5-8].

Nanofluidics have nanometer scale channels and are being used in single molecule research because of the benefit of confinement of nanometer size protein molecules [9-12]. Different aspects of nanofluidics are the subject of many studies concerning nanoscale forces, theoretical description of nanoscale features, surface energy dominant phenomena, electrical double-layer studies, nanochannel and molecule-size related phenomena. All these concerns come from substantial increase in surface-to-volume ratio and the similarity in size of nanochannels and molecules. The major applications of nanofluidics lie in separation science and single molecule studies in confined space of nanochannels. Coupled with laser fluorescence spectroscopy, nanofluidics is being used in single molecule detection to understand protein conformation and function mechanisms in biophysics [13-18].

A detailed understanding of nanodomain molecular physics is very important for a thorough comprehension of in-vivo molecular biophysics and the working mechanisms of cellular processes. Conventional single molecule spectroscopy uses a confocal microscope objective and focused laser spot to sample a volume of less than a femtoliter in a drop of dilute aqueous molecular solution. Due to Brownian diffusion, single molecules are randomly transported through this small sampling volume in 3-dimensions. It's virtually impossible to study a specific "single molecule" under such conditions because of random movement of molecules in and out of the sampling volume in solution. The advantage of utilizing nanofluidics to do single molecule detection is that it's possible to trap nanometer size molecules in nanochannels by dynamic control through electrophoresis, electro-osmosis processes and a 1-dimensional movement [12]. Hence "single" molecule detection can be realized. Nanofluidics has many

potential applications in medical research, chemical biology, biophysics, and genomics. In this paper, we present preliminary experimental results in nanochannel fabrication and characterization, fabrication of nanofluidics devices and laser fluorescence spectroscopy studies in the nanofluidics devices.

Experimental Procedure

100mm diameter and 500μm thick Fused silica (SiO_2) wafers (Mark Optics, Santa Ana, California) were used as the substrate. The wafer surface quality parameters are: surface quality: flatness<5 μm, 60-40 (scratch and dig), TTV<25μm, R_a<20Å. A general cleaning procedure is necessary for wafers before metal deposition and for glass chips before bonding. The procedure consists of ultrasonic cleaning with acetone and then isopropanol for 10 minutes each; soaking in Nano-Strip™ (Cyantek Corporation) at 60°C to remove organic and metal residues on wafers and glass chips; rinsing with filtered DI water; blowing dry with clean nitrogen. Standard photolithography and electron beam lithography (e-beam) were used to fabricate microchannels and nanochannels for the nanofluidics devices. Figure 1 shows the fabrication sequences of nanofluidics: (a) SiO_2 wafer was sputter coated with a chrome layer of 50nm (AJA International ATC2400 Sputtering System), then spin coated with a PMMA (NANO™ 495 PMMA A2) layer of 50nm thickness; (b) electron beam lithography was used to write (expose) nanometer pattern (50μm long and 20-80nm wide in CAD file definition) on PMMA on the wafer; PMMA was developed and chrome was etched chemically (CR-7S10, Cyantek Corporation); reactive ion etch (Oxford Instrument Plasmalab System 100) was performed for etching nanochannels in SiO_2; (c) all un-exposed PMMA was removed by acetone; a photoresist (Shipley™ 955cm-2.1) was spin coated on the wafer; (d) the wafer was exposed through a 127×127mm chrome mask with microchannel patterns by UV light in a contact exposure process (Quintel Mask Aligner), developed (MF™-CD-26), and the chrome layer with microchannel patterns was etched (CR-7S10, Cyantek Corporation); reactive ion etch was used to etch microchannels in SiO_2; (e) stripping of photoresist and chrome layer; via holes were machined using a CO_2 laser in superpulse mode; (f) the wafer was diced and the individual chips were bonded with blank SiO_2 wafer chips (200μm thick) to seal micro and nanochannels; low temperature bonding (up to 100°C, 12h) was used here. $CF_2CF_2CF_2CF_2$ (Octafluorocyclobutane c-C_4F_8) was used as plasma etching gas in reactive ion etch (RIE) of silicon dioxide (SiO_2). The parameters used were c-C_4F_8 45.0sccm, O_2 2.0sccm, pressure 7mtorr, RF power 200W, ICP power 2000W. For etching of nanochannels, 30-120 seconds were used; for microchannels, 8-10 minutes were taken.

