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Dohn, Søren; Greve, Anders; Svendsen, Winnie Edith; Boisen, Anja

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The influence of refractive index change and initial bending of cantilevers on the optical lever readout method

S. Dohn,[a)] A. Greve, W. E. Svendsen, and A. Boisen
Department of Micro- and Nanotechnology, Technical University of Denmark, DTU Nanotech, Building 345 East, DK-2800 Kongens Lyngby, Denmark

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It has been speculated that the initial bending of cantilevers has a major influence on the detector signal in a cantilever-based sensor using the optical lever readout method. We have investigated theoretically as well as experimentally the changes induced in the detector signal when the optical lever technique is used to monitor a cantilever with initial bending during changes in the refractive index of the surrounding media. We find that for changes in refractive index as small as 10^{-4} the detector signal is highly dependent on the initial bending of the cantilever. The findings are validated experimentally using an environmental chamber and varying the pressure. We sketch routes to circumvent the problem and formulas suitable for data treatment are given. © *2010 American Institute of Physics.* [doi:10.1063/1.3430065]

Cantilever-based sensing is a growing research area and cantilever-based sensors are applied to a variety of applications including detection of DNA,[1] RNA,[2] proteins,[3–5] the effect of drugs on methicillin-resistant bacteria[6] in water, and detection of explosives[7] and single cells[8] in air. Generally, cantilever-based sensors can be used to measure mass by monitoring their resonant frequency or to measure induced surface stress by monitoring their bending. The bending can be monitored using several different techniques including integrated piezoresistors,[9,10] optical interferometry,[11,12] and the optical lever technique.[13] The optical lever technique is a simple technique with possible resolution of less than 1 nm deflection of the cantilever.

Very often the cantilever-based sensors are operated in water or in a controlled gaseous environment, separating the optical readout and the cantilever-based sensor by a glass window. The refraction occurring at the glass interface due to index mismatch causes a change in the optical pathway, which is not related to a change in cantilever bending—in the following referred to as a spurious signal. Excellent investigations were done by Lang *et al.*[14,15] on the optical lever method in aqua solutions where the effect of refraction has been included for a fixed refractive index. Recently, Huang *et al.*[16] and Huber *et al.*[17] did similar work on the spurious signal arising from changes in the refractive index of the medium. Huber *et al.* used reference points on the cantilever chip and solid sidebars as reference signals to correct the measured signals[18,19] and thereby avoid the need for a theoretical data correction.

However, all the investigations above have been performed on cantilevers with negligible initial bending, why the influence of initial bending is not accounted for. Initial bending is mainly a problem when using very sensitive polymer-based cantilevers for biochemical detection: polymer-based cantilevers can be a factor of 20 more sensitive to surface stress changes than the traditional Si-based cantilevers due to the much lower Young's modulus. However, the sensitivity comes at the cost of increased initial bending—often in the range of 1–10 μm for a cantilever with a length of 500 μm.[20]

Moreover, the cantilevers are so sensitive to surface stress that a functionalization of individual cantilevers will change their bending with a magnitude depending on the specific surface coating and application method. Aligning to several differently functionalized cantilevers on a chip can be difficult due to differences in bending of several micrometers, which is often beyond the limit of detection for sensitive optical detection systems. Thus, using solid sidebars or the cantilever chip as a reference[18,19] can also prove very difficult.

Here, we investigate theoretically as well as experimentally the spurious signal arising from a change in refractive index when measuring on cantilevers with an initial bending. The system analyzed in the following is a cantilever-based gas sensor consisting of an environmental chamber with a glass lid and an optical lever readout. The system is chosen since the change of refractive index is easy to control and calculate from the pressure and gas composition. The results presented are equally valid to a fluidic system. We find that the spurious signals due to changes in the refractive index are highly dependent on the initial bending of the cantilever. A formula for correction of measured data depending on the refractive index and initial bending of the cantilever is given.

We consider an optical lever system as depicted in Fig. 1. The cantilever of length L is located $l_1 = 15$ mm below the optical window of an environmental chamber where the pressure and humidity can be controlled. A laser is mounted so that the laser beam hits the optical window at an angle of $\theta_1 = 40$ °C to the normal of the surface. The position of the laser can be manipulated so the focused laser beam hits the cantilever apex at an angle of $\theta_2 + \alpha_R$, where α_R is the angle of the cantilever surface to the normal of the optical window. The laser beam is reflected off the cantilever surface at an angle of

[a)]Electronic mail: soren.dohn@nanotech.dtu.dk.

