

REVIEW

Theory, fabrication and applications of microfluidic and nanofluidic biosensors

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Biosensors are a broad array of devices that detect the type and amount of a biological species or biomolecule. Several different types of biosensors have been developed that rely on changes to mechanical, chemical or electrical properties of the transduction or sensing element to induce a measurable signal. Often, a biosensor will integrate several functions or unit operations such as sample extraction, manipulation and detection on a single platform. This review begins with an overview of the current state-of-the-art biosensor field. Next, the review delves into a special class of biosensors that rely on microfluidics and nanofluidics by presenting the underlying theory, fabrication and several examples and applications of microfluidic and nanofluidic sensors.

Keywords: biosensors; microfluidics; nanofluidics; cancer; devices; fluid slip

1. Introduction

Modern, productive and advancing societies function owing to improved lifestyle and health of the constituents obtained through major improvements in environmental factors such as cleaner water, air and food. Furthermore, health and life science-related improvements have also contributed to the growth of our society. One scientific tool that has enabled these improvements is the use of sensors for detecting chemical and biological molecules that are critical to the functioning of humans and other societal constituents. Among the various sensors and detectors that have been developed for identifying chemical and biological molecules, biosensors find a special place.

Biosensors are devices typically used to detect target biomolecules such as proteins, enzymes or nucleic acids though they can have other applications such as the detection of chemical contaminants in water. Biomolecules or chemical contaminants can be sensed or detected through a variety of mechanisms but generally detection involves an interaction between the target molecule and a transducer to produce a measureable signal indicating the presence and concentration of that target molecule within a sample. In the case of a mixture

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of target molecules, the biosensor would enable the ability to discern between different species. Therefore, an ideal biosensor will provide specific information on the type and amount of the biomolecules present in a test sample.

Conventionally, biosensors were developed in two broad categories: (i) microarray type, which usually comprised cantilever or field-effect devices with adsorption of target analytes to sensing elements as the main transduction mechanism, and (ii) microfluidic and nanofluidic sensors that usually involved manipulations of small fluidic volumes (microlitres to nanolitres) leading to an optical method for detection. However, in the past decade advances in micro- and nanofabrication have led to the miniaturization of several analytical methods such as gas chromatography [1] and the integration of methods such as mass spectrometry with traditional separation techniques such as capillary electrophoresis [2]. These advancements have led to the development of integrated biosensors that bring together several unit operations (e.g. sample extraction, purification, sensing, etc.) on a single platform. Therefore, this review first discusses broad detection mechanisms, and then describes the various platforms used in biosensors including integrated systems that combine both microfluidic (or nanofluidic) and microarray types.

(a) Detection and transduction methods

Detection can be performed by a variety of methods. Common optical methods include fluorescence [3–6], optical cavity resonators [7–9] and surface-plasmon resonance or SPR [10–16] in which a surface-based chemical reaction corresponds to a change in the refractive index and thus a shift in the optical signal. Electrochemical impedance spectroscopy or EIS [17–19] detects changes in surface or nanostructure impedance owing to surface reactions or transport. In other electrically based techniques, a surface-based reaction corresponds to a change in a measured electrical signal such as current [20–23], resistance [24–26], capacitance [27] or conductance [28–34] of the test sample. In addition, detection methods could also rely on mechanical changes induced owing to adsorption of target molecules or analytes to cantilevers [35–37] or nanowires [33], changes to inherent biomolecular charge in the case of field-effect transistors, sometimes also classified as microarray-type biosensors [38–43], or electrochemical changes such as those arising from redox reactions inducing variations in current flow [44,45]. Therefore, biosensors form a complex area of research given the diversity of target molecules, need for low false positives and variety of detection platforms available [46–50] with a brief summary presented in table 1*a*.

(b) Microfluidic and nanofluidic biosensor platforms

Micro/nanofluidic-based biosensors present one platform with detection often integrated onto the same platform with different elements to control and manipulate fluidic transport as summarized in table 1*b*. Additionally, target biomolecules are typically found in some types of solution, and may require use of microarrays to immobilize target species on the surface of the biosensor [58,59]. The solvents are usually aqueous solutions to preserve the natural state, or after processing may be organic materials. The sensor should be able to perform the

Table 1. (a) Summary of various detection mechanisms in biosensors. (b) Various methods of fluid manipulation in microfluidic and nanofluidic platforms.

(a) detection method	detection mechanisms and platforms ^a	measurable response to target analyte	references
mechanical	cantilevers	bending of cantilever or change in resonant frequency	[35–37]
	nanowires	change in surface charge through protonation/deprotonation	[33]
electrical	field-effect transistors	changes in inherent biomolecular charge	[38,41–43,51]
	nanowires	changes in electrical signal such as current, resistance, capacitance or conductance	[24–34]
optical	fluorescence	intensity of fluorescent signal	[3–5]
	optical cavity resonator	shift in resonance wavelength proportional to change in mass	[7,9]
	surface-plasmon resonance	shift in refractive index	[10,11,13–16,52]
(b) sample manipulation method	underlying principle involved		references
pumps	electrokinetic pumping (including electro-osmosis)		
valves	surface modification		[53]
droplets transfer	electrically controlled surface tension drives liquid droplets		[54,55]
arrays/mixing of fluids	use of heterogeneous surfaces (e.g. use of nanoholes or modified surfaces)		[56,57]

^aAs discussed in the text, many of the mechanisms can be combined with sample manipulation techniques in table 1*b* in microfluidic or nanofluidic platforms or can be used as stand-alone methods. The development of integrated biosensors usually combines several unit operations on a single platform.

function regardless of background solvents and interfering agents. Consequently, many different types of biosensors have been developed. In this article, the focus will be on microfluidic and nanofluidic biosensors. Specifically, the review will discuss the underlying physical and operating principles, as well as the fabrication of biosensor devices that rely on microfluidics and nanofluidics.

There has been a growing desire to create portable detection devices that can perform measurements in a matter of minutes using increasingly smaller sample volumes [10,24,25,60–62] approaching the picolitre range. Several commercial examples for biosensing platforms exist including point-of-use type kits such as home pregnancy tests for detection of hormones such as human chorionic gonadotropin (hCG) for early indication of pregnancy. Another familiar example of a commercially available portable biosensor is a home blood glucose meter for diabetics [63]. It is expected that with smaller volumes, higher specificity for detection can be achieved with the eventual goal of single-molecule detection.

Such goals have driven the development first of microfluidic systems beginning in the early 1990s and now the development of nanofluidic systems starting in the late 1990s [64]. In fact, new platforms and systems are constantly being designed to detect a single or a class of (bio)chemicals, or with system-level analytical capabilities for a broad range of (bio)chemical species and have led to the emergence of a whole array of platforms known as micro total analysis systems (μ -TAS) or lab-on-chip (LoC), which have the advantage of incorporating sample handling, separation, detection and data analysis onto one platform. In common laboratory settings, the test biofluid (e.g. blood, urine, saliva or tear-drops) is injected into the chip generally using an external pump or syringe for analysis. Some devices have also been designed and built with integrated micropumps. As an example, figure 1 [41] shows a biosensor that can be incorporated in a wristwatch and a cassette-type biosensor with multiple unit operations on a single platform. Other platforms such as those modelled after compact discs (CDs) that use centrifugal forces have also been developed [65]. This integration of multiple unit operations on a single platform is the hallmark of biosensors as these are typically considered multi-functional, multi-component devices [38,66]. Furthermore, since most biosensors require working with biomolecules in an aqueous electrolyte solution to imitate the most natural environment, microfluidic and nanofluidic systems lend themselves as the most straightforward technological platforms for the development of specific biosensors.

(c) *Introduction to microfluidics in biosensing*

Microfluidics generally refers to the study of fluidic systems with critical operational lengths in the 1–100 μm range, while nanofluidics is defined as the study of fluidic systems with critical operational length scales at 1–100 nm [56,67]. These devices and systems are characterized by high surface-area-to-volume (SA/V) ratios. Practical devices with SA/V ratios of the order of 10^9 m^{-1} have already been fabricated [67]. There are two main methods that have been used for driving the flow of fluids in micro- and nanochannels: pressure-driven and electrokinetic. With decreasing channel dimensions, pressure-driven flow may become a challenge as discussed in a recent review [67]; therefore electric-field driven or electrokinetic flows are the primary method for fluid (and biochemical

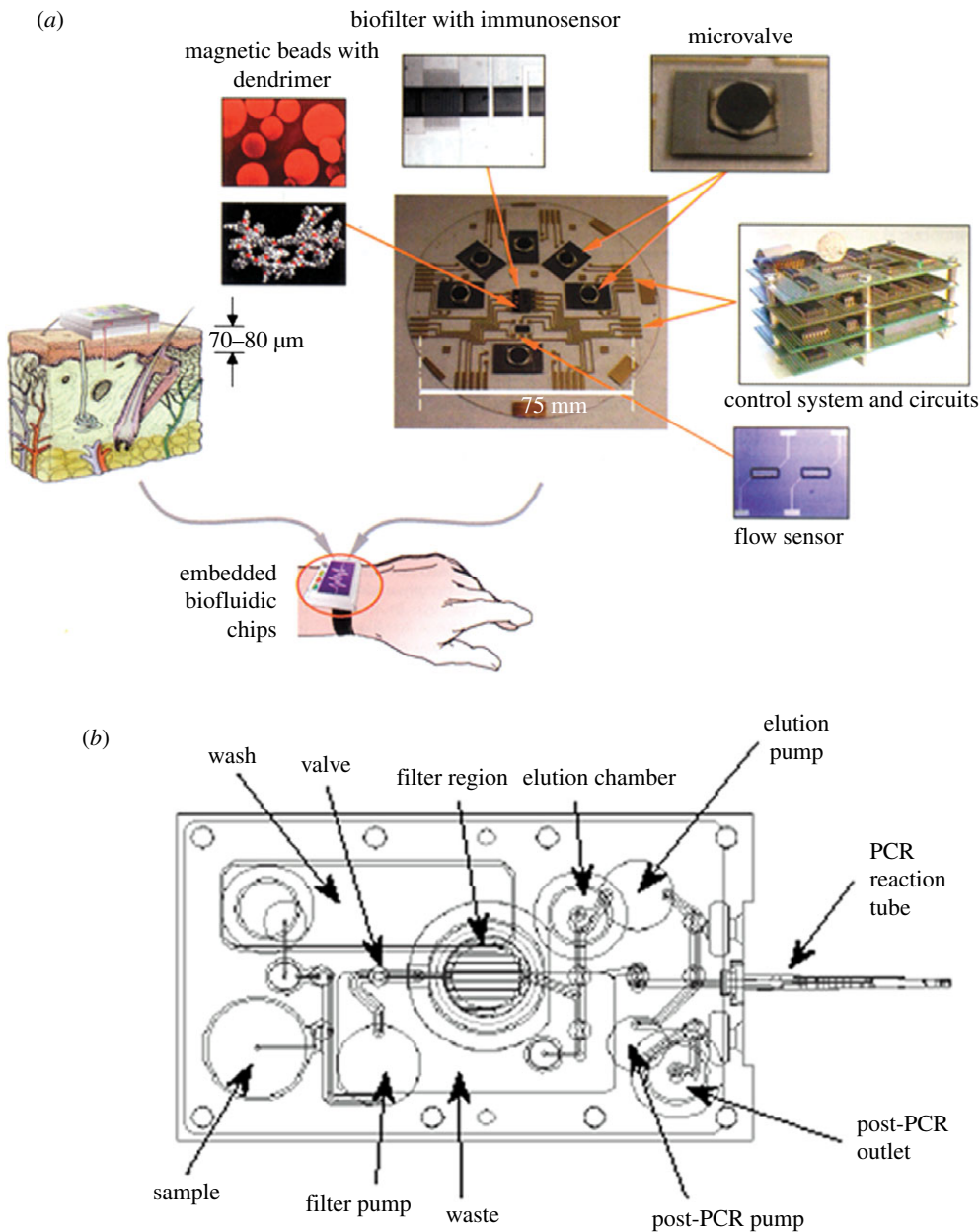


Figure 1. (a) An example of a microfluidic-based biosensor that can be incorporated onto a wristwatch. The lab-on-a-chip system relies on manipulation of small volumes of fluid in microchannels using microvalves. (b) A cassette-type biosensor used for human genomic DNA analysis. The use of micropumps and microvalves allows for fluid manipulation and multiple sample processing steps in a single cassette [41]. (Online version in colour.)

species) actuation and consequently biosensors based on electrokinetic flow have also been developed. In the so-called ‘digital based microfluidics’ based on electro-osmosis [67,68], electrically controlled surface tension is used to drive

liquid droplets [54,55]. Direct manipulation of electro-osmosis through chemical modification of surfaces to preferentially direct flows has also been demonstrated [53] thus eliminating the need for valves and pumps.

Consequently, there are reports in the literature showing that micro- and nanofluidic devices enable detection of target molecules in increasingly smaller concentrations (down to 0.2 fM) [69]. If we were to imagine a relatively large nanochannel or nanopipe with a 100 nm diameter and a length of 10 μm , the volume available would be of the order of approximately 10^{-20} m^3 (or nearly 80 al), and it could accommodate approximately 10^8 water molecules, at room temperature and pressure. Therefore, at saturation, such a nanochannel or nanopipe would be able to accommodate a 10 fM concentration of target molecules with similar size to water. In most cases, it can be expected that the target analytes maybe much larger in size than a water molecule and at least one to two orders of magnitude lower in concentration, suggesting the possibility that nanofluidics can enable detection down to the 1–100 aM (attomolar or 10^{-18} M) range. From a biosensor viewpoint, the ability to detect 10 000 or fewer molecules of a biological species presents a very lucrative idea for further research. Scaling down of length scales has already spawned extensive research activities in molecular diagnostic and analysis tools based on microfluidic and nanofluidic systems, described as LoC or $\mu\text{-TAS}$ above. Systems have already been developed for cell sorting [70], rapid PCR and DNA microarrays [71–74] and diagnostic methods based on RNA detection for applications towards early cancer detection [75], point-of-care devices in unconventional or hazardous environments, and for management of diseases during pandemics and natural disasters [76–80].

Despite these advances, fundamental physics of mass transport and reaction time places limitations on the concentration of target molecules that can be detected as well as the speed at which they can be detected. Typical characteristic time scales, t_c , for biosensors are limited by the travel time of the target molecule to the sensing site and consequently rely on the diffusion of the species. Therefore, t_c would be expected to scale as l_c^2/D with l_c being the characteristic length of the device and D being the diffusivity of the species of interest [81,82]. Therefore, with smaller channel size, i.e. lower l_c , t_c will also become smaller and enable faster detection. The direct consequence is that microfluidic and, even more so, nanofluidic sensors can use geometry to enable faster diagnostics. Often, increased detection speed and decreased minimum concentration tend to be competing interests. Increased detection speed requires increased fluid velocity which may not leave enough time for target molecules to diffuse across a channel and bind to a detection site before being transferred downstream. Therefore, even though the characteristic length scale may enable highly sensitive detection, the actual transport may be a limiting factor in achieving lower detection limits. Considerable effort has been placed on designing and constructing sensors that balance the need for these and other competing factors, as discussed throughout this review.

