Keysight Technologies
MAC Mode Imaging of Biological Molecules with Keysight 7500 AFM
Application Brief

Introduction

One of the most appealing features of atomic force microscopy (AFM) for the field of biology is that it can operate in aqueous environments, making this technique capable of monitoring or measuring biological molecules under physiological conditions. In terms of AFM imaging, the complexity of probe oscillations and tip/sample force interactions in solutions is much higher than that under ambient conditions. For instance, ordering of the liquid at a solid interface is a ubiquitous phenomenon and such liquid structuring could have a profound effect on the operation of an AFM, causing the tip to hop between different stable configurations in the ordered region of the fluid. A Keysight Technologies, Inc. patented magnetic AC (MAC) mode is developed as a better approach to AFM in liquid, in which only the cantilever part of an AFM probe is coated with a thin magnetic coating and the introduction of an alternating magnetic field will induce a direct tip oscillation. This method eliminates the spurious resonances seen in fluid tapping mode. In addition, a substantial signal-to-noise advantage is obtained for A/C mode AFM in fluids if the tip is oscillated by direct application of a magnetic force, as opposed to indirect mechanical excitation with an acoustic transducer. In this application brief, examples of high-resolution MAC mode imaging of biological samples in liquid using Keysight 7500 AFM will be demonstrated.

Instrumentation

The Keysight 7500 AFM/SPM microscope is a high-performance instrument that delivers high resolution imaging with integrated environmental control functions. Keysight's patented magnetic AC mode (MAC Mode) is offered as a system option. Switching imaging modes with the Keysight 7500 AFM/SPM microscope is quick and convenient, a result from the scanner's interchangeable, easy-to-load nose cones. Every aspect of the Keysight 7500 AFM's design and construction are optimized to reduce mechanical noise, and deliver industry leading performance. The compact and completely encapsulated scanner provides easy cantilever exchange, a slot for (optional) preamps for STM and CSAFM operation, as well as an integrated, high-reliability connector to interface with the control electronics. All 7500 AFM's come with the lowest noise closed loop position detectors to provide the ultimate convenience and performance in imaging, without sacrificing resolution and image quality.

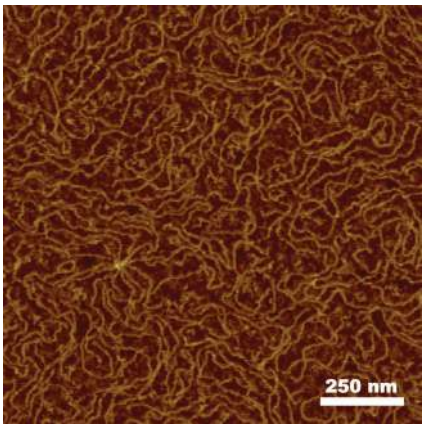

Figure 1. MAC mode imaging of lambda phage DNA in a buffer solution.

Imaging of DNA in a Buffer Solution

Lambda phase DNA is used as the first example to demonstrate the effective AFM imaging in liquid. When DNA in a buffer solution containing Ni^{++} or Mg^{++} is added to a mica substrate, these divalent cations can form a charge bridge between the negatively charged phosphate groups on the DNA backbone and the negatively charged siloxy groups on the mica. This will lead to stable immobilization of DNA molecules onto the substrate. The amount of DNA that can be adsorbed to bare mica strongly depends on the DNA concentration in the solution, soaking/reaction time, as well as the buffer composition and buffer pH. Figure 1 is an AFM

