

Nanomechanical mass sensing and stiffness spectrometry based on two-dimensional vibrations of resonant nanowires

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Nanomechanical resonators based on nanowires and nanotubes have emerged as promising candidates for mass sensors¹⁻⁶. When the resonator is clamped at one end and the atoms or molecules being measured land on the other end (which is free to vibrate), the resonance frequency of the device decreases by an amount that is proportional to the mass of the atoms or molecules. However, deposition on the free end is a situation not frequently found, and many biomolecules have sizes that are comparable to the size of the resonator, so the relationship between the added mass and the frequency shift breaks down⁷⁻¹⁰. Moreover, whereas a resonator fabricated by top-down methods can vibrate in just one dimension because it is shaped like a diving board, a perfectly axisymmetric one-dimensional nanoresonator such as a nanowire or nanotube can support vibrations with same amplitude and frequency in two dimensions¹¹. Here we propose a new approach to mass sensing and stiffness spectroscopy based on the fact that a molecule landing on a such a nanoresonator will break this symmetry and the vibration will become a superposition of two orthogonal vibrations with different frequencies. The measurement of the frequency degeneration breakage enables the determination of the adsorbate's mass and stiffness, and the azimuthal direction from which the adsorbate arrives. We experimentally demonstrate such sensing paradigm with resonant silicon nanowires, which serves to add kPa resolution in Young's modulus determination to their zeptogram mass sensitivity.

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The development of ultrasensitive mass spectrometers capable of characterizing the proteome at the single cell level with high accuracy will speed up the identification of disease biomarkers and the discovery of new drugs for the treatment of the disease^{12,13}. While resonators fabricated by top-down approaches have achieved mass sensitivities in the zeptogram range (10^{-21} g)^{8,14-16}, devices based on nanowires and nanotubes have approached the yoctogram scale (10^{-24} g)¹⁻⁶ and the ultimate detection limit corresponding to the mass of one single atom (1 Da = 1.66 yoctogram). Despite their small size, nanowires and nanotubes can be detected by optical methods, which opens a wide range of applications where the resonators can be operated in different environments without being in contact with the displacement sensors and readout circuitry^{5,6,17-19}.

In this work, we have investigated Si nanowires horizontally assembled and epitaxially clamped at the sidewalls of pre-patterned microtrenches on Si substrates by using vapour-liquid-solid growth mechanism²⁰. The stochastic Brownian displacement of the nanowires at room temperature was measured by using a home-made optical interferometer (Fig. 1a)^{5,6}. We selected nanowires protruding at 90° with respect to the sidewall where they were anchored (Fig. 1b). The length and diameter of the selected nanowires were in the range of 5-10 μm and 100-300 nm, respectively. The thermal fluctuations of the nanowires result in two close resonance peaks (Fig. 1c)^{5,6}. We have measured a frequency separation between peaks with a mean value of 0.7% (measurements in 60 different nanowires, Fig. 1c). To gain insight into the origin of the thermomechanical fluctuations and predict the changes in the resonant properties upon molecular adsorption, we adopt the Ritz formulation on basis of the linear, two dimensional small deformation elasticity theory^{21,22} (Supplementary Information). Si nanowires are axially oriented along $\langle 111 \rangle$ direction and exhibit a hexagonal cross-section formed by the $\{112\}$ planes. Theoretically, these nanowires vibrate at all planes of flexural vibration with the same frequency¹¹. However, a small imperfection in the cross-section breaks the symmetry of the nanowire and splits each flexural vibration mode in two close resonance peaks of similar amplitude. Each peak corresponds to the nanowire vibrating in two orthogonal planes. For the unloaded nanowire, it can be shown that the two orthogonal vibration modes are determined by the directions in which the cross-section area moments are maximum (I_{max}) and minimum (I_{min}).

