# Brillouin Microscopy: A Tool for 3D Imaging in Mechanobiology

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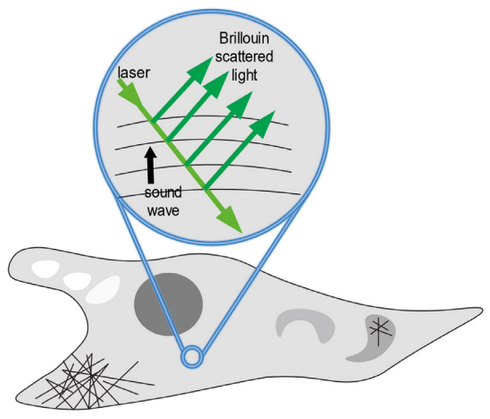
## Technology Overview

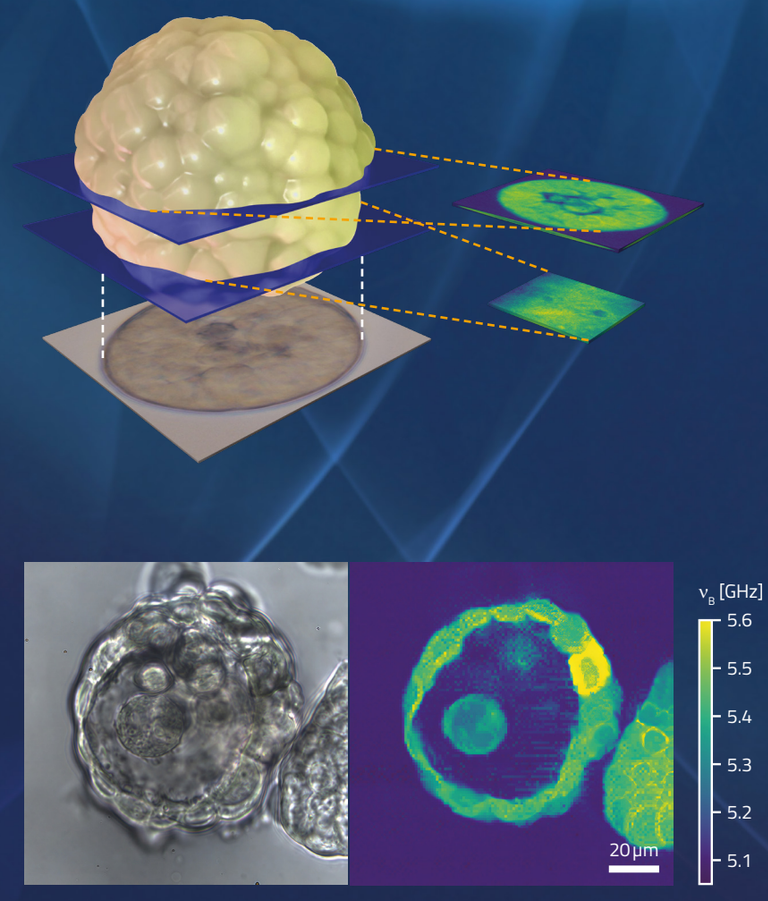
Brillouin microscopy allows non-destructive, label-free and contact-free probing of mechanical properties of biological samples in 3D (volume) by means of light.

1. Laser light scans sample and get scattered by thermally induced acoustic waves (thermal fluctuations of density) and undergoes a frequency shift – so called Brillouin-scattering.
2. Spectrum of Brillouin-scattered light is measured and analyzed for every point of the sample.
3. Characteristics of Brillouin spectra, like frequency shift due to scattering, are assigned to each point, forming a 3D-map of the sample.
4. Bigger frequency shifts corresponds to a larger longitudinal modulus of the probed volume, which can be interpreted as being mechanically 'stiffer'.

In biological workflows, Brillouin microscopy adds a non-invasive mechanical readout to existing imaging methods. It is especially useful for comparing mechanical phenotypes between biological conditions, such as control vs. treatment, healthy vs. diseased, or different cell states.




