Microfluidics and Sensors for DNA Analysis

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Abstract— *The manipulation of fluids in microchannels has been studied extensively due to its vast array of applications including genome sequencing, single cell detection, cost and time reduction with electronic microdevices. Microfluidics has the potential to influence subject areas from chemical synthesis and biological analysis to optics and information technology. The review paper introduces the advancement of microfluidics in DNA analysis. Wherever possible commercially available device information is also provided to emphasize the importance of that particular technology and its scope. It will briefly introduce you to different types of biosensor technology currently researched and one example that make the conceptual design into a reality.*

Keywords— Biosensor, DNA, Electrical, Microfluidics, PDMS.

I. INTRODUCTION

Microfluidics drives the advance technology to perform biological and chemical experiments at micro and nanometer scale, at affordable cost with minimal material consumption and optimal results. In microfluidic devices fluidic components are miniaturized and integrated together, leading to a realization of an entire "lab on a chip," in the same way that a microelectronic circuit is a whole computer on a chip [1]. There has been keen interest in achieving the full potential of this approach and, consequently, the development of many microfluidic devices and fabrication methods. Elastomeric materials such as polydimethylsiloxane (PDMS) have excellent alternatives to the silicon and glass used in new devices fabricated by MEMS (microelectromechanical systems) processes. Simplified device fabrication and the possibility of incorporating densely integrated microvalves into designs have helped microfluidics to expand into a ubiquitous technology that has found applications in many diverse fields.

Microfluidics is the science and technology of systems that process or manipulate small (10^{-9} to 10^{-6} liters) amounts of fluids, using channels with dimensions less than tens to hundreds of micrometers. Applications of microfluidic technologies offer many useful capabilities: the ability to use minuscule quantities of samples and reagents and to carry out separations and detections with high resolution and sensitivity; low cost; short times for analysis; and small footprints for the analytical devices [2]. Microfluidics exploits its most prominent characteristic, small size and less distinct characteristics of fluids in microchannels. It offers new capabilities in the control of concentrations of molecules in space and time. Microfluidics is a key to advancing molecular sensor based on bioassays including immunoassay, cell separation, DNA amplification and analysis [2]. It processes a vast number of parallel experiments rapidly with the tiny amount of reagents and chemicals. A reduction in size to the micrometer scale will usually not change the nature of molecular reactions, but laws of scale for surface per volume, molecular diffusion, and heat transport enable dramatic increases in throughput. The research for drugs demands robust and fast methods to find, refine and test a likely drug with relatively low cost. The discovery of a unique molecule with new qualities out of a nearly unlimited number of possibilities is laborious, time-consuming and relies heavily on technological resources that are available for handling small liquid volumes, automation, and high-through-put processing and analysis [1]. Initially, the concept of microfluidics solely dedicated to significantly reducing sample consumption and increasing efficiency in separation methods such as electrophoresis, but eventually low costs of mass production of microchips and automation of reaction systems for commercial use. At the time of review, there are many commercially available microfluidic devices made by prominent companies like Agilent Technologies, Evotec Technologies, Hitachi, and Fluidigm Technology [2].

II. MICROFLUIDIC DEVICE FABRICATION

Microfluidic devices are fabricated using standard photolithographic techniques using the replica molding method because it allows for simple, low-cost prototyping of microchannels. For rapid prototyping, polydimethylsiloxane-covered cover glasses are suitable for sealing devices. PDMS is spun onto a cover slip to a thickness of several microns and allowed to cure. The PDMS comes into contact with the chip containing trenches [3]. Fig. 1 shows typical processing steps of the microfluidic device fabrication.

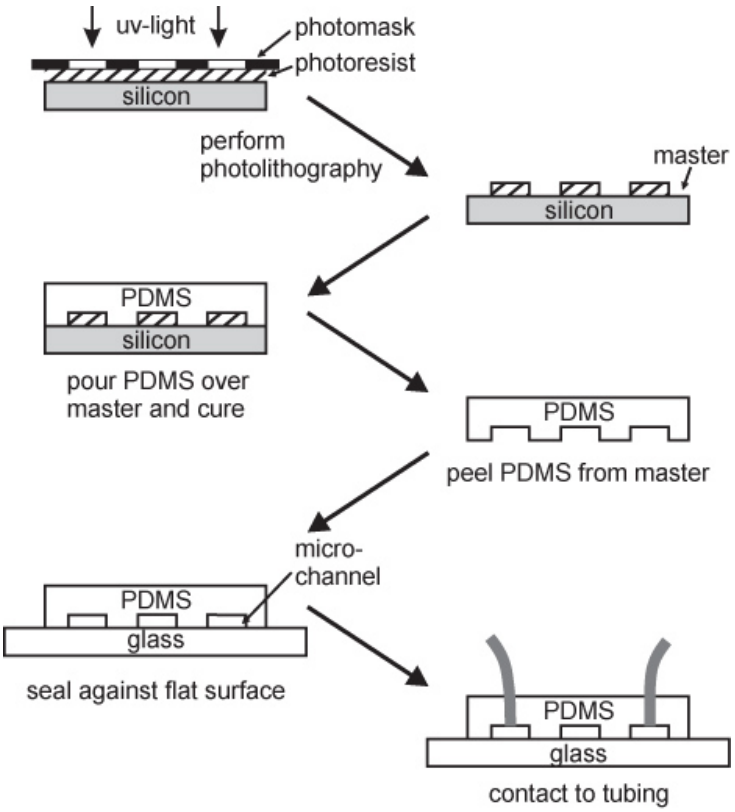

FIG. 1. MICROFLUIDIC DEVICE FABRICATION PROCESS [3]