In this review, we seek to outline the fundamental fluid mechanics that comes into play in biosensors with the goal of highlighting potential areas of concern that if resolved would enable even better design and operation of biosensors. Additionally, we will review the fabrication techniques employed for building devices at the micro- and nanoscale. Finally, we will review recent types of biosensors, the way in which they detect the presence of target molecules and the

reliability and detection limits of these current state-of-the-art devices. Therefore, the purpose of this review is to provide a detailed overview of microfluidic and nanofluidic biosensors. Given the breadth of topics discussed in this review, it is nearly impossible to include all published research. Therefore, throughout this article, the reader is directed to several other related reviews, books and additional references that provide in-depth discussions of specific topics.

2. Theory

(a) Fluid mechanics and scaling analyses for microfluidics and nanofluidics

Nanofluidic devices are typically defined as devices which have at least one critical dimension in the range of 1–100 nm. Perhaps a broader definition should include the concept that the device operation length scales are the same as the length scales for phenomena governed by small collections of molecules. For example, devices with volumes of 10^{-21} m^3 have been fabricated with the ability to confine hundreds of thousands of molecules. Compare this to Avogadro's number, $N_A = 6.023 \times 10^{23}$, which represents 1 mol of a substance at room temperature and pressure. With these small ensembles of molecules, nanofluidics enables highly sensitive devices and sensors, especially for biological species, as discussed above. Consequently, owing to the high SA/V ratio (approx. 10^9 m^{-1}), interactions with the walls become increasingly important at these length scales [56]. In the next few paragraphs, some important dimensionless parameters, their physical significance, and their use with respect to biosensors are discussed.

The Reynolds number is a non-dimensional parameter that gives the ratio of the inertial forces to the viscous forces. The Reynolds number is given by the equation

$$Re = \frac{\rho u l_c}{\mu}, \quad (2.1)$$

where ρ is the density of the fluid, μ the viscosity, l_c the characteristic length for the flow and u the average velocity. For microfluidics and nanofluidics, the critical dimension, which is often the channel height, becomes increasingly small and consequently corresponds to increasingly small Reynolds numbers. Thus, the ratio of inertial forces to viscous forces decreases, indicating that the viscous forces dominate over the inertial forces at these length scales.

In most microfluidics and nanofluidics applications, a particle (ion, colloid, biomolecule, AFM probe tip, etc.) will interact with the channel walls. Considering three important forces, electrostatic or coulombic, F_{el} , van der Waals, F_{vdw} , and hydrodynamic forces, F_h , provides better insight to surface–particle interactions and subsequent phenomena. For an infinite flat surface separated by a distance D from a particle of radius R , F_{el} is given by [83]

$$F_{\text{el}} = -\frac{2\pi R L_D}{\epsilon \epsilon_0} \left[2\sigma_S \sigma_P \exp\left(-\frac{D}{\lambda_D}\right) + (\sigma_S^2 + \sigma_P^2) \exp\left(-\frac{2D}{\lambda_D}\right) \right], \quad (2.2)$$

where λ_D is the Debye length and σ_S and σ_P are the surface charge densities of the sample and the particle, respectively. F_{vdw} is given by [83]

$$F_{\text{vdw}} = \frac{A_H R}{6D^2}, \quad (2.3)$$

where A_H is the Hamaker constant, which serves as an indicator of the interaction between the particle and the sample surface. F_h is given by [83]

$$F_h = b_s V = -f^* \frac{6\pi\mu R^2 V}{D}, \quad (2.4)$$

where V is the particle velocity, b_s is the hydrodynamic damping coefficient and μ is the viscosity of the fluid. The coefficient f^* is related to the boundary slip properties. If the no-slip assumption holds true at the solid–liquid interface, then $f^* = 1$, while if slip exists $f^* < 1$. As an aside, it should be noted that equation (2.4) is often used in the literature for determining slip-lengths using a colloidal AFM probe tip. As the operational length scales (here, characteristic length scale is separation distance, D) decrease, it can be seen from equations (2.2)–(2.4) that F_{el} displays an exponential dependence, F_{vdw} an inverse quadratic dependence and F_h an inverse linear dependence on the separation distance. Therefore, consideration of each force term at the microscale, and even more so at the nanoscale, is essential towards completely describing the importance of wall–species interactions.

(b) *The electric double layer*

The concept of the electric double layer or EDL is derived from the colloidal sciences [67,84] but finds wide use and applicability in microfluidics and nanofluidics [85–88]. We begin by a short discussion on the origin of surface charge. Surfaces in contact with an aqueous solution often obtain a net surface charge. This can occur by dissociation of surface groups, as in the dissociation of protons from surface-bound carboxylic acid groups leading to a negatively charged surface ($-\text{COOH} \rightarrow \text{COO}^- + \text{H}^+$), or through adsorption of ions from the solution [84]. Glass, which is commonly used for micro/nanofluidic-based biosensors, obtains a net negative surface charge when placed in contact with an aqueous solution at neutral pH through the dissociation of surface silanol groups. Ions with opposite polarity arrange themselves along the charged surface to form a fixed layer of counter-ions known as the Stern layer. Outside of the Stern layer is the mobile diffuse layer. The zeta (ζ) potential is measured at the slip plane located between the Stern and diffuse layers. The Stern and diffuse layers make up the electric double layer. The EDL screens the bulk solution, which is electrically neutral outside of the EDL. The screening length is known as the Debye length and is given by

$$\lambda_D = \sqrt{\frac{\epsilon_e RT}{F^2 \sum_i z_i^2 c_i}}, \quad (2.5)$$

where ϵ_e is the electric permittivity of the medium, R the gas constant, T the temperature, F Faraday's constant, z_i the valence of the i th species and c_i the concentration of the i th species. Typical Debye lengths range from about 0.1 to 100 nm with larger Debye lengths corresponding to smaller concentrations [89]. The concept is depicted schematically in figure 2a [67]. It should be noted from equation (2.5) that the Debye length is a function of the inverse square root of the bulk ionic concentration. In addition, the Debye screening length represents

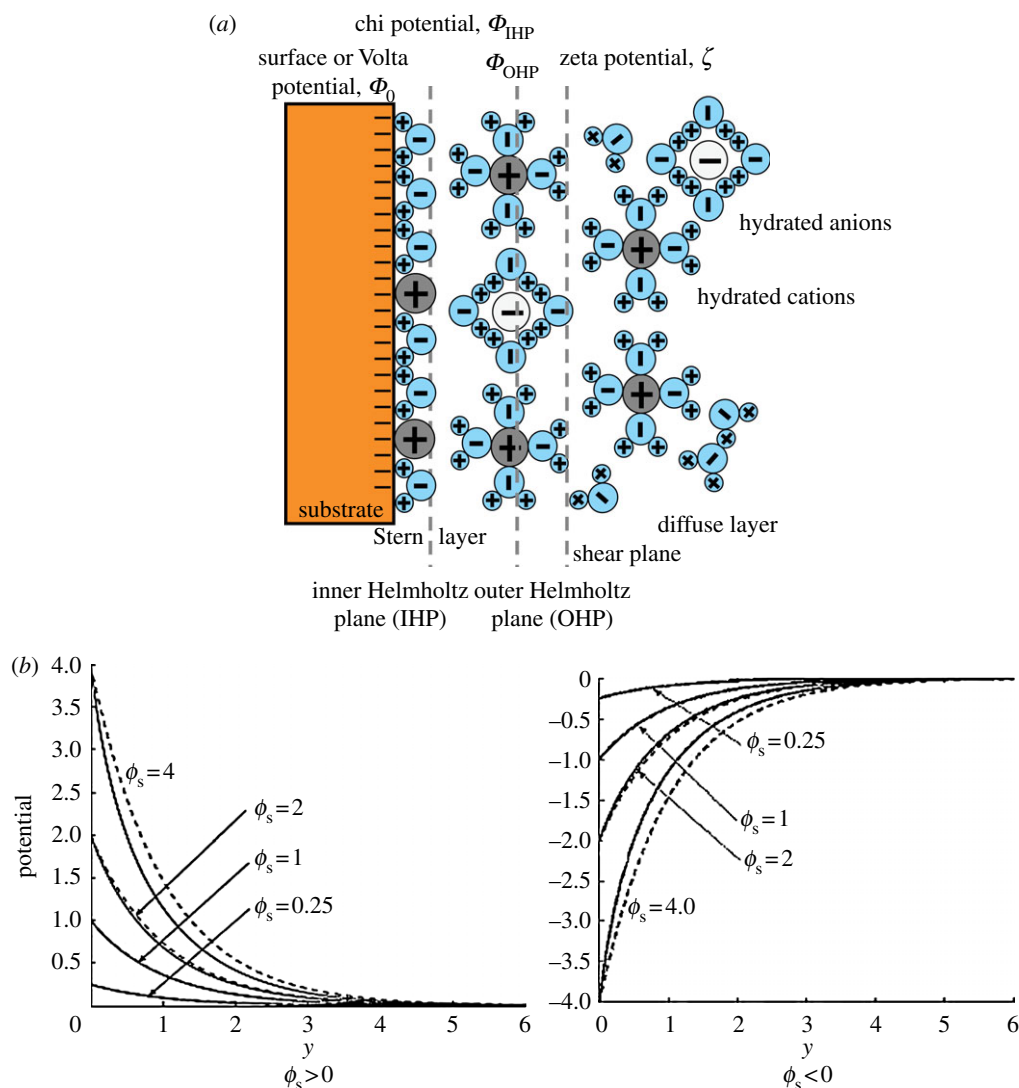


Figure 2. (a) A schematic of the electric double layer [67]. (b) The dimensionless potential is shown for a slit-like or circular channel where the Boltzmann equation applies (non-overlapping EDL). The dimensionless value y is the distance away from the channel wall over the Debye length and ϕ_s is the surface potential. A 1 : 1 electrolyte and a bulk ionic strength of 0.3 M were used to generate the plots [68]. Solid curves, analytical; dashed curves, Debye-Hückel. (Online version in colour.)

the $1/e$ (where e is the base of the natural logarithm) cut-off for the drop in surface potentials. The distance is tracked from the wall to the bulk solution, where the electrolyte solution is electrically neutral.

As channel sizes are decreased, the EDL occupies a larger portion of the channel. Depending on the channel dimensions and Debye length, the EDLs may in fact overlap and the electric potential in the bulk will no longer be neutral. The relative size of the Debye length compared with the channel plays a crucial

role in the dominant physics of ion transport. When the Debye length is small compared with the radius of the nanochannel ($\kappa a \geq 1$ where κ is the inverse Debye length and a is the radius of the channel) electromigration, the transport of ions under the influence of an electric field, is the dominant effect. If the EDL spans the width of the pore ($\kappa a < 1$) then electro-osmotic flow, the bulk fluid motion induced by an electric field, is the dominating effect [85]. The Debye length can be controlled by altering the concentration of the solution or by changing the surface charge density [85,90]. In the case of strongly overlapped EDLs, the co-ion exclusion principle [91] may occur, which can be used for permselectivity of ions. This means that only counter-ions are present in the bulk of the solution and thus co-ions are excluded from the nanochannel/nanopore. In the case of biosensors, the presence of certain molecules may interfere with the ability to detect a target molecule. For example, glucose sensors work by measuring the rate of the glucose oxidase-catalysed oxidation, which can be monitored through the rate at which hydrogen peroxide is formed. Other common molecules in biological samples such as ascorbate, urate and acetaminophen interfere with the detection of hydrogen peroxide. A permselective polymer layer is used to eliminate the interference of these molecules from measurements [92,93].

The presence of the EDL plays a significant role when using silicon nanowires for electrical detection of target molecules. The Debye layer can cause screening of the sensing area, which leads to the inability for target molecules to bind to the chemically modified sensing surface [94]. Additionally, a recent study has shown that the conductance of a silicon nanowire changes linearly with the flow velocity in the channel. The streaming potential, which refers to the movement of counter-ions in the EDL caused by an applied electric field, depends on the flow velocity. It was found that the magnitude of the conductance changes in the silicon nanowires depended on the streaming potential, ionic strength and composition of the electrolyte solution used as a solvent for target molecules. This is an important consideration for silicon nanowires since the detection of target molecules is based on conductance changes in the nanowires caused by binding events of the target molecules. This study showed that electrical properties in the buffer solution must be considered when building devices that use nanowires as biosensors. The study also demonstrated the ability to use nanowires to measure flow velocities in microchannels [95].

(c) *Electrokinetics in microfluidics and nanofluidics*

When dealing with microchannels and nanochannels, it is often preferable to use an electric field rather than a pressure gradient to drive flow through the channels. From the Hagen–Poiseuille equation, we know that the pressure drop, $|\Delta P|$, in a nanochannel is related to the volumetric flow rate Q by [96]

$$|\Delta P| = \frac{128\mu L}{\pi d^4} Q, \quad (2.6)$$

where μ is the viscosity of the fluid, L is the length of the channel and d is the channel diameter. So to achieve a flow rate of 1 pl s^{-1} for water in a channel that is $10 \mu\text{m}$ long and 10 nm in diameter would require a pressure

drop of approximately 40 GPa. The Hagen–Poiseuille equation (2.6) assumes laminar, viscous, incompressible, pressure-driven flow, where the no-slip condition holds.

The application of an external electric field across the channel results in a net force on the ions in the EDL. As the ions in the diffuse layer move under the influence of the electric field, they drag the rest of the fluid along with them resulting in bulk fluid motion known as electro-osmotic flow. The electro-osmotic flow velocity is found by solving the Navier–Stokes equation with a body force given by $\rho_e E$, where ρ_e is the net charge density and E is the applied electric field. The net charge density comes from the distribution of ions in the EDL which from Poisson’s equation is

$$\varepsilon \nabla^2 \psi = \rho_e, \quad (2.7)$$

where ε is the dielectric constant or function of the fluid and ψ is the potential distribution of the EDL. Poisson’s equation is a nonlinear second-order differential equation, which when coupled with the governing equation for the charge density gives rise to situations where an analytical solution does not exist, and the EDL field must then be described through a numerical solution. However, in certain cases, analytical solutions are available. For example, in the case of microfluidics, when the channel walls are sufficiently far apart and the net charge in the centre of the bulk region of the channel is nearly neutral, the net charge distribution outside the shear plane is described using the Boltzmann distribution [97] which has the functional form

$$\rho_e = -2zec_0 \sinh\left(\frac{ze\psi}{k_b T}\right) \quad (2.8)$$

for a symmetric, $z:z$ electrolyte. In equation (2.8), z is the valence, c_0 the concentration of the bulk electrolyte, e the charge of an electron, k_b the Boltzmann constant and T the temperature of the fluid. Therefore, by substituting equation (2.8) into equation (2.7), the governing differential equation for the potential distribution becomes

$$\nabla^2 \psi = \frac{-2zec_0}{\varepsilon} \sinh\left(\frac{ze\psi}{k_b T}\right) \quad (2.9)$$

for the case when the electric field in the fluid caused by the EDL can be assumed to be one-dimensional. This assumption holds in the case of a slit-like or circular microfluidic channel for which analytical solutions have previously been reported [98]. As an example of what the analytical solution looks like visually, the dimensionless potential is plotted for different distances y from the channel wall over the Debye length in figure 2*b* [68]. Figure 2*b* shows the exponential decay in potential starting at the channel wall and extending out into the electrically neutral bulk fluid. For a biosensor that relies on surface–species interactions, the main consequence is that large changes in potential can occur at the wall for analyte–wall interactions. Equation (2.9) is often linearized using the Debye–Hückel approximation, which is considered valid when $|ze\zeta| < k_b T$ [97], or a wall potential of nearly 26 mV. If the Debye–Hückel approximation is used, then an analytical solution has also been derived for a rectangular

microfluidic channel [98]. It should be noted that the Boltzmann distribution shown in equation (2.8) does not apply for the case of overlapping EDLs [99], since the bulk is no longer electrically neutral.

