Force Spectroscopy with the Atomic Force Microscope

Application Note

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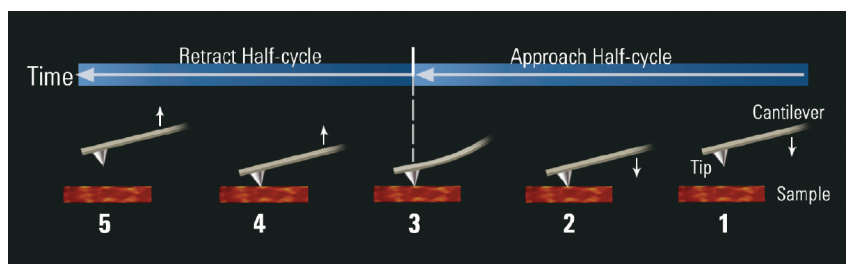*Agilent Technologies*

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Figure 1. In Force spectroscopy raster-scanning is disabled temporarily or indefinitely after the tip positions at a desired in-plane coordinate (X,Y). Then, either the sample or the cantilever (as shown here) moves in the Z-direction. Typically, an approach half-cycle is followed by a retract half-cycle, but consecutive half-cycles may have unequal durations and include different offsets in Z.

Introduction and Review

Atomic Force Microscope (AFM) Spectroscopy is an AFM based technique to measure, and sometimes control the polarity and strength of the interaction between the AFM tip and the sample. Although the tip-sample interaction may be studied in terms of the energy, the quantity that is measured first is always the tip-sample *force*, and thus the nomenclature: *force spectroscopy*. Unlike imaging, force spectroscopy is performed mostly when the servo feedback loop is deactivated. In force spectroscopy, the cantilever-tip assembly acts as a force sensor (Figure 1).

Force spectroscopy is widely used in air, liquids, and different controlled environment. It may involve functionalized tips to study specific interactions of conjugated molecules (Sidebar 1).

In order to quantitatively measure interaction forces, it is necessary to know the bending stiffness (or spring constant)

Functionalized Tips

Except in ultra high vacuum, the surfaces of the tip and the sample are nearly always covered, either partially or completely, with an adsorbed layer of molecules from the environment. Therefore, the interaction between the tip and the sample surface may be mediated through these adsorbed species. On the other hand, often the tip and (or) the sample surface are intentionally exposed to chemical or biological species by the design of the experiment. In these cases, the tip (or the sample) is said to be *functionalized*. (See for example the article in reference 1.)[1]

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of the AFM cantilever as accurately as possible. Measuring the cantilever's mechanical properties, including its bending stiffness, is itself an important research topic in AFM. There are several methods to measure cantilever's spring constant in addition to theoretical calculation. A commonly adapted convenient one is the *thermal tune* method (Sidebar 2).
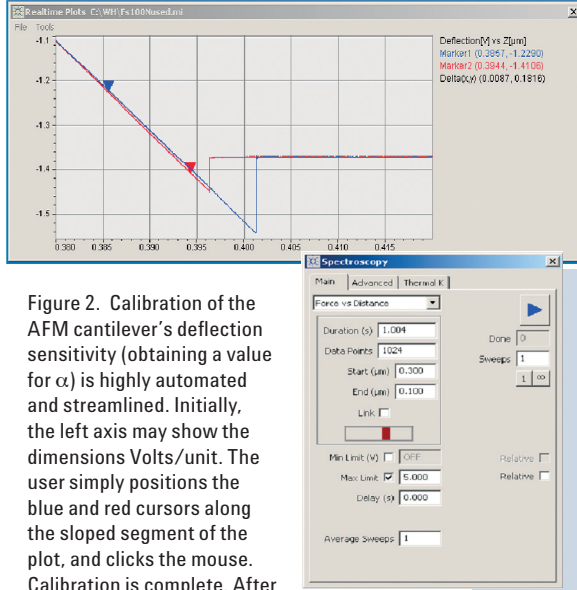

