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Original Research

Field-Effect Devices (FEDs): Detection of Charged Macromolecules, Direct Electrostatic DNA Detection and Microfluidic Modules

Mohammed Salah Mahdi Al-Hussaini[1] | Mohammed Baqer Sabah Mohammed AL Shalwah[2] | Ghufran Firas Majeed Al-Sheikhly[3]

[1,2] Hillah University College, Medical Devices Technology Engineering, Iraq

[3]Middle Technical University/Electrical Engineering Technical College, Department of Medical Devices Engineering Technology, Iraq

Abstract:

In order to provide an accurate diagnosis of many different medical issues, sensitive and precise detection of DNA biomarkers is essential. The requirement to apply an extra labelling step to the device frequently complicates the adoption of such biosensing components in integrated microfluidic devices. We highlighted recent developments in microfluidic integrated devices as a means of presenting label-free DNA biosensor technology in this review. The literature began with examples of basic biosensing methods implemented in flow-cell structures, then moved on to more complex microfluidic devices, and finally, instances of higher integration. The use of nanopore technology to sequence complete genomes on microfluidic chips was emphasised, as were recent advances in the commercialisation of label-free DNA detection devices on microfluidic chips. The technical viability of future high-throughput and high-specification portable systems is clearly demonstrated by multiplexed DNA amplification modules, droplet-based microfluidics, and nanopore-assisted sequencing. At present, nanopore technology allows for point-of-need DNA sequencing with a throughput of 10–20 G bases per 48 hours. While this kind of sequencing throughput is certainly remarkable, there are many diagnostic uses for which whole genome sequencing is superfluous. For both Point-of-Need applications in centralised healthcare systems and low-resource settings, microfluidic technologies that provide sample-in-answer-out operations with a few minute time to result may be more realistic in real-life practice. Several commercial initiatives are now showing signs of imminently launching sample-in-answer-out systems with targeted medical diagnostic applications, building on more established technologies in microfluidic DNA systems. Integrating very sensitive biosensors into portable devices, or even systems that do not require any equipment, at a reasonable cost is a significant obstacle that needs to be addressed. Due to the fact that different diagnostic companies are following different strategies, there is currently no commercially available method for

mass-producing a product that seamlessly integrates all of the necessary components. In addition to the high power demands of heating on modules that incorporate DNA amplification, the use of sensing technologies that necessitate complex or costly equipment can provide challenges in terms of portability. There is a pressing need to create assays and devices that enable multiplexed detection since most real-world applications require the simultaneous quantification of multiple DNA strands. In conclusion, it is essential to conduct early device development using real physiological samples in order to guarantee device compliance with biomedical device standards and worldwide regulatory frameworks. Finding out the findings with DNA-based FEDs and developing associated correct theoretical models requires further experiments. Here, novel transducer techniques and technologies may hold great promise for ensuring repeatable and trustworthy biosensor signals. The authors have recently presented a method that accounts for this: instead of using "conventional" field-effect-based DNA sensors, their method uses the redistribution of ion concentration within the intermolecular spaces, which is caused by DNA hybridization, as a detection mechanism. Preliminary studies and theoretical estimates with synthetic ssDNA and polyelectrolyte multilayers have the potential to provide a large sensor signal in the tens of millivolts range. Additionally, the novel biosensor idea can function in both high- and low-ionic-strength solutions. Scientists from several fields, including bio-and electrochemistry, biophysics, device engineering, and analytics, should collaborate to address the complex and multidisciplinary problem of field-effect-based DNA biosensors.

Keywords: Field-Effect Devices (FEDs), Macromolecules, Electrostatic DNA, Microfluidic Modules

Introduction:

Environmental monitoring, biomedicine, biotechnology, the food and pharmaceutical sector, process technology [1,2], security, antibioterrorism, and many more fields find their applications in the fascinating and intriguing field of (bio-)chemical sensor research. Semiconductor field-effect devices (FEDs) like ion-sensitive field-effect transistors (ISFETs), capacitive electrolyteinsulator-semiconductor structures (EISs), and light-addressable potentiometric sensors (LABSs) are now an integral part of a new wave of electronic chips that can directly measure chemicals and biological variables. Although there has been much variation in the design of these sensors across sensitivity materials, fabrication techniques, and sensor topologies [3,4], they all share the basic principle of applying an electric field to a semiconductor substrate in order to produce regions of excess charge. Using the interface potential between the analyte (test sample) and the sensor chip, FEDs allow for the external modification of the threshold voltage (Vth) or flat-band voltage (Vfb). This causes a change in the electric field within the FED's insulator, which in turn modifies the space-charge region in the semiconductor at the silicon/insulator interface (for example, altering the EIS structure's capacitance or the ISFET's inversion channel's conductance) [5,6]. A FED for (bio-)chemical sensing will typically have the same fundamental construction as the electrolyte-insulator-semiconductor system illustrated schematically in. A FED can trace its origins back to an insulated-gate field-effect transistor (IGFET) or a metal-insulator-semiconductor (MIS) capacitor, with the gate electrode substituted with an analyte and a reference electrode. FEDs are extremely sensitive to any form of electrical interaction at or near the gate insulator/electrolyte interface since they are essentially surface-charge measuring devices (they measure the charge in a capacitive way). Hence, an ISFET, capacitive EIS sensor [7,8], or LAPS may monitor almost any (bio-)chemical process that causes chemical or electrical changes at this

contact. This can only be achieved by functionally coupling appropriate chemical or biological recognition elements to the sensitive surface of the corresponding FED. The current in the ISFET's channel, the capacitance of the EIS sensor, or the photocurrent of the LAPS can be modulated as a result of changes in the chemical composition, which in turn affect the surface charge of the gate insulator and the potential drop at the electrolyte/insulator interface. In a wide variety of contexts, including those involving enzymatic reactions, pH, ion concentrations, cellular metabolisms, action potentials of live cells, etc., these devices have shown to be invaluable. The potential use of FEDs in the detection of charged macromolecules [9,10], such as DNA, proteins, and polyelectrolytes, has only now come to light. Read on for a more in-depth look at the workings of various ISFET, EIS [11,12], and LAPS kinds, as well as the principles of operation. There are numerous applications that greatly benefit from the ability to detect molecular interactions at the solid-liquid interface. These applications include biomedical implants [13,14], drug-carrier systems, biosensors, DNA arrays, and protein-chip technologies, among many others. Important physiological processes can be better understood with a thorough knowledge of the adsorption and binding of charged macromolecules onto charged surfaces, which is crucial for sensor applications as well. Genomic diagnostics is expected to be in high demand in the fields of genetics [15,16], medicine, and drug discovery, and molecular analysis of nucleic acids can detect over 400 disorders [17,18]. The standard operating procedure for DNA detection is a DNA hybridization event, in which a molecule of complementary DNA (cDNA) is used to identify an unknown strand of single-stranded DNA (ssDNA). A dsDNA helix structure with two complimentary strands is generated as a consequence of the hybridization event: The binding reactions of the base pairs (adenine-thymine, cytosine guanine) are very specific and effective, allowing the hybridization event to proceed even when other non-complementary nucleic acids are present. If the bases on the probe and the corresponding target DNA molecules are identical [19,20], the hybridization reaction will take place more effectively. In order to reliably identify even a single nucleotide polymorphism, geno-sensors and DNA chips need have a sensitivity high enough. Labelling DNA molecules (either the analyte DNA or the immobilised ssDNA) with different markers (radiochemical, enzymatic, fluorescent, redox, etc.) is necessary for the readout of the DNA-hybridization event in the methods actually used for DNA-hybridization detection. The low detection limits, high selectivity, and lengthy, costly, and complicated implementation processes are drawbacks of all these methods. Because of this, a label-free detection would be ideal. Bypassing the challenges of labelling, bio-functionalized semiconductor devices might directly detect the intrinsic charges of biomolecules electrically [21,22]. Consequently, various research groups have recently invested a lot of time and energy into developing a field-effect platform that can detect charged biomolecules (e.g., DNA, proteins, and peptides) electronically based on their intrinsic molecular charge. Additionally, experimental evidence has been presented for the potential of using a genetic field-effect transistor for potentiometric detection of single nucleotide polymorthisms. These devices present a new challenge for DNA chips with direct electrical readout that can analyse nucleic acid samples in real-time without labels [23,24]. It is fast, simple, inexpensive, and compatible with sophisticated micro- and nano-fabrication processes [25,26]. The following factors determine the variation in DNA hybridization detection results obtained with FEDs: ● the type of FED setup (capacitive EIS and MIS structure, depletion-/enhancement-mode FET, floating-gate FET with Au or Pt, extending-gate FET, FET devices with or without reference electrode, poly-Si and hydrogenated amorphous Si (a-Si:H) thin film transistor), ● the variety of gate-insulator materials (SiO2, silanized SiO2, SiO2-Si3N4, SiO2-Ta2O5, SiO2-poly-L-lysine) with varying thicknesses (ranging from 2 nm to 100 nm), and ● the method of detection itself [27,28].

