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Single molecular level analysis and processing in nanochannels

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1. ABSTRACT

Recent advances in nanofluidic technologies have enabled devices to be fabricated that integrate nanochannels with at least one dimension smaller than several hundred nanometers. Since this dimension is close to the sizes of biomolecules such as DNA, proteins, and other biomolecules, it opens up new scientific and technological fields based on analysis and manipulation of single molecules. This paper reviews the current state of knowledge regarding single molecular level analysis and processing in nanochannels, particularly experimental findings in this area. While there have been many theoretical and molecular simulation studies, this paper surveys experimental studies. Following a brief survey of techniques for fabricating nanochannels, we review fundamental studies of single molecule behavior and manipulation in nanochannels. We then discuss important transport phenomena of single molecules in nanochannels. Finally, the emerging challenge of electrical single-molecule detection and its possible applications are highlighted.

2. INTRODUCTION

Biomolecules confined in extremely small volumes with dimensions ranging from a few to hundreds of nanometers exhibit a broad range of physical behaviors (1, 2). As the characteristic dimension of such spaces is becoming smaller, the surface-to-volume ratio is increasing. At such small dimensions, the surface or interface starts to dominate the physical behaviors in confined spaces. Such behavior includes new kinds of fluid motions that not observed in bulk fluid phases, such as capillary action, commensurate–incommensurate transitions on chemisorption, extinction of the electric double layer and a corresponding mono-ion distribution. It also permits novel analysis and manipulation of biomolecules on a single-molecule level. Thus, one of the scientific and technical goals of nanofluidics is to understand the new phenomena obtained by reducing the dimensionality and by surface effects, the phenomena of which can only be realized in nanofluidics. Nanofluidic structures can be categorized into three types based on their dimensions:

zero-dimensional pores (nanocavities, nanopores), one-dimensional channels (nanochannels), and two-dimensional channels (nanoslits). Nanochannels can thus be defined as structures that extend in one or two dimensions and have structural components that have at least one cross-sectional dimension smaller than 1 μm. Nanochannels are generally classified as channels with one dimension that is smaller than several hundred nanometers, whereas microchannels are channels with one dimension between 1 μm and 1 mm; channels larger than 1 mm (for which the Reynolds number is larger than 1) are usually considered to be macro objects. When the channel width or height is of the order of the range of intermolecular forces, large frictional forces will be generated between a confined molecule and its nearest neighbors or the nanochannel surface. Consequently, intuition based on mechanical friction is not applicable; rather friction is given by the ensemble average of intermolecular forces such as electrostatic, van der Waals forces, and so on (3). Experiments performed in the last decade have confirmed these expectations (4–7).

Reducing either depth or width of a slit-shaped channel will cause the behavior of the confined system to change from being nearly three-dimensional to being two-dimensional. Reducing the diameter of a cylindrical or rectangular channel will produce one-dimensional behavior. Thus, such confined fluid flows can be used to study both finite-size effects and the effects of varying the dimensionality. In addition to being of scientific interest, a fundamental understanding of these phenomena is necessary for many potential applications. Nanoporous materials are widely used in the chemical, food, energy, and pharmaceutical industries in equipment such as reactors and separation matrices. Such processes are mainly designed using empirical methods that have little scientific basis. An improved understanding of the physics in confined nanospaces is expected to lead to significant improvements in these applications. Since nanofabrication techniques can be used to fabricate nanostructures with well-defined dimensions, artificial nanochannels are promising experimental models for studying the properties of nanopores; the results of such experiments can be compared with those of computational and theoretical modeling (8–10). Applications of single biomolecules are emerging discipline. For example, the dynamic behavior of biomolecular polymers (4, 11) and biochemical reactions that can clarify the mechanism of enzymes against its substrates (3, 12, 13) have been investigated on a single-molecule level. Furthermore, other single-molecular level phenomena are being exploited in novel analysis at single-molecule level electrochemical reactions (14, 15), nanofluidic fuel cells (16), and batteries (17, 18).

In this review, we consider whether nanofluidic technology has the potential to enable individual single biomolecules in one-dimensional nanochannels to be analyzed and manipulated. The physics in nanochannels has a characteristic size of the order of the size of the biomolecules, which give rises to strong entropic effects. When the characteristic size is of the order of the Debye length, it affects transportation of biomolecules, electrical analysis, and electrokinetic manipulation. Such physical properties have the potential to create novel platforms for studying single biomolecules. We also briefly summarize the methods for fabricating nanochannels and give an overview of the fabrication methods, dimensions, and applications that have been developed for single-biomolecule studies. The remaining sections discuss measuring the mechanical properties of a polymer of single biomolecule and electrical detection of a single biomolecule.

3. PLATFORM FOR OBSERVING SINGLE BIOMOLECULES

Nanochannels are advantageous for fluorescence microscopy. Single biomolecules have conventionally been observed using fluorescence microscopy, but it is difficult to obtain a S/N for the fluorescence intensity between the target and the surrounding medium for single molecule visualization. To increase this ratio, several techniques have been developed that reduce the background fluorescence, including two-photon (multiphoton) confocal microscopy (19, 20), near-field optics that use decaying evanescent waves to produce a sub-diffraction-limited focal volume (21–24), and super-resolution microscopy techniques such as STED, STORM, and PALM (25–29). The basic concept behind confocal and near-field techniques is localization of the excitation volume: confocal and multiphoton excitation illuminates only the focal plane to suppress background fluorescence from out-of-focus planes (Figure 1A); near-field excitation excites only regions near a surface on which target molecules are immobilized (Figure 1B). These techniques require expensive, complex, and bulky optical systems. Because nanofluidic structures such as nanochannels and nanocavities (30–32) confine a target molecule to a nanoscale volume, other molecules near the target molecule are automatically excluded, resulting in a high S/N in fluorescence observations without employing special optics (Figure 1C). Nanochannels and nanocavities are thus ideal platforms for observing single molecules.

4. METHODS FOR FABRICATING NANOCHANNELS

Many methods have been developed for fabricating nanochannels. Since excellent reviews of fabrication methods have been published (5, 33, 34), we do not discuss nanochannel fabrication in detail. This section focuses on methods that have been employed for analyzing and manipulating single biomolecules.

