

# Correlation Analysis of Bowman's Layer Microdistortions and Corneal Biomechanics Changes Evaluated by Brillouin Microscopy After KLEx

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**Purpose:** To investigate the correlation between Bowman's layer microdistortions and the changes in corneal biomechanics evaluated by Brillouin microscopy after Keratorefractive Lenticule Extraction (KLEx).

**Methods:** This study included forty-three right eyes that underwent KLEx, with a mean spherical equivalent of  $-5.89 \pm 1.44$  diopters. Metric M, the total width of the microdistortions, was calculated to indicate the quantity of Bowman's layer microdistortions according to the images measured using Fourier-domain optical coherence tomography (FD-OCT) 1 month postoperatively. Corneal biomechanical metrics were obtained and analyzed using Brillouin microscopy preoperatively and 1 month postoperatively.

**Results:** Bowman's layer microdistortions were observed using FD-OCT one month postoperatively, revealing a total width of 502 (175, 698)  $\mu\text{m}$  of the four meridians. Central, mean, minimum (Min), maximum (Max), and spatial standard deviation (SSD) Brillouin modulus (BM) quantified by Brillouin microscopy were  $2.888 \pm 0.074$  GPa,  $2.878 \pm 0.041$  GPa,  $2.783 \pm 0.059$  GPa,  $2.956 \pm 0.053$  GPa,  $0.217 \pm 0.063$  GPa before KLEx, respectively; these changed to  $2.800 \pm 0.058$  GPa ( $P < 0.001$ ),  $2.821 \pm 0.033$  GPa ( $P < 0.001$ ),  $2.734 \pm 0.040$  GPa ( $P < 0.001$ ),  $2.908 \pm 0.051$  GPa ( $P < 0.001$ ),  $0.223 \pm 0.062$  GPa ( $P = 0.666$ ) 1 month after KLEx, respectively. Metric M was correlated with value's changes of Mean BM ( $r = 0.57$ ,  $P < 0.001^*$ ) and Min BM ( $r = 0.35$ ,  $P = 0.02$ ), controlling for spherical equivalent.

**Conclusion:** In this study, the alterations in corneal biomechanics following KLEx are positively correlated with the range of Bowman's layer microdistortions. Increased Bowman's layer microdistortions may lead to a more pronounced decline in the corneal biomechanics. These findings may be useful for evaluating KLEx outcomes, detecting complications early, and optimizing patient monitoring.

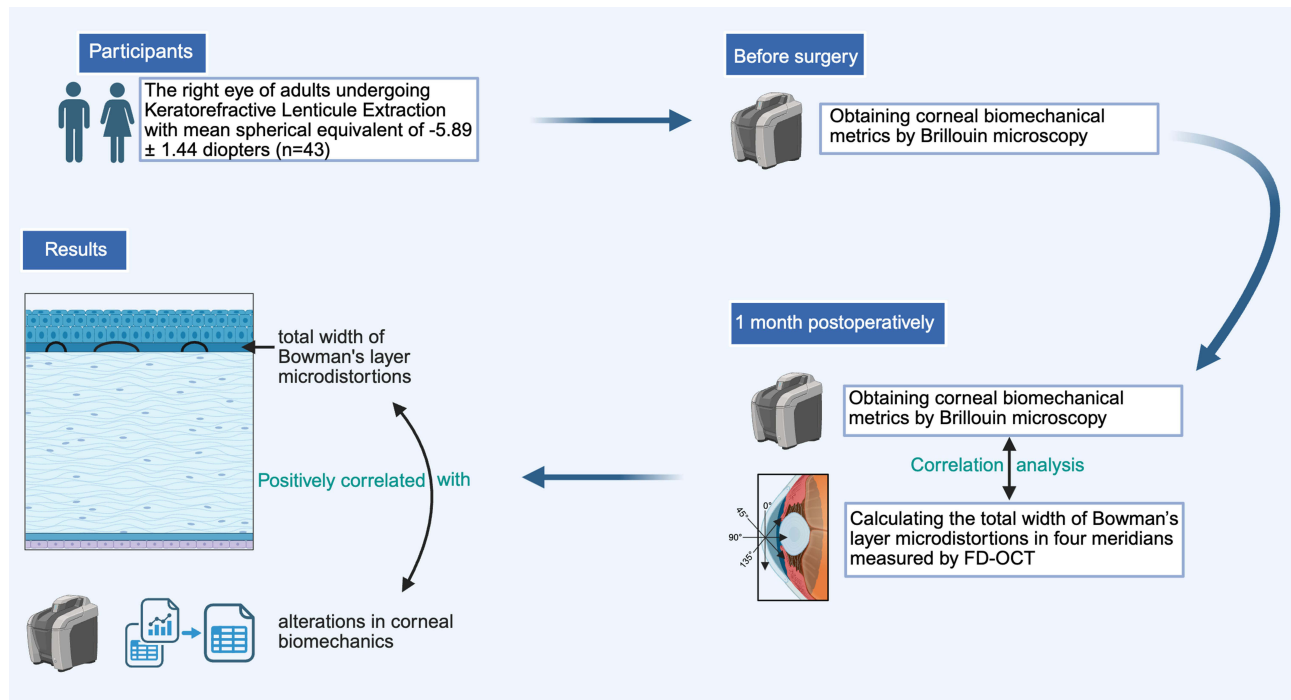
**Plain Language Summary:** After a type of corneal laser surgery: Keratorefractive Lenticule Extraction (KLEx), tiny irregularities were observed in a specific layer of the cornea (Bowman's layer). The researchers used a special imaging technique to measure corneal stiffness before and after the procedure. This study aimed to determine the relationship between small irregularities and the cornea's stiffness.