Principle of Brillouin microscopy on a cell (courtesy Prevedel lab EMBL Heidelberg)

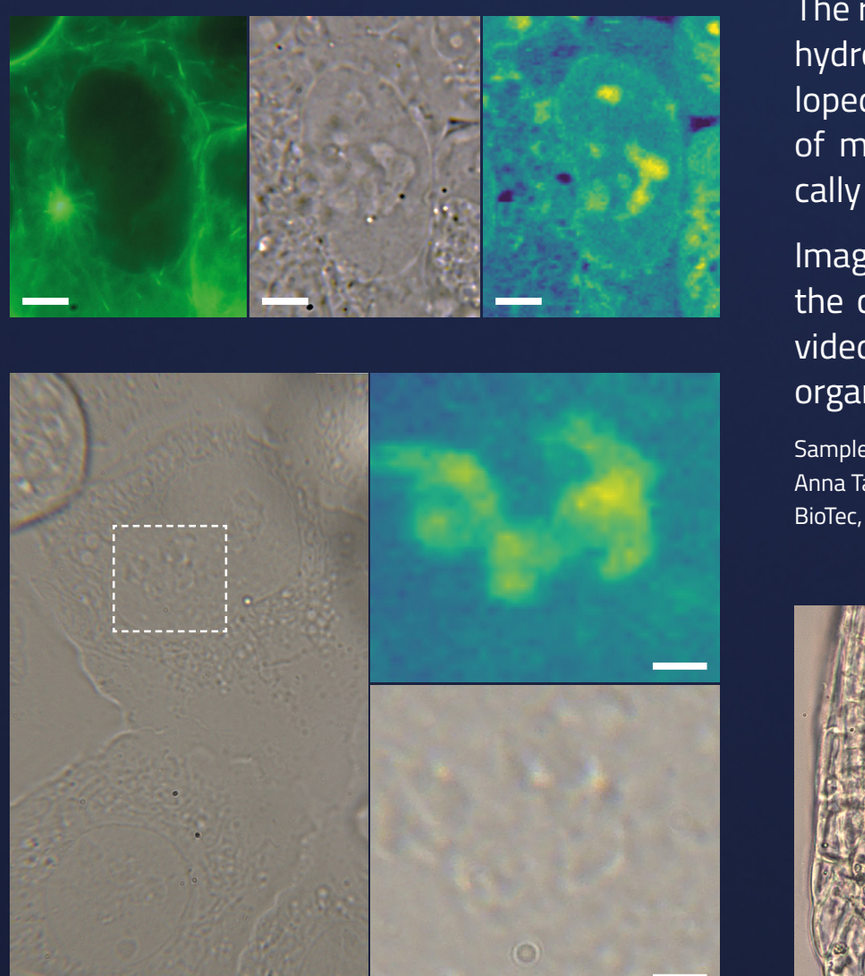


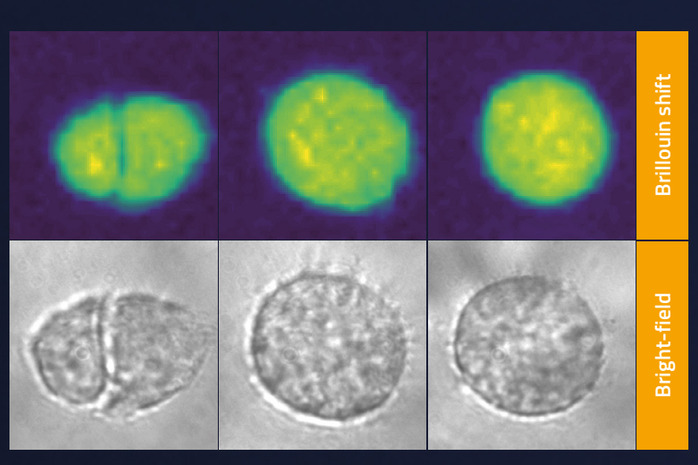

MCF-7 cells were cultured in a hydrogel for 12 days. Since the cells expressed a cell cycle reporter (FUCCI), their progression through the cell cycle could be tracked in real time.




