REVIEW ARTICLE

Low-cost optical instrumentation for biomedical measurements

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(Received 19 May 2000; accepted for publication 23 August 2000)

Low-cost instruments for measurement in medicine, biotechnology, and environmental monitoring are presented. Recent developments in optoelectronic technology enable practical compact designs. This article presents the available types of light emitters, detectors, and wavelength selection components that are used in low-cost instruments. The main spectroscopic techniques (absorption, reflectance, luminescence intensity, lifetime, and polarization, evanescent wave and surface plasmon resonance) that are used with these instruments are described. Numerous examples of devices for a broad variety of biomedical measurements are presented. © *2000 American Institute of Physics.* [S0034-6748(00)00112-X]

I. INTRODUCTION

Biomedical measurements find their applications in three broad areas—medicine, biotechnology, and environmental protection. In medicine, there is a need for rapid analysis of blood, serum, and other body fluids; biotechnology relies on measurements for bioprocess control; in environmental protection there is a requirement for information about pollutants.

Currently, the available laboratory medical analyzers are highly effective, but the cost-per-sample is low only in cases when large numbers of samples are assayed. Furthermore, they usually require special conditions for operation, specific sample preparation, and trained personnel. These conditions are met easily for the clinical lab in hospitals; however, clinical testing is also needed during surgery, in smaller medical centers, in emergency vehicles, as well as in point-of-care sites. The basic requirements for these measurements are rapidity and high reliability of the results. In biotechnology, there is a continuous need for on-line measurements of various bioprocess parameters, especially the concentrations of substrates, products, different enzymes and inhibitors, promoters, and repressors. However, typical on-line measurable parameters usually include *p*H, dissolved oxygen, and biomass concentration, and most other parameters are measured off-line, using the aforementioned conventional analyzers. In many cases, ready-made analyzers are not available, and analytes are determined using decades old analytical techniques. Furthermore, sampling often creates other problems such as the need to maintain sterility, as well as decreasing the working volume. In environmental monitoring, there are no such problems; on the other hand, the analytes there are often quite different from the other two areas—typically pollutants (oils, pesticides, herbicides) or harmful bacteria.

In their vast majority, biomedical measurements are targeted to the measurement of concentrations. The most common (and most important) analytes are *p*H, dissolved oxygen, CO_2, different ions (K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, etc.), urea, and glucose. Depending on the area, additional analytes are of interest: fatty acids, vitamins, lactate, various proteins, DNA, and RNA. In biotechnology, there is also a need for evaluation of cell mass concentration and cell parameters. In environmental protection, there is a substantial need for measurement of chemical oxygen demand (COD) and biological oxygen demand (BOD).

The range of the measurement can vary several orders of magnitude (10^{-1}–10^{-12} M). Typically, the concentration of glucose and the ions responsible for membrane potential is in the millimolar range. Concentrations of different vitamins and minerals vary from micromolar to nanomolar, while concentrations of different hormones are often picomolar. As direct physical measurement of these concentrations is not possible, a preliminary chemical reaction is often employed as ''chemical amplification.'' This reaction produces products (or involves substrates) which are easily quantified.

Ideally, all the reaction chemistry is ''packed'' in one entity (sensor), which is read out by a specific device (transducer). The transducer could employ different physical principles for the conversion process—electrochemical, heat production, mass changes, etc. In this article, we concentrate on devices employing optical transducing techniques.

Optical detection of analytes is one of the oldest and most established techniques. The newly developed devices are easy to compare with the existing methods. Optical techniques are especially desirable because of their inherent safety—the transducer does not require direct physical contact with the sensor. They could be spatially separated (the use of light guides is optional) and yet still allow reliable measurements. Another advantage of optical methods is their high efficiency—they can be designed to cover the entire

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range of biomedical concentrations, down to single molecule detection.

The cost of instrumentation for measurement in case of concentration measurement is the combined cost of the sensor and the read out device. Recent developments in semiconductor technology and especially in optoelectronics enable very low-cost read out devices, which can detect changes in a very small volume. This decreases both the required volume of chemicals and the sample.

There are several books[1–5] and reviews[6–11] on different aspects of optical sensors. Additional information may also be found in regularly published reviews on chemical sensors.[12–14] The aim of this review is to concentrate on low-cost optical devices for biomedical measurements, i.e., devices that cost approximately 10 or more times less than the conventional appliances used for the same type of measurement. Our intention is to give a picture of current optoelectronic technology that allows creation of such devices, to describe optical principles and techniques used in this instrumentation, and to present a few examples.

II. TECHNOLOGY FOR LOW-COST OPTICAL READ-OUT DEVICES

Any optical device that utilizes spectroscopic techniques (with very few exceptions) contains four basic modules: light source, light detector, wavelength selection device(s), and a signal processing unit (PU). The last module can be analog or digital (analog-to-digital converter (ADC) +microcomputer). The advances in electronics now allow the PU to consist of just 1 integrated circuit (IC). The power required by the PU is now on the order of 1–2 mW, the price is $<$\$20. However, until recently the same was not the case for light sources, light detectors, and wavelength-selection devices. Newly emerged and commercialized technologies have changed the situation, allowing a dramatic downscaling of the dimensions, power consumption, and price of the modules; furthermore, the technological solutions offer an expanded lifetime and increased reliability of the instrumentation.

A. Light sources

Until recently, the most widely used light source was a glow lamp. The advantage of a lamp is the broad range continuous emission spectrum—from UV to IR. This makes it possible to use it as an universal source by selecting a specific wavelength for operation. It also has numerous drawbacks: low power efficiency, low mechanical stability (glass!), and it is slow (for modulation it requires an external device: chopper for low frequencies, or Pockels cell for high frequencies). Another choice is a laser—typically a bulky and expensive device, which requires laboratory conditions for operation and is inefficient in power utilization.

The development of optoelectronics has resulted in the appearance of several economical types of semiconductor light sources—light emitting diodes (LEDs), laser diodes (LDs), as well as diode pumped solid-state lasers (DPSLs).

Currently available LEDs cover the entire visible, a big part of IR and part of UV spectrum. The typical LED emits in a relatively wide band—40 nm full width half-maximum (FWHM). Its optical power is usually in the range 0.1–5 mW prior to filtering (LEDs with lower power outputs are not practical for measurement applications). Other advantages are the possibility for direct electronic modulation to very high frequencies ($F_{\text{cut-off}}$ is usually in the range of 1–100 MHz, and reasonable modulation is achievable up to 300 MHz, depending on the LED type), very long life (100 000 h), small dimensions, high energy efficiency, and low cost ($\sim$\$2). One drawback is the very long ''red tail'' of the spectrum, which still imposes a requirement for filtering the output light.

Another choice for light sources are semiconductor laser diodes. Like all lasers, they emit in a very narrow spectral band (0.5–3 nm FWHM). The available optical power is in the range of 3-5 mW; however, in IR there are much more powerful LDs, with available optical power up to 10 W. An additional advantage with LDs is the fact that there is no red tail in the emission and consequently, no need for light filtering. Their lifetime is also very long—2000–100 000 h, the dimensions are still very small, and the price is still low ($\sim$\$40). However, LDs are available mostly in IR and the red region; the first blue-violet LD only recently appeared, and for the time being there are no LDs emitting in the UV, blue, green, or yellow part of the spectrum.

