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REVIEW PAPER

Nanobiosensors: optofluidic, electrical and mechanical approaches to biomolecular detection at the nanoscale

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Abstract Next generation biosensor platforms will require significant improvements in sensitivity, specificity and parallelity in order to meet the future needs of a variety of fields ranging from in vitro medical diagnostics, pharmaceutical discovery and pathogen detection. Nanobiosensors, which exploit some fundamental nanoscopic effect in order to detect a specific biomolecular interaction, have now been developed to a point where it is possible to determine in what cases their inherent advantages over traditional techniques (such as nucleic acid microarrays) more than offset the added complexity and cost involved constructing and assembling the devices. In this paper we will review the state of the art in nanoscale biosensor technologies, focusing primarily on optofluidic type devices but also covering those which exploit fundamental mechanical and electrical transduction mechanisms. A detailed overview of next generation requirements is presented yielding a series of metrics (namely limit of detection, multiplexibility, measurement limitations, and ease of fabrication/assembly) against which the various technologies are evaluated. Concluding remarks regarding the likely technological impact of some of the promising technologies are also provided.

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1 Introduction

Interest in the development of new biosensing and high-throughput screening technologies has been largely driven by recent developments in two different application areas. The first of these areas grew out of a number of advancements in proteomics and genomics research that led to an explosion in the number of biomarkers associated with specific disease states (Growdon 1999; Sander 2000; Srinivas et al. 2001, 2002) and pharmacological responses (Ross et al. 2004). While in general the number of useful, unequivocal single biomarkers available for screening healthy populations for complex diseases remains relatively small (Ward et al. 2001; Hernandez and Thompson 2004) the potential for diagnosis based on panels of multiple biomarkers (Sidransky 2002; Wulfkuhle et al. 2003) presents an exciting possibility. The second broad application area relates to the need to rapidly detect and identify emerging pathogenic threats. Viral detection [e.g. influenza (Dawson et al. 2006; Liu et al. 2006; Lu 2006), adenovirus (McCaman et al. 2001; Gu et al. 2003) and dengue hemorrhagic fever (Guzman and Kouri 2004; Zaytseva et al. 2005)] necessitates the development of sensor platforms with very low limits of detection and false alarm rates that can also track or at least account for the relatively high rates of mutagenic drift. Other threats such as water- (Straub and Chandler 2003), food- (Rasooly and Herold 2006) or air-borne pathogens (McBride et al. 2003; Stetzenbach et al. 2004) also require high sensitivity and specificity but introduce other engineering challenges such

as larger sample volumes to be processed or more complex target capture requirements. Emerging autonomous and networked biosensor systems, which are designed to operate and report findings with as little human intervention as possible, add additional constrains such as power usage and time multiplexing.

In this article we review the state of the art in nanoscale biosensing techniques with a particular focus on optofluidic approaches (i.e. those which exploit some combination of optics and micro-/nanofluidics) but also inclusive of nanoscopic electrical and mechanical devices. The goal will be to introduce the various approaches in the context of how well they will be able to address the challenges associated with the two application areas described above. In the following section a detailed overview of "next generation" biosensor requirements is presented along with a final section which describes where state of the art technologies tend to be lacking and where these emerging technologies are likely to have the biggest impact. Following this a detailed overview of the various competing formats are presented. This review is written assuming some knowledge of traditional biosensing techniques. For more in-depth information on biosensing, readers are referred to recent books by Kress-Rogers (1997) or Eggins (1996).

2 Overview of next generation biosensor requirements

Though the application areas listed in the introduction are reasonably disparate, in general the requirements for next generation biosensor and biosensor platforms can broadly be classified into three different categories. Here we provide a detailed overview of these requirements to provide context for our later discussion.

2.1 Sensitivity and specificity

Biosensor development has always attempted to push the limits of sensitivity and specificity with the goal of detecting rarer targets with greater precision. The term sensitivity is used in the literature in a number of different contexts (D'Amico and Di Natale 2001) and thus it is useful to delineate them now for clarity. Test sensitivity refers to the number of true positives a given test yields (i.e. a given test suggests the presence of a particular biomarker and its presence can be confirmed by a "gold standard" test) divided by the number of true positives and false negatives (i.e. the test suggests the presence of a given biomarker and it is not confirmed by the gold standard test). For any final technology it is ultimately this measure that is of utmost importance. As a metric which can lead to improved test sensitivity, internal sensitivity is essentially the local slope of the sensor response curve. In the case of the linear sensor response curve this reduces to change in sensor output per unit change in the quantity measured. As an example an appropriate measure for wavelength sensitive optically resonant refractive index sensor would be $\Delta\lambda$/RIU [i.e. the total expected change in the resonant wavelength per unit change in refractive index unit (RIU)]. The third measure of sensitivity which is often encountered in the literature is the limit of detection, LOD, or the smallest amount of a quantity of interest which produces a measurable output signal. This final measure is of particular interest since the lower the LOD the earlier a disease state can be diagnosed or the presence of an unexpected pathogen can be detected. Rather than simply expressing the LOD as an absolute quantity (ng) it is common to divide this number by the volume of sample which is processed (ng/mL) since this is also a the parameter of interest. In general, however, the latter of these is dependent on a much larger number of system parameters beyond just that of the sensor performance (e.g. residence time, transport speed, mixing efficiency, diffusivity of the target) thus making it more difficult to provide a direct comparison. As such in this review we will focus on the former.

2.2 Multiplexing

New sensor platforms must have inherently high degrees of multiplexing capabilities. The necessity to maximize the number of biomarkers a patient sample can be interrogated against is perhaps obvious (i.e. the greater the number of disease markers which can be probed, the more information that can be obtained). In pathogen detection multiplexing capability is required not only to screen against the large number of different pathogens which may be present in a given sample but also to provide specific subtype information. In virology for example, subtype information is particularly important for tracking emerging viruses (Dawson et al. 2006) and designing/delivering appropriate vaccines. In cases where tracking a single biomarker is of interest it may be also useful to provide time multiplexing (i.e. samples taken at regular time intervals to be interrogated against the same set of probes but at different sites so as to avoid cross concentration). This allows for time tracking of, for example viral load, against the individual base state, enabling more accurate diagnosis. This time multiplexing capability is more relevant to the development of autonomous sensor systems, where samples taken at regular intervals.

2.3 Reduction in measurement complexity and cost

In general sensor systems which can reduce the number of required sample processing steps as well as the amount of

on-chip or off chip infrastructure are likely to be more successful than those which do not. Related to the former of these is the goal of "label free" transduction methods whereby the target itself does not require tagging with, for example, a florescent label. Label free methods are typically based on a positive binding event inducing a change in the local refractive index (which is most relevant for optofluidic technologies), electrical, or mechanical conditions and form the basis of most of the techniques described herein. Techniques which successfully decrease the LOD can also serve to eliminate some processing steps such as target amplification. Nucleic acid amplification via the polymerase chain, PCR, requires the sample to be sent through a predefined thermal cycle each time roughly doubling the amount of the target sequence. Although numerous rapid chip based PCR devices have been demonstrated (Kopp et al. 1998; Zhang et al. 2006a, b) reliance on such processes limits the degree of multiplexing, which can be achieved and requires more on-chip infrastructure. In some portable cases the amount of power required to perform the cycling is also detrimental. At present, most technologies require relatively sophisticated forms of infrastructure to perform the desired measurement. As such the development of techniques that can couple high sensitivity with a simple (e.g. colorimetric) feedback technique would also be desirable. With regards to cost, biosensor elements should minimize any cost prohibitive fabrication or extensive assembly processes that would make mass-producibility unfeasible. Although many applications will be able to support higher device costs if they can meet stringent sensitivity/specificity/autonomy requirements, others, such as large scale screening efforts, it is likely that cost will be an extremely important factor.

