Focused Ion-Beam Based Nanohole Modeling, Simulation, Fabrication, and Application

There are three major steps toward the fabrication of a single-digit nanohole: (1) preparing the free-standing thin film by epitaxial deposition and electrochemical etching, (2) making submicron holes (0.2–0.02 μm) by focused ion beam (FIB), and (3) reducing the hole to less than 10 nm by FIB-induced deposition. One specific aim for this paper is to model, simulate, and control the focused ion-beam machining process to fabricate holes that can reach a single-digit nanometer scale on solid-state thin films. Preliminary work has been done on the thin film (30 nm in thickness) preparation, submicron hole fabrication, and ion-beam-induced deposition, and the results are presented.

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1 Introduction

The idea of using nanometer-sized holes to characterize biological macromolecule and other polymer molecules has been around for quite a few years, with the most exciting prospect of developing an ultrafast method for DNA sequencing [1]. To date, most work done in this area has used a protein channel in a lipid bilayer [2], and a limited amount of work has been reported on fabricating and using nanoholes in a solid-state thin film [3]. The benefits of protein nanopores include well-defined size/shape and well-established procedure for their synthesis. However, these natural nanopores have several drawbacks for the purpose of characterizing DNA and other macromolecules. The most severe of these limitations are the instability, both mechanical and chemical, and toxicity of the protein nanopores.

The use of solid-state nanopores mitigates these problems, and offers the additional advantage of tunable pore size. However, the fabrication of these nanometer-sized holes on solid-state materials imposes a great challenge, especially in the control and reproducibility of the size and shape of the nanoholes. The previously reported methods [3–5], which will be discussed in detail in Sec. 2.3, involved sophisticated instruments and complicated procedures, and the mechanisms for the hole formation in these procedures are not well understood, thus making them difficult to follow and repeat. A reliable control over the size and shape of the nanoholes has not been achieved. In the work here, we will employ a two-step approach, using both standard and innovative techniques, to fabricate nanoholes on a free-standing solid-state thin film. Theoretical modeling and Monte Carlo simulation will be carried out to elucidate the physical principles governing the nanohole fabrication process. With these concerted efforts, we will develop protocols for fabrication of solid-state nanoholes with more consistency and better control over their size and shape. Using these nanoholes, we will, through a novel approach, characterize the structural and dynamic properties of DNA and RNA molecules at the single molecule scale. Detailed nanohole applications and nanofabrication methods have been reviewed in Sec. 2. In Sec. 3, focused ion-beam (FIB) process modeling and simulation for nanohole fabrication, thin film preparation, nanohole fabrication using FIB, and setup for DNA/RNA characterization were all discussed. Some preliminary study results were shown in Sec. 4.
all the work published so far, the signal detected is the modulation of the ionic current by the passage of the DNA through the nanopore. The current is created by an applied voltage across the nanopore bearing membrane or film. Due to the structural differences, each nucleotide blocks the current by a (slightly) different amount, thus producing a characteristic modulation in the ionic current as the DNA passes the nanopore [13]. Experiments using α-hemolysin nanopores on DNA and RNA molecules have not been able to read the sequence of the molecules at the single-nucleotide resolution. However, stretches (tens of bases) of the same nucleotide can indeed be distinguished from each other, such as poly A from poly C and poly A from poly dA, by the difference in the ionic current blockade as well as the speed at which the polymer passes the nanopore [13]. The limiting factor on the resolution is that each nucleotide or base-pair passes the nanopore too fast to allow an accurate determination of the blockage current. Various proposals have been made to circumvent this problem, such as increasing the magnitude of the ion current [14] or adding a rotating electric field [15]. Experiments of investigating DNA molecules passing a solid-state nanohole were also performed, and the results were shown to be similar to that obtained using the α-hemolysin nanopore [3]. It is obvious that some major hurdles have to be overcome before rapid DNA sequencing with nanopores becomes practical.

