Nanohole Fabrication using FIB, EB and AFM for Biomedical Applications

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Although many efforts have been made in making nanometer-sized holes, there is still a major challenge in fabricating individual single-digit nanometer holes in a more controllable way for different materials, size distribution and hole shapes. In this paper we describe our efforts to use a top down approach in nano-fabrication method to make single-digit nanoholes. There are three major steps towards the fabrication of a single-digit nanohole. 1) Preparing the freestanding thin film by epitaxial deposition and electrochemical etching. 2) Making sub-micro holes (0.2 μm to 0.02μm) by focused ion beam (FIB), electron beam (EB), atomic force microscope (AFM), and others methods. 3) Reducing the hole size to less than 10 nm by epitaxial deposition, FIB or EB induced deposition and micro coating. Preliminary work has been done on thin films (30 nm in thickness) preparation, sub-micron hole fabrication, and E-beam induced deposition. The results are very promising.

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NOMENCLATURE

FIB = focused ion beam
EB = electron beam.
AFM = atomic force microscope.

1. Introduction

The progress of nanotechnology depends on the developments of novel experimental strategies, such as those that can observe events at the scales of individual molecules or atoms. The essential features of these strategies must include a device or mechanism that can provide a significant amplification of the desired interactions at a nano scale. The nanohole that we are developing will be one of such devices that can detect and characterize the structures, interactions and processes at the individual molecule scale by drawing the molecules through the hole. Up to date, most work done in this area has used a protein channel in a lipid bilayer, [1] and only limited work has been reported using nanoholes in a solid-state thin film. [2]

1.1 Methods in nanofabrication

There are two categories in nano manufacturing, one is the so-called the "bottom up" approach which builds nano structures molecule by molecule, or atom by atom by means of self-assembly and self-organization; the other is the "top down" approach which uses facilities such as electro beam (EB), focused ion beam (FIB), X-ray, deep UV, atomic force microscopy (AFM), etc., and various lithography and etching methods to make nano scale structures. In this research we will mainly concentrate on the top down methods to fabricate nanometer size holes. Nano fabrication commonly refers to an ensemble of technologies implemented in making structures with a size of less than 100 nm in at least one dimension. Sophisticated lithography methods have been developed by the semiconductor industry and these methods have kept a continuous increase in computer and electronics business.

The current fabrication process in the semiconductor industry mostly involves electron beam lithography to make designed patterns on a set of masks and then utilizes optical projection lithography for the reproduction of the mask patterns at a high throughput level, using various material pattern transfer techniques. A typical lithographic process consists of three steps: coating a substrate with irradiation-sensitive polymer layer (resist), exposing the resist with light, electron or ion beams, and developing the resist image with a suitable chemical. Exposures can be done by either scanning a focused beam pixel by pixel from a designed pattern, or exposing through a mask for parallel replication.[3] The response of the resist to image-wise exposure can be either positive or negative, depending on whether the exposed or unexposed portions will be removed from the substrate after development. The next step after lithography is the pattern transfer from the resist to the substrate. There are a number of pattern transfer techniques: selective growth of materials in the trenches of the resist, etching of the unprotected areas, and doping through the open areas of the resist by diffusion or implantation.[4] Both wet chemical etching and dry plasma etching can be used. For a high-resolution pattern transfer, dry etching is more suitable, and often requires a metallic layer as a mask. This metal mask is obtained by lift-off, i.e., by first depositing a thin metallic layer over the developed resist pattern and then dissolving the resist in order to leave only the metal portions that are directly in contact with the substrate.[5] For multilevel fabrication, lithography and pattern transfer are used for each level.

