PIUMA
NANOINDENTER

OPTICS11
ideas on the top of a fiber

# Piuma Nanoindenter Application note:

## Characterizing the mechanical properties of hydrogels

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.

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**Hydrogels are commonly used as scaffolds for regenerative medicine or tissue engineering applications. Hydrogels guide cell function and tissue development by regulating the delivery of biochemical compounds and providing mechanical stimuli to the cellular construct, having specifc elastic, viscous and degradation properties.**

## Introduction

Hydrogels can be described as (bio)polymeric materials that have the ability to take up a high degree of liquid in their matrix. Characterizing the micromechanical properties of hydrogels is traditionally performed by rheology or compression testing, techniques that focus on global, or bulk, mechanical properties, ignoring local variations thereof.

In this application note the local mechanical properties of three common hydrogels are explored by interferometry-based fiber-top nanoindentation, a novel measurement technology that is particularly suitable for exploring the local mechanical properties of soft (bio)materials. A 4% LMP agarose hydrogel and a 2.5% and 5% gelatin hydrogel are examined by the Piuma Nanoindenter, while in fully hydrated condition.

## Methods and materials

The Piuma Nanoindenter features a monolithic, glass, cantilever-based indentation probe at the end of an optical fiber [1]. The probe has a precalibrated stiffness (accuracy > 99%) and a spherical tip of known radius. For each sample a matching probe can be selected (Table 1).

| Sample | Probe stiffness | Tip radius |
|---|---|---|
| 4% LMP | 11.90 N/m | 59 µm |
| 2.5% Gelatin | 0.37 N/m | 60 µm |
| 5% Gelatin | 0.55 N/m | 70 µm |

Table 1: *Sample specific probe parameters.*

The Piuma Nanoindenter enables researchers to scan a prespecified area of any sample automatically. In this mode the Piuma detects the surface of the sample for each new point in a grid scan by using a coarse-fine approach routine, of which the stepsize can be specified. To make sure each indentation starts out of contact, the Piuma is set to hover 3 µm above the local sample surface, prior to indentation. For each indentation, the load-indentation curve is recorded from the piezo motion and cantilever bending signals.

The Oliver&Pharr model is applied to determine the Youngs Modulus based on the unloading curve of the load-displacement data, using a linear fit on all datapoints from 65% to 85% of the maximum load in the unloading phase [3,4]. Additionally the software suite allows for estimation of the Young's Modulus using the Herzian contact model applied to the linear visco-elastic regime of the stress-strain curve [2].

For these experiments, the Piuma Nanoindenter is placed on top of a regular tabletop surface; no special stabilization or dampening is used during the experiments.

**Sample preparation - 4% LMP agarose**

2g of low melting point (LMP) agarose gel powder is dissolved in 50 ml demineralized water in a 60mm petri dish. The mixture is heated in a microwave for 5 minutes at 90W. After heating the agarose is gelled during a 30 minute period at 22$^{o}$C. After setting, the agarose is immersed in water.

**Sample preparation - 2.5% Gelatin**

1.25g of gelatin powder is dissolved in 50ml of demineralized water. The mixture is heated to 50$^{o}$C and stirred using a hot stir plate for 30 minutes. After heating and mixing the liquid is poored in a 60mm petridish and, after setting for 40 minutes at 22$^{o}$C, immersed in water.

**Sample preparation - 5% Gelatin**

Equal to 2.5% gelatin using 2.5g of gelatin dissolved in 50ml of water.

## Results - Agarose

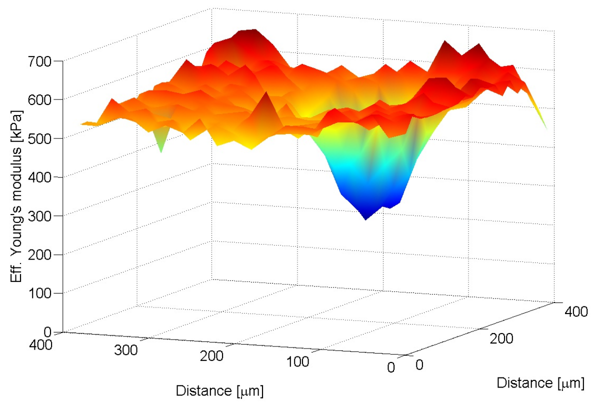


Figure 1: *3D map of a 25 x 25 point grid scan of the Young's modulus of 4% LMP agarose. The point-to-point pitch is 25 μm.*

In order to explore a local feature observed with the on-board optical microscope, a 400 X 400 μm grid scan with a 20 μm pitch in both X and Y direction is performed. The displacement profile for the indentation is set to be 15 μm at 7.5 μm/s and 1 shold time at maximum displacement.