**Biosensing.** The nanopore is not limited to analyzing nucleic acids; it can also be used as a nanoscale biosensor to detect other macromolecules, macromolecular processes, or other biological entities, e.g., bacteria and viruses. One of the intriguing aspects of the technique is that it may be developed into a biosensing method based on the unique physical characteristics of the analytes instead of their biological activities. Such a non-agent biosensing technique would greatly enhance the durability, tolerance to environments, and sensitivity. Prototype biosensors employing genetically engineered α-hemolysin pores have already been devised for the detection of a variety of substances, including proteins, metal ions, and small organic molecules [16]. A well-defined nanopore with a diameter of around 5 nm will be able to detect and perhaps identify individual globular protein molecules. Hybridized DNA should be readily distinguished from single-stranded DNA by a nanopore of appropriate size, thus providing an alternative to microarrays for assays based on hybridization. Kasianowicz et al. [17] showed that two different proteins could be detected simultaneously with the nanopore of α-hemolysin. By placing the analyte recognition site on a pore-permeate polymer, the nanopore system could be programed to detect different proteins. These authors also pointed out that a robust, artificial nanohole would allow the quantitative detection of multiple analytes. Howorka et al. [18] tethered a DNA oligonucleotide near the entrance of the α-hemolysin nanopore, and showed that the device could distinguish between polynucleotides whose sequence perfectly complements the tethered oligomer and those whose sequence imperfectly complements the tethered oligomer. Even a single-nucleotide mismatch could be detected in such a system. Subsequent developments in nanopore technology could eventually lead to devices for detecting a specific protein based on its sequence, which would be a more challenging task since there are 20 different amino acids to differentiate and the complications from post-translational modification.

**Immunosuppression and controlled drug delivery.** It has been shown [19] that cells can be encapsulated in microchambers with nanometer-scale holes in the walls that are large enough for small molecules to pass through and, at the same time, are small enough to impede the passage of large immune system molecules or viruses, so that the enclosed cells can secrete the desired proteins while avoiding the attack by viruses or the immune system. These researchers believed that microcapsules containing replacement islets of Langerhans cells could be implanted beneath the skin of some diabetes patients. This could temporarily restore the body’s delicate glucose control feedback loop without the need for powerless immunosuppressants that can leave the patient at risk of infection. The encapsulation approach can also be used for longer-term controlled drug delivery with the rate of drug release being determined by the size and the number of nanoholes in the capsule. The major advantage of using capsules made of solid-state materials as drug delivery devices over polymer and liposome capsules is that the solid-state capsules can be easily integrated with micro-electronic systems to externally control the drug delivery. Researchers at Intelligent Micro Engineered Drug Delivery (IMEDD) 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 [20].

**Nanofiltration and other applications.** The nanoholes can be used in biofluid purification, especially for viral elimination of blood products [21]. The nanofiltration process filters the protein solution through membranes with nanoholes having diameter in the range 15–40 nm. The viruses, both enveloped and non-enveloped, are filtered out based on size exclusion, while 90–95% of protein activity is recovered. Some data also suggested that the nanofiltration also removed undesired proteins [21]. Since the bacteria and viruses can change their shapes, they might be able to pass through holes that are smaller than their normal size. It would be interesting to study what size hole a particular bacterium or virus can pass, and what the processes and mechanisms are. Such information could be useful to rationally design effective filters and protective gears against the particular bacterium or virus.

Other applications of nanoholes include detecting and counting single molecules, measuring a molecule’s length [22], studying the dynamics of polymer translocation in confined spaces [23,24], and sensing a polymer’s local cross-sectional volume [13]. All the above mentioned applications of nanoholes depend on reliable fabrication methods for such holes. We are proposing to employ an interdisciplinary approach to make such holes in free-standing solid-state films. There are still many technical hurdles that need to be overcome before reliable protocols can be set up; our proposed research is intended to solve some of these problems.

### 2.2 Methods in Nanofabrication

Nanofabrication commonly refers to an ensemble of technologies implemented in making structures with a size of less than 100 nm in at least one dimension. There are generally two tactics in nanomanufacturing: one is the “bottom up” approach in which the nanostructures are assembled molecule by molecule, or atom by atom by means of self-assembly and self-organization; and the other is the “top down” approach in which nanostructures are machined using sculpture techniques, such as electron beam (EB) and FIB, X-ray, deep UV, atomic force microscopy (AFM), etc., in conjunction with various lithography and etching methods. In this research we will mainly concentrate on the top down methods to fabricate nanoholes.

