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Molecular Layer Detection on a Diffractive Optical Balance

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Abstract

Diffraction-based molecular detection is achieved by etching optical gratings into thermal oxide on silicon. The gratings perform as a stable common-path diffractive optical balance (DOB) designed to operate around a missing diffraction order. The biosensor is operated in an off-null condition with a phase bias to produce a high-contrast responsivity that is linear in accumulated molecules, but with a low background. The DOB linear responsivity is a factor of 20 larger than the reflectometric responsivity of planar thermal oxide.

Label-free interferometric biosensors have the potential for highly-multiplexed assay applications because of the absence of secondary labels ^{1, 2}. To achieve high signal-to-noise performance, interferometric optical biosensors should exhibit large linear responsivity to accumulating molecules with low background and high stability ³. Diffraction-based biosensors have intrinsically low backgrounds because the off-angle detection of diffraction orders performs as a spatial filter that eliminates the zero-order reflectance. Fourier-domain detection also has a well-known multiplex advantage for signal-to-noise performance ⁴ based on point detection of global properties. An example of a diffraction-based biosensors includes UV photo-inactivation to produce periodic stripes of antigen or antibody to detect choriogonadotropin in serum ⁵. Increasing the diffraction signal was achieved by labeling the target molecules with gold particles ⁷ or by using horseradish peroxidase ⁸ based on absorption gratings to push detection limits below 1 ng/mL. Absorptive diffraction-based biosensors have also been fabricated out of pH-sensitive gels that change absorption (color) dependent on the pH of a test sample ⁹, but these enhanced sensitivity approaches are not label free. Micromechanical (MEMS) diffraction biosensors that are label free have been constructed using optical diffraction from interdigitated cantilevers ^{10, 11}. However, the fabrication of cantilevers is complicated, and the height offsets may not be uniform.

In this paper, we describe a diffraction-based biosensor that has high stability, near-null background and hence high contrast, linear sensitivity and high signal-to-noise ratio. It is based on the principle of a missing diffraction order commonly encountered in diffraction physics that occurs when there is a balanced destructive interference of partial diffracted waves. Thermal oxide gratings on silicon provide a convenient material to realize the missing-order approach. The resulting performance is similar to near anti-reflection reflectometric biosensors ¹²⁻¹⁵, but with the added advantage of the off-angle spatial filtering that eliminates spurious reflectances from the optical system. Linear responsivity is

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achieved while retaining low background by operating in a near-null condition which is slightly shifted from complete destructive interference conditions. The performance of the diffraction optical balance is compared with an in-line reflectometric BioCD.

Label-free biosensors with quadratic responsivity (i.e. null-condition) have very low sensitivity to layers of small molecules or to sub monolayers of even large molecules³ because of the small value of $(d/\lambda)^2$ when $d = \lambda$. In the case of printed gratings of bilayers on planar surfaces, there is no direct way to increase this sensitivity other than by adding large light-scattering labels such as beads or nanoparticles⁷. In the case of the DOB, on the other hand, it is straightforward to design the device parameters to provide a bias for the first diffraction order. The bias is introduced by slightly mis-balancing the grating so that there is a non-zero initial first-order diffraction field. This locally-generated bias acts as a local oscillator that establishes a heterodyne interference condition with linear dependence on d/λ . Because the bias is generated locally, it is also a common-path configuration¹⁶ and hence is stable against mechanical vibrations. The drawback of having a bias is the introduction of a background signal that limits the signal-to-background ratio of this diffraction-based biosensor. However, a DOB can be designed and fabricated for large signal-to-background ratios, while retaining linear dependence (and hence high sensitivity) on the bilayer thickness.

The diffractive optical balance is fabricated by etching parallel periodic grooves on a surface (125 grooves/mm on a thermal-oxide silicon wafer with 65 nm deep grooves). Two features are essential to realize the near-null optical balance. First, the reflection coefficients r_1 and r_2 must be nearly equal on the grating ridges and grooves to produce the initial pseudo-zero first-order diffraction that depends on the relation $I = \eta|r_1 - r_2|^2$, where η is the diffraction coefficient. The values for r_1 and r_2 are measured at a common reference level, which is the interface between the top of the ridge and the ambient medium. Second, the ridges and grooves must have a proper height difference h (the depth of grooves) that optimizes the diffraction change after applying the bilayer.

