A Review of Nanofluidic Patents

Prashanta Dutta[1,*] and Juli Morse[2]

[1]*School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164-2920, USA,*
[2]*Office of Intellectual Property Administration and Washington State University Research Foundation, Washington State University, Pullman, WA 99163, USA*

Abstract: Nanofluidics is a relatively new area of research, generally viewed as the study of the behavior, manipulation, and control of fluids at nanometer (<100 nm) scales. At nanometer scales, fluids exhibit unique physical behaviors which are not present in larger structures. Nanofluidic structures have been successfully applied to technologies including analytical separations and the manipulation of proteins, RNA and DNA. There are increasing numbers of applications emerging, as well as innovative fabrication methods enabling the development of these applications. This review covers some of the recent and significant patents relating to nanofluidic devices and methods. We particularly focus on nanofluidic patents targeted to separate, sense and manipulate biofluids and the presence of macromolecules therein. Moreover, several important fabrication methods are reviewed relating to forming microscale and nanoscale fluidic structures. This study found that a majority of the current nanofluidic patents are intended for bioengineering and biotechnology applications, and none of these patents used gas as a working fluid. To date, the number of nanofluidic patents has been very limited, though it is expected that the nanofluidic area will grow in the near future.

Keywords: Nanofluidic, pump, valve, sensors, actuators, bioseparation, protein, DNA.

1. INTRODUCTION

The study of microfluidics and development of resulting technologies such as the micropump, microvalve, etc. has now been underway for over 20 years [1-6]. Originating in university laboratories, this area of research has studied the behavior of fluids at the micron and sub-micron scales, and has uncovered and characterized unique physical behaviors, which depart from the generally understood behaviors and properties of bulk fluids in mass. The behavior of fluids in micron and sub-micron scale devices primarily depends on the type of fluid and mean free path of the fluid molecules. For instance, no-slip velocity at the channel wall cannot be used in microscale gas flow analysis, but the no-slip velocity model is still applicable for microscale liquid flow [6]. Moreover, for microscale liquid flow, classical Navier-Stokes modeling and analysis have been very effective. At this scale, the behavior of liquids is primarily dominated by viscous effects, but the typical bulk fluid viscosity still holds [7-8]. Depending on fluid type (liquid or gas), unique flow profiles and thermodynamic behaviors can be created in these small-scale geometries, such as microchannels and microtubes.

Nanofluidics is formally classified as the study of the behavior, manipulation, and control of fluids in devices having at least one dimension less than 100 nm. In the same way that unique properties have been achieved by using nanofibers and nanoparticles in materials, unique scale-dependent fluids effects are also present in the flow of fluids in nanoscale devices [9]. In nanoscale fluidics, one starts to see a breakdown of the continuum applicability and hence, the standard Navier-Stokes model [10,11]. At this point, the

molecular size can no longer be considered infinitely small compared to the flow (i.e. bulk quantities like viscosity and density start to vary across geometries) [12,13]. Constrained by this dimension, fluids exhibit unique physical behaviors which are not present in larger structures. Regions of the fluid exhibit new properties not observed in bulk flow, such as increased viscosity near the pore wall [14], and thermodynamic property effects [15,16].

Due to the recent advances in micro and nanofabrication, there are growing research interests in the field of micro and nanofluidics. We continue to see considerable contributions to the field from academic research, evidenced in many journal publications as well as patent applications. At the same time, the value of micro and nanofluidics work has grown and reached into the commercial realm, where the design and implementation of such devices has become significant. Micro and nanofluidics technology has been implemented into inkjet print heads [17], fuel cells [18,19], as well as devices for separation, analysis and detection [20-21].

In recent years, nanofluidic structures have been successfully applied to technologies including analytical separations and the manipulation of proteins, RNA and DNA [22]. The most promising and widespread application to date is the integration of nanofluidics and microfluidics into lab-on-a-chip devices. In such devices, analytes can be detected, separated and transferred based on size, charge and mass, analyte concentration can be assessed, reactants can be efficiently mixed, and fluids can be separated based on selected characteristics. These devices conserve materials such as buffer, analytes, and reagent while greatly reducing processing time. They are also enhancing the speed of medical and biological research, detection and diagnosis, due to the ability to utilize very small sample sizes and to

*Address correspondence to this author at the School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164-2920, USA; Tel: (509) 335-7989; Fax: (509) 335-4662; E-mail: dutta@mail.wsu.edu

perform analyses at much higher rates than current capabilities.

2. PATENTS IN NANOFLUIDICS

The United States Patent and Trademark Office (USPTO) Class 977 Nanotechnology [23] section covers all disclosures related to the following categories:

i.   Nanostructure and chemical compositions of nanostructure

ii. Devices that include at least one nanostructure

iii. Mathematical algorithms and computer software specifically adapted for modeling configurations or properties of nanostructure

iv. Methods or apparatus for making, detecting, analyzing, or treating nanostructure and

v.   Specified particular uses of nanostructure

In this context, the term "nanostructure" is defined as atomic, molecular, or macromolecular structure that (a) has at least one physical dimension of approximately 1-100 nanometers; and (b) possesses a special property, provides a special function, or produces a special effect that is uniquely attributable to the structure's nanoscale physical size [23].

In addition, the USPTO has created over 250 cross-reference collection subclasses in order to improve the ability to search and examine nanotechnology-related applications. There are already over 2400 existing patent applications identified within the Class 977 (Nanotechnology section). While there is not a specific subclass for nanofluidics, patents in nanofluidics are included in many of the above categories, namely in the creation of nanostructures, channels, and tubes, and in the utilization of such structures within devices for flows and separation of chemical and biological molecules. They are also classified according to another functional area, i.e. semiconductor manufacturing process, analyte purification, etc.