III. DEOXYRIBONUCLEIC ACID (DNA)

Deoxyribonucleic Acid is one of the most used scientific terms in biotechnology. DNA is a biopolymer consisting of repeating units, i.e., four types of nucleotides, adenine (A), thymine (T), guanine (G), and cytosine (C). Each nucleotide is comprised of nucleobases and sugars as shown in fig. 2. These nucleobases are linked to ester bonds between the sugar and the phosphate groups, the backbone of DNA polymers. DNA double helix structure is a direct result of two DNA polymers with complementary base sequences paired following the Watson-Crick rule, A-T, and G-C. DNAs are the carriers of genetic information encoded by the sequence of four nucleotides, which transmits to RNA that directs protein synthesis process [4]. Each base pair is 3.4 Angstroms apart, and because each base pair is rotated 36 degrees on the previous pair, the helix repeats every 34 Angstroms. The average size of a protein-coding gene is 30 kbp, and the average mass of a base pair is 650 Da. The longest gene in the human genome is Titin (80 791 bp) while the most extended piece of synthetic DNA is the Mycoplasma genitalium bacterial genome (582 970 bp). Because of the negatively charged phosphate ions in the backbone, DNA has an overall negative charge [5].

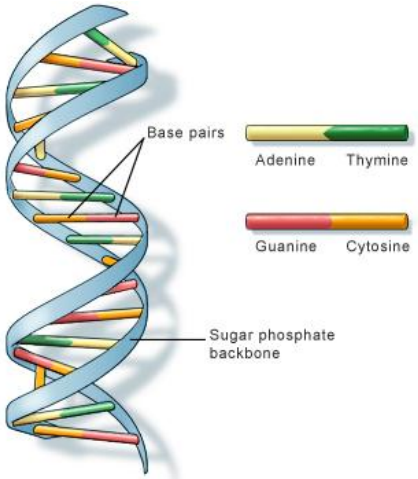

FIG. 2. DNA DOUBLE HELIX STRUCTURE FORMED WITH SUGAR-PHOSPHATE BACKBONE [4].

IV. MICROFLUIDICS FOR DNA ANALYSIS

Detection of single cells or individual DNA strands is necessary for specific diseases. Particular DNA mutations or cell disorders can now be detected using DNA analysis. There are several fundamental techniques used for DNA analysis including detection, amplification, and separation. Further advancement in the field to push for higher specificity and selectivity and the faster reaction time is ongoing. Microfluidic technologies enable one with a precise actuation of fluids and manipulation of bioparticles (e.g. DNA, RNA, proteins, and cells) at the micro scale. Fluid flow in ultra low dimensions of micrometers is laminar and precisely controlled by adjusting the flow rate. This distinct property gives rise to more efficient and accurate mass delivery to cells in controlled time and space. Also, because microfluidic platforms have scalable sizes with most biological macromolecules, cells, and blood vessels, they provide unique functionality for the design and remodeling of precise scaffolds, which mimic the physiological microenvironment [6]. DNA amplification is an essential step for DNA analysis. Micro Polymerase Chain Reaction (Micro-PCR) is used to perform DNA amplification. Micro-PCR offers many advantages including less use of reagents, rapid cooling/heating rates, decreased power consumption, and portability. PCR microfluidic devices allow simultaneous multiple reactions to occur while reducing the risk of contamination and increasing efficiency. Electrophoresis is a method of separating macromolecules such as DNAs, Proteins, and cells by charge and size. Capillary electrophoresis on microfluidic chip devices can increase throughput and streamline the process that was conventionally time-consuming. A microfluidic channel is ranging from five to two hundred microns in diameter filled with a conducting buffer solution. The potential difference creates a current through the channel. Once current begins to flow, charged particles begin to flow. Because the rate of their flow is dependent on their charge, the DNA fragments are distributed by charge along the microchannels in a way similar to a conventional gel electrophoresis. Each well connects to an electrode. A voltage is selectively applied to each pair of wells to perform sample injection and separation.

V. COMMERCIAL DEVICES

5.1 The Fluidigm BioMark System

The Fluidigm BioMark System is the first commercial system for digital PCR-based on integrated microfluidic microchips with integrated chambers and valves for partitioning samples. Quake et al. at the California Institute of Technology developed the microfabrication technique in 1998. It is one of the many microfluidic systems produced that utilizes the NanoFlex valve on an integrated microfluidic chip for single cell gene expression profiling using digital PCR, genotyping, mutant detection, as well as real-time PCR. Fig. 3 shows the Fluidigm BioMark System.

