Comparative micromorphologic in vitro porcine study of IntraLase and Femto LDV femtosecond lasers

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PURPOSE: To assess flap creation and stromal bed quality of 2 femtosecond refractive surgery lasers in laser in situ keratomileusis.

SETTING: Augenklinik am Neumarkt, Cologne, Germany.

METHODS: Corneal flaps were created in 115 freshly enucleated porcine eyes using the 60 kHz IntraLase FS laser (Advanced Medical Optics) and a prototype model of the Femto LDV femtosecond laser (Ziemer Ophthalmic Systems AG). The parameters that were evaluated included actual versus intended thickness by subtraction pachymetry, cutting and total suction time, quality of flap edges, and smoothness of flap beds. Confocal microscopy (Atos PLμ [Altos GmbH]) was used to objectively determine the root mean square (RMS) of the surface roughness of the stromal bed.

RESULTS: Cutting time was 31 seconds for the 60 kHz IntraLase FS laser and 38 seconds for the Femto LDV laser. With both lasers, the standard deviation in achieved versus intended flap thickness was small (136 μm ± 10 and 130 ± 9 μm, respectively). Under micromorphologic examination, stromal bed quality was slightly better with the IntraLase. The RMS of bed roughness was 1.6 ± 0.5 μm with the IntraLase and 2.0 ± 0.4 μm with the Femto LDV. Neither laser showed significant thermal or mechanical damage in adjacent tissue layers of the stromal bed. The laser-induced bubble layer was more pronounced with the IntraLase.

CONCLUSION: The laser cuts of the IntraLase FS and Femto LDV femtosecond lasers were equally smooth and of excellent quality. The standard deviation of the flap thickness was small and equal in both systems.

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Since the introduction of the femtosecond laser for ophthalmic applications in 2002,[1] several studies have compared the laser with mechanical microkeratomes. Most of the studies have focused on postoperative outcomes; a few have looked at aspects of the corneal changes caused by these 2 methods of flap creation and compared the differences.[2–7] The study we present was designed to evaluate the performance of 2 femtosecond lasers. It focuses on the possible induction of thermal or mechanical side-effects to adjacent tissue layers, speed of flap creation, surface smoothness, and potential variety of flap geometry settings.

MATERIALS AND METHODS

The Intralase FS (Advanced Medical Optics) and the Femto LDV (Ziemer Ophthlamic Systems AG) femtosecond lasers were used in the study. Table 1 summarizes the basic features of the 2 lasers.

Freshly enucleated porcine eyes (n = 115) were kept in balanced salt solution (BSS) at 4°C to 7°C and used within 12 hours. The intraocular pressure (IOP) in the eyes was controlled with a Schiötz tonometer, and BSS was injected into the vitreous to maintain IOP between 20 mm Hg and 30 mm Hg. Before the laser cut, the corneal thickness was measured with ultrasound pachymetry (Sonomed); the mean of 3 apical measurements was used. After the cut, the flap was lifted and pachymetry was repeated. Flap

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Table 1. Basic features of the 2 laser systems.

Feature	IntraLase	Femto LDV
Concept	Amplifier	Oscillator
Wavelength (nm)	~1060	~1040
Pulse width (fs)	>500	~250
Spot size (µm)	>1	<1
Repetition rate	60 kHz	>1 MHz
Pulse energy	~1 µJ	Some nJ
Operation speed (@ 9.5 mm diameter) (s)	31*	38
Cutting geometry/ flexibility	Very high	Limited
Size/mobility/ footprint	Bulky/fixed/ 120 cm × 125 cm	Very small/ mobile/ 70 cm × 95 cm
Environmental requirements	Constant temp; humidity required	Industrial laser; not sensitive to environment

*The manufacturer's technical specification for the commercially available Femto LDV notes 25 seconds cutting time.

thickness was determined by subtracting the corneal thickness after the flap was lifted from the total corneal thickness. The whole eye was then immersed in a fixative (paraformaldehyde 4%, glutaraldehyde 2.5%, and sodium cacodylate 1%; pH 7.2) with the flaps lifted and prepared for light microscopy (LM) and scanning electron microscopy (SEM) by the Department of Pathology at the University of Veterinary Medicine Hanover, Germany. An equal number of eyes (n = 10) were analyzed using LM (toluidine blue staining) to determine thermal damage, if any, and SEM to evaluate the edges and surface quality of the corneal bed.

