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# Noninvasive determination of optical lever sensitivity in atomic force microscopy

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Atomic force microscopes typically require knowledge of the cantilever spring constant and optical lever sensitivity in order to accurately determine the force from the cantilever deflection. In this study, we investigate a technique to calibrate the optical lever sensitivity of rectangular cantilevers that does not require contact to be made with a surface. This noncontact approach utilizes the method of Sader *et al.* [Rev. Sci. Instrum. **70**, 3967 (1999)] to calibrate the spring constant of the cantilever in combination with the equipartition theorem [J. L. Hutter and J. Bechhoefer, Rev. Sci. Instrum. **64**, 1868 (1993)] to determine the optical lever sensitivity. A comparison is presented between sensitivity values obtained from conventional static mode force curves and those derived using this noncontact approach for a range of different cantilevers in air and liquid. These measurements indicate that the method offers a quick, alternative approach for the calibration of the optical lever sensitivity. © 2006 American Institute of Physics. [DOI: 10.1063/1.2162455]

#### **I. INTRODUCTION**

Atomic force microscopy<sup>1</sup> (AFM) is commonly used for measuring forces such as colloid and surface force interactions (e.g., electrostatic and van der Waals) and inter/ intramolecular forces of single biomolecules (e.g., DNA and proteins). With AFM, the interaction forces are detected by measuring the deflection of a microfabricated cantilever, which essentially acts as a soft spring, as it interacts with the sample. Most standard commercial AFMs use the optical lever method to detect the vertical deflection of the cantilever because it achieves resolution comparable to interferometer techniques while remaining inexpensive and easy to use. This optical lever method relies on a laser beam being reflected off the back of the cantilever into a position-sensitive photodectector (PSD) that records changes in the laser PSD position as a voltage V relative to the angular cantilever

deflection.<sup>2,3</sup> The measured voltage is then converted into cantilever deflection using a measured sensitivity value  $(mV^{-1})$  or inverse optical lever sensitivity<sup>4</sup> (InvOLS). Knowledge of the cantilever deflection  $\Delta D$  and spring constant of the cantilever k then allows for simple calculation of the interaction force given by Hooke's Law, where  $F = k\Delta D$ . Several techniques exist for calibration of the spring constant, including the Cleveland method,<sup>5</sup> thermal noise method,<sup>6</sup> and Sader method.<sup>7,8</sup> For a review of these calibration methods, see Ref. 9. Calculating the InvOLS typically requires that a force measurement be performed on a hard surface to measure the voltage response of the PSD as a function of the known distance moved by the piezo. For these InvOLS measurements, the amount of cantilever deflection is known to be equivalent to the distance moved by the piezo, as the cantilever is pushed into the surface. Therefore, the tip-surface contact region in the resulting voltage versus distance curve is observed as a linear slope. The inverse of the slope can then be given as the InvOLS.

Due to the essential need and relative ease of determining the InvOLS from a force measurement, the procedure is routinely and widely used by AFM users. However, there are

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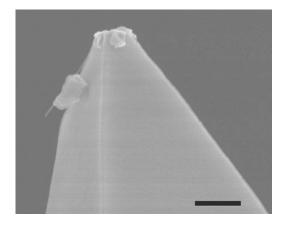


FIG. 1. Scanning electron microscope image Nanosensor EFM-PPP cantilever tip functionalized with a carbon nanotube that was damaged after performing force measurements on a hard substrate. Scale bar=600 nm.

