Applanation-free femtosecond laser processing of the cornea

Manuela Miclea,1,* Ulrich Skrzypczak,1 Frank Fankhauser,2 Sebastian Faust,3 Heinrich Graener,4 and Gerhard Seifert1
1Martin Luther University Halle-Wittenberg, Centre for Innovation Competence SiLi-nano, D-06120 Halle (Saale), Germany
2Augenzentrum Fankhauser AG, CH-3012 Bern, Switzerland
3Martin Luther University Halle-Wittenberg, D-06120 Halle (Saale), Germany
4University of Hamburg, D-20146 Hamburg, Germany
*manuela.miclea@physik.uni-halle.de

Abstract: A novel approach for applanation-free femtosecond refractive surgery with the help of a contact liquid layer is presented. A laboratory device for performing corneal procedures is described based on a femtosecond-laser system which has been tested and evaluated by processing ex vivo pig eyes. With its help, flap cuttings for different flap thicknesses were performed. The accomplished corneal surfaces are comparable to already published results. The reproducibility of the flap thicknesses is very good, with a standard deviation of 10 µm. The processing and removal of an intrastromal lenticule as thin as 30 µm could be shown. The extraction of such lenticule through a corneal side channel could also be accomplished successfully and is a promising improvement of the overall surgery procedure.

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OCIS codes: (140.3390) Laser materials processing; (170.4470) Ophthalmology.

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1. Introduction

Material processing with femtosecond (fs) lasers allows very precise ablation with strongly reduced thermal damage effects in comparison to nanosecond and picosecond lasers [1]. Due to these advantages, fs laser refractive surgery has become a common tool in medical care during the last years. In ophthalmology fs lasers are mostly used as part of the LASIK (laser in situ keratomileusis) refractive surgery [2]. The mechanical knife (microkeratome) is replaced by the fs laser resulting in significant advantages for the patients. The possibility of using the fs laser system as an all-in-one device for flap and lenticule processing is not anymore only a matter of laboratory research, but has already been applied to human eyes on a small but rapidly increasing number of the overall treatment count [3]. Still, however, most procedures in fs refractive surgery are performed by applanation of the cornea. Besides being uncomfortable for the patients, the mechanical stress on the cornea during treatment might limit the precision of the cut, in particular when not only flap cutting, but complete refractive correction by fs processing is intended.

In this paper we report on a novel approach for applanation-free fs refractive surgery with the help of a contact liquid layer. We will present a laboratory device for performing corneal surgical procedures, which has been tested and evaluated by processing ex vivo pig eyes.

2. Experiment

2.1. The fs laser surgery system

Our processing setup, as shown schematically in Fig. 1, was based on the oscillator-regenerative amplifier system Pharos (Light Conversion). This laser system is operating at a wavelength of 1030 nm, providing pulses of 300 fs duration. The repetition rate can be varied from 10 kHz to 350 kHz. For surgery in the cornea the output beam from the laser system was delivered into a \(xyz\)-scanner (Scanlab) provided with a telecentrical \(f-\vartheta\)-lens usable at a...
working distance of 80 mm. The optical system has a numerical aperture of 0.086 and focuses the laser to a beam waist of about 4 µm. This has been verified by a z-stepwise beam profiling to reconstruct the actual Gaussian beam shape. A Gaussian beam fit to this data has been used to determine the beam waist considering the M² value of the laser of 1.1.

As described in detail in the next chapter, our approach for processing of the cornea involves no applanation of the eye. The contact liquid layer used instead, however, has an individual thickness for each eye, i.e., the positioning of the laser focus cannot be done by the help of a reference surface like in other fs surgery systems on the market. Therefore we use a positioning system to find the eye vertex, which is then used as a reference point for further processing. The position of the eye vertex is measured with a confocal setup which follows the same optical path within the system as the surgery beam.

