Using Thermal K to Calibrate the Spring Constants (κ) of AFM Probes

Support Note

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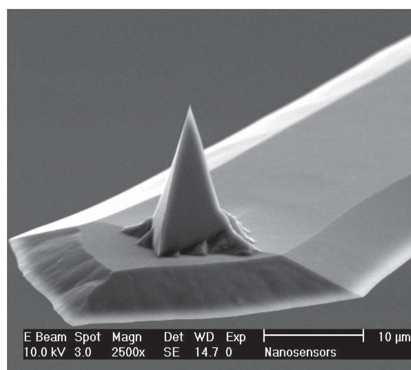

Introduction

Atomic Force Spectroscopy (AFM) is a powerful technique that can be used to quantify the elastic properties of materials or to measure single molecule unfolding or unbinding interactions on the picoNewton scale. AFM probes are composed of flexible, triangular or rectangular cantilevers with a sharp tip near the end of the cantilever. They can be manufactured from a variety of materials, but most AFM probes are made from silicon and/or silicon nitride (Si_3N_4) wafers using semiconductor-based etching processes.

An AFM probe's sensitivity, or spring constant (κ), is the force required to bend the cantilever per unit distance (usually reported in Newtons/meter; N/m). It is an important factor in AFM probe behavior and performance for imaging applications and an essential parameter when attempting to quantify intra- or inter-molecular interactions or the compliance of materials with the AFM.

Nominal spring constant values are commonly reported by AFM probe suppliers. However, because of manufacturing variability, the spring constants of individual cantilevers can vary greatly; even between probes in the same batch or from the same wafer. Consequently, in order to accurately quantify material compliance or molecular interactions with the AFM, the spring constant for each probe should be determined by empirical or semi-empirical methods.

Calculation of Cantilever Spring Constants:

Various techniques have been applied to the calculation of cantilever spring constants for AFM applications. Methods that have been used for this purpose include dimensional techniques, which are based on the cantilever's material properties, geometry, and dimensions [Sader 1995]; methods in which the spring constant is calculated by adding mass to the probe [Cleveland 1993]; techniques that require pressing the probe against a very stiff surface and subsequently against a reference spring of known compliance [Torii 1996]; and thermal methods, which treat the cantilever as a simple harmonic oscillator [Butt 1995, Hutter 1993].

Thermal K from Agilent:

Thermal K is an option for the Agilent AFM that allows the user to quickly and easily calculate the spring constant of an AFM probe. Thermal K calculates AFM probe spring constants by describing the motion of the cantilever as a harmonic oscillator through the use of the equipartition theorem from fundamental thermodynamic theory. The theory states that the kinetic energy stored in a system at a momentum coordinate, which is the deflection of a cantilever from its equilibrium position, is equal to one half of the thermal energy of the system [Cook 2006]. The deflection value from the resting state of the cantilever is considered to be small for AFM probes, and it can be assumed to be linearly related to the force required to deflect the cantilever according to Hooke's Law, F = $-\kappa \cdot x$.

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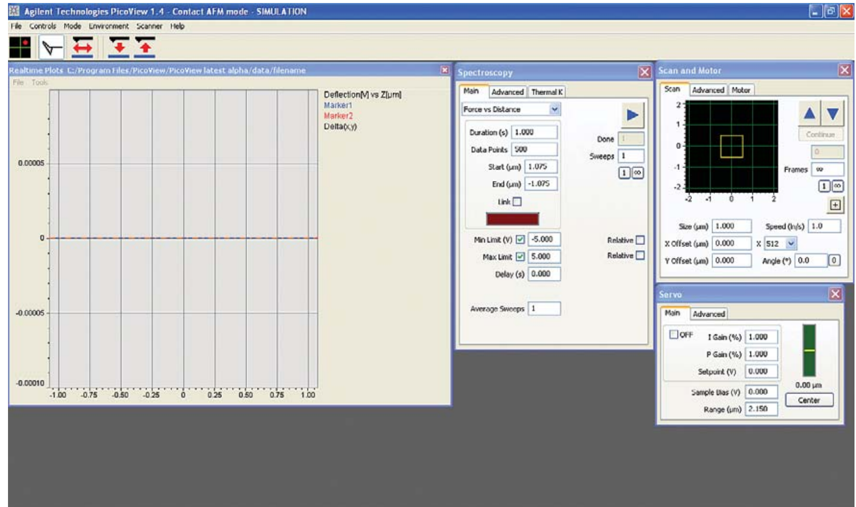
For an ideal spring with a spring constant value of κ, the thermal noise according to its position allows the spring constant to be determined. In principle, simply measuring the deflection value of the cantilever and temperature would permit its spring constant to be calculated. However, an AFM cantilever is not an ideal Hookean spring, so its potential energy can not accurately be described in such a simple manner. Consequently, the area of the resonant peak in the Power Density Spectrum (PSD) spectra of the vibrational noise permits a more accurate determination of κ [Cook 2006, Hutter 1993, Hutter 2005].

