Histological comparison between the anterior capsule integrity in femtosecond laser-assisted capsulotomy and traditional continuous curvilinear capsulotomy in cataract surgeries

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Purpose
To compare the histological edge structures of anterior lens capsule specimens obtained from laser-assisted cataract surgery capsulotomies using two different laser platforms (LenSx post-soft-fit and Victus II) with that of traditional continuous curvilinear capsulorhexis (CCC) using scanning electron microscopy (SEM).

Setting
I-Care Center, Alex Eye Center, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Design
Prospective comparative series.

Patients and methods
Anterior capsule specimens following CCC (n=15) or femtosecond laser-assisted capsulotomy using two platforms (LenSx, n=15 and Victus, n=15) were studied by SEM. Irregularity of the capsule edge was quantified using two parameters (angular second moment and contrast) using ImageJ software. The clinical features and laser parameters were correlated with angular second moment and contrast. SEM images were analyzed for the coefficient of variation (CoV) of pixilation along the capsule edge and homogeneity using gray-level co-occurrence matrix analysis. Subjective analysis for cut-surface irregularities and assessment for complications and anomalies, such as tags and misdirected laser shots, were also made.

Results
The femtosecond laser-assisted capsulotomy edge surfaces created by both laser platforms showed marked irregularity compared with the smoother edge of the CCC. The angular second moment and contrast measures for both lasers differed significantly from those obtained for CCC ($P<0.001$). There was little between-laser difference in angular second moment and contrast measures. The mean CoV values from the regression analysis showed the manual edge (16.47%) was smoother than the edges created with LenSx post-soft-fit (20.88%) and Victus II (23.04%) platforms. In the manual group, there is dishomogeneous thickness along the capsulorhexis edge, while in the femtosecond laser samples; the cuts are more homogeneous in thickness throughout the whole capsulorhexis edge. Tags occurred with a mean of 4. Misdirected pulses were seen with Victus II (5/15) cases.

Conclusion
Laser capsulotomies are approaching the smoothness of manual capsulorhexis. The LenSx post-soft-fit platform shows less anomalies and smaller difference for the CoV and homogeneity metrics compared with the Victus II platform. In the manual group, there is dishomogeneous thickness along the capsulorhexis edge, while in the femtosecond laser samples the cuts are more homogeneous regarding thickness throughout the whole capsulorhexis edge.

Keywords:
cataract, femtosecond laser, FLACS, lens capsule, phacoemulsification

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Introduction

The anterior capsulotomy or capsulorhexis is an important step in cataract surgery that can influence the position and centration of the intraocular lens. The size, shape, and centration of the capsulorhexis can vary depending on the type of cataract as well as the surgeon's experience [1].

Popularized by Gimbel and Neuhann [2], continuous curvilinear capsulorhexis (CCC) has several intraoperative and postoperative advantages, but its execution requires special attention and refined surgical skills. Obtaining a precise CCC is essential to prevent intraoculr lens (IOL) decentration, tilt, myopic shift, posterior and anterior capsule opacification, and the shrink-wrap effect [3].

Other options have been used as alternatives to conventional CCC, including the Fugo blade and bipolar diathermy radiofrequency [4,5].

Femtosecond lasers use a shorter pulse time (10^{-15} s) than other lasers and are successfully used initially in refractive surgery for flap creation in laser in-situ keratomileusis and, subsequently, in corneal transplantation surgery [6,7]. The laser cuts tissue by vaporizing it, creating plasma, and then a cavitation bubble that expands and collapses, separating the tissue. The union of these bubbles creates a cut surface. Because power is a function of energy per unit time, shorter pulse times further decrease the energy required for a given effect [8].

Recently, femtosecond lasers were introduced into cataract surgery to perform four kinds of incisions; that is, the capsulorhexis, nuclear fragmentation, tunnel creation, and corneal relaxing incisions [9].

The main advantages of femtolaser-assisted cataract surgeries are a better quality of incision with any desired geometry, position, and incision number; increased reliability and reproducibility of capsulotomies; increased stability and central position of the implanted posterior chamber lenses; and reduction of the cumulative dissipated energy and high effective phaco time during phacoemulsification [10].

Initial results with femtosecond lasers showed higher precision of the capsulorhexis in size and shape than manual techniques. A symmetric capsulotomy that completely overlies the capsulorhexis border on the IOL optic leads to better IOL positioning in the early postoperative period and to symmetrical, uniform long-term capsule fibrosis. As a consequence, less decentration was observed than in cases with an asymmetric capsulorhexis [11].

It is found that the smaller the capsulotomy, the greater the probability of a significantly reduced IOL shifts due to capsule fibrosis. Increased optical quality and reduced internal aberrations were measured in eyes with femtosecond laser-assisted capsulotomies [12].