Figure 2. Calibration of the AFM cantilever's deflection sensitivity (obtaining a value for α) is highly automated and streamlined. Initially, the left axis may show the dimensions Volts/unit. The user simply positions the blue and red cursors along the sloped segment of the plot, and clicks the mouse. Calibration is complete. After calibration, the dimensions change to nm/unit.
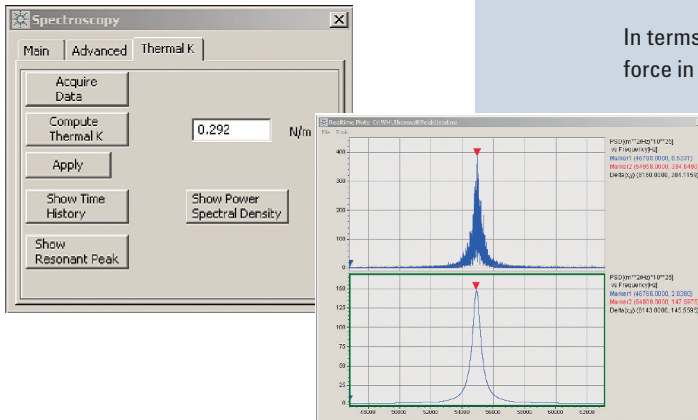

Figure 3. Thermal Tune measurement of the cantilever's spring constant is highly automated. With a few clicks of the mouse, the AFM collects data in the time-domain of the cantilever's thermal noise response (not shown), computes the frequency spectrum of the time-domain data (top plot), and extracts the resonance frequency from a fit (bottom plot) to the frequency spectrum.

Converting Cantilever Deflection into Tip-sample Force

The force F between the tip and the sample is related to the cantilever's deflection through Hooke's law:

$$F = k \cdot \alpha \cdot V \qquad \text{(Eq. 1)}$$

where k is the cantilever's spring constant, V is the measured cantilever's deflection in volt, and α is called the deflection sensitivity that converts cantilever's deflection from volt to nanometers.

These three quantities, k, α, and V are the main quantities that the AFM determines in force spectroscopy. The deflection V is measured directly with a position-sensitive split photodiode detector (PSPD). The deflection sensitivity α is determined from a force vs. distance curve by simply positioning two cursors on the contact part (See Figure 2).

The cantilever's bending stiffness or spring constant k is usually estimated by its manufacturer, but this estimate can be quite gross; 300 per cent deviation from the stated estimate is not unusual. For this reason, several methods have been developed to arrive at a more accurate estimate. In Agilent AFMs, k is determined using the *Thermal Tune* method (see Figure 3). The graphic user interface for it is highly streamlined and made intuitively easy to understand and to use for timely measurements using a few clicks of the mouse.

The spring constant k is given by[2]

$$k = 2 \pi^3 l^3 w \sqrt{\frac{\rho^3}{E}} f_0^3 \qquad \text{(Eq. 2)}$$

Where l, w, and f_0 are a (rectangular) cantilever's length, width, and fundamental resonance frequency, respectively, and E and ρ are, respectively, the Young's modulus of elasticity and mass density of the material of the cantilever.

In terms of the AFM-determined quantities, V, α, and f_0, the tip-sample force in *Eq. 1* is given by

$$F = 2 \pi^3 l^3 w \sqrt{\frac{\rho^3}{E}} f_0^3 \alpha V \qquad \text{(Eq. 3)}$$

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Applications of Force Spectroscopy

Force Pulling Measurements of Intermolecular and Intramolecular Interactions

Some of the fastest growing applications of force spectroscopy involve a method commonly referred to as Force Pulling. Here, the AFM measures the forces and from these measurements we can extract the bond energies of the interactions between two molecules (intermolecular) or between different parts of a single molecule (intramolecular). Intermolecular experiments sometimes involve a third molecule that acts as a link (for example, polyethylene glycole or PEG): one end of it attaches to the AFM tip, and the other end to one of the two molecules of interest. The second molecule of interest is on the sample surface.

