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NANOPOROUS POLYMERIC GRATING-BASED OPTICAL BIOSENSORS (PREPRINT)

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14. ABSTRACT This paper demonstrates a label-free biological sensing method using nanoporous polymer gratings. The high index modulation (0.07) of the nanoporous polymer grating structure generates a high signal-to-noise ratio, making the structure an ideal label-free biodetection platform. The fabrication process of the nanoporous polymeric grating involves holographic interference patterning and a functionalized pre-polymer syrup that facilitates the immobilization of biomolecules onto the polymeric sensor surface. The performance of the nanoporous polymeric sensor is evaluated by sequentially capturing biomolecules (biotin, streptavidin, biotinylated anti-rabbit IgG, and rabbit-IgG) onto the nanoporous regions and monitoring the changes in diffraction and transmission intensity. We have observed that diffraction intensity decreases and transmission intensity increases as biomolecules bind to the polymer structures, an observation consistent with our theoretical analysis. Furthermore, high molecular selectivity is demonstrated within this assay by immobilizing anti-rabbit IgG within the nanoporous polymer and observing the changes in the transmission and diffraction intensities upon the grating's exposure to rabbit and goat IgG (control). The two optical responses are profoundly different for each biomolecule and the selective binding of rabbit IgG is clearly evident. The nanoporous polymer grating-based biosensing method described in this paper is inexpensive, label-free, and amenable as a high-throughput assay, characteristics pertinent in many biomedical research and clinical applications.						
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Nanoporous Polymeric Grating-Based Optical Biosensors

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ABSTRACT

This paper demonstrates a label-free biological sensing method using nanoporous polymer gratings. The high index modulation (0.07) of the nanoporous polymer grating structure generates a high signal-to-noise ratio, making the structure an ideal label-free biodetection platform. The fabrication process of the nanoporous polymeric grating involves holographic interference patterning and a functionalized pre-polymer syrup that facilitates the immobilization of biomolecules onto the polymeric sensor surface. The performance of the nanoporous polymeric sensor is evaluated by sequentially capturing biomolecules (biotin, streptavidin, biotinylated anti-rabbit IgG, and rabbit-IgG) onto the nanoporous regions and monitoring the changes in diffraction and transmission intensity. We have observed that diffraction intensity decreases and transmission intensity increases as biomolecules bind to the polymer structures, an observation consistent with our theoretical analysis. Furthermore, high molecular selectivity is demonstrated within this assay by immobilizing anti-rabbit IgG within the nanoporous polymer and observing the changes in the transmission and diffraction intensities upon the grating's exposure to rabbit and goat IgG (control). The two optical responses are profoundly different for each biomolecule and the selective binding of rabbit IgG is clearly evident. The nanoporous polymer grating-based biosensing method described in this paper is inexpensive, label-free, and amenable as a high-throughput assay, characteristics pertinent in many biomedical research and clinical applications.

The sensing and monitoring of biological molecules such as proteins, enzymes, and DNA are of tremendous importance in applications such as gene mapping,¹ clinical diagnostics,² and drug discovery.³ An ideal biosensing method should be sensitive, selective, rapid, cost-effective, and label-free.⁴ A label-free biodetection method does not require a tedious, time-consuming fluorescence or radioactive labeling process,⁵ and provides a rapid, convenient bioassay by converting the molecular-recognition event into an electrochemical,⁶ optical,⁷ acoustic,⁸ or calorimetric⁹ signal. In particular, porous silicon¹⁰ has been proven to be an appealing platform in label-free optical detection due to its large internal surface area and inherent high detection sensitivity. Various analytes such as DNA,¹¹ protein,¹² enzymes,¹³ pathogens,¹⁴ and bacteria¹⁵ have been selectively detected through different immobilization protocols. Despite its merits in biosensing applications, porous silicon is limited by its chemical and mechanical instability.¹⁶ Moreover, its fabrication process involves complicated multi-step electrochemical etching and the usage of hazardous chemicals (hydrofluoric acid).

