Nano-Bio-Sensing

Sandro Carrara
Editor

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Foreword by Giovanni De Micheli

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Foreword

Much of our economy and way of living will be affected by nanotechnologies in the decade to come and beyond. Mastering materials at the molecular level and their interaction with living matter opens up unforeseeable horizons. Still much of the potentials of nano-biotechnology is untapped. Although we understand most basic principles of molecular interaction, the transformation of scientific results into robust technologies that can support health care and environment protection has still to take place. In other words, we are at the verge of a technological revolution that will bring to us a multitude of bio-electronic devices that interact with biological systems through intelligent – and possibly distributed – means of computation. The recent strong interest about cyber-physical systems is motivated by this trend.

This book reviews the principles and practice of nano-bio-sensing. It covers extensively the basic principles of interaction of living matters with detectors and the transduction principles. It reviews surface science as well as electrical and optical technologies for bio-sensing. Nano-scale effects, inducing quantum confinement, are specifically addressed along with their benefits, such as the amplification of sensing phenomena, yielding devices with higher sensitivity. Specific importance is given to low-power sensing techniques, as well as nonconventional means for powering the sensors, which may be very useful for implanted biosensors. Moreover, fault-tolerant bio-sensing systems are described. Overall, various physical, chemical, and electrical effects contribute jointly to enable the construction of a new generation of nano-bio-sensors.

The importance of this research field should not be underestimated. The healthcare sector will soon be able to benefit from real-time sensors – *in the body* and *on the body* – that can predict specific pathologies and give the opportunity of preventive treatment. Pharmacology will be positively affected by means of creating rational and personalized drugs that can be tuned to the characteristics of the patient. Nutrition science and practice will benefit from advances in sensors to enable the monitoring of the consumption of nutrients in the right combination and

quantity for the expected effort. Positive impact of these methods can be measured, for example, in the training of sportsmen and in managing the attention span of youths. A combination of electro-sensing technologies can rationalize work and living spaces, enabling better working and living conditions and specifically longer autonomy to the elderly. Similarly, these technologies can be used to monitor the environment, to protect us from infections and pollution, and raise the level of security of individuals and communities. All these important and ethical goals are addressed by some research programs, most notably by the Swiss *nano-tera.ch* initiative which I am leading.

Given the intrinsic scientific merits of nano-bio-sensing and its wide projected impact on society, I believe that this book provides the reader with an important guide through the various technologies. It represents a key reference point for both scientists and engineers.

EPFL Lausanne, 2010 Giovanni De Micheli

Contents

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Chapter 1
Introduction to Nano-Biosensing

Sandro Carrara

1.1 Introduction to Nanotechnology

Although Richard Philip Feynman envisaged nanotechnology in his famous lecture held at California Institute of Technology in 1959 [1], modern nanotechnology started when Gerd Binning and Heinrich Rorer invented the scanning tunneling microscope (STM) at the IBM laboratory in Zurich, in the early 1980s [2]. The importance of this invention was immediately recognized and they became Nobel laureates a few years later, in 1986.

The STM is a microscope based on a quantum phenomenon, the tunneling effect, which enables electrons to overcome an energy barrier even if they do not have enough energy. For that, the energy barrier has to be no higher than the electron energy and the barrier width has to be on the scale of tenths of a nanometer. Such a barrier is called a *tunneling barrier*. If an electron sea has a Fermi level below the energy of the tunneling barrier, then the large majority of the electrons do not have enough energy to overcome the barrier. However, a few of them do not have zero quantum probability to overcome the barrier and, thus, a small current flows through the system. This current is called the *tunneling current* and it is usually less than a few nanoamperes. The core of this microscope is a piezoelectric mover that ensures small shifts (with steps below tenths of nanometers) of a conductive tip, which approaches the conductive sample step-by-step until a tunneling current is measured, even if the tip and sample are not in contact. Once the tunneling current has been locked, the piezoelectric mover is driven to obtain a scan over the sample surface, as schematically shown in Fig. 1.1. A feedback system is used to keep the tunneling current constant and an image of the sample surface is acquired from the voltage used to control the feedback. Such an imaging technique is called *microscopy in constant current*.

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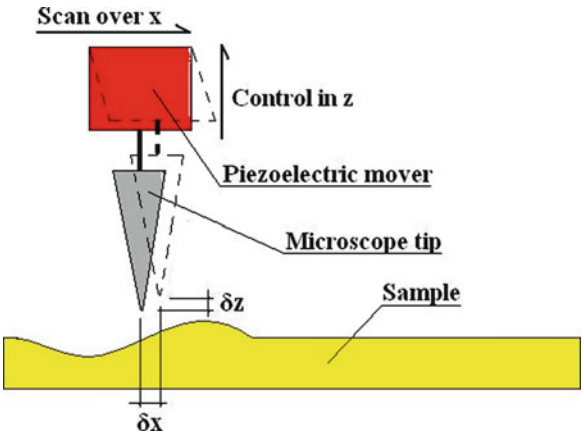

Fig. 1.1 The working principle of a scanning probe microscope, including a tip interacting with the sample and a piezoelectric mover, which scans the sample area for imaging and controls the tip–sample interaction acting on the *z*-position

In a STM, the current is of the order of picoamps, whereas the best piezoelectric mover ensures steps with spatial resolution of the order of picometers. A current control of the order of picoamps provides control of the vertical position of the tip below 1 Å (0.1 nm). So, this microscope enables nanoscale imaging to measure a single gold atom [3] or precise resolution of the interatomic distances in graphite [4]. Of course, if the lateral movement of the microscope tip stops at a sample point, then the application of a high enough tip–sample voltage modifies the sample locally. This allows for sample manipulations at the atomic scale [5].