2.4 How well do existing technologies meet these needs?

With respect to these requirements, the strength of conventional array based technologies (e.g. nucleic acid and protein microarrays or traditional ELISA approaches) has always been in addressing the multiplexing requirements. The incorporation of a suitable microfluidic element can also serve to solve some of the requirements associated with the third category above (Sect. 2.3). In general, however, the relatively low sensitivity of such approaches limits the types of targets which can be reasonably expected to be interrogated and places stricter requirements on the amount of sample processing and detection infrastructure that is required. Emerging nanotechnologies such as nanoparticles (Seydack 2005), nanowires and nanotubes (Li et al. 2005; Zheng et al. 2005), nanomechanical resonators (Majumdar 2002) and nanophotonics (Chow et al. 2004; Schmidt et al. 2004; Ouyang et al. 2006) are of interest largely to address this failing. As will be described in detail below, while many of these devices tend to not to have much greater internal sensitivity (i.e. slope of the sensor response curve in response to changes in bulk properties such as conductivity or refractive index) than traditional techniques, their inherent advantage is that the total surface area or volume that is probed tends to be much smaller. As a result the total mass required to impart a measurable transduction signal is significantly lower and the LOD is significantly improved. In general, however, the extension of these technologies to the extreme parallelity of the 2D microarray format is complicated by the challenges involved in functionalization of individual sensor and 2D optical or electrical addressing of reaction sites with sub-micrometer spacing. The proceeding sections will focus largely on how well these emerging nanotechnologies have done in terms of pushing the limits of detection and what approaches are being used to achieve the cost and parallelity afforded by the conventional techniques.

3 Optofluidic biosensors

This first section reviews the state of the art in label free optical biosensing techniques. This section is divided into three areas. The first area focuses on techniques that exploit localized changes in the refractive index, induced by biomolecular binding, in the evanescent field of a dielectric structure. The second area is an extension of the first looking more specifically at photonic crystal based devices. The final section provides a detailed review of surface plasmon resonance (SPR) based biomolecular detection.

3.1 Evanescent field based devices

While most of the optical energy is confined within the structure itself, solid core dielectric waveguides have an exponentially decaying tail of the guided optical mode, referred to as the evanescent field, that impinges a small distance (on the order of a hundred nanometers for most systems of interest here) into the surrounding medium. The majority of label-free optofluidic biosensors utilize this evanescent field to probe the surface of a sensing site for the presence of bound or absorbed analytes (Marazuela and Moreno-Bondi 2002). Binding of the target at the sensing site causes a change in the local refractive index in that region imparting a slight phase shift to the propagating optical mode. This simple phenomenon can be exploited via a number of different techniques to perform label-free optofluidic detection. In this first sub-section we present an overview of these techniques while analyzing their

advantages and limitations with regards to the criteria established above.

3.1.1 Interferometric based techniques

Interferometry can be used to detect the phase difference between two collimated light beams of a coherent light source. The simplest practical configuration for chip based integrated optofluidic biosensing is the Mach-Zehnder interferometer (MZI) such as that presented by Luff et al. (1998). Their design consisted of an input optical waveguide, which splits into two arms of equal length and then recombined to form the output optical waveguide. One arm is referred to as the reference arm while a section of the other, sensing arm, is functionalized with the desired biorecognition agent. In the absence of any surface modifications to either of the arms, the light recombining at the output port remains in phase giving rise to constructive interference and maximal light intensity at the output port. When binding occurs at the surface of the sensing arm it changes the local refractive index and the resulting phase shift causes the output power to drop due to destructive interference effects. In the aforementioned work by Luff et al., this phase shift was deduced by measuring the light intensity at the output waveguide. More recently an MZI biosensor using silicon nitride waveguides and standard CMOS processes was demonstrated (Prieto et al. 2003). It was used to detect the pesticide Carbaryl and was shown to have a refractive index LOD of around 10^{-5}. Others (Heideman and Lambeck 1999) have managed to push this limit to as low as 10^{-7} using the MZI configuration. While this technique is very sensitive, it is difficult to multiplex devices in a MZI configuration since each can consist of only one reference and one sensing arm. Another drawback is that the interaction length necessary for producing sufficient phase shifts is often on the order of a centimeter, which in comparison with some of the other techniques that will be discussed below, is very large. In a variant on the traditional approach, Lou et al. (2005) recently proposed using silica nanowire waveguides in a MZI configuration. Their theoretical estimates indicate that the sensing site length can be decreased by an order of magnitude (to around 1 mm) to achieve similar refractive index sensitivity as the previously described works.

Brandenburg (1997) demonstrated the use a Young's interferometer based sensor for refractive index measurements in liquids. Recently Ymeti et al. (2007) extended this concept to perform multiplexed label-free biosensing using the architecture shown in Fig. 1. These devices consist of a waveguide which guides monochromatic light coupled in from a laser source. This waveguide is split into two parallel waveguides by the means of a *Y*-junction. Similar to the MZI sensor, one of the waveguides is functionalized with a biorecognition agent while the other acts as a reference waveguide. The light emerging from the output ends of the two waveguides is collected by a cylindrical lens and made incident on a charge coupled device (CCD) screen. By analyzing the interference pattern generated, the phase shift caused by binding events occurring on the surface of the sensing waveguide can be inferred. In their design they were able to resolve the interference patterns between four waveguides. Thus by using one waveguide as the reference, three different targets could be detected simultaneously. In that work detection of herpes simplex virus 1 (HSV-1) was demonstrated and a device sensitivity of 10^{-7} RIUs, which corresponded to a protein mass coverage of approximately 20 fg/mm^2 was reported. Although a certain level of multiplexing has been demonstrated, it is very hard to push beyond this limit with the Young interferometer design. As in the case of MZI sensors, interaction lengths in these sensors are on the order of a centimeter.

3.1.2 Resonant cavity based techniques

As stated above, one of the chief drawbacks of evanescent-wave sensing by interferometric techniques is the long interaction length of the sensing site, which requires a relatively large amount of bound mass to make an appreciable difference in the transduction signal. Resonant cavity sensors provide a way to overcome this disadvantage by shrinking the size of devices by orders of magnitude while still retaining similar device sensitivity. Microcavities sustaining whispering gallery modes (WGMs) (Ilchenko and Matsko 2006; Matsko and Ilchenko 2006) have been a popular choice for a label-free biosensing architecture. WGMs correspond to light being confined along a circular orbit along the edge of a sphere, disk or cylinder type structure. Those wavelengths of light, which after completing one revolution return in phase, are sustained in the resonator while the other wavelengths die out due to interference effects. WGMs have been extensively studied in liquid droplets and fused silica spheres (Arnold et al. 2003) both of which can have nearly atomic scale smoothness. In such microcavities optical losses are significantly lower than in other optical resonators and the Quality-factor (Q-factor) can exceed a hundred million (Gorodetsky et al. 1996; Armani et al. 2003). Typically, light is evanescently coupled into these resonators using tapered fibers. The output spectrum observed at the end of the coupling fiber consists of a series of sharply peaked dips in transmission. Changes in the local refractive index at the surface of the resonator cause a slight perturbation to the resonance condition for the cavity, imparting a lateral shift to the peaks in the output spectrum.

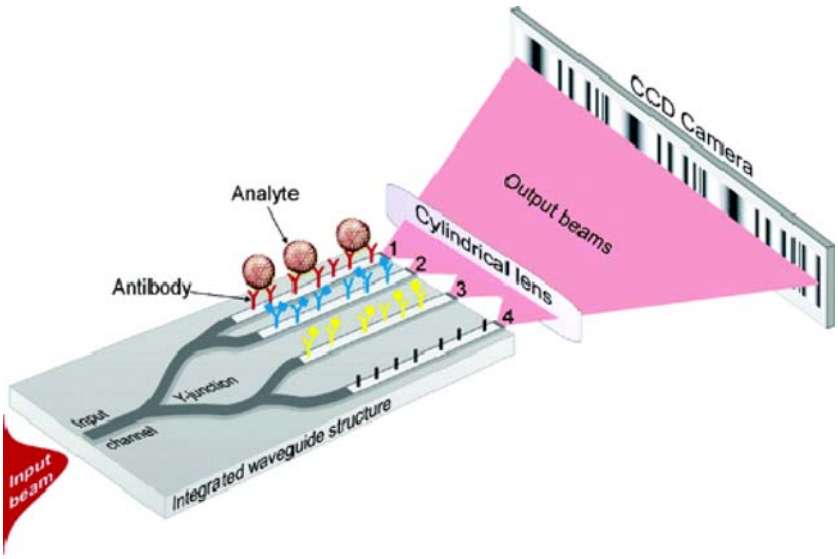

Fig. 1 Four-channel Young's interferometer based optical biosensor from Ymeti et al. (2007). Channels 1, 2, and 3 are the sensor channels which are functionalized with antibody capture probes and 4 is the reference channel. Copyright American Chemical Society. Reproduced with permission