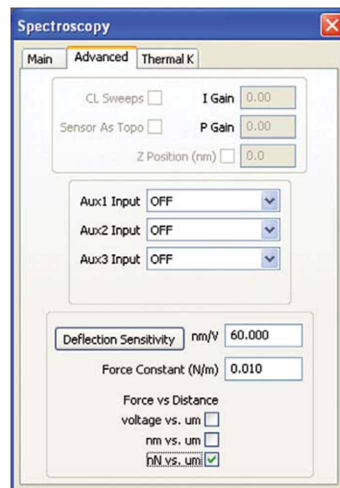
Calculating AFM Cantilever Spring Constants with Thermal K

Agilent's Thermal K option includes the Thermal K software and the necessary connector cables. A data acquisition card will also need to be installed on the user's computer. After installation is complete, Thermal K is accessed from the *'Spectroscopy'* feature in PicoView: ▶

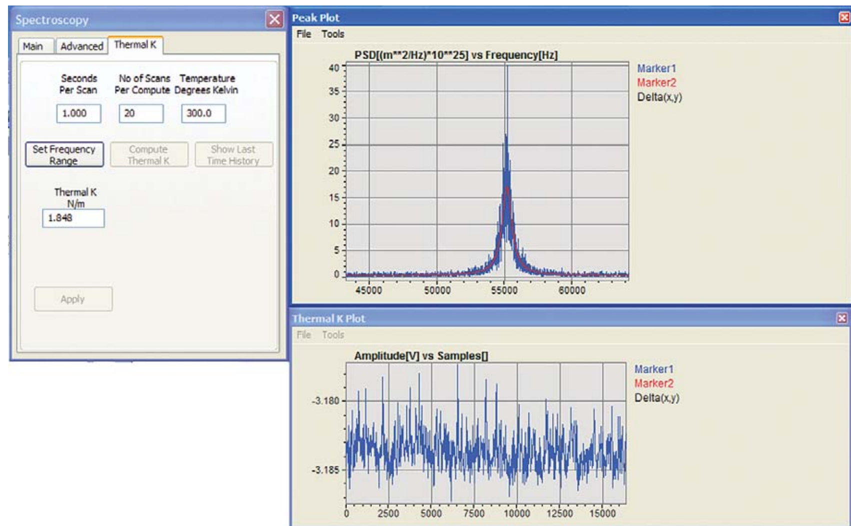


The slope of the contact portion of a Deflection vs Distance curve, which is acquired by pushing the probe against on a rigid substrate such as freshly cleaved mica, is used to calculate the cantilever's deflection calibration factor: ▶

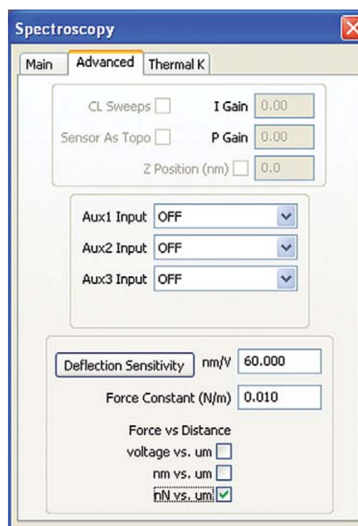
The *'Deflection Sensitivity'* of cantilever (nm/volt) is then calculated in the *'Spectroscopy-Advanced'* window: ▶



In the *'Spectroscopy-Thermal K'* window, a Power Spectral Density ('PSD') plot is obtained in order to determine the resonant frequency of the cantilever. Then, the spring constant of the cantilever (N/m) is calculated. The κ value will appear simultaneously in the *'Spectroscopy-Thermal K'* window and the *'Spectroscopy- Advanced'* window: ▶



After the spring constant has been calculated, subsequent sweeps will be displayed in units of Force vs Distance (nN/µm) units by selecting *'nN vs µm'* in the *'Spectroscopy-Advanced'* window: ▶



Note: In order to obtain accurate spring constant values with Thermal K, the z closed loop scanner is recommended. Also, in order to maintain the integrity of the tip of the AFM probe, it is often preferable to perform the calibration after all Force vs Distance sweeps have been performed.

Conclusion

Atomic Force Spectroscopy is a powerful technique that can be used to quantify material properties and molecular interactions on the single molecule level with picoNewton scale sensitivity. Because of probe to probe variability, and since cantilever sensitivity is such an important factor in many measurements with the AFM, the calculation of each cantilever's spring constant is an important step in many studies that use the AFM. Thermal K, which is an option for the Agilent AFM, allows each cantilever's spring constant value to be easily, quickly and accurately calculated.

References:

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