

## Reducing Thermal Noise in Molecular Force Spectroscopy

G.A. Shaw\*

Manufacturing Engineering Laboratory

National Institute of Standards and Technology, Gaithersburg, MD 20899

\*gordon.shaw@nist.gov

### Abstract

Molecular force spectroscopy is the practice of measuring the mechanical properties of single molecules. The precision determination of these properties requires an instrument capable of piconewton-level force measurement. The atomic force microscope (AFM) is capable of such measurements if experiments are performed carefully. One factor limiting the force measurement resolution of the AFM in wet chemical environments is the presence of a squeeze film damper between the microfabricated cantilever used for force measurement and the surface to which the molecules of interest are attached. The effect of this damping on the force sensor's thermal noise is examined, and a new method is proposed to reduce this noise at low frequency by using a micropipette.

### Introduction

In the field of molecular force spectroscopy, researchers use a variety of techniques to test the mechanical properties of single molecules. In a typical experiment, a single molecule of interest is tethered to a solid surface at two ends, and the two ends are moved apart while the force exerted is measured at one end. Among the techniques used are optical tweezers [1], magnetic tweezers [2], meniscus forces [3], and atomic force microscopy [4]. These methods have matured to the point where measurements of individual DNA molecules have been discussed as a possible standard for metrology [5]. Despite these advances, the uncertainty of the force measurements used is no less than five percent. In order to decrease uncertainty in the measured force, and establish their absolute accuracy, force measurements traceable to the international system of units (SI) are desirable in the force range used for molecular force spectroscopy (tens of piconewtons).

Of the techniques used in force spectroscopy, the AFM provides the most direct traceability path to the SI because it uses a passive mechanical force sensor in the form of a microfabricated cantilever beam. An AFM can be calibrated to measure force by using a force sensor [6], or by calibrating the spring constant of the microfabricated cantilever using a reference spring [7]. The latter method is much more common, and several studies have been devoted to the topic [8-11]. However, the AFM cantilevers used in molecular force spectroscopy require special consideration due to their low spring constants, typically on the order of 0.01 N/m. The force measurement uncertainty in these sensors is limited by thermomechanical noise.

Because every physical object is in a state of dynamic thermal equilibrium with its environment, small thermal fluctuations are evident in a wide variety of different phenomena. In mechanical structures, these fluctuations can be observed as a small amount of random motion in a particular spatial coordinate. According to the equipartition theorem, the thermal energy associated with the motion of an ideal Hookean spring-mass system can be described by

$$\frac{1}{2}k_bT = \frac{1}{2}k\langle x^2 \rangle \quad (1)$$

Where  $k_b$  is Boltzman's constant,  $T$  is absolute temperature,  $k$  is the system spring constant, and  $x$  is the spring deflection, as illustrated in Figure 1.

