

**Hardness Evaluation of Biological Tissue  
Using Nanoindentation**



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## INTRO

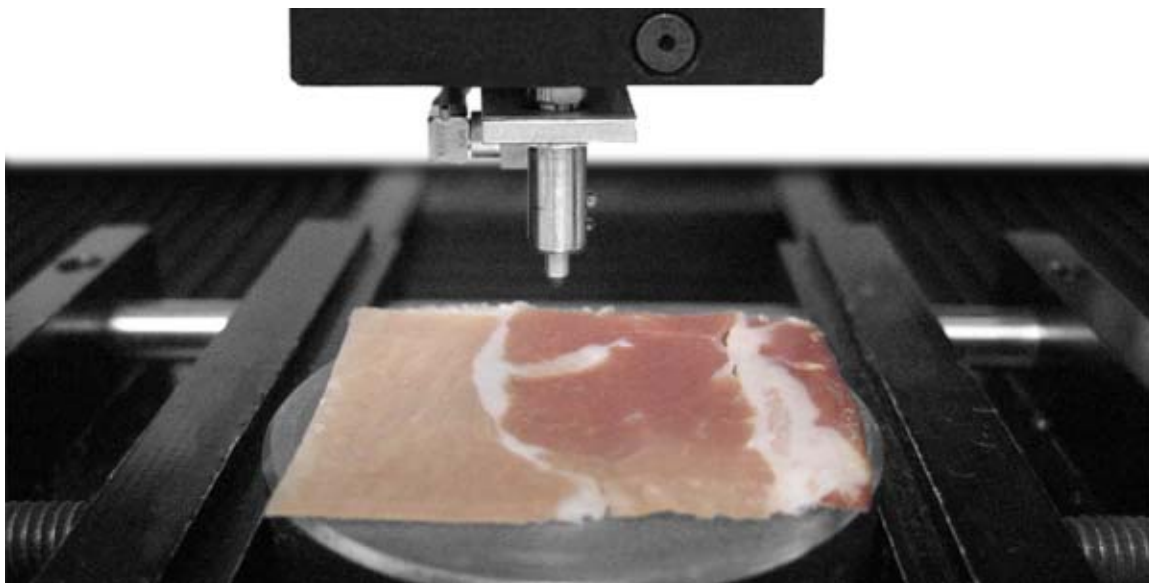
The ability to accurately measure mechanical properties in the fields of Life Science has recently become an important aspect of many current studies. In some cases, understanding the mechanical properties of soft biological surfaces have helped uncover the mechanical effects of diseases. Understanding mechanical properties provides a context for identifying the local mechanical behavior linked to specific changes. It is also critical in the development of artificial bio-materials.

## IMPORTANCE OF NANOINDENTATION FOR BIOMATERIALS

With many traditional mechanical tests (Hardness, Adhesion, Compression, Puncture, Yield Strength etc.), today's quality control environments with advanced sensitive materials, from tissue to brittle materials, now require greater precision and reliability control. Traditional mechanical instrumentation fails to provide the sensitive load control and resolution required; designed to be used for bulk hard materials. The challenges specifically associated with biomaterials have required development of mechanical testing capable of accurate load control on extremely soft materials. These materials needs very low testing loads (sub mN) associated with large depth range (100's microns). In addition, various mechanical tests can now be preformed on a single system. It can provide a range of important measurement on bio-materials including hardness, elastic modulus, loss modulus, storage modulus, creep on one side and also failure points such as scratch resistance or yield strength information.

## MEASUREMENT OBJECTIVE

In this application, the Nanovea Mechanical Tester, in Nanoindentation mode, is used to study the hardness and elastic modulus of 3 separate areas of prosciutto (fat, light meat and dark meat).



