ORAL SESSION:

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OPTICAL CHEMICAL AND BIOCHEMICAL SENSORS FOR IN-VIVO CLINICAL APPLICATIONS AT IFAC-CNR

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Abstract

Optics plays a key role in the development of optical chemical and biochemical sensors in medicine. This is particularly true in the case of invasive applications where the high degree of miniaturisation of optical fibres, their considerable geometrical versatility, and their extreme handiness enable unique performances. Moreover, the immunity to electromagnetic interference and the absence of electrical contact make them useful, not only for invasive applications, but also in the case of a non invasive application for the setting of the interrogation optoelectronic unit in a safer location, with only the sensing element close to the patient's bedside [1].

Two sensors for invasive applications have been developed, both of which are for gastroesophageal applications: one is for the detection of bile-containing refluxes, and the other is for the continuous monitoring of carbon dioxide in the stomach or in the oesophagus [2].

The optical fibre bile sensor utilizes bilirubin, the main biliary pigment, as a marker for the detection of bile-containing refluxes. It makes use of two light-emitting diodes as sources, with an emission peak at 470 nm and 565 nm for the signal and the reference, respectively. Figure 1 shows the probe currently used in clinical practice. The probe consists of a diffusing plastic reflector connected with the optical fibre bundle by means of a small, thin stainless-steel wire. The probe is completely open to the external environment, and ensures that no stagnation occurs by ensuring a fast response of the sensor and the direct removal of the mucus by the liquid content of the stomach. The fibres of the bundle are randomly mixed, so that partial covering of the bundle surface does not cause any variations in the ratio between the signal and the reference. The optical device, Bilitec 2000, was clinically validated and has been commercialized by Medtronic.

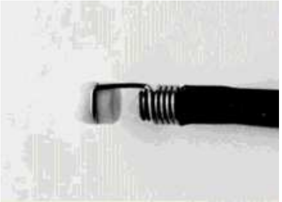

Figure 1. Photo of the optical probe for the detection of bile-containing reflux (external diameter: 4 mm; distance between fibre and reflector: 2 mm)

The optical fibre sensor for continuous monitoring of gastric CO_2 was developed in collaboration with Joanneum Research (Graz, Austria). The optical-sensitive layer consists basically of a dye/quaternary ammonium ion pair that is dissolved in a thin layer of ethylcellulose. Cresol red and tetraoctylammonium are the pH-sensitive dye and the quaternary ammonium ion used in the ion pair, respectively. The sensing membrane is covered by a silicone coating, which prevents leaching of the dye or of the quaternary ammonium salt. The dye is characterised by an absorption peak centred at 590 nm which is modulated by pCO_2: the absorption is maximum in the absence of CO_2 and decreases with the increase of CO_2. Figure 2 shows a photograph of the optical probe

Figure 2. Photo of the optical probe for continuous monitoring of gastric CO_2

which consists of a piece of black plastic (PEEK) containing the CO_2-sensitive membrane. The probe head has a diameter of 7 mm and is 9.5 mm long. Figure 3 shows a typical measurement obtained during the clinical validation where the optical fibre sensor is compared with the results achieved with the Tonocap sensor, the system based on gastric tonometry. The trend of the end-tidal CO_2 ($EtCO_2$), i.e. the CO_2 partial pressure in the expiration at the end of the expiratory phase, was followed perfectly by the gastric pCO_2. The short peak in the pCO_2 tracing of the optical sensor, not detected by Tonocap, could be ascribed to many causes, one of which could be the occurrence of a bile reflux, containing bicarbonate, from the duodenum into the stomach.