The current fabrication process in the semiconductor industry involves electron-beam lithography to make designed patterns on a set of masks and then use optical projection lithography for the reproduction of the mask patterns at a high throughput level, combined with various material pattern transfer techniques. A typical lithographic process consists of three steps: coating a substrate with an 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 [25]. The response of the resist to image-radiation 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 [26,27]. Electron-beam lithography is sophisticated and costly, and is not suitable for our proposed nanohole fabrication.

The FIB machining to make microdevices and microstructures has gained more and more attention recently [28,29]. FIB can be used as a direct milling method to make microstructures without involving complicated masks and pattern transfer processes. FIB machining has advantages of high feature resolution, and imposes no limitations on fabrication materials and geometry. Figure 1 illustrates a FIB machine architecture: Liquid-metal source feeds metal ions into an extractor, gets excited, and the ions pass through the aperture and are focused by a lens onto a spot on the substrate. Focused ion beams operate in the range 10–200 keV. As the ions penetrate the material, their energy is used to remove substrate atoms. FIB has been proven to be an essential tool for highly localized implantation doping, mixing, micromachining, controlled damage, as well as ion-induced deposition [30]. The technological challenge to fabricate nanoholes using electron-beam lithography and FIB is that the minimal feature size accessible by these techniques is typically limited to tens of nanometers; thus novel procedures must be devised.

2.3 Existing Solid-State Nanohole Fabrication Methods.

Nanometer sized holes in free-standing synthetic films have been fabricated for quite a few years for various purposes. In order to achieve stable metal point contacts (nanobridges), Rails et al. [31] made holes with diameters less than 10 nm in 50-nm-thick Si3N4 films, using anisotropic etching and electron-beam lithography. Using a combination of chemical etching and electrostopping, Apel et al. [32] made holes with diameters of nanometers on 12-μm-thick poly(ethylene terphthalate) (PET) films. With a similar etching approach, Siwy and Fulinsky [33] made holes as small as 2 nm in diameters on 12-μm-thick PET films for the purpose of ion pumping. These nanoholes, while having the desired diameters, are made on relatively thick films, so they are not appropriate for studying macromolecules.

In 2001, a group at Harvard reported the invention of an “ion-beam sculpting” technique to fabricate holes with diameters of several nanometers on Si3N4 and other solid-state films, and demonstrated that these nanoholes can detect individual molecules of 500 bp dsDNA as they were driven through a nanohole by a 120 mV bias voltage in saline solution [3]. With this technique, a 100-nm-diameter bowl-shaped cavity, or a larger hole (~100 nm), was first created on the free-standing Si3N4 films using reactive ion etching or focused ion beam, respectively. The nanohole could then be created either by ion-beam sputtering of the opposite side of the bowl-shaped cavity or by the hole-closing process from the ion-beam-induced lateral transport of matter.

Whether hole opening or closing dominated was controlled by changing the sample temperature or the ion-beam parameters. A feedback system was used to count the ion transmission rate in order to achieve the desired hole size. Later, the same research group further demonstrated that the solid-state nanoholes could be used to detect individual molecules of double-stranded DNA and to observe their folding behaviors [12]. A more sophisticated “ion-sculpting” instrument was reported recently [34].

In 2003, Storm et al. [5] reported a nanohole fabrication technique on single-crystalline silicon films. In this method, a 20 nm hole is first made in the silicon film by electron-beam lithography and anisotropic etching, and the hole is then shrunk to a single nanometer size through thermal oxidation by exposing it to a high energy electron beam. The electron beam fluidizes the silicon oxide, leading to a shrinking of the hole due to surface tension. The process used direct real time visual feedback from the electron microscope (EM) image and could make nanometer-scale sample modifications. There are several other reported studies of fabrication and modification of nanoholes for macromolecular studies. Polk et al. [35] reported the use of electrodeposition of silver at the silicon micropore edge to create openings less than hundreds of nanometers in size. Heng et al. [36] reported the fabrication of nanometer-diameter holes on nanometer-thick metal oxide semiconductor (MOS)-compatible films using electron-beam-induced sputtering. Chen et al. [37] studied the DNA translocation kinetics in ion-beam sculpted nanoholes fabricated in Si3N4 with surface properties that had been modified by an atomic layer deposition of Al2O3. The surface modification improved nanohole performance by reducing electrical noise and enhancing the DNA capture rate. A femtosecond laser was also used to ablate holes with a diameter of 300 nm and a depth of 52 nm [38].