This concept was used to demonstrate a highly sensitive refractometric sensor using fused silica microsphere resonators (Hanumegowda et al. 2005). The microspheres had a radius of 55 μm and the sensor had a LOD on the order of 10^{-7} RIU. For biosensing purposes these resonators can be coated with a suitable biorecognition agent. Vollmer et al. (2002) demonstrated detection of bovine serum albumin (BSA) and streptavidin-biotin binding using such a design. Although these systems possess the properties to make them excellent candidates for ultra-sensitive biosensors, it can be difficult to control the physical parameters of these structures during fabrication as traditional lithographic techniques typically cannot be used to create on-chip cavities with such high Q-factors. This problem was solved by Armani et al. (2003) who used a novel process to fabricate toroidal silica microresonators with a diameter of 120 μm on a chip. These structures had a Q-factor in excess of a hundred million. An extension of this work (Armani and Vahala 2006) demonstrated the use of these microresonators to detect heavy water concentration. Using an entirely different approach, Zhu et al. (2007) have proposed a technique for on-capillary refractive index detections. The circular cross section of the capillary acts as a ring resonator along which the input laser light remains confined. The interaction of the evanescent field with the contents of the liquid filled capillary allowed for non-invasive, on-capillary analysis.

While the techniques described above are well suited to perform highly sensitive detections, they lack robustness and are difficult to integrate in planar systems compatible with traditional microfluidics for performing multiplexed detections. Another drawback is that the entire surface of such devices has to be functionalized although the WGM interacts with a small fraction of this surface area. Planar microdisk (Boyd and Heebner 2001; Krioukov et al. 2002) and microring resonator structures can overcome some of these difficulties as they can easily be integrated on chip using standard semiconductor fabrication techniques (though the Q-factor is not nearly as high as those discussed above). Microring resonators consist of a ring waveguide which is adjacent to a bus waveguide. Light from a laser is coupled into the bus waveguide and this in turn evanescently couples into the ring resonator. As in the case of the silica microsphere resonators, resonance occurs for those wavelengths which are in phase after performing one round-trip around the ring and the spectrum at the output end of the bus waveguide consists of sharply peaked dips in transmission. Binding events along the surface of the microring increase the refractive index in the evanescent field, effectively lengthening the ring and causing the resonant peaks to red shift. Chao et al. (2006) demonstrated polymer microring resonators of 45 μm radius having a Q-factor of 20,000. They were able to detect an effective refractive index change of 10^{-7} RIU and had a detection limit of approximately 250 pg/mm^2 mass coverage on the microring surface. While microring resonator sensors provide a robust architecture for potentially building highly multiplexed biosensors, their binding surface area is still reasonably large. For example in the Chao et al. device mentioned above the total surface area or the ring resonator is $\sim 6.5 \times 10^{-4}$ mm^2 thus the mass LOD is ~ 160 fg.

3.2 Photonic crystal devices

Photonic crystals (Joannopoulos et al. 1995) are composed of periodic dielectric structures. One of the features this periodicity gives rise to is a range of wavelengths which

are not allowed to propagate within the structure, referred to as the photonic bandgap. The size of the bandgap and its position in the spectrum can be tuned by varying the refractive index contrast of the dielectric materials and/or the periodicity of the structure (Erickson et al. 2006). These properties of photonic crystals make them extremely useful in a number of applications, including biosensing. As an example, Skivesen et al. (2007) demonstrated a photonic-crystal waveguide biosensor. This consisted of a silicon waveguide flanked on either side by a 2D photonic crystal which caused light corresponding to the photonic bandgap to remain guided in the waveguide. As with the other optical devices, adsorption of proteins on the surface of the photonic crystal increased the local refractive index and shifted the bandgap. This was then detected by recording the spectrum at the waveguide output. A total of 0.15 μM concentration of BSA, corresponding to a surface coverage of 6 ng/mm^2, was easily detected. While being a novel technique for performing label-free sensing, this device design is hard to multiplex since the bandgap prohibits transmission over a large range of wavelengths. In the experiment mentioned above, the device exhibited a relatively low solution phase LOD although the authors argue that there is still room for optimization.

Photonic crystal resonator devices posses very high Q-factors and are very sensitive to changes in the refractive index of their structural elements. They consist of a 1D or 2D photonic crystal with a defect in the crystal structure which acts as the resonant cavity. Chow et al. (2004) demonstrated a 2D photonic crystal microcavity consisting of a periodic lattice of holes in a silicon layer with a central hole defect. Changes in the refractive index in these holes shifted the resonant peak which allowed them to measure the refractive index of the surrounding liquid medium. More recently Lee and Fauchet (2007) demonstrated a similar architecture with a Q-factor well over 5,000. The device and experimental setup is shown in Fig. 2. They were able to detect BSA with a lower detection limit of a molecule monolayer with a total mass as small as 2.5 fg. Although it is difficult to perform multiplexed detections on a single waveguide using this design, it offers the unique advantage of performing highly sensitive detections in ultra-small volumes.

3.3 Surface plasmon resonance biosensing

Surface plasmons are electromagnetic waves which propagate along metal/dielectric interfaces. As will be described below the conditions for exciting these optical modes are extremely sensitive to the dielectric environment very near this interface. As a result SPR is one of the most commonly exploited label-free optical biosensing technique in use today and forms the third optofluidic architecture we will review here. Detailed reviews of the topic are widely available in the literature (Homola et al. 1999a, b; Haes and Van Duyne 2004; Karlsson 2004) and thus here we provide just a brief introduction to the various implementations and focus on the limitations of the current state of the art in SPR biosensing in the context of multiplexing and sensitivity.

3.3.1 Angular SPR biosensing

The most common implementation of SPR biosensing is the attenuated total reflection (ATR) approach using the Kretschmann geometry shown in Fig. 3a. In this arrangement, transverse polarized light is incident on a coupling prism at a specific angle extending an evanescent

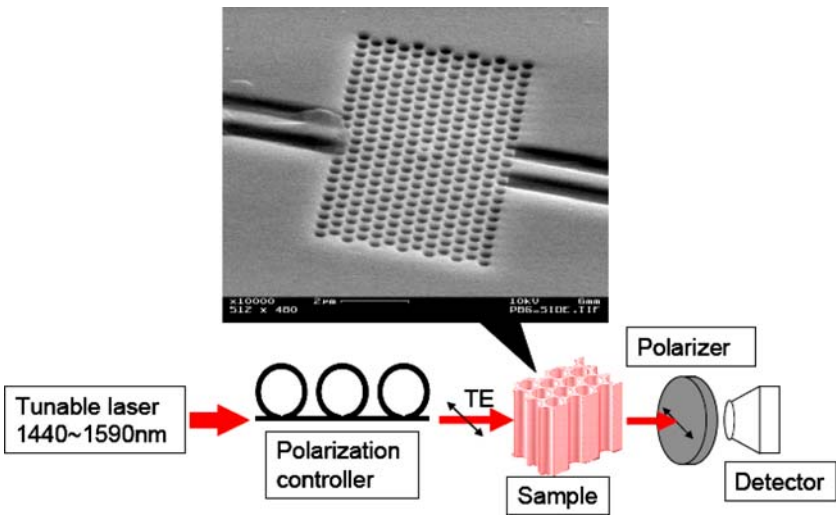

Fig. 2 Optically resonant photonic crystal biosensor from Lee and Fauchet (2007). Scanning electron microscopy photograph of a typical device used in these experiments and schematic of the experimental setup. A limit of detection (*LOD*) on the order of 2.5 fg (for bovine serum albumin) was obtained using this device. Copyright Optical Society of America. Reproduced with permission