The positioning system contains a fiber-coupled monomode laser diode at 980 nm (near the surgery laser’s fundamental wavelength) with 150 mW continuous output power. It is focused on the eye and the reflection from the cornea follows the way back through the optical setup and is then detected with a photodiode connected to a monomode fiber. It has to be noted that due to the losses in the optical system only around 10 mW reach the retina. Also, the beam focusing results in a divergent beam throughout the eye and therefore in a negligible optical intensity on the retina. The laser focus is on the eye vertex when the signal of the photodiode is maximal. The resolution of this confocal system at that point merely depends on the numerical aperture of the focusing optics and on the aberrations within the system. The axial resolution of the setup is 5 µm, limited by the relatively low numerical aperture of the f-θ-lens. Further signal peaks allow for the determination of the depth of each refractive index change given the presence of a layered system.

The z-scanning of the laser focus is achieved by a variable telescope integrated into the xyz-scanner. Using the two mirrors of the scanner (for x- and y-coordinates respectively) and the z-scan, a 3D coordinate set which is calculated in a computer program is scanned by the laser focus. The 3D profiles in the cornea, which represent cutting masks for flap, lenticule, hinge and side cut, are created digitally based on the individual properties of the eye. These properties, in particular corneal curvature, astigmatism and the distance from the vertex to the pupil centre, were obtained from the individual eye topography measured with a Scout keratometer (Opticon). The flaps were computed to follow the curvature of the cornea and the hinge could be placed wherever needed. The side cut angle in particular has been chosen such that it is always in a right angle with the tangent on the corneal surface. The right angle is appropriate in order to prevent epithelial ingrowth after folding down the flap.

Lenticule profiles were programmed depending on the intended refractive correction using the Munnerlyn formula [4]. The cuts were generated as circles starting on the eye vertex in the intended depth, increasing their diameter by changing the z-position (depth) of the focus within the cornea. The distance between the spots on a circle and the distance between circles could be arbitrarily chosen in the controlling software. During the entire procedure the eye was observed with a CCD camera which gets its image of the eye through a 2 µm thin pellicle placed in the laser beam.

For the presented studies pig eyes were used. The freshly enucleated eyes were kept at low temperature. In order to preserve the IOP during treatment, an external pressure basin with a counter pressure leveled at less than 20 mmHg was employed. Additionally, to keep the corneas fresh and transparent, a dextrane solution was added on it [5]. The eyes were usually processed within hours after the enucleation. It was necessary to remove the epithelial layer prior to processing of the cornea since it degrades in the first hours after enucleation and thus impairs the optical quality of the eye’s surface. Due to the use of the contact liquid, this removal should not have a significant impact on the beam propagation. The corneal thicknesses were measured with an ultrasound pachymeter (Tomey SP-100, operable between 150 and 1200 µm with ± 5 µm) before and after the procedure. The eyes were fixed under the scanner in a suction ring. The suction pressure was kept between 300 and 500 mbar.
It has also been verified that the application of the suction ring does not affect the eye topography and the cornea thickness significantly. All 3D profiles were programmed based on the topography measured with the eye fixed in the suction ring.

2.2. Applanation-free surgery

Most of the fs refractive surgery devices developed up to now involve either a weak or a strong applanation scheme of the eye in order to achieve a more or less plane surface of the cornea for the fs laser entrance into the cornea [6]. The cutting directly on the curved cornea surface would induce strong coma aberrations only allowing for cuts of low quality. In the case of weak applanation on one hand, an adaption piece of almost the same curvature as the cornea is brought onto the eye. In the case of strong applanation on the other hand, a full applanation is generated by pressing the eye down with a glass plate. Both applanation schemes disregard the possibility of irregularities in the cornea shape.

The novelty of the proposed processing procedure lies in the fact that a liquid (physiological saline) with a refractive index (n = 1.338) close to that of the cornea (regularly n = 1.376) is brought on top of the eye into the suction ring [7]. This liquid ensures a very good refractive index matching and thus minimizes the wave front distortion of the fs laser pulses upon entrance into cornea. The thickness of the liquid can be varied from a few hundred µm to 1 mm. Thicknesses between 200 and 300 µm were used throughout these experiments. Using this approach, an applanation-free processing of the cornea becomes possible. As the thickness of the liquid layer can be extracted from the second signal in the positioning process, a constant layer thickness is not required but will be accounted for during the 3D profile calculation process. A sketch of the suction ring is presented in Fig. 2.

