Scanning Electronic Microscopy Evaluation of the Roughness of the Stromal Bed After Deep Corneal Cut with the LDV Femtosecond Laser (Z6) (Ziemer) and the ONE Microkeratome (Moria)

Varga, Zsolt; Bergin, Ciara; Roy, Sylvain; Nicolas, Michael; Tschuor, Patrizia; Majo, François

How to cite

VARGA, Zsolt et al. Scanning Electronic Microscopy Evaluation of the Roughness of the Stromal Bed After Deep Corneal Cut with the LDV Femtosecond Laser (Z6) (Ziemer) and the ONE Microkeratome (Moria). In: Current eye research, 2016, vol. 41, n° 10, p. 1302–1309. doi: 10.3109/02713683.2015.1119281

This publication URL: https://archive-ouverte.unige.ch/unige:93153
Publication DOI: 10.3109/02713683.2015.1119281

Current Eye Research

ISSN: 0271-3683 (Print) 1460-2202 (Online) Journal homepage: http://www.tandfonline.com/loi/icey20

Scanning Electronic Microscopy Evaluation of the Roughness of the Stromal Bed After Deep Corneal Cut with the LDV Femtosecond Laser (Z6) (Ziemer) and the ONE Microkeratome (Moria)

Zsolt Varga, Ciara Bergin, Sylvain Roy, Michael Nicolas, Patrizia Tschuor & François Majo

To cite this article: Zsolt Varga, Ciara Bergin, Sylvain Roy, Michael Nicolas, Patrizia Tschuor & François Majo (2016) Scanning Electronic Microscopy Evaluation of the Roughness of the Stromal Bed After Deep Corneal Cut with the LDV Femtosecond Laser (Z6) (Ziemer) and the ONE Microkeratome (Moria), Current Eye Research, 41:10, 1302-1309, DOI: 10.3109/02713683.2015.1119281

To link to this article: http://dx.doi.org/10.3109/02713683.2015.1119281

CURRENT EYE RESEARCH
2016, VOL. 41, NO. 10, 1302–1309
http://dx.doi.org/10.3109/02713683.2015.1119281

Taylor & Francis
Taylor & Francis Group

Scanning Electronic Microscopy Evaluation of the Roughness of the Stromal Bed After Deep Corneal Cut with the LDV Femtosecond Laser (Z6) (Ziemer) and the ONE Microkeratome (Moria)

Zsolt Varga[a,b], Ciara Bergin[a], Sylvain Roy[c], Michael Nicolas[a], Patrizia Tschuor[a], and François Majo[a]

[a]Department of Ophthalmology, University of Lausanne, Fondation Asile des Aveugles, Lausanne, Switzerland; [b]Department of Clinical Neuroscience, University Hospital of Geneva, Geneva, Switzerland; [c]Private Practice, Carouge, Switzerland

ABSTRACT

Purpose: To compare the stromal bed surface quality and the accuracy of dissection depth after deep lamellar cuts using the Leonardo Da Vinci (LDV) femtosecond laser (Z6) and the ONE Microkeratome.

Methods: Deep lamellar cuts were performed on nine human donor corneoscleral buttons: five with the LDV femtosecond (FS) laser (Z6) (Ziemer) and four with the ONE Microkeratome (MK) (Moria). Corneal thickness was measured with ultrasound pachymetry before and after the dissection. The Stromal bed quality was evaluated using light microscopy (n = 4) and scanning electron microscopy (SEM) (n = 9). The surface roughness on SEM images was graded on the scale of 1 (smoothest) to 5 (roughest) by four observers, blinded to the method used. Particle analysis on the SEM images was performed in order to have an objective measure of smoothness.

Results: The achieved dissection depth using the FS laser was 496.4 ± 46.4 µm when attempting 500 µm and 474 ± 60 µm with the microkeratome when attempting 350 µm. Histological evaluation of the corneoscleral buttons by both light and electron microscopy showed significantly smoother surface using the FS laser compared to the microkeratome. There were fewer and smaller particles observed in the SEM images of FS laser cut buttons ($p < 0.001$).The average observer based score of anterior surface roughness (50×) was 2.2 for the FS laser and 3.9 for the microkeratome dissections ($p < 0.001$).

Conclusions: The LDV femtosecond laser (Z6) platform is capable of creating deep corneal lamellar dissection with smoother surface quality and with more predictable cut depth as compared to the One Microkeratome.

ARTICLE HISTORY
Received 23 May 2014
Revised 11 June 2015
Accepted 6 November 2015

KEYWORDS
Cornea; femtosecond laser; microkeratome; scanning electronic microscopy

Introduction

Lamellar grafts are now performed as the first choice graft for the majority of the patients requiring corneal transplants.[1,2] Currently, descemet stripping automated endothelial keratoplasty (DSAEK) is the most popular form of endothelial keratoplasty (EK). EK has been associated with quicker visual recovery, less postoperative astigmatism, reduced risk of infection and better wound integrity giving it clear advantages over penetrating keratoplasty.[3,4] Inherent differences or limitations of methods used to prepare of donor tissues, result in suboptimal interface and consequently lower vision and overall reduced success with EK procedures.[4,5]