So, the modern era of nanotechnology was born when technology allowed us to see and manipulate materials at the atomic scale with the invention of the STM.

1.2 Nanoscale Microscopy on Biological Systems

Instead of a current, a more general idea to monitor and control other physical parameters during an image scan over a sample surface led to further inventions in *scanning probe microscopy*. In general, the physical parameters controlled may be the tunneling current, the tip height, the tip–sample interaction forces, or the tip friction on the sample, etc. Each of them leads to a special type of microscopy or to a special operating mode of the microscope. Among the different scanning probe microscopes, the most popular is the *atomic force microscope* (AFM). This is based on the interactions between the tip and the sample and allows measurements of interactions down to 10^{-18} N [6]. In the case of imaging in constant-force mode, the image is once again obtained by the feedback voltage.

The STM, the AFM, and the other scanning probe microscopes may be applied to imaging or investigations of biological samples [7]. With the AFM and the STM,

imaging, sensing, and manipulation are performed at sub-nanometer resolution. Therefore, we are now dealing with proper *nano-biosensing* when we use the STM or the AFM to sense biological samples, such as DNA [8, 9], proteins [9], and cells [10].

Imaging of DNA by using scanning tunneling microscopy of graphite substrates was one of the first applications of scanning probe microscopes to bioimaging. Unfortunately, defects on graphite were found to mimic the image features of the DNA [11]. Therefore, the STM was abandoned in favor of the AFM for imaging of biological molecules [10]. Over the last 15 years, many examples of imaging molecules of biological relevance using the AFM have been published. It is easy to find in the literature good investigations reporting atomic force microscopy of DNA chains [12], antibodies [13], oxidases [14], cytochromes [15, 16], and photosynthetic proteins [17, 18] focused on both single molecular size and crystal structure within the protein film.

More recently, the AFM was applied to sense the single forces of interaction within biological samples. This new emerging field in nano-biosensing has the name force spectroscopy. Carlos Bustamante reported the first example of overstretching of a DNA molecule in 1996 [19]. In the following years, different research groups demonstrated the use of the AFM in force spectroscopy of single molecules. Hermann E. Gaub applied the AFM in force spectroscopy of polysaccharides and synthetic polymers [18]. Bruno Samorì investigated single-protein force spectroscopy, e.g., with angiostatin [20] and fibronectin [21]. Giovanni Dietler and Sandor Kasas proposed stiffness spectroscopy of living cells by using the AFM [22]. Very recently, James Gimzewski demonstrated that cancer cells in an early stage may be recognized by force spectroscopy even when their optical morphology appears to be similar to that of normal cells [23].

In Chap. 2, Sandor Kasas and Giovanni Dietler from EPFL, the Institute of Technology in Lausanne (Switzerland), summarize details of the technique and the most important scientific results obtained in nano-biosensing of DNA, proteins, and cells by using AFM force spectroscopy.

1.3 Nanolayer Made of Organic and Biological Materials

Single molecules of biological relevance are typically on the nanoscale. For example, thiols required for the cell membranes have a size close to 1 nm [24]; a cytochrome may be included in a sphere of 4 nm [25]; an antibody may be included in a box with size below 5 nm [26]. Then, molecular layers built onto a solid substrate with these objects have the right size to obtain nanostructured materials. From this point of view, Irvin Langmuir (Nobel laureate in chemistry in 1932) obtained the very first nanotechnology when in 1917 he provided the first report about the organization of oil films at the air/water interface [27]: this was the first investigation on monolayers obtained with fatty acids.

Three different kinds of molecular films are possible with molecules having an aliphatic chain and a hydrophilic end group. The spreading of such molecules on water produces a monolayer at the air/water interface because the hydrophilic group dips into the water, whereas the aliphatic chain floats on top of the water owing to its hydrophobic character. In the trough, a highly ordered film is obtained on the water surface when the amphiphilic molecules previously dispersed onto the water are compressed by means of moving barriers. These kinds of films are called *Langmuir films*. Once the Langmuir layer has been obtained, there are two ways to transfer the film onto a solid substrate. The substrate may be softly applied to the water and softly removed from the surface. In that manner, the monolayer at the air/water interface is transferred onto the substrate owing to interaction forces established between the hydrophobic tails of the molecules and the substrate surface, as schematically shown in Fig. 1.2a. The films obtained are called *Langmuir–Schaeffer films* in honor of Vincent Schaeffer, an eclectic man who became Langmuir's research assistant in 1932. The other way to transfer Langmuir films onto solid substrates is to dip the vertical substrate into the water and in a gentle manner. In that case, a first monolayer adheres to the substrate surface during dipping, and a second layer adheres to the first one when the substrate is removed from the water, as schematically shown in Fig. 1.2b. The result is a molecular bilayer with the two molecular layers organized in opposite directions with respect to the aliphatic chains. The films obtained are called *Langmuir–Blodgett films* in honor of Katherine Burr Blodgett, who also worked with Irvin Langmuir at General Electric Laboratories, after 1920.