For quantitative analysis of the stromal surface quality, a profilometer (Atos PLµ [Altos GmbH]) based on the principle of confocal microscopy was used.[8] The roughness of the stromal bed was defined by standard deviations of the measured mean values of the surface topography for a 200 µm × 200 µm area (overall surface area). To avoid the influence of artifacts from tissue fixation, additional measurements were performed with SEM high magnification on visually smooth surface areas of the stromal bed (smooth surface area). The roughness was graded by an observer who was masked to the laser model used.

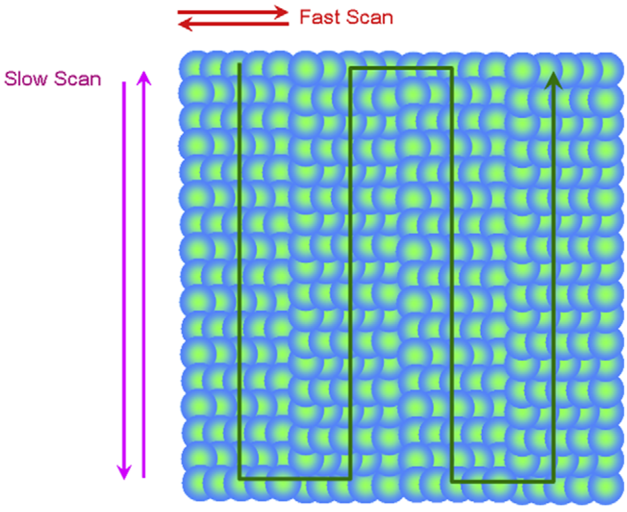

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Figure 1. The laser pulses of the Femto LDV are applied in a fast and slow mode, ensuring an overlap that helps to avoid tissue bridges.

Procedure time measurements included total suction time and cutting time during flap creation. The total suction time, which is the total time under which the eye globe is fixated by low pressure and without blood and oxygen supply, is of great clinical importance. In the IntraLase system, the suction ring is applied first, followed by introduction of the cone with the applanation plate. Hereafter, the surgeon controls the flap position, having the option to readapt the flap position on the computer interface for up to 1.0 mm, with the limitation that the initial flap diameter might decrease. The laser is then introduced for the cutting process. During the laser process, in the IntraLase system, the surgeon can visually control the laser interaction process on the cornea. With the Femto LDV, the laser is applied through a mirror arm that ends in a handpiece with an integrated suction ring. A control window allows centration of the suction ring on the center of the cornea. Once the handpiece is applied, the surgeon has no further options to control or adapt the flap position. During the laser emission and cutting process, the control window is closed, allowing no visual control of the process. Both the Femto LDV and the IntraLase applanate the cornea over a diameter of 10.0 mm and offer a maximum flap diameter of up to 9.5 mm depending on the initial settings.

In the IntraLase system, the cutting time depends mainly on the spot/line separation of the scanning pattern, which can affect the surface quality of the cut. In the performed cuts, a typical clinical setting with a spot/line separation of 8 µm/8 µm and additional "pocket" was used. The Femto LDV scans the cornea in fast and slow modes, and this overlap is designed to minimize residual tissue bridges (Figure 3).

Although in both lasers the cutting effect is initialized by photodisruption, the laser process differs in some characteristic aspects. The laser-induced gas bubble layer within the corneal interface is less pronounced in the Femto LDV. The IntraLase works with an additional side cut. The Femto LDV does not perform side cuts in a separate process; instead, it creates a simultaneous side cut and raster pass similar to the action of a mechanical microkeratome.

