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Recent Advances in Optical DNA Biosensors Technology

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Abstract: Recent advances in nucleic acids-based biosensors have led to the development of DNA biosensors for DNA hybridisation detection and for nucleic acid–ligand binding studies. This review presents a concise description and an evaluation of the optical devices that have been employed in the development of optical DNA biosensors. Such optical devices include optical fibres, planar waveguide, surface plasmon resonance, resonant mirror and surface-enhanced Raman scattering. The specificity and response of each optical DNA biosensor are discussed. Overall, a rapid growth in the development of optical DNA biosensor technology will be seen in the near future, which will lead to the establishment of optical DNA biosensor technology as a major tool of analytical biochemistry.

Keywords*:* DNA hybridisation · Genosensors · Optical DNA biosensors

1. Introduction

The revolutionary developments in medicine and biology in recent years, which have been influenced greatly by the progress of the Human Genome Project, have offered a variety of approaches for a better understanding of the molecular basis of life. Molecular diagnostics based on the analysis of genomic sequences has offered a highly sensitive and quantitative method for the detection of genetic variations, genetic mutations and infectious species at molecular level, opening up the possibility of performing reliable diagnoses even before appearance of any symptoms of a disease.

Generally, classical methods for the analysis of specific gene sequences are based on either direct sequencing or DNA hybridisation. DNA hybridisation is more commonly used in diagnostic laboratories, due to its simplicity, than the direct sequencing method [1]. In classical methods, the sequence of the target gene was identified by a DNA probe that can form a double-stranded hybrid with its complementary strand with high efficiency and extremely high specificity, even in the presence of a mixture of many different nucleic acids. Normally, nucleic acid probes were single-stranded oligonucleotides labelled with either radioactive or non-radioactive material to provide detectable signals for DNA hybridisation [2]. Radioactive labels are extremely sensitive but have obvious disadvantages, such as short shelf life, risks associated with exposure of its radiation, cost, storage and disposal problems. In contrast, non-radioactive probes, such as enzymatic or luminescence labels, are less sensitive but more flexible in terms of design and application. Therefore, using currently available technologies, large-scale and routine clinic screening based on gene diagnostics is limited. Thus, developments of faster, simpler, label-free and reliable methods are needed in order to significantly improve the applications of DNA molecular diagnostics [1].

The acceleration in the development of biosensors for the analysis of specific gene sequences has arisen from current advances in automated DNA synthesis and the convenient site-specific labelling of synthetic oligonucleotides with suitable functional moieties, coupled with advances in microelectronics. By definition, a biosensor is an analytical device that incorporates a biologically active layer as the recognition element and converts the physical parameters of the biological interaction into a measurable analytical signal proportional to target analyte concentration [3]. The signal may be further amplified, processed, or stored for later analysis. A DNA biosensor employs an immobilised synthetic sequence as recognition element. Optical DNA biosensors rely on the conversion of the base-pair recognition event into a useful optical signal. Optical devices are favoured because of their potentially easier fabrication, higher chemical stability and lower risk of external interferences, compared to sensor devices based on other transduction principles, such electrochemical devices [4], since no electrical contact or magnetic field is applied in such sensor devices.

Recently, various reviews regarding DNA biosensors with various transducers [5][6], including electrochemical ones [1][7–10], have been reported. Here, we will focus on the most recent development in optical DNA biosensors, with basic insight for the optical detection methods and optical biosensor device structure, such as optical fibres, planar waveguide, surface plasmon resonance and surface-enhanced Raman scattering. This discussion leads to an evaluation of the advantages of optical devices for the study of the various events that occur in the DNA hybridisation process. This review does not consider nanosensor technology in terms of nanobiosensor and biochips, since this field has been recently reviewed in more detail in [11][12].

2. Optical DNA Biosensor Devices

In principle, nearly all techniques known from conventional optics have been

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adapted to waveguiding or near-field sensing geometries [13] and for their potential in sensor application. The most common and promising methods can be divided into two groups. In sensors based on evanescence (also called evanescence-field), such as optical fibres and planar waveguide, the detection is based on total reflection of an incident light beam, an evanescence wave, extending some 100 nm into the lower refractive-index medium. The other sensors are also related to such optical phenomena, with detection schemes based on the change in refractive index, such as surface plasmon resonance (SPR) sensors, resonant mirrors and grating couplers. They are associated with the attractive feature of direct sensing, without necessary labels, due to the signals of this devices being directly associated with adsorbed molecular mass. Several kinds of optical devices can be employed in the development of an optical DNA biosensor. Such devices ranging from optical fibres, planar waveguide, surface plasmon resonance, resonant mirror to surface-enhanced Raman spectroscopy are discussed in detail below.

2.1. *Optical Fibres*

2.1.1. *Single Fibre-Optic Biosensors*

These biosensors employ optical fibres that serve both as waveguides for the transmission of radiation to the sensing area as well as solid supports for the immobilised biorecognition elements. Fibre-optic biosensors are based on the principle of the evanescence wave, which stimulates fluorescent labels attached or in close proximity to the surface of the fibre, as shown in a scheme of a fibre-optics biosensor given in Fig. 1a and 1b [14]. The evanescent wave is an electromagnetic wave which is generated when light is completely reflected within the sensor surface (total internal reflection phenomenon). The evanescent wave penetrates beyond the optical interface into the lower reflection index medium by the distance of a wavelength from the surface and decays exponentially.

