Nanobiosensors based on optoelectronic and nanomechanical transducers for genomic and proteomic applications

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Articulo invitado

We show the design, fabrication and testing of micro/nanobiosensor devices based on highly sensitive optoelectronic and nanomechanical transducers. Most of the devices are fabricated by standard Silicon CMOS microelectronics technology according to a precise design for achieving a high sensitivity for biosensing applications. Three biosensors have been developed: (a) a Surface Plasmon Resonance biosensor, (b) an integrated, Mach-Zehnder micro/nano interferometer device based on optical waveguides, and (c) nanomechanical biosensors based on microcantilevers.

Keywords: Biosensor; nanotechnology; optoelectronics.

En este artículo se muestra el diseño, la fabricación y la caracterización de dispositivos micro/nanobiosensores basados en transductores optoelectrónicos y nanomecánicos. La mayoría de los dispositivos se fabrican con tecnología estándar de Si compatible CMOS, después de un cuidadoso diseño de los transductores para conseguir biosensores de alta sensibilidad. Se muestran tres tipos de biosensors: (a) un biosensor de Resonancia de Plasmón Superficial (b) un micro/nano interferómetro integrado Mach-Zehnder basado en guías de ondas ópticas y (c) biosensores nanomecánicos basados en micropalancas.

Descriptores: Biosensores; nanotenologia; optica integrada.

PACS: 42.25.-p; 42.82.-m; 85.85.+j

1. Introduction

There is an increasing interest in systems based on micro/nanotechnologies for ultrasensitive and miniaturized biosensors [1]. Genomic and proteomic sensing are fields where new laboratory analysis (faster, direct, more accurate, selective, with high throughput, and cheaper than conventional methods) are required. On the other hand, new methods allowing label-free and real time measurement of simultaneous interactions (such as antigen/antibody or DNA hybridization) must be developed. A highly multidisciplinary approach including microelectronics, MEMS, micro/nanotechnologies, molecular biology, nanobiotechnology and chemistry are needed for the implementation of such new analytical devices [2]. Biosensing devices fabricated with optoelectronics and MEMS micro/nanotechnologies are powerful devices which can fulfill these requirements. In this line, our group is working on two different approaches [3]:

(A) A platform based on optoelectronic biosensors (evanescent wave detection). Two optical biosensors have already been developed: a portable Surface Plasmon Resonance Sensor (presently being marketed) and an integrated Mach-Zehnder interferometer device. For the latter, the use of standard Si microelectronics technology makes possible the integration of optical, fluidic and electrical functions on one optical sensing circuit in order to obtain a complete lab-on-a-chip. A limit of detection close to femtomolar is achievable with this sensor in a direct format [4].

(B) A platform based on nanomechanical biosensors. Microcantilever biosensors are a new class of high sensitivity biosensors capable of performing local, high resolution and label-free molecular recognition measurements [5]. Moreover, nanomechanical biosensors based on microcantilevers have recently been reported as a promising alternative to current DNA-chips, promising permiting real-time monitoring of DNA without a need for labeling. For that reason, we are working on the development of a portable multibiosensor microsystem based on an array of microcantilevers [6] able to detect analytes with femtomolar sensitivity, and capable of discerning single base variations in DNA strands.

2. Receptor immobilization at the nanometer scale

For biosensing purposes, a layer of receptor molecules (proteins, DNA,...) that are capable of binding the analyte molecules in a selective way must be previously immobilized on the transducer surface. The immobilisation of the receptor molecule on the sensor surface is a key point for the final performance of the sensor. The immobilization procedure must be stable and reproducible, and must retain the stability and activity of the receptor. One of the most promising strategies is the covalent immobilization on gold-coated surfaces using thiol self-assembled monolayers (SAM) [7]. For example, a widespread method is the functionalization of ss-DNA with an alkane chain termed in a thiol (-S-H). Sulphurs form

a strong bond with gold, and thus thiol-derivatized ssDNA spontaneously forms a single self-assembled monolayer upon immersion on clean gold surfaces. This can be applied also to silicon surfaces, using silane monolayers covalently attached the SiO_2 or Si_3N_4 sensor surfaces [7]. Several aspects must be taken into account in the development of the immobilization procedures such as the non-specific interactions, an optimized surface density of the receptor in order to prevent steric hindrance phenomenon, or the regeneration of the receptor.