Femtosecond lasers can be used during cataract surgery to create corneal wounds and capsulotomies and to perform lens fragmentation. Occasionally, despite successful docking, these steps may not be completed successful. An incomplete capsulotomy poses the risk for anterior capsule and posterior capsule tears [13].

The elastic and strength changes in the anterior capsule are independent of cataract formation. These characteristics are relevant to small incision cataract surgery, as the strength, thickness, and elasticity of the capsule opening that influence the safety of the procedure as well as the refractive outcome [14].

In addition to the greater precision, capsulorhexis created by femtosecond lasers are reported to have more capsular edge strength than a manual capsulorhexis [15].

When scanning electron microscopy (SEM) evaluated the images of the excised capsule disk edge produced by manual capsulorhexis and laser capsulotomy, it is noted that the laser-induced microgrooves [15].

Trivedi *et al.* [16] found that the morphology of the anterior capsulotomy edge seen with SEM correlated with capsular edge strength, with smooth regular edges being the strongest. They hypothesize that similar to what has been described in the literature for femtosecond laser-supported corneal surgery, low energy settings for femtosecond lasers produce a more regular and smoother cut surface in capsulorhexis creation. The purpose of this SEM study was to evaluate capsulorhexis cut quality obtained during femtosecond laser cataract surgery at different energy settings and to determine whether there are differences between that technique and a standard manual technique.

Friedman *et al.* [15] showed that decreasing laser pulse energy led to a decrease in the strength of the capsulotomy.

Patients and methods

Patients

Written informed consent was obtained from each patients according to Declaration of Helsinki Ethics Comitte at faculty of medicine approved the study. This prospective randomized comparative study of 45 eyes divided into three groups:

(1) Group 1: 15 eyes having femtosecond laser assisted cataract surgery (FLACS) using Bausch & Lomb Victus platform (Technolas Perfect Vision, Munich, Germany).
(2) Group 2: 15 eyes having FLACS by Alcon LenSx platform (Alcon Laboratories Inc., Fort Worth, Texas, United States).
(3) Group 3: 15 eyes having manual CCC.

Inclusion criteria

(1) Age more than 40 years.
(2) Cases of normal ocular examination apart from cataract. Patients had cataract of grade I–III depending on the Lens Opacities Classification System III [17].

Exclusion criteria

(1) Narrow palpebral fissure.
(2) Significant pterygia and loose conjunctiva.
(3) Extensive corneal opacities.
(4) Narrow pupil (at least 6.0 mm before laser treatment).
(5) White intumescent cataracts.
(6) Brunescent and black cataracts.
(7) Pseudoexfoliation, pigment dispersion syndrome, iritis, pigment on lens capsule, and nystagmus.
(8) Congenital cataract.
(9) Subluxated lens.
(10) Intraoperative incomplete femtosecond-assisted anterior capsulotomy.

Methods

In laser group 1, the femtosecond laser-assisted capsulotomies were created using the Victus femtosecond laser platform (Bausch & Lomb, Legacy Tower, Rochester, New York, United States); in laser group 2, they were created using the LenSx femtosecond laser platform. Anterior capsules from a control group included specimens obtained following manual CCC. A total of three skilled surgeons will perform the procedures in these three groups (one surgeon for each group).

Femtosecond laser-assisted capsulotomy procedures were performed under topical anesthesia of benoxinate hydrochloride 0.4% eye drops.

In all specimens, the depth and coordinates of the femtosecond laser-assisted capsulotomies were determined with the real-time live optical coherence tomography integrated into the laser system. The patient interface consists of an applanation lens, suction ring, and tubing. After docking is performed, the cornea is applanated and suction is activated.

In femtosecond laser-assisted capsulotomy specimens, the laser parameters include the capsule size (5–5.5 mm), pulse energy of 7 μJ, spot separation of 4 μm, and layer separation of 4 μm. Following femtosecond laser-assisted capsulotomy the capsules were carefully removed with a 2.2 mm capsulorhexis forceps.

The manual CCC was performed using the standard technique with Utrataforceps (Geuder, Heidelberg, Germany) and centripetal forces with regrasping maneuvers under an ophthalmic viscosurgical device.

The specimens were fixed by immediate immersion in 4F1G in phosphate buffer solution (pH 7.2) at 4°C for 3 h. The specimens then were postfixed in 2% OsO_4 in the same buffer at 4°C for 2 h. The specimens were then washed in the buffer and dehydrated at 4°C through a graded series of ethanol. The specimens were dried by means of the critical point method and then mounted using carbon paste on an Al-stub and coated with gold up to a thickness of 400A in a sputter-coating unit (JFC-1100E).

Observation of the specimens was performed using a Jeo JSM-5300 SEM (Jeol USA Inc., Joel Peabody, Massachusetts, United States) operated between 15 and 20 keV in the Unit of Electron Microscopy, Faculty of Science of University of Alexandria.

