Nanophotonics for Lab-on-Chip Applications

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Abstract. Optical methods are the preferred measurement techniques for biosensors and lab-on-chip applications. Their key properties are sensitivity, selectivity and robustness. To simplify the systems and their operation, it is desirable to employ label-free optical methods, requiring the functionalization of interfaces. Evanescent electromagnetic waves are probing the optical properties near the interfaces, a few 100 nm deep into the sample fluid. The sensitivity of these measurements can be improved with optical micro-resonators, in particular whispering gallery mode devices. Q factors as high as 2×10^8 have been achieved in practice. The resulting narrow-linewidth resonances and an unexpected thermo-optic effect make it possible to detect single biomolecules using a label-free biosensor principle. Future generations of biosensors and labs-on-chip for point-of-care and high-troughput screening applications will require large numbers of parallel measurement channels, necessitating optical micro-resonators in array format produced very cost-effectively.

Keywords: Nanophotonics, optical sensing, label-free biosensing, micro-resonators.

1 Introduction

The human being is a complex biochemical engine, involving more than 100,000 different species of molecules, most of which are proteins [1]. Deciphering the dynamic interplay between these molecules and investigating the interaction with other substances – pharmaceutically active or potentially toxic molecules – is a humungous task.

Optical biosensing has proven to be an invaluable tool for detecting minute concentrations of target molecules in small sample volumes. Due to the ease and low cost with which large number of photons can be generated, manipulated and detected with high sensitivity, numerous types of optical biosensing methods have been developed, and many of them are commercially available in miniaturized form such as optical biochips or optical labs-on-chip [2].

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In this article we are investigating which factors are influencing the main properties of a biosensing method – selectivity, sensitivity and robustness – and we are examining how recent advances in nanophotonics can contribute to the improvement of optical biosensing techniques. In particular, the sensitivity of label-free optical biosensing methods has been increased to the point where it has become possible to detect the presence of a single molecule [3].

2 Fundamentals of Optical Biosensing

Each cell in the human body contains thousands of molecular species [1]. As a consequence, detecting minute concentrations of a particular (target) molecule in biologically relevant environments is not only a problem of sensitivity; it is also a problem of specificity. We have to make sure that our measurement signal is caused by the target molecule and not by other molecules generating spurious signals.

2.1 The Basic Measurement Problem in Life Sciences

Several physical methods exist which are capable of detecting the presence of specific molecules in a matrix of other substances, most notably Raman scattering, infrared spectroscopy, magnetic resonance or mass spectroscopy [4]. However, these methods suffer from insufficient sensitivity, robustness and ease-of-use, and because of the high technical demands, it is difficult to achieve cost-effective parallelization.

The basic measurement problem is the precise determination of the number of molecules of a specific type in a given sample volume of aqueous solution, containing also unknown species and numbers of other molecules. The target molecules are displacing water molecules, and they are therefore changing the physical properties of the aqueous solution, for example local mass (density), polarizability (refractive index) or absorption of electromagnetic radiation. Even if a very sensitive physical method was known, capable of measuring these effects for minute concentrations of the target molecule, this would not be sufficient to determine the concentration of the target molecules because other molecules, present in unknown concentrations, are also influencing the measured effect.

It is clear, therefore, that before the measurement of any effect should take place, we have to single out – to mark – the target molecules in such a way that the measured effect is only due to the target molecules. This vital marking step is done chemically, and it plays a crucial role for the specificity of the particular method.

Finally, the practical success of a measurement method does not only depend on the **specificity** and the **sensitivity** of the method, the **robustness** of the measurement signal also influences the precision of the measured concentration values. Only if thermal fluctuations, diffusion effects, baseline shifts, pressure variations, flow inhomogeneities, none-specific binding and cross talk (influence of other molecules on the measurement) affect the measurement result merely insignificantly, is it possible to provide a reliable, precise value of the concentration of a target molecule.

2.2 Molecular Species and Concentrations of Interest

Three types of molecular species are of particular interest in life sciences: (1) small molecules with pharmaceutical actions, (2) peptides and (3) proteins. Peptides and proteins consist of amino acids, which are chained together by peptide chemical bonds. In the human body 20 different amino acids are employed for the synthesis of such chains [4]. Some relevant properties of the three types of molecules of interest are summarized in Table 1.

