Park SYSTEMS
www.parkAFM.com

Mina Hong, Gerald Pascual, Byong Kim and Keibock Lee
Park Systems, Inc., Santa Clara, USA

Collagen Fibrils Imaging In Air Using Park NX10 Atomic Force Microscope PinPoint™ Nanomechanical Mode

Abstract

Collagen is a protein that provides structure in various connective tissues in animals and can be found in ligaments, tendons, and skin. The characterization of collagen's mechanical properties at nanoscale can potentially reveal significant insights into the causes of macroscale phenomena such as the elasticity of skin and its degradation as we age. One tool that has been used to acquire nanoscale data of collagen is the atomic force microscope (AFM). Conventional AFM techniques based on force-volume spectroscopy have been used to analyze the topography and mechanical properties of collagen. However, these techniques are extremely time-consuming—acquisition of a quantifiable elasticity map can take hours to complete. A new AFM-based nanomechanical mode has been developed to address this drawback and can perform the same task significantly faster without sacrificing resolution. Our investigation revealed that our sample collagen bundles had diameters ranging from 60 to 600 nm and an average elastic modulus of about 1.9 GPa, a value in agreement with other reported research. The total time taken to acquire this data was measured in minutes as opposed to hours.

Introduction

Collagen is the most abundant protein found in mammals [1]; therefore, it is very important to characterize its structure, functions, and mechanical properties [2-5]. For instance, as people age our skin begins to lose its firmness and wrinkles start to appear. Our skin is largely made of collagen, which in turn are made up of nanofibers. Understanding how these fibers are arranged and oriented as well as how hard or soft they are is important in understanding the process of how skin wrinkles form on our bodies.

Conventional techniques to characterize these fibers based on force-volume spectroscopy were performed in past research efforts. However, these techniques have been recognized as being exceedingly slow—it takes days for these methods to acquire an elasticity map for a test sample that is quantifiable. The need for a much faster technique is very real, and finally one has been developed that is at least 100 times faster: PinPoint™ Nanomechanical Mode [6]. With PinPoint™ mode, the same quantifiable elasticity map took days to be collected can now be acquired within the space of an hour and with a correlated topography image that reveals the position and orientation of sample collagen nanofibers.

Experiments

The dehydrated collagen fibril sample provided by our collaborator at Niigata University was cut and spin-cast on a petri dish and imaged with a Park NX10 AFM in ambient condition using PinPoint™ nanomechanical mode, the basic of operation of which is detailed in Figure 1. In order to get the most accurate mechanical properties data, spring constant of the cantilever needs to be chosen properly so it can respond to any changes of the material properties on the surface. We picked FMR probes (spring constant 2.8 N/m nominal) since they met our needs in providing instant response to the surface properties. We

repeated the tests with three different probes to prove the reproducibility and consistency of this mode. In addition, we also imaged polystyrene – low density polyolefin elastomer (PS-LDPE) standard calibration sample as a reference in terms of accuracy verification of the mode. The PS-LDPE is a copolymer sample mounted on a 12 mm steel sample puck. A blend of PS and PE were spin-cast onto a silicon substrate, creating a film with different material properties. PS with elastic modulus around 2 GPa serves as the matrix while PE is the low density doping component with elastic modulus around 0.1 GPa [7].

