Intrastromal Corneal Reshaping Using a High-Intensity Femtosecond Laser: A Novel Method of Vision Correction

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Abstract

A new technology to perform a minimally invasive cornea reshaping procedure has been developed. This can eliminate the incidence of the flap-related complications of the conventional eye refractive procedures by multiphoton processes using a very high-intensity ($I \geq 10^{13}$ W/cm$^2$), but low energy ($E_p \sim 100 – 200 \mu$J) femtosecond laser pulses. Due to much lower energy than that of the nanosecond laser pulses for the thermal photoablation, the multiphoton processes cause almost no collateral damage by heat and shock wave generation.

In this method, a series of femtosecond laser pulses is used to create very narrow (< 30 µm) and sufficiently long (≥ 2.5 mm) micro-channels in the cornea. The micro-channels are oriented almost perpendicular to the eye’s optical axis. Once the micro-channel reaches a desired length, another series of femtosecond pulses with higher intensity is efficiently delivered through the micro-channel to the endpoint where a certain amount of the stromal tissue is disintegrated by the multiphoton processes. The disintegrated fragments are ejected out of the cornea via the same micro-channel, allowing the corneal surface to collapse, and changing its refractive power. This new corneal reshaping method obviates any process of damaging the corneal surface layer, while retaining the advantages of the conventional refractive procedures such as Laser in situ keratomileusis (LASIK) and Photorefractive keratectomy (PRK).

In order to demonstrate the flapless cornea reshaping procedure, we have conducted ex-vivo experiments on fresh porcine eyes. The reshaped corneas were evaluated by using optical coherence tomography (OCT). The test results have shown that this flapless intrastromal procedure can reshape the cornea as intended with almost no surface damage.

We have also performed a series of experiments to demonstrate the multiphoton processes in the corneal tissue by very high-intensity femtosecond laser pulses. Through the optical emission spectroscopy, we investigated the spectral lines of cal-
cium atom and ions from the femtosecond laser-induced plasma from the porcine corneal tissue. The experimental results have shown the intensity-dependence of ablation rate, which qualitatively verifies the characteristics of the multiphoton processes.
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This dissertation carries T-3335 in the records of the Department of Mechanical and Aerospace Engineering.
To my wife.
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Chapter 1

Introduction

1.1 Toward flapless corneal reshaping (“Flapless Femtosecond LASIK”)

Eye refractive procedures are for correcting vision defects, including myopia (near-sightedness), hyperopia (farsightedness), and astigmatism. The cornea is reshaped in the refractive correction procedures, as the curvature of the cornea plays a very significant role in determining the refractive power of the eye [1–3].

Eye refractive procedures have been improved with the development of new technologies. The advent of excimer nanosecond lasers opened a new prospect in the field along with the non-invasive medical imaging techniques such as corneal topography for mapping the thickness and curvature profile of the cornea, and optical coherence tomography (OCT) for capturing high-resolution images of the eye components. Based on the ability of the excimer lasers to precisely remove the corneal tissue, various types of laser systems have been developed for eye refractive surgeries [4–7]. Laser-assisted in situ keratomileusis (LASIK) has especially become the commonly used laser system for eye surgery. However, efforts to further improve the cornea re-
shaping procedure are still going on due to some complications related to the current laser procedures.

The most critical factor responsible for the incidence of the complications is the flap creation process in LASIK. A thin LASIK flap consisting of the anterior surface of the cornea is cut and lifted up to expose the underlying cornea layer, stroma. This layer has to be properly reshaped by means of ablation with the excimer laser beam. After the procedure, the flap is replaced. Even though the procedure of the LASIK flap creation is very reliable method to achieve the minimally invasive eye refractive surgery, the flap creation itself cannot intrinsically avoid the incidence of the flap-related complications, such as irregular / dislocated flap, epithelial ingrowth, diffuse lamellar keratitis, and corneal ectasia.

It is highly desirable that the future eye refractive procedures be capable of reshaping the cornea without the flap creation to eliminate the related complications. However, several technical difficulties have been encountered in achieving cornea reshaping without a flap. The stromal tissue, which has to be reshaped in the refractive surgery, is covered by the anterior corneal surface layers. This requires that a laser beam should ablate only the target tissue in the stromal layer without damaging a flap. Moreover, a proper way has to be followed to remove the ablation fragments out of the cornea. There have been many attempts to develop this type of flapless cornea reshaping method, however, with success so far, only in our lab. A new method of cornea reshaping was introduced by Suckewer et al. in the mid 2000s, in which multiphoton processes in tissue by very high-intensity ($I \geq 10^{13}$ W/cm$^2$) femtosecond laser pulses are used $^8$. It is a novel attempt for intrastromal tissue removal by means of micro-channels without the LASIK flap creation. Hence, we will call it here flapless cornea reshaping.

In this thesis, we describe several technologies to create micro-channels of practically required specifications and make changes in the chosen area of the cornea.
We have demonstrated the flapless corneal reshaping method through a series of ex-vivo experiments on fresh pig eyes, showing the curvatures of reshaped corneas were changed without any significant damage in the surface layers.

1.2 Current eye refractive procedures

1.2.1 Why reshape cornea?

Ideally, the refractive powers of the eyes lens and the cornea act together in focusing light onto the retina. Especially the curvature of the cornea plays a significant role in determining the refractive power of the eye. While the cornea has lower refractive index ($n_c \sim 1.376$) than the lens ($n_l \sim 1.408$), approximately two-third of the total refraction is attributed to the cornea [3,9]. This is because the cornea, with the tear film covering its surface, is exposed to air with a refractive index of 1. The tear film smoothens out micro-irregularities of the corneal surface [2]. The air-tear film-cornea interface accounts for roughly 42 diopters (capable of focusing a parallel ray of light at 1/42 meter) out of the total refractive power, approximately 59 diopters. Compared with the cornea and the lens, other parts like the aqueous humor and vitreous body of the eye have negligible contribution to the total optical refractive power of the eye. Besides the importance of the cornea in the eye’s optical property, cornea is the most easily accessible part of the eye. For this reason, the cornea is usually reshaped to adjust the total refractive power of the eye.

The human cornea consists of five layers: epithelium, Bowman’s membrane, stroma, Descemets membrane, and endothelium as shown in Figure 1.1. Among the five layers, the stroma takes approximately 85 – 90% of the overall thickness of the cornea, which ranges from $\sim 500 \mu m$ at the center to $\sim 700 \mu m$ at the periphery, and averages $\sim 540 \mu m$ [10]. As the stromal layer allows some degree of thickness change, a certain amount of stromal tissue is removed to reshape the cornea in the laser
refractive procedures. The stroma contains 200 - 250 regularly arranged lamellae of densely packed fibrils. This precise and highly organized network plays a major role in the corneal transparency and its mechanical strength [11][13].

Figure 1.1: Structure of the human cornea taken by light micrograph [9]. The thickest layer between Bowmans membrane and Descemets membrane is the stromal layer.

The LASIK flap includes the epithelium, Bowman’s membrane, and the anterior part of the stroma. It is cut and lifted during the LASIK procedure in order to provide access for the ablating laser pulses to the stroma. The epithelium – the outermost layer of the cornea – is composed of five to seven regularly arranged cell layers, and its thickness is between 40 \( \mu m \) and 50 \( \mu m \) [14]. When the epithelium layer is damaged, it immediately begins the recovering process. Cells at the edge of the wound migrate to cover the defect. The rate of the early wound coverage is 60 – 80 \( \mu m \) per hour [15]. Reproduction of the epithelial cell occurs 24 – 30 hours after injury [16]. Thus, even a small damage in this corneal surface leads to a significant wound healing process accompanied by pain.
Bowman’s membrane is a smooth non-regenerating acellular interface between the epithelium and the stromal layers. Its thickness is approximately 12 µm. Bowman’s layer consists of randomly oriented collagen fibrils that are composed of a compact feltwork, an anatomical network as neuropil, and thus, it is inelastic and indistensible [2]. Therefore, it contributes to maintaining the corneal shape and the biomechanical properties.

1.2.2 Current technologies for vision correction

A diamond knife was an initial tool used for reshaping the cornea. For example, in one of the methods, cornea was incised by a diamond knife in radial directions to rearrange the tensile forces to flatten the corneal surface (radial keratotomy) as shown in Figure 1.2 for correction of myopia. However, it was not sufficiently precise, and the incisions had to be made deep into the cornea, at almost 90% of the corneal thickness, to make an effective refractive change. It considerably weakened the corneal biomechanical properties and caused several complications as well such as corneal perforations and severe light scattering [1,17,18].

![Figure 1.2: An incision pattern in the radial keratotomy using a diamond knife for correction of myopia (a), and its cross-sectional view (b) [1].](image-url)
A major breakthrough in the eye refractive technique was made when the excimer nanosecond lasers became commercially available in the 1980s. It was found that excimer laser beams in the ultraviolet (UV) wavelength range (ArF: 193 nm, photon energy $h\nu \approx 6.4$ eV; KrF: 248 nm, $h\nu \approx 5.0$ eV; XeCl: 308 nm, $h\nu \approx 4.0$ eV) were capable of efficiently ablating material by photoablation [19–24].

Photoablation is a thermal process which typically requires a laser intensity in the range of $10^9 - 10^{11}$ W/cm$^2$. Photons in a laser pulse with a pulse duration of nanoseconds or longer are linearly absorbed by molecules in tissue. In other words, each photon is absorbed by a single molecule. It generally promotes the molecules to excited states in their vibrational-rotational bands. It is followed by non-radiative collisions between the particles in which the internal energies are converted into the heat energy. This significantly raises the local temperature causing a localized plasma, ablating tissue in the affected area.

When certain types of excimer lasers such as ArF and KrF lasers are applied, each photon energy may exceed the dissociation energies of some molecules in tissue. Then they can be dissociated by one-photon absorption and have repulsive forces against each other. This leads the particles to be ejected from the tissue. Table 1.1 shows the dissociation energies of some molecules in tissue and photon energies of applicable excimer lasers. Since the dissociation energies of typical chemical bonds in tissue range from 2.7 eV (C-S) to 7.1 eV (C=O) [25], the excimer nanosecond lasers are usually used to facilitate the photoablation process in the tissue.

As seen in Figure 1.3, the excimer lasers have shown a great precision and repeatability in removing or cutting a biological tissue [4]. The resolution of photoablation of the tissue by the excimer lasers is of the order of a micrometer, which has never been achieved by other types of tools. However, it is known that DNA strongly absorbs UV radiation at $\lambda = 240 - 260$ nm. Therefore, the KrF laser at $\lambda = 248$ nm might cause a mutagenic effect in the tissue [26]. For that reason, the ArF laser at $\lambda = 193$ nm is
Table 1.1: Dissociation energies of typical chemical bonds in a tissue\textsuperscript{25} and photon energies of applicable lasers

<table>
<thead>
<tr>
<th>Type of bond</th>
<th>Dissociation energy (eV)</th>
<th>Laser type</th>
<th>Photon energy (eV)</th>
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<tbody>
<tr>
<td>C=O</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C=C</td>
<td>6.4</td>
<td>ArF</td>
<td>6.4</td>
</tr>
<tr>
<td>O-H</td>
<td>4.8</td>
<td>KrF</td>
<td>5.0</td>
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<tr>
<td>N-H</td>
<td>4.1</td>
<td>Nd:YLF (4w)</td>
<td>4.7</td>
</tr>
<tr>
<td>C-O, C-C</td>
<td>3.6</td>
<td>XeCl</td>
<td>4.0</td>
</tr>
<tr>
<td>S-H</td>
<td>3.5</td>
<td>XeF</td>
<td>3.5</td>
</tr>
<tr>
<td>C-N</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-S</td>
<td>2.7</td>
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</table>

typically used for medical purposes. Moreover, the ArF laser provides higher ablation resolution and efficiency. Especially in the conventional laser refractive surgeries, the ArF laser is safer for the retina when the laser beam is directed approximately parallel to the vision axis due to its very shallow absorption depth to the eye tissue.

Unlike the previous methods using mechanical scalpels, the surgical laser instruments allowed cornea reshaping by excision of the corneal tissue. In other words, corneal curvature changes are made by directly and precisely removing the stromal tissue using laser ablation. Intensive researches and clinical trials led to the development of the well-known laser refractive surgeries such as LASIK and photorefractive keratectomy (PRK)\textsuperscript{27–31}. These procedures have gained a worldwide popularity since the first excimer laser was approved for the eye refractive surgery in the United States in 1995, which has been performed on millions of people around the world\textsuperscript{32}.

Conventional laser refractive procedures can be divided into several kinds according to the type of laser used to cut or ablate the corneal tissue. Excimer laser refractive surgeries include surface ablation techniques such as PRK, laser subepithelial keratomileusis (LASEK), epithelial laser in situ keratomileusis (epi-LASEK), and LASIK.
In the surface ablation techniques, the epithelium – the outermost layer of the cornea – is removed prior to laser ablation of the underlying stromal tissue. The epithelium is mechanically scraped with a blade or chemically detached from the anterior Bowmans layer – acellular interface between the epithelium and the stromal layers – by applying 18 - 20% solution of ethanol \[^{33}\]. The ethanol-assisted process can provide smooth and uniform exposed area, and it is easier and faster than the process using a mechanical blade. However, the solution can be toxic to the corneal cells \[^{34}\]. After removing the epithelium, excimer laser pulses are applied to photoablate the stromal tissue.

In PRK, the epithelial layer is removed by the mechanical or chemical method as described above, and it is not preserved. After applying the excimer laser pulses, a bandage contact lens is placed for a few days to facilitate the wound healing process of the surface layer. Since PRK has been conducted for longer than any other laser refractive procedures, its efficacy and stability for correction of low-to-moderate myopia (up to -7.00D) and hyperopia has been well demonstrated \[^{35, 36}\]. However,
because in this procedure the surface layer being destroyed, that leads to uncomfortable pain and photophobia during the healing period after surgery. In addition, there is a possible incidence of corneal haze due to interaction between the mediators for the wound healing responses (cytokines) simultaneously released from the epithelium and the stroma \[37\].

LASEK and epi-LASIK are modifications of PRK. These procedures are distinct from PRK, in that the epithelial sheet is preserved and repositioned over the stromal layer after the ablation. LASEK involves the same ethanol solution for separating the surface layer, and epi-LASIK uses an epi-keratome to mechanically cut the epithelium \[38\]. PRK, LASEK, and epi-LASIK have shown similar visual outcomes and similar level of pain during the healing period \[39\]; however, LASEK and epi-LASIK have some benefits for lower incidence of corneal haze \[40\]. These surface ablation techniques are better than LASIK for patients with thin corneas, dry eye, or risk of potential corneal ectasia \[38\].

LASIK is a very popular eye refractive surgery today. In order to avoid damaging the surface layers of the cornea and following significant wound healing process, a hinged thin corneal flap is created using a mechanical blade called a microkeratome as shown in Figure 1.4. The LASIK flap, which includes the anterior part of the cornea, is relatively much thicker than the epithelial sheet in LASEK and epi-LASIK, having a thickness of 100 – 200 µm. The hinged flap is lifted to expose the stromal layer, and then the excimer nanosecond laser pulses are delivered to the stroma in near parallel direction to the optical axis of the eye. The ablation of the stromal tissue is done in the same fashion as PRK. The flap is replaced after the ablation process. The flap immediately sticks to the remaining stromal tissue by hydrated cellular glue called Glycosaminoglycan (GAG) and dehydration pressure created by endothelial cell pump, which is responsible for regulating the amount of fluid in the stroma \[41,42\]. A schematic of LASIK process is depicted in Figure 1.5.
Figure 1.4: A schematic of a flap creation process using a microkeratome. A hinged thin corneal flap is cut and lifted back to expose the underlying stromal layer to the excimer laser beam [43].

Figure 1.5: A schematic of LASIK procedure. A flap is created by a microkeratome (a) and folded back to expose the stromal tissue (b). The excimer laser beam is applied to photoablate the tissue (c), and then the flap is replaced (d).
The corneal flap in LASIK serves as a natural and compatible bandage for expediting the postoperative wound healing process. Therefore, LASIK causes little pain and provides a much quicker vision recovery than the other methods. Furthermore, LASIK does not produce haze because the wound-healing reactions of the epithelium and the stroma are decoupled.

In fact, there exists non-negligible rate of the complications, which include the flap-related complications, under- or over-correction, postoperative regression of the vision, and the eye infections [44–47]. Among them, the flap-related complications were reported to occur up to 5.7% of the total LASIK procedures performed using a microkeratome. These involve irregular or dislocated flap, for example, by finger-to-eye contact (see Figure 1.6), epithelial ingrowth that may induce discomfort or blurred vision, dry eyes due to considerable denervation in the surface layers, diffused lamellar keratitis, and flap tears. In addition, lysing the anterior lamellae of the corneal stroma may weaken the corneal biomechanics and predispose to corneal ectasia in rare patients [44,48].