Table 1. Relevant properties of the three main types of molecules of interest in biosensing

	Small Molecules	Peptides	Proteins
# amino acids	–	< 100	100 - 30,000
Weight	≤ 1000 Da	≤ 15 kDa	10 - 3600 kDa
Extent / Size	≤ 2 nm	≤ 5 nm	≤ 1000 nm

Although giant proteins of a few 1000 kDa occur, proteins on average consist of 800 amino acids, they have a weight of about 100 kDa and a size of about 10 nm, see also references [4] and [5].

The typical concentration of a specific molecule of interest in biomedical application is in the pM to µM range. However, in particular cases such as the early detection of biomarkers for cancer, significantly smaller concentrations of molecules should be detectable.

2.3 Taxonomy of Optical Biosensing Methods

The many ways in which light interacts with matter and the simplicity of the involved measurement setups have made optical methods the most widely used techniques for biosensing. The taxonomy of the different optical effects that can be exploited for biochemical sensing is illustrated in Fig. 1, see also [6].

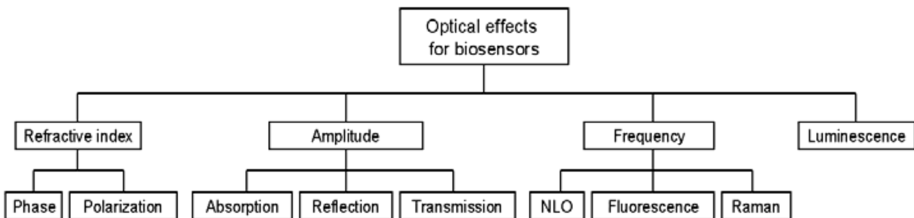

Fig. 1. Taxonomy of optical effects which can potentially be employed for biosensing

The most widely adopted optical methods for biosensing either make use of light-emitting molecules (fluorescence or occasionally luminescence), or they determine the change of refractive index employing evanescent waves (surface plasmon resonance SPR or grating couplers) [2]. In both cases, the key difficulty lies in ensuring

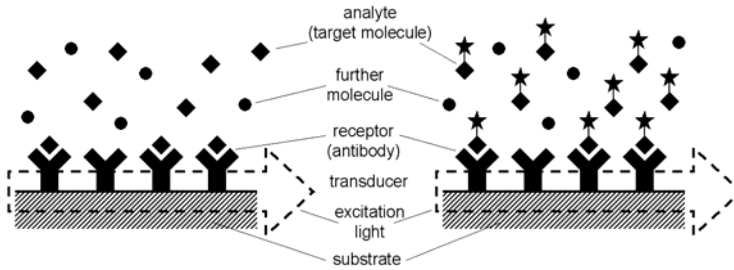

Fig. 2. Illustration of evanescent-wave based optical biosensing, making use of receptor molecules immobilized on a surface. Left: Label-free sensing principle. Right: Labeled sensing principle.

that the measured optical effect (emission of fluorescent light or change in refractive index) is really caused by the presence of the target analyte.

This problem is solved in both cases by immobilizing a highly specific receptor (antibody) molecule on a solid surface (planar substrates, porous membranes, glass or plastic beads, capillary walls, microfabricated optical resonators, etc.), as illustrated in Fig. 2. Analyte molecules, arriving in the aqueous solution in contact with the sensitized surface, will interact with the specific receptor, and they will therefore accumulate close to the surface. As can be seen in Table 1, the thickness of this layer depends on the type of target molecule, and it is typically in the range of 1-100 nm. For this reason, it is advantageous and sufficient to measure the optical parameters close to the surface. This is effectively accomplished by evanescent waves, electromagnetic waves propagating along an optical interface, where part of the wave field's energy is found outside of but close to the optical interface.

2.4 Principles of Label-Based (Fluorescence) Biosensing Methods

The highest sensitivity and selectivity in optical biosensing is routinely achieved with fluorescence-based techniques. Four types of assay configurations are possible: Sandwich, competitive, displacement and direct binding [7]. In particular, the sandwich assay achieves the highest selectivity for the target molecule, since two binding sites (epitopes) are employed, one for the immobilized receptor and one for the fluorescently labelled tracer molecule. The probability of non-specific binding of the fluorescent molecule to a molecule other than the target is much reduced because the probabilities for the two binding sites on the target multiply.

