

Supplementary Note 1: Longitudinal modulus

Longitudinal modulus is defined as the ratio of uniaxial stress to uniaxial strain (**Supplementary Fig. 1**) with zero transverse strain. A longitudinal acoustic wave involves such longitudinal stress and strain. In Brillouin interactions in the GHz frequency range, we probe the longitudinal modulus by measuring thermodynamically generated acoustic vibrations within the probed voxel. In the quasi-static limit, the longitudinal modulus, M , is related to the bulk modulus, K , and shear modulus, G , as follows: $M=K+4/3G$. The bulk modulus has little frequency dependence; on the other hand, shear and Young's moduli have been shown to increase significantly with frequency¹. In Brillouin measurements, we expect a strong contribution of the bulk modulus of water as modified by the presence of other macromolecules in the cell microenvironment.

For viscoelastic materials, these moduli are complex quantities. The real part, the storage modulus, accounts for the elastic behavior (stored elastic energy), and the imaginary part, the loss modulus, describes the viscous-like dissipation (associated with rate processes within the solid network alone and/or fluid flow with respect to the porous solid network²). The Brillouin frequency shift as previously described is given by $\Omega=2K\sqrt{M'/\rho}\sin(\theta/2)$, with K the photon wavenumber, θ the angle between incident and scattered photons, M' the real part of the material longitudinal modulus and ρ the mass density of the sample; it is therefore proportional to the propagation velocity $\sqrt{M'/\rho}$ of the gigahertz acoustic waves inside the material.

In principle, both longitudinal and shear moduli at GHz can be probed by Brillouin spectroscopy. However, the measurement of shear modulus requires the detection of scattered light at a 90-degree angle in the polarization state orthogonal to the input polarization state. Furthermore, the strength of shear Brillouin scattering is much lower than longitudinal Brillouin scattering in most materials and should be negligible for cytoplasms due to their high water content.

Supplementary Note 2: Apodized VIPA spectrometer

We have previously demonstrated a double stage spectrometer for high throughput analysis of Brillouin scattering³. Here, we introduce a novel apodization technique to improve the spectral contrast (or extinction) of the spectrometer (**Supplementary Fig. 2a**). Virtually Imaged Phased Array (VIPA)⁴ is a modified tilted Fabry-Perot etalon to which cylindrically focused light is coupled through a narrow anti-reflection coated window. Upon entering the etalon, light undergoes multiple internal reflections and the multi-beam interference induces high spectral dispersion; a Fourier transform lens then focuses the pattern on the detector. Because of the multiple partial reflections, light from a VIPA etalon exits with an exponential intensity profile along the dispersion direction (**Supplementary Fig. 2b-c**). The Fourier transform lens then focuses the exponential beam into a Lorentzian transverse profile. This Lorentzian spectral transfer function carries significant energy at the wings compared to a Gaussian profile. Considering a VIPA with a finesse of 40, the magnitude of the wing with respect to the peak transmission is about -30 dB. This extinction ratio is not sufficient to reject back-reflections or stray light in Brillouin cellular microscopy.

To improve the extinction, one of the effective strategies is to convert the exponential beam profile to a Gaussian profile. To accomplish this, we used an apodization filter that converts the exponential beam profile to a more rounded shape (**Supplementary Fig. 2d**). For this, we used a linear-variable neutral density filter (Rugate Inc, or Newport 50FS04DV). This device provided a steep transmission slope to smoothen the sharp edge of the exponential profile. The effective apodization is clearly seen in the output beam profile as imaged from the

output face of the etalon (**Supplementary Fig. 2e**, solid vs. dotted line). With the filter, extinction in the transfer function was measured to be greater than -40 dB (**Supplementary Fig. 2f**, red), improved from -30 dB before apodization (**Supplementary Fig. 2f**, black). This result agreed well with our calculation based on the measured beam profiles.