RESULTS

Both lasers achieved the intended flap thickness of 130 µm. The Femto LDV achieved a mean thickness of 130 µm ± 9 (SD) and the IntraLase achieved

Table 2. Comparison of flap thickness, suction, cutting time, and flap diameter.

Measurement	Femto LDV	IntraLase 60 kHz
Number of eyes	55	60
Intended thickness (μm)	130	130
Achieved thickness (μm)	130 ± 9	135 ± 10
Cutting time (s)	38*	31
Suction time (s ± SD)	82 ± 22	70 ± 15
Flap diameter (mm)	10.5	9.3

*The manufacturer's technical specification for the commercially available Femto LDV notes 25 seconds cutting time.

a mean thickness of 135 ± 10 μm. The cutting speed did not vary in either system but was slightly faster in the IntraLase system (31 seconds versus 38 seconds). In the IntraLase, nearly half the cutting time was consumed by the vertical side cut (12 seconds). Table 2 summarizes the results of intended versus achieved flap thickness and flap diameter, as well as cutting and suction times.

Figure 2 shows a typical Femto LDV post-laser interface together with an LM section, showing a very smooth cut without obvious thermal or mechanical damage to adjacent tissue layers. The IntraLase cut was characterized by a more pronounced gas bubble layer in the flap interface, which appeared as a white opaque layer. The Femto LDV created a corneal resection from the outside to the inside, where the side cut was performed simultaneously, allowing the bubbles to escape through the side-cut. Within minutes, the gas bubbles disappeared and transparency of the cornea was reestablished. In Figure 3, an IntraLase cut is shown in situ. The flap was not opened. No signs of thermal or mechanical damage to adjacent tissue layers are detectable in the LM section.

The flap edges differed in the 2 systems. The side-cut angle for the IntraLase was set at 90 degrees. In Figures 4 and 5, the SEM and LM sections of the flap edge after IntraLase and Femto LDV application are depicted. The bed surfaces had similar macroscopic features in both laser systems. Because of tissue bridges in the interface, the traction forces necessary to open the flap were equal in both systems, at least from the subjective experience of the experimental surgeons. The initial macroscopic appearance of the stromal bed was equally smooth in both laser systems. Examples of SEM sections of the stromal bed are shown in Figures 6 and 7.

The bed roughness was slightly better in the IntraLase system, but the difference was not significant. Table 3 summarizes the results of the confocal microscopy analysis.

DISCUSSION

The IntraLase and Femto LDV are infrared scanning-pulse femtosecond lasers emitting at 1060 nm and 1040 nm, respectively; they use photodisruption to create a corneal cut within the corneal tissue. The interaction process is based on nonlinear absorption and consecutive disruption of the tissue.[9–11] Nonlinear absorption means the corneal tissue is usually transparent for the infrared laser radiation at moderate intensities where no absorption takes place. Only at very high intensities can 4 or more infrared photons interact with the tissue at the same time and be absorbed by the tissue-like one ultraviolet photon, leading to ionization of the molecules. As this happens at the very focus of the laser beam only, it gives the user the advantage of 3-dimensional tissue processing. The absorption process is no longer limited to the surface.[12–15] Applanation is also used during flap creation with the femtosecond laser, although at lower pressure levels.[16]

Numerous studies have compared the consistency of flap thickness and visual outcomes with mechanical microkeratomes and the femtosecond laser. Most have shown that the femtosecond laser provides more predictable flap thickness than mechanical microkeratomes.[17,18]

