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Brief Title: A Study to Test the Diagnostic Potential of Brillouin Microscopy for Corneal Ectasia

I. BACKGROUND AND SIGNIFICANCE

A) HISTORICAL BACKGROUND

The overall goal of this study is to change the current paradigm to detect corneal ectasia. We aim to establish biomechanical criteria to identify early signs of keratoconus and to screen at-risk subjects for post-LASIK ectasia based on a novel Brillouin optical imaging.

Ectasia refers to thinning and bulging of the cornea, which causes severe visual degradation and frequently requires a corneal transplantation [1]. The mechanism of ectasia is known to relate to corneal biomechanics (**Fig. 1**). In a healthy cornea, collagen fibers provide the mechanical strength to withstand the outward intraocular pressure (IOP); if the corneal tissue becomes compromised and weakened, the ectasia can develop.

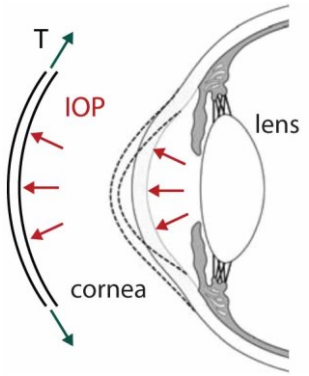

Fig. 1: Intraocular pressure (IOP) induces corneal tensile (T) load. A weak cornea, with reduced elastic modulus, cannot withstand IOP and thus thins and bulges, causing ectasia.

An abnormal loss of corneal integrity and strength can either result from degenerative ocular conditions, such as ***keratoconus***, or as a complication of refractive surgery, most notably with Laser In Situ Keratomileusis (***LASIK***).

Keratoconus is the most common corneal degeneration in the US affecting ~1/2000 of the general population [2] with a mean onset age of 15.4 years [3]. LASIK is the most frequently performed laser surgery in the US with nearly 1 million operations per year. Iatrogenic ectasia following LASIK is the most feared complication of surgery causing severe visual degradation and often requiring a corneal transplantation because of the irreparable corneal changes after the vision correction [1].

For these conditions, the early diagnosis is crucial. Keratoconus progression could be halted by promising interventions, such as corneal collagen crosslinking [4], if detected early. In addition, patients with weak corneas could be screened and counseled to avoid LASIK surgery. Ectasia due to keratoconus or after LASIK have similar biomechanical presentations [5]. In fact, unidentified keratoconus is the most likely cause for post-LASIK ectasia occurrence [6], and the early-diagnosis of keratoconus onset is widely considered as the key advance needed to dramatically reduce the occurrence of post-LASIK ectasia [6].

Current diagnostic methods are based on morphological rather than biomechanical analysis. Current diagnosis of keratoconus is clinical. The irregular morphologic patterns of the cornea can be detected by pachymetry and topography before clinical signs occur, but these tests cannot reliably differentiate truly weak or keratoconic corneas from atypical normal ones [7, 8]. The Screening protocols for LASIK that rely on indirect morphological risk factors have had limited success in the clinic. About 15% of patients seeking laser vision correction are considered at-risk and are thus denied treatment; on the other hand, patients with normal appearing pachymetry and topography

and no other risk factors have gone on to develop post-LASIK ectasia [9-11]. This situation has also led to multimillion dollar lawsuits [12] and has contributed to the decision by the FDA to reevaluate LASIK safety [13, 14].

The compelling need for improved diagnostics has triggered interest in the mechanical properties of the cornea, e.g. elastic modulus or stiffness, as their connection to the pathophysiology of the condition makes them highly suited to early identify patients at risk for developing ectasia [6, 15]. However, mechanical measurements are traditionally performed by macroscopic and destructive methods [16]. Recently, a widespread effort has been put forward to achieve non-invasive assessment of corneal mechanical properties. Corneal hysteresis measured by an ocular response analyzer [17] (ORA) has been shown to correlate with advanced keratoconus [18]. However, hysteresis is indirectly related to the elastic properties, and the clinical usefulness of the ORA device in early keratoconus patients remains highly questionable [19-21]. Other techniques based on ultrasounds [22, 23] are under development [24-27]. However, currently there is no clinical ophthalmic device capable of measuring corneal stiffness or elastic modulus.

Our Innovation: Brillouin microscopy - In this study, we will introduce an entirely novel approach for *in vivo* biomechanical analysis of the cornea via optical means. In the past years, we have invented and developed an optical technology, Brillouin microscopy, highly suited for elasticity-based screening in refractive surgery [28]. When laser light hits a sample (e.g. lens or cornea), a portion of the light is reflected due to the acoustic waves that are weak but naturally present in the material by thermal fluctuations. The acoustic waves propagate with a speed that is directly related to the sample's elastic property. We measure the acoustic speed (therefore the elastic modulus) by measuring the frequency shift of the reflected light using a specially designed spectrometer, i. e. the Doppler principles used in a laser-based speed measuring of a traveling car. The spectrometer we have developed can detect the small Brillouin frequency shift 1000 times more efficiently than existing instruments. This technique is different from ultrasound and offers much higher resolution and sensitivity. It is capable of probing the elasticity of the tissues non-invasively with microscopic resolution. We have extensively validated the Brillouin microscopy for the ophthalmic research by mapping ocular tissue elasticity, its spatial heterogeneity and relevant changes in both lens [29] and cornea [30].

Importantly, using human corneas *ex vivo*, we have recently demonstrated that *Brillouin technology can clearly differentiate ectatic from normal corneas* based on different elastic modulus. We also have developed a prototype instrument for clinical use. We have already tested the safety of the prototype system *in vivo* on five human subjects [31]. This is part of an ongoing safety study approved by PHC IRB.

B) PRE-CLINICAL AND CLINICAL STUDIES

The traditional Brillouin spectroscopy, widely used for material characterization [32], is slow in acquiring spectra (10 min to 1 hour per spectrum) thus limited to point-sample analysis. In 2008, we have introduced a novel spectrometer, that reduced the acquisition time to 1-10 seconds per spectrum [33]. Then with the multi-stage VIPA spectrometry [34, 35], we

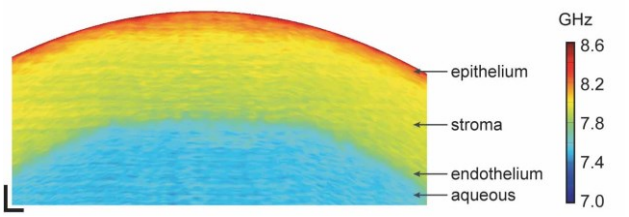

Fig. 2: (a) Cross-sectional Brillouin image of bovine cornea *ex vivo*. Color represents the measured Brillouin frequency shift.

further reduced the spectrum acquisition time by 100 times, which enabled us introducing the Brillouin confocal microscopy to obtain the first 3D elasticity imaging of the cornea (**Fig. 2**) and the lens in animals [29, 30].

As preliminary investigations for this study

1) We have performed theoretical calculations and modeling on the safety of Brillouin technology. Besides we have experimentally tested the validity of our theory and modeling.
2) In ex-vivo animal studies, we have found that the Brillouin technology reveals depth-dependent variations in corneal modulus corresponding to different stromal collagen organizations [30].
3) Through in vivo longitudinal study in mice, we have tested the safety and efficacy of our technology [29].
4) With ex-vivo human tissue studies we have shown that the Brillouin technology can measure the abnormal weakening of human cornea due to ectasia.
5) We have completed a safety study on 10 heathly human subjects *in vivo* [31].
6) In the in vivo human study, we have shown that our current Brillouin instrument provides sufficient sensitivity and reproducibility to measure corneal ectasia in humans *in vivo*.