We have developed immobilisation procedures at the nanometer-scale which seek to fulfill all the requirements described above, based on thiol-chemistry, silanization or esterification, depending on the type of sensor surface and the application [7]. Different examples will be shown in the following, in the description of each biosensor platform.

3. Optoelectronic biosensors platform

Integrated optical (IO) devices are increasingly being used as transducers for optochemical sensing applications [7]. IO devices combine high sensitivity, mechanical stability, miniaturization and the possibility of mass-production. Most of the integrated optical sensors make use of the evanescent field detection principle for sensing[8]. In an optical waveguide, the light travels inside the waveguide, confined within the structure by Total Internal Reflection (TIR). A detailed study of how light travels inside the waveguide shows that the light is transmitted through a model of the electromagnetic field called "guided modes" (as shown in Fig. 1). Although light is confined inside those modes, part of it (*evanescent field, EW*) travels through a region that extends outward, around a hundred nanometers, into the medium surrounding the waveguide (see Fig. 1). This EW field can be used for sensing purposes. When a receptor layer is immobilized onto the waveguide, as shown in Fig. 1, exposure of this surface to the partner analyte molecules produces a (bio)chemical interaction, which takes place on the surface of the waveguide and induces a change in its optical properties that is detected by the evanescent wave. Thus, evanescent wave sensors are selective, sensitive devices for the detection of very low levels of chemicals and biological substances, and for the measurement of molecular interactions in-situ and in real time [7].

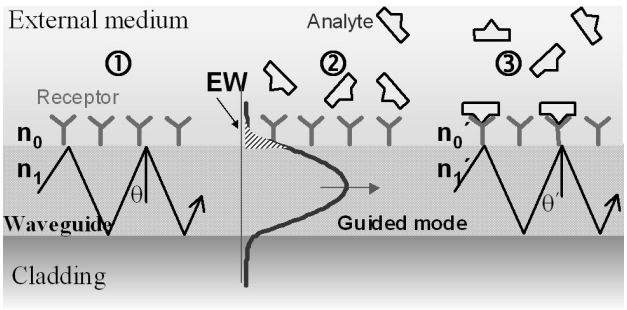

FIGURE 1. Biomolecular interaction sensing by the evanescent wave detection principle in an optical waveguide sensor.

3.1. Surface plasmon resonance biosensor

One of the best known and most developed EW biosensors is the Surface Plasmon Resonance (SPR) sensor, because of its sensibility and simplicity [9,10]. Surface plasmons are elementary excitations which result from a collective oscillation of the free-electron plasma at a metal-dielectric film interface. In a SPR sensor, a thin metal film (usually Au) is evaporated on the dielectric material surface. The sensing mechanism is based on variations of the refractive index of the medium adjacent to the metal sensor surface during the interaction of an analyte to its corresponding receptor, previously immobilized at the sensor surface in the region of the evanescent field. The recognition of the complementary molecule by the receptor causes a change in the refractive index, and the SPR sensor monitors that change. After the molecular interaction, the surface can be regenerated using a suitable reagent to remove the bound analyte without denaturing the immobilized receptor.

We have developed a home-made portable SPR sensor prototype (see Fig. 2) as a highly sensitive field analytical method for environmental monitoring. As a proof of its utility in the detection of pathogens, we have determined several pesticides, such as the chlorinated compound DDT and the neurotoxins of the carbamate (carbaryl) and organophosphorus types (chlorpyrifos). For the determination of these compounds, a binding inhibition immunoassay was applied. For DDT, the assay sensitivity was evaluated in the 0.004-3545g μg/l range of pesticide concentration by the determination of the limit of detection (0.3 g μg/l). For carbaryl, the dynamic range of the sensor is 0.12-2 g μg/l, a detection limit of 0.06 μg/l. Similarly the immunoassay for chlorpyrifos determination afforded a high sensitivity working in the 0.02-1.3 g μg/l range. Figure 3 shows the calibration curves obtained from the pesticides carbaryl and chlorpyrifos, respectively.

