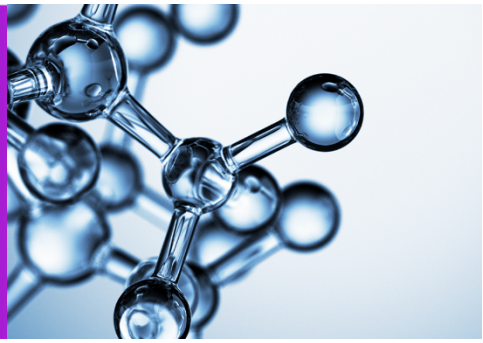
KLA INSTRUMENTS

Complex Shear Modulus of Compliant Biomaterials



Summary

In this application note, we present a new technique for measuring the local complex shear modulus of compliant biomaterials by instrumented indentation. We demonstrate the technique by testing edible gelatin as an exemplary material. With a contact diameter of about 100µm, we measure the shear modulus G′ = 2.89 ± 0.52kPa and the shear loss modulus G″ = 0.71 ± 0.36kPa (145Hz, N = 10). The same test method may be used to test other compliant biomaterials such as muscle or vascular tissue.

Introduction

Knowledge of mechanical properties is uniquely important for biomaterials because of the reciprocal relationship between function and properties. Generally, the function of tissue affects, and is affected by, its mechanical properties. Further, the hierarchical structure of tissue makes it desirable to measure mechanical properties over all the relevant length scales for the tissue. Nanoindentation seems like a promising choice for measuring small-scale mechanical properties, but the nanoindentation techniques developed for engineering materials simply do not work with biological materials. The primary difficulties are: (1) knowing the area of mutual contact between the punch and the tissue, and (2) making relevant and reliable measurements of contact stiffness and damping when the test material is very compliant and viscoelastic. This application note demonstrates how the KLA iNano® is used to test biological materials and explains the unique hardware, procedure and analyses required for such testing. Edible gelatin is used for this demonstration, because it has properties comparable to tissue, but it is widely available and can be prepared in a repeatable and controlled way.

General Theory of Complex Shear Modulus, G*

Generally, the constitutive relation which governs the viscoelasticity of biomaterials is

$$\tau^* = G^*\gamma^* \qquad (1)$$

where the complex shear modulus, G*, is the material property that relates complex shear stress, τ*, and complex shear strain, γ*. The complex shear stress may be expressed as an oscillation in time, t, having an amplitude, τ_0, and angular frequency, ω, as:

$$\tau^* = \tau_0 e^{i\omega t} \qquad (2)$$

In response to such a shear stress, we expect a complex shear strain, γ^*, having amplitude γ_0, which also oscillates at an angular frequency of ω, but lags by a phase angle, δ, or

$$\gamma^* = \gamma_0 e^{i(\omega t-\delta)} = \gamma_0 e^{i\omega t}/e^{i\delta} \qquad (3)$$

Rearranging Equation 1 to solve for G^* and substituting the expressions for complex stress and strain (Equations 2 and 3) gives the following expression for complex modulus:

$$G^* = \tau^*/\gamma^* = (\tau_0/\gamma_0)e^{i\delta} = (\tau_0/\gamma_0)(\cos\delta + i\sin\delta) \qquad (4)$$

Equation 4 clarifies the components of the complex modulus as:

$$G^* = G' + iG'', \text{ where} \qquad (5a)$$

$$G' = (\tau_0/\gamma_0)\cos\delta \qquad (5b)$$

$$G'' = (\tau_0/\gamma_0)\sin\delta \qquad (5c)$$

where G′ is known as the shear storage modulus and G″ is known as the shear loss modulus. Finally, the ratio G″/G′ is called the loss factor, because it quantifies the ability of the material to damp out energy relative to the ability to store energy:

$$\text{Loss factor} \equiv G''/G' = \tan\delta \qquad (5d)$$

Macroscopically, G′, G″ and tanδ are all measured by means of rheometry. Our goal is to use instrumented indentation testing to measure comparable values of these very same properties on a much smaller scale.

Measuring Complex Modulus by Instrumented Indentation

We employ Sneddon's contact solution[1], as developed by others[2, 3] to relate the shear storage modulus, G′, to contact stiffness, S, Poisson's ratio, ν and contact diameter, D:

$$G' = S(1-\nu)/(2D) \qquad (6a)$$

Loubet et al. proposed that in an indentation test, the shear loss modulus should be related to contact damping, $D_s\omega$, in an analogous way [4]:

$$G'' = D_s\omega(1-\nu)/(2D) \qquad (6b)$$

and the validity of this analogy is borne out by much experience [5]. Thus, in order to measure the components of the complex shear modulus, one must know the contact diameter, Poisson's ratio, and the stiffness and damping of the contact.