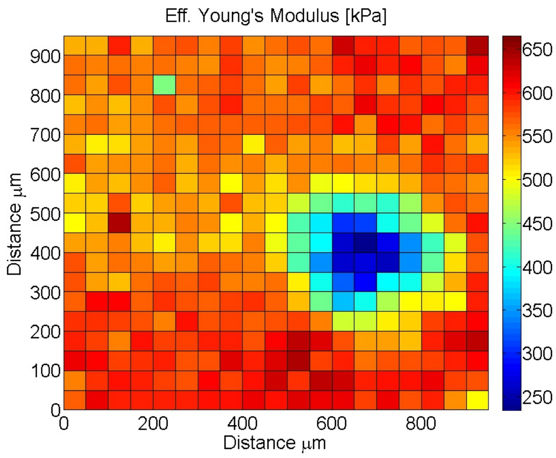


Figure 2: *2D elasticity map of a 25 X 25 point grid scan of 4% LMP agarose. The point-to-point pitch is 25 μm.*

The scan reveals a feature in the agarose hydrogel that introduces a local elasticity gradient of a factor of three. This elasticity gradient is the result of a local distortion of the hydrogel structure due to a trapped air bubble in the gel matrix 0.5 mm beneath the gel surface (Fig. 1 & 2).

As figure 3 illustrates, the effective Youngs Modulus is calculated using the Oliver & Pharr model, applied on 65% to 85% of the unloading curve.

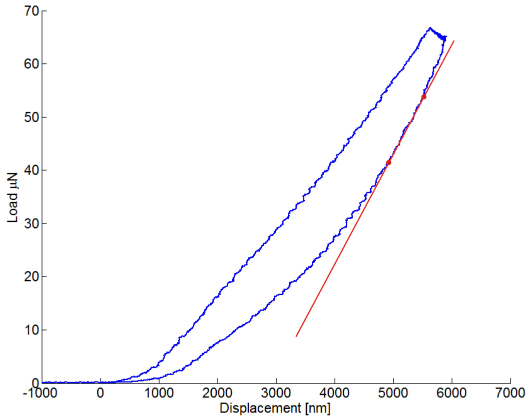


Figure 3: *Typical load-displacement curve of local indentation cycle on 4% LMP agarose.*

This section can be adjusted in the Piuma software suite. All experiment data is output in tab delimited text files, allowing researchers to model other parameters than those output by the Piuma Nanoindenter program.

## Results - Gelatin

In order to explore the mechanical consistency of fresh gelatin, a 750 X 1250 μm grid scan with a 25 μm point-to-point pitch in both X and Y direction is performed. The scan provided 1500 Effective Young's Modulus estimates, which are depicted as a histogram using 60 bins in figure 4. The median is 741 Pa, the mean 744 Pa and the standard deviation 75 Pa. A spatial rendering of the scan also reveals that the global elasticity of the gel is evenly distributed but that there exists some point-to-point variation in elasticity.

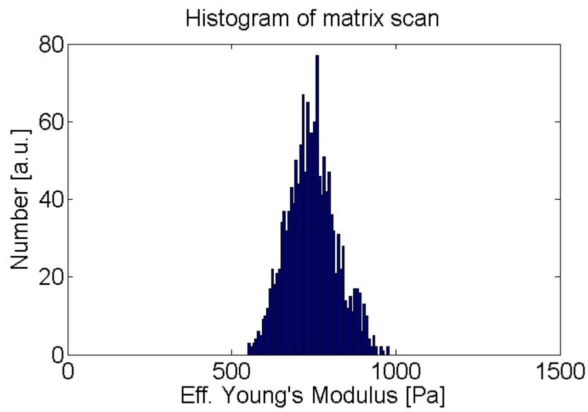


Figure 4: *Histogram of 30 X 50 point matrix scan of fresh 2.5% gelatin. The point-to-point pitch is 25 μm.*

The point-to-point variation could be explained by the dynamic mechanical properties of the gelatin, such as viscoelasticity.