Applications of Force Pulling are broad and growing. In biology and biochemistry, for example, they include advancing our knowledge of

- how macromolecules are structured
- how biomolecules assemble themselves
- how to build single-biomolecular sensors
- how viral infectious diseases work at the level of molecules

Force spectroscopy via non-specific binding is a simple and easy way to study molecular interactions. Figure 4 shows an example of the pulling experiment on the protein titin I27 [4]. Here an unfunctionized tip was brought in contact with a surface that was fully covered with titin molecules, and then was pulled away from the surface. In a good event, one end of a titin was bound to the tip. As the tip was pulled up, individual domains of the protein were unfolded sequentially, creating a regular saw-tooth pattern. The unfolding force versus pulling rate as well as details of each unfolding event may be used to study structure and function of the giant protein.
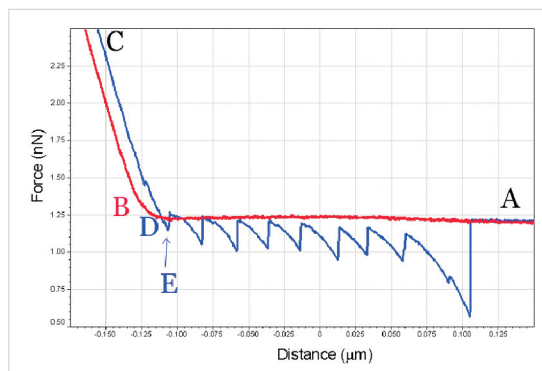

Figure 4. Force vs. distance curve of intramolecular forces for I27: a recombinant polyprotein composed of eight repetitions of the Ig module 27 domain of human titin. Red is approach, and blue is retract half-cycle. A: the equilibrium position of the cantilever. B: in approach, the tip contacts the sample and the cantilever is pushed by the sample and deflects up. C: the approach half-cycle ends and the retract half-cycle begins. D: the cantilever bends down, and passes its equilibrium position and its deflection reverses polarity from up to down, indicating the presence of an attractive interaction between the tip and the sample. E: multiple unfolding events (ruptures) of the molecule. Multiple ruptures most frequently correspond to the incremental unfolding of a macromolecule, as seen here. The final event in the retract half-cycle shown here corresponds to the cantilever breaking away from the sample completely and returning to its equilibrium position (A).[3]

Nanomechanics

Force spectroscopy provides several methods for routinely investigating, characterizing, and measuring mechanical properties of the sample surface locally, that is, with nanometer-scale XY resolution. Under some conditions, this resolution limit enhances, reaching the atomic-scale, but for the most part, the majority of applications in this area have so far involved molecular or nanometer-scale resolution.

One of the most widely explored applications is evaluating the mechanical compliance of the sample surface: How hard is the surface at a given XY location? How much harder is one location (or one sample) as compared with others?

This application is usually called nanoindentation. The tip and the sample are brought into contact, and then pushed further towards each other, forcing the cantilever and the sample into mechanical compliance under the increasing force at the tip-sample contact. Cantilever deflection and tip penetration into the sample together hold the convoluted mechanical response of the cantilever-sample system, which is usually modeled with Hertzian contact mechanics, but also with Sneddon contact mechanics.[4]

In nanoindentation experiments, it is not always straightforward to separate the response of the cantilever from that of the sample, but AFM users and manufacturers continue to devise new and sometimes improved methods for decoupling each from each, and for improving the sensitivity of nanoindentation experiments in general. For example, Agilent Technologies AFMs incorporate segmented piezoelectric scanners with low hysteresis. Closed-loop scanners are also available for hysteresis-free measurements.