In this paper, we present the utilization of an interferometrically created nanoporous polymer grating as a label-free optical biosensing platform. This method not only retains the merits of porous silicon – label-free and large internal surface area – but also circumvents its limitations. The fabrication of a nanoporous polymer biosensor is much more convenient, inexpensive, and safer than porous silicon. By mixing desired chemicals or biomolecules into the pre-polymer syrup, the chemical affinity of the porous polymer can be conveniently adjusted, thus bypassing additional surface modification procedures that are necessary for porous silicon sensors.¹⁷ In this paper, we prove that the

addition of aminopropyltriethoxysilane (APTES) into the pre-polymer syrup effectively facilitates the capture of biomolecules, such as biotin, onto the nanoporous polymer surface. Additionally, we demonstrate that such biofunctionalized nanoporous polymeric gratings can be used to monitor the binding of biological analytes of various sizes (biotin, streptavidin, biotinylated anti-rabbit IgG, and rabbit-IgG).

We recently presented an original method to create periodic nanopores encased within a polymer matrix by modifying the traditional holographic polymer dispersed liquid crystal (H-PDLC) system.^{18,19} This holographic interference patterning technique was employed to fabricate the nanoporous polymeric gratings and combines holography and laser-induced polymerization, producing a periodically modulated optical intensity profile with dimensions on the order of the laser wavelength. The nanoscale voids range in size from 20 to 100 nm. The large surface area of the nanoporous polymer gratings enables the structures' potential usage as a platform for high-throughput sensing applications.

To implement the nanoporous polymer grating as a biomolecular signal transducer, it is essential to modify the chemical affinity of the nanoporous surface while simultaneously maintaining the stability of the polymer film in an aqueous environment. To meet this requirement, we mixed APTES into the pre-polymer syrup. APTES prevented the porous polymer from cracking in aqueous solution and formed covalent bonds with biotin, serving as an adhesion promoter facilitating the attachment of biomolecules onto the nanoporous polymer surfaces.²⁰ We adjusted the APTES concentration in the pre-polymer syrup so that it was high enough to guarantee biomolecular binding onto the nanoporous surface, but low enough not to adversely

affect the formation of nanopores or periodic structures during the holographic fabrication.

The final composition of the pre-polymer syrup we employed contained 10 wt% APTES (Aldrich), 25 wt% acetone solution (Aldrich), 15 wt% TL213 liquid crystal (Merck), 40 wt% dipentaerythritol hydroxypenta acrylate (Aldrich), 1 wt% Rose Bengal (Spectra Group Limited), 2 wt% n-phenylglycine (Aldrich), and 7 wt% n-vinylpyrrolidinone (Aldrich). To fabricate the silanized nanoporous polymer structures, we first mixed the pre-polymer syrup homogeneously with a mixer and sonicator (VWR). Second, we added 20 μL of syrup onto a glass slide and covered the syrup with a second glass slide, coated with a non-reactive 100 nm gold layer. Third, we used a 514 nm Argon ion laser as the exposure source to conduct the holographic interference patterning process. In this step, the sandwiched sample was exposed to two 100 mW laser beams at the desired writing angle (30°) for one minute. Fourth, immediately following the interference patterning, we post-cured the sandwiched sample under a white light source for 24 hours. Upon separating the sample from the cover slide, we obtained a silanized nanoporous polymer grating structure situated on a glass slide. Figure 1 depicts the morphology of a silanized nanoporous polymer grating by scanning electron microscopy (SEM) and bright-field transmission electron microscopy (TEM). The images reveal the repeating parallel line pattern of the nanoporous regions (air voids) alternating with polymer regions. The size of the nanopores ranges from 20 nm to 100 nm. The periodicity of the polymeric gratings is observed to be ~ 650 nm, which is in good agreement with the predicted value of ~ 670 nm calculated from the Bragg reflection equation.²¹