1. Safety and risk analysis of the Brillouin technology for use in human.

Theory - The Brillouin technology uses illumination intensity levels comparable or smaller than equivalent ocular instruments that are already FDA-cleared and widely used in the clinical practice such as the corneal confocal scanning microscopy and the anterior-segment optical coherence tomography. The optical source within the experimental Brillouin instrument emits near-infrared light at the wavelength of 780 nm. Exposure of the human eyes to this light will be within safety limits established by the American National Standard for Safe Use of Lasers (ANSI Z136.1-2007). The ANSI standard indicates that exposure to this light at the levels we propose for imaging (<3 mW) does not pose a risk to the subjects even for continuous prolonged exposure of 8h. We expect to limit the continuous exposure time for one imaging scan to less than 10 seconds, i.e. very short within this time limit. The subject will be asked to comply with the imaging procedure 40-50 times during the session to gather spatially-resolved data within the cornea. A short break between the imaging scans will be provided to rest the eye. The imaging will not require any direct contact with the eye. The scanning procedure is not associated with any discomfort, except for the possibility of minimal discomfort arising from staring at the low intensity light in order to reduce the eye movements to a minimum, and from straining due to not blinking during the scanning. The only direct contact will be at the subject's chin and forehead with the respective rests of the standard slit-lamp interface. The chin and forehead rests will be cleaned with alcohol wipes in between the subjects.

To evaluate the potential risks associated with the use of Brillouin ocular microscopy, we can calculate the Maximum Permissible Exposure (MPE), defined as the highest power or energy density that can be admitted to the eye without causing a biohazard. MPE corresponds to 10% of the dose that has a 50% chance of creating damage in a worst-case scenario. In the Brillouin scanner, the laser light is focused in the cornea, and it is well diffused when it reaches the retina. According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the exposure limit

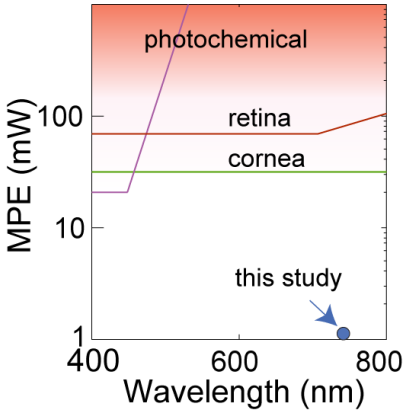

Fig. 3: Maximum permissible power for cw 30 s exposure with NA=0.1.

for the cornea-lens thermal safety is 4 W/cm^2 for continuous wave sources in the wavelength region of 400 - 1050 nm; i.e. MPE = 32 mW in a 1-mm-diameter zone (0.79 mm^2 in area) [36]. The 1-mm aperture for irradiance averaging is based on the thermal modeling [37], which shows that the temperature rise is independent of the beam size up to 1 mm due to rapid thermal conduction (dissipation ~ 0.5 W/m/^{o}C) in the cornea and lens. As for the retina, the dominant mechanism of retinal damage is thermal for an exposure time (T) longer than 0.25 s. According to the guidelines from American National Standard Institute, the exposure limit for the retinal thermal hazard has been expressed as 1.8 x 10^{-3} C_A C_E $T^{-0.25}$ [W/cm^2], where C_A = 1 - 1.5 (for λ = 400 - 800 nm), C_E = 267 (for α = 0.2; i.e. NA = 0.1), and the aperture size of 7 mm (area of 0.38 cm^2) [38]. The MPE is calculated to be 183 mW for T = 1 s and 66 mW for T = 60 s, which is consistent with the above analysis. Taken together, we conclude that the Brillouin scan in the cornea and lens does not pose a risk to the human eye.

Experiment - To experimentally test the theoretical prediction, since the lower exposure limit is predicted for corneal thermal safety, we used an infra-red high-resolution camera (FLIR SC8000) with a temperature sensitivity of 0.1°C to physically detect any possible changes in temperature of the cornea due to light illumination. In this setup, we used a porcine eyeball and we focused laser light of different power levels onto the cornea with several objective lenses providing numerical apertures (NA) from 0.05 to 0.3. At the levels of light exposure we intend to use in our pilot study (i.e. ~ 0.5mW, NA = 0.1), we have detected no increase in temperature on the corneal surface, even after prolonged exposures of greater than 60 seconds. In order to observe a detectable change in temperature, we had to shine 180 mW of light power onto the specimens for 10 seconds, which has resulted in a temperature rise less than 1°C, well below tissue damage threshold (**Fig. 4**). These results are consistent with thermal modeling prediction. Note that this exposure level is some 300 times higher than the power level we plan to use in our study. Therefore, we have experimentally verified that the thermal modeling predictions are accurate and that the Brillouin technology poses no risk to the cornea.

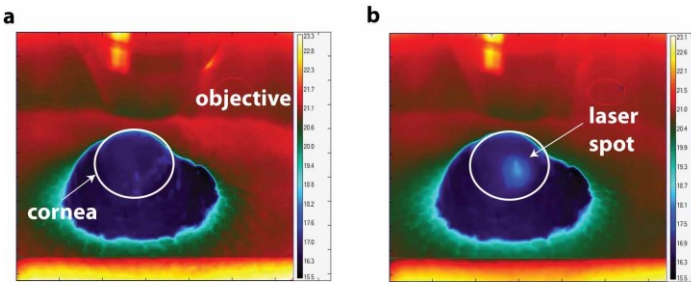

Fig. 4: Corneal temperature maps before (a) and after (b) light exposure at 180 mW for 10s, which corresponds to more than 100 times higher than the exposure levels proposed in our study. The maximum temperature elevation is less than 1°C.

2. Ex vivo animal studies :
Brillouin can detect the local depth-dependent elasticity changes due to the corneal collagen organization (bovine *ex vivo*).

The collagen content and the fibril organization are known to be responsible for the mechanical strength of the corneal stroma [39-41]. We have investigated the correlation between the internal micro-structure and the elastic modulus of cornea comparing high-resolution axial profiles of intact bovine corneas (**Fig. 5a**) with the corresponding collagen-stained (Masson's trichrome) histology images (**Fig. 5b**) and second harmonic generation (SHG) microscopy to highlight collagen fibers (**Fig. 5c**). The anterior part

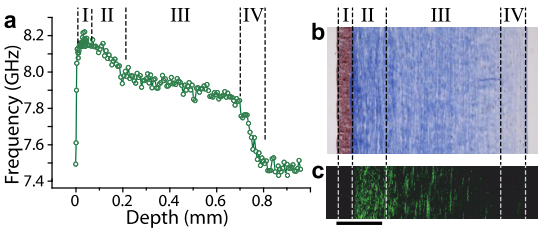

Fig. 5: (a) Brillouin depth profile of an intact bovine cornea. (b) Masson's trichrome stained image of a thin tissue slice. (c) A second-harmonic-generation image of the section. Scale bar, 0.2 mm.

of the stroma shows a markedly interwoven organization of collagen fibers and has the highest Brillouin modulus.

