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Line-scanning speeds up Brillouin microscopy

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Two new Brillouin microscopes leverage line-scanning to overcome previous limitations of the technique, enabling fast imaging, with low phototoxicity, of mechanical properties in living embryos of model organisms and tumor spheroids.

A growing body of evidence has shown the importance of cell and tissue mechanical properties and their interplay with the environment. Mechanical forces can affect several cellular properties, including proliferation, shape, differentiation, apoptosis and sorting. Furthermore, aberrant mechanical properties or an impaired mechanoresponse are associated with several diseases[1], including cancer, as well as aging[2]. Our ability to measure the mechanical properties of intact cells and tissues in situ is therefore not only key to understanding tissue morphogenesis and homeostasis but could also offer a tool for early diagnosis of disease. Several techniques have been developed to probe the mechanical properties of cells and tissues; however, most of them require invasive manipulation to the sample (for example, micropipette aspiration, tissue indentation, injections of beads), to which the cells can react, complicating interpretation of experiments. In this issue of *Nature Methods*, two teams propose line-scanning Brillouin microscopes for in situ mechanical characterization of live tissues[3,4].

Brillouin microscopy is a non-invasive, contact- and label-free technique that is based on the concept of Brillouin scattering, the inelastic scattering of light by the material's intrinsic thermal fluctuations (Fig. 1a). Since its theoretical prediction more than a century ago[5,6] and subsequent experimental observation[7], analysis of Brillouin scattered spectra has become a potent technique for the mechanical characterization of condensed matter[8]. The frequency shift between the illumination and scattered light — that is, the Brillouin shift — is dependent on the speed of light propagation in the material, which is itself related to a material's elastic properties (M') (Fig. 1b,c). Furthermore, the width of the Brillouin spectrum is related to a material's viscous properties (M''). Together, this allows the characterization

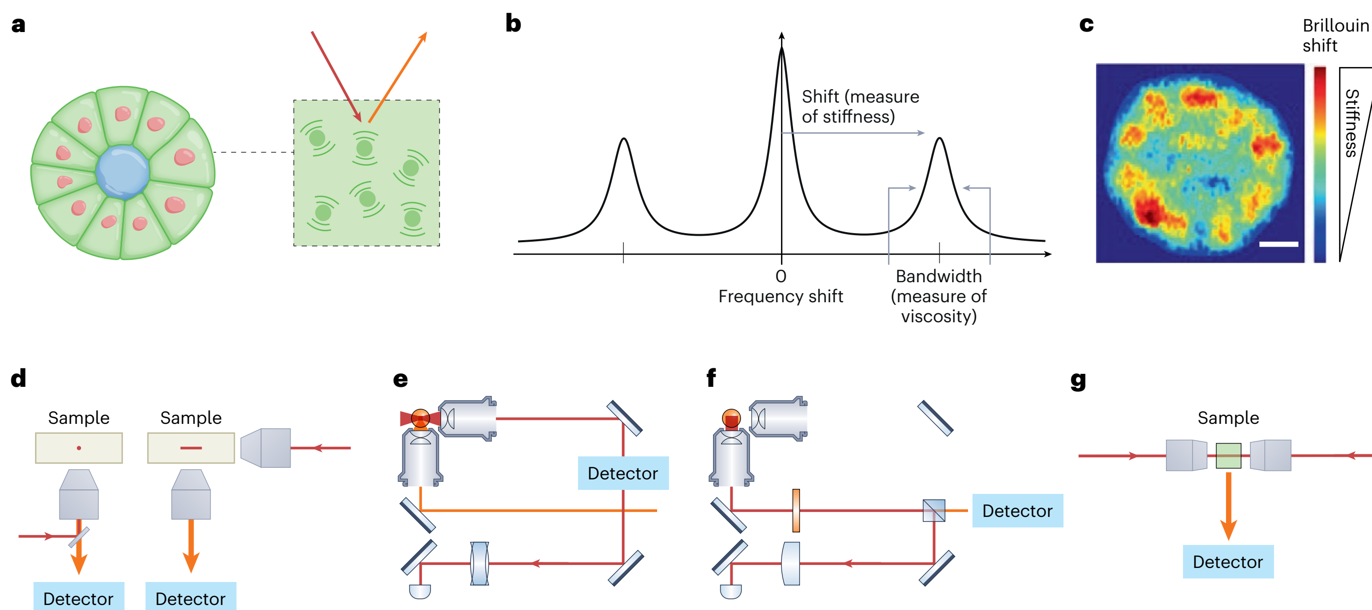

Fig. 1 | Principle of line-scanning Brillouin microscopy. a, A schematic of a spheroid. Cytoplasm is shown in green and nuclei in red. The blue region in the middle is the lumen. Illumination light (red arrow) interacts with the material's thermal fluctuations and gets scattered with a different wavelength (orange arrow). **b**, The frequency spectrum of the light scattered from material. Elastically scattered light has the same wavelength as the illumination light and therefore shows no frequency shift (highest peak). The two other peaks show the inelastic Brillouin-scattered light. The frequency shift (Brillouin shift) is related to the elastic mechanical properties of the material, with stiffer material exhibiting higher frequency shift. The line width is related to the viscous properties of the material. **c**, Example map of the Brillouin shift of the schematic spheroid. Stiffer regions such as nuclei exhibit higher Brillouin shift. **d**, Principle of multiplexing by line scanning. **e**,**f**, Illumination configuration of the orthogonal (**e**) and epi (**f**) configuration of the LSBM setup used by Bevilacqua et al.[3]. **g**, Illumination configuration of the dLSBM setup used by Zhang et al.[4]. (Panels **a** (right) and **b** adapted with permission from ref. 9, Springer Nature. Panels **c**,**g** adapted with permission from ref. 4, Springer Nature. Panel **d** adapted with permission from ref. 12, Springer Nature. Panels **e**,**f** adapted with permission from ref. 3, Springer Nature.)