![Fig. 2. Applanation-free geometry.](image)

The required removal of the epithelial layer of the pig eyes caused a significant swelling of the cornea upon contact with the liquid. A thickness increase by up to 100 µm could be measured with the ultrasound pachymeter. Therefore processing of the eye was not started before the cornea's thickness remained constant. It had to be ensured that during the processing time, which is determined by eye positioning and fs laser processing (less than 1 min.), the thickness did not change by more than 2 to 3 µm. Another point which might influence the processing is a change of the refractive index of the pig cornea with thickness due to hydration, as shown in Ref [8]. However, these problems are not relevant in case of surgery of human eyes: their epithelial layer would not be removed during the surgery procedure since vital eyes don’t degrade. The thickness of the pigs corneas varies from 600 µm up to 1000 µm (depending mostly on age), accompanied by a variation of the refractive index from 1.405 to 1.373. These changes can induce errors of up to 10 µm in the calculation of the 3D profiles where the refractive index is assumed to be constant and thus influence the
aimed cutting depth. The human cornea varies much less, from 550 µm to 600 µm, inducing an error margin of only 1 to 2 µm.

Furthermore, the nonlinear behavior of the cornea is of importance. It has recently been shown that the pig cornea has a nonlinear refractive index of $2 \times 10^{-19}$ m$^2$/W, which is relatively high, compared to water [9]. This means that a precise calculation of the focal position can only be done by taking into account self-focusing effects. Of course self-focusing in the liquid layer has to be considered as well. Because the nonlinear properties of the physiological saline are relatively weak (like water), it induces only a small nonlinear phase change in the laser wave fronts depending on the thickness of the liquid layer (which can be precisely measured with the positioning system). In other words, the liquid layer behaves in good approximation like a Kerr lens on top of the eye, which can therefore easily be included in calculating the 3D profiles. When all these effects are being considered, the calculation of the focal position for a certain flap thickness can be performed in a well controlled fashion.

3. Results

3.1. Optimization of surgery parameters

Looking for the optimum surgery parameters, it is helpful to briefly recall the fs processing mechanism in transparent corneal tissue, which has already been the subject of extensive theoretical and experimental studies [10–12]. By focusing intense fs laser pulses to a place inside the material, multiphoton absorption, subsequent photoionization, formation of a plasma with electron densities as high as $10^{21}$/cm$^3$, and finally photodisruption of the material occur. The latter process is driven by cavitation bubbles, which themselves are the product of the plasma explosion. Within a time of ~10 ns after laser pulse interaction, electron-ion recombination takes place and the ionization energy is converted to heat. The material is evaporated and a hot gas at high pressure continues to expand in the time from 10 ns to 1 µs, until it finally cools down [13,14] on a time scale up to 10 µs. Thus, even for the high laser repetition rates of a few hundred kHz applied here, cavitation bubbles from the last pulse will already be present when the next pulse arrives. If there is spatial overlap, existing bubbles can absorb successive pulses heating the gas even further and thereby screening the tissue from the surgery beam. In the case of only partial overlap, beam distortion at the interface might cause uncontrollable damage to the region already processed.

Therefore, a successful processing can take place only if laser fluence and temporal as well as spatial distance between the pulses are well adjusted [6]. For the present study this means that the laser repetition rate should be adjusted such that consecutive pulses can reach a new position where they do not hit the developed gas bubble being still in movement in the area of the preceding irradiation spot. Additionally safety requirements have to be regarded: the total energy applied has to be kept as low as possible in order to avoid retina damage; the total surgery time should be as short as possible in order not to damage the vital functions of the eye due to the suction.