Many applications might require a combination of pressure and electrically driven flows. In such cases, when the flow is being driven both by a pressure gradient and an applied potential difference, the pressure gradient induces a flow of charge in the diffuse layer and generates a finite voltage called the streaming potential. The electric field in the fluid then is no longer one-dimensional [100]. In such cases, numerical solutions are often needed. It should be noted here that in nanochannels situations regularly arise where either electroneutrality is violated or the electric field gradients are not one-dimensional; therefore for nanochannel flows, theoretical predictions usually rely on numerical simulations and advanced methods such as molecular dynamics.

In microchannels, most of the assumptions and equations described above hold well and therefore enable a broader range of analytical solutions. For example, for thin EDLs (i.e. $\lambda_D \ll h$), the method of matched asymptotics gives a velocity expressed as

$$u = -\frac{\varepsilon\psi}{\eta}E = \mu_{EO}E, \quad (2.10)$$

where the velocity u is the electro-osmotic velocity with μ_{EO} denoting the electro-osmotic mobility. Equation (2.10) is commonly referred to as the Helmholtz–Smoluchowski approximation for determining the electro-osmotic velocity. This result gives a plug-like flow distribution for electro-osmotic flow in channels that have thin double layers [68]. Notably, different ions will have different values for their electro-osmotic mobilities. If there are several different types of ions in a solution, the ions will move at different velocities under the influence of the electric field, causing them to separate. This forms the basis of many microchip-based electrophoretic techniques. By using the fundamental principles for electrokinetic phenomena described above, many different sensors and techniques have been developed and discussed in the literature as reviewed throughout this article. One recent example includes the preconcentrating of analytes in order to enable lower detection limits in nanofluidic-based biosensors [4].

(d) Analyte transport regimes

Detection of target molecules is limited by the diffusion of the molecule to the sensor as well as by the target molecules' ability to bind to the sensor. In the case of a device that has no bulk flow, the diffusion of target molecules across the width of the channel is governed by

$$J_i = -D_i \frac{\partial c_i}{\partial x}, \quad (2.11)$$

where J_i is the molar flux, D_i the diffusion coefficient and c_i the concentration of the i th species in the x -direction or usually along the length of the channel. Equation (2.11) is the one-dimensional form of Fick's first law of diffusion and generally is applied to microchannels or larger nanochannels (of the order of several hundred nanometres). However, as nanochannel dimension is decreased, diffusion becomes hindered. The Renkin equation gives the extent to which the

diffusion coefficient is reduced in a confined space [67]. Following the discussion in §1 about the characteristic measurement time, t_c , the diffusion of the molecule to the sensor, therefore, imposes a minimum amount of time necessary for the detection of a target molecule. However, in most devices, the flow is being driven through an external force and so convection of the fluid must also be considered.

The convection of target molecules in the presence of concentration gradients and diffusion is determined by the Nernst–Planck equation, which is discussed here in the one-dimensional form for convenience,

$$J_i = -D_i \frac{\partial c_i}{\partial x} + c_i u - \mu z_i c_i \frac{\partial \Phi}{\partial x}, \quad (2.12)$$

where u is the bulk fluid velocity, z_i the valence of the i th species and Φ the electrical potential which is the sum of the applied potential and the local potential from the EDL.

As target molecules are collected by the sensor, a depletion zone is created around the sensor. A recent review by Squires *et al.* [81] gives a qualitative description of the way the depletion zone develops with time. The depletion zone creates a concentration gradient along the length of the channel, and is represented by the first term in the Nernst–Planck equation (2.12).

As flow rate is increased, target molecules may not have enough time to diffuse across the width of the channel to the detector (usually located at or near the channel walls) before being transferred downstream past the detector. Therefore, the fluid mechanics should work in such a way so as to allow sufficient time for the target molecule to bind to the detector. However, if the bulk fluid velocity is too slow, the depletion zone grows increasingly larger as described previously [81]. Consequently, very few molecules may be close to the detector, which slows down detection and limits the resolution of detection. Alternatively, if the flow rate is increased in order to decrease the size of the depletion zone, the sensitivity of the detector will be reduced. Target molecules in the channel that are further away from the sensor will be swept downstream before having enough time to diffuse across the channel and bind to the detector. Figure 3 [81] shows COMSOL simulations that illustrate how the development of the depletion zone in time influences the flux of target molecules to the sensor in the case where there is no bulk fluid flow. Therefore, accounting for the molecules that pass by the sensor without binding, a larger minimum concentration will be required to detect the presence of the target molecule when there is bulk fluid motion. Notably, devices are being designed that incorporate depletion zone mixing to enhance delivery of the target molecule from the bulk solution to the sensing surface [57,101,102]. Tests are also performed to optimize the flow rate by measuring the dependence of signal strength on flow rate in many microfluidic-based biosensor devices [26,61].

(e) Considerations for slip flow

If the adhesion between the micro/nanochannel surface and the biofluid is high, biomolecules will stick to the channel surface and resist flow. In order to facilitate flow, channel surfaces with low bioadhesion are required. Fluid flow in polymer channels can produce triboelectric surface potential which may affect the flow [103]. Polymers are known to generate surface potential, and

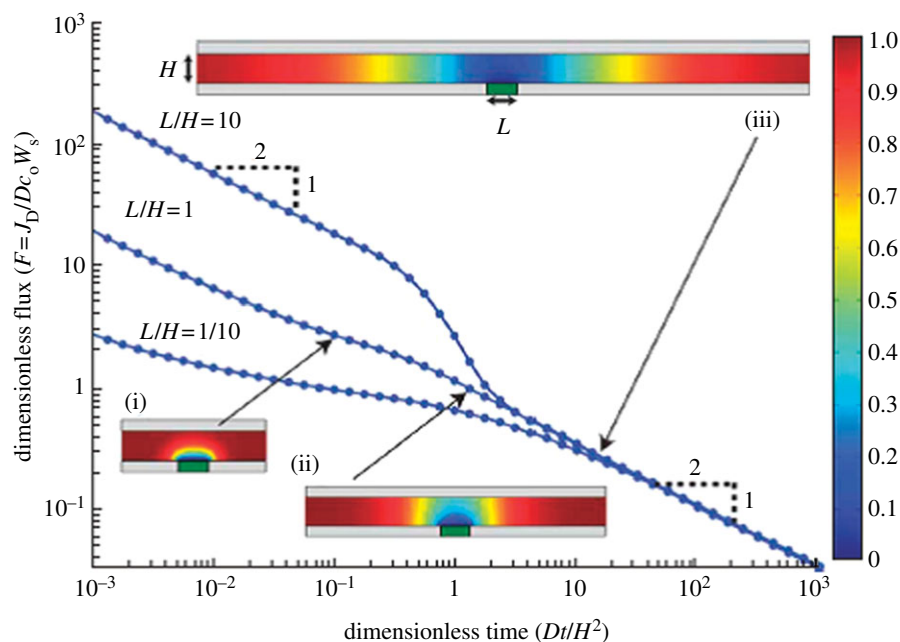


Figure 3. A COMSOL simulation that shows the dimensionless flux of target molecules to a biosensor as a function of the dimensionless time. The biosensor is assumed to be as wide as the channel. For the purposes of the simulation, the target molecules diffuse to the detector and bind immediately upon reaching it. There is no bulk flow in this case [81]. (Online version in colour.)

the magnitude of the potential varies from one polymer to another [104–106]. Conductive surface layers on the polymer could be deposited, if possible, to reduce triboelectric effects.

Hydrophobic surfaces exhibit slip which reduces fluid drag [107–109]. The study of the interactions between hydrophobic surfaces and aqueous solutions reveals that during wetting, spherical cap-shaped bubbles with sub-100 nm diameter are formed on the hydrophobic surface at the solid–liquid interface [109–111]. The nanobubbles are believed to be charged species and an EDL forms at the gas–liquid interface, which imparts electrostatic stabilization to these structures. Nanobubbles are formed readily on water-repellant hydrophobic surfaces as shown in figure 4*a* [110]. The size and propensity of nanobubbles are potentially controlled by the charged species in the liquid wetting the hydrophobic surface, bias at the surface and surface heterogeneity among other parameters. Surface roughness and chemical heterogeneity as well as pH value have been shown to have a significant influence on the tendency of nanobubble formation on surfaces of comparable hydrophobicity [111]. The presence of nanobubbles is expected to influence fluid slip at the interface (figure 4*b*) and consequently liquid adhesion, and fluid flow characteristics [110].

The fluid slip has been measured using an atomic force microscope (AFM) in tapping mode. It has been shown that water slip over hydrophilic surfaces (contact angle approx. 0°) is zero, whereas for hydrophobic (contact angle $> 90^\circ$) and superhydrophobic surfaces (contact angle approx. 167°) it is 43 and 236 nm,

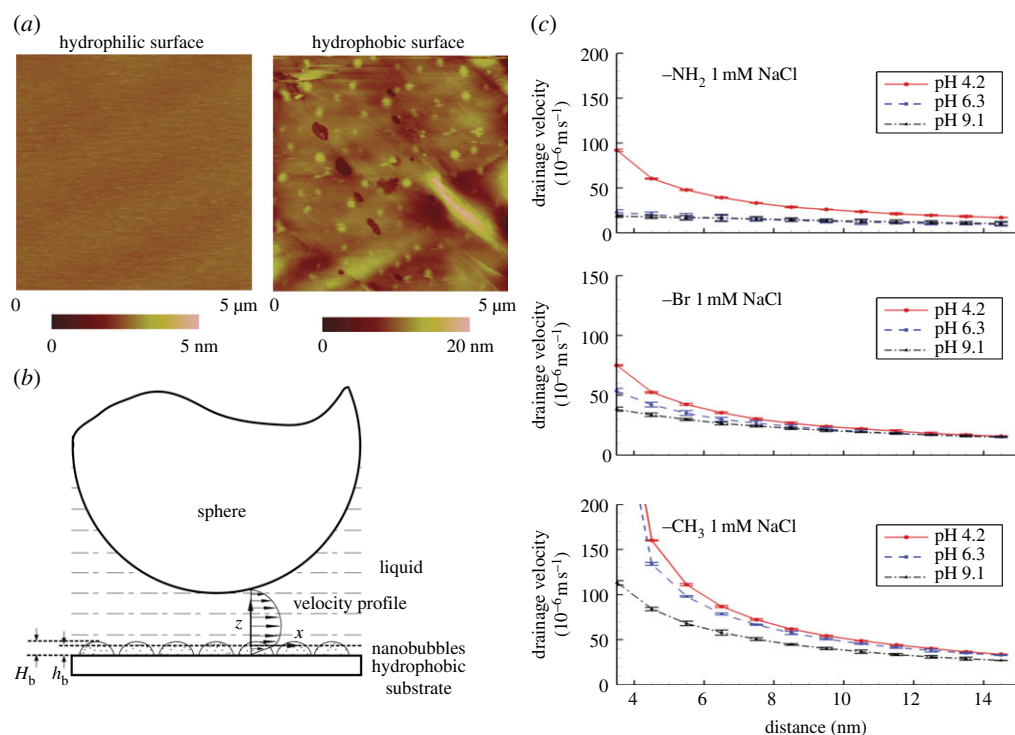


Figure 4. (a) AFM images of hydrophilic and hydrophobic surfaces. Nanobubbles with a typical diameter and height of about 150 nm and 6 nm, respectively, were observed on the hydrophobic surface [110]. (b) A schematic of a sphere–flat system with nanobubbles covering the flat surface. The presence of the nanobubbles changes the velocity profile between the sphere and the surface resulting in an increase of slip length [110]. (c) Mean drainage velocities as a function of the distance between the AFM tip and the surface. Three different functionalized surfaces were studied [83]. (Online version in colour.)

respectively [108,109]. It has been shown that any presence of electric field affects contact angle, propensity and size of nanobubbles and drag [108,109,111,112]. Figure 4c [83] shows the drainage velocity as a function of AFM tip–sample separation, comparing three different functionalized surfaces. Measurements of fluid drainage have been used in an indirect way to characterize the boundary-slip effect. Generally, higher drainage occurs at surfaces with higher contact angles. The results indicate higher pH corresponds to a higher surface charge density and a lower drainage velocity [83].

3. Fabrication

In this section, a brief discussion of common materials and techniques for fabrication is presented. It should be noted that this section does not give a comprehensive and detailed review of all fabrication methods but merely points out some of the essentials for biosensors. The reader is directed to many references including books and review articles for further details about specific methods and materials.

(a) Materials and substrates for biosensor fabrication

The most common substrates for microfluidic and nanofluidic devices are silicon, glass and a variety of polymers. Silicon has the advantage of being widely used by the semiconductor industry so its properties are well studied and fabrication methods are well developed. However, silicon is a semiconductor so a SiO₂ layer is thermally grown on the surface as an insulating layer for most silicon devices that require electrical isolation. This is particularly important for sensors that employ electrical-based detection methods since electrical effects in the bulk silicon substrate could potentially interfere with detection or provide stray noise in the sensor. Silicon with a thermally grown thin layer of SiO₂ has been used as a transducer, where an Au/Cr contact was connected to the Si chip to monitor impedance changes caused by variation in charge distribution owing to binding of DNA [30]. Glass has the advantage of being chemically stable and is transparent, which is particularly attractive when optical detection methods such as fluorescence or SPR are used. However, depending on the type of glass, there can be large amounts of impurities or trace materials that may potentially interfere with either device fabrication or sensor operation. For example, soda lime glass is one of the cheapest and most commonly used forms of glass, but can contain a large amount of contaminants such as aluminium. Pyrex or borosilicate glass is often used in microfluidic and nanofluidics devices, but is more expensive than soda lime. The best optical properties would be achieved in either fused silica or quartz, but these types of materials are significantly more expensive. One of the challenges with glass is the difficulty in bonding device layers to create sealed channels. Often high temperatures and/or large electric fields may be needed and therefore fabrication methods for biomaterials have to be adapted to meet the device-bonding requirements. Polymers are attractive materials for substrates because of their low cost, disposability and ability to adapt to several biomaterial and diagnostic applications [113]. For example, a polymer-based disposable device capable of detecting 0.25–10 ppm of anaesthetic propofol within 60 s has been already demonstrated (figure 5) [114]. Poly(dimethyl siloxane) (PDMS) is the most commonly used polymer for fabrication of fluidic channels [3,4,11,60] towards biosensor applications though poly(methyl methacrylate) (PMMA) is also used [115]. Conducting polymers such as poly(phenylene vinylene) [23], and its soluble derivatives along with polyaniline have been used for electronic components [28]. The main challenges in working with polymers relate to the relatively lower Young's modulus for these materials making polymer channels more prone to collapse and deformation than either silicon or glass. Furthermore, many polymers also show non-specific adsorption of biomaterials and can therefore pose contamination challenges for biosensors.