In this review, we will take a look at some of the recent and significant patents relating to nanofluidics, coming out of research groups in universities, as well as startups and large companies. Notably, we will focus our review on nanofluidic patents, including methods and devices for sorting, sensing and forming. The rest of the article is organized as follows. In the following section, we review patents related to fluid manipulation and sensing in nanofluidic devices. Next, we focus on bioseparation and preconcentration patents in nanofluidic structures. Then we present several important methods for forming microscale and nanoscale structures. Finally, we provide some conclusions based on current and future trends of micro and nanofluidic research and development.

3. SENSING AND ACTUATION USING NANOFLUIDIC STRUCTURES

Due to size limitations, regular scale control mechanisms such as pumping, valving, etc. cannot be easily adapted to microscale or nanoscale fluidic devices. Hence, novel approaches are proposed and developed (Table **1**) to manipulate fluids and macromolecules through nanofluidic channels. Lopez *et al.* [24] developed a method for forming a stimuli responsive polymer that can be mounted in a porous platform for sensing and actuation. The working principle of a stimuli responsive nanofluidic valve is shown in Fig. (**1**). Stimuli responsive actuators are covalently attached to the base porous material which is located within a microchannel. These actuators can be operated by changing the working environment conditions surrounding the valve, such as temperature, pH, ionic strength, intensity of light, frequency of light, etc. In Fig. (**1**), a change in temperature (either an increase or decrease based on the actuator material) causes the responsive polymers to shrink in size in such a way that openings are formed that allow flow through them. Once the environmental conditions are reversed to the actuator's original state, the actuator will revert to its original size and the valve will return to a closed position. Thus, by changing the external stimuli, one can open and close the valve repeatedly.

These stimuli responsive materials can be used as inexpensive pumping methods for liquids in *nanofluidic* systems. Fig. (**2**) shows a peristaltic pump based on stimuli actuations. This pump consists of a channel and three stimuli

Table 1.   US Patents on Fluid Manipulation in Nanodevices

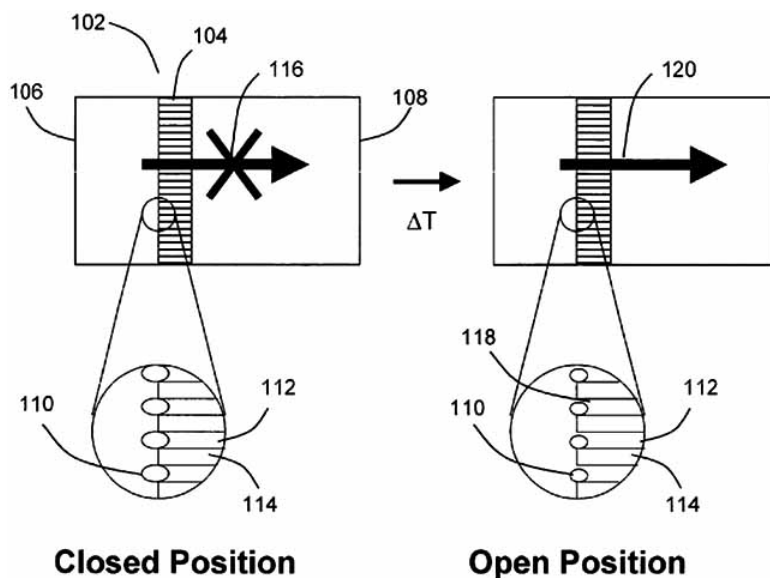| Patent No.	Title	Assignee	Inventors	Issue date
6491061	Stimuli responsive hybrid materials containing molecular actuators and their applications.	University of New Mexico	Lopez *et al.*	12/10/2002
6828786	Method and apparatus for nanomagnetic manipulation and sensing.	California Institute of Technology	Scherer and Barbic	12/07/2004
7217562	Gradient structures interfacing microfluidics and nanofluidics, methods for fabrication and uses thereof.	Princeton University	Cao *et al.*	05/15/2007
7195780	Nanoparticle delivery system.	University of Florida	Dennis *et al.*	03/27/2007
10/705776	Methods and apparatus for ink delivery to nanolithographic probe systems.	NanoInk, Inc.	Cruchon-Dupeyrat *et al.*	pending

Fig. (1). Schematic diagram of the valving principle using stimuli responsive polymer materials [24]. The valve is operated by an array of actuators located in pores. The size of actuators can be altered by changing the condition(s) surrounding the valve. Components **102**: channel, **104**: hybrid material valve, **106 & 108**: upstream and downstream of flow, **110**: actuator, **112**: pore, **114**: porous framework material, **118**: gap.
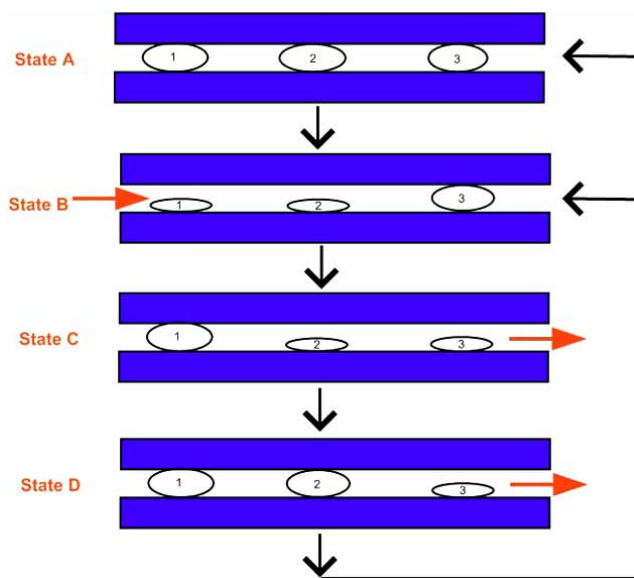

Fig. (2). Schematic view of stimuli responsive polymer based peristaltic pump [24]. States A, B, C and D show the changes in the shapes of actuators corresponding to the temperature changes. In native undeformed states (State A), all actuators extend from the top to the bottom of the channel, and no fluid can pass though the channel.

responsive polymer actuators [24]. Actuators are in close proximity to one another and covalently bonded to the bottom surface of the channel. It is noteworthy to mention that actuators may be bonded to the top surface or some of the actuators may be attached to the top of the channel, while others may be attached to the bottom of the channel. Like the valving principle mentioned in Fig. (**1**), these actuators can be activated by changing the environmental stimuli. For instance, by changing the temperature surrounding the polymeric materials, components 1, 2 and 3 can be actuated successively and/or in a coordinated manner to allow pumping of fluid (not shown) from an upstream to a downstream direction through the channel. The pumping of

the fluid can be achieved by following three steps as shown in Fig. (**2**) (State B, C, and D).