Considering the above issues, the operation parameters (i) pulse energy, (ii) axial and (iii) radial distance between the laser pulses have been optimized for intrastromal cuts. The laser pulse energy was kept as low as possible, with values between 0.85 µJ and 1.5 µJ corresponding to laser fluences for the given spot size between 1.7 J/cm$^2$ and 3 J/cm$^2$. These values are slightly above the breakdown values of cornea for 300 fs pulses, as published to be between 0.4 J/cm$^2$ and 1.8 J/cm$^2$ [11,15]. The liquid thickness above the eye was kept between 300 µm and 1000 µm. For a fast processing of the tissue, we used the fs laser at repetition rates between 200 to 350 kHz. Because of the cavitation bubbles’ generation and evolution, higher repetition rates are not necessary and also the probability to hit a bubble at the former spot position is increasing. For example, at a repetition rate of 200 kHz the processing time for a flap and the side cut at 4 µm radial and 1.5 µm axial distances between the focal points, is less than 20 s for 10 mm flap diameter, decreasing to around 10 s at 350 kHz.
We performed studies changing the spot distance in radial and axial direction in order to find the minimum spot overlap necessary for a decent flap removal as a function of laser pulse energy. In this way the approximate upper limit for usable spot distances at a given pulse energy have been determined. More than 100 pig eyes were processed at different parameters for the sake of reproducibility. The relation between these maximal spot distances at given laser energy is shown in Fig. 3; the figure can also be understood in a reversed way, then giving the minimum laser energy allowing decent flap removal at a given spot distance. From the relation of the radial and axial distances it can be seen that the produced cavitation bubbles must have had an elliptical shape, expanding more into the radial than in the axial direction. This is, in accordance with the results published in Ref [11], due to the presence of the collagen fibrils distributed alongside the radial direction. Also the linearity of the dependence is in agreement with already published results. It was shown that for ultrashort laser pulses shorter than 1 ps, the diameter of the cavitation bubbles in water at energies close to its breakdown threshold has a linear dependence on energy [14]. In the experiments an opaque bubble layer (OBL) effect was observed but vanished within less than one minute.

![Fig. 3. Relation between the radial and axial spot distances on the minimum laser energy necessary for decent flap removal.](image)

Environmental scanning electron microscopy (ESEM) images were taken after preparation of flaps with different spot distances at the same laser energy. In the ESEM images presented in Fig. 4 it is apparent that larger spot distances (Fig. 4b) lead to tissue bridges instead of the desired smooth surface (Fig. 4a).
The minimum energy necessary to remove the flap (provided an optimum choice of the spot distances) was about 0.85 µJ. At energies between 1.1 and 1.3 µJ optimal flaps were processed in a short time between 10 and 20 s. A spot separation of 4 µm radial and 2 µm axial distances was sufficient to create a flap surface without any visible tissue bridges. Also, the side cut, carried out with the same energy as the bed cut, is of very good quality so that the flap can be removed very easily. An example of a flap and a side cut is presented in Figs. 5a and 5b, respectively.

3.2. Reproducibility of the flap thickness

In order to be able to assess the reproducibility of flap thickness by the developed applanation-free surgery, about 50 pig eyes were processed using the same laser and surgery parameters. The variations of the central flap thicknesses measured after processing are presented in Fig. 6. The deviation from the programmed 150 µm flap thickness shows variations mostly in the 10-15 µm range, only a few eyes have larger variance. This is mostly due to the uncontrollable hydration because of lack of epithelial layer and the weak change of the thickness due to suction distension. A thickness of 150 µm has been chosen since this is a value usually employed in refractive surgery. Besides that, other thicknesses as in 200 or 300 µm have also been successfully tested.
The reported results in Fig. 6 are comparable to the published flap thickness reproducibility in commercially available systems. There, a standard deviation between 5 µm and 19 µm is reported with an average of 12 µm [16]. It can be stated that the system demonstrated here works similar to the commercial ones concerning the processing quality, but with the additional advantage of completely avoiding applanation of the eye.