(b) Fabrication techniques and methods

Most biosensor methods discussed here rely on fabrication methods derived from the semiconductor and microelectromechanical systems industries. Fabrication methods are broadly classified in two categories, bottom-up methods that allow for device fabrication by using manipulation of individual blocks or fundamental building matter, and top-down approaches which use conventional and advanced materials machining techniques to develop devices. Both of these

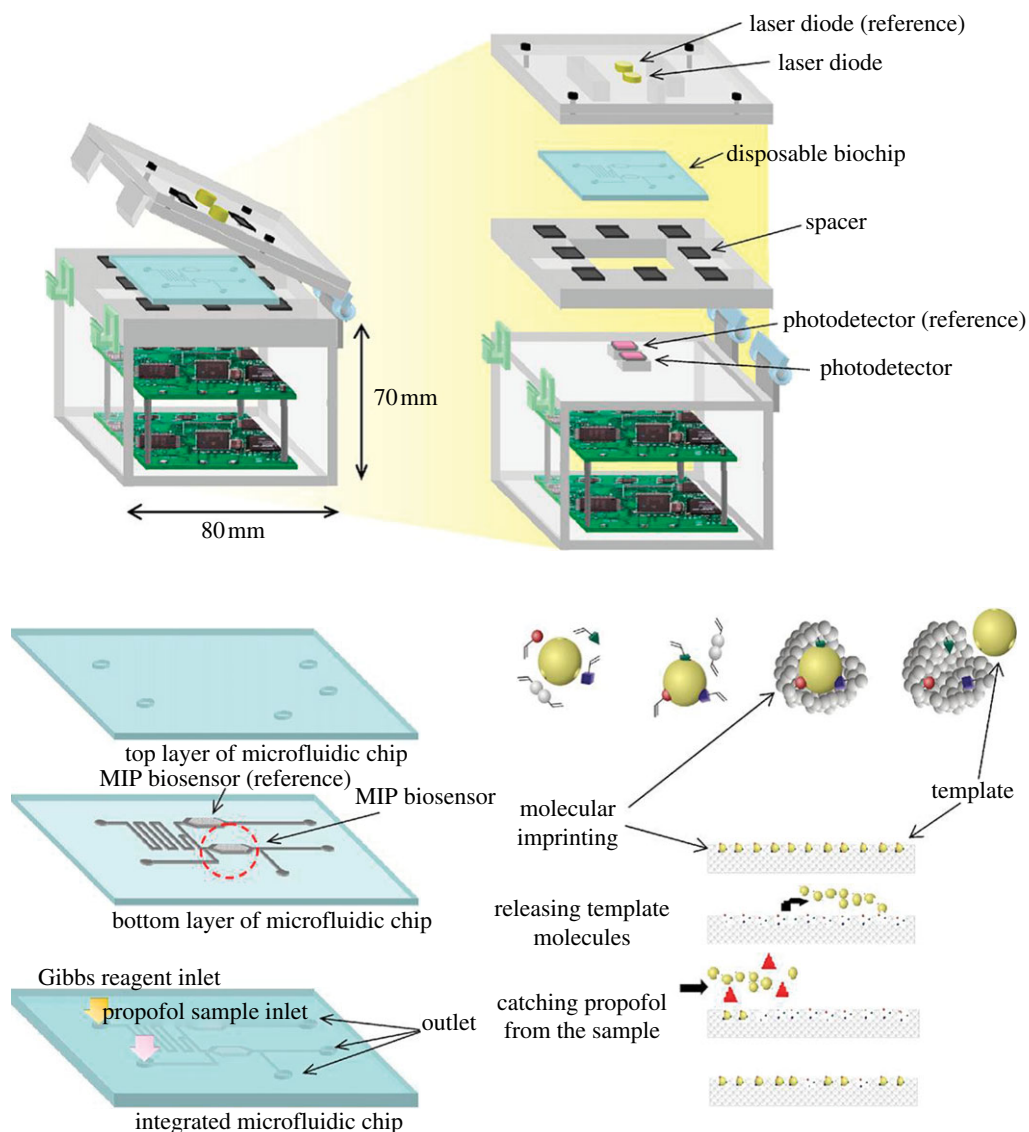


Figure 5. A disposable microfluidic biosensor that uses optical detection methods to detect the presence of anaesthetic propofol. The device is compact and highly sensitive with a detection limit of 0.25 ppm [114]. MIP, molecularly imprinted polymer. (Online version in colour.)

approaches are reviewed next. A summary of fabrication techniques is given in table 2.

(i) *Bottom-up approaches*

As described above, bottom-up approaches use material manipulation to build systems one building block at a time. For example, methods such as self-assembly can be driven by thermodynamic energy minimization processes to develop

Table 2. Summary of major pros and cons of different fabrication techniques used in biosensor fabrication.

technique	pros	cons	references
lithography	ultraviolet	lower resolution difficulties patterning on three-dimensional substrates	[116–118]
	ion or E-beam	higher cost	[116]
	nanoimprint	improved resolution/minimum feature size high throughput	
etching	wet	clean and simple helps remove particulate defects	[119,120]
	dry	anisotropic reduced undercutting user may not need to handle dangerous chemicals	
		photoresist delamination can be isotropic depending on substrate and etchant	
bonding	fusion	toxic gases requires specialized equipment	[121]
	anodic	requires extremely clean substrate may collapse owing to reflow of glass time and energy intensive generally limited to silicon–glass bonding	[121]
		high voltage causes channel bowing and collapse	
	polymer bonding	only for polymer-based device or requires polymer adhesion layer	[122]
	adhesive bonding	introduces an interfacial material layer	[123]
		works with a wide variety of substrates	

polymer structures based on phase segregation. Other self-assembly methods use molecular assembly of alkanethiols to form organized monolayers that enable surface modification [56], as discussed below. Other methods include pick-and-place approaches using advanced tools such as optical tweezers [124,125] and AFMs [41,103,126]. Most of these methods are highly specific and remain in research phases with only a few manufactured biosensors.

(ii) *Top-down methods*

Derived from processing of bulk silicon and glass for devices, these methods remain more common and popular for fabrication of devices. All device fabrication begins with definition of channels or other features on a blank substrate by methods of lithography. Lithography is followed by etching to form the actual features [116]. Other material addition (e.g. metals) may take place to provide functionality to the devices. Next, essentials of lithography and device etching are discussed.

UV lithography. Ultraviolet lithography has been widely used by the semiconductor industry. A light-sensitive material, photoresist, is spun onto a clean substrate which is then soft-baked to remove excess solvents and activate the photo-initiators in the resist. A photomask with the desired pattern is placed over the substrate and then exposed to ultraviolet light. If the photoresist is a positive-type, the photoresist in the exposed area will dissolve away when placed in a developer. In the case of a negative photoresist, the unexposed area will dissolve [116]. This process is used to define features on the substrate to create devices. Though UV lithography is a relatively cost-effective method, problems with uneven photoresist coverage often occur when patterning hierarchical nanostructures. Methods such as detachment lithography [117] and alterations to the photoresist spin step [118] have been shown to resolve this issue. This method is frequently used in micro/nanofluidic-based biosensors to fabricate fluidic channels [6,20,127] and is also used in the fabrication of silicon nanowires [24,95]. Typical feature sizes are in the several micrometres range with the smallest features of the order of 2–5 μm .

Advanced lithographic techniques. In contrast to using UV light, other methods for defining features that eventually form structures on the substrates have also been developed. For example, focused proton beam lithography uses a highly focused beam of H_2^+ ions to directly write a pattern into a polymer substrate. While the technique tends to be higher in cost when compared with conventional UV lithography, feature sizes achieved through this method range down to 22 nm. This method can also be used for high-aspect-ratio structures such as pillars [128]. This process is similar to electron beam lithography which uses a beam of high energy (around 200 keV) electrons to fabricate high-resolution patterns [129]. E-beam lithography has been employed to create highly reproducible nanofluidic hole arrays used in devices that implement depletion zone mixing to help increase detection time limited by diffusion [57]. In nanoimprint lithography, a pre-patterned mould, typically made of silicon is pressed into a flat polymer sheet often made of PMMA or PDMS. The mould is held in contact with the polymer under pressure and heated for about 30 min. The mould is then removed and the device is ready to be bonded [130]. Soft lithography begins with the fabrication of a master using standard photolithography or e-beam lithography.

A polymer such as PDMS is poured into the master and allowed to cure. Once fully cured, the PDMS mould is detached from the master and the device is ready to be bonded [11,131]. Detachment lithography is a photoresist-based contact printing technique used in the fabrication of three-dimensional structures. A pre-patterned silicon substrate is brought into contact with a flat PDMS stamp that has been coated with photoresist. After annealing and rapid peeling, the protruding areas of the silicon substrate become coated with photoresist. The substrate can then be patterned using other lithographic methods to produce more complicated structures [117]. Of these methods, nanoimprint lithography has been used by many researchers for the fabrication of PDMS fluidic channels for biosensors [3,11,25,26,45,61].

Etching techniques. Etching techniques are classified as wet or dry etching techniques depending on whether the etch medium is a liquid or a vapour. Patterns defined in lithography are physically transferred or transcribed into the substrate using wet or dry etching. Wet etching can be performed using hydrofluoric acid (HF) [31,60] or buffered oxide etch [6] for glass and KOH for silicon, respectively. HF gives isotropic wet etching thus undercutting the masking layer and resulting in semicircular sidewalls or sidewalls that taper downward. Depth of the features is controlled by the etch time. Continued exposure to HF required for etching deeper features can result in photoresist delamination. For this reason, metal masking layers of aluminium or gold with an intermediate chrome adhesion layer are used to protect the substrate [119]. In the case of silicon, KOH gives an anisotropic etch resulting in sidewalls with a mirror-like finish. Patterns can also be transferred into the substrate through reactive ion etching (RIE). RIE is often used rather than wet etching because it is anisotropic [6,57]. For glass, CF_4 or CHF_3 is typically used for etching, while Cl_3/BCl_3 plasma is typically used for silicon [132]. An example of nanochannels fabricated in glass is shown in figure 6 [118].

(c) *Surface modification*

Surface modification methods can be divided in two broad categories: physical and chemical methods. The definition of these broad categories depends on how the process actually affects the surface. Physical methods, in most cases, do not change the chemical composition of the surface, but may alter physical characteristics such as surface roughness, grain sizes and grain boundaries, and faceting. Consequently, physical modification often uses tools such as lasers, plasmas, temperature, ion beams and physical polishing and grinding to alter the surface state of a material of interest. Chemical methods are often classified as such because these methods introduce a change in the eventual chemistry or chemical composition at the surface of a material. The surface may possess chemical properties that are different from the bulk material. Among these formation of surface layers, either covalently bonded or physisorbed, has been most common. Other chemical methods include treatment with UV light and reactive plasmas. These changes can also introduce a change in the eventual surface charge density or the surface energy. As discussed above, many detection methods rely on a biosensing element immobilized on a surface. The immobilization processes are largely governed by surface–species interactions and therefore the ability to systematically control or characterize surface

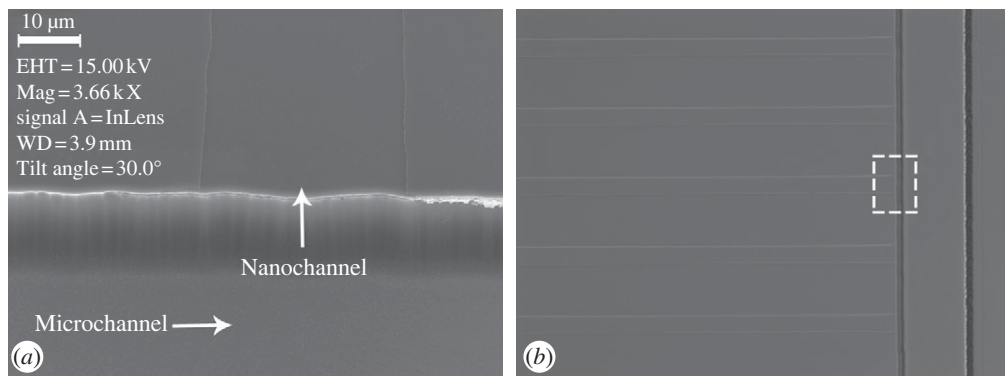


Figure 6. (a) A scanning electron microscopy image of a micro–nanochannel interface [118]. This image shows a zoomed-in view of the area outlined by the dashed rectangle in (b). (b) An optical micrograph image of a micro–nanochannel interface. The channels are etched into glass using 49% hydrofluoric acid for the microchannels and 10 : 1 buffered oxide etch for the nanochannels. The microchannels are approximately $100\text{ }\mu\text{m}$ wide and $9.5 \pm 0.4\text{ }\mu\text{m}$ deep, while the nanochannels are $38 \pm 1.5\text{ }\mu\text{m}$ wide and $50 \pm 6\text{ nm}$ deep.

properties such as surface charge presents an important element in biosensor design. As an example, the use of monolayers to generate desired surface states for biosensing is discussed below.

Surface modification has been essential for biosensors to immobilize affinity tags for binding of target molecules to surfaces [133–138]. For example, several platforms for cell, protein and DNA assays have been investigated [74,139–144]. Other examples include tissue engineering, bio-threat and environmental sensors [145–147], biomimetic surfaces [148,149], and development of myriad surface-based platforms for clinical diagnostics and applications [138,150–155]. Recent reviews have also discussed in detail the methods for surface modification [56] and operational physics behind modified surfaces [81], and in the sections to follow several examples of applied surface modification towards biosensors that use tools of surface modification are presented. For instance, microcontact printing is a fabrication technique that employs self-assembly. A polymer-based stamp, usually made of PDMS, is coated with an alkanethiol and then brought into contact with a substrate (usually silicon) that has a thin layer of gold deposited on the surface. A self-assembled monolayer (SAM) forms in the regions where the stamp comes into contact with the gold surface [156].

(d) Bonding techniques

Once device features have been fabricated on a substrate, the features must be isolated from the outside environment to protect the sample of interest from contamination or interferences towards sensing. Therefore, the features are often sealed using a cover layer with well-established bonding methods. Next, a discussion for three methods used in several biosensors is presented.

(i) Anodic bonding

Anodic bonding is used most often to bond silicon to glass in microfluidic-based biosensor devices [31]. Typically silicon devices are bonded with glass

top covers because of the optical transparency of glass which is preferred for device testing and characterization. The silicon substrate and glass top cover are brought into physical contact and bonded by applying heat (temperatures of the order of 400°C), pressure (approx. 100–500 kPa), and a voltage (approx. several kilovolts) across the device. The strong potential difference applied across the device can cause the channels to bow. If the aspect ratio for the channels is very low, the silicon and glass may come into contact in the centre of the channel causing bonding owing to elastic deformation of either the glass or the silicon. Once the surfaces come in contact, the bond expands quickly down the channel, resulting in completely sealed-off channels. Aspect ratios as low as 0.004 (channel depth of 20 nm and width of 5 μm) have been fabricated by this method [121].

