• Clinical Research •

Evaluation of corneal stromal demarcation line depth after corneal collagen cross-linking with two different UV energy levels in keratoconus: a comparative study using AS-OCT and *in vivo* confocal microscopy

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Abstract

● **AIM:** To compare *in vivo* confocal microscopy (IVCM) and anterior segment optical coherence tomography (AS-OCT) in evaluating demarcation line depth (DLD) after corneal collagen cross-linking (CXL) performed under different ultraviolet (UV) energy levels in keratoconus.

● **METHODS:** This was a retrospective case series. This study included 216 participants (328 eyes) who underwent CXL with either high (7.2 J/cm²) or low (1.8 J/cm²) UV energy. DLD was assessed by IVCM and AS-OCT at 3, 6, and ≥12mo postoperatively. The high-energy group consisted of participants with progressive keratoconus who received CXL treatment, while the low-energy group included participants with refractive errors who underwent femtosecond laser-assisted *in situ* keratomileusis (FS-LASIK) Xtra.

● **RESULTS:** The high-energy group included 111 participants (144 eyes, mean age 23.39±7.30y, 68 males), and low-energy group included 105 participants (184 eyes, mean age 22.95±5.84y, 53 males). At 3 and 6mo, as well as at the final follow-up, visibility of the demarcation line was consistently superior with IVCM compared with AS-OCT (all $P<0.001$). At 3mo, the mean DLD measured by IVCM was 382.2±91.12 μm in the high-energy group and 192.53±58.76 μm in the low-energy group, while AS-OCT recorded 354.74±78.82 μm and 196.51±55.57 μm, respectively. Both modalities demonstrated significantly deeper demarcation line with higher UV energy ($P<0.05$), and no significant difference was observed between IVCM and AS-OCT measurements within each energy group ($P>0.05$). Furthermore, a significant monotonic temporal trend toward shallower IVCM-detected DLD was observed in both energy groups.

● **CONCLUSION:** Compared with AS-OCT, IVCM provides clearer and more reliable visualization of the corneal stromal demarcation line after CXL. While both modalities yield comparable depth measurements, IVCM maintains higher visibility across different energy levels and during follow-up at various time points, making it a preferred modality for postoperative assessment in clinical practice.

● **KEYWORDS:** keratoconus; corneal collagen cross-linking; *in vivo* confocal microscopy; anterior segment optical coherence tomography; corneal stromal demarcation line

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INTRODUCTION

Corneal collagen cross-linking (CXL) is a procedure that combines riboflavin and ultraviolet (UV) light to slow the progression of keratoconus and other ectatic corneal diseases[1-2]. The conventional method, known as the Dresden protocol, involves UV irradiation at 3 mW/cm² for 30min and has been widely validated for its clinical efficacy[3-4]. In recent years, several modifications to this standard protocol have been

explored to improve efficiency and outcomes[5]. These include increasing UV energy to reduce treatment time, adjusting riboflavin concentration to enhance stromal penetration, and using transepithelial (epi-on) techniques to avoid complications related to epithelial removal[6-9].

One of the key challenges following CXL is the effective assessment and quantification of the CXL effect. Postoperatively, a hyperreflective band can often be seen in the anterior corneal stroma under slit-lamp examination[10]. The depth of this demarcation line [demarcation line depth (DLD)] can be measured using anterior segment optical coherence tomography (AS-OCT) or *in vivo* confocal microscopy (IVCM). However, the comparability of DLD measurements obtained by AS-OCT and IVCM, particularly under different CXL energy settings, remains to be clarified.

The present study was designed to address this gap. We performed a comparative analysis of DLD and visibility using AS-OCT and IVCM in patients undergoing high-energy therapeutic CXL and low-energy prophylactic CXL [femtosecond (FS)-laser-assisted *in situ* keratomileusis (LASIK) Xtra]. By quantitatively evaluating measurement differences between the two modalities, our goal was to provide an objective reference for interpreting postoperative imaging findings and to establish imaging-based evidence for optimizing individualized CXL energy protocols.

Importantly, DLD represents an imaging-derived morphological interface rather than the true depth of biomechanical stiffening, and should be interpreted as an indirect surrogate marker of the CXL effect.

PARTICIPANTS AND METHODS

Ethical Approval This study was approved by the Ethics Committee of Xi'an No.1 Hospital (No.2025-049) and conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before surgery. No monetary compensation was provided for participation in this study.