The lack of LDs which emit below 600 nm is partially compensated by diode pumped solid-state lasers. They are based on LD pumping of a nonlinear crystal, usually Nd:YAG, Nd:YLF, Nd:YVO_4, or other. The pumping LD operates in the region of 1040–1060 nm or 1300–1340 nm. By doubling or tripling the LD frequency, UV, blue, and green laser emissions are generated. A broad variety of wavelengths is available. Diode pumped solid-state lasers possess potentially all of the advantages of the normal LD; however, they require much more power for the generation of the second or third harmonic. Despite the slow decrease in price, they are significantly more expensive ($>$\$4000), as this is usually a complete laser system.

Recently, the need for high stability sources in the UV and blue region has been met by the use of scintillation light sources. They are based on long-lived radioisotope and scintillation crystals which convert the radioactive emission (typically β particles) into emission in UV[15] and blue light.[16,17] The source is extremely stable, with significantly less drift in comparison with the other light sources, and has a projected life of 20 yr without needing external power.

B. Light detectors

Photodiodes (PD) are the preferred detectors for low-cost devices. They can operate at high light levels without degradation. Depending on the semiconductor material, their spectral response varies from 180 to 2600 nm. PDs are fast (depending on the internal capacitance, the bandwidth in some cases is up to 1 GHz), robust, and inexpensive. The supply voltage is low (0–30 V); they can work even in photovoltaic mode (requiring no power). Their drawbacks are the relatively high noise and the lack of internal amplification. As their output signal is usually small, they require additional amplifier, which also introduces noise. As a result,

the photodiodes can be used at low light intensities (when possible) only by limiting of the signal bandwidth and using noise-cancellation techniques.

Diode arrays and charge-coupled devices (CCDs) are also being used in spectroscopy. While allowing simultaneous observation of the whole spectrum, the devices are significantly slower than a single photodiode. For this reason, they find application mainly in steady-state devices.

An avalanche photodiode (APD) is quite similar to a normal photodiode; however it operates with significant external bias. Both the avalanche or nonavalanche mode of operation may be exploited. The nonavalanche mode enables internal amplification of the detected signal (10 to 100 times or more than a PD). APDs are fast—they can be used at frequencies up to 10 GHz. They require from 100 to several kV for operation, their internal noise is higher than normal PDs, and they are more expensive than a normal PD ($\sim$\$200). Nevertheless, the signal-to-noise ratio of the APDs is better as compared to the PDs due to the amplification. They are becoming increasingly popular because they can tolerate intense illumination, their sensitivity is comparable with some photomultiplier tube (PMT), and new dc–dc converters allow for use of APDs with standard low-voltage power supplies.

A PMT is the ultimate choice for photo detector in case where sensitivity, speed, and minimum noise are the primary requirement for the system. The internal amplification of a PMT is up to 10^6 or more. Another advantage is the possibility of large receiving surfaces. The noise is significantly less in comparison with the solid-state devices. The drawbacks of the PMT include the need for a high-voltage power supply (600–1200 V) and the possibility of destruction by overexposure. As the device is typically made of glass, it has low mechanical stability. However, new designs of miniature PMTs (with metal enclosures, built-in power supply, and some of them include current-to-voltage converters) are likely to be used more often in low-cost instrumentation.

C. Wavelength-selection devices

There are two common types of wavelength selection devices—filters or monochromators. The filters used in spectroscopy are usually based on interference. They are used to monitor light at fixed wavelengths. The advancement of thin-film deposition technology has made available filters with FWHM 10 nm, 50 to 75% transmission at the peak wavelength, absorption at the stopband >4, and at a cost below \$20. Additionally, these filters are available mounted directly on a large-surface PIN photodiode.

Another recent development is linearly variable filters.[18] They provide the possibility of varying the central wavelength of the bandpass. In the future, this technology could be competitive with monochromators.

Monochromators are the best wavelength-selection device known until now. In a classic monochromator, the incoming light is passed through the entrance slit, goes to the grating where it is diffracted, and after that goes to the exit slit, which selects part of the resulting spectrum. The narrower the slits, the better is the spectral resolution and the lower the output intensity.

The grating is the most important part of the monochromator, as it breaks the light into a spectrum. The classical ruled gratings have high peak efficiency and throughput. However, they produce relatively high levels of stray light. Holographic gratings, which are an important development in recent years, produce much less stray light and have higher resolution; as a drawback, the efficiency is usually less in comparison with a ruled grating. There is a special exception from this rule—when the groove spacing to wavelength ratio is near one, the efficiency is virtually the same as for the ruled grating.

In the newly developed monochromators, based on diode array or CCD array, no exit slit is employed. Instead, the spectrum is directed to a linear array of photodetectors. The size of the single detector determines the spectral resolution. These monochromators are significantly cheaper, possess no moving parts and are very reliable. Additional advantages are simultaneous access to the whole spectrum and integrated optoelectronic conversion. However, their resolution is lower in comparison to their stand alone counterparts; furthermore, as they rely on diode arrays, they are mostly used at high light intensities.

III. TRANSDUCTION TECHNIQUES AND EXAMPLES

Optical sensing involves probing samples with photons. The interaction between light and matter changes the parameter(s) of the photon flux, thus giving analytical information about the processes in the sample phase.

A. Absorption

1. Basic equations

The intensity of the light I passing through an absorbing medium is determined by Beer–Lambert's law:

$$I = I_0 \times 10^{-\epsilon l C}. \tag{1}$$

Here I_0 is the intensity of the incoming light, ϵ is the molar absorption coefficient, l is the thickness of the sample, and C is the concentration. The law is generally applicable (although not followed in all cases, especially when subsequent, concentration dependent reactions occur).

Another technique is reflectance measurement. The reflectance format for measurement (the probing light and the response come from the same direction) is particularly attractive in case when optical fibers are used. The reflectivity of an absorptive sample in air is given by

$$R = \frac{(n-1)^2 + k^2}{(n+1)^2 + k^2}, \tag{2}$$

where k is the extinction of the sample, and n is its refractive index. As can be seen, the reflected light intensity increases as the absorption coefficient increases. However, the mirror type reflection proved to be difficult for detection and is generally not used in sensor-based instrumentation. Instead, diffuse reflectance is used. In this case, the sensor contains weakly absorbing sensitive species together with strongly reflective material. The interrogating light is diffusely reflected

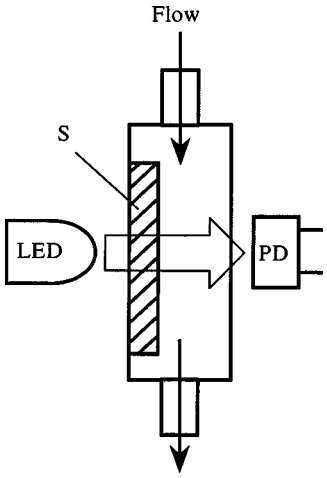

FIG. 1. Absorption sandwich type sensor based on flow-through cuvette. PD is the photodiode, *S* is the sensing membrane. The block arrow represents the direction of light.

and passed through the analyte-sensitive material. Here the intensity of the reflected light decreases with the increase in the absorption coefficient. One widely used model is that of Kubelka–Munk[19]

$$F(R)=\frac{(1-R)^2}{2R}=\frac{\epsilon C}{S}, \tag{3}$$

where R is the reflected light, S is the scattering coefficient, and C is the concentration.