The surface of the capsulotomy edge was the primary focus of the SEM. We applied for all SEM images which were taken from the edge of the specimens; in all groups the same contrast, brightness, working distance, magnification (×5000), and standard scale (5 μm) were found. The same technician took images with the same device by capturing all images at the perpendicular position on the lens capsular edge.

Objective and subjective analyses of the capsule edge were performed. En-face SEM images of the capsule edge were processed using the ImageJ software. A surface irregularity was observed in a two-dimensional image because of variation in pixel grayness.

Two parameters, contrast and angular second moment, reflected the irregularity of the capsule edge and are regular features of the ImageJ program available under the gray-level co-occurrence matrix texture plug-in. Coefficient of variation (CoV) was taken for irregularity plot profile of each SEM image of three locations of each capsule and the mean of them was measured.

The thickness of the anterior lens capsule cut edge was measured for each image in three locations of each SEM image (at the beginning of the cut edge, the end, and at the central point of the cut edge), and the mean of them was measured by use of J image program for all images of the three groups. Note, any tags or misdirected laser shots in the free floating capsulotomies at the time of surgery.

Results

Patient characteristics

In all, 45 capsules from 42 patients were included in the study and were divided into three groups, each containing 15 capsules. The mean patient age in group 1 was 65.6 years and it was 58.4 years in group 2, whereas it was 60.66 years in group 3 with no statistically significant difference. In group 1, there was six men and nine women and in group 2 there was five men and 10 women, whereas in group 3 there was five men and 10 women. There was no statistically significant difference between all groups.

The mean axial length in all groups was 24.03 mm, with no statistically significant difference. The mean *K* value varied in group 1, with a mean of 43.91 D, while they were in groups 2 and 3 with a mean of 44.25 and 43.9 D, respectively. There was no statistically significant difference between the groups. The anterior chamber depth varied with a mean of 3.18 in group 1, while they were with a mean of 3.2 and 3.02 in groups 2 and 3, respectively, with no statistically significant difference between both groups.

J image analysis of scanning electron microscopy images

Capsulorhexis geometry, cut-surface quality, and regularity of the capsule edge

Subjective

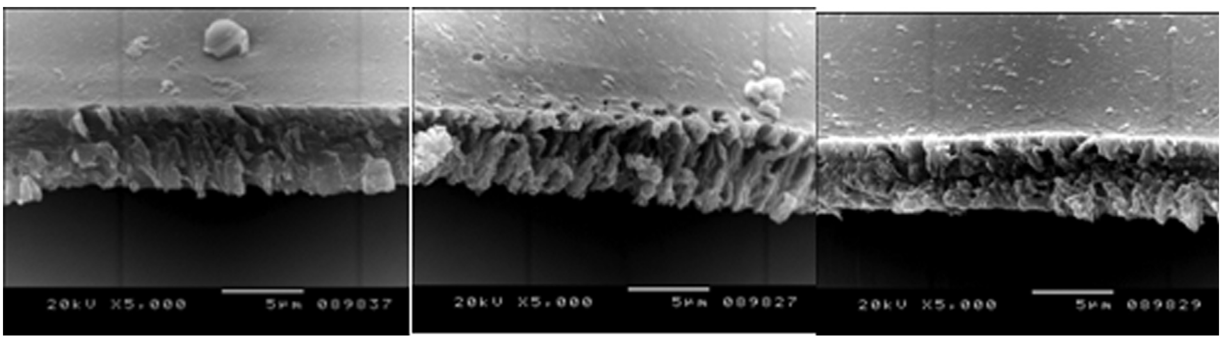
During image preprocessing using ImageJ, unwanted noise was suppressed and features as image brightness and contrast were enhanced in the digital photographs. Whereas brightness applies uniformly to a large set of pixels in the image, contrast was an important parameter in the context of measurements in this study.

Figures 1–3 show examples of SEM for each group. The capsule edges from the CCC were smooth with minimal irregularities. In contrast, the capsule edges from both femtosecond laser platforms showed marked irregularities.

Objective analysis (gray-level co-occurrence matrix parameters of J image)

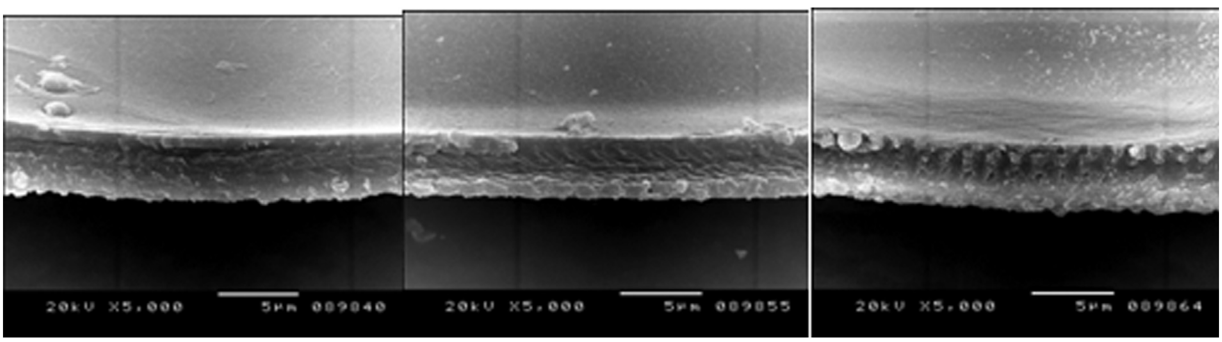
The Kruskal–Wallis test was used to compare angular second moment and contrast values between the three groups, and the Mann–Whitney *U* test was used for further pairwise comparisons. A level of significance of 0.05 was used.

