GRADIENT STRUCTURES INTERFACING MICROFLUIDICS AND NANOFLUIDICS, METHODS FOR FABRICATION AND USES THEREOF

This patent application claims the benefit of priority to U.S. Provisional Patent Application No. 60/373,407, filed on April 16, 2002 and U.S. Provisional Patent Application No. 60/419,742, filed October 18, 2002. DARPA Grant Number MDA972-00-1-0031 supported work that led to portions of the inventions described herein. Accordingly, the U.S. Government may have rights in these inventions.

Background of the Invention

1. Field of the Invention

The present invention relates to bionanotechnology and in particular to a method of fabricating a hybrid microfluidic/nanofluidic device having a gradient structure formed by a modified photolithography technique at the interface between microfluidic and nanofluidic portions of the device and uses thereof.

2. Description of the Related Art

Nanotechnology, electronics and biology are combined in the newly emerging field of bionanotechnology. Nanofabrication of extremely small fluidic structures, such as channels, can be used in bionanotechnology for the direct manipulation and analysis of biomolecules, such as DNA, and proteins at single molecule resolution. For example, the channels can be used for stretching genomic DNA and scanning for medically relevant genetic or epigenetic markers. New insights of understanding the confinement-mediated entropic behavior of biopolymers in ultra-small nanoscale fluidics have just started to emerge.

On the nanometer scale, DNA is a stiff molecule. The stiffness of the molecule is described by a parameter called the persistence length. Despite the relative stiffness of DNA for sufficiently long molecules, it tends to form a disordered tangle of compact random coils in free solution. The conformation of a polymer in free solution has been referred to as a spherical "blob" by the polymer dynamics community. The size of the blob depends on the length of the DNA molecule and the persistence length.

It has been described that in order to uniformly stretch chain-like long DNA, dimensions of nanofluidic structures should be near, in the vicinity of or smaller than the persistence length of double stranded DNA of about 50 nm to about 70 nm. Arrays of up to half millions of nanochannels fabricated over a 100 mm wafer using nanoimprinting lithography (NIL) with sealed channels having a cross section as small as 10 nm by 50 nm to stretch, align and analyze long genomic DNA in a highly parallel fashion, and the resulting have been described in Cao H., Wang J., Tegenfeldt P., Austin R.H., Chen E., Wei W. and Chou S.Y., Fabrication of 10nm Enclosed Nanofluidic Channels (2002) Applied Physics Letters, Vol. 81, No. 1, pp174. It is challenging to efficiently move long DNA arranged as a blob into the small channels, since it is energetically unfavorable for long biopolymers to spontaneously elongate and enter nanochannels directly from the environment due to the large free energy needed to overcome negative entropy change, as illustrated in Figs. 1A - 1B. For example, a double stranded T4 phage DNA molecule with a length of 169 kilobases will form a Gaussian coil with a radius of gyration ($Rg=(L\rho/6)^{1/2}$, where L is the length and ρ the persistence length of the DNA), approximately 700 nm in aqueous buffer solution which is many times the width of the opening of the nanochannels. Consequently, problems such as DNA clogging at the junction of nano- and macro-environment have arisen and undermine the performance of conventional nanofluidic devices.

U.S. Patent Application No. 2002/0160365 describes a method for separation of long strands of DNA by length by forcing the molecules to traverse a boundary between a low-force energy region and a high-force energy region. The high-force energy region is a diverse pillar region. The low-force energy region is a larger chamber formed adjacent the high-force energy region.

U.S. Patent Application No. 2002/0072243 describes fabrication techniques using a pattern of sacrificial and permanent layers to define the interior geometry of a fluidic device. A pattern for a fluidic device having microchannels and an array of retarding obstacles is defined in a resist layer. The pattern is produced using lithographic techniques. For electron beam lithography and for deep structures made with photolithography, a hard pattern mask is required to assist in pattern transfer. An inlet chamber, outlet chamber, inlet microchannel, outlet chamber and an array of holes is

formed in a sacrificial layer. A ceiling layer is deposited to cover the sacrificial layer. The ceiling layer enters the holes to form closely spaced pillars. The sacrificial layer is removed to form microchannels between the floor and ceiling layers. The pillars act as a sieve or an artificial gel filter for fluid flowing through the system. Steps needed in removing the sacrificial materials, such as heating the substrate up to 200 -400° C, limits the use of certain materials. Electron beam lithography has the flexibility to write different patterns, but has low throughput and high manufacturing costs.

It is desirable to provide an improved structure interfacing between microfluidic and nanofluidic components of a device for reducing the local entropic barrier to nanochannel entry and an improved method for fabrication thereof.

Summary of the Invention

The present invention relates to a device for interfacing nanofluidic and microfluidic components suitable for use in performing high throughput i.e., macromolecular analysis. Diffraction gradient lithography (DGL) is used to form a gradient interface between a microfluidic area and a nanofluidic area. The gradient interface area reduces the local entropic barrier to nanochannels formed in the nanofluidic area.

In one embodiment, the gradient interface area is formed of lateral spatial gradient structures for narrowing the cross section of a value from the micron to the nanometer length scale. In another embodiment, the gradient interface area is formed of a vertical sloped gradient structure. Additionally, the gradient structure can provide both a lateral and vertical gradient. The gradient structures can be used to squeeze and funnel biomolecules into a small nanofluidic area.