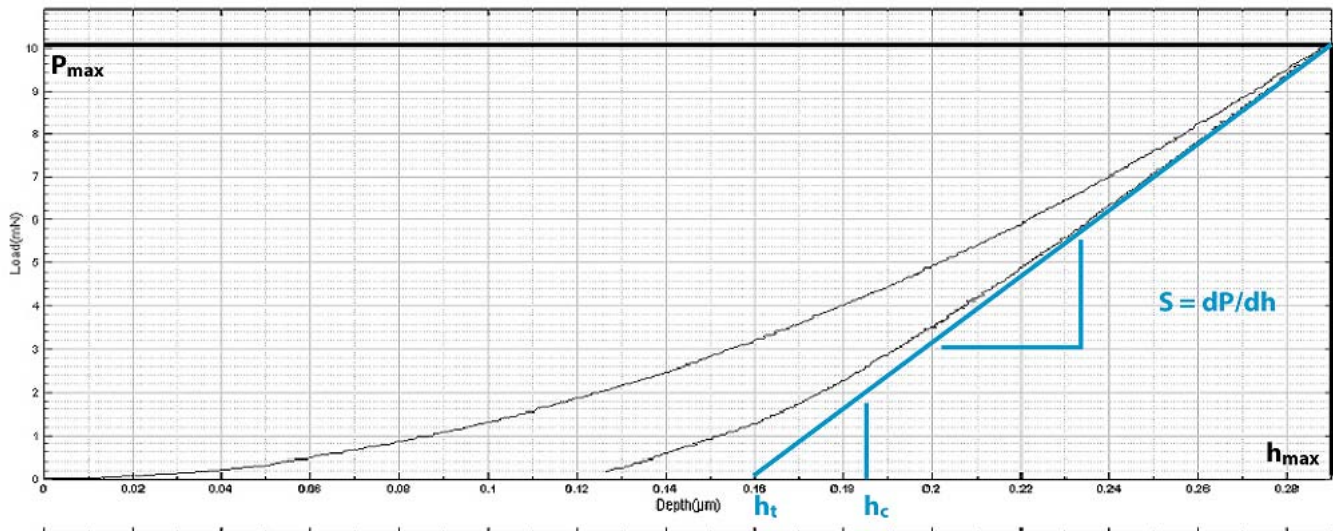
## MEASUREMENT PRINCIPAL

Nanoindentation is based on the standards for instrumented indentation, ASTM E2546 and ISO 14577. It uses an already established method where an indenter tip with a known geometry is driven into a specific site of the material to be tested, by applying an increasing normal load. When reaching a pre-set maximum value, the normal load is reduced until complete relaxation occurs. The load is applied by a piezo actuator and the load is measured in a controlled loop with a high sensitivity load cell. During the experiment the position of the indenter relative to the sample surface is precisely monitored with high precision capacitive sensor. The resulting load/displacement curves provide data specific to the mechanical nature of the material under examination. Established models are used to calculate quantitative hardness and modulus values for such data. Nanoindentation is especially suited to load and penetration depth measurements at nanometer scales and has the following specifications:

Max displacement (Dual Range 50/250 $\mu\text{m}$ )	: 250 $\mu\text{m}$
Depth Resolution (Theoretical)	: 0.003 nm
Depth Resolution (Noise Level)	: 0.4 nm
Maximum force	: 400 mN
Load Resolution (Theoretical)	: 0.03 $\mu\text{N}$
Load Resolution (Noise Floor)	: 1.5 $\mu\text{N}$

### Analysis of Indentation Curve

Following the ASTM E2546 (ISO 14577), hardness and elastic modulus are determined through load/displacement curve as for the example below.



### Hardness

The hardness is determined from the maximum load,  $P_{\text{max}}$ , divided by the projected contact area,  $A_c$ :

$$H = \frac{P_{\text{max}}}{A_c}$$

## Young's Modulus

The reduced modulus,  $E_r$ , is given by:

$$E_r = \frac{\sqrt{\pi}}{2} \frac{S}{\sqrt{A_c}}$$

Which can be calculated having derived  $S$  and  $A_c$  from the indentation curve using the area function,  $A_c$  being the projected contact area. The Young's modulus,  $E$ , can then be obtained from:

$$\frac{1}{E_r} = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i}$$

Where  $E_i$  and  $\nu_i$  are the Young's modulus and Poisson coefficient of the indenter and  $\nu$  the Poisson coefficient of the tested sample.

## How are these calculated?

A power-law fit through the upper 1/3 to 1/2 of the unloading data intersects the depth axis at  $h_t$ . The stiffness,  $S$ , is given by the slope of this line. The contact depth,  $h_c$ , is then calculated as:

$$h_c = h_{\max} - \frac{3P_{\max}}{4S}$$

The contact Area  $A_c$  is calculated by evaluating the indenter area function. This function will depend on the diamond geometry and at low loads by an area correction.