State A shows the initial or shut off position. In this state, all three actuators extend from the top to bottom of the channel, blocking the whole channel. In state B, the first and second actuators allow fluid to enter into the channel by decreasing its size from the native state, while the third actuator remains in the native state, and hence, the fluid cannot leave the channel. In state C, the first actuator closes the entry to the channel by returning to its native state, while the second and third actuators allow openings for the fluid to displace in the channel. Finally, in state D, the second

actuator returns to its native undeformed state, but the first and third actuator remain at the same shape as state C, allowing fluid to leave the system. For continuous pumping of liquid, states B to D are repeated by changing external stimuli. One of the major drawbacks of this type of pump or valve is the slow response time.

A number of US patents address methods for sensing and analyzing fluids in nanodevices. US Patent 6828786 presents a nanofluidic sensor by combining the properties of magnetic nanoparticles with the binding chemistry of biological molecules. They have demonstrated ultra-small, highly sensitive and robust biomagnetic devices by coating nanomagnetic particles with biological molecules for rapid detection of single molecules and biological agents. More recently, Cao *et al*. (US Patent 7217562) used diffraction gradient lithography to create an interface between microfluidic and nanofluidic regions in a microchip for use in high throughput macromolecular analysis. This invention also presents a method of analyzing biopolymers such as proteins, polypeptides, and nucleic acids by correlating the detected signal to at least one of the following properties: length, conformation, and chemical composition.

In one significant patent, Cruchon-Dupeyrat and co-workers (US Patent 7034854 & US patent application 11/399,621) developed a nanofluidic device to supply ink for direct-write nanolithographic printing with the use of a tip or tip array. Their device consists of (a) ink reservoirs, (b) micro/nanofluidic channels connected to the reservoirs, (c) tip or tip array, and (d) dipping wells or an array of dipping wells connected to the microfluidic channels. This application also specifies a method for preventing cross-contamination in closely spaced microchannels by forming a hydrophobic barrier layer between the microchannels. In another recent invention (US Patent 7195780), an *in vivo* delivery of bioactive materials through a nanofluidic channel is reported. The nanochannel is formed by covalently bonding a number of hollow nanotubes end to end. Possibilities of using different sizes of nanotubes and different tube materials are also reported in this patent.

4. SEPARATION USING NANOFLUIDIC STRUCTURES

The advent of micro and nanofluidic technology has revolutionized the field of analytical chemistry and molecular biosciences, as significant benefits can be realized when this technology is used in separation, sequencing and purification processes. Table **2** illustrates significant nanofluidic patents related to the preconcentration, separation and sequencing of biomolecules. US patent 6618679 presents a

Table 2.   US Patents on Bioseparation Using Nanofluidic Structures

Patent No.	Title	Assignee	Inventors	Issue date
6110339	Nanofabricated separation matrix for analysis of biopolymers and methods of making and using same.	Visible Genetics Inc.	Yager *et al*	08/29/2000
6618679	Methods for analysis of gene expression.	Althea Technologies, Inc.	Loehrlein *et al*	11/09/2003
6635163	Entropic trapping and sieving of molecules.	Cornell Research Foundation, Inc.	Han and Craighead	10/21/2003
6685841	Nanostructured devices for separation and analysis.	Science & Technology Corporation @ UNM	Lopez *et al*.	02/02/2004
6913697	Nanostructured separation and analysis devices for biological membranes.	Science & Technology Corporation @ UNM	Lopez *et al*.	07/05/2005
6905586	DNA and RNA sequencing by nanoscale reading through programmable electrophoresis and nanoelectrode-gated tunneling and dielectric detection.	UT-Battelle, LLC	Lee and Thundat	06/14/2005
7033476	Separation and counting of single molecules through nanofluidics, programmable electrophoresis, and nanoelectrode-gated tunneling and dielectric detection.	UT-Battelle, LLC	Lee and Thundat	04/25/2006
6685810	Development of a gel-free molecular sieve based on self-assembled nano-arrays.	California Institute of Technology, Brown University Research Foundation	Noca *et al*.	02/03/2004
6762059	Methods and apparatuses for characterization of single polymers.	U.S. Genomics, Inc.	Chan *et al*.	07/13/2004
7005264	Method and apparatus for nucleic acid sequencing and identification.	Intel Corporation	Su and Berlin	02/28/2006
7118661	Nanolaminate microfluidic device for mobility selection of particles.	The Regents of the University of California	Surh *et al*.	10/10/2006

novel method for DNA sequencing using miniaturized formats including both microfluidic and nanofluidic platforms. This patent claims that required unit operations such as separation, amplification, detection, etc. can be performed in a miniaturized format. The use of a micro or nanofluidic platform reduces the assay cost significantly, while increasing the system throughput enormously.

Han and Craighead (US Patent 6635163) developed a nanofluidic entropic trap for separation of DNA molecules based on the size of nucleic acids [25]. This nanofluidic trap Fig. (**3**) consists of alternating thin/restricted regions to constrain the flow of small objects, and thick/obstacle-free regions to allow molecules to relax for more efficient separation at the thin regions. The characteristic size of the thin region is significantly smaller than the size of the molecules to be sieved, while the size of the thick region should be comparable or larger than the size of target molecules to be separated. In Fig. (**3**), the mobility of molecule 'b' is higher than that of 'a' because of the larger contact area between the molecule 'b' and the top surface. This patent claims that this DNA trap can also be used for separation of cells, viruses, or other similarly-sized particles.