Measurement of Capacitance and Voltage

The field-effect transducer is used to immobilise well-defined sequences of single-stranded DNA (ssDNA) as a biological recognition layer. This layer should convert the specific recognition process between the two complementary DNA strands into a measurable signal, in this case changes in the capacitance of the functionalized EIS structure. This is how bare DNA is obtained. In order to get the EIS sensor to work, a reference electrode is used to apply a DC polarisation voltage (VG), and a modest AC voltage (V 10-50 mV) is used to measure the capacitance of the system. A bare EIS hetero-system's full AC equivalent circuit is intricate and incorporates many different components, such as the semiconductor's bulk resistance and space-charge capacitance, the gate insulator's capacitance, the electrolyte-insulator interface's double-layer capacitance, the bulk-electrolyte solution's resistance, the reference electrode's impedance, and the semiconductor, gate insulator, electrolyte, and reference electrode, respectively. Yet, the EIS structure's equivalent circuit works for the typical values of insulator thickness (30-100 nm), electrolyte solution ionic strength (104 M), and measurement frequencies (below 1 kHz). One way to simplify it is to think of it as a series connection of three variables: the insulator capacitance, Ci εi /d (where εi and d are the permittivity and thickness of the insulator, respectively), and the space-charge capacitance of the semiconductor, Csc. Ci and Csc are dependent on the voltage applied to the system and the electrolyte-gate insulator interfacial potential. The electrochemical double-layer capacitance is often ignored because it is believed to be significantly larger than Ci and Csc. Capacitances C, Ci, and Csc are typically defined per cm2 of surface area, and the equation for the total capacitance of the bare EIS structure, C, is thus comparable to that of a MIS capacitance, with the addition of the possibility of modulating the space-charge capacitance through the electrolyte solution-insulator interface potential.

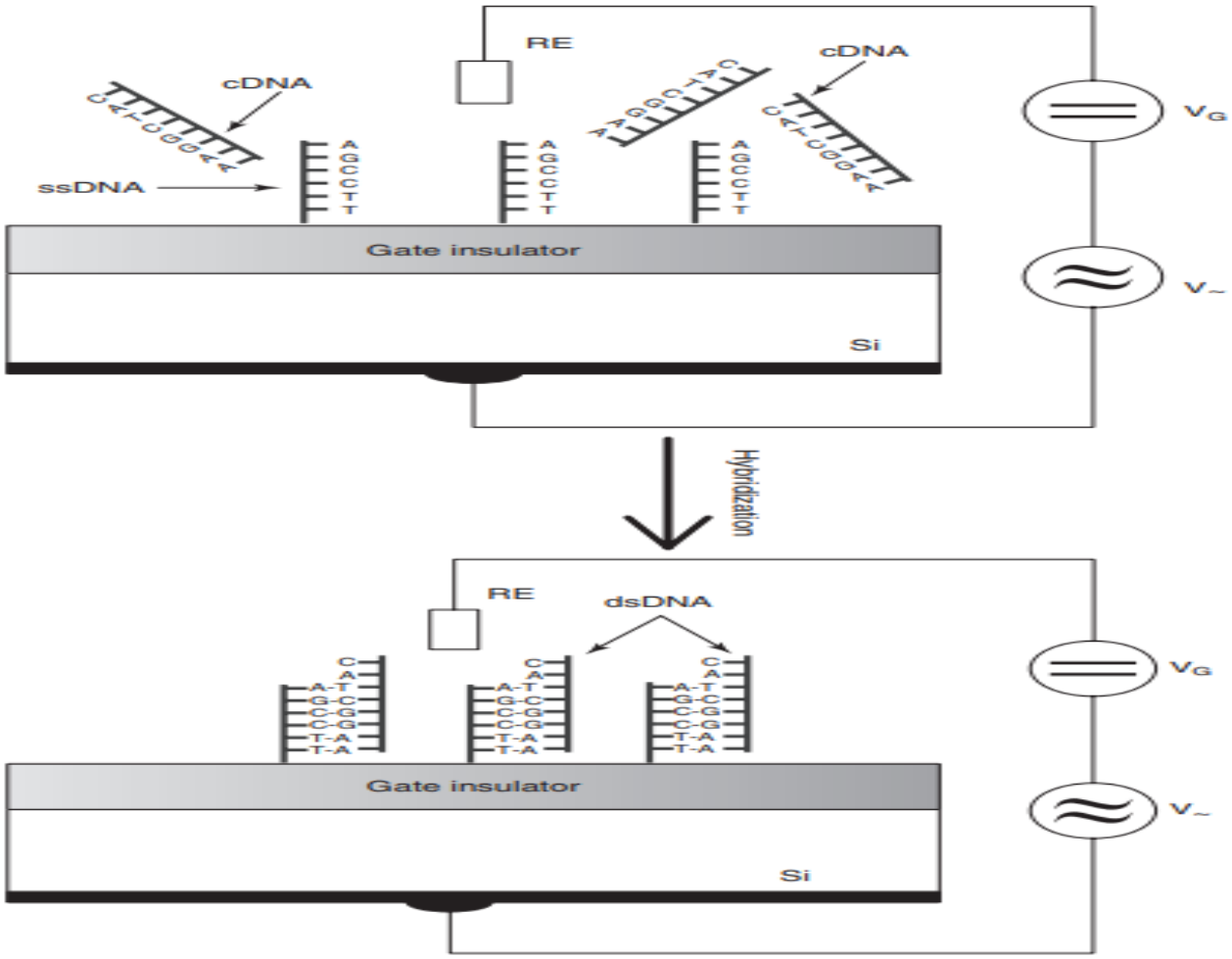

Figure 1. Diagram depicting the capacitive EIS architecture of a DNA sensor. Using the reference electrode (RE), a DC polarisation voltage (VG) is applied to set the EIS sensor's operating point, and a tiny AC voltage (V) is applied to measure the capacitance of the system. DNA can be either single-stranded (ssDNA), complementary (cDNA), or double-stranded (dsDNA).