For a perfect Berkovich and Vickers indenters, the area function is  $A_c=24.5h_c^2$  For Cube Corner indenter, the area function is  $A_c=2.60h_c^2$  For Spherical indenter, the area function is  $A_c=2\pi Rh_c$  where  $R$  is the radius of the indenter. The elastic components, as previously mentioned, can be modeled as springs of elastic constant  $E$ , given the formula:  $\sigma = E\varepsilon$  where  $\sigma$  is the stress,  $E$  is the elastic modulus of the material, and  $\varepsilon$  is the strain that occurs under the given stress, similar to Hooke's Law. The viscous components can be modeled as dashpots such that the stress-strain rate

relationship can be given as, 
$$\sigma = \eta \frac{d\varepsilon}{dt}$$
 where  $\sigma$  is the stress,  $\eta$  is the viscosity of the material, and  $d\varepsilon/dt$  is the time derivative of strain.

Since the analysis is very dependent on the model that is chosen. Nanovea provides the tool to gather the data of displacement versus depth during the creep time. The maximum creep displacement versus the maximum depth of indent and the average speed of creep in nm/s is given by the software. Creep may be best studied when loading is quicker. Spherical tip might be a better choice.

## Other tests possible includes the following:

Puncture Resistance, Stress-Strain & Yield Stress, Fracture Toughness, Compression Strength, Fatigue testing and many others.

## TEST CONDITIONS

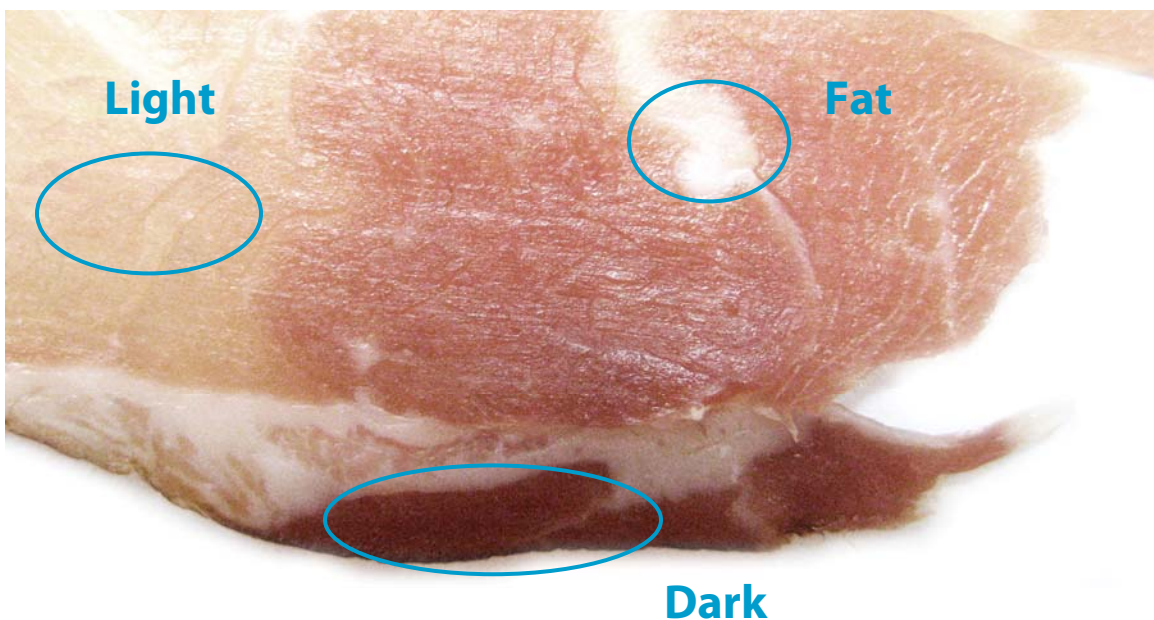
Maximum force (mN)	1
Loading rate (mN/min)	3
Unloading rate (mN/min)	3
Creep (s)	60
Computation Method	ASTM 2546 & Oliver Pharr
Indenter type	100 $\mu\text{m}$ Spherico-Conical Diamond Tip

## RESULTS

This section includes a summary table that compares the main numerical results for the different samples, followed by the full result listings, including each indentation performed, accompanied by micrographs of the indentation, when available. These full results present the measured values of Hardness and Young's modulus as the penetration depth with their averages and standard deviations. It should be considered that large variation in the results can occur in the case that the surface roughness is in the same size range as the indentation.

