



Brillouin microscopy: assessing ocular tissue biomechanics

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Purpose of review

Assessment of corneal biomechanics has been an unmet clinical need in ophthalmology for many years. Many researchers and clinicians have identified corneal biomechanics as source of variability in refractive procedures and one of the main factors in keratoconus. However, it has been difficult to accurately characterize corneal biomechanics in patients. The recent development of Brillouin light scattering microscopy heightens the promise of bringing biomechanics into the clinic. The aim of this review is to overview the progress and discuss prospective applications of this new technology.

Recent findings

Brillouin microscopy uses a low-power near-infrared laser beam to determine longitudinal modulus or mechanical compressibility of tissue by analyzing the return signal spectrum. Human clinical studies have demonstrated significant difference in the elastic properties of normal corneas versus corneas diagnosed with mild and severe keratoconus. Clinical data have also shown biomechanical changes after corneal cross-linking treatment of keratoconus patients. Brillouin measurements of the crystalline lens and sclera have also been demonstrated.

Summary

Brillouin microscopy is a promising technology under commercial development at present. The technique enables physicians to characterize the biomechanical properties of ocular tissues.

Keywords

biomechanics, Brillouin spectroscopy, cornea, keratoconus, refractive surgery

INTRODUCTION

Multiple techniques have emerged in recent years for measuring corneal biomechanics. The most common approach uses an air puff and analyzes corneal deformability against the external pressure using tonometry, topography, or optical coherence tomography (OCT). While providing clinically useful information, the output of these devices is influenced by intraocular pressure (IOP) and geometric properties of the cornea. Extracting corneal biomechanical data from such measurements relies on multiple assumptions and modeling. Another drawback of these techniques is that they do not provide spatial aspect of tissue strength as it may vary across stromal thickness, from center to periphery, and at the focal point of disease.

The study discusses a novel optical technique – Brillouin microscopy – that measures spectral shift of a probing laser beam as it scatters from a localized volume of tissue. Brillouin spectral shift has direct relationship to longitudinal modulus or mechanical compressibility of tissue. Using confocal imaging it can produce a full volumetric map of the elastic

properties. We discuss latest clinical evaluation of the technique in human studies conducted under Institutional Review Board-approved protocols.

BACKGROUND

As corneal refractive surgery had become widespread around the world, researchers and clinicians became more interested in biomechanics of the

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KEY POINTS

- Brillouin microscopy allows full-volume mapping of longitudinal elastic modulus of cornea.
- Brillouin microscopy can be used for screening and monitoring of keratoconus progression.
- Brillouin microscopy can be used for assessment of corneal cross-linking procedure effectiveness including the spatial map of tissue changes and remodeling.
- Future clinical applications of Brillouin microscopy extend into refractive surgery.
- Additional ophthalmic applications of Brillouin microscopy include measurements of crystalline lens and sclera

stroma [1]. Even with very precise modern-day excimer lasers, variability of tissue strength has been implicated in suboptimal outcomes of laser-assisted in situ keratomileusis (LASIK) and other procedures [2]. Significant differences in corneal strength have been noted between normal eyes, eyes after refractive surgery, and also eyes with keratoconus [3].

Today there are two instruments that provide some insight into corneal biomechanics: the ocular response analyzer (ORA) from Reichert and the Corvis ST from Oculus. Both are based on measurement of dynamic corneal response to a puff of air. The ORA device provides a corneal hysteresis measurement, which has been shown to correlate with known corneal pathologies such as keratoconus [4,5]. Although useful in some applications [6], corneal hysteresis values can be produced by various combinations of corneal thickness, rigidity, intraocular pressure, and hydration, and therefore do not truly represent corneal biomechanical properties. More recently available Corvis ST device provides more detailed information about corneal response by using high-speed Scheimpflug imaging. It provides a so called Corneal Biomechanical Index (CBI) which includes corneal thickness information. CBI has been shown to improve keratoconus detectability [7], and in combination with tomographic data in corneal ectasia detection [8[¶]]. Ongoing research may discover additional parameters [9]. Both ORA and Corvis ST devices have shown clinical usefulness, but do not provide direct measurement of corneal elasticity and do not allow three-dimensional mapping of these properties of stroma. The mapping of localized corneal properties has important clinical benefits. First, it allows localization of corneal weakening, such as in case of early keratoconus, which produces focal region of increased elasticity [10]. Second, it allows depth-dependent

mapping of corneal strength important in refractive surgery [11] and monitoring effectiveness of corneal cross-linking treatments [12,13].

