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Parallel optical read-out of micromechanical pillars applied to prostate specific membrane antigen detection

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ABSTRACT

Micro and nanomechanical resonators represent a promising platform for proteins label-free detection because of their extreme sensitivity, fast response and low cost. Micro-pillars are columnar resonators that can be easily arranged in dense arrays of several thousand sensors in a squared mm. To exploit such a large density, however, a method for tracking independently micropillars resonance frequency is required. Here we present a detection method based on CCD imaging and software image analysis, which can measure the resonance frequency of tens of pillars in parallel. Acquiring simultaneously the frequency shift of up to 40 sensors and applying a proper statistical analysis, we were able to overcome the variability of the single measures improving the device sensitivity at low analyte concentration range.

As a proof of concept, this method has been tested for the detection of a tumor marker, the Prostate Specific Membrane Antigen (PSMA). Pillars have been functionalized with an antibody against PSMA. The tumor marker (PSMA) has been detected in a range of concentrations between 300 pM and 100 nM, in buffer and in diluted bovine serum. The sensitivity of our method was limited only by the affinity constant of the antigen–antibody recognition. Moreover, this detection technique demonstrated to be effective in the 1–6 nM range, which is the window of PSMA concentration of clinical interest.

1. Introduction

The application of micro- and nano-electromechanical sensors was proposed at the end of the 1990s and since then experienced a constant development. Two principal strategies have been recognized involving micro and nanocantilevers or double clamped beams. The static deflection mode, in which molecules adsorbed on the sensor surface create an asymmetrical stress that results in sensor bending (Fritz et al., 2000). Instead, in the dynamic mode, the resonance frequency of the sensor is monitored and its variation upon mass adsorption is detected (Battiston et al., 2001). In the latter configuration, the main advantages are: the extremely low limit of mass detection, the small amount of sample required for operation and the label-free detection of molecular species. All these advantages come from the small size of the sensor that is often in the submicron range, thus reducing dramatically the sensor mass and increasing the surface to volume ratio.

In the last decade, applications in different fields were demonstrated (Arlett et al., 2011; Bargatin et al., 2012; Pang et al., 2012; Tamayo et al., 2013): single atom sensitivity was reached in vacuum condition (Yang et al., 2006), applications in air and liquid were proposed, with a detection limit reduced to attogram (10^{-18} g) (Verd et al., 2007) and to nanogram (10^{-9} g) (Braun et al., 2009) respectively.

Micromechanical pillars are vertical cantilevers used as mass sensors in dynamic mode (Kehrbusch et al., 2008; Melli et al., 2010). Because of the reduced lateral size, pillars can be arranged in a dense array; when nearest neighbors are close enough and the lateral surfaces are hydrophobic, a superhydrophobic Cassie–Baxter (CB) state is realized (Melli et al., 2011). When a solution is introduced in a CB pillar array, gas is trapped between the lateral walls and only the top surface of the pillars is in contact with the solution, ensuring that the biochemical recognition process occurs

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only on the top. This configuration reduces drastically device deterioration and non specific adsorption which can change not only the cantilever mass but also its stiffness, making measurement interpretation difficult (Tamayo et al., 2013). Moreover, we recently demonstrated that, at low concentration, when the biochemical recognition is diffusion limited rather than reaction limited, if the sensor area is significantly smaller than the analyte diffusion length and the pillar spacing larger, a reduction of incubation times down to three order of magnitude can be obtained (Melli et al., 2011; Nair and Alam, 2006).

Finally, pillars dense arrays can be exploited to implement a multiple sensor. However, the complexity of addressing electrically each individual resonator grows exponentially as the number of pillars increases. On the other hand, if a suitable optical detection scheme is adopted, thousands of pillars, integrated in few mm^2 areas, could be monitored in parallel. This enables sensitivity improvements over individual pillars by averaging signals coming from a multitude of devices in the array.

In this paper we report on an innovative read-out strategy which enables monitoring the frequency resonance of tens of pillars in parallel using a CCD imaging system and software image processing. As proof of principle, we detected a tumor associated antigen of Prostate Cancer (PCa), the Prostate Specific Membrane Antigen (PSMA), both in physiological solution and bovine serum.