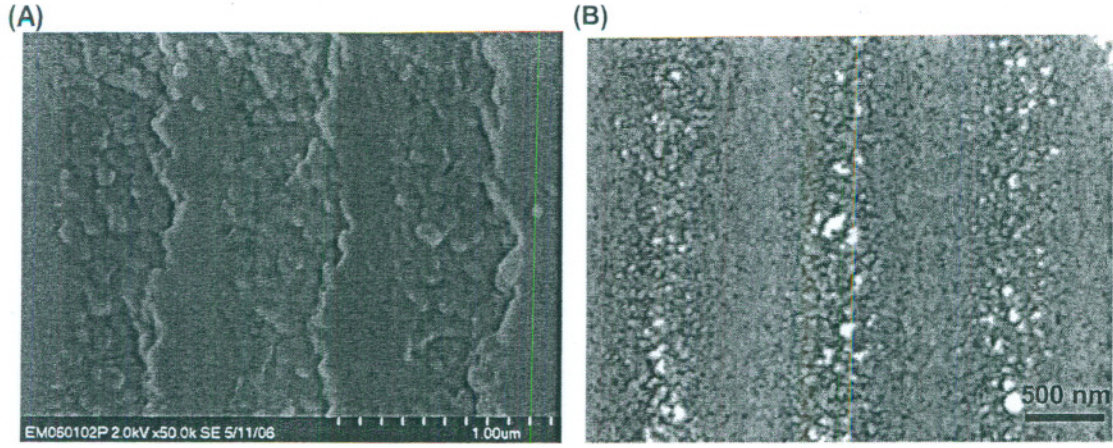


Figure 1. (A) SEM (surface) and (B) bright-field TEM (cross-section) morphology of a silanized nanoporous polymer structure (bright regions on the TEM image are air voids).

In diffraction-based bioassays,²² the high index modulation of grating structures facilitates the observation of changes in refractive index and enhances the signal-to-noise ratio. Thus it is essential to characterize the performance of the bioassay through quantitative analysis of the nanoporous polymer gratings' index modulation. A series of diffraction experiments were therefore conducted to calculate the value of the grating structures' index modulation. According to the Kogelnik coupled wave theory,²² the grating efficiency, η , of a lossless dielectric diffraction element can be expressed as

$$\eta = \sin^2(\nu^2 + \varepsilon^2)^{1/2} / (1 + \varepsilon^2 / \nu^2) \quad (1)$$

where ν and ε are determined by the following equations:

$$\nu = \pi \Delta n d / \lambda \cos \theta \quad (2)$$

$$\varepsilon = n_{ave} \pi d (\theta - \theta_{Bragg}) \sin(2\theta_{Bragg}) / \lambda \cos \theta \quad (3)$$

The thickness of the film d ($3.0\ \mu\text{m}$), the average refractive index of the syrup $n_{\text{ave}}(1.48)$,²³ the writing wavelength λ ($514\ \text{nm}$), and Bragg angle θ_{Bragg} (30°) were all obtained experimentally. By fitting the collected data of incident angle (θ) vs. grating efficiency (η) (solid squares in Figure 2) to the theoretical calculation curve (solid line in Figure 2), Δn was determined to be equal to 0.07. To our knowledge, the index modulation (Δn) achieved in this report is among the highest of all holographic polymer grating structures reported in literature.²⁴

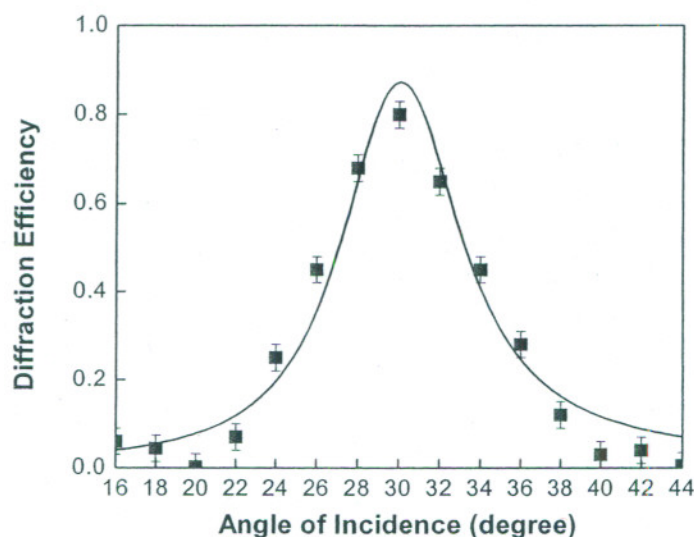


Figure 2. Nanoporous polymer grating's diffraction efficiency dependence on the incident angle of monochromatic light from a 632 nm He-Ne laser. Experimental data is depicted by solid squares; theoretical simulation is represented by a solid line.