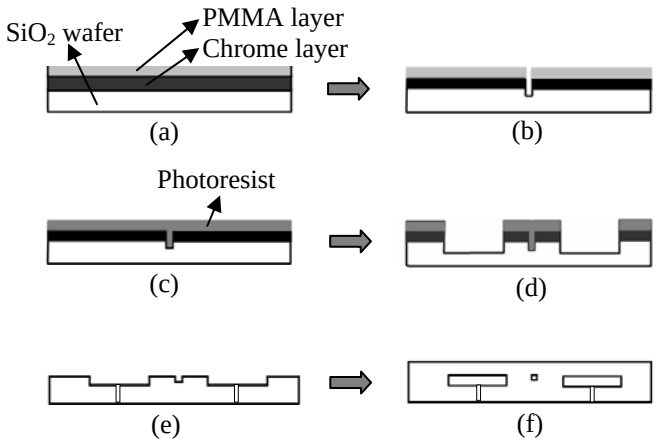


Figure 1. Schematics showing the manufacturing procedure: (a) SiO_2 wafer was deposited with chrome and PMMA; (b) electron beam lithography, chrome etch and reactive ion etch for fabricating nanochannels; (c) removal of PMMA, spin coat of photoresist; (d) contact exposure and develop, chrome etch, and reactive ion etch of microchannels; (e) stripping of photoresist and chrome layer; access hole drilling by CO_2 laser; (f) dicing and bonding of patterned chips with blank glass.

Figure 2 (a) shows a single SiO_2 wafer with microfluidics and nanofluidic chips after e-beam and photolithography fabrication and before dicing. In (b) an individual device chip (microfluidic or nanofluidic chip, see Figure 2c) is shown, with a cross-bone channel consisted of 2 "V" shape "via microchannels". Note that there is a reservoir at the end of "V" via microchannel. There are several support posts (100μm diameter) in the "V" shape microchannel to prevent channel collapse during sealing of the SiO_2 chip to the top cover during bonding. Also an array of small posts (5μm diameter) is located across the "V" microchannel for purpose of filtering micron sized particles. A CO_2 laser was used to punch access holes (20-100μm in diameter) at the reservoir center, Figure 2(b). On a single wafer, there were 2 kinds of device chips: microfluidics chips and nanofluidics chips, Figure 2(c). Nanochannels or microchannels were fabricated on the plateau (350μm×10, 20 and 30μm) in the single chip center to connect the 2 "V" via microchannels. Note that the via microchannels in the cross bone pattern had the same size for both microfluidics and nanofluidics chips. Microfluidics devices were fabricated using step (a), (c), (d), (e) and (f) in Figure 1.

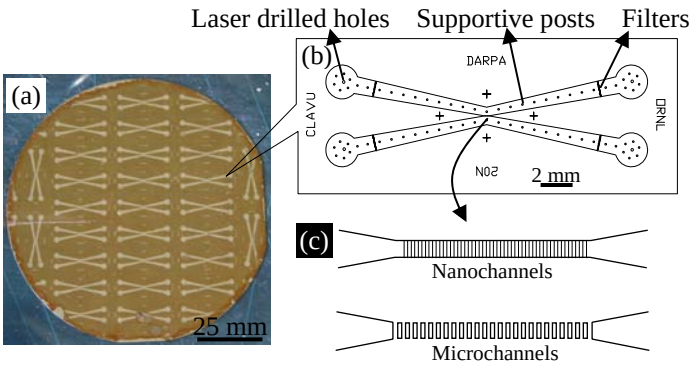


Figure 2. Schematic of (a) a SiO_2 wafer with fabricated microfluidics and nanofluidics chips; (b) an individual chip; (c) ebeam fabricated nanochannels or photolithography fabricated microchannels at the centerof a single chip.

The fused silica glass chips were sputter coated with gold for characterization by scanning electron microscopy. The depth of microchannels was measured by a profilometer. For depth measurement of nanochannels, FEI™ Nova 200 Dual Beam FIB/SEM (focused ion beam/scanning electron microscope) was used. The sample was coated with a 5-10nm carbon film before loading to the Dual Beam machine. A platinum protection coating was deposited at the position of the nano-target preparation to protect the top surface edge of dulling during the ion milling. A focused gallium-ion (Ga^+) beam was used to mill a cross section perpendicular to the nanochannels such that nanochannel cross section was revealed. A relatively large ion current of 1nA was used to mill Pt/SiO_2, followed by a decreased ion current of 30pA for fine "polishing" of sidewall for better imaging of nanochannel cross sections. The cross sections were characterized in SEM mode. Figure 3 shows SEM images of a sample at different preparation steps. The electron acceleration voltage of SEM was 5 kV to reduce charging effects in the insulating fused silica. The acceleration voltage for FIB was 30 kV.