The disadvantage of label-based biosensing is their need for a well-suited dye molecule that can either be attached directly to the target molecule or to a tracer molecule (antibody) which then attaches to the target. In both cases, the regular biochemical function of the target is impaired, since a "foreign" molecular species is attached to it. This is particularly true for peptides and small molecules, in comparison with which typical dye molecules are of relevant size.

The optical measurement in label-based biosensing consists of exciting the fluorescent molecule with suitable light pulses, and in observing the generated fluorescent light of longer wavelength. Since the fluorescent light can be detected today with

close to single-photon resolution, it is virtually possible to detect the presence of a single fluorescent molecule and, therefore, a single target molecule. If the excitation light is transmitted in the form of a travelling evanescent wave, a large fraction of the fluorescence light is generated in the vicinity of the surface, where target molecules are bound to the immobilized receptors.

It should be emphasized that the specificity of label-based biosensing methods is not due to the high quality of the optical measurement but it is rather due to the high specificity of the – direct or indirect – chemical binding of the target to the fluorescent molecule and to the immobilized receptor molecule.

2.5 Principles of Label-Free Optical Biosensing

To overcome the need for a dye and two antibody molecules, a number of label-free optical biosensing methods have been developed, relying on the measurement of changes in the local refractive index when target molecules are bound to the surface of a waveguiding structure. In practice, the two preferred structures are dielectric waveguides and metal-dielectric interfaces for the transmission of surface plasmons, as illustrated in Fig. 3.

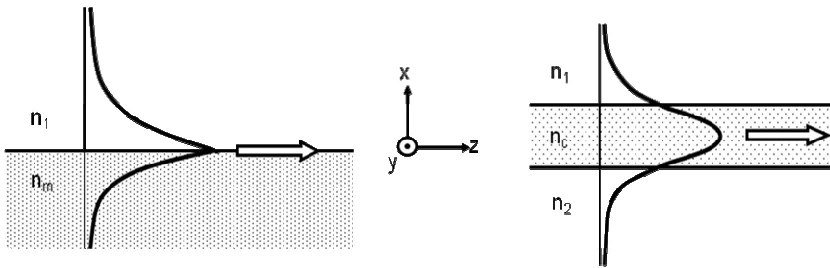

Fig. 3. Propagation of an evanescent electromagnetic wave as a surface plasmon at a metal-dielectric boundary (left) or in a dielectric slab waveguide (right)

In both cases, the presence of analyte molecules subtly changes the effective refractive index at the surface sensed by the evanescent wave. This can be measured by determining the effect of the changed refractive index on coupling light into or out of the waveguiding structure. In practice light coupling is accomplished either with a prism or a grating, as illustrated in Fig. 4. Most often, prism coupling is used for surface plasmon transmission, and grating coupling is employed for dielectric waveguides.

A change in the effective refractive index alters the resonant coupling condition for incident light, which can be adjusted either with the in- or out-coupling angle Θ or with the wavelength λ. The resonance readout principle is exploited in today's optical biochips with evanescent wave sensing: The amount of light in the guided wave is maximum for the combination of Θ and λ that fulfills the coupling condition for the actual value of the effective refractive index, which, in turn, is dependent on the analyte concentration.

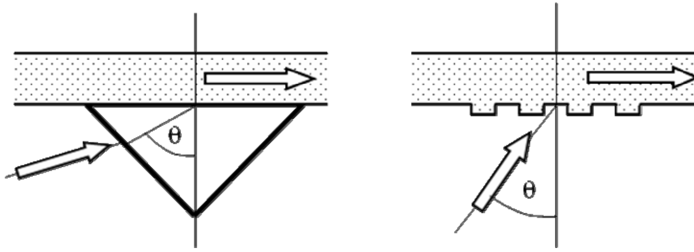

Fig. 4. Coupling of incident light into a waveguiding structure using a prism (left), or a grating coupler (right)

The most sensitive methods for measuring this resonance condition are capable of resolving changes in the refractive index of about 10^{-7} [8]. This corresponds to a mass density detection sensitivity of nearly 100 fg/mm^2. The molar concentration of the analyte can be determined with a sensitivity of as low as 10^{-11}, i.e. the concentration sensitivity can be as low as 10^{-11} mole per liter. It is important to stabilize or to compensate for temperature variations at these high measurement sensitivities, since $\partial n/\partial T \approx 10^{-4}$ K^{-1} for water at room temperature [9]. Typical compensation principles include referencing methods to monitor all non-specific effects such as temperature fluctuations.