Silanized nanoporous polymer gratings with as high a refractive index modulation as demonstrated here are excellent platforms for label-free biosensing applications. The nanoporous polymer grating's sensory capability is based on changes in the refractive index between porous region (average index = 1.40) and nonporous region (average index = 1.47) before and after immobilization of analyte. For example when biotin (index = 1.43) binds onto the nanopores, the grating's diffraction intensity decreases and

transmission intensity increases. Based upon diffraction theory, we could monitor the nanoporous polymer grating's first order diffraction and transmission intensity responses to various biotin concentrations, establishing the nanoporous polymer's ability as an effective biosensor. The biotin solution was prepared by dissolving sulfo-NHS-LC-LC-biotin (G-Bioscience) in phosphate buffered-saline (PBS) and diluting to the solution to desired concentrations. For each individual sample, the polymer grating was immersed and incubated in each respective concentration of biotin solution for one hour. Next, the sample was rinsed by complete immersion in PBS for five minutes and dried by applying a direct air flow to the substrate. The prepared sample was subjected to the optical measurement performed by a collimated He-Ne laser (632.8 nm, 5 mW, 500:1, Thorlabs) at an incident angle of 30° . Two silicon photodetectors (DET series, Thorlabs) were used to record the intensity of first order diffracted and transmitted light. A photodiode amplifier (PDA-700, Terahertz Technologies Inc.) was used to amplify the signals from the photodetectors and transfer the inputted photocurrent into a recordable output voltage.

Figure 3 shows the optical response of the grating at different biotin concentrations. The diffraction intensity decreases (Figure 3A) and the transmission intensity increases (Figure 3B) with a corresponding increase in biotin concentration. This is consistent with the proposed diffraction-based principle that the nanoporous grating is sensitive to refractive index variations caused by biotin immobilization, indicating this method's potential as a quantitative biosensor.

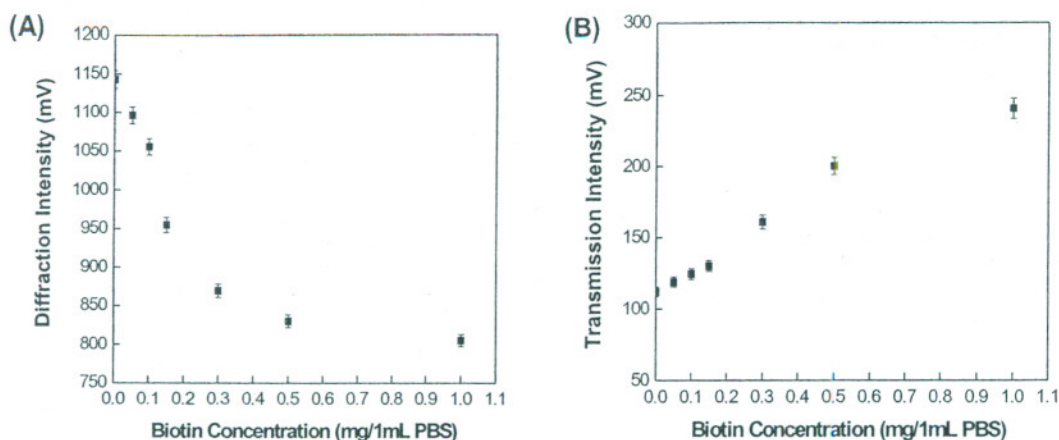


Figure 3. Dependence of (A) diffraction and (B) transmission intensity on biotin concentration.

In a control study we immersed a nanoporous sample into a pure PBS solution for a fixed period of time and observed that either a no change in diffraction and transmission intensity or a slight decrease in both intensities transpired. It is hypothesized that this occurrence is due to light scattering from the polymer surfaces during immersion in the PBS aqueous solution. The nanoporous polymer structures' diffraction and transmission decrease in aqueous solution was considered to be rather minor. We observed that a nanoporous polymer grating held in PBS solution for more than 24 hrs kept $> 90\%$ of its diffraction and transmission signal intact, indicating that aqueous environments do not have detrimental effects to the films' ability to monitor changes. The decrease in both diffraction and transmission of a PBS-immersed sample might also account for the previous observation with biotin immersion (Figure 3), in which the amount the diffraction intensity decreased was not equal to that in which transmission intensity increased. This would explain why a biotin concentration of 0.5 mg/mL led to a 20% decrease of diffraction intensity and only a 15% increase in transmittance, rather than a 20% increase. We can conclude that the optical observation of a decrease in

diffraction intensity and a corresponding increase in transmission comprise positive immobilization of biotin.