All the spectroscopic techniques described so far work with some type of indicator Ind, where [Ind] is changing following interaction with the analyte A:

$$[\mathrm{Ind}]+[\mathrm{A}]\leftrightarrow[\mathrm{Ind-A}], \tag{4}$$

$$[\mathrm{A}]=K_D\frac{[\mathrm{Ind}]}{[\mathrm{Ind-A}]}. \tag{5}$$

2. Examples

The first low-cost optical absorption devices that used sensor chemistry were described 20 years ago.[20] They were based on the direct application of the Beer-Lambert's law (Fig. 1) and typically consist of a LED as a light source, PD as a detector, and a sensing membrane with covalently bound indicator. The membrane is placed inside a flow-through cuvette. The device was capable of measuring *p*H and albumin. Using the same configuration, sensors were developed for measurement of *p*H[21–23] CO_2,[24–28] NH_3[29,30] (both gaseous and dissolved in water), glucose, urea, and penicillin,[31] dissolved oxygen,[32,33] various heavy metals,[34,35] chlorine,[36] etc. All the examples described utilize visible indicators. NIR indicators[37,38] were also developed, because of the availability of NIR light sources. The technique utilizes the standard arrangement of a photometer. However, it is often not possible to make reference measurements simultaneously; usually the measurements are performed on two or more wavelengths[39] in a ratiometric mode. This technique was employed for the detection of metal ion concentrations.[40] As expected, the variety of absorption based low-cost devices increased after the introduction of LED-based spectrometers.[41] There are commercially available battery operated pocket LED based photometers[42] for water monitoring.

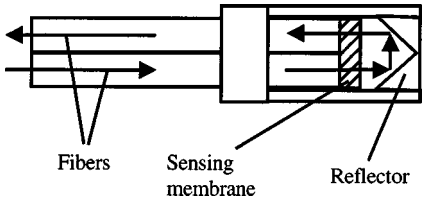

FIG. 2. Fiber-optic based absorption sensor. The reflector is positioned at some distance from the sensing membrane, allowing free access to the analyzed solution.

The standard absorption arrangement is often inconvenient. On many occasions, it is difficult, if not impossible, to pass the sample through a flow-through compartment. Instead, the sensor needs to be introduced into the sample under test. This usually requires the use of fiber optics for light transmission to and from the sample and imposes the use of specific optical configurations.

One possible solution is to use a reflector at the end of the fiber (Fig. 2). This ''folded beam'' configuration[39,43,44] is advantageous because the light passes the sensing layer twice—this allows the use of 50% thinner membranes, decreasing the response time. The light still passes the solution under the test, so the device requires sample-specific calibration. The resulting signal is measured either using a single photodiode, or CCD array for enhanced spectral analysis.

Another solution is to fold the light guides[45] (Fig. 3). In this case, the light passes the sensitive phase only, while the analyte penetrates the membrane from the side. The approach is not often in use, as it requires bending of the fibers with a very small radius, which strongly increases the losses in the bent region.

The sensor does not need to consist of solid-phase chemistry. The efforts needed to develop a new sensor often are not worth the potential benefits, especially when the target application is in the field of process control or environmental measurements. In such cases, it is more advantageous to use well-known wet chemistry, confined inside a permeable membrane tube (Fig. 4). The use of selective membranes allowed for developing effective sensors for ammonia,[46] NaOH (Ref. 47), chlorinated hydrocarbons, and heavy

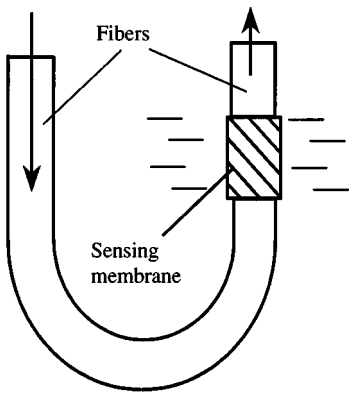

FIG. 3. Folded waveguide configuration. The arrows indicate the direction of the interrogating light.

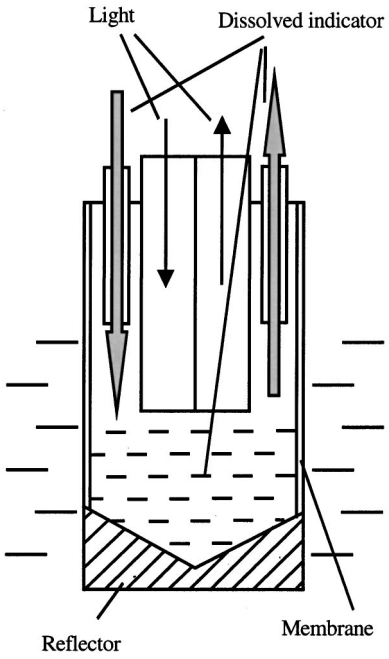

FIG. 4. Sensor based on liquid chemistry. The dissolved indicator is contained inside the reaction chamber by a semipermeable membrane. It is changed through the inlet–outlet tubes.

metals.[48,49] Such sensors are used both in a beam-through and folded beam arrangement. The use of wet chemistry allowed renewing the solutions without disassembling the sensor.

The diffuse reflectance-based systems utilize probes with a specific design. (Fig. 5). Instead of having an external reflector, the sensor chemistry is packed together with white scattering material. The interrogating light from a LED is scattered to pass the regions where the indicator is immobilized and then backscattered to the detector (semiconductor photodiode).[50] The arrangement has proven its viability, resulting in development of sensors for *p*H,[51,52] dry reagent strip for K^+ in serum,[53] chlorine gas,[54] hydrogen sulfide,[55] and formaldehyde.[56] The technique is well adapted for tissue diagnosis[57,58] and noninvasive hemoglobin oxygenation determination.[59,60]

The reflectance technique was also a basis for the first low cost, mass production blood glucose meters.[61] They have been in use for more than 30 years. The meters quantify the ratio of the reflected light from one or two different spots. The measurement requires removing the sample (wiping off the blood), which is the source of errors (insufficient blood removal, scratching the surface, etc.).[62] Although they were almost replaced by the electrochemical biosensors in the whole blood and serum testing, these devices are used for urine tests,[63,64] for determinations in cerebrospinal fluid[65] and veterinary care.[66]

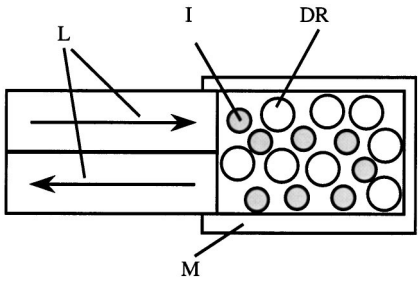

FIG. 5. Diffuse reflectance sensor configuration. *L* is the incoming and outgoing light (through optical fiber), I is the bead with immobilized indicator, DR is the diffuse reflective white bead, *M* is the dialysis membrane.