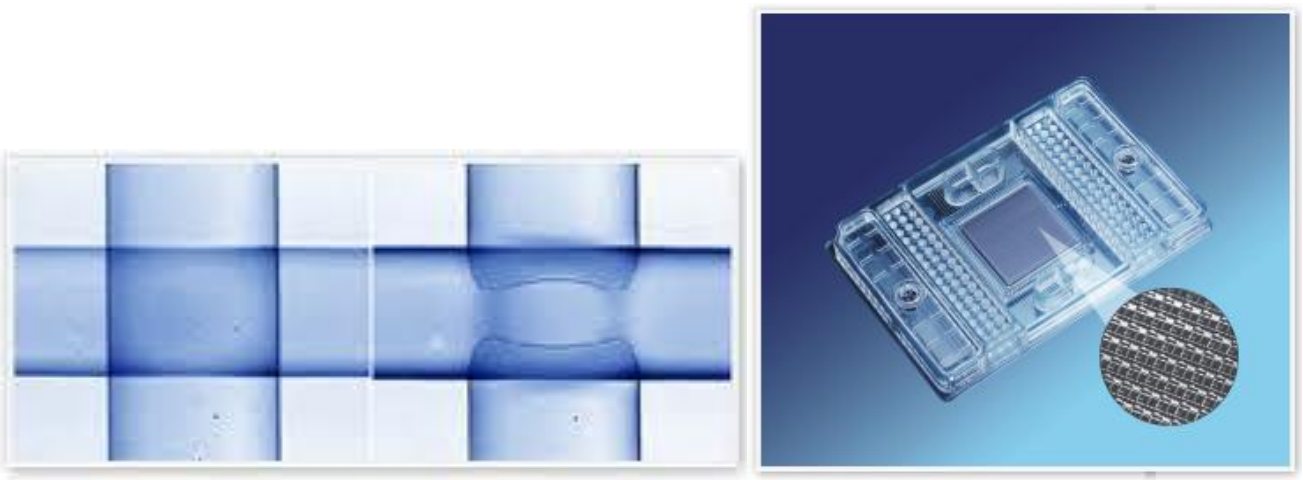

FIG. 3. THE FLUIDIGM BIOMARK SYSTEM WITH NANOFLEX VALVE [7].

5.2 Agilent LabChip

Agilent 2100 device is one of the most widely used commercial chip-based DNA analysis devices. They are manufactured from PDMS using soft lithography. The system uses interchangeable chips that interface with a bench-top device that acts as the power supply for electrophoresis and also contains the optical detection system. Fig. 4 shows the Agilent LabChip with its detailed processing steps.

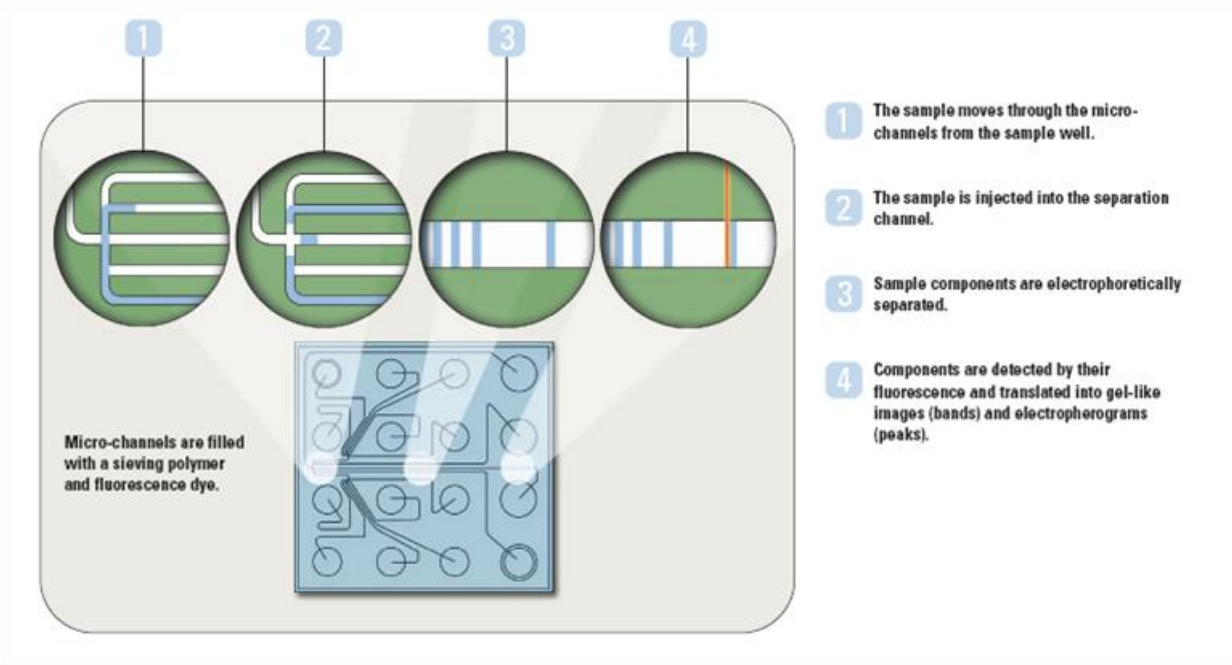

FIG. 4. AGILENT LABCHIP IS SHOWN WITH THE PROCESSING STEPS [8].

VI. DNA SENSOR METHODS

DNA sensors ought to detect, record, and indicate a physical or chemical property, with potential capability for further processing. DNA biosensor is a "compact logical device or unit incorporating a biological or biologically derived sensitive 'recognition' element integrated or associated with a physicochemical transducer [4]. Fig. 5 shows a typical structure of a biosensor. A sensor can be broken down into three elements: (1) recognition element, (2) transducer and (3) amplifier/processor. Recognition refers to binding of target molecules in our case the DNA. Binding of DNA induces the sensing effect, or the physical or chemical changes, due to a detection event. Desired characteristics for this stage include:

1. Selectivity – Only the target DNA generates sensing effect
2. Sensitivity – Large sensing effect with a small amount
3. Resolution – A good indication of the amount of captured DNA
4. Dynamic Range – Not saturated with a large quantity of DNA

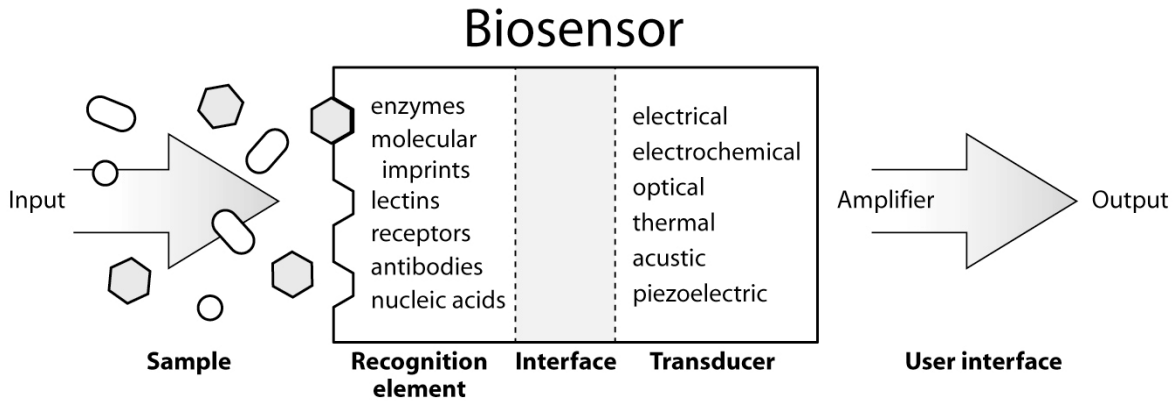

FIG. 5. THE CONFIGURATION OF A BIOSENSOR SHOWING BIO-RECOGNITION, INTERFACE, AND TRANSDUCTION ELEMENTS [4].

Transduction refers to the conversion of the detection event into a useful, measurable signal that is usually an electrical signal of either voltage or current [4]. Most transducers are physical sensors that convert the sensing effect of molecular binding into measurable electrical signals. The signal from the transducer has to be measured or recorded and interpreted as the

quantity of molecular activity. High levels or signal processing may be needed to retrieve meaningful data in most cases. Signal processing is performed mostly done by digital processors and computers [5]. Sensors through signal transductions convert the detection event into a measurable signal. Chemical, physical and biological quantities transformed into a detectable signal, mostly an electrical signal. DNA sensors fall into three types, namely optical transduction, electrical transduction, and mechanical transduction [5].

VII. OPTICAL BIOSENSOR

Optical biosensors are a powerful detection and analysis tool that has large applications in biomedical research, health- care, pharmaceuticals, environmental monitoring, and homeland security. They are immune to electromagnetic interference; capable of performing remote sensing, and can provide multiplexed detection within a single device. There are two screening protocols in optical biosensing: fluorescence-based detection and label-free detection [5]. A surface plasmon wave (SPW) is a charge density oscillation that occurs at the interface of two media with dielectric constants of opposite signs, such as a metal (gold or silver) and a dielectric. There are four basic methods to excite the SPR, as shown in Fig. 6: prism coupling, waveguide coupling, fiber optic coupling, and grating coupling. In the prism coupling configuration (Fig. 6(A)), the incident light is totally reflected at the prism–metal interface and generates an evanescent field penetrating into the metal layer. At the resonant angle or resonant wavelength, the propagation constant of the evanescent field matches that of the SPW, and as a result, the photon will have couple into the SPW [5].

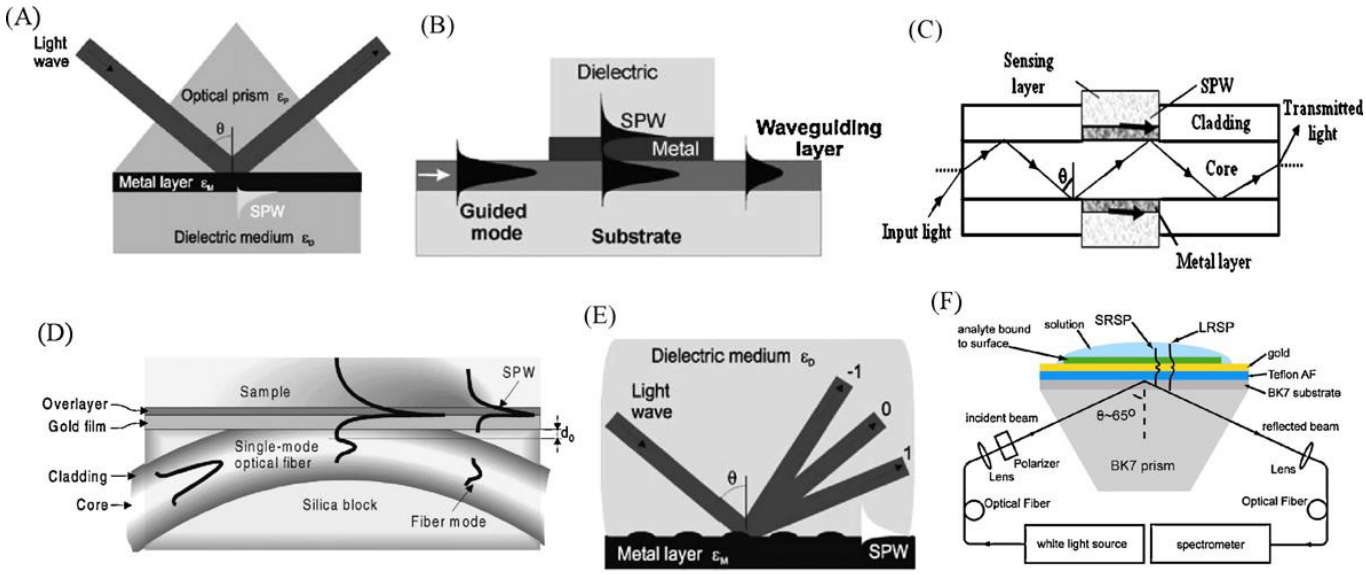

FIG. 6. VARIOUS SPR SENSOR CONFIGURATIONS. (A) PRISM COUPLING, (B) WAVEGUIDE COUPLING, (C) OPTICAL FIBER COUPLING, (D) SIDE-POLISHED FIBER COUPLING, (E) GRATING COUPLING AND (F) LONG-RANGE AND SHORT-RANGE SURFACE PLASMON [5].

The following research demonstrates a Localized Surface Plasmin Resonance (LSPR) Biosensor. The major discovery reported is that triangular silver nanoparticles fabricated by nanosphere lithography do indeed function as unexpectedly sensitive and selective nanoscale affinity biosensors. LSPR Nano sensors as shown in fig 7. possess at least two unique characteristics modified by changing nanoparticle size and shape: (1) modest refractive sensitivity on the order of 1 part in 102 and (2) a short-range, sensing length scale determined by the characteristic decay length of the local electromagnetic field. These two factors combine to yield an areal mass sensitivity of ~100-1000 pg/mm2, which is only a factor of 100 poorer than the best propagating SPR sensitivities. LSPR Nano sensors retain all of the other desirable features of SPR spectroscopy [6].

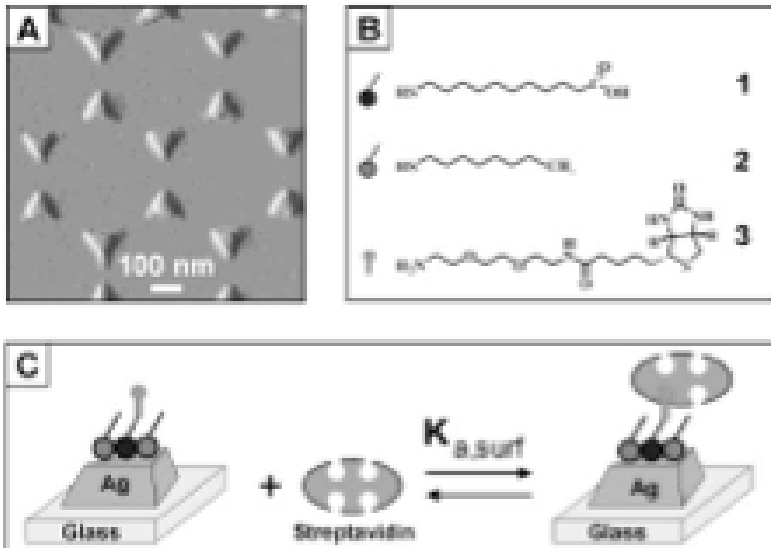

FIG. 7. LSPR NANOBIOSENSOR: (A) AFM IMAGE OF THE AG NANOPARTICLES. (B) SURFACE CHEMISTRY OF THE AG NANOBIOSENSOR. (C) SCHEMATIC REPRESENTATION OF SA BINDING TO A BIOTINYLATED AG NANOBIOSENSOR [6].