In one aspect of the invention, a method for fabricating a fluidic device by diffraction gradient lithography comprises forming a nanofluidic area on a substrate, forming a microfluidic area on the substrate and forming a gradient interface area between the nanofluidic area and the microfluidic area. The gradient interface area can be formed by using a blocking mask positioned above a photo mask and/or photoresist during photolithography. The edge of the blocking mask provides diffraction to cast a

gradient light intensity on the photoresist. In another embodiment, a system is provided for fabricating the fluidic device.

In one aspect of the invention, the nanofluidic components comprise nanoscale fluidic structures. The nanofluidic structures can include nanopillars, nanopores and nanochannel arrays.

In another aspect of the invention, a fluidic device is formed of a gradient interface between a nanofluidic area and a microfluidic area, at least one sample reservoir in fluid communication with the microfluidic area, the sample reservoir capable of releasing a fluid and at least one waste reservoir in fluid communication with the nanofluidic area, the waste reservoir capable of receiving a fluid. In another aspect a system for carrying out analysis is provided including a fluidic device is formed of a gradient interface between a nanofluidic area and a microfluidic area, at least one sample reservoir in fluid communication with the microfluidic area, the sample at least one reservoir capable of releasing a fluid and at least one waste reservoir in fluid communication with at least one of the channels the waste reservoir capable of receiving a fluid, signal acquisition and a data processor. The signal can be a photon, electrical current/impedance measurement or change in measurements. The fluidic device can be used in MEMS and NEMS devices.

In another embodiment, methods for analyzing at least one macromolecule are provided which, for example, include the steps of: providing a fluidic device formed of a gradient interface between a nanofluidic area and a microfluidic area, at least one sample reservoir in fluid communication with the microfluidic area, the at least one sample reservoir capable of releasing a fluid and at least one waste reservoir in fluid communication with the nanofluidic area, the waste reservoir capable of receiving a fluid, transporting at least one macromolecule from the microfluidic area to the nanofluidic area to elongate the at least one macromolecule, detecting at least one signal transmitted from the at least one macromolecule and correlating the detected signal to at least one property of the macromolecule.

Cartridges including a nanofluidic chip in accordance with this invention are also disclosed herein. Such cartridges are capable of being inserted into, used with and removed from a system such as those shown herein. Cartridges useful with analytical

systems other than the systems of the present invention are also comprehended by this invention.

The invention will be more fully described by reference to the following drawings.

Brief Description of the Drawings

Fig. 1A is a schematic diagram of a prior art device including nanochannels.

Fig. 1B is a graph of entropy change to the nanochannels of the device of Fig. 1A.

Fig. 2 is a schematic diagram of a device for interfacing microfluidic and nanofluidic components in accordance with the teachings of the present invention.

Fig. 3 is a graph of entropy change to the nanochannels of the device of Fig. 2.

Figs. 4A - 4D diagrammatically illustrate a process incorporating diffraction gradient lithography (DGL) to fabricate a micropost array and interface gradient structure.

Figs. 5A-5B diagrammatically illustrate a process incorporating diffraction gradient lithography (DGL) to fabricate a sloped gradient interface area.

Fig. 6A is a schematic diagram of a method for adjusting the diffraction gradient using thickness.

Fig. 6B is a schematic diagram of a method for adjusting the diffraction gradient using a variable distance.

Fig. 7 is a schematic diagram of a microfluidic/nanofluidic chip.

Fig. 8 is a schematic diagram of a system for analyzing macromolecules using the microfluidic/nanofluidic chip.

Fig. 9A is an optical image during fabrication of the device of the present invention after photoresist development, in accordance with Fig. 4B, step 4.

Fig. 9B is a scanning electronic microscope during fabrication of the device of the present invention after pattern transfer and photoresist removal, in accordance with Fig. 4C, step 5.

Fig. 10A is a scanning electronic microscope during fabrication of the device of the present invention after pattern transfer and photoresist removal using a first etching condition, in accordance with Fig. 4C, step 5.

Fig. 10B is a scanning electronic microscope during fabrication of the device of the present invention after pattern transfer and photoresist removal using a second etching condition, in accordance with Fig. 4C, step 5.

Fig. 11A is an intensified charge coupled device (CCD) image of fluorescent long DNA molecules entering the prior art nanofluidic chip shown in Fig. 1.

Fig. 11B is an intensified charge coupled device (CCD) image of fluorescent long DNA molecules entering device 10 shown in Fig. 2.

Detailed Description

Reference will now be made in greater detail to a preferred embodiment of the invention, an example of which is illustrated in the accompanying drawings. Wherever possible, the same reference numerals will be used throughout the drawings and the description to refer to the same or like parts.

Fig. 2 is a schematic diagram of device 10 for interfacing microfluidic and nanofluidic components in accordance with the teachings of the present invention. Gradient interface area 12 is positioned between microfluidic area 14 and nanofluidic area 16. Microfluidic area 14 can comprise a plurality of microposts 18 formed on substrate 19. For example, microposts 18 can have a diameter in the range of about 0.5 to about 5.0 microns and distance D_1 between microposts 18 can be in the range of about 0.5 to about 5.0 microns. In one embodiment, microposts 18 have a diameter in the range of about 1.2 to about 1.4 microns and a distance D_1 between microposts 18 in a range of about 1.5 to about 2.0 microns.