In the posterior and innermost region of the cornea, collagen fibers mostly run parallel to the corneal surface and the collagen amount decreases towards the endothelium, which correlates with the Brillouin modulus decrease [42]. These results are also consistent with previous mechanical tests on shaved-off corneas that showed decreasing elastic modulus across the corneal depth [43]. This correlation strongly supports the hypothesis that LASIK surgery, by depleting the cornea of some of its stroma with high modulus, will lead to decreased overall modulus and stiffness. The Brillouin technology will be sensitive enough to detect such change.

3. In vivo animal studies:

Safety and efficacy of the Brillouin technique in mice in longitudinal study in vivo.

A total of 12 mice (C57BL/6 strain) were tested; 11 mice up to 40 weeks of age were imaged at various ages only once each, and a single mouse was imaged weekly from day 17 after birth till 9 weeks later [44]. **Figure 6** depicts measured Brillouin frequency shift in mouse ocular lens tissue, which clearly shows an increasing trend with age. We have not observed any complications due to imaging. In addition, it is evident that the repeated measurements on the same mouse follow the trend of the curve obtained on separate mice; this further confirms the non-perturbative, non-disturbing character of our measurements.

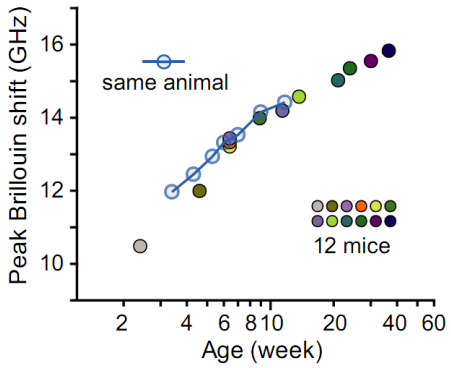

Figure 6: Peak frequency shifts of murine lens nucleus in vivo measured from individual mice at various ages (solid circles) and one mouse over time (open circles)

4. Ex vivo human studies :

Brillouin technique detects lower elastic modulus in ectatic corneas.

We have tested 4 ectatic (weakened) corneas from keratoconus patients whom underwent corneal transplantation, and 5 normal donor corneas. As shown in **Fig. 7**, the profiles of ectatic vs. normal corneas are dramatically different. Compared to normal controls, ectatic corneas have lower mean elastic modulus (**Fig. 7c**) and increased slope, i.e. the rate of elastic modulus drops across depth (**Fig. 7b**). The negative Brillouin slope of ectatic corneas was 0.94 ± 0.15 GHz/mm, showing a statistically significant increase from 0.4 ± 0.25 GHz/mm for the controls (unpaired t-test $P < 0.0025$); the mean Brillouin modulus was 2.58 ± 0.096 GPa for ectatic corneas and 2.72 ± 0.06 GPa for normal corneas (unpaired t-test $P < 0.05$). Also the stiffness, i.e. the integral under the elasticity

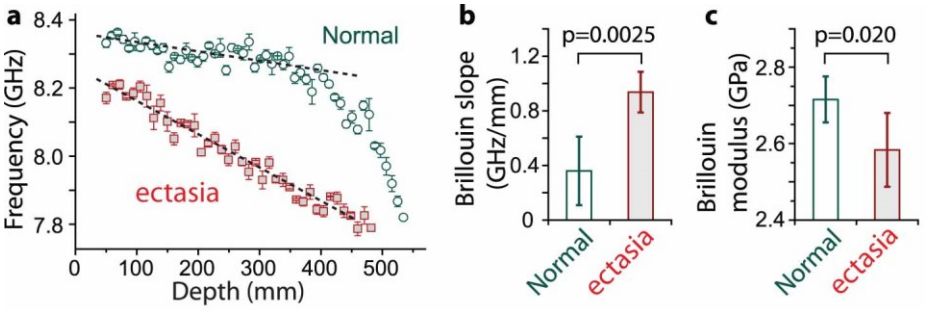

Fig. 7: (a) Brillouin profile of human corneas *ex vivo* obtained from normal and keratoconus/ectasia patients. The differences in the slope (b) and the mean modulus (c) are statistically significant.

curve (not shown), is significantly lower in corneas with ectasia. *This suggests that the Brillouin parameters, such as slope, modulus and stiffness, are appropriate and sensitive metrics to detect ectasia.*

5. In vivo human safety study

Brillouin technique can be applied to human subjects to measure cornea and lens elasticity (10 subjects).

As part of an ongoing safety study approved by PHC IRB (IRB#2008P002176), we have performed the Brillouin imaging procedure on 10 healthy subjects (20 - 60 years old), who have reported no discomfort, or complications during or after the imaging session. We have completed the study, including the 6-month follow up exam on eight of the study subjects on March 13 2014. The Brillouin test has not caused any ocular problems in this subject. Figures 8a and 8b show representative high resolution Brillouin profiles with a pixel integration time of 0.4s of the cornea and the lens, respectively. Axial resolution was estimated to be about 60 μm enabling local measurement of the elasticity of cornea and lens [31]. Lens data also suggests age-related stiffening, occurring mainly from the thickening of the stiffer nucleus portion of the lens. Such stiffening has long been suspected to be the main cause for the onset of presbyopia, but it had never been measured *in vivo*.

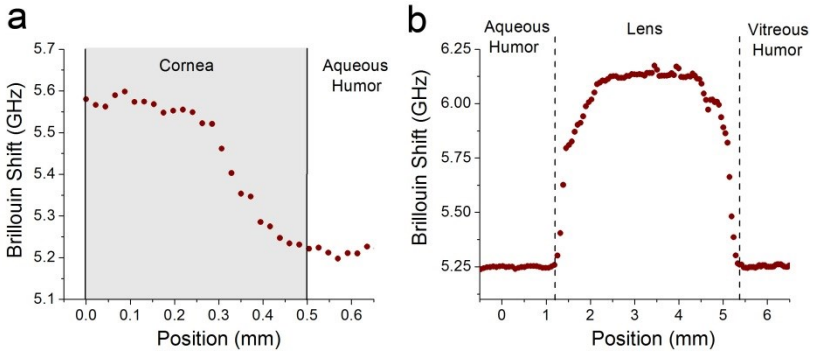

Fig. 8: In vivo Brillouin scan (at 780 nm) of the human cornea (a) and lens (b)

6. *In vivo* human study results

Brillouin measurements are reproducible and sensitive to the targeted corneal changes.

To calculate the sensitivity of the measurement, we used the *in vivo* data of the aqueous humor, which can be considered as a reference material with constant Brillouin shift; at 0.4s integration time, we computed the sensitivity of the instrument to be ~ 30MHz. *This is about 10-15 times better than the measured difference between normal and ectatic corneas.*

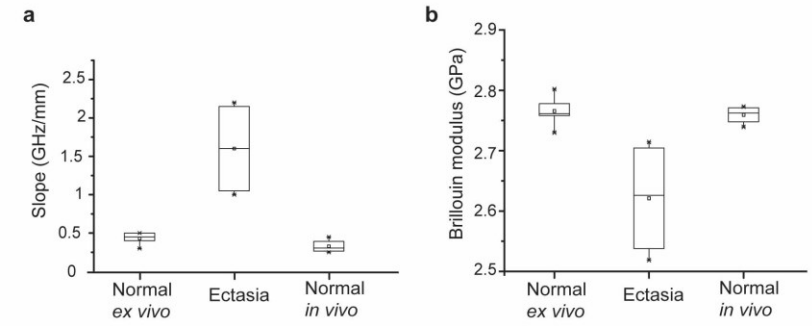

Fig. 10: Slope (a) and mean modulus (b) for the human corneas ex vivo (normal, ectasia) and in vivo (healthy individual)