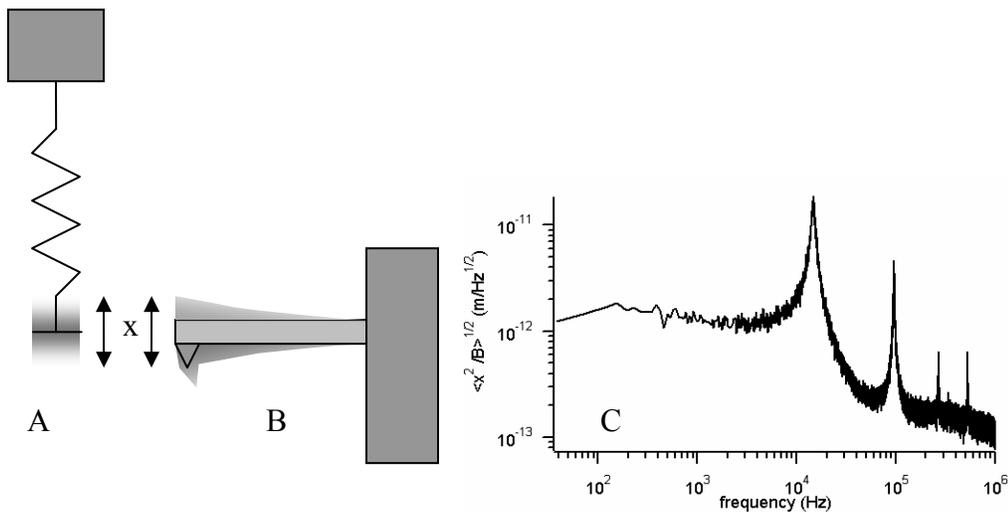


Figure 1. Thermomechanical noise of a cantilever beam. An ideal spring-mass system in thermal equilibrium with its environment is shown in A. The thermal oscillation of a cantilever beam, such as those used in AFM, is shown schematically in B. The wide-bandwidth power density spectrum of the thermal noise measured on a cantilever designed for molecular force spectroscopy is shown in C, where the vertical axis shows thermal noise per unit bandwidth,  $B$ .

These small fluctuations can be observed in microfabricated cantilever beams such as those used in an AFM. For example, the thermal noise spectrum of an AFM cantilever designed for molecular force spectroscopy experiments is shown in Figure 1. Since AFM cantilevers used in force spectroscopy are underdamped systems, even when immersed in water, the cantilevers are fabricated to have as high a resonance frequency as possible so that the thermal noise is concentrated in as high a frequency as is practical [12]. The use of a low-pass filter then allows the elimination of most of the thermal noise above its cutoff frequency. In such a design, the ability to keep the resonance frequency high is key to achieving the piconewton-level force resolution necessary for molecular force spectroscopy experiments.

The determining factors in a system's resonant frequency are its stiffness, mass, and damping properties. In general, a combination of a high resonant frequency and low stiffness are desirable, and as such, molecular force spectroscopy experiments are conducted with cantilevers that are as small as possible while still maintaining their structural integrity after submersion in water. In a passive mechanical sensor such as an AFM cantilever, stiffness is a function of the cantilever geometry and the materials from which they are fabricated. While geometry does affect a cantilever's mass and damping properties, the ambient environment also plays an important role. The system mass is affected by environment due to the static fluid layer which moves along with the surface of the AFM cantilever. The damping properties of a cantilever depend heavily upon the viscosity of the surrounding medium. This fluid damping can be treated as a superposition of two components, one based on Stokes flow and one on Reynolds flow [13]. The Stokes flow component is caused by hydrodynamic drag forces, and the magnitude of this effect is dependent on the cantilever geometry and the ambient fluid's viscosity. The Reynolds flow results from squeeze film flow in a thin gap between the AFM cantilever and a flat surface. In this case, the damping depends on cantilever geometry, fluid viscosity, and separation between the AFM cantilever and the flat surface. In molecular force spectroscopy experiments, the cantilever geometry is fixed by the fabrication process, and the fluid viscosity is fixed by the solvent appropriate for a particular molecule of interest; the solvent is typically an aqueous electrolyte solution. This leaves only the separation between the AFM cantilever and flat surface to be optimized for minimal damping, and therefore minimal thermal noise. In fact, other authors have examined the effect of surface-probe proximity on thermal noise [14, 15]. It was found that

surface damping increased markedly when the cantilever was within 5  $\mu\text{m}$  of the surface. It was also found that by cutting away part of the cantilever using a focused ion beam, the surface damping was drastically reduced.

In this work an experimental study is carried out to examine the effect of separation between an AFM cantilever and flat surface on low frequency thermal noise. Using an AFM cantilever designed specifically for molecular force spectroscopy experiments, we find behavior similar to that previously reported. In addition, an alternative method for reducing surface damping has been tested. Rather than reducing the dimensions of the cantilever, the dimensions of the measurement surface have been reduced. In most AFM force spectroscopy experiments, a molecule is attached by one end to an AFM cantilever and by the other end to a flat measurement surface. By using a glass micropipette as the measurement surface, a significant reduction in low frequency thermal noise has been observed. The use of micropipettes in molecular force spectroscopy is well-established, and protocols are available for attachment of single molecules to such structures [1]. The use of a pipette is also much less expensive and time consuming than the fabrication of custom cantilevers.