Long-range Attractive Forces

Force spectroscopy can be used to measure long-range attractive forces between the tip and the sample surface in a manner similar to force pulling applications. Here, the area of interest in the force-distance curve is often the vicinity of (immediately prior to) the tip-sample contact in the approach half-cycle, as depicted by the discontinuity in the approach curve (red) in Figure 2.

In Figure 5, the approach and retract portions of a force spectroscopy cycle appear in blue and red traces, respectively. The effect of long-range forces is seen in a typical jump-to-contact signature in the approach curve. The jump-to-contact corresponds to the tip (cantilever) accelerating into contact with the sample over a distance of approximately 5 nm. This distance is typical for many sample and tip combinations in air. With less than 10 nm of space separating the two, the long-range attractive forces are strong enough between the tip and the sample surface to overcome the cantilever's restoring force.
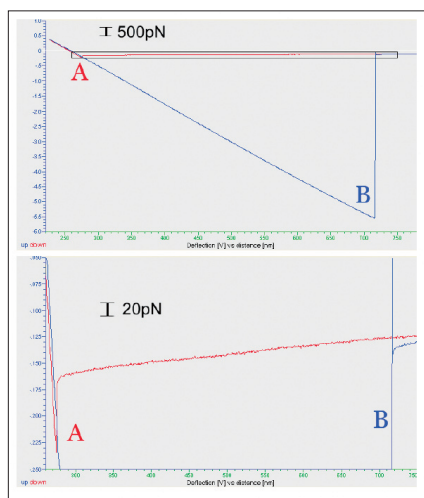

Figure 5. Bottom plot is a zoom into the black boxed area in top plot. Jump-to-contact (A) is a typical event in the approach half-cycle (red) when the tip and the sample are in air or some other gaseous environment. In this example, the tip-sample complex forms a very strong attractive force (several µNs) after contact in air; this is seen in the large deflection of the cantilever down during the retract half-cycle (blue), before snapping free of the sample (B). Zooming into the approach plot, the level of non-contact noise during the approach is revealed to be less than 5 pN peak-to-peak, with a corresponding rms value of 1.2 pN. See text.

Noise in AFM Spectroscopy

As with all AFM techniques, the limits of force sensing and force resolution in force spectroscopy are determined by the overall noise in the system, which includes the AFM, the sample, and the environment in which they are. The sources of noise from the AFM include the thermal noise of the cantilever, mechanical vibrations of the components of the AFM, and electrical and optical contributions of the corresponding components in the system. See Agilent Application note on Intrinsic Contact Noise in AFM for more about noise in AFM.

The noise determines the lower limit of the force that the AFM can detect. In the cycle recorded in Figure 5, the cantilever (tip) and the sample are apart for most of the approach half-cycle, which covers about 500 nm of travel in Z. A zoom into that data magnifies the noise during the non-contact portion of the half-cycle trace (red). This noise has an rms value of 1.2 pN. It is less than 5 pN peak-to-peak, which is exceedingly smaller than the jump-to-contact signal (A) at the far left of the trace. This low level of noise makes it possible to see a tip-sample detachment event with a force signature of just under 10 pN, recorded in the retract half-cycle in Figure 6.

Another source of noise that may be significant in force spectroscopy manifests itself in force pulling applications. This involves pulling an assembly that includes a long molecule which acts as a tether somewhere in the chain of elements between the sample surface and the AFM tip. In some cases this molecule itself is the subject of study (for example a titin molecule, in the example shown in Figure 4). In other examples, the tether molecule functions as a link, and is itself not the molecule of interest. For example, linear chains of poly ethylene glycole (PEG) are a preferred choice for this purpose, because they are chemically and physically inert, and allow for fast and free reorientation of the *tethered* molecule at the free end of the chain.[5] The added length of the tether molecule adds to the amplitude of measured noise stemming from the mechanical response of the tip-sample complex to thermal noise and to other sources of mechanical noise in the environment. Essentially, the tether molecule acts as an antenna and amplifier for mechanical noise. This increases the minimum detectable force, and lowers the force resolution.
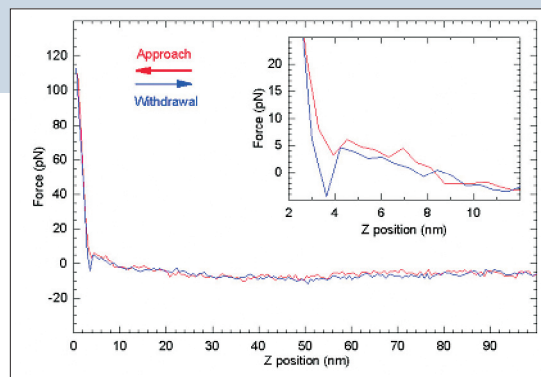

