In vivo corneal stiffness mapping by the Stress-Strain Index maps and Brillouin Microscopy

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The study of corneal stiffness in vivo has numerous clinical applications such as the measurement of intraocular pressure, the preoperative screening for iatrogenic ectasia after laser vision correction surgery and the diagnosis and treatment of corneal ectatic diseases such as keratoconus. The localised aspect of the microstructure deterioration in keratoconus leading to local biomechanical softening, corneal bulging, irregular astigmatism and ultimately loss of vision boosted the need to map the corneal stiffness to identify the regional biomechanical failure. Currently, two methods to map the corneal stiffness in vivo are integrated into devices that are either already commercially available or about to be commercialised: the stress-strain index (SSI) maps and the Brillouin Microscopy (BM). The former method produces 2D map of stiffness across the corneal surface, developed through numerical simulations using the corneal shape, its microstructure content, and the deformation behaviour under air-puff excitation. It estimates the whole stress-strain behaviour, making it possible to obtain the material tangent modulus under different intraocular pressure levels. On the other hand, BM produces a 3D map of the corneal longitudinal modulus across the corneal surface and thickness. It uses a low-power near-infrared laser beam and through a spectral analysis of the returned signal, it assesses the mechanical compressibility of the tissue as measured by the longitudinal modulus. In this paper, these two techniques are reviewed, and their advantages and limitations discussed.

Keywords: cornea, biomechanics, mapping, ectasia, keratoconus

# Introduction

In the field of ophthalmology an adequate knowledge of corneal biomechanics has become indispensable in numerous applications such as measuring the intraocular pressure, assessing preoperative risk for iatrogenic ectasia post laser vision correction, diagnosing keratoconus (KC) in its early forms, indicating, and evaluating crosslinking (CXL) treatment effectiveness, among others 1.

The cornea is the main refractive surface of the eye 2. To allow the light rays to be clearly focused on the retina, the cornea needs to keep its transparency as well as to maintain a stable aspheric shape able to resist to occasional external insults and to bear the intraocular pressure (IOP) 3. These peculiar characteristics are possible due to a highly organised collagen structure in the corneal stroma 4. The collagen fibrils are the main load-carrying components of the corneal tissue with their content and distribution clearly controlling the tissue’s biomechanical behaviour 5, 6.

Corneal ectatic diseases such as KC are characterised by a localised degradation of this collagen microstructure, causing the tissue to become unable to maintain its shape under the IOP, and leading to a localised bulging resulting, in turn, in irregular astigmatism and loss of clear vision 7. The localised nature of these diseases generated the necessity for mapping the corneal biomechanical behaviour either to improve the early detection of these specific fragilities or to direct treatments such as cross-linking to the most needed areas improving their efficacies and reducing the incidence of complications 8.

The clinical need for in vivo characterisation of corneal biomechanics led to the development of some commercial devices, the first of which employed precise measurements of corneal deformation under an air-puff pressure to provide estimates of overall corneal biomechanical behaviour 9. The Ocular Response Analyzer (ORA; Reichert, NY, USA) was the pioneer device to address this challenge 10. Using an infrared signal, the ORA indirectly monitors the corneal deformation caused by an air-puff stimulus. Also using air-puff stimulation, a later device, the Corvis ST (Oculus, Wetzlar, Germany), improved on the ORA by including an ultrafast Scheimpflug camera that allows the direct monitoring of corneal deformation 11.

Using a method that does not rely on corneal deformation, the Brillouin light-scattering microscopy (BM) employs the Brillouin frequency shift to estimate the longitudinal modulus of the tissue, for which the refractive index and material density are known 12. The main advantage of BM over air-puff-based technologies was the possibility to produce a depth-dependant, spatially-resolved mapping of the longitudinal modulus as opposed to the global biomechanics metrics provided by the ORA and Corvis ST.

However, this point was addressed in the recent development of the Corvis ST, which allowed the production of 2D maps of the stress-strain index (SSI), a material stiffness parameter allowing estimation of the tissue’s stress-strain behaviour. The maps relied on the proven link between tissue microstructure and the stiffness distribution to translate the single SSI measurement into a 2D stiffness map of the cornea 13.

In this paper the two main techniques, which are already available, or almost ready, for clinical practice, for mapping corneal stiffness are reviewed. The emphasis of the review is on the possible applications of the maps in the diagnosis and management of corneal ectatic diseases.