Figure 1



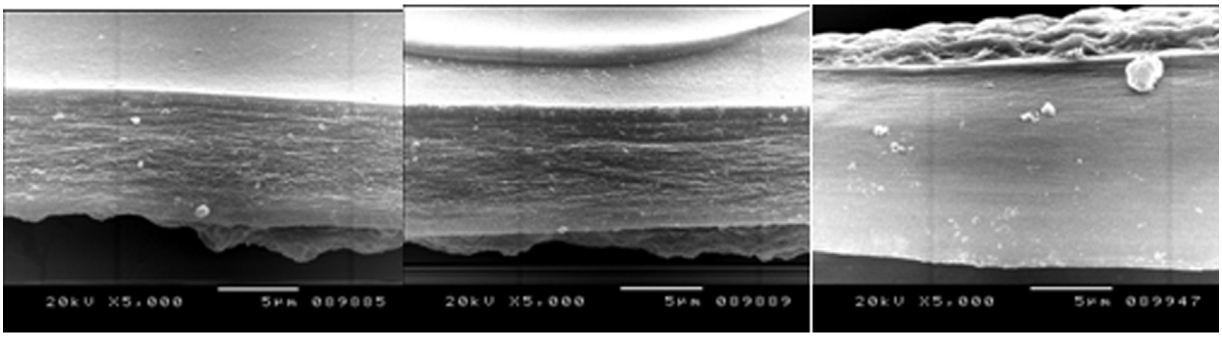
Examples of ×5000 SEM of capsule edge cut of three cases of the Victus group. SEM, scanning electron microscopy.

Figure 2



Examples of ×5000 SEM of capsule edge cut of three cases of the LenSx group. SEM, scanning electron microscopy.

Figure 3



Examples of ×5000 SEM of capsule edge cut of three cases of manual CCC (group 3). CCC, continuous curvilinear capsulorhexis; SEM, scanning electron microscopy.

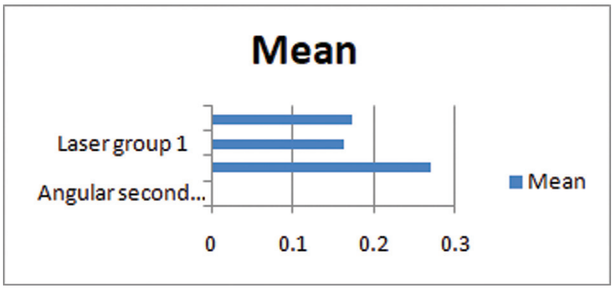
Table 1 Pairwise comparison of angular second moment and contrast values for the manual continuous curvilinear capsulotomy and the two laser platforms

	Victus	LenSx	Phaco
Angular second moment			
Range	0.00008–0.00043	0.00011–0.00025	0.00009–0.00071
Mean±SD	0.00016±0.00009	0.00017±0.00004	0.00027±0.00016
H (*P*)		21.297* (<0.001*)	
Significance between groups		P_1=0.043*, P_2<0.001*, P_3=0.014*	
Contrast			
Range	192.02–1084.56	155.70–613.52	44.57–773.50
Mean±SD	506.51±232.25	326.44±102.69	260.95±161.90
H (*P*)		29.668* (<0.001*)	
Significance between groups		P_1=0.002*, P_2<0.001*, P_3=0.036*	

H (*P*), *H* and *P* values for Kruskal–Wallis test, significance between groups was done using post-hoc tests (Dunn's multiple comparison test). P_1, *P* value for comparing between groups 1 and 2. P_2, *P* value for comparing between groups 1 and 3. P_3, *P* value for comparing between groups 2 and 3. *Statistically significant at *P* value less than or equal to 0.05.

The mean±SD for angular second moment and contrast for all groups is shown in Table 1. Both measures varied significantly between the three groups (*P*<0.001). Pairwise comparison showed that manual CCC was significantly different (*P*<0.001) from both laser groups in angular second moment and contrast. Both angular second moment and contrast were significantly different between the two laser groups. The contrast in most of Victus laser cases were little higher than that of LenSx and in most cases of Victus laser; the angular second moment were lower than that of LenSx as shown in Figs 4–7.