Figure 1.6: A dislocated LASIK flap by finger-to-eye contact after LASIK surgery [45]
A remarkable improvement of the laser refractive surgeries has been achieved by adopting very low energy femtosecond lasers at the near infrared wavelength range (NIR, $\lambda = 700 – 1000$ nm). Generally, transparent materials such as corneal tissue do not absorb visible or NIR light; however, photodisruption process may occur when the laser intensity is in the range of $10^8 – 10^9$ W/cm$^2$. This is also a thermal process, resulting in the formation of cavity bubbles or gas bubbles in tissue which are used to separate the tissue layers. The size of the bubbles depends on the applied laser energy, not intensity. While higher than 1 mJ of pulse energy is required for the nanosecond lasers to induce bubbles in the tissue, the similar intensity level can be achieved with less than 1 $\mu$J of pulse energy for the femtosecond lasers. Thus, the photodisruption process with low energy femtosecond laser pulses not only significantly reduces the collateral damage by heating and shock wave generation, but also provides much higher precision of tissue cut. For example, while the diameter of bubble generated by a nanosecond pulse is of the order of a millimeter, that by a femtosecond pulse is much smaller than 100 $\mu$m [49–54].

This new use of femtosecond laser has enabled the development of femtosecond laser-assisted in situ keratomileusis (fs-LASIK) [55–59]. In fs-LASIK, the femtosecond laser pulses are used to produce micro-bubbles in the cornea about 100 – 150 $\mu$m below surface of the cornea (below flap). By connecting the micro-bubbles to form a separating area between the lamellae in the stromal layer, femtosecond laser pulses can create the LASIK corneal flap instead of the microkeratome as seen in Figure 1.7 [60]. As mentioned above, the femtosecond lasers used in fs-LASIK have pulse energy typically below 1 $\mu$J, and the intensity significantly less than $10^{10}$ W/cm$^2$. After the flap creation, removal of the stromal tissue is conducted by using the excimer laser with 10 – 20 nanosecond pulse durations.

Fs-LASIK has replaced a significant portion of the conventional LASIK procedures with the microkeratome. It has provided a better flap quality and a considerable
Figure 1.7: Schematic of the flap creation using the photodisruption process by femto-second laser pulses \[60\]. After exposing the stromal layer, the ablation process is conducted by the excimer laser nanosecond pulses as in the conventional LASIK procedure.

decrease in the incidence of the flap complications. The flap complications, however, cannot still be ignored. There have been calls for making the procedures more reliable. In fact, there is much room for improvement. First, the fs-LASIK requires two laser suites for the creation of the flap (femtosecond laser) and for the photoablation of the eye tissue (excimer laser). Not only does it complicate to equip the whole system, but it almost doubles the cost of instrumentation. Secondly, intrinsically, the flap creation process itself cannot perfectly avoid the incidence of the related complications.

A flapless cornea reshaping by small-incision lenticule extraction (SMILE) technique using femtosecond laser pulses has been developed recently \[6,61\]. In SMILE, a small opening (2 – 3 mm) is cut by femtosecond laser pulses instead of the flap creation process. An intrastromal lenticule is created by photodisruption with femtosecond laser pulses, similar to the process of creation bubbles in the flap removal with femtosecond laser pulses. The corneal lenticule is extracted using forceps through the opening that is made by the same femtosecond laser \[61\]. Hence, SMILE is one-step
eye refractive surgery in which excimer lasers are no longer being used. The incision geometry for cutting the lenticule and the opening is illustrated in Figure 1.8. The underside of the lenticule cut (1) is created, followed by the lenticule side cut (2). Then, the cap cut including cutting of the upper side of the lenticule (3) is performed. Finally, a 2–3 mm cap opening incision (4) is created, through which the lenticule is extracted manually by using forceps. Only a small part of the corneal surface layer is damaged in the procedure instead of having a flap cut. Although there are some reports that SMILE could keep the anterior stromal lamellae stable and increase corneal nerve reinnervation [6,62], it is as yet unclear if SMILE improves corneal stability or decreases nerve damage. Also, the procedure depends significantly on the human factor because the delicate lenticule has to be extracted manually by the surgeon, which may lead to other kinds of complications, including epithelial abrasions, small tears at the incision, perforated caps, and incomplete tissue removal.

1.3 A new approach using multiphoton processes by a very high-intensity femtosecond laser

Since the mid 2000s, the fundamentally new laser-tissue interactions have been pursued by using a very high intensity \( I \geq 10^{13} \text{ W/cm}^2 \) femtosecond laser to the medical fields. In this new type of laser-tissue interactions, the tissue is reshaped by multiphoton processes. The generation of the ultrashort pulses \( \tau \sim 50 - 100 \) fs made it possible to achieve such high intensity for the multiphoton processes in tissue using relatively low energy per pulse. Due to the use of low pulse energy and the requirement of very high intensity, which tightly confines the interaction region, it become possible to modify tissue with almost no collateral damage and with a great precision.
Figure 1.8: Incision geometry in SMILE procedure to create two surface cuts for the lenticule and the opening [61].
A new approach to reshape the cornea without creation of the LASIK flap has been developed [8, 48, 63]. It is available via the multiphoton processes with very high intensity femtosecond pulses. These new type of laser-tissue interactions have been applied not only to the other ophthalmic surgeries such as treatment of presbyopia [64] and cataract removal [65], but also to dermatologic procedures such as tattoo removal [66, 67], in which much higher femtosecond laser intensities than in the cornea reshaping have been used. Such efforts to improve current medical procedures using multiphoton processes by a very high intensity femtosecond laser will be briefly described in the later part of this section.

When the laser intensity goes higher than $10^{13}$ W/cm$^2$, the multiphoton processes occur through the absorption of a significant number of photons by an individual particle in a time frame shorter than the relaxation times of the particle. Then an ultrahigh electric field is created in the vicinity of the particles, which frees the particles from the tissue. This causes an ablation-like process of the material. Hence, we will call it here “multiphoton ablation”. Typically, a femtosecond laser intensity in the range of $10^{13} - 10^{15}$ W/cm$^2$ is required to generate “multiphoton ablation” of the cornea tissue. It can be achieved with a low femtosecond pulse energy, generating almost no collateral damage by heat and shock wave generation [48, 65, 66].

Based on the optical properties of the NIR femtosecond laser pulses mentioned in the previous section, a flapless cornea reshaping method using multiphoton processes was tried in the late 1990s [60, 68]. The femtosecond laser beam was directed parallel to the optical axis of the eye and focused very tightly to a spot within the stroma using a focusing objective with a high numerical aperture. A certain amount of the particles in the target tissue was disintegrated by the multiphoton processes, and corresponding change in the corneal surface curvature was expected as schematically illustrated in Figure 1.9.
Figure 1.9: A schematic of the femtosecond laser cornea reshaping method. An NIR femtosecond laser beam was tightly focused parallel to the optical axis of the eye. A very high intensity larger than the threshold for the multiphoton processes of the corneal tissue is achieved around the focal spot, causing a change in the corneal curvature [60].

This type of approach to directly focus the femtosecond laser beam inside the cornea along the optical axis of the eye, however, could not lead to development of a practical eye refractive solution. First of all, focusing of the very high intensity pulses 100 - 200 µm below the anterior surface layer of the cornea cannot prevent damaging such layer even with a large numerical aperture (NA) objective. For correction of moderate to high degree of vision error, it is required to have very high intensities of $10^{14} - 10^{15}$ W/cm² at the focus slightly below the anterior surface layer for a large amount of ablation, for example, at the center of the stroma for treatment of myopia. Such pulses will have intensities which exceed the threshold value for the “multiphoton ablation” ($\sim 10^{13}$ W/cm²) before reaching the focal spot. As a result, the laser pulses will significantly damage the surface layer. Secondly, micro-bubbles generated in the vicinity of the focal spot will hinder the delivery of the subsequent pulses by scattering. Before reaching the threshold intensity value for the
“multiphoton ablation”, the focused femtosecond laser pulse begins to have a sufficient intensity to initiate photodisruption ($10^8 – 10^9 \text{W/cm}^2$) in the corneal tissue. Thus, it is unavoidable to have a large number of micro-bubbles around the beam path. Lastly, a proper method to remove the ablation fragments out of the cornea did not exist. The remaining ablation fragments around the treatment area may not only make it complicated to estimate the potential degree of reshaping, but also deteriorate the transparency of the cornea.

A new approach to remove eye tissue without cutting or damaging the surface of the eye has been proposed and studied by our group at Princeton University. Using a very high-intensity femtosecond laser pulses, which can be focused at the target inside the eye to have intensity range of $10^{13} \text{W/cm}^2 – 10^{15} \text{W/cm}^2$, tissue materials such as the lens or the cornea can be “multiphoton-ablated”. The crucial part of this novel approach is that the “multiphoton-ablated” tissue is removed from the eye via micro-channels extending from the surface of the eye to the vicinity of the ablation region. The micro-channels can be created also by the multiphoton processes using the same femtosecond laser.

One of our group’s early research interest was in application of this new technique for the correction of presbyopia [64]. Presbyopia is a condition associated with aging in which the lens loses its accommodation ability to adjust the lens shape to focus at various distances. It is believed that presbyopia is caused by changes in the shape of the lens with age and accompanied by hardening and thickening of the lens [64, 69, 70]. In order to alter the mechanical and optical properties of the lens, a portion of the lens tissue is “multiphoton-ablated” by focusing a high-intensity femtosecond laser beam. In the method, two or more laser beams can be cross-focused to one point from different angles to provide a higher precision of ablation as shown in Figure 1.10 If each laser beam intensity is set to be slightly below the threshold for the multiphoton process at the focal point, the ablation region would be more
tightly localized within the region of the highest-intensity of the total intensity of multi-beams. The “multiphoton-ablated” lens tissue can be removed from the eye either by using traditional cataract surgery aspiration instrumentation or through a micro-channel created by the ultrashort pulses.

Another research was dedicated to application of this new laser-tissue interaction via the multiphoton processes to the removal of cataractous tissue in the lens [65]. In this new procedure, the disintegration and removal of the cataractous tissue in the lens is somewhat similar to that of the presbyopia correction. The “multiphoton-ablated” tissue can also be removed through a micro-channel.

The multiphoton processes in tissue have also been applied for tattoo removal [66, 67]. Conventional tattoo removal procedures use thermal photoablation of tattoo
pigments in the skin by various colors of nanosecond type lasers. A nanosecond laser beam is directed through the surface of the patient’s skin and absorbed by the pigment of specific color. The pigment is heated by the laser light and broken into small particles, which diffuse into the body. Hence, this thermal photoablation process requires different laser beams for different pigment colors; besides, it is very painful due to the heating of the skin. On the other hand, the multiphoton processes by very high intensity but very low energy of femtosecond pulses have a non-thermal nature; therefore, they allow a painless tattoo removal. Especially, the tattoo removal procedure via multiphoton processes has a great advantage that it needs only one laser beam, because the multiphoton processes are practically independent of the pigment color. In a new tattoo removal procedure, a picosecond laser beam, which is part of uncompressed femtosecond laser beam, is focused to have an intensity above of $10^8$ W/cm$^2$ to create a temporary channel from the skin surface to the tattoo pigment. This channel provides an unobstructed passage for the femtosecond main beam with a very high intensity to the pigments. The pigments are broken by the multiphoton processes, and the fragments are ejected through the temporary channel or diffused into the blood stream.

The early work on application of high-intensity femtosecond laser for the various medical procedures in ophthalmology and dermatology was followed by a novel idea of reshaping the cornea by means of using additional micro-channels [8, 48]. Now initial high-power femtosecond laser beam ($I \sim 10^{13}$ W/cm$^2$) is directed normally to the optical axis of the eye, as in original cornea reshaping procedure, but it is just initial femtosecond beam to create micro-channels leading from the surface of the cornea. Femtosecond pulses of higher intensity ($10^{13} – 10^{15}$ W/cm$^2$) are then guided by the micro-channel toward the corneal center, and “ablate” the stromal tissue in the vicinity of the end point of the micro-channel via multiphoton processes. The ablation fragment can be removed via the same micro-channel. Hence, this new
method allows direct removal of a desired amount of the stromal tissue via tiny holes in the surface layer. In addition, this new method of flapless cornea reshaping can provide a fully laser-based procedure that can prevent an incidence of human error.

While the laser beams are directed parallel to the eyes optical axis in the other laser refractive procedures, our method using laser beam perpendicular to the optical axis can perfectly avoid damaging the retina. Furthermore, the femtosecond laser pulse energy is much less than that of the nanosecond lasers used in conventional refractive surgeries. Hence, much safer eye procedure can be achieved with this type of method.

The excimer lasers can be used as femtosecond-type lasers to generate the “multiphoton ablation” of tissues. For example, a powerful subpicosecond laser system was developed for generation of the soft X-ray laser (SXL) at $\lambda = 13.5$ nm in the late 1980s in our group at Princeton University \cite{71,73}. Excimer laser could generate femtosecond pulses by applying femtosecond pulses from the front end with the wavelength matched to the excimer amplifiers by using nonlinear optical techniques. It could generate UV laser pulse with a pulse duration of $\sim 200$ fs.

However, the excimer femtosecond lasers are not very appropriate to be used for the flapless cornea reshaping procedure. The corneal tissue absorbs the pulses in the UV wavelength range extremely well. For instance, the absorption depth of the cornea at $\lambda = 193$ nm of the ArF excimer laser is approximately only 0.25 $\mu$m \cite{74}. Even longer wavelength of XeCl excimer laser at $\lambda = 308$ nm cannot penetrate stroma sufficiently deep to reach its region near center of pupil at several mm from the cornea surface.

On the other hand, the Ti:Sapphire laser has the wavelength range (NIR) transparent to the eye tissue as mentioned above. Moreover, it is more compact and advantageous in the operation and the stability. The Ti:Sapphire lasers with a chirped
pulse amplification (CPA) are preferred to be used to generate ultrashort pulses of very high intensity for multiphoton ablations of the tissues.

1.4 Thesis outline

The main objective of this thesis is to develop a new flapless and computer-based eye refractive procedure that applies multiphoton processes generated by a very high laser intensity as the main mechanism for the tissue removal. Such a flapless procedure would considerably reduce the rate of the complications, convincing people who want to correct their visual disorders without any concern. The thesis is arranged in the following order.

Chapter 2 gives a detailed description of the new method for flapless cornea reshaping via multiphoton processes. Principles and new techniques for creating microchannels of the required specifications in the practical clinics are discussed. It is followed by methods to realize whole cornea reshaping forms corresponding to corrections of myopia, hyperopia, and astigmatism.

In Chapter 3, we describe ex-vivo experiments with porcine eyes for demonstration of the flapless cornea reshaping procedure via multiphoton processes. The high-power femtosecond laser system and the setup for the ex-vivo experiment on the porcine eye are presented. Furthermore, the experimental results, which were analyzed by the optical coherence tomography (OCT), are also described.

Chapter 4 provides further discussion on multiphoton processes. An experiment on demonstration of multiphoton processes in the corneal tissue is described using the spectroscopic analysis. It is followed by discussion of advantages of using the multiphoton processes for the cornea reshaping.

Chapter 5 summarizes the work in this thesis and provides thought and directions for future work.
Appendix A provides a description of preparation for in-vivo experiment of the flapless cornea reshaping on live animal eyes as the next step of this research. Experimental setup and designs of the stages for holding the animal and its eye during the procedure are presented.

In Appendix B, we discuss about how the live animal test will be performed and our expectation of the results.
Chapter 2

Flapless cornea reshaping procedure using a high-intensity femtosecond laser

2.1 Overview

In the previous chapter, we investigated various types of conventional eye refractive techniques. Laser refractive surgeries utilizing photoablation by the excimer nanosecond lasers such as LASIK have been successful and recently farther improved by relatively low-intensity femtosecond lasers for better quality of the flap creation process. However, current approaches that significantly cut or destroy the corneal surface layer have a fundamental limitation that they inevitably have the incidence of the flap-related complications and biomechanically weakened cornea. For this reason, researchers have tried to develop a new type of eye refractive procedure that could avoid damaging the corneal surface. For example, aforementioned SMILE is one of the most recent flapless eye refractive techniques. During SMILE procedure, a stromal lenticule is created by photodisruption and removed using forceps through a
small access point in the surface layer. It is too early to discuss about how the small
incision techniques would improve the quality of the eye refractive procedure.