This was a retrospective case study involving 216 patients (328 eyes) who underwent CXL at the Laser Vision Correction Center of Xi'an No.1 Hospital between March 2019 and December 2024. Based on the UV energy used during the procedure, patients were divided into two groups: a high-energy group (7.2 J/cm²) and a low-energy group (1.8 J/cm²). The high-energy group consisted of participants with progressive keratoconus who received CXL treatment, while the low-energy group included participants with refractive errors who underwent FS-LASIK Xtra. This grouping followed routine clinical indications in real-world practice, whereby high-energy CXL is typically applied for progressive keratoconus, whereas low-energy prophylactic CXL is performed in conjunction with refractive surgery. Therefore, the present analysis of DLD across energy levels is observational in nature and not intended as a causal or dose–response comparison. The inclusion criteria for the high-energy group were: a diagnosis of progressive keratoconus with a minimum corneal thickness of ≥400 μm at the thinnest point. Progression was defined as an increase in the maximum keratometry value (K) of ≥0.75 D within the last 3mo, an equivalent sphere change of ≥0.75 D in the last 12mo, or a corneal thickness reduction of ≥30 μm within the last 6mo. The inclusion criteria for the low-energy group were: age ≥18y, refractive stability over the past 2y, and a minimum of 1wk without soft contact lens wear or 1mo without rigid gas-permeable contact lens wear before surgery. Exclusion criteria included: history of ocular trauma or previous corneal surgery, other ocular diseases, systemic conditions that could affect the cornea, and pregnancy or lactation at the time of treatment.

Surgical Procedure For patients with keratoconus, the KXL system (Avedro, USA) was used to perform epithelium-off rapid CXL with a total surface dose of 7.2 J/cm^2. The preoperative calibrated expected irradiance was 30 mW/cm^2. Local anesthesia was achieved using 1% oxybuprocaine eye drops. Under sterile conditions in the operating room, a mechanical scraper was used to remove the central 9.0 mm of corneal epithelium. After epithelial removal, the corneal stroma was soaked with 0.1% riboflavin solution (Vibex Xtra, Avedro, USA) for 10min. The remaining riboflavin in the cornea and conjunctival sac was washed out using balanced salt solution (BSS). The central cornea was then irradiated with 370 nm UV light for 4min at a working distance of 5 cm. During UV irradiation, BSS was intermittently used to keep the cornea moist. The patient was instructed to fixate on the light center, and the surgeon continuously monitored the central cornea, making adjustments as needed. At the end of the procedure, the eye was irrigated with BSS, a soft bandage contact lens was placed, and a transparent eye shield was applied until complete epithelial healing.

For patients with refractive error, the LASIK-Xtra protocol was adopted, with a total surface dose of 1.8 J/cm^2. A femtosecond laser (WaveLight FS200, Alcon, USA) was used to create a corneal flap with a diameter of 8.5 mm and a cutting depth of 110 μm. Following flap elevation, stromal ablation was performed under the flap using an excimer laser (WaveLight EX500, Alcon, USA). These patients subsequently underwent accelerated CXL, with a preoperatively calibrated irradiance of 30 mW/cm^2. The corneal stroma was soaked with 0.23% riboflavin solution (Vibex Xtra, Avedro, USA) for 60s, and after washing off the remaining riboflavin with BSS, the corneal flap was returned to its original position. UV irradiation with 370 nm light was applied to the central cornea for 60s at a working distance of 5 cm. After the procedure, the