A mechanical cut is performed to prepare the posterior lamellar before implantation, it has been hypothesized that femtosecond laser (FL) creates a smoother mechanical cut and smoother stromal surface than the microkeratome (MK). To date there have been a number of articles investigating this.[6–15] Several authors have developed subjective (observer based grading) and objective (e.g. texture analysis) scores to quantify the smoothness of stromal beds using primarily scanning electron microscopy (SEM) images for comparison.[7,11,13] FL and MK dissected corneas show good stromal bed quality, with MK resulting in better overall smoothness,[11,13] however, on closer examination of the literature a relationship between smoothness and low laser pulse energy (nJ)/high laser pulse frequency/spot size has been indicated.[8,13,16,17] The Leonardo Da Vinci (LDV) FS laser from Ziemer has far greater pulse frequency (>1000 kHz) and smaller spot size (≪1 µm) than other commercially available devices (Visumax ≤500 kHz, 0.5–2.5 µm; Intralase 15–150 kHz, 0.5–8 µm; Femtec 40 kHz, 1–3 µm). This higher pulse frequency corresponds with a lower pulse energy, which has been associated with fewer/smaller intrastromal bubbles,[18] and less stromal cell apoptosis and less intrastromal fibronectin formation indicating less wound healing.[17]

The aim of this study was to examine the surface quality by subjective and objective assessment of deep lamellar cuts performed by the LDV FS laser (Z6) (Ziemer) versus the ONE Microkeratome (Moria).

CONTACT François Majo fmajo@bluewin.ch Head of Medical and Surgical Cornea, Refractive Surgery and Eye Bank, Hôpital Ophtalmique Universitaire Jules-Gonin, Avenue de France 15, 1004 Lausanne, Switzerland.

Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/icey

Materials and methods

Donor corneas and preparation of the graft

Donor corneoscleral buttons, kept in organ culture for up to 21 days (CorneaMax, Eurobio, Courtaboeuf, France) and in a deturgescent medium for 2 days prior to dissection (CorneaJet, Eurobio, Courtaboeuf, France), from the eye bank in Lausanne were used. Deep lamellar cuts were performed on 9 human donor corneoscleral buttons that were unsuitable for transplantation: 5 with the LDV femtosecond laser (Crystalline, hand piece LCS, (Z6)) (Ziemer AG, Port, Switzerland) and 4 with the ONE Microkeratome (Moria SA, Antony, France). Once the cut was completed, the entire corneoscleral button was transferred to a Coronet donor punch (Network Medical Products Ltd., Ripon, UK) to perform an 8 mm partial thickness trephination from the endothelial side. Following this, the donor cornea was placed back and secured onto the artificial anterior chamber with the endothelial side up. The posterior lamellar disk was separated from the stromal bed using Bonn forceps (Moria SA, Antony, France) and a blunt LASIK spatula to divide residual tissue bridges (Figure 1).

Femtosecond laser

A Ziemer LDV femtosecond laser (Z6) was used for the deep lamellar dissection using the LCS handpiece that was designed for lamellar surgery with a cutting depth of 500 µm. The FS laser cuts were performed on corneoscleral buttons secured on a custom made Ziemer artificial anterior chamber. The pressure in the artificial anterior chamber was controlled through an integrated irrigation port using a bottle height of 1.5 meters measured from the artificial anterior chamber to obtain a pressure of approximately 60 mm Hg. A 9 mm diameter cut was created at 500 µm depth using pulse energy of <100 nJ, a pulse frequency of >5 MHz and a spot size of <0.1 µm. We used the posterior lamellar keratoplasty femtolaser profile available with the Z6 LDV femtolaser.

Microkeratome

The microkeratome cuts were performed with the ONE Microkeratome and a 350 µm head, using a new blade for each cut.

