Shrinking of Solid-state Nanopores by Direct Thermal Heating

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Abstract

Solid-state nanopores have emerged as useful single-molecule sensors for DNA and proteins. A novel and simple technique for solid-state nanopore fabrication is reported here. The process involves direct thermal heating of 100 to 300 nm nanopores, made by focused ion beam (FIB) milling in free-standing membranes. Direct heating results in shrinking of the silicon dioxide nanopores. The free-standing silicon dioxide membrane is softened and adatoms diffuse to a lower surface free energy. The model predicts the dynamics of the shrinking process as validated by experiments. The method described herein, can process many samples at one time. The inbuilt stress in the oxide film is also reduced due to annealing. The surface composition of the pore walls remains the same during the shrinking process. The linear shrinkage rate gives a reproducible way to control the diameter of a pore with nanometer precision.

Background

The use of α-hemolysin protein nanopores inspired the fabrication of solid-state nanopores. Solid-state nanopores have emerged as novel biosensors for single molecule analysis of DNA, proteins, etc. [1-7]. Solid-state nanopores are more stable than protein nanopores under various experimental conditions like pH, salinity, and temperature [8-11]. When a single bio-molecule electrophoretically passes through a nanopore, it gives significant current blockage pulses.

The diameter of the nanopore should be almost at the same scale as the size of the translocating species. The pores fabricated with conventional processes result into initial diameters larger than the size of species of interest [12-16]. The nanopore diameter is then reduced using transmission electron microscope (TEM) or field emission scanning electron microscope (FESEM) to induce the shrinking [15,17] and FIB for the sculpting processes [18]. During the TEM shrinking process, the viscous flow of SiO₂ membrane is induced by an electron beam of optimal intensity. The nanopore shrinks or expands based on the surface-tension-driven mass flow. The nanopore, fulfilling the condition \(r < t/2\), would shrink under the electron beam at optimal conditions where \(r\) is the radius of the pore and \(t\) is the thickness of the membrane. TEM beam exposure depletes oxygen from the oxide at depletion rate of about 10% per hour [15]. Higher shrinking rates can be achieved through FESEM induced shrinking.

The FESEM induced shrinking mechanism is putatively not surface tension driven, but explained by radiolysis. The crystalline structure of the nanopore is disturbed under a high energy FESEM electron beam. This results in pore shrinkage due to the diffusion of Si and oxygen atoms toward the edge of the pore to overcome the crystalline defects present at the edge. The stoicheometry of the SiO₂ is expected to be different than a normal oxide layer due to radiolysis. Different shrinking rates were reported by using different acceleration voltages during FESEM exposure [17]. The nanopore was found to be always shrinking independent of the ratio of the pore’s diameter and membrane thickness under FESEM [17].

During the FIB sculpting process, the nanopore is exposed to an energetic ion beam. The accelerating ions drilled a nanopore in a thin oxide membrane due to sputtering of the surface, or these reduced the pore diameter due to atom diffusion or surface tension driven mass flow [18,19]. The FIB sculpting process is also dependent on the substrate temperature. Under an Argon ion beam, the pore closed at room temperature while it opened at temperatures close to 0°C [18].
Chemical composition of the material around the nanopore periphery changes during TEM or FESEM induced shrinking processes. This produces variable modifications of nanopore surface properties. These processes make the nanopore unfavorable for molecule analysis due to increased surface charge and electrical noise in the desired signal. In addition, all these shrinking processes are time consuming because they can only process one nanopore at a time. In this article, we report a simple and novel method to shrink nanopores using direct thermal heating. High temperature treatment (>1000°C), or annealing, promotes the viscous flow of the silicon dioxide (SiO₂) membrane and results in morphological changes that depend on the ratio of nanopore diameter to membrane thickness. Residual stress in the SiO₂ membrane is also reduced during high temperature annealing. Surface composition of the nanopore is maintained in this approach, as opposed to being inevitably changed in the electron or ion irradiation approaches previously reported. Annealing has been extensively used in semiconductor industry to reduce leakage current in thin films [20], to repair gate oxide damage from electrical stress [21], and to minimize residual stress in amorphous films [22].