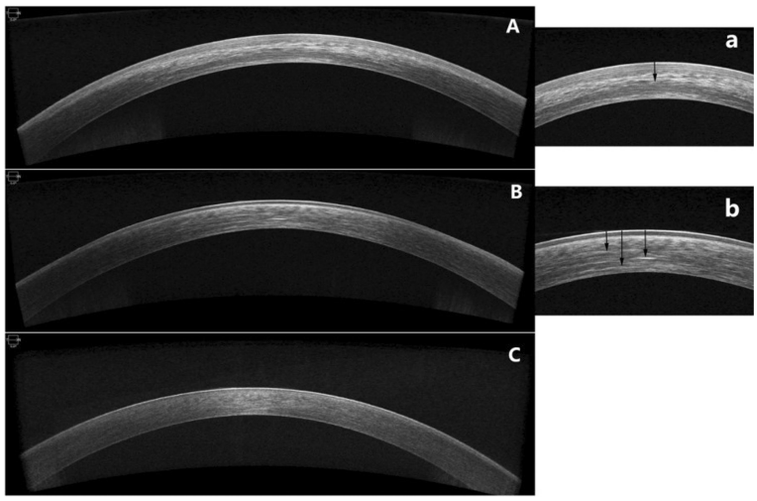

Figure 1 Evaluation of the demarcation line after corneal collagen cross-linking using AS-OCT A: AS-OCT image scored as grade 2 (clearly visible demarcation line); a: Magnified view of A showing a clearly defined demarcation line; B: AS-OCT image scored as grade 1 (visible but not clearly defined demarcation line); b: Magnified view of B, in which the demarcation line cannot be accurately identified; C: AS-OCT image scored as grade 0 (demarcation line not visible). AS-OCT: Anterior segment optical coherence tomography.

corneal flap was checked for proper repositioning, the eye was irrigated with BSS, and a soft bandage contact lens was placed with a transparent eye shield. The bandage lens was removed the following day.

IVCM and AS-OCT Examinations All patients underwent IVCM using the IVCM III RCM system (Heidelberg Engineering, Germany). A 670 nm laser beam was used to sequentially scan and obtain two-dimensional images of different corneal layers. Topical anesthesia was administered using 1% oxybuprocaine eye drops. The device was optically coupled to the cornea using a disposable sterile polymethyl methacrylate cap filled with high-viscosity carbomer gel. Patients were instructed to fixate on a target to ensure the accuracy of image acquisition. Corneal images were collected from the optical center of the cornea, and the DLD between the acellular region and the normal stromal cell layer was assessed. The transition zone was defined as the interface between the anterior stromal region showing keratocyte loss following CXL and the deeper stromal layer with normal keratocyte morphology. DLD was measured at the central cornea as the distance from the epithelial surface to this transition zone.

AS-OCT was performed using the Cirrus HD-OCT system (Carl Zeiss Meditec, Germany) equipped with an anterior segment imaging module to acquire high-resolution scans along the horizontal meridian of the cornea. Patients were instructed to fixate on the internal fixation target, and images were captured when the corneal reflex (a vertical white line along the corneal apex) became visible. In the enhanced corneal images, the demarcation line was identified and graded for visibility using the following scale: 0=not visible; 1=visible but difficult to measure; 2=clearly visible[11]. Images graded as score 1 were categorized as "not visible" for analysis because the demarcation line was insufficiently distinct for reliable depth measurement. For patients with a score of 2, central corneal DLD was measured using the built-in caliper tool provided by the manufacturer (Figure 1).

Image analysis was independently performed by two experienced ophthalmologists (Cao WJ and Zhou K) who were masked to the UV energy protocol and patients' clinical information during image evaluation. All images were assessed according to predefined and standardized assessment criteria. Disagreements between the two graders were resolved by adjudication from a senior corneal specialist (Cheng Y). This three-observer evaluation approach was applied to enhance the reliability and objectivity of image assessment.

Statistical Analysis Statistical Analysis was performed using SPSS software version 25.0 (IBM Corp., USA). Data were expressed as mean±standard deviation (SD) for continuous variables.

Normality of continuous variables was assessed using the Shapiro-Wilk test. For comparisons of continuous variables, the independent samples *t*-test was used when data followed a normal distribution; otherwise, the Mann-Whitney *U* test was applied. The visibility of the demarcation line was analyzed by incorporating follow-up time as a variable. For paired or related categorical data, the McNemar test was used.

For DLD measured by IVCM, follow-up time was also included as a variable. As the data did not follow a normal distribution, and sample sizes in some follow-up subgroups were relatively small, the Kruskal-Wallis test was used to compare differences among the three follow-up intervals (3mo, 6mo, and ≥12mo). When significant differences were detected, post hoc pairwise comparisons were performed using the Mann-Whitney *U* test with Bonferroni correction.

