CELLSENSE TECHNOLOGIES

ApplicationNote

Live cells · Organoids · Spheroids 3D cell culture · Organisms and Tissues · Plant science · Biomaterials · hydrogels soft materials · Other cool topics

3D mapping of Softwell® hydrogel wellplates from Matrigen with defined Elastic Modulus by Brillouin Microscopy

Key Question

Can mechanically defined hydrogels be distinguished non-invasively and in three dimensions under biologically relevant conditions?

Why This Question Matters

Hydrogels with defined stiffness are widely used in cell biology and mechanobiology to mimic the mechanical properties of biological tissues. By tuning the elastic modulus of the substrate, researchers can study how cells respond to changes in their mechanical environment, for example during differentiation, migration, or disease progression.

For such experiments, it is important that the mechanical properties of the hydrogel are well defined, reproducible, and stable, and that they can be accessed non-invasively in the same format used for imaging and cell culture.

Why Other Methods Fall Short for This Application

Method	Limitation for this question
Bulk rheology	Provides macroscopic reference values but lacks spatial resolution and does not capture depth-resolved variation in standard imaging formats.
Indentation / contact probing	Contact-based and typically surface-biased; integration with imaging workflows can be limited and measurement can perturb soft hydrated materials.
Indirect markers / reporters	Often provide proxy readouts and can be labeling- and model-dependent; do not directly measure mechanical properties of the gel volume.

Materials and Methods

Hydrogel samples

Brillouin measurements were performed on commercially available hydrogels with defined nominal elastic moduli, spanning a range from very soft to relatively stiff substrates. The hydrogels were bound to glass-bottom well plates and covered with buffer.

Imaging configuration

Measurements were carried out in a standard inverted microscope geometry, probing through the glass substrate. For each hydrogel, vertical Brillouin images were acquired across the full thickness of the gel.

Region-of-interest selection and averaging

To avoid interface-related effects, regions close to the glass-gel and gel-buffer boundaries were excluded from the analysis. Brillouin shifts were averaged over the remaining gel volume to obtain representative values for each stiffness condition.

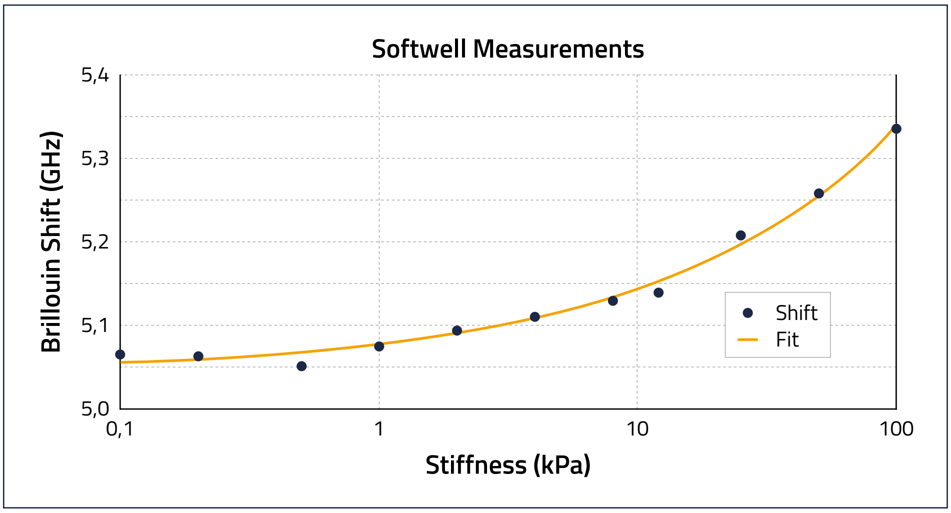

Figure 1

Brillouin shift data for Softwell gels with different nominal stiffness. The fit line follows a square root law and can be used to determine the stiffness of unknown gels.