3 Research Design and Methods

One main objective of this research is to understand the FIB working principles and to model and simulate its sputtering and deposition processes during the nanohole fabrication, so that we can effectively control the processes to make a single-digit nanoscale hole for various biomedical applications.

3.1 Modeling of the FIB Milling Process. FIB can be used to make submicron holes routinely. However, the mechanism of the FIB milling process to make a hole in a desired material is not well understood. Many questions, such as how to obtain the specified diameters and depth and what parameters (such as beam energy, beam current, incident angle, and milling time) to use, need to be answered by further research. The FIB milling involves two processes: (1) sputtering, ions with high energy displace and remove atoms of substrate material and the ions lose their energy as they go into the substrate; (2) redeposition, the displaced substrate atoms, which have gained energy from ions through energy transfer, go through a similar process, sputtering other atoms, taking their vacancy, or flying out.

To model a complete FIB hole milling process is difficult, due to that once there is an opening milled by FIB on the substrate, there will be a beam loss when partial of beam reaches the opening. We do not know exactly when this will happen and how big the opening is, since the hole diameter and depth, and substrate materials all change case by case. The following introduced two spattering and redeposition modeling mainly simulate groove, dent, or non-through-hole FIB milling process, where we do not need to consider beam loss or through-hole material loss.

3.1.1 Sputtering Modeling. When an ion beam impacts a substrate surface with an incident angle θ, the substrate material will be sputtered within a volume Vs (refer to Fig. 2). \( p_{i_{1}} \) is the ion-beam covered distance. The milling depth hs for a unit width in the y direction can be estimated by \( Vs \times (L \times 1) \). The ion-beam density follows a Gaussian distribution with a peak in the center of the spot as shown in Fig. 2. The sputtered volume rate can be calculated using the following equation [39]:
mined by a Monte Carlo Method

\[ V_{si} = \eta(\theta) \cdot \int_{x_1}^{x_3} \int_{y_1}^{y_3} \frac{I_p}{nC_e} \frac{1}{2\sigma^2} \exp\left( -\frac{x^2 + y^2}{2\sigma^2} \right) dxdy \cdot V_a \]  

(1)

where \( V_{si} \) is the sputtered volume rate of an element; \( \eta(\theta) \) is the sputtering yield (atoms/ion), and the value of \( \eta(\theta) \) can be determined by a Monte Carlo Method [40]; \( x_1 \) and \( x_3 \) are the element’s lower and upper boundaries in the \( x \) direction; \( \phi_1(x) \) and \( \phi_3(x) \) are the element’s lower and upper boundaries in the \( y \) direction; \( I_p \) is the ion-beam current; \( V_a \) is the volume of one atom; \( n \) is the charge number of one ion; \( C_e \) is the charge of one ion; and \( \sigma \) is the standard deviation of the Gaussian distribution.

### 3.1.2 Redeposition Modeling

As shown in Fig. 3, some of the sputtered particles from the element \( P_{j+1} \) are redeposited on the surface of the substrate. We assume that the direction of the sputtered particles has a cosine distribution. When considering redeposited volume, we divide the elements \( P_{j+1} \) and \( P_{j+1} \) into small sites having a thickness \( dy \) in the \( y \) direction [39].

The redeposition volume rate \( V_{pi} \) can be calculated using the following equation [39]:

\[ V_{pi} = \frac{1}{2} \frac{F(\beta) - F(\alpha)}{F(180)} \cdot \gamma \cdot V_{si} \]  

(2)

where \( F(\alpha) = \frac{\pi r^2 \cos^3 \omega - 3 \cos \omega + 2}{3} \) represents the volume of a portion of the sphere between the plane \( x-y \) and the plane whose angle to the plane \( x-y \) is \( \alpha \), in which \( r \) stands for the radius of the sputter sphere, and \( \alpha \) and \( \beta \) are the angles that the surface of the current element makes with the starting and the ending points of the other elements on the \( x-z \) plane (Fig. 3). \( \gamma/2 \cdot 180^\circ \) is called the sticking coefficient, and \( \gamma \) is the angle that the current element makes (cross hatched area in Fig. 3) projected in the \( x-y \) plane.

