Corneal Stromal Demarcation Line Depth Following Standard and a Modified High Intensity Corneal Cross-linking Protocol

George D. Kymionis, MD, PhD; Konstantinos I. Tsoulnaras, MD; Dimitrios A. Liakopoulos, MD, MSc; Christina A. Skatharoudi, MD; Michael A. Grentzelos, MD; Nikolaos G. Tsakalis, MD

ABSTRACT

PURPOSE: To compare the corneal stromal demarcation line depth using anterior segment optical coherence tomography (AS-OCT) after corneal cross-linking (CXL) using two different treatment protocols: the standard Dresden protocol (30 minutes with 3 mW/cm^2) and a modified high intensity protocol (7 minutes with 18 mW/cm^2), corresponding to a total surface dose of 5.4 and 7.5 J/cm^2, respectively.

METHODS: This prospective, comparative, interventional case series included 29 keratoconic patients (32 eyes). All patients underwent CXL using the same high intensity ultraviolet-A (UV-A) irradiation device (CCL-365; Peschke Meditrade GmbH, Huenenberg, Switzerland). Sixteen eyes were treated for 30 minutes with 3 mW/cm^2 according to the standard Dresden protocol, whereas 16 eyes were treated with a novel modified high intensity CXL protocol for 7 minutes with 18 mW/cm^2 of UV-A irradiation intensity. One month postoperatively, corneal stromal demarcation line depth was measured by two independent observers using AS-OCT.

RESULTS: There was no significant difference in corneal stromal demarcation line depth between observer measurements for both groups (P = .645, Dresden protocol group; P = .715, high intensity group). Mean corneal stromal demarcation line depth was 341.81 ± 47.02 μm for the Dresden protocol group and 313.37 ± 48.85 μm for the high intensity protocol group. There was no statistically significant difference (P = .104) in the corneal stromal demarcation line depth between the two groups. Mean endothelial cell density did not change significantly in either group (P = .090, Dresden protocol group; P = .103, high intensity group). No intraoperative or postoperative complications were noted.

CONCLUSIONS: Corneal stromal demarcation line depth using UV-A irradiance with 3 mW/cm^2 for 30 minutes and 18 mW/cm^2 for 7 minutes was similar. It seems that the current modified accelerated CXL protocol provided the same treatment depth as the standard Dresden protocol.

[*J Refract Surg.* 2016;32(4):218-222.]

Corneal cross-linking (CXL) has already proved to be a safe and effective surgical technique used to strengthen the tissue of the ectatic cornea and arrest keratoconus progression.[1,2] The standard CXL procedure requires a 30-minute dose of ultraviolet-A (UV-A) irradiation at an intended irradiance of 3.0 mW/cm^2 with total surface dose of 5.4 J/cm^2 (Dresden protocol).[1]

Currently, the most reliable clinical sign to determine treatment depth is the depth of the corneal stromal demarcation line, which is detectable on slit-lamp examination as early as 2 weeks after CXL and can be measured equally with confocal microscopy and anterior segment optical coherence tomography (AS-OCT).[3-5]

In our recently published studies, we evaluated the corneal stromal demarcation line depth (provided by the AS-OCT device) in different CXL protocols. The accelerated 10-minute protocol with UV-A irradiation intensity of 9 mW/cm^2 resulted in a significantly shallower effect than the standard Dresden protocol of 3 mW/cm^2 irradiation intensity.[6] The high intensity CXL treatment protocol of 5 minutes with UV-A irradiation intensity of 18 mW/cm^2 has proved to be even shallower than the previously mentioned protocols.[7]

Consequently, we have modified the 10-minute high UV-A intensity protocol to 14 minutes of UV-A irradiation, maintaining the irradiance to 9 mW/cm^2, which results in an increased total surface dose (7.5 J/cm^2) delivered to the cornea; the depth of the produced demarcation line was equal to the standard Dresden protocol.[8]

From Vardinoyiannion Eye Institute of Crete, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece (GDK, KIT, DAL, CAS, MAG, NGT); the 1st Department of Ophthalmology, "G. Gennimatas" Hospital of Athens, Medical School, National and Kapodistrian University of Athens, Athens, Greece (GDK); and Bascom Palmer Eye Institute, University of Miami, Miller School of Medicine, Miami, Florida (GDK).