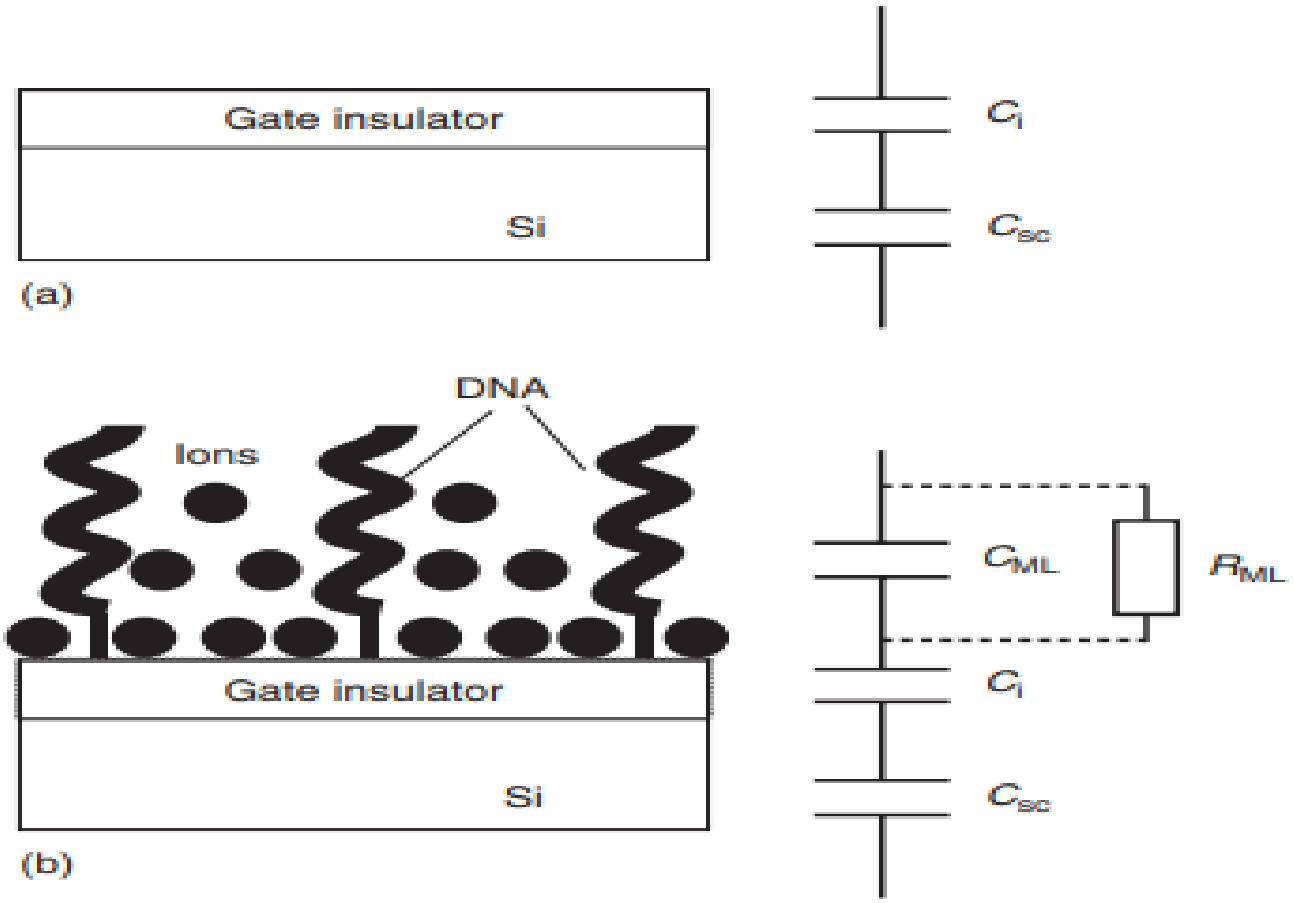

Figure 2. An EIS biosensor that has been functionalized with charged macromolecules (b) and an original, unaltered EIS structure (a) are shown in a simplified equivalent circuit. Csc represents the capacitance of the gate insulator, CML stands for the capacitance of the molecular layer, and Ci represents the capacitance of the space-charge area in the semiconductor.

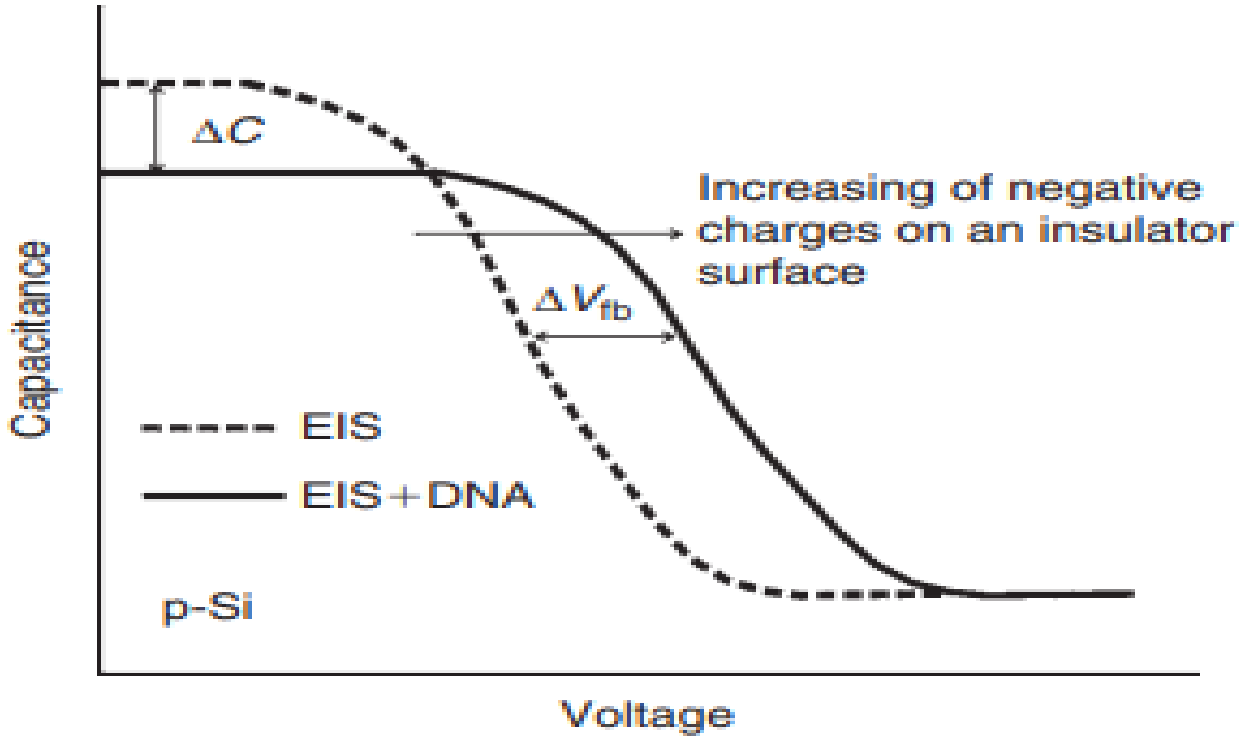

Figure 3. A naked EIS sensor and an EIS sensor coated with a molecular layer (in this case, DNA) show different capacitance-voltage (C-V) curves. The inclusion of the extra molecular layer changes the capacitance (ΔC) and voltage (ΔVfb) axes of the original EIS structure's C-V curve.