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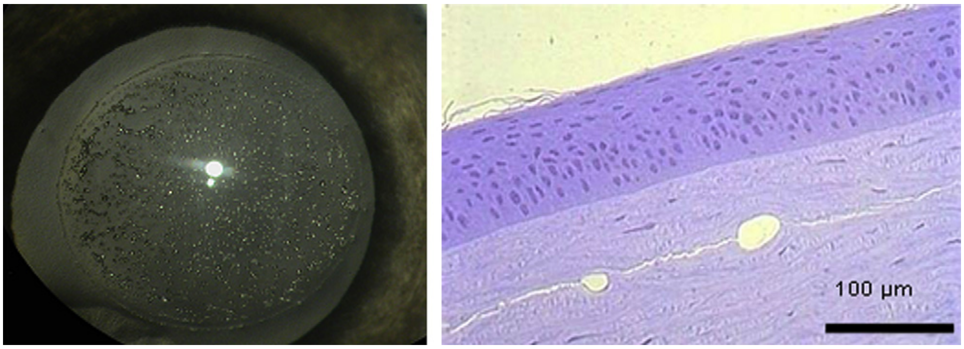

Figure 2. Corneal interface and LM section of cornea after laser in situ keratomileusis flap cut with the Femto LDV. Some of the confulated gas bubbles are visualized by immediate fixation of the cornea.

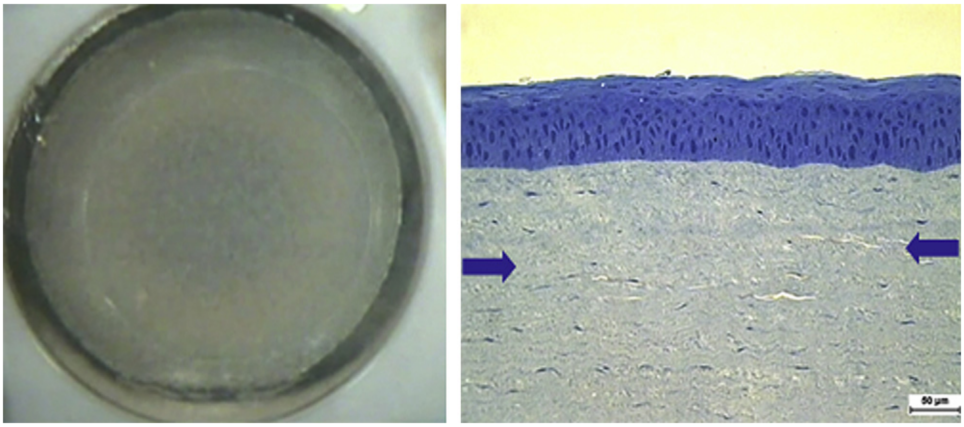

Figure 3. Corneal in situ interface after IntraLase 60 kHz flap cut. The eye was fixated after the gas bubble layer disappeared. The corneal layers are back in the original position, making it difficult to detect the dissected layer (*arrows*).

Technical Differences Between the IntraLase and Femto LDV Femtosecond Laser Systems

The IntraLase and Femto LDV femtosecond laser systems can be differentiated generally by the interaction process and beam delivery. The IntraLase uses higher pulse energies and lower pulse repetitions rates than the Femto LDV. An amplified laser system is used to deliver pulse energies in the range of 1 µJ to the cornea. The repetition rate is 60 kHz. The Femto LDV system delivers nanojoule pulse energy to the eye and uses megahertz repetition rates. Based on these laser parameters, the nature of the cutting processes of the 2 lasers is different.[19]

Operating Characteristics of the Two Systems

With the IntraLase, a flap thickness between 90 µm and 160 µm can be achieved, as well as a flap diameter of up to 9.5 mm, and the hinge width and position are adjustable. The IntraLase also allows readjustment of the flap position according to the position of the pupil visible on the interface, as well as visual control of the laser process during laser cutting. However, the IntraLase tended to cause opaque bubble layers, leading to trauma because of higher pressure applied to the surrounding corneal fibers. This did not occur with the Femto LDV because it creates a corneal resection from the outside to the inside; the bubbles formed during the cut escape through the side cut, preventing them from building up inside. Moreover, the bubble generation by the LDV system is significantly lower than the generation by the IntraLase. With the IntraLase, the lamellar portion is cut first so gas builds up and cannot escape until the side cut, which is the final portion to be cut, is accessible.