Submitted: July 19, 2015; Accepted: December 15, 2015

The authors have no financial or proprietary interest in the materials presented herein.

Correspondence: George D. Kymionis MD, PhD, Vardinoyiannion Eye Institute of Crete, University of Crete, Faculty of Medicine, 71003 Heraklion, Crete, Greece. E-mail: kymionis@med.uoc.gr

doi:10.3928/1081597X-20160216-01

The purpose of this study was to evaluate and compare the depth of the corneal stromal demarcation line using AS-OCT with two different treatment protocols: the standard Dresden CXL protocol (30 minutes with 3 mW/cm^2) versus a modified high intensity CXL protocol (7 minutes with 18 mW/cm^2) using the same high intensity UV-A irradiation device.

PATIENTS AND METHODS

Patient Population

This prospective, comparative, interventional case series included 29 patients (22 male and 7 female; 32 eyes) with progressive keratoconus. The clinical diagnosis of keratoconus was based on corneal topography data (iTrace; Tracey Technologies, Houston, TX). Inclusion criteria of the patient selection were progressive keratoconus (progression of the ectatic disorder was based on either an increase in the cone apex keratometry of 0.75 diopters [D] or on alteration of 0.75 D in the spherical equivalent refraction during the past 6 months), age older than 18 years, corneal thickness greater than 400 µm, and no other ocular pathological signs, pregnancy, or lactation. The patients included in this study were classified as stage I and II according to the Amsler–Krumeich classification.

All patients underwent uneventful CXL treatment with the use of the high intensity UV-A illuminator (CCL-365; Peschke Meditrade GmbH, Huenenberg, Switzerland). The selection of the CXL irradiation treatment protocol was made alternately; 16 eyes were treated for 30 minutes with an intended irradiance of 3 mW/cm^2 according to the Dresden protocol (Dresden protocol group), whereas 16 eyes were treated for 7 minutes with an intended irradiance of 18 mW/cm^2 (high intensity protocol group).

Data obtained from the patient records included age, sex, preoperative ultrasound corneal pachymetry (Corneo-Gage Plus; Sonogage Inc., Cleveland, OH), preoperative keratometric readings, endothelial cell density, and AS-OCT scans (Visante OCT 3.0; Carl Zeiss Meditec Inc., Jena, Germany).

Institutional review board approval from University Hospital of Heraklion was obtained and all patients were appropriately informed before their participation in the study and gave written informed consent in accordance with institutional guidelines, according to the tenets of the Declaration of Helsinki.

Surgical Technique

All procedures were performed under sterile conditions. After topical anesthesia with proxymetacaine hydrochloride 0.5% eye drops (Alcaine; Alcon Laboratories, Inc., Fort Worth, TX), corneal epithelium was mechanically removed using a rotating brush within an 8- to 9-mm diameter. After epithelial removal, riboflavin (0.1% solution of 10 mg riboflavin-5-phosphate in 10 mL dextran-T-500 20% solution; Medicross, Medio-Haus, Behrensbrook, Neudorf, Germany) was instilled on the center of the cornea every 3 minutes for approximately 30 minutes in both groups. UV-A irradiation was performed using a new high intensity UV-A optical system (CCL-365). Before treatment, an intended irradiance of 3 mW/cm^2 (Dresden protocol group) or 18 mW/cm^2 (high intensity protocol group) was calibrated using the UV-A light meter YK-35UV (Lutron Electronic, Coopersburg, PA). In the Dresden protocol group, UV-A irradiance was performed for 30 minutes according to standard Dresden protocol in an intended irradiance of 3 mW/cm^2, whereas the high intensity protocol group (modified accelerated protocol) used UV-A irradiance for 7 minutes in an intended irradiance of 18.0 mW/cm^2, corresponding to a total surface dose of 5.4 and 7.5 J/cm^2, respectively. During UV-A irradiation, riboflavin solution was applied every 3 minutes in both groups to maintain corneal riboflavin saturation. At the end of the procedure, a silicone-hydrogel bandage contact lens (14 mm in diameter, 8.6 base curvature, Dk = 140 barrers; lotrafilcon B, Air Optix; Alcon Laboratories, Inc.) was applied until full reepithelialization.