Results and discussion
A boron-doped double-side-polished Si (100) wafer was thermally oxidized to a thickness of 400 nm. Square etch-start windows were opened in the SiO₂ using standard photolithography. Free-standing SiO₂ membranes (30 × 30 μm²) were achieved using wet tetramethylammonium hydroxide (TMAH) anisotropic etching through the whole wafer thickness. The schematic in Figure 1(a) depicts the membrane formed after anisotropic etching. Bulk membrane composition was determined by energy dispersive X-ray spectroscopy (EDS). The EDS analysis showed that the membranes contained only Si and O, as shown in Figure 1(b). The EDS analysis revealed 31% Si and 69% O. This was in good agreement with the expected stoichiometric film ratio of 33.33% Si and 66.66% O in SiO₂. A FIB was then employed to drill nanopores in free-standing SiO₂ membranes operated at a 30 kV acceleration voltage [23]. A larger portion of the drilled nanopores were in the diameter range of 100 to 300 nm. The high-resolution transmission electron microscope operating at 300 kV was used to image the nanopores after FIB drilling as shown in the inset of Figure 1(b). The nanopore dyes were kept in heating furnace at specific temperature for pore shrinking. The nitrogen flow rate of 20 sccm was maintained during this process.

We observed the nanopores shrinking or expanding when subjected to high temperature (1000 to 1250°C), contradicting previous findings [15]. The nanopores having an initial diameter of 250 nm were reduced to 3 nm at 1150°C as shown in Figure 2. The nanopores were imaged with TEM after each temperature processing step to characterize the process. After loading the dyes into the furnace, the temperature was allowed to stabilize for 30 s before counting the actual processing time. After the thermal process, the dyes were unloaded from furnace and cooled down to room temperature. When the dyes were processed at temperatures below 1000°C, it was observed that there was very little or no change in the diameter of the nanopore. This can be explained by the fact that at low temperature (<1000°C), the oxide layer would not be relaxed to an extent that it would start changing pore morphology. When the nanopores were processed at a higher temperature (>1250°C), the oxide membranes either broke due to very high thermal stress or the shrinking process was too fast to control. This was especially so for pores smaller than 20 nm diameter [24]. As an example, a nanopore with initial diameter of ~270 nm, processed at 1250°C, is shown in Figure 3. The TEM images of the nanopore...
show that the nanopore closed after 4 min due to an increased shrinking rate. The shrinking or expansion rate thus increased at higher temperature. When the pore diameter was larger than the membrane thickness, the nanopore started expanding in size instead of shrinking. A 350 nm nanopore in a 300 nm thick membrane was processed at 1150°C for 50 min. The pore expanded in size to 1.5 μm (Figure 4). It is interesting to note that direct heating can be used to shrink or expand the pore based only on the ratio of initial nanopore diameter to cylindrical length of the pore. The temperature itself had no effect on whether the nanopore would shrink or expand. The pore shrinking and expanding mechanism can be explained by the surface

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**Figure 2** TEM micrographs showing thermal shrinking of silicon dioxide nanopore. (a) TEM micrograph of ~250 nm pore drilled with FIB in 300 nm thick oxide membrane. (b) TEM micrograph of the nanopore after 5 min of thermal shrinking at 1150°C. The diameter of the nanopore was ~150 nm. The wavy surface of the oxide at nanopore edges shows the shrinking process due to viscous flow of oxide. (c) Nanopore after 10 min. The diameter is ~20 nm. (d) Nanopore after another 10 min and 40 s showing the diameter of ~3 nm.

**Figure 3** High temperature shrinking process. (a) TEM micrograph of ~270 nm diameter nanopore before shrinking. (b) TEM micrograph of nanopore after 4 min of thermal shrinking at 1250°C. The pore closed in just 4 min due to high shrinkage rate. The shrinking rate was about 70 nm/min.
tension which induced viscous flow of oxide film as described below.