Figure 4



Comparison between the three studied groups regarding angular second moment.

Objective analysis (coefficient of variation of grayness by J image)

The mean CoV values from the regression analysis showed the manual edge (16.47%) was smoother than the edges created with LenSx 20.88% and Victus 23.04% platforms, as shown in Table 2 and Fig. 8.

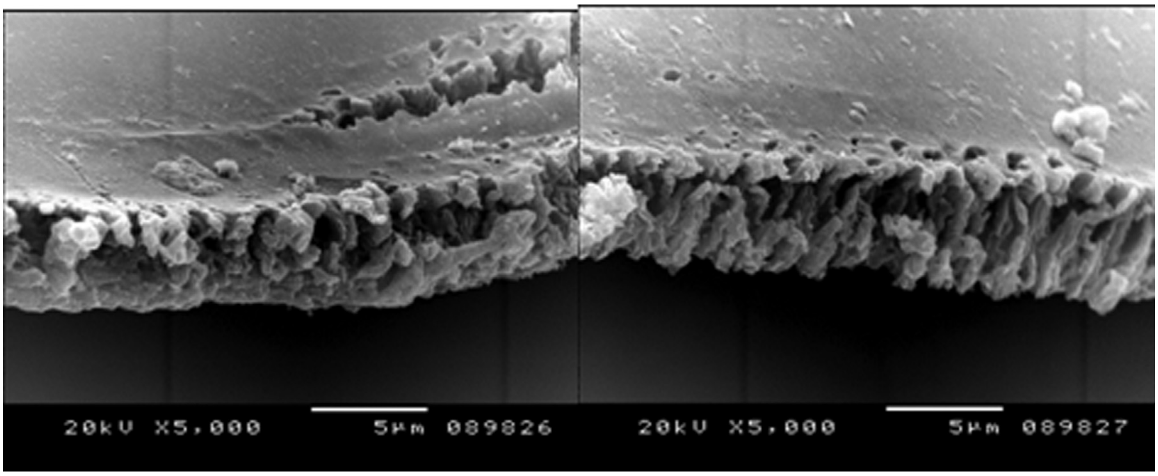
Thickness measurements of capsule cut edge

The thickness of edge cut by femtosecond laser is uniform at every point of cut with a mean of 4.79±0.62 and 4.27±0.40 µm of groups 1 and 2 laser groups, respectively. The thickness of edge cut of group 3 is less uniform with large variability with a mean of 7.70±4.13 µm, as shown in Table 3. Figures 9–11 are scatter charts of average thickness of cut edge among all groups.

Evaluation of tags and misdirected laser shots

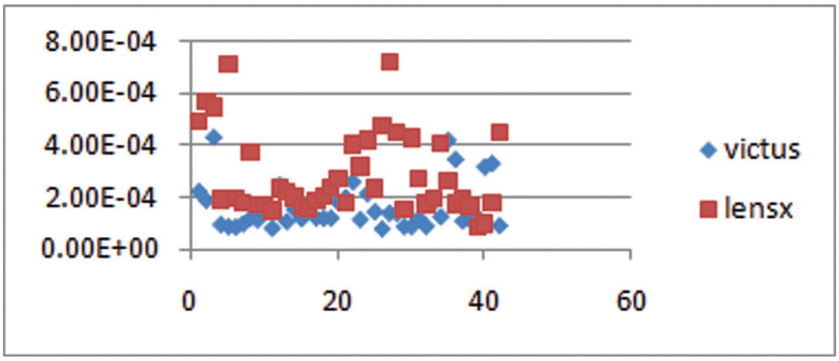
Tags as shown in Fig. 12 are present in all cases of laser groups with a mean of 4. Misdirected laser shots

Figure 5



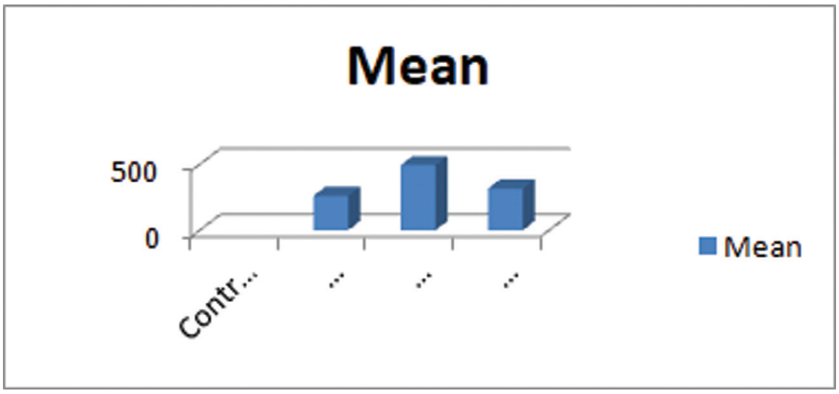
Scatter chart showing angular second moment among Victus and LenSx cases.