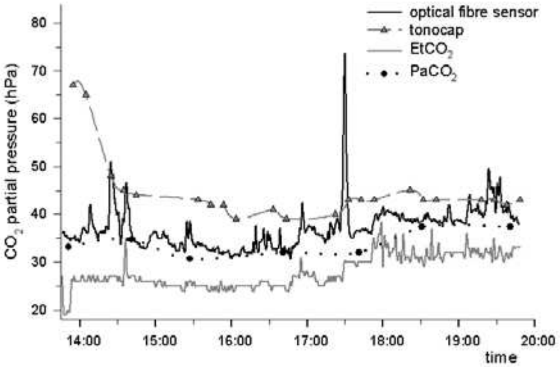

Figure 3. Clinical validation of the CO_2 sensor performed on a critically ill patient in intensive care unit with the location of the probe in the stomach. The tracing of the optical sensor is compared with the Tonocap tracing. The triangles in the Tonocap tracing are the measured values every ten minutes(the shortest response time possible with Tonocap). The end-tidal CO_2 ($EtCO_2$), and the arterial partial pressure of carbon dioxide ($PaCO_2$) values are also shown.

A minimally invasive optical fibre sensor for the on-line measurement of the pH in adipose tissue was developed, making use of a microdialysis catheter as body interface [3]. Phenol red is the acid-base indicator used for the optical transduction and it is covalently bound to the internal wall of a glass capillary which is in series with the microdialysis catheter. The glass capillary is interrogated with optical fibres connected to an optoelectronic unit which makes use of a light emitting diode at 590 nm as source and a photodiode as detector. For the clinical validation, an animal model was developed to create a situation of stress, based on drawing more than 50% of the blood in a pig and on subsequent reinjection after 1h. The microdialysis catheter is inserted in the abdomen of the pig. Blood pH values were measured roughly every 30min with a blood gas analyzer, and a glass microelectrode, inserted close to the microdialysis catheter, is used as reference. As the blood is drawn, a decrease in pH is observed both in the blood and in the adipose tissue. The recovery of the animal with a return to a more healthy state occurs simultaneously in the blood and in the adipose tissue (see Figure 4). A discrepancy of 0.1–0.2 pH units between the glass microelectrode and the optical sensor was observed and it is due to the low resolution of the electrode (0.1 pH units). The results obtained are very promising, and adipose tissue appears to be a reliable alternative site for in vivo continuous monitoring of stress conditions.

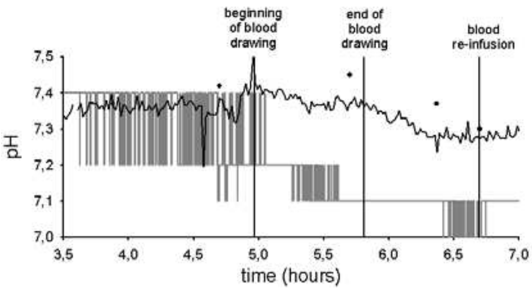

Figure 4. Clinical validation of the microdialysis-based pH sensor. The optical sensor (black solid line) is compared with the glass electrode (sold grey line). The beginning and the end of blood drawing and the blood re-infusion are indicated by the vertical lines.

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BIOSENSORS AND BIOPHOTONICS AT IMM-NA

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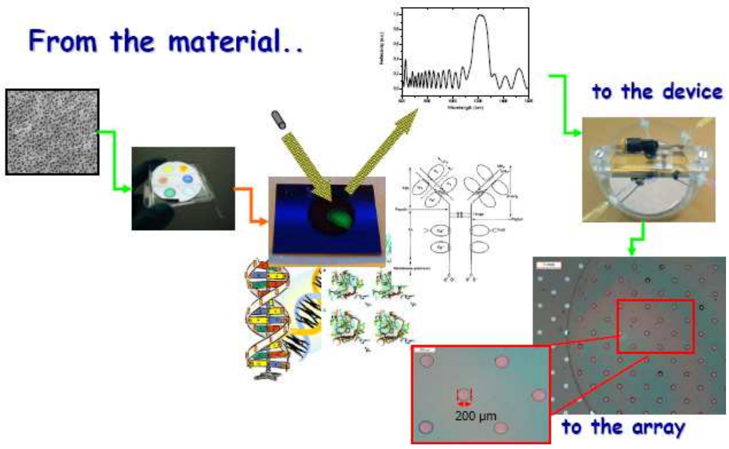
Abstract

Biosensors and biophotonics are hot topics in European current technological development, due to the innovative contents and the huge possibilities of device applications ranging from basic sciences to industrial and everyday life products. In our institute we have engaged for many years in several topics concerning both biosensors and biophotonics. In this communication, we will review the main results achieved in each of this topic.