The nanopore shrinking process was characterized at different temperatures as shown in Figure 5. The nanopore had no shrinking or expansion at 900°C. When the temperature was increased above 1000°C, the pore morphology started changing due to the diffusion and the viscous flow of oxide. The average nanopore shrinking rate was ~22 nm/min at 1150°C, which increased to 80 nm/min when the temperature was raised to 1250°C. At higher temperatures, the shrinking process was difficult to control precisely at the nano scale. When the nanopore diameter was reduced to tens of nanometers, low processing temperature (<1150°C) was used to accurately control the shrinking.

An obvious concern in the pore shrinking process is the possibility of hydrocarbon contamination that can affect pore shrinkage dynamics. All the dyes were cleaned with Piranha solution (1:1, sulfuric acid: hydrogen peroxide) before nanopore drilling with FIB. The chips were cleaned with argon-oxygen plasma for 5 min before and after each shrinking step. The chips were also cleaned with piranha solution for 5 min after TEM images to see whether the cleaning had any effect on the nanopore size. TEM images after cleaning revealed that the pore diameter remained the same. The local EDS analysis after each processing step showed no traces of hydrocarbons as shown in Table 1. Secondly, the nanopore shrank (Figure 2) or expanded (Figure 4) based on the ratio of nanopore radius to oxide membrane thickness, which is a strong indication that hydrocarbon contamination is not involved in the shrinking process. Thus, the process is not associated with hydrocarbon contamination.

The physics of nanopore shrinkage and expansion can be explained by taking into account the surface tension of the viscous oxide membrane [15]. At high temperature, the oxide membrane softens and deforms to find a structural morphology with lower surface free energy \( F \). For simplicity, the nanopore is considered cylindrical with radius \( r \) and oxide membrane thickness \( t \). The change in free energy with respect to radius can be calculated using the simple mathematical relation \( \Delta F = \gamma \Delta A = 2\pi \gamma (rt - r^2) \), where \( \gamma \) is the surface tension of the fluid and \( \Delta A \) is the change in the surface area [15,25]. From the above relation, it can be concluded that surface free energy of the nanopore having \( r < t/2 \) can be lowered by reducing \( r \), whereas for nanopores having \( r > t/2 \), their surface free energy can be lowered by
The fabrication process started by oxidizing a double-sided-polished, boron-doped silicon (100) wafer. The initial oxide thickness was 400 nm. Positive photoresist (PR) S1813 (Shipley Microposit J2 PR, Marlborough, MA, USA) was coated on one side of the wafer and square windows were opened after development. PR was coated on the other side followed by buffered hydrofluoric acid wet etching to remove oxide from square windows. The wafer was then washed with de-ionized (DI) water and dried with nitrogen. The wafer was submerged in acetone to remove the remaining PR. In order to make free-standing membranes, anisotropic etching was performed using 20% TMAH in DI water at 90°C (Mallinckrodt Baker, Inc. Phillipsburg, NJ, USA). Self-limiting etch was stopped once 30 × 30 μm² square windows were achieved in SiO₂. The thickness of the SiO₂ membranes were then reduced to 300 nm by reactive ion etching (RIE) using tetrafluoromethane at 100 W and gas flow rate of 15 sccm. The etch rate of the RIE was characterized using a reflectometer (Ocean Optic, Dunedin, FL, USA). All samples were cleaned with piranha solution before FIB (Carl Zeiss, Peabody, MA, USA) drilling. The free-standing oxide membranes were drilled with the FIB to create the initial pores. The FIB process was optimized first in terms of drilling time and milling current while the acceleration voltage of 30 kV was fixed. HRTEM (Hitachi High Technologies America, Inc., Schaumburg, IL, USA) operating at 300 kV was used to image the nanopores and to characterize their diameters.