Nanofluidic area 16 can comprise a plurality of nanochannel arrays 20 including a surface having a plurality of nanochannels 21 in the material of the surface. By "a plurality of channels" is meant more than two channels, typically more than 5, and even typically more than 10, 96, 100, 384, 1,000, 1,536, 10,000, 100,000 and 1,000,000 channels. Nanochannels 21 can be provided as a plurality of parallel linear channels across substrate 19. Nanochannels 21 can have a trench width of less than about 150 nanometers, more typically less than 100 nanometers, and even more typically less than: 75, 50, 25 and 15 nanometers. In certain embodiments, the trench width can be about 10 nanometers. In the present invention, the trench width can be at least 2 nm, and typically

at least 5 nm. Nanochannels 21 can have a trench depth of less than about 200 nanometers.

The nanochannels can have sealing material adjacent to the channel wall material. In this embodiment, the sealing material can reduce the trench width. Varying the sealing material deposition parameters can be used to narrow the trench width of the channels. The deposition parameters can be varied to provide trench widths of typically less than 100 nanometers. As more material is deposited, trench widths can be narrowed to less than 75 nanometers, and even less than: 50 nanometers, 25 nanometers, and 15 nanometers. Trench widths of about 10 nm can also be provided by the methods of the present invention. Typically, the resulting trench widths after deposition will be greater than 2 nm, and more typically greater than 5 nanometers. Trench depths of less than 175, 150, 125, 100, 75, 50, and 25 nm can also be provided by the methods of the present invention. Trench depths of about 15 nm can also be provided. Typically, the trench depths will be at least 5 nm, and more typically at least 10 nm.

In certain embodiments, the trench depth is typically less than 175 nm, and more typically less than 150 nm, 125 nm, 100 nm, 75 nm, 50 nm and 25 nm. In certain embodiments, the trench depth is about 15 nm. In certain embodiments, the trench depth is at least 2 nm, typically at least 5 nm, and more typically at least 10 nm. At least some of the nanochannels 21 can be surmounted by sealing material to render such channels at least substantially enclosed. The lengths of the channels of the nanochannel array can have a wide range.

The lengths of the channels can also be the same or different in nanochannel array 20. For carrying out macromolecular analysis using nanochannel array 20 as provided below, it is desirable that nanochannels 21 are at least about 1 millimeter (mm), 1 micrometer (μm) or longer. The length of nanochannels 21 is greater than about 1 millimeter (mm), about 1 centimeter (cm), and even greater than about 5 cm, about 15 cm, and about 25 cm. Nanochannels 21 can be fabricated with nanoimprint lithography (NIL), as described in Z. N. Yu, P. Deshpande, W. Wu, J. Wang and S. Y. Chou, Appl. Phys. Lett. 77 (7), 927 (2000); S.Y. Chou, P.R. Krauss, and P.J. Renstrom, Appl. Phys. Lett. 67 (21), 3114 (1995); Stephen Y. Chou, Peter R. Krauss and Preston J. Renstrom, Science 272, 85 (1996) and U.S. Patent No. 5,772,905 hereby each incorporated in their

entirety by reference into this application. Nanochannel 21 can be formed by nanoimprint lithography, interference lithography, self-assembled copolymer pattern transfer, spin coating, electron beam lithography, focused ion beam milling, photolithography, reactive ion-etching, wet-etching, plasma-enhanced chemical vapor deposition, electron beam evaporation, sputter deposition, and combinations thereof. Alternatively, other conventional methods can be used to form nanochannels.

In an alternate embodiment, nanofluidic area 16 can comprise nanoscale fluidic structures. For example, the nanoscale fluidic structures can comprise nanopillars and nanospheres.

Gradient interface area 12 is used to effectively stretch and align biopolymers 22 before they approach nanofluidic area 16. Biopolymers 22 can be preliminarily stretched between adjacent pairs of microposts 18 before entering nanochannels 21. Gradient interface area 12 reduces the steepness of the entrophy barrier before biopolymers 22 enter nanofluidic area 16, as shown in Fig. 3.

Referring to Fig. 2, gradient interface area 12 can comprise a plurality of gradient structures 23 formed on substrate 19. Distance D_2 between gradient structures 23 is gradually reduced towards nanofluidic area 16. For example, distance D_2 between gradient structures 23 can be reduced from about 2 microns to gradually below about 500 nm, about 400 nm, about 200 nm, about 150 nm, about 10 nm, about 5 nm and about 2 nm. In one embodiment, the distance D_2 between gradient structures 23 is reduced in a range of about a radius of gyration of biopolymer 22 to substantially a diameter of biopolymer 22. For example, diameter D_2 between gradient structures 23 can be reduced in the range of about 2 nm, a diameter of a DNA module, to about 700 nm, a radius of gyration of a T4 phage DNA molecule.

Gradient structures 23 can provide a gradual elevation of height H_1 from substrate 19. Nanofluidic area 16 can have a shallower depth DP_1 than depth DP_2 of microfluidic area 14. Accordingly, gradual elevation of height H_1 from microfluidic area 14 to nanofluidic area 16 provides improved interconnection of microfluidic area 14 with nanofluidic area 16.

Basic fabrication steps of the present invention using diffraction gradient lithography are outlined in partial, schematic perspective views in Figs. 4A - 4C, as

including processing steps 1-3. One or more nanochannels 21 were fabricated on substrate 19 in this process. Substrate 19 can be a silicon wafer substrate. Alternatively, any type of material compatible with the photolithography can be used as a substrate. Substrate 19 was coated with photoresist 32 after HMDS treatment and baked. Photomask 34 having a micron size post array can be used to pattern microfluidic area 14 and gradient interface area 12, in step 1.