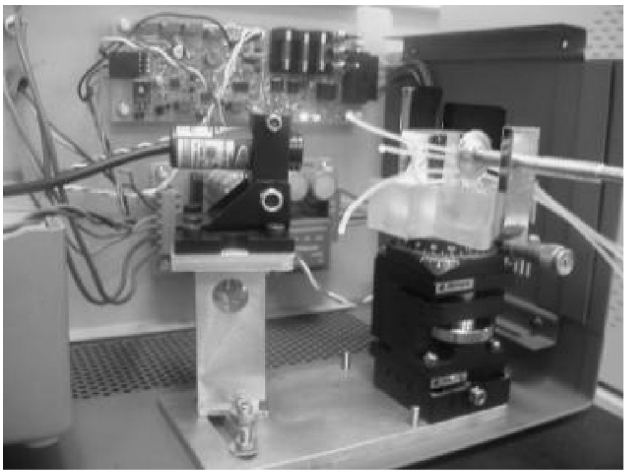

FIGURE 2. Portable SPR sensor prototype system including sensor, optics, electronics and flow delivery system.

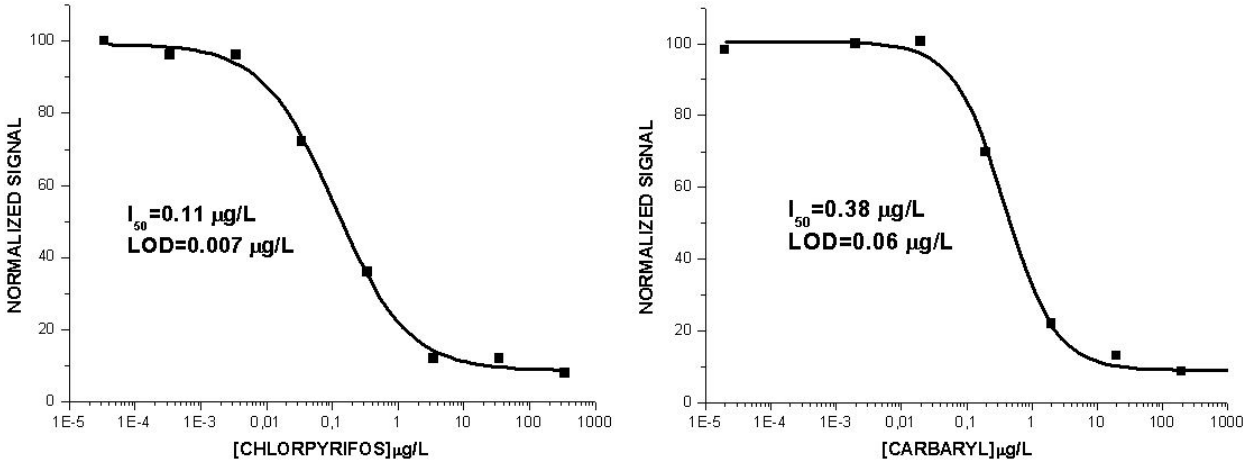

FIGURE 3. Calibration curve for the immunoassays determination by using SPR technology of (*left*) Chlorpyrifos (*right*) Carbaryl.

The reusability of the sensor was demonstrated after 250 assay cycles, without significant variations in the average maximum signal. This immunosensing technique, together with the portable surface plasmon resonance sensor developed, can be applied as a fast, cost-effective field-analytical method for the monitoring of any chemical and biological compound if the corresponding receptor is available.

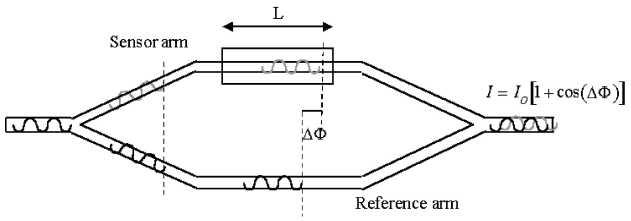

FIGURE 4. Mach-Zehnder interferometer configuration.

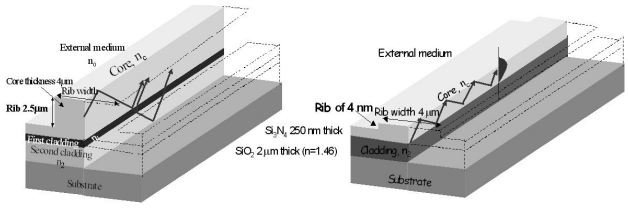

FIGURE 5. Structure of the MZI devices (*left*) ARROW-MZI (*right*) TIR-MZI.