a number of complications and disadvantages associated with this measurement that form the motivation for this study and are discussed below. Firstly, (1) if at any time during an experiment the laser spot position or deflection signal is changed, then a sensitivity measurement must be performed. This may not be practical during an experiment. (2) Particularly for biological AFM, it may be the case that the sample substrate is soft. Force measurements on soft surfaces show a nonlinear response in the tip-sample contact region and thus do not allow for clear determination of the sensitivity. This problem is overcome by performing a measurement before or after the experiment on a different substrate that is hard. However, taking a measurement before the experiment is undesirable as it may cause tip damage, especially when the end of cantilever tip has been specially functionalized (e.g., with a ligand or carbon nanotube). Likewise, performing a measurement after the experiment may cause tip damage rendering it unusable for future experiments (see Fig. 1). (3) Biological samples may also contaminate the tip with a soft material causing a nonlinearity in the contact region of the force curve, as mentioned above. In this case, any subsequent measurements on a hard substrate may be affected. (4) In contrast to static force measurements, determination of the InvOLS to calculate the oscillation amplitude for dynamic measurements is more ambiguous. This is because the contact region in the amplitude curve is not always precisely linear, and may vary depending on the percentage of the set-point amplitude relative to the free amplitude. The sample damping behavior of the oscillating cantilever (i.e., the slope of the amplitude-distance curve) also depends on the quality factor of the cantilever. For high Q values  $(> \sim 150)$  it is linear but below that, as is usually the case in fluid, this condition is not met.

Early attempts to address this problem were performed by D'Costa and Hoh<sup>10</sup> who showed that a change in the PSD voltage as a function of a fixed displacement of the PSD correlated linearly to the measured optical lever sensitivity. This result was obtained by performing a static mode force curve to measure the InvOLS with a maximized sum signal, subsequently measuring the PSD voltage change for a fixed displacement of the PSD and then repeating the measurement after changing the lateral laser position on the cantilever (i.e., parallel to the base of the beam). A plot of the PSD shift versus the InvOLS values provided a linear calibration curve for a cantilever of given length, where the slope could be used to determine the InvOLS for a different cantilever of the same length with only a knowledge of the PSD shift required. Although this method can be performed without touching the sample and is suitable for all cantilever geometries, it relies on having to obtain a calibration curve for each cantilever of a given length and is noted to be dependent on the configuration of the AFM instrument. More recently, Craig and Neto<sup>11</sup> introduced a noncontact technique to calibrate the spring constant of colloid probes using hydrodynamic forces, a concept that was later adapted to measure the InvOLS for standard cantilevers,<sup>12</sup> while other noncontact methods used typically require that specific designs and adjustments be made to the optical detection system.<sup>13</sup> Lastly, with the advent of the method of Sader et al.<sup>7,8</sup> it became apparent that the unknown InvOLS could be noninvasively measured without touching the surface,14 an approach which we investigate in this study.

Specifically, we combine two existing spring constant calibration techniques to calibrate the InvOLS, which only requires a measurement of the thermal noise spectrum of the cantilever and has the advantage of being a push-button noncontact method. To assess the accuracy of this noncontact approach, we explore different cantilevers in air and liquid. The validity of this method is established by comparison with the standard contact method discussed previously.

#### **II. PROCEDURE FOR DETERMINING INVOLS**

The procedure for determining the InvOLS of rectangular cantilevers is now described.

First, the thermal noise spectrum of the cantilever is measured from the photodetector output, and the fundamental mode is fitted to the power response function S(f) of a simple harmonic oscillator:

$$S(f) \cong P_{\text{white}} + \frac{P_{\text{dc}} f_R^4}{(f^2 - f_R^2)^2 + \frac{f^2 f_R^2}{O^2}},$$
(1)

where *f* is the frequency (Hz),  $f_R$  is the resonance frequency (Hz), *Q* is the quality factor,  $P_{dc}$  is the dc power response (V<sup>2</sup> Hz<sup>-1</sup>) of the cantilever measured from the photodetector, and  $P_{white}$  is a white-noise floor (V<sup>2</sup> Hz<sup>-1</sup>). A least-squares fitting procedure is used to determine the unknown parameters in Eq. (1).<sup>8</sup>

The equipartition theorem applied to the fundamental mode then requires

$$\frac{1}{2}k_B T = \frac{1}{2}k\langle z^2 \rangle,\tag{2}$$

where k is the spring constant of the cantilever,  $\langle z^2 \rangle$  is the mean-square deflection of fluctuations of the cantilever,  $k_B$  is the Boltzmann constant, and T is the absolute temperature.