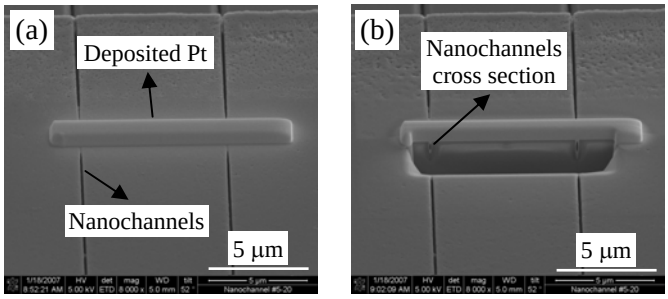


Figure 3. (a) A platinum block was deposited on SiO_2 surface with nanochannels; (b) after FIB sputtering, the cross section of nanochannels were shown.

A photo of an assembled device is shown in Figure 4(a), with the schematics of the device and laser fluorescence spectroscopy shown in 4(b). The bonded SiO_2 chip was positioned between an aluminum base plate and a plastic block. There are 4 tapped holes at the sides of the plastic piece which are connected to 4 laser drilled access holes in SiO_2. A syringe was attached through the fittings and tubing. Molecular solution was loaded into the device by a syringe. Platinum electrodes (127µm diameter wires) were attached into the plastic piece so that electric fields can be applied to the molecule solution for control and manipulation. The laser beam is delivered to the molecular solution in the fluidic device from the bottom (inverted microscope) In this epi-illuminated arrangement, the fluorescence signals travel back through the objective to be collected and analyzed.

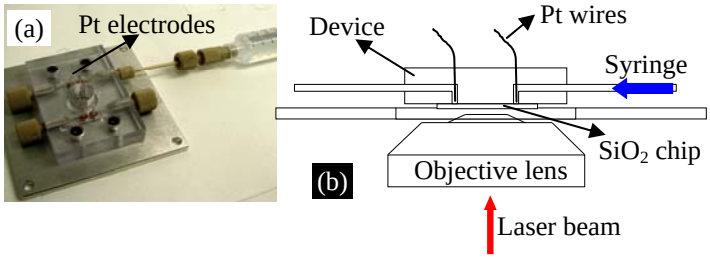


Figure 4. (a) Photo of an assembled device; (b) laser fluorescence spectroscopy setup.

Results and Discussion

The SEM pictures shown in Figure 5 present the morphology of the fused silica micro/nanofluidic device chips. The cross bone shaped via microchannels are shown in Figure 5(a). The CO_2 laser drilled holes had a diameter of 80 µm in this sample, Figure 5(b). By changing laser pulses parameters, the hole diameter can be varied from 20-100µm. During hole drilling, the laser photons were absorbed by SiO_2 and the photon energy was converted to thermal energy, resulting in melting and vaporization of SiO_2. Because superpulse mode was used, the laser irradiance of each single pulse was several orders higher than 10^6 W/cm^2, resulting in percussion drilling [19], where most of the materials were vaporized and cleared off by a gas jet (compressed dry air). Another purpose of gas jet was to prevent condensation of vaporized materials on laser lens. It can also be seen that there was a layer of debris surrounding the edge of hole with a thickness of several microns. If this layer extruding out from the reservoir bottom is taller than the supportive posts or chip surface, bonding of glass chips is impossible. Mechanical and chemical methods were taken to clean up the hole edge.

A top view of nanochannels at the plateau is shown in Figure 5(c). The pitch (spacing) of nanochannels can not be less than 2µm. In single molecule detection, the laser beam is focused to the diffraction limit. Typical wavelengths in laser

fluorescence spectroscopy are 500-700 nm. At the diffraction limit the focused beam spot size is ~ 1μm. In order to allow the laser beam to illuminate one single nanochannel at one time, the distance between 2 nanochannels should be greater than 2μm. In this sample shown here, it was about 4μm. Figure 5(d) and (e) are the morphology of filter posts and supportive posts in the via microchannels. The surface microstructure of microchannel bottom is shown in Figure 5(f). After 10 minutes of reactive ion etch, the depth of via microchannels was 2.9μm. Since the microchannels (Figure 2c) for the microfluidics devices were fabricated simultaneously with the "V" shape via microchannels, they have the same depth of 2.9μm with the via microchannels. The filter post (5μm diameter) array was for the purpose of blocking any foreign particles with a potential to clog nanochannels when molecular solution is flowing from the reservoir to the nanochannels in the center. The 100μm diameter support posts support the top glass chip during bonding.

