

Water content, not stiffness, dominates Brillouin spectroscopy measurements in hydrated materials

To the Editor: Brillouin microscopy is an optical method to map the mechanical properties of materials^{1–5}. The first Brillouin micrographs with cellular resolution were reported in *Nature Methods* in 2015⁴ and inspired a growing number of applications in biomechanics and biophotonics. In these publications, Brillouin measurements were considered a proxy for stiffness. In contrast, we show here in hydrogels that water content dominates Brillouin signals in hydrated materials.

Brillouin microscopy relies on the phenomenon of Brillouin scattering, whereby photons exchange energy with thermally driven acoustic waves, or phonons, leading to a frequency shift ω_b between the incident and scattered light, given by

$$\omega_b = \frac{2n}{\lambda} \sqrt{\frac{M}{\rho}} \sin \frac{\theta}{2} \quad (1)$$

where ρ and n are the density and refractive index of the material, and λ and θ are the in vacuo wavelength and angle between the incident and scattered wave vectors. M is the longitudinal elastic modulus and represents the compressibility of a material or, specifically, the mechanical stress necessary to compress or expand it in one direction without lateral strain.

Despite the fact that equation (1) depends on M , recent reports have interpreted Brillouin micrographs in terms of Young's modulus E for cells and tissues^{3–5}. E represents the stiffness of a material, that is, how resistive it is to deformation, and describes the stress necessary to compress or extend a material in one direction while allowing lateral strain. As biological materials are composed mostly of water, which is relatively incompressible, M is several orders of magnitude greater than E . Further, Brillouin scattering is sensitive to gigahertz frequencies where, owing to viscoelastic effects, mechanical properties may diverge from those at biologically relevant strain rates, which typically occur over seconds. Nonetheless, empirical correlations between M and E for cells⁴, hydrogels^{3,4} and other biological tissues³ have suggested that variations in M , as

measured by Brillouin scattering, reflect variations in E .

We set out to examine the relationship between Brillouin measurements and Young's modulus, accounting for the potential influence of water content ϵ , which can affect both M and E in hydrated materials. We used hydrogels as a simplified model of biological materials because both contain fluid interspersed within a flexible solid network that provides elasticity.

As the molecular weight of polyethylene oxide (PEO) increases, hydrogels change from a dilute suspension with zero Young's modulus to a semi-dilute entangled network with finite E . When the molar concentration is decreased in proportion to the increase in molecular weight, water content ϵ can be fixed while E increases. Thus, we could vary E independently of ϵ while measuring M using a custom Brillouin microscope⁵ (Supplementary Methods).

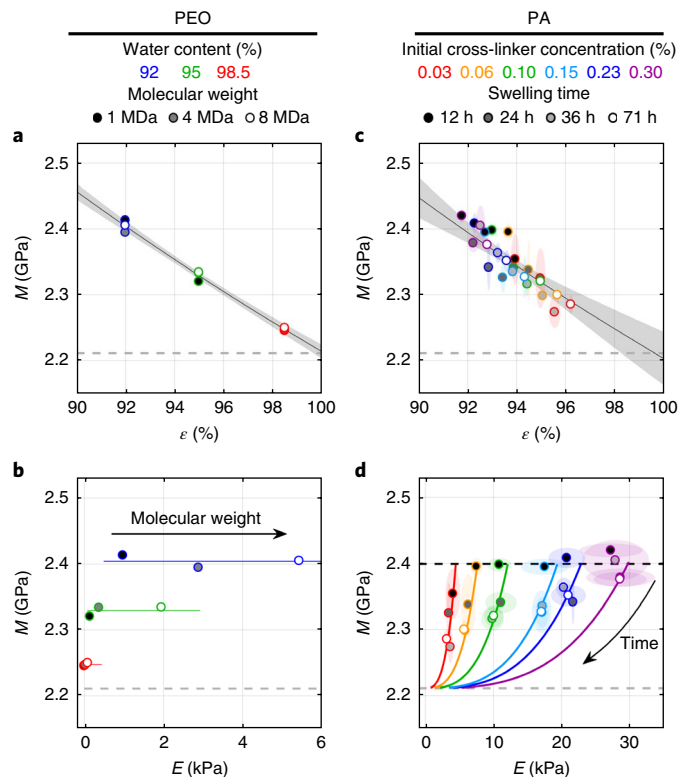


Fig. 1 | Young's modulus E , water content ϵ and longitudinal elastic modulus measured by Brillouin M for polyethylene oxide and polyacrylamide hydrogels. **a, M for PEO of the indicated molecular weight, measured at different ϵ . The fit to equation (2) is shown ($R^2 = 0.97$; one hydrogel per data point). **b**, M is plotted versus E (color code as in **a**; one hydrogel per data point). Lines represent values of constant ϵ . **c**, M over all swelling times (shading within symbols) and cross-linker concentrations (colored symbols), plotted versus ϵ for PA. The fit to equation (2) is shown ($R^2 = 0.78$; four hydrogels per data point, mean ± 2 s.d.). **d**, Relationships between M and E at various PA hydrogel compositions. Changes in M and E during swelling were consistent with predictions of equations (2) and (3) using $M_f = 2.21$ GPa and $M_s = 16.35$ GPa (curves; four hydrogels per data point, mean ± 2 s.d.). Dashed gray lines represent the value of M for pure water. The black dashed line in **d** represents the estimated initial value of M at the start of swelling when $\epsilon_0 = 0.9$ for all hydrogels. Elliptical regions surrounding data points in **c** and **d** represent 2 s.d. Shaded regions in **a** and **c** represent 95% confidence bounds on the fit by equation (2).**