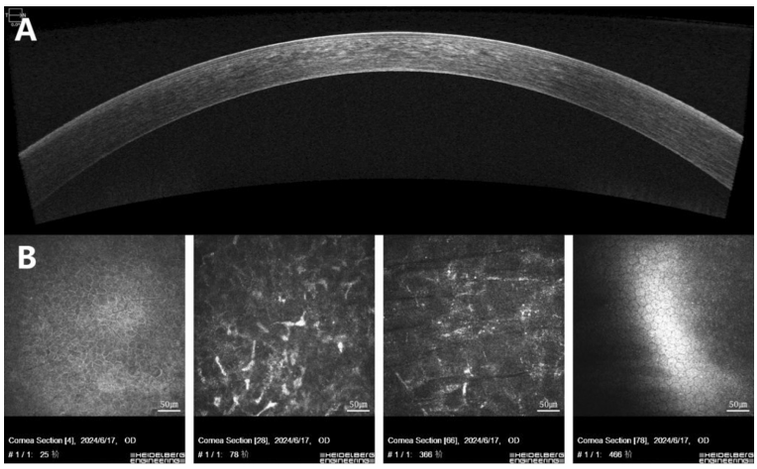

Figure 2 Comparison of AS-OCT and IVCM at 6mo after corneal cross-linking in the high-energy group A: AS-OCT image showing no visible demarcation line. B: IVCM image showing distinct transition zone between the treated and untreated stromal regions, with a measured demarcation line depth of 366 μm. The IVCM images are 400 μm x 400 μm. AS-OCT: Anterior segment optical coherence tomography; IVCM: *In vivo* confocal microscopy.

In addition, to evaluate monotonic trends in IVCM-measured DLD across ordered follow-up intervals, the Jonckheere-Terpstra nonparametric trend test was performed using R software.

RESULTS

Baseline Characteristics The high-energy group included 111 patients (144 eyes), consisting of 68 males and 43 females. The low-energy group included 105 patients (184 eyes), with 53 males and 52 females. There were no statistically significant differences in baseline characteristics between the two groups (Table 1).

Visibility The visibility of the CXL demarcation line as detected by IVCM and AS-OCT was compared at each time point. AS-OCT visibility was graded on a three-point scale: 0=not visible, 1=visible but not clearly distinguishable, 2=clearly visible. AS-OCT images graded as 2 were classified as "visible," while grades 0 and 1 were considered "not visible." At 3mo, 6mo, and at the final follow-up (≥12mo), significant differences in visibility were observed between IVCM and AS-OCT in both the high- and low-energy groups (P<0.001; Table 2, Figure 2).

Comparison of DLD Between Different Energy Groups Patients who completed the 3mo follow-up after undergoing either high- or low-energy CXL and had measurable DLD on both IVCM and AS-OCT were included for analysis. A total of 50 eyes were included in the high-energy group and 73 eyes in the low-energy group. The Mann-Whitney U test was used to compare DLD between the two energy groups for each imaging modality. Using IVCM, the mean DLD was 382.2±91.12 μm in the high-energy group and 192.53±58.76 μm in the low-energy group (Z=−8.252, P<0.001). Using AS-OCT, the mean DLD was 354.74±78.82 μm in the high-energy group and 196.51±55.57 μm in the low-energy group (Z=−8.164, P<0.001; Table 3 and Figure 3).

Table 1 Population characteristics and comparison: high-energy group *vs* low-energy group

Parameters	High-energy group	Low-energy group	*P*
Age, y (mean±SD)	23.39±7.30	22.95±5.84	0.477
Male/female, *n*	68/43	53/52	0.145
Eyes/patients, *n*			
Total	144/111	184/105	0.104
3mo	65/49	94/52	0.280
6mo	29/27	69/43	0.293
≥12mo	80/50	40/23	0.917

SD: Standard deviation.

Table 2 Comparison of IVCM and AS-OCT visibility at different follow-up time points between the high-energy and low-energy groups

Group and time point	AS-OCT (visible/total)	IVCM (visible/total)	*Z*	*P*
High-energy group				
3mo	50/65	65/65	13.067	<0.001
6mo	14/29	29/29	13.067	<0.001
≥12mo	33/80	70/80	31.610	<0.001
Low-energy group				
3mo	75/94	92/94	12.190	<0.001
6mo	29/69	69/69	38.025	<0.001
≥12mo	11/40	39/40	26.036	<0.001

AS-OCT: Anterior segment optical coherence tomography; IVCM: *In vivo* confocal microscopy. The number of eyes included at each follow-up time point varied because of differences in follow-up completion.

Comparison of DLD Between IVCM and AS-OCT Within both the high- and low-energy groups, DLD measured by IVCM and AS-OCT were compared. In the high-energy group, the mean DLD measured by IVCM and AS-OCT was not statistically significant (t=−1.869, P=0.110). The same was in the low-energy group (Z=−0.517, P=0.605; Table 3).