The problem of knowing the contact diameter is solved by using a flat-ended cylinder or cone, because for such an indenter, the contact diameter is simply that of the punch face, which doesn't change over the course of the test (note that this is in contrast to pyramidal indenters which are commonly used to test engineering materials).

Indeed, the Poisson's ratio must be known *a priori*, but for gels and biomaterials, it is safe to assume the value for incompressibility, $\nu = 0.5$, because water is the dominant component of such materials.

Thus, the problem of measuring complex modulus reduces to that of measuring contact stiffness and damping. This is an experimental challenge because the contact stiffness and damping are generally quite small. Dynamic analysis using the KLA iNano reveals that the contact stiffness and damping are determined as the directly measured values of stiffness and damping, K and $D\omega$, less the contribution of the instrument, K_i and $D_i\omega$:

$$S = K - K_i \qquad (7a)$$

$$D_s\omega = D\omega - D_i\omega \qquad (7b)$$

Thus, the problem of accurately knowing S becomes more challenging as the value of K becomes comparable to the value of K_i, which is the case when testing ultra-compliant materials (and likewise for $D\omega$ and $D_i\omega$). The iNano has been designed so that K_i and $D_i\omega$ are both minimized and measurable. Further, the iNano test method for gels and biomaterials includes an extra step with every test in which K_i and $D_i\omega$ are carefully measured. Once S and $D\omega$ are determined according to Equations 7a and 7b, then the storage and loss modulus can be known by Equation 6, and the loss factor is defined as:

$$\tan\delta = G''/G' = D_s\omega/S \qquad (8)$$

Sample Preparation

Food-grade gelatin was prepared at double concentration by mixing a pouch of gelatin powder (Knox Unflavoured Gelatine, Kraft Foods Group, Inc., USA) with 4oz (118ml) boiling water (half the prescribed amount of water). The mixture was stirred constantly until the gelatin was fully dissolved. A sample holder specifically designed for testing wet samples was filled to the brim with the gel solution, and the sample was placed in an air-tight environment to prevent dehydration while setting overnight. Just prior to testing, the sample was removed from the air-tight container and a very thin piece of glass—a section of a microscope slide coverslip—was floated on top of the gel in order to provide a relatively stiff surface on which the instrument could engage the sample. Scotch tape, sticky-side up, was adhered with epoxy to the edge of the sample holder in order to provide a surface for cleaning the indenter between tests. The sample, completely prepared for testing, is shown in Figure 1.

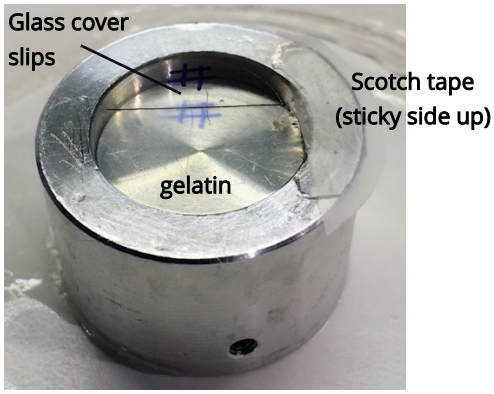


Figure 1. Gelatine sample.

Equipment and Procedure

A KLA iNano nanoindenter, configured with a flat-ended cylindrical punch tip having a diameter of 107.7nm, was used to perform ten indentations on the gel surface. Test sites were spaced by 400μm to avoid mutual interaction. The iNano test method **Complex Shear Modulus of Biomaterials** was used with the input values summarized in Table 1; each gel indentation test included the following steps:

1. Self-calibration: with the indenter not in contact with the sample, the stiffness and damping of the instrument alone (K_i and $D_i\omega$) were measured under the same conditions anticipated for the test (same indenter position, frequency, and amplitude).
2. Engagement: with the indenter over the cover slip, the whole actuator was moved down until the indenter touched the glass.
3. Approach: with the indenter over the gel, the actuator was moved down until contact with the gel was sensed by a shift in phase angle.

4. Pre-test compression: the flat face of the indenter was pressed into full contact with the gel.
5. Test: the indenter was vibrated in contact with the gel in order to measure composite stiffness and damping (K and Dω)

Following each test described above, the **Quick Touch** test method was used to quickly clean the indenter by pressing it into contact with the Scotch tape mounted on the edge of the sample holder.