As an effort to eliminate the risk from the LASIK flap creation process, we have
developed a new flapless cornea reshaping technique based on a novel approach using
“multiphoton ablation” of the corneal tissue. The damage in the corneal surface can
be most minimized with this method, even far less than in SMILE. Furthermore, it
can provide a computer-based control of the whole procedure, excluding the incidence
of complications by human errors.

This chapter provides details of the flapless femtosecond cornea reshaping proce-
dure, including the general concepts of cornea reshaping procedure for vision correc-
tion, principles for creation of the micro-channels in the cornea, and methods to form
the overall “multiphoton ablation” patterns.

2.2 General concepts of cornea reshaping method
to correct vision disorders

2.2.1 Cornea curvature modification to correct vision defects

Figure 2.1 illustrates typical vision defects, which are the result of either too much
or too little refractive power \[43\]. When the eye has too much of refractive power,
light from distant objects is focused in front of the retina. This type of vision error is
termed myopia. Myopia can be corrected by decreasing corneal refractive power by
flattening the curvature of the cornea. When the eye has too little refractive power,
the focal point of light from distant objects is behind the retina. This condition is
called hyperopia. Hyperopia can be treated by increasing corneal refractive power by
making the corneal curvature more steeply curved. Another typical vision disorder is
astigmatism (irregular vision), which is caused by irregular or asymmetric curvature
of the cornea or the lens. Astigmatism can be fixed by applying different cornea reshaping profile for different meridians, for example, reshaping the corneal surface into a form of spherical-cylindrical lens.

Figure 2.1: Schematic of typical vision disorders due to too strong refractive power (myopia), too weak refractive power (hyperopia), and irregular refractive profile (astigmatism) \[43\].

2.2.2 Calculation of corneal refractive change

Corneal thickness changes for correction of the typical vision defects can be approximately calculated by the following geometric analysis of a simplified eye. A perfect calculation of the corneal shape change is very difficult because of the gradient in the refractive index and the aspheric curvature of the corneal surface in the real eye. This simplified eye structure neglects the complexity of the real eye, assuming its homogeneous optical properties and perfectly symmetric shape. This kind of simplification can help to determine the ranges of the removal amount of stromal tissue in the refractive surgeries and corresponding laser parameters.

Figure 2.2 shows schematic cross sections of pre- and postoperative corneal surfaces in myopia correction. \( r_0 \) and \( r_1 \) are the initial and final radii of the cornea. The reshaping occurs within the treatment zone of diameter, \( d \). The thickness of the removed corneal tissue, \( h(y) \), is a function of the distance from the optical axis, \( y \). From the geometry, \( h(y) \) can be expressed as
Figure 2.2: Geometry of cornea reshaping for myopia correction: $d$ is the diameter of the treatment zone; $r_0$ and $r_1$ are radii of the pre- and postoperative corneal surfaces; $y$ is the distance from the optical axis; $h(y)$ is the thickness change at $y$.

\[
    h(y) = \sqrt{r_0^2 - y^2} + OO_1 - \sqrt{r_1^2 - y^2}
\]  

(2.1)

\[
    OO_1 = AO_1 - AO = \sqrt{r_1^2 - \frac{d^2}{4}} - \sqrt{r_0^2 - \frac{d^2}{4}}
\]  

(2.2)

By substituting Equation (2.2) in Equation (2.1), we obtain

\[
    h(y) = \sqrt{r_0^2 - y^2} + \sqrt{r_1^2 - \frac{d^2}{4}} - \sqrt{r_0^2 - \frac{d^2}{4}} - \sqrt{r_1^2 - y^2}
\]  

(2.3)

$r_1$ can be expressed in terms of the degree of myopia in diopters $D$, and the refractive index $n$ of the cornea by using the following relation [5].
\[ D = (n - 1) \left( \frac{1}{r_1} - \frac{1}{r_0} \right) \]  \hspace{2cm} (2.4)

\[ r_1 = \frac{(n - 1) r_0}{r_0 D + n - 1} \]  \hspace{2cm} (2.5)

Substituting Equation (2.5) in (2.3) leads to

\[ h(y) = \sqrt{r_0^2 - y^2} + \sqrt{\left\{ \frac{(n - 1) r_0}{r_0 D + n - 1} \right\}^2 - \frac{d^2}{4}} - \sqrt{r_0^2 - \frac{d^2}{4}} - \sqrt{\left\{ \frac{(n - 1) r_0}{r_0 D + n - 1} \right\}^2 - y^2} \]  \hspace{2cm} (2.6)

Thus, the maximum thickness change in myopia correction occurs on the optical axis, at \( y = 0 \), which is given by

\[ h(0) = r_0 + \sqrt{\left\{ \frac{(n - 1) r_0}{r_0 D + n - 1} \right\}^2 - \frac{d^2}{4}} - \sqrt{r_0^2 - \frac{d^2}{4}} - \frac{(n - 1) r_0}{r_0 D + n - 1} \]  \hspace{2cm} (2.7)

We can derive the thickness change in hyperopia correction in a similar manner. As in Figure 2.3, the radius of corneal curvature decreases after the correction; therefore, \( D \) is of the opposite sign (positive) to myopia correction. Since there is no thickness change at \( y = 0 \), i.e., \( h'(y) = 0 \),

\[ \overline{OO_1} = r_0 - r_1 \]  \hspace{2cm} (2.8)

Thus, the thickness change in hyperopia correction can be expressed as

\[ h'(y) = \sqrt{\left\{ \frac{(n - 1) r_0}{r_0 D + n - 1} \right\}^2 - y^2} + r_0 - \frac{(n - 1) r_0}{r_0 D + n - 1} - \sqrt{r_0^2 - y^2} \]  \hspace{2cm} (2.9)

The maximum thickness change occurs at the edge of the treatment zone, \( y = \frac{d}{2} \), which is nearly equal to the maximum thickness change in myopia correction for the same diopter changes.
Figure 2.3: Geometry of cornea reshaping for hyperopia correction: $d$ is the diameter of the treatment zone; $r_0$ and $r_1$ are radii of the pre- and postoperative corneal surfaces; $y$ is the distance from the optical axis; $h'(y)$ is the thickness change at $y$.

Assuming $r_0 = 7.7$ mm and $n = 1.376$, which are typically anterior radius of curvature and refractive index of the cornea, respectively [75], the maximum thickness changes for both corrections according to each treatment diameter are shown in Figure 2.4.

The larger diameter of treatment zone is considered to be favorable for preventing an abrupt change in the peripheral region and most people with refractive errors have mild to moderate degree of myopia or hyperopia ($< \pm 6$ diopters). Therefore, generally a convex spherical lens shape with the maximum thickness at the center less than 150 $\mu$m is removed for the correction of myopia, and a toroidal ring shape, also having a cross-sectional diameter less than 150 $\mu$m, is removed for hyperopia correction as depicted in Figure 2.5.
Figure 2.4: Maximum thickness changes of corneal tissue at the optical axis in myopia correction and at the edge of the treatment zone in hyperopia correction.

For correction of astigmatism, it can be achieved by giving two different radii of postoperative corneal curvatures in two different planes perpendicular to the optical axis.

### 2.3 Flapless femtosecond laser cornea reshaping procedure

In the new flapless cornea reshaping technique for correction of myopia, hyperopia, and astigmatism, very high-intensity femtosecond laser pulses \( I \geq 10^{13} \text{ W/cm}^2 \) have to be applied in order to change the corneal curvature by directly removing the stromal tissue via “multiphoton ablation” without the flap creation. A schematic of the flapless stromal tissue removal method is shown in Figure 2.6 [8].

In the beginning of the process, a high-intensity femtosecond laser beam \( I \sim 10^{13} \text{ W/cm}^2 \), called here the first laser beam, operating at wavelength \( \lambda \sim 790 \text{ nm} \) is
Figure 2.5: Forms of cornea reshaping for corrections of myopia (left) and hyperopia (right). The dashed red lines indicate changed corneal curvatures and adjusted focuses of light into the retina.

Figure 2.6: Illustration of the method for the femtosecond laser cornea reshaping. A micro-channel is created via multiphoton processes by a high-intensity femtosecond laser beam focused in the stroma of the cornea [8].
being used to create a temporary micro-channel within the cornea. The orientation of the micro-channel is normal to the optical axis of the eye. The micro-channel is created ("drilled") 100 – 150 µm beneath the anterior surface of the cornea starting from a point on the corneal surface, which is outside of the eye vision area.

Once the micro-channel reaches the stromal region to be removed, another femtosecond beam, "the second laser beam", is delivered to the end-point of the micro-channel, passing through the temporarily open micro-channel. The second laser beam is delayed in relation to the first laser beam by the order of nanosecond, having usually higher pulse intensity ($10^{13}$ – $10^{15}$ W/cm$^2$). In the vicinity of the end-point of the micro-channel, the femtosecond laser pulses of the second beam provide multiphoton ablation of the stromal tissue.

The total amount of ablated material depends on the pulse energy and the number of pulses delivered to the end-point. Because the rate of "ablation" is a function of laser intensity for given energy of pulses, the femtosecond laser pulses can "ablate" the tissue in very controlled way by adjusting number of pulses at every chosen stroma point.

"Multiphoton-ablated" stromal tissue is ejected from the eye via the same micro-channel due to the transient ablation-induced recoil pressure. The pressure level is sufficient to expel the "ablated debris", without practically any local damage in the surrounding tissue owing to the very low pulse energy, which is in range of 100 – 200 µJ.

After a desired fraction of the stromal tissue is removed, the surface layer of the cornea above the removed region then collapses, changing the curvature of the corneal surface. Hence, the cornea can be reshaped in an intended manner keeping the surface layer only minimally damaged at the micro-channels entrance holes, which are located in the area outside eye vision. The size of the holes has been measured in the experiment to be smaller than 30 µm in diameter. Since the rate of the early
wound coverage of the epithelium layer is 60 – 80 μm per hour\textsuperscript{15}, these tiny damages would not cause any significant postoperative wound healing process.

2.3.1 Principles of micro-channel creation in the cornea

The micro-channel created in the cornea provides an entry conduit for the femtosecond laser pulses in the second laser beam. The second laser beam is guided by the micro-channel to the stromal region, where it is to be ”multiphoton-ablated”. In order for this flapless refractive procedure to be used in practical clinics, creation of micro-channels with the required specifications is very crucial. Small entrance diameter less than 30 μm and relatively long length ($\geq 2.5$ mm) of micro-channels are needed to be made in the cornea. Accordingly, the laser beam for drilling the micro-channel has to have a small focal diameter and a sufficiently long focal depth. However, these conditions are mutually incompatible with each other in diffraction optics. The Rayleigh length, which is defined as the distance from the waist of the beam to the location where the area of the beam is doubled\textsuperscript{76}, is given by

$$z = \frac{\pi \omega_0^2}{\lambda}$$  \hspace{1cm} (2.10)

where $z$ is the Rayleigh length, $\omega_0$ is the beam radius at the waist, and $\lambda$ is the wavelength of the laser beam. On the other hand, the beam radius at the waist is dependent on the beam profile and approximately calculated by using

$$\omega_0 = \frac{\lambda f}{D_b}$$  \hspace{1cm} (2.11)

where $\lambda_f$ is the focal length of the lens, and $D_b$ is the size of the collimated laser beam before the lens system. These relations indicate that the focal depth is proportional to the square of the beam diameter. Therefore, a small diameter at the focus can hardly be achieved with a long focal depth. For example, the Rayleigh length of a
laser beam with a diameter of 30 µm at the waist and a wavelength of 790 nm is approximately 0.9 mm, whereas 2.5 mm or longer focal depth is required to elongate the micro-channel to the central part of the stroma. Hence, such a high aspect ratio micro-channel is not attainable using a typical diffractive laser beam propagation method.

A diffractionless beam propagation might be considered for a long beam focal depth. However, it is hard to use a diffractionless beam for this application. It is different from a beam propagation in a transparent material of a simple shape, such as a glass with a flat boundary, using an axicon lens or other beam shaping techniques [77]. If a diffractionless beam such as Bessel beam is intended to be focused inside the cornea with a few millimeters-long focal depth, almost half of the beam would propagate through the curved corneal surface boundary and the anterior chamber of the eye, while the rest of the beam would travel through air before reaching the focal point. It leads to a significant aberration along with the complicated corneal surface curvature as illustrated in Figure 2.7. Furthermore, more than two orders of magnitude higher laser intensity would be needed for the elongated focused beam to generate the multiphoton processes in the corneal tissue. Corneal reflection and scattering of the laser beam by the micro-bubbles generated around the micro-channel would also cause a considerable loss of energy during the beam propagation.

Figure 2.7: Beam aberration caused by different optical paths and the complicated corneal surface curvature when a Bessel beam profile is applied to create a micro-channel in the cornea.
Currently, the practical solution for achieving the micro-channel with the high aspect ratio is to deliver the laser pulses through the temporary micro-channels using diffractive propagation of laser pulses. We utilize a focusing lens with relatively long focal length and set the laser intensity for the micro-channel creation to be slightly higher than the threshold. Then a micro-channel with the desired specifications can be created despite a larger focal diameter than the required micro-channel size, because only a small fraction of the beam near the intensity peak can generate the multiphoton processes throughout the sufficiently long focal depth in order to reach the stromal optical axis.

2.3.2 Two-beams scheme for “multiphoton ablation” of deep stromal tissue

When a series of femtosecond laser pulses (one laser beam) are being used to create a micro-channel in the cornea, a repetition rate of 1 kHz or higher is required to keep the micro-channel open during the process, because the micro-channel may collapse in a few milliseconds. The high-intensity pulses propagating inside the temporary micro-channel interact with the ablation fragments created by the preceding pulses and with partially collapsing tissue walls. Furthermore, bubbles around created micro-channels with preceded femtosecond laser pulses may scatter upcoming femtosecond laser pulses due to cavitation and trapping of the gaseous “ablation” debris, hence disturbing creation of new micro-channels in the vicinity. This may lead to distortion of the uniformity of micro-channels and considerable energy loss. Thus, it may cause difficulties in delivering pulse energy with a high efficiency and consistency into the ablation area. Consequently, significantly higher pulse energy than the threshold value is needed to create a long micro-channel. Such too high pulse energy damages the whole micro-channel region. This results in excessive removal in the whole micro-channel, including the entrance part. However, the damage in the entrance part of the
micro-channel has to be at the minimum level in order not to induce any significant
wound healing process of the corneal surface layer.

In order to eliminate the negative effect from the bubbles and the gaseous “ablation”
debris, i.e., to clear the passage for the “ablating” laser beam before it arrives
at the micro-channel, we need another laser beam which precedes the “ablating” laser
beam in the exactly the same pathway. Once a micro-channel of a sufficient length is
created in the cornea, the preceding beam breaks bubbles and the gaseous debris in
the beam pathway, generating a plasma channel around its focus. If the “ablating”
laser beam arrives before the plasma channel collapses, in a few nanoseconds, it can
be delivered to the point in the stroma with much less scattering and energy loss.

Based on the principle described above, the two laser-beams scheme was developed
to enhance the energy efficiency and avoid the quality degradation of the micro-
channel. In the two laser-beams scheme, each laser shot consists a pair of pulses with
the overall repetition rate of 1 kHz (1 millisecond between 2 consecutive shots) or
higher. There is a time delay on the order of a nanosecond between the two pulses in
each shot. The preceding pulse (pre-pulse) is used to create the micro-channel, and
the following pulse (main pulse) is used to “multiphoton ablate” the stromal tissue.
The two laser beams can be constructed by splitting a laser beam and having one of
the two beams travel a longer optical distance via optical delay line.

One advantage of the two laser-beams scheme is highly efficient pulse energy
delivery to the deep stromal region. As mentioned above, a considerable amount of
recombined gases is generated in the beam path while creating a micro-channel. After
a sufficiently long micro-channel is made and the main pulse is turned on, the pre-
pulse, which precedes the main pulse and is focused in the void of the temporarily
open micro-channel, breaks the recombined gases and generates a plasma channel
in the beam pathway. The plasma channel acts as a clear passage for the main
pulse to be delivered to the end-point of the micro-channel, which arrives before the
recombination of the particles in the plasma. As a result, the ablation becomes much more predictable and energy-efficient.