Integrating the second term in Eq. (1) over all frequencies, and incorporating the definition of InvOLS, which is the ratio of the cantilever deflection to the measured photodetector voltage, leads to the result

TABLE I. Comparison of the mean "contact" InvOLS with the "noncontact" InvOLS values obtained in air. A comparison between the noncontact and "converted static-to-dynamic" InvOLS (indicated as "converted") is also given, with the percentage relative errors given in parentheses. The converted InvOLS represents the dynamic sensitivity obtained by correcting the contact InvOLS using the correction factor stated. Corresponding calibrated spring constant *k* values of the cantilevers are shown. *Q* and *f* are the quality factor and resonant frequency in air, respectively. Properties of air:  $\rho_f = 1.18 \text{ kg m}^{-3}$  and  $\eta = 1.86 \times 10^{-5} \text{ kg m}^{-1} \text{ s}^{-1}$ .

Cantilever No.		$L(\mu m)$	$b(\mu m)$	Aspect ratio	Q	f(kHz)	Sader k(N m <sup>-1</sup> )	Contact InvOLS (nm V <sup>-1</sup> )	Noncontact InvOLS (nm V <sup>-1</sup> )	Converted InvOLS (nm V <sup>-1</sup> )
1	Mikromash CSC 38 B	350	35	10.0	46.9	11.7	0.079	150.9±0.1	177.2±2.8	164.5(7.7%)
2	Mikromash CSC 38 C	300	35	8.6	57.8	15.7	0.123	$113.6 \pm 0.1$	137.1±1.8	123.9(10.7%)
3	Mikromash CSC 38 A	250	35	7.1	78.1	21.9	0.219	$84.7 \pm 0.2$	$98.4 \pm 1.1$	91.7(7.3%)
4	Mikromash NSC 36 C	130	35	3.7	223.1	83.2	2.12	$51.0 \pm 0.2$	$62.7 \pm 0.2$	55.6(12.8%)
5	Mikromash NSC 36 A	110	35	3.1	253.5	116.7	3.31	$39.9 \pm 0.1$	$52.1 \pm 0.1$	43.5(19.8%)
6	Mikromash NSC 36 B	90	35	2.6	338.6	171.8	6.41	33.7±0.2	44.3±0.1	36.8(20.5%)

$$\langle z^2 \rangle = \frac{\pi}{2} \text{InvOLS}^2 f_R P_{\text{dc}} Q.$$
 (3)

Substituting Eq. (3) into Eq. (2) then gives the required result

InvOLS = 
$$\sqrt{\frac{2k_BT}{\pi k f_R P_{\rm dc} Q}}$$
. (4)

In Eq. (4), the spring constant k must be known in order to calculate the InvOLS. We calculate k using the method of Sader *et al.*<sup>7,8</sup> which relies on the known parameters of  $f_R$  and Q obtained from the original simple harmonic oscillator (SHO) fit. The method of Sader *et al.*<sup>7,8</sup> typically requires that the cantilever be in air.

For the purpose of clarity, we henceforth refer to the InvOLS value obtained in Eq. (4) as being the "noncontact" InvOLS and that obtained using the conventional approach involving contact of the cantilever with the surface as the "contact" InvOLS.

#### **III. EXPERIMENTAL METHODS**

To assess the accuracy of this noncontact method, we tested six different cantilevers in both air and liquid by making a comparison with contact InvOLS values determined from force measurements taken on a hard substrate using an MFP-3D AFM (Asylum Research, Santa Barbara, CA). First, a number of static mode force curves (n=10) were taken for each cantilever in air on a cleaned glass slide, and the mean contact InvOLS was then calculated from the linear slope of the tip-sample contact region in the deflection volts versus distance curves. After taking force measurements, the cantilever was raised well above the surface, without changing the laser position or deflection signal, and the thermal noise power spectrum of the cantilever was obtained by Fourier transforming the cantilever deflection signal that had previously been digitized. In addition, it was ensured that the  $P_{dc}$ units of the thermal power spectrum remained  $V^2 Hz^{-1}$ , as required. For liquid measurements, static mode curves were taken on a freshly cleaved mica substrate in milli-Q water with a resistivity of 18.3 M $\Omega$ . A thermal power spectrum of the cantilever and subsequent SHO fitting were performed according to the methods described above.