The measurements showed that corneal stiffness decreased one month after surgery. This study found that the extent of tiny irregularities in the Bowman's layer was linked to the amount of change in corneal stiffness. Specifically, a larger area of these irregularities was associated with a larger change in stiffness measurements.

In conclusion, changes in corneal strength after KLEx surgery are related to the presence and extent of these small structural irregularities.

**Keywords:** bowman's layer microdistortions, brillouin microscopy, brillouin modulus, corneal biomechanics

## Graphical Abstract



## Introduction

As a secure and efficient treatment for correcting myopia and astigmatism, Keratorefractive Lenticule Extraction (KLEx) surgery has gained wide popularity and fast development.<sup>1</sup> Our previous studies have reported that Bowman's layer microdistortions seem to be one of the representative features of KLEx. Moreover, they do not influence intraocular scattering, and both their incidence and extent are positively related to myopic correction.<sup>2–4</sup> Preserving the Bowman's layer has been regarded as a more secure procedure to maintain corneal biomechanical strength in refractive surgeries, but whether Bowman's layer microdistortions following KLEx relate to corneal biomechanics remains unclear.<sup>3,5</sup>

Brillouin microscopy was first reported by Scarcelli,<sup>6</sup> providing an all-optical, three-dimensional mechanical mapping of the cornea without requiring air-puff stimulation or any external disturbances.<sup>7</sup> This technique utilizes near-infrared and low-power laser light, ensuring its security in clinical applications.<sup>8</sup> Based on the Brillouin scattering theory,<sup>9</sup> the Brillouin frequency shift—originating from a combination of intrinsic molecular characteristics<sup>10</sup> and corneal hydration<sup>11,12</sup> offers quantitative estimate of the longitudinal modulus, or Brillouin modulus, derived from optical spectroscopy. Brillouin metrics obtained through conversion can efficiently characterize focal corneal biomechanical changes.<sup>13–15</sup> A previous study<sup>16</sup> has validated its repeatability and accuracy, supporting its use in corneal biomechanical assessment. In clinical applications, Brillouin microscopy has proven useful in evaluating subclinical keratoconus,<sup>17</sup> where early detection of biomechanical changes is critical. While it does not entirely replace traditional methods like Scheimpflug technology, it offers a complementary advantage in detecting subtle and focal corneal biomechanical alterations, which may go unnoticed by conventional assessments.

This study aimed to investigate the correlation between postoperative Bowman's layer microdistortions and changes in corneal biomechanics by using Fourier-domain optical coherence tomography (FD-OCT) and Brillouin microscopy.

