"Torsional tapping" atomic force microscopy using T-shaped cantilevers

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Torsional oscillation of atomic force microscope cantilevers has been shown to offer increased optical lever sensitivity, quality factor, resonant frequency, and stiffness as compared to flexural oscillation. In this letter, T-shaped cantilevers are oscillated torsionally to give a tapping motion at the tip. This gives many of the advantages of small cantilevers, without the requirement for specialized detection optics. In order to demonstrate the capability of this technique, high resolution images of LH2 membrane protein crystal structures are presented. Reduced settle time and tip-sample force under error signal are also demonstrated. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3126047]

Since the invention of atomic force microscopy (AFM),[1] systems and methodologies have been employed to increase the signal-to-noise ratio and the sensitivity of the technique in order to measure smaller forces, reduce the force applied to the sample by the tip, and increase the resolution obtainable. In oscillatory AFM techniques the force sensitivity has been shown to depend critically upon the resonant frequency, amplitude, quality factor (Q), and stiffness of the cantilever used.[2,3]

Torsionally oscillating cantilevers have previously been shown to have increased resonant frequency and Q *(*Refs. 4 and 5) when compared to flexurally oscillating cantilevers. Sahin *et al.*[6] have utilized flexurally oscillating T-shaped cantilevers with laterally offset tips to give access to the higher harmonics of the flexural oscillation via the increased bandwidth of the torsional mode. Various cantilever geometries not conforming to the ubiquitous rectangular and triangular shapes commonly used for AFM (see Ref. 7, for example) have also been reported, with the aim of increased resonant frequency, reduced hydrodynamic damping, reduced spring constant, increased deflection sensitivity, or all of the above.

Here, commercially available T-shaped cantilevers designed for Harmonix™ (Ref. 8) imaging are driven in their first torsional mode to give vertical oscillation at the tip. The torsional amplitude is monitored and held constant by adjusting the tip-sample separation in the same way as the flexural amplitude is used in tapping mode. Henceforth, in this letter, this mode of operation will be referred to as "torsional tapping." The advantages of this approach are outlined below.

The minimum force gradient that may be measured by slope detection has been shown to be[3]

$$\delta F'_{\min} = (2k_1 k_B TB/\omega_0 Q\langle z_{\mathrm{osc}}^2\rangle)^{1/2}, \tag{1}$$

by assuming that thermal oscillation of the cantilever is the dominant noise source. Here $\delta F'_{\min}$ is the minimum detectable force gradient, k_1 is the cantilever spring constant, k_B is Boltzmann's constant, T is the absolute temperature, B is the bandwidth of the measurement, ω_0 is the angular resonant frequency, Q is the quality factor, and $\langle z_{\mathrm{osc}}^2\rangle$ is the mean square oscillation amplitude. From Eq. (1) it may be seen that force sensitivity is reduced by increasing the spring constant and measurement speed, but increased by increasing the resonant frequency and quality factor. It has been previously shown that the maximum possible measurement bandwidth is[3]

$$B_{\max} = \omega_0/2Q, \tag{2}$$

where $2Q/\omega_0$ is the "ring down" time constant associated with transient oscillations of the cantilever, as may be caused by perturbations in the tip-sample force.

Increases in resonant frequency and Q while decreasing k_1 have previously been affected by reducing the size of cantilever sensors;[9] however this has required the construction of dedicated optical lever detection systems with small laser spot sizes. The spring constant for torsional bending of the cantilevers used here (Mikromasch TL01) is estimated to be approximately 15 N/m.[10,11] If the temperature, amplitude, and measurement bandwidth are held constant, and the resonant frequencies and quality factors from Figs. 1(b) and 1(c) are inserted into Eq. (1); the minimum force gradient detectable with this cantilever in air in torsional tapping is a factor of 0.6 smaller than could be detected by the same cantilever in flexural tapping under the same conditions. It has also been shown that operating at higher tapping frequencies decreases the response time of the feedback electronics for typical AFM systems.[12]

For the cantilevers used here, the resonant frequency of the first torsional mode is typically increased by a factor of 10, as compared to the first flexural mode and the Q by a factor of approximately 4.5 [see Figs. 1(b) and 1(c)]. As the increase in frequency is larger than the increase in Q, Eq. (2) states that the maximum available measurement bandwidth is increased by a factor of 2.5. In order to verify this figure experimentally, a T-shaped cantilever was driven into oscillation at resonance well above the sample surface using the same setup described in Ref. 5. This comprises a homebuilt cantilever holder incorporating two magnetostrictive actua-