The relative width $\delta\lambda$ of the resonance peak is the inverse of the resonator's quality factor Q, i.e.

$$Q = \lambda / \delta\lambda . \tag{1}$$

Typical Q factors of surface plasmon resonance (SPR) devices are below 100, and for dielectric waveguides with grating couplers they can reach a few 1000. It should also be noted that the optimum interaction length for SPR devices is of the order of 10 µm, and for dielectric waveguides with grating couplers it is of the order of 100 µm; increasing the interaction length does not significantly improve the Q factor. In SPR devices these small interaction lengths are related to the absorption length in the employed metal of a few 10 µm. In contrast, the absorption length in dielectric waveguides can be several centimeters; in these devices it is rather the technological quality of the coupling grating that limits their Q factor.

The optimum interaction length is of importance for the amount of analyte substance that is necessary to be bound to the sensor's surface for a needed sensitivity. If the **total interaction surface** can be reduced, then less of the potentially valuable and scarcely synthesized analyte substance is required.

3 Nanophotonics for High-Sensitivity Label-Free Biosensing

From the previous section it becomes clear that the most relevant desired improvements of optical biosensing techniques concern the following five aspects:

- Replacement of fluorescence-based with label-free measurement techniques
- Increase of selectivity

- Increase of sensitivity
- Increase of stability
- Reduction of total interaction surface

While the first two of these improvements are essentially problems that need to be addressed in the chemical world, the last three requirements are currently being investigated by many researchers with nanophotonics.

The basic idea is to replace the “one-pass” sensing structures illustrated in Fig. 2 with resonant optical devices, where light is “recycled” and the same photon can repeatedly interact with the same receptor molecule.

The most popular of these resonant optical devices include Fabry-Perot, distributed feedback (DFB), micro-bead, capillary, planar ring/racetrack and micro-toroid/micro-disk resonators, and they are schematically illustrated in Fig. 5, see also [10]. Label-free measurements of specific analyte molecules are achieved by sensitizing the surface of the micro-resonators with suitable receptors, as illustrated in Fig. 2.

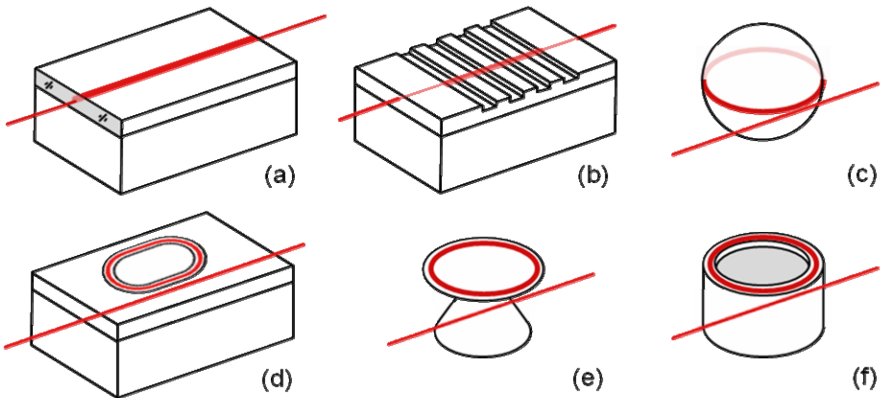

Fig. 5. Optical micro-resonators employed for biosensing with increased sensitivity and reduced total interaction surface. (a) Fabry-Perot, (b) distributed feedback (DFB), (c) micro-sphere, (d) planar ring or racetrack, (e) micro-toroid or micro-disk, (f) capillary.