C-V curve, but with the voltage polarity inverted (cumulation at positive voltage and inversion at negative voltage). The total EIS structural capacitance, Ci Csc, is equal to the system's maximum capacitance and is defined by the geometrical capacitance of the insulator, C Ci. The more practical range, however, corresponds to the C-V curve's depletion zone when applied to sensors. Various electrochemical and solid-state characteristics are included in the EIS structure's equation for the flat-band voltage Vfb, which affects where the C-V curve lies along the voltage axis. the capacitance of the brush (i.e. the diffuse layer capacitance inside the DNA layer at the underlying layer-brush interface) depends on several factors, including the amount of DNA covered, the interfacial potential, and the significantly higher ionic strength inside the brush compared to the bulk solution. This is especially true in the case of DNA brushes, which are polyelectrolyte brushes made up of charged macromolecules densely end-grafted to the surface. In comparison to the original, unaltered EIS structure [29,30], the functionalized EIS structure's C-V curve is moved along the voltage axis. The change in C-V is perpendicular to the voltage axis in. means that the surface of the gate-insulator is further negatively charged. Based on this, it can be inferred that the molecular layer can cause a change in the flat-band voltage ΔVfb on either the electrolyte side or the gate-insulator side, in addition to the gate voltage VG that is applied. In comparison to static DC measurements using the transistor structure, C-V measurements are more intriguing and instructive since the curve shifts along the capacitance and voltage axes at the same time [31,32]. If the effects of adsorption or binding of charged macromolecules are completely separate, then a single measurement can be used to determine the change in capacitance and the change in flat-band voltage. If charges are present in the gate insulator, surface, or interface states, it is important to keep this in mind. Concerning the fl at-band voltage, variations in the fl at-band voltage can be brought about by the series capacitance, which happens to coincide with the impact of modulation of the fl at-band voltage generated by the molecule charge. In what follows, we'll go over the history of potential ways that charged macromolecules can alter the fl atband voltage [33,34], with an emphasis on two main ideas: one that uses the charge redistribution within the intermolecular spaces caused by DNA hybridization, and another that relies on direct electrostatic detection of charged macromolecules by their intrinsic molecular charge.

The Use of DNA's Intrinsic Molecular Charge for Direct Electrostatic Detection

For the most part, FEDs can detect the charge of adsorbed macromolecules like DNA or the charge change caused by a hybridization event because they are capacitive surface-charge measuring devices. Among other factors, the electric field in the gate insulator is affected by the nett surface charge at the interface between the electrolyte and the insulator. Whenever there is a change in charge at the surface of an insulator, the charge density in the space-charge region of a semiconductor will change by an equal and opposite sign. As a result of the negative charge on the phosphate backbone of DNA molecules, it is anticipated that when single-stranded DNA (ssDNA) molecules hybridise with their complementary strands (cDNA) [35,36], the charge on the target molecule alters the gate's applied charge, which in turn affects the EIS sensor's at-band voltage and capacitance, the FED's threshold voltage and drain current. It is well-known from Schenck's immuno-modified FETs from 1978 that all reported results on DNA-FEDs based on the mechanism of a direct electrostatic detection by their intrinsic molecular charge have some principal limitations owing to the so-called counter-ion screening effect. The feasibility of using FETs to detect antibody-antigen affinity binding reactions has been the subject of heated controversy. Following these talks, everyone agreed that testing protein charges with the electrolyte solution's tiny inorganic counter-ions would produce layers that are almost completely uncharged under the microscope, making it impossible to assess immune-species using FETs [37,38]. In a perfect world, with immobilised immunological binding sites at a really capacitive interface, virtually full antibody coverage, strongly charged antigens, and extremely low ionic strength, the predicted signal strength would be 10 mV or lower. The proportion of DNA charge that stays in the double layer and is reflected in the space-charge region of the FED decreases as the ionic strength of the electrolyte increases. As an illustration, in physiological conditions with a λD of 0.8 nm, the majority of the DNA charge will be located at a distance greater than the Debye length from the surface, rendering its detection extremely challenging, if

not impossible. Additional linker molecules or spacers extending from the insulating layer of the FED can further immobilise ssDNA molecules, thus reducing the DNA hybridization-induced charge shifts. Alternatively, a stronger hybridization signal should be anticipated in the event that the DNA molecules are positioned more or subsurface. Another big hurdle in detecting DNA hybridization is the screening of the charge associated with the probe-or target-DNA molecules by tiny counter-ions (cations) in the solution. The counter-ion condensation theory put forth by Manning states that DNA charges are reduced by 76% and 88% by monovalent cations and divalent cations, respectively; the residual charge is compensated for by the more diffuse ionic layer. As a result, effective measurements are hindered, particularly in solutions with high ionic strengths, by the counter-ion condensation effect, which masks or reduces the expected hybridization signal. An expected consequence of increasing the electrolyte solution's salt concentration is a larger screening of the molecular charges, leading to a smaller sensor signal. During the worst possible situation. for the hybridised pair (cDNA-ssDNA) to result in a net-reduced or even zero charge, even if the charged target molecule has attached to the immobilised probe molecule. Hence, the basic field-effect transducer can't provide a detectable signal to the sensor. A first approximation to the sensor signal induced by DNA hybridization can be made by modelling the probe-target hybridization as a charge transfer from the test sample to the gate insulator surface. This will allow us to estimate the signal induced by the hybridization process. Given the need for electro-neutrality in the system, it is necessary to introduce an equal amount of the opposite charge into the system through the double layer or the inversion layer of the FET. As an example, a field-effect transistor (FET) will only reflect around 1.7% of the hybridization-induced charge, according to the typical values of the double-layer capacitance (Cdl) and the gate-insulator capacitance (Ci), which are 20 μF cm2 and 0.35 μF cm2, respectively, for a 10 nm thick SiO2 layer. In the solution, ions will balance out the residual charge. The anticipated sensor signal, which may undergo modifications due to hybridization, might be described as. The variables δ (hybridization efficiency), θ (fraction of DNA charge compensated by condensed cations), N (density of the immobilised probe molecules), b (distance between the nearest unit charges along the DNA, standard deviation of one per base pair), and θ (a neutral molecule is represented by θ = 1). Surface potential variations of several millivolts are predicted by the model calculations for an Al2O3-gate FED with charged macromolecules when the charge density of molecules is doubled following hybridization. Contrarily, FEDs featuring an uncharged gate-insulator surface are anticipated to exhibit bigger signals, measuring several tens of mV, according to the identical model. The sensitivity of the sensor can be improved by operating FEDs in solutions with extremely low ionic strength and by using a large density of immobilised probe molecules (N 1012 molecules/cm2) in order to decrease the counter-ion screening effect. However, in these instances, the likelihood of hybridization is lower, which means that the hybridization process will take longer and the sensor signals will be weaker. Herein lies the theoretical underpinning of the "large" sensor signals that have been occasionally seen in experiments. continues to be uncertain. Additionally, in order for FEDs to detect DNA by its intrinsic charge, the surface interaction must only take place between the immobilised ssDNA and its matching cDNA [39,40]. It is expected that the underlying gate surface of the FED will not be affected by any background interaction of tiny ions. The ideal condition for the immobilised ssDNA molecules to "insulate" the underlying gate insulator from the solution is for them to create a monolayer that is both densely packed and completely homogeneous, with no pores or interstitial spaces. On the other hand, the estimated values for the theoretical maximum surface coverage of 25-30% for rod-like molecules undergoing random-sequential adsorption are at odds with this demand. The notion that the molecular layers are substantially less dense enables small inorganic ions and water molecules to penetrate the interstitial space and reach the underlying layer is supported by several experimental findings. In conclusion, it appears that there are obstacles to the practical implementation of field-effect detectors (FEDs) for the pure electrostatic detection of charged macromolecules based on their intrinsic molecular charge, particularly in solutions with a high ionic strength, such physiological circumstances. In addition to the "disturbing" aspects already mentioned, there may be one more that is undesirable. When DNA molecules connect to the reference electrode in an unintended way, it might

dampen or even obscure the biosensor signal that was anticipated. Additionally, sensor drift and leakage currents can potentially produce or significantly alter a distorted sensor signal. Using differential measuring setups is always a good idea. in order to neutralise or at least reduce the impact of some of these disruptive factors. However, in order to comprehend and appropriately interpret the DNA-detection investigations conducted with FEDs, a great deal of experimental and theoretical research is required.

