

Asymmetrical twin cantilevers for single molecule detection

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Recently, there has been growing interest in sensors based on frequency shift detection of mechanical resonators, that has led to extremely sensitive diagnostic methods for medicine and biology. Here the authors propose and demonstrate a strategy capable of detecting the presence of a few macromolecules, which uses an asymmetrical cantilever structure of several microns in size, operated at room temperature, and under ordinary vacuum conditions. The idea is to detect the presence of one or more molecules by detecting the mechanical cross-talk induced by the molecular link between a short cantilever and an array of longer cantilevers facing the short one. © 2007 American Institute of Physics. [DOI: 10.1063/1.2732200]

The use of nano- and microcantilevers as molecular sensors has emerged in the last few years after the pioneering work of a Swiss group showed that the absorption of DNA molecules caused the static deflection of an array of silicon cantilevers.¹ Since then, the rush towards single molecule detection has moved the focus of the research to the dynamic mode. Decreasing the size in the nanometric range, increasing the resonance frequency to gigahertz values, and forcing environmental conditions to ultra high vacuum and very low temperatures, all proved to be crucial issues in this context. Using a single cantilever or a single doubly clamped beam, single virus, single molecule, and zeptogram sensitivity have been demonstrated.²⁻⁴

A different route to improve the resolution consists in the fabrication of a resonating structure, in which the absorption of a target molecule would change the dynamical mode structure of the resonator rather than simply adding mass and shifting the resonance of a given mode. Only very few examples of this approach have appeared until now in the literature.^{5,6}

As an improvement to our previous work,⁵ in this letter we propose an alternative approach in which the basic idea consists in the fabrication of an asymmetric couple of cantilevers facing each other with submicron separation. The two cantilevers are sized differently, so that one, the shorter or driver, shows the highest resonance frequency while the longer, or follower, has a lower resonance frequency. The actual geometry used for our experiments is shown in Fig. 1(a): notice that the follower is flanked by two identical structures in order to reduce the center of mass displacement of the fundamental mode, reduce energy dissipation, and increase the frequency sensitivity. When the two cantilevers are connected by a molecular bridge, actuating the driver

induces a displacement also on the follower. When this system is operated out of resonance, the force needed to cause a detectable displacement largely exceeds the strength of the molecular bonds. When the system is, however, operated at the resonance frequency of the follower cantilever, the required force decreases dramatically and, in principle, the presence of a single molecule can be observed. The Q factor of an oscillator can be expressed as the ratio of the energy stored in the oscillator by the amount of energy dissipated during each cycle or, in other words, the work exerted on the cantilever to keep it in oscillation,

$$Q = E/\Delta E = \frac{1}{2}kx^2/Fx, \quad F = \frac{1}{2}kx/Q.$$

Using the typical values of $k=1$ N/m and $Q \sim 10^4$, it follows that a displacement of 10 nm in a micrometer sized cantile-

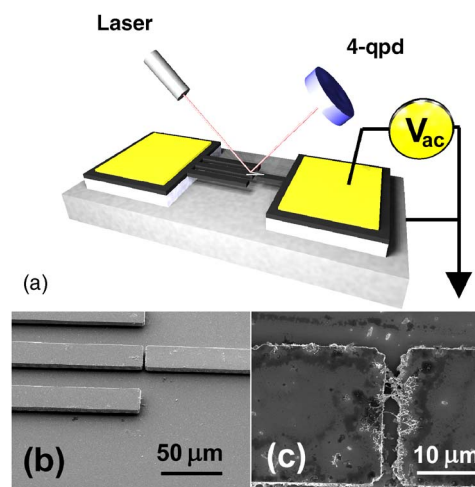


FIG. 1. (Color online) (a) Device geometry and experimental setup: cantilevers are actuated by applying an ac bias between the top layer and substrate. The response is detected by tracking the reflection of a laser with a four quadrant photodiode. The driver is sized $175 \times 20 \times 2.5 \mu\text{m}^3$ and has a nominal resonance frequency of 88 kHz. The three followers are sized $275 \times 20 \times 2.5 \mu\text{m}^3$ and have a nominal resonance frequency of 38 kHz. The separation between cantilever and substrate is determined by the thickness of the BOX layer (see text) and is about $2 \mu\text{m}$ (b) scanning electron microscopy image of the central part of the device. The gap between the two opposite cantilevers is $1.5 \mu\text{m}$. (c) An example of molecular link consisting of MWCNTs deposited by dielectrophoresis (see text).

