# Brillouin Microscopy Visualizes Centralized Corneal Edema in Fuchs Endothelial Dystrophy

*Amira M. Eltony, PhD,* Fatima Clouser, MS,* Peng Shao, PhD,* Roberto Pineda II, MD,† and Seok-Hyun Yun, PhD*‡*

**Purpose:** To investigate the feasibility of using Brillouin microscopy for assessment of corneal edema in patients with Fuchs endothelial corneal dystrophy (FECD). Brillouin microscopy analyzes the frequency shift of light inelastically scattered by naturally occurring acoustic waves in a small volume of tissue. The resulting frequency shift is a measure of the local hydromechanical properties of the tissue.

**Methods:** Participants were scanned using a clinical Brillouin imaging system (780 nm laser, 5 mW), and a color-coded map of the mean Brillouin shift laterally across the corneal stroma was created.

**Results:** Brillouin maps of normal subjects (n = 8) were relatively homogeneous, whereas maps of patients with FECD (n = 7) exhibited significantly reduced Brillouin shifts (unpaired *t* test, $P < 0.001$) centrally. The mean difference of 83 MHz corresponds to approximately 3.9% higher water content (percentage difference in volume fraction) in central corneas of the FECD group relative to normal subjects. The Brillouin scan of a patient with FECD 1 month after Descemet membrane endothelial keratoplasty measured a 62 MHz increase in Brillouin shift relative to the preoperative level, indicating normalization of corneal hydration.

**Conclusions:** All patients with FECD scanned exhibited a centralized reduction in Brillouin shift, distinct from the normal subjects measured and consistent with centralized edema characterized by pachymetry. Brillouin scans revealed substantially reduced water content after Descemet membrane endothelial keratoplasty. These results suggest that Brillouin microscopy could aid treatment planning and assessment of FECD. Moreover, corneal hydration mapping may be useful in understanding fluid pump function dynamics of the cornea and developing early interventions for FECD.

Received for publication June 17, 2019; revision received September 12, 2019; accepted September 17, 2019. Published online ahead of print October 24, 2019.

From the *Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA; †Massachusetts Eye and Ear, Boston, MA; and ‡Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA.

This study was supported by funding from the National Institutes of Health (Bethesda, MD), grants R01-EY025454, P41-EB015903, and R41-EY028820.

A.M. Eltony, P. Shao, and S-.H. Yun hold patents related to the technology. S-.H. Yun is the scientific founder of Intelon Optics, Inc., which licensed the patents.

Correspondence: Seok-Hyun Yun, PhD, Harvard Medical School, Wellman Center for Photomedicine, 65 Landsdowne Street, UP-525, Cambridge, MA 02139 (e-mail: syun@hms.harvard.edu).



**Key Words:** cornea, edema, Fuchs, endothelial disease, imaging

(*Cornea* 2020;39:168–171)

Fuchs endothelial corneal dystrophy (FECD) is a progressive condition of the cornea caused by endothelial dysfunction and inability to maintain fluid pump function, resulting in significant visual impairment in advanced stages. The characteristic clinical feature is the formation of focal projections, termed "guttae,"[1] excreted from the thickened posterior band of Descemet membrane, followed by a progressive increase in corneal stromal edema.[2] The current standard of care treats FECD during the later stages of the disease with a complete or partial corneal transplantation. However, its progression rates vary, and quantitative methods to predict progression rates are not currently available.

FECD and other eye diseases alter the water concentration in the corneal stroma,[3] as do procedures such as photorefractive keratectomy or corneal graft surgery.[4,5] Current clinical methods to assess water content are based on monitoring corneal thickness, typically using ultrasound-based or optical coherence tomography-based pachymetry or Scheimpflug camera topography, or identifying concomitant structural features using slit-lamp biomicroscopy. These are indirect measurements of water concentration and cannot take into account factors such as the natural variation in corneal thickness between people.[6]

Brillouin microscopy is an emerging technique developed to characterize the biomechanical properties of tissues and has shown potential in corneal disease diagnosis.[7] In Brillouin microscopy, laser light is focused to a small volume of tissue, and the Brillouin-scattered light from this region is collected at 180 degrees and measured with a spectrometer. The technique is based on spontaneous Brillouin scattering, which is the inelastic scattering of light by naturally occurring acoustic waves in material. The resulting frequency shift, called the Brillouin frequency shift, depends on the local hydromechanical properties of the tissue. Recent studies have established that Brillouin frequency shifts are modulated by water concentration.[8,9] Here, we investigate whether Brillouin microscopy has sufficient sensitivity to detect corneal edema in patients with FECD and to visualize quantitative, spatial patterns of water concentration in patients with FECD.