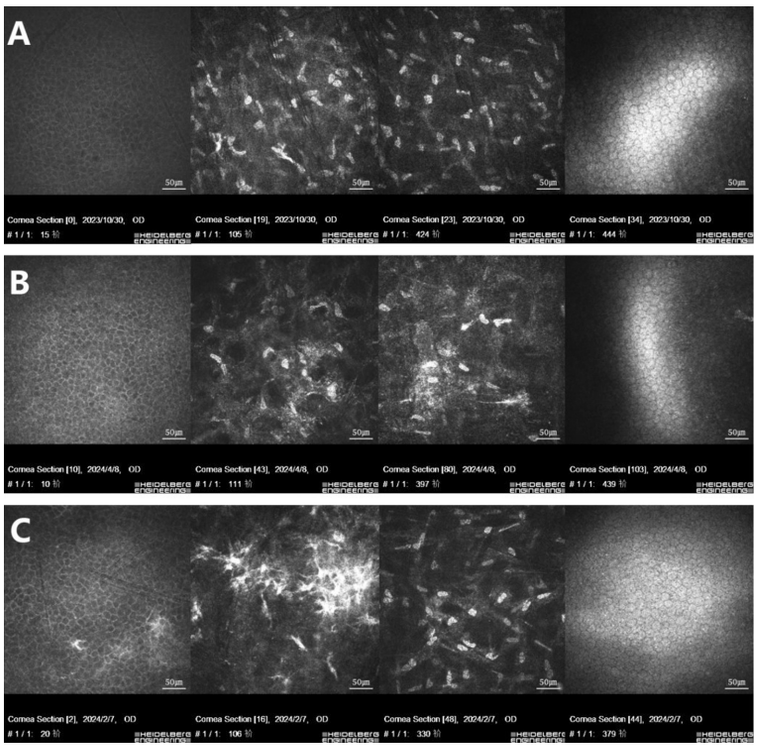

Figure 3 IVCM images comparing corneal layers in non-operated eyes, and eyes at 3mo after high- and low-energy CXL A: Images from a non-operated eye showing the epithelial layer, anterior stroma, posterior stroma, and endothelium. No stromal activation is observed. B: Images from the high-energy CXL group at 3mo postoperatively, showing the epithelial layer, anterior stroma, posterior stroma, and endothelium. Activated stromal tissue is visible in both anterior and posterior stroma, with a measured demarcation line depth of 397 μm. C: Images from the low-energy CXL group at 3mo postoperatively, showing the epithelial layer, anterior stroma, posterior stroma, and endothelium. Activated tissue is visible in the anterior stroma, but not in the posterior stroma. The measured demarcation line depth is 106 μm. The IVCM images are 400 μm × 400 μm. IVCM: *In vivo* confocal microscopy; CXL: Corneal cross-linking.

Longitudinal Comparison of IVCM-Detected DLD Within Each Energy Group To evaluate longitudinal changes in DLD, IVCM measurements at three postoperative follow-up time points were compared within the high-energy and low-energy groups. Significant differences in DLD across follow-up intervals were observed in both groups (high-energy group: H=29.666, P<0.001; low-energy group: H=15.388, P<0.001; Table 4 and Figure 4).

Post hoc pairwise comparisons using the Mann-Whitney U test with Bonferroni correction (adjusted significance level P<0.0167) revealed distinct temporal patterns between the two energy groups. In the low-energy group, a significant difference was identified between the 3mo and 6mo follow-ups (P<0.001), whereas comparisons involving ≥12mo follow-up did not reach statistical significance (3mo *vs* ≥12mo: P=0.018; 6mo *vs* ≥12mo: P=0.398). In contrast, in the high-energy group, significant differences were detected between ≥12mo follow-up and both 3mo (P<0.001) and 6mo follow-ups (P=0.001), while no significant difference was observed between 3mo and 6mo follow-ups (P=0.426).

To further assess the presence of an overall temporal trend across ordered follow-up intervals, the Jonckheere-Terpstra nonparametric trend test was performed. A significant monotonic temporal trend in IVCM-detected DLD across ordered follow-up intervals was observed in both groups [low-energy group: Jonckheere-Terpstra test (JT)=4882.5, P=0.001; high-energy group: JT=2514, P<0.001].