Some ex-vivo methods have been used to address this gap, but in-vivo imaging has been difficult. Ultrasound surface wave elastometry has been used for measurements in donor corneas [14], and recently *in vivo* [15]. OCT-based elastography has been attempted to measure variation in biomechanical strength between middle and posterior stroma [16]. Although promising, the methods require a complex finite element model of the cornea to deduce actual biomechanical moduli from available data. Other OCT elastography techniques that measure acoustic surface waves are under development in preclinical stages [17,18].

BRILLOUIN TECHNIQUE

The principle of Brillouin microscopy is illustrated in Fig. 1. Brillouin light scattering occurs by the interaction of light and intrinsic acoustic waves in tissues. The acoustic waves are naturally present in tissue. They originate from thermal fluctuations of molecules, which generate pressure fluctuations that propagate at the speed of sound (~ 1620 m/s in corneal stroma). The acoustic waves modulate the refractive index periodically, and when light is reflected from this modulation, a Doppler frequency shift occurs. The frequency shift is proportional to

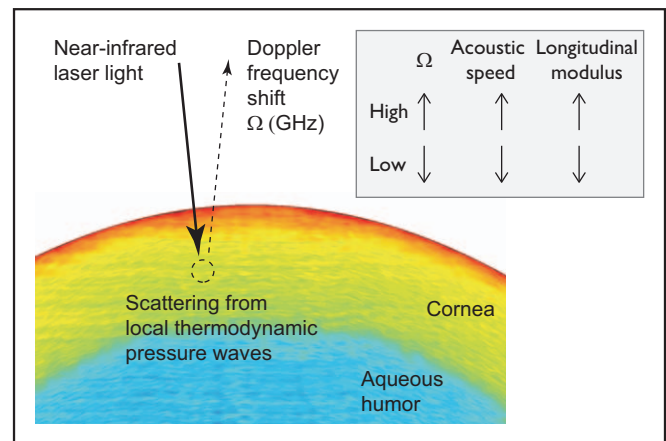


FIGURE 1. The principle of Brillouin microscopy. A low-power, narrowband NIR laser light is focused into the corneal tissue, and the Doppler Brillouin frequency shift of scattered light from the focus is analyzed by a confocal spectrometer. Spontaneous Brillouin scattering originates from thermodynamically induced pressure (acoustic) waves. The magnitude of Brillouin frequency is proportional to the acoustic propagation speed of tissue at the focus and provides a direct measurement of local longitudinal modulus of the tissue. NIR, near-infrared.

the speed of sound (Brillouin frequency = $2 \times$ acoustic speed/wavelength of light in the tissue). The acoustic speed is proportional to the square of longitudinal modulus. Longitudinal modulus is defined as the magnitude of hydrostatic pressure required to cause a fractional volume change and is approximately the inverse of the compressibility. Brillouin microscopy uses a low-power, focused laser beam and a high-resolution confocal spectrometer to measure the Brillouin frequency at the focus [19]. By scanning the beam, the spatial variation of longitudinal modulus of tissue is mapped.

A compelling advantage of Brillouin microscopy is that it provides a direct readout of the local tissue property [20]. In the sense that the primary output of Brillouin microscopy is the acoustic speed information, this technique is analogous to high-frequency ultrasound microscopy [21]. However, as an all-optical technique, Brillouin microscopy does not require any physical contact, deposits no acoustic energy in the tissue, and provides microscopic three-dimensional resolution [22].

It should be noted that longitudinal modulus does not directly correlate with the elasticity or stiffness a physician can feel by touching the tissue. The latter mechanical property is better described in terms of shear (or Young's) modulus [23]. From the mechanical perspective, shear and longitudinal moduli are two independent elastic properties and have vastly different magnitudes in soft tissues. However, it is expected that during normal physiological changes or pathologic processes *in vivo*, these two moduli are more likely to change in the same direction. For example, the thickening (thinning) of collagen fibrils in cornea stroma would increase (decrease) both moduli. Nonetheless, caution is needed when relating longitudinal and shear moduli and interpreting Brillouin outputs.

CLINICAL APPLICATIONS

Ocular biomechanics plays significant role in diagnostic and treatment of eye diseases [24]. We overview the recent clinical results obtained with Brillouin microscopy and discuss several specific applications based on the biomechanical characterizations of the cornea, crystalline lens, and the sclera.