To verify the reproducibility and non-perturbative nature of the *in vivo* measurements, we compared four axial scans of the cornea of the same human subject and quantified the variation of slope and mean modulus. We obtained the slope (normalized for comparison with *ex vivo*) of 0.36 ± 0.14 GHz/mm and modulus of 2.76 ± 0.015 GPa. The *in vivo* values are consistent with the *ex vivo* measurements in normal subjects (no statistically significant difference with one-way ANOVA) and show a statistically significant difference (unpaired t-test $P < 0.01$) with the *ex vivo* ectasia corneas (**Fig. 10**). *The measurement is clearly reproducible as the variations from scan to scan are small compared to the variation we expect to find due to ectasia.*

C) RATIONALE BEHIND THE RESEARCH, AND THE POTENTIAL BENEFITS TO SOCIETY

The Brillouin microscopy is a safe and non-contact technique that would overcome many of the current state of the art technology limitations by providing high resolution 3D spatially-resolved information of the elastic modulus of the cornea.

Significance of Brillouin microscopy for the keratoconus and the LASIK surgery

This research will introduce a novel diagnostic test for early diagnosis of keratoconus and refractive surgery screening that targets the proper biomechanical parameters. As for keratoconus, the novel diagnostic tool is expected to enable early identification and better management of disease progression as well as development/monitoring of customized treatment via the corneal crosslinking. As for LASIK surgery, this study is expected to drastically change the current status quo where many normal eyes are denied the beneficial treatment for vision correction while predisposed "weak" corneas are missed and operated upon with terrible consequences. Because of this study, early keratoconus patients that are currently not diagnosed, could be recognized and treated at a time where the progression of the disease can still be halted. Furthermore, the technique will serve as an indispensable tool to monitor and optimize the most promising treatment (currently in clinical trials in the US) to stop keratoconus progression, i.e. the corneal collagen crosslinking that is based on the stiffening of the corneal stroma and can be easily characterized by the Brillouin technology [30]. As for LASIK, because of the validation of the Brillouin technology, subjects with weak corneas could be screened before the LASIK surgery and consulted to avoid the operation. In addition, since the Brillouin instrument will provide 3D-resolved information on corneal elasticity, this research will pose the foundation to develop less invasive, possibly customizable, laser surgical procedures based on biomechanical anomalies.

The broader clinical significance - Several corneal conditions present with altered biomechanical properties (e.g. keratoglobus, and pellucidal marginal degeneration). Developing the Brillouin microscopy into a viable clinical ophthalmology exam will improve the diagnosis, management and treatment of these conditions. In addition, the current assessment of intraocular pressure (IOP) via tonometry, crucial for the glaucoma diagnosis and management, is heavily biased by the differences in corneal thickness curvature and stiffness among the general population. Only the thickness and curvature, however, can be currently accounted for [45]. The Brillouin technique will enable us measuring the corneal stiffness, which will improve the accuracy of IOP measurements thus providing an improved screening and management of glaucoma patients. Hence, the Brillouin instrument validated in this proposal can become a widespread diagnostic device for the corneal examinations.

The feedback from the clinical community - The clinical significance of development of the Brillouin microscopy for detection of the corneal ectasia has been recently recognized by the

ophthalmology community. Two highly competitive research grants have been awarded for the research involved with this pilot study by Harvard Catalyst Advanced Imaging Pilot program and by the American Society for Laser Medicine and Surgery.

The positive medical impact - This research will lead to large-scale clinical studies of Brillouin imaging for keratoconus and ectasia patients. If these are successful and the Brillouin imaging is demonstrated capable of detecting biomechanical anomalies in patients with early keratoconus and post-LASIK ectasia, improved diagnosis and screening protocols will soon follow. In addition, the comparison of the biomechanical and topographical features during the disease progression will also crucially advance our mechanistic understanding of the keratoconus and ectasia development.

II. SPECIFIC AIMS

Our *long-term goal* is to enable early, reliable identification of individuals at high risk to develop keratoconus and ectasia. The *objective* here is to quantify the biomechanical changes involved in keratoconus and in post-LASIK ectasia patients by using Brillouin optical imaging. Our *central hypothesis* is that low Brillouin modulus/stiffness is an early indicator of keratoconus progression and a predictor of post-LASIK ectasia occurrence. This hypothesis was formulated on the basis of the consensus in the literature regarding the biomechanical origin of ectasia development and our preliminary estimation about the mechanical sensitivity of Brillouin optical imaging. The *rationale* for this research is that the collected preliminary data will enable designing two important clinical studies focused on biomechanical characterization of keratoconus progression and pre-operative elasticity-based screening of LASIK patients. The proposed research consists of three *specific aims*:

Specific Aim 1: Compare biomechanical properties of eyes affected by Corneal disease vs. normal corneas

The objective of this aim is to compare the biomechanical Brillouin parameters of keratoconus patients, Fuch's Endothelial Corneal dystrophy patients, and Dry Eye Syndrome patients vs. healthy individuals to determine the diagnostic sensitivity of Brillouin measurement. Our working hypothesis is that the mechanical degradation of the corneal tissue involved in corneal disease progression can be detected by the Brillouin-derived metrics. This hypothesis is supported by previous studies *ex vivo* that have found that human keratoconus corneas to have reduced elastic modulus [46-48], an altered collagen organization [49, 50] and reduced number of crosslinks [51], and by our previous studies that have shown that the Brillouin imaging is highly sensitive to the organizational and crosslinking variations in collagen networks [42, 52]. The data collected here will allow us to design clinical studies to test the hypothesis that biomechanical changes develop early in keratoconus before irregularities in corneal morphology occur.

Specific Aim 2: Compare biomechanical properties of post-LASIK ectasia vs. normal corneas

The objective of this aim is to compare the biomechanical Brillouin parameters of patients who developed post-LASIK ectasia and patients who had no complications after LASIK surgery to determine the difference in the elasticity between normal post-operative corneas and corneas that developed the complication. The working hypothesis of this aim is that the post-LASIK ectasia patients will show lower corneal elastic moduli, lower stiffness values, and higher slope levels than subjects without complications. This hypothesis is strongly supported by our preliminary data on ectasia vs. normal cases *ex vivo*. The rationale of this aim is that quantifying the difference in corneal modulus between post-operative diseased vs. post-operative normal controls, together with the measurements of Aim 1, will determine what corneal elasticity levels pre-operatively are to be considered at risk.

Specific Aim 3: compare biomechanical properties change before and after laser vision correction surgeries

The objective of this aim is to compare the biomechanical Brillouin parameters of partients before and after laser vision correction surgeries, i.e., the photorefractive keratectomy (PRK) and LASIK surgeries. The working hypothesis of this aim is that post-operative corneas will present lower Brillouin stiffness values compared with their pre-operative status, despite Brillouin modulus remaining the same. The rational of this aim is that quantifying the difference of Brillouin stiffnes between the pre- and post-operative corneas will help us understand the biomechanical property changes induced by corneal refractive procedures, and together with aims 1 and 2, will strenghen pre-screening to exclude surgery candidates at risk.

III. SUBJECT SELECTION

Based on the biostatistical considerations (details are explained in Section VI) estimated upon our preliminary data, we have calculated that 168 patients subdivided in 8 groups are necessary and sufficient to address the specific aims of this project. Inclusion/exclusion criteria and source of subject recruitment methods are described in this section.