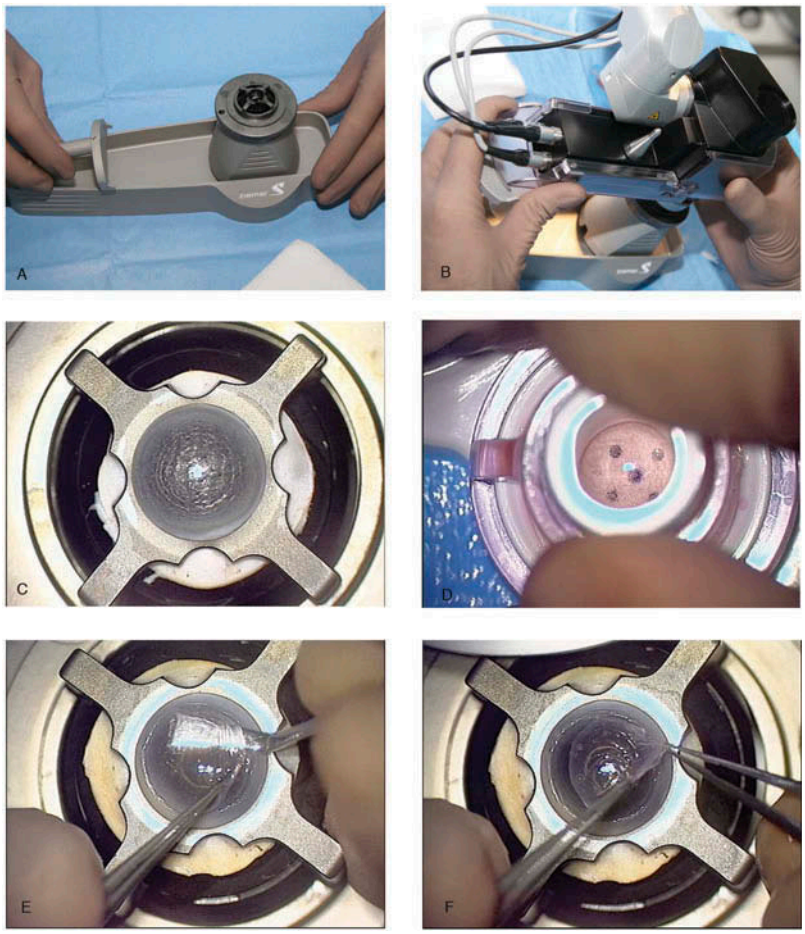

Figure 1. A customized artificial anterior chamber (aAC) has been designed for the LDV FS laser to perform the lamellar dissection on the donor cornea. The aAC was filled with an ophthalmic viscoelastic device to protect the endothelium and the corneoscleral button was secured in place with the fixation screw. Particular attention was paid to achieve good centration of the donor cornea on the aAC (A). The Ziemer LDV femtosecond laser was used to create the deep lamellar dissection with the LCS handpiece that fits securely on the aAC to ensure good stability while performing the cut. A 9 mm diameter cut was created at 500 µm depth using pulse energy of <100 nJ, a repetition rate of >5 MHz and a spot size of <1 µm (B and C). Once the cut was completed the corneoscleral button was transferred to a Coronet donor punch to perform an 8 mm diameter partial thickness trephination from the endothelial side. Following this the donor cornea was placed back on the aAC with the endothelial side up and secured as described above (D). A Bonn forceps and a blunt FemtoLASIK spatula were used to separate the endothelial graft from the stromal bed (E and F).

Post cut preparation

Corneal thickness (CT) was measured with ultrasound pachymetry (Corneo-Gage Plus, Sonogage, Cleveland) before and after the dissection. The cutting depth was calculated by subtracting the residual stromal bed thickness from the pre-cut corneal thickness. All specimens were then halved. The first half was fixed in formaldehyde, processed by paraffin sections and stained with hematoxylin and eosin for evaluation by light microscopy (LM). Scanning electron microscopy (SEM) was performed on the second half, these specimens were fixed in 1% glutaraldehyde in 0.1 M Sorensen's Phosphate Buffer pH 7.4 at 4°C for 2 hours, and post fixed with 1% osmium tetroxide in the same buffer at room temperature for 1 hour, dehydrated in graded alcohol solutions to 100% ethanol, and critical point dried over CO2 (CPD 030, Bal-Tec AG, Balzers). They were sputter coated with 15 nm platinum (MED 010, Bal-Tec) and examined with a JEOL 6300 F scanning electron microscope at 5 kV (JEOL, Tokyo, Japan). The LM and SEM images were digitally saved using SEMFORE (JEOL) in TIFF format for subsequent review. The magnifications were set at 50× and 250× for the posterior and anterior surface analysis.

Analysis of images

Subjective assessment

Four observers were asked to rate the surface smoothness of SEM images as "1 = smoothest", "2 = smooth but not best", "3 = median", "4 = rough but not worst" and "5 = roughest". A reference scale was provided to help the observer maintain consistency throughout grading (Supplementary Figures S1 and S2). The images were provided to the observers in a randomised order, with no identification of the orientation (posterior/anterior) or the cut method (FL or MK) used in corneal preparation provided. The 50× and 250× images were presented separately.

Objective assessment (Figure 2)

The image-processing technician was masked to the surface and cut type of images. The original images were imported in the ImageJ software (Figure 2A). The measure scale was calibrated in micrometers depending on magnification. A Gaussian filter was applied to each image, with a resolution of ten pixels (Figure 2B). The difference between image A and image B was calculated to isolate the small particles in each image, creating a third image (Figure 2C). The result was adjusted to the default threshold and particle analysis was performed with a minimum threshold area of 1 μm^2 (Figure 2D), the results of this analysis were exported to csv files and imported into R for analysis. To examine the large particles in an image, image B was adjusted to the default threshold (Figure 2E) and the particle analysis was also performed (Figure 2F) the results of this analysis were exported to csv files and imported into R for analysis.

Statistical analysis

Analysis was performed with R Version 2.15.1.[24] Values were tested for differences between groups by using an analysis of variance corrected for repeated measures on the same cornea. A $p < 0.05$ was considered statistically significant.

Results

The characteristics of the donors and corneas used in this study are given in Table 1.

Achieved cut with femtolaser and microkeratome

The achieved dissection depth using the FS laser was 496.4 ± 46.4 μm when attempting 500 μm and 474 ± 60 μm with the microkeratome when attempting 350 μm (Table 1).

Analysis of images

Subjective assessment: The intra-observer agreement was moderate in ×50 images, with κ greater than 0.36 (Observer 1 vs Observer 2 = 0.43; Observer 1 vs Observer 3 = 0.36; Observer 1 vs Observer 4 = 0.39). In the ×250 images the intra-observer agreement was higher with κ greater than 0.53 (Observer 1 vs Observer 2 = 0.60; Observer 1 vs Observer 3 = 0.61; Observer 1 vs Observer 4 = 0.53). Importantly all graders were within one grade of one another on all images (Table 2). The median of the four observations was used as the grade for each image and the mean of the group was calculated with respect to magnification (×50 or ×250), surface (posterior or anterior) and cut type (FL or MK), the summary is given in Table 3. The images of the MK group had significantly rougher rated images than images from the FL group, at both resolutions and on both surfaces (Table 3).