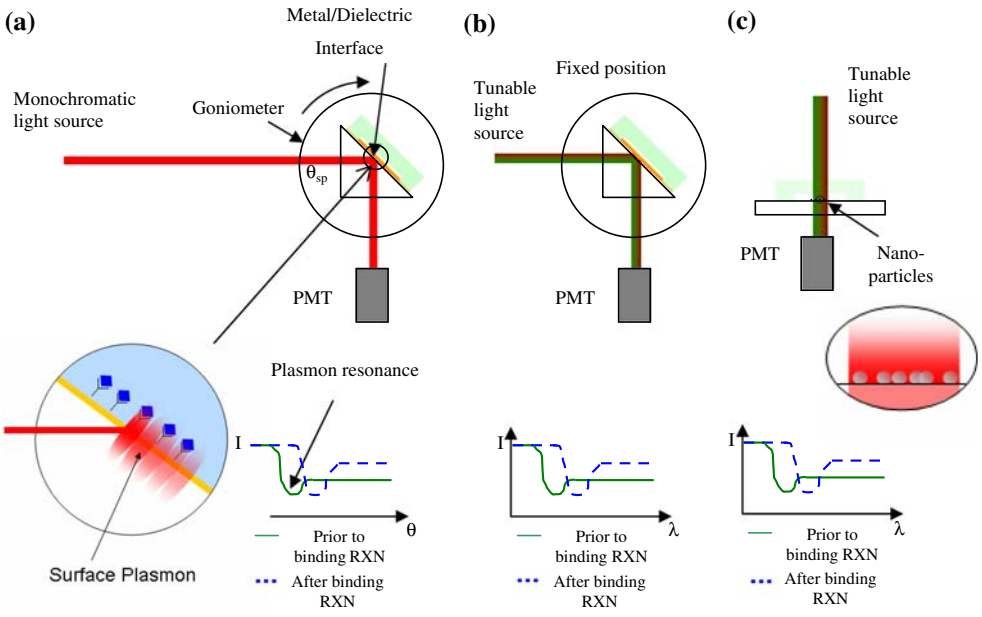

Fig. 3 Variations on surface plasmon resonance (*SPR*) detection. **a** Angular SPR, **b** spectral SPR, and **c** nanoparticle or Local SPR. Details on each of the above techniques are provided in the text

(non-propagating mode) into the metal/dielectric interface. Since the evanescent mode is non-propagating, under most conditions the incident light is reflected from the surface and very little loss in optical power is observed at the detector. At a particular angle of incidence, θ_{sp}, a momentum matching condition exists and a certain amount of the incident energy is coupled into a surface plasmon mode resulting in a reduction in the reflected power. The incident angle for this plasmon excitation is described by Eq. (1) below,

$$\sin\theta_{sp} = \frac{1}{n_p}\sqrt{\frac{\varepsilon_m(\lambda)\varepsilon_d}{\varepsilon_m(\lambda)+\varepsilon_d}} \quad (1)$$

where n_p is the refractive index of the prism, $\varepsilon_m(\lambda)$ is the dielectric constant of the metal film which varies as a function of the excitation wavelength, λ, and ε_d which represents the dielectric constants of the dielectric layer (Homola et al. 1999a, b). Since the plasmon wave extends only a few hundred nanometers into the dielectric layer only the near field region is probed optically by the technique. At fixed wavelength ε_m is constant and thus from Eq. (1) it is apparent that θ_{sp} is only a function of the dielectric constant in the region very near the interface.

For biosensing applications, this metal/dielectric interface consists of a thin metal film deposited either directly on the prism surface or an index matched slide and a solution phase "sensing" (dielectric) layer. When the metal layer (typically gold is used as it is inert to the atmosphere, but can be modified by appropriate surface chemistry to provide active sites for bonding of organic molecules) is functionalized with a series of biorecognition agents, the dielectric constant of the sensing layer is modified and a shift in θ_{sp} is observed. As solution phase targets specific to the immobilized biorecognition agents are introduced the refractive index in the sensing layer increases further and θ_{sp} again is affected. By monitoring the rate of and total change in θ_{sp}, quantitative information regarding the presence and concentration of the solution phase targets, and bioaffinity constants for the target-probe binding reaction can be obtained. A subtle variation on the above geometry uses a grating coupler to excite a plasmon wave at the interface (Unfricht et al. 2005).

3.3.2 *Spectral SPR biosensing*

A variation on the above approach, termed spectral SPR (Dostalek et al. 2005; Otsuki et al. 2005; Yuk et al. 2005), exploits the wavelength dependence of ε_m to interrogate the surface plasmon coupling conditions. The apparatus is similar to the angular approach above, the difference being that the experiment is conducted at fixed incident angle and a tunable excitation source is used to sweep over the wavelengths of interest as illustrated in Fig. 3b (alternatively a broadband excitation source could be used and the spectrum of the reflected light is recorded). It is not difficult to extract that since ε_m is a function of λ so long as the wavelength scanning range is large enough that the right hand side of Eq. (1) can be made to equal the left had side at a particular resonant wavelength. Analogous to the above, by monitoring the change in the resonant wavelength with time information regarding the concentration and bioaffinity properties of the target can be obtained.

The spectral technique has several advantages over the traditional angular approach which are usually stated as a simplification of the experimental apparatus and increased inherent sensitivity [since the wavelength dependence of ε_m is relatively weak (Homola et al. 1999a, b)]. More importantly, however, the spectral approach lends itself to imaging based data collection techniques (Otsuki et al. 2005) which are more amenable to multiplexing for high throughput screening (as will be described below). Despite these advantages the angular SPR approach is more broadly exploited than the spectral approach due to added expense of the tunable illumination source or the added complication of extracting the reflected spectrum.

3.3.3 Local SPR biosensing

As an alternative to the traditional SPR biosensing approaches outlined above, nanoparticle based SPR (or local SPR, LSPR) has recently been developed for surface phase geometries (Kelly et al. 2003). This approach relies on coupling into a plasmon mode on the surface of a sub-wavelength scale, surface immobilized, metallic nanoparticle or nanostructure (Haes and Van Duyne 2002; Nath and Chilkoti 2002; Yonzon et al. 2004) or nanohole array (Tetz et al. 2006). Similar to the spectral approach above, this results in a decrease in the transmitted power (or increase in scattered power) at a specific resonant wavelength which is strongly dependent on the environmental dielectric conditions. The most significant advantage of this approach is the simplicity of the arrangement (as shown in Fig. 3c) and the facile extension to a highly multiplexed architecture (details of how this is done will be expanded on Sect. 3.3.5).

The most extensive characterizations and implementations of this architecture have been carried out by the Van Duyne group (see a recent review by Zhao et al. 2006). The well cited study by Haes and Van Duyne (2002) provides details of the experimental apparatus and reports refractive sensitivities on the order of $\Delta n = 0.01$. Implementation in the context of the detection of biomarkers associated with Alzheimer's disease (Haes et al. 2005) using sera samples has also been demonstrated. Readers are also referred to another series of papers from this group (Haes et al. 2004; Yonzon et al. 2004) which provides a comparison between localized and propagating SPR biosensing. Interesting recent examples from other groups include the work by Kim et al. (2007) who developed an interesting LSPR architecture based on what they referred to as a "gold capped oxide nanostructure" comprising of oxide posts with gold caps. Because the spacing between the structures was relatively small the reflected light exhibited interferometric behavior that was highly sensitive to changes in the thickness of the biomolecular layer. Detection limits in the pM range (for oligonucleotides) were reported. The LOD reported in some of the earlier papers using this approach were much lower than those for traditional SPR. however. recently Dahlin et al. (2006) described a technique for improving the instrumentation and data analysis techniques for this architecture in the end reporting sensitivities on the order of 100 pg/cm^2. In a different approach Kim et al. (2006) compared the LSPR response of gold nanodot and nanoring arrays (fabricated using imprint lithography), examining the effect that nanostructure geometry has on the internal sensitivity (e.g. decreasing ring width was found to increase sensitivity).

One of the challenges with multiplexing such systems is in the development of ordered and uniform metal nanostructured arrays which are sufficiently well spaced to avoid plasmon coupling effects (Haynes et al. 2003) but sufficiently dense so that the aggregate signal can be detected. Nanofabrication techniques such as focused ion beam and electron beam lithography make precision fabrication of such arrays relatively easy but the serial nature and general expense involved, strongly conflict with the development of mass producible devices. As a result a number of alternative parallel techniques have been developed. One of the more commonly exploited techniques is nanosphere lithography (Hulteen and Vanduyne 1995; Haynes and Van Duyne 2001) whereby a monolayer of self-assembled spheres is assembled on a surface and a metallic layer deposited over the array. After chemical removal of the spheres, metal deposited through the voids in the monolayer comprise the metallic nanoparticle array. While the local quality of the array can be excellent, a challenge remains in being able to self-assemble a defect free sphere layer over a wide enough spatial distance to extend this local order to larger area arrays. An alternative technique based on nanoimprint lithography was demonstrated by Kim et al. (2006).