Baseline data of normal population

Currently available Brillouin microscopes use near-infrared (NIR) laser light at a wavelength of 780 nm [25]. At this wavelength, the Brillouin frequencies measured in normal human corneas fall in a tight range between 5.69 and 5.76 GHz, which

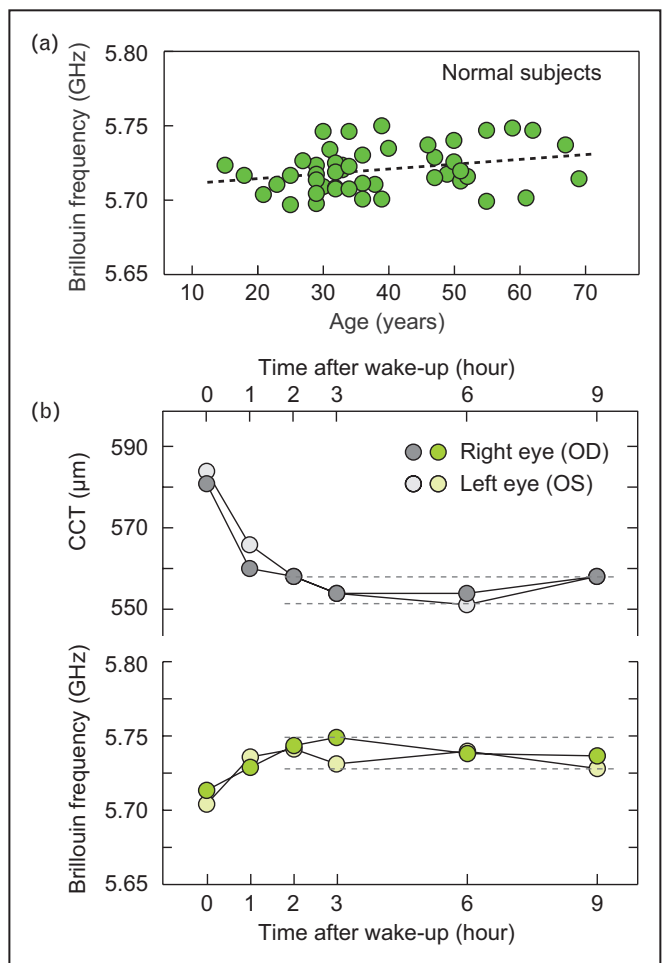


FIGURE 2. Brillouin measurements of normal population. (a) Central cornea Brillouin frequency values of normal individuals in an age range of 15–70. (b) Diurnal change of a healthy volunteer. Within 1 h after wake-up, the hydration level of corneal stroma is stabilized, so are the central corneal thickness (CCT) and Brillouin frequency. The magnitude of frequency variation during day time is less than the instrument's sensitivity of ± 10 MHz.

correspond to longitudinal modulus of 2.74–2.81 GPa (Fig. 2a).

Because the acoustic speed varies with water content in tissues [26], Brillouin frequency is sensitive to corneal hydration. This has been recently studied by measuring volunteers during daytime [27]. The time-lapse data showed the effects of hydration change in both corneal thickness and Brillouin frequency in the first 1 h after wake-up, but Brillouin frequency is stabilized after the transient period within instrument's sensitivity (Fig. 2b). The sensitivity of Brillouin frequency to hydration, not only the compressibility of collagen fibrils and extracellular matrix, must be considered in interpreting the underlying structural and molecular changes of tissue from the Brillouin frequency.