Porous silicon based micro-optical systems

Porous Silicon (PSi) is an almost ideal material as a transducer due to its porous structure with hydrogen terminated surface, having a specific area of the order of 200 – 500 m^2 cm^{-3}, so that a very effective interaction with several adsorbates is ensured. Moreover, PSi is an available and low cost material, completely compatible with standard micromachining techniques. The PSi is fabricated by the electrochemical etching of a silicon wafer in a hydrofluoridic acid solution. Since this fabrication process is self-stopping, it is possible to realise vertical structures having layers with different porosities and hence different refractive indexes. These devices exhibit peculiar responses: from the single layer, which optically acts as a Fabry-Perot interferometer, to several kind of multilayers such as Bragg reflectors or microcavities. PSi optical sensors are based on changes of reflectivity on exposure to the target analytes which could penetrate in the spongy structure and replace the air into the PSi pores. To give selectivity to the PSi optical sensor, it is possible to link on its surface the biological probes by a proper passivation process. In Figure 1 we have summarised the fabrication flow chart of a PSi biosensor starting from the as-etched material, which is the transducer element, to the sensing device after the optical structuring and the derivatization process.



Porous silicon base devices, single or in microarrays, have been used as label-free optical sensors for monitoring protein-protein, protein-ligand, DNA-DNA interactions.

Biophotonics in marine diatoms

The diatoms are aquatic unicellular organisms, showing naturally evolved complex nanostructures. In these micro algae, the organic matter, the protoplasts, is enclosed in a hydrated amorphous silica cell wall, called the frustule. Diatoms are usually grouped on the basis of the frustule symmetry: centric diatoms, which have a circular symmetry, and pennate diatoms, which are bilaterally symmetrical. Anyway, as a matter of fact, the shape and the size of frustules are extremely different among the 100.00 and more species of existing diatoms: they can be circular, oval, stick-shaped, star like, and so on, and range in dimensions from micrometres up to one millimetre. Valve surfaces exhibit specie-specific patterns of regular arrays of chambers, called areolae, developed into the frustule depth. Areolae range in diameter from few hundreds of nanometers up to few microns, and can be circular, polygonal or elongate. In addition, they are internally or externally occluded by cribra, which are thin, silica laminas pierced by pores ranging in diameter few nanometers. We have use these frustules as optical components and optical transducers for biomolecular interactions.

Digital Holography

Today, Digital Holography, in which an holographic interference pattern is digitally sampled by a CCD camera and the image numerically reconstructed by applying results from diffraction theory, offers a number of significant advantages, such as the ability to acquire images rapidly, the availability of both amplitude and the phase information, and the versatility of the processing techniques that can be applied to the complex field data acquired. In addition, advances in digital imaging devices, such as the CCD and CMOS cameras, and in computational and data storage capacities, have been central to realize real-time processing applications. Starting from these considerations, we have used microscope objectives in various interferometers to obtain high-fidelity and resolution images of biological specimens. In particular, the phase information, that is directly available by Digital Holography, has been used to obtain a direct observation and an accurate quantitative interpretation of morphological changes in transparent microscopic biological specimens. In particular, we have employed Digital Holography to obtain quantitative analysis of bovine sperm. The estimated information, about the dimensional and morphological parameters of animal sperm, and the user-friendly format of the results prospect the Digital Holography as an adequate technique for prognostic fertilization based on morphological analysis, in zootechnical industry.

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PLASMONIC AND MAGNETO-PLASMONIC NANOSTRUCTURED MATERIALS FOR SENSORS AND BIOSENSORS APPLICATION

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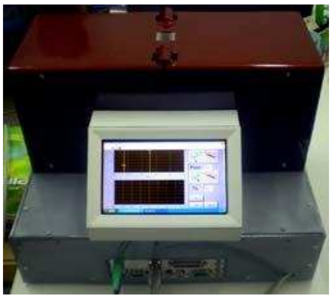
Abstract

Surface plasmon resonance (SPR) and localised SPR (LSPR) are the leading optical methods that provide easy, highly reproducible and sensitive assays for gas an bio-sensing. They relies on the changes in refractive index that occur when a target analyte binds to the metal film or nanoparticles. The advantage of these sensors is that they provide real-time information of chemical and bio-chemical interactions occurring at the interface between a thin gold film and a dielectric interface, without the need for labelling of reagents [1]. The sensitivity and limits of detection of SPR sensors can show variations depending on the method used to excite the surface plasmon (prism coupling, grating coupling, optical fibers etc.). Even though the technology has allowed to reach quite smart results in terms of sensitivity and limits of detection, the research in this field is still oriented in the improvement of these sensing features.