Various fibre-optic sensor configurations for the detection of nucleic acid hybridisation by fluorescence have been described [15–17]. A number of these biosensors for selective detection of nucleic acid sequences use fluorescent intercalating and groove binding dyes to detect hybridisation of DNA. Such an approach can provide very high sensitivity and also introduces the advantage of a high degree of selectivity for the detection of hybridisation because the dyes are activated for emission only by the presence of hybridised nucleic acid.

Hybridisation usually involves only a fraction of the ssDNA at the surface of the device. Dye solutions are usually at concentrations of about 10^{-8} M and use hundreds

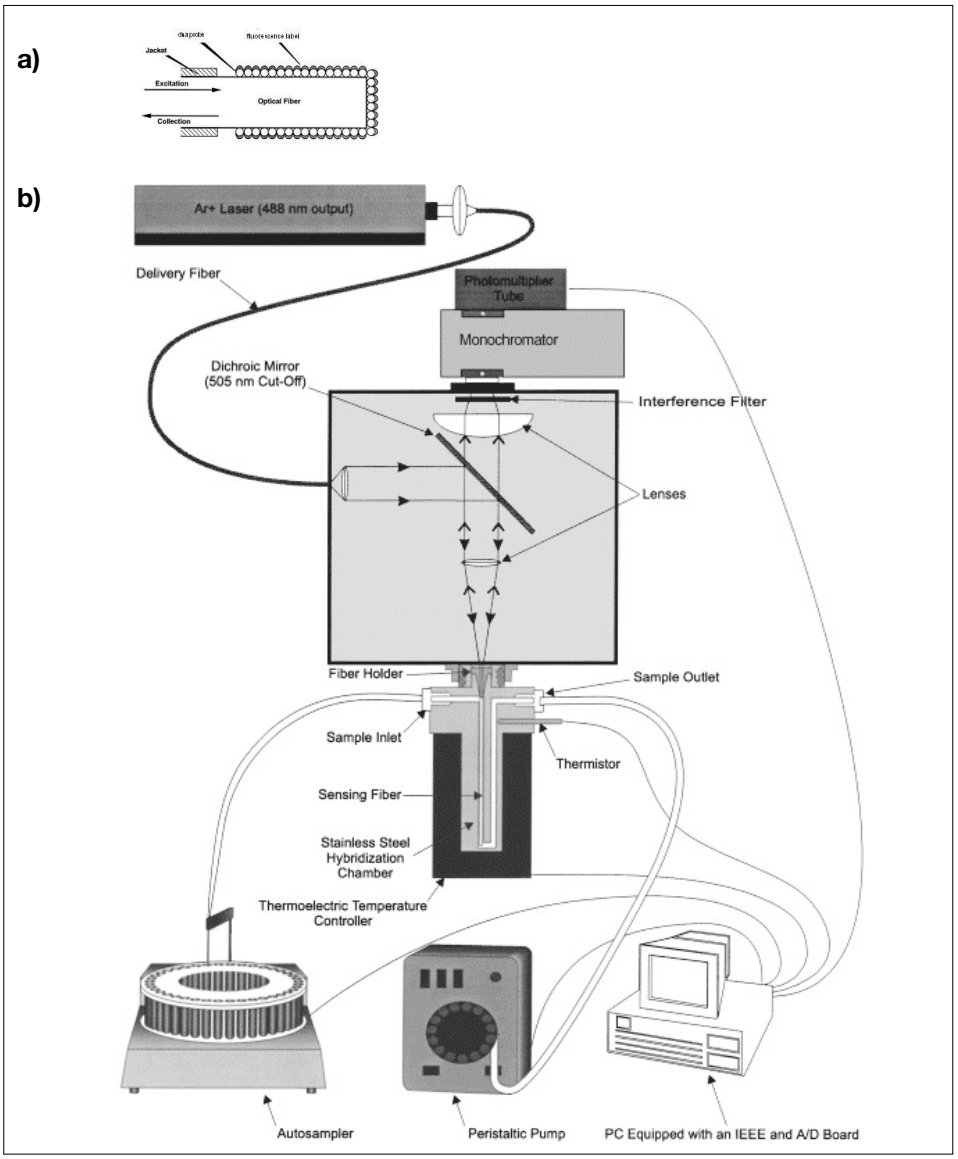

Fig. 1. A dedicated spectrofluorimeter (b) used in DNA hybridisation detection based on a single optical fibre biosensor (a) (adapted from Hanafi-Bugby *et al.* [14])

of microlitres to cover a surface, and then most methods wait for dye incorporation with double stranded DNA (dsDNA) to reach equilibrium. This means that the dye/dsDNA ratio is often greater than 1:1 in a two-dimensional plane of DNA at an interface. Almost all work investigating dyes is done in bulk solution, and most often uses the dye at a relatively low concentration with respect to DNA, but this does not reflect what is usually encountered in a device using immobilised DNA [16].