For a DOB grating, as shown in Fig. 1a, the initial reflection coefficients on the ridges and the grooves of the grating are r_1 and r_2 , respectively. Both are referenced to the top surface of the ridge. The reflection coefficient from the grooves (referenced to the groove floor) is r_3 . The relationship between r_3 and r_2 is $r_3 = r_2 e^{i\alpha}$, where $\alpha = 4\pi n_0 h \cos \theta_0 / \lambda$ is the phase delay contributed by the height difference h between the ridge and groove surfaces. After adding a molecular layer, the coefficients r_1 and r_2 change to r_1' and r_2' , where the reference surface remains the initial ridge surface. Because the molecular layer thickness satisfies $d = \lambda$, the modified reflection coefficients are¹⁷

$$\begin{aligned} r_1' &= r_1 + (r_1 + r_1)^2 i\delta \\ r_2' &= r_2 + e^{-i\alpha} (1 + r_2 e^{i\alpha})^2 i\delta \end{aligned} \quad (1)$$

where $\delta = \frac{(n_0^2 - n_p^2)}{n_0 \cos \theta_0} \frac{\pi}{\lambda} d$, d is the molecular thin film thickness, n_p is its refractive index, n_0 is the refractive index of the ambient medium, λ is the probe light wavelength, and θ_0 is the incidence angle. All reference surfaces remain at the initial ridge surface. For $\epsilon = r_1 - r_2$, the

initial first-order diffraction intensity is $I = \eta|\epsilon|^2$. After the molecular layer deposition, the diffraction intensity becomes

$$I' = |r'_1 - r'_2|^2 = I + \eta|B(r_1, h)|^2 \delta^2 + 2\eta \text{Im}[B(r_1, h)^* \epsilon] \delta \quad (2)$$

where $B(r_1, h) = 1 - e^{-i\alpha} + r_1^2(1 - e^{i\alpha})$.

At perfect balance, $\epsilon = r_1 - r_2 = 0$, and Eq. 2 is reduced to $I' = \eta|B(r_1, h)|^2 \delta^2$, which has the conventional quadratic dependence of the diffracted intensity on the thickness of the molecular layer that is characteristic of a missing diffraction order. For a DOB in air $n_0 = 1$ at normal incidence, $\alpha = 4\pi h/\lambda$ and $\delta = (1 - n_p^2)\pi d/\lambda$, the diffraction induced by a biolayer achieves a maximum when $h = \lambda/4$ and $r_1 = \pm 1$. For $r_1 = r_2 = 1$ (both referenced to the top surface of the ridge), then $r_3 = -1$ due to the half-wave delay caused by $h = \lambda/4$ in reflection. A molecular layer on the ridge surface induces a maximum phase change for the anti-nodal condition on r_1 , while a molecular layer on the groove floor induces a minimum change because of the nodal field condition on r_3 . The molecular dipoles in the groove are not excited by the field, and the molecules in the groove are essentially invisible³. Therefore, for the perfectly balanced DOB, the molecular phase on the ridge (anti-nodal surface) tips the relative phases of the quarter-wave grating and causes the emergence of the missing order.

Quadratic responsivity of a biosensor to immobilized molecules is not favorable to high sensitivity detection. Therefore, linear responsivity is achieved by introducing a phase bias on the grating. The bias is caused by the imbalance of Eq. (2) when ϵ is non-zero. For $|\delta| = |\epsilon| = 1$ the diffracted intensity is dominated by the linear dependence on the biolayer thickness through the third term in Eq. (2), which is the heterodyne term. As ϵ goes to zero, this linear term vanishes and the quadratic response is recovered. The design challenge for a DOB biosensor is to set a finite ϵ that produces sufficiently low background, but with sufficient sensitivity to submonolayer biolayer coverage.

A DOB surface based on thermal oxide on silicon requires only a single step of SiO₂ etching. Grooves with 8 μm periodicity and 65 nm height were etched on a SiO₂/Si chip with 20 $\times$ 20 mm² area on a 200 nm SiO₂ thermal oxide on silicon (shown in Fig. 1a) using photolithography. The first-order diffraction angle is 3.5° at normal incidence of a 488 nm probe laser. The theoretical biosensor linear responsivity is plotted in Fig. 1c as a function of the ridge and groove thermal oxide thicknesses d_1 and d_2 . The linear sensitivity vanishes for the perfect balance condition, but near the fabricated SiO₂ thicknesses of 205/140 nm for the ridges and grooves, respectively, demonstrated in this paper, the theoretical protein response is 40% for 1 nm protein.

A laser scanning BioCD system² was designed and constructed to perform protein array imaging of the DOB surface, shown in Fig. 2. The incident angle is tilted from the surface-normal direction so that the first-order diffraction beam from the DOB chip is diffracted counter-propagating to the path of the incidence beam. The tilted angle is 1.75° which is half of the first-order diffraction angle, and the laser divergence angle was small (1.26°) allowing

the diffraction to be separated from the reflected beam. In this configuration, the interferometry is common path and stable, and specular reflections are eliminated. A pinhole is placed after the objective lens ($f = 100$ mm) to block the other diffraction orders. The DOB chip was attached to a BioCD placed on a motor (Lincoln Laser, Inc.) spinning at 20 Hz on a linear translation stage (MM2000, Newport Corp). The motor and the linear stage form a polar coordinate system to perform 2D mapping with appropriate computer control. An avalanche photo detector (C5460, Hamamatsu Corp) with photo-sensitivity 1.5×10^6 V/W was used for light detection for a typical power of the first-order diffraction in our experiments in the range of 0.1~1 μ W for a 488 nm probe laser power around 10 mW.