Figure 6



Comparison between the three studied groups regarding contrast.

Figure 7



Scatter chart showing contrast among the three group cases.

Table 2 Comparison between the three studied groups regarding the average coefficient of variation of change of grayness in cut edges of all scanning electron microscopy images

	Group 1	Group 2	Group 3
Average CoV (%)	23.04	20.88	16.47

CoV, coefficient of variation.

Figure 8

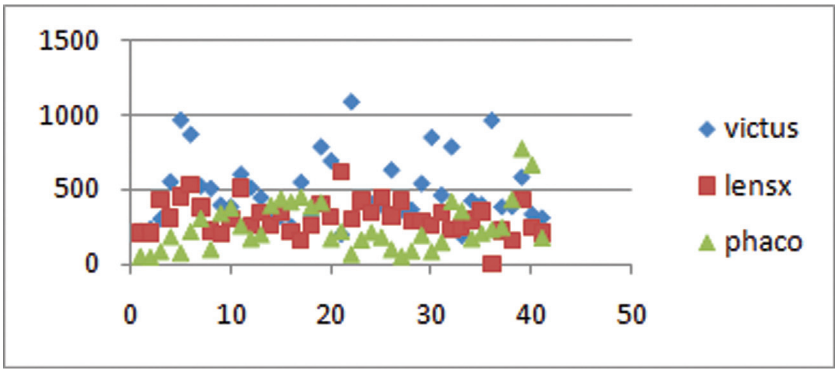


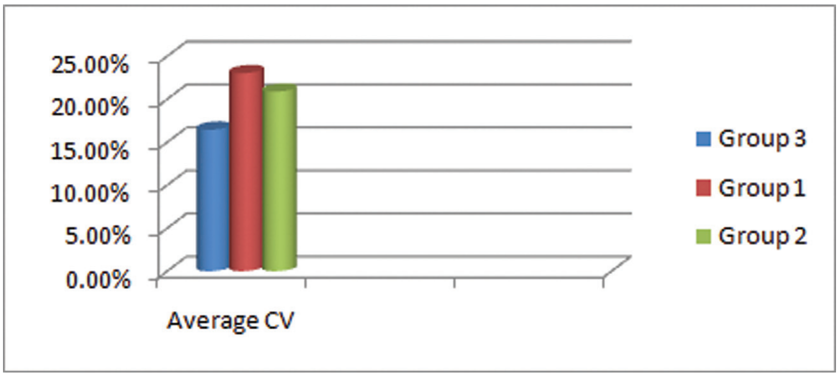
Chart showing comparison between the three studied groups regarding CoV. CoV, coefficient of variation.

Table 3 Comparison between average thickness of capsular cut edges of each scanning electron microscopy images of the three studied groups

Figures	Mean thickness		
	Victus	LenSx	Phaco
Mean±SD	4.79±0.62	4.27±0.41	7.70±4.13
F (*P*)		8.715* (0.001*)	
Significance between Groups		P_1=0.563, P_2=0.002*, P_3<0.001*	

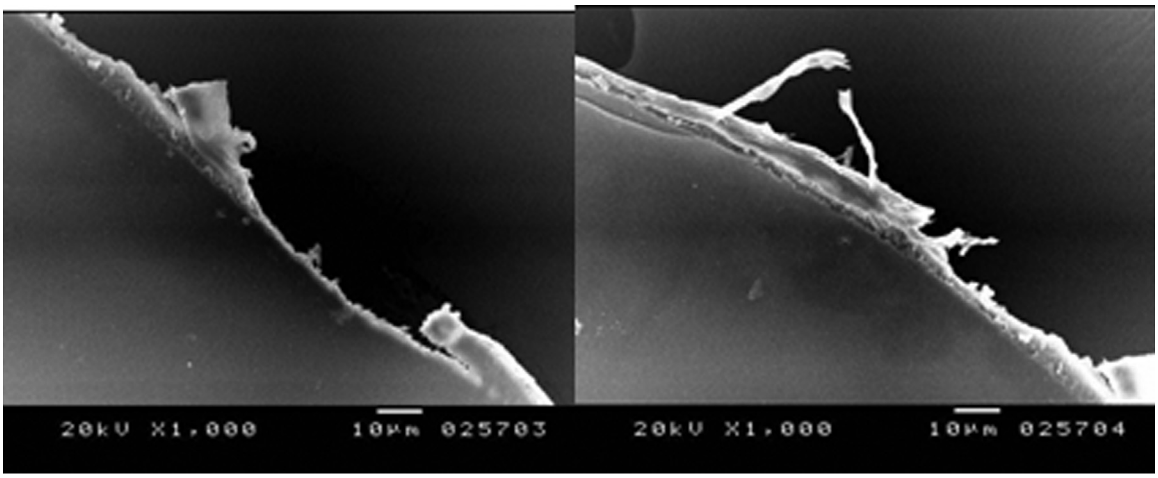
F (*P*), *F* and *P* values for analysis of variance test, significance between groups was done using post-hoc test (least significant difference). P_1, *P* value for comparing between groups 1 and 2. P_2, *P* value for comparing between groups 1 and 3. P_3, *P* value for comparing between groups 2 and 3. *Statistically significant at *P* value less than or equal to 0.05.