The normal histological architecture of the posterior stroma was preserved with no evidence of thermal damage at the cut surface following the LDV FS laser dissection (Figure 3). SEM images also showed fewer irregularities and tissue tags on the surfaces dissected by the FS laser.

Objective assessment: The large and small particle analysis results are given in Table 3. In both the anterior and posterior surface images the small particle analysis demonstrated that there were significantly more and larger particles evident in the images of MK cut corneas than in images of FL cut corneas (Figure 4). The large particle analysis was not as consistent throughout images, in anterior images at ×50 magnification significantly less and significantly larger particles were observed in the FL group than in the MK group. In anterior images at the ×250 magnification and posterior images at the ×50 and ×250 magnification a similar trend was observed with the size of the particles was significantly larger but with the number of particles was not significantly different between groups.

Discussion

The subjective and objective assessments used in this report indicate the greater smoothness when creating deep lamellar cuts of human donor corneas with the LDV femtosecond laser as compared to MK. Furthermore, light microscopy showed significantly fewer irregularities and loose collagen fibers at the stromal surface in FL specimens compared to the MK specimens. This result has important implications as it suggests that

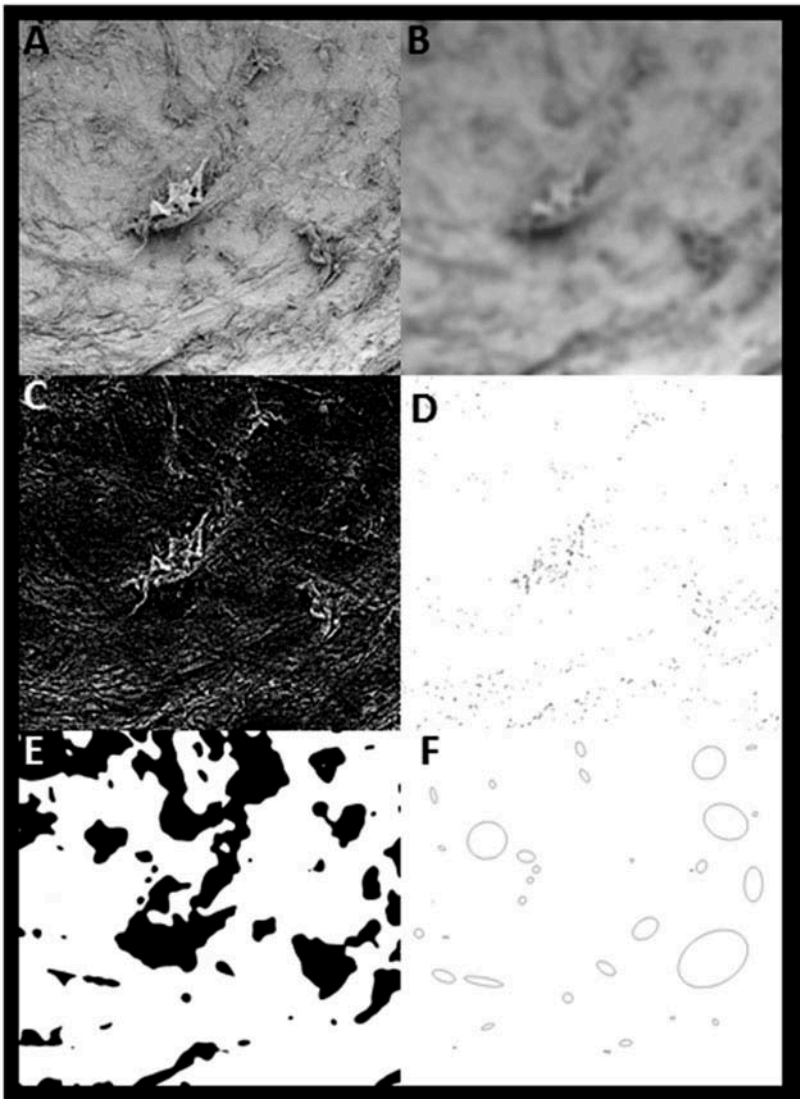

Figure 2. Objective assessments of SEM images. The SEM image at 50× magnification (A), a Gaussian filter was passed over image A (B). The difference between image A and image B was calculated to isolate the small particles in each image, creating a third image (C). The result was adjusted to the default threshold and particle analysis was performed with a minimum threshold area of 1 μm^2, (D) Image B was adjusted to the default threshold (E) and the particle analysis was also performed (F).

Table 1. Corneal donor characteristics.