It is useful, for the sake of a major understanding, to assimilate the nanowire cross-section to an ellipse with the major and minor radius given by R_{\max} and R_{\min} . The asymmetry factor defined as $\Omega \equiv \frac{R_{\max}-R_{\min}}{R_{\min}}$, can be simply obtained by calculating $\Omega = \frac{\omega_f - \omega_s}{\omega_f}$, where ω_f and ω_s are the faster and slower eigenfrequencies. If we consider the average difference of 0.7% observed in the measurements of the eigenfrequencies, this formula estimates a difference between the maximum and minimum cross-section diameter of our nanowires of only 0.7%. The observation of the frequency degeneration breakage critically depends on the mechanical energy loss. In air, the large hydrodynamic force brings about a very low quality factor (Q) of 5 that prevents the discernment of the splitting of the resonant frequency (Fig. 1c, inset). However, the Q in vacuum is of about 2000 as the suspended silicon nanowires exhibit almost defect-free structural quality, high surface smoothness and large clamp robustness. Hence a small asymmetry in the nanowire cross-section results into an observable degeneration breakage (Fig. 1c). A practical criterion to define the observation of the degeneration breakage is when the asymmetry factor $\Omega > 1/Q$. In vacuum, a small asymmetry in the cross-section of 0.1% (0.1 nm in 100 nm wide nanowires) is enough to observe the degeneration breakage.

Let us now study the effect of a molecular adsorbate on the nanowire at a longitudinal position z_0 with length $\Delta z \ll L$, and oriented with an azimuth angle β with respect to the fast vibration axis (Fig. 2a). We anticipate that the deposition will not only produce a change in the split eigenfrequencies but it will also induce a rotation of the vibration planes. These changes are derived by applying the Ritz formalism (Supplementary Information). It is useful to make the analysis of the molecular adsorption on the basis of three parameters: the sum of the relative shift of the eigenfrequencies, the difference in the relative shift of the eigenfrequencies, and the rotation angle of the vibration planes. In the case that the cross-section and thickness of the adsorbate are much smaller than those of the nanowire and the asymmetry factor is small, these parameters can respectively be written as,

$$\frac{\Delta\omega_s}{\omega_s} + \frac{\Delta\omega_f}{\omega_f} \cong \left\{ -\psi(z_0)^2 \frac{\rho_D}{\rho_{NW}} + \phi(z_0)^2 \frac{E_D}{E_{NW}} \right\} \frac{V_D}{V_{NW}} \quad (1)$$

$$\frac{\Delta\omega_f}{\omega_f} - \frac{\Delta\omega_s}{\omega_s} \cong \phi(z_0)^2 \frac{E_D}{E_{NW}} \frac{V_D}{V_{NW}} \cos(2\beta) \quad (2)$$

$$\Delta\alpha \cong \frac{\phi(z_0)^2}{2\Omega_0} \frac{E_D}{E_{NW}} \frac{V_D}{V_{NW}} \sin(2\beta) \quad (3)$$

where ψ and ϕ represents the non dimensionalized eigenmode amplitude and curvature, E is the Young's modulus, ρ is the mass density, V the volume and Ω_0 is the initial asymmetry factor. The subscripts D and NW refer to the deposited material and the nanowire, respectively. The eigenmode shape and curvature are well-known functions for beams with length to width ratio higher than 5 as it is our case (Supplementary Information)⁷. The sum of the relative frequency shifts, Eq. (1), is proportional to the volume of adsorbed material and it is independent of the azimuth deposition angle β . However, the proportionality constant depends on two opposite mechanisms, the added mass and the adsorbate stiffness that brings about a decrease and increase of the resonance frequency sum, respectively^{7,10}. The first mechanism scales up with the vibration amplitude, so that it dominates as the adsorption approaches to the free end. Conversely, the adsorbate stiffness effect is amplified by the vibration curvature, so that it is negligible near the nanowire free end and it is maximal at the fixed end. Interestingly, the difference between the relative frequency shifts, Eq. (2), only depends on the adsorbate stiffness, and it would be unaltered by a pure change in the nanowire mass. Changes in the eigenfrequency difference are more noticeable when the adsorption is near the clamping region and the deposition is along one of the vibration planes. Conversely, either adsorption near the nanowire free end or at angle close to 45 degrees with the vibration planes negligibly affect the difference between the relative frequency shifts.