Bright-field image and Brillouin microscopy scan of a live human neuromuscular organoid from a pluripotent stem cell derived neuromesodermal progenitor population. The left part of the neuromuscular organoid, as it is shown in the bright-field image on the top panel, corresponds to the tissue predominately consisting of neurons, while the right part of the organoid mainly consists of the muscular cells. The Brillouin microscopy image as a map of the Brillouin frequency shifts was acquired across the whole organoid spanning both types of tissues in a single scan (see bottom panel).

Sample courtesy: Dr. Mina Gouti, the principal investigator in the "Stem Cell Modeling of Development & Disease" group, Max Delbrück Center for Molecular Medicine, Berlin, Germany





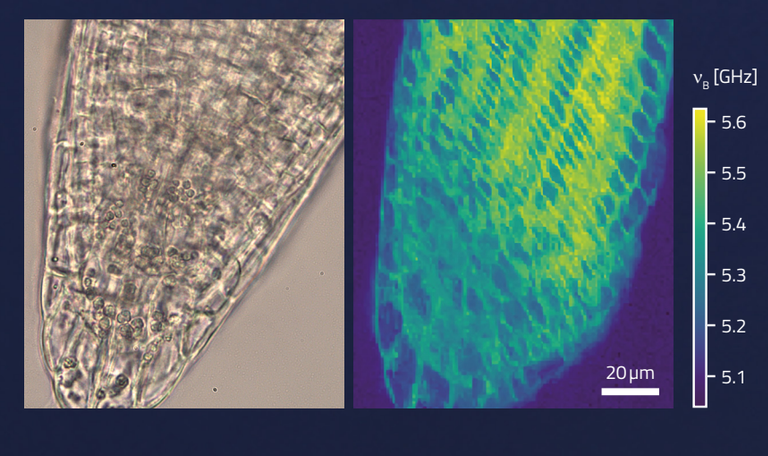

Brillouin microscopy and brightfield images of mesenchymal stromal cells in a hydrogel matrix. The upper panel shows maps of the Brillouin frequency shift across cells, while the lower panel contains corresponding colocalized bright-field images. For the measurements, each hydrogel matrix sample containing cells was placed in a glass-bottom Petri dish and maintained at 37 °C using an on-stage incubator.

Sample courtesy: Prof. Dr. Georg Duda, Director Julius Wolff Institute, Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, Germany

Examples with U2OS cells illustrate how the technique complements brightfield and fluorescence channels to reveal intracellular detail. In the first set of images, a single cell is shown in three modalities: fluorescence (left), brightfield (middle), and Brillouin microscopy (right). The Brillouin map highlights differences in intracellular mechanics, with regions of higher water content appearing blue and stiffer regions rendered in yellow. The map is displayed on a frequency shift range of 5.1-5.4 GHz (scale bar 5 µm).

The second presents a brightfield view of U2OS cells with a magnified region (white box) of the nucleus. While conventional techniques provide structural information, the Brillouin map resolves subcellular features that are not readily visible otherwise (scale bar of 2 µm).

Sample courtesy: Dr. Marketa Schmidt Cernohorska, Centre for Nanomaterials and Biotechnology, Faculty of Science, Jan Evangelista Purkyne University (UJEP), Usti nad Labem, Czech Republic




The researchers observed that in compliant hydrogels (1-2 kPa), some spheroids developed a central lumen—a structural feature of mammary morphogenesis that is typically lost in tumorigenesis.

Imaging at multiple depths, both through the center and along the outer edge, provided a detailed 3D representation of a live organoid.

Sample courtesy:
Anna Taubenberger, Oncomechanics Group,
BioTec, Dresden University of Technology

The Arabidopsis thaliana calyptra (root cap) comprises multiple specialized tissues, including columella cells involved in gravity sensing, lateral root cap cells that protect the growing root, and adjacent meristematic tissues responsible for continuous root growth.

Brillouin microscopy reveals spatially heterogeneous mechanical contrast across these structures, reflecting differences in cellular organization, wall architecture, and local physiological state within the living root tip.




## Talk to me!

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