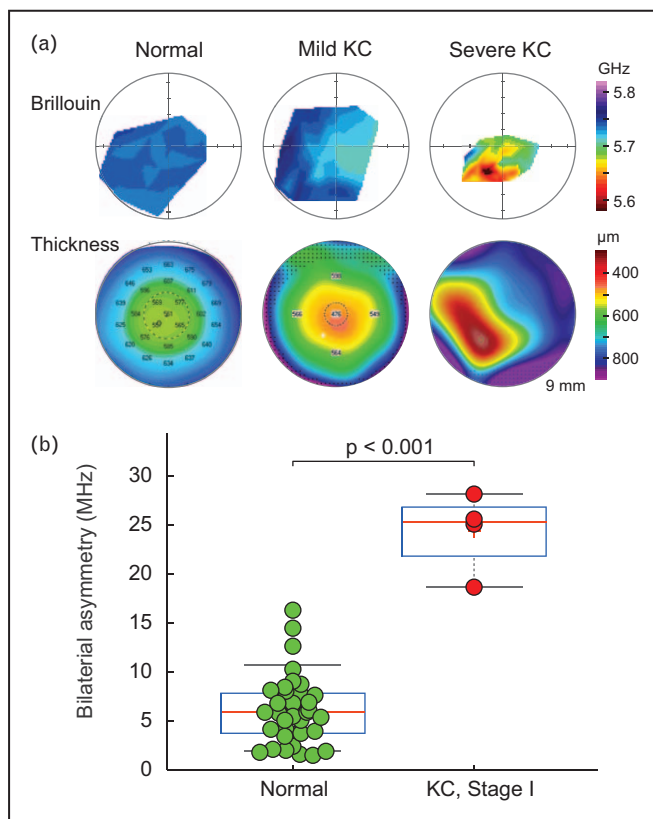


FIGURE 3. Brillouin measurement of keratoconus patients. (a) Brillouin maps and corresponding pachymetry maps by Pentacam (Oculus GmbH) of patients diagnosed with mild KC (stage 1; middle) and severe KC (right), in comparison to a normal subject (left). (b) The difference in Brillouin frequency between left and right eyes. The bilateral asymmetry is significantly higher in early-stage keratoconus.

Diagnosis and screening for corneal diseases

Due to focal nature of keratoconus, identifying local variations in elastic modulus may allow earlier detection of the disease before it manifests itself morphologically. Early clinical data show statistically significant differences between Brillouin measurements in the cone region versus in other corneal loci [28]. Distinct biomechanical features that differentiate keratoconus from normal corneas have been identified, and they may serve as diagnostic metrics for keratoconus detection in clinical practice [25^{***}]. There are three clinical findings. First, the average Brillouin frequency of keratoconus eyes is significantly lower when compared to the normal population, and the difference increases with the severity of the disease; second, the reduction of Brillouin frequency is most pronounced in the cone region, whereas the surrounding corneal tissue have normal Brillouin frequencies (Fig. 3a); and third, mild keratoconus is most clearly distinguished by comparing the difference of Brillouin frequency

between the left and right eyes (Fig. 3b). These results suggest that the biomechanical tissue properties are altered in keratoconic cones, and that the biomechanical bilateral asymmetry may serve as a diagnostic metric for detecting early-stage keratoconus and possibly identifying progressive keratoconus for early interventions. This expectation is supported by clinical experience that in many keratoconus patients, the severity of the disease is asymmetric [29].

Fuchs' dystrophy is associated the loss of endothelial cell function in water transport. The corneal thickness recovery in response to induced hydration control has been suggested as a test of endothelial function [30,31]. Brillouin microscopy may be used to measure abnormal hydration changes in patients with Fuchs' dystrophy and help monitor the progression of the disease.

Refractive procedure evaluation

Corneal screening has been a standard procedure for evaluating the eligibility of candidates for refractive surgery. Having detailed biomechanical information about the stroma may improve this screening process to decrease the risk of surgical complications. Postrefractive surgery measurements may also provide useful information about each procedure and compare residual biomechanical stability of the eye between different surgical options, such as LASIK, photorefractive keratectomy (PRK), or small incision lenticule extraction procedure (SMILE) [32]. Such comparison has been limited to theoretical models [33] or nonposition-specific evaluations [34]. Having noninvasive, spatially-resolved biomechanical mapping can improve current evaluation methods.

Personalized treatment planning potential

Several theoretical models using finite element corneal mesh have predicted differential responses of 'weak' and 'strong' corneas to the same refractive procedure [35,36]. Peripheral disruption of collagen fibers produces an in-axis flattening effect responsible for the refractive effects of astigmatic keratotomy [37]. Ablation of tissue with LASIK or PRK procedure alters the corneal shape beyond the actual material that is removed from the stroma [38]. To properly account for differential response of the cornea to the refractive procedure, models must be loaded not just with geometric and morphological stromal properties, but also with its local elastic properties [39–41].

By measuring longitudinal modulus and hydrostatic tissue properties, Brillouin microscopy may enable clinicians to create patient-specific nomograms for refractive procedures reducing outcome

variability and refractive outliers that may require secondary procedures (re-treatments). It has been shown that corneal hydration affects the excimer laser ablation rate in LASIK surgeries [42,43]. Biomechanical differences are thought to explain the variability in refractive outcomes following cataract and astigmatic keratotomy surgeries [44]. It may be possible to individually tailor ablation parameters or astigmatic keratotomy nomograms based on Brillouin measurements made prior to treatment.

Given the vastly different magnitudes and lack of a priori correlation between longitudinal and shear moduli, however, it is uncertain whether and how Brillouin data can be integrated into finite element modeling. Nevertheless, research effort is warranted to incorporate Brillouin data into patient-specific simulations of various refractive procedures to increase confidence in outcomes for both the patient and the surgeon.