Of course, it is not necessary always to have chemistry packed at the sensor tip. It also possible to use the low-cost read-out devices just for detection of the titration end point. There are several examples of low-cost devices for titrations.[67,68]

Almost all of these examples are capable of working with steady-state light sources. However, in real-life measurements, this approach will require protection of the sensor from ambient light. The standard solution of this problem (especially when LEDs are used as light sources) is to modulate the interrogation light and to detect only the ac component of the signal. As the signal levels are usually quite high, there is no need to use lock-in techniques. Instead, the LED signal is switched on and off and the difference is measured asynchronously.[39,69] A second, reference LED[39,70] is also used in a similar fashion in order to account for the changes in the optical path. The resulting signal is calculated from the measured intensities I_{signal}, $I_{\text{reference}}$, and $I_{\text{background}}$ according to Eq. (4) (Ref. 39) as

$$I_{\text{output}}=\frac{I_{\text{signal}}-I_{\text{background}}}{I_{\text{reference}}-I_{\text{background}}}. \tag{6}$$

The sensitivity of the technique can be significantly improved when ratiometric indicators are used. In this case, the LEDs are selected with peak emissions close to the corresponding absorption maxima, and two detectors with corresponding filters are used for simultaneous detection of the intensities at the signal and the reference wavelengths.[71] The output signal in this case is calculated according to Eq. (5):

$$I_{\text{output}}=\frac{\text{signal}_{\text{LED1}}}{\text{reference}_{\text{LED1}}}\times\frac{\text{reference}_{\text{LED2}}}{\text{signal}_{\text{LED2}}}. \tag{7}$$

Again, it is possible to extend the technique by the use of background correction.

All of the described devices rely on a chemical sensor that exhibits relatively high changes in absorbance. However, there are cases when the detection of very low changes ($\Delta A<0.001$) is desirable. One specific example is noninvasive detection of glucose concentration using its absorption in IR region.[72,73] The problem is that a small change needs to be detected in a high background environment. One possible solution[74] is to simultaneously use two light sources (one at peak absorption and the other at reference wavelength) that are modulated with the same frequency, but shifted at 180° in phase (Fig. 6). The amplitudes of the sources are adjusted to be equal. This results in a dc signal at the photodetector. Now, if there is a change in the absorption in one of the wavelengths, an ac signal appears, which is easily detected and amplified. This technique is capable of detection changes in absorption of magnitude 10^{-4}–10^{-5} absorbance units.

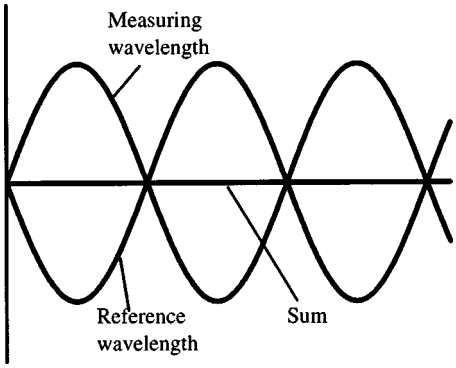

FIG. 6. Light modulation for detector of low absorptions. The intensities on the measuring and the reference wavelengths are modulated at the same frequency, but shifted at 180°. The sum of the ac of the intensities is 0. See the text for details.

B. Luminescence

Following the absorption of a photon, the excited molecules can lose energy through nonradiative relaxation, emission of a photon or energy transfer to an acceptor. The first process always occurs; the other two are usually competitive to it. The reemitted photons usually possess less energy and consequently, are shifted toward the red part of the spectrum. This difference in the wavelengths is a specific advantage in comparison with absorption spectroscopy—it strongly decreases the level of the shot noise, as only the emission is observed. Since the noise is proportional to the square root of the light intensity, very good values of the signal-to-noise ratio can be achieved. This allows measuring extremely low concentrations, down to a single-molecule detection.

1. Basic equations

The luminescence intensity F_0 is a fraction of the absorbed light. For a weakly absorbing sample

$$F_0 = (2.3) k I_0 \phi \epsilon_\lambda l C. \tag{8}$$

Here ϕ is the quantum yield of the luminophore, ϵ_λ is the molar absorptivity at λ_{ex}, l is the optical path length in the sample, C is the concentration of the luminophore, and k is a coefficient for instrumental factors. As can be seen, there are large number of factors influencing the measured intensity. For this reason, the instruments employing measurements of the absolute intensity should be extremely well designed and calibrated.

Equation (8) describes only the steady state parameters of the luminescence. In the event the excitation light is an impulse function, the luminescence L decays according to an exponential law:

$$L = L_0 \exp(-t/\tau). \tag{9}$$

Here L_0 is the initial luminescence intensity, and τ is the luminescence lifetime.

In the case where the analyte quenches the luminescence intensity, some of the aforementioned complications could be significantly reduced. If the analyte interacts with the ground state indicator according to Eq. (4), forming a nonluminescent product, the process is described as *static quenching*. The measurement is based on determination of F_0/F(F_0 is the intensity of absence of quencher, F is the intensity in presence of quencher, $[Q]$ is the concentration of the quencher)

$$\frac{F_0}{F} = 1 + K_A[Q]. \tag{10}$$

K_A is the association constant with the quencher: $K_A = [LQ]/[L][Q]$.

In this case, the measurement is based strictly on concentration changes. There are neither changes in the quantum yield ϕ of the luminophore, nor in its lifetime, i.e., $\tau_0/\tau = 1$.

Dynamic quenching is defined as the collision process in which the quencher decreases the quantum efficiency (and the lifetime) of the luminophore. The measurement is based on determination of F_0/F or τ_0/τ, accounting that

$$\frac{F_0}{F} = 1 + K_{SV}[Q], \tag{11}$$

$$\frac{\tau_0}{\tau} = 1 + K_{SV}[Q]. \tag{12}$$

K_{SV} is the Stern–Volmer constant. When luminescence changes due to dynamic quenching, it is preferable to measure the lifetime instead of the intensity. The reason for that is the fact that the lifetime does not depend either on the intensity of the excitation light, or on the concentration of the indicator.

The above spectroscopic techniques are used when the indicator exists in only one luminescent form. There is also a range of indicators that, upon association with the analyte, form another also luminescent form with different characteristics. There are two distinct, widely used cases.

(1) The absorption (and the excitation) spectra of the associated and dissociated forms are different, the emission spectrum is the same. The ratio of the luminescence intensities, in this case, depends on excitation light intensities I_{λ_1} and I_{λ_2}, parameters and the concentration of the associated and dissociated form of the indicator:

$$R = \frac{I_{\lambda_1} \phi_{\lambda_1} \epsilon_{\lambda_1} [\mathrm{Ind}]}{I_{\lambda_2} \phi_{\lambda_2} \epsilon_{\lambda_2} [\mathrm{Ind{-}A}]}. \tag{13}$$

Recalling that the total amount of the indicator [T] in the sensor is known and constant, it easy to calculate the calibration function using Eq. (5).