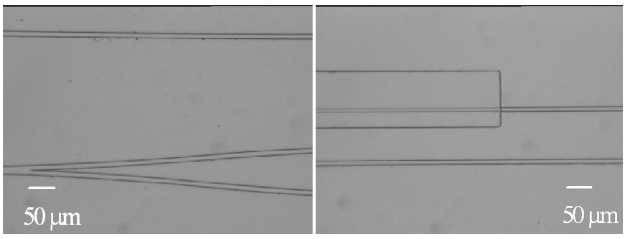

FIGURE 6. Integrated Mach-Zehnder interferometer: details of the MZI Y-junction and sensor area.

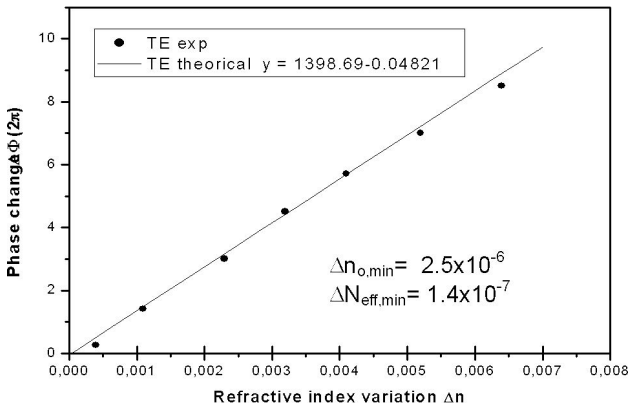

FIGURE 7. Sensitivity evaluation of a TIR-MZI nanodevice by using solutions of varying refractive indices.

3.2. Integrated Mach-Zehnder interferometers

From the different types of IO sensors[8], the interferometric arrangement is the most sensitive and the only one that provides an internal reference for the compensation of refractive-index fluctuations and unspecific adsorption. Due to the high sensitivity of the interferometer sensor, the direct detection of small molecules (even without inhibition immunoassays) would be possible with this device [11]. A detection limit of 10^{-7} in the refractive index (or better) can be achieved with these deviceswhich opens up the possibility of developing highly sensitive devices, for example, for directly determing the extreme protein (femtomolar) concentration [11].

In an interferometer (see Fig. 4) two light beams of equal intensity are made to travel across two areas of a waveguide (one is the sensor and the other is the reference) and are finally combined, creating an interference pattern. When a biochemical reaction takes place in the sensor area, only the light that travels through this arm will experience a change in its effective refractive index. At the sensor output, the intensity of the light shows a sinusoidal variation that depends on the difference of the effective refractive indexes of the sensor and reference arms (ΔN) and on the interaction length (L), and