A Revolutionary Approach to Electrical DNA Detection Without Labels

The redistribution of ions in the intermolecular spaces of the immobilised ssDNA (caused by the DNA-hybridization process) and the modification of ion sensitivity have recently been suggested as a novel method for direct label-free DNA detection with FEDs [41,42]. The ion-sensitive FED's top surface is altered in this method by arranging immobilised ssDNA probe molecules in a normal to surface orientation with an average interprobe spacing of as measured center-to-center. Interposed with the immobilised molecules is the residual surface of the ion-sensitive layer that comes into touch with the electrolyte solution. In a model similar to a DNA brush? Between the DNA and the external electrolyte, the mobile ions move freely. For optimal hybridization effectiveness and speed, the probe ssDNA molecules should be spread out across the surface with plenty of interstitial space in between. As a consequence, the concentration of cations may rise and the concentration of anion fall within the intermolecular gaps, leading to a redistribution of ions that differs significantly from the bulk electrolyte concentration, n0. Due to the roughly doubling of the dsDNA's charge during hybridization, a new distribution of ions and electrostatic potential inside the intermolecular gaps will be achieved. Hybridization causes a redistribution of ions (including proton concentration) in the intermolecular gaps of the DNA layer, which the underlying ion-sensitive FED can detect. Here, the counter-ion condensation effect is utilised to detect the DNA-hybridization event, in contrast to the FEDs discussed earlier for DNA-hybridization detection using the intrinsic molecular charge, where the screening of the molecular charge by small counter-ions is seen as a major obstacle. More importantly for sensor applications, the hybridization event's impact on the average ion concentration in the intermolecular gaps is an intriguing parameter to measure. The theoretical calculation model for the average cation concentration. anions in the gaps between molecules are depicted. A hexagonal lattice with a cell radius of Rs has been modelled using ssDNA and dsDNA as evenly negatively charged, infinitely long cylinders with radii of 0.5 nm and 1 nm, respectively. To simulate the impact of DNA hybridization, we raise the cylinder's radius from 0.5 nm to 1 nm and double its linear charge density. The model disregards the sensor surface's charge state for simplicity's sake. Anion and cation average concentration in intermolecular gaps can be determined for the model given above by applying the equations found in. The cations and anions are represented by b 0.34 nm and b 0.17 nm, respectively, along the cylinder of ssDNA and dsDNA, and an rss and a rds, respectively, are related to the charges. The electrostatic potential equations from, which linearize the Poisson-Boltzmann equation near the Donnan potential in the hexagonal DNA cell, were used to get the formulas and [43,44].

We used θ 0.8 as the fraction of ssDNA and dsDNA that were charged-compensated by the condensed cations. ● The concentration of cations and anions in the intermolecular spaces is significantly different from the bulk solution, and ● the average ion concentration in these spaces is also different after hybridization compared to before; this effect is more pronounced in electrolytes with low ionic strength and depends on the ionic strength of the bulk solution. When compared to DNA detection by intrinsic molecular charge, it is possible to generate a detectable sensor signal even in high ionic-strength solutions (0.5 M), which allow for rapid hybridization events and high hybridization efficiencies. A number of variables will affect the final biosensor signal: High hybridization signals at low densities of immobilised ssDNA necessitate solutions with lower ionic strength, a smaller nds/nss ratio is produced by a decrease in immobilised ssDNA density as RS increases (this effect is particularly pronounced in solutions with high ionic strength), and • While there is a dramatic rise in nds/nss at close proximity (due to the dense immobilised ssDNA), the amount of sensor area accessible for ion interaction falls. To generate the best hybridization signal [45,46], it is usually necessary to determine the ideal separation distance or density of the immobilised ssDNA;

however, this varies depending on the sensor design and operating conditions. In a differential measurement setup, combining a cation- and anion-sensitive FED can double the hybridization signal. All things considered, the ssDNA is shown in the aforementioned model as a rod-shaped molecule with an orientation perpendicular to the surface. More accurately, ssDNA is a molecule that resembles a flexible coil. When those molecules with a coil shape are arranged in a specific way on the FED's ion-sensitive gate, they can partially obstruct the surface sites that are used for ion-binding or ion-exchange activities and also make it harder for ions that need to be determined to reach the transducer surface. A hard rod-like dsDNA, on the other hand, is generated following the hybridization event. A new sensor signal is generated as the FED's ion sensitivity is altered by hybridization, which occurs when the surface of the FED is opened for ion contact.

Devices for Microfluidic DNA Detection Without Labels The initial goal of healthcare democratisation, which was formulated in the early 1990s, is now more within reach than ever before, thanks to the fast technology advancements in biosensors and microfluidics over the last couple of decades. Various biomarkers of clinical importance can now be detected, either qualitatively or quantitatively, using a wide range of biosensing ideas and microfluidic architectures. Researchers have been putting a lot of time and energy into developing sensitive and specific methods for detecting DNA biomarkers since they are being linked to a wide variety of clinical disorders. Current research focuses on microfluidic structure integration for sample pre-processing and automated reagent handling, with the end objective of developing small footprint [47,48], user-friendly, and inexpensive DNA diagnostic microsystems. To achieve the goal of durable and reliable diagnostic microsystems, it is essential that device functionality be as simple as possible. One way to achieve this is to simplify the biosensing assay by doing away with the requirement for any extra labelling phase. This would be a huge step forward in developing assays that are ready for widespread deployment [49,50]. Thus, the purpose of this review is to showcase the most up-to-date innovations in label-free DNA biosensors, with an emphasis on their incorporation into microfluidic systems. From the most basic method of flow-cell integration to the most complicated, we categorised the microfluidic devices according to the integrated microfluidic structure's complexity. We used the biosensing principle to subclassify them because there were so many intriguing flow-cell integrated architectures. Advanced microfluidic architectures for label-free DNA biosensing systems, higher integration illustrative cases, and cutting-edge nanofluidic DNA sequencers were subsequently covered. Finally, we cover the ongoing commercialisation initiatives in the industry. It should be mentioned that label-free detection, according to our definition, refers to methods that do not involve attaching any extra molecule to the target DNA sequence. As a result, methods that use diluted reagents in solutions for measurement still fall under this category.

Methods for Flow-Cell Biosensing

A remarkable leap from macro to micro to nanoscale has occurred in label-free biosensing during the previous 20 years. The incorporation of microfluidics was crucial to the successful miniaturisation of sensing technology. Still, biosensing has a ways to go before it can be considered commercially viable for use in bedside diagnostics. Optical, electrochemical, and mass-based sensing principles are utilised in technologies that enable label-free detection of DNA hybridization through the use of microfluidic devices to transport the analytes.