In step 2, blocking mask 35 was placed over or coated on photomask 34. Blocking mask 35 extends over portion 36 of photomask 34. Blocking mask 35 masks portion 38 of nanofluidic area 16 positioned under portion 36 of photomask 34 to protect nanochannels 21. In step 3, device 10 was exposed to incident UV light 37. Blocking mask 35 causes light diffraction along edge 39 of blocking mask 35.

Blocking mask 35 can be formed of any material which is opaque to exposing light used in optical lithography. For example, blocking mask 35 can be formed of a metal, such as aluminum foil or an opaque plastic.

Referring to Fig. 4B, in step 4, device 10 was developed using conventional techniques. Light diffraction caused by edge 39 of blocking mask 35 generates a gradient in dissolution rate of photoresist 32 by the developer. During development, exposed photoresist 32 was completely removed at portion 41 which is not blocked by blocking mask 35, exposing the substrate surface underneath. At portion 42, photoresist 32 has a gradient of undeveloped photoresist along the light diffraction area. The thickness of the gradient of undeveloped photoresist corresponds to exposure to diffracted light. At portion 43, blocking mask 35 completely blocks exposure of photoresist 32 to light.

Referring to Fig. 4C, in step 5, photoresist 32 was used as an etching mask during a reactive ion etching (RIE) process and gradient patterns in photoresist 32 were transferred into substrate 19.

A light intensity profile on photomask 34 is shown in Fig. 4D. The light intensity profile shows reduced light intensity along edge 39 of blocking mask 35. The gradient profile can be controlled by the type of photoresist, development conditions and etching conditions. For example, a low contrast resist can provide a gradual gradient profile. Edge 39 of blocking mask 35 can be varied to adjust the gradient profile. For example, edge 39 can be angled or patterned to adjust the gradient profile.

In one embodiment, gradient interface area 12 is formed as a gradual slope from microfluidic area 14 to nanofluidic area 16, as shown in Figs. 5A-5B. In this embodiment, one or more nanochannels were fabricated in substrate 19. Substrate 19 was coated with photoresist 32 after HMDS treatment and baked, in step 1. In step 2, blocking mask 35 was placed over photoresist 32. Blocking mask 35 extends over portion 36 of photomask 34. Blocking mask 35 masks portion 38 of nanofluidic area 16 to protect nanochannels 21. In step 3, device 10 was exposed to incident UV light 37. Blocking mask 35 causes light diffraction along edge 39 of blocking mask 35. In step 4, device 10 was developed using conventional techniques. Photoresist 32 was used as an etching mask during a reactive ion etching (RIE) process and gradient patterns in photoresist 32 were transferred into substrate 19. During development, the diminishing light intensity casted on photoresist 32 forms a gradient vertical slope in gradient interface area 12 which is transferred into substrate 16.

Width W_2 of blocking mask 35 and distance between photomask 34 and blocking mask 35 can be varied to determine the distance D_3 of blocking mask 35 to photoresist 32, as shown in Figs. 6A-6B. For example, blocking mask 35 can have a varying width W_2 in the range of about 1 mm to about 10 mm. W_2 can be formed of one or more additional blocking masks which are fused to blocking mask 35 for increasing Width W_2 of blocking mask 35. Blocking mask 35 can be coated on photomask 34.

In an alternate embodiment, distance D_3 of blocking mask 35 to photoresist 32 can be adjusted by adjusting the distance between blocking mask 35 and photomask 34. Blocking mask 35 can be positioned over photomask 34 using blocking mask holder 40. Photomask 34 can be positioned over photoresist 32 using aligner 42. Blocking mask holder 40 can move blocking mask in X_1, X_2, Y_1, Y_2 directions. Aligner 42 can move photomask 34 in the X_1, X_2, Y_1, Y_2 directions. Distance D_3 can be varied upon movement of blocking mask 35 towards and away from photoresist 32. Distance D_3 determines diffraction to photoresist 32. For example, a smaller distance D_3 provides a narrower diffraction zone in gradient interface area 12.

In another aspect of the invention, there is provided a microfluidic/nanofluidic chip that includes the gradient interface area for interfacing microfluidic and nanofluidic components. Referring to Fig. 7, microfluidic/nanofluidic chip 100 has microfluidic area

14, substrate 19, nanofluidic area 16, gradient interface area 12 and reservoirs 102 for handling samples and reservoirs 104 for receiving samples and sample collection. Tunnels 103 formed in substrate 19 can be used for connecting reservoirs 102 and 104 respectively to microfluidic area 14 and nanofluidic area 16.

Nanofluidic area 16 can comprise nanofluidic channels 21 as described above. Alternatively, nanofluidic area 16 and gradient interface area 12 can comprise branched channels 106. Branched channels 106 can be split into smaller and smaller branches range from about 5.0 microns to about 2 nanometers to provide decreasing lateral gradient distances between channels providing a lateral gradient. Branched channels 106 can include a gradual elevation in height formed using diffraction gradient lithography, as described above.

The reservoirs are in fluid communication with at least one of the channels, so that the sample reservoirs are capable of releasing a fluid into the channels, and the waste reservoirs are capable of receiving a fluid from the channels. Typically the fluids contain macromolecules for analysis.

In certain embodiments of the present invention, the microfluidic/nanofluidic chip contains at least one sample reservoir formed in the surface of the substrate. Reservoirs can be defined using photolithography and subsequently pattern transferred to the substrate using Reactive Ion etching (RIE), chemical etching or FIB milling directly to create reservoirs in fluid communication with nanofluidic area 16 or nanochannels 21. In this embodiment, at least one waste reservoir in fluid communication with at least one of the channels. Typically, the microfluidic/nanofluidic chip contains at least 1 sample reservoir. Alternatively, a variety of other embodiments include various numbers of reservoirs.