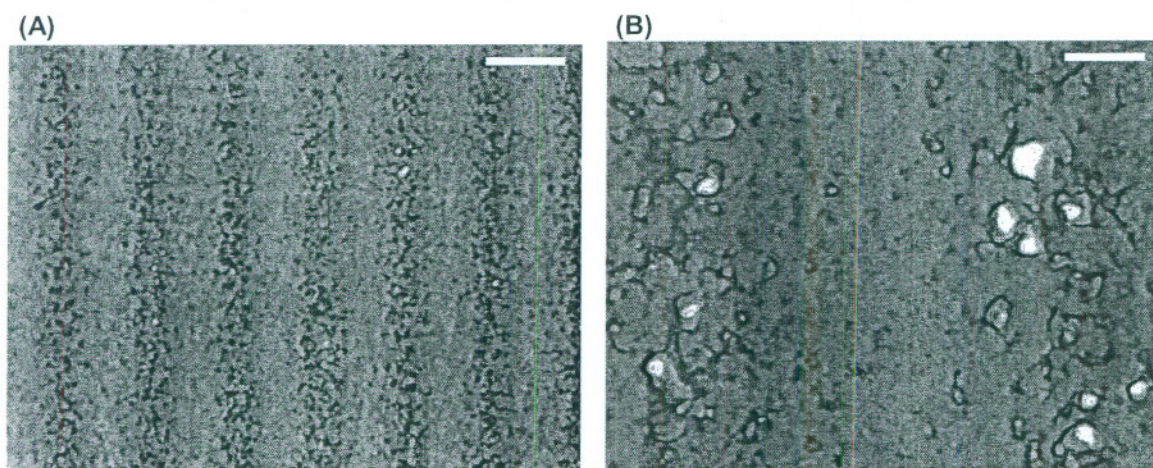


Figure 4. TEM micrograph of the cross-sectional morphology after the sample was incubated with biotin (dark outlines). The scale bar for each image is (A) 500 nm and (B) 200 nm.

The immobilization of biotin onto the nanoporous region could be directly observed through sample morphology. Figure 4 shows two TEM micrographs of the biotin-functionalized nanoporous grating. The existence of biotin binding to nanoporous region is indicated by the appearance of a dark outline surrounding the nanopores. The biotin binding reduces the size of the air voids, increasing the average index of the nanoporous region and decreasing the grating's diffraction intensity, as observed from Figure 3. The optical and morphological observations not only validate that mixing APTES into the pre-polymer syrup is an effective way to enable biotin immobilization onto the nanoporous polymer surfaces, but also proves that the optical response induced by biomolecular attachment is significant enough to be detected.

Our next goal was to establish that the nanoporous polymer grating-based biosensor could be extended to the detection of larger biomolecules and more complicated biological interactions, such as antibody/protein binding. The detection of

rabbit IgG, a commonly used biomarker responsible for many infectious diseases,²⁵ was investigated through a multi-step assay. The insert located in Figure 5A schematically illustrates the sequence of biomolecular interactions that occur within the nanopores in preparation for the Rabbit IgG assay. A silanized nanoporous polymer grating was first derivatized with biotin. Streptavidin could then be selectively captured by biotin onto the nanoporous polymer surface. The following exposure of biotinylated anti-rabbit IgG to the sensor surface resulted in its immobilization to the polymer surface through a second biotin-streptavidin interaction. Anti-rabbit IgG was then used as a probe to selectively capture and detect rabbit IgG.