Figure 9



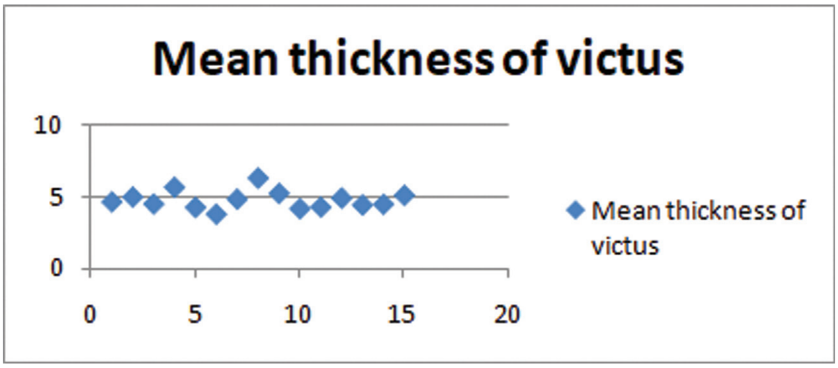
Scatter chart showing capsular thickness of cut edges among group 1 cases.

Figure 10



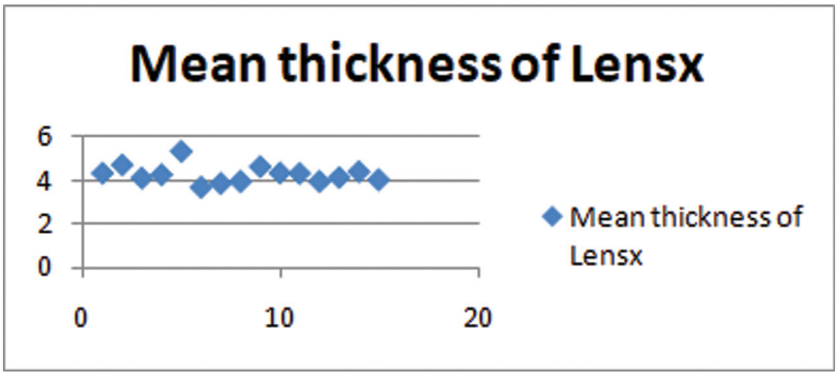
Scatter chart showing capsular thickness of cut edges among group 2 cases.

Figure 11



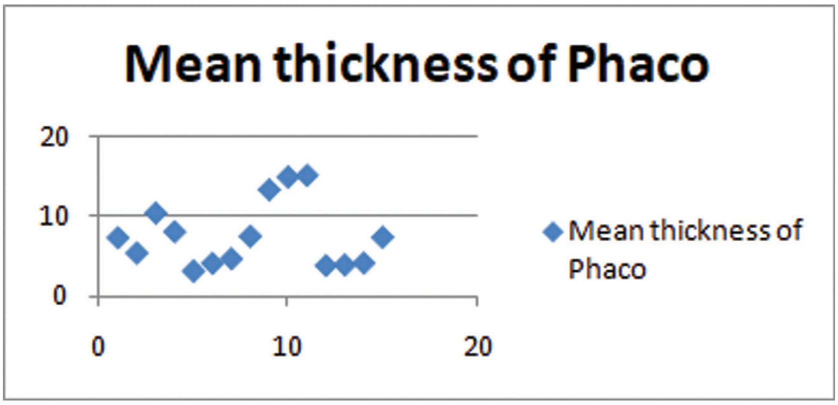
Scatter chart showing capsular thickness of cut edges among group three cases.

Figure 12



Multiple ×1000 SEM image of LACS edge cut of anterior capsule, showing tags. SEM, scanning electron microscopy.

Figure 13



SEM images of superior surface of Victus edge cut, showing misdirected laser shots. SEM, scanning electron microscopy.

present tracks of pulses that appeared to have been directed inside the capsulotomy of (5/15) of Victus laser cases only (Fig. 13).

Discussion

In laser assisted cataract surgery (LACS) capsulotomies, the photodisruptive mechanical and thermal effects contribute to the corrugating and stretching of the capsular edge, offering a mechanical basis for weakness in capsular integrity. These irregularities have been postulated to either limit the distension of the capsule or act as focal points for the concentration of stress that would increase the risk of capsular tear. Eye movements during surgery have been considered to contribute to increased capsulotomy edges' irregularities, by creating multiple, random cavitation that could compromise the integrity of the capsular edge. The implementation of robust eye tracking system in LACS platforms would greatly improve the smoothness of capsulotomy edges.