Another advantage of the two laser-beams scheme is the elongated focal depth of the main laser pulse. The main pulse may experience a waveguide effect in the plasma channel. This prevents the main laser pulse from collapsing before reaching the end-point of the micro-channel. In plasma, the refractive index can be expressed as

\[ n = \sqrt{1 - \frac{\omega_p^2}{\omega_0^2}} \]  

(2.12)

where \( \omega_p \) is the plasma frequency:

\[ \omega_p = \sqrt{\frac{n_e e^2}{\epsilon_0 m_e}} \]  

(2.13)

where \( n_e \) is the electron number density, \( e \) is the charge of electron, \( \epsilon_0 \) is the permittivity of free space, and \( m_e \) is the mass of electron. Equation (2.12) indicates that the refractive index in plasma decreases as the electron density increases. If the refractive index of a plasma medium decreases from the center toward the fringe \( (\frac{\partial n}{\partial r} < 0) \), it can construct a waveguide for intense, uniform laser pulses to be delivered over a much longer distance than the Rayleigh length.

Once the end-point of the micro-channel reaches the desired “ablation” area, 0.5 – 1.0 mm from the entrance hole, the pre-pulse, which is focused in the void of the micro-channel, keeps the micro-channel open and generates a plasma channel around its focus. The plasma channel has initially the maximum electron density along the central axis. As the plasma expands cylindrically, ionion and ionatom collisions form a shock wave propagating in radial direction from the boundary between the plasma and the surrounding neutral or weakly ionized gases [78, 79]. The expansion of the shock wave leads to radially increasing electron density, hence the electron density
would reach its maximum value at the edge of the plasma channel and reaches its minimum on channel axis. It results in smaller refractive index toward the edge of the plasma, which allows the fringe of the pulse to travel faster than the central part, distorting the wave front as illustrated in Figure 2.8. Hence, the plasma behaves like a focusing spherical lens, thereby becoming favorable to guide the subsequent main laser pulse. The main pulse is guided by the plasma waveguide until it gets to the removal region deep inside the stromal layer.

By properly choosing the delay time between the pre-pulse and the main pulse, effective and highly localized “multiphoton ablation” of the tissue at the end-point of the micro-channel is achieved. The main pulses also contribute to the extension of the micro-channels during the propagation. A comparison of the visual effect of the single laser beam and the two laser beams-schemes with the delay times of about 0.5 ns and longer between two sequential pulses is shown in Figure 2.9. The micro-channels were created in a porcine eye with a laser intensity of \( \sim 10^{13} \text{ W/cm}^2 \), and the intensity of the main pulse was \( \sim 10^{14} \text{ W/cm}^2 \). It shows that a much brighter and well-confined ablation spark occurs at the end of the micro-channel in the two-beams scheme, especially with the delay time of about 0.5 ns. Based on this result, the delay time was set to be \( \sim 0.5 \text{ ns} \) in the ex-vivo experiments with the porcine eye.

2.3.3 Local intensity control within the cornea by a focal shift

Damage in the entrance part of the micro-channel has to be minimized during the procedure in order to avoid any significant wound healing deterioration of the epithelium and causing some other complications. Meanwhile, a considerably larger amount of tissue needs to be removed at the deep part of the stroma. This can be accomplished by preventing the entrance part from having an excessive laser intensity, significantly higher than the threshold value for the nonlinear laser-tissue interactions in the cornea.
Figure 2.8: Illustration of the plasma wave guide effect; the plasma channel (red) has the maximum electron density at its edge. Solid lines indicate the wave front, while dashed arrows show the beam propagating direction.

Figure 2.9: Comparison of the single-pulse and the double-pulse schemes in delivering laser energy to the end-point of the micro-channel in the porcine cornea. The pulse intensity of the pre-and main beams were $\sim 10^{13}$ W/cm$^2$ and $\sim 10^{14}$ W/cm$^2$, respectively, and the experimental conditions except the delay time were the same.
While the focus of the pre-pulse is fixed on the corneal surface to minimize the size of the entrance hole, the focus of the main pulse is located deep inside the stromal layer, a few millimeters apart from the focus of the pre-pulse. Owing to the waveguide effect provided by the pre-pulse, the main pulse intensity can be locally controlled within the cornea.

Figure 2.10: Cross-sectional view of the local intensity control in a micro-channel. The pre-beam is focused at a point on the corneal surface to have an intensity slightly above the threshold value for the multiphoton processes, while the main beam at the micro-channel entrance has an intensity far below the threshold; it is focused down to have an enough intensity for the “multiphoton ablation” of the stromal tissue.

Figure 2.10 shows schematically the local intensity control by giving a focal shift between the pre-pulse and the main pulse. The pre-pulses with intensity of \( \sim 10^{13} \) W/cm\(^2\) are focused at a point on the corneal. This value is slightly higher than the threshold to initiate the multiphoton processes of the corneal tissue. As a result, a very narrow micro-channel can be temporarily created that can reach to the central part of the stroma. The intensity of the main pulse at the entrance part is set to be far below the threshold for the multiphoton processes. It is focused in the deep stromal layer to have an intensity in a range of \(10^{13}\) up to \(10^{15}\) W/cm\(^2\) in order to generate the “multiphoton ablation” of a desired amount of the tissue in the chosen
the location. The intensity of the main pulses at each location can be controlled by moving the focal point and/or adjusting the main pulse energy.

### 2.3.4 Applanation

It is important to remove tissue at a constant distance from the corneal anterior surface. As shown in Figure 2.11, a flat contact glass or another thin transparent material is placed on the eye as an applanator. The applanator flattens the curvature of the cornea during the procedure. Then the distance between the corneal surface and the removal region becomes uniform, which is determined by the distance between the bottom of the contact glass and the center of the focused beam.

This parallelization effect by applying the applanation is shown in Figure 2.12. Figure 2.12 shows some images of micro-channels created in a porcine cornea without (Figure 2.12 (a)) and with (Figure 2.12 (b)) applying a flat glass plate on the corneal surface. These cross-sectional images of the tested cornea were obtained by the OCT, which is described in the next chapter. Figure 2.12 (b) clearly demonstrates that the relative location of the micro-channel to the surface can be kept consistent by applying the applanator as compared with Figure 2.12 (a). As a result, the corneal surface becomes parallel to the path of the laser beam when applying an applanator on the eye. Accordingly, a micro-channel is created along the beam path. After the applanator is removed, the eye recovers its surface curvature with the micro-channel parallel to it.

As seen in Figure 2.11, the applanation also decreases the surface area projected by the laser pulses, significantly reducing the size of the micro-channel entrance hole. Figure 2.13 shows top view images of micro-channels in the porcine corneas taken immediately after the tests. The size of the hole decreased by more than a factor of two when the applanation was applied.
Besides, we can make the micro-channel parallel to the anterior corneal surface, the applanation provides a fine control of the vertical location of the ablation region, which is the flap thickness in the LASIK procedure. This can be simply achieved by adjusting the height of the applanator, for example, using a micrometer stage.

2.3.5 Cornea reshaping patterns for correction of the refractive errors

An overall procedure is carried out by repeating the process of creating a micro-channel in a way to form a whole “multiphoton ablation” pattern. The ablation patterns for correction of each vision disorder is illustrated in Figure 2.14. As discussed in the previous section of this chapter, a spherical convex lens-shaped volume of the
Figure 2.12: Cross-sectional images of the micro-channel in the porcine cornea (a) without and (b) with applying the applanator. The gray part shows the porcine cornea, and the black part within the cornea indicates the created micro-channel.

Figure 2.13: Images of the micro-channel entrance holes (in the red circles) on the porcine corneas without (a) and with (b) applying the applanation.

stromal tissue is removed to flatten the corneal curvature for correction of myopia and a toroidal ring-shaped volume to increase the corneal curvature for correction of hyperopia. A spherical-cylindrical lens-shaped volume is removed for correction of astigmatism, which can be formed by combining the two shapes above.

Two types of patterns have been considered for myopic correction. One is to form a star shape as depicted in Figure 2.14. Micro-channels are made in the cornea by rotating the beam direction about an entrance hole to remove the stromal tissue in a part of the circular removal area (the central circle in Figure 2.14 (a)). A star shape
Figure 2.14: Schematic illustration of the “multiphoton ablation” patterns in the cornea for correction of myopia (a) and (b), and hyperopia (c). The corneal reshaping patterns are constructed by creating many micro-channels.
is formed by repeating the process to cover the whole circle with a few of holes. The main advantage of this pattern is to minimize damage in the corneal surface layer by decreasing the number of the entrance holes. However, a large number of micro-bubbles due to cavitation and the remaining ablation gases are generated around a micro-channel during the process, especially in the entrance part of the micro-channel. These bubbles considerably obstruct creation of neighboring micro-channels by scattering incoming laser pulses. Therefore, higher pulse energies are required to overcome the scattering loss. As a result, the entrance part of the micro-channel could be excessively damaged, and the ablation amount becomes less predictable, as was already discussed in previous section.

To avoid the beam scattering problem, another type of pattern can be used for myopic correction as shown in Figure 2.14 (b). In this circular pattern, each micro-channel is created through its own entrance hole. By having an appropriate separation angle between neighboring micro-channels, marked as \( \theta \) in Figure 2.14 (b) and Figure 2.15, we can avoid the micro-bubbles in the beam path. Even though many entrance holes are created on the surface, each hole size can be kept very small as lower pulse energy when the scattering losses are very much decreased. The region between the micro-channels is also affected after the procedure, because the collapse of the surface layer diffuses to the vicinity. Thus, the separation angle is determined such that the laser beam is not disturbed by the micro-bubbles, and at the same time, a smooth and uniform corneal curvature change is achieved after the collapse. Another advantage of this pattern is that it can provide a very symmetric reshaping of the cornea.
Figure 2.15: Illustration of separation of neighboring micro-channels in order to avoid beam scattering by the micro-bubbles

The pattern for correcting hyperopia also consists of a number of micro-channels as seen in Figure 2.14. These micro-channels are connected with each other forming a toroidal ring around the center of the stroma. This pattern allows much shorter length of each micro-channel than in the myopic correction, for example, less than 1 mm. A larger number of micro-channels can make the reshaping region more symmetric. The number of micro-channels, however, has to be well optimized in order to avoid any significant damage to the surface of the cornea. The ablation width is needed to be uniform across the whole reshaping region. Therefore, a constant laser intensity is applied over the full micro-channel length.
Chapter 3

Ex-vivo experiment of the flapless femtosecond LASIK on animal eye tissue

3.1 Overview

We have conducted ex-vivo experiment with animal eyes for demonstration of the flapless corneal reshaping. This procedure requires much higher laser intensity than in current ophthalmologic procedures. While the highest intensity level applied in the conventional eye surgeries to cause photodisruption is approximately $10^9$ W/cm$^2$ or less, the intensity level for the multiphoton ablation of corneal tissue is higher than $10^{13}$ W/cm$^2$. In the experiment, a high-power Ti:Sapphire femtosecond laser system has been used to generate the femtosecond pulse ($\tau \sim 100$ fs) of sufficiently high energy ($> 0.5$ mJ) to achieve such high-intensities.

In this chapter, the details of the high-power femtosecond laser system are provided. Then the experimental setup for the flapless cornea reshaping on the porcine eye is described. It is followed by the experimental results and discussion.
3.2 High-power femtosecond laser system

3.2.1 Generation of femtosecond laser pulses

Ti:Sapphire laser

The Ti:Sapphire (Ti:Al₂O₃) laser is widely used ultrashort pulse solid-state laser. The Ti:Sapphire laser crystal has a broad emission bandwidth over 400 nm (λ = 670 – 1070 nm) with a relatively high gain cross-section compared to the other tunable solid-state lasers [80]. Due to the inverse relationship between the frequency bandwidth and the time duration, the Ti:Sapphire laser crystal is ideal for generating ultrashort laser pulses by using mode-locking techniques, while it can also be operated in a continuous mode tunable over such a wide range.

![Figure 3.1: Schematics of energy level of Ti:Al₂O₃](image)

Schematic energy levels of Ti:Al₂O₃, the Ti:Sapphire laser crystal, are shown in Figure 3.1 (note: the Ti:Sapphire laser can operate in a similar way for the four-level lasing system based on the molecular vibrational states [81,82]). The electronic ground state of the Ti³⁺ ion is excited by optical pumping to the excited state. The absorption transitions occur over a broad range of wavelength from 400 to 600 nm. Then the vibrational relaxation drops the ions to the lower vibrational levels within
the excited electronic state, and lasing transitions occur to the upper vibrational levels within the ground electronic state.

The absorption and emission spectra are shown in Figure 3.2 [83]. The emission spectrum has its peak around 790 nm, with a 180 nm full-width-half-maximum (FWHM) bandwidth.

![Figure 3.2: The absorption and emission spectra of Ti:sapphire lasing medium [83].](image)

**Mode-locking**

Ti:Sapphire laser can generate ultrashort pulses by using mode-locking techniques. Typically, a mode-locked Ti:Sapphire oscillator produces pulses ranging from a few femtoseconds to picoseconds, and the pulse repetition rate is from 70 to 90 MHz.

Mode-locking methods can be classified into two types: active and passive mode-locking. In active mode-locking, a loss modulator, for example, an acousto-optic modulator, is placed inside the cavity close to one of the end mirrors (Figure 3.3 [84]).

Assuming that a mode at a frequency $\nu_0$, which is at the peak of the laser gain profile, begins to oscillate first in the cavity, it develops sidebands at $\nu_0 \pm \nu_m$, where $\nu_m$ is the modulated frequency. The modulator operation frequency is at the cavity mode frequency, $\nu_r = \frac{c}{2L}$, where $c$ is the speed of light and $L$ is the cavity length. The
Figure 3.3: A cavity configuration for active mode-locking [84].

frequencies further develop sidebands by the modulator so as to construct a broad frequency bandwidth as seen in Figure 3.4 [85]. As the pulse duration is inversely correlated to the frequency bandwidth, lasers with a large gain-bandwidth profile can achieve the ultrashort pulse durations.

In time domain, the loss modulator produces a sinusoidal loss modulation with a period equal to the round trip time, $T_r = \frac{2L}{c}$. The saturated gain is constant; thus, the net gain exists only when the loss modulation is near the minimum. Therefore, it can generate a train of pulse with pulse duration much shorter than the round trip time [86].
In passive mode-locking, a saturable absorber is used to induce self-amplitude modulation of the pulse inside the laser cavity. A saturable absorber introduces
an intensity dependent loss modulation, relatively large loss for low intensity pulses and significantly smaller loss for high intensities. Thus, this loss modulation has its minimum at the peak of the pulse as illustrated in Figure 3.5.

One of the most successful passive mode-locking methods is Kerr lens mode-locking (KLM). The Kerr lens effect is induced by the nonlinear component of refractive index of the lasing medium or an additional nonlinear medium in the cavity such as a glass plate. The refractive index of a medium can be simply expressed as

\[ n(I) = n_0 + n_2 I \]  \hspace{1cm} (3.1)

where \( n_0 \) is the linear refractive index, \( n_2 \) is the second order nonlinear refractive index, and \( I \) is the light intensity. Typical values of of the nonlinear refractive index \( n_2 \) of solid-state materials are of the order of \( 10^{-16} \text{ cm}^2/\text{W} \). For example, the nonlinear index of Ti:Sapphire crystal decreases monotonically from \( \sim 3.3 \times 10^{-16} \text{ cm}^2/\text{W} \) at \( \lambda = 550 \text{ nm} \) to \( \sim 2.8 \times 10^{-16} \text{ cm}^2/\text{W} \) at \( \lambda = 1550 \text{ nm} \) \cite{87}. Therefore, a varying refractive index is induced only when such a high intensity pulse travels in a nonlinear medium. Assuming a Gaussian intensity profile of the pulse, the central part of the pulse experiences a higher refractive index, so travelling at a lower speed than the peripheral part. Therefore, the center of the pulse is retarded, being focused along the axis of propagation. This effect causes the self-phase modulation (SPM), in which the leading edge of the pulse shifts to lower frequencies (“red shift”) and the trailing edge of the pulse to higher frequencies (“blue shift”). Thus, the bandwidth the pulse is spectrally broadened by SPM, enabling generation of shorter pulse.

