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Application of holographic sub-wavelength diffraction gratings for monitoring of kinetics of bioprocesses

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ABSTRACT

In this work we present a refractive index (RI) sensor based on a sub-wavelength holographic diffraction grating. The sensor chip was fabricated by dry etching of the finely spaced (d = 428 nm) diffraction grating in SiO_x doped diamond like carbon (DLC) film. It is shown that employing a fabricated sensor chip, and using the proposed method of analysis of data, one can inspect kinetics of processes in liquids occurring in the vicinity of the grating surface. The method is based on the spectral composition analysis of polarized polychromatic light reflected from the sub-wavelength diffraction grating. The RI measurement system was tested with different model liquid analytes including 25 wt.%, 50 wt.% sugar water solutions, 10 °C, 50 °C distilled water, also Gram-positive bacteria *Bacillus subtilis* interaction with ion-permeable channels forming antibiotic gramicidin D and a murolytic enzyme lysozyme. Analysis of the data set of specular reflection spectra enabled us to follow the kinetics of the RI changes in the analyte with millisecond resolution. Detectable changes in the effective RI were not worse than $\Delta n = 10^{-4}$.

1. Introduction

In recent years, optical biosensors based on a waveguide technology have attracted increasing attention and demonstrated the ability for use in medium and high throughput applications. Some of them took the step into commercialization and have proved of value for industrial applications [1]. Several techniques that were successfully commercialized have been introduced: dual polarization interferometry; wavelength-interrogated optical sensor; colorimetric resonant grating reflection; resonant waveguide grating biosensor; waveguide evanescent fluorescent excitation; optical waveguide grating coupler sensor [1]. The mentioned techniques exploit the evanescent field interaction with the analyte that is excited by the prism, the grating or the waveguide. These techniques can be attributed to the class of optical biosensors in which the reflectance of a surface is altered by the presence of a bound analyte [2], i.e. the evanescent field is used to probe the optical properties of the solution in the vicinity of the surface.

Some applications of such optical biosensors could be mentioned: biomolecular interaction studies; living cells – kinetics of adhesion, growth and spreading; membrane protein-lipid bilayer interactions; protein-DNA/RNA interactions, nucleic acids, genosensors and many more [3]. According to [2] such biosensors show the most promising future, because they can be miniaturized and applied as ultralow cost sensors for medicine and environmental monitoring. On the contrary, for the most applications the transducer surface has to be modified to provide selectivity for particular analytes onto the sensor [3]. In this way, information can be obtained without a complete description of the adsorbed layer.

In our recent work [4,5] we have demonstrated that by exciting total internal reflection (TIR) in SiO_x containing diamond like carbon (DLC) coated photoresist diffraction grating (DG) one can obtain anomalies (dips) in the specular reflection, transmission or waveguided light spectrum. The presence of the anomalies corresponds to the evanescent wavelengths travelling on the grating surface, while the positions of the anomalies are sensitive to the refractive index (RI) of the analyte that is in contact with the diffraction grating. Moreover, the wavelength describing the position of the anomaly in the spectrum is sensitive to the angle of incidence. Therefore, the necessary wavelength, where the highest sensitivity or lowest absorption in the analyte is registered, can easily be tuned by simply tilting the DG [4,5]. When there is more than one anomaly present, the RI at two different wavelengths can be determined by a single measurement. Employment of the broadband illumination source, having precise control of the incident angles and the ability to utilize different measurement geometries provides extensive flexibility for different applications.

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In the present research we propose to use the above discussed TIR in the DG based method for monitoring of the real time changes in the RI of different liquid analytes. The employed sensor chip was dry-etched in DLC film to withstand the experiment conditions. The model liquid analytes with a variable bulk concentration and different temperatures were chosen to illustrate potential of the method. Moreover, the reaction in a bacterial suspension was investigated employing the described method.

2. Materials and methods

2.1. Fabrication of the sensor chip

The SiO_x doped DLC films were deposited at room temperature by a 1000 eV energy ion beam using a closed drift direct current ion source from a mixture of the hexamethyldisiloxane vapor with hydrogen as a feed gas. The DLC films were deposited on chemically cleaned fused silica (FS) substrates and in parallel on crystalline silicon substrates for the optical characterization of the film. The thickness (210–250 nm) and the RI of the DLC film (1.82–1.88 at λ = 632.8 nm) were measured by a laser ellipsometer Gaertner L115. From the transmittance and reflectance spectra of the DLC film on FS substrate it was calculated that the film is transparent up to 3 eV (>413.3 nm). The same transmission properties were obtained previously by us for twice thinner film [5]. Wettability of the synthesized DLC film and of the DG fabricated in the DLC film was evaluated by measuring a surface contact angle with water. More experimental details can be found in [6].

