Enhanced Transmission through Gold Nanohole Arrays Fabricated by Thermal Nanoimprint Lithography for Surface Plasmon Based Biosensors

J. Martinez-Perdiguero*, A. Retolaza, A. Juarros, D. Otaduy, S. Merino

*Corresponding author. Tel.: +34 943206744; fax: +34 943202757.
E-mail address: jmartinez@tekniker.es.

Abstract

We present the fabrication of gold nanohole arrays using thermal nanoimprint lithography and integration into a microfluidic device for detection of biological analytes. The biosensor makes use of a surface-plasmon mediated effect known as enhanced optical transmission, in which the transmission of light is modulated. The sensitivity achieved with these gold nanohole arrays has been characterized and up-to 300 RIU/nm were obtained varying the array parameters. Detection of protein biomarkers via capture by specific antibodies has been achieved demonstrating the biosensing capabilities of the fabricated devices.

Keywords: nanoimprint lithography; biosensor; nanoholes; surface plasmon resonance; enhanced transmission;

1. Introduction

Surface plasmon resonance is a widely known phenomenon and it is used in bioanalytical laboratories as a label-free real-time method to study molecular interaction studies. It can be used for concentration determination and quantitative kinetic studies and it offers high-throughput for screening in, for example, drug discovery research. Commercial systems usually work in internal-reflection based Kretschmann configuration which requires a somehow complicated optical setup. The non-classical extraordinary light transmission effect through metallic nanohole arrays [1] can be used for surface refractive index sensing.
using miniaturizable collinear optics. In addition, micrometric-size footprints allow for multiplexing with high spatial resolution.

2. Array Fabrication via Nanoimprint Lithography

Typically, manufacturing of nanohole arrays is made by focused ion beam (FIB) or electron beam lithography (EBL) techniques which are not suited for mass production. To avoid this, we fabricated the nanohole arrays using nanoimprint lithography which is a low-cost, high resolution method with a much larger throughput [2]. Up to 49 different nanohole arrays with different hole diameters (100-250 nm) and periodicities (450-800 nm) with an average footprint of 25 µm² were patterned in a silicon master using standard electron beam lithography. The master stamp was coated with tridecafluoro-(1,1,2,2)-tetrahydrooctyl-trichlorosilane as antiadhesive layer and it was used repeatedly (100 times showing no deterioration).

Initially mr-I7010R resin, that has a glass-transition temperature of 50ºC, was spin-coated at 6000 rpm over 4 inch glass wafers. After that, the substrate was embossed by the master stamp at 130ºC and demolded at 40ºC. The residual layer was then etched with oxygen plasma to subsequently deposit a 5nm titanium layer to improve adhesion of a typically 60 nm thick gold layer via e-beam evaporation. After this, the resist was lifted off to obtain the nanoholes array structures. Scanning electron microscopy (SEM) pictures of a typical array are shown in Fig 1.

![SEM images of a 50x50 gold nanohole array fabricated with thermal nanoimprint lithography (hole diameter 115nm, periodicity 450nm).](image)

3. Optical characterization

These nanohole arrays were integrated into a microfluidic cell whose inlet and outlet ports allowed addressing of the arrays with different liquids. Light from a tungsten-halogen lamp was focused with a microscope objective on the arrays and the transmission spectra recorded with an optical fiber spectrometer. The optical setup used is shown in Fig. 2.

![Optical setup used](image)

Modulation of the incident light was measured and, as expected, it does not correspond to that of the classical aperture theory [1]. Peaks at different wavelengths can only be explained using a surface...
plasmon mediated transmission (see Fig. 3a). Peak positions and sharpness are known to depend on the hole and array parameters [1]. The sensitivity of the devices was measured using solutions of sucrose in water with known index of refraction and measuring the shift of resonance peaks (Fig. 3b). Up to 300 nm/RIU were achieved.

Fig. 2. Scheme of the optical setup to collect the light transmitted through the nanohole arrays.

Fig. 3. (a) Normalized transmission spectrum of a gold nanohole array (that of Fig. 1) in air. (b) Resonance peaks shift using sucrose solutions of known index of refraction: solid line $n=1.333$, $\lambda_{\text{peak}}=645.7$ nm; dotted line $n=1.356$, $\lambda_{\text{peak}}=651.2$ nm; dashed line $n=1.381$, $\lambda_{\text{peak}}=656.1$ nm.

4. Protein biosensing.

Biofunctionalization of the chips with monoclonal antibodies was performed using standard amine coupling. Summarizing, the gold chips were immersed overnight in a ethanol solution containing 5mM mercapto-undecanoic acid to create a carboxylated self-assembled monolayer (SAM). Immobilization of
monoclonal anti-TNF-alpha antibodies was performed in an acidic solution during 1 hour after activation of the carboxyl groups via N-hydroxysuccinimide and carbodiimide (ECD) solution. To prevent non-specific binding the surfaced was blocked with ethanolamine.

After the described protocol, the gold surface was ready for TNF-alpha protein capture. The transmission spectra was recorded after and before the incubations of a buffer solution containing TNF-alpha and the shift of the peaks measured demonstrating the biosensing capabilities of the fabricated devices. The protein binding could be reversed with a 10mM pH 2.5 glycine solution to perform several cycles and experiments with the same chip.

Acknowledgements

The research leading to these results has received funding from the ETORTEK project MIBIO2 (Microtecnologías para biodispositivos y detección de biomarcadores) funded by the Basque Government.

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