Figure 2 shows a cluster map of fabrication methods of nanochannel together with their approximate channel sizes, materials, and applications. It reveals that the most commonly used material are SiO_2-based materials such as glass, quartz, and SiO_2, which are useful for optical and electrical analysis and manipulation of single biomolecules. Si is also commonly used because there have been many advances in surface/bulk fabrication techniques matured in MEMS in recent decades. It is important to note that the surfaces of nanochannels formed in Si are modified or coated with SiO_2 by natural oxidation, thermal oxidation or chemical/physical vapor deposition respectively because

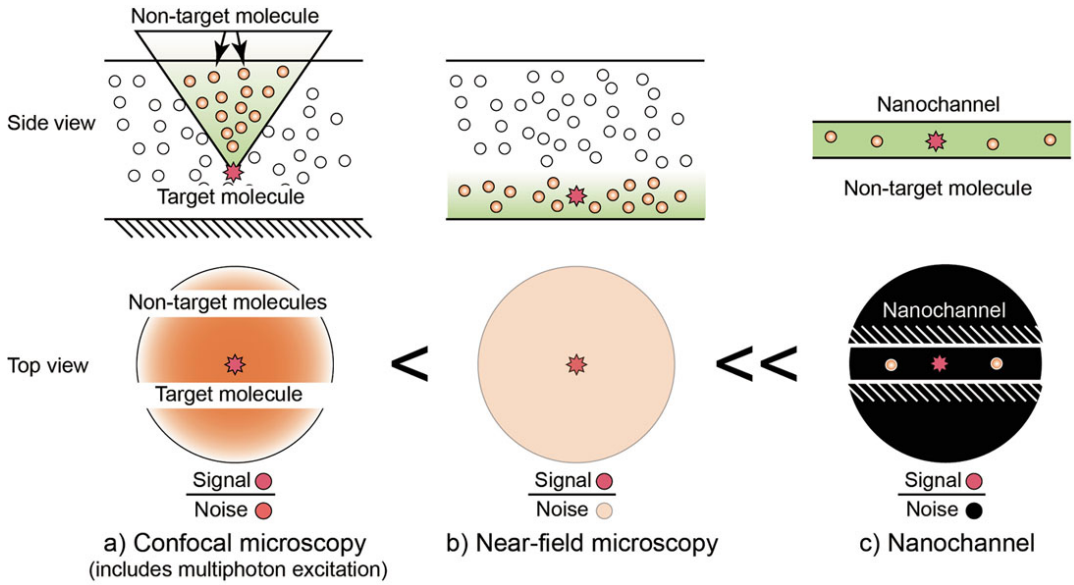


Figure 1. Schematic image of various fluorescence detection techniques for visualizing single biomolecules. The shaded green area represents the excitation light and the excitation area. The red stars represent the target single biomolecule conjugated with a fluorescent dye and the surrounding orange circles are the non-targeted biomolecules in the solution. There is no fluoresence (indicated by the black area) outside the nanochannel because there are no biomolecules outside.

SiO_2 has many advantages including biocompatibility, hydrophilicity, and chemical stability; these have been extensively investigated in many studies (6, 7). From the viewpoint of applications, nanochannel fabrication techniques should be cost-effective and they should allow the channel dimensions to be easily and precisely controlled. Thus, nanoimprint lithography is becoming increasingly popular and it is now possible to fabricate features that are smaller than 10 nm. Nanofabrication techniques can be categorized into two groups based on their methodology: top-down and bottom-up methods. Top-down methods commence with large components that are patterned and superfluous part is removed during fabrication. In contrast, bottom-up methods begin with small components such as atoms or molecules and use them to construct larger structures. This paper discusses nanochannels fabricated by top-down methods such as photolithography and various MEMS and NEMS based fabrication techniques (35, 36). Bottom-up methods are not discussed here because they have not yet matured sufficiently to allow devices to be fabricated. Top-down methods for fabricating nanochannels can be subcategorized into the following three categories: etching-based methods, sacrificial-layer methods, and nanoimprint lithography (NIL).

4.1. Etching-based fabrication techniques

In etching-based fabrication techniques, nanochannels are usually fabricated in two steps (Figure 3A). First, an open nanochannel is formed by etching a trench in a substrate (e.g., quartz or fused silica) or a film (e.g., a thin SiO_2 layer) deposited on a substrate. This is typically performed using electron beam lithography followed by wet or dry etching or sometimes direct milling using a focused ion beam. The open nanochannel is then covered by bonding a flat substrate or film. Reactive ion etching is the most commonly used dry etching method; it may be possible to control the sidewall slope from vertical to tapered in various materials regardless of their crystal structures. In the case of wet etching, isotropic etching can be realized only in specific cases. Isotropic wet etching suffers from under etching, which widens channel structures. In contrast, atomically flat sidewalls can be obtained using anisotropic wet etching. Anisotropic etching requires precisely aligning the channel axis along the crystal orientation; thus, it cannot be used to fabricate arbitrary channel patterns.

4.2. Sacrificial-layer fabrication techniques

Sacrificial layer fabrication techniques can directly fabricate hollow nanochannels so that there is no need to cover open nanochannels (37, 38). A sacrificial layer is deposited and patterned on a substrate and a top layer is then deposited on this sacrificial layer. In this method, the nanochannel pattern is defined by the pattern of the sacrificial layer. Subsequently, a nanochannel is formed by removing the sacrificial layer and leaving the bottom, side, and top layers that form an enclosed nanochannel (Figure 3B). The sacrificial layer can be removed by wet or dry etching; however, in wet etching, the nanochannel structure sometimes collapses during drying due to surface tension. The nanochannel size is generally limited by the etching time of sacrificial layer; for a long, narrow nanochannel, it takes very long time to remove the entire sacrificial layer by diffusion-limited chemical reactions in wet and dry etching processes. This method has the advantages that it is not necessary to cover the top of nanochannels and that the sacrificial layer thickness can be precisely controlled in the deposition process, allowing very thin nanochannels to be fabricated (39, 40).