**Stress-Strain Index Map**

A recent addition to the Corvis ST was the development by Eliasy et al. of an algorithm based on the results of a large simulation using finite element modelling of the human ocular globe, able to estimate the overall corneal stress-strain behaviour: the Stress-Strain Index (SSI) 14. Like most biological tissues, the corneal material is hyperplastic with the stress-strain curve assuming an almost exponential shape 7. This behaviour leads to the tangent modulus (Et, a measure of material stiffness) increasingly linearly with loads such as IOP, and therefore, a particular Et value measured under a specific IOP will not be valid under different pressure levels. The SSI was developed to consider this point and was intended to estimate the whole stress-strain behaviour, and hence Et at any IOP. Unlike previously developed indices such as the corneal biomechanical index (CBI), that used statistical methods to enhance the ability to diagnosis keratoconus 15, the SSI was developed to represent a standard mechanical property, the material stiffness. Following its development, the SSI validation studies showed the parameter to be independent of IOP and the central corneal thickness (CCT) in healthy 14 and in KC corneas 16. SSI was also observed to reduce with KC progression 17, reflecting, and quantifying, the expected changes in corneal stiffness.

By combining the mean distribution of collagen fibril density (which was found consistent in healthy eyes) with patient-specific corneal geometry, Zhang et al. developed a finite-element-based numerical modelling method to translate the single value of SSI into a 2D map, presenting the SSI variation across corneal surface 13. The method relied on the proven link between corneal microstructure and stiffness distribution 5 and the consistency in collagen fibril density distribution in healthy corneas 18-20. Studying healthy corneal specimens, Zhou et al. observed that, within the central zone with a 6 mm diameter, the standard deviations of collagen fibril densities within the 45o sectors surrounding the superior-inferior meridian, and the nasal-temporal meridian were 2.8% and 2.9%, respectively 18. The authors also observed high consistency in circumferential fibril content at 11 mm diameter with a 1.8% standard deviation 18. The collagen fibril diameter in the central cornea was also reported by Boote et al. to be consistent within healthy specimens 19. The same authors also reported as the presence of midline symmetry between left and right eyes when comparing preferentially aligned fibrils 20.

As these observations are not valid in KC corneas 21, 22, the development of keratoconic stiffness maps was possible due to some other findings. First, there was evidence that in KC cases, the microstructure features outside the diseased area were not different from those observed in healthy cases 18, 21, 22. Second, a method was developed by Eliasy et al. 23, and later clinically validated 24, which was able to delineate the diseased area (the keratoconic cone) in topography elevation maps. Third, a further method used the cone features and the cornea’s maximum curvature to estimate the magnitude of fibril density reduction inside the cone 21, 23, 24. These three methods allowed extension of the SSI mapping technology, initially developed for healthy corneas, to KC cases 13.

In brief, the method begins by building two 3D patient-specific whole eye numerical models based on the finite element method: one (Model 1) adopting homogenous material, whose stiffness at all locations is estimated by the SSI value obtained in vivo through the Corvis ST exam and the other (Model 2) adopts an anisotropic material for which the mean distribution of stiffness followed the distribution of corneal fibril density. In order to obtain the specific stiffness at each integration point of Model 2, an inverse analysis process is carried out by assuming an initial stiffness level while observing that the ratios between these stiffness levels matched the ratios between the fibril contents at the same locations, and then comparing the corneal apical displacement between Models 1 and 2. The stiffness levels in Model 2 are changed by the same percentage as the differences between the apical displacements of Models 1 and 2 and the process is repeated until there is a match judged by the following objective function:

$Root Mean Squared Error\leq \sqrt{\frac{\sum\_{i=1}^{n}(δ\_{i Model 1}- δ\_{i Model 2} )^{2}}{n}}$

where *δ* is the apical displacement, *i* refers to different IOP application steps and *n* is the total number of IOP steps.

As demonstrated by Zhang et al., the regional variation in the SSI values showed little fluctuations with IOP, CCT or corneal curvature changes, corroborating the SSI map as a robust estimation of the tissue’s material properties with little effect of the usual confounding (loading and geometric) factors 13. In the same study, the authors observed that simulated healthy eyes demonstrated only slight variations in SSI values across the corneal surface, while in keratoconic corneas there was a substantial SSI reduction inside the cone area. Further, the SSI reduction inside the cone was higher in more advanced cases of the disease.

The SSI maps have been employed to assess the effect of KC progression on corneal stiffness (Elsheikh, unpublished data). In a sample of 29 eyes of 29 patients aged 20.1 ± 7.0 years (9 – 40) that presented significant KC progression over 17.1 ± 17.1 months (1.4 – 58.4), there was a significant reduction in SSI concentrated in the cone area (-0.15 ± 0.09, range: -0.42 to -0.01, p < 0.001), while the area outside the cone underwent minimal non-significant change in SSI (0 ± 0.01, range: -0.04 to 0.01, p = 0.999). Figures 1 and 2 depict the results of a representative case of a 21-year-old male patient that progressed over 25 months from a Kmax of 54.7 D to 56.2 D, a posterior elevation at the thinnest point from 49 µm to 70 µm and corneal minimum thickness from 504 µm to 491 µm. In figure 1 the radial elevation maps in relation to the optimal sphere of the front surface (A: baseline, B: post-progression) and of the back surface (C: baseline, D: post-progression), with the estimated cone area, are plotted. In figure 2 the SSI maps show that the disease progression was concentrated mostly inside the cone area. There was a significant reduction in the SSI values within the cone region, while the SSI values remained relatively stable outside of it. A further study to evaluate the effect of cross-linking treatment on corneal stiffness distribution, as represented by SSI maps, is being conducted by the University of Liverpool group.