We first tested protein immobilization and imaging on the DOB surface. For protein immobilization we used physical adsorption based on silanization of the silica surface through treatment with chlorodimethyl-octadecylsilane ($\text{CH}_3(\text{CH}_2)_{17}\text{Si}(\text{CH}_3)_2\text{Cl}$) that binds with silanol groups on the silica surface¹⁸. The CH_3 end group of the silanes are hydrophobic and bind with protein through hydrophobic interaction, which is a relatively simple means of immobilizing proteins on the disc surface^{19, 20}. The DOB chips were soaked in 0.02 M chloro-octadecylsilane in toluene for 12 hours and rinsed with toluene, acetone, methanol and deionized water and dried with dry nitrogen gas. Rabbit IgG (I5006, Sigma Inc.) at a concentration of 100 $\mu\text{g/ml}$ in phosphate buffered saline (PBS, $\text{PH} = 7.4$) was spotted on the chip surface with a pipette in a volume of 0.5 μL . After incubation for 15 minutes, the protein spot was washed by PBST (PBS+0.05% Tween), deionized water and dried with dry nitrogen gas.

By detecting the diffraction from the DOB surface and rejecting the specular reflection, the data are automatically demodulated from the carrier frequency (the grating) through spatial filtering. The results are shown in Fig. 3b showing that the protein was successfully immobilized, and the image has been demodulated with no residual carrier frequency caused by the 8 μm periodicity of the grating when probed by a focal spot with a 15 μm radius. Because a SiO_2 surface is a general surface for gene and protein arrays, the DOB grating based on a silicon wafer is compatible with many surface chemistries for biomaterial immobilization.

To calibrate the DOB surface response, we fabricated 1 nm high SiO_2 spots (which have a refractive index similar to protein) on a DOB surface and on a conventional in-line BioCD surface, which is a 120 nm SiO_2/Si wafer. The process etched the background SiO_2 by 1 nm except in the spot regions. The spots on the DOB surface and on the in-line BioCD were processed under the same conditions, and the spot thicknesses were identical. The imaging results are shown in Fig. 3c and d. The intensity increment due to 1 nm SiO_2 is 50% on the DOB while only 2.5% on a reflectometric BioCD¹⁶ which is an improvement in linear responsivity of a factor of 20. The signal-to-noise ratio (SNR) is the signal increment on the spots divided by the standard deviation of the background. The SNR is equal to 26 for the DOB and is equal to 6 for the reflectometric BioCD. Therefore, the SNR is improved by a factor of 4, with further improvements possible through appropriate design and fabrication.

In conclusion we have presented the concept of a DOB surface, fabricated a prototype of the DOB based on a SiO_2/Si wafer, and constructed a common-path laser scanning system to

image immobilized protein using the diffraction signal. We calibrated the responsivity with SiO₂ spots for precise thickness control. A 1 nm SiO₂ layer induces a 50% signal increment, which is 20 times larger than a reflectometric in-line BioCD. The combined off-null and off-angle operation of the DOB give it low background and high contrast while simultaneously eliminating specular reflections. It is compatible with high-speed laser scanning and imaging in which the DOB chips are attached to a spinning BioCD, with the potential for high multiplexing bioassay applications in the future.

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Fig. 1.

DOB grating fabricated on thermal oxide on silicon. a) The SiO_2 thicknesses on the ridges and the grooves are 205 nm and 140 nm, respectively. A pseudo-zero 1st-order diffraction is achieved when the grating is illuminated by normal-incidence 488 nm wavelength light. b) A protein layer immobilized on both the ridges and the grooves increases the diffraction. The depth of the grooves is an essential parameter for a strong induced diffraction. c) The map of linear responsivity per 1 nm protein as a function of ridge and groove thermal oxide thicknesses.

Fig. 2.

Common-path laser scanning BioCD system based on first-order diffraction from the DOB chip. The laser is not incident perpendicular to the disc, but is tilted by half the diffraction angle. This permits the -1st diffraction order to counter-propagate through the pinhole that serves as a spatial filter to demodulate the periodic reflectivity of the grating.

Fig. 3.

a) An optical micrograph of the gratings produced by photolithography. b) An IgG antibody spot immobilized on the grating was scanned and automatically demodulated from the carrier frequency in the image. c) and d) Spot arrays created on the DOB grating and on a BioCD by a second round of photolithography with precise 1 nm SiO_2 thickness to simulate protein spots and calibrate the DOB sensitivity. The linear responsivity is 50% per nm on the DOB while 2.5% per nm on a reflectometric BioCD.