Optical here has been a lot of focus on optical methods in biosensing as a potential analytical tool for label-free DNA detection. Optical biosensing devices have many benefits, such as being naturally resistant to electromagnetic interference, having low micro and nanoscale limits of detection (LOD), and being able to detect numerous analytes on a single Lab-on-Chip (LOC) device. The best-known optical method for label-free, real-time binding kinetics and affinity characteriszation is surface plasmon resonance (SPR). It entails measuring the incident light-induced resonant oscillation of conduction electrons at the interface of materials with different permittivity levels. In 1983, Liedberg et al. demonstrated the first surface plasmon resonance (SPR) biosensing device, which solidified SPR as a significant optical technique for bio-recognition molecule detection. In the past ten years, researchers have poured a lot of time and energy into studying this widely used laboratory technique and creating several SPR devices to detect DNA hybridization. With this technique, a very sensitive limit of detection

(LOD) of 3.2 fM was attained with flow injection rates of 10 μL min−1. The immunochemical detection of DNA methylation was documented in 2015 by Kurita et al., who described the construction of a sequence-specific microfluidic device based on surface plasmon resonance (SPR) [51,52]. A group has reported using an affinity measurement within a single-base bulge area that involves a target (methyl)-cytosine. To hybridise with a biotinylated bulge-inducing DNA probe on a gold surface, the target was first passed through a microfluidic channel that contained an anti-methylcytosine antibody. They were able to distinguish between the methylation status of individual cytosines in genomic λ DNA and HCT116 human colon cancer cells, and the results demonstrated a notably high DNA methylation assessment (6 amol or 48 fg). Another strategy that has gained traction is localised surface plasmon resonance (LSPR), which uses the localized-field phenomena to its advantage by allowing for less background interference in its measurements. Because LSPR does not excite propagating plasmons using a prism or grating coupler [53,54], its instrumentation is simpler than that of SPR. Because of how simple the equipment is, LSPR sensors may be made smaller and more portable, and they are also very inexpensive. Huang et al. (2012) conducted research that used an on-chip microfluidic device to detect label-free DNA in real-time by taking advantage of the unique LSPR capabilities of gold nanorings. The sensor's surface was immobilised with single-stranded DNA (ssDNA) probes. Next, the device's waste reservoirs were traversed [55,56], and the complementary target DNA was thereafter expelled from the device via two outputs. It had previously been introduced into a sensing chamber for LSPR analysis. The use of gold nanorings in this way resulted in a 3.1 nm shift following DNA hybridization and an 8.2 nm shift upon DNA probe immobilisation. So, a label-free nanosensor method and a microfluidic platform are successfully coupled. Another possibility for label-free DNA detection is the use of interferometric techniques like bimodal [57,58], Mach-Zehnder, and micro ring resonators. The suggested microcavity sensor's interferometric spectra were very sensitive to changes in the ambient refractive index (RI) and might find use in a wide variety of biological contexts. Lab-on-chip SERS (LoC-SERS) and nano/micro/optofluidic SERS are two terms that describe microfluidic platforms that are based on surface-enhanced Raman spectroscopy (SERS) [59,60]. The remarkable sensitivity of microfluidic SERS analysis to a wide variety of targets across a wide concentration range has led to an increasing number of DNA biosensing applications that take advantage of samples with volumes as small as nL. To detect DNA hybridization with single-molecule sensitivity, Qi et al. introduced a microfluidic SERS device. The present endeavour is to enhance the SERS substrate's characteristics using nanotechnology in order to achieve targeted multiplex detection of tiny oligonucleotides [61,62].

Powerful electrochemical

Electrochemical biosensors have recently risen to prominence as a prominent and extensively researched method for biological sensing. This is because electrochemical technologies are very appealing for the creation of point-of-care diagnostic devices since they have low detection levels, minimal running costs, and simple apparatus that allows for easy miniaturisation. A field-effect transistor (FET) that can be activated by (bio)molecule binding is called a bio-FET. Due to their ability to act as an intrinsic amplifier, devices based on field-effect transistors have garnered a lot of interest. This is because, without the need for further circuitry, these devices can convert small changes in surface potential to massive increases in current. When biomolecules attach to the FET dielectric or gate electrode [63,64], it alters the charge distribution of the underlying semiconductor materials, which in turn modifies the conductance. This dielectric is called the FET gate dielectric. Additionally, Bio-FET is ideal for point-of-site detection applications like medical diagnostics, biological research, environmental monitoring, and food analysis due to its small size, low weight, compatibility with commercial planar processes for large-scale circuitry, and potential integration into microfluidic devices for Lab-on-Chip. It is also suitable for applications where labelling is not necessary. On a printed circuit board, Xu et al. demonstrated a microfluidic integrated label-free DNA FET. Graphene was produced by chemical vapour deposition (CVD) and utilised for two purposes per graphene site in an 8-channel field-

effect transistor (FET) DNA sensor array. The first is as a field-effect transistor (FET) for sensing, and the second is as an electrophoretic electrode for site-specific DNA immobilisation. Graphene was utilised as a field-effect transistor (FET), and hybridization resulted in detection of the site-specific single-stranded target DNA at the femtomolar level (LOD = 100 fM). This device is ideal for multiplex DNA detection since it uses an 8-graphene-electrode/FET array with a microfluidic channel on top [65,66]. One of the most common characteriszation techniques used in biosensing laboratories is electrochemical impedance spectroscopy (EIS). This allows researchers to uncover underlying biological recognition events by detecting the non-linear response of an electrochemical system to an applied voltage.In order to detect DNA hybridization (DNA/DNA), Ben-Yoav et al. created a microfluidic EIS-based biochip, which serves as an example of an EIS-based system. Three micro-channels mounted on an arranged electrochemical micro-chip made up the biochip. Gold and platinum were used to immobilise probe ssDNA, with mercaptohexanol (MCH) serving as a back-filler. Then, in a phosphate buffered saline (PBS) solution, the target ssDNA was subjected to the probe ssDNA while flowing through a [Fe(CN)6]3−/4− redox couple. This approach showed a selectivity of 13% when hybridising with non-complementary DNA and a limit of detection (LOD) of 3.8 nM. The change in the nett charge of an electrode surface is used to determine the open circuit potential (OCP). For the purpose of detecting PNA/DNA hybridization and sample amplification by gold nanoparticles (AuNPs), Jolly et al. used OCP and EIS in their work. Immobilised onto the surface of a gold working electrode were peptide nucleic acid (PNA) probes, chosen for their intrinsic neutral charge. Then, using a three-electrode setup, OCP and EIS were used to measure the hybridization of PNA with DNA in real-time. The present setup increased capacitance significantly with the help of AuNPs, allowing for a LOD of 1 fM. In 2015, Wu et al. showed a label-free EIS-based biosensor device that could detect DNA hybridization. Concentric double ring-single disc electrodes were used to improve the number of hybridization events, and this device was integrated with microfluidic DC-biased AC electroosmotic (ACEO) stirring. Gold disc electrodes (DE) were used to immobilise thiol-modified probe DNA, which allowed for the formation of Au-S bonds. After that, EIS and OCP were used to measure the hybridised target DNA, which was mixed under ACEO conditions. To characterise the probe-MCH layer, we utilised OCP, and EIS to quantify changes upon DNA hybridization. A linear detection range of 1 aM-10 pM and an ultrasensitive limit of detection (LOD) of 0.5 aM were both achieved using this approach. Also, in just 141 seconds, the ACEO stirring approach reached 90% hybridization saturation.