Supplementary Note 3: Calibration and analysis of Brillouin spectrum

We implemented *in situ* calibration of the spectrometer free spectral range (FSR) and the CCD-pixel to optical-frequency conversion ratio (PR) in the Brillouin microscope. To determine these parameters, we fitted the setup with two calibration arms carrying reference materials with known Brillouin shifts. These could be interrogated periodically using a pair of computer-controlled motorized shutters.

In our procedure for calibration and spectral analysis, we first consider a CCD frame captured from a sample to be measured (**Supplementary Fig. 3a**). Switching the probe light to the calibration arm, a frame exhibiting two distinct peaks corresponding to Methanol and Acrylic (both Stokes and anti-Stokes peaks of the acrylic are seen) is observed (**Supplementary Fig. 3b**). It can be shown that:

$$\frac{FSR}{2} - PR \bullet \left[P_{ACR-S} - \left(\frac{P_{ACR-S} + P_{ACR-AS}}{2} \right) \right] = \Omega_{ACR-S}$$

$$\frac{FSR}{2} - PR \bullet \left[P_{METHA} - \left(\frac{P_{ACR-S} + P_{ACR-AS}}{2} \right) \right] = \Omega_{METHA}$$

where P_{ACR-S} , P_{ACR-AS} and P_{METHA} denote the measured value of Brillouin peaks in pixel units and Ω_{ACR-S} and Ω_{METHA} represent the actual value of Brillouin peaks in GHz units. These equations can be solved for the two unknowns, FSR and PR.

The Brillouin peak in pixel units P_X is measured by Lorentzian curve fitting. Then, the Brillouin shift of sample, Ω_X , is calculated from the following expression:

$$\Omega_X = \frac{FSR}{2} - PR \bullet \left[P_X - \left(\frac{P_{ACR-S} + P_{ACR-AS}}{2} \right) \right]$$

The wavelength of acoustic phonons in water and cytoplasm is about 200 nm, which is equal to one half of the optical wavelength (532 nm / 1.33 / 2 = 200 nm). From this, the propagation speed of an acoustic phonon is determined to be 200 nm x 7.5 GHz = 1,500 m/s. The measured Brillouin spectrum has a Lorentzian profile with a spectral width of about 500 MHz in both water and cytoplasm at 37 °C. This finite linewidth is due to the damping of acoustic phonons.

Supplementary Note 4: Accuracy of Brillouin shift estimation

In Brillouin scattering spectroscopy, the induced frequency shift, Ω , is related to the longitudinal modulus M , through the relation $M = \rho \lambda^2 \Omega^2 / (4n^2)$, where ρ is the mass density, λ is the optical wavelength and n is the refractive index. Thus, the ability of the instrument to detect changes in longitudinal modulus, $\Delta M'$ is related to the measurement accuracy of the Brillouin frequency shift, $\delta \Omega$. Thanks to the apodized design, our Brillouin spectrometer is background-free, and operated in the shot noise limited regime. In this regime, the accuracy in the determination of the center of the Brillouin spectrum is ultimately limited by the signal-to-noise ratio (SNR) according to the relation⁵:

$$\delta\Omega = \frac{\sqrt{\Delta_B^2 + \Delta_{NA}^2 + p^2/12 + r^2}}{SNR}$$

where Δ_B is the intrinsic linewidth of the Brillouin spectrum; Δ_{NA} is the broadening due to high-NA collection; p is the pixel spectral dispersion, i.e. the frequency interval between two adjacent CCD pixels in the spectrometer (~ 0.15 GHz); r is the spectral resolution, determined by the Free Spectral Range (FSR=20GHz) divided by the finesse of the spectrometer (~ 40). In the epi-illumination epi-detection configuration, signal collection is maximal while spectrum broadening due to high-NA is minimal due to the cosine angle dependence of Brillouin shift. We verified experimentally that NA-induced linewidth broadening does not prevent accurate measurements: the overall spectral linewidth was measured to be ~ 0.55 GHz at NA=0.6, and ~ 0.63 GHz at NA=1.4, only marginally increased with respect to the intrinsic instrumental linewidth of ~ 0.5 GHz measured at low NA.