(ii) *Fusion bonding*

Fusion bonding is typically used for glass-to-glass bonding. Glass is heated to a temperature near to the glass transition temperature (approx. 550°C) and put under pressure to create a seal. The temperature and the aspect ratio of the channels are the main factors that determine the survival of the channels. If the bonding temperature is too high, the channels tend to deform or collapse owing to re-flow of glass. If the temperature is too low bonding will not occur [121]. A sealed channel with a depth of 6 nm was achieved in a Pyrex substrate [157]. Small aspect ratios down to 0.0004 for 25 nm deep channels in Pyrex have been achieved [121].

(iii) *Other bonding techniques*

Typically PDMS devices are bonded to glass covers by placing the glass cover in contact with the PDMS and curing the PDMS while applying pressure [60,131] or by simply applying pressure [122]. PDMS can also be used as an intermediate layer for glass-to-glass bonding. Uncured PDMS is spun onto a glass cover and then exposed to oxygen plasma. The PDMS layer and glass substrate are brought into contact and cured under an applied pressure [118]. Oxygen plasma bonding is also used for devices where fluidic channels are fabricated in PDMS and a glass top cover is bonded to the device [25,26]. In some cases, the use of adhesive layers has also been used to bond devices [123,158].

4. Applications

As discussed in the previous sections, most biosensors rely on a target molecule adhering to a detector molecule and providing a measurable signal. Consequently, several different types of sensors have been developed for the detection of target molecules. In the next few paragraphs, a broad-brushed discussion is presented for biosensors ranging from relatively common materials such as urea [159], to biomarkers for various autoimmune disorders and cancer. In this review, no specific category of biosensors is chosen since the focus here is on demonstrating the use of microfluidics and nanofluidics as a widely applicable platform for biosensing applications. Therefore, it is likely that some papers

may have been missed; however, the reader has been directed to several other related reviews, books and key references that provide in-depth discussions of specific topics.

A wide range of proteins are studied including hCG [4,14,16], which is a biomarker for tumours; β -amyloid, an important biomarker for Alzheimer's disease caused by the cleaving of the amyloid precursor protein [160]; the cancer biomarker alpha-fetoprotein [26]; and interleukin-6 which is a biomarker for autoimmune disorders [13,161]. Other target molecules include micro-RNA (miRNA) [24,28], a biomarker for cancer, DNA [15,20,25,30,34,61,122], herpes simplex-2 virus infection [5] and common drinking water contaminants including *E. coli* [45], pesticides [22,35], synthetic oestrogen in river water [21] and heavy metals [3,162–164].

Many detection methods rely on target molecule labelling and optical measurements. One such method is surface-plasmon resonance imaging (SPRI). In one study, target miRNA molecules were adsorbed onto a single-stranded locked nucleic acid microarray where poly(A) tails are added through surface reaction. DNA-coated Au nanoparticles bind to poly(A) tails which can then be analysed using SPRI. The percent reflectivity was found to vary linearly with concentration at concentrations of 10–500 fM. This study achieved a sensor that was 50 times more sensitive than a previously reported fluorescence-based microarray [165], with a detection limit of 10 fM (5 attomoles, dissolved in 500 μ l) [10].

Methods that require labelling of target molecules and optical detection methods are limited by the ability of the fluorophore labels to bind with the correct molecules. Additionally, quenching from the excitation light or from environmental effects limits the reliability of optical-based measurements. For these reasons, researchers have begun to develop label-free detection methods that do not rely on optical measurements. Recently, silicon-based nanowires have been used for the detection of miRNA, a non-protein coding RNA that regulates gene expression and has been implicated as a biomarker for certain types of cancer and neurological disorders [24]. MiRNA cells bind to peptide nucleic acids (PNA), which are immobilized on the surface of the nanowires to serve as receptors for the miRNA. Changes in resistance measured before and after the introduction of the sample indicate that miRNA has bonded to the PNA on the surface of the nanowire. This detection method allows for label-free direct detection of miRNA. Since the method is based on changes in electrical signals, this allows for quantitative determination of the miRNA present in the sample. The reported detection limit is 1 fM. A potential limitation is that certain common proteins such as bovine serum albumin also bind to PNA and may affect resistance signals, though effects were not found to be significant in a recent study [24]. Streaming potential and Debye screening may also affect electrical signals as previously discussed; however, researchers are working to minimize these effects [166]. A similar study was done using silicon nanowires to detect DNA down to 10 fM [34]. A similar detection method was developed using polymer-based nanowires. In this study, concentration of miRNA could be quantified in the range of 10 fM–20 pM with a detection limit of 5.0 fM [28].

Electrophoretic separation was used in a microfluidic analysis system to detect the presence of anthrax in 750 nl of mouse blood within 30 min (figure 7) [60]. In another study, a nanofluidic device is used to localize gold nanoparticles (60 nm) and increase the concentration of target molecules near the entrance

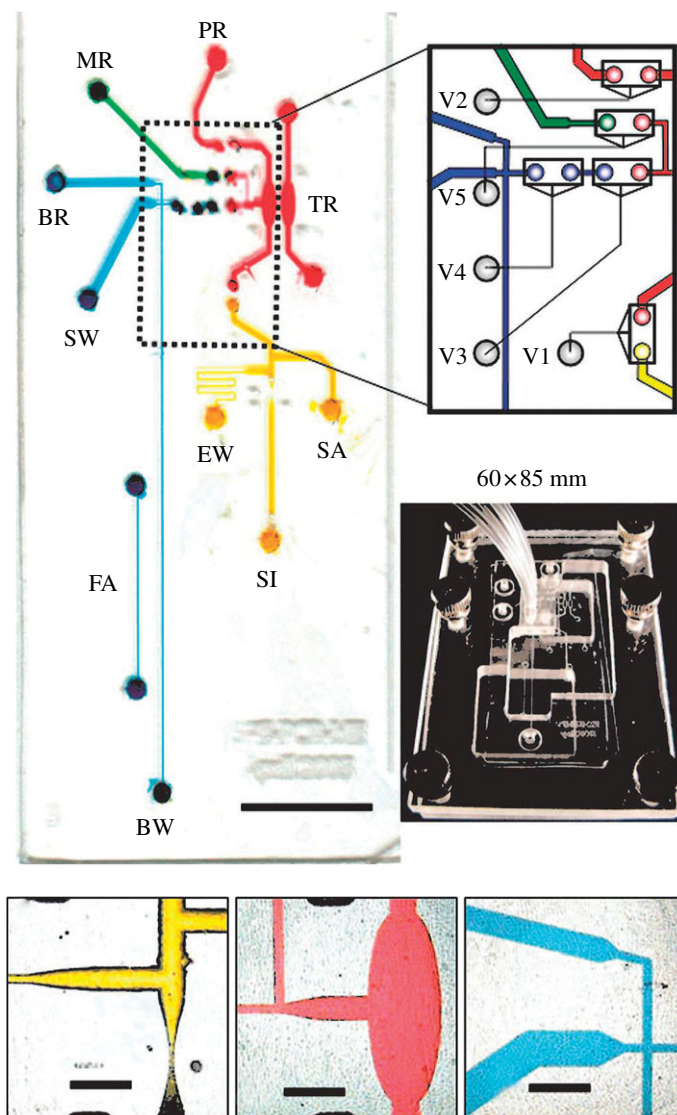


Figure 7. A multi-layer microfluidic device that employs electrophoretic separation to detect anthrax in whole mouse blood [60]. (Online version in colour.)

of the channel. Since the channel is only 40 nm tall, the particles become trapped at the entrance. Capillary forces drive the target molecules through the nanoparticle clusters where the target molecules bind creating an area that has a high concentration of target molecules. Surface-enhanced Raman spectroscopy, which depends on the excitation of molecules by infrared light, is then used to detect target molecule concentration down to 11.5 pM. The method is particularly attractive because it can distinguish between toxic β -amyloid peptide and harmless forms [160].

While many biosensors focus on the detection of biomarkers for certain diseases, biosensors also have applications for detecting environmental contaminants. The Environmental Protection Agency has set the maximum concentration allowance for the pesticide dichlorvos at 5×10^{-10} M for drinking water. A carbon nanotube-based biosensor reduced the detection limit from 1 nM to 1 pM in 10 min with a linear range of detection from 1 pM to 1 μ M. It is believed that the decrease in the detection limit is due to the selective concentration of the pesticide inside the carbon matrix [22]. A further concern for clean drinking water is the presence of heavy metals such as arsenic, which can occur naturally, or materials like uranium, which can arise from waste armaments and other industrial and military applications. The United States and Europe set the permissible level of arsenic at $10 \mu\text{g l}^{-1}$ while in other parts of the world it is $50 \mu\text{g l}^{-1}$. Microfluidics-based biosensors are capable of detecting arsenic concentrations of $10 \mu\text{g l}^{-1}$ in 75–120 min in contrast to $120\text{--}180 \mu\text{g l}^{-1}$ using fluorescence-based techniques [3]. Biosensors have also been developed for the detection of other heavy metals [162–164], synthetic oestrogen [21] and *E. coli* [45], which are all concerns for water contamination.

For all biosensors non-specific adsorption of biomolecules is a concern. Permselective membranes are one method for dealing with this issue. The principle of ion permselectivity is widely used in glucose biosensors. Glucose sensors are based on the rate of oxidation of glucose by dioxygen, which is measured by the formation of hydrogen peroxide. A permselective polymer layer is used in amperometric glucose biosensors to avoid interferences of the detection of hydrogen peroxide by ascorbate, urate and acetaminophen [167] found in blood samples. Permselective membranes are typically made of Nafion or a sol–gel material such as 3-mercaptopropyl trimethoxysilane (MPTMS) with studies indicating MPTMS is a better permselective material since signals from interfering molecules were reduced when compared with Nafion-coated biosensors [168]. Nafion permselective membranes are also used in glucose sensors to prevent reduction of sensor signal in electrically based sensors caused by dependence of the signal strength on buffer concentration. As buffer concentration increases, signal strength decreases. The use of the permselective membranes near the sensing surface reduces the influence of buffer concentration [169]. In another study, Nafion was used as the nanochannel in a microfluidic-based biosensor. Nanofluidic concentration polarization effect was used to pre-concentrate the sample before detection. The detection limit for this device was 2.72 nM with a sample size of 25 μ l and a detection time of 15 min, including the pre-concentration step [4]. A recent study shows that non-specific adsorption can be reduced by increasing SAM incubation time, decreasing surface roughness and, in the case of gold electrodes, optimizing the directional alignment of the gold crystal [170]. The device used to perform this study is shown in figure 8. Another method of dealing with the non-specific binding of proteins exploits the Vroman effect [171,172]. The Vroman effect is when a weak affinity protein bound to the surface of a substrate is displaced by a strong affinity protein. The reverse process does not occur. A protein's affinity to bind depends on the size of the protein, concentration of proteins in the solution, the binding substrate and the pH of the solution. The reactions occur so that the total entropy of the system is minimized, which is why certain proteins will replace others already bound on a surface while the reverse process will not occur [171]. The Vroman effect allows a target protein

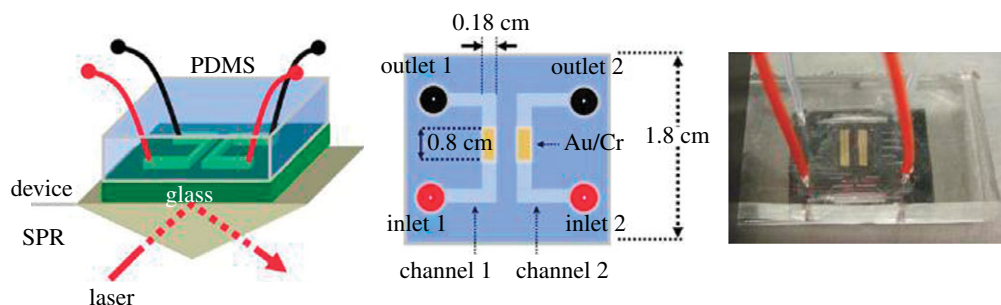


Figure 8. Schematic of a device used for studying methods to reduce non-specific adsorption. SPR is used to detect binding of target molecules. Fluidic channels are fabricated in a PDMS slab and bonded with a glass cover to allow optical access for SPR detection. Fluid inlet–outlet tubes are inserted through the PDMS slab [170]. (Online version in colour.)

with a strong affinity to be detected in the presence of several other lower affinity proteins. Displacement of proteins initially adsorbed to a surface results in a shift in reflectivity that can be measured by SPR [11].

Microfluidic biosensors offer the possibility of creating portable and potentially disposable detection devices. Recently, an electrochemical microfluidic biosensor was developed for the detection of DNA. The device uses an array of microelectrodes as a transducer and ferro/ferricyanide entrapping liposomes as signal reporters to quantify the concentration of DNA in the range of 1–50 fM in 6 min using only a simple potentiostat [122]. This type of sensing mechanism was used in the design of a miniaturized electrochemical detection system with an integrated potentiostat. The device is fully portable and relatively inexpensive (less than \$50) [20], when compared with some other microfluidics devices that can easily cost well over a few hundred dollars. This varies from fluorescence microscopy since it does not require expensive, bulky detection equipment and image processing software, though the detection limit for fluorescence-based detection has been reported to be as low as 0.2 fM [69]. Additionally, devices have been developed that have six channels in parallel and use only capillary forces to fill the channels in 8–47 min. This could potentially lead to a single device that can be used to detect several different target molecules at once [6]. Researchers have also made advances towards fabricating reusable devices by applying a low (0.9 V) DC voltage to reset the sensing surface [173].

5. Summary

The fundamental fluid mechanics and fabrication methods relevant to micro- and nanofluidic-based biosensors as well as recent types of biosensors, the way in which they detect the presence of target molecules and the reliability and detection limits have been reviewed. The critical dimensions of micro- and nanofluidic-based devices are the same as the length scales for physical phenomena governed by small collections of molecules. Minimum concentrations and the amount of time required for detection are limited by mass transport and reaction time. Reliable, fast detection is further complicated by effects such as non-specific

adsorption of molecules, interferences from composition of electrolyte solutions, Debye screening of sensing areas and streaming potential effects of electrically based biosensors. Advances have been made to overcome these challenges, but further research is required to decrease minimum concentrations and detection time and allow for simultaneous detection of multiple target molecules while having low false positives.