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ver can be obtained with a force lower than 0.5 pN, largely below the strength of any chemical bond. A typical application of the device described above consists in the detection of organic macromolecules with different functional groups at the two ends, that can selectively absorb on the two opposite cantilevers, such as DNA, proteins, or protein suprastructures. It is worth to stress here that the detection mechanism is based on the formation of a mechanical link between two oscillating structures rather than in a simple change of mass. Therefore it would apply more properly to the detection of molecules with very low mass but high stiffness rather than heavy but soft ones.

The devices are fabricated from silicon on insulator wafers composed of a top *p*-type silicon layer 2.5 μm thick, with resistivity of 0.1 $\Omega\text{ cm}$, a buried oxide (BOX) layer of 2.0 μm thick, and a *p*-type silicon substrate. We used optical lithography, inductively coupled plasma dry etching, and HF based wet etching to fabricate the structures and suspend the cantilevers. The samples are first rinsed in water, then in methanol, and then gently blown dry with N_2 to minimize stiction. A detail of a fabricated structure is reported in Fig. 1(b). The HF etching also partially removes the BOX under the cantilever supporting pads; as a consequence, the triple follower cantilevers are mechanically interacting.⁶ Ohmic contacts to the top silicon layer have been fabricated by depositing a 10 nm Ni/90 nm Al film followed by rapid thermal annealing at 500 $^\circ\text{C}$ for 30 seconds. Contact resistance less than 10 Ω has been obtained.

The actuation and detection schemes are depicted in Fig. 1(a). When a bias difference is applied between the substrate and the cantilever—which are electrically insulated from each other by the BOX layer—the latter is pulled toward the substrate.⁷ By applying an ac voltage, the cantilever is forced to oscillate at the bias frequency. In order to avoid higher harmonics effects, the driver is forced to oscillate at the resonance frequency of the follower so that the driver is operated in a quasistatic regime and the operation of the device is simplified. The response of the follower is detected using the optical lever method in conjunction with a four quadrant photodiode (4-qpd), as used in atomic force microscopy with vertical sensitivity far better than 1 nm.⁸ A wave function generator (Stanford Research System DS345) and a lock-in amplifier (Stanford Research System SR830) are used to generate the ac voltage and to acquire the signal from the 4-qpd, respectively. In order to achieve a high Q factor and be sensitive to small frequency shifts, we operated the cantilever in a vacuum chamber with base pressure of 4×10^{-5} mbar.

A molecular bridge between the driver and follower was created on purpose, in order to test the efficiency of our detection scheme. Two different strategies have been followed. GaAs nanowires have been transferred by mechanical contacts directly from the growth substrate to the cantilevers, and multiwalled carbon nanotubes (MWCNTs) have been deposited from solution by dielectrophoresis. Both ways to bridge the structures gave positive results. We will, however, restrict ourselves in the following to MWCNTs, as they are a better approximation to a molecular link. After dispersing the MWCNTs in dimethylformamide (DMF) at a highly diluted concentration, a droplet of the dispersion was placed across the driver and follower cantilevers, followed by the application of a rf signal (5 MHz; 5 Vp.p.) for 5–20 min. The samples were then rinsed in DMF and subsequently in chlo-