3.3. Intrastromal lenticular extraction

In ophthalmology it has always been highly desired to be able to perform all steps of the refractive surgery (flap and lenticule) with one device. The procedure can create a non-refractive (flap) and a refractive (lenticule) cut by processing two surfaces with different radii of curvature. After lifting the flap, the intrastromal tissue is removed. Subsequently, the flap is repositioned. Throughout this procedure, the corneal curvature is changing and perpetually a refractive correction is performed. Moreover, the possibility to skip lifting the flap and to only extract the lenticule throughout a side channel through the cornea is a possible advantage primarily concerning the wound healing. Finally, the programmable features of this system with flexibility in the z-plane enable the creation of virtually any surface inside the stroma, and thus to correct higher order visual defects.

Figure 7 presents proof-of-concept examples of flaps and lenticules. In Fig. 7a is a flap in 150 µm depth with a lenticule exhibiting a central thickness of 100 µm and a diameter of 10 mm as the flap. This corresponds to a refractive correction of −7.5 diopters. Figure 7b shows a
very thin lenticule with 10 mm diameter (a lens-like intrastromal tissue) of about 30 µm central thickness which implies a refractive correction of −1 diopters. As can be seen from this case, by striving for thin lenticules even weaker refractive corrections can be achieved. The example in Fig. 7c shows the extraction of a 7 mm diameter lenticule with a thickness of 35 µm through a lateral channel of 5 mm width.

3.4. Experimental considerations

The laser energy was kept low in order to avoid retinal irradiation damage, slightly above the breakdown energy in the cornea. Due to the highly repetitive fs-laser, the processing time of flap and edge is shorter than 20 s. Also, two different surfaces can be created within the same cornea thereby creating a lenticule as thin as 30 µm. This limit can be pushed even further by optimization of the choice of surgery parameters. The extraction of an intrastromal lenticule through a corneal side channel could also be accomplished successfully and is a promising improvement of the overall surgery procedure.

The mechanical limits of lenticule removal through a lateral tunnel are determined by the mechanical properties of the human corneal stroma and the configuration and size of the tunnel. The larger the tunnel is created the easier can be the removal of the lenticule and also the thinner will be the minimal thickness of the refractive lenticule. However, only limited experience of lenticule removal in human corneae is available at this point in time and therefore no standard values for minimal thicknesses exist. Our experiments support the feasibility of extracting a 35 µm lenticule corresponding to a refractive correction of 1 diopter which seems sufficient as a first approach towards a determined minimal limit for refractive correction by means of fs processed refractive tissue processing. For corrections at or below 1 diopter intrastromal ablation or a combination of intrastromal ablation and lenticule removal can be considered. However further investigations in human tissue are necessary to clarify these issues.

4. Conclusions

This work demonstrated the feasibility of a novel applanation-free surgery system for fs refractive surgery. It has been shown that flaps with a very good quality surface can be processed through a liquid layer without the need for painful applanation of the eye. The reproducibility and the standard deviation of the flap thicknesses are comparable to the values published by commercially available systems. Regarding the specific problems of ex vivo pig corneas, which vary significantly from individual to individual and change over time due to enucleation, the reproducibility of the cutting results for human eyes can be expected to be even better.

Finally it should be stated that the fs processing procedure obtained here cannot be immediately transferred to refractive surgery on human eyes, not only because of the peculiar problems of ex vivo pig eyes. However, the general similarity of pig and human corneal tissue could be verified: we had the chance to perform a few tests on human corneas, where it was observed that the parameters for intrastromal cuttings are similar to those needed for pig corneas. Furthermore, it may be expected that, from a merely technical point of view, in vivo operation should work even better. Of course, histology, wound healing and safety issues have to be investigated further to confirm this statement.

Acknowledgments

The authors wish to express their gratitude to the German state Saxony-Anhalt and Schwind eye-tech-solutions for financial support of this study, and to the “Tönnies” in Weissenfels, Germany for providing the pig eyes.