Athermalization in atomic force microscope based force spectroscopy using matched microstructure coupling

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The authors describe a method for athermalization in atomic force microscope (AFM) based force spectroscopy applications using microstructures that thermomechanically match the AFM probes. The method uses a setup where the AFM probe is coupled with the matched structure and the displacements of both structures are read out simultaneously. The matched structure displaces with the AFM probe as temperature changes, thus the force applied to the sample can be kept constant without the need for a separate feedback loop for thermal drift compensation, and the differential signal can be used to cancel the shift in zero-force level of the AFM. © *2009 American Institute of Physics.* [DOI: 10.1063/1.3167276]

Atomic force microscope (AFM) has been used extensively to probe the nanoscale interactions that take place in wide range of time scales, from microseconds[1,2] to minutes.[3] Long time-scale experiments require stability and control of drift to minimize the effects of changes in ambient conditions. Thermal drift of the cantilever due to ambient temperature changes is a significant source of drift in AFM systems along with mechanical vibrations,[4] material creep, and surface stress changes.[5]

The AFM cantilever is usually a bimaterial structure and is sensitive to temperature changes. In contrast, the deflection of the cantilever due to changes in ambient temperature is detrimental for AFM especially for long time-scale experiments where the rate of drift is comparable with the rate of measured interactions. Thermal drift can be corrected using correlation methods[6] and Kalman filtering[7] for imaging purposes, but a different approach is needed to address this problem for force spectroscopy experiments involving biomolecules or cells. The effect of thermal drift in these experiments is twofold: (a) the cantilever bends which causes false force reading and (b) The zero-force level shifts. These cannot be tolerated in biomolecular experiments where the samples are delicate and the precise control of both force and tip-to-sample distance is critical. Thus, effective methods for reducing thermal drift in AFM are needed to probe slow biomolecular interactions.

Wenzler *et al.*[8] reported significant reduction of thermal drift by simply removing the metal layer over the base of the cantilever. The end of the cantilever, where the deflection is read, still has the metal layer so these cantilevers are still exposed to thermally induced deflection. Instead of modifying the existing cantilevers, Beyder *et al.*[9] developed a new type of force sensing structure to effectively reduce the probe dependent thermal drift. In addition to the efforts for reducing the thermal drift with modified and new probes, researchers have also developed new techniques for existing cantilevers. Spagnoli *et al.*[3] developed a software routine where the cantilever is time-shared between the sample and the substrate for referencing. When the cantilever should be engaged on the sample for the entire experiment, the referencing can be done by reading the deflection of a reference sensor. The reference sensor, which provides distance information from the cantilever substrate-to-sample can simply be another cantilever next to the measurement one,[10,11] an interferometer,[12] or an electrostatic sensor.[4] The reference sensor provides information for compensation of drift in distance from cantilever plane to sample substrate. However, this approach does not prevent cantilever bending against a stationary surface while the cantilever is connected to the surface through a biomolecule or a cell. A typical experiment of this type is a force clamp experiment on a biomolecule.[13]

In this paper, we introduce a method for athermalization of AFM cantilevers by coupling them with thermomechanically matched microstructures. Athermalization consists of a system design such that ambient temperature fluctuations have no effect on the measurements made by AFM cantilevers. A specific case for biomolecular experiments is schematically shown in Fig. 1. Here, we use a passive bimaterial membrane as the matching microstructure designed such that it thermally deflects identically with the measurement canti-

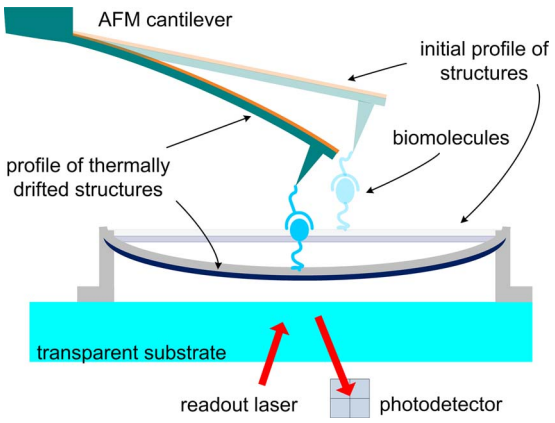

FIG. 1. (Color online) Schematic of a micromachined membrane with integrated diffraction grating interferometer coupled with AFM cantilever for athermalization of the cantilever in a biomolecular experiment. Profiles of the structures before and after thermal deflection are schematically shown.

