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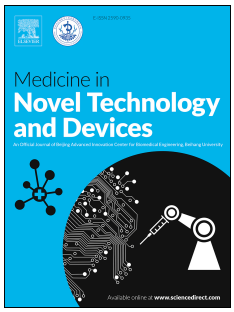

Review of In-vivo Characterisation of Corneal Biomechanics

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Review of In-vivo Characterisation of Corneal Biomechanics

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Abstract

The study of corneal biomechanics *in vivo* has been evolving fast in recent years. While an organised corneal structure is necessary for its transparency, resistance to occasional external insults and bearing the intraocular pressure (IOP), which several clinically relevant events can disturb. This review focuses on three techniques that are available for clinical use, namely the Ocular Response Analyzer (Reichert Ophthalmic Instruments, Buffalo, NY, USA), the Corvis ST (Oculus Optikgeräte GmbH, Wetzlar, Germany) and the Brillouin Optical Scattering System (Intelon Optics Inc., Lexington, MA, USA). The principles and the main parameters of each device are discussed along with their strategies to improve accuracy in the IOP measurement, corneal ectasia diagnosis, evaluation of corneal cross-linking procedures, and planning of corneal refractive surgeries.

1. Introduction

The cornea is a unique biological tissue that is responsible for clearly focusing the light rays onto the retina [1]. In order to do so, it has a delicately built aspheric shape that needs to be stiff enough to resist occasional external insults and bear the intraocular pressure (IOP) without losing its form or transparency. This is made possible by the cornea's histological structure, which confers the tissue's complex viscoelastic biomechanical properties [2] that play an important role in short-term elasticity as well as in long-term changes in stiffness like those caused by creep, stress-relaxation and changes in strain rate [3].

In respect to its structure, the cornea is composed of different layers, of which the stroma is the main load-carrying element [1], it accounts for 90% of the total tissue's thickness and is significantly stiffer than the other layers [4]. The stromal structure is highly organised, composed of approximately 200 superimposed lamellae [5], each containing parallel collagen fibrils immersed in an extracellular matrix replete with keratocytes, proteoglycans and glycoproteins [2]. Earlier research established collagen fibrils as the main load-carrying components [6] and noted a clear association between the corneal biomechanical behaviour and the content and distribution of the stromal fibrils [7].

However, the corneal structure can be disturbed by several clinically relevant events, which can be physiological as in the ageing process [8], pathological as in the development of ectatic diseases [9] or therapeutic as in corneal laser vision correction surgeries [10]. These events impact corneal biomechanical behaviour and can lead to changes in corneal geometry under IOP, and influence IOP measurement accuracy, impacting glaucoma management [11].

Corneal stiffness (or resistance to deformation under load) has been observed experimentally to increase with age [12]. Earlier studies reported stromal microstructure changes with age that were in line with increased stiffness, such as increases in the number of collagen fibrils [8], fibrillar cross-sectional areas and density of fibrillar cross-links [13], along with a decrease in inter-fibrillar spacing [14]. Clinical studies have reported similar age-related cornea stiffening. Young age has been identified as a risk factor for keratoconus and iatrogenic ectasia, conditions marked by a reduction in corneal stiffness [9, 15]. The age-dependent response was also observed in different corneal refractive surgical procedures (radial keratotomy, astigmatic keratotomy and LASIK), in which older patients were systematically over-corrected while younger patients were under-corrected [16-18].

The effects of age, diseases such as keratoconus and diabetes, treatments such as prostaglandins, and refractive surgeries on corneal biomechanical behaviour are now widely recognised. In turn, the effects of biomechanics on IOP measurement, the outcome of refractive surgeries, and the progression of ectatic diseases have been widely reported. The resulting growth in interest in corneal biomechanics has led to the development and improvement of several methods for its measurement in-vivo. Among the current technologies, there are optical/imaging systems coupled with non-contact air-puff tonometer as present in the Ocular response analyzer (ORA, Reichert Ophthalmic Instruments, Buffalo, NY), the Corvis ST (Oculus

Optikgeräte GmbH, Germany) and in experimental devices [19-21]. Other technologies include Brillouin spectroscopy, in which the frequency shift in the scattered light can be correlated with the material stiffness, optical coherence elastography, which is able to record corneal strain maps by tracking subpixel displacement, among others [22-25]. This revision focused on techniques already available for clinical use or being prepared for commercialisation.