## Experimental

An Asylum Research MFP3D AFM was used in all experiments<sup>1</sup>. This system uses an optical lever arm detection scheme in which the light from a superluminescent diode is focused on the back side of a microfabricated cantilever. The reflected light impinges on a four-quadrant photodiode, and the voltage difference between the photodiode quadrants tracks the slope of the cantilever as it deflects under the influence of an external force. This deflection signal voltage is then digitized by the AFM electronics and recorded on the computer controlling the experiment using Wavemetrics Igor Pro software. This software includes a routine for processing a voltage signal to produce a power spectral density as a function of frequency. Where noted, a DSP low pass filter was applied to the signal before processing.

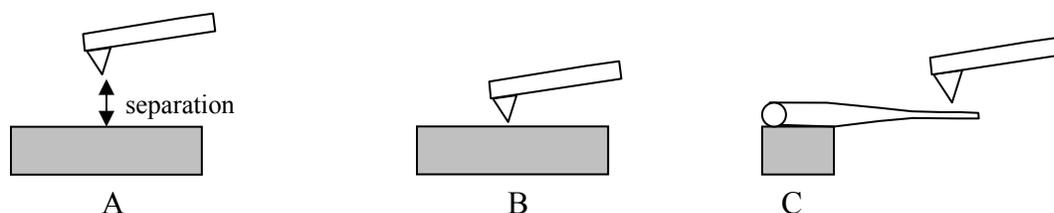


Figure 2. Schematic of the experiment. Illustration A shows an AFM cantilever which is far from a surface. The cantilever is brought into close proximity to the surface, as is shown in B, increasing low frequency thermal noise. The use of a micropipette as a measurement surface is shown in C.

Power spectral densities are calculated in  $\text{V}/\text{Hz}^{1/2}$ , and are converted to measure the deflection of the cantilever using an optical lever sensitivity, which has units of  $\text{m}/\text{V}$ . This conversion factor is separately measured for each experiment. To obtain this quantity, the AFM cantilever is pressed against a rigid surface using the AFM's calibrated motion stage. The change in photodiode voltage as the cantilever deflects in contact with the rigid surface is then calibrated to units of displacement measured by the AFM stage. This measurement was performed ten times for each experiment. Type A (statistical) uncertainty [16] of the inverse optical lever sensitivity is estimated to be the relative standard deviation of these ten measurements, and has a value of 1.1%. The electrical noise of the system was recorded with no signal input, and found to be approximately 1% of the measured signal. There are several type B uncertainties (not statistically defined,) including the effect of the AFM cantilever tip applying an off-axis force to the end of the AFM cantilever [17, 18], which contributes a relative uncertainty of 5%. Additionally, there is a type B uncertainty associated with applying the inverse optical lever sensitivity determined from a quasi-static test to a dynamic measurement of motion [19]. Assuming that the superluminescent diode beam extends the entire length of the AFM cantilever (approximately 60 or 100  $\mu\text{m}$  in the case of the cantilevers used here) this correction has a relative type B uncertainty of approximately 5%.

<sup>1</sup> Commercial equipment and materials are identified to adequately specify certain procedures. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Summing these uncertainties in quadrature yields a combined standard uncertainty of 7.2 % on the dynamic displacement measurements.

Olympus bio-lever cantilevers were used throughout. The cantilever beams have lengths of 60  $\mu\text{m}$  and 100  $\mu\text{m}$  and spring constants of 0.025 N/m and 0.0085 N/m respectively. These spring constants were measured using the thermal method [20, 21] and have uncertainties of approximately 20% [22].

For low-frequency thermal noise measurements, a cantilever was placed in the AFM, immersed in water, and allowed to equilibrate for several hours. The cantilever was then brought into contact with the surface of interest, and the optical lever sensitivity was measured. Next, the deflection signal was recorded, after low-pass filtering above 1 kHz, with a data acquisition rate of 25 kHz for 2.61 seconds. The standard deviation of this voltage signal was then determined. This process was repeated ten times, and the average of these ten standard deviations is reported as noise. The same data were also processed using the power spectral density software routine. To determine the effect of separation between the probe and the measurement surface, the cantilever was then moved away from the surface under closed-loop position control using custom written software. Determination of the exact value of separation between the tip of the AFM cantilever and measurement surface was complicated by snap-in, so these separations have a type B uncertainty of approximately 250 nm. Noise was measured at each position, and the process was repeated to measure the noise as the cantilever was brought into close proximity with the micropipette.