lever. This provides constant tip-to-membrane distance even under thermal fluctuations when the piezoactuator keeps the cantilever-to-substrate distance the same. Thus when the piezo is ramped up and down for molecular force spectroscopy experiments, the peak force exerted on the biomolecules stays the same. Maintaining the peak force at the set value is important to avoid pushing the biomolecules with the probe tip too hard so that they would not be damaged and would become the secondary source of adhesive interaction.[14] Maintaining the set peak force without the need for an external driver or feedback is a unique capability with the introduced approach when compared to the previously demonstrated methods.[4,10–12] Note that the cantilever still bends, and there is a shift in zero-force level set for the cantilever. This can be corrected by reading the displacement of the membrane. To make sure that the membrane displacement is only due to thermal fluctuations but not the biomolecular interaction forces, the membrane should be much stiffer as compared to the cantilever. The details of the experimental setup are explained elsewhere.[15]

For experimental verification, we chose a 320 μm long, triangular-shaped cantilever made of silicon nitride, chromium and gold (MLCT-C, Veeco Probes). Thermal deflection of the cantilever tip was measured to be 315 nm/K. This figure matches our calculations using an analytical thermal deflection model for multilayer structures and verifies our model.[16] Even with a temperature control device that can provide 0.1 K stability,[17] the resultant force on the selected cantilever coupled to a surface will be 315 pN due to thermal fluctuations. This is a significant limitation for long timescale biomolecular experiments since this force level is comparable to biomolecular interaction forces.

To test the concept of athermalization of AFM cantilevers by a matching microstructure, we coupled the AFM cantilever in air with an identical one using the setup schematically shown in Fig. 2(a). We mounted the reference cantilever on a diffraction grating using a 300-μm-thick spacer and read its displacement using the diffraction grating interferometer. To control the temperature of the cantilevers, we placed the substrate of the reference cantilever on a thermoelectric cooler (TEC). The temperature was monitored using a semiconductor temperature sensor (LM135, National

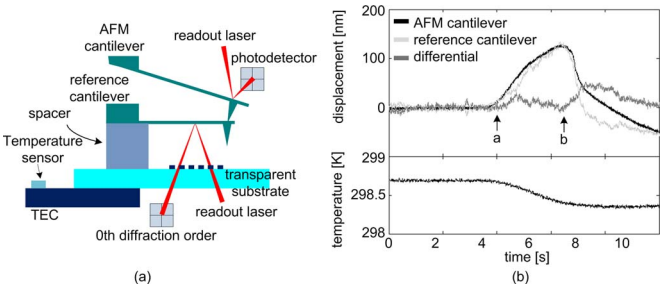

FIG. 2. (Color online) (a) Schematic of the setup with temperature control for the athermalization of an AFM cantilever using an identical one. (b) Displacements of the cantilevers recorded simultaneously together with the temperature data. Small arrows indicate when the thermal excitation was turned on and off.

Semiconductor). Figure 2(b) shows the displacement traces of the cantilevers recorded simultaneously together with the temperature data. We thermally excited the system shortly by running current through TEC from point *a* to *b*, labeled with small arrows. The temperature change was 0.4 K and both cantilevers deflected by 128 nm, which was expected from analytical calculations. The forced thermal responses of the cantilevers (from point *a* to *b*) were nearly identical and the differential displacement signal showed significant reduction in thermal deflection. However, the natural responses of the cantilevers (from point *b* to the end) were different because the thermal time constants of the cantilevers were different due to the mounting differences. This reduced the thermal deflection cancellation capability to some extent, but the differential signal still exhibited at least three times smaller change when compared with the change on AFM signal.

For biomolecular experiments we couple the cantilever with a bimaterial circular membrane as schematically shown in Fig. 3(a). We functionalize the cantilever and the membrane surface by incubation with biomolecules in a petri dish. The details for the membrane, which was made of 1.5-μm-thick silicon nitride and 0.2-μm-thick gold, are given elsewhere.[15] The expected thermal deflection at the center of the 500 μm diameter membrane was calculated to be 119 nm/K using an analytical model where the radius of curvature $(1/R)$ for a temperature change of ΔT is expressed as[18]

$$1/R = \frac{6}{h_1 + h_2} \cdot \frac{(\alpha_2 - \alpha_1)\Delta T(1 + h_1/h_2)^2}{3[1 + (h_1/h_2)]^2 + [1 + (h_1/h_2)(D_1/D_2)](h_1/h_2)^2 + \dfrac{1}{(h_1/h_2)(D_1/D_2)}}, \quad (1)$$

where h, α, and D are the thickness, coefficient of thermal expansion, and plate rigidity of the layers and the subscripts differentiate the layers.

