Piuma application note AN1501

Exploring the global and local mechanical properties of hydrogels: measuring the E-modulus of agarose and gelatin

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Hydrogels are commonly used as cell scaffolds in tissue engineering. Hydrogels guide cell function and tissue development by regulating the delivery of biochemical compounds and providing mechanical stimuli to the cellular construct, such as specific elasticity, viscosity or adhesion properties.

Introduction

Hydrogels consist of intertwined and/or cross-linked biocompatible polymers. Characterizing the micromechanical properties of hydrogels is traditionally performed by rheology or bulk indentation, methods that focus on bulk mechanical properties rather than local variations.

In this application note the local variations in elasticity of three hydrogels are mapped by fiber-top nanoindentation, a novel measurement technology for measuring the elasticity of soft materials. A 4% LMP agarose hydrogel and a 2.5% and 5% gelatin hydrogel are characterized while fully immersed in solution.

Methods and materials

The Piuma Nanoindenter features a 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, matching probe parameters are selected (Table 1).

Sample	Probe stiffness	Tip radius
4% LMP agarose	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 detects the surface of the sample automatically, for each new point in a grid scan. The surface is found with a typical accuracy of <1 μm using a piezostepper and piezostack in a coarse-fine approach routine. To make sure each indentation starts out of contact, the Piuma is programmed to hover 3 μm above the sample surface, before indenting. For each indentation, the load-indentation curve is recorded from the piezo motion and cantilever bending signals.

The reduced Young′s Modulus of an indentation is determined using the Oliver&Pharr approximation of the Youngs Modulus [3, 4] on the unloading curve of a load-displacement plot, using all datapoints from 65% to 85% of the maximum load. Additionally the software suite allows for estimation of the Young's Modulus using the Herzian contact model applied to the linear 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% agarose

2 g of low melting point (LMP) agarose gel powder is dissolved in 50 ml demineralized water. The mixture is heated in the microwave for 5 minutes in defrost mode, prepared in a 60 mm petridish and, after setting for 30 minutes at room temperature, submersed in water.

Sample preparation - 2.5% gelatin

1.25 g of gelatin powder is dissolved in 50 ml of demineralized water. The mixture is heated to 50 °C and stirred using a hot stir plate for 30 minutes, prepared in a 60 mm petridish and, after setting for 40 minutes at room temperature, submersed in demineralized water.

Sample preparation - 5% gelatin

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

Results - agarose

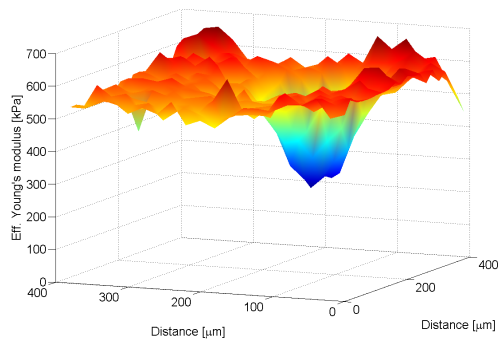

Figure 1: *3D elasticity map of a 25 x 25 point grid scan of 4% LMP agarose. The point-to-point pitch is 25 μm.*

In order to explore a local feature observed with the on-board optical microscope, a 400 × 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 s relaxation time at maximum stroke.

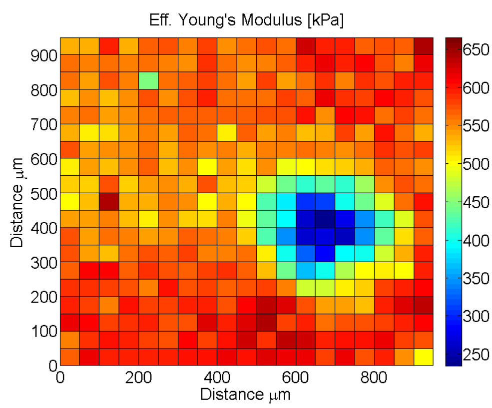

Figure 2: *2D elasticity map of a 25 × 25 point grid scan of 4% LMP agarose. The point-to-point pitch is 25 μm.*

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).