## Methods

### Participants

Forty-three right eyes of forty-three consecutive participants (21 men and 22 women) who underwent KLEx were included in this prospective, non-randomized study. The mean spherical equivalent (SE) was  $-5.89 \pm 1.44$  diopters (range:  $-2.25$  to  $-9.00$  diopters). To ensure consistency across all ocular metrics, only patients meeting the following criteria were enrolled to the cohort: patients aged 18–40 years with stable refraction for at least 2 years; all eyes' corrected distance visual acuity (CDVA) of 20/20 or better; no obvious dry eye symptoms and Schirmer's test no less than 5mm/5min; a normal corneal topography assessed by Pentacam HD; no history of previous ocular surgery, trauma or diseases like keratoconus, glaucoma that could affect corneal biomechanics. This study followed the principles of the Declaration of Helsinki and the protocols were approved by the Ethical Committee of the Eye & ENT Hospital of Fudan University (No. 2020530). All participants provided written consent after receiving comprehensive information regarding the potential benefits and risks of the study. Table 1 summarizes the corneal thickness, preoperative refraction, and lenticule thickness. Postoperative measurements were conducted exactly 4 weeks ( $\pm 2$  days) after the surgery to ensure consistency across all participants.

### KLEx Surgeries

All KLEx surgeries were performed by the same experienced surgeon (ML) using the VisuMax femtosecond laser system (Carl Zeiss Meditec, Jena, Germany) with 130 nJ pulse energy. The thickness and diameter of the cap were set to 120  $\mu\text{m}$  and 7.5 mm separately, with a 2 mm side cut at the superior position. Lenticule diameter was 6.2 to 6.8 mm and mean lenticule thickness were  $112.1 \pm 18.7\mu\text{m}$ . All surgeries were successfully performed, followed by standard postoperative pharmacological treatments including artificial tears and 0.3% levofloxacin.

### Brillouin Microscopy Measurement

Brillouin scattering is caused by the interaction between the sample's microscopic phonons and incident light, which occurs because of inherent density or pressure fluctuations.<sup>18</sup> The difference in Brillouin frequency, also named the frequency shift, between incident light and Brillouin photons is typically 1–10 GHz.<sup>10</sup> It is capable to estimates the longitudinal viscoelastic modulus from optical spectroscopy directly.<sup>7</sup> The relationship between the Brillouin shift  $\Delta\nu_B$  and the Brillouin longitudinal modulus  $M'$  (BM), can be described as

$$M = \frac{\rho\lambda^2(\Delta\nu_B)^2}{4n^2}$$

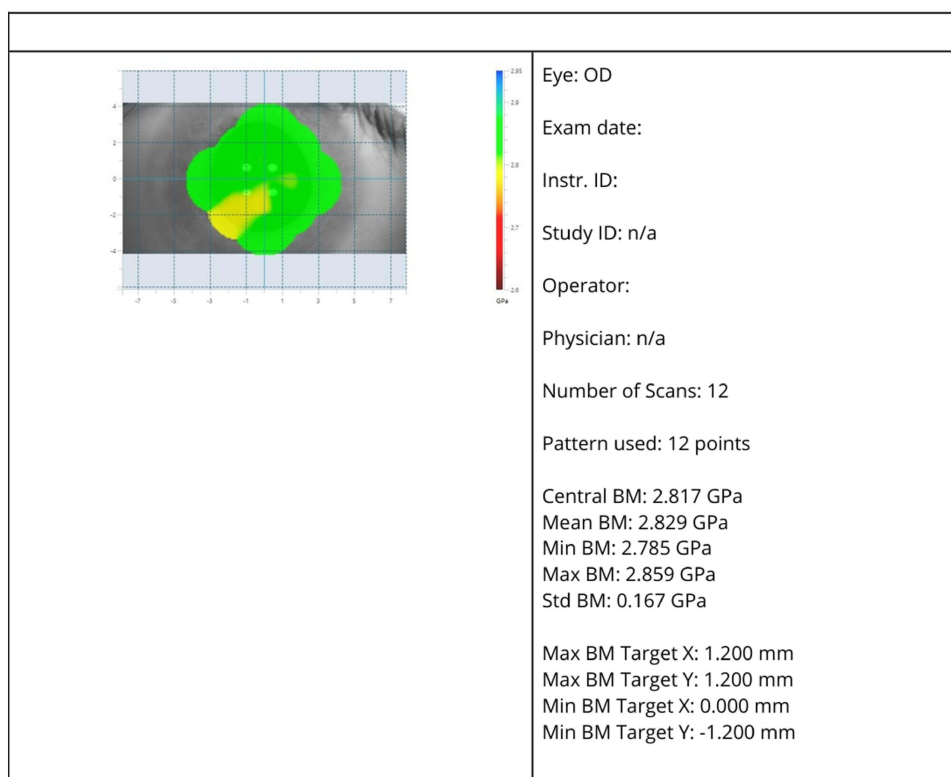
where  $\lambda$  is the wavelength of the probing laser,  $\rho$  is the total corneal density, and  $n$  is the corresponding refractive index. Despite the spatial variations in the refractive index and density within the cornea, research indicates that the ratio  $\rho/n^2$  remains approximately invariable,<sup>19,20</sup> so BM is proportionate to the Brillouin frequency shift. A standardized Brillouin microscopy report is shown in Figure 1.