1.2 Fabrication of nanometer scale holes

There are several ways that are possible to make nano scale holes and they can be classified as two groups: the natural material method, using self assembly of proteins to form a nanopore [6] or using nanoporous polymers or membranes [7, 8]; and the fabrication method, using equipment to make nano holes on solid state materials. In using the natural material method, researchers have synthesized protein nanopores (α-hemolysin) in a lipid bilayer separating two solution-filled compartments [6] and also successfully used a highly ordered alumina through-hole membrane and reactive beam etching to fabricate highly ordered arrays of GaAs and InP nanoholes, as small as 60 nm.[7] Researchers at iMEDD (intelligent Micro Engineered Drug Delivery) are developing an implantable drug delivery device based on nanoporous silicon membranes. The company has fabricated thin silicon membranes with pore sizes in the 10-100 nanometer range. [9] In the fabrication method, femtosecond laser has been used to ablate holes with a diameter of 300 nm and a depth of 52 nm.[10] Electro beam or focus ion beam lithography plus reactive etching can be used to fabricate nanostructures and holes in as small as 10-20 nm [3, 11] Researchers at Harvard University have developed a method called Ion-beam sculpting at nanometer scales that can be used to make holes in 60 nm, if with further ion beam deposition the hole can be even much smaller.[2]

Although many efforts have been made in making nano holes, there is still a big challenge in fabricating individual single-digit nanometer holes in a reproducible and more controllable way for different materials, size distribution and hole shapes. In this work we use the top down approach and nano-fabrication method to make the single-digit nano holes. Nanometer sized holes can find numerous applications in various fields. In genetic therapy and direct DNA vaccination, strategies must be devised to make the exogenous DNA pass the pores into the nucleus of a host cell. In chemistry, the forced permeation of molecules through pores or porous media is the crucial technique for separations and purifications of synthetic molecules. Nanoholes can be used to investigate the mechanisms of macromolecular translocation, as well as to characterize the physical properties of macromolecules, such as size, charge and structures, which can potentially lead to the development of novel biosensors. Rapid sequencing of individual nucleic acid molecules can lead to a wealth of potentially revolutionary change in medicine, bio-agent defense and identification of biological species. The development of reliable methods for fabricating nanometer sized holes on solid state free standing film will facilitate the implementation of aforementioned applications.

2. Research Design and Methods

2.1 Method for nanohole fabrication

The general method to make the single-digit nanoholes is described in Fig. 1. There are three major steps towards the making of a single-digit nanohole, and in each step there are details that will be discussed in the following sections.

2.1.1 Preparation of silicon epitaxial freestanding films

In order to fabricate solid state nanoholes, free standing films with appropriate thickness and mechanical properties have to be made first. There are many ways to make freestanding films. In this research for our specific requirements we have used the method of epitaxial deposition and electrochemical etching to prepare the film. Fig. 2 shows the main principle in making the freestanding film. Fig. 3 shows our microfabricated silicon nitride free standing thin film with a scale drawing and two photos of the film. The mechanical and electrical properties of the freestanding film directly affect the efficiency of the fabrication and the quality of the nanoholes, as well as the performance of the nanoholes in studying macromolecular translocation.

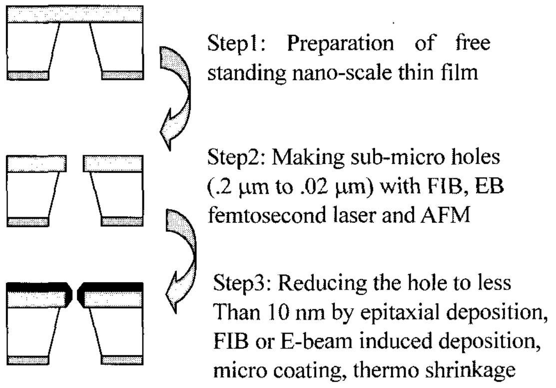


Fig. 1 The procedure to make nanoholes

2.1.2 Making sub-micro holes (0.2 to 0.02 µm) by FIB

We have tried several methods to make sub-micro holes and found out two feasible ways, using FIB, or using AFM probe with a nano pin as a puncher directly. By using FIB, a prepared free standing thin film can be positioned on the sample stage, and the ion beam can be focused to a spot as small as 30 nm (See Fig. 4). Due to proximity effect the penetrated holes have dimensions of 40 to 50 nm. The holes made with FIB milling are strongly dependent on the ionic current and milling time, in addition to the beam size. With the optimal parameter setting, it will be possible to obtain hole in the 20 nm range.