Also, the nonlinear medium acts like a spherical focusing lens due to the variable refractive index profile, higher refractive index at the center. Thus, the high intensity pulse undergoes “self-focusing” in the medium. This reduces the beam size, further increasing the pulse intensity.
One method to favor the high intensity pulse in the cavity is to introduce a hard aperture as shown in Figure 3.6. The physical aperture simply cuts off the longer pulses or continuous wave by letting only the self-focusing high intensity pulse pass through it. Another method is to use the overlap between the pulse and the pumped region of the lasing medium ("soft aperture") without any additional intracavity element. These methods can create an intensity dependent loss modulation, large loss for low-intensity pulses. Therefore, such combination of SPM and self-focusing effect in KLM provides an effect of a ‘non-resonant’ saturable absorber, which is inherently
broadband. While the shortest pulse duration achieved by ultrafast dye lasers is 27 fs with $\sim 10$ mW average power, pulses around 5 fs can be directly generated by a Ti:Sapphire laser with KLM with $\sim 100$ mW average power. As an example of the intensity increase effect by KLM, we can compare a 5 fs pulse produced by KLM in a 1.5 meter cavity, which corresponds to 10 ns round trip time ($f = 100$ MHz), with a continuous wave in the same cavity but without the Kerr lens. Considering the output powers are typically similar in both cases, the light intensity can be increased by $10^6$ times by the KLM method. However, the KLM method has a drawback that it cannot start by itself. Additional perturbation to increase laser noise or some restrictive cavity designs are needed to start the pulse modulation \[82,85\].

3.2.2 Amplification of high-intensity ultrashort pulse

Chirped Pulse Amplification (CPA)

Ti:Sapphire lasers can generate relatively high energies from modest scale laser systems. However, a high-intensity laser beam tends to self-focus destructively in the optical components due to the nonlinear characteristics of the index of refraction. It limits the laser intensity in amplifiers to be less than $10^9$ W/cm$^2$ \[88\].

Chirped pulse amplification (CPA) is a technology that removes the limitation. In CPA, a mode-locked ultrashort laser pulse, typically with the energy of the order of a nanojoule, is stretched in the time domain to reduce its peak intensity. Hence, the low-intensity pulse can be amplified without damaging the optical components. A typical single stage amplifier can produce pulses up to 5 mJ at a repetition rate of 1 kHz, and multi-stage amplifier can produce pulses up to several joules at a repetition rate of up to 10 Hz. After being amplified, the pulse is recompressed to have the ultrashort pulse duration again \[89\].

The most popular method to stretch the ultrashort pulse is using diffraction gratings. High stretching ratio up to $10^4$ can be achieved with CPA systems based on
diffraction gratings. Diffraction gratings are presently available in the order of 1 m², and they allow direct recompression of pulses to have powers more than 1 PW [90].

Figure 3.7: Schematic of a chirped pulse amplification system for amplification of ultrashort pulses [91]. The gratings for the compressor have to be much larger than those for stretcher due to much larger output pulse power in comparison to input pulse power. The beam size at the grating for the compressor is significantly magnified to decrease its peak intensity below the damage threshold of the optical components.

Figure 3.7 shows a schematic of a CPA system based on diffraction gratings. The input ultrashort pulse is incident on a diffraction grating. The arranged gratings send the higher frequency lights (blue) over a longer optical path than that of the lower frequency lights (red). The stretched pulse has a positive group velocity dispersion (GVD). An ultrashort pulse can be stretched by 10,000 times using a single grating pair. After the stretched pulse is amplified through a laser amplifier such as a regenerative amplifier, which is described below, the pulse is recompressed essentially
by reversing the stretching process. The gratings are arranged to send the higher frequency light over a shorter optical path than that of the lower frequency light so that the blue light catches up with the red lights (negative GVD). It is important to use much larger gratings for the pulse compressor than those for the pulse stretcher. Since the high power output pulse may damage the optical elements when it is focused in a small area so having a very high intensity, the beam size at the grating for the compressor has to be significantly magnified in order to reduce its peak intensity below the damage threshold of the optical components.

Other stretching systems include prism-based devices, optical fibers, and devices using the natural dispersion of bulk material or specially designed dielectric chirped mirrors for relatively small stretching and compression ratios \[86\].

**Regenerative amplification**

Regenerative amplification is considered as a very efficient method to amplify the chirped pulses in a CPA system. A single pulse selected from a train of pulse is confined in the resonator by manipulating polarization. After being amplified through a number of round trips, the pulse is ejected out from the resonator. Typically, an input pulse of a few nanojoules is amplified to have several millijoules through a single Ti:Sapphire crystal rod. Thus, an overall amplification ratio could be greater than \[10^6\] \[82\].

For example, one of the commercial Ti:Sapphire regenerative amplifiers (Quanta-Ray, TSA, Spectra-Physics Lasers) operate as follows (see Figure 3.8):

1. The stretched mode-locked pulses are injected into the resonator.

2. While Pockels Cell 1 (PC1) is deactivated, the pulses pass through the quarter-wave plate (WP1) twice; therefore, they have a \(\lambda/2\) rotation that is transmitted by the polarizer (P1). Thus, the pulses are ejected out from the resonator after a single round trip.
3. PC1 is activated with quarter-wave voltage (3500 V) as soon as the selected pulse leaves it. Then PC1 becomes effectively a quarter-wave plate that partly cancels the effect of WP1. Therefore, the pulse has a $\lambda/4$ rotation when it passes through P1, while other pulses have a $\lambda/2$ rotation being ejected out after one round trip. Therefore, only the selected pulse is trapped in the resonator.

4. After several round trips, the pulse has a gain over $10^6$. Then the same voltage is applied to Pockels Cell 2 (PC2). It negates the whole effect of WP1. Thus, the pulse with no rotation is ejected out of the resonator.

### 3.3 Flapless cornea reshaping test on the porcine eye

In Section 2.3, we have described the principles and methods for creation of micro-channels in the cornea with the required specifications, including entrance hole size smaller than 30 $\mu$m and sufficiently long length ($\geq 2.5$ mm), and the “multiphoton ablation” patterns consisting of significant number of micro-channels for cornea re-
shaping in the chosen area. In order to demonstrate the flapless cornea reshaping procedure, we have conducted ex-vivo experiments on fresh porcine eyes applying the methods introduced in Section 2.3, the one-beam scheme, the two-beams scheme, local intensity control by a focal shift of the main pulse in respect to the pre-pulse, applanation, the star-shaped and the circular reshaping patterns for decreasing the corneal curvature, and the torus-shaped reshaping pattern for increasing the corneal curvature. In this section, we provide the details of the proof-of-principle experiment and the measurement of the cornea reshaping process using the optical coherence tomography (OCT). It is followed by the experimental results showing the availability of the flapless procedure to reshape the cornea in a controlled fashion. In the last part of this section, some limitations of the ex-vivo experiment using animal eyes are discussed, which demand live animal test as the next step of this research.

3.3.1 Experimental setup

A schematic of the experimental setup for animal eye test of the flapless cornea reshaping procedure is shown in Figure 3.9. A mode-locked femtosecond pulse train (82 MHz) generated from a Ti:Sapphire laser oscillator ($\lambda = 790$ nm, $\tau = 50$ fs) is amplified through a combination of a CPA and a regenerative amplification to have an output pulse energy higher than 0.5 mJ with $\tau \sim 100$ fs at the 1 kHz repetition rate. The output laser beam is split into two beams, the pre-beam and the main beam, with the energy ratio approximately 50:50. The pulses in the pre-beam, the pre-pulses, are focused by a plano convex lens ($f = 215$ mm) at a point on the corneal surface of the cornea. The intensity and the diameter of the pulse is adjusted by the attenuators and the apertures in the beam paths.

At the beginning of the test, only the pre-beam was turned on to start creating a temporary micro-channel from a point on the corneal surface toward the center of the stroma. The minimum intensity for creating and keeping the micro-channel in the
cornea was about $1 \times 10^{13}$ W/cm². The intensity of the pre-pulse was set to be as close as possible to the threshold value to minimize the diameter of the micro-channel entrance. Only a small fraction of the focused pre-pulse near the peak intensity creates a micro-channel, while the rest of the beam only generates micro-bubbles around the channel due to the lower intensity than the threshold value. Therefore, especially in the myopic correction test, a high aspect ratio of the length to the width of the micro-channel (> 100) with an entrance diameter less than 30 µm could be formed by applying a sufficiently long Rayleigh length, more than 3 mm.

As an applanator, a transparent flat glass plate has been used. The vertical location of the micro-channel in respect to cornea surface was precisely controlled by adjusting the height of the applanator. In the test, the location of the micro-channel was set to be ~ 150 m below the surface, and the applanator was removed immediately after the procedure.

The pulses in the main beam, the main pulses, travel the optical delay line to be delayed by 0.5 ns with respect to the pre-pulse. The main pulses pass through a
combination of a diverging lens \((f = -75 \text{ mm})\) and a focusing lens \((f = 225 \text{ mm})\). The distance between the two lenses to collimate the beam can be calculated by

\[
d_1 = f_c + f_d \\
d_2 = f_m + \frac{f_c f_d}{f_c + f_d - d_1}
\]

where \(d_1\) is the distance between the converging lens and the diverging lens, \(f_c\) is the focal length of the converging lens, \(f_d\) is the focal length of the diverging lens (negative), \(d_2\) is the distance between the lens combination and the second focusing lens that is also used for focusing the pre-pulse, and \(f_m\) is the focal length of the second focusing lens. In the experiment, the distance between the two lenses in the combination was around 150 mm where the magnified beam \((\sim 3x)\) was collimated.

Due to the magnified beam with a shorter Rayleigh length, the region of high intensity for multiphoton ablation around the end point of the micro-channel could be more confined.

The main pulse became coaxial with the pre-pulse at the second beam splitter (50:50), being focused toward the cornea. Passing through the two splitters of the same energy ratio, the maximum pulse energies of the pre-pulse and the main pulse were 25% of the initial output pulse energy, respectively. It is crucial to put the focuses of the pre-pulse and the main pulse on the exactly same axis. For a precise alignment, a glass piece of low reflectivity around \(\lambda = 790 \text{ nm}\) was used to divert the very weak portion of the laser beams to a charge coupled device (CCD) camera, through which the both focal spots could be accurately aligned.

The focus of the main pulse was shifted on the same axis by adjusting \(d_1\) to have a few millimeters separation from the focus of the pre-pulse. The focus of the main pulse was located at the center of the stroma in the myopic correction test, approximately 2.5 mm apart from the focus of the pre-pulse.
The main beam was initially blocked by a shutter. Once the temporary micro-channel reached the desired length, about 0.5 – 1 mm, the main beam was turned on to generate the multiphoton ablation process around the end point of the micro-channel. The intensity of the main pulse at its focus varied between $10^{13}$ and $10^{15}$ W/cm$^2$ depending on the intended amount of tissue ablation.

Porcine corneas were used for the animal test. It is known that the eyes of pigs have very similar biomechanical properties as the eyes of human beings [92, 93]. For example, the table and curves in Figure 3.10 show the experimentally measured tensile strengths and stress-strain relations of 10 human and 10 porcine cornea specimens. The similarity between the biomechanical properties of human and porcine corneas provides a good prediction for the future clinical trials of the procedure. The porcine eyes were obtained from a local abattoir and kept in wet ice until they were tested within 12 hours after being enucleated.

In the myopic correction test, the circular “multiphoton ablation” pattern was made by repetition of the single micro-channel creation process. The separation angle between the micro-channels was $9^\circ$, which resulted in the creation of 40 micro-channels. The main pulse was focused at the center of the reshaping area. In other words, the intensity of the main pulse was the highest at the stromal center such that the maximum ablation amount at the region was intended.

In the hyperopic correction test, the beam direction was tangential to the circumference of the toroidal ring. Since the length of the micro-channel was much shorter than in the myopic correction test, the one-beam scheme could be successfully applied. The toroidal “multiphoton ablation” pattern could be formed by connecting many short micro-channels. Thus, relatively low energy pulses were used for the hyperopia correction test, leading to small entrance holes and minimal trauma to the corneal surface.
Figure 3.10: Comparisons of biomechanical properties of the human and the porcine eyes, (a) the measured tensile strength of 10 human and 10 porcine cornea specimens in MPa, and (b) the stress-strain curves for human and porcine corneas [92]
3.3.2 Optical coherence tomography (OCT) for measurement of the corneal shape

The changes of the porcine corneal shape before and after the tests were assessed by using an OCT. The OCT is a low-coherence interferometric technique, in which the coherence length is of the order of tens of micrometers or less. It is used for obtaining tomographic images of materials with a resolution of micrometers. For comparison, a conventional laser interferometry has a long coherence length of the order of meters [94–96].

In the OCT, a broadband light source is split into the reference arm and the sample arm. The light from the sample arm reflected from the material is combined with the light from the reference arm. The combined light forms an interference pattern if the difference between the optical distances is less than the coherence length. For example, an OCT scheme using simple linear scan is illustrated in Figure 3.11. By scanning the mirror in the reference arm, a tomographic image of the material can be obtained [97]. The biggest advantage of the OCT technique is that it is fast and conducts direct in-vivo imaging without the preparation of the sample.

In our experiment, Carl Zeiss Visante OCT 1000 (see Figure 3.12) was used for the measurement of the corneal shapes. The optical source of the OCT machine is superluminescent diode (SLD) with the center wavelength of 1310 nm. The axial resolution of the OCT machine, which is related to the measurement of the ablation width, is 18 µm.

3.3.3 Experimental results and analysis

We observed the ablated materials from the corneal tissue being ejected out of the eye during creation of the micro-channel. In Figure 3.13 we can see an ablation gas jet from the porcine eye when the femtosecond laser beam was turned on to “drill” a
Figure 3.11: Principle of an optical coherence tomography (OCT) scheme using simple linear scan method. The superluminescent diode (SLD) output is split, reflected, and combined by the 50/50 beam coupler [97].

Figure 3.12: Carl Zeiss Visante OCT 1000 for anterior segment imaging of the eye, which has been used in described experiments.
micro-channel. The emission of the ablation gas jet implies a certain amount of the multiphoton-ablated tissue removed from the cornea.

The main aspects that we evaluated in the experiment were the quality of the micro-channel including size of the entrance, uniformity, and ablation width profile, and the overall corneal surface curvature change after the procedure. In the following sections, the experimental results on the creation of a micro-channel and improvements of the ablation profile by adopting the new techniques introduced in the previous chapter are provided. Furthermore, the test results of forming the overall reshaping patterns for correction of myopia and hyperopia are provided and discussed.

Figure 3.13: The porcine cornea emitting an ablation gas jet during creation of a micro-channel. (a) The porcine eye under the applanator without the laser pulses, and (b) the porcine eye ejecting the ablated materials with the laser pulses turned on.

Micro-channel creation with the one-beam scheme

The initial test with the porcine corneas was conducted using the one-beam scheme. A single laser pulse train at the repetition rate of 1 kHz were focused by a convex lens (f = 215 mm) at a point on the porcine corneal surface to create micro-channels. The pulse energy was \( \sim 50 \, \mu J \) at the beginning of the process and gradually increased up to \( \sim 200 \, \mu J \) when the micro-channel reached deep inside the stroma (\( \sim 2 \, \text{mm} \)).
The distance between the surface and the micro-channel was set to be \( \sim 150 \, \mu m \) by adjusting the height of the applanator.

Figure 3.14 shows the OCT cross-sectional images of the cornea before and immediately after the test. The removed area, shown as the black region in the OCT images, can be distinguished from the intact tissues, shown as the whitening region. The length of the micro-channel was approximately 2 mm, and was parallel to the surface with a \( \sim 150 \, \mu m \) separation.

The width of ablation ranged from \( \sim 60 \, \mu m \) in the entrance part of the micro-channel up to larger than \( \sim 215 \, \mu m \) around the end point of the channel. This amount of ablation is excessive considering that the maximum ablation widths at the stromal center in the practical clinics are usually 30 – 90 \( \mu m \) for correction of mild to moderate myopia and up to \( \sim 150 \, \mu m \) for a high degree myopia. The ablation width can be reduced by attenuation of the pulse energy. However, the length of the micro-channel gets shorter (< 2 mm) as the pulse energy decreases. Such a short micro-channel cannot cover the whole removal area.

As seen in Figure 3.14 (b) and (c), the ablation profiles were neither smooth nor uniform. This is because the tissue wall partly collapses within the time interval between the consecutive pulses (1 ms) so that the high-intensity pulses repeatedly hit and interact with the tissue wall during the process. In addition, the existence of the scattering bubbles and the recombined ablation gases in the micro-channel caused further non-uniformity. In addition, these factors cause significant loss of the pulse energy during its propagation through the micro-channel, requiring application of higher pulse energy to “multiphoton ablate” the desired amount of tissue in the deep cornea. The use of higher pulse energy, however, excessively damages the whole part of the micro-channel including the surface layer.

An entrance hole to the micro-channel is shown in Figure 3.14 (b) (inside the red circle). The diameter of the hole was measured to be larger than 100 \( \mu m \). Even
Figure 3.14: OCT images of the porcine cornea before the test (a), measured in a parallel direction to a micro-channel after the test (b), and measured in a normal direction to some micro-channels after the test (c). The red circle in (b) marks the micro-channel entrance hole.
though the entrance hole was the only damage to the surface layer, it had to be much smaller in order not to induce any considerable postoperative wound healing process.