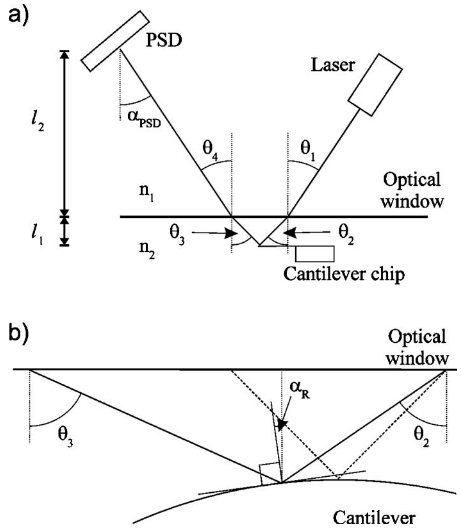

FIG. 1. Schematic of the optical lever system (a) with a close-up of the cantilever surface and (b) laser beam.

$$\theta_3 = \theta_2 + 2\alpha_R \tag{1}$$

and passes the optical window and leaves the glass at an angle of θ_4 and hits at a position s on a position sensitive diode (PSD) located at the distance $l_2=100$ mm from the optical window and rotated to an angle of $\alpha_{PSD}=40$ °C.

The position of the reflected laser beam on the PSD can be described by the simplified formula

$$s = [l_1(\tan\theta_2 + \tan\theta_3) + l_2\tan\theta_4]/\cos\alpha_{PSD}, \tag{2}$$

only neglecting the contribution from the vertical movement of the cantilever. (In the present setup this introduces an error of less than 0.1%.) Due to the index mismatch on either side of the optical window (n_1 outside and n_2 inside), the laser beam is refracted according to Snell's law

$$n_1\sin\theta_1 = n_2\sin\theta_2. \tag{3}$$

Using Eq. (3) the expression for s becomes

$$s = \frac{l_1(\tan\theta_2 + \tan\theta_3) + l_2\dfrac{\sin\theta_3}{\sqrt{(n_1/n_2)^2 - \sin^2\theta_3}}}{\cos\alpha_{PSD}}. \tag{4}$$

The refractive index of air depends heavily on the pressure, temperature, and humidity, and the theoretical conversion from pressure to refractive index is calculated from the work of Owens *et al.*,[21] using their formulas for dry CO_2-free air. The refractive index of dry CO_2-free air is to a very good approximation linear with pressure in the range of interest

$$n = 1 + 2.5834 \times 10^{-7}/\text{mbar P}. \tag{5}$$

To calculate the theoretical change in position, s, due to change in refractive index, n_2, the radius of curvature, R, of the cantilever must be known. The cantilever shape due to an induced surface stress can to a very good approximation[22] be described using Stoney's equation[23]

$$U(x) = \frac{3\sigma(1-\nu)}{E}\left(\frac{x}{t}\right)^2, \tag{6}$$

where σ is the difference in surface stress on the top and the bottom of the cantilever, E is Young's modulus of the cantilever material, ν is the Poisson ratio, t is the thickness, and x is the distance to the base of cantilever. Given a surface stress, σ, the apex of the cantilever will deflect the distance $U(L)$ (positive toward the optical window) and the angle, α_R, of the stressed cantilever tip to the initial cantilever tip will be given by

$$\tan\alpha_R = \left.\frac{\partial U(x)}{\partial x}\right|_{x=L} \approx 2\frac{U(L)}{L} = \frac{L}{R}, \tag{7}$$

where R is the cantilever radius of curvature $[R=L^2/2U(L)]$. To account for the vertical movement of the cantilever l_1 in Eq. (4) can be replaced by $l_1 - U(L)$.

The cantilevers we have tested are fabricated in the photoactive polymer SU8 having a Young's modulus of $E=4-5$ GPa and Poisson's ratio of $\nu=0.33$. The thickness of the cantilevers is $t=5.5$ μm with a length of $L=500$ μm. The cantilevers have an initial deflection in the order of $U(L)_i = \pm 10$ μm or $R_i = \pm 0.125$ m.

The optical lever system comprises an environmental chamber where pressure and humidity can be controlled, and a readout unit that can be placed on top of the chamber. The readout unit features two lasers and PSD systems that can be operated independently, thereby making it possible to measure on two cantilevers simultaneously. The PSD output signal is 64 μm/V and the voltage noise measured on a flat mirror corresponds to a deflection sensitivity of less than 1 nm. The pressure can be controlled to within ± 1 mbar and the minimum obtainable pressure in the chamber is 0.1 mbar.