3.3.4 Sensitivity and LOD of propagating and localized SPR

To date the most sensitive propagating SPR measurements are those reported by Tao et al. (1999) who demonstrated the potential for detection resolution on the order of $\Delta n = 10^{-8}$. In general, however, most systems operate with resolutions on the order of $\Delta n = 10^{-5}$. This results in LODs which are typically on the order of 100–1,000 pg/cm^2 (Jung et al. 1998; Su et al. 2005). As with some of the other devices above, the extreme sensitivity exhibited by propagating SPR at least partially relates to the relatively large interaction lengths of the plasmon field with the surface bound analyte. As such a large number of binding

events are required in order to induce a relatively small aggregate change in refractive index and rare target identification becomes more difficult. The ultimate limitation of such systems is that the achievable spatial resolution is limited by the decay length of the excited plasmons (Berger et al. 1994). For most setups this is on the order of 20 μm and we can use this to estimate the potentially achievable LOD as being on the order of 0.4 and 4 fg (i.e. 0.4×10^{-15} and 4×10^{-15} g). It is important to note, however that, as discussed by Huang et al. (2007), the propagation distance of the plasmon can be substantially reduced by optimizing the interrogation wavelength and thus there may be room to push these values farther down.

As mentioned in the introductory paragraphs, the strength of emerging nanotechnologies is not necessarily that the overall internal sensitivity to bulk measures is much greater but that the surface area that is probed is much smaller. McFarland and Van Duyne (2003) have reported zeptomole level sensitivity using single silver nanoparticles sensors. A darkfield image of the silver nanoparticles used in these experiments is shown in Fig. 4. As expanded on in the McFarland and Van Duyne (2003) work, a paper by Riboh et al. (2003) demonstrated wavelengths shifts on the order of 38 nm for adsorption of ~100 antibiotin molecules. Extrapolating then it can be argued that assuming a molecular mass of 150 kDa for antibiotin the total mass per particle is on the order of 25 ag (25×10^{-18} g). Assuming a 1 nm wavelength resolution the LOD should be on the order of 0.6 ag.

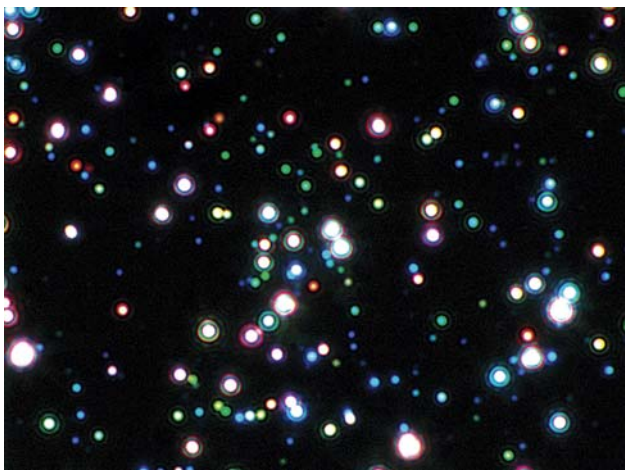

Fig. 4 Dark-field optical image of Ag nanoparticles used as single particle nanosensors in McFarland and Van Duyne (2003). The field of view in this image is approximately 130 μm × 170 μm. Copyright Optical Society of America. Reproduced with permission

3.3.5 Multiplexing SPR biosensing techniques

Surface plasmon resonance imaging (Rothenhausler and Knoll 1988) is a relatively simple technique by which the above measurements can be multiplexed to monitor 2D arrays (Homola et al. 2005). In essence the technique involves exciting a relatively broad area of the sample surface which has been pre-arrayed with a series biorecognition sites. For the propagating SPR case the reflected light is then imaged on, typically, a CCD camera (in place of the PMT shown in Fig. 3). The use of 2D imaging makes it difficult to extract the optical adsorption as a function of incident angle (Fig. 3a). As such the measurement is typically made at a fixed wavelength and by observing the changes in spatial pattern of the reflected intensity as the peak adsorption angle drifts closer to or farther away from the observation angle (Jordan et al. 1997; Nelson et al. 1999, 2001). Numerous examples of the application of this technique exist in the literature as applied to nucleic acid hybridization (Nelson et al. 2001) and detection of low molecular weight protein biomarkers (Lee et al. 2006).

While this method is relatively easy to setup and make kinetic measurements with the relatively broad adsorption peaks obtained from SPR measurements can reduce the overall sensitivity (i.e. the percent change in reflectivity in the case of a positive binding event can be quite small). To improve the overall sensitivity of the technique Fang et al. (2006) demonstrated a nanoparticle enhanced technique for low level and multiplexed detection of microRNA. A similar amplification method was used by Li et al. (2006) for single nucleotide polymorphism genotyping. Shumaker-Parry and Campbell (2004) introduced an apparatus that allowed the extraction of angular data in an imaging format. In that work 1.2 ng/cm^2 sensitivity was reported and which can be equated to a 500 fg LOD for a 200 μm spot size. As an alternative Otsuki et al. (2005) demonstrated the use of spectral SPR imaging whereby images are captured as a function of wavelength rather than angular position. The advantage of this approach is that eliminates the need for a rotating experimental setup, but increases the complexity of the apparatus as either a tunable laser source or monochromator is required.

To improve the LOD, imaging techniques based on localized SPR are also under development. As an example Endo et al. (2006) demonstrated an interesting core-shell nanoparticle imaging technique applied to the monitoring of antibody–antigen reactions. Using this technique as many as 300 separate reaction sites were monitored. The advantage of such techniques is the relative simplicity of the optical setup (a simple co-linear transmission setup can be used, rather than requiring a precise angular alignment) and the potential to achieve the single nanostructure LOD

as described in Sect. 3.3.4. One of the major remaining challenges is in the development of techniques for independently functionalizing each nanostructure.

On the commercial side SPR biosensor developers have introduced multiplexed versions of their existing platforms, namely the Spreeta from Texas Instruments (Spangler et al. 2001; Chinowsky et al. 2003) and FlexChip from BIACORE (Wassaf et al. 2006). Of those that which contains the highest degree of parallelity is the FlexCHIP platform. An example implementation of which for antibody arrays is shown in Fig. 5, from Usui-Aoki et al. (2005). This chip uses a grating coupling mechanisms and a mechanical scanning technique to image as many as 400 reactions over the course of 3 h (which is still orders of magnitude lower than microarray based assays). In these multiplexed systems, however, the sensitivity is reduced to that available from a standard microarray (see Usui-Aoki et al. 2005). As such there remains a significant need for the development of platforms which can maintain the extreme sensitivity available from traditional SPR while pushing the limits of parallelity into the 1,000s or 10s of 1,000s of binding reactions.

4 Nanoscale electrical biosensors

In this section, we focus on the use of single dimensional electrical nanostructures for biosensing. In the first section, we provide a general overview followed by detailed discussion of the advantages of semiconductor nanowire and carbon nanotube approaches. The second section addresses issues relating to device assembly and multiplexing architectures.