The collapse of microchannels in silicon during anodic bonding has been studied [20, 21]. During anodic bonding, pressure, temperature and electrical field are applied on the bonding pair to facilitate bonding of silicon and glass wafers. According to this theory, collapse of channels is affected by the mechanical properties of materials being bonded, atmosphere condition, the voltage applied and channel dimensions (width and depth). Bonding temperature plays an important role in affecting these parameters since with increasing temperature the viscosity of fused silica will decrease, which will make deformation of channels easier than at lower temperature. A low temperature of 100°C was used during bonding of SiO_2 chips in current study and no collapse of microchannels was observed [22]. The morphology of bonded nanochannels is currently being investigated by Dual Beam FIB. However, laser fluorescence experiments showed that dye solution filled the nanochannels after low temperature bonding. Due to extremely small size of the nanochannel, collapse is always a concern during even low temperature or room temperature bonding.

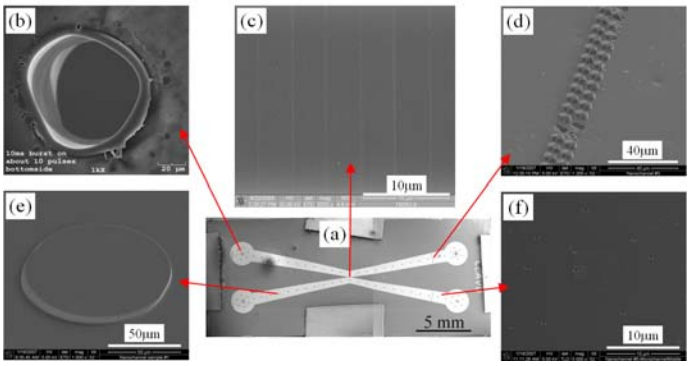
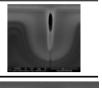
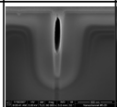
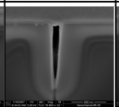
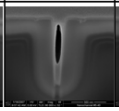
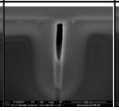
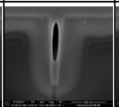
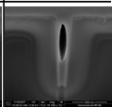


Figure 5. SEM pictures of (a) a SiO_2 nanofluidics device chip: (b) CO_2 laser punched hole in reservoirs; (c) topview of nanochannels on the plateau; (d) filter posts across the "V"-shape microchannel; (e) supportive posts in the microchannel; (f) surface morphology of microchannel.

The characterization result of nanochannels by Dual Beam FIB/SEM is shown in Table 1. Three minutes RIE etch time was used for nanochannels in Table 1.The major parameters that affected the channel dimension were (a) electron dose ($\mu C/cm^2$) during ebeam lithography, and (b) nanochannel width definition in the CAD file of e-beam computer. When the width was defined to be 20-80nm, the actual width after fabrication was in the range of 60-260nm. In Table 1, the nanochannel depth varied in 500-1200nm. No nanochannels were present for 20/30nm width definition with electron dose of $500\mu C/cm^2$, and 20nm width with $700\mu C/cm^2$. This was possibly because the chrome layer nanochannel patterns were not etched through during wet etch process, due to unsufficient chrome layer etching time after PMMA development. The wet etch process in chrome etch solution is controlled by the ions transport rate in the nanochannels in PMMA layer between bulk etch solution and chrome layer. If the PMMA nanometer scale channel was too small, i.e. in the range of 20-30nm definition, more time was needed for thourough etch of chrome layer, compared with wider PMMA mask channels. Hence during reactive ion etch of nanochannels in SiO_2, since the nanochannel patterns in chrome were not through, the SiO_2 was being protected resulting in no presence of nanochannels. Also, the eletron dose played an important role because when the dose was increased, for the same width definition, wider exposed PMMA channel pattern would result. This explains the follwing: (a) at 30nm definition, $500\mu C/cm^2$ didn't produce nanochannels, but $700\mu C/cm^2$ did produce nanochannels; (b) all nanochannels at $900\mu C/cm^2$ were successful; however the width was 3-6 times greater than original width definition. Increasing chrome etch time seems like the most reasonable way to solve this problem, but at the tradeoff of possibly increasing channel width for larger nanochannel definitions.