In our study, we used SEM to evaluate the morphology and quality of the capsulorhexis cut obtained with the LACS at certain low capsulotomy energy settings during cataract surgery by two different platforms. We compared the results with those obtained using a standard manual technique. Low-magnification images showed a perfectly circular capsulorhexis for the LACS in all samples, with no tears in the overall profile and geometry.

Our results go with the results in recent studies that reported an evaluation of cut-surface morphology with SEM and highlighted that the edge features of the LACS capsulorhexis were as smooth as those obtained with the manual method, except for laser-induced microgrooves. Evaluation of SEM high-magni-fication images indicated numerous differences in the cut-surface quality between the groups, with a smoother and more regular cut surface in the manual group than in all the LACS groups.

Mastropasqua *et al.* [18] recently described a subjective method of quantifying capsule edge irregularities using a masked grading system from 0 to 3, in which 0 indicated a smooth cut surface with minimal microgrooves, pitting, or notches; 1 indicated a slightly irregular surface with minimal microgrooves, pitting, or notches; 2 indicated an irregular surface with minimal microgrooves, pitting, or notches; and 3 indicated a very irregular surface with microgrooves, pitting, or notches.

In our study, we developed an objective algorithm to measure the surface irregularities using gray-level co-occurrence matrix parameters of the ImageJ system in the evaluation of edge roughness and irregularities of all groups. ImageJ is easy to use public domain software that runs on any operating system. The distinction between data (the pixel values) and display (the colored squares) is particularly important in understanding surface irregularities.

Two related facts can cause trouble while assessing surface irregularities. First, images that look the same can contain different pixel values and second, images that look different can contain the same pixel values. The angular second moment measurement tool in ImageJ and the contrast tool are two parameters that help us quantitatively discern such differences.

In our study, the CoV of grayness changes at the cut edge was used as a second-order objective measure. Mathematically, this variable is more easily affected by few large changes in pixilation rather than by local variations. Therefore, to the naked eye, the subjective sense of smoothness may not always parallel the objective measure CoV.

The end results of our study denoted that the change of contrast was more in Victus than LenSx cases and both the LACS groups were more than that of the CCC group. The angular second moment was more in the CCC group cases than LACS groups and the least of them were Victus cases. CoV of Victus cases is higher than LenSx cases and that of CCC cases was the lowest of them. The edges of manually torn capsules were smoother than the LACS capsulotomy edges; some measurable differences in irregularity were found between LenSx and Victus specimens. The LACS capsulotomy edges showed that in cases of LenSx, the roughness of the cut edge is lesser than of Victus cases, despite the intrinsic differences in laser settings and specific technology.

In the LenSx laser, the smoother cut edges could be obtained by placing a soft contact lens between the cornea and the curved rigid interface. The microscopic features and irregularity of the LACS capsulotomy edges can be directly related to photodisruption and eye movements.

Correlation between morphologic thickness of capsule edge cut made by two LACS laser platforms and manual continuous curvilinear capsulorhexis

In our study, in the manual group, there was dishomogenous and less uniform thickness along the capsulorhexis edge with variability and a mean of 7.70 ±4.13 μm, while in the femtosecond laser samples, the cuts were more homogeneous in thickness throughout the whole capsulorhexis edge with a mean of 4.79±0.62 and 4.27±0.40 μm of 1 and 2 laser groups, respectively. The homogeneous thickness is an important factor in the strength of cut edge of the capsule.

In Mastropasqua *et al.* [18], the thickness measured at the cut edge of the manual group and the group that used an energy power of 7 μJ had a significantly higher thickness and lower thickness, respectively, of the capsulorhexis edge than the other groups of using powers 13.5, 14, and 15 μJ.Auffarth and colleagues have found, in porcine eyes, that LACS capsulotomies resulted in a stronger anterior capsular opening than the manual CCC, offering a hypothesis that tears may originate by increased stress at the capsular edges when pulling the capsulotomy leaf. On the other hand, biomechanical data from porcine specimens cannot be translated to the human lens capsule due to intrinsic differences in elasticity between species [19].

Tags and misdirected laser shots in correlation with previous studies

On qualitative analysis, in Bala and colleagues, tags were present in 5 of 10 of LenSx pre-soft-fit cases and in 1 of 10 LenSx post-soft-fit cases. Tags were present in 1 of 4 Victus I cases and 3 of 10 Victus II cases. Several capsules generated by the Catalys (4 of 10 cases) and Victus II (4 of 10 cases) showed tracks of pulses that appeared to have been directed inside the capsulotomy. These were not seen with the other laser platforms. Appendages of capsules created by misdirected pulses were not considered to be tags [13].

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Nil.

Conflicts of interest

There are no conflicts of interest.

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