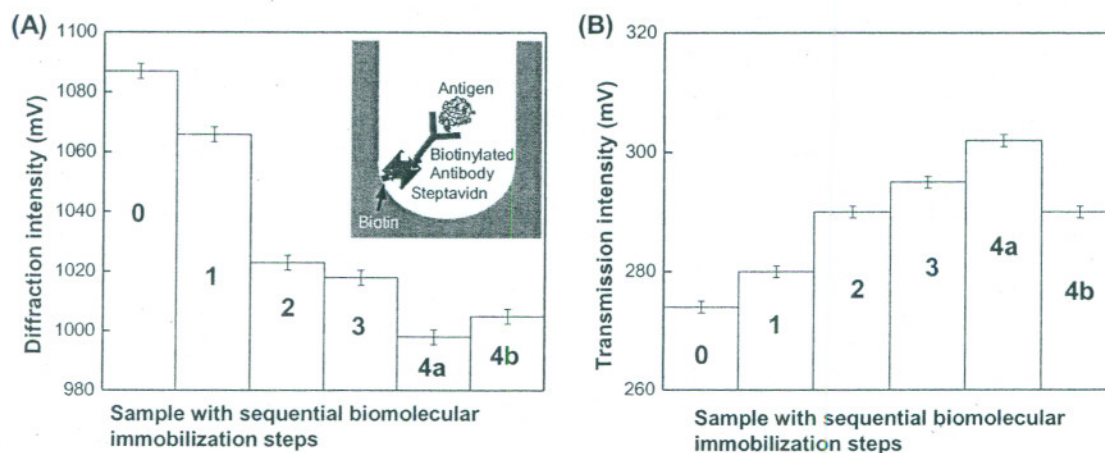


Figure 5. Intensity of (A) diffracted and (B) transmitted light with the sequential surface immobilization steps. Step 0: original sample; Step 1: sample incubated in 50 $\mu\text{g/mL}$ biotin solution; Step 2: sample incubated in 50 $\mu\text{g/mL}$ streptavidin solution; Step 3: sample incubated in 20 $\mu\text{g/mL}$ biotinylated anti-rabbit IgG solution ; Step 4a: sample incubated in 20 $\mu\text{g/mL}$ rabbit IgG solution; Step 4b: sample incubated in 20 $\mu\text{g/mL}$ goat IgG solution.

Figure 5 shows that the diffraction intensity decreases and the corresponding transmitted intensity increases, when the nanoporous polymer grating sample was incubated with biotin (step 1), streptavidin (step 2), biotinylated anti-rabbit IgG (step 3), and rabbit IgG (step 4a) solutions sequentially. In a separate control experiment, step 4a was replaced with the incubation of goat IgG, a control molecule (step 4b), and a

significantly smaller diffracted light intensity decrease was observed. In addition, instead of detecting a transmitted intensity increase as in step 4a, we observed that the transmitted light intensity slightly decreased with the control protein. This observation indicates negative immobilization, which was also observed in the previously mentioned control experiment involving pure PBS buffer as the detection target. Our experimental data indicated that the nonspecific binding between goat IgG and anti-rabbit IgG was negligible, and that the assay was highly specific.

The subsequent immobilization of the bioanalytes (steps 1 and 2) was found essential to the immobilization of biotinylated anti-rabbit IgG onto the nanoporous polymer surface. Diffraction intensity decreases and transmission increases were not observed upon the silanized porous polymer's direct exposure to streptavidin or the biotinylated anti-rabbit IgG solution. In addition, we found that a relatively low concentration of biotin and streptavidin was necessary to facilitate the further immobilization of antibodies and antigens onto the interior void interface. Higher concentrations ($>100\text{ }\mu\text{g/mL}$) of biotin and streptavidin resulted in the saturation of these two analytes on the porous region surface, preventing subsequent immobilization steps.

In summary, we have developed a biomolecular sensing approach combining a biofunctionalized nanoporous polymer grating structure and a diffraction-based method. Experimental results have shown that mixing APTES into the pre-polymer syrup is an effective way to generate biofunctionalized nanoporous polymer structures that facilitate the subsequent immobilization of biomolecules. The resulting silanized nanoporous polymer gratings possess a high refractive index modulation of 0.07, a characteristic crucial in optical biosensing applications and effective in detecting various biological

substances from small biotin to much larger rabbit IgG molecules. The fabrication and biosensing method described here is inexpensive, safe, and label-free. Moreover, the large surface area of nanoporous polymeric structures renders them inherent for high detection capability. Future work includes improving the long-term stability of polymer gratings in aqueous solutions, *in situ* monitoring of bioanalyte activities, and the development of a superior optical setup for high-sensitivity biosensing.

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