Summary table of main numerical results

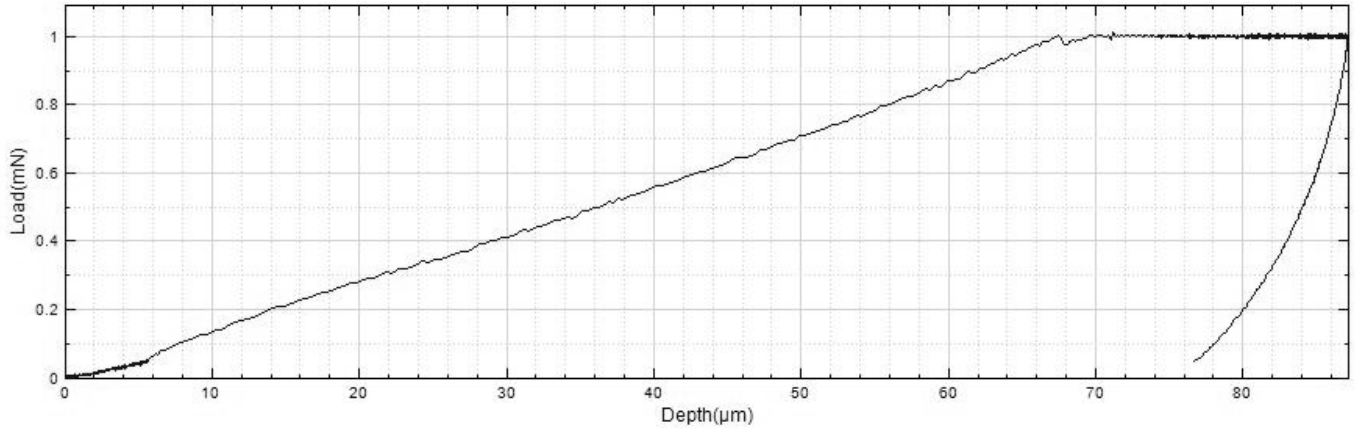
Sample	Hardness [Vickers]	Hardness [kPa]	Young's Modulus [kPa]	Max Depth [ $\mu\text{m}$ ]	Creep [ $\mu\text{m}$ ]
Fat Area	$0.00354 \pm 0.00010$	$37.5 \pm 1.1$	$548 \pm 60$	$67.5 \pm 3.3$	$20.1 \pm 3.5$
Light Area	$0.00635 \pm 0.00065$	$67.2 \pm 6.8$	$570 \pm 80$	$35.4 \pm 3.3$	$14.0 \pm 1.0$
Dark Area	$0.00899 \pm 0.00053$	$95.2 \pm 5.6$	$834 \pm 126$	$24.6 \pm 1.3$	$9.34 \pm 0.93$