A) GENERAL INCLUSION/EXCLUSION CRITERIA

This study **includes** anyone who has a clear enough cornea or clear enough media to permit imaging, and who fulfills all specific inclusion/exclusion criteria of one of the following study groups. **Excluded** from this study are specific volunteers who have occludable narrow angles (without a patent peripheral iridotomy). Also monocular volunteers will be excluded. Subjects will be excluded if they do not or cannot understand or follow the instructions for the imaging. For subjects who do not speak English, the PHRC policy on "Obtaining and Documenting Informed consent of Subjects who do not speak English" will be followed. For signing of the informed consents, volunteers who do not speak English will be given a written translation of the short form of the entire English version of the consent form in a language understandable to them and will be communicated with through a native speaking staff or the interpreter service at MEEI/MGH.

B) SPECIFIC INCLUSION/EXCLUSION CRITERIA

Group I: Normal healthy subjects (age 20 - 60, N = 15)

This group includes healthy subjects with normal appearing corneas respecting all the general inclusion/exclusion criteria, with less than ± 3 dioptres refractive correction, normal corneal topography (no irregular bow-tie, no skewed axis, no scar or other irregularities), and no history of eye diseases, except presbyobia and/or cataract.

Group II: Keratoconus subjects (age 20 - 60, N = 45)

This group includes subjects classified as patients with mild, moderate, or advanced keratoconus. Inclusion criteria are an irregular cornea determined by distorted keratometry mires, distortion of the retinoscopic or ophthalmoscopic red reflex (possibly a combination of the two) and slit-lamp biomicroscopic signs such as Vogt's striae or Fleischer's ring of over 2 mm arc or corneal scarring consistent with keratoconus. The inclusion criteria also extend to forme-fruste keratoconus, or subclinical keratoconus, i.e. fellow-eyes of mild, moderate, or advanced keratoconus. The inclusion criteria also extend to advanced keratoconus patients scheduled to receive corneal transplant of the keratoconic eye.

Group III: post-LASIK patients with no complications (age 20 - 60, N = 5)

This group includes healthy subjects who have undergone LASIK surgery in the past 12 months without complications. Inclusion criteria are normal post-operative topography and pachimetry and a clinician evaluation of the normal post-operative corneal conditions.

Group IV: post-LASIK patients with complication of ectasia (age 20 - 60, N = 3)

This group includes patients who have been diagnosed with post-LASIK ectasia after having undergone LASIK surgery more than 12 months prior to our scheduled Brillouin analysis. The number of patients in this category is reduced to 3 based on recruiting considerations due to the history of reported cases of post-LASIK ectasia at MEEI which may limit the number of eligible patients within the projected duration of this study. Our statistical considerations, however, demonstrate that imaging 3 patients will allow us to achieve the specified aims. The inclusion criterion is the clinical diagnosis of post-LASIK ectasia based on topography, pachymetry and clinician evaluation.

Group V: Patients who are scheduled for PRK surgery (age 20 - 60, N = 10)

This group includes patients who have been diagnosed with myopia and have been scheulded to undergo PRK surgery. Patients with highastigmatism > 2 diopter, prior ocular surgeries, and those patients taking any ocular medications except seasonal allergy medicine such as ketotifen or artificial tears will be excluded.

Group VI: Patients who are scheduled for LASIK surgery (age 20 – 60, N = 10)

This group includes myopic patients who are scheulded to receive LASIK surgery. Patients with high astigmatism > 2 diopter, prior ocular surgeries, and those paitnets taking any ocular medications except seasonal allergy medicine such as ketotifen and artificial tears will be excluded.

Group VII: Patients who are diagnosed with Fuch's endotheliul corneal dystrophy (FECD) (age 20-75, N = 40).

This group includes subjects who are diagnosed with Fuch's corneal dystrophy, at early, mild and advanced stages. The inclusion also extends to subjects with, and without keratoconus. But this exclude patients with any other corneal disorders other than keratoconus, and/or history of ophthalmological surgeries that may affect endothelium cell status, e.g. cataract surgeries.

Group VIII Patients who are diagnosed with Dry Eye Syndrome (Keratoconjunctivitis Sicca) age 20-60, N=40).

This group includes patients (age 20-60) who are diagnosed with Dry Eye Syndrome (Keratoconjuctivitis Sicca). This group included subjects who are diagnosed with Dry Eye Syndrome at early, mild, moderate, and advanced stages. The inclusion also extends to subjects with and without keratoconus. It excludes patients with any other corneal disorders other than keratoconus, and/or history of ophthalmological surgeries that may affect endothelium cell status such as previous corneal transplants, cataract surgeries, etc.

All subjects will be recruited by the Wellman Center for Photomedicine (MGH) through Partners HealthCare (PHC) "All Users" email announcements (studies seeking volunteers), ads posted at PHC campuses, flyers and classified internet advertisements. Group II and IV subjects will be

additionally recruited among Massachusetts Eye and Ear Infirmary (MEEI) patients through the procedures described below.

IV. SUBJECT ENROLLMENT

1) Volunteers responding to the PHC email announcement or the ads will contact a member of the study staff who is available to answer any question regarding the study. To enroll a volunteer, a member of the study staff will go through an IRB approved questionnaire on the phone to determine whether a volunteer fulfills inclusion/exclusion criteria to be recruited and go on for the routine eye exam at Massachusetts Eye and Ear Infirmary (MEEI). The member of the study staff will then schedule an appointment for the routine eye exam at MEEI based on the pre-determined time availabilities agreed among the study staff; otherwise he/she will obtain the patients permission to be contacted by the study staff in order to schedule an appointment for the routine eye exam at MEEI. The study staff will also ask permission to contact the subject via regular or electronic mail to provide: confirmation of the time and date of the appointment; address, directions and parking information regarding MEEI; and a copy of the consent form.

2) Subject recruitment can occur from the MEEI or Boston Laser (1101 Beacon Street, Suite 6 Brookline, MA 02446) patient pool. The study coordinator at MEEI or Boston Laser will access medical records, patient registries or clinical databases to identify potential subjects. To ensure that patient privacy is protected and that the individual patient is appropriate to participate in the research, the first contact with potential subjects identified through their private health information will not be made by the study investigators. Rather, the patient's primary doctor or eye specialist will be involved.
   In particular, the patient's primary doctor or eye specialist who has first hand knowledge of the patient's medical history must (1) give approval for his/her patient to be contacted for research purposes, (2) initially introduce the study to the patient and (3) obtain the patient's permission to be contacted by study staff.

Subject recruitment can occur among patients seeing Roberto Pineda (MEEI site PI), Amy Scally (co-investigator), Matthew Gardiner (co-investigator), Sheila Borboli-Gerogiannis (co-investigator), Amy Watts (co-investigator), Samir Melki (co-investigator), Ula Jurkunas, Joseph Ciolino, or Sherleen Chen. In this case, special precautions will be taken to avoid the possibility that the patients may feel obligated to participate because they are being asked by their treating physician. The study staff and treating physician will make clear that participation is voluntary, that patients do not have to participate, and the decision not to participate will not affect their care, now or in the future. Further, to minimize the possibility that patients will feel obligated to participate, patients will be

- (a) initially contacted about the research study through a recruitment letter, and/or
- (b) Patient’s primary physician will mention the research study to the subjects and give the interested subjects the contact info of the study staff so that the subjects initiate the enrollment procedure or ask additional questions about the research study; A flyer containing an introduction of the study and a picture of the imaging system will be either shown to the patients or put up in the physician’s office; and/or
- (c) Patient’s primary physician will ask permission to the subjects for them to be contacted by the study staff for further information about the study and possible enrollement; and/or

- (d) If patients are interested, enrollment and consent procedures will be preferentially administered by a member of the study staff rather than the treating physician

If a patient were to express genuine interest in participating in the study and wanted to enroll into the study, the treating physicians may provide the PHC IRB approved consent form to the patient, and if the patient consents to the study, they may perform the initial eye exam or provide contact info of the study staff to schedule the participation in the study.

