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Nanosurf

Application of Low-Force Photothermal Off-Resonance Tapping For *In Situ* AFM Imaging of Soft Virus Capsids
The atomic force microscope (AFM) is becoming an essential tool to image and measure the mechanical properties of biological samples in near-physiological conditions. Unlike optical or electron microscopy techniques, samples measured by AFM require very minimal preparation. The primary feedback mechanism in AFM is the force between the sample being measured and a probe. When working with soft biological samples, the force generated by the probe can often be enough to cause irreversible damage, or in some cases, physically remove the sample from the substrate if lateral stresses become too high. For this reason, imaging soft biological materials such as virus capsids on top of another soft substrate material such as cells or tissue continues to be a challenge for the AFM community.

One of the most popular dynamic force AFM imaging techniques (sometimes called amplitude modulation or tapping mode) works by trying to maintain the amplitude of an oscillating probe near is resonance frequency as it traces the contours of a surface. While it is simple enough to measure changes in amplitude, using this to determine how much force was applied to the surface requires complex technical modeling, which is oftentimes beyond the interest of general microscopists. More often, it takes a skilled AFM operator to instinctively know what settings to use for any specific sample. Developing this level of expertise has a steep learning curve and it is often frustrating for those new to the technique.

Photothermal off-resonance tapping mode (e.g. WaveMode) is a technique that combines the force selectivity of contact mode while at the same time minimizing sheer or lateral forces, which can displace loosely bound or soft materials. The benefits of a photothermally actuated cantilever for imaging at or near the resonance of the cantilever for biological applications has been well documented. Using the same photothermal light source to drive the cantilever off-resonance is a more novel technique. First, since the measurement is done off-resonance, the Q-factor of the cantilever is irrelevant, and thus the imaging bandwidth increases. Second, because the feedback mechanism is force-deflection via amplitude truncation and not peak-to-peak amplitude reduction, the force that is applied to the sample can be precisely controlled down to the low pico-Newton range. Third, off-resonance tapping mode is still considered an intermittent contact mode meaning that the probe only interacts with the surface for only a small portion of its oscillation, thus minimizing lateral stress while the probe is scanned across the surface.

Recently, WaveMode was used to image HSV-1 virus capsids on a soft rat-liver nuclei substrate. One of the challenges for imaging this type of sample with AFM is that the capsid is expected to be stiffer than the surrounding substrate, which could cause the capsid to be pushed into the nuclei. It has been shown that the nucleus exhibits a viscoelastic stiffness response, which increases its stiffness as the AFM indentation velocity increases. Thus, imaging in WaveMode causes the nucleus to stabilize around the capsid and allows for clean images of the complex.

Speaker
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