The high axisymmetry shown by silicon nanowires makes that a minuscule adsorption can easily rotate the vibration planes (Eq. (3)). The optical interferometry technique is sensitive to the out-of-plane component of the nanowire displacement. Since the split eigenmodes vibrate in orthogonal directions with nearly the same amplitude, the measurement of the out-of plane amplitude readily allows to determine the vibration direction of the eigenmodes with respect to the optical axis. Thus, such measurement provides a new sensing parameter in highly symmetric nanomechanical resonators such as nanowires and nanotubes. This method is in line with a growing trend in nanomechanical sensing towards using amplitude variations in eigenmodes instead of, or in addition to, eigenfrequency shifts^{23,24}. The amount of rotation is inversely proportional to the asymmetry factor (Ω_0) and it depends on the deposition angle. In the limit of small depositions, the rotation is zero for depositions along one of the vibration planes and maximum when the deposition angle is 45 degrees with respect to the vibration planes. The sign of the rotation angle is defined so that the fast vibration axis approaches to the deposition axis. Since the rotation arises from the mechanical stiffness of the adsorbate, it is enhanced as the deposition approaches to the fixed nanowire end.

The degeneration breakage of the nanowire vibration modes provides a unique asset for measuring mass with ultra high sensitivity and classifying the adsorbate on the basis of its mechanical properties. In addition, for set-ups where adsorption arises from multiple sources, the technique can discriminate the source on basis of the deposition angle. The method requires knowledge of the adsorbate longitudinal position on the nanowire, that can be derived by measuring the eigenfrequency split in several eigenmodes^{7,9,10}.

To experimentally validate the proposed sensing paradigm, discrete masses were deposited on the nanowire by focusing an e-beam of a scanning electron microscope (SEM) on the wished region of the nanowire²³. The e-beam produces the slow deposition of an amorphous carbon layer through the dissociation of organic species present in the SEM chamber (Fig. 2b & c).

Figures 2d & 2e show the effect of successive carbon depositions of about 0.6 fg near the nanowire fixed end on the orientation of the fundamental vibration axes. The deposition was performed at an angle of 45 degrees with the optical axis (Fig. 2a). Initially, the vibration plane of the fast-mode is at $\alpha=-17\pm 2$ deg with the optical axis (green arrow in Fig. 2d). Each deposition rotates the fast vibration plane 7-12 degrees towards the deposition direction. Thus, when the fast vibration axis is at 45 degrees with the deposition axis, the vibration angle has the maximum mass sensitivity of about 50 attograms per degree. Once that the fast vibration direction reaches the deposition direction, further deposition does not induce a rotation of the vibration planes. In our set-up, the position sensitivity is of about 10 pm/Hz^{1/2}. By externally driving the nanowires to obtain resonant amplitudes of about 100 nm, the angle uncertainty can be reduced to 0.01 deg, which implies mass sensitivities of the order of 100 zeptograms. Notice that the adsorption-induced rotation of the vibration planes is a mechanical effect, and hence the mass sensitivity depends on the Young's modulus of the adsorbate (Eq. (3)).

In order to further exploit the sensitivity and the spectroscopic capability of the proposed technique, we studied the singular case in which the adsorption occurs along the fast vibration axis. In this case, the vibration axes do not rotate and the difference between eigenfrequency shifts exhibits the maximum responsivity to adsorption (Eq. (2)). By using this geometry, the mass and the mechanical properties of the adsorbate can be accurately determined by measuring the sum and difference of the relative shifts of the eigenfrequencies (Eq. (1) & (2)). Figure 3 shows the sum and difference of the relative frequency shifts as a function of the longitudinal deposition position for a

mass of about 3 fg (Fig. 3). The sum of the eigenfrequency shifts goes from a maximal and positive value when the deposition is near the clamp towards a minimal and negative value when the deposition is near the nanowire free end. The difference between the eigenfrequency shifts resembles the curvature of the fundamental eigenmode shape, i.e., maximum at the clamping region and negligible near the free end. We have fitted the experimental values (symbols) to the theoretical expressions (dashed lines in Fig. 3). A significant agreement between the theory and experimental data is obtained. Considering a density of the deposited carbon of 1950 Kg/m^3 , the fitting parameters imply a Young's modulus of the deposited material of about 55 GPa.