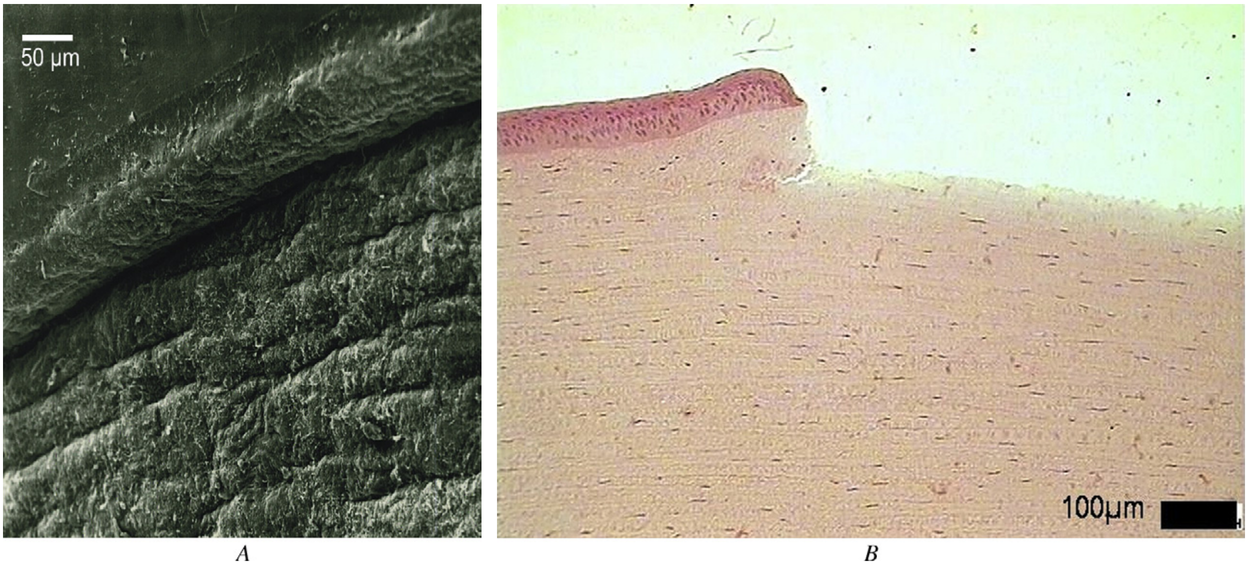

Figure 4. IntraLase flap edge with SEM (*A*) and LM (*B*). The flap edge is set perpendicular to the cutting plane but can be adjusted if needed. The flap edge is sharp, and the achieved thickness and angle of incidence meets the intended presettings.

