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Collagen fibrils imaging in air and in liquid

PARK SYSTEMS

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As the most abundant (25–35%) protein in mammals,[1] collagen is found everywhere in connective tissues, including bone, skin, and muscle. Thus characterization of the structure, compositions, and mechanical properties of collagen fibrils is crucial in understanding their performance over time.

Atomic force microscopy (AFM), as a powerful nanotechnology tool, has been widely used to determine the morphology, mechanical properties, and *in situ* self-assembly processes of collagen fibrils.[2] Conventional techniques to characterize these collagen fibrils are mainly based on AFM force-volume spectroscopy, which collects force–distance (F–d) curves at each pixel to calculate material properties. However, these techniques have been recognized as being exceedingly slow—it takes hours to acquire an elasticity map. Driven by the demand for a much faster technique, Park Systems developed the PinPoint Nanomechanical Mode to provide a solution that is at least 100 times faster than traditional techniques.[3] With this application, an elasticity map can be acquired within minutes and with a correlated topography image that reveals the position and orientation of the sample. This mode represents a new application tool for acquiring real-time topography and quantitative mechanical property maps of various materials, ranging from hard disks to soft tissues. Here, we report imaging collagen fibrils using the PinPoint Nanomechanical Mode.

Technique

Well-defined control of the XY scanner in the Park NX10 AFM system and the Park SmartScan operation software allow the PinPoint Nanomechanical Mode to provide high-speed F–d curves with accurate control of both contact force and time. The tip is lifted up before moving to the next pixel to prevent sample damage or tip wear due to the lateral force. Additionally, compared to the traditional AFM force-volume mode, which takes several hours to finish a 128 × 128 pixel image, PinPoint only takes several minutes to yield high-resolution topography images as well as maps of various mechanical properties such as stiffness, adhesion force, modulus, and deformation. The F–d curves collected at each pixel were fitted to the Hertz model, which due to the ease of use, has been extensively applied by the AFM

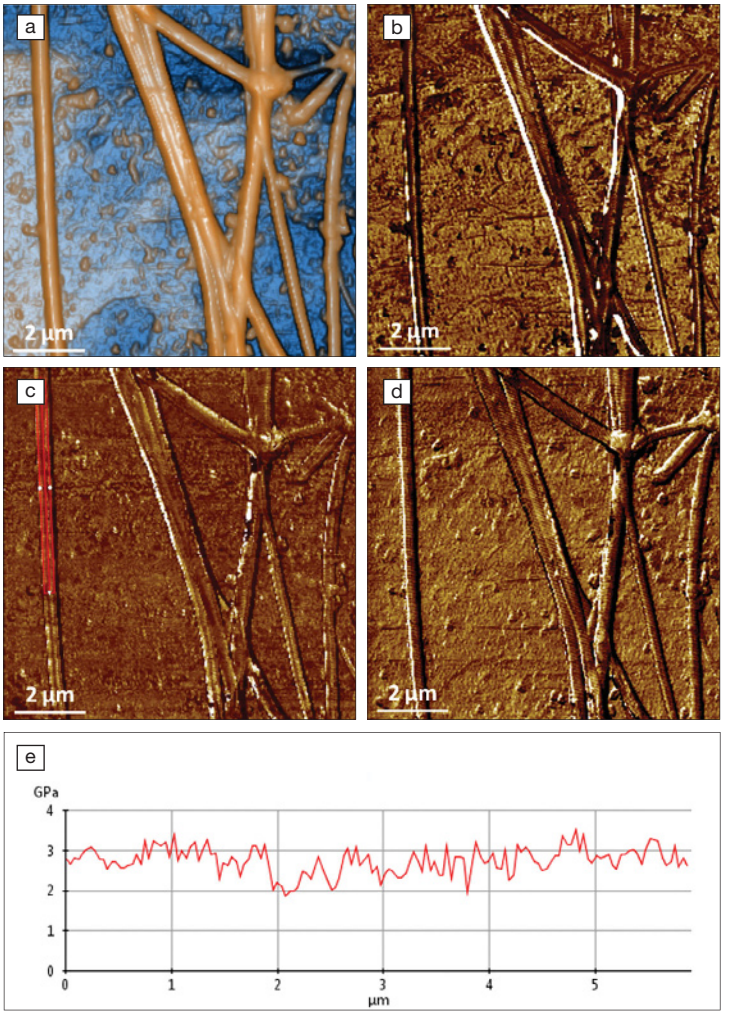

Figure 1. PinPoint Nanomechanical images of collagen for (a) contrast enhanced height, (b) adhesion force, (c) modulus, and (d) stiffness images. (e) Module line profile averaged over the red rectangular area along the length of the collagen fibril in the image (c). Images: 256 × 256 pixel and scan size 10 µm × 10 µm.