Krull and coworkers have described works in the direct analysis of DNA hybridisation at the surface of this type of device [18][19]. The method involved covalent immobilisation of ssDNA onto the optical fibre by first activating the surface of the quartz optical fibres with aminopropyltriethoxysilane (APTES) and 5'-dimethoxytrityl-2'-deoxythymidine conjugated with a long-chain aliphatic spacer arm, followed by solid-phase oligonucleotide synthesis. Detection of dsDNA at the fibre surface after hybridisation was achieved by staining the duplex with ethidium bromide (EB), a fluorescent intercalating dye. It was reported that the EB fluorescence intensity detected using this device was directly proportional to the amount of complementary DNA present in solution. A detection limit of 86 ng/ml was obtained with a sensitivity of 200% fluorescence intensity increase per 100 ng/ml. The sensor can be regenerated and sustains full activity after prolonged storage time, harsh washing conditions and sterilisation [19].

While ethidium bromide has become an established fluorescent standard for the detection of hybridisation, the recent proliferation of asymmetric cyanine dyes, which are capable of significantly greater fluorescence enhancements over EB, suggests alternatives which may provide greater sensitivity in hybridisation detection schemes. One of these alternatives is the use of thiazole orange derivatives, such as thiazole orange methoxy hydroxy ethyl (TOMEHE) as reported

by Krull and coworkers [14]. This dye was chosen for its fluorescence enhancement of up to 1000-fold when bound to dsDNA and an apparent wavelength shift of approximately 65 nm in the fluorescence emission maximum on going from association with ssDNA to dsDNA. The results demonstrated that TOMEHE displays a 10-fold greater fluorescence enhancement over EB when bound to dsDNA, but also displayed a 2.5-fold increase in fluorescence intensity over EB when associated with ssDNA. It was also apparent that TOMEHE can interact with ssDNA and provide enhanced emission. With this latter feature, measurement of the ratio between the fluorescence emissions from the two types of associations could potentially be used to determine the amount of hybridisation on the sensor surface without interferences by spectral source fluctuations, detector drift and ionic strength variations, which typically limit the accuracy and precision of single fluorescence intensity measurements.

In the last few years, a kind of drop-based optical fibre device has been developed, in which a drop itself can serve as an optical cell when a light beam probes the drop and the transmitted light or the resulting emission is detected. By using the drop-based optical detector, some problems existing in a conventional optical detector, such as scattering and background fluorescence due to the window in a fluorescence detection system, can be solved, and it has been successfully applied to efficiently detect trace amounts of DNA in flowing solution [6]. By using a flow injection-renewable fluorescence cells technique, this procedure permits a simple, fast, and inexpensive measurement with high-sensitivity and very low reagent cost. 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB-d) has been applied for the analysis of DNA. Here, the interaction between TMB-d and DNA, the most noticeable quenching TMB-d fluorescence was observed. The value of fluorescence quenching was linear over a wide concentration range of calf thymus DNA. Hence, calf thymus DNA can be determined down to the ng/ml level.

2.1.2. *Fibre-Optic Arrays*

The first generation of DNA arrays explored oligonucleotide probe sequences photopolymerised onto fibre bundle surfaces [20] or directly coupled to fibre cores [21]. The initial photopolymerised fibre-optic sensor arrays were capable of single nucleotide polymorphism (SNP) detection and sampled at extremely small (submicroliter) volumes. The arrays could be regenerated over three assays with reproducible experimental results and provided a rapid assay time with high specificity [20]. The schematic device of fibre and its image arrays is given in Fig 2a and 2b [22].