With increasing molecular weight, E measured by rheometry increased for a given ε . However, M was unaffected by the change in molecular weight, but decreased with ε (Fig. 1a and Supplementary Note). Thus, for PEO hydrogels, changes in E were uncorrelated with changes in M when controlling for water content (Fig. 1b and Supplementary Note).

To understand the relationship between M and ε , we considered a biphasic model where the aggregate compressibility is equal to the sum of the individual fluid and solid compressibilities weighted by their respective volume fractions⁶,

$$\frac{1}{M} = \frac{\varepsilon}{M_f} + \frac{1-\varepsilon}{M_s} \quad (2)$$

where M_f and M_s are the longitudinal elastic moduli (inverse compressibilities) of the fluid and solid, respectively. Equation (2) captures the relationship between M and ε for PEO (Fig. 1a and Supplementary Note).

Previously reported correlations between M and E were based on polyacrylamide (PA) hydrogels^{3,4}, which swell over time (Supplementary Fig. 1). Using different concentrations of bis-acrylamide to vary PA stiffness, we measured M , E and ε during swelling (Supplementary Figs. 2–4). All values of M collapsed onto a single relationship with ε , consistent with equation (2), despite differences in E or the extent of swelling (Fig. 1c and Supplementary Note).

During swelling of PA, E measured by unconfined compression decreased according to

$$E = E_0 Q^{-1/3} \quad (3)$$

where $Q = (1 - \varepsilon_0)/(1 - \varepsilon)$ is the swelling ratio and E_0 is the estimated Young's modulus immediately after gelation.

There was no clear relationship between M and E when considering all data. However, equations (2) and (3) predicted how M changed versus E for individual hydrogels as ε increased from $\varepsilon_0 = 0.9$ to 1. These predicted trajectories (shown by solid curves in Fig. 1d and the Supplementary Note) explain how a correlation may arise owing to a mutual dependence on ε without an explicit relationship between M and E .

In conclusion, Brillouin measurements appear insensitive to Young's modulus after accounting for the influence of water content in PEO and PA hydrogels. This suggests that Brillouin measurements applied to biological tissues may be similarly affected by water content, although differences in hydration between tissues and the role of bound water may have additional effects. This work cautions against the straightforward application of Brillouin microscopy, or Brillouin scattering in general, as a form of optical bioelastography and motivates further research into the factors influencing the Brillouin frequency shift in biological materials.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Datasets generated and analyzed during the current study are available upon reasonable request. □

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Author contributions

P.-J.W., I.V.K., C.P., P.T., J.W.R., J.M.S., I.E.D. and D.R.O. planned the study. P.-J.W. and I.K. conducted experiments. All authors participated in and contributed to data analysis. D.R.O. and P.-J.W. wrote the manuscript. All authors contributed to editing and revision of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41592-018-0076-1>.

Reply to 'Water content, not stiffness, dominates Brillouin spectroscopy measurements in hydrated materials'

Scarcelli and Yun reply—

The recent applications of Brillouin microscopy to tissues and cells following publication of our work^{1,2} have renewed interest in what physical quantity this technique measures. The Correspondence by Wu et al.³ focuses on water content and questions the

biomechanical relevance of Brillouin measurements. The physics of Brillouin light scattering has firm roots in the thermodynamic and elastic properties of materials. The dependence of Brillouin scattering on solid constituents and water content has been extensively studied for hydrated materials⁴.

The Brillouin frequency shift (ω_b) is proportional to the square root of the longitudinal modulus (M). In hydrated samples, compressibility β ($\sim 1/M$) is approximated to a concentration-weighted sum, $\beta = \varepsilon_f \beta_f + \varepsilon_s \beta_s$, where $\varepsilon_{f,s}$ and $\beta_{f,s}$ are the volume fraction and compressibility of fluid and solid constituents, respectively.