VIII. MECHANICAL BIOSENSOR

Mechanical interactions are fundamental to biology. Mechanical forces of synthetic origin determine motility and adhesion on the cellular scale and govern transport and affinity on the molecular level. Biological sensing in the mechanical devices provides unique opportunities to measure forces, displacements and mass changes from cellular and subcellular processes. A basic design of the cantilever chip is shown in fig. 8. Nanomechanical systems are particularly well matched in size with molecular interactions, and provide a basis for biological probes with single-molecule.

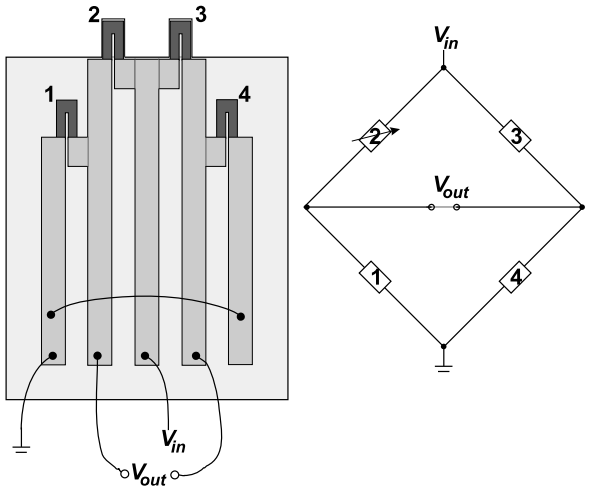

FIG. 8. THE BASIC DESIGN OF THE CANTILEVER CHIP WITH FOUR INTEGRATED PIEZORESISTORS PLACED IN AN ON-CHIP WHEATSTONE BRIDGE [9].

The following research presents a cantilever-based biochemical sensor with piezoresistive read-out as shown in figure 8, optimized for measuring surface stress. The resistors and the electrical wiring on the chip are encapsulated in Low-Pressure Chemical Vapor Deposition (LPCVD) silicon nitride so that the chip is well suited for operation in liquids. The wiring is titanium silicide, which in contrast to conventional metal wiring—is compatible with the high-temperature LPCVD coating process [9]. The 1/f noise is found from the measured spectral noise density shown in Fig. 9. The lowest curve is for no applied voltage and reflects the Johnson noise, whereas the other three curves show 1/f noise for three different supply voltages.

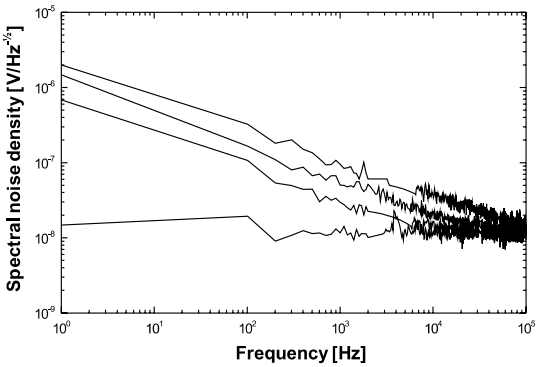

FIG. 9. SPECTRAL NOISE DENSITY CURVES AT DIFFERENT VOLTAGES [9].

IX. ELECTRICAL BIOSENSOR

Biosensors are electronic devices that produce electric signals as the result of biological interactions. A biosensor includes a natural receptor linked to an electronic transducer in such a way that biochemical activity converted into electrical activity [6]. The electronic component of the biosensor measures voltage (potentiometric), current (amperometric), light, sound, temperature, or mass (piezoelectric) [6].

The motivation of the thrombin FET biosensor came from this paper recently published, which uses functionalized nanotubes as a detection scheme. The ability to precisely control the size, shape and surface functionality of organic/inorganic materials is a necessity for contemporary developments in the fields of catalysts, batteries, electronic devices, drug delivery systems, and so forth. Recently, nanometer-sized particles with controlled shapes have aroused burgeoning interest because of the beneficial properties as a result of their high surface area and small dimensions. Over the last decade, in particular, numerous studies on the synthesis and physical properties of carbon nanotubes (CNTs) have been carried out and, in turn, have opened new avenues for advanced device applications. One notable example is the CNT-based biological sensing system [10]. A schematic representation of a nanotube sensor and corresponding sensitivity plot is shown in fig. 10.