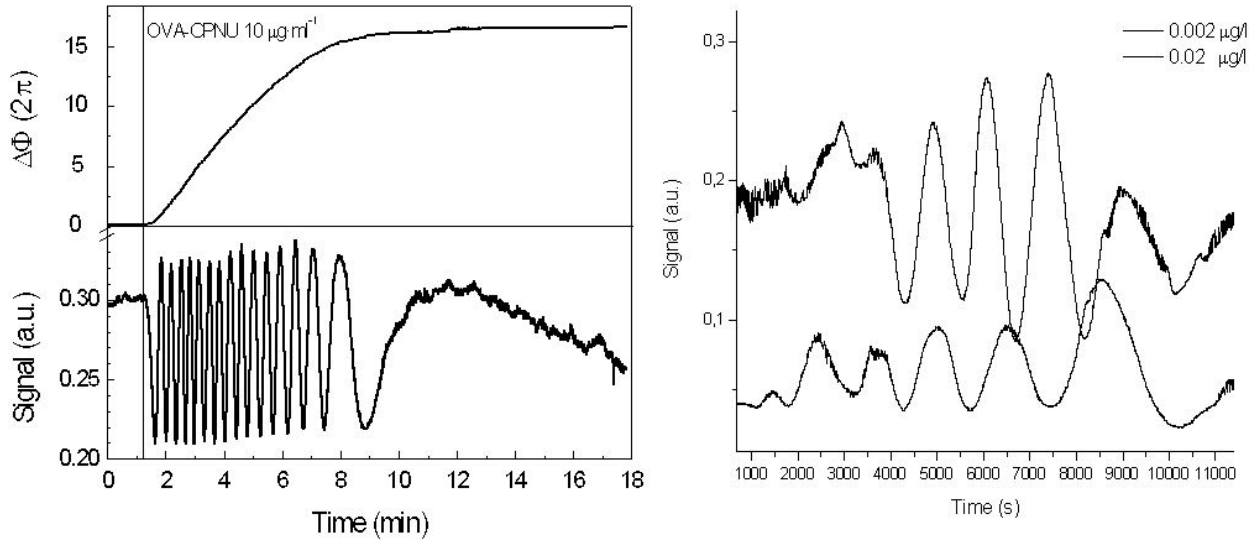

FIGURE 8. (*left*) Immobilization of the receptor on the MZI surface by covalent attachment (*right*) Immunoreaction with the specific antibody.

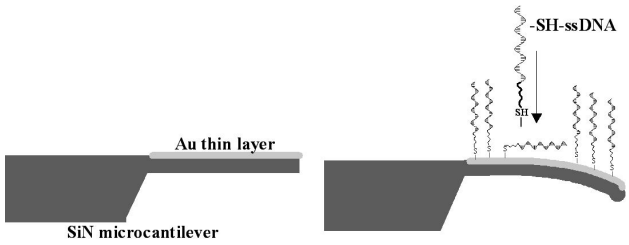

FIGURE 9. Working principle in nanomechanical cantilevers: (*left*) cantilever with a thin gold layer for covalent attachment of the receptor (*right*) bending of the cantilever due to biomolecular interaction.

can be directly related to the concentration of the analyte to be measured. We have fabricated two integrated Mach-Zehnder interferometric devices using two technologies: (a) a MZI Microdevice based on ARROW waveguide [12] and (b) a MZI Nanodevice based on TIR waveguide [13].

(a) MZI Microdevice based on ARROW waveguide:

For the development of a highly sensitive integrated optical sensor based on the Mach-Zehnder interferometer configuration, it is necessary to design optical waveguides that satisfy two conditions: monomode behaviour and high surface sensitivity. ARROW (Anti Resonant Reflecting Optical Waveguides) structures based on Silicon technology meet these requirements. This optimised waveguide consists of a rib-ARROW structure with a silicon oxide core layer (n_{core}=1.485) and thickness greater than 2 μm; a silicon oxide second cladding layer with a refractive index of 1.46 and a fixed thickness of 2 μm; and a silicon nitride first cladding layer, 0.12 μm thick, with a refractive index of 2.00. The waveguide is overcoated with a thin silicon nitride layer (n_{ov}=2.00) and with a silicon oxide layer (n=1.46) with a thickness of 2 μm. The rib depth is 60% of the core thickness and the rib width should be less than 8 μm to obtain single-mode behaviour. The structure of the ARROW device can be seen in Fig. 5.

(b) a MZI Nanodevice based on TIR waveguide.

In Fig. 5, the cross section of the MZI TIR waveguide is shown. The basis of the TIR structure is: (i) a Si wafer, (ii) a 2 μm thick thermal Silicon-Oxide layer (n=1.46), (iii) a LPCVD Silicon Nitride layer of 100 nm thickness (n=2.00), which is used as a guiding layer. To achieve monomode behavior, a rib structure must be defined with a depth of only **4 nm** by a lithographic RIE step. Finally, a Silicon-Oxide protective layer is deposited by LPCVD over the structure with a 2 μm thickness (n=1.46), which is patterned and etched by RIE to define the sensing arm of the interferometer. Photographs of one integrated MZI device can be seen in Fig. 6. Several devices, using both technologies, have been fabricated in our Clean Room facilities. The devices are implemented with a microfluidic unit, electronics, data acquisition and software for optical and biochemical testing.

For evaluating the sensor sensitivity, a calibrating curve was recorded using solutions with different refractive indexes, as depicted in Fig. 7 for a TIR-MZI device. For the TIR device, the lower detection limit measured was $\Delta n_{o,min} = 7.10^{-6}$, which means an effective refractive index of $\Delta N = 4{\cdot}10^{-7}$. For the ARROW-MZI devices, a minimum detectable refractive index variation of $\Delta n_{o,min} = 2{\cdot}10^{-5}$ was obtained.