2. Ocular response analyzer (ORA)

The Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Buffalo, NY) was the first commercial device to be made available for evaluating corneal biomechanics in vivo [19]. The device was introduced in clinical practice in 2005, it indents a central corneal area of 3-6mm diameter using a high-speed air-puff and employs an electro-optical system to monitor corneal deformation and recovery.

The air pressure is steadily increased up to a level beyond what is necessary to applanate the central cornea. During this stage, the cornea applanates at a pressure known as P_1, then takes a convex shape as the pressure increases to its highest level, P_{max}, where [26, 27]

$$P_{max} = 1.1713\ P_1 + 28.106\ (mmHg) \quad (1)$$

The pressure then decreases gradually, going through another applanation event at a pressure known as P_2, as described in Figure 1. Due to the tissue's viscoelasticity, P_2 is always smaller than P_1, and the difference between them ($P_1 - P_2$), which is attributed to the dampening response of the cornea or its energy absorption during the loading–unloading cycle, is termed corneal hysteresis (CH) [28]. Another ORA parameter is the corneal resistance factor (CRF). The CRF is also related to the viscoelasticity of the tissue, but it is weighted by elasticity due to its empirical development that maximized correlation to the central corneal thickness [29]. The CRF is also dependent on P_1 and P_2, and take the form CRF = a ($P_1 - bP_2$), where a and b are constants.

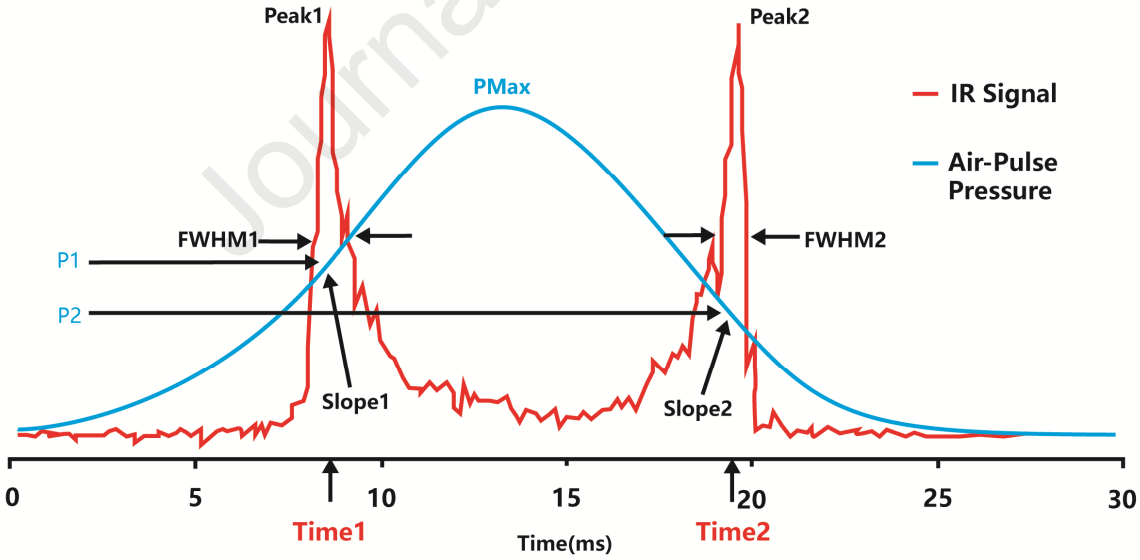

Figure 1 ORA signal and pressure application profile showing first and second applanation pressures (P1 and P2). IR: Infrared

The first parameter, CH, which was intended to provide a measure of corneal viscoelasticity, was reported to be correlated with the central corneal thickness, CCT [30-33] and age [34], but not IOP [35]. CH was also affected by medical history, showing reductions after refractive surgery [35-38] and in patients with keratoconus [35, 39], diabetes [40] and glaucoma [35, 41]. Reductions in CH was also reported with visual field progression in glaucoma [42]. However, some opposite trends were also reported including no correlation with CCT [35] or keratoconus progression [43], no significant changes after the cross-linking treatment [44] and a strong

correlation with IOP [33]. The second parameter, CRF, was less examined in the literature, but was found to be correlated with CCT [31-33] and age [34], reduced after refractive surgery (where there was a loss in CCT) [38] but saw no significant change with glaucoma [41], keratoconus (which is not expected given the reduction in CCT) [43] or the cross-linking treatment [44].