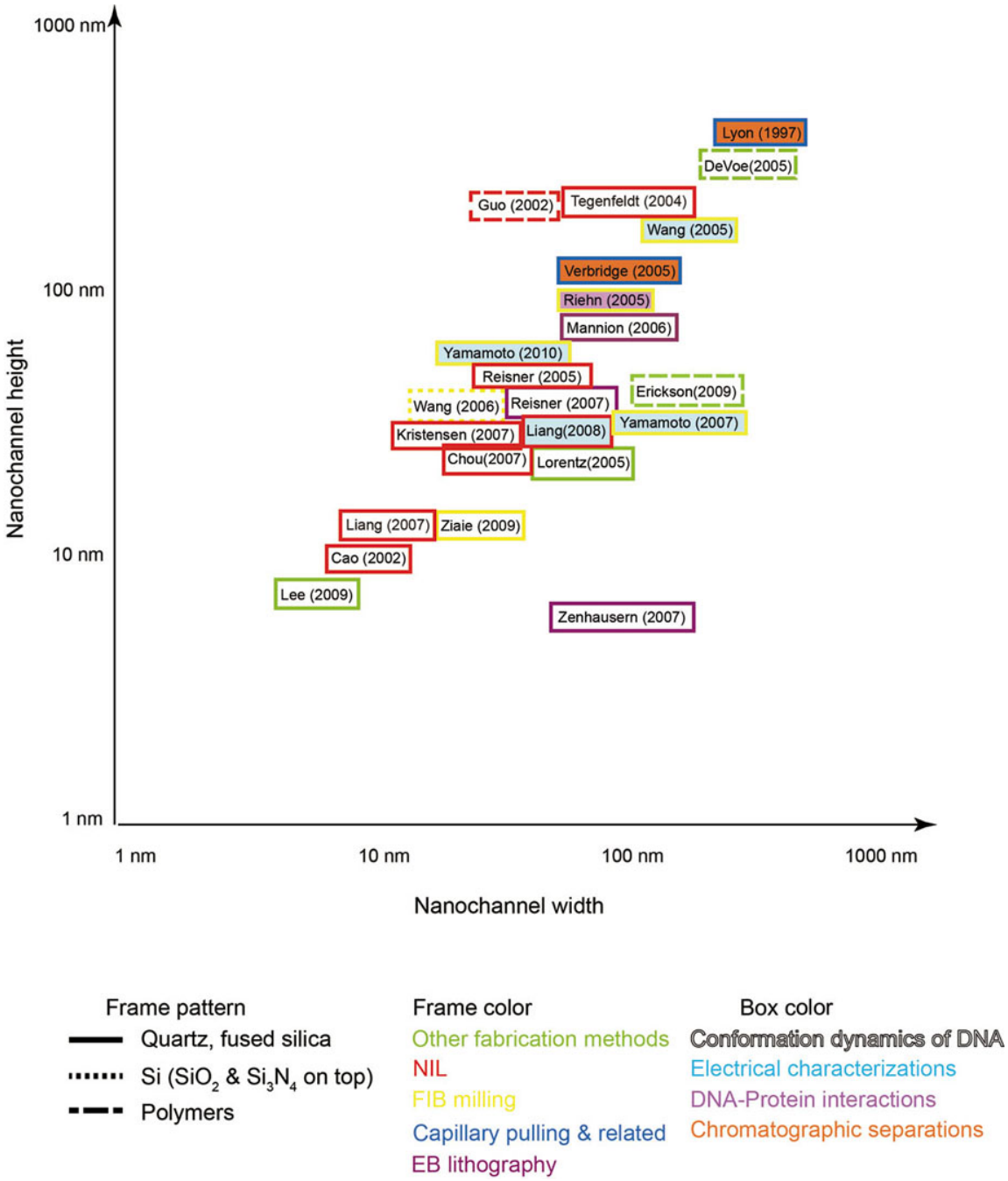


Figure 2. Fabrication and application map of nanochannels for studing single molecules. It shows the cross-sectional dimensions and the applications of the nanochannels. The horizontal and vertical axes represent the nanochannel width and height, respectively. The frame color indicates the fabrication method and the color in the box indicates the application. Quasi zero-dimensional nanopore and nanocavity studies are excluded from this map.

4.3. Nanoimprint lithography

Although Chou *et al.* (41) first developed NIL, a fine-pattern fabrication method called the molded mask method, which was similar to nanoimprint lithography, was proposed and developed at NTT Laboratories as early as the 1970s; however, all the original reports were published in Japanese (42).

NIL first involves fabricating a mold master with a negative relief of the desired nanofluidic structures. The mold is usually fabricated by the etching-based fabrication techniques described above. This implies that the same time and labor are required to fabricate a mold as etching-based nanochannel fabrication; however, once a mold has been fabricated, many molded copies can be obtained. NIL

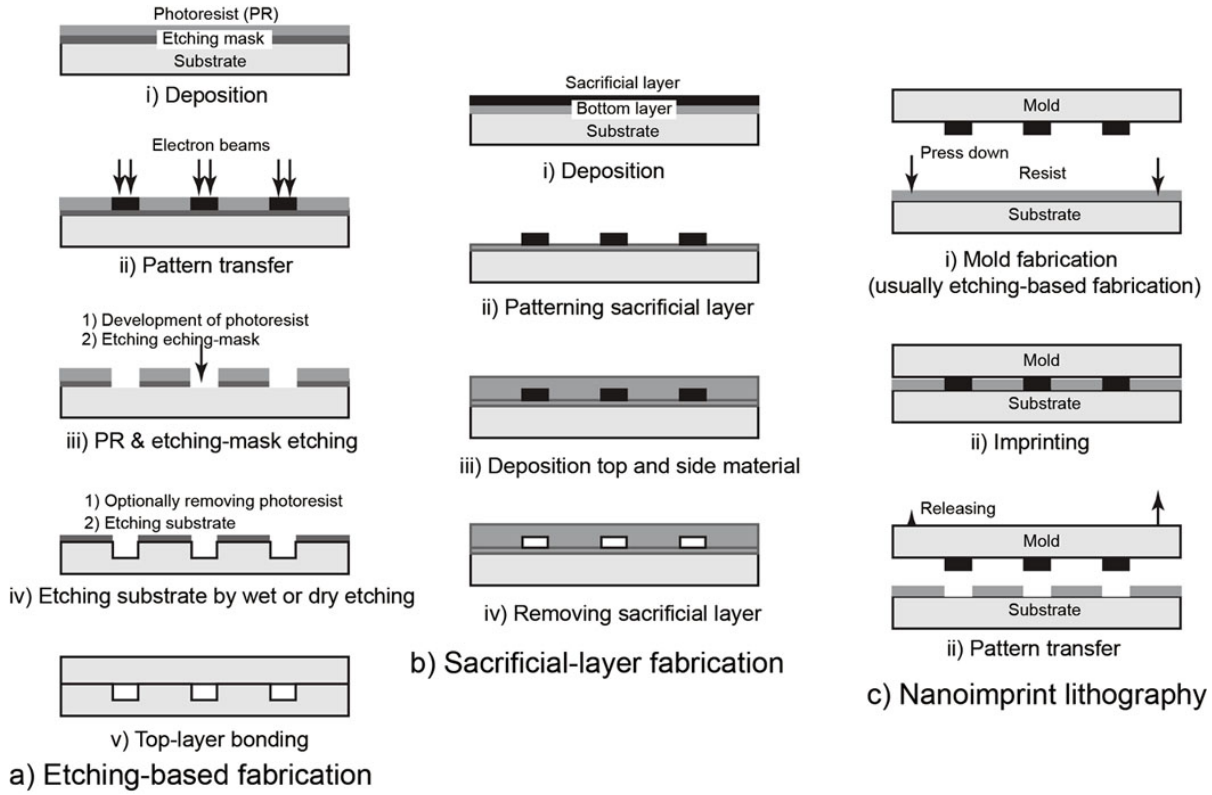


Figure 3. Methods for fabricating nanochannels: (a) etching-based methods; (b) sacrificial-layer based methods; (c) NIL.

has two basic steps (Figure 3C). The first step is the imprint step in which a mold with a negative relief of the nanostructure is pressed into a substrate. This step produces the negative relief of the mold in positive relief. The pattern transferred to the polymer may be directly used as a nanochannel or, in a second step, the pattern can be transferred to the bottom substrate by etching using the imprinted pattern as an etching mask.