(2) The emission spectra of the two forms are different. In this case, the ratio of the emission intensities (on given wavelengths) is again a function of the analyte concentration, with the advantage that the intensity of the excitation light does not participate in the calibration function:

$$R = \frac{\phi_{\lambda_1} \epsilon_{\lambda_1} [\mathrm{Ind}]}{\phi_{\lambda_2} \epsilon_{\lambda_2} [\mathrm{Ind{-}A}]}. \tag{14}$$

There are not many known ratiometric indicators. However, any intensity measurements can be converted to ratiometric measurements if a mixture of two luminophores is used. On the other hand, this creates different types of

problems—the most common one appears from the different photobleaching rates of the two luminophores.

Another possibility is to measure the average lifetime of the indicator. If it exists in two forms with largely different lifetimes, the apparent lifetime, determined by the phase shift of the harmonically excited luminescence ($\tau_{app}=\tan\varphi/\omega$), is given by

$$\tau_{app}=\frac{\dfrac{\alpha_1\tau_1^2}{1+\omega^2\tau_1^2}+\dfrac{\alpha_2\tau_2^2}{1+\omega^2\tau_2^2}}{\dfrac{\alpha_1\tau_1}{1+\omega^2\tau_1^2}+\dfrac{\alpha_2\tau_2}{1+\omega^2\tau_2^2}}. \tag{15}$$

Consequently, if the fractional intensities α of the luminescence change, this leads to a change in the apparent lifetime.

Often, it is not possible to find an appropriate luminescent indicator, but there is an absorption-based one. In this case, the effect of the resonance energy transfer (RET) is employed. This is a transfer of the excited state energy from an initially excited donor to an acceptor without the appearance of a photon. The rate of the transfer k_T depends on the extent of spectral overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the quantum yield Q_D of the donor, the relative orientation k of the donor and acceptor dipoles, and the distance r between the donor and acceptor molecules. The distance R_0 at which RET is 50% efficient (the donor intensity is decreased to one half of the intensity in absence of quencher) is called the Förster distance. The dependence of the rate on the distance r is given by

$$k_T=\frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6, \tag{16}$$

where τ_D is the decay time of the donor in absence acceptor. R_0 can be calculated from the Förster equation

$$R_0=9.78\times10^3[k^2n^{-4}Q_DJ(\lambda)]^{1/6}, \tag{17}$$

where n is the refractive index of the medium. In solutions, k^2 is accepted equal to 2/3.

If there is no covalent binding between the donor and the acceptor, the rate of RET strongly depends on the acceptor concentration. In the case of the unlinked donor and acceptor, the impulse function of the quenched intensity is given by

$$I_{DA(t)}=I_0\exp\left[-\frac{t}{\tau_D}-2\gamma_1\sqrt{\frac{t}{\tau_D}}\right]. \tag{18}$$

Here γ_1 is function of the acceptor concentration. Extensive mathematical treatment of the RET based sensors may be found in the book by Lakowicz.[5] Sensors based on RET are very useful for custom tailoring of a sensor to specific analytes. However, when the donor–acceptor pair is immobilized inside a matrix, one practical problem is the possibility for leakage of the acceptor, thus changing its concentration. A useful solution would be to make a covalent link between the two molecules.

Often it is not possible to adjust the proper distance between the donor and acceptor. However, if there is spectral overlap, there will be some decrease of the luminescence intensity due to the inner filter effect (absorption of the luminescence emission along the optical path between the excitation spot and the photodetector). The relationship between the luminescence intensity and the analyte is governed by the Beer–Lambert's law. This principle is used for intensity based sensors.

There are many occasions, when the only available indicator is intensity based. Mixing it with a luminophore with different excitation and/or emission spectrum as mentioned allows one to create a reference for ratiometric sensing. Another possibility is to use so called ''modulation sensing.'' This situation occurs when two luminophores with substantially different lifetimes ($\tau_1>1000\tau_2$) are present in the sensor. If the frequency of the excitation light is $\omega=0.1/\tau_2$,

$$A=\frac{A_1}{\sqrt{1+\omega^2\tau_1^2}}+\frac{A_2}{\sqrt{1+\omega^2\tau_2^2}}=\frac{A_1}{\sqrt{10001}}+\frac{A_2}{\sqrt{1.01}}$$
$$=0.01A_1+A_2\approx A_2. \tag{19}$$

In other words, the intensity of the long-lived luminescence is completely demodulated (dc), while the short-lived luminescence is 100% modulated. Consequently, the modulation (ac/dc) of the *total* luminescence is equal to the ratio of the amplitudes of the short- and long-lived luminescence. If one of the luminophores is sensitive to the analyte, and the other is not, the measured ratio is a function of the analyte concentration.

A special case of luminescence measurements is the detection of the anisotropy. This involves probing the sensor with polarized light and measuring the vertical and horizontal components of the luminescence emission. The anisotropy of the sensor is given by

$$r=\frac{I_\parallel-I_\perp}{I_\parallel+2I_\perp}. \tag{20}$$

Here $I_\parallel$ and $I_\perp$ represent the vertical and the horizontal component of the luminescence. The measured anisotropy r of the luminescence depends on the fundamental anisotropy of the molecule r_0 and the rate of its rotational diffusion.[5] The dependence is given by the Perrin equation:

$$\frac{r_0}{r}=1+\frac{\tau}{\theta}=1+6D\tau. \tag{21}$$

Here τ is the luminophore lifetime, θ is the rotational correlation lifetime, and D is the rotational diffusion.

There are also a significant number of other luminescence effects (i.e., twisted intramolecular charge transfer) and techniques (time resolved fluorescence assays, frequency domain gated detection, etc.). They are rarely used in sensing and almost never used in low-cost instrumentation at the present time.

2. Examples

The typical optical configurations for luminescent measurements of solutions are shown in Fig. 7. The right angle

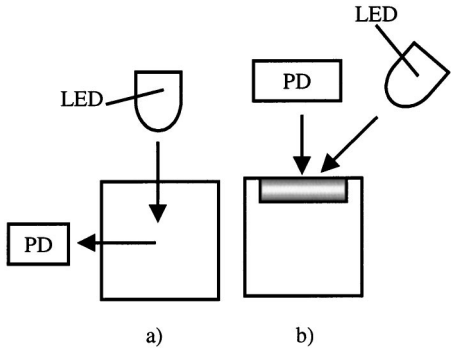

FIG. 7. Optical configurations for fluorescence measurements. (a) *L* format; (b) front face format, often used with sensing membranes. The excitation and emission filters are not shown.

format is used, when the sample is optically dilute ($A<0.1$), as this diminishes the inner filter effect. Front face geometry is employed for higher optical concentrations or when sensing membranes are used. Low-cost fluorimeters are commercially available for solution measurements.[75] Another example of direct fluorescence measurements is the newly developed corneal fluorimeter, which measures the autofluorescence of the cornea to evaluate the level of diabetic retinopathy.[76,77]

