NANOVEA

NANOVEA.COM

INFO@NANOVEA.COM
EURO@NANOVEA.COM
MEXINFO@NANOVEA.COM

BIOLOGICAL TISSUE HARDNESS EVALUATION

USING

NANOINDENTATION

Prepared by

Camille Murray, Jonathan Thomas, and Pierre Leroux

Introduction

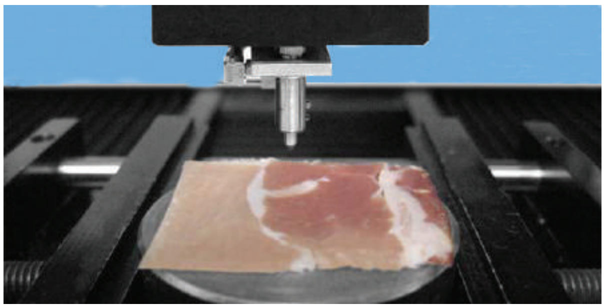
Accurate mechanical property measurement in the life sciences industry has become an important aspect of many current studies. In some cases, understanding mechanical properties of biological surfaces help uncover the effects of disease on the strength and health of biological tissue. Understanding mechanical properties provides quantitative values for identifying localized mechanical behavior, critical to the development of natural and artificial biomaterials.

Importance of Nanoindentation for Biomaterials

Traditional mechanical tests (hardness, adhesion, compression, puncture, yield strength, etc.) require greater precision and reliability in today's quality control environments with a wide range of advanced materials from tissues to brittle materials. Traditional mechanical instrumentation fails to provide the sensitive load control and resolution required for advanced materials. The challenges associated with biomaterials require developing mechanical tests capable of accurate load control on extremely soft materials. These materials require very low sub mN testing loads with large depth range to ensure proper property measurement. In addition, many different mechanical test types can be performed on a single system allowing for greater functionality. This provides a range of important measurements on biomaterials including hardness, elastic modulus, loss and storage modulus, and creep in addition to scratch resistance and yield strength failure points.

MEASUREMENT OBJECTIVE

In this application Nanovea's mechanical tester in nanoindentation mode is used to study the hardness and elastic modulus of 3 separate areas of a biomaterial substitute on fat, light meat, and dark meat regions of prosciutto.



Maximum force [mN]	1
Loading rate [mN/min]	3
Unloading rate [mN/min]	3
Creep [s]	60
Computation Method	ASTM 2546 & Oliver Pharr
Indenter type	100 µm Conical Diamond Tip

Nanoindentation is based on instrumented indentation standards ASTM E2546 and ISO 14577. It uses established methods where an indenter tip of known geometry is driven into a specific site of the test material with a controlled increasing normal load. When reaching a pre-set maximum depth, normal load is reduced until complete relaxation occurs. Load is applied by a piezo actuator and measured in a controlled loop with a high sensitivity load cell. During experiments the indenter position relative to the sample surface is monitored with a high precision capacitive sensor. The resulting load and displacement curves provide data specific to the mechanical nature of the tested material. Established models calculate quantitative hardness and modulus values with the measured data. Nanoindentation is suited to low load and penetration depth measurements at nanometer scales.