Regardless of whether the noncontact InvOLS was required in air or liquid, the spring constant in Eq. (4) was obtained using the method of Sader et al. in air. This ensured that an accurate k value was used, as the requirements for the Sader calibration method are easily achieved in air. In particular, it is important that the Q factor of the cantilever greatly exceeds unity (i.e.,  $\approx > 10$ ). Commercial cantilevers with spring constants ranging from  $\approx 0.08$  to 6.5 N/m were used for all measurements and corresponded to the use of cantilevers for various AFM applications. Nominal manufacturer's values for the length L and width b of all cantilevers were used in the calculations. All cantilevers were chosen so as to meet the required criteria for the Sader method, namely, they were rectangular in shape, had a  $Q \ge 1$ , and their length greatly exceeded the cantilever width. The latter is defined by the aspect ratio (i.e., L/b), where the lowest limit tested for the applicability of the calibration method has been 3.3.<sup>8</sup> It is noted that both cantilevers 5 and 6 used here have lower aspect ratios of 3.1 and 2.6, respectively.

#### **IV. RESULTS AND DISCUSSION**

Tables I and II show a comparison of the mean contact and noncontact InvOLS values for all cantilevers in both air and liquid, respectively. Errors in the mean contact InvOLS arise from variations in the linear slope of the tip-sample contact region (e.g., due to stick-slip motion of cantilever on the substrate) of repeated independent force curves for each cantilever. Errors given for the noncontact value are estimated by fitting errors of the SHO for each cantilever. Values for the plan view dimensions, aspect ratio, Q,  $f_R$ , and k of the cantilevers are also included in both tables. For all cantilevers, in both air and liquid, the noncontact InvOLS values were found to be greater than the measured contact values with a mean difference of  $14.4\pm1.7$  nm V<sup>-1</sup>(±SE) recorded.

Greater noncontact InvOLS values are expected due to the difference between the mode shapes of static and dynamic bending of the cantilever.<sup>15</sup> Therefore, the noncontact (i.e., thermally driven cantilever) and contact (i.e., statically end-load cantilever) InvOLSs represent the dynamic and static sensitivity of the cantilever, respectively. Based on the slopes at the end of the cantilever for each situation, a correction factor has been used to relate the two sensitivities (i.e., InvOLS<sub>dynamic</sub>=1.09InvOLS<sub>static</sub>.<sup>16</sup> A more accurate approximation of the correction factor can be determined by knowing the laser spot size diameter and its position along

TABLE II. Comparison of mean "contact" InvOLS with the "noncontact" InvOLS values obtained in liquid. A comparison between the noncontact and "converted static-to-dynamic" InvOLS (indicated as "converted") is also given, with the percentage relative errors given in parentheses. The converted InvOLS represents the dynamic sensitivity obtained by correcting the contact InvOLS using the correction factor stated. Corresponding calibrated spring constant *k* values of the cantilevers are shown. The *k* values given are those obtained from the measurements in air (see Table I). *Q* and *f* are the quality factor and resonant frequency in liquid, respectively. Properties of liquid:  $\rho_f = 997 \text{ kg m}^{-3}$  and  $\eta = 8.59 \times 10^{-4} \text{ kg m}^{-1} \text{ s}^{-1}$ .