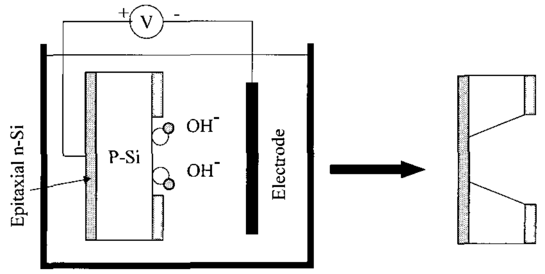
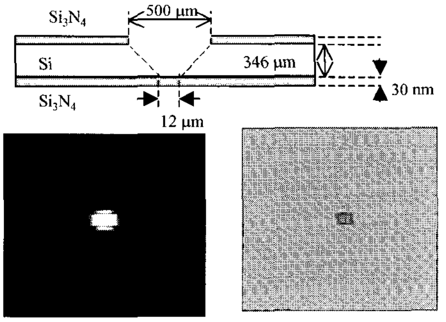


Fig. 2 Method of making a freestanding film



Top View with 500 µm window, Bottom View with 12 µm square hole

Fig. 3 Microfabricated silicon nitride thin film

How FIB makes holes can be described by an established sputtering phenomenon.[12] When ion beams with energies of 10 to 200 KeV impinge on a surface, an atomic scale erosion process, called sputtering, removes one atom from the surface for every incident ion approximately. [2, 12] However a detailed study on how to develop a scientific model for using FIB to 'drill' a nanohole, how to control the hole size and shape, and how the film material affects on the sputtering process need to be investigated further in the future.

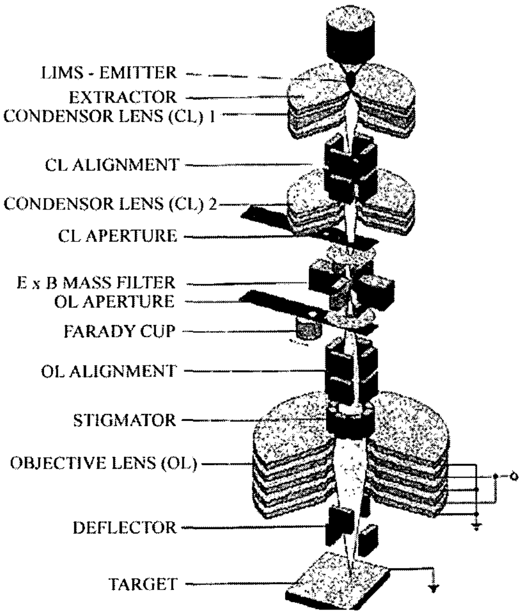


Fig. 4 Schematic of a FIB machine

2.1.3 Making sub-micro holes (0.2 to 0.02μm) by AFM probe with a nano pin or nanotube

AFM is a versatile tool capable of making atomic scale measurements. It not only can make various precision measurements, but also can be used as a force application and measurement tool, such as a puncher. We have shown that commercial available AFM probe tips can make a hole in the free-standing film, but the hole dimensions (around 2 μm) are far from the desired size (see later discussion). We are going to grow a 20 to 30 nm pin on the commercial AFM tip, and then use this pin as the puncher to penetrate the free-standing thin film to generate a hole.

A) Fabricate nano pin on the AFM tip. Electron beam has been used to deposit a nano tip, as small as 20 nm in diameter, on the AFM probe, see Fig. 5, in order to scan high aspect ratio structures .[13] We will use the same EB deposition procedure to fabricate nano pin on the AFM tip. The nano pin will have a diameter of 20 to 30 nm at the end and a height of 300 nm. Such a pin also has been shown to be mechanically robust enough to stick on the AFM tip and not be bent under the force. In doing this we must solve several problems. i) EB often has a drift problem, and it cannot be focused on one fixed spot for an extended period of time because of many disturbances from surroundings. ii) The pin should grow from the AFM tip straightly up. However, the pin often leans to a side that will affect thin film punch process greatly. iii) We still don't exactly know how large a force should be provided to the tip. To solve these problems, we are performing a systematic testing of the punching process to optimize the parameters, and develop a punch force model for thin film penetration in nano scale. Using the mechanics theory and finite element analysis, we will elucidate the effects of punch force and speed, nano pin size and geometry, and thin film materials on the nano hole formation. Based on this model, a carefully punch force calculation and control can be used to guide the nano hole fabrication process.