Langmuir–Blodgett and Langmuir–Schaeffer films are also used to obtain multilayers by repeating the transfer of a single monolayer or bilayer. Thus, they are suitable for producing complex films with different functionalities for biosensing.

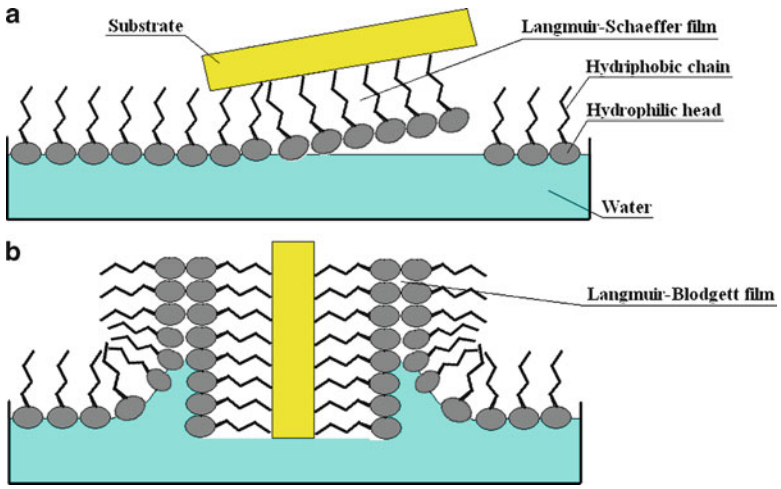

Fig. 1.2 Amphiphilic molecules with hydrophilic head groups and hydrophobic chains at the air/water interface and two methods for obtaining thin films

Although they are usually used with small aliphatic molecules, typically fatty acids, the possibility has been demonstrated to also organize both proteins [28] and polymers [29] in Langmuir–Blodgett or in Langmuir–Schaeffer films, even improving the stability of the molecular structures [30, 31].

Of course, thin films involving more functionality are also obtained by using self-assembly or layer-by-layer deposition. The self-assembly is based on chemical bonds between the layers and the substrate surface. Typically, thiols are self-assembled onto gold or other metals, whereas silane films are formed on silicon. Once the first layer has formed on the substrate surface, the next layers are assembled on the first layer with protocols that use functional groups of the first and the second layers. The repetition of similar chemical anchoring results in a highly organized multilayer with different molecules embedded within the same thin molecular film [32].

Layer-by-layer deposition is based on successive deposition of different molecular layers by using physical adsorption. Typically, a first layer is deposited onto the solid substrate on the basis of the mutual electrostatic attraction between the molecules and the substrate surface. Polar molecules present a positive molecular side chain to a surface that is negatively charged and, then, the molecules adhere to the surface. In a second step, a second molecular layer of a different kind of polar molecule is attached to the first one by using the positively charged side chain of the second molecules. In that way, the second molecules are attracted by the negatively charged side chains present in the first layer. Successive depositions following these steps result in multilayers that may include different kinds of molecules in the same sandwich [33].

The recent literature offers many examples of thin molecular films used for bioelectronics and nano-biosensing. Langmuir–Blodgett films were used by Victor Erokhin [34] for quantum devices. Langmuir–Schaeffer films were used by Frank Würthner and coworkers to study the charge transfer properties of molecular films [35]. Self-assembly was used by us for immunosensors and DNA detectors [36]. Layer-by-layer deposition was used by Reinhard Renneberg for enzyme encapsulation in polymers [37]. Franz Dickert and coworkers used protein crystals for stamping sensing layers [38]. The imprinting and patterning of molecular layer is of crucial importance to define sensing architectures. So, in Chap. 3, Franz Dickert from Vienna University (Austria) presents a valuable overview of the modern techniques of polymer nanopatterning especially dedicated to mass-sensitive biosensing.

1.4 Metallic Nanolayers and Plasmon Resonance

Quantum phenomena become more evident at the nanoscale. Charge quantization appears when electrons are confined within structures with size of the order of a few nanometers. In certain conditions, a Fermi sea confined in a conducting layer with a thickness of a few nanometers may result in coherent Schrödinger waves driving electrons to move as a unique plasmon wave. This phenomenon is observable by using a laser beam with a monochromatic wavelength and varying its incident angle to a

metal surface. Once the incident beam has reached the right angle, the plasmon is switched on and the light reflected by the surface suddenly decreases owing to the loss of the energy needed to push the plasmon. Another way to observe this quantum effect is to fix the angle of incidence and to sweep the frequency of the laser beam until the light reflected suddenly decreases. In both cases, the right incident angle combined with the right beam frequency determines the so-called *plasmon resonance*, which is the beam condition that switches on the electron plasmon within the metal surface.