Table 3 Comparison of demarcation line depth measured by IVCM and AS-OCT at 3mo postoperatively between high-energy and low-energy groups

Parameters	High-energy group (n=50)	Low-energy group (n=73)	Z	P
IVCM (μm)	382.2±91.12	192.53±58.76	-8.252	<0.001
AS-OCT (μm)	354.74±78.82	196.51±55.57	-8.164	<0.001
t/Z	-1.869	-0.517		
P	0.110	0.605		

AS-OCT: Anterior segment optical coherence tomography; IVCM: *In vivo* confocal microscopy.

DISCUSSION

CXL is widely used to halt the progression of keratoconus and other ectatic corneal diseases. The procedure involves the combined action of a photosensitizer—riboflavin (vitamin B2)—and UV light to generate reactive oxygen species. These reactive molecules induce chemical cross-linking between

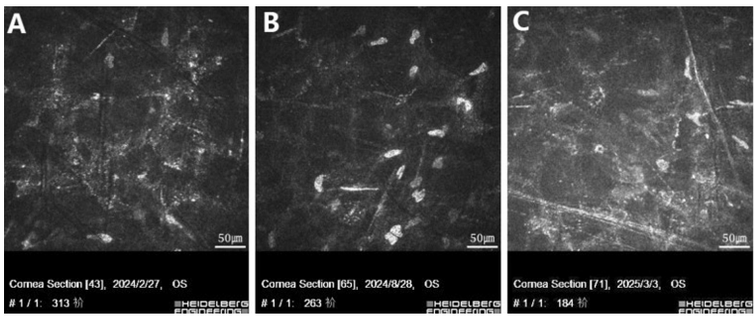

Figure 4 IVCM images showing the DLD at 12, 18, and 24mo postoperatively in the high-energy group A: Image at 12mo postoperatively, with a measured DLD of 313 μm. B: Image at 18mo postoperatively, with a measured DLD of 263 μm. C: Image at 24mo postoperatively, with a measured DLD of 184 μm. The IVCM images are 400 μm x 400 μm. IVCM: *In vivo* confocal microscopy, DLD: Demarcation line depth.

Table 4 Comparison of IVCM-measured demarcation line depth between the high- and low-energy groups at different follow-up periods

Parameters	High energy		Low-energy	
	Eyes, *n*	Depth (μm, mean±SD)	Eyes, *n*	Depth (μm, mean±SD)
3mo	65	376.15±94.15	92	180.05±58.53
6mo	29	348.28±127.56	69	150.07±48.00
≥12mo	70	280.29±107.91	39	157.31±52.02
H	-	29.666	-	15.388
P	-	<0.001	-	<0.001

Differences across follow-up intervals within each group were assessed using the Kruskal-Wallis test. The number of eyes included at each follow-up time point varied due to differences in follow-up completion. SD: Standard deviation; IVCM: *In vivo* confocal microscopy.

adjacent collagen fibrils in the corneal stroma, forming new covalent bonds. This process enhances the biomechanical strength of the cornea and helps prevent further progression of ectasia[12-13]. In corneal refractive surgery, corneal tissue is reshaped to correct refractive errors. However, in patients with high refractive errors or relatively thin corneas, postoperative biomechanical instability may occur, increasing the risk of visual regression or even postoperative ectasia. The incidence of corneal ectasia following LASIK has been reported to range from 0.04% to 0.6%[14]. Combining CXL with refractive procedures has been shown to enhance postoperative biomechanical stability, thereby reducing the risk of ectasia and refractive regression[15]. Previous studies[16-19] have reported that the UV energy typically used for prophylactic CXL in refractive surgery ranges from 1.4 to 2.7 J/cm². In the present study, we used a dose of 1.8 J/cm² for refractive patients. For keratoconus patients, the standard CXL dose is typically between 5.4 and 7.2 J/cm². According to published literature[20], a transition zone between the cross-linked and untreated stromal tissue becomes apparent approximately 2wk after CXL. This demarcation line is considered an important indicator of effective CXL. The DLD is closely associated with UV energy, irradiation time, and riboflavin penetration, and serves as a key parameter for evaluating treatment efficacy. A shallow demarcation line may indicate insufficient cross-linking, while excessive depth may raise safety concerns such as potential endothelial cell damage.