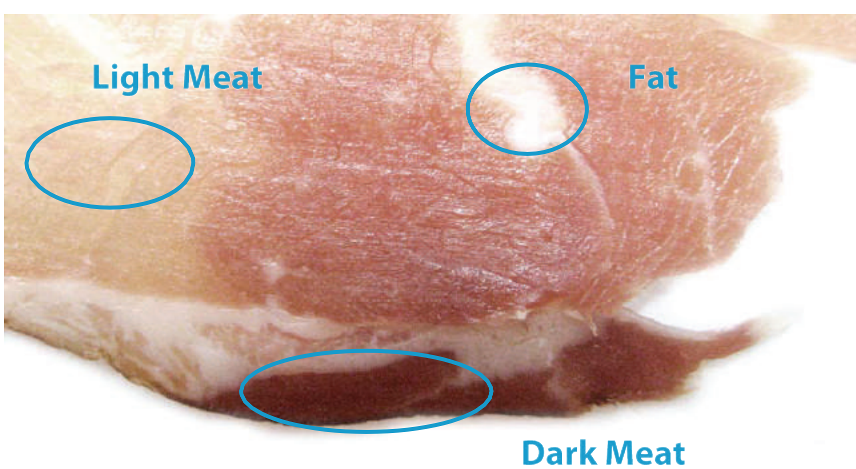


Figure 1: Biomaterial substitute (prosciutto) with test areas

RESULTS AND DISCUSSION

These tables below presents measured values of hardness and Young's modulus with averages and standard deviations. High surface roughness may cause large variations in the results due to small indentation size.

Test Area	Hardness [kPa]	Young's Modulus [kPa]	Creep [µm]
Fat	37.5 ± 1.1	548 ± 60	20.1 ± 3.5
Light Meat	67.2 ± 6.8	570 ± 80	14.0 ± 1.0
Dark Meat	95.2 ± 5.6	834 ± 126	9.3 ± 0.9

The fat area had about half the hardness of the meat areas. Meat treatment caused the darker meat area to be harder than the light meat area. Elastic modulus and hardness are in direct relation to mouth feel chewiness of the fat and meat areas. The fat and light meat area have creep continuing at a higher rate than the dark meat after 60 seconds.

DETAILED RESULTS - FAT

Test	Hardness [kPa]	Young's Modulus [kPa]	Creep [µm]
1	37.4	516	24.7
2	37.1	617	19.6
3	39.3	538	15.9
4	36.5	471	17.7
5	37.2	599	22.5
Average	**37.5**	**548**	**20.1**
Standard Deviation	**1.1**	**60**	**3.5**