### 3.2 Modeling of FIB-Induced Deposition

The principle of focused ion-beam-induced deposition is based on the principles of localized chemical vapor deposition (CVD) by a direct writing technique. The precursor gas for deposition is an organometallic gas. The deposition process is illustrated in Fig. 4; the precursor gases are sprayed on the surface by a needle (‘‘nozzle’’), where they adsorb. In a second step, the incentive ion beam decomposes the adsorbed precursor gases. Then the volatile reaction products absorb from the surface and are removed through the vacuum system, while the desired reaction product remains fixed on the surface as a thin film [41,42].

The volume rate of adsorbed molecules \( V_{\text{deposit}} \) is determined by the adsorption, desorption, and consumption due to ion-induced reaction, leading to the following equation [42]:

\[ V_{\text{deposit}} = \left[ F_g \left( 1 - \frac{N}{N_0} \right) - m s f(t) \frac{N}{N_0} \right] \times V_m \]  

(3)

The first term governs the adsorption (assuming Langmuir kinetics), where \( F \) is the gas flux, \( g \) is the sticking probability, \( N \) is the number of adsorbed molecules per surface area, and \( N_0 \) is the number of available adsorption sites. The second term accounts for the consumption of adsorbates by the ion-induced reaction, where \( m \) is the number of molecules participating in a reaction, \( s \) represents the maximum reaction yield, and \( f(t) \) is the ion flux. Finally, the third term represents spontaneous thermal desorption into the gas phase with a time constant \( \tau_{\text{des}} \), and \( V_m \) is the volume of each deposited metal atom [42].

### 3.3 Process Control of FIB Machining

To control the FIB process means that if we know the FIB milling parameters and the mill-off volume of the submicron hole, then we can compute the milling time to control the process. Also if we know the submicron hole size and the desired nanohole size, then we can compute the deposition time to control the process.

#### 3.3.1 Milling Time Calculation to Make Submicron Hole

In FIB machining, we need to provide a milling time for the FIB machine to make a submicron hole. Based on the above two modeling equations (1) and (2), we can readily calculate the FIB milling time \( t_{\text{milling}} \) to make a hole with specified diameter and depth. The total time can be calculated by the following equation:

\[ t_{\text{milling}} = \frac{V_h}{V_{si} - V_{pi}} \]  

(4)

where \( V_h \) is the total mill-off volume for the hole, which can be calculated as \( V_h = \pi r^2 d_h \), where \( d_h \) is the hole depth and \( r \) is the radius of the hole. When \( d_h/r \) is small, redeposition volume \( V_{pi} \).
can be ignored.

After reviewing Eqs. (1), (2), and (4) it can be found that most of the parameter values can be obtained either from the FIB machine’s setup or from user defined materials and conditions. However, there is a function \( \eta(\theta) \), called the sputtering yield (atoms/ion), a function of beam incident angle \( \theta \). Its value cannot be obtained readily because it depends on the type of ion and on the material to be sputtered. Biersack and Haggmark [40] suggested that the value of \( \eta(\theta) \) can be determined by a Monte Carlo method, and they developed a software package transport of ions in matter (TRIM) (see Sec. 3.4) to simulate ion trajectories and energy distributions.

3.3.2 Deposition Time Calculation. The deposition process is to use ion energy to decompose organometallic gas. After deposition, metal particles will be deposited on the substrate surface. To shrink the submicron hole \( D \) to a single-digit nanohole \( d \), ion spot size should be set a little bit larger than the submicron hole diameter to cover the hole edge and let the deposition build up. In our case the spot size is chosen as 110% \( \times D \), where \( D \) is the submicron hole diameter. The deposited material build up angle can be assumed to be \( \rho^2 \) (It might vary according to different metal crystal structures). It can be seen from Fig. 5 that with known sizes of submicron and nanoholes as \( D \) and \( d \), respectively, the deposition thickness \( t \) can be calculated (\( t = 0.5D \tan \rho \)).

From Eq. (3), the deposition time can be estimated as

\[
t_{deposition} = \frac{\pi(D/2 \times 110\%)^2 \times t}{V_{deposit}}
\]

To solve Eq. (5), we must first solve Eq. (3) and know the material buildup angle \( \rho \), which is related to \( t \); however, both of them cannot be solved analytically and they only can be obtained from experimentally determined results. In this research we will conduct such experiments to obtain these parameter values.