## MATERIALS AND METHODS

This pilot study recruited 8 subjects with normal corneas (mean ± SD age, 35 ± 9 years) and 7 subjects diagnosed with FECD (53 ± 10 years) from the Massachusetts Eye and Ear Cornea Service in Boston, MA, and the Woolfson Eye Institute in Atlanta, GA, between May 2018 and December 2018. It is noted that previous studies found no significant age dependence in corneal Brillouin shift values.[10] All subjects underwent a comprehensive ophthalmic examination by a cornea specialist including pachymetry, slit-lamp biomicroscopy, and ancillary Pentacam (Oculus, Wetzlar, Germany) Scheimpflug imaging to quantify the central corneal thickness (CCT). Exclusion criteria for participating in the study included previous refractive or intraocular surgery, dry eye syndrome, glaucoma, and diagnosis of any corneal dystrophies other than FECD. Institutional Review Board/Ethics Committee approval was obtained from the Partners Human Research Committee (Partners HealthCare Institutional Review Board) and Woolfson Eye Institute Ethics Committee. Written informed consent was obtained from all subjects before Brillouin imaging. All experiments were performed in accordance with the principles of the Declaration of Helsinki.

Participants were scanned using 2 clinical Brillouin imaging systems (780 nm laser, 5 mW) with identical specifications, which have been described previously.[10] Axial scans were taken at ~30 different transverse locations within a radius of approximately 4 mm from the corneal apex. From each axial scan, a mean Brillouin shift was computed by averaging the Brillouin shift values measured within the span of the corneal stroma. A color-coded Brillouin map was obtained by 2-dimensional interpolation of the mean Brillouin shift laterally across the corneal stroma.

## RESULTS

Brillouin maps of the 7 patients with FECD exhibit greater heterogeneity than those of the 8 normal subjects (see representative examples in Fig. 1). A pattern of centralized reduction in Brillouin shift, corresponding to increased water content in the central region of the corneal stroma, is apparent for all patients with FECD. This pattern is more pronounced in patients with advanced-stage FECD (Fig. 1), consistent with progressive edema that is characteristic of the condition.

The mean Brillouin shift in the central region—defined here as the zone <1 mm from the corneal apex—is 5731 ± 15 MHz (mean ± SD) for normal subjects and 5648 ± 42 MHz for patients with Fuchs, a statistically significant difference (unpaired $t$ test, $P < 0.001$). The average CCT for the FECD group is 607 ± 47 μm (mean ± SD), expectedly higher than 557 ± 17 μm for the normal group (unpaired $t$ test, $P < 0.05$). Figure 2A plots the central Brillouin shift versus CCT for all study subjects. A trend toward decreasing Brillouin value with increasing CCT is evident, with a fitted slope (95% confidence interval) of −0.92 (−1.42, −0.42) MHz/μm. This result is consistent with previous measurements of individual diurnal changes in healthy volunteers,[9] which reported a slope of −1.06 ± 0.2 MHz/μm.

Regional difference in Brillouin shift is less affected by interpersonal variation.[10] Similarly, we found that the ratio of corneal thickness in the peripheral region to CCT, denoted central-to-peripheral thickness ratio ("CPTR"), could be a useful measure of FECD severity.[11] We found that the CPTR is higher on average for the FECD group, 0.94 ± 0.04 versus 0.88 ± 0.01, than for the normal group and that the difference is statistically significant (unpaired $t$ test, $P < 0.005$). We computed the difference between mean Brillouin shift in the periphery of the cornea (defined here as the region >3 mm from the corneal apex) and Brillouin shift in the central area (again defined as the region <1 mm from the corneal apex) for each subject. Figure 2B shows Brillouin regional difference versus CPTR for all study subjects. A trend toward increasing Brillouin regional difference with decreasing CPTR is apparent, with a fitted slope (95% confidence interval) of 580 (400, 770) MHz/CPTR.