In order to improve the sensitivity and specificity for separating keratoconic and normal corneas, 37 additional parameters based on the ORA's infra-red waveform signal analysis were introduced and found to show lower values for keratoconic eyes [45]. Another study reported a case where the waveform signal parameters demonstrated changes with LASIK-induced ectasia even though CH remained stable [46]. The parameters were also found to significantly change after cross-linking in contrast to both CH and CRF [47].

Due to the reasonable success of the ORA parameters, including the new waveform additions, several publications describe them as biomechanical parameters. However, as these parameters could not be directly linked to the standard biomechanical properties including most notably the tangent modulus, they can only be considered as *indicators of biomechanical behaviour* rather than *intrinsic biomechanical properties*.

3. Corvis ST

Corneal Visualisation Scheimpflug Technology (Corvis ST, CVS, Oculus Optikgeräte GmbH, Germany) is a dynamic Scheimpflug analyzer that uses a concentric air puff to deform the central cornea while monitoring its deformation. The instrument uses an ultra-high-speed camera to capture 140 images of the central horizontal meridian of the cornea over 32 ms – the duration of the air puff. The images are analysed in real-time to produce several dynamic corneal response (DCR) parameters, which have been studied in detail since the instrument's introduction into clinical practice in 2010.

The Corvis ST is similar to the ORA in that both are non-contact instruments based on using an air puff with similar dimensions and profiles. However, there are several differences, the first of which is the maximum pressure of the air puff being constant in Corvis ST and variable (dependent on P_1) in ORA. Second, the magnitude of information recorded on corneal deformation is much greater in the Corvis ST, and third while ORA's main parameters rely on the applanation pressures, P_1 and P_2, Corvis ST relies instead on the DCRs.

The most prominent DCRs include [20, 48], Figure 2

- the maximum deformation amplitude (DA Max, displacement of corneal apex including eye movement),
- the maximum deflection amplitude (DeflAmpMax = DA Max – eye movement),
- DARatio2mm (DA Max divided by DA at 2mm away from the apex),
- DARatio1mm (DA Max divided by DA at 1mm away from the apex),
- The highest concavity radius (radius of corneal apex at the maximum concavity state),
- maximum inverse radius (1/radius of cornea's anterior surface at the apex at maximum concavity),
- the integrated inverse radius (IIR, the integrated sum of inverse concave radius between the first and the second applanation events),
- peak distance (distance between the two bending peaks on the cornea's anterior surface at the maximum concavity state), and
- the A1 velocity (speed of corneal apex at first applanation).

Later in 2017, two stiffness parameters were introduced; one at first applanation (SP-A1 = (adjAP1 – bIOP) / (A1DeflAmp), and one at highest concavity (SP-HC = (adjAP1 – bIOP) / (DeflAmpMax – A1DeflAmp)) [49]. The SP parameters rely on values of adjAP1, the applied air puff pressure at the time and position of first applanation, bIOP, the biomechanically-corrected IOP measurement [50], A1DeflAmp, the deflection amplitude at A1, and DeflAmpMax, the maximum deflection amplitude.