Theoretical studies have shown that the plasmon resonance conditions are related to properties of the electromagnetic wave at the prism/metal interface [39]. To have a simple understanding of the physical situation, we can think of a simplified ideal situation. When a laser beam is sent, with a certain angle, toward the interface between two light transmitting materials, the incident beam is usually split in two beams: the transmitted and the reflected ones. The angles of these two beams are related to the angle of the incident beam through the Snell equation. Of course, the angle of the transmitted beam may be reduced by varying the angle of the incident beam, depending on the ratio between the refractive indexes of the two materials. Once the so-called *critical angle* for the incident beam has been reached, the transmitted beam has zero emerging angle. This means that the transmitted beam is not transmitted any more but, instead, is now traveling parallel to the interface. We can now introduce a metallic material into this highly simplified model: we can create a similar situation between transparent and conducting materials. The typical situation is that of a glass prism on a gold surface, where a glass/metal interface is obtained. Now, the electromagnetic wave travels parallel to the interface at the critical angle and it affects the electrons in the metal surface. More precise numerical simulations provide a very clear understanding of the electromagnetic field at the interface, called an *evanescent wave*, which typically extends though the interface up to 200 nm [40]. So, the evanescent wave affects electrons in this nanometer-sized region of the metal, creating a plasmon: a coherent electron wave that it is excited by the laser beam incident to the glass/metal interface.

In the case of a glass/gold interface, the incident beam is never transmitted within the metal but is reflected every time. However, the intensity of the reflected beam is highly diminished at the angle that matches the resonance conditions. The resonance conditions depend on the laser frequency, metal nature, and glass refractive index [39]. When the surface plasmon is switched on, part of the incident energy is used to maintain the plasmon. So, the intensity of the reflected beam is suddenly diminished and we can acquire information about the resonance conditions by measuring the intensity of the reflected laser beam. Alternatively, it is possible to fix the incident angle of the primary laser beam, and to vary the frequency of the laser beam. Analogously, the intensity of the reflected beam suddenly decreases once the beam frequency reaches the critical value for the resonance condition.

The quantum phenomenon of surface plasmon resonance was discovered in the 1960s by investigating the interaction of a laser beam with metallic surfaces [41]. Metal/glass interfaces were typically obtained by using a glass prism on metal surfaces. Then, the conditions of the incident laser beam were varied to define the optimal resonance conditions by looking at the reflected beam intensity. In the

first configurations, the prism was used to focus the laser beam onto the surface of a metal in bulk.

The plasmon resonance conditions are so narrow in terms of the optimal angle or frequency that any small variation of the interface conditions results in a large variation of the plasmon and, then, in a large variation in the intensity of the reflected light. For that reason, another configuration was proposed to use the plasmon surface resonance for biosensing purposes: the so-called *Kretschmann configuration* [42]. In this system, a very thin metal layer, typically a 50-nm-thick layer of gold, is used as a support for the electron plasmon. The incident and reflected laser beams are managed by the downside of this layer. The topside of the metal layer is instead used to immobilize probe antibodies and to study the interaction with proteins and antigens by using a microfluidic system. The evanescent wave is affected by the refractive index of the topside of the system because it extends for no more than 200 nm over the glass/metal interface [40]. Then, when the probe molecules interact with target ones, the average refractive index changes. This changes the evanescent wave, which modifies the plasmon and slightly changes the resonance conditions. Therefore, acquisition of the intensity of the reflected beam registers the molecular binding or interaction on the topside in a Kretschmann system, as schematically shown by Fig. 1.3.

The invention of such a nano-biosensor led to the birth of a worldwide company, Biacore (now part of General Electrics Healthcare), that is the worldwide leader in this nano-biosensing technology. With use of its instruments, interactions between monoclonal antibodies and antigens were originally investigated by Robert Karlsson, Anne Michaelsson, and Lars Mattsson [43]. Further investigations by using antibody fragments were performed by Gabrielle Zeder-Lutz [44]. Very specific protein–protein interactions were also improved by developing special molecular precursors based on ethylene glycol monolayer by George Whitesides [45, 46] or based on LipaDEA by Inger Vikholm-Lunding and Martin Albers [47].

In Chap. 4, Inger Vikholm-Lunding and Martin Albers, from a VTT laboratory in Tampere (Finland), summarize the physics of surface plasmon resonance, the

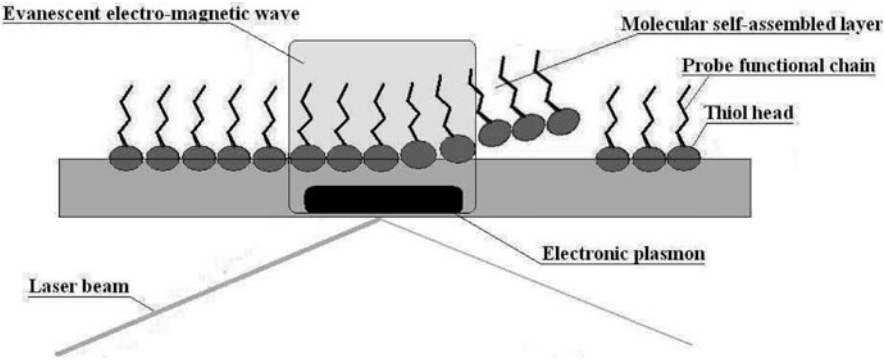

Fig. 1.3 In a small metallic layer, the incident laser beam creates the plasmon, which is affected by molecules in the other side through the evanescent wave

basic principle of the related biosensors, and recent advancements in more reliable surfaces that improve the specificity of molecular recognition.