To verify shot-noise limited operation, we measured the SNR at different illumination power levels and spectrum acquisition times. Brillouin spectra of methanol were acquired at different power levels ranging 1.5 mW and 4 mW and different acquisition times ranging from 4 ms to 100 ms. A good square root dependence of SNR on the product of acquisition time and power level demonstrating shot-noise limited operation (**Supplementary Fig. 4a**). An exemplary distribution of estimated shifts is shown for the 50 ms acquisition time and 4 mW power settings (**Supplementary Fig. 4b**). The standard deviation of this distribution can be used to estimate the accuracy in peak localization, in this case it is 8.8 MHz, or 0.15% as reported in the main text. Also the experimental accuracy in peak localization showed a good square root dependence on collected number of photons. Peak localization accuracy agreed well with Eq. (1) and was measured to be inversely proportional to SNR, yielding 4 MHz/mW/ $\sqrt{\text{Hz}}$. This relationship allows us estimating the sensitivity of our technique to elasticity changes within the cell: differentiating, we get $\Delta M/M = a\Delta E'/E'$, where ΔM and $\Delta E'$ are variation of longitudinal and Young's moduli and the coefficient a is typically found in the range of 0.02 to 0.1 in cells, tissues and biopolymers⁶. Thus, a Brillouin frequency sensitivity of 0.1% corresponds to a relative error in longitudinal modulus of $\Delta M/M \approx 0.2\%$, which indicates that the instrument is able to detect changes $\Delta E'/E'$ as little as 2 to 10%.

Supplementary Note 5: Computation of longitudinal modulus

To derive the longitudinal modulus from the Brillouin spectral shift, through the relation $M = \rho\lambda^2\Omega^2/(4n^2)$, the ratio of density to index of refraction ρ/n^2 needs to be estimated. Both density and index may vary spatially within a cell and from condition to condition, however, their ratio is found to be approximately constant⁷. We use the osmotic pressure experiments to validate this consideration (**Supplementary Fig. 5**). In the main text (**Fig. 1e**), we showed the correlation between micro-indentation Young's modulus, and longitudinal modulus computed using constant values for density (1.08 g/cm³) and index (1.37). This curve is reproduced in **Supplementary Fig. 5** with turquoise dots. For comparison, in magenta, we also show the estimated values of longitudinal modulus using density and refractive index values derived from the volume changes induced by osmotic pressure as calculated by the Boyle-Van't Hoff equation⁸. Even at the highest osmotic pressure condition, the two estimations of longitudinal modulus differ by $\sim 1\%$, which demonstrates how the longitudinal modulus can be effectively computed directly from Brillouin shift.

Supplementary Note 6: AFM-based micro-indentation to estimate Young's modulus

The AFM micro-indentation test provides a force-vs-elongation curve, which can be used to extract the overall mechanical properties of the cell. The Young's modulus of cells was obtained by using the thin-layer Hertz model⁹:

$$F = \frac{4E_Y R^{1/2}}{3(1-\nu)} \delta^{3/2} \left[1 - \frac{2\alpha_0}{\pi} \chi + \frac{4\alpha_0^2}{\pi^2} \chi^2 - \frac{8}{\pi^3} \left(\alpha_0^3 + \frac{4\pi^2}{15} \beta_0 \right) \chi^3 + \frac{16\alpha_0}{\pi^4} \left(\alpha_0^3 + \frac{3\pi^2}{5} \beta_0 \right) \chi^4 \right]$$

where

$$\alpha_0 = -\frac{1.2876 - 1.4678\nu + 1.3442\nu^2}{1-\nu}$$
$$\beta_0 = \frac{0.6387 - 1.0277\nu + 1.5164\nu^2}{1-\nu}$$