A solid state DNA sequencing chip was developed for rapid, high-resolution separation of single-stranded DNA ladder bands using a nanofabricated separation matrix (US Patent 6110339). The separation matrix consists of a number of lithographically formed posts or pillars separated by a distance of 10 to 30 nm. Lopez *et al.* (US Patent 6685841) also patented a nanofluidic separation matrix containing an array of nanostructures that provide a gradient or variation in physical properties such as size or pitch in at least one direction of the plane. This group claims that the molecular matrix with a gradient in size/pitch is more efficient, highly resolving, reproducible and cost effective than artificial gels containing regular arrays of nanopillars fabricated using electron beam and/or imprint lithography (US Patent 6913697). They presented the separation of one type of biomolecules from a mixture containing a number of biomolecules using the matrix.

High throughput is very important in developing new devices/technologies for bio-separation. Lee and Thundat (US Patent 6905586) developed a nanofluidic platform for ultra fast sequencing of DNA and RNA molecules [26]. Their device consists of (a) nanometer gaps for the passage of a single DNA or RNA molecule, (b) microfluidic channels for sample loading and delivery, (c) programmable electric fields for precise control of DNA or RNA movement, and (d) nanoelectrodes for measurement of gated tunneling
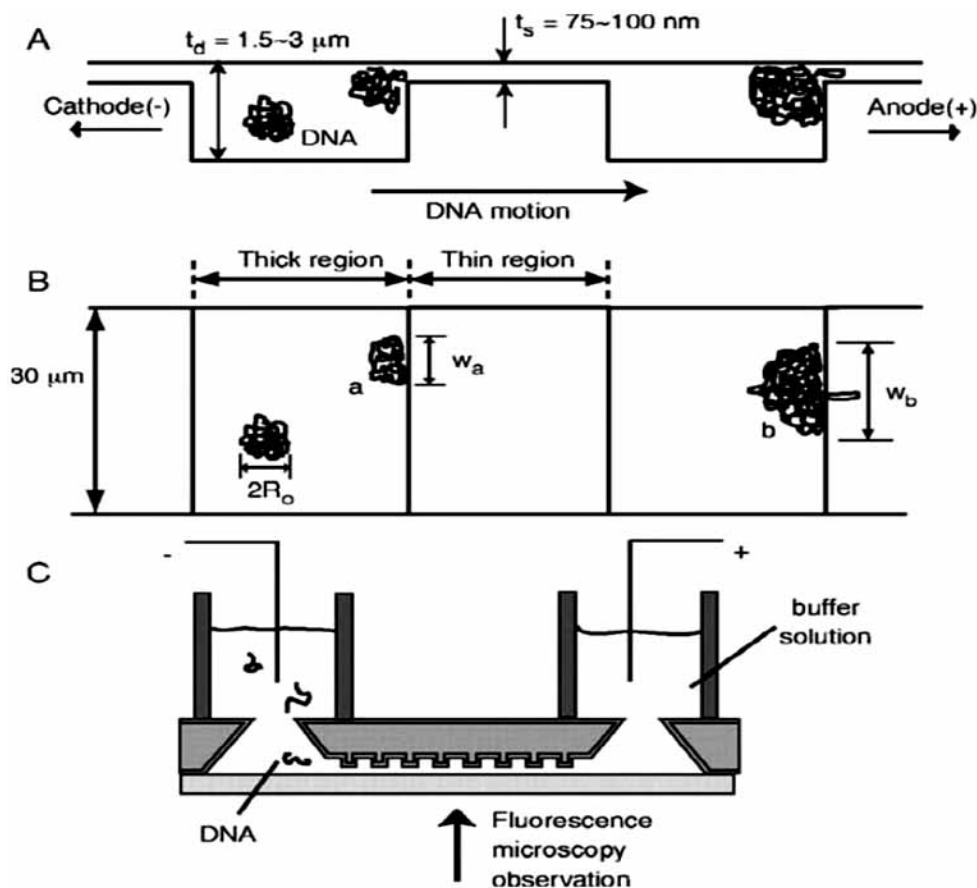

Fig. (3). Separation of DNA using entropic trapping [25]. Reprinted with permission from AAAS. (a) Cross-sectional view of a nanotrap, where t_d and t_s are the heights of the thick and thin regions, respectively. (b) Top view of the device. (c) Experimental setup for the DNA separation. DNA molecules trapped in the thin regions since their radius of gyration is larger than the height of the thinner region. In the thick regions, DNA molecules can form spherical equilibrium shapes because the channel height here is larger than their radius of gyration. Longer DNA molecules (larger radius of gyration), have a higher probability to escape the thin region because of a larger surface area in contact with the boundary.

current. Fig. (**4**) shows the schematic of the patented device along with essential components. Reliable detection of DNA/RNA molecules in this device depends on the precise control of their movement through the nanogate. The appropriate flow control is achieved by applying two perpendicular and programmable electric fields in the system.

The first electric (electrophoresis) field is parallel to the substrate (40) and is applied through a pair of electrophoresis electrodes (61) and (62), while the second electric (holding) field is perpendicular to the substrate surface and is applied using two parallel plates (not shown here) located above and below the substrate surface. The device and method proposed in this patent have the potential of performing DNA sequencing at a rate of about 1,000,000 bases per second per detection system. The same group modified the concept of single molecule detection and quantification in a follow up patent (US Patent 7033476).

A self-assembled molecular sieve for analyzing various biomolecules is presented in US Patent 6685810. This non-gel, self-assembled nano-array sieve is integrated in a microdevice to perform electrophoretic separations of polyelectrolytes such as protein, DNA, RNA, etc. in the presence of an electric field. They also incorporated an optical detector in the line-of-sight of the nano-array such that the target molecules will flow through the detector as the molecules exit the sieve. Chan *et al*. (US Patent 6762059) patented a method for measuring velocity of an elongated polymer in a nanofluidic channel. Using time correlation, the center-of-mass velocity, center-to-center velocity, and end-to-end velocity of single elongated polymers are obtained for a number of locations in the channel. They also proposed methods for determining lengths and molecular masses of single polymers.