For use in macromolecular analysis, microfluidic/nanofluidic chip 100 can provide at least a portion of nanofluidic area 16 capable of being imaged with a two-dimensional detector. Imaging of the nanofluidic area 16 is provided by presenting the nanochannels and any sealing material to suitable apparatus for the collection of emitted signals, such as optical elements for the collection of light from the nanochannels. In this embodiment, the microfluidic/nanofluidic chip is capable of transporting a plurality of

elongated macromolecules from a sample reservoir, across macrofluidic area and across the nanofluidic area.

In certain embodiments of the present invention, the microfluidic/nanofluidic chip contains an apparatus for transporting macromolecules from the sample reservoirs, through the macrofluidic area, nanofluidic area, and into the waste reservoirs. A suitable apparatus includes at least one pair of electrodes capable of applying an electric field across at least some of the channels in at least one direction. Electrode metal contacts can be integrated using standard integrated circuit fabrication technology to be in contact with at least one sample and at least one collection/waste reservoir to establish directional electric field. Alternating current (AC), direct current (DC), or both types of fields can be applied. The electrodes can be made of almost any metal, and are typically thin Al /Au metal layers deposited on defined line paths. Typically at least one end of one electrode is in contact with buffer solution in the reservoir.

In certain embodiments of the present invention, the microfluidic/nanofluidic chip contains at least two pair of electrodes, each providing an electric field in different directions. With at least two sets of independent electrodes, field contacts can be used to independently modulate the direction and amplitudes of the electric fields to move macromolecules at desired speed or directions.

In another aspect of the present invention, system 200 is used for carrying out macromolecular analysis, as shown in Fig. 8. System 200 includes a microfluidic/nanofluidic chip 100 as described herein, and an apparatus for detecting at least one signal transmitted from one or more fluids in nanochannels 21 of the microfluidic/nanofluidic chip 100.

In various embodiments of the present invention, the system further includes at least one of the following: a transporting apparatus to transport a fluid through at least microfluidic area 14 and nanochannels 21; a sample loading apparatus for loading at least one fluid to sample reservoirs in microfluidic/nanofluidic chip 100; image or signal detectors and a data processor.

Microfluidic/nanofluidic chip 100 used in system 200 is typically disposable, individually packaged, and having a sample loading capacity of 1-50,000 individual fluid samples. Microfluidic/nanofluidic chip 100 typically has sample loading openings and a

reservoir, or sample loading openings and plates connected with a sealing mechanism, such as an O-ring. Electrodes 202 are connected to electric potential generator 204 and microfluidic/nanofluidic chip 100. Electrodes 202 and electric potential generator 204 can be connected with metal contacts. Suitable metal contacts can be external contact patches that can be connected to an external scanning/imaging/electric-field tuner.

In one embodiment of the present invention, system 200 includes an apparatus to excite the macromolecules inside the channels and detect and collect the resulting signals. Laser beam 206 is focused using a focusing lens 208 to a spot on nanofluidic area 16. The generated light signal from the macromolecules inside the nanofluidic area or nanochannels (not shown) is collected by focusing/collection lens 209, and is reflected off a dichroic mirror/band pass filter 210 into optical path 212, which is fed into CCD (charge coupled device) camera 213. Alternatively, exciting light source could be passed through a dichroic mirror/band pass filter box 210 and focusing/collecting scheme from the top of the chip. Various optical components and devices can also be used in the system to detect optical signals, such as digital cameras, PMTs (photomultiplier tubes), and APDs (Avalanche photodiodes).

System 200 can include data processor 214. Data processor 214 can be used to process the signals from CCD 213 to project the digital image of nanofluidic area 16 on display 215. Data processor 214 can also analyze the digital image to provide characterization information, such as macromolecular size statistics, histograms, karyotypes, mapping, diagnostics information and display the information in suitable form for data readout 216.

Microfluidic/nanofluidic chip 100 can be encased in a suitable housing, such as plastic, to provide a convenient and commercially-ready cartridge or cassette. Typically the nanofluidic cartridges will have suitable features on or in the housing for inserting, guiding, and aligning the sample loading device with the reservoirs. Insertion slots, tracks, or both can be provided in the plastic case.

Macromolecular fluid samples that can be analyzed by the system includes fluids from a mammal (e.g., DNA, cells, blood, Serum, biopsy tissues), synthetic macromolecules such as polymers, and materials found in nature (e.g., materials derived from plants, animals, and other life forms). Such fluid samples can be managed, loaded,

and injected using automated or manual sample loading apparatus of the present invention.

In another aspect of the present invention, there is provided a method of analyzing at least one macromolecule. In this invention, the analysis includes the steps of providing a microfluidic/nanofluidic chip 100 according to the present invention, providing the at least one sample reservoir with at least one fluid, the fluid comprising at least one macromolecule; transporting the at least one macromolecule from a macrofluidic area through a gradient interface area into the at least one channel to elongate said at least one macromolecule; detecting at least one signal transmitted from the at least one elongated macromolecule; and correlating the detected signal to at least one property of the at least one macromolecule.

In one embodiment of the present invention, the method of analyzing a macromolecule includes wetting the channels using capillary action with a buffer solution or a buffer solution containing macromolecules. Macromolecules such as polymers and DNA can be introduced into nanochannel arrays by electric field, capillary action, differential surface tension by temperature or chemical gradient or differential pressure such as vacuum.