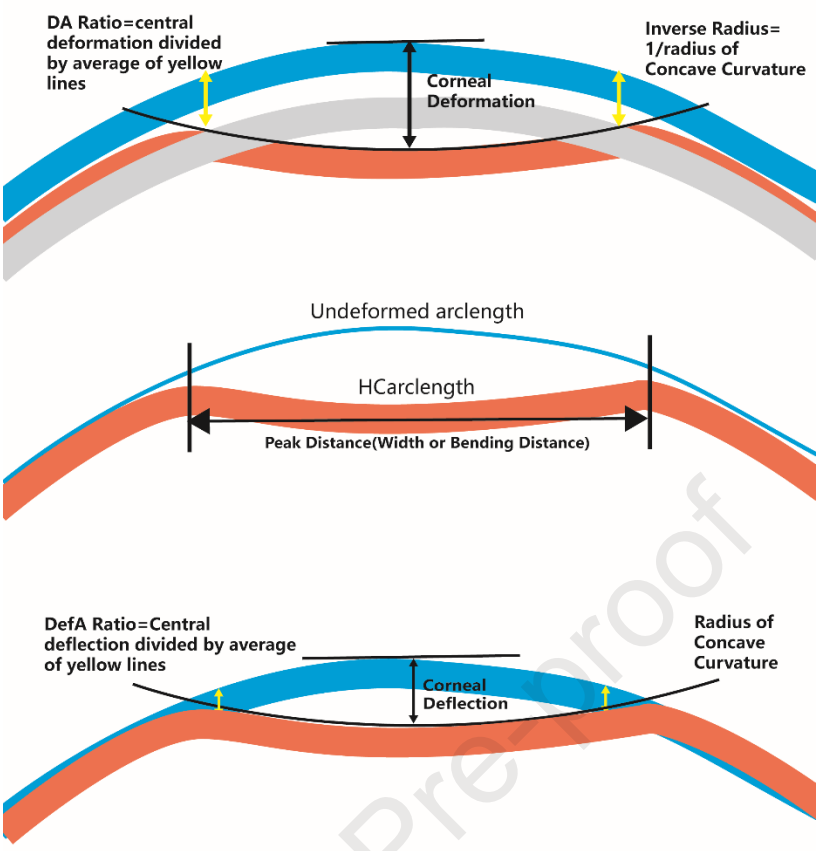

Figure 2 Corvis ST dynamic corneal response (DCR) parameters

The correlation between the two SP parameters and cornea's overall stiffness was confirmed in several studies including those showing consistent reductions in keratoconic eyes [51, 52] and eyes with normal-tension glaucoma [53]. While SP demonstrated the strongest correlation with stiffness and the clearest change with these diseases, other DCRs such as the HC radius, the inverse concave radius and the DARatio, also showed significant changes [51, 53-55]. The utility of these DCRs as overall stiffness measures was further confirmed by their correlation with CCT and age [54], both of which are important components of the cornea's stiffness [56]. The DCRs were also found to change less with transepithelial PRK than with femtosecond-assisted LASIK [57], in line with the known larger effect of the latter on corneal biomechanics [58, 59].

The success of the DCRs in providing reliable measures of corneal stiffness encouraged the development of new parameters to specifically assist the distinction between keratoconic and healthy eyes. These parameters are particularly helpful in cases where biomechanical deterioration has taken place but topographic distortion has not become apparent. The first parameter, called the Corvis Biomechanical Index (CBI), relies on the Corvis ST DCRs and was introduced in 2016 [60]. The parameter was found to be highly sensitive and specific to separate keratoconic from healthy eyes – with a cut-off value of 0.5, the CBI achieved 100% specificity, 94.1% sensitivity and an overall AUC (area under the curve) of 0.990 [51, 60]. The development of the CBI encouraged the development of the second parameter – the Tomographic and Biomechanical Index (TBI). This parameter combined Scheimpflug based corneal tomography and biomechanics for enhancing ectasia detection [61]. Consideration of tomography and biomechanics made the TBI more successful than the commonly used tomographic index from the Pentacam's (Oculus Optikgeräte GmbH, Germany) Belin-Ambrosio display (BAD-D), SP-A1 and the CBI in the detection of keratoconus [61, 62]. With an optimized TBI cut-off value of 0.29, the TBI achieved a sensitivity of 90.4% and a specificity of 96% in the fellow eyes of asymmetric keratoconus patients who had normal topography [61].

Like the CH and CRF, the Corvis ST parameters discussed above lacked direct links to the traditional biomechanical properties such as the tangent modulus (Et). For this reason, the Stress-Strain Index (SSI) was introduced as a measure of corneal material stiffness (rather than overall stiffness). The SSI is intended to provide an estimate of the whole stress-strain behaviour of the tissue, and can therefore be used to determine the tangent modulus at any stress [63]. Experimental studies have demonstrated that the variations in corneal stiffness show similar patterns (figure 3B), rather than intersecting trends (figure 3A) [12]. The approximated exponential stress-strain behaviour produces an almost linear variation of Et with stress. This characteristic results in proportional changes in Et and SSI at any stress level (figure 3C). As a material stiffness parameter, the SSI was found to be independent of IOP and CCT, but strongly correlated with age. The tangent modulus values obtained based on the SSI were also found to be close to those obtained experimentally for ex-vivo human corneas tested earlier under inflation conditions [56]. However, due to the high physiological variation of corneal material stiffness in the general population and the localised character of the keratoconus disease, this parameter cannot be used in disease diagnosis but is considered more suitable for following disease progression and assessing the efficacy of cross-linking treatment.