Assuming that the frequency sensitivity is limited by the thermomechanical noise²⁵, and using Si nanowires as those used here, the technique is able of measuring mass with zeptogram sensitivity and discriminating variations in the Young's modulus of about 0.1 kPa per femtogram of sample. Dry proteins have a Young's modulus in the 0.1-10 GPa range. Therefore the proposed technique could detect variations of 100 part per million in the Young's modulus of a single protein. This capability of resonant nanowires for measuring mechanical properties in addition to mass with ultrahigh sensitivity opens the door for relevant biomedical applications. It is increasingly clear the important role of the mechanical properties in relevant biological processes and pathogenic disorders²⁶⁻²⁸. As a paramount example, a single point mutation in the capsid protein of some viruses can significantly change the elasticity of the virus particle²⁹. In the case of the human immunodeficiency virus, its stiffness largely decreases during the maturation process, acting as mechanical switch in the infection process³⁰. We envision the application of resonant nanowires for detecting subtle protein changes at the level of single mutations that manifest as biological relevant mechanical changes.

To conclude, we have demonstrated that the small size and high cross-sectional symmetry of nanowire-based nanomechanical resonators offer a unique mechanism for weighing adsorbates and accurately classifying them by their mechanical properties. The proposed technique is based on the degeneration breakage in the vibration mode of a perfectly symmetric nanobeam when an infinitely small particle lands on the beam. Our results indicate that resonant Si nanowires meet the key requirements for developing an ultrasensitive biological spectrometer: high sensitivity, high mechanical selectivity, small size and hence small sample consumption, and capability for multiplexed detection. A technological bottle neck is the transport of intact biomolecules in vacuum to

the nanomechanical resonator. However, this difficulty has been recently circumvented by Roukes and co-workers implementing an electrospray injection system¹³.

Methods

Device fabrication. Silicon on Insulator (SOI) substrates with a <110> orientation of the 2 μm thick device layer were patterned by photolithography and reactive ion etching in order to define simple supporting microstructures at which <111> oriented sidewalls the nanowires were intended to grow. Previously to the nanowire growth, the substrates were coated with 80 or 150 nm diameter gold nanoparticles (British Biocell). Silicon nanowires were grown in an atmospheric pressure CVD reactor at 800 °C with 10% H_2/Ar as both the diluent and carrier gas. Flow rates of 270 and 45 s.c.c.m. were used respectively. The carrier gas was flown through liquid SiCl_4 bubbler kept at 0 °C in order to maintain a constant vapour pressure.

Optical interferometry. The Si die was mounted on a three-axis piezoelectric translation stage for positioning and scanning under the probe beam. The sample on the piezoelectric translation stage was placed into a small vacuum chamber pumped down to the 10^{-6} Torr range. A 5 mW He-Ne gas laser beam ($\lambda=633$ nm) was directed to the sample through an intensity filter, beamsplitter and a 50x objective with a numerical aperture of 0.55. The spot size was 0.6-0.8 μm . The optical power of the beam incident on the nanowires was of about 0.5 mW. Interferometric contrast arose between the laser beam reflected from the nanowires and that reflected from the substrate underneath. The reflected light was collected by the objective and detected by a photovoltaic silicon pin diode followed by a low-noise current to voltage preamplifier and high speed digitizer connected to a PC. The length and diameter of the investigated nanowires were 5-10 μm and 100-300 nm, respectively.

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Author contributions

J.T. and M.C. wrote this manuscript with inputs from all authors. All the authors analysed and interpreted the data. A.S. and M.F.-R built the CVD reactor and fabricated the devices. E.G. and D.R. performed the detection and mass adsorption experiments and collected the data. J.T., E.G. & D.R. developed the theoretical model. E.G., D.R. & J.T. designed the experiments. J. M. and R.G. designed the setup for SEM carbon deposition.