**Micro-channel creation with the two-beams scheme and a focal shift**

To rectify the problems caused by the rapidly collapsing tissue wall and the recombined ablation gases, the two-beams scheme was applied. The pre-pulses provided the plasma guiding channel for the subsequent main pulses. The pre-pulse energy ($E_p$) was between 45 and 55 $\mu$J, and the main pulse energy ($E_m$) was approximately the same as the pre-pulse energy at the beginning of the creation of the channel and then gradually increased up to $\sim 75$ $\mu$J when the micro-channel end point was around 3 mm away from the surface. The main pulse was turned on when the micro-channel end point was around 0.5 mm from the entrance hole. Both pulses were focused at the same point.

Figure 3.15 shows the OCT images of a micro-channel created by using the two-beams scheme. A micro-channel was successfully formed 150 $\mu$m below the surface without any damage on the surface except the entrance hole. It is remarkable that a micro-channel as long as 3 mm could be made with half of the pulse energy of the one-beam scheme. Furthermore, the ablation profile was very smooth and uniform. It is because the pre-pulses kept the tissue wall open and cleared the scattering recombined ablation gases before the main pulses arrived and passed through the plasma channel.

In the test, we intended to ablate more amount of tissue deeper inside the stromal layer. For the myopic correction, the ablation width has to be at the maximum in the stromal center in order to flatten the curvature, whereas such a variation in the ablation width is not necessary in the hyperopic correction. In Figure 3.15 (a), the maximum ablation width was less than 100 $\mu$m around the center of the stroma, which is well within the practical requirement. However, the ablation width in the entrance part of the micro-channel, including the entrance hole, was somewhat excessive. The
Figure 3.15: The corneal cross-sections tested with the double-pulse scheme taken by the OCT, (a) the tested cornea immediately after the creation of the micro-channel, and (b) the tested cornea after the surface layer fully collapsed.

diameter of the entrance hole was measured to be approximately 100 \( \mu m \). It was still too large to avoid any significant wound healing process. This excessive damage in the entrance part of the micro-channel implies that the laser intensity around the entrance part was still too high.

A few hours after the test, the surface layer above the removed region collapsed and merged with the stromal tissue as seen in Figure 3.15 (b). It formed a smooth structure with flattening of the curvature as intended. This can be seen by the boundary line along the ablated region. It demonstrates that the flapless cornea reshaping procedure is capable of removing a certain amount of the stromal tissue from the cornea and reshaping the surface curvature without creating a flap.
A focal shift for the local intensity control within the cornea has been applied to reduce size of the entrance hole. Figure 3.16 shows the OCT cross-sections of the tested corneas with the two-beams scheme and a focal shift of 2.5 mm. The intensity sum of the pre-pulse and the main pulse around the micro-channel entrance was set to be slightly above the threshold value for the multiphoton processes in the cornea. The pre-pulse energy was fixed at around 40 µJ, and the main pulse energy varied between 40 and 90 µJ. Although the main pulse energy was of the same order as the pre-pulse energy, the main pulse could be focused more tightly than the pre-pulse because of the inversely proportional relationship between the focal size and the beam diameter at the focusing lens. The diameter of the main beam was three times larger than that of the pre-beam. Hence, the intensity of the main pulse at the focus was an order of magnitude higher ($\sim 10^{14}$ W/cm$^2$) than that of the pre-pulse with a similar energy.

In Figure 3.16, we can observe that the size of the entrance hole has been significantly reduced. The entrance hole diameter was measured to be less than 30 µm. The ablation width was also kept less than 30 µm throughout the entrance region longer than 0.5 mm. Therefore, this experimental result indicates the effectiveness of the local intensity control method to minimize the corneal surface damage.

Figure 3.16 also shows the dependence of the maximum ablation width at the stromal center on the main pulse energy. The maximum ablation widths were approximately 30 µm with $E_m \sim 40$ µJ, 50 µm with $E_m \sim 65$ µJ, and 100 µm with $E_m \sim 90$ µJ. Thus, the ablation profile can be controlled by setting local intensity of the main pulse by adjusting the pulse energy, the focal depth, and the focal shift of the main pulse in respect to the pre-pulse.
Figure 3.16: OCT images of the cross-sections of the porcine cornea tested with the two-beams scheme and a focal shift of 2.5 mm between the pre-pulse and the main pulse. The pre-pulse energy was fixed at 40 $\mu$J, and the main pulse energy at each test was $\sim$ 40 $\mu$J (a), $\sim$ 65 $\mu$J (b), and $\sim$ 90 $\mu$J (c).
Cornea reshaping test with the “multiphoton ablation” patterns

The multiphoton ablation patterns consisting of many micro-channels were made for the overall reshaping of the porcine corneas. Initially, we tried the star-shaped reshaping pattern for correction of myopia, in which the corneal curvature was intended to be decreased. Figure 3.17 the images of the cornea reshaped in the star pattern with four entrance holes using the two-beams scheme. The images were taken immediately after the test and 1 day later. The bubbles in the ablation region immediately after the test (see Figure 3.17(a)) were generated by cavitation and the gaseous ablation fragments trapped by the collapsed surface layer. The bubbles dissipated to the surrounding tissue in a few hours. However, uniform delivery of the pulse energies to the whole area was not possible because of the bubble scattering problem, which was discussed in the previous chapter. It resulted in the use of higher pulse energies than in the single micro-channel creation test. Consequently, we could not obtain a consistent outcome from this star pattern test.

Figure 3.17: Top view images of the tested cornea in the star pattern taken immediately after the test (a), and a day after (b).
As the damage of the micro-channel entrance significantly reduced by controlling the local intensity of the pulses within the cornea, we could test the circular pattern containing significant number of the entrance holes. The pre-pulse energy was $\sim 40 \mu\text{J}$, and the main pulse energy was $\sim 55 \mu\text{J}$. The pre-pulses were focused at the entrance hole of the micro-channel, and the focus of the main pulse was at the stromal center, 2.5 mm away from the entrance hole.

Forty micro-channels with 9° of separation angle were created symmetrically about the center of the circular removal area as shown in Figure 3.18. Each micro-channel was “drilled” through its own entrance hole. The diameter of the whole circle was about 5 mm. The minimum separation angle, in order to avoid the bubble scattering of the laser pulses, was found to be about 5° in the experiment. However, closely packed entrance holes on the surface would cause a non-negligible wound healing process after the procedure. Thus, the separation angle was chosen to balance between the surface damage and a smooth and uniform corneal curvature change after the collapse of the surface layer.

![Figure 3.18](image)

Figure 3.18: Top view images of the cornea reshaping process in the circular pattern with 40 entrance holes taken immediately after the test (a), and 1 day later (b).
Figures 3.19 and 3.20 show the cross-sectional images of the tested cornea before the procedure (upper images) and after the collapse of the surface layer (lower images). The OCT images were taken by scanning the tested cornea from the center of the ablation area to the circumference. The separation between the scanning lines was 0.625 mm. The direction of the scanning line was randomly chosen to confirm that the collapse of the surface layer diffused to the intact region between the micro-channels.

As a result, the surface curvatures were smoothly flattened throughout the whole scanned area with the separation angle, $\theta = 9^\circ$. The thickness change at the stromal center was maximum at the center of the scanned area and gradually decreased toward the outside. There was almost no thickness change at the circumference of the ablation area. Instead, we could observe a few “small bumps” due to collapse of the micro-channel entrance holes in the absence of the wound healing process.

In order to observe the healing effect in the surface layer, we need to conduct an in-vivo experiment with a live animal such as a pig or a rabbit. Through such experiment, we will also be able to observe how the tear film smoothes out the small irregularities of the surface layer.

In Figure 3.21 the corneal images before and after the reshaping procedure were overlapped to facilitate the comparison. The green parts in the images show the original corneal curvatures at the corresponding locations, and the whitening parts indicate the reshaped cornea. The maximum thickness change was approximately 80 $\mu$m at the center of the reshaping area; the degree of the thickness change decreases as it gets farther from the center. Consequently, it is demonstrated that the cornea can be reshaped in the form of a spherical convex lens. Further improvements of the controllability of the reshaping profile over the whole ablation area and of the symmetry would realize a successful application of the flapless cornea reshaping procedure using high-intensity femtosecond laser pulses.
Figure 3.19: OCT scans of the circularly ablated porcine cornea before the test and after the collapse of the surface layer at the center (top), 0.625 mm away from the center (middle), 1.25 mm away from the center (bottom).
Figure 3.20: OCT scans of the circularly ablated porcine cornea before the test and after the collapse of the surface layer 1.875 mm away from the center (top), 2.5 mm from the center (bottom).
Figure 3.21: Overlapped images of the corneal cross-sections before the test and after the collapse of the surface layer. The black parts show the thickness differences.
We have also tested the toroidal “multiphoton ablation” pattern for correction of hyperopia. Eight micro-channels were created and connected together to form a toroidal ring-shaped ablation region (see Figure 3.22). The one-beam scheme was applied, because the length of the micro-channel was much shorter (< 1.5 mm) than in the myopic correction test. Moreover, the ablation width had to be constant along the whole removal path. Therefore, only the pre-pulses of $E_p \sim 50 \mu J$ were turned on and focused by a convex lens with $f = 215$ mm to have a sufficient Rayleigh length longer than the micro-channel length.

![Image](image1.png)

Figure 3.22: Top view images of the tested cornea in the toroidal ring pattern with eight micro-channels taken immediately after the test (a), and a day after (b).

In the cross-section of the cornea taken immediately after the test (Figure 3.23 (a)), ablation widths of approximately 90 µm were observed. After the collapse of the surface layer (Figure 3.23 (b)), the surface parts on both the sides of the torus were merged with the stromal tissue, and the corneal curvature was increased as intended. The images of before and after the reshaping procedure were overlapped for easier comparison as seen in Figure 3.23 (c). The green parts show the change of the corneal surface curvature. Unlike in the myopic correction, the diameter of the
whole ablation area for the hyperopic correction can be easily set by changing the number of micro-channels and the adjacent angle between them.

Figure 3.23: The cross-sectional OCT images of the porcine cornea ablated in the toroidal ring shape taken immediately after the test (a), after the collapse of the surface layer (b), and an overlap of the both images (c)

3.4 Conclusion

We have investigated a new method for cornea reshaping using a high-intensity femtosecond laser. The laboratory tests with fresh porcine eyes have shown that this flapless intrastromal procedure can remove the stromal tissue, keeping the surface layer
intact. The curvatures of the tested corneas, reshaped in the circular and toroidal patterns, were changed to be flattened and bent within a practical range, respectively. In the circular reshaping test for myopic correction, the two-beams scheme and the local intensity control by giving a focal shift were applied to minimize the damage in the micro-channel entrance part as well as to create a sufficiently long micro-channel. Moreover, the circular multiphoton pattern to form the chosen ablation area enabled a scattering-free and symmetric cornea reshaping.

However, there are some limitations of the ex-vivo test with the animal eye tissue. First of all, the cornea swells over time as it loses its function for regulating corneal deturgescence after death [93]. Even though we tried to conduct the tests within a few hours after the porcine eyes were collected keeping them in a saline solution at a low temperature, we could not avoid the increase of water content in the cornea, which might have resulted in a little different condition from the live tissue. Secondly, we could not observe wound healing effect of the corneal surface layer in the channel entrance region. It is very important to investigate the degree of the wound healing process induced by the small damages created during the cornea reshaping procedure, how quickly the vision is recovered after the procedure, and the existence of any other unexpected side effects, following up for a long period.

We are in process of preparing in-vivo experiment of the flapless cornea reshaping using live rabbit as a next stage of this research. The details of the preparation and expectations on the live animal test are discussed in Appendices A and B.
Chapter 4

Multiphoton processes for the flapless corneal reshaping

4.1 Overview

In the flapless cornea reshaping, we use multiphoton processes as the main mechanism to remove the corneal tissue. It requires a high laser intensity ($> 10^{13} \text{ W/cm}^2$) that can be attained at relatively low energy by the ultrashort pulse lasers such as Ti:Sapphire femtosecond laser with CPA. Even for the much higher pulse intensity, the "ultrahigh intensity", the femtosecond laser has a low pulse energy, which could be several orders of magnitude lower than that of the nanosecond laser pulses. As a result, the multiphoton processes do not cause any harmful mechanical effects, including heat and shock wave propagation to the surroundings. Actually, our new approach to the flapless cornea reshaping can be realized only by the multiphoton processes using very high-intensity pulses of the femtosecond lasers. The micro-channels of the required specifications cannot be created in the cornea by using other methods.

In this chapter, detailed description of the multiphoton processes in the corneal tissue is provided with a demonstration experiment, and the advantages of cornea
reshaping with multiphoton processes using femtosecond lasers is discussed by comparing with thermal processes using nanosecond lasers with much higher energy, but much lower intensities.

4.2 Multiphoton processes

In multiphoton processes, individual particles, such as atoms or molecules, absorb significant number of photons almost instantaneously, in a time that is faster than the particles relaxation time [63, 65, 98]. Since the cross-sections of the multiphoton processes are very small, highly intense radiation is required for the processes to occur. These can cause multiphoton ionization of the particle due to bound-free multiphoton transitions. For example, a helium atom (He) with 24.59 eV of ionization potential can be ionized by 22-photon absorption by using a 50 ps laser pulse at $\lambda = 1060$ nm ($h\nu \approx 1.17$ eV), requiring a laser intensity of $10^{14} - 10^{15}$ W/cm$^2$ [99].

In such a high electric field, for example, produced by a laser pulse with $I \sim 10^{14}$ W/cm$^2$, several hundred eV can be efficiently transferred to a single particle by the multiphoton processes. Several hundred eV is very small energy per system, but it is very large per single particle so that higher stages of ionization can be created. The electrons on the outer shell can be removed by multiple multiphoton ionization of atoms, producing multiply charged ions [98]. These higher stage of ionizations occur through sequential steps of multiphoton ionization, which means (N+1)-times charged ions are produced from multiphoton processes of N-times charged ions. As an example, Kr$^{4+}$ ions can be produced by using a 50 ps laser pulse at $\lambda = 1064$ nm ($h\nu \approx 1.16$ eV) with intensity at $\sim 10^{14}$ W/cm$^2$ [100], which indicates at least 127.81 eV energy transfer per single Kr atom.

The multiphoton processes in the case of using very high intensity laser pulses should be distinguished from successive absorption of single photons by particles
with relatively long relaxation time of intermediate quantum states in the case of using not so high intensity light. For instance, polyatomic molecules can be excited and dissociated by consecutive absorption of several photons from an infrared laser pulse with pulse durations of nanoseconds or longer. The relaxation time of the excited molecules can be comparable or longer than the pulse duration [101]. The laser intensity used for this processes is up to $10^9$ W/cm$^2$, which is many orders lower than that for the simultaneous absorption of a large number of photons in the multiphoton processes. Besides, the number of photons absorbed per molecule is mainly determined by pulse energy per unit area (energy fluence, J/cm$^2$) in this process [102], while the pulse intensity (W/cm$^2$) plays a major role in determining the number of photons absorption in the multiphoton processes.

The mechanisms governing the multiphoton processes for different field strengths and the field frequencies can be divided into three regimes as illustrated in Figure 4.1. Each regime can be characterized by the Keldysh parameter [103,104], $\gamma$,

$$\gamma = \frac{\omega}{e} \left[ \frac{mcne_0E_g}{I} \right]^2$$  \hspace{1cm} (4.1)

where $\omega$ is the laser angular frequency, $e$ is the charge of electron, $m$ is the reduced mass of electron, $c$ is the speed of light, $n$ is the refractive index of the material, $E_g$ is the band gap energy of the material, $e_0$ is the vacuum permittivity, and $I$ is the laser field intensity. The Keldysh parameter gives the ratio between the binding potential energy of the particle (for example, Coulomb potentials in atoms and ions) and the ponderomotive energy, which is the average oscillation energy of an electron in the radiation field of the laser pulse [105].

The multiphoton ionization processes mentioned above are involved in the first regime, in which the laser electric field is small with respect to the Coulomb potential. Then the electric field can be considered as a perturbation. The rate of $N$-photon process depends on the laser intensity $I$ as $I^N$. The multiphoton ionization occurs
by almost simultaneous absorption of $N$ photons if the total sum of each photon energy, $h\nu$, exceeds the ionization energy ($Nh\nu > E_i$) as illustrated in Figure 4.1 (a). In this regime, $\gamma$ is greater than 1.5. For a 790 nm femtosecond laser applied in water, which is the main constituent of the corneal tissue, a laser intensity of $10^{13}$ W/cm$^2$ and slightly lower is involved in this regime. The intensity of the pre-pulse in the flapless cornea reshaping procedure corresponds to this regime. When a micro-channel is created by the pre-pulses, the sum of the photon energies, which are almost simultaneously absorbed by a single particle, is kept just above the ionization potential of the particle. Then the free electrons do not have enough kinetic energies to ionize by collisions other particles, hence not leading to further ionization so that the diameter of the micro-channel can be minimized.