5. ANALYSIS AND MANIPULATION OF SINGLE BIOMOLECULES

Polymers are of fundamental importance in biochemistry. The genetic information of all living beings are recorded, translated, and expressed at a molecular level by specialized biopolymers such as DNA, RNA, and proteins, respectively. The so-called "-omics" sciences include genomics, proteomics, and transcriptomics, which are concerned with studying the functions of these biopolymers. Thus, biopolymers represent important materials for nanofluidic-based analysis and manipulation of single molecules.

In recent decades, there have been an increasing number of single-molecule studies using nanochannels. For example, single DNA fragments have been confined and detected in 500-nm-diameter silica capillaries (43). The interactions of single DNA and DNA binding proteins have been investigated in 120 nm × 150 nm fused silica channels (44). Single DNA digestion by restriction enzymes has been observed (45). Single rhodamine labeled cellulase was detected in 100-nm-diameter glass nanochannels (46). Single-molecule studies distinguish and identify individual molecules, which can be easily segregated by preparing extremely dilute solutions containing an average of one molecule per detection volume. This situation means that single-molecular system is not always low concentration. It is possible to realize relatively high molar concentrations because of very small detection volumes, making it possible to realize near physiological conditions for single-molecule study. This is an important advantage of single-molecule systems. By exploiting this advantage of nanocavities, impressive work has been done in studying the activity of DNA polymerase at high concentrations (32). This type of research will be related to further topic of molecular crowding; however, it lies beyond the scope of this review.

5.1. Polymer dynamics of DNA in nanochannels

By accurately controlling the dimensions of nanochannels, biomolecules such as DNA can be confined in nanochannels. This is of critical importance because it enables individual molecules to be observed in well-defined domains. A molecule can be confined in a nanochannel when its radius is smaller than the nanochannel radius. Figure 4 schematically depicts DNA confined in a nanochannel, where the confining geometry alters the polymer conformation (11, 47, 48). The physical properties of DNA have been extensively studied and are prototypical of linear macromolecules with no internal

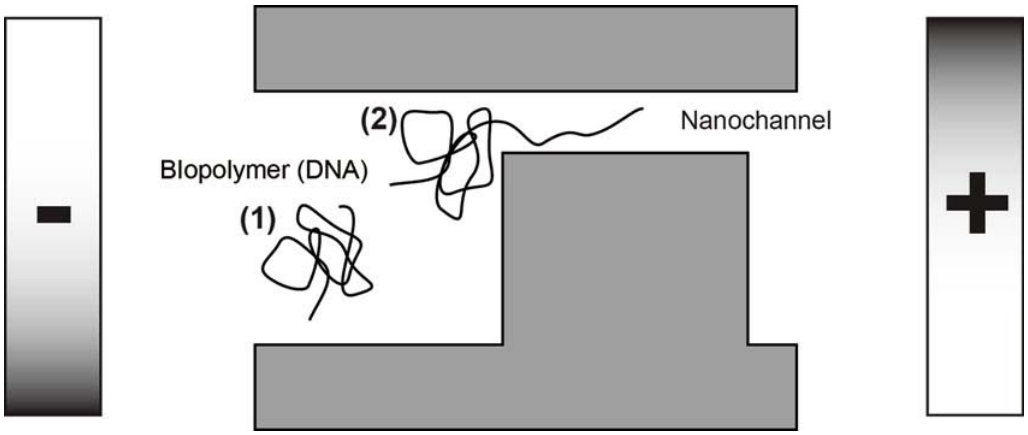


Figure 4. Cross-sectional schematic diagram of the entropic effect on DNA at the entrance of a nanochannel. DNA molecules are randomly coiled and trapped when they are in larger section (1) and they cannot enter the nanochannel section (2) because the radius of gyration of DNA is larger than the nanochannel height. When an external force generated by an electric field is applied from right to left, DNA (it has a negatively charged backbone) will enter the nanochannel with confined and stretched conformations.

interactions. Thus, it is a model polymer for investigating polymer physics. Macroscopically, DNA in aqueous solution appears as a ball-like random-coiled conformation with a characteristic radius known as the radius of gyration. At higher magnifications, the relative rigidity of a polymer chain can be measured by the persistence length. The contour length of DNA is the arc length along the backbone contour; namely, the end-to-end distance when DNA is fully stretched. Since the base pair spacing is approximately 0.34 nm, the contour length of DNA with N base pairs is $\approx 0.34 \times N$ nm. Confining DNA in nanochannels offers the following four advantage to the study of DNA dynamics: (1) nanochannels that are narrower than the radius of gyration have the potential to improve manipulation and separation of DNA; (2) studying a well-defined and well-known polymer in such geometries will be beneficial for fundamental studies of polymer physics; (3) conformational information may have important implications for the biological functions of DNA–protein interactions; (4) DNA can be used as a molecular probe for investigating the internal space of nanochannels by counterion enrichment and the exclusion of co-ions due to electrostatic interactions with charges on the backbone.

Confinement alters the conformation of DNA, which modifies its transportation properties. Most notably, when DNA is confined in geometries smaller than the radius of gyration, it is no longer hydrodynamically coupled in a spherical ball with a diameter determined by the radius of gyration; rather, it is extended in a nonspherical conformation. In this case, the viscous mobility of the stretched conformation is inversely proportional to the contour length of DNA (49, 50). This affects the electrophoresis mobility. Small, rigid molecules are governed by an entropic energy barrier generated by a gradient in the degree of confinement, leading to a size-dependent electrophoretic mobility when molecules are subject to such gradients. Large, flexible molecules such as DNA have sufficiently many degrees of freedom that their entropic forces vary along the contour of the molecule. This effect has been used in entropic trapping to separate single molecules of long DNA (Figure 5) (51, 52). Entropic trapping can be used as a normally-closed valve to confine DNA at a desired location without employing any mechanical components and at any power.

One of the earliest studies used a nanoslit to elongate DNA and it investigated the polymer dynamics of a single DNA molecule (53). Single DNA molecules were stretched into linear and U-shaped conformations around nanofabricated cylindrical posts (diameter: 2 μm; heights: 0.1–10 μm) separated by 2 μm and the corresponding degree of elongation was measured. This study found that the degree of confinement may affect the transient extension.