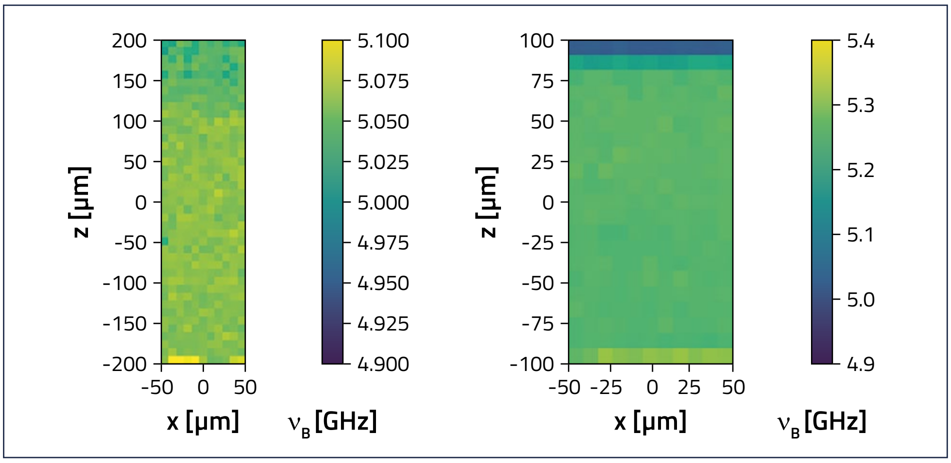

Figure 2

Brillouin images of two different gels.

Bottom/bright: glass substrate

Top/dark: PBS buffer

Note the different scales for the Brillouin shift – the right image represents a higher stiffness

Results and Discussion

Across the full stiffness range investigated, the Brillouin frequency shift increased monotonically with the nominal elastic modulus specified for the hydrogels. Despite the fact that Brillouin microscopy probes the high-frequency longitudinal response rather than Young's modulus directly, the correspondence between Brillouin shift and nominal stiffness was highly reproducible and predictable.

This behaviour is consistent with expectations for hydrated polymer networks, where changes in shear elasticity lead to measurable, though sublinear, changes in the longitudinal mechanical response[1-3]. Importantly, the observed trend was stable across repeated measurements and accessible in a fully non-contact manner.

Interpretation and Relevance

These results demonstrate that Brillouin microscopy can reliably distinguish hydrogels of different nominal stiffness, even when the mechanical contrast arises from relatively small changes in the nominal elastic modulus compared to the dominant bulk response of hydrated materials[2-4].

- Non-invasive quality control of mechanically defined cell culture substrates.
- Depth-resolved access to mechanical contrast in three dimensions, beyond surface-only measurements.
- A mechanical contrast channel that complements optical and fluorescence imaging workflows.

At the same time, Brillouin measurements should be interpreted as a relative and material-specific mechanical contrast, not as a direct readout of Young's modulus[2,3].

Key Takeaways

- Brillouin microscopy reproducibly distinguishes hydrogels with different nominal elastic moduli.
- Brillouin frequency shift shows a predictable, monotonic dependence on specified stiffness values.
- Measurements probe the high-frequency longitudinal mechanical response; interpretation is material-specific.
- The method enables non-contact, depth-resolved mechanical characterization of soft hydrogels.

References

1. Scarcelli, G. & Yun, S. H.
   Confocal Brillouin microscopy for three-dimensional mechanical imaging.
   Nature Photonics 2, 39–43 (2008)
2. Prevedel, R., Diz-Muñoz, A., Ruocco, G. & Antonacci, G.
   Brillouin microscopy: an emerging tool for mechanobiology.
   Nature Methods 16, 969–977 (2019)
3. Wu, P.-J. et al.
   Brillouin microscopy, what is it really measuring?
   arXiv:1711.03312 (2017; revised 2018)
4. Antonacci, G. et al.
   Recent progress and current opinions in Brillouin microscopy for life science applications. Biophysical Reviews 12, 615–624 (2020)
5 Mahmodi, H., Piloni, A., Utama, R. H. & Kabakova, I.
   Mechanical mapping of bioprinted hydrogel models by Brillouin microscopy.
   Bioprinting 23, e00151 (2021)

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