1.5 Quantum Systems and Electrical Charge Confinement

Nanosized structures made of metals or organic molecules are presented in Chaps. 3 and 4. In both cases, they are nanostructures but they have only one dimension on the nanoscale. As we have seen before, the confinement of the electron sea in a layer with a thickness of only 50 nm results in the appearance of the plasmon resonance. The appearance of the plasmon is due to the quantum nature of the electrons. That means we can try to observe that charge quantization through a direct current measurement. Although the theory of plasmon resonance involves some equations for the current density in the metal surface related to the plasmon [48], it is not reliable to measure directly electron current related to plasmon resonance. However, we can further reduce the layer thickness until the quantum phenomena are so predominant that their effects on the electrical flow through the nanolayer may be measured directly. The ultimate limit of this squeezing would be the single atoms in the conducting layer, which correspond to a metal layer with thickness below 1 nm.

Modern nanotechnology offers a simple method to obtain single-atom-thick and highly oriented crystals of metallic nature: graphene. Graphene has a single atomic layer obtained by mechanical [49] or chemical [50] exfoliation of graphite. The mechanical exfoliation is simply achieved by peeling the graphite, whereas the chemical exfoliation is obtained by using aggressive acids that intercalate between the graphite sheets and cause them to detach from each other. In both cases, the result is a monodispersed suspension of single graphite layers, which are called graphene. Graphene may be cast onto substrate surfaces and then characterized in terms of electrical characteristics. The electrical conductivity on a graphene single sheet is of ambipolar nature [49]. Thus, the intrinsic carrier states in graphene are due to both electrons and holes. It is interesting that even the Schrödinger equation leads to stationary states [51] in the third dimension (that normal to the graphene plane). This means that the electrons confined in the graphene layer have only quantized wavelength in the third dimension. This means we have a quantum well.

In solid-state physics, quantum wells are usually obtained by fabricating a very thin semiconducting layer between two insulating ones [52]. They have thickness on the submicron scale depending on the fabrication techniques. They are usually used for quantum lasers [53] and charge quantization confinement [54]. Usually, the quantum confinement is again measured by means of optical effects. On the other hand, current measurements are directly possible in graphene layers, which have quantum well states [55].

Ideally, graphene sheets may be rolled up to obtain carbon nanotubes. Carbon nanotubes are monoatomically flat tubes with the regular crystal structure of a graphene sheet. They are fabricated by means of arc discharge [56] or chemical vapor deposition [57]. The final product is a set of tubes that may be individual

single-walled tubes or individual multiwalled tubes, depending on the fabrication conditions. They have amazing electrical properties with orders of magnitude improvements in the electrical conductivity [58] and the mean free path [59] of the charge carriers owing to ballistic transport of electrons and holes in their walls. Thus, carrier confinement is also possible in one dimension by using carbon nanotubes because their external radius is typically 2 nm in the case of a single-walled tube. In that case, the electrons are free to move in the tube, remaining confined in its wall [60].

Analogously, an electron may be confined in a quantum region by its injection in a nanosized conducting cube surrounded by insulating material. In such a case, the Schrödinger wavelength is quantized in all three spatial dimensions and pure quantized energy states emerge in the system. So, electrons may be trapped only in steady states in the nanosized cube, whereas any interaction with them shows fully quantized energy. A system that can trap electrical charge carriers at a point is called a quantum dot [61]. Individual small clusters of metallic [62] or semiconducting [63, 64] atoms embedded in insulating materials can produce quantum dots that can work at room temperature. Another possibility is to obtain granular structures by using polysilicon [65]. This results in individual grains separated from each other by tunneling junctions. A further possibility is to embed metal clusters into an insulating material [61]. A third one is to create a monodispersed small metallic cluster in a liquid by stabilizing the metallic cores with alkanethiols [66]. A fourth one is to grow semimetallic nanoclusters within an organic matrix [62]. In all these cases, quantum confinement within the nanostructures obtained has been demonstrated by both electrical and optical characterization.

The electrical properties of graphene, carbon nanotubes, and metallic nanoparticles are highly sensitive to the chemical environment and, then, they are suitable for chemical or biomolecular sensing. Janos Fendler envisaged the used of colloidal nanoparticles for molecular recognition [67]. Smalley applied carbon nanotubes to gas sensing [68]. We obtained improved sensitivity in biosensing using both gold nanoparticles [69] and carbon nanotubes [70]. As we have seen, quantum phenomena take place in these nanosystems and they are directly observable in the electrical properties of the systems. For example, Schönenberger measured direct electron quantum trapping in a metallic nanoparticle in 1992 [62], whereas we observed a similar phenomenon in semiconducting nanoparticles a few years later [63].

In Chap. 5, I summarize the physics of these quantum systems. The electrical properties of quantum wells, wires, and dots are described and their physics is presented. Then, the use of these nanosystems to enhance the properties of electrochemical biosensors is reviewed.

1.6 Molecular Confinement by Optical Nanomanipulation

We have seen that charge confinement is easily investigated by using electromagnetic waves interacting with the confined electrons or holes. This also leads to the development of technologies that use this interaction for nano-biosensing purposes.

The confinement of electrons within 50-nm-thick metal layers leads to applications of plasmon resonance in molecular detection through the interaction of molecules with the evanescent wave of a laser beam and, thus, with the confined electrons. In general, we can say that nanotechnology plays a role in the combination of optics with microfluidics, leading to a new branch of science: integrated optofluidics [71].