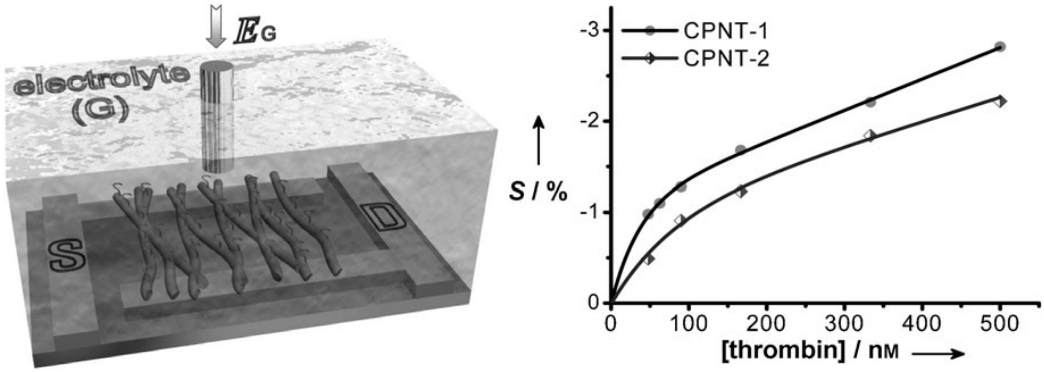

FIG. 10. SCHEMATIC REPRESENTATION OF CPPY NANOTUBE SENSOR AND GRAPH SHOWING SENSITIVITY OF THE SENSOR [10]

CPPy nanotubes with controlled chemical functionalities covalently immobilized onto the microelectrode substrate for high-quality electrical contact between polymer transducers and metal electrodes. Consecutively, thrombin aptamers readily tethered onto the nanotubes by covalent linkages with- out sophisticated surface treatment. Thus, this fabrication approach might present an efficient route for the construction of sensor platforms that based on nanoscale polymer transducers conjugated with molecular recognition elements. The FET-type sensors based on A CPPy nanotubes constructed by using liquid-ion gating. The recognition ability of thrombin aptamers, combined with the inherent charge transport property of CPPy nanotubes yielded a direct and label-free electrical readout [10].

Following research presents a novel silicon field effect transistor-based electrochemical sensor tailored to sense the protein thrombin. This novel device structure employs site-specific self-assembled silicon oxide-cladded silicon quantum dots (SiOx-Si QDs) as the gate material, replacing the conventional metal gate electrode. A functionalization process of QDs on the field effect transistor gate region proposed [11], where a single-stranded DNA (ssDNA) thrombin aptamer is covalently

attached to the QD surface, which specifically binds to thrombin as shown in figure 11. The sensing operation of the QD-gated FET operates by increasing the threshold voltage. This increase in threshold voltage was experimentally observed, in addition to the corresponding decrease of the MOSFETs drain current [11].

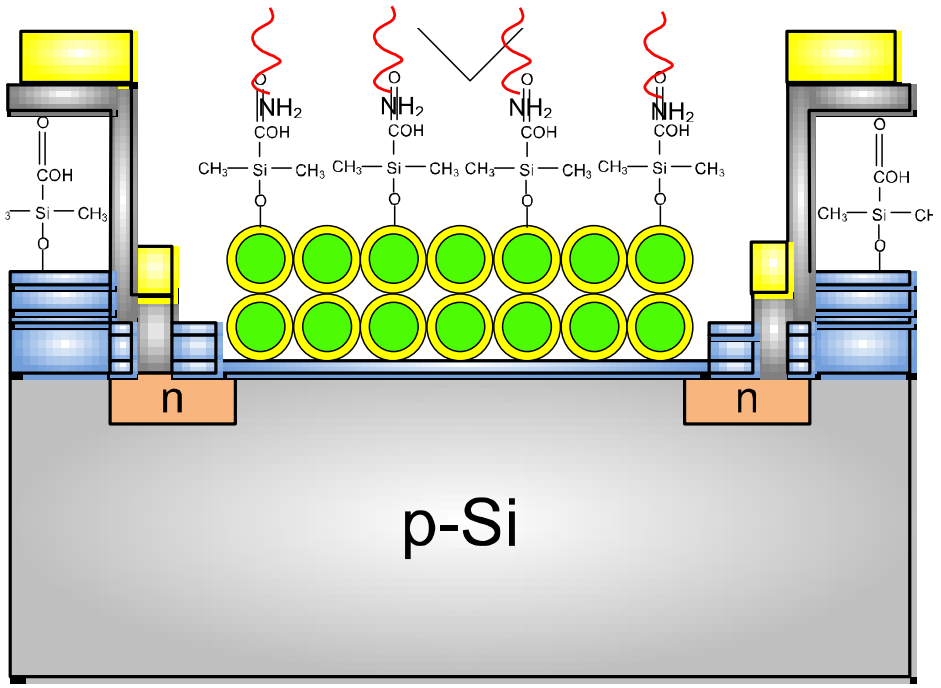

FIG. 11. QUANTUM DOT GATE FIELD EFFECT TRANSISTOR THROMBIN BIOSENSOR [11]

Figure 12 below shows the ID-VG and ID-VD transfer characteristics, respectively, of the fabricated transistor having a W/L ratio of 10µm / 26µm. As evident from the features, the substitution of the conventional metal gate electrode with site-specific self-assembled SiOx-cladded Si quantum dots atop a 40Å thermally grown gate insulator demonstrates the feasibility of configuring a QD gate FET as a biosensing device.