In the second regime, the Coulomb potential is significantly perturbed by the ultra-intense local electric field produced by a higher intensity femtosecond pulse than in the first regime. A trapped valence electron within the perturbed Coulomb potential can be raised to an upper state by absorbing energies of single photons and then ionized by a quantum tunneling as shown in Figure 4.1 (b). The value of $\gamma$ is
around 1.5 in this regime. When applying a 790 nm femtosecond laser pulse in water, an intensity between $10^{13}$ and $10^{14}$ W/cm$^2$ corresponds to this value.

In the third regime, with an even higher laser intensity, the Coulomb field is highly perturbed by significantly stronger local electric field than in the second regime. The trapped valence electron can be ionized by a quantum tunneling without absorption of any photon. The $\gamma$ is lower than 1.5 in this regime, and typically an intensity in a range of $10^{14} – 10^{15}$ W/cm$^2$ is required for this process to have high probability in the case of a 790 nm femtosecond laser pulses being applied in a water.

When a high-intensity pulse sufficient to initiate the multiphoton processes interacts with a solid material such as a tissue, the particles (for example, large molecules) in the material are disintegrated and eliminated from the material by the ablation-produced pressure. This ablation process, which is caused by the simultaneous absorption of a large number of photons, is called “multiphoton ablation” and already was explained in early part of this dissertation.

The application of the multiphoton processes to a tissue can also be used for tattoo removal [66,67]. Present tattoo removal procedures use thermal photoablation of tattoo pigments in the skin by various beam colors (beam wavelengths) of nanosecond type lasers. A nanosecond laser beam is directed through the surface of the patient’s skin and absorbed by the pigment of specific color. The pigment is heated by the laser light and broken into small particles, which diffuse into the body. Hence, this thermal photoablation process requires different laser beams for different pigment colors; besides, it is very painful due to heating and removal layer-by-layer of the skin in number of sessions. On the other hand, the multiphoton processes by very high intensity but very low energy pulses have a non-thermal nature; therefore, they allow not only a painless tattoo removal, but more importantly, removal tattoo without damage of the skin, hence make possible tattoo removal much faster than with nanosecond or sub-nanosecond type of lasers. Because the multiphoton processes are
practically independent of the pigment color, due to only weak dependence of the multiphoton absorption cross section on the color of pigments, therefore the tattoo removal procedure via multiphoton processes requires only single laser beam.

4.3 Experiment on demonstration of multiphoton processes in the cornea tissue

We performed a series of experiments to demonstrate the multiphoton processes in the corneal tissue by a high-intensity femtosecond laser pulses. Unlike as in the thermal processes by longer pulse duration lasers such as the excimer nanosecond lasers, the rate of the multiphoton processes is mainly a function of the intensity of the pulses for their constant energy. The rate of the non-resonant \( N \)-photon multiphoton ionization of an atom, \( W_N \), is given by \[ W_N = \sigma_N \left( \frac{I}{h\nu} \right)^N \] (4.2)

where \( N \) is the minimum number of photons required for ionization, \( \sigma_N \) is the generalized \( N \)-photon ionization cross-section, \( I \) is the intensity of the incident laser pulse, \( h\nu \) is the plank constant, and \( \nu \) is the laser photon energy.

We can estimate the order of magnitude of the \( N \)-photon ionization cross-section using a simple calculation. When 1-photon transition takes place in an atom, the second transition can occur if the second photon is incident within the relaxation time of the interaction. The relaxation time is of the order of \( \nu^{-1} \) \[ 108 \]. Thus, the rate for the 2-photon ionization can be approximately estimated as

\[ W_2 \simeq \sigma_1 \frac{I}{h\nu} \nu^{-1} \sigma_1 \frac{I}{h\nu} \] (4.3)
where $\sigma_1$ is the cross-section of 1-photon ionization. The estimation for the rate of $N$-photon ionization can be generalized as

$$W_N \simeq (\sigma_1^N v^{-N+1}) \left( \frac{I}{h\nu} \right)^N \quad (4.4)$$

Hence, the cross-section of the $N$-photon ionization is approximately

$$\sigma_N \simeq \sigma_1^N v^{-N+1} \quad (4.5)$$

Considering $\sigma_1$ and $v^{-1}$ are typically of the orders of $10^{-17}$ cm$^2$ and $10^{-15}$ s, respectively, the crude estimation of the 4-photon ionization cross-section results in $\sigma_4 \simeq 10^{-113}$ cm$^8$s$^3$. For example, $\sigma_4$ of calcium atom was measured to be between the orders of $10^{-112}$ and $10^{-114}$ cm$^8$s$^3$ for a laser wavelength at $\lambda = 644$ nm in an experimental work [109]. Hence, this simple calculation gives a good estimation of the non-resonant multiphoton ionization cross-sections. Further, the very low cross-sections imply that effective multiple-photon ionizations can be achieved only by high laser intensities. It should be noted that the multiphoton ionization cross-section is dependent on the incident laser frequency. If a multiple of the laser frequency coincides with any proper frequency of the atom (intermediate resonant state), a resonant appears in the multiphoton processes which significantly increases the multiphoton ionization cross-section.

In the experiment, we investigated the dependence of the ablation rate of the elements in the cornea on the laser intensity through the optical emission spectroscopy. Emission light from the femtosecond laser-induced plasma from the corneal tissue was analyzed by using a spectrometer and a two-dimensional CCD camera. The spectroscopic data provided information on the spectral line intensities from the particles (molecules, atoms, ions) realized as a gas plume from the cornea, which are quite closely related to the rate of released particles of particular elements from the cornea.
Experimental setup

A schematic of the experimental setup is represented in Figure 4.2. Mode-locked femtosecond pulses were generated from a Ti:Sapphire oscillator and amplified by a CPA system with a regenerative amplifier. The output pulses were at $\lambda = 790$ nm with pulse duration of $\sim 100$ fs and maximum pulse energy of $\sim 1.5$ mJ at 1 kHz repetition rate.

Figure 4.2: A schematic of experimental setup for demonstration of multiphoton processes in the cornea stimulated by femtosecond laser pulses and monitored using the optical spectroscopy.

The femtosecond laser pulses were focused by a convex lens (focal length $f = 75$ mm) at the surface and deep in the stroma of the porcine cornea for “multiphoton ablation” of the tissue. The plasma emission was collected and sent to the spectrometer by using a combination of focusing lenses. The slit width of the spectrometer was $100 \ \mu m$. The spectral lines were detected by a highly sensitive two-dimensional CCD camera ( Mightex monochrome 1.4MP 1/2” CCD Camera).
The porcine eye was placed on a translational stage connected to a stepper motor. During the “ablation”, the cornea was continuously moved in order to provide “multiphoton ablation” of chosen spots of the cornea so that appropriate number of the emission signals could be accumulated on the CCD. The stepper motor was controlled by a microprocessor (Arduino Uno) to provide a constant speed at 10 mm/sec. In the experiment, the exposure time of the CCD was three seconds, and thus, 3,000 emission signals were accumulated in the CCD for one spectral line.

The height of the corneal surface was varied by adjusting the vertical microstage, while the location of the laser focal spot was fixed. The relative distance between the focal point and the cornea surface determined choice of the intensity of the laser pulses. The minimum beam waist at the focus, $\omega_0$, was approximately 5 $\mu$m. Assuming a Gaussian beam profile, the beam waist, $\omega(z)$, at the distance $z$ from the laser focus is given by

$$\omega(z) = \omega_0 \sqrt{1 + z^2 \left( \frac{\lambda}{\pi \omega_0^2} \right)^2} \quad (4.6)$$

Thus, the intensity $I(z)$ at $z$ can be expressed as

$$I(z) = \frac{I_0}{1 + z^2 \left( \frac{\lambda}{\pi \omega_0^2} \right)^2} \quad (4.7)$$

where $I_0$ is the laser intensity at the focus of the beam. In the test, the value of $z$ varied from 0 to 1 mm with each step size of 40 $\mu$m.

**Experimental result and discussion**

Among many elements in the corneal tissue, we could identify the strong spectral lines of calcium, including Ca I (neutral calcium atom) line at $\lambda = 422.7$ nm ($3p^64s4p^1P_1^0 - 3p^64s^2^1S_0$ transition) and the Ca II (singly ionized calcium ion) doublet lines at $\lambda = 393.4$ nm ($3p^64p^2P_{1/2}^0 - 3p^64s^2S_{1/2}$ transition) and $396.8$ nm ($3p^64p^2P_{3/2}^0$ transition).
– 3p^6 4s^2 S_{1/2} transition) \textsuperscript{[110]}. Some of the spectral data recorded by spectrometer are shown in Figure \textsuperscript{4.3}. Even though the spectrometer with CCD camera has high efficiency in the wavelength range from 370 nm to 600 nm, no other clear atomic spectral lines were detected. It is because the intensity ratio of atomic spectral line to molecular bands is generally very small in the ablation by the femtosecond laser pulses \textsuperscript{[111]}.

![Figure 4.3: Spectral lines of calcium obtained from the multiphoton ablation of the cornea tissue; (a) the doublet lines from Ca II, and (b) the line from Ca I. The line intensities are in arbitrary units, however, both Ca I and Ca II line intensities are in the same units.](image)

Figure 4.3: Spectral lines of calcium obtained from the multiphoton ablation of the cornea tissue; (a) the doublet lines from Ca II, and (b) the line from Ca I. The line intensities are in arbitrary units, however, both Ca I and Ca II line intensities are in the same units.

It is highly probable that the Ca I line came from the recombination of the singly ionized calcium ions Ca II. The ionization energy of the neutral calcium is 6.11 eV, and the photon energy of the laser light at \( \lambda = 790 \) nm is 1.57 eV. If we use the results from the crude estimation described above, the 4-photon ionization cross-section would be of the order of \( 10^{-113} \) cm\(^8\)s\(^3\). Using Equation (4.2), the value of the intensity needed for the 4-photon ionization is estimated to be of the order of \( 10^{13} \) W/cm\(^2\). Figure \textsuperscript{4.4} shows the line intensity curves of the spectral lines of Ca I and Ca II as functions of the laser intensity. Although the line intensities are in arbitrary unit, both Ca I and Ca II line intensities are in the same units. The curve for the Ca I line intensity indicates that the corresponding laser-tissue interaction can be observed with the laser intensity approximately at \( 8 \times 10^{13} \) W/cm\(^2\), which is
quite consistent with the estimation of femtosecond laser intensity of pulses for the multiphoton processes.

Figure 4.4: Ca I and Ca II spectral line intensities from the “ablation” of the corneal tissue as a function of femtosecond laser intensity. The line intensities are in arbitrary unit. Each point in the figure is result of averaging over five experimental data points.

We can also see from Figure 4.4 that the Ca II spectral lines appeared at the intensity above $5 \times 10^{14}$ W/cm$^2$. This can be interpreted as the singly ionized calcium ions were further excited by the “simultaneous” (multiphoton) absorption of significant number of photons. The intensity for generating the Ca II line signals requires at least six photons being simultaneously absorbed by a single calcium atom in the ground state to be excited to the lowest excitation level of the singly ionized calcium ion ($3p^64p$, $\Delta E > 9.26$ eV). The order of the 6-photon ionization cross-section
can also be approximately estimated as $10^{-177}$ cm$^{12}$s$^5$. Substituting this value into Equation (4.2), the laser intensity needed for the 6-photon ionization is in the order of $10^{14}$ W/cm$^2$, which quite well fits to the experimental result. It is thought that the Ca II lines at higher intensity ($> 10^{15}$ W/cm$^2$) were rather from the recombination of the doubly ionized calcium ions, which requires more than 12-photon absorption, since the energy needed for the double ionization of calcium is 17.98 eV.

It is expected that excited states of doubly ionized calcium ions (Ca III) or triply ionized calcium ions (Ca IV) would be generated as the intensity goes $10^{16}$ W/cm$^2$. Transition from the ground state to the lowest excited state of Ca III ($3s^23p^53d$) requires at least 33-photon process process ($\Delta E \geq 51.63$ eV), and transition to Ca IV requires at least 44-photon process ($\Delta E \geq 68.90$ eV). The strong lines of Ca III are in the extreme ultraviolet spectral range (EUV), shorter than $\lambda = 100$ nm. As EUV is the most highly absorbed wavelength range in air, it requires high vacuum for transmission. However, it is not practical to place an eye in a vacuum condition, which limits study of the higher number of the multiphoton processes in the cornea. Meanwhile, significantly higher number of multiphoton processes have been used for development of recombination type X-ray laser at $\lambda = 3.37$ nm in our group. 100 fs laser pulses at $\lambda = 800$ nm ($h\nu = 1.55$ eV) with $I \sim 10^{19}$ W/cm$^2$ are used in hydrogen-like C VI ions, providing the transition energy of 490 eV per single ion. This means more than 300 photons are used for single act of ionization process [112].

Figure 4.5 shows a curve of the intensity ratio of Ca II and Ca I lines as a function of the femtosecond laser intensity. It indicates that the relatively higher number of the multiphoton processes became dominant as the laser intensity increased. While the intensity of Ca I line is saturated at laser intensity around $6 \times 10^{14}$ W/cm$^2$, the intensity of Ca II line increases as the laser intensity further increases. Along with the above discussion of the order of the intensity for each process, this intensity dependence qualitatively verifies the characteristics of the multiphoton processes.
4.4 Advantages of cornea reshaping with multi-photon processes using a high-intensity femtosecond laser

When comparing nanosecond lasers with a pulse duration of 10 – 20 ns, such as ArF, KrF excimer lasers, or solid state lasers such as Nd:YAG laser, to the Ti:Sapphire femtosecond laser that have a pulse duration of about 100 fs, the Ti:Sapphire femtosecond laser is much more effective and much more precise in cornea reshaping than any of nanosecond-type lasers being presently used in cornea reshaping procedures.
Figure 4.6: The energy fluence threshold for laser-induced optical breakdown (LIOB) of human corneal tissue as a function of laser pulse duration with a calculated curve \(113\).
Figure 4.6a, b show the experimental values of the energy fluence threshold (a, in J/cm$^2$) and intensity threshold (b, in W/cm$^2$) for ablation of human corneal tissue as a function of laser pulse duration. The nanosecond lasers with $\tau = 10 - 20$ ns has the threshold fluence of $F \approx 100$ J/cm$^2$ whereas the threshold fluence for the femtosecond lasers with $\tau \sim 100$ fs is $F \approx 1$ J/cm$^2$ [113,114]. It indicates that considerably higher pulse energy is needed for the nanosecond laser to induce ablation of the tissue than for the femtosecond laser [58,115]. This implies that ablation with nanosecond laser causes much more energy conversion to the harmful mechanical effects such as heating and shock wave propagation in tissue. For example, the pressure of the generated shock wave can be expressed as [116]

$$P_s = \frac{\sqrt{I}}{\lambda_L}$$  \hspace{1cm} (4.8)

where $P_s$ is the shock wave pressure, $I$ is the laser intensity, and $\lambda_L$ is the laser wavelength. The femtosecond laser pulse has more than $10^6$ times higher intensity; therefore, the generated shock wave pressure is at least $10^3$ times higher than that of the nanosecond laser pulse. However, the distance of the shock wave propagation is proportional to the pulse energy. In the case of ablation of the eye tissue with a nanosecond laser, the shock wave decays after expanding by a few centimeters, while the shock wave generated by a femtosecond laser propagates only a few tens of micrometers [51,58]. Therefore, the “multiphoton ablation” of tissue by femtosecond lasers with very high-intensity ($I \geq 10^{13}$ W/cm$^2$), but very low pulse energy ($E_p \sim 100 - 200 \mu J$) can provide very clean tissue removal without any significant collateral damage due to heating and shock wave propagation in the tissue at any significant distance [49,117,120]. For instance, Figure 4.7 (a) shows an experimental result of corneal tissue ablations by nanosecond pulses ($\tau = 7$ ns, $\lambda = 600$ nm, 25 pulses) with energy per pulse $E_p \approx 8.8$ mJ and the intensity $I = 1.6 \times 10^7$ W/cm$^2$. We can observe a large collateral damage around the ablation site. On the other hand, femtosecond
pulses ($\tau = 300$ fs, $\lambda = 615$ nm, 4000 pulses) with much less energy per pulse $E_p \approx 0.29$ mJ but much higher intensity $I = 1.2 \times 10^{13}$ W/cm$^2$ could create very clean ablation of tissue as shown in Figure 4.7 (b). The pulse energies in both cases could not be in the same level for a good comparison because of the different threshold energy fluences for the different pulse durations. However, much larger amount of total energy ($E_p \times$ number of pulses) delivered to the corneal tissue in the case of using the femtosecond laser implies that “multiphoton ablation” by high-intensity femtosecond pulses causes much less collateral damage than in the thermal ablation by nanosecond pulses.