Current methods for DNA sequencing are limited by the length of the nucleic acids (500~1000 base pairs) that can be sequenced. These are much shorter than the length of the functional unit of DNA, which can be tens or even hundreds of thousands of bases in length. To overcome this challenge, Su and Berlin (US Patent 7005264) developed a method for detecting long DNA molecules using a nanofluidic platform. In a recent patent (US Patent 7118661), Surh *et al*. developed multiple-interleaved nanolaminate patterns in a microfluidic device for electrophoretic separation of particles based on their mobility. The nanolaminate patterns are formed by assembling alternating layers of two materials (one conducting, one insulating), and joining specific subsets of the conducting layers together to form a single, extended electrode, interleaved with other similar electrodes. Hence, the subsets of metallic layers may be dynamically charged to create time-dependent potential fields that can trap or transport charge colloidal particles.

5. METHODS FOR FABRICATING NANOFLUIDIC STRUCTURES

There are a number of approaches to micro and nanofabrication of fluidic structures. Some processes for microfabrication have translated successfully into the nano-scale, while other innovative methods are constantly emerging to achieve new and novel nanofabrication results. Of the patented processes which are reviewed here (Table **3**), the majority were developed within the context of university research labs.

A significant advancement to microchannel fabrication was developed by Harold G. Craighead and Stephen W. Turner of Cornell University [27], and patented in US Patent 6753200. In this method, two-dimensional and three-dimensional fluidic devices and structures are fabricated
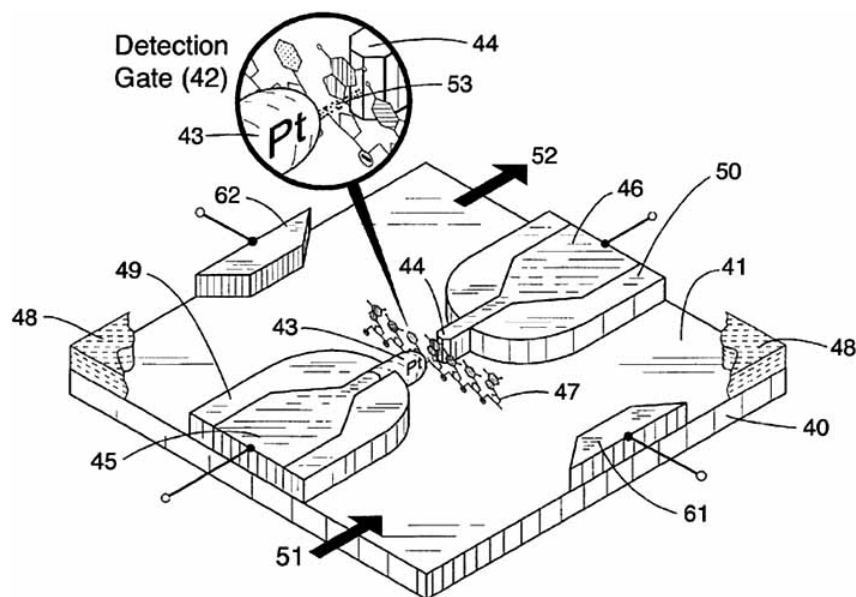

Fig. (4). Schematic of a DNA/RNA sequencing system with integrated nanoelectrode-gated molecular reading in a liquid on a hydrophilic and nonconductive substrate surface [26]. Inset figure shows the nucleotide detection gate or nanogate. Movement of DNA through the nanogate is controlled by the duration and amplitude of the electrophoresis electric field and the holding electric field. Components **40**: substrate, **41**: hydrophilic and nonconducting silicon oxide surface, **42**: nano gate, **43 & 44**: precision nanotips, **45 & 46**: detection nanoelectrodes, **47**: DNA molecule, **48**: liquid layer, **49 & 50**: nonconducting and nonhydrophilic surfaces (SiN), **51**: micro/nanofluidic injection device, **52**: sample drain, **61** electrophoresis electrode (cathode), and **62**: electrophoresis electrode (anode).

Table 3.   US Patents on Fabrication Methods for Nanodevices

Patent No.	Title	Assignee	Inventors	Issue date
6503409	Lithographic fabrication of nanoapertures.	Sandia Corporation	Fleming	1/7/2003
6743570	Method of using heat-depolymerizable polycarbonate sacrificial layer.	Cornell Research Foundation	Harnett *et al.*	6/1/2004
6753200	Monolithic nanofluid sieving structures for DNA manipulation.	Cornell Research Foundation	Craighead and Turner	6/22/2004
6818964	Selectively-etched nanochannel electrophoretic and electrochemical devices.	Regents of the University of California	Surh *et al.*	11/16/2004
7022617	Small scale wires with microelectromechanical devices.	Cornell Research Foundation	Subramanian and MacDonald	4/4/2006
7052616	Fabrication of molecular scale devices using fluidic assembly.	Penn State Research Foundation	Fonash *et al.*	5/30/2006
7067351	Selectively-etched nanochannel electrophoretic and electrochemical devices.	Regents of the University of California	Surh *et al.*	6/7/2006
7169251	Method of forming nanofluidic channels.	Regents of the University of Michigan	Guo and Cheng	1/30/2007
7189635	Reduction of a feature dimension in a nano-scale device.	Hewlett Packard Development Company	Sharma	3/13/2007
7211143	Sacrificial template method of fabricating a nanotube.	Regents of the University of California	Yang *et al.*	5/1/2007
7217562	Gradient structures interfacing microfluidics and nanofluidics.	Princeton University	Cao *et al.*	5/15/2007
7282446	Method of manufacturing self-ordered nanochannel array and method of manufacturing nanodot using nanochannel array.	Samsung Electronics Co, Ltd.	Yoo *et al.*	10/16/2007

through the use of planar processing techniques adapted from semiconductor electronics fabrication. One important aspect is the ability to create multiple fluidic devices as a monolithic unit, enabling integration of fluidic devices with other, electronic or optical, devices on a single substrate. Another aspect is the ability to nanofabricate flow channels in the 10 nm range.