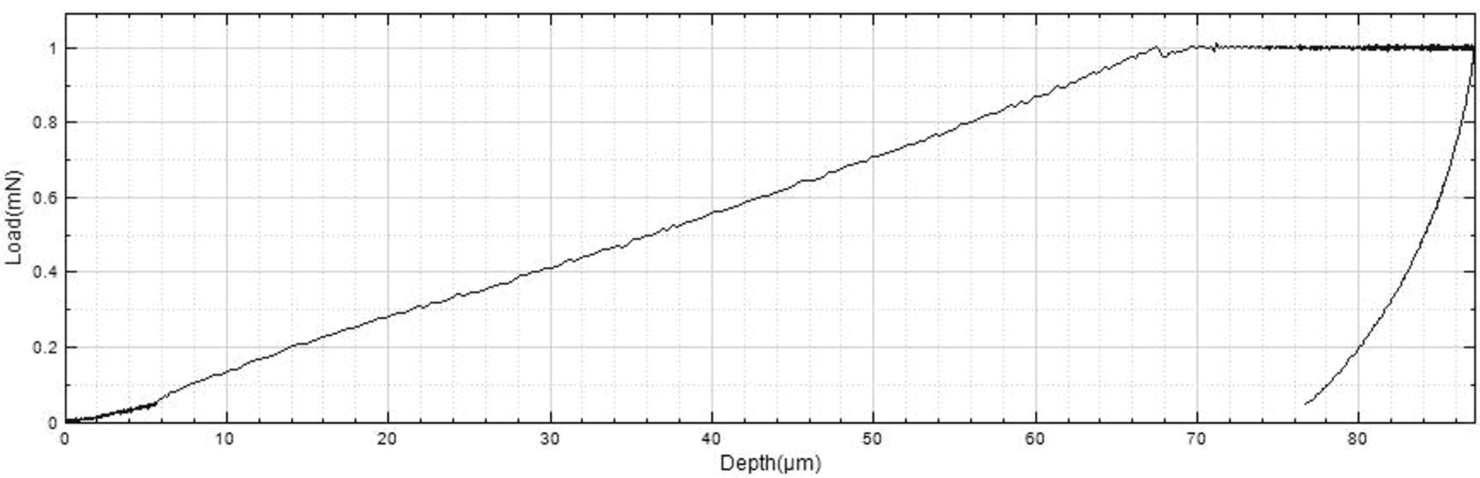


Figure 2: Loading Curve – Fat Area

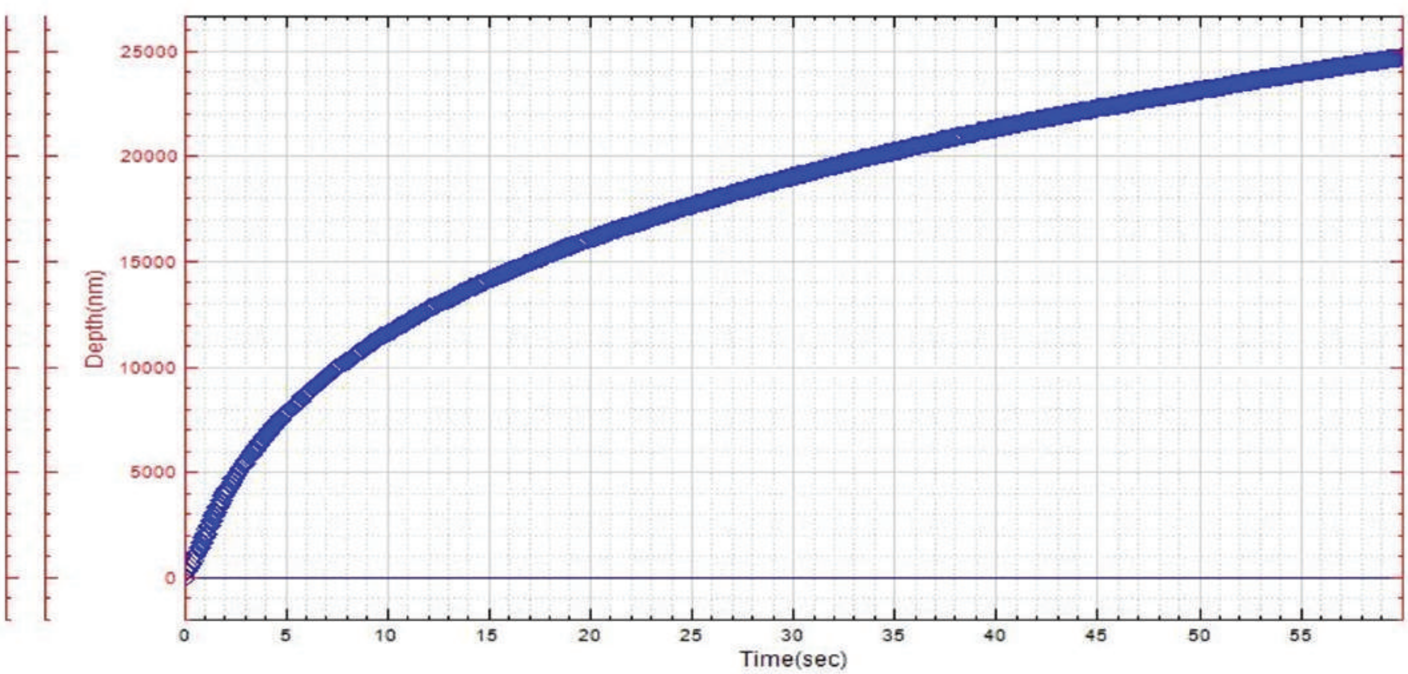


Figure 3: Creep Curve – Fat Area

DETAILED RESULTS - LIGHT MEAT

Test	Hardness [kPa]	Young's Modulus [kPa]	Creep [µm]
1	60.7	565	15.2
2	60.4	567	14.9
3	76.3	699	13.5
4	71.2	480	13.1
5	67.5	539	13.3
Average	**67.2**	**570**	**14.0**
Standard Deviation	**6.8**	**80**	**1.0**