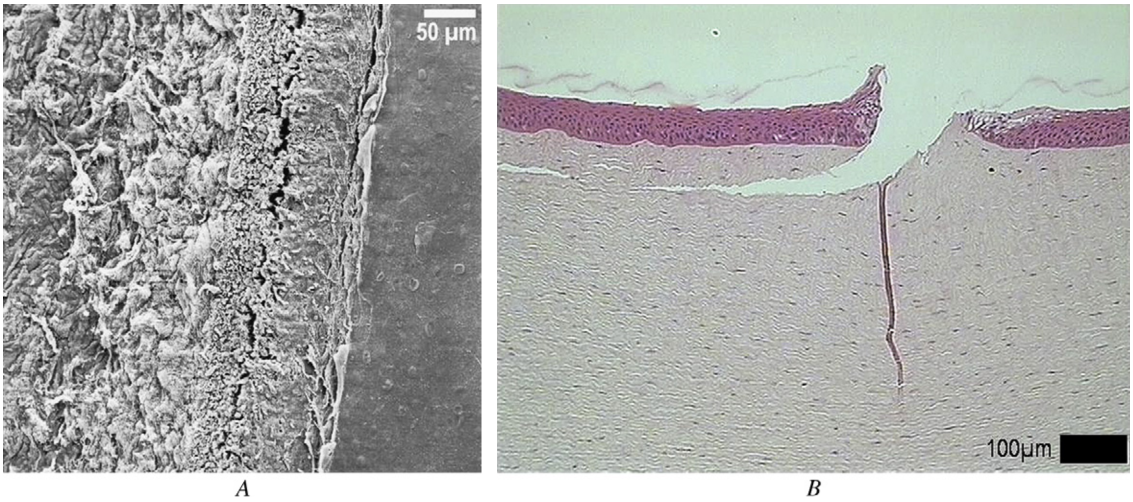

Figure 5. The Femto LDV flap edge with SEM (*A*) and LM (*B*). The angle of incidence of the flap edge is sharp and comparable to the edge of a mechanical flap cut.

With the Femto LDV, 2 flap thicknesses are available (130 and 160 μm for the preproduction prototype and 110 μm and 140 μm for the commercially available model). The device allows alignment of the suction ring and flap position on the corneal center, but repositioning is not an option. There is no visual control during the laser process and no side-cut option. However, the system does provide an electronic suction control, which ensures a controlled cutting process. Flap resistance is equivalent to that of the IntraLase, but one of the difficulties in flap entry is visualization of the flap edge since the gas bubble layer is much less intense and vanishes quickly. Additionally, since the Femto LDV does not produce a separate side cut, tiny tissue bridges are found at the flap edge; along with the pinpoint angle of incidence of the flap edge, these bridges make it difficult to find the entrance with a blunt instrument.

Morphologic and surface quality studies demonstrate that there has been a technological evolution, resulting in an improvement in corneal flap and stromal bed quality as the laser's speed has increased.[4,5] The

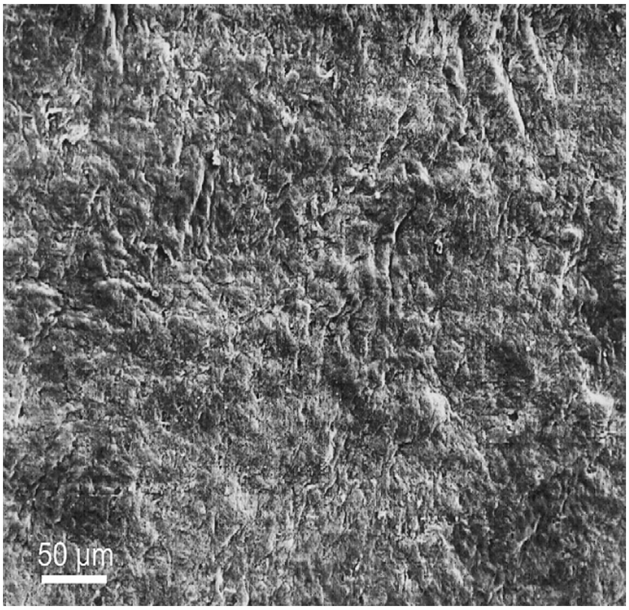

Figure 6. The IntraLase 60 kHz stromal bed (SEM; original magnification ×200).