![Image](image_url)

**Figure 4.7:** Scanning electron microscopy of an excised human cornea ablated with (a) 7 ns pulses with $\lambda = 600$ nm, 25 pulses, and $I = 1.6 \times 10^7$ W/cm$^2$, and (b) 300 fs pulses with $\lambda = 615$ nm, 4000 pulses, and $I = 1.2 \times 10^{13}$ W/cm$^2$ [120].

Moreover, the multiphoton processes by femtosecond laser pulses provide much higher precision of tissue ablation. Since the multiphoton processes occur only near the peak of the pulse intensity due to the high intensity requirement, the nonlinear laser-tissue interaction is highly localized. The interaction volume can be smaller than the focal volume of the beam when the laser intensity is close to the threshold value. For example, Table 4.1 provides corneal ablation depths by a nanosecond pulse ($\tau = 7$ ns) and a femtosecond pulse ($\tau = 300$ fs) with threshold intensities for ablation. 

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The minimum ablation depth by the nanosecond laser pulse is \( \sim 100 \mu m \), while \( \sim 250 \) times less ablation depth can be achieved by the femtosecond laser pulse. It indicates that “multiphoton ablation” by high intensity femtosecond laser pulses can provide much higher precision of tissue removal. For that reason, in the flapless cornea reshaping procedure, only femtosecond laser pulses can create sufficiently narrow and long micro-channels in the cornea.

Table 4.1: Data for corneal ablation by nanosecond and femtosecond lasers \[120\]

<table>
<thead>
<tr>
<th></th>
<th>ns laser system (7 ns, 600 nm)</th>
<th>fs laser system (300 fs, 615 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. ablation depth, ( d_{max} ) [\mu m per pulse]</td>
<td>( \approx 100 )</td>
<td>( \approx 0.4 )</td>
</tr>
<tr>
<td>Threshold fluence, ( F_{th} ) [J cm(^{-2})]</td>
<td>( \approx 60 )</td>
<td>( \approx 1 )</td>
</tr>
<tr>
<td>Threshold intensity, ( I_{th} ) [W cm(^{-2})]</td>
<td>( \approx 9 \times 10^9 )</td>
<td>( \approx 3 \times 10^{12} )</td>
</tr>
</tbody>
</table>

The total amount of ablation is proportional to the amount of energy deposited in the tissue. However, there would be no concern about the procedure time using the femtosecond lasers, because they can be operated at high repetition rate from a few kHz up to tens of MHz.

A comparison between the single-type photon process by a nanosecond laser and the multiphoton processes by a high-intensity femtosecond laser for creation of a micro-channel in the cornea is illustrated in Figure 4.8. Assuming the wavelengths of both lasers are in the NIR regime, the single-type photon process cannot create any ablation in the corneal tissue. The low intensity nanosecond laser pulses mainly transmit through the corneal tissue without absorption, and a small portion (\( \sim 10\% \)) of the pulses are reflected or scattered. On the other hand, many photons in a high-intensity femtosecond pulse can be simultaneously absorbed by a single molecule leading to its dissociation. As a result, micro-channels in the cornea can be created
by this multiphoton processes with a very high precision and almost without collateral
effect to the surrounding tissue.

Figure 4.8: Schematic of comparison between single-photon (a) and multiphoton
processes (b). Low intensity nanosecond laser pulses ($I << 10^{13}$ W/cm$^2$) transmit
through the cornea, while high intensity femtosecond pulses ($I \geq 10^{13}$ W/cm$^2$) can
create micro-channels in the cornea by the multiphoton processes.
Chapter 5

Conclusion

The goal of this research was to develop a new type of eye refractive procedure that can eliminate the incidence of complications from the present refractive surgeries such as LASIK. The major complications of the laser refractive surgeries are caused by the flap creation process in order to expose the underlying stromal layer to the laser beam, the process in which the surface layers are reshaped. Therefore, one of the best methods to improve the laser refractive procedure is to obviate such undesirable process as creation of the flap for LASIK-type procedures. This can be done by reshaping cornea by its ablation with very high intensity laser pulses. The laser pulses are propagating towards area to be ablated via very narrow and sufficiently long micro-channels in the cornea.

In this new method of flapless cornea reshaping, such micro-channels are created using the multiphoton processes by very high intensity femtosecond laser pulses ($I \sim 10^{13} \text{ W/cm}^2$). Once the micro-channel reaches a desired length (0.5 – 1.0 mm), similar or higher intensity femtosecond pulses ($I \sim 10^{13} - 10^{15} \text{ W/cm}^2$) are delivered through the micro-channel to the end point, where a certain amount of tissue is “ablated” by the multiphoton processes. Even though the laser intensities are very high, the energy per pulse is very low ($E_p \sim 100 - 200 \mu\text{J}$). Due to much lower energy of individual
femtosecond laser pulses than in the thermal photoablation by the nanosecond lasers pulses, the multiphoton processes cause almost no collateral damage by heat and shock wave generation.

The two-beams scheme was applied to eliminate the scattering micro-bubbles and ablation gases in the beam pathway as well as to provide a plasma waveguide for the main “ablating” pulses. Energy loss by scattering was significantly reduced by using this method. In order to decrease the size of the channel entrance hole, the intensity of the main pulse at the micro-channel entrance was set to be far below the threshold for the multiphoton processes in the cornea. This was achieved by shifting focal spot of the main pulse forward in direction of its propagation and away from focal spot of the pre-pulse on cornea surface. As a result, we were able to produce a high quality micro-channel with an intended ablation width profile and minimal damage in the surface layer. In the experiment with the porcine corneas, the diameter of the micro-channel entrance hole was measured to be smaller than 30 µm, and the micro-channels could be extended longer than 2.5 mm, satisfying the requirements to be applied in the practical refractive procedure.

Overall reshaping of the porcine cornea was carried out by forming a “multiphoton ablation” pattern. A star-shaped and a circular pattern were tested for myopic correction, and a toroidal pattern for hyperopic correction. Each pattern was made by creating a certain number of micro-channels. The change of the corneal shapes was measured by using the OCT after the surface layer was collapsed. The cross-sectional images of the reshaped corneas taken by the OCT showed that the surface curvatures had changed in the intended manner, improving the myopic and the hyperopic correction tests. The corneal surface was intact except the entrance holes, which were small enough to be covered up within half an hour. In addition, the reshaped corneal curvatures were uniform and smooth with an appropriate separation angle (9°) between the micro-channels.
We also performed a series of experiments to demonstrate the multiphoton processes in the corneal tissue by very high intensity femtosecond laser pulses. Through the optical emission spectroscopy, we investigated the spectral lines of Ca I and Ca II from the femtosecond laser-induced plasma from the porcine corneal tissue. The spectral line intensities were quite closely related to the rate of the released particles from the cornea. The Ca I spectral line appeared between $I \sim 10^{13} - 10^{14}$ W/cm$^2$, and its intensity increased with the laser intensity and was saturated at laser intensity around $I \sim 6 \times 10^{14}$ W/cm$^2$. On the other hand, the spectral line for the higher number of the multiphoton processes (Ca II line) appeared near the saturation laser intensity for the Ca I line. The intensity of Ca II line kept increasing as the laser intensity further increased. This dependence of ablation rate on the laser intensity qualitatively verifies the characteristics of the multiphoton processes.

In future work, more precise methods have to be devised to achieve better controlled cornea reshaping. Also, a customized ablation profile will be required in order to achieve treatment for a higher order aberration.

There are three areas to improve the cornea reshaping procedure. First, real time monitoring of the procedure using an optical microscopy is obstructed by the micro-bubbles around the micro-channels; therefore, the location of the end point of the micro-channel cannot be exactly tracked. This could be rectified by setting up an OCT guided surgery system. It would realize real time monitoring of the cross-section of the tested cornea, including the ablation profile during the procedure. In addition, the real time monitoring of the ablation process through the OCT would provide very useful feedback for the laser parameters, leading to enhancement of the controllability of the ablation profile.

Secondly, the flapless cornea reshaping procedure can be fully automated using a computer system connected with articulated arms and other optical elements. It should be readily available for our new procedure because there is no need to handle
the flap or to extract stromal lenticule by using forceps. This is a great advantage that eliminates the incidence of human error.

Lastly, the procedure time is also another issue to be resolved. It takes currently more than half an hour to complete a whole reshaping. Compared to LASIK which usually takes less than 10 minutes to treat an eye, the procedure time of the new cornea reshaping has to be significantly shortened. This may be achieved by employing multiple laser beams. For example, a high-power laser beam from the Ti:Sapphire laser with CPA can be further split into larger number of beam pairs by adding more beam splitters in the optical configuration of the system.

In order to make this technology a surgically useful modality, accurate corneal topography analysis is of critical importance. The corneal topography is a valuable technique for measuring three dimensional structure of the corneal surface. Thus, it is a very useful tool for determining the quality of vision. However, the corneal topography could not be applied in the ex-vivo experiment on the porcine eye. As the endothelial layer lost its function to pump excessive fluid out of the stroma, the dead cornea kept being thick and opaque. It obstructed measurement of the corneal thickness change over time. In the future experiments on live animals, we will be able to assess the test results using a corneal topography such as a corneal pachymetry along with the OCT technique. Since the corneal shape may slightly change during the healing period, understanding of the wound healing time course and its influence on the corneal surface curvature is needed. Observation of wound healing process of the reshaped cornea will be possible in the live animal tests. Also, the surgical system should be equipped with proper eye tracking and stabilizing technologies to secure the eye during the ablation process. Some of the designs for holding the eye for the live animal test are discussed in Appendix A.
Through further research for these improvements, we hope to see the flapless cornea reshaping procedure treating vision defects of humans without any complication in the not too far future.
Appendix A

Preparation of setup for the femtosecond flapless LASIK procedure on live animals

Experiment of our new flapless cornea reshaping procedure on a live rabbit is now in preparation. The ex-vivo experiment with the porcine corneas had some limitations. The water content in the stromal layer is not the same as in the live tissue, because the dead tissue did not have any metabolic energy to regulate the corneal deturgescence. This also caused the porcine cornea to swell over time. Therefore, a live animal eye test is required to demonstrate the procedure in a condition close to that of a clinical trial. The live animal test will provide much more reliable assessments of the vision correction and the postoperative wound healing process. Furthermore, we might be able to observe the existence of unexpected side effects.

We have designed a laser beam delivery system with articulated mirror arms as shown in Figure A.1. After a laser beam is split into two beams and combined to be coaxial with a certain delay time, the two laser beams enter into the articulated mirror arm system. The articulated mirror arms are used to rotate the beam direction and
to deliver the beam to the focusing lens. The focusing lens is connected to a micro-translational stage so that the location of the focal points can be precisely adjusted.

![Figure A.1: Schematic of the rotational laser beam delivery system with articulated mirror arms.](image)

Unlike in the ex-vivo test, the laser beam may be blocked by the face parts around the eye such as the brow or the nose, while the orientation of the beam propagation is still required to be almost perpendicular to the optical axis of the eye. This problem can be solved by adding two mirrors behind the focusing lens to significantly reduce the radius of beam rotation as shown in Figure A.1 for example, to be less than 2 cm. In particular, the holder for the end mirror has been specially designed to avoid...
a collision with the face parts during the beam rotation. A dielectric mirror with high damage threshold (> 0.40 J/cm$^2$) has been selected as the end mirror due to the high-intensity at the mirror which is \( \sim 2 \) cm away from the focus \( (I > 10^{10} \text{ W/cm}^2) \).

Figure A.2: Schematic of the stage for holding and positioning the rabbit during the cornea reshaping procedure.

We have also designed stages for holding the rabbit during the cornea reshaping procedure and measurement. Figure A.2 shows a schematic of the stage for holding the rabbit during the procedure. The rabbit will be anesthetized and laid on a bed facing upward and fixed using the straps. The angle and position of the rabbit will be roughly adjusted by the bowl supporting bed, and be aligned by adjusting the XYZ micro-stage at the bottom.

Figure A.3 shows a schematic design for the rabbit holder for the OCT and corneal pachymetry measurement. The pachymetry measurement can also be provided by Carl Zeiss OCT 1000. The stage has been designed to fit the extruded parts of the OCT machine, which are originally for supporting and fixing the patient’s chin and forehead during the measurement. The rabbit will be fixed by the straps, and its
position will be finely controlled by adjusting the XYZ micro-stage at the bottom. The angle of the scanning line can be adjusted using the OCT machine.

![Diagram of stage for holding and positioning the rabbit during OCT and corneal pachymetry measurement](image)

Figure A.3: Schematic of the stage for holding and positioning the rabbit during the OCT and corneal pachymetry measurement viewed from two different angles.

For holding the rabbit eye during the ablation process, we will adopt the designs of the conventional LASIK instruments. Figure A.4 shows an image taken during
LASIK procedure. The lid speculum is placed to keep the eye widely open. For the flapless reshaping test on animal, the shape of the speculum has to be designed to accommodate the path of the end mirror and the laser beam. Eyelids and lashes should be kept outside the path of them as well. The suction ring holds the eye by activating vacuum. In order not to block the laser beam, which is almost parallel to the upper surface of the ring, the other part of the suction ring will be placed lower than the upper surface of the ring.

Our group of Taehee Han, Michael Giglia, Dr. Hui Xia, and Prof. Szymon Suckewer will conduct the live animal experiment with the assistance of Dr. Laura Conour and her team in the department of veterinary medicine at Princeton University.

![Image of lid speculum and suction ring](image)

Figure A.4: A lid speculum and a suction ring are placed to hold the eye during LASIK procedure [121].
Appendix B

Discussion of the flapless cornea reshaping procedure on live animals

The live animal test will firstly be performed in the same condition as in the ex-vivo test. The femtosecond pulses from the Ti:Sapphire laser system will have pulse duration of $\tau \sim 100$ fs at 1 kHz repetition rate, and the energy per pulse will be between 100 and 200 $\mu$J. The intensity of the pre-pulse will be kept slightly above the threshold value ($\sim 10^{13}$ W/cm$^2$), and the intensity of the main pulse will be varied between $10^{13}$ and $10^{15}$ W/cm$^2$ according to the amount of tissue to be removed. The delay time between the pre-pulse and the main pulse will also be $\sim 0.5$ ns. The symmetric circular pattern with a separation angle of $\sim 9^\circ$ and $\sim 40$ micro-channels will be made in the live rabbit cornea for myopic correction test, and toroidal pattern with similar or less number of micro-channels for hyperopic correction test.

Besides the OCT measurement of the cross-sectional ablation profiles and surface curvature changes of the reshaped cornea, two-dimensional maps of the corneal thickness before and after the test will be obtained using the corneal pachymetry.
The Carl Zeiss Visante OCT 1000 can provide a global thickness map of the cornea as well. However, we were not able to use the corneal pachymetry in the previous ex-vivo experiment with the porcine eyes, because the dead tissue kept swelling, not allowing correct measurement of the total thickness of the cornea. In the pachymetry mode, the system automatically processes the 8 – 16 line scans acquired and creates a pachymetry map on the screen as shown in Figure B.1. Therefore, an overall change of the corneal shape will be evaluated by the corneal pachymetry, while high-resolution cross-sectional corneal images given by the OCT will give a feedback on the laser parameters related to the multiphoton ablation amount.

Figure B.1: Pachymetry map on the review screen of Carl Zeiss Visante OCT 1000.

The measurements will be conducted preoperatively, and immediately after the test in order to take the ablation profiles before the surface collapses. Then the OCT images will be taken every hour to observe how quickly the surface layer collapses and merges with the stromal layer. In particular, it will be very important to check some micro-irregularities in the micro-channels would be smoothened out due to the
collapse of the surface layer or the tear film covering. The speed of wound recovery of the micro-channel entrance holes will also be measured and compared to the conventional procedures. The postoperative examination will be performed at regular intervals up to six months. It will be focused on the degree and period of vision correction, and existence of any significant trauma in the tested eye or other unexpected complications. The results from this animal experiment will be very useful for preparation of the future clinical trials on humans.
Bibliography