3.4 Simulation. Based on the above model and the Monte Carlo method, a software package called TRIM was developed based on the work by Biersack and Haggmark in 1980 [40] to simulate the scattering and slowing down of energetic ions in amorphous solids. The program can determine the ion range and target damage distribution, and has been used for predicting the sputtering yield for different ions within a wide range of energy [28].

In TRIM simulation, over 1000 ions will make the simulation stable [28]. We have performed some simulations using TRIM. Figure 6 shows the results of 1010 gallium ions with an energy level of 30 keV impinging onto a 100-nm-thick silicon substrate. From Fig. 6 it can be seen that the penetration depth of ions is around 40 nm (the majority of ions can reach this value), and the sputtered diameter is around 30 nm. In our preliminary FIB nanohole fabrications experiments, the free-standing film thickness is 30 nm, the material is silicon nitride, and the milling ion is gallium. Using these parameters, TRIM suggested an energy level of 65 keV for making a through-hole. Figure 7 shows the simulation results of (a) gallium ion trajectories and (b) recoils of substrate atoms. It can be seen from both Figs. 7(a) and 7(b) that this FIB machining setup can make a through-hole (30 nm) with diameter around 30 nm.

TRIM simulation provides a good means for visualizing the paths of ions through the substrate material, and a method for estimating the parameters to be used in a FIB sputtering process for a specific purpose.

3.5 Methods for Nanohole Fabrication. The general approach to make the single-digit nanoholes can be described in Fig. 8. There are three major steps: preparation of free-standing thin films, making submicron holes by FIB, and reducing the size to a single-digit nanohole by FIB-induced deposition.

3.5.1 Preparation of Free-Standing Thin Film. There are many ways to make free-standing films. For our specific requirements in this research we have used the epitaxial deposition and electrochemical anisotropic etching to prepare the film. Figure 9 shows the main principle in making the free-standing film: An n-type epitaxial layer grown on a p-type wafer forms a p-n junction...
tion diode: in the electrochemical etching the provided potential is in reverse bias on the $p$-$n$ diode, such that a thin passivating silicon dioxide layer forms; hence, it is not etched. However, the $p$-type layer is electrically floating and is etched. Once the $p$-type layer is etch-removed, the etching is stopped leaving an $n$-type silicon thin film [43]. Figure 10(a) shows our microfabricated silicon nitride free-standing thin film with a dimensional drawing and two photos of the film, and Fig. 10(b) shows a scanning electron microscope (SEM) image, showing that a $12 \times 12$ $\mu$m$^2$ free-standing film is at the bottom of the inverse-pyramid shaped opening in the silicon substrate. It has been found that the mechanical and electrical properties of the free-standing film directly affect the efficiency and quality of the fabrication of the nanoholes, as well as the performance of the nanoholes in studying macromolecular translocation.

3.5.2 Making Submicron Holes (0.2–0.03 $\mu$m) by FIB. When using the FIB, a prepared free-standing thin film is positioned on the sample stage, and the ion beam can be focused on the film to a spot as small as 7–9 nm (see Fig. 1). Considering the single beam simulation result (Fig. 7) and the proximity effect, the penetrated hole is 30–40 nm in diameter.

3.5.3 Reducing the Hole to Less Than 10 nm by FIB-Induced Deposition. There are several potential ways to reduce the hole size, such as epitaxial deposition, FIB- or E-beam-induced deposition, microcoating, and thermo-assisted shrinkage. In this research we have used both FIB- and E-beam-induced depositions. We used IB as an energy source to decompose organometallic gases to deposit the metal atoms (platinum was deposited in our preliminary testing) around the rims of the large prefabricated hole to shrink it to the desired nanoscale; see Fig. 5.