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Additional information

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FIGURE CAPTIONS

Figure 1 | Optical readout of the thermal displacement fluctuations of silicon nanowires. **a**, Schematic of the optical interferometer used to detect the out of plane nanowire vibrations. The nanowire longitudinal axis and incident laser beam are orthogonal. The nanowire sample was placed in a vacuum chamber at 10^{-6} Torr range and room temperature. The spot size was of about $0.7\ \mu\text{m}$ and the incident power of $0.5\ \text{mW}$. Picometer scale modulation of the height of the nanowire above the substrate results into a measurable intensity modulation due to the interference between the light reflected from the nanowires and from the substrate. **b**, Scanning electron microscopy image of a typical nanowire used in this work. We selected nanowires normal to the trench wall where they were anchored with a length and diameter in the range of $5\text{-}10\ \mu\text{m}$ and $100\text{-}300\ \text{nm}$, respectively. **c**, Fast Fourier transform of the amplified photodetector signal that is dominated by the displacement thermal fluctuation of the nanowires. The thermal fluctuations of the Si nanowires result in a single resonance peak in air that splits into two peaks in vacuum (inset). Depending on the nanowire dimensions, the resonance frequencies are in the range from 2 to $6\ \text{MHz}$. **d**, Histogram of the relative frequency separation between the two close resonance peaks observed in vacuum. The number of studied devices was 60 .

Figure 2 | Rotation of the fundamental vibration planes of silicon nanowires induced by attogram-scale adsorption. **a**, Schematic of the electron-beam induced deposition of carbon on the nanowires. In the chosen coordinate system, the y - and z - axes are set along the optical and nanowire longitudinal axes, respectively. The nanowire fundamental flexural vibration mode splits in two close resonance peaks of similar amplitude consisting on the nanowire vibrating in two orthogonal planes. The origin of this frequency splitting is ultrascale asymmetries in the nanowire cross-section. The vibration axes in which the nanowire vibrates at the lower and higher eigenfrequencies are referred to as slow and fast axes, respectively. The azimuth angle (xy plane) between the deposition axis and the fast axis is referred to as β . **b & c**, Scanning electron microscopy images of a $100\ \text{nm}$ thick nanowire prior (**b**) and after (**c**) electron-beam induced carbon deposition near the clamped end. The e-beam produces the slow deposition of an amorphous carbon layer through the dissociation of organic species present in the SEM chamber. In nanowires with a diameter smaller than $150\ \text{nm}$, we observed that the e-beam efficiently transmits through the NW producing an almost symmetric carbon deposition on the opposite side (bottom in the image) **d**, Evolution of the frequency spectra (top to bottom) of the nanowire thermal displacement after four depositions of about $0.6\ \text{fg}$ near the nanowire clamped end. The deposition was performed at an angle of about 45 degrees with the optical axis as indicated by the blue arrow. Each deposition rotates the fast vibration plane $7\text{-}12$ degrees towards the deposition direction as indicated by the green arrows. Notice that the middle panel only shows the peak corresponding to the fast eigenmode in which the nanowire vibrates along the optical axis. In this case, the slow eigenmode peak vanishes as the nanowire vibrates parallel to the silicon substrate, and the optical interferometric signal in our configuration is only sensitive to the out-of plane displacement. On the right, the orbits of the nanowire tip in the xy -plane are plotted. **e**, Angle between the fast vibration axis and the optical axis (symbol α in **a**) as a function of the added mass on the clamped end of the nanowire. The graph includes the data shown in fig. 2d.

Figure 3 | Effect of mass deposition on the nanowire along one of the fundamental vibration axes on the eigenfrequency splitting. **a**, Schematic of the carbon deposition on the nanowire. In this case, the deposition is along the fast-mode vibration axis. In this deposition geometry, the mass and mechanical properties of the adsorbate can be disentangled from the sum and difference of the relative frequency shifts. **b**, Sum and difference of the relative shifts of the split eigenfrequencies as a function of the deposition position along the nanowire. The deposited mass was $3\ \text{fg}$. The longitudinal z -position of the deposition was normalized to the nanowire length. The symbols represent the experimental data and the dashed red line the theory based on the Ritz method applied to the beam equation in two-dimensions (see text and supplementary information).