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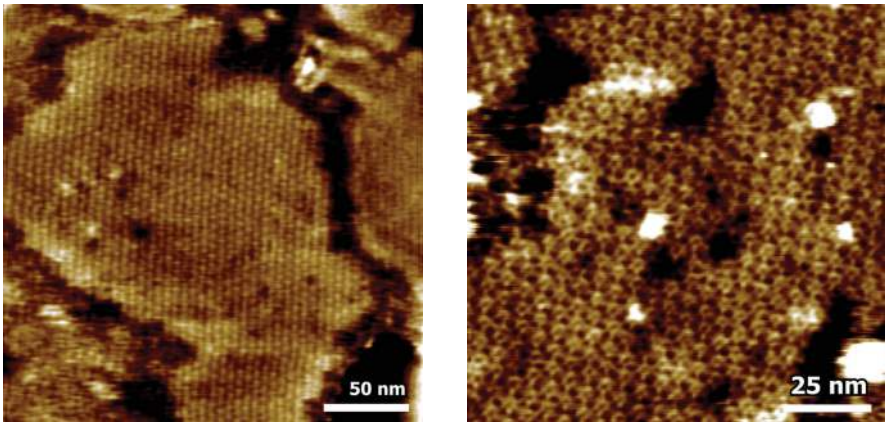

Figure 2. MAC mode imaging of purple membrane proteins in a buffer solution.

topographic image showing a case of high surface coverage of DNA molecule. Individual DNA molecules with various configurations are clearly observed.

Molecular-level Visualization of Purple Membrane Proteins via MAC Mode Imaging

Bacteriorhodopsin is a light-driven proton pump in the cell membrane of Halobacterium salinarium. The bacteriorhodopsin molecules adsorb on freshly cleaved mica substrate to form a densely packed 2-dimensional membrane. The membrane has a distinguished purple color, thus often called purple membrane (PM) in the literature. PM is stable on mica under a pH ranging from 4 to 9, and they can form patches of several hundred nm to several microns in size. The thickness of the membrane is about 5.5 nm in average and changes slightly with pH. Contact mode AFM has been used to image PM in KCL solutions of different pH level. Till today, most of the high resolution images of PM seen are achieved by contact mode AFM. However, contact-mode imaging is less suitable for weakly attached bio-sample, because bio-molecules are often disrupted by the AFM stylus during scanning. Dynamic force microscopy (DFM) methods like tapping mode and MAC mode are designed to reduce the lateral force exerted on the sample surface, thus better suited for imaging soft and weakly attached biological samples. However, tapping mode in solution is difficult to operate. Also for delicate samples like PM, the force for imaging has to be very extremely low; otherwise the lattice structure of the PM would be easily destroyed. Fortunately with MAC mode, the oscillation of the cantilever is directly driven by an oscillating electromagnetic field, thus the interaction force between the tip and the sample can be more accurately controlled. Consequently, high resolution images of the lattice structure of PM can be achieved using MAC mode in solution.

The densely-packed bacteriorhodopsin molecules form highly ordered 2-D trigonal lattices in the purple membrane. A topography image of a patch of PM of about 200 nm in size, obtained by MAC mode, is presented in Figure 2 (left). The hexagonal arrangement of the bacteriorhodopsin molecules with a repeating unit of about 6 nm and a corrugation of less than 0.8 nm is clearly revealed by the MAC mode image. At high magnification, the arrangement of bacteriorhodopsin trimers in each of the repeating unit in the membrane revealed a distinct donut-like shape, as seen in Figure 2 (right). Careful examination also shows the donut like trimer structures that are likely connected by fibrous arms. This demonstrates that MAC mode AFM is a favorable method in studying the topography of soft and weakly attached biological samples, capable of revealing single- or even sub-molecular structures with high resolution under physiological conditions.

Summary

Using imaging of DNA and purple membrane proteins in well-controlled buffer solutions as two examples, it is demonstrated that high-resolution imaging of biological samples at single molecular level can be achieved readily with Keysight 7500 AFM.

AFM Instrumentation from Keysight

Keysight Technologies offers high-precision modular AFM solutions for research industry, and education. Exceptional worldwide support is provided by experienced application scientists and technical service personnel. Keysight's leading-edge R&D laboratories are dedicated to the timely introduction and optimization of innovative and easy-to-use AFM technologies.

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