Various macromolecules can be analyzed using the present method. For analyzing DNA typical process conditions include providing dilute solutions of DNA which are stained at a ratio of 4:1 to 10:1 base pair/dye with a suitable dye. Suitable dye stains include TOTO-1, BOBO-1, BOBO-3 (Molecular Probes, Eugene, Oregon). Solutions of stained DNA can be further diluted and treated with an anti-oxidant and an anti-sticking agent.

In one embodiment of the present invention, the method of analyzing a macromolecule includes the sizing of one DNA macromolecule. One DNA macromolecule can be extracted from a single cell or spore, such as anthrax, and suitably transported (e.g., in a polymerized gel plugs) to avoid breakage.

The length of a single DNA can be detected/reported and intensity profile can be plotted. In various embodiments of the present invention, the method of analyzing a macromolecule includes correlating the detected signal to at least one of the following properties: length, conformation, and chemical composition. Various macromolecules

that can be analyzed this way include, biopolymers such as a protein, a polypeptide, and a nucleic acid such as RNA or DNA or PNA. For DNA nucleic acids, the detected signals can be correlated to the base pair sequence of said DNA.

The typical concentration of the macromolecules in the fluid will be one macromolecule, or about at least attogram per ml, more typically at least one femtogram per ml, more typically at least one picogram per ml, and even more typically at least one nanogram per ml. Concentrations will typically be less than about 5 micrograms per milliliter and more typically less than about 0.5 micrograms per milliliter.

In one embodiment of the present invention, the method of analyzing a macromolecule measures the length of macromolecules having an elongated length of greater than 150 nanometers, and typically greater than about 500 nanometers, about 1 micron, about 10 microns, about 100 microns, about 1 mm, about 1 cm, and about 10 cm long.

DNA having greater than 10 base pairs can also be analyzed using the present methods. Typically, the number of base pairs measured can be greater than 100 base pairs, greater than 1,000 base pairs, greater than 10,000 base pairs, greater than 100,000 base pairs and greater than 1,000,000 base pairs. DNA having more than 1 million, 10 million, and even 100 million basepairs can be analyzed with the present methods.

In one embodiment of the present invention, the methods can be used to analyze one or more of the following: restriction fragment length polymorphism, a chromosome, and single nucleotide polymorphism.

The invention can be further illustrated by the following examples thereof, although it will be understood that these examples are included merely for purposes of illustration and are not intended to limit the scope of the invention unless otherwise specifically indicated. All percentages, ratios, and parts herein, in the Specification, Examples, and Claims, are by weight and are approximations unless otherwise stated.

EXAMPLES

Large arrays of nanochannels were first fabricated on an entire Si substrate chip using nanoimprinting lithography, described in S. Y. Chou, P.R. Krauss, and P.J. Renstrom, Appl. Phys. Lett. 67 (21), 3114 (1995); Stephen Y. Chou, Peter R. Krauss and

Preston J. Renstrom, Science 272, 85 (1996) and U.S. Patent No. 5,772,905. This chip was spin coated with positive tone photoresist (AZ5214-E) using standard protocol at 4000 rpm for 1 min after HMDS treatment and baked at 110 °C for 2 min. A Karl Suss MA-6 contact aligner and a uniform micron feature size hexagon array photomask were used to pattern the microfluidic area. A blocking mask of a piece of aluminum foil was placed on top of the photomask. The distance between the blocking mask and the photoresist surface was about 3 mm. The chip was exposed at 400 nm UV light in hard contact mode for 35 seconds and developed with a standard procedure (AZ312 MIF: H_2O 1:1). The photoresist was used as an etching mask during a subsequent reactive ion etching (RIE) process and the gradient patterns in the photoresist were transferred into the underlying Si substrate.

Figure 9A shows a top view optical image of the actual gradient chip after photoresist development. The gaps between posts were then etched into the chip using a combination of O_2 and CHF_3 plasma followed by removal of the resist using acetone. Figure 9B shows a scanning electronic microscope (SEM) image of the interfacing zone with gradient lateral spacing between microposts after pattern transfer and photoresist removal. The area directly under the blocking mask with the prefabricated nanochannels is protected from RIE by the masking photoresist.

Figs. 10A-10B illustrate cleaved profile SEM images showing the gradual reduction of the gaps between the microposts, typically from 1.2 μm gradually to below 400 nm, and the gradual elevation of the substrate of the fluidic chip to interconnect to the shallower nanofluidic channels. The gradient profile shown in Figs. 10A and 10B is slight differently controlled by the choice of photoresist, development and etching conditions.

Fluorescently stained long DNA molecules were introduced into prior art nanofluidic chips shown in Fig. 1 and device 10 shown in Fig. 2. In Fig. 11A, DNA entered from the right side of the image, and approached and stalled at the edge of the prior art nanofluidic chip, causing fouling of the chip. In Fig. 11B, lambda phage DNA molecules or genomic BAC DNA were partially uncoiled when they entered the gradient area, and slowed down at the edge of the nanochannels due to "uphill" entrophy. Larger DNA molecules moved into the nanochannels continuously and remained stretched, with

significantly improved efficiency. Moving DNA molecules can be seen in the left part of the image as long white streaks after image integration.

It is to be understood that the above-described embodiments are illustrative of only a few of the many possible specific embodiments which can represent applications of the principles of the invention. Numerous and varied other arrangements can be readily devised in accordance with these principles by those skilled in the art without departing from the spirit and scope of the invention.

