True Non-Contact AFM for Soft Biological Samples

The Superiority of the True Non-Contact AFM of the XE–series

Truth Behind Imaging Soft Biological Samples

Imaging soft biological samples with an Atomic Force Microscope (AFM) is a very challenging task. Manufacturers often rely on devious techniques to overcome their design flaws, which prevent them from achieving the image quality their customers demand. This article will cover such devious techniques in detail and clarify the truth behind the imaging of soft samples using an AFM.

Figure 1 demonstrates the comparison of movements of the probe tip relative to the sample surface for images being acquired between True Non-Contact mode of the XE-series and tapping imaging AFM. Contact or tapping AFM uses the “physical contact” between the probe tip and the sample surface, whereas True Non-Contact AFM of the XE-series does not require this contact with the sample. True Non-Contact AFM of the XE-series is desirable because it provides a means for measuring sample topography with minimal or no contact between the tip and the sample surface. The total force between the tip and the sample in the Non-Contact regime is very weak, generally about 10^{-12} N. This weak force is useful when a biological sample or other very soft sample is being measured; the tip will also have an extended lifetime because it is not abraded during the scanning process. On the other hand, due to the small force between the tip and the sample, it is not possible to measure the deflection of the cantilever directly. Because of this, True Non-Contact AFM of the XE-series detects the changes in the phase or the vibration amplitude of the cantilever that are induced by the attractive force between the probe tip and the sample while the cantilever is mechanically oscillated near its resonant frequency.

Is True Non-Contact AFM Better than Tapping AFM for Soft Biological Samples?

True Non-Contact AFM of the XE-series does not suffer from the tip or sample degradation effects that are observed after taking numerous scans with contact or tapping AFM. Hence, as mentioned above, True Non-Contact AFM of the XE-series is preferable to contact or tapping AFM for measuring soft samples. In the case of rigid samples, contact and Non-Contact images may look the same. However, with a few monolayers of condensed water lying on the surface of a rigid sample, the images may look quite different. An AFM operating in contact or tapping imaging will penetrate the liquid layer to image the underlying surface, whereas in True Non-Contact mode an AFM will correctly image the surface of the liquid layer (see Figure 2).

Figure 1. Tapping AFM vs. True Non-Contact AFM.

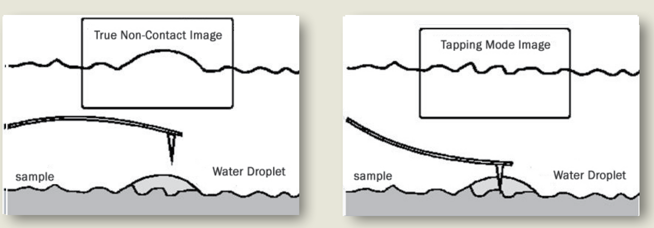

Figure 2. True Non-Contact vs. Tapping Imaging in soft sample imaging.

What About Soft Cantilevers with Ultra Low Resonance Frequency? Q-control?

Before coming to a quick conclusion about the suitability of very soft cantilevers for biological samples, one must understand how such cantilevers with ultra low resonance frequency in the range of 9 kHz -11 kHz came to existence in the first place. As previously mentioned, tapping AFM will necessarily contact the soft sample surface and even worse, penetrate into the sample, degrading the cantilever tip and the sample itself. It is precisely this reason why the tapping AFM needs a softer cantilever so that it may minimize the tapping force and sample penetration, especially for soft samples. As cantilevers get softer, the resonance frequency shifts lower and becomes susceptible to

thermal noise, effectively diffusing the resonance peak. In other words, locating the resonance peak for tapping cantilevers become problematic. The Q-control is designed to improve the signal-to-noise ratio, which helps locate the resonance peak. However, such Q-control is limited to a very specific set of sample environments to be effective.
Conclusively, Q-control is more of a trick than a solution. In the end, it is just another problem for an existing problem, rather than a fix. One must wonder if all the trouble with soft cantilevers and Q-control is worth the time and resources when True Non-Contact AFM, which directly invalidates the use of ultra low resonance frequency cantilevers and subsequent Q-control, is achievable with the use of the XE-series.

The advanced engineering of the XE-series allow for probing soft samples, disturbing neither the sample surface nor the cantilever tip.

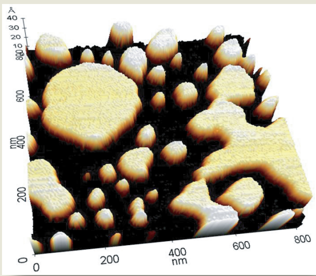

Figure 3.
True Non-Contact AFM image of phospholipid absorbed on mica (0.8 μm scan size) The fine details on the image show 3 nm thickness of supported membrane.

Figure 4.
True Non-Contact AFM image of STO feeder cells (80 μm scan size). A Cr-coated silicon nitride cantilever was used to image the sample. Its spring constant is approximately 0.32 N/m.