

## Fluid viscosity determination by means of uncalibrated atomic force microscopy cantilevers

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In this letter it has been proved that the vibrating resonance frequency of an atomic force microscope cantilever is strictly characterized by its thickness ( $\alpha$ ), while its width/thickness ratio ( $\beta$ ) appears to be a less sensitive parameter that can be approximated to a constant. We therefore propose a data analysis method that, by accounting for a constant  $\beta$ , allows for the determination of the value of  $\alpha$  and consequently to calculate  $\eta$ . This method of monitoring viscosity has the advantage of requiring short measurement times on very small sample volumes, thereby avoiding laborious, time-consuming cantilever calibration. © 2006 American Institute of Physics.

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The atomic force microscope (AFM), by imaging the surface of materials in atomic resolution, has proved to be a useful tool to investigate mechanical properties and structures in a wide range of materials such as metals, semiconductors, polymers, and synthetic and biological membranes.<sup>1,2</sup> Great attention has been paid to the use of the AFM because of its capability of measuring the interaction force, at nanonewton scale, between the microscope tip and the sample surface. In this operation mode AFM measures the forces (at the atomic level) between a sharp probing tip (which is attached to a cantilever spring) and the sample surface.

In this context AFM has also been proved to be able to measure the rheological properties of simple or complex fluids with a resolution in three dimensions on the nanometer scales.<sup>3</sup> Indeed, since the frequency spectra of cantilevers strongly depends on the fluid in which they are immersed, the drag forces acting on cantilever being directly related to kinematic viscosity,<sup>4</sup> fluid density and viscosity can be determined readily from the knowledge of the resonance frequency.<sup>5-8</sup>

This is a unique feature of such a technique since traditionally informations about viscosity is recovered from perturbative measurements performed on several milliliters of material in a mechanical rheometer by applying a small amplitude oscillatory shear strain, precluding, therefore, the study of fragile, rare, and precious materials, including many biological samples that are difficult to obtain in large quantities. Moreover, while conventional rheometers provide an average measurement of the bulk response, AFM allows for, in principle, local measurements to be carried out in inhomogeneous systems by probing the material response in micrometer length scales using tens of microliter sample volumes.

Notwithstanding the growing interest in such techniques, the determination of the relationship between the frequency response of a cantilever beam immersed in a fluid and viscosity poses a formidable challenge<sup>5,7-10</sup> and at present, few

theoretical models exist for its calculation. The most simplistic model makes the heuristic analogy with the dynamic motion of a sphere through a viscous fluid.<sup>11</sup> This model, although allowing for an analytical solution, fails on many cases since it does not take into account the geometry of the cantilevers.<sup>5</sup> A more rigorous model (inviscid model)<sup>12</sup> overcomes such an insufficiency, but since it does not account for the fluid viscosity it is still inadequate.<sup>6</sup> Finally, a model that describes the dynamic deflection of a cantilever, by rigorously accounting, in the hydrodynamic function, for the geometry of the cantilever and the fluid viscosity, has been proposed.<sup>6,7</sup> Such a model, however, while fully calculating the expected cantilever resonance frequency, thereby allowing for a numerical solution, requires a large amount of numerical computation and a detailed knowledge of the cantilever geometry. A noteworthy contribution was recently developed by introducing a numerical approximation of the hydrodynamic function.<sup>13</sup> This approximation, although considerably simplifying computation, still requires microscopic details of the cantilever geometry that strongly inhibit a wide use of the above model when using commercially available cantilevers.

If cantilever geometrical details (length, width, thickness, density, etc.) required by the above models are unknown, a possible alternative approach consists in measuring an empirical AFM-setup calibration curve by using standard liquids such as a water-glycerol mixture at different concentrations or temperatures (typically 10).<sup>14</sup>

In this context we first developed and tested an analytical expression that, by using the approximated hydrodynamic function,<sup>13</sup> allows for the recovery of samples viscosity upon cantilever calibration.

Secondly, since calibration is a highly time-consuming procedure and subject to several drawbacks (i.e., cantilevers rupture), we suggest an alternative data analysis approach allowing for the determination of the absolute value of the fluid viscosity by simply accounting for the cantilever resonance frequency measured in the sample under study and in a liquid of known viscosity used as a standard (i.e., pure water).