The dynamic polymer motion of DNA was found to result from a balance between the confinement entropic force and the stretching entropic force (54). In this study, T2 phage DNA (contour length ≈ 51 μm) was stretched to approximately 35% of its contour length. This study also demonstrated superior separation of long DNA using the confinement-induced entropic force. The degree of DNA stretching will be inversely proportional to the nanochannel dimensions. A nanochannel fabricated in polymethylmethacrylate (PMMA) by NL was used to measure the contour length of T5 phage DNA (natural contour length ≈ 35 μm) labeled with a dye and the contour length was found to be 42 μm (55). Ions surrounding DNA are also important for stretching DNA. By exploiting the fact that the persistence length of DNA is inversely proportional to the ionic strength of the surrounding medium, DNA was stretched by controlling the ion concentration (56). In addition, stretching of a DNA molecule depends on the channel width (57, 58). A critical dimension is approximately twice the persistence length of the DNA. For channels smaller than this critical dimension, the extension is not only well estimated by the Odijk model, but also by the –0.85 power of the nanochannel

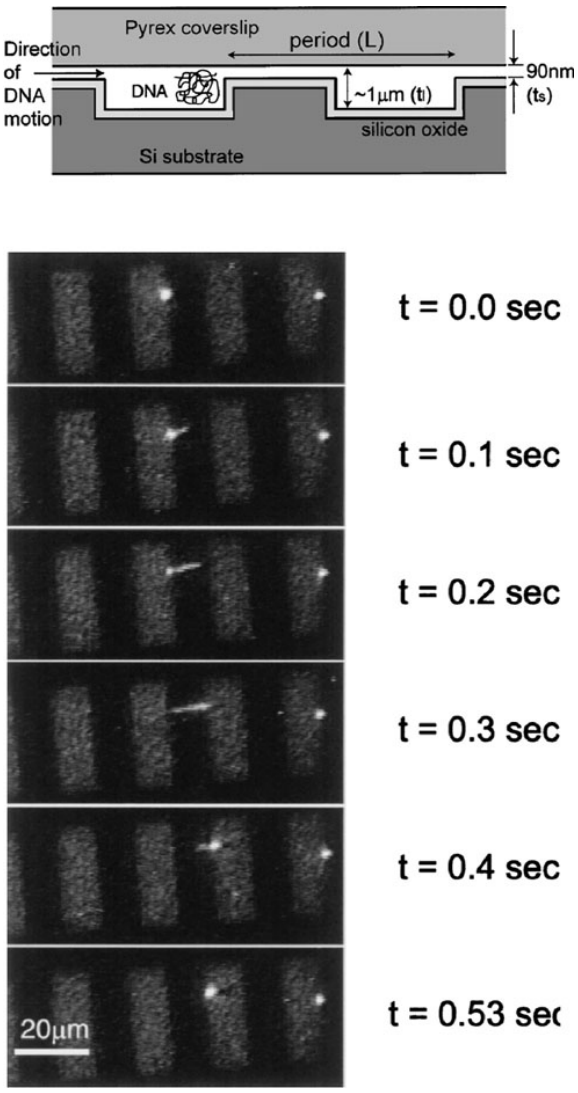


Figure 5. Schematic diagram of entropic trap and separation of single long DNA (λ-DNA) molecule. It shows a sequence of video frame images obtained in 0.1 s intervals that show the escape of λ-DNA. DNA forms nearly spherical blobs when they are entropically trapped at thick section. [Reproduced with permission from, ref. 51]

width *D*, whereas conventional de Gennes theory (59) predicts that the extension should scale as the 2/3 power of the nanochannel width *D*.

DNA relaxation in the stretched state was investigated by compressing stretched DNA into the compressed state in a nanochannel. The dynamics was explained using a variation of the de Gennes model (60). The recoil force induced by confinement was estimated by electrophoretically introducing DNA into a nanochannel array (47). In this study, the recoil process was categorized into four stages (Figure 5). The polymer response of DNA was examined under various conditions including stretching mode with subsequent relaxation, relaxed recoil, and stretched recoil.

An impressive and flexible nanochannel system in which the nanochannel size can be dynamically controlled was fabricated using elastomer nanochannels (61). The cross-sectional dimensions of the nanochannels could be controlled by applying a normal force. The nanochannels could be used as valves to trap single DNA molecules.

5.2. Electrical single-molecule analysis

To detect biomolecules that are only a few nanometers in size, devices must have very small feature sizes and ultrahigh sensitivities. Here, we review recently developed electrical single-biomolecule sensing techniques that exploit the properties of nanochannels. These techniques can directly identify the kind of biomolecule (e.g., DNA, RNA, and protein) and reveal interactions between biomolecules and their substrates without labeling with fluorescent dyes or antibodies. These devices usually have a pair of sensing electrodes that face each other and have a nanometer-sized gap or a nanochannel between them (hence, they are referred to as nanogap electrodes or nanogap detectors). Many studies have reported sensing of single biomolecules using nanopores, whereas only a few studies have used nanochannels. We report recent progress in electrical measurements using nanochannels to sense single biomolecules. We also briefly discuss the advantages and disadvantages of zero-dimensional nanopores and one-dimensional nanochannels as platforms for electrical detection and manipulation of single biomolecules.

As the surface-to-volume ratio increases with miniaturization, interfacial effects are becoming increasingly important and they are starting to govern internal physical phenomena. Among them, we discuss the electrical double layer (EDL), which is a local charge distribution at a liquid–solid interface. Localized surface charges on the solid are compensated by oppositely charged mobile counterions in solution. This condensed charge layer not electrically shields surface charges, but it also acts as a capacitor (condenser) in electrical measurements. The EDL thickness, which depends on both the strength of the surface charge and ionic strength of the solution, can vary from less than 1 nm for high ionic strengths to tens of nanometers for low ionic strengths. In a nanochannel, the EDL accounts for a high proportion of the total volume and its ionic composition can be highly asymmetric in the radial direction because the nanochannel width is comparable to or smaller than the EDL thickness. If the nanochannel width is smaller than the EDL thickness, the overall cationic and anionic concentrations will be unequal. Consequently, the counterions will be enriched and the co-ions will be excluded due to electrostatic interactions with surface charges, which are induced by reducing the nanochannel diameter (Figure 6). A high sensitivity in electrical measurements can be obtained due to disappearing the capacitance of the EDL, especially in the DC and low frequency regions; that is an important advantage of nanochannels for electrical measurements. On the other hand, electrical measurements in small spaces need to consider depletion of carrier ions. The electrical current in the liquid is supplied by carrier ions. If all the ions between the electrodes are transported to the