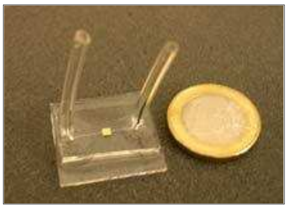
Recently, it has been proposed in the literature a novel Magneto-Optic Surface Plasmon Resonance (MOSPR) sensor [2] which sensor performances can be greatly enhanced with respect to traditional SPR sensors (an improvement by a factor of 3 in the limit of detection is demonstrated in Ref. 2). The novel device is based on the combination of the magneto-optic (MO) effects of the magnetic materials and the surface plasmon resonance. This combination can be achieved by introducing into the sensing transducer layer a magnetic thin film whose magnetization state change direction alternately during the surface plasmon excitation. By this way, a great enhancement of the magneto-optic effects in the p-polarized light is produced when the resonant condition is satisfied [3]. Such enhancement is strongly localized at the surface plasmon resonance and strongly depends on the refractive index of the dielectric medium, allowing its use for optical sensing. Since this MOSPR sensor seems very promising, we have undertaken the analysis of this configuration both for biosensing purposes (as in the mentioned ref. [2]) and for gas sensing (for the first time in a MOSPR sensor). The experimental set-up of the MOSPR is very similar to that for standard SPR sensors, the only difference is the realization of a transducing sensing layer constituted of a magnetic Co layer, which in our case is introduced in a multilayer Au/Co/Au deposited onto glass substrates. Moreover, a magnetic actuator is used to control the magnetization state of the magnetic layer in the transversal configuration, and the relative variations of the reflectivity are detected. Since the MO effects are very localized, a very sharp curve can be obtained; as a consequence, small variations of the refractive index will induce large changes in the MO response, allowing to greatly improve the sensitivity of the MOSPR sensor.

In this work, some preliminary results for both the gas sensing and biosensing schemes are shown. As concerns gas sensing, TiO_2 nanocrystals prepared by chemical route has been deposited in thin

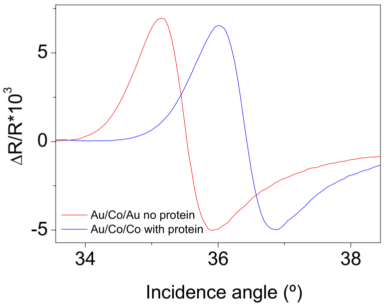
film form by spin coating onto the gold surface in contact with the medium to be monitored and its interaction with Volatile Organic Compounds has been monitored both in a standard SPR and in MOSPR configurations in order to compare their sensing performances. For biosensing applications, the response of the MOSPR device for adsorption of Bovine Serum Albumin proteins onto the gold surface and its interaction with its specific antibody has been testes. In both cases a smart enhancement in sensitivity of the MOSPR sensor with respect to the SPR sensor can be evidenced.



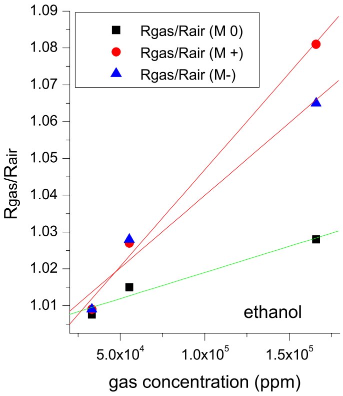
SPR device realised at IMM Institute for liquid and gas measurements in the presence of magnetic field.



Biochip for SPR gas and liquid measurements



MOSPR signals relative to the Au/Co/Au multilayer before and after BSA protein immobilization



SPR Calibration curves obtained with and without magnetic field

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