B) Mounting nanotubes on the AFM tip. Carbon nanotubes have superior mechanical properties and have been used as AFM probes.[14-17] The ultimate needle that can be used to make nanoholes will be a single walled carbon nanotube. Various methods have been reported on mounting carbon nanotubes onto AFM probes. Hafner et al. [14] swept an AFM tip across a silicon chip covered with vertical single walled nanotubes (SWNT), causing some of them to detach and bond to the AFM tip. Hafner et al. also have grown nanotubes directly onto the AFM probe tip by chemical vapor deposition.[15] Dai et al. demonstrated a method for manual attachment of nanotube material onto the end of AFM probes. [16] Gommans et al. developed a novel method of manually attaching single walled nanotubes to the end of an AFM probe tip using fibers of SWNT pulled from a dispersion of N,N-dimethylformamide (DMF).[17] We will follow the approach of Gommans et al [17] to mount SWNT on the AFM tip.

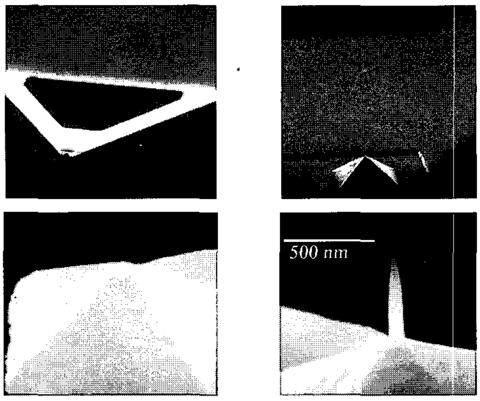


Fig. 5 EB induced deposition for a nano tip on an AFM probe (After D. Kel ler and C. Chou [13])

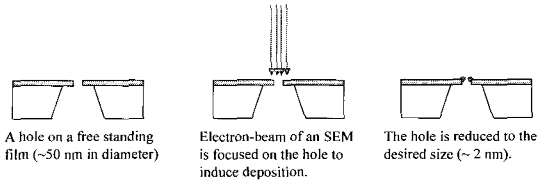
Briefly, the procedure starts with suspending the SWNT in N, N-dimethylformamide (DMF), and then the SWNTs are attracted to a carbon fiber via electrostatic interaction. The nanotubes adhere to each other and to the highly graphitized carbon fiber by van der Waals forces, leading to a fine fiber of SWNT material formed on the end of the carbon fiber. The AFM probe tip is brought into contact with the SWNT fiber at the end of the carbon fiber and attached by van der Waals attraction using the micromanipulator. The SWNT fiber is cut to a length of 2 μm by a focused laser. In order to prevent disruption of the weak van der Waals force holding the SWNT fiber to the tip, the nanotube modified AFM tip is then coated with a thin layer of polymer. After coating, the SWNT fiber is shortened by laser cutting and is exposed at the tip of the fiber as the polymer burned off at a faster rate than the nanotubes.

Using the nanotube tip, holes with diameters of several nanometers can be made on the freestanding film using direct punch by exerting a controlled force. The critical issue will be how much force to use and how fast to increase the force. The length of the nanometer tip will also be important to make the punching more precise. Experiments will be carried out to optimize these parameters so that holes can be made with a minimum size without mechanically damage the film. These holes should be much smaller than that can be made using the FIB milling, so it will be easier to precise control the process of shrinking the size of the hole to the desired size using the method discussed below.

3. Reducing the hole to less than 10 nm

There are several potential ways to reduce the hole size such as epitaxial depostion, FIB or E-beam induced deposition, micro coating, thermo-assisted shrinkage. In this research we will mainly use FIB and EB induced deposition since we have the necessary equipment and experience, and have done preliminary work. Focused electron beam in an SEM has been used to deposit ultra-small tips for AFM [13];

the same approach will be used to shrink the size of the hole to desired nano-scale. We propose to adjust an electro beam to a diameter of 100 nm then point the beam to a pre-made ~50 nm hole. After a specified amount of time, carboneous materials (mainly from the diffusion pump oil) in the EM chamber will be deposited on the edge of the hole, leading to a deduction of the hole diameter to the desired size, see Fig. 6. When there is not sufficient carboneous material in the EM chamber for the e-beam induced deposition, a tiny amount of diffusion pump oil-acetone mixture will be introduced in the chamber.