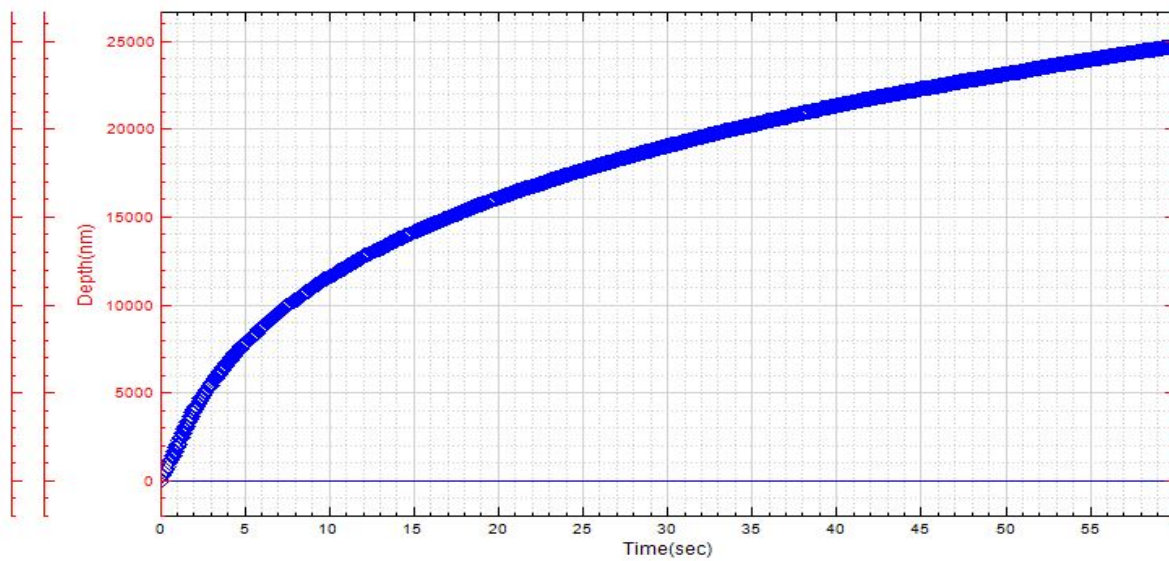


## Detailed Results- Fat Area

Fat Area					
	Hardness [Vickers]	Hardness [kPa]	Young's Modulus [kPa]	Max Depth [ $\mu\text{m}$ ]	Creep [ $\mu\text{m}$ ]
1	0.00354	37.4	516	67.5	24.7
2	0.00350	37.1	617	67.6	19.6
3	0.00371	39.3	538	62.4	15.9
4	0.00345	36.5	471	71.6	17.7
5	0.00352	37.2	599	67.9	22.5
Average	<b>0.00354</b>	<b>37.5</b>	<b>548</b>	<b>67.4</b>	<b>20.1</b>
Standard Deviation	<b>0.00010</b>	<b>1.1</b>	<b>60</b>	<b>3.3</b>	<b>3.5</b>



