# THERMAL FLUCTUATION ANALYSIS OF A SURFACE-COUPLED AFM CANTILEVER

**Jianhua Wu, Ying Fang and Cheng Zhu**

Woodruff School of Mechanical Engineering and Joint Emory/Georgia Tech Coulter School of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0363

## ABSTRACT

The Brownian fluctuations of an atomic force microscope (AFM) cantilever with its tip coupled elastically to a surface have been analyzed. Measuring the changes in the tip fluctuations in the presence and absence of coupling thus enables determination of the elastic modulus of the coupling element.

## INTRODUCTION

AFM has been widely used to measure mechanical properties of biomolecules and cells. Typically, such measurements involve monitoring the sample deformations in response to controlled applied forces. Because biomolecules and cells are usually highly compliant, extremely soft cantilevers must be used to apply ultra-low level of forces. Consequently, such experiments are susceptible to thermal excitations, manifesting as force and displacement fluctuations that reduce measurements accuracy.

One can try to take advantage of thermal excitations rather than being limited by them. This is exemplified by the thermal fluctuation method, which has been commonly used for calibration of cantilever spring constant [1]. The method is based on the analysis of the cantilever response to thermal excitations. Coupling a molecule or a cell to the cantilever tip, either by stretching or by indentation, will alter its response characteristics. An understanding of the dependence of the system responses on the mechanical properties of the coupling element thus provides an alternative mean for measuring such mechanical properties. We have solved such dependence for the simple case in which the coupling element can be treated as elastic.

## GOVERNING EQUATIONS

Commercial cantilevers are either rectangular or V-shaped, as depicted in Fig. 1 (not to scale). To be as general as possible, let us consider an arbitrarily shaped plate with uniform thickness $h$ and

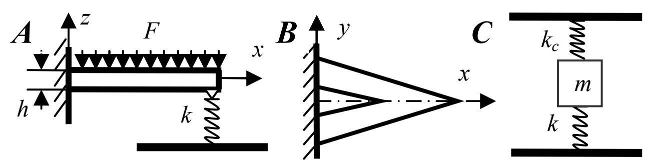


**Figure 1. Schematic of a surface-coupled V-shaped AFM cantilever loaded by thermal excitations. The coupling of the cantilever tip and the surface is modeled by a string with a spring constant *k*. *A* Side-view. *B* Top-view. *C* Mass-spring model.**

Young's modulus $E$. The equation of motion in the lateral direction for small deflection $z$ of the cantilever can be written as

$$\rho \frac{\partial^2}{\partial t^2} z(\mathbf{p},t) + 2\zeta \frac{\partial}{\partial t} z(\mathbf{p},t) + D\Delta^2 z(\mathbf{p},t) + k\delta(\mathbf{p}-\mathbf{p}_0) z(\mathbf{p},t) = F(\mathbf{p},t) \quad (1)$$

where $t$ is time, $\mathbf{p}$ denotes a point in the $x$-$y$ plane (Fig. 1 $B$), $\Delta^2 \equiv \partial^2/\partial x^2 + \partial^2/\partial y^2$ is the LaPlace operator, $\rho$ is the density, $\zeta$ is the damping coefficient, and $D = Eh^3/[12(1-\nu^2)]$ where $\nu$ is the Poisson's ratio, is the flexural rigidity. The external load $F$ represents thermal excitations and satisfies the definition for white noises:

$$< F(\mathbf{p}_1,t_1) F(\mathbf{p}_2,t_2) >= \sigma^2 \delta(t_1 - t_2) \delta(\mathbf{p}_1 - \mathbf{p}_2) \quad (2)$$

where $<\cdot>$ denotes averaging over time and space and $\sigma$ denotes the strength of the thermal excitations. The cantilever tip $\mathbf{p}_0$ is coupled to the surface by a spring, which is included in Eq. 1 as a point load whose magnitude is proportional to the deflection $z$ with the proportionality constant being the spring constant $k$. For the boundary conditions, a built-in edge is on the opposite side of the tip and the remaining edges are free.

## RESULTS

The total potential energy of the system can be written as the sum of two terms: the bending energy of the plate and the elastic energy of the spring.

$$U = \frac{D}{2}\int_S z(\mathbf{p},t)\Delta^2 z(\mathbf{p},t)dS + \frac{1}{2}kz^2(\mathbf{p}_0,t) \quad (3)$$

Modeling the continuous system by a discrete single degree-of-freedom mass-spring system using the tip deflection as the only dependent variable, the potential energy can be expressed as $\frac{1}{2}Kz^2(\mathbf{p}_0,t)$ where $K$ is an equivalent spring constant. If the tip deflection is solely caused by thermal excitations, at thermodynamic equilibrium the equipartition theorem applies, which requires

$$\frac{1}{2}K < z^2(\mathbf{p}_0,t) >= \frac{1}{2}k_B T \quad (4)$$

where $T$ is the absolute temperature and $k_B$ is the Boltzmann constant. For rectangular and V-shaped cantilevers in the absence of tip coupling, it has been shown that $K = k_c \equiv F_0/z(\mathbf{p}_0)$ where $F_0$ is a constant load applied to the tip and $z(\mathbf{p}_0)$ is the corresponding static deflection [2, 3]. In other words, $K$ is identical to the static cantilever spring constant, which is the basis for calibration of the cantilever spring constant [1].