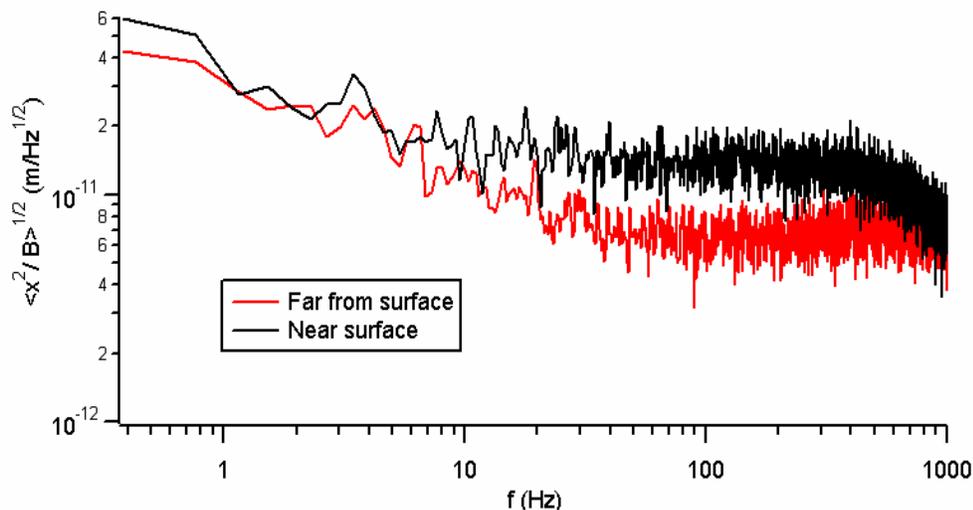


Figure 3. Effect of cantilever proximity to surface on thermal noise. The low-frequency power spectral noise density of the 100  $\mu\text{m}$  long AFM cantilever is shown for the case when the cantilever is near the measurement surface (i.e., less than 500 nm separation) and far from the measurement surface (i.e., greater than 60  $\mu\text{m}$  separation.) Not shown is the spectrum for the experiment in which the AFM cantilever is brought into close proximity with a micropipette instead of a flat surface. The power spectral noise density for this case is not shown for clarity, as it is almost identical in magnitude to that of the cantilever when far from a flat surface.

## Results and Discussion

Figure 1 shows the wideband thermal noise spectra of the 100  $\mu\text{m}$  long AFM cantilevers in an air ambient. The first mode resonance is clearly visible, and higher modes are also visible. The amplitude of the thermal noise increases drastically when the ambient is changed to water. Figure 3 shows the low frequency thermal noise of the same cantilever in water. The contribution of  $1/f$  noise can be seen below 10 Hz, but between this frequency and 1 kHz, the cantilever's thermal noise when near the surface (i.e., within 500 nm) is twice as large as the thermal noise when the cantilever is greater than 60  $\mu\text{m}$  from the flat sample surface.

A similar result can be observed for the shorter cantilever, as seen in Figure 4. In both cases, the amount of thermal noise increases significantly as the cantilevers are brought into proximity with the flat measurement surface. In the molecular force spectroscopy experiments where short molecules are to be examined, this effect will decrease the signal-to-noise ratio of the measurement. A typical single molecule force spectroscopy experiment in AFM is measurement of the overstretch transition of a DNA molecule.[4] The force displacement curves generated in this measurement show a force plateau that indicates the overstretch transition is occurring. This plateau occurs at approximately 65 piconewtons of force which requires measuring a 2 nm deflection if a cantilever with a 0.03 N/m stiffness is used. Minimizing deflection uncertainty caused by thermal noise is therefore a stringent requirement.