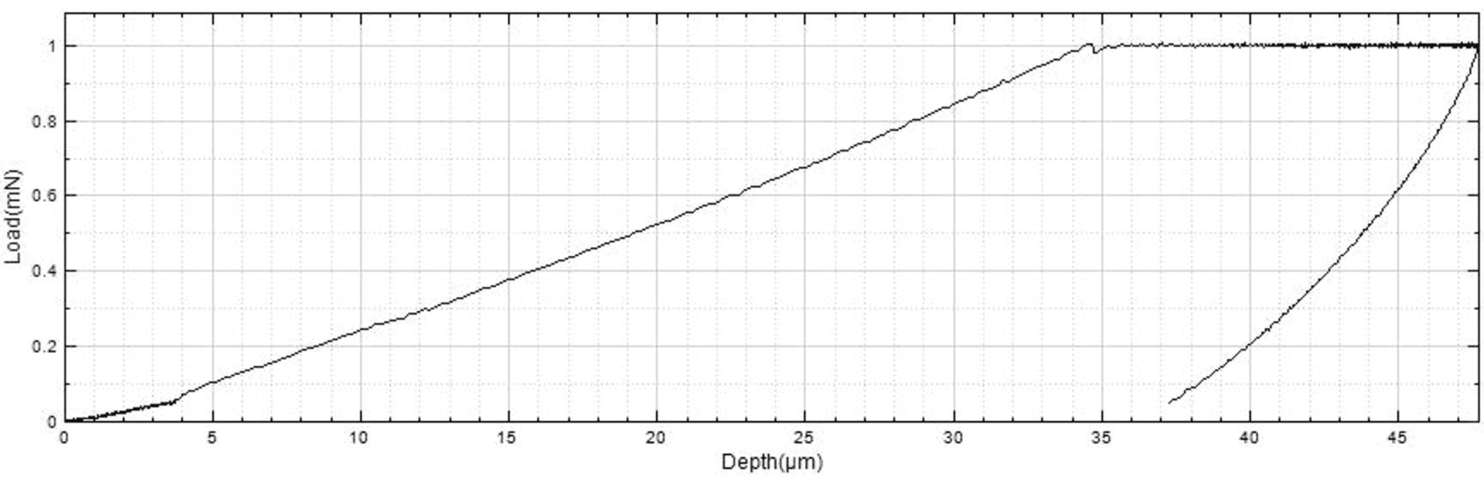


Figure 4: Loading Curve – Light Meat

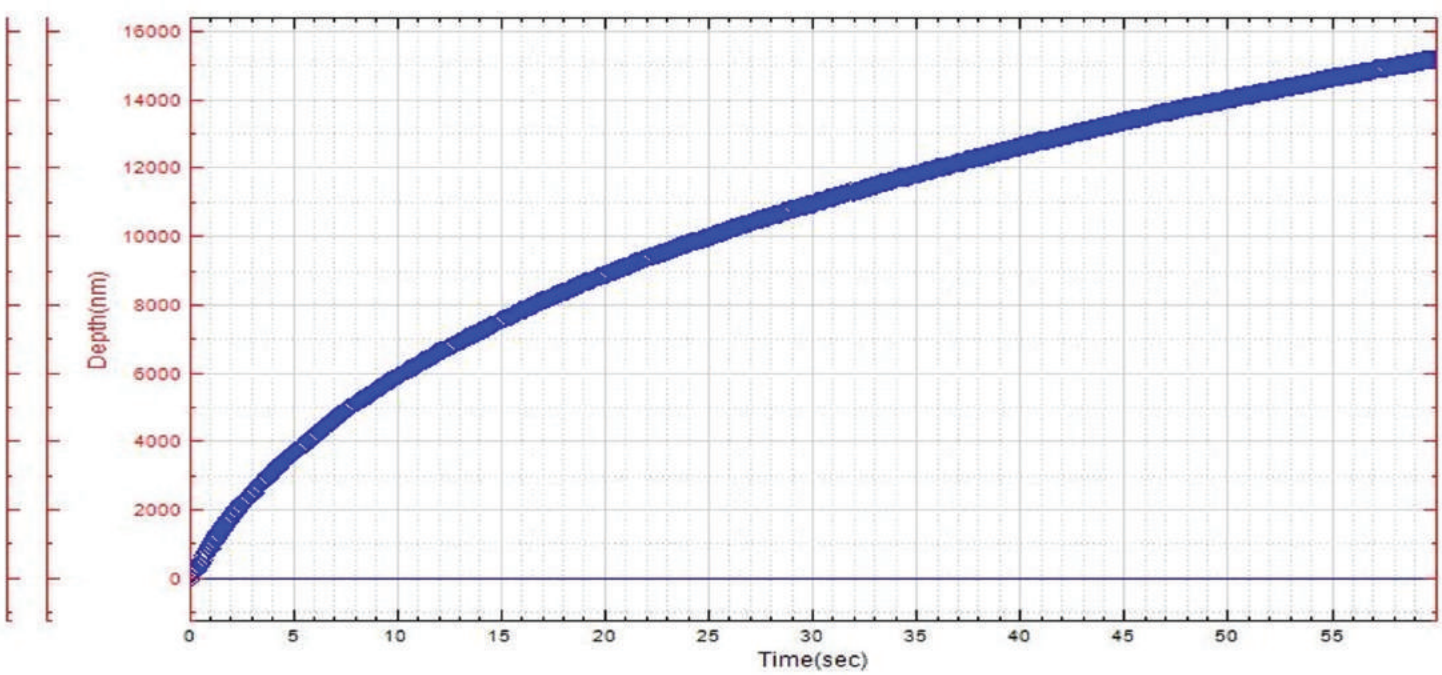


Figure 5: Creep Curve – Light Meat

DETAILED RESULTS - DARK MEAT

Test	Hardness [kPa]	Young's Modulus [kPa]	Creep [µm]
1	101.7	684	8.8
2	90.1	742	10.6
3	89.5	848	9.6
4	100.1	891	9.6
5	94.3	1005	8.1
Average	**95.2**	**834**	**9.3**
Standard Deviation	**5.6**	**126**	**0.9**