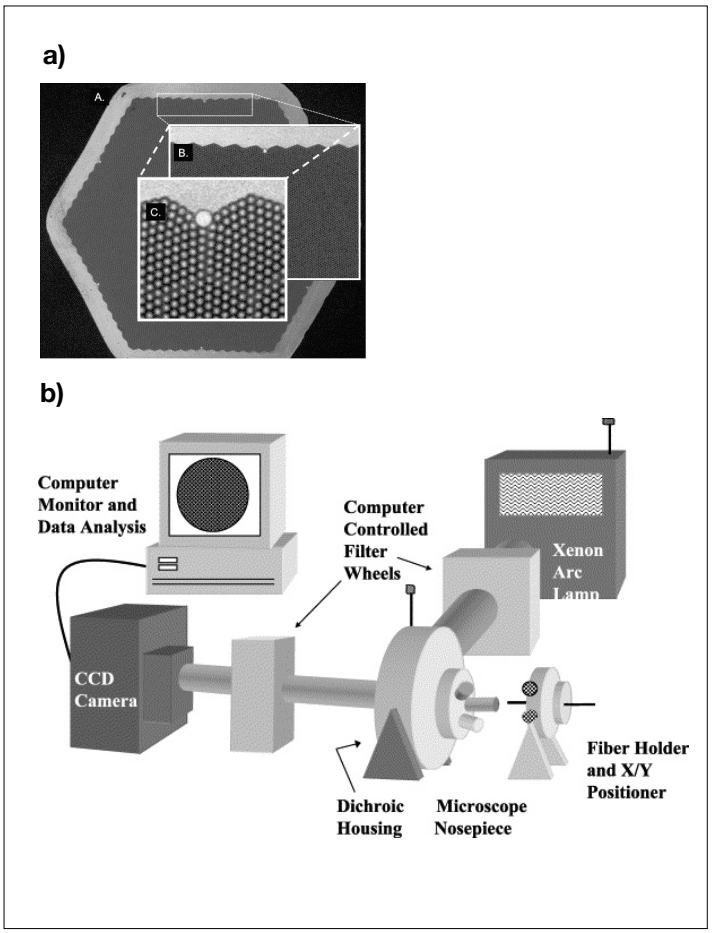

Fig. 2. A custom-built epifluorescence imaging system (b) to be used with fibre-optic arrays as shown in (a) (adapted from Epstein *et al.* [22]). (a): White light images of a 1 mm diameter fibre bundle. (A) The entire hexagonally packed bundle. (B, C) Higher magnifications of the fibre bundle illustrating the miniature feature sizes (3 μm) and close-packed arrangement. The large circle is a positional marker built into the array.

The derivative fibre core experiments were able to detect unlabeled (non-fluorescent) target solutions by competitive hybridisation with fluorescent target samples. In this method, fluorescent synthetic target complements were synthesised and initially hybridised to the array to saturate the array probe elements. The unlabeled target solution was then hybridised to the same array, competing with the pre-hybridised synthetic targets. The presence of unlabeled target was determined by a fluorescence decrease caused by displacement of the fluorescent synthetic target by the unlabeled species. This procedure eliminates the need to incorporate fluorescence into the target and allows quantitative measurements to be performed.

The second generation of fibre-optic arrays incorporated microspheres into its sensing element design [23][24]. The microspheres were localised into the etched wells of a fibre optic imaging bundle. Microsphere-based sensing elements provided the experimenter more control over the array design. The number of identical microspheres present in an array influences the assay parameters [23]. The signal-to-noise ratio increases by the square root of the number of identical sensors examined. In contrast, fewer identical microspheres present in an array offer a concentration advantage. Assuming that hybridisation goes to completion, the same number of target molecules hybridised to fewer numbers of microspheres, yields more target molecules per microsphere and results in an increased signal. Using this methodology, zeptomolar concentrations of DNA target molecules were detected (10^{-21} mol, ~ 600 total target molecules) [23]. Multiplexed arrays were fabricated to monitor specific hybridisations in real time, enabling quantitative analysis of unknown target concentrations [25]. Fibre-optic microsphere based gene arrays demonstrated allelic discrimination or identification of RNA isoforms originating from a single gene [26][27]. Employing microspheres containing addressable sequences for decoding, a single series of microsphere arrays can serve as a universal platform for multiple genomic applications [22][28].

A third generation of microsphere-based arrays employs a different approach by changing the nucleic acid probe design

[29]. Detection using molecular beacons [30] has provided another level of nucleic acid interaction not established with other platforms.

Molecular beacons probe sequences contain a reporter fluorophore, which is quenched by an adjoining quenching molecule. The quenching molecule can be either a non-fluorescent species that non-radioactively captures the reporter fluorophores energy or another fluorophore with an excitation wavelength overlapping the emission wavelength of the reporter fluorophore. In the absence of target molecules, the quencher and reporter fluorophore are close to one another due to the self-complementary DNA sequences, or stem structures present in the molecular beacon molecule. When proximal, the fluorescence energy is transferred from the molecular beacon reporter fluorophore and a low signal is measured. The probe sequence connecting the two self-complementary stems is termed a loop sequence. The interaction between the molecular beacon loop sequence and the correct target molecule causes a conformational change in the molecular beacon structure, spatially separating the two stems as well as the reporter fluorophore and quencher [30].

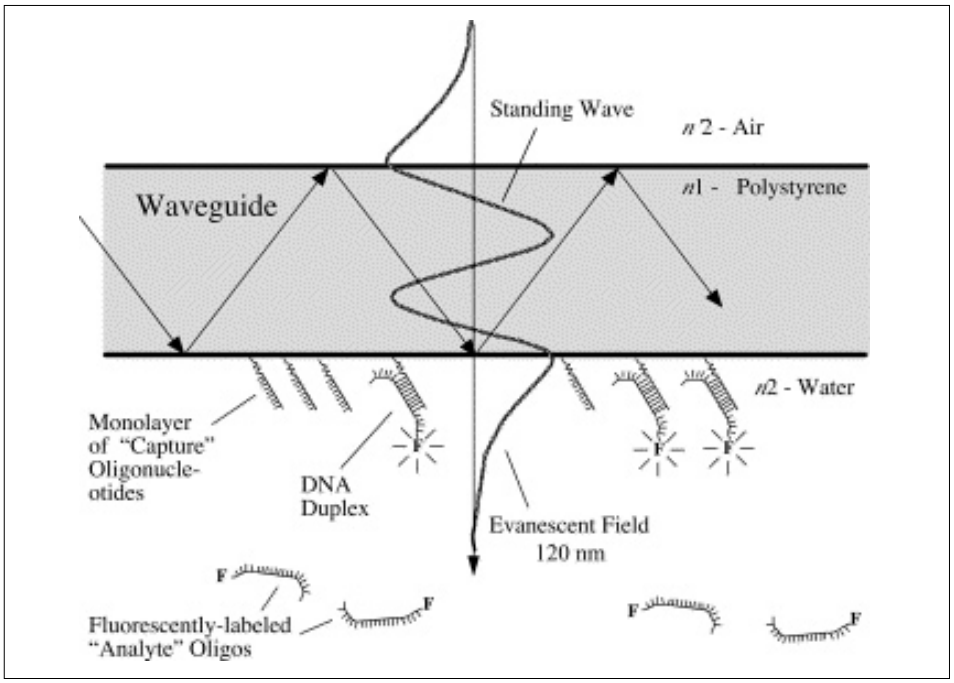

Fig. 3. Illustration of the planar waveguide DNA biosensor showing the evanescent field created by reflected light, stretching *ca.* 110 nm from waveguide surface to excite bound fluorescent molecules (adapted from Tolley *et al.* [31])

2.2. *Planar Waveguide*

2.2.1. *Fluorescence-based Biosensor*

Planar waveguides, as used in the total internal reflection fluorescence (TIRF) technique, offer advantages in optical detection, such as an increased sensitivity due to multiple reflections of light within the waveguide, and the potential to perform hundreds, or even thousands, of different hybridisation assays on a single device. Affinity assays, such as DNA hybridisation, are inherently sensitive because the energetic of base pair formation is the mechanism of selectivity, rather than subtle changes in conformation within a single-stranded DNA molecule. Furthermore, TIRF is a suitable technology for performing affinity assays due to its inherent high sensitivity and rapid assay speed. In TIRF assays, a planar waveguide (an optical substrate) presents an attached DNA probe (stationary phase) for capture oligonucleotides that will bind to their complementary strands of DNA. The evanescent wave generated by the substrate will excite only fluorescently labelled analyte DNA molecules that have bound to the stationary capture oligonucleotides, thereby eliminating the need for a washing step. The assay system, combining DNA hybridisation with planar waveguide fluorescent biosensor technology to detect single nucleotides, is given in Fig. 3 [31].

Peter *et al.* [32] reported the use of such techniques based on evanescent field excitation of fluorophore-labelled DNA-targets specifically binding to immobilised DNA probes, for real-time analysis of hybridisation events. Oligonucleotide probes are directly immobilised on the surface of a disposable sensor chip *via* biotin/neutravidin linkage and hybridise to complementary Cy5-labelled target DNA in the sample; this is recorded as an increase in the fluorescence signal. Under optimised conditions, the hybridisation rate was constant and directly proportional to the target concentration. When an 18-mer oligonucleotide was used as a probe, a linear calibration curve was obtained for a 56-mer single-stranded DNA target derived from the neomycin phosphotransferase gene, a selection marker in a variety of genetically modified plants, with an estimated limit of detection of 0.21 nmol l^{-1}. No cross-hybridisation to a 51-mer non-complementary sequence was observed and even a single-nucleotide mismatch led to a negligible signal.

A more recent work on fluorescent planar waveguide was reported in [31]. The technique has been employed to detect single nucleotide polymorphisms (SNPs) using a simple hybridisation assay with the complementary strand (DNA probe/capture oligonucleotide) immobilised on the waveguide. Under normal conditions, both the wild-type sequence and the SNP-containing sequence will hybridise with the capture oligonucleotide, but with different reaction kinetics and equilibrium duplex concentrations. A 'design of experiments' approach was used to maximise the differences in the kinetics profiles of the two. Nearly perfect discrimination can be achieved at short times (2 min) with temperatures that destabilise or melt the heteroduplex while maintaining the stability of the homoduplex. The counterion content of the solvent was shown to have significant effect not only on the melting point of the heteroduplex and the homoduplex, but also on the hybridisation rate. Changes in both the stability and the difference between the hybridisation rates of the hetero- and homoduplex were observed with different concentrations of three different cations (Na^+, K^+, Mg^{2+}). With the difference in hybridisation rates maximised, discrimination between the hetero- and the homoduplex can be obtained at lower, less rigorous temperatures at hybridisation times of 7.5 min or longer.

2.2.2. *Grating Coupler*

The optical grating coupler biosensor (OGCB) is a relative newcomer in the field of integrated optics, *i.e.* the science of light guided in structures smaller than the wavelength of the light. As a technique for investigating processes at the solid/liquid interface, it pushes the sensitivity (as well as the convenience and wealth of obtainable information) to levels even higher than the already impressive achievements of surface plasmon resonance (SPR) [33]. By incorporating a grating in a planar optical waveguide one creates a device with which the spectrum of guided lightmodes can be measured. When the surface of the waveguide is exposed to different solutions, the peaks in the spectrum shift due

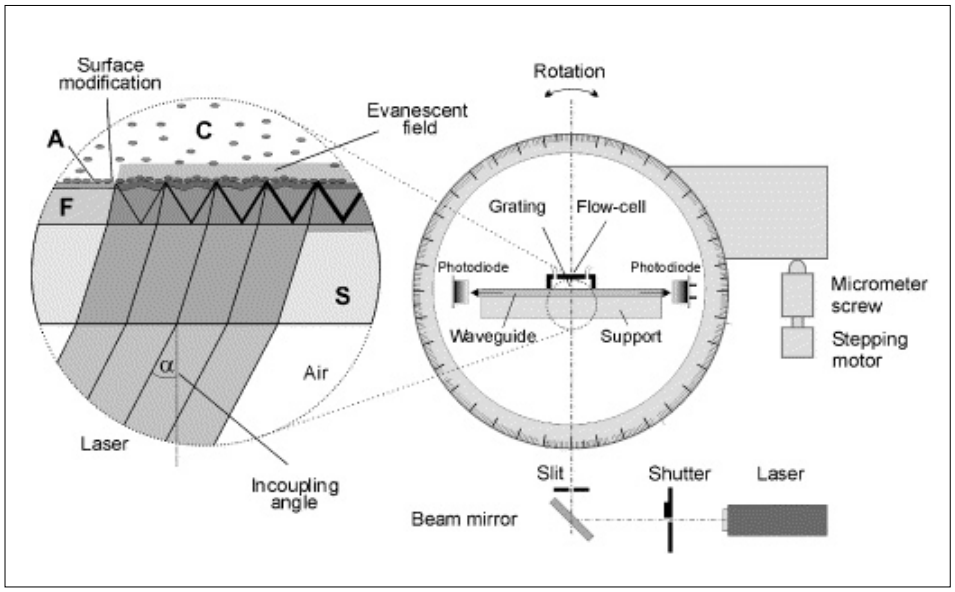

Fig. 4. The optical setup of an optical grating coupler planar waveguide instrument. Light from a He–Ne laser is diffracted by an optical grating at the surface and starts to propagate *via* total internal reflection inside the waveguiding film (F). At a well-defined incident angle (α) the phase shift during one internal reflection equals zero (constructive interference) and a guided mode is excited, which generates an evanescent field penetrating into the bulk solution (C) up to a distance of about 100–200 nm. Changes in the refractive index at the surface (*e.g.* formation of an adlayer (A) of adsorbed biomolecules, such as DNA) can then be monitored by precise measurement of the incoupling angle (α) as a function of time (adapted from Voros *et al.* [33]).

to molecular interactions with the surface. Optical waveguide lightmode spectroscopy (OWLS) is a highly sensitive technique that is capable of real-time monitoring of these interactions. Fig. 4 shows a schematic diagram of the optical setup of an OWLS instrument. Since this integrated optical method is based on the measurement of the polarisability density (*i.e.* refractive index) in the vicinity of the waveguide surface, radioactive, fluorescent or other kinds of labels are not required. In addition, measurement of at least two guided modes enables the absolute mass of adsorbed molecules to be determined [33].

2.3. *Surface Plasmon Resonance*

A surface plasmon is a quantised excitation of the electron gas coupled to the electromagnetic field at the interface between a metal and a dielectric medium. Under suitable conditions, it can be used as a very sensitive optical monitor of the changes in the metal surfaces, for example by detecting the dielectric constant changes induced by the adsorption of biomolecules at metal surfaces [34]. Surface plasmon resonance (SPR) detects changes in the refractive index caused by variation of the mass on the sensor chip surface, *e.g.* when the analyte binds to the immobilised ligand on the surface [35]. These changes are proportional to the mass of molecules bound to the surface, and stoichiometric and kinetic data for the interaction can be determined. In most SPR studies, reaction between macromolecules such as protein–protein [36], drug with affinity for nucleic acids [37] and protein–DNA interaction [38] were detected. Direct (label less) monitoring of hybridisation reactions has been demonstrated with surface plasmon resonance (SPR) [39–44].

Several commercial SPR instruments have been introduced that detect the real-time binding of molecular interactions: Biacore (Biacore, Uppsala, Sweden), IBIS (Hengelo, The Netherlands) and Spreeta (Texas Instruments, US). Two of them have been intensively reported in literature for detection of DNA–DNA interactions: the Biacore system as mostly employed in many DNA hybridisation detections and the Spreeta which is described as a portable device.

The Biacore device uses SPR which arises in thin metal films under conditions of total internal reflection. In the sensing element of this instrument, the gold chip surface can be modified with a dextran matrix on which the biological probe is immobilised (often *via* avidin–biotin links). Oligonucleotides are introduced within a fluid flow system. Hybridisation is carried out at controlled temperature and positive signals are obtained within several minutes. Since the Biacore system is particularly efficient for analysis of binding and dissociation kinetics, real time DNA–protein binding measurement have also been reported for interaction between immobilised DNA templates with enzymes, such as endonuclease, ligase and polymerase [45].

Spreeta is a low-cost SPR based biosensing platform enabling real-time, quantitative, affinity and kinetic analysis of biomolecular interactions. Spreeta's revolutionary approach to SPR incorporates the otherwise costly and technically challenging optical system inside the sensor. It is easily incorporated into instruments and provides additional flexibility through its onboard memory, customised software and fluidics options [46]. Light from a LED is reflected from a gold surface and the angle and intensity of the SPR minimum is measured. This measurement is used to calculate the effective refractive index (RI) at the gold surface. When molecules bind to the gold surface, the measured RI changes. The gold surface can be covered with a biofilm, customised for essentially any molecule for which detection is desired, such as DNA hybridisation [47][48].

2.4. *Resonant Mirror*

The resonant mirror (RM) is an evanescent wave sensor which has been designed to combine the simple construction of SPR devices with the enhanced sensitivity of wave guiding devices, due to multiple strikes of a photon producing an evanescence wave with an increase of the attenuation of the light or ratio of phase-change light. RM differs from SPR in two aspects: firstly, there is no significant variation in the intensity of reflected light with angle in the RM, but a phase shift occurs in some of the reflected light which is translated into an intensity peak at the resonant angle using phase optics. Secondly, in the RM, light propagating along the waveguide strikes the sample–waveguide interface many times over long interaction distance, whereas in SPR there is a single point of interaction. Fig. 5 shows a block diagram of a resonant mirror system. Applications of this device in biological analysis have been directed to the detection of enzyme–substrate, antibody–antigen [49] and protein–cell interaction [50] as well as for rapid and direct detection of DNA hybridisation [51].

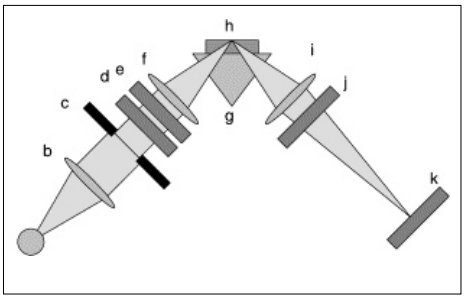

Fig. 5. Block diagram of instrument configuration (a) light source (LED or tungsten–halogen lamp); (b) condenser lens; (c) aperture stop; (d) removable filter; (e) polariser; (f) plano-convex cylindrical lens; (g) 60° SF10 prism; (h) RM chip, where DNA hybridisation can be detected directly; (i) polariser; (j) focusing lens and (k) CCD detector

Commercial devices such as BIOS-1 and IAsys are resonant mirror instruments. The BIOS-1 (Artificial Sensor Instruments, Switzerland) comprises a glass or plastic diffraction grating coated with a planar waveguide overlayer, which comprised various metal oxides, depending on the required refractive index. The IAsys system (Affinity Sensor, UK) uses a waveguide instead of a diffraction grating and it employs an integrated prism with a low-index coupling layer, high-index resonant layer, and a range of derivatised sensor surfaces. Here, the gold film is replaced by titanium or hafnium, acting as a dielectric resonant layer of high refractive index (resonant mirror) [52]. The resonant mirror method has been used to detect DNA hybridisation with an estimated limit of detection in the femtomolar range. Regeneration of the surface-immobilised probe was possible, allowing reuse without a significant loss of hybridisation activity [53].

2.5. *Surface-enhanced Raman Scattering*

Raman is a very useful spectroscopic technique, allowing structural fingerprinting due to its narrow and highly resolved bands (as compared to a fluorescence bandwidth of 10–50 nm). Laser-induced fluorescence has traditionally been the technique of choice for optical detection of trace-level analytes (femtomolar or lower). For high quantum yield fluorophores, the effective fluorescence cross-sections can be as high as 10^{-16} cm^2 per molecule. In contrast, the cross-sections for Raman scattering are extremely small, ranging from 10^{-30} to 10^{-25} cm^2 per molecule, producing low intensity scattered radiation, and consequently requiring a higher analyte concentration. However, very large enhancements of the Raman scattering (>10^8) make the surface-enhanced Raman scattering (SERS) technique sensitive enough for trace analysis [12]. The enhancement mechanism for SERS comes from intense localised fields arising from surface plasmon resonance in metallic (*e.g.* Ag, Cu, Au) nanostructures with sizes in the order of tens of nanometres. This performance makes the technique as competitive as fluorescence for certain examples of trace analysis, such as DNA. The schematic device and its enhancement principle are given in Fig. 6a and 6b.

By using this technique, the development of a new type of DNA probe based on SERS detection has been reported by Vo-Dinh *et al.* [12] as a surface-enhanced Raman gene (SERG) probe. The SERG probe does not require the use of radioactive labels and has at the same time a great potential for sensitivity and selectivity. Results showed that the SERG probe could have a wide variety of applications