4.1 Single dimension electrical nanostructures

One-dimensional nanostructures (Xia et al. 2003; Lieber and Wang 2007), such as carbon nanotubes and semiconductor or polymer nanowires, represent an interesting and relatively new paradigm for biochemical sensing (Cui et al. 2001). Though a number of different implementation architectures exist, those which have been the most successful mimic the basic field effect transistor, FET (Cunningham 1998). In this arrangement, a single nanowire (or nanotube) is placed between two lithographically patterned microscale contact pads which are referred to as the source and drain respectively. It is possible to functionalize the nanowire with the appropriate capture probes prior to assembly into the FET structure, however, due to the complexity involved in the assembly process, it can be easier to first assemble the wires into the device structure and then functionalize them using traditional (e.g. spotting) techniques. During sensing, the conductivity of the wire is measured by monitoring the current flow between the electrically excited contacts. When binding of a charged species occurs, the local charge field is modified leading to an accumulation or depletion of carriers in the nanowire, reflected by a change in its conductivity (analogous to applying a gate voltage). An example of this working principal is shown in Fig. 6 for the nanowire virus detection device presented by Patolsky et al. (2004). The

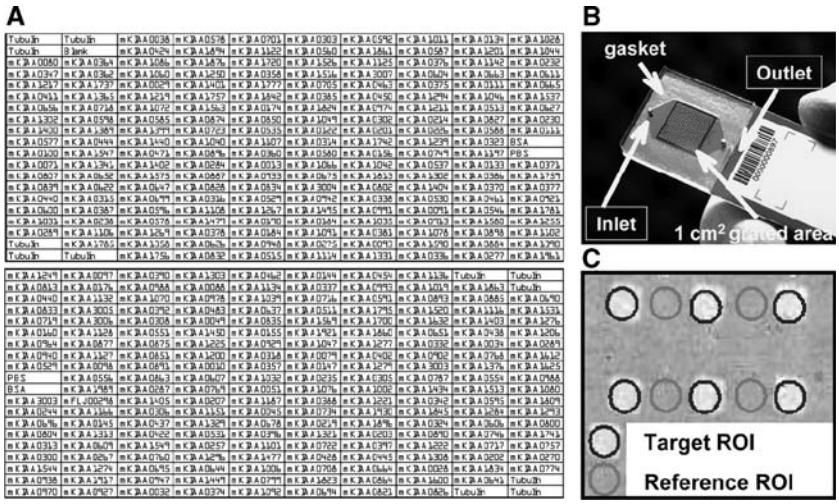

Fig. 5 Surface plasmon resonance antibody array chip from Usui-Aoki et al. (2005). Antibody microarray layout. SPR signals from this array were measured by a FLEXCHIP^{TM} Kinetic Analysis System. **a** Layout of the array containing 400 reaction sites which could be monitored in parallel. Array details are available in the aforementioned reference. **b** Overview of the affinity chip where all reaction sites are located in a 1 cm × 1 cm. A flow cell with a volume of 47 μL is used to transport the sample over the array. **c** Visualization of immobilized antibodies. Copyright Wiley-VCH Verlag GmbH & Co KGaA. Reproduced with permission

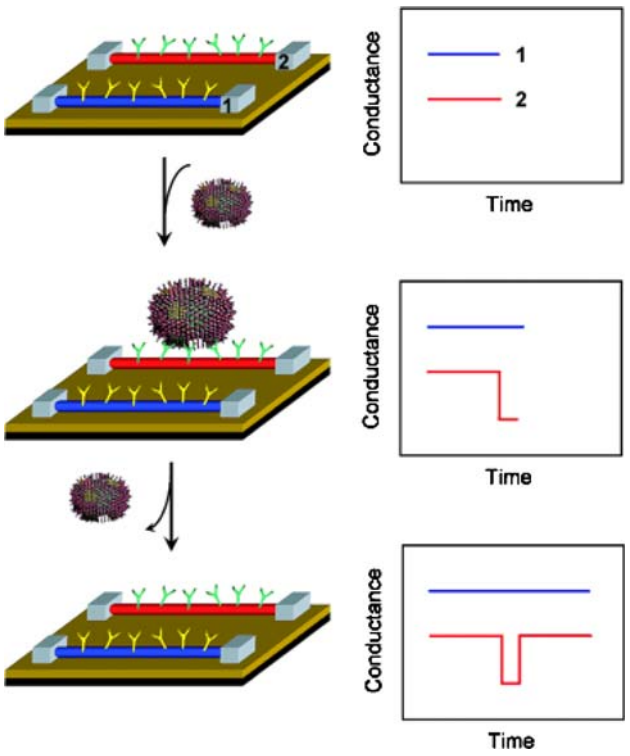

Fig. 6 Technique for semiconductor nanowire based detection of single viruses from Patolsky et al. (2004). In this figure, two nanowire devices (labeled *1* and *2*) are functionalized with different antibodies. The antibodies on the second wire are specific to the target virus. When introduced, the virus binds to the second nanowire and a change in the conductance is observed and taken to be indicative of the presence of the virus in solution. When the virus unbinds from the nanowire, the conductance returns to its original value. Figure is Copyright 2004 National Academy of Sciences, USA

advantage of incorporating a nanowire into the FET is that its cross-sectional area is of the same spatial scale as the charge field surrounding the bound molecule and thus a small number of close proximity targets can have significant impact on the measured conductivity. The result is an extremely sensitive device, capable of obtaining both static and dynamic information through a very simple electrical measurement.

4.1.1 Semiconductor nanowire based devices

Semiconductor nanowires have two main advantages over carbon nanotubes when used in biosensing devices. First, the material properties can be more precisely controlled by manipulating the conditions during synthesis and using well-developed semiconductor doping techniques. Second, the native oxide layer that forms on the outside of silicon nanowires allows the use of a broad class of already well-developed functionalization and blocking chemistries. Generally speaking, nanowire devices represent a suitable technology for liquid phase measurements, so long as the ionic strength of the sample buffer is relatively low. The reason for this is that the spatial extent to which the charge field of a bound target can expect to interact with the nanowire is limited by the Debye screening length (Cheng et al. 2006). This length is strongly dependent on the ionic strength of the transport buffer with lower ionic strength buffers yielding longer screening lengths (Hunter 1981). Physiological systems such as human sera have relatively high ionic strengths leading to screening lengths on the order of 1 nm. As such, most devices require some dilution of the physiological system in order to increase the Debye length and detect binding events which may occur a fair distance from the wire itself. Given this limitation, architectures have been used for a number of label free sensing applications (see Li et al. 2004; Wang et al. 2005) including the detection of single viruses (Patolsky et al. 2004). Of particular note is the work of Hahm and Lieber (2004) who demonstrated that a time dependent conductance increase enabled the identification of fully complementary versus mismatched DNA samples. For more detailed information, biosensing applications are covered in a number of broader reviews of semiconductor nanowire devices (Bauer et al. 2004; Patolsky et al. 2006; Wanekaya et al. 2006; Wang et al. 2007).

4.1.2 Carbon nanotube based devices

The competitive technology to the above is carbon nanotube field effect transistors (NT-FETs). Comprehensive reviews are available for the synthesis and sensing of carbon nanotube architectures (see Wang 2005; Sinha et al. 2006; Mahar et al. 2007). The two main advantages single walled carbon nanotubes provide over silicon nanowires are higher electron mobilities (McEuen et al. 2002) and diameters in the sub nanometer range (Kong et al. 1998). This makes it possible to, in principle, detect lower charge densities. In addition to the limitations discussed above for nanowires, an additional limitation of existing nanotube devices is that the synthesis cannot be as precisely controlled as with nanowire devices. Recent advancements, however, enable oriented long single walled structures with nearly homogeneous mechanical and electrical properties (Huang et al. 2004) which may serve to solve this problem.

One of the first effective sensing architectures comprised of NT-FETs was presented by Kong et al. (2000), where they were able to detect toxic gaseous molecules in NH3 and NO2 down to concentrations as low as 1 ppm with low response times (around 5 min) at room temperature, which constituted a huge leap over the current technology (Shimizu and Egashira 1999). It was also found that an electrolyte gate is roughly an order of magnitude

more effective in modulating the conductance of a nanotube when compared to a standard silicon back gate referenced to vacuum, making NT-FETs ideal for biological applications (Rosenblatt et al. 2002). Likely the first NT-FET displaying real time biological and pH sensing capabilities was established by (Besteman et al. 2003) in which they were able to measure glucose oxidase activity by measuring changes in nanotube conductance. Part of this breakthrough came as a result of using an effective linker molecule between nanotube and the analyte. Subsequent research by Larrimore et al. (2006) displayed how nanotubes can function as ultra sensitive reference electrodes to redox reactions, effectively giving rise to nanoscale electrochemical cells. In parallel, single stranded DNA was immobilized on aligned carbon nanotubes by He and Dai (2004), who opened the route for sequence specific DNA diagnosis using NT-FETs. In that work cyclic voltammetry (Bard 2001) was used to analyze the binding activity of two sets of complementary strands. NT-FET sensors also present novel avenues for proteomic research. Boussaad et al. (2003) were able to detect as few as 20 molecules of cythochrome c ($\sim$0.5 ag) adsorbed onto individual nanotubes. In addition, Chen et al. (2004) were able to accurately characterize protein binding and inhibition down to 100 nM solution phase concentration range.