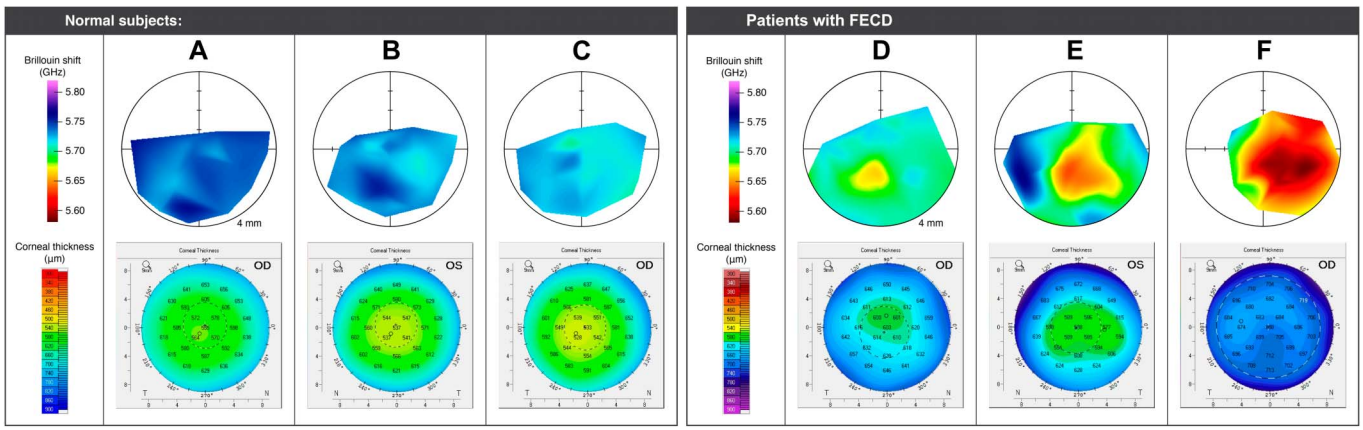


**FIGURE 1.** Representative examples of Brillouin maps (top row; radius of the outer circle is 4 mm) and corneal topographies (bottom row) for normal subjects (A, B, C) and patients with FECD (D, E, F) scanned in this study. Normal subjects A and B both had a CPTR of 0.86. Subject C had a CPTR of 0.89. FECD subject D had the lowest CPTR of the patients with FECD measured (0.89) and presented with central guttae with areas of pigmented confluence. Subject E had a CPTR of 0.94 and presented with central guttae with patchy confluence. Subject F had the largest CPTR of the patients with FECD measured (0.99) and presented with centrally confluent guttae and haziness.