Modern optofluidics addresses new functionalities in optical and fluidic systems to develop new methods to manipulate, assemble, pattern, and sense cells or biological molecules in a fluidic environment [72]. The optical laser beam provides new tools for biomanipulation, whereas the fluidic system provides environments similar to native ones for biological systems.

To manipulate objects, highly intense and focused laser beams create gradient forces to trap the objects, as shown in Fig. 1.4. These forces move and trap molecules or cells owing to their electrical charges usually being nonuniformly distributed on the surface [73, 74]. So, gradients focused to a point are used to concentrate cell samples, whereas separation of molecules is obtained thanks to different polarizations in different compounds. Liquid samples are moved along a complex pathway to separate target molecules from interfering ones, to obtain interactions with probe molecules, to mix the complexes obtained with labels, and to obtain the final spots for sensing [75]. Therefore, optical tweezers [76] are a unique tool for nano-biosensing. However, they have some drawbacks owing to their intrinsic physical properties. First, highly intense optical beams might damage the objects, especially in case of soft materials. Moreover, the use of a single laser beams limits manipulations in large-scale as well as high-throughput detection.

Merging electronics with optofluidics offers another tool: optoelectronic tweezers [77, 78]. In this case, charges induced in sample regions of a photoconductive

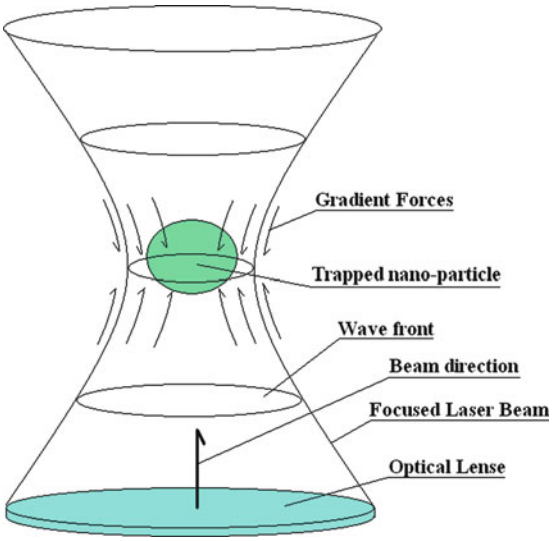

Fig. 1.4 In optical tweezers, gradient forces generated with a focused laser beam trap colloidal nanoparticles at a point

substrate create dielectrophoretic pathways. These charged regions create electrical force gradients that trap biological objects. Once the objects have been trapped, dynamical reconfiguration of the charged regions moves and manipulates the objects along complex pathways [77] even for parallel manipulation [78]. This technique proposes something similar to a "light-induced dielectrophoresis," offering all the advantages of the well-known electrophoresis, which is widely used in biology. An advantage of optoelectronic tweezers is that they use an optical power intensity that is up to 5 orders of magnitudes lower than that required by conventional optical tweezers. Moreover, they are more suitable for large-scale nanomanipulations and high throughput with biological objects. Optical tweezers are also used to permanently assemble molecules in complex structures [79] and this is done at the nanoscale owing to their capability to manipulate single objects at a time.

Very recent years have seen in-depth investigations and extensive developments of optical and optoelectronic tweezers. Different groups have provided exciting results. For example, Yen-Heng Lin and Gwo-Bin Lee demonstrated the feasibility of light-induced concentration and fusion of nanoparticles to assist structure formation by self-assembly [80]. Syoji Ito, Hiroyaki Yoshikawa, and Hiroshi Masuhara obtained photochemical fixation on polymers by optical patterning [81]. Misdy Lee and Philippe Fauchet used photonic crystals for sensing proteins [82]. Ming C. Wu and coworkers invented the NanoPen [83], an innovative technique for dynamic patterning of nanoparticles, and demonstrated its feasibility in cell manipulation.

In Chap. 6, Ming C. Wu from Berkeley University (USA) describes all these exciting findings by introducing the physics of optoelectronic tweezers, and by summarizing the most relevant results published in the field.

1.7 Sensing Biochip Made Using Nanoscale Electronics

In the previous sections, we saw that nano-biosensing is possible by using plasmon resonance, as well as electrochemical detection or atomic force spectroscopy. We have seen that patterning is possible with nanoscale precision both for mass-sensitive as well as for optical biosensing. The very large scale of integration (VLSI) of modern microelectronics offers the possibility to integrate all these nano-biosensing techniques in a single biochip. Modern electronics is able to integrate memory storage with a capacity of more of 200 GB in a single pen drive with a readout speed of up to more than 200 MB per second [84], computation with 147.6×10^9 instructions per second with the novel Core i7 from Intel [85], and a data communication rate of terabits per second in optical fibers [86]. That means there are many opportunities to achieve so-called *distributed diagnostics*. For example, high-throughput DNA detection with a capability similar to that provided by the Affymetrix technology may be replicated onto a silicon biochip that can provide faster and label-free assays with fully electronic readers [87]. This is highly useful to address in-field large screenings of populations. Continuous monitoring of some important metabolites, such as glucose for diabetic patients [88, 89], might be replicated in a fully implantable [90], subcutaneous

biochip [91] providing real-time monitoring and data transmission [92] to open up the human metabolism for remote monitoring. Electrocardiogram acquisition with accuracy similar to that of current hospital instruments was proposed in the form of wearable patches [93] worn under clothes during normal daily life.