The Brillouin Optical Scan System (BOSS; Intelon, USA) was used to evaluate the Brillouin biomechanical parameters, including Central, Mean, maximum (Max), minimum (Min), and spatial standard deviation (SSD). Central

**Table 1** Preoperative Parameters

Parameter	Value	Range
Age (y)	$26.9 \pm 6.2$	18 to 38
Refractive sphere (D)	$-5.34 \pm 1.44$	$-8.00$ to $-1.50$
Refractive cylinder (D)	$-0.75$ ( $-1.63, -0.25$ )	$-2.50$ to $0$
Spherical equivalent (D)	$-5.89 \pm 1.44$	$-9.00$ to $-2.25$
Corneal thickness ( $\mu\text{m}$ )	$547.0$ (539.0, 567.5)	512 to 602
Lenticule thickness ( $\mu\text{m}$ )	$112.10 \pm 18.70$	68 to 157

**Abbreviation:** D, diopters.

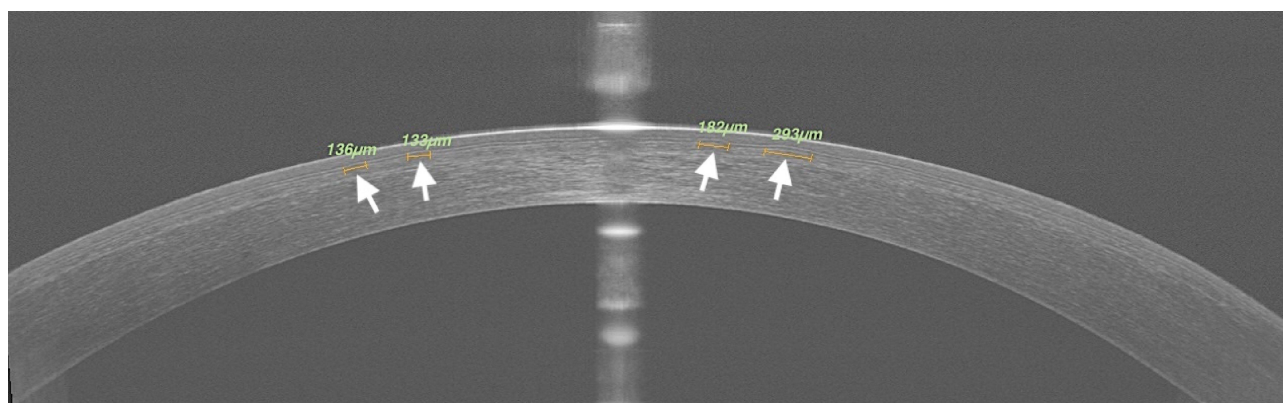


**Figure 1** The Heatmap and Point report of Brillouin microscopy in the mode of “12 points” in the distance of 1.2 mm away from the center of the cornea postoperatively.

BM was the average BM of the closest point to the center of cornea; Mean BM was the weighted average of the average BM of every point; Min BM was the minimum of the average BM of every point; Max BM was the maximum of the average BM of every point; SSD is the square root of the sum of the Standard BM of every point. The same experienced physician performed Brillouin microscopy measurements, and all patients underwent this examination in a dark room, where the temperature was between 26°C and 28°C. To stabilize the degree of corneal hydration,<sup>12</sup> the period of the daily examination period was 9 am to 5 pm, at least 2 h after waking up. Each measurement took 10 minutes in the absence of any errors. During the examination, all participants were required to focus on the indicator light and avoid movement or frequent blinking. In the “12 points” mode and setting of 1.2 mm, the Brillouin microscopy measures values at a distance of 1.2 mm and 2.4 mm away from the pupil center horizontally in the nasal and temporal directions, vertically in the superior and inferior directions and both 1.2 mm in the temporal and superior, nasal and superior, temporal and inferior, nasal and inferior directions. By identifying the central cornea or pupil, the system tracks eye movements. If a participant blinked, the instrument automatically discarded the data. Our analysis only included data with a quality index categorized as “OK”.

