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A Fresnel zone plate biosensor for signal amplification with enhanced signal-to-noise ratio†

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We describe a signal amplified biosensor based on self-assembled optical diffraction grating of a Fresnel zone plate structure. Diffracted light rays passed through gratings are interfered constructively at the focal point, resulting in the enhanced signal amplification without sacrificing signal-to-noise ratio (SNR).

There has been an increasing need for development of biosensors that can detect clinically important biomolecules in a rapid and label-free way, for diagnosis of disease[1] and drug discovery.[2] In particular, biosensors based on optical diffraction can provide simple, fast, and inexpensive platforms to detect biomolecules of interest.[3] In this context, such optical diffraction biosensors, for the detection of biomarkers of cancer,[4–6] bacteria,[7] and glucose,[8] have been reported. Basically, a diffraction-based biosensor simply detects the target molecules by diffraction gratings formed by modulated refractive index (RI) when the target molecules only bind to the periodically patterned capture molecules. Since the diffraction intensity depends on the difference in RI, most approaches have been explored to amplify the signals by increasing effective heights of microfabricated gratings. For example, Loo *et al.* selectively increased heights of gratings by enzymatically precipitating the patterned substrate.[9] In another way, Bailey *et al.* reported that Au nanoparticles (NP) conjugated to target DNA molecules can also modulate RI, accompanying multicolor diffraction gratings by surface plasmon resonance upon hybridization of AuNP conjugated DNA to capture the probe.[10] Similar to this, despite the disadvantage of an extra immobilization step to prepare the analyte, Savran *et al.* improved the detection sensitivity by conjugating target cancer markers to microbeads[5,11] and further, by increasing the number of microbeads with the aid of rolling circle amplification method,[6] all of which were selectively self-assembled onto capture molecule-immobilized periodic patterns. However, the approach of designing grating patterns has rarely progressed for high performance of biosensors.

Compared with the aforementioned works, in this study, we introduced a Fresnel zone plate (FZP) structure in order to amplify the signal intensity without sacrificing the SNR as schematically shown in Fig. 1. The FZP structure consists of a set of symmetric rings, radially alternating between opaque and transparent zones. The light passed through the FZP diffracts near the opaque zones and constructively interferes at the designed focal point. In contrast to conventional one-dimensional patterns of diffraction gratings, a properly designed two-dimensional pattern of FZP gratings not only diffracts the signal light but also enables the diffracted light to converge constructively at a single point. It is different from the study wherein the diffraction signal is focused by an extra optical lens because the intensity of noise is also increased.[12] Therefore the signal as well as SNR of optical diffraction biosensors can be improved by lens-embedded structural gratings.

In this study, we describe the fabrication of a FZP-biosensor by using a model of streptavidin coated microbeads (SA-MBs) and biotin. The theoretical analysis by optical simulation of

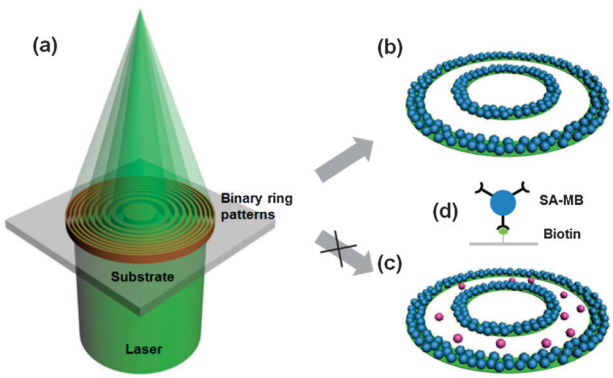

Fig. 1 (a) Schematic illustration of the signal amplified optical biosensor based on the Fresnel zone plate. (b) FZP pattern of microbeads by incubating streptavidin-coated microbeads on the biotin pattern. (c) Deteriorated diffraction pattern by non-specific binding of microbeads denoted by purple color. (d) Cross-sectional illustration of microbeads pattern.

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wave propagation was also conducted on the experimental results of the focal length and the SNR of the FZP-biosensor.