For the envisaged new revolution in technology to succeed, modern microelectronics needs to integrate many different functions on the same chip: sample manipulation, biosensing, data acquisition, data elaboration, data verification, data storage, data transmission, energy harvesting. This creates new challenges for system integration design for heterogeneous systems [94]. New biochip heterogeneous systems need to integrate organic molecules onto silicon, including probes (e.g., proteins) and nanostructures (e.g., carbon nanotubes). They need to integrate analog front ends with analog-to-digital converters, microcontrollers with random-access memory and erasable programmable read-only memory (EPROM), radio-frequency transceivers, batteries, large capacitors, and subsystems for energy scavenging.

Each of these chip features requires special efforts to succeed in distributing diagnostics from hospitals to homes. For example, highly packed self-assembled monolayer films with organic molecules with special functions for biosensing purposes have been investigated on gold [95], and now are required for silicon. Analog front ends with very low power consumption [96] are now required for remotely powered biochips. Low-noise front ends [97] are necessary to obtain signals from low-intensity samples. CMOS imagers also integrating a digital signal processing core for image postprocessing [98] are indispensable for real-time acquisition and detail quantification. Of course, the final expected result is solutions that can provide a fully integrated lab-on-chip in systems similar to credit cards [99] or to bring ELISA tests into our hands [100].

In recent years, we have demonstrated improved reliable sensing of DNA and proteins by integrating nanostructured thin-film precursors onto the chip electrodes [32, 36]. Roland Thewes and partners have shown the possibility of fully electronic detection of DNA on a chip [87, 101]. Yuki Maruyama applied CMOS photodetectors to DNA detection on a chip [102], whereas Edoardo Charbon showed the possibility for a large-scale CMOS imager based on *single-photon avalanche diode* lab-on-chip applications [103]. In Chap. 7, Edoardo Charbon and Yuki Maruyama, now both at the Technical Institute of Delft (The Netherlands), summarize the most advanced results in the field of VLSI chips for biosensing by discussing the new 90-nm technology node that nowadays provides higher possible scales of integration.

1.8 Quantized Energy and Its Harvesting

As we saw in the previous section, an innovative biochip needs system integration of biological and organic molecules with silicon substrates, analog front ends with digital memories, and radio-communication transceivers with systems for remote

powering. In general, remote powering [104] and energy scavenging [105] are two key features of the modern biochip to obtain autonomous nodes in distributed diagnostics monitoring. The key role of energy scavenging and remote powering is due to weight constraints for wearable or implantable medical systems. These systems are required to be extremely small and light for their use during normal human daily life. If we want to develop innovative systems for remote monitoring at home of chronic patients and healthy people, then we need to develop systems that can be worn on the skin or implanted under the skin without any pain or any trouble. Patches on the chest for continuous electrocardiogram monitoring [93] should use extremely light and small batteries for the power supply. A subcutaneous implant for measuring glucose in the interstitial tissues should be remotely powered by radio-frequency energy [106]. A similar system for measuring glucose within a blood vessel might recover energy from glucose consumption [107] or from vibrations [108] induced by a fluidic flux.

Energy scavenging or remote powering for autonomous nanosensors and intelligent nodes has been extensively investigated in recent years. Inductive links have been investigated in depth for powering implanted sensors [109]. A system for an inductive link consists of two coils: the primary coil placed on the skin, and a second coil located under the skin. The first generates a variable magnetic field by means of an alternating current flowing through it; the variation of the magnetic flux through the implanted coil generates an electromotive force, in accordance with the Faraday–Neumann–Lenz law. In that way, the energy is transferred from the first to the second coil, and the remote powering is obtained.

Human movements also provide power that we can transform into electrical energy. This energy harvesting is of three different kinds – electromagnetic, electrostatic, and piezoelectric – depending on the method chosen to transduce the kinetic energy. As known, kinetic harvesting by using electromagnetic transducers is used for high-power applications, typically with synchronous machines in power plants. It is also used for powering small biosystems as well as quartz wristwatches such as the Seiko Kinetic [110]. In that case, the watch is able to automatically recharge itself by means of the wrist movements by means of a magnetic rotor.

Body thermal gradients also generate energy [111]. The physical phenomenon used is the Seebeck effect [112]: in the presence of a temperature difference between two different metals or semiconductors, a voltage drop is created across them. The core element of this kind of scavenger is a thermocouple.

A fuel cell is an electrochemical device that generates current through the reaction between two chemical species, one reduced at the anode and the other oxidized at the cathode [113]. The main difference from a classic battery is that the fuel cell can produce energy continuously, as long as the reactants continue to be present. So, an enzymatically catalyzed fuel cell based on the glucose redox reaction might be used for remote powering of a biochip in veins and arteries, where glucose is in sufficient quantity [114].

Alternatively, solar energy is converted into electrical energy by using photovoltaic cells. In the case of miniaturized photovoltaic cells, light and small

systems for energy scavenging from solar light are used for powering biomedical wearable diagnostics tools [115].