Traditionally, AS-OCT has been the preferred modality for assessing the postoperative demarcation line following CXL. Its advantages include non-invasiveness, speed, convenience, and the ability to directly visualize the location of the demarcation line. However, in clinical practice, the demarcation line is not always visible after surgery. Even when visible, the depth is sometimes difficult to determine with precision. As shown in Figure 1C, the AS-OCT image of a patient at 6mo postoperatively revealed no distinct hyperreflective line, making it impossible to evaluate the CXL effect. In Figure 1B, although several hyperreflective lines of varying lengths and depths are present, no clear boundary is identifiable, rendering precise depth measurement unfeasible. Liu *et al*[21] compared demarcation line visibility after modified biphasic high-dose transepithelial CXL (10.8 J/cm² at the cone apex and 7.2 J/cm² in surrounding areas) versus conventional transepithelial CXL (7.2 J/cm²). At 2wk postoperatively, AS-OCT revealed a visible demarcation line in 81.3% (26/32) of eyes in the modified group, compared to only 15.6% (5/32) in the conventional group. In most conventional cases, the line appeared poorly defined and discontinuous, indicating that lower CXL energy reduces demarcation line visibility on AS-OCT. Similarly, Doors *et al*[22] reported that among 29 eyes treated with the standard CXL protocol for keratoconus or post-LASIK ectasia, the demarcation line was visible by AS-OCT in 75% of cases at 1mo post-operative. However, by 3mo, only 5 of 20 eyes had a visible line, all of which were too faint for accurate measurement. By 6mo, the demarcation line was no longer detectable in any of the treated eyes. Consistent

with these findings, our study also showed that demarcation line visibility on AS-OCT decreased with lower energy levels and longer postoperative follow-up intervals. However, in contrast to AS-OCT, IVCM demonstrated superior visibility under both conditions—among patients in the low-energy group and at extended follow-up times—suggesting its potential as a more reliable imaging modality in these contexts. IVCM evaluates the corneal response after CXL by identifying a cellular-level transition zone, separating the anterior stromal region with keratocyte depletion from the deeper stroma where keratocytes are preserved or undergoing repopulation, thereby providing high-resolution microstructural information. In contrast, AS-OCT visualizes the demarcation line as a hyperreflective band within the corneal stroma, representing an optical interface rather than a direct cellular change. A key consideration is whether the transition zone identified by IVCM corresponds, in terms of depth and location, to the demarcation line visualized on AS-OCT. Importantly, this demarcation line represents an imaging-derived morphological interface rather than the true depth of biomechanical stiffening induced by CXL, and the two imaging modalities reflect different structural and biological aspects of the stromal response after CXL. Previous studies have reported a good degree of correspondence between the two modalities under specific conditions. Kymionis *et al*[23] reported no statistically significant differences between IVCM and AS-OCT in measuring CXL depth one month postoperatively. Similarly, Thorsrud *et al*[24] found strong correlation between the two methods in keratoconus patients. In our study, we compared DLD at 3mo postoperatively in both high- and low-energy groups and found no significant difference between IVCM and AS-OCT measurements in either group. However, demarcation line visibility was markedly better with IVCM. In the low-energy group, visibility at 3mo was 97.87% with IVCM versus 79.79% with AS-OCT. At ≥12mo, visibility remained high with IVCM (97.50%) but dropped significantly with AS-OCT (27.50%). These findings indicate that AS-OCT may have limitations in detecting the demarcation line in cases with lower UV energy or during long-term follow-up. For example, in the patient shown in Figure 2, imaged 6mo after high-energy CXL, the demarcation line was not visible on AS-OCT, whereas IVCM clearly revealed activated stromal tissue and measurable depth. Therefore, IVCM appears to provide a more robust assessment of cross-linking-related stromal changes, especially in low-energy protocols and at extended follow-up intervals.

