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Large-scale arrays of nanomechanical sensors for biomolecular fingerprinting

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ABSTRACT

A review of activities involving the development of large arrays of nanomechanical resonators is presented. This review includes demonstration of the use of these arrays for the detection of biological targets. Both top-down and bottom-up approaches to the realization of such arrays were developed. Using a top-down approach, a nanomachining method for the fabrication of large arrays of doubly-clamped silicon carbonitride (SiCN) resonators with width as narrow as 16 nm and a yield approaching 100% was developed. The specific detection of protein-A using such resonator arrays functionalized with single domain antibody fragments (sdAb) was also demonstrated with femtogram-level mass sensitivity. A nano-imprinting based fabrication of these resonator arrays was also realized, opening up their potential for cost-effective manufacturing. On a bottom-up approach, resonant silicon nanowires were also produced using directed chemical vapor deposition methods. These bottom-up resonant nanowires were in turn successfully employed for the specific detection of streptavidin with attogram-level mass sensitivity.

1. Introduction

Detection of biological and chemical agents is critical to many areas of the life sciences including: disease diagnosis, drug molecule screening, and rapid analysis of various molecular systems. Technologies currently used for such assaying include mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), and enzyme-linked immunosorbent assay (ELISA). ELISA [1] is a widely employed, array-based, analytical technique for the parallel analysis of antigens or antibodies. This technique however requires fluorescent tagging, which may disrupt the biochemical properties being investigated. Other platforms, such as quartz crystal microbalance (QCM), surface acoustic wave sensors (SAW), and surface plasmon resonance sensors (SPR) offer tagless alternatives for the analysis of molecular mixtures. Neither of these techniques is however well-suited for the detection of a large number of analytes [2].

Micro- and nanoresonators have been shown to be promising platforms for the tagless array-based detection of molecular systems. The binding of the analyte onto the sensor surface is detected through a shift of resonant frequency induced by its added mass. The mass sensitivity of mechanical resonators scales favorably as their mass is reduced, offering a compelling path for the development of large arrays of sensors of exceptional sensitivities. Sub 100 nm-wide nanoelectromechanical systems (NEMS) were first reported by Carr et al. [3]. The properties of silicon in addition to stiction issues inherent to this process however limited the fabrication yield to less than 25% for widths below 50 nm. Sacrificial layer processes also typically require critical point drying (CPD) following immersion of the resonator in order to prevent stiction. Such critical point drying is known to leave contamination on the sensor surface that may interfere with the detection of biological analytes [4].

While Dalton range ($\sim 10^{-24}$ g) mass sensitivity has been proposed as the ultimate limit of nanoresonator-based detection [5], femtogram (1 fg = 10^{-15} g) and attogram (1 ag = 10^{-18} g) level sensitivities have been widely reported [6–11]. For instance, Waggoner et al. demonstrated the detection of prostate specific antigen (PSA) with sensitivities down to 50 fg/mL using trampoline-like nanomechanical resonators in conjuction with a secondary mass labeling technique [12]. More recently, zeptogram (1 zg = 10^{-21} g) level mass resolutions have been demonstrated [13,14]. However, these state-of-the-art mass sensitivities were achieved only at cryogenic temperatures, and the employed fabrication techniques are not readily scalable for large array-based detection.

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A review of activities involving the development of alternatives to traditional sacrificial layer nanomachining process for the realization of large arrays of nanomechanical resonators is here presented. These development efforts include both top-down and bottom-approach to the realization of such arrays. Using a top-down approach, we developed a novel nanomachining method for the fabrication of large arrays of doubly-clamped SiCN resonators with width as narrow as 16 nm with a yield approaching 100% [15]. This novel process combines the surface machining of a SiCN glassy layer with bulk machining techniques for the release of the device [15]. This process allows the realization of sub-20 nm wide and tens of micron long suspended structures with a yield approaching 100% without the need of critical point drying [15]. This process has also more recently produced the first sub-10 nm wide suspended structures ever realized by surface machining [16]. The specific detection of protein-A using these resonator arrays is now here reported. In addition, a nanoimprinting based approach to the fabrication of these devices was also realized, opening up the potential of this technology to cost-effective manufacturing [17]. A bilayer resist consisting of PMMA 495/LOR 3A allowed high fabrication yields for resonators of widths ranging from 120 nm to 300 nm, thicknesses of 40 nm and 70 nm, and a length of 14 μm. To our knowledge, these 120 nm resonators are the narrowest suspended structures ever fabricated via nanoimprinting.