Mass-Based

Biosensing via mass-based signal transduction techniques (e.g., surface acoustic waves, quartz crystal microbalance, and others) has been the subject of less recent microfluidic research. The mass change that occurs when a target analyte attaches to an immobilised probe is the basis for these novel biosensing technologies. One of the main benefits of mass-based sensors is their ability to detect biological recognition events at the micro and nano level. These sensors are highly sensitive and resilient. In 2010, Hong et al. demonstrated the reliability of mass-based sensors for the label-free detection of viral hemorrhagic septicaemia (VHS) using a QCM biosensor that was coupled with piezoelectric wafer layer wells and syringe pumps. The sensor's gold-coated quartz surface was modified with various thiol, amine, and biotin 3′ alterations to immobilise DNA probes, maximising sensitivity. During the measurement, a syringe pump was used to flow 25 µL/min of both target complementary DNA and mismatched DNA through the wells [67,68]. The results demonstrated that biotin immobilisation was the most effective for target DNA immobilisation and detection, with a LOC of 1.6 nM. Even after 32 regenerations, the system that used biotinylated probe DNA continued to generate a strong signal, demonstrating its excellent stability. A DNA sensor based on quartz

crystal microbalance (QCM) that uses fluid circulation for high selectivity and limit of detection (LOD) was created by García-Martinez et al. at a frequency of 50 MHz. High resolution (7.1 ng/cm2) was achieved under flow circumstances in this study by using oscillator circuits based on Miller topology to calibrate and identify background frequency noise. Immobilised onto the gold-quartz surface within a 0.5 M NaCl solution were DNA-disulfide probes. Following the DNA target's hybridization under flow, a dehybridization solution containing 0.5 M NaOH and 3 M NaCl was added. With a limit of detection (LOD) of 50 ng/mL, the research demonstrated that the QCM oscillator could detect DNA biosensing. A piezoelectric crystal's surface is used to generate mechanical waves in surface acoustic wave (SAW) devices. It has been demonstrated that sensors based on SAW are quite selective when it comes to bio-recognition events. Nevertheless, there is a dearth of research that makes use of SAW to detect DNA at highly optimised frequencies. However, with the potential commercialisation of medical devices that demand extremely sensitive, selective, and cost-effective analysis, SAW-based devices may have a chance to become a big player. Zhang et al [69,70]. recently introduced mass-sensitive SAW technology as a means of detecting amplified and sequence-specific DNA hybridization by in situ silver nanoparticle manufacturing and enzyme-mediated DNA elongation.The DNA that was being targeted was hybridised with probe DNA oligonucleotides that were immobilised with gold at a flow rate of 40 µL/min. The target DNA was extended through interactions with terminal deoxynucleoside transferase (TdT) and deoxy-ribonucleoside triphosphate (dNTP). Following this, the DNA template for prolongation binds to the DNA that binds Ag+. Next, AgNPs were formed by nucleating the accumulated Ag+ ions. A discernible rise in mass and the production of AgNPs with a corresponding decrease in LOD were the results of these two reactions [71,72]. The results of the single nucleotide polymorphism (SNP) tests indicated a high level of sequence specificity, even when dealing with DNA that was mismatched on one strand. Thanks to the signal amplification caused by AgNPs, a limit of detection (LOD) of 0.8 pM was attained.

Preparing Samples using Microfluidic Modules

Flow cell chambers, which are channels that enable the continuous flow examination of a pre-processed DNA sample, are the simplest microfluidic devices integrated with label-free DNA biosensors. This approach has already been discussed. When the required modules can be integrated onto a chip, and the sample can be pre-processed there, the real benefit of combining microfluidic components with a DNA biosensor becomes apparent. The extraction of the target DNA sequence is the initial stage in DNA detection sample preparation. The need for a DNA amplification stage is proportional to the target DNA concentration in the sample and the biosensor's sensitivity. Additionally, droplet microfluidic devices have demonstrated great promise in applications requiring extremely high throughput analysis, since they enable the execution of the entire experiment inside a few nL liquid compartments.

Extracting DNA

DNA extraction microfluidic modules employ a number of methods to free the desired DNA from the target cells. At present, a fundamental laboratory technique in molecular biology is DNA extraction. using one of many available commercial kits designed for routine laboratory work. The procedure consists of four main stages that can be adjusted to suit different samples and their subsequent uses: (i) Destroying cells; (ii) Extracting proteins, lipids, and other nucleic acids from membranes; (iii) Purifying and binding bulk nucleic acids; and (iv) Concentrating nucleic acids. Using microfluidics for these operations has several advantages, such as automating tedious and time-consuming laboratory procedures and reducing the likelihood of cross-contamination and human handling errors. This paves the way for a sample-in-answer-out Lab-on-Chip device to be realised by their integration with DNA detection modules. Adapting DNA extraction procedures for use in a microfluidic device was

the first focus of DNA extraction microfluidic modules developments. Handling several chemical reagents in multistep tests and transferring chemicals between solid and liquid phases were the primary obstacles encountered by these early examples. It should be noted that when selecting the extraction reagents, caution must be exercised to ensure that they do not impede any later processes of amplification or detection. Using magnetic particles is another fascinating application of microfluidics. To illustrate the point, Karle et al. isolated and linked the bacterial DNA to the surfaces of magnetic beads after cultivating Escherichia coli in a suspension of these beads with lysis and binding buffers. Then, a revolving permanent magnet may be used to guide the DNA-carrying beads inside the chip as needed. The intended chip and fluidic handling stages are greatly simplified with this approach. When it comes to DNA extraction microfluidic modules, the GeneXpert MTB/RIF is among the most commercially successful implementations. This method involves separating the target cells from the rest of the material using filtration and then releasing their DNA using sonication.