What is claimed is:

1. A method for fabricating a fluidic device comprising the steps of:

forming a nanofluidic area on a substrate;

forming a microfluidic area on said substrate; and

forming a gradient interface area between said nanofluidic area and said microfluidic area.

2. The method of claim 1 wherein said gradient interface area comprises a plurality of gradient structures, and a lateral distance between said gradient structures is decreased towards said nanofluidic area.

3. The method of claim 2 wherein said distance between said gradient structures is reduced below about 500 nanometers.

4. The method of claim 2 wherein said distance between said gradient structures is reduced below about 10 nm.

5. The method of claim 2 wherein said distance between said gradient structures is reduced to a distance substantially of a diameter of a biopolymer.

6. The method of claim 2 wherein said gradient structures have a gradual vertical elevation from said microfluidic area to said nanofluidic area.

7. The method of claim 2 wherein said gradient structures are branched channels.

8. The method of claim 1 wherein said gradient interface provides a gradual increase in vertical elevation from said microfluidic area to said nanofluidic area.

9. The method of claim 1 wherein said steps of forming said gradient interface area and forming said microfluidic area are formed simultaneously by the steps of:

coating photoresist over said substrate;

providing a photomask over said photoresist, said photomask patterning said microfluidic area and said gradient interface area;

providing a blocking mask over said photomask, said blocking mask extending over a portion of said photomask applied over said nanofluidic area; and

exposing said photomask to light.

10. The method of claim 9 wherein said blocking mask causes light diffraction along an edge of said blocking mask.

11. The method of claim 10 further comprising the step of:

selecting said edge of blocking mask for controlling said light diffraction.

12. The method of claim 9 wherein said blocking mask is formed of a material which is opaque to light.

13. The method of claim 9 wherein said blocking mask is formed of a metal.

14. The method of claim 9 wherein said blocking mask is formed of aluminum foil.

15. The method of claim 9 further comprising the step of:

developing said photoresist after said step of placing said blocking mask over said photomask,

wherein said photoresist has a gradient of undeveloped photoresist along a light diffraction area, said light diffraction area caused by an edge of said blocking mask.

16. The method of claim 9 wherein said photomask has a thickness in a range of about 1 mm to about 10 mm.

17. The method of claim 9 wherein said blocking mask has a thickness in the range of about 1 mm to about 12 mm.

18. The method of claim 9 wherein said step of providing a blocking mask over said photomask further comprises the step of:

controlling a distance between said blocking mask and said photomask, wherein said distance controls an amount light diffraction along an edge of said blocking mask.

19. The method of claim 1 wherein said nanofluidic area comprises a nanofluidic structure selected from the group consisting of nanopillars, nanopores and nanochannels.

20. The method of claim 19 wherein said nanofluidic structure comprises nanochannels, said nanochannels being formed by: nanoimprint lithography, interference lithography, self-assembled copolymer pattern transfer, spin coating, electron beam lithography, focused ion beam milling, photolithography, reactive ion-etching, wet-etching, plasma-enhanced chemical vapor deposition, electron beam evaporation, sputter deposition, and combinations thereof.

21. A fluidic device formed by the method of claim 1.

22. A method for forming a microfluidic/nanofluidic device comprising the steps of:

forming a nanofluidic area on a substrate;

coating photoresist on said substrate;

providing a blocking mask over said photoresist, said blocking mask extending over a portion of said photoresist; and

exposing said blocking mask to light, wherein said photoresist has a gradient of undeveloped photoresist along a light diffraction area forming a gradient interface area, said light diffraction area caused by an edge of said blocking mask.

23. The method of claim 22 wherein after said step of coating photoresist on said substrate further comprising the step of:

providing a photomask over said photoresist, said photomask patterning said microfluidic area and said gradient interface area.

24. The method of claim 23 wherein in said step of providing a blocking mask, said blocking mask is coated on said photoresist.

25. The method of claim 23 wherein said step of providing a blocking mask over said photomask further comprises the step of:

controlling a distance between said blocking mask and said photomask, wherein said distance controls an amount light diffraction.

26. A fluidic device formed by the method of claim 22.

27. A system for fabricating a fluidic device comprising:

means for forming a nanofluidic area on a substrate;

means for forming a microfluidic area on said substrate; and

means for forming a gradient interface area between said nanofluidic area and said microfluidic area.

28. The system of claim 27 wherein said gradient interface area comprises a plurality of gradient structures, and a lateral distance between said gradient structures is decreased towards said nanofluidic area.

29. The system of claim 28 wherein said distance between said gradient structures is reduced below 500 nanometers.

30. The system of claim 28 wherein said distance between said gradient structures is reduced below about 10 nm.

31. The system of claim 28 wherein said distance between said gradient structures is reduced to a distance substantially a diameter of a biopolymer.

32. The system of claim 28 wherein said gradient structures are branched channels.

33. The system of claim 28 wherein said gradient structures have a gradual vertical elevation from said substrate to said nanofluidic area.

34. The system of claim 28 wherein said gradient interface provides a gradual increase in vertical elevation from said microfluidic area to said nanofluidic area.

35. The system of claim 27 wherein said means for forming a gradient interface area comprises:

means for applying photoresist over said substrate;

means for applying a photomask over said photoresist;

means for providing a blocking mask over said photomask, said blocking mask extending over a portion of said nanofluidic area; and

means for exposing said photomask to light.