Upon enrollment, each volunteer will be registered as patient at MEEI. A member of the study staff will use a PHC IRB approved consent form and one of the co-investigator physicians at MEEI will perform the initial eye exam. They will also confirm the eligibility of each volunteer to participate in the study. The subject then will then be accompanied by study staff to Wellman Center at MGH main campus for the subsequent Brillouin imaging, or will schedule the Brillouin imaging session for a later date with a member of the study staff.

A copy of the signed consent form will be given to the subject to keep for her/his records.

V. STUDY PROCEDURES

The study procedures involve an initial eye examination at MEEI to validate the subject eligibility plus the study imaging session at Wellman Center (MGH). The detailed description of which is provided below.

A) EYE EXAM AT MEEI

On appointment day at MEEI, a member of the study staff will explain the study to the volunteer and obtain consent at MEEI prior to the eye exam. The standard eye exam may include medical and ocular history taking, manifest refraction test, slit lamp biomicroscopy of anterior segment, intraocular pressure measurement, and corneal topography (Pentacam). After the Pentacam, a member of the study staff will confirm which eye(s) are eligible for the study by marking and signing the consent form.

Before the Brillouin imaging session, the study staff will obtain the signed consent form and a copy of the pentacam of the study subject after all the identifying information has been deleted.

For the Brillouin imaging session, the subject will be escorted by a member of the study staff to Wellman Center (MGH); alternatively, the Brillouin imaging session can be scheduled for a later date at the convenience of the subject; in this case, an appointment can be taken on that day or the study staff can obtain permission to contact the study subject for scheduling purposes.

If an unexpected condition were to be found during the eye examination, the volunteer would be informed and recommended to follow up with her/his regular eye doctor. If the volunteer does not have a regular eye doctor, Dr. Pineda will refer her/him to an appropriate specialist for treatment. If treatment for the condition were needed, this would certainly take precedence, and the volunteer would not be enrolled in the study.

New subjects will be recruited instead after amending the protocol for recruiting additional subjects for the corresponding group.

B) STUDY IMAGING SESSION AT WELLMAN CENTER (MGH)

At MGH, the subject undergoes Brillouin imaging on one eye only. The study procedure, Brillouin microscopy, will be explained to the subject again when he/she arrives at the Wellman Center, and will be given opportunity to ask questions. If the study subject decides to go on with the study, the determination of which eye will be imaged will follow.

Determination of which eye is eligible for Brillouin microscopy examination. Subjects of Group I will be given the choice to decide which eye they want to be examined by Brillouin microscopy. The subjects, however, will be encouraged to choose the eye they do NOT usually use for monocular activities in their daily lives such as taking pictures. Subjects of Group II – VIII will also be given the choice to decide which eye they want to be examined by Brillouin microscopy if in the eye screening visit both of their eyes were deemed eligible for the study. This will occur if they have bilateral keratoconus (Group II), if they had LASIK surgery on both eyes (Group III), if they are diagnosed with post-LASIK ectasia in both eyes (Group IV), if they are diagnosed with Fuch's dystrophy in both eyes (Group VII), and if they are diagnosed with Dry Eye Syndrome (Group VIII). The subjects can also choose to have both eyes scanned. However, if in the eye screening process, only one of the subject's eyes (corneas) is deemed eligible for our study, the patient will be informed that the test could be performed only on the eligible eye. At this time, the subject will have an opportunity to consent to the procedure on that eye or withdraw from the study. If the subject consents to the procedure, he/she will circle the Left or Right eye segment on the consent form and sign the consent form recording the date and time before the imaging procedure. The MGH co-investigator, either Dr. Eltony, or Dr. Shao will also confirm the designation of the study eye on the consent form, and sign it with date and time as well. If, on his/her own, the patient expresses interest in having both eyes examined and both eyes are found eligible for Brillouin microscopy examination, then he/she will circle both the Left and Right eye segment on the consent form and sign the consent form recording the date and time before the imaging procedure. The MGH co-investigator, either Dr. Eltony, or Dr. Shao will also confirm the designation of the study eye on the consent form, and sign it with date and time as well.

Brillouin imaging session During the imaging session of the cornea the subject will be asked to sit calmly in a chair with his/her chin and forehead on a chin and forehead rest, respectively. These subject contact areas will be cleansed with alcohol wipes in-between volunteers. Additionally, the subject may be asked to use a "bite bar" during the Brillouin imaging because of its great sensitivity to motion in high resolution mode. A bite bar is a piece of Plexiglas covered with sterile dental-impression material. The subject will make an impression of his teeth in the bite bar, and rest his teeth in this impression for the duration of each scanning to help immobilize his head. The not-to-image eye will be covered with an eye patch. At operator's instructions, the subject will fix his/her eye on a small red or green blinking light, or onto a projected object such as a Maltese cross. No mechanical devices for direct immobilization of the eye will be used. The operator will adjust the chin rest, the head position, and the orientation of the Brillouin instrument in order to create the best accessible angle to start imaging of the cornea. The localization of the near infrared laser in the eye, and the eye gaze angle will be recorded with the white-light bright field microscopy. Then, the subject will be asked to hold still while fixing the study eye on the blinking light or projected object for up to 5 to 10 s during which the instrument gathers the Brillouin spectrum. The subject will be asked to comply with this repeated procedure, 5-50 times per eye to gather in-line depth data in a spatially-resolved pattern on the cornea. Each line scan will acquire about 15 to 40 points across the corneal depth and will take no longer than 10 s. Short break between the imaging scans will be provided to rest and to blink the eye. The entire imaging or scanning session may take up to 60

minutes in total. The subject may ask for breaks or stopping the imaging session at any point. During the pause between imaging sessions, the subject will be asked whether they have felt any discomfort related to their positioning, with the imaging near infrared light (λ=780 nm), or with the imaging procedure.

Follow-up Brillouin imaging sessions for patient in Group V and VI Two follow-up Brillouin imaging sessions will be conducted at: 1 week, and 4-10 weeks after the surgery for patients in both Group V and VI. The imaging sessions will be accomplished after the patients' follow-up checks at MEEI with their physician and the same imaging protocols will be used, as described above. At the follow-up checks at MEEI, the patients' eye of interest will be imaged with both Pentacam, and an optical-cohernece tomography imaging system by Dr. Amy Scally to measure the corneal thickness. The Brillouin imaging sessions will be subject to the physician's confirmation of the patient's recovery status and eligibility to continue the study.

If a volunteer that enrolls in the research study has undergone an eye exam comparable to the one that would be given at the beginning of the research study (STEP A) within the past year, the subject may elect to waive the initial eye exam (STEP A). In this case, the subject will be asked written permission that the file of his previous visit, and in particular his/her topography and pachymetry exams, can be reviewed by Dr. Pineda or Amy Scally to confirm eligibility. For the purpose of this study, the information needed is a routine eye exam for eligibility purposes; therefore a baseline eye exam undergone in a few-month window before the study is sufficient to provide the required information.