## Anterior Segment Measurements

The anterior segment of FD-OCT (RTVue, version 6.2; Fremont, CA) was used to measure microdistortions in the Bowman’s layer. Four meridians (0, 45, 90, and 135°) of the cornea were obtained using linear scanning. The normal Bowman layer appeared to have a smooth linear structure. The twisted segments of Bowman’s layer were defined as microdistortions, as previously reported.<sup>2,3</sup> The protrusion of each microdistortion above the Bowman’s layer baseline was designated as a peak, and the width of each peak was subsequently measured. The peak count in each eye was recorded and the total width of all peaks detected in the four-line scans of each eye was summed as metric M, representing the quantitative assessment of microdistortions in the corneal Bowman layer for statistical analysis (Figure 2). One examiner (XQ) conducted the measurements and one blinded investigator (CZ) analyzed the data.



**Figure 2** The illustration of metric M of Bowman's layer microdistortions. The white arrows show the microdistortions in Bowman's layer that were taken into account in the vertical Meridian. The yellow line shows the width of each microdistortion to be 136  $\mu\text{m}$ , 133  $\mu\text{m}$ , 182  $\mu\text{m}$  and 293  $\mu\text{m}$ , respectively. The total width of microdistortions in horizontal meridian is  $136 + 133 + 182 + 293 = 744 \mu\text{m}$ . Metric M is the sum of the total width of microdistortions in four meridians ( $0^\circ$ ,  $45^\circ$ ,  $90^\circ$ , and  $135^\circ$ ) of the cornea.

## Data Analysis

All statistical analyses and visualizations were conducted using the R software (version 3.6.3, Vienna, Austria). Continuous data are presented as mean, standard deviation, median, interquartile range, minimum and maximum values, and their normality was tested using the Shapiro–Wilk Normality test. Normality-approximating variables are described as mean  $\pm$  standard deviation (SD), whereas non-normal variables are expressed as median (interquartile range [IQR]). Categorical data were characterized using frequency counts and percentages. Paired *t*-tests were used for pre- and post-surgery comparisons of normally or nearly normally distributed quantitative data. Partial correlation analysis was employed to examine the correlation between Brillouin values and Bowman's layer microdistortions, controlling for spherical equivalent (SE) variables. Statistical significance was set at  $P < 0.05$ .

## Results

All patients included in this study exhibited stable or improved visual outcomes at 1 month postoperatively, with CDVA equal to or greater than their preoperative values. They reported satisfactory postoperative visual quality and did not experience significant dry eye symptoms. The incidence and range of microdistortions in the Bowman's layer 1 month postoperatively are shown in Table 2. The number of Bowman's layer microdistortions in the four meridians was 3 (1, 4) (range: 0 to 6), and the total width of the microdistortions (metric M) was 502 (175, 698)  $\mu\text{m}$  (range: 0 to 1060).

Table 3 shows the changes in various corneal biomechanical metrics measured preoperatively and 1 month postoperatively using Brillouin microscopy, including the Central, Mean, minimum (Min), maximum (Max), and spatial standard deviation (SSD) Brillouin modulus (BM). They were  $2.888 \pm 0.074$  GPa,  $2.878 \pm 0.041$  GPa,  $2.783 \pm 0.059$  GPa,  $2.956 \pm 0.053$  GPa,  $0.217 \pm 0.063$  GPa before KLEx, respectively, which changed to  $2.800 \pm 0.058$  GPa,  $2.821 \pm 0.033$  GPa,  $2.734 \pm 0.040$  GPa,  $2.908 \pm 0.051$  GPa,  $0.223 \pm 0.062$  GPa 1 month after KLEx, respectively. All four Brillouin modulus, except SSD, showed a significant decrease postoperatively ( $P < 0.001$ ), while SSD was similar before and after KLEx ( $P = 0.67$ ).

**Table 2** Total Number of Peaks and Metric M of Microdistortions in Bowman's Layer

Parameter	1 Month	Range
Total peaks <sup>a</sup>	3 (1, 4)	0 to 6
Metric M <sup>b</sup> ( $\mu\text{m}$ )	502 (175, 698)	0 to 1060

Notes: <sup>a</sup>Total peaks = total number of Bowman's layer microdistortions of four meridians ( $0^\circ$ ,  $45^\circ$ ,  $90^\circ$ , and  $135^\circ$ ); <sup>b</sup>Metric M = total width of microdistortions of four meridians.