Postoperative medications included chloramphenicol/dexamethasone drops (Dispersadron; Thea Laboratories, Inc., Clermont-Ferrand, France) four times daily until the removal of the bandage contact lens. After the removal of the contact lens, patients received corticosteroid drops four times daily (FML, fluorometholone 0.1%; Falcon Pharmaceuticals, Fort Worth, TX) with tapering for the next 3 weeks. Patients were encouraged to use artificial tears at least six times per day for at least 3 months postoperatively.

Measurements of the demarcation line depth with the AS-OCT scans were taken by two independent observers 1 month postoperatively, as analytically reported in our previous studies.[4-8] Endothelial cell density was measured 1 month postoperatively and compared with preoperative values due to increased total UV-A energy dose delivered by the cornea in the current modified accelerated protocol as we performed in a previous modified protocol.[8]

Statistical Analysis

All statistical analyses were performed with SPSS for Windows software (version 20; SPSS, Inc., Chicago, IL). Normality of distribution of all measurements was confirmed using the Shapiro–Wilk test, appropriate for small sample sizes of fewer than 50 participants.

TABLE 1

Preoperative Patient Parameters for CXL in the Dresden Protocol (30 Minutes With 3 mW/cm^2) and High Intensity Protocol (7 Minutes With 18 mW/cm^2) Groups

Parameter	Dresden Protocol	High Intensity Protocol	*P*[a]
Age (y)	27.56 ± 6.20 (19 to 41)	25.06 ± 6.34 (18 to 39)	.268
Minimum corneal thickness (μm)	465.19 ± 29.92 (415 to 534)	482.69 ± 31.60 (422 to 530)	.118
Mean steep K (D)	49.82 ±3.89 (44.08 to 57.41)	47.81 ± 2.61 (43.99 to 51.97)	.097
Mean flat K (D)	45.55 ± 3.12 (40.92 to 51.20)	43.98 ± 2.16 (41.53 to 47.58)	.108

CXL = corneal cross-linking; K = keratometric values; D = diopters
[a]*t test.*

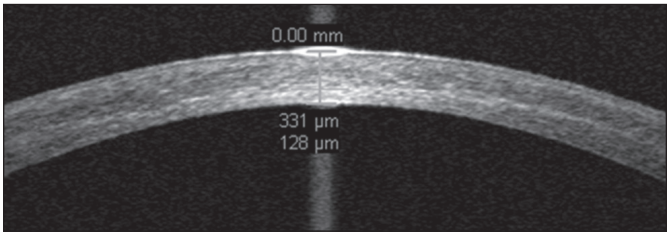

Figure 1. High-resolution anterior segment corneal optical coherence tomography scan visualizing the corneal stromal demarcation line 1 month after corneal collagen cross-linking with a 30-minute ultraviolet-A irradiation time period at an intended irradiance of 3 mW/cm^2 (standard Dresden protocol); central corneal demarcation line depth was 331 μm.

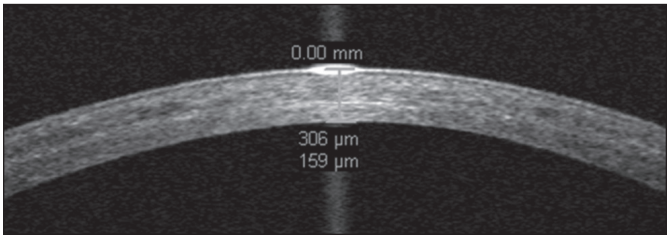

Figure 2. High-resolution anterior segment corneal optical coherence tomography scan visualizing the corneal stromal demarcation line 1 month after corneal collagen cross-linking with a 7-minute ultraviolet-A irradiation time period at an intended irradiance of 18 mW/cm^2 (modified accelerated protocol); central corneal demarcation line depth was 306 μm.

All values are expressed as mean ± standard deviation. A *P* value of less than .05 was considered statistically significant. To avoid bias and ensure the comparability of the two groups, the baseline characteristics between the groups were compared. The agreement between the two observers (DAL and CAS) was studied using the Bland–Altman method and the 95% limits of agreement were computed.

RESULTS

The study enrolled 32 eyes of 29 patients (22 male and 7 female; 16 eyes in each group). The two groups were similar in age, preoperative corneal thickness, and steep and flat keratometric readings (**Table 1**). No intraoperative or postoperative complications were observed in any of the patients. The mean time of corneal reepithelialization was 4.0 ± 0.89 days (range: 3 to 6 days) in the Dresden protocol group and 3.5 ± 0.82 days (range: 2 to 5 days) in the high intensity protocol group, with no statistically significant difference in the duration of epithelial healing between groups (*P* = .109).