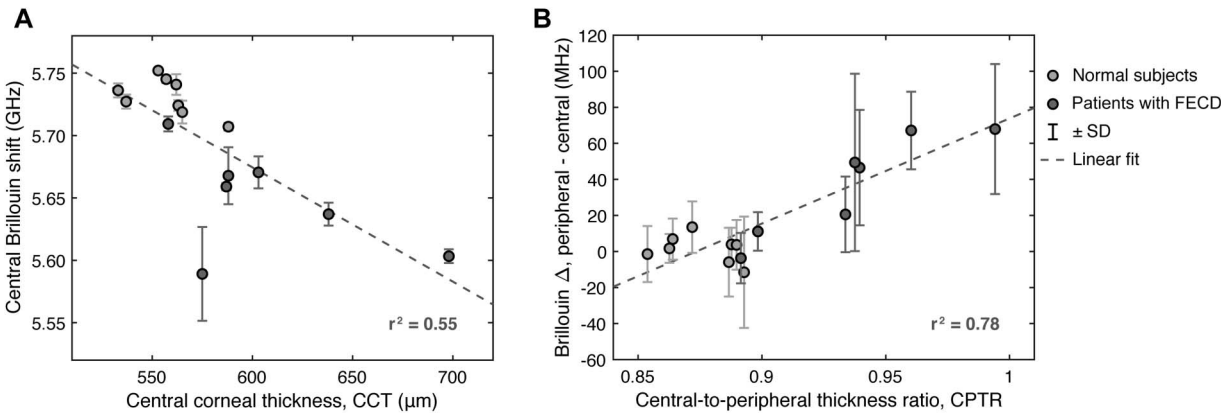


**FIGURE 2.** A, Plot of Brillouin shift in the central region (<1 mm from the corneal apex) versus CCT for normal subjects (orange) and patients with FECD (blue). The dotted line shows a linear fit to the data with a fitted slope [95% confidence interval] of −0.92 [−1.42, −0.42] MHz/μm. B, Plot of Brillouin regional difference (peripheral minus central) versus CPTR for normal subjects (orange) and patients with FECD (blue). The dotted line shows a linear fit to the data with a fitted slope (95% confidence interval) of 580 (400, 770) MHz/CPTR.

One patient with FECD was scanned just before a partial thickness corneal transplant (Descemet membrane endothelial keratoplasty [DMEK]) and then scanned again 1 month postoperatively. This case study can be seen in Figure 3. The mean Brillouin shift in the patient's central corneal region increased significantly from 5637 ± 9 MHz (mean ± SD) just before DMEK to 5699 ± 8 MHz 1 month after DMEK (unpaired $t$ test, $P < 0.001$), consistent with the expected reduction in corneal edema after treatment. The Brillouin value after DMEK is slightly less than the lowest value measured in the normal group, 5702 ± 21 MHz. The CPTR improved from 0.96 to 0.87, close to 0.88 for the normal group.

## DISCUSSION

Previous work has established a relationship between Brillouin shift and corneal hydration.[9] Assuming there is a typical ~0.78 volume fraction of water in the healthy cornea, we estimate that there is 3.9% higher water content