**Table 3** Preoperative and Postoperative Corneal Biomechanical Parameters Evaluated by Brillouin Microscopy

Parameter (GPa)	Preoperative		Postoperative			
	Value	Range	Value	Range	t <sup>a</sup>	P
Central BM	2.888 ± 0.074	2.709 to 3.080	2.800 ± 0.058	2.648 to 2.920	7.37	<0.001*
Mean BM	2.878 ± 0.041	2.813 to 3.007	2.821 ± 0.033	2.745 to 2.889	8.66	<0.001*
Min BM	2.783 ± 0.059	2.671 to 2.939	2.734 ± 0.040	2.648 to 2.849	4.25	<0.001*
Max BM	2.956 ± 0.053	2.859 to 3.080	2.908 ± 0.051	2.790 to 3.012	5.04	<0.001*
SSD	0.217 ± 0.063	0.089 to 0.374	0.223 ± 0.062	0.099 to 0.377	-0.43	0.666

**Notes:** BM, Brillouin Modulus; Central BM is the Average BM of the closest point to the center of cornea; Mean BM is the weighted average of the Average BM of the 12 points; Min BM is the minimum of the Average BM of the 12 points; Max BM is the maximum of the Average BM of the 12 points; SSD is the square root of the sum of the Standard BM of the 12 points. \*Statistical significance is set at P < 0.05. <sup>a</sup> Paired t-test.

**Table 4** Correlation Between Metric M of Bowman's Layer Microdistortions and Changes of Brillouin Modulus

Parameter <sup>a</sup>	r <sup>b</sup>	P
Central BM	0.21	0.18
Mean BM	0.57	<0.001*
Min BM	0.35	0.02*
Max BM	0.23	0.14
SSD	0.16	0.32

**Notes:** BM, Brillouin Modulus; Central BM is the Average BM of the closest point to the center of cornea; Mean BM is the weighted average of the Average BM of the 12 points; Min BM is the minimum of the Average BM of the 12 points; Max BM is the maximum of the Average BM of the 12 points; SSD is the square root of the sum of the Standard BM of the 12 points. <sup>a</sup>Preoperative BM value minus postoperative BM value. <sup>b</sup>Partial correlation analysis; Spherical equivalent was controlled to analyze. \*Statistical significance is set at P < 0.05.

To further analyze the correlation between corneal biomechanical metrics and metric M, we conducted a partial correlation analysis controlling for spherical equivalent (Table 4). Metric M was positively correlated with value's decrease in the Mean BM ( $r = 0.57$ ,  $P < 0.001$ ) and Min BM ( $r = 0.35$ ,  $P = 0.02$ ). However, no significant associations were found between metric M and changes in Central BM ( $r = 0.21$ ,  $P = 0.18$ ), Max BM ( $r = 0.23$ ,  $P = 0.14$ ), or SSD ( $r = 0.16$ ,  $P = 0.32$ ) (Supplemental Figure 1).

## Discussion

The human cornea is perfectly transparent with five layers, and the Bowman's layer is a fibrous meshwork beneath the epithelium and above the stroma, which is non-renewable but the strongest layer in the cornea.<sup>21</sup> In KLEx, by making three femtosecond laser cuts and a 2 mm opening incision, a cylindrical lenticule is created, freed from the surrounding stroma using a manual spatula, and extracted from the incision.<sup>22</sup> Because the integrity of the corneal flap and anterior corneal stroma is preserved, KLEx has several advantages, including avoiding flap-related complications, minimizing ocular surface disruption, and improving short- and long-term biomechanical stability, which has the greatest potential.<sup>23,24</sup>

Bowman's layer microdistortions after KLEx were found and confirmed by FD-OCT, which was able to observe subtle changes at the microscopic level of the cornea that were not easily discernible by the naked eye in several reports.<sup>2-4,25</sup> The microdistortions are considered to be caused by mechanical factors, as previous studies have demonstrated that high myopia correction with thicker lenticule thickness eyes have more noticeable microdistortions.<sup>2,4</sup> Following the removal of the lenticule, the stromal bed is flatter than the posterior surface of the cornea, and the correction of high myopia results in a greater difference in the consistency of the adhesive surfaces. After KLEx, the length of the microdistortions decreased as the two layers gradually achieved better alignment, but they persisted and remained stable over an extended period.<sup>4,26</sup> Our current study is the first to investigate the relationship between microdistortions in Bowman's layer following KLEx and changes in corneal biomechanics.