The corneal stromal demarcation line was clearly visible in AS-OCT images in all eyes included in the study by both observers. The average corneal stromal demarcation line depth measurements between the two independent observers were used in the statistical analysis. The limits of agreement between the two observers were -19.62 to 22.12 (Dresden protocol group) and -22.62 to 24.86 (high intensity protocol group) (**Figure A**, available in the online version of this article). There was no statistically significant difference in demarcation line depth measurements between the two observers (*P* = .645 for Dresden protocol group and *P* = .715 for high intensity protocol group). Mean depth of corneal stromal demarcation line was 341.81 ± 47.02 μm (range: 248.00 to 400.00 μm) for the Dresden protocol group and 313.37 ± 48.85 μm (range: 230.50 to 393.50 μm) for the high intensity protocol group. There was no statistically significant difference in the corneal stroma demarcation line depth between the two groups (*P* = .104, *t* test for unpaired data). **Figures 1-2** show high-resolution AS-OCT scans of the corneal stroma demarcation line representative of each group.

The mean endothelial cell density did not change statistically significantly in either group. The endothelial cell density decreased from 2,590 ± 125 to 2,530 ± 134 cells/mm^2 (*P* = .090) in the Dresden protocol group and from 2,641 ± 157 to 2,578 ± 165 cells/mm^2 (*P* = .103) in the high intensity protocol group.

DISCUSSION

The standard Dresden CXL protocol is already proved to be safe and effective in arresting progression

of ectatic disorders such as keratoconus.[9] However, its extensive duration regarding surgical procedure (more than an hour in total) has driven the investigation of alternative "equally effective" treatment procedures with higher irradiance and lower treatment times.

The concept of the accelerated CXL was based on the photochemical law of Bunsen–Roscoe (law of reciprocity), which demonstrates the inverse relationship between intensity and duration of light (irradiation) that determines the reaction of a light-sensitive material and is used in photochemistry and chemical photography.[10] However, there are reported studies concerning photochemical effect in inert materials demonstrating that the law of reciprocity could be altered, an effect called "reciprocity law failure."[11-13] In addition, Schindl et al. summarized that ultraviolet irradiation could possibly not follow the Bunsen–Roscoe law in cell and tissue samples and this law seems to be restricted to rather narrower limits for most photobiologic reactions.[14]

Moreover, concerns have already been raised regarding the effectiveness of high intensity CXL in biomechanically strengthening the cornea, due to the possible oxygen dependency of the CXL procedure.[15] Wernli et al. showed in vitro that efficacy of CXL with high UV-A intensities is significantly decreased.[16] We demonstrated that the accelerated CXL protocols provided shallower treatment depth as recorded by the demarcation line depth.[6,7] Thus, the 10-minute CXL protocol produced a significantly shallower demarcation line in contrast to the standard Dresden protocol,[6] whereas the 5-minute CXL protocol seemed to be even shallower as independently studied.[7] Moreover, Brittingham et al. demonstrated that the accelerated CXL protocol had shallower demarcation line depth and weaker effect on the keratometric values compared to the standard Dresden protocol.[17] Also, Hashemi et al. presented better corneal flattening achieved with standard CXL protocol than with the accelerated protocol.[18]

Our findings indicated that the accelerated CXL protocols possibly provided less effective treatment outcomes and additional modifications could be made to the time and irradiation intensity settings to achieve the same photochemical-therapeutic effect with the successful Dresden CXL protocol. Consequently, we have modified the accelerated 10-minute CXL protocol by increasing the UV-A irradiation time to 14 minutes, keeping the same intended UV-A irradiance of 9 mW/cm^2, corresponding to the increased total surface dose of 7.5 J/cm^2. Thus, we achieved the same treatment effect (provided by demarcation line depth) with the clinically successful standard Dresden 30-minute protocol and we proposed that our modified 14-minute protocol could replace the classic Dresden protocol in terms of its efficacy.[8]

In the current study, we modified the high intensity (5 minutes with 18 mW/cm^2 of UV-A) CXL protocol in a similar fashion as we did in our previous study.[8] Consequently, the UV-A duration time was increased from 5 to 7 minutes, which corresponds to a 40% increase in time and in total surface UV-A energy dose (7.5 J/cm^2).