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References

- 1 Radadia, A. D., Morgan, R. D., Masel, R. I. & Shannon, M. A. 2009 Partially buried microcolumns for micro-gas analyzers. *Anal. Chem.* **81**, 3471–3477. (doi:10.1021/ac8027382)
- 2 Nemes, P., Knolhoff, A. M., Rubakhin, S. S. & Sweedler, J. V. 2011 Metabolic differentiation of neuronal phenotypes by single-cell capillary electrophoresis-electrospray ionization-mass spectrometry. *Anal. Chem.* **83**, 6810–6817. (doi:10.1021/ac2015855)
- 3 Buffi, N., Merulla, D., Beutier, J., Barbaud, F., Beggah, S., von Lintel, H., Renauda, P. & van der Meer, J. R. 2011 Development of a microfluidics biosensor for agarose-bead immobilized *Escherichia coli* bioreporter cells for arsenite detection in aqueous samples. *Lab Chip* **11**, 2369–2377. (doi:10.1039/c1lc20274j)
- 4 Lee, J. H. & Han, J. 2010 Concentration-enhanced rapid detection of human chorionic gonadotropin as a tumor marker using nanofluidic preconcentrator. *Microfluid. Nanofluid.* **9**, 973–979. (doi:10.1007/s10404-010-0598-z)
- 5 Zubair, A., Burbelo, P. D., Vincent, L. G., Iadarola, M. J., Smith, P. D. & Morgan, N. Y. 2011 Microfluidic LIPS serum antibody detection: demonstration of a rapid test for HSV-2 infection. *Biomed. Microdevices* **13**, 1053–1062. (doi:10.1007/s10544-011-9575-x)
- 6 Gervais, L., Hitzbleck, M. & Delamarque, E. 2011 Capillary-driven multiparametric microfluidic chips for one-step immunoassays. *Biosens. Bioelectron.* **27**, 64–70. (doi:10.1016/j.bios.2011.06.016)
- 7 Hu, J., Sun, X., Agarwal, A. & Kimerling, L. C. 2009 Design guidelines for optical resonator biochemical sensors. *J. Opt. Soc. Am. B* **26**, 1032–1041. (doi:10.1364/JOSAB.26.001032)
- 8 Fukuyama, M. *et al.* 2010 Selective detection of antigen-antibody reaction using Si ring optical resonators. *Jpn J. Appl. Phys.* **49**, 04DL09. (doi:10.1143/JJAP.49.04DL09)
- 9 White, I. M., Zhu, H., Suter, J. D., Fan, X. & Zourob, M. 2009 Label-free detection with the liquid core optical ring resonator sensing platform. *Methods Mol. Biol.* **503**, 139–165. (doi:10.1007/978-1-60327-567-5_7)
- 10 Fang, S., Lee, H. J., Wark, A. W. & Corn, R. M. 2006 Attomole microarray detection of microRNAs by nanoparticle-amplified SPR imaging measurements of surface polyadenylation reactions. *J. Am. Chem. Soc.* **128**, 14044–14046. (doi:10.1021/ja065223p)
- 11 Choi, S. & Chae, J. 2009 Surface plasmon resonance biosensor based on Vroman effect: towards cancer biomarker detection. In *Proc. IEEE 15th Int. Mixed-Signals, Sensors, and Systems Test Workshop, Scottsdale, AZ, 10–12 June 2009*. (doi:10.1109/IMS3TW.2009.5158683)
- 12 Metzger, J., von Landenberg, P., Kehrel, M., Buhl, A., Lackner, K. J. & Lippa, P. B. 2007 Biosensor analysis of 2-Glycoprotein I-reactive autoantibodies: evidence for isotype-specific binding and differentiation of pathogenic from infection-induced antibodies. *Clin. Chem.* **53**, 1137–1143. (doi:10.1373/clinchem.2006.079632)
- 13 Chou, T.-H., Chuang, C.-Y. & Wu, C.-M. 2010 Quantification of Interleukin-6 in cell culture medium using surface plasmon resonance biosensors. *Cytokine* **51**, 107–111. (doi:10.1016/j.cyto.2010.04.004)
- 14 Piliarik, M., Bockova, M. & Homola, J. 2010 Surface plasmon resonance biosensor for parallelized detection of protein biomarkers in diluted blood plasma. *Biosens. Bioelectron.* **26**, 1656–1661. (doi:10.1016/j.bios.2010.08.063)

- 15 Malic, L., Veres, T. & Tabrizian, M. 2011 Nanostructured digital microfluidics for enhanced surface plasmon resonance imaging. *Biosens. Bioelectron.* **26**, 2053–2059. (doi:10.1016/j.bios.2010.09.001)
- 16 Springer, T., Piliarik, M. & Homola, J. 2010 Real-time monitoring of biomolecular interactions in blood plasma using a surface plasmon resonance biosensor. *Anal. Bioanal. Chem.* **398**, 1955–1961. (doi:10.1007/s00216-010-4159-9)
- 17 Pei, R., Cheng, Z., Wang, E. & Yang, X. 2001 Amplification of antigen–antibody interactions based on biotin labeled protein–streptavidin network complex using impedance spectroscopy. *Biosens. Bioelectron.* **16**, 355–361. (doi:10.1016/S0956-5663(01)00150-6)
- 18 Vitarelli, M. J., Prakash, S. & Talaga, D. S. 2011 Determining nanocapillary geometry from electrochemical impedance spectroscopy using a variable topology network circuit model. *Anal. Chem.* **83**, 533–541. (doi:10.1021/ac102236k)
- 19 Rodriguez, M. C., Kawde, A. N. & Wang, J. 2005 Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of the surface charge. *Chem. Commun.* 4267–4269. (doi:10.1039/b506571b)
- 20 Kwayke, S., Goral, V. N. & Baeumner, A. J. 2006 Electrochemical microfluidic biosensor for nucleic acid detection with integrated minipotentiostat. *Biosens. Bioelectron.* **21**, 2217–2223. (doi:10.1016/j.bios.2005.11.017)
- 21 Martinez, N. A., Schneider, R. J., Messina, G. A. & Raba, J. 2010 Modified paramagnetic beads in a microfluidic system for the determination of ethinylestradiol (EE2) in river water samples. *Biosens. Bioelectron.* **25**, 1376–1381. (doi:10.1016/j.bios.2009.10.031)
- 22 Sotiropoulou, S. & Caniotakis, N. A. 2005 Lowering the detection limit of the acetylcholinesterase biosensor using a nanoporous carbon matrix. *Anal. Chim. Acta* **530**, 199–204. (doi:10.1016/j.aca.2004.09.007)
- 23 Chen, L., McBranch, D. W., Wang, H.-L., Helgeson, R., Wudl, F. & Whitten, D. G. 1999 Highly sensitive biological and chemical sensors based on reversible fluorescence quenching in a conjugated polymer. *Proc. Natl Acad. Sci. USA* **96**, 12287–12292. (doi:10.1073/pnas.96.22.12287)
- 24 Zhang, G.-J., Chua, J. H., Chee, R.-E., Agarwal, A. & Wong, S. M. 2009 Label-free direct detection of MiRNAs with silicon nanowire biosensors. *Biosens. Bioelectron.* **24**, 2504–2508. (doi:10.1016/j.bios.2008.12.035)
- 25 Javanmard, M. & Davis, R. W. 2011 A microfluidic platform for electrical detection of DNA hybridization. *Sens. Actuators B* **154**, 22–27. (doi:10.1016/j.snb.2010.03.067)
- 26 Maeng, J. H., Lee, B. C., Ko, Y. J., Cho, W., Ahn, Y., Cho, N. G., Lee, S. H. & Hwang, S. Y. 2008 A novel microfluidic biosensor based on an electrical detection system for alpha-fetoprotein. *Biosens. Bioelectron.* **23**, 1319–1325. (doi:10.1016/j.bios.2007.11.019)
- 27 Moreno-Hagelsieb, L., Lobert, P. E., Pampin, R., Bourgeois, D., Remacle, J. & Flandre, D. 2004 Sensitive DNA electrical detection based interdigitated Al/Al₂O₃ microelectrodes. *Sens. Actuators B* **98**, 269–274. (doi:10.1016/j.snb.2003.10.036)
- 28 Fan, Y., Chen, X., Trigg, A. D., Tung, C.-h., Kong, J. & Gao, Z. 2007 Detection of MicroRNAs using target-guided formation of conducting polymer nanowires in nanogaps. *J. Am. Chem. Soc.* **129**, 5437–5443. (doi:10.1021/ja067477g)
- 29 Iqbal, S. M., Balasundaram, G., Ghosh, S., Bergstrom, D. E. & Bashir, R. 2005 Direct current electrical characterization of DS-DNA in nanogap junctions. *Appl. Phys. Lett.* **86**, 153901. (doi:10.1063/1.1900315)
- 30 Macanovic, A., Marquette, C., Polychronakos, C. & Lawrence, M. F. 2004 Impedance-based detection of DNA sequences using a silicon transducer with PNA as the probe layer. *Nucleic Acids Res.* **32**, e20. (doi:10.1093/nar/gnh003)
- 31 Durand, N. F. Y. & Renaud, P. 2009 Label-free determination of protein–surface interaction kinetics by ionic conductance inside a nanochannel. *Lab Chip* **9**, 319–324. (doi:10.1039/b811006a)
- 32 Chen, R., Bangsaruntip, S., Drouvalakis, K. A., Kam, N. W. S., Shim, M., Li, Y., Kim, W., Utz, P. J. & Dai, H. 2003 Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc. Natl Acad. Sci. USA* **100**, 4984–4989. (doi:10.1073/pnas.0837064100)

- 33 Cui, Y., Wei, Q., Park, H. & Lieber, C. M. 2001 Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science* **293**, 1289–1292. (doi:10.1126/science.1062711)
- 34 Hahm, J. & Lieber, C. M. 2004 Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors. *Nano Lett.* **4**, 51–54. (doi:10.1021/nl034853b)
- 35 Alvarez, M., Calle, A., Tamayo, J., Lechuga, L., Abad, A. & Montoya, A. 2003 Development of nanomechanical biosensors for detection of the pesticide DDT. *Biosens. Bioelectron.* **18**, 649–653. (doi:10.1016/S0956-5663(03)00035-6)
- 36 Waggoner, P. S., Tan, C. P. & Craighead, H. G. 2010 Microfluidic integration of nanomechanical resonators for protein analysis in serum. *Sens. Actuators B* **150**, 550–555. (doi:10.1016/j.snb.2010.08.041)
- 37 Hwang, K. S., Lee, S.-M., Kim, S. K., Lee, J. H. & Kim, T. S. 2009 Micro- and nanocantilever devices and systems for biomolecule detection. *Annu. Rev. Anal. Chem.* **2**, 77–98. (doi:10.1146/annurev-anchem-060908-155232)
- 38 Lee, S. & Bhushan, B. 2010 Biological molecules in therapeutic nanodevices. In *Springer handbook of nanotechnology* (ed. B. Bhushan), pp. 453–484. Berlin, Germany: Springer.
- 39 Olthius, W., Bergveld, P. & Kruijs, J. 1997 The exploitation of ISFETs to determine acid-base behaviour of proteins. *Electrochim. Acta* **43**, 3483–3488. (doi:10.1016/S0013-4686(98)00095-4)
- 40 Schoning, M. J. & Poghosian, A. 2002 Recent advances in biologically sensitive field-effect transistors (BioFETs). *Analyst* **127**, 1137–1151. (doi:10.1039/b204444g)
- 41 Bhushan, B. 2010 MEMS/NEMS and BioMEMS/BioNEMS: materials, devices, and biomimetics. In *Springer handbook of nanotechnology* (ed. B. Bhushan), pp. 1663–1740. Berlin, Germany: Springer.
- 42 Bhushan, B., Tokachichu, D., Keener, M. T. & Lee, S. C. 2006 Nanoscale adhesion, friction, and wear studies of biomolecules on silicon-based surfaces. *Acta Biomater.* **2**, 39–49. (doi:10.1016/j.actbio.2005.08.010)
- 43 Bhushan, B., Tokachichu, D. R., Keener, M. T. & Lee, S. C. 2005 Morphology and adhesion of biomolecules on silicon based surfaces. *Acta Biomater.* **1**, 327–341. (doi:10.1016/j.actbio.2005.01.002)
- 44 Wang, J. 1999 Towards genelectronics: electrochemical biosensing of DNA hybridization. *Chem. Eur. J.* **5**, 1681–1685. (doi:10.1002/(SICI)1521-3765(19990604)5:6<1681::AID-CHEM1681>3.0.CO;2-U)
- 45 Mannoor, M. S., Zhang, S., Link, A. J. & McAlpine, M. C. 2010 Electrical detection of pathogenic bacteria via immobilized antimicrobial peptides. *Proc. Natl Acad. Sci. USA* **107**, 19 207–19 212. (doi:10.1073/pnas.1008768107)
- 46 Tsai, H.-K. A., Moschou, E. A., Kulinsky, L., Daunert, S. & Madou, M. J. 2009 Integrating biosensors and drug delivery: a step closer toward scalable responsive drug delivery systems. *Adv. Mater.* **21**, 656–660. (doi:10.1002/adma.200801770)
- 47 Watts, A. S., Urbas, A. A., Moschou, E., Gavalas, V. G., Zoval, J. V., Madou, J. & Bachas, L. G. 2007 Centrifugal microfluidics with integrated sensing microdome optodes for multiion detection. *Anal. Chem.* **79**, 8046–8054. (doi:10.1021/ac0709100)
- 48 Nelson, W. C., Peng, I., Lee, G.-A., Loo, J. A., Garrell, R. L. & Kim, C.-J. 2010 Incubated protein reduction and digestion on an electrowetting-on-dielectric digital microfluidic chip for MALDI-MS. *Anal. Chem.* **82**, 9932–9937. (doi:10.1021/ac101833b)
- 49 Shah, G. J., Veale, J. L., Korin, Y., Reed, E. F., Gritsch, H. A. & Kim, C.-J. 2010 Specific binding and magnetic concentration of CD8+ T-lymphocytes on electrowetting-on-dielectric platform. *Biomicrofluidics* **4**, 044106. (doi:10.1063/1.3509457)
- 50 Lang, H. P., Hegner, M. & Gerber, C. 2005 Cantilever array sensors. *Mater. Today* **4**, 30–36. (doi:10.1016/S1369-7021(05)00792-3)
- 51 Olthius, W., Bergveld, P. & Kruijs, J. 1997 The exploitation of ISFETs to determine acid-base behaviour of proteins. *Electrochim. Acta* **43**, 3483–3488. (doi:10.1016/S0013-4686(98)00095-4)
- 52 Metzger, J., von Landenberg, P., Kehrel, M., Buhl, A., Lackner, K. J. & Lippa, P. B. 2007 Biosensor analysis of 2-glycoprotein I-reactive autoantibodies: evidence for isotype-specific binding and differentiation of pathogenic from infection-induced antibodies. *Clin. Chem.* **53**, 1137–1143. (doi:10.1373/clinchem.2006.079632)