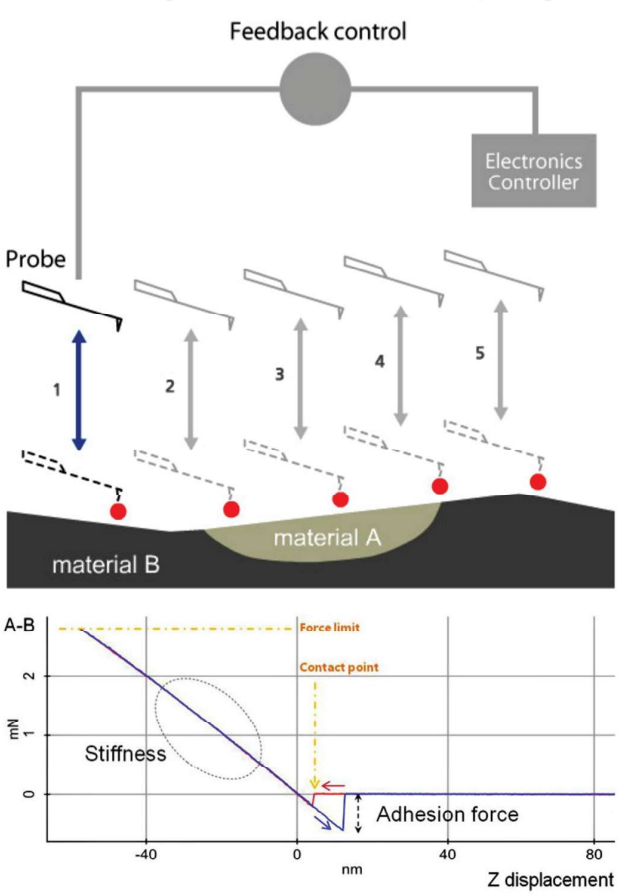


Figure 1. Working mechanism of PinPoint™ nanomechanical mode by Park Systems. The probe is moved from point 1 to 5, and at each point force-distance curves are taken to calculate the nanomechanics each point.

Results and Discussions

Combined with the SmartScan software, the operation of PinPoint ™ nanomechanical mode is super user friendly and it is also identical in both air and liquid conditions. Figure 2 exhibits a set of images of the PS-LDPE sample (pixel size 96 × 96 and scan size 1.6μm×1.6μm) collected within 3mins. The unambiguous and high contrast adhesion force (Figure 2b), modulus (Figure 2c) and stiffness (Figure 2d) images were captured in real time with the topography image (Figure 2a). With the XEI software, the cross section profile of an interesting area in modulus image (red line in Figure 2b) can be shown as in Figure 3.The modulus we produced of PS and PE is around 2.7 GPa and 0.3 GPa respectively, quite comparable to the 2 GPa and 0.1 GPa claimed by the calibration sample supplier considering reasonable errors, for example, the cantilevers we used (the difference in force constant, tip radius, tip's Poisson ratio), the models we used to fit the force curves to produce the mechanical data and the conditions the samples were kept.

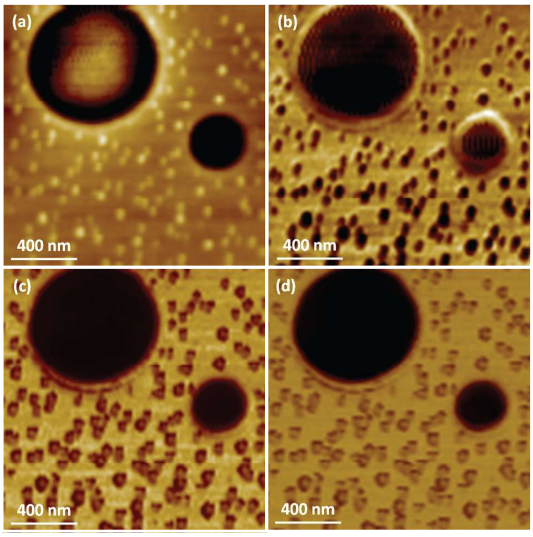


Figure 2. PinPoint™ nanomechanical images of PS-LDPE standard sample include (a) height, (b) adhesion force, (c) modulus and (d) stiffness. Images pixel 96×96 and scan size 1.6μm×1.6μm.

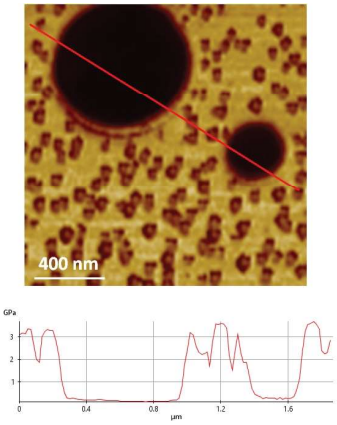
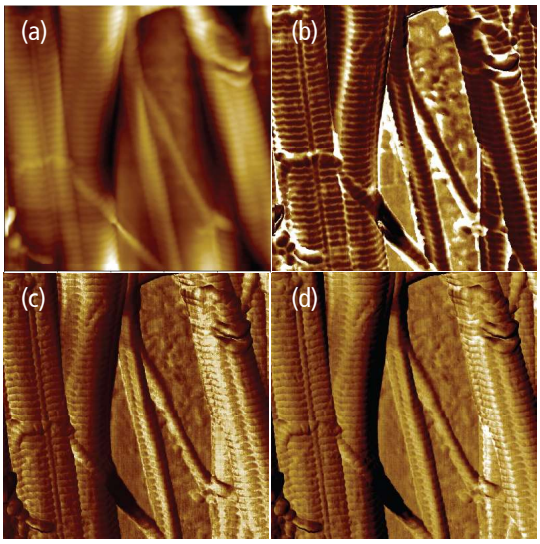


Figure 3. Cross section line profile of Figure 2(b) exhibiting that the modulus of PS and PE is around 2.7GPa and 0.3Gpa, comparable with the 2 GPa and 0.1 GPa

Figure 4 shows similar data of collagen fibrils as of Figure 2 for PS-LDPE. The repeatability and consistency of the image quality are well kept among repeated experiments with different cantilevers (same type).The images clearly reveal distinct collagen fibrils of various sizes in diameter from the substrate. In addition, all small segments, positioned perpendicular to the fibrils' longitude direction and formed due to the self-assembly process of individual triple helices of fibrillar collagen, can be clearly seen in all images. The diameter of the collagen bundles we observed varies from ~150nm to ~ 600nm, corresponding to the composition of hundreds to thousands microfibrils respectively [8]. From Figure 4d, we measured the average collagen elastic modulus to be around 1.94 GPa. This is

in great agreement with the work reported by Gautieriet al. that the Young's modulus from wet (~300 MPa) to dry (~1.8-2.25 GPa) collagen was significantly increased [9].The strengthen mechanism will not be discussed in detail here. In all, since collagen fibrils are a very comprehensive and representative model for studying protein properties, we are confident that PinPoint™ nanomechanical mode can be very useful in providing quantitative and high quality topography and mechanical properties mapping.



Summary

The topography and mechanical properties of PS-LDPE and collagen fibril standard samples have been efficiently and accurately imaged using Park NX10 AFM, PinPoint™ nanomechanical mode. One can characterize the mechanical properties on a surface or at cross section easily. PinPoint™ nanomechanical mode can effectively minimize the lateral force on the probe and protect the sample from and relative damages. Force-distance curves are taken and analyzed at each pixel, which are further turned into quantitative and low noise mechanical mapping over a wide range of numbers (MPa - GPa). High contrast mapping of mechanical properties including adhesion force, modulus, stiffness and deformation are taken real time with high resolution height image. In all, PinPoint™ nanomechanical mode will successfully provide researchers with critical material property information to enable better understanding of their samples at the nanoscale

References

1. Di Lullo, G.A., et al., Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. Journal of Biological Chemistry, 2002. 277(6): p. 4223-4231.
2. Narayanan, B., et al., Self-assembly of collagen on flat surfaces: The interplay of collagen–collagen and collagen–substrate interactions. Langmuir, 2014. 30(5): p. 1343-1350.
3. Su, H., et al., The ultrastructure of type I collagen at nanoscale: large or small D-spacing distribution? Nanoscale, 2014. 6(14): p. 8134-8139.
4. Berenguer de la Cuesta, F., et al., Coherent X-ray diffraction from collagenous soft tissues. Proceedings of the National Academy of Sciences, 2009. 106(36): p. 15297-15301.
5.Wenger, M.P.E., et al., Mechanical properties of collagen fibrils. Biophysical Journal, 2007. 93(4): p. 1255-1263.
6. Park Systems Introduces PinPoint™ Nanomechanical Mode to Characterize Nano Mechanical Properties of Materials and Biological Cells. Available from: http://www.parkafm.com/index.php/company/news/press-release/450-nanomechanical-mode-to-characterize-nano-mechanical.
7.PS-LDPE-12M Product Description. Available from: http://www.brukerafmprobes.com/a-3724-ps-ldpe-12m.aspx.
8. Layton, B.E. and A.M. Sastry, Equal and local-load-sharing micromechanical models for collagens: Quantitative comparisons in response of non-diabetic and diabetic rat tissue. Acta Biomaterialia, 2006. 2(6): p. 595-607.
9. Gautieri, A., et al., Hierarchical Structure and Nanomechanics of Collagen Microfibrils from the Atomistic Scale Up. Nano Letters, 2011. 11(2): p. 757-766.