Our results showed that the demarcation line depth of CXL (using AS-OCT) was similar between the standard Dresden protocol and the modified high intensity 7-minute protocol with 18 mW/cm^2. In our study, corneal stromal demarcation line depth is measured as the absolute depth of demarcation line in the corneal stroma, which is the established methodology used in the majority of the published studies.[5,19] Also, another methodology of demarcation line depth estimation has been suggested, presenting the dermarcation line depth as the fraction of the demarcation line depth to the total corneal thickness.[17] This methodology seems to provide further valuable information and could be evaluated in forthcoming studies. No intraoperative or early postoperative complications were noted. The epithelial healing process after CXL was the same in both groups. endothelial cell density did not change significantly in both groups 1 month postoperatively. However, there are some limitations in our study, such as the short follow-up period and the limited population.

The current study supports that the Bunsen–Roscoe law is not directly applicable to the living human cornea and modifications in accelerated protocols should be made to maintain the same efficacy levels as in the Dresden protocol. Even if the exact amount of stromal tissue that needs to be cross-linked to stabilize the progression of an ectatic disorder is not yet clarified in the literature, the long-term clinical validity of the Dresden protocol remains the gold standard approach and every modifying approach has to be referred and compared to this protocol. Therefore, with this study we proposed a modification to the high (5-minute) UV-A intensity CXL settings to have comparable photochemical results with the standard Dresden protocol. Further studies are necessary in larger population groups and with longer follow-up to establish the relationship between time intensity settings and efficacy levels for the CXL treatment.

AUTHOR CONTRIBUTIONS

Study concept and design (GDK, KIT); data collection (GDK, DAL, CAS, NGT); analysis and interpretation of data (GDK, KIT, DAL, MAG); writing the manuscript (GDK, KIT, DAL, NGT); critical revision of the manuscript (GDK, KIT, CAS, MAG); statistical expertise (DAL); administrative, technical, or material support (KIT, DAL); supervision (GDK)

REFERENCES

1. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol.* 2003;135:620-627.
2. Kymionis GD, Grentzelos MA, Liakopoulos DA et al. Long-term follow-up of corneal collagen cross-linking for keratoconus–the Cretan study. *Cornea.* 2014;33:1071-1079.
3. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. *Cornea.* 2006;25:1057-1059.
4. Kymionis GD, Grentzelos MA, Plaka AD, et al. Evaluation of the corneal collagen cross-linking demarcation line profile using anterior segment optical coherence tomography. *Cornea.* 2013;32:907-910.
5. Kymionis GD, Grentzelos MA, Plaka AD, et al. Correlation of the corneal collagen cross-linking demarcation line using confocal microscopy and anterior segment optical coherence tomography in keratoconic patients. *Am J Ophthalmol.* 2014;157:110-115.
6. Kymionis GD, Tsoulnaras KI, Grentzelos MA, et al. Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. *J Cataract Refract Surg.* 2014;40:736-740.
7. Kymionis GD, Tsoulnaras KI, Liakopoulos DA, et al. Corneal stromal demarcation line determined with anterior segment optical coherence tomography following a very high intensity corneal collagen cross-linking protocol. *Cornea.* 2015;34:664-667.
8. Kymionis GD, Tsoulnaras KI, Grentzelos MA, et al. Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. *Am J Ophthalmol.* 2014;158:671-675.
9. Raiskup F, Theuring A, Pillunat LE, Spoerl E. Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: ten-year results. *J Cataract Refract Surg.* 2015;41:41-46.
10. Bunsen R, Roscoe HE, Photochemische Untersuchungen. *Poggendorff's Ann Chem Phys.* 1857;100:43-88, 481-516, 1857;101:235-263.
11. Johnson KM, Hesselink L, Goodman JW. Holographic reciprocity law failure. *Appl Opt.* 1984;23:218.
12. de Almeida A, Sobol WT, Barnes GT. Characterization of the reciprocity law failure in three mammography screen-film systems. *Med Phys.* 1999;26:682-688.
13. Grossman LW, Van Tuinen RJ, Kruger JB, Scholz KL. Film reciprocity law failure in scintillation camera imaging. *Radiology.* 1981;138:697-700.
14. Schindl A, Rosado-Schlosser B, Trautinger F. Reciprocity regulation in photobiology: an overview [article in German]. *Hautarzt.* 2001;52:779-785.
15. Richoz O, Hammer A, Tabibian D, Gatzioufas Z, Hafezi F. The biomechanical effect of corneal collagen cross-linking (CXL) with riboflavin and UV-A is oxygen dependent. *Transl Vis Sci Technol.* 2013;2:6.
16. Wernli J, Schumacher S, Spoerl E, Mrochen M. The efficacy of corneal cross-linking shows a sudden decrease with very high intensity UV light and short treatment time. *Invest Ophthalmol Vis Sci.* 2013;54:1176-1180.
17. Brittingham S, Tappeiner C, Frueh BE. Corneal cross-linking in keratoconus using the standard and rapid treatment protocol: differences in demarcation line and 12-month outcomes. *Invest Ophthalmol Vis Sci.* 2014;55:8371-8376.
18. Hashemi H, Miraftab M, Seyedian MA, et al. Long-term results of an accelerated corneal cross-linking protocol (18 mW/cm(2)) for the treatment of progressive keratoconus. *Am J Ophthalmol.* 2015;160:1164-1170.
19. Doors M, Tahzib NG, Eggink FA, Berendschot TT, Webers CA, Nuijts RM. Use of anterior segment optical coherence tomography to study corneal changes after collagen cross-linking. *Am J Ophthalmol.* 2009;148:844-851.