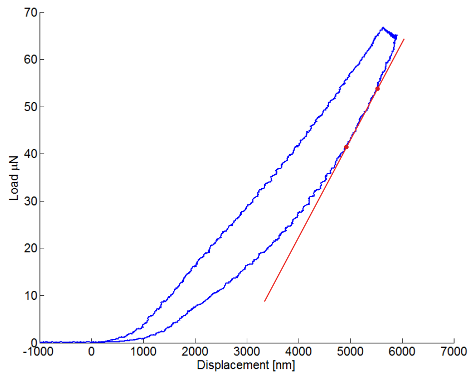

Figure 3: *Typical load-displacement curve of local indentation cycle on 4% LMP agarose.*

As figure 3 illustrates, the effective Youngs Modulus is calculated using the Oliver&Pharr compliance method on 65% to 85% of the unloading curve. This can be alternated in the Piuma software suite. All experiment data is output in open access text files, allowing researchers to model other parameters than those output by the Piuma Nanoindenter program.

Results - gelatin

In order to explore the global homogeneity of freshly produced gelatin, a 750 × 1250 μm grid scan with a 25 μm 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.

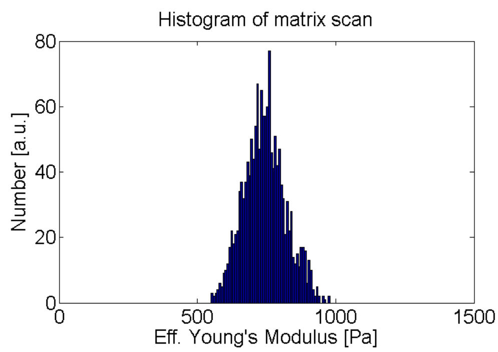

Figure 4: *Histogram of 30 × 50 point matrix scan of fresh 2.5% gelatin. The point-to-point pitch is 25 μm.*

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.

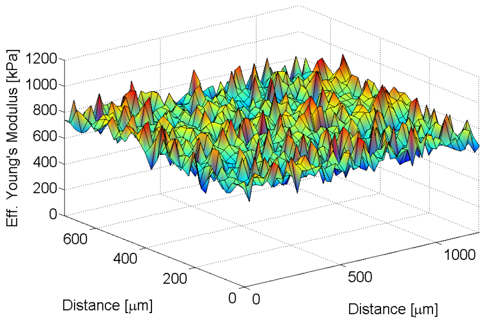

Figure 5: *3D figure of 50 × 30 grid scan of 2.5% gelatin. Mean = 0.744 kPa, std = 0.075 kPa.*

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 Modulus estimates 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.

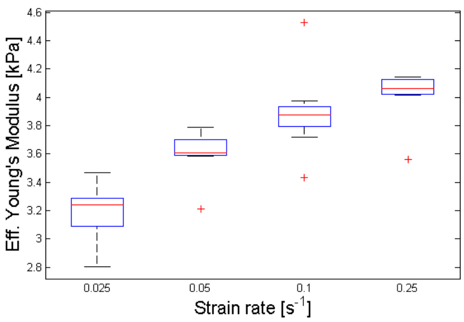

Figure 6: *Boxplot of effective Young's Modulus estimates for different strain rates. Strain rates 0.025, 0.05, 0.1 and 0.25 s^{-1} correspond to 0.92, 1.82, 3.64 and 9.13 µm/s displacement velocities, respectively.*

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 µN), 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.

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References

[1] Gruca, G., De Man, S., Slaman, M., Rector, J. H., Iannuzzi, D., *Ferrule-top micromachined devices: design, fabrication, performance. Measurement Science and Technology* Vol. **21** No. **9** (2010)

[2] Hertz, H. R., 1882, Ueber die Beruehrung elastischer Koerper (On Contact Between Elastic Bodies), in Gesammelte Werke (Collected Works), Vol. **1**, Leipzig, Germany, (1895)

[3] W.C. Oliver, G.M. Pharr, *An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation J.Mater. Res.* Vol. **7** No. **6** 1564 (1992)

[4] W.C. Oliver, G.M. Pharr, *Measurement of hardness and elastic modulus by instrumented indentation: Advances in understanding and refinements to methodology J.Mater. Res.* Vol. **19** No. **1** 3 (2004)