A hole on a free standing film (~50 nm in diameter)

Electron-beam of an SEM is focused on the hole to induce deposition.

The hole is reduced to the desired size (~ 2 nm).

Fig. 6 Proposed EB induced deposition to reduce the nanohole size

Another approach of shrinking the hole size will be the FIB induced deposition of materials. Several researchers have used FIB to sculpt and sputter nano structures [2, 12, 18] We will use FIB induced deposition on a sub-micron hole to reduce the hole size to single-digit nanometer.

A preliminary model has been proposed to explain the hole-closing phenomenon by Li et al. [2] in which incident ions both create and annihilate excess, independent and, mobile surface 'adatom' (for example, atoms or molecular clusters) that can diffuse to the hole. The concentration of surface adatoms C(r, t), is governed by a two-dimensional diffusion equation:

$$\frac{\partial}{\partial t}C(r,t) = FY_a - FC\sigma - \frac{C}{\tau_{trap}} + D\nabla^2 C \tag{1}$$

where r and t are surface position and time; D is the adatom surface diffusion coefficient and F the incident ion-beam flux; Y_a is the incoming flux factor, σ is the annihilation number and τ_{trap} is the annihilation rate due to trapping at surface defects.

From steady-state solutions to Equation (1), the diffusion flux into closing the hole can be obtained. A characteristics distance from the hole edge, X_m, within which adatoms are more likely to reach the hole than be annihilated by traps or ion erosion:

$$\frac{1}{X_m^2} = \frac{1}{D\tau_{trap}} + \frac{\sigma}{D}F \tag{2}$$

Adatoms beyond X_m are more likely to be annihilated before they reach the hole rim. By using this model and solution we can study how to reduce the hole to our desired nano size.

4. Preliminary experimental results

Using available facilities and resources, we have carried out a series of preliminary studies to assess the feasibility of the research.

4.1 Thin film fabrication

The prerequisite of making nanoholes is the ability to fabricate solid state free-standing thin films. We have used the epitaxial deposition and electrochemical etching (details described in the Section 2 of Research Design and Methods) method to make thin (~30 nm), free-standing films on a 350 μm thick silicon substrate. The film is made of silicon nitride and has a dimension of 12μm x 12μm. Fig. 7(A) shows an Electro Microscope (EM) photo of the geometry of one of the thin films.

4.2 Making nanoholes using the focused ion beam (FIB) milling

A FEI DB235 Dual Beam FIB is used for this work. It is an ideal tool for patterning surfaces with nanometer scale resolution. The electron beam (SEM) is used to image and orient samples with respect to the ion beam. Imaging with the SEM reduces damage to the sample due to exposure to the ion beam. The FIB control software contains predefined shapes (circle, rectangle, line, and, polygon). These shapes are inserted into the image window. The dimensions of each pattern element are controlled in the patterning dialog box. The user can define the length, width, and depth of individual features. The user also defines the milling parameters such as dwell time and percent overlap. The spot size and current density of the ion beam are also controlled by the user. Etching efficiency is dependent on sample material, ion beam current density, dwell time and percent overlap in the position of the beam. Using the focused ion beam milling method, we have been able to make holes of various sizes (30 nm to 200 nm) on the Si3N4 free-standing film. The critical parameters that dictate the size of the holes are the beam current and the diameter of the pattern selection, and the milling time. Fig. 7(B) shows five holes in a thin film that were made with different parameter settings of the focused ion beam machine. There are four through holes and the big central one is a not through dent. The smallest hole is 30-40 nm.

4.3 Batch production of nanoholes using pattern and automation features from the Dual Beam FIB

Users can also create patterns such as an array of circles or squares and save the pattern as a file. Pattern data consisting of arrangements of geometric elements can be stored and used alone or in combination. For example, arrays of squares of known size and spacing can be stored as a pattern. Using the SEM a nitride membrane can be imaged and its location can be stored as a stage position. A single pattern or multiple patterns can be defined on the surface of the membrane and then pores can be manually or automatically milled using the ion beam. For multiple membranes an AUTO TEM script can be prepared which will automatically move the stage to predefined locations and mill the same pattern at each location. The automation of the technique will be particularly useful for batch production of nanometer-scale pores over large surface areas or on multiple samples. To demonstrate the batch production and automation features Fig. 7 (C) shows two rows of holes with square and circle shapes and different sizes. Fig. 7 (D) shows one column of same diameter holes.