The force spectroscopy experiment was carried out with the membrane incubated with 10–20 μl of antihuman IgG and the AFM cantilever incubated with 10 μl of human IgG (10 μg/ml) for 15–20 min at room temperature. Using the piezoactuator, we brought the cantilever in and out of contact with the membrane and recorded the displacement of the structures simultaneously, as shown in Fig. 3(b). The recorded displacement traces in these experiments can be used to extract information regarding to the molecules used; such as unbinding force strength between the molecules. To observe the effect of thermal drift, we thermally excited the system shortly by 0.3 K at point *a* labeled with a small arrow. The peak deflection of the cantilever was 60 nm. This

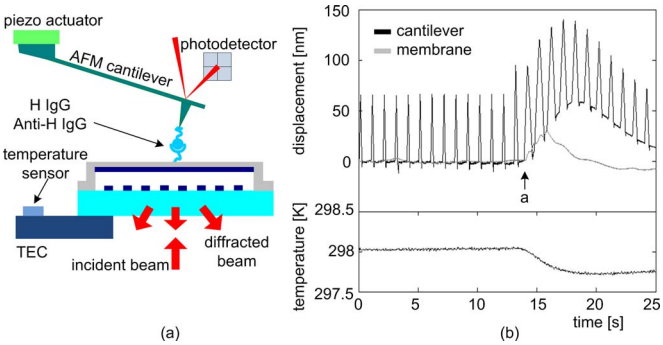

FIG. 3. (Color online) (a) Schematic of the setup with temperature control for biomolecular force spectroscopy. The AFM cantilever is engaged on a membrane that thermally displaces with the cantilever and reduces the effect of thermal fluctuations. (b) Displacements of the cantilever and the membrane recorded simultaneously together with the temperature data. Small arrow indicates when the thermal excitation was turned on.

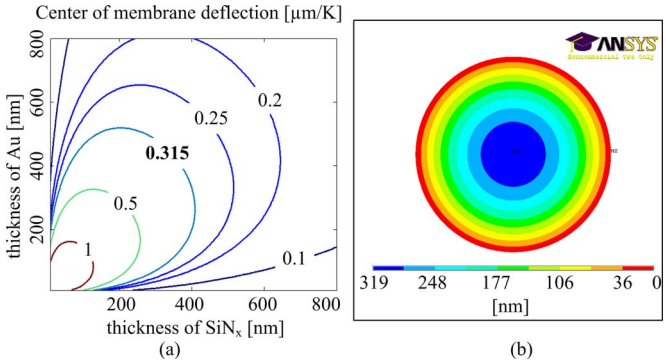

FIG. 4. (Color online) (a) Design space showing the center deflection of a 150 μm radius membrane made of silicon nitride and gold per Kelvin temperature difference. (b) FEM simulation showing the displacement profile of the membrane made of 150-nm-thick silicon nitride and 200-nm-thick gold.

was measured when the structures were out of contact, which corresponds to the shift in zero-force level of the cantilever. The membrane, on the other hand, deflected 30 nm. The measured deflection at the center of the membrane was in good agreement with the analytical deflection model of Eq. (1).

The shift in zero-force level was reduced with a differential signal using the recorded membrane displacement as a reference, but the complete cancellation of thermal drift requires a membrane that exhibits the same deflection with the cantilever. The delay seen in deflection curves of the structures can be explained with the differences between thermal time constants of the structures. Note that the introduced thermal disturbance was abrupt. Consequently, the responses of the structures were dominated by their time constants. However, the change in temperature in a typical force spectroscopy experiment is very slow, and a well designed membrane could match both the thermal deflection and the time constant of the cantilever as will be discussed below.

The second effect of thermal disturbance was the change in the peak force. This was reduced when the cantilever was coupled with the bimaterial membrane since both structures deflected in the same direction. Again, complete cancellation requires perfectly matching membrane. Note that the membrane used was 3000 times stiffer than the cantilever. This ensured the membrane displacement due to the biomolecular interactions was insignificant.

Based on the experimental data we obtained using the available membrane, we designed an ideal membrane for this particular cantilever. The layer thicknesses were determined to match the thermal time constants of the structures. For immersed structures, thermal paths from the structure areas to the fluid will have higher conductivity. Based on this assumption, and using water as working fluid, we designed a membrane with 270-nm-thick gold and 400-nm-thick silicon nitride. If the radius of this membrane is set to 150 μm [the design space for thermal deflection is given in Fig. 4(a)], the analytical model of Eq. (1) predicts that the membrane center deflects by 320 nm/K. Thus it matches the thermal deflection of the selected cantilever. This figure was verified with the finite element simulation (FEM) using ANSYS software, as shown in Fig. 4(b). Moreover, Fig. 4(b) shows the possibility of using this membrane with different cantilevers by coupling them at different locations on the constant displacement contours on the membrane to match their thermal displacements. Note that this membrane is 300 times stiffer than the selected cantilever, and hence the membrane deflection due to biomolecular interactions will still be insignificant.

In summary, we present a method for athermalization in AFM especially for biomolecular experiments. This method uses microstructures that thermomechanically match cantilevers. We demonstrate the concept of athermalization by coupling a measurement cantilever with an identical one. We also present athermalization in AFM for biomolecular experiments using a micromachined membrane. We also provide the design of a membrane to match the cantilevers for perfect cancellation of thermal drift.

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