4.2 Assembly and multiplexing techniques

Although nanowire fabrication and synthesis techniques are very well developed (Wang 2003), one of the more challenging aspect of incorporating them into a multiplexed sensing architecture has classically been the lack of a simple technique for assembling the wires into a device structure with the desired electrical connectivity. Typically "hybrid" assembly approaches are used which involve lithographically patterning the electrical infrastructure and then using an active assembly procedure to attract and position the 1D nanostructure. Assembly techniques based on a number of different mechanisms have been developed include magnetic (Hangarter and Myung 2005), dielectrophoretic (Smith et al. 2000; Evoy et al. 2004) and fluidic (Huang et al. 2001) positioning. The advantage of an active approach (the former two) is that it enables one to, in principle, capture different types (e.g. p-doped vs. n-doped for nanowires, metallic vs. dielectric for nanotubes) or even differently functionalized nanowires at specific locations, thereby increasing the flexibility of the device. The challenge with this approach is the same as with most directed assembly procedures in that although they may be autonomous, the amount of time required to complete a scaled up assembly process (with say 10 s of thousands of reaction sites) quickly becomes excessive. Passive assembly approaches can be much quicker, but lack the positioning specificity.

At present the most highly developed multiplexed sensing architecture based on these "hybrid" assembly procedures is that presented by the Lieber group (Zheng et al. 2005) based on their pioneering semiconductor nanowire studies (Cui and Lieber 2001; Cui et al. 2001; Huang et al. 2001). The architecture as developed comprises a 1D array of approximately 200 electrically independent nanowire devices and was used to perform low-level detection of a series of serum-borne cancer antigens. Very low solution level LODs were reported, on the order of 0.9 pg/mL. Antibody attachment was carried out using a well-developed functionalization chemistry with traditional microarray spotting techniques used to discriminate between different wires. Though only demonstrated in 1D, an extension to 2D should be a relatively simple matter simply involving slightly more involved onboard electronics. To avoid the use of these relatively complex assembly procedures, there is significant interest in being able to develop nanowire sensing platforms compatible with traditional CMOS fabrication. Such an approach was recently demonstrated by Stern et al. (2007) where nanowires were fabricated as part of a CMOS process using ultra-thin SOI wafers and enabled through a unique tetramethylammonium hydroxide wet etching procedure (traditional RIE type etches were observed to degrade sensing performance). A LOD on the order of 100 fM (solution phase antibody concentration) was reported.

5 Nanoscale mechanical biosensors

The next broad class of nanoscopic biosensor we will cover here are those which exploit mechanical effects. We separate the discussion into two sections, the first focusing on cantilever based approaches and the second emphasizing acoustic techniques.

5.1 Cantilever based devices

The high mechanical quality factors associated with resonant micro- and nano-mechanical systems (Craighead 2000; Majumdar 2002; Ekinci et al. 2004; Ekinci and Roukes 2005) have proven useful in a number of application areas ranging from signal processing (Shim et al. 2007), to electrometry (Cleland and Roukes 1998), to, as is most relevant to this review, mass sensing. Recent works have demonstrated the ability of doubly clamped very high-frequency nanomechanical cantilever systems to accurately weigh as little as a handful of xenon atom (Yang

et al. 2006) with a noise floor of $\sim$20 zg (LOD) and resolution of 7 zg or 7×10^{-21} g. As label free biosensors such systems have been applied to the detection of viruses (Ilic et al. 2004a, b; Gupta et al. 2006), single cells (Ilic et al. 2001) and individual strands of DNA (Ilic et al. 2005). In these "dynamic mode" systems the amount of bound mass is typically determined by observing changes in the resonant frequency of the oscillator. Cantilever readout is often done optically (Ilic et al. 2000) in which case spatial multiplexing is relatively easy. The limitation being that the cantilevers must be sufficiently well spaced that they can be independently functionalized. While the LOD of these biosensor can be as low as a few attograms (Ilic et al. 2004a, b), see Fig. 7 for SEM images of the devices used in that work, such measurements must normally be made in reasonably high vacuum environments (or at least in air) due to the effect of viscous damping on the mechanical oscillations. As a result, they must usually be removed from the aqueous or physiological sensing environment (the exception being the detection of air-borne pathogens) before detection can be performed.

To avoid this problem several groups have developed "static mode" deflection based systems (Wu et al. 2001; Zhang et al. 2006a, b), whereby the bending of a mechanical structure is measured in response to binding events along one side of the cantilever. Detection of solution phase concentrations are on the order of pM, which is roughly equivalent to that achievable using florescent labeling, have been reported. The LOD of these devices tends to be lower since binding occurs along an entire surface of the cantilever (which is on the order of 500 μm long × 100 μm wide). An alternative solution to this problem has been presented by Burg and Manalis (2003) who demonstrated the integration of microfluidic channels inside a vibrating cantilever beam. The advantage of this is that the cantilever itself can vibrate in a vacuum environment, while the binding sites simultaneously remain in contact with the liquid. An integrated version of the device was recently reported (Burg et al. 2006) to have an LOD on the order of 10^{-9} g/cm^2 with eight independently addressable sensor sites. With their reported surface area (53,000 μm^2) this yields an absolute LOD on the order of 0.5 pg. Of additional note is a recent work by Braun et al. (2005) which proposed a direct solution to the vibrating cantilever in a physiological environment problem, analytically demonstrating than and LOD of 25 fg should be possible. To our knowledge, however, this has yet to be demonstrated experimentally.

5.2 Acoustic biosensors

Acoustic devices form a second class of mechanical biosensor, the most common implementations of which exploit bulk acoustic waves, BAW, such as the quartz crystal microbalance (Marx 2003). Analogous to the above, these devices measure changes in the resonant frequency of a piezoelectric crystal in response to changes in surface adsorbed mass. Limits of detection of such devices are on the order of ng/cm^2 and recent lithographically fabricated devices (Rabe et al. 2003; Zhang and Kim 2005) have demonstrated on-chip multiplexing with balance sizes on the order of a few 10 s of micrometers in radius. A variant on these BAW techniques are Surface Acoustic Wave, SAW, devices which exploit shear surface waves (Josse et al. 2001; Lange et al. 2006). Sensitivities are typically of the same order (Kalantar-Zadeh et al. 2003) as QCM type devices. Although the ultimate size of the interrogation area is limited by the required spatial distance between excitation electrodes, some recent works have discussed techniques for exploit nanostructures to increase the overall sensitivity (Rao and Zhang 2006). For more information readers are referred to a recent review article by Lucklum and Hauptmann (2006).

6 Homogeneous (solution) phase biosensors

Unlike the devices described above, mobile or solution phase sensors typically incorporate functionalized nanoparticles, which have been suspended in the sensing volume to act as both the binding and detection platform.

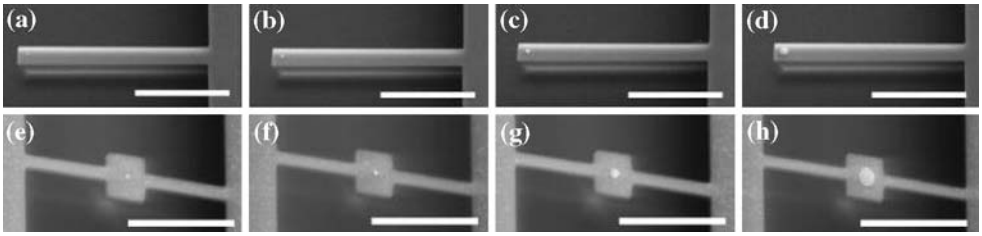

Fig. 7 Nanomechanical resonators for ultralow LOD mass sensors from Ilic et al. (2004a, b). Oblique-angle SEM micrographs of **a–d** cantilevers (scale bar = 5 μm) and **e–h** bridge oscillators (scale bar = 2 μm). The diameters of the Au pads were 50, 100, 200, and 400 nm, from left to right. Reused with permission from (Ilic et al. 2004a, b). Copyright 2004, American Institute of Physics

The advantage of such techniques is the ability to concentrate sensing units within a volume region as opposed to a surface, which allows for faster and more efficient binding of targets (as the diffusive transport length scale is reduced). Such systems are also very amenable to in vivo sensing applications and have the potential for very simple feedback mechanisms. As will be discussed below, the current challenges in this area revolve around the synthesis of bio-compatible nanoparticles for these in vivo applications and the differentiation of different nanoparticles within a single solution for multiplexed analysis.