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Resonance spectra of cantilevers immersed in 100  $\mu$ l droplets of glycerol/water mixtures (ranging from 0% to 80%) placed over a glass slide of a rectangular coverslip were detected by using an SPMagic SX atomic force microscopy (Elbatech, Italy). Resonance frequencies were recovered by fitting the response of a simple harmonic oscillator to resonance spectra. Four rectangular ultrasharp silicon nitride cantilevers of different force constant were used. (A) 40 N/m (NSC16, MikroMash), (B) 0.15 N/m (CSC17, MikroMash), (C) 48 N/m (NCLR, Nanosensor), and 5.5 N/m (NSG01, NT-MDT). Temperature was controlled at  $T_1 = (303.1 \pm 0.1)$  K and  $T_2 = (293.1 \pm 0.1)$  K.

The vacuum resonant frequency  $\omega_0$  of a rectangular cantilever is related to the resonance frequency  $\omega$  in a fluid having density  $\rho$  by<sup>5</sup>

$$\omega_0 = \omega \sqrt{1 + \frac{\pi \rho W}{4 \rho_0 T} \Gamma'(\omega)}, \quad (1)$$

where  $L$ ,  $W$ ,  $T$ , and  $\rho_0$  are length, width, thickness, and density of the cantilever.  $\Gamma'$ , and  $\Gamma''$  are the real and the complex part, respectively, of the hydrodynamic function  $\Gamma = \Gamma' + i\Gamma''$  for the oscillating cantilever. The explicit expression for  $\Gamma'$  and  $\Gamma''$  are hardly manageable combinations of the Bessel functions and make the use of Eq. (1) prohibitively. To account for the influence of the fluid motion on the oscillating behavior of the cantilever an analytical approximation of both the real and the complex part of the hydrodynamic function has been given.<sup>13</sup> Accordingly,

$$\Gamma' = a_1 + a_2 \frac{\delta}{W}, \quad (2)$$

$$\Gamma'' = b_1 \frac{\delta}{W} + b_2 \left( \frac{\delta}{W} \right)^2,$$

where  $a_1 = 1.0553$ ,  $a_2 = 3.7997$ ,  $b_1 = 3.8018$ ,  $b_2 = 2.7364$ , and  $\delta$  is the length giving the thickness of the thin viscous layer surrounding the cantilever in which the velocity has dropped by a factor of  $1/e$  and is defined by

$$\delta = \sqrt{\frac{2\eta}{\rho\omega}}. \quad (3)$$

By substituting Eq. (2) in Eq. (1), an explicit expression for  $\eta$  can be obtained,

$$\frac{\eta}{\omega} = \alpha \left[ \left( \frac{\omega_0^2 - \omega^2}{\omega^2} \right) - \beta \right]^2, \quad (4)$$

where  $\alpha$  and  $\beta$  are a combination of cantilever beam geometry and structure parameters ( $L$ ,  $W$ ,  $T$ ,  $\rho$ , and  $\rho_0$ ),

$$\alpha = \frac{8}{\pi^2} \frac{\rho_0^2 T^2}{\rho} \frac{1}{a_2^2}, \quad \beta = \frac{\pi}{4} \frac{\rho}{\rho_0} \frac{W}{T} a_1. \quad (5)$$

Therefore, by adopting the approximated expression for  $\Gamma$  [Eq. (2)], a new explicit expression is obtained [Eq. (4)] capable of recovering the sample's viscosity, as soon as geometrical details of the specific cantilever used are known.