### Methods

#### Nanopore fabrication and characterization process

The fabrication process started by oxidizing a double-sided-polished, boron-doped silicon (100) wafer. The initial oxide thickness was 400 nm. Positive photoresist (PR) S1813 (Shipley Microposit J2 PR, Marlborough, MA, USA) was coated on one side of the wafer and square windows were opened after development. PR was coated on the other side followed by buffered hydrofluoric acid wet etching to remove oxide from square windows. The wafer was then washed with de-ionized (DI) water and dried with nitrogen. The wafer was submerged in acetone to remove the remaining PR. In order to make free-standing membranes, anisotropic etching was performed using 20% TMAH in DI water at 90°C (Mallinckrodt Baker, Inc. Phillipsburg, NJ, USA). Self-limiting etch was stopped once 30 × 30 μm² square windows were achieved in SiO₂. The thickness of the SiO₂ membranes were then reduced to 300 nm by reactive ion etching (RIE) using tetrafluoromethane at 100 W and gas flow rate of 15 sccm. The etch rate of the RIE was characterized using a reflectometer (Ocean Optic, Dunedin, FL, USA). All samples were cleaned with piranha solution before FIB (Carl Zeiss, Peabody, MA, USA) drilling. The free-standing oxide membranes were drilled with the FIB to create the initial pores. The FIB process was optimized first in terms of drilling time and milling current while the acceleration voltage of 30 kV was fixed. HRTEM (Hitachi High Technologies America, Inc., Schaumburg, IL, USA) operating at 300 kV was used to image the nanopores and to characterize their diameters.

### High temperature shrinking process

The heating furnace was first turned on to raise the temperature to the desired range. All samples were put together in a horizontal carrier inside the furnace. The samples were allowed to heat up for 30 s before starting the actual processing time. The nitrogen flow rate of 20 sccm was maintained throughout the shrinking process. After the desired amount of time, the samples were taken out of furnace to cool down to room temperature. All the samples were cleaned with argon-oxygen plasma for 5 min before and after every thermal processing step to avoid hydrocarbon contamination.

### Conclusions

We demonstrated a new technique to shrink nanopores in oxide membranes with nanometer precision. The shrinking process is controlled and repeatable. In contrast to TEM or FESEM shrinking methods, our process can be used to shrink many nanopore dyes in parallel. We processed 5 to 10 dyes in one run and achieved similar shrinking rates. Our technique has an additional advantage in that it did not change the chemical composition of the pore walls. The oxide layer is softened under high temperature and is allowed to diffuse due to surface diffusion of viscous oxide.

### Abbreviations

DI: de-ionized; EDS, energy dispersive X-ray spectroscopy; FESEM: field emission scanning electron microscope; FIB: focused ion beam; HRTEM: high-resolution transmission electron microscope.
high-resolution transmission electron microscope; PR: photoresist; RIE: reactive ion etching; TEM: transmission electron microscope; TMAH: tetramethylammonium hydroxide.

Acknowledgements
We are thankful to the staff at Nanotechnology Research and Teaching Facility for their help in fabrication. Partial chip characterization was carried out at UTA Characterization Center for Materials and Biology (C²MB). The work was supported by grants from The Metropolex Research Consortium for Electronic Devices and Materials (MRCEDM), Arlington, Texas and National Science Foundation CAREER grant number ECCS-0849569.

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Authors’ contributions
WA fabricated the nanopores and carried out the thermal shrinking process. WA and AI did the characterization and imaging of the nanopores. WA and SM developed the conceptual framework and wrote the paper. SM designed the experiments and supervised the work.

Competing interests
The authors declare that they have no competing interests.

Received: 11 February 2011 Accepted: 4 May 2011

Published: 4 May 2011

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doi:10.1186/1556-276X-6-372
Cite this article as: Asghar et al.: Shrinking of Solid-state Nanopores by Direct Thermal Heating. Nanoscale Research Letters 2011 6:372.