**Brillouin Microscopy**

Brillouin Microscopy (BM) is an imaging modality based on the inelastic scattering that arises from the interaction of light with the medium’s inherent acoustic phonons – or density fluctuations 25. The Doppler effect that arises from the reflection of light waves by these progressive inherent sound waves denominates the Brillouin shift 25. Given that the refractive index and density of the material are known, the Brillouin frequency shift can be explicitly converted to the sample’s longitudinal modulus (M’) using the following relationship:

. $M^{'}= \frac{ρΩ^{2}λ^{2}}{4n^{2}}$

Where *Ω* is the frequency shift of the scattered light, *λ* is the wavelength of the incident photons, *ρ* is the density of the material and *n* is the refractive index of the material 26. The method to estimate the longitudinal modulus assumes mechanical isotropy of corneal tissue 26.

The technique was first used in ocular tissues in the early 1980s 27-30. However, the long measurement time – up to one hour – limited the assessment to single spatial points. More recently, with the development of a parallel spectrometer based on a virtually imaged phased array, the acquisition time was reduced to 1s or less allowing BM to provide a 3D spatially resolved map of the corneal longitudinal modulus 12. It should be noted that the longitudinal modulus cannot be directly related to the elastic or shear moduli. The latter two moduli are the ones that provide a measure of the stiffness of the material under direct and shear loads, respectively. Nevertheless, a strong correlation (R2 = 0.98) has been found between the Brillouin-derived longitudinal modulus of elasticity and tangent modulus of porcine corneas at very low strain (< 10%) 31.

BM has been used to map regional softening in KC cases. In an ex-vivo study, Scarcelli et al. showed that the mean Brillouin shift inside the cone area (7.99 ± 0.10 GHz) was significantly lower than those observed in the corresponding areas of healthy corneas (8.17 ± 0.06 GHz, p < 0.001) 32. The Brillouin shift in areas outside the cone was also significantly higher than inside the cone area (8.19 ± 0.04 GHz, p < 0.001), but no difference was observed between the non-cone areas in KC cases and corresponding areas in healthy cases. These results were corroborated in an in vivo study comparing healthy to advanced KC cases, in which the healthy corneas presented a Brillouin shift that was uniformly distributed across the anterior central region, while the KC cases showed significant reductions inside the cone area (p < 0.001), with no difference between the area outside the cone and corresponding areas in healthy cases 33. The in vivo measurements, however, showed large interpersonal variability in both healthy and KC cases. Most of the variability has been hypothesised to stem from natural personal variation in the collagen composition and the hydration level of corneal tissue 34-36. In order to overcome this issue, regional variations of the Brillouin shift have been studied in vivo 34. The healthy cases presented a relatively uniform distribution of the Brillouin shift across their 4-mm-wide scanned areas, while in KC cases (including those with mild disease), there was a linear increase from the cone to the cornea periphery. In addition, the rate of regional variation increased with disease severity 34.

Seiler et al. have observed that the Brillouin shift at the point of maximum posterior elevation was better correlated with geometry-derived keratoconus indices such as minimum corneal thickness (MCT) and maximum anterior curvature (Kmax) 37. The authors also showed that the Brillouin shift at this point progressively decreased from mild to severe cases. However, its diagnostic ability was significantly lower than both MCT and Kmax. Shao et al. observed in a small sample of 4 mild KC cases that the Brillouin asymmetry between the left and right cone regions was significantly higher than the asymmetry observed in healthy cases with no overlap between the groups 34. Even though the sample size was small, this new metric is promising in detecting early KC cases and worth further investigation.

The effect of CXL was initially studied with the BM in porcine corneas, showing significant stiffening that was higher in the anterior portion of corneal tissue 38. The BM’s ability to estimate the longitudinal modulus across corneal thickness allowed a study to show that, in porcine corneas, accelerated protocols had their effect significantly reduced through tissue thickness 39. It has also been shown using BM in porcine corneas that the stiffening effect of localised CXL extended beyond the treated area suggesting that custom treatments should also account for this effect on the surrounding tissue 40. In a cross-sectional, in vivo study, Shao et al. compared a group of 8 untreated KC eyes with 16 CXL-treated cases 41. The untreated group showed a mildly significant lower Brillouin shift (but with large overlap) than the post-CXL KC cases (p < 0.05). The latter group also showed non-significant differences when compared to a healthy control group. The cross-sectional study design, the small sample size and the big overlap between the groups suggest that these results should be taken with caution.