The binary-type FZP was designed to have a focal length of 60.2 mm at a wavelength of 532 nm; the radius and width of the outermost zone were 889.6 μm and 18 μm, respectively ($f = 2R_N\Delta R_N/\lambda$, where f, R_N, ΔR_N, N, and λ are the focal length, radius and width of the N-th zone, number of circles, and wavelength, respectively, $N = 25$). A photomask was prepared by printing the set of black and white circles on the transparent plastic film. With this FZP pattern, a theoretical SNR of over 100 was calculated by simulation of wave propagation with home-made MATLAB® code. This SNR value is almost 10 times higher than the highest ideal value, theoretically obtained from conventional one-dimensional patterns of diffraction gratings.[13] In this study, we defined SNR as a ratio of light intensity at the focal point to the averaged light intensity of background noise. A more detailed procedure of theoretical simulation is also explained in Fig. S1 (ESI†). In order to examine the performance of the FZP-biosensor, we prepared FZP patterns of microbeads through specific interaction between SA-MBs and biotin molecules as schematically presented in Fig. 2. Firstly, an FZP pattern of biotin was fabricated by typical photolithography as follows: a piranha-etched glass substrate was dipped in the (3-aminopropyl) trimethoxysilane solution (Aldrich Co., 2 wt% in anhydrous toluene) in order to introduce amine groups on the surface of the substrate. Then, the negative tone of photoresist solution (DNR-L300, Dongjin Semichem) was spin coated on the substrate, followed by a baking process. After exposure to UV light (365 nm) with the aforementioned photomask, the substrate was baked again and then, chemically developed to give an FZP pattern of PR. The PR pattern was successfully confirmed by examination with optical microscope as shown in Fig. S2 (ESI†). By dipping the substrate into biotin solution (NHS–PEG_4–biotin from Pierce Biotechnology, 0.2 wt% in 0.01 M PBS buffer solution) for 20 min at room temperature, biotin was covalently bonded to the exposed amine groups between PR patterns *via* amide bond, resulting in an FZP pattern of biotin.

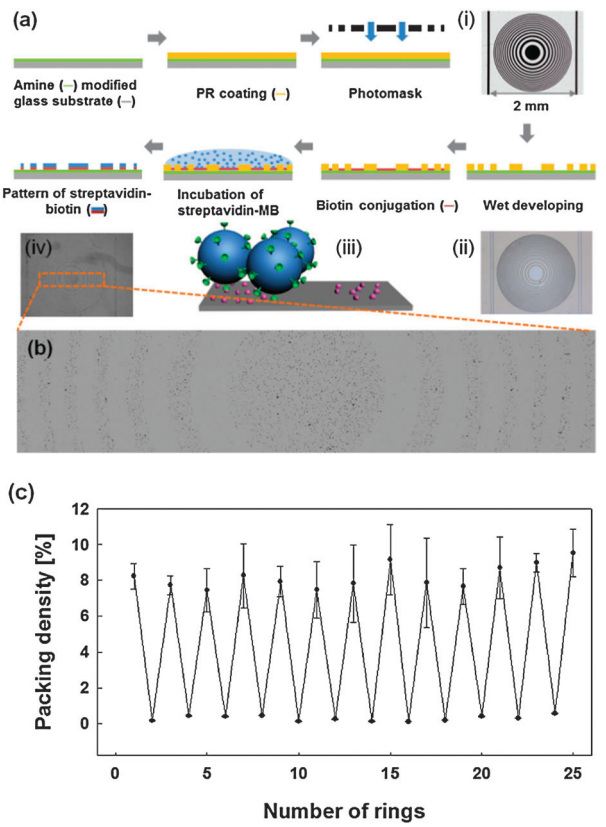

Fig. 2 (a) Fabrication process of the FZP biosensor and optical microscopic images of (i) photomask, (ii) PR pattern, (iv) SA-MB pattern. (iii) Schematic illustration of incubation process between surface patterned biotin and SA-MB (pink dot: biotin, blue sphere: MB, green rod: streptavidin). (b) Magnified optical image of the FZP pattern of SA-MBs. (c) Packing density of attached microbeads across the rings.

The SA-MBs (Spherotech Inc., diameter of 0.7–0.9 μm, 5×10^{10} beads per ml) were incubated on the prepared FZP pattern of biotin for 10 min at room temperature by dropping 10 μl of SA-MB solution on the biotin pattern. At this stage, biotin molecules were only introduced onto the odd rings of FZP whereas the even rings were covered with PR. This aided the prevention of non-specific binding of streptavidin to the substrate as shown in Fig. 1c, which is beneficial to elaborate diffraction gratings. The residual PR and unbounded SA-MBs were removed by washing the substrate with ethanol and deionized water thoroughly for three times and dried gently with nitrogen gas. The FZP pattern of microbeads was successfully confirmed by optical microscopy as shown in Fig. 2b. The entire image is also presented in Fig. S3 (ESI†). To calculate the amount of SA-MBs specifically binding to biotin, we analyzed the packing density of microbeads with 9 samples through microscopic image analysis by home-made MATLAB® code as shown in Fig. 2c. Briefly, the gray level of the microscopic image was converted to a binary type black and white image with a proper threshold, which is shown in Fig. S4 (ESI†). The packing density was evaluated by dividing the number of black pixels by the total number of pixels in the specific circles. As shown in Fig. 2c, the average packing densities of odd and even rings were 8.2% and 0.3%, respectively. It is obvious that the undesired binding of SA-MBs on the even rings was prevented successfully as expected, which is critical for diffraction efficiency as well as SNR.