and F is the applied force, δ is the indentation depth, E_Y is the Young's modulus, R is the probe radius, ν is the Poisson's ration, $\chi = \sqrt{R\delta}/h$, and h is the cell height. The force and indentation depth were obtained from force-displacement curves of AFM based indentation. In the model we used average cell height $h = 7.5 \mu\text{m}$ for all conditions, estimated via high-resolution confocal fluorescence microscopy, by staining the plasma membrane. Fluorescence images were interpolated, thresholded and processed to identify cell edges and measure typical dimensions using ImageJ software. The Poisson's ratio was assumed to be $\nu = 0.4$ based on previous studies on chondrocytes¹⁰ and fibroblasts¹¹.

The overall results obtained from AFM micro-indentation are shown in **Supplementary Fig. 6**. The force-displacement curves for several cells in different sucrose concentrations are shown in **Supplementary Fig. 7**. Typical force-displacement curve and the best-fit curve from the thin-layer Hertz model are shown in **Supplementary Fig. 8**.

Supplementary Note 7: Brillouin-mechanical validation in polyacrylamide gels.

To further understand and validate the relationship between Brillouin-derived high-frequency longitudinal modulus and quasi-static traditional Young's or shear moduli, we have developed polyacrylamide (PA) gels of varying water content (5% to 12% polymer wt%) and varying bis-acrylamide concentrations (0.01% to 0.3% polymer wt%) to cover a range of substrate stiffness between ~600 Pa and 20 kPa that are generally used as a cell substrates¹². Using these gels we have compared longitudinal modulus as measured with our Brillouin apparatus with shear modulus measured with a rheometer. Gels were generally created in 6-well plates; after gel polymerization the well-plates were placed under the Brillouin microscope and Brillouin shift values were recorded at different locations. High-frequency longitudinal moduli were computed from the Brillouin shift values using values of mass density and index of refraction computed from the composition of the gels. Then, a biopsy punch was used to extract disks (typically 3 disks per gel composition of 4 mm diameter, 2-5 mm height) from the polyacrylamide gels. To obtain the shear modulus, the PA disks were measured with an AR-G2 stress-controlled rheometer (TA Instruments) with 8-mm-diameter parallel plate geometry. We used 100 μm pre-compression, performed frequency sweeps from 0.1 to 10 Hz with 0.1% strain amplitude at 23°C and used the 1 Hz values for comparison with Brillouin. High correlation ($R^2 > 0.94$) was obtained in curve fit to a log-log linear relationship: $\log(M) = a \log(G') + b$, where the fitting parameters were $a=0.023$ and $b=9.27$ (**Supplementary Fig. 9**). Within this range of water content and crosslinker formulations, the effect of changing the water content within the gels or

increasing number of crosslinks leads to similar correlations between high-frequency longitudinal modulus and quasi-static shear modulus, thus demonstrating that our method is sensitive to changes in polymer concentration and crosslinking.

Supplementary Note 8: Brillouin shift of cells at different plating times

After plating cells into a culture dish, cells undergo a slow process that starts with attachment onto the substrate and ends with the cells fully spread. During this time, cells progressively change their morphology from round in suspension to stretched onto a substrate. In this process, the projected cell area grows and the actin cytoskeleton polymerizes. To investigate the cell internal stiffness as a function of time from plating, we plated NIH 3T3 fibroblasts onto polyacrylamide gels (12%) functionalized with collagen (see **Online Methods**). We measured several conditions starting at 8 hours after plating. We chose this time point because at earlier time points strong variations in the nucleus to cytoplasm volume may bias the measurements. A highly statistically significant difference (unpaired t-test, $p < 0.001$) was observed between the 8-hour and 12-hour conditions (**Supplementary Fig. 11**), indicating that as cells grow and stabilize their cytoskeleton, also their internal stiffness increases, as one would expect. For comparison we also show the average value on cells plated by more than 18 hours and 24 hours, which indicates that after 12 hours the cells are fully spread and cell stiffness reaches a plateau value.

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