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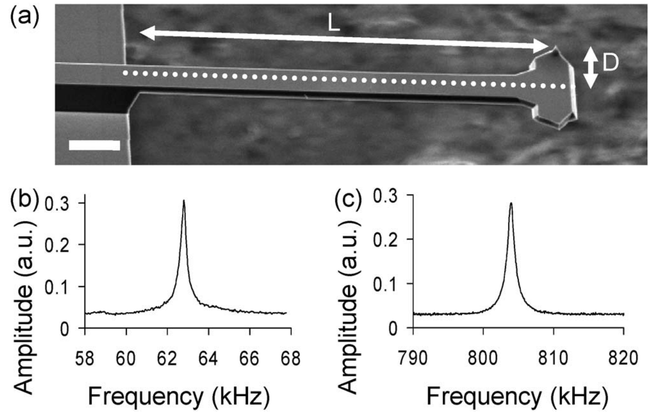

FIG. 1. (a) Scanning electron micrograph of T-shaped cantilevers used in this work (Mikromasch TL01), showing the cantilever length (l) and tip offset (d). The dotted line marks the torsion axis. Scale bar 10 μm. (b) The first flexural and (c) torsional resonances of a T-shaped cantilever. The resonant frequencies are 62.8 and 804 kHz and quality factors are 150 and 672, respectively.

tors under oppositely poled dc magnetic fields and a small solenoid carrying an ac current at the drive frequency. The cell was mounted in a Veeco Multimode AFM with a NanoScope IIIa controller, Extender electronics, and Signal Access Module. All data presented here were taken using the above instrument. At time $t=0$ the drive signal was switched off and the resulting amplitude decay was recorded using a high speed analog-to-digital converter (National Instruments USB5133). The experiment was conducted separately for both vertical and torsional excitation and the results are displayed in Fig. 2. For the cantilever used, the ring-down time is a factor of approximately 3.5 less for the torsional mode, in agreement with Eq. (2).

Further to the increase in force sensitivity and reduced settle time, the optical lever detection system is more effective in torsion. This is due to the fact that the measured deflection voltage at the photodiode is proportional to the angle through which the cantilever is deflected.[13] Increase in the cantilever deflection angle has been shown to give a linear increase in signal to noise ratio for optical lever systems, regardless of the geometry of the optical beam path.[14] For flexural deflection in the small angle limit the deflection angle of the cantilever may be approximated to the tip deflection divided by the length. In the case of a T-shaped cantilever under torsional bending, the angle is given by the tip deflection divided by the tip offset from the torsion axis. For the cantilevers used here, the ratio of the cantilever

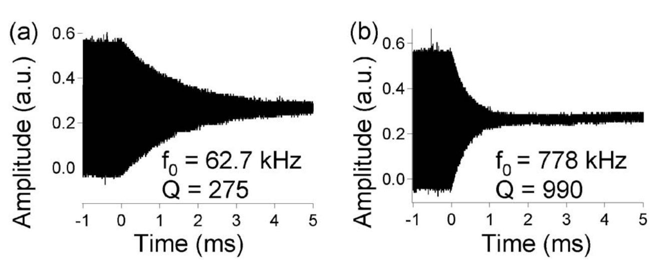

FIG. 2. Amplitude decay of (a) flexurally oscillating and (b) torsionally oscillating T-shaped cantilever after the drive signal is switched off at $t=0$. The times taken for the amplitudes to decay to $1/e$ of their initial values are approximately 1.4 and 0.4 ms, respectively. These values match the decay time calculated from the resonant frequency and Q (shown) of the respective mode used.

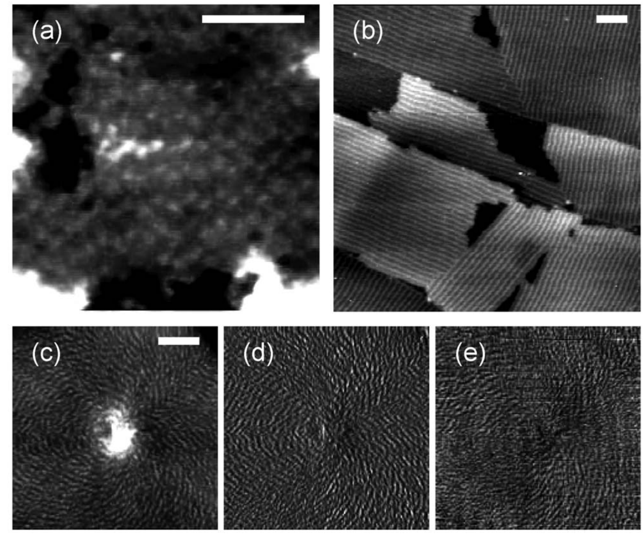

FIG. 3. Torsional tapping images of various test samples obtained in air. (a) Height image of 2D crystals of the photosynthetic bacterial light harvesting protein LH2 taken in air using a "whisker" probe. The scale bar represents 50 nm and the grayscale is 5 nm. (b) Height image of $C_{60}H_{122}$ nanocrystal. The scale bar represents 50 nm and the grayscale is 3 nm. (c) Height (second order plane fitted to remove large scale curvature of the spherulite), (d) amplitude (feedback error signal) and (e) flexural deflection images of polyethylene spherulite crystals. Scale bar 2 μm, grayscale (c) 350 nm, (d) 1.5 V and (e) 50 mV.