3.1 Optical Micro-resonators for Biosensing with Enhanced Sensitivity

The micro-resonators illustrated in Fig. 5 can be fabricated with stable processes well known from silicon technology. Very high Q factors of more than 10^6 are routinely achieved, corresponding to a resonance peak resolution $\delta\lambda$ of about 0.0005 nm [10].

Since it is not difficult to locate the centroid of a resonance to $\delta\lambda/100$, this very high measurement precision of the resonance peak allows the significant increase of the sensitivity of optical biosensing methods. While the lowest reported limit of detection (LOD) of the surface density of bound biomolecule mass is nearly 100 fg/mm^2 measureable with SPR, the LOD achieved with optical micro-cavities is about one order of magnitude lower [10].

These LOD values can be surpassed with micro-cavities offering much increased Q factors. The highest Q factor reported to date is 2×10^8, achieved with micro-toroids fabricated on a silicon wafer [3].

3.2 Whispering Gallery Mode Biosensing for Single-Molecule Detection

Micro-resonators with circular topology are called Whispering Gallery Mode (WGM) resonators [10]. Actually, most of the optical micro-cavities employed for biosensing use WGM recirculation. Their much improved Q factors when compared to SPR or to grating-coupled dielectric waveguides allow a significant increase in sensitivity of biosensing. Surprisingly, the sensitivities achieve in practice are even much higher than predicted by the simple “reactive” theory. This is caused by the actual interaction of the analytes with the oscillating evanescent field at the biosensor’s surface: Not only does the electromagnetic field polarize the bound biomolecule reactively (in phase with the oscillating field), the biomolecule can also absorb energy from the field, effectively creating a “nanoscopic” heat source at the surface of the optical micro-cavity. This, in turn, causes a red-shift of the resonant wavelength through the thermo-optic effect because the WGM material is locally heated by the biomolecule. As an example, the optothermal constant $\partial n/\partial T$ of silica is 1.3×10^{-5} K^{-1}.

The expected wavelength shift $\Delta\lambda$ due to the thermo-optic effect produced by a single biomolecule bound at the intensity maximum has been calculated in [3] to be

$$\Delta\lambda \propto \frac{\lambda^2 \sigma}{n^2 \kappa V} \frac{\partial n}{\partial T} Q P \qquad (2)$$

with the absorption cross section σ of the biomolecule, the effective refractive index n of the micro-cavity, the thermal conductivity κ, the optical mode volume V and the coupled optical power P.

In this way, biosensing with WGM optical micro-resonators obtains a double benefit from the ultra-high Q factors: Firstly, the wavelength red-shift is proportional to Q, as shown in equation (2), and secondly, the relative width of the resonance peak is inversely proportional to Q, as seen in equation (1), implying that the precision with which a wavelength shift can be measured is proportional to Q.

Functionalized micro-toroids have been successfully employed in this fashion as label-free, single-molecule biosensors, exploiting an ultra-high Q factor of 10^8 and a coupled power of 1 mW [3].

3.3 Ultra-Low-Noise Photodetection with CMOS/CCD Image Sensors

The advances in ultra-high Q optical micro-resonators for biosensing are complemented by progress in silicon-based ultra-low-noise photosensing [11]. In particular, three achievements are of major importance in this domain: (1) The successful fabrication of silicon-based image sensors with extremely low dark current density at room temperature of less than 1 pA/cm^2 for charge coupled devices (CCD) and of less than 10 pA/cm^2 for CMOS-based image sensors; (2) the design and fabrication methodology for avalanche photodetector (APD) arrays in Geiger mode, capable of detecting the arrival of each individual photon in the visible and near infrared spectral range;

(3) the architecture of CMOS image sensors (CIS) with optimized analog and digital signal processing (correlated double sampling, bandwidth optimization, in-pixel gain, column-parallel low-pass filtering, etc.).

The result of these successes is the availability of cost-effective image sensors exhibiting readout noise levels of less than 1 electron (r.m.s.) at room temperature and video readout rates [12]. Since silicon has an external quantum efficiency of close to 100% in the visible and near infrared spectral range, it has become possible today to detect virtually every incident photon in an optical experiment, and the photosensitive surface of such ultra-low-noise image sensors can be as large as several square centimeters.