If a patient of MEEI or Dr. Pineda's or Amy Scally's that enrolls in the research study is scheduled to undergo standard check-up examinations at MEEI, as part of his routine eye care, within a period of two years following the imaging session, the subject will be asked permission that the information regarding the pachymetry tests or Pentacam can be analyzed by the study staff, in order to correlate the Brillouin signatures with the longitudinal evolution of the patient's cornea topography.

If a patient expresses interest in a follow-up visit and/or Brillouin examination, they will be scheduled at the end of the first visit or the patient will be contacted by the study staff for scheduling.

A remuneration of $200 will be offered for the completion of the Brillouin imaging study. If the subject is determined to be ineligible to participate in the study after the routine eye exam, he/she will receive the reimbursement of $25 for the screening exam. The payment by check will be mailed to the subject.

The volunteers will not be charged for the visit or for Brillouin imaging session. Subjects may receive reimbursement for transportation and a parking voucher or $5 reimbursement for MBTA tickets. If the subject is coming from more than 200 miles away for the imaging, then we will reimburse airfare, meals, and transportation expenses to and from the airport.

A study subject may contact study PIs for any concerns or questions he/she may have after the study procedures are completed. The contact person at the Wellman Center (MGH) is Andy (SH) Yun, PhD, Phone # 617-768-8698, and the contact person at the MEEI is Roberto Pineda, MD Phone # 617-573-3234 or 617-573-4393.

VI. BIOSTATISTICAL ANALYSIS

A) SPECIFIC DATA VARIABLES COLLECTED FOR THE STUDY

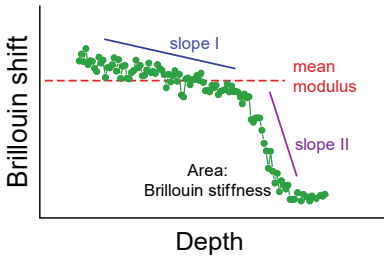

Fig. 11: Definition of Brillouin elasticity parameters.

Axial scans in different locations of the cornea may be performed: one scan in the center, and four in cross pattern at 2-3 mm distance from the center. In particular, one scan covers the region where keratoconus/ectasia is most prominently developing, and another axial scan diametrically 180° opposite to it. Axial scanning is performed by electronically moving the objective lens with speeds ranging between 30 and 80 μm/s. The Brillouin spectra are recorded with frame integration times between 0.1 and 1 s. The laser power at the sample will be below 3 mW. The beam scanning, CCD acquisition and spectral analysis are synchronized with LABVIEW and MATLAB programs. The axial profile of the Brillouin shift is plotted against the corneal depth (**Fig. 11**) and calculated using the refractive index of the anterior chamber measured by the pre-test eye exam (low-coherence interferometry).

Elasticity-based metrics derived from Brillouin images.

Axial analysis: **Fig. 11** shows a typical Brillouin axial scan of a cornea and pictorial representation of several parameters that can be extracted with their respective definitions. Mean Brillouin modulus is defined as the spatial average (Pa); it can be considered the closest quantity compared to the macroscopic modulus measured in traditional mechanical tests. The slope (GHz/mm) is the rate of change of modulus across depth. Brillouin stiffness is the integral of the modulus over depth, i.e. the area under the curve (Pa·mm). All the parameters can be calculated for the entire cornea or for a specific depth region. *Regional variations:* From Brillouin measurements, we also expect to capture the biomechanical differences between ectatic and normal areas within the same cornea. These measurements represent an ideal intracorneal positive control for our technology assessment.

B) STATISTICAL METHODS AND POWER ANALYSIS

Specific Aim 1: Biomechanical comparison of eyes affected by Corneal disease vs. Normal corneas

Hypothesis: Keratoconic corneas have lower (reduced) Brillouin modulus and stiffness compared to normal. Fuch's Endothelial Corneal Dystrophy corneas and Dry Eye Syndrome corneas will exhibit different biomechanical properties due to corneal hydration changes.

Subjects: (Group I: Normal, N = 15); (Group II: Keratoconus, N = 25); (Group VII: Fuch's Endothelial Corneal Dystrophy, N=40); (Group VIII: Dry Eye Syndrome, N=40).

Analysis Plan: The relationship between normal vs. eyes affected by corneal disease (dichotomous explanatory variable) and Brillouin metrics (continuous outcome variable) will be investigated using unpaired t-tests.

Sample size and Power: Advanced keratoconic corneas have been previously measured elsewhere to have a Young's modulus of 28 ± 4.7 MPa compared to 57 ± 4.1 MPa of normal corneas [47]. Using the Young's-to-longitudinal modulus conversion [42, 52], we expect to observe (acquire) Brillouin moduli of 3.57 ± 0.02 GPa and 3.66 ± 0.01 GPa for keratoconic and normal corneas, respectively. If the true difference is comparable or greater than this estimation, a sample size of 5 keratoconus vs. 5 healthy controls allows rejecting the null hypothesis that the population means of the experimental and the control groups are equal with the power of 0.9. The Type I error probability (p-value) associated with this test equals 0.05. The stiffness parameters that include the thinning

effect of keratoconus are expected to have higher separation and thus will have sufficient power and type I error probability.
An additional number of 40 patients is added to establish a correlation between corneal stiffness and progression of the disease. As independent variable we will use the K-max obtained from pentacam evaluation which varies from ~40D in mild cases to ~70D in advanced keratoconus. As preliminary results from this study, a slope of -4 MHz/D and a correlation coefficient of 0.87 was found between the minimal Brillouin modulus and K-max (σ_x=13.82D) in moderate to advanced keratoconus. If the true slope of the line obtained by regressing Brillouin modulus against K-max is the same for mild cases, the use of 40 patients will enable to reject the null hypothesis that this slope equals zero with power of 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. Furthermore, the additional number of subjects will inform us on the different mechanical patterns associated with the progression of keratoconus vs normal corneas.

Specific Aim 2: Biomechanical comparison of post-LASIK ectasia vs. normal corneas
Hypothesis: Patients with post-LASIK ectasia will present lower Brillouin modulus/stiffness and higher axial slope than post-LASIK patients without complication.
Subjects: Post-LASIK ectasia, N = 3 (Group III); Post-LASIK no complications, N = 5 (Group III)
Analysis Plan: The relationship between ectasia vs. normal post-operative conditions (dichotomous explanatory variable) and Brillouin parameters (continuous outcome variable) will be investigated using unpaired t-tests.
Sample size and Power: Assuming standard deviation comparable to preliminary data, the sample size of 3 post-operative ectasia cases vs. 5 post-operative no-complication controls allows to detect a true difference of < 0.4 GHz/mm (slope) and < 0.12 GPa (mean modulus) with 0.05 one-tailed alpha and > 80% power. From preliminary data, we estimate the separation between normal and ectasia cases to be 0.58 GHz/mm in slope and 0.14 GPa in mean modulus. Therefore, the sample size is expected to detect the desired difference with sufficient power and type I error. The stiffness parameter has shown higher separations in preliminary data, and it is expected to have sufficient power and type I error for this analysis as well.