We have applied the MZI biosensors for the detection of the insecticide carbaryl [2]. The introduction of different concentrations of the specific monoclonal antibodies porduces an immunoreaction proportional to this concentration, as observed in Fig. 8 (right).

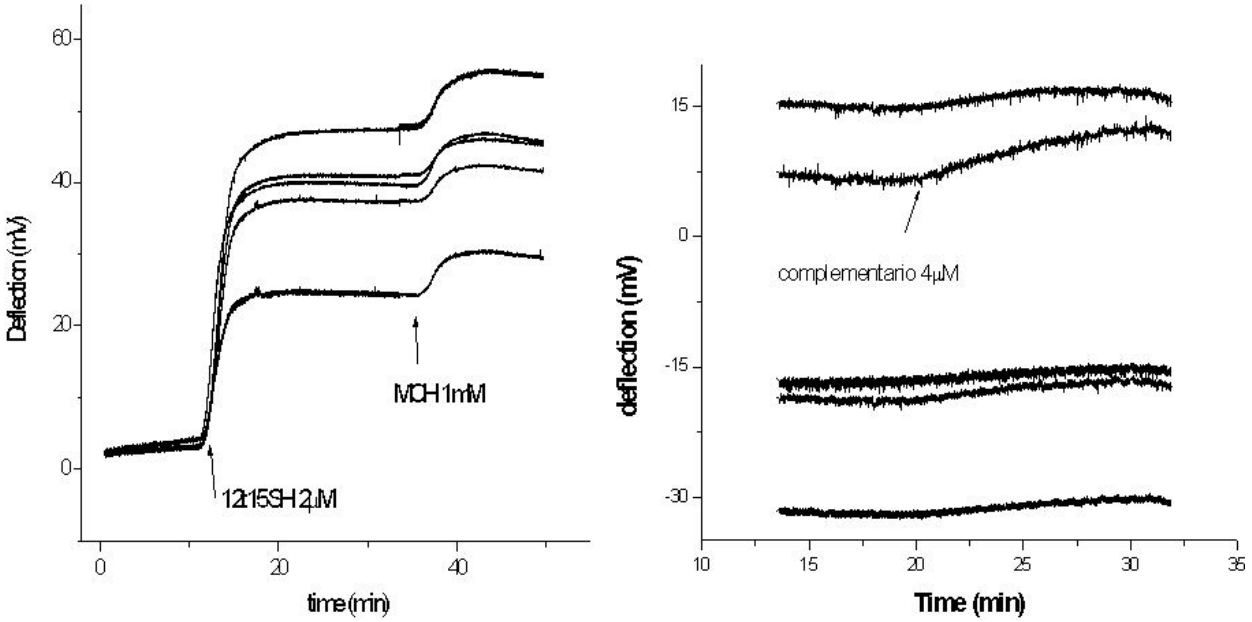

FIGURE 10. Real time simultaneously monitoring of five microcantilevers. (*left*) Immobilization and blocking treatment, and (*right*) hybridisation bending signal.

Nanomechanical biosensor platform

Microcantilevers, such as those used in Atomic Force Microscopes, have been recently employed as this new class of biosensors [14]. The so-called nanomechanical biosensors have demonstrated that they are capable of detecting single-base mismatches in oligonucleotide hybridization without labeling [5], as well as performing protein recognition [15] with extreme sensitivity. The working principle for nanomechanical biosensors relies on the induced surface stress produced when molecules bind to a surface (see Fig. 9). Surface stress mainly arises from electrostatic, van der Waals, steric interactions, etc., between the adsorbed molecules.

Cantilever bending (deflection) measurements are carried out by using the well-known optical beam deflection method. A laser beam is focused on the free end of the cantilever and the deflection of the reflected beam is measured with a four-segment photodetector. For example, for the DNA hybridization detection, nucleic acids are immobilized on one side of the micromachined lever. Exposure of the cantilever to a sample containing complementary nucleic acid gives rise to a cantilever bending (deflection) of a few nanometers.

To test the reliability of such approach, we have used arrays of Si cantilevers fabricated in our Clean Room for the real-time detection of (a) immobilization of DNA strands and (b) hybridization with the corresponding complementary DNA strands, as shown in Fig. 10.

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