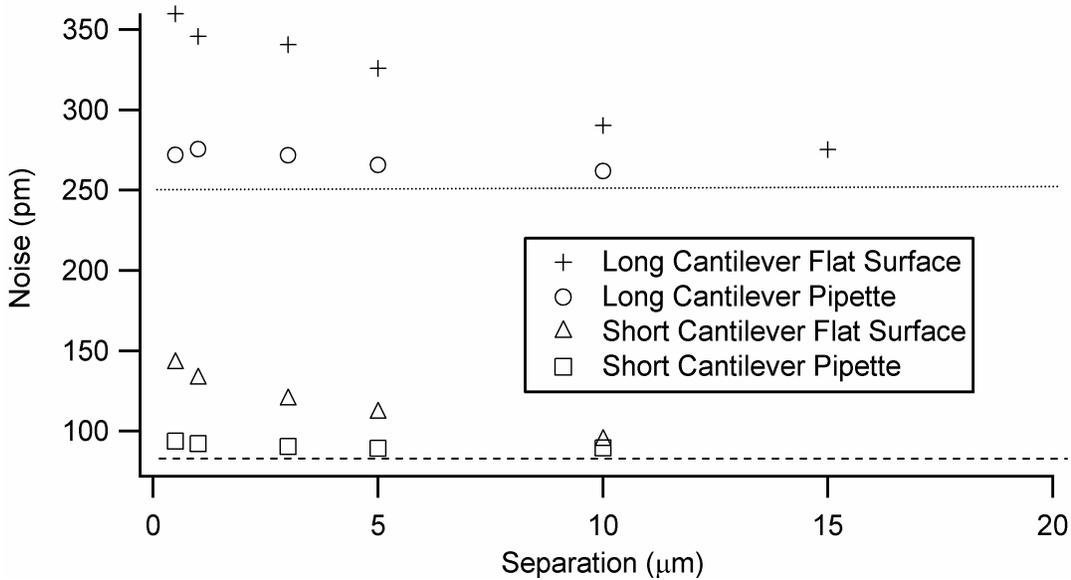


Figure 4. Effect of surface proximity on measured noise for AFM cantilevers immersed in water. The long cantilever is the one described in the text having length 100 μm, likewise for the short cantilever with length 60 μm. Whereas noise increases drastically when the cantilevers are brought near to a flat surface, the increase is much less when they are brought near a micropipette. Dashed lines show the noise recorded when the cantilevers were more than 60 μm from the surface.

The thermomechanical noise floor can be estimated using equation 2, which shows the noise floor is proportional to the square roots of measurement bandwidth and system damping[15].

$$N = \frac{\sqrt{4k_b T \gamma B}}{k} \quad (2)$$

Here,  $N$  denotes the cantilever displacement noise floor,  $\gamma$  is system damping,  $k$  is the AFM cantilever spring constant, and  $B$  is the measurement system bandwidth. The introduction of a low-pass filter into the measurement signal chain therefore has the desired effect of reducing noise in the measured signal. An additional and more subtle difference can be realized by using a measurement surface that minimizes surface damping. In the case of the current work, such a reduction has been measured using a glass micropipette as a measurement surface. Figure 4 compares the measured noise as the cantilever approaches a flat surface or a micropipette. Whereas the noise increases markedly when approaching the flat surface, it remains nearly unchanged when approaching the micropipette. In the case of the shorter cantilever, the noise is reduced by as much as 50% by using the pipette tip rather than the flat surface. Since the micropipette has much less surface area in contact with the fluid near the cantilever, there may be less friction between the fluid and the measurement surface, resulting in less dissipation from the interaction between the static fluid layers of the

cantilever and measurement surface. This effect could cause a reduction in damping of the AFM cantilever's thermal vibrations, and would provide a mechanism for the decreased noise observed.

## Conclusion

A series of experiments have been carried out to demonstrate the impact of fluid damping on AFM cantilevers designed for molecular force spectroscopy. It was shown that low-frequency thermomechanical noise increases as these cantilevers were brought into close proximity with a flat surface. It was also shown that this effect was mitigated when the cantilevers were brought close to a micropipette, with the largest improvements observed when the cantilever and measurement surface were less than 1  $\mu\text{m}$  apart. This result may be of particular interest to those seeking to minimize thermomechanical noise in force spectroscopy measurements performed on molecules with contour lengths of less than 1  $\mu\text{m}$ .

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