Figure 6. Extremely low noise AFM is capable of detecting pN-scale forces, such as the tip-sample detachment depicted here in the retract half-cycle (blue). This data was taken with the tip and the sample in water.

The constituents of the fluid layers adsorbed onto the sample surface and onto the tip apex play an important role in changing the apparent distance between the solid materials of the sample and the tip. They also determine the magnitude of the force that holds the tip and sample together prior to detachment during the retract half-cycle.

The jump-to-contact phenomenon and its connection with the adsorbed fluid layers is a complex subject that has provided a rich and growing volume of published research literature. It is an active area of work despite being on the oldest in AFM spectroscopy. The AFM is the only instrument that is able to routinely and easily create this type of data for analysis of forces at the nanometer-scale.

Advanced Features in Agilent AFMs

Volume Spectroscopy

Volume spectroscopy combines AFM imaging and force spectroscopy. The final recorded data set in Volume Spectroscopy contains not only the three dimensional image, but also force spectroscopy data for all the user-selected points in the image.

Agilent's PicoScan features two options for volume spectroscopy, Topograph&FlexGrid and Topograph&SPS (Figure 7a). FlexGrid allows marking up to 25 locations (XY coordinates) anywhere in an AFM image (Figure 7b), and recording at those locations one or more force-distance curves from a single or multiple approach-retract cycles of force spectroscopy during scanning. Topograph&SPS allows volume spectroscopy on every nth data point in each scan line.

SPS Images

Volume spectroscopy offers a powerful method for extending the utility of force spectroscopy, by making it easy to extract useful information from multiple force spectroscopy data sets in a graphic user interface.

In Topograph&SPS, an image type complimentary to the topography image is available for viewing and analysis. This image type maps one of 4 different measured quantities, all of which are based on the force spectroscopy data. These quantities are

1) The Z value corresponding to the point in time when the retract half cycle starts, triggered by the cantilever deflection reaching a user-selected trigger level.

2) The Z value corresponding to the point in the retract half-cycle when the cantilever's downward deflection reached its maximum value (after which the cantilever snaps free of the sample surface and bounces back up to reach its equilibrium position—event B in Figure 5).

3) The cantilever deflection value corresponding to 2 above (event B in Figure 5).

4) The slope of the cantilever deflection data at the lowest values of the deflection during the approach half-cycle.

Agilent has developed these four image types to address the growing demand in Force Pulling applications to rapidly capture and analyze multiple force spectroscopy data sets (especially for adhesion and contact mechanics studies), in a way that can easily and graphically be correlated to topographic features of the corresponding AFM image.
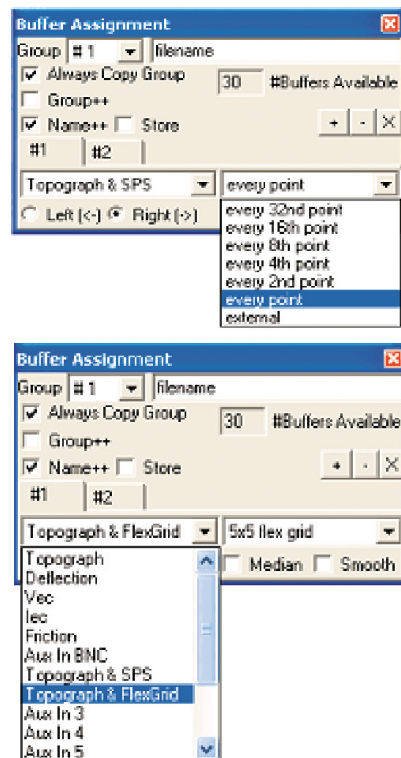