Cantilever No.		$L(\mu m)$	$b(\mu m)$	Aspect ratio	Q	f(kHz)	Sader k(N m <sup>-1</sup> )	Contact InvOLS (nm V <sup>-1</sup> )	Noncontact InvOLS (nm V <sup>-1</sup> )	Converted InvOLS (nm V <sup>-1</sup> )
1	Mikromash CSC 38 B	350	35	10.0	2.2	3.5	0.079	88.1±0.3	$105.2 \pm 3.3$	96.0(9.6%)
2	Mikromash CSC 38 C	300	35	8.6	2.4	5.1	0.123	$79.6 \pm 0.9$	$97.9 \pm 2.8$	86.9(12.6%)
3	Mikromash CSC 38 A	250	35	7.1	2.8	7.7	0.219	$58.1 \pm 0.4$	$71.9 \pm 1.2$	63.4(13.5%)
4	Mikromash NSC 36 C	130	35	3.7	4.5	36.1	2.12	$52.6 \pm 0.1$	63.6±0.7	57.3(11.0%)
5	Mikromash NSC 36 A	110	35	3.1	5.3	53.1	3.31	$27.9 \pm 0.2$	$35.7 \pm 0.3$	30.4(17.3%)
6	Mikromash NSC 36 B	90	35	2.6	6.1	83.3	6.41	$21.5 \pm 0.2$	$28.8 \pm 0.2$	23.4(22.8%)

the length of the cantilever.<sup>4</sup> We assumed the correction factor shown here to convert the contact InvOLS values into a dynamic sensitivity in order to make a direct comparison with the noncontact InvOLS, since the laser spot diameter was smaller than the cantilever length and laser spot was positioned at the very end of each cantilever.<sup>4</sup> The converted value shall henceforth be referred to as the "converted staticto-dynamic" InvOLS. In doing so, we found good agreement between the converted static-to-dynamic and noncontact InvOLS values for all cantilevers in both air and liquid (last column in Tables I and II). The noncontact values revealed a mean difference of  $8.5 \pm 0.8$  nm V<sup>-1</sup>( $\pm$ SE) from the converted values, with all cantilevers agreeing to within approximately <20%. Obviously, the noncontact or dynamic InvOLS can be converted to a contact or statically measured InvOLS. Thus, both the dynamic and static sensitivities can be obtained using the approach outlined here. It is noted that increased errors for cantilevers 5 and 6 (approximately double) most likely arise due to their lower aspect ratio values, which are considered to be low for the applicability of the spring constant calibration method. For these two cantilevers, the spring constant would be underestimated which causes an overestimation of the noncontact InvOLS. In addition, the accuracy of the noncontact InvOLS is governed by the error ( $\approx 10\%$ ) in spring constant calibration measurements. We used nominal manufacturer's values for the cantilever dimensions, which introduce potential errors of 5% and 10% for the length and width, respectively. In addition, small errors in the resonance frequency and Q arise due to error in the SHO fitting. For the method described here, we are combining two calibration measurements (i.e., Sader and thermal noise methods), thus the observed errors may be additive in origin of the two methods.

Finally, Fig. 1 shows a scanning electron microscope image of a cantilever tip functionalized with a multiwall carbon nanotube that was severely damaged after performing amplitude and static mode force measurements on a hard substrate in liquid. Damage such as this is most prevalent for sharp, delicate silicon tips with high spring constant cantilevers (i.e., noncontact levers), while it is possible that biomolecule and/or self-assembled monolayers coated on softer cantilevers typically used for biological applications may also be adversely affected if the part of the tip end is removed or contaminated. This observation clearly highlights the potential for tip damage when performing these sorts of InvOLS force measurements and subsequent possibility of rendering tips useless for further experiments. The advantage of the present noncontact approach is thus self-evident in totally eliminating such damage.

In conclusion, this method provides a quick, noncontact approach to calibrate the dynamic and static InvOLS in both air and liquid. Although other noncontact methods exist, this noncontact method does not require any prior invasive calibration measurements or adjustment of the optical stage (i.e., laser position or PSD). After calibrating the cantilever spring constant in air, the optical lever sensitivity can be determined at any time in both air and liquid for a cantilever that is in position ready for immediate imaging or force measurements. We stress that the prescribed method is suitable for levers with spring constants that can be accurately determined using the calibration technique used here, although it can be extended to other cantilevers provided the spring constant can be accurately and independently calibrated. The only requirement for this method is a measurement of the thermal power spectrum of the cantilever, which is now becoming a standard feature on many commercial AFMs, such as the one used in this study. This method should be of general use to the AFM community, particularly those researchers undertaking force measurements in liquid on deformable or biological systems.

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