Most of the recently described instruments use chemical sensors. The devices for intensity measurements usually utilize fiber optics for light delivery [Fig. 8(a)]. The excitation is delivered through one of the shoulders of a Y-type optical fiber to the reagent phase and the emission is observed through the other shoulder. It is also possible to use a single fiber format [Fig. 8(b)]. Using variations of this setup, sensors for *p*H,[78,79] oxygen,[80,81] CO_2,[82,83] NH_3,[84,85] sulfides[86]

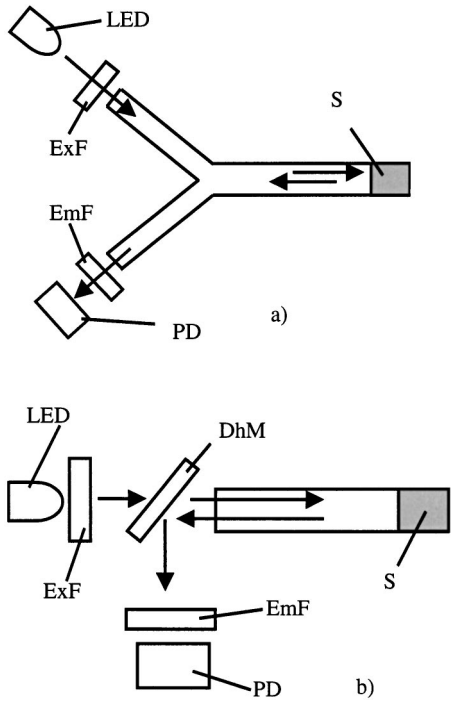

FIG. 8. Frequently used fiber-optic configurations for luminescence measurements. (a) *Y*-type fiber; (b) single fiber. ExF is the excitation filter, EmF is the emission filter, PD is the photodiode, DhM is the dichroic mirror.

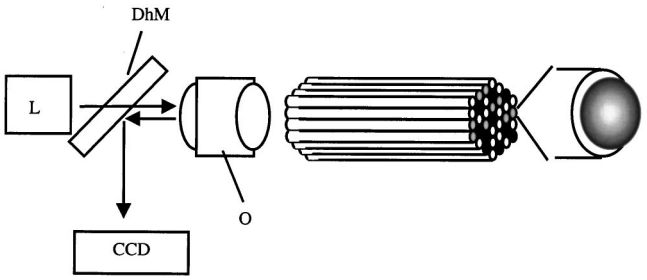

FIG. 9. Multifiber multisensor array. In the exploded view, a bead with immobilized indicator attached to the end of the fiber is shown. *O* is the microscope objective, *L* is the light source, DhM is the dichroic mirror. CCD–CCD camera.

Na^+, K^+,[87,88] Cl^-,[89] F^-,[90] NO_2,[91] Cd^{2+},[92] ricin,[93] cholesterol,[94] glucose,[95–98] and DNA[99] have been developed. There are also examples of sensors for NH_3,[100] Na^+,[101] and K^+,[102] based on inner filter effects. Some of the RET-based sensors are also used as intensity sensors.[103,104]

The aforementioned examples are just some of the most recent developments, and the number of sensors for different analytes continues to grow. Multianalyte sensors are also being developed.[105–107] The increased number of analytes that can be monitored has led to the development of a new class of sensors (Fig. 9), based on multifiber bundles.[108–111]

Optical fibers are not the only used light guide. Another often used carrier is a glass slab or capillary[112] (Fig. 10). When used together with an immobilized sensing phase on the surface, it allows for very effective decoupling of the excitation and emission. Capillaries are used simultaneously as waveguides, carriers of the sensing chemistry, and sample containers in different commercially available medical devices for blood gas analysis.[113]

In low-cost applications, when photodiodes are used for detection, the sensitivity of the fluorimeter is limited by the noise performance of the electronics. This imposes a requirement for use of noise cancellation techniques, such as lock-in detection[114] or a special shaping of the signal with long times of integration and an additional cycle for the cancellation of the ambient light.[7]

Ratiometric measurements are preferable in comparison to intensity based, as they rely on comparison with an internal standard—the ratio of two intensities. This eliminates many of the possible sources of error. Ratiometric sensors have been developed for *p*H,[115–119] Na^+,[120,121] K^+,[122,123]

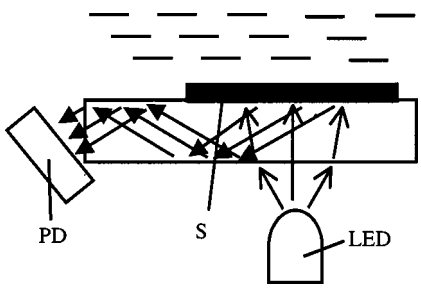

FIG. 10. Slab (or capillary) waveguide configuration. The incident angle of the excitation light is greater than the one for total internal reflection, so only a very small amount reaches the detector. The emission is well coupled to the waveguide.

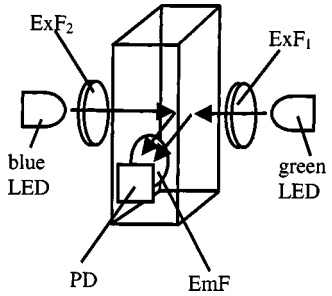

FIG. 11. Dual excitation configuration for ratiometric measurements ExF_1, ExF_2 are the excitation filters, EmF is the emission filter, PD is the photodiode. If at the place of the photodiode a LED is used, and vice versa, the set up becomes dual emission configuration.

Ca^{2+},[124,125] Mg^{2+},[126] and CO_2.[127] The most recent developments are the design of ratiometric sensors for Cl^-[128] and oxygen.[129] Most of them are compatible for use with the commercially available UV and blue LEDs. This allows the use of low-cost devices for intensity measurements. Ratiometric measuring devices have been also described.[130] In one of the designs,[131] the fluorometer uses blue and green LEDs for excitation in a T format (Fig. 11). The emission intensities of a *p*H sensitive dye are asynchronously detected and converted in duty ratio of a square wave. The device is also capable of measuring the luminophore concentration. It is easily adapted for dual excitation or dual emission mode.[132] Another low-cost design is a laser diode based sensor for field measurements of the F685/F730 chlorophyll fluorescence ratio.[133]

A variety of instrumentation is based on lifetime measurements. Like ratiometric measurements, lifetimes are also insensitive to the variations of the excitation light, optical path, and concentration of the luminophore. The lifetime can be measured using impulse or harmonically modulated excitation.[5] However, a flash lamp or laser pulse is required for impulse excitation, especially for measurements of short lifetimes (especially in the nanosecond region). Until recently, there were no low-cost laser sources in the blue-violet region. This precluded the use of the impulse method in low-cost instrumentation. Given the availability of the blue-violet laser diode[134] and the emerging frequency doubled blue laser[135], one can expect increased use of impulse detection in low-cost devices.

A method of choice for lifetime detection in low-cost devices is frequency domain fluorimetry.[5] It is based on the detection of the phase shift between the excitation and emission (Fig. 12).