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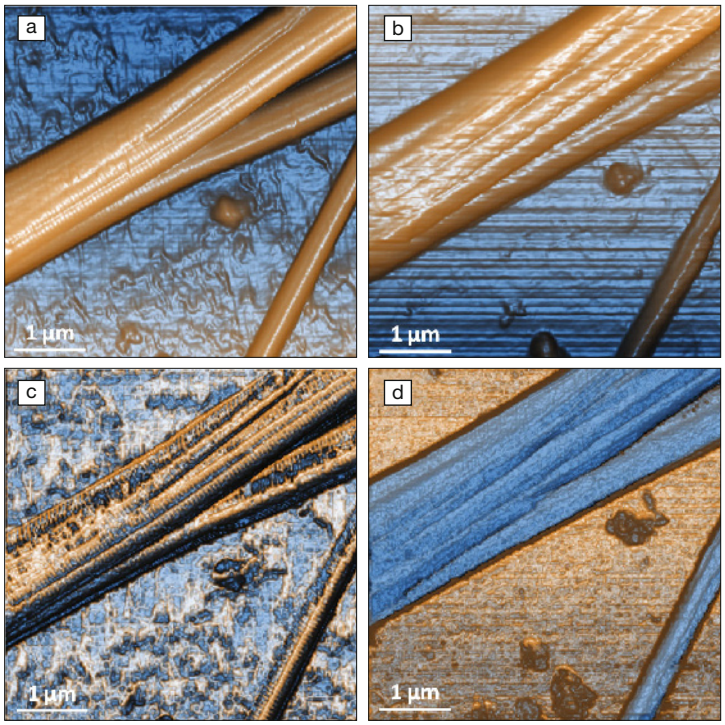

Figure 2. Contrast-enhanced height images of collagen in (a) air and (b) liquid, and modulus mapping in (c) air and (d) liquid showing that collagen significantly softens when hydrated. Images: 256 × 256 pixel and scan size 5 µm × 5 µm.

community to quantify the mechanical properties of biological samples.[4]

Experiments and results

Niigata University provided dehydrated collagen fibril samples that were cut and spin-cast on a petri dish and imaged with a Park NX10 AFM in ambient conditions using the PinPoint Nanomechanical Mode. To get proper response, the spring constant of the AFM tip needs to be chosen carefully. Medium stiffness (ferromagnetic resonance [FMR]) probes[5] were selected based on the relative stiffness of the cantilever compared to that of the sample. In addition, the collagen fibril sample was also imaged in phosphate buffered saline solution, provided by Thermo Fisher Scientific, to test the capability of this mode in liquid. Combined with the Park SmartScan software, the operation of the PinPoint Nanomechanical Mode is easy and nearly identical in both air and liquid conditions, with only a few parameters adjusted. XEI image processing software from Park Systems was used for image postprocessing. Cross-section profiles of interesting areas in an image can be shown to get information across that plane.

Figure 1 shows high-quality topography and nanomechanical property mappings of collagen. The repeatability and consistency of the PinPoint Nanomechanical Mode are reproduced among repeated experiments with different FMR cantilevers. All images clearly reveal distinct differences between the collagen fibrils and substrate. The thinness of the fibrils, on the order of tens of nm, and the tiny segments that form them can be clearly identified in all images. The diameter of the collagen bundles ranges from ~60 nm to ~600 nm, depending on the number of nanofibrils the bundle is comprised of, which vary from tens to thousands of nanofibrils.[6] The collagen's elastic modulus was measured, and the average value was about 2–3 GPa (Figure 1c and e). This is in agreement with the values reported by Gautieri et al. (~1.8–2.25 GPa).[7] To verify the function of this mode in liquid, an area of interest was selected and imaged both in air and liquid to compare the two. The data are shown in **Figure 2**, and the diameter of the bundle increased from 1.31 µm in air (Figure 2a) to 1.68 µm in liquid (Figure 2b) due to the hydration of the protein. In addition, the modulus of the fully hydrated collagen in liquid significantly dropped to only 4–12 MPa, reasonable numbers for liquid collagen samples based on previous reports.[8] The individual fibrils and small segments became hard to visualize compared to those in air. One possible reason is that the FMR cantilever is too stiff for soft collagen fibrils in liquid. During the drying process of the collagen, the sample's modulus was observed to increase with time, in a strong positive correlation to the extent to which the fibrils were dehydrated.

Summary

The topography and mechanical properties of collagen fibril samples have been efficiently and accurately imaged both in air and liquid using the Park NX10 AFM system and the PinPoint Nanomechanical Mode. Since the cantilever is lifted and moved from pixel to pixel, PinPoint mode minimizes the lateral force on the probe and protects both the probe and sample from damage. F–d curves were turned into quantitative and low-noise mechanical mapping over a wide range from MPa to GPa. Using this mode, high-contrast mapping of mechanical properties, including adhesion force, modulus, stiffness, and deformation, are taken in real time with high-resolution height imaging to help researchers better understand their samples at the nanoscale.

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