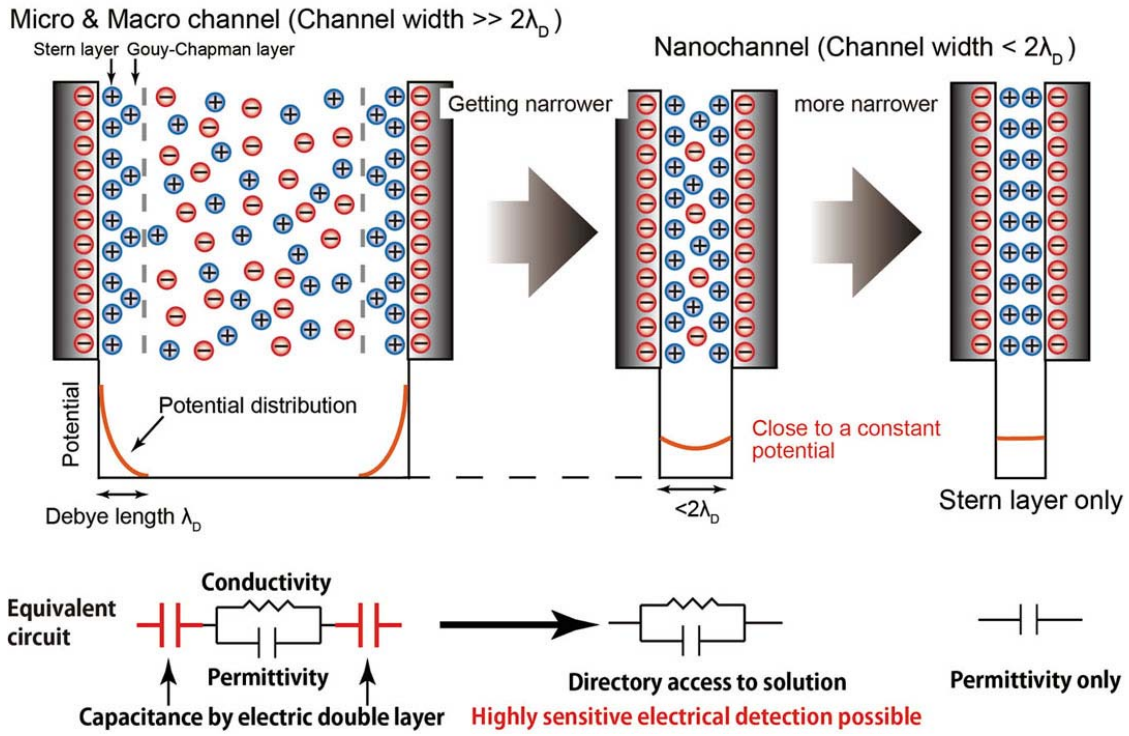


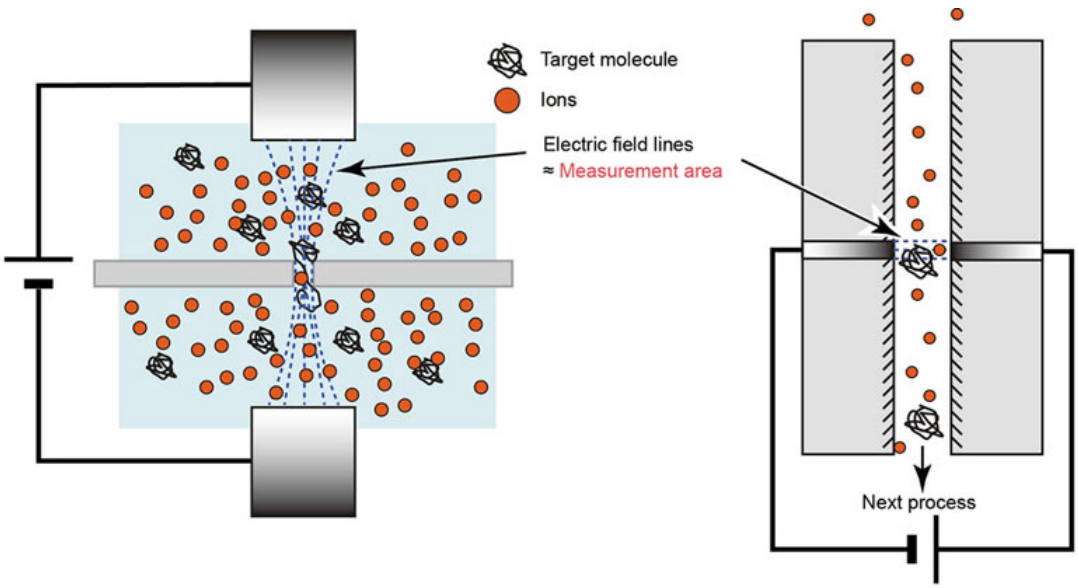
Figure 6. Schematic image of EDL in a nanochannel.

oppositely charged electrode, the current will stop. Therefore, DC electrical measurements in a small space can only be performed in a short period for batch environments, whereas long periods are possible in continuous-flow environments such as nanochannels since a continuous supply of carrier ions can be provided.

Two electrical detection schemes are used for analyzing single biomolecules in nanobiotechnology: nanopores and nanochannels. Nanopore-based detection is currently much more popular than nanochannel-based detection. Figure 7 illustrates the differences between these two detection schemes. For a nanopore detector, the detected biomolecule will diffuse away and disappear after detection. Thus, it is not possible to reuse biomolecules after measurements by nanopore detection or conduct sequential processing of single biomolecules. On the other hand, it is the same that only a single molecule can pass through a nanochannel at one time when the nanochannel crosssection is close to the biomolecule size. Thus, a one-dimensional single-molecule stream is automatically created in a nanochannel, making nanochannels ideal platforms for sequential processing of single molecules, because the detected biomolecule will remain inside nanochannel. This is a huge advantage of nanochannel over nanopore detection. Since biomolecules move about their barycenters due to thermal agitation by the surrounding molecules, their time-averaged diameters are larger than their actual diameters. Thus, the crosssectional size of a nanochannel need not be slightly larger than the biomolecules to realize a one-dimensional single-biomolecular stream.

The label-free electrical detection of a single biomolecule in a nanochannel was first demonstrated by visualizing the motion of relatively large biomolecules of λ-DNA (62). However, no obvious electrical measurement data was given at that time.

One of the anticipated applications on electrical single DNA measurement lies on DNA sequencing. In the case of nanopore sensing, nanopore device in which a single strand of DNA is threaded through a nanopore could be used to efficiently sequence DNA. However, there are various issues will have to be solved to realize this approach, including controlling the DNA translocation rate, suppressing stochastic nucleobase motions, and resolving the signal overlap between different nucleobases (63). On the other hand, nanochennel sensing will be overcome those problems as above mentioned. The feasibility of DNA sequencing in nanochannel with a grapheme nanoribbon using an electrode was theoretically demonstrated by density function theory coupled to non-equilibrium Green function theory (64). The first successful electrical measurement of a biomolecule in a nanochannel was demonstrated using a small DNA fragment toward realizing DNA sequencing (65) (Figure 8). This device consists of a nanochannel with a pair of Au nanogap electrodes with a minimum gap length of 9 to 18 nm. It measures the change in the electrical current as DNA passes through the gap. The current change caused by 1.1 kbp DNA was measured, but since the relative position between the flowing DNA and the nanogap electrodes was not vizualized, it is unclear whether the current change was due to the targeted DNA molecule. The authors suggested



	Nanopore	Nanochannel
Volume & Number	Large detection volume. e.g. 10 mm cubic = 1 mL 10 mM in 1 mL ≈ 10^{18} molecules	Quite small detection volume. e.g. 50 nm cubic = 1.25 × 10^{-22} L 10 mM in 1.25 × 10^{-22} L ≈ 0.75 molecules
Noise	There are Avogadro's number of molecules, which is noise source in both upper and lower chambers.	Only a small volume in nanogap.
Measurement & Manipulation	Electric field for measurement and manipulation interferes each other.	It is possible to separate the electric field for measurement and manipulation.
Pre & Post processing	Almost impossible. The measured molecules diffuse away after measurement.	Possible. 1-D channel format makes sequential processing possible.
Purpose	Basically for measurement.	Measurements and single-molecular sequential processing.