The approach taken by this technology eliminates the laminate bonding steps used in the prior work and allows precise definition of the interior size aspects of working fluidic channels. As shown in Fig. (**5**), this process creates a working fluid gap between a floor layer and ceiling layer by first creating an intermediate sacrificial layer. The sacrificial layer can be patterned in an array of nanoholes, followed by chemical vapor deposition of silicon nitride over the sacrificial layer and into the holes to produce pillars. The sacrificial layer is removed by a wet chemical etch through access holes in the ceiling layer, and the holes are sealed. In this way, one or more layers can be fabricated by successive application and pattern removal. The empty spaces (channels) left behind can be enabled to perform different functions.

Several approaches to creating nanodimensional fluid handling structures further make use of sacrificial layers of material. US Patent 6743570 utilizes a heat-depolymerizable polycarbonate layer, removable from underneath other layers by heating, and leaving behind over-hanging structures such as tubes [28]. In a separate application, US Patent 7211143 provides methods for fabricating uniform nanotubes by use of a sacrificial ZnO nanowires layer over which GaN is grown. The ZnO template is removed by way of thermal reduction and evaporation. A combination approach is described in US Patent 7189635. This is a method for reducing feature dimensions of a nano-scale device. This method involves consuming the surface of a device substrate (which is pre-patterned with nanowires), therein reducing the dimension of the pre-existing nanowires and forming a second set of nanowires spaced in trenches between the first set.

Another common and well-established approach to micro-forming is lithography. Lithographic fabrication techniques are described in US Patent 6503409 and functionalized to the nanoscale to fabricate 1-2 nm characteristics (apertures) using silicon lithographic techniques. These nanostructures (nanopores, nanochannels, and nanoslits) have applications in chemically selective devices for DNA sequencing and electronic, ionic, and molecular tunneling. A device and methods for interfacing nanofluidics and microfluidics components for high throughput macromolecular analysis, using diffraction gradient lithography, are
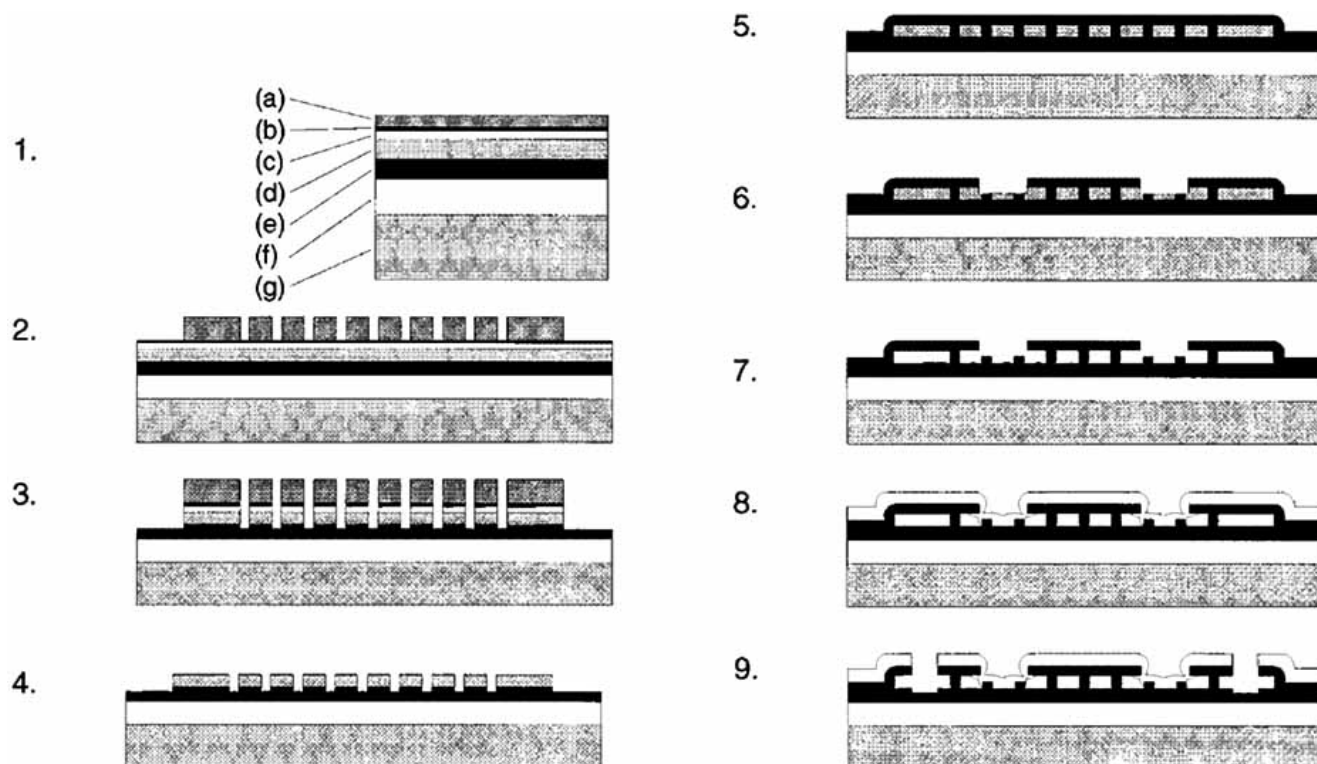
Fig. (5). Process steps used to fabricate the nanodevices. Reprinted with permission from [27]. Copyright 1998, American Vacuum Society. **Step 1:** The process begins with a thin film stack composed as follows: (a) PMMA, (b) aluminum, (c) silicon dioxide, (d) polysilicon, (e) silicon nitride, (f) silicon dioxide and (g) silicon. **Step 2:** Use electron beam lithography (EBL) to pattern the resist. **Step 3:** Transfer the pattern in three etch steps to the polysilicon sacrificial layer. **Step 4:** Remove the patterning layers down to the polysilicon. **Step 5:** Deposit silicon nitride. **Step 6:** Etch access holes in the nitride. **Step 7:** Remove the sacrificial layer with a wet etch. **Step 8:** Reseal the device with very low temperature oxide silicon dioxide. **Step 9:** Etch loading windows at both ends of the device.

claimed in recent US Patent 7217562. Fig. (**6**) shows the steps involved in the process: coating photoresist over the substrate, providing a photomask over the photoresist to pattern the microfluidics and gradient area, placing a blocking mask over the photomask to cover the nanofluidics area, and exposing the photomask to light [29]. The aforementioned gradient interface area reduces the local entropic barrier to nanochannels formed in the nanofluidics area.