4 Outlook: More Nanophotonics in Label-Free Biosensing ?

There are several examples where affordable high-sensitivity (approaching single-molecule) detection biosensors would be of large practical relevance, in particular for the early point-of-care detection of biomarkers of wide-spread diseases (cancer, diabetes, etc.), high-throughput screening (HTS) of lead compounds in drug discovery or for supporting basic life science research studying cell signaling pathways and cell membrane biophysics.

However, in all of these applications, high sensitivity must also be coupled with high selectivity and small bio-interaction surface. For this reason, many parallel, small-sized measurement channels must be co-integrated on the same biosensor in array-format. It is not obvious which type of optical micro-resonator is best suited for this task: Stable optical coupling to the 3D structures of undercut micro-disks or micro-toroids [3] is more difficult than to planar photonic crystal (nano-) cavities [13] or to micro-ring ("race-track") resonators [14]. As an example, micro-disk arrays have been successfully fabricated, in which the micro-disks are coupled to waveguide buses with suitable multiplexing [15]. In any case, practical fabrication aspects will decisively influence the choice of technique for highly parallel array biosensing.

It can even be envisaged that miniature optical micro-resonators with a surface of only a few square micrometers could be manufactured and encapsulated in biocompatible micro-capsules such as lipid vesicles. These micro-biosensors would be powered and read out non-invasively with suitable near-infrared laser light, and they would either be circulating freely through the cardiovascular system or they would be residing as "smart tattoos" near the surface of various body parts [16].

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References

1. van Beek, J.H.G.M.: Channeling the Data Flow: Handling Large-Scale Biological Measurements in Silico. Proceedings of the IEEE 94, 692–709 (2006)
2. Seitz, P.: Optical Biochips. In: Biophotonics, pp. 217–226. Springer, Berlin (2008)

3. Armani, A.M., Kulkarni, R.P., Fraser, S.E., Flagan, R.C., Vahala, K.J.: Label-Free, Single-Molecule Detection with Optical Microcavities. Science 317, 783–787 (2007)
4. Jain, K.K.: The Handbook of Nanomedicine. Humana Press, Totowa (2008)
5. Hunter, P.J.: Modeling Human Physiology: The IUPS/EMBS Physiome Project. Proceedings of the IEEE 94, 678–691 (2006)
6. Prasad, P.N.: Introduction to Biophotonics. Wiley, Hoboken (2003)
7. Ligler, F.S..: Fluorescence-Based Optical Biosensors. In: Biophotonics, pp. 199–215. Springer, Berlin (2008)
8. Lambeck, P.V.: Integrated optical sensors for the chemical domain. Meas. Sci. Technol. 17, 93–116 (2006)
9. Lide, D.R. (ed.): CRC Handbook of Chemistry and Physics, 87th edn. Taylor & Francis, Bota Raton (2006)
10. Vollmer, F., Arnold, S.: Whispering-gallery-mode biosensing: label-free detection down to single molecules. Nature Methods 5, 591–596 (2008)
11. Seitz, P.: Smart Pixel Arrays. In: Encyclopedia of Modern Optics, Elsevier, Oxford (2005)
12. Lotto, Ch., Seitz, P.: Synchronous and Asynchronous Detection of Ultra-Low Light Levels. In: Proceedings of the 2009 International Image Sensor Workshop, Bergen, Norway (2009)
13. Lee, M., Fauchet, P.M.: Two-dimensional silicon photonic crystal based biosensing platform for protein detection. Optics Express 15, 4530–4535 (2007)
14. De Vos, K., Bartoluzzi, I., Schacht, E., Bienstman, P., Baets, R.: Silicon-on-Insulator microring resonator for sensitive and label-free biosensing. Optics Express 15, 7610–7615 (2007)
15. Choi, S.J., Peng, Z., Yang, Q., Choi, S.J., Dapkus, P.D.: Eight-Channel Microdisk CW Laser Arrays Vertically Coupled to Common Output Bus Waveguides. IEEE Photonics Technology Letters 16, 356–358 (2004)
16. Chinnayelka, S., McShane, N.J.: Competitive binding assays in microcapsules as smart tattoo biosensors. Proceedings of IEEE Sensors 2005, 1304–1307 (2005)