Micro-rheology of soft biological samples using optical tweezers

Introduction

With the rise of mechanobiology and its implications in broad fields such as cancer, developmental biology and regenerative medicine, growing interest has focused on measuring the mechanical properties of living cells, tissues and biological scaffolds. The stiffness of cells and their surrounding environment ranges from hundreds of Pa (brain tissue) to hundreds of kPa (bone). In addition, most biological tissue is viscoelastic, that is, it behaves neither as a perfectly elastic solid nor as a flowing viscous liquid, but rather as a combination of both. Interestingly, in pathological states such as cancer and inflammation, cells and tissues change not only their stiffness, but also the balance between their elastic and viscous responses to external forces [1,2].

Typically, nanotechnologies such as Optical Tweezers and Atomic Force Microscopy are used to measure mechanical properties in indentation experiments, with an optically-trapped bead or the tip of a cantilever brought into contact with the surface of a sample and then forced to indent it. While this method offers good local estimates of stiffness at the sample's surface, it can't be used to probe the interior of samples. Such an approach would require an optical trapping setup with a reliable trap calibration which holds valid for particles of any shape and inside media with any optical properties. Finally, any solution to assess how the viscoelastic properties of cells or tissues change with different probing frequencies, a technique known as micro-rheology, would require an instrument capable of applying oscillatory forces and measuring bead displacements at a broad range of probing frequencies (several decades).

In this application note, we show how the micro-rheology module of the *SENSOCELL™* optical tweezers system can be used to measure the viscoelastic properties of extracellular matrices or living cells, with stiffnesses ranging from tens of Pa to several kPa and at probing frequencies up to the kHz regime.

Our unique solution is based on *Impetux's* distinctive and patented technology [3], which makes the repeated calibration of the trap unnecessary, provides a remarkable ease of use for non-experts and eliminates time-consuming calibrations required for traditional systems. ***SENSOCELL™*** is thus the only commercial optical tweezers system that can carry out direct force measurements inside living cells.

Methods

All experiments presented here have been carried out using polyacrylamide hydrogels of different stiffnesses. As probes, we have used 3 μm diameter latex beads embedded within the gels *(Fig. 1)*.

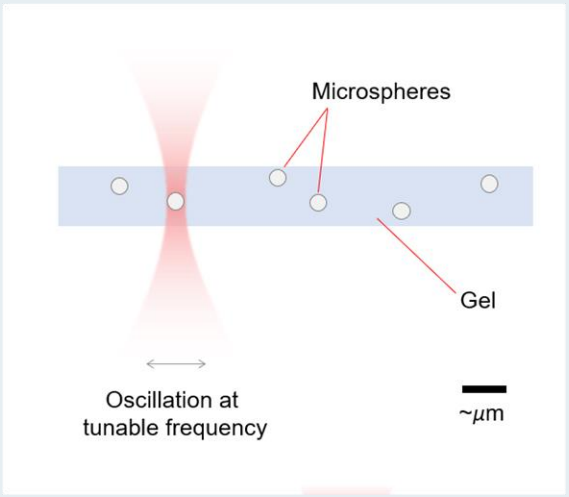

Fig. 1 - ***Top***. A schematic view showing optical trapping of spherical beads embedded within a soft hydrogel. ***Bottom***. Image acquired via the SENSOCELL™ control software (LightAce) showing a 3μm-diameter trapped bead. Note there is also an additional non-trapped bead out of focus to the right.

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Laser power at the sample was set to 150 mW, the optical trap position was oscillated with 500nm amplitude and at frequencies ranging from 3 Hz to 4500 Hz.

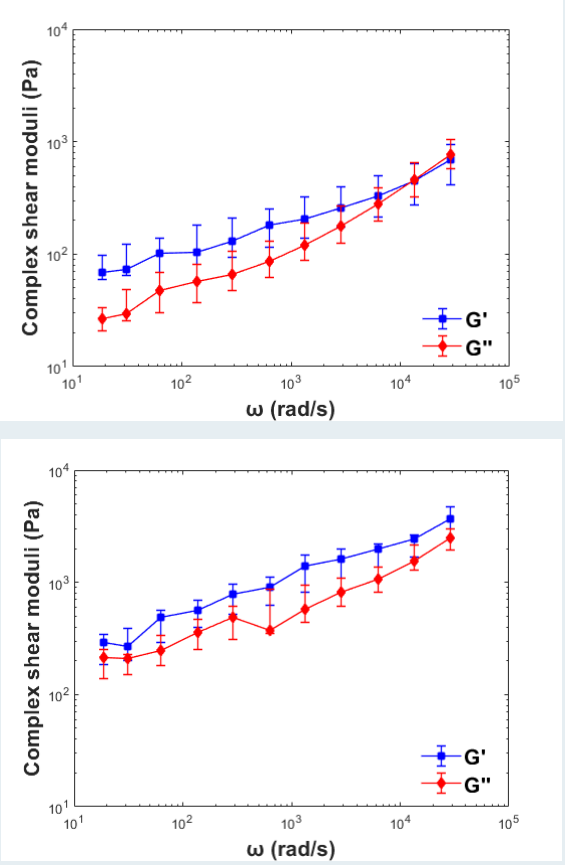

Fig. 2 - Two examples of the frequency-dependent behavior of the complex shear moduli of soft polyacrylamide gels. Blue symbols indicate storage modulus (G') and red symbols indicate loss modulus (G''). Symbols are median values and error bars indicate Q1 and Q3 ranges. N = 13 beads probed for each gel.