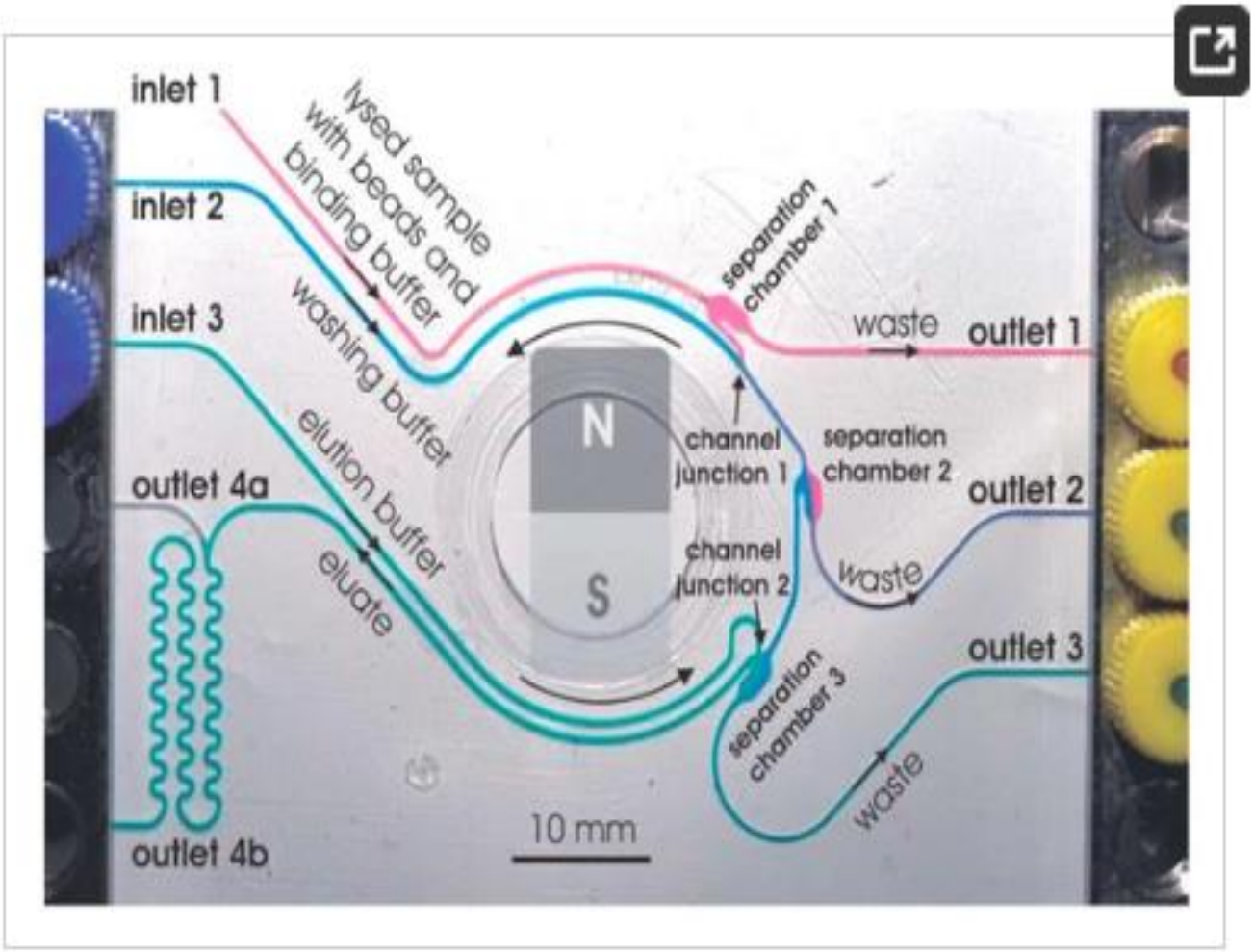

Figure 4. Photograph of magnetic particle assisted DNA extraction chip.

Results of the Measurement Using Synthetic DNA and Polyelectrolyte Layers

This work presents illustrative experiments with capacitive EIS sensors that have been used for detecting hybridization and DNA immobilisation and for monitoring the layer-by-layer adsorption of polyelectrolytes such as anionic poly(sodium 4-styrene sulfonate) (PSS) and cationic poly(allylamine hydrochloride—PAH. The effects of charged macromolecule adsorption on FEDs can be studied using polyelectrolyte (PE) multilayers as a model system. When dissolved in an appropriate polar solvent, such as water, these linear macromolecule chains contain a great number of charged or chargeable groups. Polyetheretherketone (PE) multilayers are created

by sequentially adsorbed alternating-charge polyions. The charge overcompensation effect causes the surface charge to flip with each adsorption step of a PE layer that is already charged. Strong electrostatic forces stabilise a PE multilayer, which is the final outcome of the subsequent deposition. There is a detailed description of the laboratory setup, including the FED preparation, experimental conditions, and methods for detecting DNA immobilisation and hybridization and layer-by-layer adsorption of polyelectrolyte multilayers. Atomic force microscopy, capacitance-voltage, constant-capacitance, and impedance-spectroscopy techniques have been used to systematically characterise the attachment of these charged macromolecules to the FED surfaces. The impact of the DNA hybridization event on the shift of the capacitance-voltage curves along the capacitance and voltage axes, as well as the quantity and polarity of the polyelectrolyte layers, have both been studied. C-V measurements, enable the observation of changes in the flat-band voltage (ŔVfb) and the maximum capacitance (ŔC) in the C-V curve's accumulation zone, caused by charged macromolecules. The direction of the voltage axis shifts, which are clearly visible in the zoomed graph in the depletion zone (60% of the Cmax), is determined by the sign of the charge on the PE layer. Once each layer of polycation and polyanion has been adsorbed, alternating potential changes of around 30-90 mV have been noted. While the direction of the potential change after adsorption of the positively charged PAH is consistent with an additional positive charge on the Ta2O5 surface of the FED, the direction of the potential change after adsorption of the negatively charged PSS is consistent with a negative charge on the Ta2O5 surface. Simultaneously, there are minor shifts in the maximum (or geometrical) capacitance within the C-V curve's accumulation range (a drop of up to 2.5%). The results show that the extra eight PE layers should have produced a somewhat bigger geometrical capacitance effect, in line with the assumption that the adsorbed PE layers are homogenous dielectric films with a fixed thickness and dielectric constant.

Conclusion:

Recent advances in microfluidic-assisted label-free DNA biosensing devices were discussed in this paper. Our primary emphasis was on highly integrated biosensor devices that do not require labels. These devices are ideal for point-of-site detection in fields including medicine, biology, environmental science, and food analysis. There are a number of label-free biosensing methods being investigated for microfluidic integration at the moment, including optical, electrochemical, and mass-based approaches. While mass-based approaches excel in detecting larger molecules, they may struggle to keep up with smaller molecules like short chain oligonucleotides. Optical methods, in contrast, can provide extremely low-limits of detection for incredibly tiny molecules; but, they are hindered in terms of platform miniaturisation by their complex apparatus requirements. One benefit of electrochemical biosensors is that they need very little power and have very low limits of detection. Although each of the microfluidic modules used for sample preparation—cell separation, DNA extraction, and DNA amplification—has been proven in isolation in the literature, there have been very few reports of attempts to integrate several modules. The technical viability of future high-throughput and high-specification portable systems is clearly demonstrated by multiplexed DNA amplification modules, droplet-based microfluidics, and nanopore-assisted sequencing. At present, nanopore technology allows for point-of-need DNA sequencing with a throughput of 10–20 G bases per 48 hours. While this kind of sequencing throughput is certainly remarkable, there are many diagnostic uses for which whole genome sequencing is superfluous. For both Point-of-Need applications in centralised healthcare systems and low-resource settings, microfluidic technologies that provide sample-in-answer-out operations with a few minute time to result may be more realistic in real-life practice. Several commercial initiatives are now showing

signs of imminently launching sample-in-answer-out systems with targeted medical diagnostic applications, building on more established technologies in microfluidic DNA systems. Integrating very sensitive biosensors into portable devices, or even systems that do not require any equipment, at a reasonable cost is a significant obstacle that needs to be addressed. Due to the fact that different diagnostic companies are following different strategies, there is currently no commercially available method for mass-producing a product that seamlessly integrates all of the necessary components. In addition to the high power demands of heating on modules that incorporate DNA amplification, the use of sensing technologies that necessitate complex or costly equipment can provide challenges in terms of portability. There is a pressing need to create assays and devices that enable multiplexed detection since most real-world applications require the simultaneous quantification of multiple DNA strands. In conclusion, it is essential to conduct early device development using real physiological samples in order to guarantee device compliance with biomedical device standards and worldwide regulatory frameworks.

Biomedical research, clinical diagnostics, drug screening, genetics, environmental monitoring, food analysis, and a plethora of other fields could benefit from field-effect-based DNA chips, which offer a novel method for the concurrent examination of many nucleic acids. One major benefit of FEDs is their active microelectronic device integration capacity; another is that they can combine the two possibilities of label-free direct electrical detection of DNA hybridization (e.g., active area arrays up to 105 cm2 are achievable). Meanwhile, it's clear that developing FEDs to detect DNA and other charged macromolecules by their intrinsic molecular charge without labels is more challenging than anticipated. Despite how intriguing the results are, they are rather varied and occasionally contradictory. Factors that impact the immobilisation and hybridization detection of DNA by FEDs include: ● the material of the FED's gate (metal, insulator, polymer) and any surface charges or modifications it may have, ● the method of ssDNA immobilisation (passive or electrochemical adsorption, covalent binding, binding via cross-linker), ● the conditions of immobilisation and hybridization, including pH, ionic strength, temperature, ● the density and length of the immobilised ss-DNA, the length of the spacer or linker molecule, etc. As a result, biosensor applications and the basic characteriszation of numerous important physiological processes both benefit greatly from a thorough knowledge of the adsorption and interaction of charged macromolecules like DNA onto (charged) surfaces of FEDs.

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