Several hybrid methods have been presented, utilizing lithography techniques in the presence of additional processes, for the creation of fluidic devices. US Patent 7052616 presents such a method for producing fluidic devices, involving the use and removal of sacrificial layers on a substrate. In this method, the process also makes use of lithography techniques, enabling the depositing and patterning of electrode materials on a substrate. US Patent 7169251 claims a method of forming enclosed nanofluidic channels via lithography and etching. The method is comprised of a first substrate and material layer, a second substrate, formation of a nanoscale slot on the second substrate via nanolithography and etching, and bonding the first substrate to the second aligned with the nanoscale slot such that a nanofluidic channel is enclosed.

Etching is another approach for surface modifications and nanostructure fabrication. A process for creating

dimensional sidewalls in fluid channels is covered by US Patent 7022617. This process cycles between etching and polymerizing chemistries, creating ripples corresponding to the cycles, and ultimately resulting in fluidic channels formed by oxidizing the rippled sidewalls. A method for forming silicon wires in a single crystal silicon substrate is also claimed. US Patent 7067351 pertains to nanochannel electrophoretic and electrochemical devices having selectively-etched nanolaminates located in the fluid transport channel. Particularly, this patent covers methods for making such devices. The nanolaminate surfaces are selectively-etched to form trenches and baffles, and when implemented in electrochemical detection devices, these features prove advantageous in increasing the sensitivity of devices to low concentrations of analyte, improving the plug flow characteristic of the channel, and allowing additional discrimination of colloidal particles during cyclic voltammetry. This patent relates to a previous US Patent 6818964 covering the apparatus itself, devices made from the nanofabrication process. Finally, a fabrication method involving etching which was developed at Samsung Electronics was reviewed (US Patent 7282446). This is a method of creating small and highly ordered nanochannel-arrays using self-alignment techniques. The fabrication takes place via a series of anodizing and etching steps, with masking and compression molding. The invention provides a
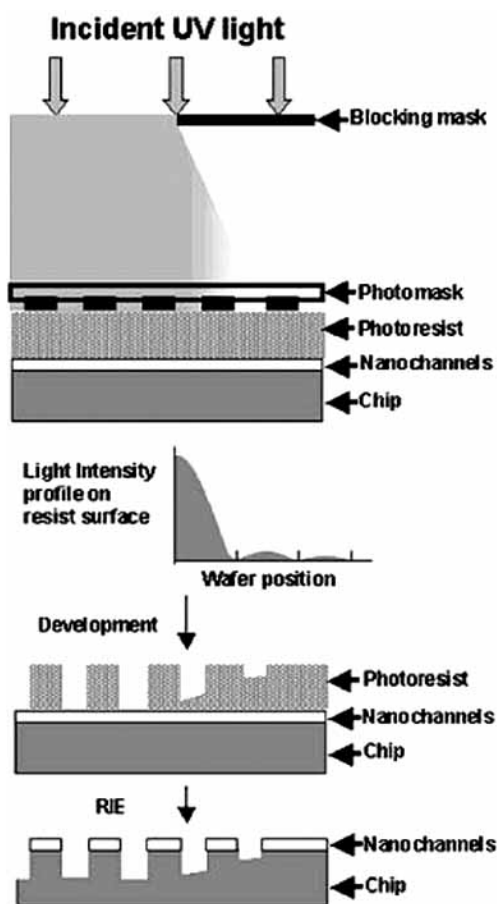
Fig. (6). Schematic representation of the diffraction gradient lithography (DGL) setup. Reprinted with permission from [29]. Copyright 2002, American Institute of Physics. The substrate chip under the photoresist already has nanochannels on the surface, and was fabricated using nanoimprinting lithography (NIL).

method of manufacturing an array of vertical nanochannels and a method of manufacturing a nanodot. Specifically, this illustrates forming a self-ordered nanochannel array by two-step anodizing and fabricating a nanodot using the nanochannel-array.

6. CURRENT & FUTURE DEVELOPMENTS

Nanofluidics, generally viewed as the study of the behavior, manipulation, and control of fluids at nanometer scales, is a relatively new but growing field of research. Thus far, nanofluidic structures have been successfully applied to technologies including analytical separations and the manipulation of proteins, RNA and DNA. There are increasing numbers of applications emerging, as well as innovative fabrication methods enabling the development of these applications. In this review, we covered some of the recent and significant patents relating to nanofluidic devices and methods with a focus on separation, sensing and manipulation of biofluids. We also reviewed several important fabrication methods related to forming microscale and nanoscale fluidic structures. A majority of the current nanofluidic patents are directed towards bioengineering and

biotechnology applications. This trend is expected to continue.

This study found that the number of patents in nanofluidics is still relatively limited. For instance, while there are more than 2000 issued patents in microfluidics, there are less than 100 specifically related to nanofluidics. This may be due to several factors. First, very few institutes have access to the nanofabrication equipment necessary to create nanoscale features. Although bottom-up techniques have been around for several decades, they have not shown much potential for device level contributions. Second, it is very difficult to pass fluid (especially liquid and complex biofluids) through nanofluidic channels using mechanical pumping. The high pressure required for pumping in nanoscale channels makes them structurally unstable. A nanofluidic device is practically useless unless an innovative pumping technique can be integrated within the device. Third, although some research has successfully utilized fabrication processes developed for nanoelectronics and protonics, appropriate integration of individual components and surfaces for nanofluidics remains a challenge.