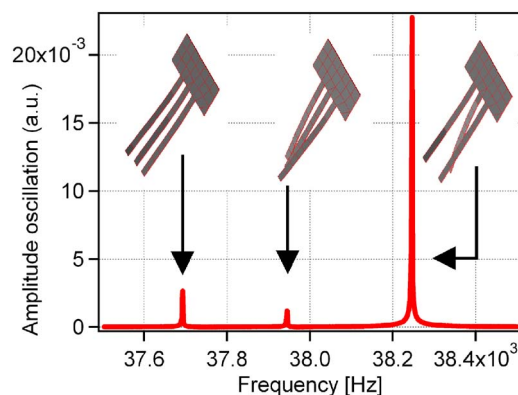


FIG. 2. (Color online) Typical frequency response of a device without any molecular link. The corresponding modes are drawn in the insets. The lower resonance is related to a mode where all three cantilevers move in phase, in the middle resonance the central cantilever does not move, while the left and right ones move in antiphase and finally, in the higher resonance, the central cantilever moves in antiphase with respect to the left and right ones. The third mode shows the highest amplitude because of the smallest displacement of the center of mass.

reform, in order to minimize the stiction of the suspended structures, and then blown dry with nitrogen. The amount of deposited MWCNT varied with solution concentration, solvent, and time of rf application, and only in few samples we deposited a countable number of MWCNT. However, we never observed deposition of MWCNT other than between the two extremities of the cantilevers. Examples of MWCNT molecular bridges are displayed in Fig. 1(c).

Let us consider first the behavior of the follower trio when actuated directly by applying an ac voltage between cantilevers and substrate. Because of the etching under the pad, the three nominally identical and degenerate oscillation modes split into three different modes with well defined symmetry. The frequency response of the follower trio measured on the central cantilever is shown in Fig. 2. The insets sketch the motion of the three modes. Notice that the mode at higher frequency is more pronounced because in this configuration the motion of the center of mass is very small and the dissipation at the mechanical constraints is minimized. The middle mode should not be detectable when measured on the central cantilever. However, small imperfections in the fabrication process may remove the symmetry and allow for a finite oscillation amplitude.

In Fig. 3(a) we display the frequency response of the follower trio of Fig. 1(c) measured on the central cantilever when actuated through the nearby driver. First of all we notice that the follower cantilevers indeed move because of the presence of the molecular link, while no motion was ever observed before the MWCNT deposition. Secondly we notice that the oscillation amplitudes are of the same magnitude for the three modes, which are consistent with the mechanical presence of a molecular link inhibiting high amplitude oscillations.

We then focused our attention on the evolution of the system under continuous “long term” actuation. Even if the force on the molecules is very small and the chemical bond with the surface or within the molecules cannot be broken, the molecules were subjected to thousands of push-pulls per second⁹ and eventually the molecules may detach from one of the two cantilever ends. Indeed in Fig. 3(b) we display the resonance curves at the second, third, and fifth experimental runs. The system clearly evolves toward narrower features

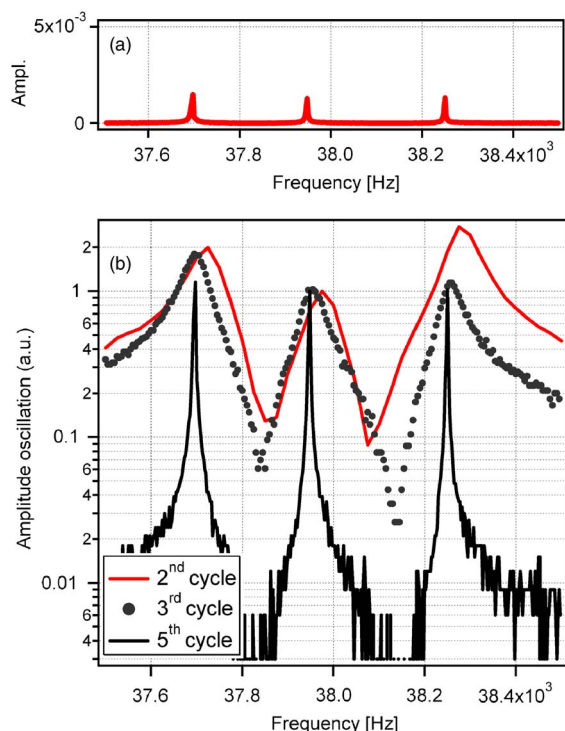


FIG. 3. (Color online) (a) Frequency response of the device in (c). The presence of the molecular link significantly attenuates the first and third modes. (b) Evolution of the frequency response after several measurement cycles.