Currently, the Ocular Response Analyzer (ORA)<sup>27</sup> and the Corneal Visualization Scheimpflug Technology (Corvis-ST)<sup>28</sup> are the main devices used to measure corneal biomechanics in clinical practice. These biomechanical parameters recorded by them provide useful insights into corneal biomechanical performance<sup>29</sup> and thoroughly quantifying corneal stiffness.<sup>30</sup> However, these parameters were revealed to be influenced by intraocular pressure (IOP)<sup>30</sup> and central corneal thickness (CCT),<sup>27,31</sup> and studies have proven their limited utility in discriminating subtle subclinical diseases.<sup>32,33</sup> Moreover, they are incapable of providing a detailed analysis of the local or focal corneal biomechanical properties.<sup>9</sup>

In our study, we employed Brillouin microscopy, a novel technique based on the principles of Brillouin scattering, to measure the Brillouin modulus. This approach enables the quantification of corneal biomechanics without external stimuli or corneal deformation.<sup>7</sup> Furthermore, Brillouin microscopy has the unique ability to detect depth-dependent variations across the different layers of the cornea, which creates three-dimensional maps that illustrate the distribution of the Brillouin longitudinal modulus (BM) throughout the cornea.<sup>15</sup> Our results indicated a significant decrease in Central BM, Mean BM, Min BM, and Max BM post-KLEx compared to preoperative levels ( $P < 0.001$ ) but no notable distinction in spatial standard deviation (SSD) values ( $P = 0.67$ ), confirming that the extraction of lenticule weakened corneal biomechanics, which was consistent with previous findings using Pentacam HD and Corvis ST measurements.<sup>34,35</sup> This might be due to the reduced corneal thickness after KLEx, which directly weakened the structural integrity and deformation resistance of the cornea. Additionally, surgery typically involves central corneal femtosecond laser cutting, which might result in variable biomechanical properties across different regions of the cornea, ultimately affecting overall corneal biomechanics.

The present study explored the correlation between Bowman's layer microdistortions and changes in corneal biomechanics evaluated using Brillouin microscopy. As previous study has identified a significant relationship between microdistortions and myopic correction,<sup>2</sup> we employed partial correlation analysis, controlling for SE, to analyze the data. Metric M was positively correlated with a decrease in the Mean BM and Min BM. This might be attributed to the fact that the Bowman's layer was formed by a dense network of randomly interwoven collagen fibers, possessing the highest average stiffness values.<sup>36</sup> Following KLEx, corneal cap partial compression might be necessary to match its larger posterior surface area with the smaller residual stromal bed, which induced Bowman's layer microdistortions and consequently affected corneal biomechanics.

Our study had two limitations. First, the sample size was relatively limited, primarily because of the lengthy measurement acquisition time required for Brillouin microscopy, resulting in many patients being unable to endure and cooperate. Therefore, we focused on the easily obtainable Brillouin metrics of the entire cornea, some of which revealed a significant correlation between microdistortions in the Bowman's layer and corneal biomechanical changes. Nevertheless, in future research, a larger number of patients, a randomized controlled design with bilateral inclusion and complex depth-related Brillouin parameters for in-depth topography are required to fully verify these findings. Extended follow-up periods like 12 months or more are warranted to assess whether these biomechanical changes are sustained over time. Second, the measurement of microdistortions in this study was semi-quantitative. Future studies employing more advanced models and higher-resolution optical coherence tomography (OCT) techniques could provide a more precise and efficient evaluation of Bowman's layer microdistortions.

In summary, alterations in corneal biomechanics after KLEx are positively associated with the extent of Bowman's layer microdistortions. Increased microdistortions in the Bowman's layer may lead to a more pronounced reduction in

corneal biomechanical properties. These findings may be useful for evaluating KLEx outcomes, detecting complications early, and optimizing patient monitoring.

## Data Sharing Statement

The data that support the findings of this study are not available due to privacy reasons but available from the corresponding author upon reasonable request at limeiyan@fudan.edu.cn.

## Ethics Statement

This study was adhered to the tenets of the Declaration of Helsinki and approved by the Ethics Committee of the Eye & ENT Hospital of Fudan University (No. 2020530). Written informed consent was obtained from all recruited participants before their participation in the study.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

Chi Zhang and Xinyi Quan are co-first authors for this study. The authors declare no competing interest in this work.

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