Nevertheless, nanofluidics is a very promising research area. Funding agencies around the world have invested heavily in nanotechnology research over the last few years. According to the U.S. National Nanotechnology Initiative (NNI) [30], US government funding of nanotechnology research has increased each of the past 10 years, and this trend is expected to continue. In addition, global government investments in nanotechnology research have been lead by Europe, Israel, North America and Japan, but countries such as Russia, China, Brazil, Turkey and India are also beginning to make investments into this sector. As both public and private investments continue to support nanotechnology research, we expect to see growth in the development and application of nanofluidics. The number of patents in nanofluidics and the increasing breadth of applications are expected to continue to grow in the near and foreseeable future.

REFERENCES

[1]   Harley JC, Huang YF, Bau HH, Zemel JN, Gas-flow in microchannels. J Fluid Mech 1995; 284: 257-274.
[2]   Beskok A, Karniadakis GE, Trimmer W. Rarefaction and compressibility effects in gas microflows. J Fluids Eng-Transactions ASME 1996; 118: 448-456.
[3]   Santiago JG, Wereley ST, Meinhart CD, Beebe DJ, Adrian RJ. A particle image velocimetry system for microfluidics. Exp Fluids 1998; 25: 316-319.
[4]   Dutta P, Beskok A. Analytical solution of combined electroosmotic/pressure driven flows in two-dimensional straight channels: Finite Debye layer effects. Anal Chem 2001; 73: 1979-1986.
[5]   Whitesides GM. The origins and the future of microfluidics. Nature 2006; 442: 368-373.
[6]   Karniadakis G, Beskok A, Aluru N. Microflows and Nanoflows: Fundamentals and Simulation. New York: Springer 2005.
[7]   Qiao R, Aluru NR. Ion concentrations and velocity profiles in nanochannel electroosmotic flows. J Chem Phy 2003; 118: 4692-4701.
[8]   Travis KP, Gubbins KE. Poiseuille flow of Lennard-Jones fluids in narrow slit pores. J Chem Phy 2000; 112: 1984-1994.
[9]   Whitesides, GM, Stroock AD. Flexible methods for microfluidics. Phys Today 2001; 54: 42-48.
[10] Gad-el-Hak, M. The fluid mechanics of microdevices-the Freeman scholar lecture. J Fluids Eng- Transactions ASME 1999; 121: 5-33.

[11] Eijkel JCT, van den Berg A. Nanofluidics: what is it and what can we expect from it? Microfluid Nanofluid 2005; 1: 249-267.

[12] Travis KP, Todd BD, Evans DJ. Departure from Navier-Stokes hydrodynamics in confined liquids. Phys Rev E 1997; 55: 4288-4294.

[13] Koplik J, Banavar, JR. Continuum deductions from molecular hydrodynamics. Annu Rev Fluid Mech 1995; 27: 257-292.

[14] Eijkel JCT. Liquid slip in micro- and nanofluidics: Recent research and its possible implications. Lab Chip 2007; 7: 299-301.

[15] Xuan X, Xu B, Sinton D, Li D. Electroosmotic flow with Joule heating effects. Lab Chip 2004; 4: 230-236.

[16] Hu G, Li D. Multiscale phenomena in microfluidics and nanofluidics. Chem Eng Sci 2007; 62: 3443-3454.

[17] Field, L.A., Donald, D.K., Barth, P.W., Servaites, J., Hoen, S.T., Harley, J.A .: US20016299673 (**2001**).

[18] Hidrovo, CH, Kramer TA, Wang EN, *et al.* Two-phase microfluidics for semiconductor circuits and fuel cells. Heat Transfer Eng 2006; 27: 53-63.

[19] Jayashree RS, Mitchell M, Natarajan D, Markoski LJ, Kenis PJA. Microfluidic hydrogen fuel cell with a liquid electrolyte. Langmuir 2007; 23: 6871-6874.

[20] Cui H, Horiuchi K, Dutta P, Ivory CF. Isoelectric Focusing in a Poly-di-methyl-siloxane microfluidic chips. Anal Chem 2005; 77: 1303-1309.

[21] Cui H, Horiuchi K, Dutta P, Ivory CF. Multistage isoelectric focusing in polymeric microchips. Anal Chem 2005; 77: 7878-7886.

[22] Tegenfeldt JO, Prinz C, Cao H, *et al.* Micro- and nanofluidics for DNA analysis. Ana Bioanal Chem 2004; 378: 1678-1692.

[23] http://www.uspto.gov/go/classification/uspc977/defs977.htm.

[24] Lopez, G., Chilkoti, A., Atanassov, P., Goparaju, V.: US20026491061 (**2002**).

[25] Han J, Craighead HG. Separation of long DNA molecules in a microfabricated entropic trap array. Science 2000; 5468: 1026-1029.

[26] Lee, J.W., Thundat, T.G.: US20056905586 (**2005**).

[27] Turner SW, Perez AM, Lopez, A, Craighead, HG. Monolithic nanofluid sieving structures for DNA manipulation. J Vac Sci Technol B 1998; 6: 3835-3840.

[28] Harnett CK, Coates GW, Craighead HG. Heat-depolymerizable polycarbonates as electron beam patternable sacrificial layers for nanofluidics. J Vac Sci Technol B 2001; 19: 2842-2845.

[29] Cao H, Tegenfeldt JO, Austin RH, Chou SY. Gradient Structures Interfacing Microfluidics and Nanofluidics. Appl Phys Lett 2002; 81: 3058-3060.

[30] http://www.plunkettresearch.com/Industries/Nanotechnology MEMs/NanotechnologyMEMs Statistics/tabid/219/Default.aspx.