#### SUPPLEMENTAL MATERIALS

It has been shown that, for Thermomicroscopes (Sunnyvale, CA) cantilevers, the inclination and deflection at the cantilever tip are proportional to each other, i.e.,  $z(L) = \alpha L [dz/dx]_{x=L}$ , which allows the definition of virtual deflection, i.e.,  $z^*(L,t) = \alpha L [\partial z/\partial x]_{x=L}$  (Wu et al., 2005). In addition, mean square virtual deflections to the mean square real deflections are proportional to each other, and the proportionality constant is a linear function of the ratio of the molecular spring constant to the cantilever spring constant:

$$\langle z^{*2} \rangle / \langle z^{2} \rangle = a(k/k_{c}) + b$$
 (S1)

The constants  $\alpha$ , a, and b are listed in Table 1 for Thermomicroscopes cantilevers.

Cantilever	А	В	С	D	Е	F	Theoretical
Shape	V-shaped	Rectangular	V-shaped	V-shaped	V-shaped	V-shaped	Rectangular
α	0.670137	0.665266	0.685908	0.661468	0.652268	0.584174	2/3
а	0.3943	0.3024	0.3879	0.4068	0.4188	0.4315	1/3
b	1.3072	1.2969	1.3831	1.3738	1.3834	1.3349	4/3

### Table 1

It has also shown that, due to the bandwidth limitations, errors will arise if one directly uses Eq. S1 in the equipartition theorem [i.e.,  $\frac{1}{2}(k_c + k) = \frac{1}{2}k_BT$ ] to evaluate the spring constants of the cantilever and the molecular linker according to the following (Wu et al., 2005).

$$k = k_c \frac{bk_B T - k_c < z^{*2} >}{k_c < z^{*2} > -ak_B T}.$$
(S2)

Depending the number (N) of vibration modes measurable from experiment, the following approximation will substantially reduce the errors.

$$k \approx c_N k_c \frac{b_N k_B T - k_c \sum_{n=1}^{N} \langle z_n^{*2} \rangle}{k_c \sum_{n=1}^{N} \langle z_n^{*2} \rangle + a_N k_B T},$$
(S3)

The coefficients  $a_N$ ,  $b_N$ , and  $c_N$  sing for N = 1, 2, and 3 are listed in Table 2 for the indicated Thermomicroscopes cantilevers.

## Table 2

Cantilever B					
	N=1 $N=2$ $N=3$				
$a_N$	0.1445	-0.0757	-0.1589		
$b_N$	0.8170	1.0694	1.1556		
$c_N$	0.9583	0.9813	0.9861		

Cantilever C					
	N=1 $N=2$ $N=3$				
$a_N$	0.1842	0.0045	-0.0991		
$b_N$	0.6920	0.9727	1.0902		
$c_N$	0.8697	0.9694	0.9838		

# Cantilever D

	N = 1	N=2	N=3
$a_N$	0.2067	0.0460	-0.0648
$b_N$	0.6946	0.9912	1.1226
$c_N$	0.8452	0.9655	0.9814

### Cantilever E

	N = 1	N = 2	N=3
$a_N$	0.2104	0.0626	-0.0487
$b_N$	0.6796	0.9915	1.1307
$c_N$	0.8098	0.9517	0.9750