This study compared corneal DLD under different UV energy levels within the constraints of routine clinical practice. Because current clinical consensus and ethical considerations preclude the use of multiple CXL energy protocols within the same patient cohort, a real-world clinical scenario was analyzed in which high-energy CXL was applied in patients with keratoconus and low-energy CXL in patients undergoing refractive surgery. This design enabled evaluation of the imaging characteristics and temporal evolution of DLD under different energy conditions. Within this clinical framework, lower UV energy resulted in shallower CXL-induced stromal changes. These findings are consistent with results reported by Liu *et al*[21], who demonstrated that the central DLD measured by IVCM was significantly greater in the high-energy group (248.3±25.0 μm) than in the low-energy transepithelial CXL group (136.5±15.6 μm, $P<0.001$). In our study, patients with keratoconus underwent CXL at 7.2 J/cm², resulting in a mean IVCM-measured depth of 382.2±91.12 μm. In contrast, patients undergoing combined FS-LASIK with low-energy CXL at 1.8 J/cm² had significantly shallower DLD, averaging 192.53±58.76 μm. This difference reflects the differing clinical goals of CXL in these two contexts. For keratoconus patients, CXL is used therapeutically to halt disease progression, necessitating deeper and more robust cross-linking. In contrast, when used adjunctively with refractive surgery, the goal of CXL is prophylactic—to enhance biomechanical stability and reduce the risk of postoperative ectasia or refractive regression. Given the potential complications associated with deeper cross-linking, such as stromal haze or endothelial cell damage, lower UV energy protocols are often preferred in the prophylactic setting.

Given the consistently high visibility of IVCM across different follow-up intervals, we further analyzed changes in DLD over time within both the high- and low-energy groups. Our findings revealed statistically significant differences in DLD at different follow-up points in both groups, with an overall trend toward decreasing depth over time, as illustrated by the representative patient shown in Figure 4. This temporal reduction was more pronounced in the high-energy group, which may reflect differences in stromal remodeling and wound-healing responses following CXL performed with different energy levels. High-energy CXL induces a deeper initial zone of keratocyte apoptosis and collagen cross-linking. During postoperative healing, progressive keratocyte repopulation, extracellular matrix turnover, and stromal remodeling may occur, leading to a gradual reduction in the morphologically detectable DLD on imaging[25-26], without necessarily implying attenuation of the underlying biomechanical effect. In contrast, low-energy CXL produces a more superficial initial effect, which may be associated with a less pronounced and more heterogeneous remodeling process. Accordingly, in the low-energy group, the IVCM-detected DLD decreased at 6mo compared with 3mo follow-up, followed by a modest increase at ≥12mo; however, the mean depth at ≥12mo remained

lower than that observed at 3mo. Compared with the high-energy group, the temporal change in DLD in the low-energy group was less pronounced. This relatively attenuated and less uniform pattern is more likely attributable to greater heterogeneity in stromal remodeling responses associated with low-energy or prophylactic CXL protocols. Under such conditions, limited riboflavin penetration, oxygen availability, and spatially heterogeneous photochemical reactions may result in a shallower and less uniform imaging-detected DLD, leading to variability in the morphologically detectable transition zone on imaging over time. Previous studies[27-28] have similarly reported that epi-on or low-penetration CXL protocols tend to produce a shallower and less distinct demarcation line compared with conventional epi-off or higher-energy protocols, and that postoperative stromal reflectivity and remodeling may remain inhomogeneous during mid- to long-term follow-up. In this context, longitudinal assessments based on IVCM primarily reflect modality-specific, imaging-detected stromal remodeling processes over time, rather than serving as direct surrogates for long-term biomechanical efficacy. Building on these findings, we plan to conduct a prospective study with a larger sample size to dynamically investigate the relationship between IVCM-detected DLD and long-term clinical outcomes following CXL.

This study has several limitations. First, its retrospective design may introduce inherent selection bias and limits causal inference. Second, the two study groups differed in underlying corneal pathology (keratoconus *vs* post-LASIK corneas), reflecting real-world clinical indications but precluding direct causal comparison of different CXL energy protocols. Third, biomechanical outcomes were not directly assessed, as corneal biomechanical measurements (such as Corvis ST or Brillouin microscopy) were not available; therefore, the present analysis relied on imaging-derived surrogates rather than direct biomechanical endpoints. Fourth, formal inter-observer agreement metrics [such as κ or intraclass correlation coefficient (ICC)] were not calculated, although images were independently graded by two masked observers with adjudication by a senior specialist to enhance consistency. Finally, demarcation line depth represents an imaging-based morphological marker and may not fully capture the spatial extent or magnitude of biomechanical stiffening induced by CXL. Prospective studies integrating standardized biomechanical assessments are warranted to further elucidate the relationship between imaging-detected stromal changes and functional biomechanical outcomes.

In summary, while both AS-OCT and IVCM allow postoperative assessment of DLD, IVCM demonstrates consistently superior visibility across different energy levels and follow-up intervals, making it more suitable for long-term imaging-based evaluation in clinical practice.

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