Prior to measuring the focal length, we conducted semi-theoretical simulation of wave propagation to conjecture the focal length of the FZP pattern of PR, as described in Fig. S5 (ESI†). Fig. 3 shows the highly converged light at a distance of 60.3 mm above the surface of the pattern, which is slightly different from the designed value (60.2 mm) due to deteriorated fidelity of pattern transfer in the photolithography process. It is also interesting that the signal light is interfered strongly at a shorter focal length, $f/3$, which can be implemented for the compact device. The detailed result of simulation is also found in ESI.†

Then, we observed the real wave propagation of the FZP pattern of SA-MBs near the expected focal length by capturing the image plane by CCD with a home-made system as illustrated in Fig. 4a. Briefly, we first located the objective lens to have a clear view of the FZP pattern on the substrate. Then the objective lens was precisely lifted up to the pre-calculated focal point, 60.3 mm. The objective lens was shifted down again from the focal point to 5 mm and the image was

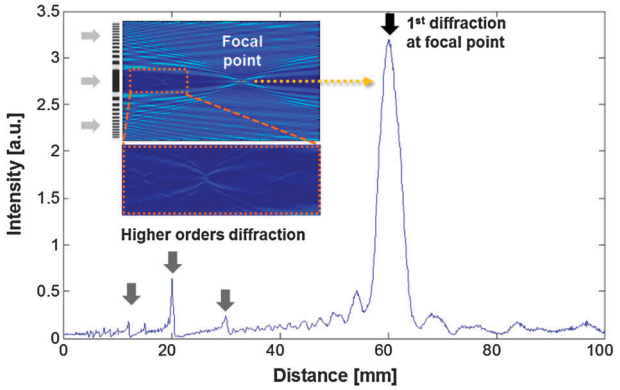

Fig. 3 Semi-theoretically simulated wave propagation of the fabricated PR pattern, as shown in the inset image. The light passed through the FZP pattern constructively interferes at a focal point (60.3 mm) above the substrate surface. Other focal points are also expected at *f*/2, *f*/3, *f*/5 originated from higher order diffractions.

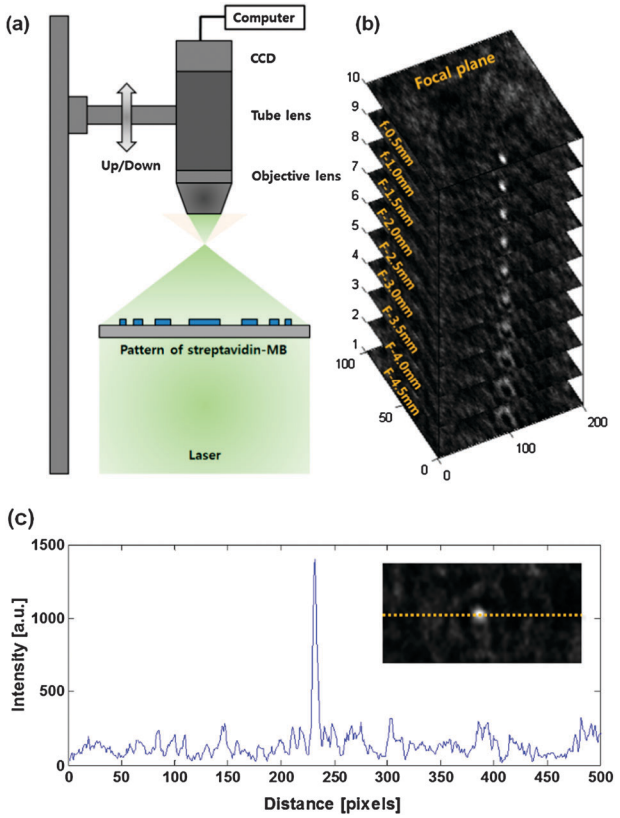

Fig. 4 (a) Experimental apparatus for measuring the wave propagation of light passed through the FZP pattern. (b) Measured light propagation map along the vertical direction, which shows concentrated signals at the focal length. (c) Profile of light intensity at the focal plane across the horizontal direction denoted as a yellow dash line (scale: 0.9374 μm per pixel).

captured as the lens was moved up at an interval distance of 20 μm for 10 mm. As shown in Fig. 4b, it is obvious that the light passing through the substrate was converged at the expected focal length by diffraction on the FZP pattern of microbeads. The whole set of optical images and its intensity profile is also provided in Fig. S6 (ESI†). Additionally, we evaluated the SNR of the 1st diffraction order signal with the optical image at the focal plane. It showed that the averaged SNR from 5 samples was 10.4 (SD: 0.58), which is as high as the ideal value, theoretically obtained for the conventional diffraction biosensor (Fig. 4c). This inspired result is explained by the immensely concentrated signal light at a single point without an increase in the background noise whereas using an extra optical lens concentrates the signal as well as noise level. In our study, the light rays except diffracted ones are scattered and therefore, signal can be amplified without sacrificing SNR. Considering the low packing density of 8.2%, it is noteworthy that relatively low concentration of analytes also can be detected with our FZP biosensor.

We described the design, fabrication, and performance of an optical diffraction biosensor based on a Fresnel zone plate structure. This biosensor improved the signal amplification of the optical diffraction biosensor with an enhanced SNR of 10 by concentrating the signals at a single point. The wave propagation simulations also provided the design rule of the FZP and analysis of the focal length as well as SNR. The FZP biosensor would have great potential to offer a platform for various label-free optical biosensors based on the diffraction gratings with enhanced SNR.

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