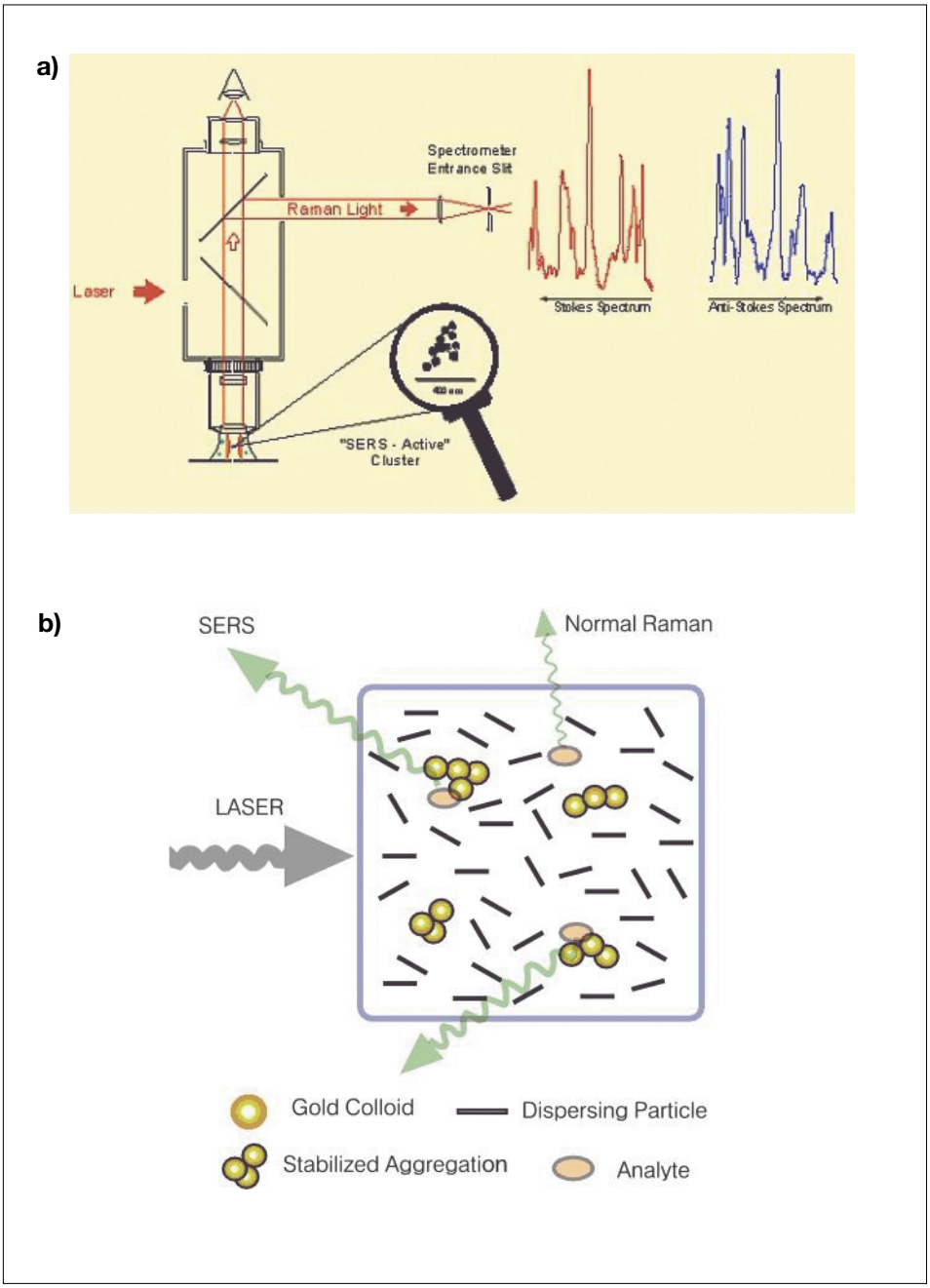

Fig. 6. Schematic diagram of the optical setup for the SERS sensor (a) and its surface enhancement principle (b)

in nucleic acid identification; ssDNA can be attached to SERG probes and the resulting probe may be used to identify specific genes or detect bacterial and viral components. Use in detection of PCR DNA samples is also feasible.

SERS was reported for monitoring DNA hybridisation of a fragment of the *BRCA1* breast cancer susceptibility gene on modified silver surfaces [54]. Rhodamine B was covalently attached to a 5′-amino-labeled oligonucleotide sequence (23-mer) through a succinimidyl ester intermediate in methanol. The silver surfaces were prepared by depositing a discontinuous layer (9.0 nm) of silver onto glass slides, which had been etched with HF to form a microwell platform, and subsequently modified with a monolayer of mercaptoundecanoic acid. The complementary probe was covalently attached to the silver surfaces using a succinimidyl ester intermediate in acetonitrile. Hybridisation with the SERS-active recognition probe was performed at 37 ºC for 16 h using a buffered saline medium. Spectra were obtained using the 632.8 nm laser line from a He–Ne laser, and an acquisition time of 60 s. The silver island substrate allows a very large enhancement of the Raman signal of the DNA-Rhodamine B, and clear distinction between hybridised samples and controls on a microwell array sampling platform.

3. Concluding Remarks

As described above, over the last decade there has been a great surge in optical DNA biosensor technology. Much of this work has focused on optical fibres, planar waveguide, surface plasmon resonance (SPR) and surface-enhanced Raman scattering (SERS) techniques. Optical transducer-based biosensors are receiving increasing attention since they match with nanosensor technology. Commercial devices, such as Biacore, IAsys and Spreeta, represent reliable and desirable tools for DNA biosensor configuration.

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