Figure 7a. Volume Spectroscopy. Flex Grid and SPS record force spectroscopy data at multiple user-selected locations, and link that data to the image. See text.
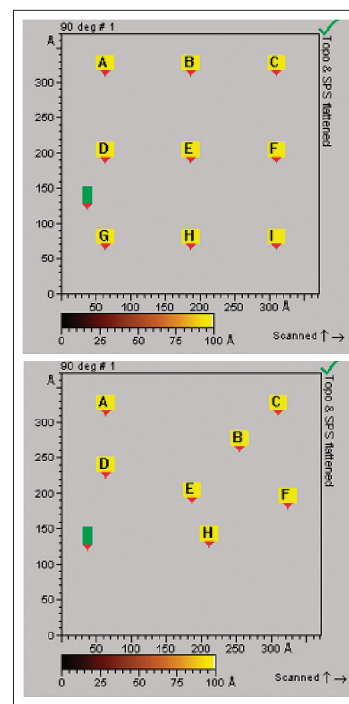

Figure 7b. The FlexGrid markers for force spectroscopy can be positioned anywhere in the AFM image field.
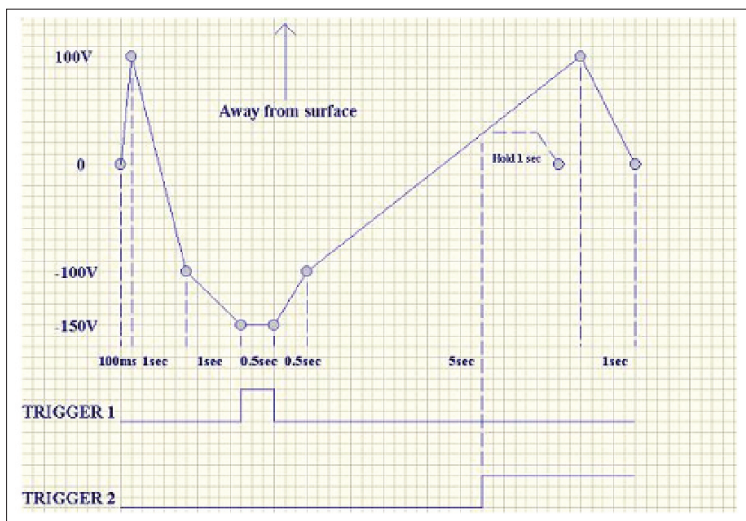
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Figure 8. Sweep voltage and trigger signals that control the movement of the probe (or the sample) in Z in Agilent's Custom Force Spectroscopy.
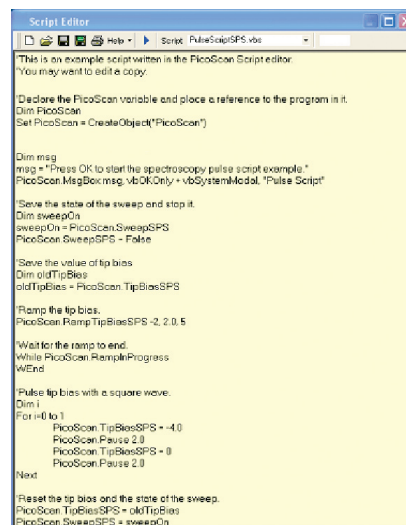

Figure 9. The Script Editor window in Agilent's PicoScan software can be used to write programs to run highly customized imaging and force spectroscopy operations.