Since those details are often roughly known, in spite of scanning electron microscopy (SEM) or transmission electron microscopy (TEM) analysis being involved, an alternative approach to recover  $\eta$  consists in characterizing the can-

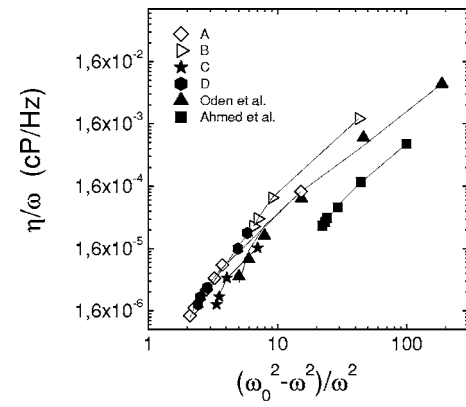


FIG. 1. The  $\eta/\omega$  ratio is reported vs the adimensional parameter  $(\omega_0^2 - \omega^2)/\omega^2$  for all cantilevers employed and for two sets of data already published (Refs. 9 and 10). The best fit of Eq. (4) to each data set (full lines) gives the  $\alpha$  and  $\beta$  coefficients reported in Table I.

tiliver's elastic behavior. Indeed, by calibrating the cantilever with standards of references,  $\alpha$  and  $\beta$  can be recovered.

In Fig. 1 values of the ratio  $\eta/\omega$  as a function of the adimensional parameter  $\omega_0^2 - \omega^2/\omega^2$  are presented both for all the cantilevers employed and for two sets of data already published<sup>9,10</sup> and used to increase the range of the cantilever's elastic and geometric characteristics investigated. In all the measurements shown  $\omega_0$  is the resonance frequency in air, since its value differs less than a few percent from that in the vacuum.<sup>6</sup> From the fitting of Eq. (4) to the experimental data (full lines) coefficients  $\alpha$  and  $\beta$  are determined for each cantilever. While  $\beta$  remains essentially unaltered in the limit of the experimental accuracy,  $\alpha$  greatly differs from cantilever to cantilever (Table I). Accordingly by defining the adimensional parameter  $Y$ ,

$$Y = \frac{\eta}{\omega \alpha}, \quad (6)$$

Eq. (4) can be written as

$$Y = \left[ \left( \frac{\omega_0^2 - \omega^2}{\omega^2} \right) - \beta \right]^2. \quad (7)$$

In Fig. 2 values of  $Y$ , calculated via Eq. (6) where  $\alpha$ 's are those reported in Table I, are reported versus the adimensional quantity  $\omega_0^2 - \omega^2/\omega^2$  for all the cantilevers and standards employed in Fig. 1. All data almost collapse into a single master curve. The best fit of Eq. (7) (solid line) well captures the experimental data with  $\beta = 1.0 \pm 0.3$ .

TABLE I. Values of  $\alpha$  determined by fitting Eq. (4) to experimental data and calculated by using Eq. (8), for all the cantilevers investigated.

Cantilever	$\alpha \pm \Delta\alpha (\times 10^{-7} \text{g/cm})$	
	Fitted	Calc.
A	$5.9 \pm 0.3$	$5.9 \pm 0.3$
B	$9.9 \pm 0.5$	$10 \pm 0.3$
C	$3.5 \pm 0.4$	$3.3 \pm 0.3$
D	$7.1 \pm 0.8$	$6.7 \pm 0.3$
Oden <i>et al.</i>	$3.2 \pm 0.7$	$3 \pm 0.3$
Ahmed <i>et al.</i>	$0.81 \pm 0.08$	$0.9 \pm 0.3$

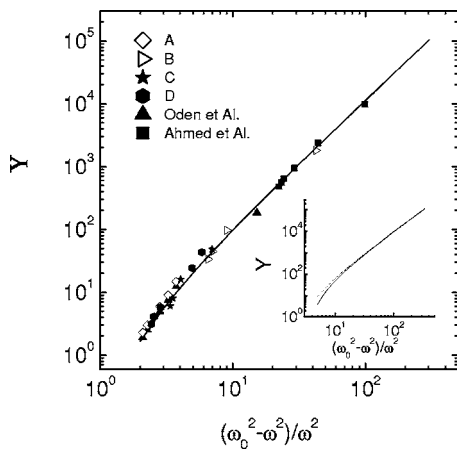


FIG. 2. The adimensional parameter  $Y$  is reported vs  $(\omega_0^2 - \omega^2)/\omega^2$  for all the cantilevers and standards employed in Fig. 1. All data almost collapse into a single master curve. The best fit of Eq. (7) (solid line) well capture the experimental data.