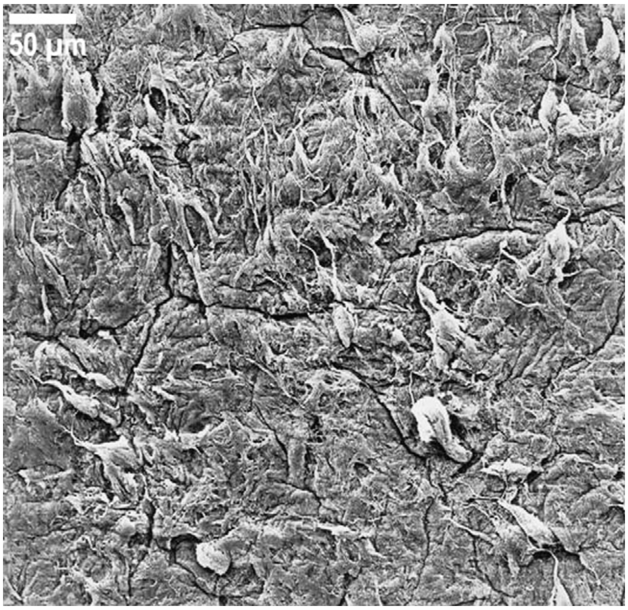

Figure 7. The Femto LDV stromal bed (SEM).

Table 3. Stromal bed roughness.

Femtosecond Laser	Overall Surface Area (µm)	Smooth Surface Area (µm)
Femto LDV	3.0 ± 0.3	2.0 ± 0.4
60 kHz IntraLase	2.7 ± 0.6	1.6 ± 0.5

most recent study[5] compared the 60 kHz IntraLase femtosecond laser and the Zyoptix XP microkeratome (Bausch & Lomb). Using SEM images of corneal beds created with the 2 devices, masked observers graded the images. The images were also assessed using computerized software designed for roughness analysis. The results show that the 60 kHz IntraLase femtosecond laser produced smoother quality stromal beds than the Zyoptix XP microkeratome, noting that the higher speed allows lower energy use and a tighter spot/line separation.[6,7] Other studies have involved human cadaver models, but we used porcine eyes so we would have access to a sufficient number of eyes for statistical significance concerning flap geometry. It must be considered that both lasers have been optimized for use in human eyes and were applied on the same biological material (porcine cornea). At the same time, interpretation of the quality of the laser–tissue interaction in application to human corneas is somewhat limited.

Convenience is an important issue when comparing the performance of the 2 lasers. The Femto LDV is a small, compact system that fits under all excimer laser systems, so patients are not required to move from 1 bed to another. Additionally, it is not affected by changes in the environment such as temperature and humidity because of the laser oscillator. IntraLase microjoule pulses need an amplified laser system consisting of a laser oscillator that generates femtosecond laser pulses with several nanojoules of pulse energy because there is insufficient energy for the system to cut inside the cornea. Using a laser oscillator makes the laser more sensitive to misalignment and even temperature changes, but the other cutting and scanning principle of the Femto LDV allows the surgeon to discount the amplifier and use the oscillator only. This system is potentially more resistant to changes in temperature, humidity, or vibration. The disadvantage of the Femto LDV is that the scanning device has to be implemented into the handpiece, hindering the surgeon's control of the cutting process.

Analysis of the results of this in vitro investigation seem to confirm the high quality of femtosecond laser flap creations reported in earlier studies. The smoothness of the stromal beds was good, with no thermal damage and little standard deviation in flap geometry. The ability to customize the flap parameters of edge angle, hinge position, and flap thickness provide an additional benefit for both systems. The hinge position can be patient specific with the choice of nasal, superior, temporal, or inferior. This study confirms that the technological evolution from the 15 kHz to 30 kHz to the current 60 kHz model has enabled the femtosecond laser to more effectively compete with mechanical microkeratomes in terms of speed of flap creation and ease of use. The 60 kHz laser has significant advantages with regard to flap geometry and positioning. This study also demonstrates the superiority of the 60 kHz laser in stromal bed smoothness. Although the total suction time remains longer than with the mechanical microkeratome used in this study, the biologic rehabilitation time of neurosensory tissue is in the range of 3 to 8 minutes, which is much longer than the total suction time seen in this study with the 60 kHz femtosecond laser (total suction time = 70 seconds [mean]). The mean total suction time for the Femto LDV was 50 seconds. Future developments are needed to speed up the procedure without endangering the laser–tissue interaction, particularly with regard to thermal injury or increased dosage of infrared radiation.

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