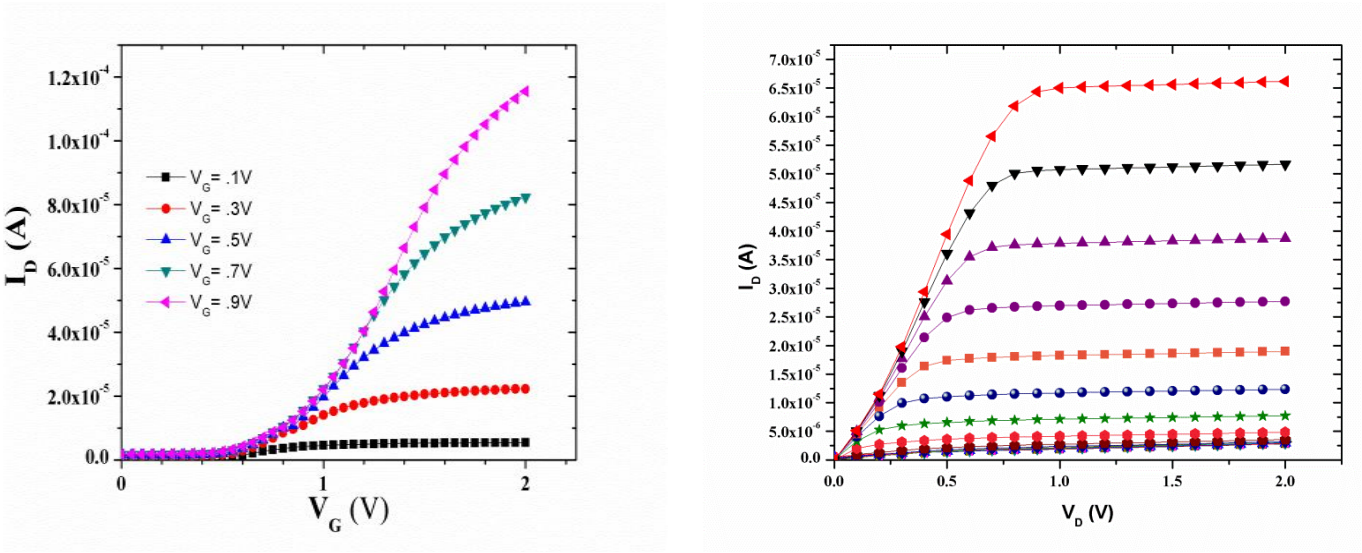

FIG. 12. ID-VG AND ID-VD TRANSFER CHARACTERISTICS OF THE LIQUID TOP-GATED QUANTUM DOT FET [11].

As evident from Figure 13, there is a definite increase in threshold voltage as higher Thrombin concentration is present at the gate, according to the threshold voltage equation for an NMOS transistor.

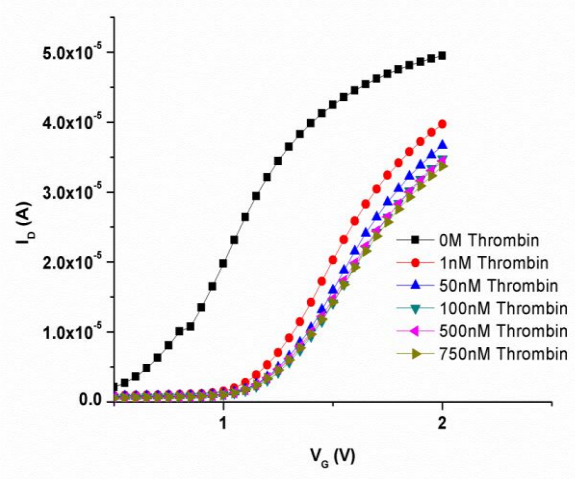

FIG. 13. ID-VG TRANSFER CHARACTERISTICS OF THE SSDNA THROMBIN APTAMER FUNCTIONALIZED QDG GET WITH ADDITIONS OF THROMBIN PROTEIN [11]

X. CONCLUSION

Microfluidics offers revolutionary new capabilities for the future of DNA sensing. The manipulation of small volumes of fluid with precise dynamic control over concentrations provides the key to advancement. The paper gives a detailed insight into the world of novel biosensors and it's feasibility. It also introduces the audience to the importance of microfluidics and its application in DNA sensing technologies. Commercially available devices for DNA separation and analysis are explored. Nanotechnology enables development of vast types of sensors to analyze metabolites that in turn drives the diagnostic methods in medicine and research.

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