36. The system of claim 35 wherein said blocking mask causes light diffraction along an edge of said blocking mask.

37. The system of claim 36 wherein said edge of blocking mask is selected to control said light diffraction.

38. The system of claim 35 wherein said blocking mask is formed of a material which is opaque to light.

39. The system of claim 35 wherein said blocking mask is formed of a metal.

40. The system of claim 35 wherein said blocking mask is formed of aluminum foil.

41. The system of claim 35 further comprising:

means for developing said photoresist, wherein said photoresist has a gradient of undeveloped photoresist along a light diffraction area, said light diffraction area caused by an edge of said blocking mask.

42. The system of claim 35 wherein said photomask has a thickness in a range of about 1 mm to about 10 mm.

43. The system of claim 35 wherein said nanofluidic area comprises nanofluidic structures selected from the group consisting of nanopillars, nanopores and nanochannels.

44. The system of claim 43 wherein said plurality of channels are formed by: nanoimprint lithography, interference lithography, self-assembled copolymer pattern transfer, spin coating, electron beam lithography, focused ion beam milling, photolithography, reactive ion-etching, wet-etching, plasma-enhanced chemical vapor deposition, electron beam evaporation, sputter deposition, and combinations thereof.

45. A fluidic chip comprising:

a surface having a nanofluidic area formed in the material of the surface;

a microfluidic area on said surface;

a gradient interface area between said nanofluidic area and said microfluidic area,

at least one sample reservoir in fluid communication with said microfluidic area, said sample reservoir capable of receiving a fluid; and

at least one waste reservoir in fluid communication with at least one of said channels, said waste reservoir capable of receiving a fluid.

46. The fluidic chip of claim 45 wherein said gradient interface area comprises branched fluidic channels having reduced lateral distance between adjacent channels toward said nanofluidic area.

47. A method of analyzing at least one macromolecule, comprising the steps of:

providing a surface having a nanofluidic area formed of a plurality of channels in the material of the surface;

a microfluidic area on said surface;

a gradient interface area between said nanofluidic area and said microfluidic area,

at least one sample reservoir in fluid communication with said microfluidic area, said sample reservoir capable of receiving a fluid;

at least one waste reservoir in fluid communication with said nanofluidic area, said waste reservoir capable of receiving a fluid;

providing the at least one sample reservoir with at least one fluid, said fluid comprising at least one macromolecule;

transporting the at least one macromolecule between said microfluidic area and said nanofluidic area to elongate said at least one macromolecule;

detecting at least one signal transmitted from the at least one elongated macromolecule; and

correlating the detected signal to at least one property of the at least one macromolecule.

48. The method according to claim 47 wherein the detected signal is correlated to at least one of the following properties: length, conformation, physical and chemical attachment such as a bound marker or tagging and chemical composition.

49. The method according to claim 47 wherein the macromolecule is a synthetic polymer or biopolymer.

50. The method of claim 49 wherein the biopolymer is at least one of: a protein, a polypeptide, and a nucleic acid.

51. The method of claim 50 wherein the nucleic acid is DNA and the detected signals are correlated to the base pair sequence of said DNA.

52. The method of claim 49 wherein the biopolymers are at least substantially unfolded in the channels.

53. The method of claim 47 wherein the concentration of the macromolecules in the fluid is at least one attogram per milliliter.

54. The method of claim 47 wherein the concentration of the macromolecules in the fluid is at least one femtogram per milliliter.

55. The method of claim 47 wherein the concentration of the macromolecules in the fluid is at least one picogram per milliliter.

56. The method of claim 47 wherein the concentration of the macromolecules in the fluid is less than 5 micrograms per milliliter.

57. The method of claim 47 wherein the concentration of the macromolecules in the fluid is less than 0.5 micrograms per milliliter.

58. The method of claim 47 wherein the macromolecules have an elongated length in the channels of greater than 150 nanometers.

59. The method of claim 47 wherein the macromolecules have an elongated length in the channels of greater than 500 nanometers.

60. The method of claim 47 wherein the macromolecules have an elongated length in the channels of greater than 1 micron.

61. The method of claim 47 wherein the macromolecules have an elongated length in the channels of greater than 10 microns.

62. The method of claim 47 wherein the macromolecules are DNA having greater than 100 base pairs.

63. The method of claim 47 wherein the macromolecules are DNA having greater than 1,000 base pairs.

64. The method of claim 47 wherein the macromolecules are DNA having greater than 10,000 base pairs.

65. The method of claim 47 wherein the macromolecules are DNA having greater than 100,000 base pairs.

66. The method of claim 47 wherein the macromolecules are DNA having greater than 1,000,000 base pairs.

67. The method of claim 47 wherein the at least one macromolecule is a chromosome.

68. The method of claim 67 wherein the at least one chromosome is analyzed to determine the presence of at least one single nucleotide polymorphism.

69. A cartridge comprising at least one fluidic chip, said cartridge capable of being inserted and removed from a system for carrying out macromolecular analysis, said at least one fluidic chip comprising at least one nanochannel array, said nanochannel array comprising:

a surface having a nanofluidic area formed in the material of the surface;

a microfluidic area on said surface;

a gradient interface area between said nanofluidic area and said microfluidic area,

at least one sample reservoir in fluid communication with said microfluidic area, said sample reservoir capable of receiving a fluid;

at least one waste reservoir in fluid communication with at least one of said channels, said waste reservoir capable of receiving a fluid; and

an apparatus for detecting at least one signal transmitted from the at least one fluid in said at least one channel.