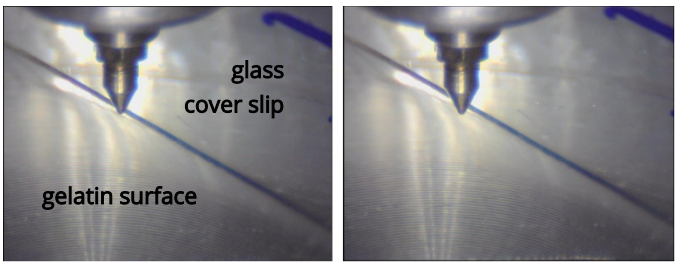


Figure 2. Still shots from real-time video of indentation process: (left) flat-punch indenter approaching the sample surface; (right) measuring the complex shear modulus of the gel.

Table 1. Summary of Method Inputs

Input	Value	Units
Punch diameter	107.7	µm
Poisson's ratio	0.5	None
Pre-test compression	10	µm
Target frequency	145	Hz
Phase change for contact	0.5	degrees

Results and Discussion

Figure 3 shows the gel surface, which is quite smooth, and the circular mark left by the flat face of the punch. The mark confirms that the instrument properly sensed contact and applied the pre-test compression so that the full face of the indenter was in contact with the gel.

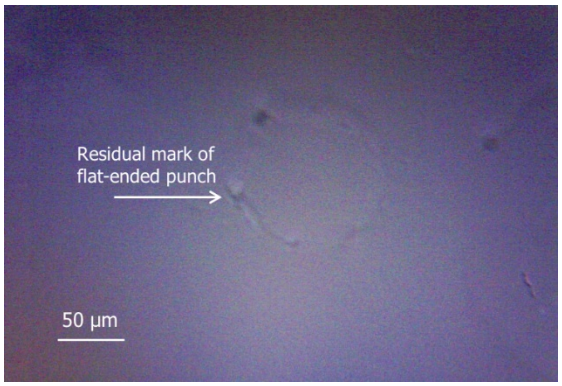


Figure 3 - Indentation residual mark after test.

Table 2 summarizes the results of all ten tests on the gelatin. The values for storage modulus, G', and loss modulus, G", are reasonable for this material, although the test-to-test variation is higher than expected for nanoindentation measurements of a smooth, uniform material. The reader should take note of the very small values for contact stiffness and damping; the contact stiffness is on the order of 1N/m, and the contact damping is even less. The integrated self-calibration process is essential for accurate measurement of such small values.

Nevertheless, the observed test-to-test variation is primarily due to measurement uncertainty, not true point-to-point variation in material properties. Repeated measurements of the instrument stiffness under these conditions give a standard deviation of 0.25N/m. This standard deviation of instrument stiffness is 20% of the mean contact stiffness. This degree of uncertainty fully accounts for the observed relative variation in G' of 18%. The relative variation in loss modulus, G", has a similar explanation: the standard deviation in instrument damping is 33% of the mean contact damping.

The influence of measurement uncertainty may be mitigated by using a larger punch to increase the size of the contact. From Equation 6a, we see that the contact stiffness, S, depends proportionally on the storage modulus, G', and the contact diameter, D. Thus, if we want to increase the value of the contact stiffness, relative to our uncertainty of 0.25N/m, then we must increase the diameter of the punch. Doubling the size of the punch should cut in half the relative variation in G'. The same can be said of damping: doubling the diameter of the punch should cut in half the relative variation in G".

Table 2. Storage modulus G' and loss modulus G" of edible gelatin (double concentration), measured at 145Hz, 22.8°C, and 34.9% humidity.

Test	G'	G"	Tanδ	S	$D_s\omega$
Units	Pa	Pa	-	N/m	N/m
1	3163	802	0.25	1.36	0.35
2	2996	587	0.20	1.29	0.25
3	3458	820	0.24	1.49	0.35
4	3862	1611	0.42	1.66	0.69
5	2968	993	0.34	1.28	0.43
6	3081	496	0.16	1.33	0.21
7	2394	455	0.19	1.03	0.20
8	2485	545	0.22	1.07	0.24
9	2352	482	0.21	1.01	0.21
10	2103	324	0.15	0.91	0.14
Mean	2886	712	0.24	1.24	0.31
Std. Dev.	521	356	0.08	0.22	0.15
% Std. Dev.	18.1	50.0	32.7	18.1	50.0

Conclusions

The KLA iNano system and the test method **Complex Shear Modulus of Biomaterials** were used to measure the complex shear modulus of food-grade gelatin, an exemplary biomaterial. With a flat punch having a diameter of only 100µm, the shear storage modulus G' measured 2.89 ± 0.52kPa and the shear loss modulus G" measured 0.71 ± 0.36kPa (N = 10). Using a larger-diameter punch would improve the relative uncertainty in measured properties, but at the sacrifice of spatial resolution in the measurement.

References

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