Figure 7. Comparison of physical and electrical characteristics of nanopores and nanochannels.

that the detection resolution for DNA sequencing could be improved by reducing the nanogap dimensions and the DNA flow speed to minimize the fluctuation time.

In addition, manipulation of single biomolecules will enable sequential processing of single biomolecules. One example is sorting of single biomolecules that identifies and separates single biomolecules one by one (66). The study used a multi-branched nanochannel that had a single inlet and multiple outlets in which a pair of sensing electrodes and multiple electrodes for manipulations were fabricated. A single flowing biomolecule was identified at the sensing electrodes and it was guided to an appropriate outlet channel by switching the flowing path at a branch in the nanochannel. Figure 9A shows snapshots taken at 3.3-ms intervals of a 15.0-kbp sized DNA molecule flowing through the electrode gap. The relative position of the DNA to the electrodes is clearly visualized while performing electrical measurements. Figure 9B shows the electric current measured as the single DNA molecule passed through the electrode gap. The presence of DNA in the electrode gap will reduce the ionic current path between the electrodes and thus will reduce the electric current (Figure 9B). The results clearly show that there is a certain baseline current in the absence of DNA that decreases when DNA is present. The baseline ionic

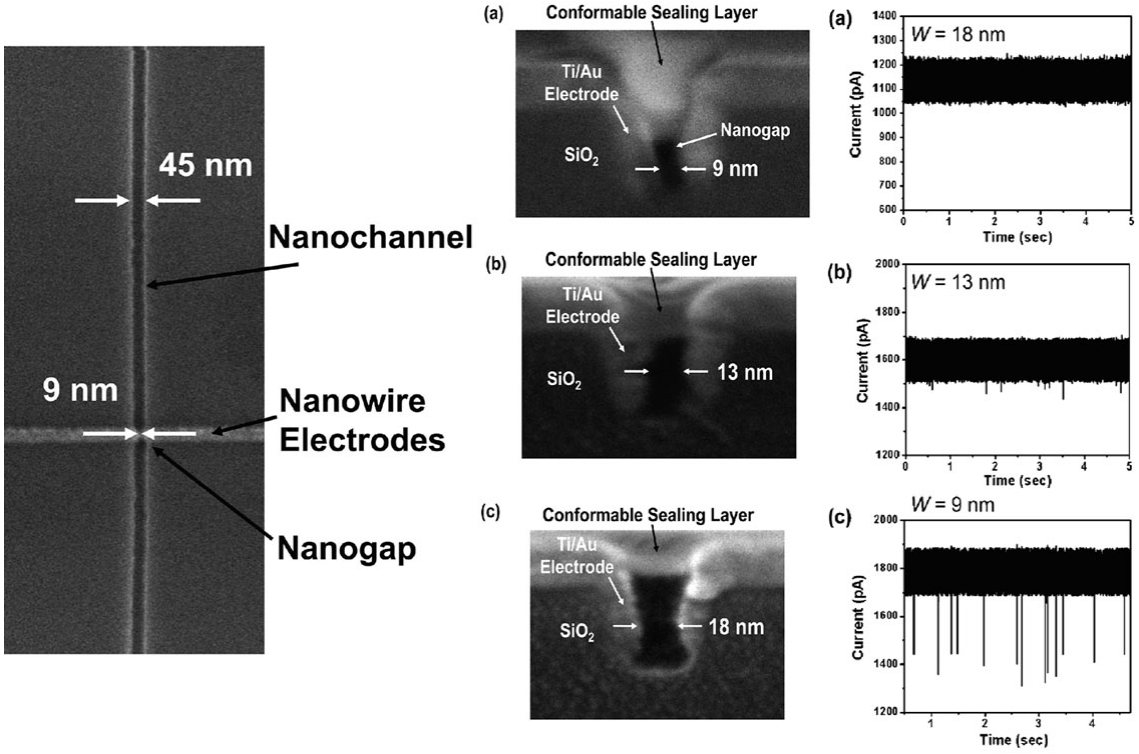


Figure 8. Scanning electron microscopy image of nanogap detector and typical measurement data. (a) Top view of nanogap detector. (b) Magnified images of three different cross sections. c) Electrical measurement of 1.1 kbp DNA in three nanogap detectors with different gap sizes. [Reproduced with permission from, ref. 65]