For each trapped bead, the micro-rheology module of ***SENSOCELL™*** computes the complex shear modulus G*(ω) of the sample, where the storage modulus G'(ω) (real part) accounts for the energy stored in the sample, and the loss modulus G''(ω) (imaginary part) accounts for the energy dissipated. Subsequently, the data is fitted using a structural damping model, where the power-law exponent (α) and the viscosity of the sample (μ) are obtained using the following fitting formula (1):

$$G^*=G_0\,(1+i\tan(\alpha\,\pi/2))\,(\omega/\omega_0)^{\alpha}+i\mu\omega \quad (1)$$

Here, the parameter α describes whether a sample tends to display a solid-like behavior (α = 0) or a liquid-like behavior (α = 1).

Results

For all gels and beads tested, G' and G'' strongly increased with probing frequency *(Fig. 2)*. Similarly, G' was typically larger than G'', except at very high frequencies, where the viscosity of the sample is expected to dominate the mechanical response. This viscoelastic frequency response of highly diluted polyacrylamide gels is very similar to that measured by others using a rheometer [4].

Interestingly, we found a strong correlation between the storage modulus of the gels and the computed power-law exponent values, with the softest gels displaying an almost liquid-like behavior (α = 0.85) and the stiffer gels displaying an exponent value that saturated towards 0.3 *(Fig. 3)*.

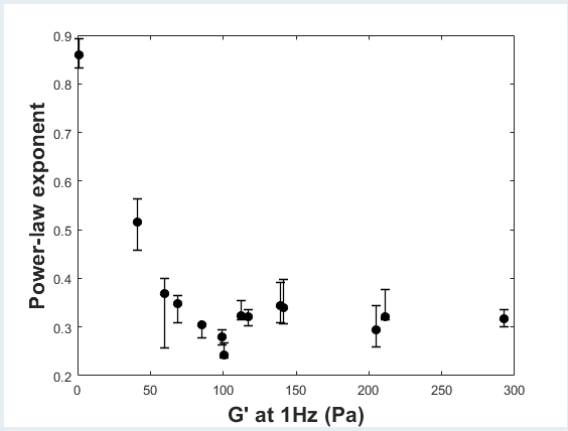

Fig. 3 - Power-law exponent obtained by fitting a structural damping model (Eq. 1) to the micro-rheology data for polyacrylamide gels. The softest gels display a liquid-like behavior while for the stiffer gels the power-law exponent saturates at 0.3. Symbols are median values and error bars indicate Q1 and Q3 ranges. N = 13 beads probed for each gel.

The large moduli values displayed by the gels at the highest frequencies allowed us to explore the limits of our system in terms of reliability and repeatability for micro-rheology measurements. Of note, for moduli values in the order of kPa and the experimental conditions used here, the oscillation of the bead is expected to be in the sub-nanometer range. Nevertheless, as shown by *Fig. 4*, the ***SENSOCELL™*** system offers repeatable values (as computed by the coefficient of variance CoV) for stiffness at least up to 6 kPa.

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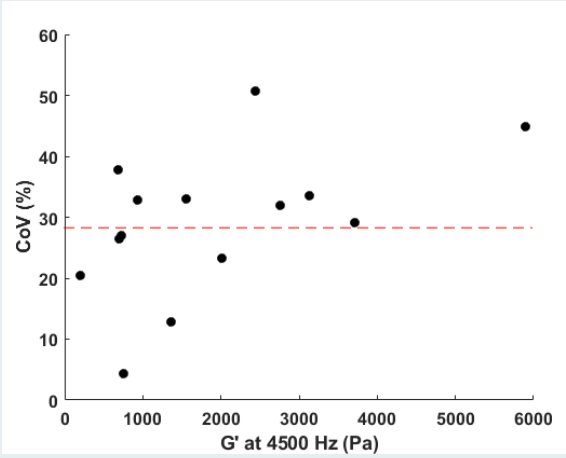

Fig. 4 - Coefficient of variance (CoV) displayed by gels at the highest frequency probed (where the measured G' is largest). Red dashed line corresponds to the average CoV for all gels tested. N = 13 beads probed for each gel.

Conclusions

Measurements like those presented here show the potential of ***SENSOCELL™*** for micro-rheology applications inside soft hydrogels and living cells. The distinctive technology used by ***SENSOCELL™*** allows carrying out mechanical characterization inside soft samples in a seamless, quick and calibration-free manner. The results obtained display also large accuracy and repeatability for ranges up to kPa in stiffness and kHz in probing frequency. Many other mechanobiology experiments can be carried out using ***SENSOCELL™***, the only commercial optical tweezers system that can carry out direct force measurements within living cells.

References

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