A cantilever with an initial bending of $U(L) = -7$ μm was inserted in the setup with an angle $\alpha_i \approx 0$, and the environmental chamber was evacuated to $P=5$ mbar and flooded with nitrogen to $P=800$ mbar—this procedure was repeated 20 times to initialize the chamber. Afterward, the procedure was repeated 50 times while the voltage of the PSD preamplifier was recorded.

The results of the measurements on the polymer cantilever are shown in Fig. 2 for five repetitions of evacuation and purging. From the measurements it is obvious that several effects are present. The measured laser position on the PSD displays a repeatable dependence on the pressure and thereby refractive index of the atmosphere in the environmental chamber [Eq. (5)]. A linear trend between the refractivity and the position can be seen but there is also a clear hysteresis [best seen in Fig. 2(b)]. The hysteresis is due to the polymer material of the cantilever that absorbs and degasses during changes in pressure change and humidity.[24]

Thus, the cantilever bending is sensitive to pressure and humidity, and in order to separate the effect of the polymer absorption and desorption from the spurious signal arising from changes to the refractive index, optical mirrors are used as model systems. An optical mirror was inserted in the setup with an angle $\alpha_i \approx 0$ and the procedure described above was repeated for a flat mirror, a concave mirror with a radius of curvature of $R=-0.1$ m, and a convex mirror with a radius of curvature of $R=0.034$ m.

The result of the measurements on the flat mirror is shown in Fig. 3. The change in position is linear with the refractivity and pressure, but plotted logarithmic for clarity. Linear fits to each cycle of the measured data gives an average change in slope of $\beta_{av}=10.0$ nm/mbar with a standard deviation of $\sigma=0.29$ nm/mbar. Similar results for the convex and concave mirror are collected in Table I. The theoret-

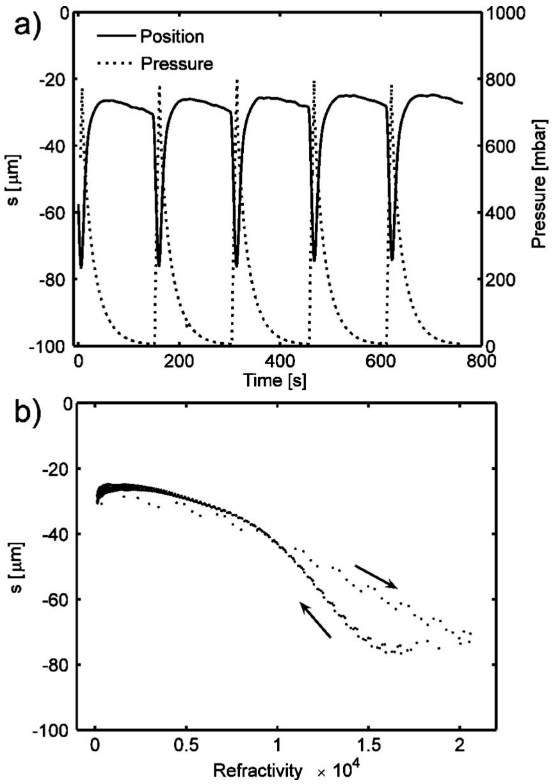

FIG. 2. The measured position on the PDS, s, as a function of the pressure measured using a noncoated polymer cantilever shown for five series of evacuation and purging with (a) nitrogen. The change in position for the same measurement as a function of the (b) refractivity (n_2-1).

ical change in position on the PDS, Δs, as a function of cantilever radius of curvature, R, when changing the refractivity from $n-1=1.3\times10^{-6}$ to $n-1=2.1\times10^{-4}$ (corresponding to a change in pressure from $P=5$ mbar to $P=800$ mbar) is plotted in Fig. 4. The results obtained from the measurements on the mirrors and cantilever are also plotted and a reasonable agreement is observed.

From Fig. 4 it is evident that even for a perfectly straight cantilever a change in the refractive index will be followed by a change in PSD position. Huang *et al.*[16] showed that by optimizing the setup this effect can be minimized for straight cantilevers. However, the change in PSD position can increase dramatically if the cantilever has an initial bending and there is no systematic way to avoid this. Cantilevers with a length of 500 μm and a bending of $U(L)=\pm10$ μm ($1/R=\pm80$ m^{-1}) will result in a signal change of more than 60 μm on the PSD with a change in refractive index smaller than 0.2%—nearly an order of magnitude more than for the perfectly straight cantilevers. This change in position on the PSD from the cantilever with an initial bending of $U(L)=\pm10$ μm corresponds to a real change in bending of the cantilever of roughly 50 nm in the present setup. Using cantilevers with an initial bending in measurements like the ones described by Huber *et al.*[17] where the change in refractive index is up to 0.9%, the spurious signal will be on the order of 0.5 μm.