	Femtolaser						Microkeratome				
Donor characteristics	C1	C2	C3	C4	C5	Mean	C6	C7	C8	C9	Mean
Age	80	64	74	79	79		89	64	63	81	74.7
ECD	<2000	<2000	1950	<2000	<2000	<2000	2000	2000	2435	2555	2247
% of endothelial cell loss	N/A	N/A	N/A	N/A	N/A		9.1	14.5	N/A	N/A	
Previous surgery	phaco			phaco	phaco		phaco				
CCT with epithelium	756	770	622	771	614	707	745	800	652	589	670
Intended cut	500	500	500	500	500	500	350	350	350	350	350
Achieved cut	489	493	509	539	452		534	455	451	456	
Residual thickness	267	277	113	232	162	210	211	345	201	133	222

the LDV FS laser may produce a smoother graft-host interface, which has been associated with better visual outcomes.[17,19]

With the LDV FS laser the attempted dissection depth was closer to achieved depth and more predictable than with the ONE Microkeratome. These results correspond with the results of Price et al. who found that ONE Microkeratome with the 350 μm head the achieved dissection depth approximately 100 μm greater than intended.[20]

A comparison of the stromal bed quality reported here with the outcomes available in the literature is confounded by the large range of experimental designs used. There are large differences between femtosecond laser devices (Table 4), in terms of spot size, frequency, and energy which will affect smoothness and safety. Depending on the report, outcomes on smoothness vary in favor of FL[13,14,21] or MK,[5,11,22] but FL devices with lower energy and small

Table 2. Subjective roughness score intra-observer agreement.

×50				
Grade observer 1		Observer2	Observer 3	Observer 4
1	Exact	2	2	2
	Within 1 grade	0	0	0
2	Exact	7	3	6
	Within 1 grade	6	10	7
3	Exact	11	7	11
	Within 1 grade	6	10	6
4	Exact	9	11	9
	Within 1 grade	4	2	4
5	Exact	6	6	5
	Within 1 grade	4	4	5
Total	Exact	35	29	33
	Within 1 grade	20	26	22
×250				
Grade observer 1		Observer2	Observer 3	Observer 4
1	Exact	2	3	3
	Within 1 grade	1	0	0
2	Exact	9	8	8
	Within 1 grade	5	6	6
3	Exact	9	10	9
	Within 1 grade	6	5	6
4	Exact	10	9	7
	Within 1 grade	5	6	8
5	Exact	8	8	8
	Within 1 grade	0	0	0
Total	Exact	38	38	35
	Within 1 grade	17	17	20

spot separation create a dissection with comparable smoothness to MK devices (Table 5).[14,21] LDV femtosecond laser has very low pulse energy and high frequency with the smallest spot size separation (Table 4). Until now the LDV device has not been compared to MK devices in terms of stromal bed smoothness. However, a recent report by Riau et al. demonstrated similar stromal bed quality between the Visumax (μJ) and LDV (nJ) femtosecond laser, suggesting that the increase in smoothness has an upper limit, i.e., that there is a pulse energy below which smoothness is no longer improved.[17]

In terms of safety, Mehta et al. reported that no significant detectable thermal damage at the cut stromal surface using the 40 kHz Femtec system and Cheng et al. found that endothelial cell viability was not affected by the femtosecond laser dissection.[23,24] However examining the tissue responses between the Visumax (μJ) and LDV (nJ) devices, Riau et al. found a significant difference, with apoptotic cells and fibronectin present in the central incision site of μJ but not the nJ.[17] This suggests that the LDV device has reduced impact at the cellular level.[17]

There may be additional settings that affect the cut, several groups have examined the relationship between depth of the cut and quality of the interface.[5,11,13,15,25–27] Peyrot et al. have shown the reduced stromal bed quality with increased dissection depth is due to increased optical aberrations of the laser beam focus on deeper stromal layers.[16] The LDV FS laser has a larger numerical aperture compared to the other femtosecond laser platforms,[8] this makes this platform more suitable for deep corneal dissection as the laser energy is focused to a smaller area in the Z-axial plane achieving less scattering in the posterior stroma.

The effect of these device settings on surgical outcomes are difficult to interpret due to inconsistencies between reports. Rousseau et al. and Kunert et al. both demonstrated that cut settings selected on the Intralase femtosecond laser, significantly affected the quality of the stromal interface achieved, with higher pulse frequency achieving smoother stromal beds.[12,25] Perhaps suboptimal laser settings resulted in the poorer visual outcomes observed with EK prepared with the Visumax (200 kHz) than with Amadeus II microkeratome (Ziemer Ophthalmic Systems AG, Port, Switzerland).[28]

Scanning electron microscopy (SEM) images have been used several times to assess the smoothness of lamellar cuts. Initially, subjective grading of roughness by one or more observers was utilized, however it has been shown that this grading is susceptible to variations in contrast.[9] Objective measures suffer similarly as a change between gray scale values within and between SEM images is not directly related to changes in height; it also represents changes in tilt of the image, surface contamination and enhanced emission from edges and ridges. As such traditional texture analysis or mean grayscale variation methods are not suitable summary measures of the local variation or surface roughness. Marian et al. have previously described a methodology where a 3D surface is reconstructed and the image flattened to isolate the local variation.[9] However, this methodology requires stereo SEM images and it does not address the issue of enhanced emission from edges. In this article we have used an alternative methodology, the subtraction of the background,

Table 3. Summary of comparative results from the MK and FL prepared cornea anterior and posterior dissections at the 50× and 250× magnifications.