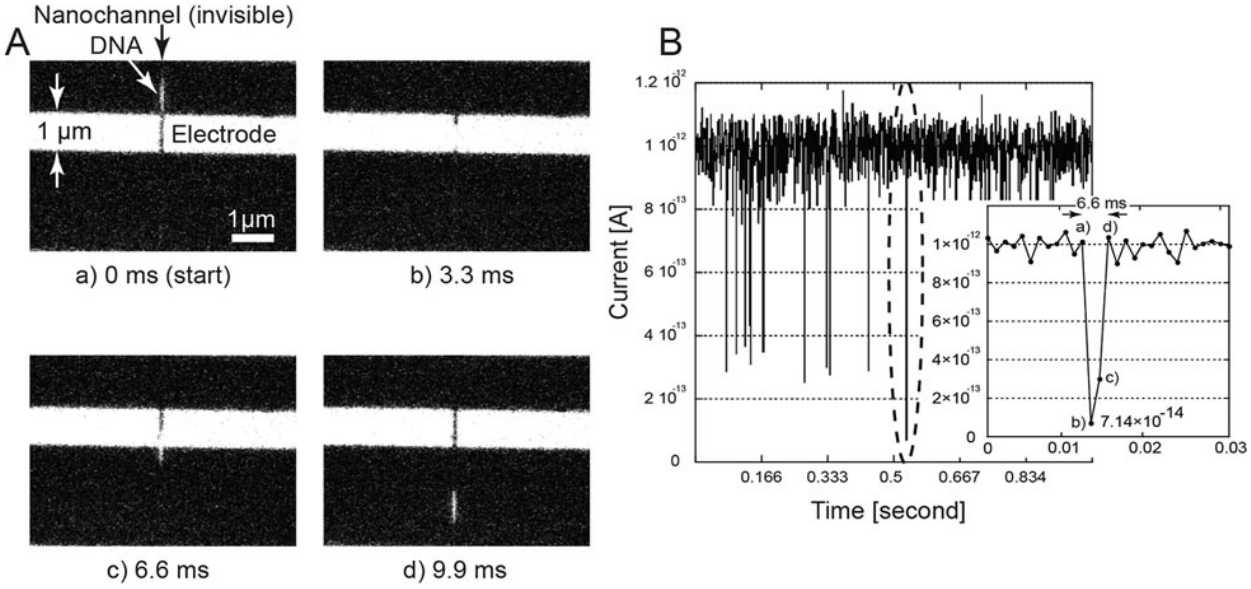


Figure 9. Electrical measurements of a flowing single DNA molecule with visualization of the relative position between the DNA and the electrodes. A. Snapshots of the motion and relative position of the flowing DNA and the measurement electrodes. The time interval between frames a) to d) is 3.3 ms and the video frame rate is 30 frames/s. In each photograph, the white vertical line is the fluorescently labeled flowing DNA and the lateral white stripe is the measurement electrode. The nanochannel, which is located vertically in the middle, is invisible in these photographs. B. Graph of electric signal measured when DNA flows through the electrode gap. The interval between the points is determined the video frame rate. The inserted graph shows a detailed profile of the current drop indicated by the dashed circle. Alphabetically labeled points correspond to the measured points in photos a) to d) in figure A. [Reproduced with permission from, ref. 66]

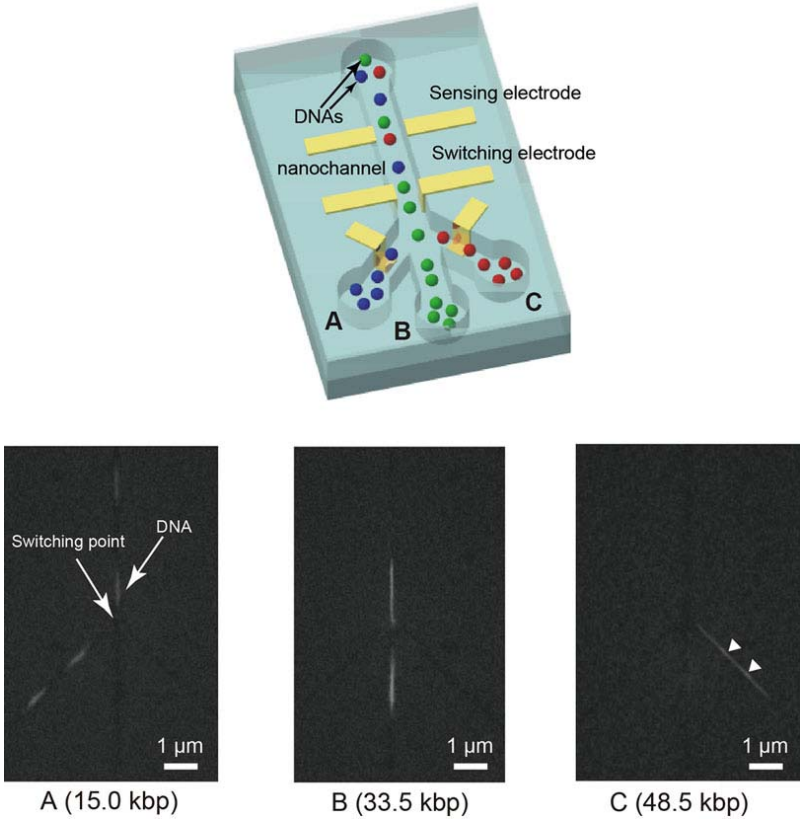


Figure 10. Fluorescence images during sorting of three sizes of single DNA molecules. The photographs show fluorescence images of flowing single DNA at the switching junction. Three sizes of DNA (A: 15.0 kbp; B: 33.5 kbp DNA; C: 48.5 kbp DNA) are shown. The apparent lengths of these DNA molecules estimated by de Gennes and Flory–Pincus scaling theory are approximately 1.1, 2.4, and 3.6 μm, respectively. Brighter white domains, which are indicated by the white triangles, were observed for 48.5 kbp DNA; they can be attributed to local conformational changes that may aggregate in the region. [Reproduced with permission from IOP (66)]

current is about 1 pA and it drops to about 300 fA (greatest drop: 70 fA) when a DNA molecule is in the electrode gap.

Figure 10 shows the measured electric current for different sizes of DNA and the fluorescence image taken during the flow path switching at a branch in the nanochannel network. 15.0-kbp DNA was programmed to flow to the left channel-A, 33.5-kbp DNA to the middle channel-B, and 48.5-kbp DNA to the right channel-C. The switching was performed by electrophoresis, which was generated by the electrodes in the nanochannel (including one cathode in the inlet channel and two anodes in the outlet channels-A and -C). Each DNA molecule was directed to the correct outlet channel, as shown in the figure.

6. CONCLUSIONS

We evaluated whether nanofluidic technology has the potential to analyze and manipulate single biomolecules and found that nanochannels are an ideal tool for visualizing, analyzing, and manipulating biomolecules with a single biomolecule resolution. In particular, nanochannels are currently the only method available for sequentially processing single biomolecules. Single biomolecule research is still in an emerging stage but various experiments have been successfully performed. The concept of single molecular study is expected to be extended to other fields besides life science. Currently, single biomolecule studies are mainly conducted on DNA; however, proteins could be used as target molecules to investigate phenomena such as protein–protein interactions, the polymer dynamics of polypeptides with inter molecular interactions, and post-translational events. Achievable goals include highly sensitive sensing and technology for separating biomolecules, which would lead to innovations in both life science and industrial applications.

In addition to analysis and manipulation, peripheral technologies are important for realizing single biomolecule processing systems. While various nanofluidic devices for single biomolecule analysis are continuously being developed, several technical hurdles need to be overcome. For example, preprocesses for preparing clean samples are very important because nanochannels can easy become clogged by particulate contamination in the sample

solution. In all nanofluidic systems, it is very important to control flow in the nanochannels. Conventional macroscale pumps and valves are not suitable for nanochannels; thus, novel methods are required control fluid flow in nanochannels.

The field of nanofluidics is still in an experimental stage and it is currently difficult to realize practical applications. However, single molecular level processing has a great potential to realize highly accurate analysis and processing and it can only be realized by nanofluidic technologies at the moment. If all the required technologies can be realized, nanofluidic-based single molecular processing will lead to future innovations.

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Abbreviations: S/N: signal-to-noise ratio; STED: stimulated-emission depletion microscopy, STORM: stochastic optical reconstruction microscopy, PALM: photoactivated localization microscopy, MEMS: microelectromechanical systems, NEMS: nanoelectromechanical systems, EB: electron beam, FIB: focused ion beam; RIE: reactive ion etching, NIL: nanoimprint lithography; PMMA: polymethylmethacrylate; EDL: electrical double layer, DC: direct current

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