3.6 Studying the Mechanical and Structural Properties of DNA and RNA Using the Nanohole. The studies up to date using nanohole technology all measure the variation of ionic currents and the translocation time of the polynucleotides during the translocation of the molecule through the nanohole, driven by an applied electric field. The capability of this approach has been shown by distinguishing long stretches of the same nucleotides, such as 30 adenines followed by 70 cytosines [13], and by the distinction between individual DNA hairpins that differ by one base-pair by driving the molecule through an $\alpha$-hemolysin nanopore [44]. However, to use this method for sequencing real DNA at a single-nucleotide resolution, there is still much work to be done both experimentally and theoretically. The complications come from the short translocation time, the thermal fluctuations, and the secondary structure interference of the measured signal. We are proposing an alternative and complementary approach to study the process of the translocation of DNA by exerting a mechanical force on the polymer during their translocation through the nanohole. Comparing with other techniques for applying and measuring low forces (approximately in piconewtons), such as laser tweezers, the AFM has the advantage of a more precise position control. In the AFM, the movements of the cantilever can be controlled with angstrom spatial resolution. This capability will be crucial in obtaining the structural and sequence information on the nucleic acid at a near single base resolution. In the force measurements, the signal is not sensitive to electrical noise, and the speed of translocation can be varied over a large range. Our goal is to use the mechanical force to complement the ionic current measurements, and to obtain new information for theoretical studies in elucidating the requirements that will allow sequencing of nucleotides at the single-nucleotide resolution.

3.6.1 Experimental Setup. We will use the AFM cantilever as the force sensor to apply and measure forces on the DNA as the molecule passes through a nanohole. A schematic of the experimental setup is shown in Fig. 11. The electrical components will be added to the AFM liquid chamber to induce the entry of the DNA into the nanohole, and to make experimental measurements in which the current and force are both employed as control parameters. The experiments will be performed in several different ways. One method will place the end-biotinylated DNA (or RNA) molecules in the lower chamber and allow to diffuse, or driven by a small ($\sim 1$ mV) voltage, into the upper chamber. In the meantime the AFM tip is placed near the nanohole to monitor if a DNA molecule has emerged and attached to the tip, as indicated by the deflection and the vibration spectrum of the cantilever. As soon as a molecule is detected, a reverse voltage will be applied to drive any excess portion of the DNA molecule back to the lower chamber. The cantilever will then be moved away from the surface and pull the DNA over to the upper chamber, with or without the reverse voltage. Another method will be to attach the DNA molecules to the tip before placing the cantilever onto the AFM. The DNA is then threaded through the nanohole down to the lower chamber by a voltage, and the pulling follows. This may be a more efficient way to do the experiment, but the DNA molecules...
immobilized on the tip need to be sparsely distributed in order to ensure no intermolecular interaction. This can be checked by fluorescent microscopy. In the experiments proposed here we will use nanoholes that are less than 2 nm in diameter.

4 Preliminary Studies

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

(A) Thin film fabrication. A prerequisite for making nanoholes is the ability to fabricate solid-state free-standing thin films. We have used epitaxial deposition and electrochemical etching (see Fig. 9 and Sec. 3.5.1) 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 dimensions of 12×12 μm². Figure 10(b) shows an EM photo of the geometry of one of the thin films.

(B) Making submicron holes using FIB milling. The FEI DB235 dual beam FIB 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 beam damage to the sample due to exposure to the stronger energy ion beam. The FIB control software contains predefined patterns (circle, rectangle, line, and, polygon). These shapes are inserted into the image window. The dimension of each pattern element is controlled in the patterning dialog box. The user can define the length, width, and depth of the individual features. The user also defines the milling parameters, such as the 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 the 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 were able to make holes of various sizes (30–200 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. Figure 12(a) 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 dent. The smallest hole is 30–40 nm.

(C) Batch production of nanoholes using pattern and automation features from the dual beam FIB. Users can 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 film and then holes 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. 12(b) shows two rows of holes with square and circle shapes and different sizes. Figure 12(c) shows one column of the same diameter holes.

(D) Reducing the hole size by FIB-induced deposition. As mentioned before both FIB- and EB-induced depositions can reduce the hole size from hundreds of nanometers to a single-digit nanometer. To assess the feasibility the following testing was made. Figure 12(d) shows two holes of the same size originally (330 nm in the long diameter), with the size of one hole reduced to around 180 nm after FIB deposition. Figures 13(a)–13(d) show that, originally, four squares and four circles were milled (a), after 100 nm platinum deposition the lower left hole was partially filled in (b), after 200 nm Pt deposition the lower left hole was closed, and then by using the milling function a small hole opened again (d).

References