Perhaps the most popular in this group are the oxygen sensors, based on dynamic quenching. Oxygen quenches the most known long-lifetime luminophores ($\tau > 5$ ns) quite efficiently. The appearance of the first stable probes in the microsecond range[136,137] allowed the development of oxygen sensors that are easily excitable with blue LEDs, require relatively low excitation frequency (50–100 kHz),[138] and have extremely stable performance. Currently, there are a large number of publications on lifetime oxygen sensors, utilizing a wide range of stable dyes[139–142] with lifetimes up to 1 ms, as well as different examples of low-cost readout devices.[143–145] The sensors have been also successfully commercialized.[146]

Other often measured quenchers are Cl^-[147,148] and SO_2.[149] Their sensing is also based on dynamic quenching. However, the lifetimes of the fluorophores are shorter and the modulation frequencies are higher.

Lifetime detection was also successfully used for measurement of *p*H (both using long[150] and short[151] lifetime sensors), Na^+,[152] K^+,[153] Ca^{2+},[154] and Mg^{2+}.[155] The detection is based on two-state indicators, and the apparent lifetime is used for measurements. RET-based sensors are also frequently used in lifetime detection. Energy transfer sensors were used for measurement of *p*H,[156] CO_2,[157] NH_3,[158] and glucose.[159] Changes in the lifetime due to changes of polarity have been used for measurements of the water concentration[160] in nonpolar media. Fluorescent lifetimes have also been used for measurements of temperature.[161]

Measurements of the phase shift are performed using different techniques. At low frequencies, it is possible to compare the output of simple zero-crossing detectors;[145] however, the accuracy of such systems is low. Significantly better results are obtained using lock-in techniques[162] (using solid-state integrated circuits for synchronous detection); however the accuracy of the lifetime determination varies with the lifetime. The best accuracy is achieved using phase-locked detection,[163] but the technique was used only for detection of very long lifetimes.[164] Low-cost devices were not available for measurements of nanosecond lifetimes; the use of a lock-in amplifier for lifetime measurements at this time scale has only recently been reported.[165] There is a significant commercial interest in the development of lifetime devices, as can be concluded from the recent patent literature.[166–169]

Modulation sensing is used when there is a need for a reference measurements of the luminescence. When the ratiometric mixture is used, there is always a spectral overlap between the emissions of the luminophores so the signal on the wavelength ratio is always the (variable) sum of the two emissions. When modulation sensing is used, it is possible to completely suppress the long-lifetime emission, thus achieving better discrimination between the emissions. Modulation sensing has been used for detection of *p*H and calcium[170] and for drug compliance monitoring.[171,172] However, the standard form of modulation sensing is not suitable for most applications, as it requires complete exclusion of the ambient light. Recently, a modification of the technique using two different excitation frequencies was used to design an oxygen sensor that can tolerate ambient light.[173] Given the uniqueness of the technique and the fact that it is generic, more applications can be expected soon.

Polarization-based sensors traditionally rely on the association of macromolecules. When a small molecule labeled with a fluorescent marker binds to a much bigger one (typical example is the antibody–antigen reaction), its rotational correlation time significantly increases. This increases the fluorescence anisotropy. Antibody–antigen assays are widely used in biophysics and clinical chemistry.[174,175] Recently, the approach was use to design assays for Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, and Zn^{2+}.[176–178] This allowed simple reversible detec-

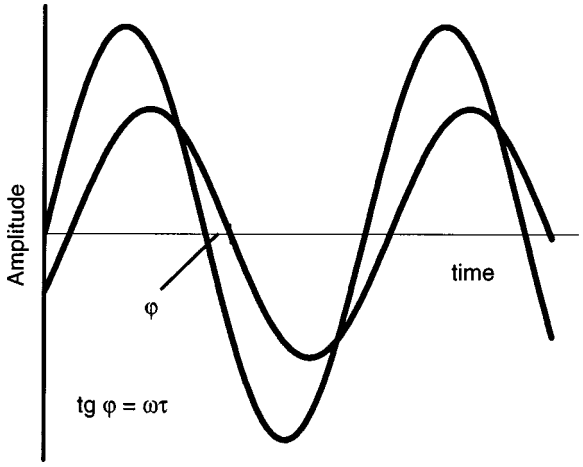

FIG. 12. Illustration of the phase shift φ between the excitation light and luminescence emission. It depends on the excitation frequency ω and the lifetime τ of the luminophore.

tion in the nano- and picomolar range (Fig. 13).

Fluorescence anisotropy is basically a ratiometric technique, as it relies on the ratio of the vertical and horizontal components of the luminescence. If a sensor consists of dyes with polarized and unpolarized emissions, any change in the intensity of the unpolarized emission will result in change of the total anisotropy. This effect was recently used to create polarization-based sensors for *p*H, oxygen, glucose, and for drug compliance monitoring.[179–181]

C. Waveguide effects

1. Basic equations

Fluorescence or lifetime spectroscopy, in principle, could be performed without the use of light guide. In the case when a light guide is employed, additional measurement techniques become available: refractomery, evanescent wave, and surface plasmon resonance.

Determination of the refractive index of liquids is based on Snell's law. Some parameter related to the angle of refraction at the waveguide-to-liquid interface is measured. The simplest measurement is to measure the intensity of the transmitted or reflected light through the waveguide, as the light that exceeds the critical angle is lost into the liquid phase. The transmittance T is function of the ratio of the refractive indexes of the waveguide n_w and the liquid n_l:

$$T=f\left(\frac{n_l}{n_w}\right). \tag{22}$$

However, the refractive index depends strongly on the temperature (10^{-3} to 10^{-4} /°C), so temperature correction and stabilization are critical for achieving high sensitivities.

When the light travels along the waveguide, not all the energy is confined to the guide. The electric field amplitude E of the light outside of the waveguide decays exponentially with the distance x into the rare medium (Fig. 14):

$$E=E_0\exp(-x/d_p), \tag{23}$$

where E_0 is the amplitude at the surface of the guide. The part of the light that travels outside the guide is called the

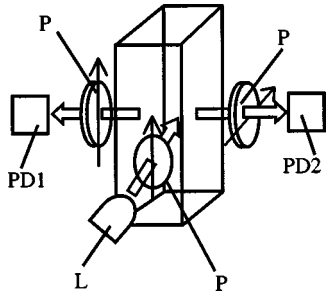

FIG. 13. Optical arrangement for detection of fluorescence anisotropy. *L* is the light source, PD1, PD2 are the photodetectors, *P* is the polarizers. Excitation and emission filters are not shown. Block arrows show the direction of the light, thin arrows show the orientation of the polarizers.

evanescent wave (EW). The depth of penetration, d_p, is defined as the distance for the electric field amplitude to fall to 1/e of this value at the surface:[3]

$$d_p=\frac{\lambda}{2\pi n_1[\sin^2\theta-(n_1/n_2)^2]^{1/2}}, \tag{24}$$

where n_1 and n_2 are the indexes of the guide and the surrounding medium, and θ is the angle of propagation in the guide. Penetration depths are typically 50–1000 nm for visible light ($d_p<\lambda$).[182] This allows one to perform absorption spectrometry or to excite luminescence without passing the interrogation light through the sample. The low depth of penetration allows discrimination against the interferences in the bulk medium.