4.4 Reducing the hole size by FIB deposition

As mentioned before both FIB and EB induced deposition can reduce hole size from hundreds nm to single digit nm. To find the feasibility the following tests were made. Fig. 7 (E) and (F) both originally have two same size holes (around 330 nm long dia.), after FIB deposition the hole sizes reduced to around 180 nm. Fig. 7 (G) to (J) show that originally 4 squares and 4 circles were milled (G), after 100 nm platinum deposition the lower left hole filling in (H), after 200 nm Pt deposition the lower left hole was closed, and then by using milling function a small hole opened again.

4.5 Making nanoholes using the AFM tips

Another method that we have tried to create nanoholes on the free-standing film is to puncture the film directly with a nanometer size tip. The AFM was used to perform the task by pushing the tip against the film with an increasing force, until the film is pierced. Initial tests using the tapping mode silicon tip (from Veeco Metrology, Santa Barbara, CA, USA) showed that the film can be pierced without breakdown at a force of several micro-newtons, see Fig. 8. The only problem is that the hole size is too big (~2 μm) and the hole edge is not smooth. We will add a nano pin (~30 – 40 nm) or nanotube (~1-2 nm) on the AFM tip to puncture the thin film to make holes.

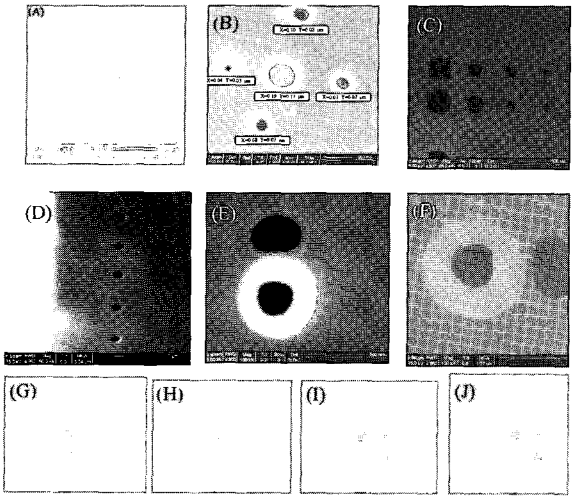


Fig. 7 Fabrication of nanoholes on Si3N4 free-standing film (A) The 12μm x 12μm free-standing film is at the bottom of the inverse-pyramid shaped opening in the silicon substrate (B) The holes made using FIB milling with different parameter settings (ion beam current = 10 and 30 pA and the milling time was changed in the range of 0.5 to 7.0 sec) (C) Two rows of holes with square and circle shapes and different sizes (D) One column of same diameter holes (E) & (F) Two holes in each picture originally had same size, after FIB deposition one hole size was reduced (G) to (J) show the lower left hole fill-in, closed and opened again

4.6 Other methods tested

Using the facilities at the Regional Laser and Biomedical Technology Laboratories (RLBL), located in the Chemistry Department at the University of Pennsylvania, we have tested the possibility of using femtosecond laser to create nanohole in the silicon nitride thin film. The results were not satisfactory since even the smallest holes created this way are too large (more than 3 μm). We also tried to use the electron beam to create the holes inside an SEM, but no holes were made even after focusing the electron bean on the film for an hour (at 30 keV).