Table 1. Cross section of nanochannels (SEM pictures)
Dimension mark: ▬ 400nm

Electron dose ($\mu C/cm^2$)	Nanochannel width definition (nm)					
	20	30	40	50	60	80
500	No channel	No channel				
700	No channel					
900						

To decrease depth of nanochannels, a shorter reactive ion etch time is needed. For 30s RIE etch, depth of nanochannels were decreased to 200nm, Figure 6. From Table 1 and Figure 6, it can be seen that the etch of nanochannles was anisotropic.

High aspect ratio channels (depth/width) can be fabricated by RIE [22].

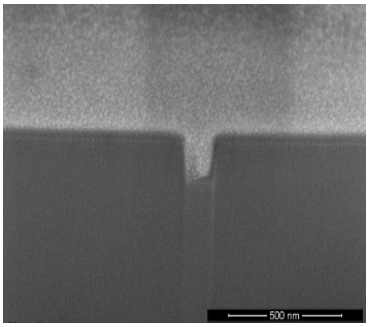
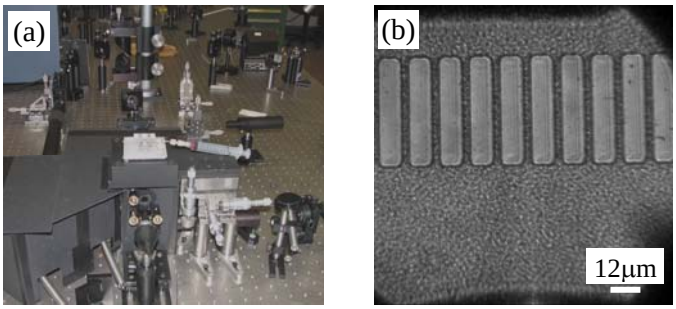


Figure 6. Cross section of a nanochannel with optimized dimension.

In order to verify that microfluidic and nanofluidic devices had through channels after bonding, a laser fluorescence experiment was performed, shown in Figure 7. A syringe was used to feed Rhodamine B fluorescent dye into the devices. Since the fused silica surface is hydrophilic, once the dye solution reached the via microchannels through the access holes, it traveled through the channels in seconds by wetting the channel surface. Some pressure was also used to facilitate wetting process and to flush the channel to remove trapped air bubbles. Figure 7 (a) shows the experimental setup. A CCD camera and white light source were used to image the channels in the device and the location of laser beam in the channel area. In (b) is shown the microchannels in a microfluidics device. The wavelength of laser was 532nm and beam size was around 30μm. It was observed that there was strong fluorescence from the dye in the microchannels (Figure 7c). The fluorescence was weaker in the nanochannels, Figure 7(d), compared with microchannels due to the reduced width of the channel. These images show that the nanochannels were preserved during bonding process. Further single molecule trapping and detection experiments are underway using 1μm probe size on a single nanochannel in the device.



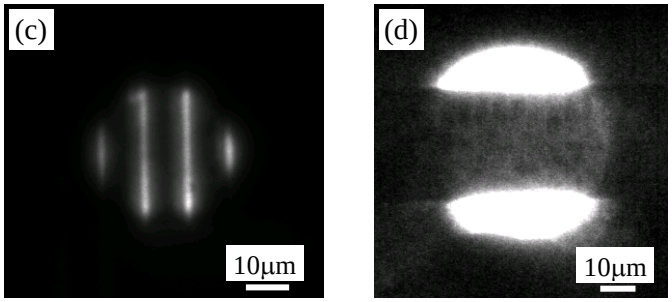


Figure 7. (a) Experimental setup for the single molecule detection using the nanofluidics device; (b) microfluidics device in experiment under a white light source; (c) fluorescence image of 4 microchannels; (d) fluorescence image of 9 nanochannels.

Acknowledgments

The project is financially supported by DARPA (Defense Advanced Research Projects Agency) and the Center for Laser Applications at the University of Tennessee Space Institute. A portion of this research was conducted at the Center for Nanophase Materials Sciences and the SHaRE User Facility, which are sponsored by the Division of Scientific User Facilities, Office of Basic Energy Sciences, U.S. Department of Energy. Claus Daniel acknowledges financial support from the Eugene P. Wigner Fellowship Program at Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725. Invaluable technical help from CNMS personnel is appreciated greatly: Scott T. Retterer for electron beam lithography; Darrell K. Thomas for photolithography, and Dale K. Hensley for characterization.

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