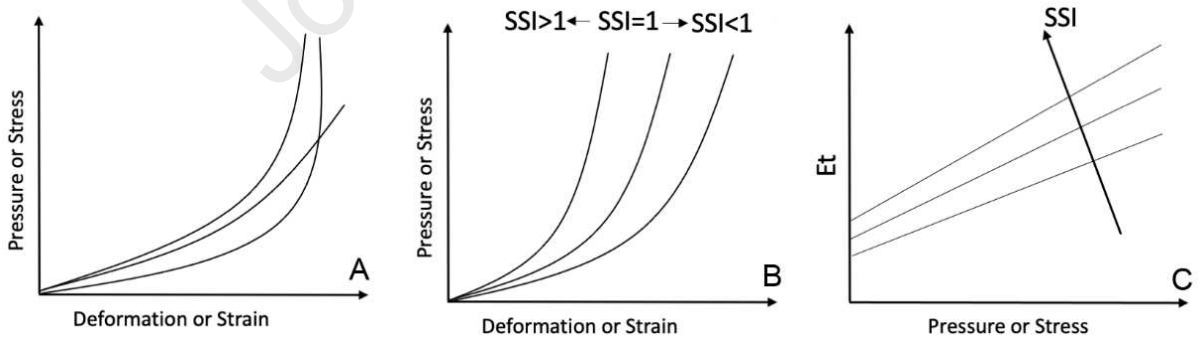

Figure 3 Explanation of SSI developed by Corvis ST, A. Material behaviour in which stress-strain curves intersect, B. Material behaviour in which stress-strain curves follow a similar pattern, C. The almost linear increase in Et with SSI increases for all levels of applied pressure or stress, corresponding to the curves presented in (B). SSI: Stress-Strain Index; Et: tangent modulus.

4. Brillouin microscopy

Originally described by Brillouin at 1922 [64] and extensively used to study condensed matter in material science [65], Brillouin scattering or Brillouin spectroscopy is a technique of near a century. It probes local mechanical properties of the material based on the inelastic scattering of light when it interacts with microscopic sound waves (acoustic phonons) that are inherently present in the material. The energy exchange between photons and phonons causes frequency shift in the scattered light which reflects the phonons' properties (i.e. speed) that in turn are

closely related to the biomechanical properties of the material. The measured frequency shift can be explicitly converted to the longitudinal modulus of the material given that the refractive index and density of the material are known [66].

Demonstration of Brillouin scattering in biology and ophthalmology was made in the 1980s [67-70], but it remained a point-sampling technique largely due to the long measurement time, and only became an imaging modality (Brillouin microscopy, BM) about a decade ago thanks to the adoption of virtually-imaged phased array (VIPA) etalons [22]. Recent advances have made BM capable of obtaining 3D, high-resolution elastic mapping of the material in a non-contact, label-free fashion [66].

While most BM studies in the early 2010s have focused on the crystalline lens [22, 71-75], the cornea has been attracting more attention in recent years, in line with the global awareness of the important role of corneal biomechanics in disease diagnosis, refractive surgery planning and ocular tonometry. The advantages of BM over ORA or Corvis ST mainly lie in its ability to provide spatially resolved map of corneal biomechanics. In keratoconus (KC), BM studies have confirmed regional softening in the cone area both ex vivo and in vivo [76-79], and recent studies have demonstrated the potential of the Brillouin-based metrics in differentiating KC from normal corneas, with the bilateral asymmetry of the Brillouin shift (between individual left and right eyes) being the most promising metric [79, 80]. However, the diagnosis power of the Brillouin frequency shift is yet to prevail over the established tomography-based indices [81].

Further, the spatial resolving capability of BM has enabled a more comprehensive evaluation of corneal cross-linking (CXL) procedures in stiffening the cornea [79, 82-88], allowing 3D assessment of their biomechanical efficacy across corneal surface and thickness, and promoting customised procedures such as the localised CXL [84, 87].