On a 5% gelatin sample the viscoelastic behavior is studied in more detail. By varying the velocity of the displacement of the indentation probe, and thus the strain rate, the material's local elasticity as a function of time can be studied. The Piuma Nanoindenter allows for a customization of the indentation profile, including a parametrization of the indentation velocity.

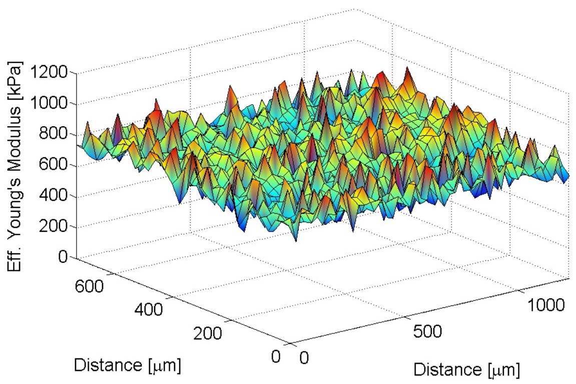


Figure 5: 3D figure of 50 X 30 grid scan of 2.5% gelatin. Mean = 0.744 kPa, std = 0.075 kPa.

As figure 6 illustrates, the indentation series corresponding to strain rates of 0.025 $s^{-1}$, 0.05 $s^{-1}$, 0.1 $s^{-1}$ and 0.25 $s^{-1}$ show corresponding Young's Moduli of 3.19 kPa (+/- 0.19 kPa), 3.61 kPa (+/- 0.16 kPa), 3.89 kPa (+/- 0.27 kPa) and 4.02 kPa (+/- 0.17 kPa), respectively. This confirms that the 5% gelatin sample shows viscoelastic behavior.

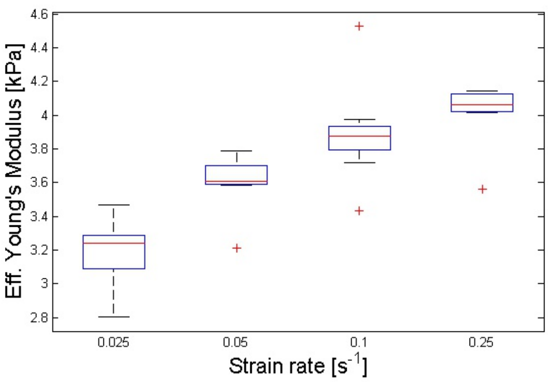


Figure 6: Boxplot of effective Young's Modulus estimates for different strain rates. Strain rates 0.025, 0.05, 0.1 and 0.25 $s^{-1}$ correspond to 0.92, 1.82, 3.64 and 9.13 μm/s displacement velocities, respectively.

**Conclusion**

The agarose hydrogel scan clearly shows a gradual decline in elasticity towards the center of the trapped air bubble. Furthermore, the global material properties surrounding the feature area appear relatively homogeneous. The global elasticity of 2.5% gelatin shows the elasticity over the entire scan area is comparable, but with some point-to-point variation. The viscosity analysis on 5% gelatin shows that gelatin possesses viscoelastic properties, which could explain the point-to-point variability in the previous grid scan.

The Piuma Nanoindenter appears a powerful tool to investigate the local micro-mechancial properties of hydrogels. Elasticity gradients as a result of hydrogel structure or chemical composition can be investigated with great accuracy while immersed, and mapped with the push of a button. Easy sample mounting of almost any container size, the high sensitivity (0.1 N), the broad operating range (<100 Pa up to 1 GPa) and the ability to measure in liquids without any special requirements makes the system widely deployable in any lab that works with hydrogels and soft (bio)materials.

References

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