Specific Aim 3: Biomechanical comparison of before and after laser vision correction surgeries
Hypothesis: Patients with post-LASIK ectasia will present lower Brillouin modulus/stiffness and higher axial slope than post-LASIK patients without complication.
Subjects: Patients scheduled to receive PRK surgery, N = 10 (Group V) and LASIK surgery, N = 10 (Group VI)
Analysis Plan: The relationship between pre- vs. post-operative conditions (dichotomous explanatory variable) and Brillouin parameters (continuous outcome variable) will be investigated using paired t-tests.
Sample size and Power:
Assuming PRK and LASIK surgeries have similar effects on biomechanical properties of human cornea, and that standard deviation comparable to preliminary data, the sample size of 10 patients allows to detect a true difference of < 0.03 GPa (mean modulus) with 0.05 alpha and > 90% power. This is about 1% of the mean Brillouin modulus of normal cornea according to our preliminary data. Other studies reported 10 – 20% change of biomechanical properties in samples received LASIK treatment [68]. Therefore, the sample size is expected to detect the desired difference with sufficient power and type I error.

C) STUDY ENDPOINTS

1) The studies in Aim 1 will provide *biomechanical metrics for keratoconus diagnosis, Fuch's Endothelial Corneal Dystrophy diagnosis, Dry Eye Syndrome diagnosis and progression analysis.* If the data of Aim 1 show statistically significant differences in Brillouin parameters between normal and corneal disease subjects, we will compute the performances of a Brillouin diagnostic test for keratoconus, Fuch's Endothelial Corneal Dystrophy, and Dry Eye Syndrome. For this, Brillouin metrics will be treated as continuous predictors of dichotomous disease outcome. Receiver operating characteristic (ROC) curves will be traced and one- or two-parameter cutoff criteria for optimal sensitivity/specificity will be calculated. These criteria will be tested in a *future clinical study* including subjects with subclinical corneal disease (i.e. fellow eyes of patients). The data collected here will also provide sufficient information on effect size to design a clinical study aimed at characterizing the biomechanical progression of keratoconus, Fuch's Endothelial Corneal Dystrophy, and Dry Eye Syndrome. In addition, the analysis of the regional variation may provide preliminary data for a clinical study where Brillouin vs. topographical maps are compared to evaluate mechanical and morphological changes in corneal disease.
2) If the t-test comparison in Aim 2 shows statistically significant differences in Brillouin parameters (slope, modulus and stiffness) between the ectasia vs. healthy post-operative conditions, we can evaluate the performance of Brillouin microscopy as a diagnostic test for post-op ectasia. To do this, we will analyze the same datasets using Brillouin parameters as continuous predictors of disease outcome. Assuming the data to be distributed normally, the power calculation of the previous section remains valid for the receiver operating characteristic (ROC) curves [53]. In addition to the one-parameter ROC curves based on single Brillouin metric, we will trace ROC curves employing two parameters, slope plus modulus or stiffness. These ROC curves will establish optimal cut-off criteria for maximum sensitivity and specificity of the Brillouin test of ectasia in post-operative conditions. To translate these results in preoperative conditions and evaluate the optimal cut-off criteria for a Brillouin screening test of ectasia, we will account for the "baseline" mechanical change of LASIK operations taking into account the measurements in healthy individuals, i.e. calculating the reduction of corneal stiffness and modulus that LASIK operation induces in normal patients without complications. In this way, we will be able to estimate a lower bound of corneal modulus/stiffness that can be considered at risk to develop ectasia pre-operatively.

VII. RISKS AND DISCOMFORTS

COMMON: Risks are minimal as the Brillouin Ocular Scanner (BOS) light source power is within ANSI and ISO standards, and it is also *less than that of the current commercially available systems that use similar light sources*. The Brillouin Ocular Scanner has been approved by the radiation safety office and biomedical engineering office at Massachusetts General Hospital. In case of any emergency, a mechanical shutter will block the near infrared laser beam by the push of an emergency button. NO eye dilatation is needed for Brillouin imaging, and discomfort from eye straining due to not being able to blink during the scanning. Subjects will be escorted by study staff from MEEI to MGH. They will be constantly monitored and asked about their well-being. Risks of infection are minimal as the BOS obtains images without direct contact to the eye itself. Cleansing the chin and forehead rests with alcohol wipes in between patients minimizes the risk of infection from subject to subject. The dental impression material of the bite bar will be discarded after each imaging session.

UNCOMMON: We are not aware of any uncommon risks. To ensure compliance with the ANSI 2007 standards, the light source power used in the Brillouin imaging is measured before each imaging session. The values are logged into the experiment book for each imaging session and kept on record. If any adjustments are made to the system during a session, the power will be measured and recorded again before continuing the imaging session.

VIII. POTENTIAL BENEFITS

The volunteers who participate in the study may benefit from the eye examination performed at MEEI, which may confirm that the individual has normal eyesight or which may uncover a disorder. Should this technology prove effective in measuring corneal elasticity at high sensitivity, there will be a potential benefit to the subjects enrolled in the study to gather information about the elasticity of their cornea. This can be beneficial if and when decisions have to be made regarding the opportunity to undergo refractive surgery. In addition, this could give more accurate Intra-Ocular Pressure (IOP) reading by correcting the elasticity bias. If successful, Brillouin Microscopy may become a new gold standard to diagnose the onset and the progression of eye diseases such as keratoconus, and may detect at-risk patients for LASIK surgery.

IX. MONITORING AND QUALITY ASSURANCE

The laser light exposure in this study is within documented safe limits set by the American National Standards Institute (ANSI 2007) and ISO 15004-2:2007. In addition, Brillouin technology operates at light exposure levels that are less than that of the current commercially available systems that use similar light sources.

The principal investigators will be available to discuss and monitor any changes in the health of the subject's eyes after the imaging sessions. If any problem related to the study imaging session is depicted, the study will be immediately altered or stopped, and actions will be taken depending on the severity of the injury (see below). All forthcoming appointments will be cancelled by contacting the volunteers by phone. The results from the Brillouin imaging study will be monitored bi-weekly during a research personnel meeting. A full review of all study procedures and data will be due every 3 months.

If during the study or later, the subject wishes to discuss his/her rights as a research subject, his/her participation in the study and/or concerns about the study, or a research-related injury, with someone not directly involved in the study, or if the volunteer feels under any pressure to enroll in this study or to continue to participate in this study, he/she is asked to contact a representative of the Human Research Committee at MGH (617) 424-4100, or at MEEI (617) 573-3446.

Any adverse event during the subject's participation in the study will be documented whether or not considered to be related to the study procedures. The following will be included in all safety reports: Subject identification number and initials, primary investigator's name, subject's date of birth, gender, and ethnicity, signs and symptoms and severity, date of onset, date of resolution or death, relationship of the event to the study, and all actions taken. All adverse events observed by the co-investigators, a professional colleague, or the subject himself/herself will be recorded.

Reports of unanticipated problems involving risks to subjects or others will be reported to the Partners Human Research Committee in accordance with PHRC unanticipated problems including adverse event reporting guidelines through Insight/eIRB within 5 working days/7 calendar days. In case of Adverse Event, a report will be submitted containing a detailed description of the adverse

event; the basis for determining that the event is unexpected; the basis for determining that the event is possibly related to the research procedures; the basis for determining that the research places subjects at an increased risk of harm; and, whether any changes to the research or other corrective actions are warranted. In this case, amendments to the protocol to incorporate possible changes and/or corrective actions will be considered.

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