The present work extended these previous results to the general case of arbitrarily shaped cantilever in the presence of an elastic coupling at the tip. Using eigenmode expansion it can be shown that

$$K = k_c + k \quad (5)$$

In words, under thermal excitations, the system behaves as if the cantilever spring and the coupling spring are in parallel, as depicted in Fig. 1 *C*. It follows from Eqs. 4 and 5 that

$$k = k_B T\left(\frac{1}{< z^2(\mathbf{p}_0,t) >_p} - \frac{1}{< z^2(\mathbf{p}_0,t) >_a}\right) \quad (6)$$

where the subscripts $a$ and $p$ denote, respectively, the deflections measured in the absence and present of coupling.

Because most AMF monitor changes in the positions of the laser light that is reflected from the cantilever tip, it is usually the tip rotation $(\partial/\partial x)z(\mathbf{p}_0)$, not the tip deflection $z(\mathbf{p}_0)$, that is directly measured. Under static loads, the two can be related; and the relationship depends on the cantilever shape. For instance, in the absence of tip coupling and when the static load is applied to the tip, $z(\mathbf{p}_0) = (2/3)L(\partial/\partial x)z(\mathbf{p}_0)$, where $L$ is the length of the rectangular cantilever. Under dynamic loads, both deflection and rotation become functions of time and the static relationship defines a virtual deflection $z^* \equiv (2/3)L(\partial/\partial x)z(\mathbf{p}_0)$. In general, the mean square deflection differs from the mean square virtual deflection. For surface-coupled rectangular cantilevers under thermal excitations, we have shown that

$$< z^{*2} >= \frac{4}{3}\left(1 + \frac{1}{4}\frac{k}{k_c}\right) < z^2 > \quad (7)$$

Setting $k = 0$ or $k \to \infty$ respectively reduces Eq. 7 to two special cases, which are the cases of free or pined end studied by Butt and Jaschke [2]. It follows from Eqs. 4-7 that, for rectangular cantilever, the spring constant of the coupling element can be expressed as

$$k = \frac{4k_B T}{3 < z^{*2} >_a}\left(\frac{< z^{*2} >_a - < z^{*2} >_p}{< z^{*2} >_p - 0.25 < z^{*2} >_a}\right) \quad (8)$$

The cantilever is continuously being loaded by thermal excitations that add energy to the system. For it to be in thermodynamic equilibrium with a bounded mean potential energy, the energy has to be dissipated to the surroundings via viscous damping. This implies a relationship between the damping coefficient $\zeta$ and the strength of the thermal excitations $\sigma$, which we have shown to be

$$\sigma^2 = 4k_B T\zeta \quad (9)$$

Equation 9 is a special form of the fluctuation-dissipation theorem.

## DISCUSSION AND CONCLUSION

The present analysis has provided the theoretical basis for measuring the elastic property $k$ of a molecule or a cell that couples the AFM cantilever tip to a rigid surface from the changes in the mean square fluctuations of the tip deflection. We have recently used this method to measure the molecular elasticities of P-selectin bound to two forms of P-selectin glycoprotein ligand 1 (PSGL-1) and several anti-P-selectin antibodies [4]. The results so obtained are in good agreement with those obtained by using controlled force to stretch these molecular complexes. Thus, the present method has been validated experimentally.

Equation 5 indicates the parallel arrangement equivalency of the cantilever spring and the coupling spring under thermal excitations. In many AFM experiments, a periodic excitation is applied to the cantilever and the changes in the tip responses in the presence and absence of coupling is used to determine the viscoelasticity of the sample that the cantilever tip probes [5, 6]. It was generally assumed that under such situation, the cantilever spring and the coupling spring were in parallel, based on a single degree-of-freedom mass-spring model [7]. This assumption is invalid in general. However, it is approximate when the excitation frequencies are close to the system resonant frequencies and when the spring constant ratio $k/k_c$ is small (<1). We have shown that Eq. 5 is valid because the excitations are of the properties of white noises, which contain broad frequencies and are spatially and temporal independent (cf. Eq. 2). Moreover, the equipartition theorem is only applicable to thermally excited systems.

## ACKNOWLEDGMENTS

This work was supported by NIH grants AI38282 and AI44902.

## REFERENCES


[1] Levy, R. and Maaloum, M., 2002, “Measuring the spring constant of atomic force microscope cantilever: thermal fluctuations and other methods,” *Nanotechnology*, Vol. 13, pp. 33-37.

[2] Butt, H-J. and Jaschke, M., 1995, “Calculation of thermal noise in atomic force microscopy,” *Nanotechnology* Vol. 6, pp. 1-7.

[3] Stark, R.W., Drobek, T. and Heckl, W. M., 2001, “Thermal-mechanical noise of a free v-shaped cantilever for atomic-force microscopy,” *Ultramicroscopy* Vol. 86, pp. 207-215

[4] Marshall, B.T., 2001, “Force and Bond Lifetime Relationship of the P-selectin/PSGL-1 Interaction,” Ph.D. Thesis, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

[5] Rabe, U., Janser, K. and Arnold, W., 1996, “Vibrations of free and surface-coupled atomic force microscope cantilever: Theory and experiment, ” *Rev. Sci. Instrum*. Vol. 67, pp. 3281-3293

[6] Turner, J.A., Hirsekorn, S., Rabe, U. and Arnold, W., 1997, “High-frequency response of atomic-force microscope cantilevers, ” J. Appl. Phys. Vol. 82, pp. 966-979

[7] Burnham, N.A., Kulik, A.J., Gremaud, G., Gallo, P.-J and Oulevey, F., 1996, “Scannning Local-Acceleration Microscopy,” *J. Vac. Sci. Technol*. Vol. B14, pp. 794-799.