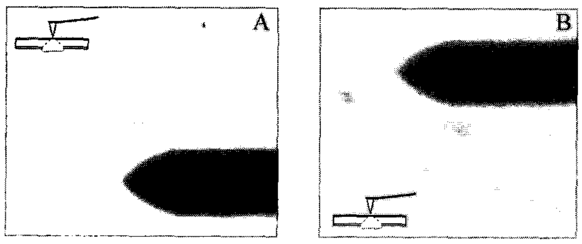


Fig. 8 (A) Optical microscope image of the top view of the AFM cantilever and the free-standing film before a hole is punched (B) After a hole is punched The size of the film is 12μm x 12μm, see the central square with a tiny hole. The black spots outside the square are dusts/debris The inset is the schematic of the side view of the setup

5. Conclusions

Several nanohole fabrication methods by using top down manufacturing techniques have been introduced, discussed, and tested. Free standing thin films with a thickness of 30 nm have been made by epitaxial deposition and electrochemical etching. FIB, EB and AFM were all used in making nano holes, and the results are promising. Further study is needed to get the hole to single digit nano holes and to develop the process in production scale.

REFERENCES

1. Sauer-Budge, A. F., Nyamwanda, J. A., Lubensky, D. K. and Branton, D., "Unzipping Kinetics of double-stranded DNA in a nanopore," Phys Rev Let, 90, 238101-1 to 238101-4, 2003.
2. Li, J., Stein, D., McMullan, C., Branton, D., Aziz, M. J. and Golovchenko, J. A., "Ion-beam sculpting at nanometer length scales," Nature 412: 166-169, 2001.
3. Yong, C. and Pepin, A., "Nanofabrication: Conventional and nonconventional methods," Electrophoresis, 22, 187-207, 2001.
4. Smith, H. I. and Craighead, H. G., "Nanofabrication," Phys. Today 43, 24-30, 1990.
5. Sze, S. M., "Semiconductor Devices: Physics and Technology," John Wiley, New York, 1985.
6. Deamer, D. W. and Branton, D., "Characterizing of nucleic acids by nanopore analysis," Acc. Chem. Res. 35, 817-825, 2002.
7. Nakao, M., Oku, S., Tamamura, T., Yasui, K. and Masuda, H., "GaAs and InP Nanohole arrays fabricated by reactive beam etching using highly ordered alumna membranes," Japanese J. of Apply. Phys., 38, 1052-1055, 1999.
8. Bhatia, S. and Nicholson, D., "Molecular transport in nanopores," J Chem. Phys., 119. 1719-1730, 2003.
9. Pores Help Nanogate Deliver, MEDICAL MATERIALS UPDATE, http://www.buscom.com/letters/mmupromo/mmu/mmu.html
10. Pronko, P. P., Dutta, S. K., Squier, R. J. V., Du, D. and Mourou, G., "Machining of sub-micron holes using a femtosecond laser at 800 nm," Optics Commun., 114, 106-110, 1995.
11. Siwy, Z. and Fulinski, A., "Fabrication of a Synthetic nanopore ion pump," Phys. Rev. Lett., 89, 198103-1 to 198103-198104, 2002.
12. Johnson, R. E. and Shou, J., "Sputtering of inorganic insulators," K. Danske Vidensk. Selsk. Mat.-fys. Meddr., 43, 403-494, 1993.
13. Keller, D. and Chou, C., "Imaging Steep, High Structures by Scanning Force Microscopy with Electron Beam Deposited Tip," Surf. Sci., 268, 333-339, 1992.
14. Hafner, J. H., Cheung, C. L., Oosterkamp, T. H. and Lieber, C. M., "High-Yield Assembly of Individual Single-Walled Carbon Nanotube Tips for Scanning Probe Microscopes," J. Phys. Chem. B 105, 743-747, 2001.
15. Hafner, J. H., Cheung, C. L. and Lieber, C. M., "Direct growth of single-walled carbon nanotube scanning probe microscopy tips," J. Amer Chem Soci, 121, 9750-9751, 1999.
16. Dai, H. J., Hafner, J. H., Rinzler, A. G., Colbert, D. T. and Smalley, R. E., "Nanotubes as nanoprobes in scanning probe microscopy," Nature, 384, 147-150, 1996.
17. Gommans, H. H., Alldredge, J. W., Tashiro, H., Park, J., Magnuson, J. and Rinzler, A. G., "Fibers of aligned single-walled carbon nanotubes: Polarized Raman spectroscopy," J. Appl. Phys. 88, 2509, 2000.
18. Li, J., Gershowl, M., Stein, D., Brandin, E. and Golovchenko, J. A., "DNA molecules and configurations in a solid-state nanopore microscope," Nature Materials 2, 611-615, 2003.