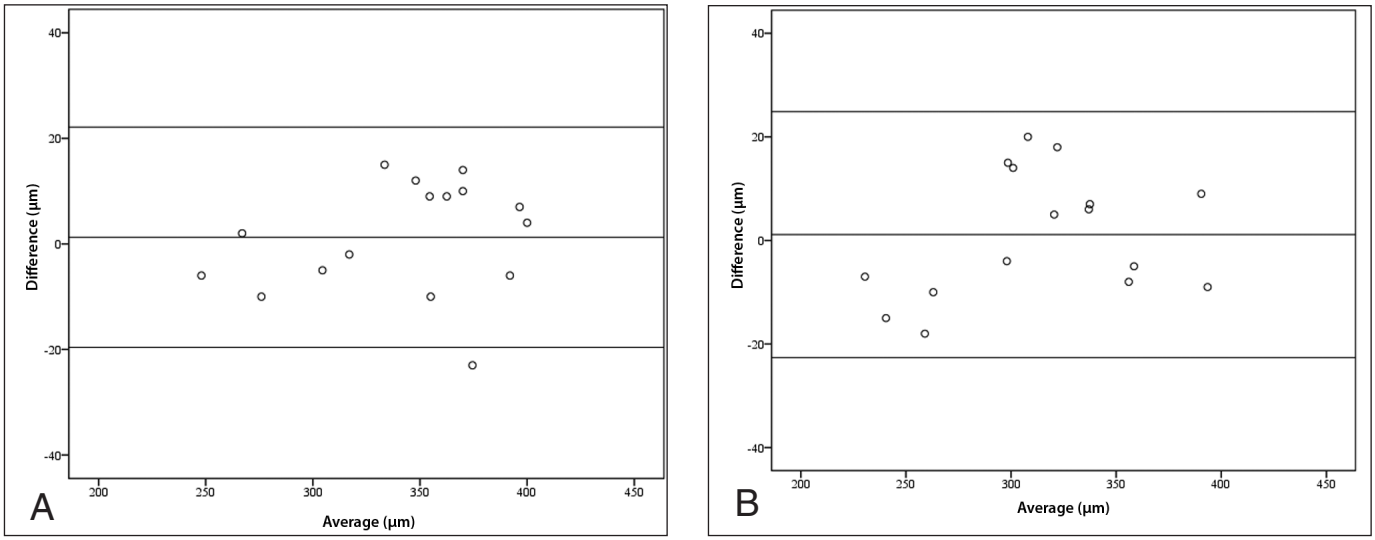

Figure A. Bland–Altman plots of agreement between the two observers for anterior segment optical coherence tomography measurements of the corneal stromal demarcation line depth in the (A) Dresden protocol group (30 minutes with 3 mW/cm^2) and the (B) high intensity protocol group (7 minutes with 18 mW/cm^2). The horizontal lines represent the mean and 95% limits of agreement.