- 53 Prakash, S. & Karacor, M. B. 2010 Surface mediated flows in glass nanofluidic channels. In *Proc. 13th Solid-State Sensors, Actuators and Microsystems Workshop*. Hilton Head Island, SC: Transducer Research Foundation.
- 54 Cho, S. K., Moon, H. & Kim, C.-J. 2003 Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic-based circuits. *J. Microelectromech. Syst.* **12**, 70–80. (doi:10.1109/JMEMS.2002.807467)
- 55 Wheeler, A. R., Moon, H., Bird, C. A., Loo, R. R. O., Kim, C.-J., Loo, J. A. & Garrell, R. L. 2005 Digital microfluidics with in-line sample purification for proteomics analysis with MALDI-MS. *Anal. Chem.* **77**, 534–540. (doi:10.1021/ac048754+)
- 56 Prakash, S., Karacor, M. B. & Banerjee, S. 2009 Surface modification in microsystems and nanosystems. *Surf. Sci. Rep.* **64**, 233–254. (doi:10.1016/j.surfrep.2009.05.001)
- 57 Yanik, A. A., Huang, M., Artar, A., Chang, T.-Y. & Altug, H. 2010 Integrated nanoplasmonic-nanofluidic biosensors with targeted delivery of analytes. *Appl. Phys. Lett.* **96**, 021101. (doi:10.1063/1.3290633)
- 58 Bhushan, B., Kasai, T., Kulik, G., Barbieri, L. & Hoffman, P. 2005 AFM study of perfluorosilane and alkylsilane self-assembled monolayers for anti-stiction in MEMS/NEMS. *Ultramicroscopy* **105**, 176–188. (doi:10.1016/j.ultramic.2005.06.034)
- 59 Raiteri, R., Grattarola, M., Butt, M. & Skladal, P. 2001 Micromechanical cantilever-based biosensor. *Sens. Actuators B* **79**, 115–126. (doi:10.1016/S0925-4005(01)00856-5)
- 60 Easley, C. J. *et al.* 2006 A fully integrated microfluidic genetic analysis system with sample-in–answer-out capability. *Proc. Natl Acad. Sci. USA* **103**, 19 272–19 277. (doi:10.1073/pnas.0604663103)
- 61 Chen, I. J. & White, I. M. 2011 High-sensitivity electrochemical enzyme linked assay on a microfluidic interdigitated microelectrode. *Biosens. Bioelectron.* **26**, 4375–4381. (doi:10.1016/j.bios.2011.04.044)
- 62 Srinivasan, V., Pamula, V., Pollack, M. & Fair, R. 2003 A digital microfluidic biosensor for multianalyte detection. In *Proc. IEEE 16th Annu. Int. Conf. Micro Electro Mechanical Systems, 19–23 January 2003*, pp. 327–330. (doi:10.1109/MEMSYS.2003.1189752)
- 63 Newman, J. & Turner, A. 2005 Home blood glucose biosensors: a commercial perspective. *Biosens. Bioelectron.* **20**, 2435–2453. (doi:10.1016/j.bios.2004.11.012)
- 64 Auroux, P.-A., Iossifidis, D., Reyes, D. R. & Manz, A. 2002 Micro total analysis systems. 2. Analytical standard operations and applications. *Anal. Chem.* **74**, 2637–2652. (doi:10.1021/ac020239t)
- 65 Noroozi, Z., Kido, H., Peytavi, R., Nakajima-Sasaki, R., Jasinkas, A., Micic, M., Felgner, P. L. & Madou, M. J. 2011 A multiplexed immunoassay system based upon reciprocating centrifugal microfluidics. *Rev. Sci. Instrum.* **82**, 064303. (doi:10.1063/1.3597578)
- 66 Rogers, K. 2000 Principles of affinity-based biosensors. *Mol. Biotechnol.* **14**, 109–129. (doi:10.1385/MB:14:2:109)
- 67 Prakash, S., Piruska, A., Gatimu, E. N., Bohn, P. W., Sweedler, J. V. & Shannon, M. A. 2008 Nanofluidics: systems and applications. *IEEE Sens. J.* **8**, 441–450. (doi:10.1109/JSEN.2008.918758)
- 68 Conlisk, A. T. 2012 *Essentials of micro and nanofluidics with applications to the biological and chemical sciences*. Cambridge, UK: Cambridge University Press.
- 69 Zaytseva, N. V., Montagna, R. A. & Baeumner, A. J. 2005 Microfluidic biosensor for the serotype-specific detection of Dengue virus. *Anal. Chem.* **77**, 7520–7527. (doi:10.1021/ac0509206)
- 70 Huang, L. R., Cox, E. C., Austin, R. H. & Sturm, J. C. 2004 Continuous particle separation through deterministic lateral displacement. *Science* **304**, 987–990. (doi:10.1126/science.1094567)
- 71 Belgrader, P., Bennett, W., Hadley, D., Richards, J., Stratton, P., Mariella, R. & Milanovich, F. 1999 Infectious disease: PCR detection of bacteria in seven minutes. *Science* **284**, 449–450. (doi:10.1126/science.284.5413.449)
- 72 Blazej, R. G., Kumaresan, P. & Mathies, R. A. 2006 Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing. *Proc. Natl Acad. Sci. USA* **103**, 7240–7245. (doi:10.1073/pnas.0602476103)

- 73 Bliss, C. L., McMullin, J. N. & Backhouse, C. J. 2007 Rapid fabrication of a microfluidic device with integrated optical waveguides for DNA fragment analysis. *Lab Chip* **7**, 1280–1287. (doi:10.1039/b708485d)
- 74 Tegenfeldt, J. O., Prinz, C., Cao, H., Huang, R. L., Austin, R. H., Chou, S. Y., Cox, E. C. & Sturm, J. C. 2004 Micro- and nanofluidics for DNA analysis. *Anal. Bioanal. Chem.* **318**, 1678–1692. (doi:10.1007/s00216-004-2526-0)
- 75 Srinivas, P. R., Verma, M., Zhao, Y. & Srivastava, S. 2002 Proteomics for cancer biomarker discovery. *Clin. Chem.* **48**, 1160–1169.
- 76 Jain, K. K. 2004 Applications of biochips: from diagnostics to personalized medicine. *Curr. Opin. Drug Discov. Dev.* **7**, 5507–5512.
- 77 Yager, P., Edwards, T., Fu, E., Helton, K., Nelson, K., Tam, M. R. & Weigl, B. H. 2006 Microfluidic diagnostic technologies for global public health. *Nature* **442**, 412–418. (doi:10.1038/nature05064)
- 78 Gardeniers, H. & Van Den Berg, A. 2004 Micro- and nanofluidic devices for environmental and biomedical applications. *Int. J. Environ. Anal. Chem.* **84**, 809–819. (doi:10.1080/03067310310001626678)
- 79 Jain, K. K. 2005 Nanotechnology in clinical laboratory diagnostics. *Clin. Chim. Acta* **358**, 37–54. (doi:10.1016/j.cccn.2005.03.014)
- 80 Jain, K. K. 2005 The role of nanobiotechnology in drug discovery. *Drug Discov. Today* **10**, 1435–1442. (doi:10.1016/S1359-6446(05)03573-7)
- 81 Squires, T. M., Messinger, R. J. & Manalis, S. R. 2008 Making it stick: convection, reaction and diffusion in surface-based biosensors. *Nat. Biotechnol.* **26**, 417–426. (doi:10.1038/nbt1388)
- 82 Piruska, A., Gong, M., Sweedler, J. V. & Bohn, P. W. 2010 Nanofluidics in chemical analysis. *Chem. Soc. Rev.* **39**, 1060–1072. (doi:10.1039/b900409m)
- 83 Wu, Y., Misra, S., Karacor, M. B., Prakash, S. & Shannon, M. A. 2010 Dynamic response of AFM cantilevers to dissimilar functionalized silica surfaces in aqueous electrolyte solutions. *Langmuir* **26**, 16 963–16 972. (doi:10.1021/la103005c)
- 84 Israelachvili, J. N. 1992 *Intermolecular and surface forces*, 2nd edn. San Diego, CA: Academic Press.
- 85 Kemery, P. J., Steehler, J. K. & Bohn, P. W. 1998 Electric field mediated transport in nanometer diameter channels. *Langmuir* **14**, 2884–2889. (doi:10.1021/la980147s)
- 86 Burgeen, D. & Nakache, F. R. 1964 Electrokinetic flow in ultrafine capillary slits. *J. Phys. Chem.* **68**, 1084–1091. (doi:10.1021/j100787a019)
- 87 Li, D. 2004 *Electrokinetics in microfluidics*. New York, NY: Academic Press.
- 88 Rice, C. L. & Whitehead, R. 1965 Electrokinetic flow in a narrow cylindrical capillary. *J. Phys. Chem.* **69**, 4017–4024. (doi:10.1021/j100895a062)
- 89 Abgrall, P. & Nguyen, N. T. 2008 Nanofluidic devices and their applications. *Anal. Chem.* **80**, 2326–2341. (doi:10.1021/ac702296u)
- 90 Kuo, T.-C., Sloan, L. A., Sweedler, J. V. & Bohn, P. W. 2001 Manipulating molecular transport through nanoporous membranes by control of electrokinetic flow: effect of surface charge density and debye length. *Langmuir* **17**, 6298–6303. (doi:10.1021/la010429j)
- 91 Donnan, F. G. 1995 Theory of membrane equilibria and membrane potentials in the presence of non-dialysing electrolytes: a contribution to physical-chemical physiology. *J. Membr. Sci.* **100**, 45–55. (doi:10.1016/0376-7388(94)00297-C)
- 92 Zhang, Y., Hu, Y., Wilson, G. S., Moatti-Sirat, D., Poitout, V. & Reach, G. 1994 Elimination of the acetaminophen interference in an implantable glucose sensor. *Anal. Chem.* **66**, 1183–1188. (doi:10.1021/ac00079a038)
- 93 Matsumoto, T., Furusawa, M., Fujiwara, H., Matsumoto, Y. & Ito, N. 1998 A micro-planar amperometric glucose sensor unsusceptible to interference species. *Sens. Actuators B* **49**, 68–72. (doi:10.1016/S0925-4005(98)00145-2)
- 94 Stern, E., Wagner, R., Sigworth, F. J., Breaker, R., Fahmy, T. M. & Reed, M. A. 2007 Importance of the Debye screening length on nanowire field effect transistor sensors. *Nano Lett.* **7**, 3405–3409. (doi:10.1021/nl071792z)
- 95 Kim, D. R., Lee, C. H. & Zheng, X. 2009 Probing flow velocity with silicon nanowire sensors. *Nano Lett.* **9**, 1984–1988. (doi:10.1021/nl900238a)

- 96 Tamaki, E., Hibara, A., Kima, H.-B., Tokeshi, M. & Kitamori, T. 2006 Pressure-driven flow control system for nanofluidic chemical process. *J. Chromatogr. A* **1137**, 256–262. (doi:10.1016/j.chroma.2006.10.097)
- 97 Erikson, D. & Li, D. 2003 Analysis of alternating electroosmotic flows in a rectangular microchannel. *Langmuir* **19**, 5421–5430. (doi:10.1021/la027035s)
- 98 Mala, G. M., Yang, C. & Li, D. 1998 Electrical double layer potential distribution in a rectangular microchannel. *Colloid Surf. A* **135**, 109–116. (doi:10.1016/S0927-7757(97)00215-X)
- 99 Qu, W. & Li, D. 2000 A model for overlapped EDL fields. *J. Colloid Interf. Sci.* **224**, 397–407. (doi:10.1006/jcis.1999.6708)
- 100 Erikson, D. & Li, D. 2003 Three-dimensional structure of electroosmotic flow over heterogeneous surfaces. *J. Phys. Chem. B* **107**, 12 212–12 220. (doi:10.1021/jp027724c)
- 101 Golden, J. P., Floyd-Smith, T. M., Mott, D. R. & Ligler, F. S. 2007 Target delivery in a microfluidic immunosensor. *Biosens. Bioelectron.* **22**, 2763–2767. (doi:10.1016/j.bios.2006.12.017)
- 102 Vijayendran, R. A., Motsegood, K. M., Beebe, D. J. & Leckband, D. E. 2003 Evaluation of a three-dimensional micromixer in a surface-based biosensor. *Langmuir* **19**, 1824–1828. (doi:10.1021/la0262250)
- 103 Bhushan, B. 2010 Nanotribology, nanomechanics, and materials characterization. In *Springer handbook of nanotechnology* (ed. B. Bhushan), p. 789–856. Berlin, Germany: Springer.
- 104 Henniker, J. 1962 Triboelectricity in polymers. *Nature* **196**, 474. (doi:10.1038/196474a0)
- 105 Sakaguchi, M. & Kashiwabara, H. 1992 A generation mechanism of triboelectricity due to the reaction of mechaniradicals with mechanoions which are produced by mechanical fracture of solid polymer. *Colloid Polym. Sci.* **270**, 621–626. (doi:10.1007/BF00654038)
- 106 Freeman, G. R. & March, N. H. 1999 Triboelectricity and some associated phenomena. *Mater. Sci. Technol.* **15**, 1454–1458.
- 107 Vinogradova, O. I. & Belyaev, A. V. 2010 Wetting, roughness and hydrodynamic slip. In *Nanoscale liquid interfaces*, ch. 1. Singapore: Pan Stanford Publishing Pte Ltd.
- 108 Wang, Y. & Bhushan, B. 2010 Liquid microdroplet sliding on hydrophobic surfaces in the presence of electric field. *Langmuir* **26**, 4013–4017. (doi:10.1021/la903460a)
- 109 Wang, Y. & Bhushan, B. 2010 Boundary slip and nanobubble study in micro/nanofluidics with atomic force microscope. *Soft Matter* **6**, 29–66. (doi:10.1039/b917017k)
- 110 Wang, Y., Bhushan, B. & Maali, A. 2009 Atomic force microscopy measurement of boundary slip on hydrophilic, hydrophobic, and superhydrophobic surfaces. *J. Vac. Sci. Technol.* **27**, 754–760. (doi:10.1116/1.3086637)
- 111 Mazumder, M. & Bhushan, B. 2011 Propensity and geometrical distribution of surface nanobubbles: effect of electrolyte, roughness, pH, and substrate bias. *Soft Matter* **7**, 9184–9196. (doi:10.1039/C1SM05560G)
- 112 Bhushan, B. & Pan, Y. 2011 Role of electric field on surface wetting of polystyrene surface. *Langmuir* **27**, 9425–9429. (doi:10.1021/la201636g)
- 113 Siegrist, J., Gorkin, R., Bastien, M., Stewart, G., Peytavi, R., Kido, H., Bergeron, M. & Madou, M. J. 2010 Validation of a centrifugal microfluidic sample lysis and homogenization platform for nucleic acid extraction with clinical samples. *Lab Chip* **10**, 363–371. (doi:10.1039/b913219h)
- 114 Hong, C. C., Chang, P. H., Lin, C. C. & Hong, C. L. 2010 A disposable microfluidic biochip with on-chip molecularly imprinted biosensors for optical detection of anesthetic propofol. *Biosens. Bioelectron.* **25**, 2058–2064. (doi:10.1016/j.bios.2010.01.037)
- 115 Chikkaveeraiah, B. V., Liu, H., Mani, V. & Papadimitrakopoulos, F. 2009 A microfluidic electrochemical device for high sensitivity biosensing: detection of nanomolar hydrogen peroxide. *Electrochem. Commun.* **11**, 819–822. (doi:10.1016/j.elecom.2009.02.002)
- 116 Madou, M. 1997 *Fundamentals of microfabrication*. Boca Raton, FL: CRC Press LLC.
- 117 Yeom, J. & Shannon, M. A. 2010 Detachment lithography of photosensitive polymers: a route to fabricating three-dimensional structures. *Adv. Funct. Mater.* **20**, 289–295. (doi:10.1002/adfm.200900686)
- 118 Pinti, M. & Prakash, S. 2011 A two-step wet etch process for the facile fabrication of hybrid micro-nanofluidic devices. In *Proc. 2011 ASME Int. Mechanical Engineering Congress and Exposition, Denver, CO, 11–17 November 2011*, IMECE2011-64508.