TABLE I. Measured slope of the variations in PSD position as a function of the refractivity (n_2-1) β_{av}, with the spread in the values σ, and the corresponding calculated values β_{cal} for different mirrors.

Mirror	$1/R$ (m^{-1})	β_{av} (m)	σ (m)	β_{cal} (m)
Flat	0	0.039	0.001	0.042
Convex	29	0.167	0.002	0.170
Concave	−10	0.005	0.001	−0.002

The generated change in surface stress for a typical DNA immobilization is 4 mN/m,[25] and this change in surface stress will give rise to a cantilever bending of roughly 15 nm. This number is comparable to the signal obtained when changing the refractive index of the environment by only 10^{-4}—a change that easily happens in liquids due to changes in buffer solution or due to variations in temperature. Therefore, it is crucial to have control of pressure, humidity, and temperature during measurements in order to avoid spurious signals from the cantilever-based sensor.

The initial bending with rather small radius of curvature gives rise to large error signals when the refractive index of the environment is changed. The effect can be calculated and subtracted from the measurement data to obtain the real signal using Eq. (4) and inserting from Eqs. (1), (3), and (7). However, the method requires measurements of the refractive index of the environment or monitoring of gas compo-

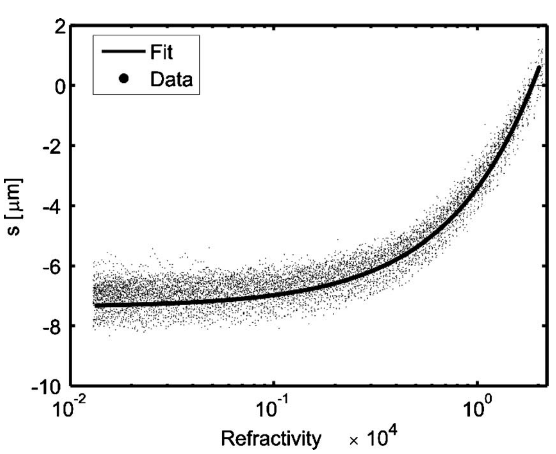

FIG. 3. The measured change in position on the PDS, Δs, as a function of the refractivity using a flat optical mirror. The full line is the fitted average change in position due to the change in refractive index.

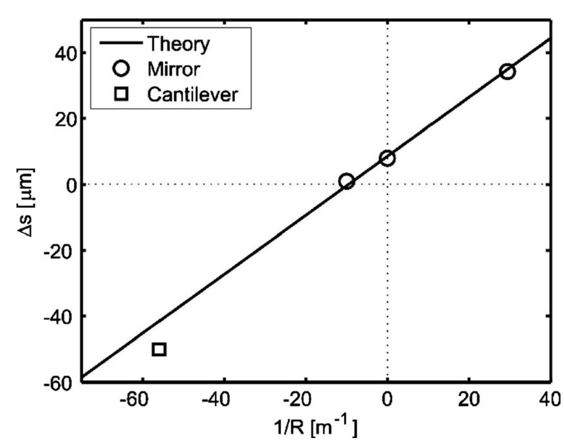

FIG. 4. The theoretical change in position on the PDS, Δs, as a function of cantilever radius of curvature, R, when changing the refractivity from $n-1=1.3\times10^{-6}$ to $n-1=2.1\times10^{-4}$. The corresponding measured values for the three different mirrors are plotted as well as the value for the cantilever.

sition, temperature, and pressure. Using this approach, it is necessary to measure the initial bending of the cantilever, which could be done by scanning the full cantilever[18,26] or by simply measuring the bending using an optical microscope before the measurement. A second approach is to use a reference cantilever with the same initial bending and subtract the signals as done by Jeon and co-workers.[19,27] However, this is not straightforward to obtain using polymer cantilevers if the measurement and reference cantilever are functionalized differently. A third approach is to use soft cantilevers with a stiff reflecting pad at the apex as described by Yue *et al.*[28] With a design like this, the $1/R \approx 0$ and the effect of changes to the refractive index will always be similar to that of the perfectly straight cantilever regardless of initial bending.

We have investigated theoretically as well as experimentally the changes induced in the detector signal when the optical lever technique is used to monitor cantilever bending during changes to the refractive index of the surrounding gas. We have shown that the use of cantilevers with significant initial bending ($R < 0.1$ m) as a cantilever-based sensor poses some challenges when the refractive index is changed as little as 10^{-4}. We have sketched some routes to circumvent the problems by changing design or by subtracting a calculated spurious signal from the measurement results.

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