length to the tip offset is eight. Hence the measured deflection voltage of the cantilever for a given displacement is increased by the same factor in the torsional configuration. The signal to noise ratio is also increased by the increase in operating frequency due to a larger number of oscillation cycles being averaged in the acquisition time for 1 pixel if the measurement bandwidth is held constant (this is the same factor that gives the dependence of force sensitivity upon resonant frequency in Eq. (1)—the more oscillation cycles that may be averaged per pixel, the lower the uncertainty in the measurement).

In order to demonstrate the ability of this method to produce high sensitivity, low noise images, two-dimensional (2D) crystals of the photosynthetic bacterial light harvesting protein LH2 were imaged under ambient conditions. These samples have previously been extensively studied by contact mode AFM under buffer solution.[15] LH2 crystals were synthesized using a method similar to that outlined in Ref. 16 and prepared for imaging by pipetting approximately 10 μl of LH2 crystals in buffer solution onto a freshly cleaved muscovite mica surface with 50 μl of tris-KCl buffer and allowed to bind for 10 min. The sample was then rinsed thoroughly with buffer solution before washing with deionized water and drying under a stream of dry nitrogen.

The LH2 samples were imaged using Mikromasch TL01 Hi'Res cantilevers with diamondlike carbon "whiskers" (radius of curvature <1 nm) grown at the tip apex. Stable imaging was achieved with setpoint ratios varying at 50%–95%. Figure 3(a) shows a membrane patch with LH2 protein crystals, taken with a setpoint of approximately 90%. The well characterized zigzag and rectangular packing reported elsewhere[17] is clearly visible, however the ringlike structure of the individual protein units reported when imaging in buffer[15–18] is not observed. This is consistent with the results reported in Ref. 18 when imaging in air. As the rise and fall time of the features observed on the sample surface is considerably less than the inner diameter of the LH2 ring, ring structures should be clearly resolved if they are present. The absence of rings may indicate that the conformation of the individual proteins is different in air to in liquid environments. Hexacontane ($C_{60}H_{122}$) nanocrystals were also imaged in torsional tapping using whisker tips. Thin films were prepared by spin casting on HOPG substrates from a saturated toluene solution, followed by annealing at 150 °C for one hour. Imaging was performed at a setpoint of approximately 95%. Figure 3(b) shows a height image of such a crystal. Individual lamellae are clearly resolved, as previously reported.[19]

Finally, in order to demonstrate the ability of this technique to track the surface of highly topographic samples, polyethylene (PE) spherulite semicrystalline aggregates were imaged using torsional tapping under ambient conditions. The samples were prepared by dissolving PE (molecular weight: 60 kDa) in *p*-xylene at 120 °C and a concentration of 0.5% (w/w), then pipetting the solution onto a clean silicon wafer. The solvent was evaporated at 160 °C and the sample was quenched to room temperature. Mikromasch TL01 cantilevers with conventional silicon tips were used with a setpoint ratio of approximately 85% and an amplitude of around 10 nm. Figures 3(c)–3(e) are the height, amplitude, and flexural deflection (respectively) of the cantilever captured during a scan of a spherulite crystal. As can be seen from Figs. 3(c) and 3(d), the flexural deflection of the cantilever correlates well with the amplitude (feedback error signal). This indicates that under error signal, the cantilever is deflected flexurally, thus limiting the maximum force applied between the tip and the sample. Hence, the torsional configuration allows stiff, high frequency cantilevers to be used while maintaining a low spring constant in the Z direction. The maximum peak-to-valley deflection in Fig. 3(d) is approximately 35 mV, corresponding to a variation in force of approximately 3 nN. The dc component of the torsional deflection signal was also recorded, but showed no measurable variation above the noise level.