and the peak position shifts toward lower resonance frequencies. The first effect can be explained considering that the molecular link is far from being ideal, indeed it is a highly dissipative link. Therefore the fewer the MWCNT across the gap, the lower the dissipation and the higher the Q factor. In order to understand the second effect, we should consider that the presence of MWCNTs on the cantilevers implies the added mass and added rigidity to the system.⁵ The two factors modify the frequency in opposite directions, but in our case the second contribution is dominant and the progressive reduction of the number of molecular links causes the decrease of the resonance frequency. The evolution of the Q factor and of the resonance frequency shift as a function of the number of cycles is plotted separately in Figs. 4(a) and 4(b). A dashed line indicates the Q factor and resonance frequency values relative to the device without MWCNTs.

Due to the unknown nature of the bond between CNT and silicon, that is, indeed the weaker point in the cantilever-CNT-cantilever link, it is rather difficult to know how many CNTs are present in each experimental run and therefore to propose an absolute number for the sensitivity of our device. A finite element analysis was, however, performed on this system, simulating the link between the cantilevers with small nanowires of different sizes and stiffnesses. Frequency shift of the order of those obtained experimentally in the third, fourth, and fifth runs has been reproduced by using a single nanowire with diameter of 20 nm and Young's modulus of 1 GPa. This parameter fits the typical values of polymers or structures made of proteins¹⁰ much better than those made with MWCNT, indicating that the behavior of our system is probably dominated by the MWCNT-silicon contact. In order to compare our results with mass-sensitive techniques, we estimate the amount of MWCNT in Fig. 1(c) to be less than $0.1 \mu\text{m}^3$ in volume and 260 fg in mass; this

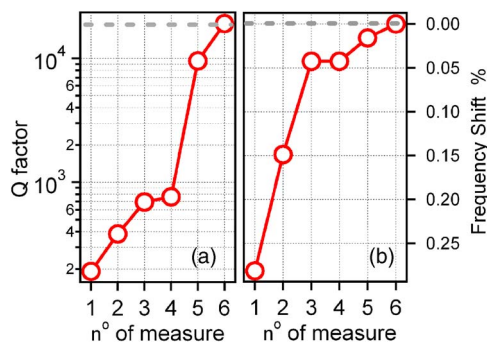


FIG. 4. (Color online) (a) Evolution of the resonance Q factor after several measurement cycles. (b) Plot of the frequency shift with respect of the first measurement for several measurement cycles. In both cases the gray dashed line refers to the device without molecular link.

compares favorably with the smallest feature—150 pg—measured with the mode localization approach.⁶ When comparing with mass detection based only on frequency shift, we rather take into account the ratio between the detected and the cantilever masses: in our case it is less than 1.6×10^{-5} while, for instance, in Ref. 3, it is 0.8×10^{-5} indicating that our strategy is at least equivalent.

In conclusion, we have demonstrated that, using an asymmetric cantilever system operated at room temperature and under normal vacuum conditions, in a driver-follower scheme, it is possible to detect the presence of a few molecules and to follow their evolution as they change in number or strength. We believe that with a proper control of the bonding chemistry, the sensitivity of our system could be improved to reach the single molecule level.

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⁹To give an estimation of the number of push-pull events in a single measurement run, the resonance spectrum of the third run in Fig. 3(a) consists of 200 points and each of them was integrated in 100 ms. Therefore the total number of push-pull events is greater than 1.5×10^6 even if not all have the same intensity.

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