The existence of a master curve is an important physical issue of our investigation, implying that  $\alpha$  strictly characterizes the different cantilevers while  $\beta$  appears to be a less sensitive parameter that can be approximated to a constant. Such a result should not surprise the reader. Indeed, while  $\beta$  depends essentially on the cantilever width to thickness ratio [all the other parameters are constant in Eq. (5)] that has been found to be nearly constant in a large number of commercially available cantilevers,  $\alpha$  depends quadratically on thickness.<sup>6,15</sup> Therefore a knowledge of  $\alpha$  for the selected cantilever allows for the recovery, via Eq. (7), of sample viscosity by simply measuring the cantilever resonance frequency in air and in the sample.

An even a more relevant consequence comes from simulating a large spread of  $\beta$  values. Indeed, varying  $\beta$  from 1 to 3 does not much affect the value of  $\eta$  (see inset in Fig. 2). Therefore, by setting  $\beta$  to 1, it is possible to determine  $\alpha$  by using a liquid of known viscosity (i.e., pure water  $\eta_{\text{H}_2\text{O}}$ ). Indeed, from Eq. (6), accounting for the cantilever resonance frequency in pure water  $\omega_{\text{H}_2\text{O}}$ ,  $\alpha$  gives

$$\alpha = \frac{\eta_{\text{H}_2\text{O}}}{\omega_{\text{H}_2\text{O}}} \left[ \left( \frac{\omega_0^2 - \omega_{\text{H}_2\text{O}}^2}{\omega_{\text{H}_2\text{O}}^2} \right) - 1 \right]^{-2}. \quad (8)$$

The values of  $\alpha$ , calculated through Eq. (8), are reported in Table I. The agreement between the calculated  $\alpha$ 's with those obtained by fitting Eq. (4) to the calibration curves of Fig. 1 strongly supports the assumption of setting  $\beta$  to a constant. Similarly (i.e., by setting  $\beta$  to 1) the explicit expression for the sample viscosity  $\eta_s$  can be obtained by combining Eq. (6) with Eq. (8),

$$\eta_s = \frac{Y_s}{Y_{\text{H}_2\text{O}}} \frac{\omega_s}{\omega_{\text{H}_2\text{O}}} \eta_{\text{H}_2\text{O}}, \quad (9)$$

where  $Y_{\text{H}_2\text{O}}$  and  $Y_s$  are calculated through Eq. (7) by using  $\omega_{\text{H}_2\text{O}}$  and  $\omega_s$ , the cantilever resonance frequency in the sample. Equation (9) allows, therefore, for the recovery of  $\eta_s$ , so avoiding the classical calibration necessary to recover  $\alpha$  (Fig. 1) and drastically reducing both the time required to calibrate the cantilever and the risk of its rupturing.

In conclusion, we show that by using analytical approximations of the hydrodynamic functions<sup>13</sup> an explicit expression relating viscosity and cantilever resonance frequency can be developed [Eq. (4)]. Equation (4), while well capturing data behavior, allows for easy determination of the sample viscosity once the cantilever specifications are known. We also show that it is possible to override the knowledge of cantilever geometry by accounting for the cantilever resonance frequency in a reference liquid [Eq. (9)].

This method of monitoring viscosity should be considered as a crucial improvement in the applicability of AFM techniques since it has distinct advantages over other approaches that make this method elective in studying rare and precious materials such as biological samples. The possibility of performing measurements in small volumes ( $\sim$ microliters) and of recovering viscosity in the micrometer length scale is highly attractive for the study of highly purified (or recombinant) protein solutions or heterogeneous gels and macromolecular aggregates.<sup>16–19</sup> Because of the short measurement times required (a few seconds) streaming process such as gelation or macromolecular aggregation kinetics can be followed. Finally, since time-consuming and potentially perilous cantilever calibrations are avoided, the investigation of large sets of samples, by using different cantilevers, is made possible.

In conclusion, we would note that although all the above results are strictly valid for all those samples whose viscosity is larger than that of pure water, used as a standard of reference, this is far from being a major limitation, since this condition is often satisfied at least in a large part of biological samples.

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