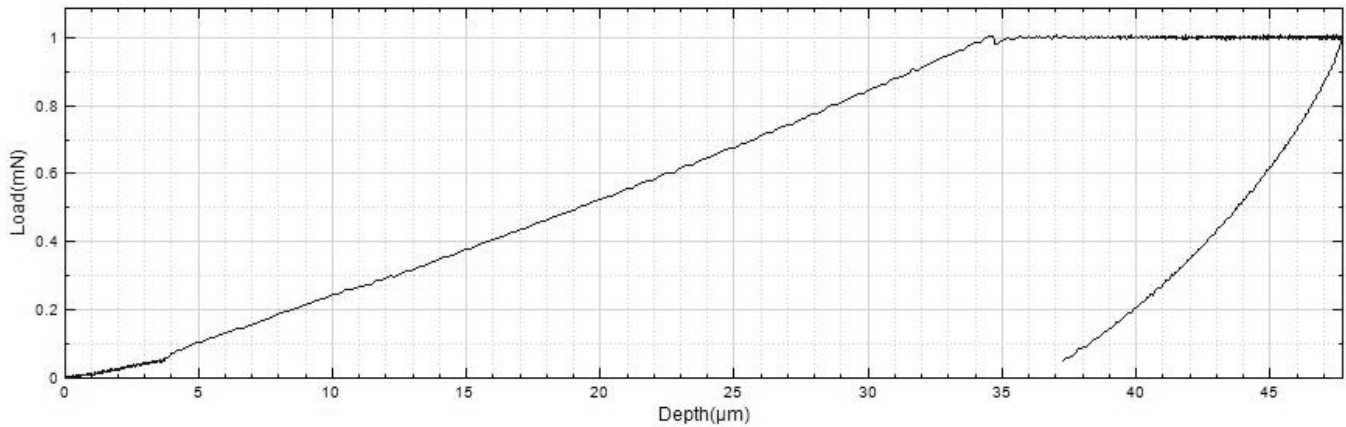
**Loading Curve -Fat Area**



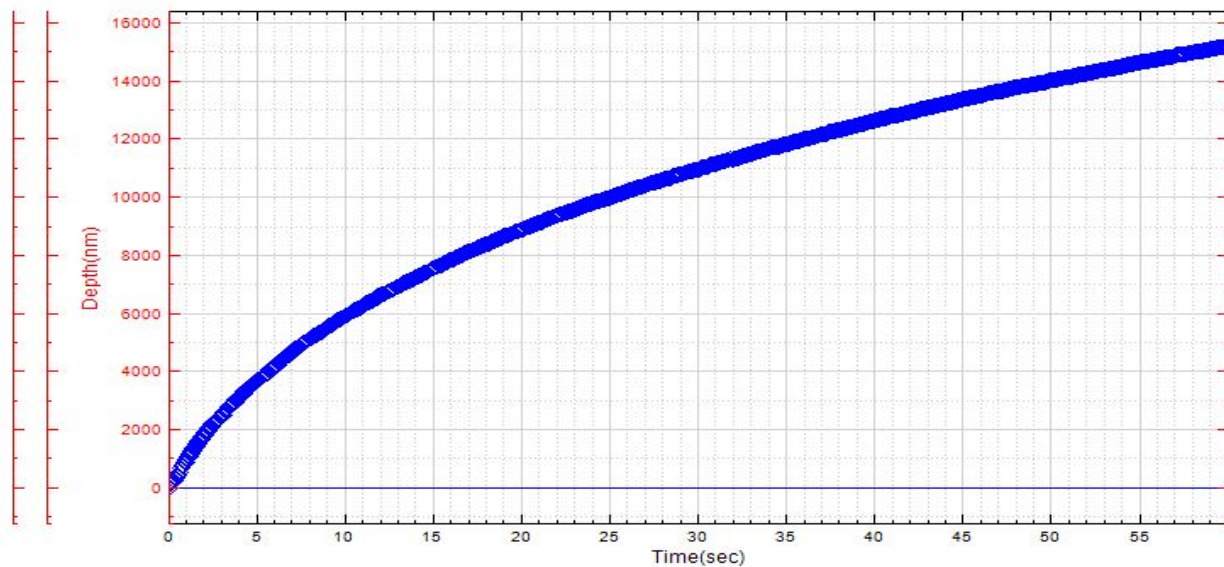
**Creep Curve -Fat Area**

## Detailed Results – Light Meat Area

Light Meat Area					
	Hardness [Vickers]	Hardness [kPa]	Young's Modulus [kPa]	Max Depth [μm]	Creep [μm]
1	0.00574	60.7	565	38.3	15.2
2	0.00571	60.4	567	38.5	14.9
3	0.00721	76.3	699	30.3	13.5
4	0.00673	71.2	480	34.5	13.1
5	0.00638	67.5	539	35.2	13.3
Average	<b>0.00635</b>	<b>67.2</b>	<b>570</b>	<b>35.4</b>	<b>14.0</b>
Standard Deviation	<b>0.00065</b>	<b>6.8</b>	<b>80</b>	<b>3.3</b>	<b>1.0</b>