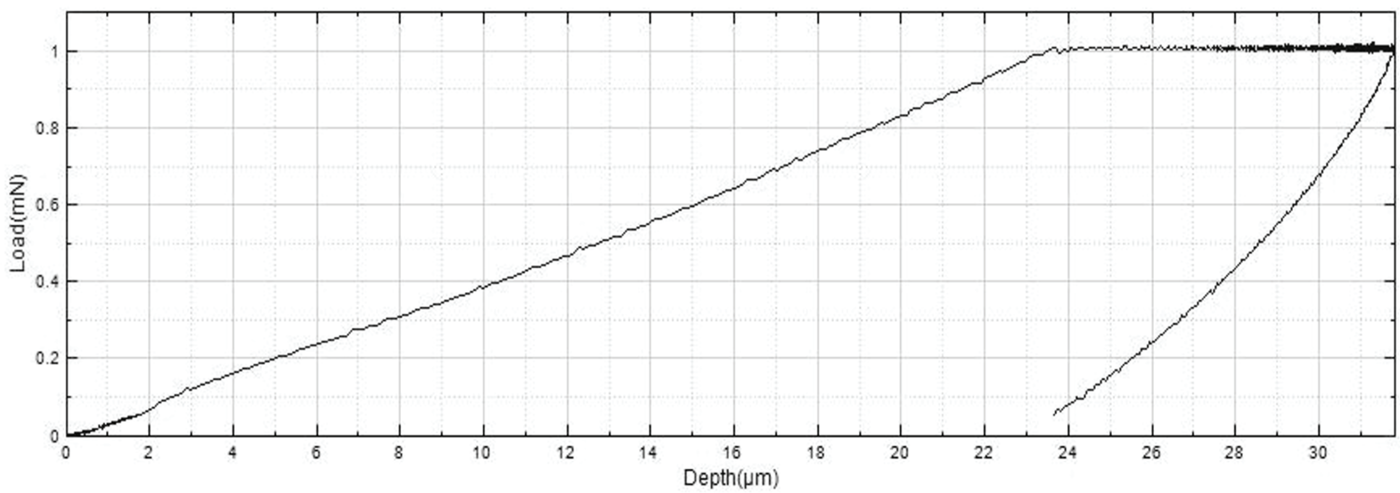


Figure 6: Loading Curve – Dark Meat

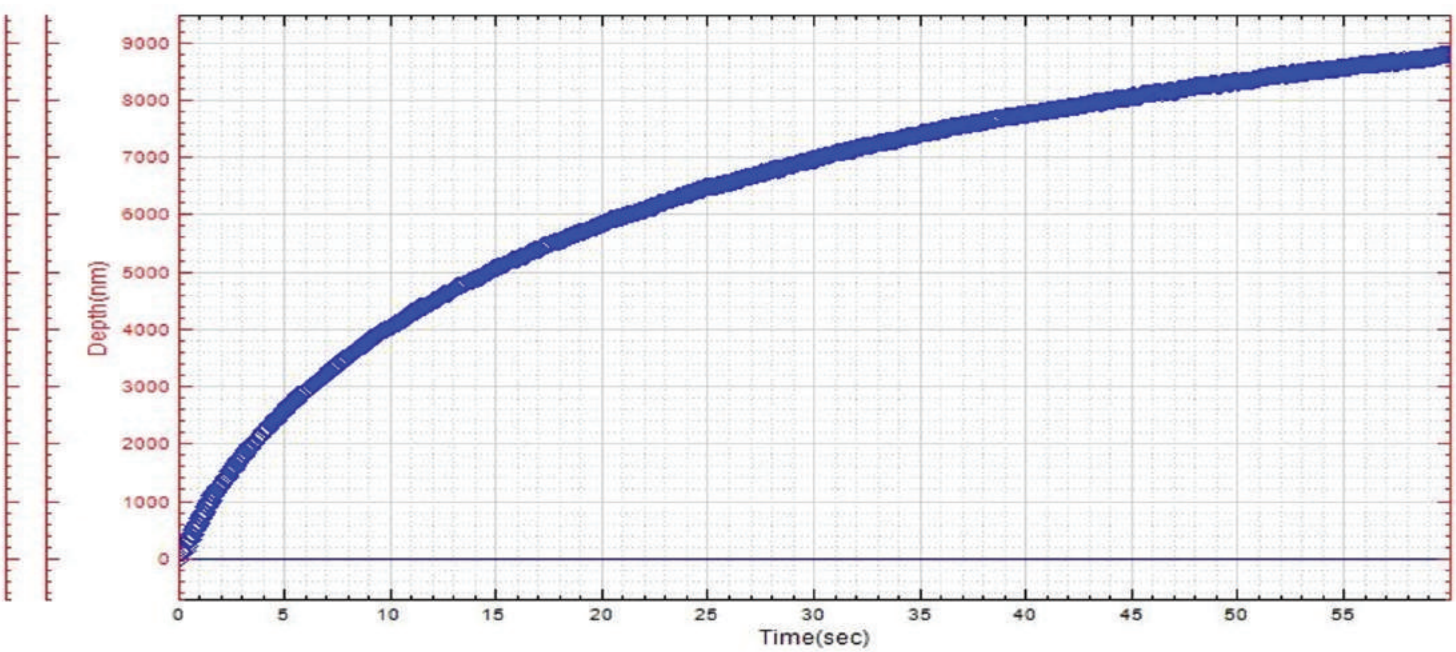


Figure 7: Creep Curve – Dark Meat

Conclusion

In this application, Nanovea's mechanical tester in nanoindentation mode reliably determined mechanical properties of the fat and meat areas while overcoming high sample surface roughness. The system simultaneously provides precise mechanical property measurements on extremely hard materials and soft biological tissues.

The load cell in closed loop control with the piezo table ensures precise measurement of hard or soft gel materials from 1 to 5kPa. Using the same system, it is possible to test biomaterials at higher loads up to 400N. Multi-cycle loading can be used for fatigue testing and yield strength information in each zone can be obtained using a flat cylindrical diamond tip. In addition, with Dynamic Mechanical Analysis (DMA), the viscoelastic properties loss and storage moduli can be evaluated with high accuracy using the closed loop load control. Tests at various temperatures and under liquids are also available on the same system.

Nanovea's mechanical tester continues to be the superior tool for biological and soft polymer/gel applications.