Cycling Rate and Delays

Agilent AFMs' force spectroscopy allow the user to control the time delays introduced at various points during a force spectroscopy cycle, and the rate of the approach-retract cycle. These features are important because for some applications, the duration of a delay (typically at a point like C in Figure 4) can change the strength of the interaction between the tip and the sample, and the rate of cycling can affect the tip-sample binding strength: the tip-sample force F during the retract half-cycle is no longer a function simply of the distance traveled, but of the product of cycling velocity and cycling time, both of which the AFM user controls.

Statistical Data Analysis

Another highly desirable tool for extracting bond force (and binding energy) information from force pulling data is easy statistical analysis. In the language of bond rupture and bond energies, one is often concerned with the probability that a given bond will break at a given rate of cycling. Therefore, individual force-distance curves are insufficient to address such an issue. Multiple cycles are needed to extract statistically meaningful information. To make the process of data analysis on multiple force-distance curves faster, Agilent's AFMs include features that automatically identify and measure features of interest (such as a rupture event) on multiple data sets.

Custom Spectroscopy

For complex force spectroscopy, Agilent's PicoScan features a collection of controls and options in its Custom Spectroscopy graphic user interface. Here, the user can set up a spectroscopy experiment that is not limited to the choices in regular spectroscopy. For example, the user can define an approach half-cycle without a retract, or vice versa. The user can define how an approach or retract sweep is to be initiated (for example, triggered when the cantilever deflection reaches a certain value); how long the sweep is to last; how far the Z must travel; when to stop to sweep (at a given time after start, or for a given cantilever deflection); and how much of a delay if any to introduce and at what point in the sweep to include it. Figure 8 shows an example.

Scripting

For the most demanding custom applications, Agilent offers Scripting. This is an extremely flexible tool that allows the user to customize imaging and force spectroscopy measurements using a large number of built-in functions. In Agilent's PicoScan Script Editor, using the Visual BASIC programming language, the user can write programs that control the AFM. The software includes examples of scripting programs (Figure 9).

Point-and-Shoot with Closed-loop XY Feedback

Agilent's AFMs control the position and movement of the AFM tip with closed-loop feedback. This is a significant advantage over open-loop scanner AFMs, in which the position and movement of the tip is determined essentially using a look-up table. The open-loop scanner AFM takes an instruction for moving and positioning the tip to a given XY coordinate by using a formula to convert those coordinates into voltages that are expected to actuate the scanner to the desired XY coordinate. The problem with the open-loop scanner is the inherent nonlinearity, hysteresis, and creep of the piezo.

Closed-loop scanners use sensors to correct the intended versus the actual position of the tip in real time, so that the tip is exactly where the user wants it to be at any time, especially over a large scan area. This allows the user to take an AFM image, and having set up the force spectroscopy parameters, simply point the mouse on a given location on the AFM image, click, and capture force spectroscopy at that location. This so-called point-and-shoot feature provides convenient accurate positioning followed by spectroscopy measurements.

Summary

AFM is one of the most powerful tools for visualizing and measuring surfaces in three dimensions at high resolution. In addition to imaging, AFM force spectroscopy is gaining more and more attentions as it enables a whole new and fast-proliferating set of non-imaging applications.

Force spectroscopy allows the AFM user to observe and measure small forces, down to the pN range, between entities as small as individual molecules. This information is vitally needed for advancing nanoscience to realize the visions of nanotechnology across all areas of science. In fact, one rapidly expanding application of force spectroscopy is to study the energies that hold molecules together, or different parts of a molecule.

This application note is intended to be an introduction to force spectroscopy and a brief on available features in Agilent AFM for force measurements. Applications of force spectroscopy cover a wide range of different fields, including biology, chemistry, surface and interface science, and materials science. Specific in-depth applications on force spectroscopy can be found in future or already published application notes [7].[6]

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