	×50			×250		
Anterior	FELK	Moria	*p*-value	FELK	Moria	*p*-value
Number of images	15	12		15	12	
Average observer rating	2.2	3.9	<0.001	2.2	4.2	<0.001
Particle analysis (L)						
Mean no of particles	40.1	64.7	0.006	48.5	56.7	0.27
Mean particle size	130.7	116.9	0.01	134.9	108.3	<0.001
Particle analysis (S)						
Mean no of particles	318.2	438.8	0.07	36.5	132.1	<0.001
Mean particle size	13.7	30.6	<0.001	13.7	20.3	<0.001
Posterior						
Number of images	20	8		20	8	
Average observer rating	2.8	4.0	<0.001	3.2	3.8	<0.001
Particle analysis (L)						
Mean no of particles	38.1	30.3	0.53	49.3	40.8	0.93
Mean particle size	121.9	110.5	0.01	116.3	106.3	<0.001
Particle analysis (S)						
Mean no of particles	380.1	516.4	0.009	72.3	105.1	0.001
Mean particle size	14.8	16.7	<0.001	12.8	12.9	<0.001

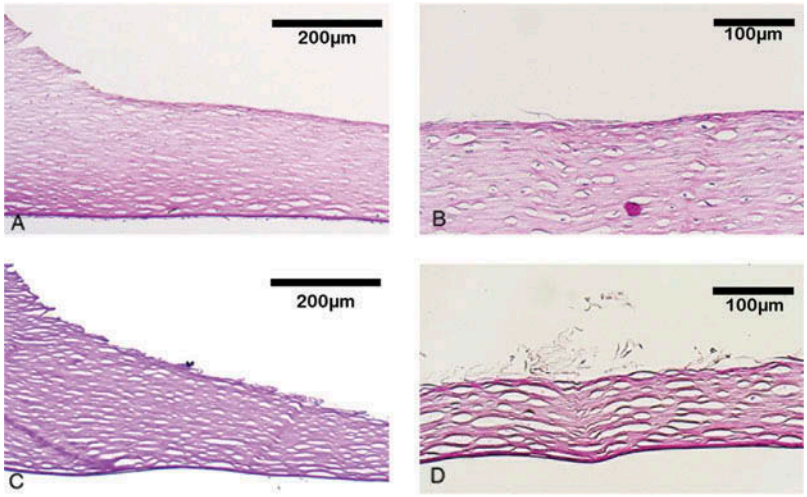

Figure 3. Histology. (A) Peripheral corneal surface following Ziemer LDV femtosecond laser dissection (Scale bar equals 200 µm). (B) Central corneal surface following Ziemer LDV femtosecond dissection (Scale bar equals 100 µm). (C) Peripheral corneal surface following microkeratome dissection (same scale as A). (D) Central corneal surface following microkeratome dissection (same scale as B). Nuclei of keratocytes can be clearly identified in blue color in each section.

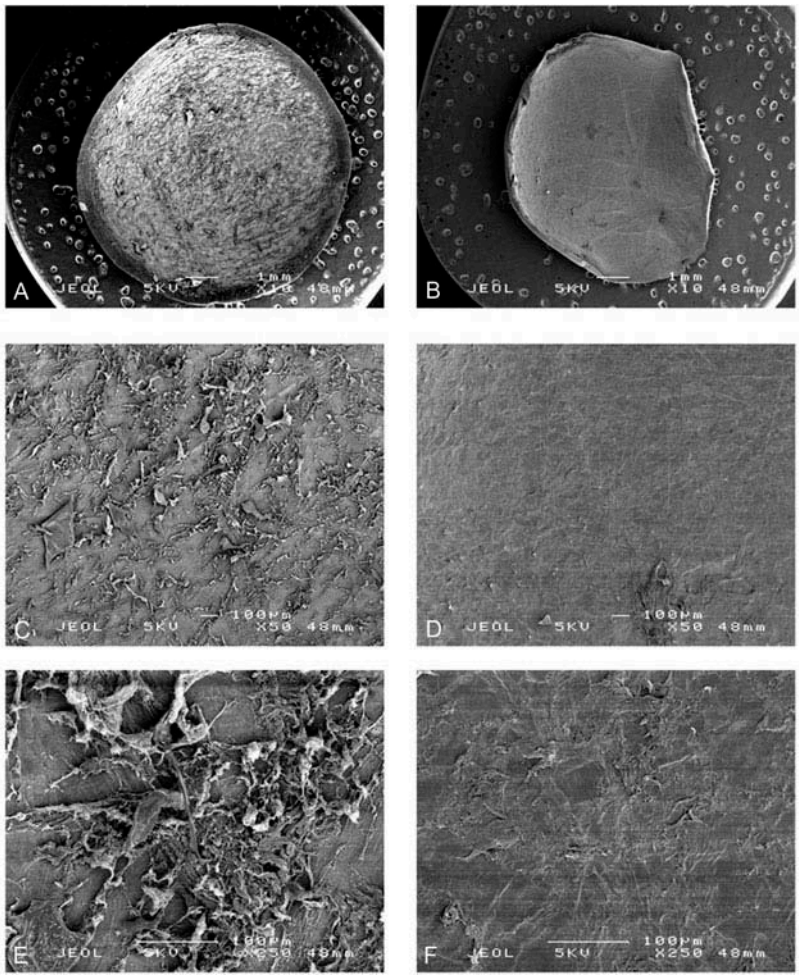

Figure 4. Scanning electron microscopy. Stromal bed images shown in the left column were cut with manual Moria microkeratome (A: 10×, C: 50×, E: 250×) and stromal bed images shown in the right column were cut with a Ziemer LDV femtosecond laser (B: 10×, D: 50×, F: 250×).

