BRILLOUIN MICROSCOPY FOR CELL AND TISSUE BIOMECHANICS

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Abstract: We present Brillouin microscopy, an all-optical imaging modality to map mechanical properties of material based on Brillouin light scattering. Brillouin microscopy has been used to evaluate ocular tissue biomechanics as well as to characterize cell and nucleus stiffness.Conventional tests to characterize material mechanical properties are widely used and well established but are generally invasive and based on contacting the sample; thus they are difficult to apply in biomedical settings *in vivo* and/or *in situ*. The ability to measure the mechanical properties of biological tissue and biomaterials *in vivo* would have a significant impact in many areas of biomedical research and clinical application. At the cellular scale, the biomechanical component of the interaction between cells and their local microenvironment has emerged as a crucial regulator of cellular function. However, current instruments to assess cell elasticity, including atomic force microscopy and microrheology, require contact or are limited to the analysis of few points at random locations. As a result, it is difficult to map cellular elasticity when cells are not physically accessible such as in microfluidics chips or in 3D microenvironments.An intriguing solution to this challenge comes from Brillouin light scattering where, thanks to the interaction of light with intrinsic mechanical vibrations (phonons) of material, the mechanical information can be read out optically via the spectral analysis of the scattered light. Brillouin spectroscopy has long been employed as a non-contact method for mechanical testing[1] but its poor temporal resolution limited the analysis to few points per sample.In the past years, by developing a spectrometer with several orders of magnitude improved throughput and extinction[2, 3], we created a Brillouin imaging technology, where contrast is based on elastic modulus[4, 5], and we demonstrated its application *in vivo* at the tissue level[6, 7] and recently at the cell level[8].

At the tissue level, we have focused on ocular tissue biomechanics[9-12], in particular to a corneal ectatic disease, named keratoconus. Keratoconus is the most common corneal degeneration in the US. It is an ectatic disorder characterized by the progressive thinning and bulging of the cornea, which assumes the shape of a cone. Corneal biomechanics is central in the development of the disease. In a healthy cornea, collagen fibers provide the strength to balance the intraocular pressure (IOP); if the cornea is weakened or compromised, this mechanical balance is disrupted resulting in progressive thinning and bulging. Therefore the biomechanical characterization of the cornea is crucial to understand the progression of keratoconus and thus improve its early diagnosis and management. Recently, we have been able to use Brillouin microscopy to differentiate keratoconic corneas from normal corneas[11, 13]. Brillouin images show remarkable biomechanical features distinctively different between corneas of healthy subjects and keretoconic corneas. Overall, keratoconic corneas presented lower elastic modulus than healthy corneas. Importantly, Brillouin imaging showed that the mechanical loss is primarily concentrated within the area of the keratoconic cone. Outside the cone, the Brillouin shift was comparable to that of normal corneas. Our results demonstrate the potential of Brillouin microscopy for diagnosis and treatment monitoring of keratoconus.Beyond tissue applications, we recently developed high-resolution Brillouin microscopy to map intracellular elasticity. Transitioning to the cell level required >100x reduction in scattering volume and >10x higher elasticity sensitivity compared to tissue-level instruments. This was achieved through increased spectrometer sensitivity and extinction as well as with efficient collection of Brillouin scattering at high resolution. Here, we show that Brillouin cellular microscopy is able to map the longitudinal elastic modulus with sub-cellular three-dimensional resolution in a non-contact and non-invasive fashion. We also validated Brillouin microscopy against gold-standard mechanical tests and known elasticity features of cells thus showing that the novel technology is sensitive to the relevant mechanical changes occurring within a cell.

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