- 119 Zhu, H., Holl, M., Ray, T., Bhushan, S. & Meldrum, D. R. 2009 Characterization of deep wet etching of fused silica glass for single cell and optical sensor deposition. *J. Micromech. Microeng.* **19**, 065013. (doi:10.1088/0960-1317/19/6/065013)
- 120 Ventra, M. D., Evoy, S. & Hefflin, J. R. (eds) 2004 *Introduction to nanoscale science and technology*, p. 632. Boston, MA: Kluwer Academic Publishers.
- 121 Mao, P. & Han, J. 2005 Fabrication and characterization of 20 nm planar nanofluidic channels by glass–glass and glass–silicon bonding. *Lab Chip* **5**, 837–844. (doi:10.1039/b502809d)
- 122 Goral, V. N., Zaytseva, N. V. & Baeumner, A. J. 2006 Electrochemical microfluidic biosensor for the detection of nucleic acid sequences. *Lab Chip* **6**, 414–421. (doi:10.1039/b513239h)
- 123 Flachsbarth, B. R., Wong, K., Iannacone, J. M., Abante, E. N., Vlach, R. L., Rauchfuss, P. A., Bohn, P. W., Sweedler, J. V. & Shannon, M. A. 2006 Design and fabrication of a multilayered polymer microfluidic chip with nanofluidic interconnects via adhesive contact printing. *Lab Chip* **6**, 667–674. (doi:10.1039/b514300d)
- 124 Svoboda, K. & Block, S. M. 1994 Biological applications of optical forces. *Annu. Rev. Biophys. Biomol. Struct.* **23**, 247–285. (doi:10.1146/annurev.bb.23.060194.001335)
- 125 Ashkin, A. & Dziedzic, J. M. 1989 Internal cell manipulation using infrared laser traps. *Proc. Natl Acad. Sci. USA* **86**, 7914–7918. (doi:10.1073/pnas.86.20.7914)
- 126 Stavis, C. *et al.* 2011 Functionalization of thin-film diamond for highly stable and selective biological interfaces. *Proc. Natl Acad. Sci. USA* **108**, 983–988. (doi:10.1073/pnas.1006660107)
- 127 Qiang, L., Vaddiraju, A., Rusling, J. F. & Papadimitrakopoulos, F. 2010 Highly sensitive and reusable Pt-black microfluidic electrodes for long-term electrochemical sensing. *Biosens. Bioelectron.* **26**, 682–688. (doi:10.1016/j.bios.2010.06.064)
- 128 Wang, L. P., Shao, P. G., von Kan, J. A., Ansari, K., Bettiol, A. A., Pan, X. T., Wohland, T. & Watt, F. 2007 Fabrication of nanofluidic devices utilizing proton beam writing and thermal bonding techniques. *Nucl. Instrum. Meth. B* **260**, 450–454. (doi:10.1016/j.nimb.2007.02.062)
- 129 Moritz, J., Dieny, B., Nozieres, J. P., Landis, S., Lebib, A. & Chen, Y. 2002 Domain structure in magnetic dots prepared by nanoimprint and e-beam lithography. *J. Appl. Phys.* **91**, 7314–7316. (doi:10.1063/1.1452260)
- 130 Abgrall, P., Lowb, L.-N. & Nguyen, N.-T. 2007 Fabrication of planar nanofluidic channels in a thermoplastic by hot-embossing and thermal bonding. *Lab Chip* **7**, 520–522. (doi:10.1039/b616134k)
- 131 Park, S.-m., Huh, Y. S., Craighead, H. G. & Erickson, D. 2009 A method for nanofluidic device prototyping using elastomeric collapse. *Proc. Natl Acad. Sci. USA* **106**, 15 549–15 554. (doi:10.1073/pnas.0904004106)
- 132 Zhang, Z. L. & MacDonald, N. C. 1992 An RIE process for submicron, silicon electromechanical structures. *J. Micromech. Microeng.* **2**, 31. (doi:10.1088/0960-1317/2/1/007)
- 133 Abbasi, F., Mirzadeh, H. & Katbab, A. A. 2001 Modification of polysiloxane polymers for biomedical applications: a review. *Polym. Int.* **50**, 1279–1287. (doi:10.1002/pi.783)
- 134 De Campos, A. M., Sanchez, A., Gref, R., Calvo, P. & Alonso, M. J. 2003 The effect of a PEG versus a chitosan coating on the interaction of drug colloidal carriers with the ocular mucosa. *Eur. J. Pharm. Sci.* **20**, 73–81. (doi:10.1016/S0928-0987(03)00178-7)
- 135 Deng, L., Wang, Y., Shang, L., Wen, D., Wang, F. & Dong, S. 2008 A sensitive NADH and glucose biosensor tuned by visible light based on thionine bridged carbon nanotubes and gold nanoparticles multilayer. *Biosens. Bioelectron.* **24**, 951–957. (doi:10.1016/j.bios.2008.07.066)
- 136 Krenkova, J. & Foret, F. 2004 Immobilized microfluidic enzymatic reactors. *Electrophoresis* **25**, 3550–3563. (doi:10.1002/elps.200406096)
- 137 Pijanowska, D. G., Remiszewska, E., Lysko, J. M., Jazwinski, J. & Torbicz, W. 2003 Immobilization of bioreceptors for microreactors. *Sens. Actuators B* **91**, 152–157. (doi:10.1016/S0925-4005(03)00081-9)
- 138 Rosi, N. R. & Mirkin, C. A. 2005 Nanostructures in biodiagnostics. *Chem. Rev.* **105**, 1547–1562. (doi:10.1021/cr030067f)
- 139 Ayhan, H., Cicek, H. & Tuncel, S. A. 2003 Investigation of surface properties of biodegradable albumin microspheres via phagocytosis phenomena. *J. Bioact. Compat. Polym.* **18**, 273–282. (doi:10.1177/088391103036043)

- 140 Falconnet, D., Csucs, G., Grandin, H. M. & Textor, M. 2006 Surface engineering approaches to micropattern surfaces for cell-based assays. *Biomaterials* **27**, 3044–3063. (doi:10.1016/j.biomaterials.2005.12.024)
- 141 Franks, W., Tosatti, S., Heer, F., Seif, P., Textor, M. & Hierlemann, A. 2007 Patterned cell adhesion by self-assembled structures for use with a CMOS cell-based biosensor. *Biosens. Bioelectron.* **22**, 1426–1433. (doi:10.1016/j.bios.2006.06.031)
- 142 Gray, J. J. 2004 The interaction of proteins with solid surfaces. *Curr. Opin. Struc. Biol.* **14**, 110–115. (doi:10.1016/j.sbi.2003.12.001)
- 143 Sauberlich, S., Klee, D., Richter, E. J., Hocker, H. & Spiekermann, H. 1999 Cell culture tests for assessing the tolerance of soft tissue to variously modified titanium surfaces. *Clin. Oral Implan. Res.* **10**, 379–393. (doi:10.1034/j.1600-0501.1999.100505.x)
- 144 Yang, W. S. *et al.* 2002 DNA-modified nanocrystalline diamond thin-films as stable, biologically active substrates. *Nat. Mater.* **1**, 253–257. (doi:10.1038/nmat779)
- 145 Cirino, N. M., Musser, K. A. & Egan, C. 2004 Multiplex diagnostic platforms for detection of biothreat agents. *Expert Rev. Mol. Diagn.* **4**, 841–857. (doi:10.1586/14737159.4.6.841)
- 146 Danielsson, B. 1990 Calorimetric biosensors. *J. Biotechnol.* **15**, 187–200. (doi:10.1016/0168-1656(90)90026-8)
- 147 Palchetti, I. & Mascini, M. 2008 Nucleic acid biosensors for environmental pollution monitoring. *Analyst* **133**, 846–854. (doi:10.1039/b802920m)
- 148 Wu, L. Q., Ghodssi, R., Elabd, Y. A. & Payne, G. F. 2005 Biomimetic pattern transfer. *Adv. Funct. Mater.* **15**, 189–195. (doi:10.1002/adfm.200400279)
- 149 Bhushan, B. 2009 Biomimetics: lessons from nature - an overview. *Phil. Trans. R. Soc. A* **367**, 1445–1486. (doi:10.1098/rsta.2009.0011)
- 150 Bogunia-Kubik, K. & Sugisaka, M. 2002 From molecular biology to nanotechnology and nanomedicine. *BioSystems* **65**, 123–138. (doi:10.1016/S0303-2647(02)00010-2)
- 151 Ciardelli, G., Rechichi, A., Sartori, S., D'Acunto, M., Caporale, A., Peggion, E., Vozzi, G. & Giusti, P. 2006 Bioactive polyurethanes in clinical applications. *Polym. Adv. Technol.* **17**, 786–789. (doi:10.1002/pat.781)
- 152 Freitas, R. A. 2005 What is nanomedicine? *Nanomed. Nanotechnol. Biol. Med.* **1**, 2–9. (doi:10.1016/j.nano.2004.11.003)
- 153 Grayson, A. C. R., Shawgo, R. S., Johnson, A. M., Flynn, N. T., Li, Y. W., Cima, M. J. & Langer, R. 2004 A BioMEMS review: MEMS technology for physiologically integrated devices. *Proc. IEEE* **92**, 6–21. (doi:10.1109/JPROC.2003.820534)
- 154 Herrmann, M., Roy, E., Veres, T. & Tabrizian, M. 2007 Microfluidic ELISA on non-passivated PDMS chip using magnetic bead transfer inside dual networks of channels. *Lab Chip* **7**, 1546–1552. (doi:10.1039/b707883h)
- 155 Langer, R. & Peppas, N. A. 2004 Advances in biomaterials, drug delivery, and bionanotechnology. *AIChE J.* **49**, 2990–3006. (doi:10.1002/aic.690491202)
- 156 Jackman, R. J., Wilbus, J. L. & Whitesides, G. M. 1995 Fabrication of submicrometer features on curved substrates by microcontact printing. *Science* **269**, 664–666. (doi:10.1126/science.7624795)
- 157 van Delft, K. M., Eijkel, J. C. T., Mijatovic, D., Druzhinina, T. S., Rathgen, H., Tas, N. R., van den Berg, A. & Mugele, F. 2007 Micromachined Fabry–Pérot interferometer with embedded nanochannels for nanoscale fluid dynamics. *Nano Lett.* **7**, 345–350. (doi:10.1021/nl062447x)
- 158 Prakash, S., Yeom, J., Jin, N., Adesida, I. & Shannon, M. A. 2007 Characterization of ionic transport at the nanoscale. *Proc. Inst. Mech. Eng. Part N J. Nanoeng. Nanosyst.* **220**, 45–52. (doi:10.1243/17403499JNN91)
- 159 Singh, M., Verma, N., Garg, A. K. & Redhu, N. 2008 Urea biosensors. *Sens. Actuators B* **134**, 345–351. (doi:10.1016/j.snb.2008.04.025)
- 160 Chou, I. H., Benford, M., Beier, H. T. & Cote, G. L. 2008 Nanofluidic biosensing for beta-amyloid detection using surface enhanced Raman spectroscopy. *Nano Lett.* **8**, 1729–1735. (doi:10.1021/nl0808132)
- 161 Messina, G. A., Panini, N. V., Martinez, N. A. & Raba, J. 2008 Microfluidic immunosensor design for the quantification of interleukin-6 in human serum samples. *Anal. Biochem.* **380**, 262–267. (doi:10.1016/j.ab.2008.05.055)

- 162 Zhang, X.-B., Kong, R.-M. & Lu, Y. 2011 Metal ion sensors based on DNazymes and related DNA molecules. *Annu. Rev. Anal. Chem.* **4**, 105–128. (doi:10.1146/annurev.anchem.111808.073617)
- 163 Wang, Z., Lee, J. H. & Lu, Y. 2008 Fluorescent sensor for Hg^{2+} in aqueous solution based on structure-switching DNA. *Chem. Commun.* 6005–6007. (doi:10.1039/b812755g)
- 164 Lan, T., Furuya, K. & Lu, Y. 2010 A highly selective lead sensor based on a classic lead DNzyme. *Chem. Commun.* **46**, 3896–3898. (doi:10.1039/b926910j)
- 165 Shingara, J., Keiger, K., Shelton, J., Laosinchai-Wolf, W., Powers, P., Conrad, R., Brown, D. & Labourier, E. 2005 An optimized isolation and labeling platform for accurate microRNA expression profiling. *RNA* **11**, 1461–1470. (doi:10.1261/rna.2610405)
- 166 Bunimovich, Y. L., Shin, Y. S., Yeo, W. S., Amori, M., Kwong, G. & Heath, J. R. 2006 Quantitative real-time measurements of DNA hybridization with alkylated nonoxidized silicon nanowires in electrolyte solution. *J. Am. Chem. Soc.* **128**, 16 323–16 331. (doi:10.1021/ja065923u)
- 167 Chen, X., Matsumoto, N., Hu, Y. & Wilson, G. S. 2002 Electrochemically mediated electrodeposition/electropolymerization to yield a glucose microbiosensor with improved characteristics. *Anal. Chem.* **74**, 368–372. (doi:10.1021/ac015628m)
- 168 Yang, Y. L., Tseng, T. F. & Lou, S. L. 2007 Using MPTMS as permselective membranes of biosensors. In *Proc. 29th Annu. Int. Conf. of the IEEE Engineering in Medicine and Biology Society, 22–26 August 2007*, pp. 6624–6627. (doi:10.1109/IEMBS.2007.4353878)
- 169 Soldatkin, A. P., El'skaya, A. V., Shul'ga, A. A., Jdanova, A. S., Dzyadevich, S. V., Jaffrezic-Renault, N., Martelet, C. & Clechet, P. 1994 Glucose sensitive conductometric biosensor with additional Nafion membrane: reduction of influence of buffer capacity on the sensor response and extension of dynamic range. *Anal. Chim. Acta* **288**, 197–203. (doi:10.1016/0003-2670(93)E0627-J)
- 170 Choi, S. & Chae, J. 2010 Methods of reducing non-specific adsorption in microfluidic biosensors. *J. Micromech. Microeng.* **20**, 075015. (doi:10.1088/0960-1317/20/7/075015)
- 171 Vroman, L. & Adams, A. L. 1969 Findings with the recording ellipsometer suggesting rapid exchange of specific plasma proteins at liquid solid interfaces. *Surf. Sci.* **16**, 438–446. (doi:10.1016/0039-6028(69)90037-5)
- 172 Jung, S.-Y., Lim, S.-M., Albertorio, F., Kim, G., Gurau, M. C., Yang, R. D., Holden, M. A. & Cremer, P. S. 2003 The Vroman effect: a molecular level description of fibrinogen displacement. *J. Am. Chem. Soc.* **125**, 12 782–12 786. (doi:10.1021/ja037263o)
- 173 Choi, S. & Chae, J. 2009 A regenerative biosensing surface in microfluidics using electrochemical desorption of short-chain self-assembled monolayer. *Microfluid. Nanofluid.* **7**, 819–827. (doi:10.1007/s10404-009-0440-7)