Surface plasmon resonance (SPR) occurs when the waveguide is covered with a thin metal film. If the momentum k_{SP} of the photons in the film plane matches the momentum of the surface plasmons on the opposite surface of the field, the reflection coefficient drastically changes. This occurs at some critical angle of incidence θ_{SP}, which is dependent on the dielectric constants of the waveguide ϵ_2 and the metal ϵ_m, as well as on the wavelength of the light λ:

$$k_{SP}=\frac{2\pi}{\lambda}n_1\sin\theta_{SP}=\frac{2\pi}{\lambda}\left(\frac{1}{\epsilon_2}+\frac{1}{\epsilon_m}\right)^{1/2}. \tag{25}$$

Here n_1 is the refraction coefficient of the support.

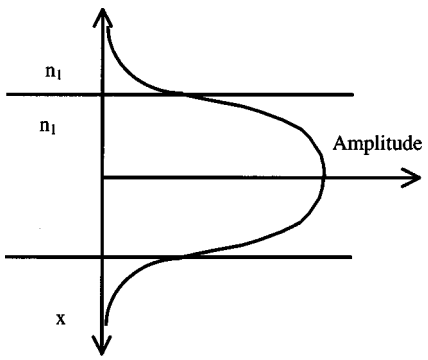

FIG. 14. Distribution of the light energy inside and outside the waveguide. The portion of the light that travels outside the guide is referred to an evanescent wave.

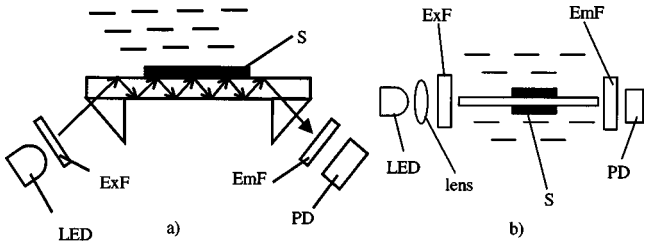

FIG. 15. Optical set ups for evanescent wave sensing. (a) Slab based version; (b) fiber based version. ExF is the excitation filter, EmF is the emission filter, PD is the photodiode and *S* is the sensing membrane.

SPR is extremely sensitive to changes in the refractive index in the medium in contact with the metal film. At constant λ, the SPR angle varies significantly with very small alteration of the index.

2. Examples

Refractometry sensors may be based on fiber optic or planar configurations. Different geometries have been tested to increase the sensitivity. For example, the fiber could be just a straight fiber without cladding,[183] bent fiber (U shape[184] or spiral[185]), twisted fibers,[186] etc. The refraction changes could take place in the surrounding media, or in a thin film,[187] covering the waveguide. The device could also work in reflection mode.[188] Ion selective films have been used together with refractometric sensor,[189,190] extending the possibilities and sensitivity of the technique. Refractometry has also been tried in glucose determination in interstitial fluid.[191] However, the variation of the refractive index is very low—from 1.3334 to 1.3337 in the diabetic range, making a practical sensor difficult.

EW sensors and SPR sensors are used usually for detection of affinity reactions. High sensitivity is of great importance for antigen–antibody interactions and DNA hybridization.[10] EW sensors usually rely on the excitation of fluorescent markers near the waveguide surface. Their fluorescence is preferentially back coupled by tunneling.[192] This significantly increases the intensity of the collected fluorescence from the surface in comparison with the one from the solution under test. The design of the sensor could be based on a slab or fiber lightguide (Fig. 15). The slab version usually requires prisms or grating for the input of the interrogating light.[193,194] The fiber versions need focusing lenses.[195] Fiber optic versions are typically low-cost, using LED[195] or LD[196,197] for fluorescence excitation, and photodiodes for emission detection. EW based sensors are capable of detection of concentrations from nanomolar[198] to the femtomolar range.[199] They have been used for rapid detection of fibrin degradation products,[200] hormones,[194] DNA hybridization,[198,201] cocaine in metabolites[202] and leaves,[203] toxins,[204–206] harmful bacteria (*E. coli*,[196] *H. pilori*,[197] *L. donovani*[207]), polymerase chain reaction (PCR) amplification,[208] polyaromatic hydrocarbons,[209] and ozone.[210]

SPR sensors measure the changes in the refractive index close to the metal surface. Any change will create a significant response. Because of very high sensitivity, the sensors are used to monitor complex formation–dissociation during an affinity reaction, or through changes of ϵ of indicator chromophores.[211] The first process is wavelength independent. SPR sensors are built around Kretschmann[212] or cylindrical prism, or use miniaturized fiberoptic low-cost versions[213] (Fig. 16). Recently, a fully integrated low-cost SPR detector was shown to be useful in a variety of biological, environmental, and industrial applications.[214] SPR allows characterization of binding reaction in real time without labeling. However, the experimental conditions (temperature, blank buffer) should be well defined.[215] Special attention must be paid to reaction surface,[216] referencing,[217] and regeneration.[218] Additionally, mathematical treatment of the results is often required.[219] SPR sensors are used for determining the active concentration of molecules,[220] kinetics reaction rates,[221] and affinity constants.[222] The ability to monitor the binding of low molecular mass compounds to immobilized macromolecules is used in HIV studies[223] and drug binding.[224] Other applications are direct detection of antigen-antibody reactions,[225] and DNA hybridization.[226] The sensors have been used for hemoglobin,[227] myoglobin[228] and heparin[229] quantification, in high throughput screening,[230] virus-cell docking,[231] insulin-like growth factors,[232] PCR products,[233,234] simazine,[235] as well as monitoring recombinant proteins in process media.[236]

In conclusion, there is a great diversity of optical devices. A great variety of detection techniques, optical configurations, and chemical indicators are being used. Many of them have been successfully commercialized. However, there is a continuing need for the development of accurate and reliable sensors. The development of new, small, low-cost devices will continue due to an increasing need for biomedical and other measurements. In particular, advances in genomics and proteomics will require novel low-cost sensor applications.

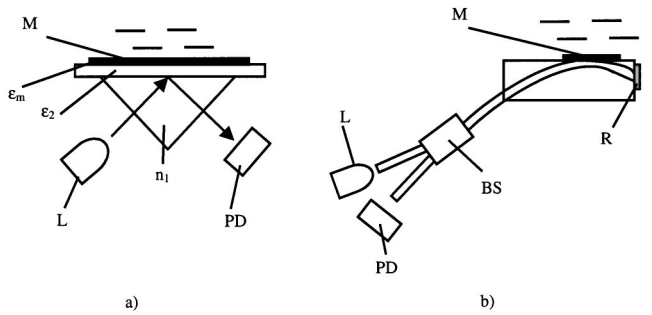

FIG. 16. Optical setups for SPR sensing. (a) Kretschmann prism based version; (b) fiber based version. *M* is the metal layer, *L* is the laser diode, PD is the photodiode, BS is the beam splitter, *R* is the reflector, ϵ_m is the dielectric constant of the metal, ϵ_2 is the dielectric constant of the waveguide, and n_1 is the refraction index of the prism.

ACKNOWLEDGMENTS

One of the authors (Y.K.) thanks Peter Harms for helpful discussions and suggestions. This work was supported by grants from NSF, NIH, and unrestricted matching funds from Genentech, Merck, and Pfizer.

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