2. Material and methods

2.1. Pillars fabrication

Pillars are obtained by a deep dry etching of a patterned Si wafer. Details of the fabrication procedure are reported elsewhere (Borin et al., 2014). Briefly, we started from a well cleaned Si(100) wafer. A 20 nm thick Nickel mask was patterned by electron beam lithography and electron beam evaporation, creating an hexagonal lattice of 2 μm x 3 μm rectangles, corresponding to the top area of the micropillars, with a center-to-center distance of 12 μm. The vertical structure was obtained by Induction Coupled Plasma (ICP) Deep Reactive Ion Etching (DRIE), using a BOSCH™-like approach with SF_6, Ar, and C_4F_8. The number of cycles defines the height of the micropillars (around 12 μm). The recipe was optimized in order to obtain a controlled undercut (approximately 2–3°), that reduces the base of the pillar to almost 700 nm and improves the oscillation amplitude of the resonator. An example of the so fabricated matrix is reported in Fig. 1a. Fig. 1b shows a magnified view of a single pillar with the typical scallops created by the cyclical etching process. In order to increase the sensitive area and the oscillation amplitude, "T" shaped pillars were also fabricated starting from Si(100) wafer coated with a 500 nm thick Si_3N_4 layer, defining a larger top area of 3 μm × 4.5 μm. The fabrication process differs from the one described above for the presence of a last isotropic and selective SF_6 dry etching step which thins the silicon structure but not the topmost Si_3N_4 layer. The resulting structure is exemplified in Fig. 1c. This geometry offers a larger active layer, with only a slight increase of the overall pillar mass, a larger oscillation amplitude and smaller resonance frequency, making them easy to detect at the cost of a slightly lower sensitivity.

Finally the Ni layer was stripped from the top of the pillar, the sample was cleaned in piranha solution, thermally oxidized to relax possible residual stress and a layer of 5 nm Cr/20 nm Au was deposited for further chemical reactions.

The described process results in 5 mm × 5 mm silicon chips with a 300 μm × 300 μm patterned area in the center containing an array of 640 pillars. The obtained chips can be handled with tweezers and mounted on the read-out chamber. The production costs of these devices, also in a prototypal stage, are extremely low, therefore the devices were used as produced and disposed after use.

Finally, in spite of the parallel fabrication process, pillars showed different resonance frequency. This is due to several sources of inhomogeneities in the lithographic process. Arrays of micro- and nano-mechanical resonators were already reported to have a rather broad distribution of resonance frequencies (Bargatin et al., 2012; Martinez et al., 2010; Sampathkumar et al., 2011). A characterization of the pillar resonance frequency distribution is discussed in Supplementary Information S2.

2.2. Pillars hydrophobization

In order to achieve permanent superhydrophobic properties (Cassie–Baxter state), pillar walls were coated with a hydrophobic layer as discussed in detail in (Borin et al., 2014). In particular we formed an alkanosilane coating through octadecyltrichlorosilane (OTS) deposition from a 1 mM solution in dry toluene for 1 h. The

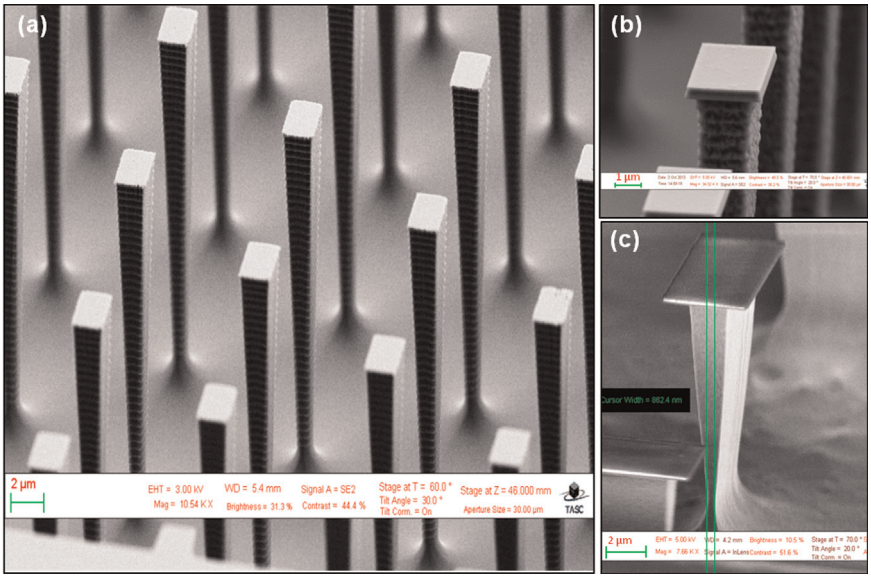

Fig. 1. (a) SEM image of a micropillars array obtained from deep plasma etching of a patterned silicon wafer, the tapered shape is visible. (b) Magnified view of a single pillar with the typical scallops created by the etching process. (c) SEM image of a "T" shaped pillar obtained adding an isotropic and selective dry etching step to the process.