of a material's complex longitudinal modulus $M = M' + iM''$, which is on the order of gigahertz. The difference in the range of frequencies probed with Brillouin microscopy (gigahertz, versus hertz to kilohertz for atomic force microscopy), together with the definition of the longitudinal modulus, which is different from (but related to) the more commonly used Young's modulus, means that the measures of elasticity obtained by Brillouin microscopy (gigapascal) are much higher than those obtained by other techniques (kilopascal).

Despite its growing use in biological studies[9,10], the slow acquisition speed (typically more than tens of minutes for a single 2D snapshot) and consequent phototoxicity of the method has prevented its application, especially for more dynamic and/or photosensitive biological processes. Bevilacqua et al.[3] and Zhang et al.[4] present line-scanning Brillouin microscopes that address these limitations and expand the scope of applicability of this technique. Both setups leverage multiplexing via line-scanning (Fig. 1d), which allows faster acquisition times than in point-scanning techniques, and a near infrared laser to reduce phototoxicity.

Bevilacqua et al. present a line-scan Brillouin microscope (LSBM) with two different configurations for imaging: the orthogonal-line LSBM (O-LSBM) uses two separate objectives positioned at 90 degrees for illumination and detection while the epi-line LSBM (E-LSBM) uses a single objective to illuminate the sample with a focused line and subsequently detect the 180° backscattered signal (Fig. 1e,f). The former method has the least photodamage and best axial resolution, and can therefore be used for more sensitive or larger samples with longer total illumination times. However, due to the 90-degree positioning of the two objectives, the sample needs to be accessible from both sides. The E-LSBM does not have this requirement, and minimizes the effects of scattering and optical aberrations. An LSBM can also be combined with selective plane illumination microscopy (SPIM) to acquire fluorescence images of the sample. In addition, the authors also developed a GPU-accelerated numerical fitting routine for real-time analysis and visualization of data. The LSBM requires as little as ~10 s per 2D image slice (1 ms per pixel), and the authors used the setup to image mechanical properties of the highly dynamic *Drosophila melanogaster* gastrulation over ~30 mins, as well as slower developmental processes in *Phallusia mammillata* and mouse embryos over 14 and 46 hours, respectively.

Zhang et al. implement line scanning in a dual-line illumination configuration (dLSBM) with orthogonal collection (Fig. 1g), which, coupled with an image fusion algorithm, reduces artifacts caused by heterogeneous refractive index distribution in thick samples. The authors used dLSBM to perform 3D Brillouin mapping of living spheroids and show that their technique substantially reduces the light dose and photodamage compared to those in standard confocal Brillouin microscopy, improving sample viability and proliferation. dLSBM can acquire a 2D image of a spheroid in 20 seconds (1 ms per pixel), whereas the same image takes 16 minutes on a conventional Brillouin scanning microscope. 3D scanning is achieved by rotating the spheroid in the *y*–*z* plane and can be performed in less than 4 min, which is unprecedented relative to previous Brillouin setups. This faster acquisition time enabled the authors to capture dynamic processes in living tissues, which they demonstrate by imaging the mechanical responses of tumor spheroids to external perturbations such as osmotic shocks.

These breakthroughs in the development of Brillouin microscopy substantially expand its range of applicability to mechanical measurements in live 3D tissues and organisms, making it an attractive tool for the fields of cell and developmental biology and beyond. In addition, many biomechanical studies have focused on characterization of the material's elastic properties using Young's modulus as its measure. However, recent studies are highlighting the importance of viscous properties in different biological processes[11]. Brillouin microscopy can simultaneously measure the elastic and viscous properties of biological samples and is therefore a useful tool for mechanical characterization of tissues in development and homeostasis. In medicine, with more evidence showing the importance of mechanical changes of tissues in various diseases, the method could potentially also be used as a tool for early disease diagnosis.

Looking ahead, several challenges remain to be addressed before Brillouin microscopy becomes a routine tool for the biomechanical characterization of biological samples. The difference between the longitudinal modulus (measured by Brillouin microscopy) and Young's modulus has so far kept Brillouin microscopy studies separate from those obtained via other techniques. Thus, more systematic studies are needed to better understand the longitudinal modulus, determine its origins in biological samples and cross-compare it to elasticity measurements obtained with other techniques. This would facilitate the biological interpretation of the results and provide a more thorough understanding of the mechanical properties of biological tissues. It will also be crucial to further develop the technology so that large fields of view can be imaged at greater depths, expanding the set of samples for which Brillouin microscopy will be useful. Finally, another challenge will be to make these improved microscopes user-friendly and accessible to the wider research community, perhaps through international advanced imaging centers or open-access microscopy projects such as OpenSPIM.

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Competing interests

The authors declare no competing interests.