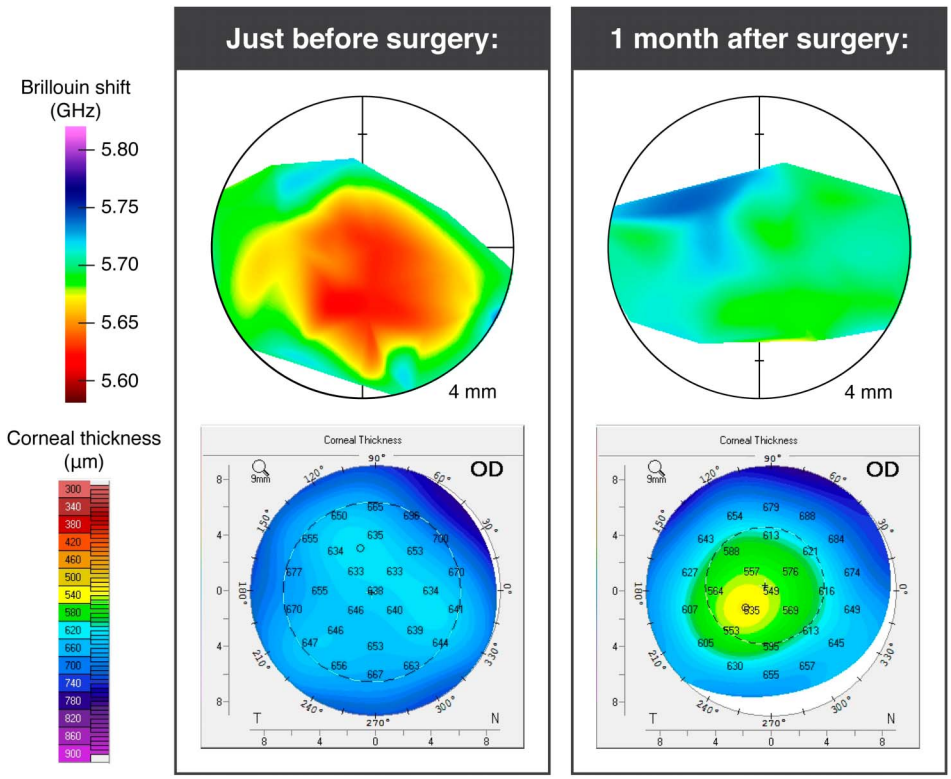


**FIGURE 3.** Case study of a patient with FECD scanned just before DMEK surgery and again 1 month post-operatively. The top row shows the Brillouin shift map, and the bottom row shows corneal thickness. Before surgery, the patient had a CPTR of 0.96 and presented with confluent guttae. One month after surgery, the patient's CPTR was 0.87 (with the graft).

on average in the central corneas of the FECD group compared with the normal group (percentage difference in volume fraction). The Brillouin spatial maps of the normal subjects are relatively uniform, with negligible difference (1.3 ± 7.7 MHz) on average between peripheral and central corneal regions. By contrast, patients with FECD exhibit a centralized reduction in Brillouin shift, with an average peripheral-to-central difference of 37 ± 28 MHz. This corresponds to an average of 1.5% higher water content (percentage difference in volume fraction) in the central regions relative to the peripheral regions of the patients with FECD.

Brillouin microscopy allows for noncontact mapping of corneal water content (volume fraction) with a sensitivity of approximately ±0.2%.[9] The results of this pilot study of patients with FECD suggest that this technique may be useful for assessment of FECD or other dystrophies that cause fluid accretion or reduction in the cornea stroma. Further study is warranted to investigate what correlation exists between Brillouin shifts and guttae in early stage FECD and to determine whether the patterns of edema identified by Brillouin measurements can be applied to predict the rate of FECD progression and aid in surgical planning. Because of its sensitivity to water content, Brillouin microscopy may also prove beneficial for understanding the role that fluid dynamics play in different corneal disorders and possibly for monitoring corneal grafts after transplantation.

## ACKNOWLEDGMENTS

*The authors thank Dr. R. Doyle Stulting for assistance with the study taking place at the Woolfson Eye Institute in Atlanta, GA.*

## REFERENCES

1. Eghrari AO, Riazuddin SA, Gottsch JD. Fuchs corneal dystrophy. *Prog Mol Biol Transl Sci.* 2015;134:79–97.
2. Wilson SE, Bourne WM. Fuchs' dystrophy. *Cornea.* 1988;7:2–18.
3. Sherwin JC, Kokavec J, Thornton SN. Hydration, fluid regulation and the eye: in health and disease. *Clin Exp Ophthalmol.* 2015;43:749–764.
4. Fields CR, Taylor SM, Barker FM. Effect of corneal edema upon the smoothness of excimer laser ablation. *Optom Vis Sci.* 1994;71:109–114.
5. Dougherty PJ, Wellish KL, Maloney RK. Excimer laser ablation rate and corneal hydration. *Am J Ophthalmol.* 1994;118:169–176.
6. Doughty MJ, Zaman ML. Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. *Surv Ophthalmol.* 2000;44:367–408.
7. Yun SH, Chernyak D. Brillouin microscopy: assessing ocular tissue biomechanics. *Curr Opin Ophthalmol.* 2018;29:299–305.
8. Wu P-J, Kabakova IV, Ruberti JW, et al. Water content, not stiffness, dominates Brillouin spectroscopy measurements in hydrated materials. *Nat Methods.* 2018;15:561–562.
9. Shao P, Seiler TG, Eltony AM, et al. Effects of corneal hydration on Brillouin microscopy in vivo. *Investig Opthalmology Vis Sci.* 2018;59:3020–3027.
10. Shao P, Eltony AM, Seiler TG, et al. Spatially-resolved Brillouin spectroscopy reveals biomechanical abnormalities in mild to advanced keratoconus in vivo. *Sci Rep.* 2019;9:7467.
11. Repp DJ, Hodge DO, Baratz KH, et al. Fuchs' endothelial corneal dystrophy: subjective grading versus objective grading based on the central-to-peripheral thickness ratio. *Ophthalmology*. 2013;120:687–694.