The last few years have also seen the development of alternate "bottom-up" techniques for the fabrication of nanodevices. Bottom-up approaches offer the advantages of higher throughput, and potentially lower manufacturing costs. For example, silicon nanowires have been used to detect biological and chemical species [18]. These sensors operate by monitoring changes of electrical conductivity associated with the binding of the analyte to the nanowire. A single-walled carbon nanotube (SWNT) resonator has been described by Sazonova et al. [19]. Also, mechanical resonators have been produced from 43 nm diameter platinum [20] and 75 nm diameter silicon nanowires [14]. Both these structures were tested using magnetomotive actuation, which requires ultra-high vacuum and cryogenic cooling. We have developed cantilevered nanomechanical silicon resonators using a directed chemical vapor deposition method. We have recently reported the analysis of such wires with diameters as small as 40 nm using a simpler and cost-effective room-temperature interferometry technique [21]. Given that those dimensions compare to the mean free path of air molecules at ambient pressure, we specifically observed exceptionally high quality factors, as high as 7000, at atmospheric pressure, which is only a factor of ~3 lower than that in vacuum [21]. We here report the successful use of these nanowire resonators for the specific detection of proteins with attogram-level mass sensitivity.

2. Experimental

2.1. Fabrication of nanoresonator arrays

The SiCN nanoresonators were initially realized via a combination of electron beam lithography and surface nanomachining. A 50 nm thick SiCN layer was deposited by plasma-enhanced chemical vapor deposition (PECVD) onto single-crystal, (1 0 0), silicon wafers (500 μm-thick, 100 mm-diameter) [15]. The SiCN-coated wafers were then annealed in a tube furnace at 500 °C for 6 h which resulted in a tensile stress of ~200 MPa. Next, the resonator beams and the supporting pads were patterned using electron beam lithography (EBL). A 30 nm thick Cr film, deposited by thermal evaporation and subsequently lifted off in acetone, was used as a mask for reactive ion etching. Finally, the resonators were released by anisotropic etching in KOH solution (35%) saturated with IPA [15,22].

This SiCN technology was subsequently employed in conjunction with nanoimprint lithography using a bilayer of PMMA 495/LOR as imprint resist. Two bilayer thicknesses were investigated. A 150 nm/150 nm bilayer was initially employed, and reproducibly yielded suspended beams as narrow as 300 nm. A 100 nm/100 nm bilayer was then employed, which yielded released devices as narrow as 120 nm. The imprint itself was performed using a Nanonex NX-2500 system at a temperature of 190 °C, a pressure of 200 psi, and a hold time of 2 min. An oxygen plasma was then employed to remove any resist residue at the bottom of the pattern. This oxygen plasma cleaning was performed in a Trion reactive-ion etch (RIE) system, and at low pressures to ensure etch anisotropy (10 mT, 7 sccm O_2, 60 W). The etch time employed varied from 60 s to 120 s given that the residual layer height was slightly different for each imprint.

Bottom-up cantilevered silicon nanowires were grown onto a silicon-on-insulator (SOI) wafer that consisted of a 7 μm thick (1 1 0)-oriented device layer, and a 100 nm thick buried oxide layer [23]. Vertical {1 1 1} sidewalls were formed by patterning and etching trenches in the (1 1 0) top silicon device layer using KOH. Gold catalyst particles were then deposited from colloidal suspension using a dip-and-dry process. The CVD growth was performed using silane, HCl, and B_2H_6 (the boron dopant source) as precursors. Silane preferentially decomposes on the gold surface at a growth temperature of $T = 680$ °C. The deposited silicon dissolves in the gold, eventually forming a super-saturated Au–Si liquid alloy. Silicon atoms then precipitate from this alloy to form a nanowire whose diameter is about that of the gold particle. The nanowires are epitaxially grown horizontally from the vertical sidewall surface, and are thus rigidly anchored (covalently bonded) at their base.

2.2. Interferometric setup

The experimental optical interferometry setup used for assaying of the resonance frequencies of the nanoresonators is shown in Fig. 1 [15,22]. The resonator arrays were mounted onto a piezoelectric element which was actuated by the tracking output of a spectrum analyzer (Agilent model 4411B). The beam of a laser diode ($\lambda = 655$ nm) was directed through a beamsplitter and focused onto the substrate using a NA = 0.45 microscope objective. At resonance, motion of the nanoresonators relative to the substrate created a moving fringe pattern that was reflected back through the microscope objective, was redirected by the beamsplitter, and impinged on an AC-coupled photodetector (New Focus model 1601).

2.3. Biofunctionalization

In order to utilize such SiCN nanoresonators for the specific detection of biological targets, analyte-specific functional layers need to be immobilized onto their surface. The binding affinity of these layers is then assessed by monitoring the resonant frequency shifts related to any added mass on each individual device. The specific detection of protein-A using single domain antibody fragments (sdAb) was here chosen as model system. Protein-A is a 56 kDa surface protein originally found in the cell wall of *Staphylococcus aureus*. The protein has found widespread use in biochemical research because of its ability to bind immunoglobulins.

The resonator chips were cleaned in cold piranha (3:1 H_2SO_4:H_2O_2) just prior to the experiments and stored in DI water. The resonance frequency of all devices was assessed prior to the start of the functionalization procedure. The samples were then transferred into 100% ethanol for 2 min in a petri dish. This was followed by silanization in 2% 3-aminopropyl(triethoxysilane) (APTES) in ethanol (pH 5 adjusted using glacial acetic acid for 2 min). The samples were washed again in 100% ethanol for 5 min followed by blow drying and curing in an incubator @ 80 °C for