**Method comparisons and limitations**

The two described methods possess different advantages and limitations. The SSI maps estimate the whole stress-strain behaviour across the whole surface of the corneal tissue (2D), allowing measurement of the material stiffness under different IOP values 13, which, due to the non-linear nature of the tissue, has a direct impact on the tangent modulus 7, 42. Since the SSI map relies on the corneal microstructure and there is no method to directly describe individuals’ microstructures in vivo, it was necessary to assume that all healthy corneas and the areas outside the cone in KC eyes shared the same mean microstructure. However, the slight variations (under 3% SD) observed in ex-vivo microstructure studies and the use of each individual’s specific corneal thickness and curvature profiles considerably mitigates this limitation 18. Another limitation of the SSI maps was the method used to link the cone geometric features to the fibril density reduction within the cone 21. It was developed using the microstructure of 7 KC corneas, which was all that was available to the authors at the time but with the inclusion of more KC cases, the method is expected to gradually improve 21.

On the other hand, the BM can estimate the longitudinal modulus across both corneal surface and thickness (3D) 43. The BM measurements are usually acquired at a constant stress state, thus differ from the SSI maps in that it does not account for the tissue’s non-linearity. Further, the longitudinal modulus – a measurement of the tissue’s compressibility – assessed by the BM is dependent on the corneal hydration levels 34-36, and does not directly correlate with the tangent modulus 43. Even though it is expected that both metrics would change in a similar fashion in response to corneal physiological or pathological processes. In earlier studies, quadratic 35 and log-log linear 44 relationships were reported between Brillouin-derived moduli and the moduli derived from conventional methods – these relationships were determined empirically and explicit links are still unavailable. Table 1 provides an overview and comparison of both methods.

The most important clinical applications of in-vivo stiffness mapping are the early diagnosis and treatment of corneal ectasia. Both methods were able on one hand to detect the localised stiffness reduction with KC progression and on the other, the stiffening promoted by CXL. The body of evidence is constantly growing with the initial clinical studies showing important populational trends. The wider availability of the methods in clinical practice increasing their contribution to the multimodal management of corneal ectatic disease, could allow the development of patient-specific cut-offs for early diagnosis, progression and treatment efficacy.

Both described methods are undergoing further improvements. On the SSI maps, it includes the refinement of the estimation of fibril density reduction in KC cases through the analysis of a bigger sample and a more detailed inclusion of the contribution of covalent bonds throughout the cornea to the tissue’s stiffness. On the Brillouin Microscopy side, dealing with the low intensity of the scattered Brillouin light and the small frequency shifts makes the measurement system very sensitive to alignment, vibration, temperature, and humidity. New designs to overcome these challenges and facilitate its use in clinics are being developed. Along with further improvements that both described methods are undergoing, some other technologies are also being developed using ultrasound and optical coherence tomography (OCT) 45-47. The OCT elastography is being extensively studied and it is expected that in the future it could also be part of the tools available in clinical practice to map corneal stiffness 48-53.

**Summary**

Mapping the corneal stiffness in vivo allows a better understanding of corneal ectatic diseases that affect the biomechanics of the localised areas of pathology. Both methods covered in this review have been shown to be effective in accomplishing this task. The SSI map method estimates the whole stress-strain behaviour of the corneal tissue, and its variation, across the corneal surface, while the BM method estimates the longitudinal modulus in 3D. The initial results suggest that both methods would aid in the diagnosis of KC, following-up its progression and in customising the CXL treatment.

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Figure 1. Case example of elevation maps in a progressive keratoconus case.

Figure 1A: Front surface: baseline radial elevation map. Dotted line: estimated cone region.

Figure 1B: Front surface: post-progression (in 25 months) radial elevation map. Dotted line: estimated cone region.

Figure 1C: Back surface: baseline radial elevation map. Dotted line: estimated cone region.

Figure 1D: Back surface: post-progression (in 25 months) radial elevation map. Dotted line: estimated cone region.

Figure 2. Case example of Stress-Strain Index (SSI) maps used in keratoconus progression.

Figure2A: Baseline SSI map exam.

Figure2B: SSI map after 25 months.

Figure 2C: Differential map, showing the stiffness decrease mostly concentrated inside the cone area.

**Declaration of Interest**

Prof Elsheikh is a consultant to Oculus, Wetzlar, Germany