Table 4. Summary of the setting of the commercially available femtosecond devices.

Device	Intralase	Femtec	Visumax	LDV
Concept:	**Amplifier**	**Amplifier**	**Amplifier**	**Oscillator**
Wavelength:	**≈1053 nm**	**≈1053 nm**	**≈1053 nm**	**≈1040 nm**
Pulse Width:	**>500 fs**	**>500 fs**	**≈400 fs**	**≈250 fs**
Spot Size:	**0.5–8 µm**	**1–3 µm**	**0.5–2.5 µm**	**<<1 µm**
Rep Rate:	**15–150 kHz**	**10–40 kHz**	**100–500 kHz**	**1–5 MHz**
Pulse Energy:	**≈1 µJ**	**>1 µJ**	**<1 µJ**	**≈.003 µJ**

Table 5. Summary of available articles comparing stromal bed quality of microkeratome and femtosecond laser dissected human corneas.

Author	Microkeratome			Femtosecond laser		Outcome
	Device	Method	Head	Device	Energy	Roughness
Jones	Moria	Manual	350 µm	Intralase 15 kHz	7.4 µJ	NA
Sarayba	Hanastome	Manual	160 µm	Intralase 15–30 kHz	1.2–2.0 µJ	MK>FL
Mootha	Moria	CBm	300 µm	Intralase 60 kHz	2.0 µJ	FL>MK
	Horizon		300 µm	Intralase 60 kHz	2.0 µJ	FL>MK
Dickman	Moria	Manual rotary	350–400 µm	Intralase 60 kHz	4 different	FL>MK
	Gebauer	Motor	300–550 µm	Intralase 60 kHz	4 different	FL>MK
Serrao	Hanastome	Motorized	160 µm	Intralase 150 kHz	0.45–0.75 µJ	Low energy and small spot separation FL ≈ MK
Lombardo	Moria	Manual	350 µm	Intralase 150 kHz	0.5–1.0 µJ	Low energy FL ≈ MK

which in addition to removing the curvature and tilt information, also removed enhanced emission from edges and ridges, isolating the local variation.

The strengths of our study were that the samples were processed in a regular Eye Bank for human therapy, also that the samples were imaged and the evaluation of the quality of the dissection surface was performed at two different magnifications using light and electron microscopy. On another hand, this study is limited by the small number of human cornea available and that our study was limited to ex vivo experiments without grafting and clinical follow-up. Only functional evaluation will determine if there is true superiority of nJ FS laser for preparing lamellar grafts.

Today, MK is the more frequently used device to perform EK (Moria SA or Gebauer AG). Comparing all the femtolasers available on the market, our results support the use of the LDV FS laser for the preparation of lamellar grafts for endothelial keratoplasty. A comparative clinical trial will be required in order to examine the effect of this device on visual outcomes, and to the best device to perform corneal lamellar tissue dissection.

Declaration of interests

Dr. Majo is a consultant for Ziemer. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Supplemental material

Supplemental data for this article can be accessed at www.tandfonline.com/icey.

References

1. Dapena I, Ham L, Melles GR. Endothelial keratoplasty: DSEK/DSAEK or DMEK–the thinner the better? Curr Opin Ophtalmol 2009;20(4):299–307.
2. Ting DS, Sau CY, Srinivasan S, Ramaesh K, Mantry S, Roberts F. Changing trends in keratoplasty in the West of Scotland: a 10-year review. Br J Ophthalmol 2012;96(3):405–408.
3. Nieuwendaal CP, Lapid-Gortzak R, van der Meulen IJ, Melles GJ. Posterior lamellar keratoplasty using descemetorhexis and organ-cultured donor corneal tissue (Melles technique). Cornea 2006;25(8):933–936.
4. Price MO, Giebel AW, Fairchild KM, Price FW, Jr. Descemet's membrane endothelial keratoplasty: prospective multicenter study of visual and refractive outcomes and endothelial survival. Ophthalmol 2009;116(12):2361–2368.
5. Jones YJ, Goins KM, Sutphin JE, Mullins R, Skeie JM. Comparison of the femtosecond laser (IntraLase) versus manual microkeratome (Moria ALTK) in dissection of the donor in endothelial keratoplasty: initial study in eye bank eyes. Cornea 2008;27(1):88–93.
6. Dickman MM, van Maris MP, van Marion FW, Schuchard Y, Steijger-Vermaat P, Van den Biggelaar FJ, et al. Surface metrology and 3-dimensional confocal profiling of femtosecond laser and mechanically dissected ultrathin endothelial lamellae. Invest Ophthalmol Vis Sci 2014;55(8):5183–5190.
7. Kymionis GD, Kontadakis GA, Naoumidi I, Kankariya VP, Panagopoulou S, Manousaki A, et al. Comparative study of stromal bed of LASIK flaps created with femtosecond lasers (IntraLase FS150, WaveLight FS200) and mechanical microkeratome. Br J Ophthalmol 2014;98(1):133–137.
8. Lubatschowski H. Overview of commercially available femtosecond lasers in refractive surgery. J Refract Surg 2008;24(1):S102–S107.
9. Marian A, Nada O, Legare F, Meunier J, Vidal F, Roy S, et al. Smoothness assessment of corneal stromal surfaces. J Cataract Refract Surg 2013;39(1):118–127.
10. Monterosso C, Galan A, Böhm E, Zampini A, Parekh M, Caretti L. Effect of 60 kHz and 150 kHz femtosecond lasers on corneal stromal bed surfaces: a comparative study. ISRN Ophthalmol 2013. doi:10.1155/2013/971451.
11. Mootha VV, Heck E, Verity SM, Petroll WM, Lakshman N, Muftuoglu O, et al. Comparative study of descemet stripping automated endothelial keratoplasty donor preparation by Moria CBm microkeratome, horizon microkeratome, and Intralase FS60. Cornea 2011;30(3):320–324.
12. Rousseau A, Bensalem A, Garnier V, Savoldelli M, Saragoussi J-J, Renard G, et al. Interface quality of endothelial keratoplasty buttons obtained with optimised femtosecond laser settings. Br J Ophthalmol 2012;96(1):122–127.
13. Sarayba MA, Ignacio TS, Binder PS, Tran DB. Comparative study of stromal bed quality by using mechanical, IntraLase femtosecond laser 15- and 30-kHz microkeratomes. Cornea 2007;26(4):446–451.