The sinusoidal profile mask was formed in a positive tone maP-1205 photoresist (Micro resist technology GmbH) spincoated on the DLC coated FS substrate employing laser interference lithography. The laser beam from a HeCd laser (λ = 441.6 nm) was expanded and collimated employing a micro objective aperture and a lens. The collimated beam was divided into two beams employing a beam splitter. Using two mirrors, the beams were directed and intersected on the photoresist coated substrate fixed in a sample holder. The optical setup and more experimental details can be found in [7]. The fabricated photoresist pattern was used as an etching mask after exposing to the interference pattern and developing in a MF-26A developer. Residuals of the resist in the grooves were removed by bombarding them with 300 eV energy oxygen ion beam (processing duration 3 min) in a multi-cell closed drift Hall-current ion source beam etcher. The following plasma etching of SiO_x doped DLC was performed at room temperature in a parallel electrode unit using CF_4/O_2 (80%/20%) gas mixture RF plasma (process pressure 60–62 Pa, plasma power density 0.75 W/cm^2, etching duration 3 min). After the etching, the photoresist mask was removed in a photoresist remover mr-Rem 660.

The surface and linear dimensions of the DG etched in DLC were investigated with a FEI Quanta 200 FEG scanning electron microscope (SEM) working in a low vacuum mode. The SEM images were taken for tilted sample (20°) seeking an enhanced spatial impression. The period of the produced DG $d = 427.76 \pm 0.83$ nm was also estimated by optical means measuring the angles of diffraction with a laser diode (λ = 405 nm) [8].

2.2. Measurement setup

The reflected light from the DG was analysed in a simple optical setup consisting of a white light source (halogen lamp), collimating optics (three FS lenses) and a Glan-Taylor polarizing prism (Fig. 1). TM polarization was used in the experiments. A fluid cell together with the sensor chip was attached to a goniometric stage (resolution 1′). The temperature of the analyte was controlled via the thermoelectric (TEC) Peltier element glued instead of the back side

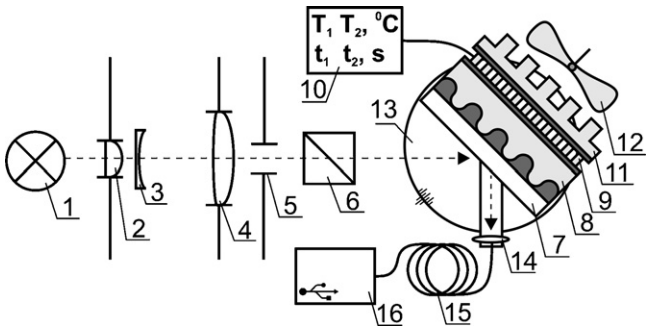

Fig. 1. Principle scheme of the measurement setup: 1 light source (halogen lamp); 2, 3, 4 FS collimating lenses; 5 aperture; 6 Glan-Taylor polarizer; 7 sensor chip; 8 analyte with a temperature control sensor; 9 TEC; 10 TEC controller; 11 heat sink; 12 fan; 13 goniometric stage; 14 lens coupling light to the spectrometer fiber (15); 16 spectrometer connected to a personal computer via USB connection.

of the fluid cell. The heat sink was attached on the opposite side of the TEC element. To avoid mechanical vibration, a fan was affixed separately from the optical system. Depending on the chosen heating/cooling mode the microprocessor operated TEC controller was keeping the temperature constant or cyclically changed the temperature between two preset values keeping it for the desired time intervals. The TEC controller was controlled according to a temperature sensor Pt100 present in the vicinity of the TEC, while the absolute temperature in the cell was calibrated by monitoring resistance of a negative temperature coefficient thermistor. More technical details about the used TEC system can be found elsewhere [9]. A quartz lens detecting the reflection spectra was fixed on a platform that can be rotated independently around the same axis as the sensor chip with a fluid cell. The light was coupled to the optical fiber and the data was collected with the AvaSpec-2048 spectrometer (Avantes, spectral range: 360–860 nm, resolution 1.2 nm). The driver interface package with automated spectra acquisition program, enabling automatic saving of each spectrum in controlled time intervals (≥16.5 ms/spectra) as separate text file, was applied.

2.3. Data analysis

The measured spectra were normalized to a lamp spectrum. The spectra were collected at a constant angle of incidence ($\alpha = 14^\circ$). According to the shape of the reflection spectrum ($R(\lambda)$), as described in [10], the critical wavelength positions (λ_{cr}) at $dR(\lambda)/d\lambda = 0$ (or $R_{max}(\lambda_{cr})$) were selected and used for the calculations of effective RI of the analyte:

$$n = \frac{m_{cr}\lambda_{cr}}{d} - \sin\alpha_{cr} \quad (1)$$

where n – effective RI, m_{cr} is order of diffraction (e.g. ±1) obeying the threshold situation where the light undergoes TIR, λ_{cr} – critical wavelength, d – period of the DG, α_{cr} – angle of incidence [10].

The obtained set of spectra were analyzed with the developed automated spectra analysis algorithm retrieving λ_{cr} values automatically. To enhance the signal, the first derivative of the reflection coefficient ($dR(\lambda)/d\lambda$) was calculated employing the Savitzky–Golay smoothing procedure, whereby the data was fitted by a second order polynomial expansion and the derivative was obtained by using a 51 point filter [11].

2.4. Analytes

Distilled water of varying temperature and sugar-water (SW) solutions of different concentrations were chosen as model analyte materials. One cycle of cooling and heating of the distilled water included exposure of the analyte at two target temperatures: