PIUMA NANOINDENTER

Optics11 B.V.
De Boelelaan 1081
1081 HV Amsterdam
The Netherlands

For more information,
please visit our website:
www.optics11.com

or contact us at:
info@optics11.com

Office telephone:
+31 (0)20 598 79 17

Piuma Nanoindenter Application note:

Characterizing the mechanical properties of immersed PNIPAAm hydrogel micropillars

The Piuma Nanoindenter is specifically developed to non-destructively characterize the mechanical properties of soft materials, such as tissues, biomaterials, hydrogels and cells. Using a novel proprietary optical fiber-top technology, the Piuma provides great force sensitiviy, accuracy and precision while remaining very easy to use.

Application note AN-1601, written by Ernst Breel & Thomas van der Wardt, Optics11 BV
Published on 9 March 2016 on www.optics11.com

First synthesized in the 1950s, Poly(N-isopropylacrylamide), also known as PNIPAAm, has been of interest to many researchers for applications in the field of active polymers and tissue engineering. The reason for this interest is due to its thermosensitivity: the polymer displays a lower critical solution temperature (LCST) of 32°C, above which it changes from a swollen hydrated state to a shrunken dehydrated state [1]. When switching from one state to another the mechanical properties of PNIPAAm change.

This note describes the mechanical characterization of PNIPAAm pillars immersed in solution using the Piuma Nanoindenter.

In cell culturing, PNIPAAm can be used to modify the surface of traditional polystyrene culture dishes, and facilitate cell growth by simulating a physiological stiffness and cell harvesting by enabling cell release by switching from 37°C to below 32°C. This feature combined with a high degree of biocompatibility and biostability make PNIPAAm hydrogels very promising for tissue engineering and cell culturing applications [2].

As it is possible to modify PNIPAAm hydrogels to alter (bio)chemical and mechanical properties, a method for the local mechanical characterization of this material while in physiological conditions is relevant: not only to assess the resemblance of the hydrogels' mechanical properties with the properties of native extracellular matrix, but also to examine the switching behavior or crosslinking consistency of the material itself.

Methods and materials

The Piuma Nanoindenter is fitted with a 60.6 N/m cantilever-based probe with a tip radius of 27 µm, mounted on an extended fiber. The displacement profile is first set to displace 18 µm in 2 seconds, with a 1 second holding time.

Following the Herz model, the data in the loading section of the load-displacement curve is used to determine the Young's Modulus, using a fit of all data points from the contact point to 40% of the maximum load point [3].

Sample preparation

PNIPAAm is synthesized by reacting the monomer N-isopropylacrylamide with the crosslinker N,N'-methylene-bis-acrylamide, in the presence of the initiator potassium persulfate. Using a molding technique, 300 x 300 x 100 µm (l x w x h) micropillar structures are formed.

Results

A 10 x 10 grid with a 15 µm step size, covering the pillar center, is configured so the mechanical distribution within a single pillar can be obtained. Additionally the topography of one PNIPAAm pillar is extracted from the dataset.

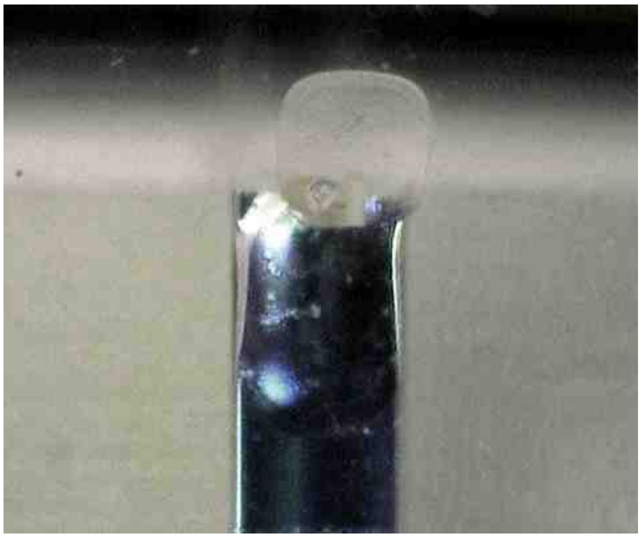

Figure 1: inverted camera view of PNIPAAm micropillar and cantilever.

A single PNIPAAm micropillar exhibits a gaussian-like distribution in stiffness with a local highest modulus in the center of the pillar:

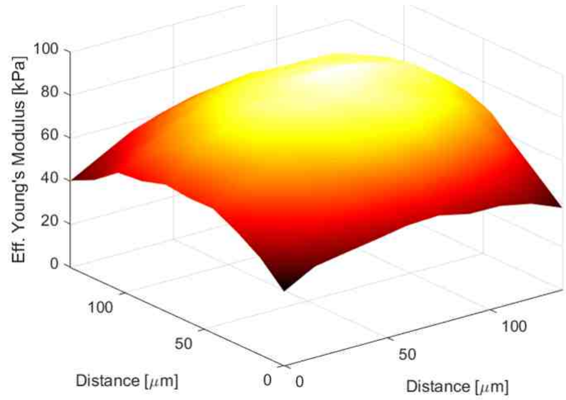

Figure 2: 140 x 140 m surface scan of single PNIPAAm pillar center region.

To investigate the correlation with the topography of the sample the surface topography is extracted from the dataset following the contact point determination.

This concludes the topography of the measured spot on the micropillar is relatively flat:

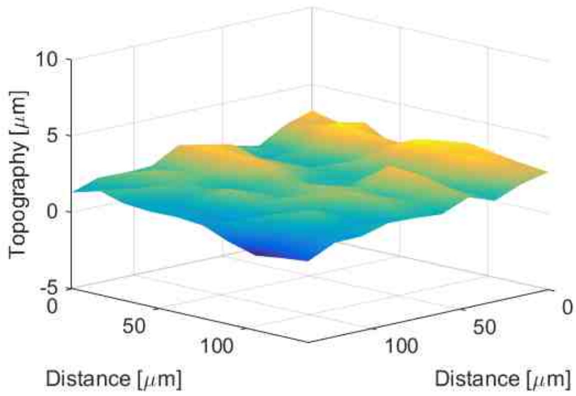

Figure 3: topography of PNIPAAm micropillar following a Hertzian contact point determination.

Repeating experiments on other pillars of different production batches show the same results, indicating that a stiffness gradient is introduced in the microstructures prior, during or after the synthesis.

The stiffness change induced by the change in temperature is also measured by using the Piuma Nanoindenter: the sample stage is heated to 40°C while the sample is immersed in water using a glass petri-dish. After the water has reached the threshold temperature of 32°C, the PNIPAAm micropillars shrink and turn opaque. When stabilized in the shrunk state the Young's Modulus is measured to have increased a ten-fold.

Conclusion

The mechanical properties of PNIPAAm material and micropillar structures can be studied with the Piuma Nanoindenter. By enabling the characterization of the local mechanical properties, a stiffness gradient in the PNIPAAm micropillars can be revealed.

By correlating the local stiffness with the topography the relation of topography with mechanical properties can be examined. In this case, the stiffness gradient within the PNIPAAm micropillars cannot be explained by the micropillar shape, following the topography.

By enabling the characterization of the local mechanical properties while the sample remains in a fully hydrated state, the Piuma provides useful information for improving material or structure synthesis parameters.

References

[1] Schild, H.G. *"Poly(N-isopropylacrylamide): experiment, theory and application"*, Progress in Polymer Science, 17 (2), 163-249 (1992)

[2] Galperin, A., Long, T.J., Ratner, B.D., *Degradable, thermo-sensitive poly(N-isopropyl acrylamide)-based scaffolds with controller porosity for tissue engineering applications*, Biomacromolecules 11, 2583-2592 (2010)

[3] Hertz, H. R., *Ueber die Beruehrung elastischer Koerper (On Contact Between Elastic Bodies), in Gesammelte Werke (Collected Works)*, Vol. 1, Leipzig, Germany, (1895)