Brillouin shift is sensitive to changes in water content, $\Delta\epsilon_f$, and solid constituents, $\Delta\beta_s$, via the following equation:

$$\frac{\Delta\beta}{\beta} = \left(1 - \frac{\beta_s}{\beta}\right) \frac{\Delta\epsilon_f}{\epsilon_f} + (1 - \epsilon_f) \frac{\Delta\beta_s}{\beta}$$

For tissues, where $\epsilon_f \approx 75\%$ and $\beta_s/\beta_f \approx 0.25$ (ref. ⁵), we found⁶ the following:

$$\frac{\Delta\omega_b}{\omega_b} \approx -0.4 \frac{\Delta\epsilon_f}{\epsilon_f} - 0.04 \frac{\Delta\beta_s}{\beta_s}$$

Therefore, in terms of relative changes, Brillouin frequency is about tenfold more sensitive to water content than to solid-part compressibility, consistent with the findings of Wu et al.³. However, state-of-the-art instruments² can measure $\Delta\omega_b/\omega_b = 0.1\%$, that is, they have the detection sensitivity of $\Delta\epsilon_f/\epsilon_f \approx 0.25\%$ for water and $\Delta\beta_s/\beta_s \approx 2.5\%$ for solid parts. Directional Brillouin spectroscopy has reported anisotropic compressibility of hydrated biopolymer fibers⁵, while water has isotropic properties—evidence that this technique can reveal solid-part compressibility.

In live biological samples, the water content changes relatively little. The density of cells varies normally by <1%. Corneal hydration is tightly regulated by endothelial cells, changing by <0.2% during the daytime⁶. Wu et al.³ investigated highly hydrated gels and an artificially large variation of water content of 92–98%. For such samples, the sensitivity to solid constituents vanishes:

at $\epsilon_f = 98\%$ the minimum detectable $\Delta\beta_s/\beta_s$ is $\sim 40\%$. Therefore, generalizing their results to biological samples needs caution. It is worthwhile to repeat the study with samples with higher polymer concentration and physiologically relevant water content variation. In some samples with higher polymer concentration, the exchanged water may be bound to the polymer chains instead of being free in the interstitial space, in which case the sensitivity to hydration decreases several-fold and can be comparable to the sensitivity to solid parts⁴.

A relevant question is how much $\Delta\beta_s$ and $\Delta\epsilon_f$ are involved in the specific process under investigation. Compressibility can differ among different polymers by as much as 400%, but for most biological samples, the difference in solid compressibility is likely to be smaller. For example, actin polymerization or collagen degradation can change solid-part compressibility, and these processes could be detected if $\Delta\beta_s/\beta_s > \sim 2.5\%$. These processes may also accompany structural compaction and osmotic pressure changes, which can also lead to measurable Brillouin frequency change $\Delta\omega_b$ through interactions between water and solid parts (not considered in the above simplified equations or in Wu et al.³). Judicious interpretation is therefore required to connect Brillouin data to underlying structural and biological processes, and vice versa.

The longitudinal modulus is not directly related to the Young's modulus or the shear modulus, as we had stated in our earlier paper². Nevertheless,

phenomenological correlations may appear for many, if not all, physiological and pathologic processes in which the underlying biomechanical changes, including water transport⁷, alter both moduli in the same direction². However, such correlations need to be calibrated a priori if one wants to relate the two moduli.

In conclusion, the Brillouin frequency shift can be used to measure changes in water content and solid-part compressibility and, under some circumstances, may serve as a proxy for stiffness. □

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Competing interests

The authors declare the following competing interests: G.S. is a scientific advisor for and A.Y. is the scientific founder of Intelon Optics, Inc.

BraCeR: B-cell-receptor reconstruction and clonality inference from single-cell RNA-seq

To the Editor: Reconstruction of antigen-receptor sequences from single-cell RNA-seq (scRNA-seq) data allows the linking of antigen-receptor use to the full transcriptomic identities of individual B lymphocytes, without the need for additional targeted repertoire sequencing (Rep-seq). Here we report BraCeR (freely available at <https://github.com/teichlab/bracer/> and as Supplementary Software), an extension of TraCeR¹, for the reconstruction of paired full-length B-cell-receptor (BCR) sequences and inference of clonality from scRNA-seq data (Supplementary Note 1).

BraCeR builds on the well-verified pipeline of TraCeR for the assembly of BCR sequences from paired-end or single-end reads, with modifications to account for somatic hypermutations (SHMs) and isotype switching (Supplementary Notes 2 and 3 and Supplementary Data). In addition, a ‘Build’ mode facilitates the creation of resource files for the analysis of species other than humans and mice.

BraCeR was tested on experimental human and mouse scRNA-seq data with various SHM rates and repertoire diversities^{2,3}. Its reconstruction accuracy

was similar to that of BASIC, a previously reported tool for BCR reconstruction². Compared with that tool, BraCeR yielded a reconstruction efficiency that was somewhat superior for the long (125 bp) reads and similar for short (50 bp) reads (Supplementary Note 4 and Supplementary Tables 1–7). However, BraCeR allows for the reconstruction of additional heavy and light chains present in a cell, and can identify nonproductively rearranged chains; we validated BCR reconstruction (including that of additional sequences) through direct comparison with targeted BCR sequencing