In summary, T-shaped cantilevers have been excited and monitored torsionally to give tapping mode imaging under a different geometry. Due to the increased Q and resonant frequency outweighing the increase in torsional spring constant for this configuration, the minimum force gradient detectable in dynamic AFM is decreased while increasing the available measurement bandwidth. The geometry of the T-shaped cantilever also improves the sensitivity of the optical lever detection system over conventional cantilevers. Hence torsional tapping offers an increase in force sensitivity and signal to noise ratio over conventional cantilevers but does not require specialized detection optics. Alternatively, small tapping amplitudes can be used without a significant decrease in signal-to-noise ratio, due to the increased optical lever sensitivity. An obvious application of this technique is for high resolution dynamic mode imaging in liquid, upon which we hope to report in the near future.

This configuration also offers an advantage of having a low force constant in the Z direction when compared to the cantilevers usually used in tapping mode in air, thus decreasing the likelihood of tip or sample damage when the feedback error signal is large and negative. Further to the acquisition of high resolution, high sensitivity images at increased scan rates it would also be possible to reduce the Q via electronic Q control[20] in order to acquire images with the same force sensitivity as typically obtainable in conventional tapping-mode AFM but at higher scan rates.

It should be noted that the cantilevers used here were designed for use in Harmonix™ imaging and not optimized for torsional tapping in any way. It is conceivable that cantilevers specifically designed for this application could yield considerably better results.

[1]G. Binnig, C. F. Quate, and Ch. Gerber, Phys. Rev. Lett. **56**, 930 (1986).
[2]Y. Martin, C. C. Williams, and H. K. Wickramasinghe, J. Appl. Phys. **61**, 4723 (1987).
[3]T. R. Albrecht, P. Grütter, D. Horne, and D. Rugar, J. Appl. Phys. **69**, 668 (1991).
[4]L. Huang and C. Su, Ultramicroscopy **100**, 277 (2004).
[5]N. Mullin and J. Hobbs, Appl. Phys. Lett. **92**, 053103 (2008).
[6]O. Sahin, S. Magonov, C. Su, C. F. Quate, and O. Solgaard, Nat. Nanotechnol. **2**, 507 (2007).
[7]A. Beyder and H. Sachs, Ultramicroscopy **106**, 838 (2006).
[8]Harmonix imaging is commercially available on Veeco AFM systems: www.veeco.com.
[9]T. E. Schäffer, J. P. Cleveland, F. Ohnesorge, D. A. Walters, and P. K. Hansma, J. Appl. Phys. **80**, 3622 (1996).
[10]Two methods were used to calculate this figure: the torsional Sader method (Ref. 11) allows the calculation of the spring constant (in units of newton-metres per radian) for a rectangular cantilever of the same dimensions, but without the crossbar. This may then be converted into a spring constant in newtons per metre by multiplying by the square of the tip offset. This method would be expected to underestimate the spring constant. The cantilever may also be treated as two springs in series. Assuming that there is minimal cross-talk between the torsional and flexural deflection signals, and that the inverse optical lever sensitivity is determined by the geometry of the cantilever, it is possible to calculate the spring constants by taking an approach curve while monitoring the vertical and lateral photodiode signals. Estimates from these two methods are in broad agreement.
[11]C. P. Green, H. Lioe, J. P. Cleveland, R. Proksch, P. Mulvaney, and J. E. Sader, Rev. Sci. Instrum. **75**, 1988 (2004).
[12]T. Sulchek, G. G. Yaralioglu, C. F. Quate, and S. C. Minne, Rev. Sci. Instrum. **73**, 2928 (2002).
[13]G. Meyer and N. M. Amer, Appl. Phys. Lett. **53**, 2400 (1988).
[14]C. A. J. Putman, B. G. De Grooth, N. F. Van Hulst, and J. Greve, J. Appl. Phys. **72**, 6 (1992).
[15]S. Scheuring, F. Reiss-Husson, A. Engel, J. L. Rigaud, and J. L. Ranck, EMBO J. **20**, 3029 (2001).
[16]M. Chami, G. Pehau-Arudet, O. Lambert, J. L. Ranck, D. Levy, and J. L. Rigaud, J. Struct. Biol. **133**, 64 (2001).
[17]S. Bahatyrova, R. N. Frese, K. O. van der Werf, C. Otto, C. N. Hunter, and J. D. Olsen, J. Biol. Chem. **279**, 21327 (2004).
[18]A. Stamouli, S. Kafi, D. C. G. Klein, T. H. Oosterkamp, J. W. M. Frenken, R. J. Cogdell, and T. J. Aartsma, Biophys. J. **84**, 2483 (2003).
[19]S. N. Magonov and N. A. Yerina, Langmuir **19**, 500 (2003).
[20]T. Sulchek, R. Hsieh, J. D. Adams, G. G. Yaralioglu, S. C. Minne, C. F. Quate, J. P. Cleveland, and A. Atalar, Appl. Phys. Lett. **76**, 1473 (2000).