Recently, Teresa Meng showed that coils with a size of only 1 mm may be used to remotely power a subcutaneous brain electrode [116]. Sven Kerzenmacher reported the possibility to recover energy from electrochemical transformation of glucose in a fuel cell [114]. Koushik Maharatna and Bashir Al-Hashimi explored structural variability of carbon nanotubes with the aim of developing photovoltaic devices [117]. In Chap. 8, Koushik Maharatna and Bashir Al-Hashimi concentrate on harvesting from solar energy as enhanced by using carbon nanotubes and focus on powering body-worn nanosensors.

1.9 Toward Error-Free and Fault-Tolerant Nano-Biosensing Chips

As we have seen, we can obtain nano-biosensing chips for distributed diagnostics by integrating state-of-the-art technologies in the fields of nanotechnology, biotechnology, and microelectronics. Microelectronics already provides reliable silicon chip production with the technology node at 90 nm and it is now setting the new chip generation at 45 nm. This means a tremendous scale of integration. Biotechnology already provides recombinant DNA sequences and antibodies usable as molecular probes in biosensors for detection specificity. Nanotechnology already offers low-cost methods for producing metallic nanoparticles and carbon nanotubes that help in enhancing detection sensitivity and in lowering detection limits. So, system integration is now possible by merging contributions from different branches of science and technology. However, system integration has to deal with a new challenge to succeed in widely distributed biomedical systems: error-free and fault-tolerant biosensors.

Error-free systems already exist in information and communication theory as well as in microelectronics. In information theory, error detection and correction, or error control, are methods enabling error management to provide reliable data from unreliable noisy channels or communication systems. Noise affects all communication channels and, thus, errors are inevitably added to signals during their transfer from transmitters to receivers. Error detection enables identification of errors in the data received, whereas error correction enables reconstruction of the original transmitted data. Error correction is usually performed in two different ways: the so-called *automatic repeat request* (ARQ) [118] and the *forward error correction* (FEC) [119]. In the ARQ method, a verification-code sequence is added to the data received and the data are transmitted back to the data source with a data-retransmission request. Once the data have been received back, they are checked against the verification code and the data are retransmitted if the check fails. In the FEC method, data redundancy is used to improve information on the data sent. The transmitter adds a verification code to the transmitted data. Once the data have been received, the verification code is used to verify or reconstruct data that are most

likely similar to the original data. Of course, the two methods are often merged to obtain hybrid automatic protocols for error-free data transmission.

Very similar strategies for error correction are often used in flash memory. Flash memory is a nonvolatile memory usually used in memory cards and USB pen drives. It is a specific type of EPROM where data are managed (written and deleted) in blocks. In such a memory, an error-correcting code is written in a few bytes in each memory block. That code is used as a checksum each time data integrity is verified. The idea is to check for errors that might be accidentally introduced during writing, copying, or storing operations. The checksum may be recalculated any time and compared with that stored in the check bytes previously registered in the data block. If the calculated checksum does not match the stored one, the data are corrupted.

Another very important concept introduced in computer science in the 1970s is that of fault-tolerant systems. The fault tolerance is the ability of a system to tolerate faults without stopping its working functions. In 1983, Algirdas Avižienis introduced at the annual International Symposium on Computer Architecture the concept of fault-tolerant system design by introducing the ideas of system pathology, fault detection, and proper recovery algorithms [120]. The fault-tolerance concept was initially considered only for single processors. Nowadays, the concept is largely used also in multicore computing [121], multimachine interactions [122], and network-on-chip [123]. In general, fault tolerance is the ability of a machine to continue operating in the event of failure of one of its components. Of course, the quality of fault-survived working functions depends on how many components fail and how severe the fault events are. But, in any case, the machine has the possibility to remain operating even only partially, which is so important in comparison with a not-fault-tolerant system that goes into total breakdown in the case of a small failure of a single component.

To succeed in distributed diagnostics, present development of nano-biosensing needs to take into account the strategies of fault-tolerant and error-free systems to ensure biochips are robust enough to be used at home by patients and healthy people who may have little knowledge of science and technology. In Chap. 9, Yang Liu and Shantanu Chakrabartty present the new concept of error-free biosensors and describe new lines of development toward robust and reliable nano-biosensing VLSI chips.

1.10 Conclusion

In this first chapter, we have seen different aspects of nano-biosensing. New detection methods are offered by nanotechnology for unexpected scales of sensitivity. Atomic force spectroscopy enables detection of piconewton forces on samples scanned with picometer resolution. Surface plasmon resonance allows molecular detection of very few nanomoles in protein–protein interactions. Mass-sensitive biodetection can also extend to the range to sub-nanomoles. In addition, optical

tweezers enable manipulation in a liquid at the level of a single cell and a single molecule, even allowing nanopattering of the substrate surface. Electrochemical sensing offers fully electronic readers for biosensing, with the possibility to directly integrate silicon chips, organic nanoenhancers, and molecular probes. With the 90-nm technology node fully operating now and the newly envisaged technology node at 45 nm, modern VLSI electronics provide a previously impossible scale of integration and new ideas for energy harvesting and the development of error-free biochips will lead to a monitoring nano-biosensing chip that is autonomous, robust, and reliable enough for daily use at home or in the field.

Scientific and technical details making it possible that distributed diagnostics might became a reality in the next 10–15 years are presented and discussed in the next pages of this book.

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