BM has also been used to evaluate the biomechanical effects of LASIK (the most common corneal refractive surgery modality) [85, 89]. These studies showed that the biomechanical properties of the stromal residual bed did not undergo noticeable changes but the flap (anterior) region experienced significant stiffness reductions. More recently, BM was used to evaluate the mechanical properties of biocompatible corneal substitutes [90] and assess corneal oedema in patients with Fuchs endothelial corneal dystrophy [91].

Despite its advantages and great potential, interpretation of BM measurements is still debatable [92-94] because BM probes biomechanics at a much higher frequency compared to conventional methods such as extensometry and inflation tests (GHz vs quasi-static) [66, 95]. To date, log-log linear [72] and quadratic [96] relations between Brillouin-derived moduli with moduli by conventional methods were empirically determined, but explicit relations are still unavailable, preventing its application in patient-specific modelling of refractive surgery and tonometry. Further, as BM measurements are usually obtained at a constant stress state, it has difficulty to reveal the nonlinearity (i.e. hyperelasticity) of corneal tissue. On one hand, the longitudinal bulk modulus derived from the Brillouin frequency shift is shown to be independent of the tissue's in-plane tension in contrast to the tangent modulus obtained using extensometry [96]. On the other hand, reduced Brillouin frequency shift was found in the LASIK flap after its creation, suggesting reduced Brillouin shift with released tissue tension [85]. Another drawback of BM lies in its lack of capacity in evaluating the viscoelasticity of the corneal tissue. Despite that viscosity can be evaluated based on the linewidth on the Brillouin spectrum in theory [66], this has not been systematically studied with corneal tissue and the connection of Brillouin-derived viscoelastic properties with those assessed by conventional methods (characterised by creep, stress relaxation, strain rate dependency and hysteresis [97]) remain unexplored.

Although it remains challenging to fully understand the complicated mechanics of corneal tissue at high frequencies and its explicit connection with conventional moduli, BM has indeed provided significant insights into corneal biomechanical responses against corneal disease and various surgical procedures. Advances in BM techniques have already led to a clinically compatible system called BOSS (Brillouin Optical Scattering System, Intelon Optics Inc., Lexington, MA,

USA) which is currently being prepared for commercialisation [98], and future development in this field [99] is expected to benefit our understanding of corneal biomechanics.

5. Summary

Due to its important clinical applications, the study of corneal biomechanics in-vivo has been evolving fast during the last two decades. While the first devices and currently the only commercially available, ORA and Corvis ST, have been successful in providing reasonably accurate compensations for the corneal biomechanical impact on IOP measurement, their initial parameters could not be directly linked to the standard biomechanical properties. This point was addressed with the recent development of the stress-strain index of the Corvis ST, which was intended to provide an estimate of Et at any stress or IOP level. Meanwhile, the study of Brillouin microscopy over the last decade culminated in the development of a clinically compatible device that is being prepared for commercialisation. There are promises and hurdles to be overcome by this new technology, and its clinical value is to be elucidated in the following years. Table 1 summarises the main features of the three technologies discussed in this paper. The huge recent progress made is expected to continue and accelerate in the near future with technologies that are able to measure standard biomechanical measures with less influence of intrinsic ocular parameters such as the corneal geometry and the intraocular pressure and extrinsic factors such as surface humidity and atmospheric pressure.

Table 1: Brief analysis of ORA, Corvis ST and Brillouin Microscopy

	Pros	Cons
ORA	• Pioneer device • Compensation for the corneal biomechanical impact on IOP measurement	• Indirect assessment of corneal deformation • Parameters cannot be directly linked to the standard biomechanical properties • CRF is dependent mainly on corneal thickness rather than overall corneal mechanical stiffness
Corvis ST	• Newly developed parameter to measure corneal material stiffness • Corneal biomechanical compensation for IOP measurement in healthy and ectatic corneas	• Records restricted to the central horizontal meridian • Corneal material stiffness measure is given as a single value and does not consider stiffness variation across corneal surface or through the thickness
Brillouin Microscopy	• Spatially resolved map of corneal biomechanics	• The Brillouin shift is not directly linked to corneal stiffness • Dependence on tissues hydration levels

ORA: Ocular Response Analyzer; IOP: intraocular pressure.

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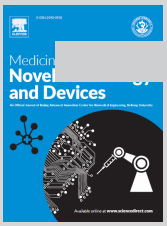

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