14. Serrao S, Buratto L, Lombardo G, De Santo MP, Ducoli P, Lombardo M. Optimal parameters to improve the interface quality of the flap bed in femtosecond laser-assisted laser in situ keratomileusis. J Cataract Refract Surg 2012;38(8):1453–1459.
15. Terry MA, Ousley PJ, Will B. A practical femtosecond laser procedure for DLEK endothelial transplantation: cadaver eye histology and topography. Cornea 2005;24(4):453–459.
16. Peyrot DA, Aptel F, Crotti C, Deloison F, Lemaire S, Marciano T, et al. Effect of incident light wavelength and corneal edema on light scattering and penetration: laboratory study of human corneas. J Refract Surg 2010;26(10):786–795.
17. Riau AK, Liu Y-C, Lwin NC, Ang HP, Tan NY, Yam GH, et al. Comparative study of nJ-and μJ-energy level femtosecond lasers: evaluation of flap adhesion strength, stromal bed quality, and tissue responses. Invest Ophthalmol Vis Sci 2014;55(5):3186–3194.
18. Kermani O, Oberheide U. Comparative micromorphologic in vitro porcine study of IntraLase and Femto LDV femtosecond lasers. J Cataract Refract Surg 2008;34(8):1393–1399.
19. Hjortdal J, Nielsen E, Vestergaard A, Sondergaard A. Inverse cutting of posterior lamellar corneal grafts by a femtosecond laser. Open Ophthalmol J 2012;6:19–22.
20. Price MO, Price FW, Jr., Stoeger C, Soper M, Locke GD, Bavuso T. Central thickness variation in precut DSAEK donor grafts. J Cataract Refract Surg 2008;34(9):1423–1424.
21. Lombardo M, De Santo MP, Lombardo G, Lomoriello DS, Desiderio G, Ducoli P, et al. Surface quality of femtosecond dissected posterior human corneal stroma investigated with atomic force microscopy. Cornea 2012;31(12):1369–1375.
22. Price MO, Price FW, Jr. Descemet's stripping with endothelial keratoplasty: comparative outcomes with microkeratome-dissected and manually dissected donor tissue. Ophthalmol 2006;113(11):1936–1942.
23. Mehta JS, Parthasarthy A, Por YM, Cajucom-Uy H, Beuerman RW, Tan D. Femtosecond laser-assisted endothelial keratoplasty: a laboratory model. Cornea 2008;27(6):706–712.
24. Cheng YY, Schouten JS, Tahzib NG, Wijdh RJ, Pels E, van Cleynenbreugel H, et al. Efficacy and safety of femtosecond laser-assisted corneal endothelial keratoplasty: a randomized multicenter clinical trial. Transplantation 2009;88(11):1294–1302.
25. Kunert KS, Blum M, Duncker GI, Sietmann R, Heichel J. Surface quality of human corneal lenticules after femtosecond laser surgery for myopia comparing different laser parameters. Graefes Arch Clin Exp Ophthalmol 2011;249(9):1417–1424.
26. Seitz B, Langenbucher A, Hofmann-Rummelt C, Schlotzer-Schrehardt U, Naumann GO. Nonmechanical posterior lamellar keratoplasty using the femtosecond laser (femto-plak) for corneal endothelial decompensation. Am J Ophthalmol 2003;136(4):769–772.
27. Mehta JS, Shilbayeh R, Por YM, Cajucom-Uy H, Beuerman RW, Tan DT. Femtosecond laser creation of donor cornea buttons for Descemet-stripping endothelial keratoplasty. J Cataract Refract Surg 2008;34(11):1970–1975.
28. Vetter JM, Butsch C, Faust M, Schmidtmann I, Hoffmann EM, Sekundo W, et al. Irregularity of the posterior corneal surface after curved interface femtosecond laser-assisted versus microkeratome-assisted descemet stripping automated endothelial keratoplasty. Cornea 2013;32(2):118–124.