6.1 Solution phase SPR

Similar LSPR techniques to those described in Sect. 3.3.3 are also popular in solution phase systems (Storhoff et al. 1998) since they have the ability to provide a very simple colorimetric feedback (i.e. a change in the local dielectric conditions surrounding a solution phase nanoparticle results in a change in the peak scattered light which is interpreted as a change in solution color by the observer). Examples of implementation include detection of glucose (Aslan et al. 2004), protein conformation changes (Chah et al. 2005), specific sequences of genomic DNA (Li and Rothberg 2004) and cholera toxin (Schofield et al. 2007). As alluded to above, however, the relatively broad absorption spectrum associated with plasmon coupling and the inability to spatially localize the probes complicates multiplexed detection. Yu and Irudayaraj (2007) reported the use of functionalized gold nanorods for the detection of shifts in plasmon waves due to molecular binding of proteins on the surface of the nanorods. Use of controlled aggregation of the gold nanorods allows for magnified shifts in the plasmon peak. The plasmon bands show significant sensitivity to the aspect ratio of the nanorods, allowing for differentiation and increases the potential for multiplexed analysis. In addition, spectra of nanorods with multiple detection sites were shown to have differentiation for single, double, and triple binding of targets. For more information and a more in depth comparison with surface phase techniques readers are referred to Haes et al. (2004).

6.2 Encoded quantum dots

Quantum dots (QDs) are very small (2–10 nm, not inclusive of solubility ligands and functionalization chemistries) semiconductor nanostructures which confine electrons to discrete energy levels. The spacing between these energy levels is strictly governed by the quantum confinement conditions enforced by the dot size. As such the wavelength at which a photon is emitted as the electron falls from a higher to a lower energy state can be precisely controlled through careful QD synthesis. Advantages of QDs over most florescent dyes include: a wide absorption spectrum coupled with a very narrow emission spectrum, a high quantum yield, and a resistance to photobleaching and optical or chemical degradation (Caruso 2004).

One of the most interesting approaches to multiplexing solution phase systems uses a QD encoding scheme. This approach involves the use of micro- or nanoparticles that contain within themselves a series of embedded QDs of different sizes. As such the emission spectrum of such a particle comprises the summation of the emissions of the QD particles embedded within. A particle then is designed to have a specific colorimetric code which differs from other particles made with a different ratio of colored QDs. Quantum dots are particularly useful for this application as they can all be excited with the same light source. Han et al. (2001) used CdSe/ZnS QDs to demonstrate the encoding of polymeric microbeads using a three-color scheme. Cao et al. (2006) was able to encapsulate QDs within a silica shell nanoparticle for improved biocompatibility. In both studies, the nanoparticles were functionalized for the detection of fluorescently tagged DNA. One of the advantages to this is the ability to simultaneously detect both the coding and target spectrum. Although a separate peak can be detected for a target signal, there is some overlap between the target FITC signal and blue QD emissions. A review article by Medintz et al. (2005) discusses many available functionalization methods for labeling and sensing applications.

An example of research in multiplexed assays using encoded QDs exploits flow cytometry for a fast, efficient, and low-cost spectral analysis (Xu et al. 2003). Gao and Nie (2004) demonstrated a system using flow cytometry capable of reading 1,000 encoded particles per second. Ideally, a sample would be mixed with a suspension of QD embedded nanoparticles to bind targets to receptors. The mixture would then be fed to a flow cytometry system where the coding and target signals could be recorded for later analysis.

6.3 Alternative solution phase approaches

Before closing this section we briefly mention a couple of alternative approaches to multiplexed solution phase sensing which do not fit into the previous two categories but are important for comprehensiveness. Wang and Tan (2006) recently developed a coding scheme based upon fluorescence resonance energy transfer (FRET) using varying ratios of inorganic and organic dyes. This particular method is valuable to biological in vivo applications where toxicity concerns may prohibit the use of QDs.

Pregibon et al. (2007) demonstrated the use of microfluidics combined with photolithography to create graphically encoded polymer nanoparticles that contain regions for a grid identification system and analyte binding. The unique identification scheme presented does not rely upon wavelength or intensity based differentiation. Furthermore, these particles due to the photolithographic synthesis method have a potentially more stable and efficient yield.

7 Summary and conclusions

The previous four sections have provided a cross-sectional overview of the state of the art in nanobiosensors, with particular attention paid to how well each technology meets the broad application requirements as outlined in Sect. 2. Before closing this review we will attempt to condense as much of that information as possible down into a few short summary statements.

As was originally mentioned in section two and referred to throughout this review, the primary advantage of nanoscopic biosensors in not necessarily that they exhibit higher internal sensitivity to bulk measures, such as refractive index changes, but rather that the surface area or volume which is probed is much smaller. Given that most of the applications described above are concerned with pushing the limits of how few molecules can be detected in a given volume of solution, nanobiosensors are inherently useful so long as the entire platform (inclusive of the biosensor and associated micro-/nanofluidics) are designed in such a way that the entire sample volume can be interrogated by the sensor. With this in mind, to our knowledge mechanically resonant devices have demonstrated the lowest limits of detection, so long as the measurements can be made in an environment where viscous damping is minimized (usually vacuum but also potentially in air). In liquid environments, where viscous damping is high, the 1D nanostructure electrical detection technologies have, to date, best demonstrated the ability to push the limits of sensitivity. Since most biomolecular detection strategies involve the "chaining" together of a series of molecules (namely a linker, probe and finally target) to perform the measurement, the spatial limitations on the charge field disturbance in moderate ionic strength solutions can in some cases prove to be a significant limitation. As mentioned above, reducing the run buffer ionic strength can help to increase the double layer thickness, but can also serve to impede binding due to enhanced electrostatic repulsion. Optical devices have a similar spatial probing limitation, governed by the thickness of the evanescent field. This tends to be somewhat less restrictive since the evanescent field can be as thick as a few hundred nanometers (Saleh and Teich 1991). The tradeoff is that the thicker the evanescent field, the more the exposed optical energy is diluted and the greater the probed volume. As such a greater amount of bound mass is required to produce a measurable change in the transduction signal. For systems like the zero-dimensional LSPR nanoparticles shown in Fig. 4, the total surface area is so small that this is more than compensated for.

Surface plasmon resonance imaging techniques as described in Sect. 3.3.5 almost certainly represent the most well developed and cheapest technique for performing label free, highly multiplexed, biosensing. The simplest implementations, however, lack the LOD strength of the nanosensor technologies. As such platforms which incorporate the LOD advantages of the LSPR techniques while maintaining this level of multiplexing are likely to be very successful. The advanced optical devices such as the photonic structures described in Sects. 3.1 and 3.2 have the advantage of being able to operate in a planar format (meaning the excitation source, device, fluidics, and detector can all be in the same plane) make such devices useful for more chip-based devices. To date, however, the LOD and measurement parallelity of these devices has not been as well developed as some other techniques. An advantage of such devices is that the fabrication is not particularly difficult, typically requiring only single layer lithography. One-dimensional nanostructure arrays are particularly promising given the demonstrated sensitivity (subject to the limitations described above) and the inherent ease of using an electrical measurement scheme. The challenges in terms of assembly of nanowires into a device structure are somewhat of a bottle neck at present, however, as the semiconductor industry continues to push the limits of lithography, direct patterning will become more and more feasible (beyond what has already been demonstrated).

Another major challenge to the further development of particle nanosensor technology is one that is not often addressed in the literature, namely the ability to target and confine chemical functionalization to the sensor site itself. For example, sensors which probe only a small area but require functionalization of a larger one (say due to the use of traditional spotting techniques) do not really improve the LOD as binding occurs everywhere and not preferentially at the sensor site. In cases where the background surface chemistry is much different than the sensor itself (say a gold nanoparticle on a silica surface) specific functionalization can be relatively easy. It is more difficult for devices where the surface of the sensor itself is not much different than the background surface. An example of such a case would be silicon on insulator optical devices (SOI) where it is difficult to find chemistries which will work on silicon but not the underside oxide layer.

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