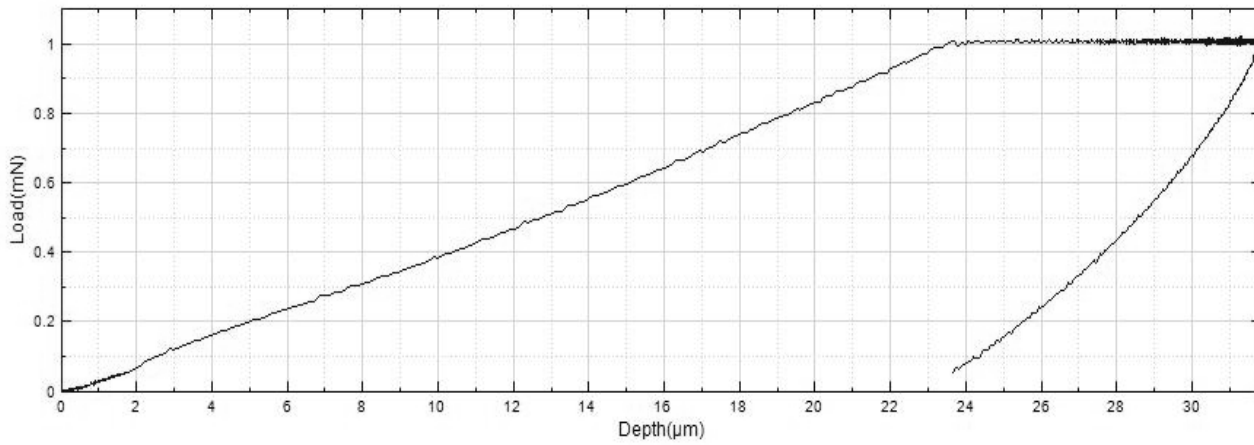
**Loading Curve –Light Meat Area**



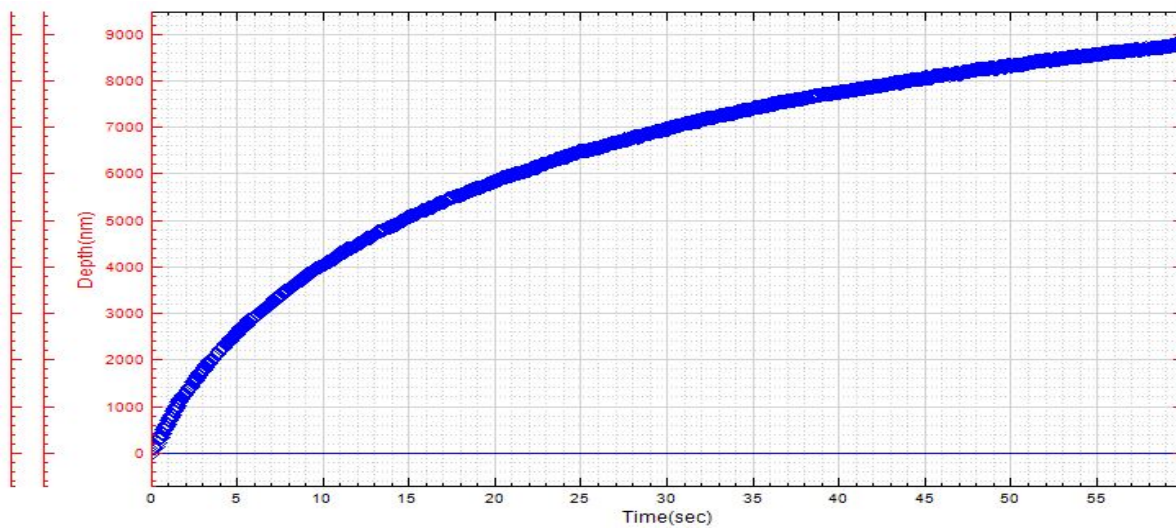
**Creep Curve –Light Meat Area**

## Detailed Results – Dark Meat Area

Dark Meat Area					
	Hardness [Vickers]	Hardness [kPa]	Young's Modulus [kPa]	Max Depth [ $\mu\text{m}$ ]	Creep [ $\mu\text{m}$ ]
1	0.00961	101.7	684	24.6	8.79
2	0.00852	90.1	742	26.1	10.6
3	0.00846	89.5	848	25.6	9.59
4	0.00946	100.1	891	23.2	9.64
5	0.00891	94.3	1005	23.7	8.13
Average	<b>0.00899</b>	<b>95.2</b>	<b>834</b>	<b>24.6</b>	<b>9.34</b>
Standard Deviation	<b>0.00053</b>	<b>5.6</b>	<b>126</b>	<b>1.3</b>	<b>0.93</b>



**Loading Curve –Dark Meat Area**



**Creep Curve –Dark Meat Area**



## CONCLUSION

Even with the relatively rough surface the Nanovea Mechanical Tester, in Nanoindentation mode, reliably determined the mechanical properties of the fat and meat areas. The fat area was about half the hardness of the meat areas. The response in the fat area was also more plastic than what was observed in the meat area. It is interesting to see that the darker meat area is harder than the light meat area because of the meat treatment. Elastic modulus and hardness are in direct relation to mouth feel chewiness of the meat/fat areas. It is also interesting to see that the fat and the light meat area have creep that continues at a higher rate even after 60 seconds while the dark meat area slows down comparatively. In conclusion, this demonstrates the wide and unmatched capability of the Nanovea Mechanical Tester. In addition to providing the precise measurement of mechanical properties on extremely hard materials the same system can also be used for soft biological tissue with low kPa hardness values.

The precision load cell in closed loop control with the piezo table ensures accuracy 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 200N. Multi-cycle loading tests can be used for fatigue testing. Using a flat cylindrical diamond tip can give yield strength information in each zone. In addition, with DMA "Dynamic Mechanical Analysis", the viscoelastic properties, including loss and storage moduli, can be evaluated with high accuracy because of the close loop load control.

With the additional extended 50mm Z motorized table and the possibility of using a large probe of 25mm in diameter, the Nanovea Mechanical Tester truly has been and continues to be the superior tool for biological and soft polymer/gel applications. Tests at various temperatures and under liquids are also available on the same system.

To learn more about [Nanovea Nanoindentation](#).