Monitoring effectiveness of corneal cross-linking procedures

Corneal cross-linking (CXL) is one of the effective options to treat keratoconus and post-LASIK ectasia [45] by reinforcing the collagen and proteoglycans network in the corneal stroma. After ex-vivo studies [46,47,48], clinical Brillouin measurements of patients were conducted to evaluate biomechanical changes of corneal properties before and after CXL. The first clinical study on CXL patients has begun in collaboration with Dr Theo Seiler at IROC, Zurich, Switzerland, using a rack-mounted Brillouin system. Prior to the procedure, significant differences between keratoconus patients and controls were observed in the mean measurements of the Brillouin longitudinal modulus. After the procedure, the longitudinal moduli of the cross-linked corneas returned to the normal range [25]. Furthermore, measurement of a cohort of keratoconus patients who had received CXL at IROC in the past 4 years revealed a trend of increasing Brillouin frequencies after the treatment [49].

Measuring postoperative treatment penetration depth is difficult and imprecise with existing methods such as slit-lamp exam [12] or OCT [50]. Brillouin spectroscopy has the potential to more accurately demarcate treated zones. Having detailed preoperative assessment of corneal strength in keratoconus patient may also lead to improved treatment designs that are customized for a given individual [51,52] as opposed to a generic cross-linking protocol.

Characterization of the crystalline lens

In addition to corneal imaging, Brillouin microscopy can be used to evaluate the crystalline lens *in vivo*. Early studies have demonstrated feasibility of

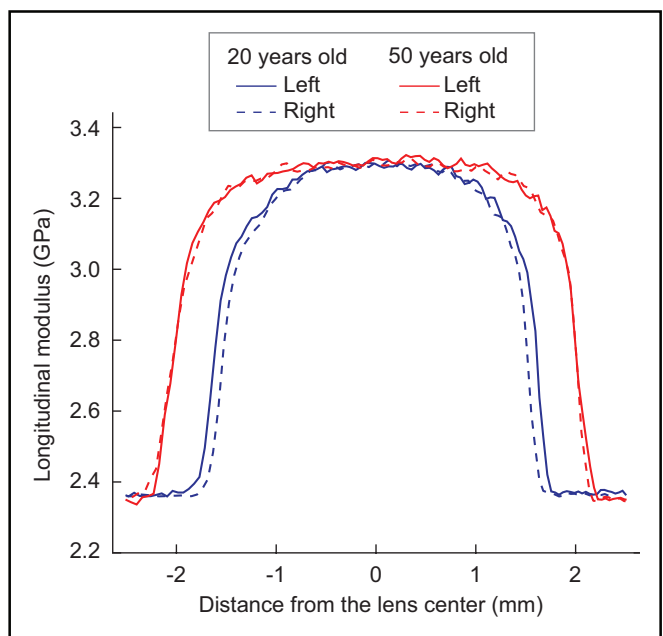


FIGURE 4. Brillouin axial profiles of the crystalline lens in two healthy volunteers with different ages.

the approach in animal models [53,54]. Brillouin data showed remarkable correlation to Young's modulus obtained by other conventional mechanical techniques. Recently, human measurements have shown positional changes in Brillouin elastic modulus that are consistent with known lens anatomy (nucleus and cortex) as well as age-related changes in the central region of the lens associated with growth [55]. Figure 4 shows typical axial Brillouin lens profiles for two subjects. Such data can prove useful in characterizing the crystalline lens for age-related presbyopia and optimization of surgical or pharmacological procedures to reduce its symptoms.

Characterization of the sclera

Brillouin spectroscopy measurements of sclera are more challenging due to highly scattering properties of this tissue. However, an optical method for improving the resolution and signal for scleral measurements was developed and tested *ex vivo* [56]. Using additional optical filtering allows for imaging of sclera and conjunctiva of porcine eyes. Scleral measurements may help with detecting and monitoring of myopia progression due to scleral weakening and pave the way to early prevention and treatments such as scleral cross-linking [57].

CONCLUSION

Brillouin microscopy provides safe and noncontact method for measuring longitudinal elastic modulus

of ocular tissues. It provides three-dimensional resolution of the imaged tissue revealing spatial variations in mechanical properties. Its clinical usefulness has already been demonstrated in corneal diagnostic and treatment monitoring and may eventually be a valuable tool for surgical planning of refractive and therapeutic procedures. Beyond corneal applications, crystalline lens imaging may help clinicians in assessing presbyopia and effectiveness of various treatments for restoring its elasticity; imaging of the sclera may determine predisposition to progressive myopia in patients and allow for earlier intervention before refractive error manifests itself.

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Conflicts of interest

S.Y. has patents on related technologies. D.C. is a full-time executive employee of Intelon Optics, Inc.

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